The Ministry of Health, Labour and Welfare Ministerial Notification No. 47

Pursuant to Paragraph 1, Article 41 of the Pharmaceutical Affairs Law (Law No. 145, 1960), we hereby revise a part of the Japanese Pharmacopoeia (Ministerial Notification No. 65, 2011) as follows*. However, in the case of drugs which are listed in the Japanese Pharmacopoeia (hereinafter referred to as ''previous Pharmacopoeia'') [limited to those in the Japanese Pharmacopoeia whose standards are changed in accordance with this notification (hereinafter referred to as ''new Pharmacopoeia'')] and drugs which have been approved as of February 28, 2014 as prescribed under Paragraph 1, Article 14 of the law [including drugs the Minister of Health, Labour and Welfare specifies (the Ministry of Health and Welfare Ministerial Notification No. 104, 1994) as of February 27, 2014 as those exempted from marketing approval pursuant to Paragraph 1, Article 14 of the law (hereinafter referred to as ''drugs exempted from approval'')], the Name and Standards established in the previous Pharmacopoeia (limited to part of the Name and Standards for the drugs concerned) may be accepted to conform to the Name and Standards established in the new Pharmacopoeia before and on September 30, 2015. In the case of drugs which are listed in the new Pharmacopoeia (excluding those listed in the previous Pharmacopoeia) and drugs which have been approved as of February 28, 2014 as prescribed under the Paragraph 1 of the same law (including those exempted from approval), they may be accepted as those being not listed in the new Pharmacopoeia before and on September 30, 2015.

Norihisa Tamura
The Minister of Health, Labour and Welfare

February 28, 2014

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

*The term ''as follows'' here indicates the content of Supplement II to the Japanese Pharmacopoeia Sixteenth Edition from General Notice to Ultraviolet-visible Reference Spectra (pp. 2619 – 2813).

CONTENTS

PREFACE

The 16th Edition of the Japanese Pharmacopoeia (JP) was promulgated by Ministerial Notification No.65 of the Ministry of Health, Labour and Welfare (MHLW) on March 24, 2011.

In July 2011, the Committee on JP established the basic principles for the preparation of the JP 17th Edition, setting out the roles and characteristics of the JP, the definite measures for the revision, and the date of the revision.

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

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

The JP has been prepared with the aid of the knowledge and experience of many professionals in the pharmaceutical field. Therefore, the JP should have the characteristics of an official standard, which might be widely used by all parties concerned, and it should play an appropriate role of providing information and understanding about the quality of drugs to the public. Moreover, as a pharmaceutical quality standard, it should contribute promoting and maintaining of advancedness as well as international consistency and harmonization of technical requirements in the international community.

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

The target date for the publication of JP 17th Edition (the Japanese edition) was set as April 2016.

JP Expert Committees are organized with the following committees: Expert Committee; Sub-expert Committee; Sub-committee on Manufacturing Process-related Matters; Committee on Chemicals; Committee on Antibiotics; Committee on Biologicals; Committee on Crude Drugs; Committee on Pharmaceutical Excipients; Committee on Physico-Chemical Methods; Committee on Drug Formulation; Committee on Biological Methods; Committee on Nomenclature for Pharmaceuticals; Committee on International Harmonization and Committee on Reference Standards. Furthermore, working groups are established under the Committee on Pharmaceutical Excipients; Committee on Physico-Chemical Methods; Committee on Drug Formulation; Committee on Biological Methods and Committee on International Harmonization to expedite discussion on revision drafts.

In the Committee on JP, Takao Hayakawa took the role of chairman from July 2003 to December 2010, and Mitsuru Hashida from January 2011 to February 2014.

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

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

Draft revisions covering subjects in General Notices, General Rules for Crude Drugs, General Rules for Preparations, General Tests and Monographs, for which discussions were finished between April 2012 and September 2013, were prepared for a supplement to the JP 16. They were examined by the Committee on JP in October 2013, followed by the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) in December 2013, and then submitted to the Minister of Health, Labour and Welfare.

Numbers of discussions in the committees to prepare the supplement drafts were as follows: Expert Committee (5); Sub-committee on Manufacturing Process-related Matters (6); Committee on Chemicals (16); Committee on Antibiotics (3); Committee on Biologicals (8); Committee on Crude Drugs (16); Committee on Pharmaceutical Excipients (12); Committee on Physico-Chemical Methods (9); Committee on Drug Formulation (14); Committee on Biological Methods (13); Committee on Nomenclature for Pharmaceuticals (4); Committee on International Harmonization (10); and Committee on Reference Standards (1).

It should be noted that in the preparation of the drafts for the supplement, generous cooperation was given by the Pharmaceutical Technology Committee of the Osaka Pharmaceutical Manufacturers Association, the Pharmacopeia and CMC Committee of the Pharmaceutical Manufacturer's Association of Tokyo, the Tokyo Crude Drugs Association, the International Pharmaceutical Excipients Council Japan, the Japan Kampo Medicines Manufacturers Association, the Japan Flavor and Fragrance Materials Association, the Japan Medical Plants Federation, the Japan Pharmaceutical Manufacturers Association, the Federation of Pharmaceutical Manufacturers' Association of Japan, the Parenteral Drug Association Japan Chapter, the Japan Reagent Association, the Japan Oilseeds Processors Association, the Home Medicine Association of Japan, the Association of Membrane Separation Technology of Japan, the External Pharmaceutical Association, the Japan Alcohol Association and the Pharmacopoeial Drug Society.

In consequence of this revision, the JP 16th Edition carries 1896 articles, owing to the addition of 60 articles and the deletion of 1 article.

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

1. The Supplement II to JP 16th Edition comprises the following items, in order: Notification of MHLW; Contents; Preface; General Notices; General Rules for Crude Drugs; General Rules for Preparations; General Tests, Processes and Apparatus; Official Monographs; then followed by Infrared Reference Spectra and Ultraviolet-visible Reference Spectra; General Information; and as an appendix a Cumulative Index containing references to the MHLW Ministerial Notification No. 190, the main volume, the Supplement I and the Supplement II.

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

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

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

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

(1) Alcohol number
(2) Absorbance
(3) Congealing point
(4) Refractive index
(5) Osmolar ratio
(6) Optical rotation
(7) Constituent amino acids
(8) Viscosity
(9) pH
(10) Content ratio of the active ingredients
(11) Specific gravity
(12) Boiling point
(13) Melting point
(14) Acid value
(15) Saponification value

(16) Ester value
(17) Hydroxyl value
(18) Iodine value

5. Identification tests comprise the following items, which are generally put in the order given below:
(1) Coloration reactions
(2) Precipitation reactions
(3) Decomposition reactions
(4) Derivatives
(5) Infrared and/or ultraviolet-visible absorption spectrometry
(6) Nuclear magnetic resonance spectrometry
(7) Chromatography
(8) Special reactions
(9) Cations
(10) Anions

6. Purity tests comprise the following items, which are generally put in the order given below, except that unnecessary items are omitted depending on the nature of drug:
(1) Color
(2) Odor
(3) Clarity and/or color of solution
(4) Acidity or alkalinity
(5) Acidity
(6) Alkalinity
(7) Chloride
(8) Sulfate
(9) Sulfite
(10) Nitrate
(11) Nitrite
(12) Carbonate
(13) Bromide
(14) Iodide
(15) Soluble halide
(16) Thiocyanate
(17) Selenium
(18) Cationic salts
(19) Ammonium
(20) Heavy metals
(21) Iron
(22) Manganese
(23) Chromium
(24) Bismuth
(25) Tin
(26) Aluminum
(27) Zinc
(28) Cadmium
(29) Mercury
(30) Copper
(31) Lead
(32) Silver
(33) Alkaline earth metals
(34) Arsenic
(35) Free phosphoric acid
(36) Foreign matters
(37) Related substances
(38) Isomer
(39) Optical isomer
(40) Polymer
(41) Residual solvent
(42) Other impurities
(43) Residue on evaporation
(44) Readily carbonizable substances

7. Paragraph 23 of General Notices was revised as follows:

Paragraph 23: The definition of ''weigh accurately'' was changed to mean to weigh to one decimal place lower for accuracy corresponding to ultramicro-chemical balances.

8. To Paragraph 1 of General Rules for Crude Drugs the following items were added:
(1) Cistanche Herb
(2) Prepared Glycyrrhiza

9. The General Rule for Preparations was revised as follows:
(1) [2] Monographs for Preparations, 1. Preparations for Oral Administration: In the definition of (i) Enteric-coated (delayed-release) preparations under (2) Modified-release dosage forms, ''Enteric-coated preparations are included in a group of delayed-release preparations'' was added at the end.
(2) [2] Monographs for Preparations, 3.1. Injections: ''Among the suspensions for injection in unit-dose containers, the preparations that could impair the uniform dispersion upon standing have an appropriate uniformity'' was added under the item (17) next to (16). The previous item (17) to (21) were changed to item (18) to (22), respectively.
(3) [2] Monographs for Preparations, 5.1.2 Inhalation Liquids and Solutions, 6.1. Ophthalmic Liquids and Solutions, and 8.1.2. Nasal Liquids and Solutions: The titles were changed from Inhalation Solutions, Ophthalmic Preparations, and Nasal Solutions, respectively.

10. The following item in General Tests, Processes and Apparatus was added:

(1) 2.61 Turbidimetric Test

11. The following items in General Tests, Processes and Apparatus were revised:
(1) 1.11 Arsenic Limit Test
(2) 2.25 Infrared Spectrophotometry
(3) 5.01 Crude Drugs Test
(4) 6.02 Uniformity of Dosage Units
(5) 6.06 Foreign Insoluble Matter Test for Injections
(6) 7.02 Test Methods for Plastic Containers
(7) 7.03 Test for Rubber Closure for Aqueous Injections

12. The following Reference Sandards were added:
Clopidogrel Sulfate
Docetaxel
Insulin Glargine
Leuprorelin Acetate
D-Mannitol
Olmesartan Medoxomil
Paroxetine Hydrochloride
Pitavastatin Methylbenzylamine
Pranlukast
Sivelestat

13. The following Reference Standard was revised the name:
Spiramycin II Acetate

14. The following Standard Solution was added according to the revision of Arsenic Limit Test:
Certified Standard Arsenic Solution

15. The following substances were newly added to the Official Monographs:
Aciclovir Granules
Aciclovir Ophthalmic Ointment
Aciclovir Tablets
Azelnidipine Tablets
Bepotastine Besilate
Bepotastine Besilate Tablets
Brotizolam Tablets
Calcium Sodium Edetate Hydrate
Candesartan Cilexetil and Amlodipine Besylate Tablets
Clonazepam Fine Granules
Clonazepam Tablets
Clopidogrel Sulfate
Clopidogrel Sulfate Tablets
Colestimide Granules
Cyclophosphamide Tablets
Docetaxel Hydrate
Docetaxel for Injection
Docetaxel Injection
Fluconazole Capsules
Fudosteine
Fudosteine Tablets
Ifenprodil Tartrate Fine Granules
Ifenprodil Tartrate Tablets
Insulin Glargine (Genetical Recombination)
Insulin Glargine (Genetical Recombination) Injection
Insulin Human (Genetical Recombination) Injection
Iopamidol Injection
Leuprorelin Acetate
Losartan Potassium and Hydrochlorothiazide Tablets
Loxoprofen Sodium Tablets
Mecobalamine Tablets
Mequitazine Tablets
Naftopidil
Naftopidil Orally Disintegrating Tablets
Naftopidil Tablets
Olmesartan Medoxomil
Olmesartan Medoxomil Tablets
Olopatadine Hydrochloride
Olopatadine Hydrochloride Tablets
Paroxetine Hydrochloride Hydrate
Paroxetine Hydrochloride Tablets
Pilsicainide Hydrochloride Capsules
Pilsicainide Hydrochloride Hydrate
Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets
Pitavastatin Calcium Hydrate
Pitavastatin Calcium Tablets
Pranlukast Hydrate
Sivelestat Sodium Hydrate
Sivelestat Sodium for Injection
Tacalcitol Ointment
Tazobactam and Piperacillin for Injection
Telmisartan
Telmisartan Tablets
Belladonna Total Alkaloids
Cistanche Herb
Daisaikoto Extract
Kakkontokasenkyushin'i Extract
Maoto Extract
Otsujito Extract
Prepared Glycyrrhiza

16. The following monographs were revised:
Alprostadil Alfadex
Dried Aluminum Hydroxide Gel Fine Granules
Beclometasone Dipropionate

Betamethasone
Bisacodyl Suppositories
Calcitonin Salmon
Precipitated Calcium Carbonate Fine Granules
Calcium Pantothenate
Carmellose
Cefaclor Fine Granules
Cefcapene Pivoxil Hydrochloride Fine Granules
Cefdinir
Cefdinir Fine Granules
Cefditoren Pivoxil Fine Granules
Cefmetazole Sodium
Cefotaxime Sodium
Cefpodoxime Proxetil
Ceftazidime Hydrate
Cefteram Pivoxil
Cefteram Pivoxil Fine Granules
Cefuroxime Axetil
Celmoleukin (Genetical Recombination)
Chlordiazepoxide Powder
Corn Starch
Cortisone Acetate
Daunorubicin Hydrochloride
Dexamethasone
Dobutamine Hydrochloride
Donepezil Hydrochloride Fine Granules
Droperidol
Droxidopa Fine Granules
Epoetin Alfa (Genetical Recombination)
Ethanol
Anhydrous Ethanol
Etizolam Fine Granules
Etizolam Tablets
Filgrastim (Genetical Recombination)
Flomoxef Sodium
Fluocinolone Acetonide
Fluocinonide
Fluoxymesterone
Fursultiamine Hydrochloride
L-Glutamic Acid
Glycerin
Concentrated Glycerin
Glycine
Haloperidol Fine Granules
Heparin Calcium
Heparin Sodium
Heparin Sodium Injection
L-Histidine
Hydrocortisone
Hydrocortisone Acetate
Hydrocortisone Sodium Phosphate
Hydrocortisone Sodium Succinate
Hydrocortisone Succinate
Hypromellose
Indometacin
Insulin Human (Genetical Recombination)
Iodamide
Iohexol Injection
Irsogladine Maleate Fine Granules
L-Lysine Hydrochloride
D-Mannitol
D-Mannitol Injection
Maprotiline Hydrochloride
Mecobalamine
Meropenem for Injection
Methylcellulose
Methylprednisolone Succinate
Metildigoxin
Metoprolol Tartrate
Mexiletine Hydrochloride
Morphine Hydrochloride Hydrate
Nartograstim (Genetical Recombination)
Panipenem
Peplomycin Sulfate
Piroxicam
Polysorbate 80
Potato Starch
Pravastatin Sodium Fine Granules
Prednisolone
Prednisolone Acetate
Probucol Fine Granules
Progesterone
Propylene Glycol
Rice Starch
Risperidone Fine Granules
Roxithromycin
Sarpogrelate Hydrochloride Fine Granules
Purified Sodium Hyaluronate
Spiramycin Acetate
Spironolactone
Stearic Acid
Tegafur
Thiamine Chloride Hydrochloride
Triamcinolone
Triamcinolone Acetonide
Troxipide Fine Granules
Sterile Water for Injection in Conteiners
Wheat Starch
Zaltoprofen Tablets
Zidovudine
Acacia
Powdered Acacia
Apricot Kernel
Areca
Atractylodes Lancea Rhizome
Powdered Atractylodes Lancea Rhizome

Atractylodes Rhizome
Powdered Atractylodes Rhizome
Benincasa Seed
Brown Rice
Chrysanthemum Flower
Clove Oil
Coptis Rhizome
Powdered Coptis Rhizome
Cornus Fruit
Crataegus Fruit
Daiokanzoto Extract
Ephedra Herb
Exsiccated Gypsum
Gardenia Fruit
Powdered Gardenia Fruit
Gentian
Powdered Gentian
Glycyrrhiza
Powdered Glycyrrhiza
Hangekobokuto Extract
Hochuekkito Extract
Japanese Gentian
Jujube Seed
Kakkonto Extract
Kamishoyosan Extract
Koi
Lonicera Leaf and Stem
Magnolia Bark
Powdered Magnolia Bark
Mallotus Bark
Mentha Herb
Mentha Oil
Moutan Bark
Powdered Moutan Bark
Nutmeg
Orange Oil
Orenngedokuto Extract
Peach Kernel
Peony Root
Powdered Peony Root
Perilla Herb
Peucedanum Root
Phellodendron Bark
Pogostemon Herb
Processed Aconite Root
Powdered Processed Aconite Root
Rhubarb
Powdered Rhubarb
Royal Jelly
Saibokuto Extract
Saikokeishito Extract
Saireito Extract
Schisandra Fruit
Scutellaria Root
Powdered Scutellaria Root
Senna Leaf
Powdered Senna Leaf
Sesame
Shosaikoto Extract
Shoseiryuto Extract
Swertia Herb
Powdered Swertia Herb
Toad Venom
Wood Creosote

17. The following monograph was deleted:
Thiotepa

18. 'Particle size' was deleted from the following monographs:
Dried Aluminum Hydroxide Gel Fine Granules
Precipitated Calcium Carbonate Fine Granules
Cefaclor Fine Granules
Cefcapene Pivoxil Hydrochloride Fine Granules
Cefdinir Fine Granules
Cefditoren Pivoxil Fine Granules
Cefteram Pivoxil Fine Granules
Donepezil Hydrochloride Fine Granules
Droxidopa Fine Granules
Etizolam Fine Granules
Haloperidol Fine Granules
Irsogladine Maleate Fine Granules
Pravastatin Sodium Fine Granules
Probucol Fine Granules
Risperidone Fine Granules
Sarpogrelate Hydrochloride Fine Granules
Troxipide Fine Granules

19. The descriptions of following monographs were revised according to the provision of crystal forms:
Beclometasone Dipropionate
Betamethasone
Calcium Pantothenate
Cortisone Acetate
Dexamethasone
Doroperidol
Fluocinolone Acetonide
Fluocinonide
Fluoxymesterone
Fursultiamine Hydrochloride
L-Glutamic acid
Glycine
L-Histidine
Hydrocortisone
Hydrocortisone Acetate

Hydrocortisone Sodium Phosphate
Hydrocortisone Sodium Succinate
Hydrocortisone Succinate
Indometacin
Iodamide
L-Lysine Hydrochloride
D-Mannitol
Maprotiline Hydrochloride
Methylprednisolone Succinate
Metildigoxin
Metoprolol Tartrate
Mexiletine Hydrochloride
Piroxicam
Prednisolone
Prednisolone Acetate
Progesterone
Spironolactone
Tegafur
Thiamine Chloride Hydrochloride
Triamcinolone
Triamcinolone Acetonide
Zidovidine

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*: Chairman, the Committee on JP
**: Acting Chairman, the Committee on JP

GENERAL NOTICES

Change the paragraph 23 as follows:

23. The term ''weigh accurately'' means to weigh down to the degree of 0.1 mg, 10 μg, 1 μg or 0.1 μg by taking into account the purpose of the test and using a relevant weighing device. The term ''weigh exactly'' means to weigh to the given decimal places.

—Abbreviations—
CS: Colorimetric Stock Solution
RS: Reference Standard
TS: Test Solution
VS: Refer to a solution listed in Standard Solutions for Volumetric Analysis <*9.21*>

GENERAL RULES FOR CRUDE DRUGS

Change the paragraph 1 as follows:

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

Acacia, Achyranthes Root, Agar, Akebia Stem, Alisma Rhizome, Aloe, Alpinia Officinarum Rhizome, Aluminum Silicate Hydrate with Silicon Dioxide, Amomum Seed, Anemarrhena Rhizome, Angelica Dahurica Root, Apricot Kernel, Aralia Rhizome, Areca, Artemisia Capillaris Flower, Artemisia Leaf, Asiasarum Root, Asparagus Tuber, Astragalus Root, Atractylodes Lancea Rhizome, Atractylodes Rhizome, Bear Bile, Bearberry Leaf, Belladonna Root, Benincasa Seed, Benzoin, Bitter Cardamon, Bitter Orange Peel, Brown Rice, Bupleurum Root, Burdock Fruit, Calumba, Capsicum, Cardamon, Cassia Seed, Catalpa Fruit, Cherry Bark, Chrysanthemum Flower, Cimicifuga Rhizome, Cinnamon Bark, Cistanche Herb, Citrus Unshiu Peel, Clematis Root, Clove, Cnidium Monnieri Fruit, Cnidium Rhizome, Coix Seed, Condurango, Coptis Rhizome, Cornus Fruit, Corydalis Tuber, Crataegus Fruit, Cyperus Rhizome, Digenea, Dioscorea Rhizome, Dolichos Seed, Eleutherococcus Senticosus Rhizome, Ephedra Herb, Epimedium Herb, Eucommia Bark, Euodia Fruit, Fennel, Forsythia Fruit, Fritillaria Bulb, Gambir, Gardenia Fruit, Gastrodia Tuber, Gentian, Geranium Herb, Ginger, Ginseng, Glehnia Root and Rhizome, Glycyrrhiza, Gypsum, Hemp Fruit, Honey, Houttuynia Herb, Immature Orange, Imperata Rhizome, Ipecac, Japanese Angelica Root, Japanese Gentian, Japanese Valerian, Jujube, Jujube Seed, Koi, Leonurus Herb, Lilium Bulb, Lindera Root, Lithospermum Root, Longan Aril, Longgu, Lonicera Leaf and Stem, Loquat Leaf, Lycium Bark, Lycium Fruit, Magnolia Bark, Magnolia Flower, Mallotus Bark, Malt, Mentha Herb, Moutan Bark, Mulberry Bark, Nelumbo Seed, Notopterygium, Nuphar Rhizome, Nutmeg, Nux Vomica, Ophiopogon Tuber, Oriental Bezoar, Oyster Shell, Panax Japonicus Rhizome, Peach Kernel, Peony Root, Perilla Herb, Peucedanum Root, Pharbitis Seed, Phellodendron Bark, Picrasma Wood, Pinellia Tuber, Plantago Herb, Plantago Seed, Platycodon Root, Pogostemon Herb, Polygala Root, Polygonatum Rhizome, Polygonum Root, Polyporus Sclerotium, Poria Sclerotium, Powdered Acacia, Powdered Agar, Powdered Alisma Rhizome, Powdered Aloe, Powdered Amomum Seed, Powdered Atractylodes Lancea Rhizome, Powdered Atractylodes Rhizome, Powdered Calumba, Powdered Capsicum, Powdered Cinnamon Bark, Powdered Clove, Powdered Cnidium Rhizome, Powdered Coix Seed, Powdered Coptis Rhizome, Powdered Corydalis Tuber, Powdered Cyperus Rhizome, Powdered Dioscorea Rhizome, Powdered Fennel, Powdered Gambir, Powdered Gardenia Fruit, Powdered Gentian, Powdered Geranium Herb, Powdered Ginger, Powdered Ginseng, Powdered Glycyrrhiza, Powdered Ipecac, Powdered Japanese Angelica Root, Powdered Japanese Gentian, Powdered Japanese Valerian, Powdered Longgu, Powdered Magnolia Bark, Powdered Moutan Bark, Powdered Oyster Shell, Powdered Panax Japonicus Rhizome, Powdered Peach Kernel, Powdered Peony Root, Powdered Phellodendron Bark, Powdered Picrasma Wood, Powdered Platycodon Root, Powdered Polygala Root, Powdered Polyporus Sclerotium, Powdered Poria Sclerotium, Powdered Processed Aconite Root, Powdered Rhubarb, Powdered Rose Fruit, Powdered Scutellaria Root, Powdered Senega, Powdered Senna Leaf, Powdered Smilax Rhizome, Powdered Sophora Root, Powdered Sweet Hydrangea Leaf, Powdered Swertia Herb, Powdered Tragacanth, Powdered Turmeric, Powdered Zanthoxylum Fruit, Prepared Glycyrrhiza, Processed Aconite Root, Processed Ginger, Prunella Spike, Pueraria Root, Quercus Bark, Red Ginseng, Rehmannia Root, Rhubarb, Rose Fruit, Rosin, Royal Jelly, Safflower, Saffron, Saposhnikovia Root and Rhizome, Sappan Wood, Saussurea Root, Schisandra Fruit, Schizonepeta Spike, Scopolia Rhizome, Scutellaria Root, Senega, Senna Leaf, Sesame, Sinomenium Stem and Rhizome, Smilax Rhizome, Sophora Root, Sweet Hydrangea Leaf, Swertia Herb, Toad Cake, Tragacanth, Tribulus Fruit, Trichosanthes Root, Turmeric, Uncaria Hook, Zanthoxylum Fruit, Zedoary.

GENERAL RULES FOR PREPARATIONS

[2] Monographs for Preparations

Change the paragraph (2) (i) under 1. Preparations for Oral Administration as follows:

1. Preparations for Oral Administration

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

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

(i) Delayed-release (enteric-coated) preparations

Delayed-release preparations are designed to release the bulk of the active substance(s) not in stomach but mainly in small intestine, in order to prevent degradation or decomposition of the active substance(s) in stomach or to decrease the irritation of the active substance(s) on stomach. Delayed-release preparations are generally coated with an acid-insoluble enteric film. Delayed-release preparations are included in a group of modified-release dosage forms that delay the start to release active substance(s).

(ii) Extended-release preparations

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

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

Change the paragraphs (17) to (22) under 3-1. Injections as follows:

3-1. Injections

(17) Among the suspensions for injection in unit-dose containers, the preparations that could impair the uniform dispersion upon standing have an appropriate uniformity.

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

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

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

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

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

(iii) Name and quantity of stabilizers, preservatives, and diluents if added. In the case where nitrogen or carbon dioxide is filled in the container to replace the air inside, a statement of this replacement is not required.

(21) For ampoules or other containers of 2 mL or less, the designations ''injection'', ''for injection'' and ''aqueous suspension for injection'' may be replaced by ''inj.'', ''for inj.'' and ''aq. susp. for inj.'', respectively.

For ampoules or other containers of more than 2 mL and not exceeding 10 mL, made of glass or similar materials, the designations ''injection'', ''for injection'' and ''aqueous suspension for injection'' may be abbreviated in the same way as above, when the information is printed directly on the surface of ampoules or containers.

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

Change the title of sections 5-1-2, 6-1 and 8-1-2 as follows, respectively:

5-1-2. Inhalation Liquids and Solutions
6-1. Ophthalmic Liquids and Solutions
8-1-2. Nasal Liquids and Solutions

[*Note: These section titles have been revised in the Japanese edition, but they do not give any effect to those of sections 6-1 and 8-1-2. However, these are posted here for the consistency with the Japanese edition.*]

GENERAL TESTS, PROCESSES AND APPARATUS

1.11 Arsenic Limit Test

Change the 3. Test solutions as follows:

3. Test solutions

(i) Absorbing solution for hydrogen arsenide: Dissolve 0.50 g of silver *N*,*N*-diethyldithiocarbamate in pyridine to make 100 mL. Preserve this solution in a glass-stoppered bottle protected from light, in a cold place.

(ii) Standard Arsenic Stock Solution: Weigh exactly 0.100 g of finely powdered arsenic (III) trioxide dried at 105°C for 4 hours, and add 5 mL of sodium hydroxide solution (1 in 5) to dissolve. Add dilute sulfuric acid to neutralize, add further 10 mL of dilute sulfuric acid, add freshly boiled and cooled water to make exactly 1000 mL, and preserve in a glass-stoppered bottle.

(iii) Standard Arsenic Solution: Pipet 10 mL of Standard Arsenic Stock Solution, add 10 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 mL. Each mL of the solution contains 1 μg of arsenic (III) trioxide (As_2O_3). Prepare Standard Arsenic Solution just before use.

In the case where the preparation of Standard Arsenic Stock Solution is difficult, Certified Standard Arsenic Solution may be used to prepare Standard Arsenic Solution as follows: Pipet 15 mL of Certified Standard Arsenic Solution, add 1 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 100 mL. Pipet 5 mL of this solution, add 1 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 100 mL. Prepare just before use.

(iv) Certified Standard Arsenic Solution: JCSS Arsenic Standard Solution (100 mg/L) Each mL of this solution contains 0.1 mg of arsenic (A_S).

JCSS (Japan Calibration Service System) is a registration system of calibration service.

2.25 Infrared Spectrophotometry

Change as follows:

Infrared Spectrophotometry is a method of measurement of the extent, at various wave numbers, of absorption of infrared radiation when it passes through a layer of a substance. In the graphic representation of infrared spectra, the plot usually shows units of wave numbers as the abscissa and units of transmittance or absorbance as the ordinate. Wave number and transmittance or absorbance at each absorption maximum may be read graphically on an absorption spectrum and/or obtained by a data-processor. Since the wave number and the respective intensity of an absorption maximum depend on the chemical structure of a substance, this measurement can be used to identify or determine a substance.

1. Instrument and adjustment

Several models of dispersive infrared spectrophotometers or Fourier-transform infrared spectrophotometers are available.

The instruments, adjusted according to the instruction manual of each individual instrument, should comply with the following test for resolving power, transmittance reproducibility and wave number reproducibility. When the spectrum of a polystyrene film about 0.04 mm thick is recorded, the depth of the trough from the maximum absorption at about 2850 cm^{-1} to the minimum at about 2870 cm^{-1} should be not less than 18% transmittance and that from the maximum at about 1583 cm^{-1} to the minimum at about 1589 cm^{-1} should be not less than 12% transmittance.

The wave number (cm^{-1}) scale is usually calibrated by the use of several characteristic absorption wave numbers (cm^{-1}) of a polystyrene film shown below. The number in parentheses indicates the permissible range.

3060.0 (±1.5)	2849.5 (±1.5)	1942.9 (±1.5)
1601.2 (±1.0)	1583.0 (±1.0)	1154.5 (±1.0)
1028.3 (±1.0)		

When the dispersive infrared spectrophotometer is used, the permissible range of the absorption wave numbers at 1601.2 cm^{-1} and at 1028.3 cm^{-1} should be both within ±2.0 cm^{-1}.

As the repeatability of transmittance and wave number, the difference of transmittance should be within 0.5% when the spectrum of a polystyrene film is measured twice at several wave numbers from 3000 to 1000 cm^{-1}, and the difference of wave number should be within 5 cm^{-1} at about 3000 cm^{-1} and within 1 cm^{-1} at about 1000 cm^{-1}.

2. Preparation of samples and measurement

Unless otherwise specified, when it is directed to perform the test "after drying the sample", use a sample dried under the conditions specified in the monograph. Prepare the specimen for the measurement according to one of the following procedures. Because the amount of specimen or mixture described is as an example and that depends on the measurement conditions, prepare it so that the transmittance of most of the absorption bands is in the range of 5% to 80%. If the sample is a salt it should be noted that the salt exchange can be occurred between added potassium bromide

or potassium chloride. As a general rule in the disk method or the diffuse reflectance method, potassium chloride is used for a hydrochloride sample. For other salts, correspondence such as to try the paste method is needed.

Single crystals of sodium chloride, potassium bromide, etc. are available for the optical plate.

Generally, the reference cell or material is placed in the reference beam for double-beam instruments, while for single-beam instruments, it is placed in the same optical path in place of the specimen and measured separately under the same operating conditions. The composition and preparation of the reference depend on the sample preparation methods, and sometimes the background absorption of the atmosphere can be utilized.

Unless otherwise specified in the monograph, the spectrum is usually recorded between 4000 cm^{-1} and 400 cm^{-1}. The spectrum should be scanned using the same instrumental conditions as were used to ensure compliance with the requirements for the resolving power and for the precision of wave number scale and of wave numbers.

2.1. Potassium bromide disk or potassium chloride disk method

Powder 1 to 2 mg of a solid sample in an agate mortar, triturate rapidly with 0.10 to 0.20 g of potassium bromide for infrared spectrophotometry or potassium chloride for infrared spectrophotometry with precautions against moisture absorption, and compress the mixture with a press in a suitable die (disk-forming container) to make the sample disk. Adjust the amount of sample, potassium bromide or potassium chloride according to the size of the disk. Prepare a potassium bromide reference disk or a potassium chloride reference disk in the same manner as the sample disk. If necessary to obtain a transparent disk, press the mixture under reduced pressure not exceeding 0.67 kPa in a die with pressure applied to the die of 50 to 100 kN (5000 – 10,000 kg) per cm^2 for 5 to 8 minutes.

2.2. Solution method

Place the sample solution prepared by the method directed in each monograph in a fixed cell for liquid, and usually measure the spectrum against the reference solvent used for preparing the sample solution. The solvent used in this method should not show any interaction or chemical reaction with the specimen to be examined and should not damage the optical plate. The thickness of the fixed cell is usually 0.1 mm or 0.5 mm.

2.3. Paste method

Powder 5 to 10 mg of a solid specimen in an agate mortar, and, unless otherwise specified, triturate the specimen with 1 to 2 drops of liquid paraffin to give a homogeneous paste. After spreading the paste to make a thin film in the center of an optical plate, place the plate upon another optical plate with precautions against intrusion of air, bubbles in the film, and examine its absorption spectrum.

2.4. Liquid film method

Examine 1 to 2 drops of a liquid specimen as a thin film held between two optical plates. When the absorption intensity is not sufficient, place spacers of aluminum foil, etc., between the two optical plates to make a thicker liquid film.

2.5. Film method

Examine a thin film just as it is or a prepared thin film as directed in each monograph.

2.6. Gas sampling method

Put a sample gas in a gas cell previously evacuated under the pressure directed in the monograph, and examine its absorption spectrum. The path length of the gas cell is usually 5 cm or 10 cm, but, if necessary, may exceed 1 m.

2.7. ATR method

Place a specimen in close contact with an attenuated total reflectance (ATR) prism, and examine its reflectance spectrum.

2.8. Diffuse reflectance method

Powder 1 to 3 mg of a solid specimen into a fine powder of not more than about 50 μm particle size in an agate mortar, and triturate rapidly with 0.05 to 0.10 g of potassium bromide for infrared spectrophotometry or potassium chloride for infrared spectrophotometry with precautions against moisture absorption. Place the mixture in a sample cup, and examine its reflectance spectrum.

3. Identification

When the spectrum of a specimen and the Reference Spectrum of the substance expected to be found or the spectrum of the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the substance expected to be found. Furthermore, when several specific absorption wave numbers are specified in the monograph, the identification of a specimen with the substance expected to be found can be confirmed by the appearance of absorption bands at the specified wave numbers.

3.1. Identification by the use of a Reference Standard

When the spectra of a specimen and the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance as the Reference Standard. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with that of the Reference Standard, treat the specimen being examined and the Reference Standard in the same manner as directed in the monograph, then repeat the measurement.

3.2. Identification by the use of a Reference Spectrum

When the spectra of a specimen and the Reference Spectrum exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance associated with the Reference Spectrum. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with the Reference Spectrum, treat the specimen being examined as directed in the monograph, then repeat the measurement. Infrared Reference Spectra, in the range between 4000 cm^{-1} and 400 cm^{-1}, are shown in the section ''Infrared Reference Spectra'' for the monographs requiring the identification test by Infrared Spectrophotometry, except for monographs in which ''Identification by absorption wave number'' is specified.

3.3. Identification by the use of absorption wave number

When several specific absorption wave numbers of the substance being examined are specified in the monograph, a specimen can be identified as being the same substance as the expected substance by confirmation of clear appearance of the absorption bands at all the specified wave numbers.

Add the following:

2.61 Turbidity Measurement

Turbidity measurement is used to determine the turbidity (degree of opalescence) for the decision whether the article to be examined complies with the clarity requirement stated in the Purity.

As a rule, the visual method is specified for the requirement in individual monograph.

1. Visual method

This is used to determine the degree of opalescence with white (or faintly-colored) fine particles. So the degree of opalescence of a colored sample is liable to be determined lower that it is difficult to compare the degree correctly without using similarly colored reference suspension.

1.1. Reference suspensions

Pipet 5 mL, 10 mL, 30 mL and 50 mL of formazin opalescence standard solution, dilute them separately to exactly 100 mL with water, and use these solutions so obtained as Reference suspensions I, II, III and IV, respectively. Shake before use. Degrees of opalescence of Reference suspensions I, II, III and IV are equivalent to 3 NTU, 6 NTU, 18 NTU and 30 NTU, respectively.

1.2. Procedure

Place sufficient of the test solution, water or the solvent to prepare the test solution and, where necessary, newly prepared Reference suspensions in separate flat-bottomed test tubes, 15 – 25 mm in inside diameter and of colorless and transparent, to a depth of 40 mm, and compare the contents of the tubes against a black background by viewing in diffused light down the vertical axes of the tubes. The diffused light must be such that Reference suspension I can be readily distinguished from water, and that Reference suspension II can readily be distinguished from Reference suspension I.

In this test Reference suspensions are used when the clarity of the test solution is obscurely and it is not easy to determine that its degree of opalescence is similar or not similar to water or to the solvent used to prepare the test solution.

1.3. Interpretation

A liquid is considered ''clear'' when its clarity is the same as that of water or of the solvent used to prepare the liquid or its turbidity is not more pronounced than that of Reference suspension I. If the turbidity of the liquid is more than that of Reference suspension I, consider as follows: When the turbidity is more than that of Reference suspension I but not more than that of Reference suspension II, express ''it is not more than Reference suspension II''. In the same way, when the turbidity is more than that of Reference suspension II but not more than that of Reference suspension III, express ''it is not more than Reference suspension III'', and when the turbidity is more than that of Reference suspension III but not more than that of Reference suspension IV, express ''it is not more than Reference suspension IV''. When the turbidity is more than that of Reference suspension IV, express ''it is more than Reference suspension IV''.

1.4. Reagent solutions

Formazin opalescence standard solution: To exactly 3 mL of formazin stock suspension add water to make exactly 200 mL. Use within 24 hours after preparation. Shake thoroughly before use. Degrees of opalescence of this standard solution is equivalent to 60 NTU.

2. Photoelectric photometry

The turbidity can also be estimated by instrumental measurement of the light absorbed or scattered on account of submicroscopic optical density inhomogeneities of opalescent solutions and suspensions. The photoelectric photometry is able to provide more objective determination than the visual method. Though they can determine the turbidity by measuring the scattered or transmitted light, the measuring system and light source must be specified in individual test method, and for the comparison of observed data, the same measuring system and light source should be used.

In each case, the linear relationship between turbidity and concentration must be demonstrated by constructing a calibration curve using at least 4 concentrations. For colored samples, the turbidity value is liable to be estimated lower because of attenuating both incident and scattered lights due to the absorption by the color, and the transmission-dispersion method is principally used.

2.1. Turbidimetry

When a light passes through a turbid liquid the transmitted light is decreased by scattering with the particles dispersed in the liquid. A linear relationship is observed between turbidity and concentration when the particles with a constant size are uniformly dispersed, the size is small and the suspension is not higher concentration. The turbidity can be measured by Ultraviolet-visual Spectrophotometry <*2.24*> using spectrophotometer or photoelectric photometer. The turbidity of the sample in higher concentration can also be measured, however, it is susceptible to the color of the sample, and the measurement is usually performed at around 660 nm to avoid possible disturbance occurred from the absorption by the color.

2.2. Nephelometry

When a suspension is viewed at right angles to the direction of the incident light, it appears opalescent due to the refraction of light from the particles of the suspension (Tyndall effect). A certain portion of the light entering a turbid liquid is transmitted, another portion is absorbed and the remaining portion is scattered by the suspended particles. The scattered light measuring method shows the linear relationship between the nephelometric turbidity units (NTU) values and relative detector signals in a low turbidity range. As the degree of turbidity increases, not all the particles are exposed to the incident light and the scattered radia-

tion of other particles is hindered on its way to the detector.

2.3. Ratio Turbidimetry

This method measures both scattered and transmitted light values at the same time, and the turbidity is determined from the ratio of the scattered light value to the transmitted light value. This procedure compensates for the light that is diminished by the color of the sample and eliminates the influence of the color. When the measurement is performed by using an integrating sphere, it is particularly called the integrating sphere method, which measures the total transmitted light value as well as the scattered light value occurred with the suspended particles, and the turbidity can be determined from the ratio of them.

2.4. Application of photoelectric photometry for monograph requirements

The turbidity of the test solution, determined by the photoelectric photometry, can be used as an indicating standard for the conformity to the clarity requirements by converting into NTU by using turbidity known reference solutions such as Reference suspensions I – IV, if needed, and water or the solvent used. In an automatically compensable apparatus being calibrated with turbidity known reference solutions, the measuring result is given in NTU and it can be compared directly with required specified value.

NTU is often used as the unit in the turbidity determinations. It is the unit used in the case when the turbidity is estimated by the instrument which measures the 90 ± 30° scattered light against the incident light intensity, using tungsten lump, and in the case the estimation is performed by the instrument which measures the 90 ± 2.5° scattered light against the incident light intensity using 860 nm infrared light, FNU is used as the unit. FNU is equivalent to NTU at a range of smaller measurements (less than 40 NTU). For the unit of formazin concentration, FTU is also used, which is defined as a suspension of 1 mg formazin in 1L of purified water is 1 FTU.

5.01 Crude Drugs Test

Add the following next to 9.1. Essential oil determination:

10. Assay of Marker Compounds for the Assay of Crude Drugs and Extracts of Kampo Formulations Utilizing Nuclear Magnetic Resonance (NMR) Spectroscopy

10.1. Principle of Quantitative Analytical Technique Utilizing Nuclear Magnetic Resonance (NMR) Spectroscopy

The spectra obtained by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy after dissolving the substance to be measured in a solution, are frequently used as a powerful analytical method for determining the chemical structure of the substance from the following reasons: the resonance signals appear at different chemical shifts depending on the chemical structure of the substance measured; the signals are split by spin-spin interactions through chemical bonds mainly depending on the number of ^{1}H bonded to adjacent carbon atoms; the signal intensities (areas) are proportional to the number of ^{1}H resonating at the same frequency; etc.

In the ^{1}H-NMR spectra, the proton nuclei (^{1}Hs) in different chemical environments within the same molecule are observed as the separate signals having different chemical shifts depending on their resonance frequencies. Accordingly, we can compare the intensities of 2 signals having different chemical shifts each other. The intensity of the signal S_i would be given by the following equation (1);

$$S_i \propto N_i \frac{m}{VM} p \sin\beta \frac{1 - e^{-T_r/T_{1i}}}{1 - e^{-T_r/T_{1i}} \cos\beta} M_0 \qquad (1)$$

where N_i is the number of resonating ^{1}H which gives the signal, V is the volume of the sample solution, m is the mass of the sample, M is the molecular mass of the substance measured, p is the purity of the sample, β is the excitation pulse angle, T_{1i} is the spin-lattice relaxation time of ^{1}H which gives the signal, T_r is the repetition time, M_0 is the equilibrium magnetization and the subscript i indicates the independent signal. The relaxation time of a ^{1}H is different depending on the environments of the ^{1}Hs. Since the sensitivity of NMR is not so good, the signal-to-noise ratio (S/N ratio) of signals should generally be improved by measuring it repeatedly and averaging noises. When the NMR measurement is performed under the condition with the repetition time T_r sufficiently longer than the longest T_1 among the T_{1s} of the signals observed for the analyte compound, the condition of $1 - e^{-T_r/T_1} \approx 1$ for all of the signals of the analyte compounds would be satisfied and quantitative analysis utilizing NMR (quantitative NMR) can be performed. On the other hand, when NMR is used for the structural determination, priority is given to improve detection sensitivity, and the condition for increasing the S/N ratio of signals by using repeated measurements is usually used. Under this condition, since the repetition time is not long enough to ensure quantitative NMR, the proportion of signal intensity to the number of each equivalent ^{1}H nuclei in the measured molecule is not obtained precisely.

However, when NMR is measured under the conditions, which ensure quantitative performance, the signal intensity ratio proportional to each number of equivalent molecule is obtained.

When the intensity of two signals having different chemical shifts in the same molecule are compared under the quantitative conditions which ensure quantitative performance, the following equation (2) is obtained and the signal intensities S_i and S_j are found to be proportional to the number of resonating ^{1}Hs.

$$\frac{S_i}{S_j} = \frac{N_i}{N_j} \qquad (2)$$

This proportionality between the signal area and number of resonating ^{1}H can be applied to the signals from 2 different molecules. In this case, since it is considered that the excitation pulse angle and the volume of the sample solution used for the measurement can be kept constant independent of the substance measured, the following equation (3), in which the observed signal area S is proportional only to the

purity, molecular mass and mass used for the measurement of analyte compound, can be obtained. (a and s indicate the signals of the analyte compound and a reference substance (internal standard), respectively.)

$$p_a = \frac{S_a N_s M_a m_s}{S_s N_a M_s m_a} p_s \quad (3)$$

Although there are some prerequisites to be met, such that each molecule should not interact (such as react) with other molecules in the solution and the molecule should have separate signals at different chemical shifts from others, we will be able to evaluate the purity of the analyte compound by measuring its ^{1}H-NMR under the conditions which ensure quantitative performance, if we have a standard material with known purity and use it as an internal standard for the measurement. In other words, if a standard material whose molecular mass and accurate purity are known would be provided as the superior standard, we can evaluate the purity of the substances coexisting in the solution of the standard material by measuring ^{1}H-NMR of the solution. In this case, when traceability of the measurement to the International System of Units (SI) is guaranteed for the standard material, purity of the analyte compound can be calculated indirectly as the SI traceable value by using the standard material as the superior standard. In such a measurement, it is necessary to dissolve the sample and the standard material in a solution. Thus, it is practically important for precise evaluation of the purity of analyte compound that both of the sample and the standard material should be weighed accurately, and dissolved in a solvent for NMR measurement.

10.2. Supply of Reference Materials and Software for Quantitative NMR

From among certified reference materials supplied from public institutions (NMIJ CRM), those with SI traceable pricing have been marketed as internal reference materials. Easy-to-use solid-state compounds include 1,4-bis(trimethylsilyl)benzene-d_4 (BTMSB-d_4), methanol, and dimethylsulfoxide for organic solvent use and 3-(trimethylsilyl)-1-propanesulfonic acid-d_6-sodium salt (DSS-d_6), maleic acid and dimethyl sulfone for aquatic use which both exhibit a sharp peak for specific chemical shifts in ^{1}H-NMR. In addition, such measurement software capable of performing quantification (qNMR) easier based on the above-mentioned principle is also supplied by NMR manufacturers.

10.3. Marker Compounds for Assay and Preparations for Quantitative Analysis of Crude Drugs and Extracts of Kampo Formulations in the JP

If it is possible to price a reagent used as a quantitative index component in a crude medicine with a correct content using qNMR based on the above-mentioned principle, it also becomes possible to use the reagent as a preparation for analysis with assured metrological traceability. According to a result of a validation experiment, in case of a compound with molecular mass of around 300 to be measured, it is possible to perform pricing at an ordinary laboratory level by using about 10 mg of the compound for the measurement while ensuring two significant figures even if including errors between used devices. As content of quantitative index component in a crude medicine is just a few percent at most in general and the minimum unit of regulation value is 0.1%, two significant figures is believed to be enough to ensure accuracy of content of preparation for quantitative analysis in consideration of variation for each crude medicine as a natural substance.

Such reagents priced with SI traceable quantitative value (degree of purity) by qNMR that have been defined in a paragraph for reagent and test solution are available as Japanese Pharmacopoeia regents for quantitative analysis. Further, in cases where a reagent priced by qMNR is used as a preparation for quantitative analysis such as HPLC and involved in a calculation of quantitative value of subject compound after converting degree of purity (%) of the priced regent, it becomes possible to use the resulting quantitative value as a SI traceable value. In addition, in cases where a reagent priced by qNMR is used as a reference material for a quantitative analysis based on HPLC, condition of the quantitative analysis is based on an assumption that no impurity is recognized at any peak of a component of the regent to be quantified, which is required to be confirmed separately by a device such as photodiode array detector or mass spectrometer.

10.4. Precautions for Performing qNMR

In order to perform qNMR, such device is required that is capable of gated decoupling for ^{13}C-NMR with higher accuracy in a magnetic field with a resonance frequency of at least 400 MHz or higher for ^{1}H-NMR in consideration of resolution required for separation of impurities from peaks and even detection sensitivity as well. Further, it is also required to perform measurement under a condition that receiving sensitivity of the receiver is appropriate with optimally adjusted probe tuning and shim.

In terms of reagents for quantification for which qNMR is performed, amounts of reagents and internal reference materials to be taken are defined in paragraph 9.41 Reagents, Test Solutions. As high accuracy is required for both of them, it is required to use an ultramicro balance to take the amounts by the minimum weight of the balance or higher. Defined amounts to be taken for both of them are those described as validated realistic minimum amounts. Therefore, in cases where both of them are completely dissolved, SN ratio of spectrum is improved when measured after increasing the amounts while keeping the quantitative ratio, resulting in measurements with higher accuracy in most cases. Even though SN ratio is even more improved when a measurement is performed by integrating as many times as possible resulting in a measurement result with higher accuracy, it is required to consider stability of the magnetic field and the devices if the measurement lasts more than a few hours. Sensitivity is also improved, albeit a little, by using deuterated solvent with higher deuteration rate. In some cases, impurity signal may be detected on spectrum which has not been observed before, by further improving SN ratio. In cases where any existence of signal derived from such impurities has been made clear, the range of chemical shift where such signal exists should not be integrated. In addition, as signals of small amount of impurities have been

observed also in deuterated solvent for NMR measurement or BTMSB-d_4 or DSS-d_6 as internal reference materials, it is important to recognize the range of these impurities signals before qNMR measurement. Moreover, qNMR measurement should be performed immediately after sample preparation, since impurity signals have been confirmed to increase albeit a little by little if samples are kept in the solvent for measurement for long hours. Even though it is not necessary to measure NMR under qNMR condition for confirming impurity signal, it is easier to distinguish it from satellite signal by performing a measurement under a condition of decoupling of ^{13}C NMR without performing spinning. While BTMSB-d_4 and DSS-d_6, which are used for qNMR as internal reference materials, have chemical shift values at around 0.2 ppm and 0.1 ppm respectively when tetramethylsilane (in organic solvent) or DDS (in deuterated water) is used as the reference for chemical shifts (δ), chemical shifts of other signals are indicated by regarding the chemical shifts of these internal reference materials as 0 ppm for convenience when measuring qNMR.

6.02 Uniformity of Dosage Units

Change the paragraphs 1. Content Uniformity and 2. Mass Variation as follows:

1. Content Uniformity

Select not less than 30 units, and proceed as follows for the dosage form designated.

Where different procedures are used for assay of the preparation and for the content uniformity test, it may be necessary to establish a correction factor to be applied to the results of the latter.

(i) Solid dosage forms: Assay 10 units individually using an appropriate analytical method. Calculate the acceptance value (see Table 6.02-2).

(ii) Liquid or Semi-Solid dosage forms: Assay 10 units individually using an appropriate analytical method. Carry out the assay on the amount of well-mixed material that is removed from an individual container in conditions of normal use and express the results as delivered dose. Calculate the acceptance value (see Table 6.02-2.).

1.1. Calculation of Acceptance Value

Calculate the acceptance value by the formula:

$$|M - \overline{X}| + k_s,$$

in which the terms are as defined in Table 6.02-2.

[*Note: A part of the paragraph 1. Content Uniformity will be revised in the Japanese edition, but it does not give any effect to English text. This is daringly posted here for the consistency with the Japanese edition.*]

2. Mass Variation

◆*Mass Variation* is carried out based on the assumption that the concentration (mass of drug substance per mass of dosage unit) is uniform in a lot.◆

Carry out an assay for the drug substance(s) on a representative sample of the batch using an appropriate analytical method. This value is result A, expressed as % of label claim (see *Calculation of the Acceptance Value*). Select not less than 30 dosage units, and proceed as follows for the dosage form designated.

(i) Uncoated or Film-coated Tablets: Accurately weigh 10 tablets individually. Calculate the content, expressed as % of label claim, of each tablet from the mass of the individual tablets and the result of the assay. Calculate the acceptance value.

(ii) Hard Capsules: Accurately weigh 10 capsules individually, taking care to preserve the identity of each capsule. Remove the contents of each capsule by suitable means. Accurately weigh the emptied shells individually, and calculate for each capsule the net mass of its contents by subtracting the mass of the shell from the respective gross mass. Calculate the drug substance content of each capsule from the mass of the individual capsules and the result of the assay. Calculate the acceptance value.

(iii) Soft Capsules: Accurately weigh the 10 intact capsules individually to obtain their gross masses, taking care to preserve the identity of each capsule. Then cut open the capsules by means of a suitable clean, dry cutting instrument such as scissors or a sharp open blade, and remove the contents by washing with a suitable solvent. Allow the occluded solvent to evaporate from the shells at room temperature over a period of about 30 minutes, taking precautions to avoid uptake or loss of moisture. Weigh the individual shells, and calculate the net contents. Calculate the drug substance content in each capsule from the mass of product removed from the individual capsules and the result of the assay. Calculate the acceptance value.

(iv) Solid dosage forms other than tablets and capsules: Proceed as directed for *Hard Capsules*, treating each dosage unit as described therein. Calculate the acceptance value.

(v) Liquid dosage forms: Accurately weigh the amount of liquid that is removed from each of 10 individual containers in conditions of normal use. If necessary, compute the equivalent volume after determining the density. Calculate the drug substance content in each container from the mass of product removed from the individual containers and the result of the assay. Calculate the acceptance value.

2.1. Calculation of Acceptance Value

Calculate the acceptance value as shown in *Content Uniformity*, except that ◆the value of $\overline{X}$ is replaced with A, and that◆ the individual contents of the dosage units are replaced with the individual estimated contents defined below. x_1, $x_2 \ldots x_n$ = individual estimated contents of the dosage units tested, where

$$x_i = w_i \times \frac{A}{\overline{W}}$$

w_1, $w_2 \ldots w_n$ = individual masses of the dosage units tested,
A = content of drug substance (% of label claim) obtained using an appropriate analytical method.
$\overline{W}$ = mean of individual masses (w_1, $w_2 \ldots$, w_n).

Table 6.02-2

Variable	Definition	Conditions	Value
$\overline{X}$	mean of individual contents (x_1, x_2, ..., x_n) expressed as a percentage of the label claim		
$x_1, x_2, \ldots, x_n$	individual contents of the dosage units tested, expressed as a percentage of the label claim		
n	sample size (number of dosage units in a sample)		
k	acceptability constant	If $n = 10$, then	2.4
		If $n = 30$, then	2.0
s	sample standard deviation		$\sqrt{\dfrac{\sum_{i=1}^{n}(x_i - \overline{X})^2}{n-1}}$
RSD	relative standard deviation (the sample standard deviation expressed as a percentage of the mean)		$\dfrac{100s}{\overline{X}}$
M (case 1) To be applied when $T \leq 101.5$	reference value	If $98.5\% \leq \overline{X} \leq 101.5\%$, then	$M = \overline{X}$ $(AV = ks)$
		If $\overline{X} < 98.5\%$, then	$M = 98.5\%$ $(AV = 98.5 - \overline{X} + ks)$
		If $\overline{X} > 101.5\%$, then	$M = 101.5\%$ $(AV = \overline{X} - 101.5 + ks)$
M (case 2) To be applied when $T > 101.5$	reference value	If $98.5\% \leq \overline{X} \leq T$, then	$M = \overline{X}$ $(AV = ks)$
		If $\overline{X} < 98.5\%$, then	$M = 98.5\%$ $(AV = 98.5 - \overline{X} + ks)$
		If $\overline{X} > T$, then	$M = T\%$ $(AV = \overline{X} - T + ks)$
Acceptance Value (AV)			general formula: $\lvert M - \overline{X} \rvert + ks$ [Calculations are specified above for the different cases.]
$L1$	maximum allowed acceptance value		$L1 = 15.0$ unless otherwise specified.
$L2$	maximum allowed range for deviation of each dosage unit tested from the calculated value of M	On the low side, no dosage unit result can be less than $0.75M$ while on the high side, no dosage unit result can be greater than $1.25M$ (This is based on an $L2$ value of 25.0.)	$L2 = 25.0$ unless otherwise specified.
T	target content per dosage unit at time of manufacture, expressed as the percentage of the label claim. Unless otherwise stated, T is 100.0%, or T is the manufacturer's approved target content per dosage unit.		

6.06 Foreign Insoluble Matter Test for Injections

Change as follows:

Foreign Insoluble Matter Test for Injections is a test method to examine foreign insoluble matters in injections.

1. Method 1.

This method is applied to either injections in solution, suspension or emulsion, and vehicles for solid injections to be dissolved or suspended before use.

Clean the exterior of containers, and inspect with the unaided eyes at a position of light intensity of approximately 1000 lx under an incandescent lamp: Injections or vehicles must be free from readily detectable foreign insoluble matters. As to Injections in plastic containers for aqueous injections, the inspection should be performed with the unaided eyes at a position of light intensity of approximately 8000 to 10,000 lx, with an incandescent lamp at appropriate distances above and below the container.

2. Method 2.

This method is applied to solid injections to be dissolved or suspended before use.

Clean the exterior of containers, and dissolve or suspend the contents with vehicles attached to the preparations or with Water for Injection carefully, avoiding any contamination with extraneous foreign substances. The solution thus constituted must be free from foreign insoluble matters that is clearly detectable when inspected with the unaided eyes at a position of light intensity of approximately 1000 lx, right under an incandescent lamp.

7.02 Test Methods for Plastic Containers

Change the section of 1.7 as follows:

1.7. Cytotoxicity test

The following test methods are designed to detect cytotoxic substances in plastic materials by evaluating the cytotoxicity of the culture medium extracts from plastic containers for pharmaceutical products. Other appropriate standard methods of cytotoxicity testing may be used for the evaluation, if appropriate. However, the final decision shall be made based upon the test methods given here, if the test results obtained according to the other methods are questionable. Other than those of the culture medium, reagents and test solutions being specified for the test may be used if they meet for the purpose of the test.

1.7.1. Cell lines

The recommended cell lines are L929 cells (ATCC. CCL1) and V79 cells (JCRB0603). In addition, other established cell lines may be used when it is confirmed that they form well-defined colonies reproducibly, with characteristics comparable to those of L929 cells and V79 cells.

1.7.2. Culture medium

(i) Medium for L929 cells: To Eagle's minimum essential medium add fetal calf serum (FCS) to make 10 vol% FCS.

(ii) Medium for V79 cells: M05 medium prepared by adding 10 mL each of nonessential amino acid TS and 100 mmol/L sodium pyruvate TS to 1000 mL of Eagle's minimum essential medium, then adding fetal calf serum (FCS) to make 5 vol% FCS. Medium for L929 cells may be used instead if it gives equivalent sensitivity.

1.7.3. Reference materials and control substances

(i) Negative reference material: high-density polyethylene film

(ii) Positive reference material (A): polyurethane film containing 0.1% zinc diethyldithiocarbamate

(iii) Positive reference material (B): polyurethane film containing 0.25% zinc dibutyldithiocarbamate

(iv) Control substances: zinc diethyldithiocarbamate or zinc dibutyldithiocarbamate

1.7.4. Test procedure

(i) Sample preparation: When the material of the container consists of a single homogeneous layer, subdivide the cut pieces of a container into pieces of the size of approximately 2 × 15 mm and subject the pieces to the test. When the material of the container has multiple layers, such as laminated and coated materials, prepare cut pieces with a surface area of one side of 2.5 cm^2 and subject the pieces to the test without subdividing them into smaller pieces.

(ii) Preparation of sample solutions: Transfer an appropriate amount of the sample to a screw-capped glass bottle or a sterile disposable centrifuge tube. Cap the bottle or tube loosely and cover the cap with clean aluminum foil. Sterilize the bottle or tube by autoclaving at 121°C for 15 minutes. When the material of the sample is not resistant to heat during autoclaving, gas sterilization with ethylene oxide (EO) may be used. In the case of EO sterilization, sufficient aeration should be achieved to avoid an additional toxic effect of residual EO in the test results. To the bottle or tube add the culture medium in a proportion of 1 mL per 2.5 cm^2 (one side) or 10 mL per 1 g of the sample, loosely cap the bottle or tube, and allow to stand in an incubator maintaining 5% carbon dioxide at 37°C for 24 hours. Transfer the culture medium extract, which is designated 100% sample solution, to a sterilized screw-capped glass bottle or a sterile disposable centrifuge tube. Dilute the 100% sample solution with fresh culture medium using a dilution factor of two to prepare serial dilutions having extract concentrations of 50%, 25%, 12.5%, 6.25%, 3.13% and so on.

(iii) Preparation of cell suspension: Remove the culture medium from the maintained cell culture vessel (flask or dish), and add gently a suitable volume of phosphate buffer solution for cytotoxicity test. Rinse the cells by gentle rotation of the cell culture vessel two or three times, and discard the phosphate buffer solution. Add a sufficient volume of trypsin solution to cover the cell layer. Cap the vessel and place in an incubator maintaining 5% carbon dioxide at 37°C for 1 to 2 minutes. After confirming detachment of the cell layer from the bottom surface of the vessel by using a

microscope and by gently tapping of the vessel, add an appropriate volume of the fresh culture medium and gently pipet the cells completely out of the vessel. Transfer the pipetted cell suspension into a sterile disposable centrifuge tube and centrifuge. Discard the supernatant liquid, resuspend the cells in an appropriate volume of flesh phosphate buffer solution for cytotoxicity test by pipetting, and centrifuge the tube again. Discard the supernatant liquid, and add an appropriate volume of fresh culture medium to the tube. Resuspend the cells by gentle pipetting and make a homogeneous cell suspension. Determine the cell concentration using a hemocytometer.

(iv) Cytotoxicity test: Dilute the cell suspension prepared according to procedure (iii) with culture medium to adjust the cell concentration to 100 cells/mL. Place a 0.5 mL aliquot of the diluted cell suspension on each well of a sterile disposable multiple well plate (24 wells). Incubate the plate in the incubator maintaining 5% carbon dioxide at 37°C for 4 – 24 hours to attach the cells to the bottom surface of the well. Discard the medium from each well, and add a 0.5 mL aliquot of the sample solution or fresh medium to at least 3 wells each. Place the plate immediately in the incubator and incubate the plate for the appropriate period: 7 – 9 days for L929 cells; 6 – 7 days for V79 cells. After the incubation, discard the medium from the plate, add an appropriate volume of methanol or dilute formaldehyde TS to each well and allow the plate to stand for about 30 minutes to fix the cells. Discard the methanol or dilute formaldehyde TS from each well and add an appropriate volume of dilute Giemsa's TS to each well. After ensuring good staining of the colonies, discard the stain solution from the wells, wash with water, dry, and count the number of colonies in each well. Calculate a mean number of colonies for each concentration of the sample solution, and divide the mean by the mean number of colonies for the fresh medium to obtain the relative plating efficiency (%) for each extract concentration of the sample solution. Plot the extract concentration (%) of the sample solution on a logarithmic scale and the relative plating efficiency on an ordinary scale on semilogarithmic graph paper to obtain a colony formation inhibition curve of the container. Read the 50% inhibition concentration, IC_{50} (%), at which the colony number is half that in the control group, from the inhibition curve.

It is recommended to check the sensitivity and the reproducibility of the test system by the use of suitable reference materials or control substances in the test system, if necessary.

7.03 Test for Rubber Closure for Aqueous Infusions

Change as follows:

The rubber closure for aqueous infusions means a rubber closure (containing material coated or laminated with the stuff like plastics) used for a container for aqueous infusion having a capacity of 100 mL or more, and is in direct contact with the contained aqueous infusion. The rubber closure when in use does not interact physically or chemically with the contained medicament to alter any property or quality, does not permit the invasion of microbes, does not disturb the use of the contained infusion, and meets the following requirements.

1. Cadmium

Wash the rubber closures with water, dry at room temperature, cut into minute pieces, mix well, place 2.0 g of them in a crucible of platinum or quartz, moisten them with 2 mL of sulfuric acid, heat gradually to dryness, and ignite between 450°C and 500°C until the residue is incinerated. When incineration was insufficient, moisten the residue with 1 mL of sulfuric acid, heat to dryness, and ignite again. Repeat the above-mentioned procedure if necessary. Cool the crucible, moisten the residue with water, add 2 to 4 mL of hydrochloric acid, heat on a water bath to dryness, add 1 to 5 mL of hydrochloric acid, and dissolve by heating. Then add 0.5 to 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1) and 0.5 to 1 mL of a warmed solution of ammonium acetate (2 in 5). When any insoluble residue remains, filter through a glass filter. To the solution thus obtained add 10 mL of a solution of diammonium hydrogen citrate (1 in 4), 2 drops of bromothymol blue TS and ammonium TS until the color of the solution changes from yellow to green. Then add 10 mL of ammonium sulfate solution (2 in 5) and water to make 100 mL. Next, add 20 mL of a solution of sodium *N*,*N*-diethyldithiocarbamate trihydrate (1 in 20), mix, allow to stand for a few minutes, add 20 mL of 4-methyl-2-pentanone, and mix by vigorous shaking. Allow to stand to separate the 4-methyl-2-pentanone layer from the solution, filter if necessary, and use as the sample solution. On the other hand, to exactly 10 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry <*2.23*> under the following conditions, using the sample solution and the standard solution. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: Cadmium hollow-cathode lamp.
Wavelength: 228.8 nm.

2. Lead

To exactly 1 mL of the Standard Lead Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, proceed as directed for the sample solution under **1**, and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry <*2.23*> under the following conditions, using the sample solution and the standard solution obtained in **1**. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: Lead hollow-cathode lamp.
Wavelength: 283.3 nm.

3. Extractable substances

Wash the rubber closures with water, and dry at room temperature. Place an amount of them, equivalent to about 150 cm^2 in surface area, in a glass vessel, add 2 mL of water per cm^2 of the sample, stopper adequately, heat at 121°C for 1 hour in an autoclave, take out the glass vessel, allow to cool to room temperature, then remove immediately the rubber closures, and use the remaining solution as the test solution. Prepare the blank solution with water in the same manner. Perform the following tests with the test solution and the blank solution.

3.1. Description

The test solution is clear and colorless. Read the transmittance of the test solution at 430 nm and 650 nm (10 mm), using the blank solution as the blank. Both of them are not less than 99.0%.

3.2. pH <*2.54*>

To 20 mL each of the test solution and the blank solution add 1 mL each of potassium chloride solution, prepared by dissolving 1.0 g of potassium chloride in water to make 1000 mL. The difference of pH between the two solutions is not more than 1.0.

3.3. Zinc

To exactly 10 mL of the test solution add diluted dilute nitric acid (1 in 3) to make exactly 20 mL, and use this solution as the sample solution. Further, to exactly 1 mL of Standard Zinc Solution for atomic absorption spectrophotometry add diluted nitric acid (1 in 3) to make exactly 20 mL, and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry <*2.23*>, using these solutions, under the following conditions. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Zinc hollow-cathode lamp.
Wavelength: 213.9 nm.

3.4. Potassium Permanganate-reducing substances

Measure 100 mL of the test solution in a glass-stoppered, Erlenmyer flask, add 10 mL of 0.002 mol/L potassium permanganate VS, then add 5 mL of dilute sulfuric acid, and boil for 3 minutes. After cooling, add 0.10 g of potassium iodide, stopper, mix by shaking, then allow to stand for 10 minutes, and titrate <*2.50*> with 0.01 mol/L sodium thiosulfate VS (indicator: 5 drops of starch TS). Perform the blank test in the same manner, using 100 mL of the blank solution. The difference in mL of 0.002 mol/L potassium permanganate VS required between the tests is not more than 2.0 mL.

3.5. Residue on evaporation

Measure 100 mL of the test solution, evaporate on a water bath to dryness, and dry the residue at 105°C for 1 hour. The mass of the residue is not more than 2.0 mg.

3.6. UV spectrum

Read the absorbance of the test solution between 220 nm and 350 nm against the blank solution as directed under Ultraviolet-visible Spectrophotometry <*2.54*>: it is not more than 0.20.

4. Cytotoxicity test

The following test methods are designed to detect cytotoxic substances in rubber materials by evaluating the cytotoxicity of the culture medium extracts from rubber closure for aqueous infusion. Other appropriate standard methods of cytotoxicity testing may be used for the evaluation, if appropriate. However, the final decision shall be made based upon the test methods given here, if the test results obtained according to the other methods are questionable. Other than those of the culture medium, reagents and test solutions being specified for the test may be used if they meet for the purpose of the test.

4.1. Cell lines

The recommended cell lines are L929 cells (ATCC. CCL1) and V79 cells (JCRB0603). In addition, other established cell lines may be used when it is confirmed that they form well-defined colonies reproducibly, with characteristics comparable to those of L929 cells and V79 cells.

4.2. Culture medium

(i) Medium for L929 cells: To Eagle's minimum essential medium add fetal calf serum (FCS) to make 10 vol% FCS.

(ii) Medium for V79 cells: M05 medium prepared by adding 10 mL each of nonessential amino acid TS and 100 mmol/L sodium pyruvate TS to 1000 mL of Eagle's minimum essential medium, then adding fetal calf serum (FCS) to make 5 vol% FCS. Medium for L929 cells may be used instead if it gives equivalent sensitivity.

4.3. Reference materials and control substances

(i) Negative reference material: Highdensity polyethylene film

(ii) Positive reference material (A): polyurethane film containing 0.1% zinc diethyldithiocarbamate

(iii) Positive reference material (B): Polyurethane film containing 0.25% zinc dibutyldithiocarbamate

(iv) Control substances: Zinc diethyldithiocarbamate (reagent grade) or zinc dibutyldithiocarbamate

4.4. Test procedure

(i) Sample preparation: Rubber closure is subjected to the test without cutting into pieces. Reference material is divided into pieces of approximately 2 × 15 mm and subjected to the test.

(ii) Preparation of sample solutions: Transfer an appropriate amount of the sample to a screw-capped glass bottle or a sterile disposable centrifuge tube. Cap the bottle or tube loosely and cover the cap with clean aluminum foil. Sterilize the bottle or tube by autoclaving at 121°C for 15 minutes. When the material of the sample is not resistant to heat during autoclaving, gas sterilization with ethylene oxide (EO) may be used. In the case of EO sterilization, sufficient aeration should be achieved to avoid an additional toxic effect of residual EO in the test results. To the bottle or tube add the culture medium in a proportion of 60 cm^2 surface

area or 10 mL per 1 g of the sample, loosely cap the bottle or tube, and allow to stand in an incubator maintaining 5% carbon dioxide at 37°C for 24 hours. To the reference material add 10 mL of the culture medium per 1 g and extract in the same manner. Transfer the culture medium extract, which is designated 100% sample solution, to a sterilized screw-capped glass bottle or a sterile disposable centrifuge tube. Dilute the 100% sample solution with fresh culture medium using a dilution factor of two to prepare serial dilutions having extract concentrations of 50%, 25%, 12.5%, 6.25%, 3.13% and so on.

(iii) Preparation of cell suspension: Remove the culture medium from the maintained cell culture vessel (flask or dish), and add gently a suitable volume of phosphate buffer solution for cytotoxicity test. Rinse the cells by gentle rotation of the cell culture vessel two or three times, and discard the phosphate buffer solution. Add a sufficient volume of trypsin solution to cover the cell layer. Cap the vessel and place in an incubator maintaining 5% carbon dioxide at 37°C for 1 to 2 minutes. After confirming detachment of the cell layer from the bottom surface of the vessel by using a microscope and by gently tapping of the vessel, add an appropriate volume of the fresh culture medium and gently pipet the cells completely out of the vessel. Transfer the pipetted cell suspension into a sterile disposable centrifuge tube and centrifuge. Discard the supernatant liquid, resuspend the cells in an appropriate volume of flesh phosphate buffer solution for cytotoxicity test by pipetting, and centrifuge the tube again. Discard the supernatant liquid, and add an appropriate volume of fresh culture medium to the vessel. Resuspend the cells by gentle pipetting and make a homogeneous cell suspension. Determine the cell concentration using a hemocytometer.

(iv) Cytotoxicity test: Dilute the cell suspension prepared according to procedure (iii) with culture medium to adjust the cell concentration to 100 cells/mL. Place a 0.5 mL aliquot of the diluted cell suspension on each well of a sterile disposable multiple well plate (24 wells). Incubate the plate in the incubator maintaining 5% carbon dioxide at 37°C for 4 – 24 hours to attach the cells to the bottom surface of the well. Discard the medium from each well, and add a 0.5 mL aliquot of the sample solution or fresh medium to at least 3 wells each. Place the plate immediately in the incubator and incubate the plate for the appropriate period: 7 – 9 days for L929 cells; 6 – 7 days for V79 cells. After the incubation, discard the medium from the plate, add an appropriate volume of methanol or dilute formaldehyde TS to each well and allow the plate to stand for about 30 minutes to fix the cells. Discard the methanol or dilute formaldehyde TS from each well and add an appropriate volume of dilute Giemsa's TS to each well. After ensuring good staining of the colonies, discard the stain solution from the wells, wash with water, dry, and count the number of colonies in each well. Calculate a mean number of colonies for each concentration of the sample solution, and divide the mean by the mean number of colonies for the fresh medium to obtain the relative plating efficiency (%) for each extract concentration of the sample solution. Plot the extract concentration (%) of the sample solution on a logarithmic scale and the relative plating efficiency on an ordinary scale on semilogarithmic graph paper to obtain a colony formation inhibition curve of the rubber closure. Read the 50% inhibition concentration, IC_{50} (%), at which the colony number is half that in the negative control group, from the inhibition curve.

It is recommended to check the sensitivity and the reproducibility of the test system by the use of suitable reference materials or control substances in the test system, if necessary.

4.5. Interpretation

IC_{50} (%) is not less than 90%.

5. Acute systemic toxicity

This test is performed when the sample solution does not meet the requirements of the cytotoxicity test.

The sample solution meets the requirements, when examined under the following conditions against the blank solution.

5.1. Preparation of the sample solution and the blank solution

Wash the rubber closures with water and Water for Injection successively, and dry under clean conditions at room temperature. Transfer the rubber closures to a glass container. Add isotonic sodium chloride solution of 10 times the mass of the test material, stopper adequately, heat in an autoclave at 121°C for 1 hour, take out the glass container, and allow to cool to room temperature. The solution thus obtained is used as the sample solution. The blank solution is prepared in the same manner.

5.2. Test procedures

(i) Test animals: Use healthy male or female mice of inbred strain or from a closed colony, weighing 17 to 25 g.

(ii) Procedure: Separate the animals into two groups of 5 mice, and inject intravenously 50 mL each of the solutions per kg body mass. From the viewpoint of animal rights, it is recommended to start the test with small size animal groups first, such as with 3 animals, and then add 2 animals to each group if the acceptable result was obtained.

5.3. Interpretation

Observe the animals for 72 hours after injection: During the observation period, none of the animals treated with the sample solution show any weight loss, abnormality or death.

9.01 Reference Standards

Change the name of Spiramycin Acetate II RS under section (2) as follows:

Spiramycin II Acetate RS

Add the following to section (1):

Clopidogrel Sulfate RS
Docetaxel RS
Insulin Glargine RS
Leuprorelin Acetate RS
D-Mannitol RS

Olmesartan Medoxomil RS
Paroxetine Hydrochloride RS
Pitavastatin Methylbenzylamine RS
Pranlukast RS
Sivelestat RS

9.21 Standard Solutions for Volumetric Analysis

Add the following:

Sulfuric Acid, 0.02 mol/L

1000 mL of this solution contains 1.9616 g of sulfuric acid (H_2SO_4: 98.08).

Preparation—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 2.5 times the initial volume.

9.22 Standard Solutions

Change the following as follows:

Formazin stock suspension To 25 mL of hexamethylenetetramine TS add 25 mL of hydrazinium sulfate TS, mix, and use after allowing to stand at room temperature for 24 hours. Store in a glass container free from surface defects. Use within 2 months. Shake thoroughly before use. The turbidity of this suspension is equivalent to 4000 NTU.

Standard Zinc Solution for Atomic Absorption Spectrophotometry To exactly 10 mL of Standard Zinc Stock Solution add water to make exactly 1000 mL. Prepare before use. Each mL of this solution contains 0.01 mg of zinc (Zn).

Add the following:

Certified Standard Arsenic Solution See Arsenic Limit Test <*1.11*>.

Standard Nickel Stock Solution Dissolve exactly 4.48 g of nickel (II) sulfate hexahydrate in water to make exactly 1000 mL.

Standard Nickel Solution for Atomic Absorption Spectrophotometry To exactly 10 mL of Standard Nickel Stock Solution add water to make exactly 1000 mL. Prepare before use. Each mL of this solution contains 0.01 mg of nickel (Ni).

9.41 Reagents, Test Solutions

Change the following as follows:

Atractylodin TS for assay Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 5 mg of atractylodin for assay, and dissolve in methanol to make exactly 1000 mL.

Dilute formaldehyde TS See formaldehyde TS, dilute.

Dilute Giemsa's TS See Giemsa's TS, dilute.

Ethanol, dilute To 1 volume of ethanol (95) add 1 volume of water.

(*E*)-Ferulic acid $C_{10}H_{10}O_4$ White to light yellow, crystals or crystalline powder. Freely soluble in methanol, soluble in ethanol (99.5), and practically insoluble in water. Melting point: 173 – 176°C.

Identification—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 215 nm and 219 nm, between 231 nm and 235 nm, and between 318 nm and 322 nm.

Purity Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 1 mg of (*E*)-ferulic acid in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (20:12:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365 nm): no spot appears other than the principle spot at the *R*f value of about 0.6.

Formaldehyde TS, dilute Dilute formaldehyde solution to 10 times its volume with water.

Geniposide for assay $C_{17}H_{24}O_{10}$ Use geniposide for thin-layer chromatography meeting the following additional specifications, 1) Geniposide for assay 1 or 2) Geniposide for assay 2 (Purity value by quantitative NMR). The former is used after drying (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 24 hours), and the latter is corrected its content based on the amount (%) obtained in the Assay.

1) Geniposide for assay 1

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (240 nm): 249 – 269 [10 mg dried in a desiccator (reduced pressure of not exceeding 0.67 kPa, phosphorus (V) oxide) for 24 hours, diluted methanol (1 in 2), 500 mL].

Purity Related substances—Dissolve 5 mg of geniposide for assay in 50 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and measure each peak area by the automatic integration method: the total area of the peaks other than geniposide obtained from the sample solution is not larger than the peak area of geniposide obtained from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating condi-

tions in the Assay under Gardenia Fruit.

Time span of measurement: About 3 times as long as the retention time of geniposide, beginning after the solvent peak.

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Gardenia Fruit.

Test for required detectability: Pipet 1 mL of the standard solution, add diluted methanol (1 in 2) to make exactly 20 mL. Confirm that the peak area of geniposide obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 10 μL of the standard solution.

2) Geniposide for assay 2 (Purity value by quantitative NMR)

Unity of peak—Dissolve 5 mg of geniposide for assay 2 in 50 mL of diluted methanol (1 in 2). To 1 mL of this solution add diluted methanol (1 in 2) to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of geniposide peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Gardenia Fruit.

Detector: A photodiode array detector (wavelength: 240 nm, measuring range of spectrum: 220 – 400 nm).

System suitability

System performance: Proceed as directed in the system suitability in the Assay under Gardenia Fruit.

Assay—Weigh accurately 10 mg of geniposide for assay 2 and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> and Crude Drugs Test <*5.01*> according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the internal reference compound. Calculate the resonance intensities, A1 (equivalent to 1 hydrogen) and A2 (equivalent to 1 hydrogen), of the signals around δ 3.93 ppm and δ 4.06 ppm assuming the signal of the internal reference compound as δ 0 ppm.

$$\text{Amount (\%) of geniposide } (C_{17}H_{24}O_{10}) = M_S \times I \times P/(M \times N) \times 1.7147$$

M: Amount (mg) of geniposide for assay 2

M_S: Amount (mg) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy

I: Sum of the signal resonance intensities, A1 and A2, based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000

N: Sum of number of the hydrogen derived from A1 and A2

P: Purity (%) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: An apparatus of nuclear magnetic resonance spectrum measurement having ^{1}H resonance frequency of not less than 400 MHz.

Target nucleus: ^{1}H.

Digital resolution: 0.25 or lower.

Measuring spectrum range: 20 ppm and upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the two signals of around δ 3.93 ppm and δ 4.06 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals of around δ 3.93 ppm and δ 4.06 ppm are not overlapped with any signal of obvious foreign substance, and the ratios of the resonance intensities, A1/A2, of each signal around δ 3.93 ppm and δ 4.06 ppm are between 0.99 and 1.01, respectively.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity, A1 or A2, to that of the internal reference is not more than 1.0%.

Geniposide for thin-layer chromatography $C_{17}H_{24}O_{10}$ White crystals or crystalline powder. Freely soluble in water and in methanol, and soluble in ethanol (99.5). Melting point: about 160°C.

Purity Related substances—Dissolve 1.0 mg of geniposide for thin-layer chromatography in exactly 1 mL of methanol, and perform the test with 20 μL of this solution as directed in the Identification (2) under Gardenia Fruit: no spot other than the principal spot at an *R*f value of about 0.3 is observed.

Giemsa's TS, dilute Dilute Giemsa's TS to about 50 times its volume with a solution prepared by dissolving 4.54 g of potassium dihydrogen phosphate and 4.75 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL, and filter with a filter paper. Prepare before use.

Glutamine TS Dissolve 2.92 g of L-glutamine in water to make 100 mL, and sterilize by filtration through a mem-

brane filter with a pore size not exceeding 0.22 μm.

Hexamethylenetetramine TS Dissolve exactly 2.5 g of hexamethylenetetramine in exactly 25 mL of water.

Hydrazinium sulfate TS Dissolve exactly 1.0 g of hydrazinium sulfate in exactly 100 mL of water. Use after standing for 4 – 6 hours.

3-(3-Hydroxy-4-methoxyphenyl)-2-(*E*)-propenic acid $C_{10}H_{10}O_4$ White to light yellow, crystals or crystalline powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 230°C (with decomposition).

Identification—Determine the absorption spectrum of a solution of 3-(3-hydroxy-4-methoxyphenyl)-2-(*E*)-propenic acid in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 215 nm and 219 nm, between 238 nm and 242 nm, between 290 nm and 294 nm, and between 319 nm and 323 nm.

Purity Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 1 mg of 3-(3-hydroxy-4-methoxyphenyl)-2-(*E*)-propenic acid in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer chromatography <*2.03*>. Spot 2 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (20:12:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): no spot appears other than the principle spot at the *R*f value of about 0.6.

Iodine monobromide IBr Blackish brown, crystals or masses. It dissolves in water, in ethanol (95), in acetic acid (100), in diethyl ether and in carbon disulfide.

Melting point <*2.60*>: 37 – 43°C

Storage—Preserve in light-resistant glass containers, in a cold place.

Magnolol for assay $C_{18}H_{18}O_2$ Use magnolol for thin-layer chromatography meeting the following additional specifications, 1) magnolol for assay 1 or 2) magnolol for assay 2 (Purity valve by quantitative NMR). The former is used after drying in a desiccator (silica gel) for 1 hour, and the latter is corrected the content based on the amount (%) obtained in the Assay.

1) Magnolol for assay 1

Absorbance <*2.24*> $E_{1\,cm}^{1\%}$ (290 nm): 270 – 293 (10 mg, methanol, 500 mL). Use the sample dried in a desiccator (silica gel) for not less than 1 hour.

Purity Related substances—Dissolve 5.0 mg of magnolol for assay in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the area of each peak from these solutions by the automatic integration method: the total area of the peaks other than magnolol obtained from the sample solution is not larger than the peak area of magnolol obtained from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Magnolia Bark.

Time span of measurement: About 3 times as long as the retention time of magnolol.

System suitability

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay under Magnolia Bark.

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of magnolol obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

2) Magnolol for assay 2 (Purity value by quantitative NMR)

Unity of peak—Dissolve 5 mg of magnolol for assay 2 in 10 mL of the mobile phase. To 1 mL of this solution add the mobile phase to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of magnolol peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Magnolia Bark.

Detector: A photodiode array detector (wavelength: 289 nm, measuring range of spectrum: 220 – 400 nm).

System suitability

System performance: Proceed as directed in the system suitability in the Assay under Magnolia Bark.

Assay—Weigh accurately 5 mg of magnolol for assay 2 and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated chloroform for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> and Crude Drugs Test <*5.01*> according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the internal reference compound. Calculate the resonance intensities, A1 (equivalent to 2 hydrogen) and A2 (equivalent to 2 hydrogen), of the signals around δ 6.70 ppm and δ 6.81 ppm assuming the signal of the internal reference compound as δ 0 ppm.

$$\text{Amount (\%) of magnolol } (C_{18}H_{18}O_2) = M_S \times I \times P/(M \times N) \times 1.1758$$

M: Amount (mg) of magnolol for assay 2
M_S: Amount (mg) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy
I: Sum of the signal resonance intensities, A1 and A2, based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000
N: Sum of numbers of the hydrogen derived from A1 and A2
P: Purity (%) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: An apparatus of nuclear magnetic resonance spectrum measurement having ^{1}H resonance frequency of not less than 400 MHz.

Target nucleus: ^{1}H.

Digital resolution: 0.25 or lower.

Measuring spectrum range: 20 ppm and upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the two signals of around δ 6.70 ppm and δ 6.81 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals of around δ 6.70 ppm and δ 6.81 ppm are not overlapped with any signal of obvious foreign substance, and the ratios of the resonance intensities, A1/A2, of each signal around δ 6.70 ppm and δ 6.81 ppm are between 0.99 and 1.01, respectively.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity, A1 or A2, to that of the internal reference is not more than 1.0%.

Paeonol for assay $C_9H_{10}O_3$ Use paeonol for thin-layer chromatography meeting the following additional specifications, 1) Paeonol for assay 1 or 2) Paeonol for assay 2 (Purity value by quantitative NMR). The former is used after drying in a desiccator (silica gel) for 1 hour, and the latter is corrected the content based on the amount (%) obtained in the Assay.

1) Paeonol for assay 1

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (274 nm): 853 – 934 [5 mg after drying in a desiccator (silica gel) for 1 hour or more, methanol, 1000 mL].

Purity Related substances—Dissolve 5.0 mg of paeonol for assay in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 μL each of the sample solution and standard solution (1) as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and measure each peak area of these solutions by the automatic integration method: the total area of the peaks other than paeonol obtained from the sample solution is not larger than the peak area of paeonol obtained from the standard solution (1).

Operating conditions

Proceed as directed in the operating conditions in the Assay under Moutan Bark except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of paeonol obtained with 10 μL of the standard solution (2) can be measured, and the peak height of paeonol obtained with 10 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of paeonol beginning after the solvent peak.

2) Paeonol for assay 2 (Purity value by quantitative NMR)

Unity of peak—Dissolve 5 mg of paeonol for assay 2 in 50 mL of the mobile phase. To 1 mL of this solution add the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and compare the absorption spectra of at least 3 points including the top of paeonol peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Moutan Bark.

Detector: A photodiode array detector (wavelength: 274 nm, measuring range of spectrum: 220 – 400 nm).

System suitability

System performance: Proceed as directed in the system suitability in the Assay under Moutan Bark.

Assay—Weigh accurately 5 mg of paeonol for assay 2 and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩ and Crude Drugs Test ⟨*5.01*⟩ according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the internal reference compound. Calculate the resonance intensities, A1 (equivalent to 2 hydrogen) and A2 (equivalent to 1 hydrogen), of the signals around δ 6.17 – 6.25 ppm and δ 7.54 ppm assuming the signal of the internal reference

compound as δ 0 ppm.

$$\text{Amount (\%) of paeonol } (C_9H_{10}O_3) = M_S \times I \times P/(M \times N) \times 0.7336$$

M: Amount (mg) of paeonol for assay 2
M_S: Amount (mg) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy
I: Sum of the signal resonance intensities, A1 and A2, based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000
N: Sum of numbers of the hydrogen derived from A1 and A2
P: Purity (%) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: An apparatus of nuclear magnetic resonance spectrum measurement having ^1H resonance frequency of not less than 400 MHz.

Target nucleus: ^1H.

Digital resolution: 0.25 or lower.

Measuring spectrum range: 20 ppm and upper, including between -5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time not less than 60 seconds.

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

Measuring temperature: A constant temperature between 20°C and 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, SN ratio of the two signals of around δ 6.17 – δ 6.25 ppm and δ 7.54 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals of around δ 6.17 – δ 6.25 ppm and δ 7.54 ppm are not overlapped with any signal of obvious foreign substance, and the ratios of the resonance intensities, (A1/2)/A2, of each signal around δ 6.17 – δ 6.25 ppm and δ 7.54 ppm are between 0.99 and 1.01, respectively.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the resonance intensity, A1 or A2, to that of the internal reference is not more than 1.0%.

Polysorbate 20 Chiefly consists of addition polymer of sorbitan monolaurate and ethylene oxide. Pale yellow to yellow liquid, having a faint, characteristic odor.

Identification (1) To 0.5 g of polysorbate 20 add 10 mL of water and 10 mL of sodium hydroxide TS, boil for 5 minutes, and acidify with dilute hydrochloric acid: an oily fraction is separated.

(2) To 0.5 g of polysorbate 20 add 10 mL of water, shake, and add 5 drops of bromine TS: the red color of the test solution does not disappear.

(3) Place 0.1 g of polysorbate 20 in a flask, dissolve in 2 mL of a solution of sodium hydroxide in methanol (1 in 50), and heat under a reflux condenser for 30 minutes. Add 2 mL of boron trifluoride-methanol TS through the condenser, and heat for 30 minutes. Then, add 4 mL of heptane through the condenser, and heat for 5 minutes. After cooling, add 10 mL of saturated sodium chloride solution, shake for about 15 seconds, then add sufficient saturated sodium chloride solution such that the upper layer of the content reaches to the neck of the flask. Take 2 mL of the upper layer, wash 3 times with each 2-mL portion of water, dry with anhydrous sodium sulfate, and use this solution as the sample solution. Separately, dissolve 50 mg of methyl laurate for gas chromatography, 50 mg of methyl palmitate for gas chromatography, 80 mg of methyl stearate and 100 mg of methyl oleate for gas chromatography in heptane to make 50 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions: the retention time of the principal peak obtained from the sample solution is the same with that of the peak of methyl laurate obtained from the standard solution.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated the inside surface with polyethylene glycol 20 M for gas chromatography 0.5 μm in thickness.

Column temperature: Inject at a constant temperature of 80°C, raise the temperature at the rate of 10°C per minute to 220°C, and maintain the temperature at 220°C for 40 minutes.

Injection port temperature: A constant temperature of about 250°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of the peak of methyl laurate is about 10 minutes.

Split ratio: 1:50.

System suitability

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, methyl laurate, methyl palmitate, methyl stearate and methyl oleate are eluted in this order, and the resolution between the peaks of methyl stearate and methyl oleate is not less than 2.0.

Acid value <*1.13*>: not more than 4.0.

Saponification value <*1.13*>: 43 – 55

Loss on drying <*2.41*>: not more than 3.0% (5 g, 105°C, 1 hour).

Residue on ignition—Weigh accurately about 3 g of polysorbate 20, heat gently at first, and ignite gradually (800 – 1200°C) until the residue is completely incinerated. If any carbonized substance remains, extract with hot water, filter

through a sheet of filter paper for quantitative analysis (5C), and ignite the residue with the filter paper. Add the filtrate to it, evaporate to dryness, and ignite carefully until the carbonized substance does not remain. If any carbonized substance still remains, add 15 mL of ethanol (95), crush the carbonized substance with a glass rod, burn the ethanol, and ignite carefully. Cool in a desiccator (silica gel), and weigh the residue accurately: not more than 1.0%.

Sodium glycocholate for thin-layer chromatography $C_{26}H_{42}NNaO_6$ White to pale brown, crystalline powder or powder. Freely soluble in water and in methanol, and slightly soluble in ethanol (99.5). Melting point: about 260°C (with decomposition).

Identification—Determine the infrared absorption spectrum of sodium glycocholate for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 2940 cm^{-1}, 1599 cm^{-1}, 1398 cm^{-1}, 1309 cm^{-1}, 1078 cm^{-1}, 1040 cm^{-1}, 982 cm^{-1} and 915 cm^{-1}.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +25 – +35° (60 mg, methanol, 20 mL, 100 mm).

Purity Related substances—Dissolve 5 mg of sodium glycocholate for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 mL of the sample solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Proceed with 5 μL each of the sample solution and standard solution as directed in the Identification under Bear Bile: the spots other than the principal spot with an *R*f value of about 0.2 obtained from the sample solution are not more intense than the spot obtained from the standard solution.

Sodium tauroursodeoxycholate for thin-layer chromatography $C_{26}H_{44}NNaO_6S$ White to pale brown, crystalline powder or powder. Freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5).

Identification—Determine the infrared absorption spectrum of sodium tauroursodeoxycholate for thin-layer chromamatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 2930 cm^{-1}, 1645 cm^{-1}, 1556 cm^{-1}, 1453 cm^{-1}, 1215 cm^{-1} and 1049 cm^{-1}.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +40 – +50° (40 mg, methanol, 20 mL, 100 mm).

Purity Related substances—Dissolve 10 mg of sodium tauroursodeoxycholate for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 0.2 mL of the sample solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Perform the test with 5 μL each of the sample solution and standard solution as directed in the Identification under Bear Bile: the spots other than the principal spot with an *R*f value of about 0.2 obtained from the sample solution are not more intense than the spot obtained from the standard solution.

Starch, soluble A potato starch, dried after treating with acid, neutralizing and washing with water. A white powder. Practically insoluble in ethanol (99.5). Soluble by heating after addition of water.

pH <*2.54*>: To 2.0 g of soluble starch add 90 mL of freshly boiled and cooled water, and heat to dissolve. After cooling, add freshly boiled and cooled water to make 100 mL: pH of this solution, measured at 25°C, is 4.0 – 7.5.

Purity: Iron—Place 1.0 g of soluble starch in a crucible, moisten with a little amount of sulfuric acid, and heat gradually at a temperature as lower as possible to carbonize completely. After allowing to cool, moisten the residue with a little amount of sulfuric acid, heat gradually until white fumes are on longer evolved, then ignite at 600 ± 50°C until the residue is completely incinerated. After cooling, dissolve the residue by adding 1 mL of 7.5 mol/L hydrochloric acid TS and a suitable amount of water, and evaporate to dryness on a water bath. Dissolve the residue in 4 mL of 7.5 mol/L hydrochloric acid TS, and add water to make 40 mL. To 10 mL of this solution add water to make 15 mL, and use this solution as the test solution. Separately, to 1.0 mL of Standard Iron Solution add 7.5 mol/L hydrochloric acid TS to make 15 mL, and use this solution as the control solution. To the test solution and the control solution add 1 mL of a solution of hydroxylammonium chloride (1 in 10), mix, and allow them to stand for 5 minutes, and add 1 mL of a solution of 1,10-phenanthrolinium chloride monohydrate (7 in 2500) and 5 mL of a solution of ammonium acetate (1 in 4), and add water to make 25 mL. After allowing to stand at 20 – 30°C for 15 minutes, compare the color of both solution against a white background: the solution obtained from the test solution is not more colored than the solution obtained from the control solution (not more than 40 ppm).

Loss on drying <*2.41*>—Not more than 20% (1 g, 105°C, 2 hours).

Sensitivity—Mix thoroughly 2.0 g of soluble starch with 10 mL of water, then add 90 mL of hot water, and boil for 2 minutes while stirring to dissolve. After allowing to cool to room temperature, to 2.5 mL of this solution add 97.5 mL of water and an amount of 0.005 mol/L iodine VS: a blue to blue-purple color appears, and the color disappears on the addition of 0.01 mol/L sodium thiosulfate VS.

Zinc dibutyldithiocarbamate $[(C_4H_9)_2NCSS]_2Zn$ A white powder. Melting point: 106 – 110°C.

Content: Not less than 95.0%. Assay—Weigh accurately about 1.0 g of zinc dibutyldithiocarbamate, add 10 mL of water and 5 mL of hydrochloric acid, and evaporate to dryness on a hot plate. To the residue add 15 mL of diluted hydrochloric acid (1 in 3), dissolve by warming, then add 50 mL of water and 40 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to blue (indicator: 0.1 mL of eriochrome black T TS).

Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 47.41 mg of $[(C_4H_9)_2NCSS]_2Zn$

Zinc diethyldithiocarbamate $[(C_2H_5)_2NCSS]_2Zn$ A white to pale yellow powder. Melting point: 177 – 182°C.

Content: 94.0 – 108.0 %. Assay—Weigh accurately about 0.8 g of zinc diethyldithiocarbamate, add 50 mL of water and 15 mL of diluted hydrochloric acid (1 in 3), and boil to dissolve. After cooling, add 40 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate ⟨*2.50*⟩ with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to blue (indicator: 0.1 mL of eriochrome black T TS).

Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 36.19 mg of $[(C_2H_5)_2NCSS]_2Zn$

Add the following:

2 mol/L Acetic acid TS To 12 g of acetic acid (100) add water to make 100 mL.

Acteoside for thin-layer chromatography See Verbascoside for thin-layer chromatography.

Ammonium pyrrolidinedithiocarbamate $C_5H_{12}N_2S_2$ A white or light yellow, crystalline powder. Sparingly soluble in water, and very slightly soluble in ethanol (95). Reserve in a vessel together with a fragment of ammonium carbonate put in a muslin bag.

L-Asparagine monohydrate $C_4H_8N_2O_3.H_2O$ [K8021, Special class]

Azelnidipine for assay $C_{33}H_{34}N_4O_6$ [Same as the monograph Azelnidipine. When dried, it contains not less than 99.5% of azelnidipine ($C_{33}H_{34}N_4O_6$).]

Bepotastine besilate for assay $C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$ [Same as the monograph Bepotastine Besilate. However, it contains not less than 99.5% of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$), calculated on the anhydrous basis and corrected on the amount of the residual solvent.]

1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy $C_{12}H_{18}D_4Si_2$ 1,4-Bis(trimethylsilyl)benzene-d_4 that the traceability to the international unit system was secured.

Brotizolam for assay $C_{15}H_{10}BrClN_4S$ [Same as the monograph Brotizolam. When dried, it contains not less than 99.0% of brotizolam ($C_{15}H_{10}BrClN_4S$).]

Butyl parahydroxybenzoate for resolution check $C_{11}H_{14}O_3$ Colorless crystals or a white crystalline powder. Very soluble in methanol, freely soluble in ethanol (95) and in acetone, and practically insoluble in water. Melting point: 68 – 71°C.

Identification—Determine the infrared absorption spectrum of butyl parahydroxybenzoate for resolution check as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum of Butyl Parahydroxybenzoate or the spectrum of Butyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Related substances—Dissolve 50 mg of butyl parahydroxybenzoate for resolution check in 2.5 mL of methanol, and add the mobile phase to make 50 mL. To 10 mL of this solution add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: total area of the peaks other than butyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of butyl parahydroxybenzoate obtained from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Butyl Parahydroxybenzoate.

Time span of measurement: About 1.5 times as long as the retention time of butyl parahydroxybenzoate.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of butyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 3.5% to 6.5% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of butyl parahydroxybenzoate are not less than 2500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of butyl parahydroxybenzoate is not more than 5.0%.

Clonazepam for assay $C_{15}H_{10}ClN_3O_3$ [Same as the monograph Clonazepam]

Cyclophosphamide hydrate for assay $C_7H_{15}Cl_2N_2O_2P.H_2O$ [Same as the monograph Cyclophosphamide Hydrate. It contains not less than 99.0% of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$).]

2,2′-dinaphthylether $C_{20}H_{14}O$ White crystals.
Melting point ⟨*2.60*⟩: 102 – 107°C

Docetaxel hydrate $C_{43}H_{53}NO_{14}.3H_2O$ [Same as the namesake monograph]

Doxepin hydrochloride $C_{19}H_{21}NO.HCl$ White crystals or crystalline powder. Melting point: 185 – 191°C.

DSS-d_6 for nuclear magnetic resonance spectroscopy $C_6H_9D_6NaO_3SSi$ Sodium 3-(trimethylsilyl)-1-propanesulfonate-d_6 that the traceability to the International System of

Units has been secured.

Eagle's minimum essential medium Dissolve 6.80 g of sodium chloride, 400 mg of potassium chloride, 115 mg of anhydrous sodium dihydrogen phosphate, 93.5 mg (as anhydrous) of magnesium sulfate, 200 mg (as anhydrous) of calcium chloride, 1.00 g of glucose, 126 mg of L-arginine hydrochloride, 73.0 mg of L-lysine hydrochloride, 31.4 mg of L-cysteine hydrochloride monohydrate, 36.0 mg of L-tyrosine, 42.0 mg of L-histidine hydrochloride monohydrate, 52.0 mg of L-isoleucine, 52.0 mg of L-leucine, 15.0 mg of methionine, 32.0 mg of phenylalanine, 48.0 mg of L-threonine, 10.0 mg of L-tryptophan, 46.0 mg of L-valine, 75.0 mg of succinic acid, 100 mg of sodium succinate hexahydrate, 1.8 mg of choline bitartrate, 1.0 mg of folic acid, 2.0 mg of myoinositol, 1.0 mg of nicotinamide, 1.0 mg of calcium D-pantothenate, 1.0 mg of pyridoxal hydrochloride, 0.1 mg of riboflavin, 1.0 mg of thiamine chloride hydrochloride, 20 μg of biotin and 6.0 mg of phenol red in 1000 mL of water, heat in an autoclave at 121°C for 15 minutes and cool to room temperature, then add separately sterilized 22 mL of 10% sodium hydrogen carbonate TS and 10 mL of glutamine TS.

Ephedrine hydrochloride for assay of crude drugs $C_{10}H_{15}NO.HCl$ Ephedrine hydrochloride for assay or the substance that complies with the following requirements.

White crystals or crystalline powder. Freely soluble in water, and soluble in ethanol (95).

Identification—Determine the infrared absorption spectrum of ephedrine hydrochloride for assay of crude drugs, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum of Ephedrine Hydrochloride: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -33.0 – $-36.0°$ (after drying, 0.1 g, water, 2 mL, 100 mm).

Melting point <*2.60*>: 218 – 222°C

Purity Related substances—Dissolve 10 mg of ephedrine hydrochloride for assay of crude drugs in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than ephedrine obtained from the sample solution is not larger than the peak area of ephedrine obtained from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Ephedra Herb.

Time span of measurement: About 3 times as long as the retention time of ephedrine, beginning after the solvent peak.

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay under Ephedra Herb.

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of ephedrine obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 10 μL of the standard solution.

Loss on drying <*2.41*>: Not more than 0.5% (0.1 g, 105°C, 3 hours).

Ethanol (99.5) for liquid chromatography C_2H_5OH A clear, colorless liquid, miscible with water.

Purity Ultraviolet absorbing substance—Perform the test as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the blank: the absorbances at 210 nm, at 220 nm, at 230 nm, at 240 nm, at 254 nm and at 260 nm are not more than 0.70, 0.40, 0.20, 0.10, 0.02 and 0.01, respectively.

Ethylamine hydrochloride $C_2H_5NH_2.HCl$ White to light yellowish brown, crystals or crystalline powder, having a deliquescency.

Ethylene oxide A colorless flammable gas. Use ethylene oxide from a metal cylinder.

Boiling point <*2.57*>: 9 – 12°C

Factor IIa A lyophilized factor IIa purified from human plasma. A white to pale yellowish powder. It contains not less than 2000 IU per mg of protein.

Fatty acid methyl esters mixture TS Dissolve 0.50 g of a mixture of methyl myristate for gas chromatography, methyl palmitate for gas chromatography, methyl palmitoleate for gas chromatography, methyl stearate for gas chromatography, methyl oleate for gas chromatography, methyl linoleate for gas chromatography and methyl linolenate for gas chromatography, corresponding to the composition of Polysorbate 80, in heptane to make 50.0 mL.

Fluconazole for assay $C_{13}H_{12}F_2N_6O$ [Same as the monograph Fluconazole]

Fudosteine for assay $C_6H_{13}NO_3S$ [Same as the monograph Fudosteine]

Glycerin for gas chromatography $C_3H_8O_3$ [K 8295, Special class] When perform the test as directed in the Purity (11) under Concentrated Glycerin, it does not show any peak at the retention times corresponding to ethylene glycol and diethylene glycol.

High-density polyethylene film Prepared for cytotoxicity test. It does not show cytotoxicity.

Human anti-thrombin A serine protease inhibitor obtained from healthy human plasma. A protein that inhibits activities of activated blood coagulation factor II (thrombin) and activated blood coagulation factor X. It contains not less than 6 IU per mg of protein.

Hydrochlorothiazide $C_7H_8ClN_3O_4S_2$ [Same as the namesake monograph]

Ifenprodil tartrate for assay $(C_{21}H_{27}NO_2)_2.C_4H_6O_6$ [Same as the monograph Ifenprodil Tartrate. It contains not less than 99.5% of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6]$, calculated on the anhydrous basis, and meets the following additional requirement.]

Purity Related substances—Dissolve 20 mg of ifenprodil tartrate for assay in 200 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than ifenprodil obtained from the sample solution is not larger than 1/2 times the peak area of ifenprodil obtained from the standard solution. For this calculation, use the area of the peak, having the relative retention time of about 0.55 to ifenprodil, after multiplying by the relative response factor, 7.1.

Operating conditions

Detector, column, column temperature, and flow rate: Proceed as directed in the operating conditions in the Assay under Ifenprodil Tartrate Fine Granules.

Mobile phase A: Dissolve 6.8 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 6.5 with potassium hydroxide TS, and add water to make 1000 mL. To 420 mL of this solution, add 320 mL of methanol for liquid chromatography and 260 mL of acetonitrile for liquid chromatography.

Mobile phase B: Methanol for liquid chromatography.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0.0 – 15.0	100	0
15.0 – 15.1	100 → 0	0 → 100
15.1 – 35.0	0	100

Time span of measurement: For 35 minutes after injection of the sample solution.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, add the mobile phase A to make exactly 10 mL. Confirm that the peak area of ifenprodil obtained from 20 μL of this solution is equivalent to 7 to 13% of that of ifenprodil obtained from the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ifenprodil are not less than 3500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ifenprodil is not more than 2.0%.

Immature orange [Same as the namesake monograph]

Iodine bromide (II) TS Dissolve 20 g of iodine monobromide in acetic acid (100) to make 1000 mL. Store protected from light.

Iopamidol for assay $C_{17}H_{22}I_3N_3O_8$ [Same as the monograph Iopamidol]

Isomalt $C_{12}H_{24}O_{11}$ White powder or grain. Very soluble in water, and practically insoluble in ethanol (99.5).

Lithium hydroxide monohydrate $LiOH.H_2O$ White, crystals or crystalline powder, having a hygroscopicity.

Magnoflorine iodide for assay $C_{20}H_{24}INO_4$ White to light yellowish white, crystals or crystalline powder. Slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5). Melting point: about 250°C (with decomposition).

It is used after correcting with the amount of magnoflorine iodide obtained in the Assay.

Identification (1) Determine the absorption spectrum of a solution of magnoflorine iodide for assay in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 221 nm and 225 nm.

(2) Determine the infrared absorption spectrum of magnoflorine iodide for assay as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 3170 cm^{-1}, 3000 cm^{-1}, 2840 cm^{-1}, 1459 cm^{-1}, 1231 cm^{-1}, 1122 cm^{-1} and 833 cm^{-1}.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (223 nm): 1066 – 1132 (5 mg, methanol, 1000 mL).

Purity Related substances—Dissolve 5 mg of magnoflorine iodide for assay in 2 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and formic acid (5:3:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, air-dry the plate, and spray evenly sodium nitrite TS: the spot other than the principal spot at the *R*f value of about 0.3 obtained from the sample solution is not more intense than the spot obtained from the standard solution.

Unity of peak: Dissolve 5 mg of magnoflorine iodide for assay in 10 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following condi-

tions, and compare the absorption spectra of at least 3 points including the top of magnoflorine peak and around the two middle peak heights of before and after the top: no difference is observed in the shape between their spectra.

Operating conditions

Column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay (4) under Kakkontokasenkyushin'i Extract.

Detector: A photodiode array detector (wavelength: 303 nm; measuring range of spectrum: 220 – 400 nm).

Flow rate: Adjust the flow rate so that the retention time of magnoflorine is about 20 minutes.

System suitability

System performance: To 1 mL of the sample solution add a mixture of water and methanol (1:1) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of magnoflorine are not less than 5000 and not more than 1.5, respectively.

System repeatability: To 1 mL of the sample solution add a mixture of water and methanol (1:1) to make 100 mL. When the test is repeated 6 times with 10 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of magnoflorine is not more than 1.5%.

Assay—Weigh accurately 5 mg of magnoflorine iodide for assay and 1 mg of DSS-d_6 for nuclear magnetic resonance spectroscopy by using an ultramicro balance, dissolve in 1 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution in an NMR tube 5 mm in outer diameter, and measure ^{1}H-NMR spectrum as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> and Crude Drugs Test <*5.01*> according to the following conditions, using DSS-d_6 for nuclear magnetic resonance spectroscopy as the internal reference compound. Calculate the signal integrated intensity A (equivalent to 3 hydrogen) around δ 6.94 – δ 7.05 ppm [the integrated intensities A1 (equivalent to 2 hydrogen) and A2 (equivalent to 1 hydrogen) of the signals around δ 6.96 ppm and δ 7.04 ppm], assuming the signal of the internal reference compound as δ 0 ppm.

$$\text{Amount (\%) of magnoflorine iodide } (C_{20}H_{24}INO_4) = M_S \times I \times P/(M \times N) \times 2.0918$$

M: Amount (mg) of magnoflorine iodide for assay
M_S: Amount (mg) of DSS-d_6 for nuclear magnetic resonance spectroscopy
I: The signal integrated intensity, A, based on the signal integrated intensity of DSS-d_6 for nuclear magnetic resonance spectroscopy as 9.000
N: Number of hydrogen derived from A.
P: Purity (%) of DSS-d_6 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: A nuclear magnetic resonance spectroscopy apparatus having ^{1}H resonance frequency of not less than 400 MHz.

Target nuclei: ^{1}H.

Digital resolution: 0.25 or lower.

Measuring spectrum width: 20 ppm or upper, including between −5 ppm and 15 ppm.

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time 60 seconds or longer.

Integrating times: Not less than 8 times.

Dummy scanning: Not less than 2 times.

Measuring temperature: A constant temperature of 20 – 30°C.

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of the signal around δ 6.94 – δ 7.05 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals of around δ 6.96 – δ 7.04 ppm are not overlapped with any signal of obvious foreign substance, and the ratio of the integrated intensity of each signal (A1/2)/A2 is between 0.99 and 1.01.

System repeatability: When the test is repeated 6 times with the sample solution under the above operating conditions, the relative standard deviation of the ratio of the integrated intensity A to that of the internal reference is not more than 1.0%.

Magnolia flower [Same as the namesake monograph]

Maltitol $C_{12}H_{24}O_{11}$ A white crystalline powder. Very soluble in water, and practically insoluble in ethanol (99.5).

Mequitazine for assay $C_{20}H_{22}N_2S$ [Same as the monograph Mequitazine. When dried, it contains not less than 99.5% of mequitazine ($C_{20}H_{22}N_2S$).]

4′-Methoxyacetophenone $C_9H_{10}O_2$ White to light brown, crystals or crystalline powder.

Melting point <*2.60*>: 34 – 39°C

Methyl arachidate for gas chromatography $C_{21}H_{42}O_2$ White to light yellow, crystals or crystalline masses.

Melting point <*2.60*>: 45 – 50°C

Methyl eicosenoate for gas chromatography $C_{21}H_{40}O_2$ A clear and colorless, liquid.

Methyl laurate for gas chromatography $C_{13}H_{26}O_2$ A colorless to yellow, liquid.

Refractive index <*2.45*> n_D^{20}: 1.431 – 1.433

Specific gravity <*2.56*> d_{20}^{20}: 0.870 – 0.872

Methyl lignocerate for gas chromatography $C_{25}H_{50}O_2$ A white, crystalline powder.

Melting point <*2.60*>: 58 – 61°C

Methyl linoleate for gas chromatography $C_{19}H_{34}O_2$ A colorless to light yellow, liquid.

Specific gravity <*2.56*> d_{20}^{20}: 0.880 – 0.889

Methyl linolenate for gas chromatography $C_{19}H_{32}O_2$ A colorless to light yellow, liquid.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 0.890 – 0.901

Methyl myristate for gas chromatography $C_{15}H_{30}O_2$ A colorless to light yellow, liquid.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: about 0.866 – 0.874

Methyl oleate for gas chromatography $C_{19}H_{36}O_2$ A clear, colorless to light yellow, liquid.

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 0.866 – 0.882

Methyl palmitate for gas chromatography $C_{17}H_{34}O_2$ White, crystals or waxy masses.

Congealing point ⟨*2.42*⟩: 25 – 31°C

Methyl palmitoleate for gas chromatography $C_{17}H_{32}O_2$

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 0.876 – 0.881

Methyl parahydroxybenzoate for resolution check $C_8H_8O_3$ Colorless crystals or a white crystalline powder. Freely soluble in methanol, in ethanol (95) and in acetone, and very slightly soluble in water. Melting point: 125 – 128°C.

Identification—Determine the infrared absorption spectrum of methyl parahydroxybenzoate for resolution check as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum of Methyl Parahydroxybenzoate or the spectrum of Methyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Related substances—Dissolve 50 mg of methyl parahydroxybenzoate for resolution check in 2.5 mL of methanol, and add the mobile phase to make 50 mL. To 10 mL of this solution add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: total area of the peaks other than methyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of methyl parahydroxybenzoate obtained from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Methyl Parahydroxybenzoate.

Time span of measurement: About 5 times as long as the retention time of methyl parahydroxybenzoate.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of methyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 3.5% to 6.5% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of methyl parahydroxybenzoate are not less than 2500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methyl parahydroxybenzoate is not more than 5.0%.

Methyl stearate for gas chromatography $C_{19}H_{38}O_2$ White, crystals or crystalline masses.

Melting point ⟨*2.60*⟩: 36 – 42°C

Naftopidil for assay $C_{24}H_{28}N_2O_3$ [Same as the monograph Naftopidil. When dried, it contains not less than 99.5% of naftopidil ($C_{24}H_{28}N_2O_3$).]

Nickel (II) sulfate hexahydrate $NiSO_4.6H_2O$ [K 8989, Special class]

Nitrilotriacetic acid $C_6H_9NO_6$ A white crystalline powder. Melting point: about 240°C (with decomposition).

Identification—Determine the infrared absorption spectrum of nitrilotriacetic acid as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 1718 cm^{-1}, 1243 cm^{-1}, 1205 cm^{-1}, 968 cm^{-1}, 903 cm^{-1}, 746 cm^{-1} and 484 cm^{-1}.

Loss on drying ⟨*2.41*⟩: not more than 0.5% (1 g, 105°C, 3 hours).

Content: not less than 97.0%. Assay—Weigh accurately about 0.2 g of nitrilotriacetic acid, dissolve in 50 mL of water by heating, and titrate ⟨*2.50*⟩ after cooling with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 9.557 mg of $C_6H_9NO_6$

Nonessential amino acid TS Dissolve 89 mg of L-alanine, 150 mg of L-asparagine monohydrate, 133 mg of L-aspartic acid, 147 mg of L-glutamic acid, 75 mg of glycine, 115 mg of L-proline and 105 mg of L-serine in 100 mL of water, and sterilize by filtration through a membrane filter with a pore size not exceeding 0.22 μm.

Olopatadine hydrochloride for assay $C_{21}H_{23}NO_3.HCl$ [Same as the monograph Olopatadine Hydrochloride. When dried, it contains not less than 99.5% of olopatadine hydrochloride ($C_{21}H_{23}NO_3.HCl$).]

***H*-D-phenylalanyl-L-pipecolyl-L-arginyl-*p*-nitroanilide dihydrochloride** A white powder. Slightly soluble in water.

Absorbance ⟨*2.24*⟩ $E_{1\,cm}^{1\%}$ (316 nm): 192 – 214 (10 mg, water, 300 mL).

Pilsicainide hydrochloride hydrate for assay $C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$ [Same as the monograph Pilsicainide Hydrochloride Hydrate. It contains not less than 99.3% of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$).]

Piperacillin hydrate $C_{23}H_{27}N_5O_7S.H_2O$ [Same as the namesake monograph]

Phosphate buffer solution for cytotoxicity test Dissolve 0.20 g of potassium chloride, 0.20 g of potassium dihydrogen phosphate, 8.00 g of sodium chloride and 1.15 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL, and sterilize in an autoclave at 121°C for 15 minutes.

Propyl parahydroxybenzoate for resolution check $C_{10}H_{12}O_3$ Colorless crystals or a white crystalline powder. Freely soluble in methanol, in ethanol (95) and in acetone, and very slightly soluble in water. Melting point: 96 – 99°C.

Identification—Determine the infrared absorption spectrum of propyl parahydroxybenzoate for resolution check as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum of Propyl Parahydroxybenzoate or the spectrum of Propyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Related substances—Dissolve 50 mg of propyl parahydroxybenzoate for resolution check in 2.5 mL of methanol, and add the mobile phase to make 50 mL. To 10 mL of this solution add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: total area of the peaks other than propyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of propyl parahydroxybenzoate obtained from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Propyl Parahydroxybenzoate.

Time span of measurement: About 2.5 times as long as the retention time of propyl parahydroxybenzoate.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of propyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 3.5% to 6.5% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propyl parahydroxybenzoate are not less than 2500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propyl parahydroxybenzoate is not more than 5.0%.

Propylene glycol for gas chromatography $C_3H_8O_2$ [K 8837, Special class] When perform the test as directed in the Purity (7) under Propylene Glycol, it does not show any peak at the retention times corresponding to ethylene glycol and diethylene glycol.

Raponticin for thin-layer chromatography $C_{21}H_{24}O_9$ A white to pale yellow-brown crystalline powder, having no odor. Slightly soluble in methanol, and practically insoluble in water and in ethanol (99.5).

Identification—Determine the infrared absorption spectrum of raponticin for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1612 cm^{-1}, 1577 cm^{-1}, 1513 cm^{-1}, 948 cm^{-1}, 831 cm^{-1} and 798 cm^{-1}.

Purity Related substances—Dissolve 4 mg of raponticin for thin-layer chromatography in 2 mL of methanol, and use this as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed in the Purity (3) under Rhubarb: the spot other than the principal spot that appears at an *R*f value of about 0.3 obtained with the sample solution is not more intense than the spot obtained with the standard solution.

Rutin for thin-layer chromatography $C_{27}H_{30}O_{16}$ Pale yellow to yellow-green, crystals or crystalline powder, having no odor. Soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of rutin for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 255 nm and 259 nm, and between 356 nm and 360 nm.

(2) Determine the infrared absorption spectrum of rutin for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1655 cm^{-1}, 1600 cm^{-1}, 1507 cm^{-1} and 1363 cm^{-1}.

Purity *Related substances*—Dissolve 10 mg of rutin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed in Identification 1) under Crataegus Fruit: the spot other than the principal spot appeared at an *R*f value of about 0.3 obtained with the sample solution is not more intense than the spot obtained with the standard solution.

Sivelestat sodium hydrate $C_{20}H_{21}N_2NaO_7S.4H_2O$ [Same as the namesake monograph]

10% Sodium hydrogen carbonate TS Dissolve 10 g of sodium hydrogen carbonate in water to make 100 mL, and sterilize in a tight container in an autoclave at 121°C for 15 minutes or by filtration through a membrane filter with a

pore size not exceeding 0.22 μm.

Sodium pyruvate $CH_3COCOONa$ A white to pale yellow crystalline powder. Freely soluble in water, and slightly soluble in ethanol (99.5) and in acetone.

Identification (1) Determine the infrared absorption spectrum of sodium pyruvate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1710 cm^{-1}, 1630 cm^{-1}, 1410 cm^{-1}, 1360 cm^{-1}, 1190 cm^{-1}, 1020 cm^{-1}, 980 cm^{-1}, 830 cm^{-1},750 cm^{-1}, 630 cm^{-1} and 430 cm^{-1}.

(2) A solution of sodium pyruvate (1 in 20) responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

Content: Not less than 97.0%. Assay—Weigh accurately about 0.4 g of sodium pyruvate, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution into an iodine bottle, cool to 10°C or lower, add exactly 40 mL of 0.05 mol/L iodine VS, then add 20 mL of a solution of sodium hydroxide (17 in 100), and allow to stand at a dark place for 2 hours. Then add 15 mL of diluted sulfuric acid (1 in 6), and titrate <*2.50*> with 0.1 mol/L sodium thiosulfate VS (indicator: starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 1.834 mg of $C_3H_3NaO_3$

100 mmol/L Sodium pyruvate TS Dissolve 1.1 g of sodium pyruvate in water to make 100 mL, and sterilize by filtration through a membrane filter with a pore size not exceeding 0.22 μm.

Telmisartan for assay $C_{33}H_{30}N_4O_2$ [Same as the monograph Telmisartan]

1 mol/L Tris buffer solution, pH 7.5 Dissolve 12.11 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 90 mL of water, adjust to pH 7.5 with hydrochloric acid, and add water to make 100 mL.

Trypsin Obtained from bovine or hog pancreas, and prepared for biochemistry or to meet the following requirements. White to light yellowish crystals or powder.

Loss on drying: Not more than 5.0% (60°C, in vacuum, 4 hours).

Content: Not less than 220 trypsin units per mg. Assay (i) Sample solution—Weigh accurately about 20 mg of the substance to be assayed, and dissolve in 0.001 mol/L hydrochloric acid TS so that each mL contains about 3000 trypsin units. To a suitable amount of this solution add 0.001 mol/L hydrochloric acid TS so that each mL contains about 40 trypsin units, and use this solution as the sample solution.

(ii) Diluting solution—Dissolve 4.54 g of potassium dihydrogen phosphate in water to make exactly 500 mL (Solution I). Dissolve 4.73 g of anhydrous disodium hydrogen phosphate in water to make exactly 500 mL (Solution II). To 80 mL of Solution II add a suitable amount of Solution I to adjust to pH 7.6.

(iii) Substrate solution—Dissolve 85.7 mg of *N*-α-benzoyl-L-ethylarginine hydrochloride in water to make exactly 100 mL, and use this solution as the substrate stock solution. Pipet 10 mL of the substrate stock solution add diluting solution to make exactly 100 mL, and use this solution as the substrate solution. The substrate solution gives an absorbance of between 0.575 and 0.585 at 253 nm when determined as directed under Ultraviolet-visible spectrophotometry <*2.24*> using water as the blank. If necessary adjust the absorbance by addition of the diluting solution or substrate stock solution.

(iv) Procedure—Transfer exactly 3 mL of the substrate solution, previously warmed to 25 ± 0.1°C, into a 1-cm quartz cell, add exactly 0.2 mL of the sample solution, immediately start the timer, and determine the change of the absorbance at 253 nm at 25 ± 0.1°C for 5 minutes, using the control prepared by adding exactly 0.2 mL of 0.001 mol/L hydrochloric acid TS to exact 3 mL of the substrate solution. Obtain the variation per minute of the absorbance, A, from the part where the changing rate of the absorbance is constant for at least 3 minutes.

(v) Calculation—Calculate trypsin unit per mg using the following equation. Where, one trypsin unit is the quantity of enzyme that gives the variation of the absorbance 0.003 per minute.

$$\text{Trypsin unit per mg} = A/0.003 \times 1/M$$

M: Amount (mg) of the substance to be assayed in 0.2 mL of the sample solution

Storage At a cold place.

Trypsin TS Dissolve 0.5 g of trypsin and 0.2 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in phosphate buffer solution for cytotoxicity test to make 1000 mL, and sterilize by filtration through a membrane filter with a pore size not exceeding 0.22 μm.

Verbascoside for thin-layer chromatography $C_{29}H_{36}O_{15}$ A white to very pale yellow, odorless crystalline powder or powder. Soluble in methanol, sparingly soluble in ethanol (99.5), and slightly soluble in water.

Identification Determine the infrared absorption spectrum of verbascoside for thin-layer chromatography as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1604 cm^{-1}, 1446 cm^{-1}, 1272 cm^{-1} and 815 cm^{-1}.

Purity Related substances—Dissolve 10 mg of verbascoside for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed in the Identification under Cistanche Herb: the spot other than the principal spot that appears at an *R*f value of about 0.35 obtained from the sample solution is not more intense than the spot obtained from the standard solution.

V8 protease for insulin glargine A protease obtained

from *Staphylococcus aureus* strain. When an amount of the enzyme which hydrolyzes 1 μmol of carbobenzoxy-phenylalanyl-leucyl-glutamyl-4-nitroanilide in 1 minute at pH 7.8 and 25°C is defined as 1 unit, it contains not less than 20 units per mg.

9.42 Solid Supports/Column Packings for Chromatography

Add the following:

Cellulose derivative-bonded silica gel for liquid chromatography Prepared for liquid chromatography.

β-Cyclodextrin binding silica gel for liquid chromatography A silica gel bound with β-cyclodextrin, prepared for liquid chromatography.

Graphite carbon for liquid chromatography Prepared for liquid chromatography.

Octadecylsilanized porous glass for liquid chromatography Prepared for liquid chromatography.

Ovomucoid-chemically bonded amino silica gel for liquid chromatography Prepared for liquid chromatography.

9.43 Filter Papers, Filters for filtration, Test Papers, Crucibles, etc.

Add the following:

Sintered glass filter for cupric oxide filtration A glass filter with a pore size of 10 – 16 μm.

9.62 Measuring Instruments, Appliances

Change the Balances and weights, and Volumetric measures as follows:

Balances and weights **(1)** Chemical balances—Use balances readable to the extent of 0.1 mg.

(2) Semimicrobalances—Use balances readable to the extent of 10 μg.

(3) Microbalances—Use balances readable to the extent of 1 μg.

(4) Ultramicrobalances—Use balances readable to the extent of 0.1 μg.

(5) Weights—Use calibrated weights.

Volumetric measures Use volumetric flasks, transfer pipets, piston pipets, burets and measuring cylinders conforming to the Japanese Industrial Standard.

Official Monographs

Add the following:

Aciclovir Granules

アシクロビル顆粒

Aciclovir Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of aciclovir ($C_8H_{11}N_5O_3$: 225.20).

Method of preparation Prepare as directed under Granules, with Aciclovir.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 254 nm and 258 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: Aciclovir Granules in single-unit containers meet the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Aciclovir Granules, add 100 mL of dilute sodium hydroxide TS, agitate with the aid of ultrasonic waves with occasional shaking, and add dilute sodium hydroxide TS to make exactly 200 mL. Filter this solution, discard the first 20 mL of the filtrate, pipet V mL of the subsequent filtrate, add dilute sodium hydroxide TS to make exactly V' mL so that each mL contains about 1 mg of aciclovir ($C_8H_{11}N_5O_3$). Pipet 15 mL of this solution, add 50 mL of water and 5.8 mL of 2 mol/L hydrochloric acid TS, add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of acyclovir ($C_8H_{11}N_5O_3$)
$= M_S \times A_T/A_S \times V'/V \times 8$

M_S: Amount (mg) of Aciclovir RS, calculated on the anhydrous basis

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Aciclovir Granules is not less than 85%.

Start the test with an accurately weighed amount of Aciclovir Granules, equivalent to about 0.4 g of aciclovir ($C_8H_{11}N_5O_3$), withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Aciclovir RS (separately determine the water <*2.48*> in the same manner as Aciclovir), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 252 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of aciclovir ($C_8H_{11}N_5O_3$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 1800$

M_S: Amount (mg) of aciclovir RS, calculated on the anhydrous basis
M_T: Amount (g) of Aciclovir Granules
C: Labeled amount (mg) of aciclovir ($C_8H_{11}N_5O_3$) in 1 g

Assay Powder Aciclovir Granules, and weigh accurately a portion of the powder, equivalent to about 0.1 g of aciclovir ($C_8H_{11}N_5O_3$), add 60 mL of dilute sodium hydroxide TS, agitate with the aid of ultrasonic waves for 15 minutes, then add dilute sodium hydroxide TS to make exactly 100 mL, and filter. Discard the first 20 mL of filtrate, pipet 15 mL of the subsequent filtrate, add 50 mL of water and 5.8 mL of 2 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Aciclovir RS (separately determine the water <*2.48*> in the same manner as Aciclovir), and dissolve in dilute sodium hydroxide TS to make exactly 25 mL. Pipet 15 mL of this solution, add 50 mL of water and 5.8 mL of 2 mol/L hydrochloric acid TS, add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using 0.1 mol/L hydrochloric acid TS as the blank.

Amount (mg) of acyclovir ($C_8H_{11}N_5O_3$)
$= M_S \times A_T/A_S \times 4$

M_S: Amount (mg) of Aciclovir RS, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Add the following:

Aciclovir Ophthalmic Ointment

アシクロビル眼軟膏

Aciclovir Ophthalmic Ointment contains not less than 90.0% and not more than 110.0% of the labeled amount of aciclovir ($C_8H_{11}N_5O_3$: 225.20).

Method of preparation Prepare as directed under Ophthalmic Ointments, with Aciclovir.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 254 nm and 258 nm.

Metal Particles <*6.01*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Particle diameter Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Assay Weigh accurately a portion of Aciclovir Ophthalmic Ointment, equivalent to about 15 mg of aciclovir ($C_8H_{11}N_5O_3$), add exactly 20 mL of hexane and exactly 20 mL of dilute sodium hydroxide TS, and shake vigorously. Centrifuge this mixture, discard the upper layer, pipet 1 mL of the lower layer, add 70 mL of water and 5 mL of 2 mol/L hydrochloric acid TS, then add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of Aciclovir RS (separately determine the water <*2.48*> in the same manner as Aciclovir), and dissolve in dilute sodium hydroxide TS to make exactly 20 mL. Pipet 1 mL of this solution, add 70 mL of water and 5 mL of 2 mol/L hydrochloric acid TS, then add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank.

Amount (mg) of acyclovir ($C_8H_{11}N_5O_3$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Aciclovir RS, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Add the following:

Aciclovir Tablets

アシクロビル錠

Aciclovir Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of aciclovir ($C_8H_{11}N_5O_3$: 225.20).

Method of preparation Prepare as directed under Tablets, with Aciclovir.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 254 nm and 258 nm.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Aciclovir Tablets is not less than 80%.

Start the test with 1 tablet of Aciclovir Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 8.9 μg of aciclovir ($C_8H_{11}N_5O_3$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of aciclovir RS (separately determine the water <*2.48*> in the same manner as Aciclovir), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 252 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Dissolution rate (%) with respect to the labeled amount of aciclovir ($C_8H_{11}N_5O_3$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of Acyclovir RS, calculated on the anhydrous basis

C: Labeled amount (mg) of aciclovir ($C_8H_{11}N_5O_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Aciclovir Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of aciclovir ($C_8H_{11}N_5O_3$), add 60 mL of dilute sodium hydroxide TS, and agitate for 15 minutes with the aid of ultrasonic waves, then add dilute sodium hydroxide TS to make exactly 100 mL, and filter. Discard the first 20 mL of filtrate, pipet 15 mL of the subsequent filtrate, add 50 mL of water and 5.8 mL of 2 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this

solution as the sample solution. Separately, weigh accurately about 25 mg of Aciclovir RS (separately determine the water <2.48> in the same manner as Aciclovir), and dissolve in dilute sodium hydroxide TS to make exactly 25 mL. Pipet 15 mL of this solution, add 50 mL of water and 5.8 mL of 2 mol/L hydrochloric acid TS, add water to make exactly 100 mL. Pipet 5 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 255 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 0.1 mol/L hydrochloric acid TS as the blank.

$$\text{Amount (mg) of acyclovir } (C_8H_{11}N_5O_3) = M_S \times A_T/A_S \times 4$$

M_S: Amount (mg) of Aciclovir RS, calculated on the anhydrous basis

Containers and storage Containers—Well-closed containers.

Alprostadil Alfadex

アルプロスタジル　アルファデクス

Delete the following item:

Expiration date

Dried Aluminum Hydroxide Gel Fine Granules

乾燥水酸化アルミニウムゲル細粒

Delete the following item:

Particle size

Add the following:

Azelnidipine Tablets

アゼルニジピン錠

Azelnidipine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of azelnidipine ($C_{33}H_{34}N_4O_6$: 582.65).

Method of preparation Prepare as directed under Tablets, with Azelnidipine.

Identification Powder Azelnidipine Tablets. Weigh a portion of the powder, equivalent to 4 mg of Azelnidipine, add 150 mL of ethanol (99.5), treat with ultrasonic waves for 15 minutes, then add ethanol (99.5) to make 200 mL. Centrifuge this solution, filter the supernatant liquid through a glass wool filter with a pore size not exceeding 0.7 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 253 nm and 257 nm and between 339 nm and 346 nm.

Purity Related substances—Conduct this procedure using light-resistant vessels. Powder Azelnidipine Tablets. Weigh a portion of the powder, equivalent to 10 mg of Azelnidipine, add 10 mL of a mixture of acetonitrile and water (4:1), agitate gently, then disperse to fine particles with the aid of ultrasonic waves for 15 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Pipet 2 mL of this solution, add a mixture of acetonitrile and water (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks, having the relative retention times of about 0.10, about 0.13, about 0.50, and about 1.42 to azelnidipine, obtained from the sample solution, are not larger than 9/20 times, 1/5 times, 2/5 times, and 2/5 times the peak area of azelnidipine obtained from the standard solution, respectively, the area of the peak, other than azelnidipine and the peaks mentioned above, is not larger than 1/10 times the peak area of azelnidipine from the standard solution. Furthermore, the total area of these peaks other than azelnidipine is not larger than 1.75 times the peak area of azelnidipine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Azelnidipine.

Time span of measurement: About 2 times as long as the retention time of azelnidipine.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile and water (4:1) to make exactly 20 mL. Confirm that the peak area of azelnidipine obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of azelnidipine are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of azelnidipine is not more than 1.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Azelnidipine Tablets add exactly 1 mL of

the internal standard solution per 2 mg of azelnidipine ($C_{33}H_{34}N_4O_6$), and add a mixture of acetonitrile and water (4:1) to make 32 mL. Disintegrate the tablet with occasional shaking, and treat with ultrasonic waves for 10 minutes. Centrifuge this solution, pipet V mL of the supernatant liquid, equivalent to 2.5 mg of azelnidipine ($C_{33}H_{34}N_4O_6$), add a mixture of acetonitrile and water (4:1) to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of azelnidipine } (C_{33}H_{34}N_4O_6) = M_S \times Q_T/Q_S \times 8/5V$$

M_S: Amount (mg) of azelnidipine for assay

Internal standard solution—A solution of 2,2′-dinaphthylether in a mixture of acetonitrile and water (4:1) (1 in 1000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Azelnidipine Tablets is not less than 75%.

Start the test with 1 tablet of Azelnidipine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 8.9 μg of azelnidipine ($C_{33}H_{34}N_4O_6$), and use this solution as the sample solution. Separately, weigh accurately about 45 mg of azelnidipine for assay, previously dried in vacuum at 70°C for 5 hours, dissolve in ethanol (99.5) to make exactly 25 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 270 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the blank.

$$\text{Dissolution rate (\%) with respect to the labeled amount of azelnidipine } (C_{33}H_{34}N_4O_6) = M_S \times A_T/A_S \times V'/V \times 1/C \times 18$$

M_S: Amount (mg) of azelnidipine for assay
C: Labeled amount (mg) of azelnidipine ($C_{33}H_{34}N_4O_6$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Azelnidipine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of azelnidipine ($C_{33}H_{34}N_4O_6$), add exactly 25 mL of the internal standard solution, add 50 mL of a mixture of acetonitrile and water (4:1). After treating with ultrasonic waves for 10 minutes, add a mixture of acetonitrile and water (4:1) to make 100 mL. Centrifuge this solution, to 5 mL of the supernatant liquid add a mixture of acetonitrile and water (4:1) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of azelnidipine for assay, previously dried in vacuum at 70°C for 5 hours, dissolve in exactly 25 mL of the internal standard solution, and add a mixture of acetonitrile and water (4:1) to make 100 mL. To 5 mL of this solution add a mixture of acetonitrile and water (4:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of azelnidipine to that of the internal standard.

$$\text{Amount (mg) of azelnidipine } (C_{33}H_{34}N_4O_6) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of azelnidipine for assay

Internal standard solution—2,2′-dinaphthylether in a mixture of acetonitrile and water (4:1) (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 0.9 g of potassium dihydrogen phosphate in 300 mL of water, add 700 mL of acetonitrile, then adjust to pH 6.0 with dilute sodium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of azelnidipine is about 13 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, azelnidipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of azelnidipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Beclometasone Dipropionate

ベクロメタゾンプロピオン酸エステル

Change the Description as follows:

Description Beclometasone Dipropionate occurs as a white to pale yellow powder.

It is soluble in methanol, sparingly soluble in ethanol (95) and in 1,4-dioxane, and practically insoluble in water.

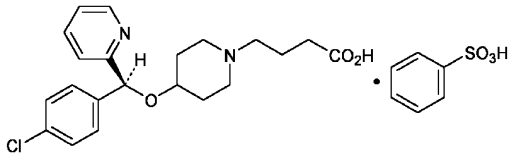
Melting point: about 208°C (with decomposition).

It shows crystal polymorphism.

Add the following:

Bepotastine Besilate

ベポタスチンベシル酸塩



$C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$: 547.06

(*S*)-4-{4-[(4-Chlorophenyl)(pyridin-2-yl)methoxy]piperidin-1-yl}butanoic acid monobenzenesulfonate

[*190786-44-8*]

Bepotastine Besilate contains not less than 99.0% and not more than 101.0% of $C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$, calculated on the anhydrous basis and corrected on the amount of the residual solvent.

Description Bepotastine Besilate occurs as white to pale yellowish white, crystals or crystalline powder.

It is very soluble in acetic acid (100), and sparingly soluble in water and in ethanol (99.5).

The pH of a solution of 1 g of Bepotastine Besilate in 100 mL of water is about 3.8.

Identification **(1)** Determine the absorption spectrum of a solution of Bepotastine Besilate (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bepotastine Besilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Bepotastine Besilate as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

(4) Mix well 30 mg of Bepotastine Besilate with 0.1 g of sodium nitrate and 0.1 g of anhydrous sodium carbonate, and gradually ignite. After cooling, dissolve the residue in 2 mL of dilute hydrochloric acid and 10 mL of water, filter if necessary, and add barium chloride TS: a white precipitate is produced.

Melting point <*2.60*> 159–163°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Bepotastine Besilate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 10 mg of Bepotastine Besilate in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having a relative retention time of about 2.5 to bepotastine, obtained from the sample solution is not larger than the peak area of bepotastine obtained from the standard solution, and the area of the peak other than bepotastine and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of bepotastine from the standard solution. Furthermore, the total area of the peaks other than bepotastine from the sample solution is not larger than the peak area of bepotastine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium 1-pentane sulfonate in a mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and acetonitrile (7:3) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of bepotastine is about 6 minutes.

Time span of measurement: About 5 times as long as the retention time of bepotastine, beginning after the peak of benzenesulfonic acid.

System suitability—

Test for required detectability: Pipet 2.5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of bepotastine obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bepotastine are not less than 3000 and 0.8–1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bepotastine is not more than 2.0%.

(3) Optical isomer—Dissolve 5.0 mg of Bepotastine Besilate in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the area of each peak by the automatic integration method: the area of the peak, having a relative retention time of about 0.9 to

bepotastine obtained from the sample solution, is not larger than the peak area of bepotastine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with β-cyclodextrin binding silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogen phosphate TS and acetonitrile (3:1).

Flow rate: Adjust the flow rate so that the retention time of bepotastine is about 17 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bepotastine are not less than 3000 and 0.8–1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bepotastine is not more than 5.0%.

(4) Residual solvent—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Water ⟨*2.48*⟩ Not more than 0.1% (0.3 g, coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.8 g of Bepotastine Besilate, dissolve in 60 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 54.71 mg of $C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$

Containers and storage Containers—Tight containers.

Add the following:

Bepotastine Besilate Tablets

ベポタスチンベシル酸塩錠

Bepotastine Besilate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$: 547.06).

Method of preparation Prepare as directed under Tablets, with Bepotastine Besilate.

Identification To an amount of powdered Bepotastine Besilate Tablets, equivalent to 2 mg of Bepotastine Besilate, add 40 mL of water, shake thoroughly, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 260 nm and 264 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Bepotastine Besilate Tablets add exactly $V/5$ mL of the internal standard solution, then add the mobile phase to make V mL so that each mL contains about 0.4 mg of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$), shake vigorously for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, to 2 mL of the subsequent filtrate add the mobile phase to make 10 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$)
$= M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of bepotastine besilate for assay, calculated on the anhydrous basis and corrected on the amount of the residual solvent

Internal standard solution—A solution of ethyl parahydroxybenzoate in acetonitrile (1 in 4500).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Bepotastine Besilate Tablets is not less than 85%.

Start the test with 1 tablet of Bepotastine Besilate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 2.2 μg of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$), and use this solution as the sample solution. Separately, weigh accurately about 0.11 g of bepotastine besilate for assay (separately determine the water ⟨*2.48*⟩ and the residual solvent in the same manner as Bepotastine Besilate), and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of bepotastine in each solution.

Dissolution rate (%) with respect to the labeled amount of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/5$

M_S: Amount (mg) of bepotastine besilate for assay, calculated on the anhydrous basis and corrected on the amount of the residual solvent

C: Labeled amount (mg) of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bepotastine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bepotastine is not more than 1.5%.

Assay Weigh accurately the mass of not less than 20 tablets of Bepotastine Besilate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of bepotastine besilate ($C_{21}H_{25}ClN_2O_3.C_6H_6O_3S$), add exactly 5 mL of the internal standard solution, then add 20 mL of the mobile phase, shake thoroughly for 10 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, to 2 mL of the subsequent filtrate add the mobile phase to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of bepotastine besilate for assay (separately determine the water <*2.48*> and the residual solvent in the same manner as Bepotastine Besilate), add exactly 10 mL of the internal standard solution, and dissolve in the mobile phase to make 50 mL. To 2 mL of this solution add the mobile phase to make 10 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of bepotastine to that of the internal standard.

$$\text{Amount (mg) of bepotastine besilate } (C_{21}H_{25}ClN_2O_3.C_6H_6O_3S) = M_S \times Q_T/Q_S \times 1/2$$

M_S: Amount (mg) of bepotastine besilate for assay, calculated on the anhydrous basis and corrected on the amount of the residual solvent

Internal standard solution—A solution of ethyl parahydroxybenzoate in acetonitrile (1 in 4500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A solution of sodium 1-pentanesulfonate in a mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and acetonitrile (7:3) (1 in 1000).

Flow rate: Adjust the flow rate so that the retention time of bepotastine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, bepotastine and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bepotastine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Betamethasone

ベタメタゾン

Change the Description as follows:

Description Betamethasone occurs as a white to pale yellowish white, crystalline powder.

It is sparingly soluble in methanol, in ethanol (95) and in acetone, and practically insoluble in water.

Melting point: about 240°C (with decomposition).

It shows crystal polymorphism.

Bisacodyl Suppositories

ビサコジル坐剤

Change the Uniformity of dosage units as follows:

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 suppository of Bisacodyl Suppositories add a suitable amount of tetrahydrofuran, warm to 40°C, and shake to dissolve. After cooling, add tetrahydrofuran to make exactly V mL so that each mL contains about 0.2 mg of bisacodyl ($C_{22}H_{19}NO_4$). Pipet 5 mL of this solution, and proceed as directed in the Assay.

$$\text{Amount (mg) of bisacodyl } (C_{22}H_{19}NO_4) = M_S \times Q_T/Q_S \times V/50$$

M_S: Amount (mg) of Bisacodyl RS

Internal standard solution—A solution of ethyl parahydroxybenzoate in acetonitrile (3 in 100,000).

Add the following:

Brotizolam Tablets

ブロチゾラム錠

Brotizolam Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of brotizolam ($C_{15}H_{10}BrClN_4S$: 393.69).

Method of preparation Prepare as directed under Tablets, with Brotizolam.

Identification Shake a quantity of powdered Brotizolam Tablets, equivalent to 0.1 mg of Brotizolam, with 10 mL of methanol, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 239 nm and 243 nm.

Purity Related substances—Use the sample solution obtained in the Assay. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than brotizolam obtained from the sample solution is not larger than 1.5 times the peak area of brotizolam obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of brotizolam, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 10 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of brotizolam obtained with 40 μL of this solution is equivalent to 7 to 13% of that obtained with 40 μL of the standard solution.

System performance: When the procedure is run with 40 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of brotizolam are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of brotizolam is not more than 2.0%.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Brotizolam Tablets add exactly V mL of the mobile phase so that each mL contains about 25 μg of brotizolam ($C_{15}H_{10}BrClN_4S$), shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of brotizolam } (C_{15}H_{10}BrClN_4S) = M_S \times A_T/A_S \times V/1000$$

M_S: Amount (mg) of brotizolam for assay

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Brotizolam Tablets is not less than 85%.

Start the test with 1 tablet of Brotizolam Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 0.14 μg of brotizolam ($C_{15}H_{10}BrClN_4S$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of brotizolam for assay, previously dried at 105°C for 3 hours, dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 200 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of brotizolam in each solution.

$$\text{Dissolution rate (\%) with respect to the labeled amount of brotizolam } (C_{15}H_{10}BrClN_4S) = M_S \times A_T/A_S \times V'/V \times 1/C \times 9/20$$

M_S: Amount (mg) of brotizolam for assay

C: Labeled amount (mg) of brotizolam ($C_{15}H_{10}BrClN_4S$) in 1 tablet

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of water and acetonitrile (63:37).

Flow rate: Adjust the flow rate so that the retention time of brotizolam is about 7 minutes.

System suitability—

System performance: When the procedure is run with 200 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of brotizolam are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 200 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of brotizolam is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Brotizolam Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.25 mg of brotizolam ($C_{15}H_{10}BrClN_4S$), add exactly 10 mL of the mobile phase, and shake for 15 minutes. Centrifuge this solution,

and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of brotizolam for assay, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of brotizolam in each solution.

$$\text{Amount (mg) of brotizolam } (C_{15}H_{10}BrClN_4S) = M_S \times A_T/A_S \times 1/100$$

M_S: Amount (mg) of brotizolam for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.1 g of ammonium carbonate in 1000 mL of water. To 600 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of brotizolam is about 3 minutes.

System suitability—

System performance: When the procedure is run with 40 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of brotizolam are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of brotizolam is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Calcitonin(Salmon)

カルシトニン(サケ)

Change the title and Japanese title as follows:

Calcitonin Salmon

カルシトニン　サケ

Precipitated Calcium Carbonate Fine Granules

沈降炭酸カルシウム細粒

Delete the following item:

Particle size

Calcium Pantothenate

パントテン酸カルシウム

Change the Description as follows:

Description Calcium Pantothenate occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

The pH of a solution prepared by dissolving 1.0 g of Calcium Pantothenate in 20 mL of water is between 7.0 and 9.0.

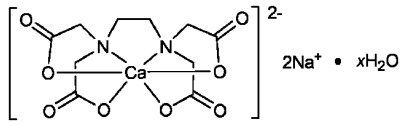
It is hygroscopic.

It shows crystal polymorphism.

Add the following:

Calcium Sodium Edetate Hydrate

エデト酸カルシウムナトリウム水和物



$C_{10}H_{12}CaN_2Na_2O_8.xH_2O$

Disodium [{*N*,*N*′-ethane-1,2-diylbis[*N*-(carboxymethyl)glycinato]}(4-)-*N*,*N*′,*O*,*O*′,O^N,$O^{N'}$]calciate(2-) hydrate

[*23411-34-9*]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Calcium Sodium Edetate Hydrate contains not less than 98.0% and not more than 102.0% of calcium disodium edetate ($C_{10}H_{12}CaN_2Na_2O_8$: 374.27), calculated on the anhydrous basis.

◆**Description** Calcium Sodium Edetate Hydrate occurs as white, powder or particles.

It is freely soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

It is hygroscopic.◆

Identification (1) Dissolve 2 g of Calcium Sodium

Edetate Hydrate in 10 mL of water, add 6 mL of a solution of lead (II) nitrate (33 in 1000), shake, and add 3 mL of potassium iodide TS: no yellow precipitate is formed. Make this solution alkaline by the addition of diluted ammonia solution (28) (7 in 50), and add 3 mL of ammonium oxalate TS: a white precipitate is formed.

◆**(2)** Determine the infrared absorption spectrum of Calcium Sodium Edetate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

(3) A solution of Calcium Sodium Edetate Hydrate (1 in 20) responds to the Qualitative Tests <*1.09*> (2) for sodium salt.

pH <*2.54*> The pH of a solution of 2.0 g of Calcium Sodium Edetate Hydrate in 10 mL of water is 6.5 to 8.0.

Purity ◆**(1)** Clarity and color of solution—Dissolve 0.25 g of Calcium Sodium Edetate Hydrate in 10 mL of water: the solution is clear and colorless.◆

(2) Chloride <*1.03*>—Dissolve 0.70 g of Calcium Sodium Edetate Hydrate in water to make 20 mL. To this solution add 30 mL of dilute nitric acid, allow to stand for 30 minutes, and filter. To 10 mL of the filtrate add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.10%).

◆**(3)** Heavy metals <*1.07*>—Proceed with 1.0 g of Calcium Sodium Edetate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).◆

(4) Disodium edetate—Dissolve 1.00 g of Calcium Sodium Edetate Hydrate in 50 mL of water, add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> with 0.01 mol/L magnesium chloride VS until the color of the solution changes from blue to red-purple (indicator: 40 mg of eriochrome black T-sodium chloride indicator): the amount of 0.01 mol/L magnesium chloride VS consumed is not more than 3.0 mL (not more than 1.0%).

◆**(5)** Nitrilotriacetic acid—Conduct this procedure using light-resistant vessels. Dissolve 0.100 g of Calcium Sodium Edetate Hydrate in diluting solution to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 40.0 mg of nitrilotriacetic acid in diluting solution to make exactly 100 mL. Pipet 1 mL of this solution, add 0.1 mL of the sample solution, then add diluting solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of nitrilotriacetic acid in each solution: A_T is not larger than A_S (not more than 0.1%).

Diluting solution: Dissolve 10.0 g of iron (III) sulfate *n*-hydrate in 20 mL of 0.5 mol/L sulfuric acid TS and 780 mL of water, adjust to pH 2.0 with sodium hydroxide TS, and add water to make 1000 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with graphite carbon for liquid chromatography (mean pore size: 25 nm, specific surface: 120 m^2/g, 5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 50.0 g of iron (III) sulfate *n*-hydrate in 50 mL of 0.5 mol/L sulfuric acid TS, add 750 mL of water, adjust to pH 1.5 with 0.5 mol/L sulfuric acid TS or sodium hydroxide TS, and add 20 mL of ethylene glycol and water to make 1000 mL.

Flow rate: 1.0 mL per minute (the retention time of nitrilotriacetic acid is about 5 minutes.).

System suitability—

Test for required detectability: When perform the test with 20 μL of the standard solution under the above operating conditions, the SN ratio of the peak of nitrilotriacetic acid is not less than 50.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, nitrilotriacetic acid and edetic acid are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nitrilotriacetic acid is not more than 1.0%.◆

Water <*2.48*> 5.0 – 13.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.5 g of Calcium Sodium Edetate Hydrate, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution, add 80 mL of water, adjust to pH 2 – 3 with dilute nitric acid, and titrate <*2.50*> with 0.01 mol/L bismuth nitrate VS until the color of the solution changes from yellow to red (indicator: 2 drops of xylenol orange TS).

Each mL of 0.01 mol/L bismuth nitrate VS
= 3.743 mg of $C_{10}H_{12}CaN_2Na_2O_8$

◆**Containers and storage** Containers—Tight containers.◆

Add the following:

Candesartan Cilexetil and Amlodipine Besylate Tablets

カンデサルタン シレキセチル·アムロジピンベシル酸塩錠

Candesartan Cilexetil and Amlodipine Besylate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of candesartan

cilexetil ($C_{33}H_{34}N_6O_6$: 610.66) and amlodipine besylate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$: 567.05).

Method of preparation Prepare as directed under Tablets, with Candesartan Cilexetil and Amlodipine Besylate.

Identification **(1)** Shake thoroughly a quantity of powdered Candesartan Cilexetil and Amlodipine Besylate Tablets, equivalent to 8 mg of Candesartan Cilexetil, with 20 mL of 0.01 mol/L hydrochloric acid TS, and centrifuge. Remove the supernatant liquid, to the residue add 20 mL of 0.01 mol/L hydrochloric acid TS, shake thoroughly, and centrifuge. Remove the supernatant liquid, to the residue add 40 mL of methanol, shake thoroughly, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 5 mL of the filtrate add methanol to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 252 nm and 256 nm, and between 302 nm and 307 nm.

(2) Shake thoroughly a quantity of powdered Candesartan Cilexetil and Amlodipine Besylate Tablets, equivalent to 2.5 mg of Amlodipine Besylate, with 20 mL of 0.01 mol/L hydrochloric acid TS, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. To 5 mL of the filtrate add methanol to make 25 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 236 nm and 240 nm, and between 360 nm and 364 nm.

Purity Related substances—Shake vigorously for 20 minutes a quantity of powdered Candesartan Cilexetil and Amlodipine Besylate Tablets, equivalent to 8 mg of Candesartan Cilexetil, with 20 mL of diluting solution, and filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add diluting solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.8 to candesartan cilexetil, obtained from the sample solution is not larger than 1.5 times the peak area of candesartan cilexetil obtained from the standard solution, the area of the peaks, having a relative retention time of about 0.9, about 1.1 and about 1.2 from the sample solution is not larger than 1/2 times the peak area of candesartan cilexetil from the standard solution, the area of the peak, having a relative retention time of about 1.4, is not larger than the peak area of candesartan cilexetil from the standard solution, and the area of the peak other than candesartan cilexetil and the peaks mentioned above is smaller than 1/10 times the peak area of candesartan cilexetil from the standard solution. Furthermore, the total area of the peaks other than candesartan cilexetil is not larger than 4 times the peak area of candesartan cilexetil from the standard solution.

Diluting solution: To 3.5 mL of triethylamine add water to make 500 mL, and adjust to pH 3.0 with phosphoric acid. To 400 mL of this solution add 600 mL of acetonitrile.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 253 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, acetonitrile and trifluoroacetic acid (4000:1000:1).

Mobile phase B: A mixture of acetonitrile, water and trifluoroacetic acid (4000:1000:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 15	100 → 50	0 → 50
15 – 50	50 → 0	50 → 100
50 – 60	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: For 60 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add diluting solution to make exactly 50 mL. Confirm that the peak area of candesartan cilexetil obtained with 20 μL of this solution is equivalent to 1.4 to 2.6% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of candesartan cilexetil are not less than 100,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of candesartan cilexetil is not more than 2.0%.

Uniformity of dosage units <*6.02*> Perform the test according to the following methods: it meets the requirements of the Content uniformity test.

(1) Candesartan cilexetil—To 1 tablet of Candesartan Cilexetil and Amlodipine Besylate Tablets add exactly 20 mL of diluting solution, shake for 20 minutes to disintegrate the tablet, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add exactly V'/5 mL of the internal standard solution, then add diluting solution to make V' mL so that each mL contains about 0.16 mg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), and use this solu-

tion as the sample solution. Then, proceed as directed in the Assay (1).

Amount (mg) of candesartan cilexetil ($C_{33}H_{34}N_6O_6$)
$= M_S \times Q_T/Q_S \times V'/V \times 2/25$

M_S: Amount (mg) of candesartan cilexetil for assay, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in diluting solution (1 in 2500).

Diluting solution: To 3.5 mL of triethylamine add water to make 500 mL, and adjust to pH 3.0 with phosphoric acid. To 400 mL of this solution add 600 mL of acetonitrile.

(2) Amlodipine besylate—To 1 tablet of Candesartan Cilexetil and Amlodipine Besylate Tablets add exactly 20 mL of diluting solution, shake for 20 minutes to disintegrate the tablet, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add exactly $V'/5$ mL of the internal standard solution, then add diluting solution to make V' mL so that each mL contains about 70 μg of amlodipine besylate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), and use this solution as the sample solution. Then, proceed as directed in the Assay (2).

Amount (mg) of amlodipine besylate
($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$)
$= M_S \times Q_T/Q_S \times V'/V \times 1/25$

M_S: Amount (mg) of Amlodipine Besylate RS, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in diluting solution (1 in 2500).

Diluting solution: To 3.5 mL of triethylamine add water to make 500 mL, and adjust to pH 3.0 with phosphoric acid. To 400 mL of this solution add 600 mL of acetonitrile.

Dissolution ⟨*6.10*⟩ **(1)** Candesartan cilexetil—When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of a solution, prepared by dissolving 1 g of polysorbate 80 in 2nd fluid for dissolution test to make 1000 mL, as the dissolution medium, the dissolution rate in 45 minutes of Candesartan Cilexetil and Amlodipine Besylate Tablets is not less than 80%.

Start the test with 1 tablet of Candesartan Cilexetil and Amlodipine Besylate Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 8.9 μg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), and use this solution as the sample solution. Separately, weigh accurately about 45 mg of candesartan cilexetil for assay (separately, determine the water ⟨*2.48*⟩ in the same manner as Candesartan Cilexetil), and dissolve in acetonitrile to make exactly 50 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of candesartan cilexetil in each solution.

Dissolution rate (%) with respect to the labeled amount of candesartan cilexetil ($C_{33}H_{34}N_6O_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of candesartan cilexetil for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of candesartan cilexetil ($C_{33}H_{34}N_6O_6$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (57:43:1).

Flow rate: Adjust the flow rate so that the retention time of candesartan cilexetil is about 6.5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of candesartan cilexetil are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of candesartan cilexetil is not more than 1.0%.

(2) Amlodipine besylate—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, as the dissolution medium, the dissolution rate in 30 minutes of Candesartan Cilexetil and Amlodipine Besylate Tablets is not less than 80%.

Start the test with 1 tablet of Candesartan Cilexetil and Amlodipine Besylate Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 3.9 μg of amlodipine besylate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), and use this solution as the sample solution. Separately, weigh accurately about 39 mg of Amlodipine Besylate RS (separately, determine the water ⟨*2.48*⟩ in the same manner as Amlodipine Besylate), and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, add acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly

50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of amlodipine in each solution.

Dissolution rate (%) with respect to the labeled amount of amlodipine besylate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of Amlodipine Besylate RS, calculated on the anhydrous basis

C: Labeled amount (mg) of amlodipine besylate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$) in 1 tablet

Operating conditions—

Detector, column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay (1).

Flow rate: Adjust the flow rate so that the retention time of amlodipine is about 4 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of amlodipine are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amlodipine is not more than 1.0%.

Assay **(1)** Candesartan cilexetil—Weigh accurately the mass of not less than 20 Candesartan Cilexetil and Amlodipine Besylate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 8 mg of candesartan cilexetil ($C_{33}H_{34}N_6O_6$), add exactly 20 mL of diluting solution, shake vigorously for 20 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add diluting solution to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of candesartan cilexetil for assay (separately, determine the water <2.48> in the same manner as Candesartan Cilexetil), dissolve in diluting solution to make exactly 100 mL, and use this solution as the candesartan cilexetil standard stock solution. Pipet 10 mL of the candesartan cilexetil standard stock solution, add exactly 5 mL of the internal standard solution, then add diluting solution to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of candesartan cilexetil to that of the internal standard.

Amount (mg) of candesartan cilexetil ($C_{33}H_{34}N_6O_6$)
$= M_S \times Q_T/Q_S \times 1/5$

M_S: Amount (mg) of candesartan cilexetil for assay, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in diluting solution (1 in 2500).

Diluting solution: To 3.5 mL of triethylamine add water to make 500 mL, and adjust to pH 3.0 with phosphoric acid. To 400 mL of this solution add 600 mL of acetonitrile.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 7 mL of triethylamine add water to make 1000 mL, and adjust to pH 6.5 with phosphoric acid. To 800 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of candesartan cilexetil is about 31 minutes.

System suitability—

System performance: Mix 10 mL of the candesartan cilexetil standard stock solution and 5 mL of the amlodipine besylate standard stock solution prepared in the Assay (2), add exactly 5 mL of the internal standard solution, then add diluting solution to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, amlodipine, the internal standard and candesartan cilexetil are eluted in this order and the resolution between the peaks of the internal standard and candesartan cilexetil is not less than 15.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of candesartan cilexetil to that of the internal standard is not more than 1.0%.

(2) Amlodipine besylate—Weigh accurately the mass of not less than 20 Candesartan Cilexetil and Amlodipine Besylate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 3.5 mg of amlodipine besylate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), add exactly 20 mL of diluting solution, shake vigorously for 20 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add diluting solution to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 35 mg of Amlodipine Besylate RS (separately, determine the water <2.48> in the same manner as Amlodipine Besylate), dissolve in diluting solution to make exactly 100 mL, and use this solution as the amlodipine besylate standard stock solution. Pipet 5 mL of the amlodipine besylate standard stock solution, add exactly 5 mL of the internal standard solution, then add diluting solution to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of amlodipine to that

of the internal standard.

$$\text{Amount (mg) of amlodipine besylate } (C_{20}H_{25}ClN_2O_5.C_6H_6O_3S) = M_S \times Q_T/Q_S \times 1/10$$

M_S: Amount (mg) of Amlodipine Besylate RS, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in diluting solution (1 in 2500).

Diluting solution: To 3.5 mL of triethylamine add water to make 500 mL, and adjust to pH 3.0 with phosphoric acid. To 400 mL of this solution add 600 mL of acetonitrile.

Operating conditions—

Detector, column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay (1).

Flow rate: Adjust the flow rate so that the retention time of amlodipine is about 2.5 minutes.

System suitability—

System performance: Mix 10 mL of the candesartan cilexetil standard stock solution prepared in the Assay (1) and 5 mL of the amlodipine besylate standard stock solution, add exactly 5 mL of the internal standard solution, then add diluting solution to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, amlodipine, the internal standard and candesartan cilexetil are eluted in this order and the resolution between the peaks of amlodipine and the internal standard is not less than 15.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of amlodipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Carmellose

カルメロース

Change the origin/limits of content and the Purity as follows:

Carmellose is partly *O*-carboxymethylated cellulose.

Purity **(1)** Chloride—Shake well 0.8 g of Carmellose with 50 mL of water, add 10 mL of sodium hydroxide TS to dissolve, and add water to make 100 mL. Heat 20 mL of this solution with 10 mL of dilute nitric acid in a water bath until a flocculent precipitate is produced, cool, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuge each time, combine the supernatant liquid and the washings, and add water to make 100 mL. Take 25 mL of this solution in a Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the test solution. Separately, to 0.40 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution. To the test solution and the control solution add 1 mL each of silver nitrate TS, ◆mix,◆ and allow to stand protected from light for 5 minutes. Compare the opalescence developed in both solutions ◆against a black background by viewing downward or transversely◆. The opalescence in the test solution is not more intense than that in the control solution (not more than 0.36%).

(2) Sulfate—Shake well 0.40 g of Carmellose with 25 mL of water, add 5 mL of sodium hydroxide TS to dissolve, and add 20 mL of water. Heat this solution with 2.5 mL of hydrochloric acid in a water bath until a flocculent precipitate is produced, cool, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuge each time, combine the supernatant liquid and the washings, and add water to make 100 mL. Filter this solution, discard the first 5 mL of the filtrate, take 25 mL of the subsequent filtrate in a Nessler tube, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the test solution. Separately, to 1.5 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the control solution. To the test solution and the control solution add 2 mL each of barium chloride TS, mix, and allow to stand for 10 minutes. Compare the opalescence developed in both solutions ◆against a black background by viewing downward or transversely◆. The opalescence in the test solution is not more intense than that in the control solution (not more than 0.72%).

◆**(3)** Heavy metals <*1.07*>—Proceed with 1.0 g of Carmellose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).◆

Cefaclor Fine Granules

セファクロル細粒

Delete the following item:

Particle size

Cefcapene Pivoxil Hydrochloride Fine Granules

セフカペン　ピボキシル塩酸塩細粒

Delete the following item:

Particle size

Cefdinir

セフジニル

Change the Purity (2) as follows:

Purity

(2) Related substances—Dissolve about 0.1 g of Cefdinir in 10 mL of 0.1 mol/L phosphate buffer solution, pH 7.0. To 3 mL of this solution add tetramethylammonium hydroxide TS, pH 5.5, to make 20 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of their peaks by the area percentage method: the amount of the peaks, having the relative retention time of about 0.7, about 1.2 and about 1.5 to cefdinir, is not more than 0.7%, not more than 0.3% and not more than 0.8%, respectively, the total amount of the peaks, having the relative retention time of about 0.85, about 0.93, about 1.11 and about 1.14 to cefdinir, is not more than 0.4%, and the amount of the peak other than cefdinir and the peaks mentioned above is not more than 0.2%. And the total amount of the peaks other than cefdinir is not more than 3.0 %.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: To 1000 mL of tetramethylammonium hydroxide TS, pH 5.5, add 0.4 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

Mobile phase B: To 500 mL of tetramethylammonium hydroxide TS, pH 5.5, add 300 mL of acetonitrile for liquid chromatography and 200 mL of methanol, and add 0.4 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 2	95	5
2 – 22	95 → 75	5 → 25
22 – 32	75 → 50	25 → 50
32 – 37	50	50

Flow rate: 1.0 mL per minute (the retention time of cefdinir is about 22 minutes).

Time span of measurement: For 37 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add tetramethylammonium hydroxide TS, pH 5.5, to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add tetramethylammonium hydroxide TS, pH 5.5, to make exactly 10 mL. Confirm that the peak area of cefdinir obtained from 10 μL of this solution is equivalent to 7 to 13% of that obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 30 mg of Cefdinir RS and 2 mg of cefdinir lactam ring-cleavage lactones in 3 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, add tetramethylammonium hydroxide TS, pH 5.5, to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, peak 1 and peak 2 of cefdinir lactam ring-cleavage lactones separated into 4 peaks, cefdinir, peak 3 and peak 4 of remaining cefdinir lactam ring-cleavage lactones are eluted in this order. Relative retention time of peak 3 of cefdinir lactam ring-cleavage lactone to cefdinir is about 1.11. The number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 7000 and not more than 3.0, respectively.

System repeatability: When the test is repeated 3 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefdinir is not more than 2.0%.

Cefdinir Fine Granules

セフジニル細粒

Delete the following item:

Particle size

Cefditoren Pivoxil Fine Granules

セフジトレン　ピボキシル細粒

Delete the following item:

Particle size

Cefmetazole Sodium

セフメタゾールナトリウム

Change the Purity and Assay as follows:

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefmetazole Sodium in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To a mixture of exactly 0.5 mL of

Cobalt (II) Chloride CS and exactly 5 mL of Iron (III) Chloride CS add water to make exactly 50 mL. To exactly 15 mL of this solution add water to make exactly 20 mL.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Cefmetazole Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of Cefmetazole Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Cefmetazole Sodium in 10 mL of water, and use this solution as the sample solution. Pipet 4 mL, 2 mL, 1 mL, 0.5 mL and 0.25 mL of the sample solution, add water to them to make exactly 100 mL, and use these solutions as the standard solutions (1), (2), (3), (4) and (5), respectively. Separately, dissolve 0.10 g of 1-methyl-1*H*-tetrazole-5-thiol in water to make exactly 100 mL, and use this solution as the standard solution (6). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solutions (1) to (6) on a plate of silica gel for thin-layer chromatography. Develop with a mixture of 1-buthanol, water and acetic acid (100) (4:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spot obtained from the sample solution corresponding to the spot obtained from the standard solution (6) is not more intense than the spot obtained from the standard solution (6), and the spots other than this spot and other than the principal spot are not more intense than the spot from the standard solution (1). Furthermore, the total amount of the spots other than the principal spot from the sample solution, calculated by the comparison with the spots from the standard solutions (1), (2), (3), (4) and (5), is not more than 8.0%.

Assay Weigh accurately an amount of Cefmetazole Sodium and Cefmetazole RS, equivalent to about 50 mg (potency), and dissolve each in the mobile phase to make exactly 25 mL. Pipet 1 mL each of these solutions, add exactly 10 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of cefmetazole to that of the internal standard.

Amount [μg (potency)] of cefmetazole ($C_{15}H_{17}N_7O_5S_3$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cefmetazole RS

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.75 g of ammonium dihydrogenphosphate in 700 mL of water, add 280 mL of methanol, 20 mL of tetrahydrofuran and 3.2 mL of 40% tetrabutylammonium hydroxide TS, and adjust to pH 4.5 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of cefmetazole is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefmetazole and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefmetazole to that of the internal standard is not more than 1.0%.

Cefotaxime Sodium

セフォタキシムナトリウム

Change the origin/limits of content and Purity (1) as follows:

Cefotaxime Sodium contains not less than 916 μg (potency) and not more than 978 μg (potency) per mg, calculated on the dried basis. The potency of Cefotaxime Sodium is expressed as mass (potency) of cefotaxime ($C_{16}H_{17}N_5O_7S_2$: 455.47).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefotaxime Sodium in 10 mL of water: the solution is clear, and its absorbance at 430 nm, determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.40.

Cefpodoxime Proxetil

セフポドキシム　プロキセチル

Change the Purity (2) and Isomer ratio as follows:

Purity

(2) Related substances—Dissolve 50 mg of Cefpodoxime Proxetil in 50 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the

amount of the peak, having the relative retention time of about 0.8 to the isomer B of cefpodoxime proxetil, is not more than 2.0%, the amount of the peak other than cefpodoxime proxetil is not more than 1.0%, and the total amount of the peaks other than cefpodoxime proxetil is not more than 6.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 22°C.

Mobile phase A: A mixture of water, methanol and a solution of formic acid (1 in 50) (11:8:1).

Mobile phase B: A mixture of methanol and a solution of formic acid (1 in 50) (19:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 65	95	5
65 – 145	95 → 15	5 → 85
145 – 155	15	85

Flow rate: 0.7 mL per minute (the retention time of the isomer B of cefpodoxime proxetil is about 60 minutes).

Time span of measurement: For 155 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 5 mL of the sample solution add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 200 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add the mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make exactly 100 mL. Confirm that the peak areas of the isomer A and the isomer B of cefpodoxime proxetil obtained from 20 μL of this solution are equivalent to 1.4 to 2.6% of them obtained from 20 μL of the solution for system suitability test, respectively.

System performance: When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the isomer A and the isomer B of cefpodoxime proxetil are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of the isomer A and the isomer B of cefpodoxime proxetil is not more than 2.0%.

Isomer ratio Perform the test with 5 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas of the two isomers of cefpodoxime proxetil, A_a, for the isomer having the smaller retention time, and A_b, for the isomer having the larger retention time, by the automatic integration method: $A_b/(A_a + A_b)$ is between 0.50 and 0.60.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution obtained in the Assay under the above operating conditions, the internal standard, the isomer A and the isomer B of cefpodoxime proxetil are eluted in this order with the resolution between the peaks of the isomers being not less than 4.

System repeatability: When the test is repeated 5 times with 5 μL of the standard solution obtained in the Assay under the above operating conditions, the relative standard deviation of the ratio of the peak area of the isomer B of cefpodoxime proxetil to that of the internal standard is not more than 1.0%.

Ceftazidime Hydrate

セフタジジム水和物

Change the origin/limits of content, Optical rotation and Purity as follows:

Ceftazidime Hydrate contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ceftazidime Hydrate is expressed as mass (potency) of ceftazidime ($C_{22}H_{22}N_6O_7S_2$: 546.58).

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −28 – −34° (0.5 g calculated on the anhydrous basis, phosphate buffer solution, pH 6.0, 100 mL, 100 mm).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ceftazidime Hydrate in 10 mL of a solution obtained by dissolving 5 g of anhydrous disodium hydrogen phosphate and 1 g of potassium dihydrogen phosphate in water to make 100 mL: the solution is clear, and its absorbance at 420 nm, determined as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, is not more than 0.20.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Ceftazidime Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances (i) Trityl-*t*-butyl substance and *t*-butyl substance—Dissolve 0.10 g of Ceftazidime Hydrate in 2 mL of diluted disodium hydrogen phosphate TS (1 in 3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted disodium hydrogenphosphate TS (1 in 3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these

solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of acetic acid (100), *n*-butyl acetate, acetate buffer solution, pH 4.5 and 1-butanol (16:16:13:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots which appear upper in position than the principal spot obtained from the sample solution are not more intense than the spot obtained from the standard solution.

(ii) Other related substances—Dissolve 20 mg of Ceftazidime Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ceftazidime obtained from the sample solution is not larger than that of ceftazidime obtained from the standard solution, and the total of peak areas other than ceftazidime is not larger than 5 times the peak area of ceftazidime from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.0 g of ammonium dihydrogenphosphate in 750 mL of water, adjust to pH 3.5 with phosphoric acid, and add water to make 870 mL. To this solution add 130 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ceftazidime is about 4 minutes.

Time span of measurement: About 3 times as long as the retention time of ceftazidime, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add the mobile phase to make exactly 5 mL, and confirm that the peak area of ceftazidime obtained with 5 μL of this solution is equivalent to 15% to 25% of that obtained with 5 μL of the standard solution.

System performance: Dissolve about 10 mg each of Ceftazidime Hydrate and acetanilide in 20 mL of the mobile phase. When the procedure is run with 5 μL of this solution under the above operating conditions, ceftazidime and acetanilide are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ceftazidime is not more than 2.0%.

(4) Free pyridine—Weigh accurately about 50 mg of Ceftazidime Hydrate, dissolve in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pyridine, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak heights, H_T and H_S, of pyridine in each solution: the amount of free pyridine is not more than 0.3%.

$$\text{Amount (mg) of free pyridine} = M_S \times H_T/H_S \times 1/1000$$

M_S: Amount (mg) of pyridine

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.88 g of ammonium dihydrogenphosphate in 500 mL of water, add 300 mL of acetonitrile and water to make 1000 mL, and adjust to pH 7.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of pyridine is about 4 minutes.

System suitability—

System performance: Dissolve 5 mg of Ceftazidime Hydrate in 100 mL of a solution of pyridine in the mobile phase (1 in 20,000). When the procedure is run with 10 μL of this solution under the above operating conditions, ceftazidime and pyridine are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak height of pyridine is not more than 5.0%.

Delete the following item:

Loss on drying

Add the following next to the Purity:

Water <2.48> 13.0 – 15.0% (0.1 g, volumetric titration, direct titration).

Change the Assay as follows:

Assay Weigh accurately an amount of Ceftazidime Hydrate, equivalent to about 0.1 g (potency), and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0, to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL

of the internal standard solution, then add 0.05 mol/L phosphate buffer solution, pH 7.0, to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Ceftazidime RS, equivalent to about 20 mg (potency), dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0, to make exactly 20 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add 0.05 mol/L phosphate buffer solution, pH 7.0, to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of ceftazidime to that of the internal standard.

Amount [μg (potency)] of ceftazidime ($C_{22}H_{22}N_6O_7S_2$)
$= M_S \times Q_T/Q_S \times 5000$

M_S: Amount [mg (potency)] of Ceftazidime RS

Internal standard solution—A solution of dimedon in 0.05 mol/L phosphate buffer solution, pH 7.0 (11 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with hexasilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.26 g of anhydrous disodium hydrogen phosphate and 2.72 g of potassium dihydrogen phosphate in 980 mL of water, and add 20 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ceftazidime is about 4 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and ceftazidime are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftazidime to that of the internal standard is not more than 1.0%.

Cefteram Pivoxil

セフテラム　ピボキシル

Change the Purity (2) as follows:

Purity

(2) Related substances—Dissolve 50 mg of Cefteram Pivoxil in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the each area of the peaks, having the relative retention time of about 0.2 and about 0.9 to cefteram pivoxil, obtained from the sample solution is not larger than 1/2 times and 1.25 times the peak area of cefteram pivoxil obtained from the standard solution, respectively, the area of the peak other than cefteram pivoxil and the peaks mentioned above is not larger than 1/4 times the peak area of cefteram pivoxil from the standard solution, and the total area of the peaks other than cefteram pivoxil is not larger than 2.75 times the peak area of cefteram pivoxil from the standard solution. For this calculation, use the area of the peak, having the relative retention time of about 0.1 to cefteram pivoxil, after multiplying by its relative response factor, 0.74.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of cefteram pivoxil.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefteram pivoxil obtained from 10 μL of this solution is equivalent to 7% to 13% of that obtained from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefteram pivoxil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefteram pivoxil is not more than 3.0%.

Cefteram Pivoxil Fine Granules

セフテラム　ピボキシル細粒

Delete the following item:

Particle size

Cefuroxime Axetil

セフロキシム　アキセチル

Change the Purity as follows:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Cefuroxime Axetil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard

Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 25 mg of Cefuroxime Axetil in 4 mL of methanol, add a solution of ammonium dihydrogen phosphate (23 in 1000) to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 40 mL of methanol and a solution of ammonium dihydrogen phosphate (23 in 1000) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cefuroxime axetil obtained from the sample solution is not larger than 1.5 times the total area of the two peaks of cefuroxime axetil obtained from the standard solution, and the total area of the peaks other than cefuroxime axetil is not larger than 4 times the total area of the two peaks of cefuroxime axetil from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of the peak having the larger retention time of the two peaks of cefuroxime axetil, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add 4 mL of methanol and a solution of ammonium dihydrogenphosphate (23 in 1000) to make exactly 10 mL. Confirm that the total area of the two peaks of cefuroxime axetil obtained with 2 μL of this solution is equivalent to 7% to 13% of that obtained with 2 μL of the standard solution.

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the resolution between the two peaks of cefuroxime axetil is not less than 1.5.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the total area of the two peaks of cefuroxime axetil is not more than 2.0%.

(3) Acetone—Weigh accurately about 1 g of Cefuroxime Axetil, add exactly 0.2 mL of the internal standard solution and dimethylsulfoxide to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of acetone, and add dimethylsulfoxide to make exactly 100 mL. Pipet 0.2 mL of this solution, add exactly 0.2 mL of the internal standard solution and dimethylsulfoxide to make 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of acetone to that of the internal standard: the amount of acetone is not more than 1.3%.

$$\text{Amount (\%) of acetone} = M_S/M_T \times Q_T/Q_S \times 1/5$$

M_S: Amount (g) of acetone
M_T: Amount (g) of Cefuroxim Axetil

Internal standard solution—A solution of 1-propanol in dimethylsulfoxide (1 in 200).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography coated with a mixture of polyethylene glycol 600 for gas chromatography and polyethylene glycol 1500 for gas chromatography (1:1) in the ratio of 20% (125 – 150 μm in particle diameter).

Column temperature: A constant temperature of about 90°C.

Temperature of injection port: A constant temperature of about 115°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 4 minutes.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, acetone and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acetone to that of the internal standard is not more than 5.0%.

Celmoleukin (Genetical Recombination)

セルモロイキン(遺伝子組換え)

Change the Identification (2) as follows:

Identification

(2) When hydrolyze the substance to be examined according to Method 1 and Method 4 described in ''1. Hydrolysis of Protein and Peptide'', and perform the test according to Method 1 described in ''2. Methodologies of Amino Acid Analysis'' under Amino Acid Analysis of Proteins <*2.04*>, there are glutamic acid (or glutamine) 17 or 18, threonine 11 to 13, aspartic acid (or asparagine) 11 or 12, lysine 11, isoleucine 7 or 8, serine 6 to 9, phenylalanine 6, alanine 5, proline 5 or 6, arginine 4, methionine 4, cysteine 3 or 4, valine 3 or 4, tyrosine 3, histidine 3, glycine 2, and tryptophan 1.

Procedure

(i) Hydrolysis Based on the results of the Assay (1), place an amount of Celmoleukin (Genetical Recombination), equivalent to about 50 μg as the total protein in two hydrolysis tubes, and evaporate to dryness under vacuum.

To one of the hydrolysis tubes add 100 μL of a mixture of diluted hydrochloric acid (59 in 125), mercapto acetic acid and phenol (100:10:1), and shake. Place this hydrolysis tube in a vial and humidify the inside of the vial with 200 μL of the mixture of diluted hydrochloric acid (59 in 125), mercapto acetic acid and phenol (100:10:1). Replace the vial interior with inert gas or reduce the pressure, and heat at about 115°C for 24 hours. After drying under vacuum, dissolve in 0.5 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution (1). To the other hydrolysis tube, add 100 μL of ice cold performic acid, oxidize for 1.5 hours on ice, add 50 μL of hydrobromic acid, and dry under vacuum. Add 200 μL of water, repeat the dry under vacuum procedure two more times, place the hydrolysis tube in a vial, and humidify the inside of the vial with 200 μL of diluted hydrochloric acid (59 in 125). Replace the vial interior with inert gas or reduce the pressure, and heat at about 115°C for 24 hours. After drying under vacuum, dissolve in 0.5 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution (2). Separately, weigh exactly 60 mg of L-aspartic acid, 100 mg of L-glutamic acid, 17 mg of L-alanine, 23 mg of L-methionine, 21 mg of L-tyrosine, 24 mg of L-histidine hydrochloride monohydrate, 58 mg of L-threonine, 22 mg of L-proline, 14 mg of L-cystine, 45 mg of L-isoleucine, 37 mg of L-phenylalanine, 32 mg of L-arginine hydrochloride, 32 mg of L-serine, 6 mg of glycine, 18 mg of L-valine, 109 mg of L-leucine, 76 mg of L-lysine hydrochloride, and 8 mg of L-tryptophan, dissolve with 0.1 mol/L hydrochloric acid TS to make exactly 500 mL, and use this solution as the standard solution. Transfer 40 μL each of the standard solution to two hydrolysis tubes, evaporate to dryness under vacuum, and proceed in the same way for each respective sample solution to make the standard solutions (1) and (2).

(ii) Amino acid analysis Perform the test with exactly 250 μL each of the sample solutions (1) and (2) and standard solutions (1) and (2) as directed under Liquid Chromatography <*2.01*> according to the following conditions, and from the peak areas for each amino acid obtained from the sample solutions (1) and (2) and standard solutions (1) and (2) calculate the molar number of the amino acids contained in 1 mL of the sample solutions (1) and (2). Furthermore, calculate the number of amino acids assuming there are 22 leucine residues in one mole of celmoleukin.

Operating conditions—

Detector: A visible absorption photometer [wavelength: 440 nm (proline) and 570 nm (amino acids other than proline)].

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with strongly acidic ion-exchange resin (sulfonic acid group bound divinylbenzene-polystyrene) for liquid chromatography (Na type) (5 μm in particle diameter).

Column temperature: Maintaining a constant temperature of about 48°C for 28 minutes after sample injection, then a constant temperature of about 62°C until 121 minutes after the injection.

Reaction temperature: A constant temperature of about 135°C.

Color developing time: About 1 minute.

Mobile phases A, B, C and D: Prepare according to the following table.

Mobile phase	A	B	C	D
Citric acid monohydrate	17.70 g	10.50 g	6.10 g	—
Trisodium citrate dihydrate	7.74 g	15.70 g	26.67 g	—
Sodium chloride	7.07 g	2.92 g	54.35 g	—
Sodium hydroxide	—	—	2.30 g	8.00 g
Ethanol (99.5)	40 mL	—	—	—
Benzyl alcohol	—	10 mL	5 mL	—
Thiodiglycol	5 mL	5 mL	5 mL	—
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL	4 mL
Caprylic acid	0.1 mL	0.1 mL	0.1 mL	0.1 mL
Water	a sufficient quantity	a sufficient quantity	a sufficient quantity	a sufficient quantity
Total	1000 mL	1000 mL	1000 mL	1000 mL

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A, B, C and D as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)	Mobile phase D (vol%)
0 – 35	100	0	0	0
35 – 60	0	100	0	0
60 – 111	0	0	100	0
111 – 121	0	0	0	100

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for 10 minutes while passing a current of nitrogen, and assign as Solution A. Separately, to 1957 mL of 1-methoxy-2 propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for 30 minutes while passing a current of nitrogen, and assign as Solution B. Mix Solutions A and B before use.

Flow rate of mobile phase: Adjust the flow rate so that the retention times of serine and leucine are about 30 minutes and 73 minutes, respectively (about 0.21 mL per minute).

Flow rate of reaction reagent: About 0.25 mL per minute.

System suitability—

System performance: To 2 mL of the standard solution add 0.02 mol/L hydrochloric acid TS to make 25 mL. When the procedure is run with 250 μL of this solution under the above operating conditions, the resolution between the peaks of threonine and serine is not less than 1.2.

System repeatability: To 2 mL of the standard solution add 0.02 mol/L hydrochloric acid TS to make 25 mL. When the test is repeated 3 times with 250 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of aspartic acid, serine, arginine and

proline is not more than 2.4%.

Chlordiazepoxide Powder

クロルジアゼポキシド散

Add the following next to the Purity:

Dissolution ⟨*6.10*⟩ When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 60 minutes of Chlordiazepoxide Powder is not less than 70%.

Start the test with an accurately weighed amount of Chlordiazepoxide Powder, equivalent to about 3.3 mg of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), withdraw not less than 15 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 12 mg of Chlordiazepoxide RS, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, dissolve in 20 mL of 0.1 mol/L hydrochloric acid TS, and add the dissolution medium to make exactly 200 mL. Pipet 3 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 260 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of chlordiazepoxide ($C_{16}H_{14}ClN_3O$)

$= M_S/M_T \times A_T/A_S \times 1/C \times 27$

M_S: Amount (mg) of Chlordiazepoxide RS
M_T: Amount (g) of Chlordiazepoxide Powder
C: Labeled amount (mg) of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) in 1 g

Add the following:

Clonazepam Fine Granules

クロナゼパム細粒

Clonazepam Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$: 315.71).

Method of preparation Prepare as directed under Granules, with Clonazepam.

Identification Powder Clonazepam Fine Granules. To a portion of the powder, equivalent to 1 mg of Clonazepam, add an appropriate volume of methanol and shake for 10 minutes, add methanol to make 100 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 307 nm and 311 nm.

Dissolution Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Assay Powder Clonazepam Fine Granules. Weigh accurately a portion of the powder, equivalent to about 2.4 mg of clonazepam ($C_{15}H_{10}ClN_3O_3$), add exactly 30 mL of a mixture of methanol and water (7:3), and shake for 15 minutes. Centrifuge this solution, pipet 5 mL of the supernatant liquid, add a mixture of methanol and water (7:3) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of clonazepam for assay, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of methanol and water (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas of clonazepam, A_T and A_S, in each solution.

Amount (mg) of clonazepam ($C_{15}H_{10}ClN_3O_3$)
$= M_S \times A_T/A_S \times 3/25$

M_S: Amount (mg) of clonazepam for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 310 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and methanol (4:3:3).

Flow rate: Adjust the flow rate so that the retention time of clonazepam is about 5 minutes.

System suitability—

System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clonazepam are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clonazepam is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Clonazepam Tablets

クロナゼパム錠

Clonazepam Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$: 315.71).

Method of preparation Prepare as directed under Tablets, with Clonazepam.

Identification Powder Clonazepam Tablets. To a portion of the powder, equivalent to 1 mg of Clonazepam, add an appropriate volume of methanol and shake for 10 minutes, then add methanol to make 100 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 307 nm and 311 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Clonazepam Tablets, add $V/10$ mL of methanol, shake for 15 minutes, add 2-propanol to make exactly V mL so that each mL contains about 10 μg of clonazepam ($C_{15}H_{10}ClN_3O_3$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of clonazepam for assay, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 200 mL. Pipet 10 mL of this solution, add 2-propanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 312 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a mixture of 2-propanol and methanol (9:1) as the control.

Amount (mg) of clonazepam ($C_{15}H_{10}ClN_3O_3$)
$= M_S \times A_T/A_S \times V/2000$

M_S: Amount (mg) of clonazepam for assay

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of 0.5-mg tablet and 1-mg tablet is not less than 80%, and that of 2-mg tablet is not less than 75%.

Start the test with 1 tablet of Clonazepam Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 0.56 μg of clonazepam ($C_{15}H_{10}ClN_3O_3$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of clonazepam for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution and add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of clonazepam in each solution.

Dissolution rate (%) with respect to the labeled amount of clonazepam ($C_{15}H_{10}ClN_3O_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/4$

M_S: Amount (mg) of clonazepam for assay
C: Labeled amount (mg) of clonazepam ($C_{15}H_{10}ClN_3O_3$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clonazepam are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clonazepam is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Clonazepam Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 2.5 mg of clonazepam ($C_{15}H_{10}ClN_3O_3$), add exactly 50 mL of a mixture of methanol and water (7:3), and shake for 15 minutes. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of clonazepam for assay, previously dried at 105°C for 4 hours, dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add a mixture of methanol and water (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas of clonazepam, A_T and A_S, in each solution.

Amount (mg) of clonazepam ($C_{15}H_{10}ClN_3O_3$)
$= M_S \times A_T/A_S \times 1/10$

M_S: Amount (mg) of clonazepam for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 310 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and methanol (4:3:3).

Flow rate: Adjust the flow rate so that the retention time of clonazepam is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clonazepam are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clonazepam is not more than 1.0%.

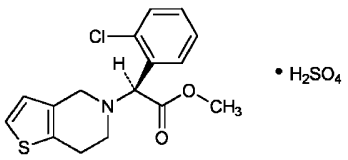
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Clopidogrel Sulfate

クロピドグレル硫酸塩



$C_{16}H_{16}ClNO_2S.H_2SO_4$: 419.90

Methyl (2*S*)-2-(2-chlorophenyl)-2-[6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-yl]acetate monosulfate

[*120202-66-6*]

Clopidogrel Sulfate contains not less than 97.0% and not more than 101.5% of $C_{16}H_{16}ClNO_2S.H_2SO_4$, calculated on the anhydrous basis.

Description Clopidogrel Sulfate occurs as a white to pale yellowish white, crystalline powder or powder.

It is freely soluble in water and in methanol, and soluble in ethanol (99.5).

It gradually develops a brown color on exposure to light.

Melting point: about 177°C (with decomposition).

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Clopidogrel Sulfate in methanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Clopidogrel Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clopidogrel Sulfate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Clopidogrel Sulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Clopidogrel Sulfate, or each of Clopidogrel Sulfate and Clopidogrel Sulfate RS in ethanol (99.5), respectively. Then evaporate the ethanol to dryness, and repeat the test on the residues dried in vacuum.

(3) Perform the test with Clopidogrel Sulfate as directed under Flame Coloration Test <*1.04*> (2): a green color appears.

(4) A solution of Clopidogrel Sulfate in a mixture of water and methanol (1:1) (1 in 100) responds to the Qualitative Tests <*1.09*> (1) for sulfate.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Clopidogrel Sulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 65 mg of Clopidogrel Sulfate in 10 mL of a mixture of acetonitrile for liquid chromatography and mobile phase A (3:2), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of acetonitrile for liquid chromatography and the mobile phase A (3:2) to make exactly 100 mL. Pipet 2.5 mL of this solution, add a mixture of acetonitrile for liquid chromatography and the mobile phase A (3:2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.5 and about 1.1 to clopidogrel, obtained from the sample solution is not larger than 2 times the peak area of clopidogrel obtained from the standard solution, the area of the peak other than clopidogrel and the peaks mentioned above from the sample solution is not larger than the peak area of clopidogrel from the standard solution, and the total area of the peaks other than clopidogrel from the sample solution is not larger than 5 times the peak area of clopidogrel from the standard solution.

Operating conditions—

Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: Dissolve 0.87 g of sodium 1-pentanesufonate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 950 mL of this solution add 50 mL of methanol.

Mobile phase B: A mixture of acetonitrile for liquid chromatography and methanol (19:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 3	89.5	10.5
3 – 48	89.5 → 31.5	10.5 → 68.5
48 – 68	31.5	68.5

Flow rate: 1.0 mL per minute.

Time span of measurement: For 68 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add a mixture of acetonitrile for liquid chromatography and the mobile phase A (3:2) to make exactly 20 mL. Confirm that the peak area of clopidogrel obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clopidogrel are not less than 60,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clopidogrel is not more than 2.0%.

(3) Optical isomer—Dissolve 0.10 g of Clopidogrel Sulfate in 25 mL of ethanol (99.5) for liquid chromatography, add heptane for liquid chromatography to make 50 mL, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add a mixture of ethanol (99.5) for liquid chromatography and heptane for liquid chromatography (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of ethanol (99.5) for liquid chromatography and heptane for liquid chromatography (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of the optical isomer, having the relative retention time of about 0.6 to clopidogrel, obtained from the sample solution is not greater than the peak area of clopidogrel obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with cellulose derivative-bonded silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of heptane for liquid chromatography and ethanol (99.5) for liquid chromatography (17:3).

Flow rate: Adjust the flow rate so that the retention time of clopidogrel is about 18 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clopidogrel are not less than 3500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clopidogrel are not more than 2.0%.

(4) Residual solvents—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Water <*2.48*> Not more than 0.5% (1 g, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 45 mg each of Clopidogrel Sulfate and Clopidogrel Sulfate RS (separately, determine the water <*2.48*> in the same manner as Clopidogrel Sulfate), and dissolve them separately in the mobile phase to make exactly 50 mL. Take exactly 7 mL of each solution, add to each of them the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of clopidogrel in each solution.

$$\text{Amount (mg) of } C_{16}H_{16}ClNO_2S.H_2SO_4 = M_S \times A_T/A_S$$

M_S: Amount (mg) of Clopidogrel Sulfate RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 0.87 g of sodium 1-pentanesufonate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 950 mL of this solution add 50 mL of methanol. To 600 mL of this solution, add 400 mL of a mixture of acetonitrile for liquid chromatography and methanol (19:1).

Flow rate: Adjust the flow rate so that the retention time of clopidogrel is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clopidogrel are not less than 4500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clopidogrel is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Add the following:

Clopidogrel Sulfate Tablets

クロピドグレル硫酸塩錠

Clopidogrel Sulfate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of clopidogrel ($C_{16}H_{16}ClNO_2S$: 321.82).

Method of preparation Prepare as directed under Tablets, with Clopidogrel Sulfate.

Identification To a quantity of powdered Clopidogrel Sulfate Tablets, equivalent to 75 mg of clopidogrel ($C_{16}H_{16}ClNO_2S$), add 50 mL of methanol, and after treating with ultrasonic waves with occasional shaking, add methanol to make 100 mL. To 10 mL of this solution add methanol to make 30 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 269 nm and 273 nm, and between 276 nm and 280 nm.

Purity Related substances—Keep the sample solution and the standard solution at 5°C or below and use within 24 hours. Take a quantity of Clopidogrel Sulfate Tablets equivalent to 0.15 g of clopidogrel ($C_{16}H_{16}ClNO_2S$), add 120 mL of the mobile phase, treat with ultrasonic waves with occasional shaking until the tablets are disintegrated, and add the mobile phase to make 200 mL. Centrifuge this solution, to 10 mL of the supernatant liquid add the mobile phase to make 30 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention times of about 0.3, about 0.5 and about 0.9 to clopidogrel, obtained from the sample solution is not larger than 3/10 times the peak area of clopidogrel obtained from the standard solution. The area of the peak having the relative retention time of about 2.0 from the sample solution is not larger than 1.2 times the peak area of clopidogrel from the standard solution. The area of the peak other than clopidogrel and the peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of clopidogrel from the standard solution. The total area of the peaks other than clopidogrel from the sample solution is not larger than 1.7 times the peak area of clopidogrel from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column of 4.6 mm in inside diameter and 15 cm in length, packed with ovomucoid-chemically bonded amino silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and to 750 mL of this solution add 250 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of clopidogrel is about 6 minutes.

Time span of measurement: About 2.5 times as long as the retention time of clopidogrel, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of clopidogrel obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clopidogrel are not less than 2500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clopidogrel is not more than 2.0%.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Clopidogrel Sulfate Tablets add a suitable amount of the mobile phase, treat with ultrasonic waves with occasional shaking until the tablet is disintegrated, and add the mobile phase to make exactly 50 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add exactly $V/5$ mL of the internal standard solution, and add the mobile phase to make V mL so that each mL contains about 0.1 mg of clopidogrel ($C_{16}H_{16}ClNO_2S$). Use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of clopidogrel } (C_{16}H_{16}ClNO_2S) = M_S \times Q_T/Q_S \times V/10 \times 0.766$$

M_S: Amount (mg) of Clopidogrel Sulfate RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 1500).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate of 25-mg tablet in 30 minutes is not less than 70%, and that of 75-mg tablet in 45 minutes is not less than 80%.

Start the test with 1 tablet of Clopidogrel Sulfate Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 28 μg of clopidogrel ($C_{16}H_{16}ClNO_2S$), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Clopidogrel Sulfate RS (separately determine the water ⟨*2.48*⟩ in the same manner as Clopidogrel Sulfate), dissolve in 5 mL of methanol, and add water to make exactly 100 mL. Pipet 6 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 240 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as a blank.

Dissolution rate (%) with respect to the labeled amount of clopidogrel ($C_{16}H_{16}ClNO_2S$)

$= M_S \times A_T/A_S \times V'/V \times 1/C \times 108 \times 0.766$

M_S: Amount (mg) of Clopidogrel Sulfate RS, calculated on the anhydrous basis

C: Labeled amount (mg) of clopidogrel ($C_{16}H_{16}ClNO_2S$) in 1 tablet

Assay To 20 tablets of Clopidogrel Sulfate Tablets add 400 mL of the mobile phase, treat with ultrasonic waves with occasional shaking until the tablets are disintegrated, add the mobile phase to make exactly 500 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add the mobile phase to make exactly V mL so that each mL contains about 0.5 mg of clopidogrel ($C_{16}H_{16}ClNO_2S$). Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 33 mg of Clopidogrel Sulfate RS (separately determine the water ⟨*2.48*⟩ in the same manner as Clopidogrel Sulfate), and dissolve in the mobile phase to make exactly 50 mL. Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clopidogrel to that of the internal standard.

Amount (mg) of clopidogrel ($C_{16}H_{16}ClNO_2S$) in 1 tablet of Clopidogrel Sulfate Tablets

$= M_S \times Q_T/Q_S \times V/10 \times 0.766$

M_S: Amount (mg) of Clopidogrel Sulfate RS, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl parahydoxybenzoate in the mobile phase (1 in 1500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column of 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 0.87 g of sodium 1-pentanesulfonate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 950 mL of this solution add 50 mL of methanol. To 600 mL of this solution add 400 mL of a mixture of acetonitrile for liquid chromatography and methanol (19:1).

Flow rate: Adjust the flow rate so that the retention time of clopidogrel is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and clopidogrel are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of clopidogrel to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Colestimide Granules

コレスチミド顆粒

Colestimide Granules contain not less than 87.0% and not more than 113.0% of the labeled amount of colestimide.

Method of preparation Prepare as directed under Granules, with Colestimide.

Identification Determine the infrared absorption spectrum of powdered Colestimide Granules as directed in the potassium chloride disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 1587 cm^{-1}, 1528 cm^{-1} and 1262 cm^{-1}.

Uniformity of dosage units ⟨*6.02*⟩ Colestimide Granules in single-unit containers meet the requirement of the Mass variation test.

Disintegration ⟨*6.09*⟩ Carry out the test for 10 minutes with 0.09 – 0.11 g of Colestimide Granules in six glass tubes

of the apparatus: it meets the requirement.

Assay Weigh accurately about 4.5 g of sodium cholate hydrate (separately determine the water), dissolve in water to make exactly 1000 mL, and use this solution as the sodium cholate standard stock solution. Take out the contents of not less than 20 single-unit containers of Colestimide Granules, weigh accurately an amount of the contents, equivalent to about 0.2 g of colestimide, add exactly 200 mL of the sodium cholate standard stock solution, shake for 1 hour, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Then, proceed as directed in the Assay (2) under Colestimide.

$$\text{Amount (mg) of colestimide} = M_S \times (Q_S - Q_T)/Q_S \times 1/5 \times 1/2.2 \times 0.947$$

M_S: Amount (mg) of sodium cholate hydrate, calculated on the anhydrous basis

2.2: Quantity (g) of the cholic acid exchange per mg of colestimide

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 80,000).

Containers and storage Containers—Tight containers.

Corn Starch

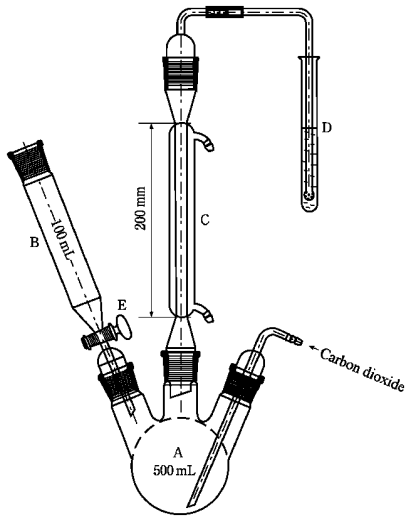
トウモロコシデンプン

Change the Purity (3) as follows:

Purity

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the following figure.



A: Three-necked round-bottom flask (500 mL)
B: Cylindrical dropping funnel (100 mL)
C: Condenser
D: Test tube
E: Tap

(ii) Procedure Introduce 150 mL of water into the three-necked round-bottom flask, close the tap of the cylindrical dropping funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Corn Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

$$\text{Amount (ppm) of sulfur dioxide} = V/M \times 1000 \times 3.203$$

M: Amount (g) of Corn Starch

V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

Cortisone Acetate

コルチゾン酢酸エステル

Change the Description as follows:

Description Cortisone Acetate occurs as white, crystals or crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

Melting point: about 240°C (with decomposition).

It shows crystal polymorphism.

Add the following:

Cyclophosphamide Tablets

シクロホスファミド錠

Cyclophosphamide Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$: 279.10).

Method of preparation Prepare as directed under Tablets, with Cyclophosphamide Hydrate.

Identification To Cyclophosphamide Tablets add 1 mL of water for every 53 mg of Cyclophosphamide Hydrate, shake vigorously for 5 minutes, add 6 mL of methanol for every 53 mg of Cyclophosphamide Hydrate, and shake vigorously for 10 minutes. To this solution add methanol so that each mL contains about 5.3 mg of Cyclophosphamide Hydrate, and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard not less than 3 mL of the first filtrate, and use the subsequent filtrate as the sample solution. Separately, dissolve 53 mg of cyclophosphamide hydrate for assay in 10 mL of a mixture of methanol and water (9:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol and water (8:1) to a distance of about 10 cm, and air-dry the plate. Heat the plate at 130°C for 15 minutes. After cooling, spray evenly ninhydrin-butanol TS on the plate, and after air-drying heat at 130°C for 10 minutes: the principal spot obtained from the sample solution and the spot obtained from the standard solution show a red-purple color and the same *R*f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cyclophosphamide Tablets add $3V/5$ mL of a mixture of water and methanol (3:2), and shake vigorously to homogenously disperse the tablet. To this solution add a mixture of water and methanol (3:2) to make exactly V mL so that each mL contains about 1.1 mg of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$), and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$)
$= M_S \times A_T/A_S \times V/50$

M_S: Amount (mg) of cyclophosphamide hydrate for assay

Dissolution <*6.10*> When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Cyclophosphamide Tablets is not less than 80%.

Start the test with 1 tablet of Cyclophosphamide Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 59 μg of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$) and use this solution as the sample solution. Separately, weigh accurately about 30 mg of cyclophosphamide hydrate for assay, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions and determine the peak areas, A_T and A_S, of cyclophosphamide in each solution.

Dissolution rate (%) with respect to the labeled amount of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180$

M_S: Amount (mg) of cyclophosphamide hydrate for assay
C: Labeled amount (mg) of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clophosphamide are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clophosphamide is not more than 2.0%.

Assay To 10 tablets of Cyclophosphamide Tablets add $13V/20$ mL of a mixture of water and methanol (3:2), and shake vigorously to homogenously disperse the tablets. To this solution add a mixture of water and methanol (3:2) to make exactly V mL so that each mL contains about 2.7 mg of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$), and centrifuge. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add a mixture of water and methanol (3:2) to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 53 mg of cyclophosphamide hydrate for assay, dissolve in a mixture of water and methanol (3:2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak area, A_T and A_S, of cyclophosphamide in each solution.

Amount (mg) of cyclophosphamide hydrate ($C_7H_{15}C_{l2}N_2O_2P.H_2O$)
$= M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of cyclophosphamide hydrate for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (3:2).

Flow rate: Adjust the flow rate so that the retention time of cyclophosphamide is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cyclophosphamide are not less than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cyclophosphamide is not more than 1.0%.

Containers and storage Containers—Tight containers.

Daunorubicin Hydrochloride

ダウノルビシン塩酸塩

Change the Purity (3) as follows:

Purity

(3) Related substances—Weigh accurately about 50 mg of Daunorubicin Hydrochloride, dissolve in diluted acetonitrile (43 in 100) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Daunorubicin Hydrochloride RS, and dissolve in diluted acetonitrile (43 in 100) to make exactly 50 mL. Pipet 1 mL of this solution, add diluted acetonitrile (43 in 100) to make exactly 200 mL, and use this solution as the standard solution (1). Separately, weigh accurately about 5 mg of Doxorubicin Hydrochloride RS, and dissolve in diluted acetonitrile (43 in 100) to make exactly 100 mL. Pipet 1 mL of this solution, add diluted acetonitrile (43 in 100) to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 5 μL each of the sample solution and the standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of related substances by the following equations: each amount of each peak, having a relative retention time of about 0.3, about 0.6, about 0.7, about 0.8, about 1.7 and about 2.0 to daunorubicin, is not more than 1.3%, not more than 1.0%, not more than 0.3%, not more than 0.5%, not more than 0.4%and not more than 0.5%, respectively, and the amount of doxorubicin is not more than 0.4%. Furthermore, the total amount of the peaks, other than daunorubicin and the peaks mentioned above, is not more than 0.4%. For this calculation use the area of the peak, having a relative retention time of about 0.3 to daunorubicin, after multiplying by its relative response factor 0.7.

Each amount (%) of related substances other than doxorubicin
$= M_{S1}/M_T \times A_T/A_{S1} \times 1/2$

M_{S1}: Amount (mg) of Daunorubicin Hydrochloride RS
M_T: Amount (mg) of Daunorubicin Hydrochloride
A_{S1}: Peak area of daunorubicin obtained from the standard solution (1)
A_T: Peak area of each related substance obtained from the sample solution

Amount (%) of doxorubicin $= M_{S2}/M_T \times A_T/A_{S2} \times 5$

M_{S2}: Amount (mg) of Doxorubicin Hydrochloride RS
M_T: Amount (mg) of Daunorubicin Hydrochloride
A_{S2}: Peak area of doxorubicin obtained from the standard solution (2)
A_T: Peak area of doxorubicin obtained from the sample solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.88 g of sodium lauryl sulfate and 2.25 g of phosphoric acid in water to make 1000 mL. To 570 mL of this solution add 430 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of daunorubicin is about 26 minutes.

Time span of measurement: About 2 times as long as the retention time of daunorubicin.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution (1) add diluted acetonitrile (43 in 100) to make exactly 10 mL. Confirm that the peak area of daunorubicin obtained with 5 μL of this solution is equivalent to 7 to 13% of that obtained with 5 μL of the standard solution (1).

System performance: Dissolve 5 mg each of Daunorubicin Hydrochloride and doxorubicin hydrochloride in 25 mL of diluted acetonitrile (43 in 100). To 1 mL of this solution add diluted acetonitrile (43 in 100) to make 10 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, doxorubicin and daunorubicin are eluted in this order with the resolution between these peaks being not less than 13.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of daunorubicin is not more than 3.0%.

Dexamethasone

デキサメタゾン

Change the Description as follows:

Description Dexamethasone occurs as white to pale yellow, crystals or crystalline powder.

It is sparingly soluble in methanol, in ethanol (95) and in acetone, slightly soluble in acetonitrile, and practically insoluble in water.

Melting point: about 245°C (with decomposition).

It shows crystal polymorphism.

Dobutamine Hydrochloride

ドブタミン塩酸塩

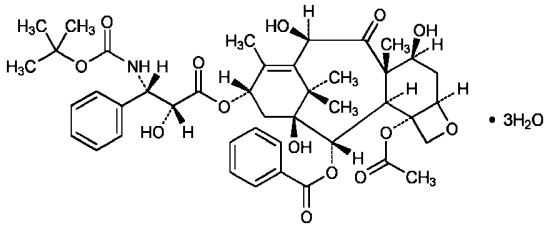
Change the Melting point as follows:

Melting point ⟨*2.60*⟩ 188 – 192°C

Add the following:

Docetaxel Hydrate

ドセタキセル水和物



$C_{43}H_{53}NO_{14}.3H_2O$: 861.93

(1*S*,2*S*,3*R*,4*S*,5*R*,7*S*,8*S*,10*R*,13*S*)-4-Acetoxy-2-benzoyloxy-5,20-epoxy-1,7,10-trihydroxy-9-oxotax-11-en-13-yl (2*R*,3*S*)-3-(1,1-dimethylethyl)oxycarbonylamino-2-hydroxy-3-phenylpropanoate trihydrate

[*148408-66-6*]

Docetaxel Hydrate contains not less than 97.5% and not more than 102.0% of docetaxel ($C_{43}H_{53}NO_{14}$: 807.88), calculated on the anhydrous basis and corrected on the amount of the residual solvent.

Description Docetaxel Hydrate occurs as a white crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide and in ethanol (99.5), soluble in methanol and in dichloromethane, and practically insoluble in water.

It decomposes on exposure to light.

Identification **(1)** Determine the absorption spectrum of a solution of Docetaxel Hydrate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Docetaxel Hydrate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 60 mg of Docetaxel Hydrate in 1 mL of dichloromethane. Perform the test with this solution as directed in the solution method under Infrared Spectrophotometry ⟨*2.25*⟩ using a fixed cell composed of potassium bromide optical plates with the cell length of 0.1 mm, and compare the spectrum with the Reference Spectrum or the spectrum of Docetaxel Hydrate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −39 – −41° (0.2 g calculated on the anhydrous basis and corrected on the amount of residual solvent, methanol, 20 mL, 100 mm).

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Docetaxel Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay, as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak, having the relative retention time of about 0.97, about 1.08, and about 1.13 to docetaxel, is not more than 0.50%, not more than 0.30%, and not more than 0.30%, respectively, the amount of each peak other than docetaxel and the peaks mentioned above is not more than 0.10%, and the total amount of the peaks other than docetaxel is not more than 1.0%. For this calculation use the area of the peak, having the relative retention time of about 0.97 to docetaxel, after multiplying by the relative response factor 1.6.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: For 39 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make 100 mL. To 1 mL of this solution add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make 10 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 10 mL. Confirm that the peak area of docetaxel obtained with 10 μL of this solution is equivalent to 35 to 65% of that obtained with 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above

operating conditions, the number of theoretical plates and the symmetry factor of the peak of docetaxel are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of docetaxel is not more than 2.0%.

(3) Residual solvent—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Water <2.48> 5.0 – 7.0% (50 mg, coulometric titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Docetaxel Hydrate and Docetaxel RS (separately determine the water <2.48> and the residual solvent in the same manner as Docetaxel Hydrate), dissolve them separately in 2.5 mL of ethanol (99.5), add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of docetaxel in each solution.

Amount (mg) of docetaxel ($C_{43}H_{53}NO_{14}$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Docetaxel RS, calculated on the anhydrous basis and corrected on the amount of the residual solvent

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase A: Water.

Mobile phase B: Acetonitrile for liquid chromatography.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 9	72	28
9 – 39	72 → 28	28 → 72

Flow rate: 1.2 mL per minute.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of docetaxel are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of docetaxel is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Docetaxel for Injection

注射用ドセタキセル

Docetaxel for Injection is a preparation for injection which is dissolved before use.

It contains not more than 93.0% and not less than 105.0% of the labeled amount of docetaxel ($C_{43}H_{53}NO_{14}$: 807.88).

Method of preparation Prepare as directed under Injections, with Docetaxel Hydrate.

Description Docetaxel for Injection occurs as a clear and yellow to orange-yellow, viscous liquid.

Identification To an amount of Docetaxel for Injection, equivalent to 20 mg of docetaxel ($C_{43}H_{53}NO_{14}$), add 50 mL of methanol, and use this solution as the sample solution. Separately, dissolve 4 mg of docetaxel hydrate in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Then develop the plate with a mixture of ethyl acetate, heptane and ethanol (99.5) (12:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the *R*f value of the spot obtained from the sample solution and the standard solution is the same.

pH Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Purity **(1)** Related substances—Perform the test with 20 μL of the sample solution obtained in the Assay, as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak, having the relative retention time of about 0.27, about 1.05, about 1.08, about 1.13, and about 1.18 to docetaxel, is not more than 0.30%, not more than 1.3%, not more than 1.5%, not more than 0.50%, and not more than 0.50%, respectively, the amount of each peak other than docetaxel, the peak having the relative retention time of about 0.97 and the peaks mentioned above is not more than 0.20%, and the total amount of the peaks other than docetaxel and the peak having the relative retention time of about 0.97 is not more

than 3.5%. For this calculation use the area of the peak, having the relative retention time of about 0.27 to docetaxel, after multiplying by the relative response factor 0.67.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Docetaxel Hydrate.

Time span of measurement: For 39 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 100 mL. Confirm that the peak area of docetaxel obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of docetaxel are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of docetaxel is not more than 2.0%.

(2) Residual solvent—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Bacterial endotoxins ⟨*4.01*⟩ Less than 2.5 EU/mg.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test. (T: 120.0%)

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately an amount of Docetaxel for Injection, equivalent to about 20 mg of docetaxel ($C_{43}H_{53}NO_{14}$), add 5 mL of ethanol (99.5), further add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Docetaxel RS (separately determine the water ⟨*2.48*⟩ and the residual solvent in the same manner as Docetaxel Hydrate), dissolve in 20 mL of ethanol (99.5), add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of docetaxel in each solution.

Amount (mg) of docetaxel ($C_{43}H_{53}NO_{14}$) in 1 mL of Docetaxel for Injection

$= M_S/M_T \times A_T/A_S \times d \times 1/2$

M_S: Amount (mg) of Docetaxel RS, calculated on the anhydrous basis and corrected on the amount of the residual solvent

M_T: Amount (mg) of Docetaxel for Injection

d: Density (g/mL) of Docetaxel for Injection

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Docetaxel Hydrate.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of docetaxel are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of docetaxel is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Add the following:

Docetaxel Injection

ドセタキセル注射液

Docetaxel Injection is a hydrophilic injection.

It contains not more than 93.0% and not less than 105.0% of the labeled amount of docetaxel ($C_{43}H_{53}NO_{14}$: 807.88).

Method of preparation Prepare as directed under Injections, with Docetaxel Hydrate.

Description Docetaxel Injection occurs as a clear and pale yellow to yellowish orange, liquid.

Identification To a volume of Docetaxel Injection, equivalent to 20 mg of docetaxel ($C_{43}H_{53}NO_{14}$), add 50 mL of methanol, and use this solution as the sample solution. Separately, dissolve 4 mg of docetaxel hydrate in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Then develop the plate with a mixture of ethyl acetate, heptane and ethanol (99.5) (12:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main

wavelength: 254 nm): the *R*f value of the spot from the sample solution and the standard solution is the same.

pH Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Purity Related substances—Perform the test with 20 μL of the sample solution obtained in the Assay, as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each peak, having the relative retention time of about 0.27, about 1.05, about 1.08, about 1.13, and about 1.18 to docetaxel, is not more than 0.30%, not more than 1.3%, not more than 1.5%, not more than 0.50%, and not more than 0.50%, respectively, the amount of each peak other than docetaxel, the peak having the relative retention time of about 0.97 and the peaks mentioned above is not more than 0.20%, and the total amount of the peaks other than docetaxel and the peak having the relative retention time of about 0.97 is not more than 3.5%. For this calculation, use the area of the peak, having the relative retention time of about 0.27 to docetaxel, after multiplying by the relative response factor 0.67.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Docetaxel Hydrate.

Time span of measurement: For 39 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 100 mL. Confirm that the peak area of docetaxel obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 20 μL of the solution for system suitability test.

System performance: When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of docetaxel are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of docetaxel is not more than 2.0%.

Bacterial endotoxins <*4.01*> Less than 2.5 EU/mg.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To exactly a volume of Docetaxel Injection, equivalent to about 20 mg of docetaxel ($C_{43}H_{53}NO_{14}$), add 5 mL of ethanol (99.5), further add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Docetaxel RS (separately determine the water <*2.48*> and the residual solvent in the same manner as Docetaxel Hydrate), dissolve in 20 mL of ethanol (99.5), add a mixture of water, acetonitrile for liquid chromatography and acetic acid (100) (1000:1000:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of docetaxel in each solution.

$$\text{Amount (mg) of docetaxel } (C_{43}H_{53}NO_{14}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Docetaxel RS, calculated on the anhydrous basis and corrected on the amount of the residual solvent

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Docetaxel Hydrate.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of docetaxel are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of docetaxel is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Donepezil Hydrochloride Fine Granules

ドネペジル塩酸塩細粒

Delete the following item:

Particle size

Droperidol

ドロペリドール

Change the Description as follows:

Description Droperidol occurs as a white to light yellow powder.

It is freely soluble in acetic acid (100), soluble in dichloromethane, slightly soluble in ethanol (99.5), and practically insoluble in water.

It is gradually colored by light.

It shows crystal polymorphism.

Droxidopa Fine Granules

ドロキシドパ細粒

Delete the following item:

Particle size

Epoetin Alfa (Genetical Recombination)

エポエチン　アルファ（遺伝子組換え）

Change the Containers and storage as follows:

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature not exceeding −70°C.

Ethanol

エタノール

Change the Purity (4) as follows:

Purity

(4) Other impurities (absorbance)—Determine the absorption spectrum of Ethanol between 235 nm and 340 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, in a 5-cm cell using water as a blank: the absorbances at 240 nm, between 250 nm and 260 nm and between 270 nm and 340 nm are not more than 0.40, 0.30, and 0.10, respectively, and the spectrum shows a steadily rising curve with no observable peaks or shoulders.

Anhydrous Ethanol

無水エタノール

Change the Purity (4) as follows:

Purity

(4) Other impurities (absorbance)—Determine the absorption spectrum of Anhydrous Ethanol between 235 nm and 340 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, in a 5-mL cell using water as a blank: the absorbances at 240 nm, between 250 nm and 260 nm and between 270 nm and 340 nm are not more than 0.40, 0.30, and 0.10, respectively, and the spectrum shows a steadily rising curve with no observable peaks or shoulders.

Etizolam Fine Granules

エチゾラム細粒

Delete the following item:

Particle size

Etizolam Tablets

エチゾラム錠

Change the Identification, Uniformity of dosage units, Dissolution and Assay as follows:

Identification **(1)** To a quantity of powdered Etizolam Tablets, equivalent to 5 mg of Etizolam, add 10 mL of methanol, shake, and filter. Evaporate the filtrate to dryness on a water bath, and dissolve the residue in 2 mL of sulfuric acid. The solution gives off a light yellow-green fluorescence when exposed to ultraviolet light (main wavelength: 365 nm).

(2) To a quantity of powdered Etizolam Tablets, equivalent to 1 mg of Etizolam, add 80 mL of 0.1 mol/L hydrochloric acid TS, shake, and then filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of this filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits absorption maxima between 249 nm and 253 nm, and between 292 nm and 296 nm when perform the measurement within 10 minutes.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Etizolam Tablets add 2.5 mL of water, and stir until the tablet is disintegrated. Add 20 mL of methanol, stir for 20 minutes, add methanol to make exactly 25 mL, and centrifuge. Pipet V mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add diluted methanol (9 in 10) to make 25 mL so that each mL contains

about 8 μg of etizolam ($C_{17}H_{15}ClN_4S$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of etizolam } (C_{17}H_{15}ClN_4S) = M_S \times Q_T/Q_S \times 1/V \times 1/20$$

M_S: Amount (mg) of etizolam for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (9 in 10) (1 in 10,000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Etizolam Tablets is not less than 70%.

Start the test with 1 tablet of Etizolam Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 0.28 μg of etizolam ($C_{17}H_{15}ClN_4S$). Pipet 2 mL of this solution, add exactly 2 mL of acetonitrile, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of etizolam for assay, previously dried at 105°C for 3 hours, dissolve in 50 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of acetonitrile, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of etizolam in each solution.

$$\text{Dissolution rate (\%) with respect to the labeled amount of etizolam } (C_{17}H_{15}ClN_4S) = M_S \times A_T/A_S \times V'/V \times 1/C \times 9/10$$

M_S: Amount (mg) of etizolam for assay
C: Labeled amount (mg) of etizolam ($C_{17}H_{15}ClN_4S$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 243 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of etizolam is about 7 minutes.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of etizolam are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etizolam is not more than 2.0%.

Assay To 20 Etizolam Tablets add 50 mL of water, and stir until they disintegrate. Add 400 mL of methanol, stir for 20 minutes, add methanol to make exactly 500 mL, and centrifuge. Pipet an amount of the supernatant liquid, equivalent to about 0.2 mg of etizolam ($C_{17}H_{15}ClN_4S$), add exactly 2 mL of the internal standard solution, add diluted methanol (9 in 10) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 100 mg of etizolam for assay, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (9 in 10) to make exactly 100 mL. Pipet 2 mL of this solution, and add diluted methanol (9 in 10) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution, add diluted methanol (9 in 10) to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of etizolam to that of the internal standard.

$$\text{Amount (mg) of etizolam } (C_{17}H_{15}ClN_4S) = M_S \times Q_T/Q_S \times 1/500$$

M_S: Amount (mg) of etizolam for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in diluted methanol (9 in 10) (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of etizolam is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and etizolam are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of etizolam to that of the internal standard is

not more than 1.0%.

Filgrastim (Genetical Recombination)

フィルグラスチム(遺伝子組換え)

Change the origin/limits of content as follows:

Filgrastim (Genetical Recombination) is an aqueous solution in which a desired product is a recombinant N-methionyl human granulocyte colony-stimulating factor consisting of 175 amino acid residues. It has a stimulating effect on neutrophil production.

It contains not less than 0.45 mg and not more than 0.55 mg of protein per mL, and not less than 1.0×10^8 units per mg of protein.

Add the following next to the Purity:

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.25 EU/mL.

Change the Assay (1) as follows:

Assay (1) Protein content—Perform the test with exactly 200 μL each of Filgrastim (Genetical Recombination) and Filgrastim RS as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S of filgrastim.

Amount (mg) of protein in 1 mL of Filgrastim (Genetical Recombination)
$= C \times A_T/A_S$

C: Protein concentration (mg/mL) of Filgrastim RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, 1-propanol and trifluoroacetic acid (699:300:1).

Mobile phase B: A mixture of 1-propanole, water and trifluoroacetic acid (800:199:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 2	90	10
2 – 13	90 → 70	10 → 30
13 – 15	70 → 0	30 → 100
15 – 18	0	100

Flow rate: Adjust the flow rate so that the retention time of filgrastim is about 15 minutes.

System suitability—

System performance: When the procedure is run with 200 μL of a solution prepared by dissolving 1 mg of uracil and 2 mg of diphenyl in 100 mL of a mixture of water, 1-propanol and trifluoroacetic acid (649:350:1) under the above operating conditions, uracil and diphenyl are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 200 μL of Filgrastim RS under the above operating conditions, the relative standard deviation of the peak area of filgrastim is not more than 2.5%.

Flomoxef Sodium

フロモキセフナトリウム

Change the Purity (4) as follows:

Purity

(4) 1-(2-Hydroxyethyl)-1*H*-tetrazol-5-thiol—Use the sample solution obtained in the Assay as the sample solutions. Weigh accurately about 20 mg of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard: the amount of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol is not more than 1.0% of the amount of Flomoxef Sodium calculated on the anhydrous basis.

Amount (mg) of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol ($C_3H_6N_4OS$)
$= M_S \times Q_T/Q_S \times 1/10$

M_S: Amount (mg) of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol

Internal standard solution—A solution of *m*-cresol (3 in 1000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 20 mL. Confirm that the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol obtained with 5 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 5 μL of the standard solution.

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 3 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard is not more than 1.0%.

Add the following:

Fluconazole Capsules

フルコナゾールカプセル

Fluconazole Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$: 306.27).

Method of preparation Prepare as directed under Capsules, with Fluconazole.

Identification To an amount of powdered contents of Fluconazole Capsules, equivalent to 25 mg of Fluconazole, add 0.01 mol/L hydrochloric acid-methanol TS to make 100 mL, shake for 30 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 259 nm and 263 nm and between 265 nm and 269 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To the total amount of the content of 1 capsule of Fluconazole Capsules add the mobile phase to make exactly 100 mL. Disperse the particles with the aid of ultrasonic waves, stir for 30 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 50 μg of fluconazole ($C_{13}H_{12}F_2N_6O$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of fluconazole ($C_{13}H_{12}F_2N_6O$)
$= M_S \times A_T/A_S \times V'/V \times 1/5$

M_S: Amount (mg) of fluconazole for assay

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rates in 90 minutes of 50-mg capsule and 100-mg capsule are not less than 80% and not less than 70%, respectively.

Start the test with 1 capsule of Fluconazole Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 28 μg of fluconazole ($C_{13}H_{12}F_2N_6O$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of fluconazole for assay, previously dried at 105°C for 4 hours, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of fluconazole in each solution.

Dissolution rate (%) with respect to the labeled amount of fluconazole ($C_{13}H_{12}F_2N_6O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of fluconazole for assay
C: Labeled amount (mg) of fluconazole ($C_{13}H_{12}F_2N_6O$) in 1 capsule

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fluconazole are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fluconazole is not more than 1.0%.

Assay Take out the contents from not less than 20 Fluconazole Capsules, weigh accurately, and powder, if necessary. Weigh accurately a quantity of the contents, equivalent to about 50 mg of fluconazole ($C_{13}H_{12}F_2N_6O$), and add the mobile phase to make exactly 100 mL. Disperse the particles with the aid of ultrasonic waves, stir for 30 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of fluconazole for assay, previously dried at 105°C for 4 hours, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to

make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of fluconazole in each solution.

$$\text{Amount (mg) of fluconazole } (C_{13}H_{12}F_2N_6O) = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of fluconazole for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 261 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 0.82 g of anhydrous sodium acetate in 1000 mL of water, and adjust to pH 5.0 with acetic acid (100). To 700 mL of this solution add 200 mL of methanol and 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of fluconazole is about 4 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fluconazole are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fluconazole is not more than 1.0%.

Containers and storage Containers—Tight containers.

Fluocinolone Acetonide

フルオシノロンアセトニド

Change the Description as follows:

Description Fluocinolone Acetonide occurs as white, crystals or crystalline powder.

It is freely soluble in acetic acid (100) and in acetone, soluble in ethanol (99.5), sparingly soluble in methanol, and practically insoluble in water.

Melting point: 266 – 274°C (with decomposition).

It shows crystal polymorphism.

Fluocinonide

フルオシノニド

Change the Description as follows:

Description Fluocinonide occurs as white, crystals or crystalline powder.

It is sparingly soluble in chloroform, slightly soluble in acetonitrile, in methanol, in ethanol (95) and in ethyl acetate, and practically insoluble in water.

It shows crystal polymorphism.

Fluoxymesterone

フルオキシメステロン

Change the Description as follows:

Description Fluoxymesterone occurs as white, crystals or crystalline powder.

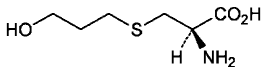
It is sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water.

It shows crystal polymorphism.

Add the following:

Fudosteine

フドステイン



$C_6H_{13}NO_3S$: 179.24

(2*R*)-2-Amino-3-(3-hydroxypropylsulfanyl)propanoic acid

[*13189-98-5*]

Fudosteine, when dried, contains not less than 99.0% and not more than 101.0% of $C_6H_{13}NO_3S$.

Description Fudosteine occurs as white, crystals or crystalline powder.

It is freely soluble in water, slightly soluble in acetic acid (100), and practically insoluble in ethanol (99.5).

It dissolves in 6 mol/L hydrochloric acid TS.

Melting point: about 200°C (with decomposition).

Identification **(1)** To 5 mL of a solution of fudosteine (1 in 1000) add 2 mL of sodium hydroxide TS, shake well, add 0.3 mL of sodium pentacyanonitrosylferrate (III) TS, and shake well again. After allowing to stand at 40°C for 10 minutes, cool the solution in an ice bath for 2 minutes, add 2 mL of dilute hydrochloric acid, and shake: a red-orange color develops.

(2) Determine the infrared absorption spectrum of Fudosteine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and com-

pare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −7.4 – −8.9° (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

Purity **(1)** Chloride ⟨*1.03*⟩—Dissolve 0.20 g of Fudosteine in 10 mL of water and 20 mL of nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of nitric acid and water to make 50 mL (not more than 0.044%).

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Fudosteine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead solution (not more than 10 ppm).

(3) Arsenic ⟨*1.11*⟩—Prepare the test solution with 2.0 g of Fudosteine according to Method 3, and perform the test (not more than 1 ppm).

(4) L-Cystine—Dissolve exactly 0.25 g of Fudosteine in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve exactly 25 mg of L-cystine in 2 mL of 1 mol/L hydrochloric acid TS, then add the mobile phase to make exactly 50 mL. Pipet 2.5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: the peak area of L-cystine obtained from the sample solution is not larger than the peak area of L-cystine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A solution of sodium 1-hexanesulfonate in diluted phosphoric acid (1 in 1000) (1 in 1250).

Flow rate: Adjust the flow rate so that the retention time of fudosteine is about 8 minutes.

System suitability—

System performance: Dissolve 25 mg of L-cystine in 2 mL of 1 mol/L hydrochloric acid TS, add 25 mg of Fudosteine, and add the mobile phase to make 50 mL. Take 2.5 mL of this solution, add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, L-cystine and fudosteine are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of L-cystine is not more than 2.0%.

(5) Related substances—Dissolve 0.25 g of Fudosteine in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, pipet 2.5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than fudosteine obtained from the sample solution is not larger than the peak area of fudosteine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 55°C.

Mobile phase: Diluted phosphoric acid (1 in 1000).

Flow rate: Adjust the flow rate so that the retention time of fudosteine is about 3 minutes.

Time span of measurement: About 10 times as long as the retention time of fudosteine, beginning after the peak of fudosteine.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fudosteine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fudosteine is not more than 2.0%.

(6) Residual solvent—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Loss on drying ⟨*2.41*⟩—Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition ⟨*2.44*⟩—Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Fudosteine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.92 mg of $C_6H_{13}NO_3S$

Containers and storage Containers—Well-closed containers.

Add the following:

Fudosteine Tablets

フドステイン錠

Fudosteine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of fudosteine ($C_6H_{13}NO_3S$: 179.24).

Method of preparation Prepare as directed under Tablets, with Fudosteine.

Identification Powder Fudostine Tablets. To a portion of the powder, equivalent to 88 mg of Fudosteine, add 10 mL of a mixture of water and methanol (1:1), shake, centrifuge, and use the supernatant liquid as the sample soluion. Separately, dissolve 90 mg of fudosteine for assay in 10 mL of a mixture of water and methanol (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2.5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the principal spot obtained from the sample solution and the spot obtained from the standard solution show a red-purple color and have the same *R*f value.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Dissolution <*6.10*> When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 20 minutes of Fudosteine Tablets is not less than 85%.

Start the test with 1 tablet of Fudosteine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard 5 mL of the first filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 55.6 μg of fudosteine ($C_6H_{13}NO_3S$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of fudosteine for assay, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of fudosteine in each solution.

Dissolution rate (%) with respect to the labeled amount of fudosteine ($C_6H_{13}NO_3S$)

$$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$$

M_S: Amount (mg) of fudosteine for assay

C: Labeled amount (mg) of fudosteine ($C_6H_{13}NO_3S$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of fudosteine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of fudosteine is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Fudosteine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.5 g of fudosteine ($C_6H_{13}NO_3S$), add 70 mL of the mobile phase, shake vigorously for 15 minutes, add the mobile phase to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of fudosteine for assay, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of fudosteine to that of the internal standard.

Amount (mg) of fudosteine ($C_6H_{13}NO_3S$)

$$= M_S \times Q_T/Q_S \times 10$$

M_S: Amount (mg) of fudosteine for assay

Internal standard solution—A solution of L-methionine in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A solution of sodium 1-hexanesulfonate in diluted phosphoric acid (1 in 1000) (1 in 1250).

Flow rate: Adjust the flow rate so that the retention time of fudosteine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20

μL of the standard solution under the above operating conditions, fudosteine and the internal standard are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of fudosteine to that of the internal standard is not more than 1.0%.

Containers and storage containers—Tight containers.

Fursultiamine Hydrochloride

フルスルチアミン塩酸塩

Change the Description as follows:

Description Fursultiamine Hydrochloride occurs as white, crystals or crystalline powder. It is odorless or has a characteristic odor, and has a bitter taste.

It is freely soluble in water, in methanol and in ethanol (95).

It shows crystal polymorphism.

L-Glutamic Acid

L-グルタミン酸

Change the Description as follows:

Description L-Glutamic acid occurs as white, crystals or crystalline powder. It has a slight characteristic and acid taste.

It is slightly soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in 2 mol/L hydrochloric acid TS.

It shows crystal polymorphism.

Glycerin

グリセリン

Change the Purity (11) as follows:

Purity

(11) Ethylene glycol, diethylene glycol and related substances—Weigh accurately about 5.88 g of Glycerin, mix with methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g each of ethylene glycol and diethylene glycol, mix with methanol to make exactly 100 mL. Pipet 5 mL of this solution and transfer into a 100-mL volumetric flask. Separately, weigh 5.0 g of glycerin for gas chromatography, mix with a suitable amount of methanol and put in the volumetric flask, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas, A_{T1} and A_{S1}, of ethylene glycol and, A_{T2} and A_{S2}, of diethylene glycol by the automatic integration method. The amounts of ethylene glycol and diethylene glycol, calculated by the following equations, are not more than 0.1%, respectively. The amount of the peak other than glycerin, ethylene glycol and diethylene glycol obtained from the sample solution, calculated by the area percentage method, is not more than 0.1%, and the total amount of the peaks other than glycerin is not more than 1.0%.

$$\text{Amount (\%) of ethylene glycol} = M_{S1}/M_T \times A_{T1}/A_{S1} \times 5$$

$$\text{Amount (\%) of diethylene glycol} = M_{S2}/M_T \times A_{T2}/A_{S2} \times 5$$

M_{S1}: Amount (g) of ethylene glycol
M_{S2}: Amount (g) of diethylene glycol
M_T: Amount (g) of Glycerin

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused-silica column 0.32 mm in inside diameter and 30 m in length, coated the inner surface with 14% cyanopropylphenyl-86% dimethyl silicone polymer for gas chromatography 1 μm in thickness.

Column temperature: Inject at a constant temperature of about 100°C, raise the temperature at the rate of 7.5°C per minute to 220°C, and maintain at a constant temperature of about 220°C.

Injection port temperature: A constant temperature of about 220°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium.

Flow rate: about 38 cm per second.

Split ratio: 1:20.

Time span of measurement: About 3 times as long as the retention time of glycerin, beginning after the solvent peak.

System suitability—

System performance: Mix 50 mg each of ethylene glycol, diethylene glycol and glycerin for gas chromatography with 100 mL of methanol. When the procedure is run with 1 μL of this solution under the above operating conditions, ethylene glycol, diethylene glycol and glycerin are eluted in this order, and the resolution between the peaks of ethylene glycol and diethylene glycol is not less than 40, and between the peaks of diethylene glycol and glycerin is not less than 10.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ethylene glycol and diethylene glycol is not more than 10%, respectively.

Concentrated Glycerin

濃グリセリン

Change the Purity (11) as follows:

Purity

(11) Ethylene glycol, diethylene glycol and related substances—Weigh accurately about 5 g of Concentrated Glycerin, mix with methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g each of ethylene glycol and diethylene glycol, mix with methanol to make exactly 100 mL. Pipet 5 mL of this solution and transfer into a 100-mL volumetric flask. Separately, weigh 5.0 g of glycerin for gas chromatography, mix with a suitable amount of methanol and put in the volumetric flask, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas, A_{T1} and A_{S1}, of ethylene glycol and, A_{T2} and A_{S2}, of diethylene glycol by the automatic integration method. The amounts of ethylene glycol and diethylene glycol, calculated by the following equations, are not more than 0.1%, respectively. The amount of the peak other than glycerin, ethylene glycol and diethylene glycol obtained from the sample solution, calculated by the area percentage method, is not more than 0.1%, and the total amount of the peaks other than glycerin is not more than 1.0%.

$$\text{Amount (\%) of ethylene glycol} = M_{S1}/M_T \times A_{T1}/A_{S1} \times 5$$

$$\text{Amount (\%) of diethylene glycol} = M_{S2}/M_T \times A_{T2}/A_{S2} \times 5$$

M_{S1}: Amount (g) of ethylene glycol
M_{S2}: Amount (g) of diethylene glycol
M_T: Amount (g) of Glycerin

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused-silica column 0.32 mm in inside diameter and 30 m in length, coated the inner surface with 14% cyanopropylphenyl-86% dimethyl silicone polymer for gas chromatography 1 μm in thickness.

Column temperature: Inject at a constant temperature of about 100°C, raise the temperature at the rate of 7.5°C per minute to 220°C, and maintain at a constant temperature of about 220°C.

Injection port temperature: A constant temperature of about 220°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium.

Flow rate: about 38 cm per second.

Split ratio: 1:20.

Time span of measurement: About 3 times as long as the retention time of glycerin, beginning after the solvent peak.

System suitability—

System performance: Mix 50 mg each of ethylene glycol, diethylene glycol and glycerin for gas chromatography with 100 mL of methanol. When the procedure is run with 1 μL of this solution under the above operating conditions, ethylene glycol, diethylene glycol and glycerin are eluted in this order, and the resolution between the peaks of ethylene glycol and diethylene glycol is not less than 40, and between the peaks of diethylene glycol and glycerin is not less than 10.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ethylene glycol and diethylene glycol is not more than 10%, respectively.

Glycine

グリシン

Change the Description as follows:

Description Glycine occurs as white, crystals or crystalline powder. It has a sweet taste.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (95).

It shows crystal polymorphism.

Haloperidol Fine Granules

ハロペリドール細粒

Delete the following item:

Particle size

Heparin Calcium

ヘパリンカルシウム

Change the origin/limits of content as follows:

Heparin Calcium is the calcium salt of sulfated glycosaminoglycans composed of disaccharide units of D-glucosamine and uronic acid (L-iduronic acid or D-glucuronic acid) obtained from the intestinal mucosa of healthy edible swine.

It prolongs the clotting time of blood.

It contains not less than 180 Heparin Units (anti-factor IIa activity) per mg, calculated on the dried basis, and not less than 8.0% and not more than 12.0% of calcium (Ca: 40.08).

Add the following next to the Bacterial endotoxins:

Anti-factor Xa activity to anti-factor IIa activity ratio The ratio of the anti-factor Xa activity determined by the following method to the anti-factor IIa activity obtained in the Assay, calculated by dividing the former with the later, is 0.9 – 1.1.

Anti-factor Xa activity determination

(i) Substrate solution: Dissolve 25 mg of *N*-benzoyl-L-isoleucyl-L-glutamyl(γ-OR)-glycyl-L-arginyl-*p*-nitroanilide hydrochloride in 33.3 mL of water.

(ii) Anti-thrombin solution: Proceed as directed in the Assay (1).

(iii) Factor Xa solution: To 1200 μL of factor Xa TS add 1200 μL of buffer solution.

(iv) Buffer solution: Proceed as directed in the Assay (1).

(v) Stopping solution: Proceed as directed in the Assay (1).

(vi) Heparin standard solutions: Proceed as directed in the Assay (1). However, the standard solutions are prepared based on anti-factor Xa activity Unit instead of Heparin Unit.

(vii) Heparin sample solutions: Proceed as directed in the Assay (1). However, the sample solutions are prepared based on anti-factor Xa activity Unit instead of Heparin Unit.

(viii) Procedure: Transfer separately two 50-μL portions of each different dilution of the heparin standard solutions and the heparin sample solutions and five 50-μL portions of buffer solution as the blank to 1.5-mL tubes. Warm these 21 tubes, anti-thrombin solution, factor Xa solution and substrate solution at 37°C all together. Start the following procedure at 2 minutes after warming in the order: buffer solution, S_1, S_2, S_3, S_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, S_1, S_2, S_3, S_4, and buffer solution. To each tube add 50 μL of anti-thrombin solution, mix, and warm at 37°C for exactly 4 minutes, add 100 μL of factor Xa solution, mix, and incubate for exactly 12 minutes. Then, add 100 μL of substrate solution, mix, incubate for exactly 4 minutes, add 50 μL of stopping solution to each tube, and mix immediately. Separately, to 50 μL of stopping solution add 100 μL of substrate solution, 100 μL of factor Xa solution, 50 μL of anti-thrombin solution and 50 μL of buffer solution, mix, and use this solution as a control. Determine the absorbance of each solution at 405 nm against the control. Confirm that the relative standard deviation of the reading of the blank is not more than 10%.

(ix) Calculations: When the regression expression, $y = I_c + A_{Xs} + B_{Xt}$, is obtained using y as log of the absorbance values, $_{Xs}$ as the concentration of the heparin standard solutions and $_{Xt}$ as the concentration of the heparin sample solutions, the potency ratio R is B/A.

I_c: Common intercept

A: Slope of regression expression of the heparin standard solution

B: Slope of regression expression of the heparin sample solution

Calculate anti-factor Xa activity per mg of Heparin Calcium by the following formula.

$$\text{Anti-factor Xa activity per mg of Heparin Calcium} = 100 \times R \times V/M$$

V: Total volume (mL) of the solution (the sample stock solution) prepared as containing about 100 anti-factor Xa activity Units per mL

M: Amount (mg) of Heparin Calcium to make the sample stock solution

However, when a 90% confidence interval of D of the regression expression $y = I'_c + A'_{Xs} + B'_{Xt} + D$, where D is a constant term showing the difference between the blank and the intercept assumed from the two lines, is not in the range of between −0.2 and 0.2, analyze by excluding the measurements of the blank.

The criteria for the test suitability are performed as directed in the Assay (1). When these criteria are not satisfied, repeat the test after changing the dilution rate so that the potency ratio becomes about 1 using the obtained potency as reference.

Change the Assay (1) as follows:

Assay (1) Heparin

(i) Substrate solution: Dissolve 25 mg of *H*-D-phenylalanyl-L-pipecolyl-L-arginyl-*p*-nitroanilide dihydrochloride in 32.0 mL of water.

(ii) Anti-thrombin solution: Dissolve human anti-thrombin in water so that each mL contains 1 IU. To 150 μL of this solution add 2250 μL of buffer solution.

(iii) Factor IIa solution: Dissolve factor IIa in buffer solution so that each mL contains 20 IU. To 150 μL of this solution add 150 μL of buffer solution and 300 μL of water.

(iv) Buffer solution: Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 10.2 g of sodium chloride, 2.8 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate and 1.0 g of polyethylene glycol 6000 in 800 mL of water, adjust to pH 8.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

(v) Stopping solution: To 2 mL of acetic acid (100) add water to make 10 mL.

(vi) Heparin standard solutions: Dissolve Heparin Sodium RS in water so that each mL contains 100 Heparin Units, and use this solution as the standard stock solution. Dilute the standard stock solution with buffer solution so that each mL contains exactly 0.1 Heparin Units, and use this solution as the standard solution. Make heparin standard solutions S_1, S_2, S_3 and S_4 respectively by adding the standard solution to buffer solution as directed in the following table.

Heparin standard solution		Buffer solution (μL)	Standard solution (μL)
No.	Heparin concentration (Unit/mL)		
S_1	0.005	950	50
S_2	0.010	900	100
S_3	0.015	850	150
S_4	0.020	800	200

(vii) Heparin sample solutions: Weigh accurately an appropriate amount of Heparin Calcium, dissolve in water so that each mL contains about 100 Heparin Units, and use this solution as the sample stock solution. Dilute exactly the sample stock solution with buffer solution so that each mL contains 0.1 Heparin Units, and use this solution as the sample solution. Make heparin sample solutions T_1, T_2, T_3 and T_4 respectively by adding the sample solution to buffer solution as directed in the following table.

Heparin sample solution		Buffer solution (μL)	Sample solution (μL)
No.	Heparin concentration (Unit/mL)		
T_1	0.005	950	50
T_2	0.010	900	100
T_3	0.015	850	150
T_4	0.020	800	200

(viii) Procedure: Transfer separately two 50-μL portions of each dilution of the heparin standard solutions and the heparin sample solutions and five 50-μL portions of buffer solution as the blank to 1.5-mL tubes. Warm these 21 tubes, anti-thrombin solution, factor IIa solution and substrate solution at 37°C all together. Start the following procedure at 2 minutes after warming in the order: buffer solution, S_1, S_2, S_3, S_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, S_1, S_2, S_3, S_4, and buffer solution. To each tube add 100 μL of anti-thrombin solution, mix, and warm at 37°C for exactly 4 minutes, add 25 μL of factor IIa solution, mix, and incubate for exactly 4 minutes. Then, add 50 μL of substrate solution, mix, incubate for exactly 4 minutes, add 50 μL of stopping solution to each tube, and mix. Separately, to 50 μL of stopping solution add 50 μL of substrate solution, 25 μL of factor IIa solution, 100 μL of anti-thrombin solution and 50 μL of buffer solution, mix, and use this solution as a control. Determine the absorbance of each solution at 405 nm against the control. Confirm that the relative standard deviation of the reading of the blank is not more than 10%.

(ix) Calculations: When the regression expression, $y = I_c + A_{Xs} + B_{Xt}$, is obtained using y as log of the absorbance values, $_{Xs}$ as the concentration of the heparin standard solutions and $_{Xt}$ as the concentration of the heparin sample solutions, the potency ratio R is B/A.

I_c: Common intercept
A: Slope of regression expression of the heparin standard solution
B: Slope of regression expression of the heparin sample solution

Calculate Heparin Unit (anti-factor IIa activity) per mg of Heparin Calcium by the following formula.

Heparin Unit (anti-factor IIa activity) per mg of Heparin Calcium
$= 100 \times R \times V/M$

V: Total volume (mL) of the solution (the sample stock solution) prepared as containing about 100 Heparin Units (anti-factor IIa activity) per mL
M: Amount (mg) of Heparin Calcium to make the sample stock solution

However, when a 90% confidence interval of D of the regression expression $y = I'_c + A'_{Xs} + B'_{Xt} + D$, where D is a constant term showing the difference between the blank and the intercept assumed from the two lines, is not in the range of between −0.2 and 0.2, analyze by excluding the measurements of the blank.

The criteria for the test suitability are the following 3 items, (1), (2) and (3).

(1) Judgment on consistence of the intercept assumed from the two lines

When the regression expression, $y = I_s + A''_{Xs} + B''_{Xt} + I_{t\text{-}s}$, is obtained from the data of the heparin standard solutions and the heparin sample solutions except of the blank solution, a 90% confidence interval of the constant term, $I_{t\text{-}s}$, is between −0.2 and 0.2.

I_s: Intercept of the regression expression of the heparin standard solution
$I_{t\text{-}s}$: Difference of the intercepts assumed from the two lines

(2) Judgment on linearity

When the regression expression, $y = I_c + A'''_{Xs} + B'''_{Xt} + Q_{sXs}{}^2 + Q_{tXt}{}^2$, is obtained from the data of the heparin standard solutions and the heparin sample solutions, a 90% confidence interval of the secondary coefficients, Q_s and Q_t, is between −1000 and 1000.

Q_s: Secondary coefficient of the regression expression of the heparin standard solution
Q_t: Secondary coefficient of the regression expression of the heparin sample solution

(3) Judgment by checking if the relative potency obtained is within the range previously validated on this test method

The potency ratio obtained is not less than 0.8 and not more than 1.2.

When these criteria are not satisfied, repeat the test after changing the dilution rate so that the potency ratio becomes about 1 using the obtained potency as reference.

Heparin Sodium

ヘパリンナトリウム

Change the origin/limits of content as follows:

Heparin Sodium is a sodium salt of sulfated glycosaminoglycans composed of disaccharide units of D-glucosamine and uronic acid (L-iduronic acid or D-glucuronic acid) obtained from the intestinal mucosa of healthy edible swine.

It prolongs the clotting time of blood.

It contains not less than 180 Heparin Units (anti-factor IIa activity) per mg, calculated on the dried basis.

Delete the following item:

Pyrogen

Add the following two items next to the Residue on ignition:

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.0030 EU/Heparin Unit.

Anti-factor Xa activity to anti-factor IIa activity ratio The ratio of the anti-factor Xa activity determined by the following method to the anti-factor IIa activity obtained in the Assay, calculated by dividing the former with the later, is 0.9 – 1.1.

Anti-factor Xa activity determination

(i) Substrate solution: Dissolve 25 mg of *N*-benzoyl-L-isoleucyl-L-glutamyl(*γ*-OR)-glycyl-L-arginyl-*p*-nitroanilide hydrochloride in 33.3 mL of water.

(ii) Anti-thrombin solution: Proceed as directed in the Assay.

(iii) Factor Xa solution: To 1200 μL of factor Xa TS add 1200 μL of buffer solution.

(iv) Buffer solution: Proceed as directed in the Assay.

(v) Stopping solution: Proceed as directed in the Assay.

(vi) Heparin standard solutions: Proceed as directed in the Assay. However, the standard solutions are prepared based on anti-factor Xa activity Unit instead of Heparin Unit.

(vii) Heparin sample solutions: Proceed as directed in the Assay. However, the sample solutions are prepared based on anti-factor Xa activity Unit instead of Heparin Unit.

(viii) Procedure: Transfer separately two 50-μL portions of each dilution of the heparin standard solutions and the heparin sample solutions and five 50-μL portions of buffer solution as the blank to 1.5 mL-tubes. Warm these 21 tubes, anti-thrombin solution, factor Xa solution and substrate solution at 37°C all together. Start the following procedure at 2 minutes after warming in the order: buffer solution, S_1, S_2, S_3, S_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, S_1, S_2, S_3, S_4, and buffer solution. To each tube add 50 μL of anti-thrombin solution, mix, and warm at 37°C for exactly 4 minutes, add 100 μL of factor Xa solution, mix, and incubate for exactly 12 minutes. Then, add 100 μL of substrate solution, mix, incubate for exactly 4 minutes, add 50 μL of stopping solution to each tube, and mix immediately. Separately, to 50 μL of stopping solution add 100 μL of substrate solution, 100 μL of factor Xa solution, 50 μL of anti-thrombin solution and 50 μL of buffer solution, mix, and use this solution as a control. Determine the absorbance of each solution at 405 nm against the control. Confirm that the relative standard deviation of the reading of the blank is not more than 10%.

(ix) Calculations: When the regression expression, $y = I_c + A_{X_S} + B_{X_t}$, is obtained using y as log of the absorbance values, $_{X_S}$ as the concentration of the heparin standard solutions and $_{X_t}$ as the concentration of the heparin sample solutions, the potency ratio R is B/A.

I_c: Common intercept
A: Slope of regression expression of the heparin standard solution
B: Slope of regression expression of the heparin sample solution

Calculate anti-factor Xa activity per mg of Heparin Sodium by the following formula.

$$\text{Anti-factor Xa activity per mg of Heparin Sodium} = 100 \times R \times V/M$$

V: Total volume (mL) of the solution (the sample stock solution) prepared as containing about 100 anti-factor Xa activity Units per mL
M: Amount (mg) of Heparin Sodium to make the sample stock solution

However, when a 90% confidence interval of D of the regression expression $y = I'_c + A'_{X_S} + B'_{X_t} + D$, where D is a constant term showing the difference between the blank and the intercept assumed from the two lines, is not in a range of between −0.2 and 0.2, analyze by excluding the measurements of the blank.

The criteria for the test suitability are performed as directed in the Assay. When these criteria are not satisfied, repeat the test after changing the dilution rate so that the potency ratio becomes about 1 using the obtained potency as reference.

Change the Assay as follows:

Assay

(i) Substrate solution: Dissolve 25 mg of *H*-D-phenylalanyl-L-pipecolyl-L-arginyl-*p*-nitroanilide dihydrochloride in 32.0 mL of water.

(ii) Anti-thrombin solution: Dissolve human anti-thrombin in water so that each mL contains 1 IU. To 150 μL of this solution add 2250 μL of buffer solution.

(iii) Factor IIa solution: Dissolve factor IIa in buffer solution so that each mL contains 20 IU. To 150 μL of this solution add 150 μL of buffer solution and 300 μL of water.

(iv) Buffer solution: Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 10.2 g of sodium chloride, 2.8 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate and 1.0 g of polyethylene glycol 6000 in 800 mL of water, adjust to pH 8.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

(v) Stopping solution: To 2 mL of acetic acid (100) add water to make 10 mL.

(vi) Heparin standard solutions: Dissolve Heparin Sodium RS in water so that each mL contains 100 Heparin Units, and use this solution as the standard stock solution. Dilute the standard stock solution with buffer solution so that each mL contains exactly 0.1 Heparin Units, and use this solution as the standard solution. Make heparin standard solutions S_1, S_2, S_3 and S_4 respectively by adding the standard solution to buffer solution as directed in the following table.

Heparin standard solution		Buffer solution (μL)	Standard solution (μL)
No.	Heparin concentration (Unit/mL)		
S_1	0.005	950	50
S_2	0.010	900	100
S_3	0.015	850	150
S_4	0.020	800	200

(vii) Heparin sample solutions: Weigh accurately an appropriate amount of Heparin Sodium, dissolve in water so that each mL contains about 100 Heparin Units, and use this solution as the sample stock solution. Dilute exactly the sample stock solution with buffer solution so that each mL contains 0.1 Heparin Units, and use this solution as the sample solution. Make heparin sample solutions T_1, T_2, T_3 and T_4 respectively by adding the sample solution to buffer solution as directed in the following table.

Heparin sample solution		Buffer solution (μL)	Sample solution (μL)
No.	Heparin concentration (Unit/mL)		
T_1	0.005	950	50
T_2	0.010	900	100
T_3	0.015	850	150
T_4	0.020	800	200

(viii) Procedure: Transfer separately two 50-μL portions of each dilution of the heparin standard solutions and the heparin sample solutions and five 50-μL portions of buffer solution as the blank to 1.5 mL-tubes. Warm these 21 tubes, anti-thrombin solution, factor IIa solution and substrate solution at 37°C all together. Start the following procedure at 2 minutes after warming in the order: buffer solution, S_1, S_2, S_3, S_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, T_1, T_2, T_3, T_4, buffer solution, S_1, S_2, S_3, S_4, and buffer solution. To each tube add 100 μL of anti-thrombin solution, mix, and warm at 37°C for exactly 4 minutes, add 25 μL of factor IIa solution, mix, and incubate for exactly 4 minutes. Then, add 50 μL of substrate solution, mix, incubate for exactly 4 minutes, add 50 μL of stopping solution to each tube, and mix. Separately, to 50 μL of stopping solution add 50 μL of substrate solution, 25 μL of factor IIa solution, 100 μL of anti-thrombin solution and 50 μL of buffer solution, mix, and use this solution as a control. Determine the absorbance of each solution at 405 nm against the control. Confirm that the relative standard deviation of the reading of the blank is not more than 10%.

(ix) Calculations: When the regression expression, $y = I_c + A_{X_S} + B_{X_t}$, is obtained using y as log of the absorbance values, $_{X_S}$ as the concentration of the heparin standard solutions and $_{X_t}$ as the concentration of the heparin sample solutions, the potency ratio R is B/A.

I_c: Common intercept
A: Slope of regression expression of the heparin standard solution
B: Slope of regression expression of the heparin sample solution

Calculate Heparin Unit (anti-factor IIa activity) per mg of Heparin Sodium by the following formula.

Heparin Unit (anti-factor IIa activity) per mg of Heparin Sodium
$= 100 \times R \times V/M$

V: Total volume (mL) of the solution (the sample stock solution) prepared as containing about 100 Heparin Units (anti-factor IIa activity) per mL
M: Amount (mg) of Heparin Sodium to make the sample stock solution

However, when a 90% confidence interval of D of the regression expression $y = I'_c + A'_{X_S} + B'_{X_t} + D$, where D is a constant term showing the difference between the blank and the intercept assumed from the two lines, is not in the range of between -0.2 and 0.2, analyze by excluding the measurements of the blank.

The criteria for the test suitability are the following 3 items, (1), (2) and (3).

(1) Judgment on consistence of the intercept assumed from the two lines

When the regression expression, $y = I_s + A''_{X_S} + B''_{X_t} + I_{t\text{-}s}$, is obtained from the data of the heparin standard solution and the heparin sample solution except of the blank solution, a 90% confidence interval of the constant term, $I_{t\text{-}s}$, is between -0.2 and 0.2.

I_s: Intercept of the regression expression of the heparin standard solution
$I_{t\text{-}s}$: Difference of the intercepts assumed from the two lines

(2) Judgment on linearity

When the regression expression, $y = I_c + A'''_{X_S} + B'''_{X_t} +$

$Q_{sXs}^2 + Q_{tXt}^2$, is obtained from the data of the heparin standard solution and the heparin sample solution, a 90% confidence interval of the secondary coefficients, Q_s and Q_t, is between -1000 and 1000.

Q_s: Secondary coefficient of the regression expression of the heparin standard solution
Q_t: Secondary coefficient of the regression expression of the heparin sample solution

(3) Judgment by checking if the relative potency obtained is within the range previously validated on this test method

The potency ratio obtained is not less than 0.8 and not more than 1.2.

When these criteria are not satisfied, repeat the test after changing the dilution rate so that the potency ratio becomes about 1 using the obtained potency as reference.

Heparin Sodium Injection

ヘパリンナトリウム注射液

Change the Assay as follows:

Assay Proceed as directed in the Assay under Heparin Sodium, replacing (vii) Heparin sample solutions and (ix) Calculations with the following.

(vii) Heparin sample solutions: Take exactly an appropriate amount of Heparin Sodium Injection, dilute exactly with buffer solution so that each mL contains 0.1 Heparin Units, and use this solution as the sample solution. Make heparin sample solutions T_1, T_2, T_3 and T_4 respectively by adding the sample solution to buffer solution as directed in the following table.

Heparin sample solution		Buffer solution (μL)	Sample solution (μL)
No.	Heparin concentration (Unit/mL)		
T_1	0.005	950	50
T_2	0.010	900	100
T_3	0.015	850	150
T_4	0.020	800	200

(ix) Calculations: When the regression expression, $y = I_c + A_{Xs} + B_{Xt}$, is obtained using y as log of the absorbance values, $_{Xs}$ as the concentration of the heparin standard solutions and $_{Xt}$ as the concentration of the heparin sample solutions, the potency ratio R is B/A.

I_c: Common intercept
A: Slope of regression expression of the heparin standard solution
B: Slope of regression expression of the heparin sample solution

Calculate Heparin Units (anti-factor IIa activity) in 1 mL of Heparin Sodium Injection by the following formula.

Heparin Units (anti-factor IIa activity) in 1 mL of Heparin Sodium Injection
$= 0.1 \times R \times V/a$

V: Total volume (mL) of the sample solution prepared as containing 0.1 Heparin Units (anti-factor IIa activity) per mL
a: Amount (mL) of Heparin Sodium Injection to make the sample solution

However, when a 90% confidence interval of D of the regression expression $y = I'_c + A'_{Xs} + B'_{Xt} + D$, where D is a constant term showing the difference between the intercepts assumed from the measurement of the blank and the two lines, is not in the range of between -0.2 and 0.2, analyze by excluding the measurements of the blank.

The criteria for the test suitability are followed as directed in the Assay under Heparin Sodium. When these criteria are not satisfied, repeat the test after changing the dilution rate so that the potency ratio becomes about 1 using the obtained potency as reference.

L-Histidine

L-ヒスチジン

Change the Description as follows:

Description L-Histidine occurs as white, crystals or crystalline powder, having a slight bitter taste.

It is freely soluble in formic acid, and soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in 6 mol/L hydrochloric acid TS.

It shows crystal polymorphism.

Hydrocortisone

ヒドロコルチゾン

Change the Description as follows:

Description Hydrocortisone occurs as a white crystalline powder.

It is sparingly soluble in methanol, in ethanol (95) and in 1,4-dioxane, and very slightly soluble in water.

Melting point: 212 – 220°C (with decomposition).

It shows crystal polymorphism.

Hydrocortisone Acetate

ヒドロコルチゾン酢酸エステル

Change the Description as follows:

Description Hydrocortisone Acetate occurs as white, crys-

tals or crystalline powder.

It is sparingly soluble in 1,4-dioxane, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Melting point: about 220°C (with decomposition).

It shows crystal polymorphism.

Hydrocortisone Sodium Phosphate

ヒドロコルチゾンリン酸エステルナトリウム

Change the Description as follows:

Description Hydrocortisone Sodium Phosphate occurs as a white to light yellow powder.

It is freely soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (95).

It is hygroscopic.

It shows crystal polymorphism.

Hydrocortisone Sodium Succinate

ヒドロコルチゾンコハク酸エステルナトリウム

Change the Description as follows:

Description Hydrocortisone Sodium Succinate occurs as white, powder or masses.

It is freely soluble in water, in methanol and in ethanol (95).

It is hygroscopic.

It is gradually colored by light.

It shows crystal polymorphism.

Hydrocortisone Succinate

ヒドロコルチゾンコハク酸エステル

Change the Description as follows:

Description Hydrocortisone Succinate occurs as a white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (99.5), and practically insoluble in water.

It shows crystal polymorphism.

Hypromellose

ヒプロメロース

Change the Viscosity and the pH as follows:

Viscosity ⟨*2.53*⟩

(i) Method I: Apply to Hypromellose having a labeled viscosity of less than 600 mPa·s. Put an exact amount of Hypromellose, equivalent to 4.000 g calculated on the dried basis, in a tared, wide-mouth bottle, add hot water to make 200.0 g, stopper the bottle, stir by mechanical means at 350-to 450-revolutions per minute for 10 to 20 minutes to get a homogeneous dispersion. If necessary, take off the sample attached on the walls of the bottle, put them in the dispersed solution, and dissolve by continuing the stirring in a water bath not exceeding 10°C for 20 to 40 minutes. Add cooled water, if necessary, to make 200.0 g, and use this solution as the sample solution. Centrifuge the solution if necessary to expel any entrapped air bubbles. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method I under Viscosity Determination: not less than 80% and not more than 120% of the labeled viscosity.

(ii) Method II: Apply to Hypromellose having a labeled viscosity of not less than 600 mPa·s. Put an exact amount of Hypromellose, equivalent to 10.00 g calculated on the dried basis, in a tared, wide-mouth bottle, add hot water to make 500.0 g, stopper the bottle, and prepare the sample solution in the same manner as directed in Method I. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method II under Viscosity Determination, using a single cylinder-type rotational viscometer, according to the following operating conditions: not less than 75% and not more than 140% of the labeled viscosity.

Operating conditions—

Apparatus: Brookfield type viscometer LV model.

Rotor No., rotation frequency, and conversion factor: According to the following table, depending on the labeled viscosity.

Labeled viscosity (mPa·s)				Rotor No.	Rotation frequency /min	Conversion factor
Not less than 600 and less than 1400				3	60	20
〃	1400	〃	3500	3	12	100
〃	3500	〃	9500	4	60	100
〃	9500	〃	99,500	4	6	1000
〃	99,500			4	3	2000

Procedure of apparatus: Read value after 2 minutes of rotation, and stop the rotation for at least 2 minutes. Repeat this procedure more two times, and average three observed values.

pH ⟨*2.54*⟩ The pH of the sample solution obtained in the Viscosity, measured after 5 minutes immersing the electrode in the sample solution, is between 5.0 and 8.0.

Add the following:

Ifenprodil Tartrate Fine Granules

イフェンプロジル酒石酸塩細粒

Ifenprodil Tartrate Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of ifenprodil tartrate [$(C_{21}H_{27}NO_2)_2.C_4H_6O_6$:

800.98].

Method of preparation Prepare as directed under Granules, with Ifenprodil Tartrate.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 274 nm and 278 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: Ifenprodil Tartrate Fine Granules in single-unit containers meet the requirement of the Content uniformity test.

To the total amount of the content of 1 container of Ifenprodil Tartrate Fine Granules, add 10 mL of water and a suitable amount of a mixture of ethanol (99.5) and water (3:1), shake thoroughly, and add a mixture of ethanol (99.5) and water (3:1) to make exactly V mL so that each mL contains about 0.1 mg of ifenprodil tartrate [$(C_{21}H_{27}NO_2)_2.C_4H_6O_6$]. Filter through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of ifenprodil tartrate [$(C_{21}H_{27}NO_2)_2.C_4H_6O_6$] $= M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of ifenprodil tartrate for assay, calculated on the anhydrous basis

Dissolution Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Assay Powder Ifenprodil Tartrate Fine Granules, and weigh accurately a portion of the powder, equivalent to about 10 mg of ifenprodil tartrate [$(C_{21}H_{27}NO_2)_2.C_4H_6O_6$], add 5 mL of water and a suitable amount of a mixture of ethanol (99.5) and water (3:1), shake thoroughly, and add a mixture of ethanol (99.5) and water (3:1) to make exactly 100 mL. Filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of ifenprodil tartrate for assay (separately determine the water <*2.48*> in the same manner as Ifenprodil Tartrate), add 10 mL of water and a mixture of ethanol (99.5) and water (3:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ifenprodil in each solution.

Amount (mg) of ifenprodil tartrate [$(C_{21}H_{27}NO_2)_2.C_4H_6O_6$] $= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of ifenprodil tartrate for assay, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 224 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 6.5 with potassium hydroxide TS, and add water to make 1000 mL. To 420 mL of this solution add 320 mL of methanol for liquid chromatography and 260 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of ifenprodil is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ifenprodil are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ifenprodil is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Ifenprodil Tartrate Tablets

イフェンプロジル酒石酸塩錠

Ifenprodil Tartrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of ifenprodil tartrate [$(C_{21}H_{27}NO_2)_2.C_4H_6O_6$: 800.98].

Method of preparation Prepare as directed under Tablets, with Ifenprodil Tartrate.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 274 nm and 278 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ifenprodil Tartrate Tablets, add $V/20$ mL of water, and shake until the tablet is completely disintegrated. Then, add $7V/10$ mL of a mixture of ethanol (99.5) and water (3:1), shake thoroughly, and add a mixture of ethanol (99.5) and water (3:1) to make exactly V mL so that each mL contains about 0.1 mg of ifenprodil tartrate [$(C_{21}H_{27}NO_2)_2.C_4H_6O_6$]. Filter through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 10

mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6]$
$= M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of ifenprodil tartrate for assay, calculated on the anhydrous basis

Dissolution Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Assay Weigh accurately the mass of not less than 20 Ifenprodil Tartrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2C_4H_6O_6]$, add 5 mL of water and a suitable amount of a mixture of ethanol (99.5) and water (3:1), shake thoroughly, and add a mixture of ethanol (99.5) and water (3:1) to make exactly 100 mL. Filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of ifenprodil tartrate for assay (separately determine the water <*2.48*> in the same manner as Ifenprodil Tartrate), add 10 mL of water and a mixture of ethanol (99.5) and water (3:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of ifenprodil tartrate in each solution.

Amount (mg) of ifenprodil tartrate $[(C_{21}H_{27}NO_2)_2.C_4H_6O_6]$
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of ifenprodil tartrate for assay, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 224 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 6.5 with potassium hydroxide TS, and add water to make 1000 mL. To 420 mL of this solution, add 320 mL of methanol for liquid chromatography and 260 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of ifenprodil is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ifenprodil are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ifenprodil is not more than 1.0%.

Containers and storage containers—Tight containers.

Indometacin

インドメタシン

Change the Description as follows:

Description Indometacin occurs as a white to light yellow, fine, crystalline powder.

It is sparingly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

It is colored by light.

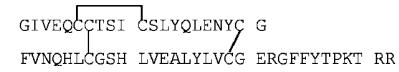
Melting point: 155 – 162°C

It shows crystal polymorphism.

Add the following:

Insulin Glargine (Genetical Recombination)

インスリン　グラルギン(遺伝子組換え)



$C_{267}H_{404}N_{72}O_{78}S_6$: 6062.89

[*160337-95-1*]

Principle of Insulin Glargine (Genetical Recombination) is an analogue of human insulin (genetical recombination), being substituted asparagine residue with glycine residue at 21st of A chain and added two arginine residues at C-terminal of B chain. It is a peptide composed with A chain consisting of 21 amino acid residues and B chain consisting of 32 amino acid residues. It has an activity to reduce the blood glucose level.

It contains not less than 94.0% and not more than 105.0% of insulin glargine ($C_{267}H_{404}N_{72}O_{78}S_6$), calculated on the anhydrous basis. 0.0364 mg of Insulin Glargine (Genetical Recombination) is equivalent to 1 Insulin Unit.

Description Insulin Glargine (Genetical Recombination) occurs as a white powder.

It is practically insoluble in water and in ethanol (99.5).

It is sparingly soluble in 0.01 mol/L hydrochloric acid TS.

It is hygroscopic.

It is gradually decomposed by light.

Identification Keep the sample solution and standard solu-

tion at 2 – 8°C. Weigh a suitable amount of Insulin Glargine (Genetical Recombination), and dissolve in 0.01 mol/L hydrochloric acid TS so that each mL contains 10.0 mg. Transfer 5 μL of this solution into a clean test tube, add 1 mL of 1 mol/L tris buffer solution, pH 7.5 and 100 μL of a solution of V8 protease for insulin glargine in 1 mol/L tris buffer solution, pH 7.5 (20 units/mL), allow to react at 35 – 37°C for 3 hours, then add 2 μL of phosphoric acid to stop the reaction, and use this solution as the sample solution. Separately, proceed with Insulin Glargine RS in the same manner as above, and use the solution so obtained as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compere the chromatograms obtained from these solutions: the similar peeks appear in the both chromatograms at each corresponding retention time.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 3 mm in inside diameter and 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: To 930 mL of a solution, prepared by dissolving 11.6 g of phosphoric acid and 42.1 g of sodium perchloric acid in 1600 mL of water, adjusting to pH 2.3 with triethylamine and adding water to make 2000 mL, add 70 mL of acetonitrile for liquid chromatography.

Mobile phase B: To 430 mL of a solution, prepared by dissolving 11.6 g of phosphoric acid and 42.1 g of sodium perchloric acid in 1600 mL of water, adjusting to pH 2.3 with triethylamine and adding water to make 2000 mL, add 570 mL of acetonitrile for liquid chromatography.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	90 → 20	10 → 80
30 – 35	20	80

Flow rate: 0.55 mL per minute.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the symmetry factors of the first two bigger peaks, they appear after a peak of just after the solvent peak, are not more than 1.5, respectively, and the resolution between these peaks is not less than 3.4.

Purity **(1)** Related substances—Perform the test with 5 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of these peaks by the area percentage method: the amount of the peak other than insulin glargine is not more than 0.4%, and the total amount of the peaks other than insulin glargine is not more than 1.0%.

Operating conditions—

Detector, column, column temperature, mobile phases A and B, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: For 40 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL. Confirm that the peak area of insulin glargine obtained with 5 μL of this solution is equivalent to 5 to 15% of that obtained with 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 μL of the standard solution obtained in the Assay under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of insulin glargine is not less than 20,000 and not more than 1.8, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution obtained in the Assay under the above operating conditions, the relative standard deviation of the peak area of insulin glargine is not more than 2.0%.

(2) High-molecular mass proteins—Keep the sample solution at 2 – 8°C. Dissolve 15 mg of Insulin Glargine (Genetical Recombination) in 1.5 mL of 0.01 mol/L hydrochloric acid TS, add water to make 10 mL, and use this solution as the sample solution. Perform the test with 100 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the total amount of the peaks other than insulin glargine is not more than 0.3%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 276 nm).

Column: Two stainless steel columns connected in series of 8 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 400 mL of water add 300 mL of acetonitrile for liquid chromatography and 200 mL of acetic acid (100), adjust to pH 3.0 with ammonia solution (28), and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of insulin glargine is about 35 minutes.

Time span of measurement: From the retention time corresponding to the exclusion volume of the size-exclusion

column to the completion of the elution of insulin glargine.
System suitability—

Test for required detectability: To 1 mL of the sample solution add 0.01 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL. Confirm that the peak area of insulin glargine obtained with 100 μL of this solution is equivalent to 5 to 15% of that obtained with 100 μL of the solution for system suitability test.

System performance: Heat 15 mg of Insulin Glargine (Genetical Recombination) at 100°C for 1.5 – 3 hours, then dissolve in 1.5 mL of 0.01 mol/L hydrochloric acid TS, and add water to make exactly 10 mL. When the procedure is run with 100 μL of this solution under the above operating conditions, the high-molecular mass protein and insulin glargine are eluted in this order with the resolution between these peaks is not less than 1.5.

System repeatability: When the test is repeated 6 times with 100 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of insulin glargine is not more than 2.0%.

(3) Other product-related impurities—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

(4) Host cell proteins—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

(5) DNA—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Zinc content Weigh accurately about 45 mg of Insulin Glargine (Genetical Recombination), dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 10 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, take exactly a suitable amount of Standard Zinc Solution for Atomic Absorption Spectrophotometry, dilute with 0.01 mol/L hydrochloric acid TS to make three solutions containing 0.20 μg, 0.40 μg and 0.60 μg of zinc (Zn: 65.38) in mL, respectively, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and calculate the amount of zinc in the sample solution using a calibration curve obtained from the absorbances of the standard solutions: not more than 0.80% of zinc (Zn: 65.38), calculated on the anhydrous basis.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Zinc hollow-cathode lamp.
Wavelength: 213.9 nm.

Water <*2.48*> Not more than 8.0% (90 mg, coulometric titration).

Bacterial endotoxins <*4.01*> Less than 10 EU/mg.

Assay Keep the sample solution and standard solution at 2 – 8°C. Weigh accurately about 15 mg of Insulin Glargine (Genetical Recombination), dissolve in 1.5 mL of 0.01 mol/L hydrochloric acid TS, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve Insulin Glargine RS in 0.01 mol/L hydrochloric acid TS so that each mL contains 10 mg of insulin glargine, then exactly dilute with water so that each mL contains about 1.5 mg of insulin glargine, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of insulin glargine in each solution.

Amount (mg) of insulin glargine ($C_{267}H_{404}N_{72}O_{78}S_6$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of insulin glargine in 1 mL of the standard solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 3 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: Dissolve 20.7 g of anhydrous sodium dihydrogen phosphate in 900 mL of water, adjust to pH 2.5 with phosphoric acid, and add water to make 1000 mL. To 250 mL of this solution add 250 mL of acetonitrile for liquid chromatography, dissolve 18.4 g of sodium chloride in this solution, and add water to make 1000 mL.

Mobile phase B: Dissolve 20.7 g of anhydrous sodium dihydrogen phosphate in 900 mL of water, adjust to pH 2.5 with phosphoric acid, and add water to make 1000 mL. To 250 mL of this solution add 650 mL of acetonitrile for liquid chromatography, dissolve 3.2 g of sodium chloride in this solution, and add water to make 1000 mL.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 20	96 → 83	4 → 17
20 – 30	83 → 63	17 → 37
30 – 40	63 → 96	37 → 4

Flow rate: 0.55 mL per minute (the retention time of insulin glargine is about 21 minutes).

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of insulin glargine are not less than 20,000

and not more than 1.8, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of insulin glargine is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding −15°C.

Add the following:

Insulin Glargine (Genetical Recombination) Injection

インスリン　グラルギン(遺伝子組換え)注射液

Insulin Glargine (Genetical Recombination) Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled Insulin Unit.

Method of preparation Prepare as directed under Injections, with Insulin Glargine (Genetical Recombination).

Description Insulin Glargine (Genetical Recombination) Injection occurs as a clear, colorless liquid.

Identification **(1)** Insulin Glargine (Genetical Recombination) Injection forms a precipitate when adjusted to pH 5.7 – 6.5 by addition of dilute sodium hydroxide TS, and the precipitate disappears when adjusted to pH 3.5 – 4.5 by addition of 0.1 mol/L hydrochloric acid TS.

(2) Determine the retention times of insulin glargine obtained from the sample solution and the standard solution in the test of the Assay: the relative retention time of insulin glargine obtained from the sample solution with respect to that obtained from the standard solution is 0.95 – 1.05.

pH Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Purity **(1)** Related substances—Keep the sample solution at 2 – 8°C. Perform the test with 5 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peak other than insulin glargine is not more than 0.5%, and the total amount of the peaks other than insulin glargine is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, mobile phases A and B, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Insulin Glargine (Genetical Recombination).

Time span of measurement: For 40 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL. Confirm that the peak area of insulin glargine obtained with 5 μL of this solution is equivalent to 5 to 15% of that with 5 μL of the solution for system suitability test.

System performance: When the procedure is run with 5 μL of the standard solution obtained in the Assay under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of insulin glargine are not less than 20,000 and not more than 1.8, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution obtained in the Assay under the above operating conditions, the relative standard deviation of the peak area of insulin glargine is not more than 2.0%.

(2) High-molecular mass proteins—To a suitable amount of Insulin Glargine (Genetical Recombination) Injection add water so that each mL contains 40 Insulin Units, and use this solution as the sample solution. Then, proceed as directed in the Purity (2) under Insulin Glargine (Genetical Recombination).

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Zinc content Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Assay To a suitable amount of Insulin Glargine (Genetical Recombination) Injection add exactly water so that each mL contains 40 Insulin Units, and use this solution as the sample solution. Then, proceed as directed in the Assay under Insulin Glargine (Genetical Recombination).

Amount (Insulin Unit) of insulin glargine ($C_{267}H_{404}N_{72}O_{78}S_6$) in 1 mL

$= M_S \times A_T/A_S \times d \times 1/0.0364$

M_S: Amount (mg) of insulin glargine in 1 mL of the standard solution

d: Dilution factor of the sample solution

0.0364: Mass (mg) of insulin glargine equivalent to 1 Insulin Unit

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, at a temperature of 2 – 8°C avoiding freezing.

Insulin Human (Genetical Recombination)

Change the Japanese title as follows:

インスリン　ヒト(遺伝子組換え)

Add the following:

Insulin Human (Genetical Recombination) Injection

インスリン　ヒト(遺伝子組換え)注射液

Insulin Human (Genetical Recombination) Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled Insulin Unit.

Method of preparation Prepare as directed under Injections, with Insulin Human (Genetical Recombination) suspended in Water for Injection then dissolved by addition of Hydrochloric Acid or Sodium Hydroxide.

Description Insulin Human (Genetical Recombination) Injection occurs as a clear, colorless liquid, and slightly a fine precipitate may be observable upon storage.

Identification Insulin Human (Genetical Recombination) Injection forms a precipitate when adjusted to pH 5.3 – 5.5 by addition of dilute hydrochloric acid, and the precipitate disappears when adjusted to pH 2.5 – 3.5 by further addition of the acid.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

pH Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Purity (1) Desamide substance—Perform the test with 20 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <*2.01*>, according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peak, having the relative retention time of about 1.3 to human insulin, is not more than 1.5%.

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Insulin Human (Genetical Recombination).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay under Insulin Human (Genetical Recombination).

Test for required detectability: Pipet 1 mL of the sample solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of human insulin obtained with 20 μL of this solution is equivalent to 1.4 to 2.6% of that obtained with 20 μL of the sample solution.

System repeatability: Dissolve Human Insulin RS in 0.01 mol/L hydrochloric acid TS so that each mL contains about 4 Insulin Units. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of human insulin is not more than 2.0%.

(2) High-molecular proteins—For each mL of Insulin Human (Genetical Recombination) Injection add 4 μL of 6 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test with 100 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the total amount of the peaks other than human insulin is not more than 2.0%.

Operating conditions—

Detector, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Insulin Human (Genetical Recombination).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography.

Time span of measurement: From the retention time corresponding to the exclusion volume of the size-exclusion column to the completion of the elution of human insulin.

System suitability—

System performance: Proceed as directed in the system suitability in the Purity (2) under Insulin Human (Genetical Recombination).

Test for required detectability: Pipet 1 mL of the sample solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of human insulin obtained with 100 μL of this solution is equivalent to 1.4 to 2.6% of that obtained with 100 μL of the sample solution.

Zinc content To an exact volume of Insulin Human (Genetical Recombination) Injection, equivalent to 300 Insulin Units, add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. If necessary, further dilute with 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, take exactly a suitable amount of Standard Zinc Solution for Atomic Absorption Spectrophotometry, dilute with 0.01 mol/L hydrochloric acid TS to make three solutions containing 0.20 μg, 0.60 μg and 1.20 μg of zinc (Zn: 65.38) in mL, respectively, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, using the 0.01 mol/L hydrochloric acid TS as the blank, and calculate the amount of zinc in the sample solution using a calibration curve obtained from the absorbances of the standard solutions: 10 – 40 μg of zinc (Zn: 65.38) per 100 Insulin Units.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Zinc hollow cathode lamp.
Wavelength: 213.9 nm.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.80 EU/Insulin Unit. Apply to the preparations intended for intravenous administration.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method1: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To exactly 10 mL of Insulin Human (Genetical Recombination) Injection add exactly 40 μL of 6 mol/L hydrochloric acid TS. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay under Insulin Human (Genetical Recombination).

Amount (Insulin Unit) of human insulin ($C_{257}H_{383}N_{65}O_{77}S_6$) in 1 mL
$= M_S \times F/D \times (A_{T1}+A_{TD})/(A_{S1}+A_{SD}) \times 1.004 \times 5/2$

M_S: Amount (mg) of Human Insulin RS
F: Labeled unit (Insulin Unit/mg) of Human Insulin RS
D: Volume (mL) of 0.01 mol/L hydrochloric acid TS used to dissolve Human Insulin RS

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant, at a temperature of 2 – 8°C avoiding freezing.

Iodamide

ヨーダミド

Change the Description as follows:

Description Iodamide occurs as a white crystalline powder.
It is slightly soluble in water and in ethanol (95).
It dissolves in sodium hydroxide TS.
It is gradually colored by light.
It shows crystal polymorphism.

Iohexol Injection

イオヘキソール注射液

Change the Assay and the Containers and storage as follows:

Assay To an exactly measured volume of Iohexol Injection, equivalent to about 1.5 g of iohexol ($C_{19}H_{26}I_3N_3O_9$), add water to make exactly 25 mL. Pipet 10 mL of this solution, add 25 mL of a solution of sodium hydroxide (1 in 20) and 0.5 g of zinc powder, and boil under a reflux condenser for 30 minutes. After cooling, wash down the inside of the condenser with 20 mL of water, and filter. Then, proceed as directed in the Assay under Iohexol.

Each mL of 0.1 mol/L silver nitrate VS
= 27.37 mg of $C_{19}H_{26}I_3N_3O_9$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Add the following:

Iopamidol Injection

イオパミドール注射液

Iopamidol Injection is an aqueous injection.
It contains not less than 95.0% and not more than 105.0% of the labeled amount of iopamidol ($C_{17}H_{22}I_3N_3O_8$: 777.09).

Method of preparation Prepare as directed under Injections, with Iopamidol.

Description Iopamidol Injection occurs as a clear, colorless or faint yellow, liquid, having slight viscosity.
It is gradually colored to faint yellow by light.

Identification (1) To a volume of Iopamidol Injection, equivalent to 0.3 g of Iopamidol, add 0.2 mL of sulfuric acid, and mix. When heat the solution over a flame, the color of the solution changes from colorless to purplish brown, and a purple gas is evolved.

(2) To a volume of Iopamidol Injection, equivalent to 0.6 g of Iopamidol, add water to make 100 mL, and use this solution as the sample solution. Separately, dissolve 60 mg of iopamidol for assay in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 4 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, 2-butanone and ammonia solution (28) (2:2:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultra-violet light (main wavelength: 254 nm): the Rf value of the principal spot obtained from the sample solution is the same as that obtained from the standard solution.

pH Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Purity (1) Primary aromatic amines—To a volume of Iopamidol Injection, equivalent to 0.18 g of Iopamidol, add 6 mL of water and mix. Add 1 mL of sodium nitrite solution (1 in 50) and 12 mL of 2 mol/L hydrochloric acid TS, shake

the solution and allow to stand for 2 minutes. Add 1 mL of a solution of ammonium amidosulfate (1 in 10), shake well, and allow to stand for 1 minute. Add 1 mL of naphthylethylenediamine TS and water to make exactly 50 mL. Determine the absorbance of this solution at 495 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using a blank solution prepared in the same manner, as the reference solution: the absorbance is not more than 0.18.

(2) Iodine—Take a volume of Iopamidol Injection, equivalent to 2.0 g of Iopamidol, and add 2 mL of 1 mol/L sulfuric acid TS and 1 mL of toluene. Then shake well and allow to stand: the toluene layer is colorless.

(3) Free iodine ion—To exactly 10 mL of Iopamidol Injection add a suitable amount of water, and adjust the pH to about 4.5 with diluted 0.25 mol/L sulfuric acid TS (1 in 10). Titrate <*2.50*> with 0.001 mol/L silver nitrate VS (potentiometric titration): the amount of iodine ion contained in Iopamidol Injection is not more than 40 μg per mL.

Each mL of 0.001 mol/L silver nitrate VS = 0.1269 mg of I

Bacterial endotoxins <*4.01*> Less than 1.5 EU/mL.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To exactly 1 mL of Iopamidol Injection add water to make exactly 200 mL. Take exactly V mL of this solution, add water to make exactly V' mL so that each mL contains about 80 μg of iopamidol ($C_{17}H_{22}I_3N_3O_8$), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of iopamidol for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 10 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of iopamidol in each solution.

$$\text{Amount (mg) of iopamidol } (C_{17}H_{22}I_3N_3O_8) = M_S \times A_T/A_S \times V'/V \times 4/5$$

M_S: Amount (mg) of iopamidol for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: Water.

Mobile phase B: A mixture of water and methanol (3:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 6	92	8
6 – 18	92 → 65	8 → 35
18 – 30	65 → 8	35 → 92
30 – 34	8	92

Flow rate: 1.5 mL per minute.

System suitability—

System performance: Dissolve 1 mg each of iopamidol for assay and *N*,*N*′-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide in water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, *N*,*N*′-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide and iopamidol are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of iopamidol is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Storage—Light-resistant.

Irsogladine Maleate Fine Granules

イルソグラジンマレイン酸塩細粒

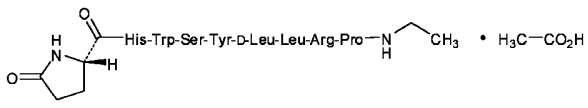
Delete the following item:

Particle size

Add the following:

Leuprorelin Acetate

リュープロレリン酢酸塩



$C_{59}H_{84}N_{16}O_{12}.C_2H_4O_2$: 1269.45

5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide monoacetate

[*74381-53-6*]

Leuprorelin Acetate contains not less than 96.0%

and not more than 102.0% of leuprorelin ($C_{59}H_{84}N_{16}O_{12}$: 1209.40), calculated on the anhydrous and de-acetic acid basis.

Description Leuprorelin Acetate occurs as a white to yellowish white, powder.

It is very soluble in water and in acetic acid (100), freely soluble in methanol, and sparingly soluble in ethanol (99.5).

It is hygroscopic.

Identification Determine the infrared absorption spectrum of Leuprorelin Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Leuprorelin Acetate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -38 – $-41°$ (0.25 g calculated on the anhydrous and de-acetic-acid basis, diluted acetic acid (100) (1 in 100), 25 mL, 100 mm).

pH <*2.54*> The pH of a solution of 0.10 g of Leuprorelin Acetate in 10 mL of water is 5.5 to 7.5.

Constituent amino acids When hydrolyzed by Method 1 described in ''1. Hydrolysis of Protein and Peptide'' and performed the test by Method 1 described in ''2. Methodologies of Amino Acid Analysis'' under Amino Acid Analysis of Proteins <*2.04*>, histidine, glutamic acid, proline, tyrosine and arginine is 1 and leucine is 2, respectively.

Procedure

(i) Hydrolysis Weigh accurately about 50 mg of Leuprorelin Acetate, and dissolve in 1 mL of water. Put 0.1 mL of this solution in a test tube for hydrolysis, freeze-dry the content, and add 2 mL of a solution of phenol in 6 mol/L hydrochloric acid (1 in 200). Freeze the solution, seal the tube in vacuum, and heat the tube at 110°C for 24 hours. After cooling, open the tube, take out 0.1 mL of the hydrolyzate, add 1 mL of water, and freeze-dry. Dissolve the residue in 7.8 mL of diluting solution, and use this solution as the sample solution. Separately, weigh exactly 0.45 mg of L-alanine, 0.66 mg of L-aspartic acid, 1.05 mg of L-arginine hydrochloride, 0.74 mg of L-glutamic acid, 0.38 mg of glycine, 1.05 mg of L-histidine hydrochloride monohydrate, 0.66 mg of L-isoleucine, 0.66 mg of L-leucine, 0.58 mg of L-proline, 0.53 mg of L-serine, 0.60 mg of L-threonine and 0.91 mg of L-tyrosine, dissolve in diluting solution to make exactly 100 mL, and use this solution as the standard solution (1). Separately, dissolve 1 mg of L-tryptophan and 0.4 mg of ethylamine hydrochloride in diluting solution to make 100 mL, and use this solution as the standard solution (2).

(ii) Amino acid analysis Perform the test with exactly 100 μL each of the sample solution and the standard solutions (1) and (2) as directed under Liquid Chromatography <*2.01*> according to the following conditions: the peaks of histidine, glutamic acid, leucine, proline, tyrosine, arginine, serine and tryptophan appear on the chromatogram obtained from the sample solution. Apart from this, calculate the molar content of each constituent amino acid in 1 mL of the sample solution from the peak area of each amino acid obtained from the sample solution and standard solution (1), and further calculate the number of the constituent amino acids assuming that the sum of each molar content of histidine, glutamic acid, leucine, proline, tyrosine and arginine in 1 mole of leuprorelin acetate is 7.

Diluting solution: Dissolve 6.29 g of lithium hydroxide monohydrate and 10.51 g of citric acid monohydrate in water to make 1000 mL, and adjust to pH 2.2 with hydrochloric acid.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 6 cm in length, packed with strongly acidic ion-exchange resin (Na type) for liquid chromatography (3 μm in particle diameter).

Column temperature: Retain a constant temperature of about 58°C for 18 minutes, then retain a constant temperature of about 70°C for a further 20 minutes.

Reaction vessel temperature: A constant temperature of about 135°C.

Mobile phase: Prepare the mobile phases A, B, C, D and E according to the following table, then add 0.1 mL of caprylic acid to each mobile phase.

	Mobile phase A	Mobile phase B	Mobile phase C	Mobile phase D	Mobile phase E
Citric acid monohydrate	19.80 g	22.00 g	12.80 g	6.10 g	—
Trisodium citrate dihydrate	6.19 g	7.74 g	13.31 g	26.67 g	—
Sodium chloride	5.66 g	7.07 g	3.74 g	54.35 g	—
Sodium hydroxide	—	—	—	—	8.00 g
Ethanol (99.5)	130 mL	20.0 mL	4.0 mL	—	100 mL
Thiodiglycol	5.0 mL	5.0 mL	5.0 mL	—	—
Benzyl alcohol	—	—	—	5.0 mL	—
Lauromacrogol solution (1 in 4)	4.0 mL	4.0 mL	4.0 mL	4.0 mL	4.0 mL
Water	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount
Total amount	1000 mL	1000 mL	1000 mL	1000 mL	1000 mL

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A, B, C, D and E as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)	Mobile phase D (vol%)	Mobile phase E (vol%)
0 – 1.6	100	0	0	0	0
1.6 – 4.5	0	100	0	0	0
4.5 – 13.5	0	0	100	0	0
13.5 – 27.0	0	0	0	100	0
27.0 – 33.0	0	0	0	0	100

Reaction reagent: Dissolve an appropriate amount of lithium acetate dihydrate, acetic acid (100) and 1-methoxy-2-propanol in water to make 1000 mL, and use this solution as solution A. Separately, dissolve an appropriate amount of ninhydrin and sodium borohydride in 1-methoxy-2-propanol to make 1000 mL, and use this solution as solution B. Mix equal parts of solutions A and B before use.

Flow rate of mobile phase: About 0.40 mL per minute.

Flow rate of reaction reagent: About 0.35 mL per minute.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution (1) under the above operating conditions, the resolutions between the peaks of threonine and serine, glycine and alanine, and isoleucine and leucine are not less than 1.2, respectively.

System repeatability: When the test is repeated 5 times with 100 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of arginine, aspartic acid, proline and serine is not more than 4.0%.

Purity Related substances—Dissolve 0.10 g of Leuprorelin Acetate in the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks, having the relative retention time of about 0.65, about 0.77, about 0.78 and about 0.90 to leuprorelin, obtained from the sample solution is not larger than 1/2 times the peak area of leuprorelin obtained from the standard solution, and the total area of the peaks other than leuprorelin from the sample solution is not larger than 2 times the peak area of leuprorelin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of leuprorelin, beginning after the solvent peak.

System suitability—

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of leuprorelin obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 20 μL of the standard solution.

Water <*2.48*> Not more than 5.0% (0.1 g, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.2% (0.5 g).

Acetic acid Weigh accurately about 0.1 g of Leuprorelin Acetate, dissolve in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of acetic acid (100), add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of acetic acid in each solution, and calculate the amount of acetic acid by the following equation: 4.7 – 8.0%.

$$\text{Amount (\%) of acetic acid} = M_S/M_T \times A_T/A_S \times 10$$

M_S: Amount (g) of acetic acid (100)

M_T: Amount (g) of Leuprorelin Acetate

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 0.7 mL of phosphoric acid add water to make 1000 mL, and adjust to pH 3.0 with a solution of sodium hydroxide (21 in 50). To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is 3 to 4 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the symmetry factor of the peak of acetic acid is not more than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of acetic acid is not more than 2.0%.

Assay Weigh accurately about 0.1 g each of Leuprorelin Acetate and Leuprorelin Acetate RS (separately determine the water <*2.48*> and acetic acid in the same manner as Leuprorelin Acetate), dissolve separately in the mobile phase to make exactly 100 mL. To exactly 5 mL each of these solutions add the mobile phase to make them exactly 100 mL, and use so obtained solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to

the following conditions, and determine the peak areas, A_T and A_S, of leuprorelin in each solution.

$$\text{Amount (mg) of leuprorelin } (C_{59}H_{84}N_{16}O_{12}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Leuprorelin Acetate RS, calculated on the anhydrous and de-acetic acid basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 15.2 g of triethylamine in 800 mL of water, adjust to pH 3.0 with phosphoric acid, and add water to make 1000 mL. To 850 mL of this solution add 150 mL of a mixture of acetonitrile and 1-propanol (3:2).

Flow rate: Adjust the flow rate so that the retention time of leuprorelin is 41 to 49 minutes (1.0 – 1.5 mL per minute).

System suitability—

System performance: Dissolve about 0.1 g of Leuprorelin Acetate RS in 100 mL of the mobile phase. To 5 mL of this solution add water to make 50 mL. To 5 mL of this solution add 0.1 mL of sodium hydroxide TS, stopper the vessel, shake vigorously, then heat at 100°C for 60 minutes. After cooling, add 50 μL of 1 mol/L phosphoric acid solution, and shake vigorously. When the procedure is run with 20 μL of this solution under the above operating conditions, a peak having the relative retention time of about 0.90 to leuprorelin and leuprorelin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of leuprorelin is not more than 1.5%.

Containers and storage Containers—Hermetic containers.

Add the following:

Losartan Potassium and Hydrochlorothiazide Tablets

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Losartan Potassium and Hydrochlorothiazide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$: 461.00) and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$: 297.74).

Method of preparation Prepare as directed under Tablets, with Losartan Potassium and Hydrochlorothiazide.

Identification (1) Shake well a portion of powdered Losartan Potassium and Hydrochlorothiazide Tablets, equivalent to 50 mg of Losartan Potassium, with 10 mL of methanol, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 50 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of losartan potassium in methanol to make 10 mL. To 5 mL of this solution add methanol to make 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and acetic acid (100) (75:25:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the two spots obtained from the sample solution and the spot obtained from the standard solution show the same *R*f value.

(2) Shake well a portion of powdered Losartan Potassium and Hydrochlorothiazide Tablets, equivalent to 12.5 mg of Hydrochlorothiazide, with 10 mL of methanol, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 50 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of hydrochlorothiazide in methanol to make 10 mL. To 5 mL of this solution add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and acetic acid (100) (75:25:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the two spots obtained from the sample solution and the spot obtained from the standard solution show the same *R*f value.

Uniformity of dosage units <*6.02*> Perform the test according to the following methods: it meets the requirement of the Content uniformity test.

(1) Losartan potassium—To 1 tablet of Losartan Potassium and Hydrochlorothiazide Tablets add *V*/2 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), and stir for 60 minutes to disintegrate the tablet, add sodium dihydrogen phosphate TS, pH 2.5, to make exactly *V* mL so that each mL contains about 0.5 mg of losartan potassium ($C_{22}H_{22}ClKN_6O$). Pipet 10 mL of this solution, add 45 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), add sodium dihydrogen phosphate TS, pH 2.5, to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 46 mg of Losartan Potassium RS (separately determine the water <*2.48*> in the same manner as Losartan Potassium), and dissolve in 50 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), add sodium dihydrogen phosphate TS, pH 2.5,

to make exactly 100 mL, and use this solution as the losartan potassium standard stock solution. Pipet 12 mL of the losartan potassium standard stock solution, add 44 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), add sodium dihydrogen phosphate TS, pH 2.5, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of losartan in each solution.

Amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$) $= M_S \times A_T/A_S \times 3V/250$

M_S: Amount (mg) of Losartan Potassium RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 2.5 with phosphoric acid, and add water to make 1000 mL. To 900 mL of this solution add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of losartan is about 5 minutes.

System suitability—

System performance: To 12 mL of the losartan potassium standard stock solution and 4 mL of the hydrochlorothiazide standard stock solution obtained in (2), add 42 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), and add sodium dihydrogen phosphate TS, pH 2.5, to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrochlorothiazide and losartan are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of losartan is not more than 1.0%.

(2) Hydrochlorothiazide—To 1 tablet of Losartan Potassium and Hydrochlorothiazide Tablets add $V/2$ mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), and stir for 60 minutes to disintegrate the tablet, add sodium dihydrogen phosphate TS, pH 2.5, to make exactly V mL so that each mL contains about 0.125 mg of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$). Pipet 10 mL of this solution, add 45 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), add sodium dihydrogen phosphate TS, pH 2.5, to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 35 mg of Hydrochlorothiazide RS (separately determine the loss on drying <2.41> under the same conditions as Hydrochlorothiazide), and dissolve in 50 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), add sodium dihydrogen phosphate TS, pH 2.5, to make exactly 100 mL, and use this solution as the hydrochlorothiazide standard stock solution. Pipet 4 mL of the hydrochlorothiazide standard stock solution, add 48 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), add sodium dihydrogen phosphate TS, pH 2.5, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of hydrochlorothiazide in each solution.

Amount (mg) of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) $= M_S \times A_T/A_S \times V/250$

M_S: Amount (mg) of Hydrochlorothiazide RS, calculated on the dried basis

Operating conditions—

Proceed as directed in the operating conditions in (1).

System suitability—

System performance: To 12 mL of the losartan potassium standard stock solution obtained in (1) and 4 mL of the hydrochlorothiazide standard stock solution, add 42 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), and add sodium dihydrogen phosphate TS, pH 2.5, to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrochlorothiazide and losartan are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hydrochlorothiazide is not more than 1.0%.

Dissolution <6.10> (1) Losartan potassium—When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Losartan Potassium and Hydrochlorothiazide Tablets is not less than 85%.

Start the test with 1 tablet of Losartan Potassium and Hydrochlorothiazide Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg of losartan potassium ($C_{22}H_{22}ClKN_6O$), and use this solution as the sample solution. Separately, weigh accurately about 46 mg of Losartan Potassium RS (separately determine the water <2.48> in the same manner as Losartan Potassium), and dissolve in water to make exactly 100 mL, and use this solution

as the losartan potassium standard stock solution. Pipet 12 mL of the losartan potassium standard stock solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of losartan in each solution.

Dissolution rate (%) with respect to the labeled amount of losartan potassium ($C_{22}H_{22}ClKN_6O$)

$= M_S \times A_T/A_S \times V'/V \times 1/C \times 108$

M_S: Amount (mg) of Losartan Potassium RS, calculated on the anhydrous basis

C: Labeled amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Uniformity of dosage units (1).

System suitability—

System performance: To 12 mL of the losartan potassium standard stock solution and 8 mL of the hydrochlorothiazide standard stock solution obtained in (2), add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrochlorothiazide and losartan are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of losartan is not more than 1.0%.

(2) Hydrochlorothiazide—When the test is performed at 100 revolutions per minute according to the Basket method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Losartan Potassium and Hydrochlorothiazide Tablets is not less than 80%.

Start the test with 1 tablet of Losartan Potassium and Hydrochlorothiazide Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 13.9 μg of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$), and use this solution as the sample solution. Separately, weigh accurately about 35 mg of Hydrochlorothiazide RS (separately determine the loss on drying <2.41> under the same conditions as Hydrochlorothiazide), dissolve in 20 mL of methanol, and add water to make exactly 200 mL, and use this solution as the hydrochlorochiazide standard stock solution. Pipet 8 mL of hydrochlorothiazide standard stock solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of hydrochlorothiazide in each solution.

Dissolution rate (%) with respect to the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$)

$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of Hydrochlorothiazide RS, calculated on the dried basis

C: Labeled amount (mg) of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Uniformity of dosage units (1).

System suitability—

System performance: To 12 mL of the losartan potassium standard stock solution obtained in (1) and 8 mL of the hydrochlorothiazide standard stock solution, add water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrochlorothiazide and losartan are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hydrochlorothiazide is not more than 1.0%.

Assay **(1)** Losartan potassium—To 10 Losartan Potassium and Hydrochlorothiazide Tablets add $21V/25$ mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), stir for 60 minutes to disintegrate the tablets, add sodium dihydrogen phosphate TS, pH 2.5, to make exactly V mL so that each mL contains about 2 mg of losartan potassium ($C_{22}H_{22}ClKN_6O$), and treat with ultrasonic waves for 2 minutes. Pipet 10 mL of this solution, add 10 mL of acetonitrile, and add sodium dihydrogen phosphate TS, pH 2.5, to make exactly 50 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of Losartan Potassium RS (separately determine the water <2.48> in the same manner as Losartan Potassium), and dissolve in 30 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), add sodium dihydrogen phosphate TS, pH 2.5, to make exactly 50 mL, and use this solution as the losartan potassium standard stock solution. Pipet 10 mL of the losartan potassium standard stock solution, add 4 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), add sodium dihydrogen phosphate TS, pH 2.5, to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of losartan in each solution.

Amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$) in 1 tablet

$= M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of Losartan Potassium RS, calculated

on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: Dissolve 1.25 g of potassium dihydrogen phosphate and 1.5 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL. To 930 mL of this solution add 70 mL of acetonitrile.

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 12	100 → 92	0 → 8
12 – 28	92 → 38	8 → 62

Flow rate: Adjust the flow rate so that the retention time of losartan is about 20 minutes.

System suitability—

System performance: To 25 mL of the losartan potassium standard stock solution and 10 mL of the hydrochlorothiazide standard stock solution obtained in (2), add sodium dihydrogen phosphate TS, pH 2.5, to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrochlorothiazide and losartan are eluted in this order, and the number of theoretical plates of the peak of hydrochlorothiazide and the symmetry factor of the peak of losartan are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of losartan is not more than 1.0%.

(2) Hydrochlorothiazide—To 10 Losartan Potassium and Hydrochlorothiazide Tablets add $21V/25$ mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), stir for 60 minutes to disintegrate the tablets, add sodium dihydrogen phosphate TS, pH 2.5, to make exactly V mL so that each mL contains about 0.5 mg of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$), and treat with ultrasonic waves for 2 minutes. Pipet 10 mL of this solution, add 10 mL of acetonitrile, and add sodium dihydrogen phosphate TS, pH 2.5, to make exactly 50 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Hydrochlorothiazide RS (separately determine the loss on drying <2.41> under the same conditions as Hydrochlorothiazide), and dissolve in a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), to make exactly 50 mL, and use this solution as the hydrochlorothiazide standard stock solution. Pipet 20 mL of the hydrochlorothiazide standard stock solution, add 30 mL of a mixture of acetonitrile and sodium dihydrogen phosphate TS, pH 2.5 (3:2), add sodium dihydrogen phosphate TS, pH 2.5, to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of hydrochlorothiazide in each solution.

Amount (mg) of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) in 1 tablet

$= M_S \times A_T/A_S \times V/500$

M_S: Amount (mg) of Hydrochlorothiazide RS, calculated on the dried basis

Operating conditions—

Proceed as directed in the operating conditions in (1).

System suitability—

System performance: To 25 mL of the losartan potassium standard stock solution obtained in (1) and 10 mL of the hydrochlorothiazide standard stock solution, add sodium dihydrogen phosphate TS, pH 2.5, to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, hydrochlorothiazide and losartan are eluted in this order, and the number of theoretical plates of the peak of hydrochlorothiazide and the symmetry factor of the peak of losartan are not less than 4000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hydrochlorothiazide is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Loxoprofen Sodium Tablets

ロキソプロフェンナトリウム錠

Loxoprofen Sodium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of loxoprofen sodium ($C_{15}H_{17}NaO_3$: 268.28).

Method of preparation Prepare as directed under Tablets, with Loxoprofen Sodium Hydrate.

Identification To a quantity of powdered Loxoprofen Sodium Tablets, equivalent to 60 mg of loxoprofen sodium ($C_{15}H_{17}NaO_3$), add 20 mL of methanol, shake vigorously for 10 minutes, and centrifuge. To 1 mL of the supernatant liquid add methanol to make 20 mL. To 2 mL of this solution add methanol to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum be-

tween 221 nm and 225 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Loxoprofen Sodium Tablets add exactly V mL of the internal standard solution so that each mL contains about 3 mg of loxoprofen sodium ($C_{15}H_{17}NaO_3$). After treating with ultrasonic waves for 10 minutes with occasional shaking, centrifuge the solution. To 2 mL of the supernatant liquid add diluted methanol (3 in 5) to make 100 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of loxoprofen sodium ($C_{15}H_{17}NaO_3$)
$= M_S \times Q_T/Q_S \times V/10 \times 1.089$

M_S: Amount (mg) of Loxoprofen RS

Internal standard solution—A solution of ethyl benzoate in diluted methanol (3 in 5) (3 in 2000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Loxoprofen Sodium Tablets is not less than 85%.

Start the test with 1 tablet of Loxoprofen Sodium Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 13 μg of loxoprofen sodium ($C_{15}H_{17}NaO_3$). Use this solution as the sample solution. Separately, weigh accurately about 31 mg of Loxoprofen RS, previously dried in vacuum at 60°C for 3 hours, dissolve in 5 mL of ethanol (99.5), and add water to make exactly 250 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 223 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as the control.

Dissolution rate (%) with respect to the labeled amount of loxoprofen sodium ($C_{15}H_{17}NaO_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36 \times 1.089$

M_S: Amount (mg) of Loxoprofen RS
C: Labeled amount (mg) of loxoprofen sodium ($C_{15}H_{17}NaO_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Loxoprofen Sodium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 60 mg of loxoprofen sodium ($C_{15}H_{17}NaO_3$), add exactly 20 mL of the internal standard solution, and shake vigorously for 15 minutes. Centrifuge this solution, and to 2 mL of the supernatant liquid add diluted methanol (3 in 5) to make 100 mL. Use this solution as the sample solution. Separately, weigh accurately about 30 mg of Loxoprofen RS, previously dried in vacuum at 60°C for 3 hours, and dissolve in exactly 10 mL of the internal standard solution. To 2 mL of this solution add diluted methanol (3 in 5) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of loxoprofen to that of the internal standard.

Amount (mg) of loxoprofen sodium ($C_{15}H_{17}NaO_3$)
$= M_S \times Q_T/Q_S \times 2 \times 1.089$

M_S: Amount (mg) of Loxoprofen RS

Internal standard solution—A solution of ethyl benzoate in diluted methanol (3 in 5) (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 222 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol, water, acetic acid (100) and triethylamine (600:400:1:1).

Flow rate: Adjust the flow rate so that the retention time of loxoprofen is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, loxoprofen and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of loxoprofen to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

L-Lysine Hydrochloride

L-リシン塩酸塩

Change the Description as follows:

Description L-Lysine Hydrochloride occurs as a white powder. It has a slight, characteristic taste.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (95).

It shows crystal polymorphism.

D-Mannitol

D-マンニトール

Add the following paragraph on the International Harmonization next to the CAS registry number, and change the origin/limits of content and below as follows:

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

D-Mannitol contains not less than 97.0% and not more than 102.0% of $C_6H_{14}O_6$, calculated on the dried basis.

◆**Description** D-Mannitol occurs as white, crystals, powder or grain. It has a sweet taste with a cold sensation.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in sodium hydroxide TS.

It shows crystal polymorphism.◆

Identification Determine the infrared absorption spectrum of D-Mannitol as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of D-Mannitol RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, put 25 mg each of D-Mannitol and D-Mannitol RS in glass vessels, dissolve in 0.25 mL of water without heating, dry them in a 600 – 700 W microwave oven for 20 minutes or in a drying chamber at 100°C for 1 hour, then further dry by gradual reducing pressure, and perform the same test as above with so obtained non-sticky white to pale yellow powders: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 165 – 170°C

Purity **(1)** Clarity and color of solution—Dissolve 5.0 g of D-Mannitol in water to make 50 mL: the solution is clear, and its clarity is the same as that of water or its turbidity is not more than that of reference suspension I, and its color is not more intense than the following control solution.

Control solution: To 3.0 mL of Cobalt (II) Chloride CS, 3.0 mL of Iron (III) Chloride CS and 2.4 mL of Copper (II) Sulfate CS, add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

◆**(2)** Heavy metals <*1.07*>—Proceed with 5.0 g of D-Mannitol according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm)◆.

(3) Nickel—Shake 10.0 g of D-Mannitol with 30 mL of 2 mol/L acetic acid TS, and add water to make exactly 100 mL. Add 2.0 mL of a saturated solution of ammonium pyrrolidinedithiocarbamate (about 10 g/L) and 10.0 mL of water-saturated 4-methyl-2-pentanone, and shake for 30 seconds without exposure to light. Allow the layers to separate, and use the 4-methyl-2-pentanone layer as the sample solution. Separately, put 10.0 g each of D-Mannitol in three vessels, add 30 mL of 2 mol/L acetic acid TS to them, shake, add a suitable amount of water and exactly 0.5 mL, 1.0 mL and 1.5 mL respectively of Standard Nickel Solution for Atomic Absorption Spectrophotometry, and add water to make them exactly 100 mL. Then, proceed in the same manner as the sample solution, and use so obtained three 4-methyl-2-pentanone layers as the standard solutions. Additionally, prepare a 4-methyl-2-pentanone layer by proceeding in the same manner as the sample solution without using D-Mannitol, and use this layer as the blank solution. Perform the test with the sample solution and standard solutions as directed in the standard addition method under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions. Set the zero of the instrument using the blank solution, and between each measurement, rinse with water and ascertain that the readings return to zero with the blank solution: amount of nickel is not more than 1 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.

Lamp: Nickel hollow-cathode lamp.

Wavelength: 232.0 nm.

(4) Related substances—Dissolve 0.50 g of D-Mannitol in water to make 10 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 0.5 mL of the standard solution (1), add water to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution and the standard solutions (1) and (2) as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the peak area of D-sorbitol, having the relative retention time of about 1.2 to D-mannitol, obtained from the sample solution is not larger than that of D-mannitol obtained from the standard solution (1) (not more than 2.0%), the total peak area of maltitol, having the relative retention time of about 0.69, and isomalt, having the relative retention times of about 0.6 and about 0.73, is not larger than the peak area of D-mannitol from the standard solution (1) (not more than 2.0%), and the area of the peak other than D-mannitol and the peaks mentioned above is not larger than 2 times the peak area of D-mannitol from the standard solution (2) (not more than 0.1%). Furthermore, the total area of the peak other than D-mannitol from the sample solution is not larger than the peak area of D-mannitol from the standard solution (1) (not more than 2.0%). For these calculations exclude the peak which area is not larger than the peak area of D-mannitol from the standard solution (2) (not more than 0.05%).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 1.5 times as long as the

retention time of D-mannitol.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

◆Test for required detectability: Confirm that the peak area of D-mannitol obtained with 20 μL of the standard solution (2) is equivalent to 1.75 to 3.25% of that obtained with 20 μL of the standard solution (1).

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of D-mannitol is not more than 1.0%.◆

(5) Glucose—To 7.0 g of D-Mannitol add 13 mL of water and 40 mL of Fehling's TS, boil gently for 3 minutes, and allow to stand for 2 minutes to precipitate copper (I) oxide. Separate the supernatant liquid, filter through a sintered glass filter for cupric oxide filtration coated with siliceous earth or a sintered glass filter (G4). Wash the precipitates with 50 – 60°C hot water until the washing no longer alkaline, and filter the washings through the filter described above. Discard all the filtrate at this step. Immediately, dissolve the precipitate with 20 mL of iron (III) sulfate TS, filter through the filter described above in a clean flask, and wash the filter with 15 – 20 mL of water. Combine the filtrate and the washings, heat to 80°C, and titrate ⟨*2.50*⟩ with 0.02 mol/L potassium permanganate VS until the green color turns to light red and the color persists at least 10 seconds: not more than 3.2 mL is required to change the color of the solution (not more than 0.1% expressed as glucose).

Conductivity ⟨*2.51*⟩ Dissolve 20.0 g of D-Mannitol in a freshly boiled and cooled water prepared from distilled water by heating to 40 – 50°C, add the same water to make 100 mL, and use this solution as the sample solution. After cooling, measure the conductivity of the sample solution at 25 ± 0.1°C while gently stirring with a magnetic stirrer: not more than 20 $\mu S \cdot cm^{-1}$.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.5 g each of D-Mannitol and D-Mannitol RS (separately determine the loss on drying ⟨*2.41*⟩ under the same conditions as D-Mannitol), dissolve separately in water to make exactly 10 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of D-mannitol in each solution.

$$\text{Amount (g) of } C_6H_{14}O_6 = M_S \times A_T/A_S$$

M_S: Amount (g) of D-Mannitol RS, calculated on the dried basis

Operating conditions—

Detector: A differential refractometer maintained at a constant temperature (40°C for example).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (calcium type) composed with a sulfonated polystyrene cross-linked with 8 % of divinylbenzene (9 μm in particle diameter).

Column temperature: 85 ± 2°C.

Mobile phase: water.

Flow rate: 0.5 mL per minute (the retention time of D-mannitol is about 20 minutes).

System suitability—

System performance: Dissolve 0.25 g each of D-Mannitol and D-sorbitol in water to make 10 mL, and use this solution as the solution for system suitability test (1). Separately, dissolve 0.5 g each of maltitol and isomalt in water to make 100 mL. To 2 mL of this solution add water to make 10 mL, and use this solution as the solution for system suitability test (2). When proceed with 20 μL each of the solution for system suitability test (1) and the solution for system suitability test (2) as directed under the above operating conditions, isomalt (first peak), maltitol, isomalt (second peak), D-mannitol and D-sorbitol are eluted in this order, the relative retention time of isomalt (first peak), maltitol, isomalt (second peak) and D-sorbitol to D-mannitol is about 0.6, about 0.69, about 0.73 and about 1.2, respectively, and the resolution between the peaks of D-mannitol and D-sorbitol is not less than 2.0. Coelution of maltitol and the second peak of isomalt may be observed.

◆System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of D-mannitol is not more than 1.0%.◆

◆**Containers and storage** Containers—Well-closed containers.◆

D-Mannitol Injection

D-マンニトール注射液

Change the Identification and Assay as follows:

Identification Concentrate D-Mannitol Injection on a water bath to make a saturated solution. To 5 drops of this solution add 1 mL of iron (III) chloride TS and 5 drops of a solution of sodium hydroxide (1 in 5): a yellow precipitate is produced. Shake this solution vigorously: a clear solution is produced. On addition of a solution of sodium hydroxide (1 in 5), no precipitate is produced.

Assay Measure exactly a volume of D-Mannitol Injection, equivalent to about 5 g of D-mannitol ($C_6H_{14}O_6$), and add water to make exactly 250 mL. To exactly 10 mL of this solution add water to make exactly 100 mL. Measure exactly 10 mL of this solution into an iodine flask, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. After cooling, add 2.5 g of potassium iodide, stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a

blank determination in the same manner.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of $C_6H_{14}O_6$

Maprotiline Hydrochloride

マプロチリン塩酸塩

Change the Description as follows:

Description Maprotiline Hydrochloride occurs as a white crystalline powder.

It is soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water.

Melting point: about 244°C (with decomposition).

It shows crystal polymorphism.

Mecobalamin

メコバラミン

Change the origin/limits of content and the Description as follows:

Mecobalamin contains not less than 98.0% and not more than 101.0% of $C_{63}H_{91}CoN_{13}O_{14}P$, calculated on the anhydrous basis.

Description Mecobalamin occurs as dark red, crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

It decomposes on exposure to light.

Add the following:

Mecobalamin Tablets

メコバラミン錠

Mecobalamin Tablets contain not less than 92.0% and not more than 108.0% of the labeled amount of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$: 1344.38).

Method of preparation Prepare as directed under Tablets, with Mecobalamin.

Identification **(1)** Conduct this procedure without exposure to light, using light-resistant vessels. To a quantity of powdered Mecobalamin Tablets, equivalent to 1 mg of Mecobalamin, add 10 mL of hydrochloric acid-potassium chloride buffer solution, pH 2.0, treat with ultrasonic waves, and add hydrochloric acid-potassium chloride buffer solution, pH 2.0 to make 20 mL. Centrifuge this solution, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.8 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 262 nm and 266 nm, between 303 nm and 307 nm, and between 461 nm and 465 nm.

(2) Conduct this procedure without exposure to light, using light-resistant vessels. To a quantity of powdered Mecobalamin Tablets, equivalent to 1 mg of Mecobalamin, add 10 mL of phosphate buffer solution, pH 7.0, treat with ultrasonic waves, and add phosphate buffer solution, pH 7.0 to make 20 mL. Centrifuge this solution, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.8 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 264 nm and 268 nm, between 339 nm and 343 nm, and between 520 nm and 524 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure without exposure to light, using light-resistant vessels. Take 1 tablet of Mecobalamin Tablets, and disintegrate the tablet by adding $V/5$ mL of water. Add methanol to make exactly V mL so that each mL contains about 25 μg of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$). After shaking for 5 minutes, allow to stand for not less than 10 minutes. Filter thus obtained supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Mecobalamin RS (separately determine the water <*2.48*> in the same manner as Mecobalamin), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add 5 mL of water and methanol to make exactly 50 mL. Use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of mecobalamin in each solution.

Amount (mg) of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$)
$= M_S \times A_T/A_S \times V/1000$

M_S: Amount (mg) of Mecobalamin RS, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Mecobalamin.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mecobalamin are not less than 2000 and 0.8 to 1.1, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mecobalamin is not more than 1.0%.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of Mecobalamin Tablets is not less than 80%.

Conduct this procedure without exposure to light, using light-resistant vessels. Start the test with 1 tablet of Mecobalamin Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add water to make exactly V' mL so that each mL contains about 0.28 μg of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$). Use this solution as the sample solution. Separately, weigh accurately about 28 mg of Mecobalamin RS (separately determine the water ⟨*2.48*⟩ in the same manner as Mecobalamin), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of mecobalamin in each solution.

Dissolution rate (%) with respect to the labeled amount of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$)

$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/10$

M_S: Amount (mg) of Mecobalamin RS, calculated on the anhydrous basis

C: Labeled amount (mg) of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 264 nm).

Column: A stainless steel column of 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust to pH 3.0 of a solution of 6.0 g of L-tartaric acid in 1000 mL of water with a solution of 14.3 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water. To 630 mL of this solution add 370 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of mecobalamin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mecobalamin are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mecobalamin are not more than 2.0%.

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Disintegrate 20 tablets of Mecobalamin Tablets with $V/5$ mL of water. Add methanol to make exactly V mL so that each mL contains about 50 μg of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$). After shaking for 5 minutes, allow to stand for not less than 10 minutes. Filter thus obtained supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Mecobalamin RS (separately determine the water ⟨*2.48*⟩ in the same manner as Mecobalamin), and dissolve in water to make exactly 100 mL. To exactly 10 mL of this solution add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of mecobalamin in each solution.

Amount (mg) of mecobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$) in 1 tablet

$= M_S \times A_T/A_S \times V/10000$

M_S: Amount (mg) of Mecobalamin RS, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Mecobalamin.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of mecobalamin are not less than 3000 and 0.8 to 1.1, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mecobalamin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Mequitazine Tablets

メキタジン錠

Mequitazine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of mequitazine ($C_{20}H_{22}N_2S$: 322.47).

Method of preparation Prepare as directed under Tablets, with Mequitazine.

Identification Powder Mequitazine Tablets. To a portion of the powder, equivalent to 3 mg of Mequitazine, add 50 mL of ethanol (95), shake thoroughly, and add ethanol (95) to make 100 mL. Centrifuge, if necessary, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.5 μm. Discard 10 mL of the first filtrate, to 4 mL of the subsequent filtrate add ethanol (95) to make 25 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 253 nm and 257 nm and between 301 nm and 311 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Mequitazine Tablets add 50 mL of a mixture of methanol and water (4:3), and disperse to fine particles with the aid of ultrasonic waves. Shake this solution thoroughly, and add methanol to make exactly 100 mL. Centrifuge, if necessary, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.5 μm. Discard 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add methanol to make exactly V' mL so that each mL contains about 4.8 μg of mequitazine ($C_{20}H_{22}N_2S$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of mequitazine ($C_{20}H_{22}N_2S$)
$= M_S \times A_T/A_S \times V'/V \times 1/50$

M_S: Amount (mg) of mequitazine for assay

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Mequitazine Tablets is not less than 70%.

Start the test with 1 tablet of Mequitazine Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 3.3 μg of mequitazine ($C_{20}H_{22}N_2S$), and use this solution as the sample solution. Separately, weigh accurately about 15 mg of mequitazine for assay, previously dried in vacuum at 60°C using phosphorous (V) oxide as the desiccant for 3 hours, dissolve in 50 mL of methanol, and add the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 253 nm as directed under Ultraviolet-visible Sprctrophotometry <*2.24*>, using the dissolution medium as the control.

Dissolution rate (%) with respect to the labeled amount of mequitazine ($C_{20}H_{22}N_2S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45/2$

M_S: Amount (mg) of mequitazine for assay

C: Labeled amount (mg) of mequitazine ($C_{20}H_{22}N_2S$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Mequitazine Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 3 mg of mequitazine ($C_{20}H_{22}N_2S$), add 50 mL of a mixture of methanol and water (4:3), shake thoroughly, and add methanol to make exactly 100 mL. Centrifuge, if necessary, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.5 μm. Discard 10 mL of the first filtrate, pipet 4 mL of the subsequent filtrate, add methanol to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 24 mg of mequitazine for assay, previously dried in vacuum at 60°C using phosphorous (V) oxide as the desiccant for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 254 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of mequitazine ($C_{20}H_{22}N_2S$)
$= M_S \times A_T/A_S \times 1/8$

M_S: Amount (mg) of mequitazine for assay

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Meropenem for Injection

注射用メロペネム

Change the Purity (2) and Assay as follows:

Purity

(2) Related substances—Dissolve an amount of Meropenem for Injection, equivalent to 0.10 g (potency) of Meropenem Hydrate, in triethylamine-phosphate buffer solution, pH 5.0 to make 25 mL, and use this solution as the sample solution. Prepare the sample solution before use. Pipet 1 mL of the sample solution, add triethylamine-phosphate buffer solution, pH 5.0 to make exactly 100 mL. Pipet 5 mL of this solution, add triethylamine-phosphate buffer solution, pH 5.0 to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the peak area of ring-opened meropenem and meropenem dimer, respectively having the relative retention time of about 0.5 and about 2.2 to meropenem obtained from the sample solution is not larger than the peak area of meropenem obtained from the standard solution, the area of the peak, other than meropenem and the peaks mentioned above, is not larger than 1/5 times the peak area of meropenem obtained from the standard solution, and the total area of the peaks other than meropenem

is not larger than 3 times the peak area of meropenem obtained from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Meropenem Hydrate.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add triethylamine-phosphate buffer solution, pH 5.0, to make exactly 25 mL. Confirm that the peak area of meropenem obtained with 10 μL of this solution is equivalent to 16 to 24% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the sample solution, previously allowed to stand at 60°C for 30 minutes, under the above operating conditions, the ring-opened meropenem, meropenem and the meropenem dimer are eluted in this order, and the resolution between the peaks of the ring-opened meropenem and meropenem is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of meropenem is not more than 1.5%.

Assay Weigh accurately the mass of the contents of not less than 10 containers of Meropenem for Injection. Weigh accurately an amount of the contents, equivalent to about 50 mg (potency) of Meropenem Hydrate, dissolve in exactly 10 mL of the internal standard solution, add triethylamine-phosphate buffer solution, pH 5.0, to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Meropenem RS, equivalent to about 50 mg (potency), dissolve in exactly 10 mL of the internal standard solution, add triethylamine-phosphate buffer solution, pH 5.0, to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Meropenem Hydrate.

Amount [mg (potency)] of meropenem ($C_{17}H_{25}N_3O_5S$)
$= M_S \times Q_T/Q_S$

M_S: Amount [mg (potency)] of Meropenem RS

Internal standard solution—A solution of benzyl alcohol in triethylamine-phosphate buffer solution, pH 5.0 (1 in 300).

Methylcellulose

メチルセルロース

Change the Viscosity and the pH as follows:

Viscosity <*2.53*>

(i) Method I: Apply to Methylcellulose having a labeled viscosity of less than 600 mPa·s. Put an exact amount of Methylcellulose, equivalent to 4.000 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 200.0 g, stopper the bottle, stir by mechanical means at 350-to 450-revolutions per minute for 10 to 20 minutes to get a homogeneous dispersion. If necessary, take off the sample attached on the walls of the bottle, put them in the dispersed solution, and dissolve by continuing the stirring in a water bath not exceeding 5°C for 20 to 40 minutes. Add cooled water, if necessary, to make 200.0 g, and use this solution as the sample solution. Centrifuge the solution if necessary to expel any entrapped air bubbles. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method I under Viscosity Determination: not less than 80% and not more than 120% of the labeled viscosity.

(ii) Method II: Apply to Methylcellulose having a labeled viscosity of not less than 600 mPa·s. Put an exact amount of Methylcellulose, equivalent to 10.00 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 500.0 g, stopper the bottle, and prepare the sample solution in the same manner as directed in Method I. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method II under Viscosity Determination, using a single cylindertype rotational viscometer, according to the following operating conditions: not less than 75% and not more than 140% of the labeled viscosity.

Operating conditions—

Apparatus: Brookfield type viscometer LV model.

Rotor No., rotation frequency, and conversion factor: According to the following table, depending on the labeled viscosity.

Labeled viscosity (mPa·s)				Rotor No.	Rotation frequency /min	Conversion factor
Not less than 600 and less than 1400				3	60	20
〃	1400	〃	3500	3	12	100
〃	3500	〃	9500	4	60	100
〃	9500	〃	99,500	4	6	1000
〃	99,500			4	3	2000

Procedure of apparatus: Read value after 2 minutes of rotation, and stop the rotation for at least 2 minutes. Repeat this procedure more two times, and average three observed values.

pH <*2.54*> The pH of the sample solution obtained in the Viscosity, measured after 5 minutes immersing the electrode in the sample solution, is between 5.0 and 8.0.

Methylprednisolone Succinate

メチルプレドニゾロンコハク酸エステル

Change the Description as follows:

Description Methylprednisolone Succinate occurs as a white, crystals or crystalline powder.

It is soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 235°C (with decomposition).

It shows crystal polymorphism.

Metildigoxin

メチルジゴキシン

Change the Description as follows:

Description Metildigoxin occurs as a white to light yellowish white, crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, in pyridine and in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) and in acetone, and very slightly soluble in water.

It shows crystal polymorphism.

Metoprolol Tartrate

メトプロロール酒石酸塩

Change the Description as follows:

Description Metoprolol Tartrate occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in methanol, in ethanol (95) and in acetic acid (100).

Optical rotation $[\alpha]_D^{20}$: +7.0 – +10.0° (after drying, 1 g, water, 50 mL, 100 mm).

It shows crystal polymorphism.

Mexiletine Hydrochloride

メキシレチン塩酸塩

Change the Description and the Identification (2) as follows:

Description Mexiletine Hydrochloride occurs as a white powder.

It is freely soluble in water and in ethanol (95), and slightly soluble in acetonitrile.

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of Mexiletine Hydrochloride (1 in 20) shows no optical rotation.

Mexiletine Hydrochloride shows crystal polymorphism.

Identification

(2) Determine the infrared absorption spectrum of Mexiletine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Mexiletine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize Mexiletine Hydrochloride from ethanol (95), filter, dry the crystals, and perform the test with the crystals.

Morphine Hydrochloride Hydrate

モルヒネ塩酸塩水和物

Change the Purity (4) as follows:

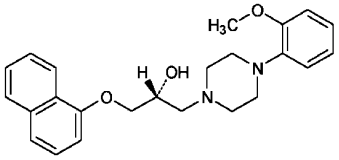
Purity

(4) Related substances—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 10 mL of diluted methanol (4 in 5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (4 in 5) to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add diluted methanol (4 in 5) to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, ethanol (99.5) and ammonia solution (28) (21:14:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot having a Rf value of about 0.17 obtained with the sample solution is not more intense than the spot obtained with the standard solution (1), and the spots other than the principal spot, the spot mentioned above and the spot of the starting point are not more intense than the spot with the standard solution (2).

Add the following:

Naftopidil

ナフトピジル



and enantiomer

$C_{24}H_{28}N_2O_3$: 392.49

(2*RS*)-1-[4-(2-Methoxyphenyl)piperazin-1-yl]-3-(naphthalen-1-yloxy)propan-2-ol

[*57149-07-2*]

Naftopidil, when dried, contains not less than 99.0% and not more than 101.0% of $C_{24}H_{28}N_2O_3$.

Description Naftopidil occurs as a white crystalline powder.

It is very soluble in acetic anhydride, freely soluble in *N*,*N*-dimethylformamide and in acetic acid (100), slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is gradually colored to light brown by light.

A solution of Naftopidil in *N*,*N*-dimethylformamide (1 in 10) shows no optical rotation.

Identification **(1)** Dissolve 50 mg of Naftopidil in 5 mL of acetic acid (100), and add 0.1 mL of Dragendorff's TS: orange colored precipitates are produced.

(2) Determine the absorption spectrum of a solution of Naftopidil in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Naftopidil, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 126 – 129°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Naftopidil according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Naftopidil in 60 mL of methanol, add diluted 0.1 mol/L potassium dihydrogen phosphate TS, pH 2.0 (1 in 2) to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and water (3:2) to make exactly 100 mL. Pipet 4 mL of this solution, add a mixture of methanol and water (3:2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by automatic integration method: each peak area other than naftopidil obtained from the sample solution is not larger than 3/4 times the peak area of naftopidil obtained from the standard solution, and the total area of the peaks other than naftopidil from the sample solution is not larger than 2.5 times the peak area of naftopidil from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.80 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 4.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL. To 450 mL of this solution add 550 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of naftopidil is about 10 minutes.

Time span of measurement: About 2 times as long as the retention time of naftopidil, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2.5 mL of the standard solution, add a mixture of methanol and water (3:2) to make exactly 10 mL. Confirm that the peak area of naftopidil obtained with 10 μL of this solution is equivalent to 17.5% to 32.5% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of naftopidil are not less than 2500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of naftopidil is not more than 3.0%.

(3) Residual solvents—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Naftopidil, previously dried, dissolve in 50 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.25 mg of $C_{24}H_{28}N_2O_3$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Add the following:

Naftopidil Orally Disintegrating Tablets

ナフトピジル口腔内崩壊錠

Naftopidil Orally Disintegrating Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of naftopidil ($C_{24}H_{28}N_2O_3$: 392.49).

Method of preparation Prepare as directed under Tablets, with Naftopidil.

Identification Powder Naftopidil Orally Disintegrating Tablets. To a portion of the powder, equivalent to 25 mg of Naftopidil add 100 mL of methanol, shake thoroughly, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 6 mL of the filtrate add methanol to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 281 nm and 285 nm, and

between 318 nm and 322 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of Content uniformity test.

To 1 tablet of Naftopidil Orally Disintegrating Tablets add $V/10$ mL of water, disintegrate and disperse the tablet with the aid of ultrasonic waves. To this solution add $V/2$ mL of methanol, shake thoroughly, then add methanol to make exactly V mL so that each mL contains about 0.25 mg of naftopidil ($C_{24}H_{28}N_2O_3$), and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 6 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of naftopidil for assay, previously dried at 105°C for 3 hours, dissolve in methanol to make exactly 100 mL. Pipet 3 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 283 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of naftopidil ($C_{24}H_{28}N_2O_3$)
$= M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of naftopidil for assay

Disintegration Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolution per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, as the dissolution medium, the dissolution rate in 30 minutes of Naftopidil Orally Disintegrating Tablets is not less than 75%.

Start the test with 1 tablet of Naftopidil Orally Disintegrating Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 28 μg of naftopidil ($C_{24}H_{28}N_2O_3$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of naftopidil for assay, previously dried at 105°C for 3 hours, dissolve in 50 mL of methanol, then add the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 283 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the control.

Dissolution rate (%) with respect to the labeled amount of naftopidil ($C_{24}H_{28}N_2O_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of naftopidil for assay

C: Labeled amount (mg) of naftopidil ($C_{24}H_{28}N_2O_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Naftopidil Orally Disintegrating Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of naftopidil ($C_{24}H_{28}N_2O_3$), add 30 mL of methanol, shake thoroughly, add diluted 0.1 mol/L potassium dihydrogen phosphate TS, pH 2.0 (1 in 2) to make exactly 50 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 10 mL of the internal standard solution, add a mixture of methanol and water (3:2) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of naftopidil for assay, previously dried at 105°C for 3 hours, dissolve in 30 mL of methanol, add diluted 0.1 mol/L potassium dihydrogen phosphate TS, pH 2.0 (1 in 2) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add a mixture of methanol and water (3:2) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S of the peak area of naftopidil to that of the internal standard.

Amount (mg) of naftopidil ($C_{24}H_{28}N_2O_3$) $= M_S \times Q_T/Q_S$

M_S: Amount (mg) of naftopidil for assay

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of methanol and water (3:2) (3 in 2000).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Naftopidil.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, naftopidil and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of naftopidil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Add the following:

Naftopidil Tablets

ナフトピジル錠

Naftopidil Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of naftopidil ($C_{24}H_{28}N_2O_3$: 392.49).

Method of preparation Prepare as directed under Tablets, with Naftopidil.

Identification Powder Naftopidil Tablets. To a portion of the powder, equivalent to 25 mg of Naftopidil, add 100 mL of methanol, shake thoroughly, and centrifuge, if necessary. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. To 6 mL of the filtrate add methanol to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 281 and 285 nm, and between 318 and 322 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Naftopidil Tablets add V/10 mL of water, disintegrate and disperse the tablet with the aid of ultrasonic waves. To this dispersed solution add V/2 mL of methanol, shake thoroughly, add methanol to make exactly V mL so that each mL contains about 0.25 mg of naftopidil ($C_{24}H_{28}N_2O_3$). Centrifuge this solution, if necessary, filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 6 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of naftopidil for assay, previously dried at 105°C for 3 hours, dissolve in methanol to make exactly 100 mL. Pipet 3 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 283 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of naftopidil ($C_{24}H_{28}N_2O_3$)
$= M_S \times A_T/A_S \times V/200$

M_S: Amount (mg) of naftopidil for assay

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, as the dissolution medium, the dissolution rate in 15 minutes of 25-mg and 50-mg tablet and in 30 minutes of 75-mg tablet is not less than 75%.

Start the test with 1 tablet of Naftopidil Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 28 μg of naftopidil ($C_{24}H_{28}N_2O_3$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of naftopidil for assay, previously dried at 105°C for 3 hours, dissolve in 50 mL of methanol, and add the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 283 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the dissolution medium as the control.

Dissolution rate (%) with respect to the labeled amount of naftopidil ($C_{24}H_{28}N_2O_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of naftopidil for assay
C: Labeled amount (mg) of naftopidil ($C_{24}H_{28}N_2O_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Naftopidil Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of naftopidil ($C_{24}H_{28}N_2O_3$), add 30 mL of methanol, shake thoroughly, and add diluted 0.1 mol/L potassium dihydrogen phosphate TS, pH 2.0 (1 in 2) to make exactly 50 mL. Centrifuge this solution, if necessary, filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 10 mL of the internal standard solution, add a mixture of methanol and water (3:2) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of naftopidil for assay, previously dried at 105°C for 3 hours, dissolve in 30 mL of methanol, add diluted 0.1 mol/L potassium dihydrogen phosphate TS, pH 2.0 (1 in 2) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add a mixture of methanol and water (3:2) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S of the peak area of naftopidil to that of the internal standard.

Amount (mg) of naftopidil ($C_{24}H_{28}N_2O_3$) $= M_S \times Q_T/Q_S$

M_S: Amount (mg) of naftopidil for assay

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of methanol and water (3:2) (3 in 2000).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Naftopidil.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, naftopidil and the internal standard are eluted in

this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of naftopidil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Nartograstim (Genetical Recombination)

ナルトグラスチム(遺伝子組換え)

Change the origin/limits of content as follows:

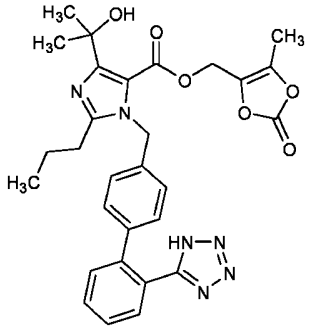
Nartograstim (Genetical Recombination) is an aqueous solution in which a desired product is a recombinant human granulocyte colony-stimulating factor (G-CSF) analog. It is N-methionylated, and threonine, leucine, glycine, proline and cysteine residues at the positions, 1, 3, 4, 5 and 17 of G-CSF are substituted by alanine, threonine, tyrosine, arginine and serine, respectively. It is a glycoprotein consisting of 175 amino acid residues. It has a stimulating effect on neutrophil production.

It contains not less than 0.9 mg and not more than 2.1 mg of protein per mL, and not less than 4.0×10^8 units per mg of protein.

Add the following:

Olmesartan Medoxomil

オルメサルタン メドキソミル



$C_{29}H_{30}N_6O_6$: 558.59

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-1*H*-imidazole-5-carboxylate

[*144689-63-4*]

Olmesartan Medoxomil contains not less than 98.5% and not more than 101.5% of $C_{29}H_{30}N_6O_6$, calculated on the anhydrous basis and corrected on the amount of the residual solvent.

Description Olmesartan Medoxomil occurs as a white to pale yellowish white, crystalline powder.

It is slightly soluble in acetonitrile and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Olmesartan Medoxomil in acetonitrile (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Olmesartan Medoxomil RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Olmesartan Medoxomil as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Olmesartan Medoxomil RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <*1.07*>—Proceed with 2.0 g of Olmesartan Medoxomil according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Olmesartan Medoxomil in 20 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks, having the relative retention times of about 0.2 and about 1.6 to olmesartan medoxomil, obtained from the sample solution are not larger than 2/5 times and 3/10 times the peak area of olmesartan medoxomil obtained from the standard solution, respectively, the area of the peaks other than olmesartan medoxomil and the peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of olmesartan medoxomil from the standard solution, and the total area of these peaks is not larger than 3/10 times the peak area of olmesartan medoxomil from the standard solution. In addition, the total area of the peaks other than olmesartan medoxomil from the sample solution is not larger than 4/5 times the peak area of olmesartan medoxomil from the standard solution. For these calculations use the areas of the peaks, having the relative retention times of about 0.7 and about 3.4 to olmesartan medoxomil, after multiplying by their relative response factors 0.65 and 1.39, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octylsilanized silica gel for liquid chromatography (3.5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 2.04 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 3.5 with a solution prepared by dissolving 1.73 g of phosphoric acid in water to make 1000 mL. To 400 mL of this solution add 100 mL of acetonitrile.

Mobile phase B: Dissolve 2.04 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 3.5 with a solution prepared by dissolving 1.73 g of phosphoric acid in water to make 1000 mL. To 100 mL of this solution add 400 mL of acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	75	25
10 – 35	75 → 0	25 → 100
35 – 45	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: For 45 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add acetonitrile to make exactly 20 mL. Confirm that the peak area of olmesartan medoxomil obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of olmesartan medoxomil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of olmesartan medoxomil is not more than 2.0%.

(3) Residual solvent—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Water ⟨*2.48*⟩ Not more than 0.5% (0.5 g, coulometric titration).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Olmesartan Medoxomil and Olmesartan Medoxomil RS (separately determine the water ⟨*2.48*⟩ and the residual solvent in the same manners as Olmesartan Medoxomil), dissolve them separately in a mixture of acetonitrile and water (4:1) to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add a mixture of water and acetonitrile (3:2) to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of olmesartan medoxomil to that of the internal standard.

$$\text{Amount (mg) of } C_{29}H_{30}N_6O_6 = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Olmesartan Medoxomil RS, calculated on the anhydrous basis and corrected on the amount of the residual solvent

Internal standard solution—A solution of isobutyl parahydroxybenzoate in a mixture of water and acetonitrile (3:2) (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.04 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 3.4 with a solution prepared by dissolving 1.73 g of phosphoric acid in water to make 1000 mL. To 330 mL of this solution add 170 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of olmesartan medoxomil is about 16 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, olmesartan medoxomil and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of olmesartan medoxomil to that of the internal standard is not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Add the following:

Olmesartan Medoxomil Tablets

オルメサルタン　メドキソミル錠

Olmesartan Medoxomil Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$: 558.59).

Method of preparation Prepare as directed under Tablets, with Olmesartan Medoxomil.

Identification To a quantity of powdered Olmesartan Medoxomil Tablets, equivalent to 20 mg of Olmesartan Medoxomil, add 60 mL of a mixture of acetonitrile and water (3:2), agitate for 10 minutes with the aid of ultrasonic waves, and add a mixture of acetonitrile and water (3:2) to make 100 mL. Centrifuge this solution, to 5 mL of the supernatant liquid add a mixture of acetonitrile and water (3:2) to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 255 nm and 259 nm.

Purity Related substances—To a quantity of powdered Olmesartan Medoxomil Tablets, equivalent to 20 mg of Olmesartan Medoxomil, add 20 mL of a mixture of acetonitrile and water (9:1), agitate for 15 minutes with the aid of ultrasonic waves, centrifuge, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peaks, having the relative retention time of about 0.2 and about 1.6 to olmesartan medoxomil, obtained from the sample solution is not larger than 3/5 times the peak area of olmesartan medoxomil obtained from the standard solution, and the area of the peak other than olmesartan medoxomil and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of olmesartan medoxomil from the standard solution. Furthermore, the total area of the peaks other than olmesartan medoxomil from the sample solution is not larger than 1.4 times the peak area of olmesartan medoxomil from the standard solution. For these calculations use the areas of the peaks, having the relative retention time of about 0.7 and about 3.4 to olmesartan medoxomil, after multiplying by their relative response factors, 0.65 and 1.39, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2) under Olmesartan Medoxomil.

Time span of measurement: For 45 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile and water (9:1) to make exactly 20 mL. Confirm that the peak area of olmesartan medoxomil obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of olmesartan medoxomil are not less than 5500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of olmesartan medoxomil is not more than 2.0%.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Olmesartan Medoxomil Tablets add $5V/7$ mL of a mixture of acetonitrile and water (3:2) and exactly $V/10$ mL of the internal standard solution. Agitate for 10 minutes with the aid of ultrasonic waves with occasional stirring, and add a mixture of acetonitrile and water (3:2) to make exactly V mL so that each mL contains about 0.2 mg of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$). Centrifuge this solution, to 5 mL of the supernatant liquid add a mixture of acetonitrile and water (3:2) to make 25 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) $= M_S \times Q_T/Q_S \times V/200$

M_S: Amount (mg) of Olmesartan Medoxomil RS, calculated on the anhydrous basis and corrected on the amount of the residual solvent.

Internal standard solution—A solution of isobutyl parahydroxybenzoate in a mixture of acetonitrile and water (3:2) (1 in 1000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rates in 30 minutes of 5-mg, 10-mg and 20-mg tablets are not less than 80%, and that of 40-mg tablet is not less than 75%.

Start the test with 1 tablet of Olmesartan Medoxomil Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 6 μg of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$), and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Olmesartan Medoxomil RS (separately, determine the water <*2.48*> and the residual solvent in the same manner as Olmesartan Medoxomil), dissolve in 15 mL of ethanol (99.5) by warming at 50 – 60°C, and after cooling add ethanol (99.5) to make exactly 20 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 50 mL. Then, pipet 5 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 257 nm of the

sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24> using the dissolution medium as the control.

Dissolution rate (%) with respect to the labeled amount of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45/4$

M_S: Amount (mg) of Olmesartan Medoxomil RS, calculated on the anhydrous basis and corrected on the amount of the residual solvent
C: Labeled amount (mg) of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Olmesartan Medoxomil Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$), add 70 mL of a mixture of acetonitrile and water (3:2) and exactly 10 mL of the internal standard solution. Agitate for 15 minutes with the aid of ultrasonic waves with occasional stirring, and add a mixture of acetonitrile and water (3:2) to make 100 mL. Centrifuge this solution, to 5 mL of the supernatant liquid add a mixture of acetonitrile and water (3:2) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Olmesartan Medoxomil RS (separately determine the water <2.48> and the residual solvent in the same manner as Olmesartan Medoxomil), dissolve in 60 mL of a mixture of acetonitrile and water (3:2), add exactly 20 mL of the internal standard solution, then add a mixture of acetonitrile and water (3:2) to make 100 mL. To 5 mL of this solution add a mixture of acetonitrile and water (3:2) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of olmesartan medoxomil to that of the internal standard.

Amount (mg) of olmesartan medoxomil ($C_{29}H_{30}N_6O_6$)
$= M_S \times Q_T/Q_S \times 1/2$

M_S: Amount (mg) of Olmesartan Medoxomil RS, calculated on the anhydrous basis and corrected on the amount of the residual solvent

Internal standard solution—A solution of isobutyl parahydroxybenzoate in a mixture of acetonitrile and water (3:2) (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.04 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 3.4 with a solution prepared by dissolving 1.73 g of phosphoric acid in water to make 1000 mL. To 330 mL of this solution add 170 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of olmesartan medoxomil is about 16 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, olmesartan medoxomil and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

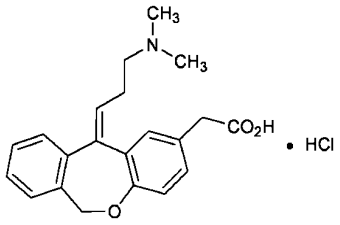
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviations of the ratio of the peak area of olmesartan medoxomil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Olopatadine Hydrochloride

オロパタジン塩酸塩



$C_{21}H_{23}NO_3$.HCl: 373.87
{11-[(1*Z*)-3-(Dimethylamino)propylidene]-6,11-dihydrodibenzo[*b*,*e*]oxepin-2-yl}acetic acid monohydrochloride [*140462-76-6*]

Olopatadine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{21}H_{23}NO_3$.HCl.

Description Olopatadine Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in formic acid, sparingly soluble in water, and very slightly soluble in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

The pH of a solution obtained by dissolving 1.0 g of Olopatadine Hydrochloride in 100 mL of water is 2.3 to 3.3.

Melting point: about 250°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Olopatadine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Olopatadine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry

<2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Olopatadine Hydrochloride (1 in 100) add 1 mL of dilute nitric acid: this solution responds to the Qualitative Tests <1.09> (2) for chloride.

Purity **(1)** Heavy metals <1.07>—Proceed with 2.0 g of Olopatadine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Olopatadine Hydrochloride in 100 mL of a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than olopatadine obtained from the sample solution is not larger than 1/10 times the peak area of olopatadine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 299 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.3 g of sodium lauryl sulfate in a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (11:9) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of olopatadine is about 11 minutes.

Time span of measurement: About 2 times as long as the retention time of olopatadine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (3:2) to make exactly 20 mL. Confirm that the peak area of olopatadine obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of olopatadine are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of olopatadine is not more than 2.0%.

(3) Residual solvent—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Loss on drying <2.41> Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Olopatadine Hydrochloride, previously dried, dissolve in 3 mL of formic acid, add 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.39 mg of $C_{21}H_{23}NO_3$.HCl

Containers and storage Containers—Well-closed containers.

Add the following:

Olopatadine Hydrochloride Tablets

オロパタジン塩酸塩錠

Olopatadine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of olopatadine hydrochloride ($C_{21}H_{23}NO_3$.HCl: 373.87).

Method of preparation Prepare as directed under Tablets, with Olopatadine Hydrochloride.

Identification Shake well a quantity of powdered Olopatadine Hydrochloride Tablets, equivalent to 5 mg of Olopatadine Hydrochloride, with 100 mL of 0.01 mol/L hydrochloric acid TS, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 295 nm and 299 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Olopatadine Hydrochloride Tablets add $4V/5$ mL of a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (3:2). To this solution add exactly $V/10$ mL of the internal standard solution, shake well, and add a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (3:2) to make V mL so that each mL contains about 50 μg of olopatadine hydrochloride ($C_{21}H_{23}NO_3$.HCl). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, and use this filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of olopatadine hydrochloride ($C_{21}H_{23}NO_3$.HCl)
$= M_S \times Q_T/Q_S \times V/1000$

M_S: Amount (mg) of olopatadine hydrochloride for assay

Internal standard solution—A solution of doxepin hydrochloride in a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (3:2) (7 in 20,000).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 15 minutes of Olopatadine Hydrochloride Tablets is not less than 85%.

Start the test with 1 tablet of Olopatadine Hydrochloride Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 2.8 μg of olopatadine hydrochloride ($C_{21}H_{23}NO_3$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of olopatadine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL. Then pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of olopatadine in each solution.

Dissolution rate (%) with respect to the labeled amount of olopatadine hydrochloride ($C_{21}H_{23}NO_3$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of olopatadine hydrochloride for assay
C: Labeled amount (mg) of olopatadine hydrochloride ($C_{21}H_{23}NO_3$.HCl) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of olopatadine are not less than 10,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of olopatadine is not more than 1.5%.

Assay Weigh accurately the mass of not less than 20 Olopatadine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of olopatadine hydrochloride ($C_{21}H_{23}NO_3$.HCl), add 80 mL of a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (3:2), and add exactly 10 mL of the internal standard solution. Shake well for 10 minutes, add a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (3:2) to make 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, and use this filtrate as the sample solution. Separately, weigh accurately about 50 mg of olopatadine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (3:2) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (3:2) to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of olopatadine to that of the internal standard.

Amount (mg) of olopatadine hydrochloride ($C_{21}H_{23}NO_3$.HCl)
$= M_S \times Q_T/Q_S \times 1/10$

M_S: Amount (mg) of olopatadine hydrochloride for assay

Internal standard solution—A solution of doxepin hydrochloride in a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (3:2) (7 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 299 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.3 g of sodium lauryl sulfate in a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (11:9) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of olopatadine is about 11 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, olopatadine and the internal standard are eluted in this order with the resolution between these peaks being not less than 13.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of olopatadine is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Panipenem

パニペネム

Change the Identification and below as follows:

Identification **(1)** Dissolve 20 mg of Panipenem in 2 mL of water, add 1 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2) Determine the absorption spectrum of a solution of Panipenem in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Panipenem as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +55 – +65° (0.1 g calculated on the anhydrous basis and corrected on the amount of the residual solvent, 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0, 10 mL, 100 mm).

pH <*2.54*> Dissolve 0.5 g of Panipenem in 10 mL of water: the pH of the solution is between 4.5 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve 0.30 g of Panipenem in 40 mL of water, and observe immediately: the solution is clear and its absorbance at 400 nm determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*> is not more than 0.4.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Panipenem according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Keep the sample solution at 5°C or below. Dissolve 50 mg of Panipenem in 50 mL of water, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of the peak other than panipenem is not more than 2.0%, and the total amount of the peaks other than panipenem is not more than 6.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized porous glass for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 3.12 g of sodium dihydrogen phosphate dihydrate in 700 mL of water, adjust to pH 8.0 with dilute sodium hydroxide TS, then add water to make 1000 mL, and add 20 mL of acetonitrile.

Mobile phase B: Dissolve 3.12 g of sodium dihydrogen phosphate dihydrate in 700 mL of water, adjust to pH 8.0 with dilute sodium hydroxide TS, then add water to make 750 mL, and add 250 mL of acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 15	100	0
15 – 50	100 → 0	0 → 100

Flow rate: 1.0 mL per minute (the retention time of panipenem is about 16 minutes).

Time span of measurement: For 50 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Use a solution of Panipenem (1 in 100,000) as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add water to make exactly 10 mL. Confirm that the peak area of panipenem obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above conditions, the number of theoretical plates and the symmetry factor of the peak of panipenem are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above conditions, the relative standard deviation of the peak area of panipenem is not more than 2.0%.

(4) Residual solvents—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Water Weigh accurately about 0.5 g of Panipenem, transfer to a 15-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the sample solution. Separately, weigh accurately 2 g of water, and add the internal standard solution to make exactly 100 mL. Pipet 5 mL and 10 mL of this solution, add the internal standard solution to make exactly 20 mL, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with 1 μL of the sample solution and standard solutions (1) and (2) as directed under Gas Chromatography <*2.02*> according to the following condition, and calculate the ratios, Q_T, Q_{S1} and Q_{S2} of the peak area of water to that of the internal standard. Calculate the amount of water by the following formula: water is not

more than 5.0%.

$$\text{Amount of water (\%)} = M_S/M_T \times (Q_T + Q_{S2} - 2Q_{S1})/2(Q_{S2} - Q_{S1}) \times 1/100 \times 100$$

M_S: Amount (g) of water
M_T: Amount (g) of Panipenem

Internal standard solution—A solution of acetonitrile in methanol (1 in 100).
Operating conditions—
Detector: A thermal conductivity detector.
Column: A glass column 3 mm in inside diameter and 2 m in length, packed with porous ethyl vinylbenzene-divinylbenzene copolymer for gas chromatography (150 to 180 μm in particle diameter).
Column temperature: A constant temperature of about 125°C.
Carrier gas: Helium.
Flow rate: Adjust the flow rate so that the retention time of acetonitrile is about 8 minutes.
System suitability—
System performance: When the procedure is run with 1 μL of the standard solution (2) under the above operating conditions, water, methanol, and the internal standard are eluted in this order with the resolution between the peaks of water and internal standard being not less than 10.
System repeatability: When the test is repeated 6 times with 1 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak area of water to that of the internal standard is not more than 5.0%.

Residue on ignition ⟨*2.44*⟩ Not more than 0.5% (1 g).

Assay Conduct this procedure within 30 minutes after preparation of the sample and standard solutions. Weigh accurately an amount of Panipenem and Panipenem RS, equivalent to about 0.1 g (potency), dissolve them separately in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0, to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0, to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of panipenem to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of panipenem } (C_{15}H_{21}N_3O_4S) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Panipenem RS

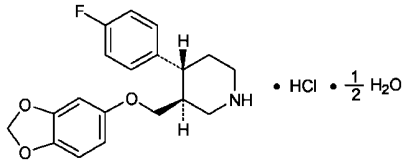
Internal standard solution—A solution of sodium *p*-styrenesulfonate in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 1000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 280 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silicone polymer coated silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: A mixture of 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 8.0 and acetonitrile (50:1).
Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 12 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, panipenem and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of panipenem to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.
Storage—At a temperature not exceeding −10°C.

Add the following:

Paroxetine Hydrochloride Hydrate

パロキセチン塩酸塩水和物



$C_{19}H_{20}FNO_3.HCl.½H_2O$: 374.83
(3*S*,4*R*)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine monohydrochloride hemihydrate
[*110429-35-1*]

Paroxetine Hydrochloride Hydrate contains not less than 98.5% and not more than 101.5% of paroxetine hydrochloride ($C_{19}H_{20}FNO_3.HCl$: 365.83), calculated on the anhydrous basis.

Description Paroxetine Hydrochloride Hydrate occurs as a white crystalline powder.
It is freely soluble in methanol, soluble in ethanol (99.5), and slightly soluble in water.
Optical rotation $[\alpha]_D^{20}$: −83 – −93°(0.1 g calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).
Melting point: about 140°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Paroxetine Hydrochloride Hydrate in ethanol

(99.5) (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Paroxetine Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Paroxetine Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Paroxetine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Paroxetine Hydrochloride Hydrate (1 in 500) responds to the Qualitative Tests <*1.09*> for chloride.

Purity (1) Heavy metals <*1.07*>—Proceed with 1.0 g of Paroxetine Hydrochloride Hydrate according to Method 4, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 30). Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) 4-(4-Fluorophenyl)-1-methyl-1,2,3,6-tetrahydropyridine—Dissolve 0.42 g of Paroxetine Hydrochloride Hydrate in 10 mL of a mixture of water and acetonitrile (4:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (4:1) to make exactly 100 mL. Pipet 1 mL of this solution, and add a mixture of water and acetonitrile (4:1) to make exactly 100 mL. Pipet 2 mL of this solution, add a mixture of water and acetonitrile (4:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 75 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.8 to paroxetine, obtained from the sample solution is not larger than the peak area of paroxetine obtained from the standard solution. For this calculation use the area of the peak, having the relative retention time of about 0.8 to paroxetine, after multiplying by the relative response factor 0.86.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 242 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: Dissolve 30 g of sodium perchlorate monohydrate in 900 mL of water, add 3.5 mL of phosphoric acid, 2.4 mL of triethylamine and water to make 1000 mL, and then adjust to pH 2.0 with phosphoric acid or triethylamine.

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 20	85 → 80	15 → 20
20 – 27	80 → 55	20 → 45
27 – 36	55	45

Flow rate: 1.5 mL per minute.

System suitability—

System performance: When the procedure is run with 75 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of paroxetine are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 75 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paroxetine is not more than 5.0%.

(3) Related substances—Dissolve 20 mg of Paroxetine Hydrochloride Hydrate in 20 mL of a mixture of water and tetrahydrofuran (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and tetrahydrofuran (9:1) to make exactly 100 mL. Pipet 1 mL of this solution, and add a mixture of water and tetrahydrofuran (9:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than paroxetine obtained from the sample solution is not larger than the peak area of paroxetine obtained from the standard solution. For these calculations use the areas of the peaks, having the relative retention time of about 0.29, about 0.66, about 0.73, about 0.85, about 0.91, about 1.14, about 1.51, and about 1.84 to paroxetine, after multiplying by their relative response factors 0.46, 0.82, 1.10, 0.95, 0.93, 0.82, 1.55, and 1.54, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: A mixture of water, tetrahydrofuran and trifluoroacetic acid (180:20:1).

Mobile phase B: A mixture of acetonitrile, tetrahyrofuran and trifluoroacetic acid (180:20:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	80	20
30 – 50	80 → 20	20 → 80
50 – 60	20	80

Flow rate: 1.0 mL per minute.

Time span of measurement: For 60 minutes after injection, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of paroxetine are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paroxetine is not more than 2.0%.

(4) Optical isomer—Dissolve 0.10 g of Paroxetine Hydrochloride Hydrate in 20 mL of methanol, add a solution of sodium chloride (29 in 1000) to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 10 mL of methanol, and add a solution of sodium chloride (29 in 1000) to make exactly 50 mL. Pipet 2 mL of this solution, add 4 mL of methanol, and add a solution of sodium chloride (29 in 1000) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak of the optical isomer, having the relative retention time of about 0.4 to paroxetine, obtained from the sample solution is not larger than the peak area of paroxetine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 295 nm).

Column: A stainless steel column 4 mm in inside diameter and 10 cm in length, packed with α_1-acid glycoprotein-binding silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 18°C.

Mobile phase: A mixture of sodium chloride solution (29 in 1000) and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of paroxetine is about 22 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of paroxetine are not less than 500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paroxetine is not more than 2.0%.

(5) Residual solvent—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Water <*2.48*> 2.0 – 3.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Paroxetine Hydrochloride Hydrate and Paroxetine Hydrochloride RS (separately determine the water <*2.48*> in the same manner as Paroxetine Hydrochloride Hydrate), dissolve them separately in water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paroxetine in each solution.

$$\text{Amount (mg) of paroxetine hydrochloride } (C_{19}H_{20}FNO_3.HCl) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Paroxetine Hydrochloride RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 295 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with trimethylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 3.85 g of ammonium acetate in 1000 mL of water, and adjust to pH 4.5 with acetic acid (100). To 600 mL of this solution, add 400 mL of acetonitrile and 10 mL of triethylamine, then adjust to pH5.5 with acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of paroxetine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of paroxetine are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paroxetine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Paroxetine Hydrochloride Tablets

パロキセチン塩酸塩錠

Paroxetine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of paroxetine ($C_{19}H_{20}FNO_3$: 329.37).

Method of preparation Prepare as directed under Tablets, with Paroxetine Hydrochloride Hydrate.

Identification Powder Paroxetine Hydrochloride Tablets. To a portion of the powder, equivalent to 10 mg of paroxetine ($C_{19}H_{20}FNO_3$), add 140 mL of ethanol (99.5), treat with the aid of ultrasonic waves for 5 minutes, add ethanol (99.5) to make 200 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 233 nm and 237 nm, between 263 nm and 267 nm, between 269 nm and 273 nm, and between 293 nm and 297 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Paroxetine Hydrochloride Tablets add $V/5$ mL of 0.1 mol/L hydrochloric acid TS, disintegrate with the aid of ultrasonic waves for 10 minutes, add $3V/5$ mL of a mixture of water and 2-propanol (1:1), and treat with the ultrasonic waves for 20 minutes. To this solution add a mixture of water and 2-propanol (1:1) to make exactly V mL so that each mL contains about 0.2 mg of paroxetine ($C_{19}H_{20}FNO_3$), filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of paroxetine } (C_{19}H_{20}FNO_3) = M_S \times A_T/A_S \times V/100 \times 0.900$$

M_S: Amount (mg) of Paroxetine Hydrochloride RS, calculated on the anhydrous basis

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of 5-mg and 10-mg tablet is not less than 80%, and of 20-mg tablet is not less than 75%.

Start the test with 1 tablet of Paroxetine Hydrochloride Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 5.6 μg of paroxetine ($C_{19}H_{20}FNO_3$), and use this solution as the sample solution. Separately, weigh accurately about 11 mg of Paroxetine Hydrochloride RS (separately determine the water <*2.48*> in the same manner as Paroxetine Hydrochloride Hydrate), and dissolve in the dissolution medium to make exactly 100 mL. Pipet 3 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paroxetine in each solution.

$$\text{Dissolution rate (\%) with respect to the labeled amount of paroxetine } (C_{19}H_{20}FNO_3) = M_S \times A_T/A_S \times V'/V \times 1/C \times 54 \times 0.900$$

M_S: Amount (mg) of Paroxetine Hydrochloride RS, calculated on the anhydrous basis

C: Labeled amount (mg) of paroxetine ($C_{19}H_{20}FNO_3$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of paroxetine are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paroxetine is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Paroxetine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of paroxetine ($C_{19}H_{20}FNO_3$), add 20 mL of 0.1 mol/L hydrochloric acid TS, treat with the aid of ultrasonic waves for 10 minutes. To this solution add 60 mL of a mixture of water and 2-propanol (1:1), and treat with the aid of ultrasonic waves for 20 minutes. Then add a mixture of water and 2-propanol (1:1) to make exactly 100 mL, filter through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 23 mg of Paroxetine Hydrochloride RS (separately determine the water <*2.48*> in the same manner as Paroxetine Hydrochloride Hydrate), and dissolve in 20 mL of 0.1 mol/L hydrochloric acid TS, add a mixture of water and 2-propanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paroxetine in each solution.

$$\text{Amount (mg) of paroxetine } (C_{19}H_{20}FNO_3) = M_S \times A_T/A_S \times 0.900$$

M_S: Amount (mg) of Paroxetine Hydrochloride RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 295 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with trimethylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 3.85 g of ammonium acetate in 1000 mL of water, and adjust to pH 4.5 with acetic acid (100). To 600 mL of this solution, add 400 mL of acetonitrile and 10 mL of triethylamine, then adjust to pH 5.5 with acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of paroxetine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of paroxetine are not less than 5000 and not more than 3.0, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paroxetine is not more than 2.0%.

Containers and storage Containers—Well-closed containers.

Peplomycin Sulfate

ペプロマイシン硫酸塩

Change the Identification and Assay as follows:

Identification **(1)** To 4 mg of Peplomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Peplomycin Sulfate RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Peplomycin Sulfate as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Peplomycin Sulfate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 10 mg each of Peplomycin Sulfate and Peplomycin Sulfate RS in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: the retention time of the principal peak obtained from the sample solution is the same as that obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase stock solution, mobile phase A, mobile phase B, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (3).

(4) A solution of Peplomycin Sulfate (1 in 200) responds to the Qualitative Tests <*1.09*> (1) and (2) for sulfate.

Assay Weigh accurately an amount of Peplomycin Sulfate and Peplomycin Sulfate RS, both previously dried, equivalent to about 50 mg (potency), dissolve them separately in the mobile phase to make exactly 100 mL. Pipet 4 mL each of these solutions, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 1 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of peplomycin to that of the internal standard.

Amount [μg (potency)] of peplomycin sulfate ($C_{61}H_{88}N_{18}O_{21}S_2.H_2SO_4$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Peplomycin Sulfate RS

Internal standard solution—A solution of 1-aminonaphthalene in mobile phase (1 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (2.2 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 0.96 g of sodium 1-pentane sulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water, add 5 mL of acetic acid (100), and adjust to pH 4.3 with ammonia TS. To 650 mL of this solution add 350 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of peplomycin is about 3 minutes.

System suitability—

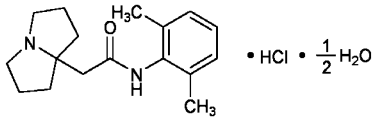
System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, peplomycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of peplomycin to that of the internal standard is not more than 1.0%.

Add the following:

Pilsicainide Hydrochloride Hydrate

ピルシカイニド塩酸塩水和物



$C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$: 317.85

N-(2,6-Dimethylphenyl)tetrahydro-1*H*-pyrrolizin-7a(5*H*)-ylacetamide monohydrochloride hemihydrate [*88069-49-2*, anhydride]

Pilsicainide Hydrochloride Hydrate contains not less than 99.0% and not more than 101.0% of $C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$.

Description Pilsicainide Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is very soluble in acetic acid (100), and freely soluble in water, in methanol and in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification **(1)** Determine the absorption spectrum of a solution of Pilsicainide Hydrochloride Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pilsicainide Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pilsicainide Hydrochloride Hydrate (1 in 100) responds to the Qualitative Tests <*1.09*> (2) for chloride.

pH <*2.54*> Dissolve 1.0 g of Pilsicainide Hydrochloride Hydrate in 50 mL of water: the pH of this solution is between 5.3 and 6.1.

Melting point <*2.60*> 210.5 – 213.5°C (Heat the bath to 160°C in advance).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Pilsicainide Hydrochloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 40 mg of Pilsicainide Hydrochloride Hydrate in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 20 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks other than pilsicainide obtained from the sample solution is not larger than the peak area of pilsicainide obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 750 mL of water add 5 mL of triethylamine, adjust to pH 4.0 with phosphoric acid, and add water to make 1000 mL. To this solution add 200 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of pilsicainide is about 5 minutes.

Time span of measurement: About 5 times as long as the retention time of pilsicainide, beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pilsicainide are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pilsicainide is not more than 2.0%.

(3) Residual solvents—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Water <*2.48*> 2.5 – 3.3% (50 mg, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Pilsicainide Hydrochloride Hydrate, dissolve it in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <*2.50*> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.79 mg of $C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$

Containers and storage Containers—Tight containers.

Add the following:

Pilsicainide Hydrochloride Capsules

ピルシカイニド塩酸塩カプセル

Pilsicainide Hydrochloride Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$: 317.85).

Method of preparations Prepare as directed under Capsules, with Pilsicainide Hydrochloride Hydrate.

Identification Take out the contents of Pilsicainide Hydrochloride Capsules, to a quantity of the content, equivalent to 50 mg of Pilsicainide Hydrochloride Hydrate, add 10 mL of water, and shake well. Centrifuge this solution, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 μm. To 1 mL of the filtrate, add 1 mL of 1 mol/L hydrochloric acid TS and 8 mL of water. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 261 nm and 265 nm, and between 268 nm and 272 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Pilsicainide Hydrochloride Capsules, add water, and shake to disperse the content of the capsule uniformly while warming in a water bath. After cooling, add exactly V mL of the internal standard solution so that 0.2 mL of the internal standard solution is added for each mg of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$), then, add water so that each mL contains about 0.5 mg of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$). To 5 mL of this solution, add water to make 50 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$)
$= M_S \times Q_T/Q_S \times V/10$

M_S: Amount (mg) of pilsicainide hydrochloride hydrate for assay

Internal Standard Solution—Dissolve 2.5 g of lidocaine for assay in 20 mL of 0.5 mol/L hydrochloric acid TS, and add water to make 1000 mL.

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of water as the dissolution medium, the dissolution rate in 30 minutes of Pilsicainide Hydrochloride Capsules is not less than 85%.

Start the test with 1 capsule of Pilsicainide Hydrochloride Capsules, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 28 μg of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of pilsicainide hydrochloride hydrate for assay, dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of pilsicainide in each solution.

Dissolution rate (%) with respect to the labeled amount of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of pilsicainide hydrochloride hydrate for assay

C: Labeled amount (mg) of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$) in 1 capsule

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pilsicainide are not less than 4000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pilsicainide is not more than 1.0%.

Assay Take out the contents of not less than 20 Pilsicainide Hydrochloride Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$), add 50 mL of water and shake well. After adding exactly 10 mL of the internal standard solution, add water to make 100 mL. To 5 mL of this solution add water to make 50 mL, and filter the solution. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of pilsicainide hydrochloride hydrate for assay, dissolve in exactly 10 mL of the internal standard solution, and add water to make 100 mL. To 5 mL of this solution add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pilsicainide to that of the internal standard.

Amount (mg) of pilsicainide hydrochloride hydrate ($C_{17}H_{24}N_2O.HCl.\frac{1}{2}H_2O$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of pilsicainide hydrochloride hydrate for assay

Internal Standard Solution—Dissolve 2.5 g of lidocaine for assay in 20 mL of 0.5 mol/L hydrochloric acid TS, and add water to make 1000 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of around 40°C.

Mobile phase: To 750 mL of water add 5 mL of triethylamine, adjust the pH to 4.0 with phosphoric acid, and add water to make 1000 mL. To this solution, add 200 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of pilsicainide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and pilsicainide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pilsicainide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets

ピオグリタゾン塩酸塩·メトホルミン塩酸塩錠

Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$: 392.90) and metformin hydrochloride ($C_4H_{11}N_5.HCl$: 165.62).

Method of preparation Prepare as directed under Tablets, with Pioglitazone Hydrochloride and Metformin Hydrochloride.

Identification **(1)** Shake vigorously a quantity of powdered Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets, equivalent to 0.33 mg of Pioglitazone Hydrochloride, with 10 mL of water, and filter through a membrane filter with a pore size not exceeding 0.45 μm. After washing the membrane filter with 10 mL of water, dissolve the retained substance on the filter by running through 10 mL of 0.1 mol/L hydrochloric acid TS, and determine the absorption spectrum of the filtrate so obtained as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 267 nm and 271 nm.

(2) Shake vigorously a quantity of powdered Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets, equivalent to 20 mg of Metformin Hydrochloride, with 50 mL of water, and filter through a membrane filter with a pore size not exceeding 0.45 μm. To 1 mL of the filtrate add water to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 230 nm and 234 nm.

Uniformity of dosage units <*6.02*> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

(1) Pioglitazone hydrochloride—To 1 tablet of Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets add 40 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously for 10 minutes, add 40 mL of methanol, and shake. To this solution add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add exactly $V'/20$ mL of the internal standard solution, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly V' mL so that each mL contains about 16.5 μg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$), and use this solution as the sample solution. Then, proceed as directed in the Assay (1).

Amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$)
$= M_S \times Q_T/Q_S \times V'/V \times 1/20$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) (1 in 2500).

(2) Metformin hydrochloride—To 1 tablet of Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets add 40 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously for 10 minutes, add 40 mL of methanol, and shake. To this solution add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add exactly $V'/20$ mL of the internal standard solution, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly V' mL so that each mL contains about 0.25 mg of met-

formin hydrochloride ($C_4H_{11}N_5$.HCl), and use this solution as the sample solution. Then, proceed as directed in the Assay (2).

Amount (mg) of metformin hydrochloride ($C_4H_{11}N_5$.HCl)
$= M_S \times Q_T/Q_S \times V'/V \times 1/2$

M_S: Amount (mg) of metformin hydrochloride for assay

Internal standard solution—A solution of 4′-methoxyacetophenone in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) (1 in 2000).

Dissolution ⟨*6.10*⟩ **(1)** Pioglitazone hydrochloride—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution, prepared by mixing 50 mL of 0.2 mol/L hydrochloric acid TS and 150 mL of potassium chloride solution (3 in 20), adding water to make 1000 mL and adjusting to pH 2.0 with 5 mol/L hydrochloric acid TS, as the dissolution medium, the dissolution rate in 30 minutes of Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 18.4 μg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 37 mg of Pioglitazone Hydrochloride RS (separately, determine the water ⟨*2.48*⟩ in the same manner as Pioglitazone Hydrochloride), and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of pioglitazone in each solution.

Dissolution rate (%) with respect to the labeled amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis
C: Labeled amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay (1).
System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pioglitazone are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pioglitazone is not more than 1.0%.

(2) Metformin hydrochloride—When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of the dissolution medium used in (1), the dissolution rate in 30 minutes of Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 0.56 mg of metformin hydrochloride ($C_4H_{11}N_5$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of metformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in the dissolution medium to make exactly 50 mL, use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of metformin in each solution.

Dissolution rate (%) with respect to the labeled amount of metformin hydrochloride ($C_4H_{11}N_5$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 1800$

M_S: Amount (mg) of metformin hydrochloride for assay
C: Labeled amount (mg) of metformin hydrochloride ($C_4H_{11}N_5$.HCl) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay (2).
System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of metformin are not less than 6000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metformin is not more than 1.0%.

Assay **(1)** Pioglitazone hydrochloride—Weigh accurately the mass of not less than 20 Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 33 mg of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S$.HCl), add 40 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously for 10 minutes, add 40 mL of methanol, and shake. Add a mix-

ture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 33 mg of Pioglitazone Hydrochloride RS (separately determine the water <2.48> in the same manner as Pioglitazone Hydrochloride), and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pioglitazone to that of the internal standard.

Amount (mg) of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S.HCl$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Pioglitazone Hydrochloride RS, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.2 g of sodium lauryl sulfate in 1000 mL of a mixture of a solution of ammonium dihydrogen-phosphate (23 in 4000) and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of pioglitazone is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, pioglitazone and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pioglitazone to that of the internal standard is not more than 1.0%.

(2) Metformin hydrochloride—Weigh accurately the mass of not less than 20 Pioglitazone Hydrochloride and Metformin Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.5 g of metformin hydrochloride ($C_4H_{11}N_5.HCl$), add 40 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously for 10 minutes, add 40 mL of methanol, and shake. Add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of metformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make exactly 10 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of metformin to that of the internal standard.

Amount (mg) of metformin hydrochloride ($C_4H_{11}N_5.HCl$)
$= M_S \times Q_T/Q_S \times 10$

M_S: Amount (mg) of metformin hydrochloride for assay

Internal standard solution—A solution of 4′-methoxyacetophenone in a mixture of 0.1 mol/L hydrochloric acid TS and methanol (1:1) (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 255 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.2 g of sodium lauryl sulfate in 1000 mL of a mixture of a solution of ammonium dihydrogen-phosphate (23 in 4000) and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of metformin is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, metformin and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of metformin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Piroxicam

ピロキシカム

Change the Description as follows:

Description Piroxicam occurs as a white to pale yellow crystalline powder.

It is slightly soluble in acetonitrile and in ethanol (99.5), and practically insoluble in water.

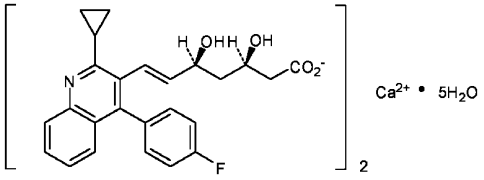
Melting point: about 200°C (with decomposition).

It shows crystal polymorphism.

Add the following:

Pitavastatin Calcium Hydrate

ピタバスタチンカルシウム水和物



$C_{50}H_{46}CaF_2N_2O_8.5H_2O$: 971.06

Monocalcium bis{(3*R*,5*S*,6*E*)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3,5-dihydroxyhept-6-enoate} pentahydrate

[*147526-32-7*, anhydride]

Pitavastatin Calcium Hydrate contains not less than 98.0% and not more than 102.0% of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$: 880.98), calculated on the anhydrous basis.

Description Pitavastatin Calcium Hydrate occurs as a white to pale yellow powder.

It is slightly soluble in methanol, very slightly soluble in water and in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Pitavastatin Calcium Hydrate in methanol (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pitavastatin Calcium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of 3400 – 3300 cm^{-1}, about 1560 cm^{-1}, 1490 cm^{-1}, 1219 cm^{-1}, 1066 cm^{-1} and 766 cm^{-1}.

(3) Dissolve 0.25 g of Pitavastatin Calcium Hydrate in 5 mL of dilute hydrochloric acid, neutralize with ammonia TS, and filter: the filtrate responds to the Qualitative Tests <*1.09*> (1), (2), and (3) for calcium.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +22.0 – +24.5° (0.1 g calculated on the anhydrous basis, a mixture of water and acetonitrile (1:1), 10 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—To 1.0 g of Pitavastatin Calcium Hydrate in a quartz crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) and mix well, then fire the ethanol to burn, and heat gradually to carbonize. After cooling, moisten the residue with 1.5 mL of sulfuric acid, heat carefully, then ignite at 550°C until the residue is incinerated. After cooling, moisten the residue with 1.5 mL of nitric acid, heat carefully, then ignite at 550°C until the residue is completely incinerated. After cooling, dissolve the residue in 3 mL of hydrochloric acid, and evaporate the solvent to dryness on a water bath. Moisten the residue with 3 drops of hydrochloric acid, dissolve in 10 mL of hot water with the aid of gentle heat, and filter. Wash the residue with 20 mL of water, and pour the filtrates and washings into a Nessler tube. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, then add 2 mL of dilute acetic acid, add water to make 50 mL, and use this solution as the test solution. The control solution is prepared as follows: Take 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. Hereafter, proceed as for the test solution, then add 2.0 mL of Standard Lead Solution, 2 mL of acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Related substances—Conduct this procedure using light-resistant vessels. Dissolve 0.10 g of Pitavastatin Calcium Hydrate in 100 mL of a mixture of acetonitrile and water (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 1.1 to pitavastatin, obtained from the sample solution is not more than 1/2 times the peak area of pitavastatin obtained from the standard solution, and the area of the peak other than pitavastatin and the peak, having the relative retention time of about 1.1, from the sample solution is not more than 1/10 times the peak area of pitavastatin from the standard solution. Furthermore, the total area of the peaks other than pitavastatin from the sample solution is not larger than the peak area of pitavastatin from the standard solution. For this calculation, use the area of the peak, having the relative retention time of about 1.4 to pitavastatin, after multiplying by the relative response factor, 1.8.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: To 10 mL of dilute acetic acid add water to make 1000 mL. To 800 mL of this solution add diluted so-

dium acetate TS (1 in 100) to adjust to pH 3.8.

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 20	60	40
20 – 40	60 → 10	40 → 90
40 – 60	10	90

Flow rate: Adjust the flow rate so that the retention time of pitavastatin is about 23 minutes.

Time span of measurement: About 2.5 times as long as the retention time of pitavastatin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add a mixture of acetonitrile and water (3:2) to make exactly 20 mL. Confirm that the peak area of pitavastatin obtained with 10 μL of this solution is equivalent to 4 to 6% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pitavastatin are not less than 17,000 and not more than 1.3, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pitavastatin is not more than 2.0%.

(3) Residual solvent—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Water ⟨*2.48*⟩ 9.0 – 13.0% (0.2 g, volumetric titration, direct titration. Use a mixture of pyridine for water determination and ethylene glycol for Karl Fischer method (83:17) instead of methanol for water determination).

Assay Conduct this procedure using light-resistant vessels.

Weigh accurately about 0.1 g of Pitavastatin Calcium Hydrate, dissolve in a mixture of acetonitrile and water (3:2) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of acetonitrile and water (3:2) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Pitavastatin Methylbenzylamine RS (separately determine the water ⟨*2.48*⟩ by coulometric titration using 0.1 g), dissolve in a mixture of acetonitrile and water (3:2) to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add a mixture of acetonitrile and water (3:2) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pitavastatin to that of the internal standard.

Amount (mg) of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$) $= M_S \times Q_T/Q_S \times 4 \times 0.812$

M_S: Amount (mg) of Pitavastatin Methylbenzylamine RS, calculated on the anhydrous basis

Internal standard solution—Butyl parahydroxybenzoate in a mixture of acetonitrile and water (3:2) (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 10 mL of dilute acetic acid add water to make 1000 mL. To 350 mL of this solution add 650 mL of methanol, and dissolve 0.29 g of sodium chloride in this solution.

Flow rate: Adjust the flow rate so that the retention time of pitavastatin is about 17 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and pitavastatin are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pitavastatin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Pitavastatin Calcium Tablets

ピタバスタチンカルシウム錠

Pitavastatin Calcium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$: 880.98).

Method of preparation Prepare as directed under Tablets, with Pitavastatin Calcium Hydrate.

Identification Powder Pitavastatin Calcium Tablets. Weigh a portion of the powder, equivalent to 4 mg of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$), add 10 mL of methanol and shake well, and centrifuge. To 1 mL of the su-

pernatant liquid, add methanol to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 242 nm and 246 nm.

Purity Related substances—Conduct this procedure using light-resistant vessels. Take a quantity of Pitavastatin Calcium Tablets, equivalent to 20 mg of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$), add 60 mL of a mixture of acetonitrile and water (3:2), and disintegrate the tablets with the aid of ultrasonic waves. To this dispersed solution, add a mixture of acetonitrile and water (3:2) to make 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the peaks by the area percentage method: the amount of the peak, having the relative retention time of about 1.1 and about 1.7 to pitavastatin, obtained from sample solution is not more than 0.5%, the amount of the peak other than pitavastatin and the peaks mentioned above is not more than 0.1%, and the total amount of the peaks other than pitavastatin is not more than 1.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: To 10 mL of dilute acetic acid add water to make 1000 mL. To 800 mL of this solution add diluted sodium acetate TS (1 in 100) to adjust to pH 3.8.

Mobile phases B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 20	60	40
20 – 40	60 → 30	40 → 70
40 – 65	30	70

Flow rate: Adjust the flow rate so that the retention time of pitavastatin is about 23 minutes.

Time span of measurement: About 2.7 times as long as the retention time of pitavastatin, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add a mixture of acetonitrile and water (3:2) to make 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add a mixture of acetonitrile and water (3:2) to make exactly 50 mL. Confirm that the peak area of pitavastatin obtained with 50 μL of this solution is equivalent to 7 to 13% of that obtained with 50 μL of the solution for system suitability test.

System performance: When the procedure is run with 50 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pitavastatin are not less than 7500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of pitavastatin is not more than 2.0%.

Uniformity of dosage unit <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Pitavastatin Calcium Tablets add exactly V mL of the internal standard solution so that each mL contains about 0.2 mg of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$), and add V mL of a mixture of acetonitrile and water (3:2), shake well until the tablet is disintegrated completely. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of pitavastatin calcium } (C_{50}H_{46}CaF_2N_2O_8) = M_S \times Q_T/Q_S \times V/100 \times 0.812$$

M_S: Amount (mg) of Pitavastatin Methylbenzylamine RS, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of acetonitrile and water (3:2) (3 in 10,000).

Dissolution <6.10> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 15 minutes of Pitavastatin Calcium Tablets is not less than 85%.

Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Pitavastatin Calcium Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard not less than 5 mL of the first filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 1.1 μg of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$), and use this solution as the sample solution. Separately, weigh accurately about 24 mg of Pitavastatin Methylbenzylamine RS (separately determine the water), and dissolve in a mixture of acetonitrile and water (3:2) to make exactly 200 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following

conditions, and determine the peak areas, A_T and A_S, of pitavastatin in each solution.

Dissolution rate (%) with respect to the labeled amount of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/2 \times 0.812$

M_S: Amount (mg) of Pitavastatin Methylbenzylamine RS, calculated on the anhydrous basis

C: Labeled amount (mg) of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pitavastatin are not less than 4500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pitavastatin is not more than 2.0%.

Assay Conduct this procedure using light-resistant vessels. Weigh accurately the mass of not less than 20 Pitavastatin Calcium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$), add 30 mL of a mixture of acetonitrile and water (3:2), and treat with the ultrasonic waves for 10 minutes. To this solution, add a mixture of acetonitrile and water (3:2) to make exactly 50 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Pipet 5 mL of this filtrate, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 24 mg of Pitavastatin Methylbenzylamine RS (separately determine the water <2.48> by coulometric titration using 0.1 g), and dissolve in a mixture of acetonitrile and water (3:2) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pitavastatin to that of the internal standard.

Amount (mg) of pitavastatin calcium ($C_{50}H_{46}CaF_2N_2O_8$)
$= M_S \times Q_T/Q_S \times 1/2 \times 0.812$

M_S: Amount (mg) of Pitavastatin Methylbenzylamine RS, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of acetonitrile and water (3:2) (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 10 mL of dilute acetic acid add water to make 1000 mL. To 350 mL of this solution add 650 mL of methanol, and dissolve 0.29 g of sodium chloride in this solution.

Flow rate: Adjust the flow rate so that the retention time of pitavastatin is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and pitavastatin are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pitavastatin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Change the following as follows:

Polysorbate 80

ポリソルベート**80**

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Polysorbate 80 is a mixture of partial esters of fatty acids, mainly oleic acid, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

Description Polysorbate 80 is a colorless or brownish yellow, clear or slightly opalescent, oily liquid.

It is miscible with water, with methanol, with ethanol (99.5) and with ethyl acetate.

It is practically insoluble in fatty oils and in liquid paraffin.

Viscosity: about 400 mPa·s (25°C).

Specific gravity d_{20}^{20}: about 1.10

Identification It meets the requirements of the Composition of fatty acids.

Composition of fatty acids Dissolve 0.10 g of Polysorbate 80 in 2 mL of a solution of sodium hydroxide in methanol (1 in 50) in a 25-mL conical flask, and boil under a reflux condenser for 30 minutes. Add 2.0 mL of boron trifluoride-

methanol TS through the condenser, and boil for 30 minutes. Add 4 mL of heptane through the condenser, and boil for 5 minutes. After cooling, add 10.0 mL of saturated sodium chloride solution, shake for about 15 seconds, and add a quantity of saturated sodium chloride solution such that the upper phase is brought into the neck of the flask. Collect 2 mL of the upper phase, wash with three 2-mL portions of water, dry with anhydrous sodium sulfate, and use this solution as the sample solution. Perform the test with 1 μL each of the sample solution and fatty acid methyl esters mixture TS as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. Identify each peaks obtained with the sample solution using the chromatogram obtained with fatty acid methyl esters mixture TS. Determine each peak area with the sample solution by the automatic integration method, and calculate the composition of fatty acids by the area percentage method: myristic acid is not more than 5.0%, palmitic acid is not more than 16.0%, palmitoleic acid is not more than 8.0%, stearic acid is not more than 6.0%, oleic acid is not less than 58.0%, linoleic acid is not more than 18.0% and linolenic acid is not more than 4.0%.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated the inside surface with polyethylene glycol 20 M for gas chromatography 0.5 μm in thickness.

Column temperature: Inject at a constant temperature of about 80°C, rise the temperature at the rate of 10°C per minute to 220°C, and maintain at 220°C for 40 minutes.

Injection port temperature: A constant temperature of about 250°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium.

Flow rate: 50 cm per second.

System suitability—

Test for required detectability: Dissolve 0.50 g of the mixture of fatty acid methyl esters described in the following table in heptane to make 50.0 mL, and use this solution as the solution for system suitability test. To 1.0 mL of the solution for system suitability test add heptane to make 10.0 mL. When the procedure is run with 1 μL of this solution under the above operating conditions, the SN ratio of methyl myristate is not less than 5.

Mixture of fatty acid methyl esters	Composition (%)
Methyl myristate for gas chromatography	5
Methyl palmitate for gas chromatography	10
Methyl stearate for gas chromatography	15
Methyl arachidate for gas chromatography	20
Methyl oleate for gas chromatography	20
Methyl eicosenoate for gas chromatography	10
Methyl behenate	10
Methyl lignocerate for gas chromatography	10

System performance: When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, ◆methyl stearate and methyl oleate are eluted in this order,◆ the resolution between these peaks is not less than 1.8, and the number of theoretical plates of the peak of methyl stearate is not less than 30,000.

◆**Acid value** ⟨*1.13*⟩ Not more than 2.0 (using ethanol (95) instead).◆

Saponification value Introduce about 4 g of Polysorbate 80 into a 250-mL borosilicate glass flask. Add exactly 30 mL of 0.5 mol/L potassium hydroxide-ethanol VS and a few glass beads. Attach a reflux condenser, and heat for 60 minutes. Add 1 mL of phenolphthalein TS and 50 mL of ethanol (99.5), and titrate ⟨*2.50*⟩ immediately with 0.5 mol/L hydrochloric acid VS. Perform a blank determination in the same manner. Calculate the saponification value by the following equation: 45 – 55.

$$\text{Saponification value} = (a - b) \times 28.05/M$$

M: Amount (g) of sample
a: Volume (mL) of 0.5 mol/L hydrochloric acid VS required for blank determination
b: Volume (mL) of 0.5 mol/L hydrochloric acid VS required for sample determination

Hydroxyl value Introduce about 2 g of Polysorbate 80 into a 150-mL round bottom flask, add exactly 5 mL of acetic anhydride-pyridine TS, and attach an air condenser. Heat the flask in a water bath for 1 hour keeping the level of the water about 2.5 cm above the level of the liquid in the flask. Withdraw the flask and allow to cool. Add 5 mL of water through the condenser. If a cloudiness appears add sufficient pyridine to clear it, noting the volume added. Shake the flask, and heat in the water bath for 10 minutes. Withdraw the flask and allow to cool. Rinse the condenser and the walls of the flask with 5 mL of neutralized ethanol, and titrate ⟨*2.50*⟩ with 0.5 mol/L potassium hydroxide-ethanol VS (indicator: 0.2 mL of phenolphthalein TS). Perform a blank determination in the same manner. Calculate the hydroxyl value by the following equation: 65 – 80.

$$\text{Hydroxyl value} = (a - b) \times 28.05/M + \text{acid value}$$

M: Amount (g) of sample
a: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS required for blank determination

b: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS required for sample determination

Purity ◆**(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Polysorbate 80 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).◆

(2) Ethylene oxide and 1,4-dioxane—Transfer exactly 1.00 g of Polysorbate 80 into a 10-mL headspace vial, add exactly 2 mL of water, seal the vial immediately with a septum of silicon rubber coated the surface with fluororesin and an aluminum cap. Mix carefully, and use the content as the sample solution. Separately, pipet 0.5 mL of a solution, prepared by dissolving ethylene oxide in dichloromethane so that each mL contains 50 mg, and add water to make exactly 50 mL. Allow to stand to reach room temperature. Pipet 1 mL of this solution, add water to make exactly 250 mL, and use this solution as ethylene oxide stock solution. Separately, pipet 1 mL of 1,4-dioxane, add water to make exactly 200 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as 1,4-dioxane stock solution. To exact 6 mL of ethylene oxide stock solution and exact 2.5 mL of 1,4-dioxane stock solution add water to make exactly 25 mL, and use this solution as ethylene oxide-1,4-dioxane standard stock solution. Separately, transfer exactly 1.00 g of Polysorbate 80 into a 10-mL headspace vial, add exactly 2 mL of ethylene oxide-1,4-dioxane standard stock solution, seal the vial immediately with a septum of silicon rubber coated the surface with fluororesin and an aluminum cap. Mix carefully, and use the content as the standard solution. Perform the test with the sample solution and standard solution as directed in the head-space method under Gas Chromatography ⟨*2.02*⟩ according to the following conditions. The amounts of ethylene oxide and 1,4-dioxane, calculated by the following equations, are not more than 1 ppm and not more than 10 ppm, respectively.

$$\text{Amount (ppm) of ethylene oxide} = 2 \times C_{EO} \times A_a/(A_b - A_a)$$

C_{EO}: Concentration (μg/mL) of added ethylene oxide in the standard solution

A_a: Peak area of ethylene oxide obtained with the sample solution

A_b: Peak area of ethylene oxide obtained with the standard solution

$$\text{Amount (ppm) of 1,4-dioxane} = 2 \times 1.03 \times C_D \times A'_a/(A'_b - A'_a)$$

C_D: Concentration (μg/mL) of added 1,4-dioxane in the standard solution

1.03: Density (g/mL) of 1,4-dioxane

A'_a: Peak area of 1,4-dioxane obtained with the sample solution

A'_b: Peak area of 1,4-dioxane obtained with the standard solution

Head-space injection conditions—

Equilibration temperature in vial: A constant temperature of about 80°C.

Equilibration time in vial: 30 minutes.

Carrier gas: Helium.

Injection volume of sample: 1.0 mL.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.53 mm in inside diameter and 50 m in length, coated the inside surface with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography 5 μm in thickness.

Column temperature: Inject at a constant temperature of about 70°C, rise the temperature at the rate of 10°C per minute to 250°C, and maintain the temperature at 250°C for 5 minutes.

Injection port temperature: A constant temperature of about 85°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium.

Flow rate: 4.0 mL per minute.

Split ratio: 1:3.5.

System suitability—

System performance: Introduce 0.100 g of acetaldehyde in a 100-mL volumetric flask, and add water to make 100 mL. To exact 1 mL of this solution add water to make exactly 100 mL. Transfer exactly 2 mL of this solution and exactly 2 mL of ethylene oxide stock solution into a 10-mL headspace vial, seal the vial immediately with a fluororesin coated silicon septum and an aluminum cap. Mix carefully, and use the content as the solution for system suitability test. When perform the test with ◆the standard solution and◆ the solution for system suitability test under the above conditions, acetaldehyde, ethylene oxide and 1,4-dioxane are eluted in this order, and the resolution between the peaks of acetaldehyde and ethylene oxide is not less than 2.0.

(3) Peroxide value—Introduce about 10 g of Polysorbate 80, accurately weighed, into a 100-mL beaker, dissolve in 20 mL of acetic acid (100). Add 1 mL of saturated potassium iodide solution and allow to stand for 1 minute. Add 50 mL of fleshly boiled and cooled water, and titrate ⟨*2.50*⟩ with 0.01 mol/L sodium thiosulfate VS, while stirring with a magnetic stirrer (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction. Calculate peroxide value by the following equation: not more than 10.0.

$$\text{Peroxide value} = (a - b) \times 10/M$$

M: Amount (g) of sample

a: Volume (mL) of 0.01 mol/L sodium thiosulfate VS required for sample determination

b: Volume (mL) of 0.01 mol/L sodium thiosulfate VS required for blank determination

Water ⟨*2.48*⟩ Not more than 3.0% (1 g, volumetric titration, direct titration).

Residue on ignition Heat a quartz or platinum crucible to redness for 30 minutes, allow to cool in a desiccator (silica gel or other appropriate desiccants), and weigh accurately. Evenly distribute 2.00 g of Polysorbate 80 in the crucible,

dry at 100 – 105°C for 1 hour, ◆and gradually heat with as lower temperature as possible to carbonize completely.◆ Then after igniting to constant mass in an electric furnace at 600 ± 25°C, allow the crucible to cool in a desiccator, and weigh the mass accurately. Flames should not be produced at any time during the procedure. If after prolonged ignition the ash still contains black particles, take up the ash with hot water, filter through a filter paper for quantitative analysis, and ignite the residue and the filter paper. Combine the filtrate with the ash, carefully evaporate to dryness, and ignite to constant mass: not more than 0.25%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Potato Starch

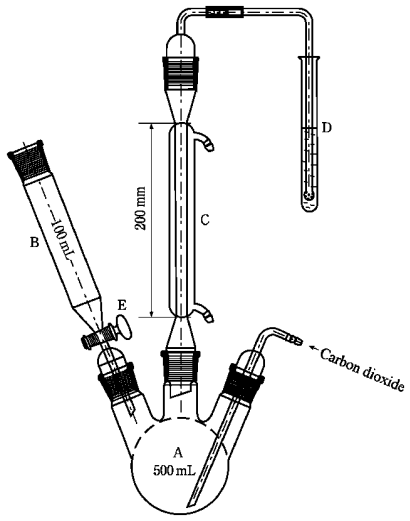
バレイショデンプン

Change the Purity (3) as follows:

Purity

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.



A: Three-necked round-bottom flask (500 mL)
B: Cylindrical dropping funnel (100 mL)
C: Condenser
D: Test tube
E: Tap

(ii) Procedure Introduce 150 mL of water into the three-necked round-bottom flask, close the tap of the cylindrical dropping funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Potato Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

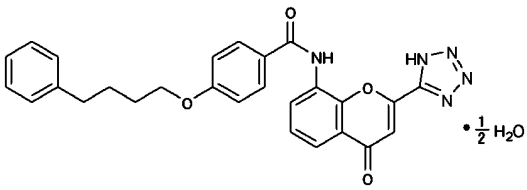
$$\text{Amount (ppm) of sulfur dioxide} = V/M \times 1000 \times 3.203$$

M: Amount (g) of Potato Starch
V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

Add the following:

Pranlukast Hydrate

プランルカスト水和物



$C_{27}H_{23}N_5O_4.½H_2O$: 490.51
N-[4-Oxo-2-(1*H*-tetrazol-5-yl)–4*H*-chromen-8-yl]-4-(4-phenylbutyloxy)benzamide hemihydrate
[*150821-03-7*]

Pranlukast Hydrate contains not less than 98.0% and not more than 101.0% of pranlukast ($C_{27}H_{23}N_5O_4$: 481.50), calculated on the anhydrous basis.

Description Pranlukast Hydrate occurs as a white to light yellow, crystalline powder.

It is very slightly soluble in ethanol (99.5), and practically insoluble in water.

Melting point: about 233°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Pranlukast Hydrate in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pranlukast RS prepared in the same manner as the sample solution: both

spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pranlukast Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Pranlukast RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Suspend 1.0 g of Pranlukast Hydrate in 10 mL of *N*,*N*-dimethylformamide, proceed according to Method 4, and perform the test. Prepare the control solution with 10 mL of *N*,*N*-dimethylformamide in the same manner as preparation of the test solution, and add 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Suspend 1.0 g of Pranlukast Hydrate in 10 mL of *N*,*N*-dimethylformamide, then proceed according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 20 mg of Pranlukast Hydrate in 50 mL of a mixture of acetonitrile and dimethylsulfoxide (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and dimethylsulfoxide (3:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time about 1.5 to pranlukast, obtained from the sample solution is not larger than 1/2 times that of pranlukast obtained from the standard solution, the area of the peak other than pranlukast and the peak mentioned above from the sample solution is not larger than 1/5 times that of pranlukast from the standard solution, and the total area of the peaks other than pranlukast from the sample solution is not larger than the peak area of pranlukast from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of pranlukast, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, add a mixture of acetonitrile and dimethylsulfoxide (3:1) to make exactly 50 mL. Confirm that the peak area of pranlukast obtained with 10 μL of this solution is equivalent to 7 to 13% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pranlukast are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pranlukast is not more than 2.0%.

(4) Residual solvent—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Water <*2.48*> 1.5 – 2.2% (50 mg, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 20 mg each of Pranlukast Hydrate and Pranlukast RS (separately determine the water <*2.48*> in the same manner as Pranlukast Hydrate), dissolve them separately in a mixture of acetonitrile and dimethylsulfoxide (3:1) to make exactly 50 mL. To exactly 5 mL each of these solutions add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 4 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pranlukast to that of the internal standard.

$$\text{Amount (mg) of pranlukast } (C_{27}H_{23}N_5O_4) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Pranlukast RS, calculated on the anhydrous basis

Internal standard solution—A solution of isoamyl parahydroxybenzoate in a mixture of acetonitrile and dimethylsulfoxide (3:1) (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogen phosphate TS, acetonitrile and methanol (5:5:1).

Flow rate: Adjust the flow rate so that the retention time of pranlukast is about 10 minutes.

System suitability—

System performance: When the procedure is run with 4 μL of the standard solution under the above operating conditions, pranlukast and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 4 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pranlukast to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Pravastatin Sodium Fine Granules

プラバスタチンナトリウム細粒

Delete the following item:

Particle size

Prednisolone

プレドニゾロン

Change the Description as follows:

Description Prednisolone occurs as a white crystalline powder.

It is soluble in methanol and in ethanol (95), slightly soluble in ethyl acetate, and very slightly soluble in water.

Melting point: about 235°C (with decomposition).

It shows crystal polymorphism.

Prednisolone Acetate

プレドニゾロン酢酸エステル

Change the Description as follows:

Description Prednisolone Acetate occurs as a white crystalline powder.

It is slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Melting point: about 235°C (with decomposition).

It shows crystal polymorphism.

Probucol Fine Granules

プロブコール細粒

Delete the following item:

Particle size

Progesterone

プロゲステロン

Change the Description as follows:

Description Progesterone occurs as white, crystals or crystalline powder.

It is soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It shows crystal polymorphism.

Propylene Glycol

プロピレングリコール

Add the following next to the Purity (6) as follows:

Purity

(7) Ethylene glycol, diethylene glycol and related substances—Weigh accurately about 5 g of Propylene Glycol, mix with methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g each of ethylene glycol and diethylene glycol, and mix with methanol to make exactly 100 mL. Pipet 5 mL of this solution, and transfer to a 100-mL volumetric flask. Separately, weigh 5.0 g of propylene glycol for gas chromatography, mix with a suitable amount of methanol and put in the 100-mL volumetric flask, dilute with methanol to volume, and use this solution as the standard solution. Perform the test with exactly 1 μL each of the sample solution and standard solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and determine the peak areas, A_{T1} and A_{S1}, of ethylene glycol and, A_{T2} and A_{S2}, of diethylene glycol by the automatic integration method. The amounts of ethylene glycol and diethylene glycol calculated by the following equations are not more than 0.1%, respectively. The amount of the peak other than propylene glycol, ethylene glycol and diethylene glycol obtained from the sample solution, calculated by the area percentage method, is not more than 0.1%, and the total amount of the peaks other than propylene glycol is not more than 1.0%.

$$\text{Amount (\%) of ethylene glycol} = M_{S1}/M_T \times A_{T1}/A_{S1} \times 5$$

$$\text{Amount (\%) of diethylene glycol} = M_{S2}/M_T \times A_{T2}/A_{S2} \times 5$$

M_{S1}: Amount (g) of ethylene glycol
M_{S2}: Amount (g) of diethylene glycol
M_T: Amount (g) of Propylene Glycol

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica tube 0.32 mm in inside diameter and 30 m in length, coated the inside surface 1 μm in thickness with 14% cyanopropylphenyl-86% dimethyl silicone polymer for gas chromatography.

Column temperature: Inject at a constant temperature of about 100°C, rise the temperature at the rate of 7.5°C per minute to 220°C, and maintain at a constant temperature of about 220°C.

Injection port temperature: A constant temperature of about 220°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium.

Flow rate: about 38 cm per second.

Split ratio: 1:20.

Time span of measurement: About 3 times as long as the retention time of propylene glycol, beginning after the solvent peak.

System suitability—

System performance: Mix 50 mg each of ethylene glycol, diethylene glycol and propylene glycol for gas chromatography with 100 mL of methanol. When the procedure is run with 1 μL of this mixture under the above operating conditions, ethylene glycol, propylene glycol and diethylene glycol are eluted in this order, and the resolution between the peaks of ethylene glycol and propylene glycol is not less than 5, and that between the peaks of propylene glycol and diethylene glycol is not less than 50.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ethylene glycol and diethylene glycol is not more than 10%.

Rice Starch

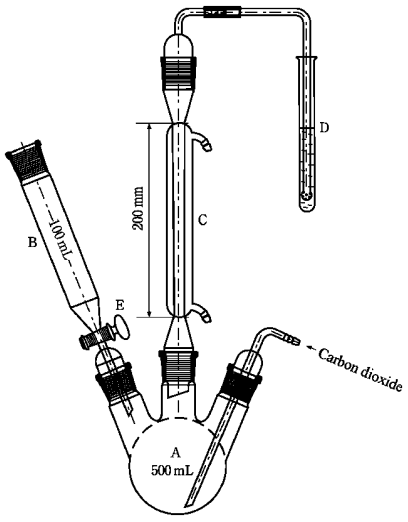
コメデンプン

Change the Purity (3) as follows:

Purity

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.



A: Three-necked round-bottom flask (500 mL)
B: Cylindrical dropping funnel (100 mL)
C: Condenser
D: Test tube
E: Tap

(ii) Procedure Introduce 150 mL of water into the three-necked round-bottom flask, close the tap of the cylindrical dropping funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Rice Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test tube with the aid of a little water to a wide-necked conical flask. Heat on a water bath for 15 minutes and allow to cool. Add 0.1 mL of bromophenol blue TS, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination in the same manner, and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

$$\text{Amount (ppm) of sulfur dioxide} = V/M \times 1000 \times 3.203$$

M: Amount (g) of Rice Starch
V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

Risperidone Fine Granules

リスペリドン細粒

Delete the following item:

Particle size

Roxithromycin

ロキシスロマイシン

Change the Assay as follows:

Assay Weigh accurately an amount of Roxithromycin and Roxithromycin RS, equivalent to about 20 mg (potency), dissolve them separately in the mobile phase to make exactly 10 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of roxithromycin.

$$\text{Amount [}\mu\text{g (potency)] of roxithromycin } (C_{41}H_{76}N_2O_{15}) = M_S \times A_T/A_S \times 1000$$

M_S: Amount [mg (potency)] of Roxithromycin RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 200 mL of a solution of ammonium dihydrogenphosphate (17 in 100) add 510 mL of water, and adjust to pH 5.3 with 2 mol/L sodium hydroxide TS. To this solution add 315 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of roxithromycin is about 21 minutes.

System suitability—

System performance: Dissolve 5 mg each of Roxithromycin RS and *N*-demethylroxithromycin in the mobile phase to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, *N*-demethylroxithromycin and roxithromycin are eluted in this order with the resolution between these peaks being not less than 6 and the symmetry factor of the peak of roxithromycin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of roxithromycin is not more than 1.0%.

Sarpogrelate Hydrochloride Fine Granules

サルポグレラート塩酸塩細粒

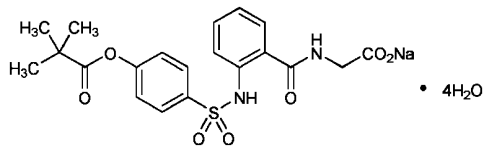
Delete the following item:

Particle size

Add the following:

Sivelestat Sodium Hydrate

シベレスタットナトリウム水和物



$C_{20}H_{21}N_2NaO_7S.4H_2O$: 528.51

Monosodium *N*-{2-[4-(2,2-dimethylpropanoyloxy)phenylsulfonylamino]benzoyl}aminoacetate tetrahydrate

[*201677-61-4*]

Sivelestat Sodium Hydrate contains not less than 98.0% and not more than 102.0% of sivelestat sodium ($C_{20}H_{21}N_2NaO_7S$: 456.44), calculated on the anhydrous basis.

Description Sivelestat Sodium Hydrate occurs as a white crystalline powder.

It is freely soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Melting point: about 190°C (with decomposition, after drying in vacuum, 60°C, 2 hours).

Identification (1) Determine the absorption spectrum of a solution of Sivelestat Sodium Hydrate in boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0 (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Sivelestat Sodium Hydrate as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 50 mg of Sivelestat Sodium Hydrate in 5 mL of water with one drop of ammonia TS: the solution responds to the Qualitative Tests <*1.09*> for sodium salt.

Purity (1) Heavy metals <*1.07*>—Proceed with 2.0 g of Sivelestat Sodium Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 10 mg of Sivelestat Sodium Hydrate in 10 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 1.2 to sivelestat, obtained from the sample solution is not larger than 1/2 times the peak area of sivelestat obtained from the standard solution, the areas of the peaks, having the relative retention time of about 0.25, about 0.60, and about 2.7 to sivelestat, from the sample solution is not larger than 3/10 times the peak area of sivelestat from the standard solution, the area of the peaks other than sivelestat and peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of sivelestat from the standard solution, and the total area of the peaks other than sivelestat from the sample solution is not larger than the peak area of sivelestat from the standard solution.

Operating conditions—

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Time span of measurement: About 4 times as long as the retention time of sivelestat, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of water and acetonitrile (1:1) to make exactly 20 mL. Confirm that the peak area of sivelestat obtained with 10 μL of this solution is equivalent to 4 to 6% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of sivelestat are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sivelestat is not more than 2.0%.

(3) Residual solvent—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Water ⟨*2.48*⟩ 12.0 – 14.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 50 mg of Sivelestat Sodium Hydrate, dissolve in a mixture of water and acetonitrile (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add exactly 5 mL of the internal standard solution. To 4 mL of this solution, add 7 mL of acetonitrile and 9 mL of water, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Sivelestat RS, previously dried (in vacuum, 60°C, 2 hours), and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, and add exactly 5 mL of the internal standard solution. To 2 mL of this solution, add 3 mL of acetonitrile and 5 mL of water, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of sivelestat to that of the internal standard.

Amount (mg) of sivelestat sodium ($C_{20}H_{21}N_2NaO_7S$)
$= M_S \times Q_T/Q_S \times 1.051$

M_S: Amount (mg) of Sivelestat RS

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.44 g of potassium dihydrogen phosphate in water to make 1000 mL, then adjust to pH 3.5 with phosphoric acid. To 5 volumes of this solution, add 4 volumes of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of sivelestat is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and sivelestat are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of sivelestat to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Sivelestat Sodium for Injection

注射用シベレスタットナトリウム

Sivelestat Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of Sivelestat Sodium Hydrate ($C_{20}H_{21}N_2NaO_7S.4H_2O$: 528.51).

Method of preparation Prepare as directed under Injections, with Sivelestat Sodium Hydrate.

Description Sivelestat Sodium for Injection occurs as white, masses or powder.

Identification (1) Dissolve an amount of Sivelestat Sodium for Injection, equivalent to 0.1 g of Sivelestat Sodium Hydrate, in 10 mL of water. To 1 mL of this solution add boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0, to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 311 nm and 315 nm.

(2) Take an amount of Sivelestat Sodium for Injection, equivalent to 0.1 g of Sivelestat Sodium Hydrate, add 10 mL of methanol, and shake. Take 1 mL of the supernatant liquid, add methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of sivelestat sodium hydrate in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (20:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultravio-

let light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same *R*f value.

pH Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Purity Related substances—Dissolve an amount of Sivelestat Sodium for Injection, equivalent to 1.0 g of Sivelestat Sodium Hydrate, in water to make 100 mL. To 1 mL of this solution add 9 mL of a mixture of acetonitrile and water (5:4), and use the solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.25 to sivelestat, obtained from the sample solution is not larger than 3 times the peak area of sivelestat obtained from the standard solution.

Operating conditions—

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay under Sivelestat Sodium Hydrate.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

System suitability—

Proceed as directed in the system suitability in the Purity (2) under Sivelestat Sodium Hydrate.

Bacterial endotoxins <*4.01*> Less than 25 EU/mg.

Uniformity of dosage units <*6.02*> It meets the requirement of the Mass variation test.

Foreign insoluble matter <*6.06*> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <*6.07*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take a number of Sivelestat Sodium for Injection, equivalent to about 1 g of sivelestat sodium hydrate ($C_{20}H_{21}N_2NaO_7S.4H_2O$), and dissolve all the contents in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and 5 mL of acetonitrile. To 2 mL of this solution add 3 mL of a mixture of water and acetonitrile (1:1), and use the solution as the sample solution. Then, proceed as directed in the Assay under Sivelestat Sodium Hydrate.

Amount (mg) of sivelestat sodium hydrate ($C_{20}H_{21}N_2NaO_7S.4H_2O$)
$= M_S \times Q_T/Q_S \times 20 \times 1.216$

M_S: Amount (mg) of Sivelestat RS

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 2500).

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Purified Sodium Hyaluronate

精製ヒアルロン酸ナトリウム

Change the Microbial limit as follows:

Microbial limit <*4.05*> The acceptance criteria of TAMC and TYMC are 10^2 CFU/g and 10^1 CFU/g, respectively. In the case of the sample of a nominal average molecular mass between 500,000 and 1,200,000, perform the test with 1 g, and of a nominal average molecular mass between 1,500,000 and 3,900,000, perform the test with 0.3 g.

Spiramycin Acetate

スピラマイシン酢酸エステル

Change the the origin/limits of content, Content ratio of the active principle and Assay as follows:

Spiramycin Acetate is a derivative of a mixture of macrolide substances having antibacterial activity produced by the growth of *Streptomyces ambofaciens*.

It contains not less than 900 μg (potency) and not more than 1450 μg (potency) per mg, calculated on the dried basis. The potency of Spiramycin Acetate is expressed as mass (potency) of spiramycin II acetate ($C_{47}H_{78}N_2O_{16}$: 927.13). One mg (potency) of Spiramycin Acetate is equivalent to 0.7225 mg of spiramycin II acetate ($C_{47}H_{78}N_2O_{16}$).

Content ratio of the active principle Dissolve 25 mg of Spiramycin Acetate in 25 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the areas, A_{II}, A_{III}, A_{IV}, A_V, A_{VI} and A_{VII}, of the peaks of spiramycin II acetate, spiramycin III acetate, spiramycin IV acetate, spiramycin V acetate, spiramycin VI acetate and spiramycin VII acetate, respectively, by the automatic integration method, and calculate the ratios of the amounts of A_{II}, A_{IV} and the total of A_{III} and A_V to the total amount of all these peaks: the amount of A_{II} is 30 – 45%, A_{IV} is 30 – 45%, and the total of A_{III} and A_V is not more than 25%. The relative retention times of spiramycin III acetate, spiramycin IV acetate, spiramycin V acetate, spiramycin VI acetate and spiramycin VII acetate with respect to spiramycin II acetate are about 1.3, about 1.7, about 2.3, about 0.85 and about 1.4, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile, 0.02 mol/L potassium dihydrogen phosphate TS and a solution of dipotassium hydrogen phosphate (87 in 25,000) (26:7:7).

Flow rate: Adjust the flow rate so that the retention time of spiramycin II acetate is about 10 minutes.

System suitability—

System performance: Dissolve 25 mg of Spiramycin II Acetate RS in the mobile phase to make 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of spiramycin II acetate are not less than 14,500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of spiramycin II acetate is not more than 2.0%.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <*4.02*> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Spiramycin II Acetate RS, equivalent to about 50 mg (potency), dissolve in 20 mL of methanol, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Spiramycin Acetate, equivalent to about 50 mg (potency), dissolve in 20 mL of methanol, and add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration sample solution and the low concentration sample solution, respectively.

[Note: "origin/limits of content" and "Assay" of this monograph have been revised in the Japanese edition, but they do not give any effect to the English text. However, these items are posted here for the consistency with the Japanese edition.]

Spironolactone

スピロノラクトン

Change the Description as follows:

Description Spironolactone occurs as a white to light yellow-brown fine powder.

It is freely soluble in chloroform, soluble in ethanol (95), slightly soluble in methanol, and practically insoluble in water.

Melting point: 198 – 207°C (Insert the capillary tube into a bath at about 125°C, and continue the heating so that the temperature rises at a rate of about 10°C per minute in the range between 140°C and 185°C, and when the temperature is near the expected melting range, reduce the heating so that the temperature rises at a rate of about 3°C per minute.)

It shows crystal polymorphism.

Stearic Acid

ステアリン酸

Change to read other than the monograph title:

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Stearic Acid is a mixture consisting mainly of stearic acid ($C_{18}H_{36}O_2$: 284.48) and palmitic acid ($C_{16}H_{32}O_2$: 256.42) obtained from fats or oils of vegetable or animal origin.

It occurs as three types, stearic acid 50, stearic acid 70 and stearic acid 95, composed with different fatty acid composition. Each type contains respectively the amount of stearic acid and the sum of stearic acid and palmitic acid as shown in the following table.

Type	Fatty acid composition	
	Stearic acid (%)	Sum of stearic acid and palmitic acid (%)
Stearic acid 50	40.0 – 60.0	not less than 90.0
Stearic acid 70	60.0 – 80.0	not less than 90.0
Stearic acid 95	not less than 90.0	not less than 96.0

The label states the type of Stearic Acid.

◆**Description** Stearic acid occurs as white, unctuous masses, crystalline masses or powder. It has a faint, fatty odor.

It is soluble in ethanol (99.5), and practically insoluble in water.◆

Congealing point The apparatus consists of a test tube about 25 mm in diameter and 150 mm long placed inside a test tube about 40 mm in diameter and 160 mm long. The in-

ner tube is closed by a stopper which carries a thermometer about 175 mm long and graduated in 0.2°C fixed so that ◆ the upper end of◆ the bulb is about 15 mm above the bottom of the tube. The stopper has a hole allowing the passage of the stem of a stirrer made from a glass rod or other suitable material formed at one end into a loop of about 18 mm overall diameter at right angles to the rod. The inner tube with its jacket is supported centrally in a 1-L beaker containing a suitable cooling liquid to within 20 mm of the top. A thermometer is supported in the cooling bath.

Place in the inner tube sufficient quantity of the liquid or previously melted substance to be examined, to cover the thermometer bulb and determine the approximate freezing point by cooling rapidly. Place the inner tube in a bath about 5°C above the approximate congealing point until all but the last traces of crystals are melted. Fill the beaker with water or a saturated solution of sodium chloride, at a temperature about 5°C lower than the expected congealing point, insert the inner tube into the outer tube, ensuring that some seed crystals are present, and stir thoroughly until solidification takes place. Note the highest temperature observed during solidification. The congealing point of stearic acid 50 is 53 – 59°C, of stearic acid 70 is 57 – 64°C, and of stearic acid 95 is 64 – 69°C.

Acid value ⟨*1.13*⟩ 194 – 212

Iodine value Introduce about 1 g of Stearic Acid, weighed accurately, into a 250-mL flask fitted with a ground-glass stopper and previously dried or rinsed with acetic acid (100), and dissolve it in 15 mL of chloroform unless otherwise prescribed. Add very slowly exactly 25 mL of iodine bromide TS. Close the flask and keep it in the dark for 30 minutes unless otherwise prescribed, shaking frequently. Add 10 mL of a solution of potassium iodine (1 in 10) and 100 mL of water. Titrate ⟨*2.50*⟩ with 0.1 mol/L sodium thiosulfate VS, shaking vigorously until the yellow color is almost discharged. Add 5 mL of starch TS and continue the titration adding the 0.1 mol/L sodium thiosulfate VS dropwise until the color is discharged. Perform a blank determination in the same manner. When the iodine value is calculated by the following equation, that of stearic acid 50 and 70 is not more than 4.0, and of stearic acid 95 is not more than 1.5.

$$\text{Iodine value} = (a - b) \times 1.269/M$$

M: Amount (g) of Stearic Acid
a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination
b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the test

Purity **(1)** Acidity—Melt 5.0 g, shake for 2 minutes with 10 mL of hot carbon dioxide-free water, cool slowly and filter. To the filtrate add 0.05 mL of methyl orange TS: no red color develops.

◆(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Stearic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).◆

◆Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).◆

Assay Place 0.100 g of Stearic Acid in a ◆small◆ conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, ◆shake, and◆ boil under reflux for about 10 minutes ◆to dissolve.◆ Add 4 mL of heptane through the condenser, and boil again under reflux for 10 minutes. Allow to cool, add 20 mL of a saturated solution of sodium chloride, shake and allow the layers to separate. Remove 2 mL of the separated heptane layer, and dry it over about 0.2 g of anhydrous sodium sulphate, ◆previously washed with heptane.◆ Take 1.0 mL of the dried heptane layer in a 10-mL volumetric flask, add heptane to make up to 10 mL, and use this solution as the sample solution. Perform the test with 1 μL of the sample solution as directed under Gas Chromatography ⟨*2.02*⟩ according to the following conditions, and determine the peak area of methyl stearate, A, and the area of all of fatty acid ester peaks, B, and calculate the content (%) of stearic acid in the fatty acid fraction by the following equation.

$$\text{Content (\%) of stearic acid} = A/B \times 100$$

In the same way, calculate the content (%) of palmitic acid, and calculate the sum (%) of stearic acid and palmitic acid.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated the inside surface with a layer about 0.5 μm thick of polyethylene glycol 20 M for gas chromatography.

Column temperature: Maintain at 70°C for 2 minutes after injection, raise the temperature at a rate of 5°C per minute to 240°C, and maintain at 240°C for 5 minutes.

Injection port temperature: A constant temperature of about 220°C.

Detector temperature: A constant temperature of about 260°C.

Carrier gas: Helium.

Flow rate: 2.4 mL per minute.

◆Split ratio: Split less.◆

◆Time span of measurement: For 41 minutes after sample injection, beginning after the solvent peak.◆

System suitability—

◆Test for required detectability:◆ Put 50 mg each of stearic acid for gas chromatography and palmitic acid for gas chromatography in a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, then proceed as the same manner for the sample solution, and use the solution so obtained as the solution for system suitability test. ◆Pipet 1 mL of the solution for system suitability test, add heptane to make exactly 10 mL. Pipet 1 mL of this solution, add heptane to make exactly 10 mL. Again, pipet 1 mL of this solution, and add heptane to make 10 mL. Confirm that the peak area of methyl stearate obtained with 1 μL of this solution is equivalent to 0.05 to 0.15% of that obtained with 1 μL of the solu-

tion for system suitability test.◆

System performance: When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, the relative retention time of methyl palmitate to methyl stearate is about 0.9, and the resolution between these peaks is not less than 5.0.

System repeatability: When the test is repeated 6 times with 1 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of methyl palmitate and methyl stearate is not more than 3.0%. Furthermore, the relative standard deviation of the ratio of the peak area of methyl palmitate to the peak area of methyl stearate obtained from the 6-time repetition is not more than 1.0%.

◆**Containers and storage** Containers—Well-closed containers.◆

Add the following:

Tacalcitol Ointment

タカルシトール軟膏

Tacalcitol Ointment contains not less than 90.0% and not more than 115.0% of the labeled amount of tacalcitol ($C_{27}H_{44}O_3$: 416.64).

Method of preparation Prepare as directed under Ointments, with Tacalcitol Hydrate.

Identification When the test is performed with 30 μL each of the sample solution and standard solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions, the retention times of the main peak of the sample solution and standard solution are the same. The absorption spectra of their peaks exhibit similar intensities of absorption at the same wavelengths.

Operating conditions—

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: A photodiode array detector (wavelength: 265 nm, spectrum range of measurement: 210 – 400 nm).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

Purity Related substances—This test is only applied to the preparations of 20 μg/g.

Conduct this procedure using light-resistant vessels. To an amount of Tacalcitol Ointment, equivalent to about 20 μg of tacalcitol ($C_{27}H_{44}O_3$), add 5 mL of hexane and 5 mL of methanol, shake thoroughly for 15 minutes, and centrifuge. Discard the upper layer, pipet 5 mL of the lower layer, and evaporate the solvents in vacuum. Dissolve the residue in 1 mL of methanol, filter this solution through a membrane filter with a pore size not exceeding 0.2 μm, and use the filtrate as the sample solution. Perform the test with 30 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peak other than tacalcitol and pre-tacalcitol, having a relative retention time of about 0.83 to tacalcitol, is not more than 0.8%, and the total amount of the peaks other than tacalcitol and pre-tacalcitol is not more than 2.0%.

Operating conditions—

Detector, column and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase A: Water.

Mobile phase B: Acetonitrile for liquid chromatography.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	40	60
30 – 50	40 → 0	60 → 100
50 – 60	0	100

Flow rate: Adjust the flow rate so that the retention time of tacalcitol is about 24 minutes.

Time span of measurement: For 60 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 0.5 mL of the sample solution add methanol to make 50 mL, and use this solution as the solution for system suitability test. Pipet 4 mL of the solution for system suitability test, and add methanol to make exactly 10 mL. Confirm that the peak area of tacalcitol obtained with 30 μL of this solution is equivalent to 28 to 52% of that obtained with 30 μL of the solution for system suitability test.

System performance: When the procedure is run with 30 μL of the sample solution under the above operating conditions, pre-tacalcitol and tacalcitol are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 30 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of tacalcitol is not more than 10%.

Assay Weigh accurately an amount of Tacalcitol Ointment, equivalent to about 2 μg of tacalcitol ($C_{27}H_{44}O_3$), add exactly 5 mL of hexane, exactly 4 mL of methanol, and exactly 1 mL of the internal standard solution, shake thoroughly for 15 minutes, and centrifuge. Filter the lower layer through a membrane filter with a pore size not exceeding 0.2 μm, and use the filtrate as the sample solution. Separately, weigh accurately about 1 mg of Tacalcitol RS (separately determine the water <*2.48*> in the same manner as Tacalcitol Hydrate), and dissolve in methanol to make exactly 20 mL. Pipet 1 mL of this solution, and add methanol to make ex-

actly 100 mL. Pipet 4 mL of this solution, add exactly 1 mL of the internal standard solution and exactly 5 mL of hexane, shake thoroughly for 15 minutes, and centrifuge. Filter the lower layer through a membrane filter with a pore size not exceeding 0.2 μm, and use the filtrate as the standard solution. Perform the test with 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of tacalcitol to that of the internal standard.

$$\text{Amount } (\mu g) \text{ of tacalcitol } (C_{27}H_{44}O_3) = M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of Tacalcitol RS, calculated on the anhydrous basis

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (3 in 2,500,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of acetonitrile for liquid chromatography and diluted 0.25 mol/L acetic acid TS (1 in 10) (13:7).

Flow rate: Adjust the flow rate so that the retention time of tacalcitol is about 18 minutes.

System suitability—

System performance: When the procedure is run with 30 μL of the standard solution under the above operating conditions, the internal standard and tacalcitol are eluted in this order with the resolution between these peaks being not less than 14.

System repeatability: When the test is repeated 6 times with 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tacalcitol to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Tazobactam and Piperacillin for Injection

注射用タゾバクタム·ピペラシリン

Tazobactam and Piperacillin for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of tazobactam ($C_{10}H_{12}N_4O_5S$: 300.29) and not less than 95.0% and not more than 105.0% of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

Method of preparation Prepare as directed under Injections, with Tazobactam, Piperacillin Hydrate and Sodium Hydrogen Carbonate.

Description Tazobactam and Piperacillin for Injection occurs as white to pale yellowish white, masses or powder.

Identification (1) Determine ^1H spectrum of a solution of Tazobactam and Piperacillin for Injection in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 4.2 ppm, a multiple signal B at δ 7.3 – 7.5 ppm, a double signal C at around δ 7.8 ppm and a double signal D at around δ 8.1 ppm. The ratio of integrated intensity of these signals, A:B and C:D, is about 1:5 and about 1:1, respectively.

(2) Tazobactam and Piperacillin for Injection responds to the Qualitative Tests <*1.09*> (1) for sodium salt.

pH <*2.54*> The pH of a solution of an amount of Tazobactam and Piperacillin for Injection, equivalent to 4.0 g (potency) of Piperacillin Hydrate, in 40 mL of water is 5.1 to 6.3.

Purity (1) Clarity and color of solution—A solution of an amount of Tazobactam and Piperacillin for Injection, equivalent to 4.0 g (potency) of Piperacillin Hydrate, in 40 mL of water is clear and colorless.

(2) Related substances—Keep the sample solution at 5°C. Dissolve an amount of Tazobactam and Piperacillin for Injection, equivalent to 0.1 g (potency) of Piperacillin Hydrate, in 100 mL of dissolving solution, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dissolving solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.06 to piperacillin, obtained from the sample solution is not larger than 1.3 times the peak area of tazobactam obtained from the standard solution, the area of the peak, having the relative retention time of about 0.05, about 0.07, about 0.19, about 0.45 and about 0.53 to piperacillin, from the sample solution is not larger than 1/10 times the peak area of tazobactam from the standard solution, and the total area of the peaks, having the relative retention time of about 0.05, about 0.06, about 0.07, about 0.19, about 0.45 and about 0.53 to piperacillin, from the sample solution is not larger than 1.5 times the peak area of tazobactam from the standard solution. Furthermore, the area of the peak, having the

relative retention time of about 1.20 and about 1.36 to piperacillin, from the sample solution is not larger than 1/5 times the peak area of piperacillin from the standard solution, the area of the peak, having the relative retention time of about 0.15 and about 0.63 to piperacillin, from the sample solution is not larger than 3/10 times the peak area of piperacillin from the standard solution, the area of the peak, having the relative retention time of about 0.91 and about 1.53 to piperacillin, from the sample solution is not larger than 2/5 times the peak area of piperacillin from the standard solution, the total area of the peaks eluted between the relative retention time of about 0.85 and about 0.87 to piperacillin, from the sample solution is not larger than 1/2 times the peak area of piperacillin from the standard solution, the total area of the peaks, having the relative retention time of about 0.85 and about 0.87 to piperacillin, from the sample solution is not larger than 1.5 times the peak area of piperacillin from the standard solution, and the area of the peak other than tazobactam, piperacillin and the peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of piperacillin from the standard solution. The total area of the peaks other than tazobactam, piperacillin and the peaks, having the relative retention time of about 0.05, about 0.06, about 0.07, about 0.19, about 0.45 and about 0.53 to piperacillin, from the sample solution is not larger than 4.0 times the peak area of piperacillin from the standard solution. For these calculations use the area of the peaks, having the relative retention time of about 0.05, about 0.06, about 0.07, about 0.15, about 0.19, about 0.45, about 0.53, about 0.63, about 0.68, about 0.79, about 0.91 and about 1.53 to piperacillin, after multiplying by their relative response factors 2.09, 0.70, 0.92, 0.42, 0.69, 0.56, 0.19, 1.37, 1,93, 1.64, 1.73 and 1.29, respectively, and for the total area of the peaks having the relative retention time of about 0.85 and about 0.87 to piperacillin and the total area of the peaks that are eluted between the peaks having the relative retention time of about 0.85 and about 0.87 to piperacillin, use after multiplying by their relative response factors, 1.79 and 2.50, respectively.

Dissolving solution: To 950 mL of diluted 1 mol/L dipotassium hydrogen phosphate TS for buffer solution (1 in 100) adjusted to pH 6.5 with phosphoric acid, add 50 mL of acetonitrile.

Operating conditions—

Detector, column, column temperature, mobile phase A, mobile phase B, flowing of the mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (1).

Time span of measurement: For 36 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add dissolving solution to make exactly 20 mL. Confirm that the peak area of tazobactam obtained with 20 μL of this solution is equivalent to 3.5% to 6.5% of that obtained with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, tazobactam and piperacillin are eluted in this order with the resolution between these peaks being not less than 50, and the number of theoretical plates and the symmetry factor of the peak of tazobactam are not less than 40,000 and not more than 1.5, respectively, and those of piperacillin are not less than 150,000 and not more than 1.5, respectively. Furthermore, when warm the sample solution at 40°C for 60 minutes and proceed with 20 μL of this solution under the above conditions, the resolution between the two peaks, having the relative retention time of about 0.85 and about 0.87 to piperacillin, is not less than 2.9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above conditions, the relative standard deviations of the peak area of tazobactam and piperacillin are not more than 2.0%, respectively.

Water ⟨*2.48*⟩ Weigh accurately the mass of the content of 1 container of Tazobactam and Piperacillin for Injection, dissolve in 20 mL of methanol for Karl Fisher method, and perform the test with this solution according to the direct titration of Volumetric titration: not more than 0.6%. Perform a blank determination in the same manner, and make any necessary correction.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.07 EU/mg (potency) of Piperacillin Hydrate.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

Sterility ⟨*4.06*⟩ Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (1) Tazobactam—Dissolve the contents of 10 containers of Tazobactam and Piperacillin for Injection in a suitable amount of dissolving solution. Washout these empty containers with dissolving solution, combine the washings and the former solution, and add dissolving solution to make exactly V mL so that each mL contains about 5 mg (potency) of Tazobactam. Pipet 5 mL of this solution, add dissolving solution to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg (potency) of Tazobactam RS, dissolve in 10 mL of acetonitrile, dilute with an amount of diluted 1 mol/L dipotassium hydrogen phosphate TS for buffer solution (1 in 100) adjusted to pH 6.5 with phosphoric acid to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions and determine the peak areas, A_T and A_S, of tazobactam in each solution.

Amount [g (potency)] of tazobactam ($C_{10}H_{12}N_4O_5S$) in 1 container of Tazobactam and Piperacillin for Injection
$= M_S \times A_T/A_S \times V/50{,}000$

M_S: Amount [mg (potency)] of Tazobactam RS

Dissolving solution: To 950 mL of diluted 1 mol/L dipotassium hydrogen phosphate TS for buffer solution (1 in 100) adjusted to pH 6.5 with phosphoric acid, add 50 mL of acetonitrile.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: Dissolve 1.74 g of dipotassium hydrogen phosphate in 1000 mL of water, and adjust to pH 2.6 with phosphoric acid.

Mobile phase B: Acetonitrile.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 5	100	0
5 – 15	100 → 76	0 → 24
15 – 25	76 → 65	24 → 35
25 – 36	65	35

Flow rate: 1.5 mL per minute.

System suitability—

System performance: Dissolve 50 mg (potency) of piperacillin hydrate in the standard solution to make 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, tazobactam and piperacillin are eluted in this order with the resolution between these peaks being not less than 50, and the number of theoretical plates and the symmetry factor of the peak of tazobactam are not less than 25,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above conditions, the relative standard deviations of the peak area of tazobactam is not more than 1.0%.

(2) Piperacillin—Dissolve the contents of 10 containers of Tazobactam and Piperacillin for Injection in a suitable amount of dissolving solution. Washout these empty containers with dissolving solution, combine the washings and the former solution, and add dissolving solution to make exactly V mL so that each mL contains about 40 mg (potency) of Piperacillin Hydrate. Pipet 5 mL of this solution, add dissolving solution to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg (potency) of Piperacillin RS, dissolve in 2.5 mL of acetonitrile, dilute with an amount of diluted 1 mol/L dipotassium hydrogen phosphate TS for buffer solution (1 in 100) adjusted to pH 6.5 with phosphoric acid to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of piperacillin in each solution.

Amount [g (potency)] of piperacillin ($C_{23}H_{27}N_5O_7S$) in 1 container of Tazobactam and Piperacillin for Injection
$= M_S \times A_T/A_S \times V/12{,}500$

M_S: Amount [mg (potency)] of Piperacillin RS

Dissolving solution: To 950 mL of diluted 1 mol/L dipotassium hydrogen phosphate TS for buffer solution (1 in 100) adjusted to pH 6.5 with phosphoric acid, add 50 mL of acetonitrile.

Operating conditions—

Proceed as directed in the operating conditions in the Assay (1).

System suitability—

System performance: When the procedure is run with 20 μL of the solution for system suitability test obtained in the Assay (1) under the above operating conditions, tazobactam and piperacillin are eluted in this order with the resolution between these peaks being not less than 50, and the number of theoretical plates and the symmetry factor of the peak of piperacillin are not less than 100,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above conditions, the relative standard deviations of the peak area of piperacillin is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Tegafur

テガフール

Change the Description as follows:

Description Tegafur occurs as a white crystalline powder.

It is soluble in methanol, and sparingly soluble in water and in ethanol (95).

It dissolves in dilute sodium hydroxide TS.

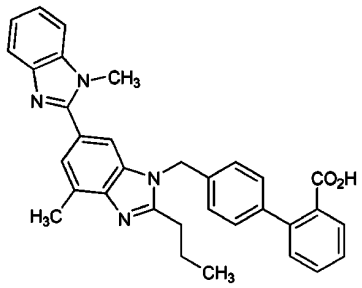
A solution of Tegafur in methanol (1 in 50) shows no optical rotation.

It shows crystal polymorphism.

Add the following:

Telmisartan

テルミサルタン



$C_{33}H_{30}N_4O_2$: 514.62

4′-{[4-Methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl}biphenyl-2-carboxylic acid [*144701-48-4*]

Telmisartan, when dried, contains not less than 99.0% and not more than 101.0% of $C_{33}H_{30}N_4O_2$.

Description Telmisartan occurs as a white to pale yellow crystalline powder.

It is freely soluble in formic acid, slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in water.

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Telmisartan in methanol (7 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Telmisartan as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Telmisartan in ethanol (95) by warming, and cool in ice. Collect the crystals formed, dry, and perform the test with the crystals.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Telmisartan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead solution (not more than 10 ppm).

(2) Related substances—To 25 mg of Telmisartan add 5 mL of methanol and 0.1 mL of sodium hydroxide TS, and dissolve with the aid of ultrasonic waves. To this solution add methanol to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 1.7 to telmisartan, obtained from the sample solution is not larger than 1/5 times the peak area of telmisartan obtained from the standard solution, the area of the peak other than telmisartan and the above mentioned peak from the sample solution is not larger than 1/10 times the peak area of telmisartan from the standard solution, and the total area of the peaks other than telmisartan from the sample solution is not larger than the peak area of telmisartan from the standard solution. For this calculation use the area of the peak, having the retention time of about 0.7 to telmisartan, after multiplying by its relative response factor 1.2.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 12.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 2.0 g of potassium dihydrogen phosphate and 3.4 g of sodium 1-pentanesulfonate in 1000 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (1 in 10).

Mobile phase B: A mixture of acetonitrile and methanol (4:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 25	70 → 20	30 → 80

Flow rate: 1.0 mL per minute.

Time span of measurement: About 2 times as long as the retention time of telmisartan, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, add methanol to make exactly 100 mL. Confirm that the peak area of telmisartan obtained with 2 μL of this solution is equivalent to 3.5 to 6.5% of that obtained with 2 μL of the standard solution.

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of telmisartan are not less than 45,000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of telmisartan is not more than 5%.

(3) Residual solvent—Being specified separately when

the drug is granted approval based on the Pharmaceutical Affairs Law.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition ⟨*2.44*⟩ Not more than 0.1% (1 g).

Assay Weigh accurately about 0.19 g of Telmisartan, previously dried, dissolve in 5 mL of formic acid, add 75 mL of acetic anhydride, and titrate ⟨*2.50*⟩ with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 25.73 mg of $C_{33}H_{30}N_4O_2$

Containers and storage Containers—Tight containers.

Add the following:

Telmisartan Tablets

テルミサルタン錠

Telmisartan Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$: 514.62).

Method of preparation Prepare as directed under Tablets, with Telmisartan.

Identification Powder Telmisartan Tablets. To a portion of the powder, equivalent to 0.7 mg of Telmisartan, add 100 mL of methanol, shake well, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 226 nm and 230 nm and between 295 nm and 299 nm.

Uniformity of dosage units ⟨*6.02*⟩ Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Telmisartan Tablets add $4V/5$ mL of a mixture of water and methanol (1:1), disintegrate the tablet using ultrasonic waves, and add a mixture of water and methanol (1:1) to make exactly V mL so that each mL contains about 0.8 mg of telmisartan ($C_{33}H_{30}N_4O_2$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard 10 mL of the first filtrate, pipet 5 mL of the subsequent filtrate, add a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of telmisartan ($C_{33}H_{30}N_4O_2$)
$= M_S \times A_T/A_S \times V/25$

M_S: Amount (mg) of telmisartan for assay

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes of Telmisartan Tablets is not less than 85%.

Start the test with 1 tablet of Telmisartan Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard not less than 5 mL of the first filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 11 μg of telmisartan ($C_{33}H_{30}N_4O_2$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of telmisartan for assay, previously dried at 105°C for 4 hours, add 10 mL of a solution of meglumine in methanol (1 in 500), dissolve with the aid of ultrasonic waves, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 296 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the control.

Dissolution rate (%) with respect to the labeled amount of telmisartan ($C_{33}H_{30}N_4O_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of telmisartan for assay
C: Labeled amount (mg) of telmisartan ($C_{33}H_{30}N_4O_2$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Telmisartan Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 80 mg of telmisartan ($C_{33}H_{30}N_4O_2$), add 80 mL of a mixture of water and methanol (1:1), shake thoroughly, and add a mixture of water and methanol (1:1) to make exactly 100 mL. Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of telmisartan for assay, previously dried at 105 °C for 4 hours, add 10 mL of a solution of meglumine in a mixture of water and methanol (1:1) (1 in 500), dissolve by shaking well, and add a mixture of water and methanol (1:1) to make exactly 25 mL. Pipet 5 mL of this solution, add a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of telmisartan in each solution.

Amount (mg) of telmisartan ($C_{33}H_{30}N_4O_2$)
$= M_S \times A_T/A_S \times 4$

M_S: Amount (mg) of telmisartan for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 295 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2 g of diammonium hydrogenphosphate in 1000 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (1 in 10). To 300 mL of this solution add 700 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of telmisartan is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of telmisartan are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of telmisartan is not more than 1.0%.

Containers and storage Containers—Tight containers.

Thiamine Chloride Hydrochloride

チアミン塩化物塩酸塩

Change the Description as follows:

Description Thiamine Chloride Hydrochloride occurs as white, crystals or crystalline powder. It is odorless or has a slight, characteristic odor.

It is freely soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).

Melting point: about 245°C (with decomposition).

It shows crystal polymorphism.

Delete the following Monograph:

Thiotepa

チオテパ

Triamcinolone

トリアムシノロン

Change the Description as follows:

Description Triamcinolone occurs as a white crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Melting point: about 264°C (with decomposition).

It shows crystal polymorphism.

Triamcinolone Acetonide

トリアムシノロンアセトニド

Change the Description as follows:

Description Triamcinolone Acetonide occurs as a white, crystalline powder.

It is sparingly soluble in acetone and in 1,4-dioxane, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Melting point: about 290°C (with decomposition).

It shows crystal polymorphism.

Troxipide Fine Granules

トロキシピド細粒

Delete the following item:

Particle size

Sterile Water for Injection in Containers

注射用水(容器入り)

Delete the following item:

Extractable volume

Wheat Starch

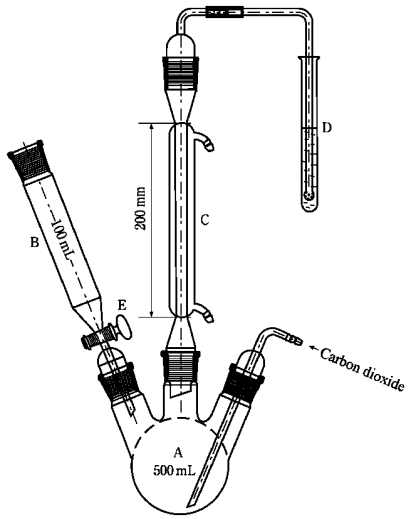
コムギデンプン

Change the Purity (3) as follows:

Purity

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.



A: Three-necked round-bottom flask (500 mL)
B: Cylindrical dropping funnel (100 mL)
C: Condenser
D: Test tube
E: Tap

(ii) Procedure Introduce 150 mL of water into the three-necked round-bottom flask, close the tap of the cylindrical dropping funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Wheat Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

$$\text{Amount (ppm) of sulfur dioxide} = V/M \times 1000 \times 3.203$$

M: Amount (g) of Wheat Starch
V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

Zaltoprofen Tablets

ザルトプロフェン錠

Change the Identification as follows:

Identification Powder a suitable amount of Zaltoprofen Tablets. To a portion of the powder, equivalent to 80 mg of Zaltoprofen, add 30 mL of ethanol (99.5), shake well, and centrifuge. To 1 mL of the supernatant liquid add ethanol (99.5) to make 20 mL. To 2 mL of this solution add ethanol (99.5) to make 25 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 227 nm and 231 nm and between 329 nm and 333 nm, and a shoulder between 238 nm and 248 nm.

Zidovudine

ジドブジン

Change the Description as follows:

Description Zidovudine occurs as a white to pale yellowish white, powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and sparingly soluble in water.

It gradually turns yellow-brown on exposure to light.

Melting point: about 124°C.

It shows crystal polymorphism.

Crude Drugs

Acacia

アラビアゴム

Change the Identification as follows:

Identification To 1 g of pulverized Acacia add 25 mL of water and 1 mL of sulfuric acid, and heat under a reflux condenser in a boiling water bath for 60 minutes. After cooling, add gently 2.0 g of anhydrous sodium carbonate. To 1 mL of this solution add 9 mL of methanol, mix well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg each of D-galactose, L-arabinose and L-rhamnose monohydrate in 1 mL water separately, add methanol to make 10 mL, and use these solutions as the standard solutions (1), (2) and (3), respectively. Perform the test with these solutions as directed under Thin-layer chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, acetic acid (100) and water (12:3:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 2 minutes: the three spots obtained from the sample solution are the same with the spots of D-galactose, L-arabinose and L-rhamnose obtained from the standard solution in the color tone and the *R*f value, respectively.

Powdered Acacia

アラビアゴム末

Change the Identification as follows:

Identification To 1 g of Powdered Acacia add 25 mL of water and 1 mL of sulfuric acid, and heat under a reflux condenser in a boiling water bath for 60 minutes. After cooling, add gently 2.0 g of anhydrous sodium carbonate. To 1 mL of this solution add 9 mL of methanol, mix well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg each of D-galactose, L-arabinose and L-rhamnose monohydrate in 1 mL water, add methanol to make 10 mL, and use these solutions as the standard solutions, (1), (2) and (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, acetic acid (100) and water (12:3:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 2 minutes: the three spots obtained from the sample solution are the same with the spots of D-galactose, L-arabinose and L-rhamnose obtained from the standard solution in the color tone and the *R*f value, respectively.

Apricot Kernel

キョウニン

Change the Purity (2) as follows:

Purity

(2) Foreign matter <*5.01*>—When perform the test with not less than 250 g of Apricot Kernel, it contains not more than 0.10% of fragments of endocarp.

Areca

ビンロウジ

Change the Identification as follows:

Identification To 1.0 g of pulverized Areca add 5 mL of 0.01 mol/L hydrochloric acid TS and 5 mL of ethyl acetate, shake for 15 minutes, centrifuge, and remove the upper layer. To the water layer add 1 mL of sodium hydroxide TS and 5 mL of ethyl acetate, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of arecoline hydrobromide for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and acetic acid (100) (10:6:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS, air-dry, then spray evenly sodium nitrite TS: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the brown spot obtained from the standard solution. The color of this spot fades immediately and then disappears after air-drying.

Atractylodes Lancea Rhizome

ソウジュツ

Add the following next to the Description:

Identification To 2.0 g of pulverized Atractylodes Lancea Rhizome add 5 mL of hexane, shake for 5 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and acetic acid (100) (10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes: a grayish green spot appears at an *R*f value of about 0.5.

Delete the following item:

Purity (3)

Powdered Atractylodes Lancea Rhizome

ソウジュツ末

Add the following next to the Description:

Identification To 2.0 g of Powdered Atractylodes Lancea Rhizome add 5 mL of hexane, shake for 5 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and acetic acid (100) (10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes: a grayish green spot appears at an *R*f value of about 0.5.

Delete the following item:

Purity (3)

Atractylodes Rhizome

ビャクジュツ

Change the Description, Identification and Purity as follows:

Description 1) Wa-byakujutsu—Periderm-removed rhizome is irregular masses or irregularly curved cylinder, 3 – 8 cm in length, 2 – 3 cm in diameter; externally light grayish yellow to light yellowish white, with scattered grayish brown parts. The rhizome covered with periderm is externally grayish brown, often with node-like protuberances and coarse wrinkles. Difficult to break, and the fractured surface is fibrous. A transverse section, with fine dots of light yellow-brown to brown secrete.

Odor, characteristic; taste, somewhat bitter.

Under a microscope <*5.01*>, a transverse section reveals periderm with stone cell layers; fiber bundles in the parenchyma of the cortex, often adjoined to the outside of the phloem; oil sacs containing light brown to brown substances, situated at the outer end of medullary rays; in the xylem, radially lined vessels, surrounding large pith, and distinct fiber bundle surrounding the vessels; in pith and in medullary rays, oil sacs similar to those in cortex, and in parenchyma, crystals of inulin and small needle crystals of calcium oxalate.

2) Kara-byakujutsu—Irregularly enlarged mass, 4 – 8 cm in length, 2 – 5 cm in diameter; externally grayish yellow to dark brown, having sporadic, knob-like small protrusions. Difficult to break; fractured surface has a light brown to dark brown xylem remarkably fibrous.

Odor, characteristic; taste, somewhat sweet, but followed by slight bitterness.

Under a microscope <*5.01*>, a transverse section usually reveals periderm with stone cells, absence of fibers in the cortex; oil sacs containing yellow-brown contents in phloem ray and also at the outer end of it; xylem with radially lined vessels surrounding large pith, and distinct fiber bundle surrounding the vessels; pith and medullary ray exhibit oil sacs as in cortex; parenchyma contains crystals of inulin and small needle crystals of calcium oxalate.

Identification To 2.0 g of pulverized Atractylodes Rhizome add 5 mL of hexane, shake for 5 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetic acid (100) (10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot appears at an *R*f value of about 0.6.

Purity (1) Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Atractylodes Rhizome according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Atractylodes Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Atractylodes lancea rhizome—When proceed as directed in the Identification, using exactly 5 mL of hexane, any grayish green spot does not appear at an *R*f value of about 0.5, immediately below the red-purple spot appeared at an *R*f value of about 0.6.

Powdered Atractylodes Rhizome

ビャクジュツ末

Change the Identification and Purity as follows:

Identification To 2.0 g of Powdered Atractylodes Rhizome add 5 mL of hexane, shake for 5 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetic acid (100) (10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot appears at an *R*f value of about 0.6.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Powdered Atractylodes Rhizome according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Atractylodes Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Atractylodes lancea rhizome—When proceed as directed in the Identification, using exactly 5 mL of hexane, any grayish green spot does not appear at an *R*f value of about 0.5, immediately below the red-purple spot appeared at an *R*f value of about 0.6.

Add the following:

Belladonna Total Alkaloids

ベラドンナ総アルカロイド

Belladonna Total Alkaloids contains not less than 95.0% and not more than 99.0% of hyoscyamine ($C_{17}H_{23}NO_3$: 289.37), not less than 1.3% and not more than 3.9% of scopolamine ($C_{17}H_{21}NO_4$: 303.35), and not less than 99.0% and not more than 102.0% of the total alkaloids (hyoscyamine and scopolamine), calculated on the dried basis.

Method of preparation Belladonna Total Alkaloids is prepared by purification of the extract from Belladonna Root with water or aqueous ethanol.

Description Belladonna Total Alkaloids occurs as white, crystals or crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (99.5), and slightly soluble in water.

Identification Dissolve 2 mg of Belladonna Total Alkaloids in 1 mL of ethanol (95), and use this solution as the sample solution. Then proceed as directed in the Identification under Belladonna Root.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -18.5 – $-22.0°$ (after drying, 1 g, ethanol (99.5), 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Place 1.0 g of Belladonna Total Alkaloids in a porcelain crucible, and mix with 1.2 mL of dilute hydrochloric acid. Mix with 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and after evaporating the solvent on a boiling water bath, carbonize by gradual heating. Then proceed according to Method 4, and perform the test. The control solution is prepared as follows: Mix 1.2 mL of dilute hydrochloric acid with 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and evaporate the solvent on a boiling water bath. After cooling, add 1 mL of sulfuric acid, then proceed according to Method 4, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Belladonna Total Alkaloids according to Method 4, and perform the test (not more than 1 ppm).

(3) Residual solvent—Being specified separately when the drug is granted approval based on the Pharmaceutical Affairs Law.

Loss on drying <*2.41*> Not more than 1.0% (1 g, in vacuum, 60°C, 6 hours).

Residue on ignition <*2.44*> Not more than 0.2% (0.5 g).

Assay Weigh accurately about 25 mg of Belladonna Total Alkaloids, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 3 mL of the internal standard solution and the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate RS (separately determine the loss on drying <*2.41*> under the same conditions as Atropine Sulfate Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as the standard stock solution (1). Also, weigh accurately about 25 mg of Scopolamine Hydrobromide RS (separately determine the loss on drying <*2.41*> under the same conditions as Scopolamine Hydrobromide Hydrate), and dissolve in the mobile phase to make exactly 25 mL. Pipet 3 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard stock solution (2). Take exactly 5 mL of standard stock solution (1), add exactly 2 mL of the standard stock solution (2), and add exactly 3 mL of the internal standard solution. To this solution add the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_{TA} and Q_{SA}, of the peak area of hyoscyamine (atropine) to that of the internal standard and the ratios, Q_{TS} and Q_{SS}, of the peak area of scopolamine to that of the internal standard. Then calculate the amounts of hyoscyamine and scopolamine using the following equations. The amount of the total alkaloids is obtained as the sum of them.

The amount (mg) of hyoscyamine ($C_{17}H_{23}NO_3$)
$= M_{SA} \times Q_{TA}/Q_{SA} \times 0.855$

The amount (mg) of scopolamine ($C_{17}H_{21}NO_4$)
$= M_{SS} \times Q_{TS}/Q_{SS} \times 6/125 \times 0.789$

M_{SA}: The amount (mg) of Atropine Sulfate RS, calculated on the dried basis

M_{SS}: The amount (mg) of Scopolamine Hydrobromide RS, calculated on the dried basis

Internal standard solution: A solution of brucine *n*-hydrate in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of around 20°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 900 mL of water, add 10 mL of triethylamine, adjust to pH 3.5 with phosphoric acid, and add water to make 1000 mL. To 900 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of atropine is about 14 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, scopolamine, atropine and the internal standard are eluted in this order, and the resolutions between scopolamine and atropine, and atropine and the internal standard are not less than 11 and not less than 4, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of scopolamine to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Benincasa Seed

トウガシ

Change the Description as follows:

Description 1) *Benincasa cerifera* origin—Flattened, ovate to orbicular ovate seed, 10 – 13 mm in length, 6 – 7 mm in width, about 2 mm in thickness; slightly acute at base; hilum and germ pore form two protrusions; externally light grayish yellow to light yellowish brown; prominent band along with marginal edge of seed; under a magnifying glass, surface of the seed is with fine wrinkles and minute hollows.

Odorless; bland taste and slightly oily.

Under a microscope <*5.01*>, a transverse section reveals the outermost layer of seed coat composed of a single-layered and palisade like epidermis, the epidermis obvious at prominent band along with marginal edge of seed; hypodermis composed of slightly sclerified parenchyma beneath epidermis; inside of the parenchyma several layers of stone cells lie; the innermost layer of seed coat composed of parenchyma several cells thick; perisperm coated with cuticle, composed of parenchyma several cells thick; endosperm composed of a row of compressed cells; cotyledon contains oil drops and aleurone grains, occasionally starch grains.

2) *Benincasa cerifera* forma *emarginata* origin—Flattened, ovate to ellipsoidal seed, 9 – 12 mm in length, 5 – 6 mm in width, about 2 mm in thickness; hilum and germ pore form two protrusions as in 1); externally light grayish yellow, smooth, no prominent band along with marginal edge of seed.

Odorless; bland taste and slightly oily.

Under a microscope <*5.01*>, a transverse section reveals the outermost layer composed of a single-layered epidermis coated with cuticle, often detached; hypodermis composed of slightly sclerified parenchyma beneath epidermis; inside of the parenchyma several layers of stone cells lie; the innermost layer of seed coat composed of parenchyma several cells thick; perisperm coated with cuticle, composed of parenchyma several cells thick; endosperm composed of a row of compressed cells; cotyledon contains oil drops and aleurone grains, occasionally starch grains.

Brown Rice

コウベイ

Change the Identification (2) as follows:

Identification

(2) To 1 g of pulverized Brown Rice add 5 mL of ethyl acetate, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of cycloartenyl ferulate for thin-layer chromatography in 1 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (5:2) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple fluorescent spot obtained from the standard solution.

Chrysanthemum Flower

キクカ

Change the Description and Identification as follows:

Description **1)** *Chrysanthemum morifolium* origin—Capitulum, 15 – 40 mm in diameter; involucre consisting of 3 to 4 rows of involucral scales; the outer involucral scale linear to lanceolate, inner involucral scale narrow ovate to ovate; ligulate flowers are numerous, white to yellow; tubular flowers in small number, light yellow-brown; tubular flowers occasionally degenerate; outer surface of involucre green-brown to brown; light in texture and easy to break.

Odor, characteristic; taste, slightly bitter.

2) *Chrysanthemum indicum* origin—Capitulum, 3 – 10 mm in diameter; involucre consisting of 3 to 5 rows of involucral scales; the outer involucral scale linear to lanceolatae, inner involucral scale narrow ovate to ovate; ligulate flower is single, yellow to light yellow-brown; tubular flowers in numerous, light yellow-brown; outer surface of involucre yellow-brown to brown; light in texture and easy to break.

Odor, characteristic; taste, slightly bitter.

Identification To 1 g of pulverized Chrysanthemum Flower add 20 mL of methanol, shake for 10 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of methanol, and use this as the sample solution. Separately, dissolve 1 mg of luteolin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, 2-butanone, water and formic acid (25:3:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark green spot obtained from the standard solution.

Add the following:

Cistanche Herb

Cistanchis Herba

ニクジュヨウ

Cistanche Herb is stout stem of 1) *Cistanche salsa* G. Beck, 2) *Cistanche deserticola* Y. C. Ma or 3) *Cistanche tubulosa* Wight (*Orobanchaceae*), spadix removed in case flowers open.

Description **1)** *Cistanche salsa* origin—Flatly cylindrical, 5 – 25 cm in length, 1 – 2.5 cm in diameter; the one end mostly slightly narrow and curved; external surface brown to blackish brown, covered with thick scales; fleshy and solid, slightly soft and oily, hardly broken; fractured surface yellow-brown to brown, vascular bundles light brown and arranged in a wavy ring.

Odor, characteristic; taste, slightly sweet, followed by slight bitterness.

Under a microscope <*5.01*> a transverse section of middle part reveals the outermost part is a single layered epidermis coated with cuticle; cortex composed of parenchyma; collateral vascular bundles fusiform or rhombic and arranged in a wavy ring in the inner portion of cortex; groups of cells with slightly thickened cell walls sometimes attached outside of phloem of collateral vascular bundles, and exhibit tail like form; pith composed of parenchyma; parenchyma contains starch grains or gelatinized starch.

2) *Cistanche deserticola* origin—Flatly cylindrical, and approximate to 1), but large in size, 5 – 50 cm in length, 1 – 8 cm in diameter.

Odor, characteristic; taste, slightly sweet, followed by slight bitterness.

Under a microscope <*5.01*> a transverse section of middle part reveals, approximate to 1).

3) *Cistanche tubulosa* origin—Flatly fusiform to cylindrical, slightly curved, 5 – 25 cm in length, 2 – 9 cm in diameter; external surface brown to blackish brown, covered with thick scales; solid in texture and firm, hardly broken; fractured surface light grayish brown to yellow-brown, vascular bundles yellow-white and scattered throughout the surface.

Odor, characteristic; taste, slightly sweet, followed by slight bitterness.

Under a microscope <*5.01*> a transverse section of middle part reveals, approximate to 1) and 2), but collateral vascular bundles distributed throughout the parenchyma from marginal region to the center of transverse section; cells with slightly thickened cell walls observed sometimes around collateral vascular bundles, but exhibit no tail like form;

Identification To 1 g of pulverized Cistanche Herb add 5 mL of water and 5 mL of 1-butanol, shake for 15 minutes, centrifuge, and use the 1-butanol layer as the sample solution. Separately, dissolve 1 mg of verbascoside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chlolo-1,4-benzoquinone monoimine TS on the plate, and allow to stand in an ammonia gas: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of

pulverized Cistanche Herb according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Cistanche Herb according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <*5.01*> Not more than 20.0%.

Total ash <*5.01*> Not more than 11.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 35.0%.

Containers and storage Containers—Well-closed containers.

Clove Oil

チョウジ油

Delete the following item:

Optical rotation

Change the Purity as follows:

Purity (1) Clarity of solution—Dissolve 1.0 mL of Clove Oil in 2.0 mL of diluted ethanol (7 in 10): the solution is clear.

(2) Water-soluble phenols—To 1.0 mL of Clove Oil add 20 mL of boiling water, shake vigorously, filter the aqueous layer after cooling, and add 1 to 2 drops of iron (III) chloride TS: a yellow-green, but no blue or violet, color develops.

(3) Heavy metals <*1.07*>—Proceed with 1.0 mL of Clove Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

(4) Optical rotation <*2.49*> α_D^{20}: 0 – −1.5° (100 mm).

Coptis Rhizome

オウレン

Change the origin/limits of content and Purity as follows:

Coptis Rhizome is the rhizome of *Coptis japonica* Makino, *Coptis chinensis* Franchet, *Coptis deltoidea* C. Y. Cheng et Hsiao or *Coptis teeta* Wallich (*Ranunculaceae*), from which the roots have been removed practically.

It contains not less than 4.2% of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81)], calculated on the basis of dried material.

For Coptis Rhizome used only for extracts or infusions and decoctions, the label states the restricted utilization forms.

Purity (1) Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Coptis Rhizome according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm). When the decision is difficult by this method, perform the test as directed under Atomic Absorption Spectrophotometry <*2.23*> using the test solution as the sample solution. The standard solution is prepared by adding purified water to 1.0 mL of Standard Lead Solution to make exactly 100 mL, and perform the test with these solutions according to the following conditions: the absorbance with the sample solution is not more than that with the standard solution (not more than 5 ppm). If necessary, the standard and sample solutions may be used after extracting with a solvent after addition of a chelating agent, and concentrating.

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.
Lamp: A lead hollow-cathode lamp.
Wavelength: 283.3 nm.

The procedure and permissible limit for Coptis Rhizome labeled to be used for extracts or infusions and decoctions are as follows.

To 4.0 g of moderately fine cuttings of Coptis Rhizome add 80 mL of water, and heat until the amount becomes about 40 mL with occasional stirring. After cooling, filter, and proceed with the filtrate according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Coptis Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Powdered Coptis Rhizome

オウレン末

Change the Purity as follows:

Purity (1) Phellodendron bark—Under a microscope <*5.01*>, crystal cell rows or mucilage masses are not observable. Stir 0.5 g of Powdered Coptis Rhizome with 2 mL of water: the solution does not become gelatinous.

(2) Curcuma—Place Powdered Coptis Rhizome on a filter paper, drop diethyl ether on it, and allow to stand. Remove the powder from the filter paper, and drop 1 drop of potassium hydroxide TS: no red-purple color develops. Under a microscope <*5.01*>, Powdered Coptis Rhizome does not contain gelatinized starch or secretory cells containing yellow-red resin.

(3) Heavy metals <*1.07*>—Proceed with 1.0 g of Powdered Coptis Rhizome according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm). When the decision is difficult by this method, perform the test as directed under Atomic Absorption Spectrophotometry <*2.23*> using

the test solution as the sample solution. The standard solution is prepared by adding purified water to 1.0 mL of Standard Lead Solution to make exactly 100 mL, and perform the test with these solutions according to the following conditions: the absorbance with the sample solution is not more than that with the standard solution (not more than 5 ppm). If necessary, the standard and sample solutions may be used after extracting with a solvent after addition of a chelating agent, and concentrating.

Gas: Combustible gas—Acetylene or hydrogen.
Supporting gas—Air.

Lamp: A lead hollow-cathode lamp.

Wavelength: 283.3 nm.

(4) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Coptis Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Cornus Fruit

サンシュユ

Change the Identification as follows:

Identification To 1 g of coarse cuttings of Cornus Fruit add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of loganin for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with a red-purple spot obtained from the standard solution. Further, a spot, slightly different in color tone from the above-mentioned spot, is found immediately below of the spot.

Crataegus Fruit

サンザシ

Change the Description and Identification as follows:

Description

1) *Crataegus cuneata* origin—Nearly spherical fruits, 8 – 14 mm in diameter; externally yellow-brown to grayish brown, with fine reticulated wrinkles, remained dent of 4 – 6 mm in diameter at one end, often the base of calyx around the dent, short peduncle or scar at the other end.True fruits, usually five loculus, often split five, mericarp, 5 – 8 mm in length, light brown, usually, containing one seed into each mericarp.

Almost odorless; taste, slightly acid.

Under a microscope <*5.01*>, a transverse section of central parts reveals in the outermost layer composed of epidermis to be covered with comparatively thick cuticle layer, cuticle intrude into lateral cell walls of epidermis, and reveal wedge-like. Cell of the epidermis or 2- to 3-layer of parenchyma cells beneath these observed contents of yellow-brown to red-brown in color followed these appeared parenchyma. Vascular bundles and numerous stone cells appear single or gathered 2 to several cells scattered on the parenchyma, and observed solitary crystals and clustera crystals of calcium oxalate. Pericarp of true fruits composed of mainly sclerenchyma cells, seed covered with seed coats, perisperm, endosperm, cotyledon observed inside seed coats; sclerenchyma cells of true fruits and cells of seed coats containing solitary crystals of calcium oxalate.

2) *Crataegus pinnatifida* var. *major* origin—Approximate to 1), but it is large in size, 17 – 23 mm in diameter, the outer surface red-brown and lustrous, spot-like scars of hairs are distinct. At one end remained dent, 7 – 9 mm in diameter, mericarp, 10 – 12 mm in length, yellow-brown in color, usually ripe seeds are absent.

Odor, characteristic; taste, acid.

Under a microscope <*5.01*>, a transverse section of the central parts approximate to 1), but it contains a few stone cells in parenchyma.

Identification

1) *Crataegus cuneata* origin—To 1.0 g of pulverized Crataegus Fruit add 5 mL of methanol, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of rutin for thin-layer chromatography in 20 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, 2-butanone, water and formic acid (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the green fluorescent spot obtained from the standard solution, and one or two similar green fluorescent spots are found at an *R*f value of about 0.5. These spots disappear gradually by allowing to cool, and appear again by heating.

2) *Crataegus pinnatifida* var. *major* origin—To 1 g of pulverized Crataegus Fruit add 5 mL of methanol, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of hyperoside for thin-layer chromatography in 20 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer

chromatography, develop the plate with a mixture of ethyl acetate, 2-butanone, water and formic acid (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the green fluorescent spot obtained from the standard solution, and a similar fluorescent spot is found just above the spot. These spots disappear gradually by allowing to cool, and appear again by heating.

Daiokanzoto Extract

大黄甘草湯エキス

Change the Identification (1) as follows:

Identification **(1)** To 1.0 g of Daiokanzoto Extract add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the orange fluorescent spot obtained from the standard solution (Rhubarb).

Add the following:

Daisaikoto Extract

大柴胡湯エキス

Daisaikoto Extract contains not less than 1.8 mg and not more than 7.2 mg of saikosaponin b_2, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 26 mg and not more than 78 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)	5)
Bupleurum Root	6 g	6 g	6 g	6 g	6 g
Pinellia Tuber	4 g	4 g	4 g	3 g	4 g
Scutellaria Root	3 g	3 g	3 g	3 g	3 g
Peony Root	3 g	3 g	3 g	3 g	3 g
Jujube	3 g	3 g	3 g	3 g	3 g
Immature Orange	2 g	2 g	2 g	2 g	2 g
Ginger	1 g	1 g	2 g	1 g	1.5 g
Rhubarb	1 g	2 g	1 g	1 g	2 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 5), using the crude drugs shown above.

Description Daisaikoto Extract occurs as light yellow-brown to brown powder or blackish brown viscous extract, having a slightly order, and a hot first, then a bitter taste.

Identification **(1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown to grayish brown spot obtained from the standard solution (Scutellaria Root).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sam-

ple solution. Separately, dissolve 1 mg of Paeoniflorin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzoaldehyde-sulfuric acid TS on the plate, heat at 105°C for 2 minutes, and examine: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple to purple spot obtained from the standard solution (Peony Root).

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 1.0 g of powdered immature orange add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in an ammonia gas: two consecutive spots at *R*f values of about 0.7 obtained from the sample solution have respectively the same color tone and *R*f value with the blue-green spot and blue spot underneath obtained from the standard solution (Immature Orange).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot obtained from the standard solution (Ginger).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the orange fluorescent spot obtained from the standard solution (Rhubarb).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed in Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 11.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 9.0%, calculated on the dried basis.

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, separate the supernatant liquid, combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of saikosaponin b_2 for assay, previously dried in a desiccator (silica gel) for 24 hours or more, and dissolve in 50 mL of methanol, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of saikosaponin b_2 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of saikosaponin b_2 for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute (the retention time of saikosaponin b_2 is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.1 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water), dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Baicalin RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, elute with 20 mL of water, add 1 mL of acetic acid (100), to the effluent, then add water to make exactly 25 mL, and use this as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S \times 5/8$$

M_S: Amount (mg) of Paeoniflorin RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

Containers and storage Containers—Tight containers.

Ephedra Herb

マオウ

Change the origin/limits of content as follows:

Ephedra Herb is the terrestrial stem of *Ephedra sinica* Stapf, *Ephedra intermedia* Schrenk et C.A. Meyer or *Ephedra equisetina* Bunge (*Ephedraceae*).

Ephedra Herb contains not less than 0.7% of total alkaloids [as ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], calculated on the basis of dried material.

Add the following next to the Purity:

Loss on drying ⟨*5.01*⟩ Not more than 12.5% (6 hours).

Change the Assay as follows:

Assay Weigh accurately about 0.5 g of moderately fine powder of Ephedra Herb, place in a glass-stoppered centrifuge tube, add 20 mL of diluted methanol (1 in 2), shake for 30 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure twice with the residue using 20-mL portion of diluted methanol (1 in 2). Combine all the extracts, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of ephedrine hydrochloride for assay of crude drugs, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 20 mL. Pipet 2 mL of the solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine (the relative retention time to ephedrine is about 0.9) obtained from the sample solution, and the peak area, A_S, of ephedrine from the standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]

$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drugs

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, and add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: Adjust the flow rate so that the retention time of ephedrine is about 27 minutes.

System suitability—

System performance: Dissolve 1 mg of ephedrine hydrochloride for assay of crude drugs and 1 mg of pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

Gardenia Fruit

サンシシ

Change the Identification (2) and Assay as follows:

Identification

(2) To 1.0 g of pulverized Gardenia Fruit add 20 mL of methanol, warm for 3 minutes on a water bath, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and methanol (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot obtained from the standard solution.

Assay Weigh accurately about 0.5 g of pulverized Gardenia Fruit, transfer into a glass-stoppered centrifuge tube, add 40 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and take the supernatant liquid. To the residue add 40 mL of diluted methanol (1 in 2), and repeat the same procedure as above. Combine the extracts so obtained, and add diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 20 mL, use this solution as the sample solution. Separately, weigh accurately about 10 mg of geniposide for assay, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine

the peak areas of geniposide, A_T and A_S, in each solution.

$$\text{Amount (mg) of geniposide} = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of geniposide for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (22:3).

Flow rate: Adjust the flow rate so that the retention time of geniposide is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of geniposide for assay and caffeine in methanol to make 15 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, caffeine and geniposide are eluted in this order with the resolution between these peaks being not less than 3.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

Powdered Gardenia Fruit

サンシシ末

Change the Identification (2) and Assay as follows:

Identification

(2) To 1.0 g of Powdered Gardenia Fruit add 20 mL of methanol, warm for 3 minutes on a water bath, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and methanol (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot obtained from the standard solution.

Assay Weigh accurately about 0.5 g of Powdered Gardenia Fruit, transfer into a glass-stoppered centrifuge tube, add 40 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and take the supernatant liquid. To the residue add 40 mL of diluted methanol (1 in 2), and repeat the same procedure as above. Combine the extracts so obtained, and add diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 20 mL, use this solution as the sample solution. Separately, weigh accurately about 10 mg of geniposide for assay, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas of geniposide, A_T and A_S, in each solution.

$$\text{Amount (mg) of geniposide} = M_S \times A_T/A_S \times 2$$

M_S: Amount (mg) of geniposide for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (22:3).

Flow rate: Adjust the flow rate so that the retention time of geniposide is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of geniposide for assay and caffeine in methanol to make 15 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, caffeine and geniposide are eluted in this order with the resolution between these peaks being not less than 3.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

Gentian

ゲンチアナ

Change the Purity as follows:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of pulverized Gentian according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Gentian according to Method 4, and perform the test (not more than 5 ppm).

Powdered Gentian

ゲンチアナ末

Change the Purity as follows:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Powdered Gentian according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of Powdered Gentian according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, stone cell and fiber are not observed.

Glycyrrhiza

カンゾウ

Change the Assay as follows:

Assay Weigh accurately about 0.5 g of pulverized Glycyrrhiza in a glass-stoppered centrifuge tube, add 70 mL of dilute ethanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 25 mL of dilute ethanol, and proceed in the same manner. Combine all the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Glycyrrhizic Acid RS (previously determine the water), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: Use a column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of glycyrrhizic acid is about 10 minutes.

System suitability—

System performance: Dissolve 1 mg of propyl parahydroxybenzoate for resolution check in 20 mL of the standard solution. When the procedure is run with 20 μL of this solution under the above operating conditions, glycyrrhizic acid and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Powdered Glycyrrhiza

カンゾウ末

Change the Assay as follows:

Assay Weigh accurately about 0.5 g of Powdered Glycyrrhiza in a glass-stoppered centrifuge tube, add 70 mL of dilute ethanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 25 mL of dilute ethanol, and proceed in the same manner. Combine all the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Glycyrrhizic Acid RS (separately determine the water), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: Use a column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of glycyrrhizic acid is about 10 minutes.

System suitability—

System performance: Dissolve 1 mg of propyl parahydroxybenzoate for resolution check in 20 mL of the standard solution. When the procedure is run with 20 μL of this solution under the above operating conditions, glycyrrhizic acid and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Exsiccated Gypsum

焼セッコウ

Add the following latin name next to the title:

Gypsum Exsiccatum

Hangekobokuto Extract

半夏厚朴湯エキス

Change the Assay (1) as follows:

Assay (1) Magnolol—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of magnolol for assay, and dissolve in diluted methanol (7 in 10) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of magnolol in each solution.

$$\text{Amount (mg) of magnolol} = M_S \times A_T/A_S \times 1/8$$

M_S: Amount (mg) of magnolol for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: 1.0 mL per minute (the retention time of magnolol is about 15 minutes).

System suitability—

System performance: Dissolve 1 mg each of magnolol for assay and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, honokiol and magnolol are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnolol is not more than 1.5%.

Hochuekkito Extract

補中益気湯エキス

Change the Identification (7) and (11) as follows:

Identification

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(11) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Use 3-(3-hydroxy-4-methoxyphenyl)-2-(*E*)-propenic acid-(*E*)-ferulic acid TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (20:12:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the light yellowish white fluorescent spot obtained from the standard solution (Cimicifuga Rhizome).

Japanese Gentian

リュウタン

Change the Identification as follows:

Identification To 0.5 g of pulverized Japanese Gentian add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and the same *R*f value with the dark purple spot obtained from the standard solution.

Jujube Seed

サンソウニン

Change the Identification and Extract content as follows:

Identification To 2 g of pulverized Jujube Seed add 10 mL of methanol, and heat under a reflux condenser for 10 minutes. After cooling, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots appear at the *R*f value of about 0.3 and about 0.4, and these spots exhibit a fluorescence when examined under ultraviolet light (main wavelength: 365 nm) after spraying evenly dilute sulfuric acid on the plate and heating at 105°C for 5 minutes.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 8.5%.

Kakkonto Extract

葛根湯エキス

Change the Identification (2) and Assay (1) as follows:

Identification

(2) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (4:4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot is observed at an *R*f value of about 0.5 (Ephedra Herb).

Assay (1) Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay of crude drugs, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine obtained with the sample solution, and the peak area, A_S, of ephedrine with the standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]

$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drugs

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, and add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay of crude drugs and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

Add the following:

Kakkontokasenkyushin'i Extract

葛根湯加川芎辛夷エキス

Kakkontokasenkyushin'i Extract contains not less than 9.5 mg and not more than 28.5 mg (for preparation prescribed 3 g of Ephedra Herb) or not less than 13 mg and not more than 39 mg (for preparation prescribed 4 g of Ephedra Herb) of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 17 mg and not more than 51 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 18 mg and not more than 54 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), and not less than 1.5 mg and not more than 6 mg (for preparation prescribed 2 g of Magnolia Flower) or not less than 2 mg and not more than 8 mg (for preparation prescribed 3 g of Magnolia Flower) of magnoflorine [magnoflorine iodide ($C_{20}H_{24}INO_4$: 469.31)], per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Pueraria Root	4 g	4 g
Ephedra Herb	4 g	3 g
Jujube	3 g	3 g
Cinnamon Bark	2 g	2 g
Peony Root	2 g	2 g
Glycyrrhiza	2 g	2 g
Ginger	1 g	1 g
Cnidium Rhizome	3 g	2 g
Magnolia Flower	3 g	2 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Kakkontokasenkyushin'i Extract occurs as light brown to blackish brown, powder or viscous extract, having a characteristic order, and a sweet first, then a bitter and hot taste.

Identification **(1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Puerarin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (Pueraria Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (4:4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot is observed at an *R*f value of about 0.5 (Ephedra Herb).

(3) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot obtained from the standard solution.

(ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycin-

namaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution.

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzoaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple to purple spot obtained from the standard solution (Peony Root).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot obtained from the standard solution (Ginger).

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 15 mL of water and 5 mL of 0.1 mol/L hydrochloric acid TS, and then shake with 25 mL of diethyl ether. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (Cnidium Rhizome).

(8) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, to 1 g of pulverized Magnolia Flower add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the brown spot (*R*f value: about 0.4) obtained from the standard solution (Magnolia Flower).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed in Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis.

Assay (1) Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the aqueous layer add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and separate the supernatant liquid. In addition, repeat twice in the same manner for the aqueous layer using 1.0 mL of ammonia TS and 20 mL of diethyl ether. Combine the supernatant liquids, evaporate the solvent under reduced pressure, dissolve the residue in diluted methanol (1 in 2) to make exactly 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay of crude drugs, previously dried at 105°C for 3 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine with the sample solution, and peak area, A_S, of ephedrine with standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]
$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drugs

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, then add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay of crude drugs and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

(2) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, elute with 20 mL of water, add 1 mL of acetic acid (100) to the effluent, then add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

Amount (mg) of paeoniflorin ($C_{23}H_{28}O_{11}$)
$= M_S \times A_T/A_S \times 5/8$

M_S: Amount (mg) of Paeoniflorin RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(3) Glycyrrhizinic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh

accurately about 10 mg of Glycyrrhizinic Acid RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizinic acid in each solution.

Amount (mg) of glycyrrhizinic acid ($C_{42}H_{62}O_{16}$)
$= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of Glycyrrhizinic Acid RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizinic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizinic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizinic acid is not more than 1.5%.

(4) Magnoflorine—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether, shake, add 3.0 mL of diluted sodium hydroxide TS (1 in 10), shake for 10 minutes, centrifuge, and remove the upper layer. Add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 3.0 mL of 0.1 mol/L hydrochloric acid TS and 20 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2) shake for 15 minutes, centrifuge, and separate the supernatant liquid. Combine the previous supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of magnoflorine iodide for assay, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of magnoflorine in each solution.

Amount (mg) of magnoflorine [as magnoflorine iodide ($C_{20}H_{24}INO_4$)]
$= M_S \times A_T/A_S \times 1/20$

M_S: Amount (mg) of magnoflorine iodide for assay, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 303 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, then add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of magnoflorine is about 20 minutes).

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of magnoflorine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnoflorine is not more than 1.5%.

Containers and storage Containers—Tight containers.

Kamishoyosan Extract

加味逍遙散エキス

Change the Identification (5) and Assay (2) as follows:

Identification

(5) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly

4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

Assay

(2) Geniposide—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of geniposide for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of geniposide in each solution.

$$\text{Amount (mg) of geniposide} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of geniposide for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (900:100:1).

Flow rate: 1.0 mL per minute (the retention time of geniposide is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of geniposide are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

Koi

コウイ

Change the Identification as follows:

Identification Dissolve exactly 0.50 g of Koi in a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve exactly 20.0 mg of maltose hydrate in a mixture of water and methanol (1:1) to make exactly 5 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography in equal size of circular spot each other. Develop the plate with a mixture of 2-butanone, water and acetic acid (100) (3:1:1) to a distance of about 7 cm, and dry at 105°C for 10 minutes the plate. Spray evenly 2,3,5-triphenyl-2*H*-tetrazolium chloride-methanol TS for spraying on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the orange spot obtained from the standard solution, and it is larger and more intense than the spot obtained from the standard solution.

Lonicera Leaf and Stem

ニンドウ

Change the Identification as follows:

Identification To 1 g of pulverized Lonicera Leaf and Stem add 5 mL of methanol, shake for 5 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of chlorogenic acid for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution (1). Separately, dissolve 1 mg of loganin for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thinlayer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (1). Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution (2).

Magnolia Bark

コウボク

Change the Assay as follows:

Assay Weigh accurately about 0.5 g of pulverized Magnolia Bark, add 40 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 20 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of diluted methanol (7 in 10). Combine the whole

filtrates, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of magnolol for assay, dissolve in diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of magnolol in each solution.

$$\text{Amount (mg) of magnolol} = M_S \times A_T/A_S$$

M_S: Amount (mg) of magnolol for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: Adjust the flow rate so that the retention time of magnolol is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg each of magnolol for assay and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, honokiol and magnolol are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnolol is not more than 1.5%.

Powdered Magnolia Bark

コウボク末

Change the Assay as follows:

Assay Weigh accurately about 0.5 g of Powdered Magnolia Bark, add 40 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 20 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of diluted methanol (7 in 10). Combine the whole filtrates, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of magnolol for assay, dissolve in diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of magnolol in each solution.

$$\text{Amount (mg) of magnolol} = M_S \times A_T/A_S$$

M_S: Amount (mg) of magnolol for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: Adjust the flow rate so that the retention time of magnolol is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg each of magnolol for assay and honokiol in diluted methanol (7 in 10) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, honokiol and magnolol are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of magnolol is not more than 1.5%.

Mallotus Bark

アカメガシワ

Change the Identification as follows:

Identification To 0.5 g pulverized Mallotus Bark add 10 mL of methanol, warm on a water bath for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of bergenin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (100:17:13) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the dark blue spot obtained from the standard solution.

Add the following:

Maoto Extract

麻黄湯エキス

Maoto Extract contains not less than 15 mg and not more than 45 mg of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 48 mg and not more than 192 mg of amygdalin, and not less than 14 mg and not more than 42 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)
Ephedra Herb	5 g
Apricot Kernel	5 g
Cinnamon Bark	4 g
Glycyrrhiza	1.5 g

Prepare a dry extract or viscous extract as directed under Extracts, or prepare a dry extract by adding Light Anhydrous Silicic Acid to the extractive prepared as directed under Extracts, according to the prescription 1), using the crude drugs shown above.

Description Maoto Extract occurs as light brown to blackish brown, powder or viscous extract, having a slightly order, and a sweet and bitter, then a slightly astringent taste.

Identification **(1)** Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (4:4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot is observed at an *R*f value of about 0.5 (Ephedra Herb).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate and water (4:4:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the green-brown spot obtained from the standard solution (Apricot Kernel).

(3) Perform the test according to the following (i) or (ii) (Cinnamon Bark).

(i) Put 10 g of the dry extract (or 30 g of viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot obtained from the standard solution.

(ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution.

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution

(Glycyrrhiza).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed in Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 9.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 13.0%, calculated on the dried basis. However, for the dry extract prepared by adding Light Anhydrous Silicic Acid, between 10.0% and 22.0%.

Assay **(1)** Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to 0.5 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the aqueous layer add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and separate the supernatant liquid. In addition, repeat twice in the same manner for the aqueous layer using 1.0 mL of ammonia TS and 20 mL of diethyl ether. Combine all the supernatant liquids, evaporate the solvent under reduced pressure, dissolve the residue in diluted methanol (1 in 2) to make exactly 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay of crude drugs, previously dried at 105°C for 3 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine obtained from the sample solution, and peak area, A_S, of ephedrine from the standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]

$$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drugs

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, then add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay of crude drugs and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

(2) Amygdalin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, then elute with water to make exactly 20 mL, and use this effluent as the sample solution. Separately, weigh accurately about 10 mg of amygdalin for assay, previously dried in a desiccator (silica gel) for 24 hours or more, and dissolve in diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of amygdalin in each solution.

$$\text{Amount (mg) of amygdalin} = M_S \times A_T/A_S \times 4$$

M_S: Amount (mg) of amygdalin for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and methanol (5:1).

Flow rate: 0.8 mL per minute (the retention time of amygdalin is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating con-

ditions, the number of theoretical plates and the symmetry factor of the peak of amygdalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amygdalin is not more than 1.5%.

(3) Glycyrrhizinic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizinic Acid RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizinic acid in each solution.

Amount (mg) of glycyrrhizinic acid ($C_{42}H_{62}O_{16}$) $= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of Glycyrrhizinic Acid RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizinic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizinic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizinic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Mentha Herb

ハッカ

Change the Total ash as follows:

Total ash <*5.01*> Not more than 12.0%.

Mentha Oil

ハッカ油

Change the Optical rotation as follows:

Optical rotation <*2.49*> α_D^{20}: −17.0 – −36.0° (100 mm).

Moutan Bark

ボタンピ

Change the Identification and Assay as follows:

Identification To 2.0 g of pulverized Moutan Bark add 10 mL of hexane, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 1 mL of hexane, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Assay Weigh accurately about 0.3 g of pulverized Moutan Bark, add 40 mL of methanol, heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of methanol. Combine the whole filtrates, add methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of paeonol for assay, dissolve in methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeonol in each solution.

Amount (mg) of paeonol $= M_S \times A_T/A_S \times 1/2$

M_S: Amount (mg) of paeonol for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile, and acetic acid (100) (65:35:2).

Flow rate: Adjust the flow rate so that the retention time of paeonol is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg of paeonol for assay and 5 mg of butyl parahydroxybenzoate for resolution check in methanol to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, paeonol and butyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeonol is not more than 1.5%.

Powdered Moutan Bark

ボタンピ末

Change the Identification (1) and Assay as follows:

Identification **(1)** To 2.0 g of Powdered Moutan Bark add 10 mL of hexane, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 1 mL of hexane, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Assay Weigh accurately about 0.5 g of Powdered Moutan Bark, add 40 mL of methanol, heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of methanol. Combine the whole filtrates, add methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of paeonol for assay, dissolve in methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeonol in each solution.

$$\text{Amount (mg) of paeonol} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of paeonol for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile, and acetic acid (100) (65:35:2).

Flow rate: Adjust the flow rate so that the retention time of paeonol is about 14 minutes.

System suitability—

System performance: Dissolve 1 mg of paeonol for assay and 5 mg of butyl parahydroxybenzoate for resolution check in methanol to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, paeonol and butyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeonol is not more than 1.5%.

Nutmeg

ニクズク

Change the Identification as follows:

Identification To 1 g of pulverized Nutmeg add 5 mL of methanol, allow to stand for 10 minutes with occasional shaking, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of myristicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (9:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Orange Oil

オレンジ油

Change the Optical rotation as follows:

Optical rotation <*2.49*> α_D^{20}: +43 – +50° (50 mm).

Orengedokuto Extract

黄連解毒湯エキス

Change the Assay (3) as follows:

Assay

(3) Geniposide—Weigh accurately about 0.2 g of the dry extract (or an amount of the viscous extract, equivalent to 0.2 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of geniposide for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of geniposide in each solution.

$$\text{Amount (mg) of geniposide} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of geniposide for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (900:100:1).

Flow rate: 1.0 mL per minute (the retention time of geniposide is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of geniposide are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

Add the following:

Otsujito Extract

乙字湯エキス

Otsujito Extract contains not less than 1.2 mg and not more than 4.8 mg of saikosaponin b_2, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 17 mg and not more than 51 mg (for preparation prescribed 2 g of Glycyrrhiza) or not less than 25 mg and not more than 75 mg (for preparation prescribed 3 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)
Japanese Angelica Root	6 g	6 g	6 g
Bupleurum Root	5 g	5 g	5 g
Scutellaria Root	3 g	3 g	3 g
Glycyrrhiza	2 g	2 g	3 g
Cimicifuga Rhizome	1.5 g	1 g	1 g
Rhubarb	1 g	0.5 g	1 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 3), using the crude drugs shown above.

Description Otsujito Extract occurs as light brown to brown powder or blackish brown viscous extract, having a slightly order, and a hot and slight sweet taste.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 10 mL of diethyl ether, shake, and centrifuge. Separate the diethyl ether layer, add 10 mL of sodium hydroxide TS, shake, centrifuge, separate the diethyl ether layer, and use this layer as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of butyl acetate and hexane (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot obtained from the standard solution (Japanese Angelica Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use

this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown to grayish brown spot obtained from the standard solution (Scutellaria Root).

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution (Glycyrrhiza).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Use 3-(3-hydroxy-4-methoxyphenyl)-2-(*E*)-propenic acid-(*E*)-ferulic acid TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (20:12:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the light yellowish white fluorescent spot obtained from the standard solution (Cimicifuga Rhizome).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the orange fluorescent spot obtained from the standard solution (Rhubarb).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of the dry extract (or an amount of the viscous extract, equivalent to 1.0 g of dried substance) as directed under Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of the dry extract (or an amount of the viscous extract, equivalent to 0.67 g of dried substance) according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> The dry extract: Not more than 9.5% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.5%, calculated on the dried basis.

Assay **(1)** Saikosaponin b_2—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, separate the supernatant liquid, combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of saikosaponin b_2 for assay, previously dried in a desiccator (silica gel) for 24

hours or more, and dissolve in 50 mL of methanol, and add water to make exactly 100 mL. Pipet 10 mL of this solution, and add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of saikosaponin b_2 in each solution.

$$\text{Amount (mg) of saikosaponin } b_2 = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of saikosaponin b_2 for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL per minute (the retention time of saikosaponin b_2 is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of the dry extract (or an amount of the viscous extract, equivalent to 0.1 g of the dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water), dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of Baicalin RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute (the retention time of baicalin is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Glycyrrhizinic acid—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, separate the supernatant liquid, combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizinic Acid RS (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizinic acid in each solution.

$$\text{Amount (mg) of glycyrrhizinic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Glycyrrhizinic Acid RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in

15) and acetonitrile (13:7).

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizinic acid is about 12 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizinic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizinic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Peach Kernel

トウニン

Change the Purity (2) as follows:

Purity

(2) Foreign matter <*5.01*>—When perform the test with not less than 250 g of Peach Kernel, it contains not more than 0.10% of broken pieces of endocarp.

Peony Root

シャクヤク

Change the Identification (2) as follows:

Identification

(2) To 2 g of pulverized Peony Root add 10 mL of methanol, warm on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution.

Powdered Peony Root

シャクヤク末

Change the Identification (2) as follows:

Identification

(2) To 2 g of Powdered Peony Root add 10 mL of methanol, warm on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the purple spot obtained from the standard solution.

Perilla Herb

ソヨウ

Change the origin/limits of content and Identification as follows:

Perilla Herb is the leaves and the tips of branches of *Perilla frutescens* Britton var. *crispa* W. Deane (*Labiatae*).

It contains not less than 0.08% of perillaldehyde, calculated on the basis of dried material.

Identification To 0.6 g of pulverized Perilla Herb, add 10 mL of diethyl ether, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of perillaldehyde for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid-ethanol TS for spray on the plate, and heat at 105°C for 2 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple spot obtained from the standard solution.

Peucedanum Root

ゼンコ

Change the origin/limits of content, Description and Identification as follows:

Peucedanum Root is the root of **1)** *Peucedanum praeruptorum* Dunn (Peucedanum Praeruptorum Root) or **2)** *Angelica decursiva* Franchet et Savatier (*Peucedanum decursivum* Maximowicz) (*Umbelliferae*) (Angelica Decursiva Root).

Description **1)** Peucedanum Praeruptorum Root—Slender obconical to cylindrical root, occasionally dichotomized at the lower part 3 – 15 cm in length, 0.8 – 1.8 cm in diameter at the crown; externally light brown to dark brown; ring-node-like wrinkles numerous at the crown, sometimes with hair-like remains of petioles; the root having somewhat deep longitudinal wrinkles and scars of cutting off of lateral roots; transverse section surface light brown to whitish in color; brittle in texture.

Odor, characteristic; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section reveals the outermost layer composed of a cork layer, inner tangential walls of some cork cells thickened; collenchyma just inside of the cork layer; in cortex numerous oil canals scattered and intercellular air spaces observed; occasionally phloem fibers observed at the terminal portion of phloem; vessels and scattered oil canals in xylem; starch grains in parenchyma, 2 to 10 several-compound grains.

2) Angelica Decursiva Root—Similar to 1), but without hair-like remains of petioles at the crown.

Under a microscope <*5.01*>, a transverse section reveals, similar to 1), but cell wall of cork cells not thickened, phloem fibers not observed at the terminal portion of phloem, nor oil canals observed in xylem.

Identification **(1)** Peucedanum Praeruptorum Root—To 1 g of pulverized Peucedanum Root add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (±)-praeruptorin A for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and hexane (3:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the fluorescent spot obtained from the standard solution.

(2) Angelica Decursiva Root—To 1 g of pulverized Peucedanum Root add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of nodakenin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (12:2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the fluorescent spot obtained from the standard solution.

Phellodendron Bark

オウバク

Change the Description as follows:

Description Flat or rolled semi-tubular pieces of bark, 2 – 4 mm in thickness; externally grayish yellow-brown to grayish brown, with numerous traces of lenticels; the internal surface yellow to dark yellow-brown in color, with fine vertical lines, and smooth; fractured surface fibrous and bright yellow.

Odor, slight; taste, extremely bitter; mucilaginous; it colors the saliva yellow on chewing.

Under a microscope <*5.01*>, a transverse section reveals primary ray expands outward and looks fan shaped in secondary cortex, and sometimes ray differentiated later converges outward; groups of stone cells yellow and scattered in primary ray; groups of phloem fibers light yellow to yellow, lined alternately with the other tissue of phloem between rays, and then these tissues show obviously latticework; solitary crystals of calcium oxalate, single and compound starch grains observed in parenchyma.

Pogostemon Herb

カッコウ

Change the Identification as follows:

Identification To 0.5 g of pulverized Pogostemon Herb, add 5 mL of methanol, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (9:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes; a blue-purple spot appears at an *R*f value of about 0.4.

Add the following:

Prepared Glycyrrhiza

Glycyrrhizae Radix Praeparata

シャカンゾウ

Prepared Glycyrrhiza is prepared by roasting Glycyrrhiza.

It contains not less than 2.5% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), calculated on the basis of dried material.

Description Usually cut; external surface dark brown to dark red-brown and with longitudinal wrinkles; cut surface brown to light yellow-brown; in case periderm fallen off, external surface brown to light yellow-brown and fibrous; on transversely cut surface cortex and xylem almost distinctly defined, and exhibits radial structure; sometimes radial cleft observed.

Odor, fragrant; taste sweet, followed by slight bitterness.

Identification To 2.0 g of pulverized Prepared Glycyrrhiza add 10 mL of ethyl acetate, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Shake the residue with 5 mL of ethyl acetate and 5 mL of 0.1 mol/L hydrochloric acid TS for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (7:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 3 minutes, and allow to cool: a red-purple spot is observed at an *R*f value of about 0.6.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Prepared Glycyrrhiza according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.40 g of pulverized Prepared Glycyrrhiza according to Method 4, and perform the test (not more than 5 ppm).

(3) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively.

Loss on drying <*5.01*> Not more than 8.0% (6 hours).

Total ash <*5.01*> Not more than 7.0%.

Acid-insoluble ash <*5.01*> Not more than 2.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 25.0%.

Assay Weigh accurately about 0.5 g of pulverized Prepared Glycyrrhiza in a glass-stoppered centrifuge tube, add 70 mL of dilute ethanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 25 mL of dilute ethanol, and proceed in the same manner. Combine all the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Glycyrrhizic Acid RS (separately determine the water), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Glycyrrhizic Acid RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of glycyrrhizic acid is about 10 minutes.

System suitability—

System performance: Dissolve 1 mg of propyl parahydroxybenzoate for resolution check in 20 mL of the standard solution. Proceed with 20 μL of this solution under the above operating conditions, glycyrrhizic acid and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Processed Aconite Root

ブシ

Change the Identification as follows:

Identification To 3 g of pulverized Processed Aconite Root in a glass-stoppered centrifuge tube add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and take the diethyl ether layer. Evaporate the layer to dryness under reduced pressure, dissolve the residue in 1 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chro-

matography in 5 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia water (28) (40:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, air-dry the plate, and spray evenly sodium nitrite TS: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution.

Powdered Processed Aconite Root

ブシ末

Change the Identification as follows:

Identification To 3 g of Powdered Processed Aconite Root in a glass-stoppered centrifuge tube add 2 mL of ammonia TS and 20 mL of diethyl ether, shake for 10 minutes, centrifuge, and take the diethyl ether layer. Evaporate the layer to dryness under reduced pressure, dissolve the residue in 1 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 5 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia water (28) (40:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, air-dry the plate, and spray evenly sodium nitrite TS: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-brown spot obtained from the standard solution.

Rhubarb

ダイオウ

Change the Identification and Purity (3) as follows:

Identification To 1.0 g of pulverized Rhubarb add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography<*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow spot obtained from the standard solution, and the spot develops a red color on spraying sodium carbonate TS.

Purity

(3) Raponticin—To 0.1 g of pulverized Rhubarb add exactly 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of raponticin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl formate, 2-butanon, water and formic acid (10:7:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the chromatogram obtained with the sample solution shows no spot having the same color tone and *R*f value with the blue fluorescent spot obtained with the standard solution.

Powdered Rhubarb

ダイオウ末

Change the Identification and Purity (3) as follows:

Identification To 1.0 g of Powdered Rhubarb add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow spot obtained from the standard solution, and the spot develops a red color on spraying sodium carbonate TS.

Purity

(3) Raponticin—To 0.1 g of Powdered Rhubarb add exactly 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of raponticin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography.

Develop the plate with a mixture of ethyl formate, 2-butanon, water and formic acid (10:7:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the chromatogram obtained with the sample solution shows no spot having the same color tone and *R*f value with the blue fluorescent spot obtained with the standard solution.

Royal Jelly

ローヤルゼリー

Change the Identification as follows:

Identification To a portion of Royal Jelly, equivalent to 0.2 g of dried substance, add 5 mL of water, 1 mL of dilute hydrochloric acid and 10 mL of diethyl ether, shake for 15 minutes, and centrifuge. Take the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 2 mg of 10-hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of 1-propanol and ammonia solution (28) (7:3) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot obtained from the sample solution has the same color tone and *R*f value with the dark purple spot obtained from the standard solution.

Saibokuto Extract

柴朴湯エキス

Change the Identification (1) as follows:

Identification (1) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-buthanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

Saikokeishito Extract

柴胡桂枝湯エキス

Change the Identification (1) as follows:

Identification (1) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-buthanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

Saireito Extract

柴苓湯エキス

Change the Identification (1) as follows:

Identification (1) To 2.0 g of Saireito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

Schisandra Fruit

ゴミシ

Change the Identification as follows:

Identification To 1.0 g of pulverized Schisandra Fruit add 10 mL of methanol, warm on a water bath for 3 minutes with shaking, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of schisandrin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-violet spot obtained from the standard solution.

Scutellaria Root

オウゴン

Change the Assay as follows:

Assay Weigh accurately about 0.5 g of pulverized Scutellaria Root, add 30 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 30 minutes, and cool. Transfer the mixture to a glass-stoppered centrifuge tube, centrifuge, and separate the supernatant liquid. Wash the vessel for the reflux extraction with 30 mL of diluted methanol (7 in 10), transfer the washings to the glass-stoppered centrifuge tube, centrifuge after shaking for 5 minutes, and separate the supernatant liquid. To the residue add 30 mL of diluted methanol (7 in 10), shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the extracts, add diluted methanol (7 in 10) to make exactly 100 mL, then pipet 2 mL of this solution, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 5$$

M_S: Amount (mg) of Baicalin RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 146) and acetonitrile (18:7).

Flow rate: Adjust the flow rate so that the retention time of baicalin is about 6 minutes.

System suitability—

System performance: Dissolve 1 mg of Baicalin RS and 2 mg of methyl parahydroxybenzoate for resolution check in methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, baicalin and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

Powdered Scutellaria Root

オウゴン末

Change the Assay as follows:

Assay Weigh accurately about 0.5 g of Powdered Scutellaria Root, add 30 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 30 minutes, and cool. Transfer the mixture to a glass-stoppered centrifuge tube, centrifuge, and separate the supernatant liquid. Wash the vessel for the reflux extraction with 30 mL of diluted methanol (7 in 10), transfer the washings to the glass-stoppered centrifuge tube, centrifuge after shaking for 5 minutes, and separate the supernatant liquid. To the residue add 30 mL of diluted methanol (7 in 10), shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the extracts, add diluted methanol (7 in 10) to make exactly 100 mL, then pipet 2 mL of this solution, add diluted methanol (7 in 10) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of baicalin in each solution.

Amount (mg) of baicalin ($C_{21}H_{18}O_{11}$)
$= M_S \times A_T/A_S \times 5$

M_S: Amount (mg) of Baicalin RS, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 146) and acetonitrile (18:7).

Flow rate: Adjust the flow rate so that the retention time of baicalin is about 6 minutes.

System suitability—

System performance: Dissolve 1 mg of Baicalin RS and 2 mg of methyl parahydroxybenzoate for resolution check in methanol to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, baicalin and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

Senna Leaf

センナ

Change the Identification (2) as follows:

Identification

(2) To 2 g of pulverized Senna Leaf add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the aqueous layer with undissolved sodium chloride, and adjust to pH 1.5 with 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, shake with 30 mL of tetrahydrofuran for 10 minutes, separate the tetrahydrofuran layer, and use the separated tetrahydrofuran layer as the sample solution. Separately, dissolve 1 mg of Sennoside A RS in 1 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red fluorescent spot obtained from the standard solution.

Powdered Senna Leaf

センナ末

Change the Identification (2) as follows:

Identification

(2) To 2 g of Powdered Senna Leaf add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the aqueous layer with undissolved sodium chloride, and adjust to pH 1.5 with 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, shake with 30 mL of tetrahydrofuran for 10 minutes, separate the tetrahydrofuran layer, and use the separated tetrahydrofuran layer as the sample solution. Separately, dissolve 1 mg of Sennoside A RS in 1 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the red fluorescent spot obtained from the standard solution.

Sesame

ゴマ

Change the Identification as follows:

Identification Grind a suitable amount of Sesame. To 1.0 g of the ground add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of sesamin for thin-layer chromatography in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (10:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the

brown spot obtained from the standard solution.

Shosaikoto Extract

小柴胡湯エキス

Change the Identification (1) as follows:

Identification (1) Shake 2.0 g of dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot obtained from the standard solution (Bupleurum Root).

Shoseiryuto Extract

小青竜湯エキス

Change the Identification (1) and Assay (1) as follows:

Identification (1) Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol and shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid(100) (4:4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot is observed at an *R*f value of about 0.5 (Ephedra Herb).

Assay (1) Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay of crude drugs, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine obtained from the sample solution, and the peak area, A_S, of ephedrine obtained from the standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]

$$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drugs

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, and add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay of crude drugs and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

Swertia Herb

センブリ

Change the Identification as follows:

Identification To 1 g of pulverized Swertia Herb add 10 mL of ethanol (95), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of Swertiamarin RS in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate

with a mixture of ethyl acetate, 1-propanol and water (6:4:3) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Powdered Swertia Herb

センブリ末

Change the Identification as follows:

Identification To 1 g of Powdered Swertia Herb add 10 mL of ethanol (95), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of Swertiamarin RS in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol and water (6:4:3) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Toad Venom

センソ

Change the Identification as follows:

Identification To 0.3 g of pulverized Toad Venom add 3 mL of acetone, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of resibufogenin for thin-layer chromatography in 2 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of cyclohexane and acetone (3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with the spot obtained from the standard solution.

Wood Creosote

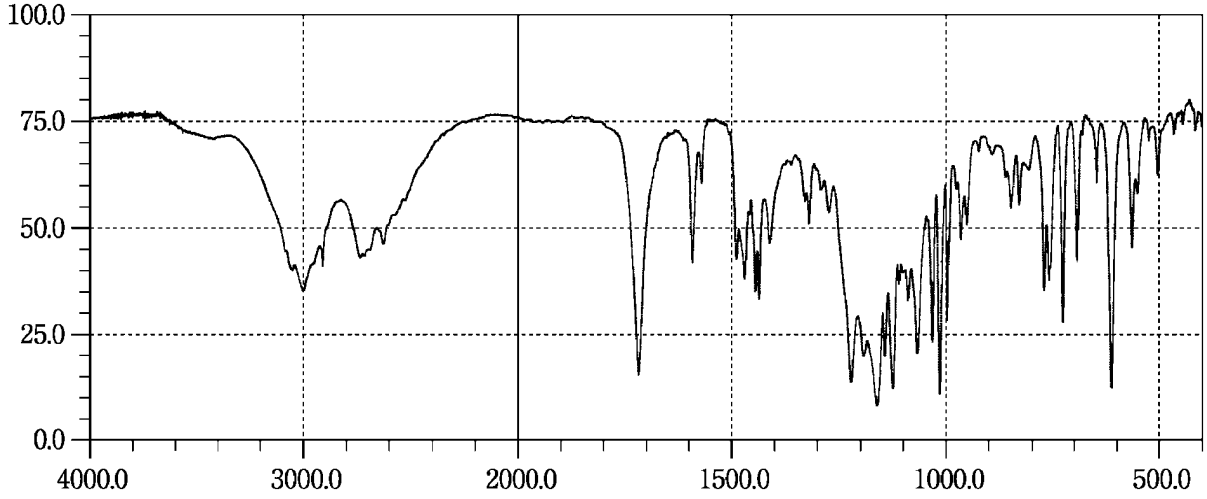
木クレオソート

Add the following latin name next to the title:

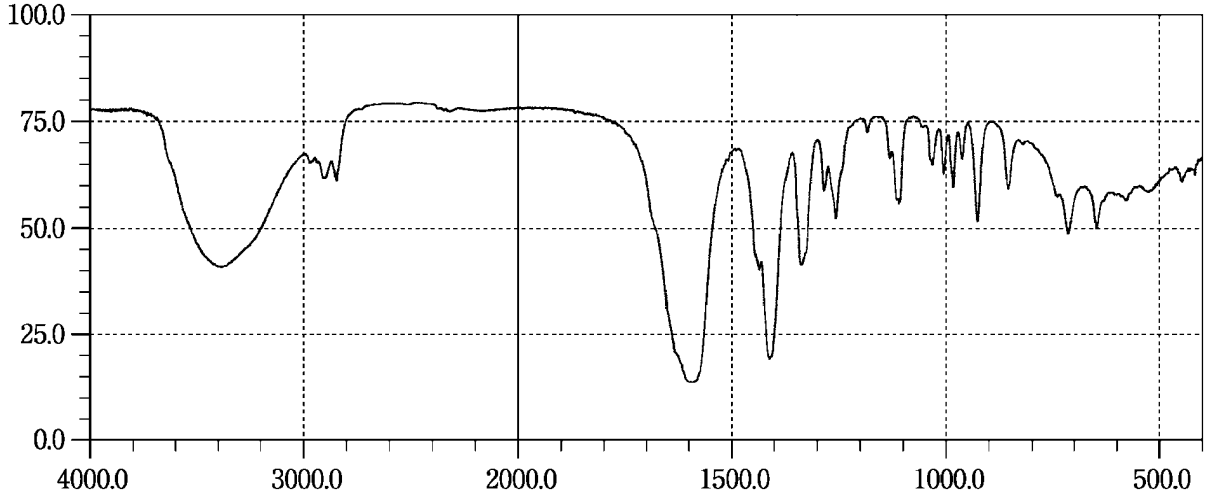
Creosotum Ligni

Add the following 17 spectra:

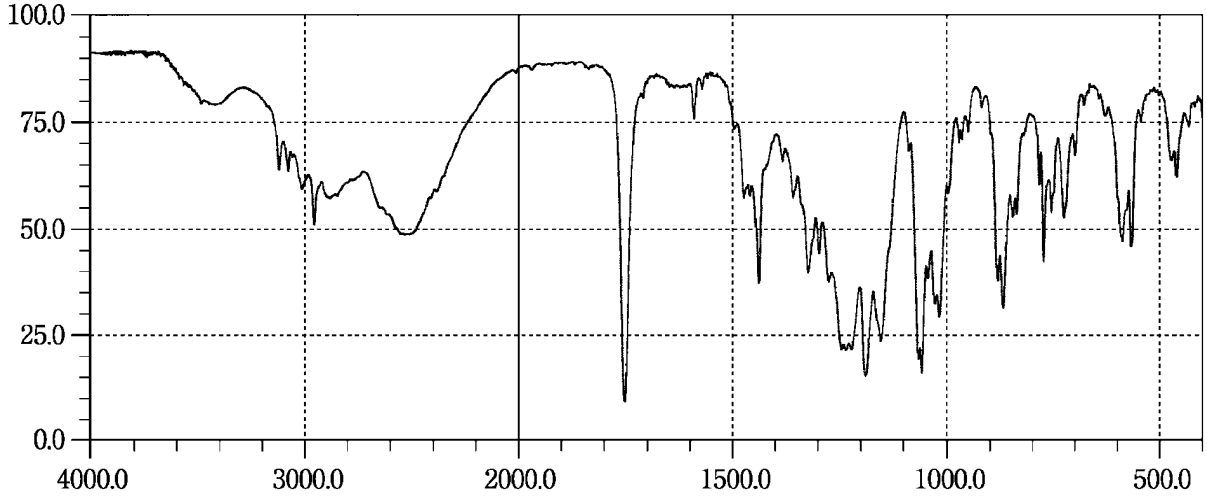
Bepotastine Besilate



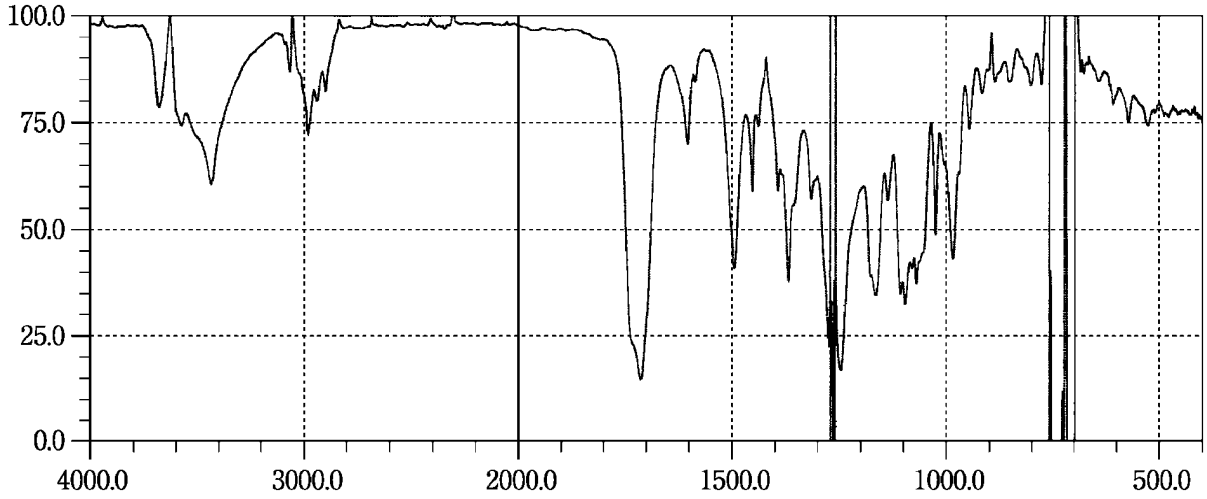
Calcium Sodium Edetate Hydrate



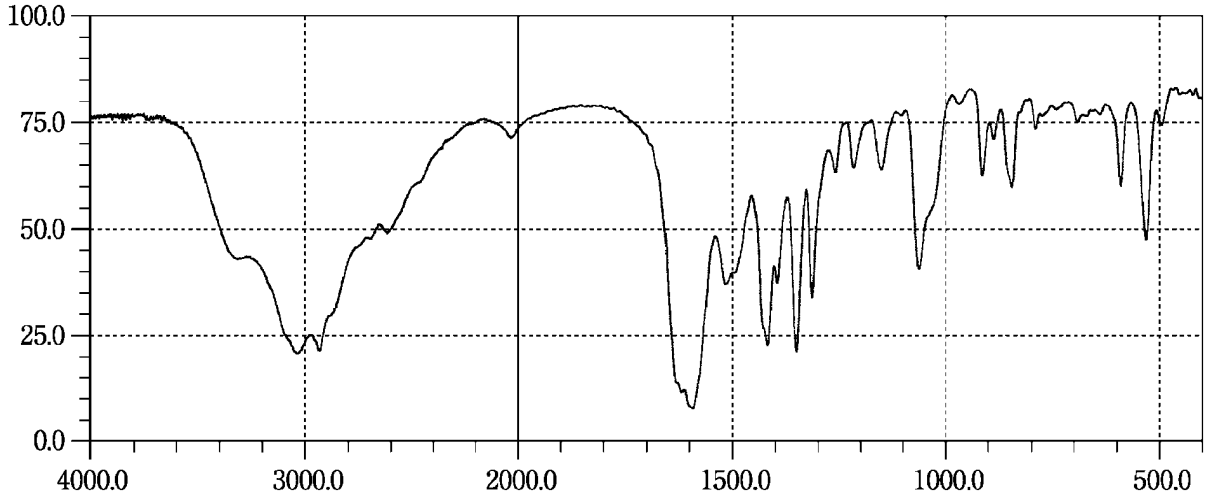
Clopidogrel Sulfate



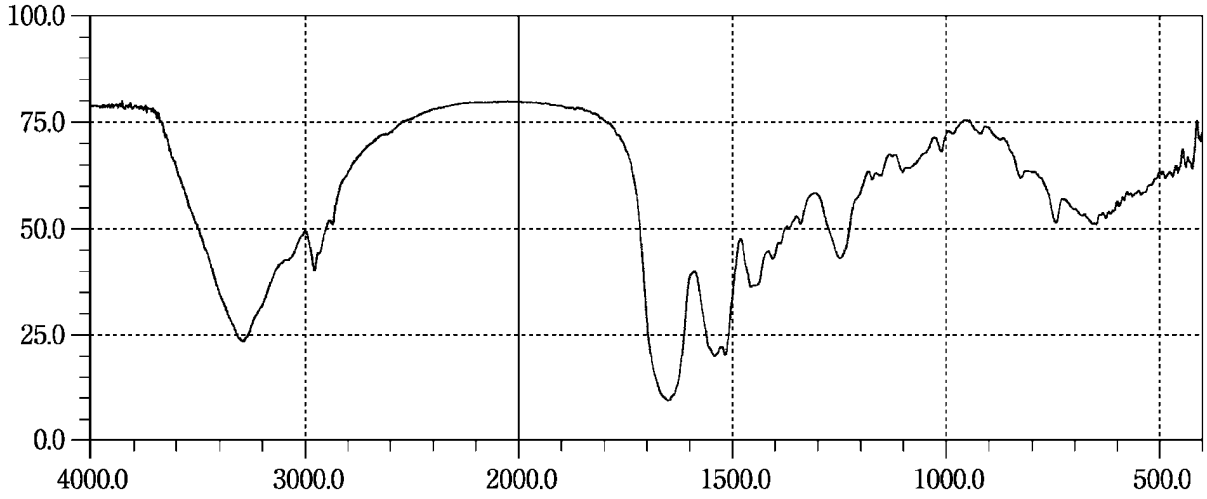
Docetaxel Hydrate



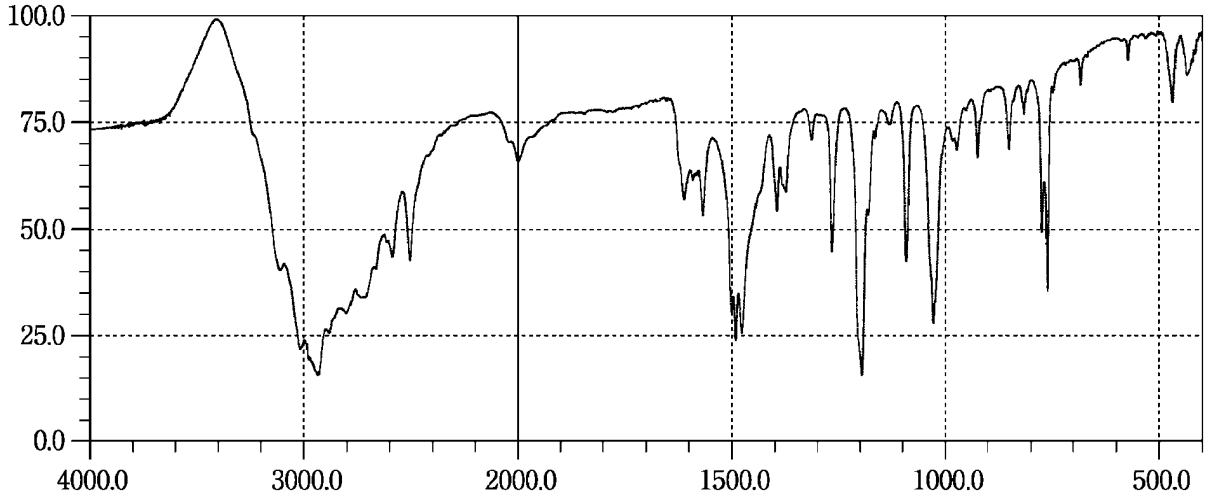
Fudosteine



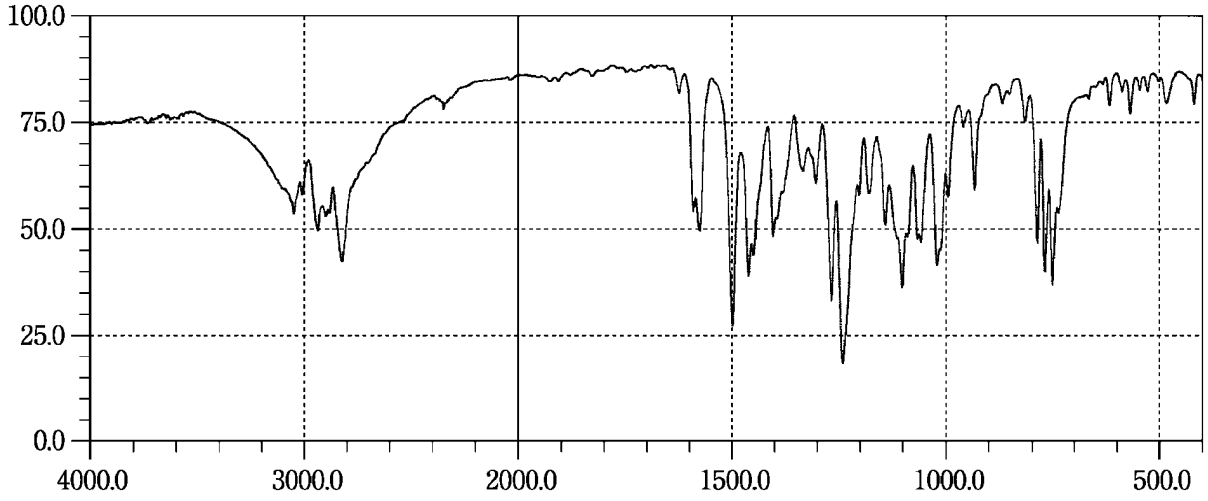
Leuprorelin Acetate



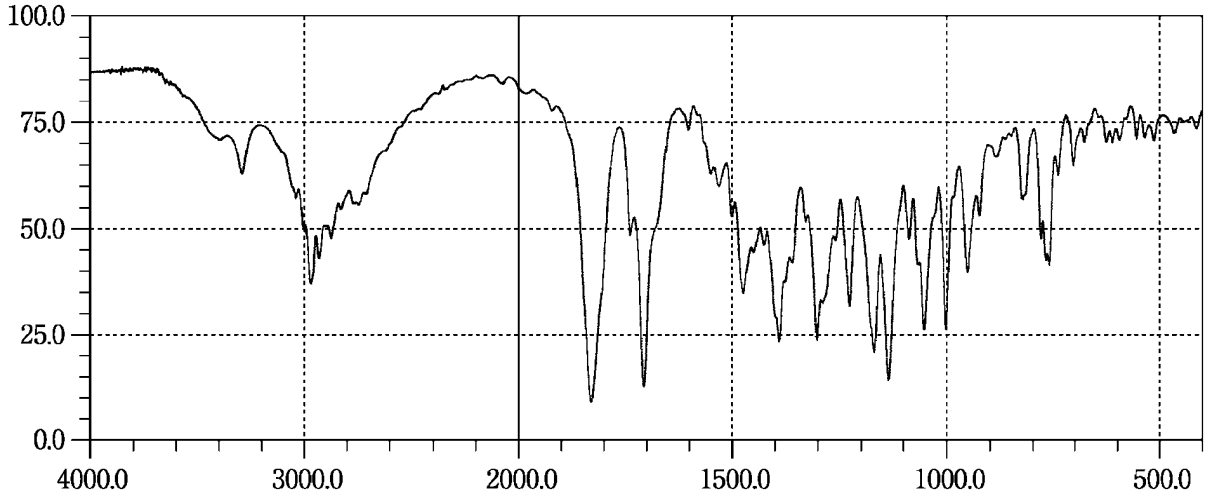
Mexiletine Hydrochloride



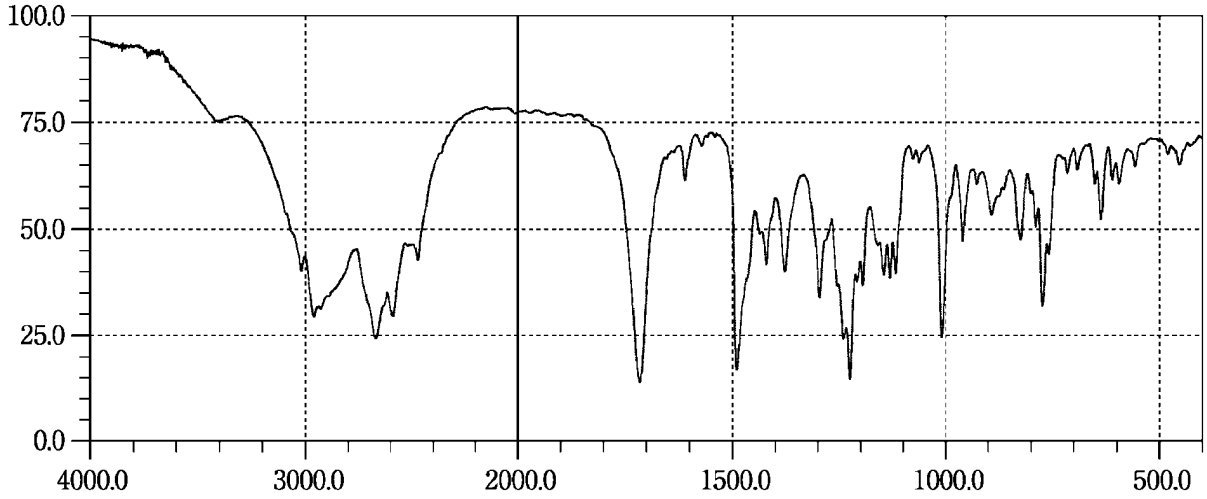
Naftopidil



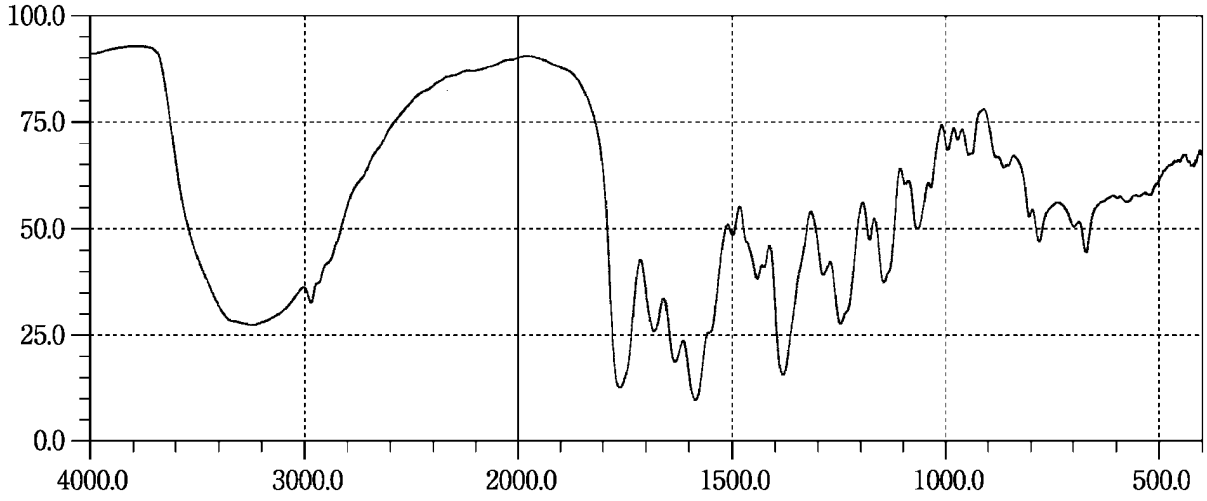
Olmesartan Medoxomil



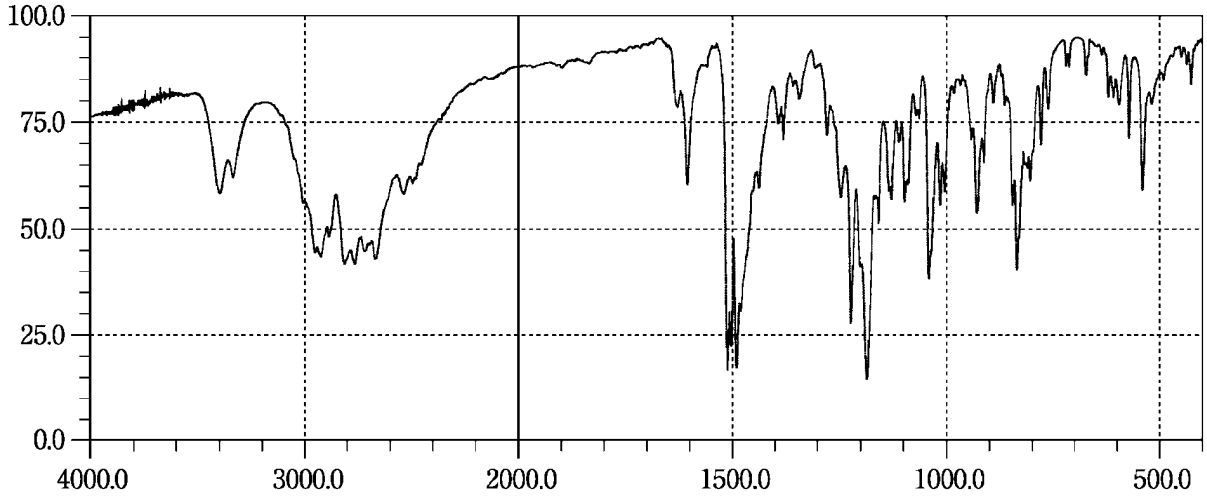
Olopatadine Hydrochloride



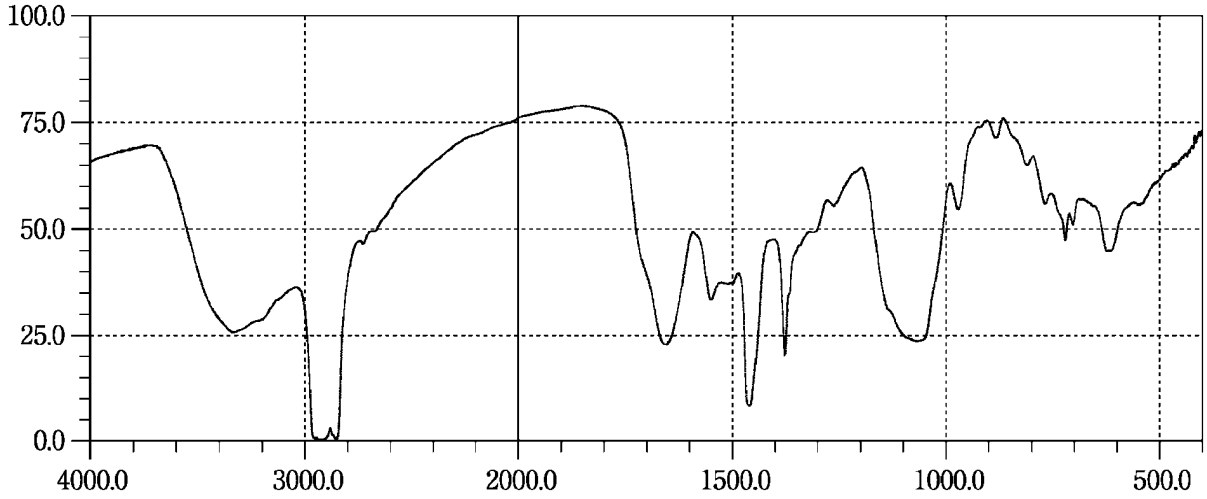
Panipenem



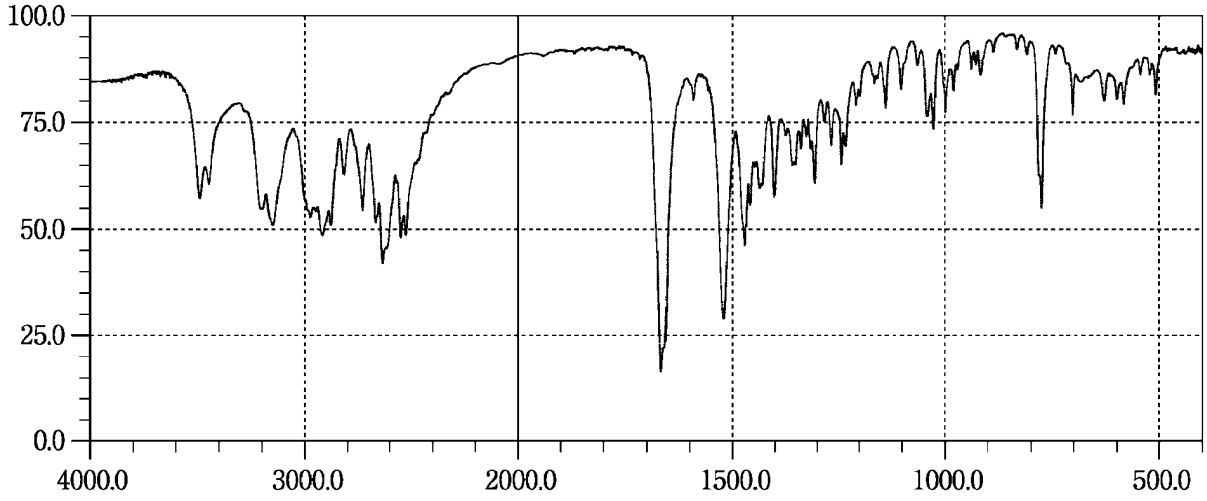
Paroxetine Hydrochloride Hydrate



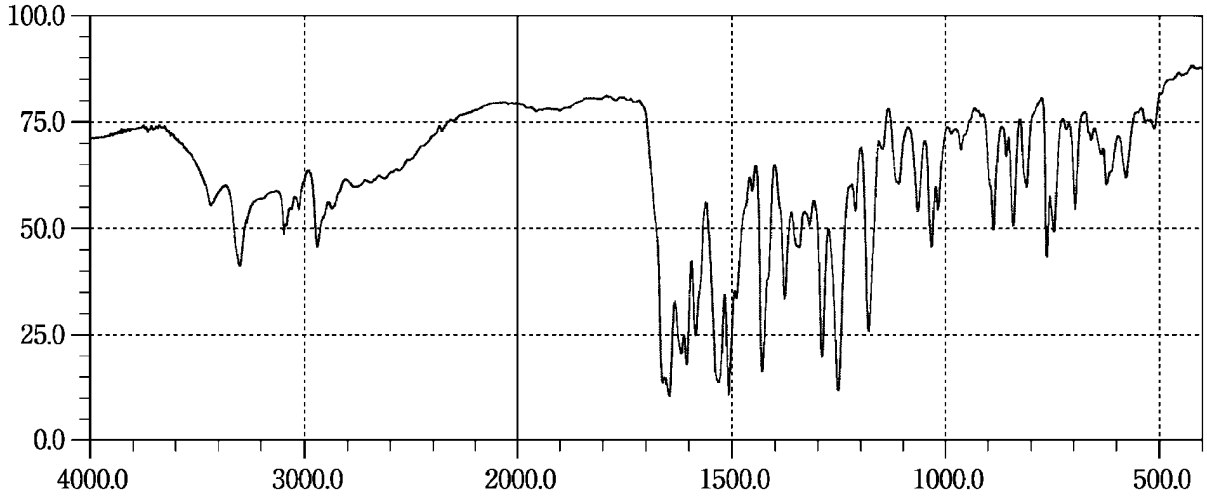
Peplomycin Sulfate



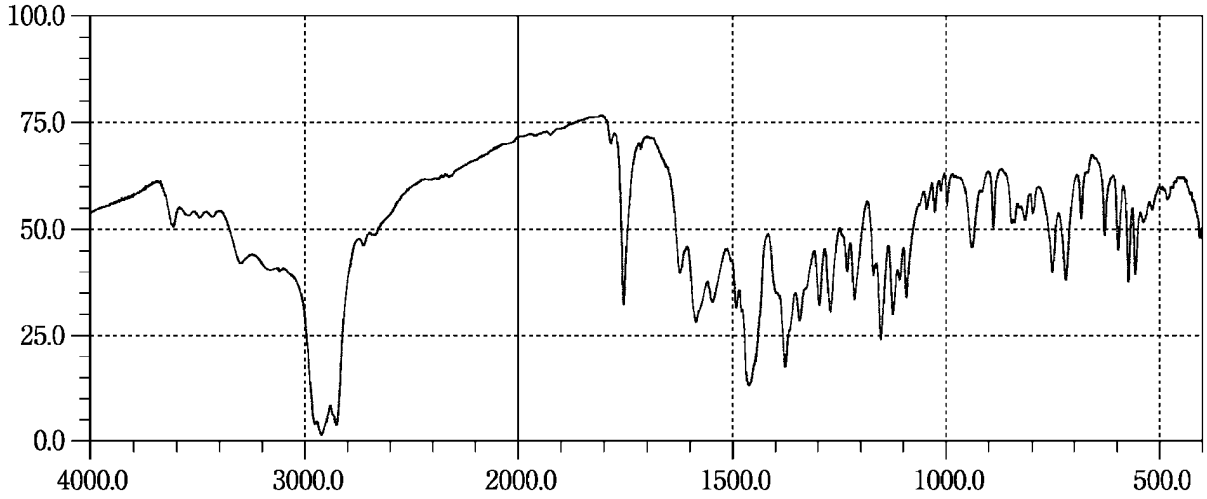
Pilsicainide Hydrochloride Hydrate



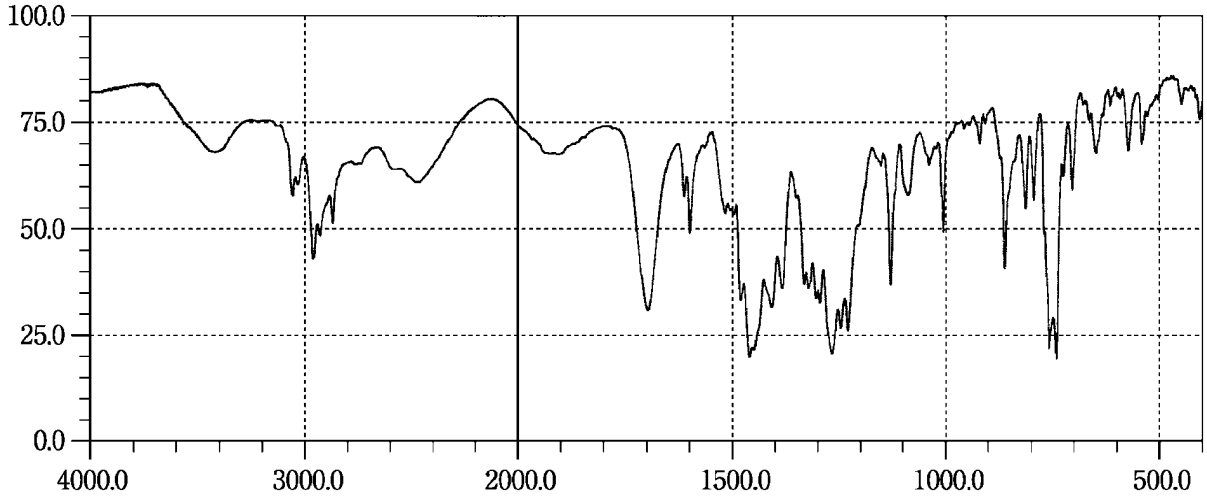
Pranlukast Hydrate



Sivelestat Sodium Hydrate

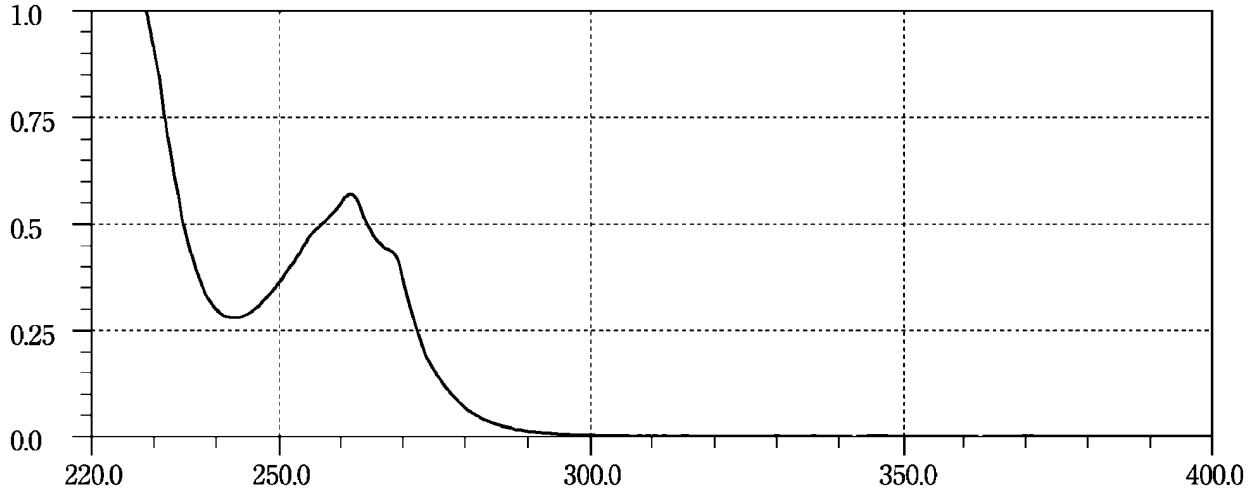


Telmisartan

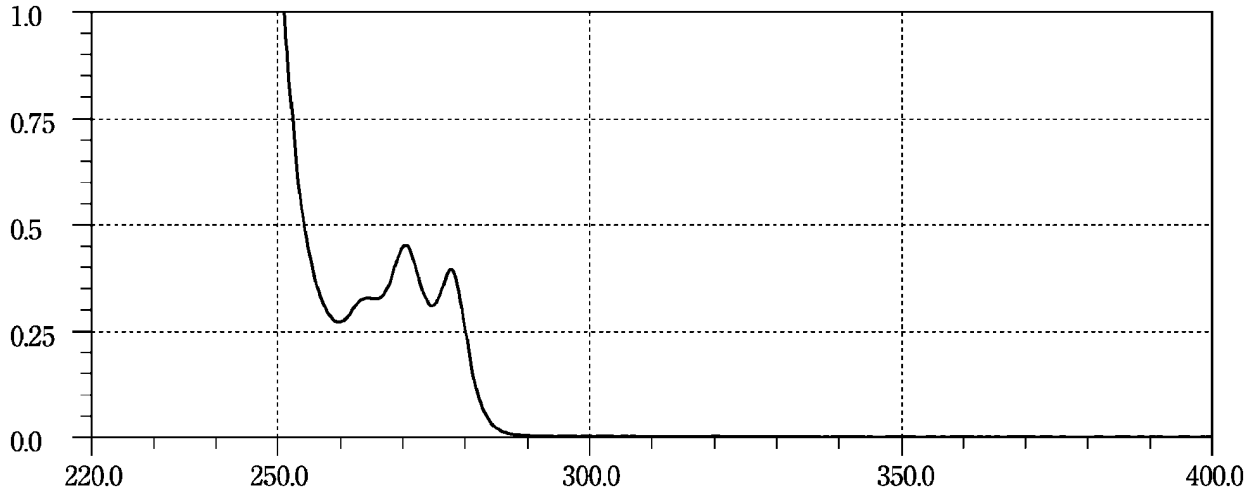


Add the following 13 spectra:

Bepotastine Besilate



Clopidogrel Sulfate



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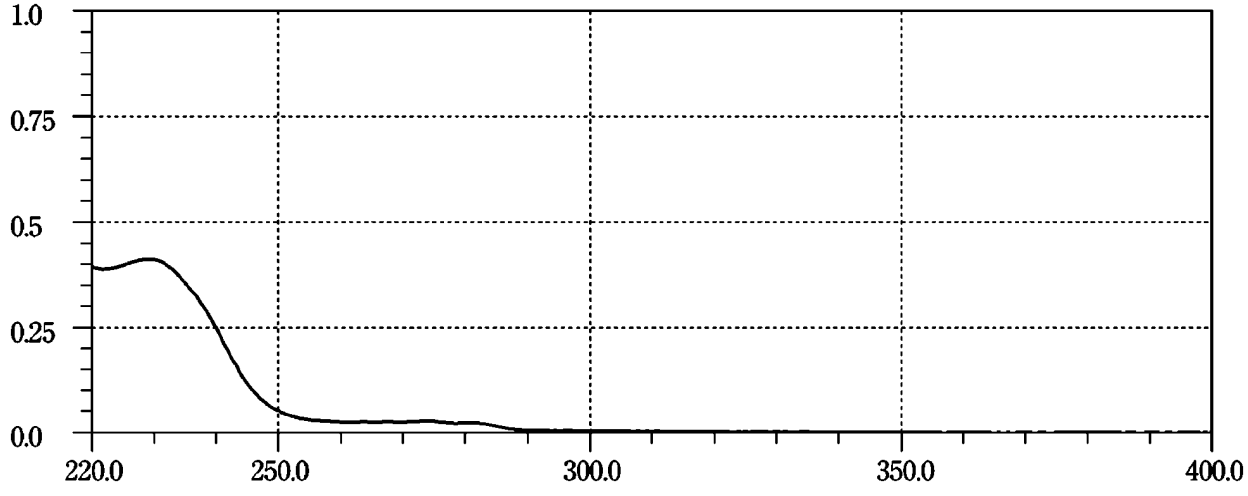

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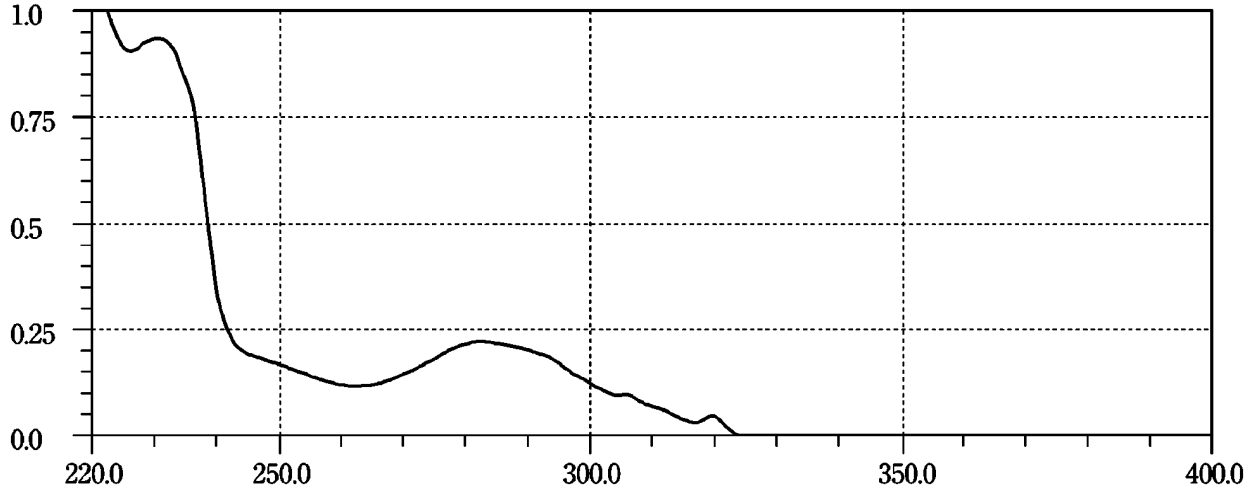

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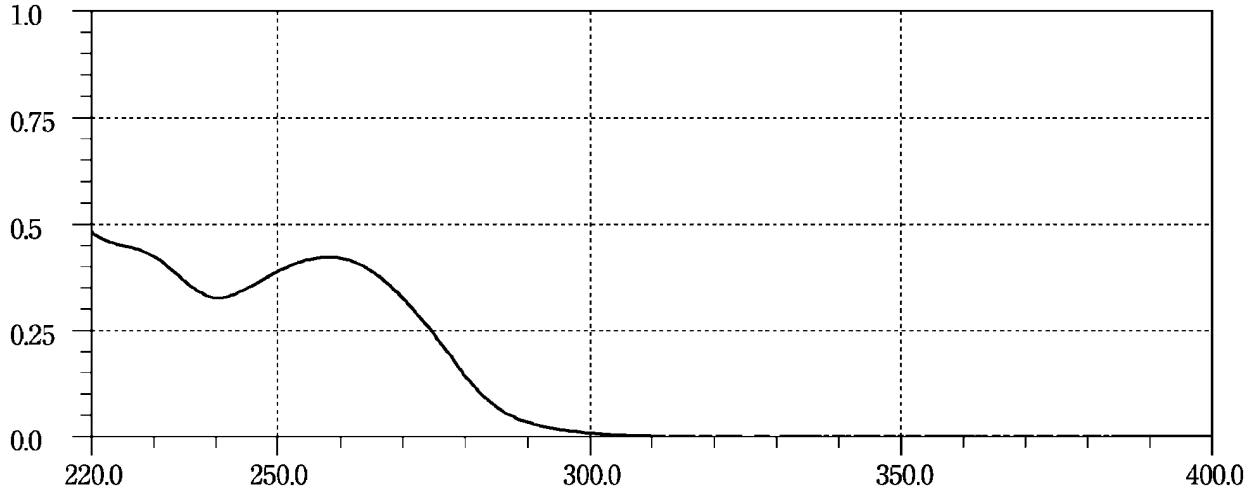

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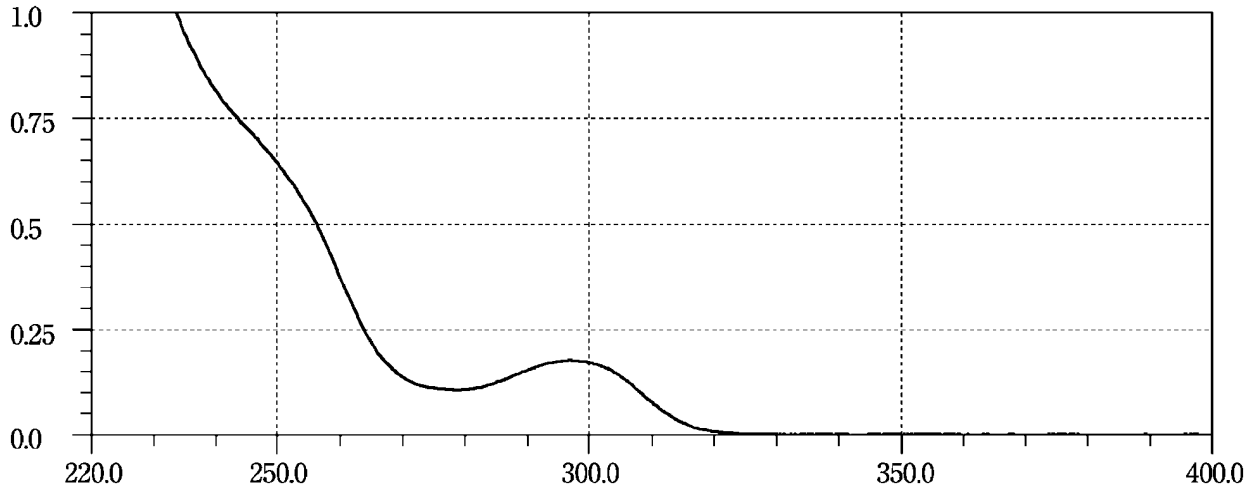

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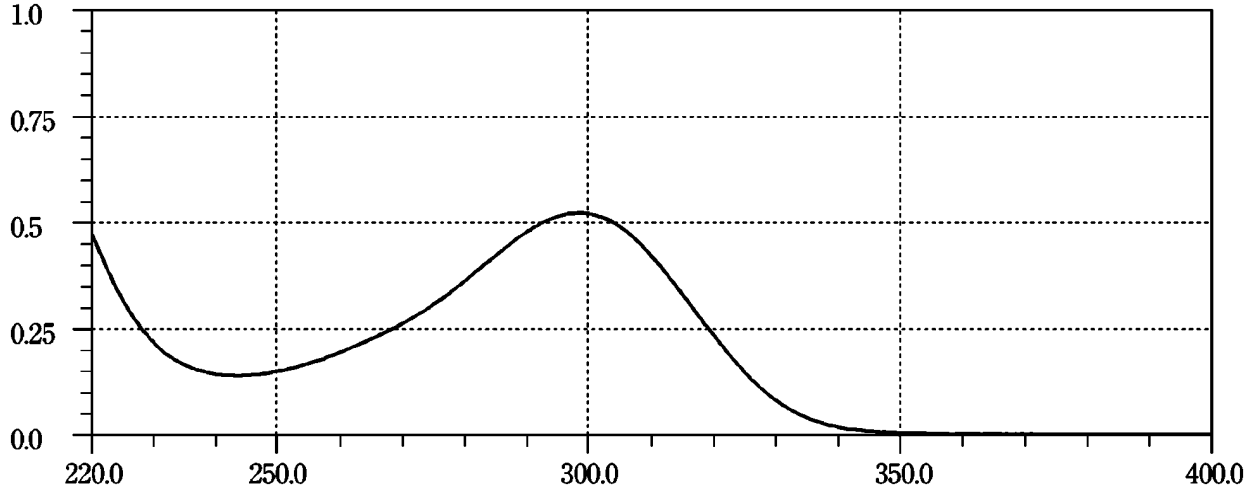

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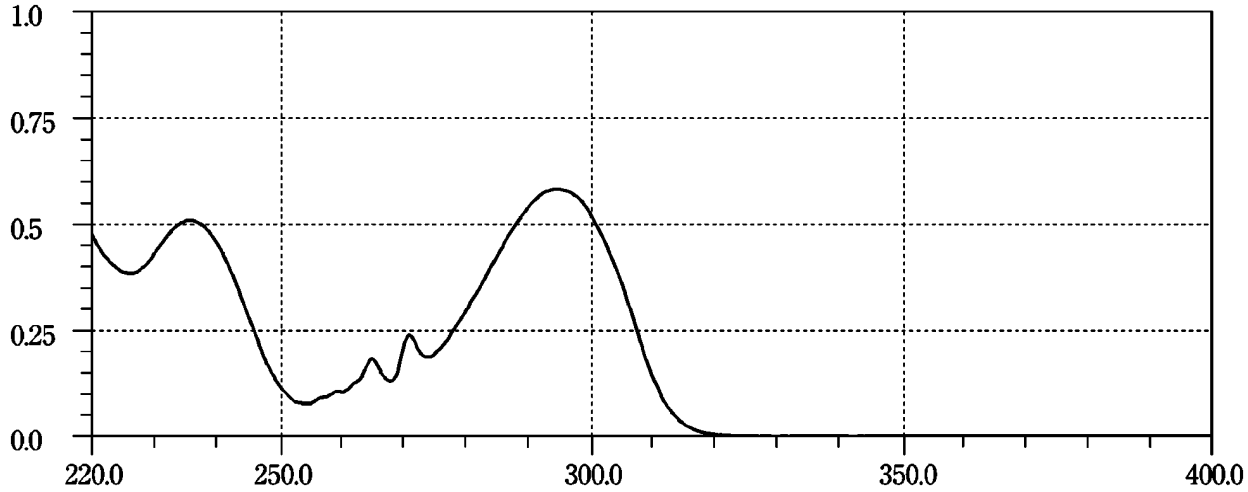

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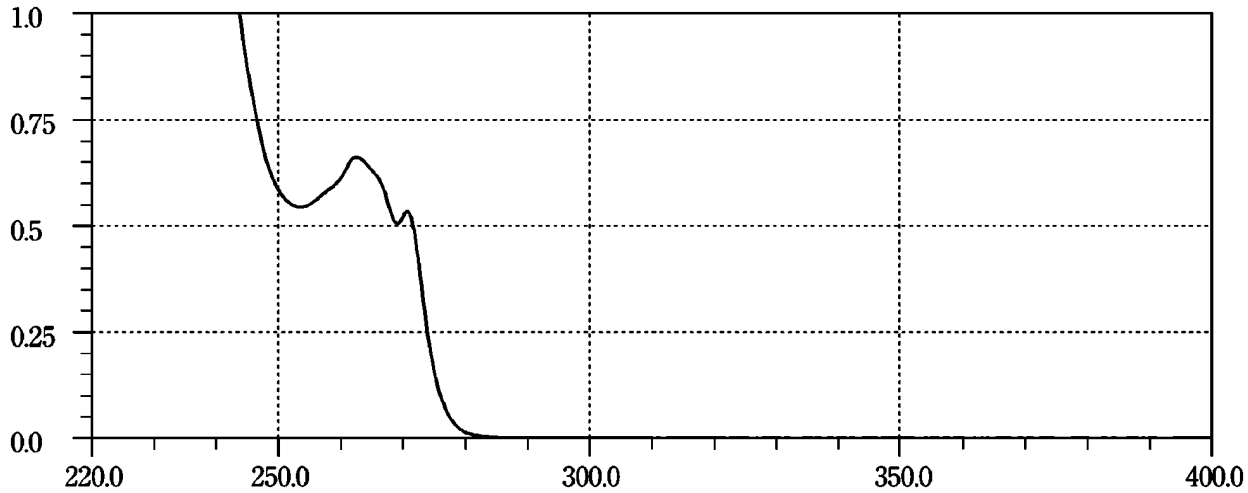

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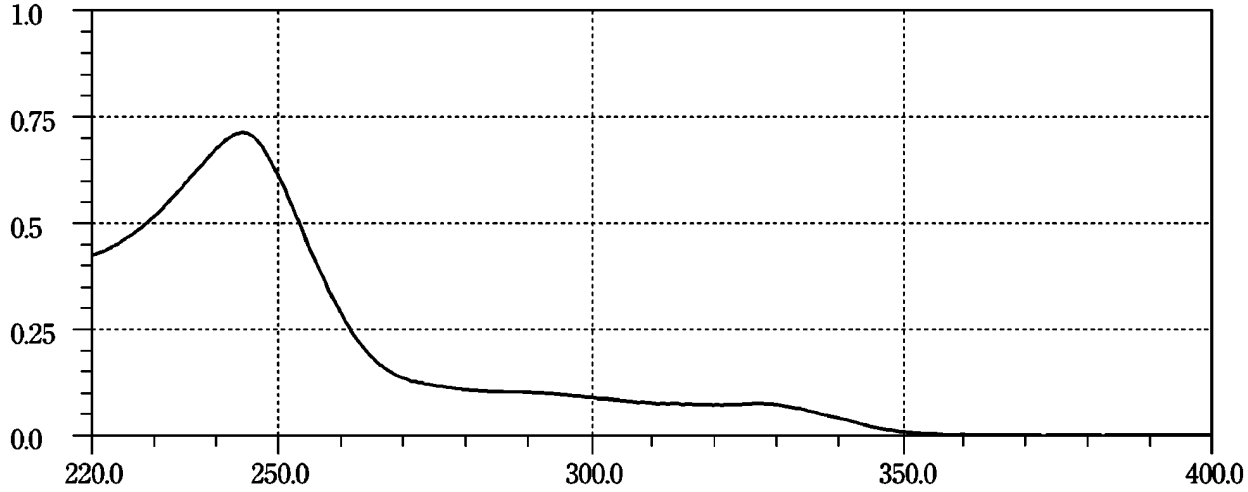

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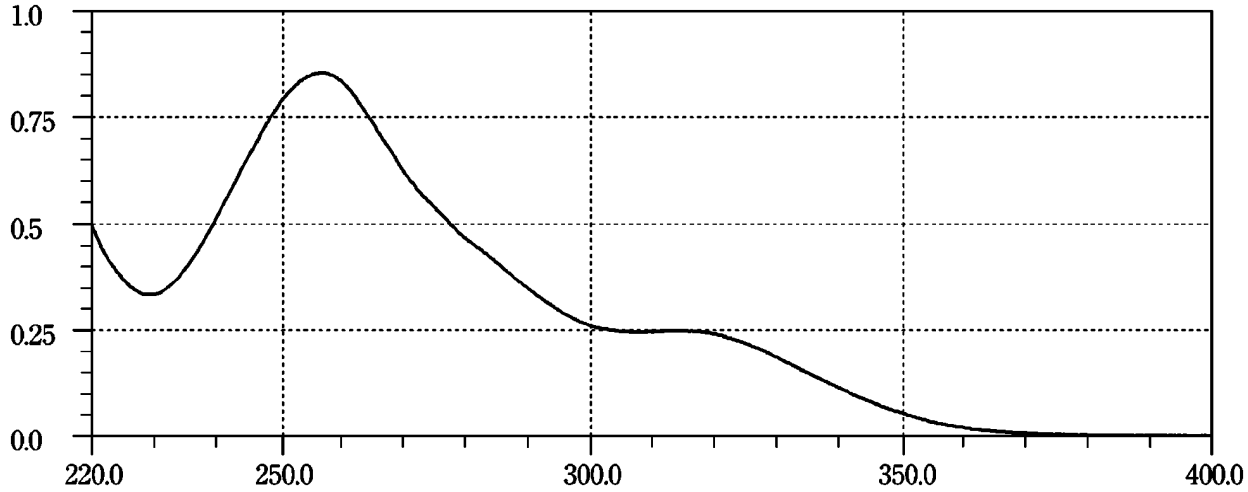
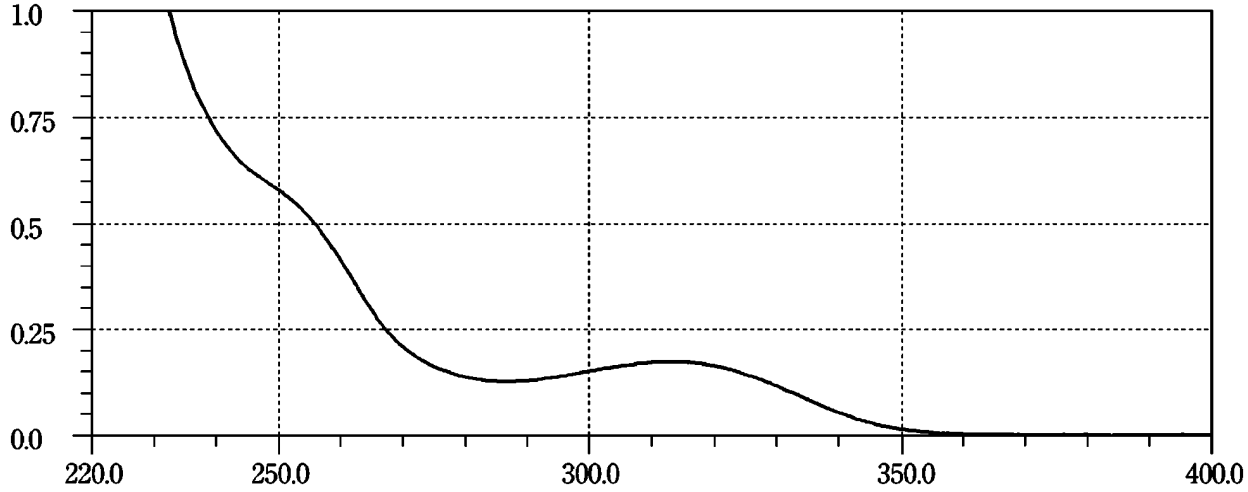


Sivelestat Sodium Hydrate



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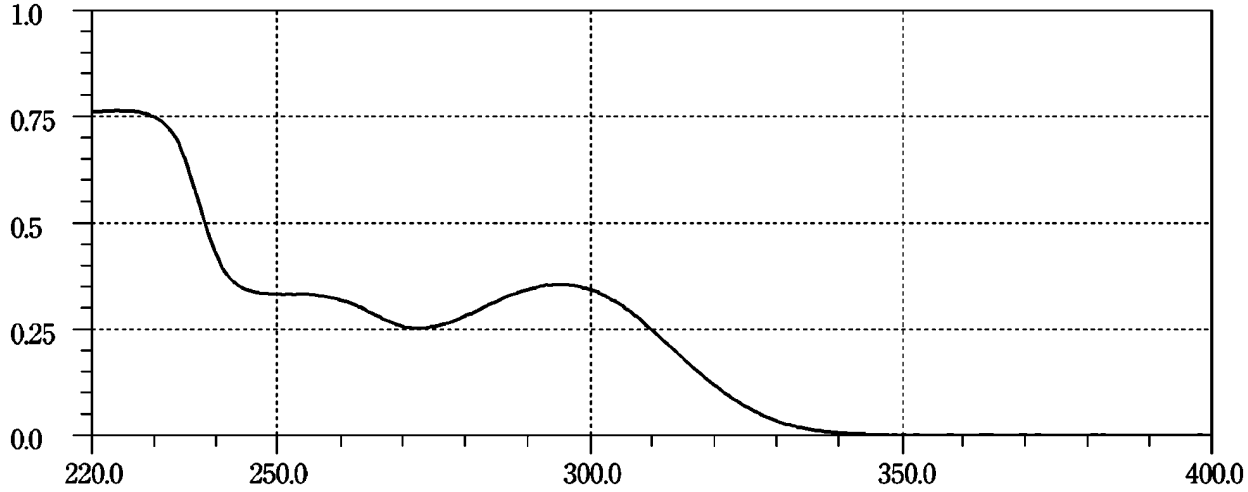


GENERAL INFORMATION

G4 Microorganisms

Media Fill Test (Process Simulation)

Change the paragraph 1.1 as follows:

1. Frequency of media fills

1.1. Initial performance qualification

Initial performance qualification should be conducted for each new facility, item of equipment, filling line, and container design (except for multiple sizes of the same container design), etc. As referring to Table 1, a sufficient number of units should be used to simulate aseptic manufacturing process. A minimum of three consecutive separate successful runs should be performed on each separate day. However, the action as shown in Table 1 may be performed at the time when the contamination is found.

Table 1 Initial performance qualification

Minimum number of simulations	Number of units filled per simulation	Contaminated units in any of the three simulations	Action
3	<5000	$\geqq 1$	Investigation, corrective measures, restart validation
3	5000 – 10000	1	Investigation, consideration of repeat of one media fill
		>1	Investigation, corrective measures, restart validation
3	>10000	1	Investigation
		>1	Investigation, corrective measures, restart validation

Terminal Sterilization and Sterilization Indicators

Change to read as follows:

Sterilization and Sterilization Indicators

Sterilization refers to the destruction or removal of all forms of viable microorganisms in items. This reference information applies to cases where sterilization is required as well as the manufacture of sterile products. When sterilization is applicable, an appropriate sterilization method should be selected in accordance with the items being sterilized (such as products, or equipment, instrumentation, or materials that must be sterilized), including the packaging, after full consideration of the advantages and disadvantages of each sterilization method.

After installation of a sterilizer (including design and development of the sterilization process), an equipment maintenance and inspection program must be established based on qualification evaluation to ensure that the sterilization process is being properly performed as designed on the basis of sufficient scientific evidence. A quality system must also be established for manufacturing in general at manufacturing facilities where sterile pharmaceutical products are manufactured. For example, all operation potentially affecting quality, including sterility after sterilization, must be clearly identified, and any operating procedures that are needed to prevent microbial contamination of products must be established and properly enforced.

In order to establish sterilization conditions and ensure sterility after sterilization, the bioburden before sterilization of the items being sterilized must be evaluated periodically or on the basis of batches. For bioburden test method, refer to 4.05 Microbial Limit Test, etc.

Representative sterilization methods are presented in this reference information, but other sterilization methods can also be used, provided that they meet the following requirements and do not have any deleterious affect on the item being sterilized.

- The mechanism of sterilization is well established
- The critical physical parameters of the sterilization process are clear, controllable, and measurable
- The sterilization procedure can be performed effectively and reproducibly

1. Definitions

The terms used in this text are defined as follows.

- Filter integrity test: A non-destructive test for demonstrat-

ing the correlation with the microbial removal performance data of filters.

- Bioburden: Population of viable microorganisms in an item to be sterilized.
- D value: The value represents exposure time (decimal reduction time) to achieve 90% reduction of a population of the test microorganism, and resulted that 10% of the original organisms remain.
- F_H value: The unit of lethality indicating the measure of the microbial inactivation capacity of a process in dry heat sterilization, expressed as the equivalent time (minutes) at 160°C for microbes with a z value (the number of degrees that are required for a 10-fold change in the D value) of 20°C.
- F_0 value: The unit of lethality indicating the measure of the microbial inactivation capacity of a process in moist heat sterilization, expressed as the equivalent time (minutes) at 121.1°C for microbes with a z value (the number of degrees that are required for a 10-fold change in the D value) of 10°C.
- Sterility assurance level (SAL): Probability of a single viable microorganism surviving in a product after sterilization, expressed as 10^{-n}.
- Dose of irradiation (absorbed dose): Quantity of ionizing radiation energy imparted per unit mass of the item, expressed in units of gray (Gy).
- Critical parameter: A measureable parameter that is inherently essential to the sterilization process.
- Loading pattern: A specified combination of the numbers, orientation and distribution of the item(s) to be sterilized within the sterilization chamber or irradiation container.

2. Sterilization

2.1. Heat method

In the heat method, microorganisms are killed by heat.

2.1.1. Moist-heat sterilization

Moist-heat sterilization includes widely used saturated steam sterilization and other types of moist-heat sterilization. The control points, utilities, and control devices in moist-heat sterilization are provided as reference in Table 1.

Saturated steam sterilization is a method for killing microorganisms with high pressure saturated steam. Critical parameters in this method are temperature, pressure, and exposure time at the specified temperature. Therefore, the temperature, pressure, and exposure time in routine sterilization process control should be continuously monitored and measured, and measuring equipment for that purpose should be included in the sterilization equipment specifications.

Other types of moist-heat sterilization may include steam pressurization cycles, water dispersion cycles, water immersion cycles, and the like, which are used when the items being sterilized is sterilized in a hermetically sealed container. Critical parameters in such methods are the temperature in the container and the exposure time at the specified temperature.

2.1.2. Dry-heat sterilization

Dry-heat sterilization is a method for destructing microorganisms with dry heated air. This method is usually conducted in a batch or continuous (tunnel-type) dry heat sterilizer. Attention must be paid to the cleanliness of the air that flows into the sterilizer in either case. The control points, utilities, and control devices in dry-heat sterilization are provided as reference in Table 2. This method is suitable for when the item to be sterilized is highly heat-resistant, such as glass,

Table 1 Control points, utilities, and control devices in moist-heat sterilization (reference)

	Saturated steam sterilization	Other types of moist-heat sterilization
Control point	• Temperature profile (usually indicated by F_0 value) • Temperature (drain or the like as needed) • Pressure (in sterilizer) • Exposure time at specified temperature • Loading pattern of items being sterilized • Steam quality (degree of superheat, dryness, non-condensable gas concentration, and chemical purity, as needed) • Quality of air that is introduced to the sterilizer for vacuum break. • Quality of cooling water • Other requirements	• Temperature profile (usually indicated by F_0 value) • Temperature (drain and the like as needed) • Pressure, as needed (in sterilizer) • Exposure time at specified temperature • Loading pattern of item being sterilized • Quality of air that is introduced to the sterilizer for vacuum break. • Quality of cooling water • Other requirements
Utilities and control devices that should be controlled	• Steam • Air introduced to the sterilizer for vacuum break • Cooling water • Temperature control device • Pressure control device • Time control device • Other	• Steam • Hot water • Air introduced to the sterilizer for vacuum break • Cooling water • Temperature control device • Pressure control device • Time control device • Conveyor for when a continuous sterilizer is used • Other

Table 2 Control points, utilities, and control devices in dry-heat sterilization (reference)

	Batch-type dry heat sterilizer	Tunnel-type dry heat sterilizer
Control point	• Temperature profile (usually indicated by F_H value) • Temperature • Exposure time at specified temperature • Pressure differential between inside and outside of container • Loading pattern of items being sterilized • Quality of air (heating air, cooling air) • Other requirements	• Temperature profile (usually indicated by F_H value) • Temperature • Belt speed (exposure time) • Pressure differential between inside and outside of equipment • Loading density • Quality of air (heating air, cooling air) • Other requirements
Utilities and control devices that should be controlled	• Air (heating air, cooling air) • Temperature control device • Time control device • Internal differential pressure gauge • HEPA filter • Other	• Air (heating air, cooling air) • Temperature control device • Time control device • Internal differential pressure gauge • HEPA filter • Cooler (if needed) • Other

ceramic or metal, or is thermo-stable, such as mineral oils, fatty oils, or solid pharmaceutical products.

Critical parameters in this method are temperature and the exposure time at the specified temperature (belt speed). Dry-heat sterilization requires higher temperatures and longer exposure times than does moist-heat sterilization even though the sterilization in both methods may be based on the same heating temperature. The temperature and exposure time in routine sterilization process control should be continuously monitored and measured, and measuring equipment for that purpose should be included in the sterilization equipment specifications.

2.1.3. Microwave sterilization

When substances to be sterilized such as chemical solutions are exposed to microwaves, the polar molecules of the substance being sterilized vibrate as they attempt to change orientation due to the absorbed microwaves, and energy is released by the friction between the molecules. The method of killing microorganisms by the heat (microwave heat) generated at this time is called the microwave sterilization. A frequency of 2450 ± 50 MHz is ordinarily used.

Microwave devices are composed of a heating irradiation component which produces radiofrequency radiation to generate heat using a magnetron, a component for maintaining the sterilization temperature using an infrared heater or the like, and a cooling component for cooling the item being sterilized. Such devices continuously sterilize the item at ordinary pressure. The control points, utilities, and control devices in microwave sterilization are provided as reference in Table 3.

This method is applied to liquid products or products with high water content in hermetically sealed containers.

Critical parameters in this method include the temperature of the items being sterilized and processing time. Therefore, the temperature of the items being sterilized and the processing time in routine sterilization process control should be continuously monitored and measured, and measuring equipment for that purpose should be included in the sterilization equipment specifications.

Table 3 Control points, utilities, and control devices in microwave sterilization (reference)

Control point	• Temperature profile (usually indicated by F_0 value) • Temperature • Processing time • Belt speed • Configuration of items being sterilized • Other requirements
Utilities and control devices that should be controlled	• High frequency control device • External heater (if needed) • Cooler (if needed) • Temperature monitoring device • Time monitoring device • Other

Microwave heating characteristically allows rapid sterilization at high temperatures to be continuously carried out with excellent thermal efficiency and responsiveness. However, the ease of heat transfer in the items being sterilized sometimes makes it difficult to ensure uniform heating. Attention must also be paid to the pressure resistance and uniformity of the containers that are used because the heating takes place at ambient pressure, resulting in increases in internal pressure.

2.2. Gas method

The gas method kills microorganisms through contact with a sterilization gas or vapor. Microorganisms can be sterilized at lower temperatures than in heat methods, and the items being sterilized generally sustain little thermal damage. This method is therefore often applied to plastic containers and the like which are not very resistant to heat.

In the most common gas sterilization methods, adequate washing and drying are important to prevent contamination and moisture from compromising the sterilization effect.

Table 4 Control points, utilities, and control devices in EO gas sterilization (reference)

Control point	• Pressure increase, injection time, and final pressure for the injection of sterilization gas • Temperature (in sterilizer and items being sterilized) • Humidity • EO gas concentration (gas concentration in sterilizer should be directly analyzed, but the following alternatives are acceptable when direct analysis is not feasible) i) Weight of gas used ii) Volume of gas used iii) Use of conversion formula based on initial reduced pressure and gas injection pressure • Operating time (exposure time) • Loading pattern of items being sterilized • Biological indicator placement points and cultivation results • Preconditioning conditions (temperature, humidity, time, etc.) • Aeration conditions (temperature, time, etc.) • Other requirements
Utilities and control devices that should be controlled	• EO gas • Injected vapor or water • Air replaced after completion of sterilization • Temperature control device • Pressure control device • Humidity control device • Time control device • Other

Table 5 Control points, utilities, and control devices in hydrogen peroxide sterilization (reference)

	Hydrogen peroxide sterilization	Hydrogen peroxide low temperature gas plasma sterilization
Control point	• Concentration (the concentration in the sterilizer should be directly analyzed, but a method based on evidence of sterilizer performance uniformity in the chamber is an acceptable alternative when direct analysis is not feasible) • Time • Temperature • Humidity • Pressure • Quality of hydrogen peroxide • Consumption of hydrogen peroxide • Residual moisture of substance being sterilized • Loading pattern of items being sterilized • Biological indicator placement points and cultivation results • Chemical indicator placement points and results • Other requirements	• Concentration (the concentration in the sterilizer should be directly analyzed, but a method based on evidence of sterilizer performance uniformity in the chamber is an acceptable alternative when direct analysis is not feasible) • Time • Temperature • Humidity • Pressure • Quality of hydrogen peroxide • Consumption of hydrogen peroxide • Residual moisture of substance being sterilized • Loading pattern of items being sterilized • Biological indicator placement points and cultivation results • Chemical indicator placement points and results • Other requirements
Utilities and control devices that should be controlled	• Hydrogen peroxide • Pressure gauge • Hydrogen peroxide injector • Other	• Hydrogen peroxide • Pressure gauge • Hydrogen peroxide injector • High frequency generator • Other

The sterilization effect may also be compromised if the gas is absorbed by the item being sterilized.

2.2.1. Ethylene oxide (EO) gas sterilization

EO gas sterilization kills microorganisms by altering the proteins and nucleic acids of microorganisms. Since EO gas is explosive, it is usually diluted 10 to 30% with carbon dioxide. EO gas is also a strongly reactive alkylating agent and therefore cannot be used to sterilize products which are likely to react with or absorb it.

The sterilization process consists of preconditioning, sterilization cycles, and aeration. EO gas is toxic (mutagenic, for example), and the substance being sterilized must there-

fore be aerated to ensure that the residual concentration of EO gas or other secondarily generated toxic gases (such as ethylene chlorohydrin) is at or below safe levels. Gas emissions must also be treated in compliance with regulations. The control points, utilities, and control devices in EO gas sterilization are provided as reference in Table 4.

Critical parameters in this method include temperature, humidity, gas concentration (pressure), and time. Therefore, the temperature, humidity, gas concentration (pressure), and time in routine sterilization process control should be continuously monitored and measured, and measuring equipment for that purpose should be included in the sterilization equipment specifications.

2.2.2. Hydrogen peroxide sterilization

Sterilization with hydrogen peroxide is a method for killing microorganisms through the oxidative power of hydrogen peroxide or the oxidation caused by radicals that are produced upon the generation of hydrogen peroxide plasma. Although items can be sterilized at lower temperatures than in heat methods, this method is not suitable for the sterilization of objects that absorb hydrogen peroxides, such as cellulose-based disposable garment and membrane filters because the sterilization effect will be compromised. The control points, utilities, and control devices in hydrogen peroxide sterilization are provided as reference in Table 5.

Critical parameters in this method include the concentration, time, and temperature. The control of a radio frequency device is also important when substances are sterilized with the use of plasma. The residual moisture of the substance being sterilized and the humidity in the sterilization environment may affect sterilization and should therefore be controlled when necessary.

2.3. Radiation method

2.3.1. Radiation sterilization

Radiation sterilization includes γ-ray radiation for killing microorganisms through the exposure of the items that are to be sterilized to γ-rays emitted from ^{60}Co, and electron beam radiation for killing microorganisms through exposure to an electron beam emitted from an electron beam accelerator. To select this method of sterilization, it must first be ensured that it is compatible with the items to be sterilized, including whether the quality of the substance could potentially deteriorate.

In γ-ray radiation sterilization, microorganisms are killed by secondarily produced electrons, whereas in electron beam radiation sterilization, microorganisms are directly killed by electrons. Although this kind of electron-based direct action is available, indirect action is also available, where sterilization is accomplished through the production of radicals and the like and damage to the DNA of microorganisms when γ-rays or electron beams react with water molecules.

Since sterilization can take place at room temperature, both methods can be applied to heat-labile items, and items can be sterilized while packaged because the radiation rays will penetrate the packaging. γ-Ray sterilization is suitable primarily for high density products such as metals, water, and powder because the penetration is better than that of electron beams. Electron beam radiation sterilization has a higher radiation dose per unit time (dose rate) compared with γ-rays and therefore has a shorter processing time. The control points, utilities, and control devices in radiation sterilization are provided as reference in Table 6.

2.4. Filtration method

The filtration method is a method for physically removing microorganisms in liquids or gas using a sterilization filter. It can therefore be applied to items that are unstable against heat or radiation. Filtration sterilization is for microorganisms which can be removed by a 0.2 μm membrane filter, and is not suitable for *Mycoplasma* spp., *Leptospira* spp., or viruses. The control points, utilities, and control devices in filtration sterilization are provided as reference in Table 7.

The critical parameters affecting the removal of microorganisms by the filter in liquid filtration sterilization include filtration time, filtration capacity, filtration flow rate, filtration differential pressure, and temperature. The critical parameters in gas filtration sterilization include filtration differential pressure and temperature. When a liquid is to be sterilized, the removal of microorganisms by a filter will be affected by the physical and chemical properties of the liquid that is undergoing filtration (such as viscosity, pH, and surfactant action). The microbial trapping performance of a sterilizing filter can generally be validated when a sterilizing filter challenged with more than 10^7 CFU microorganisms of a strain of *Brevundimonas diminuta* (ATCC 19146, NBRC 14213), cultured under the appropriate conditions, per square centimeter of effective filter area, provides a sterile

Table 6 Control points, utilities, and control devices in radiation sterilization (reference)

	γ-Ray radiation sterilization	Electron beam radiation sterilization
Control point	• Absorbed dose • Loading pattern (density) of items being sterilized • Exposure time (conveyor speed or cycle time) • Other requirements	• Absorbed dose • Loading pattern (density) of items being sterilized • Electron beam properties (mean electron beam current, electron beam energy, scanning width) • Other requirements
Utilities and control devices that should be controlled	• Belt conveyor • Dose measurement system • Other	• Electron beam measurement device • Belt conveyor • Dose measurement system • Other

Table 7 Control points, utilities, and control devices in filtration sterilization (reference)

	Liquid filtration sterilization	Gas filtration sterilization
Control point	• Filtration time • Filtration capacity • Filtration flow rate • Filtration differential pressure • Temperature • Filter integrity • In cases involving multiple use: expiration period and number of times the filter can be used for sterilization • Other requirements	• Filtration differential pressure • Temperature, if needed • Filter integrity • Expiration period • Number of sterilizations of times the filter can be used for sterilization • Direction of gas current (for bidirectional flow) • Other requirements
Utilities and control devices that should be controlled	• Pressure gauge • Flow rate meter • Integrity tester • Other	• Pressure gauge • Flow rate meter • Integrity tester • Other

Table 8 List of typical indicators by sterilization method

Sterilization method	Species	Strain name	*D* value, etc. (reference)
Moist-heat sterilization	*Geobacillus stearothermophilus*	ATCC 7953, NBRC 13737	≥1.5 min (121°C)
Dry-heat sterilization	*Bacillus atrophaeus*	ATCC 9372, NBRC 13721	≥2.5 min (160°C)
EO gas sterilization	*Bacillus atrophaeus*	ATCC 9372, NBRC 13721	≥2.5 min (54°C) ≥12.5 min (30°C) Gas concentration: 600 mg/L ± 30 mg/L; relative humidity: 60% RH
Hydrogen peroxide sterilization	*Geobacillus stearothermophilus*	ATCC 12980, ATCC 7953, NBRC 12550	—

effluent. The bioburden of liquids prior to filtration will affect filtration sterilization performance and should therefore be controlled.

3. Sterilization Indicators

3.1. Biological indicators (BI)

3.1.1. Introduction

A BI is an indicator prepared from the spores of a microorganism resistant to the specified sterilization process, and is used to develop and/or validate a sterilization process.

Indicators are classified based on configuration into the ''paper strip type,'' ''the type that is inoculated on or into the surface of metal or the like,'' the ''liquid type,'' and ''the self-contained type'' in which a medium and paper strip are pre-encapsulated. They are also classified by carrier, where one type comprises a carrier of paper, glass, stainless steel, plastic or the like that is inoculated with bacterial spores and packaged, and another type comprises the product or simulated product as the carrier, which is inoculated with bacterial spores. Typical examples of indicators by sterilization method are shown in Table 8.

3.1.2. Labeling of commercially available BI

Users of commercially available BI produced in accordance with ISO11138-1 must check the following information provided by the BI manufacturer to users.

- Traceability (microorganism, carrier, labeling, etc.)
- Species name
- Nominal bacterial spore count
- Resistance
- Method used
- Storage conditions (temperature, expiration date, etc.)
- Culture conditions (temperature, time, medium, etc.)
- Disposal method

Parameters determining BI performance include ''species,'' ''resistance,'' and ''bacterial count.'' Resistance varies, even for the same species, depending on the nature and configuration of the carrier or packaging, and evaluation must therefore include the packaging.

3.1.3. Control during use of commercially available BI

BI must be handled in accordance with the storage conditions, time to start of culture after sterilization, culturing conditions, disposal method, and the like provided by the BI manufacturer. Because the storage conditions in particular

affect BI performance, precautions must be taken to prevent a BI from being allowed to stand for a long period of time until use after being removed from the packaging.

The BI should be set up to enable evaluation of the entire items being sterilized. The BI should be set up in places where the sterilization effect is expected to be low in any given method, such as cold spots in heat sterilization. Care should be taken to avoid damaging the BI packaging or carrier when recovered. Predetermined procedures for preventing microbial contamination should be in place in case bacteria are released or spread if the packaging does end up becoming damaged.

When using a BI that has been purchased, the user should measure the spore count or the like when received as needed to make sure there are no significant differences with the nominal count provided by the BI manufacturer.

3.1.4. Precautions for when sterilization indicators are prepared by the user

The following must be evaluated prior to use when users prepare indicators themselves using the bioburden collected from the items being sterilized or the manufacturing environment rather than purchasing a BI for use.

- Species name
- Bacterial spore count
- Resistance (*D* value at sterilization temperature or sterilization gas concentration)
- Storage conditions (temperature, expiration date, etc.)
- Culture conditions (temperature, incubation time, medium, etc.)

An evaluation program must be established to continuously show that the resistance of picked bacteria is the most resistant of the bioburden.

3.1.5. Precautions when commercially available BI are modified by users

When a BI that has been purchased is removed from the packaging and is used to inoculate an item such as drug solution or materials, the bacterial spore count or resistance will vary and must therefore be assessed prior to use.

ISO11138 or USP<55> can be used for reference for such evaluation. Resistance can be evaluated by using a biological indicator evaluation resistometer (BIER) or the capillary method with oil bath. When such self-assessment is unfeasible, a third-party testing facility can be used.

3.2. Chemical indicator (CI)

A CI is an indicator that chemically or physically changes due to exposure to heat, gas, radiation, or the like. Such indicators are produced by being applied to or printed on a piece of paper, for example. Because the principals involved in such changes will depend on the sterilization method, a CI that is suitable for the intended sterilization method must be used. CI is classified into the following six classes based on the intended application. The classes shown here are unrelated to level of performance.

A CI indicates the progress of a sterilization step or of a number of critical parameters, but is not used to assure sterilization effect or sterility and therefore cannot be used as an alternative to a BI.

Class 1: Process indicators

These are intended to distinguish whether an item being sterilized has passed through a sterilization step. They respond to one or more critical parameters.

Class 2: Indicators for use in specific tests

These are used in tests of the exhaust capacity and vapor penetration of a vacuum-type high-pressure steam sterilizer as specified in the ISO11140 series. They correspond to the Bowie-Dick type.

Class 3: Single-variable indicators

These respond to only one critical parameter. They show exposure in a sterilization step based on a specified value for the designated parameter.

Class 4: Multi-variable indicators

These respond to two or more critical parameters. They show exposure in a sterilization step based on specified values for the designated parameters.

Class 5: Integrating indicators

These respond to all critical parameters. Their performance is equal to or greater than that required of BI in the ISO11138 series.

Class 6: Emulating indicators

These respond to all critical parameters of a specified sterilization cycle. The specifications are critical parameters of the designated sterilization step.

3.3. Dosimeter

3.3.1. Types of dosimeters

The dosimeter in a radiation process is an instrument or system which reads the absorbed dose based on changes caused by the absorption of the radiation, for which ''reproducibility'' and ''response permitting radiation to be measured'' are required. Most dosimeters are susceptible to environmental conditions (process parameters) such as temperature and dose rate before, during, and after exposure to the facilities being used, and caution is therefore required. The choice of dosimeter and calibration guidelines for radiation processes have been specified (ISO/ASTM 51261) as reference for the selection and use of dosimeters. Dosimeters for measuring the absorbed dose of radiation are shown in Table 9. γ-Ray dosimeters are not normally suitable for sterilization process control involving the use of electron beams of less than 3 MeV energy.

3.3.2. Dosimeter use

Dosimeters are used when dose distribution is measured to determine the conditions of radiation and to evaluate the absorbed dose of an items being sterilized during ordinary

Table 9 Types of dosimeters

Type of radiation	Dosimeter
γ-ray	Dyed polymethyl methacrylate dosimeter Clear polymethyl methacrylate dosimeter Ceric-cerous dosimeter Alanine – EPR dosimeter
γ-ray, electron beam	Cellulose acetate dosimeter Radiochromic film dosimeter

radiation sterilization. In the former, dosimeters are set up in advance in the object being sterilized and are then recovered after radiation for measurement in the measurement system to find the absorbed dose at each location. The dosimeters should be arranged in a broad range of vertical and horizontal directions because it is necessary to determine the relationship between minimum/maximum exposure and the process parameters as well as to verify the appropriateness of the packaging configuration based on the variation in radiation penetration and dose. In the latter, there is no need to arrange the dosimeters in the locations characterized by the maximum or minimum dose in the object being sterilized. Control points where dosimeters are easily arranged and recovered should be selected, and the absorbed dose of the object being sterilized should be ensured based on the absorbed dose at the control points. Therefore, in the measurement of dose distribution, the quantitative relationship between the control points and the locations of maximum/minimum exposure should be determined, and the passing dose range at the control points should also be calculated.

Newly purchased dosimeters should be calibrated prior to use, and dosimeters should be calibrated every time a batch is changed and at least once a year.

4. Establishment of Sterilization Conditions

4.1. Half-cycle method

In the half-cycle method, a sterilization time twice as long as that required to inactivate all of the 10^6 CFU bacteria included in the BI is used, regardless of the bioburden count on the object being sterilized or the resistance of the test microorganisms to sterilization. This method is primarily used to establish the conditions of EO or other gas sterilization.

4.2. Overkill method

In the overkill method, a sterilization condition to achieve an SAL of 10^{-6} or better is used, regardless of bioburden count on the object being sterilized or the resistance of the test microorganisms to sterilization.

This means a level of sterilization of 12 D in steam sterilization. However, a level $\geq F_0$ 12 is also referred to as the overkill method.

4.3. Combination of BI and bioburden method

In the combined bioburden/BI method, the maximum bioburden count is determined based on the results of extensive bioburden analysis, and the sterilization time (or radiation dose) is calculated using an appropriate commercially available BI with a test microorganism count $\geq$ the maximum bioburden count based on the target SAL.

When this procedure is used, the bioburden count of the object being sterilized must be tested on a daily basis, and the resistance of the test microorganisms to sterilization must be periodically measured.

If the bioburden testing reveals a microorganism more resistant than the BI microorganism, it should be used as the indicator. The sterilization conditions must also be revised as needed.

$$\text{Sterilization time (or radiation dose)} = D \times \log (N_0/N)$$

D: D value of BI

N: Target sterility assurance level (SAL)

N_0: Maximum bioburden count in object being sterilized

4.4. Absolute bioburden method

In the absolute bioburden method, the sterilization resistance of the microorganisms found in the object being sterilized or environment is measured, and the sterilization conditions are determined, in the case of moist-heat sterilization, by employing the D value of the most resistant microorganism based on the bioburden count of the object being sterilized.

The bioburden count should be determined by extensive bioburden analysis. When this procedure is used, the microorganism count and the resistance of the detected microorganisms to sterilization must be assessed on a daily basis in routine bioburden control.

Radiation sterilization may be performed in accordance with ISO11137–2.

5. References

- ISO 11138-1 (2006): Sterilization of health care products-Biological indicators-Part1: General requirements
- ISO 11137-2 (2006): Sterilization of health care products-Radiation- Part2: Establishing the sterilization dose
- ISO/ASTM 51261 (2002): Guide for selection and calibration of dosimetry systems for radiation processing
- ISO 11140–1 (2005): Sterilization of health care products-Chemical indicators- Part1: General requirements
- USP <55> BIOLOGICAL INDICATORS-RESISTANCE PERFORMANCE TEST

G5 Crude Drugs

Quantitative Analytical Technique Utilizing Nuclear Magnetic Resonance (NMR) Spectroscopy and Its Application to Reagents in the Japanese Pharmacopoeia

Change as follows:

1. Marker Compounds for the Assay of Crude Drugs in the JP and Establishment of Reference Standards for Quantitative Analyses

When the quantitative assay values are specified in the monographs of crude drugs and extracts of Kampo formulations in the JP, it is more difficult to establish and prepare their JP Reference Standards than those for synthetic chemical pharmaceutical substances, because the marker compounds for their assay are derived from natural sources.

Unlike the synthetic chemical pharmaceutical substances, crude drugs and extracts of Kampo formulations are mixtures of a great deal of compounds. Although it is necessary to choose a substance contained at the level of 0.1% to

several % in the crude drugs and the extracts of Kampo formulations as the marker compounds for their quantitative assay, the synthesis of such compounds is not so easy in most cases. Therefore, the marker compound would be separated from natural materials and be isolated to have sufficient purity. However, the preparation of the reference substance in such a way would require high economical cost and a great deal of effort. In addition, the composition of impurities contained in the reference substance prepared in such a way would be different batch by batch according to the difference of raw materials and their processes of extraction, isolation and purification. Accordingly, the difference among batches of reference materials is much larger than that of synthetic substances, and the control of their purity as the official reference standards is very difficult. Furthermore, in many cases of substances of natural origin, the greatest impurity would be water. For determining water contents precisely, it is necessary to use Karl Fischer method, and as the result, a quantity of the valuable reference standard would be consumed.

Because there are such bottlenecks mentioned above in many cases of monographs of crude drugs and extracts of Kampo formulations, the establishment of the JP Reference Standard is difficult. Instead, reagents, which are commercially available or ready to put into the market, are designated as the reference substances for the quantitative assay, and the method and the content specification using the reagent are specified in monographs of crude drugs and extracts of Kampo formulations. In these cases, the specifications of their marker substances are defined in the section of Reagents and Test Solutions of the JP. However, in a strict sense, since the assay values obtained in this manner are not certified metrologically, the reliability of the analytical value obtained by using them is somewhat ambiguous.

2. Application of Quantitative NMR to Reference Substances Used in the Assay of Crude Drugs and Extracts of Kampo Formulations

The application of quantitative NMR can solve the issue on the purity of reagents derived from natural source. These reagents are used as the reference substances with metrological traceability, when the precise contents of these reagents are determined metrologically by using quantitative NMR based on the idea shown in 10.1 Principle of Quantitative Analytical Technique Utilizing Nuclear Magnetic Resonance (NMR) Spectroscopy under <*5.01*> Crude Drugs Test.

Currently, the quantitative NMR is being carried out for these reagents defined for the quantitative assay of crude drugs in the JP and a report in which the points to practically consider at determination of absolute purities of the reagents by using quantitative NMR are discussed has been published.[1)] In addition, a validation study of quantitative NMR has also been performed using the substances which will be used with high possibility as the reference standards for HPLC quantitative analysis. For the analyte compound having molecular mass of around 300, when about 10 mg of the compound was used for the quantitative NMR measurement, it was demonstrated that an accuracy of 2 significant digits for the determined value was achieved at the ordinary laboratory level, even when the error among the NMR instruments used was included.[2)] Usually, the contents of marker compounds for the quantitative assay of crude drugs are several % at the maximum, and the minimum unit for the content specification is at the level of 0.1%. Therefore, when variability of content in crude drugs is considered, the assurance of 2 significant digits for accuracy seems sufficient for the reference standards, which are used for the quantitative assay of crude drugs.

When discussion above is considered, the ambiguity of analytical values obtained by the use of the reagents derived from natural source as the reference substances for the quantitative assay of crude drugs can be avoided practically, by using the reagents certified by quantitative NMR as the reference standards in HPLC, etc., and by incorporating the certified purity of such reagent into the calculation of the quantitative value of the sample. For example, for Gardenia Fruit in the JP, the content of geniposide is specified at not less than 3.0% based on the HPLC analysis. The report cited above[1)] demonstrated that the absolute purity of geniposide used as the reference substance in the quantitative assay of Gardenia Fruit is determined to be about 92% by quantitative NMR. Therefore, in the case that the quantitative value of 3.0% in Gardenia Fruit sample is obtained as a result of HPLC analysis by using this reagent as the reference standard assuming its purity as 100%, the true value for the sample is evaluated to be 2.8% taking it into consideration of the absolute purity determined by quantitative NMR with the assurance of metrological traceability.

3. Supply of Certified Reagents by Using Quantitative NMR

Currently, in the accreditation system of the International Accreditation Japan (IA Japan), the National Institute of Technology and Evaluation (ASNITE), a feasibility study how the accreditation should be given to the organization which performs the assay certification of the reagents using calibrated NMR apparatus has been in progress. In addition, in the IA Japan, addition of ''Quantitative NMR'' to the test method categories is scheduled. Therefore, in the near future, the reagent manufacturers will become able to perform the assay certification of the reagent after having this accreditation. Under such situation, the user of the reagent would not be required to perform qualitative NMR individually to obtain the purity value with SI traceability. Furthermore, the inter-institutional errors (including inter-instrumental errors) would become negligible, and we will be able to carry out more precise and accurate quantitation assay of the sample by incorporating the labeled certified value on the reagent into the calculation of the quantitative value of the sample.

The certified reference materials (NMIJ CRM) to be used for the SI traceable metrological determination of the internal reference compounds are supplied from the National Metrology Institute of Japan, National Institute of Advanced Industrial Science and Technology (NMIJ AIST).

4. Reference

1) J. Hosoe, *et al.*, Pharmaceutical and Medical Device Regulatory Science, **41**, 960 – 970 (2010)
2) J. Hosoe, *et al.*, Pharmaceutical and Medical Device Regulatory Science, **43**, 182 – 193 (2012)

G7 Containers and Package

Delete the following item:

Plastic Containers for Pharmaceutical Products

Add the following:

Basic Requirements for Plastic Containers for Pharmaceutical Use and Rubber Closures for Containers for Aqueous Infusions

In this chapter, there describe basic requirements for plastic containers for pharmaceutical use and rubber closures for containers for aqueous infusions, and the methods to evaluate the toxicity of containers at design stage.

Containers for pharmaceutical use should not have the properties to deteriorate the efficacy, safety or stability of the pharmaceutical products to be packed in the container. The compatibility of plastic containers with pharmaceutical products should be judged for each combination of container and the specific pharmaceutical product to be contained therein. Such judgment should be performed through verification that the container for the pharmaceutical preparation can comply with the essential requirements for the container, i.e., the design specifications, based on the data from the experiments on the prototype products of the container and/or information from scientific documentation, etc. In addition, such compatibility must be ensured based upon an appropriate quality assurance system.

1. Basic Requirements in Designing Containers for Pharmaceutical Use

The quality of the pharmaceutical products packed in the container must not deteriorate during storage. The concentration of the pharmaceuticals must not be decreased by more than a certain level due to the adsorption of the pharmaceuticals on the surface of the container, the migration of the pharmaceuticals into the inside of the material of the container, or the loss of pharmaceuticals through the container. Also, the pharmaceutical products contained therein must not be degraded by an interaction with the material of the container.

The container should not be deformed, should not deteriorate and should not be degraded by the pharmaceutical products contained therein. Unacceptable loss of function of the container should not result from a possible high temperature or low temperature or their repetitions encountered during storage or transportation.

The leachable or migrants from the container should not deteriorate the efficacy or stability of the pharmaceutical products contained therein. In addition, the possible toxic hazards due to the leachable or migrants should not exceed a given level. Furthermore, the amounts of leachable or migratable chemical substances, such as monomers and additives, from the containers to the pharmaceutical products contained therein must be sufficiently small from the viewpoint of safety.

In the case of pharmaceutical products which must be sterilized, it is required to satisfy the above-mentioned essential requirements of the container after the sterilization, if there is a possibility that the quality of the container may change after the sterilization. There should not be any residue or generation of new toxic substances of more than certain risk level after the sterilization. In addition, the container should not have any inappropriate structure and/or material that might result in any bacterial contamination of the pharmaceutical products contained therein during storage and transportation after sterilization.

1.1. Plastic containers for pharmaceutical use

The plastic material used for the container should be of high quality. Therefore, recycled plastic materials, which are of unknown constitution, must not be used.

In the case of pharmaceutical products which are unstable to light, the container should provide a sufficient level of light shielding. In the case of pharmaceutical products which are easily oxidized, the container material should not allow the permeation of oxygen. In the case of aqueous pharmaceutical products and pharmaceutical products that must be kept dry, the container material should not allow the permeation of water vapor. In addition, it is necessary to pay attention to the permeability of solvents other than water through the container.

The container should have a certain level of physical properties such as hardness, flexibility, shock resistance, tensile strength, tear strength, bending strength, heat resistance and the like, in accordance with its intended usage. The container should be of a required level of transparency, when it is necessary to examine foreign insoluble matter and/or turbidity of the pharmaceutical products by visual observation.

Furthermore, in introducing a plastic container, it is desirable that proper disposal method after use is taken into consideration.

1.2. Rubber closures for containers for aqueous infusions

For the rubber closures for container, the recycled rubber materials, which have the possibility to cause an allergic response, should not be used.

As the closure systems, the appropriate materials should be used to prevent the permeation of oxygen, water vapor and solvents.

Further, the rubber closure should have a certain level of physical properties such as air tightness, hermetic seal, penetrability of a needle, coring-resistance and self-sealing after penetration, in accordance with its intended usage.

2. Toxicity Evaluation of Container at Design Stage

For design verification, the toxicity of the container should be evaluated. For the toxicity evaluation, it is desirable to select appropriate test methods and acceptance criteria for the evaluation, and to explain the rationale for the selection clearly. The tests should be conducted using samples of the whole or a part of the prototype products of the container. If the container consists of plural parts of different materials, each part should be tested separately. Such materials as laminates, composites, and the like are regarded as a single material. To test containers made of such materials, it is recommended to expose the inner surface of the container, which is in contact with the pharmaceutical products contained therein, to the extraction media used in the tests as far as possible.

It is recommended to select the test items and the test methods for the evaluation of the toxicity of the containers, depending on their application site, in accordance with the standard test methods on medical devices and materials published in Japan, a notice entitled Basic Principle of Biological Safety Evaluation Required for Application for Approval to Market Medical Devices (MHLW Notification by Director, OMDE Yakusyokuki 0301 No.20 on March 1, 2012).

3. Test Results to be recorded per Production Unit for Plastic containers for pharmaceutical use and Rubber closures for containers for aqueous Infusions

3.1. Plastic containers for pharmaceutical use

At the commercial production phase, it is required to establish acceptance criteria on at least the test items listed below and to record the test results of each production unit of plastic containers for pharmaceutical products. In addition, it is desirable to explain the rationale for setting the acceptance criteria clearly. However, these requirements should not be applied to orally administered preparations except for liquid preparations.

(i) Combustion Tests: Residue on ignition, heavy metals. If necessary, the amounts of specified metals (lead, cadmium, etc.)

(ii) Extraction Tests: pH, UV spectrum, potassium permanganate-reducing substances, foaming test, residue on evaporation

(iii) Cytotoxicity Test

(iv) Any other tests necessary for the specific container for aqueous infusions.

3.2. Rubber closures for containers for aqueous infusions

At the commercial production phase of rubber closures, it is required to establish acceptance criteria on the test items that should be controlled other than those specified in the general chapter of <*7.03*> Test for Rubber Closure for Aqueous Infusions. And the test results of each production unit of rubber closures for containers for aqueous infusions should be recorded. In addition, it is desirable to explain the rationale for setting the acceptance criteria.

G9 Others

International Harmonization Implemented in the Japanese Pharmacopoeia Sixteenth Edition

Add the following:

Nov. 2005

Harmonized items	JP16 (Supplement II)	Remarks
Calcium Disodium Edetate	**Calcium Sodium Edetate Hydrate**	
Definition	limits of content	
Identification (1)	Identification (1)	
Identification (2)	Identification (3)	
pH	pH	
Purity (1) Chloride	Purity (2) Chloride	
Purity (2) Disodium edetate	Purity (4) Disodium edetate	
Water	Water	
Assay	Assay	

Add the following:

Jun. 2008

Harmonized items	JP16 (Supplement II)	Remarks
Stearic Acid	**Stearic Acid**	
Definition	limits of content	
Identification A	not specified as Identification	
Identification B	not specified as Identification	Being specified as Acid value
Identification C	not specified as Identification	
Appearance	not specified	
Acidity	Purity (1) Acidity	
Iodine value	Iodine value	
Freezing point	Congealing point	
Assay	Assay	
Labelling (type of stearic acid)	origin	

Add the following:

Jun. 2008/Oct. 2009 (Corr.1)

Harmonized items	JP16 (Supplement II)	Remarks
Polysorbate 80	**Polysorbate 80**	
Definition	origin	
Characters	Description	
Identification (Composition of fatty acids)	Identification	
Acid value	Acid value	JP's particular description: Applying Fats and Fatty Oils Test <*1.13*>, using ethanol (95) as the solvent.
Hydroxyl value	Hydroxyl value	
Peroxide value	Purity (3) Peroxide value	
Saponification value	Saponification value	
Composition of fatty acids	Composition of fatty acid	
Ethylene oxide and dioxan	Purity (2) Ethylene oxide and 1,4-dioxane	
Water	Water	
Total ash	Residue on ignition	
Storage	Containers and storage	

Add the following:

Jun. 2012

Harmonized items	JP16 (Supplement II)	Remarks
Mannitol	**D-Mannitol**	
Definition	limits of content	
Identification by IR	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Conductivity	Conductivity	
Melting point	Melting point	
Reducing sugars	Purity (5) Glucose	
Related substances	Purity (4) Related substances	
Nickel	Purity (3) Nickel	
Loss on drying	Loss on drying	
Microbial contamination	not specified	
Bacterial endotoxins	not specified	
Assay	Assay	
Labelling	not specified	

Change to read:

Nov. 2012 (Rev. 2)

Harmonized items	JP16 (Supplement II)	Remarks
Ethanol	**Ethanol**	
Definition	limits of content	Setting specific gravity at 15°C.
Identification A	not specified as Identification	Setting Specific gravity as specification.
Identification B	Identification	
Appearance	Purity (1) Clarity and color of solution	
Acidity or alkalinity	Purity (2) Acidity or alkalinity	
Relative density	Specific gravity	Setting specific gravity at 15°C.
Absorbance	Purity (4) Other impurities (absorbance)	
Volatile impurities	Purity (3) Volatile impurities	
Residue on evaporation	Purity (5) Residue on evaporation	
Storage	Containers and storage	

Change to read:

Nov. 2012 (Rev. 2)

Harmonized items	JP16 (Supplement II)	Remarks
Ethanol, Anhydrous	**Anhydrous Ethanol**	
Definition	limits of content	Setting specific gravity at 15°C.
Identification A	not specified as Identification	Setting Specific gravity as specification.
Identification B	Identification	
Appearance	Purity (1) Clarity and color of solution	
Acidity or alkalinity	Purity (2) Acidity or alkalinity	
Relative density	Specific gravity	Setting specific gravity at 15°C.
Absorbance	Purity (4) Other impurities (absorbance)	
Volatile impurities	Purity (3) Volatile impurities	
Residue on evaporation	Purity (5) Residue on evaporation	
Storage	Containers and storage	

Change to read:

Nov. 2011 (Rev. 1)

Harmonized items	JP16 (Supplement II)	Remarks
Carmellose	**Carmellose**	
Definition	origin	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Purity (1) Chloride	Purity (1) Chloride	
Purity (2) Sulfate	Purity (2) Sulfate	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	

Change to read:

Jun. 2011 (Rev. 2)

Harmonized items	JP16 (Supplement II)	Remarks
Wheat Starch	**Wheat Starch**	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
pH	pH	
Iron	Purity (1) Iron	
Total protein	not specified	
Oxidising substances	Purity (2) Oxidizing substances	
Sulphur dioxide	Purity (3) Sulfur dioxide	
Loss on drying	Loss on drying	
Sulphated ash	Residue on ignition	
Microbial contamination	not specified	

Change to read:

Jun. 2012 (Rev. 3)

Harmonized items	JP16 (Supplement II)	Remarks
Corn Starch	**Corn Starch**	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
pH	pH	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Limit of iron	Purity (1) Iron	
Limit of oxidizing substances	Purity (2) Oxidizing substances	
Limit of sulfur dioxide	Purity (3) Sulfur dioxide	
Microbial limits	not specified	

***Change to read*:**

Jun. 2011 (Rev. 2)

Harmonized items	JP16 (Supplement II)	Remarks
Potato Starch	**Potato Starch**	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
pH	pH	
Iron	Purity (1) Iron	
Oxidising substances	Purity (2) Oxidizing substances	
Sulphur dioxide	Purity (3) Sulfur dioxide	
Loss on drying	Loss on drying	
Sulphated ash	Residue on ignition	
Microbial contamination	not specified	

Change to read:

Jun. 2012 (Rev. 1)

Harmonized items	JP16 (Supplement II)	Remarks
Hypromellose	**Hypromellose**	
Definition	limits of content of methoxy group and hydroxypropoxy group	
Labeling	labeling of viscosity	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Identification (3)	Identification (3)	
Identification (4)	Identification (4)	
Identification (5)	Identification (5)	
Viscosity	Viscosity	
Method 1	Method I	
Method 2	Method II	
pH	pH	
Heavy metals	Purity Heavy metals	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Assay	Assay	

Change to read:

Jun. 2012 (Rev. 2)

Harmonized items	JP16 (Supplement II)	Remarks
Methylcellulose	**Methylcellulose**	
Definition	limits of content of methoxy group	
Labeling	labeling of viscosity	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Identification (3)	Identification (3)	
Identification (4)	Identification (4)	
Identification (5)	Identification (5)	
Viscosity	Viscosity	
Method 1	Method I	
Method 2	Method II	
pH	pH	
Heavy metals	Purity Heavy metals	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Assay	Assay	

Partial Revision of the Japanese Pharmacopoeia (May 31, 2013, the Ministry of Health, Labour and Welfare Ministerial Notification No. 190)

○ The Ministry of Health, Labour and Welfare Ministerial Notification No. 190

Pursuant to Paragraph 1, Article 41 of the Pharmaceutical Affairs Law (Law No. 145, 1960), we hereby revise a part of the Japanese Pharmacopoeia (Ministerial Notification No. 65, 2011) as follows. However, in the case of drugs which are listed in the Japanese Pharmacopoeia (hereinafter referred to as ''previous Pharmacopeia'') [limited to those in the Japanese Pharmacopoeia whose standards are changed in accordance with this notification (hereinafter referred to as ''new Pharmacopoeia'')] and drugs which have been approved as of May 31, 2013 as prescribed under Paragraph 1, Article 14 of the law [including drugs the Minister of Health, Labour and Welfare specifies (the Ministry of Health and Welfare Ministerial Notification No. 104, 1994) as of May 30, 2013 as those exempted from marketing approval pursuant to Paragraph 1, Article 14 of the law, the Name and Standards established in the previous Pharmacopoeia (limited to part of the Name and Standards for the drugs concerned) may be accepted to conform to the Name and Standards established in the new Pharmacopoeia before and on November 30, 2014.

May 31, 2013

Norihisa Tamura
The Minister of Health, Labour and Welfare

6.02 Uniformity of Dosage Units

Change 1. Content Uniformity, 3. Criteria and Table 6.02-2 as follows:

1. Content Uniformity

Select not less than 30 units, and proceed as follows for the dosage form designated.

Where different procedures are used for assay of the preparation and for the content uniformity test, it may be necessary to establish a correction factor to be applied to the results of the latter.

(i) Solid dosage forms: Assay 10 units individually using an appropriate analytical method. Calculate the acceptance value (see Table 6.02-2).

(ii) Liquid or Semi-Solid dosage forms: Assay 10 units individually using an appropriate analytical method. Carry out the assay on the amount of well-mixed material that is removed from an individual container in conditions of normal use and express the results as delivered dose. Calculate the acceptance value (see Table 6.02-2.).

1.1. Calculation of Acceptance Value

Calculate the acceptance value by the formula:

$$|M - \bar{X}| + ks,$$

in which the terms are as defined in Table 6.02-2.

3. Criteria

Apply the following criteria, unless otherwise specified.

(i) Solid, Semi-Solid and Liquid dosage forms: The requirements for dosage uniformity are met if the acceptance value of the first 10 dosage units is less than or equal to $L1$ %. If the acceptance value is greater than $L1$%, test the next 20 dosage units and calculate the acceptance value. The requirements are met if the final acceptance value of the 30 dosage units is less than or equal to $L1$% and no individual content of the dosage unit is less than $(1 - L2 \times 0.01)M$ nor more than $(1 + L2 \times 0.01)M$ in *Calculation of Acceptance Value* under *Content Uniformity* or under *Mass Variation*. Unless otherwise specified, $L1$ is 15.0 and $L2$ is 25.0.

Table 6.02-2

Variable	Definition	Conditions	Value
$\bar{X}$	mean of individual contents (x_1, x_2, …, x_n) expressed as a percentage of the label claim		
x_1, x_2, …, x_n	individual contents of the dosage units tested, expressed as a percentage of the label claim		
n	sample size (number of dosage units in a sample)		
k	acceptability constant	If $n = 10$, then	2.4
		If $n = 30$, then	2.0
s	sample standard deviation		$\sqrt{\dfrac{\sum_{i=1}^{n}(x_i - \bar{X})^2}{n-1}}$
RSD	relative standard deviation (the sample standard deviation expressed as a percentage of the mean)		$\dfrac{100s}{\bar{X}}$
M (case 1)	reference value	If 98.5% ≦ $\bar{X}$ ≦ 101.5%, then	$M = \bar{X}$ ($AV = ks$)
To be applied when T ≦ 101.5		If $\bar{X} < 98.5\%$, then	$M = 98.5\%$ ($AV = 98.5 - \bar{X} + ks$)
		If $\bar{X} > 101.5\%$, then	$M = 101.5\%$ ($AV = \bar{X} - 101.5 + ks$)
M (case 2)	reference value	If 98.5% ≦ $\bar{X}$ ≦ T, then	$M = \bar{X}$ ($AV = ks$)
To be applied when $T > 101.5$		If $X < 98.5\%$, then	$M = 98.5\%$ ($AV = 98.5 - \bar{X} + ks$)
		If $\bar{X} > T$, then	$M = T\%$ ($AV = \bar{X} - T + ks$)
Acceptance Value (AV)			general formula: $\lvert M - \bar{X} \rvert + ks$ [Calculations are specified above for the different cases.]
$L1$	maximum allowed acceptance value		$L1 = 15.0$ unless otherwise specified.
$L2$	maximum allowed range for deviation of each dosage unit tested from the calculated value of M	On the low side, no dosage unit result can be less than $0.75M$ while on the high side, no dosage unit result can be greater than $1.25M$ (This is based on an $L2$ value of 25.0.)	$L2 = 25.0$ unless otherwise specified.
T	Target content per dosage unit at time of manufacture, expressed as the percentage of the label claim. Unless otherwise stated, T is 100.0%, or T is the manufacturer's approved target content per dosage unit.		

Add the following to 9.22 Standard Solutions:

Standard Chromium Solution for Atomic Absorption Spectrophotometry Weigh exactly 0.283 g of potassium dichromate (standard reagent), dissolve in water to make exactly 1000 mL. Each mL contains 0.10 mg of chromium (Cr).

Standard Hydrogen Peroxide Stock Solution To an amount of hydrogen peroxide (30) add water to make a solution so that each mL contains 0.30 g of hydrogen peroxide (H_2O_2:34.01). Pipet 1 mL of this solution, add water to make exactly 10 mL, pipet 1 mL of this solution, transfer it to a flask containing 10 mL of water and 10 mL of dilute sulfuric acid, and titrate <*2.50*> with 0.02 mol/L potassium permanganate VS until the color of the solution changes to slightly red. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.02 mol/L potassium permanganate VS
= 1.701 mg of H_2O_2

Standard Hydrogen Peroxide Solution To exactly 10 mL of Standard Hydrogen Peroxide Stock Solution add water to make exactly 100 mL. Prepare before use. Each mL contains 30 mg of hydrogen peroxide (H_2O_2:34.01).

Standard Iron Solution (2) for Atomic Absorption Spectrophotometry To exactly 2 mL of Standard Iron Stock Solution add water to make exactly 250 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL. Prepare before use. Each mL contains 8 μg of iron (Fe).

Add the following to 9.43 Filter Papers, Filters for filtration, Test Papers, Crucibles, etc.:

Peroxide test strip A strip that is prepared to be able to assay the concentration of hydrogen peroxide in the range of 0 to 25 ppm. The test strips have the suitable color scale covering the range from 0 to 25 ppm hydrogen peroxide.

Add the following:

Gelatin

ゼラチン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Gelatin is a purified protein obtained from collagen of animals by partial alkaline and/or acid hydrolysis, or by thermal hydrolysis. The hydrolysis leads to gelling or non-gelling grades.

It is the gelling grade.

The label states the gel strength (Bloom value).

◆**Description** Gelatin occurs as colorless or white to light yellow-brown sheets, shreds, granules or powder.

It is freely soluble in hot water, and practically insoluble in ethanol (95).

It does not dissolve in water, but slowly swells and softens when immersed in it, gradually absorbing water 5 to 10 times its own mass.

Gelatin derived from an acid-treated collagen exhibits an isoelectric point between pH 7.0 and 9.0, and Gelatin derived from an alkali-treated collagen exhibits an isoelectric point between pH 4.5 and 5.0.◆

Identification **(1)** Dissolve 1.00 g of Gelatin in freshly boiled and cooled water at about 55°C to make 100 mL, and use this solution as the sample solution. To 2 mL of the sample solution keeping at about 55°C add 0.05 mL of copper (II) sulfate TS. Mix and add 0.5 mL of 2 mol/L sodium hydroxide TS: a violet color is produced.

(2) In a test tube about 15 mm in diameter, place 0.5 g of Gelatin, add 10 mL of water, and allow to stand for 10 minutes. Heat at 60°C for 15 minutes, then keep the tube upright at 0°C for 6 hours, and invert the tube: the contents do not flow out immediately.

Gel strength (Bloom value) Determine the mass (g) necessary to produce the force which, applied to a plunger 12.7 mm in diameter, makes a depression 4 mm deep in a gel having a concentration of 6.67% and matured at 10°C.

(i) Apparatus Texture analyzer or gelometer with a cylindrical piston 12.7 ± 0.1 mm in diameter with a plane pressure surface and a sharp bottom edge, and with a bottle 59 ± 1 mm in internal diameter and 85 mm high (jelly cup).

(ii) Procedure Place 7.5 g of Gelatin in a jelly cup, add 105 mL of water, close the cup, and allow to stand for 1 to 4 hours. Heat in a water bath at 65 ± 2°C for 15 minutes. While heating, stir gently with a glass rod. Ensure that the solution is uniform and any condensed water on the inner walls of the cup is incorporated. Allow to cool at room temperature for 15 minutes and transfer the cup to a thermostatically controlled bath at 10.0 ± 0.1°C, and fitted with a device to ensure that the platform on which the cup stands is perfectly horizontal. Close the cup, and allow to stand for 17 ± 1 hours. Remove the sample cup from the bath and quickly wipe the water from the exterior of the cup. Center the cup on the platform of the apparatus so that the plunger contacts the sample as nearly at its midpoint as possible, and start the measurement with 4 mm depression distance and 0.5 mm/second test speed: 80 to 120% of the labeled nominal value.

pH <*2.54*> pH at 55°C of the sample solution obtained in Identification (1) is 3.8 – 7.6.

Purity ◆**(1)** Heavy metals <*1.07*>—Proceed with 0.5 g of Gelatin according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).◆

(2) Iron—To 5.00 g of Gelatin, in a glass-stoppered flask, add 10 mL of hydrochloric acid, close the flask, and place in a water bath at 75 – 80°C for 2 hours. If necessary for proper solubilization, the gelatin may be allowed to swell after addition of the acid and before heating, the heating time may be prolonged and a higher temperature may be used. After cooling, adjust the content of the flask to 100.0 g with water, and use this solution as the sample solution. Separately, place 5.00 g each of Gelatin in three glass-stoppered flasks, proceed with them in the same manner as the sample solution, then add 10 mL, 20 mL and 30 mL of Standard Iron Solution (2) for Atomic Absorption Spectrophotometry exactly to each flask separately. Adjust the content of these flasks to 100.0 g each with water, and use these solutions as the standard solutions. Perform the test with the sample solution and the standard solutions as directed in the standard addition method under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and determine the content of iron: not more than 30 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Iron hollow cathode lamp.
Wavelength: 248.3 nm.

(3) Chromium—Use the sample solution obtained in (2) as the sample solution. Separately, place 5.00 g each of Gelatin in three glass-stoppered flasks, proceed with them in the same manner as the sample solution, then add 0.25 mL, 0.50 mL and 0.75 mL of Standard Chromium Solution for Atomic Absorption Spectrophotometry exactly to each flask separately. Adjust the content of these flasks to 100.0 g each with water, and use these solutions as the standard solutions. Perform the test with the sample solution and the standard solutions as directed in the standard addition method under Atomic Absorption Spectrophotomety <*2.23*> according to the following conditions, and determine the content of chromium: not more than 10 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Chromium hollow cathode lamp.
Wavelength: 357.9 nm.

(4) Zinc—Use the sample solution obtained in (2) as the sample solution. Separately, place 5.00 g each of Gelatin in three glass-stoppered flasks, proceed with them in the same

manner as the sample solution, then add 7.5 mL, 15 mL and 22.5 mL of Standard Zinc Solution for Atomic Absorption Spectrophotometry exactly to each flask separately. Adjust the content of these flasks to 100.0 g each with water, and use these solutions as the standard solutions. Perform the test with the sample solution and the standard solutions as directed in the standard addition method under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions, and determine the content of zinc: not more than 30 ppm.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Zinc hollow cathode lamp.
Wavelength: 213.9 nm.

◆**(5)** Arsenic <*1.11*>—Take 15.0 g of Gelatin in a flask, add 60 mL of diluted hydrochloric acid (1 in 5), and dissolve by heating. Add 15 mL of bromine TS, heat until the excess of bromine is expelled, neutralize with ammonia TS, add 1.5 g of disodium hydrogen phosphate dodecahydrate, and allow to cool. To this solution add 30 mL of magnesia TS, allow to stand for 1 hour, and collect the precipitates. Wash the precipitates with five 10-mL portions of diluted ammonia TS (1 in 4), and dissolve in diluted hydrochloric acid (1 in 4) to make exactly 50 mL. Perform the test with 5 mL of this solution: the solution has no more color than the following color standard.

Color standard: Proceed with 15 mL of Standard Arsenic Solution, instead of Gelatin, in the same manner (not more than 1 ppm).◆

(6) Peroxides—

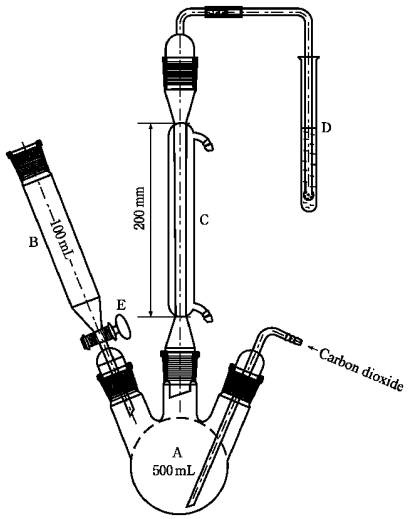
(i) Enzyme reaction: Peroxidase transfers oxygen from peroxides to an organic redox indicator which is converted to a blue oxidation product. The intensity of the color obtained is proportional to the quantity of peroxide and can be compared with a color scale provided with the test strips, to determine the peroxide concentration.

(ii) Procedure: Weigh 20.0 ± 0.1 g of Gelatin in a beaker, add 80.0 ± 0.2 mL of water, and stir to moisten all the gelatin. Allow to stand at room temperature for 1 – 3 hours. Cover the beaker with a watch-glass, and heat the beaker for 20 ± 5 minutes in a water bath at 65 ± 2°C for dissolving the sample. Stir the contents of the beaker with a glass rod to achieve a homogeneous solution, and use this as the sample solution. Dip a peroxide test strip for 1 second into the sample solution, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid, and compare the reaction zone after 15 seconds with the color scale provided. Multiply the concentration read from the color scale by a factor of 5 to calculate the concentration of peroxide in the test substance: not more than 10 ppm.

(iii) Suitability test: To exactly 10 mL of Standard Hydrogen Peroxide Solution add water to make exactly 300 mL. Pipet 2 mL of this solution, add water to make exactly 1000 mL (2 ppm). Dip a peroxide test strip for 1 second into this solution, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid and compare the color of the reaction zone after 15 seconds with the color scale: the color of the zone is equivalent to 2 ppm of the color scale.

(7) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.



A: Three-necked round-bottomed flask (500 mL)
B: Cylindrical dropping funnel (100 mL)
C: Condenser
D: Test tube
E: Tap

(ii) Procedure Introduce 150 mL of water into the three-necked round-bottomed flask and pass carbon dioxide through the whole system at a rate of 100 mL per minute. Place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test tube. After 15 minutes, remove the cylindrical dropping funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the three-necked round-bottomed flask about 25.0 g of Gelatin with the aid of 100 mL of water. Pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the three-necked round-bottomed flask ◆and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide.◆ Place the three-necked round-bottomed flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test tube with the aid of a little water to a 200 mL wide-necked conical flask. Heat the flask in a water bath for 15 minutes and cool. Add 0.1 mL of bromophenol blue TS and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide from the following expression: it is not more than 50 ppm.

$$\text{Amount (ppm) of sulfur dioxide} = V/M \times 1000 \times 3.203$$

M: Amount (g) of Gelatin
V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

Conductivity <*2.51*> Perform the test at 30 ± 1.0°C with the sample solution obtained in Identification (1), without

temperature compensation: not more than 1 $mS \cdot cm^{-1}$.

Loss on drying <*2.41*> Not more than 15.0% (5 g, 105°C, 16 hours).

Microbial limit <*4.05*> The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 10^2 CFU/g, respectively. *Salmonella* and *Escherichia coli* are not observed.

Containers and storage ◆Containers—Tight containers.◆ Storage—Protect from heat and moisture.

GENERAL INFORMATION

International Harmonization Implemented in the Japanese Pharmacopoeia Sixteenth Edition

Add the following:

November 2012 (Corr. 1)

Harmonized items	JP16 (Partial revision)	Remarks
Gelatin (Gelling Grade)	**Gelatin**	
Definition	Definition	In JP, ''Purified protein obtained from collagen of animals by enzymatic hydrolysis'' is not included.
Identification A	Identification (1)	
Identification B	Identification (2)	
pH	pH	
Conductivity	Conductivity	
Sulphur dioxide	Purity (7) Sulfur dioxide	
Peroxides	Purity (6) Peroxides	
Gel strength (Bloom value)	Gel strength (Bloom value)	
Iron	Purity(2) Iron	
Chromium	Purity (3) Chromium	
Zinc	Purity (4) Zinc	
Loss on drying	Loss on drying	
Microbial contamination	Microbial limit	
Storage	Containers and storage	
Labelling	Definition	

Change the following:

November 2010 (Rev. 1)

Harmonized items	JP16 (Partial revision)	Remarks
Uniformity of Dosage Units	**6.02 Uniformity of Dosage Units**	
(Introduction)	(Introduction)	JP's particular description: Additional explanation on Liquids. Additional explanation for the part not containing drug substance.
Content uniformity	1. Content Uniformity	
Solid dosage forms	(i) Solid dosage forms	
Liquid or Semi-Solid dosage forms	(ii) Liquid or Semi-Solid dosage forms	
Calculation of acceptance value	1.1. Calculation of Acceptance Value	
Mass variation	2. Mass Variation	JP's particular description: Assuming that the concentration of drug substance is uniform in each lot.
Uncoated or film-coated tablets	(i) Uncoated or film-coated Tablets	
Hard capsules	(ii) Hard Capsules	
Soft capsules	(iii) Soft Capsules	
Solid dosage forms other than tablets and capsules	(iv) Solid dosage forms other than tablets and capsules	
Liquid dosage forms	(v) Liquid dosage forms	The phrase ''in conditions of normal use. If necessary, compute the equivalent volume after determining the density.'' is deleted.
Calculation of acceptance value	2.1. Calculation of Acceptance Value	
Criteria	3. Criteria	
Solid, Semi-Solid and Liquid dosage forms	(i) Solid, Semi-Solid and Liquid dosage forms	
Table 1 Application of content uniformity (CU) and mass variation (MV) test for dosage forms	Table 6.02-1 Application of Content Uniformity (CU) and Mass Variation (MV) Test for Dosage Forms	JP's particular description: Addition of ''(divided forms, lyophilized forms)'' and ''(true solution)''.
Table 2	Table 6.02-2	

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