The Ministry of Health, Labour and Welfare Ministerial Notification No. 519

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*, and the revised Japanese Pharmacopoeia shall come into effect on October 1, 2012. However, in the case of drugs which are listed in the Japanese Pharmacopoeia (hereinafter referred to as ''previous Pharmacopoeia'') [limited to those listed 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 October 1, 2012 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 September 30, 2012 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 March 31, 2014. 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 October 1, 2012 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 March 31, 2014.

Yoko Komiyama
The Minister of Health, Labour and Welfare

September 27, 2012

(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 I to the Japanese Pharmacopoeia Sixteenth Edition from General Notice to Ultraviolet-visible Reference Spectra (pp. 2321 – 2545).

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; 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 Physical Methods; Committee on Biological Methods; Committee on Nomenclature for Pharmaceuticals; Committee on International Harmonization; Committee on Pharmaceutical Water; and Committee on Reference Standards. Furthermore, working groups are established under the Committee on Physico-Chemical Methods, Committee on Drug Formulation and Committee on Biological Methods 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 September 2012.

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 2010 and March 2012, were prepared for a supplement to the JP 16. They were examined by the Committee on JP in May 2012, followed by the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) in June 2012, and then submitted to the Minister of Health, Labour and Welfare.

Numbers of discussions in the committees to pre-

pare the supplement drafts were as follows: Expert Committee (8); Sub-expert Committee (4), Committee on Chemicals (22), Committee on Antibiotics (5); Committee on Biologicals (9); Committee on Crude Drugs (21); Committee on Pharmaceutical Excipients (12); Committee on Physico-Chemical Methods (14); Committee on Drug Formulation (19); Committee on Physical Methods (7); Committee on Biological Methods (13); Committee on Nomenclature for Pharmaceuticals (7); Committee on International Harmonization (8); and Committee on Pharmaceutical Water (7).

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 Parenteral Drug Association Japan Chapter, the Japan Reagent Association, the Japan Oilseeds Processors Association, the Home Medicine Association of Japan, and the Association of Membrane Separation Technology of Japan.

In consequence of this revision, the JP 16th Edition carries 1837 articles, owing to the addition of 77 articles and the deletion of 4 articles.

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

1. The Supplement I 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 main volume and the Supplement I.

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 4 of General Notices was revised as follows:

Paragraph 4: On the definition of items to be included in the Official Monographs, Crude Drugs, ''Crude Drugs being applied the requirements of the General Rules for Crude Drugs'' was changed to ''Crude Drugs''.

8. To Paragraph 1 of General Rules for Crude Drugs the following items were added:
(1) Artemisia Leaf
(2) Cherry Bark
(3) Malt

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

2–2. Liquids and Solutions for Oro-mucosal Application was added under 2. Preparations for Oro-mucosal Application. The ''Preparations for Gargles'' was classified under the number 2–2–1..

10. The following items in General Tests, Processes and Apparatus were revised:
(1) 2.22 Fluorometry
(2) 2.47 Osmolarity Determination
(3) 2.49 Optical Rotation Determination
(4) 3.01 Determination of Bulk and Tapped Densities
(5) 4.01 Bacterial Endotoxins Test
(6) 6.10 Dissolution Test

11. The following items in General Tests, Processes and Apparatus were added:
(1) 2.62 Mass Spectrometry
(2) 2.63 Inductively Coupled Plasma-Atomic Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry

12. The following Reference Standards were added:
Auranofin
Butyl Parahydroxybenzoate
Calcium Pantothenate

Carboplatin
Cetotiamine Hydrochloride
Dorzolamide Hydrochloride
Epalrestat
Epoetin Alfa
Epoetin Beta
Ethyl Parahydroxybenzoate
Filgrastim
Lenograstim
Methyl Parahydroxybenzoate
Nartograstim
Propyl Parahydroxybenzoate
Quetiapine Fumarate
Tacalcitol
Valsartan

13. The preface and reagents under 9.41 Reagents, Test Solutions were revised to allow use of the certified reference materials in addition to JIS K 8005 (Standard reagent for volumetric analysis) standard substances.

14. The following substances were newly added to the Official Monographs:
Aciclovir for Injection
Aciclovir Ointment
Aldioxa Granules
Aldioxa Tablets
Amlodipine Besilate Orally Disintegrating Tablets
Auranofin
Auranofin Tablets
Azelnidipine
Brotizolam
Bupivacaine Hydrochloride Hydrate
Carboplatin
Carboplatin Injection
Cefpodoxime Proxetil Tablets
Cetotiamine Hydrochloride Hydrate
Colestimide
Colestimide Tablets
Crospovidone
L-Cystine
Dorzolamide Hydrochloride
Dorzolamide Hydrochloride Ophthalmic Solution
Edaravone
Edaravone Injection
Emedastine Fumarate
Emedastine Fumarate Extended-release Capsules
Epalrestat
Epalrestat Tablets
Epoetin Alfa (Genetical Recombination)
Epoetin Beta (Genetical Recombination)
Fexofenadine Hydrochloride Tablets
Filgrastim (Genetical Recombination)
Filgrastim (Genetical Recombination) Injection
Hypromellose Acetate Succinate
Ibuprofen Piconol
Ibuprofen Piconol Cream
Ibuprofen Piconol Ointment
Iohexol
Iohexol Injection
Isosorbide Mononitrate 70%/Lactose 30%
Isosorbide Mononitrate Tablets
Lafutidine
Lafutidine Tablets
Lenograstim (Genetical Recombination)
Levofloxacin Fine Granules
Levofloxacin Ophthalmic Solution
Levofloxacin Tablets
Lobenzarit Sodium
Losartan Potassium Tablets
Morphine Sulfate Hydrate
Nartograstim (Genetical Recombination)
Nartograstim for Injection (Genetical Recombination)
Nifedipine Extended-release Capsules
Nifedipine Enteric Fine Granules
Nifedipine Fine Granules
Omeprazole Enteric-coated Tablets
Pemirolast Potassium Ophthalmic Solution
Pilocarpine Hydrochloride Tablets
Quetiapine Fumarate
Quetiapine Fumarate Fine Granules
Quetiapine Fumarate Tablets
Simvastatin Tablets
Tacalcitol Hydrate
Tacalcitol Lotion
Taltirelin Hydrate
Taltirelin Orally Disintegrating Tablets
Taltirelin Tablets
Tranilast
Tranilast Capsules
Tranilast Fine Granules
Tranilast for Syrup
Tranilast Ophthalmic Solution
Valsartan
Valsartan Tablets
Artemisia Leaf
Cherry Bark
Hangeshashinto Extract
Malt
Tokishakuyakusan Extract

15. The following monographs were revised:
Acrinol Hydrate
Acrinol and Zinc Oxide Oil

Actinomycin D
Aldioxa
Amiodarone Hydrochloride Tablets
Atorvastatin Calcium Hydrate
Azathioprine Tablets
Aztreonam
Benzyl Alcohol
Bisoprolol Fumarate Tablets
Butyl Parahydroxybenzoate
Calcium Pantothenate
Anhydrous Dibasic Calcium Phosphate
Dibasic Calcium Phosphate Hydrate
Candesartan Cilexetil
Cefazolin Sodium
Cefdinir
Cefditoren Pivoxil Fine Granules
Cefoperazone Sodium
Cefteram Pivoxil
Ceftibuten Hydrate
Cellacefate
Chlordiazepoxide Tablets
Chlorpheniramine Maleate Powder
Anhydrous Citric Acid
Citric Acid Hydrate
Clindamycin Hydrochloride
Clomifene Citrate
Clomifene Citrate Tablets
1% Codeine Phosphate Powder
10% Codeine Phosphate Powder
Corn Starch
Daunorubicin Hydrochloride
Dibekacin Sulfate
Diethylcarbamazine Citrate Tablets
1% Dihydrocodeine Phosphate Powder
10% Dihydrocodeine Phosphate Powder
Donepezil Hydrochloride
10% Ephedrine Hydrochloride Powder
Ethanol
Anhydrous Ethanol
Ethanol for Disinfection
Ethyl Parahydroxybenzoate
Fexofenadine Hydrochloride
Glimepiride Tablets
Human Menopausal Gonadotrophin
Heparin Calcium
Heparin Sodium
Heparin Sodium Injection
Hydralazine Hydrochloride Powder
Josamycin
Josamycin Propionate
Kanamycin Sulfate
Anhydrous Lactose
Magnesium Stearate
Mefloquine Hydrochloride
10% *dl*-Methylephedrine Hydrochloride Powder
Methyl Parahydroxybenzoate
Mizoribine
Nateglinide
Norethisterone
Parnaparin Sodium
Piperacillin Sodium
Potato Starch
Pravastatin Sodium Fine Granules
Pravastatin Sodium Tablets
Propyl Parahydroxybenzoate
Rabeprazole Sodium
0.1% Reserpine Powder
Riboflavin Powder
Ribostamycin Sulfate
Rice Starch
Sarpogrelate Hydrochloride
Streptomycin Sulfate
Streptomycin Sulfate for Injection
Sucrose
Titanium Oxide
Trichlormethiazide Tablets
Vasopressin Injection
Voglibose Tablets
Wheat Starch
Zolpidem Tartrate
Akebia Stem
Alisma Tuber (Former name: Alisma Rhizome)
Powdered Alisma Tuber (Former name: Powdered Alisma Rhizome)
Alpinia Officinarum Rhizome
Powdered Amomum Seed
Apricot Kernel
Aralia Rhizome
Asparagus Tuber
Powdered Atractylodes Lancea Rhizome
Atractylodes Rhizome
Powdered Atractylodes Rhizome
Bearberry Leaf
Belladonna Root
Benincasa Seed
Bupleurum Root
Powdered Capsicum
Chrysanthemum Flower
Cinnamon Bark
Citrus Unshiu Peel
Powdered Clove
Cnidium Monnieri Fruit
Cnidium Rhizome
Powdered Cnidium Rhizome
Powdered Coix Seed
Condurango

Coptis Rhizome
Powdered Coptis Rhizome
Corydalis Tuber
Powdered Corydalis Tuber
Crataegus Fruit
Digenea
Ephedra Herb
Epimedium Herb
Powdered Fennel
Fritillaria Bulb
Powdered Gambir
Powdered Gardenia Fruit
Gentian
Powdered Geranium Herb
Ginger
Powdered Ginger
Ginseng
Powdered Ginseng
Glycyrrhiza
Powdered Glycyrrhiza
Hemp Fruit
Ipecac
Powdered Ipecac
Japanese Angelica Root
Powdered Japanese Angelica Root
Leonurus Herb
Lycium Bark
Magnolia Bark
Powdered Magnolia Bark
Powdered Moutan Bark
Orengedokuto Extract
Panax Japonicus Rhizome
Powdered Panax Japonicus Rhizome
Peach Kernel
Powdered Peach Kernel
Peucedanum Root
Pharbitis Seed
Phellodendron Bark
Powdered Phellodendron Bark
Picrasma Wood
Plantago Herb
Polygala Root
Powdered Polygala Root
Polygonum Root
Processed Ginger
Pueraria Root
Red Ginseng
Rehmannia Root
Powdered Rose Fruit
Saireito Extract
Schizonepeta Spike
Scutellaria Root
Powdered Scutellaria Root
Powdered Senega
Senna Leaf
Powdered Senna Leaf
Shosaikoto Extract
Sinomenium Stem and Rhizome
Smilax Rhizome
Powdered Smilax Rhizome
Sweet Hydrangea Leaf
Powdered Sweet Hydrangea Leaf
Swertia Herb
Toad Cake (Former name: Toad Venom)
Turmeric
Powdered Turmeric
Uncaria Hook
Zanthoxylum Fruit
Powdered Zanthoxylum Fruit

16. The following monographs were deleted:
Amobarbital Sodium for Injection
Flurazepam
Flurazepam Capsules
Trimethadione Tablets

17. The descriptions of following monographs were revised according to the provision of crystal forms:
Atorvastatin Calcium Hydrate
Candesartan Cilexetil
Donepezil Hydrochloride
Fexofenadine Hydrochloride
Nateglinide
Rabeprazole Sodium
Sarpogrelate Hydrochloride

Those who were engaged in the preparation of the Supplement I to JP 16 are as follows:

Fumiaki Akahori
Teruo Amagasa
Mitsuo Aoki
Nobuo Aoyagi**
Hiroyuki Arai
Keiko Arimoto
Yuichi Arimoto
Naoki Aruga
Naoki Asai
Hiroshi Asama
Toshiki Asano
Kazuhide Ashizawa
Yukio Aso
Toshio Azuma
Takashi Bamba
Yosuke Demizu
Makoto Emura
Hiroyuki Fuchino
Kiyoshi Fukuhara
Yukihiro Goda
Takashi Goto
Noriaki Habasaki
Yuji Haishima
Takashi Hakamatsuka
Kentaro Hanada
Ruri Hanajiri
Toshikazu Harada
Akira Harazono
Mitsuru Hashida*
Noritaka Hashii
Rika Hatano
Takao Hayakawa*
Masahiro Hayashi
Yoshinori Hayashi

Kenji Higuchi
Yuuki Hirata
Yukio Hiyama
Masatoshi Hori
Naoki Hosono
Kenji Hosoya
Masashi Hyuga
Nobukazu Igoshi
Kazuhiko Ikegami
Koichi Inubushi
Akiko Ishii
Tsuneo Ishizuka
Shigeru Itai
Chizuko Itoh
Takashi Itoh
Kenichi Izutsu
Kazuaki Kakehi
Takemine Kanai
Norio Karibe
Hirohito Katayama
Haru Kato
Kumiko Kato
Yoshiaki Kato
Noriko Katori
Nobuo Kawahara
Urao Kawakami
Toru Kawanishi
Yoshihiko Kawarasaki
Nana Kawasaki
Keiji Kijima
Yutaka Kikuchi
Yuuichi Kikuchi
Kimio Kiryu
Yasuhiro Kishimoto
Mitsukazu Kitada
Atsushi Kittaka
Fumiyuki Kiuchi
Junko Kizu
Yukari Koga
Masayoshi Kohase
Tatsuo Koide
Shigeo Kojima
Hiroyasu Kokubo
Katsuko Komatsu
Toshifumi Konda
Kenji Kondo
Seizo Kondo
Kenichi Kumasaka
Masaaki Kurihara
Fumiyo Kusu
Midori Makita
Eiichi Mikami
Takao Mitsuhashi
Naoki Miyata
Tamaki Miyazaki
Mitsuo Mori
Nobuaki Moriguchi
Seiki Morimoto
Katsuhiro Morisawa
Osamu Morita
Takashi Morita
Toshimi Murai
Masashi Muroi
Takashi Nagashima
Takahiro Naito
Shinsaku Nakagawa
Emi Nakajima
Hiroshi Nakamura
Tatsuya Nakano
Tatsumi Nakashima
Mitsuo Nanaura
Masao Nasu
Shingo Niimi
Yasuhiko Nishi
Yutaka Nishihara
Atsushi Nukanobu
Sumiaki Oba
Yasumitsu Ogura
Ryozo Oishi
Akira Okonogi
Tsuneo Okubo
Haruhiro Okuda
Takamasa Okugawa
Yuko Osumi
Masami Otsuka
Tadashi Ouchi
Yukio Saito
Yoshikazu Sakagami
Eiji Sakai
Tomoaki Sakamoto
Shingou Sakurai
Tomofumi Santa
Hiroshi Sasaki
Kunio Sasaki
Tomoko Sasaki
Tsuguo Sasaki
Kazumichi Sasaoki
Kyoko Sato
Michiko Sekiguchi
Setsuko Sekita
Yasuo Shimada
Kozo Shimoda
Osamu Shirokizawa
Osamu Shirota
Takuji Shoda
Junya Souma
Keiichi Sudo
Naoki Sugimoto
Daisuke Sugiura
Mikio Suzuki
Sumiko Suzuki
Shinichi Tadaki
Nobuo Taguchi
Wataru Takada
Yoshikazu Takahashi
Kunihiro Takai
Masaki Takao
Kikuo Takatera
Osami Takeda
Tadahiro Takeda
Hirohumi Takeuchi
Toyoshige Tanabe
Masakazu Tanaka
Toshihiro Tanaka
Kenichi Tanamoto
Tsuyoshi Tanimoto
Susumu Terabayashi
Katsuhide Terada
Reiko Teraoka
Hiroshi Tokunaga
Kiyoshi Tomioka
Hiroyuki Tomitsuka
Shigeki Tsuda
Hideya Tsuge
Hiroyuki Tsujimoto
Eriko Uchida
Yoshimasa Uehara
Atsuhiro Uetake
Eiji Watanabe
Takehiko Yajima
Toshiyasu Yamada
Teruhide Yamaguchi
Tetsuji Yamaguchi
Hiroki Yamaji
Keiji Yamamoto
Tosuke Yamamoto
Chikamasa Yamashita
Takeshi Yamazaki
Masato Yasuhara
Shiho Yasuo
Hikaru Yoden
Chikako Yomota
Etsuo Yonemochi
Kumi Yoshida

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

GENERAL NOTICES

Change the paragraph 4 as follows:

4. ''Crude Drugs and related drugs'' are placed together in the posterior part of the Official Monographs. These include: Extracts, Powders, Tinctures, Syrups, Spirits, Fluidextracts or Suppositories containing Crude Drugs as the active ingredient, and combination preparations containing Crude Drugs as the principal active ingredient.

—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, 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 Polypourus Sclerotium, Powderd 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, 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 Venom, Tragacanth, Tribulus Fruit, Trichosanthes Root, Turmeric, Uncaria Hook, Zanthoxylum Fruit, Zedoary.

GENERAL RULES FOR PREPARATIONS

Change the paragraphs 2-2 to 2-4 under [2] Monographs for Preparations as follows:

2-2. Liquids and Solutions for Oro-mucosal Application

(1) Liquids and Solutions for Oro-mucosal Application are preparations in liquid form or flowable and viscous gelatinous state, intended for oral cavity application.

(2) Liquids and Solutions for Oro-mucosal Application are usually prepared by mixing active substance(s) with suitable excipients and Purified Water or suitable vehicles to dissolve homogenously or to emulsify or suspend, and by filtering if necessary.

(3) For Liquids and Solutions for Oro-mucosal Application which are apt to deteriorate, prepare before use.

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

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

2-2-1. Preparations for Gargles

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

(2) Solid type preparations to be dissolved in water before use are prepared as directed under 1-1. Tablets or 1-3. Granules.

2-3. Sprays for Oro-mucosal Application

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

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

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

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

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

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

2-4. Semi-solid Preparations for Oro-mucosal Application

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

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

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

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

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

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

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

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

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

GENERAL TESTS, PROCESSES AND APPARATUS

Change the introduction as follows:

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

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

2.22 Fluorometry

Change the beginning part of the text as follows:

Fluorometry is a method to measure the intensity of fluorescence emitted from a solution of fluorescent substance irradiated with an exciting light in a certain wavelength range. This method is also applied to the phosphorescent substances.

Fluorescence intensity F in a dilute solution is proportional to the concentration c in mol per liter of the solution and the pathlength l of light through the solution in centimeter.

$$F = kI_0\phi\varepsilon cl$$

k: Constant
I_0: Intensity of exciting light
ϕ: Quantum yield of fluorescence or phosphorescence

$$\text{Quantum yield of fluorescence or phosphorescence} = \frac{\text{number of quanta as fluorescence or phosphorescence}}{\text{number of quanta absorbed}}$$

ε: Molar extinction coefficient of the substance at the excitation wavelength

2.47 Osmolarity Determination

Change 2. Procedure and 5. Osmolar ratio as follows:

2. Procedure

A fixed volume of the test solution is introduced into the sample cell, as indicated for the individual apparatus.

The apparatus must first be calibrated by the two-point calibration method by using osmolal standard solutions. For the calibration, select two different standard solutions just covering the expected osmolar concentration of a sample solution. Other than the indicated osmolal standard solutions in the *Table* below, water can also be used as a standard so-

lution (0 mOsm) for measuring low osmolar sample solutions (0 – 100 mOsm). Next, after washing the sample cell and the thermistor as indicated for the individual apparatus, measure the degree of the freezing-point depression caused by a sample solution. Using the above-mentioned relation of osmolar concentration m and ΔT, the osmolarity of a sample solution can be obtained, and it is assumed to be numerically equal to the osmolarity.

In the case of higher osmolar solutions over 1000 mOsm, dilute the sample with water and prepare n'/n times diluted sample solution (n in n'). Measure the osmolarity of the diluted solution, as described above. In this case, it is necessary to state that the calculated osmolarity for the sample (see below) is an apparent osmolarity obtained by the dilution method. When the determination is performed using n'/n times diluted solution, the dilution number should be selected so that the osmolar concentration is near but not exceeding 1000 mOsm, and dilute in one step.

In the case of solid samples, such as freeze-dried medicines, prepare a sample solution by dissolving the solid using the indicated solution for dissolution.

5. Osmolar ratio

In this test method the osmolar ratio is defined as the ratio of osmolarity of a sample solution to that of the isotonic sodium chloride solution. The ratio can be used as a measure of isotonicity of sample solution. Since the osmolarity of the isotonic sodium chloride solution (NaCl 0.900 g/100 mL) c_S (mOsm) is assumed to be constant (286 mOsm), the osmolar ratio of a sample solution, of which the osmolarity is c_T (mOsm), can be calculated by means of the following equation,

$$\text{Osmolar ratio} = c_T/c_S$$

c_S: 286 mOsm

When the measurement is done by the dilution method, because the sample has an osmolarity over 1000 mOsm, the apparent osmolarity of the sample solution c_T can be calculated as $n'/n \cdot c'_T = c_T$, in which n'/n is the dilution number and c'_T is the measured osmolarity for the diluted solution. In this calculation, a linear relation between osmolarity and solute concentration is assumed. The dilution must be made in one step. Thus when the dilution measurement is performed, the dilution number is stated as (n in n').

2.49 Optical Rotation Determination

Add the following item to the end of the text:

Verification for accuracy of apparatus

Accuracy of the scale of apparatus is verified by using a solution of sucrose for optical rotation if the reading indicates the value of its known specific optical rotation. For daily verification a traceable quartz plate may be used. When the observed value is not meet the specification of the quarty plate, repeat the test after refurbishing the apparatus.

Add the following:

2.62 Mass Spectrometry

Mass spectrometry (MS) is a method to separate and detect the ions generated from the ionization of molecules according to their m/z values, and it is used for the identification and purity test of the substances. The m/z value is the dimensionless parameter obtained from dividing the relative mass (m) of the ion (the ratio of the mass of the ion to the unified atomic mass unit) by the charge number (z) of the ion. The unified atomic mass unit is defined as one-twelfth of the mass of a ^{12}C atom in its ground state, and it is used to express masses of atoms, molecules and ions. The result of measurement is shown as the mass spectrum in which the m/z values of the ions and the relative intensities of the signals corresponding to the ions are shown on the x-axis and the y-axis, respectively.

The precise mass of a molecule or an ion consisting of only a single isotope (usually, the isotope with the greatest natural abundance) of each element comprising a sample molecule is referred to as the "monoisotopic mass." Usually not only a monoisotopic ion but also its isotopic ions are seen in the mass spectrum. The molecular mass of the sample substance can be determined from the m/z value of the molecular ion. When the fragment ions are observed, the molecular structure of the sample substance can be estimated and confirmed based on the masses of the fragment ions and the mass differences among the molecular ion and the fragment ions. In tandem mass spectrometry (MS/MS), the product ions, generated by the dissociation of the selected precursor ion with m/z value, are used for the mass spectrometry. The structural estimation and confirmation of the precursor ion can be performed using the m/z value of the product ion observed in the measurement. The schematic diagram of the MS and the MS/MS is shown in Fig. 2.62-1.

1. Mass Spectrometer

A mass spectrometer usually consists of a sample introduction unit, an ionization unit (ion source), a mass analyzer, a detector and data processor, and an exhaust

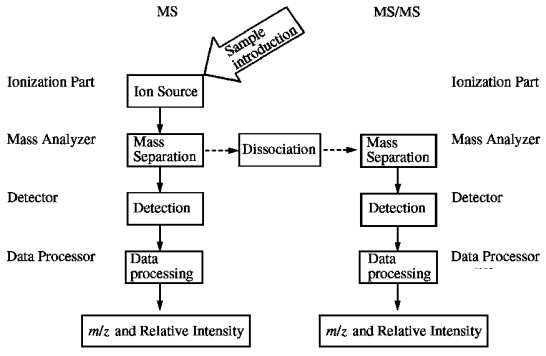


Fig. 2.62-1 Schematic diagram of mass spectrometry (MS) and tandem mass spectrometry (MS/MS)

system to maintain the mass analyzer under high vacuum (Fig. 2.62-1).

1.1. Sample Introduction

For the introduction of the sample into the ion source, the following methods are used; Direct infusion method, in which solution samples are injected into the ion source by using a syringe pump or capillary tip, for example; Direct inlet method, in which a liquid or solid sample is placed in a glass tube or other appropriate vessel and introduced into the vicinity of the electron beams or reactant ion atmosphere of the ion source. In addition, the method, in which each component separated by the chromatographic technique such as gas chromatography or liquid chromatography and capillary electrophoresis is introduced into the ion source successively, is also used.

1.2. Ion Source

When the sample substances are introduced into the mass spectrometer, ions with a positive or negative charge are generated from the substance in the ion source. There are various ionization methods in mass spectrometry, and it is important to select the most suitable ionization method according to the polarity and molecular mass of the sample substance to be measured and the purpose of the measurement. Typical ionization methods are as follows.

1.2.1. Electron Ionization (EI) Method

In the EI method, the vaporized sample molecule (M) is ionized by receiving the energy of thermal electrons (usually, 70 eV), and the molecular ion ($M^{+\cdot}$) and fragment ions with the structural information of the sample molecule are generated. This method is suitable for ionizing nonpolar molecules such as volatile or gaseous samples with low molecular mass up to approximately 1000. It is used for the identification of substances using a data library or other source, because mass spectra with reproducible fragmentation patterns can be obtained by this method.

1.2.2. Chemical Ionization (CI) Method

In the CI method, the vaporized sample molecules are ionized through ion/molecule reactant with reaction ions generated from reagent gases such as methane, isobutane and ammonia. When a reagent gas is introduced into the ionization chamber, protonated ions of the molecules $[M + H]^+$, deprotonated ions of the molecules $[M - H]^-$ or reactant ion adducts of the molecules are generated. Since the ions generated by the CI method have internal energy values that are much lower than those obtained by the EI method, the fragmentation of sample molecules hardly occurs.

1.2.3. Electrospray Ionization (ESI) Method

When the sample solution is sprayed through a capillary with a tip to which high voltage is applied, atomized charged droplets are produced. Subsequently, the sample molecules will be ionized when the charge density of the droplets increases, accompanied by the evaporation of the solvent; $[M + H]^+$, $[M - H]^-$, or alkali metal ion adduct of the molecules is thus generated. This method is used for the ionization of sample substances from those with low molecular mass and relatively high polarity to those with high molecular mass. The ESI method can also be applied for the measurement of biopolymers such as peptides, proteins and polysaccharides, because the method makes it easy to generate multiply-charged ions such as $[M + nH]^{n+}$ and $[M - nH]^{n-}$.

1.2.4. Atmospheric Pressure Chemical Ionization (APCI) Method

In the APCI method, the sample solution is sprayed and vaporized by passing through a heated capillary using nitrogen as the carrier, and the corona discharge is induced at the time with a high-voltage needle electrode, and the solvent molecules are thus ionized. The sample molecules will be ionized through the ion/molecule reaction with the solvent ions, and $[M + H]^+$, $[M - H]^-$, or alkali metal ion adduct of the molecules will be generated. This method is suitable for ionizing nonpolar to highly polar compounds with a molecular mass up to approximately 1500.

1.2.5. Matrix-assisted Laser Desorption/Ionization (MALDI) Method

When a mixture of the sample and a matrix such as α-cyano-4-hydroxycinnamic acid or sinapinic acid is irradiated with a pulsed laser, the sample molecules will be vaporized quickly and ionized, accompanied by the electronic excitation of the matrix. At that time, the proton transfer occurs between the matrix and the sample molecules, and $[M + H]^+$, $[M - H]^-$, or alkali metal ion adduct of the molecules is generated. With the MALDI method, it is possible to ionize the compounds from low molecular mass of several hundreds to high molecular mass of several hundred thousand by selecting the appropriate matrix. Since the amount of the sample required for the measurement is very small, this method is used for the ionization of samples of biological origin such as peptides and proteins.

1.2.6. Other Ionization Methods

Various other ionization methods have been developed, including the field ionization (FI) method, the field desorption (FD) method, the fast atom bombardment (FAB) method, the secondary ion mass spectrometry (SIMS) method, the atmospheric pressure photoionization (APPI) method, and an ionization method in which the volatile substances on the material surface can be directly ionized using the ionization by the collision reaction with helium in the excited state in the open space.

1.2.7. Sample Introduction Method and Ionization Method

Each ionization technique is closely related to the sample introduction methods. In the case of the gas chromatography mass spectrometry (GC-MS), vaporized substances separated by a capillary column are directly introduced into a high-vacuum ion source and ionized by the EI method or CI method, for example. In the case of liquid chromatography mass spectrometry (LC-MS), the liquid phase containing sample substances separated by the LC column is sprayed under atmospheric pressure, and the sample substances are ionized by an ionization method described above at the interface to introduce the ions to the high-vacuum mass analyzer. At that time, it is necessary to ensure that the mobile phase to be used has an appropriate composition for both the column separation and the ionization. In the

case of capillary electrophoresis mass spectrometry, the flow rate is usually adjusted by adding an appropriate solution to the electrolyte at the end of the capillary, and the sample substance is ionized by the ESI method or other ionization method.

1.3. Mass Analyzers

In a mass analyzer, the ions generated in the ion source are separated according to their m/z values. As a result, the mass and the relative abundance of the ions derived from the samples to be analyzed can be measured. The following mass analyzers are commonly used for MS.

1.3.1. Quadrupole (Q) Analyzer

The quadrupole (Q) analyzer has four rod electrodes set parallel to each other, to which high-frequency alternating current voltage is applied and on which direct current voltage is superimposed. The ions that enter this space oscillate according to their m/z values, and only ions with a specific m/z value have a stable trajectory and will be able to pass through the space. The ions with different m/z values can also become able to pass through the analyzer with a change in the applied voltage, and thereby the mass spectrum can be obtained. The mass resolving power of a Q analyzer is generally low, but Q analyzers are widely used for the qualitative and quantitative analyses as general-purpose equipment, since they have a relatively wide dynamic range and simple composition that can be downsized.

1.3.2. Ion-trap (IT) Analyzers

An ion-trap (IT) analyzer is made of an electric field or magnetic field or a combination of field, and is used to trap the ions in a space. The three most commonly used IT analyzers are as follows.

1.3.2.1. Paul Ion-trap

The Paul ion-trap is a synonym for quadrupole ion-trap (QIT). Although it is similar to the quadrupole analyzer in principle, it is able to trap ions stably by using ring electrodes and end-capped electrodes instead of rod electrodes. The trapped ions are discharged into the detector according to their m/z values by a scan of the high-frequency voltage, and thereby the mass spectrum can be obtained. This method is frequently used for qualitative analyses such as structure analysis because multiple-stage mass spectrometry (MS^n) can be achieved by using only one analyzer. The instrument with the sensitivity and dynamic range improved by using four electrodes with a hyperbolic surface is referred to as a linear ion-trap (LIT) analyzer.

1.3.2.2. Kingdon Trap

In the Kingdon trap analyzer, ions are trapped while rotating around a spindle-shaped electrode. The image current induced by the ions oscillating according to their m/z values is measured. The mass spectrum is obtained by Fourier-transforming the measured waveform data on the time axis to those on the frequency axis. This analyzer is used for qualitative analyses such as structure analyses because it has extremely high mass resolving power and mass accuracy.

1.3.2.3. Penning Ion-trap

The Penning ion-trap is used for Fourier transform-ion cyclotron resonance (FT-ICR). The ions that enter into the strong magnetic field formed by a superconducting magnet show cyclotron movement due to the effect of the Lorenz force. Here, the angular frequency (ω) can be expressed by the following equation.

$$\omega = qB/m$$

where m is the mass in atomic mass units of the ion, q is the electric charge of the ion, and B is the magnetic flux density. When the high-frequency electric field with this frequency is applied to the magnetic field, the ions move along the spiral orbital. These rotating ion groups induce the electric current, which changes periodically according to its respective m/z value in the detecting electrode. The mass spectrum can be obtained by Fourier-transforming the signals measured above and further converting the frequencies to the m/z values. The Penning ion-trap is used for precise structural studies in combination with various dissociation techniques for precursor ions, since an FT-ICR analyzer has extremely high mass resolving power and mass accuracy.

1.3.3. Time-of-flight (TOF) Analyzer

In the time-of-flight (TOF) analyzer, the ions are separated based on the difference of the flight times necessary for reaching the detector. For the ions with the mass m accelerated by the constant voltage V, the time t necessary for the ions to fly a distance L and reach the detector can be expressed by the following equation.

$$t = \sqrt{m/z} \times \frac{L}{\sqrt{2eV}}$$

The time of flight t is proportional to the square root of the m/z value, and consequently, the ions with smaller mass reach the detector faster. In the reflector mode in which the ions are reflected by the reflectron with the electrodes arranged side by side, high mass resolving power can be obtained by bringing the distribution of the kinetic energy of the ions into focus and doubling the flight distance of the ions. TOF analyzers are used for the analyses of high-molecular-mass compounds such as proteins in combination with the MALDI method and other techniques, since the mass range measurable by this method does not have a margin, theoretically. It is also frequently used for the qualitative analyses of low-molecular-mass substances, since it has high mass resolving power.

1.3.4. Magnetic Sector Analyzer

The ions that enter a magnetic sector analyzer are deflected by the Lorenz force of the magnetic field perpendicular to the ion current. At that time, ions with different m/z values (with the velocity v) fly into the magnetic field with different radii of curvature r according to the following equation.

$$r = \frac{mv}{qB}$$

Only ions with a specific m/z value are able to pass through the slit placed on the path of the ions. The mass spectrum can be obtained by scanning the magnetic flux density B, and introducing the ions with different m/z values passed through the slit into the detector in order. A magnetic sector analyzer is usually used as a double-focusing-type instrument in which the electric sector is combined with the

magnetic sector, and the analyzer is used for both qualitative and quantitative analyses, since it has high mass resolving power and is also highly quantitative.

1.4. Detectors

Ions that have passed through a mass analyzer are usually transduced to the electric signal by releasing the electrons at the detector. The following detectors are in current use. In Fourier-transform-type instruments, the electric current induced by the movement of the ions at the detector is identified with a detection electrode.

1.4.1. Secondary Electron Multiplier (SEM)

A secondary electron multiplier (SEM) has a multistage arrangement of electrodes called dynodes. The secondary electrons emitted by the collision of the ions that enter the multiplier to the first dynode are sequentially multiplied, and finally transduced to the electric signal and recorded. This multiplying effect of the secondary electrons enables the detection of small amounts of ions.

1.4.2. Channel Electron Multiplier (CEM)

A channel electron multiplier (CEM) has a pipe-shaped channel configuration, and the secondary electrons are emitted by the collision of the ions that entered the multiplier to the inner wall of the channel. Multiple amplification is achieved by repeating this process at every opposite side of the inner wall. A CEM is simpler compared to SEMs, and with a CEM it is possible to downsize.

1.4.3. Microchannel Plate (MCP)

A microchannel plate (MCP) has a configuration in which many very small CEMs are accumulated to form a detector. It is used for the detector of TOF-type instruments, since an MCP has a wide ion-receiving surface, and the time dispersion of the secondary electrons is small because of the very thin structure of the MCP.

1.4.4. Faraday Cup (FC)

A Faraday cup (FC) is a simple detector that receives the charge of the ions that have entered an ion detector, and it transduces the charge to the electric current. It has a cup-shaped configuration so that the secondary electrons emitted from the ions can be captured.

2. Tandem Mass Spectrometers

Tandem mass spectrometry (TMS) is a technique in which precursor ions are selected from the fragment ions of the sample substance at the first-stage mass analyzer, and the product ions generated by dissociating the precursor ions are separated and detected at the second-stage mass analyzer. TMS is used for (1) the structural estimation and confirmation of fragment ions, and (2) specific and high sensitive analyses. There are two categories of TMS: TMA in space, and TMS in time.

With TMS in space, the selection of the precursor ions, the dissociation of the precursor ions, and the separation of the product ions are conducted at the first-stage mass analyzer, the intermediate region, and the second stage mass analyzer, respectively. With TMS in time, the selection/dissociation/separation of the ions are conducted at the different time zones in the same mass analyzer. TMS in space includes the triple quadrupole-type, quadrupole/time-of-flight-type, and time-of-flight/time of flight-type mass analyzers. The latter includes the ion-trap type mass analyzer, with which multiple-stage mass spectrometry (MS^n) can be performed by repeating the selection and dissociation of the precursor ions and the separation of the product ions multiple times.

2.1. Dissociation of Precursor Ions

2.1.1. Collision-induced Dissociation (CID)

In this dissociation method, a part or all of the collision energy is converted to the internal energy of the ions by the collision of the accelerated ions with the neutral collision gases (He, Ar, N_2, etc.), and subsequently the ions obtaining excess internal energy are excited and dissociated.

2.1.2. Post-source Decay (PSD)

In the MALDI method, the ions generated at the ionization source are dissociated during the interval between leaving the accelerating region and reaching the detector, due to the excess internal energy of the ions themselves or the collision with the residual gas. PSD is used for MS/MS using a reflectron time-of-flight mass spectrometer.

2.1.3. Others

Other dissociation methods are electron capture dissociation, electron transfer dissociation, infrared multiphoton dissociation, and surface-induced dissociation.

2.2. Constitutions of Principal Tandem Mass Spectrometers

2.2.1. Triple Quadrupole Mass Spectrometer (Q-q-Q)

A triple quadrupole mass spectrometer (Q-q-Q) has a configuration in which three quadrupoles are tandemly connected so that the first quadrupole is used for the selection of the precursor ions, the second quadrupole is used as the collision chamber for the dissociation of the precursor ions, and the third quadrupole is used for the mass separation of the product ions. Various scanning methods can be employed, and this type of spectrometer is frequently used for quantitative analyses in particular.

2.2.2. Quadrupole Time-of-flight Mass Spectrometer (Q-TOF)

A quadrupole time-of-flight mass spectrometer (Q-TOF) has a configuration in which the third quadrupole in the Q-q-Q is replaced with a TOF mass analyzer. The precursor ions are selected at the first quadrupole, and the separation of generated ions is conducted by the orthogonal-type TOF. Measurement with high sensitivity and high resolution is possible.

2.2.3. Time-of-flight Time-of-flight Mass Spectrometer (TOF-TOF)

A time-of-flight time-of-flight mass spectrometer (TOF-TOF) consists of a TOF analyzer in which the precursor ions are selected, the collision chamber, and a TOF analyzer in which the mass separation of the product ions is performed. It is used for MALDI-TOF-TOF mass spectrometry.

2.2.4. Other Mass Spectrometers

The mass spectrometers other than those described above are the four-sector mass spectrometer with the configuration in which two double-focusing instruments are connected, and the LIT-Kingdon trap and QIT-TOF, in which an in-

time-type mass analyzer is used.

3. Methods Used for Measurement

3.1. Mass Spectrometry

The following measurement methods are used with mass spectrometry. An outline of the data obtained by each method is also described.

3.1.1. Total Ion Monitoring (TIM)

Total ion monitoring (TIM) is also known as the full-scan mode. It is the technique in which the mass spectrometer is operated so that all ions within the selected m/z range are detected and recorded, and the integrated value of the amounts of ions observed in each scanning is called the total ion current (TIC).

The chromatogram in which the total ion current obtained from the mass spectrum measured in LC-MS and GC-MS is plotted against the retention time is called the total ion current chromatogram (TICC), and the chromatogram in which the relative intensity at the specific m/z value is expressed as the function of time is called the extracted ion chromatogram (EIC).

3.1.2. Selected Ion Monitoring (SIM)

In selected ion monitoring (SIM), the mass spectrometer is operated so that only the ions with a specific m/z value are continuously detected and recorded instead of measuring the mass spectrum. SIM is used for the assay and high-sensitivity detection of sample substances in LC-MS and GC-MS.

3.2. Tandem Mass Spectrometry (TMS)

The following methods are used for measurements using TMS. An outline of the data obtained by each method is also described.

3.2.1. Product Ion Analysis

Product ion analysis is used to detect the product ions generated from the precursor ions with a selected m/z value, and with this method the sample's qualitative information can be obtained.

3.2.2. Precursor Ion Scan

Precursor ion scan is a method for scanning the precursor ions from which the product ions with a specific m/z value are generated by dissociation, and it is used for the specific detection of a substance with a specified substructure in the sample.

3.2.3. Constant Neutral Loss Scan

In constant neutral loss scan, the precursor ions that undergo the loss of specified mass (desorption of neutral species) due to dissociation are scanned. This method is used for the specific detection of substances with a specified substructure in the sample.

3.2.4. Selected Reaction Monitoring (SRM)

Selected reaction monitoring (SRM) detects product ions with a specific m/z value generated by the dissociation of the precursor ions with a specified m/z value, and it is used for the quantitative detection of trace amounts of substances present in a complex matrix. Although this method is similar to SIM, the specificity is improved by using the product ions generated from the precursor ions for the detection.

4. Application to Various Tests

In pharmaceutical analyses, mass spectrometry is used for the identification and purity tests of molecules as a specific detection method based on the mass and the structural information of the molecules.

4.1. Optimization of Instruments

In mass spectrometry, in order to obtain a good shape, sensitivity, and mass accuracy of the ion peak it is necessary to pre-optimize the measurement parameters of each component unit of the instrument by using an appropriate standard material in accord with the ionization method and mass range.

4.1.1. Tuning

The shape, sensitivity, and relative intensity of the ion peak detected are optimized by adjusting the parameters such as the gas pressure, temperature, and voltage of the ion source, mass analyzer, and detector. The various parameters of the ion source affect the generation of ion species, the species transported to the mass analyzer, and the relative intensity. The parameters related to the mass analyzer influence the peak width, mass accuracy, resolving power, and sensitivity, and the detector parameters affect the signal intensity and system sensitivity.

4.1.2. Calibration

The mass calibration of a mass spectrometer is carried out based on the mass of standard material. The reproducibility of the measurement mass values is affected by the electrical variation of the instrument, the surface cleanliness of each component unit such as the ion source, and the room temperature. There are the external and internal standard techniques for mass calibration. The number of points for the calibration differs according to the type of mass spectrometer.

4.1.3. Mass Resolving Power

The ability to separate two adjacent ion peaks from each other is referred to as the mass resolving power. Higher mass resolving power capacitates to separate and detect the ion peaks with a small mass difference. In magnetic-sector mass spectrometry, the mass resolving power R is calculated by the following equation when two peaks with the mass of M and $M + \Delta M$ overlap each other to 10% of either peak height.

$$R = M/\Delta M$$

When an instrument other than a magnetic-sector mass spectrometer is used, such as a quadrupole mass spectrometer or a time-of-flight mass spectrometer, the mass resolving power can usually be calculated by the method using peak width at half-height. When the width of the ion peak with the mass of m is Δm, the mass resolving power is calculated by $R = m/\Delta m$, and is discriminated from that of the magnetic-sector mass spectrometer.

4.2. Test for Identification

The identification of a test substance using mass spectrometry is usually performed by the confirmation of the mass of the test substance molecule. The test should be performed after confirming in advance that the measurement value is within the range specified in the monograph using the standard solutions defined in the monograph, or the specified ion can be detected. According to the mass resolving power

of the instrument and the mass of the test substance molecule, the mass of the test substance molecule obtained from mass spectrometry can be adjusted to the monoisotopic mass or the average mass.

In general, the mass of the molecule consisting of only principle isotopes should be obtained from the monoisotopic peak. However, when the monoisotopic peak cannot be identified because, for example, the molecular mass is high or the resolution is not sufficient, the average mass should be calculated from the weighted average of the peak. When samples with high molecular mass such as proteins are analyzed by ESI/MS, the average mass should be calculated by the deconvolution technique, because the ESI mass spectra would show a series of multiply charged ions with different charge states. It may be combined with the detection of the fragment ions or the product ions generated from the test substance molecule, which includes characteristic partial structural information.

4.3. Purity test

The purity test of a test substance using mass spectrometry is usually performed in combination with a separation technique such as chromatography using a standard solution with a concentration corresponding to the specified limit of the impurity in the sample. The peak responses of the molecular ions or the characteristic fragment ions and product ions generated from the specified impurity in the sample solution should be compared with those of the ions generated from the substance in the standard solution. To obtain more precise values, the method in which the stable isotope-labeled compound of the analyte is added to the sample solution as the internal standard is also important. When the test is performed using mass spectrometry in combination with, for example, chromatography, a system suitability test should also be required in accord with the chromatography.

Add the following:

2.63 Inductively Coupled Plasma-Atomic Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry

Inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS) are elemental analysis methods in which inductively coupled plasma (ICP) is used as the excitation source or the ion source.

ICP is an excitation source composed of high-temperature argon plasma with intense thermal energy, which is formed by the inductive coupling method. The atoms contained in the sample solution are excited when the solution is sprayed into the plasma. ICP-AES is the method used to measure the atomic emission spectrum of the light emitted from the plasma at the time and to identify and analyze the contents of elements contained in the sample by determining the wavelength and intensity of its spectral lines. Since ICP is also a good ionization source, the atoms in the sample solution are ionized when the solution is sprayed into the plasma. ICP-MS is the method used to measure the mass spectrum of the element ions generated by ICP at the time by separating the element ions into those with each m/z value and counting the intensities of ion peaks using a mass spectrometer as the detector.

When intense energy is added to an atom from the outside, the peripheral electrons of the atom would transit to an excited state by absorbing certain energy. The electron in the excited state would release the absorbed energy as the light when it returns to its ground state. The light released at the time has a frequency ν (or wavelength λ) characteristic of each element. When h is Planck's constant and c is the velocity of light, the energy ΔE of the released light is expressed by the following equation.

$$\Delta E = h\nu = hc/\lambda$$

Since there are many excited states with various energy levels to which peripheral electrons might transit, many emission lines with various levels of energy can generate from one element, although some lines are strong and others are weak. However, there is only a limited number of emission lines observed in the ultraviolet/visible region and with enough sensitivity for the qualitative and quantitative analyses of each element. Since each element exhibits its own spectral line with a characteristic frequency (or wavelength) in the atomic emission spectrum, the elements contained in the sample solution can be identified by determining the wavelengths of spectral lines in the spectrum. Quantitative analyses of the elements in the sample solution can also be performed by determining the intensity of the spectral line characteristic of each element. The elemental analysis method using this principle is ICP-AES.

ICP-MS is the elemental analysis method alternative to optical analysis methods such as atomic absorption spectrometry and ICP-AES. In ICP-MS, the element ions generated by the ICP are separated into those with each m/z value and the intensities of the separated ions are counted with a mass spectrometer. Compared to ICP-AES, ICP-MS is higher sensitive, and with it an isotope analysis can be performed.

ICP-AES and ICP-MS are both excellent trace analysis methods specific for the inorganic impurities or coexisting elements in drug substances and drug products. Therefore, using these methods, qualitative and quantitative analyses can be performed not only for alkaline/alkaline-earth metals and heavy metals, but also for many elements for which adequate control is required to ensure the safety of pharmaceutical products. It would be useful for the quality assurance of drug substances to apply these methods to the profile analyses of inorganic elements contained in the substances, because these methods enable the simultaneous analysis of many elements.

1. Instruments

1.1. Instrument Configuration of ICP-AES

An ICP-AES is composed of an excitation source, a sam-

ple introduction system, a light emission unit, a spectroscope, a photometer and a data processor.

The excitation source consists of a high-frequency power generator, a control circuit to supply and control the electric energy to the light emission unit, and a gas source. The sample introduction system, the main components of which are a nebulizer and a spray chamber, is used for introducing sample solutions to the light emission unit after nebulizing the solutions.

The main components of the light emission unit, in which the elements containing in the sample solution are atomized and excited to induce light emission, are a torch and a high-frequency induction coil. The torch has a triple tube structure, and the sample solution is introduced through the central tube. Argon gas is used to form the plasma and to transport the sample solution. For the observation method of the light emitted from the light emission unit, there are two viewing modes: the lateral viewing mode in which the radial light of the plasma is observed, and the axial viewing mode in which the central light of the plasma is observed.

The spectroscope separates the light from the light emission unit to the spectral lines, and is composed of optical devices such as a light-converging system and diffraction grating. There are two types of spectrometers: wavelength-scanning spectrometers (monochromators) and simultaneously measuring spectrometers (polychromators) of the wavelength-fixed type. In addition, it is necessary to form a vacuum or to substitute the air in the chamber of the photometer with argon or nitrogen gas, when it is required to measure the spectral lines of the vacuum ultraviolet region (190 nm or shorter).

The photometer, which consists of a detector and a signal processing system, transduces the light energy of incident light to the electric signal proportional to the intensity of the light. For the detector, a photomultiplier or a semiconductor detector is used.

The data processor is used to process the data obtained by the measurements, and it displays the calibration curves and measurement results.

1.2. Instrument Configuration of ICP-MS

An ICP-MS system is composed of an excitation source, a sample introduction system, an ionization port, an interface, an ion lens, a mass analyzer, an ion detector and a data processor.

The excitation source, sample introduction system and ionization port have the same configuration as their counterparts in an ICP-AES system.

The interface is the boundary component for introducing the ions generated by the plasma under atmospheric pressure into a high-vacuum mass analyzer, and is composed of the sampling cone and skimmer cone.

The ion lens brings the ions introduced via the interface into focus and helps introduce the focused ions into the mass analyzer efficiently.

For the mass analyzer, a common choice is a quadrupole mass analyzer. The interference caused by the polyatomic ions described later can be suppressed by placing a collision/reaction cell within the vacuum region before the mass analyzer, and introducing a gas such as hydrogen, helium, ammonia or methane into the cell.

The ion detector transduces the energy of the ions that reached the detector to an electric signal which is amplified by the multiplier. The data processor is used to process the data of the electric signal from the ion detector, and to display the calibration curves and measurement results, etc.

2. Pretreatment of Sample

When the samples to be analyzed are organic compounds such as pharmaceutical drug substances, they are usually digested and ashed by the dry ash method or the wet digestion method, and the sample solutions for ICP-AES or ICP-MS are prepared by dissolving the residues in small quantities of nitric acid or hydrochloric acid. When a sample is difficult to digest in the usual manner, the sample can be sealed in a closed, pressurized container and digested using microwave digestion equipment. Although liquid samples containing small amounts of organic solvents can be introduced directly into an ICP-AES or ICP-MS instrument without pretreatment, another alternative is introducing oxygen as the option gas to prevent the build-up of carbon generated from the solvent onto the torch and the interface by contributing to the incineration of organic solvents.

3. Operation of ICP-AES

The operation of an ICP-AES system is as follows. The argon plasma is formed by setting the argon gas flow at the specified rate and turning the high-frequency power source on. After confirmation that the state of the plasma is stable, a quantity of the sample solution or the standard solution prepared by the method prescribed in the monograph is introduced into the instrument via the sample injection port, and the emission intensity of the analytical line specified for the element is measured. When it is necessary to perform a test for the confirmation or identification of some elements, the emission spectrum in the wavelength range in which analytical lines specified for the elements that appear is measured.

3.1. Performance Evaluations of Spectrometers

Since each spectrometer requires its own calibration method that accords with its properties, a wavelength calibration must be performed according to the procedure indicated by the manufacturer.

For expressing the wavelength-resolving power of a spectrometer, the half height width of the analytical line in the emission spectrum of a specified element is usually defined in the form of ''not more than xxx nm (a constant value).'' The following emission lines, from the line with a low wavelength to that with a high wavelength, are usually selected for the above purpose: arsenic (As: 193.696 nm), manganese (Mn: 257.610 nm), copper (Cu: 324.754 nm) and barium (Ba: 455.403 nm).

3.2. Optimization of Operating Conditions

The operating conditions usually adopted are as follows. The operating conditions of the instrument should be optimized after stabilizing the state of the plasma by warming up the instrument for 15 – 30 min. The operating parameters should usually be set as follows: high frequency power, 0.8 –

1.4 kW; argon gas flow rate, 10 – 18 L/min for the coolant gas (plasma gas), 0 – 2 L/min for the auxiliary gas, and 0.5 – 2 L/min for the carrier gas. In the lateral viewing mode, the point for measuring the light emitted from the plasma should be set within the range of 10 – 25 mm from the top edge of the induction coil, and the aspiration rate of the sample solution should be set at 0.5 – 2 mL/min. In the axial viewing mode, the optical axis should be adjusted so that the maximum value can be obtained for the intensity of emission line measured. The integration time should be set within the range of one to several tens of seconds, taking the stability of the intensity of the emission line measured into account. When a test using an ICP-AES system is defined in a JP monograph, the operating conditions such as the analytical line (nm), high-frequency power (kW), and argon gas flow rate (L/min) should be prescribed in the monograph. However, it is necessary to optimize the operating conditions individually for each instrument and for each viewing mode used for the measurement.

3.3. Interference and Its Suppression or Correction

In the term ICP-AES, the word ''interference'' is used as a general term that indicates the influence of the coexisting components or matrix on the measurement results. Various interferences are roughly classified as either non-spectral interference (such as physical interference and ionization interference) or spectral interference. Their effects can be eliminated or reduced by applying the appropriate suppression or correction methods for the measurement.

Physical interference means that the measurement results are influenced by the difference between the spray efficiencies of the sample solution and the standard solution used for its calibration in the light emission unit, when the physical properties (such as viscosity, density and surface tension) of the solutions differ. The effective methods for eliminating or reducing this type of physical influence are as follows. The sample solution should be diluted to the level at which such interference will not occur; the properties between the sample solution and the standard solution used for its calibration should be matched as much as possible (matrix-matching method); and the internal standard method (intensity ratio method) or the standard addition method should be used.

Ionization interference indicates the influence due to the change in the ionization rate caused by the increase of electron density in the plasma, which is induced by a large number of electrons generated from the elements coexisting in the sample solution at a high concentration. The suppression or correction method against the ionization interference is essentially the same as the method used in the case of physical interference. The measurement conditions with low ionization interference can also be set by the selection and adjustment of the observation method of emitted light, the height for viewing, high-frequency power and carrier gas flow rate, and so on.

Spectral interference is the phenomenon which influences the analytical results of the sample by overlapping the various emission lines and/or the light with a continuous spectrum with the analytical line of the analyte element. To avoid this type of interference, it is necessary to select another analytical line which will not suffer from the spectral interference. However, when no suitable analytical lines can be found, it is necessary to carry out the correction of the spectral interference. In addition, when the pretreatment of the organic samples is not sufficient, the molecular band spectra (NO, OH, NH, CH, etc.) derived from nitrogen, oxygen, hydrogen and carbon remaining in the sample solution might appear at the wavelength close to the analytical line of the analyte element, and could interfere with the analysis.

4. Operation of ICP-MS

In the operation of an ICP-MS system, after the confirmation that the state of the plasma is stable, the optimization of the instrument is performed and the system's suitability is confirmed. A quantity of the sample solution or the standard solution prepared by the method prescribed in the monograph is introduced, and the ion count numbers of the signal at the m/z value specified for the analyte element are determined. When it is necessary to perform a test for the confirmation or identification of some elements, the mass spectrum in the m/z value range specified for the analyte elements is measured.

4.1. Performance Evaluation of Mass Spectrometer

The performance evaluation items for mass spectrometers are the mass accuracy and the mass resolving power. The mass accuracy should be adjusted by matching the m/z value of the mass axis of the mass analyzer to that of the standard element in the standard solution for the optimization specified in the operating conditions section of the monograph. With quadrupole mass spectrometers, it is preferable that the mass accuracy be within ± 0.2. For the mass resolving power, it is preferable that the peak width at 10% of the peak height in the observed ion peak is not more than 0.9.

4.2. Optimization of Operating Conditions

When a limit test or a quantitative test is performed, the sensitivity, background and generation ratio of oxide ions and doubly charged ions defined below should be optimized previously to assure that the performance of the instrument is suitable. For the optimization of operating conditions, the solutions of the elements which represent the low mass number elements, intermediate mass number elements and high mass number elements and are unlikely to be contaminated from the environment (e.g., ^{7}Li, ^{9}Be, ^{59}Co, ^{89}Y, ^{115}In, ^{140}Ce, ^{205}Tl and ^{209}Bi) are usually used as the standard solutions after adjusting to adequate concentrations.

The sensitivity is evaluated by the ion count numbers per second of integration time (cps). When a limit test or quantitative test is performed, it is preferable to have the sensitivity of several tens of thousands cps per 1 μg/L (ppb) for each element with a low mass number, intermediate mass number or high mass number.

For the background, it is preferable to be not more than 10 cps, when the measurement is performed at the m/z value at which no elements exist naturally (e.g., m/z value of 4, 8 or 220).

For the generation ratio of oxide ions and doubly charged

ions, the count numbers of oxide ions (e.g., ^{140}Ce: $^{140}Ce^{16}O^+$, m/z 156), doubly charged ions ($^{140}Ce^{2+}$, m/z 70) and monovalent ions ($^{140}Ce^+$, m/z 140) should be measured, and the generation ratios are calculated by dividing the ion count number of the oxide ions and doubly charged ions by that of the monovalent ions. It is preferable that the generation ratio of oxide ions (i.e., $^{140}Ce^{16}O^+/^{140}Ce^+$) is not more than 0.03 and that of doubly charged ions (i.e., $^{140}Ce^{2+}/^{140}Ce^+$) is not more than 0.05.

4.3. Interferences and their Suppression or Correction

In measurements using ICP-MS, attention must be paid to spectral interference and non-spectral interference.

Spectral interference includes isobaric interference and the interference caused by overlapping the mass spectrum of the analyte element with those of polyatomic ions or doubly charged ions. Isobaric interference is the interference by the isobaric element with the atomic mass adjacent to that of the analyte element, for example, the overlap of ^{40}Ar with ^{40}Ca and ^{204}Hg with ^{204}Pb. Since argon plasma is used as the ionization source, the polyatomic ions such as $^{40}Ar^{16}O$, $^{40}Ar^{16}O^1H$, $^{40}Ar_2$ might be generated, and they would interfere with the measurements of ^{56}Fe, ^{57}Fe and ^{80}Se, respectively. When an instrument equipped with a collision/reaction cell is used, these polyatomic ions can be decreased in the cell. Doubly charged ions are the ions exhibiting their ion peaks at 1/2 the m/z value of the corresponding monovalent ions, and the interference might occur when the element with an isotope with the mass number twice that of the analyte element might be present in the sample solution.

Non-spectral interference includes not only the physical interference and the ionization interference as in the case of the ICP-AES, but also the matrix interference unique to ICP-MS. Matrix interference is the phenomenon in which the ion count numbers of every analyte element generally decrease when large amounts of other elements might co-exist in the sample solution. This tendency becomes more significant when the mass number of a co-existing element is larger and its concentration is higher, and when the mass number of the analyte element is smaller. The extent of non-spectral interference can be estimated based on the recovery rate obtained by adding a known amount of the analyte element to the unknown sample. When it is found that the recovery rate is low and the reliability of the analysis is not assured, the correction should be carried out by using the internal standard method or the standard addition method. For ICP-MS in particular, the influence of non-spectral interference can be reduced by using the isotope dilution method.

5. System Suitability

When a limit test or quantitative test is performed using these methods, it is necessary to confirm that the performance of the instrument is suitable by carrying out a system suitability test as defined below in advance of the limit test or quantitative test.

5.1. Evaluation for Required Detectability and Linearity

In an evaluation of an ICP-MS system for the required detectability and linearity, a solution is prepared in which the analyte element is not contained and the standard solution with the concentration of the specification limit of the analyte element, and these solutions are used as the blank solution and the solution for the system suitability test, respectively. The spectra obtained with these solutions are measured according to the test conditions optimized individually for each instrument, and it must be confirmed whether the emission line (or ion peak) of the analyte element is clearly observed at the specified wavelength (or m/z value) in the solution for system suitability test when compared with the blank solution. In this regard, the limit of the analyte element should be specified at the concentration of more than the quantitation limit (10σ). The test for required detectability is not required in the assay.

For the evaluation of linearity, it should be confirmed that the correlation coefficient of the calibration curve prepared by the procedure described in the section below, ''6.2. Quantitative Analysis'' is not less than 0.99. The confirmation of linearity is not required in quantitative analyses in the section 6.1. or when isotope dilution in the section 6.2. is performed.

5.2. Evaluation for System Repeatability

Unless otherwise specified, when the test is repeated six times using the solution with the lowest concentration among those used for plotting the calibration curve according to the test conditions optimized individually for each instrument, it should be confirmed that the relative standard deviation of the observed values for the emission intensity (or ion count number) of the analyte element is not more than the specified value (e.g., not more than 3% for an assay, and not more than 5% for a purity test).

6. Qualitative and Quantitative Analyses

6.1. Qualitative Analyses

In ICP-AES, when the wavelengths and relative emission intensities of multiple emission lines from the sample solution conform to those of the emission lines from the elements contained in the standard solution, the presence of the elements can be confirmed. In addition, the library of ICP-emission spectra attached to each instrument or the wavelength table of the spectra can also be used instead of the standard solution. Since the mass number region covering all the elements can be scanned in a short time in ICP-MS, the elements contained in the sample solution can be analyzed qualitatively based on the m/z value of the ion peak in the mass spectrum obtained from the sample solution.

It would be feasible to list the metal catalysts and inorganic elements that might be contained in the sample as impurities, and for some elements (such as arsenic and lead) it might be necessary to monitor them in a routine manner from the point of view of safety, and to carry out the profile of these inorganic impurities as a part of the manufacturing controls for a drug substance. In addition, the standard solution of each element should be prepared at an appropriate concentration considering the acceptance limit of each element to be specified separately.

6.2. Quantitative Analyses

The quantitative analysis of an inorganic element in the sample solution is usually performed by one of the following methods based on the emission intensity or ion count numbers obtained by the integration of measurement data in a specified time.

(i) Calibration curve method: Prepare standard solutions for plotting a calibration curve with different concentrations (four or more) of the analyte element. Using these standard solutions, the emission intensities at the analytical line specified for the analyte element by ICP-AES or the ion count numbers at the m/z value specified for the analysis of the element by ICP-MS are measured. The data obtained are then plotted against the concentrations, and this plot is used as the calibration curve. The concentration of the analyte element in the sample solution is determined by using this calibration curve.

(ii) Internal standard method: Prepare standard solutions for plotting a calibration curve with a fixed concentration of the internal standard element and different concentrations (four or more) of the analyte element. Using these standard solutions, the ratios of the emission intensities (or ion count numbers) of the analyte element to those of the internal standard element are determined. The data obtained are plotted against the concentrations, and this plot is used as the calibration curve. The internal standard element is also added to the sample solution, so that the concentration of internal standard element in the solution becomes the same as that in the standard solution. The concentration of the analyte element in the sample solution is determined by using the calibration curve plotted above.

Before this method is applied, it is necessary to verify that the internal standard element to be added is not contained in the sample solution. If the internal standard element to be added is present in the sample solution, it is necessary to verify that the contaminated amount of standard element is negligible compared to the amount to be added. In addition, in ICP-AES, the following requirements are to be met for the internal standard element: the changes in the emission intensity due to the measurement conditions and properties of the solution should be similar to those of the analyte element, and the emission line which does not cause spectral interference to the analytical line of the analyte element should be selected for the analysis. In contrast, in ICP-MS, it is preferable to select an internal standard element which does not cause spectral interference to the analyte element and has the ionization efficiency and mass number equivalent to the analyte element.

(iii) Standard addition method: Take 4 portions or more of the sample solution with the same volume, and prepare the following solutions; the solution in which the analyte element is not added; the standard solutions for plotting calibration curve in which the analyte element is added at different concentrations (3 or more). Measure the emission intensities at the specified analytical line or the ion count numbers at the specified m/z value for these solutions. Plot the obtained data against the concentrations calculated from the added amount of the analyte element. Calibrate the concentration of the analyte element in the sample solution from the absolute value of the horizontal axis (concentration)-intercept of the regression line.

In ICP-AES, this method is useful for the correction of non-spectral interference caused by coexisting substances in the sample solution, and it is applicable only to the cases in which spectral interference does not exist, or the background and the spectral interference are exactly corrected and the relationship between the emission intensity and the concentration shows good linearity. In ICP-MS, this method is useful for the correction of non-spectral interference caused by coexisting substances in the sample solution, and it is applicable only to the cases in which the spectral interference is exactly corrected and the relationship between the ion count number and the concentration shows good linearity down to the low concentration region.

(iv) Isotope dilution method: Isotope dilution method is applicable only to the ICP-MS. The concentration of the analyte element is determined from the change of the isotope composition ratio of the element by adding a substance containing a concentrated isotope with a known isotope composition that is different from the natural composition to the sample solution. It is applicable only to the element which has two or more stable isotopes naturally and is able to perform the isotope analysis. It is the feature of this method that the analytical precision is high and is not influenced by non-spectral interference, because the quantitation can be performed only by adding an adequate amount of a substance containing a concentrated isotope and measuring the isotope composition ratio of the sample solution.

7. Note

Water and reagents and the standard solutions used in this test are as follows.

(i) For water, water for an ICP analysis should be used. It should be verified prior to the test that the impurities contained in the water do not interfere with the analysis of the analyte element. Here, the water for an ICP analysis has the electric conductivity of 1 $\mu S \cdot cm^{-1}$ or less (25°C).

(ii) Reagents that are suitable for ICP analyses and are of high quality should be used.

(iii) For argon gas, either liquefied argon or compressed argon gas with the purity of 99.99 vol% or higher should be used.

(iv) For the standard solutions, they should be prepared by diluting the Standard Solution (e.g., the Standard Solution defined in the JP, or a standard solution with a concentration certified by a public institution or scientific organization) to the specified concentration using the water for ICP analysis. However, in cases in which interference with the analysis might occur, it is preferable to match the properties of the standard solution to those of the sample solution.

(v) When a standard solution containing multiple elements is prepared, a combination of the test solutions and elements should be selected so that precipitation and/or mutual interference does not occur.

3.01 Determination of Bulk and Tapped Densities

Change the paragraph 1.2.1 and 2.1.2 as follows:

1.2.1. Apparatus

The apparatus(1) (Fig. 3.01-1) consists of a top funnel fitted with a 1.0 mm sieve. The funnel is mounted over a baffle box containing four glass baffle plates over which the powder slides and bounces as it passes. At the bottom of the baffle box is a funnel that collects the powder and allows it to pour into a cup mounted directly below it. The cup may be cylindrical (25.00 ± 0.05 mL volume with an inside diameter of 30.00 ± 2.00 mm) or cubical (16.39 ± 0.20 mL volume with inside dimensions of 25.400 ± 0.076 mm).

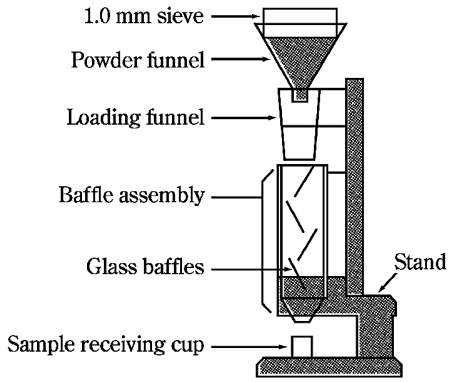


Fig. 3.01-1 Volumeter

2.1.2. Procedure

Proceed as described above for the determination of the bulk volume (V_0).

Secure the cylinder in the holder. Carry out 10, 500 and 1250 taps on the same powder sample and read the corresponding volumes V_{10}, V_{500} and V_{1250} to the nearest graduated unit. If the difference between V_{500} and V_{1250} is less than or equal to 2 mL, V_{1250} is the tapped volume. If the difference between V_{500} and V_{1250} exceeds 2 mL, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than or equal to 2 mL. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped density (g/mL) using the formula m/V_f in which V_f is the final tapped volume. Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results.

If it is not possible to use a 100 g test sample, use a reduced amount and a suitable 100 mL graduated cylinder (readable to 1 mL) weighing 130 ± 16 g and mounted on a holder weighing 240 ± 12 g. If the difference between V_{500} and V_{1250} is less than or equal to 1 mL, V_{1250} is the tapped volume. If the difference between V_{500} and V_{1250} exceeds 1 mL, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than or equal to 1 mL. The modified test conditions are specified in the expression of the results.

4.01 Bacterial Endotoxins Test

Change from the beginning up to the paragraph 2.3 as follows:

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia.

Bacterial Endotoxins Test is a test to detect or quantify bacterial endotoxins of gram-negative bacterial origin using an amoebocyte lysate prepared from blood corpuscle extracts of horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). There are two types of techniques for this test: the gel-clot techniques, which are based on gel formation by the reaction of the lysate TS with endotoxins, and the photometric techniques, which are based on endotoxin-induced optical changes of the lysate TS. The latter include turbidimetric techniques, which are based on the change in lysate TS turbidity during gel formation, and chromogenic techniques, which are based on the development of color after cleavage of a synthetic peptide-chromogen complex.

Proceed by any one of these techniques for the test. In the event of doubt or dispute, the final decision is made based on the limit test of the gel-clot techniques, unless otherwise indicated.

The test is carried out in a manner that avoids endotoxin contamination.

1. Apparatus

Depyrogenate all glassware and other heat-stable materials in a hot-air oven using a validated process. Commonly used minimum time and temperature settings are 30 minutes at 250°C. If employing plastic apparatus, such as multi-well plates and tips for micropipettes, use only that which has been shown to be free of detectable endotoxin and which does not interfere with the test.

2. Preparation of Solutions

2.1. Standard Endotoxin Stock Solution

Prepare Standard Endotoxin Stock Solution by dissolving Japanese Pharmacopoeia Reference Standard Endotoxin that has been calibrated to the current WHO International Standard for Endotoxin, using water for bacterial endotoxins test (BET). Endotoxin is expressed in Endotoxin Units (EU). One EU is equal to one International Unit (IU) of endotoxin.

2.2. Standard Endotoxin Solution

After mixing Standard Endotoxin Stock Solution thoroughly, prepare appropriate serial dilutions of Standard Endotoxin Solution, using water for BET. Use dilutions as soon as possible to avoid loss of activity by adsorption.

2.3. Sample Solutions

Unless otherwise specified, prepare sample solutions by dissolving or diluting drugs, using water for BET. By the

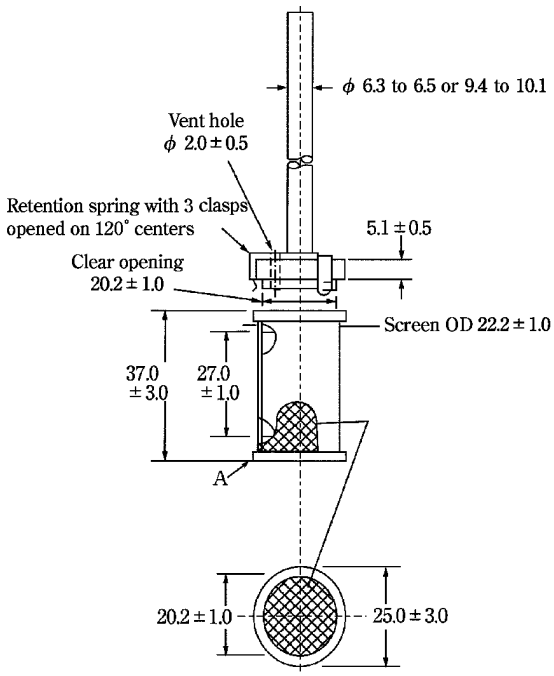
sample, an aqueous solution other than water for BET may be used to dissolve or dilute. If necessary, adjust the pH of the sample solution so that the pH of the mixture of the lysate TS and sample solution falls within the specified pH range for the lysate to be used. The pH of the sample solution may be in the range of 6.0 to 8.0. For adjustment of pH, acid, base or a suitable buffer solution may be used. The acid and base are prepared from their concentrated solutions or solids using water for BET, and then stored in containers free of detectable endotoxin. The buffer solutions must be validated to be free of detectable endotoxin and interfering factors.

6.10 Dissolution Test

Change Fig. 6.10-1 as follows: Basket stirring element under 1.1 Apparatus for Basket Method:

1.1. Apparatus for Basket Method (Apparatus 1)

The assembly consists of the following: a vessel, which may be covered, made of glass or other inert, transparent material*1; a motor; a drive shaft; and a cylindrical basket. The vessel is partially immersed in a suitable water bath of any convenient size or heated by a suitable device such as a heating jacket. The water bath or heating device permits



All dimensions are expressed in mm.
ϕ=diameter

A: Note–Maximum allowable runout at "A" is ±1.0 mm when the part is rotated on center line axis with basket mounted.
B: Screen with welded seam, 0.22–0.31 mm wire diameter with wire openings of 0.36–0.44 mm [Note–After welding, the screen may be slightly altered.]

Fig. 6.10-1 Apparatus 1, Basket stirring element

holding the temperature inside the vessel at 37 ± 0.5°C during the test and keeping the bath fluid in constant, smooth motion. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smoothly rotating stirring element. Make the apparatus to permit observation of the specimen and stirring element during the test. The vessel is cylindrical, with a hemispherical bottom and a capacity of 1 liter. Its height is 160 mm to 210 mm and its inside diameter is 98 mm to 106 mm. Its sides are flanged at the top. Use a fitted cover to retard evaporation.*2 The shaft is positioned so that its axis is not more than 2 mm at any point from the vertical axis of the vessel and rotates smoothly and without significant wobble that could affect the results. Adjust a speed-regulating device to maintaine the shaft rotation speed at a specified rate, within ±4%.

Shaft and basket components of the stirring element shown in Fig. 6.10-1 are fabricated of stainless steel (SUS316) or other inert material. A basket having a gold coating of about 0.0001 inch (2.5 μm) thick may be used. The dosage unit is placed in a dry basket at the beginning of each test. The distance between the inside bottom of the vessel and the bottom of the basket is maintained at 25 ± 2 mm during the test.

9.01 Reference Standards

Add the following to 9.01 Reference Standards (1):

Auranofin RS
Butyl Parahydroxybenzoate RS
Calcium Pantothenate RS
Carboplatin RS
Cetotiamine Hydrochloride RS
Dorzolamide Hydrochloride RS
Epalrestat RS
Epoetin Alfa RS
Epoetin Beta RS
Ethyl Parahydroxybenzoate RS
Filgrastim RS
Lenograstim RS
Methyl Parahydroxybenzoate RS
Nartograstim RS
Propyl Parahydroxybenzoate RS
Quetiapine Fumarate RS
Tacalcitol RS
Valsartan RS

9.22 Standard Solutions

Add the following to 9.22 Standard solutions:

Standard Sulfite Solution Dissolve exactly 3.150 g of anhydrous sodium sulfite in freshly prepared distilled water to make exactly 100 mL. Pipet 0.5 mL of this solution, add

freshly prepared distilled water to make exactly 100 mL. Each mL of this solution contains 80 μg of sulfur dioxide (SO_2). Prepare before use.

9.41 Reagents, Test Solutions

Change the introduction as follows:

Reagents are the substances used in the tests of the Pharmacopoeia. The reagents that are described as ''Standard reagent for volumetric analysis'', ''Special class'', ''First class'', ''For water determination'', etc. in square brackets meet the corresponding requirements of the Japan Industrial Standards (JIS). The tests for them are performed according to the test methods of JIS. The reagents that are described as ''Certified reference material'' are those noted a certificate on the basis of JIS Q 0030 and guaranteed the traceability of the international system of units. These reference materials are provided by the Metrology Management Center, National Institute of Advanced Industrial Science and Technology (AIST) and manufacturers of the certified reference materials. In the case where the reagent name in the Pharmacopoeia differs from that of JIS, the JIS name is given in the brackets. The reagents for which a monograph's title is given in the brackets meet the requirements of the corresponding monograph. In the case of the reagents that are described merely as test items, the corresponding test method of the Pharmacopoeia is applied.

Test Solutions are the solutions prepared for use in the tests of the Pharmacopoeia.

Change the following:

Albiflorin $C_{23}H_{28}O_{11}$ White powder having no odor. Freely soluble in water, in methanol and in ethanol (99.5).

Identification—Determine the absorption spectrum of a solution of albiflorin in diluted methanol (1 in 2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry *<2.24>*: it exhibits a maximum between 230 nm and 234 nm.

Purity (1) Related substances 1—Dissolve 1 mg of albiflorin in 1 mL of methanol, and perform the test with 10 μL of this solution as directed in the Identification (2) under Peony Root: any spot other than the principal spot with an *R*f value of about 0.2 does not appear.

(2) Related substances 2—Dissolve 1 mg of albiflorin in 10 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed in the Assay under Peony Root: when measure the peak areas for 2 times the retention time of paeoniflorin, the total area of the peaks other than albiflorin obtained from the sample solution is not larger than 1/10 times the total area of the peaks other than the solvent peak.

Amidosulfuric acid (standard reagent) $HOSO_2NH_2$ In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

14-Anisoylaconine hydrochloride for assay
$C_{33}H_{47}NO_{11}.HCl$ White crystalline powder or powder. Freely soluble in methanol, sparingly soluble in water and in ethanol (99.5). Melting point: about 210°C (with decomposition).

Absorbance *<2.24>* $E^{1\%}_{1\,cm}$ (258 nm): 276 – 294 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

Purity (1) Related substances—To 1.0 mg of 14-anisoylaconine hydrochloride for assay add exactly 1 mL of ethanol (99.5). Perform the test with 5 μL of this solution as directed in the Identification under Processed Aconite Root: any spot other than the principle spot with an *R*f value of about 0.5 does not appear.

(2) Related substances—Dissolve 5.0 mg of 14-anisoylaconine hydrochloride for assay in 5 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 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 of both solutions by the automatic integration method: the total area of the peaks other than 14-anisoylaconine obtained from the sample solution is not larger than the peak area of 14-anisoylaconine from the standard solution.

Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Time span of measurement: About 4 times as long as the retention time of 14-anisoylaconine.

System suitability

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 14-anisoylaconine obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of 14-anisoylaconine from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard TS for assay under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of aconitum monoester alkaloids standard TS for assay under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are not more than 1.5%, respectively.

Arbutin for assay $C_{12}H_{16}O_7$ Use arbutin for thin-layer chromatography meeting the following additional specifications.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (280 nm): 70 – 76 [4 mg, previously dried in a desiccator (in vaccum, silica gel) for 12 hours, water, 100 mL].

Purity Related substances—Dissolve 40 mg of arbutin for assay in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water 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 determine each peak area of the both solutions by the automatic integration method: the total area of the peaks other than arbutin from the sample solution is not larger than the peak area of arbutin from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Assay under Bearberry Leaf except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add water to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of arbutin obtained from 10 μL of the standard solution (2) can be measured by the automatic integration method and the peak height of arbutin obtained from 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 arbutin beginning after the solvent peak.

Arbutin for thin-layer chromatography $C_{12}H_{16}O_7$

Colorless to white crystals or crystalline powder, and odorless. Freely soluble in water, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in ethyl acetate and in chloroform.

Melting point ⟨*2.60*⟩: 199 – 201°C

Purity Related substances—Dissolve 1.0 mg of arbutin for thin-layer chromatography in exactly 1 mL of a mixture of ethanol (95) and water (7:3). Perform the test with 20 μL of this solution as directed in the Identification (2) under Bearberry Leaf: any spot other than the main spot with an *R*f value of about 0.4 does not appear.

Atractylenolide III for thin-layer chromatography

$C_{15}H_{20}O_3$ White crystals or crystalline powder. Freely soluble in methanol, soluble in ethanol (99.5), and practically insoluble in water. Melting point: 193 – 196°C

Identification—(1) Determine the absorption spectrum of a solution of atractylenolide III for thin-layer chromatography in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 217 nm and 221 nm.

(2) Determine the infrared absorption spectrum of atractylenolide III 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 3350 cm^{-1}, 1742 cm^{-1}, 1641 cm^{-1} and 1384 cm^{-1}.

Purity Related substances—Dissolve 2 mg of atractylenolide III 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 these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Proceed the test with 5 μL each of the sample solution and standard solution as directed in the Identification (3) under Tokishakuyakusan Extract: the spot other than the principal spot with an *R*f value of about 0.5 obtained from the sample solution is not more intense than the spot from the standard solution.

Benzoylhypaconine hydrochloride for assay

$C_{31}H_{43}NO_9.HCl$ White crystals or crystalline powder. Freely soluble in methanol, soluble in water, and sparingly soluble in ethanol (99.5). Melting point: about 230°C (with decomposition).

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (230 nm): 225 – 240 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

Purity (1) Related substances—To 1.0 mg of benzoylhypaconine hydrochloride for assay add exactly 1 mL of ethanol (99.5). Perform the test with 5 μL of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principal spot with an *R*f value of about 0.5 appears.

(2) Related substance—Dissolve 5.0 mg of benzoylhypaconine hydrochloride for assay in 5 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 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 of both solutions by the automatic integration method: the total area of the peaks other than benzoylhypaconine obtained from the sample solution is not larger than the peak area of benzoylhypaconine from the standard solution.

Operating conditions

Column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay (3) under Goshajinkigan Extract.

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

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

System suitability

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 benzoylhypaconine obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of benzoylhypaconine from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard TS for assay under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less

than 4, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of aconitum monoester alkaloids standard TS for assay under the above operating conditions, the relative standard deviations of the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are not more than 1.5%, respectively.

Benzoylmesaconine hydrochloride for thin-layer chromatography $C_{31}H_{43}NO_{10}$.HCl White crystals or crystalline powder. Soluble in water and in ethanol (99.5) and sparingly soluble in methanol. Melting point: about 250°C (with decomposition).

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (230 nm): 217 – 231 (5 mg calculated on the anhydrous basis, methanol, 200 mL).

Purity Related substances—Dissolve 1.0 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in exactly 1 mL of ethanol (99.5). Perform the test with 5 μL of this solution as directed in the Identification under Processed Aconite Root: no spot other than the principal spot with an *R*f value of about 0.4 appears.

Benzyl parahydroxybenzoate $C_{14}H_{12}O_3$ White, fine crystals or crystalline powder. Freely soluble in ethanol (95), and very slightly soluble in water.

Melting point ⟨*2.60*⟩: 109 – 112°C

Residue on ignition ⟨*2.44*⟩: not more than 0.1%.

Content: not less than 99.0%. Assay—Weigh accurately about 1 g of benzyl parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate ⟨*2.50*⟩ the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 228.2 mg of $C_{14}H_{12}O_3$

Bovine serum albumin-isotonic sodium chloride solution Dissolve 0.1 g of bovine serum albumin in 100 mL of isotonic sodium chloride solution. Prepare before use.

Bromocresol green-sodium hydroxide-ethanol TS Dissolve 50 mg of bromocresol green in 0.72 mL of 0.1 mol/L sodium hydroxide VS and 20 mL of ethanol (95), and add water to make 100 mL.

Test for sensitivity—To 0.2 mL of the bromocresol green-sodium hydroxide-ethanol TS add 100 mL of freshly boiled and cool water: the solution is blue, and not more than 0.2 mL of 0.02 mol/L hydrochloric acid VS is required to change the color of this solution to yellow.

Color change: pH 3.6 (yellow) to pH 5.2 (blue).

Cephaeline hydrobromate $C_{28}H_{38}N_2O_4$.2HBr A white or light-yellow crystalline powder.

Purity—Dissolve 10 mg of cephaeline hydrobromate 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 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 in the Assay under Ipecac: when measure the peak areas for 2 times the retention time of emetine, the total area of the peaks other than cephaeline obtained from the sample solution is not larger than the peak area of cephaeline from the standard solution.

Chikusetsusaponin IV for thin-layer chromatography $C_{47}H_{74}O_{18}$ White crystalline powder. Freely soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether. Melting point: about 215°C (with decomposition).

Purity Related substances—Dissolve 2 mg of chikusetsusaponin IV for thin-layer chromatography in 1 mL of methanol, and perform the test with 5 μL of this solution as directed in the Identification under Panax Japonicus Rhizome: any spot other than the principal spot with an *R*f value of about 0.4 does not appear.

(*E*)-Chlorogenic acid for thin-layer chromatography $C_{16}H_{18}O_9$ A white powder. Freely soluble in methanol and in ethanol (99.5), and sparingly soluble in water. Melting point: about 205°C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of (*E*)-chlorogenic acid for thin-layer chromatography in 2 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 10 μL of the sample 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. Examine under ultraviolet light (main wavelength: 365 nm): no spot other than the principal spot with an *R*f value of about 0.5 appears.

Cinobufagin for assay $C_{26}H_{34}O_6$ A white crystalline powder. It is odorless.

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1\,cm}$ (295 nm): 125 – 137 (10 mg, methanol, 250 mL). Use the sample dried in a desiccator (silica gel) for 24 hours for the test.

Purity Related substances—Proceed with 40 mg of cinobufagin for assay as directed in the Purity under bufalin for assay.

Content: not less than 98.0%. Assay—Weigh accurately about 10 mg of cinobufagin for assay, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 10 mL, 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 amount of cinobufagin by the area percentage method.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 295 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

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

Mobile phase: A mixture of water and acetonitrile (1:1).

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

Selection of column: Dissolve 10 mg each of cinobufagin for assay, bufalin for assay and resibufogenin for assay in methanol to make 200 mL. Proceed with 20 μL of this solution under the above operating conditions. Use a column giving elution of bufalin, cinobufagin and resibufogenin in this order, and clearly dividing each peak.

Detection sensitivity: Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of cinobufagin obtained from 20 μL of the standard solution (2) can be measured by the automatic integration method, and the peak height of cinobufagin from 20 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of cinobufagin beginning after the solvent peak.

Copper (standard reagent) Cu In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

Diethyl phthalate $C_6H_4(COOC_2H_5)_2$ A colorless, clear liquid.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.500 – 1.505

Eleutheroside B for liquid chromatography $C_{17}H_{24}O_9$ A white crystalline powder. Sparingly soluble in methanol, slightly soluble in water, and very slightly soluble in ethanol (99.5). Melting point: 190 – 194°C

Identification—Determine the absorption spectrum of a solution of eleutheroside B for liquid chromatography in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 261 nm and 265 nm.

Purity Related substances—Dissolve 1.0 mg of eleutheroside B for liquid chromatography in 10 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 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 eleutheroside B obtained with the sample solution is not larger than the peak area of eleutheroside B with the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Identification under Eleutherococcus Senticosus Rhizome.

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

System suitability

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

System performance: Proceed as directed in the system suitability in the Identification under Eleutherococcus Senticosus Rhizome.

Emetine hydrochloride for assay $C_{29}H_{40}N_2O_4.2HCl$ A white or light-yellow crystalline powder. Soluble in water.

Absorbance ⟨*2.24*⟩ $E_{1\,cm}^{1\%}$ (283 nm): 116 – 127 (10 mg, diluted methanol (1 in 2), 400 mL). [after drying in a desiccator (reduced pressure below 0.67 kPa, phosphorus (V) oxide, 50°C) for 5 hours].

Melting point ⟨*2.60*⟩: about 250°C [with decomposition, after drying in a desiccator (reduced pressure below 0.67 kPa, phosphorus (V) oxide, 50°C) for 5 hours].

Purity Related substances—Dissolve 10 mg of emetine hydrochloride 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 (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. Determine the peak areas from both solutions by the automatic integration method: the total area of peaks other than emetine from the sample solution is not larger than the peak of emetine from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Assay under Ipecac except the 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 sensitivity so that the peak area of emetine obtained from 10 μL of the standard solution (2) can be measured by the automatic integration method, and the peak height of emetine obtained from 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 emetine.

2-Ethylhexyl parahydroxybenzoate $C_{15}H_{22}O_3$ Pale yellow, clear viscous liquid. Miscible with methanol (99.5). Practically insoluble in water.

Content: not less than 98.0%. Assay—Weigh accurately about 1 g of 2-ethylhexyl parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate ⟨*2.50*⟩ the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Per-

form a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 250.3 mg of $C_{15}H_{22}O_3$

Ginsenoside Rc $C_{53}H_{90}O_{22}$ A white crystalline powder. It is odorless.

Purity—Dissolve 1 mg of ginsenoside Rc in diluted methanol (3 in 5) to make 10 mL. Perform the test with 10 μL of this solution as directed under Liquid Chromatography <*2.01*> according to the conditions directed in the Assay (2) under Ginseng until ginsenoside Rc is eluted: the total area of the peaks other than ginsenoside Rc and solvent peak is not larger than 1/10 times the total peak area excluding the peak area of the solvent.

Ginsenoside Re $C_{48}H_{82}O_{18}$ A white crystalline powder. It is odorless.

Purity—Dissolve 1.0 mg of ginsenoside Re in diluted methanol (3 in 5) to make 10 mL. Perform the test with 10 μL of this solution as directed under Liquid Chromatography <*2.01*> according to the conditions directed in the Assay (1) under Ginseng until ginsenoside Re is eluted: the total area of the peaks other than ginsenoside Re and solvent peak is not larger than 1/10 times the total peak area excluding the peak area of the solvent.

Glycyrrhizic acid for thin-layer chromatography $C_{42}H_{62}O_{16}$ A white, crystalline powder, having a characteristic sweet taste. Freely soluble in hot water and in ethanol (95), and practically insoluble in diethyl ether. Melting point: 213 – 218°C (with decomposition).

Purity Related substances—Dissolve 10 mg of glycyrrhizic acid for thin-layer chromatography in 5 mL of dilute ethanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 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 in the Identification under Glycyrrhiza: the spots other than the principal spot with an *R*f value of about 0.3 from the sample solution are not more intense than the spot from the standard solution.

Hexyl parahydroxybenzoate $C_{13}H_{18}O_3$ White crystals or crystalline powder.

Melting point <*2.60*>: 49 – 53°C

Content: not less than 98.0%. Assay—Weigh accurately about 1 g of hexyl parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <*2.50*> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 222.3 mg of $C_{13}H_{18}O_3$

Honokiol $C_{18}H_{18}O_2$ Odorless white, crystals or crystalline powder.

Purity—Dissolve 1 mg of honokiol in the mobile phase to make 10 mL, and use this solution as the sample solution. Perform the Liquid Chromatography <*2.01*> with 10 μL of the sample solution as directed in the Assay under Magnolia Bark: when measure the peak areas for 2 times as long as the retention time of magnorole, the total area of peaks other than honokiol is not larger than 1/10 times the total area of the peaks other than the solvent peak.

Hydrazinum sulfate TS Dissolve 1.0 g of hydrazinum sulfate in water to make 100 mL, and allow to stand for 4 – 6 hours.

Isobutyl parahydroxybenzoate $C_{11}H_{14}O_3$ Colorless crystals or white crystalline powder. Freely soluble in ethanol (95), and practically insoluble in water.

Melting point <*2.60*>: 75 – 78°C

Residue on ignition <*2.44*>: not more than 0.1%.

Content: not less than 99.0%. Assay—Weigh accurately about 1 g of isobuthyl parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <*2.50*> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 194.2 mg of $C_{11}H_{14}O_3$

Isopropyl parahydroxybenzoate $C_{10}H_{12}O_3$ Colorless fine crystals, or white, crystalline powder. Freely soluble in ethanol (95), and very slightly soluble in water.

Melting point <*2.60*>: 84 – 86°C

Residue on ignition <*2.44*>: not more than 0.1%.

Content: not less than 99.0%. Assay—Weigh accurately about 1 g of isopropyl parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <*2.50*> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 180.2 mg of $C_{10}H_{12}O_3$

Liquiritin for thin-layer chromatography $C_{21}H_{22}O_9$ White crystals or crystalline powder. Sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water. Melting point: about 210°C (with decomposition).

Identification—Determine the absorption spectrum of a solution of liquiritin for thin-layer chromatography in diluted methanol (1 in 2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 215 nm and 219 nm, and between 275 nm and 279 nm.

Purity Related substances—Dissolve 1.0 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and perform the test with 1 μL of this solution as directed in the Identification (5) under Kakkonto Extract: no spot other than the principal spot with an *R*f value of about 0.4 ap-

pears.

Naringin for thin-layer chromatography $C_{27}H_{32}O_{14}$ White to light yellow crystalline powder. Freely soluble in ethanol (95) and in acetone, and slightly soluble in water. Melting point: about 170°C (with decomposition).

Optical rotation <2.49> $[\alpha]_D^{20}$: −87 – −93° (0.1 g, ethanol (95), 10 mL, 100 mm).

Purity Related substances—Proceed with 10 μL of a solution, prepared by dissolving 10 mg of naringin for thin-layer chromatography in 10 mL of ethanol (95), as directed in the Identification under Bitter Orange Peel: any spot other than the principal spot with an *R*f value of about 0.4 does not appear.

Paeoniflorin for thin-layer chromatography $C_{23}H_{28}O_{11}$ Colorless, odorless powder. Freely soluble in water and in methanol, and practically insoluble in diethyl ether. Melting point: 123 – 125°C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of paeoniflorin for thin layer chromatography in exactly 1 mL of methanol. Perform the test with 20 μL of this solution as directed in the Identification (2) under Peony Root: any spot other than the principal spot with an *R*f value of about 0.3 does not appear.

Palmatin chloride $C_{21}H_{22}ClNO_4$ A yellow-brown crystalline powder.

Purity Related substances—Dissolve 1 mg of palmatin chloride in 10 mL of methanol, and use this solution as the sample solution. Proceed with 20 μL of the sample solution as directed in the Assay under Phellodendron Bark: when measure the peak areas for 2 times the retention time of berberine, the total area of the peaks other than palmatin is not larger than 1/10 times the total area except the area of solvent peak.

Potassium dichromate (standard reagent) $K_2Cr_2O_7$ In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

Potassium hydrogen phthalate (standard reagent) $C_6H_4(COOK)(COOH)$ In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

Potassium iodate (standard reagent) KIO_3 In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

Resibufogenin for assay $C_{24}H_{32}O_4$ Odorless white crystalline powder.

Absorbance <2.24> $E_{1\,cm}^{1\%}$ (300 nm): 131 – 145 (10 mg, methanol, 250 mL), dried in a desiccator (silica gel) for 24 hours.

Purity Related substances—Weigh accurately 40 mg of resibufogenin for assay and proceed as directed in the Purity under bufalin for assay.

Content: not less than 98.0%. Assay—Weigh accurately about 10 mg of resibufogenin for assay, previously dried in a desiccator (silica gel) for 24 hours, add methanol to make exactly 10 mL, 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 amount of resibufogenin by the area percentage method.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 300 nm).

Column: A stainless steel column about 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 mm in particle diameter).

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

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of resibufogenin is about 9 minutes.

Selection of column: Dissolve 10 mg each of resibufogenin for assay, bufalin for assay and cinobufagin for assay in methanol to make 200 mL. Perform the test with 20 μL of this solution according to the above operating conditions, and calculate the resolution. Use a column giving elution of bufalin, cinobufagin and resibufogenin in this odor, and clearly dividing each peak.

Detection sensitivity: Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of resibufogenin obtained from 20 μL of standard solution (2) can be measured by the automatic integration method and the peak height of resibufogenin from 20 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of resibufogenin beginning after the peak of solvent.

Resibufogenin for thin-layer chromatography $C_{24}H_{32}O_4$ White crystalline powder having no odor. It is freely soluble in acetone and in methanol.

Purity Related substances—Dissolve 5.0 mg of resibufogenin for thin-layer chromatography in exactly 5 mL of acetone. Perform the test with 5 μL of this solution as directed in the Identification under Toad Venom: no spot other than the principal spot with an *R*f value of about 0.4 appear.

Sodium carbonate (standard reagent) Na_2CO_3 In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

Sodium chloride (standard reagent) NaCl In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

Sodium fluoride (standard reagent) NaF In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

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}, 1640 cm^{-1}, 1545 cm^{-1}, 1450 cm^{-1}, 1210 cm^{-1}, 1050 cm^{-1}, and 600 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 from the standard solution.

Sodium oxalate (standard reagent) $C_2Na_2O_4$ In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

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 the absorption at the wave numbers of about 2940 cm^{-1}, 1600 cm^{-1}, 1410 cm^{-1}, 1305 cm^{-1}, 1195 cm^{-1}, 1080 cm^{-1}, 1045 cm^{-1}, 980 cm^{-1}, 950 cm^{-1}, 910 cm^{-1} and 860 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 at an *R*f value of about 0.2 obtained from the sample solution are not more intense than the spot from the standard solution.

Zinc (standard reagent) Zn In addition to JIS K 8005 standard reagent for volumetric analysis, certified reference material which can be used for volumetric analysis may be used.

Add the following:

***o*-Acetanisidide** $C_9H_{11}NO_2$ White to light brownish crystals or crystalline powder. Freely soluble in ethanol (99.5) and in acetonitrile, and slightly soluble in water. Melting point: 86 – 89°C

0.05 mol/L Acetate buffer solution, pH 4.0 To 3.0 mL of acetic acid (100) add 900 mL of water, adjust to pH 4.0 with sodium hydroxide TS, and add water to make 1000 mL.

Acetyl chloride CH_3COCl A clear and colorless liquid.

***N*-Acetylgalactosamine** $C_8H_{15}NO_6$ White, crystals or crystalline powder.

Content: not less than 98.0%. Assay—Dissolve 36 mg of *N*-acetylgalactosamine in 1 mL of water. Perform the test with 15 μL of this 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.

Operating conditions

Detector: A differential refractometer (Detector temperature: a constant temperature of about 40°C).

Column: A stainless steel column 8 mm in inside diameter and 30 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 80°C.

Mobile phase: Water.

Flow rate: 0.5 mL per minute.

Time span of measurement: About 3 times as long as the retention time of *N*-acetylgalactosamine.

***N*-Acetylneuraminic acid** $C_{11}H_{19}NO_9$ White, crystals or crystalline powder.

Content: not less than 98.0%. Assay—Dissolve 30 mg of *N*-acetylneuraminic acid in 1 mL of the mobile phase. Perform the test with 15 μL of this 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.

Operating conditions

Detector: A differential refractometer (detector temperature: a constant temperature of about 40°C).

Column: A stainless steel column 8 mm in inside diameter and 30 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (6 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: 10 mmol/L perchloric acid.

Flow rate: 0.5 mL per minute.

Time span of measurement: About 3 times as long as the retention time of *N*-acetylneuraminic acid.

***N*-Acetylneuraminic acid for epoetin alfa** $C_{11}H_{19}NO_9$ White needle crystalline powder.

0.4 mmol/L *N*-Acetylneuraminic acid TS Weigh accurately about 15.5 mg of *N*-acetylneuraminic acid for epoetin alfa, dissolve in water to make exactly 50 mL. To exactly *V* mL of this solution add water to make exactly 100 mL.

$$V\,(\mathrm{mL}) = 309.3 \times 2/\text{amount (mg) of } N\text{-acetylneuraminic acid}$$

Aldioxa for assay $C_4H_7AlN_4O_5$ [Same as the monograph Aldioxa. When dried, it contains not less than 67.3% and not more than 71.0% of allantoin ($C_4H_6N_4O_3$) and not less than 11.6% and not more than 12.5% of aluminum (Al).]

Ammonium sulfate TS Dissolve 39.6 g of ammonium sulfate in 70 mL of water, adjust to pH 8.0 with sodium hydroxide TS, and add water to make 100 mL (3 mol/L).

Artemisia·argyi for purity test Powder of the leaf and twig of *Artemisia argyi* H. Léveillé et Vaniot.

Identification—To 0.5 g of Artemisia·argyi for purity test add 5 mL of a mixture of methanol and water (3:2), shake for 10 minutes, 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 10 μL of the sample solution on a plate of octadecylsilanized silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and water (4:1) to a distance of about 7 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): two green fluorescent spots appear at *R*f values of about 0.3 and about 0.4 (eupatilin and jaceosidin).

Atractylenolide III for assay $C_{15}H_{20}O_3$ Use atractylenolide III for thin-layer chromatography. It meets the following additional specifications.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (219 nm): 446 – 481 (5 mg, methanol, 500 mL).

Purity Related substances—Dissolve 5 mg of atractylenolide III for assay in 50 mL of methanol, and use this solution as the sample solution. To exactly 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 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 total area of the peaks other than atractylenolide III obtained from the sample solution is not larger than the peak area of atractylenolide III from the standard solution.

Operating conditions

Column, column temperature and mobile phase: Proceed as directed in the operating conditions in the Assay (3) under Tokishakuyakusan Extract.

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

Flow rate: Adjust the flow rate so that the retention time of atractylenolide III is about 11 minutes.

Time span of measurement: About 5 times as long as the retention time of atractylenolide III, beginning after the solvent peak.

System suitability

Test for required detectability: To exactly 1 mL of the standard solution add methanol to make exactly 20 mL. Confirm that the peak area of atractylenolide III obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that 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 atractylenolide III 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 atractylenolide III is not more than 1.5%.

Atractylodin for assay $C_{13}H_{10}O$ White to pale yellowish crystals. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 54°C.

Identification—Conduct this procedure without exposure to light, using light-resistant vessels. Determine the absorption spectrum of a solution of atractylodin for assay in methanol (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 256 nm and 260 nm, between 270 nm and 274 nm, between 332 nm and 336 nm and between 352 nm and 356 nm.

Absorbance <*2.24*> $E^{1\%}_{1\,cm}$ (272 nm): 763 – 819 (2 mg, methanol, 250 mL). Conduct this procedure without exposure to light, using light-resistant vessels.

Purity Related substances—

(i) Conduct this procedure without exposure to light, using light-resistant vessels.

Dissolve 2 mg of atractylodin for assay in 2 mL of methanol, and use this solution as the sample solution. To exactly 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 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 and immediately develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for splaying on the plate, and heat at 105°C for 5 minutes: the spot other than the principle spot which appears at an *R*f value of about 0.4 obtained from the sample solution is not more intense than the

spot from the standard solution.

(ii) Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 5 mg of atractylodin for assay in 250 mL of methanol, and use this solution as the sample solution. To exactly 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 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 total area of the peaks other than atractylodin obtained from the sample solution is not larger than the peak area of atractylodin from the standard solution.

Operating conditions

Detector, column, column temperature and mobile phase: Proceed as directed in the operating conditions in the Assay (4) under Tokishakuyakusan Extract.

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

Time span of measurement: About 5 times as long as the retention time of atractylodin, beginning after the solvent peak.

System suitability

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

System performance: Put a suitable amount of the standard solution in a colorless vessel, and expose to ultraviolet light (main wavelength: 365 nm) for about 1 minute. When the procedure is run with 20 μL of this solution under the above operating conditions, a peak of an isomer is found in addition to the peak of atractylodin, and the isomer and atractylodin 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 atractylodin is not more than 1.5%.

Atractylodin TS for assay Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve exactly 5.0 mg of atractylodin for assay in methanol to make exactly 1000 mL.

Avidin-biotin TS To 15 mL of phosphate-buffered sodium chloride TS add 2 drops each of avidin TS and biotinylated peroxidase TS, and mix.

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt $C_{18}H_{16}N_4O_6S_4.(NH_4)_2$ A bluish green crystalline powder.

Melting point <*2.60*>: about 330°C (with decomposition).

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt TS Dissolve 5.3 g of citric acid monohydrate in water to make 500 mL. To this solution add a solution prepared by dissolving 7.1 g of anhydrous disodium hydrogen phosphate in water to make 500 mL to adjust to pH 4.3. To 20 mL of this solution add 15 mg of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt. To this solution add 14 μL of hydrogen peroxide TS before use.

Blocking TS for epoetin alfa Used for Western blotting.

Blocking TS for nartograstim test Dissolve 1.0 g of bovine serum albumin in phosphate-buffered sodium chloride TS to make 100 mL.

Blotting TS Dissolve 5.81 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 2.93 of glycine and 0.38 g of sodium lauryl sulfate in a suitable amount of water, add 200 mL of methanol, and add water to make 1000 mL.

Bovine serum albumin for gel filtration molecular mass marker Albumin obtained from bovine serum. For gel filtration chromatography.

0.1 w/v% Bovine serum albumin-sodium chloride-phosphate buffer solution Dissolve 8.0 g of sodium chloride, 0.2 g of potassium chloride, 1.15 g of anhydrous disodium hydrogen phosphate and 0.2 g of potassium dihydrogen phosphate in water to make 1000 mL. To this solution add a solution of bovine serum albumin dissolved 1.0 g in 10 mL of water.

Bovine serum albumin TS for nartograstim test Dissolve 0.5 g of bovine serum albumin and 0.5 mL of polysorbate 20 in phosphate-buffered sodium chloride TS to make 500 mL.

Buffer solution for enzyme digestion Dissolve 0.30 g of urea in a mixture of 100 μL of 2-amino-2-hydroxymethyl-1,3-propanediol solution containing 6.06 g in 100 mL of water, 100 μL of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride solution containing 7.88 g in 100 mL of water, 100 μL of methylamine hydrochloride solution containing 2.70 g in 100 mL of water, 50 μL of dithiothreitol in solution containing 30.9 mg in 1 mL of water and 420 μL of water.

Buffer solution for epoetin alfa sample Dissolve 1.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 3.2 g of sodium lauryl sulfate in a suitable amount of water, adjust to pH 6.8 with 6 mol/L hydrochloric acid TS, 1 mol/L hydrochloric acid TS or 0.1 mol/L hydrochloric acid TS, add 32 mg of bromophenol blue and 16 mL of glycerin, and add water to make 40 mL. Before use, dissolve 50 mg of dithiothreitol in 10 mL of this solution.

Buffer solution for filgrastim sample Dissolve 1.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 3.2 g of sodium lauryl sulfate in a suitable amount of water, adjust to pH 6.8 with 6 mol/L hydrochloric acid TS, 1 mol/L hydrochloric acid TS or 0.1 mol/L hydrochloric acid TS, add 32 mg of bromophenol blue and 16 mL of glycerin, and add water to make 40 mL.

Buffer solution for nartograstim sample Mix 0.8 mL of sodium lauryl sulfate solution (1 in 10), 0.5 mL of 0.5 mol/L tris buffer solution, pH 6.8, 0.4 mL of glycerin and 0.1 mL

of bromophenol blue solution (1 in 200). Prepare before use.

Buffer solution for SDS polyacrylamide gel electrophoresis Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 14.4 g of glycine and 1.0 g of sodium lauryl sulfate in water to make 1000 mL.

Carboplatin $C_6H_{12}N_2O_4Pt$ [Same as the namesake monograph]

32D Clone3 cells A cloned cell line established by culturing mouse bone marrow origin 32D cell line in the presence of G-CSF.

3-Chloro-1,2-propanediol $C_3H_7ClO_2$ A clear and colorless viscous liquid.

Purity Dissolve 0.20 g of 3-chloro-1,2-propanediol in 100 mL of diethyl ether, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diethyl ether 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 Gas Chromatography <*2.02*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than 3-chloro-1,2-propanediol obtained from the sample solution is not larger than 2 times the peak area from the standard solution.

Operating conditions

Proceed as directed in the operating conditions in the Purity (6) under Iohexol except the time span of measurement.

Time span of measurement: About 5 times as long as the retention time of 3-chloro-1,2-propanediol, beginning after the solvent peak.

System suitability

Test for required detectability: To exactly 5 mL of the standard solution add diethyl ether to make exactly 20 mL. Confirm that the peak area of 3-chloro-1,2-propanediol obtained with 5 μL of this solution is equivalent to 20 – 30% of that with 5 μL of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Purity (6) under Iohexol.

Chlorotrimethylsilane $(CH_3)_3SiCl$ A colorless or practically colorless liquid, having a pungent odor. Evolves fumes in a damp atmosphere. Very soluble in diethyl ether, and reactable with water or with ethanol. Boiling point: about 58°C.

Chymotrypsinogen for gel filtration molecular mass marker A chymotrypsinogen obtained from bovine spleen. For gel filtration chromatography.

Cyclobutanecarboxylic acid $C_5H_8O_2$ A clear and colorless liquid. Congealing point: −7.5°C

1,1-Cyclobutanedicarboxylic acid $C_6H_8O_4$ White crystals.

Melting point <*2.60*>: 159 – 163°C

Purity Related substances—Dissolve 20 mg of 1,1-cyclobutanedicarboxylic acid in 100 mL of the mobile phase used in the Purity (1) under Carboplatin, and use this solution as the sample solution. Perform the test with 25 μL of the sample solution as directed in the Purity (1) under Carboplatin. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the total amount of the peaks other than 1,1-cyclobutanedicarboxylic acid is not more than 2%. However, the time span of measurement for this calculation is about 2 times as long as the retention time of 1,1-cyclobutanedicarboxylic acid, beginning after the solvent peak.

Content: not less than 99.0%. Assay—Dissolve about 30 mg of 1,1-cyclobutanedicarboxylic acid, accurately weighed, in 50 mL of water, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 7.207 mg of $C_6H_8O_4$

Dehydrocorydaline nitrate for thin-layer chromatography $C_{22}H_{24}N_2O_7$ Yellow, crystals or crystalline powder. Sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5). Melting point: about 240°C (with decomposition).

Purity Related substances—Dissolve 5.0 mg of dehydrocorydaline nitrate for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 0.5 mL of the sample 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 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 immediately with a mixture of methanol, a solution of ammonium acetate (3 in 10) and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm) and then spray Dragendorff's TS on the plate: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution in either case.

Diclofenac sodium $C_{14}H_{10}Cl_2NNaO_2$ [Same as the namesake monograph]

Dilution fluid for particle counter A fluid used for blood dilution.

2,6-Dimethylaniline $C_8H_{11}N$ A clear liquid. Soluble in ethanol (95), and sparingly soluble in water. Specific gravity d_{20}^{20}: about 0.98.

Dimethylpolysiloxane for gas chromatography Prepared for gas chromatography.

Disodium hydrogen phosphate-citric acid buffer solution, pH 5.5 To 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS add an amount of a solution, prepared by dis-

solving 5.25 g of citric acid monohydrate in water to make 1000 mL, to adjust to pH 5.5.

Doxorubicin hydrochloride $C_{27}H_{29}NO_{11}.HCl$ [Same as the namesake monograph]

Edaravone for assay $C_{10}H_{10}N_2O$ [Same as the monograph Edaravone. When dried, it contains not less than 99.5% of edaravone ($C_{10}H_{10}N_2O$).]

Emedastine fumarate for assay $C_{17}H_{26}N_4O.2C_4H_4O_4$ [Same as the monograph Emedastine Fumarate. When dried it contains not less than 99.5% of emedastine fumarate ($C_{17}H_{26}N_4O.2C_4H_4O_4$).]

Ethyl formate $HCOOC_2H_5$ A clear and colorless liquid. Miscible with ethanol (95) and with acetone, and soluble in water.

Identification—Determine the infrared absorption spectrum of ethyl formate as directed in the liquid film method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 2980 cm^{-1}, 2930 cm^{-1}, 1718 cm^{-1}, 1470 cm^{-1}, 1449 cm^{-1}, 1387 cm^{-1}, 1302 cm^{-1}, 1181 cm^{-1}, 1004 cm^{-1}, 840 cm^{-1} and 747 cm^{-1}.

Purity—(1) Perform the test with 1 μL of ethyl formate as directed under Gas Chromatography <*2.02*> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of ethyl formate by the area percentage method: not less than 97.0%.

Operating conditions

Detector: A thermal conductivity detector.

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

Column temperature: Maintain at 50°C for 1 minute after injecting sample, then rise to 150°C at the rate of 10°C per minute, and maintain at 150°C for 1 minute.

Carrier gas: Helium.

Flow rate: 41 cm per second.

Split ratio: 1:110.

Time span of measurement: About 5 times as long as the retention time of ethyl formate.

(2) Acid (as formic acid) Dissolve 0.5 g of potassium iodate and 5 g of potassium iodide in 50 mL of water, and add 2 g of ethyl formate. After allowing to stand for 10 minutes, add 2 drops of starch TS and 1.30 mL of 0.1 mol/L sodium thiosulfate VS: the solution is colorless (not more than 0.3%).

Water <*2.48*>: not more than 0.5% (1 g, coulometric titration).

***N*-Ethylmorpholine** $C_6H_{13}NO$ A colorless to yellow-brown liquid.

Refractive index <*2.45*> n_D^{20}: 1.439 – 1.443

Specific gravity <*2.56*> d_4^{20}: 0.908 – 0.916

FBS-IMDM Dissolve an amount of the powder for 1 L of Iscove's modified Dulbecco's powder medium, 0.1 g of kanamycin sulfate (not less than 600 μg potency/mg), 3.0 g of sodium hydrogen carbonate and 36 μL of 2-mercapto ethanol solution (1 in 10) in water to make 1000 mL, and sterilize by filtration. To this solution add fetal bovine serum, previously heated at 56°C for 30 minutes, so that the concentration of the serum is 10 vol%.

(*E*)-Ferulic acid for assay $C_{10}H_{10}O_4$ Use (*E*)-ferulic acid. It meets the following additional requirements.

Absorbance <*2.24*> $E_{1\,cm}^{1\%}$ (320 nm): 878 – 969 (5 mg, methanol, 1000 mL).

Purity Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 5 mg of (*E*)-ferulic acid for assay in 10 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 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 each peak by the automatic integration method: the total area of the peaks other than (*E*)-ferulic acid obtained from the sample solution is not larger than the peak area of (*E*)-ferulic acid 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 (1) under Tokishakuyakusan Extract.

Time span of measurement: About 6 times as long as the retention time of (*E*)-ferulic acid, beginning after the solvent peak.

System suitability

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of water and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of (*E*)-ferulic acid obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that 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 (*E*)-ferulic 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 (*E*)-ferulic acid is not more than 1.5%.

Fluorescence TS Mix 400 μL of sodium dithionite solution containing 6.27 g in 200 mL of water, 210 μL of 2-mercaptoethanol, 321 μL of acetic acid (100), 400 μL of 1,2-diamino-4,5-methylenedioxybenzene solution containing 31.1 mg in 1.0 mL of water and 2669 μL of water. Prepare before use.

Fluorogenic substrate TS A solution containing oxidation-reduction indicator.

Formazin opalescence standard solution To 15 mL of formazin opalescence stock solution add water to make 1000 mL. Use within 24 hours after preparation. Shake thor-

oughly before use.

Fructose for thin-layer chromatography $C_6H_{12}O_6$ Colorless to white crystals or crystalline powder. Very soluble in water, and sparingly soluble in ethanol (99.5). It is deliquescence with the atmospheric moisture.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: -88 – $-94°$ (1 g, diluted ammonia solution (28) (1 in 1000), 100 mL, 100 mm. Previously, dried over silica gel as the desiccant for 3 hours).

Purity Related substances—Dissolve 2 mg of Fructose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), 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 2-propanol, water and methanol (3:2:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS to the plate, and heat at 105°C for 10 minutes: any spot other than the principle spot with an *R*f value of about 0.6 does not appear.

***N*-Glycolylneuraminic acid** $C_{11}H_{19}NO_{10}$ White needle crystalline powder.

0.1 mmol/L *N*-Glycolylneuraminic acid TS Weigh accurately about 16.5 mg of *N*-glycolylneuraminic acid, and dissolve in water to make exactly 50 mL. To exactly *V* mL of this solution add water to make exactly 100 mL.

$$V\,(\mathrm{mL}) = 325.3 \times 0.5/\text{amount (mg) of } N\text{-glycolylneuraminic acid}$$

1,1,1,3,3,3-Hexamethyldisilazane $(CH_3)_3SiNHSi(CH_3)_3$ A colorless or practically colorless, liquid. Very soluble in diethyl ether, and reactable with water or with ethanol. Boiling point: about 125°C.

1-Hexanol $C_6H_{14}O$ A clear and colorless liquid.
Specific gravity d_{20}^{20}: 1.415 – 1.420
Boiling point 156 – 158°C

Ibuprofen piconol $C_{19}H_{23}NO_2$ [Same as the namesake monograph]

Ibuprofen piconol for assay $C_{19}H_{23}NO_2$ [Same as the monograph Ibuprofen Piconol. It contains not less than 99.0% of ibuprofen piconol ($C_{19}H_{23}NO_2$), calculated on the anhydrous basis, and meets the following additional requirement.]

Purity Related substances—Dissolve 0.15 g of ibuprofen piconol for assay in the mobile phase to make 100 mL. To 10 mL of this solution add the mobile phase to make 30 mL, and use this 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 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 by the automatic integration method: the total area of the peaks other than ibuprofen piconol obtained from the sample solution is not larger than the peak area of ibuprofen piconol from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Perform as directed in the operating conditions in the Assay under Ibuprofen Piconol Ointment.

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

System suitability

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 ibuprofen piconol obtained with 5 μL of this solution is equivalent to 3.5 – 6.5% of that 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, the number of theoretical plates and the symmetry factor of the peak of ibuprofen piconol are not less than 5000 and not more than 1.3, 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 ibuprofen piconol is not more than 2.0%.

Iodoacetic acid ICH_2COOH White or practically white crystals.

Iscove's modified Dulbecco's fluid medium for filgrastim A fluid medium for cell culture, containing 0.165 g of anhydrous calcium chloride, 97.67 mg of anhydrous magnesium sulfate, 0.330 g of potassium chloride, 76 μg of potassium nitrate, 4.5 g of sodium chloride, 0.125 g of sodium dihydrogen phosphate monohydrate, 17.3 μg of sodium selenite pentahydrate, 30 mg of glycine, 25 mg of L-alanine, 84 mg of L-arginine hydrochloride, 25 mg of L-asparagine, 30 mg of L-aspartic acid, 91.4 mg of L-cystine dihydrochloride, 75 mg of L-glutamic acid, 0.584 g of L-glutamine, 42 mg of L-histidine hydrochloride monohydrate, 0.105 g of L-isoleucine, 0.105 g of L-leucine, 0.146 g of L-lysine hydrochloride, 30 mg of L-methionine, 66 mg of L-phenylalanine, 40 mg of L-proline, 42 mg of L-serine, 95 mg of L-threonine, 16 mg of L-tryptophan, 0.104 g of disodium L-tyrosine, 94 mg of L-valine, 13 μg of biotin, 4 mg of choline chloride, 4 mg of calcium D-pantothenate, 4 mg of folic acid, 4 mg of nicotinic acid amide, 4 mg of pyridoxal hydrochloride, 0.4 mg of riboflavin, 4 mg of thiamine hydrochloride, 13 μg of cyanocobalamin, 7.2 mg of myoinositol, 4.5 g of glucose, 5.958 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 15 mg of phenol red, 0.110 g of sodium pyruvate and 3.024 g of sodium hydrogen carbonate in 1 L.

Iscove's modified Dulbecco's powder medium A powder to make fluid medium for cell culture, containing 0.165 g of anhydrous calcium chloride, 97.67 mg of anhydrous magnesium sulfate, 0.330 g of potassium chloride, 76 μg of potassium nitrate, 4.5 g of sodium chloride, 0.125 g of sodium dihydrogen phosphate hydrate, 17.3 μg of sodium selenite pentahydrate, 30 mg of glycin, 25 mg of L-alanine, 84 mg of L-arginine hydrochloride, 25 mg of L-asparagine,

30 mg of L-aspartic acid, 91.4 mg of L-cystine dihydrochloride, 75 mg of L-glutamic acid, 0.584 g of L-glutamine, 42 mg of L-histidine hydrochloride monohydrate, 0.105 g of L-isoleucine, 0.105 g of L-leucine, 0.146 g of L-lysine hydrochloride, 30 mg of L-methionine, 66 mg of phenylalanine, 40 mg of L-proline, 42 mg of L-serine, 95 mg of L-threonine, 16 mg of L-tryptophan, 0.104 g of disodium L-tyrosine, 94 mg of L-valine, 13 μg of biotin, 4 mg of choline chloride, 4 mg of calcium D-pantothenate, 4 mg of folic acid, 4 mg of nicotinic acid amide, 4 mg of pyridoxal hydrochloride, 0.4 mg of riboflavin, 4 mg of thiamine hydrochloride, 13 μg of cyanocobalamin, 7.2 mg of myoinositol, 4.5 g of glucose, 5.958 g of *N*-2-hydroxyethylpyperadine-*N*-2-ethanesulfonate, 15 mg of phenol red and 0.110 g of sodium pyruvate in each L.

Isosorbide mononitrate for assay $C_6H_9NO_6$ Odorless white crystals.

Method of purification: To Isosorbide Mononitrate 70%/Lactose 30% add not less than 3-fold volume of ethyl acetate, shake vigorously, filter through a membrane filter with a pore size not exceeding 0.5 μm, and evaporate the filtrate to dryness on a water bath under reduced pressure. Recrystallize the residue from a mixture of hexane and ethyl acetate (3:2), and dry under reduced pressure on silica gel for 4 hours.

Identification: Determine the infrared absorption spectrum of isosorbide mononitrate for assay, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of between 3210 cm^{-1} and 3230 cm^{-1}, and about 1651 cm^{-1}, 1635 cm^{-1}, 1282 cm^{-1}, 1093 cm^{-1} and 852 cm^{-1}.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +171 – +176° (after drying, 1 g, ethanol (95), 100 mL, 100 mm).

Melting point <*2.60*>: 89 – 92°C

Purity Related substances—Dissolve 50 mg of isosorbide mononitrate for assay in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 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 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 isosorbide mononitrate obtained from the sample solution is not larger than the peak area of isosorbide mononitrate from the standard solution, and the total area of the peaks other than isosorbide mononitrate from the sample solution is not larger than 2 times the peak area of isosorbide mononitrate from the standard solution. For these calculations use the area of the peak, having a relative retention time of about 4.5 with respect to isosorbide mononitrate, after multiplying by its relative response factor, 0.62.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Isosorbide Mononitrate 70%/Lactose 30%.

Time span of measurement: About 5 times as long as the retention time of isosorbide mononitrate, beginning after the solvent peak.

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 isosorbide mononitrate are not less than 2000 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 isosorbide mononitrate is not more than 2.0%.

Loss on drying <*2.41*>: not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Content: not less than 99.0%. Assay—Weigh accurately about 0.2 g of previously dried isosorbide mononitrate for assay, put in a Kjeldahl flask, dissolve in 10 mL of water, add 3 g of Devarda's alloy and 40 mL of water, and set the flask on the apparatus as shown in the figure under Nitrogen Determination <*1.08*>. Put exactly 25 mL of 0.05 mol/L sulfuric acid VS and 5 drops of bromocresol green-methyl red TS in a absorption flask, and set to the apparatus to immerse the lower end of the condenser. Add 15 mL of sodium hydroxide solution (1 in 2) through the funnel, rinse cautiously the funnel with 20 ml of water, immediately close the clamp attached to the rubber tubing, then begin the distillation with steam, and continue until the distillate measures about 100 mL. Remove the absorption flask from the lower end of the condenser, rinse the end part of the condenser with a small quantity of water, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from red to light blue-green through a light red-purple. Perform a blank determination in the same manner.

Each mL of 0.05 mol/L sulfuric acid VS
= 19.11 mg of $C_6H_9NO_6$

Lafutidine for assay $C_{22}H_{29}N_3O_4S$ [Same as the monograph Lafutidine. When dried, it contains not less than 99.5% of lafutidine ($C_{22}H_{29}N_3O_4S$).]

Lecithin A pale yellow to yellow-brawn, powder or grains, having a characteristic odor.

It is emulsified with water.

It is hygroscopic.

Levofloxacin hydrate for assay $C_{18}H_{20}FN_3O_4.\frac{1}{2}H_2O$ [Same as the monograph Levofloxacin Hydrate]

Losartan potassium $C_{22}H_{22}ClKN_6O$ [Same as the namesake monograph]

Lysyl endopeptidase A protease obtained from *Lysobacter enzymogenes*. It contains about 150 units per mg, where 1 unit is an enzyme amount which hydrolyzes 1 μmol of tosyl-glycyl-prolyl-lysine-4-nitroanilide acetate per minute at pH 7.7 and 25°C.

Manninotriose for thin-layer chromatography $C_{18}H_{32}O_{16}$ A white powder. Very soluble in water, and practically insoluble in ethanol (99.5). It is hygroscopic. It is deliquescence with the atmospheric moisture.

Optical rotation <2.49> $[\alpha]_D^{20}$: +159 – +170° (50 mg calculated on the anhydrous basis, diluted ammonia solution (28) (1 in 1000), 5 mL, 100 mm).

Purity Related substances—Dissolve 3 mg of manninotriose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), 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 2-propanol, water and methanol (3:2:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS to the plate, and heat at 105°C for 10 minutes: a spot other than the principle spot with an *R*f value of about 0.4 is not observed.

2-Mercaptoethanol for epoetin beta $HSCH_2CH_2OH$ Prepared for study of sulfoprotein.

4-Methoxybenzaldehyde-sulfuric acid-acetic acid TS To 50 mL of acetic acid (100) add 1 mL of sulfuric acid and 0.5 mL of 4-methoxybenzaldehyde, and stir well. Prepare before use.

4-Methylbenzophenone $C_{14}H_{12}O$ White crystals.

Microplate for antigen antibody reaction test A plate made from polystyrene, and prepared for antigen antibody reaction test.

Performance: Coefficient of variation of the binding capacity of immunoglobulin G is not more than 5%, and the binding capacity of each well is within 10% of the mean value.

Molecular mass marker for epoetin alfa A solution containing about 0.4 mg each of white egg albumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme in 200 μL.

Molecular mass marker for nartograstim test A solution containing the following proteins. Ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme.

Molecular mass standard stock solution Dissolve 1.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 3.2 g of sodium lauryl sulfate in a suitable amount of water, adjust to pH 6.8 with 6 mol/L hydrochloric acid TS, 1 mol/L hydrochloric acid TS or 0.1 mol/L hydrochloric acid TS, add 32 mg of bromophenol blue and 16 mL of glycerin, and add water to make 40 mL. To 500 μL of this solution add 100 μL of molecular mass marker for epoetin alfa and 1400 μL of water, and heat at 100°C for 5 minutes. It meets the following requirement.

Identification—Dissolve 0.1 mg each of white egg albumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme in 250 μL of buffer solution for epoetin alfa sample, add water to them to make 1 mL and heat at 100°C for 5 minutes, and use these solutions as each standard solution. When perform the test with the solution to be examined and each standard solution by the SDS-polyacrylamide gel electrophoresis as directed in the Identification under Epoetin Alfa (Genetical recombination), the each band in the chromatogram obtained from the solution to be examined shows the same mobility as the band corresponding to white egg albumin, carbonic anhydrase, soybean trypsin inhibitor or lysozyme obtained from each standard solution.

Mouse anti-epoetin alfa monoclonal antibody A solution of the monoclonal antibody in phosphate-buffered sodium chloride TS, which is obtained from mouse immunized with a synthetic peptide having the amino acid sequence corresponding to N-terminal 20 residues of epoetin alfa (genetical recombination). When perform the Western blotting against Epoetin Alfa RS, it is reactable.

Myoinositol $C_6H_6(OH)_6$ White, crystals or crystalline powder.

Naphazoline hydrochloride $C_{14}H_{14}N_2.HCl$ [Same as the namesake monograph]

NFS-60 cell Prepared from leukemia mouse, infected with retrovirus (Cas-Br-M). After conditioning with a suitable medium, preserve the strain established by J. N. Ihle, *et al*. (*Proc. Natl. Acad. Sci. USA*, 1985, 82, 6687) at not exceeding −150°C in conveniently sized packets.

NADH peroxidase One unit indicates an amount of the enzyme which consumes 1 μmol of β-NADH in 1 minute at 25°C and pH 8.0 using β-nicotinamide adenine dinucleotide (β-NADH) and hydrogen peroxide as the substrate.

NADH peroxidase TS Suspend NADH peroxidase in ammonium sulfate TS so that each mL contains 10 units of the activity.

Storage—Between 0 and 8°C.

β-Nicotinamide adenine dinucleotide reduced form (β-NADH) $C_{21}H_{27}N_7O_{14}P_2.Na_2$ A white to light yellowish white powder.

Absorbance ratio: Determine the absorbances at 260 nm and at 340 nm, A_{260} and A_{340}, of a solution of β-nicotinamide adenine dinucleotide reduced form (β-NADH) in pH 7.4 phosphate buffer solution (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: the result of A_{260}/A_{340} is between 2.2 and 2.4.

Water <2.48>: not more than 8.0% (0.3 g, volumetric titration, direct titration).

β-Nicotinamide adenine dinucleotide reduced form TS Dissolve 0.4 mg of β-nicotinamide adenine dinucleotide reduced form (β-NADH) in 1 mL of 0.6 mol/L 2,2′,2″-nitrilotriethanol hydrochloride buffer solution, pH 8.0. Prepare before use.

Nifedipine for assay $C_{17}H_{18}N_2O_6$ [Same as the monograph Nifedipine. When dried, it contains not less than 99.0% of nifedipine ($C_{17}H_{18}N_2O_6$) and meets the following requirement.]

Purity Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 25 mg of nifedipine for assay 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 10 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 25 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 area by the automatic integration method: the total area of the peaks other than nifedipine obtained from the sample solution is not larger than the peak area of nifedipine from the standard solution.
Operating conditions
Detector: An ultraviolet absorption photometer (wavelength: 230 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: Adjust to pH 6.1 of a mixture of methanol and diluted 0.05 mol/L disodium hydrogen phosphate TS (1 in 5) (11:9) with phosphoric acid.
Flow rate: Adjust the flow rate so that the retention time of nifedipine is about 6 minutes.
Time span of measurement: About 2 times as long as the retention time of nifedipine, 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 20 mL. Confirm that the peak area of nifedipine obtained with 10 μL of this solution is equivalent to 18 to 32% of that 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 nifedipine are not less than 4000 and not more than 1.2, 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 nifedipine is not more than 2.0%.

Ninhydrin-ethanol TS for spraying Dissolve 1 g of ninhydrin in 50 mL of ethanol (95).

2,2′,2″-Nitrilotriethanol hydrochloride $(CH_2CH_2OH)_3N.HCl$ White, crystals or powder.
Purity Clarity of solution—A solution (1 in 20) is clear.
Content: not less than 98%. Assay—Dissolve 0.3 g of 2,2′,2″-nitrilotriethanol hydrochloride, accurately weighed, in 50 mL of water, add 5 mL of diluted nitric acid (1 in 3), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS
= 18.57 mg of $(CH_2CH_2OH)_3N.HCl$

0.6 mol/L 2,2′,2″-Nitrilotriethanol hydrochloride buffer solution, pH 8.0 Dissolve 5.57 g of 2,2′,2″-nitrilotriethanol hydrochloride in 40 mL of water, adjust to pH 8.0 with dilute sodium hydroxide TS, and add water to make 50 mL.

Omeprazole for assay $C_{17}H_{19}N_3O_3S$ [Same as the monograph Omeprazole]

Particle counter apparatus An apparatus that is able to count the fine particles derived from reticulocyte similar cells.

Pemirolast potassium $C_{10}H_7KN_6O$ [Same as the namesake monograph]

Peroxidase labeled anti-rabbit antibody It is prepared as follows: Immunize small animals with rabbit immunoglobulin G to obtain the antiserum. From the obtained antiserum the specific antibody is separated by the affinity chromatography using a column coupled with rabbit immunoglobulin G, and the specific antibody is labeled with peroxidase by the periodic acid method.

Peroxidase labeled anti-rabbit antibody TS Dissolve 0.10 g of bovine serum albumin in phosphate-buffered sodium chloride TS to make 100 mL. To 15 mL of this solution add 5 μL of peroxidase labeled anti-rabbit antibody. Prepare before use.

1,3-Phenylenediamine hydrochloride $C_6H_8N_2.2HCl$ A white or faintly reddish crystalline powder. It is colored to red or brown by light.
Identification—To 3 mL of a solution of 1,3-phenylenediamine hydrochloride (1 in 6000) add 0.5 mL of a solution of sodium nitrite (3 in 20,000), then add 2 to 3 drops of hydrochloric acid: a yellow color is produced.

(*S*)-1-Phenylethyl isocyanate $C_6H_5CH(CH_3)NCO$ Colorless to light yellow, clear liquid, having a characteristic odor.
Optical rotation <2.49> α_D^{20}: -8.5 – $11.5°$ (100 mm).
Specific gravity <2.56> d_4^{20}: 1.040 – 1.050

Phosphate buffer solution, pH 4.0 Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 4.0 with diluted phosphoric acid (1 in 10).

0.02 mol/L Phosphate buffer solution, pH 7.5 Dissolve 2.72 g of potassium dihydrogen phosphate in 900 mL of water, adjust to pH 7.5 with 0.2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Phosphate buffer solution for epoetin alfa Dissolve 0.247 g of sodium dihydrogen phosphate dihydrate, 0.151 g of disodium hydrogen phosphate decahydrate and 8.77 g of sodium chloride in water to make 1000 mL.

Pilocarpine hydrochloride for assay $C_{11}H_{16}N_2O_2.HCl$ [Same as the monograph Pilocarpine Hydrochloride. It

meets the following additional requirements.]

Purity Related substances—Dissolve 40 mg of pilocarpine hydrochloride for assay in 100 mL of phosphate buffer solution, pH 4.0 and use this solution as the sample solution. Pipet 1 mL of the sample solution, add phosphate buffer solution, pH 4.0 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 peaks, having the relative retention time of about 0.78 and about 0.92 to pilocarpine obtained from the sample solution, is not lager than 1/2 times the peak area of pilocarpine from the standard solution, the area of the peak, other than pilocarpine and the peaks mentioned above, is not larger than 1/5 times the peak area of pilocarpine from the standard solution, and the total area of the peaks other than pilocarpine is not larger than the peak area of pilocarpine from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operation conditions in the Assay under Pilocarpine Hydrochloride Tablets.

Time span of measurement: About 1.3 times as long as the retention time of pilocarpine, beginning after the solvent peak.

System suitability

Proceed as directed in the system suitability in the Purity under Pilocarpine Hydrochloride Tablets.

Polyacrylamide gel for epoetin alfa A polyacrylamide gel composed with the resolving gel 12.5% in acrylamide concentration.

Polyacrylamide gel for filgrastim A polyacrylamide gel composed with the resolving gel 15% in acrylamide concentration.

Polyacrylamide gel for nartograstim A polyacrylamide gel composed with the resolving gel 14% in acrylamide concentration.

Polysorbate 20 for epoetin beta A clear to slightly turbid, yellow-brown liquid.

Viscosity <*2.53*>: 300 – 500 mPa·s

Acid value <*1.13*>: not more than 3.

Saponification value <*1.13*>: 40 – 50

Hydroxyl value <*1.13*>: 95 – 110

Water <*2.48*>: not more than 5.0%.

Polyvinylidene fluoride membrane For Western blotting.

Potency measuring medium for nartograstim test Dissolve 10.4 g of RPMI-1640 medium in a suitable amount of water, add 16 mL of sodium hydrogen carbonate solution (3 in 40), then add water to make 1000 mL, adjust to pH 7.0 by passing carbon dioxide through the solution, and sterilize by filtration. To 90 mL of this solution add 10 mL of fetal bovine serum, previously heat at 56°C for 30 minutes, 1 mL of a solution dissolved 1.0×10^5 units of potassium benzylpenicillin and 0.1 g (potency) of streptomycin sulfate in 10 mL of isotonic sodium chloride solution, and add 5 μL of 2-mercaptoethanol solution (9 in 125). Sterilize this solution by filtration.

Primary antibody TS To a mixture of 1.5 mL of blocking TS for epoetin alfa and 13.5 mL of sodium azide-phosphate-buffered sodium chloride TS add a volume of mouse anti-epoetin alfa monoclonal antibody corresponding to 100 μg of protein, 50 μL of a solution of aprotinin containing 1×10^5 units in 5 mL of water and 100 μL of phenylmethylsulfonyl fluoride solution containing 1.74 mg in 100 mL of methanol.

Rabbit anti-nartograstim antibody Dissolve the antibody obtained from rabbit antiserum, prepared by immunizing with Nartograstim (Genetical Recombination), in tris-acetic acid buffer solution, pH 8.0, so that each mL contains 1 mg of rabbit anti-nartograstim antibody. Storage at −80°C.

Performance test: When perform the test by Ouchterlony method, a precipitation line is appeared against Nartograstim (Genetical Recombination).

Protein concentration: Determine the absorbance at 280 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and calculate the protein concentration using the specific absorbance $E^{1\%}_{1\,cm}$ 15.

Rabbit anti-nartograstim antibody TS To rabbit anti-nartograstim antibody add bovine serum albumin TS for nartograstim test so that each mL contains 0.2 μg of rabbit anti-nartograstim antibody. Prepare before use.

Reduction buffer solution for nartograstim sample Mix 0.8 mL of sodium lauryl sulfate solution (1 in 10), 0.5 mL of 0.5 mol/L tris buffer solution, pH 6.8, 0.4 mL of glycerin, 0.3 mL of 2-mercaptoethanol and 0.1 mL of bromophenol blue solution (1 in 200). Prepare before use.

Reference suspension 1 To 5.0 mL of formazin opalescence standard solution add 95.0 mL of water. Mix and shake before use.

Resazurin solution Prepared for the test for measurement of living cell.

Resorcinol-copper (II) sulfate TS Dissolve 0.1 g of resorcinol in 5 mL of water, add 125 μL of 0.1 mol/L copper (II) sulfate solution, 24 mL of hydrochloric acid, and add water to make 50 mL. Prepare this TS at least 4 hours before the time of use.

Scopoletin for thin-layer chromatography $C_{10}H_8O_4$

White or light brown, crystalline powder or powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 206°C.

Identification—(1) Determine the absorption spectrum of a solution of scopoletin for thin-layer chromatography in methanol (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 226 nm and 230 nm, between 295 nm and 299 nm and between

343 nm and 347 nm.

(2) Determine the infrared absorption spectrum of scopoletin 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 3340 cm^{-1}, 1702 cm^{-1}, 1566 cm^{-1}, 1436 cm^{-1} and 923 cm^{-1}.

Purity Related substances—Dissolve 1.0 mg of scopoletin for thin-layer chromatography in 10 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 5 μL each of the sample solution and standard solution as directed in the Identification under Artemisia Leaf: the spot other than the principal spot, having an *R*f value of about 0.4, obtained with the sample solution is not more intense than the spot obtained with the standard solution.

Secondary antibody TS To a mixture of 1.5 mL of blocking TS for epoetin alfa and 13.5 mL of sodium azide-phosphate-buffered sodium chloride TS, add 1 drop of biotinylated equine anti-mouse IgG antibody.

Sodium azide-phosphate-buffered sodium chloride TS Dissolve 8.0 g of sodium chloride, 0.2 g of potassium chloride, 2.9 g of disodium hydrogen phosphate dodecahydrate and 0.2 g of potassium dihydrogen phosphate in water to make 1000 mL. Dissolve 0.25 g of sodium azide in this solution.

Sodium cholate hydrate $C_{24}H_{39}O_5Na.H_2O$ A white powder.

Identification—Determine the infrared absorption spectrum of sodium cholate hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3400 cm^{-1}, 2940 cm^{-1}, 1579 cm^{-1}, 1408 cm^{-1} and 1082 cm^{-1}.

Water <2.48>: 3.5 – 5.0% (40 mg, coulometric titration).

Content: not less than 99.0% of sodium cholate ($C_{24}H_{39}O_5Na$), calculated on the anhydrous basis. Assay—Weigh accurately about 0.35 g of sodium cholate hydrate, 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
= 43.06 mg of $C_{24}H_{39}O_5Na$

Sodium fluoride-hydrochloric acid TS Dissolve 0.5 g of sodium fluoride in 100 mL of 0.5 mol/L hydrochloric acid TS. Prepare before use.

Sodium gluconate $C_6H_{11}NaO_7$ A white or pale yellowish brown, crystalline powder.

Purity Clarity and color of solution—A solution obtained by dissolving 1.0 g of sodium gluconate in 10 mL of water is clear and colorless or pale yellow.

5 mol/L Sodium hydroxide TS Dissolve 210 g of sodium hydroxide in water to make 1000 mL. Preserve in a polyethylene bottle.

Stachyose for thin-layer chromatography $C_{24}H_{42}O_{21}$ A white powder. Very soluble in water, and practically insoluble in ethanol (99.5). It is deliquescence with the atmospheric moisture.

Optical rotation <2.49> $[\alpha]_D^{20}$: + 144 – + 154° (50 mg calculated on the anhydrous basis, diluted ammonia solution (28) (1 in 1000), 5 mL, 100 mm).

Purity Related substances—Dissolve 2 mg of stachyose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), 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 2-propanol, water and methanol (3:2:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS to the plate, and heat at 105°C for 10 minutes: a spot other than the principle spot with an *R*f value of about 0.5 is not observed.

Subculture medium for nartograstim test Dissolve an amount of Nartograstim (Genetical Recombination), equivalent to 0.20 mg in 20 mL of phosphate-buffered sodium chloride TS. To 0.1 mL of this solution add 100 mL of potency measuring medium for nartograstim test.

Substrate TS for epoetin alfa Dissolve 30 mg of 4-chloro-1-naphthol in 10 ml of methanol, and use as Solution A. Mix 30 μL of hydrogen peroxide (30) and 50 mL of 0.02 mol/L tris buffer solution, pH 7.5, and use as Solution B. Mix Solutions A and B before use.

Succinic acid $C_4H_6O_4$ Colorless or white crystalline powder. Very soluble in hot water, soluble in water and in ethanol (99.5), and sparingly soluble in diethyl ether.

Melting point <2.60>: About 185°C.

Residue on ignition <2.44>: not more than 0.02% (1 g).

Content: not less than 99.5%. Assay—Weigh accurately about 1 g of succinic acid, dissolve in 50 mL of water, add 5 drops of phenolphthalein TS, and titrate <2.50> with 1 mol/L sodium hydroxide VS. Perform a blank titration in the same manner, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 59.05 mg of $C_4H_6O_4$

Sucrose for optical rotation $C_{12}H_{22}O_{11}$ [K 8383, Sucrose, Special class]

Sulfite oxidase One unit indicates an amount of the enzyme which consumes 1 μmol of oxygen in 1 minute at 25°C and pH 8.0 using sulfur dioxide and oxygen as the substrate.

Sulfite oxidase TS Suspend sulfite oxidase in ammonium sulfate TS so that each mL contains 2.5 units of the activity.

Storage—Between 0 and 8°C.

System suitability test solution for filgrastim Filgrastim (Genetical Recombination) containing about 2% charge

isomer.

Taltirelin hydrate for assay $C_{17}H_{23}N_7O_5.4H_2O$ [Same as the monograph Taltirelin Hydrate. It contains not less than 99.0% of taltirelin ($C_{17}H_{23}N_7O_5$), calculated on the anhydrous basis.]

Thermolysin It has the activity of 50 – 100 units per mg protein. Origin: *Bacillus thermoproteolyticus rokko*.

Tranilast for assay $C_{18}H_{17}NO_5$ [Same as the monograph Tranilast. When dried, it contains not less than 99.5% of tranilast ($C_{18}H_{17}NO_5$).]

Trichloroethylene C_2HCl_3 [K 8666, Special class]

Triethylamine for epoetin beta $(C_2H_5)_3N$ A clear and colorless liquid.

Specific gravity ⟨*2.56*⟩ d_4^{20}: 0.724 – 0.730

Water ⟨*2.48*⟩: not more than 0.2%.

Trifluoroacetic acid for epoetin beta CF_3COOH

A clear and colorless liquid.

Purity: When determine the absorbance of 50 vol% solution of trifluoroacetic acid for epoetin beta as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: not more than 0.10 at 270 nm, not more than 0.02 at 280 nm, and not more than 0.01 between 300 nm and 400 nm.

Triphenylmethane $C_{19}H_{16}$ A white to pale yellowish, crystalline powder.

Melting point ⟨*2.60*⟩: 93 – 95°C

Tris-acetic acid buffer solution, pH 8.0 Dissolve 1.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 800 mL of water, adjust to pH 8.0 with acetic acid (100), and add water to make 1000 mL.

0.02 mol/L Tris buffer solution, pH 7.4 Dissolve 2.4 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 800 mL of water, adjust to pH 7.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

0.02 mol/L Tris buffer solution, pH 7.5 Dissolve 2.4 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 29.2 g of sodium chloride in a suitable amount of water, adjust to pH 7.5 with hydrochloric acid, and add water to make 1000 mL.

0.1 mol/L Tris buffer solution, pH 7.3 Dissolve 2.42 g of 2-amino-2-hydroxymethyl-1,3-propanediol in a suitable amount of water, adjust to pH 7.3 with hydrochloric acid or 6 mol/L hydrochloric acid TS, and add water to make 200 mL.

0.5 mol/L Tris buffer solution, pH 8.1 Dissolve 12.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 160 mL of water, adjust to pH 8.1 with 1 mol/L hydrochloric acid TS, and add water to make 200 mL.

Tris-calcium chloride buffer solution, pH 6.5 Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 15 mg of calcium chloride dihydrate in 800 mL of water, adjust to pH 6.5 with dilute hydrochloric acid, and add water to make 1000 mL.

Tris-sodium chloride buffer solution, pH 8.0 Dissolve 2.42 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 1.64 g of sodium chloride in 900 mL of water, adjust to pH 8.0 with dilute hydrochloric acid, and add water to make 1000 mL.

Trypsin TS for epoetin alfa Dissolve 0.5 mg of trypsin for epoetin alfa liquid chromatography in 2.5 mL of water.

Trypsin for epoetin alfa liquid chromatography Bovine pancreas origin. It has not less than 180 units per mg, as 1 unit is equivalent to the amount of enzyme necessary to hydrolysis 1 μmol of *p*-toluenesulfonyl-L-arginine methyl ester per minute at 25°C, pH 8.2.

Umbelliferone for thin-layer chromatography $C_9H_6O_3$ White or light brown, powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 232°C.

Identification—(1) Determine the absorption spectrum of a solution of umbelliferone for thin-layer chromatography in methanol (1 in 300,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 214 nm and 218 nm, and between 322 nm and 326 nm.

(2) Determine the infrared absorption spectrum of umbelliferone 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 3160 cm^{-1}, 1681 cm^{-1}, 1604 cm^{-1}, 1323 cm^{-1}, 990 cm^{-1} and 903 cm^{-1}.

Purity Related substances – Dissolve 1.0 mg of umbelliferone for thin-layer chromatography in 10 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 5 μL each of the sample solution and standard solution as directed in the Identification under Artemisia Leaf: the spot other than the principal spot having an *R*f value of about 0.5 obtained with the sample solution is not more intense than the spot obtained with the standard solution.

Urea-EDTA TS Dissolve 48.0 g of urea and 0.2 g of disodium ethylenediamine tetraacetate dihydrate in 0.5 mol/L tris buffer solution, pH 8.1 to make 100 mL.

4-Vinylpyridine C_7H_7N A pale yellow to blackish brown liquid.

Refractive index ⟨*2.45*⟩ n_D^{20}: 1.5500 – 1.5530

Specific gravity ⟨*2.56*⟩ d_{20}^{20}: 0.9850 – 0.9880

Washing fluid for nartograstim test Dissolve 1 mL of polysorbate 20 in phosphate-buffered sodium chloride TS to make 1000 mL.

Water for ICP analysis See Inductively Coupled Plasma-Atomic Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry ⟨*2.63*⟩.

Delete the following:

Glucose-pepton medium for sterility test

Naringin dihydrate for thin-layer chromatography

9.42 Solid Supports/Column Packings for Chromatography

Add the following:

α_1-Acid glycoprotein binding silica gel for liquid chromatography Silica gel bond α_1-acid glycoprotein, prepared for liquid chromatography.

Butylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Gel type strong basic ion-exchange resin for liquid chromatography Prepared for liquid chromatography.

Phenylhexylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Porous styrene-divinylbenzene copolymer for gas chromatography (0.3 – 0.4 μm in mean pore size, not exceeding 50 m^2/g) Prepared for gas chromatography.

Strongly acidic ion-exchange non-porous resin for liquid chromatography Prepared for liquid chromatography.

Triacontylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Official Monographs

Add the following:

Aciclovir for Injection

注射用アシクロビル

Aciclovir 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 aciclovir ($C_8H_{11}N_5O_3$: 225.20).

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

Description Aciclovir for Injection occurs as white to pale yellowish white, light masses or powder.

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.

pH Being specified separately.

Purity Clarity and color of solution—Dissolve an amount of Aciclovir for Injection, equivalent to 0.25 g of Aciclovir, in 10 mL of water: the solution is clear and is not more colored than the following control solution.

Control solution: To 2.5 mL of Matching Fluid for Color F add diluted dilute hydrochloric acid (1 in 10) to make 100 mL.

Water <*2.48*> Not more than 7.5% (0.1 g, volumetric titration, direct titration).

Bacterial endotoxins <*4.01*> Less than 0.25 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 Weigh accurately the mass of the contents of not less than 10 Aciclovir for Injection. Weigh accurately an amount of the contents, equivalent to about 0.1 g of aciclovir ($C_8H_{11}N_5O_3$), and dissolve in dilute sodium hydroxide TS to make exactly 100 mL. Pipet 15 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. 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 20 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 15 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. 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.

$$\text{Amount (mg) of aciclovir } (C_8H_{11}N_5O_3) = M_S \times A_T/A_S \times 5$$

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

Containers and storage Containers—Hermetic containers.

Add the following:

Aciclovir Ointment

アシクロビル軟膏

Aciclovir Ointment contains 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 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.

Assay Weigh accurately an amount of Aciclovir Ointment, equivalent to about 10 mg of aciclovir ($C_8H_{11}N_5O_3$), add 25 mL of dilute sodium hydroxide TS, warm if necessary, and dissolve by shaking. After cooling, add water to make exactly 100 mL. Pipet 15 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 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 10 mL of this solution, and add 15 mL of dilute sodium hydroxide TS and water to make exactly 100 mL. Pipet 15 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 200 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 aciclovir ($C_8H_{11}N_5O_3$)
$= M_S \times A_T/A_S \times 1/2$

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

Containers and storage Containers—Tight containers.

Acrinol Hydrate

アクリノール水和物

Change the CAS registry number as follows:

[*6402-23-9*]

Acrinol and Zinc Oxide Oil

アクリノール・チンク油

Add the following next to the Japanese title:

Acrinol and Zinc Oxide Oil contains not less than 44.6% and not more than 54.4% of zinc oxide (ZnO: 81.38).

Change the Method of preparation as follows:

Method of preparation

Acrinol Hydrate, very finely powdered	10 g
Zinc Oxide Oil	990 g
To make	1000 g

Prepare by mixing the above ingredients. Acrinol Hydrate may be mixed after being dissolved in a little amount of warmed Purified Water or Purified Water in Containers. Instead of Zinc Oxide Oil adequate amounts of Zinc Oxide and vegetable oil may be used, and an adequate amount of Castor Oil or polysorbate 20 may be substituted for a part of the vegetable oil.

Add the following next to the Identification:

Assay Transfer about 0.8 g of well-mixed Acrinol and Zinc Oxide Oil, accurately weighed, to a crucible, heat, gradually raising the temperature until the mass is throughly charred, then strongly heat until the residue becomes yellow. After cooling, dissolve the residue by addition of 1 mL of water and 1.5 mL of hydrochloric acid, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add 80 mL of water, then add sodium hydroxide solution (1 in 50) until slightly precipitates appear, and add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7. Titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
$= 4.069$ mg of ZnO

Actinomycin D

アクチノマイシン D

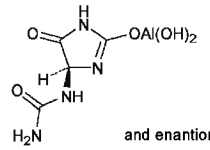
Change the Optical rotation as follows:

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -293 – $-329°$ (after drying, 10 mg, methanol, 10 mL, 100 mm).

Aldioxa

アルジオキサ

Change the structural formula, the chemical name, the Description, the Identification (1) and the Purity as follows:



and enantiomer

Dihydroxo[(4*RS*)-5-oxo-4-ureido-4,5-dihydro-1*H*-imidazol-2-yl]oxoaluminium

Description Aldioxa occurs as a white powder.

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

It dissolves in dilute hydrochloric acid.

A solution of Aldioxa in sodium fluoride-hydrochloric acid TS (1 in 100) shows no optical rotation.

Melting point: about 230°C (with decomposition).

Identification (1) Determine the infrared absorption spectrum of Aldioxa, 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.

Purity (1) Chloride <*1.03*>—To 0.10 g of Aldioxa add 6 mL of dilute nitric acid, boil to dissolve with shaking for 5 minutes, cool, and add water to make 50 mL. 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 0.142%).

(2) Heavy metals <*1.07*>—To 1.0 g of Aldioxa add 3 mL of hydrochloric acid and 3 mL of water, heat gently to boil with shaking, and evaporate on a water bath to dryness. To the residue add 30 mL of water, shake under warming, cool, filter, and to the filtrate add 2 mL of dilute acetic acid (31)

and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 3 mL of hydrochloric acid add 3 mL of water, evaporate on a water bath to dryness, and add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid (31) and water to make 50 mL (not more than 20 ppm).

Add the following:

Aldioxa Granules

アルジオキサ顆粒

Aldioxa Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of aldioxa ($C_4H_7AlN_4O_5$: 218.10).

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

Identification **(1)** 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 221 nm and 225 nm.

(2) To a quantity of powdered Aldioxa Granules, equivalent to 0.2 g of Aldioxa, add 10 mL of dilute hydrochloric acid, boil for 5 minutes, and filter: the cooled filtrate responds to the Qualitative Tests <*1.09*> for aluminum salt.

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

To the total content of 1 container of Aldioxa Granules add 80 mL of sodium fluoride-hydrochloric acid TS, shake for 20 minutes, add sodium fluoride-hydrochloric acid TS to make exactly 100 mL, and filter. Pipet V mL of the filtrate, add diluted ammonia-ammonium chloride buffer solution, pH 10.0 (1 in 10) to make exactly V' mL so that each mL contains about 20 μg of aldioxa ($C_4H_7AlN_4O_5$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of aldioxa ($C_4H_7AlN_4O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/25$

M_S: Amount (mg) of aldioxa 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 Aldioxa Granules is not less than 85%.

Start the test with an accurately weighed amount of Aldioxa Granules, equivalent to about 0.1 g of aldioxa ($C_4H_7AlN_4O_5$), 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 10 mL of the subsequent filtrate, add diluted ammonia-ammonium chloride buffer solution, pH 10.0 (1 in 10) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of aldioxa for assay, previously dried at 105°C for 2 hours, and dissolve in sodium fluoride-hydrochloric acid TS to make exactly 25 mL. Pipet 1 mL of this solution, add diluted ammonia-ammonium chloride buffer solution, pH 10.0 (1 in 10) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 223 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 aldioxa ($C_4H_7AlN_4O_5$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 360$

M_S: Amount (mg) of aldioxa for assay
M_T: Amount (g) of Aldioxa Granules
C: Labeled amount (mg) of aldioxa ($C_4H_7AlN_4O_5$) in 1 g

Assay Weigh accurately an amount of powdered Aldioxa Granules, equivalent to about 0.1 g of aldioxa ($C_4H_7AlN_4O_5$), add 80 mL of sodium fluoride-hydrochloric acid TS, shake for 20 minutes, add sodium fluoride-hydrochloric acid TS to make exactly 100 mL, and filter. Pipet 2 mL of the filtrate, add diluted ammonia-ammonium chloride buffer solution, pH 10.0 (1 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of aldioxa for assay, previously dried at 105°C for 2 hours, and dissolve in sodium fluoride-hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add diluted ammonia-ammonium chloride buffer solution, pH 10.0 (1 in 10) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 223 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of aldioxa ($C_4H_7AlN_4O_5$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of aldioxa for assay

Containers and storage Containers—Tight containers.

Add the following:

Aldioxa Tablets

アルジオキサ錠

Aldioxa Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of aldioxa ($C_4H_7AlN_4O_5$: 218.10).

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

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 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 Aldioxa Tablets add 80 mL of sodium fluoride-hydrochloric acid TS, shake for 20 minutes, add sodium fluoride-hydrochloric acid TS to make exactly 100 mL, and filter. Pipet V mL of the filtrate, add diluted ammonia-ammonium chloride buffer solution, pH 10.0 (1 in 10) to make exactly V' mL so that each mL contains about 20 μg of aldioxa ($C_4H_7AlN_4O_5$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of aldioxa ($C_4H_7AlN_4O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/25$

M_S: Amount (mg) of aldioxa 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 rates in 15 minutes of 50-mg tablet and in 30 minutes of 100-mg tablet are not less than 80% and not less than 70%, respectively.

Start the test with 1 tablet of Aldioxa 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 diluted ammonia-ammonium chloride buffer solution, pH 10.0 (1 in 10) to make exactly V' mL so that each mL contains about 22 μg of aldioxa ($C_4H_7AlN_4O_5$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of aldioxa for assay, previously dried at 105°C for 2 hours, and dissolve in sodium fluoride-hydrochloric acid TS to make exactly 25 mL. Pipet 1 mL of this solution, add diluted ammonia-ammonium chloride buffer solution, pH 10.0 (1 in 10) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 223 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 aldioxa ($C_4H_7AlN_4O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 72$

M_S: Amount (mg) of aldioxa for assay
C: Labeled amount (mg) of aldioxa ($C_4H_7AlN_4O_5$) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Aldioxa Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of aldioxa ($C_4H_7AlN_4O_5$), add 80 mL of sodium fluoride-hydrochloric acid TS, shake for 20 minutes, add sodium fluoride-hydrochloric acid TS to make exactly 100 mL, and filter. Pipet 2 mL of the filtrate, add diluted ammonia-ammonium chloride buffer solution, pH 10.0 (1 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of aldioxa for assay, previously dried at 105°C for 2 hours, and dissolve in sodium fluoride-hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add diluted ammonia-ammonium chloride buffer solution, pH 10.0 (1 in 10) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 223 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of aldioxa ($C_4H_7AlN_4O_5$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of aldioxa for assay

Containers and storage Containers—Tight containers.

Amiodarone Hydrochloride Tablets

アミオダロン塩酸塩錠

Change the Dissolution as follows:

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of acetic acid-sodium acetate buffer solution, pH 4.0 as the dissolution medium, the dissolution rate in 30 minutes of Amiodarone Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Amiodarone 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 exactly V mL of methanol, then add a mixture of the dissolution medium and methanol (1:1) to make exactly V' mL so that each mL contains about 11 μg of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of amiodarone hydrochloride for assay, previously dried at 50°C for 4 hours under reduced pressure not exceeding 0.3 kPa, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the dissolution medium, then add a mixture of the dissolution medium and methanol (1:1) 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 241 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a mixture of the dissolution medium and methanol (1:1) as the blank.

Dissolution rate (%) with respect to the labeled amount of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of amiodarone hydrochloride for assay
C: Labeled amount (mg) of amiodarone hydrochloride ($C_{25}H_{29}I_2NO_3$.HCl) in 1 tablet

Add the following:

Amlodipine Besilate Orally Disintegrating Tablets

アムロジピンベシル酸塩口腔内崩壊錠

Amlodipine Besilate Orally Disintegrating Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$: 567.05).

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

Identification To an amount of powdered Amlodipine Besilate Orally Disintegrating Tablets, equivalent to 7 mg of Amlodipine Besilate, add 200 mL of 0.01 mol/L hydrochloric acid-methanol TS, treat with ultrasonic waves, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 358 nm and 362 nm.

Purity Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and the mobile phase A (3:2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 30 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 0.45 to amlodipine obtained from the sample solution is not lager than the peak area of amlodipine from the standard solution, the area of the peak having the relative retention time of about 4.5 to amlodipine from the sample solution is not lager than 1.8 times the peak area of amlodipine from the standard solution, and the area of the peak having the relative retention time of about 0.16 to amlodipine and the peaks other than mentioned above from the sample solution is not lager than 2/5 times the peak area of amlodipine from the standard solution. Furthermore, the total area of the peaks other than amlodipine and the peak having the relative retention time of about 0.16 to amlodipine from the sample solution is not larger than 2.8 times the peak area of amlodipine from the standard solution. For these calculations, use the areas of the peaks having the relative retention time of about 0.45 and about 4.5 to amlodipine after multiplying by their relative response factors, 2.0 and 1.9, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 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: Dissolve 4.1 g of potassium dihydrogen phosphate in 1000 mL of water, adjust to pH 6.0 with a solution of 5.4 g of disodium hydrogen phosphate dodecahydrate in 500 mL of water. To 500 mL of this solution add 500 mL of methanol.

Mobile phase B: Dissolve 4.1 g of potassium dihydrogen phosphate in 1000 mL of water, adjust to pH 6.0 with a solution of 5.4 g of disodium hydrogen phosphate dodecahydrate in 500 mL of water. To 50 mL of this solution add 950 mL of methanol.

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	80	20
10 – 35	80 → 0	20 → 100
35 – 50	0	100

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

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

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add a mixture of methanol and the mobile phase A (3:2) to make exactly 50 mL. Confirm that the peak area of amlodipine obtained with 30 μL of this solution is equivalent to 14 to 26% of that with 30 μL of the standard solution.

System performance: When the procedure is run with 30 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 1.5, respectively.

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 peak area of amlodipine 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 Amlodipine Besilate Orally Disintegrating Tablets add $4V/5$ mL of a mixture of the mobile phase and methanol (1:1), disperse the particles with the aid of ultrasonic waves, add a mixture of the mobile phase and methanol (1:1) to make exactly V mL so that each mL of the solution contains about 0.14 mg of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$). Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of amlodipine besilate } (C_{20}H_{25}ClN_2O_5.C_6H_6O_3S) = M_S \times A_T/A_S \times V \times 1/250$$

M_S: Amount (mg) of Amlodipine Besilate RS, calculated

on the anhydrous basis

Disintegration Being specified separately.

Dissolution Being specified separately.

Assay Accurately weigh the mass of not less than 20 Amlodipine Besilate Orally Disintegrating Tablets, and powder them. Weigh accurately a portion of this powder, equivalent to about 7 mg of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), add 40 mL of a mixture of the mobile phase and methanol (1:1), disperse the particles with the aid of ultrasonic waves, and add a mixture of the mobile phase and methanol (1:1) to make exactly 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 35 mg of Amlodipine Besilate RS (separately, determine the water <2.48> in the same manner as Amlodipine Besilate), add 150 mL of a mixture of the mobile phase and methanol (1:1), dissolve with the aid of ultrasonic waves, then add a mixture of the mobile phase and methanol (1:1) to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, and determine the peak areas, A_T and A_S, of amlodipine from both solutions.

Amount (mg) of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$)
$= M_S \times A_T/A_S \times 1/5$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 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 4.1 g of potassium dihydrogen phosphate in 1000 mL of water, adjust to pH 6.0 with a solution of 5.4 g of disodium hydrogen phosphate dodecahydrate in 500 mL of water. To 400 mL of this solution add 600 mL of methanol.

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

System suitability—

System performance: When the procedure is run with 30 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 30 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%.

Containers and storage Containers—Tight containers.

Delete the following Monograph:

Amobarbital Sodium for Injection

注射用アモバルビタールナトリウム

Atorvastatin Calcium Hydrate

アトルバスタチンカルシウム水和物

Change the Description as follows:

Description Atorvastatin Calcium Hydrate occurs as a white to pale yellowish white crystalline powder.

It is very soluble in methanol, freely soluble in dimethylsulfoxide, and very slightly soluble in water and in ethanol (99.5).

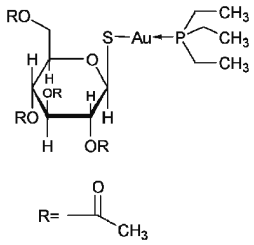
It gradually turns yellowish white on exposure to light.

It shows crystal polymorphism.

Add the following:

Auranofin

オーラノフィン



$C_{20}H_{34}AuO_9PS$: 678.48
(2,3,4,6-Tetra-*O*-acetyl-1-thio-*β*-D-glucopyranosato)(triethylphosphine)gold
[*34031-32-8*]

Auranofin, when dried, contains not less than 98.0% and not more than 102.0% of $C_{20}H_{34}AuO_9PS$.

Description Auranofin occurs as a white crystalline powder.

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

It shows crystal polymorphism.

Identification (1) To 50 mg of Auranofin add 3 mL of water, 3 mL of nitric acid and 3 mL of sulfuric acid, shake, and allow to stand: golden colored suspended matters are produced.

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

(3) Prepare the test solution with 1 mg of Auranofin as directed under Oxygen Flask Combustion Method <*1.06*>, using 10 mL of water as the absorbing liquid. Wash out the test solution into a Nessler tube with water to make 30 mL. Add 10 mL of dilute sulfuric acid, 3 mL of hexaammonium heptamolybdate-sulfuric acid TS and 0.1 mL of tin (II) chloride TS, shake, and allow to stand for 10 to 15 minutes: a blue color is developed.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -54.0 – $-62.0°$ (after drying, 0.2 g, methanol, 20 mL, 100 mm).

Melting point <*2.60*> 113 – 116°C

Purity **(1)** Chloride <*1.03*>—Put 0.5 g of Auranofin in a porcelain crucible, add 0.25 g of anhydrous sodium carbonate, mix well, and ignite until the carbonized substance is disappeared. After cooling, add 20 mL of water, heat, and filter after cooling. Wash the residue with 20 mL of water, combine the filtrate and the washings, neutralize with dilute nitric acid, then add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: Dissolve 0.25 g of anhydrous sodium carbonate in 20 mL of water, neutralize with dilute nitric acid, add 0.50 mL of 0.01 mol/L hydrochloric acid, 6 mL of dilute nitric acid, and water to make 50 mL (not more than 0.036%).

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Auranofin 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*>—Put 0.5 g of Auranofin in a Kjeldahl flask, add cautiously 2 mL of sulfuric acid and 5 mL of nitric acid, and heat until the solution becomes almost colorless. After cooling, add 15 mL of a saturated solution of ammonium oxalate monohydrate, heat until white fumes are evolved, and concentrate to 1 to 2 mL. Then, add 3 mL of water and 1 drop of methyl orange TS, neutralize with ammonia solution (28), filter, and perform the test using the filtrate as the test solution: the color is not darker than that of the following control solution.

Control solution: Heat a mixture of 2 mL of sulfuric acid and 5 mL of nitric acid until white fumes are no longer evolved. After cooling, add 15 mL of a saturated solution of ammonium oxalate monohydrate, heat until white fumes are evolved, and concentrate to 1 to 2 mL. Add 3 mL of water and 1 drop of methyl orange TS, neutralize with ammonia solution (28), and filter. To the filtrate add 2.0 mL of Standard Arsenic Solution, then proceed in the same manner as for the test solution (not more than 4 ppm).

(4) Related substances—Dissolve 50 mg of Auranofin in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 100 mL. To exactly 3 mL of this solution add chloroform 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*>. 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 chloroform and acetone (4:1) to a distance of about 10 cm, and air-dry the plate. Dry, furthermore, at 80°C for 30 minutes. After cooling, allow the plate to stand in a iodine vapor for 30 minutes: the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution.

(5) Residual solvent Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 20 mg each of Auranofin and Auranofin RS, both previously dried, dissolve each in 10 mL of a mixture of water and acetonitrile (1:1), and add exactly 5 mL each of the internal standard solution. Then add a mixture of water and acetonitrile (1:1) 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 auranofin to that of the internal standard.

$$\text{Amount (mg) of } C_{20}H_{34}AuO_9PS = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Auranofin RS

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of water and acetonitrile (1:1) (3 in 1250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 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 sodium dihydrogen phosphate dihydrate solution (1 in 100), tetrahydrofuran and acetonitrile (12:5:3).

Flow rate: Adjust the flow rate so that the retention time of auranofin 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, auranofin and the internal standard 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 ratio of the peak area of auranofin to that of the internal standard is

not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Auranofin Tablets

オーラノフィン錠

Auranofin Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of auranofin ($C_{20}H_{34}AuO_9PS$: 678.48).

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

Identification Put an amount of powdered Auranofin Tablets, equivalent to 11 mg of Auranofin, in a porcelain crucible, and heat weakly to carbonize. After cooling, add 2 mL of nitric acid and 5 drops of sulfuric acid, heat cautiously at first then incinerate by ignition. After cooling, add 4 mL of aqua regia to the residue, dissolve by warming, and add 16 mL of water. To 5 mL of this solution add 0.5 mL of tin (II) chloride TS: a purple to red-brown color is developed.

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 Auranofin Tablets add 2 mL of water, disintegrate the tablet with the aid of ultrasonic waves, add exactly 2 mL of the internal standard solution for every 3 mg of auranofin ($C_{20}H_{34}AuO_9PS$), and add 2 mL of a mixture of water and acetonitrile (1:1). Shake for 15 minutes, then add a mixture of water and acetonitrile (1:1) to make V mL so that each mL contains 0.3 mg of auranofin ($C_{20}H_{34}AuO_9PS$), centrifuge, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of auranofin ($C_{20}H_{34}AuO_9PS$)
$= M_S \times Q_T/Q_S \times V/100$

M_S: Amount (mg) of Auranofin RS

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (9 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 15 minutes is not less than 85%.

Start the test with 1 tablet of Auranofin 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 3.3 μg of auranofin ($C_{20}H_{34}AuO_9PS$), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Auranofin RS, previously dried at 105°C for 3 hours, and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. 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*> under the following conditions, and determine the peak areas, A_T and A_S, of auranofin from both solutions.

Dissolution rate (%) with respect to the labeled amount of auranofin ($C_{20}H_{34}AuO_9PS$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of Auranofin RS
C: Labeled amount (mg) of auranofin ($C_{20}H_{34}AuO_9PS$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay under Auranofin.
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 auranofin are not less than 5000 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 auranofin is not more than 1.0%.

Assay Accurately weigh the mass of not less than 20 Auranofin Tablets, and powder them. Weigh accurately a portion of the powder, equivalent to about 60 mg of auranofin ($C_{20}H_{34}AuO_9PS$), add 40 mL of water, disperse the particles with the aid of ultrasonic waves, then add exactly 40 mL of the internal standard solution, add 40 mL of a mixture of water and acetonitrile (1:1), and shake for 15 minutes. To this solution add a mixture of water and acetonitrile (1:1) to make 200 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 30 mg of Auranofin RS, previously dried at 105°C for 3 hours, dissolve in 60 mL of a mixture of water and acetonitrile (1:1), add exactly 20 mL of the internal standard solution, then add water 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 auranofin to that of the internal standard.

Amount (mg) of auranofin ($C_{20}H_{34}AuO_9PS$)
$= M_S \times Q_T/Q_S \times 2$

M_S: Amount (mg) of Auranofin RS

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (9 in 10,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Auranofin.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, auranofin and the internal standard 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 ratio of the peak area of auranofin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Azathioprine Tablets

アザチオプリン錠

Add the following next to the Uniformity of dosage units:

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 Azathioprine Tablets is not less than 80%.

Start the test with 1 tablet of Azathioprine 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, add water to make exactly V' mL so that each mL contains about 11 μg of azathioprine ($C_9H_7N_7O_2S$), and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Azathioprine RS, previously dried at 105°C for 5 hours, and dissolve in 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 280 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 azathioprine ($C_9H_7N_7O_2S$)

$= M_S \times A_T/A_S \times V'/V \times 1/C \times 108$

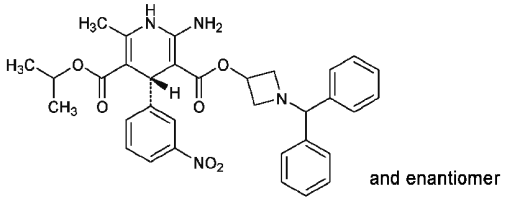
M_S: Amount (mg) of Azathioprine RS

C: Labeled amount (mg) of azathioprine ($C_9H_7N_7O_2S$) in 1 tablet

Add the following:

Azelnidipine

アゼルニジピン



$C_{33}H_{34}N_4O_6$: 582.65

3-[1-(Diphenylmethyl)azetidin-3-yl] 5-(1-methylethyl) (4*RS*)-2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate

[*123524-52-7*]

Azelnidipine contains not less than 99.0% and not more than 101.0% of $C_{33}H_{34}N_4O_6$, calculated on the dried basis.

Description Azelnidipine occurs as a light yellow to yellow, crystalline powder or powder containing masses.

It is freely soluble in ethanol (99.5) and in acetic acid (100), and practically insoluble in water.

A solution of Azelnidipine in ethanol (99.5) (1 in 100) shows no optical rotation.

Azelnidipine shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Azelnidipine in ethanol (99.5) (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.

(2) Determine the infrared absorption spectrum of Azelnidipine 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.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Azelnidipine according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Azelnidipine in a mixture of acetonitrile and water (4:1) to make 100 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add a mixture of acetonitrile and water (4:1) 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. Determine each peak area by the automatic integration method: the areas of the peak, having the relative retention time of about 0.50 and about 1.42 to azelnidipine, obtained from the sample solution are not larger than 1/5 times and 3/10 times the peak area of azelnidipine from

the standard solution, respectively, the area of the peak other than azelnidipine and the peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of azelnidipine from the standard solution, and the total area of the peaks other than azelnidipine from the sample solution is not larger than 7/10 times the peak area of azelnidipine 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 octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Dissolve 1.05 g of potassium dihydrogen phosphate in 350 mL of water, add 650 mL of a mixture of acetonitrile and methanol (7:3), and adjust to pH 5.5 with diluted phosphoric acid (1 in 10).

Flow rate: Adjust the flow rate so that the retention time of azelnidipine is about 36 minutes.

Time span of measurement: About 2 times as long as the retention time of azelnidipine, 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 (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 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 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 azelnidipine is not more than 1.0%.

(3) Residual solvent Being specified separately.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, in vacuum, 70°C, 5 hours).

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

Assay Weigh accurately about 0.4 g of Azelnidipine, 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
= 29.13 mg of $C_{33}H_{34}N_4O_6$

Containers and storage Containers—Tight containers.

Aztreonam

アズトレオナム

Change the Purity as follows:

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Aztreonam in 20 mL of dimethylsulfoxide: the solution is clear, and its absorbance at 420 nm, determined as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, is not more than 0.06.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Aztreonam 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) Related substances—Dissolve 40 mg of Aztreonam in 100 mL of water, 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. 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 each peak area of both solutions by the automatic integration method: the area of the peak other than aztreonam obtained from the sample solution is not larger than the peak area of aztreonam from the standard solution, and the total area of peaks other than aztreonam from the sample solution is not larger than 2.5 times the peak area of aztreonam 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: 254 nm).

Time span of measurement: About 4 times as long as the retention time of aztreonam beginning after the solvent peak.

System suitability—

Test for required detectability: To 5 mL of the standard solution add water to make 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of aztreonam obtained from 25 μL of this solution is equivalent to 7 to 13% of that obtained from 25 μL of the solution for system suitability test.

System performance: When the procedure is run under the above operating conditions with 25 μL of the standard solution obtained in the Assay, the internal standard and aztreonam 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 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of aztreonam is not more than 2.0%.

Benzyl Alcohol

ベンジルアルコール

Change the Assay as follows:

Assay Weigh accurately about 0.9 g of Benzyl Alcohol, add exactly 15 mL of a freshly prepared mixture of anhydrous pyridine and acetic anhydride (7:1), and heat on a water bath under a reflux condenser for 30 minutes. Cool, add 25 mL of water, and titrate <*2.50*> the excess acetic acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 108.1 mg of C_7H_8O

Bisoprolol Fumarate Tablets

ビソプロロールフマル酸塩錠

Add the following next to the Identification:

Purity Related substances—This is applied to 0.625-mg tablets. Shake vigorously for 10 minutes a portion of powdered Bisoprolol Fumarate Tablets, equivalent to 5 mg of Bisoprolol Fumarate, with exactly 20 mL of a mixture of water and acetonitrile (3:1), filter 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. 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 amount of the peak other than bisoprolol and the peak having the relative retention time of about 0.8 to bisoprolol by the area percentage method: the amount of the two peaks, having relative retention time of about 1.2 and about 3.8 to bisoprolol, are not more than 1.0%, respectively, the amount of the peak other than the peaks mentioned above is not more than 0.2%, and the total amount of the peaks other than bisoprolol is not more than 2.5%. For this calculation, use the area of the peak, having the relative retention time of about 1.2 to bisoprolol, after multiplying by the relative response factor 5.

Operating conditions—

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

Mobile phase: Dissolve 4.08 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 2.5 with phosphoric acid. To 750 mL of this solution add 250 mL of acetonitrile.

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

System suitability—

Test for required detectability: To 1 mL of the sample solution add a mixture of water and acetonitrile (3:1) to make 100 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add a mixture of water and acetonitrile (3:1) to make exactly 20 mL. Confirm that the peak area of bisoprolol obtained with 20 μL of this solution is equivalent to 7 to 13% of that 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 bisoprolol 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of bisoprolol is not more than 1.5%.

Change the Uniformity of dosage units and Dissolution 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.

Take 1 tablet of Bisoprolol Fumarate Tablets, disintegrate by adding 8 mL of water, and add water to make exactly 10 mL, and then filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 3 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 62.5 μg of bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4]$, and use as the sample solution. Separately, weigh accurately about 20 mg of bisoprolol fumarate for assay, previously dried under reduced pressure at 80°C for 5 hours, using phosphorus (V) oxide as a dessicant, and dissolve in water to make exactly 200 mL. Pipet 15 mL of this solution, add water to make exactly 25 mL, and use as the standard solution. Determine the absorbances, A_T and A_S, at 271.5 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4]$
$= M_S \times A_T/A_S \times V'/V \times 3/100$

M_S: Amount (mg) of bisoprolol fumarate 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 Bisoprolol Fumarate Tablets is not less than 85%.

Start the test with 1 tablet of Bisoprolol Fumarate 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 0.7 μg of bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4]$, and use this solution as

the sample solution. Separately, weigh accurately about 14 mg of bisoprolol fumarate for assay, previously dried in vacuum at 80°C for 5 hours, using phosphorus (V) oxide as a dessicant, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 1 mL of this solution, add the dissolution medium to make exactly 200 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*>, and determine the bisoprolol peak areas, A_T and A_S, of both solutions.

Dissolution rate (%) with respect to the labeled amount of bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4]$

$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/2$

M_S: Amount (mg) of bisoprolol fumarate for assay

C: Labeled amount (mg) of bisoprolol fumarate $[(C_{18}H_{31}NO_4)_2.C_4H_4O_4]$ in 1 tablet

Operating conditions—

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

Mobile phase: Dissolve 4.08 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 2.5 with phosphoric acid. To 750 mL of this solution add 250 mL of acetonitrile.

System suitability—

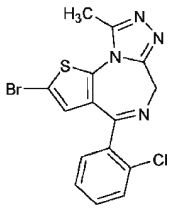
System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bisoprolol are not less than 3000 and not more than 2.0, respectively.

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 bisoprolol is not more than 2.0%.

Add the following:

Brotizolam

ブロチゾラム



$C_{15}H_{10}BrClN_4S$: 393.69

2-Bromo-4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine [*57801-81-7*]

Brotizolam, when dried, contains not less than 98.5% and not more than 101.0% of $C_{15}H_{10}BrClN_4S$.

Description Brotizolam occurs as a white or pale yellowish crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Brotizolam 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.

(2) Determine the infrared absorption spectrum of Brotizolam 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*> 208 – 212°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Brotizolam 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 Brotizolam in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add acetonitrile to make exactly 100 mL. Pipet 1 mL of this solution, add acetonitrile to make exactly 10 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. Determine each peak area by the automatic integration method: each peak area other than brotizolam from the sample solution is not larger than 1/2 times the peak area of brotizolam from the standard solution, and the total area of the peaks other than the peak of brotizolam from the sample solution is not larger than the peak area of brotizolam from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 242 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 40°C.

Mobile phase A: Dissolve 1.84 g of sodium 1-heptanesulfonate in 1000 mL of water.

Mobile phase B: Dissolve 0.46 g of sodium 1-heptanesulfonate in 250 mL of water and 750 mL of acetonitrile.

Flowing of 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 – 4	63	37
4 – 15	63 → 12	37 → 88

Flow rate: About 2 mL per minute.

Time span of measurement: About 2 times as long as the retention time of brotizolam, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of brotizolam obtained with 5 μL of this solution is equivalent to 18 to 32% of that 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, the number of theoretical plates and the symmetry factor of the peak of brotizolam are not less than 5000 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 brotizolam is not more than 2.0%.

(3) Residual solvent Being specified separately.

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 0.15 g of Brotizolam, previously dried, dissolve in 75 mL of a mixture of acetic anhydride and acetic acid (100) (2:1), 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
= 19.68 mg of $C_{15}H_{10}BrClN_4S$

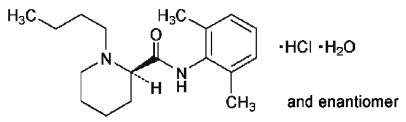
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Bupivacaine Hydrochloride Hydrate

ブピバカイン塩酸塩水和物



$C_{18}H_{28}N_2O.HCl.H_2O$: 342.90

(2*RS*)-1-Butyl-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide monohydrochloride monohydrate

[*14252-80-3*]

Bupivacaine Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of bupivacaine hydrochloride ($C_{18}H_{28}N_2O.HCl$: 324.89), calculated on the anhydrous basis.

Description Bupivacaine Hydrochloride Hydrate occurs as a white crystalline powder.

It is freely soluble in acetic acid (100), and soluble in water, in methanol and in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of 0.5 g of Bupivacaine Hydrochloride Hydrate in 50 mL of a mixture of ethanol (99.5), water and 5 mol/L sodium hydroxide TS (34:15:1) shows no optical rotation.

Melting point: about 252°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Bupivacaine Hydrochloride Hydrate in 0.01 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 Bupivacaine 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 Bupivacaine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests ⟨*1.09*⟩ for chloride.

pH ⟨*2.54*⟩ The pH of a solution obtained by dissolving 1.0 g of Bupivacaine Hydrochloride Hydrate in 100 mL of freshly boiled and cooled water is between 4.5 to 6.0.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Bupivacaine Hydrochloride Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Bupivacaine 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 20 ppm).

(3) 2,6-Dimethylaniline—Dissolve exactly 0.50 g of Bupivacaine Hydrochloride Hydrate in 10 mL of methanol. To 2 mL of this solution add 1 mL of a freshly prepared solution of 4-dimethylaminobenzaldehyde in methanol (1 in 100) and 2 mL of acetic acid (100), and allow to stand for 10 minutes: the color of the solution is not more colored than the following control solution.

Control solution: Prepare by proceeding in the same manner as above, using 2 mL of a solution of 2,6-dimethylaniline in methanol (1 in 200,000).

(4) Related substances—Dissolve 50 mg of Bupivacaine Hydrochloride Hydrate in 2.5 mL of water, add 2.5 mL of 2 mol/L sodium hydroxide TS and 5 mL of the internal standard, shake, collect the lower layer, filter, and use the filtrate as the sample solution. Pipet 1 mL of the sample solution, and add the internal standard solution to make exactly 100 mL. Pipet 1 mL of this solution, add the internal standard solution to make exactly 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 determine each peak area by the automatic integration method: the ratio of the area of the peak other than bupivacaine to the peak area of the internal standard obtained from the sample solution is not larger than the ratio of the peak area of bupivacaine to that of the internal standard from the standard solution.

Internal standard solution—A solution of methyl behenate in dichloromethane (1 in 20,000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A quartz tube 0.32 mm in inside diameter and 30 m in length, coated the inside surface with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography 0.25 μm in thickness.

Column temperature: Rise the temperature from 180°C to 230°C at the rate of 5°C per minute, and maintain at 230°C for 5 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 bupivacaine is about 10 minutes.

Split ratio: 1:12.

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

System suitability—

System performance: To 1 mL of the sample solution add the internal standard solution to make 100 mL, and use this solution as the solution for system suitability test. When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, bupivacaine 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 6 times with 1 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratio of the peak area of bupivacaine to that of the internal standard is not more than 2.0%.

(5) Residual solvent Being specified separately.

Water <*2.48*> 4.0 – 6.0% (0.25 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Bupivacaine Hydrochloride Hydrate, dissolve in 20 mL of acetic acid (100), add 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
= 32.49 mg of $C_{18}H_{28}N_2O.HCl$

Containers and storage Containers—Tight containers.

Butyl Parahydroxybenzoate

パラオキシ安息香酸ブチル

Change after the Description as follows:

◆**Description** Butyl Parahydroxybenzoate occurs as colorless crystals or white, crystalline powder.

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

Identification Determine the infrared absorption spectrum of Butyl Parahydroxybenzoate 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 Butyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 68 – 71°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Butyl Parahydroxybenzoate in ethanol (95) to make 10 mL: the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Acidity—To 2 mL of the solution of Butyl Parahydroxybenzoate obtained in (1) add 3 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mol/L sodium hydroxide VS until the solution shows a blue color: the volume of 0.1 mol/L sodium hydroxide VS used does not exceed 0.1 mL.

◆**(3)** Heavy metals <*1.07*>—Dissolve 1.0 g of Butyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute

acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).◆

(4) Related substances—Dissolve 50 mg of Butyl Parahydroxybenzoate in 2.5 mL of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 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 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 parahydroxybenzoic acid having a relative retention time of about 0.1 to butyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of butyl parahydroxybenzoate from the standard solution (0.5%). For this calculation use the peak area of parahydroxybenzoic acid after multiplying by the relative response factor, 1.4. Furthermore, the area of the peak other than butyl parahydroxybenzoate and parahydroxybenzoic acid from the sample solution is not larger than the peak area of butyl parahydroxybenzoate from the standard solution (0.5%), and the total area of the peaks other than butyl parahydroxybenzoate and parahydroxybenzoic acid is not larger than 2 times the peak area of butyl parahydroxybenzoate from the standard solution (1.0%). For this calculation the peak area not larger than 1/5 times the peak area of butyl parahydroxybenzoate from the standard solution is excluded (0.1%).

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 butyl parahydroxybenzoate.

System suitability—

◆Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of butyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.◆

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

◆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 2.0%.◆

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Butyl Parahydroxybenzoate and Butyl Parahydroxybenzoate RS, dissolve separately in 2.5 mL each of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase 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 butyl parahydroxybenzoate of each solution.

$$\text{Amount (mg) of butyl parahydroxybenzoate } (C_{11}H_{14}O_3) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Butyl Parahydroxybenzoate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 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 35°C.

Mobile phase: A mixture of methanol and potassium dihydrogen phosphate solution (17 in 2500) (1:1).

Flow rate: 1.3 mL per minute.

System suitability—

System performance: Dissolve 5 mg each of Butyl Parahydroxybenzoate, propyl parahydroxybenzoate and parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the solution for system suitability test (1). Separately, dissolve 5 mg of isobutyl parahydroxybenzoate in the mobile phase to make exactly 100 mL. Pipet 0.5 mL of this solution, add the standard solution to make exactly 50 mL, and use this solution as the solution for system suitability test (2). When the procedure is run with 10 μL each of the solution for system suitability test (1) and (2) under the above operating conditions, parahydroxybenzoic acid, propyl parahydroxybenzoate, isobutyl parahydroxybenzoate and butyl parahydroxybenzoate are eluted in this order, the relative retention times of parahydroxybenzoic acid, propyl parahydroxybenzoate and isobutyl parahydoxybenzoate to butyl parahydroxybenzoate are about 0.1, about 0.5 and about 0.9, respectively, the resolution between the peaks of propyl parahydroxybenzoate and butyl parahydroxybenzoate is not less than 5.0, and the resolution between the peaks of isobutyl parahydroxybenzoate and butyl parahydroxybenzoate 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 butyl parahydroxybenzoate is not more than 0.85%.

◆**Containers and storage** Containers—Well-closed containers.◆

Calcium Pantothenate

パントテン酸カルシウム

Change to read except the structural formula and chemical name:

Calcium Pantothenate contains not less than 98.0% and not more than 102.0% of $C_{18}H_{32}CaN_2O_{10}$, calculated on the dried basis.

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.

Identification **(1)** Determine the infrared absorption spectrum of previously dried Calcium Pantothenate 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 dried Calcium Pantothenate RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample and the Reference Standard separately in water, evaporate water, dry the residues in vacuum for 24 hours using silica gel as a desiccant, and perform the test using these residues.

(2) A solution of Calcium Pantothenate (1 in 10) responds to the Qualitative Tests <*1.09*> (1), (2) and (3) for calcium salt.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +25.0 – +28.5° (1 g calculated on the dried basis, water, 20 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Calcium Pantothenate according to Method 1, 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 0.30 g of Calcium Pantothenate in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water 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 time of about 0.6 to pantothenic acid is not larger than 1.2 times the peak area of pantothenic acid from the standard solution, the area of the peak, having the relative retention time of about 0.8 is not larger than the peak area of pantothenic acid from the standard solution, the area of the peak, having the relative retention time of about 1.5 is not larger than 3/5 times the peak area of pantothenic acid from the standard solution, and the area of the peak other than pantothenic acid and the peaks mentioned above is not larger than 3/10 times the peak area of pantothenic acid from the standard solution. Additionally, the total area of the peaks other than pantothenic acid is not larger than 2.4 times the peak area of pantothenic acid from the standard solution. For these calculations use the areas of the peaks, having the relative retention time of about 0.6 and about 0.8 to pantothenic acid, after multiplying by their relative response factors, 19 and 13, respectively.

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 pantothenic acid, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 10 mL. Confirm that the peak area of pantothenic acid obtained with 10 μL of this solution is equivalent to 14 – 26% of that 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 pantothenic acid are not less than 10,000 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 pantothenic acid is not more than 2.0%.

(3) Alkaloids—Dissolve 50 mg of Calcium Pantothenate in 5 mL of water, add 0.5 mL of hexaammonium heptamolybdate TS and 0.5 mL of a solution of phosphoric acid (1 in 10): no white turbidity is produced.

Loss on drying <*2.41*> Not more than 5.0% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 20 mg each of Calcium Pantothenate and Calcium Pantothenate RS (separately determine the loss on drying <*2.41*> in the same conditions as Calcium Pantothenate), dissolve each 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 pantothenic acid.

Amount (mg) of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Calcium Pantothenate RS, calculated on the dried basis

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 40°C.

Mobile phase: Dissolve 0.81 g of sodium 1-heptanesulfonate and 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 2.1 with phosphoric acid. To 980 mL of this solution add 10 mL of acetonitrile and 10 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of pantothenic acid 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 pantothenic acid are not less than 10,000 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 pantothenic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Anhydrous Dibasic Calcium Phosphate

無水リン酸水素カルシウム

Change the Purity (2), (3) and Assay as follows:

Purity

(2) Chloride—To 0.20 g of Anhydrous Dibasic Calcium Phosphate add 20 mL of water and 13 mL of dilute nitric acid, dissolve by warming, if necessary, add water to make 100 mL, and filter, if necessary. Put 50 mL of this solution in a Nessler tube, and use this as the test solution. Transfer 0.70 mL of 0.01 mol/L hydrochloric acid VS to another Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution. Add 1 mL of silver nitrate TS to the test solution and the control solution, mix well, and allow to stand for 5 minutes protecting from light. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely. The opalescence developed in the test solution is not more than that of the control solution. (not more than 0.25%)

(3) Sulfate—Dissolve 0.50 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of water and 5 mL of dilute hydrochloric acid, add water to make 100 mL, and filter, if necessary. Put 20 mL of this solution in a Nessler tube, add 1 mL of dilute hydrochloric acid, and add water to make 50 mL, and use this as the test solution. Transfer 1.0 mL of 0.005 mol/L sulfuric acid VS to another Nessler tube, add 1 mL of dilute hydrochloride acid and water to make 50 mL, and use this solution as the control solution. Add 2 mL of barium chloride TS to the test solution and the control solution, mix well, and allow to stand for 10 minutes. Compare the white turbidity produced in both solutions against a black background by viewing downward or transversely. The turbidity produced in the test solution is not thicker than that of the control solution. (not more than 0.48%)

Assay Weigh accurately about 0.4 g of Anhydrous Dibasic Calcium Phosphate, dissolve in 12 mL of dilute hydrochloric acid by warming on a water bath, if necessary, and add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <*2.50*> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.02 mol/L zinc sulfate VS (indicator: 25 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination in the same manner.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.721 mg of $CaHPO_4$

Dibasic Calcium Phosphate Hydrate

リン酸水素カルシウム水和物

Change the Purity (2), (3) and Assay as follows:

Purity

(2) Chloride—To 0.20 g of Dibasic Calcium Phosphate Hydrate add 20 mL of water and 13 mL of dilute nitric acid, dissolve by warming, if necessary, add water to make 100 mL, and filter, if necessary. Put 50 mL of this solution in a Nessler tube, and use this as the test solution. Transfer 0.70 mL of 0.01 mol/L hydrochloric acid VS to another Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution. Add 1 mL of silver nitrate TS to the test solution and the control solution, mix well, and allow to stand for 5 minutes protecting from light. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely. The opalescence developed in the test solution is not more than that of the control solution. (not more than 0.25%)

(3) Sulfate—Dissolve 0.50 g of Dibasic Calcium Phosphate Hydrate in 5 mL of water and 5 mL of dilute hydrochloric acid, add water to make 100 mL, and filter, if necessary. Put 20 mL of this solution in a Nessler tube, add 1 mL of dilute hydrochloric acid, and add water to make 50 mL, and use this as the test solution. Transfer 1.0 mL of 0.005 mol/L sulfuric acid VS to another Nessler tube, add 1 mL of dilute hydrochloride acid and water to make 50 mL, and use this solution as the control solution. Add 2 mL of barium chloride TS to the test solution and the control solution, mix well, and allow to stand for 10 minutes. Compare the white turbidity produced in both solutions against a

black background by viewing downward or transversely. The turbidity produced in the test solution is not thicker than that of the control solution. (not more than 0.48%)

Assay Weigh accurately about 0.4 g of Dibasic Calcium Phosphate Hydrate, dissolve in 12 mL of dilute hydrochloric acid by warming on a water bath, if necessary, and add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.02 mol/L zinc sulfate VS (indicator: 25 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination in the same manner.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 3.442 mg of $CaHPO_4.2H_2O$

Candesartan Cilexetil

カンデサルタン シレキセチル

Change the Description as follows:

Description Candesartan Cilexetil occurs as white crystals or a white crystalline powder.

It is soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

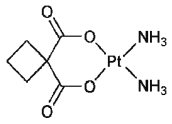
A solution of Candesartan Cilexetil in methanol (1 in 100) shows no optical rotation.

Candesartan Cilexetil shows crystal polymorphism.

Add the following:

Carboplatin

カルボプラチン



$C_6H_{12}N_2O_4Pt$: 371.25
(*SP*-4-2)-Diammine[cyclobutan-1,1-dicarboxylato(2-)-*O*,*O'*]platinum
[*41575-94-4*]

Carboplatin contains not less than 98.5% and not more than 101.0% of $C_6H_{12}N_2O_4Pt$, calculated on the dried basis.

Description Carboplatin occurs as white crystals or crystalline powder.

It is sparingly soluble in water, and very slightly soluble in ethanol (99.5).

Melting point: about 200°C (with decomposition).

Identification **(1)** To 2 mL of a solution of Carboplatin (1 in 100) add 2 to 3 drops of diluted tin (II) chloride TS (1 in 15), and allow to stand for 30 minutes: a yellowish brown precipitate is formed.

(2) Determine the infrared absorption spectrum of Carboplatin 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 Carboplatin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 0.10 g of Carboplatin in 10 mL of water: the pH of this solution is 5.0 to 7.0.

Purity **(1)** 1,1-Cyclobutanedicarboxylic acid—Weigh accurately about 40 mg of Carboplatin, dissolve in the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of 1,1-cyclobutanedicarboxylic acid, and dissolve in the mobile phase to make exactly 100 mL. Pipet 4 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 25 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 1,1-cyclobutanedicarboxylic acid of these solutions, and calculate the amount of 1,1-cyclobutanedicarboxylic acid by the following formula: it is not more than 0.2%.

Amount (%) of 1,1-cyclobutanedicarboxylic acid
$= M_S/M_T \times A_T/A_S \times 8/5$

M_S: Amount (mg) of 1,1-cyclobutanedicarboxylic acid
M_T: Amount (mg) of Carboplatin

Operating conditions—

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

Column: A stainless steel column 4.0 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 8.5 g of tetrabutylammonium hydrogensulfate in 80 mL of water, add 3.4 mL of phosphoric acid, and adjust to pH 7.5 with a solution of sodium hydroxide (43 in 100). To 10 mL of this solution add 430 mL of water and 60 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of 1,1-cyclobutanedicarboxylic acid is about 5 minutes.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of 1,1-cyclobutanedicarboxylic acid obtained with 25 μL of this solution is equivalent to 14 to 26% of that with 25 μL of the standard solution.

System performance: Dissolve 25 mg each of 1,1-cy-

clobutanedicarboxylic acid and cyclobutanecarboxylic acid in 100 mL of water. To 10 mL of this solution add the mobile phase to make 25 mL. When the procedure is run with 25 μL of this solution under the above operating conditions, cyclobutanecarboxylic acid and 1,1-cyclobutanedicarboxylic acid are eluded in this order with the resolution between these peaks being not less than 3.

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 1,1-cyclobutanedicarboxylic acid is not more than 2.0%.

(2) Related substances—Dissolve 25 mg of Carboplatin in 25 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, 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 0.8 to carboplatin, is not more than 0.25%, the amount of the peak other than carboplatin and the peak mentioned above is not more than 0.1%, and the total amount of these peaks other than carboplatin is not more than 0.5%.

Operating conditions—

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

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 – 35	100 → 0	0 → 100
35 – 50	0	100

Time span of measurement: About 2.5 times as long as the retention time of carboplatin, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add water 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, and add water to make exactly 20 mL. Confirm that the peak area of carboplatin obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the solution for system suitability test.

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

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 carboplatin is not more than 2.0%.

(3) Residual solvent Being specified separately.

Loss on drying <*2.41*> Not more than 0.1% (0.5 g, 105°C, 4 hours).

Assay Weigh accurately about 25 mg each of Carboplatin and Carboplatin RS (separately determine the loss on drying <*2.41*> under the same conditions as Carboplatin), dissolve separately in water to make exactly 25 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 carboplatin of these solutions.

$$\text{Amount (mg) of } C_6H_{12}N_2O_4Pt = M_S \times A_T/A_S$$

M_S: Amount (mg) of Carboplatin RS, calculated on the dried basis

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 phenylhexylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 27°C.

Mobile phase A: Dissolve 8.5 g of tetrabutylammonium hydrogensulfate in 80 mL of water, add 3.4 mL of phosphoric acid, and adjust to pH 7.5 with a solution of sodium hydroxide (43 in 100). To 20 mL of this solution add water to make 1000 mL.

Mobile phase B: Dissolve 8.5 g of tetrabutylammonium hydrogensulfate in 80 mL of water, add 3,4 mL of phosphoric acid, and adjust to pH 7.5 with a solution of sodium hydroxide (43 in 100). To 20 mL of this solution add water to make 800 mL, and add 200 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 – 35	100 → 0	0 → 100

Flow rate: 0.5 mL per minute.

System suitability—

System performance: To 9 mL of the standard solution add 1 mL of diluted hydrogen peroxide TS (1 in 60), and allow to stand at room temperature for not less than 1 hour. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of carboplatin and the peak having the relative retention time about 0.93 to carboplatin is not less than 1.2.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operat-

ing conditions, the relative standard deviation of the peak area of carboplatin is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Add the following:

Carboplatin Injection

カルボプラチン注射液

Carboplatin Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of carboplatin ($C_6H_{12}N_2O_4Pt$: 371.25).

Method of preparation Prepare as directed under Injections, with Carboplatin.

Description Carboplatin Injection is a clear, colorless to pale yellow liquid.

Identification (1) To an amount of Carboplatin Injection, equivalent to 20 mg of Carboplatin, add 2 to 3 drops of diluted tin (II) chloride TS (1 in 15), and allow to stand for 30 minutes: a yellowish brown precipitate is formed.

(2) Evaporate to dryness a volume of Carboplatin Injection, equivalent to 10 mg of Carboplatin, in a water bath at not exceeding 30°C under vacuum. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 3270 cm^{-1}, 2990 cm^{-1}, 2960 cm^{-1}, 1645 cm^{-1}, 1610 cm^{-1}, 1381 cm^{-1} and 1348 cm^{-1}.

pH Being specified separately.

Purity (1) 1,1-Cyclobutanedicarboxylic acid—To an exact volume of Carboplatin Injection, equivalent to 20 mg of Carboplatin, add the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of 1,1-cyclobutanedicarboxylic acid, and dissolve in the mobile phase to make exactly 100 mL. Pipet 4 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 25 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 1,1-cyclobutanedicarboxylic acid of these solutions, and calculate the amount of 1,1-cyclobutanedicarboxylic acid by the following formula: it is not more than 0.7%.

$$\text{Amount (\%) of 1,1-cyclobutanedicarboxylic acid} = M_S \times A_T/A_S \times 1/25$$

M_S: Amount (mg) of 1,1-cyclobutanedicarboxylic acid

Operating conditions—

Proceed as directed in the operating conditions in the Purity (1) under Carboplatin.

System suitability—

Proceed as directed in the system suitability in the Purity (1) under Carboplatin.

(2) Related substances—To a volume of Carboplatin Injection, equivalent to 10 mg of Carboplatin, add water to make 10 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 determine each peak area by the automatic integration method. Calculate the amounts of these peaks by the area percentage method: the total amount of the peaks other than carboplatin is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, mobile phases A and B, and flow rate: Proceed as directed in the operating conditions in the Assay under Carboplatin.

Flowing of the mobile phase, and time span of measurement: Proceed as directed in the operating conditions in the Purity (2) under Carboplatin.

System suitability—

Test for required detectability, and system repeatability: Proceed as directed in the system suitability in the Purity (2) under Carboplatin.

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

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.2 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 an exact volume of Carboplatin Injection, equivalent to about 20 mg of carboplatin ($C_6H_{12}N_2O_4Pt$), add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Carboplatin RS (separately determine the loss on drying ⟨*2.41*⟩ under the same conditions as Carboplatine), dissolve in water to make exactly 25 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 carboplatin of these solutions.

$$\text{Amount (mg) of carboplatin } (C_6H_{12}N_2O_4Pt) = M_S \times A_T/A_S \times 4/5$$

M_S: Amount (mg) of Carboplatin RS, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 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 (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 8.5 g of tetrabutylammonium hydrogensulfate in 80 mL of water, add 3.4 mL of phosphoric acid, and adjust to pH 7.5 with a solution of sodium hydroxide (43 in 100). To 10 mL of this solution add 880 mL of water and 10 mL of acetonitrile.

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

System suitability—

System performance: To a solution of 25 mg of carboplatin in 20 mL of water add 2.5 mL of a solution of 65 mg of 1,3-phenylenediamine hydrochloride in 50 mL of water, and add water to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, carboplatin and 1,3-phenylenediamine 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 carboplatin is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Expiration date 24 months after preparation.

Cefazolin Sodium

セファゾリンナトリウム

Change the Assay as follows:

Assay Weigh accurately an amount of Cefazolin Sodium and Cefazolin RS, equivalent to about 20 mg (potency), dissolve each in the internal standard solution to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of these solutions 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 cefazolin to that of the internal standard.

Amount [μg (potency)] of cefazolin ($C_{14}H_{14}N_8O_4S_3$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Cefazolin RS

Internal standard solution—A solution of *p*-acetanisidide in 0.1 mol/L phosphate buffer solution, pH 7.0 (11 in 20,000).

Operating conditions—

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

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

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

Mobile phase: Dissolve 2.27 g of disodium hydrogen phosphate dodecahydrate and 0.47 g of citric acid monohydrate in water to make 935 mL, and add 65 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefazolin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cefazolin 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefazolin to that of the internal standard is not more than 1.0%.

Cefdinir

セフジニル

Delete the Absorbance:

Cefditoren Pivoxil Fine Granules

セフジトレン　ピボキシル細粒

Change the Dissolution as follows:

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 15 minutes of Cefditoren Pivoxil Fine Granules is not less than 80%.

Start the test with an accurately weighed amount of Cefditoren Pivoxil Fine Granules, equivalent to about 0.1 g (potency) of Cefditoren Pivoxil, 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 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefditoren Pivoxil RS, equivalent to about 22 mg (potency), dissolve in 20 mL of diluted acetonitrile (3 in 4), and add the dissolution medium to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 272 nm of the sample solution and

standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control.

Dissolution rate (%) with respect to the labeled amount of cefditoren pivoxil ($C_{25}H_{28}N_6O_7S_3$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 450$

M_S: Amount [mg(potency)] of Cefditoren Pivoxil RS
M_T: Amount (g) of Cefditoren Pivoxil Fine Granules
C: Labeled amount [mg(potency)] of cefditoren pivoxil ($C_{25}H_{28}N_6O_7S_3$) in 1 g

Cefoperazone Sodium

セフォペラゾンナトリウム

Change the origin/limits of content, the Purity (1) and the Assay as follows:

Cefoperazone Sodium contains not less than 871 μg (potency) and not more than 986 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefoperazone Sodium is expressed as mass (potency) of cefoperazone ($C_{25}H_{27}N_9O_8S_2$: 645.67).

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Cefoperazone Sodium in 10 mL of water: the solution is clear, and its absorbance at 400 nm, determined as directed under Ultraviolet-visible Spectrophotometry <2.24>, is not more than 0.18.

Assay Weigh accurately an amount of Cefoperazone Sodium equivalent to about 0.1 g (potency), and dissolve in water 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 sample solution. Separately, weigh accurately an amount of Cefoperazone RS equivalent to about 20 mg (potency), dissolve in 1 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and add water to make exactly 20 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 cefoperazone to that of the internal standard.

Amount [μg (potency)] of cefoperazone ($C_{25}H_{27}N_9O_8S_2$)
$= M_S \times Q_T/Q_S \times 5000$

M_S: Amount [mg (potency)] of Cefoperazone RS

Internal standard solution—A solution of acetanilide in a mixture of water and acetonitrile (43:7) (3 in 8000).
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 35°C.

Mobile phase: To 57 mL of acetic acid (100) add 139 mL of triethylamine and water to make 1000 mL. To 20 mL of this solution add 835 mL of water, 140 mL of acetonitrile and 5 mL of dilute acetic acid.

Flow rate: Adjust the flow rate so that the retention time of cefoperazone 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 cefoperazone 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 ratios of the peak area of cefoperazone to that of the internal standard is not more than 1.0%.

Add the following:

Cefpodoxime Proxetil Tablets

セフポドキシム　プロキセチル錠

Cefpodoxime Proxetil Tablets contain not less than 93.0% and not more than 107.0% of the labeled potency of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$: 427.46).

Method of preparation Prepare as directed under Tablets, with Cefpodoxime Proxetil.

Identification Powder Cefpodoxime Proxetil Tablets. To a portion of the powder, equivalent to 65 mg (potency) of Cefpodoxime Proxetil, add 25 mL of acetonitrile, shake thoroughly, and centrifuge. To 2 mL of the supernatant liquid add acetonitrile to make 50 mL. To 5 mL of this solution add acetonitrile to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 232 nm and 236 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 Cefpodoxime Proxetil Tablets, add exactly 20 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), agitate with the aid of ultrasonic waves for 10 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 the subsequent V mL of the filtrate, equivalent to 30 mg (potency) of Cefpodoxime Proxetil, add exactly 6 mL of the internal standard solution, then add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefpodoxime Proxetil RS,

equivalent to about 60 mg (potency), dissolve in 60 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), add exactly 12 mL of the internal standard solution, then add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefpodoxime Proxetil.

Amount [mg (potency)] of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$)
$= M_S \times (Q_{T1} + Q_{T2})/(Q_{S1} + Q_{S2}) \times 10/V$

M_S: Amount [mg (potency)] of Cefpodoxime Proxetil RS

Internal standard solution—Dissolve 0.1 g of ethyl parahydroxybenzoate in a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 100 mL.

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 Cefpodoxime Proxetil Tablets is not less than 70%.

Start the test with 1 tablet of Cefpodoxime Proxetil 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 a solution of citric acid monohydrate in the mobile phase (1 in 2000) to make exactly V' mL so that each mL contains about 11 μg (potency) of Cefpodoxime Proxetil, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefpodoxime Proxetil RS, equivalent to about 22 mg (potency), and dissolve in a solution of citric acid monohydrate in the mobile phase (1 in 2000) to make exactly 100 mL. Pipet 5 mL of this solution, add a solution of citric acid monohydrate in the mobile phase (1 in 2000) 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 areas of separated two peaks, one has the retention time of about 24 minutes, A_{Ta} and A_{Sa}, and another one has the retention time of about 30 minutes, A_{Tb} and A_{Sb}, of both solutions.

Dissolution rate (%) with respect to the labeled amount of cefpodoxime proxetil ($C_{21}H_{27}N_5O_9S_2$)
$= M_S \times (A_{Ta} + A_{Tb})/(A_{Sa} + A_{Sb}) \times V'/V \times 1/C \times 45$

M_S: Amount [mg (potency)] of Cefpodoxime Proxetil RS
C: Labeled amount [mg (potency)] of cefpodoxime proxetil ($C_{21}H_{27}N_5O_9S_2$) in 1 tablet

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 and methanol (11:9).

Flow rate: Adjust the flow rate so that the retention time of one of the two peaks that elutes firster is about 24 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the resolution between the two peaks of cefpodoxime proxetil is 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 total area of the two peaks of cefpodoxime proxetil is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Cefpodoxime Proxetil Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.3 g (potency) of Cefpodoxime Proxetil, add 80 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), agitate for 10 minutes with the aid of ultrasonic waves, and add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make exactly 100 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 10 mL of the subsequent filtrate, add exactly 6 mL of the internal standard solution, then, add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefpodoxime Proxetil RS, equivalent to about 60 mg (potency), dissolve in 60 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), add exactly 12 mL of the internal standard solution, then add a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefpodoxime Proxetil.

Amount [mg (potency)] of cefpodoxime ($C_{15}H_{17}N_5O_6S_2$)
$= M_S \times (Q_{T1} + Q_{T2})/(Q_{S1} + Q_{S2}) \times 5$

M_S: Amount [mg (potency)] of Cefpodoxime Proxetil RS

Internal standard solution—Dissolve 0.1 g of ethyl parahydroxybenzoate in a mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make 100 mL.

Containers and storage Containers—Tight containers.

Cefteram Pivoxil

セフテラム　ピボキシル

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

Cefteram Pivoxil contains not less than 743 μg (potency) and not more than 824 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefteram Pivoxil is expressed as mass (potency) of cefteram ($C_{16}H_{17}N_9O_5S_2$: 479.49).

Identification **(1)** Determine the absorption spectrum of a solution of Cefteram Pivoxil in 0.05 mol/L hydrochloric acid-methanol TS (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.

(2) Determine the infrared absorption spectrum of Cefteram Pivoxil 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) Determine the ^{1}H spectrum of a solution of Cefteram Pivoxil in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals A, B and C, at around δ 1.2 ppm, at around δ 2.5 ppm and at around δ 4.0 ppm, respectively. The ratio of the integrated intensity of these signals, A:B:C, is about 3:1:1.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Cefteram Pivoxil 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—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 area of the peak, having the relative retention time of about 0.9 with respect to cefteram pivoxil from the sample solution is not larger than 1.25 times the peak area of cefteram pivoxil from the standard solution, the area of the peak, having the relative retention time of about 0.1 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 the above calculation, use the area of the peak, having the relative retention time of about 0.1, 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: Measure exactly 1 mL of the standard solution, and 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 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%.

Ceftibuten Hydrate

セフチブテン水和物

Change the Purity (2) and the Assay as follows:

Purity

(2) Related substances—(i) Keep the sample solution and the standard solution at not exceeding 5°C and use within 2 hours after preparation. Dissolve 25 mg of Ceftibuten Hydrate in 20 mL of 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0. To 4 mL of this solution add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make 20 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 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 each peak area by the automatic integration method: the area of the peak other than ceftibuten obtained from the sample solution is not larger than 1/5 times the peak area of ceftibuten from the standard solution, and the total area of the peaks other than ceftibuten from the sample solution is not larger than the peak area of ceftibuten 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 1.7 times as long as the retention time of ceftibuten, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL. Confirm that the peak area of ceftibuten obtained from 5 μL of this solution is equivalent to 7 to 13% of that of ceftibuten obtained from 5 μL of the standard solution.

System performance: Dissolve 5 mg of Ceftibuten Hydrate in 20 mL of 0.1 mol/L hydrochloric acid TS, and allow to stand at 40°C for 1 hour. To 4 mL of this solution add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make 25 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, trans-isomer of ceftibuten and ceftibuten are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 5 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ceftibuten is not more than 2.0%.

(ii) Keep the sample solution at not exceeding 5°C, and use within 24 hours after preparation. To 5 mg of Ceftibuten Hydrate add 20 mL of the mobile phase, agitate with the aid of ultrasonic waves, if necessary, then shake to dissolve, filter 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 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 their amounts by the area percentage method: the total amount of the peaks that are eluted faster than ceftibuten is not more than 5.0%. For these calculations use the areas of these peaks after multiplying by their relative response factors, 1.63, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with glycol etherified silica gel for liquid chromatography (10 μm in particle diameter).

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

Mobile phase: Dissolve 1.05 g of disodium hydrogen phosphate dodecahydrate and 0.58 g of potassium dihydrogen phosphate in water to make 1000 mL.

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

Time span of measurement: About 1.6 times as long as the retention time of ceftibuten.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase to make 20 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 mobile phase to make exactly 20 mL. Confirm that the peak area of ceftibuten obtained from 10 μL of this solution is equivalent to 7 to 13% of that of ceftibuten obtained from 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 ceftibuten are not less than 10,000 and 0.8 – 1.2, respectively.

System repeatability: When the test is repeated 5 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 ceftibuten is not more than 1.7%.

Assay Keep the sample solution and the standard solution at not exceeding 5°C and use within 2 hours after preparation. Weigh accurately an amount of Ceftibuten Hydrate and Ceftibuten Hydrochloride RS, equivalent to about 10 mg (potency), dissolve each in 36 mL of 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0, add exactly 4 mL each of the internal standard solution, shake, and use these solutions as the sample solution and the standard solution, respectively. 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 ceftibuten to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of ceftibuten } (C_{15}H_{14}N_4O_6S_2) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Ceftibuten Hydrochloride RS

Internal standard solution—A solution of methyl parahydroxybenzoate in acetonitrile (3 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

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

Mobile phase: A mixture of 0.005 mol/L *n*-decyl trimethylammonium bromide TS and acetonitrile (4:1).

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

System suitability—

System performance: Dissolve 5 mg of Ceftibuten Hydrate in 20 mL of 0.1 mol/L hydrochloric acid TS, and allow to stand at 40°C for 1 hour. To 4 mL of this solution add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make 25 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, trans-isomer of ceftibuten and ceftibuten 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ceftibuten to that of the internal standard is not more than 1.0%.

Change to read:

Cellacefate

Cellulose Acetate Phthalate

セラセフェート

[*9004-38-0*]

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 (◆ ◆).

Cellacefate is a reaction product of phthalic anhydride and partially acetylated cellulose.

It contains not less than 21.5% and not more than 26.0% of acetyl group (-$COCH_3$: 43.04), and not less than 30.0% and not more than 36.0% of carboxybenzoyl group (-COC_6H_4COOH: 149.12), calculated on the anhydrous and free acid-free basis.

◆Description Cellacefate occurs as a white powder or grain.

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

Identification Determine the infrared absorption spectrum of Cellacefate 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 Cellacefate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Viscosity <*2.53*> Weigh accurately a quantity of Cellacefate, equivalent to 15 g calculated on the anhydrous basis, dissolve in 85 g of a mixture of acetone and water (249: 1 in mass), and use this solution as the sample solution. Perform the test with the sample solution at 25 ± 0.2°C as directed in Method 1 to obtain the kinematic viscosity v. Separately, determine the density, ρ, of the sample solution as directed under Determination of Specific Gravity and Density <*2.56*>, and calculate the viscosity of the sample solution, η, as $\eta = \rho v$: not less than 45 mPa·s and not more than 90 mPa·s.

Purity (1) ◆Heavy metals <*1.07*>—Proceed with 2.0 g of Cellacefate 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) Free acids—Weigh accurately about 3 g of Cellacefate, put in a glass-stoppered conical flask, add 100 mL of diluted methanol (1 in 2), stopper tightly, and filter after shaking for 2 hours. Wash both the flask and residue with two 10-mL portions each of diluted methanol (1 in 2), combine the washes to the filtrate, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (indicator: 2-3 drops of phenolphthalein TS). Perform the blank determination with 120 mL of diluted methanol (1 in 2), and make any necessary correction.

$$\text{Amount (\%) of free acids} = 0.8306A/M$$

A: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

M: Amount (g) of Cellacefate, calculated on the anhydrous basis

The amount of free acids is not more than 3.0%, calculated as phthalic acid ($C_8H_6O_4$: 166.13).

Water <*2.48*> Not more than 5.0% (0.5 g, volumetric titration, direct titration, using a mixture of ethanol (99.5) and dichloromethane (3:2) instead of methanol for Karl Fischer method).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay (1) Carboxybenzoyl group—Weigh accurately about 1 g of Cellacefate, dissolve in 50 mL of a mixture of ethanol (95) and acetone (3:2), and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (indicator: 2 – 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

$$\text{Content (\%) of carboxybenzoyl group } (C_8H_5O_3) = \frac{\dfrac{1.491 \times A}{M} - (1.795 \times B)}{100 - B} \times 100$$

A: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

B: Amount (%) of free acids obtained in the Purity (2) Free acids

M: Amount (g) of Cellacefate, calculated on the anhydrous basis

(2) Acetyl group—Weigh accurately about 0.1 g of Cellacefate, put in a glass-stoppered conical flask, add exactly 25 mL of 0.1 mol/L sodium hydroxide VS, and boil for 30 minutes under a reflux condenser. After cooling, add 2 – 3 drops of phenolphthalein TS, and titrate <*2.50*> the excess of sodium hydroxide with 0.1 mol/L hydrochloric acid VS. Perform a blank determination.

$$\text{Content (\%) of free acids and bound acetyl group } (C_2H_3O) = 0.4305A/M$$

A: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed, corrected by the blank determination

M: Amount (g) of Cellacefate, calculated on the anhydrous basis

$$\text{Content (\%) of acetyl group } (C_2H_3O) = 100 \times (P - 0.5182B)/(100 - B) - 0.5772C$$

B: Amount (%) of free acids obtained in the Purity (2) Free acids

C: Content (%) of carboxybenzoyl group

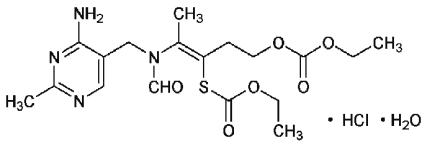
P: Content (%) of free acids and bound acetyl group (C_2H_3O)

◆Containers and storage Containers—Tight containers.◆

Add the following:

Cetotiamine Hydrochloride Hydrate

セトチアミン塩酸塩水和物



$C_{18}H_{26}N_4O_6S.HCl.H_2O$: 480.96

(3*Z*)-4-{*N*-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-*N*-formylamino}-3-(ethoxycarbonylsulfanyl)pent-3-enyl ethyl carbonate monohydrochloride monohydrate

[*616-96-6*, anhydride]

Cetotiamine Hydrochloride Hydrate contains not less than 98.0% and not more than 102.0% of cetotiamine hydrochloride ($C_{18}H_{26}N_4O_6S.HCl$: 462.95), calculated on the anhydrous basis.

Description Cetotiamine Hydrochloride Hydrate occurs as white, crystals or crystalline powder. It is odorless or has a faint characteristic odor.

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

It dissolves in 0.01 mol/L hydrochloric acid TS.

Melting point: about 132°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Cetotiamine Hydrochloride Hydrate in 0.01 mol/L hydrochloric acid TS (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 Cetotiamine 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 Cetotiamine Hydrochloride 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 Cetotiamine Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Cetotiamine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cetotiamine Hydrochloride Hydrate in 10 mL of water is clear and has no more color than the following control solution.

Control solution: Mix exactly 1.5 mL of Cobalt (II) Chloride CS, exactly 36 mL of Iron (III) Chloride CS and exactly 12.5 mL of diluted dilute hydrochloric acid (1 in 10). Pipet 1 mL of this mixture, and add diluted dilute hydrochloric acid (1 in 10) to make exactly 100 mL.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Cetotiamine 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 20 ppm).

(3) Related substances—Dissolve 50 mg of Cetotiamine Hydrochloride Hydrate 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 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 other than cetotiamine from the sample solution is not larger than the peak area of cetotiamine from the standard solution, and the total area of the peaks other than the peak of cetotiamine from the sample solution is not larger than 2 times the peak area of cetotiamine 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 cetotiamine, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of cetotiamine obtained with 10 μL of this solution is equivalent to 7 to 13% of that 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 cetotiamine are not less than 3000 and 0.7 – 1.0, 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 cetotiamine is not more than 2.0%.

(4) Residual solvent Being specified separately.

Water <*2.48*> 3.0 – 5.0% (40 mg, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 30 mg each of Cetotiamine Hydrochloride Hydrate and Cetotiamine Hydrochloride RS (separately determine the water <*2.48*> in the same manner as Cetotiamine Hydrochloride Hydrate), add exactly 10 mL each of the internal standard solution, then add a mixture of water and methanol (1:1) to make 50 mL. To 2 mL each of these solutions add a mixture of water and methanol (1:1) to make 10 mL, and use these solutions as the sample solution and the standard solution, respectively. 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 cetotiamine to that of the internal standard.

Amount (mg) of cetotiamine hydrochloride ($C_{18}H_{26}N_4O_6S.HCl$)
$= M_S \times Q_T/Q_S$

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

Internal standard solution—A solution of propyl parahydroxybenzoate in a mixture of water and methanol (1:1) (1 in 800).

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 (5 μm in particle diameter).

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

Mobile phase: Dissolve 1.0 g of sodium 1-heptanesulfonate in diluted acetic acid (100) (1 in 100) to make 1000 mL. To 1 volume of this solution add 1 volume of methanol.

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

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cetotiamine 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cetotiamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Chlordiazepoxide Tablets

クロルジアゼポキシド錠

Change the Uniformity of dosage units and Dissolution 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.

Conduct this procedure without exposure to light using light-resistant vessels. To 1 tablet of Chlordiazepoxide Tablets add 1 mL of water, shake to disintegrate the tablet, then add 20 mL of methanol, shake, add methanol to make exactly 25 mL, and filter through a membrane filter with a pore size not exceeding 0.5 μm. Discard the first 5 mL of the filtrate, take exactly V mL of the subsequent filtrate equivalent to about 2 mg of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), add exactly 1 mL of the internal standard solution, then add methanol to make 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of chlordiazepoxide ($C_{16}H_{14}ClN_3O$)
$= M_S \times Q_T/Q_S \times 5/V$

M_S: Amount (mg) of Chlordiazepoxide RS

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

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 Tablets is not less than 70%.

Conduct this procedure without exposure to light using light-resistant vessels. Start the test with 1 tablet of Chlordiazepoxide Tablets, withdraw not less than 30 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 the subsequent V mL, add the dissolution medium to make exactly V' mL so that each mL contains about 3.7 μg of chlordiazepoxide ($C_{16}H_{14}ClN_3O$), and use this solution as the sample solution. Separately, weigh accurately about 12 mg of Chlordiazepoxide RS, previously dried for 4 hours under reduced pressure with phosphorus (V) oxide as a dessicant, dissolve in 5 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 ($C_{16}H_{14}ClN_3O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 27$

M_S: Amount (mg) of Chlordiazepoxide RS

C: Labeled amount (mg) of chlordiazepoxide ($C_{16}H_{14}ClN_3O$) in 1 tablet

Chlorpheniramine Maleate Powder

クロルフェニラミンマレイン酸塩散

Add the following next to the Identification:

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 Chlorpheniramine Maleate Powder is not less than 85%.

Start the test with an accurately weighed amount of Chlorpheniramine Maleate Powder, equivalent to about 4 mg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$), 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, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 22 mg of

Chlorpheniramine Maleate RS, previously dried at 105°C for 3 hours, and dissolve in 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 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 chlorpheniramine of each solution.

Dissolution rate (%) with respect to the labeled amount of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$)

$= M_S/M_T \times A_T/A_S \times 1/C \times 18$

M_S: Amount (mg) of Chlorpheniramine Maleate RS

M_T: Amount (g) of the Chlorpheniramine Maleate Powder

C: Labeled amount (mg) of chlorpheniramine maleate ($C_{16}H_{19}ClN_2.C_4H_4O_4$) in 1 g

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 chlorpheniramine are not less than 2000 and not more than 2.5, 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 chlorpheniramine is not more than 2.0%.

Anhydrous Citric Acid

無水クエン酸

Change the Description and the rest as follows:

◆**Description** Anhydrous Citric Acid occurs as colorless crystals, white granules or crystalline powder.

It is very soluble in water, and freely soluble in ethanol (99.5).◆

Identification Determine the infrared absorption spectrum of Anhydrous Citric Acid, previously dried at 105°C for 2 hours, 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.

Purity **(1)** Clarity and color of solution—Dissolve 2.0 g of Anhydrous Citric Acid in water to make 10 mL: the solution is clear and colorless or has no more color than the following control solutions (1), (2) or (3).

Control solution (1): To 1.5 mL of Cobalt (II) Chloride CS and 6.0 mL of Iron (III) Chloride CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

Control solution (2): To 2.5 mL of Cobalt (II) Chloride CS, 6.0 mL of Iron (III) Chloride CS and 1.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

Control solution (3): To 0.15 mL of Cobalt (II) Chloride CS, 7.2 mL of Iron (III) Chloride CS and 0.15 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Sulfates—Dissolve 2.0 g of Anhydrous Citric Acid in water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted ethanol (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minute. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: the solution has no more turbidity than the following control solution (not more than 150 ppm).

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as above using this solution instead of the sample solution.

(3) Oxalic acid—Dissolve 0.80 g of Anhydrous Citric Acid in 4 mL of water, add 3 mL of hydrochloric acid and 1 g of zinc, and boil for 1 minute. After allowing to stand for 2 minutes, take the supernatant liquid, add 0.25 mL of a solution of phenylhydrazinium chloride (1 in 100), heat to boil, and then cool quickly. To this solution add the equal volume of hydrochloric acid and 0.25 mL of a solution of potassium hexacyanoferrate (III) (1 in 20), mix, and allow to stand for 30 minutes: the solution has no more color than the following control solution prepared at the same time (not more than 360 ppm expressed as oxalic anhydride).

Control solution: To 4 mL of a solution of oxalic acid dihydrate (1 in 10,000) add 3 mL of hydrochloric acid and 1 g of zinc, and proceed in the same manner as the test solution.

◆**(4)** Heavy metals <*1.07*>—Proceed with 2.0 g of Anhydrous Citric Acid 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).◆

(5) Readily carbonizable substances—Place 1.0 g of Anhydrous Citric Acid in a Nessler tube, add 10 mL of sulfuric acid, immediately heat in a 90 ± 1°C water bath for 60 minutes, and cool quickly. Compare the color of 2.0 mL each of this solution and Matching Fluid K, using test tubes 12 mm in outside diameter, from a side against white background: the solution is not more colored than the matching fluid.

Water <*2.48*> Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.55 g of Anhydrous Citric Acid, dissolve in 50 mL of water, and titrate with 1 mol/L sodium hydroxide VS (indicator: 1 drop of phenolphthalein

TS).

Each mL of 1 mol/L sodium hydroxide VS
= 64.04 mg of $C_6H_8O_7$

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

Citric Acid Hydrate

クエン酸水和物

Change the Description and the rest as follows:

◆**Description** Citric Acid Hydrate occurs as colorless crystals, white granules or crystalline powder.

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

It is efflorescent in dry air.◆

Identification Determine the infrared absorption spectrum of Citric Acid Hydrate, previously dried at 105°C for 2 hours, 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.

Purity **(1)** Clarity and color of solution—Dissolve 2.0 g of Citric Acid Hydrate in water to make 10 mL: the solution is clear and colorless or has no more color than the following control solutions (1), (2) or (3).

Control solution (1): To 1.5 mL of Cobalt (II) Chloride CS and 6.0 mL of Iron (III) Chloride CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

Control solution (2): To 2.5 mL of Cobalt (II) Chloride CS, 6.0 mL of Iron (III) Chloride CS and 1.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

Control solution (3): To 0.15 mL of Cobalt (II) Chloride CS, 7.2 mL of Iron (III) Chloride CS and 0.15 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Sulfates—Dissolve 2.0 g of Citric Acid Hydrate in water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted ethanol (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minute. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: the solution has no more turbidity than the following control solution. (not more than 150 ppm).

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as above using this solution instead of the sample solution.

(3) Oxalic acid—Dissolve 0.80 g of Citric Acid Hydrate in 4 mL of water, add 3 mL of hydrochloric acid and 1 g of zinc, and boil for 1 minute. After allowing to stand for 2 minutes, take the supernatant liquid, add 0.25 mL of a solution of phenylhydrazinium chloride (1 in 100), heat to boil, and then cool quickly. To this solution add the equal volume of hydrochloric acid and 0.25 mL of a solution of potassium hexacyanoferrate (III) (1 in 20), mix, and allow to stand for 30 minutes: the solution has no more color than the following control solution prepared at the same time (not more than 360 ppm expressed as oxalic anhydride).

Control solution: To 4 mL of a solution of oxalic acid dihydrate (1 in 10,000) add 3 mL of hydrochloric acid and 1 g of zinc, and proceed in the same manner as the test solution.

◆**(4)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Citric Acid Hydrate 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).◆

(5) Readily carbonizable substances—Place 1.0 g of Citric Acid Hydrate in a Nessler tube, add 10 mL of sulfuric acid, immediately heat in a 90 ± 1°C water bath for 60 minutes, and cool quickly. Compare the color of 2.0 mL each of this solution and Matching Fluid K, using test tubes 12 mm in outside diameter, from a side against white background: the solution is not more colored than the matching fluid.

Water ⟨*2.48*⟩ Not less than 7.5% and not more than 9.0% (0.5 g, volumetric titration, direct titration).

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

Assay Weigh accurately about 0.55 g of Citric Acid Hydrate, dissolve in 50 mL of water, and titrate ⟨*2.50*⟩ with 1 mol/L sodium hydroxide VS (indicator: 1 drop of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 64.04 mg of $C_6H_8O_7$

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

Clindamycin Hydrochloride

クリンダマイシン塩酸塩

Change the Purity as follows:

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Clindamycin Hydrochloride 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—Use the sample solution obtained in the Assay 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 peak area of clindamycin B, having the relative retention

time of about 0.7 to clindamycin, and that of 7-epiclindamycin, having the relative retention time of about 0.8 to clindamycin, obtained from the sample solution are not larger than 2 times the peak area of clindamycin from the standard solution, the area of the peak other than clindamycin and the peaks mentioned above is not larger than the peak area of clindamycin from the standard solution, and the total area of the peaks other than clindamycin from the sample solution is not larger than 4 times the peak area of clindamycin 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 clindamycin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of clindamycin obtained from 20 μL of this solution is equivalent to 7 to 13% of that of clindamycin obtained from 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 clindamycin are not less than 6000 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 clindamycin is not more than 2.0%.

Clomifene Citrate

クロミフェンクエン酸塩

Change the Isomer ratio as follows:

Isomer ratio To 10 mg of Clomifene Citrate add 10 mL of water and 1 mL of sodium hydroxide TS, and shake to uniformly disperse. Add 10 mL of ethyl acetate, shake vigorously for 5 minutes, allow to stand for 5 minutes, and use the upper layer 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. Determine the areas of two adjacent peaks, A_a and A_b, having the retention time of about 8 minutes, where A_a is the peak area of shorter retention time and A_b is the peak area of longer retention time: $A_b/(A_a + A_b)$ is between 0.3 and 0.5.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 15 m in length, coated the inside surface with a layer about 0.1 μm thick of dimethylpolysiloxane for gas chromatography.

Column temperature: A constant temperature of about 230°C.

Injection port temperature: A constant temperature of about 270°C.

Detector temperature: A constant temperature of about 300°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of the first peak of clomifene citrate is about 7.5 minutes.

Split ratio: 1:50.

System suitability—

System performance: When the procedure is run with 1 μL of the sample solution under the above operating conditions, the resolution between the two adjacent peaks having the retention time of about 8 minutes is not less than 5.

System repeatability: When the test is repeated 6 times with 1 μL of the sample solution under the above operating conditions, the relative standard deviation of the result of $A_b/(A_a + A_b)$ is not more than 1.0%.

Clomifene Citrate Tablets

クロミフェンクエン酸塩錠

Change the Identification as follows:

Identification Weigh a portion of powdered Clomifene Citrate Tablets, equivalent to 50 mg of Clomifene Citrate, shake vigorously with 50 mL of methanol for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Clomifene Citrate RS 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. Develop the plate with a mixture of 2-propanol, toluene and diethylamine (10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and standard solution show the same *R*f value.

1% Codeine Phosphate Powder

コデインリン酸塩散 1%

Add the following next to the Identification:

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 1% Codeine Phosphate Powder is not less than 85%.

Start the test with about 2 g of 1% Codeine Phosphate Powder, accurately weighed, 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 ex-

ceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of codeine phosphate hydrate for assay (separately determine the water <2.48> in the same manner as Codeine Phosphate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 50 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 codeine of each solution.

Dissolution rate (%) with respect to the labeled amount of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$)
$= M_S/M_T \times A_T/A_S \times 36/5 \times 1.023$

M_S: Amount (mg) of codeine phosphate hydrate for assay, calculated on the anhydrous basis
M_T: Amount (g) of 1% Codeine Phosphate Powder

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 codeine 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 codeine is not more than 2.0%.

10% Codeine Phosphate Powder

コデインリン酸塩散10%

Add the following next to the Identification:

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 10% Codeine Phosphate Powder is not less than 85%.

Start the test with about 0.2 g of 10% Codeine Phosphate Powder, accurately weighed, 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, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of codeine phosphate hydrate for assay (separately determine the water <2.48> in the same manner as Codeine Phosphate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 50 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 codeine of each solution.

Dissolution rate (%) with respect to the labeled amount of codeine phosphate hydrate ($C_{18}H_{21}NO_3.H_3PO_4.\frac{1}{2}H_2O$)
$= M_S/M_T \times A_T/A_S \times 18/25 \times 1.023$

M_S: Amount (mg) of codeine phosphate hydrate for assay, calculated on the anhydrous basis
M_T: Amount (g) of 10% Codeine Phosphate Powder

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 codeine 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 codeine is not more than 2.0%.

Add the following:

Colestimide

コレスチミド

[*95522-45-5*]

Colestimide is an anion exchange resin, composed of a copolymer of 2-methylimidazole and 1-chloro-2,3-epoxypropane.

It contains not less than 18.0% and not more than 20.0% of chlorine (Cl: 35.45), calculated on the dried basis.

Each g of Colestimide, calculated on the dried basis, exchanges with not less than 2.0 g and not more than 2.4 g of cholic acid ($C_{24}H_{39}O_5$: 407.56).

Description Colestimide occurs as a white to pale yellowish white powder.

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

It is hygroscopic.

Identification Determine the infrared absorption spectrum of Colestimide, previously dried, 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.

Purity **(1)** Heavy metals <1.07>—Take 2.0 g of Colestimide in a porcelain or platinum crucible, and carbonize by weakly heating. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) and

5 mL of hydrogen peroxide (30), and ignite the ethanol. After cooling, add 1 mL of sulfuric acid, then, proceed according to Method 4, and perform the test. Prepare the control solution as follows: To 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) add 5 mL of hydrogen peroxide (30), and ignite the ethanol. After cooling, add 1 mL of sulfuric acid, then, proceed in the same manner as for the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 10 ppm).

(2) Related substances—To exactly 0.50 g of Colestimide add exactly 20 mL of water, shake for 1 hour, centrifuge, and use the supernatant liquid as the sample solution. Determine the absorbance of the sample solution at 210 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance is not more than 0.50.

(3) Residual solvent Being specified separately.

Loss on drying <*2.41*> Not more than 10.0% (1 g, in vacuum, 105°C, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Degree of swelling Weigh accurately about 1 g of Colestimide, put in a 25-mL glass stoppered measuring cylinder (about 11 mm in inside diameter), add 23 mL of water, shake for 2 minutes, and add water to make 25 mL. After standing for 2 hours, measure the volume of the resin layer, and determine the volume per g, calculated on the dried basis: the volume is 12 – 18 mL/g.

Assay **(1)** Chlorine—Weigh accurately about 0.2 g of Colestimide, add 50 mL of water, and shake. Add 1 mL of nitric acid and 25 mg of potassium nitrate, shake, and titrate <*2.50*> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 3.545 mg of Cl

(2) Exchange capacity—Weigh accurately about 0.45 g of sodium cholate hydrate (separately determine the water), dissolve in water to make exactly 100 mL, and use this solution as the sodium cholate standard stock solution. Separately, weigh accurately about 30 mg of Colestimide, add exactly 30 mL of the sodium cholate standard stock solution, shake for 1 hour, and centrifuge or filter through a membrane filter with a pore size not exceeding 0.8 μm. Pipet 5 mL of the supernatant liquid or the filtrate, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, pipet 5 mL of the sodium cholate standard stock 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 cholic acid to that of the internal standard.

Exchanged amount (g) of cholic acid per g of Colestimide, calculated on the dried basis

$$= M_S/M_T \times (Q_S - Q_T)/Q_S \times 3/10 \times 0.947$$

M_S: Amount (mg) of sodium cholate hydrate, calculated on the anhydrous basis

M_T: Amount (mg) of sample, calculated on the dried basis

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 80,000).

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 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 diluted phosphoric acid (1 in 1000) and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of cholic acid 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, cholic acid 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cholic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Colestimide Tablets

コレスチミド錠

Colestimide Tablets 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 Tablets, with Colestimide.

Identification Powder Colestimide Tablets. Determine the infrared absorption spectrum of a portion of the powder 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}, 1262 cm^{-1}, 1102 cm^{-1} and 1035 cm^{-1}.

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

Disintegration <*6.09*> When carry out the test for 10 minutes, it meets the requirement.

Assay Weigh accurately about 0.45 g of sodium cholate hydrate (separately determine the water), dissolve in water to make exactly 100 mL, and use this solution as the sodium cholate standard stock solution. Separately, weigh accurately the mass of not less than 20 Colestimide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 30 mg of colestimide, add exactly 30 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. Separately, pipet 5 mL of the sodium cholate standard stock 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 cholic acid to that of the internal standard.

Amount (mg) of colestimide
$= M_S \times (Q_S - Q_T)/Q_S \times 3/10 \times 1/2.2 \times 0.947$

M_S: Amount (mg) of sodium cholate hydrate, calculated on the anhydrous basis
2.2: Exchanged amount (g) of cholic acid per g of colestimide, calculated on the dried basis

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 80,000).
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 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 diluted phosphoric acid (1 in 1000) and acetonitrile (1:1).
Flow rate: Adjust the flow rate so that the retention time of cholic acid 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, cholic acid 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cholic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Corn Starch

トウモロコシデンプン

Delete the latin name:

Add the following:

Crospovidone

クロスポビドン

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 (◆ ◆).

Crospovidone is a cross-linked polymer of 1-vinyl-2-pyrrolidone.

It contains not less than 11.0% and not more than 12.8% of nitrogen (N: 14.01), calculated on the dried basis.

Two types of Crospovidone are available, depending on the particle size: type A and type B.

◆The label states the type.◆

◆**Description** Crospovidone occurs as a white to pale yellowish powder.

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

It is hygroscopic.◆

Identification **(1)** Suspend 1 g of Crospovidone in 10 mL of water, add 0.1 mL of iodine TS, shake for 30 seconds, then add 1 mL of starch TS, and shake: a blue color is not produced within 30 seconds.

(2) When add 0.1 g of Crospovidone to 10 mL of water, shake to suspend, and allow the suspension to stand, a clear liquid is not produced within 15 minutes.

Particle size Weigh accurately about 20 g of Crospovidone, place in a 1000-mL conical flask, add 500 mL of water, shake for 30 minutes, and pour onto an accurately tared No. 235 (63 μm) sieve, previously washed with hot water and dried at 105°C for a night, and wash the residue with water until the passing water is clear. Dry the residue together with the sieve in a drying machine at 105°C for 5 hours without air-circulation. After cooling down in a desiccator for 30 minutes, weigh the mass of the residue with sieve, and calculate the amount of the residue on the sieve by the following equation: Type A is more than 15%, and type B is not more than 15%.

Amount (%) of the residue of Crospovidone on No. 235 (63 μm) sieve
$= (M_1 - M_3)/M_2 \times 100$

M_1: The mass (g) of the residue with sieve after 5 hours drying

M_2: Amount (g) of Crospovidone, calculated on the dried basis

M_3: Mass (g) of the sieve

Purity ◆**(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Crospovidone 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) Water-soluble substances—Place 25.0 g of Crospovidone in a 400-mL beaker, add 200 mL of water, and stir for 1 hour. Transfer the suspension to a 250-mL volumetric flask, rinsing with water, and dilute to volume with water. Allow the bulk of the solids to settle. Filter about 100 mL of the almost clear supernatant liquid through a 0.45 μm membrane filter, protected by superimposing a 3 μm membrane filter. Transfer exactly 50 mL of the clear filtrate to a tared 100-mL beaker, evaporate to dryness and dry at 105 – 110°C for 3 hours: the mass of the residue is not more than 75 mg.

(3) 1-Vinyl-2-pyrrolidone—To 1.250 g of Crospovidone add exactly 50 mL of methanol, and shake for 60 minutes. Leave bulk to settle, filter through a 0.2 μm membrane filter, and use the filtrate as the sample solution. Separately, dissolve 50 mg of 1-vinyl-2-pyrrolidone in methanol to make exactly 100 mL. Pipet 1 mL of this solution, and add methanol to make exactly 100 mL. To exactly 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 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: the peak area of 1-vinyl-2-pyrrolidone obtained from the sample solution is not larger than that obtained from the standard solution (not more than 10 ppm).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: Two stainless steel columns, one is 4 mm in inside diameter and 25 mm in length and the other is 4 mm in inside diameter and 250 mm in length, they are packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter), and used them as the pre-column and the separation column, respectively.

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

Mobile phase: A mixture of water and acetonitrile (9:1).

Flow rate: 1.0 mL per minute.

Washing of pre-column: After each injection of the sample solution, wash the pre-column by passing the mobile phase backwards, at the same flow rate as applied in the test, for 30 minutes.

System suitability—

System performance: Dissolve 10 mg of 1-vinyl-2-pyrrolidone and 0.50 g of vinyl acetate in methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, 1-vinyl-2-pyrrolidone and vinyl acetate 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 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 1-vinyl-2-pyrrolidone is not more than 2.0%.

(4) Peroxides—

Method 1: Apply to the sample labeled as type A. Suspend 4.0 g of Crospovidone in 100 mL of water, and use as the sample suspension. To 25 mL of the sample suspension add 2 mL of titanium (III) chloride-sulfuric acid TS, allow to stand for 30 minutes, and filter. Determine the absorbance of the filtrate at 405 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the control, prepared by filtrating the sample suspension and adding 2 mL of diluted sulfuric acid (13 in 100) to 25 mL of this filtrate: not more than 0.35 (not more than 400 ppm expressed as hydrogen peroxide).

Method 2: Apply to the sample labeled as type B. Suspend 2.0 g of Crospovidone in 50 mL of water, and use as the sample suspension. To 10 mL of the sample suspension add water to make 25 mL, add 2 mL of titanium (III) chloride-sulfuric acid TS, allow to stand for 30 minutes, and filter. Determine the absorbance of the filtrate at 405 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the control, prepared by filtrating the sample suspension, adding water to 10 mL of this filtrate to make 25 mL and 2 mL of diluted sulfuric acid (13 in 100): not more than 0.35 (not more than 1000 ppm expressed as hydrogen peroxide).

Loss on drying <*2.41*> Not more than 5.0% (0.5 g, 105°C, constant mass).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of Crospovidone, place in a Kjeldahl flask, add 5 g of a powdered mixture of 33 g of potassium sulfate, 1 g of copper (II) sulfate pentahydrate and 1 g of titanium (IV) oxide, and 3 glass beads. Wash any adhering particles from the neck into the flask with a small quantity of water. Add 7 mL of sulfuric acid, allowing it to run down the inside wall of the flask. Gradually heat the flask until the solution has a clear, yellowish-green color, and the inside wall of the flask is free from carbonized material, and then heat for a further 45 minutes. After cooling, cautiously add 20 mL of water, and connect the flask to the distillation apparatus previously washed by passing steam through it. To the absorption flask add 30 mL of a solution of boric acid (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube. Add 30 mL of a solution of sodium hydroxide (21 in 50) through a funnel, cautiously rinse the funnel with 10 mL of water, immediately close the clamp attached to the rubber tube, then start the distillation with steam to obtain 80 – 100 mL of distillate. Remove the absorption flask from the lower end of the condenser tube, rinsing the end part with a small quantity of water, and titrate <*2.50*> the distillate with 0.025 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish-blue to pale grayish red-purple. Carry out a blank determination and make any necessary correction.

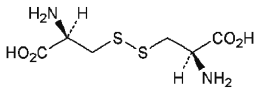
Each mL of 0.025 mol/L sulfuric acid VS = 0.7003 mg of N

Containers and storage Containers—Tight containers.

Add the following:

L-Cystine

L-シスチン



$C_6H_{12}N_2O_4S_2$: 240.30
3,3′-Disulfanediylbis[(2*R*)-2-aminopropanoic acid]
[*56-89-3*]

L-Cystine, when dried, contains not less than 99.0% and not more than 101.0% of $C_6H_{12}N_2O_4S_2$.

Description L-Cystine occurs as white crystals or crystalline powder.

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

It dissolves in 1 mol/L hydrochloric acid TS.

Identification Determine the infrared absorption spectrum of L-Cystine 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*> $[\alpha]_D^{20}$: −215 – −225° (after drying, 1 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of L-Cystine in 10 mL of 2 mol/L hydrochloric acid TS is clear and colorless.

(2) Chloride <*1.03*>—Dissolve 0.5 g of L-Cystine in 10 mL of dilute nitric acid, add 10 mL of hydrogen peroxide (30), and heat in a water bath for 10 minutes. After cooling, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <*1.14*>—Dissolve 0.6 g of L-Cystine in 5 mL of dilute hydrochloric acid, add water to make 45 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 5 mL of dilute hydrochloric acid and water to make 45 mL. To both the test and control solutions add 5 mL of barium chloride TS (not more than 0.028%).

(4) Ammonium <*1.02*>—Perform the test with 0.25 g of L-Cystine, using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <*1.07*>—Proceed with 1.0 g of L-Cystine according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <*1.10*>—Prepare the test solution with 1.0 g of L-Cystine according to Method 3, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.20 g of L-Cystine in 20 mL of 1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 10 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 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 and ammonia solution (28) (67:33) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100) on the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

(8) Residual solvent Being specified separately.

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 30 mg of L-Cystine, previously dried, and perform the test as directed under Nitrogen Determination <*1.08*>.

Each mL of 0.005 mol/L sulfuric acid VS
= 1.202 mg of $C_6H_{12}N_2O_4S_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Daunorubicin Hydrochloride

ダウノルビシン塩酸塩

Change the origin/limits of content, Identification and Purity (3) as follows:

Daunorubicin Hydrochloride is the hydrochloride of an anthracycline substance having antitumor activity produced by the growth of *Streptomyces peucetius* or *Streptomyces coeruleorubidus*.

It contains not less than 940 μg (potency) and not more than 1050 μg (potency) per mg, calculated on the dried basis. The potency of Daunorubicin Hydrochloride is expressed as mass (potency) of daunorubicin hydrochloride ($C_{27}H_{29}NO_{10}$.HCl).

Identification **(1)** Determine the absorption spectrum of a solution of Daunorubicin Hydrochloride in methanol (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 Daunorubicin 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 Daunorubicin Hydrochloride 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 Daunorubicin Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Daunorubicin Hydrochloride (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for chloride.

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 the peaks, 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 0.1%. 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 from 5 μL of this solution is equivalent to 7 to 13% of that of daunorubicin obtained from 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%.

Dibekacin Sulfate

ジベカシン硫酸塩

Change the Purity (1) as follows:

Purity (1) Clarity and color of solution—Dissolve 3.0 g of Dibekacin Sulfate in 10 mL of water: the solution is clear. Determine the absorbance of this solution at 400 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: not more than 0.15.

Diethylcarbamazine Citrate Tablets

ジエチルカルバマジンクエン酸塩錠

Add the following next to the Uniformity of dosage units:

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 Diethylcarbamazine Citrate Tablets is not less than 80%.

Start the test with 1 tablet of Diethylcarbamazine Citrate 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 56 μg of diethylcarbamazine citrate ($C_{10}H_{21}N_3O.C_6H_8O_7$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Diethylcarbamazine Citrate RS, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 25 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*⟩, and determine the peak areas, A_T and A_S, of diethylcarbamazine from each solution.

Dissolution rate (%) with respect to the labeled amount of diethylcarbamazine citrate ($C_{10}H_{21}N_3O.C_6H_8O_7$)

$= M_S \times A_T/A_S \times V'/V \times 1/C \times 225$

M_S: Amount (mg) of Diethylcarbamazine Citrate RS

C: Labeled amount (mg) of diethylcarbamazine citrate ($C_{10}H_{21}N_3O.C_6H_8O_7$) in 1 tablet

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: 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 diethylcarbamazine 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 diethylcarbamazine is not more than 2.0%.

1% Dihydrocodeine Phosphate Powder

ジヒドロコデインリン酸塩散 1%

Add the following next to the Identification:

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 1% Dihydrocodeine Phosphate Powder is not less than 85%.

Start the test with about 1 g of 1% Dihydrocodeine Phosphate Powder, accurately weighed, 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 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of dihydrocodeine phosphate for assay (separately determine the loss on drying ⟨*2.41*⟩ at 105°C for 4 hours), and dissolve in 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 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 dihydrocodeine of each solution.

Dissolution rate (%) with respect to the labeled amount of dihydrocodeine phosphate ($C_{18}H_{23}NO_3.H_3PO_4$)

$= M_S/M_T \times A_T/A_S \times 9/5$

M_S: Amount (mg) of dihydrocodeine phosphate for assay, calculated on the dried basis

M_T: Amount (g) of 1% Dihydrocodeine Phosphate Powder

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 dihydrocodeine 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 dihydrocodeine is not more than 2.0%.

10% Dihydrocodeine Phosphate Powder

ジヒドロコデインリン酸塩散10%

Add the following next to the Identification:

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 10% Dihydrocodeine Phosphate Powder is not less than 85%.

Start the test with about 0.1 g of 10% Dihydrocodeine Phosphate Powder, accurately weighed, 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, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 22 mg of dihydrocodeine phosphate for assay (separately determine the loss on drying ⟨*2.41*⟩ at 105°C for 4 hours), and dissolve in water to make exactly 100 mL. Pipet 5 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 dihydrocodeine of each solution.

Dissolution rate (%) with respect to the labeled amount of dihydrocodeine phosphate ($C_{18}H_{23}NO_3.H_3PO_4$)

$= M_S/M_T \times A_T/A_S \times 9/20$

M_S: Amount (mg) of dihydrocodeine phosphate for assay, calculated on the dried basis

M_T: Amount (g) of 10% Dihydrocodeine Phosphate Powder

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 dihydrocodeine 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 dihydrocodeine is not more than 2.0%.

Donepezil Hydrochloride

ドネペジル塩酸塩

Change the Description as follows:

Description Donepezil Hydrochloride occurs as a white crystalline powder.

It is soluble in water, and slightly soluble in ethanol (99.5).

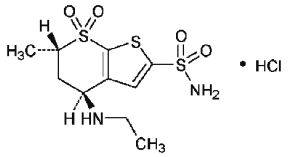
A solution of Donepezil Hydrochloride (1 in 100) shows no optical rotation.

Donepezil Hydrochloride shows crystal polymorphism.

Add the following:

Dorzolamide Hydrochloride

ドルゾラミド塩酸塩



$C_{10}H_{16}N_2O_4S_3.HCl$: 360.90

(4*S*,6*S*)-4-Ethylamino-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide monohydrochloride

[*130693-82-2*]

Dorzolamide Hydrochloride contains not less than 99.0 % and not more than 101.0 % of $C_{10}H_{16}N_2O_4S_3.HCl$, calculated on the anhydrous basis.

Description Dorzolamide Hydrochloride occurs as a white crystalline powder.

It is soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (99.5).

It dissolves in diluted ammonia solution (28) (13 in 400).

Optical rotation $[\alpha]_{404.7}^{25}$: -16.0 – $-17.5°$ (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Dorzolamide Hydrochloride shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Dorzolamide Hydrochloride in a solution of hydrochloric acid in methanol (9 in 1000) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Dorzolamide 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 Dorzolamide Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spec-

trum or the spectrum of Dorzolamide Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Dorzolamide Hydrochloride (1 in 100) responds to the Qualitative Tests <*1.09*> for chloride.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Dorzolamide 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 30 mg of Dorzolamide Hydrochloride in 50 mL of a mixture of water and methanol (4:1), 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 conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peaks other than dorzolamide is not more than 0.1%.

Operating conditions—

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

Mobile phase A: Adjust to pH 4.5 of a mixture of water and acetic acid (100) (1000:1) with 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 – 10	100	0
10 – 30	100 → 50	0 → 50

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

System suitability—

Test for required detectability: Pipet 2 mL of the sample solution, and add a mixture of water and methanol (4:1) to make exactly 100 mL. Pipet 1 mL of this solution, add a mixture of water and methanol (4:1) to make exactly 20 mL, and use this solution as the solution for system suitability test. Confirm that the peak area of dorzolamide obtained with 10 μL of the solution for system suitability test is equivalent to 0.07 to 0.13% of that with 10 μL of the sample solution.

System performance: To 1 mL of the sample solution add 2 mL of a mixture of water and methanol (4:1). 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 dorzolamide are not less than 4000 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 operating conditions, the relative standard deviation of the peak area of dorzolamide is not more than 7%.

(3) Optical isomer—Dissolve 20 mg of Dorzolamide Hydrochloride in 4 mL of diluted ammonia solution (28) (13 in 400), and extract this solution with two 4-mL portions of ethyl acetate. Combine the extracts, and evaporate the ethyl acetate at 50°C under a current of nitrogen. Dissolve the residue in 3 mL of acetonitrile, add 3 drops of (*S*)-1-phenylethyl isocyanate, and allow to stand at 50°C for 10 minutes. Evaporate at 50°C under a current of nitrogen, dissolve the residue in 10 mL of a mixture of *tert*-butylmethyl ether, acetic acid (100) and acetonitrile (873:100:27), 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 peak areas of dorzolamide, A_2, and that of the optical isomer, having the relative retention time of about 1.5 to dorzolamide, A_1, by the automatic integration method: the result of $A_1/(A_1 + A_2)$ is not more than 0.005.

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 silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: To a mixture of 30 mL of acetonitrile and 3 mL of water add *tert*-butylmethyl ether to make 1000 mL. To 650 mL of this solution add 350 mL of heptane.

Flow rate: Adjust the flow rate so that the retention time of dorzolamide is about 8 minutes.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add a mixture of *tert*-butylmethyl ether, acetic acid (100) and acetonitrile (873:100:27) to make exactly 200 mL, and use this solution as the solution for system suitability test. Confirm that the peak area of dorzolamide obtained with 5 μL of the solution for system suitability test is equivalent to 0.4 to 0.6% of that with 5 μL of the sample solution.

System performance: When the procedure is run with 5 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of dorzolamide are not less than 4000 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of dorzolamide is not more than 7%.

(4) Residual solvent Being specified separately.

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 20 mg each of Dorzolamide Hydrochloride and Dorzolamide Hydrochloride RS (separately, determine the water <*2.48*> in the same manner as Dorzolamide Hydrochloride), dissolve in a mixture of water and methanol (4:1) to make exactly 100 mL, and use these solutions as the sample solution and the standard solu-

tion, 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 dorzolamide in both solutions.

Amount (mg) of dorzolamide hydrochloride ($C_{10}H_{16}N_2O_4S_3$.HCl)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of Dorzolamide Hydrochloride 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 8.3 cm in length, packed with octylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

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

Mobile phase: Adjust to pH 4.5 of a mixture of water and acetic acid (100) (1000:1) with triethylamine.

Flow rate: Adjust the flow rate so that the retention time of dorzolamide 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 dorzolamide are not less than 4000 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 dorzolamide is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Add the following:

Dorzolamide Hydrochloride Ophthalmic Solution

ドルゾラミド塩酸塩点眼液

Dorzolamide Hydrochloride Ophthalmic Solution is an aqueous ophthalmic preparations.

It contains not less than 95.0% and not more than 107.0% of the labeled amount of dorzolamide ($C_{10}H_{16}N_2O_4S_3$: 324.44).

Method of preparation Prepare as directed under Ophthalmic preparations, with Dorzolamide Hydrochloride.

Description Dorzolamide Hydrochloride Ophthalmic Solution occurs as a clear and colorless liquid.

Identification To a volume of Dorzolamide Hydrochloride Ophthalmic Solution, equivalent to about 1.2 mg of dorzolamide ($C_{10}H_{16}N_2O_4S_3$), add 0.1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 252 nm and 256 nm.

pH Being specified separately.

Purity cis-Isomer—Use the sample solution obtained in the Assay 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, and determine the peak area of dorzolamide, A_2, and that of cis-isomer, having the relative retention time of about 1.1 to dorzolamide, A_1, by the automatic integration method: $A_1/(A_1 + A_2)$ is not larger than 0.020.

Diluting solution: To 2 mL of phosphoric acid add 900 mL of water, adjust to pH 3.0 with triethylamine, then add water to make 1000 mL.

Operating conditions—

Proceed as directed in the operation conditions in the Assay.

System suitability—

Test for required detectability: To exactly 2 mL of the sample solution add the diluting solution to make exactly 100 mL. Pipet 1 mL of this solution, add the diluting solution to make exactly 20 mL, and use this solution as the solution for system suitability test. Confirm that the peak area of dorzolamide obtained with 20 μL of the solution for system suitability test is equivalent to 0.07 to 0.13% of that with 20 μL of the sample solution.

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

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 dorzolamide is not more than 7%.

Foreign insoluble matter <*6.11*> It meets the requirement.

Insoluble particulate matter <*6.08*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Direct inoculation method, using the culture medium containing 0.7% polysorbate 80 and 0.1% of lecithin: it meets the requirement.

Assay Weigh accurately a portion of Dorzolamide Hydrochloride Ophthalmic Solution, equivalent to about 5 mg of dorzolamide ($C_{10}H_{16}N_2O_4S_3$), add the diluting solution to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Dorzolamide Hydrochloride RS (separately determine the water <*2.48*> in the same manner as Dorzolamide Hydrochloride), dissolve in the diluting solution 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 area of dorzolamide, A_T and A_S, of these solutions.

Diluting solution: To 2 mL of phosphoric acid add 900 mL of water, adjust to pH 3.0 with triethylamine, then add water to make 1000 mL.

$$\text{Amount (mg/mL) of dorzolamide } (C_{10}H_{16}N_2O_4S_3) = M_S/M_T \times A_T/A_S \times 1/4 \times d \times 0.899$$

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

M_T: Amount (g) of Dorzolamide Hydrochloride Ophthalmic Solution

d: Density (g/mL) of Dorzolamide Hydrochloride Ophthalmic Solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 253 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 25°C.

Mobile phase: A mixture of the diluting solution and acetonitrile (19:1).

Flow rate: Adjust the flow rate so that the retention time of dorzolamide 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 dorzolamide are not less than 6000 and not more than 1.8, respectively.

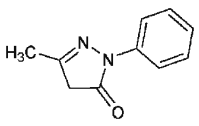
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dorzolamide is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Edaravone

エダラボン



$C_{10}H_{10}N_2O$: 174.20

5-Methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one

[*89-25-8*]

Edaravone, when dried, contains not less than 99.0% and not more than 101.0% of $C_{10}H_{10}N_2O$.

Description Edaravone occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in ethanol (99.5) and in acetic acid (100), and slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Edaravone (1 in 200,000) 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 Edaravone, 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.

pH <2.54> The pH of a solution obtained by dissolving 20 mg of Edaravone in 20 mL of water is between 4.0 and 5.5.

Melting point <2.60> 127 – 131°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Edaravone 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 Edaravone in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase 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. Determine each peak area by the automatic integration method: the area of the peak other than edaravone obtained from the sample solution is not larger than the peak area of edaravone from the standard solution.

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, methanol and acetic acid (100) (100:100:1).

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

Time span of measurement: About 7 times as long as the retention time of edaravone, beginning after the solvent peak.

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 edaravone are not less than 1500 and

not more than 1.4, 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 peak area of edaravone is not more than 2.0%.

(3) Residual solvent Being specified separately.

Loss on drying ⟨*2.41*⟩ Not more than 0.1% (1 g, in vacuum, phosphorus (V) oxide, 3 hours).

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

Assay Weigh accurately about 0.2 g of Edaravone, previously dried, dissolve in 40 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
= 17.42 mg of $C_{10}H_{10}N_2O$

Containers and storage Containers—Well-closed containers.

Add the following:

Edaravone Injection

エダラボン注射液

Edaravone Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of edaravone ($C_{10}H_{10}N_2O$: 174.20).

Method of preparation Prepare as directed under Injections, with Edaravone.

Description Edaravone Injection occurs as a clear and colorless liquid.

Identification To a volume of Edaravone Injection, equivalent to 1.5 mg of Edaravone, add water to make 50 mL. To 5 mL of this solution add water to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 238 nm and 242 nm.

pH Being specified separately.

Purity Related substance—(i) Use Edaravone Injection as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase 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. Determine each peak area by the automatic integration method: the area of the peak other than edaravone obtained from the sample solution is not larger than 2 times the peak area of edaravone 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 Edaravone.

Time span of measurement: About 7 times as long as the retention time of edaravone, beginning after the peak of edaravone.

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 edaravone are not less than 1500 and not more than 1.4, 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 peak area of edaravone is not more than 2.0%.

(ii) Use Edaravone Injection as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase 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. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.3 to edaravone, obtained from the sample solution is not larger than 4 times the peak area of edaravone from the standard solution, the area of the peak, having the relative retention time of about 0.4 to edaravone, is not larger than the peak area of edaravone from the standard solution, and the area of the peak other than edaravone and the peaks mentioned above is not larger than 2 times the peak area of edaravone from the standard solution.

Operating conditions—

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

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

Flow rate: Adjust the flow rate so that the retention time of edaravone is about 11 minutes.

Time span of measurement: About 2.5 times as long as the retention time of edaravone, beginning after the solvent peak.

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 edaravone are not less than 2000 and not more than 1.4, 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 peak area of edaravone is not more than 2.0%.

Bacterial endotoxins ⟨*4.01*⟩ Less than 5.0 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 an exact volume of Edaravone Injection, equivalent to about 3 mg of edaravone ($C_{10}H_{10}N_2O$) add exactly 10 mL of the internal standard solution, add methanol to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 75 mg of edaravone for assay, previously dried in vacuum for 3 hours using phosphorus (V) oxide as a desiccant, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, add methanol to make 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 under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of edaravone to that of the internal standard.

$$\text{Amount (mg) of edaravone } (C_{10}H_{10}N_2O) = M_S \times Q_T/Q_S \times 1/25$$

M_S: Amount (mg) of edaravone for assay

Internal standard solution—A solution of ethyl aminobenzoate in methanol (1 in 500).

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 50°C.

Mobile phase: A mixture of diluted dilute acetic acid (1 in 100) and methanol (3:1), adjusted to pH 5.5 with diluted ammonia solution (28) (1 in 20).

Flow rate: Adjust the flow rate so that the retention time of edaravone is about 8 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, edaravone and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

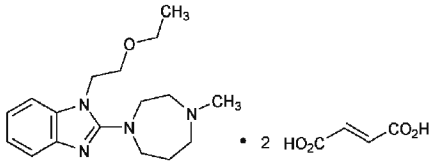
System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of edaravone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Add the following:

Emedastine Fumarate

エメダスチンフマル酸塩



$C_{17}H_{26}N_4O.2C_4H_4O_4$: 534.56

1-(2-Ethoxyethyl)-2-(4-methyl-1,4-diazepan-1-yl)-1*H*-benzoimidazole difumarate

[*87233-62-3*]

Emedastine Fumarate, when dried, contains not less than 98.5% and not more than 101.0% of $C_{17}H_{26}N_4O.2C_4H_4O_4$.

Description Emedastine Fumarate occurs as a white to pale yellow crystalline powder.

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

It shows crystal polymorphism.

Identification **(1)** Dissolve 10 mg of Emedastine Fumarate in 10 mL of water. To 2 mL of this solution add 1 mol/L hydrochloric acid TS 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: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Emedastine Fumarate 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 30 mg of Emedastine Fumarate in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 10 mg of fumaric acid 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropyl ether, formic acid and water (90:7:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the spot on the starting point from the sample solution and the spot from the standard solution show the same *R*f value.

Melting point ⟨*2.60*⟩ 149 – 152°C

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 2.0 g of Emedastine Fumarate 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 Emedastine Fumarate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 1 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 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 emedastine and fumaric acid obtained from the sample solution is not larger than the peak area of emedastine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 6.0 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 40°C.

Mobile phase: Dissolve 3.9 g of sodium dihydrogen phosphate dihydrate and 2.5 g of sodium lauryl sulfate in 1000 mL of water, and adjust to pH 2.4 with phosphoric acid. To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of emedastine is about 18 minutes.

Time span of measurement: About 2 times as long as the retention time of emedastine, beginning after the solvent peak.

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 emedastine are not less than 10,000 and not more than 1.2, 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 emedastine is not more than 2.0%.

(3) Residual solvent Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (0.5 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 Emedastine Fumarate, previously dried, dissolve in 80 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
= 26.73 mg of $C_{17}H_{26}N_4O.2C_4H_4O_4$

Containers and storage Containers—Tight containers.

Add the following:

Emedastine Fumarate Extended-release Capsules

エメダスチンフマル酸塩徐放カプセル

Emedastine Fumarate Extended-release Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of emedastine fumarate ($C_{17}H_{26}N_4O.2C_4H_4O_4$: 534.56).

Method of preparation Prepare as directed under Capsules, with Emedastine Fumarate.

Identification (1) Powder the content of Emedastine Fumarate Extended-release Capsules. To a portion of the powder, equivalent to 10 mg of Emedastine Fumarate, add 10 mL of water, shake thoroughly, and filter. Spot the filtrate on a filter paper, and spray Dragendorff's TS for spraying on the filter: the spot shows an orange color.

(2) To 2 mL of the filtrate obtained in (1) add 1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 278 nm and 282 nm, and between 284 nm and 288 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 Emedastine Fumarate Extended-release Capsules add 40 mL of the mobile phase, agitate with the aid of ultrasonic waves for 30 minutes while occasional vigorous shaking, and add the mobile phase to make exactly V mL so that each mL contains about 20 μg of emedastine fumarate ($C_{17}H_{26}N_4O.2C_4H_4O_4$). Centrifuge this solution, pipet 10 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.

Amount (mg) of emedastine fumarate ($C_{17}H_{26}N_4O.2C_4H_4O_4$)
$= M_S \times Q_T/Q_S \times V/1000$

M_S: Amount (mg) of emedastine fumarate for assay

Internal standard solution—A solution of 4-methyl-benzophenone in the mobile phase (1 in 40,000).

Dissolution Being specified separately.

Assay Weigh accurately the content of not less than 20 Emedastine Fumarate Extended-release Capsules, and powder. Weigh accurately a portion of the powder, equivalent to about 2 mg of emedastine fumarate ($C_{17}H_{26}N_4O.2C_4H_4O_4$), add 10 mL of the mobile phase, agitate with the aid of ultrasonic waves for 30 minutes while occasional vigorous shak-

ing, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of emedastine fumarate for assay, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make 100 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 50 mL. Then, pipet 10 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 emedastine to that of the internal standard.

$$\text{Amount (mg) of emedastine fumarate } (C_{17}H_{26}N_4O.2C_4H_4O_4) = M_S \times Q_T/Q_S \times 1/10$$

M_S: Amount (mg) of emedastine fumarate for assay

Internal standard solution—A solution of 4-methylbenzophenone in the mobile phase (1 in 40,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 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 3.9 g of sodium dihydrogen phosphate dihydrate and 2.5 g of sodium lauryl sulfate in 1000 mL of water, and adjust to pH 2.4 with phosphoric acid. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of emedastine 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, emedastine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

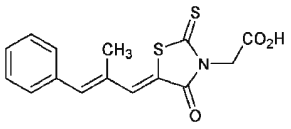
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of emedastine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Epalrestat

エパルレスタット



$C_{15}H_{13}NO_3S_2$: 319.40

2-{(5*Z*)-5-[(2*E*)-2-Methyl-3-phenylprop-2-en-1-ylidene]-4-oxo-2-thioxothiazolidin-3-yl}acetic acid

[*82159-09-9*]

Epalrestat, when dried, contains not less than 98.0% and not more than 101.0% of $C_{15}H_{13}NO_3S_2$.

Description Epalrestat occurs as yellow to orange, crystals or crystalline powder.

It is soluble in *N*,*N*-dimethylformamide, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It gradually fades the color and decomposes on exposure to light.

It shows crystal polymorphism.

Identification (1) Determine the absorption spectrum of a solution of Epalrestat in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Epalrestat 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 Epalrestat 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 Epalrestat RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the Reference Standard by separately specified method, dry them, and perform the test.

Melting point <*2.60*> 222 – 227°C

Purity (1) Heavy metals <*1.07*>—Proceed with 2.0 g of Epalrestat 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—Conduct this procedure using light-resistant vessels. Dissolve about 20 mg of Epalrestat in 8 mL of *N*,*N*-dimethylformamide, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add *N*,*N*-dimethylformamide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 3 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 epalrestat obtained from the sample solution is not larger than 1/5 times the peak area of epalrestat from the standard solution, and the total area of the peaks other than epalrestat obtained from the sample solution is not larger than the peak area of epalrestat 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 epalrestat, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add *N*,*N*-dimethylformamide to make exactly 10 mL. Confirm that the peak area of epalrestat obtained with 3 μL of this solution is equivalent to 7 to 13% of that with 3 μL of the standard solution.

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of epalrestat are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of epalrestat is not more than 2.0%.

(3) Residual solvent Being specified separately.

Loss on drying ⟨*2.41*⟩ Not more than 0.2% (1 g, in vacuum, silica gel, 60°C, 3 hours).

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

Assay Conduct this procedure using light-resistant vessels. Weigh accurately about 20 mg each of Epalrestat and Epalrestat RS, both previously dried, and separately dissolve in 8 mL of *N*,*N*-dimethylformamide, and add exactly 2 mL of the internal standard solution. To 2 mL each of these solutions add *N*,*N*-dimethylformamide to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 3 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 epalrestat to that of the internal standard.

$$\text{Amount (mg) of epalrestat } (C_{15}H_{13}NO_3S_2) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Epalrestat RS

Internal standard solution—A solution of propyl parahydroxybenzoate in *N*,*N*-dimethylformamide (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 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: To 0.05 mol/L potassium dihydrogen phosphate TS add 0.05 mol/L disodium hydrogen phosphate TS so that the pH of this mixture is 6.5. To 2 volumes of this mixture add 1 volume of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of epalrestat is about 12 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the standard solution under the above operating conditions, epalrestat and the internal standard 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 3 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of epalrestat to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Epalrestat Tablets

エパルレスタット錠

Epalrestat Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of epalrestat ($C_{15}H_{13}NO_3S_2$: 319.40).

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

Identification **(1)** Powder Epalrestat Tablets. To a portion of the powder, equivalent to 50 mg of Epalrestat, add 100 mL of methanol, shake thoroughly, and filter. To 1 mL of the filtrate add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 235 nm and 239 nm, between 290 nm and 294 nm, and between 387 nm and 391 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 using light-resistant vessels. To 1 tablet of Epalrestat Tablets add exactly 30 mL of *N*,*N*-dimethylformamide, shake thoroughly to completely disintegrate the tablet, and centrifuge. Pipet 1 mL of the supernatant liquid, and add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet *V* mL of this solution, add exactly *V′* mL of *N*,*N*-dimethylformamide so that each mL contains about 4.2 μg of epalrestat ($C_{15}H_{13}NO_3S_2$), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Epalrestat RS, previously dried in vacuum at 60°C for 3 hours with silica gel as a desiccant, and dissolve

in exactly 30 mL of *N*,*N*-dimethylformamide. Pipet 1 mL of this solution, add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet 5 mL of this solution, add *N*,*N*-dimethylformamide to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 392 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of epalrestat ($C_{15}H_{13}NO_3S_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/4$

M_S: Amount (mg) of Epalrestat RS

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 Epalrestat Tablets is not less than 70%.

Conduct this procedure using light-resistant vessels. Start the test with 1 tablet of Epalrestat 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 epalrestat ($C_{15}H_{13}NO_3S_2$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Epalrestat RS, previously dried in vacuum at 60°C for 3 hours with silica gel as a desiccant, dissolve in 10 mL of *N*,*N*-dimethylformamide, 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, at 398 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 epalrestat ($C_{15}H_{13}NO_3S_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45/2$

M_S: Amount (mg) of Epalrestat RS
C: Labeled amount (mg) of epalrestat ($C_{15}H_{13}NO_3S_2$) in 1 tablet

Assay Conduct this procedure using light-resistant vessels. Weigh accurately the mass of not less than 20 Epalrestat Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of epalrestat ($C_{15}H_{13}NO_3S_2$), add 20 mL of *N*,*N*-dimethylformamide, add exactly 5 mL of the internal standard solution, shake, and centrifuge. To 2 mL of the supernatant liquid add *N*,*N*-dimethylformamide to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Epalrestat RS, previously dried in vacuum at 60°C for 3 hours with silica gel as a desiccant, dissolve in 8 mL of *N*,*N*-dimethylformamide, add exactly 2 mL of the internal standard solution, and shake. To 2 mL of this solution add *N*,*N*-dimethylformamide to make 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Epalrestat.

Amount (mg) of epalrestat ($C_{15}H_{13}NO_3S_2$)
$= M_S \times Q_T/Q_S \times 5/2$

M_S: Amount (mg) of Epalrestat RS

Internal standard solution—A solution of propyl parahydroxybenzoate in *N*,*N*-dimethylformamide (1 in 100).

Containers and storage Containers—Tight containers.

10% Ephedrine Hydrochloride Powder

エフェドリン塩酸塩散10%

Add the following next to the Identification:

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 10% Ephedrine Hydrochloride Powder is not less than 85%.

Start the test with about 0.25 g of 10% Ephedrine Hydrochloride Powder, accurately weighed, 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, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, and 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 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 ephedrine of each solution.

Dissolution rate (%) with respect to the labeled amount of ephedrine hydrochloride ($C_{10}H_{15}NO.HCl$)
$= M_S/M_T \times A_T/A_S \times 9/10$

M_S: Amount (mg) of ephedrine hydrochloride for assay
M_T: Amount (g) of 10% Ephedrine Hydrochloride Powder

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (4) under Ephedrin Hydrochloride.

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 ephedrine are not less than 10,000 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

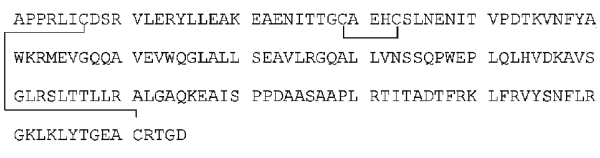
area of ephedrine is not more than 2.0%.

Add the following:

Epoetin Alfa (Genetical Recombination)

エポエチン　アルファ（遺伝子組換え）

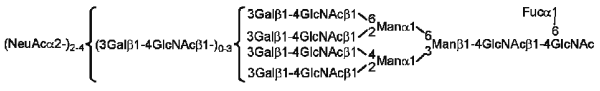
Protein moiety



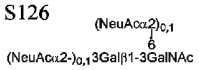
N24, N38, N83 and S126 : glycosylation

Carbohydrate moiety (structure of major glycans)

N24, N38 and N83



S126



$C_{809}H_{1301}N_{229}O_{240}S_5$: 18235.70 (Protein moiety)
[*113427-24-0*]

Epoetin Alfa (Genetical Recombination) is an aqueous solution in which a desired product is a recombinant human erythropoietin produced in Chinese hamster ovary cells. It is a glycoprotein (molecular weight: ca. 37,000 to 42,000) consisting of 165 amino acid residues. It has stimulatory effects for the differentiation and proliferation of erythroid precursor.

It contains not less than 1.1 mg and not more than 1.5 mg of protein per mL, and not less than 1.5×10^5 units per mg of protein.

Description Epoetin Alfa (Genetical Recombination) occurs as a clear and colorless liquid.

Identification Dilute a suitable volume of Epoetin Alfa (Genetical Recombination) and Epoetin Alfa RS with water. To 3 volume of these solutions add 1 volume each of buffer solution for epoetin alfa sample, heat at 100°C for 5 minutes, and use these solutions as the sample solution and the standard solution, respectively. Transfer a volume of the sample solution and the standard solution, equivalent to 0.7 μg of protein, into each sample well of the polyacrylamide gel for epoetin alfa, and start the SDS-polyacrylamide gel electrophoresis using a vertical discontinuous buffer solution system. After the electrophoresis, immerse the gel, a polyvinylidene fluoride membrane and a filter paper in the blotting TS. Set them on a semi-dry blotting apparatus, and transcribe for about 1 hour with a constant electric current of 0.7 – 0.9 mA/cm^2 depending on the dimension of the filter paper. Then, immerse the polyvinylidene fluoride membrane in the blocking TS for epoetin alfa for more than 1 hour while shaking, remove the blocking TS for epoetin alfa and add the primary antibody TS, then shake for a night or allow to stand at 4°C for 3 nights. Remove the primary antibody TS, wash the membrane with phosphate-buffered sodium chloride TS, add the secondary antibody TS, and shake for more than 1 hour. Remove the secondary antibody TS, wash the membrane with phosphate-buffered sodium chloride TS, add the avidin-biotin TS, and shake for more than 1 hour. Remove the avidin-biotin TS, wash the membrane with phosphate-buffered sodium chloride TS, and add the substrate TS for epoetin alfa for developing the color image: the main stained bands obtained from the sample solution appear as similar migrating image as those obtained from the standard solution.

Peptide mapping Evaporate to dryness under reduced pressure a volume of Epoetin Alfa (Genetical Recombination) and Epoetin Alfa RS, equivalent to about 35 μg of protein, and dissolve these residues in 100 μL of 0.1 mol/L tris buffer solution, pH 7.3. To these solutions add 5 μL of trypsin TS for epoetin alfa, warm at 37°C for 6 hours, then cool in ice, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 45 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the chromatograms obtained from these solutions: both chromatograms show the similar peaks at the corresponding retention time.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 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 45°C.

Mobile phase A: A mixture of water and trifluoroacetic acid (5000:3).

Mobile phase B: A mixture of acetonitrile, water and trifluoroacetic acid (4000:1000:3).

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	98	2
5 – 95	98 → 35	2 → 65

Flow rate: 0.75 mL per minute.

System suitability—

System performance: When the procedure is run with 45 μL of the standard solution under the above conditions, the chromatogram shows the similar pattern with the chromatogram of Epoetin Alfa RS obtained in the Peptide mapping.

Sugar chain profile Being specified separately.

Sialic acid content To an exact volume of Epoetin Alfa (Genetical Recombination), equivalent to about 1 nmol of protein, add water to make exactly 45 μL. Add exactly 5 μL of sodium hydroxide TS, allow to stand in ice water for 90 minutes, and add exactly 5 μL of dilute acetic acid. Add exactly 45 μL of water and exactly 100 μL of a mixture of water and acetic acid (100) (27:8), and warm at 80°C for 210 minutes. After cooling, add exactly 200 μL of the fluorescence TS, and warm at 60°C for 2 hours avoiding exposure to light. After cooling, add exactly 200 μL of sodium hydroxide TS, and use this solution as the sample solution. Separately, just before starting the test, to exactly 250 μL of 0.4 mmol/L *N*-acetylneuraminic acid TS add exactly 20 μL of 0.1 mmol/L *N*-glycolylneuraminic acid TS and exactly 180 μL of water. Proceed with exactly 45 μL of this solution in the same manner as for the sample solution, and use the solution so obtained 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 of *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid, A_{T1} and A_{T2}, obtained from the sample solution, and the peak areas of those, A_{S1} and A_{S2}, obtained from the standard solution. Calculate the content of sialic acid in Epoetin Alfa (Genetical Recombination) by the following equation: between 10 mol/mol and 12 mol/mol.

$$\text{Content (mol/mol) of sialic acid} = (A_{T1}/A_{S1} \times 10 + A_{T2}/A_{S2} \times 1/5)/a$$

a: Number (nmol) of moles of sample

where, molar concentration (mmol/L) of Epoetin Alfa (Genetical Recombination) is calculated by the following equation, using the absorbance A at 280 nm obtained in the Assay (1).

$$\text{Molar concentration (mmol/L) of Epoetin Alfa (Genetical Recombination)} = A \times 10^3/22{,}430$$

22,430: Molar absorbance coefficient ε

Operating conditions—

Detector: A fluorophotometer (excitation wavelength: 373 nm, fluorescence wavelength: 448 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 A: A mixture of water, acetonitrile and methanol (84:9:7).

Mobile phase B: A mixture of water and methanol (1:1).

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 – 20	100	0
20 – 20.1	100 → 0	0 → 100
20.1 – 27	0	100

Flow rate: 0.6 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, *N*-glycolylneuraminic acid and *N*-acetylneuraminic acid 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 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of *N*-glycolylneuraminic acid and *N*-acetylneuraminic acid are not more than 2.0%, respectively.

Molecular mass Use the sample solution obtained in the Identification as the sample solution. Separately, to 20 μL of molecular mass standard stock solution add 6.7 μL of the buffer solution for epoetin alfa sample, heat at 100°C for 5 minutes, and use this solution as the molecular mass standard solution. Transfer a volume of the sample solution, equivalent to 3.5 μg of protein and the total volume of the molecular mass standard solution into each sample well of the vertical discontinuous buffer solution system SDS-polyacrylamide gel, composed with resolving and stacking gels, and perform the electrophoresis. After the electrophoresis, immerse the gel in a solution of Coomassie brilliant blue R-250, containing 1.25 g in a mixture of 450 mL of methanol, 100 mL of acetic acid (100) and sufficient amount of water making up to 1000 mL. Determine the relative mobilities of the stained bands of egg albumin (Mm: about 45,000), carbonic anhydrase (Mm: about 31,000), soybean trypsin inhibitor (Mm: about 21,500) and lysozyme (Mm: 14,400), and prepare a calibration curve by linear regression against the logarithm of the molecular masses. Determine the relative mobility of the center of the main band obtained from the sample solution, and calculate the molecular mass of Epoetin Alfa (Genetical Recombination) from the calibration curve: it is between 37,000 and 42,000.

pH <2.54> 5.7 – 6.7

Purity (1) Oligomers—Perform the test with a volume of Epoetin Alfa (Genetical Recombination), equivalent to 50 μg of protein, 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 total amount of the peaks other than epoetin alfa is not more than 2%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 7.5 mm in inside di-

ameter and 60 cm in length, packed with hydrophilic silica gel for liquid chromatography.

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

Mobile phase: Dissolve 91 mg of disodium hydrogen phosphate dodecahydrate, 0.27 g of sodium dihydrogen phosphate dihydrate and 8.77 g of sodium chloride in water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of epoetin alfa is about 16 minutes.

Time span of measurement: From the time corresponding to the exclusion volume of the size-exclusion column until the elution of epoetin alfa is finished.

System suitability—

Test for required detectability: To 1 volume of Epoetin Alfa (Genetical Recombination) add 49 volumes of the mobile phase, and use this solution as the solution for system suitability test. Confirm that the peak area of epoetin alfa obtained with a volume, equivalent to 1 μg of protein, of the solution for system suitability test is equivalent to 1.5 to 2.5 % of that obtained with the same volume of Epoetin Alfa (Genetical Recombination).

System performance: Dissolve 40 mg of bovine serum albumin for gel filtration molecular mass marker and 20 mg of chymotrypsinogen for gel filtration molecular mass marker in 100 mL of the mobile phase. When the procedure is run with 50 μL of this solution under the above operating conditions, bovine serum albumin and chymotrypsinogen 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 a volume of Epoetin Alfa (Genetical Recombination), equivalent to 50 μg of protein, under the above operating conditions, the relative standard deviation of the area of the principal peak of epoetin alfa is not more than 2.0%.

(2) Host-derived proteins Being specified separately.

(3) DNA Being specified separately.

Assay (1) Protein content—Take a suitable amount of Epoetin Alfa (Genetical Recombination), dilute with phosphate buffer solution for epoetin alfa, if necessary, so that each mL contains 0.5 – 0.8 mg protein and use as the sample solution. Determine the absorbance, A, at 280 nm of the sample solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the phosphate buffer solution for epoetin alfa as the blank.

Amount (mg) of protein in 1 mL of Epoetin Alfa (Genetical Recombination)
$= A \times d \times 0.909$

d: Dilution factor for the sample solution
0.909: Reciprocal number of absorption coefficient ($E_{1\,cm}^{0.1\%}$) of epoetin alfa protein

(2) Specific activity

(i) Animals: Select healthy 6 to 8 weeks female mice (B6D2F1, etc.). Keep the mice for not less than a week before use, providing an appropriate uniform diet and water.

(ii) Standard solutions: To Epoetin Alfa RS add the bovine serum albumin-saline solution so that each mL contains exactly 10 – 40 units, and designate this solution as the high-dose standard solution, S_H. Dilute S_H exactly 4 times with the bovine serum albumin-saline solution, and designate this solution as the low-dose standard solution, S_L.

(iii) Sample solutions: To Epoetin Alfa (Genetical Recombination) add the bovine serum albumin-saline solution to make two sample solutions, the high-dose sample solution, T_H, which contains the Units per mL equivalent to S_H and the low-dose sample solution, T_L, which contains the Units per mL equivalent to S_L.

(iv) Procedure: Divide the animals into 4 equal groups of not less than 5 animals each. On the 1st, 2nd and 3rd days, inject exactly 0.2 mL each of the standard solutions and the sample solutions into each animal subcutaneously as indicated in the following design:

First group	S_H	Third group	T_H
Second group	S_L	Fourth group	T_L

On the 4th day, take a sufficient blood sample to perform the test from each animal. To 10 mL of the dilution fluid for particle counter add exactly 20 μL of the blood sample, mix, add 100 μL of the appropriate hemolysis agent, stir for 5 minutes, and determine the count of particles derived from hemolytic-resistant erythroid cells.

(v) Calculation: Logarithmic converted counts of the fine particles obtained with S_H, S_L, T_H and T_L in (iv) are symbolized as y_1, y_2, y_3 and y_4, respectively. Sum up individual y_1, y_2, y_3 and y_4 to obtain Y_1, Y_2, Y_3 and Y_4, respectively.

Specific activity (unit/mg protein) of Epoetin Alfa (Genetical Recombination)
= activity (unit/mL) of Epoetin Alfa (Genetical Recombination)/C

Activity (unit/mL) of Epoetin Alfa (Genetical Recombination)
= antilog M × unit in 1 mL of S_H × d

$M = \log 4 \times Y_a/Y_b$
$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$
$Y_b = Y_1 - Y_2 + Y_3 - Y_4$

d: Dilution factor for T_H
C: Concentration (mg/mL) of protein obtained in Assay (1)

F' computed by the following equation should be smaller than F shown in the table against n with which s^2 is calculated. Calculate L (p = 0.95) by use of the following equation: L should be not more than 0.3. If F' exceeds F, or if L exceeds 0.3, repeat the test, arranging the assay conditions.

$F' = (Y_1 - Y_2 - Y_3 + Y_4)^2/4fs^2$

f: Number of animals per group, which should be the same for each group and not less than 5.

$s^2 = (\Sigma y^2 - Y/f)/n$

Σy^2: The sum of the squares of each y_1, y_2, y_3 and y_4.
$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$
$n = 4\,(f - 1)$

$$L = 2\sqrt{(C-1)\{CM^2 + (\log 4)^2\}}$$

$$C = Y_b^2/(Y_b^2 - 4fs^2t^2)$$

$F\ (= t^2)$ values against n

n	$t^2 = F$	n	$t^2 = F$	n	$t^2 = F$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, at not exceeding 10°C avoiding freezing.

Add the following:

Epoetin Beta (Genetical Recombination)

エポエチン　ベータ(遺伝子組換え)

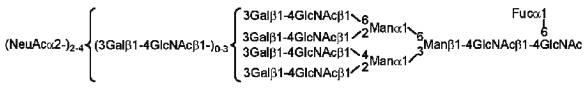
Protein moiety

APPRLICDSR VLERYLLEAK EAENITTGCA EHCSLNENIT VPDTKVNFYA
WKRMEVGQQA VEVWQGLALL SEAVLRGQAL LVNSSQPWEP LQLHVDKAVS
GLRSLTTLLR ALGAQKEAIS PPDAASAAPL RTITADTFRK LFRVYSNFLR
GKLKLYTGEA CRTGD

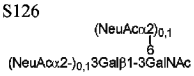
N24, N38, N83 and S126: glycosylation

Carbohydrate moiety (structure of major glycans)

N24, N38 and N83



S126



$C_{809}H_{1301}N_{229}O_{240}S_5$: 18235.70 (Protein moiety)
[*122312-54-3*]

Epoetin Beta (Genetical Recombination) is an aqueous solution in which a desired product is a recombinant human erythropoietin produced in Chinese hamster ovary cells. It is a glycoprotein (molecular weight: ca. 30,000) consisting of 165 amino acid residues. It has stimulatory effects for the differentiation and proliferation of erythroid progenitor cell.

It contains not less than 0.5 mg and not more than 1.5 mg of protein per mL, and not less than 1.5×10^5 units per mg of protein.

Description Epoetin Beta (Genetical Recombination) occurs as a clear and colorless liquid.

Identification (1) Use Epoetin Beta (Genetical Recombination) and Epoetin Beta RS as the sample solution and the standard solution, respectively. When perform a capillary electrophoresis with the sample solution and standard solution according to the following conditions, the mobility of each peak obtained from both solutions is the same and their migrating images are similar each other.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 200 nm).

Column: A silica capillary tube 50 μm in inside diameter and about 50 cm in length, chemically coated inner surface with amino groups (about 40 cm in effective length).

Electrolyte solution: Dissolve 32.8 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL, and adjust to pH 4.5 with a solution, prepared by dissolving 75.2 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. To 19 volumes of this solution add 1 volume of ethanol (99.5).

Running temperature: A constant temperature of about 20°C.

Running conditions: Migration current (a constant current of about 45 μA), migration time (30 minutes).

Injection of sample and standard solutions: 5 seconds (pressurization: 0.5 psi).

Time span of measurement: From 10 minutes to 30 minutes after injection (excluding the peak of solvent origin).

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, more than 4 major peaks of epoetin beta are detected, and the resolution between the first and second eluted major peaks is not less than 0.8.

System repeatability: When the test is repeated 3 times with the standard solution under the above operating conditions, the relative standard deviation of the migration time of the first eluted major peak is not more than 2.0%.

(2) Desalt a volume each of Epoetin Beta (Genetical Recombination) and Epoetin Beta RS, equivalent to 600 μg of protein, by a suitable method, and term them as the desalted sample and the desalted reference standard, respectively. Dissolve the desalted sample and the desalted reference standard in 600 μL each of a solution, prepared by dissolving 2.3 g of *N*-ethylmorpholine in 100 mL of water and adjusting to pH 8.0 with acetic acid (100), and use these solutions as the desalted sample solution and the desalted reference standard solution, respectively. To 500 μL each of the desalted sample solution and the desalted reference standard solution add 3.3 μL of triethylamine for epoetin beta and 1.5 μL of 2-mercaptoethanol for epoetin beta, and react at 37°C for 1 hour. After cooling, add 5.5 μL of 4-vinylpyri-

dine to them, and react at 25°C for 1 hour. To these solutions add 50 μL of diluted trifluoroacetic acid for epoetin beta (1 in 10) to stop the reaction, remove the reagents by a suitable method, and use the substances so obtained as the pyridylethylated sample and the pyridylethylated reference substance, respectively. Dissolve the pyridylethylated sample and the pyridylethylated reference substance separately in 500 μL of sodium hydrogen carbonate solution (21 in 2500). To 400 μL each of these solutions add 16 μL of a solution of lysyl endopeptidase in sodium hydrogen carbonate solution (21 in 2500) (1 in 50,000), and react at 37°C for 24 hours. While this reaction, additional two 16-μL portions of a solution of lysyl endopeptidase in sodium hydrogen carbonate (21 in 2500) (1 in 50,000) are added at 4 hours and 20 hours after starting the reaction. Then, stop the reaction by adding 100 μL of diluted trifluoroacetic acid for epoetin beta (1 in 10), and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the chromatograms obtained from these solutions: both chromatograms show the similar peaks at the corresponding retention times.

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 A: A mixture of water and trifluoroacetic acid for epoetin beta (1000:1).

Mobile phase B: A mixture of acetonitrile for liquid chromatography, water, and trifluoroacetic acid for epoetin beta (900:100:1).

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 – 10	90	10
10 – 30	90 → 80	10 → 20
30 – 50	80	20
50 – 130	80 → 40	20 → 60
130 – 140	40 → 10	60 → 90
140 – 150	10	90

Flow rate: Adjust the flow rate so that the retention time of the first peak, which appears after the solvent peak, is about 17 minutes.

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, nine major peptide peaks are appeared after the solvent peak, and the resolution between the peaks eluted at the fifth and the sixth is not less than 3.

(3) To exactly 100 μL of Epoetin Beta (Genetical Recombination) add 1 mL of resorcinol-copper (II) sulfate TS, and heat on a water bath for 30 minutes. After ice-cooling, add 2 mL of a mixture of *n*-butyl acetate and 1-buthanol (4:1), shake vigorously, and use the upper layer as the sample solution. Separately, dissolve *N*-acetylneuraminic acid in water to make three solutions, containing 0.1 mg, 0.2 mg and 0.3 mg of *N*-acetylneuraminic acid in each mL, and use these solutions as the standard stock solution (1), the standard stock solution (2) and the standard stock solution (3), respectively. Pipet 100 μL each of these standard stock solutions, add 1 mL of resorcinol-copper (II) sulfate TS to them, then proceed in the same way as for the sample solution, and use these solutions so obtained as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. Determine the absorbances of the sample solution and the standard solutions (1), (2) and (3) at 625 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Determine the amount of sialic acid (mg/mL) in the sample solution, by using the calibration curve obtained from the standard solutions, and calculate the amount of sialic acid in Epoetin Beta (Genetical Recombination) by the following equation: between 10 mol/mol and 13 mol/mol.

$$\text{Amount of sialic acid (mol/mol of epoetin beta protein)} = A/C \times 18{,}236/309.27$$

A: Amount (mg/mL) of sialic acid in the sample solution
C: Amount (mg/mL) of protein in Epoetin Beta (Genetical Recombination)
18,236: Molecular mass of protein moiety of epoetin beta
309.27: Molecular mass of *N*-acetylneuraminic acid

(4) Sugar chain profile Being specified separately.

pH <*2.54*> 7.0 – 8.0

Purity **(1)** Related substances—Perform the test with 20 μL of Epoetin Beta (Genetical Recombination) 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 other than the solvent peak by the area percentage method: the total area of the peaks other than epoetin beta is not more than 1.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with porous silica gel for liquid chromatography (10 μm in particle diameter).

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

Mobile phase: Dissolve 1.6 g of sodium dihydrogen phosphate dihydrate and 16.1 g of sodium sulfate decahydrate in water to make 1000 mL, and adjust to pH 6.8 with a solution, prepared by dissolving 16.1 g of sodium sulfate decahydrate in 0.01 mol/L sodium hydroxide TS to make 1000

mL.

Flow rate: Adjust the flow rate so that the retention time of epoetin beta is about 18 minutes.

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

System suitability—

Test for required detectability: When the procedure is run with 20 μL of diluted Epoetin Beta RS with water containing 0.05 vol% polysorbate 20 for epoetin beta (1 in 1000) under the above conditions, the peak of epoetin beta is detectable.

System performance: When the procedure is run with Epoetin Beta RS under the above conditions, the number of theoretical plates of the peak of epoetin beta is not less than 600.

System repeatability: When the test is repeated 6 times with 20 μL of Epoetin Beta RS under the above operating conditions, the relative standard deviation of the peak area of epoetin beta is not more than 1.0%.

(2) Host-derived proteins Being specified separately.

(3) DNA Being specified separately.

Assay (1) Protein content—Use Epoetin Beta (Genetical Recombination) and Epoetin Beta RS as the sample solution and the standard solution, respectively. 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 total area, A_T and A_S, of the main peak and the sub-peak of epoetin beta.

Amount (mg) of protein in 1 mL of Epoetin Beta (Genetical Recombination)

$= C_S \times A_T/A_S$

C_S: Protein concentration (mg/mL) of Epoetin Beta RS

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 butylsilanized 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 for liquid chromatography and trifluoroacetic acid for epoetin beta (400:100:1).

Mobile phase B: A mixture of acetonitrile for liquid chromatography, water and trifluoroacetic acid for epoetin beta (400:100:1).

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 – 18	65 → 50	35 → 50
18 – 33	50 → 0	50 → 100
33 – 43	0	100

Flow rate: Adjust the flow rate so that the retention time of the main peak of epoetin beta is about 22 minutes.

System suitability—

System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the main peak and the sub-peak of epoetin beta are eluted in this order, and the number of theoretical plates of the main peak is not less than 600.

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 total area of the main peak and the sub-peak of epoetin beta is not more than 4.0%.

(2) Specific activity—To Epoetin Beta (Genetical Recombination) add 0.1 w/v% bovine serum albumin-sodium chloride-phosphate buffer solution to make three solutions so that each mL contains epoetin beta equivalent to 5, 10 and 20 units (estimate), and use these solutions as the sample solutions (1), (2) and (3), respectively. Separately, to Epoetin Beta RS add 0.1 w/v% bovine serum albumin-sodium chloride-phosphate buffer solution to make three solutions so that each mL contains epoetin beta equivalent to 5, 10 and 20 units, and use these solutions as the standard solutions (1), (2) and (3), respectively. Divide ICR strain mice into 6 equal groups of not less than 5 mice. Inject exactly 0.2 mL each of the sample solutions and the standard solutions to ICR strain mice of each group subcutaneously on the 1st, 2nd and 3rd days. On the 4th day, collect the blood from the mice, put 20 μL each of the collected blood in 9.94 mL of blood dilution liquid, mix, and use these mixtures as the dilute blood solution. To each of the dilute blood solution add 100 μL of a hemolytic agent, mix gently to hemolyze, and count the particles of hemolytic agent-resistant red cell by using a particle counter.

Determine the potency ratio (P_r) of the sample solution to the standard solution, and calculate the unit per mg protein of Epoetin Beta (Genetical Recombination) by the following equation.

$$P_r = 10^M$$

$$M = 4/3 \times i \times T_a/T_b$$

$$i = \log 2$$

$$T_a = -S_1 - S_2 - S_3 + U_1 + U_2 + U_3$$

$$T_b = -S_1 + S_3 - U_1 + U_3$$

U_1: Sum of the responses obtained from the sample solution (1)

U_2: Sum of the responses obtained from the sample solution (2)

U_3: Sum of the responses obtained from the sample solution (3)

S_1: Sum of the responses obtained from the standard solu-

tion (1)
S_2: Sum of the responses obtained from the standard solution (2)
S_3: Sum of the responses obtained from the standard solution (3)

Specific activity (unit/mg of protein) of Epoetin Beta (Genetical Recombination)
$= S \times P_r \times D_T/D_S/C$

S: Potency (unit/mL) of Epoetin Beta RS
D_T: Dilution factor for the sample solution (3)
D_S: Dilution factor for the standard solution (3)
C: Protein amount (mg/mL) of Epoetin Beta (Genetical Recombination)

Containers and storage Containers—Tight containers.
Storage—Not exceeding −20°C.

Ethanol

エタノール

Change the Purity (4) to read:

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 smooth absorption curve.

Add the following next to the Containers and storage:

◆**Expiration date** In not glass containers: Unless otherwise specified, 24 months after preparation.◆

Anhydrous Ethanol

無水エタノール

Change the Purity (4) to read:

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 smooth absorption curve.

Add the following next to the Containers and storage:

◆**Expiration date** In not glass containers: Unless otherwise specified, 24 months after preparation.◆

Ethanol for Disinfection

消毒用エタノール

Change the Purity as follows:

Purity Proceed as directed in the Purity under Ethanol, with the exception of (4), which is changed as follows.

(4) Other impurities (absorbance)—Perform the test with Ethanol for Disinfection as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: 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. The absorption spectrum determined in a 5-cm cell using water as a blank shows a smooth absorption curve between 235 nm and 340 nm.

Ethyl Parahydroxybenzoate

パラオキシ安息香酸エチル

Change the Description and below as follows:

◆**Description** Ethyl Parahydroxybenzoate occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetone, and very slightly soluble in water.◆

Identification Determine the infrared absorption spectrum of Ethyl Parahydroxybenzoate 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 Ethyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 115 – 118°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Ethyl Parahydroxybenzoate in ethanol (95) to make 10 mL: the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Acidity—To 2 mL of the solution of Ethyl Parahydroxybenzoate obtained in (1) add 3 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mol/L sodium hydroxide VS until the solution shows a blue color: the volume of 0.1 mol/L sodium hydroxide VS used does not exceed 0.1 mL.

◆**(3)** Heavy metals <*1.07*>—Dissolve 1.0 g of Ethyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).◆

(4) Related substances—Dissolve 50 mg of Ethyl Parahydroxybenzoate in 2.5 mL of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 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 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 parahydroxybenzoic acid having a relative retention time of about 0.5 to ethyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of ethyl parahydroxybenzoate from the standard solution (0.5%). For this calculation use the peak area of parahydroxybenzoic acid after multiplying by the relative response factor, 1.4. Furthermore, the area of the peak other than ethyl parahydroxybenzoate and parahydroxybenzoic acid from the sample solution is not larger than the peak area of ethyl parahydroxybenzoate from the standard solution (0.5%), and the total area of the peaks other than ethyl parahydroxybenzoate is not larger than 2 times the peak area of ethyl parahydroxybenzoate from the standard solution (1.0%). For this calculation the peak area not larger than 1/5 times the peak area of ethyl parahydroxybenzoate from the standard solution is excluded (0.1%).

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 4 times as long as the retention time of ethyl parahydroxybenzoate.

System suitability—

◆Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ethyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.◆

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

◆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 ethyl parahydroxybenzoate is not more than 2.0%.◆

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Ethyl Parahydroxybenzoate and Ethyl Parahydroxybenzoate RS, dissolve separately in 2.5 mL each of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase 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 ethyl parahydroxybenzoate of each solution.

Amount (mg) of ethyl parahydroxybenzoate ($C_9H_{10}O_3$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Ethyl Parahydroxybenzoate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 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 35°C.

Mobile phase: A mixture of methanol and potassium dihydrogen phosphate solution (17 in 2500) (13:7).

Flow rate: 1.3 mL per minute.

System suitability—

System performance: Dissolve 5 mg each of Ethyl Parahydroxybenzoate, methyl parahydroxybenzoate and parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, parahydroxybenzoic acid, methyl parahydroxybenzoate and ethyl parahydroxybenzoate are eluted in this order, the relative retention times of parahydroxybenzoic acid and methyl parahydroxybenzoate to ethyl parahydroxybenzoate are about 0.5 and about 0.8, respectively, and the resolution between the peaks of methyl parahydroxybenzoate and ethyl parahydroxybenzoate is 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 ethyl parahydroxybenzoate is not more than 0.85%.

◆**Containers and storage** Containers—Well-closed containers.◆

Fexofenadine Hydrochloride

フェキソフェナジン塩酸塩

Change the Description as follows:

Description Fexofenadine Hydrochloride occurs as a white crystalline powder.

It is very soluble in methanol, soluble in ethanol (99.5), and slightly soluble in water.

A solution of Fexofenadine Hydrochloride in methanol (3

in 100) shows no optical rotation.

Fexofenadine Hydrochloride shows crystal polymorphism.

Add the following:

Fexofenadine Hydrochloride Tablets

フェキソフェナジン塩酸塩錠

Fexofenadine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl: 538.12).

Method of preparation Prepare as directed under Tablets, with Fexofenadine Hydrochloride.

Identification To an amount of powdered Fexofenadine Hydrochloride Tablets, equivalent to 40 mg of Fexofenadine Hydrochloride, add 100 mL of methanol, and shake well. Filter, discard the first 10 mL of the filtrate, and determine the absorption spectrum of the subsequent filtrate as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 257 nm and 261 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 Fexofenadine Hydrochloride Tablets add $V/5$ mL of diluted acetic acid (100) (17 in 10,000), shake until the tablet is disintegrated. Add $3V/5$ mL of acetonitrile for liquid chromatography, shake well, add a mixture of acetonitrile for liquid chromatography and diluted acetic acid (100) (17 in 10,000) (3:1) to make exactly V mL so that each mL contains about 0.3 mg of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl). Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and filter this solution 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 50 mg of Fexofenadine Hydrochloride RS (separately determine the water <*2.48*> in the same manner as Fexofenadine Hydrochloride), and dissolve in a mixture of acetonitrile for liquid chromatography and diluted acetic acid (100) (17 in 10,000) (3:1) to make exactly 200 mL. Pipet 6 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl)
$= M_S \times A_T/A_S \times 3V/500$

M_S: Amount (mg) of Fexofenadine 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 water as the dissolution medium, the dissolution rate in 30 minutes of Fexofenadine Hydrochloride Tablets is not less than 80%.

Start the test with 1 tablet of Fexofenadine 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 water to make exactly V' mL so that each mL contains about 30 μg of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Fexofenadine Hydrate RS (separately determine the water <*2.48*> in the same manner as Fexofenadine Hydrochloride), dissolve in 5 mL of methanol, add water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 50 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 fexofenadine in each solution.

Dissolution rate (%) with respect to the labeled amount of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

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

C: Labeled amount (mg) of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl) in 1 tablet

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 (5 μm in particle diameter).

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

Mobile phase: Dissolve 1.1 g of sodium dihydrogen phosphate dihydrate, 0.3 mL of phosphoric acid and 0.5 g of sodium perchlorate in 300 mL of water, add 700 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of fexofenadine is about 3.5 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 fexofenadine 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 fexofenadine is not more than 2.0%.

Assay To 20 Fexofenadine Hydrochloride Tablets add $V/5$

mL of diluted acetic acid (100) (17 in 10,000), and shake until the tablets are disintegrated. Then, add $3V/5$ mL of acetonitrile for liquid chromatography, shake well, and add a mixture of acetonitrile for liquid chromatography and diluted acetic acid (100) (17 in 10,000) (3:1) to make exactly V mL so that each mL contains about 1.2 mg of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl). Pipet 15 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase 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 45 mg of Fexofenadine Hydrochloride RS (separately determine the water <2.48> in the same manner as Fexofenadine Hydrochloride), and dissolve in a mixture of acetonitrile for liquid chromatography and diluted acetic acid (100) (17 in 10,000) (3:1) to make exactly 200 mL. Pipet 20 mL of this solution, add the mobile phase to make exactly 250 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 fexofenadine of each solution.

Amount (mg) of fexofenadine hydrochloride ($C_{32}H_{39}NO_4$.HCl) in 1 tablet
$= M_S \times A_T/A_S \times V/750$

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

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 phenylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 1000 mL of diluted acetic acid (100) (17 in 10,000) add 15 mL of a mixture of triethylamine and acetonitrile for liquid chromatography (1:1), and adjust to pH 5.25 with phosphoric acid. To 16 volumes of this solution add 9 volumes of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of fexofenadine 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, the number of theoretical plates and the symmetry factor of the peak of fexofenadine are not less than 7000 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 fexofenadine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Filgrastim (Genetical Recombination)

フィルグラスチム(遺伝子組換え)

MTPLGPASSL PQSFLLKCLE QVRKIQGDGA ALQEKLCATY KLCHPEELVL
LGHSLGIPWA PLSSCPSQAL QLAGCLSQLH SGLFLYQGLL QALEGISPEL
GPTLDTLQLD VADFATTIWQ QMEELGMAPA LQPTQGAMPA FASAFQRRAG
GVLVASHLQS FLEVSYRVLR HLAQP

$C_{845}H_{1339}N_{223}O_{243}S_9$: 18798.61
[*121181-53-1*]

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.

Description Filgrastim (Genetical Recombination) occurs as a clear and colorless liquid.

Identification Take a volume of Filgrastim (Genetical Recombination), equivalent to 5 to 10 μg of protein depending on the size of polyacrylamide gel for filgrastim, and add 10 μL of water. To 3 volumes of this solution add 1 volume of buffer solution for filgrastim sample, and use this solution as the sample solution. Separately, take a volume of Filgrastim RS which contains equal amount of protein to Filgrastim (Genetical Recombination) used above, proceed as directed for the sample solution, and use the solution so obtained as the standard solution. Set a polyacrylamide gel for filgrastim up to the electrophoresis apparatus, and put a necessary amount of buffer solution for SDS-polyacrylamide gel electrophoresis in the upper and lower reservoirs. Pipet the all amount of the sample solution and standard solution into each well of the gel, and start the electrophoresis setting the electrode of the lower reservoir as the anode. Stop the electrophoresis when the bromophenol blue band has been migrated to about the lower end of the gel. When stain the gel with a staining solution, which is prepared by dissolving 1.25 g of Coomassie brilliant blue R250 in a mixture of 450 mL of methanol, 100 mL of acetic acid (100) and water to make 1000 mL, stained bands obtained from the sample solution appear as similar migrating image at the same position as those obtained from the standard solution.

Peptide mapping Take a volume of Filgrastim (Genetical Recombination) and Filgrastim RS, equivalent to about 80 μg of protein, add 200 μL of the buffer solution for enzyme digestion, and add water to make 390 μL. To each of these solution add 10 μL of a solution containing 50 μg of V8 protease in 250 μL of water, incubate at 25°C for 17 to 19 hours, then add 18 μL of a mixture of water and trifluoroa-

cetic acid (19:1) to stop the reaction, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 70 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. When the chromatograms obtained from these solutions are compared, both chromatograms show the similar peaks at the same retention time, and the ratio of the area of the peaks that appear respectively at 8th among the major peaks obtained from the sample solution and standard solution, is 80 to 120%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 2.1 mm in inside diameter and 25 cm in length, packed with butylsilanized 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 and trifluoroacetic acid (1000:1).

Mobile phase B: A mixture of acetonitrile, water and trifluoroacetic acid (9000:1000:9).

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	98	2
2 – 30	98 → 70	2 → 30
30 – 85	70 → 50	30 → 50
85 – 90	50 → 2	50 → 98
90 – 100	2	98

Flow rate: 0.20 mL per minute.

System suitability—

System performance: When the procedure is run with 70 μL of the standard solution under the above conditions, the resolutions between each adjacent peakpair of the major 8 peaks, which are eluted after the solvent peak appeared within 10 minutes, are not less than 1.5.

pH <2.54> 3.7 – 4.3

Purity **(1)** Oligomers—Perform the test with 250 μL of Filgrastim (Genetical Recombination) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area by the automatic integration method. When their amounts are determined by the area percentage method; the total amount of the peaks other than filgrastim is not more than 2%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with hydrophilic silica gel for liquid chromatography.

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

Mobile phase: Dissolve 5.8 g of sodium chloride in 10 mL of dilute acetic acid and 900 mL of water, adjust to pH 5.5 with sodium hydroxide TS, then add 250 mg of sodium lauryl sulfate, and add water to make 1000 mL.

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

Time span of measurement: From the retention time corresponding to the exclusion volume of the size-exclusion column to the time when the elution of filgrastim is completed.

System suitability—

Test for required detectability: Measure exactly 10 μL of Filgrastim (Genetical Recombination), and add the mobile phase to make exactly 1000 μL. Confirm that the peak area of filgrastim obtained with 250 μL of this solution is 0.7 to 1.3% of that with 250 μL of Filgrastim (Genetical Recombination).

System performance: When the procedure is run with 10 μL of a solution containing 12.5 mg of egg albumin and 12.5 mg of myoglobin in 5 mL of water under the above operating conditions, egg albumin and myoglobin are eluted in this order with the resolution between these peaks being not less than 1.7.

System repeatability: When the test is repeated 6 times with 250 μL of Filgrastim (Genetical Recombination) under the above operating conditions, the relative standard deviation of the peak area of filgrastim is not more than 2.5%.

(2) Charge isomer—Perform the test with 100 μL of Filgrastim (Genetical Recombination) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure the each peak area by the automatic integration method. When their amounts are determined by the area percentage method; the amount of charge isomer, having the relative retention time of about 0.87 to filgrastim, is not more than 3%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 35 mm in length, packed with strongly acidic ion-exchange non-porous resin for liquid chromatography (2.5 μm in particle diameter).

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

Mobile phase A: To 900 mL of water add 1.14 mL of acetic acid (100), adjust to pH 5.4 with sodium hydroxide TS, and add water to make 1000 mL.

Mobile phase B: Dissolve 5.84 g of sodium chloride in 1.14 mL of acetic acid (100) and 900 mL of water, adjust to pH 5.4 with sodium hydroxide TS, 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 – 2	100	0
2 – 10	100 → 40	0 → 60
10 – 11	40 → 100	60 → 0
11 – 20	100	0

Flow rate: Adjust the flow rate so that the retention time of filgrastim is about 14 minutes.

Time span of measurement: From 6 minutes to 17 minutes.

System suitability—

Test for required detectability: Confirm that when perform the test with 100 μL of the system suitability test solution for filgrastim under the above operating conditions, the content of charge isomer is between 1.4 to 2.6%.

System performance: When the procedure is run with 100 μL of the system suitability test solution for filgrastim under the above operating conditions, charge isomer peak and filgrastim 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 100 μL of Filgrastim (Genetical Recombination) under the above operating conditions, the relative standard deviation of the peak area of filgrastim is not more than 2.5%.

(3) Host-derived proteins Being specified separately.

(4) DNA Being specified separately.

Assay (1) Protein content—Take exactly 200 μL each of Filgrastim (Genetical Recombination) and Filgrastim RS, perform the test 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 conditions except using a mixture of the mobile phase A and the mobile phase B (9:1) as the mobile phase, 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%.

(2) Specific activity—

(i) Test cell: 32D clone3 cell.

(ii) Sample dilution solution for assay: To Iscove's modified Dulbecco's fluid medium for filgrastim add 200 mmol/L L-glutamine solution and fetal calf serum to make 1 vol% and 5 vol% solution, respectively, and sterilize by filtration.

(iii) Standard solutions Dilute Filgrastim RS by the sample dilution solution for assay to prepare not less than 5 serial dilutions started from any concentration S_H so that all of their protein concentrations are within the range of 0.5 to 6 ng/mL, and use them as the standard solutions.

(iv) Sample solutions Dilute Filgrastim (Genetical Recombination) by the sample dilution solution for assay to prepare not less than 5 serial dilutions in equal ratio started from any concentration U_H so that all of their protein concentrations are within the range of 0.5 to 6 ng/mL, and use them as the sample solutions.

(v) Procedure The procedure before stopping the incubation should be performed under aseptic condition.

Transfer exactly 100 μL of each concentration of the standard solutions and sample solutions to the wells of 96-well flat bottom microplates. Not less than three plates are prepared for both standard solutions and sample solutions. Add exactly 100 μL of a test cell suspension containing 1×10^5 cells per mL in the sample dilution solution for assay to each well, and incubate under atmosphere of 5% carbon dioxide at 37 ± 2°C for 21 to 27 hours. After incubation, add 40 μL of fluorogenic substrate TS to each well, incubate under the same conditions as above for 21 to 51 hours, and measure fluorescence intensities at excitation wavelength 530 to 560 nm and at measurement wavelength 590 nm, using fluorescence microplate reader. Use the data from at least 3 plates and not less than 3 concentrations of the standard solution and sample solution for the calculation.

(vi) Calculation Transform each concentration of the sample solutions and standard solutions to common logarithm, and name them as x_U and x_S, respectively, and their totals are named as X_U and X_S, respectively. The fluorescence intensities obtained from the sample solution and the standard solution are named as y_U and y_S, and their totals are named as Y_U and Y_S, respectively. The numbers of the concentrations of the sample solution and the standard solution are named as n_U and n_S, respectively, the number of the plate is r. Calculate the specific activity of Filgrastim (Genetical Recombination) by the following equation, using the protein content (mg/mL) obtained in the Assay (1).

Specific activity (unit/mg) of Filgrastim (Genetical Recombination)

$$= \text{antilog } M \times \text{biological activity of Filgrastim RS (unit/mL)} \times \frac{\text{dilution factor for } U_H}{\text{dilution factor for } S_H} \times \frac{U_H}{S_H} \times \frac{1}{\text{protein content (mg/mL) obtained in the Assay (1)}}$$

$$M = X_S/n_S - X_U/n_U - (\Sigma Y_S/n_S r - \Sigma Y_U/n_U r)/b$$

$$b = (Sxy_S + Sxy_U)/(Sxx_S + Sxx_U)$$

$$Sxy_S = \Sigma x_S Y_S - X_S \Sigma Y_S/n_S$$

$$Sxy_U = \Sigma x_U Y_U - X_U \Sigma Y_U/n_U$$

$$Sxx_S = r\Sigma x_S^2 - rX_S^2/n_S$$

$$Sxx_U = r\Sigma x_U^2 - rX_U^2/n_U$$

The necessary requirements for validity of the test are following three items:

1) F'_S is not less than F_1 against $m = n_S(r - 1)$ shown in the table below, and F'_U is not less than F_1 against $m = n_U(r - 1)$ shown in the table.

$$F'_S = V_{RS}/V_{ES}$$

$$V_{RS} = Sxy_S^2/Sxx_S$$

$$V_{ES} = (\Sigma y_S^2 - \Sigma(Y_S^2/r))/(n_S(r - 1))$$

$$F'_U = V_{RU}/V_{EU}$$

$$V_{RU} = Sxy_U^2/Sxx_U$$

$$V_{EU} = \{\Sigma y_U^2 - \Sigma(Y_U^2/r)\}/\{n_U(r - 1)\}$$

2) F' is smaller than F_1 against $m = (n_S + n_U)(r - 1)$ shown in the table below.

$$F' = V_P/V_E$$

$$V_P = Sxy_S^2/Sxx_S + Sxy_U^2/Sxx_U - (Sxy_S + Sxy_U)^2/(Sxx_S + Sxx_U)$$

$$V_E = \{\Sigma y_S^2 + \Sigma y_U^2 - \Sigma(Y_S^2/r) - \Sigma(Y_U^2/r)\}/\{(n_S + n_U)(r - 1)\}$$

3) $L \leqq 0.3$

$$L = \frac{2/b(1 - g)\sqrt{V_E F_1\{(1 - g)(1/n_S r + 1/n_U r) + (\Sigma Y_S/n_S r - \Sigma Y_U/n_U r)^2/b^2(Sxx_S + Sxx_U)\}}}{}$$

F_1: Value against $m = (n_S + n_U)(r - 1)$ shown in the table.

$$g = V_E F_1/b^2(Sxx_S + Sxx_U)$$

Value of F_1 against m

m	F_1	m	F_1	m	F_1
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Hermetic containers. Storage—Not exceeding 10°C, avoiding freezing.

Add the following:

Filgrastim (Genetical Recombination) Injection

フィルグラスチム(遺伝子組換え)注射液

Filgrastim (Genetical Recombination) Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of filgrastim (genetical recombination) ($C_{845}H_{1339}N_{223}O_{243}S_9$: 18798.61).

Method of preparation Prepare as directed under Injections, with Filgrastim (Genetical Recombination).

Description Filgrastim (Genetical Recombination) Injection is a clear and colorless liquid.

Identification Take a volume of Filgrastim (Genetical Recombination) Injection, equivalent to 5 to 10 μg of Filgrastim (Genetical Recombination) depending on the size of polyacrylamide gel for filgrastim, and add 0 to 16 μL of water. To 3 volumes of this solution add 1 volume of buffer solution for filgrastim sample so that each mL contains about 0.19 mg of protein, and use this solution as the sample solution. Then, proceed as directed in the Identification under Filgrastim (Genetical Recombination).

Osmotic pressure ratio Being specified separately.

pH Being specified separately.

Purity Oligomers—Proceed as directed in the Purity (1) under Filgrastim (Genetical Recombination) using a volume of Filgrastim (Genetical Recombination) Injection, equivalent to about 125 μg of Filgrastim (Genetical Recombination). Where, the test for required detectability and the system repeatability under the system suitability are tested using Filgrastim RS.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.25 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.

Biological activity Calculate the biological activity in 1 ampoule or syringe of Filgrastim (Genetical Recombination) Injection by the following equation, using the biological actively in 1 mL of Filgrastim (Genetical Recombination) Injection determined as directed in the Assay (2) under Filgrastim (Genetical Recombination) and the labeled volume of Filgrastim (Genetical Recombination) Injection: it is not less than 70% and not more than 140% of the target biological activity (unit).

Biological activity (unit) in 1 ampoule or syringe of Filgrastim (Genetical Recombination) Injection
= antilog M × biological activity (unit/mL) of Filgrastim RS × dilution factor for U_H/dilution factor for S_H × U_H/S_H × labeled volume (mL) of Filgrastim (Genetical Recombination) Injection

where, the target biological activity (unit) is calculated by the following formula.

Target biological activity (unit)
= 1.5×10^8 (unit/mg) × labeled amount (mg) of Filgrastim (Genetical Recombination) in labeled volume (mL)

Assay Perform the test with an exact volume each of Filgrastim (Genetical Recombination) Injection and Filgrastim RS, equivalent to about 100 μg of Filgrastim (Genetical Recombination), as directed in the Assay (1) under Filgrastim (Genetical Recombination).

Calculate the amount of filgrastim in 1 mL of Filgrastim (Genetical Recombination) Injection by following formula.

Amount (mg) of filgrastim in 1 mL
$= C \times A_T/A_S \times V_S/V_T$

C: Protein concentration (mg/mL) of Filgrastim RS
V_S: Amount (μL) of Filgrastim RS
V_T: Amount (μL) of Filgrastim (Genetical Recombination) Injection

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant, not exceeding 10°C avoiding freezing.

Delete the following two Monographs:

Flurazepam

フルラゼパム

Flurazepam Capsules

フルラゼパムカプセル

Glimepiride Tablets

グリメピリド錠

Change the Uniformity of dosage units and the Dissolution 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 tablet of Glimepiride Tablets add V/10 mL of water, disintegrate, add V/2 mL of a mixture of acetonitrile for liquid chromatography and water (4:1), and shake. To this solution add exactly V/5 mL of the internal standard solution, add a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly V mL so that each mL contains about 100 μg of glimepiride ($C_{24}H_{34}N_4O_5S$), and centrifuge. To 2.5 mL of the supernatant liquid add a mixture of acetonitrile for liquid chromatography and water (4:1) to make 5 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Glimepiride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Glimepiride), and dissolve in a mixture of acetonitrile for liquid chromatography and water (4:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, add a mixture of acetonitrile for liquid chromatography and water (4:1) to make 20 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

Amount (mg) of glimepiride ($C_{24}H_{34}N_4O_5S$)
$= M_S \times Q_T/Q_S \times V/200$

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

Internal standard solution—A solution of butyl parahydroxybenzoate in a mixture of acetonitrile for liquid chromatography 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 disodium hydrogen phosphate-citric acid buffer solution, pH 7.5, as the dissolution medium, the dissolution rate in 15 minutes of 0.5-mg and 1-mg tablets is not less than 75%, and that in 30 minutes of 3-mg tablet is not less than 70%.

Start the test with 1 tablet of Glimepiride 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 0.56 μg of glimepiride ($C_{24}H_{34}N_4O_5S$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Glimepiride RS (separately determine the water <2.48> in the same manner as Glimepiride), and dissolve in acetonitrile for liquid chromatography to make exactly 100 mL. Pipet 2 mL of this solution, add 8 mL of acetonitrile for liquid chromatography, and add the dissolution medium to make exactly 200 mL. Pipet 5 mL of this solution, add the dissolution medium 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 glimepiride from each solution.

Dissolution rate (%) with respect to the labeled amount of glimepiride ($C_{24}H_{34}N_4O_5S$)

$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/4$

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

C: Labeled amount (mg) of glimepiride ($C_{24}H_{34}N_4O_5S$) in 1 tablet

Operating conditions—

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

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).

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 glimepiride are not less than 3000 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 operations conditions, the relative standard deviation of the peak area of glimepiride is not more than 1.5%.

Human Menopausal Gonadotrophin

ヒト下垂体性性腺刺激ホルモン

Change the Purity (1) (ii) and the Assay (ii) and (v) as follows:

Purity

1. Seminal vesicle weight assay

(ii) Standard solutions—Dissolve Menopausal Gonadotrophin RS in bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare three kinds of solutions, containing 10, 20 and 40 interstitial cell-stimulating hormone (luteinizing hormone) units per 1.0 mL, respectively. Inject these solutions into three groups consisting of five test animals each, and weigh their seminal vesicles as directed in (iv). According to the result of the test, designate the concentration of the reference standard, which will make the mass of the seminal vesicle 20 to 35 mg, as the high-dose standard solution, S_H. Dilute the S_H to 1.5 to 2.0 times the initial volume with the bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, and designate this solution as the low-dose standard solution, S_L.

Assay

(ii) Standard solutions—Dissolve Human Menopausal Gonadotrophin RS in human chorionic gonadotrophin TS to make three solutions which contain 0.75, 1.5 and 3.0 follicle-stimulating hormone Units per 1.0 mL, respectively. Inject these solutions into three groups consisting of five test animals each, and weigh their ovaries, as directed in (iv). According to the result of the test, designate the concentration of the reference standard, which will make the mass of the ovary about 120 to 160 mg, as the high-dose standard solution, S_H. Dilute the S_H to 1.5 to 2.0 times the initial volume with the human chorionic gonadotrophin TS, and designate the solution as the low-dose standard solution, S_L.

(v) Calculation—Designate the mass of ovaries by S_H, S_L, T_H and T_L as y_1, y_2, y_3 and y_4, respectively. Sum up y_1, y_2, y_3 and y_4 on each set to obtain Y_1, Y_2, Y_3 and Y_4.

Units per mg of Human Menopausal Gonadotrophin
$= \text{antilog } M \times (\text{units per mL of } S_H) \times b/a$

$M = IY_a/Y_b$

$I = \log(S_H/S_L) = \log(T_H/T_L)$
$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$
$Y_b = Y_1 - Y_2 + Y_3 - Y_4$

a: Mass (mg) of Human Menopausal Gonadotrophin

b: Total volume (mL) of the high dose of the test solution prepared by diluting with human chorionic gonadotrophin TS

F' computed by the following equation should be smaller than F_1 against n when s^2 is calculated. And compute L (P = 0.95) by the following equation: L should be not more than 0.3. If F' exceeds F_1, or if L exceeds 0.3, repeat the test in-

creasing the number of the test animals or arranging the assay method in a better way until F' is smaller than F_1 or L is not more than 0.3.

$F' = (Y_1 - Y_2 - Y_3 + Y_4)^2/(4fs^2)$

f: Number of test animals per group

$s^2 = \{\Sigma y^2 - (Y/f)\}/n$

Σy^2: The sum of the squares of each y_1, y_2, y_3 and y_4

$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$

$n = 4(f - 1)$

$L = 2\sqrt{(C - 1)(CM^2 + I^2)}$

$C = Y_b^2/(Y_b^2 - 4fs^2t^2)$

t^2: Value shown in the following table against n used to calculate s^2

n	$t^2 = F_1$	n	$t^2 = F_1$	n	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Heparin Calcium

ヘパリンカルシウム

Change the Purity (7) to (9) as follows:

Purity

(7) Protein—(i) Sodium carbonate solution To 4 volumes of a mixture of sodium hydroxide solution (1 in 100) and anhydrous sodium carbonate solution (1 in 20) (1:1) add 1 volume of water.

(ii) Copper sulfate solution To 4 volumes of a mixture of copper (II) sulfate pentahydrate solution (1 in 80) and sodium tartrate dihydrate solution (149 in 5000) (1:1) add 1 volume of water.

(iii) Alkaline copper solution for heparin Mix 50 volumes of the sodium carbonate solution and 1 volume of the copper sulfate solution. Prepare before using.

(iv) Procedure Use a solution of Heparin Calcium (1 in 200) as the sample solution. Use a solution of bovine serum albumin (1 in 40,000) as the standard solution. To exactly 1 mL each of the sample solution and the standard solution add exactly 5 mL of the alkaline copper solution for heparin, mix, and allow them to stand at room temperature for 10 minutes. To each of these solutions add exactly 0.5 mL of diluted Folin's TS (1 in 2), shake, allow them to stand at room temperature for 30 minutes, and centrifuge at room temperature. Determine the absorbances at 750 nm of the supernatant liquids as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the blank: the absorbance of the solution obtained from the sample solution is not larger than that of the solution obtained from the standard solution.

(8) Nucleic acid—Dissolve 40 mg of Heparin Calcium in 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (93 in 50,000), and determine the absorbance of this solution at 260 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance is not more than 0.15.

(9) Over-sulfated chondroitin sulfate—Dissolve 20 mg of Heparin Calcium in 0.60 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). Determine the spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> (^{1}H) in accordance with the following conditions, using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits no signal corresponding to *N*-acetyl proton of over-sulfated chondroitin sulfate at δ 2.18 ± 0.05 ppm, the signal disappears when determining the spectrum of the sample solutions as directed under ^{1}H with ^{13}C-decoupling.

Operating conditions—

Spectrometer: (1) FT-NMR, Not less than 400 MHz.

Temperature: 25°C.

Spinning: off.

Number of data points: 32,768.

Spectral range: Signal of DHO ±6.0 ppm.

Flip angle: 90°.

Delay time: 20 seconds.

Dummy scans: 4.

Number of scans: S/N ratio of the signal of *N*-acetyl proton signal of heparin is not less than 1000.

Window function: Exponential function (Line broadening factor = 0.2 Hz).

System suitability—

System performance: Dissolve 20 mg of Heparin Calcium in 0.40 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 1.0 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). To the solution of heparin calcium add 0.20 mL of the solution of Over-sulfated Chondroitin Sulfate RS. When determining the spectrum of this solution under the above operating conditions, it exhibits the signal of *N*-acetyl proton of heparin and the signal of *N*-acetyl proton of over-sulfated chondroitin sulfate at δ 2.04 ± 0.02 ppm and δ 2.18 ± 0.05 ppm, respectively.

(10) Related substances—Dissolve 2.0 mg of Heparin Calcium in 0.1 mL of water, and perform the test with exactly 20 μL of this solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: it exhibits no peaks after the heparin peak.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 202 nm).

Column: A stainless steel column 2.0 mm in inside diameter and 7.5 cm in length, packed with diethylaminoethyl group bound to synthetic polymer for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (1 in 10).

Mobile phase B: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate and 106.4 g of lithium perchlorate in 1000 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (1 in 10).

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	90	10
3 – 15	90 → 0	10 → 100

Flow rate: 0.2 mL per minute.

Time span of measurement: About 2 times as long as the retention time of heparin, beginning after the solvent peak.

System suitability—

Test for required detectability: Dissolve 10 mg of Heparin Sodium for Physicochemical Tests RS in 0.40 mL of water, and use this solution as the heparin sodium standard stock solution. Separately, dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 0.20 mL of water, and use this solution as the over-sulfated chondroitin sulfate standard solution. To 60 μL of the heparin sodium standard stock solution add 3 μL of the over-sulfated chondroitin sulfate standard solution and 12 μL of water, and mix. When the procedureis run with 20 μL of the mixture under the above operating conditions, it exhibits an over-sulfated chondroitin sulfate peak.

System performance: To 120 μL of the Heparin Sodium standard stock solution add 30 μL of the over-sulfated chondroitin sulfate standard solution, mix 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, heparin and over-sulfated chondroitin sulfate 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of over-sulfated chondroitin sulfate is not more than 2.0%.

Heparin Sodium

ヘパリンナトリウム

Change the origin/limits of content and Purity (4) to (7) 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 130 Heparin Units per mg.

It calculated on the dried basis, contains not less than 90% and not more than 110% of the labeled Units.

Purity

(4) Protein—(i) Sodium carbonate solution To 4 volumes of a mixture of sodium hydroxide solution (1 in 100) and anhydrous sodium carbonate solution (1 in 20) (1:1) add 1 volume of water.

(ii) Copper sulfate solution To 4 volumes of a mixture of copper (II) sulfate pentahydrate solution (1 in 80) and sodium tartrate dihydrate solution (149 in 5000) (1:1) add 1 volume of water.

(iii) Alkaline copper solution for heparin Mix 50 volumes of the sodium carbonate solution and 1 volume of the copper sulfate solution. Prepare before using.

(iv) Procedure Use a solution of Heparin Sodium (1 in 200) as the sample solution. Use a solution of bovine serum albumin (1 in 40,000) as the standard solution. To exactly 1 mL each of the sample solution and the standard solution add exactly 5 mL of the alkaline copper solution for heparin, mix, and allow them to stand at room temperature for 10 minutes. To each of these solutions add exactly 0.5 mL of diluted Folin's TS (1 in 2), shake, and allow them to stand at room temperature for 30 minutes. Determine the absorbances at 750 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using water as the blank: the absorbance of the solution obtained from the sample solution is not larger than that of the solution obtained from the standard solution.

(5) Nucleic acid—Dissolve 40 mg of Heparin Sodium in 10 mL of water, and determine the absorbance of this solution at 260 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: the absorbance is not more than 0.15.

(6) Over-sulfated chondroitin sulfate—Dissolve 20 mg of Heparin Sodium in 0.60 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). Determine the spectrum of this solution as directed under Nuclear Magnetic Resonance

Spectroscopy <2.21> (^{1}H) in accordance with the following conditions, using sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits no signal corresponding to *N*-acetyl proton of over-sulfated chondroitin sulfate at δ 2.15 ± 0.02 ppm, or the signal disappears when determining the spectrum of the sample solutions as directed under ^{1}H with ^{13}C-decoupling.

Operating conditions—

Spectrometer: (1) FT-NMR, Not less than 400 MHz

Temperature: 25°C.

Spinning: off.

Number of data points: 32,768.

Spectral range: Signal of DHO ±6.0 ppm.

Flip angle: 90°.

Delay time: 20 seconds.

Dummy scans: 4.

Number of scans: S/N ratio of the signal of *N*-acetyl proton signal of heparin is not less 1000.

Window function: Exponential function (Line broadening factor = 0.2 Hz).

System suitability—

System performance: Dissolve 20 mg of Heparin Sodium for Physicochemical Tests RS in 0.40 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 1.0 mL of a solution of sodium 3-trimethylsilylpropionate-d_4 for nuclear magnetic resonance spectroscopy in heavy water for nuclear magnetic resonance spectroscopy (1 in 10,000). To the solution of Heparin Sodium for Physicochemical Tests RS add 0.2 mL of the solution of Over-sulfated Chondroitin Sulfate RS. When determining the spectrum of this solution under the above operating conditions, it exhibits the signal of *N*-acetyl proton of heparin and the signal of *N*-acetyl proton of over-sulfated chondroitin sulfate at δ 2.04 ± 0.02 ppm and δ 2.15 ± 0.02 ppm, respectively.

(7) Related substances—Dissolve 2.0 mg of Heparin Sodium in 0.1 mL of water and perform the test with exactly 20 μL of this solution as directed under Liquid Chromatography <2.01> according to the following conditions: it exhibits no peaks after the heparin peak.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 202 nm).

Column: A stainless steel column 2.0 mm in inside diameter and 7.5 cm in length, packed with diethylaminoethyl group bound to synthetic polymer for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase A: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust to pH 3.0 with diluted phosphoric acid (1 in 10).

Mobile phase B: Dissolve 0.4 g of sodium dihydrogen phosphate dihydrate and 106.4 g of lithium perchlorate in 1000 mL of water, and adjust to a pH of 3.0 with diluted phosphoric acid (1 in 10).

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	90	10
3 – 15	90 → 0	10 → 100

Flow rate: 0.2 mL per minute.

Time span of measurement: About 2 times as long as the retention time of heparin, beginning after the solvent peak.

System suitability—

Test for required detectability: Dissolve 10 mg of Heparin Sodium for Physicochemical Tests RS in 0.40 mL of water, and use this solution as the heparin sodium standard stock solution. Separately, dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS in 0.20 mL of water, and use this solution as the over-sulfated chondroitin sulfate standard solution. To 60 μL of the heparin sodium standard stock solution add 3 μL of the over-sulfated chondroitin sulfate standard solution and 12 μL of water, and mix. When the procedure is run with 20 μL of the mixture under the above operating conditions, it exhibits a peak for over-sulfated chondroitin sulfate.

System performance: To 120 μL of the heparin sodium standard stock solution add 30 μL of the over-sulfated chondroitin sulfate standard solution, mix 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, heparin and over-sulfated chondroitin sulfate 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of over-sulfated chondroitin sulfate is not more than 2.0%.

(8) Galactosamine—Dissolve 2.4 mg of Heparin Sodium in 1.0 mL of a mixture of water and hydrochloric acid (7:5), and use this solution as the heparin sodium stock solution. Dissolve 8.0 mg of D-glucosamine hydrochloride in a mixture of water and hydrochloric acid (7:5) to make exactly 10 mL. Dissolve 8.0 mg of D-galactosamine hydrochloride in a mixture of water and hydrochloric acid (7:5) to make exactly 10 mL. To 99 volumes of the solution of D-glucosamine add 1 volume of the solution of D-galactosamine, and use this solution as the standard stock solution. Transfer 500 μL each of the heparin sodium stock solution and the standard stock solution to a glass-stoppered test tube, stopper tightly, and heat at 100°C for 6 hours. After cooling to room temperature, evaporate 100 μL each of the reaction solutions to dryness. Add 50 μL of methanol to each of the residues and evaporate to dryness at room temperature. Dissolve each of the residues in 10 μL of water, add 40 μL of aminobenzoate derivatization TS, and heat at 80°C for 1 hour. After cool-

ing to room temperature, evaporate the reaction solutions to dryness. Add 200 μL of each of water and ethyl acetate to each of the residues, shake vigorously, and then centrifuge. After remove the upper layers, add 200 μL of ethyl acetate to each of the lower layers, shake vigorously, and then centrifuge. These lower layers are used as the sample solution and 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: the peak area ratio of galactosamine to glucosamine of the sample solution is not larger than that of the standard solution.

Operating conditions—

Detector: A fluorescence photometer (excitation wavelength: 305 nm; emission wavelength: 360 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 45°C.

Mobile phase: To 100 mL of a mixture of water and trifluoroacetic acid (1000:1) add 100 mL of acetonitrile. Add 140 mL of the solution to 860 mL of a mixture of water and trifluoroacetic acid (1000:1).

Flow rate: 1.0 mL per minute.

Time span of measurement: About 50 minutes after injected.

System suitability—

Test for required detectability: Dissolve 8.0 mg of D-mannosamine hydrochloride in 10 mL of a mixture of water and hydrochloric acid (7:5), and use this solution as the mannosamine standard solution. Transfer 500 μL of a mixture of the standard stock solution and the mannosamine standard solution (100:1) to a glass-stoppered test tube, stopper tightly, and heat at 100°C for 6 hours. After cooling this solution to room temperature, evaporate 100 μL of the reaction solution to dryness. Add 50 μL of methanol to the residue and evaporate to dryness at room temperature. Dissolve the residue in 10 μL of water, add 40 μL of aminobenzoate derivatization TS, and heat at 80°C for 1 hour. After cooling to room temperature, evaporate the reaction solution to dryness. Add 200 μL each of water and ethyl acetate to the residue, shake vigorously, and then centrifuge. After removing the upper layer, add 200 μL of ethyl acetate to the lower layer, shake vigorously, and then centrifuge. The lower layer is used as the solution for system suitability test. When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, the ratio of the peak area of galactosamine to that of glucosamine is 0.7 – 2.0%.

System performance: When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, glucosamine, mannosamine and galactosamine are eluted in this order with the resolutions between the peaks of glucosamine and mannosamine and between the peaks of mannosamine and galactosamine being not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratio of the peak area of galactosamine to that of glucosamine is not more than 4.0%.

Heparin Sodium Injection

ヘパリンナトリウム注射液

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

Heparin Sodium Injection is an aqueous solution for injection.

It contains not less than 90% and not more than 110% of the labeled heparin Units.

Purity Barium—Measure exactly a portion of Heparin Sodium Injection, equivalent to 3000 Units of Heparin Sodium, add water to make 3.0 mL and use this solution as the sample solution. To 1.0 mL of the sample solution add 3 drops of dilute sulfuric acid, and allow to stand for 10 minutes: no turbidity is produced.

Hydralazine Hydrochloride Powder

ヒドララジン塩酸塩散

Add the following next to the Identification:

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 Hydralazine Hydrochloride Powder is not less than 85%.

Start the test with an accurately weighed amount of Hydralazine Hydrochloride Powder, equivalent to about 50 mg of hydralazine hydrochloride ($C_8H_8N_4$.HCl), 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 0.5 μm. Discard the first 5 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of hydralazine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 2 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 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 hydralazine hydrochloride ($C_8H_8N_4$.HCl)

$= M_S/M_T \times A_T/A_S \times 1/C \times 180$

M_S: Amount (mg) of hydralazine hydrochloride for assay

M_T: Amount (g) of the Hydralazine Hydrochloride Powder
C: Labeled amount (mg) of hydralazine hydrochloride ($C_8H_8N_4$.HCl) in 1 g

Add the following:

Hypromellose Acetate Succinate

ヒプロメロース酢酸エステルコハク酸エステル

[*71138-97-1*]

Hypromellose Acetate Succinate is an acetic acid and monosuccinic acid mixed ester of hypromellose.

It contains not less than 12.0% and not more than 28.0% of methoxy group (-OCH_3: 31.03), not less than 4.0 and not more than 23.0% of hydroxypropoxy group (-OC_3H_6OH: 75.09), not less than 2.0% and not more than 16.0% of acetyl group (-$COCH_3$: 43.04), and not less than 4.0% and not more than 28.0% of succinyl group (-COC_2H_4COOH: 101.08), calculated on the dried basis.

Its viscosity is expressed in millipascal second (mPa·s).

Description Hypromellose Acetate Succinate occurs as a white to yellowish white, powder or granules.

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

It dissolves in sodium hydroxide TS.

It is hygroscopic.

Identification Determine the infrared absorption spectrum of Hypromellose Acetate Succinate as directed in the ATR method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 2840 cm^{-1}, 1737 cm^{-1}, 1371 cm^{-1}, 1231 cm^{-1} and 1049 cm^{-1}.

Viscosity <*2.53*> To 2.00 g of Hypromellose Acetate Succinate, previously dried, add dilute sodium hydroxide TS to make 100.0 g, stopper tightly, and dissolve by shaking for 30 minutes. Perform the test with this solution at 20°C according to Method 1: 80 – 120% of the labeled viscosity.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Hypromellose Acetate Succinate 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) Free acetic acid and free succinic acid—Weigh accurately about 0.1 g of Hypromellose Acetate Succinate, add exactly 4 mL of 0.02 mol/L phosphate buffer solution, pH 7.5, stopper tightly, and stir for 2 hours. Then add exactly 4 mL of diluted phosphoric acid (1 in 500), turn the test tube upside down several times, centrifuge, and use the supernatant liquid as the sample solution. Separately, place 20 mL of water in a 100-mL volumetric flask, weigh the mass of the flask accurately, then add 2.0 mL of acetic acid (100), weigh the mass of the flask to calculate the accurate mass of added acetic acid, and dilute with water to volume. Pipet 6 mL of this solution, add water to make exactly 100 mL, and use this solution as the acetic acid stock solution. Separately, weigh accurately about 0.13 g of succinic acid, dissolve in water to make exactly 100 mL, and use this solution as the succinic acid stock solution. Pipet exactly 4 mL each of the acetic acid stock solution and the succinic acid stock solution, add the mobile phase to make exactly 25 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 of acetic acid and succinic acid of both solutions, A_{TA}, A_{TS} and A_{SA}, A_{SS}, and calculate the amount of free acetic acid and free succinic acid by the following expressions: the total amount is not more than 1.0%.

$$\text{Amount (\%) of free acetic acid } (C_2H_4O_2) = M_{SA}/M_T \times A_{TA}/A_{SA} \times 48/625$$

$$\text{Amount (\%) of free succinic acid } (C_4H_6O_4) = M_{SS}/M_T \times A_{TS}/A_{SS} \times 32/25$$

M_{SA}: Amount (mg) of acetic acid (100)
M_{SS}: Amount (mg) of succinic acid
M_T: Amount (mg) of Hypromellose Acetate Succinate, calculated on the dried basis

Operating conditions—

Proceed as directed in the operating conditions in the Assay (1)

System suitability—

Test for required detectability: To 3 mL of the standard solution add the mobile phase to make 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak areas of acetic acid and succinic acid obtained with 10 μL of this solution are equivalent to 7 to 13% of corresponding those with 10 μL of the solution for system suitability test.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (1).

Loss on drying <*2.41*> Not more than 5.0% (1 g, 105°C, 1 hour).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay **(1)** Acetyl group and succinyl group—Weigh accurately about 30 mg of Hypromellose Acetate Succinate, add exactly 10 mL of sodium hydroxide TS, stopper tightly, and stir for 4 hours. Add exactly 10 mL of diluted phosphoric acid (1 in 50), turn the test tube upside down several times, and filter the solution through a membrane filter with a pore size 0.22 μm. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, place 20 mL of water in a 100-mL volumetric flask, weigh the mass of the flask accurately, then add 2.0 mL of acetic acid (100), weigh the mass of the flask to calculate the accurate mass of added acetic acid, and dilute with water to volume. Pipet 6 mL of this solution, add water to make exactly 100 mL, and use this solution as the acetic acid stock

solution. Separately, weigh accurately about 0.13 g of succinic acid, dissolve in water to make exactly 100 mL, and use this solution as the succinic acid stock solution. Pipet 4 mL each of the acetic acid stock solution and the succinic acid stock solution, add the mobile phase to make exactly 25 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_{TA}, A_{TS} and A_{SA}, A_{SS}, of acetic acid and succinic acid of both solutions.

Amount (%) of acetyl group (C_2H_3O)
$= (M_{SA}/M_T \times A_{TA}/A_{SA} \times 24/125 - A_{free}) \times 0.717$

Amount (%) of succinyl group ($C_4H_5O_3$)
$= (M_{SS}/M_T \times A_{TS}/A_{SS} \times 16/5 - S_{free}) \times 0.856$

M_{SA}: Amount (mg) of acetic acid (100)
M_{SS}: Amount (mg) of succinic acid
M_T: Amount (mg) of Hypromellose Acetate Succinate, calculated on the dried basis
A_{free}: Amount (%) of free acetic acid obtained in the Purity (2)
S_{free}: Amount (%) of free succinic acid obtained in the Purity (2)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 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: 0.02 mol/L potassium dihydrogen phosphate TS, adjusted to pH 2.8 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of succinic acid 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, acetic acid and succinic acid 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 areas of acetic acid and succinic acid is not more than 2.0%.

(2) Methoxy group and hydroxypropoxy group

(i) Apparatus—Reaction bottle: A 5 mL pressure-tight glass vial, having 20 mm in outside diameter, 50 mm in height, the neck 20 mm in outside diameter and 13 mm in inside diameter, equipped with a septum of butyl rubber processed the surface with fluoroplastics, which can be fixed tightly to vial with aluminum cap, or equivalent.

Heater: A square-shaped aluminum block, having holes 20 mm in inside diameter and 32 mm in depth, adopted to the reaction bottle. Capable of stirring the content of the bottle by means of magnetic stirrer or reciprocal shaker about 100 times per minute.

(ii) Procedure—Weigh accurately about 65 mg of Hypromellose Acetate Succinate, place in the reaction bottle, add 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Stir or shake for 60 minutes while heating so that the temperature of the bottle content is 130 ± 2°C. In a case where the magnetic stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5 minute intervals by hand. Allow the bottle to cool, and again weigh accurately. If the loss in weight is not more than 0.50% or the content leakage is not found, use the upper layer of the mixture as the sample solution. Separately, put 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid in a reaction bottle, stopper the bottle immediately, and weigh accurately. Add 45 μL of iodomethane for assay and 15 to 22 μL of isopropyl iodide for assay through the septum using a microcylinge with weighing accurately every time, stir thoroughly, and use the upper layer of the mixture as the standard solution. Perform the test with 1 to 2 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 of the peak areas of iodomethane and isopropyl iodide to the peak area of the internal standard, Q_{Ta}, Q_{Tb} and Q_{Sa}, Q_{Sb}.

Amount (%) of methoxy group (CH_3O)
$= M_{Sa}/M_T \times Q_{Ta}/Q_{Sa} \times 21.86$

Amount (%) of hydroxypropoxy group ($C_3H_7O_2$)
$= M_{Sb}/M_T \times Q_{Tb}/Q_{Sb} \times 44.17$

M_{Sa}: Amount (mg) of iodomethane for assay
M_{Sb}: Amount (mg) of isopropyl iodide for assay
M_T: Amount (mg) of Hypromellose Acetate Succinate, calculated on the dried basis

Internal standard solution—A solution of *n*-octane in *o*-xylene (3 in 100).

Operating conditions—

Detector: A thermal conductivity detector or hydrogen flame-ionization detector

Column: A glass tube 3 – 4 mm in inside diameter and 1.8 – 3 m in length, packed with siliceous earth for gas chromatography, 120 to 150 μm in diameter coated with methyl silicon polymer for gas chromatography in 10 – 20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium for the thermal conductivity detector, and helium or nitrogen for the hydrogen flame-ionization detector.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 10 minutes.

System suitability—

System performance: When the procedure is run with 1 – 2 μL of the standard solution under the above operating conditions, iodomethane, isopropyl iodide and the internal standard are eluted in this order with the resolution between

each peak being not less than 5.

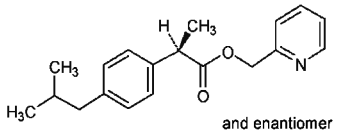
System repeatability: When the test is repeated 6 times with 1 – 2 μL of the standard solution under the above operating conditions, the relative standard deviations of the ratio of the peak area of iodomethane and isopropyl iodide to that of the internal standard are not more than 2.0%, respectively.

Containers and storage Containers—Tight containers.

Add the following:

Ibuprofen Piconol

イブプロフェンピコノール



$C_{19}H_{23}NO_2$: 297.39

Pyridin-2-ylmethyl (2*RS*)-2-[4-(2-methylpropyl)phenyl]propanoate

[*64622-45-3*]

Ibuprofen Piconol contains not less than 98.5% and not more than 101.0% of $C_{19}H_{23}NO_2$, calculated on the anhydrous basis.

Description Ibuprofen Piconol occurs as a clear, colorless to pale yellowish liquid. It is odorless or has a slight characteristic odor.

It is miscible with methanol, with ethanol (95), with acetone and with acetic acid (100).

It is practically insoluble in water.

It decomposes on exposure to light.

It shows no optical rotation.

Identification **(1)** Dissolve 10 mg of Ibuprofen Piconol in 250 mL of ethanol (95). Determine the absorption spectrum of this solution 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 Ibuprofen Piconol as directed in the liquid film 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.

Refractive index <*2.45*> n_D^{20}: 1.529 – 1.532

Specific gravity <*2.56*> d_{20}^{20}: 1.046 – 1.050

Purity **(1)** Chloride <*1.03*>—Dissolve 0.5 g of Ibuprofen Piconol in 20 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Sulfate <*1.14*>—Dissolve 0.5 g of Ibuprofen Piconol in 20 mL of acetone, add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 20 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.038%).

(3) Heavy metals <*1.07*>—Proceed with 4.0 g of Ibuprofen Piconol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(4) Related substances—Dissolve 0.10 g of Ibuprofen Piconol in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 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 hexane, ethyl acetate, acetic acid (100) and methanol (30:10:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (95) (1 in 10) on the plate, and heat at 170°C for 10 minutes: the number of spots other than the dark brown principal spot obtained from the sample solution is two or less, and they are not more intense than the dark brown spot from the standard solution.

(5) Residual solvent Being specified separately.

Water <*2.48*> Not more than 0.1% (5 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Ibuprofen Piconol, 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
= 29.74 mg of $C_{19}H_{23}NO_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Add the following:

Ibuprofen Piconol Cream

イブプロフェンピコノールクリーム

Ibuprofen Piconol Cream contains not less than 95.0% and not more than 105.0% of the labeled amount of ibuprofen piconol ($C_{19}H_{23}NO_2$: 297.39).

Method of preparation Prepare as directed under Creams, with Ibuprofen Piconol.

Identification To an amount of Ibuprofen Piconol Cream, equivalent to 50 mg of Ibuprofen Piconol, add 10 mL of methanol, warm in a water bath, mix well, filter after cooling, and use the filtrate as the sample solution. Separately, dissolve 50 mg of ibuprofen piconol 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. Develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (15:5:1) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot from the standard solution show the same *R*f value.

pH Being specified separately.

Assay Weigh accurately an amount of Ibuprofen Piconol Cream, equivalent to about 15 mg of ibuprofen piconol ($C_{19}H_{23}NO_2$), add 10 mL of tetrahydrofuran for liquid chromatography, shake vigorously, and add exactly 10 mL of the internal standard solution. Then, add methanol to make 30 mL, shake vigorously, 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 0.15 g of ibuprofen piconol for assay (separately determine the water <*2.48*> in the same manner as Ibuprofen Piconol), and dissolve in tetrahydrofuran for liquid chromatography to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add methanol to make 30 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 ibuprofen piconol to that of the internal standard.

$$\text{Amount (mg) of ibuprofen piconol } (C_{19}H_{23}NO_2) = M_S \times Q_T/Q_S \times 1/10$$

M_S: Amount (mg) of ibuprofen piconol for assay, calculated on the anhydrous basis

Internal standard solution—A solution of triphenylmethane in methanol (1 in 200).

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 methanol and acetic acid-sodium acetate buffer solution, pH 4.0 (3:1).

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

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, ibuprofen piconol and the internal standard 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ibuprofen piconol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Ibuprofen Piconol Ointment

イブプロフェンピコノール軟膏

Ibuprofen Piconol Ointment contains not less than 95.0% and not more than 105.0% of the labeled amount of ibuprofen piconol ($C_{19}H_{23}NO_2$: 297.39).

Method of preparation Prepare as directed under Ointments, with Ibuprofen Piconol.

Identification To an amount of Ibuprofen Piconol Ointment, equivalent to 50 mg of Ibuprofen Piconol, add 10 mL of methanol, warm at 60°C in a water bath, mix well, and filter after cooling. Use the filtrate as the sample solution. Separately, dissolve 50 mg of ibuprofen piconol 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. Develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (15:5:1) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot from the standard solution show the same *R*f value.

Assay Weigh accurately an amount of Ibuprofen Piconol Ointment, equivalent to about 15 mg of ibuprofen piconol ($C_{19}H_{23}NO_2$), add 10 mL of tetrahydrofuran for liquid chromatography, shake vigorously, and add exactly 10 mL of the internal standard solution. Then, add methanol to make 30 mL, shake vigorously, 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 0.15 g of ibuprofen piconol for assay (separately determine the water <*2.48*> in the same manner as Ibuprofen Piconol), and dissolve in tetrahydrofuran for liquid chromatography to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add methanol to make exactly 30 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 ibuprofen piconol to that of the internal standard.

Amount (mg) of ibuprofen piconol ($C_{19}H_{23}NO_2$)
$= M_S \times Q_T/Q_S \times 1/10$

M_S: Amount (mg) of ibuprofen piconol for assay, calculated on the anhydrous basis

Internal standard solution—A solution of triphenylmethane in methanol (1 in 200).

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 methanol and acetic acid-sodium acetate buffer solution, pH 4.0 (3:1).

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

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, ibuprofen piconol and the internal standard 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ibuprofen piconol to that of the internal standard is not more than 1.0%.

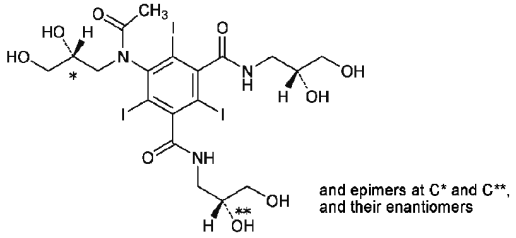
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Iohexol

イオヘキソール



$C_{19}H_{26}I_3N_3O_9$: 821.14

5-{Acetyl[(2*RS*)-2,3-dihydroxypropyl]amino}-*N*,*N*′-bis[(2*RS*)-2,3-dihydroxypropyl]-2,4,6-triiodobenzene-1,3-dicarboxamide

5-{Acetyl[(2*RS*)-2,3-dihydroxypropyl]amino}-*N*-[(2*RS*)-2,3-dihydroxypropyl]-*N*′-[(2*SR*)-2,3-dihydroxypropyl]-2,4,6-triiodobenzene-1,3-dicarboxamide

5-{Acetyl[(2*RS*)-2,3-dihydroxypropyl]amino}-*N*,*N*′-bis[(2*SR*)-2,3-dihydroxypropyl]-2,4,6-triiodobenzene-1,3-dicarboxamide

[*66108-95-0*]

Iohexol is a mixture of endo- and exo-products of iohexol.

It contains not less than 98.5% and not more than 101.0% of $C_{19}H_{26}I_3N_3O_9$, calculated on the anhydrous basis.

Description Iohexol occurs as a white powder.

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

It dissolves in a solution of sodium hydroxide (1 in 20).

A solution of Iohexol (1 in 20) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Iohexol (13 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 Iohexol, previously dried at 105°C for 6 hours, 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) Dissolve 0.1 g of Iohexol in 10 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 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 1-buthanol, water and acetic acid (100) (50:25:11) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of principal spots obtained from the sample solutions is two, and their *R*f values are about 0.2 and about 0.3, respectively.

Purity **(1)** Clarity and color of solution—A solution obtained by dissolving 1.0 g of Iohexol in 5 mL of water is clear and colorless.

(2) Aromatic primary amine—Conduct this procedure using light-resistant vessels. Dissolve 0.20 g of Iohexol in 15 mL of water, cool in ice for 5 minutes, add 1.5 mL of 6 mol/L hydrochloric acid TS and 1 mL of a solution of sodium nitrite (1 in 50), prepared before use, stir, and cool in ice for 4 minutes. Add 1 mL of a solution of amidosulfuric acid (standard reagent) (1 in 25), stir, and cool in ice for 1 minute. Then, add 0.5 mL of a solution, prepared by dissolving 0.3 g of *N*-1-naphthylethylenediamine dihydrochloride in diluted propylene glycol (7 in 10) to make 100 mL, and add water to make exactly 25 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*> within 20 minutes, using a solution prepared in the same manner with 15 mL of water as the blank: the absorbance at 495 nm is not more than 0.21.

(3) Chloride <*1.03*>—Perform the test with 2.0 g of Iohexol. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

(4) Iodine and iodide—Dissolve 1.0 g of Iohexol in 4 mL of water, add 1 mL of dilute sulfuric acid, and allow to stand for 10 minutes while occasional shaking. Add 5 mL of chloroform, shake well, and allow to stand: the chloroform layer is colorless. Then, add 1 mL of sodium nitrite solution (1 in 50), shake, allow to stand, and determine the absorbance of collected chloroform layer as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a chloroform layer prepared in the same manner with 4.0 mL of water as the blank: the absorbance at 510 nm is not larger than that of chloroform layer obtained from the following control solution.

Control solution: Dissolve exactly 0.131 g of potassium iodide in water to make exactly 100 mL. Pipet 1 mL of this solution, and add water to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 1 mL of water and 1 mL of dilute sulfuric acid, then proceed in the same manner.

(5) Heavy metals <*1.07*>—Proceed with 2.0 g of Iohexol 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).

(6) 3-Chloro-1,2-propanediol—To exactly 1.0 g of Iohexol, add exactly 2 mL of diethyl ether, and treat with ultrasonic waves for 10 minutes under cooling. Centrifuge, and use the diethyl ether layer as the sample solution. Separately, dissolve exactly 0.50 g of 3-chloro-1,2-propanediol in diethyl ether to make exactly 50 mL. Pipet 1 mL of this solution, and add diethyl ether to make exactly 100 mL. Pipet 5 mL of this solution, add diethyl ether to make exactly 25 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 Gas Chromatography <*2.02*>, and determine the peak areas, A_T and A_S, of 3-chloro-1,2-propanediol of each solution: A_T is not more than 2.5 times A_S.

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 a layer about 0.25 μm thick of 5% diphenyl-95% dimethylpolysiloxane for gas chromatography.

Column temperature: A constant temperature of about 70°C.

Injection port and detector temperature: A constant temperature of about 230°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of 3-chloro-1,2-propanediol is about 7 minutes.

Split ratio: 1:40.

System suitability—

System performance: To 1 mL of a solution of 3-chloro-1,2-propanediol in diethyl ether (1 in 200) and 1 mL of a solution of 1-hexanol in diethyl ether (1 in 800) add diethyl ether to make 200 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, 1-hexanol and 3-chloro-1,2-propanediol are eluted in this order with the resolution between these peaks being not less than 20.

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 3-chloro-1,2-propanediol is not more than 15%.

(7) Related substance (i) Dissolve 1.0 g of Iohexol in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 25 mL. Pipet 1 mL of this solution, add the methanol to make exactly 20 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 with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, 2-propanol, ammonia solution (28) and methanol (10:7:4:4) to a distance about 14 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot, other than the principal spot from the sample solution, appears at the relative *R*f value of 1.4 with respect to the spot from the standard solution, is not more intense than the spot from the standard solution.

(ii) Dissolve 0.15 g of Iohexol in water 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. Determine the peak area by the automatic integration method, and calculate the amounts by the area percentage method: the total amount of *O*-alkyl substances, having the relative retention time between 1.2 and 1.5 with respect to the second principal peak (having bigger retention time) among the two principal peaks of iohexol, is not more than 0.6%, the amount of the peaks, which are eluted after the peak of iohexol and other than *O*-alkyl substances, is not more than 0.1%, respectively, and the total amount of the peaks, which are eluted after iohexol and other than *O*-alkyl substances, is not more than 0.3%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 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 A: Acetonitrile.

Mobile phase B: Water.

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 – 1	1	99
1 – 46	1 → 10	99 → 90

Flow rate: Adjust the flow rate so that the retention time of the second principal peak (iohexol exo-product) is about 19 minutes.

Time span of measurement: About 2 times as long as the retention time of iohexol exo-product.

System suitability—

Test for required detectability: To 1 mL of the sample solution add water 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, and add water to make exactly 20 mL. Confirm that the peak area of iohexol exo-product obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that from 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 resolution between the adjacent two peaks, which appear at the retention time of about 18 minutes, is not less than 1.5.

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 area of iohexol exo-product is not more than 3.0%.

(8) Residual solvent Being specified separately.

Water ⟨*2.48*⟩ Not more than 4.0% (0.3 g, volumetric titration, direct titration).

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

Assay Weigh accurately about 0.5 g of Iohexol, dissolve in 25 mL of a solution of sodium hydroxide (1 in 20), add 0.5 g of zinc powder, boil under a reflux condenser for 30 minutes, and filter after cooling. Wash the flask and filter paper with 200 mL of water, combine the washings and filter, add 5 mL of acetic acid (100), and titrate ⟨*2.50*⟩ with 0.1 mol/L silver nitrate VS (indicator: 1 mL of tetrabromophenolphthalein ethyl ester TS) until the color of the precipitate changes from yellow to green.

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—Tight containers.

Add the following:

Iohexol Injection

イオヘキソール注射液

Iohexol Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of iohexol ($C_{19}H_{26}I_3N_3O_9$: 821.14).

Method of preparation Prepare as directed under Injections, with Iohexol.

Description Iohexol Injection is a clear and colorless liquid.

Identification To a volume of Iohexol Injection, equivalent to 0.65 g of Iohexol, add water to make 500 mL. To 1 mL of this solution add water 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 243 nm and 247 nm.

pH Being specified separately.

Purity (1) Aromatic primary amine—Conduct this procedure using light-resistant vessels. To a volume of Iohexol Injection, equivalent to 0.20 g of Iohexol add 15 mL of water, cool in ice for 5 minutes, add 1.5 mL of 6 mol/L hydrochloric acid TS and 1 mL of freshly prepared solution of sodium nitrite (1 in 50), shake, and cool in ice for 4 minutes. Then, proceed as directed in the Purity (2) under Iohexol: the absorbance of a solution so obtained is not more than 0.23.

(2) Iodine and iodide—To a volume of Iohexol Injection, equivalent to 1.0 g of Iohexol, add 4 mL of water and 1 mL of dilute sulfuric acid, and allow to stand for 10 minutes while occasional shaking. Then, proceed as directed in the Purity (4) under Iohexol: the absorbance of a chloroform layer so obtained is not more than 0.14.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.47 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 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 heat on a water bath 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.

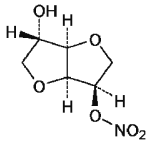
Each mL of 0.1 mol/L silver nitrate VS
= 27.37 mg of $C_{19}H_{26}I_3N_3O_9$

Containers and storage Containers—Hermetic containers.

Add the following:

Isosorbide Mononitrate 70%/Lactose 30%

70%一硝酸イソソルビド乳糖末



$C_6H_9NO_6$: 191.14
1,4:3,6-Dianhydro-D-glucitol 5-nitrate
[*16051-77-7*, Isosorbide mononitrate]

Isosorbide Mononitrate 70%/Lactose 30%, when dried, contains not less than 68.0% and not more than 72.0% of $C_6H_9NO_6$.

Description Isosorbide Mononitrate 70%/Lactose 30% occurs as a white, powder, crystalline powder, or masses.

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

Identification **(1)** Shake thoroughly 1 g of Isosorbide Mononitrate 70%/Lactose 30% with 30 mL of ethyl acetate, and filter. Wash the residue with a small quantity of ethyl acetate, combine the filtrate and the washings, evaporate to dryness on a water bath, then dry in vacuum at room temperature for 4 hours. Determine the infrared absorption spectrum of the crystals obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum of isosorbide mononitrate: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dry the residue obtained in (1) at 80°C for 2 hours. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum of Lactose Hydrate or the spectrum of Lactose RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +116 – +124° (after drying, 1 g, water, 100 mL, 100 mm).

Purity **(1)** Nitrate Dissolve an exact quantity of Isosorbide Mononitrate 70%/Lactose 30%, equivalent to 50 mg of isosorbide mononitrate ($C_6H_9NO_6$), in water to make exactly 100 mL. Pipet 25 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, to exactly 5 mL of Standard Nitric Acid Solution add water to make exactly 150 mL. Pipet 25 mL of this solution, add water to make exactly 150 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 area of nitric acid of each solution by the automatic integration method: the peak area of nitric acid obtained from the sample solution is not larger than the peak area of nitric acid from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with gel type strong basic ion-exchange resin for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 16.0 g of sodium gluconate, 18.0 g of boric acid, 25.0 g of sodium tetraborate decahydrate, and 250 mL of glycerin in water to make 1000 mL. To 20 mL of this solution add 20 mL of 1-butanol, 120 mL of acetonitrile, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of nitric acid is about 5.3 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 nitric acid are not less than 800 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 nitric acid is not more than 2.0%.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Isosorbide Mononitrate 70%/Lactose 30% according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Isosorbide To an amount of Isosorbide Mononitrate 70%/Lactose 30%, equivalent to 1.0 g of isosorbide mononitrate ($C_6H_9NO_6$), add 10 mL of acetone, shake well, centrifuge, and filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.5 μm. To the residue add 2 mL of acetone and proceed in the same manner, and combine the filtrates. Evaporate the combined filtrate to dryness on a water bath, and further dry the residue in vacuum for 30 minutes. Dissolve the residue in the mobile phase to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this

solution, add the mobile phase to make exactly 25 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 by the automatic integration method: the peak area of isosorbide, having the relative retention time of about 0.2 to isosorbide mononitrate, obtained from the sample solution is not larger than the peak area of isosorbide mononitrate from the standard solution.

Operating conditions—

Detector: A differential refractometer.

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: A mixture of water and methanol (9:1).

Flow rate: Adjust the flow rate so that the retention time of isosorbide mononitrate is about 16 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 isosorbide mononitrate 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 isosorbide mononitrate is not more than 4.0%.

(4) Related substances—Dissolve an amount of Isosorbide Mononitrate 70%/Lactose 30%, equivalent to 50 mg of isosorbide mononitrate ($C_6H_9NO_6$), in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water 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 other than isosorbide mononitrate obtained from the sample solution is not larger than 1/2 times the peak area of isosorbide mononitrate from the standard solution, and the total area of the peaks other than isosorbide mononitrate obtained from the sample solution is not larger than the peak area of isosorbide mononitrate from the standard solution. For these calculations use the area of the peak, having a relative retention time of about 4.5 with respect to isosorbide mononitrate, after multiplying by its relative response factor, 0.62.

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 isosorbide mononitrate, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of isosorbide mononitrate obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

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

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 isosorbide mononitrate is not more than 2.0%.

(5) Residual solvent Being specified separately.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Water <2.48> Between 1.0 and 2.0% (0.4 g, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately an amount of Isosorbide Mononitrate 70%/Lactose 30%, previously dried, equivalent to about 0.2 g of isosorbide mononitrate ($C_6H_9NO_6$), and dissolve in water to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 20 mL of the internal standard solution, then add water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of isosorbide mononitrate for assay, previously dried, and dissolve in 60 mL of water, add exactly 20 mL of the internal standard solution, then, add water 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 isosorbide mononitrate to that of the internal standard.

$$\text{Amount (mg) of isosorbide mononitrate } (C_6H_9NO_6) = M_S \times Q_T/Q_S \times 5$$

M_S: Amount (mg) of isosorbide mononitrate for assay

Internal standard solution—A solution of benzyl alcohol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 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 1000) and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of isosorbide mononitrate is about 4.5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, isosorbide mononitrate 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 isosorbide mononitrate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Isosorbide Mononitrate Tablets

一硝酸イソソルビド錠

Isosorbide Mononitrate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$: 191.14).

Method of preparation Prepare as directed under Tablets, with Isosorbide Mononitrate 70%/Lactose 30%.

Identification Shake well a portion of pulverized Isosorbide Mononitrate Tablets, equivalent to 50 mg of isosorbide mononitrate ($C_6H_9NO_6$), with 5 mL of acetone, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of isosorbide mononitrate for assay in 1 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 20 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 (2:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly a solution of potassium permanganate in potassium hydroxide TS (1 in 50), and allow to stand for about 50 minutes: the principal spot obtained with the sample solution and the spot with the standard solution are yellow, and their *R*f values are the same.

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 Isosorbide Mononitrate Tablets add 30 mL of water, allow standing to disintegrate the tablet, and disperse the fine particles with the aid of ultrasonic waves. Add exactly $V/10$ mL of the internal standard solution, and add water to make V mL so that each mL contains about 0.2 mg of isosorbide mononitrate ($C_6H_9NO_6$). Centrifuge this solution, 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, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of isosorbide mononitrate for assay, previously dried in vacuum (silica gel) for 4 hours, add 30 mL of water and exactly 10 mL of the internal standard solution, then add water to make 100 mL, and use this solution as the standard solution, Then, proceed as directed in the Assay.

Amount (mg) of isosorbide mononitrate ($C_6H_9NO_6$)
$= M_S \times Q_T/Q_S \times V/100$

M_S: Amount (mg) of isosorbide mononitrate for assay

Internal standard solution—A solution of benzyl alcohol (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 water as the dissolution medium, the dissolution rate in 15 minutes of Isosorbide Mononitrate Tablets is not less than 85%.

Start the test with 1 tablet of Isosorbide Mononitrate 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 11 μg of isosorbide mononitrate ($C_6H_9NO_6$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of isosorbide mononitrate for assay, previously dried in vacuum (silica gel) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water 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, A_T and A_S, of isosorbide mononitrate of each solution.

Dissolution rate (%) with respect to the labeled amount of isosorbide mononitrate ($C_6H_9NO_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of isosorbide mononitrate for assay
C: Labeled amount (mg) of isosorbide mononitrate ($C_6H_9NO_6$) 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 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of isosorbide mononitrate are not less than 2000 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 isosorbide mononitrate is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Isosorbide Mononitrate Tablets, and powder. Weigh ac-

curately a portion of the powder, equivalent to about 20 mg of isosorbide mononitrate ($C_6H_9NO_6$), add 30 mL of water, and disperse the fine particles with the aid of ultrasonic waves. Add exactly 10 mL of the internal standard solution and water to make 50 mL. Centrifuge this solution, and 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, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of isosorbide mononitrate for assay, previously dried, and dissolve in 30 mL of water, add exactly 10 mL of the internal standard solution, then, add water 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 isosorbide mononitrate to that of the internal standard.

$$\text{Amount (mg) of isosorbide mononitrate } (C_6H_9NO_6) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of isosorbide mononitrate for assay

Internal standard solution—A solution of benzyl alcohol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 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 1000) and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of isosorbide mononitrate is about 4.5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, isosorbide mononitrate 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 isosorbide mononitrate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Josamycin

ジョサマイシン

Change the chemical name as follows:

(3*R*,4*S*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[2,6-dideoxy-4-*O*-(3-methylbutanoyl)-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide

Josamycin Propionate

ジョサマイシンプロピオン酸エステル

Change the chemical name as follows:

(3*R*,4*S*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[2,6-dideoxy-4-*O*-(3-methylbutanoyl)-3-*C*-methyl-α-L-*ribo*-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino-β-D-glucopyranosyloxy]-6-formylmethyl-4-methoxy-8-methyl-9-propanoyloxyhexadeca-10,12-dien-15-olide

Kanamycin Sulfate

カナマイシン硫酸塩

Change the Purity (1) as follows:

Purity (1) Clarity and color of solution—Dissolve 1.5 g of Kanamycin Sulfate in 5 mL of water: the solution is clear. Determine the absorbance of this solution at 400 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: not more than 0.15.

Anhydrous Lactose

無水乳糖

Change to read except the structural formula, chemical name, origin/limits of content and Description:

Identification Determine the infrared absorption spectrum of Anhydrous Lactose, previously dried, 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 Anhydrous Lactose RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +54.4 – +55.9° Weigh ac-

curately about 10 g of Anhydrous Lactose, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Anhydrous Lactose in 10 mL of boiling water, and allow to cool: the solution is clear, and colorless or nearly colorless and has no more color than the following control solution. Determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the control solution: not more than 0.04.

Control solution: To a mixture of 2.5 mL of Cobalt (II) Chloride CS, 6.0 mL of Iron (III) Chloride CS and 1.0 mL of Copper (II) Sulfate CS, add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Acidity or alkalinity—Dissolve 6 g of Anhydrous Lactose by heating in 25 mL of freshly boiled and cooled water, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless, and not more than 0.4 mL of 0.1 mol/L sodium hydroxide VS is required to produce a pink or red color.

◆**(3)** Heavy metals <*1.07*>—Proceed with 4.0 g of Anhydrous Lactose according to Method 2, and perform the test. Prepare the control solution with 2 mL of Standard Lead Solution (not more than 5 ppm).◆

(4) Proteins and light absorbing substances—Dissolve 1.0 g of Anhydrous Lactose in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 80°C, 2 hours).

Water <*2.48*> Not more than 1.0% (1 g, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Microbial limit <*4.05*> The total viable aerobic microbial count is not more than 10^2 CFU per g, ◆and the total count of fungi and yeast is not more than 5×10^1 CFU per g,◆ and ◆ *Salmonella* and ◆ *Escherichia coli* should not be observed.

Isomer ratio Place 10 mg of Anhydrous Lactose in a screw capped reaction vial for gas chromatography, add 4 mL of a mixture of pyridine, trimethylsilylimidazole and dimethylsulfoxide (117:44:39), stopper, and exposure to ultrasonic waves at room temperature for 20 minutes. After cooling, transfer 400 μL of this solution into a vial for injection, add 1 mL of pyridine, stopper tightly, mix, and use this fluid as the sample solution. Perform the test with 0.5 μL of the sample solution as directed under Gas Chromatography <*2.02*> according to the following conditions. Determine the peak areas of α-lactose and β-lactose, A_a and A_b, and calculate the contents (%) of α-lactose and β-lactose in Anhydrous Lactose by the following equations.

$$\text{Content (\%) of } \alpha\text{-lactose} = A_a/(A_a + A_b) \times 100$$

$$\text{Content (\%) of } \beta\text{-lactose} = A_b/(A_a + A_b) \times 100$$

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 15 m in length, coated the inside surface with 5% diphenyl-95% dimethylpolysiloxane in 0.25 μm thickness. Use a middle polar inertness fused silica column 0.53 mm in inside diameter and 2 m in length as a guard column.

Column temperature: Keep at 80°C for 1 minute after injection, then rise to 150°C with 35°C per minute, then rise to 300°C with 12°C per minute, and keep 300°C for 2 minutes.

Injection port temperature: A constant temperature of about 275°C, or use cold-on column injection.

Detector temperature: A constant temperature of about 325°C.

Carrier gas: Helium.

Flow rate: 2.8 mL per minute (Retention time of β-lactose is about 12 minutes).

Sprit ratio: Spritless.

System suitability—

System performance: Prepare a solution with 10 mg of a mixture of α-lactose and β-lactose (1:1) in the same manner as for preparing the sample solution, and proceed with 0.5 μL of this solution under the above operating conditions, and determine the retention times of the peaks of α-lactose and β-lactose: the relative retention time of α-lactose with respect to that of β-lactose is about 0.9 with the resolution between these peaks being not less than 3.0.

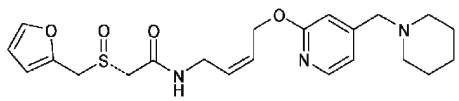
◆System repeatability: When the test is repeated 6 times with 0.5 μL of the solution used in the system performance under the above operating conditions, the relative standard deviation of the peak area of β-lactose is not more than 1.0%.◆

◆**Containers and storage** Containers—Well-closed containers.◆

Add the following:

Lafutidine

ラフチジン



$C_{22}H_{29}N_3O_4S$: 431.55

2-[(*RS*)-Furan-2-ylmethylsulfinyl]-*N*-{4-[4-(piperidin-1-ylmethyl)pyridin-2-yl]oxy-(2*Z*)-but-2-en-1-yl}acetamide

[*206449-93-6*]

Lafutidine, when dried, contains not less than 99.0% and not more than 101.0% of $C_{22}H_{29}N_3O_4S$.

Description Lafutidine occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Lafutidine in methanol (1 in 100) shows no optical rotation.

Lafutidine shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Lafutidine in methanol (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 Lafutidine, 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.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Lafutidine 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 0.10 g of Lafutidine in 100 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 5 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 0.85 to lafutidine, from the sample solution is not larger than 3/10 times the peak area of lafutidine from the standard solution, the area of the peak other than lafutidine and the peak mentioned above is not larger than 1/10 times the peak area of lafutidine from the standard solution, and the total area of the peaks other than lafutidine is not larger than 2/5 times the peak area of lafutidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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: Dissolve 0.87 g of sodium 1-pentanesulfonate in 1000 mL of diluted phosphoric acid (1 in 1000). To 850 mL of this solution add 150 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of lafutidine is about 15 minutes.

Time span of measurement: About 6 times as long as the retention time of lafutidine.

System suitability—

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 lafutidine obtained with 5 μL of this solution is equivalent to 3.5 to 6.5% of that 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, the number of theoretical plates and the symmetry factor of the peak of lafutidine are not less than 8000 and not more than 1.5, 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 lafutidine is not more than 2.0%.

(3) Residual solvent Being specified separately.

Loss on drying <*2.41*> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 4 hours).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Lafutidine, 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
= 21.58 mg of $C_{22}H_{29}N_3O_4S$

Containers and storage Containers—Tight containers.

Add the following:

Lafutidine Tablets

ラフチジン錠

Lafutidine Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of lafutidine ($C_{22}H_{29}N_3O_4S$: 431.55).

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

Identification Powder Lafutidine Tablets. To a portion of the powder, equivalent to 10 mg of Lafutidine, add 10 mL of methanol, shake thoroughly, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits an absorption maximum between 271 nm and 275 nm.

Purity Related substances—To 10 Lafutidine Tablets add $4V/5$ mL of the mobile phase, disintegrate the tablets with the aid of ultrasonic waves, then shake vigorously for not less than 30 minutes, and add the mobile phase to make V mL so that each mL contains about 1 mg of lafutidine ($C_{22}H_{29}N_3O_4S$). Centrifuge, and use the supernatant liquid 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 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 lafutidine and the peak having the relative retention time of about 0.85 to lafutidine, from the sample solution is not larger than 1/5 times the peak area of lafutidine from the standard solution, and the total area of the peaks, other than lafutidine and the peak having the relative retention time of about 0.85 to lafutidine, from the sample solution is not larger than 3/5 times the peak area of lafutidine 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 6 times as long as the retention time of lafutidine.

System suitability—

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 lafutidine obtained with 5 μL of this solution is equivalent to 3.5 to 6.5% of that 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, the number of theoretical plates and the symmetry factor of the peak of lafutidine are not less than 8000 and not more than 1.5, 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 lafutidine 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 Lafutidine Tablets add exactly V mL of the internal standard solution so that each mL contains about 2 mg of lafutidine ($C_{22}H_{29}N_3O_4S$), disintegrate the tablet with the aid of ultrasonic waves, then shake vigorously for 30 minutes. Centrifuge this solution, filter the supernatant liquid 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 0.1 g of lafutidine for assay, previously dried under a reduced pressure (not exceeding 0.67 kPa) using phosphorus (V) oxide as dessicant for 4 hours, dissolve in exactly 50 mL of the internal standard solution, and use this solution as the standard solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of lafutidine } (C_{22}H_{29}N_3O_4S) = M_S \times Q_T/Q_S \times V/50$$

M_S: Amount (mg) of lafutidine for assay

Internal standard solution—A solution of ethyl aminobenzoate in a mixture of acetonitrile and water (4:1) (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 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 15 minutes of Lafutidine Tablets is not less than 75%.

Start the test with 1 tablet of Lafutidine 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 lafutidine ($C_{22}H_{29}N_3O_4S$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of lafutidine for assay, previously dried under a reduced pressure (not exceeding 0.67 kPa) using phosphorus (V) oxide as dessicant for 4 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 2 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 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 lafutidine.

Dissolution rate (%) with respect to the labeled amount of lafutidine ($C_{22}H_{29}N_3O_4S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of lafutidine for assay
C: Labeled amount (mg) of lafutidine ($C_{22}H_{29}N_3O_4S$) 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 lafutidine are not less than 7000 and not more than 1.5, 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 lafutidine is not more than 2.0%.

Assay To 20 Lafutidine Tablets add $4V/5$ mL of the internal standard solution, disintegrate the tablets with the aid of ultrasonic waves, then shake vigorously for 30 minutes. Add the internal standard solution to make exactly V mL so that each mL contains about 2 mg of lafutidine ($C_{22}H_{29}N_3O_4S$), centrifuge, filter the supernatant liquid 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 0.1 g of lafutidine for assay, previously dried under a reduced pressure (not exceeding 0.67 kPa) using phosphorus (V) oxide as dessicant for 4 hours, dissolve in the internal standard solution to make exactly 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 lafutidine to that of the internal standard.

Amount (mg) of lafutidine ($C_{22}H_{29}N_3O_4S$) in 1 tablet
$= M_S \times Q_T/Q_S \times V/1000$

M_S: Amount (mg) of lafutidine for assay

Internal standard solution—A solution of ethyl aminobenzoate in a mixture of acetonitrile and water (4:1) (3 in 10,000)
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 275 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: Dissolve 0.87 g of sodium 1-pentanesulfonate in 1000 mL of diluted phosphoric acid (1 in 1000). To 850 mL of this solution add 150 mL of acetonitrile.
Flow rate: Adjust the flow rate so that the retention time of lafutidine is about 15 minutes.
System suitability—
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, lafutidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.
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 ratio of the peak area of lafutidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Lenograstim (Genetical Recombination)

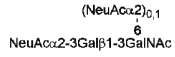
レノグラスチム（遺伝子組換え）

Protein moiety

TPLGPASSLP QSFLLKCLEQ VRKIQGDGAA LQEKLCATYK LCHPEELVLL
GHSLGIPWAP LSSCPSQALQ LAGCLSQLHS GLFLYQGLLQ ALEGISPELG
PTLDTLQLDV ADFATTIWQQ MEELGMAPAL QPTQGAMPAF ASAFQRRAGG
VLVASHLQSF LEVSYRVLRH LAQP

T133: glycosylation site

Carbohydrate moiety (structure of major glycans)



$C_{840}H_{1330}N_{222}O_{242}S_8$: 18667.41 (Protein moiety)
[*135968-09-1*]

Lenograstim (Genetical Recombination) is an aqueous solution in which a desired product is a recombinant human granulocyte colony-stimulating factor produced in Chinese hamster ovary cells. It is a glycoprotein (molecular mass: ca. 20,000) consisting of 174 amino acid residues. It has a neutrophilic leucocyte induction activity.

It contains not less than 0.40 mg and not more than 0.60 mg of protein per mL, and not less than 1.02×10^8 units per mg of protein.

Description Lenograstim (Genetical Recombination) occurs as a clear and colorless liquid.

Identification **(1)** Use Lenograstim (Genetical Recombination) and Lenograstim RS as the sample solution and the standard solution, respectively. Perform the test with a volume each of the sample solution and standard solution, equivalent to 20 μg of protein, as directed under Liquid Chromatography <*2.01*> according to the following conditions: the retention times of the two peaks of lenograstim

obtained from the sample solution and of those from the standard solution are the same.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 7.5 cm in length, packed with diethylaminoethyl group binding synthetic polymer for liquid chromatography (10 μm in particle diameter).

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

Mobile phase A: 0.02 mol/L tris buffer solution, pH 7.4.

Mobile phase B: 0.02 mol/L tris buffer solution, pH 7.4 containing 0.5 mol/L sodium chloride.

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 – 35	100 → 80	0 → 20
35 – 40	80	20

Flow rate: Adjust the flow rate so that the retention time of the first appeared peak of lenograstim is about 27 minutes.

System suitability—

System performance: When the procedure is run with a volume of the standard solution, equivalent to 20 μg of protein, under the above operating conditions, the resolution between the two peaks of lenograstim is not less than 4.

(2) Desalt 2 mL each of Lenograstim (Genetical Recombination) and Lenograstim RS by a suitable method, and assign them as the desalted sample and the desalted reference standard, respectively. Add the desalted sample and the desalted reference standard in 100 μL each of a mixture of water and 1-propanol (3:2), add 4 mL of urea-EDTA TS, and allow them to stand at 37°C for 18 hours. Then, add 10 μL of 2-mercaptoethanol to them, and allow to stand at 37°C for 4 hours. To these solutions add a solution of 27 mg of iodoacetic acid in 150 μL of sodium hydroxide TS, and react at 37°C for 15 minutes, avoiding exposure to light. Remove the reagents from these reaction solution by a suitable method, and assign obtained these substances as the reduced carboxymethylated sample and the reduced carboxymethylated reference standard. To these substances add 100 μL each of a mixture of water and 1-propanol (3:2), and add 1 mL of 0.05 mol/L ammonium hydrogen carbonate solution. Add 20 μL each of a solution of V8 protease in 0.05 mol/L ammonium hydrogen carbonate solution (1 in 1000), and react at 37°C for 18 hours. To each reaction solution add 50 μL of diluted trifluoroacetic acid (1 in 10) to stop the reaction, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 100 μL – 150 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the chromatograms from these solutions: both chromatograms show the similar peaks at the corresponding retention time.

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 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 for liquid chromatography and trifluoroacetic acid (950:50:1).

Mobile phase B: A mixture of acetonitrile for liquid chromatography, water, and trifluoroacetic acid (800:200:1).

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 – 120	100 → 20	0 → 80
120 – 140	20 → 0	80 → 100
140 – 150	0	100

Flow rate: Adjust the flow rate so that the retention time of the first appeared peak is about 33 minutes.

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, the resolution between the first appeared peak and the second appeared peak is not less than 15.

(3) Put exactly 2 mL of Lenograstim (Genetical Recombination) into a precolumn, packed with 0.36 g of octadecylsilanized silica gel for pretreatment, wash the column with 5 mL of a mixture of water, acetonitrile and trifluoroacetic acid (600:400:1), then elute with a mixture of acetonitrile, water and trifluoroacetic acid (800:200:1), and collect exactly 5 mL of the first eluate. Pipet 1.5 mL of the eluate in a test tube, add exactly 20 μL of the internal standard solution, and lyophilize. Dissolve the lyophilized substance in 250 μL of a mixture of methanol and acetyl chloride (9:1), seal the tube, and heat at 90°C for 2 hours. After cooling, open the tube, and dry the content under reduced pressure. To the residue add 200 μL of methanol, and evaporate to dryness under reduced pressure. Dissolve the residue in 200 μL of a solution of pyridine in methanol (1 in 10) and 50 μL of acetic anhydride, stopper the tube tightly, and allow to stand for 10 minutes. Evaporate the solution to dryness at about 50°C under reduced pressure, add 200 μL of methanol to the residue, and evaporate to dryness at 50°C under reduced pressure. To the residue add 50 μL of a mixture of pyridine, 1,1,1,3,3,3-hexamethyldisilazane and chlorotrimethylsilane (10:2:1), stopper tightly, shake vigorously for 30 seconds, and warm at 50°C for 10 minutes. After cooling, add 300 μL of pentane, stir gently, then add 300 μL of water, and stir gently. Separate the upper layer, evaporate

to concentrate to about 10 μL under a stream of nitrogen, and use this as the sample solution. Separately, weigh accurately about 54 mg of D-galactose and about 33 mg of *N*-acetylgalactosamine, dissolve them separately in water to make exactly 20 mL each, and use these solutions as D-galactose solution and *N*-acetylgalactosamine solution, respectively. Weigh accurately about 9.3 mg of *N*-acetylneuraminic acid, add exactly 1 mL of the D-galactose solution and exactly 2 mL of the *N*-acetylgalactosamine solution to dissolve, and add water to make exactly 20 mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution, and freeze-dry 40 μL of this solution. Dissolve the freeze-dried substance in 250 μL of a mixture of methanol and acetyl chloride (9:1), then proceed in the same manner as the sample solution, and use the solution obtained as the monosaccharide standard solution. Perform the test with 2 μL each of the sample solution and the monosaccharide standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios of each major peak area of D-galactose, *N*-acetylgalactosamine and *N*-acetylneuraminic acid to that of the internal standard, Q_T and Q_S. Calculate the amount (mol/mol of lenograstim) of each monosaccharide by the following formula: the amounts of D-galactose, *N*-acetylgalactosamine and *N*-acetylneuraminic acid are between 0.7 and 1.2, between 0.7 and 1.2, and between 1.0 and 2.0, respectively.

$$\text{Amount (mol/mol of lenograstim) of each monosaccharide} = M/(M_m \times D_S) \times Q_T/Q_S \times 18{,}667/C \times 5/3$$

M: Amount (mg) of each monosaccharide
M_m: Molecular mass of each monosaccharide
D-galactose: 180.16
N-acetylgalactosamine: 221.21
N-acetylneuraminic acid: 309.27
D_S: Dilution rate of each monosaccharide
D-galactose: 20,000
N-acetylgalactosamine and: 10,000
N-acetylneuraminic acid: 1000
C: Protein concentration (mg/mL) of Lenograstim (Genetical Recombination)
18,667: Molecular mass of protein moiety of lenograstim

Internal standard solution—Dissolve 48 mg of myoinositol in water to make 50 mL. To 1 mL of this solution add water to make 20 mL.

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 7% cyanopropyl-7% phenyl-methyl silicon polymer for gas chromatography 0.25 μm in thickness.

Column temperature: Rise the temperature at a rate of 10°C per minute from 110°C to 185°C, then at a rate of 2°C per minute to 210°C, and to 260°C at a rate of 8°C per minute, and maintain 260°C for 15 minutes.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 24 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the monosaccharide standard solution under the above operating conditions, D-galactose, the internal standard, *N*-acetylgalactosamine and *N*-acetylneuraminic acid are eluted in this order, and the resolution between the peaks of the internal standard and *N*-acetylgalactosamine is not less than 10.

pH <2.54> 7.7 – 8.3

Purity **(1)** Related substances—Perform the test with a volume of Lenograstim (Genetical Recombination), equivalent to 30 μg of protein, 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 excluding the area of the solvent peak: the total amount of the peaks other than lenograstim is not more than 1.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with porous silica gel for liquid chromatography (10 μm in particle diameter).

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

Mobile phase: Dissolve 1.4 g of anhydrous disodium hydrogen phosphate and 5.8 g of sodium chloride in water to make 1000 mL (Solution A). Separately, dissolve 1.6 g of sodium dihydrogen phosphate dihydrate and 5.8 g of sodium chloride in water to make 1000 mL (Solution B). Adjust the pH of Solution A to 7.4 with Solution B.

Flow rate: Adjust the flow rate so that the retention time of lenograstim is about 21 minutes.

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

System suitability—

Test for required detectability: When the procedure is run with 60 μL of diluted Lenograstim RS with the solvent of Lenograstim (Genetical Recombination) containing 0.1 vol% polysorbate 20 (1 in 500) under the above operating conditions, the peak of lenograstim is detectable.

System performance: When the procedure is run using Lenograstim RS under the above operating conditions, the number of theoretical plates of the peak of lenograstim is not less than 2700.

(2) Host-derived protein Being specified separately.

(3) DNA Being specified separately.

Assay **(1)** Protein—Use Lenograstim (Genetical Recombination) and Lenograstim RS as the sample solution and the standard solution, respectively. Perform the test with exactly 30 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 lenograstim.

Amount (mg) of protein in 1 mL of Lenograstim (Genetical Recombination)
$= C_S \times A_T/A_S$

C_S: Concentration (mg/mL) of protein in Lenograstim RS

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 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 for liquid chromatography and trifluoroacetic acid (600:400:1).

Mobile phase B: A mixture of acetonitrile for liquid chromatography, water and trifluoroacetic acid (800:200:1).

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 – 40	80 → 30	20 → 70

Flow rate: Adjust the flow rate so that the retention time of lenograstim is about 35 minutes.

System suitability—

System performance: When the procedure is run with 30 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of lenograstim is not less than 2900.

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 peak area of lenograstim is not more than 4.0%.

(2) Specific activity—Dilute Lenograstim (Genetical Recombination) with FBS-IMDM so that each mL contains an estimate amount of 7.69 units, 10.0 units and 13.0 units, and name them as the sample solution (1), the sample solution (2) and the sample solution (3), respectively. Separately, dilute Lenograstim RS with FBS-IMDM so that each mL contains 7.69 units, 10.0 units and 13.0 units, and name them as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. Put exactly 100 μL each of the sample solutions and the standard solutions in wells of a sterile disposable multiple well plate, add 50 μL each of NFS-60 cell suspension (prepared by adding FBS-IMDM so the each mL contains about 5×10^5 cells) to each well and mix to make homogenize, and place the plate in a CO_2 incubator at 37°C. After incubation for 22 hours, add 15 μL of resazurin solution to each well, and determine the absorbances at 570 nm, A_{T1} and A_{S1}, and at 600 nm, A_{T2} and A_{S2}. From the reaction values at each concentration of the standard solution and sample solution [deference of absorbance ($A_{S1} - A_{S2}$ and $A_{T1} - A_{T2}$)], determine the rate of potency (Pr) of the sample solution to the standard solution by the parallel assay, and calculate the potency (unit) per 1 mg of protein of Lenograstim (Genetical Recombination).

$Pr = \text{anti ln}\ (M)$
$M = (P_T - P_S)/\text{db}$
$P_T = T_1 + T_2 + T_3$
$P_S = S_1 + S_2 + S_3$
$\text{b} = H_L(L_S + L_T)/\text{Inh}$
$H_L = 12\text{n}/(\text{d}^3 - \text{d})$
$L_S = 1S_1 + 2S_2 + 3S_3 - 1/2(\text{d} + 1)P_S$
$L_T = 1T_1 + 2T_2 + 3T_3 - 1/2(\text{d} + 1)P_T$
$\text{d} = 3$
$\text{I} = \ln 1.3$
$\text{n} = 3$
$\text{h} = 2$
T_1: Mean of reaction values of the sample solution (1)
T_2: Mean of reaction values of the sample solution (2)
T_3: Mean of reaction values of the sample solution (3)
S_1: Mean of reaction values of the standard solution (1)
S_2: Mean of reaction values of the standard solution (2)
S_3: Mean of reaction values of the standard solution (3)

Specific activity (unit/mg of protein) of lenograstim
$= S \times Pr \times D_T/D_S/C$

S: Potency (unit/mL) of Lenograstim RS
D_T: Dilution rate of the sample solution (3)
D_S: Dilution rate of the standard solution (3)
C: Concentration (mg/mL) of protein of sample

Containers and storage Containers—Tight containers.
Storage—At a temperature not exceeding −20°C.

Add the following:

Levofloxacin Fine Granules

レボフロキサシン細粒

Levofloxacin Fine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$: 361.37).

Method of preparation Prepare as directed under Granules, with Levofloxacin Hydrate.

Identification To an amount of Levofloxacin Fine Granules, equivalent to 50 mg of levofloxacin ($C_{18}H_{20}FN_3O_4$), add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make 50 mL, and stir for 20 minutes. 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 to 1 mL of the subsequent filtrate add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 225 nm and 229 nm and between 292 nm and 296 nm, and a shoulder between 321 nm and 331 nm.

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

To the total amount of the content of 1 container of Levofloxacin Fine Granules add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly V mL so that each mL of the solution contains about 1 mg of levofloxacin ($C_{18}H_{20}FN_3O_4$), and stir for 20 minutes. 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 1 mL of the subsequent filtrate, add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of levofloxacin hydrate for assay (separately determine the water <*2.48*> in the same manner as Levofloxacin Hydrate), and dissolve in diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 50 mL. Pipet 2 mL of this solution, add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 327 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of levofloxacin ($C_{18}H_{20}FN_3O_4$)
$= M_S \times A_T/A_S \times V/25$

M_S: Amount (mg) of levofloxacin hydrate for assay, calculated on the anhydrous basis

Dissolution <*6.10*> When the tests are performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes of Levofloxacin Fine Granules is not less than 70%.

Start the test with an accurately weighed amount of the content of Levofloxacin Fine Granules, equivalent to about 0.1 g of levofloxacin ($C_{18}H_{20}FN_3O_4$), 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 0.45μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of levofloxacin hydrate for assay (separately determine the water <*2.48*> in the same manner as Levofloxacin Hydrate), and dissolve in 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. Determine the absorbances, A_T and A_S, at 289 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 levofloxacin ($C_{18}H_{20}FN_3O_4$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 360$

M_S: Amount (mg) of levofloxacin hydrate for assay, calculated on the anhydrous basis
M_T: Amount (g) of Levofloxacin Fine Granules
C: Labeled amount (mg) of levofloxacin ($C_{18}H_{20}FN_3O_4$) in 1 g

Assay Weigh accurately an amount of Levofloxacin Fine Granules, powder if necessary, equivalent to about 50 mg of levofloxacin ($C_{18}H_{20}FN_3O_4$), add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 50 mL, stir for 20 minutes, and 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 diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of levofloxacin hydrate for assay (separately determine the water <*2.48*> in the same manner as Levofloxacin Hydrate), and dissolve in diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 50 mL. Pipet 5 mL of this solution, add diluted 3 mol/L hydrochloric acid TS (1 in 100) 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 levofloxacin of each solution.

Amount (mg) of levofloxacin ($C_{18}H_{20}FN_3O_4$)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of levofloxacin hydrate for assay, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 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: Dissolve 1.00 g of copper (II) sulfate pentahydrate, 1.41 g of L-valine and 6.17g of ammonium acetate in 800 mL of water, and add 200 mL of methanol.

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

System suitability—

System performance: Dissolve 10 mg of ofloxacin in 20 mL of diluted 3 mol/L hydrochloric acid TS (1 in 100). To 1 mL of this solution add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, levofloxacin and an enantiomer 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 levofloxacin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Levofloxacin Ophthalmic Solution

レボフロキサシン点眼液

Levofloxacin Ophthalmic Solution is an aqueous ophthalmic solution.

It contains not less than 95.0% and not more than 107.0% of the labeled amount of levofloxacin hydrate ($C_{18}H_{20}FN_3O_4.\frac{1}{2}H_2O$: 370.38).

Method of preparation Prepare as directed under Ophthalmic Preparations, with Levofloxacin Hydrate.

Description Levofloxacin Ophthalmic Solution occurs as a clear, pale yellow to yellow liquid.

Identification (1) To an amount of Levofloxacin Ophthalmic Solution, equivalent to 5 mg of Levofloxacin Hydrate, add 0.01 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution add 0.01 mol/L hydrochloric acid TS to make 20 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 225 nm and 229 nm, and between 292 nm and 296 nm.

(2) To an amount of Levofloxacin Ophthalmic Solution, equivalent to 5 mg of Levofloxacin Hydrate, add a mixture of water and methanol (1:1) to make 5 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of levofloxacin hydrate 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 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 peaks obtained from the sample solution and the standard solution is the same.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 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: Dissolve 1.25 g of copper (II) sulfate pentahydrate, 1.76 g of L-valine and 7.71 g of ammonium acetate in water to make 1000 mL, and add 250 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of levofloxacin is about 22 minutes.

System suitability—

System performance: Dissolve 10 mg of ofloxacin in 20 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add a mixture of water and methanol (1:1) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak of levofloxacin and the peak having the relative retention time of about 1.2 to levofloxacin is not less than 3.

Osmotic pressure ratio Being specified separately.

pH Being specified separately.

Foreign insoluble matter <*6.11*> It meets the requirement.

Insoluble particulate matter <*6.08*> It meets the requirement.

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

Assay To an accurately weighed amount of Levofloxacin Ophthalmic Solution, equivalent to about 5 mg of levofloxacin hydrate ($C_{18}H_{20}FN_3O_4.\frac{1}{2}H_2O$) add exactly 2 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of levofloxacin hydrate for assay (separately determine the water <*2.48*> in the same manner as Levofloxacin Hydrate), and dissolve in water to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution, then add the mobile phase 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 levofloxacin to that of the internal standard.

$$\text{Amount (mg) of levofloxacin hydrate } (C_{18}H_{20}FN_3O_4.\tfrac{1}{2}H_2O) = M_S \times Q_T/Q_S \times 1/5 \times 1.025$$

M_S: Amount (mg) of levofloxacin hydrate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of naphazoline hydrochloride in the mobile phase (3 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 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 13.61 g of potassium dihydrogen phosphate and 0.77 g of ammonium acetate in 900 mL of water, adjust to pH 3.0 with 1 mol/L hydrochloric acid TS, 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 levofloxacin 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, levofloxacin 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of levofloxacin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Add the following:

Levofloxacin Tablets

レボフロキサシン錠

Levofloxacin Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of levofloxacin ($C_{18}H_{20}FN_3O_4$: 361.37).

Method of preparation Prepare as directed under Tablets, with Levofloxacin Hydrate.

Identification To an amount of powdered Levofloxacin Tablets, equivalent to 0.1 g of levofloxacin ($C_{18}H_{20}FN_3O_4$), add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make 100 mL, and stir for 20 minutes. 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 to 1 mL of the subsequent filtrate add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 225 nm and 229 nm and between 292 nm and 296 nm, and a shoulder between 321 nm and 331 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 Levofloxacin Tablets add about 70 mL of diluted 3 mol/L hydrochloric acid TS (1 in 100), agitate to disintegrate the tablet with the aid of ultrasonic waves, add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 100 mL, and stir for 20 minutes. Pipet V mL the solution, add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly V' mL so that each mL of the solution contains about 50 μg of levofloxacin ($C_{18}H_{20}FN_3O_4$), and 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. Then, proceed as directed in the Assay.

Amount (mg) of levofloxacin ($C_{18}H_{20}FN_3O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/5$

M_S: Amount (mg) of levofloxacin hydrate for assay, calculated on the anhydrous basis

Dissolution <*6.10*> **(1)** For a 100-mg Tablet When the tests are performed at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 90 minutes is not less than 80%.

Start the test with 1 tablet of Levofloxacin 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 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of levofloxacin hydrate for assay (separately determine the water <*2.48*> in the same manner as Levofloxacin Hydrate), and dissolve in 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. Determine the absorbances, A_T and A_S, at 289 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 levofloxacin hydrate ($C_{18}H_{20}FN_3O_4.½H_2O$)
$= M_S \times A_T/A_S \times 18/5 \times 1.025$

M_S: Amount (mg) of levofloxacin hydrate for assay, calculated on the anhydrous basis

(2) For a 250-mg Tablet and 500-mg Tablet When the tests are 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 is not less than 80%.

Start the test with 1 tablet of Levofloxacin 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 11.2 μg of levofloxacin ($C_{18}H_{20}FN_3O_4$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of levofloxacin hydrate for assay (separately determine the water <*2.48*> in the same manner as Levofloxacin Hydrate), and dissolve in the dissolution medium to make exactly 50 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 287 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 levofloxacin ($C_{18}H_{20}FN_3O_4$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of levofloxacin hydrate for assay, calculated on the anhydrous basis
C: Amount (mg) of levofloxacin ($C_{18}H_{20}FN_3O_4$) in 1 g

Assay Accurately weigh the mass of not less than 20 Levofloxacin Tablets, and powder them. Weigh accurately a portion of the powder, equivalent to about 1 g of levofloxacin ($C_{18}H_{20}FN_3O_4$), add 150 mL of diluted 3 mol/L

hydrochloric acid TS (1 in 100), agitate with the aid of ultrasonic waves for 5 minutes, and add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 200 mL, and stir for 10 minutes. Pipet 2 mL of this solution, add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 200 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. Separately, weigh accurately about 25 mg of levofloxacin hydrate for assay (separately determine the water <*2.48*> in the same manner as Levofloxacin Hydrate), and dissolve in diluted 3 mol/L hydrochloric acid TS (1 in 100) to make exactly 50 mL. Pipet 2 mL of this solution, add diluted 3 mol/L hydrochloric acid TS (1 in 100) 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 levofloxacin of each solution.

$$\text{Amount (mg) of levofloxacin } (C_{18}H_{20}FN_3O_4) = M_S \times A_T/A_S \times 40$$

M_S: Amount (mg) of levofloxacin hydrate for assay, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 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: Dissolve 1.00 g of copper (II) sulfate pentahydrate, 1.41 g of L-valine and 6.17g of ammonium acetate in 800 mL of water, and add 200 mL of methanol.

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

System suitability—

System performance: Dissolve 10 mg of ofloxacin in 20 mL of diluted 3 mol/L hydrochloric acid TS (1 in 100). To 1 mL of this solution add diluted 3 mol/L hydrochloric acid TS (1 in 100) to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, levofloxacin and an enantiomer are eluted in this order with the resolution between these peaks being not less than 3.

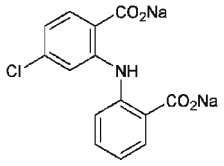
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of levofloxacin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Lobenzarit Sodium

ロベンザリットナトリウム



$C_{14}H_8ClNNa_2O_4$: 335.65

Disodium 2-[(2-carboxylatophenyl)amino]-4-chlorobenzoate [*64808-48-6*]

Lobenzarit Sodium, when dried, contains not less than 98.0% and not more than 101.0% of $C_{14}H_8ClNNa_2O_4$.

Description Lobenzarit Sodium occurs as a white to pale yellowish white crystalline powder.

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

Identification **(1)** A solution of Lobenzarit Sodium (1 in 50) responds to the Qualitative Tests <*1.09*> (1) for chloride.

(2) Determine the absorption spectrum of a solution of Lobenzarit Sodium (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 Lobenzarit Sodium, 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.

(4) A solution of Lobenzarit Sodium (1 in 50) responds to the Qualitative Tests <*1.09*> (2) for sodium salt.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Lobenzarit 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).

(2) Arsenic <*1.11*>—Prepare the test solution with 2.0 g of Lobenzarit Sodium according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 50 mg of Lobenzarit Sodium in 2.5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 20 mL, 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 tetrahydrofuran, water and triethylamine (50:15:8) to a distance

of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

(4) Residual solvent Being specified separately.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 2 hours).

Assay Weigh accurately about 0.1 g of Lobenzarit Sodium, previously dried, dissolve in exactly 40 mL of water, add exactly 60 mL of a mixture of diethyl ether and tetrahydrofuran (1:1), and titrate ⟨*2.50*⟩ with 0.1 mol/L hydrochloric acid VS while well shaking (indicator: 10 drops of bromophenol blue TS) until the blue color of the water layer changes to a persistent light blue-green. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L hydrochloric acid VS
= 16.78 mg of $C_{14}H_8ClNNa_2O_4$

Containers and storage Containers—Tight containers.

Add the following:

Losartan Potassium Tablets

ロサルタンカリウム錠

Losartan Potassium 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).

Method of preparation Prepare as directed under Tablets, with Losartan Potassium.

Identification To an amount of powdered Losartan Potassium Tablets, equivalent to 25 mg of losartan potassium, add 10 mL of methanol, shake well, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 25 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of losartan potassium in 10 mL of methanol. 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 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, methanol and acetic acid (100) (75:25:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show 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 Losartan Potassium Tablets add diluted 0.1 mol/L phosphate buffer solution, pH 8.0 (1 in 10) to make exactly 100 mL, and stir until the tablet is completely disintegrated. Pipet 5 mL of this solution, add diluted 0.1 mol/L phosphate buffer solution, pH 8.0 (1 in 10) to make exactly V mL so that each mL contains about 50 μg of losartan potassium ($C_{22}H_{22}ClKN_6O$), centrifuge, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$)
$= M_S \times A_T/A_S \times V/25$

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

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute for 25-mg and 50-mg tablets and at 75 revolutions per minute for 100-mg tablet according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 45 minutes of 25-mg and 50-mg tablets, and in 30 minutes of 100-mg tablet is not less than 85%, respectively.

Start the test with 1 tablet of Losartan Potassium 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 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 22 μg of losartan potassium ($C_{22}H_{22}ClKN_6O$), and use this solution as the sample solution. Separately, weigh accurately about 50 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. Pipet 5 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 256 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 losartan potassium ($C_{22}H_{22}ClKN_6O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

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

Assay To 20 Losartan Potassium Tablets add diluted 0.1 mol/L phosphate buffer solution, pH 8.0 (1 in 10) to make exactly 1000 mL, and stir until the tablets are completely disintegrated. Pipet 5 mL of this solution, add diluted 0.1 mol/L phosphate buffer solution, pH 8.0 (1 in 10) to make exactly V mL so that each mL contains about 50 μg of losartan potassium ($C_{22}H_{22}ClKN_6O$), centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of Losartan Potassium RS (separately determine the water ⟨*2.48*⟩ in the same manner as Losartan Potassium), dissolve in diluted 0.1 mol/L phosphate buffer solution, pH 8.0 (1 in 10) to make exactly 500 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 of these solutions.

Amount (mg) of losartan potassium ($C_{22}H_{22}ClKN_6O$) in 1 tablet
$= M_S \times A_T/A_S \times V/50$

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 600 mL of this solution add 400 mL of acetonitrile

Flow rate: Adjust the flow rate so that the retention time of losartan 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 losartan 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 losartan is not more than 1.0%.

Containers and storage Containers—Tight containers.

Magnesium Stearate

ステアリン酸マグネシウム

Change to read:

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 (◆ ◆).

Magnesium Stearate is a compound of magnesium with a mixture of solid fatty acids, and consists chiefly of variable proportions of magnesium stearate and magnesium palmitate obtained from sources of vegetable or animal origin.

It contains not less than 4.0% and not more than 5.0% of magnesium (Mg: 24.31), calculated on the dried basis.

◆**Description** Magnesium Stearate occurs as a white, light, bulky powder.

It is smooth to the touch and sticky to the skin. It has no odor or a faint, characteristic odor.

It is practically insoluble in water and in ethanol (99.5).◆

Identification Mix 5.0 g of Magnesium Stearate with 50 mL of peroxide-free diethyl ether, 20 mL of dilute nitric acid, and 20 mL of water in a round-bottom flask, and heat to dissolve completely under a reflux condenser. After cooling, transfer the contents of the flask to a separator, shake, allow the layers to separate, and transfer the aqueous layer to a flask. Extract the diethyl ether layer with two 4-mL portions of water, and combine these extracts to the main aqueous extract. After washing the combined aqueous extract with 15 mL of peroxide-free diethyl ether, transfer to a 50-mL volumetric flask, add water to make 50 mL, and use this solution as the sample solution. To 1 mL of the sample solution add 1 mL of ammonia TS: A white precipitate is formed that dissolves on addition of 1 mL of ammonium chloride TS. By further addition of 1 mL of a solution of disodium hydrogen phosphate dodecahydrate (4 in 25) a white crystalline precipitate is formed.

Purity **(1)** Acidity or alkalinity—Heat 1.0 g of Magnesium Stearate in 20 mL of freshly boiled and cooled water on a water bath for 1 minute while shaking, cool, and filter. Add 0.05 mL of bromothymol blue TS to 10 mL of the filtrate: not more than 0.05 mL of 0.1 mol/L hydrochloric acid VS or 0.1 mol/L sodium hydroxide VS is required to change the color of the solution.

(2) Chloride <1.03>—Perform the test with 10.0 mL of the sample solution obtained in Identification. Prepare the control solution with 1.4 mL of 0.02 mol/L hydrochloric acid VS (not more than 0.1%).

(3) Sulfate <1.14>—Perform the test with 6.0 mL of the sample solution obtained in Identification. Prepare the control solution with 3.0 mL of 0.02 mol/L sulfuric acid VS (not more than 1.0%).

◆**(4)** Heavy metals <1.07>—Heat 1.0 g of Magnesium Stearate weakly first, then incinerate at about 500 ± 25°C. After cooling, add 2 mL of hydrochloric acid, evaporate on a water bath to dryness, add 20 mL of water and 2 mL of dilute acetic acid to the residue, and heat for 2 minutes. After cooling, filter this solution through a filter paper, wash the filter paper with 15 mL of water, and combine the washing with the filtrate. To the filtrate add water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution as follows: evaporate 2 mL of hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).◆

Loss on drying <2.41> Not more than 6.0% (2 g, 105°C, constant mass).

◆**Microbial limit** <4.05> The acceptance criteria of TAMC and TYMC are 10^3 CFU/g and 5×10^2 CFU/g, respectively. *Salmonella* and *Escherichia coli* are not observed.◆

Relative content of stearic acid and palmitic acid Transfer

0.10 g of Magnesium Stearate to a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and reflux for 10 minutes to dissolve the solids. Add 4 mL of heptane through the condenser, and reflux for 10 minutes. After cooling, add 20 mL of saturated sodium chloride solution, shake, and allow the layers to separate. Pass the heptane layer through about 0.1 g of anhydrous sodium sulfate, previously washed with heptane, into another flask. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with heptane to volume, 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 area, A, of the methyl stearate peak and the sum of the areas, B, of all of the fatty acid ester peaks. Calculate the percentage of stearic acid in the fatty acid fraction of Magnesium Stearate by the following formula.

$$\text{Content (\%) of stearic acid} = A/B \times 100$$

Similarly, calculate the percentage of palmitic acid in the portion of Magnesium Stearate taken. The methyl stearate peak, and the sum of the stearate and palmitate peaks are not less than 40% and not less than 90% of the total area of all fatty acid ester peaks, respectively.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica capillary column 0.32 mm in inside diameter and 30 m in length, the inside coated with a 0.5-μm layer of polyethylene glycol 15000-diepoxide for gas chromatography.

Column temperature: Maintain at 70°C for 2 minutes after injection, then program to increase the temperature at the rate of 5°C per minute to 240°C and to maintain 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: Splitless.

◆Time span of measurement: For 41 minutes after the solvent peak.◆

System suitability—

◆Test for required detectability:◆ Place about 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, and proceed in the same manner as directed for the preparation of the sample solution, and use the solution so obtained as the solution for system suitability test. ◆To exactly 1 mL of the solution add heptane to make exactly 10 mL. To exactly 1 mL of this solution add heptane to make exactly 10 mL. Further, to exactly 1 mL of this solution add heptane to make exactly 10 mL. Confirm that the peak area of methyl stearate obtained from 1 μL of this solution is equivalent to 0.05 to 0.15% of that from 1 μL of the solution 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 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 are not more than 3.0%, respectively, and the relative standard deviation of the ratios of the peak area of methyl palmitate to methyl stearate is not more than 1.0%.

Assay Transfer about 0.5 g of Magnesium Stearate, accurately weighed, to a 250-mL flask, add 50 mL of a mixture of ethanol (99.5) and 1-butanol (1:1), 5 mL of ammonia solution (28), 3 mL of ammonium chloride buffer solution, pH 10, 30.0 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and 1 to 2 drops of eriochrome black T TS, and mix. Heat at 45 – 50°C to make the solution clear, and after cooling, titrate <*2.50*> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.1 mol/L zinc sulfate VS until the solution changes from blue to violet in color. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.431 mg of Mg

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

Mefloquine Hydrochloride

メフロキン塩酸塩

Change the origin/limits of content and Identification (3) as follows:

Mefloquine Hydrochloride contains not less than 99.0% and not more than 101.0% of $C_{17}H_{16}F_6N_2O$. HCl, calculated on the anhydrous basis.

Identification

(3) Determine the infrared absorption spectrum of Mefloquine Hydrochloride, previously dried at 105°C for 2 hours, 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.

Delete the Loss on drying:

Add the following next to the Purity:

Water <*2.48*> Not more than 3.0% (1 g, volumetric titration, direct titration).

Change the Assay as follows:

Assay Weigh accurately about 0.5 g of Mefloquine Hydrochloride, dissolve in 100 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
= 41.48 mg of $C_{17}H_{16}F_6N_2O.HCl$

10% *dl*-Methylephedrine Hydrochloride Powder

dl-メチルエフェドリン塩酸塩散10%

Add the following next to the Identification:

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 10% *dl*-Methylephedrine Hydrochloride Powder is not less than 85%.

Start the test with about 0.5 g of 10% *dl*-Methylephedrine Hydrochloride Powder, accurately weighed, 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 2 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add exactly 2 mL of the mobile phase, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of *dl*-methylephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 25 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the mobile phase, 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 methylephedrine of each solution.

Dissolution rate (%) with respect to the labeled amount of *dl*-methylephedrine hydrochloride ($C_{11}H_{17}NO.HCl$)
$= M_S/M_T \times A_T/A_S \times 9/4$

M_S: Amount (mg) of *dl*-methylephedrine hydrochloride for assay
M_T: Amount (g) of 10% *dl*-Methylephedrine Hydrochloride Powder

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).

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 methylephedrine 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 methylephedrine is not more than 2.0%.

Methyl Parahydroxybenzoate

パラオキシ安息香酸メチル

Change the Description and below as follows:

◆**Description** Methyl Parahydroxybenzoate, occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetone, and slightly soluble in water.◆

Identification Determine the infrared absorption spectrum of Methyl Parahydroxybenzoate 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 Methyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point ⟨*2.60*⟩ 125 – 128°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Methyl Parahydroxybenzoate in ethanol (95) to make 10 mL: the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Acidity—To 2 mL of the solution of Methyl Parahydroxybenzoate obtained in (1) add 3 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mol/L sodium hydroxide VS until the solution shows a blue color: the volume of 0.1 mol/L sodium hydroxide VS used does not exceed 0.1 mL.

◆**(3)** Heavy metals ⟨*1.07*⟩—Dissolve 1.0 g of Methyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).◆

(4) Related substances—Dissolve 50 mg of Methyl Parahydroxybenzoate in 2.5 mL of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sam-

ple solution, and add the mobile phase to make exactly 20 mL. Pipet 1 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 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 parahydroxybenzoic acid having a relative retention time of about 0.6 to methyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of methyl parahydroxybenzoate from the standard solution (0.5%). For this calculation use the peak area of parahydroxybenzoic acid after multiplying by the relative response factor, 1.4. Furthermore, the area of the peak other than methyl parahydroxybenzoate and parahydroxybenzoic acid from the sample solution is not larger than the peak area of methyl parahydroxybenzoate from the standard solution (0.5%), and the total area of the peaks other than methyl parahydroxybenzoate is not larger than 2 times the peak area of methyl parahydroxybenzoate from the standard solution (1.0%). For this calculation the peak area not larger than 1/5 times the peak area of methyl parahydroxybenzoate from the standard solution is excluded (0.1%).

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 methyl parahydroxybenzoate.

System suitability—

◆Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of methyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.◆

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

◆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 2.0%.◆

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Methyl Parahydroxybenzoate and Methyl Parahydroxybenzoate RS, dissolve separately in 2.5 mL each of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase 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 methyl parahydroxybenzoate of each solution.

Amount (mg) of methyl parahydroxybenzoate ($C_8H_8O_3$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Methyl Parahydroxybenzoate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 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 35°C.

Mobile phase: A mixture of methanol and potassium dihydrogen phosphate solution (17 in 2500) (13:7).

Flow rate: 1.3 mL per minute.

System suitability—

System performance: Dissolve 5 mg each of Methyl Parahydroxybenzoate and parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, parahydroxybenzoic acid and methyl parahydroxybenzoate are eluted in this order, the relative retention time of parahydroxybenzoic acid to methyl parahydroxybenzoate is about 0.6, and the resolution between these peaks is 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 methyl parahydroxybenzoate is not more than 0.85%.

◆**Containers and storage** Containers—Well-closed containers.◆

Mizoribine

ミゾリビン

Change the Containers and storage as follows:

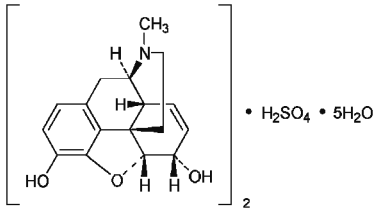
Containers and storage Containers—Tight containers.

Storage—At a temperature between 2 and 8°C.

Add the following:

Morphine Sulfate Hydrate

モルヒネ硫酸塩水和物



$(C_{17}H_{19}NO_3)_2.H_2SO_4.5H_2O$: 758.83

(5*R*,6*S*)-4,5-Epoxy-17-methyl-7,8-didehydromorphinan-3,6-diol hemisulfate hemipentahydrate

[*6211-15-0*]

Morphine Sulfate Hydrate contains not less than 98.0% and not more than 102.0% of morphine sulfate $[(C_{17}H_{19}NO_3)_2.H_2SO_4$: 668.75], calculated on the anhydrous basis.

Description Morphine Sulfate Hydrate occurs as a white, crystals or crystalline powder.

It is very soluble in formic acid, soluble in water, slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

It dissolves in dilute sodium hydroxide TS.

Identification **(1)** Determine the absorption spectrum of a solution of Morphine Sulfate Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Determine the absorption spectrum of a solution of Morphine Sulfate Hydrate in dilute sodium hydroxide TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Morphine Sulfate 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) A solution of Morphine Sulfate Hydrate (1 in 25) responds to the Qualitative Tests <*1.09*> (1) and (3) for sulfate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -107 – $-112°$ (0.2 g, calculated on the anhydrous basis, water, 20 mL, 100 mm).

Purity **(1)** Acidity—Dissolve 0.5 g of Morphine Sulfate Hydrate in 15 mL of water, add 2 drops of methyl red TS, and neutralize with 0.02 mol/L sodium hydroxide VS: the necessary volume of 0.02 mol/L sodium hydroxide VS is not more than 0.50 mL.

(2) Ammonium Being specified separately.

(3) Chloride—Dissolve 0.10 g of Morphine Sulfate Hydrate in 10 mL of water, add 1 mL of dilute nitric acid, then add 1 mL of silver nitrate TS: no turbidity is produced.

(4) Meconic acid—Dissolve 0.20 g of Morphine Sulfate Hydrate in 5 mL of water, add 5 mL of dilute hydrochloric acid and 2 drops of iron (III) chloride TS: no red color develops.

(5) Related substances—Dissolve 0.20 g of Morphine Sulfate 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 at *R*f value of about 0.17 obtained with the sample solution is not more intense than the spot with the standard solution (1), and the spot other than the principle spot, the spot at *R*f value of about 0.17 and the spot at original point is not more intense than the spot with the standard solution (2).

(6) Residual solvent Being specified separately.

Water <*2.48*> 11.0 – 13.0% (0.1 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Morphine Sulfate Hydrate, dissolve in 3 mL of formic acid, add 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <*2.50*> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 33.44 mg of $(C_{17}H_{19}NO_3)_2.H_2SO_4$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Nartograstim (Genetical Recombination)

ナルトグラスチム(遺伝子組換え)

MAPTYRASSL PQSFLLKSLE QVRKIQGDGA ALQEKLCATY KLCHPEELVL
LGHSLGIPWA PLSSCPSQAL QLAGCLSQLH SGLFLYQGLL QALEGISPEL
GPTLDTLQLD VADFATTIWQ QMEELGMAPA LQPTQGAMPA FASAFQRRAG
GVLVASHLQS FLEVSYRVLR HLAQP

$C_{850}H_{1344}N_{226}O_{245}S_8$: 18905.65
[*134088-74-7*]

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.

Description Nartograstim (Genetical Recombination) occurs as a clear and colorless liquid.

Identification **(1)** To a suitable amount of Nartograstim (Genetical Recombination) add tris-sodium chloride buffer solution, pH 8.0 so that each mL contains 1 μg of protein, and use this solution as the sample solution. Put 0.1 mL of the sample solution in the well of a microplate for antigen-antibody reaction test, allow to stand at 5°C for not less than 10 hours, then remove the liquid, and wash the well. Then to the well add 0.25 mL of blocking TS for nartograstim test, and allow to stand at room temperature for 1 hour. Remove the blocking TS, add 0.1 mL of rabbit anti-nartograstim antibody TS to the well, and stir gently at room temperature for 3 hours. Remove the rabbit antinartograstim antibody TS, and wash the well. Then, add 0.1 mL of peroxidase labeled anti-rabbit antibody TS, stir gently at room temperature for 2 hours, remove the TS, and wash the well. Then, add 0.1 mL of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt TS, allow to stand at room temperature for 10 minutes, add 0.1 mL of a solution of oxalic acid dihydrate (1 in 50), and name this well as the sample well. Separately, proceed with 0.1 mL of tris-sodium chloride buffer solution, pH 8.0 in the same manner as for the sample solution, and name the well so obtained as the control well. When compere the sample well and the control well, the sample well reveals a green color, while the control well reveals no color.

Washing procedure of well: To the well add 0.25 mL of washing fluid for nartograstim test, allow to stand for 3 minutes, and remove the washing fluid. Repeat this procedure 2 times more.

(2) To a suitable amount of Nartograstim (Genetical Recombination) add water so that each mL contains 1 mg of protein. Replace the solvent of 2 mL of this solution with tris-calcium chloride buffer solution, pH 6.5. To 0.5 mL of the solution so obtained add 0.5 mL of tris-calcium chloride buffer solution, pH 6.5 and 5 μL of thermolysin solution (1 in 1000), allow to stand at 37°C for 21 hours, and use this solution as the sample solution. Separately, proceed with 2 mL of Nartograstim RS in the same manner as for the sample solution, and use the solution so obtained 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 compare these chromatograms: the similar peaks appear at the same retention times.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 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 35°C.

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

Mobile phase B: A mixture of acetonitrile, water and trifluoroacetic acid (900:100:1).

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 – 5	100	0
5 – 90	100 → 40	0 → 60

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above conditions, the number of the peak which shows not less than 1.6 of the resolution between the adjacent peaks is not less than 15.

pH <*2.54*> 7.0 – 7.5

Purity **(1)** Related substances—To a suitable amount of Nartograstim (Genetical Recombination) add the buffer solution for nartograstim sample so that each mL contains about 0.5 mg of protein, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the buffer solution for nartograstim sample 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 by SDS polyacrylamide gel electrophoresis, using buffer solution for SDS polyacrylamide gel electrophoresis and polyacrylamide gel for nartograstim. After elec-

trophoresis, immerse the gel in a solution of coomassie brilliant blue R-250 in a mixture of water, ethanol (95) and acetic acid (100) (5:4:1) (1 in 1000), and stir gently at room temperature for not less than 12 hours. Then, remove the color from the gel in a mixture of water, ethanol (95) and acetic acid (100) (13:5:2), and dry the gel under reduced pressure. Determine the areas of the colored bands obtained from the sample solution and standard solution by a densitometer at the measure wavelength 560 nm and the control wavelength 400 nm: the total area of the band other than the principal band obtained from the sample solution is not larger than the band area obtained from the standard solution.

(2) Host-derived protein Being specified separately.

(3) DNA Being specified separately.

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.62 EU/μg.

Molecular mass To a suitable amount of Nartograstim (Genetical Recombination) add reduction buffer solution for nartograstim sample so that each mL contains about 0.5 mg of protein, and use this solution as the sample solution. Separately, to 50 μL of molecular mass marker for nartograstim test add reduction buffer solution for nartograstim sample to make 1.0 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution, both previously warmed at 40°C for 15 minutes, by SDS polyacrylamide gel electrophoresis, using buffer solution for SDS polyacrylamide gel electrophoresis and polyacrylamide gel for nartograstim. After electrophoresis, immerse the gel in a solution of coomassie brilliant blue R-250 in a mixture of water, ethanol (95) and acetic acid (100) (5:4:1) (1 in 1000), and stir gently at room temperature for not less than 12 hours. Then, destain the gel with a mixture of water, ethanol (95) and acetic acid (100) (13:5:2), and dry the gel under reduced pressure. Prepare a calibration curve from the migration distance of the molecular mass markers of the standard solution by plotting the migration distance on the horizontal axis and logarithm of the molecular mass on the vertical axis. Calculate the molecular mass of the sample solution from the calibration curve: the molecular mass of the main band is between 17,000 and 19,000.

Compositions ratio of related substance Being specified separately.

Assay (1) Protein content—To exactly V_1 mL of Nartograstim (Genetical Recombination) add exactly V_2 mL of water so that each mL contains about 0.5 mg of protein, and centrifuge. Determine the absorbance, A, of the supernatant liquid at the absorption maximum at about 280 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of protein in 1 mL of Nartograstim (Genetical Recombination)

$$= A/8.71 \times (V_1 + V_2)/V_1 \times 10$$

8.71: Specific absorbance

(2) Specific activity—To a suitable exact amount of Nartograstim (Genetical Recombination) add potency measuring medium for nartograstim test so that the potency is equivalent to 50% to 150% of the relative potency of the standard solution according to the expected potency, and use this solution as the sample solution. Separately, to a suitable exact amount of Nartograstim RS add an exact amount of the potency measuring medium for nartograstim test so that each mL contains exactly 1.2×10^4 units of nartograstim, and use this solution as the standard solution. Culture NFS-60 cells with subculture medium for nartograstim test, centrifuge the medium, remove the supernatant liquid by suction, and wash the cells with the potency measuring medium for nartograstim test. Repeat the washing procedure twice more, prepare two cell suspensions, containing 8×10^5 cells per mL and 4×10^5 cells per mL in the potency measuring medium for nartograstim test, and use these solutions as the cell suspension (1) and (2), respectively. In 8 wells of the 12th column of a 8×12 well-microplate put 50 μL each of the cell suspension (1), and in all wells of the 1st to 11th columns put 50 μL each of the cell suspension (2). Where, the wells of the 1st and 8th lines are not used for the test. To the wells of the 2nd to 4th lines of the 12th column add 50 μL each of the standard solution, and to the wells of 5th to 7th lines of the 12th column add 50 μL each of the sample solution. From the wells of the 12th column take 50 μL each of the content liquid and transfer to the corresponding wells of the 1st column. Then, from the wells of the 1st column take 50 μL each of the content liquid and transfer to the corresponding wells of the 2nd column. Proceed in the same way sequentially to the 10th column to prepare two-fold serial dilution wells. The wells of the 11th column are not performed any process. Incubate the plate under the atmosphere of 5 vol% carbon dioxide at 37°C for about 40 hours. After incubation, add to the all wells 10 μL each of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide TS, and allow to stand under the atmosphere of 5 vol% carbon dioxide at 37°C for 4 – 6 hours. Add 0.125 mL of dimethylsulfoxide, shake for 5 to 10 minutes, then determine the absorbances of all wells at 550 nm and 660 nm, A_1 and A_2, using a spectrophotometer for microplate, and calculate the difference, $(A_1 - A_2)$. Divide by 6 the total of the differences $(A_1 - A_2)$ of six wells of the 11th and the 1st column, which were added the standard solution, and use the value so obtained as the 50% absorbance, A_M. Determine the dilution index numbers (column number) of the two serial wells of the sample solution and standard solution, they are corresponding to just the before and after of the 50% absorbance (A_M), n_{T1}, n_{T2} and n_{S1}, n_{S2}, respectively, where $n_{T1} < n_{T2}$ and $n_{S1} < n_{S2}$. Differences of the absorbance of the serial wells are named as A_{T1}, A_{T2} and A_{S1}, A_{S2}, respectively. Calculate the relative potencies of each sample solution by the following equation using the mean value of the three standard solutions, and average them. Perform the same procedure by reversing the place of the sample solution and the standard solution. Then, calculate the mean relative potency by averaging both values.

$$\text{Relative potency of the sample solution} = \frac{2^a}{\Sigma 2^b \times \frac{1}{3}}$$

a: $n_{T1} + (A_{T1} - A_M)/(A_{T1} - A_{T2})$
b: $n_{S1} + (A_{S1} - A_M)/(A_{S1} - A_{S2})$

Obtain the potency per mL by the following equation, and calculate the potency per mg of protein using the protein content obtained in (1).

Amount (unit) of nartograstim per mL of Nartograstim (Genetical Recombination)
$= S \times$ mean relative potency of the sample solution $\times d$

S: Concentration (unit/mL) of the standard solution
d: Dilution factor for the sample solution

System suitability—

The absorbance difference of the individual wells of the standard solution of the 3rd column should be not less than A_M, and that of the individual wells of the 8th column should be not more than A_M. If they do not meet the requirements, prepare the standard solution of the range of 1.0×10^3 to 1.6×10^4 units, and perform the test.

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and at not exceeding −20°C.

Add the following:

Nartograstim for Injection (Genetical Recombination)

注射用ナルトグラスチム(遺伝子組換え)

Nartograstim for Injection (Genetical Recombination) is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of nartograstim (genetical recombination) ($C_{850}H_{1344}N_{226}O_{245}S_8$: 18905.65).

Method of preparation Prepare as directed under Injections, with Nartograstim (Genetical Recombination).

Description Nartograstim for Injection (Genetical Recombination) occurs as white, masses or powder.

Identification Dissolve the content of 1 container of Nartograstim for Injection (Genetical Recombination) in 1 mL of tris-sodium chloride buffer solution, pH 8.0. To a suitable amount of this solution add tris-sodium chloride buffer solution, pH 8.0 so that each mL contains 1 μg of Nartograstim (Genetical Recombination), and use this solution as the sample solution. Then, proceed with the sample solution as directed in the Identification (1) under Nartograstim (Genetical Recombination).

pH ⟨*2.54*⟩ The pH of a solution of Nartograstim for Injection (Genetical Recombination) in water, containing 100 μg of Nartograstim (Genetical Recombination) in each mL, is 4.0 to 5.5.

Purity **(1)** Clarity and color of solution—A solution of Nartograstim for Injection (Genetical Recombination) in water, containing 100 μg of Nartograstim (Genetical Recombination) in each mL, is clear and colorless.

(2) Lactose conjugate—Being specified separately.

Water ⟨*2.48*⟩ Not more than 3.0% (50 mg, coulometric titration).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.62 EU/μg.

Uniformity of dosage unit ⟨*6.02*⟩ It meets the requirement of the Mass variation test.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2, using 3 mL of water for injection per 1 Nartograstim for Injection (Genetical Recombination) to dissolve the content: 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, using the sample solution prepared by dissolving the sample in water in a concentration to be used for the injection: it meets the requirement.

Specific activity Nartograstim for Injection (Genetical Recombination), when perform the assay and the following test, contains not less than 4.0×10^8 units of nartograstim (genetical recombination) per mg nartograstim (genetical recombination).

Wash out each content of 10 Nartograstim for Injection (Genetical Recombination) with a suitable amount of potency measuring medium for nartograstim test, wash the empty containers with the same medium, combine all washings, and add the same medium to make exactly 50 mL. To an exact amount of this solution add the same medium so that the concentration of nartograstim (genetical recombination) is equivalent to 50% to 150% of that of the standard solution, and use this solution as the sample solution. Separately, weigh accurately a suitable amount of Nartograstim RS, dissolve in the potency measuring medium for nartograstim test so that each mL contains exactly 1.2×10^4 units of nartograstim according to the labeled unit, and use this solution as the standard solution. Then, determine the nartograstim potency (unit) in 1 Nartograstim for Injection (Genetical Recombination) by proceeding as directed in the Assay (2) under Nartograstim (Genetical Recombination), and calculate the ratio against the amount of nartograstim obtained in the Assay.

Nartograstim (genetical recombination) potency (unit) in 1 Nartograstim for Injection (Genetical Recombination)
$= S \times$ mean relative potency of the sample solution $\times d \times 5$

S: Concentration (unit/mL) of the standard solution
d: Dilution factor for the sample solution
5: Amount (mL) of the medium used to dissolve per 1

sample

$$\text{Relative activity of sample solution} = \frac{2^a}{\Sigma 2^b \times \frac{1}{3}}$$

a: $n_{T1} + (A_{T1} - A_M)/(A_{T1} - A_{T2})$
b: $n_{S1} + (A_{S1} - A_M)/(A_{S1} - A_{S2})$

System suitability—

Proceed as directed in the system suitability in the Assay (2) under Nartograstim (Genetical Recombination).

Assay Weigh accurately the mass of each content of not less than 10 Nartograstim for Injection (Genetical Recombination). Weigh accurately an amount of the content, equivalent to about 0.25 mg of Nartograstim (Genetical Recombination) according to the labeled amount, dissolve in exactly 5 mL of the mobile phase, and use this solution as the sample solution. Separately, dissolve a suitable amount of Nartograstim RS in the mobile phase so that each mL contains about 50 μg of nartograstim, 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 nartograstim of these solutions.

Amount (μg) of nartograstim (genetical recombination) in 1 Nartograstim for Injection (Genetical Recombination)
$= M_S \times A_T/A_S \times M/M_T \times 5$

M_S: Amount (μg) of nartograstim in 1 mL of the standard solution
M: Mean mass (mg) of each content
M_T: Amount (mg) of Nartograstim for Injection (Genetical Recombination)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with porous silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate and 1.0 g of sodium lauryl sulfate in 700 mL of water, adjust to pH 6.5 with sodium hydroxide TS, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of nartograstim is about 16 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 nartograstim 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 nartograstim is not more than 1.5%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, and at a temperature not exceeding 10°C.

Nateglinide

ナテグリニド

Change the Description as follows:

Description Nateglinide occurs as a white crystalline powder.

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

It dissolves in dilute sodium hydroxide TS.

It shows crystal polymorphism.

Add the following:

Nifedipine Extended-release Capsules

ニフェジピン徐放カプセル

Nifedipine Extended-release Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$: 346.33).

Method of preparation Prepare as directed under Capsules, with Nifedipine.

Identification Conduct this procedure without exposure to light, using light-resistant vessels. Take out the content of Nifedipine Extended-release Capsules, and powder. To an amount of the powder, equivalent to 3 mg of Nifedipine, add 100 mL of methanol, shake for 15 minutes, and centrifuge. Determine the absorption spectrum of the supernatant liquid so obtained as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a broad absorption maximum between 335 nm and 356 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. To the total content of 1 capsule of Nifedipine Extended-release Capsules, add 50 mL of a mixture of methanol and water (9:1), agitate for 15 minutes with the aid of ultrasonic waves with occasional shaking, and shake for further 15 minutes. Then, add methanol to make exactly V mL so that each mL contains about 0.1 mg of nifedipine ($C_{17}H_{18}N_2O_6$). 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 methanol to make exactly 10 mL, and use this

solution as the sample solution. Separately, weigh accurately about 50 mg of nifedipine for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6) = M_S \times A_T/A_S \times V/500$$

M_S: Amount (mg) of nifedipine for assay

Dissolution Being specified separately.

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Take out the contents of not less than 20 Nifedipine Extended-release Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of nifedipine ($C_{17}H_{18}N_2O_6$), add 50 mL of a mixture of methanol and water (9:1), shake vigorously for 15 minutes, and add methanol 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 methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nifedipine for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol 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 nifedipine of each solution.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6) = M_S \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of nifedipine for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 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: Adjust to pH 6.1 of a mixture of methanol and diluted 0.05 mol/L disodium hydrogen phosphate TS (1 in 5) (11:9) with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of nifedipine 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 nifedipine are not less than 4000 and not more than 1.2, 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 nifedipine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Nifedipine Enteric Fine Granules

ニフェジピン腸溶細粒

Nifedipine Enteric Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$: 346.33).

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

Identification Conduct this procedure without exposure to light, using light-resistant vessels. Shake for 15 minutes a quantity of powdered Nifedipine Enteric Fine Granules, equivalent to 3 mg of Nifedipine, with 100 mL of methanol, and filter. Determine the absorption spectrum of the filtrate so obtained as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a broad absorption maximum between 335 nm and 356 nm.

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

Conduct this procedure without exposure to light, using light-resistant vessels. To the total content of 1 container of Nifedipine Enteric Fine Granules add 50 mL of a mixture of methanol and water (9:1), agitate for 15 minutes with the aide of ultrasonic waves with occasional shaking, and shake for further 15 minutes. Then, add methanol to make exactly V mL so that each mL contains about 0.1 mg of nifedipine ($C_{17}H_{18}N_2O_6$). 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 methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nifedipine for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6) = M_S \times A_T/A_S \times V/500$$

M_S: Amount (mg) of nifedipine for assay

Dissolution <*6.10*> When the tests are performed at 50 revolutions per minute according to the Paddle method, using 900 mL each of 1st and 2nd fluids for dissolution test as the dissolution medium, the dissolution rate in the test using

the 1st fluid for dissolution test in 60 minutes is not more than 15%, and that in the test using the 2nd fluid for dissolution test in 30 minutes is not less than 75%.

Conduct this procedure without exposure to light, using light-resistant vessels. Start the test with an accurately weighed amount of Nifedipine Enteric Fine Granules, equivalent to about 20 mg of nifedipine ($C_{17}H_{18}N_2O_6$), 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 5 mL of the subsequent filtrate, add the dissolution medium to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of nifedipine for assay, previously dried at 105°C for 2 hours, dissolve in 50 mL of methanol, and add the dissolution medium to make exactly 100 mL. Pipet 2 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 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*>, and determine the peak areas, A_T and A_S, of nifedipine of each solution.

Dissolution rate (%) with respect to the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$)

$$= M_S/M_T \times A_T/A_S \times 1/C \times 72$$

M_S: Amount (mg) of nifedipine for assay
M_T: Amount (g) of Nifedipine Enteric Fine Granules
C: Labeled amount (mg) of nifedipine ($C_{17}H_{18}N_2O_6$) in 1 g

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 nifedipine are not less than 4000 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 nifedipine is not more than 1.0%.

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately a portion of powdered Nifedipine Enteric Fine Granules, equivalent to about 10 mg of nifedipine ($C_{17}H_{18}N_2O_6$), add 50 mL of a mixture of methanol and water (9:1), shake vigorously for 15 minutes, and add methanol 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 methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nifedipine for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol 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 nifedipine of each solution.

Amount (mg) of nifedipine ($C_{17}H_{18}N_2O_6$)

$$= M_S \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of nifedipine for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 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: Adjust to pH 6.1 of a mixture of methanol and diluted 0.05 mol/L disodium hydrogen phosphate TS (1 in 5) (11:9) with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of nifedipine 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 nifedipine are not less than 4000 and not more than 1.2, 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 nifedipine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Nifedipine Fine Granules

ニフェジピン細粒

Nifedipine Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of nifedipine ($C_{17}H_{18}N_2O_6$: 346.33).

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

Identification Conduct this procedure without exposure to light, using light-resistant vessels. Shake for 15 minutes a quantity of powdered Nifedipine Fine Granules, equivalent to 6 mg of Nifedipine, with 200 mL of methanol, and centrifuge. Determine the absorption spectrum of the supernatant liquid so obtained as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a broad absorption maximum between 335 nm and 356 nm.

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

Conduct this procedure without exposure to light, using light-resistant vessels. To the total content of 1 container of Nifedipine Fine Granules add 50 mL of a mixture of methanol and water (9:1), agitate for 15 minutes with the aide of ultrasonic waves with occasional shaking, and shake for further 15 minutes. Then, add methanol to make exactly V mL so that each mL contains about 0.1 mg of of nifedipine ($C_{17}H_{18}N_2O_6$). 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 methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nifedipine for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6) = M_S \times A_T/A_S \times V/500$$

M_S: Amount (mg) of nifedipine 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 Nifedipine Fine Granules is not less than 85%.

Conduct this procedure without exposure to light, using light-resistant vessels. Start the test with an accurately weighted amount of Nifedipine Fine Granules, equivalent to about 10 mg of nifedipine ($C_{17}H_{18}N_2O_6$), 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, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of nifedipine for assay, previously dried at 105°C for 2 hours, dissolve in 50 mL of methanol, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 50 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 nifedipine of these solutions.

$$\text{Dissolution rate (\%) with respect to the labeled amount of nifedipine } (C_{17}H_{18}N_2O_6) = M_S/M_T \times A_T/A_S \times 1/C \times 36$$

M_S: Amount (mg) of nifedipine for assay
M_S: Amount (g) of Nifedipine Fine Granules
C: Labeled amount (mg) of nifedipine ($C_{17}H_{18}N_2O_6$) in 1 g

Operating conditions—

Proceed as directed in the operating conditions under 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 nifedipine are not less than 4000 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 nifedipine is not more than 1.0%.

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately a protein of powdered Nifedipine Fine Granules, equivalent to about 10 mg of nifedipine ($C_{17}H_{18}N_2O_6$), add 50 mL of a mixture of methanol and water (9:1), shake vigorously for 15 minutes, and add methanol 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 methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nifedipine for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol 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 nifedipine of these solutions.

$$\text{Amount (mg) of nifedipine } (C_{17}H_{18}N_2O_6) = M_S \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of nifedipine for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 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: Adjust to pH 6.1 of a mixture of methanol and diluted 0.05 mol/L disodium hydrogen phosphate TS (1 in 5) (11:9) with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of nifedipine 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 nifedipine are not less than 4000 and not more than 1.2, 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 nifedipine is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Norethisterone

ノルエチステロン

Change the Identification (2) as follows:

Identification

(2) Determine the infrared absorption spectrum of Norethisterone 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.

Add the following:

Omeprazole Enteric-coated Tablets

オメプラゾール腸溶錠

Omeprazole Enteric-coated Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$: 345.42).

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

Identification Powder Omeprazole Enteric-coated Tablets. To a portion of the powder, equivalent to 10 mg of Omeprazole, add 10 mL of ethanol (95), shake for 10 minutes, and centrifuge. To 1 mL of the supernatant liquid add phosphate buffer solution, pH 7.4 to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 273 nm and 277 nm, and between 299 nm and 303 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 Omeprazole Enteric-coated Tablets add $V/20$ mL of a solution of sodium tetraborate decahydrate (19 in 5000), and shake thoroughly to disintegrate the tablet. Then, proceed as directed in the Assay.

Amount (mg) of omeprazole ($C_{17}H_{19}N_3O_3S$)
$= M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of omeprazole for assay

Internal standard solution—A solution of 1,2-dinitrobenzene in ethanol (95) (1 in 400).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL each of 1st fluid for dissolution test and 2nd fluid for dissolution test as the dissolution medium, the dissolution rates of 10-mg tablet and 20-mg tablet in 120 minutes of the test using the 1st fluid for dissolution test are not more than 5%, respectively, and those of 10-mg tablet in 20 minutes and 20-mg tablet in 15 minutes of the test using the 2nd fluid for dissolution test are not less than 85%, respectively.

Start the test with 1 tablet of Omeprazole Enteric-coated 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 11 μg of omeprazole ($C_{17}H_{19}N_3O_3S$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of omeprazole for assay, previously dried in vacuum at 50°C using phosphorus (V) oxide as desiccant for 2 hours, and dissolve in ethanol (95) 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. Determine the absorbances, A_T and A_S, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*> at 323 nm when the test is performed using the 1st fluid as the dissolution medium and at 293 nm when the test is performed using the 2nd fluid as the dissolution medium, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of omeprazole ($C_{17}H_{19}N_3O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of omeprazole for assay
C: Labeled amount (mg) of omeprazole ($C_{17}H_{19}N_3O_3S$) in 1 tablet

Assay To 20 Omeprazole Enteric-coated Tablets add $V/20$ mL of a solution of sodium tetraborate decahydrate (19 in 5000), shake to disintegrate. To this solution add $3V/5$ mL of ethanol (95), shake for 15 minutes, then add ethanol (95) to make exactly V mL so that each mL contains about 0.4 mg of omeprazole ($C_{17}H_{19}N_3O_3S$), and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 4 mL of the internal standard solution, add a mixture of ethanol (95) and a solution of sodium tetraborate decahydrate (19 in 5000) (19:1) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of omeprazole for assay, previously dried in vacuum at 50°C with phosphorus (V) oxide as the desiccant for 2 hours, dissolve in a mixture of ethanol (95) and a solution of sodium tetraborate decahydrate (19 in 5000) (19:1), add exactly 20 mL of the internal standard solution, add a mixture of ethanol (95) and a solution of sodium tetraborate decahydrate (19 in 5000) (19:1) to make 100 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 omeprazole to that of the internal standard.

Amount (mg) of omeprazole ($C_{17}H_{19}N_3O_3S$) in tablet
$= M_S \times Q_T/Q_S \times V/1000$

M_S: Amount (mg) of omeprazole for assay

Internal standard solution—A solution of 1,2-dinitrobenzene in ethanol (95) (1 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 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 25°C.

Mobile phase: Dissolve 2.83 g of disodium hydrogen phosphate dodecahydrate and 0.21 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL, and adjust to pH 7.6 with diluted phosphoric acid (1 in 100). To 290 mL of this solution add 110 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of omeprazole is about 8 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, omeprazole 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of omeprazole to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Parnaparin Sodium

パルナパリンナトリウム

Change the origin/limits of content as follows:

Parnaparin Sodium is a low-molecular heparin sodium obtained by depolymerization, with hydrogen peroxide and copper (II) acetate or with sodium hypochlorite, of heparins sodium from the healthy edible porcine intestinal mucosa. The mass-average molecular mass ranges between 4500 and 6400.

The potency is not less than 70 low-molecular-mass-heparin units and not more than 95 low-molecular-mass-heparin units of anti-factor Xa activity per milligram calculated with reference of the dried substance.

Add the following:

Pemirolast Potassium Ophthalmic Solution

ペミロラストカリウム点眼液

Pemirolast Potassium Ophthalmic Solution is an aqueous ophthalmic preparations.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pemirolast potassium ($C_{10}H_7KN_6O$: 266.30).

Method of preparation Prepare as directed under Ophthalmic Preparations, with Pemirolast Potassium.

Description Pemirolast Potassium Ophthalmic Solution is a clear, colorless liquid.

Identification To a volume of Pemirolast Potassium Ophthalmic Solution, equivalent to 1 mg of Pemirolast Potassium, add diluted 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 (1 in 10) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 255 nm and 259 nm, and between 355 nm and 359 nm.

Osmotic pressure ratio Being specified separately.

pH Being specified separately.

Purity Related substances—To a volume of Pemirolast Potassium Ophthalmic Solution, equivalent to 2 mg of Pemirolast Potassium, add 1 mL of methanol and diluted 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 (1 in 10) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 20 mL of methanol and diluted 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 (1 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. Determine each peak area by the automatic integration method: the area of the peak other than pemirolast obtained from the sample solution is not larger than 3/10 times the peak area of pemirolast from the standard solution, and the total area of the peaks other than pemirolast obtained from the sample solution is not larger than the peak area of pemirolast from the standard solution.

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 260 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: A mixture of trifluoroacetic acid TS and methanol (4:1).

Mobile phase B: A mixture of methanol and trifluoroacetic acid TS (3:2).

Flowing of 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 – 60	100 → 0	0 → 100

Flow rate: Adjust the flow rate so that the retention time of pemirolast is about 19 minute.

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

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add diluted 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 (1 in 10) to make exactly 20 mL. Confirm that the peak area of pemirolast obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: Dissolve 10 mg of pemirolast potassium in 10 mL of diluted 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 (1 in 10), transfer this solution to a colorless test tube, and illuminate with a D65 fluorescent lamp (3000 lx) for 72 hours. To 2 mL of this solution add 1 mL of methanol and diluted 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 (1 in 10) to make 5 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak, having the relative retention time about 0.9 to pemirolast, and the peak of pemirolast is 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 pemirolast is not more than 2.0%.

Foreign insoluble matter ⟨*6.11*⟩ It meets the requirement.

Insoluble particulate matter ⟨*6.08*⟩ It meets the requirement.

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

Assay Pipet a volume of Pemirolast Potassium Ophthalmic Solution, equivalent to 2 mg of pemirolast potassium ($C_{10}H_7KN_6O$), add exactly 2 mL of the internal standard solution, then add a mixture of diluted 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 (1 in 10) and methanol (3:2) to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Pemirolast Potassium RS (separately determine the water ⟨*2.48*⟩ in the same manner as Pemirolast Potassium), and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, then add a mixture of diluted 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 (1 in 10) and methanol (3:2) 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. Calculate the ratios, Q_T and Q_S of the peak area of pemirolast to that of the internal standard.

Amount (mg) of pemirolast potassium ($C_{10}H_7KN_6O$)
$= M_S \times Q_T/Q_S \times 1/25$

M_S: Amount (mg) of Pemirolast Potassium RS, calculated on the anhydrous basis.

Internal standard solution—A solution of ethyl aminobenzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 260 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 40°C.

Mobile phase: A mixture of water, methanol and acetic acid (100) (30:20:1).

Flow rate: Adjust the flow rate so that the retention time of pemirolast is about 4 minute.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, pemirolast and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

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 pemirolast to that of the internal standard is not more than 1.0%.

Containers and storage Container—Tight containers.

Add the following:

Pilocarpine Hydrochloride Tablets

ピロカルピン塩酸塩錠

Pilocarpine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2$. HCl: 244.72).

Method of preparation Prepare as directed under Tablets, with Pilocarpine Hydrochloride.

Identification Perform the test with 10 μL each of the sample solution and the standard solution, both obtained in the assay, as directed under Liquid Chromatography ⟨*2.01*⟩ ac-

cording to the following conditions: the principal peaks obtained from the sample solution and standard solution show the same retention time, and both spectra of these 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: Photodiode array detector (wavelength: 215 nm; spectrum range of measurement: 200 – 370 nm).

System suitability—

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

Purity Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add phosphate buffer solution, pH 4.0 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 two peaks, having the relative retention time of about 0.78 and about 0.92 to pilocarpine, obtained from the sample solution is not larger than the peak area of pilocarpine from the standard solution, the area of the peak other than pilocarpine and the peaks mentioned above is not larger than 1/5 times the peak area of pilocarpine from the standard solution, and the total area of the peaks other than pilocarpine is not larger than 2 times the peak area of pilocarpine 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 1.3 times as long as the retention time of pilocarpine, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add phosphate buffer solution, pH 4.0 to make exactly 20 mL. Confirm that the peak area of pilocarpine obtained with 10 μL of this solution is equivalent to 7 – 13% of that 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 pilocarpine 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 pilocarpine 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 Pilocarpine Hydrochloride Tablets add a suitable amount of phosphate buffer solution, pH 4.0, shake until the tablet is completely disintegrated, then add phosphate buffer solution, pH 4.0 to make exactly V mL so that each mL contains about 0.2 mg of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2$.HCl), and filter 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. Separately, weigh accurately about 40 mg of pilocarpine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in phosphate buffer solution, pH 4.0 to make exactly 100 mL. Pipet 5 mL of this solution, add phosphate buffer solution, pH 4.0 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, and determine the peak areas, A_T and A_S, of pilocarpine of each solution.

$$\text{Amount (mg) of pilocarpine hydrochloride } (C_{11}H_{16}N_2O_2.HCl) = M_S \times A_T/A_S \times V/200$$

M_S: Amount (mg) of pilocarpine hydrochloride for assay

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 pilocarpine 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 pilocarpine 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 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 30 minutes is not less than 80%.

Start the test with 1 tablet of Pilocarpine 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 3 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 pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of pilocarpine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 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 pilocarpine of both solutions.

Dissolution rate (%) with respect to the labeled amount of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

M_S: Amount (mg) of pilocarpine hydrochloride for assay
C: Labeled amount (mg) of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2$.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 pilocarpine 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 pilocarpine is not more than 1.0%.

Assay To 20 Pilocarpine Hydrochloride Tablets add a suitable amount of phosphate buffer solution, pH 4.0, shake until the tablets are completely disintegrated, then add phosphate buffer solution, pH 4.0 to make exactly V mL so that each mL contains about 0.4 mg of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2$.HCl), and filter 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. Separately, weigh accurately about 40 mg of pilocarpine hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in phosphate buffer solution, pH 4.0 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 pilocarpine of each solution.

Amount (mg) of pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2$.HCl) in 1 tablet
$= M_S \times A_T/A_S \times V/2000$

M_S: Amount (mg) of pilocarpine hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with phenylated silica gel for liquid chromatography (10 μm in particle diameter).

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

Mobile phase: To 1000 mL of 0.05 mol/L potassium dihydrogen phosphate TS add phosphoric acid to adjust to pH 2.5. To this solution add 5.0 mL of triethylamine, and adjust to pH 2.5 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of pilocarpine 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 pilocarpine 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 pilocarpine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Piperacillin Sodium

ピペラシリンナトリウム

Change the origin/limits of content and Purity (4) as follows:

Piperacillin Sodium contains not less than 863 μg (potency) and not more than 978 μg (potency) per mg, calculated on the anhydrous basis.

The potency of Piperacillin Sodium is expressed as mass (potency) of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

Purity

(4) Related substances—Dissolve 0.10 g of Piperacillin Sodium in 50 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, and determine the areas of each peak by the automatic integration method: the area of the peak of ampicillin appeared at the retention time of about 7 minutes from the sample solution is not larger than 1/2 times that of piperacillin from the standard solution, the total area of related compounds 1 appeared at the retention times of about 17 minutes and about 21 minutes is not larger than 2 times of the peak area of piperacillin from the standard solution, the peak area of related compound 2 appeared at the retention time of about 56 minutes is not larger than that of piperacillin from the standard solution, and the total area of the peaks other than piperacillin is not larger than 5 times of the peak area of piperacillin from the standard solution. The peak areas of ampicillin, related compound 1 and related compound 2 are used after multiplying by their relative response factors, 1.39, 1.32 and 1.11, respectively.

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 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 0.2 mol/L potassium dihydrogen phosphate (45:4:1).

Mobile phase B: A mixture of acetonitrile, water and 0.2 mol/L potassium dihydrogen phosphate (25:24:1).

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 – 7	100	0
7 – 13	100 → 83	0 → 17
13 – 41	83	17
41 – 56	83 → 20	17 → 80
56 – 60	20	80

Flow rate: 1.0 mL per minute. (the retention time of piperacillin is about 33 minutes).

Time span of measurement: About 1.8 times as long as the retention time of piperacillin beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase A to make exactly 20 mL. Confirm that the peak area of piperacillin obtained from 20 μL of this solution is equivalent to 7 to 13% of that of piperacillin obtained from 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 piperacillin are not less than 15,000 and not more than 1.5, respectively.

System repeatability:When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of piperacillin is not more than 2.0%.

Potato Starch

バレイショデンプン

Delete the latin name:

Pravastatin Sodium Fine Granules

プラバスタチンナトリウム細粒

Change the Purity as follows:

Purity Related substances—The sample solution and the standard solution are stored at not exceeding 5°C after preparation. To an amount of Pravastatin Sodium Fine Granules, equivalent to 25 mg of Pravastatin Sodium, add 25 mL of a mixture of water and methanol (1:1), agitate for 15 minutes with the aid of ultrasonic waves, and centrifuge. Filter the supernatant liquid, 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 water and methanol (1:1) 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 peaks, having the relative retention time of about 0.36 and about 1.9 to pravastatin, obtained from the sample solution is not larger than 1/2 times and 3 times the peak area of pravastatin from the standard solution, respectively, the area of the peak other than pravastatin and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin from the sample solution is not larger than 4.5 times the peak area of pravastatin from the standard solution. For this calculation, use the area of the peaks, having the relative retention time of about 0.28, about 0.36 and about 0.88 to pravastatin, after multiplying by their relative response factors, 1.16, 1.72 and 1.22, respectively.

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 238 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, methanol, acetic acid (100) and triethylamine (750:250:1:1).

Mobile phase B: A mixture of methanol, water, acetic acid (100) and triethylamine (650:350:1:1).

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 – 50	50	50
50 – 75	50 → 0	50 → 100

Flow rate: 1.3 mL per minute.

Time span of measurement: For 75 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 water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin obtained with 20 μL of this solution is equivalent to 7 to 13% of that 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 pravastatin are not less than 3500 and not more than 1.6, 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 pravastatin is not more than 1.5%.

Pravastatin Sodium Tablets

プラバスタチンナトリウム錠

Change the Purity as follows:

Purity Related substances—The sample solution and the standard solution are stored at not exceeding 15°C after preparation. To an amount of powdered Pravastatin Sodium Tablets, equivalent to 50 mg of Pravastatin Sodium, add 40 mL of a mixture of water and methanol (1:1), agitate with the aid of ultrasonic waves, then add a mixture of water and methanol (1:1) to make 50 mL, centrifuge, and use the supernatant liquid 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 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 peaks, having the relative retention time about 0.36 and about 1.9 to pravastatin obtained from the sample solution is not larger than 3/10 times and 2 times the peak area of pravastatin from the standard solution, respectively, the area of the peak other than pravastatin and the peak mentioned above from the sample solution is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin from the sample solution is not larger than 3 times the peak area of pravastatin from the standard solution. For this calculation, use the area of the peaks, having the relative retention time about 0.28, about 0.36 and about 0.88, after multiplying by their relative response factors, 1.16, 1.72 and 1.22, respectively.

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 238 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, methanol, acetic acid (100) and triethylamine (750:250:1:1).

Mobile phase B: A mixture of methanol, water, acetic acid (100) and triethylamine (650:350:1:1).

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 – 50	50	50
50 – 75	50 → 0	50 → 100

Flow rate: 1.3 mL per minute.

Time span of measurement: For 75 minutes after injection, 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 methanol (1:1) to make exactly 10 mL. Confirm that the peak area of pravastatin obtained with 20 μL of this solution is equivalent to 7 to 13% of that 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 pravastatin are not less than 3500 and not more than 1.6, 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 pravastatin is not more than 1.5%.

Propyl Parahydroxybenzoate

パラオキシ安息香酸プロピル

Change the Description and below as follows:

◆**Description** Propyl Parahydroxybenzoate occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetone, and very slightly soluble in water.◆

Identification Determine the infrared absorption spectrum of Propyl Parahydroxybenzoate 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 Propyl Parahydroxybenzoate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <*2.60*> 96 – 99°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Propyl Parahydroxybenzoate in ethanol (95) to make 10 mL: the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of Cobalt (II) Chloride CS, 12.0 mL of Iron (III) Chloride CS and 2.0 mL of Copper (II) Sulfate CS add diluted dilute hydrochloric acid (1 in 10) to make 1000 mL.

(2) Acidity—To 2 mL of the solution of Propyl Para-

hydroxybenzoate obtained in (1) add 3 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mol/L sodium hydroxide VS until the solution shows a blue color: the volume of 0.1 mol/L sodium hydroxide VS used does not exceed 0.1 mL.

◆**(3)** Heavy metals <*1.07*>—Dissolve 1.0 g of Propyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).◆

(4) Related substances—Dissolve 50 mg of Propyl Parahydroxybenzoate in 2.5 mL of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 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 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 parahydroxybenzoic acid having a relative retention time of about 0.3 to propyl parahydroxybenzoate obtained from the sample solution is not larger than the peak area of propyl parahydroxybenzoate from the standard solution (0.5%). For this calculation use the peak area of parahydroxybenzoic acid after multiplying by the relative response factor, 1.4. Furthermore, the area of the peak other than propyl parahydroxybenzoate and parahydroxybenzoic acid from the sample solution is not larger than the peak area of propyl parahydroxybenzoate from the standard solution (0.5%), and the total area of the peaks other than propyl parahydroxybenzoate is not larger than 2 times the peak area of propyl parahydroxybenzoate from the standard solution (1.0%). For this calculation the peak area not larger than 1/5 times the peak area of propyl parahydroxybenzoate from the standard solution is excluded (0.1%).

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 2.5 times as long as the retention time of propyl parahydroxybenzoate.

System suitability—

◆Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of propyl parahydroxybenzoate obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.◆

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

◆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 2.0%.◆

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Propyl Parahydroxybenzoate and Propyl Parahydroxybenzoate RS, dissolve separately in 2.5 mL each of methanol, and add the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase 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 propyl parahydroxybenzoate of each solution.

Amount (mg) of propyl parahydroxybenzoate ($C_{10}H_{12}O_3$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Propyl Parahydroxybenzoate RS

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 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 35°C.

Mobile phase: A mixture of methanol and potassium dihydrogen phosphate solution (17 in 2500) (13:7).

Flow rate: 1.3 mL per minute.

System suitability—

System performance: Dissolve 5 mg each of Propyl Parahydroxybenzoate, ethyl parahydroxybenzoate and parahydroxybenzoic acid in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, parahydroxybenzoic acid, ethyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order, the relative retention times of parahydroxybenzoic acid and ethyl parahydroxybenzoate to propyl parahydroxybenzoate are about 0.3 and about 0.7, respectively, and the resolution between the peaks of ethyl parahydroxybenzoate and propyl parahydroxybenzoate is not less than 3.0.

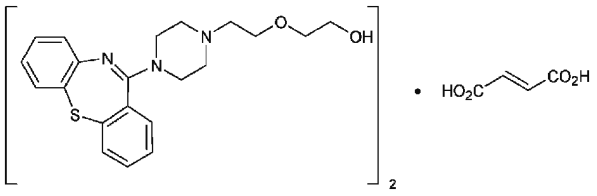
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propyl parahydroxybenzoate is not more than 0.85%.

◆**Containers and storage** Containers—Well-closed containers.◆

Add the following:

Quetiapine Fumarate

クエチアピンフマル酸塩



$(C_{21}H_{25}N_3O_2S)_2.C_4H_4O_4$: 883.09

2-[2-(4-Dibenzo[*b*,*f*][1,4]thiazepin-11-ylpiperazin-1-yl)ethoxy]ethanol hemifumarate

[*111974-72-2*]

Quetiapine Fumarate contains not less than 98.0% and not more than 102.0% of $(C_{21}H_{25}N_3O_2S)_2.C_4H_4O_4$, calculated on the anhydrous basis.

Description Quetiapine Fumarate occurs as a white powder.

It is sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

Identification **(1)** Determine the absorption spectrum of a solution of Quetiapine Fumarate in a mixture of water and acetonitrile (1:1) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Quetiapine Fumarate 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 Quetiapine Fumarate 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 Quetiapine Fumarate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 40 mg of Quetiapine Fumarate and 10 mg of fumaric acid for thin-layer chromatography in separate 10 mL of methanol, and use these solutions as the sample solution and the standard solution, respectively. 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 isopropyl ether, formic acid and water (90:7:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot having a larger *R*f value among the spots obtained with the sample solution and the spot with the standard solution show the same *R*f value.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Quetiapine Fumarate 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 (i) To 20 mg of Quetiapine Fumarate add 30 mL of the mobile phase, dissolve with the aid of ultrasonic waves, add the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 50 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. Determine each peak area by the automatic integration method, and calculate the amount of each related substance by the following equation: the amount is not more than 0.10%. For these calculations use the area of the peak, having a relative retention time of about 0.5 and about 0.9 with respect to quetiapine, after multiplying by their relative response factors, 0.6 and 0.9, respectively.

Amount (%) of each related substance = $A_T/A_S \times 1/2$

A_S: Peak area of quetiapine obtained with the standard solution

A_T: Each peak area other than quetiapine obtained with the sample 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 1.8 times as long as the retention time of quetiapine, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of quetiapine obtained with 50 μL of this solution is equivalent to 7 to 13% of that with 50 μL of the standard solution.

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 quetiapine are not less than 6000 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 quetiapine is not more than 2.0%.

(ii) To 20 mg of Quetiapine Fumarate add 30 mL of a mixture of acetonitrile, water and the mobile phase (2:1:1), dissolve with the aid of ultrasonic waves, add the same mixture to make 50 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add the same mixture to make exactly 100 mL. Pipet 5 mL of this solution, add the same mixture to make exactly 50 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. Determine each peak area by the automatic integration method, and calculate the

amount of each related substance by the following equation: the amount is not more than 0.10%. For these calculations use the area of the peak, having a relative retention time of about 1.9 with respect to quetiapine, after multiplying by its relative response factor, 0.8.

Amount (%) of each related substance = $A_T/A_S \times 1/2$

A_S: Peak area of quetiapine obtained from the standard solution

A_T: Each peak area other than quetiapine obtained from the sample solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 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 25°C.

Mobile phase: A mixture of methanol, diammonium hydrogen phosphate solution (33 in 12,500) and acetonitrile (70:21:9).

Flow rate: Adjust the flow rate so that the retention time of quetiapine is about 3.5 minutes.

Time span of measurement: About 8 times as long as the retention time of quetiapine, beginning from about 1.2 times the retention time of quetiapine.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add a mixture of acetonitrile, water and the mobile phase (2:1:1) to make exactly 50 mL. Confirm that the peak area of quetiapine obtained with 50 μL of this solution is equivalent to 7 to 13% of that with 50 μL of the standard solution.

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 quetiapine 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 quetiapine is not more than 2.0%.

(iii) The total amount of the related substances obtained in (i) and (ii) is not more than 0.5%.

(3) Residual solvent Being specified separately.

Water ⟨*2.48*⟩ Not more than 0.5% (Weigh accurately about 0.1 g of Quetiapine Fumarate, transfer to a centrifuge tube, add exactly 4 mL of methanol for Karl Fisher method, shake vigorously for 1 minute, and centrifuge at 2000 round per minute for 5 minutes. Pipet 1 mL of the supernatant liquid and perform the test. Perform a blank determination in the same manner, and make any necessary correction. Coulometric titration).

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

Assay Weigh accurately about 20 mg each of Quetiapine Fumarate and Quetiapine Fumarate RS (separately determine the water ⟨*2.48*⟩ in the same manner as Quetiapine Fumarate), add 60 mL of the mobile phase to them, dissolve with the aid of ultrasonic waves, and add the mobile phase to make exactly 100 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. 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 quetiapine.

Amount (mg) of quetiapine fumarate $[(C_{21}H_{25}N_3O_2S)_2.C_4H_4O_4]$ $= M_S \times A_T/A_S$

M_S: Amount (mg) of Quetiapine Fumarate 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 (5 μm in particle diameter).

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

Mobile phase: Dissolve 2.6 g of diammonium hydrogen phosphate in 1000 mL of water, and adjust to pH 6.5 with phosphoric acid. To 39 volumes of this solution add 54 volumes of methanol and 7 volumes of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of quetiapine is about 15 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 quetiapine are not less than 6000 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 quetiapine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Quetiapine Fumarate Fine Granules

クエチアピンフマル酸塩細粒

Quetiapine Fumarate Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$: 383.51).

Method of preparation Prepare as directed under Granules, with Quetiapine Fumarate.

Identification Powder Quetiapine Fumarate Fine Granules. To a portion of the powder, equivalent to 12.5 mg of quetiapine ($C_{21}H_{25}N_3O_2S$), add 60 mL of a mixture of water and acetonitrile (1:1), shake, then add the same mixture to make 100 mL, and filter. To 3 mL of the filtrate add the same mixture to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 290 nm and 296 nm.

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 Quetiapine Fumarate Fine Granules is not less than 80%.

Start the test with an accurately weighed amount of Quetiapine Fumarate Fine Granules, equivalent to about 0.1 g of quetiapine ($C_{21}H_{25}N_3O_2S$), 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 1.0 μm. Discard the first 5 mL of the filtrate, pipet 4 mL of the subsequent filtrate, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 32 mg of Quetiapine Fumarate RS (separately determine the water <*2.48*> in the same manner as Quetiapine Fumarate), and dissolve in water to make exactly 50 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 289 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 quetiapine ($C_{21}H_{25}N_3O_2S$)

$$= M_S/M_T \times A_T/A_S \times 1/C \times 360 \times 0.869$$

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

M_T: Amount (g) of Quetiapine Fumarate Fine Granules

C: Labeled amount (mg) of quetiapine ($C_{21}H_{25}N_3O_2S$) in 1 g

Assay To an accurately weighed amount of Quetiapine Fumarate Fine Granules, equivalent to about 0.25 g of quetiapine ($C_{21}H_{25}N_3O_2S$), add 10 mL of water, and allow to stand for 15 minutes. Add 100 mL of the mobile, shake for 15 minutes, then add the mobile phase to make exactly 200 mL, and stir the solution thoroughly. After standing for 15 minutes, pipet 6 mL of the supernatant liquid, add the mobile phase to make exactly 50 mL, and filter 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 17 mg of Quetiapine Fumarate RS (separately determine the water <*2.48*> in the same manner as Quetiapine Fumarate), add 60 mL of the mobile phase, dissolve with the aid of ultrasonic waves, then add the mobile phase 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 quetiapine.

Amount (mg) of quetiapine ($C_{21}H_{25}N_3O_2S$)

$$= M_S \times A_T/A_S \times 50/3 \times 0.869$$

M_S: Amount (mg) of Quetiapine Fumarate 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 (5 μm in particle diameter).

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

Mobile phase: A mixture of methanol, diammonium hydrogen phosphate solution (33 in 12,500) and acetonitrile (54:39:7).

Flow rate: Adjust the flow rate so that the retention time of quetiapine is about 15 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 quetiapine are not less than 7000 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 quetiapine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Quetiapine Fumarate Tablets

クエチアピンフマル酸塩錠

Quetiapine Fumarate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of quetiapine ($C_{21}H_{25}N_3O_2S$: 383.51).

Method of preparation Prepare as directed under Tablets, with Quetiapine Fumarate.

Identification Powder Quetiapine Fumarate Tablets. To a portion of the powder, equivalent to about 12.5 mg of quetiapine ($C_{21}H_{25}N_3O_2S$), add 5 mL of water, shake, add 60 mL of a mixture of water and acetonitrile (1:1), shake, then add the same mixture to make 100 mL, and filter. To 3 mL of the filtrate add the same mixture to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 290 nm and 296 nm.

Purity Related substances—To 10 Quetiapine Fumarate Tablets add 10 mL of water, allow to stand for 15 minutes,

then shake for 25 minutes, and add a mixture of water and acetonitrile (1:1) to make exactly 200 mL. Stir this solution for 4 hours, and allow to stand for 15 minutes. Pipet 3 mL of this solution, add the mobile phase so that each mL contains about 0.15 mg of quetiapine ($C_{21}H_{25}N_3O_2S$), and filter 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 the mobile phase 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. Determine each peak area by the automatic integration method: the area of the peak with the relative retention time of about 0.6 to quetiapine obtained from the sample solution is not larger than 1/5 times the peak area of quetiapine from the standard solution, the area of the peak other than quetiapine and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of quetiapine from the standard solution, and the total area of the peaks other than quetiapine and the peak with the relative retention time of about 0.6 to quetiapine is not larger than 1/5 times the peak area of quetiapine 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 2.3 times as long as the retention time of quetiapine, beginning after the peak of fumaric acid.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of quetiapine obtained with 50 μL of this solution is equivalent to 7 to 13% of that with 50 μL of the standard solution.

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 quetiapine are not less than 7000 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 quetiapine 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 Quetiapine Fumarate Tablets add 5 mL of water, allow to stand for 15 minutes, then shake for 25 minutes, add 30 mL of a mixture of water and acetonitrile (1:1), shake, and add the same mixture to make exactly 50 mL. Stir this solution for 4 hours, and allow to stand for 15 minutes. To exactly 8 mL of this solution, add the mobile phase to make exactly V mL so that each mL contains about 0.16 mg of quetiapine ($C_{21}H_{25}N_3O_2S$), and filter 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 18 mg of Quetiapine Fumarate RS (separately determine the water <*2.48*> in the same manner as Quetiapine Fumarate), add 60 mL of the mobile phase, dissolve with the aid of ultrasonic waves, then add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of quetiapine } (C_{21}H_{25}N_3O_2S) = M_S \times A_T/A_S \times V/16 \times 0.869$$

M_S: Amount (mg) of Quetiapine Fumarate 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 Quetiapine Fumarate Tablets is not less than 75%.

Start the test with 1 tablet of Quetiapine Fumarate 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 5 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 14 μg of quetiapine ($C_{21}H_{25}N_3O_2S$), and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Quetiapine Fumarate RS (separately determine the water <*2.48*> in the same manner as Quetiapine Fumarate), add 60 mL of the mobile phase, agitate with the aid of ultrasonic waves to dissolve, and add the mobile phase to make exactly 100 mL. Pipet 8 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 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 quetiapine.

$$\text{Dissolution rate (\%) with respect to the labeled amount of quetiapine } (C_{21}H_{25}N_3O_2S) = M_S \times A_T/A_S \times V'/V \times 1/C \times 72 \times 0.869$$

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

C: Labeled amount (mg) of quetiapine ($C_{21}H_{25}N_3O_2S$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 mm in inside diameter and 8 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 methanol, a solution of diammonium hydrogen phosphate (33 in 12,500) and acetonitrile

(54:39:7).

Flow rate: Adjust the flow rate so that the retention time of quetiapine 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 quetiapine are not less than 1400 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 quetiapine is not more than 2.0%.

Assay To 20 Quetiapine Fumarate Tablets add 20 mL of water, allow to stand for 15 minutes, shake for 25 minutes, and add a mixture of water and acetonitrile (1:1) to make exactly 500 mL. Stir the solution for 4 hours. After standing for 15 minutes, pipet 4 mL of this solution, and add the mobile phase to make exactly V mL so that each mL contains about 0.16 mg of quetiapine ($C_{21}H_{25}N_3O_2S$). 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. Separately, weigh accurately about 18 mg of Quetiapine Fumarate RS (separately determine the water <*2.48*> in the same manner as Quetiapine Fumarate), add 60 mL of the mobile phase, dissolve with the aid of ultrasonic waves, then add the mobile phase 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 quetiapine.

Amount (mg) of quetiapine ($C_{21}H_{25}N_3O_2S$) in 1 tablet of Quetiapine Fumarate Tablets
$= M_S \times A_T/A_S \times V/16 \times 0.869$

M_S: Amount (mg) of Quetiapine Fumarate 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 (5 μm in particle diameter).

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

Mobile phase: A mixture of methanol, diammonium hydrogen phosphate solution (33 in 12,500) and acetonitrile (54:39:7).

Flow rate: Adjust the flow rate so that the retention time of quetiapine is about 15 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 quetiapine are not less than 7000 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 quetiapine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Rabeprazole Sodium

ラベプラゾールナトリウム

Change the Description as follows:

Description Rabeprazole Sodium occurs as a white to pale yellowish white powder.

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

It dissolves in 0.01 mol/L sodium hydroxide TS.

It is hygroscopic.

A solution of Rabeprazole Sodium (1 in 20) shows no optical rotation.

Rabeprazole Sodium shows crystal polymorphism.

0.1% Reserpine Powder

レセルピン散0.1%

Add the following next to the Identification:

Dissolution Being specified separately.

Riboflavin Powder

リボフラビン散

Add the following next to the Purity:

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 60 minutes of Riboflavin Powder is not less than 80%.

Conduct this procedure without exposure to light. Start the test with an accurately weighed amount of Riboflavin Powder, equivalent to about 5 mg of riboflavin ($C_{17}H_{20}N_4O_6$), 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, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 22 mg of Riboflavin RS, previously dried at 105°C for 2 hours, dissolve in water by warming and add water to make exactly 200 mL after cooling. Pipet 5 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 445 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spec-

trophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 45/2$

M_S: Amount (mg) of Riboflavin RS
M_T: Amount (g) of the Riboflavin Powder
C: Labeled amount (mg) of riboflavin ($C_{17}H_{20}N_4O_6$) in 1 g

Ribostamycin Sulfate

リボスタマイシン硫酸塩

Change the Purity (1) as follows:

Purity **(1)** Clarity and color of solution—Dissolve 2.9 g of Ribostamycin Sulfate in 10 mL of water: the solution is clear. Determine the absorbance of this solution at 400 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: not more than 0.10.

Rice Starch

コメデンプン

Delete the latin name:

Sarpogrelate Hydrochloride

サルポグレラート塩酸塩

Change the Description and the Identification (2) as follows:

Description Sarpogrelate Hydrochloride occurs as a white crystalline powder.

It is slightly soluble in water and in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of Sarpogrelate Hydrochloride (1 in 100) shows no optical rotation.

Sarpogrelate Hydrochloride shows crystal polymorphism.

Identification

(2) Determine the infrared absorption spectrum of Sarpogrelate Hydrochloride 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 Sarpogrelate Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the Reference Standard separately with acetone by heating and suspending, filter and dry the crystals at 50°C for 1 hour, and perform the test with the crystals.

Add the following:

Simvastatin Tablets

シンバスタチン錠

Simvastatin Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of simvastatin ($C_{25}H_{38}O_5$: 418.57).

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

Identification To an amount of powdered Simvastatin Tablets, equivalent to about 2.5 mg of Simvastatin, add 25 mL of acetonitrile, treat with ultrasonic waves for 15 minutes, and centrifuge. To 2 mL of the supernatant liquid add acetonitrile to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 229 nm and 233 nm, between 236 nm and 240 nm, and between 245 nm and 249 nm.

Purity Related substances—Powder not less than 20 Simvastatin Tablets. To a portion of the powder, equivalent to about 50 mg of Simvastatin, add 200 mL of a mixture of acetonitrile and 0.05 mol/L acetate buffer solution, pH 4.0 (4:1), and agitate with the aid of ultrasonic waves for 15 minutes. After cooling, add the same mixture to make 250 mL, and centrifuge. To 5 mL of the supernatant liquid add the same mixture to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and 0.05 mol/L acetate buffer solution, pH 4.0 (4:1) 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. Determine each peak area by the automatic integration method: the area of the peak with the relative retention time of about 0.5 to simvastatin obtained from the sample solution is not larger than 1.6 times the peak area of simvastatin from the standard solution, the area of the peak with the relative retention time of about 2.0 from the sample solution is not larger than the peak area of simvastatin from the standard solution, and the total area of the peaks other than simvastatin is not larger than 4 times the peak area of simvastatin 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 2.5 times as long as the retention time of simvastatin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of simvastatin obtained with 10 μL of this solution is equivalent to 14 to 26% of that 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 simvastatin are not less than 6000 and 0.9 – 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 simvastatin 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 Simvastatin Tablets add V/20 mL of water, and disintegrate the tablet with the aid of ultrasonic waves. Add a mixture of acetonitrile and 0.05 mol/L acetate buffer solution, pH 4.0 (4:1) to make $3V/4$ mL, and agitate with the aid of ultrasonic waves for 15 minutes. After cooling, add the same mixture to make exactly V mL so that each mL contains about 0.1 mg of simvastatin ($C_{25}H_{38}O_5$), centrifuge, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of simvastatin } (C_{25}H_{38}O_5) = M_S \times A_T/A_S \times V/200$$

M_S: Amount (mg) of Simvastatin RS, calculated on the dried basis

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution of polysorbate 80, prepared by dissolving 3 g in water to make 1000 mL, as the dissolution medium, the dissolution rate in 45 minutes of Simvastatin Tablets is not less than 70%.

Start the test with 1 tablet of Simvastatin 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 5.6 μg of simvastatin ($C_{25}H_{38}O_5$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Simvastatin RS (separately determine the loss on drying ⟨*2.41*⟩ under the same conditions as Simvastatin), and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase 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 simvastatin.

$$\text{Dissolution rate (\%) with respect to the labeled amount of simvastatin } (C_{25}H_{38}O_5) = M_S \times A_T/A_S \times V'/V \times 1/C \times 45/2$$

M_S: Amount (mg) of Simvastatin RS, calculated on the dried basis

C: Labeled amount (mg) of simvastatin ($C_{25}H_{38}O_5$) in 1 tablet

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 50°C.

Mobile phase: A mixture of methanol and 0.02 mol/L potassium dihydrogen phosphate TS (4:1).

Flow rate: Adjust the flow rate so that the retention time of simvastatin 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 simvastatin 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 simvastatin is not more than 1.0%.

Assay Weigh accurately the mass of not less than 20 Simvastatin Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of simvastatin ($C_{25}H_{38}O_5$), add 200 mL of a mixture of acetonitrile and 0.05 mol/L acetate buffer solution, pH 4.0 (4:1), and treat with ultrasonic waves for 15 minutes. After cooling, add the same mixture to make exactly 250 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add the same mixture to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Simvastatin RS (separately determine the loss on drying ⟨*2.41*⟩ under the same conditions as Simvastatin), dissolve in a mixture of acetonitrile and 0.05 mol/L acetate buffer solution, pH 4.0 (4:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of simvastatin.

$$\text{Amount (mg) of simvastatin } (C_{25}H_{38}O_5) = M_S \times A_T/A_S \times 5/2$$

M_S: Amount (mg) of Simvastatin RS, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 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

45°C.

Mobile phase: Dissolve 3.90 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 4.5 with sodium hydroxide TS or phosphoric acid, and add water to make 1000 mL. To 700 mL of this solution add 1300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of simvastatin 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 simvastatin are not less than 6000 and 0.9 – 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 simvastatin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Streptomycin Sulfate

ストレプトマイシン硫酸塩

Change the Purity (1) as follows:

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Streptomycin Sulfate in 5 mL of water: the solution is clear, and its absorbance at 400 nm determined as directed under Ultraviolet-visible Spectrophotometry <*2.24*> is not more than 0.17.

Streptomycin Sulfate for Injection

注射用ストレプトマイシン硫酸塩

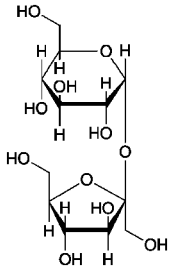
Change the pH as follows:

pH <*2.54*> The pH of a solution prepared by dissolving an amount of Streptomycin Sulfate for Injection, equivalent to 2.0 g (potency) of Streptomycin Sulfate, in 10 mL of water is 4.5 to 7.0.

Sucrose

精製白糖

Change to read:



$C_{12}H_{22}O_{11}$: 342.30

β-D-Fructofuranosyl α-D-glucopyranoside

[*57–50–1*]

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 (◆ ◆).

Sucrose contains no additives.

For Sucrose used for preparation of the parenteral infusions, the label states the purpose.

◆**Description** Sucrose is a white crystalline powder, or lustrous colorless or white crystals.

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

◆**Identification** Determine the infrared absorption spectrum of Sucrose 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*> $[\alpha]_D^{20}$: +66.3 – +67.0° (26 g, water, 100 mL, ◆100 mm◆).

Purity (1) Color value—Dissolve 50.0 g of Sucrose in 50.0 mL of water, filter through a membrane filter with 0.45 μm in pore size, degas, and use this solution as the sample solution. Measure the absorbance of the sample solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a cell of at least 4 cm (a cell length of 10 cm or more is preferred), and calculate the color value by the following equation: not more than 45.

$$\text{Color value} = A \times 1000/b/c$$

A: Absorbance measured at 420 nm

b: Path length (cm)

c: Concentration (g/mL) of Sucrose in the sample solution, calculated from the refractive index (n_D^{20}) obtained as directed under Refractive Index Determination <*2.45*>. Use the following table and interpolate the value, if necessary.

n_D^{20}	c (g/mL)
1.4138	0.570
1.4159	0.585
1.4179	0.600
1.4200	0.615
1.4221	0.630
1.4243	0.645
1.4264	0.661

System suitability—

System repeatability: When the test is repeated 2 times with the sample solution, the difference between 2 results is not larger than 3.

(2) Clarity of solution—Dissolve 50.0 g of Sucrose in water to make 100 mL, and use this solution as the sample solution: the sample solution is clear, and its clarity is not different from water, or its opalescence is not more than that of reference suspension 1.

(3) Sulfite

(i) Enzyme reaction: Sulfite is oxidized by sulfite oxidase to sulfuric acid and hydrogen peroxide which in turn is reduced by nicotinamide adenine dinucleotide peroxidase in the presence of nicotinamide adenine dinucleotide reduced form (NADH). The amount of NADH oxidized is proportional to the amount of sulfite. Calculate the amount of oxidized NADH from the degree of reduction of the absorbance at 340 nm. A suitable kit may be used.

(ii) Procedure: Dissolve 4.0 g of Sucrose in freshly prepared distilled water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 4.0 g of Sucrose in freshly prepared distilled water, add exactly 0.5 mL of Standard Sulfite Solution, then add freshly prepared distilled water to make exactly 10 mL, and use this solution as the standard solution. Use freshly prepared distilled water as a blank. Separately, introduce 2.0 mL each of the sample solution, the standard solution and the blank in 10-mm cells, add 1.00 mL of β-nicotinamide adenine dinucleotide reduced form TS and 10 μL of NADH peroxidase TS, stir with a plastic stirring rod, and allow to stand at 20 – 25°C for 5 minutes. Measure the absorbance of these solutions at 340 nm, A_{T1}, A_{S1} and A_{B1}, as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the blank. Then, to these solutions add 50 μL each of sulfite oxidase TS, stir, allow to stand at 20 – 25°C for 30 minutes, then measure the absorbance of these solutions in the same manner as above, A_{T2}, A_{S2} and A_{B2}: the result of $(A_{T1} - A_{T2}) - (A_{B1} - A_{B2})$ is not larger than half the result of $(A_{S1} - A_{S2}) - (A_{B1} - A_{B2})$ (not more than 10 ppm expressed as SO_2).

(4) Reducing sugars—Transfer 5 mL of the sample solution obtained in (2) to a test-tube about 150 mm long and about 16 mm in diameter, add 5 mL of water, 1.0 mL of 1 mol/L sodium hydroxide VS and 1.0 mL of methylene blue TS, mix, and heat in a water bath. After exactly 2 minutes, take the tube out of the bath, and examine the solution immediately: the blue color does not disappear completely. Ignore any blue color at the air and solution interface.

Conductivity <*2.51*> Dissolve 31.3 g of Sucrose in freshly prepared distilled water to make 100 mL, and use this solution as the sample solution. Measure the conductivity of the sample solution (κ_1 ($\mu S \cdot cm^{-1}$)) while gently stirring with a magnetic stirrer. Measure the conductivity of the water used for preparing the sample solution (κ_2 ($\mu S \cdot cm^{-1}$)) in the same manner as above. The measured conductivity must be stable within 1% in the rate of change per 30 seconds. Calculate the corrected conductivity of the sample solution (κ_C) by the following expression: k_C is not more than 35 $\mu S \cdot cm^{-1}$.

$$\kappa_C\ (\mu S \cdot cm^{-1}) = \kappa_1 - 0.35\kappa_2$$

Loss on drying <*2.41*> Not more than 0.1% (2 g, 105°C, 3 hours).

Dextrins For Sucrose used to prepare parenteral infusions, to 2 mL of the sample solution obtained in the Purity (2) add 8 mL of water, 0.05 mL of 2 mol/L hydrochloric acid and 0.05 mL of iodine TS: the solution remains yellow.

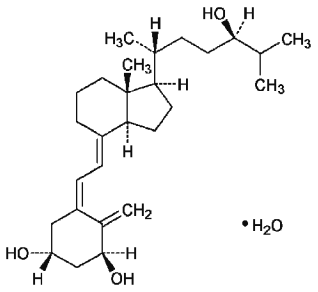
Bacterial endotoxins <*4.01*> Less than 0.25 EU/mg, for Sucrose used to prepare parenteral infusions.

◆**Containers and storage** Containers—Well-closed containers.◆

Add the following:

Tacalcitol Hydrate

タカルシトール水和物



$C_{27}H_{44}O_3.H_2O$: 434.65

(1*S*,3*R*,5*Z*,7*E*,24*R*)-9,10-Secocholesta-5,7,10(19)-triene-1,3,24-triol monohydrate

[*93129-94-3*]

Tacalcitol Hydrate contains not less than 97.0% and not more than 103.0% of tacalcitol ($C_{27}H_{44}O_3$: 416.64), calculated on the anhydrous basis.

Description Tacalcitol Hydrate occurs as white, crystals or crystalline powder.

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

It decomposes on exposure to light.

Melting point: about 100°C. Place Tacalcitol Hydrate in a capillary tube and immediately flame-seal, put the tube in a bath heated at a temperature of about 10°C below the predicted melting point, then start the determination by ris-

ing the temperature at the rate of 1°C per minute.

Identification **(1)** Determine the absorption spectrum of a solution of Tacalcitol 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 Tacalcitol 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 Tacalcitol 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 Tacalcitol RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +58 – +63° (25 mg calculated on the anhydrous basis, ethanol (99.5), 5 mL, 100 mm).

Purity **(1)** 1α,24(*S*)-Dihydroxycolecalciferol — Conduct this procedure avoiding contact to the air as possible and using light-resistant vessels. Dissolve 1 mg of Tacalcitol Hydrate in 20 mL of methanol, and use this solution 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 the peak area of tacalcitol, A_a, and the area of a peak having the relative retention time of about 1.1 to tacalcitol, A_b, by the automatic integration method: $A_b/(A_a + A_b)$ is not more than 0.02.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with triacontylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 15°C.

Mobile phase: A mixture of acetonitrile and water (3:2).

Flow rate: Adjust the flow rate so that the retention time of tacalcitol is about 26 minutes.

System suitability—

Test for required detectability: To 2 mL of the sample solution add methanol to make 20 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 20 mL. Confirm that the peak area of tacalcitol obtained with 30 μL of this solution is equivalent to 15 to 25% of that with 30 μL of the solution for system suitability test.

System performance: Dissolve 1 mg of Tacalcitol Hydrate in ethanol (99.5) to make 20 mL. Put 1 mL of this solution in a glass ampoule, flame-seal, heat at 100°C for 1 hour, and cool quickly to room temperature. Open the ampoule, evaporate to dryness the content under the nitrogen stream. Dissolve the residue with 1 mL of methanol. When the procedure is run with 30 μL of this solution under the above operating conditions, the resolution between the peaks corresponding to pre-tacalcitol having the relative retention time of about 0.85 to tacalcitol and tacalcitol is not less than 4.

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 2.0%.

(2) Related substances—Dissolve 1 mg of Tacalcitol Hydrate in 0.2 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 50 μL of the sample solution, add ethanol (99.5) 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 20 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 toluene and acetone (4:3) to a distance of about 15 cm, and air-dry the plate. Spray evenly a mixture of sulfuric acid and methanol (1:1) on the plate, and heat at 105°C for 5 minutes: the spot other than the principal spot obtained from the sample solution is not more than one, and not more intense than the spot from the standard solution.

(3) Residual solvent Being specified separately.

Water <*2.48*> 3.7 – 4.6% (10 mg, coulometric titration).

Assay Conduct this procedure avoiding contact to the air as possible and using light-resistant vessels. Weigh accurately about 1 mg each of Tacalcitol Hydrate and Tacalcitol RS (separately determine the water <*2.48*> in the same manner as Tacalcitol Hydrate), and dissolve each in methanol to make exactly 50 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. 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 area of tacalcitol, A_T and A_S, of each solution.

$$\text{Amount (mg) of tacalcitol } (C_{27}H_{44}O_3) = M_S \times A_T/A_S$$

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

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 35°C.

Mobile phase: A mixture of acetonitrile and water (3:1).

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

System suitability—

System performance: When the procedure is run with 40 μL of the standard solution under the above operating con-

ditions, the number of theoretical plates and the symmetry factor of the peak of tacalcitol are not less than 1500 and not more than 1.5, 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 tacalcitol is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature of 2 – 8°C.

Add the following:

Tacalcitol Lotion

タカルシトールローション

Tacalcitol Lotion contains not less than 90.0% and not more than 110.0% of tacalcitol ($C_{27}H_{44}O_3$: 416.64).

Method of preparation Prepare as directed under Lotions, with Tacalcitol Hydrate.

Identification Perform the test with 30 μL each of the sample solution and standard solution, both are obtained in the Assay, as directed under Liquid Chromatography <*2.01*> according to the following conditions: the retention time of the principal peaks obtained from the sample solution and the standard solution is the same, and both adsorption spectra of these 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.

Assay Weigh accurately an amount of Tacalcitol Lotion, equivalent to about 2 μg of tacalcitol ($C_{27}H_{44}O_3$), add exactly 4 mL of methanol and exactly 1 mL of the internal standard solution, and shake. Add 5 mL of hexane, shake thoroughly for 30 minutes, centrifuge at 4°C, 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 exactly 100 mL. Pipet 4 mL of this solution, add exactly 1 mL of the internal standard solution, shake, add 5 mL of hexane and shake well for 30 minutes, then centrifuge at 4°C, 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%.

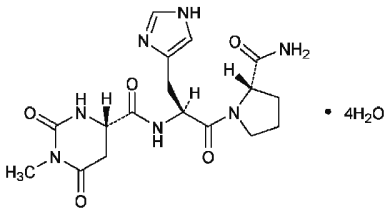
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Taltirelin Hydrate

タルチレリン水和物



$C_{17}H_{23}N_7O_5.4H_2O$: 477.47

N-[(4*S*)-1-Methyl-2,6-dioxohexahydropyrimidine-4-carbonyl]-L-histidyl-L-prolinamide tetrahydrate

[*201677-75-0*]

Taltirelin Hydrate contains not less than 98.5% and not more than 101.0% of taltirelin ($C_{17}H_{23}N_7O_5$: 405.41), calculated on the anhydrous basis.

Description Taltirelin Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in water, in ethanol (99.5) and in acetic acid (100), and soluble in methanol.

It dissolves in 1 mol/L hydrochloric acid TS.

It shows crystal polymorphism.

Identification **(1)** Dissolve 30 mg of Taltirelin Hydrate in 10 mL of water. To 0.5 mL of this solution add 2 mL of a solution of 4-nitrobenzenediazonium fluoroborate (1 in 2000) and 3 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0: a red color is produced.

(2) Determine the infrared absorption spectrum of Taltirelin 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.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −22.5 – −24.5° (1 g calculated on the anhydrous basis, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Taltirelin 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 Taltirelin Hydrate in 20 mL of the mobile phase, 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 their amounts by the area percentage method: the amount of the peak other than taltirelin from the sample solution is not more than 0.1%, and the total amount of the peaks other than taltirelin is not more than 0.5%.

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: Dissolve 3.4 g of potassium dihydrogen phosphate in 1000 mL of water, adjust to pH 2.5 with phosphoric acid, and add 1.7 g of sodium 1-octanesulfonate. To 900 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of taltirelin is about 15 minutes.

Time span of measurement: About 1.5 times as long as the retention time of taltirelin, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase 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, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of taltirelin obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that 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 taltirelin are not less than 7000 and not more than 1.5, 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 taltirelin is not more than 2.0%.

(3) Residual solvent Being specified separately.

Water <*2.48*> 14.0 – 15.5% (0.2 g, volumetric titration, direct titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Taltirelin Hydrate, dissolve in 70 mL of acetic acid (100), and titrate <*2.50*> with 0.1 mol/L perchloric acid VS until the color of solution changes from violet through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 40.54 mg of $C_{17}H_{23}N_7O_5$

Containers and storage Containers—Well-closed containers.

Add the following:

Taltirelin Orally Disintegrating Tablets

タルチレリン口腔内崩壊錠

Taltirelin Orally Disintegrating Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$: 477.47).

Method of preparation Prepare as directed under Tablets, with Taltirelin Hydrate.

Identification Powder Taltirelin Orally Disintegrating Tablets. To a portion of the powder, equivalent to 30 mg of Taltirelin Hydrate, add 10 mL of water, shake for 5 minutes, and filter. To 0.5 mL of the filtrate add 2 mL of a solution of 4-nitrobenzenediazonium fluoroborate (1 in 2000) and 3 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0: a red color is produced.

Purity Related substances—Powder Taltirelin Orally Disintegrating Tablets. To a portion of the powder, equivalent to 5 mg of Taltirelin Hydrate, add 20 mL of the mobile phase, shake for 5 minutes, 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. 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 their amounts by the area percentage method: the amount of the peak with the relative retention time of about 0.7 to taltirelin is not more than 0.7% and the peaks with the relative retention time of about 0.8 and 0.9, respectively, are not more than 0.3%, and the peak other than taltirelin and other than the peaks mentioned above is not more than 0.1%. And the total amount of the peaks other than taltirelin is not more than 1.0%.

Operating conditions—

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

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate in 1000 mL of water, adjust to pH 2.5 with phosphoric acid, and add 1.7 g of sodium 1-octanesulfonate. To 900 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of taltirelin is about 15 minutes.

Time span of measurement: About1.5 times as long as the retention time of taltirelin, beginning 1/3 times the retention time of taltirelin.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase 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, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of taltirelin obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that 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 taltirelin are not less than 7000 and not more than 1.5, 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 taltirelin 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 Taltirelin Orally Disintegrating Tablets add $V/2$ mL of the mobile phase and exactly $V/10$ mL of the internal standard solution, and shake vigorously for 5 minutes. Then, add the mobile phase to make V mL so that each mL contains about 0.1 mg of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$), 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. Then, proceed as directed in the Assay.

Amount (mg) of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$)
$= M_S \times Q_T/Q_S \times V/500 \times 1.178$

M_S: Amount (mg) of taltirelin hydrate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of *o*-acetanisidide (1 in 2500).

Disintegration Being specified separately.

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 Taltirelin Orally Disintegrating Tablets is not less than 85%.

Start the test with 1 tablet of Taltirelin 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 water to make exactly V' mL so that each mL contains about 5.6 μg of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of taltirelin hydrate for assay (separately determine the water <*2.48*> in the same manner as Taltirelin Hydrate), and dissolve in 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 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 taltirelin.

Dissolution rate (%) with respect to the labeled amount of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$)

$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18 \times 1.178$

M_S: Amount (mg) of taltirelin hydrate for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$) 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 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of taltirelin 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 taltirelin is not more than 2.0%.

Assay Weigh accurately, and powder not less than 20 Taltirelin Orally Disintegrating Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$), add 25 mL of the mobile phase and exactly 5 mL of the internal standard solution, shake for 5 minutes, add the mobile phase to make 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 50 mg of taltirelin hydrate for assay (separately determine the water <2.48> in the same manner as Taltirelin Hydrate), 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 taltirelin to that of the internal standard.

Amount (mg) of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$)

$= M_S \times Q_T/Q_S \times 1/10 \times 1.178$

M_S: Amount (mg) of taltirelin hydrate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of *o*-acetanisidide (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 about 40°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate in 1000 mL of water, adjust to pH 2.5 with phosphoric acid, and add 1.7 g of sodium 1-octanesulfonate. To 850 mL of this solution add 150 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of taltirelin 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, taltirelin 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of taltirelin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Taltirelin Tablets

タルチレリン錠

Taltirelin Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$: 477.47).

Method of preparation Prepare as directed under Tablets, with Taltirelin Hydrate.

Identification Powder Taltirelin Tablets. To a portion of the powder, equivalent to 30 mg of Taltirelin Hydrate, add 10 mL of water, shake for 15 minutes, and filter. To 0.5 mL of the filtrate add 2 mL of a solution of 4-nitrobenzendiazonium fluoroborate (1 in 2000) and 3 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0: a red color is produced.

Purity Related substances—Powder Taltirelin Tablets. To a portion of the powder, equivalent to 5 mg of Taltirelin Hydrate, add 20 mL of the mobile phase, shake for 20 minutes, 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. 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 these amounts by the area percentage method: the amount of the peak with the relative retention time of about 0.7 to taltirelin is not more than 0.7% and the peaks with the relative retention time of about 0.8 and about 0.9, respectively, are not more than 0.3%, and the peak other than taltirelin and other than the peaks mentioned above is not more than 0.1%. And the total amount of the peaks other than taltirelin is not more than 1.0%.

Operating conditions—

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

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate in 1000 mL of water, adjust to pH 2.5 with phosphoric acid, and add 1.7 g of sodium 1-octanesulfonate. To 900 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of taltirelin is about 15 minutes.

Time span of measurement: About1.5 times as long as the retention time of taltirelin, beginning from 1/3 times the retention time of taltirelin.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase 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, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of taltirelin obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that 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 taltirelin are not less than 7000 and not more than 1.5, 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 taltirelin 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 Taltirelin Tablets add $V/2$ mL of the mobile phase and exactly $V/10$ mL of the internal standard solution, and agitate with the aid of ultrasonic waves for 10 minutes while occasional shaking. Then, add the mobile phase to make V mL so that each mL contains about 0.1 mg of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$), 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. Then, proceed as directed in the Assay.

Amount (mg) of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$)
$= M_S \times Q_T/Q_S \times V/500 \times 1.178$

M_S: Amount (mg) of taltirelin hydrate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of *o*-acetanisidide (1 in 2500).

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 Taltirelin Tablets is not less than 85%.

Start the test with 1 tablet of Taltirelin 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 5.6 μg of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of taltirelin hydrate for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Taltirelin Hydrate), and dissolve in 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 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 taltirelin.

Dissolution rate (%) with respect to the labeled amount of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18 \times 1.178$

M_S: Amount (mg) of taltirelin hydrate for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$) 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 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of taltirelin 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 taltirelin is not more than 2.0%.

Assay Weigh accurately, and powder not less than 20 Taltirelin Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$), add 25 mL of the mobile phase and exactly 5 mL of the internal standard solution, shake for 20 minutes, add the mobile phase to make 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 50 mg of taltirelin hydrate for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Taltirelin Hydrate), 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 taltirelin to that of the internal standard.

Amount (mg) of taltirelin hydrate ($C_{17}H_{23}N_7O_5.4H_2O$)
$= M_S \times Q_T/Q_S \times 1/10 \times 1.178$

M_S: Amount (mg) of taltirelin hydrate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of *o*-acetanisidide (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 about 40°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate in 1000 mL of water, adjust to pH 2.5 with phosphoric acid, and add 1.7 g of sodium 1-octanesulfonate. To 850 mL of this solution add 150 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of taltirelin 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, taltirelin 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of taltirelin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Titanium Oxide

酸化チタン

Change the Purity (3) as follows:

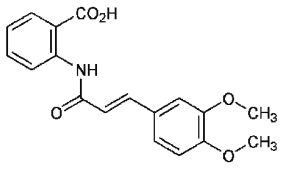
Purity

(3) Water-soluble substances—Shake thoroughly 4.0 g of Titanium Oxide with 50 mL of water, and allow to stand overnight. Shake thoroughly with 2 mL of ammonium chloride TS, add further 2 mL of ammonium chloride TS if necessary, and allow titanium oxide to settle. Add water to make 200 mL, shake thoroughly, and filter through double filter paper. Discard the first 10 mL of the filtrate, evaporate 100 mL of the clear filtrate on a water bath, and heat strongly at 800°C to constant mass: the mass of the residue is not more than 5.0 mg.

Add the following:

Tranilast

トラニラスト



$C_{18}H_{17}NO_5$: 327.33

2-{[(2*E*)-3-(3,4-Dimethoxyphenyl)prop-2-enoyl]amino}benzoic acid [*53902-12-8*]

Tranilast, when dried, contains not less than 99.0% and not more than 101.0% of $C_{18}H_{17}NO_5$.

Description Tranilast occurs as light yellow crystals or crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, slightly soluble in acetonitrile, in methanol and in ethanol (99.5), and practically insoluble in water.

It gradually becomes light yellow-brown on exposure to light.

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Tranilast in methanol (1 in 200,000) 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 Tranilast, 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*> 207 – 210°C

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Tranilast 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—Conduct this procedure without exposure to light using light-resistant vessels. Dissolve 50 mg of Tranilast in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 50 mL. Pipet 1 mL of this solution, add acetonitrile to make exactly 20 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. Determine each peak area by the automatic integration method: the area of the peak other than tranilast obtained from the sample solution is not larger than the peak area of tranilast from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 255 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 diluted acetic acid (100) (1 in 100) and acetonitrile (3:2).

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

Time span of measurement: About 4 times as long as the retention time of tranilast, beginning after the solvent peak.

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 tranilast are not less than 5000 and not more than 1.5, 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 tranilast is not more than 3.0%.

(3) Chloroform—Weigh accurately about 1 g of Tranilast, dissolve in exactly 5 mL of a solution, prepared by adding *N*,*N*-dimethylformamide to exactly 1 mL of the internal standard solution to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 3 g of chloroform, and add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution, add *N*,*N*-dimethylformamide to make 100 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 chloroform to that of the internal standard: the amount of chloroform is not more than 0.006%.

$$\text{Amount (\%) of chloroform} = M_S/M_T \times Q_T/Q_S \times 1/20$$

M_S: Amount (g) of chloroform
M_T: Amount (g) of Tranilast

Internal standard solution—A solution of trichloroethylene in *N*,*N*-dimethylformamide (1 in 50).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1 m in length, packed with porous styrene-divinylbenzene copolymer for gas chromatography (0.3 – 0.4 μm in mean pore size, not exceeding 50 m^2/g) (150 – 180 μm in particle diameter).

Column temperature: A constant temperature of about 160°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of chloroform is about 2 minutes.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, chloroform 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 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chloroform to that of the internal standard is not more than 1.0%.

(4) Residual solvent Being specified separately.

Loss on drying <*2.41*> Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition <*2.44*> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.4 g of Tranilast, previously dried, dissolve in 25 mL of *N*,*N*-dimethylformamide, add 25 mL of water, and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS, until a 30-seconds persistent light-red color is obtained (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 32.73 mg of $C_{18}H_{17}NO_5$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Add the following:

Tranilast Capsules

トラニラストカプセル

Tranilast Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of tranilast ($C_{18}H_{17}NO_5$: 327.33).

Method of preparation Prepare as directed under Capsules, with Tranilast.

Identification To an amount of the content of Tranilast Capsules, equivalent to 0.1 g of Tranilast, add 180 mL of diethyl ether, shake thoroughly, filter, and evaporate the filtrate to dryness on a water bath. Determine the absorption spectrum of a solution of the residue in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 333 nm and 337 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 using light-resistant vessels. Shake

the contents and the empty capsule shell of 1 Tranilast Capsules with a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) to make exactly V mL so that each mL contains about 0.5 mg of tranilast ($C_{18}H_{17}NO_5$), 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, then add a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of tranilast } (C_{18}H_{17}NO_5) = M_S \times Q_T/Q_S \times V/50$$

M_S: Amount (mg) of tranilast for assay

Internal standard solution—Ethyl parahydroxybenzoate in a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) (1 in 5000).

Dissolution ⟨*6.10*⟩ When the test is performed at 75 revolutions per minute according to the Paddle method using the sinker, using 900 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 5.5 as the dissolution medium, the dissolution rate in 60 minutes of Tranilast Capsules is not less than 75%.

Conduct this procedure without exposure to light using light-resistant vessels. Start the test with 1 capsule of Tranilast 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 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 5.6 μg of tranilast ($C_{18}H_{17}NO_5$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of tranilast for assay, previously dried at 105°C for 3 hours, and dissolve in 2nd fluid for dissolution test to make exactly 100 mL. Pipet 5 mL of this solution, and add 2nd fluid for dissolution test to make exactly 50 mL. Then, pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 332 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

$$\text{Dissolution rate (\%) with respect to the labeled amount of tranilast } (C_{18}H_{17}NO_5) = M_S \times A_T/A_S \times V'/V \times 1/C \times 18$$

M_S: Amount (mg) of tranilast for assay
C: Labeled amount (mg) of tranilast ($C_{18}H_{17}NO_5$) in 1 capsule

Assay Conduct this procedure using light-resistant vessels. Weigh accurately the mass of the contents of not less than 20 Tranilast Capsules, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of tranilast ($C_{18}H_{17}NO_5$), shake with a suitable amount of a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3), then add the same mixture to make exactly 200 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, then add a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of tranilast for assay, previously dried at 105°C for 3 hours, and dissolve in a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) to make exactly 50 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 7.0 and acetonitrile (7:3) 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 tranilast to that of the internal standard.

$$\text{Amount (mg) of tranilast } (C_{18}H_{17}NO_5) = M_S \times Q_T/Q_S \times 4$$

M_S: Amount (mg) of tranilast for assay

Internal standard solution—Ethyl parahydroxybenzoate in a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 255 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 diluted acetic acid (100) (1 in 100) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of tranilast is about 7 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 tranilast 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tranilast to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Tranilast Fine Granules

トラニラスト細粒

Tranilast Fine Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of tranilast ($C_{18}H_{17}NO_5$: 327.33).

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

Identification To an amount of Tranilast Fine Granules, equivalent to 0.1 g of Tranilast, add 180 mL of diethyl ether, shake thoroughly, filter, and evaporate the filtrate to dryness on a water bath. Determine the absorption spectrum of a solution of the residue in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 333 nm and 337 nm.

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

Conduct this procedure using light-resistant vessels. Shake the total content of 1 container of Tranilast Fine Granules with a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) to make exactly V mL so that each mL contains about 0.5 mg of tranilast ($C_{18}H_{17}NO_5$), 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, then add a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of tranilast ($C_{18}H_{17}NO5$)
$= M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of tranilast for assay

Internal standard solution—Ethyl parahydroxybenzoate in a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) (1 in 5000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 5.5 as the dissolution medium, the dissolution rate in 30 minutes of Tranilast Fine Granules is not less than 75%.

Conduct this procedure without exposure to light using light-resistant vessels. Start the test with an accurately weighed amount of Tranilast Fine Granules, equivalent to about 0.1 g of tranilast ($C_{18}H_{17}NO_5$), 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 5 mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of tranilast for assay, previously dried at 105°C for 3 hours, and dissolve in 2nd fluid for dissolution test to make exactly 100 mL. Pipet 5 mL of this solution, and add 2nd fluid for dissolution test to make exactly 50 mL. Then, pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 332 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 tranilast ($C_{18}H_{17}NO_5$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 360$

M_S: Amount (mg) of tranilast for assay
M_T: Amount (g) of Tranilast Fine Granules
C: Labeled amount (mg) of tranilast ($C_{18}H_{17}NO_5$) in 1 g

Assay Conduct this procedure using light-resistant vessels. Powder Tranilast Fine Granules. Weigh accurately a portion of the powder, equivalent to about 0.1 g of tranilast ($C_{18}H_{17}NO_5$), shake with a suitable amount of a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3), then add the same mixture to make exactly 200 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, then add a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of tranilast for assay, previously dried at 105°C for 3 hours, and dissolve in a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) to make exactly 50 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 7.0 and acetonitrile (7:3) 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 tranilast to that of the internal standard.

Amount (mg) of tranilast ($C_{18}H_{17}NO_5$)
$= M_S \times Q_T/Q_S \times 4$

M_S: Amount (mg) of tranilast for assay

Internal standard solution—Ethyl parahydroxybenzoate in a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 255 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 diluted acetic acid (100) (1 in 100) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of tranilast is about 7 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 tranilast 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tranilast to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Tranilast for Syrup

シロップ用トラニラスト

Tranilast for Syrup is a preparation for syrup, which is suspended before use.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of tranilast ($C_{18}H_{17}NO_5$: 327.33).

Method of preparation Prepare as directed under Syrups, with Tranilast.

Identification To an amount of Tranilast for Syrup, equivalent to 0.1 g of Tranilast, add 180 mL of diethyl ether, shake thoroughly, filter, and evaporate the filtrate to dryness on a water bath. Determine the absorption spectrum of a solution of the residue in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 333 nm and 337 nm.

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

Conduct this procedure using light-resistant vessels. Shake the total content of 1 container of Tranilast for Syrup with a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3), then add the same mixture to make exactly V mL so that each mL contains about 0.5 mg of tranilast ($C_{18}H_{17}NO_5$), 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, then add a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of tranilast ($C_{18}H_{17}NO_5$)
$= M_S \times Q_T/Q_S \times V/50$

M_S: Amount (mg) of tranilast for assay

Internal standard solution—Ethyl parahydroxybenzoate in a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) (1 in 5000).

Dissolution <*6.10*> When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 5.5 as the dissolution medium, the dissolution rate in 60 minutes of Tranilast for Syrup is not less than 75%.

Conduct this procedure without exposure to light using light-resistant vessels. Start the test with an accurately weighed amount of Tranilast for Syrup, equivalent to about 0.1 g of tranilast ($C_{18}H_{17}NO_5$), 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 5 mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of tranilast for assay, previously dried at 105°C for 3 hours, and dissolve in 2nd fluid for dissolution test to make exactly 100 mL. Pipet 5 mL of this solution, and add 2nd fluid for dissolution test to make exactly 50 mL. Then, pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, at 332 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 tranilast ($C_{18}H_{17}NO_5$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 360$

M_S: Amount (mg) of tranilast for assay
M_T: Amount (g) of Tranilast for Syrup
C: Labeled amount (mg) of tranilast ($C_{18}H_{17}NO_5$) in 1 g

Assay Conduct this procedure using light-resistant vessels. Powder Tranilast for Syrup. Weigh accurately the a portion of the powder, equivalent to about 0.1 g of tranilast ($C_{18}H_{17}NO_5$), shake with a suitable amount of a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3), then add the same mixture to make exactly 200 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, then add a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of tranilast for assay, previously dried at 105°C for 3 hours, and dissolve in a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) to make

exactly 50 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 7.0 and acetonitrile (7:3) to make exactly 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 tranilast to that of the internal standard.

$$\text{Amount (mg) of tranilast } (C_{18}H_{17}NO_5) = M_S \times Q_T/Q_S \times 4$$

M_S: Amount (mg) of tranilast for assay

Internal standard solution—Ethyl parahydroxybenzoate in a mixture of 0.05 mol/L phosphate buffer solution, pH 7.0 and acetonitrile (7:3) (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 255 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 diluted acetic acid (100) (1 in 100) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of tranilast is about 7 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 tranilast 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tranilast to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Add the following:

Tranilast Ophthalmic Solution

トラニラスト点眼液

Tranilast Ophthalmic Solution is an aqueous ophthalmic preparation.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of tranilast ($C_{18}H_{17}NO_5$: 327.33).

Method of preparation Prepare as directed under Ophthalmic preparations, with Tranilast.

Description Tranilast Ophthalmic Solution occurs as a clear and pale yellow liquid.

Identification When add 2 mL of dilute hydrochloric acid to a volume of Tranilast Ophthalmic Solution, equivalent to about 50 mg of Tranilast, a white precipitate is produced. Collect the precipitate by filtration, wash the precipitate with two 10-mL portions of water, and dry at 105°C for 3 hours. Dissolve 5 mg of the precipitate in methanol to make 100 mL. To 5 mL of this solution add methanol 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 333 nm and 337 nm.

Osmotic pressure ratio Being specified separately.

pH Being specified separately.

Foreign insoluble matter <*6.11*> It meets the requirement.

Insoluble particulate matter <*6.08*> It meets the requirement.

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

Assay Conduct this procedure using light-resistant vessels. To exactly a volume of Tranilast Ophthalmic Solution, equivalent to about 5 mg of tranilast ($C_{18}H_{17}NO_5$), add exactly 10 mL of the internal standard solution, then add ethanol (99.5) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of tranilast for assay, previously dried at 105°C for 3 hours, and dissolve in ethanol (99.5) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add ethanol (99.5) 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 tranilast to that of the internal standard.

$$\text{Amount (mg) of tranilast } (C_{18}H_{17}NO_5) = M_S \times Q_T/Q_S \times 1/5$$

M_S: Amount (mg) of tranilast for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in ethanol (99.5) (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 255 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 diluted acetic acid (100) (1 in 100) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of tranilast is about 7 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 tranilast 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 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of tranilast to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Trichlormethiazide Tablets

トリクロルメチアジド錠

Change the Uniformity of dosage units and Assay 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 tablet of Trichlormethiazide Tablets add V/5 mL of diluted phosphoric acid (1 in 50), and disintegrate the tablet. Add $2V$/5 mL of acetonitrile, shake vigorously for 15 minutes, add the mobile phase to make exactly V mL so that each mL contains about 40 μg of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm, discard the first 4 mL of the filtrate, and use the subsequent filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$)
$= M_S \times A_T/A_S \times V/500$

M_S: Amount (mg) of Trichlormethiazide RS

Assay To 10 Trichlormethiazide Tablets add V/10 mL of diluted phosphoric acid (1 in 50), and disintegrate the tablets. Add V/2 mL of acetonitrile, shake vigorously for 15 minutes, add the mobile phase to make exactly V mL so that each mL contains about 0.2 mg of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$), and centrifuge. Pipet 5 mL of the supernatant liquid, add the mobile phase to make exactly 25 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 4 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of Trichlormethiazide RS, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 25 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 trichlormethiazide.

Amount (mg) of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$)
in 1 tablet
$= M_S \times A_T/A_S \times V/1000$

M_S: Amount (mg) of Trichlormethiazide RS

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Trichlormethiazide.

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 trichlormethiazide are not less than 5000 and not more than 1.2, 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 trichlormethiazide is not more than 1.0%.

Delete the following Monograph:

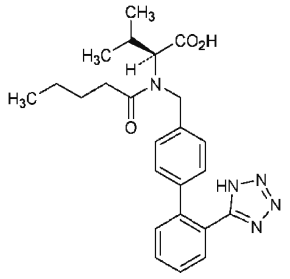
Trimethadione Tablets

トリメタジオン錠

Add the following:

Valsartan

バルサルタン



$C_{24}H_{29}N_5O_3$: 435.52

(2*S*)-3-Methyl-2-(*N*-{[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}pentanamido)butanoic acid

[*137862-53-4*]

Valsartan contains not less than 98.0% and not more than 102.0% of $C_{24}H_{29}N_5O_3$, calculated on the anhydrous basis and corrected on the amount of the residual solvent.

Description Valsartan occurs as a white powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Valsartan in methanol (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Valsartan 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 Valsartan 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 Valsartan RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -64 – $-69°$ (0.5 g calculated on the anhydrous and corrected on the amount of the residual solvent, methanol, 50 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Valsartan 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 50 mg of Valsartan in 100 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 each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.8 to valsartan, obtained from the sample solution is not larger than 1/5 times the peak area of valsartan from the standard solution, the area of the peak other than valsartan and the peak mentioned above is not larger than 1/10 times the peak area of valsartan from the standard solution, and the total area of the peaks other than valsartan is not larger than 3/10 times the peak area of valsartan 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 6 times as long as the retention time of valsartan, beginning after the solvent peak.

System suitability—

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 valsartan obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that 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 valsartan are not less than 1500 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 valsartan is not more than 2.0%.

(3) Optical isomer—Dissolve 75 mg of Valsartan in 100 mL of the mobile phase. To 5 mL of this solution add the mobile phase to make 25 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 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 valsartan, obtained from the sample solution is not larger than the peak area of valsartan from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 227 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 35°C.

Mobile phase: Dissolve 14.68 g of disodium hydrogen phosphate dodecahydrate and 3.81 g of potassium dihydrogen phosphate in 1000 mL of water. To 490 mL of this solution add 10 mL of 2-propanol.

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

System suitability—

System performance: Dissolve about 75 mg of Valsartan, previously allowed to stand at 105°C for 30 minutes, in the mobile phase to make 100 mL. To 5 mL of this solution add the mobile phase to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the optical isomer and valsartan 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 valsartan is not more than 5%.

(4) Residual solvent Being specified separately.

Water <*2.48*> Not more than 2.0% (0.1 g, coulometric titration).

Residue on ignition <*2.44*> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Valsartan and Valsartan RS (separately determine the water <*2.48*> and the residual solvent in the same manner as Valsartan), and dissolve them separately in the mobile phase to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 3 mL of the internal standard solution, and 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 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 valsartan to that of the internal standard.

Amount (mg) of valsartan ($C_{24}H_{29}N_5O_3$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Valsartan RS, calculated on the anhydrous basis and corrected on the amount of the residual solvent

Internal standard solution—A solution of diclofenac sodium in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 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 (5 μm in particle diameter).

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

Mobile phase: A mixture of water, acetonitrile, and acetic acid (100) (500:500:1).

Flow rate: Adjust the flow rate so that the retention time of valsartan 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, valsartan 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of valsartan to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Valsartan Tablets

バルサルタン錠

Valsartan Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$: 435.52).

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

Identification Determine the absorption spectra of the sample solution and the standard solution in the range 220 to 350 nm, which are obtained in the Uniformity of dosage units, as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrums with each other: both spectra exhibit similar intensities of absorption at the same wavelengths.

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 Valsartan Tablets add $V/10$ mL of water, and shake until the tablet is disintegrated. Add $V/2$ mL of methanol, shake thoroughly, add methanol to make exactly V mL so that each mL contains about 0.4 mg of valsartan ($C_{24}H_{29}N_5O_3$) for 20-mg tablet and 40-mg tablet, or contains about 0.8 mg of valsartan ($C_{24}H_{29}N_5O_3$) for 80-mg tablet and 160-mg tablet, and centrifuge. Pipet V' mL of the supernatant liquid, equivalent to 0.8 mg of valsartan ($C_{24}H_{29}N_5O_3$), add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Valsartan RS (separately determine the water <*2.48*> and the residual solvent in the same manner as Valsartan), dissolve in 10 mL of water, and add methanol to make exactly 100 mL. Pipet 2 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 250 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of valsartan ($C_{24}H_{29}N_5O_3$)
= $M_S \times A_T/A_S \times V/V' \times 1/50$

M_S: Amount (mg) of Valsartan RS, calculated on the anhydrous basis and corrected on the amount of the residual solvent

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 a 20-mg tablet, 40-mg tablet and 80-mg tablet in 30 minutes are not less than 75%, 75% and 80%, respectively, and of a 160-mg tablet in 45 minutes is not less than 75%.

Start the test with 1 tablet of Valsartan 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 22 μg of valsartan ($C_{24}H_{29}N_5O_3$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Valsartan RS (separately determine the water <*2.48*> and the residual solvent in the same manners as Valsartan), and dissolve in methanol to make exactly 50 mL. Pipet 5 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 250 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using water as the control.

Dissolution rate (%) with respect to the labeled amount of valsartan ($C_{24}H_{29}N_5O_3$)
= $M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of Valsartan RS, calculated on the anhydrous basis and corrected on the amount of the residual solvent

C: Labeled amount (mg) of valsartan ($C_{24}H_{29}N_5O_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Valsartan Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 50 mg of valsartan ($C_{24}H_{29}N_5O_3$), add 60 mL of the mobile phase, shake thoroughly, add the mobile phase to make exactly 100 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 3 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 Valsartan RS (separately, determine the water <2.48> and the residual solvent in the same manner as Valsartan), and dissolve in the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 3 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 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 valsartan to that of the internal standard.

Amount (mg) of valsartan ($C_{24}H_{29}N_5O_3$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of Valsartan RS, calculated on the anhydrous basis and corrected on the amount of the residual solvent

Internal standard solution—A solution of diclofenac sodium in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 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 (5 μm in particle diameter).

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

Mobile phase: A mixture of water, acetonitrile, and acetic acid (100) (500:500:1).

Flow rate: Adjust the flow rate so that the retention time of valsartan 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, valsartan 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of valsartan to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Vasopressin Injection

バソプレシン注射液

Change the Assay (ii) as follows:

Assay

(ii) Standard stock solution: Dissolve 2000 Units of Vasopressin RS, according to the labeled Units, in exactly 100 mL of diluted acetic acid (100) (1 in 400). Pipet 1 mL of this solution, and add diluted acetic acid (100) (1 in 400) to make exactly 10 mL.

Voglibose Tablets

ボグリボース錠

Add the following next to the Uniformity of dosage units:

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 Voglibose Tablets is not less than 85%.

Start the test with 1 tablet of Voglibose 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 0.11 μg of voglibose ($C_{10}H_{21}NO_7$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of voglibose for assay (separately determine the water <2.48> in the same manner as Voglibose), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 20 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 voglibose.

Dissolution rate (%) with respect to the labeled amount of voglibose ($C_{10}H_{21}NO_7$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/20$

M_S: Amount (mg) of voglibose for assay
C: Labeled amount (mg) of voglibose ($C_{10}H_{21}NO_7$) in 1 tablet

Operating conditions—

Apparatus, detector, column, column temperature, reaction coil, cooling coil, reaction reagent, reaction temperature, and flow rate of reaction reagent: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 1.56 g of sodium dihydrogen phosphate dihydrate in 500 mL of water. To this solution add a suitable amount of a solution, prepared by dissolving 3.58 g of disodium hydrogen phosphate decahydrate in 500 mL of water, to adjust to pH 6.5. To 500 mL of this solution add 500 mL of acetonitrile.

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

Flow rate of mobile phase: Adjust the flow rate so that the retention time of voglibose is about 6 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 voglibose are not less than 2000 and not more than1.5, 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 voglibose is not more than 3.0%.

Wheat Starch

コムギデンプン

Delete the latin name:

Zolpidem Tartrate

ゾルピデム酒石酸塩

Change the Identification (4) as follows:

Identification

(4) A solution of Zolpidem Tartrate, dissolved 1 g in 10 mL of methanol by warming, responds to the Qualitative Tests <*1.09*> (3) for tartrate.

Crude Drugs

Akebia Stem

モクツウ

Change the Description as follows:

Description Circular or ellipsoidal sections 0.2 – 0.3 cm in thickness, and 1 – 3 cm in diameter; phloem on both fractured surfaces is dark grayish brown; xylem reveals light brown vessel portions and grayish white medullary rays lined alternately and radially; pith light grayish yellow, and distinct; flank grayish brown, and with circular or transversely elongated elliptical lenticels.

Almost odorless; slightly acrid taste.

Under a microscope ⟨*5.01*⟩, a transverse section reveals ring layers mainly consisting of fiber bundles with crystal cells and stone cell groups and surrounding the outside of the phloem in arc shape. Medullary rays of the phloem consisting of sclerenchyma cells containing solitary crystals; portion near cambium is distinct; cells around the pith remarkably thick-walled; xylem medullary rays and parenchyma cells around the pith contain solitary crystals of calcium oxalate and starch grains less than 8 μm in diameter.

Alisma Rhizome

タクシャ

Change the title of the monograph and Latin name as follows:

Alisma Tuber

Alismatis Tuber

Powdered Alisma Rhizome

タクシャ末

Change the title of the monograph and Latin name as follows:

Powdered Alisma Tuber

Alismatis Tuber Pulveratum

Alpinia Officinarum Rhizome

リョウキョウ

Change the Description and Identification as follows:

Description Alpinia Officinarum Rhizome is a slightly curved and cylindrical rhizome, sometimes branched; 2 – 8 cm in length, 0.6 – 1.5 cm in diameter; externally red-brown to dark brown with fine striped lines, grayish white nodes and several traces of rootlet; hard to break; fracture surface, light brown in color and thickness of cortex is approximately the same as that of stele.

Odor, characteristic; taste, extremely pungent.

Under a microscope ⟨*5.01*⟩, a transverse section reveals epidermal cells often containing oil-like substances; cortex, endodermis and stele present beneath the epidermis; cortex and stele divided by endodermis; vascular bundles surrounded by fibers, scattered throughout the cortex and stele, cortex and stele composed of parenchyma interspersed with oil cells; parenchyma cells containing solitary crystals of calcium oxalate and starch grains, starch grains generally simple (sometimes 2- to 8-compound), narrowly ovate, ellipsoidal or ovate, 10 – 40 μm in diameter and with an eccentric navel.

Identification To 0.5 g of pulverized Alpinia Officinarum Rhizome add 5 mL of acetone, 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 5 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 cyclohexane, ethyl acetate and acetic acid (100) (12:8:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots appear at an *R*f value of about 0.4.

Powdered Amomum Seed

シュクシャ末

Change the Description as follows:

Description Powdered Amomum seed occurs as a grayish brown powder, and has a characteristic aroma and an acrid taste.

Under a microscope ⟨*5.01*⟩, Powdered Amomum Seed reveals fragments of wavy perisperm cells filled with starch grains and containing in each cell a calcium oxalate crystal; yellow and long epidermal cells of seed coat and fragments

of thin-walled tissue perpendicular to them; fragments of groups of brown, thick-walled polygonal stone cells.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Apricot Kernel

キョウニン

Change the Description and Identification as follows:

Description Flattened, somewhat asymmetric ovoid seed, 1.1 – 1.8 cm in length, 0.8 – 1.3 cm in width, 0.4 – 0.7 cm in thickness; sharp at one end and rounded at the other end where chalaza situated; seed coat brown and its surface being powdery with rubbing easily detachable stone cells of epidermis; numerous vascular bundles running from chalaza throughout the seed coat, appearing as thin vertical furrows; seed coat and thin semitransparent white albumen easily separate from cotyledon when soaked in boiling water; cotyledon, white in color.

Almost odorless; taste, bitter and oily.

Under a microscope <*5.01*>, surface of epidermis reveals stone cells on veins protruded by vascular bundles, forming round polygon to ellipse and approximately uniform in shape, with uniformly thickened cell walls, and 60 – 90 μm in diameter; in lateral view, stone cell appearing obtusely triangular and its cell wall extremely thickened at the apex.

Identification **(1)** When Apricot Kernel is knocked and ground together with water, the odor of benzaldehyde is produced.

(2) To 1.0 g of ground Apricot Kernel add 10 mL of methanol, immediately heat under a reflux condenser on a water bath for 10 minutes, cool, filter, and use the filtrate 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 20 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 (20:5:4) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a spot with a bluish white fluorescence appears at an *R*f value of about 0.7. Spray evenly thymol-sulfuric acid-methanol TS for spraying upon the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the red-brown spot from the standard solution.

Aralia Rhizome

ドクカツ

Change the Identification as follows:

Identification To 1 g of pulverized Aralia Rhizome add 10 mL of methanol, 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 5 μL of the sample 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) (30:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a purple spot appears at an *R*f value of about 0.5.

Add the following:

Artemisia Leaf

Artemisiae Folium

ガイヨウ

Artemisia leaf is the leaf and twig of *Artemisia princeps* Pampanini or *Artemisia montana* Pampanini (*Compositae*).

Description Wrinkled leaves and their fragments, frequently with thin stems. The upper surface of leaf dark green, the lower surface covered densely with grayish white cotton-like hairs. When smoothed by immersion in water, unfolded laminas 4 – 15 cm long, 4 – 12 cm wide, 1- to 2-pinnately cleft or pinnately parted. Segments in 2 to 4 pairs, oblong-lanceolate to oblong, apex acuminate sometimes obtuse, margins irregularly lobed or entire. Small sized leaves tri-cleft or entire, lanceolate.

Order, characteristic; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section of leaf reveals several-cells-layered collenchyma beneath epidermis of midvein; vascular bundles at the central portion of midvein, occasionally fiber bundles adjacent to phloem and xylem; laminas composed of upper epidermis, pallisad tissue, spongy tissue and lower epidermis, long soft hairs, T-shaped hairs and glandular hairs on epidermis of laminas; epidermal cells contain tannin-like substances, parenchyma cells contain oil-like substances and tannin-like substances.

Identification To 0.5 g of pulverized Artemisia Leaf (the parts like a floccose substance which are not easily pulverized may be removed) add 5 mL of a mixture of methanol and water (3:2), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg each of umbelliferone for thin-layer chromatography and scopoletin for thin-layer chromatography in 10 mL each of methanol, and use these solutions as the

standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL each of the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (20:10:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): two of the spots among the several spots obtained from the sample solution have the same color tone and *R*f value with the corresponding bluish white fluorescent spot obtained respectively from the standard solutions (1) and (2).
System suitability—(Ultraviolet light (main wavelength: 365 nm)) To 1 mL of the standard solution (1) add methanol to make 10 mL. Confirm that when perform the test with 1 μL of this solution under the above conditions, a bluish white fluorescent spot is detectable.

Purity *Artemisia argyi*—To 0.5 g of powdered Artemisia Leaf (the parts like a floccose substance which are not easily pulverized may be removed) add 5 mL of a mixture of methanol and water (3:2), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 0.5 g of artemisia·argyi for purity test add 5 mL of a mixture of methanol and water (3:2), shake for 10 minutes, 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 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, hexane and acetic acid (100) (20:10:1) to a distance of about 7 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): no spot appears from the sample solution at the position of the green fluorescent spot (*R*f value of about 0.5) obtained from the standard solution.

Loss on drying <*5.01*> Not more than 14.0%.

Total ash <*5.01*> Not more than 13.0%.

Acid-insoluble ash <*5.01*> Not more than 3.0%.

Extract content <*5.01*> Dilute ethanol-soluble extract: not less than 16.0%.

Asparagus Tuber

テンモンドウ

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

Asparagus Tuber is the tuber of *Asparagus cochinchinensis* Merrill (*Liliaceae*), from which most of the cork layer is removed after being passed through hot water or steamed.

Identification To 1 g of the coarse cutting of Asparagus Tuber add 5 mL of a mixture of 1-butanol and water (40:7), shake for 30 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 1-butanol, water and acetic acid (100) (10:6:3) 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 2 minutes: the spot of a red-brown at first then changes to brown color appears at an *R*f value of about 0.4.

Powdered Atractylodes Lancea Rhizome

ソウジュツ末

Change the Description as follows:

Description Powdered Atractylodes Lancea Rhizome occurs as a yellow-brown powder. It has a characteristic odor, and a slightly bitter taste.

Under a microscope <*5.01*>, Powdered Atractylodes Lancea Rhizome reveals mainly parenchyma cells, spherocrystals of inulin, fragments of parenchyma cells containing fine needle crystals of calcium oxalate as their contents; and further fragments of light yellow thick-walled fibers, stone cells and cork cells; a few fragments of reticulate and scalariform vessels, and small yellow-brown secreted masses or oil drops; starch grains absent.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Atractylodes Rhizome

ビャクジュツ

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

Atractylodes Rhizome is the rhizome of 1) *Atractylodes japonica* Koidzumi ex Kitamura (*Compositae*) (Wa-byakujutsu) or 2) *Atractylodes macrocephala* Koidzumi (*Atractylodes ovata* De Candolle) (*Compositae*) (Kara-byakujutsu).

Description 1) *Atractylodes japonica* Koidzumi ex Kitamura—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) *Atractylodes macrocephala* Koidzumi—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.

Powdered Atractylodes Rhizome

ビャクジュツ末

Change the Description as follows:

Description Powdered Atractylodes Rhizome occurs as a light brown to yellow-brown powder, and has a characteristic odor and a slightly bitter or slightly sweet taste, followed by a slightly bitter aftertaste.

Under a microscope <*5.01*>, Powdered Atractylodes Rhizome reveals mainly parenchyma cells, crystals of inulin and fragments of parenchyma cells containing small needle crystals of calcium oxalate; fragments of light yellow thick-walled fibers, stone cells and cork cells; a few fragments of reticulate and scalariform vessels; small yellow-brown secrete masses or oil droplets; starch grains absent.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Bearberry Leaf

ウワウルシ

Change the Identification (2) as follows:

Identification (2) To 0.2 g of pulverized Bearberry Leaf add 10 mL of a mixture of ethanol (95) and water (7:3), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of arbutin for thin-layer chromatography in 1 mL of a mixture of ethanol (95) 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 ethyl formate, water and formic acid (8:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid upon the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots from the sample solution and the spot from the standard solution show a yellow-brown to blackish brown color and the same *R*f value.

Belladonna Root

ベラドンナコン

Change the Identification as follows:

Identification Place 2.0 g of pulverized Belladonna Root in a glass-stoppered centrifuge tube, add 30 mL of ammonia TS, and centrifuge after irradiation of ultrasonic waves for 5 minutes. Transfer the supernatant liquid to a separator, add 40 mL of ethyl acetate, and shake. Drain off the ethyl

acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 2 mg of Atropine Sulfate 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 5 μL each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia water (28) (90:7:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate: the principal spot from the sample solution is the same in color tone and *R*f value with a yellow-red spot from the standard solution.

Benincasa Seed

トウガシ

Change the origin/limits of content, Description and Identification as follows:

Benincasa seed is the seed of 1) *Benincasa cerifera* Savi or 2) *Benincasa cerifera* Savi forma *emarginata* K. Kimura et Sugiyama (*Cucurbitaceae*).

Description **1)** *Benincasa cerifera* Savi—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* Savi forma *emarginata* K. Kimura et Sugiyama—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.

Identification To 0.5 g of pulverized Benincasa Seed add 10 mL of a mixture of methanol and water (4:1), shake for 10 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 20 μL of the sample 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) (8:6:3) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): two bluish white fluorescent spots appear at an *R*f value of about 0.4, and the spot having the smaller *R*f value shows more intense fluoresence.

Bupleurum Root

サイコ

Change the Description as follows:

Description Single or branched root of long cone or column shape, 10 – 20 cm in length, 0.5 – 1.5 cm in diameter; occasionally with remains of stem on the crown; externally light brown to brown and sometimes with deep wrinkles; easily broken, and fractured surface somewhat fibrous.

Odor, characteristic, and taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section reveals the thickness of cortex reaching 1/3 ~ 1/2 of the radius, tangentially extended clefts in cortex; and cortex scattered with a good many oil canals 15 – 35 μm in diameter; in xylem, vessels lined radially or stepwise, and fiber groups scattered; in the pith at the crown, the same oil canals as in the cortex; parenchyma cells containing starch grains and oil droplets. Starch grains composed of simple grains, 2 – 10 μm in diameter, or compound grains.

Powdered Capsicum

トウガラシ末

Change the Description as follows:

Description Powdered Capsicum occurs as a yellow-red powder. It has a slight, characteristic odor and a hot, acrid taste.

Under a microscope <*5.01*>, Powdered Capsicum reveals fragments of parenchyma containing oil droplets and yellow-red chromoplasts; fragments of epidermis from outer surface of pericarp with thick cuticle; fragments of stone

cells from inner surface of pericarp, with wavy curved side walls; fragments of thin vessels; fragments of seed coat with thick wall, and fragments of parenchyma consisting of small cells of endosperm containing fixed oil and aleuron grains.

Add the following:

Cherry Bark

Pruni Cortex

オウヒ

Cherry Bark is the bark of *Prunus jamasakura* Siebold ex Koidzumi or *Prunus verecunda* Koehne (*Rosaceae*).

Description Flat or semi-tubular pieces of bark; 3 – 6 mm thick, externally light brown to brown, internal surface smooth, grayish brown to brown, occasionally periderm peeled off; the bark with periderm externally rough and lenticels observed; internal surface with many fine longitudinal lines; transversely cut surface grayish brown to brown, fibrous.

Odor, slightly characteristics; taste, slightly bitter and astringent.

Under a microscope <*5.01*>, a transverse section reveals cork layer containing solitary crystals and rosette aggregates of calcium oxalate in the bark with periderm; in cortex many stone cells and idioblasts arranged irregularly and parenchyma cells containing solitary crystals and rosette aggregates of calcium oxalate dotted; groups of phloem fibers lined alternately with the other tissue of phloem between rays.

Identification Shake 1 g of pulverized Cherry Bark with 10 mL of dilute hydrochloric acid, and heat in a boiling water bath for 10 minutes. After cooling, add 5 mL of diethyl ether, shake for 10 minutes, centrifuge, and use the diethyl ether layer 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 ethyl acetate, hexane and acetic acid (100) (20:20:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a crimson spot appears at an *R*f value of about 0.5.

Loss on drying <*5.01*> Not more than 13.0% (6 hours).

Total ash <*5.01*> Not more than 6.5%.

Acid-insoluble ash <*5.01*> Not more than 0.5%.

Containers and storage Containers—Well-closed containers.

Chrysanthemum Flower

キクカ

Change the Description as follows:

Description **1)** *Chrysanthemum morifolium* Ramatulle—Capitulum, 15 – 40 mm in diameter; involucre consisting of 3 – 4 rows of involucral scales; the outer involucral scale linear to lanceolate, inner involucral scale narrowly 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* Linné—Capitulum, 3 – 10 mm in diameter; involucre consisting of 3 – 5 rows of involucral scales; the outer involucral scale linear to lanceolatae, inner involucral scale narrowly 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.

Cinnamon Bark

ケイヒ

Change the Description as follows:

Description Usually semi-tubular or tubularly rolled pieces of bark, 0.1 – 0.5 cm in thickness, 5 – 50 cm in length, 1.5 – 5 cm in diameter; the outer surface dark red-brown, and the inner surface red-brown and smooth; brittle; the fractured surface is slightly fibrous, red-brown, exhibiting a light brown, thin layer.

Characteristic aroma; taste, sweet and pungent at first, later rather mucilaginous and slightly astringent.

Under a microscope <*5.01*>, a transverse section of Cinnamon Bark reveals a primary cortex and a secondary cortex divided by an almost continuous ring consisting of stone cells; nearly round bundles of fibers in the outer region of the ring; cell wall of each stone cell often thickened in a U-shape; secondary cortex lacking stone cells, and with a small number of sclerenchymatous fibers coarsely scattered; parenchyma scattered with oil cells, mucilage cells and cells containing starch grains; medullary rays with cells containing fine needles of calcium oxalate.

Citrus Unshiu Peel

チンピ

Change the Latin name as follows:

Citri Unshiu Pericarpium

Powdered Clove

チョウジ末

Change the Description as follows:

Description Powdered Clove occurs as a dark brown powder. It has a strong, characteristic odor and a pungent taste, followed by slight numbness of the tongue.

Under a microscope <*5.01*>, Powdered Clove reveals epidermal tissue with stomata, collenchyma, parenchyma with oil sacs, and spongy parenchyma or its fragments; furthermore, a few fusiform thick-walled fibers, spiral vessels 6 – 10 μm in diameter, anther and pollen grains, and rosette aggregates of calcium oxalate 10 – 15 μm in diameter. Epidermis of anther shows characteristically reticulated walls; pollen grains tetrahedral 10 – 20 μm in diameter; rosette aggregates of calcium oxalate arranged in crystal cell rows, or contained in collenchyma cells and parenchyma cells.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Cnidium Monnieri Fruit

ジャショウシ

Change the Identification as follows:

Identification To 1 g of pulverized Cnidium Monnieri Fruit add 10 mL of ethyl acetate, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of osthole 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 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 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 from the sample solution has the same color tone and the *R*f value with the bluish white fluorescent spot from the standard solution.

Cnidium Rhizome

センキュウ

Change the Description as follows:

Description Irregular massive rhizome, occasionally cut lengthwise; 5 – 10 cm in length, and 3 – 5 cm in diameter; externally grayish brown to dark brown, with gathered nodes, and with knobbed protrusions on the node; margin of the vertical section irregularly branched; internally grayish white to grayish brown, translucent and occasionally with hollows; dense and hard in texture.

Odor, characteristic; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section reveals cortex and pith with scattered oil canals; in the xylem, thick-walled and lignified xylem fibers appear in groups of various sizes; starch grains usually gelatinized, but rarely remaining as grains of 5 – 25 μm in diameter; crystals of calcium oxalate not observable.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Powdered Cnidium Rhizome

センキュウ末

Change the Description as follows:

Description Powdered Cnidium Rhizome occurs as a gray to light grayish brown powder. It has a characteristic odor and a slightly bitter taste.

Under a microscope <*5.01*>, Powdered Cnidium Rhizome reveals colorless and gelatinized starch masses, and fragments of parenchyma containing them; fragments of scalariform and reticulate vessels 15 – 30 μm in diameter; fragments of thick-walled and lignified xylem fibers 20 – 60 μm in diameter; fragments of yellow-brown cork tissue; fragments of secretory tissue.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Powdered Coix Seed

ヨクイニン末

Change the Purity as follows:

Purity Foreign matter—Under a microscope <*5.01*>, Powdered Coix Seed reveals no fragments of tissue having silicified cell wall, no stone cells, no fragments of other thick-walled and lignified cells, no fragments of reticulate, scalariform and pitted vessels, no fragments of fibers and

hairs, and no large starch grains, more than 10 μm in diameter, appearing blue-purple upon addition of iodine TS.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Condurango

コンズランゴ

Change the Description as follows:

Description Tubular or semi-tubular pieces of bark, 0.1 – 0.6 cm in thickness, 4 – 15 cm in length; outer surface grayish brown to dark brown, nearly smooth and with numerous lenticels, or more or less scaly and rough; inner surface light grayish brown and longitudinally striate; fractured surface fibrous on the outer region and generally granular in the inner region.

Odor, slight; taste, bitter.

Under a microscope <*5.01*>, a transverse section reveals a cork layer composed of several layers of thin-walled cells; primary cortex with numerous stone cell groups; secondary cortex with phloem fiber bundles scattered inside the starch sheath consisting of one-cellular layer; articulate latex tubes scattered in both cortices; parenchyma cells containing starch grains or rosette aggregates of calcium oxalate; starch grain 3 – 20 μm in diameter.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Coptis Rhizome

オウレン

Change the Description and Identification (2) as follows:

Description Irregular, cylindrical rhizome, 2 – 4 cm, rarely up to 10 cm in length, 0.2 – 0.7 cm in diameter, slightly curved and often branched; externally grayish yellow-brown, with ring nodes, and with numerous remains of rootlets; generally remains of petiole at one end; fractured surface rather fibrous; cork layer light grayish brown, cortex and pith are yellow-brown to reddish yellow-brown, xylem is yellow to reddish yellow in color.

Odor, slight; taste, extremely bitter and lasting; it colors the saliva yellow on chewing.

Under a microscope <*5.01*>, a transverse section of Coptis Rhizome reveals a cork layer composed of thin-walled cork cells; cortex parenchyma usually exhibiting groups of stone cells near the cork layer and yellow phloem fibers near the cambium; xylem consisting chiefly of vessels, tracheids and xylem fibers; medullary ray distinct; pith large; in pith, stone cells or stone cells with thick-walled and lignified cells are sometimes recognized; parenchyma cells contain minute starch grains.

Identification

(2) To 0.5 g of pulverized Coptis Rhizome add 20 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride 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 1-butanol, water and acetic acid (100) (7:2:1) 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 from the sample solution and a yellow to yellow-green fluorescence spot from the standard solution show the same color tone and the same *R*f value.

Powdered Coptis Rhizome

オウレン末

Change the Description and Identification (2) as follows:

Description Powdered Coptis Rhizome occurs as a yellow-brown to grayish yellow-brown powder. It has a slight odor and an extremely bitter, lasting taste, and colors the saliva yellow on chewing.

Under a microscope <*5.01*>, almost all elements are yellow in color; it reveals mainly fragments of vessels, tracheids and xylem fibers; parenchyma cells containing starch grains; polygonal cork cells. Usually, round to obtuse polygonal stone cells and their groups, and phloem fibers, 10 – 20 μm in diameter, and fragments of their bundles. Sometimes, polygonal and elongated epidermal cells, originated from the petiole, having characteristically thickened cell walls. Starch grains are single grains 1 – 7 μm in diameter.

Identification

(2) To 0.5 g of Powdered Coptis Rhizome add 20 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride 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 1-butanol, water and acetic acid (100) (7:2:1) 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 from the sample solution and a yellow to yellow-green fluorescence spot from the standard solution show the same color tone and the same *R*f value.

Corydalis Tuber

エンゴサク

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

Corydalis Tuber is the tuber of *Corydalis turtschaninovii* Basser forma *yanhusuo* Y. H. Chou et C. C. Hsu (*Papaveraceae*), usually after being passed through hot water.

It contains not less than 0.08% of dehydrocorydaline (as dehydrocorydaline nitrate), calculated on the basis of dried material.

Identification To 2 g of pulverized Corydalis Tuber add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of dehydrocorydaline nitrate 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 methanol, ammonium acetate solution (3 in 10) and acetic acid (100) (20:1:1) 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 from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution, and a yellow fluorescent spot appears at the lower side of the spot. Separately, spray evenly Dragendorff's TS for spraying on the plate, air-dry, and then spray sodium nitrite TS: a brown spot appears at an *R*f value of about 0.6.

Powdered Corydalis Tuber

エンゴサク末

Change the Identification as follows:

Identification To 2 g of Powdered Corydalis Tuber add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of dehydrocorydaline nitrate 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 methanol, ammonium acetate solution (3 in 10) and acetic acid (100) (20:1:1) 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 from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution, and a yellow fluorescent spot appears at the lower side of the spot. Separately, spray evenly Dragendorff's TS for spraying on the plate, air-dry, and then spray sodium nitrite TS: a brown spot appears at an *R*f value of about 0.6.

Crataegus Fruit

サンザシ

Change the Description as follows:

Description 1) *Crataegus cuneata* Siebold et Zuccarini—Nearly spherical fruits, 8 – 14 mm in diameter; externally yellowish 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. In epidermal cell and 2- to 3-layers of parenchyma cells beneath these observed contents of yellowish 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 rosette aggregates 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* Bunge var. *major* N. E. Brown—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, yellowish 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 a few stone cells in parenchyma.

Digenea

マクリ

Change the Identification as follows:

Identification To 2 g of pulverized Digenea add 10 mL of dilute ethanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of kainic acid in 10 mL of dilute ethanol, 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 formate, water and formic acid (5:1:1) 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: 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-red spot from the standard solution.

Ephedra Herb

マオウ

Change the Identification as follows:

Identification To 0.5 g of pulverized Ephedra Herb add 10 mL of methanol, shake for 2 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 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying, and heat the plate at 105°C for 5 minutes: a red-purple spot appears at an *R*f value of about 0.35.

Epimedium Herb

インヨウカク

Change the Identification as follows:

Identification To 2 g of pulverized Epimedium Herb add 20 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of icariin 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 from the sample solution has the same color tone and *R*f value with the spot from the standard solution.

Powdered Fennel

ウイキョウ末

Change the Description as follows:

Description Powdered Fennel occurs as a greenish light brown to greenish brown, and is a characteristic odor and taste.

Under a microscope <*5.01*>, Powdered Fennel reveals fragments of parenchyma cells of perisperm containing aleurone grain, fragments of parenchyma cells of endosperm containing fatty oil, fragments of sclerenchyma with characteristic simple pits, fragments of oil canal within yellow-brown material, fragments of endocarp shown scalariform, spiral vessels, fragments of epidermis or epidermis with stomata.

Fritillaria Bulb

バイモ

Change the Description as follows:

Description Fritillaria Bulb is a depressed spherical bulb, 2 – 3 cm in diameter, 1 – 2 cm in height, consisting of 2 thickened scaly leaves often separated; externally and internally white to light yellow-brown in color; inside base is in a slightly dark color; the bulb sprinkled with lime before drying is dusted with white powder; fractured surface, white in color and powdery.

Odor, slight and characteristic; taste, bitter.

Under a microscope <*5.01*>, a transverse section reveals the outermost layer (epidermis) to be composed of a single layer of cells; numerous vascular bundles scattered throughout the parenchyma inside of the epidermis; parenchyma filled with starch grains; starch grains are mainly simple (rarely 2- to 3-compound), 5 – 60 μm in diameter, narrowly ovate to ovate or triangular to obovate, stratiform figure obvious; epidermal cells and parenchyma cells near the vessels contain solitary crystals of calcium oxalate.

Powdered Gambir

アセンヤク末

Change the Description as follows:

Description Powdered Gambir occurs as a red-brown to dark brown powder. It has a slight odor, and an extremely astringent and bitter taste.

Under a microscope <*5.01*>, Powdered Gambir, immersed in olive oil or liquid paraffin, consists of masses of needle crystals or yellow-brown to red-brown angular fragments, and reveals epidermal tissue and thick-walled hairs.

Powdered Gardenia Fruit

サンシシ末

Change the Description as follows:

Description Powdered Gardenia Fruit occurs as a yellow-brown powder, and has a slight odor and a bitter taste.

Under a microscope <*5.01*>, Powdered Gardenia Fruit reveals fragments of yellow-brown epidermis consisting of polygonal epidermal cells in surface view; unicellular hairs, spiral and ring vessels, stone cells often containing crystals of calcium oxalate; fragments of thin-walled parenchyma containing yellow pigments, oil drops and rosette aggregates of calcium oxalate (the above elements from fruit receptacle and pericarp); fragments of large and thick-walled epidermis of seed coat, containing a red-brown substance; fragments of endosperm filled with aleuron grains (the above elements from seed).

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Gentian

ゲンチアナ

Change the Description as follows:

Description Nearly cylindrical pieces, 10 – 50 cm in length, 2 – 4 cm in diameter; externally dark brown; the rhizome short, with fine, transverse wrinkles, and sometimes with buds and remains of leaves at the upper edge. The root longitudinally and deeply wrinkled, and more or less twisted; fractured surface yellow-brown and not fibrous, and a cambium and its neighborhood tinged dark brown.

Odor, characteristic; taste, sweet at first, later persistently bitter.

Under a microscope <*5.01*>, a transverse section of the root reveals several layers of collenchyma adjoined internally to 4 to 6 layers of thin-walled cork; secondary cortex of the parenchyma with irregularly distributed phloem; xylem consisting chiefly of parenchyma, with individual or clustered vessels and tracheids, and exhibiting some sieve tubes of xylem; parenchyma of the xylem and the cortex containing oil droplets, minute needle crystals of calcium oxalate and very rarely starch grains 10 – 20 μm in diameter.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Powdered Geranium Herb

ゲンノショウコ末

Change the Description as follows:

Description Powdered Geranium Herb occurs as a grayish green to light yellow-brown powder. It has a slight odor and an astringent taste.

Under a microscope <*5.01*>, Powdered Geranium Herb reveals mainly fibers, spiral vessels, pitted vessels, and unicellular hairs; furthermore, multicellular glandular hairs, epidermis with stomata, fragments of palisade tissue, rosette aggregates of calcium oxalate, and starch grains. Fiber is thick-walled, with somewhat distinct pits; unicellular hair shows small point-like protrusions on the surface; palisade tissue consisting of circular parenchyma cells in surface view, each cell containing one rosette aggregate of calcium oxalate which is about 20 μm in diameter. Starch grains consisting of simple grains but rarely of 2-compound grains, ovoid to spherical, 5 – 30 μm in diameter, with distinct hilum.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Ginger

ショウキョウ

Change the origin/limits of content, Description and Identification as follows:

Ginger is the rhizome, with (unpeeled) or without (peeled) the periderm, of *Zingiber officinale* Roscoe (*Zingiberaceae*).

It contains not less than 0.3% of [6]-gingerol ($C_{17}H_{26}O_4$: 294.39), calculated on the basis of dried material.

Description Irregularly compressed and often branched massive rhizome or a part of it; the branched parts are slightly curved ovoid or oblong-ovoid, 2 – 4 cm in length, and 1 – 2 cm in diameter; external surface grayish white to light grayish brown, and often with white powder; fractured surface is somewhat fibrous, powdery, light yellowish brown; under a magnifying glass, a transverse section reveals cortex and stele distinctly divided; vascular bundles and secretes scattered all over the surface as small dark brown dots.

Odor, characteristic; taste, extremely pungent.

Under a microscope <*5.01*>, a transverse section reveals cork layer, cortex, endodermis and stele in this order from the outside, cork layer often peeled off; cortex and stele, divided by a single-layered endodermis, composed of parenchyma; vascular bundles surrounded by fibers scattered in cortex and stele; oil cells contain yellow oily substances,

scattered in parenchyma; parenchyma cells contain solitary crystals of calcium oxalate; starch grains in parenchyma cells mainly simple, ovoid, triangular ovoid, ellipsoidal or spherical, with abaxial hilum, usually 10 – 30 μm in long axis.

Identification To 2 g of pulverized Ginger add 5 mL of diethyl ether, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of [6]-gingerol 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 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 from the sample solution and the spot from the standard solution show the same color tone and *R*f value.

Add the following next to the Total ash:

Assay Weigh accurately about 1 g of pulverized Ginger (separately determine the loss on drying <*5.01*>, at 105°C for 5 hours), place in a centrifuge tube, add 30 mL of a mixture of methanol and water (3:1), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue add 30 mL of a mixture of methanol and water (3:1), and repeat the extraction twice more. To the combined all extracts add a mixture of methanol and water (3:1) to make exactly 100 mL, use this solution as the sample solution. Separately, weigh accurately about 5 mg of [6]-gingerol for assay, dissolve in a mixture of methanol and water (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, and determine the peak areas, A_T and A_S, of [6]-gingerol.

$$\text{Amount (mg) of [6]-gingerol} = M_S \times A_T/A_S$$

M_S: Amount (mg) of [6]-gingerol 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 40°C.

Mobile phase: A mixture of water and acetonitrile and phosphoric acid (3800:2200:1).

Flow rate: Adjust the flow rate so that the retenton time of [6]-gingerol is about 19 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 [6]-gingerol 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 [6]-gingerol is not more than 1.5%.

Powdered Ginger

ショウキョウ末

Change the origin/limits of content, Description and Identification as follows:

Powdered Ginger is the powder of Ginger.

It contains not less than 0.20% of [6]-gingerol ($C_{17}H_{26}O_4$: 294.39), calculated on the basis of dried material.

Description Powdered Ginger occurs as a light grayish brown to light grayish yellow powder. It has a characteristic odor and an extremely pungent taste.

Under a microscope <*5.01*>, Powdered Ginger reveals mainly starch grains and parenchyma cells containing them; also, parenchyma cells containing yellow-brown to dark brown oily substances or single crystals of calcium oxalate; fragments of fibers with distinct pits; fragments of spiral, ring and reticulate vessels, and rarely fragments of cork tissue; starch grains composed of simple, compound or half-compound grains, ovoid, triangular ovoid, ellipsoidal or spherical, with abaxial hilum, usually 10 – 30 μm in long axis.

Identification To 2 g of Powdered Ginger add 5 mL of diethyl ether, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of [6]-gingerol 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 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 from the sample solution and the spot from the standard solution show the same color tone and *R*f value.

Add the following next to the Total ash:

Assay Weigh accurately about 1 g of Powdered Ginger (separately determine the loss on drying <*5.01*>, at 105°C for 5 hours), place in a centrifuge tube, add 30 mL of a mixture of methanol and water (3:1), shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue

add 30 mL of a mixture of methanol and water (3:1), and repeat the extraction twice more. To the combined all extracts add a mixture of methanol and water (3:1) to make exactly 100 mL, use this solution as the sample solution. Separately, weigh accurately about 5 mg of [6]-gingerol for assay, dissolve in a mixture of methanol and water (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, and determine the peak areas, A_T and A_S, of [6]-gingerol.

$$\text{Amount (mg) of [6]-gingerol} = M_S \times A_T/A_S$$

M_S: Amount (mg) of [6]-gingerol 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 40°C.

Mobile phase: A mixture of water and acetonitrile and phosphoric acid (3800:2200:1).

Flow rate: Adjust the flow rate so that the retenton time of [6]-gingerol is about 19 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 [6]-gingerol 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 [6]-gingerol is not more than 1.5%.

Ginseng

ニンジン

Change the Identification (2) as follows:

Identification

(2) To 2.0 g of pulverized Ginseng add 10 mL of water and 10 mL of 1-butanol, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ginsenoside Rg_1 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, methanol and water (14:5:4) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the spot from the standard solution.

Powdered Ginseng

ニンジン末

Change the Description and Identification as follows:

Description Powdered Ginseng occurs as a light yellowish white to light yellowish-brown powder. It has characteristic odor and is a slight sweet taste followed by a slight bitterness.

Under a microscope <*5.01*>, Powdered Ginseng reveals round to rectangular parenchyma cells containing starch grains, occasionally gelatinized starch, vessels, secretory cell, sclerenchyma cell, big and thin-walled cork cell; crystals of calcium oxalate and starch. Vessels are reticulate vessel fragments, scalariform vessel and spiral vessel, 15 – 40 μm in diameter. Secretory cell containing a mass of yellow glistened contents; rosette aggregate of calcium oxalate, 20 – 60 μm in diameter, and 1 – 5 μm in diameter, rarely up to 30 μm in diameter of its single crystal; sclerenchymatous cells and thin-walled cork cells. Starch grains are observed in simple grain and 2 to 6-compound grain, simple grain, 3 – 20 μm in diameter.

Identification To 2.0 g of Powdered Ginseng add 10 mL of water and 10 mL of 1-butanol, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ginsenoside Rg_1 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, methanol and water (14:5:4) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 10 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the spot from the standard solution.

Glycyrrhiza

カンゾウ

Change the Description and Identification as follows:

Description Nearly cylindrical pieces, 0.5 – 3 cm in diameter, over 1 m in length. Glycyrrhiza is externally dark

brown to red-brown, longitudinally wrinkled, and often has lenticels, small buds and scaly leaves; peeled Glycyrrhiza is externally light yellow and fibrous. The transverse section reveals a rather clear border between phloem and xylem, and a radial structure which often has radiating splits; a pith in Glycyrrhiza originated from stolon, but no pith from root.

Odor, slight; taste, sweet.

Under a microscope <*5.01*>, a transverse section reveals several layers of yellow-brown cork layers, and 1- to 3-cellular layer of cork cortex inside the cork layer; the cortex exhibiting medullary rays and obliterated sieve portions radiated alternately; the phloem exhibiting groups of phloem fibers with thick but incompletely lignified walls and surrounded by crystal cells; peeled Glycyrrhiza some times lacks periderm and a part of phloem; the xylem exhibiting large yellow vessels and medullary rays in 3 to 10 rows radiated alternately; the vessels accompanied with xylem fibers surrounded by crystal cells, and with xylem parenchyma cells; the parenchymatous pith only in Glycyrrhiza originated from stolon. The parenchyma cells contain starch grains and often solitary crystals of calcium oxalate.

Identification To 2 g of pulverized Glycyrrhiza add 10 mL of a mixture of ethanol (95) and water (7:3), heat by shaking on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of Glycyrrhizinic Acid RS in 1 mL of a mixture of ethanol (95) 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 2 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-butanol, water and acetic acid (100) (7:2:1) 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 from the sample solution and a spot from the standard solution show the same color tone and the same *R*f value.

Powdered Glycyrrhiza

カンゾウ末

Change the Description and Identification as follows:

Description Powdered Glycyrrhiza is light yellow-brown or light yellow to grayish yellow (powder of peeled Glycyrrhiza) in color. It has a slight odor and a sweet taste.

Under a microscope <*5.01*>, Powdered Glycyrrhiza reveals mainly yellow sclerenchymatous fiber bundles accompanied with crystal cell rows; vessels, 80 – 200 μm in diameter, with pitted, reticulate and scalariform pits, and with round perforations; parenchyma cells, containing starch grains and solitary crystals of calcium oxalate, their fragments, and cork tissues; but powder of peeled Glycyrrhiza shows no cork tissue; if any, a very few. Starch grains are simple grains, 2 – 20 μm in diameter; solitary crystals of calcium oxalate, 10 – 30 μm in a diameter.

Identification To 2 g of Powdered Glycyrrhiza add 10 mL of a mixture of ethanol (95) and water (7:3), heat by shaking on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of Glycyrrhizinic Acid RS in 1 mL of a mixture of ethanol (95) 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 2 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-butanol, water and acetic acid (100) (7:2:1) 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 from the sample solution and a spot from the standard solution show the same color tone and the same *R*f value.

Add the following:

Hangeshashinto Extract

半夏瀉心湯エキス

Hangeshashinto Extract contains not less than 70 mg and not more than 210 mg (for preparation prescribed 2.5 g of Scutellaria Root) or not less than 80 mg and not more than 240 mg (for preparation prescribed 3 g of Scutellaria Root) of baicalin ($C_{21}H_{18}O_{11}$: 446.36), not less than 22 mg and not more than 66 mg (for preparation prescribed 2.5 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), and not less than 7 mg and not more than 21 mg of berberine [expressed as berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81)], per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)
Pinellia Tuber	5 g	6 g	5 g
Scutellaria Root	2.5 g	3 g	2.5 g
Processed Ginger	2.5 g	3 g	—
Ginger	—	—	2.5 g
Ginseng	2.5 g	3 g	2.5 g
Glycyrrhiza	2.5 g	3 g	2.5 g
Jujube	2.5 g	3 g	2.5 g
Coptis Rhizome	1 g	1 g	1 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1), 2) or 3), using the crude drugs shown above.

Description Hangeshashinto Extract is a yellow-brown to blackish brown, powder or viscous extract. It has a slightly

odor and a hotter, bitter and slightly sweet 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 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue. 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 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, 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 spot from the standard solution (Scutellaria Root).

(2) For preparation prescribed Processed Ginger—Shake 1.0 g of dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer 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]-shogaol 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 1 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (2:1) 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 spot from the standard solution (Processed Ginger).

(3) For preparation prescribed Ginger—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. Take the diethyl ether layer, evaporate the layer 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 spot from the standard solution (Ginger).

(4) 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 Ginsenoside Rg_1 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 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, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol 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 purple spot from the standard solution (Ginseng).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 5 mL of 1-buthanol, 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 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, 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 from the standard solution (Glycyrrhiza).

(6) Shake 0.5 g of the dry extract (or 1.5 g of the viscous extract) with 10 mL of methanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of coptisine chloride 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 ethyl acetate, ammonia solution (28) and methanol (15:1:1) 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 yellow fluorescent spot from the standard solution (Coptis Rhizome).

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 the 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 the 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 10.0%, calculated on the dried basis.

Assay (1) 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), and 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%.

(2) Glycyrrhizic 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 Glycyrrhizic 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 glycyrrhizic acid in each solution.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S \times 1/2$$

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 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 glycyrrhizic 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 glycyrrhizic 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 glycyrrhizic acid is not more than 1.5%.

(3) Berberine—Weigh accurately about 0.2 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.2 g of the dried substance), add exactly 50 mL of the mobile phase, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride RS (separately determine the water ⟨*2.48*⟩ in the same manner as Berberine Chloride Hydrate), dissolve in 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 areas, A_T and A_S, of berberine in each solution.

$$\text{Amount (mg) of berberine chloride } (C_{20}H_{18}ClNO_4) = M_S \times A_T/A_S \times 1/2$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 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 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: 1.0 mL per minute (the retention time of berberine is about 8 minutes).

System suitability—

System performance: Dissolve 1 mg each of Berberine Chloride RS and palmatine chloride in the mobile phase to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, palmatine and berberine 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 berberine is not more than 1.5%.

Containers and storage Containers—Tight containers.

Hemp Fruit

マシニン

Change the Identification as follows:

Identification To 0.3 g of pulverized Hemp Fruit add 3 mL of methanol, shake for 10 minutes, 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 hexane and ethyl acetate (9:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat at 105°C for 5 minutes: a dark blue-purple spot appears at an *R*f value of about 0.6.

Ipecac

トコン

Change the Description and Purity as follows:

Description Slender, curved, cylindrical root, 3 – 15 cm in length, 0.3 – 0.9 cm in diameter; mostly twisted, and sometimes branched; outer surface gray, dark grayish brown, red-brown in color and irregularly annulated; when root fractured, cortex easily separable from the xylem; the cortex on the fractured surface is grayish brown, and the xylem is light brown in color: thickness of cortex up to about two-thirds of radius in thickened portion. Scales in rhizome opposite.

Odor, slight; powder irritates the mucous membrane of the nose; taste, slightly bitter and unpleasant.

Under a microscope <*5.01*>, a transverse section of Ipecac reveals a cork layer, consisting of brown thin-walled cork cells; in the cortex, sclerenchyma cells are absent; in the xylem, vessels and tracheids arranged alternately; parenchyma cells filled with starch grains and sometimes with raphides of calcium oxalate.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of pulverized Ipecac 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 Ipecac according to Method 4, and perform the test (not more than 5 ppm).

Powdered Ipecac

トコン末

Change the Purity as follows:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of Powdered Ipecac 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 Powdered Ipecac according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <*5.01*>, groups of stone cells and sclerenchymatous fibers are not observed.

Japanese Angelica Root

トウキ

Change the Description as follows:

Description Thick and short main root, with numerous branched roots, nearly fusiform; 10 – 25 cm in length; externally dark brown to red-brown, with longitudinal wrinkles and horizontal protrusions composed of numerous scars of fine rootlets; fractured surface is dark brown to yellow-brown in color, and smooth; and with a little remains of leaf sheath at the crown.

Odor, characteristic; taste, slightly sweet, followed by slight pungency.

Under a microscope <*5.01*>, a transverse section reveals 4 to 10 layers of cork, with several layers of collenchyma inside of the layer; the cortex exhibits many oil canals surrounded by secretory cells and often large hollows appear; boundary of phloem and xylem is distinct; in the xylem, numerous vessels radiate alternately with medullary rays; vessels in the outer part of the xylem are singly or in several

groups, and disposed rather densely in a cuneiform pattern, but vessels in the region of the center are scattered very sparsely; starch grains are simple grains, not more than 20 μm in diameter, and rarely 2- to 5-compound grains, some times up to 25 μm in diameter; starch grains often gelatinized.

Powdered Japanese Angelica Root

トウキ末

Change the Description as follows:

Description Powdered Japanese Angelica Root occurs as a light grayish brown powder. It has a characteristic odor and a slight, sweet taste with a slightly pungent aftertaste.

Under a microscope <*5.01*>, Powdered Japanese Angelica Root reveals starch grains or masses of gelatinized starch, and fragments of parenchyma containing them; fragments of light yellow-brown cork tissue; fragments of rather thick-walled collenchyma and phloem tissue; fragments of oil canal surrounded by secretory cells; fragments, 20 – 60 μm in diameter, of scalariform and reticulate vessels with simple perforation; starch grains composed of simple grains not more than 20 μm in diameter, and rarely 2- to 5-compound grains, sometimes comes up to 25 μm.

Leonurus Herb

ヤクモソウ

Change the Identification as follows:

Identification To 1 g of pulverized Leonurus Herb add 10 mL of methanol, shake for 10 minutes, 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 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of water and methanol (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS followed by immediate spraying of sodium nitrite TS on the plate: a grayish brown spot appears at an *R*f value of about 0.5, which color fades soon and then disappears after air-drying the plate.

Lycium Bark

ジコッピ

Change the Description and Identification as follows:

Description Tubular to semitubular bark, 1 – 6 mm in thickness; externally light brown to light yellow-brown, periderm peeled easily as scale; internally grayish brown, longitudinally striate; brittle in texture; fractured surface, grayish white, not fibrous.

Odor, weak and characteristic; taste, slightly sweet at first.

Under a microscope <*5.01*>, a transverse section reveals periderm composed of a cork layer of several layers of thin walled cork cells; in cortex parenchyma cells containing sandy crystals of calcium oxalate sparsely distributed, occasionally a few fibers observed; parenchyma cells contain starch grains, 1 – 10 μm in diameter; stone cells very rare.

Identification To 1.0 g of pulverized Lycium Bark add 10 mL of methanol, shake for 15 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 1-butanol, ammonium acetate solution (1 in 20) and acetic acid (100) (2:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, heat at 105°C for 2 minutes, then spray evenly sodium nitrite TS, and allow to stand for 5 minutes: a dark brown principal spot appears at an *R*f value of about 0.4.

Magnolia Bark

コウボク

Change the Description and Identification as follows:

Description Plate-like or semi-tubular bark, 2 – 7 mm in thickness; externally grayish white to grayish brown, and rough, sometimes cork layer removed, and externally red-brown; internally light brown to dark purplish brown; cut surface extremely fibrous, and light red-brown to purplish brown.

Odor, slight; taste, bitter.

Under a microscope <*5.01*>, a transverse section reveals a thick cork layer or several thin cork layers, and internally adjoining the circular tissue of stone cells of approximately equal in diameter; primary cortex thin; fiber groups scattered in the pericycle; groups of phloem fibers lined alternately with the other tissue of phloem between medullary rays in the secondary cortex, and then these tissues show a latticework; oil cells scattered in the primary and secondary cortex, but sometimes observed in the narrow medullary rays.

Identification To 1.0 g of pulverized Magnolia Bark add 10 mL of methanol, stir for 10 minutes, 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 20 μL of the sample 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) (4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate: a yellow

spot appears at an *R*f value of about 0.3.

Powdered Magnolia Bark

コウボク末

Change the Identification as follows:

Identification To 1.0 g of Powdered Magnolia Bark add 10 mL of methanol, shake for 10 minutes, 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 20 μL of the sample 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) (4:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate: a yellow spot appears at an *R*f value of about 0.3.

Add the following:

Malt

Fructus Hordei Germinatus

バクガ

Malt is the dried ripe cariopsis of *Hordeum vulgare* Linné (*Gramineae*), after being germinated.

Description Oval caryopsis, 10 mm in length, 3 – 4 mm in width, furrowed on one surface; externally light yellow, sometimes with plumule at one end, with hairs and sometimes with roots at the other end; cross section of caryopsis white and powdery; easily broken and light in texture.

Odor, slight; taste, slightly sweet.

Under a microscope <*5.01*>, a transverse section of the caryopsis reveals glume, pericarp, seed coat and endosperm in this order from the outside; 2 – 4 layered aleurone layers on the circumference of endosperm; endosperm filled with starch grains; starch grains as spheroidal or ellipsoidal, large grains about 20 μm and small grains about 2 μm in diameter mixed together.

Identification To 3.0 g of pulverized Malt add 5 mL of methanol, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Liquid 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 methanol, water and acetic acid (100) (8:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly a solution of 0.1 g of 2,3-indolinedione in 50 mL of acetone 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.

Loss on drying <*5.01*> Not more than 11.0%.

Total ash <*5.01*> Not more than 2.6%.

Acid-insoluble ash <*5.01*> Not more than 0.8%.

Extract content <*5.01*> Dilute ethanol-soluble extract: Not less than 15.0%.

Containers and storage Containers—Well-closed containers.

Powdered Moutan Bark

ボタンピ末

Change the Description and Purity (3) as follows:

Description Powdered Moutan Bark occurs as a light grayish yellow-brown powder. It has a characteristic odor and a slight, pungent and bitter taste.

Under a microscope <*5.01*>, Powdered Moutan Bark reveals starch grains and fragments of parenchyma containing them; fragments of cork tissue containing tannin; fragments of somewhat thick-walled collenchyma, medullary rays, and phloem parenchyma; rosette aggregates of calcium oxalate and also fragments of parenchyma cells containing them. Starch grains are simple or 2- to 10-compound grains, 10 – 25 μm in diameter; rosette aggregates are 20 – 30 μm in diameter.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Purity

(3) Foreign matter—Under a microscope <*5.01*>, usually vessels and other sclerenchymatous cells are not observable.

Orengedokuto Extract

黄連解毒湯エキス

Change the Assay (2) as follows:

Assay (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 dried substance), add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, 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 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 exactly10 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.

Amount (mg) of baicalin ($C_{21}H_{18}O_{11}$) = $M_S \times A_T/A_S$

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%.

Panax Japonicus Rhizome

チクセツニンジン

Change the Identification as follows:

Identification Shake 0.5 g of pulverized Panax Japonicus Rhizome with 10 mL of methanol for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of chikusetsusaponin IV 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, water and formic acid (5:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 5 minutes: one of the spot among the several spots obtained from the sample solution shows the same color tone and *R*f value with the purple-red spot from the standard solution.

Powdered Panax Japonicus Rhizome

チクセツニンジン末

Change the Description and Identification as follows:

Description Powdered Panax Japonicus Rhizome occurs as a light grayish yellow-brown powder, and has a slight odor and a slightly bitter taste.

Under a microscope <*5.01*>, Powdered Panax Japonicus Rhizome reveals mainly starch grains or gelatinized starch masses, and fragments of parenchyma cells containing them; also fragments of cork tissue, somewhat thick-walled collenchyma, phloem tissue, and reticulate vessels; rarely fragments of scalariform vessels with a simple perforation, fibers and fiber bundles, rosette aggregates of calcium oxalate, and parenchyma cells containing them; yellow to orange-yellow resin; starch grains consisting of simple grains or 2- to 4-compound grains, simple grains, 3 – 18 μm in diameter; rosette aggregates of calcium oxalate are 20 – 60 μm in diameter.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Identification Shake 0.5 g of Powdered Panax Japonicus Rhizome with 10 mL of methanol for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of chikusetsusaponin IV 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, water and formic acid (5:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 5 minutes: one of the spot among the several spots obtained from the sample solution shows the same color tone and *R*f value with the purple-red spot from the standard solution.

Peach Kernel

トウニン

Change the Description as follows:

Description Flattened, asymmetric ovoid seed, 1.2 – 2.0 cm in length, 0.6 – 1.2 cm in width, and 0.3 – 0.7 cm in thickness; somewhat sharp at one end, and round at the other end with chalaza; seed coat red-brown to light brown; externally, its surface being powdery by easily detachable stone cells of epidermis; numerous vascular bundles running

and rarely branching from chalaza through the seed coat, and, appearing as dented longitudinal wrinkles; when soaked in boiling water and softened, the seed coat and thin, translucent, white albumen easily separated from the cotyledone; cotyledone white in color.

Almost odorless; taste, slightly bitter and oily.

Under a microscope <*5.01*>, the outer surface of seed coat reveals polygonal, long polygonal, or obtuse triangular stone cells on the protrusion from vascular bundles, shape of which considerably different according to the position, and their cell walls almost equally thickened; in lateral view, appearing as a square, rectangle or obtuse triangle.

Powdered Peach Kernel

トウニン末

Change the Description as follows:

Description Powdered Peach Kernel occurs as a reddish-light brown to light brown powder. It is almost odorless and is oily and has slightly a bitter taste.

Under a microscope <*5.01*>, Powdered Peach Kernel fragments of outer seed coat epidermis; elliptical to ovoid, containing yellow-brown compound 50 – 80 μm in diameter and stone cell; cap-like shape to ovoid, yellow-brown in color. The stone cell is element of epidermis, 50 – 80 μm in diameter and 70 – 80 μm in height, cell wall of the top, 12 – 25 μm thickness, the base 4 μm in thickness, with obvious and numerous pits. Inner seed coat, yellow-brown, irregular and somewhat long polygon, 15 – 30 μm in diameter; and fragments of cotyledon and albumen containing aleurone grains and fatted oil, Aleurone grains are almost spherical grains, 5 – 10 μm in diameter.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Peucedanum Root

ゼンコ

Change the Description and Identification as follows:

Description **1)** *Peucedanum praeruptorum* Dunn—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; cross 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* Franchet et Savatier—Approximate to 1), but without hair-like remains of petioles at the crown.

Under a microscope <*5.01*>, a transverse section reveals, approximate 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* Dunn—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 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the blue-purple fluorescent spot from the standard solution.

2) *Angelica decursiva* Franchet et Savatier—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 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the purple fluorescent spot from the standard solution.

Pharbitis Seed

ケンゴシ

Change the Description as follows:

Description Longitudinally quartered or sexpartite globe, 4 – 6 mm in length, 3 – 5 mm in width; externally black to grayish red-brown or grayish white, smooth, but slightly shrunken and coarsely wrinkled. The transverse section

almost fan-shaped, light yellow-brown to light grayish brown, and dense in texture. Under a magnifying glass, the surface of the seed coat reveals dense, short hairs; dented hilum at the bottom of the ridge. Seed coat thin, the outer layer dark gray, and the inner layer light gray; two irregularly folded cotyledons in the transverse section at one end; two thin membranes from the center of the dorsal side to the ridge separating cotyledons but unrecognizable in the transverse section of the other end having hilum; dark gray secretory pits in the section of the cotyledon. 100 seeds weighing about 3.5 g.

When cracked, odor, slight; taste, oily and slightly pungent.

Phellodendron Bark

オウバク

Change the Description and Identification (2) 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 lenticel; 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 outer portion of cortex thin, with yellow scattered stone cells; inner portion of cortex thick; primary rays expanding its width towards the outer end, the phloem appearing as a nearly triangular part between these rays in secondary cortex, and many secondary rays gathering to the tip of the triangle; groups of phloem fibers light yellow to yellow, lined alternately with the other tissue of phloem between rays, and then these tissues show obviously a latticework.

Identification

(2) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 1 mg of Berberine Chloride 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 1-butanol, water and acetic acid (100) (7:2:1) 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 from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same *R*f value.

Powdered Phellodendron Bark

オウバク末

Change the Description and Identification (2) as follows:

Description Powdered Phellodendron Bark occurs as a bright yellow to yellow powder. It has a slight odor and an extremely bitter taste, is mucilaginous, and colors the saliva yellow on chewing.

Under a microscope <*5.01*>, Powdered Phellodendron Bark reveals fragments of yellow, thick-walled fiber bundles or fibers, and fibers often accompanied by crystal cell rows; fewer groups of stone cells together with idioblasts; fragments of parenchyma cells containing starch grains and oil droplets; fragments of medullary ray and phloem; mucilage cells and mucilage masses. Numerous solitary crystals of calcium oxalate, 7 – 20 μm in diameter; starch grains, simple grains and 2- to 4-compound grains, simple grain, 2 – 6 μm in diameter; oil droplets, stained red with sudan III TS.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Identification

(2) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 1 mg of Berberine Chloride 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 1-butanol, water and acetic acid (100) (7:2:1) 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 from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same *R*f value.

Picrasma Wood

ニガキ

Change the Description as follows:

Description Light yellow chips, slices or short pieces of wood; a transverse section reveals distinct annual rings and thin medullary rays; tissue dense in texture.

Odorless; taste, extremely bitter and lasting.

Under a microscope <*5.01*>, it reveals medullary rays consisting of 1 – 5 cells wide for transverse section, and 5 – 50 cells high for longitudinal section; vessels of spring wood up to about 150 μm in diameter, but those of autumn wood only one-fifth as wide; vessels, single or in groups, scattered in the xylem parenchyma; wall of wood fibers extremely thickened; medullary rays and xylem parenchyma cells con-

tain rosette aggregates of calcium oxalate and starch grains. Vivid yellow or red-brown, resinous substance often present in the vessels.

Plantago Herb

シャゼンソウ

Change the Identification as follows:

Identification To 2.0 g of pulverized Plantago Herb add 10 mL of methanol, warm on a water bath for 3 minutes, cool, 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 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride TS on the plate: a dark blue spot appears at an *R*f value of about 0.55.

Polygala Root

オンジ

Change the origin/limits of content as follows:

Polygala Root is the root or the root bark of *Polygala tenuifolia* Willdenow (*Polygalaceae*).

Powdered Polygala Root

オンジ末

Change the Description as follows:

Description Powdered Polygala Root occurs as a light grayish yellow-brown powder. It has a slight odor and a slightly acrid taste.

Under a microscope <*5.01*>, Powdered Polygala Root reveals fragments of cork layers, pitted vessels, reticulate vessels and tracheids; fragments of xylem fibers and xylem parenchyma cells with a small number of simple pits; fragments of parenchyma cells containing substances such as oil droplets, rosette aggregates and solitary crystals of calcium oxalate. Oil drop-like contents stained red with sudan III TS.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Polygonum Root

カシュウ

Change the Identification as follows:

Identification To 1 g of pulverized Polygonum Root add 10 mL of methanol, shake for 15 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 2 mL of methanol, and use this 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 ethyl acetate, water, methanol and acetic acid (100) (200:10:10:3) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a fluorescent bluish white spot appears at an *R*f value of about 0.3.

Processed Ginger

カンキョウ

Change the origin/limits of content, Description and Identification as follows:

Processed Ginger is the rhizome of *Zingiber officinale* Roscoe (*Zingiberaceae*), after being passed through hot water or being steamed.

It contains not less than 0.10% of [6]-shogaol ($C_{17}H_{24}O_3$: 276.37), calculated on the basis of dried material.

Description Irregularly compressed and often branched massive rhizome; branched parts slightly curved ovoid or oblong- ovoid, 2 – 4 cm in length, and 1 – 2 cm in diameter; external surface grayish yellow to grayish yellow-brown, with wrinkles and ring node; fractured surface brown to dark brown, transparent and horny; under a magnifying glass, a transverse section reveals cortex and stele distinctly divided; vascular bundles scattered throughout the surface.

Odor, characteristic; taste, extremely pungent.

Under a microscope <*5.01*>, a transverse section reveals cork layer, cortex and stele in this order from the outside; cortex and stele, divided by a single-layered endodermis, composed of parenchyma; vascular bundles surrounded by fibers scattered in cortex and stele; oil cells contain yellow oily substances, scattered in parenchyma; parenchyma cells contain solitary crystals of calcium oxalate, and gelatinized starch.

Identification To 2 g of pulverized Processed Ginger add 5 mL of diethyl ether, shake for 10 minutes, filter, and use the filtrate as the sample solution (1). To the residue add 5 mL of methanol, proceed in the same manner as above, and use so obtained solution as the sample solution (2). Separately, dissolve 1 mg of [6]-shogaol for thin-layer chromatography

in 2 mL of methanol, and use this solution as the standard solution (1). Separately, dissolve 1 mg of sucrose 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 (1) and standard solution (1) 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 from the sample solution (1) has the same color tone and *R*f value with the spot from the standard solution (1). Spot 10 μL each of the sample solution (2) and standard solution (2) on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (8:5:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution (2) has the same color tone and *R*f value with the spot from the standard solution (2).

Add the following next to the Extract content:

Assay Weigh accurately about 1 g of pulverized Processed Ginger, place in a centrifuge tube, add 30 mL of the mobile phase, shake for 20 minutes, centrifuge, and separate the supernatant liquid. To the residue add 30 mL of the mobile phase, and repeat the extraction twice more. To the combined all extracts add the mobile phase to make exactly 100 mL, use this solution as the sample solution. Separately, weigh accurately about 5 mg of [6]-shogaol for assay, dissolve in 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 areas, A_T and A_S, of [6]-shogaol.

$$\text{Amount (mg) of [6]-shogaol} = M_S \times A_T/A_S$$

M_S: Amount (mg) of [6]-shogaol for assay

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 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 acetonitrile and water (3:2).

Flow rate: Adjust the flow rate so that the retenton time of [6]-shogaol 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, the number of theoretical plates and the symmetry factor of the peak of [6]-shogaol 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 [6]-shogaol is not more than 1.5%.

Pueraria Root

カッコン

Change the Identification as follows:

Identification To 2 g of pulverized Pueraria Root add 10 mL of methanol, shake for 3 minutes, filter, and use the filtrate 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 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 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 from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution.

Red Ginseng

コウジン

Change the Identification (2) as follows:

Identification

(2) To 2.0 g of pulverized Red Ginseng add 10 mL of water and 10 mL of 1-butanol, shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ginsenoside Rg_1 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, methanol and water (14:5:4) to a distance of about 7 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying 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 spot from the standard solution.

Rehmannia Root

ジオウ

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

Rehmannia Root is the root of *Rehmannia glutinosa* Liboschitz var. *purpurea* Makino or *Rehmania glutinosa* Liboschitz (*Scrophulariaceae*), with the application of steaming (prepared one: Juku-jio) or without it (non-prepared one: Kan-jio).

Description **1)** Kan-jio—Massive or fusiform root, narrow at one or both ends, 5 – 10 cm in length, 0.5 – 3.0 cm in diameter, sometimes broken or markedly deformed in shape; externally yellow-brown, blackish brown or black, with deep, longitudinal wrinkles and constrictions; soft in texture; transversely cut surface yellow-brown, blackish brown, or black and peripheral portion darker.

Odor, characteristic; taste, slightly sweet at first, followed by a slight bitterness.

Under a microscope <*5.01*>, a transverse section reveals 7 – 15 layers of cork; cortex composed entirely of parenchyma; cells containing brown secretes scattered in cortex; xylem practically filled with parenchyma; vessels radially lined, mainly reticulate vessels.

2) Juku-jio—Irregularly massive root, or massive or fusiform root, narrow at one or both ends, 5 – 10 cm in length, 0.5 – 3.0 cm in diameter; externally black, usually lustrous, with deep, longitudinal wrinkles and constrictions; soft in texture and mucous; transversely cut surface black.

Odor, characteristic; taste, sweet at first, followed by a slight bitterness.

Under a microscope <*5.01*>, a transverse section reveals 7 – 15 layers of cork; cortex composed entirely of parenchyma; cells containing brown secretes scattered in cortex; xylem practically filled with parenchyma, often parenchyma partially broken and gaps observed; vessels radially lined, mainly reticulate vessels.

Add the following next to the Description:

Identification **1)** Kan-jio—Sake 0.5 g of the fine cutting of Rehmannia Root with 5 mL of water, add 20 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of stachyose for thin-layer chromatography in 1 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 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 2-propanol, water and methanol (3:2:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS on the plate, 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 from the standard solution. When further heat for more than 5 minutes, a blue spot is not observed at just lower than the spot mentioned above, or even appears it is only few.

2) Juku-jio—Sake 0.5 g of the fine cutting of Rehmannia Root with 5 mL of water, add 20 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of fructose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the standard solution (1). Separately, dissolve 3 mg of manninotriose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, water and methanol (3:2:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS on the plate, heat at 105°C for 10 minutes: the principal spot obtained from the sample solution has the same color tone and *R*f value with the spot from the standard solution (1). Furthermore, one of the spot from the several spots obtained from the sample solution has the same color tone and *R*f value with the blue spot from the standard solution (2).

Powdered Rose Fruit

エイジツ末

Change the Description as follows:

Description Powdered Rose Fruit occurs as a grayish yellow-brown powder. It has a slight odor, and has a slightly mucilaginous, astringent, bitter, and slightly acid taste.

Under a microscope <*5.01*>, Powdered Rose Fruit reveals fragments of extremely thick-walled hairs 35 – 70 μm in diameter, fragments of epidermis and hypodermis containing brown tannin masses, fragments of thin-walled fundamental tissue containing grayish brown substances, fragments of fine vessels, and solitary or twin crystals or rosette agregates of calcium oxalate (components of receptacle); fragments of sclerenchyma, fiber groups, fine vessels, and fragments of epidermis containing brown tannin and mucilage (components of pericarp); fragments of endosperm composed of polygonal cells containing aleuron grains and fatty oil, fragments of outer epidermis composed of polygonal cells containing tannin, and fragments of inner epidermis composed of elongated cells having wavy lateral walls (components of seed).

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Saireito Extract

柴苓湯エキス

Change the Assay (2) as follows:

Assay

(2) Baicalin—Weigh accurately about 0.1 g of Saireito Extract, 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), and 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 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%.

Schizonepeta Spike

ケイガイ

Change the Identification as follows:

Identification To 1 g of pulverized Schizonepeta Spike add 10 mL of ethyl acetate, shake for 15 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 ethyl acetate (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 5 minutes. After cooling for more than 10 minutes under an adequate humidity, examine under ultraviolet light (main wavelength: 365 nm): two spots, one is a bluish fluorescent spot with an *R*f value of about 0.5 and the another is a yellowish fluorescent spot with an *R*f value of about 0.1, are observed.

Scutellaria Root

オウゴン

Change the Description, Identification (2) and Assay as follows:

Description Cone-shaped, cylindrical, semitubular or flattened root, 5 – 20 cm in length, 0.5 – 3 cm in diameter; externally yellow-brown, with coarse and marked longitudinal wrinkles, and with scattered scars of lateral root and remains of brown periderm; scars of stem or remains of stem at the crown; sometimes central portion of xylem rotted, often forming a hollow; hard in texture and easily broken; fractured surface fibrous and yellow in color.

Almost odorless; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section reveals 6 – 20 layered cork remaining, cortex composed of parenchyma, sclerencyma cells scattered in cortex; xylem composed of parenchyma, vessels and small amount of xylem fibers observed in xylem; vessels usually in groups and arranged in tangential direction, radial direction or in irregular form; in case where central portion of xylem rotted, cork layer observed around hollow; parenchyma cells of cortex and xylem contain simple and compound starch grains.

Identification

(2) To 1 g of pulverized Scutellaria Root add 25 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Baicalin 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 1-butanol, water and acetic acid (100) (4:2:1) 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 from the sample solution has the same color tone and *R*f value with the dark green spot from the standard solution.

Assay Weigh accurately about 0.5 g of pulverized Scutellaria Root, add 30 mL of diluted methanol (7 in 10), heat un-

der 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 the extract, 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 exactly100 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. 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 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 Description, Identification (2) and Assay as follows:

Description Powdered Scutellaria Root occurs as a yellow-brown powder. It is almost odorless, and has a slight, bitter taste.

Under a microscope <*5.01*>, Powdered Scutellaria Root reveals fragments of parenchyma cells containing small amount of simple and compound starch grains, fragments of short reticulate vessel elements and fusiform, stick-like and ellipsoidal to spherical sclerenchyma cells; also a few fragments of spiral vessels and xylem fibers are observed.

Identification

(2) To 1 g of Powdered Scutellaria Root add 25 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Baicalin 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 1-butanol, water and acetic acid (100) (4:2:1) 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 from the sample solution has the same color tone and *R*f value with the dark green spot from the standard solution.

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 the extract, 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 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. 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 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 Senega

セネガ末

Change the Description as follows:

Description Powdered Senega occurs as a light brown powder, and has a characteristic odor resembling the aroma of methyl salicylate; taste, sweet at first, but later acrid.

Under a microscope <*5.01*>, Powdered Senega reveals fragments of pitted vessels, reticulate vessels and tracheids; fragments of xylem fibers with oblique pits; fragments of xylem parenchyma cells with simple pits; fragments of phloem parenchyma containing oily droplets; fragments of exodermis often composed of cells suberized and divided into daughter cells; oily droplets stained red by sudan III TS. The parenchyma cells of Powdered Senega do not contain starch grains and crystals of calcium oxalate.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Senna Leaf

センナ

Change the Description as follows:

Description Lanceolate to narrowly lanceolate leaflets, 1.5 – 5 cm in length, 0.5 – 1.5 cm in width, light grayish yellow to light grayish yellow-green in color; margin entire, apex acute, base asymmetric, petiole short; under a magnifying glass, vein marked, primary lateral veins running toward the apex along the margin and joining the lateral vein above; lower surface having slight hairs.

Odor slight; taste, bitter.

Under a microscope <*5.01*>, a transverse section of Senna Leaf reveals epidermis with thick cuticle, with numerous stomata, and with thick-walled, warty unicellular hairs; epidermal cells are often separated into two loculi by a septum which is in parallel with the surface of the leaf, and contain mucilage in the inner loculus; palisade of a single layer under each epidermis; spongy tissue, consisting of 3 to 4 layers, and containing clustered or solitary crystals of calcium oxalate; cells adjacent to vascular bundle, forming crystal cell rows.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Powdered Senna Leaf

センナ末

Change the Description as follows:

Description Powdered Senna Leaf occurs as a light yellow to light grayish yellow-green powder. It has a slight odor and a bitter taste.

Under a microscope <*5.01*>, Powdered Senna Leaf reveals fragments of vessels and vein tissue accompanied with crystal cell rows; fragments of thick-walled, bent, unicellular hairs; fragments of palisade and spongy tissue; clustered and solitary crystals of calcium oxalate, 10 – 20 μm in diameter.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Shosaikoto Extract

小柴胡湯エキス

Change the Assay (2) as follows:

Assay

(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 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), and 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%.

Sinomenium Stem and Rhizome

ボウイ

Change the Description as follows:

Description Round or elliptic sections, 0.2 – 0.4 cm in thickness, 1 – 4.5 cm in diameter; cortex on both fractured surfaces, light brown to dark brown; in xylem, grayish brown vessel portions and dark brown medullary rays lined alternately and radially; flank, dark gray, with longitudinal wrinkles and warty protrusions.

Almost odorless; taste, bitter.

Under a microscope <*5.01*>, a transverse section reveals extremely thick-walled stone cells in primary cortex and pericycle; irregular-sized vessels lined nearly stepwise in the vessel portion; cells of medullary ray mostly not lignified, and extremely thick-walled and large stone cells scattered here and there; primary cortex containing needle crystals of calcium oxalate; medullary rays containing starch gains, mainly simple grain, 3 – 20 μm in diameter, and small needle crystals of calcium oxalate.

Smilax Rhizome

サンキライ

Change the Description as follows:

Description Flattened and irregular cylindrical tuber, often with node-like branches; usually 5 – 15 cm in length, 2 – 5 cm in diameter; the outer surface grayish yellow-brown to yellow-brown, and the upper surface scattered with knotty remains of stem; transverse section irregular elliptical to obtuse triangular, consisting of extremely thin cortical layer and mostly of stele.

Odor, slight; almost tasteless.

Under a microscope <*5.01*>, a transverse section reveals a 2- to 3-cell-wide cork layer, with extremely narrow cortical layer, usually consisting of a 2- to 4-cell-wide, thick-walled parenchyma cells, showing large mucilage cells here and there; mucilage cell containing raphides of calcium oxalate; stele consisting chiefly of parenchyma cells, and scattered with vascular bundles; parenchyma cells containing starch grains composed mostly of simple grains, 12 – 36 μm in diameter, and sometimes mixed with 2- to 4-compound grains.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Powdered Smilax Rhizome

サンキライ末

Change the Purity (3) as follows:

Purity

(3) Foreign matter—Under a microscope <*5.01*>, Powdered Smilax Rhizome does not show a large quantity of stone cells or thick-walled fibers.

[Note: This item has been revised in the Japanese edition, but the revision does not require any change to the English text stated in the English edition.]

Sweet Hydrangea Leaf

アマチャ

Change the origin/limits of content as follows:

Sweet Hydrangea Leaf is the leaf and twig of *Hydrangea macrophylla* Seringe var. *thunbergii* Makino (*Saxifragaceae*), usually crumpled.

Powdered Sweet Hydrangea Leaf

アマチャ末

Change the Description as follows:

Description Powdered Sweet Hydrangea Leaf occurs as a dark yellow-green powder, and has a faint odor and a characteristic, sweet taste.

Under a microscope <*5.01*>, Powdered Sweet Hydrangea Leaf reveals fragments of epidermis with wavy lateral cell wall; stomata with two subsidiary cells; unicellular and thin-walled hair with numerous protrusions of the surface, 150 – 300 μm in length; fragments of palisade tissue and spongy tissue; fragments of vascular bundle and mucilage cells containing raphides of calcium oxalate 50 – 70 μm in length.

Swertia Herb

センブリ

Change the Description as follows:

Description Herb, 10 – 50 cm in length, having flowers, opposite leaves, stems, and, usually, with short, lignified roots; stems square, about 2 mm in diameter, often with branches; the leaves and stems dark green to dark purple or yellow-brown in color; the flowers white to whitish, and the roots yellowbrown. When smoothed by immersing in water, leaves, linear or narrow lanceolate, 1 – 4 cm in length, 0.1 – 0.5 cm in width, entire, and sessile; corolla split deeply as five lobes; the lobes narrow, elongated ellipse shape, and under a magnifying glass, with two elliptical nectaries juxtaposed at the base of the inner surface; the margin of lobe resembles eyelashes; the five stamens grow on the tube of the corolla and stand alternately in a row with corolla-lobes; peduncle distinct.

Odor, slight; taste, extremely bitter and persisting.

Toad Venom

Bufonis Venenum

センソ

Change the title of the monograph, the Latin name and the origin/limits of content as follows, and replace the term "Toad Venom" used anywhere in the Pharmacopoeia with "Toad Cake".

Toad Cake

Bufonis Crustum

センソ

Toad Cake is the parotoid secretion of *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider (*Bufonidae*).

When dried, it contains not less than 5.8% of bufo steroid.

Add the following:

Tokishakuyakusan Extract

当帰芍薬散エキス

Tokishakuyakusan Extract contains not less than 0.6 mg and not more than 2.4 mg of (*E*)-ferulic acid, not less than 34 mg and not more than 102 mg (for preparation prescribed 4 g of Peony Root) or not less than 51 mg and not more than153 mg (for preparation prescribed 6 g of Peony Root) of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 0.4 mg of atractylenolide III (for preparation prescribed Atractylodes Rhizome) or not less than 0.1 mg of atractylodin (for preparation prescribed Atractylodes Lancea Rhizome), per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)
Japanese Angelica Root	3 g	3 g	3 g	3 g
Cnidium Rhizome	3 g	3 g	3 g	3 g
Peony Root	6 g	6 g	4 g	4 g
Poria Sclerotium	4 g	4 g	4 g	4 g
Atractylodes Rhizome	4 g	4 g	4 g	—
Atractylodes Lancea Rhizome	—	—	—	4 g
Alisma Phizome	4 g	5 g	4 g	4 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the preparation 1) to 4), using the crude drugs shown above.

Description Tokishakuyakusan Extract is a light brown to blackish brown, powder or viscous extract. It has a characteristic odor, and a slight sweet taste at first and a bitter taste later.

Identification **(1)** 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, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer 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 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution (Japanese Angelica Root; Cnidium Rhizome).

(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-buthanol, 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 water (20:3:2) 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 from the sample solution has the same color tone and *R*f value with the purple spot from the standard solution (Peony Root).

(3) (For preparation prescribed Atractylodes Rhizome) 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, shake, and take the diethyl ether layer. Evaporate the layer 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 atractylenolide III 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 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 hexane (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 from the sample solution has the same color tone and *R*f value with the bluish white fluorescent spot from the standard solution (Atractylodes Rhizome).

(4) (For preparation prescribed Atractylodes Lancea Rhizome) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 0.5 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 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 hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot observed at an *R*f value of about 0.4. The spot shows greenish brown color after splaying evenly 4-dimethylaminobenzaldehyde TS for splaying, heating at 105°C for 5 minutes and allowing to cool (Atractylodes Lancea Rhizome).

(5) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium carbonate TS, add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of alisol 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 ethyl acetate, hexane and acetic acid (100) (10:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid TS 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 from the sample solution has the same color tone and *R*f value with the yellowish fluorescent spot from the standard solution (Alisma Rhizome).

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 the 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 the 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 10.0%, calculated on the dried basis.

Assay (1) (*E*)-Ferulic acid—Conduct this procedure without exposure to light using light-resistant vessels. 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 (*E*)-ferulic acid for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 2 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_T and A_S, of (*E*)-ferulic acid in each solution.

$$\text{Amount (mg) of (}E\text{)-ferulic acid} = M_S \times A_T/A_S \times 1/50$$

M_S: Amount (mg) of (*E*)-ferulic acid for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 320 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 7.8 g of sodium dihydrogen phosphate in 1000 mL of water, and add 2 mL of phosphoric acid. To 850 mL of this solution add 150 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of (*E*)-ferulic acid 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 (*E*)-ferulic 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 (*E*)-ferulic acid 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, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin 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 paeoniflorin in each solution.

$$\text{Amount (mg) of paeoniflorin } (C_{23}H_{28}O_{11}) = M_S \times A_T/A_S \times 1/2$$

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 of albiflorin in 10 mL of the standard solution. 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) Atractylenolide III—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 atractylenolide III for assay, previously dried in a desiccator (silica gel) for more than 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 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 atractylenolide III in each solution.

$$\text{Amount (mg) of atractylenolide III} = M_S \times A_T/A_S \times 1/40$$

M_S: Amount (mg) of atractylenolide III 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 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (550:450:1).

Flow rate: 1.0 mL per minute (the retention time of atractylenolide III 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 atractylenolide III 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 atractylenolide III is not more than 1.5%.

(4) Atractylodin—Conduct this procedure without exposure to light using light-resistant vessels. 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 methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with exactly 10 μL each of the sample solution and atractylodin TS for assay as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of atractylodin in each solution.

$$\text{Amount (mg) of atractylodin} = C_S \times A_T/A_S \times 50$$

C_S: Concentration (mg/mL) of atractylodin in atractylodin TS for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 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 330 mL of a mixture of water and phosphoric acid (55:1) add 670 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of atractylodin is about 13 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of atractylodin TS for assay under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atractylodin 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 atractylodin TS for assay under the above operating conditions, the relative standard deviation of the peak area of atractylodin is not more than 1.5%.

Containers and storage Containers—Tight containers.

Turmeric

ウコン

Change the Identification (1) as follows:

Identification (1) To 0.5 g of pulverized Turmeric, add 20 mL of methanol, shake for 15 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 ethyl acetate, hexane and acetic acid (100) (11:9:1) to a distance about 7 cm, and air-dry the plate: a yellow spot appears at an *R*f value of about 0.4.

Powdered Turmeric

ウコン末

Change the Identification (1) as follows:

Identification (1) To 0.5 g of Powdered Turmeric, add 20 mL of methanol, shake for 15 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 ethyl acetate, hexane and acetic acid (100) (11:9:1) to a distance about 7 cm, and air-dry the plate: a yellow spot appears at an *R*f value of about 0.4.

Uncaria Hook

チョウトウコウ

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

Uncaria Hook is, hook or the hook-bearing stem of *Uncaria rhynchophylla* Miquel, *Uncaria sinensis* Haviland or *Uncaria macrophylla* Wallich (*Rubiaceae*), sometimes after being passed through hot water or steamed.

Uncaria Hook contains not less than 0.03% of total alkaloids (rhynchophylline and hirstine), calculated on the dried basis.

Description Uncaria Hook is uncinate hook or short stem with opposite or single hook; the hook, 1 – 4 cm in length, curved and acuminate; externally red-brown to dark brown or grayish brown, some one with hairs, the transverse section oblong to elliptical, light brown; stem thin and prismatic square to cylindrical, 2 – 5 mm in diameter, externally, red-brown to dark brown or grayish brown; the transverse section, square to elliptical; the pith light brown, square to

elliptical; hard in texture.

Odorless and practically tasteless.

Under a microscope <*5.01*>, a transverse section of the hook reveals vascular bundles in the cortex, unevenly distributed and arranged in a ring. Parenchyma cells in the secondary cortex containing sand crystals of calcium oxalate.

Zanthoxylum Fruit

サンショウ

Change the Identification as follows:

Identification To 2 g of pulverized Zanthoxylum Fruit add 10 mL of water, shake for 5 minutes, add 5 mL of diethyl ether, 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 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 ethyl acetate, hexane, methanol and acetic acid (100) (20:20:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot with an *R*f value of about 0.3 is observed.

Powdered Zanthoxylum Fruit

サンショウ末

Change the Description and Identification as follows:

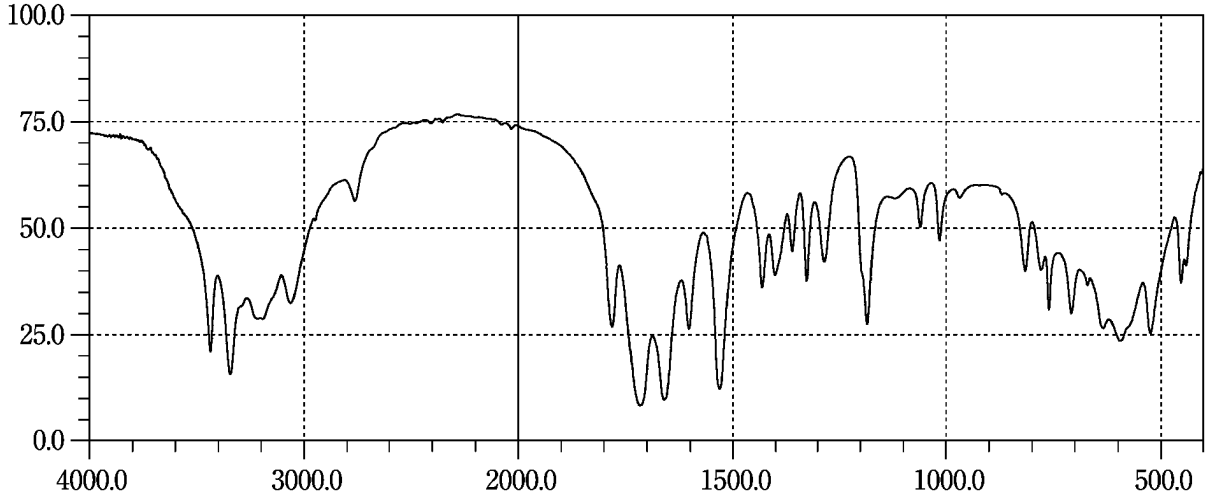
Description Powdered Zanthoxylum Fruit occurs as a dark yellow-brown powder. It has a strong, characteristic aroma and an acrid taste leaving a sensation of numbness on the tongue.

Under a microscope <*5.01*>, Powdered Zanthoxylum Fruit reveals fragments of inner tissue of pericarp consisting of stone cells with cell walls about 2.5 μm in thickness; fragments of spiral and ring vessels 10 – 15 μm in diameter; fragments of oil sacs containing essential oil or resin; fragments of epidermal cells, polygonal in surface view, containing tannin; numerous oil drops; masses of tannin, colored red by adding vanillin-hydrochloric acid TS.

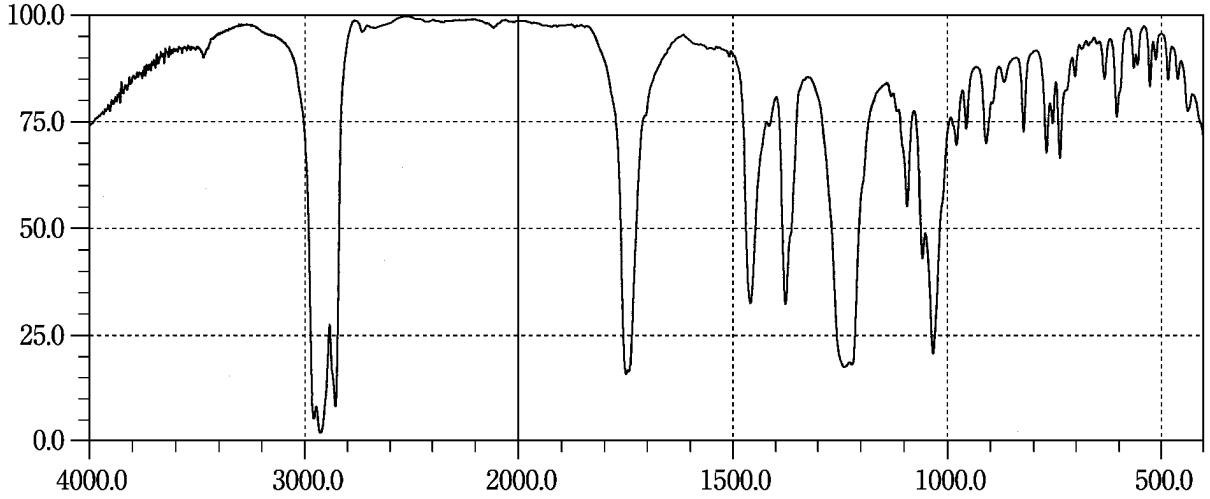
Identification To 2 g of Powdered Zanthoxylum Fruit add 10 mL of water, shake for 5 minutes, add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol and acetic acid (100) (20:20:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot with an *R*f value of about 0.3 is observed.

Add the following 29 spectra:

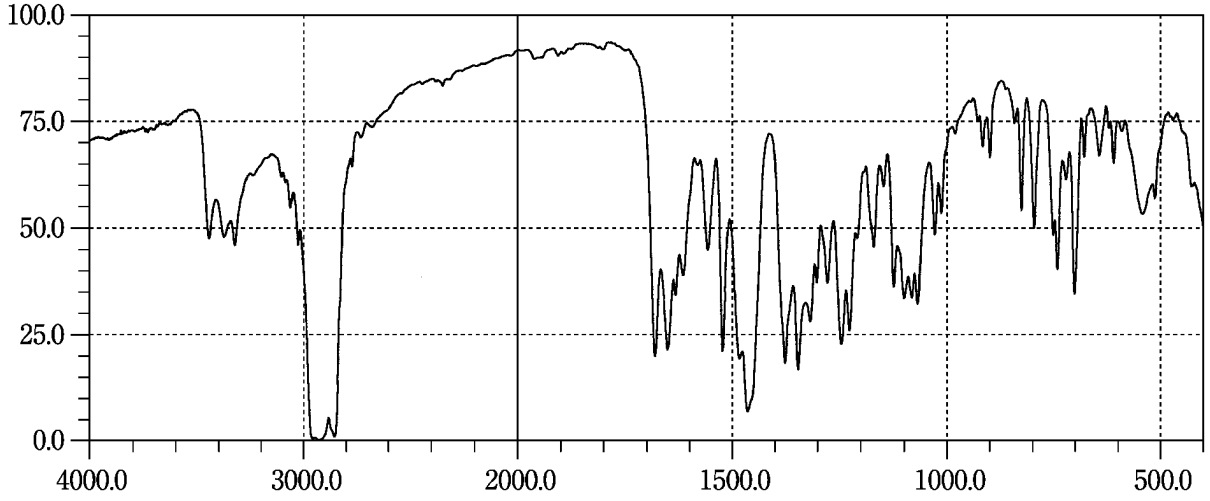
Aldioxa



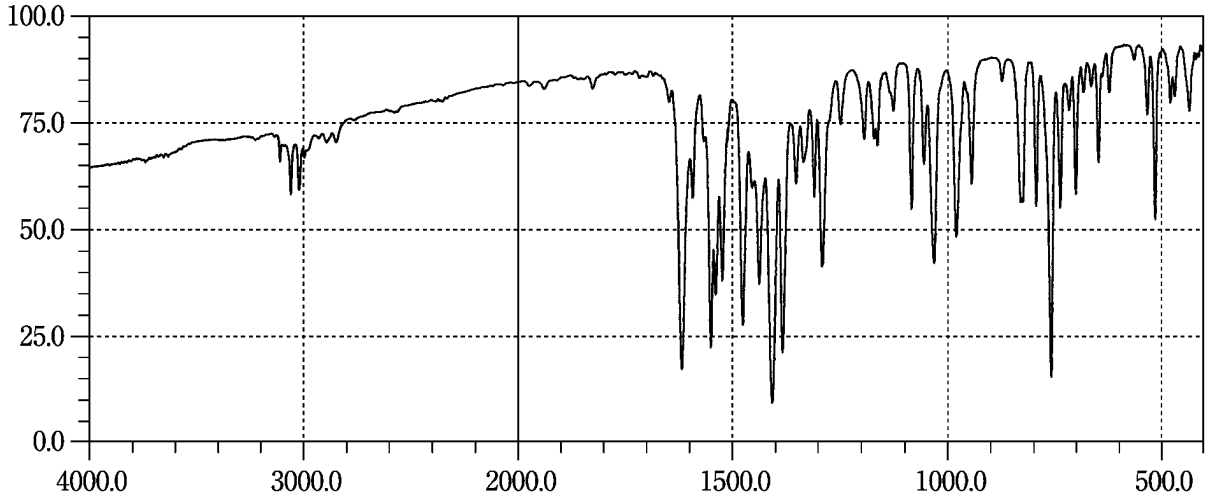
Auranofin



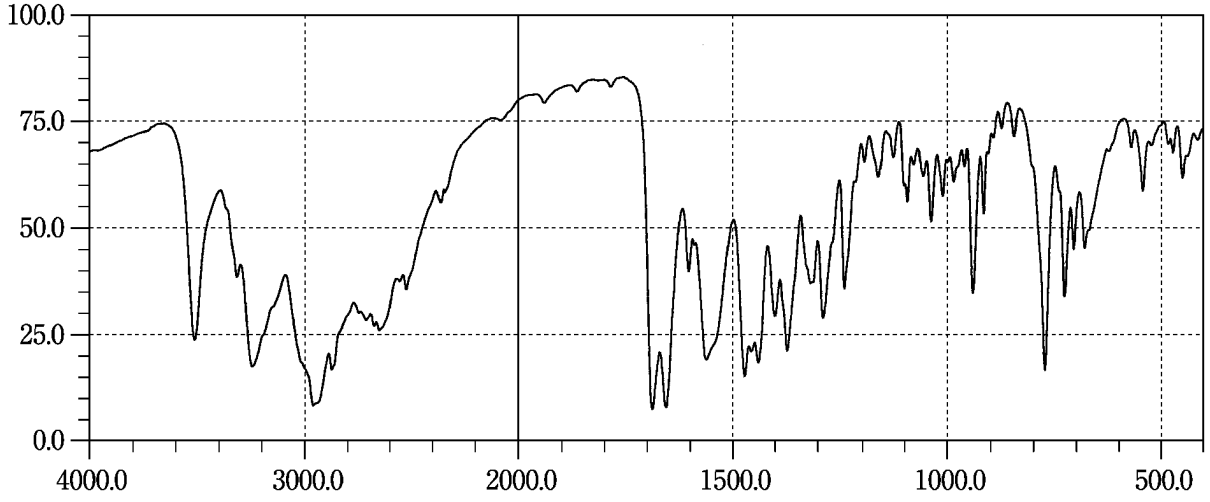
Azelnidipine



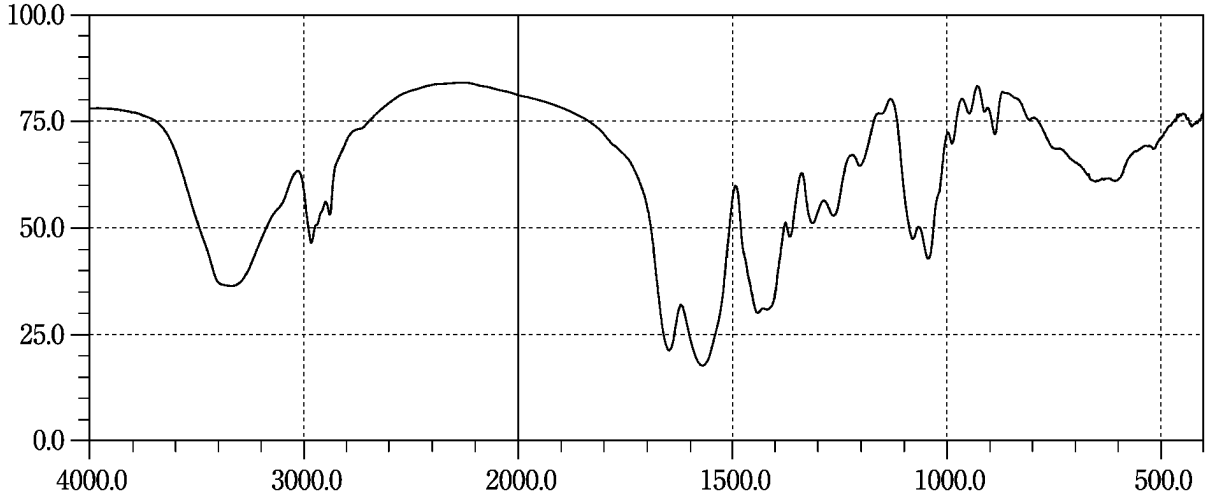
Brotizolam



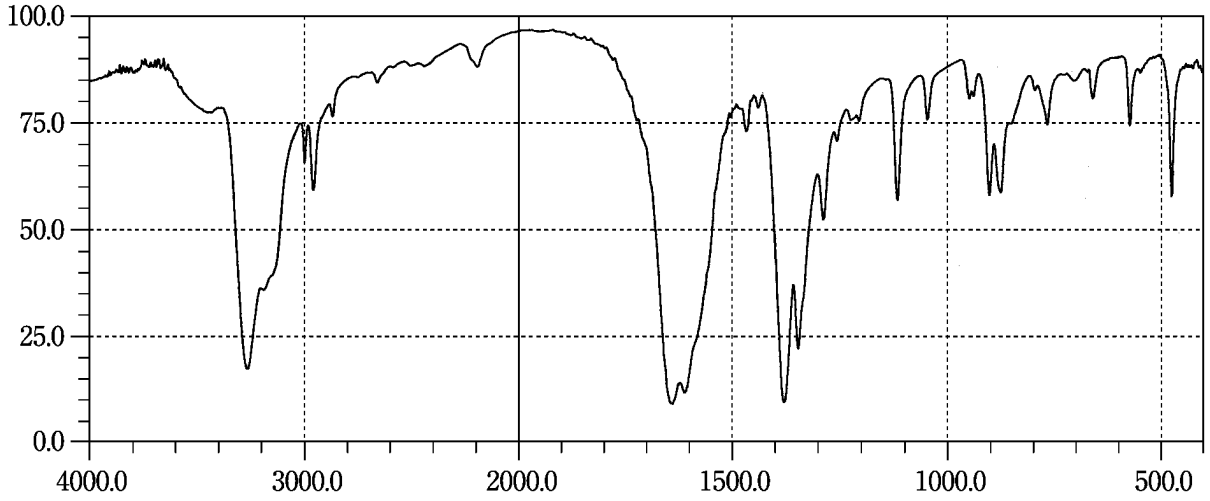
Bupivacaine Hydrochloride Hydrate



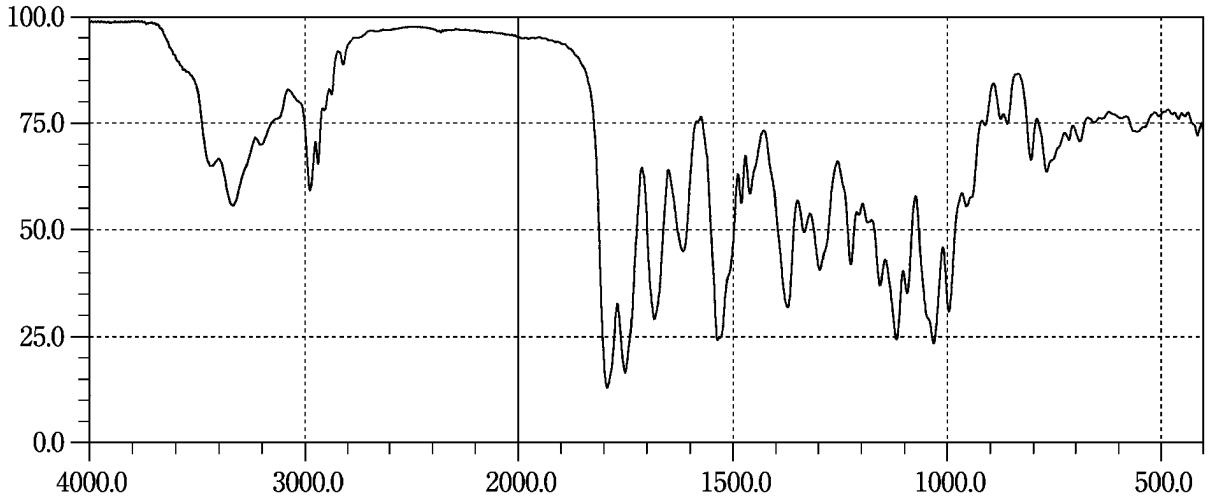
Calcium Pantothenate



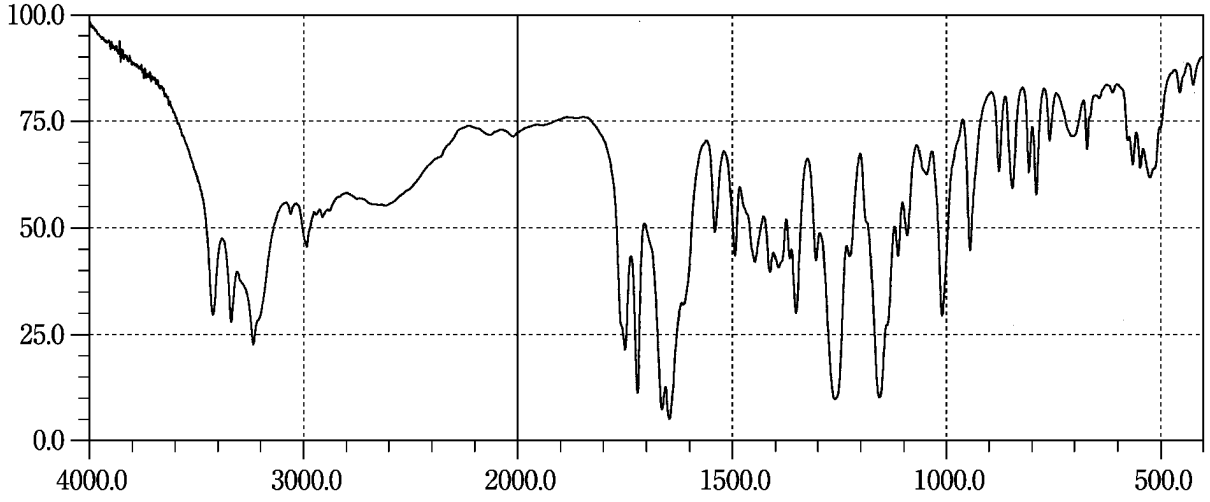
Carboplatin



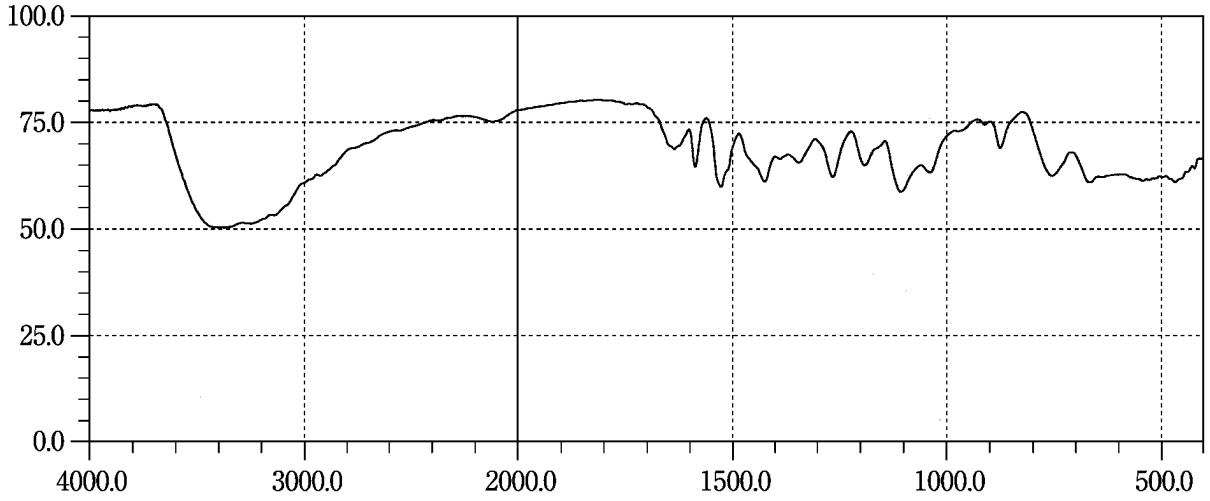
Cefteram Pivoxil



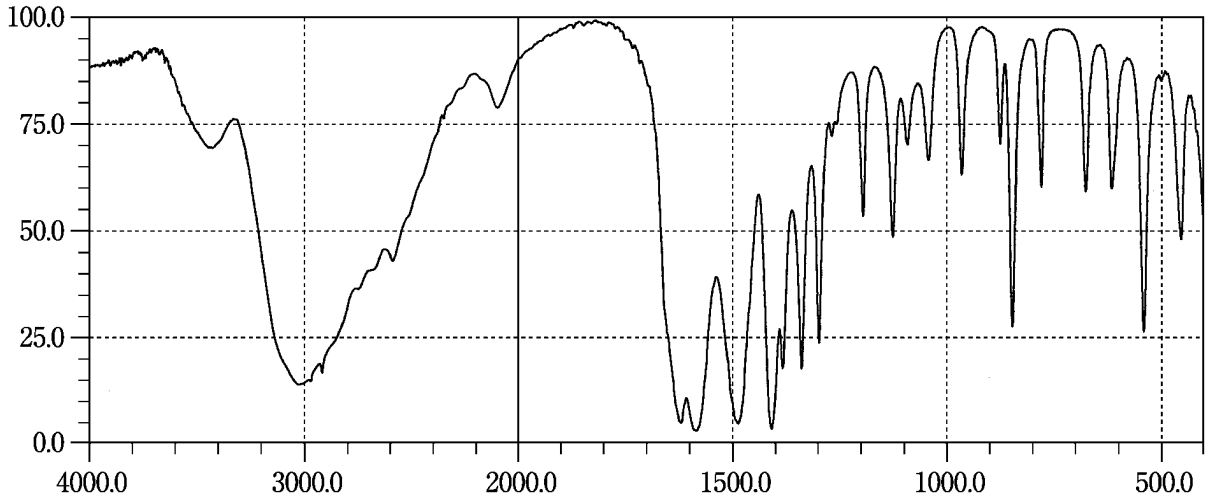
Cetotiamine Hydrochloride Hydrate



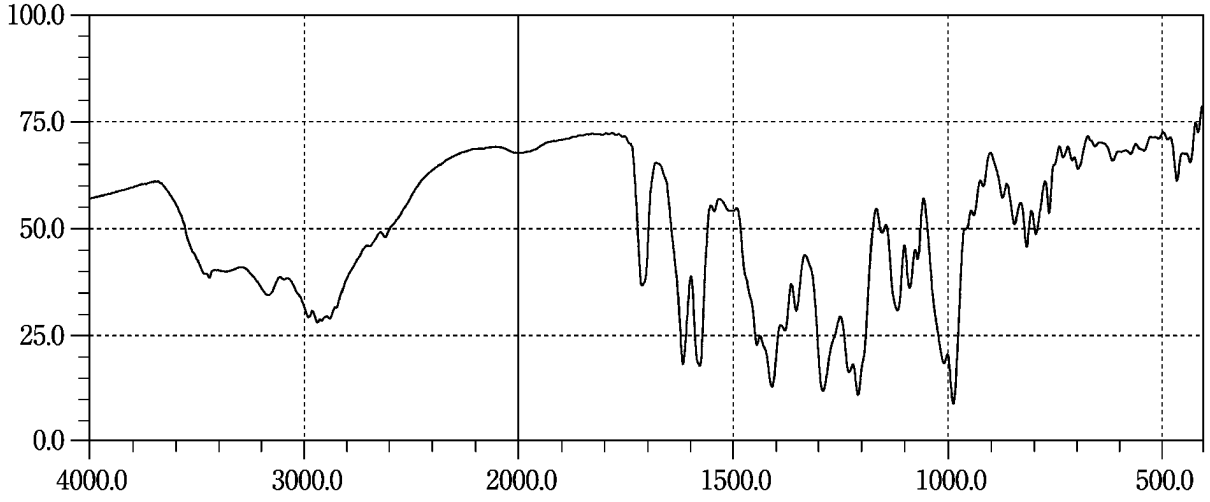
Colestimide



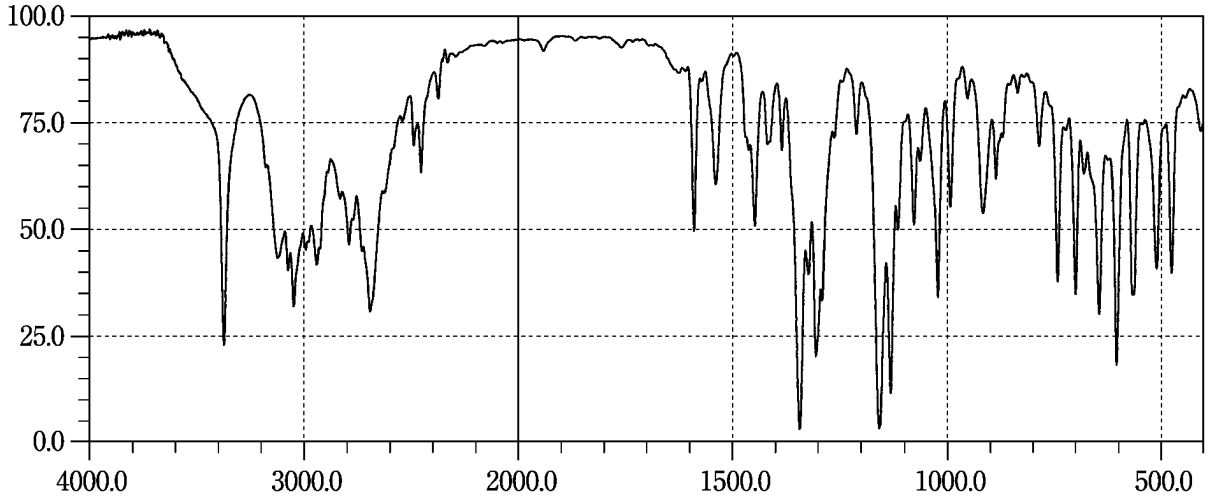
L-Cystine



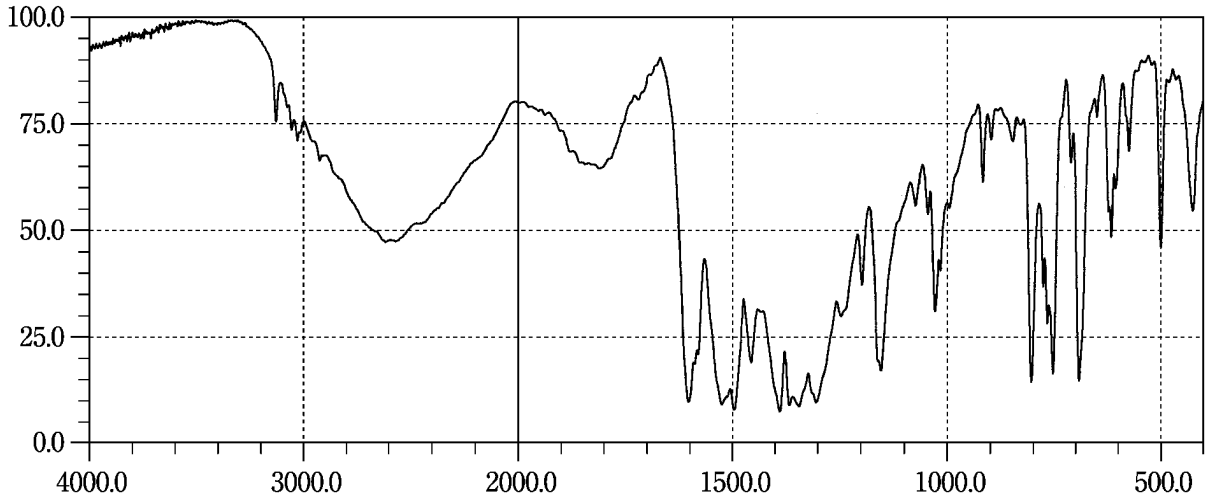
Daunorubicin Hydrochloride



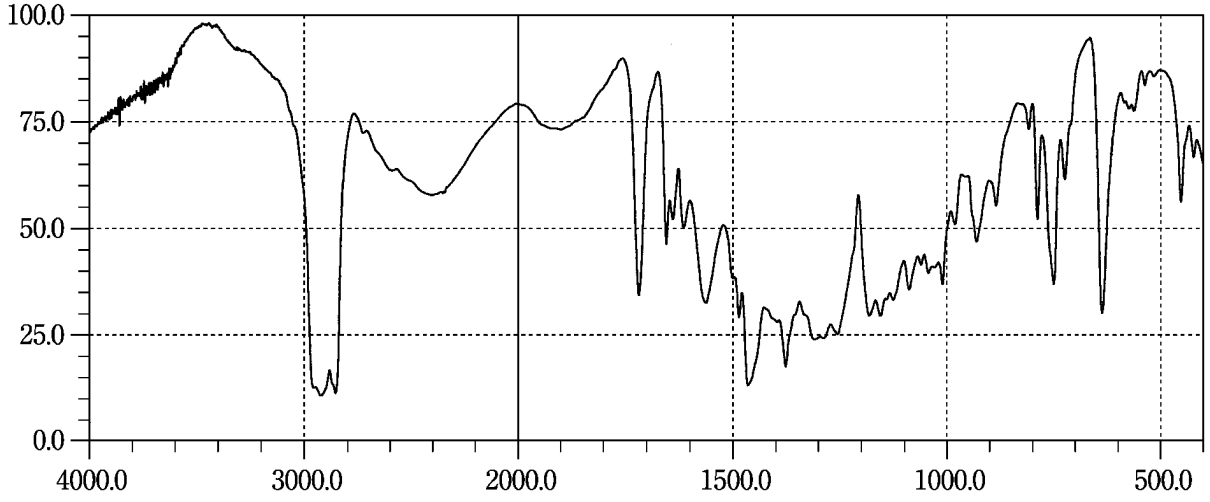
Dorzolamide Hydrochloride



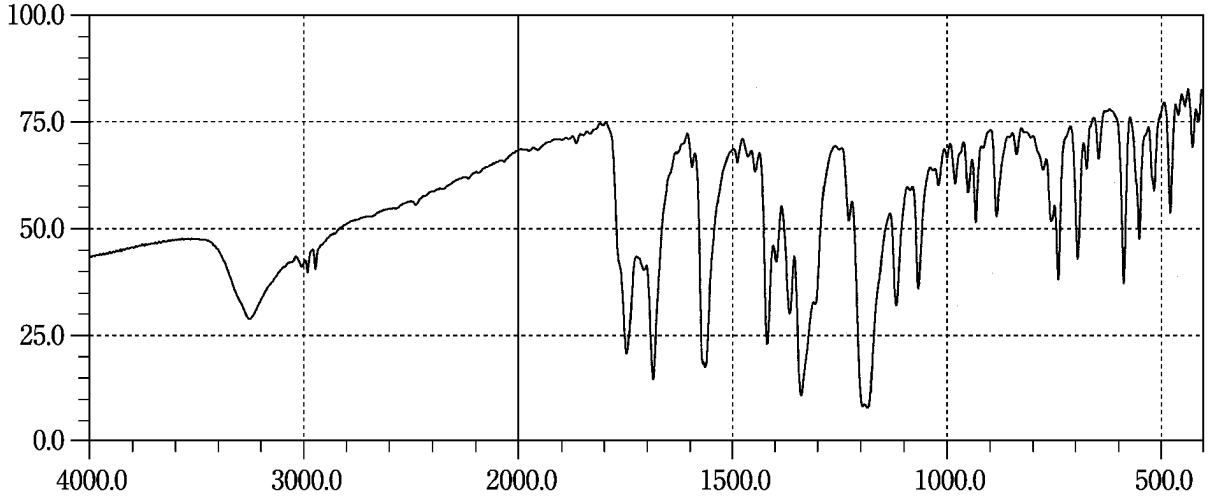
Edaravone



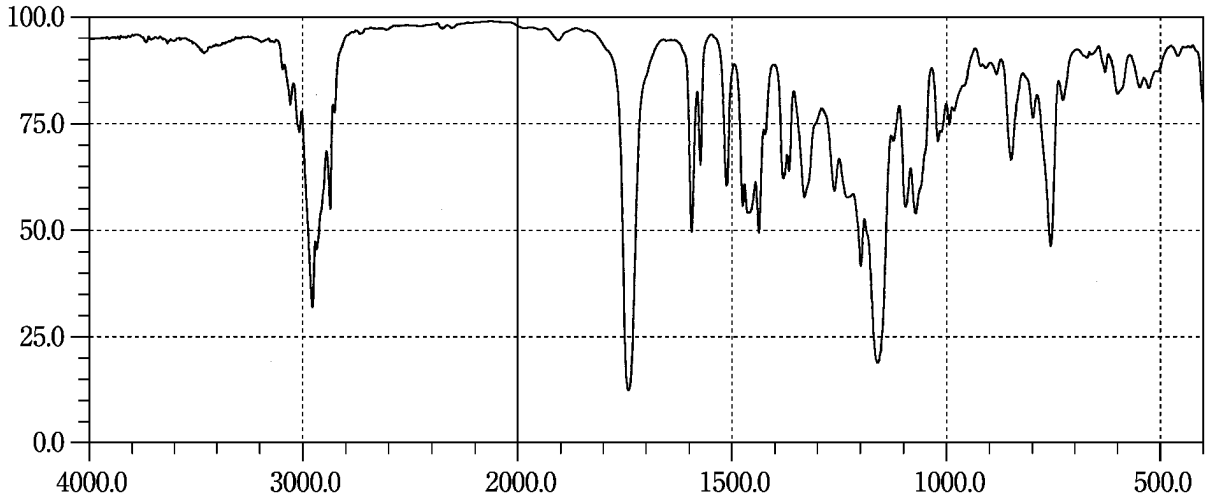
Emedastine Fumarate



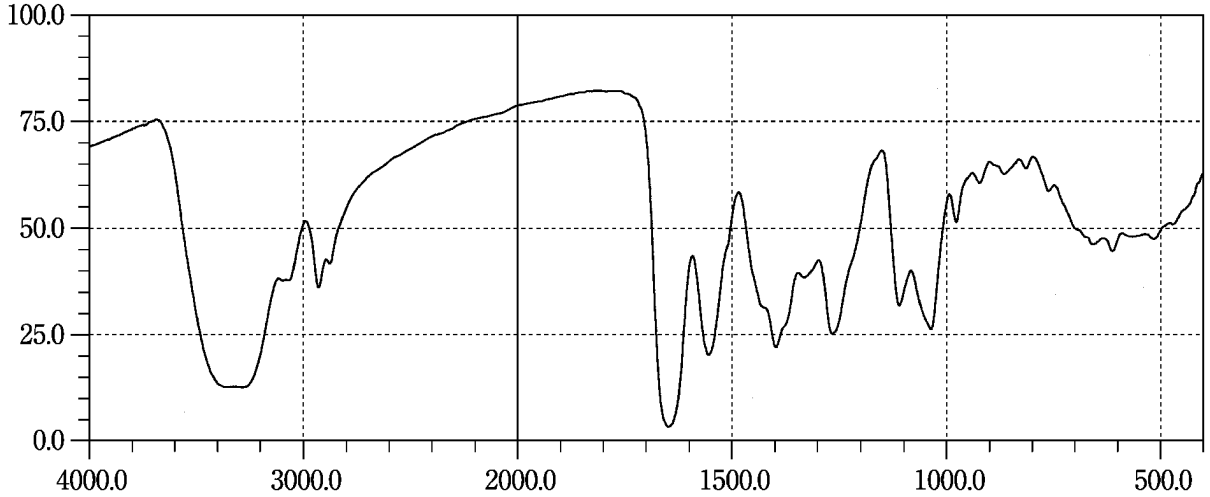
Epalrestat



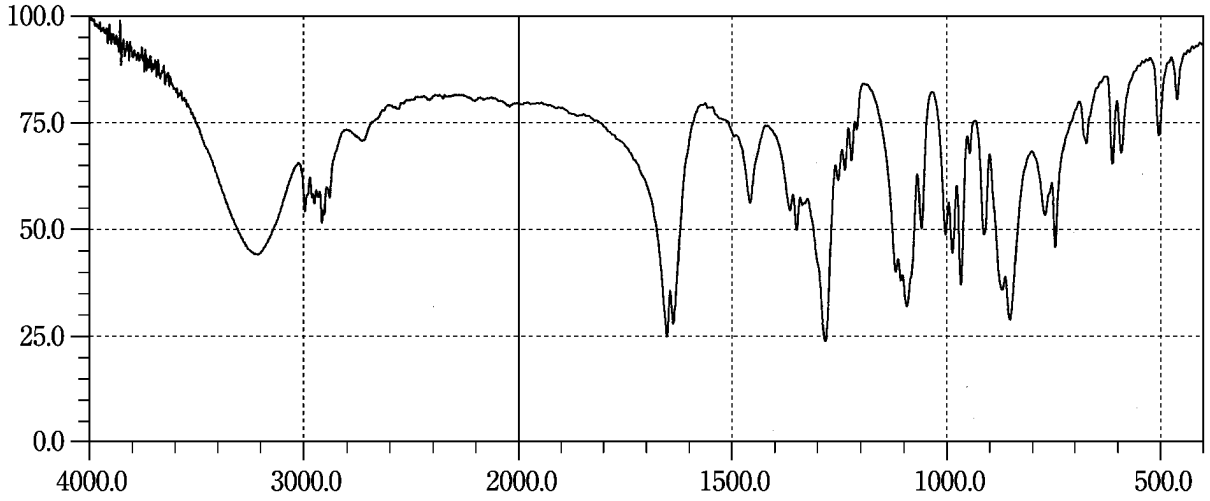
Ibuprofen Piconol



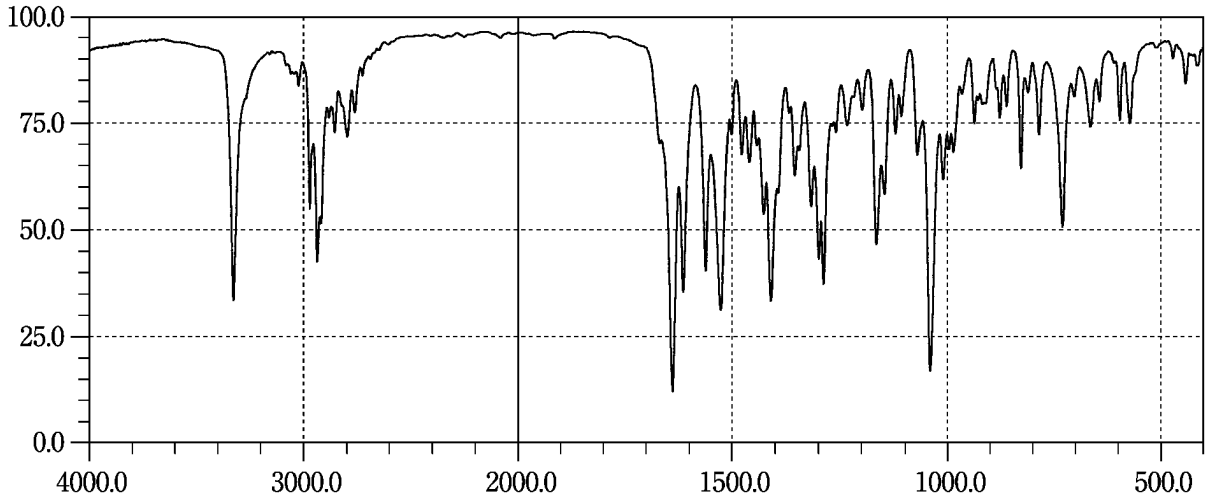
Iohexol



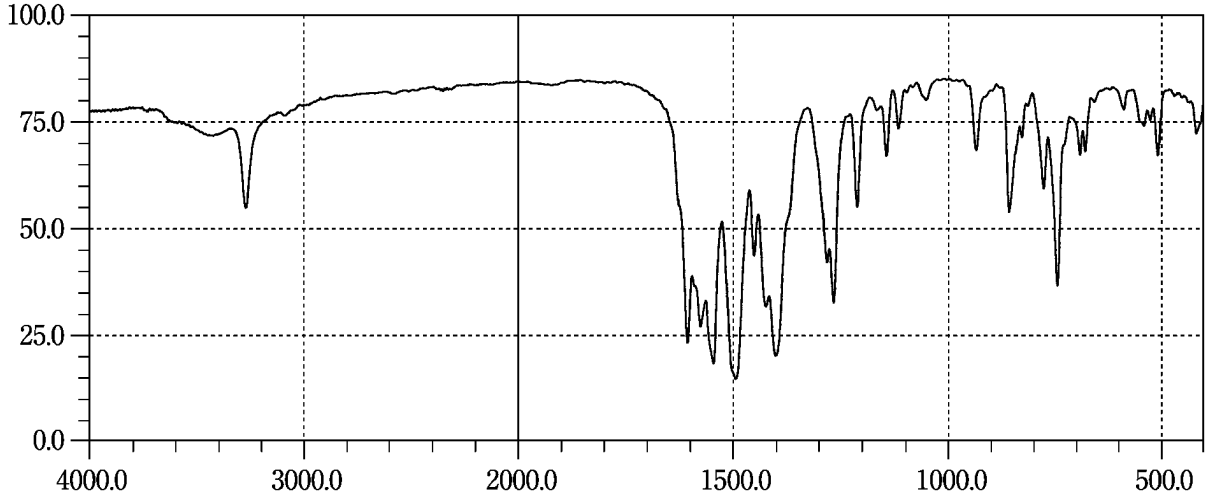
Isosorbide Mononitrate



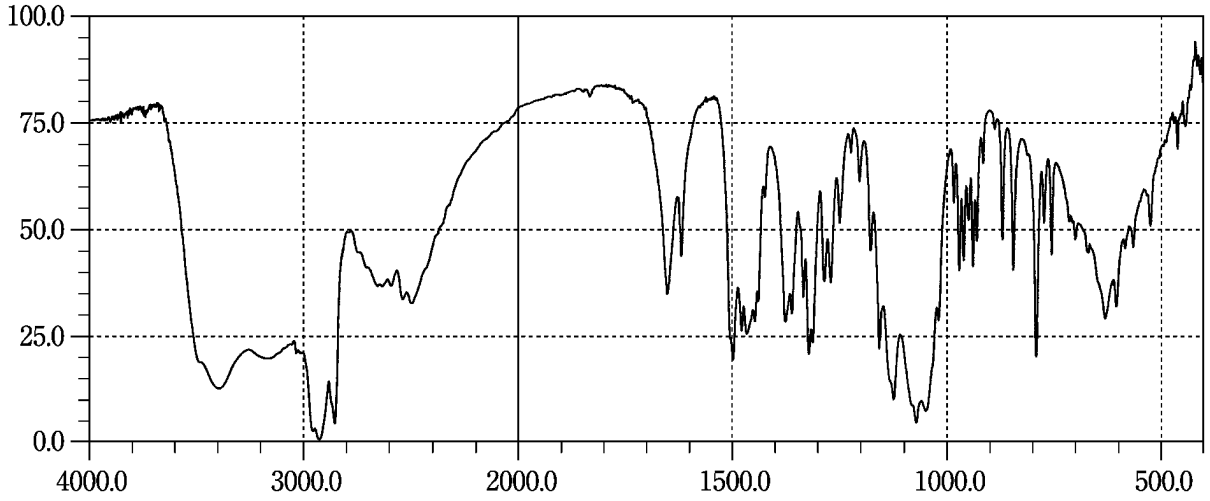
Lafutidine



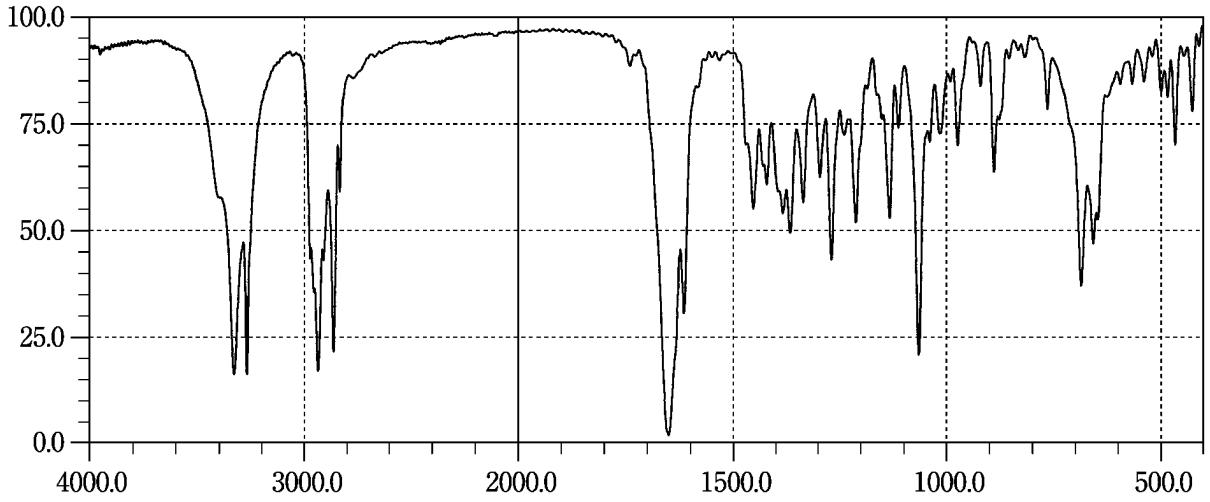
Lobenzarit Sodium



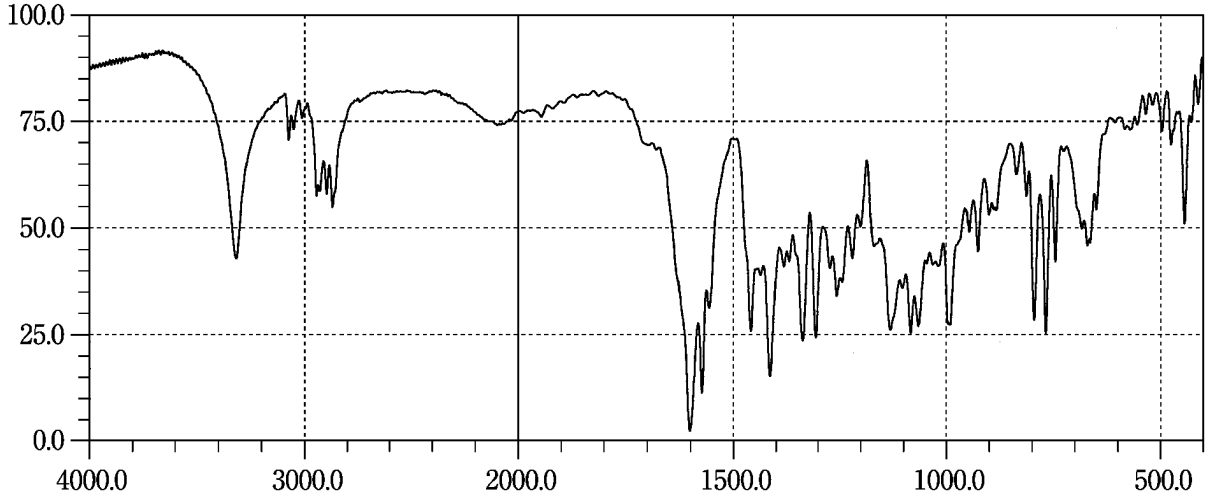
Morphine Sulfate Hydrate



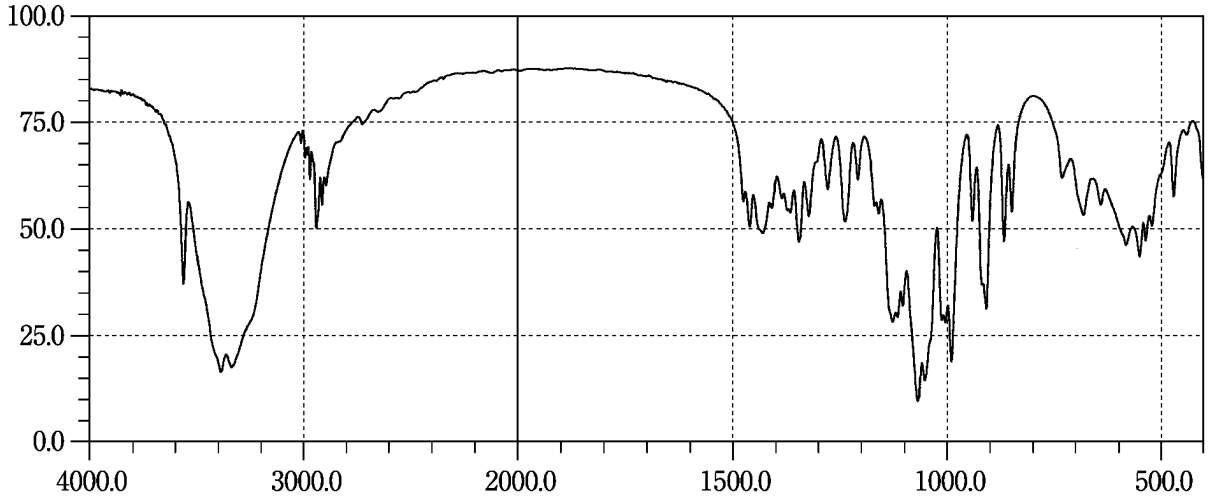
Norethisterone



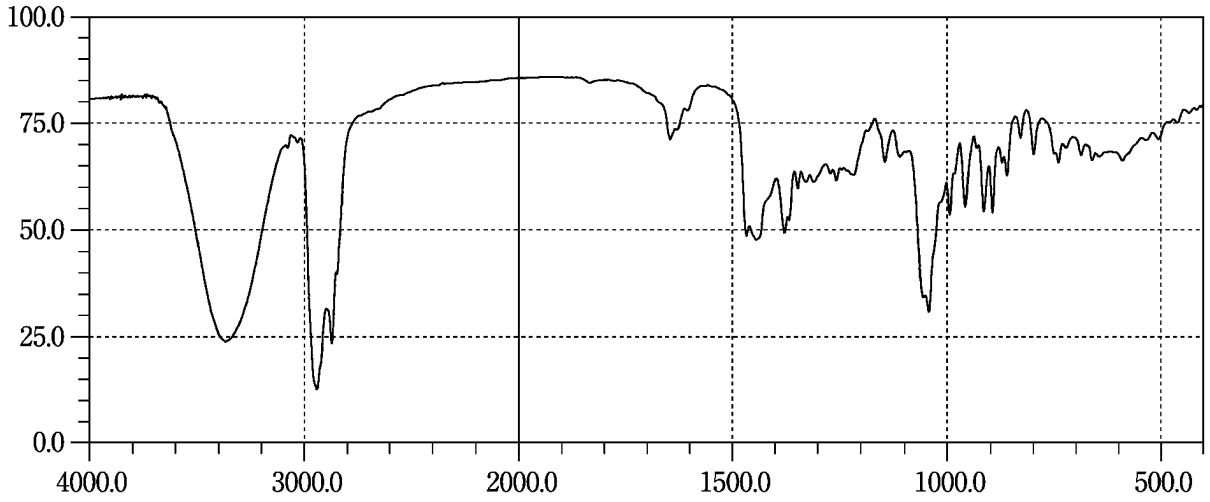
Quetiapine Fumarate



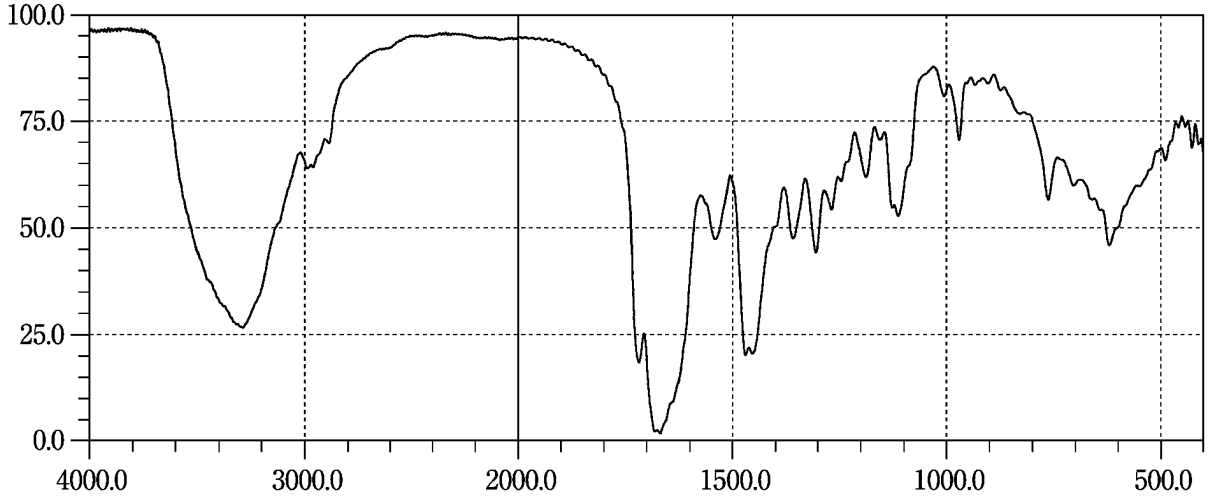
Sucrose



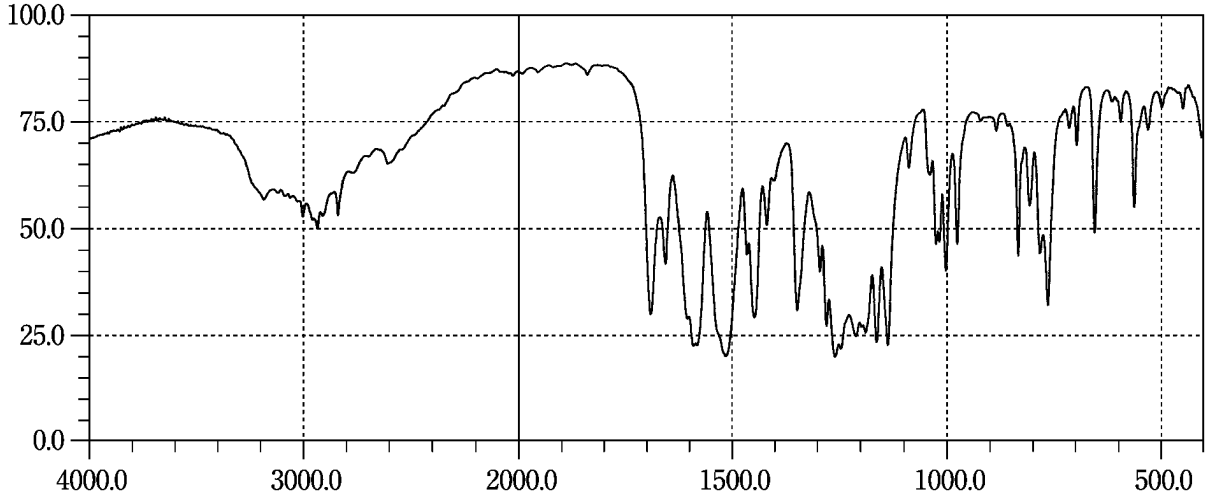
Tacalcitol Hydrate



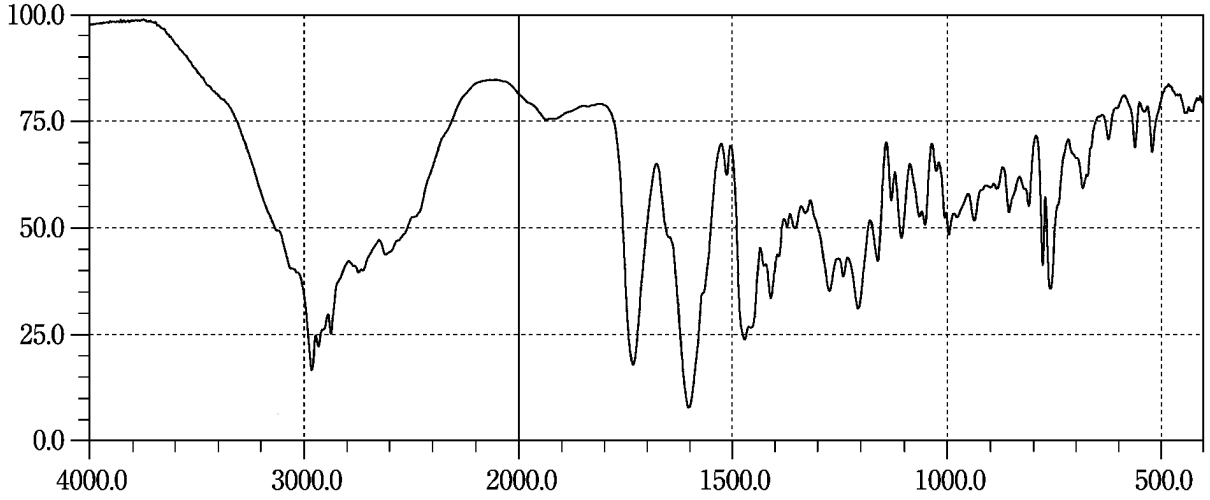
Taltirelin Hydrate



Tranilast



Valsartan



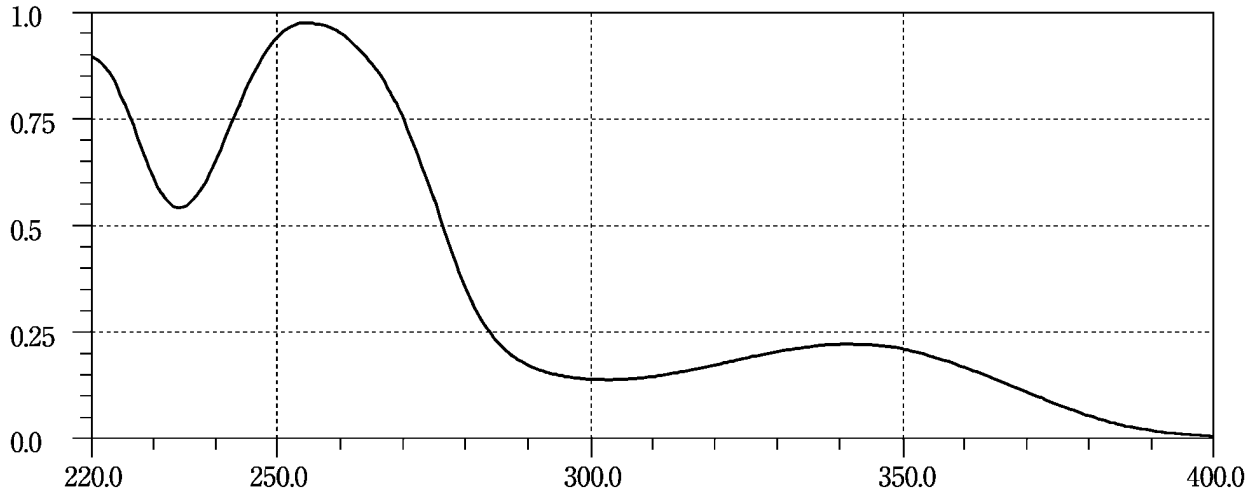
Delete the following Ultraviolet-visible Reference Spectra:

Flurazepam 1

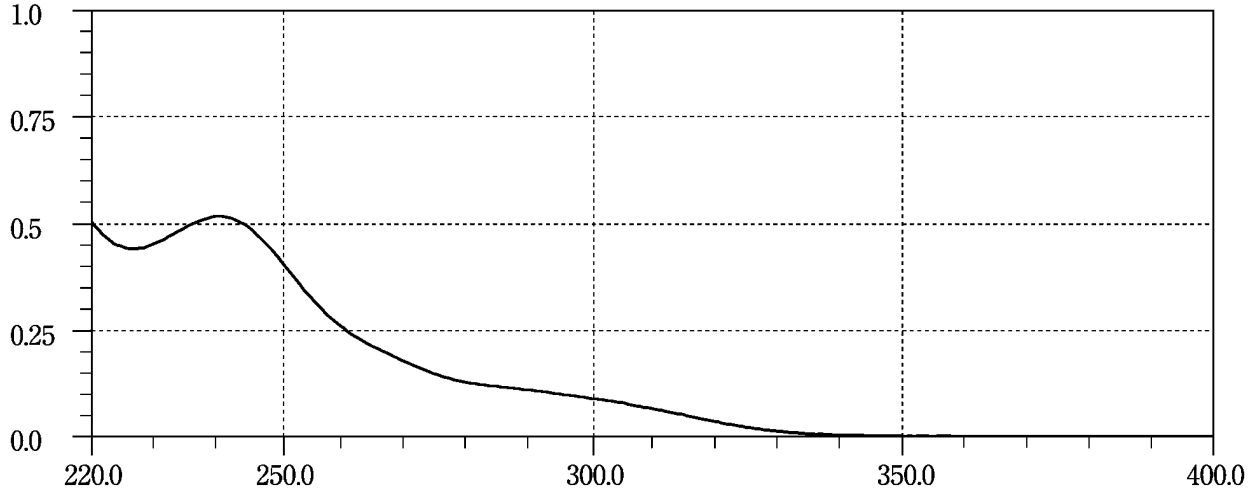
Flurazepam 2

Add the following 18 spectra:

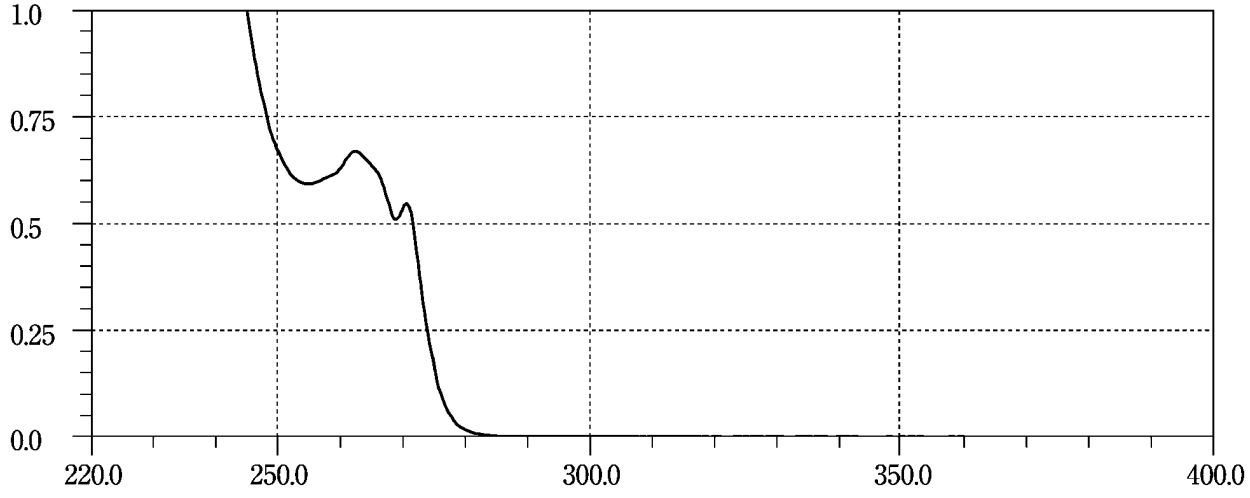
Azelnidipine



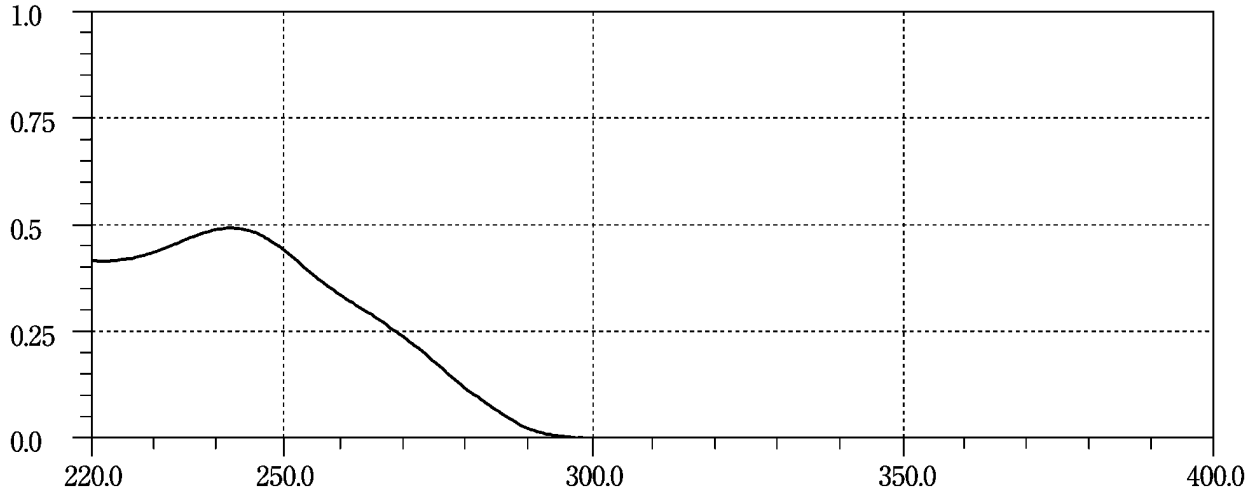
Brotizolam



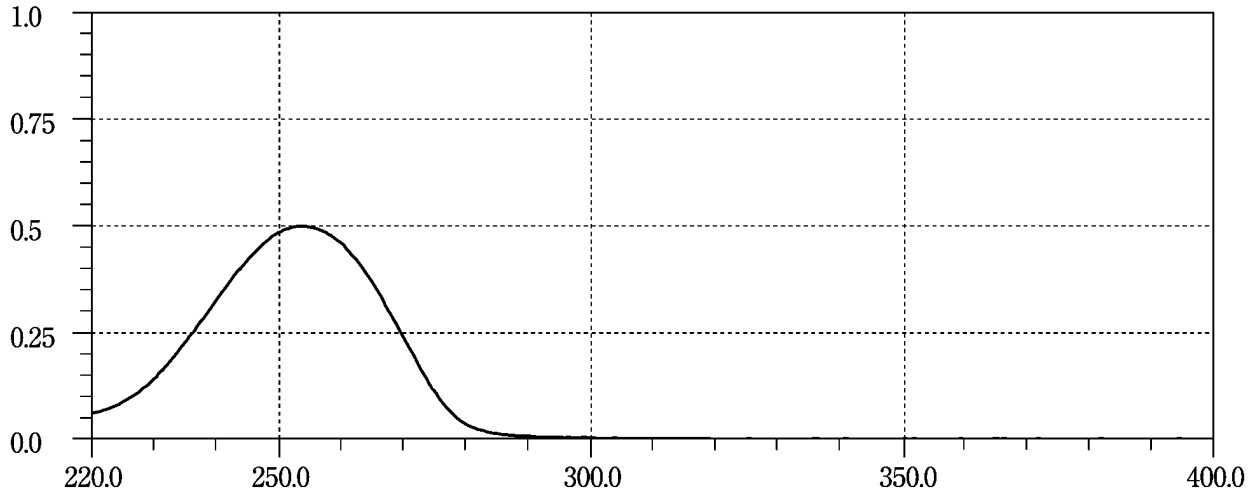
Bupivacaine Hydrochloride Hydrate



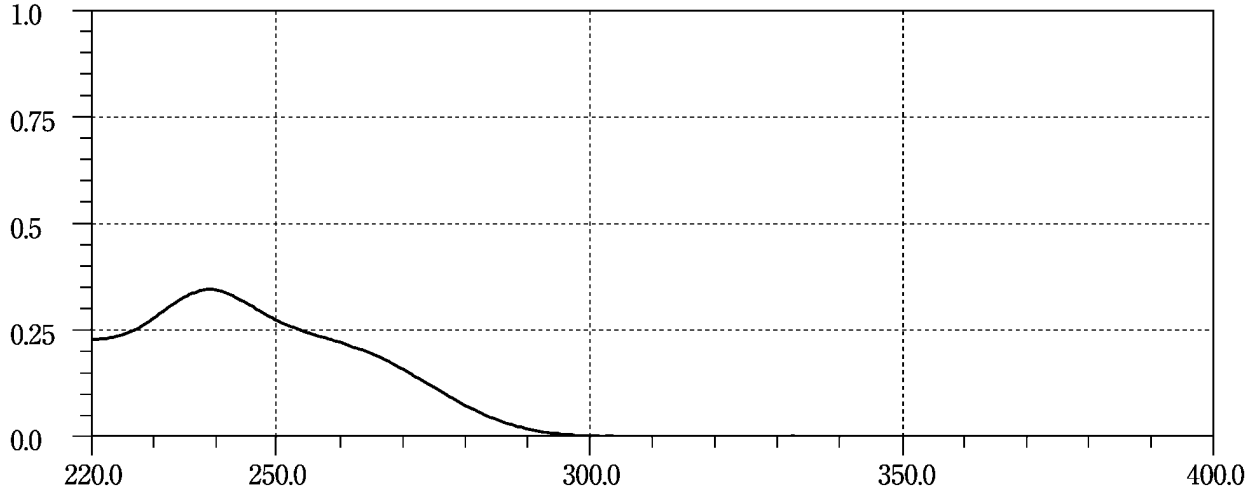
Cetotiamine Hydrochloride Hydrate



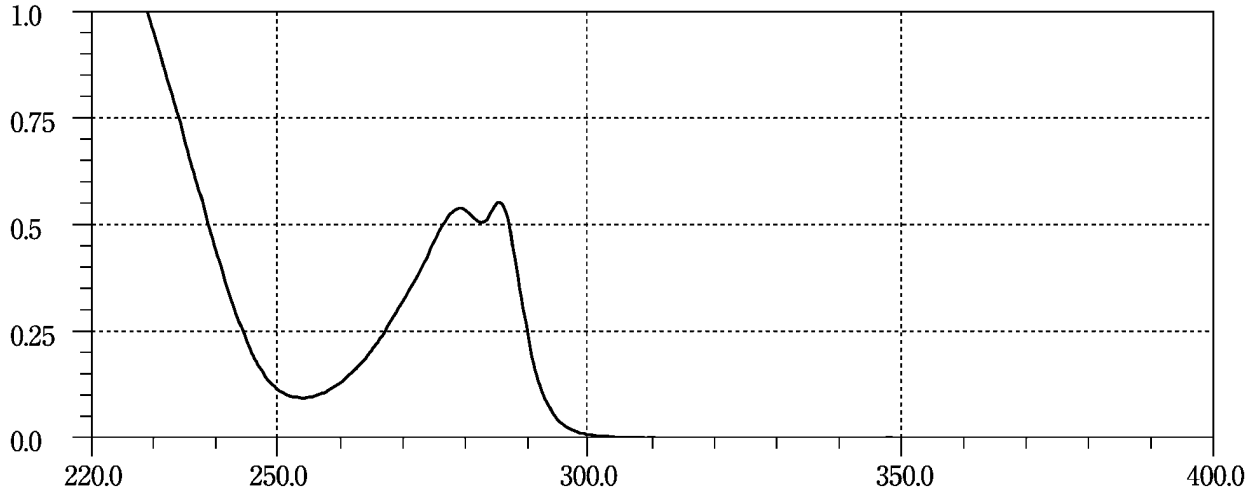
Dorzolamide Hydrochloride



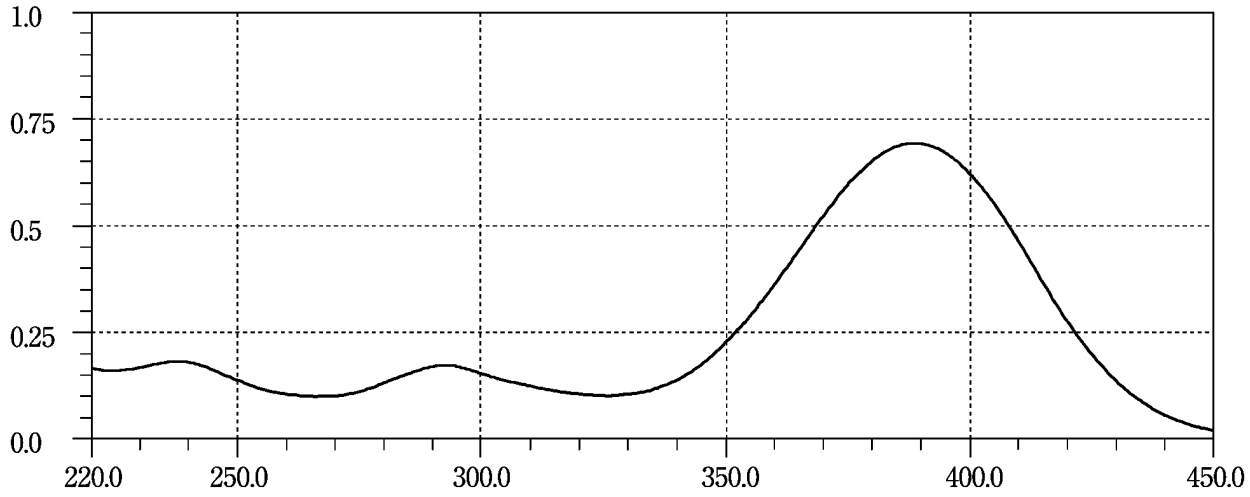
Edaravone



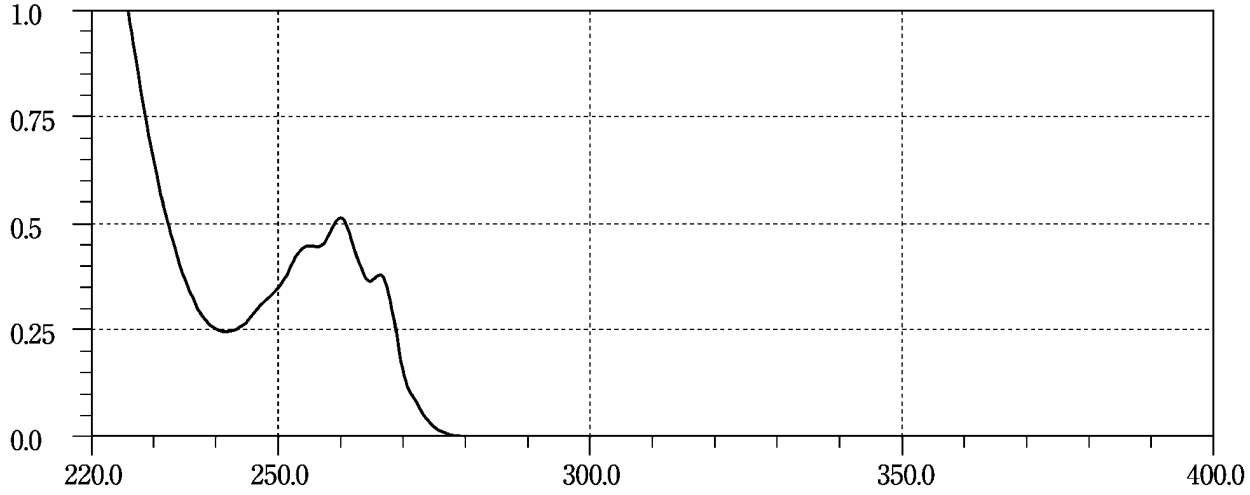
Emedastine Fumarate



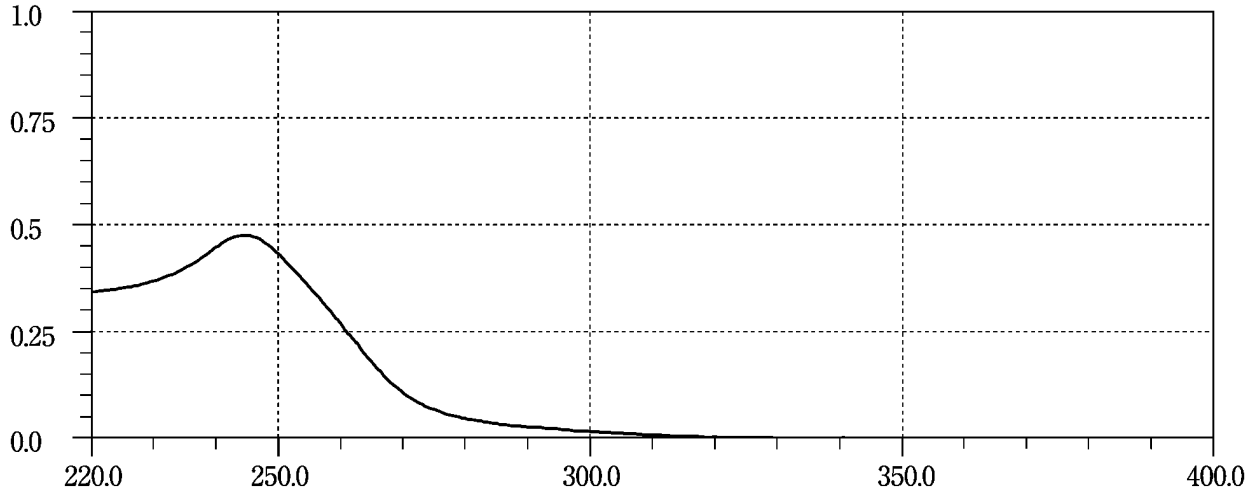
Epalrestat



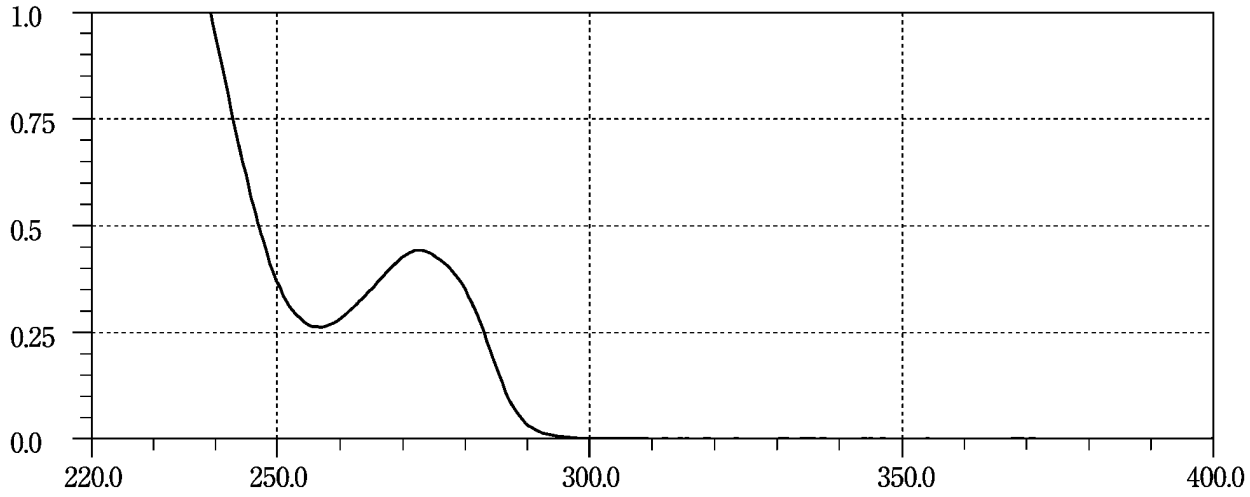
Ibuprofen Piconol



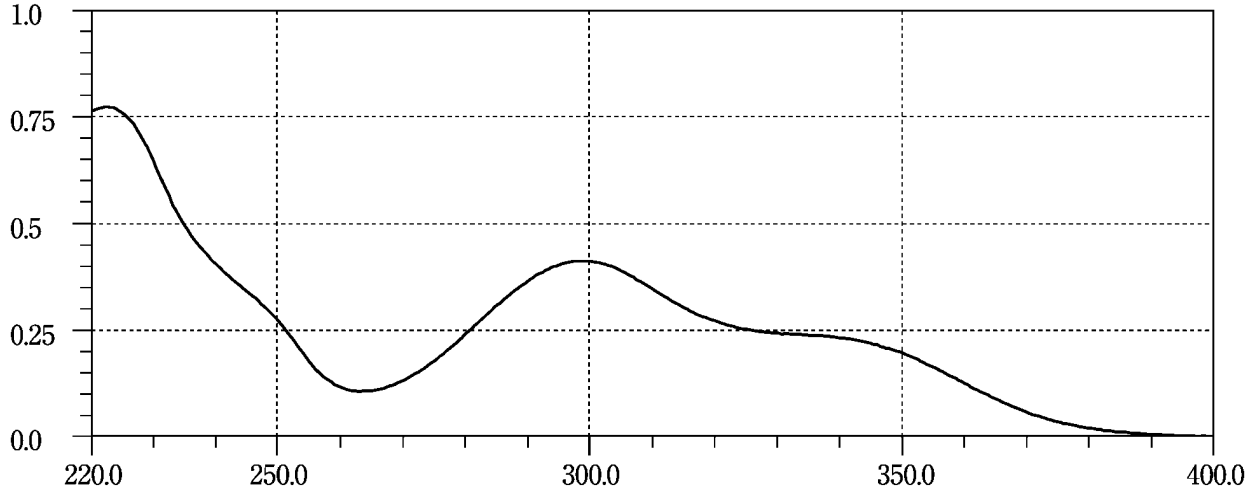
Iohexol



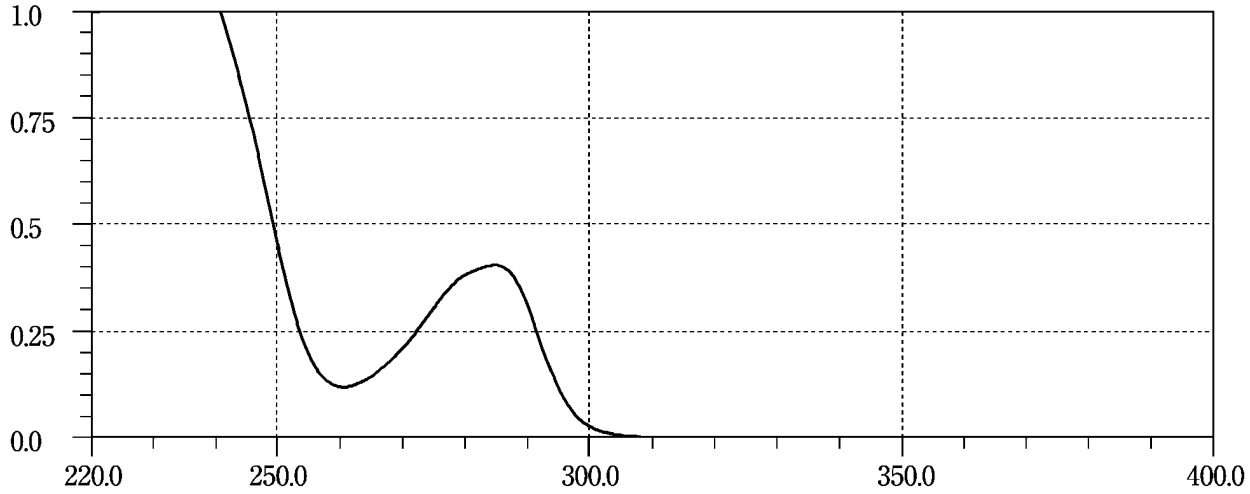
Lafutidine



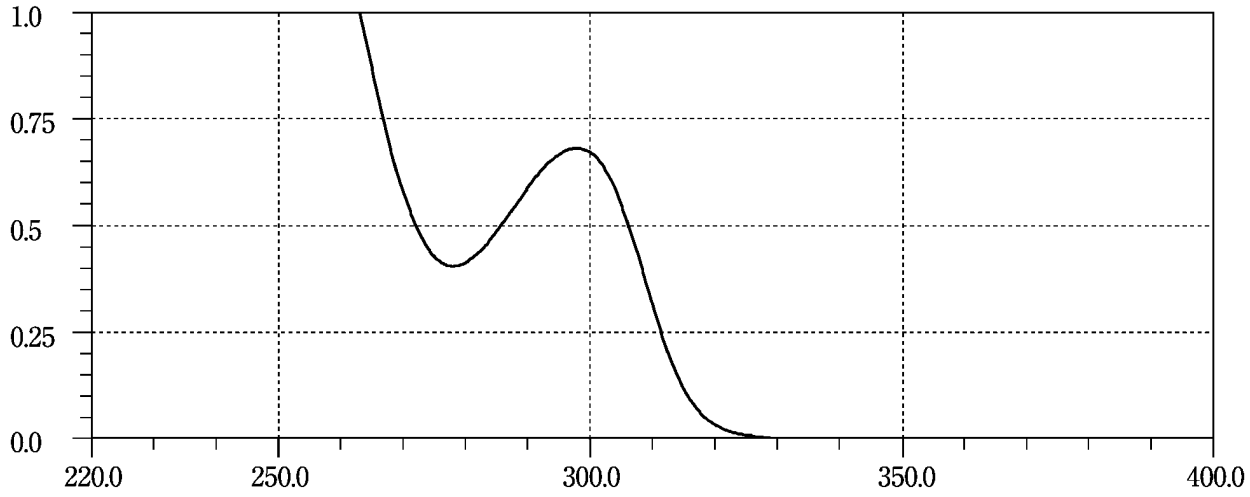
Lobenzarit Sodium



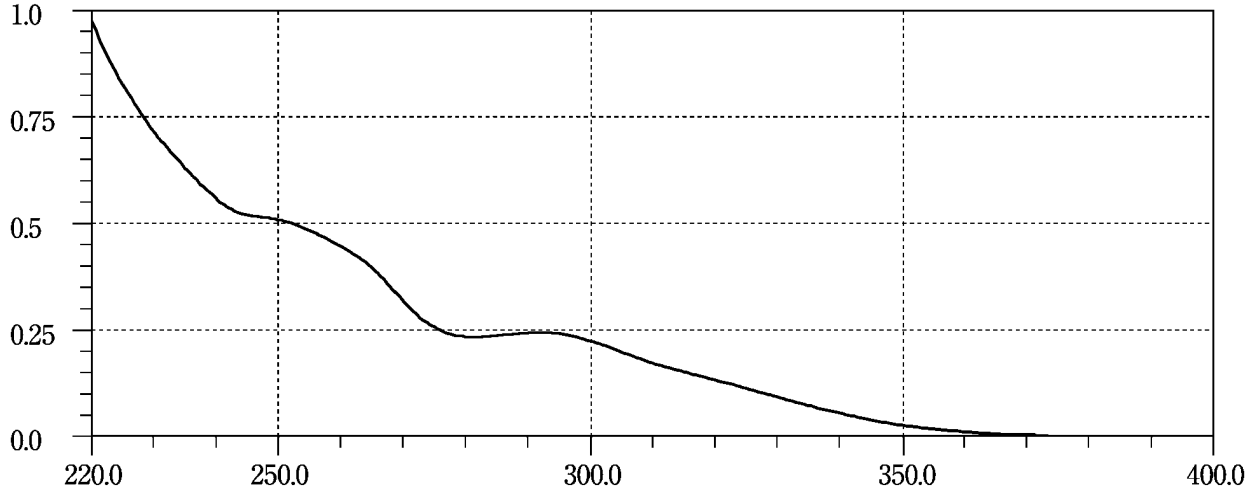
Morphine Sulfate Hydrate 1



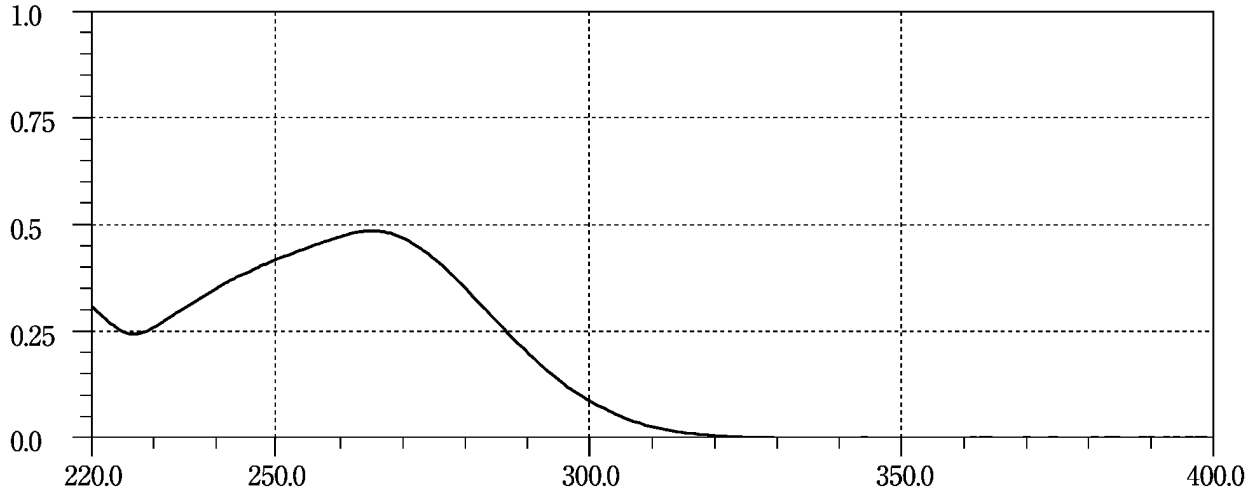
Morphine Sulfate Hydrate 2



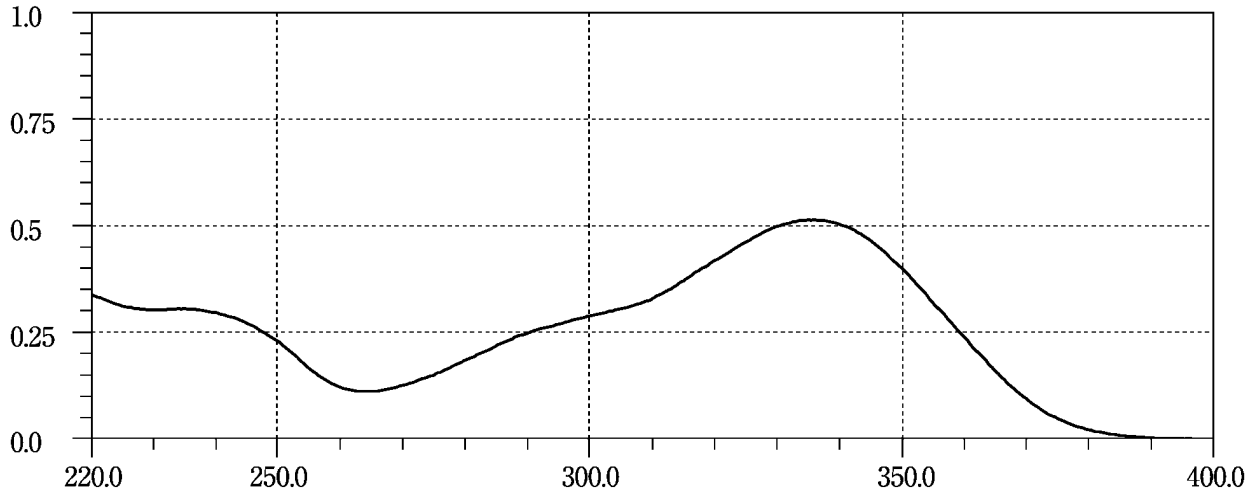
Quetiapine Fumarate



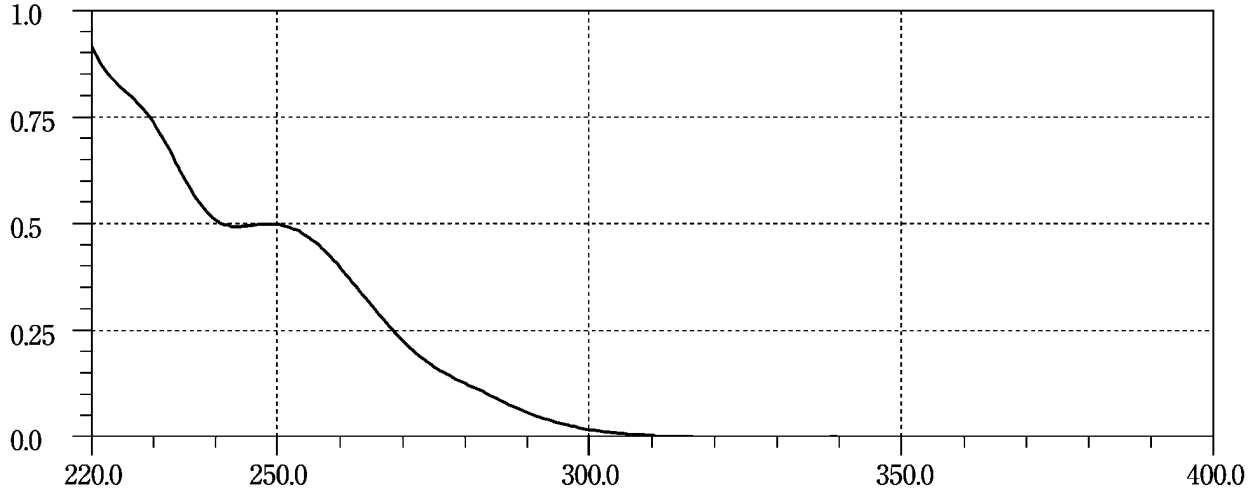
Tacalcitol Hydrate



Tranilast



Valsartan



GENERAL INFORMATION

G1 Physics and Chemistry

Delete the following:

Inductively Coupled Plasma Atomic Emission Spectrometry

Near Infrared Spectrometry

Change to read as follows:

Near infrared spectrometry (NIR) is one of spectroscopic methods used to qualitatively and quantitatively evaluate substances from analysis of data obtained by determining their absorption spectrum of light in the near-infrared range.

The near-infrared range lies between the visible light and infrared light, typically of wavelengths (wave numbers) between 750 and 2500 nm (13,333 – 4000 cm^{-1}). The absorption of near-infrared light occurs due to harmonic overtones from normal vibration or combination tones in the infrared range (4000 to 400 cm^{-1}), primarily absorption of O-H, N-H, C-H and S-H that involve hydrogen atoms, in particular. For instance the asymmetrical stretching vibration of N-H occurs in the vicinity of 3400 cm^{-1}, but the absorption due to the first harmonic overtone occurs in the vicinity of 6600 cm^{-1} (wavelength 1515 nm), which is near double 3400 cm^{-1}.

Absorption in the near-infrared range is far weaker than absorption due to normal vibration that occurs in the infrared range. Furthermore, in comparison with visible light, near-infrared light has longer wavelength, which makes it possible for the light to penetrate to a depth of several mm into solid specimens including fine particles. This method is often utilized as a nondestructive analysis, as changes occurring with absorbed light spectrum (transmitted light or reflected light) in this process provide physical and chemical information pertaining to specimens.

Conventional spectrometry, such as calibration curve method, is used as a method for analyzing near-infrared absorption spectrum whenever applicable. Ordinarily, however, chemometrics methods are used for analysis. Chemometrics ordinarily involve quantification of chemical data, as well as numerical and statistical procedures for computerization of information. Chemometrics for near-infrared spectrometry includes various types of multivariate analysis such as multiple regression analysis, to perform qualitative or quantitative evaluation of active substances.

Near-infrared spectrometry is used as a rapid and nondestructive method of analysis that replaces conventional and established analysis methods for water determinations or substance verifications. It is necessary to perform a comparison test to evaluate this method against an existing analysis method, to verify that this method is equivalent to such existing analysis method, before using this analysis method as a quality evaluation test method in routine tests.

Applications of near-infrared spectrometry in the pharmaceutical field include qualitative or quantitative evaluation of ingredients, additives or water contents of active substances or preparations. Furthermore, near-infrared spectrometry can also be used for evaluation of physical conditions of substances, such as crystal forms, crystallinity, particle diameters. It is also possible to perform spectrometry on samples that are located in a remote location away from equipment main units, without sampling, by using optical fibers. It can therefore be used as an effective means to perform pharmaceutical manufacturing process control online (or in-line).

1. Equipment

Near-infrared spectrophotometers can either be a distributed near-infrared spectrophotometer or a Fourier transform near-infrared spectrophotometer[1)]. Interference filter-type near-infrared spectrophotometers that use interference filter in the spectrometry section are also available, however, this type of equipment is hardly used in the field of pharmaceutical quality control.

1.1. Distributed near-infrared spectrophotometer

This equipment is comprised of light source section, sample section, spectrometry section, photometry section, signal processing section, data processing section, display-record-output section. Halogen lamps, tungsten lamps, light emitting diodes and other such devices that can emit high intensity near-infrared light in a stable manner and used in the light source section. The sample section is comprised of a sample cell and a sample holder. Equipment that have an optical fiber section that is comprised of optical fibers and a collimator are equipped with a function for transmitting light to sample section, which is remotely located away from the spectrophotometer main unit. Quartz is ordinarily used as material for optical fibers

The spectrometry section is intended to extract light of required wavelength, using dispersive devices and is comprised of slits, mirrors and dispersive devices. Potential dispersive devices include prisms, diffraction grating, acousto-optical tunable filters (AOTF), or liquid crystal tunable filters (LCTF).

The photometry section is comprised of detectors and amplifiers. Sensors include semiconductor detectors (silicon, lead sulfide, indium-gallium-arsenic, indium-antimony), as well as photomultiplier tubes. Detecting methods that use semiconductor detectors generally perform detections with

single elements, but there are also occasions where arraytype detectors that use multiple elements are used. Such detectors are capable of simultaneously detecting multiple wavelengths (wave numbers). The signal processing section separates signals required for measurements from output signals fed by amplifiers and then outputs such isolated signals. The data processing section performs data conversions and spectral analysis, etc. The display-record-output section outputs data, analysis results and data processing results to a printer.

1.2. Fourier transform near-infrared spectrophotometer

The configuration of the equipment is fundamentally same as that of the distributed-type equipment described in Section 1.1., except for the spectrometry section and the signal processing section.

The spectrometry section is comprised of interferometers, sampling signal generators, detectors, amplifiers, A/D conversion devices, etc. Interferometers include Michelson interferometers, transept interferometers and polarization interferometers. The signal processing section is equipped with functions that are required for spectrometer, as well as a function for translating acquired interference waveform (interferogram) into absorption spectrum by Fourier transformation.

2. Determination

There are three types of measurement methods that are used with near-infrared spectrometry: transmittance method, diffuse reflectance method and transmittance reflectance method. The section of measurement methods relies on the shape of samples and applications. The transmittance method or diffuse reflectance method is used for solid samples, including fine particles. The transmittance method or transmittance reflectance method is used for liquid samples.

2.1. Transmittance method

The degree of decay for incident light intensity as the light from a light source passes through a sample, is represented as transmittance rate T (%) or absorbance A with the transmittance method. A sample is placed in the light path between a light source and a detector, the arrangement of which is ordinarily same as that of the spectroscopic method.

$$T = 100t$$
$$t = I/I_0 = 10^{-\alpha cl}$$

I_0: Incident light intensity
I: Transmitted light intensity
α: Absorptivity
c: Solution concentration
l: Layer length (sample thickness)

$$A = -\log t = \log (1/t) = \log (I_0/I) = \alpha cl$$

This method is applied for taking measurements of samples that are liquids and solutions. Quartz glass cells and flow cells are used, with the layer length of 1 – 5 mm along. Furthermore, this method can also be applied for taking measurements of samples that are solids, including fine particles. It is also known as diffuse transmittance method. Selecting appropriate layer length is critical for this method, since the transmitted light intensity varies depending on grain sizes and surface condition of samples.

2.2. Diffuse reflectance method

The ratio of the reflection light intensity I, emitted from the sample in a wide reflectance range and a control reflection light intensity I_r emitted from surface of a substance, is expressed as reflectance R (%) with the diffuse reflectance method. The near-infrared light penetrates to a depth of several mm into solid samples, including fine particles. In that process, transmission, refraction, reflection and dispersion are repeated, and diffusion takes place, but a portion of the diffused light is emitted again from the surface of the sample and captured by a detector. The spectrum for the diffuse reflectance absorbance (A_r) can ordinarily be obtained by plotting logarithm of inverse numbers for reflectance ($1/r$) against wavelengths (wave numbers).

$$R = 100r$$
$$r = I/I_r$$

I: Reflection light intensity of light, diffuse reflected off the sample
I_r: Control reflection light intensity of light emitted from surface of reference substance

$$A_r = \log (1/r) = \log (I_r/I)$$

The intensity of diffuse reflectance spectrum can also be expressed with the Kubelka-Munk (K-M) function. The K-M function is derived, based on the existence of a sample with sufficient thickness, and expressed in terms of light scattering coefficient, which is determined by absorptivity, grain size, shape and fill condition (compression).

This method is applied to solid samples, including fine particles, and requires a diffuse reflector.

2.3. Transmittance reflectance method

The transmittance reflectance method is a combination of the transmittance method and reflectance method. A mirror is used to re-reflect a light that has passed through a sample in order to take a measurement of transmittance reflectance rate, T^* (%). Light path must be twice the thickness of the sample. On the other hand, the light reflected off a mirror and enters into a detector is used as the control light. When this method is applied to suspended samples, however, a metal plate or a ceramic reflector with rough surface that causes diffuse reflectance is used instead of a mirror.

Transmittance reflectance absorbance (A^*) is obtained by the following formula with this method:

$$T^* = 100t^*$$
$$t^* = I/I_T$$

I: Intensity of transmitted and reflected light, in cases where a sample is placed
I_T: Intensity of reflected light, in cases where is no sample

$$A^* = \log (1/t^*)$$

This is a method that is applied to solid samples, including fine particles, as well as liquids and suspended samples. The

thickness of a sample must be adjusted when applying this method to a solid sample. Ordinarily adjustment is made by setting absorbance to 0.1 – 2 (transmittance of 79 – 1%), which provides the best linearity and *S/N* ratio of detector. A cell with appropriate layer length, according to the grain size of the fine particle, must be selected when applying the method to a fine particle sample.

3. Factors that affect spectrum

Following items must be considered as factors that can affect spectrum when applying near-infrared spectrometry, particularly when conducting quantitative analysis.

(i) Sample temperature: A significant change (wavelength shift, for example) can occur when the temperature varies by a several degree (°C). Care must be taken, particularly when the sample is a solution or contains water.

(ii) Water or residual solvent: Water or residual solvent contents of a sample, as well as water (humidity) in the environment wherein measurements are taken, can potentially significantly affect absorption band of the near-infrared range.

(iii) Sample thickness: The thickness of a sample is a factor for spectral changes and therefore needs to be controlled at a certain thickness. A sample may be considered to be of adequate thickness for the diffuse reflectance method, however, if the thickness is less than a certain amount, for example, the sample may have to be placed on a support plate with high reflectance to take measurements by the transmittance reflectance method.

(iv) Fill condition of sample: The condition of sample fill can potentially affect spectrum, when taking measurements of samples that are solids or fine particles. Care must be taken with filling samples in a cell, to ensure that a certain amount is filler through a specific procedure.

(v) Optical characteristics of samples: When a sample is physically, chemically or optically uneven, relatively large beam size must be used, multiple samples must be used, measurements must be taken at multiple points on the same samples, or a sample must be pulverized to ensure averaging of sample. Grain size, fill condition, as well as roughness of surface can also affect fine particle samples.

(vi) Crystal forms: Variations in crystal structures (crystal forms) can also affect spectrum. In cases where multiple crystal forms exist, it is necessary to have consideration for characteristics of samples to be considered and care must be taken to ensure that even standard samples for calibration curve method have diversified distributions similar to that of samples that are subject to analysis.

(vii) Temporal changes in characteristics of samples: Samples can potentially undergo chemical, physical or optical property changes, due to passing of time or storage after sampling, and such changes affect spectrum in a subtle manner. For instance even with identical samples, if elapsed times differ, then their characteristics of near-infrared spectrum can vary significantly. In creating calibration curves, therefore, measurements must be taken offline in a laboratory or online in manufacturing process (or inline) and samples for calibration curves must be prepared with adequate considerations for the passing of time before measurements are taken.

4. Control of equipment performance[2,3]

4.1. Accuracy of wavelengths (wave numbers)

The accuracy of wavelengths (wave numbers) of an equipment is derived from the deviation of substances for which peak absorption wavelengths (wave numbers) have been defined, such as polystyrene, mixture of rare earth oxides (dysprosium, holmium and erbium; 1:1:1) or steam, from the figures indicated on the equipment. Tolerance figures in the vicinity of 3 peaks are ordinarily set in the following manner, though appropriate tolerance figures can be set, depending on the intended purpose:

1200 ± 1 nm (8300 ± 8 cm^{-1})
1600 ± 1 nm (6250 ± 4 cm^{-1})
2000 ± 1.5 nm (5000 ± 4 cm^{-1})

Since the location of absorption peaks vary, depending on the substance used as reference, absorption peaks of wavelengths (wave numbers) that are closest to the above 3 peaks are selected for suitability evaluations. A mixture of rare earth oxides, for instance, would indicate characteristic absorption peaks at 1261 nm, 1681 nm and 1971 nm.

Absorption peaks at 1155 nm, 1417 nm, 1649 nm, 2352 nm (layer length: 1.0 nm) can be used, when taking measurements with transmittance method that involve the use of dichloromethane as reference. The absorption peak of steam at 7306.7 cm^{-1} can be used with a Fourier transformation-type spectrophotometer, as its wave number resolution ability is high.

Other substances can also be used as reference, so long as their adequacy for the purpose can be verified.

4.2. Spectroscopic linearity

Appropriate standard plates, such as plate-shaped polymer impregnated with varying concentrations of carbon (Carbon-doped polymer standards), can be used to evaluate spectroscopic linearity. In order to verify linearity, however, standard plates with no less than 4 levels of concentration within the reflectance of 10 – 90% must be used. When measurements are expected to be taken with absorbance of no less than 1.0, it is necessary to add standard plates with reflectance of either 2% or 5% or both.

In order to plot absorbance (A_{OBS}) of such standard plates at locations in the vicinity of wavelengths 1200 nm, 1600 nm and 2000 nm against absorbance (A_{REF}) assigned to each standard plate, verifications must be made to ensure that the gradient of linearity obtained are ordinarily within the range 1.0 ± 0.05 for each of these wavelengths and 0 ± 0.05 for ordinate intercept. Depending on the intended purpose, appropriate tolerance figures can be set.

4.3. Spectrophotometric noise

The spectrophotometric noise of the equipment can be checked using appropriate reflectance standard plates, such as white-colored reflecting ceramic tiles or reflective thermoplastic resin (such as polytetrafluoroethylene).

4.3.1. High flux noise

Spectrophotometric noise is evaluated by using standard

plates with high reflectance, such as reflectance of 99%. Standard plates are used to take measurements for both samples and control samples. Generally, the average value obtained from calculation of mean square root (*RMS*) of noise for each 100 nm segments in the wavelength range of 1200 – 2200 nm ordinarily must not be more than 0.3×10^{-3} and individual values must not exceed 0.8×10^{-3}. Depending on the intended purpose, appropriate tolerance figures can be set.

$$RMS = \{1/N \cdot \Sigma (A_i - A_m)^2\}^{1/2}$$

N: Number of measurement points per segment
A_i: Absorbance at each measurement point of segment
A_m: Average absorbance for segment

4.3.2. Low flux noise

Spectrophotometric noise is evaluated by using standard plates with low reflectance, such as reflectance of 10%, when the amount of light is low. In such cases, light source, optical system, detector and electronic circuit systems all have some impact on noise. Similar to the cases of high flux noise, generally, the average value obtained from calculation of *RMS* for each 100 nm segments in the wavelength range of 1200 – 2200 nm ordinarily must not be more than 1.0×10^{-3} and individual values must not exceed 2.0×10^{-3}. Depending on the intended purpose, appropriate tolerance figures can be set.

5. Application of qualitative or quantitative analysis

Unlike in the infrared range, mainly harmonic overtones and combinations manifest as spectrum in the near-infrared range. Such absorbance spectrums are often observed as overlay of absorption bands of functional groups and atomic groups. The near-infrared spectrometry, therefore, differs from conventional analysis methods that correspond to each application, by preparing model analysis methods using methodologies of chemometrics, such as multivariate analysis.

Characteristics of near-infrared absorption spectrum must be emphasized and effects of complexities of spectrums, as well as overlay of absorption bands must be reduced by performing mathematical preprocesses, such as primary or secondary spectral differentiation processes or normalizations, which becomes one of vital procedures in establishing analysis methods that use methodologies of chemometrics. While there are many chemometrics methodologies and mathematical preprocessing methods for data, appropriate combinations must be selected that suit the purposes of intended analysis.

Evaluation of validity based on analysis parameters is ordinarily required for the analysis validation when establishing a near-infrared analysis method. Selection of parameters that are appropriate for applications must be made for its intended use. Furthermore, following issues must be considered, in conformity with attributes of the near-infrared spectrometry.

(i) Whether or not wavelengths (wave numbers) intended for the particular analysis method, are suitable for evaluation of characteristics of a sample in performing analysis under given conditions.

(ii) Whether or not the method is adequately robust to deal with variables such as handling of samples (for instance fill condition for fine particle samples, etc.) and configuration matrix.

(iii) Whether or not about the same level of accuracy or precision can be obtained, in comparison with the existing and established analysis methods, which are available as standards.

(iv) Sustaining and managing performance of an analysis method, once established, are critical. Continuous and systematic maintenance and inspection work must therefore be implemented. Furthermore, it must be determined whether or not appropriate evaluation procedures are available to deal with change controls or implementation of revalidation on changes made in manufacturing processes or raw materials, as well as changes arising from replacement of major components in equipment.

(v) Whether or not there are appropriate evaluation procedures in place to verify validity of transferring implementation of an analysis, which presupposed the use of a specific equipment, from such originally intended equipment to another equipment (model transfer) for the purpose of sharing the analysis method.

5.1. Qualitative analysis

Qualitative analysis, such as verification of substances, is performed after preparing a reference library that includes inter-lot variations within tolerance range and chemometrics methodologies, such as multivariate analysis, have been established. Minute quality characteristic variations between lots can also be established by using this method.

Furthermore, multivariate analysis includes direct analysis methods that consider wavelengths (wave numbers) and absorption as variables, such as wavelength correlation method, residual sum of squares, range sum of squares, along with factor analysis method, cluster analysis method, discriminant analysis method, as well as SIMCA (Soft independent modeling of class analogy).

It is also possible to consider the overall near-infrared absorption spectrum as a single pattern and to identify parameters obtained by applying multivariate analysis methods or characteristic wavelength (wave number) peaks of the sample substance as indices for monitoring, for the purpose of manufacturing process control for active substances or preparations.

5.2. Quantitative analysis

Quantitative analysis uses spectrums of sample groups and analysis values obtained through the existing and established analysis methods, to obtain quantitative models with methodologies of chemometorics. These are used to calculate concentrations of individual ingredients and material values of samples being measured, using conversion formulas. Chemometrics methodologies for obtaining quantitative models include multiple regression analysis method, main ingredient regression analysis method and PLS (Partial least squares) regression analysis method.

In cases where the composition of a sample is simple, concentrations of ingredients in the sample that are subject to

analysis can be calculated, by plotting a calibration curve using the absorbance of a specific wavelength (wave number) or the correlating relationship between the parameters and concentration, using samples for preparation of calibration curves with known concentrations (calibration curve method).

References

1) General Rules for Near-infrared Spectrophotometric Analysis, JIS K 0134 (2002), Japanese Industrial Standards
2) Near-Infrared Spectrophotometry, 2.2.40, European Pharmacopoeia 5.0 (2005)
3) Near-Infrared Spectrophotometry, <1119>, US Pharmacopoeia 30 (2007)

G2 Solid-state Properties

Add the following:

Measurement of the Diameter of Particles Dispersed in Liquid by Dynamic Light Scattering

This method is used for measuring average particle diameter and particle diameter distribution of submicron-sized particles dispersed in a liquid by means of dynamic light scattering.

The average particle diameter and the particle diameter distribution obtained by this method are important characteristics mainly of colloidal dispersion formulations, such as emulsion injections, suspension injections, and liposome formulations.

There are two ways of analyzing the detected signals in dynamic light scattering: photon correlation spectroscopy and frequency analysis. Dynamic light scattering is applied to the analysis of particles whose diameters range from nm scale to approximately 1 μm or particles free from the influence of sedimentation.

1. Principle

When particles in Brownian motion in solution or in suspension are irradiated with laser light, scattered light from the particles fluctuates depending on their diffusion coefficients. The intensity of the scattered light from larger particles fluctuates more slowly, because the larger particles move more slowly. On the other hand, the intensity of the scattered light from smaller particles fluctuates more rapidly, because they move faster.

In dynamic light scattering measurements, the particle diameter is determined by applying the Stokes-Einstein equation to analysis of the detected fluctuations of scattered light intensity, which reflect the diffusion coefficient of the particles.

$$d = \frac{kT}{3\pi\eta D} \times 10^{12}$$

d: particle diameter (nm)
k: Boltzmann constant (1.38×10^{-23} J·K^{-1})
T: absolute temperature (K)
η: viscosity (mPa·s)
D: diffusion coefficient (m^2·s^{-1})

In photon correlation spectroscopy, the time-dependent changes (fluctuation) in the scattered light intensity, namely the observed signals of the scattered light intensity, are transmitted to the correlator. The average particle diameter and the polydispersity index are obtained from the autocorrelation function of the scattered light intensity, which is calculated based on the data processed by the correlator.

In frequency analysis, the average particle diameter and the polydispersity index are obtained from the frequency power spectrum, which is the Fourier transform of the frequency components included in the signals of the scattered light intensity.

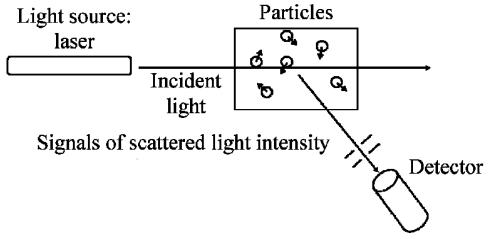


Fig. 1 Schematic illustration of the measurement principle

Major terms used in this method are as follows.

(i) Average particle diameter: dynamic light scattering harmonic intensity-weighted arithmetic averaged particle diameter, whose unit is nanometer (nm).

(ii) Polydispersity index: dimensionless indicator of the broadness of the particle diameter distribution.

(iii) Scattering volume: observation volume defined by the light-receiving optics and the incident laser light. This value may be given in the specifications of the instrument. Its order of magnitude is typically 10^{-12} m^3.

(iv) Count rate: number of the photon pulses per second detected in the light-receiving optics in photon correlation spectroscopy. This value is proportional to the detected scattered light intensity. The unit is cps (count per second).

(v) Signal of scattered light fluctuation: signal detected by the light-receiving optics in the frequency analysis. The signal is proportional to the scattered light intensity, and includes frequency components depending on the distribution of the particle diameter.

2. Apparatus

2.1. Constitution of the apparatus

The measuring apparatus generally consists mainly of a laser, sample holder, light-receiving optics and detector, and correlator or spectrum analyzer. There are two types of optical detection according to the optical arrangements: (a) homodyne detection in which only the scattered light is measured, and (b) heterodyne detection in which the scattered light and a portion of the incident light are measured simultaneously.

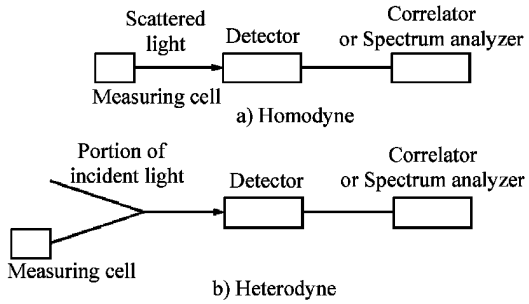


Fig. 2 Different optical arrangements of the apparatus

(i) Laser: a monochromatic laser polarized with its electric field component perpendicular to the plane formed by the incident light and light-receiving optical axes (vertical polarization).

(ii) Sample holder: a holder whose temperature can be measured and controlled within an accuracy of ±0.3°C.

(iii) Measuring cell: a rectangular or cylindrical cell made of optical glass or optical plastic, which can be placed in the sample holder. The cell is integrated with the sample holder in some apparatus.

(iv) Light-receiving optics and detector: light optics and detector which capture the scattered light from the sample at a single scattering angle between 90° to 180° and convert the captured light to a photon pulse (digitized signal). In the case that a polarization analyzer is included, it shall be positioned so that the transmittance of the vertically polarized light is maximized.

(v) Correlator: a device which calculates the autocorrelation function from the number of photon pulses in a certain time.

(vi) Spectrum analyzer: a device which calculates the frequency power spectrum by performing Fourier transformation of the frequency components present in the scattered light fluctuation signals.

(vii) Computation unit: data processor for determining the particle diameter distribution from the autocorrelation function obtained by the correlator or from the frequency power spectrum. Some computation units also function as a correlator or spectrum analyzer.

2.2. Validation and reproducibility of the instrument

Because the particle diameter obtained by dynamic light scattering is not a relative value calculated using standard particles but an absolute value based on a fundamental principle, calibration of the value is unnecessary.

However, it is necessary to confirm the performance of the instrument by using particles with certified diameter, when the instrument is first installed or if abnormal performance is suspected. In addition, it is desirable to confirm the proper performance of the instrument at least every year thereafter.

As standard particles of known diameter, polystyrene latex particles with a narrow distribution of diameter shall be used, whose average particle size is certified to be approximately 100 nm as determined by dynamic light scattering. The measured average diameter of these particles must be within 2% of the stated diameter range, and the relative standard deviation must be less than 2%. In addition, the measured polydispersity index must be less than 0.1.

3. Measurement

3.1. Choice of the dispersion liquid

The dispersion liquid shall fulfill all of the following requirements.

(i) It shall be non-absorbing at the wavelength of the laser.

(ii) It shall not cause damage such as corrosion to the materials of the instrument.

(iii) It shall not dissolve, swell or coagulate the particles.

(iv) It shall have a refractive index different from that of the particulate material.

(v) Its refractive index and viscosity shall be known within an accuracy of 0.5%.

(vi) It shall be clean enough not to interfere with the measurements.

3.2. Cleaning the measuring cell

The degree of cell washing required depends on the conditions of the measurement.

When an individually packaged clean disposable cell is used, cleaning by blowing off dust with compressed clean air is sufficient. When a cell is intended to be washed rigorously, the cell is fully rinsed beforehand with water to remove water-rinsable adhesion substances and is washed with a nonabrasive detergent.

3.3. Sample preparation

It is necessary to prepare a sample whose concentration is within an appropriate range to eliminate the influence of the multiple scattering of light. In addition, it is important to remove dust, which may affect the measurement, and to prevent their re-introduction during the preparation.

When the sample is shaken, dust-laden air is entrapped in the sample and air is dissolved in the solvent. The invisible small air bubbles scatter light more strongly than do the sample particles to be measured. It is necessary not to shake the sample violently after preparation, but to swirl it gently. A homogeneous sample solution can be prepared quickly by adding diluent to the concentrated sample droplet rather than dropping the sample droplet into the diluent.

3.4. Measurement procedure

1) Switch the instrument on and allow it to warm up. A period of approximately 30 minutes is typically required for stabilizing the laser intensity and bringing the sample holder to the desired temperature.
2) Choose the appropriate dispersion liquid, and record the count rate or the amplitude of the signals of scattered light fluctuation from the dispersion liquid.
3) Place the sample containing the dispersed particles in the instrument, and wait until temperature equilibrium is established between the sample and the sample holder. It is desirable to control and measure the temperature within an accuracy of ±0.3°C.
4) Perform a preliminary measurement of the sample, and set the particle concentration within the appropriate range based on 5.2.
5) Perform the measurement with the appropriate meas-

uring time and number of integrations.

6) Record the average particle diameter and the polydispersity index for each measurement.
7) If the measured values are dependent on the particle concentration, adopt the extrapolated infinite dilution values of the average particle diameter and the polydispersity index (or the measured values at the lowest particle concentration).
8) Confirm that no significant sedimentation has occurred in the sample at the end of the measurement. The presence of sediment indicates that the sample may have aggregated or precipitated, or that the sample may be unsuitable for measurement by dynamic light scattering.
9) Perform the measurement for each sample at least three times.

3.5. Repeatability

The repeatability of the determination of the average particle diameter, evaluated in terms of relative standard deviation, must be less than 5%.

4. Data analysis

The dispersion that is the target for the measurement is irradiated with the laser light. Phases of the light scattered by each particle fluctuate because the dispersed particles are in Brownian motion. The observed scattered intensity, which is the sum of the scattered light (result of interference), fluctuates along the time axis. Analyzing the fluctuation of the scattered light intensity as a function of time provides information on the motion of the dispersed particles.

Analysis by photon correlation spectroscopy is performed using the autocorrelation function of the scattered light intensity. This autocorrelation function depend only on the time difference (correlation time) and is independent of the time at which the measurement is started. For a large number of monodisperse particles in Brownian motion in a scattering volume, the autocorrelation function of the scattered light intensity is basically an exponential decay function of the correlation time. Polydispersity index is a parameter indicating the distribution of the decay constant, and is also a scale indicating the broadness of the distribution of particle diameter.

Frequency analysis is performed using the frequency power spectrum calculated from the scattered light intensity. The amplitude of the frequency power spectrum is proportional to the scattered light intensity and the concentration of the sample, and the characteristic frequency is inversely proportional to the particle diameter. The decay constant and the characteristic frequency are related to the translational diffusion coefficient of homogeneous spherical particles in Brownian motion. The diffusion constant of the spherical particles dispersed in the dispersion liquid is related to the particle diameter according to the Stokes-Einstein equation in the absence of inter-particle interaction. The polydispersity index determined by frequency analysis is a measure of the broadness of the particle diameter distribution calculated from the particle diameter distribution based on the scattered light intensity, and might differ from the polydispersity index determined by photon correlation spectroscopy.

Records of data shall include the average particle diameter and polydispersity index, and in addition, shall also state the principle of measurement (photon correlation spectroscopy or frequency analysis), optical configuration (homodyne or heterodyne), observation angle, temperature of the sample, refractive index and viscosity of the dispersion liquid, measuring time or number of integrations, and sample concentration.

5. Points to note regarding the measurement

5.1. Shape of particles

The particles are assumed to be homogeneous and spherical in the data analysis of dynamic light scattering.

5.2. Particle concentration

For measurement, it is necessary to prepare a sample whose concentration falls in the range satisfying the following conditions.

(i) The sample consists of dispersion liquid and particles well-dispersed in the liquid.

(ii) The range of the particle concentration is determined so that consistent results can be obtained in particle diameter measurements. The range is determined beforehand based on measurements of systematically diluted samples.

5.3. Purification of the dispersion liquid

Scattered light signals from the dispersion liquid used for sample dilution must normally be undetected or very weak. If the situations described in cases (i) or (ii) below are found, particulate substances are likely to have become mixed in the sample, and in such cases the dispersion liquid shall be further purified (by filtration, distillation, and so on) before use. The lower limit of the particle concentration is determined mainly so that scattered light from the dispersion liquid and contaminating substances will not affect the measurement. When water is chosen as the dispersion liquid, use of fresh distilled water (prepared by quartz-glass distillation) or desalted and filtered (pore size 0.2 μm) water is recommended.

(i) Large fluctuations of the count rate or of the amplitude of the scattered light fluctuation signals, accompanied by abnormally strong signals, are recorded.

(ii) Light spots appear in the path of the laser light in the sample.

5.4. Others

(i) When particles are highly charged with electricity, long-range interactions between the particles may affect the measurement result, and in such cases, a small amount of salt (for example, sodium chloride: around 10^{-2} mol/L) may be added to the dispersion liquid to reduce the effect.

(ii) Traceable polystyrene latex particles for use in the validation of the instrument are commercially available.

Reference

1) JIS Z8826: 2005 Particle size analysis—Photon correlation spectroscopy
2) ISO 13321: 1996 Particle size analysis—Photon correlation spectroscopy
3) ISO 22412: 2008 Particle size analysis—Dynamic light scattering (DLS)

Add the following:

Water-Solid Interactions: Determination of Sorption-Desorption Isotherms and of Water Activity

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

◆The powder of drug as drug substances or preparations often contacts with water during the production process or storage. For the assessment of the water-solid interactions, determinations of sorption-desorption isotherms and water activity are used. Water can be interacted physically with solid in two ways, by a sorption onto the surface of solid or an absorption permeating into the solid. In the case where both the sorption and absorption are occurred, the term ''absorption'' is usually used.◆

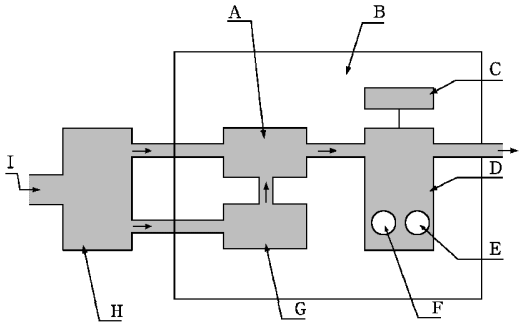
1. Determination of Sorption-Desorption Isotherms

1.1. Principle

The tendency to take up water vapour is best assessed by measuring sorption or desorption as a function of relative humidity, at constant temperature, and under conditions where sorption or desorption is essentially occurring independently of time, i.e. equilibrium. Relative humidity, *RH*, is defined by the following equation:

$$RH = (P_c/P_0) \times 100$$

P_c: pressure of water vapour in the system
P_0: saturation pressure of water vapour under the same conditions



A. Humidity controller
B. Temperature controlled chamber
C. Balance module
D. Humidity regulated module
E. Reference
F. Sample
G. Vapour humidifier
H. Flow control module
I. Dry gas

Fig. 1 Example of an apparatus for the determination of the water sorption (other designs are possible)

The ratio P_c/P_0 is referred to as the relative pressure. Sorption or water uptake is best assessed starting with dried samples and subjecting them to a known relative humidity. Desorption is studied by beginning with a system already containing sorbed water and reducing the relative humidity. As the name indicates, the sorption-desorption isotherm is valid only for the reference temperature, hence a special isotherm exists for each temperature. Ordinarily, at equilibrium, moisture content at a particular relative humidity must be the same, whether determined from sorption or desorption measurements. However, it is common to see sorption-desorption hysteresis.

1.2. Methods

Samples may be stored in chambers at various relative humidities. The mass gained or lost for each sample is then measured. The major advantage of this method is convenience, while the major disadvantages are the slow rate of reaching constant mass, particularly at high relative humidities, and the error introduced in opening and closing the chamber for weighing. Dynamic gravimetric water sorption systems allow the on-line weighing of a sample in a controlled system to assess the interaction of the material with moisture at various programmable levels of relative humidity at a constant temperature. The major benefit of a controlled system is that isothermal conditions can be more reliably established and that the dynamic response of the sample to changing conditions can be monitored. Data points for the determination of the sorption isotherm (e.g. from 0 per cent to approximately 95 per cent *RH*, non condensing) are only taken after a sufficiently constant signal indicates that the sample has reached equilibrium at a given level of humidity. In some cases (e.g. deliquescence), the maximum time may be restricted although the equilibrium level is not reached. The apparatus must adequately control the temperature to ensure a good baseline stability as well as accurate control of the relative humidity generation. The required relative humidities can be generated, e.g. by accurately mixing dry and saturated vapour gas with flow controllers. The electrostatic behaviour of the powder must also be considered. The verification of the temperature and the relative humidity (controlled with, for example, a certified hygrometer, certified salt solutions or deliquescence points of certified salts over an adequate range), must be consistent with the instrument specification. The balance must provide a sufficient mass resolution and long term stability.

It is also possible to measure amounts of water uptake not detectable gravimetrically using volumetric techniques. In the case of adsorption, to improve sensitivity, one can increase the specific surface area of the sample by reducing particle size or by using larger samples to increase the total area. It is important, however, that such comminution of the solid does not alter the surface structure of the solid or render it more amorphous or otherwise less ordered in crystallinity. For absorption, where water uptake is independent of specific surface area, only increasing sample size will help. Increasing sample size, however, will increase the time to establish some type of equilibrium. To establish accurate values, it is important to get desolvation of the sample as

thoroughly as possible. Higher temperatures and lower pressures (vacuum) facilitate this process; however, one must be aware of any adverse effects this might have on the solid such as dehydration, chemical degradation or sublimation. Using higher temperatures to induce desorption, as in a thermogravimetric apparatus, likewise must be carefully carried out because of these possible pitfalls.

1.3. Report and interpretation of the data

Sorption data are usually reported as a graph of the apparent mass change in per cent of the mass of the dry sample as a function of relative humidity or time. Sorption isotherms are reported both in tabular form and as a graph. The measurement method must be traceable with the data.

Adsorption-desorption hysteresis can be interpreted, for example, in terms of the porosity of the sample, its state of agglomeration (capillary condensation), the formation of hydrates, polymorphic change, or liquefying of the sample. Certain types of systems, particularly those with microporous solids and amorphous solids, are capable of sorbing large amounts of water vapour. Here, the amount of water associated with the solid as relative humidity is decreased, is greater than the amount that originally sorbed as the relative humidity was increased. For microporous solids, vapour adsorption-desorption hysteresis is an equilibrium phenomenon associated with the process of capillary condensation. This takes place because of the high degree of irregular curvature of the micropores and the fact that they ''fill'' (adsorption) and ''empty'' (desorption) under different equilibrium conditions. For non-porous solids capable of absorbing water, hysteresis occurs because of a change in the degree of vapour-solid interaction due to a change in the equilibrium state of the solid, e.g. conformation of polymer chains, or because the time scale for structural equilibrium is longer than the time scale for water desorption. In measuring sorption-desorption isotherms, it is therefore important to establish that something close to an equilibrium state has been reached. Particularly with hydrophilic polymers at high relative humidities, the establishment of water sorption or desorption values independent of time is quite difficult, since one is usually dealing with a polymer plasticised into its ''fluid'' state, where the solid is undergoing significant change.

In the case of crystal hydrate formation, the plot of water uptake versus pressure or relative humidity will in these cases exhibit a sharp increase in uptake at a particular pressure and the amount of water taken up will usually exhibit a stoichiometric mole:mole ratio of water to solid. In some cases, however, crystal hydrates will not appear to undergo a phase change or the anhydrous form will appear amorphous. Consequently, water sorption or desorption may appear more like that seen with adsorption processes. X-ray crystallographic analysis and thermal analysis are particularly useful for the study of such systems.

For situations where water vapour adsorption occurs predominantly, it is very helpful to measure the specific surface area of the solid by an independent method and to express adsorption as mass of water sorbed per unit area of solid surface. This can be very useful in assessing the possible importance of water sorption in affecting solid properties. For example, 0.5 per cent *m/m* uptake of water could hardly cover the bare surface of 100 m^2/g, while for 1.0 m^2/g this amounts to 100 times more surface coverage. In the case of pharmaceutical solids which have a specific surface area in the range of 0.01 m^2/g to 10 m^2/g, what appears to be low water content could represent a significant amount of water for the available surface. Since the ''dry surface area'' is not a factor in absorption, sorption of water with amorphous or partially amorphous solids can be expressed on the basis of unit mass corrected for crystallinity, when the crystal form does not sorb significant amounts of water relative to the amorphous regions.

2. Determination of The Water Activity

2.1. Principle

Water activity, A_w, is the ratio of vapour pressure of water in the product (P) to saturation pressure of water vapour (P_0) at the same temperature. It is numerically equal to 1/100 of the relative humidity (*RH*) generated by the product in a closed system. *RH* can be calculated from direct measurements of partial vapour pressure or dew point, or from indirect measurement by sensors whose physical or electric characteristics are altered by the *RH* to which they are exposed. Ignoring activity coefficients, the relationship between A_w and equilibrium relative humidity (*ERH*) are represented by the following equations:

$$A_w = P/P_0$$

$$ERH \text{ (per cent)} = A_w \times 100$$

2.2. Method

The water activity is determined by placing the sample in a small airtight cup inside which the equilibrium between the water in the solid and the headspace can be established. The volume of the headspace must be small in relation to the sample volume in order not to change the sorption state of sample during the test. The equilibration as a thermodynamic process takes time but may be accelerated by forced circulation within the cell. The acquired water activity value is only valid for the simultaneously determined temperature. This requires a precise temperature-measuring device as part of the equipment. Furthermore, the probe must be thermally insulated to guarantee a constant temperature during the test. The sensor measuring the humidity of the headspace air above the sample is a key component. Theoretically, all types of hygrometers can be used, but for analytical purposes miniaturisation and robustness are a precondition. The A_w measurement may be conducted using the dew point/chilled mirror method[1]. A polished, chilled mirror is used as a condensing surface. The cooling system is electronically linked to a photoelectric cell into which light is reflected from the condensing mirror. An air stream, in equilibrium with the test sample, is directed at the mirror which cools until condensation occurs on the mirror. The temperature at which this condensation begins is the dew point from which the *ERH* is determined. Commercially available instruments using the dew point/chilled mirror method or other technologies need to be evaluated for suitability, validated, and

Table 1 Standard saturated salt solutions

Saturated salts solutions at 25°C	*ERH* (per cent)	A_w
Potassium sulphate (K_2SO_4)	97.3	0.973
Barium chloride ($BaCl_2$)	90.2	0.902
Sodium chloride (NaCl)	75.3	0.753
Magnesium nitrate ($Mg(NO_3)_2$)	52.9	0.529
Magnesium chloride ($MgCl_2$)	32.8	0.328
Lithium chloride (LiCl)	11.2	0.112

calibrated when used to make water activity determinations.

These instruments are typically calibrated over an adequate range, for example, using some saturated salt solutions at 25 °C such as those listed in Table 1.

[1] AOAC International Official Method 978.18.

G3 Biotechnological/Biological Products

Mass Spectrometry of Peptides and Proteins

Change to read as follows:

Mass spectrometry (MS) is based on the ionization of molecules and separation of the electrically charged ions according to the dimensionless quantity, *m/z* value, which is obtained by dividing the relative mass (*m*) of the ion to unified atomic mass unit by the charge number (*z*) of the ion. The unified atomic mass unit is defined as one twelfth of the mass of ground state ^{12}C and used to express the mass of atom, molecule and ion. The results are expressed as a mass spectrum with *m/z* values of the ions on the x-axis and signal intensity of the ions on the y-axis. The mass of the molecule calculated from the *m/z* values is expressed in unified atomic mass units (u) or daltons (Da). Tandem mass spectrometry (MS/MS) is based on the fragmentation of the precursor ion selected in the first stage mass analysis and measurement of the product ions in the second stage mass analysis. This technique provides useful information for structural analysis of the molecule. Information obtained in MS is qualitative and is sometimes used for qualification. MS and MS/MS are useful for measuring masses of peptides and proteins and for confirming amino acid sequences and post-translational modifications. Both methods are therefore used for identification of pharmaceutical peptides and proteins.

1. Instrument

A mass spectrometer is composed of an ion source, an analyzer, an ion detector, and a data system (Fig. 1). A peptide and protein sample introduced into the ion source is ionized by soft-ionization methods, such as matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). The charged and gas phased ions are sorted according to the *m/z* ratio under a vacuum in the analyzer, which may be a quadrupole, time-of-flight, ion trap or Fourier transform ion cyclotron resonance analyzer. The ion flux collected in the detector is converted to an electric signal. Then the signal is recorded as a mass spectrum. MS/MS is carried out by using two mass spectrometers connected in series, an ion-trap mass spectrometer and Fourier transform ion cyclotron resonance mass spectrometer. The precursor ions are generally fragmented by collision-induced dissociation, post-source decay, electron capture dissociation, etc.

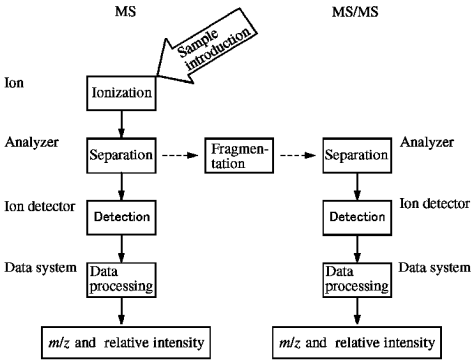


Fig. 1 Schematic diagram of mass spectrometry (MS) and tandem mass spectrometry (MS/MS)

2. Analytical mode

2.1. MS

There are two useful modes for MS:

(1) Total ion monitoring

The signals of the entire ion are acquired over the chosen range of *m/z* value. This mode provides information on the masses of the molecule of interest and different species.

(2) Selected ion monitoring

The signals of the ion at chosen *m/z* value are acquired. This mode is useful for the sensitive measurement of the chosen molecule.

2.2. MS/MS

There are four essential modes for MS/MS:

(1) Product ion analysis

The signals of all the product ions produced from the precursor at chosen *m/z* value are acquired. This mode provides structural information on the substrates and various co-existing species.

(2) Precursor ion scan mode

The signals of the precursor that yields the product ion at chosen *m/z* value are monitored. This mode is used for sorting the molecules containing a component of interest.

(3) Constant neutral loss scan mode

The signals of the precursor that loses the fragment at chosen *m/z* value are monitored. This mode is useful to sort the molecules containing a component of interest.

(4) Selected reaction monitoring

The signals of product ions at chosen *m/z* value that are produced from the precursor at chosen *m/z* value are monitored. This mode allows for sensitive and selective measure-

ment and is used for quantification of a molecule in a complex mixture.

3. Analytical procedure

3.1. MS

In advance, it should be conformed if the detectability and the difference between the calculated mass and observed mass meet the criteria stated in the monograph by mass measuring using a test solution specified in the system suitability in the monograph. If they do not meet the criteria, the system should be optimized by adjustment of the voltage of the ion source, analyzer and detector, as well as by calibration using appropriate mass calibrator. MS is performed according to the sample preparation and operating conditions indicated in the monograph. The general procedure is described as follows.

(1) Matrix-assisted laser desorption/ionization (MALDI)

A desalted peptide and protein sample is dissolved in an appropriate solvent, e.g., an aqueous solution of trifluoroacetic acid. A suitable matrix, such as α-ciano-4-hydroxycinnamic acid, 2,5-dihydroxybenzoic acid, or sinapic acid, is dissolved in an aqueous solution containing acetonitrile and trifluoroacetic acid. A mixture of sample solution and matrix solution is deposited on a sample plate and dried. The sample on the plate is set in the ion source, and ionized by a laser beam at suitable intensity.

(2) Electrospray ionization (ESI)

A desalted peptide and protein sample is dissolved in a suitable solvent, such as an aqueous solution containing acetic acid and methanol or acetonitrile. The sample solution is sprayed through a needle tip and held at a potential of several kilovolts. The sample is introduced by using a syringe or HPLC.

3.2. MS/MS

System suitability is tested by MS/MS of the test sample specified in the monograph. The detectability and system performance should be confirmed based on the detection of the product ions specified in the monograph. The sample is ionized in the same way as for MS, and the chosen precursor is fragmented by the suitable conditions specified in the monograph. The signals are recorded as a mass spectrum. A peptide containing disulfide bonds is generally reduced by dithiothreitol, 2-mercaptethanol and tris (2-carboxyethyl) phosphine. The reduced peptides are derivatized with monoiodoacetic acid, iodoacetamide, and 4-binylpyridine.

4. Identification test

4.1. Mass of the molecule

The monoisotopic mass of the peptide is preferably acquired. If the monoisotopic mass is not detectable, the average mass is calculated from the top of the ion peak. Deconvolution is effective for calculating the average mass of multiply-charged ions from proteins. The mass should meet the criteria specified in the monograph.

4.2. Amino acid sequence

After measuring the mass of the sample peptide, the presence of the specified product ions that arise from the selected precursor is confirmed according to the conditions indicated in the monograph. Digestion of sample proteins with a suitable enzyme followed by MS/MS is sometimes effective for sequencing of the high-molecular weight proteins which provide insufficient product ions. Details of the digestion procedure are provided in the section on peptide mapping.

5. Glossary

Ion-trap (IT)

Ion-trap refers to the quadrupole ion trap mass analyzer in a restricted sense. Ions stored in the analyzer by applying radio frequency voltage to ring electrodes are separated by subsequent ejection of the ions from the analyzer by varying the voltage on the ring electrodes. This allows multiple stage MS in which a selected ion is repeatedly trapped, fragmented and ejected.

Electrospray ionization (ESI)

The sample in solution is sprayed through a needle tip and held at high-voltage at atmospheric pressure. The sample is ionized by a formation of charged liquid droplets. High-molecular mass proteins are detected as multiply-charged ions. The analyzer can be connected with HPLC.

Quadrupole (Q)

The analyzer is composed of four parallel electrodes which have a hyperboloidal or cylindrical cross-section. The ions transmitted to the analyzer are separated by varying the potential of direct and radio frequency components applied to the rods so that the filter for sorting the m/z values of ions is changed.

Collision-induced dissociation (CID)

When an ion collides with a neutral atom or molecule (He, Ar, N_2 and so on), some of the translational energy of the collision is converted into internal energy, thereby causing dissociation. The terms low-energy CID and high-energy CID refer to those CIDs for which the translational energy of the precursor ions is lower than 1000 eV and higher than 1000 eV, respectively.

Electron capture dissociation (ECD)

Multiply-charged positive ions interact with low energy electrons producing charge-reduced radical ions, which readily dissociate. This method is primarily used for MS/MS in FT-ICR MS or IT MS.

Time-of-flight (TOF)

The ionized sample is accelerated at high-voltage and separated based on the time required for an ion to travel to the detector. There are two types of analyzer, a linear type in which ions travel linearly from the ion source to the detector, and a reflectron type where ions are inverted by a reflectron. The latter type allows high-resolution measurement by correction of the variation in the initial energy of ions.

Fourier transform ion cyclotron resonance (FT-ICR)

The analyzer is based on the principle that the cyclotron frequency of the ions in a magnetic field is inversely proportional to its m/z value. Ions are excited to a larger radius orbit using radio frequency energy and their image current is detected on receiver plates. The resulting data are devolved

by applying a Fourier transform to give a mass spectrum.

Post-source decay (PSD)

Metastable ion decay occurs by excess internal energy and collision with residual gas during ion acceleration out of the MALDI ion source and prior to reaching the detector. This method is used for MS/MS by using MALDI-TOF MS with a reflectron mode.

Matrix-assisted laser desorption/ionization (MALDI)

The sample, which is mixed with a suitable matrix and deposited on a target plate, is ionized by irradiation with nanosecond laser pulses. Proteins, carbohydrates, oligonucleotides, and lipids can be ionized without any dissociation. Singly-charged ions are mainly detected.

G4 Microorganisms

Microbiological Evaluation of Processing Areas for Sterile Pharmaceutical Products

Change to read as follows:

Microbiological Environmental Monitoring Methods of Processing Areas for Sterile Pharmaceutical Products

This chapter describes the methods for the evaluation of air-cleanliness and the recommended limits for environmental microorganisms. The main purposes of this chapter are 1) to confirm that the designed cleanliness levels and microbial limits are attained and maintained in processing areas for sterile pharmaceutical products, and 2) to confirm that the number of particulates and microorganisms are appropriately controlled in the processing environment for sterile pharmaceuticals.

In reference to the evaluation methods and the recommended limits described in this chapter, a risk assessment should be formed for each manufacturing facility and acceptance criteria should be established based on the identified risks. Alternative measuring methods can be applied on rational grounds.

1. Definitions

For the purposes of this chapter, the following definitions apply:

(i) Action level: An established number of objects to be monitored (and species of microorganisms, if appropriate) that requires immediate investigation and corrective action based on the investigation when exceeded.

(ii) Alert level: An established number of objects to be monitored (and species of microorganisms, if appropriate) that gives early warning of potential problems.

(iii) Aseptic processing: Filling of sterile products and other operations performed under the environmental conditions in which air supply, materials, equipment, and personnel are regulated to control microbial and particulate contamination to acceptable levels.

(iv) Aseptic processing area: The classified part of a facility in which air supply, materials, equipment, and personnel are highly regulated to control microbial and particulate contamination to acceptable levels. The area is classified into two categories: Grade A and Grade B.

(v) Microorganisms: General term for bacteria, fungi, protozoa, viruses, etc. In this chapter, microorganisms indicate only bacteria and fungi.

(vi) Shift: Scheduled period of work or production during which operations are conducted by a single or defined group of workers.

(vii) Risk assessment: A series of processes including identification, analysis, and evaluation of hazards that may cause harm in accordance with ICH Q9, ''Quality Risk Management.'' In this chapter, ''harm'' indicates contamination of products or manufacturing areas; ''hazards'' indicates possible causes of the contamination, such as personnel, environment, or operations carried out. Risk is expressed as a combination of the probable incidence and severity of the harm.

(viii) Calibration: The act of establishing the relationship between values indicated by a measuring instrument and the values represented by a material measure, by comparison with the corresponding known values of a standard instrument or a standard reference material and of adjusting the accuracy of the measuring instrument for the proper use.

(ix) At rest: The state in which production equipment is installed and operating, with no operating personnel present.

(x) In operation: The state in which the installed equipment is functioning in the defined operating mode with the specified number of personnel working.

2. Processing areas

Processing areas refer to areas in which actions such as cultivation, extraction/purification, washing and drying of containers and stoppers, weighing of raw materials, preparation of solutions, sterilization, filling, sealing, and packaging are performed, including the gowning area.

The processing areas for sterile pharmaceutical products are maintained and controlled to prevent containers, raw materials, and in-process products from microbial and particulate contamination.

Personnel engaged in such activities in the areas should receive necessary training in hygiene, microbiology, manufacturing technology, and clothing.

2.1. Classification of processing areas

(i) Grade A: A local area in which operational activities to prevent contamination risks of products at a high level are conducted. For pharmaceutical products prepared aseptically, the area is designed to preserve sterility of sterilized pharmaceutical products, containers, and closures that are exposed within it. In this area, manipulations of sterile materials prior to filling operation (e.g. aseptic connections,

sterile ingredient additions), filling, and closing operations are conducted.

(ii) Grade B: A multipurpose area in which operational activities to prevent contamination risks of products at a comparably high level are conducted. For pharmaceutical products prepared aseptically, the area is used as a route to load sterilized containers, raw materials, and in-process products that are stored to preserve sterility. Areas in which personnel, equipment and apparatuses that directly come into aseptic processing areas exist are also classed as Grade B. In a general clean room, this is the surrounding environment for the Grade A area. When contamination risks of microorganisms derived from the environment are low, for example, where isolators are installed so that the levels of human intervention and exposure are low, the surrounding area dose not necessary qualify as Grade B.

(iii) Grades C, D: Areas to prevent contamination risks of products at a comparably low level. Activities conducted in such areas include operational activities of non-sterile containers, raw materials, and in-process products that are exposed to the surrounding environment, and cleaning of equipment and apparatuses for aseptic processing. When contamination risks of microorganisms derived from the environment are low, for example, where isolators are installed so that the levels of human intervention and the exposure are low, these areas can be used as the surrounding areas.

2.2. Environmental control level by processing area

Airborne particulates in areas used for processing of pharmaceutical products may be a key indicator to monitor performance of air-conditioning systems. They may act physically as a source of insoluble particulates in the products, and biologically as a carrier of microorganisms.

In areas used for the processing of pharmaceutical products, therefore, the number of airborne particulates, as well as the number of microorganisms, should be controlled within the specified limits. Air volume, airflow pattern, frequency of ventilation, and material and personnel flow are appropriately designed so that airborne particulates that exist in the areas can be effectively discharged.

The air-cleanliness and the recommended limits for environmental microorganisms for each grade area are shown in Table 1 and Table 2.

Compared with the classifications in ISO/DIS 14644-1, (2010) the maximum number of airborne particulates in Grade A, B, and C (in operation) are almost identical to those of ISO 5, ISO 7, and ISO 8, respectively.

When the number of sampling points is determined in order to classify manufacturing areas based on the defined cleanliness levels, Table 3 can be used as a reference. Sampling points that are evenly distributed throughout the area to be monitored should be selected. The height at which operational activities are conducted in the area should be also considered. Addition of sampling points can also be effective, based on the risks.

The sampling points specified in ISO/DIS 14644-1 (2010) are shown in Table 3.

For design qualification of Grade A area, a minimum sample volume of 1 m^3 should be taken per measurement.

The monitoring of $\geq 5.0\ \mu m$ airborne particulates and airborne microorganisms on settle plates are performed, if necessary.

3. Environmental monitoring program

For the manufacture of sterile pharmaceutical products, it is necessary to predict potential deterioration of the processing environment before it occurs and to prevent any adverse effect on the quality of products. The environmental monitoring program should include all necessary items to verify whether air-cleanliness in each production area is constantly maintained. The items included in the program should be determined in reference to Sections 3.1 to 3.6 in

Table 1 Air-cleanliness

Grade	Maximum permitted number of airborne particulates (number/m^3)			
	at rest*1		in operation	
Size	$\geq 0.5\ \mu m$	$\geq 5.0\ \mu m$	$\geq 0.5\ \mu m$	$\geq 5.0\ \mu m$
A	3520	20	3520	20
B	3520	29	352000	2900
C	352000	2900	3520000	29000
D	3520000	29000	....*2	....*2

*1 The number of particulates given in the table for the ''at rest'' condition should be achieved 15 – 20 minutes after the completion of operations.

*2 The number will depend on the nature of the operation carried out.

Table 2 Recommended Limits for Environmental Microorganisms (in operation)*1

Grade	Airborne microorganisms		Microorganisms on surfaces	
	air sample (CFU/m^3)	settle plates*2 (CFU/plate)	contact plates (CFU/24 – 30 cm^2)	gloves (CFU/5 fingers)
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	....
D	200	100	50	....

*1 These are average values.

*2 The exposure time of each plate should be less than four hours. Monitoring should be performed throughout operations.

this chapter. An environmental monitoring program should be prepared for each facility. All personnel engaged in the environmental monitoring program should receive sufficient training in hygiene control, microbiology, measurement principles, measurement procedures, and gowning procedures.

3.1. Applicability

Microorganisms and airborne particulates should be monitored. Microorganisms to be monitored are bacteria and fungi, and airborne particulates to be monitored are those $\geq 0.5\ \mu m$ in size.

Table 3 Minimum sampling points based on an area of clean rooms

Area of clean rooms (m^2) equal to or less than	Minimum sampling points
1	1
2	1
4	2
6	3
8	4
10	5
24	6
28	7
32	8
36	9
52	10
56	11
64	12
68	13
72	14
76	15
104	16
108	17
116	18
148	19
156	20
192	21
232	22
276	23
352	24
436	25
500	26

3.2. Frequency of monitoring

In the processing areas used for sterile pharmaceutical products, monitoring of airborne particulates and microorganisms is required. The Grade A area, in which sterile products are in contact with environmental air, should be monitored during every operational shift. Recommended frequencies of monitoring during operation are given in Table 4. The frequencies are set for general and conventional aseptic processing. In individual cases, appropriate monitoring frequency should be determined based on the results of risk assessment. In particular, the risks of contamination of products should be considered when determining monitoring frequency of airborne microorganisms in Grade A and B areas. The monitoring frequency should be adequate for the assessment of potential effect. When high contamination risks of products are concerned, for example, where products are exposed to the environment for a long time or operational activities are frequently performed in a Grade A area, such areas should be more frequently monitored.

In contrast, in manufacturing operations in which isolator, RABS (Restricted Access Barrier System), or brow/fill/seal units are used, monitoring frequency may be reduced due to lower contamination risks to the products from human and the environment.

3.3. Monitoring points

The items to be monitored include air, floors, walls, equipment surfaces, gloves, and gowns in the processing areas. When selecting monitoring points to be included in the environmental monitoring program, the following points should be included: the points where critical operations are performed, where a contamination risk is considered high, and points that represent the cleanliness levels of the manufacturing area.

Regular monitoring points in the manufacturing area should be determined based on the risk assessment and the data obtained in monitoring for cleanliness classification;

Table 4 Recommended Frequency of Environmental Monitoring

Grade		airborne particulates	airborne microorganisms	microorganisms on surfaces	
				instruments, walls etc.	gloves, gowns
A		In operation	For each shift	At the completion of each operation	At the completion of each operation
B		In operation	For each shift	At the completion of each operation	At the completion of each operation
C, D*	Areas in which products and containers are exposed to the surrounding environment	Once a month	Twice a week	Twice a week	----
	Other areas	Once a month	Once a week	Once a week	----

* When a contamination risk is low, for example, where products are not exposed to the surrounding environment, monitoring frequency may be reduced accordingly.

e.g., the near vicinity (e.g. within 30 cm) of a site where products are exposed to the surrounding environment, a site that is prone to potential sources of contamination due to frequent human interventions and traffic or due to susceptibility to lower cleanliness levels, or a site regarded as worst-point based on the airflow analysis.

3.4. Monitoring methods

Appropriate methods should be selected according to the items to be monitored. Consideration should be given to potential contamination risks increased by interventions of personnel who are involved in sampling and disturbance of airflow during sampling.

For monitoring of airborne microorganisms, there are two types of microbial sampling methods: active sampling methods and passive sampling methods. Various types of culture medium and culture methods are available for different types of microorganisms to be monitored. For details, refer to Section 5, ''Measurement of microorganisms'' in this chapter.

3.5. Environmental control criteria

Establishment of an alert level for each item to be monitored can lead to early detection of performance degradation of facilities. It is also useful to control risks. In environmental monitoring, it is important to evaluate whether the specified cleanliness level for a monitored object is constantly maintained. The measured values obtained by environmental monitoring are averaged. The contamination risks are evaluated based on the averaged values and should not be underestimated.

In a case where bacteria are detected in a Grade A area, assessment of potential effect on the products should be carried out. Surfaces and personnel should be monitored after the completion of critical operations.

Measurement of $\geq 5.0\,\mu$m airborne particulates in Grade A and B areas is useful for early detection of abnormalities in the environment. When $\geq 5.0\,\mu$m airborne particulates are continuously or frequently detected, even if the number is low, further investigation should be encouraged due to possible abnormalities that may have impact on the environment.

3.6. Evaluation of monitoring data and measures to be taken when the limit is exceeded

Environmental monitoring data should be evaluated both in the short-term and the long-term. The following items should be included in the evaluation:

(i) Changes in numbers of microorganisms and airborne particulates over a period of time

(ii) Changes in detected species of microorganisms

(iii) Changes of monitoring points

(iv) Review of the validity of alert and action levels

(v) Review of frequency of positive results from each operator

(vi) Changes that may impact the monitoring results during the monitoring periods

Trend analysis of environmental monitoring data will provide information required to predict potential deterioration of the manufacturing environment before it occurs and to determine its probable causes. Information that may impact the environment, such as monitoring location, date and time, product manufactured during the monitoring period, batch number, personnel in operation, is also important.

In the event of any deviation found in the environmental monitoring data, actions to be taken for the products manufactured and measures to be taken to recover the required cleanliness of the environment should be determined with consideration of the nature of activities performed at the time, distance between the product and the site where the deviation was found, and the severity of the deviation.

4. Measurement of particulates

For measurement of particulates, particle counters that can detect particulates of different sizes are used. In general, a particle counter is composed of an air suction pump, a sensor that discerns particle size from variations in the reflection of a laser beam, and a converter unit. When there is distance between a counter and a sampling point, sampling tubing is used. To measure distribution of particulates precisely, the inlet of the sampling probe is positioned parallel to airflow, and air is aspirated at the same velocity as the airflow.

For measurement of particulates, calibrated devices should be used. Consideration should be given to length, diameter of the tubing, and radii of any bends in the tubing, as well as to the device itself. Calibration items include flow rate, counting efficiency, false counts, and counting loss.

There are three types of methods for particulates monitoring; an independent particle counter is placed at individual monitoring point; a network of sequentially accessed monitoring points is connected by manifold to a single particle counter; or a combination of the two. In any method, the concentration of particulates in the predetermined particulate size range according to the cleanliness level of the area to be monitored should be indicated or recorded. When monitoring $\geq 5.0\,\mu$m particulates, a short length of sample tubing should be used, because of the relatively higher rate of precipitation of particulates of large size. For particulate monitoring, the selection of the monitoring system may take into account any health risks of operators presented by the materials used in the manufacturing operation (e.g. pathogens, radiopharmaceuticals, or strong sensitizers).

In general, continuous monitoring is recommended in a Grade A area. Sampling volumes that can be accurately converted to volume per m^3 should be applied.

5. Measurement of microorganisms

Measurement methods of microorganisms for environmental monitoring include active microbial sampling methods, measurement methods for microorganisms on surfaces, and settling plates. Various types of sampling devices and measurement methods are available for the sampling and measurement of microorganisms in the air and on surfaces. Appropriate samplers and measuring methodology should be selected according to the purpose of monitoring and the items to be monitored.

5.1. Measurement by cultivation

5.1.1. Active microbial sampling methods

Methods in which a fixed volume of air is aspirated and

the number of microorganisms in the air sampled is counted. There are filtration-type sampling devices and impact-type sampling devices.

Both methods have advantages and disadvantages. Capabilities of an air sampling device (air sample volume capacity, performance of microorganism collection, etc.) should be confirmed before use. When a device is used in a Grade A area, the following points needs to be confirmed before use: the device can effectively collect air; it is easily decontaminated or sterilized; it does not disturb unidirectional airflow.

An appropriate air sampling volume for active microbial measurement should be comprehensively determined on reasonable grounds, such as cleanliness level of the area to be monitored and monitoring frequency, etc. In a Grade A area, the air sample volume should be 1 m^3 at each sampling.
(i) Impact-type sampling method: When an impact-type sampling device is used, the speed at which the collected air strikes the culture medium surface must be sufficient to capture the microorganisms, but must not have an adverse effect on the collected microorganisms. The volume of air collected must not cause a significant change in the physical or chemical properties of the culture medium.

The most commonly used samplers are as follows: i) slit sampler, ii) Andersen sampler, iii) pinhole sampler, and iv) centrifugal sampler. Each sampler has specific characteristics. The slit sampler is a device to trap microorganisms in a known volume of air that is passed through a standardized slit. The air is impacted on a slowly revolving Petri dish containing a nutrient agar. The rotation rate of the Petri dish and the distance from the slit to the agar surface are adjustable, and it is possible to estimate the number of microorganisms in the air that passes through the device for a period of up to 1 hr. The Andersen sampler consists of a perforated cover and several Petri dishes containing a nutrient agar, and a known volume of air that is passed through the perforated cover impacts on the agar medium in the Petri dishes. The sampler is suitable for the determination of the distribution of size ranges of microorganism particulates in the air. The pinhole sampler resembles the slit sampler, but has pinholes in place of the slit. Microorganisms are collected by spraying a known volume of air through several pinholes onto agar medium in a slowly revolving Petri dish. The centrifugal sampler consists of a propeller that pulls a known volume of air into the device and then propels the air outward to impact on a tangentially placed nutrient agar strip. The sampler is portable and can be used anywhere, but the sampling volume of air is limited.
(ii) Filter-type sampling method: With the filter-type sampling devices, the desired volume of air can be collected by appropriately changing the air intake rate or the filter size. However, care must be taken to ensure that sterility is maintained while the filter is placed in and removed from the holder. There are two types of filters: wet-type with gelatin filters and dry-type with membrane filters. With the dry-type filters, static electricity effects can make it impossible to quantitatively collect microorganisms on the filter.

5.1.2. Measurement methods for microorganisms on surfaces

The area to be surface-sampled should be designated according to the condition and shape of the object to be monitored.
(i) Contact plates: A contact plate is used with an appropriate contact surface and sufficient area. In principle, the recommended sampling area for equipment or apparatuses is 24 – 30 cm^2.

The culture medium surface should be brought into contact with the sampling site for several seconds by applying uniform pressure without circular or linear movement. After contact and removal, the plates are covered, and as soon as possible, incubated under appropriate culture conditions. After a contact plate has been used, the site to which the plate was applied must be wiped aseptically to remove any adherent culture medium.
(ii) Swabs: A sterilized, lint-free swab that is suitable for collecting microorganisms is premoistened with an appropriate rinse fluid, and then sampling is conducted by swabbing the defined sampling area in a slow circular movement or in closely parallel strokes while changing direction. After sampling, the swab is agitated in a specified amount of an appropriate sterilized rinse fluid, and the rinse fluid is assayed for viable organisms according to Section 4.05 of the Microbiological Examination of Non-sterile Products.
(iii) Adhesive sheets: An adhesive sheet for sampling is evenly applied to the surface of the item to be monitored and removed. This process should be repeated several times for one sampling area. Microorganisms captured on the adhesive sheet are counted in an appropriate manner. Ultrasonic treatment can be applied to recover microorganisms into solution.

5.1.3. Settling plates (passive microbial sampling method)

Petri dishes of a specified diameter (petri dishes 9 cm in diameter are commonly used) containing a suitable culture medium are placed at the measurement location, and the cover is removed. The plates are exposed for a specified time and the microorganisms deposited from the air onto the agar surface are enumerated after incubation. This method is not effective for quantitative monitoring of total airborne microorganisms, because it does not detect microorganisms that do not settle onto the surface of the culture media, and the settling velocity of aggregates of microorganisms is affected by air currents and disturbances in airflow. Although the results obtained by the settle plate method are only qualitative or semi-quantitative, this method is suitable for long-term evaluation of possible contamination of products or devices by airborne microorganisms.

Before using this method, it should be ensured that the growth of microorganisms is not inhibited due to dryness of the agar surface after lengthy exposure. The data obtained by settling plates can be useful when considered in combination with results from active sampling methods.

5.1.4. Cultivation

Culture conditions under which microorganisms can grow with high reproducibility should be applied in environmental monitoring. Growth promotion testing should be performed

Table 5 Media (examples)

Microorganisms to be detected	Media	Temperature and Incubation Time
Aerobes, Yeast and fungi	SCD agar medium SCDLP agar medium SCDL agar medium	25 – 30°C More than 5 days
Aerobes	SCD agar medium SCDLP agar medium SCDL agar medium	30 – 35°C More than 5 days
Yeast and fungi	SCD agar medium SCDLP agar medium SCDL agar medium Sabouraud glucose agar medium Potato dextrose agar medium Glucose peptone agar medium	20 – 25°C More than 5 days
Anaerobes	Reinforced clostridial agar medium SCD agar medium	30 – 35°C More than 5 days (under an anaerobic culture condition)
Extraction Liquids	Saline solution Phosphate buffered saline solution Phosphate buffered solution, pH 7.2 Buffered sodium chloride-peptone solution, pH 7.0 Peptone saline solution Peptone solution	

on all lots of prepared media. Inactivating agents may be used to negate or inhibit the effects of disinfectants or antibacterial agents that are used or manufactured in the area to be monitored.

Culture media and conditions will depend on types of target microorganisms. Examples of culture media and conditions are shown in Table 5. Liquid media as well as agar media listed in the table can be used according to the measurement method.

Media and extraction liquids should be sterilized in an appropriate manner.

In general, minimum incubation time is 5 days. If the counted number of colonies in shorter incubation time is reliable, the number may be adopted for viable count.

For detection of anaerobes, an appropriate culture medium and conditions should be applied.

(i) SCDLP agar medium

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Lecithin	1.0 g
Polysorbate 80	7.0 g
Agar	15.0 g
Water	1000 mL

Adjust pH to 7.1 – 7.5 at 25°C after sterilization in an autoclave using a validated cycle.

(ii) SCDL agar medium

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Lecithin	1.0 g
Agar	15 g
Water	1000 mL

Adjust pH to 7.1 – 7.5 at 25°C after sterilization in an autoclave using a validated cycle.

(iii) Glucose peptone agar medium

Peptone	5.0 g
Yeast extract	2.0 g
Glucose	20.0 g
Magnesium sulfate heptahydrate	0.5 g
Potassium dihydrogen phosphate	1.0 g
Agar	15.0 g
Water	1000 mL

Adjust pH to 5.6 – 5.8 at 25°C after sterilization in an autoclave using a validated cycle.

(iv) Reinforced clostridial agar medium

Beef extract	10.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Soluble starch	1.0 g
Dextrose monohydrate	5.0 g
Cystein hydrochloride monohydrate	0.5 g
Sodium chloride	5.0 g
Sodium acetate	3.0 g
Agar	15.0 g
Water	1000 mL

Adjust pH to 6.6 – 7.0 at 25°C after sterilization in an autoclave using a validated cycle.

(v) Phosphate buffered solution

Potassium dihydrogen phosphate	0.0425 g
Sodium chloride	8.5 g
Water	1000 mL

(vi) Buffered sodium chloride-peptone solution

Peptone	1.0 g
Sodium chloride	8.5 g
Water	1000 mL

(vii) Peptone solution

Peptone	10.0 g
Sodium chloride	5.0 g
Water	1000 mL

5.1.5. Identification

Identification of microorganisms detected in Grade A and B areas to the species level is recommended. Genotypic methods are more accurate and precise than traditional biochemical and phenotypic techniques. These results can be used for investigations into contaminants found in sterility tests or process simulations. See ''Rapid Identification Method of Microorganisms by Gene Analysis'' for additional information on gene analysis.

5.2. Rapid test methods

Rapid test methods can provide results in a shorter time compared with traditional culture methods.

In general, scientifically validated devices should be used for the following aspects of the identification process:

(i) Collecting method (filtration, impact, adhesion, or air aspiration etc.)

(ii) Detection signal (fluorescence, luminescence etc.)

(iii) Detection device

In many cases, the detection thresholds in the rapid test methods are higher than those in traditional methods. Sufficient consideration should be given to qualification of equipment and calibration methods when introducing rapid test methods. In addition, as the measurement principles are different from those in cultural methods, acceptance criteria for each method should be established based on scientific rationale. The acceptance criteria for rapid test methods should be equivalent to or more stringent than those for traditional methods.

6. References

(i) PIC/S GUIDE TO GOOD MANUFACTURING PRCTICE FOR MEDICAL PRODUCTS ANNEXES: Annex 1-Manufacture of sterile medicinal products (September 2009)

(ii) ISO/DIS 14644-1 (2010): Cleanrooms and associated controlled environments—Part 1: Classification of air cleanliness by particle concentration

G5 Crude Drugs

Add the following:

Quantitative Analytical Technique Utilizing Nuclear Magnetic Resonance (NMR) Spectroscopy and Its Application to Reagents in the Japanese Pharmacopoeia

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[1)] 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 ^{1}H nuclei 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.

$$\frac{S_i}{S_j} = \frac{N_i}{N_j} \tag{2}$$

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.)

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

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.

2. Supply of Reference Materials and Software for Quantitative NMR

Recently, it has become easy to obtain the SI traceable reference materials of 1,4-bis(trimethylsilyl)benzene-d_4 (BTMSB-d_4) (for organic solution) and 3-(trimethylsilyl)-1-propane sulfonic acid-d_6-sodium salt (DSS-d_6) (for aqueous solution) as solid substances, which are easy to handle. The SI traceability of these materials is guaranteed from the certified reference materials (NMIJ CRM) of 1,4-dichlorobenzene supplied from the National Metrology Institute of Japan, the National Institute of Advanced Industrial Science and Technology (NMIJ AIST), the national metrology organization of Japan. Either BTMSB-d_4 or DSS-d_6 shows a single sharp peak at the characteristic chemical shift in its ^{1}H-NMR spectrum. In addition, softwares for quantitative NMR (qNMR) based on the principle described above, have been supplied from NMR instrument manufacturers. As a result, quantitative NMR can be performed easily for the reagents used in the Japanese Pharmacopoeia (JP).

3. 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, the valuable reference standard would be consumed not for its initial purpose but for the determination of water content.

Because there are such bottlenecks mentioned above in many cases of monographs of crude drugs, 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 value is somewhat ambiguous.

4. 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. That is to say, the 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 principles described above.

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 abolute purities of the reagents by using quantitative NMR are discussed has been published.[2)] 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.[3)] 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[2)] 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.

5. 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 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.

6. Reference

1) T. Saito, et al., Accred. Qual. Assur. **14**, 79 – 86 (2009)
2) J. Hosoe, et al., Pharmaceutical and Medical Device Regulatory Science, **41**, 960 – 970 (2010)
3) J. Hosoe, et al., Pharmaceutical and Medical Device Regulatory Science, **43**, 182 – 193 (2012)

G8 Water

Quality Control of Water for Pharmaceutical Use

Change to read as follows:

Water used for manufacturing pharmaceutical products and for cleaning their containers and equipments used in the manufacture of the products is referred to as ''pharmaceutical water.'' For assuring the quality of pharmaceutical water consistently, it is important to verify through appropriate process validation of water processing system that water with the quality suitable for its intended use is produced and supplied, and to keep the quality of produced water through routine works for controlling the water processing system.

1. Types of Pharmaceutical Water

1.1. ***Water***

The specification for ''*Water*'' is prescribed in the Japanese Pharmacopoeia (JP) monograph. It is required for *Water* to meet the Quality Standards for Drinking Water provided under the Article 4 of the Japanese Water Supply Law. In the case that *Water* is produced at individual facili-

ties using well water or industrial water as source water, it is necessary for produced water to meet the Quality Standards for Drinking Water and an additional requirement for ammonium of "not more than 0.05 mg/L." Furthermore, when *Water* is to be used after storing for a period of time, it is necessary to prevent microbial proliferation.

Water is used as source water for *Purified Water* and *Water for Injection*. It is also used for manufacturing intermediates of active pharmaceutical ingredients (APIs), and for pre-washing of the equipments used in the manufacture of pharmaceutical products.

1.2. *Purified Water*

The specifications for "*Purified Water*" and "*Purified Water in Containers*" are prescribed in the JP monographs. *Purified Water* is prepared by distillation, ion-exchange, reverse osmosis (RO), ultrafiltration (UF) capable of removing microorganisms and substances with molecular masses of not less than approximately 6000, or a combination of these processes from *Water*, after applying some adequate pretreatments if necessary. For the production of *Purified Water*, appropriate control of microorganisms is required. Particularly, in the case that *Purified Water* is prepared by ion-exchange, RO or UF, it is necessary to apply the treatments adequate for preventing microbial proliferation, or to sanitize the system periodically.

When *Purified Water* is treated with chemical agents for sterilizing, preventing microbial proliferation, or maintaining the endotoxin level within an appropriate control range, a specification suitable for the intended use of treated water should be established individually, and a process control for keeping the quality of treated water in compliance with the specification thus established should be performed.

"*Purified Water in Containers*" is prepared from *Purified Water* by introducing it in a tight container.

1.3. *Sterile Purified Water*

The specification for "*Sterile Purified Water in Containers*" (its alternative name is *Sterile Purified Water*) is prescribed in the JP monograph.

Sterile Purified Water in Container is prepared from *Purified Water* by 1) introducing it into a hermetic container, sealing up the container, then sterilizing the product, or 2) making it sterile by using a suitable method, introducing the sterilized water into a sterile hermetic container by applying aseptic manipulation, then sealing up the container.

Plastic containers for aqueous injections may be used in place of hermetic containers.

1.4. *Water for Injection*

The specifications for "*Water for Injection*" and "*Sterile Water for Injection in Containers*" are prescribed in the JP monographs. *Water for Injection* is prepared by distillation or reverse osmosis and/or ultrafiltration (RO/UF), either from *Water* after applying some adequate pretreatments such as ion exchange, RO, etc., or from *Purified Water*.

In the case of water processing systems based on distillation, it is necessary to take care for avoiding contamination of produced water by the impurities accompanied with the entrain.

In the case of water processing system based on RO/UF, it is required to provide water with equivalent quality to that prepared by distillation consistently, based on substantial process validation through long-term operation and elaborate routine control of the system. It is essential to ensure consistent production of water suitable for *Water for Injection* by the entire water processing system including pretreatment facilities, in any systems based on RO/UF. For the water supplied to the system, it is also required to keep the quality suitable as source water through adequate validation and routine control on the water.

For the water processing system based on RO/UF, routine control should be performed by analyzing water specimens, monitoring some quality attributes using in-line apparatus and checking the volume of water passed through the system. In addition, it is recommended to carry out periodical appearance observation and air-leak test on the membranes being currently used. It is also recommended to establish protocols for keeping the performance of membrane modules within appropriate control ranges and for estimating the timing to exchange the modules, through diagnosis on the degree of deterioration based on the results of tensile strength test on the used membrane modules, and visual observation on those modules whether any leakages of membranes have occurred or not, and to what extent they have occurred. Furthermore, it is desirable to establish the frequency of membrane exchange considering with its actual condition of use.

In the case that *Water for Injection* is stored in the water processing system temporarily, a stringent control for microorganisms and endotoxins should be taken. An acceptable criterion of lower than 0.25 EU/mL for endotoxins is specified in the JP monograph of *Water for Injection*.

"*Sterile Water for Injection in Container*" is prepared from *Water for Injection* by 1) introducing it into a hermetic container, sealing up the container, then sterilizing the product, or 2) making it sterile by using a suitable method, introducing the sterilized water into a sterile hermetic container by applying aseptic manipulation, then sealing up the container.

Plastic containers for aqueous injections may be used in place of hermetic containers.

2. Reverse Osmosis and/or Ultrafiltration (RO/UF)

RO/UF are the methods for refining water by using membrane modules based on either reverse osmosis or ultrafiltration, or the modules combining them, and used as the alternative methods for distillation in the production of *Purified Water* or *Water for Injection*.

When *Water for Injection* is produced by RO/UF, a water processing system equipped with pretreatment facilities, facilities for producing *Water for Injection* and facilities for supplying *Water for Injection* is usually used. The pretreatment facilities are used to remove solid particles, dissolved salts and colloids in source water, and placed before the facilities for producing *Water for Injection* so as to reduce the load on the facilities for producing *Water for Injection*. They consist of apparatus properly selected from aggregation apparatus, precipitation-separation apparatus, filtra-

tion apparatus, chorine sterilization apparatus, oxidation-reduction apparatus, residual chlorine-removing apparatus, precise filtration apparatus, reverse osmosis apparatus, ultrafiltration apparatus, ion exchange apparatus, etc., depending on the quality of source water. The facilities for producing *Water for Injection* consist of apparatus for supplying pretreated water, sterilization apparatus with ultraviolet rays, heat exchange apparatus, membrane modules, apparatus for cleaning and sterilizing the facilities, etc. The facilities for supplying *Water for Injection* consist of a reservoir tank for storing *Water for Injection* in the facilities temporarily, pipe lines, heat exchange apparatus, a pump for circulating *Water for Injection* in the facilities, pressure control apparatus, etc.

In the case that *Water for Injection* is stored in the water processing system temporarily, it should usually be circulated in a loop consisting of a reservoir tank and pipe line at a temperature not lower than 80°C for preventing microbial proliferation.

When *Purified Water* is produced by RO/UF, basic composition of water processing system is almost the same as that for *Water for Injection* described above.

When RO/UF is utilized for preparing pharmaceutical water, it is necessary to select the most appropriate combination of membrane modules in consideration of the quality of source water and the quality of produced water required for its intended use. When the ultrafiltration membrane is used to prepare *Purified Water* or *Water for Injection*, membrane modules capable of removing microorganisms and substances with molecular masses not less than approximately 6000 should be used.

3. Selection of Pharmaceutical Water

Depending on the intended use of pharmaceutical water, the water suitable for assuring the quality of final products without causing any trouble during their manufacturing processes, should be selected from the above 4 types (1.1 – 1.4) of pharmaceutical water specified in the JP. Table 1 exemplifies a protocol for such selection (in the case of pharmaceutical water used for the manufacture of drug products).

Sterile Purified Water in Containers or *Water for Injection* (or *Sterile Water for Injection in Containers*) may be used in place of *Purified Water* (or *Purified Water in Containers*).

3.1. Drug Products

For the manufacture of sterile drug products such as Injections, for which endotoxins together with microorganisms should be severely controlled, *Water for Injection* (or *Sterile Water for Injection in Containers*) should be used. For the manufacture of sterile drug products such as Ophthalmic Preparations and Ophthalmic Ointments, for which contamination with microorganisms should be paid attention, *Purified Water* (or *Purified Water in Containers*), which viable count level is specified at low, can also be used.

For the manufacture of non-sterile drug products, water with a quality not lower than that of *Purified Water* (or *Purified Water in Containers*) should be used. For the Inhalations, Ear Preparations and Nasal Preparations, appropriately controlled *Purified Water* (or *Purified Water in Containers*) in vial count level should be used, and for Liquids and Solutions among Inhalations, strictly controlled *Purified Water* (or *Purified Water in Containers*) in vial count level should be used. For the Liquids and Solutions for Oral Administration, Syrups, Suppositories for Vaginal Use, Ointments and Creams, which require care against microbiological contamination, *Purified Water* (or *Purified Water in Containers*) adequately controlled from microbiological viewpoints should be used in consideration of the possible impacts of preservatives formulated in the drug products. For the manufacture of products containing crude drugs, it is recommended to select adequate type of water considering viable counts of the crude drugs used for manufacturing the product and microbial limit required for the product.

Water used for pre-washing of containers or equipment surfaces that comes in direct contact with the drug products should have the quality not lower than that of *Water*. Water used for final rinsing should have an equivalent quality to that of water used for manufacturing drug products.

3.2. Active Pharmaceutical Ingredient (API)

Water used for manufacturing active pharmaceutical ingredient (API) should be selected in consideration of the characteristics of drug product for which the API is to be used, and its manufacturing process, so that the quality of the final drug product is assured.

Water used for manufacturing API or for cleaning containers or equipment surfaces that come in direct contact with the raw materials or API intermediates, should have the quality not lower than that of *Water* adequately controlled from the chemical and microbiological viewpoints, even if the water is used at an earlier stage of synthetic or extraction process in the manufacture of API. Water used in the final purification process should have the quality equal to or higher than that of *Purified Water* (or *Purified Water in Containers*). Water used for final rinsing of containers or equipment surfaces that comes in direct contact with the APIs should have an equivalent quality to that of water used for manufacturing the APIs.

For manufacturing sterile API, *Sterile Purified Water in Containers* or *Water for Injection* (or *Sterile Water for Injection in Containers*) should be used. Similarly, for manufacturing APIs used for drug products where endotoxin control is required and there are no subsequent processes capable of removing endotoxins, *Water for Injection* (or *Sterile Water for Injection in Containers*), or *Purified Water* (or *Purified Water in Containers*) for which endotoxins are controlled at a low level, should be used.

4. Quality Control of Pharmaceutical Water

4.1. Outline

Verification that water with the quality required for its intended use has been produced by the pharmaceutical water processing system through substantial validation studies at an earlier stage of its operation, is the prerequisite for conducting quality control on pharmaceutical water in a routine

Table 1 An Exemplified Protocol for Selecting Pharmaceutical Water
(Water Used in the Manufacture of Drug Products or APIs)

Classification	Class of Pharmaceutical Water	Application	Remarks
Drug Product	*Water for Injection* or *Sterile Water for Injection in Containers*	Injections, Dialysis Agents (Peritoneal Dialysis Agents and Hemodialysis Agents)	For Hemodialysis Agents, unless otherwise specified, *Water for Injection*, *Water for Injection in Containers*, or water suitable for the dialysis.
	Purified Water or *Purified Water in Containers*	Ophthalmic Preparations, Ophthalmic Ointments, Inhalations, Ear Preparations, Nasal Preparations	For the sterile drug products, such as Ophthalmic Preparations and Ophthalmic Ointments, for which precautions should be taken against microbial contamination, *Purified Water* (or *Purified Water in Containers*) kept its viable counts at low levels may be used. For Inhalations, Ear Preparations and Nasal Preparations, *Purified Water* (or *Purified Water in Containers*) of which viable counts are controlled at an appropriate level should be used. However, for the Inhalation Liquid Preparations among Inhalations, *Purified Water* (or *Purified Water in Containers*) that vial count is strictly controlled should be used.
		Preparations for Oral Administration, Preparations for Oro-mucosal Application, Preparations for Rectal Application, Preparations for Vaginal Application, Preparations for Cutaneous Application, and Tinctures and Aromatic Waters among Preparations Related to Crude Drugs.	For Liquids and Solutions for Oral Administration, Syrups, Suppositories for Vaginal Use, Ointments, Creams and so on for which precautions should be taken against microbial contamination, *Purified Water* (or *Purified Water in Containers*) adequately controlled from microbiological viewpoints should be used, taking in mind the affection of containing preservatives.
	Water	Among Preparations Related to Crude Drugs: Extracts, Pills, Infusions and Decoctions, Teabags, Fluidextracts	The viable counts in crude drugs and the objective microbial limits of product should be considered in selecting water to be used.
Active Pharmaceutical Ingredient (API)	*Water for Injection* or *Sterile Water for Injection in Containers*	Sterile APIs	
	Purified Water or *Purified Water in Containers*	APIs	In the manufacture of APIs used for products to be rendered sterile in the formulation process and have no subsequent processes capable of removing endotoxins, *Purified Water* (or *Purified Water in Containers*) controlled endotoxins in an appropriate level should be used.
	Water	API Intermediates	

and periodical manner. If this prerequisite is fulfilled, the following methods are applicable for quality control of pharmaceutical water.

For routine control, it is very useful to control quality of produced water based on the monitoring of electrical conductivity (conductivity) and total organic carbon (TOC). In addition, items to be monitored periodically, such as some specified impurities, viable counts, endotoxins, insoluble particulate matters, etc., should be determined according to the intended use of pharmaceutical water. The frequency of measurement should be determined considering with the variation in the quality of water to be monitored.

The following are points to consider in controlling the quality of produced water from microbiological and physicochemical (conductivity and TOC) viewpoints. It is necessary to monitor other items if necessary, and to confirm that they meet the specifications established individually.

4.2. Sampling

Monitoring should be conducted at an adequate frequency to ensure that the pharmaceutical water processing system is well-controlled and that water with acceptable quality is continuously produced and supplied. Specimens should be col-

lected at the representative locations in the facilities for producing and supplying water, with particular care so that collected specimens reflect the operating condition of the pharmaceutical water processing system. An adequate protocol for the control of microorganisms at the sampling site should be established considering with the situation around the site.

Sampling frequency should be established based on the data from validation studies on the system. For microbiological monitoring, it is adequate to use the water specimens for the test within 2 hours after sampling. In the case that it is not possible to test within 2 hours, the specimens should be kept at 2 – 8°C and be used for the test within 12 hours.

4.3. Alert and Action Levels

In producing pharmaceutical water using a water processing system, microbiological and physicochemical monitoring is usually carried out to assure that water with required quality is being continuously produced when the system is operating as it designed. The operating condition of the system can be estimated by the comparison of monitoring data thus obtained against the alert level, action level, other levels for controlling the system, and acceptance criteria specified for the water required for its intended use, and also by the trend analysis of monitoring data through plotting them in a control chart. In this manner, the alert level and action level are used for controlling the process of water production, and not used for judging pass/fail of produced water.

4.3.1. Definition of Alert Level

''Alert level'' indicates that, when exceeded it, the system is threatening to deviate from its normal operating range. Alert levels are used for giving a warning, and exceeding them does not necessarily require a corrective action. Alert level is generally established either at a mean + 2σ on the basis of past trend analysis, or at a level of 70% (50% for viable counts) of action level, whichever is lower.

4.3.2. Definition of Action Level

''Action level'' indicates that, when exceeded it, the system has deviated from its normal operating range. Exceeding it indicates that corrective action must be taken to bring the system back within its normal operating range.

Alert and action levels should be established within the specified acceptance criteria of the water required for its intended use in consideration of available technologies and the quality required for the water. Consequently, exceeding an alert or action level does not necessarily indicate that the quality of produced water has become inadequate for its intended use.

4.4. Microbiological Monitoring

The main purpose of microbiological monitoring program for pharmaceutical water processing system is to foresee any microbiological quality deterioration of the produced water, and to prevent any adverse effects on the quality of pharmaceutical products. Consequently, detecting all of the microorganisms present in the water to be monitored may not be necessary. However it is required to adopt a monitoring technique able to detect a wide range of microorganisms, including slow growing microorganisms.

The following indicate incubation-based microbiological monitoring techniques for pharmaceutical water processing systems. To adopt a rapid microorganism detection technique, it is necessary to confirm in advance that the microbial counts obtained by such techniques are equivalent to those obtained by the incubation-based monitoring techniques.

4.4.1. Media and Incubation Conditions

There are many mesophilic bacteria of heterotrophic type that are adapted to poor nutrient water environments. Heterotrophic bacteria may form bio-films in many pharmaceutical water processing systems, and to cause quality deterioration of the produced water. Therefore, it is useful to monitor microbiological quality of water by using the R2A Agar Medium, which is excellent for growing bacteria of oligotrophic type.

Table 2 shows examples of measurement methods, minimum sample sizes, media, and incubation periods for estimating viable counts.

Table 2 Methods for Assessment of Viable Counts in Pharmaceutical Water

Method	Pharmaceutical Water		
	Water	*Purified Water*	*Water for Injection*
Measurement Method	Pour Plate Method or Membrane Filtration	Pour Plate Method or Membrane Filtration	Membrane Filtration
Minimum Sample Size	1.0 mL	1.0 mL	100 mL
Media	R2A Agar Medium Standard Agar Medium	R2A Agar Medium	R2A Agar Medium
Incubation Period	R2A Agar Medium: 4 – 7 days (or longer) Standard Agar Medium: 48 – 72 hours (or longer)	4 – 7 days (or longer)	4 – 7 days (or longer)
Incubation Temperature	R2A Agar Medium: 20 – 25°C or 30 – 35°C Standard Agar Medium: 30 – 35°C	20 – 25°C or 30 – 35°C	20 – 25°C or 30 – 35°C

The media shown in Table 2 are as follows.

(i) Standard Agar Medium

Casein peptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize by heating in an autoclave at 121°C for 15 – 20 minutes. pH after sterilization: 6.9 – 7.1.

(ii) R2A Agar Medium

Peptone (casein and animal tissue)	0.5 g
Casamino acid	0.5 g
Yeast extract	0.5 g
Sodium pyruvate	0.3 g
Glucose	0.5 g
Magnesium sulfate heptahydrate	0.05 g
Soluble starch	0.5 g
Dipotassium hydrogen phosphate	0.3 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize by heating in an autoclave at 121°C for 15 – 20 minutes. pH after sterilization: 7.1 – 7.3.

The following reagents should be used for preparing the R2A Agar Medium.

(i) Casamino acid Prepared for microbial test, by the acid hydrolysis of casein.

Loss on drying ⟨*2.41*⟩: Not more than 8% (0.5 g, 105°C, constant mass).

Residue on ignition ⟨*2.44*⟩: Not more than 55% (0.5 g).

Nitrogen content ⟨*1.08*⟩: Not less than 7% (105°C, constant mass, after drying).

(ii) Sodium pyruvate $CH_3COCOONa$ 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 as directed in the potassium bromide disk method under *Infrared Spectrophotometry* ⟨*2.25*⟩: it exhibits absorption maxima at the wave numbers around 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 (1 in 20) responds to the *Qualitative Tests* ⟨*1.09*⟩ for sodium salt (1).

Content: Not less than 97.0%. Assay—Weigh accurately 0.4 g of sodium pyruvate and dissolve in 200 mL of water. Transfer 20 mL of this solution into an iodine bottle, and cool to 10°C or lower. Add 40 mL of 0.05 mol/L iodine VS and 20 mL of sodium hydroxide solution (17 in 100), then allow to stand in a dark place for 2 hours, and add 15 mL of diluted sulfuric acid (1 in 6). 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 $C_3H_3NaO_3$

4.4.2. Media Growth Promotion Test

In the media growth promotion test with the R2A Agar Medium, use the strains listed below or other strains considered equivalent to these strains. Prior to the test, inoculate these strains into sterile purified water and starve them at 20 – 25°C for 3 days.

Methylobacterium extorquens: NBRC 15911

Pseudomonas fluorescens: NBRC 15842, ATCC 17386, etc.

Dilute the fluid containing the strain starved with sterile purified water to prepare a fluid containing about 5×10^1 – 2×10^2 CFU/mL of viable counts. When pipetting 1 mL of the diluted fluid onto the R2A Agar Medium and incubating at 20 – 25°C for 4 – 7 days, sufficient proliferation of the inoculated strain must be observed.

In the media growth promotion test with the Standard Agar Medium, use the strains listed below or other strains considered equivalent to these strains. Prepare the fluid containing the strain according to the procedure prescribed in the *Microbiological Examination of Non-sterile Products* ⟨*4.05*⟩. When pipetting 1 mL of the fluid onto the Standard Agar Medium and incubating at 30 – 35°C for 48 hours, sufficient proliferation of the inoculated strain must be observed.

Staphylococcus aureus: ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276

Pseudomonas aeruginosa: ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275

Colon bacillus (*Escherichia coli*): ATCC 8739, NCIMB 8545, CIP 53.126 or NBRC 3972

4.4.3. Action Levels for Microorganisms for Pharmaceutical Water Processing System

The following action levels are considered appropriate and generally applicable to pharmaceutical water processing systems.

Action Levels for viable counts in various types of pharmaceutical water

Water: 10^2 CFU/mL* (Acceptance criterion prescribed in the Quality Standards for Drinking Water provided under the Article 4 of the Water Supply Law)

Purified Water: 10^2 CFU/mL**

Water for Injection: 10^1 CFU/100 mL**

(*Viable counts obtained using the Standard Agar Medium, ** Viable counts obtained using the R2A Agar Medium)

Although the action level for *Purified Water* shown above is set at the same level as that for *Water*, it is recommended for each facility to perform a higher level of microbiological control of water processing system based on the action level established individually.

When actual counts in validation studies or routine control exceed the above action levels, it is necessary to isolate and identify the microorganisms present in the water, and to sanitize or disinfect the affected system.

4.5. Physicochemical Monitoring

Physicochemical monitoring of a pharmaceutical water processing system is usually performed using conductivity

and TOC as the indicators for water quality. By monitoring conductivity, total amounts of inorganic salts present in the water can be estimated, and by monitoring TOC, total amount of organic compounds present in the water can be estimated. Normally, the *Conductivity Measurements* <2.51> and the *Test for Total Organic Carbon* <2.59> specified in the General Tests, Processes and Apparatus of the JP should be applied to these physicochemical monitoring. However, since tests for monitoring are performed in the situations different from those for judging pass/fail to the acceptance criteria prescribed in the monographs, supplements necessary to cover the situations to which the JP general tests cannot be applied, are described below.

To adopt the monitoring using conductivity and TOC as the indicators for inorganic and organic impurities at individual facility, appropriate alert and action levels, and countermeasures against unexpected apparatus failures should be established for each indicator.

4.5.1. Monitoring of Conductivity as the Indicator for Inorganic Impurities

Measurement of conductivity for monitoring is usually conducted continuously using an in-line apparatus with a flow-through type or pipe-insertion type cell. Alternatively, offline batch testing may be performed using a dip type cell with water specimens taken at point-of-use sites or other appropriate locations of the pharmaceutical water processing system. For the operation control of a pharmaceutical water processing system, guides for judging whether it is adequate to continue the operation of the system or not based on the results from monitoring of conductivity, are shown below, both for the cases of monitoring at the standard temperature (20°C) by applying *Conductivity Measurements* <2.51> of the JP and monitoring at temperatures other than 20°C by applying <645> *WATER CONDUCTIVITY* of the United States Pharmacopeia (USP) with some modifications.

4.5.1.1. Monitoring of Conductivity by applying the *Conductivity Measurements* <2.51> of the JP

When the monitoring of the conductivity of *Purified Water* and *Water for Injection* is performed at the standard temperature (20°C), measure the conductivity after confirming that the measure temperature is within a range of 20 ± 1°C. In this case, the recommended allowable conductivity (action level) for *Purified Water* and *Water for Injection* is as follows.

• Action Level 1.1 $\mu S \cdot cm^{-1}$ (20°C)

Since the above allowable conductivity is established for in-line monitoring, an alternative action level may be used for the monitoring based on offline batch testing.

4.5.1.2. Monitoring of Conductivity by applying the <645> *WATER CONDUCTIVITY* of the USP with some modification

Usually, it is somewhat difficult to control the temperature exactly in in-line conductivity monitoring. Therefore, the following approach can be applied for the monitoring at temperatures other than the standard temperature (20°C) of the JP. This approach is based on the Stages 1 and 2 of the three-stage approach described in ''<645> *WATER CONDUCTIVITY*'' of the USP and in the monographs being associated with water for pharmaceutical use (''Purified Water'', ''Highly Purified Water'' and ''Water for Injections'') of the European Pharmacopoeia (EP).

Table 3 Stage 1 Allowable Conductivity for Different Temperatures*

Temperature (°C)	Allowable Conductivity ($\mu S \cdot cm^{-1}$)	Temperature (°C)	Allowable Conductivity ($\mu S \cdot cm^{-1}$)
0	0.6		
5	0.8	55	2.1
10	0.9	60	2.2
15	1.0	65	2.4
20	1.1	70	2.5
25	1.3	75	2.7
30	1.4	80	2.7
35	1.5	85	2.7
40	1.7	90	2.7
45	1.8	95	2.9
50	1.9	100	3.1

* Applicable only to non-temperature-compensated conductivity measurements.

Stage 1 (In-line Measurement)

(i) Determine the temperature and the conductivity of the water specimens using a non-temperature-compensated conductivity reading.

(ii) From the Table 3, find the temperature value equal to or just lower than the measured temperature. Adopt the corresponding conductivity value on this table as the allowable conductivity at the measured temperature.

(iii) If the observed conductivity is not greater than the allowable conductivity adopted above, the water tested meets the requirement for monitoring conductivity. If the observed conductivity exceeds the allowable conductivity, proceed with Stage 2.

Stage 2 (Off-line Measurement)

(i) Measure the conductivity of the water specimen, by transferring it into a container and agitating it vigorously in order to attain equilibrium between the water specimen and the atmosphere on absorbing/desorbing carbon dioxide.

(ii) Transfer a sufficient amount of water to be tested into a suitable container, and stir the water specimen. Adjust the temperature to 25 ± 1°C, and begin agitating the water specimen vigorously, while observing the conductivity periodically. When the change in conductivity, due to the uptake of atmospheric carbon dioxide, becomes not greater than 0.1 $\mu S \cdot cm^{-1}$ per 5 minutes, adopt the observed value as the conductivity (25°C) of the water specimen.

(iii) If the conductivity of the water specimen at 25°C obtained above is not greater than 2.1 $\mu S \cdot cm^{-1}$, the water tested meets the requirement for monitoring conductivity. If the observed value exceeds 2.1 $\mu S \cdot cm^{-1}$, it should be judged that the water tested does not meet the requirement for monitoring conductivity.

4.5.2. Monitoring of TOC as the Indicator for Organic Impurities

The acceptance criterion of TOC is specified as ''not greater than 0.50 mg/L (500 ppb)'' in the monographs of *Purified Water* and *Water for Injection*. However it is recommended for each facility preparing pharmaceutical water to conduct operation control of pharmaceutical water processing system through TOC monitoring on produced water based on its own alert and action levels for TOC determined individually. The following are the recommended action levels for TOC.

- Action Level: ≦300 ppb (in-line)
  ≦400 ppb (off-line)

The Quality Standards for Drinking Water provided under the Article 4 of the Japanese Water Supply Law require that TOC should be ''not greater than 3 mg/L (3 ppm)''. Taking the above recommended action levels into consideration, it is also recommended for each facility to conduct quality control of source water through TOC monitoring based on its own alert and action levels for TOC determined individually.

The JP specifies the *Test for Total Organic Carbon* <*2.59*>, and normally, TOC measurement should be conducted using an apparatus which meets the requirements described in the JP method. However, if a TOC apparatus conforms to the apparatus suitability test requirements described in ''<643> *TOTAL ORGANIC CARBON*'' of the USP, or those described in the ''*Methods of Analysis 2.2.44. TOTAL ORGANIC CARBON IN WATER FOR PHARMACEUTICAL USE*'' of the EP, the apparatus can be used for the monitoring of pharmaceutical water processing system, if sufficiently pure water not contaminated with ionic organic substances, or organic substances having nitrogen, sulfur, phosphorus or halogen atoms in their chemical structures, is used as the source water supplied to the system.

A TOC apparatus, characterized by calculating the amount of organic carbon from the difference in conductivity before and after the decomposition of organic substances without separating carbon dioxide from the sample solution, may be influenced negatively or positively, when applied to the water specimens containing ionic organic substances, or organic substances having nitrogen, sulfur, phosphorus or halogen atoms in their chemical structures. Therefore, the apparatus used for TOC monitoring should be selected appropriately in consideration of the purity of the water to be monitored and the contamination risk in the case of apparatus failure.

4.6. Storage of *Water for Injection*

In storing *Water for Injection* temporarily, adequate measures able to prevent microbial proliferation stringently, such as circulating it in a loop at a high temperature must be taken, and an appropriate storage time should also be established based on the validation studies, in consideration of the risks of contamination and quality deterioration.

5. Points to Consider for Assuring the Quality of Pharmaceutical Water in Containers

There are some specific points to consider for assuring the quality of pharmaceutical water in containers (*Purified Water in Containers*, *Sterile Purified Water in Containers* and *Sterile Water for Injection in Containers*), which are available as commercially products.

5.1. Methods for Preparing Sterile Pharmaceutical Water in Containers and Their Sterilization Validation

The following 2 different preparation methods are described in the monographs of *Sterile Purified Water in Containers* and *Sterile Water for Injection in Containers*.

(i) Introduce *Purified Water* or *Water for Injection* into a hermetic container, seal up the container, then sterilize the product.

(ii) Make *Purified Water* or *Water for Injection* sterile by using a suitable method, introduce the sterilized water into a sterile hermetic container by applying aseptic manipulation, then seal up the container.

For assuring the sterility of pharmaceutical water products, only the validation of final sterilization process is required in the case of preparation method (i), whereas validations of all the processes are indispensable in the case of preparation method (ii), since the latter is based on the idea to assure the sterility of pharmaceutical water products by ''aseptically'' introducing *Purified Water* (or *Water for Injection*) treated in advance with filtration sterilization, etc. into a sterile hermetic container, and sealing it up.

5.2. Deterioration of Water Quality during the Storage in Containers

5.2.1. Conductivity (as the indicator for inorganic impurities)

The conductivity of pharmaceutical water in containers may increase to some higher levels due to the absorption of carbon dioxide from the atmosphere at the time of its preparation and that passed through plastic layer of the containers during storage, and also due to ionic substances released from the containers, even if the conductivity of *Purified Water* or *Water for Injection* used for its production is maintained at the level not more than 1.0 $\mu S \cdot cm^{-1}$. Particularly in the cases of pharmaceutical water products packed in small scale glass containers, it is necessary to pay attention to the change of conductivity during storage.

5.2.2. Potassium Permanganate-reducing Substances or Total Organic Carbon (TOC) (as the indicator for organic impurities)

JP specifies the classical test of potassium permanganate-reducing substances in the monographs of *Purified Water in Containers*, *Sterile Purified Water in Containers* and *Sterile Water for Injection in Containers* for controlling organic impurities in pharmaceutical water in containers. It forms a remarkable contrast to the specifications of *Purified Water* and *Water for Injection*, in which JP requires to control organic impurities in pharmaceutical water in bulk based on the test of TOC (acceptance criterion: not more than 0.5 mg/L (500 ppb)). This is because that it is considered difficult to establish the specification of pharmaceutical water in containers for organic impurities based on the test of TOC from the facts that there were many cases of remarkable increases in TOC values after storage of water in containers. Particularly in the cases of pharmaceutical water

products packed in small scale plastic containers, it is necessary to pay attention to the increase of materials released from containers during storage.

The test of potassium permanganate-reducing substances is retained in the specifications of pharmaceutical water in containers, not as the most suitable method for the test of organic impurities present in the water in containers, but as a counter measure for performing the test of the water in containers with the same test method despite of the material (glass, polyethylene, polypropylene, etc.) and the size (0.5 – 2000 mL) of the containers, and the duration of storage. Therefore, it is recommended to adopt the test of TOC as the alternative for the test of potassium permanganate-reducing substances, and to perform quality control of pharmaceutical water in containers based on TOC measurements under the responsibility of each manufacturer, if possible.

In such cases, it is recommended to adopt the following values as the levels preferable to attain.

For products containing not more than 10 mL of water:
TOC not greater than 1500 ppb
For products containing more than 10 mL of water:
TOC not greater than 1000 ppb

As for the pharmaceutical water packed in the plastic containers made of polyethylene, polypropylene, etc., in addition to the concern for the release of materials such as monomer, oligomers, plasticizers, etc. from plastics, it is necessary to pay attention to the storage environment of the products to avoid the contaminations with low molecular volatile organics such as ethanol, or low molecular air pollutants such as nitrogen oxides, since these plastics have the properties of permeating various gases.

5.2.3. Microbial Limit (Total Aerobic Viable Counts)

For *Purified Water in Containers*, it is not required to assure the sterility, but it is necessary to produce it by using sanitary or aseptic processes in order to meet the acceptance criterion of ''10^2 CFU/mL'' for total aerobic viable counts throughout the period of their storages. It is also necessary to take special care against microbial contamination during its circulation. In addition, it is recommended to use them as soon as possible after opening their seals.

The acceptance criterion of ''10^2 CFU/mL'' for total aerobic viable counts of *Purified Water in Container* is at the same level as the action level for viable counts in the production of *Purified Water* (in bulk). However, different from the case of microbiological monitoring of *Purified Water*, Soybean-Casein Digest Agar Medium is used for the test of total aerobic viable counts of *Purified Water in Containers* to detect microorganisms contaminated from the surroundings during its storage and circulation.

5.3. Points to consider in the case that commercially available products of pharmaceutical water in containers are used for the manufacture of pharmaceutical products

It is allowable to use commercially available products of pharmaceutical water in containers (*Purified Water in Containers*, *Sterile Purified Water in Containers* and *Sterile Water for Injection in Containers*) for the manufacture of pharmaceutical products and products for clinical trial, and for the tests of pharmaceutical products. In such cases, it is necessary to consider the following points.

(i) When such products are used for manufacturing pharmaceutical products, it is recommended to use them soon after confirming their compliances to the requirements of JP monograph from the test results at the time of its receipt or those offered from the supplier of the products.

(ii) In the case that such products are used for manufacturing pharmaceutical products, it is necessary to validate the process in which the water was used as a part of process validation of pharmaceutical products. In the case that they are used for manufacturing products for clinical trial, it is necessary to confirm that the water doesn't give any adverse effects on the quality of the products.

(iii) The products of sterile pharmaceutical water in containers should be used only once after opening their seals, and it must be avoided to use them again after storage.

(iv) It is recommended to prepare a standard operation practice (SOP) adequate for its intended use, considering that the contamination and quality deterioration of the water due to human and laboratory environmental origins might go on rapidly immediately after opening the product seal.

Water to be used in the Tests of Drugs

Change to read as follows:

The water to be used in the tests of drugs is defined as ''the water suitable for performing the relevant test'' in the paragraph 20 under General Notices of the JP. Therefore, it is necessary to confirm that the water to be used in a test of a drug is suitable for the purpose of the relevant test before its use.

Unless otherwise specified in the individual test method, Purified Water, Purified Water in Containers or the water produced by an appropriate process, such as ion exchange or ultrafiltration, may be used for these purposes. Water produced for these purposes at other individual facilities may also be used.

Examples of the water for tests specified in General Tests in the JP are as follows:

- Water for ammonium limit test: <*1.02*> Ammonium Limit Test (Standard Ammonium Solution)
- Water used for measuring organic carbon (water for measurement): <*2.59*> Test for Total Organic Carbon
- Water for ICP analysis: <*2.63*> Inductively Coupled Plasma-Atomic Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry
- Water for bacterial endotoxins test: <*4.01*> Bacterial Endotoxins Test
- Water for particulate matter test (for injections): <*6.07*>

Insoluble Particulate Matter Test for Injections
- Water for particulate matter test (for ophthalmic solutions): <*6.08*> Insoluble Particulate Matter Test for Ophthalmic Solutions
- Water for particulate matter test (for plastic containers): <*7.02*> Test Methods for Plastic Containers

The water for tests specified in General Information in the JP is as follows:
- Water for aluminum test: Test for Trace Amounts of Aluminum in Trans Parenteral Nutrition (TPN) Solutions

The term ''water'' described in the text concerning tests of drugs means ''the water to be used in the tests of drugs'' as defined in the paragraph 20 under General Notices.

G9 Others

International Harmonization Implemented in the Japanese Pharmacopoeia Sixteenth Edition

Add the following:

Nov. 2010

Harmonized items	JP16 (Supplement I)	Remarks
Crospovidone	**Crospovidone**	
Definition	limits of content	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Particle size	
Peroxides	Purity (4) Peroxides	
Water-soluble substances	Purity (2) Water-soluble substances	
Impurity A	Purity (3) 1-Vinyl-2-pyrrolidone	
Loss on drying	Loss on drying	
Sulphated ash	Residue on ignition	
Assay	Assay	
Storage	Containers and storage	

Add the following:

Jun. 2012 (Corr.2)

Harmonized items	JP16 (Supplement I)	Remarks
Magnesium Stearate	**Magnesium Stearate**	
Definition	limits of content	
Identification A	Identification	
Identification B		Not specified because of a part of ''Relative content of stearic acid and palmitic acid''.
Acidity or alkalinity	Purity (1) Acidity or alkalinity	
Loss on drying	Loss on drying	
Limit of chloride	Purity (2) Chloride	
Limit of sulfate	Purity (3) Sulfate	
Limit of cadmium	not specified	
Limit of lead	not specified	
Limit of nickel	not specified	
Relative content of stearic acid and palmitic acid	Relative content of stearic acid and palmitic acid	
Assay	Assay	

Add the following:

Oct. 2007

Harmonized items	JP16 (Supplement I)	Remarks
Sucrose	**Sucrose**	
Definition	origin	
Appearance of solution	Purity (2) Clarity of solution	
Conductivity	Conductivity	
Specific optical rotation	Optical rotation	
Colour value	Purity (1) Color value	
Dextrins	Dextrins	
Reducing sugars	Purity (4) Reducing sugars	
Sulphite	Purity (3) Sulfite	
Loss on drying	Loss on drying	
Bacterial endotoxins	Bacterial endotoxins	
Labelling	origin	

Add the following:

Oct. 2009

Harmonized items	JP16 (Supplement I)	Remarks
	General Information	
Water-Solids Interactions	**Water-Solids Interactions : Determinations of the Sorption-Desorption Isotherms and the Water Activity**	
	(Introduction)	JP's particular description: Explanation on the test method.
Introduction	not specified	
Physical states of sorbed water	not specified	
Rates of water uptake	not specified	
Determination of Sorption-desorption Isotherms	1. Determination of Sorption-desorption Isotherms	
Principle	1.1 Principle	
Methods	1.2 Methods	
Report and interpretation of the data	1.3 Report and interpretation of the data	
Determination of the water activity	2. Determination of the water activity	
Principle	2.1 Principle	
Method	2.2 Method	
Figure 1 Example of an apparatus for the determination of the water sorption (other designs are possible)	Fig. 1 Example of an apparatus for the determination of the water sorption (other designs are possible)	
Table 1 Standard saturated salt solutions	Table 1 Standard saturated salt solutions	

Change to read:

Jun. 2011 (Rev.2)

Harmonized items	JP16 (Supplement I)	Remarks
Bulk Density and Tapped Density of Powders	**3.01 Determination of Bulk and Tapped Densities**	
	(Introduction)	JP's particular description: Explanation of the test method.
Bulk density	1. Bulk density	
Method 1: Measurement in a graduated cylinder	1.1. Method 1: Measurement in a graduated cylinder	
Procedure	1.1.1. Procedure	
Method 2: Measurement in a volumeter	1.2. Method 2: Measurement in a volumeter	
Apparatus	1.2.1. Apparatus	
Procedure	1.2.2. Procedure	
Method 3: Measurement in a vessel	1.3. Method 3: Measurement in a vessel	
Apparatus	1.3.1. Apparatus	
Procedure	1.3.2. Procedure	
Tapped density	2. Tapped density	
Method 1	2.1. Method 1	
Apparatus	2.1.1. Apparatus	
Procedure	2.1.2. Procedure	
Method 2	2.2. Method 2	
Procedure	2.2.1. Procedure	
Method 3	2.3. Method 3	
Procedure	2.3.1. Procedure	
Measures of powder compressibility	3. Measures of powder compressibility	
Figure 1 Volumeter	Fig. 3.01-1 Volumeter	
Figure 2 Measuring vessel (left) and cap (right)	Fig. 3.01-2 Measuring vessel (left) and cap (right)	
Figure 3	Fig. 3.01-3	

Change to read:

Jun. 2011 (Rev.2)

Harmonized items	JP16 (Supplement I)	Remarks
Bacterial Endotoxins Test	**4.01 Bacterial Endotoxins Test**	
(Introduction)	(Introduction)	
Apparatus	1. Apparatus	
	2. Preparation of solutions	
Preparation of standard endotoxin stock solution	2.1. Preparation of standard endotoxin stock solution	
Preparation of standard endotoxin solution	2.2. Preparation of standard endotoxin solution	
Preparation of sample solutions	2.3. Preparation of sample solutions	

Determination of maximum valid dilution	3. Determination of maximum valid dilution	
Gel-clot technique	4. Gel-clot techniques	
(1) Preparatory testing	4.1. Preparatory testing	
(i) Test for confirmation of labeled lysate sensitivity	4.1.1. Test for confirmation of labeled lysate sensitivity	
(ii) Test for interfering factors	4.1.2. Test for interfering factors	
(2) Limit test	4.2. Limit test	
(i) Procedure	4.2.1. Procedure	
(ii) Interpretation	4.2.2. Interpretation	
(3) Quantitative test	4.3. Quantitative test	
(i) Procedure	4.3.1. Procedure	
(ii) Calculation and interpretation	4.3.2. Calculation and interpretation	
Photometric quantitative techniques	5. Photometric quantitative techniques	
(1) Turbidimetric technique	5.1. Turbidimetric technique	
(2) Chromogenic technique	5.2. Chromogenic technique	
(3) Preparatory testing	5.3. Preparatory testing	
(i) Assurance of criteria for the standard curve	5.3.1. Test for assurance of criteria for the standard curve	
(ii) Test for interfering factors	5.3.2. Test for interfering factors	
(4) Test	5.4. Quantitative test	
(i) Procedure	5.4.1. Procedure	
(ii) Calculation	5.4.2. Calculation of endotoxin concentration	
(iii) Interpretation	5.4.3. Interpretation	
Reagents, test solutions		These reagents and test solution are being defined in ''9.41 Reagents, Test Solutions''.
Amoebocyte lysate		
Lysate TS		
Water for bacterial endotoxins test (BET)		
Table 1	Table 4.01-1	
Table 2	Table 4.01-2	
Table 3	Table 4.01-3	
Table 4	Table 4.01-4	

Change to read:

Jun. 2010 (Rev.3)

Harmonized items	JP16 (Supplement I)	Remarks
Dissolution	**6.10 Dissolution Test**	JP's particular description: The test also aims at preventing significant bioinequivalence.
Apparatus	1. Apparatus	
Apparatus 1 (Basket apparatus)	1.1. Apparatus for Basket Method (Apparatus 1)	

Apparatus 2 (Paddle apparatus)	1.2. Apparatus for Paddle Method (Apparatus 2)	JP's particular description: The sinker is allowed to use in case only when specified in the monograph.
Apparatus 3 (Reciprocating cylinder)	not specified	
Apparatus 4 (Flow-through cell)	1.3. Apparatus for Flow-Through Cell Method (Apparatus 3)	
Apparatus suitability	2. Apparatus suitability	
Procedure	3. Procedure	
Apparatus 1 or 2	3.1. Basket Method or Paddle Method	
Immediate-release dosage forms	3.1.1. Immediate-release Dosage Forms	
Procedure	(i) Procedure	
Dissolution medium	(ii) Dissolution Medium	
Time	(iii) Time	
Extended-release dosage forms	3.1.2. Extended-release Dosage Forms	
Procedure	(i) Procedure	
Dissolution medium	(ii) Dissolution Medium	
Time	(iii) Time	
Delayed-release dosage forms	3.1.3. Delayed-release Dosage Forms	
Procedure	(i) Procedure	In the Harmonized text: Alternative usage of Method A or B.
Method A		
Method B		
	(ii) Dissolution Medium	JP's particular description.
Time	(iii) Time	JP's particular description: Time is specified each for the 1st and 2nd fluids.
Apparatus 3	not specified	
Immediate-release dosage forms		
Procedure		
Dissolution medium		
Time		
Extended-release dosage forms		
Procedure		
Dissolution medium		
Time		
Delayed-release dosage forms		
Procedure		
Time		
Apparatus 4	3.2. Flow-Through Cell Method	
Immediate-release dosage forms	3.2.1. Immediate-release Dosage Forms	
Procedure	(i) Procedure	
Dissolution medium	(ii) Dissolution Medium	
Time	(iii) Time	

Extended-release dosage forms	3.2.2. Extended-release Dosage Forms	
Procedure	(i) Procedure	
Dissolution medium	(ii) Dissolution Medium	
Time	(iii) Time	
Delayed-release dosage forms		
Procedure		
Time		
Interpretation	4. Interpretation	JP's particular description: Follow Interpretation 1 when the value Q is specified in the individual monograph, otherwise follow Interpretation 2.
Immediate-release dosage forms	4.1. Immediate-release Dosage Forms	JP's particular description: Setting of Interpretation 2.
	4.1.1. Interpretation 1	
	4.1.2. Interpretation 2	
Extended-release dosage forms	4.2. Extended-release Dosage Forms	JP's particular description: Setting of Interpretation 2.
	4.2.1. Interpretation 1	
	4.2.2. Interpretation 2	
Delayed-release dosage forms	4.3. Delayed-release Dosage Forms	Nonharmonized items: Different dissolution medium. Deletion of disharmonized part on the value Q. JP's particular description: Setting of Interpretation 2.
	4.3.1. Interpretation 1	The value Q is specified in the individual monograph.
	4.3.2. Interpretation 2	
Acceptance Table 1	Acceptance Table 6.10-1	
Acceptance Table 2	Acceptance Table 6.10-2	
Acceptance Table 3	Acceptance Table 6.10-3	
Acceptance Table 4	Acceptance Table 6.10-4	
Figure 1 Apparatus1 Basket stirring element	Fig. 6.10-1 Apparatus 1 Basket stirring element	
Figure 2 Paddle stirring element	Fig. 6.10-2 Apparatus 2 Paddle stirring element	
Figure 2a Alternative sinker	Fig. 6.10-2a Alternative sinker	
Figure 3 Apparatus 3	not specified	
Figure 4 Apparatus 4 (top) large cell for tablets and capsules (bottom) tablet holder for the large cell	Fig. 6.10-3 Apparatus 3 (top) large cell for tablets and capsules (bottom) tablet holder for the large cell	
Figure 5 Apparatus 4 (top) small cell for tablets and capsules (bottom) tablet holder for the small cell	Fig. 6.10-4 Apparatus 3 (top) small cell for tablets and capsules (bottom) tablet holder for the small cell	

Change to read:

Jun. 2010 (Rev.2)

Harmonized items	JP16 (Supplement I)	Remarks
Citric Acid, Anhydrous	**Anhydrous Citric Acid**	
Definition	limits of content	
Identification	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Readily carbonisable substances	Purity (5) Readily carbonizable substances	
Oxalic acid	Purity (3) Oxalic acid	
Sulphates	Purity (2) Sulfates	
Aluminium	not specified	
Water	Water	
Sulphated ash	Residue on ignition	
Assay	Assay	

Change to read:

Jun. 2010 (Rev.2)

Harmonized items	JP16 (Supplement I)	Remarks
Citric Acid Monohydrate	**Citric Acid Hydrate**	
Definition	limits of content	
Identification	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Readily carbonisable substances	Purity (5) Readily carbonizable substances	
Oxalic acid	Purity (3) Oxalic acid	
Sulphates	Purity (2) Sulfates	
Aluminium	not specified	
Water	Water	
Sulphated ash	Residue on ignition	
Assay	Assay	

Change to read:

Jun. 2012 (Rev.1, Corr.1)

Harmonized items	JP16 (Supplement I)	Remarks
Cellacefate	**Cellacefate**	
Definition	Content of the acetyl and carboxybenzoyl groups	
Identification	Identification	
Viscosity	Viscosity	
Water	Water	
Residue on ignition	Residue on ignition	
Limit of free acid	Purity (2) Free acids	
Phthalyl content	Assay (1) Carboxybenzoyl group	
Content of acetyl	Assay (2) Acetyl group	

Change to read:

Nov. 2010 (Rev.4)

Harmonized items	JP16 (Supplement I)	Remarks
Anhydrous Lactose	**Anhydrous Lactose**	
Definition	origin	
Infrared absorptions spectrophotometry	Identification	
Clarity and color of solution	Purity (1) Clarity and color of solution	
Specific rotation	Optical rotation	
Acidity or alkalinity	Purity (2) Acidity or alkalinity	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Water	Water	
Protein and light-absorbing impurities	Purity (4) Protein and light absorbing substances	
Content of alpha and beta anomers	Isomer ratio	
Microbial contamination	Microbial limit	

Change to read:

Nov. 2011 (Rev.1, Corr.1)

Harmonized items	JP16 (Supplement I)	Remarks
Ethyl Parahydroxybenzoate	**Ethyl Parahydroxybenzoate**	
Definition	limits of content	
Identification A	Melting point	
Identification B	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Acidity	Purity (2) Acidity	
Related substances	Purity (4) Related substances	JP's particular description: Test for required detectability, System repeatability.
Sulphated ash	Residue on ignition	
Assay	Assay	

Change to read:

Jun. 2010 (Rev.1)

Harmonized items	JP16 (Supplement I)	Remarks
Butyl Parahydroxybenzoate	**Butyl Parahydroxybenzoate**	
Definition	limits of content	
Identification A	Melting point	
Identification B	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Acidity	Purity (2) Acidity	
Related substances	Purity (4) Related substances	JP's particular description: Test for required detectability, System repeatability.
Sulphated ash	Residue on ignition	
Assay	Assay	

Change to read:

Nov. 2011 (Rev.1, Corr.1)

Harmonized items	JP16 (Supplement I)	Remarks
Propyl Parahydroxybenzoate	**Propyl Parahydroxybenzoate**	
Definition	limits of content	
Identification A	Melting point	
Identification B	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Acidity	Purity (2) Acidity	
Related substances	Purity (4) Related substances	JP's particular description: Test for required detectability, System repeatability.
Sulphated ash	Residue on ignition	
Assay	Assay	

Change to read:

Nov. 2011 (Rev.1, Corr.1)

Harmonized items	JP16 (Supplement I)	Remarks
Methyl Parahydroxybenzoate	**Methyl Parahydroxybenzoate**	
Definition	limits of content	
Identification A	Melting point	
Identification B	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Acidity	Purity (2) Acidity	
Related substances	Purity (4) Related substances	JP's particular description: Test for required detectability, System repeatability.
Sulphated ash	Residue on ignition	
Assay	Assay	

Change to read:

Jun. 2011 (Rev.2, Corr.1)

Harmonized items	JP16 (Supplement I)	Remarks
Benzyl Alcohol	**Benzyl Alcohol**	
Definition	limits of content	
Refractive index	Refractive index	
Acidity	Purity (2) Acidity	
Benzaldehyde and other related substances	Purity (3) Benzaldehyde and other related substances	
Peroxide value	Purity (4) Peroxide value	
Residue on evaporation	Purity (5) Residue on evaporation	
Assay	Assay	

Change to read:

Jun. 2011 (Rev.1)

Harmonized items	JP16 (Supplement I)	Remarks
Anhydrous Dibasic Calcium Phosphate	**Anhydrous Dibasic Calcium Phosphate**	
Definition	limits of content	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Acid-insoluble substances	Purity (1) Acid-insoluble substances	
Chloride	Purity (2) Chloride	
Sulfate	Purity (3) Sulfates	
Carbonate	Purity (4) Carbonate	
Barium	Purity (6) Barium	
Loss on ignition	Loss on ignition	
Assay	Assay	

Change to read:

Jun. 2011 (Rev.1)

Harmonized items	JP16 (Supplement I)	Remarks
Dibasic Calcium Phosphate	**Dibasic Calcium Phosphate Hydrate**	
Definition	limits of content	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Acid-insoluble substances	Purity (1) Acid-insoluble substances	
Chloride	Purity (2) Chloride	
Sulfate	Purity (3) Sulfates	
Carbonate	Purity (4) Carbonate	
Barium	Purity (6) Barium	
Loss on ignition	Loss on ignition	
Assay	Assay	

INDEX

A

B

C

D

E

F

G

H

I

J

K

L

M

N

Q

R

S

T

U

V

W

X

Y

Z

INDEX IN LATIN NAME

A

B

C

D

E

F

G

H

INDEX IN JAPANESE

ア

イ

ウ

エ

オ

カ

キ

ク

ケ

コ

サ

シ

ス

セ

ソ

タ

チ

ツ

テ

ト

ナ

ニ

ネ

ノ

ハ

ヒ

フ

へ

ホ

マ

ミ

ム

メ

モ

ヤ

ユ

ヨ

ラ

リ

レ

ロ

ワ