SUPPLEMENT I
TO
THE JAPANESE PHARMACOPOEIA
SEVENTEENTH EDITION

Official from December 1, 2017

English Version

THE MINISTRY OF HEALTH, LABOUR AND WELFARE

Notice: This *English Version* of the Japanese Pharmacopoeia is published for the convenience of users unfamiliar with the Japanese language. When and if any discrepancy arises between the Japanese original and its English translation, the former is authentic.

Printed in Japan

The Ministry of Health, Labour and Welfare Ministerial Notification No. 348

Pursuant to Paragraph 1, Article 41 of the Law on Securing Quality, Efficacy and Safety of Products including Pharmaceuticals and Medical Devices (Law No. 145, 1960), we hereby revise a part of the Japanese Pharmacopoeia (Ministerial Notification No. 64, 2016) as follows*. However, in the case of drugs which are listed in the 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 December 1, 2017 as prescribed under Paragraph 1, Article 14 of the same law [including drugs the Minister of Health, Labour and Welfare specifies (the Ministry of Health and Welfare Ministerial Notification No. 104, 1994) as of November 30, 2017 as those exempted from marketing approval pursuant to Paragraph 1, Article 14 of the same 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 May 31, 2019. 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 December 1, 2017 as prescribed under the Paragraph 1, Article 14 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 May 31, 2019.

Katsunobu Kato
The Minister of Health, Labour and Welfare

December 1, 2017

(The text referred to by the term ''as follows'' are omitted here. All of them are made available for public exhibition at the Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health 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 Seventeenth Edition from General Notice to Ultraviolet-visible Reference Spectra (pp. 2631 – 2811).

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PREFACE

The 17th Edition of the Japanese Pharmacopoeia (JP) was promulgated by Ministerial Notification No.64 of the Ministry of Health, Labour and Welfare (MHLW) on March 7, 2016.

In July 2016, the Committee on JP established the basic principles for the preparation of the JP 18th 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) Further promoting internationalization in response to globalization of drug market; 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 18th Edition (the Japanese edition) was set as April 2021.

JP drafts are discussed in the following committees that were established in the Pharmaceuticals and Medical Devices Agency: Expert Committee; Subexpert Committee; Sub-committee on Manufacturing Process-related Matters; Committee on Chemicals; Committee on Antibiotics; Committee on Biologicals; Committee on Crude Drugs; Committee on Pharmaceutical Excipients; Committee on Physico-Chemical Methods; Committee on Drug Formulation; Committee on Physical Methods; Committee on Biological Methods; Committee on Nomenclature for Pharmaceuticals; Committee on International Harmonization; and Committee on Reference Standards. Furthermore, working groups are established under the Committee on Pharmaceutical Excipients, Committee on Physico-Chemical Methods and Committee on Drug Formulation.

In the Committee on JP, Mitsuru Hashida took the role of chairman from January 2011 to November 2017.

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 August 2015 and March 2017, were prepared for a supplement to the JP 17. They were examined by the Committee on JP in April 2017, followed by the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) in June 2017, 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-committee on Manufacturing Process-related Matters (9), Committee on Chemicals (20), Committee on Antibiotics (5); Committee on Biologicals (8); Committee on Crude Drugs (17); Committee on Pharmaceutical Excipients (10); Committee on Physico-Chemical Methods (14, including a working group); Committee on Drug Formulation (27, including working groups); Committee on Physical Methods (8); Committee on Biological Methods (6); Committee on Nomenclature for Pharmaceuticals (7); Committee on International Harmonization (6); and Committee on Reference Standards (4).

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 Home Medicine Association of Japan, the Japan Kampo Medicines Manufacturers Association, the Japan Flavor and Fragrance Materials Association, the Japan Medical Plants Federation, the Japan Pharmaceutical Manufacturers Association, the Federation of Pharmaceutical Manufacturers' Association of Japan, the Parenteral Drug Association Japan Chapter, the Japan Reagent Association, the Japan Oilseeds Processors Association, the Japan Analytical Instruments Manufacturers' Association, and the Asian Society of Innovative Packaging Technology.

In consequence of this revision, the JP 17th Edition carries 1977 articles, owing to the addition of 32 articles and the deletion of 17 article.

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

1. The Supplement I to JP 17th Edition comprises the following items, in order: Notification of MHLW; Contents; Preface; 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) Chemical Abstracts Service (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) Manufacture
(14) Description
(15) Identification tests
(16) Specific physical and/or chemical values
(17) Purity tests
(18) Potential adulteration
(19) Loss on drying or Ignition, or Water
(20) Residue on ignition, Total ash or Acid-insoluble ash
(21) Tests being required for pharmaceutical preparations
(22) Other special tests
(23) Assay
(24) Containers and storage
(25) Shelf life
(26) 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. The following paragraphs were newly added to the General Rules for Preparations:
(1) The following items were newly added to the [3] Monographs for Preparations according to increase of commercial products:
1.8. Films for Oral Administration
1.8.1 Orally Disintegrating Films

8. The General Rules for Preparations was revised as follows in general:
(1) [1] General Notices for Preparations (9): Microbial Limit Test for Crude Drugs and Preparations containing Crude Drugs as Main Ingredient <*5.02*> was added as a test to be applied as necessary for non-sterile preparations to prevent contamination.
(2) [3] Monographs for Preparations: ''5-1-1. Dry Powder Inhalers'' and ''5-1-3. Metered-dose Inhalers'': the requirements for general tests of Uniformity of Delivered Dose for Inhalations <*6.14*> and Aerodynamic Particle Size Measurement for Inhalations <*6.15*> were prescribed.
(3) [3] Monographs for Preparations, ''9-1. Suppositories for Rectal Application'' and ''10-2. Suppositories for Vaginal Use'': suppositories for rectal application and vaginal use using lipophilic bases were revised to be able to apply the evaluation of meltability instead of active substance release tests to assure appropriate drug release.
(4) [3] Monographs for Preparations, ''11-2. Liquids and Solutions for Cutaneous Application'': the specification that Transdermal Systems in single-dose packages among Liquids and Solutions for Cutaneous Application is required to meet the requirements of Uniformity of Dosage Units <*6.02*> was added.

9. The following items were newly added to General Tests, Processes and Apparatus:

(1) 3.06 Laser Diffraction Measurement of Particle Size
(2) 6.14 Uniformity of Delivered Dose for Inhalations
(3) 6.15 Aerodynamic Particle Size Measurement for Inhalations

10. The following items in General Tests, Processes and Apparatus were revised:

(1) 2.24 Ultraviolet-visible Spectrophotometry
(2) 2.46 Residual Solvents
(3) 4.03 Digestion Test
(4) 6.02 Uniformity of Dosage Units
(5) 6.04 Test for Acid-neutralizing Capacity of Gastrointestinal Medicines
(6) 9.01 Reference Standards
(7) 9.21 Standard Solutions for Volumetric Analysis
(8) 9.41 Reagents, Test Solutions
(9) 9.42 Solid Supports/Column Packings for Chromatography

11. The following Reference Standards were newly added:

Entacapone RS
Entacapone Related Substance A RS for System Suitability
Glucose RS
Insulin Aspart RS
Pazufloxacin Mesilate RS
Pyridoxal Phosphate RS
Saccharin Sodium RS for Identification
Zonisamide RS

12. The following Reference Standards were revised the name:

Adrenaline Bitartrate RS for Purity
p-Aminobenzoyl Glutamic Acid RS for Purity
Anhydrous Lactose RS for Identification
Cellacefate RS for Identification
Gitoxin RS for Purity
Heparin Sodium RS for Identification
Lactose RS for Identification
Over-sulfated Chondroitin Sulfate RS for System Suitability
Povidone RS for Identification
Tyrosine RS for Digestion Test

13. The following Reference Standards were deleted from the list of 9.01 Reference Standards:

Aceglutamide RS
Diclofenamide RS
Digitoxin RS
Fluoxymesterone RS
Gramicidin RS
Lanatoside C RS
Rokitamycin RS
Tolazamide RS
Zinostatin Stimalamer RS

14. The following substances were newly added to the Official Monographs:

Azosemide
Azosemide Tablets
Calcium Levofolinate Hydrate
Cefoperazone Sodium for Injection
Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution
Clomipramine Hydrochloride Tablets
Clotiazepam Tablets
Entacapone
Entacapone Tablets
Glucose Hydrate
Purified Glucose
Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension
Biphasic Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension
Insulin Aspart (Genetical Recombination)
Irbesartan Tablets
Irbesartan and Amlodipine Besilate Tablets
Magnesium Aluminosilicate
Magnesium Aluminometasilicate
Mesalazine
Mesalazine Extended-release Tablets
Methotrexate Tablets
Montelukast Sodium Granules
Pazufloxacin Mesilate
Pazufloxacin Mesilate Injection
Pentobarbital Calcium Tablets
Pyridoxal Phosphate Hydrate
Roxithromycin Tablets
Tramadol Hydrochloride
Voriconazole for Injection
Zonisamide
Zonisamide Tablets
Goreisan Extract

15. The following monographs were revised:

Amoxicillin Hydrate
Ampicillin Hydrate
Bacitracin
Benzylpenicillin Potassium
Cefixime Hydrate

Ceftizoxime Sodium
Cellacefate
Chloramphenicol Sodium Succinate
Clarithromycin
Cloxacillin Sodium Hydrate
Colistin Sodium Methanesulfonate
Demethylchlortetracycline Hydrochloride
Dextran 40
Digoxin
Doxorubicin Hydrochloride
Doxycycline Hydrochloride Hydrate
Edaravone Injection
Epalrestat
Erythromycin
Ethanol
Anhydrous Ethanol
Folic Acid
Fosfomycin Calcium Hydrate
Fosfomycin Sodium
Gentamicin Sulfate
Glucose Injection
Heparin Calcium
Heparin Sodium
Hydrocortisone Acetate
Hydrocortisone Butyrate
Hydroxocobalamin Acetate
Hydroxypropylcellulose
Low Substituted Hydroxypropylcellulose
Hypromellose
Iohexol Injection
Isosorbide Mononitrate 70%/Lactose 30%
Anhydrous Lactose
Lactose Hydrate
D-Mannitol
Methylcellulose
Noradrenaline
Oxytetracycline Hydrochloride
Pentobarbital Calcium
Polymixin B Sulfate
Polysorbate 80
Povidone
Saccharin Sodium Hydrate
Sodium Lauryl Sulfate
Spiramycin Acetate
Sulbactam Sodium
Sultamicillin Tosilate Hydrate
Suxamethonium Chloride Injection
Teicoplanin
Tetracycline Hydrochloride
Thrombin
Tobramycin
Vasopressin Injection
Verapamil Hydrochloride
Verapamil Hydrochloride Tablets
Vinblastine Sulfate
Vincristine Sulfate
Powdered Alisma Tuber
Artemisia Capillaris Flower
Bakumondoto Extract
Bofutsushosan Extract
Boiogito Extract
Chotosan Extract
Cornus Fruit
Curcuma Rhizome
Cyperus Rhizome
Powdered Cyperus Rhizome
Daiokanzoto Extract
Daisaikoto Extract
Euodia Fruit
Gardenia Fruit
Glycyrrhiza Extract
Crude Glycyrrhiza Extract
Goshajinkigan Extract
Hachimijiogan Extract
Hangekobokuto Extract
Hangeshashinto Extract
Hochuekkito Extract
Jujube
Jujube Seed
Juzentaihoto Extract
Kakkonto Extract
Kakkontokasenkyushin'i Extract
Kamikihito Extract
Kamishoyosan Extract
Keishibukuryogan Extract
Maoto Extract
Mukoi-Daikenchuto Extract
Orengedokuto Extract
Oriental Bezoar
Otsujito Extract
Platycodon Root
Rape Seed Oil
Rikkunshito Extract
Ryokeijutsukanto Extract
Saibokuto Extract
Saikokeishito Extract
Saireito Extract
Saposhnikovia Root and Rhizome
Shakuyakukanzoto Extract
Shimbuto Extract
Shosaikoto Extract
Shoseiryuto Extract
Powdered Sweet Hydrangea Leaf
Tokakujokito Extract
Tokishakuyakusan Extract
Trichosanthes Root

Turmeric
Powdered Turmeric
Yokukansan Extract

16. The following monographs were deleted:
Aceglutamide Aluminum
Diclofenamide
Diclofenamide Tablets
Digitoxin
Digitoxin Tablets
Fluoxymesterone
Gramicidin
Lanatoside C
Lanatoside C Tablets
Mercurochrome
Mercurochrome Solution
Rokitamycin
Rokitamycin Tablets
Serrapeptase
Tolazamide
Zinostatin Stimalamer
Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder

17. The following articles were newly added to Ultraviolet-visible Reference Spectra:
Azosemide
Calcium Levofolinate Hydrate
Doxycycline Hydrochloride Hydrate
Entacapone
Mesalazine
Pazufloxacin Mesilate
Pyridoxal Phosphate Hydrate
Sultamicillin Tosilate Hydrate
Tramadol Hydrochloride
Zonisamide

18. The following articles in Ultraviolet-visible Reference Spectra were deleted:
Diclofenamide
Fluoxymesterone
Gramicidin
Rokitamycin
Tolazamide

19. The following articles were newly added to Infrared Reference Spectra:
Azosemide
Calcium Levofolinate Hydrate
Entacapone
Low Substituted Hydroxypropylcellulose
Mesalazine
Oxytetracycline Hydrochloride
Pazufloxacin Mesilate
Pyridoxal Phosphate Hydrate
Tramadol Hydrochloride
Zonisamide

20. The following articles in Infrared Reference Spectra were deleted:
Diclofenamide
Fluoxymesterone
Rokitamycin
Saccharin Sodium Hydrate
Tolazamide

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Supplement I to The Japanese Pharmacopoeia Seventeenth Edition

GENERAL RULES FOR PREPARATIONS

[1] General Notices for Preparations

Change the paragraphs (9) as follows:

(9) Even non-sterile preparations should be prepared with precautions to prevent contamination and growth of microorganisms, and the test of Microbiological Examination of Non-sterile Products <*4.05*> or Microbial Limit Test for Crude Drugs and Preparations containing Crude Drugs as Main Ingredient <*5.02*> is applied to them, if necessary.

[3] Monographs for Preparations

Add the following next to section 1-7. Jellies for Oral Administration:

1-8. Films for Oral Administration

(1) Films for Oral Administration are preparations in film form, intended for oral administration.

(2) Films for Oral Administration are usually prepared by spreading to dry a solution, composed of active substance(s) and a mixture of water-soluble polymer and other additives as a base, or by melting the mixture of active substances(s) and the base to form. Layers different in additive compositions may be stacked in appropriate manner to form the films.

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

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

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

1-8-1. Orally Disintegrating Films

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

(2) Orally Disintegrating Films show an appropriate disintegration.

Change the paragraphs (3) and (4) under 5-1-1. Dry Powder Inhalers as follows:

5-1-1. Dry Powder Inhalers

(3) Metered-dose types among Dry Powder Inhalers meet the requirements of Uniformity of Delivered Dose for Inhalations <*6.14*>, unless otherwise specified.

(4) Dry Powder Inhalers meet the requirements of Aerodynamic Particle Size Measurement for Inhalations <*6.15*>, unless otherwise specified.

Change the paragraphs (3) and (4) under 5-1-3. Metered-dose Inhalers as follows:

5-1-3. Metered-dose Inhalers

(3) Metered-dose Inhalers meet the requirements of Uniformity of Delivered Dose for Inhalations <*6.14*>, unless otherwise specified.

(4) Metered-dose Inhalers meet the requirements of Aerodynamic Particle Size Measurement for Inhalations <*6.15*>, unless otherwise specified.

Change the paragraphs (5) under 9-1. Suppositories for Rectal Application as follows:

9-1. Suppositories for Rectal Application

(5) Suppositories for Rectal Application show an appropriate release. Release of Suppositories for Rectal Application prepared using a lipophilic base can be evaluated by melting behavior of suppositories in place of release of active substances. When the melting behavior of Suppositories for Rectal Application is measured according to Method 2 under Melting Point Determination <*2.60*> unless otherwise specified, it shows an appropriate melting temperature.

Change the paragraphs (5) under 10-2. Suppositories for Vaginal Use as follows:

10-2. Suppositories for Vaginal Use

(5) Suppositories for Vaginal Use show an appropriate release. Release of Suppositories for Vaginal Use prepared using a lipophilic base can be evaluated by melting behavior of suppositories in place of release of active substances. When the melting behavior of Suppositories for Vaginal Use is measured according to Method 2 under Melting Point Determination <*2.60*> unless otherwise specified, it shows an appropriate melting temperature.

Change the paragraphs (3) under 11-2. Liquids and Solutions for Cutaneous Application as follows:

11-2. Liquids and Solutions for Cutaneous Application

(3) Unless otherwise specified, Liquids and Solutions for Cutaneous Application in single-dose packages such as Transdermal Systems meet the requirements of Uniformity of Dosage Units <*6.02*>.

GENERAL TESTS, PROCESSES AND APPARATUS

Change the introduction to read:

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, aerodynamic particle size measurement for inhalations, 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 liquids and solutions, gas chromatography, glycosylation analysis of glycoprotein, 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 liquids and solutions, iron determination, laser diffraction measurement of particle size, liquid chromatography, loss on drying determination, loss on ignition determination, mass spectrometry, melting point determination, methanol determination, methods for color matching, methods of adhesion testing, 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, release test for preparations for cutaneous application, residual solvents, 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, turbidity measurement, ultravioletvisible spectrophotometry, uniformity of delivered dose for inhalations, 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, essential oil content of crude drugs and assay of marker compounds for the assay of crude drugs and extracts of Kampo Formulations utilizing nuclear magnetic resonance (NMR) spectroscopy 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.

2.24 Ultraviolet-visible Spectrophotometry

Change the following as follows:

1. Apparatus and adjustment

A spectrophotometer or a photoelectric photometer is used for the measurement of absorbance.

After adjusting the spectrophotometer or photoelectric photometer based on the operation manual of the apparatus, it should be confirmed that the wavelength and the transmission rate meet the specifications of the tests described below.

The calibration of wavelength should be carried out as follows. Using an optical filter for wavelength calibration, measure the transmission rate in the vicinity of the standard wavelength value shown in the test results form, under the test conditions given in the test results form attached to each of the filters. When performing a test to determine the wavelength which shows minimal transmission rate, the difference between the measured wavelength and the standard wavelength value should be within ± 0.5 nm. When the measurement is repeated three times, each value obtained should be within the mean ± 0.2 nm. It is also possible to carry out the test using a deuterium discharge lamp at bright line wavelengths of 486.00 nm and 656.10 nm. In the case of these tests, the difference between the measured wavelength and the wavelength of the bright line should be within ±

0.3 nm. When the measurement is repeated three times, each value obtained should be within the mean ± 0.2 nm.

The calibration of transmission rate or absorbance should be carried out as follows. Using an optical filter for transmission rate calibration, determine the transmission rate at the standard wavelength value under the test conditions given in the test results form attached to each of the filters. The difference between the measured transmission rate and the standard transmission rate value should be within the range of from 1% larger of the upper limit to 1% smaller of the lower limit for the relative accuracy shown in the test results form. When the measurement is repeated three times, each absorbance obtained (or calculated from the transmission rate) should be within the mean ± 0.002 when the absorbance is not more than 0.500, and within the mean ± 0.004 when the absorbance is more than 0.500. In addition, it will be desirable to confirm the linearity of transmission rate at the same wavelength using several optical filters for calibration of transmission rate with different transmission rates.

2.46 Residual Solvents

Change the following as follows:

The chapter of residual solvents describes the control, identification and quantification of organic solvents remaining in drug substances, excipients and drug products.

I. Control of residual solvents

1. Introduction

Residual solvents in pharmaceuticals (except for crude drugs and their preparations) are defined here as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. The solvents are not completely removed by practical manufacturing techniques. Appropriate selection of the solvent for the synthesis of drug substance may enhance the yield, or determine characteristics such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical parameter in the synthetic process. The test method described in this chapter does not address solvents deliberately used as excipients nor does it address solvates. However, the content of solvents in such products should be evaluated and justified.

Since there is no therapeutic benefit from residual solvents, all residual solvents should be reduced to the extent possible to meet product specifications, good manufacturing practices, or other quality-based requirements. Drug products should contain no higher levels of residual solvents than can be supported by safety data. Some solvents that are known to cause unacceptable toxicities (Class 1, Table 2.46-1) should be avoided in the production of drug substances, excipients, or drug products unless their use can be strongly justified in a risk-benefit assessment. Some solvents associated with less severe toxicity (Class 2, Table 2.46-2) should be limited in order to protect patients from potential adverse effects. Ideally, less toxic solvents (Class 3, Table 2.46-3) should be used where practical.

Testing should be performed for residual solvents when production or purification processes are known to result in the presence of such solvents. It is only necessary to test for solvents that are used or produced in the manufacture or purification of drug substances, excipients, or drug products. Although manufacturers may choose to test the drug product, a cumulative method may be used to calculate the residual solvent levels in the drug product from the levels in the ingredients used to produce the drug product. If the calculation results in a level equal to or below that recommended in this chapter, no testing of the drug product for residual solvents needs to be considered. If, however, the calculated level is above the recommended level, the drug product should be tested to ascertain whether the formulation process has reduced the relevant solvent level to within the acceptable amount. Drug product should also be tested if a solvent is used during its manufacture.

The limit applies to all dosage forms and routes of administration. Higher levels of residual solvents may be acceptable in certain cases such as short term (30 days or less) or topical application. Justification for these levels should be made on a case by case basis.

2. General principles

2.1. Classification of residual solvents by risk assessment

The term ''PDE'' (Permitted Daily Exposure) is defined in this chapter as a pharmaceutically acceptable daily intake of residual solvents. Residual solvents assessed in this chapter were evaluated for their possible risk to human health and placed into one of three classes as follows:

(i) Class 1 solvents: Solvents to be avoided in manufacture of pharmaceuticals

Known human carcinogens, strongly suspected human carcinogens, and environmental hazards. Class 1 solvents are listed in Table 2.46-1.

(ii) Class 2 solvents: Solvents to be limited in pharmaceuticals

Non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity. Solvents suspected of other significant but reversible toxicities. Class 2 solvents are listed in Table 2.46-2.

(iii) Class 3 solvents: Solvents with low toxic potential

Solvents with low toxic potential to man; no health-based exposure limit is needed. Class 3 solvents are listed in Table 2.46-3 and have PDEs of 50 mg or more per day.

2.2. Option for describing limits of Class 2 solvents

Two options are available when setting limits for Class 2 solvents.

2.2.1. Option 1

The concentration limits in ppm can be calculated using equation (1) below by assuming a product mass of 10 g administered daily.

$$\text{Concentration limit (ppm)} = \frac{1000 \times PDE}{\text{dose}} \qquad (1)$$

Here, PDE is given in terms of mg per day and dose is

given in g per day.

These limits are considered acceptable for all substances, excipients, or products. Therefore this option may be applied if the daily dose is not known or fixed. If all excipients and drug substances in a formulation meet the limits given in Option 1, then these components may be used in any proportion. No further calculation is necessary provided the daily dose does not exceed 10 g. Products that are administered in doses greater than 10 g per day should be considered under Option 2.

2.2.2. Option 2

It is not considered necessary for each component of the drug product to comply with the limits given in Option 1. The PDE in terms of mg per day as stated in Table 2.46-2 can be used with the known maximum daily dose and equation (1) above to determine the concentration of residual solvent allowed in drug product. Such limits are considered acceptable provided that it has been demonstrated that the residual solvent has been reduced to the practical minimum. The limits should be realistic in relation to analytical precision, manufacturing capability, reasonable variation in the manufacturing process, and the limits should reflect contemporary manufacturing standards.

Option 2 may be applied by adding the amounts of a residual solvent present in each of the components of the drug product. The sum of the amounts of solvent per day should be less than that given by the PDE.

3. Analytical procedures

Residual solvents are typically determined using chromatographic techniques such as gas chromatography. The analytical method should be validated adequately. If only Class 3 solvents are present, a nonspecific method such as loss on drying may be used.

4. Reporting levels of residual solvents

Manufacturers of drug products need certain information about the content of residual solvents in excipients or drug substances. The following statements are given as acceptable examples of the information.

(i) Only Class 3 solvents are likely to be present. Loss on drying is not more than 0.5%.

(ii) Only Class 2 solvents are likely to be present. Name the Class 2 solvents that are present. All are not more than the Option 1 limit.

(iii) Only Class 2 solvents and Class 3 solvents are likely to be present. Residual Class 2 solvents are not more than the Option 1 limit and residual Class 3 solvents are not more than 0.5%.

If Class 1 solvents are likely to be present, they should be identified and quantified. ''Likely to be present'' refers to the solvents that were used in the final manufacturing step and to the solvents that were used in earlier manufacturing steps and not always possible to be excluded even in a validated process.

If solvents of Class 2 or Class 3 are present at greater than their Option 1 limits or 0.5%, respectively, they should be identified and quantified.

5. Limits of residual solvents

5.1. Solvents to be avoided in manufacture of pharmaceuticals

Solvents in Class 1 should not be employed in the manufacture of drug substances, excipients, and drug products because of their unacceptable toxicity or their deleterious environmental effect. However, if their use is unavoidable in order to produce a drug product with a significant therapeutic advance, then their levels should be restricted as shown in Table 2.46-1, unless otherwise justified. 1,1,1-Trichloroethane is included in Table 2.46-1 because it is an environmental hazard. The stated limit of 1500 ppm shown in Table 2.46-1 is based on a review of the safety data.

5.2. Solvents to be limited in pharmaceuticals

Solvents in Table 2.46-2 should be limited in drug products because of their inherent toxicity. PDEs are given to the nearest 0.1 mg per day, and concentrations are given to the nearest 10 ppm. The stated values do not reflect the necessary analytical precision of determination. Precision should be determined as part of the validation of the method.

5.3. Solvents with low toxic potential

Solvents in Class 3 shown in Table 2.46-3 may be regarded as less toxic and of lower risk to human health. Class 3 includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. The amounts of these residual solvents of 50 mg per day or less (corresponding to 5000 ppm or 0.5% under Option 1) would be acceptable without justification. Higher amounts may also be acceptable provided they are realistic in relation to manufacturing capability and good manufacturing practice.

5.4 Solvents for which no adequate toxicological data was found

The following solvents (Table 2.46-4) may also be of interest to manufacturers of excipients, drug substances, or drug products. However, no adequate toxicological data on which to base a PDE was found. Manufacturers should supply justification for residual levels of these solvents in drug products.

Table 2.46-1 Class 1 solvents in drug products (solvents that should be avoided).

Solvent	Concentration Limit (ppm)	Concern
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

Table 2.46-2 Class 2 Solvents which residual amount should be limited in drug products

Solvent	PDE (mg/day)	Concentration limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cumene	0.7	70
Cyclohexane	38.8	3880
1,2-Dichloroethene	18.7	1870
Dichloromethane	6.0	600
1,2-Dimethoxyethane	1.0	100
N,*N*-Dimethylacetamide	10.9	1090
N,*N*-Dimethylformamide	8.8	880
1,4-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyethanol	0.5	50
Methyl butyl ketone	0.5	50
Methylcyclohexane	11.8	1180
N-Methylpyrrolidone	5.3	530
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetrahydrofuran	7.2	720
Tetralin	1.0	100
Toluene	8.9	890
1,1,2-Trichloroethene	0.8	80
Xylene*	21.7	2170

* Usually 60% *m*-xylene, 14% *p*-xylene, 9% *o*-xylene with 17% ethylbenzene

Table 2.46-3 Class 3 solvents which should be limited by GMP or other quality-based requirements.

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
n-Butyl acetate	Methyl ethyl ketone
tert-Butyl methyl ether	Methyl isobutyl ketone
Dimethylsulfoxide	2-Methyl-1-propanol
Ethanol	Pentane
Ethyl acetate	1-Pentanol
Diethyl ether	1-Propanol
Ethyl formate	2-Propanol
Formic acid	Propyl acetate

Table 2.46-4 Solvents for which no adequate toxicological data was found.

1,1-Diethoxypropane	Methyl isopropyl ketone
1,1-Dimethoxymethane	Methyltetrahydrofuran
2,2-Dimethoxypropane	Petroleum ether
Isooctane	Trichloroacetic acid
Isopropyl ether	Trifluoroacetic acid

II. Identification and quantification of residual solvents

Whenever possible, the substance under test needs to be dissolved to release the residual solvent. Because drug products, as well as active ingredients and excipients are treated, it may be acceptable that in some cases, some of the components of the formulation will not dissolve completely. In those cases, the drug product may first need to be pulverized into a fine powder so that any residual solvent that may be present can be released. This operation should be performed as fast as possible to prevent the loss of volatile solvents during the procedure.

In the operating conditions of gas chromatography and headspace described below, parameters to be set and their description may be different depending on the apparatus. When setting these conditions, it is necessary to change them according to the apparatus used, if it is confirmed that they meet the system suitability.

In addition to the reagents specified to be used for the test, those that meet the purpose of the test can be used.

1. Class 1 and Class 2 residual solvents

The following procedures are useful to identify and quantify residual solvents when the information regarding which solvents are likely to be present in the material is not available. When the information about the presence of specific residual solvents are available, it is not necessary to perform Procedure A and Procedure B, and only Procedure C or other appropriate procedure is needed to quantify the amount of residual solvents.

A flow chart for the identification of residual solvents and the application of limit and quantitative tests is shown in Fig. 2.46-1.

1.1. Water-soluble articles

1.1.1. Procedure A

The test is performed by gas chromatography <*2.02*> according to the following conditions.

Class 1 standard stock solution: Pipet 1 mL of Residual Solvents Class 1 RS, dissolve in about 9 mL of dimethylsulfoxide, and add water to make exactly 100 mL. Pipet 1 mL of this solution in a volumetric flask, previously filled with about 50 mL of water and add water to make exactly 100 mL. Pipet 10 mL of this solution in a volumetric flask, previously filled with about 50 mL of water and add water to make exactly 100 mL.

Class 1 standard solution: Pipet 1 mL of Class 1 standard stock solution in an appropriate head space vial containing exactly 5 mL of water, apply the stopper, cap, and mix.

Class 2 standard stock solution A: Pipet 1 mL of Residual

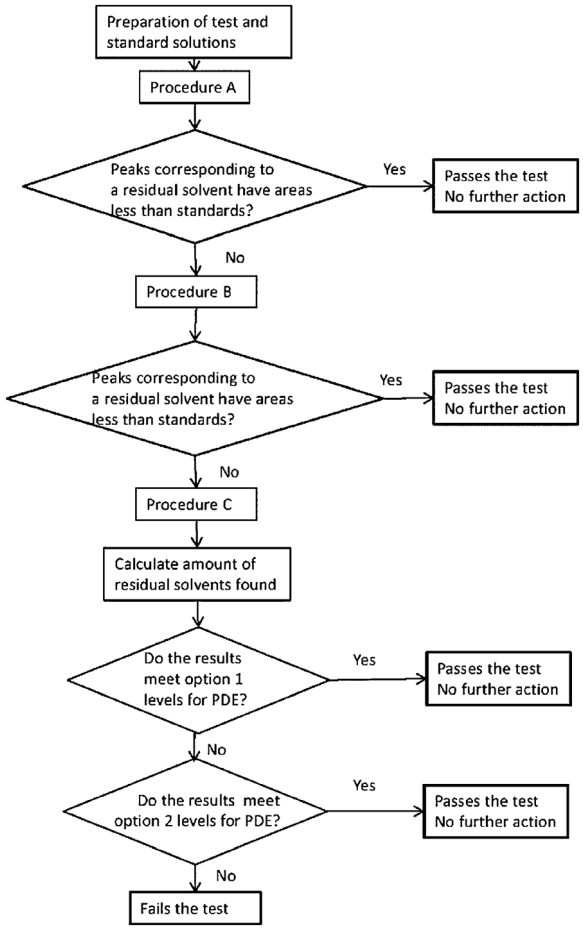


Fig. 2.46-1 Flow chart for the identification of residual solvents and the application of limit and qualification tests.

Solvents Class 2A RS, add water to make exactly 100 mL.

Class 2 standard stock solution B: Pipet 1 mL of Residual Solvents Class 2B RS, add water to make exactly 100 mL.

Class 2 standard solution A: Pipet 1 mL of Class 2 standard stock solution A in an appropriate headspace vial, add exactly 5 mL of water, and apply the stopper, cap, and mix.

Class 2 standard solution B: Pipet 5 mL of Class 2 standard stock solution B in an appropriate headspace vial, add exactly 1 mL of water, and apply the stopper, cap, and mix.

Test stock solution: Dissolve 0.25 g of the article under test in water, and add water to make exactly 25 mL.

Test solution: Pipet 5 mL of test stock solution in an appropriate headspace vial, add exactly 1 mL of water, and apply the stopper, cap, and mix.

Class 1 system suitability solution: Pipet 1 mL of Class 1 standard stock solution in an appropriate headspace vial, add exactly 5 mL of test stock solution, and apply the stopper, cap, and mix.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column (or a wide-bore column) 0.32 mm (or 0.53 mm) in inside diameter and 30 m in length, coated inside with 6% cyanopropylphenylmethyl silicon polymer for gas chromatography in a thickness of 1.8 μm (or 3.0 μm).

Column temperature: Maintain at 40°C for 20 minutes after injection, then raise the temperature to 240°C at a rate of 10°C per minute, and maintain at 240°C for 20 minutes.

Injection port temperature: 140°C.

Detector temperature: 250°C.

Carrier gas: Nitrogen or Helium.

Flow rate: About 35 cm per second.

Split ratio: 1:5. (Note: The split ratio can be modified in order to optimize sensitivity.)

System suitability—

Test for required detectability: When the procedure is run with Class 1 standard solution and Class 1 system suitability solution under the above operating conditions, the signal to noise (SN) ratio of 1,1,1-trichloroethane in Class 1 standard solution is not less than 5, and the SN ratio of each peak in Class 1 system suitability solution is not less than 3.

System performance: When the procedure is run with Class 2 standard solution A or the solution for system suitability under the above operating conditions, the resolution between acetonitrile and dichloromethane is not less than 1.0. Pipet 1 mL of a solution of Residual Solvents RS for System Suitability (1 in 100) in an appropriate headspace vial, add exactly 5 mL of water, and apply the stopper, cap, and mix, and use this solution as the solution for system suitability.

System repeatability: When the test is repeated 6 times with Class 1 standard solution under the above operating conditions, the relative standard deviation of each peak area is not more than 15%.

Separately inject (following one of the headspace operating parameter sets described in Table 2.46-5) equal volumes of headspace (about 1.0 mL) of Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, and test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If a peak response of any peak, other than a peak for 1,1,1-trichloroethane, in the test solution is greater than or equal to a corresponding peak in either Class 1 standard solution, Class 2 standard solution A or Class 2 standard solution B, or a peak response of 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in Class 1 standard solution, proceed to Procedure B to verify the identity of the peak; otherwise the article meets the requirements of this test.

1.1.2. Procedure B

The test is performed by gas chromatography <*2.02*> according to the following conditions.

Class 1 standard stock solution, Class 1 standard solution, Class 1 system suitability solution, Class 2 standard stock solutions A and B, Class 2 standard solutions A and B, test stock solution and test solution: Prepare as directed for Procedure A.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column (or a wide-bore column) 0.32 mm (or 0.53 mm) in inside diameter and 30 m in

length, coated inside with polyethylene glycol for gas chromatography in a thickness of 0.25 μm.

Column temperature: Maintain at 50°C for 20 minutes after injection, then raise the temperature to 165°C at a rate of 6°C per minute, and maintain at 165°C for 20 minutes.

Injection port temperature: 140°C.

Detector temperature: 250°C.

Carrier gas: Nitrogen or Helium.

Flow rate: About 35 cm per second.

Split ratio: 1:5. (Note: The split ratio can be modified in order to optimize sensitivity.)

System suitability—

Test for required detectability: When the procedure is run with Class 1 standard solution and Class 1 system suitability solution under the above operating conditions, the SN ratio of benzene in Class 1 standard solution is not less than 5, and the SN ratio of each peak in Class 1 system suitability solution is not less than 3.

System performance: When the procedure is run with Class 2 standard solution A or the solution for system suitability under the above operating conditions, the resolution between acetonitrile and *cis*-1,2-dichloroethene is not less than 1.0. Pipet 1 mL of a solution of Residual Solvents RS for System Suitability (1 in 100) in an appropriate headspace vial, add exactly 5 mL of water, and apply the stopper, cap, and mix, and use this solution as the solution for system suitability.

System repeatability: When the test is repeated 6 times with Class 1 standard solution under the above operating conditions, the relative standard deviation of each peak area is not more than 15%.

Separately inject (following one of the headspace operating parameter sets described in Table 2.46-5) equal volumes of headspace (about 1.0 mL) of Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, and test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If the peak response(s) in the test solution of the peak(s) identified in Procedure A is/are greater than or equal to a corresponding peak(s) in either Class 1 standard solution, Class 2 standard solution A or Class 2 standard solution B, proceed to Procedure C to quantify the peak(s); otherwise the article meets the requirements of this test.

1.1.3. Procedure C

The test is performed by gas chromatography <2.02> according to the following conditions.

Class 1 standard stock solution, Class 1 standard solution, Class 2 standard stock solution A, Class 2 standard solution A and Class 1 system suitability solution: Prepare as directed for Procedure A.

Standard stock solution (Note: Prepare a separate standard stock solution for each peak identified and verified by Procedures A and B. For Class 1 solvents other than 1,1,1-trichloroethane, prepare the first dilution as directed for the first dilution under Class 1 standard stock solution in Procedure A.): Transfer an accurately measured volume of each individual solvent corresponding to each residual solvent peak identified and verified by Procedures A and B to a suitable container, and dilute quantitatively and stepwise if necessary, with water to obtain a solution having a final concentration of 1/20 of the value stated in Table 2.46-1 or Table 2.46-2.

Standard solution: Pipet 1 mL of standard stock solution in an appropriate headspace vial, add exactly 5 mL of water, and apply the stopper, cap, and mix.

Test stock solution: Weigh accurately about 0.25 g of the article under test, dissolve in water, and add water to make exactly 25 mL.

Test solution: Pipet 5 mL of test stock solution in an appropriate headspace vial, add exactly 1 mL of water, and apply the stopper, cap, and mix.

Spiked test solution (Note: prepare a separate spiked test solution for each peak identified and verified by Procedure A and B): Pipet 5 mL of test stock solution in an appropriate headspace vial, add exactly 1 mL of standard stock solution, and apply the stopper, cap, and mix.

Operating conditions and system suitability fundamentally follow the procedure A. If the results of the chromatography from Procedure A are found to be inferior to those found with Procedure B, the operating conditions from Procedure B may be substituted.

Separately inject (following one of the headspace operating parameters described in Table 2.46-5) equal volumes of headspace (about 1.0 mL) of the standard solution, test solution, and spiked test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of each residual solvent found in the article under test by the formula:

$$\text{Residual solvent (ppm)} = 5(C/M)\ \{A_T/(A_S - A_T)\}$$

C: Concentration (μg/mL) of the appropriate Reference Standard in the standard stock solution

M: Amount (g) of the article under test taken to prepare the test stock solution

A_T: Peak responses of each residual solvent obtained from the test solution

A_S: Peak responses of each residual solvent obtained from the spiked test solution

1.2. Water-insoluble articles

1.2.1. Procedure A

The test is performed by gas chromatography <2.02> according to the following conditions. Dimethylsulfoxide may be substituted as an alternative solvent to *N*,*N*-dimethylformamide.

Class 1 standard stock solution: Pipet 1 mL of Residual Solvents Class 1 RS, dissolve in about 80 mL of *N*,*N*-dimethylformamide, and add *N*,*N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution in a volumetric flask, previously filled with about 80 mL of *N*,*N*-dimethylformamide and add *N*,*N*-dimethylformamide to make exactly 100 mL (this solution is the intermediate dilution prepared from Residual Solvents Class 1 RS and use it for preparation of Class 1 system suitability solution). Pipet 1 mL of this solution, and add *N*,*N*-dimethylformamide to make exactly 10 mL.

Class 1 standard solution: Pipet 1 mL of Class 1 standard stock solution in an appropriate head space vial containing exactly 5 mL of water, apply the stopper, cap, and mix.

Class 2 standard stock solution A: Pipet 1 mL of Residual Solvents Class 2A RS, dissolve in about 80 mL of *N*,*N*-dimethylformamide, and add *N*,*N*-dimethylformamide to make exactly 100 mL.

Class 2 standard stock solution B: Pipet 0.5 mL of Residual Solvents Class 2B RS, add *N*,*N*-dimethylformamide to make exactly 10 mL.

Class 2 standard solution A: Pipet 1 mL of Class 2 standard stock solution A in an appropriate headspace vial, add exactly 5 mL of water, and apply the stopper, cap, and mix.

Class 2 standard solution B: Pipet 1 mL of Class 2 standard stock solution B in an appropriate headspace vial, add exactly 5 mL of water, and apply the stopper, cap, and mix.

Test stock solution: Dissolve 0.5 g of the article under test in *N*,*N*-dimethylformamide, and add *N*,*N*-dimethylformamide to make exactly 10 mL.

Test solution: Pipet 1 mL of test stock solution in an appropriate headspace vial, add exactly 5 mL of water, and apply the stopper, cap, and mix.

Class 1 system suitability solution: Pipet 5 mL of test stock solution and 0.5 mL of the intermediate dilution prepared from Residual Solvents Class 1 RS, and mix. Pipet 1 mL of this solution in an appropriate headspace vial, add exactly 5 mL of water, and apply the stopper, cap, and mix.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A wide-bore column 0.53 mm in inside diameter and 30 m in length, coated inside with 6% cyanopropylphenylmethyl silicon polymer for gas chromatography in a thickness of 3.0 μm.

Column temperature: Maintain at 40°C for 20 minutes after injection, then raise the temperature to 240°C at a rate of 10°C per minute, and maintain at 240°C for 20 minutes.

Injection port temperature: 140°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: About 35 cm per second.

Split ratio: 1:3. (Note: The split ratio can be modified in order to optimize sensitivity.)

System suitability—

Test for required detectability: When the procedure is run with Class 1 standard solution and Class 1 system suitability solution under the above operating conditions, the SN ratio of 1,1,1-trichloroethane in Class 1 standard solution is not less than 5, and the SN ratio of each peak in Class 1 system suitability solution is not less than 3.

System performance: When the procedure is run with Class 2 standard solution A or the solution for system suitability under the above operating conditions, the resolution between acetonitrile and dichloromethane is not less than 1.0. Pipet 1 mL of the *N*,*N*-dimethylformamide solution of Residual Solvents RS for System Suitability (1 in 100) in an appropriate headspace vial, add exactly 5 mL of water, and apply the stopper, cap, and mix, and use this solution as the solution for system suitability.

System repeatability: When the test is repeated 6 times with Class 1 standard solution under the above operating conditions, the relative standard deviation of each peak areas is not more than 15%.

Separately inject (use headspace operating parameters in column 3 of Table 2.46-5) equal volumes of headspace (about 1.0 mL) of Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, and test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If a peak response of any peak, other than a peak for 1,1,1-trichloroethane, in the test solution is greater than or equal to a corresponding peak in either Class 1 standard solution, Class 2 standard solution A or Class 2 standard solution B, or a peak response of 1,1,1-trichloroethane is greater than or equal to 150 times the peak response corresponding to 1,1,1-trichloroethane in Class 1 standard solution, proceed to Procedure B to verify the identity of the peak; otherwise, the article meets the requirements of this test.

1.2.2. Procedure B

The test is performed by gas chromatography <*2.02*> according to the following conditions.

Class 1 standard stock solution, Class 1 standard solution, Class 1 system suitability solution, Class 2 standard stock solutions A and B, Class 2 standard solutions A and B, test stock solution, and test solution: Proceed as directed for Procedure A.

Proceed as directed for Procedure B under Water-soluble articles with a split ratio of 1:3. (Note: The split ratio can be modified in order to optimize sensitivity.) The solution for system suitability: Proceed as directed for Procedure A.

Separately inject (use headspace operating parameters in Table 2.46-5) equal volumes of headspace (about 1.0 mL) of Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, and test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If the peak response(s) in test solution of the peak(s) identified in Procedure A is/are greater than or equal to a corresponding peak(s) in either Class 1 standard solution, Class 2 standard solution A or Class 2 standard solution B, proceed to Procedure C to quantify the peak; otherwise, the article meets the requirements of this test.

1.2.3 Procedure C

The test is performed by gas chromatography <*2.02*> according to the following conditions.

Class 1 standard stock solution, Class 1 standard solution, Class 1 system suitability solution, Class 2 standard stock solution A, and Class 2 standard solution A: Proceed as directed for Procedure A.

Standard stock solution (Note: Prepare a separate standard stock solution for each peak identified and verified by Procedures A and B. For Class 1 solvents other than 1,1,1-trichloroethane, prepare the first dilution as directed for the first dilution under Class 1 standard stock solution in Procedure A.): Transfer an accurately measured volume of each individual solvent corresponding to each residual solvent peak identified and verified by Procedures A and B to

a suitable container, and dilute quantitatively and stepwise if necessary, with water to obtain a solution having a final concentration of 1/20 of the value stated in Table 2.46-1 or Table 2.46-2.

Standard solution: Pipet 1 mL of standard stock solution in an appropriate headspace vial, add exactly 5 mL of water, and apply the stopper, cap, and mix.

Test stock solution: Weigh accurately about 0.5 g of the article under test, and add *N*,*N*-dimethylformamide to make exactly 10 mL.

Test solution: Pipet 1 mL of test stock solution in an appropriate headspace vial, add exactly 5 mL of water, and apply the stopper, cap, and mix.

Spiked test solution (Note: prepare a separate spiked test solution for each peak identified and verified by Procedure A and B): Pipet 1 mL of test stock solution in an appropriate headspace vial, add exactly 4 mL of water, and apply the stopper, cap, and mix.

Operating conditions and system suitability fundamentally follow the procedure A. If the results of the chromatography from Procedure A are found to be inferior to those found with Procedure B, the operating conditions from Procedure B may be substituted.

Separately inject (use headspace operating parameters in Table 2.46-5) equal volumes of headspace (about 1.0 mL) of the standard solution, test solution, and spiked test solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of each residual solvent found in the article under test by the formula:

$$\text{Residual solvent (ppm)} = 10(C/M)\ \{A_T/(A_S - A_T)\}$$

C: Concentration (μg/mL) of the appropriate Reference Standard in the standard stock solution

M: Amount (g) of the article under test taken to prepare the test stock solution

A_T: Peak responses of each residual solvent obtained from the test solution

A_S: Peak responses of each residual solvent obtained from the spiked test solution

1.3. Headspace operating parameters and other considerations

Examples of headspace operating parameters are shown in Table 2.46-5.

These test methods describe the analytical methods using the headspace gas chromatography. The following Class 2 residual solvents are not readily detected by the headspace injection conditions: 2-ethoxyethanol, ethylene glycol, formamide, 2-methoxyethanol, *N*-methylpyrrolidone, and sulfolane. Other appropriate validated procedures are to be employed for the quantification of these residual solvents. In the headspace methods, *N*,*N*-dimethylformamide and *N*,*N*-dimethylacetoamide are often used as solvents. As not only 6 solvents described above but these two solvents are not included in the Residual Solvents Class 2A RS and/or the Residual Solvents Class 2B RS, appropriate validated procedures are to be employed for these residual solvents as necessary.

Table 2.46-5 Headspace operating parameters

	Headspace Operating Parameter Sets		
	1	2	3
Equilibration temperature (°C)	80	105	80
Equilibration time (min.)	60	45	45
Transfer-line temperature (°C)	85	110	105
Syringe temperature (°C)	80 – 90	105 – 115	80 – 90
Carrier gas: nitrogen or helium at an appropriate pressure			
Pressurization time (s)	≧ 60	≧ 60	≧ 60
Injection volume (mL)*	1	1	1

* Or follow the instrument manufacture's recommendations, as long as the method criteria are met. Injecting less than this amount is allowed as long as adequate sensitivity is achieved.

2. Class 3 residual solvents

Perform the test according to 1. Otherwise an appropriate validated procedure is to be employed. Prepare appropriately standard solutions, etc. according to the residual solvent under test.

If only Class 3 solvents are present, the level of residual solvents may be determined by loss on drying <2.41>. However when the value of the loss on drying is more than 0.5%, or other solvents exist, the individual Class 3 residual solvent or solvents present in the article under test should be identified using the procedures as described above or other appropriate procedure, and quantified as necessary.

3. Reference Standards

(i) Residual Solvents Class 1 RS (A mixture of benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethene and 1,1,1-trichloroethane)

(ii) Residual Solvents Class 2A RS (A mixture of acetonitrile, chlorobenzene, cumene, cyclohexane, 1,2-dichloroethene (*cis*-1,2-dichloroethene, *trans*-1,2-dichloroethene), dichloromethane, 1,4-dioxane, methanol, methylcyclohexane, tetrahydrofuran, toluene and xylene (*m*-xylene, *p*-xylene, *o*-xylene, ethylbenzene))

(iii) Residual Solvents Class 2B RS (A mixture of chloroform, 1,2-dimethoxyethane, hexane, methyl butyl ketone, nitromethane, pyridine, tetralin and 1,1,2-trichloroethene)

(iv) Residual Solvents RS for System Suitability (A mixture of acetonitrile, *cis*-1,2-dichloroethene and dichloromethane)

Add the following:

3.06 Laser Diffraction Measurement of Particle Size

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

The laser light diffraction technique used for the determination of particle-size distribution is based on the analysis of the diffraction pattern produced when particles are exposed to a beam of monochromatic light. Historically, the early laser diffraction instruments only used scattering at small angles. However, the technique has since been broadened to include laser light scattering in a wider range and application of the Mie theory, in addition to the Fraunhofer approximation and anomalous diffraction.

The technique cannot distinguish between scattering by primary particles and scattering by clusters of primary particles, i.e. by agglomerates or aggregates. As most particulate samples contain agglomerates or aggregates and as the focus of interest is generally on the size distribution of primary particles, the clusters are usually dispersed into primary particles before measurement.

For non-spherical particles, an equivalent sphere-size distribution is obtained because the technique assumes spherical particles in its optical model. The resulting particle-size distribution may differ from those obtained by methods based on other physical principles (e.g. sedimentation, sieving).

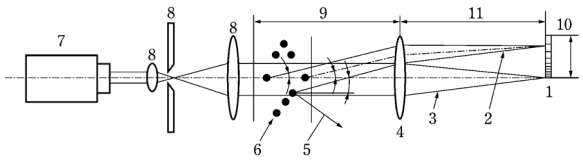
This chapter provides guidance for the measurement of size distributions of particles in different dispersed systems, for example, powders, sprays, aerosols, suspensions, emulsions, and gas bubbles in liquids, through analysis of their angular light-scattering patterns. It does not address specific requirements of particle-size measurement of specific products. This technique complies with ISO13320-1 (1999) and 9276-1 (1998).

1. Instrument

The instrument is located in an environment where it is not affected by electrical noise, mechanical vibrations, temperature fluctuations, humidity or direct bright light. An example of a set-up of a laser light diffraction instrument is given in Fig. 3.06-1. Other equipment may be used.

The instrument comprises a laser light source, beam processing optics, a sample measurement region (or cell), a Fourier lens, and a multi-element detector for measuring the scattered light pattern. A data system is also required for deconvolution of the scattering data into a volumetric size distribution and associated data analysis and reporting.

The particles can enter the laser beam in 2 positions. In the conventional case the particles enter the parallel beam before the collecting lens and within its working distance. In socalled reversed Fourier optics the particles enter behind the collecting lens and thus, in a converging beam. The advantage of the conventional set-up is that a reasonable path length for the sample is allowed within the working distance



1. Obscuration detector
2. Scattered beam
3. Direct beam
4. Fourier lens
5. Scattered light not collected by lens (4)
6. Particle ensemble
7. Light source laser
8. Beam processing unit
9. Working distance of lens (4)
10. Multi-element detector
11. Focal distance of lens (4)

Fig. 3.06-1 Example of a set-up of a laser light diffraction instrument

of the lens. The second set-up allows only small path lengths but enables measurement of scattered light at larger angles, which is useful when submicron particles are present.

The interaction of the incident light beam and the ensemble of dispersed particles results in a scattering pattern with different light intensities at various angles. The total angular intensity distribution, consisting of both direct and scattered light, is then focused onto a multi-element detector by a lens or a series of lenses. These lenses create a scattering pattern that, within limits, does not depend on the location of the particles in the light beam. Hence, the continuous angular intensity distribution is converted into a discrete spatial intensity distribution on a set of detector elements.

It is assumed that the measured scattering pattern of the particle ensemble is identical to the sum of the patterns from all individual single scattering particles presented in random relative positions. Note that only a limited angular range of scattered light is collected by the lens(es) and, therefore, by the detector.

2. Development of the method

The measurement of particle size by laser diffraction can give reproducible data, even in the sub-micron region, provided the instrument used and the sample tested are carefully controlled to limit variability of the test conditions (e.g. dispersion medium, method of preparation of the sample dispersion).

Traditionally, the measurement of particle size using laser diffraction has been limited to particles in the range of approximately 0.1 μm to 3 mm. Because of recent advances in lens and equipment design, newer instruments are capable of exceeding this range routinely. With the validation report the user demonstrates the applicability of the method for its intended use.

2.1. Sampling

The sampling technique must be adequate to obtain a representative sample of a suitable volume for the particle size measurement. Sample splitting techniques such as rotating riffler or the cone and quartering method may be applied.

2.2. Evaluation of the dispersion procedure

Inspect the sample to be analyzed, visually or with the aid of a microscope, to estimate its size range and particle shape. The dispersion procedure must be adjusted to the purpose of the measurement. The purpose may be such that it is preferable to deagglomerate clusters into primary particles as far as possible, or it may be desirable to retain clusters as intact as possible. In this sense, the particles of interest may be either primary particles or clusters.

For the development of a method it is highly advisable to check that comminution of the particles does not occur, and conversely, that dispersion of particles or clusters is satisfactory. This can usually be done by changing the dispersing energy and monitoring the change of the particle-size distribution. The measured size distribution must not change significantly when the sample is well dispersed and the particles are neither fragile nor soluble. Moreover, if the manufacturing process (e.g. crystallization, milling) of the material has changed, the applicability of the method must by verified (e.g. by microscopic comparison).

Sprays, aerosols and gas bubbles in a liquid should be measured directly, provided that their concentration is adequate, because sampling or dilution generally alters the particle-size distribution.

In other cases (such as emulsions, pastes and powders), representative samples may be dispersed in suitable liquids. Dispersing aids (wetting agents, stabilizers) and/or mechanical forces (e.g. agitation, sonication) are often applied for deagglomeration or deaggregation of clusters and stabilization of the dispersion. For these liquid dispersions, a recirculating system is most commonly used, consisting of an optical measuring cell, a dispersion bath usually equipped with stirrer and ultrasonic elements, a pump, and tubing. Non-recirculating, stirred cells are useful when only small amounts of a sample are available or when special dispersion liquids are used.

Dry powders can also be converted into aerosols through the use of suitable dry powder dispersers, which apply mechanical force for deagglomeration or deaggregation. Generally, the dispersers use the energy of compressed gas or the differential pressure of a vacuum to disperse the particles to an aerosol, which is blown through the measuring zone, usually into the inlet of a vacuum unit that collects the particles. However, for free flowing, coarser particles or granules the effect of gravity may be sufficient to disperse the particles adequately.

If the maximum particle size of the sample exceeds the measuring range of the instrument, the material that is too coarse can be removed by sieving and the mass and percentage of removed material are reported. However, after presieving, note that the sample is no longer representative, unless otherwise proven.

2.3. Optimization of the liquid dispersion

Liquids, surfactants, and dispersing aids used to disperse powders must:

(i) be transparent at the laser wavelength and practically free from air bubbles or particles;

(ii) have a refractive index that differs from that of the test material;

(iii) be non-solvent of the test material (pure liquid or pre-filtered, saturated solution);

(iv) not alter the size of the test materials (e.g. by solubility, solubility enhancement, or recrystallization effects);

(v) favor easy formation and stability of the dispersion;

(vi) be compatible with the materials used in the instrument (such as O-rings, gaskets, tubing, etc.);

(vii) possess a suitable viscosity to facilitate recirculation, stirring and filtration.

Surfactants and/or dispersing aids are often used to wet the particles and to stabilize the dispersion. For weak acids and weak bases, buffering of the dispersing medium at low or high pH respectively can assist in identifying a suitable dispersant.

A preliminary check of the dispersion quality can be performed by visual or microscopic inspection. It is also possible to take fractional samples out of a well-mixed stock dispersion. Such stock dispersions are formed by adding a liquid to the sample while mixing it with, for example, a glass rod, a spatula or a vortex mixer. Care must be taken to ensure the transfer of a representative sample and that settling of larger particles does not occur. Therefore a sample paste is prepared or sampling is carried out quickly from a suspension maintained under agitation.

2.4. Optimization of the gas dispersion

For sprays and dry powder dispersions, a compressed gas free from oil, water and particles may be used. To remove such materials from the compressed gas, a dryer with a filter can be used. Any vacuum unit should be located away from the measurement zone, so that its output does not disturb the measurement.

2.5. Determination of the concentration range

In order to produce an acceptable SN ratio in the detector, the particle concentration in the dispersion must exceed a minimum level. Likewise, it must be below a maximum level in order to avoid multiple scattering. The concentration range is influenced by the width of the laser beam, the path length of the measurement zone, the optical properties of the particles, and the sensitivity of the detector elements.

In view of the above, measurements must be performed at different particle concentrations to determine the appropriate concentration range for any typical sample of material. (Note: in different instruments, particle concentrations are usually represented by differently scaled and differently named numbers, e.g. obscuration, optical concentration, proportional number of total mass).

2.6. Determination of the measuring time

The time of measurement, the reading time of the detector and the acquisition frequency is determined experimentally in accordance with the required precision. Generally, the time for measurement permits a large number of detec-

tor scans or sweeps at short time intervals.

2.7. Selection of an appropriate optical model

Most instruments use either the Fraunhofer or the Mie theory, though other approximation theories are sometimes applied for calculation of the scattering matrix. The choice of the theoretical model depends on the intended application and the different assumptions (size, absorbance, refractive index, roughness, crystal orientation, mixture, etc.) made for the test material. If the refractive index values (real and imaginary parts for the used wavelength) are not exactly known, then the Fraunhofer approximation or the Mie theory with a realistic estimate of the refractive index can be used. The former has the advantages that it is simple and it does not need refractive index values; the latter usually provides less-biased particle-size distributions for small particles. For instance, if the Fraunhofer model is used for samples containing an appreciable amount of small, transparent particles, a significantly large amount of small particles may be calculated. In order to obtain traceable results, it is essential to document the refractive index values used, since small differences in the values assumed for the real and imaginary part of the complex refractive index may cause significant differences in the resulting particle-size distributions. Small values of the imaginary part of the refractive index (about $0.01 - 0.1i$) are often applied to allow the correction of the absorbance for the surface roughness of the particles. It should be noted, in general, that the optical properties of the substance to be tested, as well as the structure (e.g. shape, surface roughness and porosity) bear upon the final result.

2.8. Validation

Typically, the validity of a procedure may be assessed by the evaluation of its specificity, linearity, range, accuracy, precision and robustness. In particle-size analysis by laser light diffraction, specificity as defined by ICH (Validation of Analytical Procedures) is not applicable as it is not possible to discriminate different components into a sample, as is neither possible to discriminate between agglomerates from dispersed particles unless properly complemented by microscopic techniques. Exploring a linear relationship between concentration and response, or a mathematical model for interpolation, is not applicable to this procedure. Rather than evaluating linearity, this method requires the definition of a concentration range within which the result of the measurements does not vary significantly. Concentrations below that range produce an error due to a poor SN ratio, while concentrations above that range produce an error due to multiple scattering. The range depends mostly in the instrument hardware. Accuracy should be confirmed through an appropriate instrument qualification and comparison with microscopy, while precision may be assessed by means of a repeatability determination.

The attainable repeatability of the method mainly depends on the characteristics of the material (milled/not milled, robust/fragile, width of its size distribution, etc.), whereas the required repeatability depends on the purpose of the measurement. Mandatory limits cannot be specified in this chapter, as repeatabilities (different sample preparations) may vary appreciably from one substance to another. However, it is good practice to aim at acceptance criteria for repeatability such as RSD (%) $\leqq$ 10% [$n=6$] for any central value of the distribution (e.g. for x_{50}). Values at the sides of the distribution (e.g. x_{10} and x_{90}) are oriented towards less stringent acceptance criteria such as RSD $\leqq$ 15% [$n=6$]. Below 10 μm, these values must be doubled. Robustness may be tested during the selection and optimization of the dispersion media and forces. The change of the dispersing energy may be monitored by the change in the particle-size distribution.

3. Measurement

A representative sample, dispersed at an adequate concentration in a suitable liquid or gas, is passed through a beam of monochromatic light, usually a laser. The light scattered by the particles at various angles is measured by a multi-element detector. Numerical values representing the scattering pattern are then recorded for analysis. These scattering pattern values are then transformed, using an appropriate optical model and mathematical procedure, to yield the proportion of total volume to a discrete number of size classes, forming a volumetric particle-size distribution.

3.1. Precautions

(i) never look into the direct path of the laser beam or its reflections;

(ii) earth all instrument components to prevent ignition of solvents or dust explosions;

(iii) check the instrument set-up (e.g. warm-up, required measuring range and lens, appropriate working distance, position of the detector, no direct bright daylight);

(iv) in the case of wet dispersions, avoid air bubbles, evaporation of liquid, schlieren or other inhomogeneities in the dispersion; similarly, avoid improper mass-flow from the disperser or turbulent air-flow in the case of dry dispersions; such effects can cause erroneous particle-size distributions.

3.2. Measurement of the light scattering of dispersed sample(s)

After proper alignment of the optical part of the instrument, a blank measurement of the particle-free dispersion medium must be performed using the same method as that used for the measurement of the sample. The background signal must be below an appropriate threshold. The detector data are saved in order to substract them later from the data obtained with the sample. The sample dispersion is measured according to the developed method.

For each detector element, an average signal is calculated, sometimes together with its standard deviation. The magnitude of the signal from each detector element depends upon the detection area, the light intensity and the quantum efficiency. The co-ordinates (size and position) of the detector elements together with the focal distance of the lens determine the range of scattering angles for each element. Most instruments also measure the intensity of the central (unscattered) laser beam. The ratio of the intensity of a dispersed sample to that in its absence (the blank measurement) indicates the proportion of scattered light and

hence the particle concentration.

3.3. Conversion of scattering pattern into particle-size distribution

This deconvolution step is the inverse of the calculation of a scattering pattern for a given particle-size distribution. The assumption of spherical particle shape is particularly important as most algorithms use the mathematical solution for scattering from spherical particles. Furthermore, the measured data always contain some random and systematic errors, which may vitiate the size distributions. Several mathematical procedures have been developed for use in the available instruments. They contain some weighting of deviations between measured and calculated scattering patterns (e.g. least squares), some constraints (e.g. non-negativity for amounts of particles), and/or some smoothing of the size distribution curve.

The algorithms used are specific to each maker and model of equipment, and are proprietary. The differences in the algorithms between different instruments may give rise to differences in the calculated particle-size distributions.

3.4. Replicates

The number of replicate measurements (with individual sample preparations) to be performed, depends on the required measurement precision. It is recommended to set this number in a substance-specific method.

4. Reporting of results

The particle-size distribution data are usually reported as cumulative undersize distribution and/or as density distribution by volume. The symbol x is used to denote the particle size, which in turn is defined as the diameter of a volume-equivalent sphere. $Q3(x)$ denotes the volume fraction undersize at the particle size x. In a graphical representation, x is plotted on the abscissa and the dependent variable $Q3(x)$ on the ordinate. Most common characteristic values are calculated from the particle-size distribution by interpolation. The particle sizes at the undersize values of 10%, 50%, and 90% (denoted as x_{10}, x_{50}, and x_{90} respectively) are frequently used. x_{50} is also known as the median particle size. It is recognized that the symbol d is also widely used to designate the particle size, thus the symbol x may be replaced by d.

Moreover, sufficient information must be documented about the sample, the sample preparation, the dispersion conditions, and the cell type. As the results depend on the particular instrument, data analysis program, and optical model used, these details must also be documented.

5. Control of the instrument performance

Use the instrument according to the manufacturer's instructions and carry out the prescribed qualifications at an appropriate frequency, according to the use of the instrument and substances to be tested.

5.1. Calibration

Laser diffraction systems, although assuming idealized properties of the particles, are based on first principles of laser light scattering. Thus, calibration in the strict sense is not required. However, it is still necessary to confirm that the instrument is operating correctly. This can be undertaken using any certified reference material that is acceptable in industrial practice. The entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, measurement, and the deconvolution procedure. It is essential that the total operational procedure is fully described.

The preferred certified reference materials consist of spherical particles of a known distribution. They must be certified as to the mass-percentage size distribution by an absolute technique, if available, and used in conjunction with an agreed, detailed operation procedure. It is essential that the real and imaginary parts of the complex refractive index of the material are indicated if the Mie theory is applied in data analysis. The representation of the particle-size distribution by volume will equal that of the distribution by mass, provided that the density of the particles is the same for all size fractions.

The response of a laser diffraction instrument is considered to meet the requirements if the mean value of x_{50} from at least 3 independent measurements does not deviate by more than 3% from the certified range of values of the certified reference material. The mean values for x_{10} and x_{90} must not deviate by more than 5% from the certified range of values. Below 10 μm, these values must be doubled.

Although the use of materials consisting of spherical particles is preferable, non-spherical particles may also be employed. Preferably, these particles have certified or typical values from laser diffraction analysis performed according to an agreed, detailed operating procedure. The use of reference values from methods other than laser diffraction may cause a significant bias. The reason for this bias is that the different principles inherent in the various methods may lead to different sphere-equivalent diameters for the same non-spherical particle.

Although the use of certified reference materials is preferred, other well-defined reference materials may also be employed. They consist of substances of typical composition and particle-size distribution for a specified class of substances. Their particle-size distribution has proven to be stable over time. The results must comply with previously determined data, with the same precision and bias as for the certified reference material.

5.2. Qualification of the system

In addition to the calibration, the performance of the instrument must be qualified at regular time intervals or as frequently as appropriate. This can be undertaken using any suitable reference material as mentioned in the previous paragraph.

The qualification of the system is based on the concept that the equipment, electronics, software and analytical operations constitute an integral system, which can be evaluated as an entity. Thus the entire measurement procedure is examined, including sample collection, sample dispersion, sample transport through the measuring zone, and the measurement and deconvolution procedure. It is essential that the total operational procedure is fully described.

In general, unless otherwise specified in the individual monograph, the response of a laser diffraction instrument is

considered to meet the requirements if the x_{50} value does not deviate by more than 10% from the range of values of the reference material. If optionally the values at the sides of the distribution are evaluated (e.g. x_{10} and x_{90}), then these values must not deviate by more than 15% from the certified range of values. Below 10 μm, these values must be doubled. For calibration of the instrument stricter requirements are laid down in 5.1. Calibration.

4.03 Digestion Test

Change the 2.2. Tyrosine Calibration Curve as follows:

2.2. Tyrosine Calibration Curve

Weigh exactly 50 mg of Tyrosine RS for Digestion Test, previously dried at 105°C for 3 hours, and dissolve in 0.2 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 1 mL, 2 mL, 3 mL and 4 mL of this solution separately, and add 0.2 mol/L hydrochloric acid TS to each solution to make exactly 100 mL. Pipet 2 mL of each solution, and add exactly 5 mL of 0.55 mol/L sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 3) to each solution, shake immediately, then stand them at 37 ± 0.5°C for 30 minutes. Determine the absorbances, A_1, A_2, A_3 and A_4, of these solutions at 660 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared with exactly 2 mL of 0.2 mol/L hydrochloric acid TS in the same manner as the blank. Then, draw a calibration curve with the absorbances, A_1, A_2, A_3 and A_4 as the ordinate, and with the amount (μg) of tyrosine in 2 mL of each solution as the abscissa. Obtain the amount (μg) of tyrosine for the absorbance difference of 1.

6.02 Uniformity of Dosage Units

Change as follows:

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

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

The term ''Uniformity of dosage unit'' is defined as the degree of uniformity in the amount of the drug substance among dosage units. Therefore, the requirements of this chapter apply to each drug substance being comprised in dosage units containing one or more drug substances, unless otherwise specified elsewhere in this Pharmacopoeia.

To ensure the consistency of dosage units, each unit in a batch should have a drug substance content within a narrow range around the label claim. Dosage forms such as tablets, capsules, packets of powders or granules, ampoules, contain a single dose or a part of a dose of a drug substance in each dosage unit. The Uniformity of Dosage Units specification is not intended to apply to suspensions, emulsions, or gels in unit-dose containers intended for external, cutaneous administration.

The uniformity of dosage units can be demonstrated by either of two methods, *Content uniformity* or *Mass variation* (see Table 6.02-1). The test for *Content Uniformity* of preparations presented in dosage units is based on the assay of the individual contents of drug substance(s) of a number of dosage units to determine whether the individual contents are within the limits set. The *Content Uniformity* method may be applied in all cases.

The test for *Mass Variation* is applicable for the following dosage forms:

(i) solutions enclosed in unit-dose containers and into soft capsules ◇in which all components are perfectly dissolved◇;

(ii) solids (including powders, granules and sterile solids) that are packaged in single-dose packages and contain no active or inactive added substances;

(iii) solids (including sterile solids) that are packaged in single-dose packages, with or without active or inactive added substances, that have been prepared from true solutions ◇in which all components are perfectly dissolved◇ and freeze-dried in the final packages and are labeled to indicate this method of preparation; and

(iv) hard capsules, uncoated tablets, or film-coated tablets, containing 25 mg or more of a drug substance comprising 25% or more, by weight, of the dosage unit or, in the case of hard capsules, the capsule contents, ◇or in the case of film-coated tablets, the pre-coated tablets,◇ except that uniformity of other drug substances present in lesser proportions is demonstrated by meeting Content Uniformity requirements.

The test for *Content Uniformity* is required for all dosage forms not meeting the above conditions for the *Mass Variation* test. Alternatively, products listed in item (iv) above that do not meet the 25 mg/25% threshold limit may be tested for uniformity of dosage units by *Mass Variation* instead of the *Content Uniformity* test if the concentration relative standard deviation (RSD) of the drug substance in the final dosage units is not more than 2%, based on process validation data and development data, and if there has been regulatory approval of such a change. The concentration RSD is the RSD of the concentration per dosage unit (w/w or w/v), where concentration per dosage unit equals the assay result per dosage unit divided by the individual dosage unit weight. See the RSD formula in Table 6.02-2.

1. Content Uniformity

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

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

Table 6.02-1 Application of Content Uniformity (CU) and Mass Variation (MV) Test for dosage forms

Dosage Forms	Type	Sub-type	Dose and ratio of drug substance	
			≥ 25 mg & ≥ 25%	< 25 mg or < 25%
Tablets	uncoated		MV	CU
	coated	Film	MV	CU
		Others	CU	CU
Capsules	hard		MV	CU
	soft	Sus., eml., gel	CU	CU
		Solutions	MV	MV
Solids in single-dose packages ◇(divided forms, lyophilized forms, et al.)◇	Single component		MV	MV
	Multiple components	Freeze-dried from solutions in final container	MV	MV
		Others	CU	CU
Solutions ◇(perfectly dissolved)◇ enclosed in unit-dose containers			MV	MV
Others—Among the preparations not classified as the above dosage forms in this table, suppositories, percutaneous absorption type preparations (patches), semi-solid dosage forms intended for application of active pharmaceutical ingredients to the skin for the purpose of systemic action, and the like.			CU	CU

Sus.: suspension; eml.: emulsion;

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

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

1.1. Calculation of Acceptance Value

Calculate the acceptance value by the formula:

$$|M - \bar{X}| + ks,$$

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

2. Mass Variation

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

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

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

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

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

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

(v) Liquid dosage forms: Accurately weigh the amount

Table 6.02-2

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

of liquid that is removed from each of 10 individual containers in conditions of normal use. If necessary, compute the equivalent volume after determining the density. Calculate the drug substance content in each container from the mass of product removed from the individual containers and the result of the assay. Calculate the acceptance value.

2.1. Calculation of Acceptance Value

Calculate the acceptance value as shown in *Content Uniformity*, except that ◇the value of $\overline{X}$ is replaced with A, and that◇ the individual contents of the dosage units are replaced with the individual estimated contents defined below.

x_1, x_2 … x_n: individual estimated contents of the dosage units tested

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

w_1, w_2 … w_n: Individual masses of the dosage units tested

A: Content of drug substance (% of label claim) obtained using an appropriate analytical method

$\overline{W}$: Mean of individual masses (w_1, w_2 … w_n)

3. Criteria

Apply the following criteria, unless otherwise specified.

(i) Solid, Semi-Solid and Liquid dosage forms: The requirements for dosage uniformity are met if the acceptance value of the first 10 dosage units is less than or equal to

$L1\%$. If the acceptance value is greater than $L1\%$, test the next 20 dosage units and calculate the acceptance value. The requirements are met if the final acceptance value of the 30 dosage units is less than or equal to $L1\%$ and no individual content of the dosage unit is less than $(1 - L2 \times 0.01)M$ nor more than $(1 + L2 \times 0.01)M$ in *Calculation of Acceptance Value* under *Content Uniformity* or under *Mass Variation*. Unless otherwise specified, $L1$ is 15.0 and $L2$ is 25.0.

6.04 Test for Acid-neutralizing Capacity of Gastrointestinal Medicines

Change the following as follows:

2. Procedure

Take an amount of the sample so that '*a*' in the equation falls between 20 mL and 30 mL, and perform the test.

Accurately weigh the sample of the crude material or preparation, and place it in a glass-stoppered 200-mL flask. Add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper tightly, shake at 37 ± 2°C for 1 hour, and filter. Take precaution against gas to be generated on the addition of 0.1 mol/L hydrochloric acid VS, and stopper tightly. After cooling, filter the solution again, if necessary. Pipet 50 mL of the filtrate, and titrate <*2.50*> the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination <*2.54*>, end point: pH 3.5). Perform a blank determination in the same manner.

For liquid preparations, pipet the sample in a 100-mL volumetric flask, add water to make 45 mL, then add exactly 50 mL of 0.2 mol/L hydrochloric acid VS while shaking. Add water again to make the solution 100 mL. Transfer the solution to a glass-stoppered 200-mL flask, wash the residue with 20.0 mL of water, stopper tightly, shake at 37 ± 2°C for 1 hour, and filter. Pipet 60 mL of the filtrate, and titrate <*2.50*> the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination <*2.54*>, end point: pH 3.5). Perform a blank determination in the same manner.

Acid-neutralizing capacity (amount of 0.1 mol/L hydrochloric acid VS consumed per g or daily dose) (mL)
$= (b - a) \times 2 \times (t/s)$

a: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed
b: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed in the blank determination
t: 1000 mg of crude material, or daily dose of preparation (in mg of solid preparation, mL of liquid preparation)
s: Amount of the sample taken (in mg of crude material and solid preparation, mL of liquid preparation)

Add the following:

6.14 Uniformity of Delivered Dose for Inhalations

This test is used to quantitatively evaluate the uniformity of the amount of active substances sprayed or discharged from inhalations (metered-dose inhalers and dry powder inhalers). Uniformity of the amount of active substances which are administered to patients from these preparations is necessary, and is confirmed by this test. Examples for the evaluation is shown as follows. Select a suitable test method from the following, according to the characteristic of preparations. Original methods are able to be set, including the test that can evaluate intra and inter-inhalers dose uniformity simultaneously.

1. Test methods for metered-dose inhalers

Metered-dose inhalers usually operate in a valve-down position. For inhalers that operate in a valve-up position, an equivalent test is applied using methods that ensure the complete collection of the delivered dose.

The dose collection apparatus must be capable of quantitatively capturing the delivered dose.

The following apparatus (Fig. 6.14-1) and procedure may be used.

The apparatus consists of a filter-support base with an

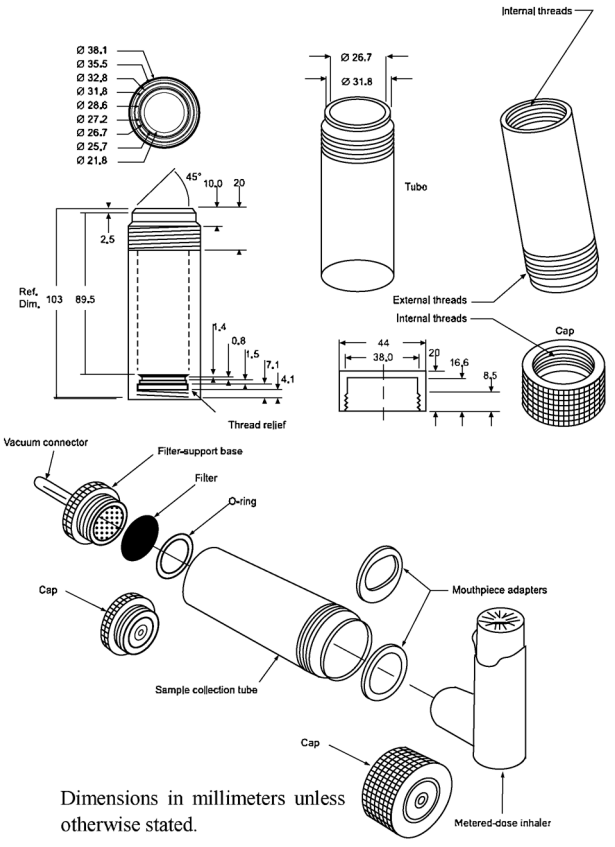


Fig. 6.14-1 Dose collection apparatus for metered-dose inhalers

open-mesh filter-support, such as a stainless steel screen, a collection tube that is clamped or screwed to the filter-support base, and a mouthpiece adapter to ensure an airtight seal between the collection tube and the mouthpiece. Use a mouthpiece adapter that ensures that the front face of the inhaler's mouthpiece is flush with the front face or the 2.5 mm indented shoulder of the sample collection tube, as appropriate. The vacuum connector is connected to a system comprising a vacuum source and a flow regulator. The source is adjusted to draw air through the complete assembly, including the filter and the inhaler to be tested, at 28.3 L per minute (±5%). Air should be drawn continuously through the apparatus to avoid loss of the active substance into the atmosphere. The filter-support base is designed to accommodate 25 mm diameter filter disks. The filter disk and other materials used in the construction of the apparatus must be compatible with the active substance and solvents that are used to extract the active substance from the filter. One end of the collection tube is designed to hold the filter disk tightly against the filter-support base. When assembled, the joints between the components of the apparatus are airtight so that when a vacuum is applied to the base of the filter, all of the air drawn through the collection tube passes through the inhaler.

1.1. Test method 1: evaluation of intra-inhaler dose uniformity

Take one inhaler, and perform the test. Unless otherwise prescribed in the instructions to the patient, shake the inhaler for 5 seconds and discharge one delivery to waste. Discharge the inverted inhaler into the apparatus, depressing the valve for a sufficient time to ensure complete discharge. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance as the delivered dose.

Repeat the procedure for a further 2 doses.

Discharge the inhaler to waste, waiting not less than 5 seconds between actuations, until $(n/2) + 1$ deliveries remain, where n is the number of deliveries stated on the label. Collect 4 doses using the procedure described above.

Discharge the inhaler to waste, waiting not less than 5 seconds between actuations, until 3 doses remain. Collect these 3 doses using the procedure described above. Determine 10 delivered doses per one inhaler, i.e., 3 doses at the beginning, 4 doses at the middle and 3 doses at the end by the above process.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

The mean of the delivered doses or the delivered dose stated on the label is used as the limit for judgement.

The preparation complies with the test if 9 out of 10 results lie between 75% and 125% of the limit and all lie between 65% and 135% of the limit. If 2 or 3 values lie outside the limits of 75 to 125%, repeat the test for 2 more inhalers, and obtain 30 values as the total. Not more than 3 of the 30 values lie outside the limits of 75 to 125% and no value lies outside the limits of 65 to 135%.

In justified cases, these ranges may be extended. But no value should be less than 50% or more than 150% of the limit.

The mean value must be within 85 to 115% of the label claim for delivered dose.

1.2. Test method 2: evaluation of inter-inhaler dose uniformity

Take one inhaler, and perform the test. Unless otherwise prescribed in the instructions to the patient, shake the inhaler for 5 seconds and discharge one delivery to waste. Discharge the inverted inhaler into the apparatus, depressing the valve for a sufficient time to ensure complete discharge. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance as the delivered dose.

Repeat the procedure for a further 9 inhalers. Determine 10 delivered doses, each 1 dose at the beginning for 10 inhalers, by the above process.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

The mean of the delivered doses or the delivered dose stated on the label is used as the limit for judgement.

The preparation complies with the test if 9 out of 10 results lie between 75% and 125% of the limit and all lie between 65% and 135% of the limit. If 2 or 3 values lie outside the limits of 75 to 125%, repeat the above procedure for 20 more inhalers, and obtain 30 values as the total. Not more than 3 of the 30 values lie outside the limits of 75 to 125% and no value lies outside the limits of 65 to 135%.

In justified cases, these ranges may be extended. But no value should be less than 50% or more than 150% of the limit.

The mean value must be within 85 to 115% of the label claim for delivered dose.

2. Test method for dry powder inhalers

The dose collection apparatus must be capable of quantitatively capturing the delivered dose. A dose collection apparatus similar to that described for the valuation of metered-dose inhalers may be used provided that the dimensions of the tube and the filter can accommodate the measured flow rate. A suitable tube is defined in Table 6.14-1. Connect the tube to a flow system according to the scheme specified in Table 6.14-1 and Fig. 6.14-2.

Unless otherwise specified, determine the test flow rate and duration using the dose collection tube, the associated flow system, a suitable differential pressure meter and a suitable volumetric flowmeter, calibrated for the flow leaving the meter, according to the following procedure.

Prepare the inhaler for use according to the instructions to the patient and connect it to the inlet of the apparatus using a mouthpiece adapter to ensure an airtight seal. Use a mouthpiece adapter that ensures that the front face of the inhaler mouthpiece is flush with the front face of the sample

Table 6.14-1 Specifications of the apparatus described in Fig. 6.14-2

Code	Component	Description
A	Sample collection tube	Dimensions of 34.85 mm internal diameter × 12 cm length
B	Filter	47 mm glass fiber filter
C	Connector	Internal diameter ≧ 8 mm (e.g., short metal coupling, with low-diameter branch to P3)
D	Vacuum tubing	A length of suitable tubing having an internal diameter ≧ 8 mm and an internal volume of 25 ± 5 mL.
E	Two-way solenoid valve	A 2-way, 2-port solenoid valve having a minimum airflow resistance orifice with internal diameter ≧ 8 mm and an opening time ≦ 100 ms
F	Vacuum pump	Pump must be capable of drawing the required flow rate through the assembled apparatus with the dry powder inhaler in the mouthpiece adapter. Connect the pump to the 2-way solenoid valve using short and/or wide (≧ 10 mm internal diameter) vacuum tubing and connectors to minimize pump capacity requirements.
G	Timer	Timer capable of driving the solenoid valve for the required time period.
P1	Pressure tap	2.2 mm internal diameter, 3.1 mm outer diameter, flush with internal surface of the sample collection tube, centered and burr-free, 59 mm from its inlet. The pressure tap P1 must never be open to the atmosphere. Differential pressure to the atmosphere is measured at P1.
P2, P3	Pressure measurements	Absolute pressure
H	Flow control valve	Adjustable regulating valve with maximum Cv ≧ 1.

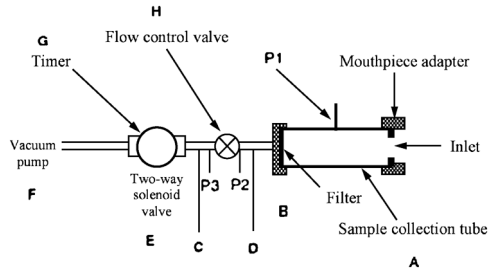


Fig. 6.14-2 Apparatus suitable for measuring the uniformity of delivered dose for dry powder inhalers

collection tube. Connect one port of a differential pressure meter to the pressure reading point P1 in Fig. 6.14-2, and let the other be open to the atmosphere. Switch on the pump, open the 2-way solenoid valve and adjust the flow control valve until the pressure drop across the inhaler is 4.0 kPa (40.8 cm H_2O) as indicated by the differential pressure meter. Remove the inhaler from the mouthpiece adapter and, without touching the flow control valve, connect a flowmeter to the inlet of the sampling apparatus. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter (Q_{out}) using the ideal gas law. For a meter calibrated for the entering volumetric flow (Q_{in}), use the following expression.

$$Q_{out} = \frac{Q_{in} \times P_0}{P_0 - \Delta P}$$

P_0: atmospheric pressure
ΔP: pressure drop over the meter

If the flow rate is above 100 L per minute adjust the flow control valve to obtain a flow rate of 100 L per minute (±5%). Note the volumetric airflow rate exiting the meter and define this as the test flow rate, $Q_{out'}$, in L per minute. Define the test flow duration, T, in seconds so that a volume of 4 L of air is drawn from the mouthpiece of the inhaler at the test flow rate, $Q_{out'}$. Ensure that critical flow occurs in the flow control valve by the following procedure: with the inhaler in place and the test flow rate $Q_{out'}$, measure the absolute pressure on both sides of the control valve (pressure reading points P2 and P3 in Fig. 6.14-2); a ratio P3/P2 of less than or equal to 0.5 indicates critical flow; switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

Dry powder inhalers contain two types of inhalers, pre-metered inhalers where powders for one emission are pre-dispensed in capsules or other suitable dosage forms and device-metered inhalers where powders for one emission are metered within the inhalers. Perform the test by the following methods depending on each function of the pre-metered inhalers or device-metered inhalers.

2.1. Pre-metered inhalers

Connect the inhaler to the apparatus using an adapter that ensures a good seal. Draw air through the inhaler under the predetermined conditions. Repeat the procedure until

the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance as the delivered dose.

Repeat the procedure for a further 9 doses. The sampling procedure to obtain 10 values of delivered doses is prescribed individually in considering the discharge mechanism of each preparation.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

The mean of the delivered doses or the delivered dose stated on the label is used as the limit for judgement.

The preparation complies with the test if 9 out of 10 results lie between 75% and 125% of the limit and all lie between 65% and 135% of the limit. If 2 or 3 values lie outside the limits of 75 to 125%, repeat the above procedure for 20 more delivered doses, and obtain 30 values as the total. Not more than 3 of the 30 values lie outside the limits of 75 to 125% and no value lies outside the limits of 65 to 135%.

In justified cases, these ranges may be extended. But no value should be less than 50% or more than 150% of the limit.

The mean value must be within 85 to 115% of the label claim for delivered dose.

2.2. Device-metered inhalers

2.2.1. Test method 1: evaluation of intra-inhaler dose uniformity

Take one inhaler, and perform the test. Connect the inhaler to the apparatus using an adapter that ensures a good seal. Draw air through the inhaler under the predetermined conditions. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance as the delivered dose.

Repeat the procedure for a further 2 doses.

Discharge the inhaler to waste until $(n/2) + 1$ deliveries remain, where n is the number of deliveries stated on the label. If necessary, store the inhaler to discharge electrostatic charges. Collect 4 doses using the procedure described above.

Discharge the inhaler to waste until 3 doses remain. If necessary, store the inhaler to discharge electrostatic charges. Collect 3 doses using the procedure described above. Determine 10 delivered doses per one inhaler, i.e., 3 doses at the beginning, 4 doses at the middle and 3 doses at the end by the above process.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

The mean of the delivered doses or the delivered dose stated on the label is used as the limit for judgement.

The preparation complies with the test if 9 out of 10 results lie between 75% and 125% of the limit and all lie between 65% and 135% of the limit. If 2 or 3 values lie outside the limits of 75 to 125%, repeat the test for 2 more inhalers, and obtain 30 values as the total. Not more than 3 of the 30 values lie outside the limits of 75 to 125% and no value lies outside the limits of 65 to 135%.

In justified cases, these ranges may be extended. But no value should be less than 50% or more than 150% of the limit.

The mean value must be within 85 to 115% of the label claim for delivered dose.

2.2.2. Test method 2: evaluation of inter-inhaler dose uniformity

Take one inhaler, and perform the test. Connect the inhaler to the apparatus using an adapter that ensures a good seal. Draw air through the inhaler under the predetermined conditions. Repeat the procedure until the number of deliveries that constitute the minimum recommended dose have been sampled. Quantitatively collect the contents of the apparatus and determine the amount of active substance as the delivered dose.

Repeat the procedure for a further 9 inhalers. Determine 10 delivered doses, each 1 dose at the beginning for 10 inhalers, by the above process.

For preparations containing more than one active substance, carry out the test for uniformity of delivered dose for each active substance.

The mean of the delivered doses or the delivered dose stated on the label is used as the limit for judgement.

The preparation complies with the test if 9 out of 10 results lie between 75% and 125% of the limit and all lie between 65% and 135% of the limit. If 2 or 3 values lie outside the limits of 75 to 125%, repeat the above procedure for 20 more inhalers, and obtain 30 values as the total. Not more than 3 of the 30 values lie outside the limits of 75 to 125% and no value lies outside the limits of 65 to 135%.

In justified cases, these ranges may be extended. But no value should be less than 50% or more than 150% of the limit.

The mean value must be within 85 to 115% of the label claim for delivered dose.

Add the following:

6.15 Aerodynamic Particle Size Measurement for Inhalations

This test is used to evaluate the fine particle characteristics of the aerosol clouds generated by preparations for inhalation, and is performed using one of the following apparatuses and test procedures. If justified, modified equipment or test procedure may be used.

1. Stage mensuration

The most reliable calibration for the separation characteristic of each impaction stage is performed in terms of the relationship between the stage collection efficiency and the aerodynamic diameter of particles and droplets passing through it as an aerosol.

Calibration is usually performed by examination of the

jet nozzle dimensions, the spatial arrangement of the jet nozzle and its collection part, and the airflow rate passing through it.

Because jet nozzles can corrode and wear over time, the critical dimensions of each stage must be measured on a regular basis to confirm them being within required ranges.

Only apparatuses that conform to specifications are used for aerodynamic particle size measurement for inhalations. An alternate validated and justified method of mensuration may be used.

2. Re-entrainment

When using the apparatuses 2 and 3, the selected technique should seek to minimize particle re-entrainment (from an upper to a lower impaction stage) where this may affect the amounts of drug collected. For example, minimizing the number of sampled doses and coating the particle collection surfaces are used to minimize particle re-entrainment. Glycerol, silicone oil or similar high viscosity liquid are used to coat particle collection surfaces. Plate coating must be part of method validation and may be omitted where it is demonstrated that the aerodynamic particle size is not influenced by the coating.

3. Inter-stage drug losses (wall losses)

Wall losses should be considered in method development and validation. If the wall losses affect the recovery rate (mass balance) of drugs, they should be controlled. Wall losses will be dependent upon a number of factors including the impactor type, operating conditions, formulation type and discharged amount to an impactor. How the wall loss is reflected within the calculation of the aerodynamic diameter of particles should be judged based up on the level and variability of the wall loss. For example, in cases where wall losses are low or have a low level of variability, the aerodynamic particle size is calculated by the assay of the drug recovered from the collection plate. In cases where wall losses are high or variable, it may be necessary to collect the wall loss drug separately and take it into account for calculation of the aerodynamic particle size.

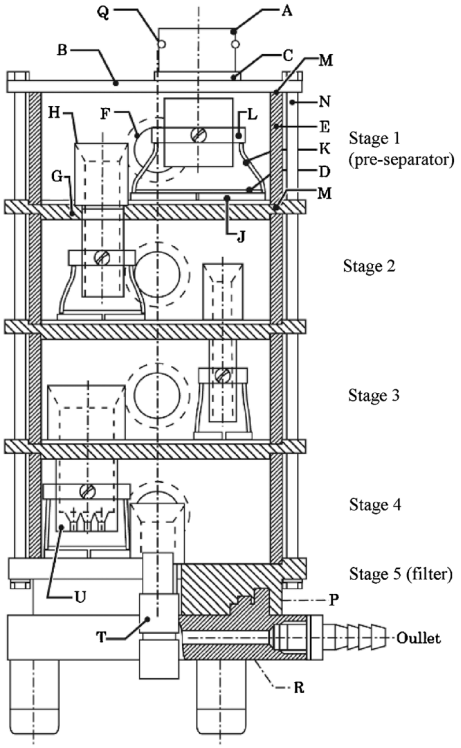
4. Recovery rate of drugs (mass balance)

In addition to the size distribution, good analytical practice dictates that a mass balance be performed in order to confirm that the amount of the drug discharged from the inhaler, which is collected in the mouthpiece adapter and the apparatus, is within an acceptable range around the expected value. The total mass of drug collected in all of the components of the mouthpiece adapter and the apparatus divided by the minimum recommended dose described in the dosage and administration is not less than 75% and not more than 125% of the average delivered dose determined under Uniformity of Delivered Dose for Inhalations <*6.14*>. This mass balance is necessary to ensure that the test results of particle size distributions are valid.

5. Measurement of fine particle dose and particle size distribution

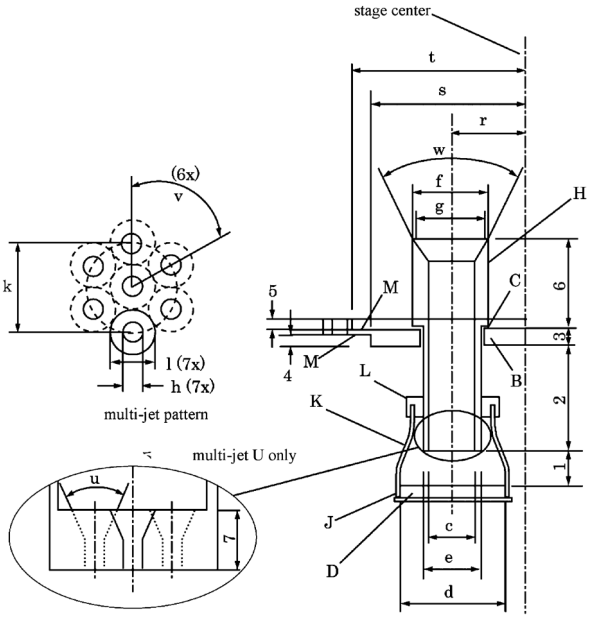
5.1. Multi-stage liquid impinger method (Apparatus 1)

The apparatus used for the multi-stage liquid impinger method (apparatus 1) is shown in Fig. 6.15-1. The apparatus 1 consists of impaction stages 1 (pre-separator), 2, 3 and 4 and an integral filter stage (stage 5), see Figures 6.15-1 to 6.15-3. An impaction stage comprises an upper horizontal metal partition wall (B) through which a metal inlet jet tube (A) with its collection plate (D) is protruding. A glass cylinder (E) with sampling port (F) forms the vertical wall of the stage, and the stage is connected to the next lower stage by the tube (H) through a lower horizontal metal partition wall (G). The tube into stage 4 (U) ends in a multi-jet arrangement. The collection plate (D) is secured in a metal frame (J) which is fastened by two wires (K) to a sleeve (L) secured on the jet tube. The horizontal face of the collection plate is perpendicular to the axis of the jet tube and centrally aligned. The upper surface of the collection plate is slightly raised above the edge of the metal frame. A recess around the perimeter of the horizontal partition wall guides the position of the glass cylinder. The glass cylinders are sealed against the horizontal partition walls with gaskets (M) and clamped together by six bolts (N). The sampling ports are sealed by stoppers. The bottom-side (back) of the lower partition wall of stage 4 has a concentrical protrusion fitted with a rubber O-ring (P) which seals against the edge of a filter placed in the filter holder. The filter holder (R) is constructed as a basin with a concentrical recess in which a perforated filter support (S) is flush-fitted. The filter holder is dimensioned for 76 mm diameter filters. The assembly of impaction stages is clamped onto the filter holder by two snap-locks (T). Connect an induction port (see Fig. 6.15-4)



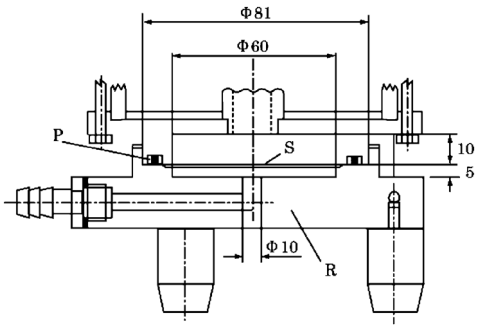
Capital letters refer to Table 6.15-1.

Fig. 6.15-1 Multi-stage liquid impinger (Apparatus 1)



Inserts show end of multi-jet tube U leading to stage 4.
Numbers and lowercase letters refer to Table 6.15-2 and capital letters refer to Table 6.15-1.

Fig. 6.15-2 Apparatus 1: Details of jet tube and collection plate



Numbers refer to dimensions (ϕ : diameter). Capital letters refer to Table 6.15-1. Dimensions in mm.

Fig. 6.15-3 Apparatus 1: Details of the filter stage (stage 5)

onto the stage 1 inlet jet tube of the impinger. A rubber O-ring on the jet tube provides an airtight connection to the induction port. A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port.

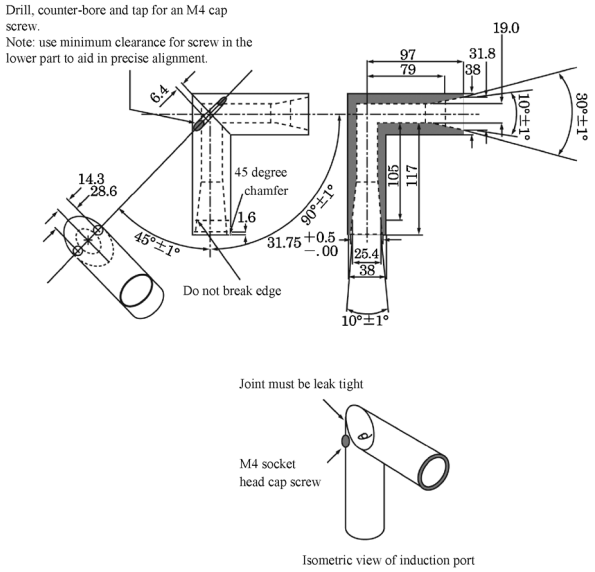
5.1.1. Procedure for metered-dose inhalers

Dispense 20 mL of a solvent, capable of dissolving the active substance, into each of stages 1 to 4 and replace the stoppers. Tilt the apparatus to wet the stoppers, thereby neutralizing electrostatic charge. Place a suitable filter capable of quantitatively collecting the active substance in stage 5 and assemble the apparatus. Place a suitable mouthpiece adapter in position at the end of the induction port. The mouthpiece end of the inhaler, when inserted to the

Table 6.15-1 Component specification for apparatus 1 in Figures 6.15-1, 2 and 3

Code*	Item	Description	Dimensions**
A, H	Jet tube	Metal tube screwed onto partition wall sealed by gasket (C), polished inner surface	see Fig. 6.15-2
B, G	Partition wall	Circular metal plate —diameter —thickness	 120 see Fig. 6.15-2
C	Gasket	e.g. polytetrafluoroethylene	to fit jet tube
D	Collection plate	Porosity 0 sintered-glass disk —diameter	 see Fig. 6.15-2
E	Glass cylinder	Plane polished cut glass tube —height, including gaskets —outer diameter —wall thickness —sampling port (F) diameter —stopper in sampling port	 46 100 3.5 18 ISO24/25
J	Metal frame	L-profiled circular frame with slit —inner diameter —height —thickness of horizontal section —thickness of vertical section	 to fit collection plate 4 0.5 2
K	Wire	Steel wire interconnecting metal frame and sleeve (2 for each frame) —diameter	 1
L	Sleeve	Metal sleeve secured on jet tube by screw —inner diameter —height —thickness	 to fit jet tube 6 5
M	Gasket	e.g. silicone	to fit glass cylinder
N	Bolt	Metal bolt with nut (6 pairs) —length —diameter	 205 4
P	O-ring	Rubber O-ring —diameter × thickness	 66.34 × 2.62
Q	O-ring	Rubber O-ring —diameter × thickness	 29.1 × 1.6
R	Filter holder	Metal housing with stand and outlet	see Fig. 6.15-3
S	Filter support	Perforated sheet metal —diameter —hole diameter —distance between holes (center-points)	 65 3 4
T	Snap-locks		
U	Multi-jet tube	Jet tube (H) ending in multi-jet arrangement	see Fig. 6.15-2

* Refers to Fig. 6.15-1.
** Measures in mm with tolerances according to JIS B 0405 unless otherwise stated.



Dimensions in mm unless otherwise stated.

Note

(1) Material may be aluminium, stainless steel or other suitable material.

(2) Machine from 38 mm bar stock.

(3) Bore 19 mm hole through bar.

(4) Cut tube to exact 45° as shown.

(5) The inner bores and tapers should be smooth – surface roughness Ra approx. 0.4 μm.

(6) Mill joining cads of stock to provide a liquid tight leak-free seal.

(7) Set up a holding fixture for aligning the inner 19 mm bore and for drilling and tapping M4 × 0.7 threads. There must be virtually no mismatch of the inner bores in the miter joint.

Fig. 6.15-4 Induction port

Table 6.15-2 Dimensions[1] of jet tube with collection plate of apparatus 1

Type	Code[2]	Stage 1	Stage 2	Stage 3	Stage 4	Filter (Stage 5)
Distance (Length)	1	9.5 (− .0 + .5)	5.5 (− .0 + .5)	4.0 (− .0 + .5)	6.0 (− .0 + .5)	n.a.
Distance (Length)	2	26	31	33	30.5	0
Distance (Length)	3	8	5	5	5	5
Distance (Length)	4	3	3	3	3	n.a.
Distance (Length)	5	0	3	3	3	3
Distance (Length)	6[3]	20	25	25	25	25
Distance (Length)	7	n.a.	n.a.	n.a.	8.5	n.a.
Diameter	c	25	14	8.0 (± .1)	21	14
Diameter	d	50	30	20	30	n.a.
Diameter	e	27.9	16.5	10.5	23.9	n.a.
Diameter	f	31.75 (− .0 + .5)	22	14	31	22
Diameter	g	25.4	21	13	30	21
Diameter	h	n.a.	n.a.	n.a	2.70 (± .5)	n.a.
Diameter	l	n.a.	n.a.	n.a	6.3	n.a.
Diameter	k	n.a.	n.a.	n.a	12.6	n.a.
Radius[4]	r	16	22	27	28.5	0
Radius	s	46	46	46	46	n.a.
Radius	t	n.a.	50	50	50	50
Angle	w	10°	53°	53°	53°	53°
Angle	u	n.a.	n.a.	n.a.	45°	n.a.
Angle	v	n.a.	n.a.	n.a.	60°	n.a.

(1) Measures in mm with tolerances according to JIS B 0405 unless otherwise stated.

(2) Refer to Fig. 6.15-2

(3) Including gasket

(4) Relative centerline of stage compartment

n.a.: not applicable

mouthpiece adapter, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use. Connect a suitable vacuum pump to the outlet of the apparatus and adjust the air flow through the apparatus, as measured at the inlet to the induction port, to 30 L per minute (±5%). Switch off the pump.

Unless otherwise prescribed in the patient instructions, shake the inhaler for 5 seconds and discharge one delivery to waste. Switch on the pump to the apparatus, locate the mouthpiece end of the inhaler in the adapter and discharge the inhaler into the apparatus, actuating the inhaler for a sufficient time to ensure complete discharge. Wait for 5 seconds before removing the assembled inhaler from the adapter. Repeat the procedure. The number of discharges should be minimized and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of the fine particle dose. After the final discharge, wait for 5 seconds and then switch off the pump.

Dismantle the filter stage of the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove the induction port and mouthpiece adapter from the apparatus and extract the active substance into an aliquot of the solvent. If necessary, rinse the inside of the inlet jet tube to stage 1 with the solvent, allowing the solvent to flow into the stage. Extract the active substance from the inner walls and the collection plate of each of the 4 upper stages of the apparatus into the solution in the respective stage by carefully tilting and rotating the apparatus, observing that no liquid transfer occurs between the stages.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent. Calculate the fine particle dose (see 6. Calculations).

5.1.2. Procedure for dry powder inhalers

Place a suitable low resistance filter capable of quantitatively collecting the active substance in stage 5 and assemble the apparatus. Connect the apparatus to a flow system according to the scheme specified in Fig. 6.15-5 and Table 6.15-3. Unless otherwise prescribed, conduct the test at the flow rate, Q_{out}, used in Uniformity of Delivered Dose for Inhalations <*6.14*>, drawing 4 L of air from the mouthpiece of the inhaler and through the apparatus.

Connect a flowmeter to the induction port. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter (Q_{out}) using the ideal gas law. For a meter calibrated for the entering volumetric flow (Q_{in}), use the following expression:

$$Q_{out} = \frac{Q_{in} \times P_0}{P_0 - \Delta P}$$

P_0: atmospheric pressure
ΔP: pressure drop over the meter

Adjust the flow control valve to achieve steady flow through the system at the required rate, Q_{out} (±5%). Ensure that critical flow occurs in the flow control valve by the following procedure. Switch off the pump.

With the inhaler in place and the test flow rate established, measure the absolute pressure on both sides of the control valve (pressure reading points P2 and P3 in Fig. 6.15-5). A ratio *P3/P2* of less than or equal to 0.5 indicates critical flow. Switch to a more powerful pump and re-measure the test flow rate if critical flow is not indicated.

Dispense 20 mL of a solvent, capable of dissolving the active substance, into each of the 4 upper stages of the apparatus and replace the stoppers. Tilt the apparatus to wet the stoppers, thereby neutralizing electrostatic charge. Place a suitable mouthpiece adapter in position at the end of the induction port. The mouthpiece end of the inhaler, when inserted to the mouthpiece adapter, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use.

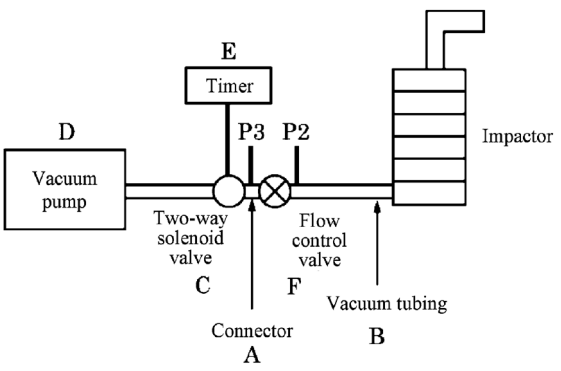
Prepare the dry powder inhaler for use according to the patient instructions. With the pump running and the 2-way solenoid valve closed, locate the mouthpiece of the inhaler in the mouthpiece adapter. Discharge the powder into the apparatus by opening the valve for the required time, T (±5%). Repeat the discharge procedure. The number of discharges should be minimized and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of fine particle dose.

Dismantle the filter stage of the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove the induction port and mouthpiece adapter from the apparatus and extract the active substance into an aliquot of the solvent. If necessary, rinse the inside of the inlet jet tube to stage 1 with the solvent, allowing the solvent to flow into the stage. Extract the active substance from the inner walls and the collection plate of each of the 4 upper stages of the apparatus into the solution in the respective stage by carefully tilting and rotating the apparatus, observing that no liquid transfer occurs between the stages.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent. Calculate the fine particle dose (see 6. Calculations).

5.2. Andersen cascade impactor method (Apparatus 2)

The apparatus used for Andersen cascade impactor



Capital letters refer to Table 6.15-3.

Fig. 6.15-5 Experimental set-up for testing dry powder inhalers

Table 6.15-3 Component specification for Fig. 6.15-5

Code*	Item	Description
A	Connector	ID ≧ 8 mm, e.g., short metal coupling, with low-diameter branch to P3.
B	Vacuum tubing	A length of suitable tubing having an ID ≧ 8 mm and an internal volume of 25 ± 5 mL.
C	Two-way solenoid valve	A 2-way, 2-port solenoid valve having a minimum airflow resistance orifice with ID ≧ 8 mm and an opening time ≦ 100 ms.
D	Vacuum pump	Pump must be capable of drawing the required flow rate through the assembled apparatus with the inhaler in the mouthpiece adapter, or equivalent. Connect the pump to the 2-way solenoid valve using short and/or wide (ID ≧ 10 mm) vacuum tubing and connectors to minimize pump capacity requirements.
E	Timer	Timer capable to drive the 2-way solenoid valve for the required duration, or equivalent.
P2, P3	Pressure measurements	Determine under steady-state flow condition with an absolute pressure transducer.
F	Flow control valve	Adjustable regulating valve with maximum C_v ≧ 1.

* Refer to Fig. 6.15-5.
ID: inner diameter

method (apparatus 2) is shown in Fig. 6.15-6. The apparatus 2 consists of 8 stages together with a final filter. Material of construction may be aluminium, stainless steel or other suitable material. The stages are clamped together and sealed with O-rings. Critical dimensions of apparatus 2 are provided in Table 6.15-4. In use, some occlusions and wear of nozzles will occur. In-use mensuration tolerances need to be justified.

The configuration used for metered-dose inhalers is shown in Fig. 6.15-6. The entry cone (see Fig. 6.15-7b) of the impactor is connected to an induction port (see Fig. 6.15-4). A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port.

In the configuration for dry powder inhalers, a pre-separator is placed above the top stage to collect large masses of non-respirable powder. The top of the pre-separator shown in Fig. 6.15-7a is used to adapt the pre-separator to the induction port. To accommodate high flow rates through the impactor, the outlet nipple, used to connect the impactor to the vacuum system, is enlarged to have an internal diameter greater than or equal to 8 mm.

5.2.1. Procedure for metered-dose inhalers

Assemble the Andersen cascade impactor with a suitable filter in place. Ensure that the system is airtight by a suitable method. Place a suitable mouthpiece adapter in position at the end of the induction port. The mouthpiece end of the inhaler, when inserted to the mouthpiece adapter, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler unit is positioned in the same orientation as the intended use. Connect a suitable pump to the outlet of the apparatus and adjust the air flow through the apparatus, as measured at the inlet to the induction port, to 28.3 L per minute (±5%). Switch off the pump.

Unless otherwise prescribed in the patient instructions, shake the inhaler for 5 seconds and discharge one delivery to waste. Switch on the pump to the apparatus, locate the mouthpiece end of the inhaler in the adapter and discharge the inhaler into the apparatus, actuating the inhaler for a sufficient time to ensure complete discharge. Wait for 5 seconds before removing the assembled inhaler from the adapter. Repeat the procedure. The number of discharges should be minimized and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate

Table 6.15-4 Critical dimensions for apparatus 2

Description	Number of nozzle	Dimension (mm)
Stage 0 nozzle diameter	96	2.55 ± 0.025
Stage 1 nozzle diameter	96	1.89 ± 0.025
Stage 2 nozzle diameter	400	0.914 ± 0.0127
Stage 3 nozzle diameter	400	0.711 ± 0.0127
Stage 4 nozzle diameter	400	0.533 ± 0.0127
Stage 5 nozzle diameter	400	0.343 ± 0.0127
Stage 6 nozzle diameter	400	0.254 ± 0.0127
Stage 7 nozzle diameter	201	0.254 ± 0.0127

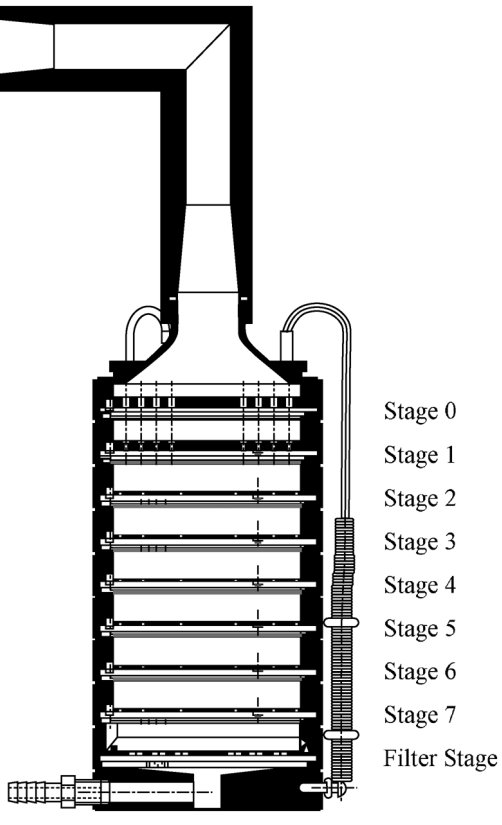


Fig. 6.15-6 Andersen cascade impactor used for metered-dose inhalers (Apparatus 2)

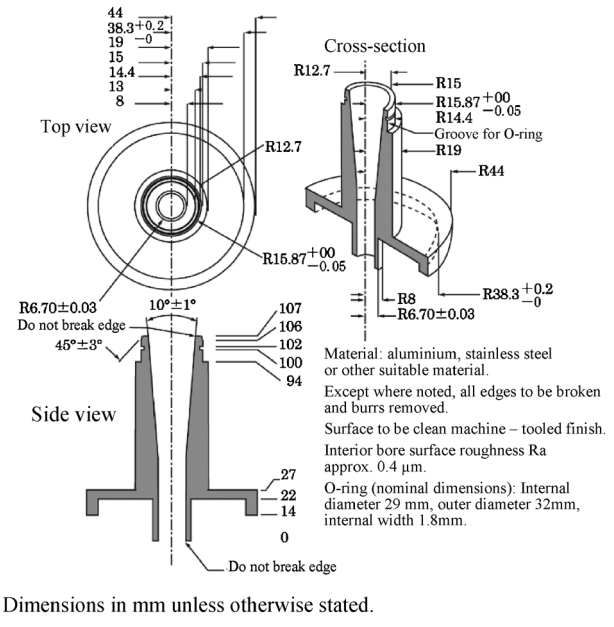
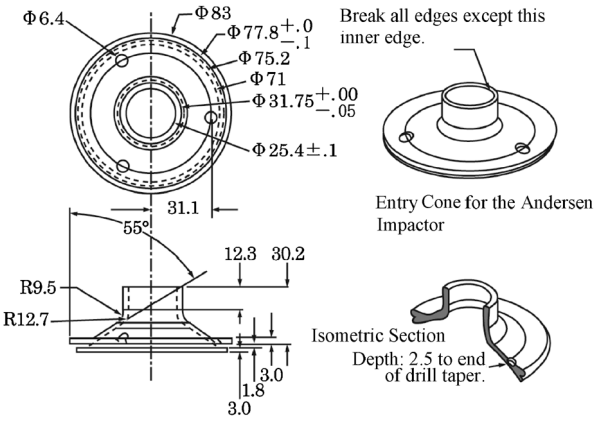


Fig. 6.15-7a Expanded view of top for the Andersen pre-separator adapted to the induction port



Material may be aluminum, stainless steel, or other suitable material. Surface roughness (Ra) should be approximately 0.4 μm. Dimensions in mm unless otherwise stated.

Fig. 6.15-7b Expanded view of the entry cone for mounting induction port on the Andersen cascade impactor without pre-separator.

and precise determination of the fine particle dose. After the final discharge, wait for 5 seconds and then switch off the pump.

Dismantle the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove the induction port and mouthpiece adapter from the apparatus and extract the active substance into an aliquot of the solvent. Extract the active substance from the inner walls and the collection plate of each of the stages of the apparatus into aliquots of the solvent.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent. Calculate the fine particle dose (see 6. Calculations).

5.2.2. Procedure for dry powder inhalers

The aerodynamic cut-off diameters of the individual stages of this apparatus are currently not well-established at flow rates other than 28.3 L per minute. Users must justify and validate the use of the impactor in the chosen conditions, when flow rates different from 28.3 L per minute are selected.

Assemble the Andersen cascade impactor with the pre-separator and a suitable filter in place and ensure that the system is airtight. Depending on the product characteristics, the pre-separator may be omitted, where justified. Stages 6 and 7 may also be omitted at high flow rates, if justified. The pre-separator may be coated in the same way as the collection plates or may contain 10 mL of a suitable solvent. Connect the apparatus to a flow system according to the scheme specified in Fig. 6.15-5 and Table 6.15-3.

Unless otherwise prescribed, conduct the test at the flow rate, Q_{out}, used in Uniformity of Delivered Dose for Inhalers <*6.14*> drawing 4 L of air from the mouthpiece of the inhaler and through the apparatus.

Connect a flowmeter to the induction port. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter (Q_{out}) using the ideal gas law. For a meter calibrated for the entering volumetric flow (Q_{in}), use the following expression:

$$Q_{out} = \frac{Q_{in} \times P_0}{P_0 - \Delta P}$$

P_0: atmospheric pressure
ΔP: pressure drop over the meter

Adjust the flow control valve to achieve steady flow through the system at the required rate, Q_{out} (±5%). Ensure that critical flow occurs in the flow control valve by the procedure described in 5.1.2. Procedure for dry powder inhalers. Switch off the pump.

Place a suitable mouthpiece adapter in position at the end of the induction port. The mouthpiece end of the inhaler, when inserted to the mouthpiece adapter, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use.

Prepare the dry powder inhaler for use according to the patient instructions. With the pump running and the 2-way solenoid valve closed, locate the mouthpiece of the inhaler in the mouthpiece adapter. Discharge the powder into the apparatus by opening the valve for the required time, T (±5%). Repeat the discharge sequence. The number of discharges should be minimized and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of fine particle dose.

Dismantle the apparatus. Carefully remove the filter and extract the active substance into an aliquot of the solvent. Remove the pre-separator, induction port and mouthpiece adapter from the apparatus and extract the active substance into an aliquot of the solvent. Extract the active substance from the inner walls and the collection plate of each of the stages of the apparatus into aliquots of the solvent.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent. Calculate the fine particle dose (see 6. Calculations).

5.3. Next generation impactor method (Apparatus 3)

The apparatus used for next generation impactor method (apparatus 3) is shown in Fig. 6.15-8. The apparatus 3 is a cascade impactor with 7 stages and a micro-orifice collector (MOC). Over the flow rate range of 30 to 100 L per minute the 50%-collection efficiency cut-off diameters (D_{50} values) range between 0.24 μm and 11.7 μm, evenly spaced on a logarithmic scale. In this flow range, there are always at least 5 stages with D_{50} values between 0.5 μm and 6.5 μm. The collection efficiency curves for each stage are sharp and minimize overlap between stages.

Material of construction may be aluminium, stainless steel or other suitable material.

The impactor configuration has removable impaction cups with all the cups in one plane (Figures 6.15-8 to

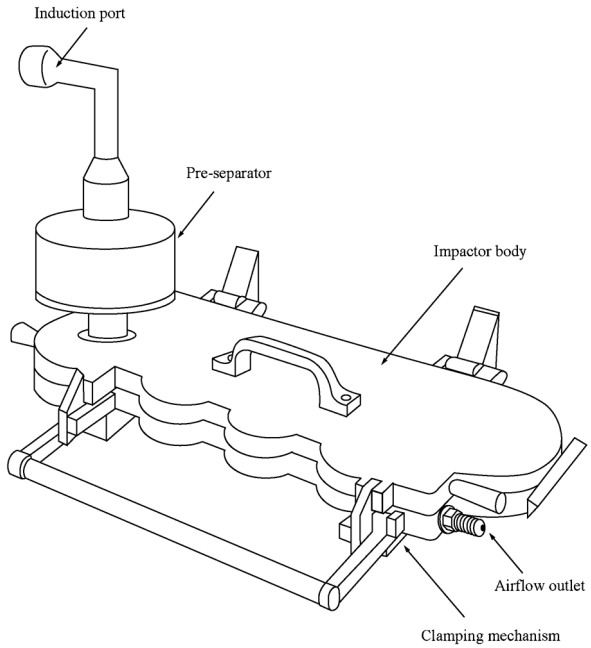


Fig. 6.15-8 Next generation impactor (shown with the pre-separator in place) (Apparatus 3)

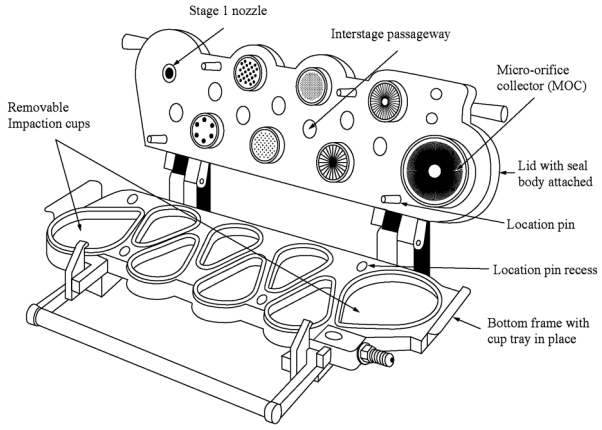


Fig. 6.15-9 Apparatus 3 showing component parts

Table 6.15-5 Critical dimensions for apparatus 3

Description	Dimension (mm)
Pre-separator (dimension a—see Fig. 6.15-12)	12.8 ± 0.05
Stage 1* nozzle diameter	14.3 ± 0.05
Stage 2* nozzle diameter	4.88 ± 0.04
Stage 3* nozzle diameter	2.185 ± 0.02
Stage 4* nozzle diameter	1.207 ± 0.01
Stage 5* nozzle diameter	0.608 ± 0.01
Stage 6* nozzle diameter	0.323 ± 0.01
Stage 7* nozzle diameter	0.206 ± 0.01
MOC*	aprox. 0.070
Cup depth (dimension b—see Fig. 6.15-11)	14.625 ± 0.10
Collection cup surface roughness (Ra)	0.5 to 2 μm
Stage 1 nozzle to seal body distance** —dimension c	0 ± 1.18
Stage 2 nozzle to seal body distance** —dimension c	5.236 ± 0.736
Stage 3 nozzle to seal body distance** —dimension c	8.445 ± 0.410
Stage 4 nozzle to seal body distance** —dimension c	11.379 ± 0.237
Stage 5 nozzle to seal body distance** —dimension c	13.176 ± 0.341
Stage 6 nozzle to seal body distance** —dimension c	13.999 ± 0.071
Stage 7 nozzle to seal body distance** —dimension c	14.000 ± 0.071
MOC nozzle to seal body distance** —dimension c	14.429 to 14.571

* See Fig. 6.15-10
** See Fig. 6.15-11

6.15-11). There are three main sections to the impactor; the bottom frame that holds the impaction cups, the seal body that holds the jets and the lid that contains the interstage passageways (Figures 6.15-8 and 6.15-9). Multiple nozzles are used at all but the first stage (Fig. 6.15-10). The flow passes through the impactor in a saw-tooth pattern.

Critical dimensions are provided in Table 6.15-5.

In routine operation, the seal body and lid are held together as a single assembly. The impaction cups are accessible when this assembly is opened at the end of an inhaler test. The cups are held in a support tray, so that all cups can be removed from the impactor simultaneously by lifting out the tray.

An induction port with internal dimensions defined in Fig. 6.15-4 connects to the impactor inlet. A pre-separator can be added when required, typically with dry powder inhalers, and connects between the induction port and the impactor. A suitable mouthpiece adapter is used to provide an airtight seal between the inhaler and the induction port.

Apparatus 3 contains a terminal MOC that for most preparations will eliminate the need for a final filter as determined by method validation. The MOC is a collection plate with nominally 4032 holes, each approximately 70 μm in diameter. Most particles not captured on stage 7 of the impactor will be captured on the cup surface below the MOC. For impactors operated at 60 L per minute, the MOC is capable of collecting 80% of 0.14 μm particles. For preparations with a significant fraction of particles not cap-

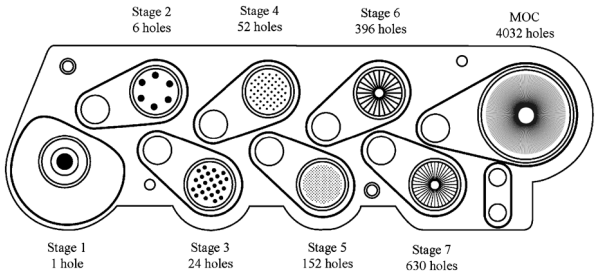


Fig. 6.15-10 Apparatus 3: nozzle configuration

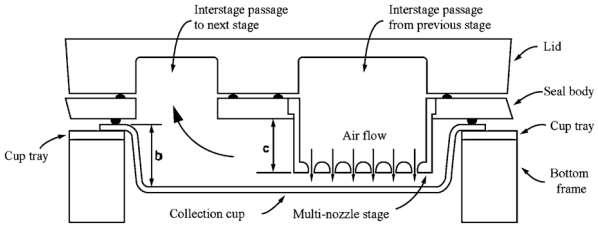


Fig. 6.15-11 Apparatus 3: configuration of interstage passageways

tured by the MOC, there is an optional filter holder that can replace the MOC or be placed downstream of the MOC (a glass fiber filter is suitable).

5.3.1. Procedure for metered-dose inhalers

Place cups into the apertures in the cup tray. Insert the cup tray into the bottom frame, and lower into place. Close the impactor lid with the seal body attached and operate the handle to lock the impactor together so that the system is airtight.

Connect an induction port with internal dimensions defined in Fig. 6.15-4 to the impactor inlet. Place a suitable mouthpiece adapter in position at the end of the induction port. The mouthpiece end of the inhaler to the mouthpiece adapter, when inserted, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use. Connect a suitable pump to the outlet of the apparatus and adjust the air flow through the apparatus, as measured at the inlet to the induction port, to 30 L per minute (±5%). Switch off the pump.

Unless otherwise prescribed in the patient instructions, shake the inhaler for 5 seconds and discharge one delivery to waste. Switch on the pump to the apparatus, locate the mouthpiece end of the inhaler in the adapter and discharge the inhaler into the apparatus, actuating the inhaler for a sufficient time to ensure a complete discharge.

Wait for 5 seconds before removing the assembled inhaler from the adapter. Repeat the procedure. The number of discharges should be minimized, and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of the fine particle dose. After the final discharge, wait for 5 seconds

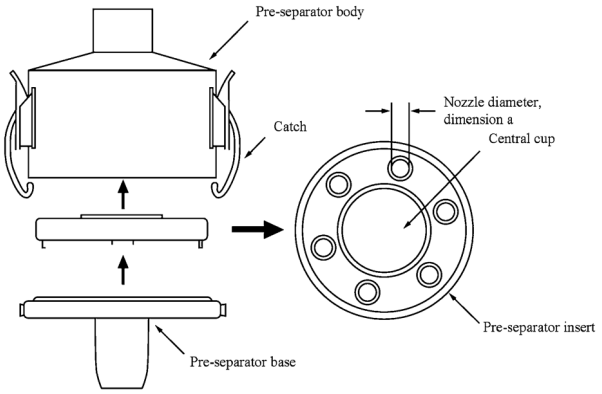


Fig. 6.15-12 Apparatus 3: pre-separator configuration

and then switch off the pump.

Dismantle the apparatus and recover the active substance as follows. Remove the induction port and mouthpiece adapter from the apparatus and extract the deposited active substance into an aliquot of the solvent. Open the impactor by releasing the handle and lifting the lid. Remove the cup tray, with the collection cups, and extract the active substance in each cup into an aliquot of the solvent.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent. Calculate the fine particle dose (see 6. Calculations).

5.3.2. Procedure for dry powder inhalers

Assemble the apparatus with the pre-separator (Fig. 6.15-12). Depending on the product characteristics, the pre-separator may be omitted, where justified.

Place cups into the apertures in the cup tray. Insert the cup tray into the bottom frame, and lower into place. Close the impactor lid with the seal body attached and operate the handle to lock the impactor together so that the system is airtight.

When used, the pre-separator should be assembled as follows. Assemble the pre-separator insert into the pre-separator base. Fit the pre-separator base to the impactor inlet. Add 15 mL of the solvent used for active substance recovery to the central cup of the pre-separator insert. Place the pre-separator body on top of this assembly and close the two catches.

Connect an induction port with internal dimensions defined in Fig. 6.15-4 to the impactor inlet or pre-separator inlet. Connect the apparatus to a flow system according to the scheme specified in Fig. 6.15-5 and Table 6.15-3.

Unless otherwise prescribed, conduct the test at the flow rate, Q_{out}, used in Uniformity of Delivered Dose for Inhalations <*6.14*> drawing 4 L of air from the mouthpiece of the inhaler and through the apparatus. Connect a flowmeter to the induction port. Use a flowmeter calibrated for the volumetric flow leaving the meter, or calculate the volumetric flow leaving the meter (Q_{out}) using the ideal gas law. For a meter calibrated for the entering volumetric flow (Q_{in}), use the following expression:

$$Q_{out} = \frac{Q_{in} \times P_0}{P_0 - \Delta P}$$

P_0: atmospheric pressure
ΔP: pressure drop over the meter

Adjust the flow control valve to achieve steady flow through the system at the required rate, Q_{out} (±5%). Ensure that critical flow occurs in the flow control valve by the procedure described in 5.1.2. Procedure for dry powder inhalers. Switch off the pump.

Place a suitable mouthpiece adapter in position at the end of the induction port. The mouthpiece end of the inhaler, when inserted to the mouthpiece adapter, lines up along the horizontal axis of the induction port. The front face of the inhaler mouthpiece must be flush with the front face of the induction port. When attached to the mouthpiece adapter, the inhaler is positioned in the same orientation as intended for use.

Prepare the dry powder inhaler for use according to the patient instructions. With the pump running and the 2-way solenoid valve closed, locate the mouthpiece of the inhaler in the mouthpiece adapter. Discharge the powder into the apparatus by opening the valve for the required time, T (±5%). Repeat the discharge sequence. The number of discharges should be minimized and typically would not be greater than 10. The number of discharges is sufficient to ensure an accurate and precise determination of fine particle dose.

Dismantle the apparatus and recover the active substance as follows.

Remove the induction port and mouthpiece adapter from the pre-separator, when used, and extract the deposited active substance into an aliquot of the solvent. When used, remove the pre-separator from the impactor, being careful to avoid spilling the cup liquid into the impactor. Recover the active substance from the pre-separator.

Open the impactor by releasing the handle and lifting the lid. Remove the cup tray, with the collection cups, and extract the active substance in each cup into an aliquot of the solvent.

Using a suitable method of analysis, determine the amount of active substance contained in each of the aliquots of solvent. Calculate the fine particle dose (see 6. Calculations).

6. Calculations

From the analysis of the solutions, calculate the mass of active substance deposited on each stage per discharge and the mass of active substance per discharge deposited in the induction port, mouthpiece adapter and when used, the pre-separator.

Starting at the collection site (filter or MOC) close to the airflow outlet of the apparatus, derive a table of cumulative mass versus cut-off diameter of the respective stage (see Table 6.15-6 for Apparatus 1, Table 6.15-7 for Apparatus 2, Table 6.15-8 for Apparatus 3). Calculate the Fine Particle Dose (FPD) by interpolation of the mass of the active substance less than or equal to 5 μm. Or it is possible to calculate the FPD as the mass of the active substance deposited on the stages corresponding to the cut-off diameter of 5 μm and less.

If necessary, and where appropriate (e.g., where there is a log-normal distribution), determine values for the Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) from the cumulative fraction of active substance versus cut-off diameter (see Tables 6.15-6 to 6.15-8). Appropriate computational methods may also be used.

Table 6.15-6 Calculations for Apparatus 1

Cut-off diameter (μm)	Mass of active substance deposited on stage per discharge	Cumulative mass of active substance per discharge	Cumulative fraction of active substance (%)
$d_4 = 1.7 \times q$	Mass from filter stage (m_5*)	$c_4 = m_5$	$f_4 = (c_4/c) \times 100$
$d_3 = 3.1 \times q$	Mass from stage 4 (m_4)	$c_3 = c_4 + m_4$	$f_3 = (c_3/c) \times 100$
$d_2 = 6.8 \times q$	Mass from stage 3 (m_3)	$c_2 = c_3 + m_3$	$f_2 = (c_2/c) \times 100$
	Mass from stage 2 (m_2)	$c = c_2 + m_2$	100

* Stage 5 is the filter stage.
$q = \sqrt{(60/Q)}$, Q: the test flow rate in L per minute (Q_{out} for dry powder inhalers).

Table 6.15-7 Calculations for Apparatus 2 when used at a flow rate of 28.3 L per minute

Cut-off diameter (μm)	Mass of active substance deposited on stage per discharge	Cumulative mass of active substance per discharge	Cumulative fraction of active substance (%)
$d_7 = 0.4$	Mass from filter stage (m_8)	$c_7 = m_8$	$f_7 = (c_7/c) \times 100$
$d_6 = 0.7$	Mass from stage 7 (m_7)	$c_6 = c_7 + m_7$	$f_6 = (c_6/c) \times 100$
$d_5 = 1.1$	Mass from stage 6 (m_6)	$c_5 = c_6 + m_6$	$f_5 = (c_5/c) \times 100$
$d_4 = 2.1$	Mass from stage 5 (m_5)	$c_4 = c_5 + m_5$	$f_4 = (c_4/c) \times 100$
$d_3 = 3.3$	Mass from stage 4 (m_4)	$c_3 = c_4 + m_4$	$f_3 = (c_3/c) \times 100$
$d_2 = 4.7$	Mass from stage 3 (m_3)	$c_2 = c_3 + m_3$	$f_2 = (c_2/c) \times 100$
$d_1 = 5.8$	Mass from stage 2 (m_2)	$c_1 = c_2 + m_2$	$f_1 = (c_1/c) \times 100$
$d_0 = 9.0$	Mass from stage 1 (m_1)	$c_0 = c_1 + m_1$	$f_0 = (c_0/c) \times 100$
	Mass from stage 0 (m_0)	$c = c_0 + m_0$	100

Table 6.15-8 Calculations for Apparatus 3

Cut-off diameter (μm)	x	Mass of active substance deposited on stage per discharge	Cumulative mass of active substance per discharge	Cumulative fraction of active substance (%)
$d_7 = 0.34 \times q$	0.67	Mass from MOC or terminal filter (m_8)	$c_7 = m_8$	$F_7 = (c_7/c) \times 100$
$d_6 = 0.55 \times q$	0.60	Mass from stage 7 (m_7)	$c_6 = c_7 + m_7$	$F_6 = (c_6/c) \times 100$
$d_5 = 0.94 \times q$	0.53	Mass from stage 6 (m_6)	$c_5 = c_6 + m_6$	$F_5 = (c_5/c) \times 100$
$d_4 = 1.66 \times q$	0.47	Mass from stage 5 (m_5)	$c_4 = c_5 + m_5$	$F_4 = (c_4/c) \times 100$
$d_3 = 2.82 \times q$	0.50	Mass from stage 4 (m_4)	$c_3 = c_4 + m_4$	$F_3 = (c_3/c) \times 100$
$d_2 = 4.46 \times q$	0.52	Mass from stage 3 (m_3)	$c_2 = c_3 + m_3$	$F_2 = (c_2/c) \times 100$
$d_1 = 8.06 \times q$	0.54	Mass from stage 2 (m_2)	$c_1 = c_2 + m_2$	$F_1 = (c_1/c) \times 100$
		Mass from stage 1 (m_1)	$c = c_1 + m_1$	100

$q = (60/Q)^x$, Q: the test flow rate in L per minute, x: listed in the table

9.01 Reference Standards

Change the following under section (1) as follows:

Adrenaline Bitartrate RS for Purity

***p*-Aminobenzoyl Glutamic Acid RS for Purity**

Anhydrous Lactose RS for Identification

Cellacefate RS for Identification

Gitoxin RS for Purity

Heparin Sodium RS for Identification

Lactose RS for Identification

Over-sulfated Chondroitin Sulfate RS for System Suitability

Povidone RS for Identification

Tyrosine RS for Digestion Test

Add the following to section (1):

Entacapone RS

Entacapone Related Substance A RS for System Suitability

Glucose RS

Insulin Aspart RS

Pazufloxacin Mesilate RS

Pyridoxal Phosphate RS

Saccharin Sodium RS for Identification

Zonisamide RS

Delete the following under section (1):

Aceglutamide RS

Diclofenamide RS

Digitoxin RS

Fluoxymesterone RS

Lanatoside C RS

Tolazamide RS

Delete the following under section (2):

Gramicidin RS

Rokitamycin RS

Zinostatin Stimalamer RS

9.21 Standard Solutions for Volumetric Analysis

Add the following:

Benzethonium chloride, 0.004 mol/L

1000 mL of this solution contains 1.7923 g of benzethonium chloride ($C_{27}H_{42}ClNO_2$: 448.08).

Preparation—Dissolve 1.792 g of benzethonium chloride for assay, previously dried at 105°C for 4 hours, in water to make exactly 1000 mL, and standardize the solution as follows:

Standardization—Pipet 10 mL of the prepared benzethonium chloride solution, adjust to pH between 2.6 and 3.4 by adding dropwise diluted dilute hydrochloric acid (1 in 2), add 1 drop of methyl orange TS, and titrate <*2.50*> with 0.02 mol/L sodium tetraphenylboron VS until the color of the solution becomes red. Calculate the molarity factor.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 8.962 mg of $C_{27}H_{42}ClNO_2$

Zinc sulfate, 0.05 mol/L

1000 mL of this solution contains 14.378 g of zinc sulfate heptahydrate ($ZnSO_4.7H_2O$: 287.55).

Preparation—Before use, dilute 0.1 mol/L zinc sulfate VS with water to make exactly twice the initial volume.

9.41 Reagents, Test Solutions

Add the following:

Aniline sulfate $(C_6H_5NH_2)_2.H_2SO_4$ A white to grayish white crystalline powder.

Purity Clarity and color of solution—Dissolve 1.0 g of aniline sulfate in 50 mL of water: the solution is clear and colorless.

3-Aminobenzoic acid $C_7H_7NO_2$ White crystals.

Melting point ⟨*2.60*⟩: About 174°C

2-Aminophenol C_6H_7NO Pale yellow-brown crystals. Soluble in ethanol (99.5), and sparingly soluble in water.

Melting point ⟨*2.60*⟩: About 172°C

4-Aminophenol C_6H_7NO A white to yellowish white crystalline powder. Soluble in ethanol (99.5), and sparingly soluble in water.

Melting point ⟨*2.60*⟩: About 186°C

Azosemide for assay $C_{12}H_{11}ClN_6O_2S_2$ [Same as the monograph Azosemide]

Baicalein for resolution check $C_{15}H_{10}O_5$ Yellow, crystals or crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification—Determine the absorption spectrum of a solution of baicalein for resolution check in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 213 nm and 217 nm, between 273 nm and 277 nm, and between 321 nm and 325 nm.

Purity Related substances—Dissolve 1 mg of baicalein for resolution check in 50 mL of methanol, 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: the total area of the peaks other than baicalein from the sample solution is not larger than 1/10 times the total area of all peaks other than the solvent peak.

Operating conditions

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

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

System Suitability

System performance: When the procedure is run with 10 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalein 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 sample solution under the above operating conditions, the relative standard deviation of the peak area of baicalein is not more than 1.5%.

Clomipramine hydrochloride for assay $C_{19}H_{23}ClN_2.HCl$ [Same as the monograph Clomipramine Hydrochloride. When dried, it contains not less than 99.0% of clomipramine hydrochloride ($C_{19}H_{23}ClN_2.HCl$).]

Clotiazepam for assay $C_{16}H_{15}ClN_2OS$ [Same as the monograph Clotiazepam. When dried, it contains not less than 99.0% of clotiazepam ($C_{16}H_{15}ClN_2OS$).]

Deoxycholic acid for thin-layer chromatography $C_{24}H_{40}O_4$ A white powder. Soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 175°C (with decomposition).

Identification—Determine the infrared absorption spectrum of deoxycholic acid 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 2930 cm^{-1}, 1716 cm^{-1}, 1447 cm^{-1} and 1042 cm^{-1}.

Purity Related substances—Dissolve 20 mg of deoxycholic acid 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 100 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 Oriental Bezoar: the spots other than the principal spot at an *R*f value of about 0.5 obtained from the sample solution are not more intense than the spot from the standard solution.

Dimidium bromide $C_{20}H_{18}BrN_3$ Red to dark brown, crystalline powder or powder.

Identification—(1) Determine the infrared absorption spectrum of dimidium bromide as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorption at the wave numbers of about 3300 cm^{-1}, 1619 cm^{-1}, 1489 cm^{-1}, 1470 cm^{-1}, 1422 cm^{-1} and 1316 cm^{-1}.

(2) A solution of dimidium bromide (1 in 1000) responds to Qualitative Tests ⟨*1.09*⟩ (1) for bromide.

Dimidium bromide-patent blue TS Dissolve each 0.5 g of dimidium bromide and 0.25 g of patent blue in 30 mL of a warmed mixture of water and ethanol (99.5) (9:1), combine the solutions, and add a mixture of water and ethanol (99.5) (9:1) to make 250 mL. To 20 mL of this solution add 270 mL of diluted sulfuric acid (7 in 675) and water to make 500 mL.

Storage—Preserve in light-resistant containers.

Gentisic acid $C_7H_6O_4$ Light yellow crystals.

Melting point ⟨*2.60*⟩: About 200°C

Imidazole hydrobromide $C_3H_4N_2.HBr$ White to pale yellow crystals. Melting point: about 221°C.

Irbesartan for assay $C_{25}H_{28}N_6O$ [Same as the monograph Irbesartan]

Mesalazine for assay $C_7H_7NO_3$ [Same as the monograph Mesalazine. When dried, it contains not less than

99.0% of mesalazine ($C_7H_7NO_3$).]

Patent blue $C_{27}H_{31}N_2NaO_6S_2$ Red-purple-brown to dark red-brown, crystalline powder to powder, or masses.

Identification—(1) To 5 mg of patent blue add 20 mL of ethanol (99.5): a dark blue color develops.

(2) Determine the infrared absorption spectrum of patent blue as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 1580 cm^{-1}, 1420 cm^{-1}, 1340 cm^{-1}, 1180 cm^{-1}, 1150 cm^{-1}, 1070 cm^{-1}, 1030 cm^{-1}, 910 cm^{-1}, 790 cm^{-1}, 700 cm^{-1} and 620 cm^{-1}.

Phthalate buffer solution (pH 5.8) Dissolve 100.0 g of potassium hydrogen phthalate in about 800 mL of water, adjust to pH 5.8 with a solution of sodium hydroxide (1 in 2), and add water to make 1000 mL.

0.01 mol/L sodium dihydrogen phosphate TS (pH 7.5) Dissolve 1.56 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust to pH 7.5 with sodium hydroxide TS, and add water to make 1000 mL.

0.2 mol/L Tris buffer solution (pH 8.1) Dissolve 24.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 1000 mL, and adjust to pH 8.1 with hydrochloric acid.

Voriconazole $C_{16}H_{14}F_3N_5O$ [Same as the namesake monograph]

Change the following as follows:

Aprotinin A clear and colorless liquid containing aprotinin extracted from the lung or parotid gland of a healthy cattle. The pH is between 5.0 and 7.0.

Content: not less than 15,000 KIE Units and not more than 25,000 KIE Units of aprotinin per mL. Assay—(i) Trypsin solution: Weigh an amount of crystalline trypsin equivalent to about 250 FIP Units of trypsin according to the labeled FIP Units, and dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 10 mL. Prepare before use, and preserve in ice.

(ii) Sample solution: To a suitable quantity of aprotinin add sodium tetraborate-calcium chloride buffer solution (pH 8.0) so that each mL contains 800 KIE Units of aprotinin, and use this solution as the sample solution.

(iii) Apparatus: Use a glass bottle as a reaction reservoir, 20 mm in inside diameter and 50 mm in height, stopper with a rubber stopper equipped with a glass/silver-silver chloride electrode for pH determination, a nitrogen-induction tube and an exhaust port. Fix the reaction reservoir in a thermostat, and keep the temperature of the bath at 25 ± 0.1°C by means of a precise thermoregulator.

(iv) Procedure: To 5.0 mL of *N*-α-benzoyl-L-arginine ethyl ester TS add 45.0 mL of sodium tetraborate-calcium chloride buffer solution (pH 8.0), and use this solution as the substrate solution. Pipet 1 mL of the trypsin solution, add sodium tetraborate-calcium chloride buffer solution (pH 8.0) to make exactly 10 mL, and use this solution as the test solution I. Transfer 10.0 mL of the substrate solution to the reaction reservoir, adjust the pH of the solution to 8.00 by adding dropwise 0.1 mol/L sodium hydroxide VS while stirring and passing a current of nitrogen, add exactly 1 mL of the test solution I previously allowed to stand at 25 ± 0.1°C for 10 minutes, then immediately add dropwise 0.1 mol/L sodium hydroxide VS by a 50-μL micropipet (minimum graduation of 1 μL), while stirring, to keep the reaction solution at pH 8.00, and read the amount of 0.1 mol/L sodium hydroxide VS consumed and the reaction time when the pH reached 8.00. Continue this procedure up to 6 minutes. Separately, pipet 2 mL of the trypsin solution and 1 mL of the sample solution, add sodium tetraborate-calcium chloride buffer solution (pH 8.0) to make exactly 10 mL, and use this solution as the test solution II. Transfer 10.0 mL of the substrate solution to the reaction reservoir, adjust the pH of the solution to 8.00, while stirring and passing a current of nitrogen, add exactly 1 mL of the test solution II, previously allowed to stand at 25 ± 0.1°C for 10 minutes, and proceed in the same manner. Separately, transfer 10.0 mL of the substrate solution to the reaction reservoir, adjust the pH of the solution to 8.00, while stirring and passing a current of nitrogen, add 1.0 mL of sodium tetraborate-calcium chloride buffer solution (pH 8.0), previously allowed to stand at 25 ± 0.1°C for 10 minutes, and perform a blank determination in the same manner.

(v) Calculation: Plot the amount of consumption (μL) of 0.1 mol/L sodium hydroxide VS against the reaction time (minutes), select linear reaction times, t_1 and t_2, designate the corresponding consumption amount of 0.1 mol/L sodium hydroxide VS as v_1 and v_2, respectively, and designate μmol of sodium hydroxide consumed per minute as D.

$$D\ (\mu\text{mol NaOH/minute}) = \frac{v_2 - v_1}{t_2 - t_1} \times \frac{1}{10}$$

$$\text{KIE Units per mL of aprotinin} = \frac{2\,(D_A - D_0) - (D_B - D_0)}{L} \times n \times 32.5$$

L: Amount (mL) of the sample solution added to the test solution II

n: Dilution coefficient of aprotinin

D_A: μmol of sodium hydroxide consumed in 1 minute when the test solution I is used

D_B: μmol of sodium hydroxide consumed in 1 minute when the test solution II is used

D_0: μmol of sodium hydroxide consumed in 1 minute when the solution for blank determination is used

32.5: Equivalent coefficient for calculation of KIE Units from FIP Units

One KIE Unit means an amount of aprotinin making a reduction of 50% off the potency of 2 Units of kallidinogenase at pH 8.0 and room temperature for 2 hours.

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

Crystalline trypsin To trypsin obtained from bovine pancreas add an appropriate amount of trichloroacetic acid to precipitate the trypsin, and recrystallize in ethanol (95).

White to yellow-white, crystals or powder. It is odorless. Freely soluble in water and in sodium tetraborate-calcium chloride buffer solution (pH 8.0).

Content: not less than 45 FIP Units of trypsin per mg.

Assay – (i) Sample solution: Weigh accurately an appropriate amount of crystalline trypsin, dissolve in 0.001 mol/L hydrochloric acid TS to prepare a solution containing 50 FIP Units per mL, and use this solution as the sample solution. Prepare before use, and preserve in ice.

(ii) Apparatus: Use a glass bottle as a reaction reservoir, 20 mm in inside diameter and 50 mm in height, stopper with a rubber stopper equipped with a glass/silver-silver chloride electrode for pH determination, a nitrogen-induction tube and an exhaust port. Fix the reaction reservoir in a thermostat, and keep the temperature of the both at 25 ± 0.1°C by means of a precise thermoregulator.

(iii) Procedure: Pipet 1.0 mL of *N*-α-benzoyl-L-arginine ethyl ester TS, transfer to the reaction reservoir, and add 9.0 mL of sodium tetraborate-calcium chloride buffer solution (pH 8.0). Allow to stand in the thermostat for 10 minutes to make the temperature of the contents reach to 25 ± 0.1°C, adjust the pH of the solution to 8.00 by adding dropwise 0.1 mol/L sodium hydroxide VS while stirring and passing a current of nitrogen, add exactly 0.05 mL of the sample solution previously allowed to stand at 25 ± 0.1°C, then immediately add dropwise 0.1 mol/L sodium hydroxide VS by a 50 μL-micropipet (minimum graduation of 1 μL) while stirring, to keep the reaction solution at pH 8.00, and read the amount of 0.1 mol/L sodium hydroxide VS consumed and the reaction time when the pH reached 8.00. Continue this procedure up to 8 minutes. Separately, transfer 10 mL of sodium tetraborate-calcium chloride buffer solution (pH 8.0), and perform a blank determination in the same manner.

(iv) Calculation: Plot the amount of consumption (μL) of 0.1 mol/L sodium hydroxide VS against the reaction time (minutes), select linear reaction times, t_1 and t_2, designate the corresponding consumption amount of 0.1 mol/L sodium hydroxide VS as v_1 and v_2, respectively, and designate μmol of sodium hydroxide consumed per minute as D (FIP Unit).

$$D\ (\mu\text{mol NaOH/min}) = \frac{v_2 - v_1}{t_2 - t_1} \times \frac{1}{10}$$

$$\text{FIP Units per mL of crystalline trypsin} = \frac{(D_1 - D_0) \times T}{L \times M}$$

D_1: μmol of sodium hydroxide consumed in 1 minute when the sample solution is used

D_0: μmol of sodium hydroxide consumed in 1 minute when the solution for blank determination is used

M: Amount (mg) of crystalline trypsin taken

L: Amount (mL) of the sample solution put in the reaction reservoir

T: Total volume (mL) of the sample solution

One FIP Unit is an amount of enzyme which decomposes 1 μmol of *N*-α-benzoyl-L-arginine ethyl ester per minute under the conditions described in the Assay.

Storage—Preserve in a cold place.

[6]-Gingerol for assay $C_{17}H_{26}O_4$ [6]-Gingerol for thin-layer chromatography. It meets the requirements of the following 1) [6]-Gingerol for assay 1 or 2) [6]-Gingerol for assay 2 (Purity value by quantitative NMR). The latter is used with correction for its amount based on the result obtained in the Assay.

1) [6]-Gingerol for assay 1

Absorbance ⟨*2.24*⟩ $E^{1\%}_{1cm}$ (281 nm): 101 – 112 [7 mg, ethanol (99.5), 200 mL].

Purity Related substances—Dissolve 5 mg of [6]-gingerol for assay 1 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 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 total area of the peaks other than [6]-gingerol from the sample solution is not larger than the peak area of [6]-gingerol 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 (3) under Hangekobokuto Extract.

Time span of measurement: About 6 times as long as the retention time of [6]-gingerol.

System suitability

System performance: Proceed as directed in the system suitability in the Assay (3) under Hangekobokuto Extract.

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of [6]-gingerol 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 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%.

2) [6]-Gingerol for assay 2 (Purity value by quantitative NMR)

Unity of peak—Dissolve 5 mg of [6]-gingerol for assay 2 in 5 mL of methanol, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and compare the absorption spectra of at least 3 points including the top of [6]-gingerol peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

Operating conditions

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

Detector: A photodiode array detector (wavelength: 282

nm, measuring range of spectrum: 220 – 400 nm).

System suitability

System performance: Proceed as directed in the system suitability in the Assay (3) under Hangekobokuto Extract.

Assay—Weigh accurately 5 mg of [6]-gingerol for assay 2 and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve both in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> and Crude Drugs Test <*5.01*> according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the internal reference compound. Calculate the resonance intensities, $A1$ (equivalent to 3 hydrogens) and $A2$ (equivalent to 1 hydrogen), of the signals around δ 3.56 ppm and δ 6.52 ppm assuming the signal of the internal reference compound as δ 0 ppm.

$$\text{Amount (\%) of [6]-gingerol } (C_{17}H_{26}O_4) = M_S \times I \times P/(M \times N) \times 1.2997$$

M: Amount (mg) of [6]-gingerol for assay 2 taken

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

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

N: Sum of the numbers of the hydrogen derived from $A1$ and $A2$

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

Operating conditions

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

Target nucleus: ^{1}H.

Digital resolution: 0.25 Hz or lower.

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

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

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

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

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

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around δ 3.56 ppm and δ 6.52 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals around δ 3.56 ppm and δ 6.52 ppm are not overlapped with any signal of obvious foreign substance, and the ratio of the resonance intensities, $(A1/3)/A2$, of each signal around δ 3.56 ppm and δ 6.52 ppm is between 0.99 and 1.01.

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

[6]-Gingerol for thin-layer chromatography

$C_{17}H_{26}O_4$ A yellow-white to yellow, liquid or solid. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification—Determine the absorption spectrum of a solution of [6]-gingerol for thin-layer chromatography in ethanol (99.5) (7 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 279 nm and 283 nm.

Purity Related substances—Dissolve 1.0 mg of [6]-gingerol for thin-layer chromatography in exactly 2 mL of methanol. Perform the test with 10 μL of this solution as directed in the Identification under Ginger: any spot other than the principal spot at the Rf value of about 0.3 does not appear.

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

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 0.3 g of hexyl parahydroxybenzoate, dissolve in 50 mL of diluted *N*,*N*-dimethylformamide (4 in 5), and titrate <*2.50*> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L sodium hydroxide VS} = 22.23 \text{ mg of } C_{13}H_{18}O_3$$

Isopromethazine hydrochloride for thin-layer chromatography $C_{17}H_{20}N_2S.HCl$ White, crystalline powder. Odorless. Freely soluble in water, in ethanol (95) and in chloroform, and practically insoluble in diethyl ether.

Melting point <*2.60*>: 186 – 195°C

Purity Related substances—Dissolve 5.0 mg of isopromethazine hydrochloride for thin-layer chromatography in exactly 25 mL of ethanol (95), and perform the test with this solution as directed in the Purity (3) under Promethazine Hydrochloride: any spot other than the principal spot at the Rf value of about 0.65 does not appear.

Loganin for assay $C_{17}H_{26}O_{10}$ Loganin for thin-layer chromatography. It meets the requirement of the following 1) Loganin for assay 1 or 2) Loganin for assay 2 (Purity value by quantitative NMR). The former is used after dry-

ing in a desiccator (silica gel) for 24 hours, and the latter is used with correction for its amount based on the result obtained in the Assay.

1) Loganin for assay 1

Absorbance ⟨*2.24*⟩ $E_{1cm}^{1\%}$ (235 nm): 275 – 303 [5 mg after drying in a desiccator (silica gel) for 24 hours, methanol, 500 mL]

Purity Related substances—Dissolve 2 mg of loganin for assay 1 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 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 loganin from the sample solution is not larger than the peak area of loganin 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 Goshajinkigan Extract.

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

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (1) under Goshajinkigan Extract.

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 loganin obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

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

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

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Goshajinkigan Extract.

Detector: A photodiode array detector (wavelength: 238 nm, measuring range of spectrum: 220 – 400 nm).

System suitability

System performance: Proceed as directed in the system suitability in the Assay (1) under Goshajinkigan Extract.

Assay—Weigh accurately 5 mg of loganin for assay 2 and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩ and Crude Drugs Test ⟨*5.01*⟩ according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the internal reference compound. Calculate the resonance intensity A (equivalent to 1 hydrogen) of the signal around δ 7.14 ppm assuming the signal of the internal reference compound as δ 0 ppm.

$$\text{Amount (\%) of loganin } (C_{17}H_{26}O_{10}) = M_S \times I \times P/(M \times N) \times 1.7235$$

M: Amount (mg) of loganin for assay 2 taken

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

I: Signal resonance intensity A based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000

N: Number of the hydrogen derived from A

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

Operating conditions

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

Target nucleus: ^{1}H.

Digital resolution: 0.25 Hz or lower.

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

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

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

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

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

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around δ 5.02 ppm and δ 7.14 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals around δ 5.02 ppm and δ 7.14 ppm are not overlapped with any signal of obvious foreign substance. Furthermore, when determined the resonance intensities $A1$ and A, both equivalent to 1 hydrogen, of each signal around δ 5.02 ppm and δ 7.14 ppm, the ratio of them, $A1/A$, is between 0.99 and 1.01.

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

Methanol, anhydrous CH_4O To 1000 mL of methanol

add 5 g of magnesium powder. After the evolving of a gas is stopped, distillate the solution, and preserve the distillate protecting from moisture. Water content per mL is not more than 0.3 mg.

Neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) Condensate of neocarzinostatin and styrene-maleic acid alternating copolymer partial butyl ester in a rate of 2:3 by amide bond. Average molecular mass: about 28,400. A pale yellow powder.

Identification—Dissolve 4 mg of the substance to be examined in 0.05 mol/L phosphate buffer solution (pH 7.0) to make 10 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 266 nm and 270 nm, and shoulders between 257 nm and 262 nm, between 286 nm and 291 nm and between 318 nm and 348 nm.

Absorbance <*2.24*> $E^{1\%}_{1cm}$ (268 nm): 13.0 – 17.5 [4 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution (pH 7.0), 10 mL].

Purity (i) Test solutions

Solution A: Dissolve 36.6 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.23 mL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine and water to make 100 mL.

Solution B: Dissolve 33.3 g of acrylamide and 0.89 g of *N*,*N*′-methylenebisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution C: Dissolve 5.98 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.46 mL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine and water to make 100 mL.

Solution D: Dissolve 10.0 g of acrylamide and 2.5 g of *N*,*N*′-methylenebisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution E: Dissolve 4 mg of riboflavin in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution F: Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.4 g of glycine in water to make 500 mL.

Buffer solution for sample: To 50 mL of Solution C add 20 mL of water and 10 mL of glycerin solution (3 in 5).

(ii) Gels

Resolving gel: Mix 2.5 mL of Solution A and 7.5 mL of Solution B. Mix the mixture with 10 mL of freshly prepared ammonium peroxodisulfate solution (7 in 5000) after degassing under reduced pressure. Pour this mixture into a glass tube, 5 mm in inside diameter and 10 cm in length, to make 7 cm height, put water gently on the upper surface of the mixture, and allow to polymerize for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

Stacking gel: Mix 1 mL of Solution C, 2 mL of Solution D, 1 mL of Solution E and 4 mL of water, pour 0.2 mL of the mixture on the resolving gel, put water gently on the upper surface of the mixture, and allow to polymerize under a fluorescent light for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

iii) Sample solution Dissolve 3.0 mg of the substance to be examined in the buffer solution for sample to make 10 mL.

(iv) Procedure Mount the gel in electrophoresis apparatus. Add a mixture of 200 mL of Solution F and 2 mL of bromophenol blue solution (1 in 100,000) to the upper reservoir (cathode) and 300 mL of Solution F to the lower reservoir (anode). Introduce carefully exactly 100 μL of the sample solution onto the surface of the gel, and allow electrophoresis at room temperature to take place with a current of 2 mA per tube as a bromophenol blue band is passing in the stacking gel and then increase the current to 4 mA per tube as the bromophenol blue band is passing in the resolving gel, and stop the current when the band reached 5 cm from the upper end of the gel.

(v) Staining and decolorization Dissolve 0.1 g of Coomassie brilliant blue G-250 in 100 mL of trichloroacetic acid solution (1 in 2), and mix 1 volume of this solution and 2 volumes of water before using. Immerse the gels for 15 hours in this mixture, and transfer into about 20 mL of acetic acid (100) solution (7 in 100) to remove the excess of dye. Replace the acetic acid (100) solution until the back ground of the gel becomes colorless.

(vi) Determination Determine the peak area, A_T, of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) and the total area, A, of the peaks other than neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3), based on the absorbance at 600 nm of the gel determined by using a densitometer. Calculate the amount of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3) by the following formula: not less than 90.0%.

Amount (%) of neocarzinostatin-styrene-maleic acid alternating copolymer partial butyl ester condensate (2:3)
$= A_T/(A_T + A) \times 100$

Water <*2.48*> Not more than 12.0% (10 mg, coulometric titration).

Polyvinyl alcohol I Colorless to white or pale yellow, granules or powder. It is odorless, or has a faint odor of acetic acid. It is tasteless. Practically insoluble in ethanol (95) and in diethyl ether. To polyvinyl alcohol I add water, and heat: a clear, viscous solution is obtained. Polyvinyl alcohol I is hygroscopic.

Viscosity <*2.53*> 25.0 – 31.0 mm^2/s. Weigh 4.000 g of polyvinyl alcohol I, previously dried, add 95 mL of water, allow to stand for 30 minutes, and heat to dissolve on a water bath under a reflux condenser for 2 hours while stirring. After cooling, add water to make 100.0 g, and mix. Allow to stand still to remove bubbles, and perform the test at 20 ± 0.1°C as directed in Method 1.

pH <*2.54*>—The pH of a solution of 1.0 g of polyvinyl alcohol I in 25 mL of water is between 5.0 and 8.0.

Purity Clarity and color of solution—To 20 mL of

water add 1.0 g of polyvinyl alcohol I, disperse by thorough stirring, warm between 60°C and 80°C for 2 hours, and cool: the solution is colorless and clear.

Saponification value 98.0 – 99.0 mol%. Weigh accurately about 3.0 g of polyvinyl alcohol I, previously dried, transfer to a glass-stoppered conical flask, add 100 mL of water, and dissolve by heating on a water bath. After cooling, add exactly 25 mL of 0.1 mol/L sodium hydroxide VS, stopper tightly, and allow to stand for 2 hours. Then add exactly 30 mL of 0.05 mol/L sulfuric acid VS, shake thoroughly, and titrate ⟨*2.50*⟩ with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction. However, when the volume of 0.1 mol/L sodium hydroxide VS consumed in the test is 25 mL or more, use about 2.0 g of the sample.

$$\text{Saponification value (mol\%)} = 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A = \frac{0.6005 \times (a - b)}{\text{amount (g) of polyvinyl alcohol I taken}}$$

a: Volume (mL) of 0.1 mol/L sodium hydroxide VS consumed in the test

b: Volume (mL) of 0.1 mol/L sodium hydroxide VS consumed in the blank test

Polyvinyl alcohol II Colorless to white or pale yellow, granules or powder. It is odorless, or has a faint odor of acetic acid. It is tasteless. Practically insoluble in ethanol (95) and in diethyl ether. To polyvinyl alcohol II add water, and heat: a clear, viscous solution is obtained. Polyvinyl alcohol II is hygroscopic.

Viscosity ⟨*2.53*⟩ 4.6 – 5.4 mm^2/s. Weigh 4.000 g of polyvinyl alcohol II, previously dried, add 95 mL of water, allow to stand for 30 minutes, and dissolve by stirring on a water bath between 60°C and 80°C for 2 hours. After cooling, add water to make 100.0 g, and mix. Allow to stand still to remove bubbles, and perform the test at 20 ± 0.1°C as directed in Method 1.

pH ⟨*2.54*⟩—The pH of a solution of 1.0 g of polyvinyl alcohol II in 25 mL of water is between 5.0 and 8.0.

Purity Clarity and color of solution—To 20 mL of water add 1.0 g of polyvinyl alcohol II, disperse by thorough stirring, heat on a water bath for 2 hours, and cool: the solution is clear and colorless.

Saponification value 86.5 – 89.5 mol%. Weigh accurately about 2 g of polyvinyl alcohol II, previously dried, transfer to a glass-stoppered conical flask, add 100 mL of water, and warm while stirring for 2 hours. After cooling, add exactly 25 mL of 0.5 mol/L sodium hydroxide VS, stopper tightly, and allow to stand for 2 hours. Then add exactly 30 mL of 0.25 mol/L sulfuric acid VS, shake thoroughly, and titrate ⟨*2.50*⟩ with 0.5 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

$$\text{Saponification value (mol\%)} = 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A = \frac{3.0025 \times (a - b)}{\text{amount (g) of polyvinyl alcohol II taken}}$$

a: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test

b: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank test

[6]-Shogaol for assay $C_{17}H_{24}O_3$ [6]-Shogaol for thin-layer chromatography. It meets the requirement of the following 1) [6]-Shogaol for assay 1 or 2) [6]-Shogaol for assay 2 (Purity value by quantitative NMR). The latter is used with correction for its amount based on the result obtained in the Assay.

1) [6]-Shogaol for assay 1

Absorbance ⟨*2.24*⟩ $E_{1cm}^{1\%}$ (225 nm): 727 – 781 [5 mg, ethanol (99.5), 500 mL].

Purity Related substances—Dissolve 5 mg of [6]-shogaol for assay 1 in 10 mL of a mixture of acetonitrile and water (2:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (2:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than [6]-shogaol from the sample solution is not larger than the peak area of [6]-shogaol 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 (2) under Mukoi-Daikenchuto Extract.

Time span of measurement: 3 times as long as the retention time of [6]-shogaol, beginning after the solvent peak.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add a mixture of acetonitorile and water (2:1) to make exactly 20 mL. Confirm that the peak area of [6]-shogaol 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 [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%.

2) [6]-Shogaol for assay 2 (Purity value by quantitative NMR)

Unity of peak—Dissolve 5 mg of [6]-shogaol for assay 2 in 10 mL of a mixture of acetonitrile and water (2:1), 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, and compare the absorption spectra of at least 3 points including the top of [6]-shogaol peak and around the two middle peak heights of before and after the top: no difference in form is observed among their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (2) under Mukoi-Daikenchuto Extract.

Detector: A photodiode array detector (wavelength: 225 nm, measuring range of spectrum: 220 – 400 nm).

System suitability

System performance: Proceed as directed in the system suitability in the Assay (2) under Mukoi-Daikenchuto Extract.

Assay—Weigh accurately 5 mg of [6]-shogaol for assay 2 and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, and measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01> according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the internal reference compound. Calculate the resonance intensity A (equivalent to 3 hydrogens) of the signal around δ 3.57 ppm assuming the signal of the internal reference compound as δ 0 ppm.

$$\text{Amount (\%) of [6]-shogaol } (C_{17}H_{24}O_3) = M_S \times I \times P/(M \times N) \times 1.2202$$

M: Amount (mg) of [6]-shogaol for assay 2 taken
M_S: Amount (mg) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy taken
I: Signal resonance intensity A based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000
N: Number of the hydrogen derived from A
P: Purity (%) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy

Operating conditions

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

Target nucleus: ^{1}H.

Digital resolution: 0.25 Hz or lower.

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

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

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

Integrating times: 8 or more times.

Dummy scanning: 2 or more times.

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

System suitability

Test for required detectability: When the procedure is run with the sample solution under the above operating conditions, the SN ratio of each signal around δ 3.57 ppm and δ 6.37 – 6.43 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the two signals around δ 3.57 ppm and δ 6.37 – 6.43 ppm are not overlapped with any signal of obvious foreign substance. Furthermore, when determined the resonance intensities, A (equivalent to 3 hydrogens) and $A1$ (equivalent to 2 hydrogens) of each signal around δ 3.57 ppm and δ 6.37 – 6.43 ppm, the ratio of the resonance intensities, $(A1/2)/(A/3)$, of each signal around δ 3.57 ppm and δ 6.37 – 6.43 ppm is between 0.99 and 1.01.

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

[6]-Shogaol for thin-layer chromatography $C_{17}H_{24}O_3$

A pale yellow, clear liquid. Miscible with methanol and with ethanol (99.5), and practically insoluble in water.

Purity Related substances—Dissolve 1.0 mg of [6]-shogaol 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 and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: any spot other than the principal spot at an Rf value of about 0.5 does not appear.

Styrene-maleic acid alternating copolymer partial butyl ester Polymerize styrene and maleic anhydride using cumene as solvent, and add 1-butanol or water to the maleic anhydride groups. Average molecular mass: about 1600. A white to pale yellowish white powder.

Identification—Dissolve 5 mg of the substance to be examined in sodium hydrogen carbonate solution (1 in 15) to make 10 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 256 nm and 260 nm, and a shoulder between 251 nm and 256 nm.

Absorbance <2.24> $E^{1\%}_{1cm}$ (258 nm): 6.3 – 7.3 [5 mg calculated on the anhydrous basis, sodium hydrogen carbonate solution (1 in 15), 10 mL].

Purity (i) Test solutions

Solution A: Dissolve 36.6 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.23 mL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine and water to make 100 mL.

Solution B: Dissolve 33.3 g of acrylamide and 0.89 g of *N*,*N*′-methylenebisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution C: Dissolve 5.98 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 48 mL of 1 mol/L hydrochloric acid TS, 0.46 mL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine and water to make 100 mL.

Solution D: Dissolve 10.0 g of acrylamide and 2.5 g of *N*,*N*′-methylenebisacrylamide in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution E: Dissolve 4 mg of riboflavin in water to make 100 mL. Preserve in a cold place, avoiding exposure to light.

Solution F: Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.4 g of glycine in water to make 500 mL.

Buffer solution for sample: To 50 mL of Solution C add 20 mL of water and 10 mL of glycerin solution (3 in 5).

(ii) Gels

Resolving gel: Mix 2.5 mL of Solution A and 7.5 mL of Solution B. Mix the mixture with 10 mL of freshly prepared ammonium peroxodisulfate solution (7 in 5000) after degassing under reduced pressure. Pour this mixture into a glass tube, 5 mm in inside diameter and 10 cm in length, to make 7 cm height, put water gently on the upper surface of the mixture, and allow to polymerize for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

Stacking gel: Mix 1 mL of Solution C, 2 mL of Solution D, 1 mL of Solution E and 4 mL of water, pour 0.2 mL of the mixture on the resolving gel, put water gently on the upper surface of the mixture, and allow to polymerize under a fluorescent light for 60 minutes. After polymerization, remove the water from the upper surface of the gel.

(iii) Sample solution Dissolve 3.0 mg of the substance to be examined in the buffer solution for sample to make 20 mL.

(iv) Procedure Mount the gel in an electrophoresis apparatus. Add a mixture of 200 mL of Solution F and 2 mL of bromophenol blue solution (1 in 100,000) to the upper reservoir (cathode) and 300 mL of Solution F to the lower reservoir (anode). Introduce carefully exactly 100 μL of the sample solution onto the surface of the gel, and allow electrophoresis at room temperature to take place with a current of 2 mA per tube as a bromophenol blue band is passing in the stacking gel and then increase the current to 4 mA per tube as the bromophenol blue band is passing in the resolving gel, and stop the current when the band reached 5 cm from the upper end of the gel.

(v) Staining and decolorization Dissolve 0.1 g of Coomassie brilliant blue G-250 in 100 mL of trichloroacetic acid solution (1 in 2), and mix 1 volume of this solution and 2 volumes of water before using. Immerse the gels for 15 hours in this mixture, and transfer into about 20 mL of acetic acid (100) solution (7 in 100) to remove the excess of dye. Replace the acetic acid (100) solution until the back ground of the gel becomes colorless.

(vi) Determination Determine the peak area, A_T, of styrene-maleic acid alternating copolymer partial butyl ester and the total area, A, of the peaks other than styrene-maleic acid alternating copolymer partial butyl ester, based on the absorbance at 600 nm of the gel determined by using a densitometer. Calculate the amount of styrene-maleic acid alternating copolymer partial butyl ester by the following formula: not less than 98.0%.

$$\text{Amount (\%) of styrene-maleic acid alternating copolymer partial butyl ester} = A_T/(A_T + A) \times 100$$

Water ⟨*2.48*⟩: Not more than 10.0% (10 mg, coulometric titration).

***p*-Toluenesulfonamide** $CH_3C_6H_4SO_2NH_2$ White, crystals or crystalline powder. Melting point: about 137°C.

Purity Related substances—Dissolve 30 mg of *p*-toluenesulfonamide in acetone to make exactly 200 mL. Spot 10 μL of this solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, cyclohexane and diluted ammonia solution (28) (10 in 11) (200:100:60:23) to a distance of about 12 cm, and air-dry the plate. Heat the plate at 110°C for 10 minutes, and immediately expose to chlorine for 2 minutes. Expose the plate to cold wind until a very pale blue color develops when 1 drop of potassium iodide-starch TS is placed on a site below the starting line on the plate. Spray evenly potassium iodide-starch TS on the plate: no spot other than the principal spot at an *R*f value of about 0.6 appears.

Delete the following:

2-Acetamidoglutarimide

Mercury (II) chloride TS

PBS containing bovine serum

PBS containing bovine serum albumin

Peroxidase-labeled rabbit anti-ECP antibody Fab′ TS

Thimerosal

Thymine

Trichloroacetic acid TS for serrapeptase

9.42 Solid Supports/Column Packings for Chromatography

Add the following:

14% Cyanopropylphenyl-86% dimethyl silicone polymer for gas chromatography Prepared for gas chromatography.

Octadecylsilanized monolithic silica for liquid chromatography Prepared for liquid chromatography.

Human albumin chemically bonded silica gel for liquid chromatography Prepared for liquid chromatography.

Official Monographs

Delete the following Monograph:

Aceglutamide Aluminum

アセグルタミドアルミニウム

Amoxicillin Hydrate

アモキシシリン水和物

Change the Purity (3) as follows:

Purity

(3) Related substances—Dissolve 0.10 g of Amoxicillin Hydrate in 50 mL of a solution of boric acid (1 in 200), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of boric acid (1 in 200) 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 of both solutions by the automatic integration method: the area of the peak other than amoxicillin obtained from the sample solution is not larger than the peak area of amoxicillin from the standard solution. Furthermore, the total area of the peaks other than amoxicillin from the sample solution is not larger than 3 times the peak area of amoxicillin from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4 mm in inside diameter and 30 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 1.36 g of sodium acetate trihydrate in 750 mL of water, adjust to pH 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust so that the retention time of amoxicillin is about 8 minutes.

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

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a solution of boric acid (1 in 200) to make exactly 10 mL. Confirm that the peak area of amoxicillin 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 amoxicillin are not less than 2500 and not more than 1.5, respectively.

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

Ampicillin Hydrate

アンピシリン水和物

Change the Purity (3) as follows:

Purity

(3) Related substances—Dissolve 50 mg of Ampicillin 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 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 other than ampicillin obtained from the sample solution is not larger than the peak area of ampicillin from the standard solution, and the total area of the peaks other than ampicillin from the sample solution is not larger than 2 times the peak area of ampicillin 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 10 times as long as the retention time of ampicillin.

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 ampicillin 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 ampicillin are not less than 5000 and not more than 1.5, respectively.

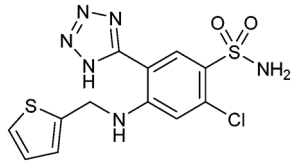
System repeatability: When the test is repeated 6 times

with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ampicillin is not more than 1.0%.

Add the following:

Azosemide

アゾセミド



$C_{12}H_{11}ClN_6O_2S_2$: 370.84
2-Chloro-5-(1*H*-tetrazol-5-yl)-4-[(thien-2-ylmethyl)amino]benzenesulfonamide
[*27589-33-9*]

Azosemide, when dried, contains not less than 99.0% and not more than 101.0% of azosemide ($C_{12}H_{11}ClN_6O_2S_2$).

Description Azosemide occurs as a white to yellow-white crystalline powder.

It is freely soluble in *N*,*N*-dimethylformamide, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

It is gradually colored to yellow by light.

Melting point: about 226°C (with decomposition).

Identification **(1)** Determine the absorption spectrum of a solution of Azosemide in dilute sodium hydroxide TS (3 in 500,000) 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 Azosemide, 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 1.0 g of Azosemide add 60 mL of dilute sodium hydroxide TS, dissolve by warming. After cooling, add 0.5 mL of nitric acid and filter. To 30 mL of the filtrate 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 with 0.45 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.032%).

(2) Heavy metal <*1.07*>—Proceed with 1.0 g of Azosemide 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) Primary aromatic amines—Dissolve 20 mg of Azosemide in 5 mL of *N*,*N*-dimethylformamide, add 12 mL of water, 1.0 mL of a solution of sodium nitrite (1 in 200) and 2.0 mL of diluted hydrochloric acid (1 in 10) under ice-cooling, shake, and allow to stand for 3 minutes. Add 1.0 mL of ammonium amidosulfate TS, shake thoroughly, allow to stand for 3 minutes, and add 1.0 mL of a solution of *N*-1-naphthylethylenediamine dihydrochloride (1 in 200). Shake this solution, and add *N*,*N*-dimethylformamide to make exactly 50 mL. Determine the absorbance of this solution at 540 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared in the same manner with 5 mL of *N*,*N*-dimethylformamide as the blank: the absorbance is not more than 0.15.

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 0.6 g of Azosemide, previously dried, dissolve in 50 mL of *N*,*N*-dimethylformamide, and titrate <*2.50*> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow to yellow-green (indicator: 10 drops of thymol blue-*N*,*N*-dimethylformamide TS). Perform a blank determination with a solution prepared by adding 15 mL of ethanol (95) to 50 mL of *N*,*N*-dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 37.08 mg of $C_{12}H_{11}ClN_6O_2S_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Add the following:

Azosemide Tablets

アゾセミド錠

Azosemide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of azosemide ($C_{12}H_{11}ClN_6O_2S_2$: 370.84).

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

Identification To a quantity of powdered Azosemide Tablets, equivalent to 60 mg of Azosemide, add dilute sodium hydroxide TS to make 100 mL, shake, and filter. To 1 mL of the filtrate add dilute sodium hydroxide TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 234 nm and 238 nm, between 272 nm and 276 nm and between 324 nm and 330 nm.

Purity Primary aromatic amines—To a quantity of powdered Azosemide Tablets, equivalent to 20 mg of Azosemide, add 5 mL of *N*,*N*-dimethylformamide, and allow to stand with occasional shaking. Add 12 mL of water, 1.0 mL

of a solution of sodium nitrite (1 in 200) and 2.0 mL of diluted hydrochloric acid (1 in 10) under ice-cooling, shake, and allow to stand for 3 minutes. Add 1.0 mL of ammonium amidosulfate TS, shake thoroughly, and allow to stand for 3 minutes. Add 1.0 mL of a solution of *N*-1-naphthylethylenediamine dihydrochloride (1 in 200), and shake. Add *N*,*N*-dimethylformamide to make exactly 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Determine the absorbance of the sample solution at 540 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared in the same manner with 5 mL of *N*,*N*-dimethylformamide as the blank: the absorbance is not more than 0.15.

Uniformity of dosage unit <*6.02*> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Azosemide Tablets add dilute sodium hydroxide TS to make exactly V mL so that each mL contains about 0.6 mg of azosemide ($C_{12}H_{11}ClN_6O_2S_2$), shake thoroughly, and centrifuge. Pipet 10 mL of the supernatant liquid, and add dilute sodium hydroxide TS to make exactly 100 mL. Pipet 10 mL of this solution, add dilute sodium hydroxide TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of azosemide for assay, previously dried at 105°C for 3 hours, and dissolve in dilute sodium hydroxide TS to make exactly 100 mL. Pipet 10 mL of this solution, and add dilute sodium hydroxide TS to make exactly 100 mL. Pipet 10 mL of this solution, add dilute sodium hydroxide TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 274 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>.

Amount (mg) of azosemide ($C_{12}H_{11}ClN_6O_2S_2$)
$= M_S \times A_T/A_S \times V/100$

M_S: Amount (mg) of azosemide for assay taken

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 60 minutes of 30-mg tablet and in 90 minutes of 60-mg tablet are not less than 70%, respectively.

Start the test with 1 tablet of Azosemide 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 or more of the filtrate, pipet V mL of the subsequent filtrate, and add the dissolution medium to make exactly V' mL so that each mL contains about 33 μg of azosemide ($C_{12}H_{11}ClN_6O_2S_2$). Pipet 8 mL of this solution, add 0.2 mol/L sodium hydroxide TS to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of azosemide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.2 mol/L sodium hydroxide TS to make exactly 100 mL. Pipet 5 mL of this solution, and add 0.2 mol/L sodium hydroxide TS to make exactly 50 mL. Pipet 15 mL of this solution, add the dissolution medium to make exactly 25 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 274 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using a solution prepared by adding 0.2 mol/L sodium hydroxide TS to 8 mL of the dissolution medium to make 20 mL as the blank.

Dissolution rate (%) with respect to the labeled amount of azosemide ($C_{12}H_{11}ClN_6O_2S_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 135$

M_S: Amount (mg) of azosemide for assay taken
C: Labeled amount (mg) of azosemide ($C_{12}H_{11}ClN_6O_2S_2$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 tablets of Azosemide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 60 mg of azosemide ($C_{12}H_{11}ClN_6O_2S_2$), add dilute sodium hydroxide TS to make exactly 100 mL, shake thoroughly, and filter. Discard the first 5 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 10 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of azosemide for assay, previously dried at 105°C for 3 hours, and dissolve in dilute sodium hydroxide TS to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, 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 azosemide to that of the internal standard.

Amount (mg) of azosemide ($C_{12}H_{11}ClN_6O_2S_2$)
$= M_S \times Q_T/Q_S$

M_S: Amount (mg) of azosemide for assay taken

Internal standard solution—A solution of propyl parahydroxybenzoate in the mobile phase (3 in 5000).

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: A mixture of 0.03 mol/L potassium dihydrogen phosphate solution, acetonitrile and methanol (55:27:18).

Flow rate: Adjust so that the retention time of azosemide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating con-

ditions, azosemide and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

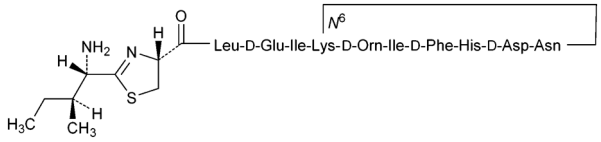
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of azosemide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Bacitracin

バシトラシン

Add the following next to Japanese name:



Bacitracin A
$C_{66}H_{103}N_{17}O_{16}S$: 1422.69
[*22601-59-8*]

Change the CAS No. as follows:

[*1405-87-4*, Bacitracin]

Benzylpenicillin Potassium

ベンジルペニシリンカリウム

Change the Purity (4) and Assay as follows:

Purity

(4) Related substances—Dissolve 40 mg of Benzylpenicillin Potassium 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 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than benzylpenicillin obtained from the sample solution is not larger than the peak area of benzylpenicillin from the standard solution, and the total area of the peaks other than benzylpenicillin from the sample solution is not larger than 3 times the peak area of benzylpenicillin from the standard solution.

Operating conditions—

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

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

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add water to make exactly 100 mL. Confirm that the peak area of benzylpenicillin 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 benzylpenicillin are not less than 4000 and 0.7 to 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 benzylpenicillin is not more than 1.0%.

Assay Weigh accurately amounts of Benzylpenicillin Potassium and Benzylpenicillin Potassium RS, equivalent to about 6×10^4 Units, dissolve each in water to make exactly 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of benzylpenicillin in each solution.

$$\text{Amount (unit) of benzylpenicillin potassium } (C_{16}H_{17}KN_2O_4S) = M_S \times A_T/A_S$$

M_S: Amount (unit) of benzylpenicillin potassium RS taken

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 (7 μm in particle diameter).

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

Mobile phase: A mixture of diammonium hydrogen phosphate solution (33 in 5000) and acetonitrile (19:6), adjusted to pH 8.0 with phosphoric acid.

Flow rate: Adjust so that the retention time of benzylpenicillin is about 7.5 minutes.

System suitability—

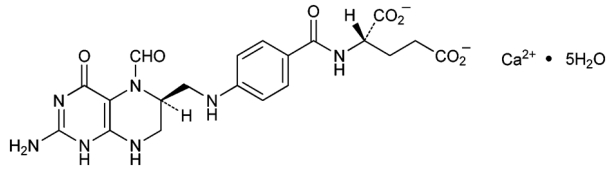
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benzylpenicillin are not less than 2000 and not more than 3.0, respectively.

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 benzylpenicillin is not more than 1.0%.

Add the following:

Calcium Levofolinate Hydrate

レボホリナートカルシウム水和物



$C_{20}H_{21}CaN_7O_7.5H_2O$: 601.58

Monocalcium *N*-[4-({[(6*S*)-2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl]methyl}amino)benzoyl]-L-glutamate pentahydrate

[*419573-16-3*]

Calcium Levofolinate Hydrate contains not less than 97.0% and not more than 102.0% of calcium levofolinate ($C_{20}H_{21}CaN_7O_7$: 511.50), calculated on the anhydrous and residual solvent-free basis.

Description Calcium Levofolinate Hydrate occurs as a white to light yellow crystalline powder.

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

It is hygroscopic.

Optical rotation $[\alpha]_D^{25}$: -10 – $-15°$ (0.25 g calculated on the anhydrous and residual solvent-free basis, 0.2 mol/L tris buffer solution (pH 8.1), 25 mL, 100 mm).

Identification **(1)** Determine the absorption spectrum of a solution of Calcium Levofolinate Hydrate (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 Calcium Levofolinate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Calcium Levofolinate Hydrate (1 in 200) responds to Qualitative Tests ⟨*1.09*⟩ (2) and (3) for calcium salt.

pH ⟨*2.54*⟩ To 0.4 g of Calcium Levofolinate Hydrate add 50 mL of freshly boiled and cooled water, and warm to 40°C, if necessary, to dissolve: the pH of the solution is between 7.0 and 8.5.

Purity **(1)** Clarity and color of solution—To 0.4 g of Calcium Levofolinate Hydrate add 50 mL of water, and warm to 40°C, if necessary, to dissolve: the solution is clear, and its absorbance at 420 nm determined as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ is not more than 0.25.

(2) Chloride—To 0.300 g of Calcium Levofolinate Hydrate add 50 mL of water, warm to 40°C, if necessary, to dissolve, add 10 mL of 2 mol/L nitric acid TS, and titrate ⟨*2.50*⟩ with 0.005 mol/L silver nitrate VS (potentiometric titration) (not more than 0.5%).

Each mL of 0.005 mol/L silver nitrate VS
= 0.177 mg of Cl

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

(4) Platinum—Being specified separately when the drug is granted approval based on the Law (not more than 5 ppm).

(5) Related substances—Dissolve 20 mg of Calcium Levofolinate Hydrate in 25 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 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than levofolinate obtained from the sample solution is not larger than the peak area of levofolinate from the standard solution, and the total area of the peaks other than levofolinate from the sample solution is not larger than 5 times the peak area of levofolinate 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 levofolinate, beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add water to make exactly 25 mL. Confirm that the peak area of levofolinate obtained with 20 μL of this solution is equivalent to 14 to 26% 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 levofolinate are not less than 1500 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 levofolinate is not more than 2.0%.

(6) Diastereomer—Dissolve 50 mg of Calcium Levofolinate Hydrate in 100 mL of water, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peak of the diastereomer, having the relative

retention time of about 2.0 to levofolinate, is not more than 0.3%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 286 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with human albumin chemically bonded 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 sodium dihydrogen phosphate dihydrate in 870 mL of water, adjust to pH 4.9 with sodium hydroxide TS or phosphoric acid, and add 110 mL of 2-propanol and 20 mL of acetonitrile.

Flow rate: Adjust so that the retention time of levofolinate is about 16 minutes.

System suitability—

Test for required detectability: Dissolve 10 mg of Calcium Folinate RS in water to make 50 mL. To 1 mL of this solution add the sample solution to make 20 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 the diastereomer obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, levofolinate and the diastereomer 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of the diastereomer is not more than 2.0%.

Water ⟨*2.48*⟩ 12.0 – 17.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 10 mg each of Calcium Levofolinate Hydrate and Calcium Folinate RS (separately determine the water ⟨*2.48*⟩ in the same manner as Calcium Folinate), and dissolve each 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 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak area, A_T, of levofolinate with the sample solution, and the peak area, A_S, of folinate with the standard solution.

Amount (mg) of calcium levofolinate ($C_{20}H_{21}CaN_7O_7$) $= M_S \times A_T/A_S$

M_S: Amount (mg) of Calcium Folinate RS taken, 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 45°C.

Mobile phase: Adjust the pH of a mixture of diluted 0.05 mol/L disodium hydrogen phosphate TS (4 in 25), methanol and tetrabutylammonium hydroxide TS (385:110:4) to 7.5 with phosphoric acid.

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

System suitability—

System performance: Dissolve 10 mg of folic acid in 50 mL of the mobile phase. To 5 mL of this solution add 5 mL of the standard solution. When the procedure is run with 20 μL of this solution under the above operating conditions, folinate and folic acid are eluted in this order with the resolution between these peaks being not less than 10.

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

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefixime Hydrate

セフィキシム水和物

Change the Identification (3), Purity and Assay as follows:

Identification

(3) Dissolve 50 mg of Cefixime Hydrate in 0.5 mL of a mixture of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy and heavy water for nuclear magnetic resonance spectroscopy (4:1). Determine the ^1H spectrum of this solution, as directed under Nuclear Magnetic Resonance Spectroscopy ⟨*2.21*⟩, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a singlet signal A at around δ 4.7 ppm, and a multiplet signal B between δ 6.5 ppm and δ 7.4 ppm. The ratio of integrated intensity of these signals, A:B, is about 1:1.

Purity Dissolve 0.1 g of Cefixime Hydrate in 100 mL of 0.1 mol/L phosphate buffer solution (pH 7.0), 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 peak other than cefixime is not more than

1.0%, and the total amount of the peaks other than cefixime is not more than 2.5%.

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 cefixime, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add 0.1 mol/L phosphate buffer solution (pH 7.0) to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 10 mL. Confirm that the peak area of cefixime obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefixime is not more than 2.0%.

Assay Weigh accurately an amount of Cefixime Hydrate, equivalent to about 0.1 g (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 100 mL. Pipet 10 mL of this solution, add 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefixime RS, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution (pH 7.0) to make exactly 20 mL. Pipet 10 mL of this solution, add 0.1 mol/L phosphate buffer solution (pH 7.0) 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 cefixime in each solution.

$$\text{Amount [}\mu\text{g (potency)] of cefixime } (C_{16}H_{15}N_5O_7S_2) = M_S \times A_T/A_S \times 5000$$

M_S: Amount [mg (potency)] of Cefixime RS taken

Operating conditions—

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

Column: A stainless steel column 4 mm in inside diameter and 125 mm 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: To 25 mL of a solution of tetrabutylammonium hydroxide TS (10 in 13) add water to make 1000 mL, and adjust to pH 6.5 with diluted phosphoric acid (1 in 10). To 300 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust so that the retention time of cefixime 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 cefixime are not less than 4000 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 peak area of cefixime is not more than 2.0%.

Add the following:

Cefoperazone Sodium for Injection

注射用セフォペラゾンナトリウム

Cefoperazone Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled potency of cefoperazone ($C_{25}H_{27}N_9O_8S_2$: 645.67).

Method of preparation Prepare as directed under Injections, with Cefoperazone Sodium.

Description Cefoperazone Sodium for Injection occurs as a white to yellowish white, crystalline powder or masses.

Identification Determine the absorption spectrum of a solution of Cefoperazone Sodium for Injection (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 226 nm and 230 nm, and between 263 nm and 267 nm.

pH <*2.54*> The pH of a solution prepared by dissolving an amount of Cefoperazone Sodium for Injection, equivalent to 1.0 g (potency) of Cefoperazone Sodium, in 4 mL of water is between 4.5 and 6.5.

Purity **(1)** Clarity and color of solution—Dissolve an amount of Cefoperazone Sodium for Injection, equivalent to 1.0 g (potency) 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 0.22.

(2) Related substances—Dissolve an amount of Cefoperazone Sodium for Injection, equivalent to 0.1 g (potency) of Cefoperazone Sodium, 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 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each

peak area by the automatic integration method: the peak area of related substance I, having the relative retention time of about 0.8 to cefoperazone, obtained from the sample solution is not larger than 2.5 times the peak area of cefoperazone from the standard solution, the peak area of related substance II, having the relative retention time of about 1.7, from the sample solution are not larger than 3/4 times the peak area of cefoperazone from the standard solution. Furthermore, the total area of the peaks other than cefoperazone from the sample solution is not larger than 3.5 times the peak area of cefoperazone from the standard solution. For the peak areas of the related substances I and II, multiply their relative response factors, 0.90 and 0.75, respectively.

Operating conditions—

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

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

System suitability—

Proceed as directed in the system suitability in the Purity (4) under Cefoperazone Sodium.

Water ⟨*2.48*⟩ Not more than 1.0% (3 g, volumetric titration, direct titration).

Bacterial endotoxins ⟨*4.01*⟩ Less than 0.05 EU/mg (potency).

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 Cefoperazone Sodium for Injection. Weigh accurately a portion of the content, equivalent to about 0.1 g (potency) of Cefoperazone Sodium, 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. Then, proceed as directed in the Assay under Cefoperazone Sodium.

Amount [mg (potency)] of cefoperazone ($C_{25}H_{27}N_9O_8S_2$)
$= M_S \times Q_T/Q_S \times 5$

M_S: Amount [mg (potency)] of Cefoperazone RS taken

Internal standard solution—A solution of acetanilide in a mixture of water and acetonitrile (43:7) (3 in 8000).

Containers and storage Containers—Hermetic containers.

Storage—In a cold place.

Shelf life 24 months after preparation.

Ceftizoxime Sodium

セフチゾキシムナトリウム

Change the Purity (1) and Assay as follows:

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ceftizoxime Sodium in 10 mL of water: the solution is clear. Perform the test with this solution as directed under Method for Color Matching ⟨*2.65*⟩: the color is not more colored than Matching Fluid M.

Assay Weigh accurately an amount of Ceftizoxime Sodium and Ceftizoxime RS, equivalent to about 25 mg (potency), dissolve each in 0.1 mol/L phosphate buffer solution (pH 7.0), add exactly 20 mL of the internal standard solution, then add 0.1 mol/L phosphate buffer solution (pH 7.0) to make 50 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 ceftizoxime to that of the internal standard.

Amount [μg (potency)] of ceftizoxime ($C_{13}H_{13}N_5O_5S_2$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Ceftizoxime RS taken

Internal standard solution—A solution of 3-hydroxybenzoic acid in 0.1 mol/L phosphate buffer solution (pH 7.0) (3 in 400).

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 (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2.31 g of disodium hydrogen phosphate dodecahydrate and 1.42 g of citric acid monohydrate in 1000 mL of water, and adjust to pH 3.6 with diluted phosphoric acid (1 in 10) or dilute sodium hydroxide TS. To 450 mL of this solution add 50 mL of acetonitrile.

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

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, ceftizoxime and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.0 and the symmetry factor of each peak is not more 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 ceftizoxime to that of the internal stand-

ard is not more than 1.0%.

Cellacefate

セラセフェート

Change the Identification as follows:

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 for Identification: both spectra exhibit similar intensities of absorption at the same wave numbers.

Chloramphenicol Sodium Succinate

クロラムフェニコールコハク酸エステルナトリウム

Change the Purity as follows:

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Chloramphenicol Sodium Succinate in 10 mL of water: the solution is clear, and the absorbance at 420 nm of the solution determined as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ is not more than 0.30.

(2) Heavy metals ⟨*1.07*⟩—Proceed with 1.0 g of Chloramphenicol Sodium Succinate 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).

Add the following:

Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution

クロラムフェニコール·コリスチンメタンスルホン酸ナトリウム点眼液

Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution is an aqueous ophthalmic preparation.

It contains not less than 90.0% and not more than 120.0% of the labeled potency of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13) and labeled Units of colistin A ($C_{53}H_{100}N_{16}O_{13}$: 1169.46).

Method of preparation Prepare as directed under Ophthalmic Liquids and Solutions, with Chloramphenicol and Colistin Sodium Methanesulfonate.

Description Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution is a clear, colorless to pale yellow liquid.

Identification **(1)** To a volume of Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution, equivalent to about 2.5 mg (potency) of Chloramphenicol, and add water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using water as a blank: it exhibits a maximum between 276 nm and 280 nm.

(2) To a volume of Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution, equivalent to about 5×10^5 Units of Colistin Sodium Methanesulfonate add 0.5 mL of ninhydrin TS, boil for 1 minute, and cool: a blue color develops.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

pH ⟨*2.54*⟩ 6.0 – 8.0

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 Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics ⟨*4.02*⟩ according to the following conditions.

(1) Chloramphenicol

(i) Test organism—*Kocuria rhizophila* ATCC 9341

(ii) Agar media for base layer and seed layer—Use the medium ii in 3) under (1) Agar media for seed and base layer.

(iii) Agar medium for transferring test organisms—Use the medium i in 2) under (2) Agar media for transferring test organisms.

(iv) Liquid medium for suspending test organisms—Use the medium (2) Liquid media for suspending test organisms of 3.2. Culture media.

(v) Standard solutions—Weigh accurately an amount of Chloramphenicol RS, equivalent to about 20 mg (potency), dissolve in 2 mL of ethanol (95), add phosphate buffer solution (pH 6.0) to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 15°C or below, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 100 μg (potency) and 25 μg (potency), and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(vi) Sample solutions—Weigh accurately an amount of Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution, equivalent to about 10 mg (potency) of Chloramphenicol, add phosphate buffer solution (pH 6.0) to make exactly 100 mL, and filter, if necessary. Take exactly a suitable amount of this solution, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 100 μg (potency) and 25 μg (potency), and use these solutions as the high concentration sample solution

and the low concentration sample solution, respectively.

(2) Colistin Sodium Methanesulfonate

(i) Test organism—*Bordetella bronchiseptica* ATCC 4617

(ii) Agar medium for base layer—

Casein peptone 17.0 g
Sodium chloride 5.0 g
Glucose 2.5 g
Soybean peptone 3.0 g
Dipotassium hydrogen phosphate 2.0 g
Agar 20.0 g
Water 1000 mL

Mix all the ingredients, then add a suitable amount of sodium hydroxide TS so that the pH of the medium will be 7.2 to 7.3 after sterilization, and sterile.

(iii) Agar medium for seed layer—

Casein peptone 17.0 g
Glucose 2.5 g
Soybean peptone 3.0 g
Sodium chloride 5.0 g
Polysorbate 80 10.0 g
Dipotassium hydrogen phosphate 2.5 g
Agar 12.0 g
Water 1000 mL

Mix all the ingredients, then add a suitable amount of sodium hydroxide TS so that the pH of the medium will be 7.2 to 7.3 after sterilization, and sterile.

(iv) Agar medium for transferring test organisms—Use the medium i in 2) under (2) Agar media for transferring test organisms.

(v) Preparation of test organism and seeded agar layer—Cultivate the test organism on the slant of the agar medium for transferring test organism at 32 to 37°C for 16 to 24 hours. Subcultures at least three times. Cultivate the grown organism on the slant of the agar medium for transferring test organism at 32 to 37°C for 16 to 24 hours, add a suitable amount of water to the grown organism, and suspend. Adjust the suspension so that the transmittance at 660 nm is 60% as directed under Ultraviolet-visible Spectrophotometry <*2.24*> using a spectrophotometer or a photoelectric photometer, and use this suspension as the test organism suspension. Keep the test organism suspension at 15°C or below, and use within 3 days. Before use, dissolve 0.13 mL of the test organism suspension, add it to 100 mL of agar medium for seed previously cooled at 48°C, mix thoroughly, and use this as the seeded agar layer.

(vi) Standard solutions—Weigh accurately an amount of Colistin Sodium Methanesulfonate RS, equivalent to about 1×10^6 Units, dissolve in phosphate buffer solution (pH 6.0) to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 10°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution (pH 6.0) to make solutions so that each mL contains 1000 Units and 250 Units, and use these solutions as the high concentration standard solution and the low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately an amount of Chloramphenicol and Colistin Sodium Methanesulfonate Ophthalmic Solution, equivalent to about 1×10^5 Units of Colistin Sodium Methanesulfonate, add phosphate buffer solution (pH 6.0) to make a solution so that each mL contains 1000 Units, and use this solution as the high concentration sample solution. Pipet 5 mL of the high concentration sample solution, add phosphate buffer solution (pH 6.0) to make a solution so that each mL contains 250 Units, and use this solution as the low concentration sample solution.

Containers and storage Containers—Tight containers.

Storage—At a temperature between 2°C and 8°C.

Clarithromycin

クラリスロマイシン

Delete the Identification (4) and change the Optical rotation, Purity, and Assay as follows:

Optical rotation <*2.49*> $[\alpha]_D^{20}$: −96 – −106° (0.25 g calculated on the anhydrous basis, acetone, 25 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 2.0 g of Clarithromycin 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—Weigh accurately about 0.1 g of Clarithromycin, dissolve in the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Clarithromycin RS, dissolve in 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, and determine the each peak area by the automatic integration method: the amount of each related substance calculated on the anhydrous basis is not more than 2.0%, and the total amount of them is not more than 5.0%. For these calculations, exclude any peak with an area of less than 0.05%.

Amount (%) of each related substance calculated on the anhydrous basis
$= M_S/M_T \times A_T/A_S \times 100$

Total amount (%) of the related substances calculated on the anhydrous basis
$= M_S/M_T \times \Sigma A_T/A_S \times 100$

M_S: Amount (mg) of Clarithromycin RS taken
M_T: Amount (mg) of Clarithromycin taken, calculated on the anhydrous basis
A_S: Peak area of clarithromycin obtained with the standard solution
A_T: Peak area of each related substance obtained with the sample solution
ΣA_T: Total area of the peaks other than clarithromycin 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 5 times as long as the retention time of the main peak, beginning from 2 minutes after injection of the sample solution.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, add the mobile phase to make exactly 10 mL. Pipet 2.5 mL of this solution, add the mobile phase to make exactly 10 mL. Confirm that the peak area of clarithromycin obtained with 10 μL of this solution is equivalent to 0.25 – 0.75% of that with 10 μL of the standard solution.

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 clarithromycin are not less than 2500 and not more than 2.5, 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 clarithromycin is not more than 3.0%.

Assay Weigh accurately an amount of Clarithromycin and Clarithromycin RS, equivalent to about 50 mg (potency), and dissolve each in the mobile phase to make exactly 10 mL. Pipet 2 mL each of these solutions, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of clarithromycin to that of the internal standard.

Amount [μg (potency)] of clarithromycin ($C_{38}H_{69}NO_{13}$)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Clarithromycin RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (1 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 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 50°C.

Mobile phase: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 3) and acetonitrile (13:7).

Flow rate: Adjust so that the retention time of clarithromycin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, clarithromycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clarithromycin to that of the internal standard is not more than 2.0%.

Add the following:

Clomipramine Hydrochloride Tablets

クロミプラミン塩酸塩錠

Clomipramine Hydrochloride Tablets contain not less than 92.0% and not more than 108.0% of the labeled amount of clomipramine hydrochloride ($C_{19}H_{23}ClN_2$.HCl: 351.31).

Method of preparation Prepare as directed under Tablets, with Clomipramine Hydrochloride.

Identification To a portion of powdered Clomipramine Hydrochloride Tablets, equivalent to 50 mg of Clomipramine Hydrochloride, add a suitable amount of 0.1 mol/L hydrochloric acid TS, shake thoroughly, and add 0.1 mol/L hydrochloric acid TS to make 250 mL. Centrifuge this solution, and to 10 mL of the supernatant liquid 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 250 nm and 254 nm.

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

To 1 tablet of Clomipramine Hydrochloride Tablets add $V/5$ mL of a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1), sonicate to disintegrate the tablet, and shake thoroughly for 30 minutes. To this solution add $3V/5$ mL of methanol, shake for 15 minutes, and add methanol to make exactly V mL so that each mL contains about 0.1 mg of clomipramine hydrochloride ($C_{19}H_{23}ClN_2$.HCl). Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of clomipramine hydrochloride ($C_{19}H_{23}ClN_2$.HCl)
$= M_S \times A_T/A_S \times V/250$

M_S: Amount (mg) of clomipramine hydrochloride for assay taken

Dissolution <*6.10*> When the test is performed at 50 revo-

lutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 45 minutes of 10-mg tablet and in 90 minutes of 25-mg tablet are not less than 80%, respectively.

Start the test with 1 tablet of Clomipramine 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 or more 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 clomipramine hydrochloride ($C_{19}H_{23}ClN_2$.HCl), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of clomipramine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 252 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of clomipramine hydrochloride ($C_{19}H_{23}ClN_2$.HCl)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 36$

M_S: Amount (mg) of clomipramine hydrochloride for assay taken
C: Labeled amount (mg) of clomipramine hydrochloride ($C_{19}H_{23}ClN_2$.HCl) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Clomipramine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of clomipramine hydrochloride ($C_{19}H_{23}ClN_2$.HCl), add 50 mL of a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1), sonicate, and shake thoroughly for 30 minutes. To this solution add 150 mL of methanol, shake for 15 minutes, and add methanol to make exactly 250 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of clomipramine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in 50 mL of a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1), add methanol 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 clomipramine in each solution.

Amount (mg) of clomipramine hydrochloride ($C_{19}H_{23}ClN_2$.HCl)
$= M_S \times A_T/A_S$

M_S: Amount (mg) of clomipramine hydrochloride for assay taken

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 (10 μm in particle diameter).

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

Mobile phase: Dissolve 2 g of sodium 1-octanesulfonate in 300 mL of water, and add 450 mL of methanol, 250 mL of acetonitrile and 1 mL of 0.5 mol/L sulfuric acid TS.

Flow rate: Adjust so that the retention time of clomipramine is about 13 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 clomipramine 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 clomipramine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Clotiazepam Tablets

クロチアゼパム錠

Clotiazepam Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of clotiazepam ($C_{16}H_{15}ClN_2OS$: 318.82).

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

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 260 nm and 264 nm.

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

To 1 tablet of Clotiazepam Tablets add 35 mL of 0.1 mol/L hydrochloric acid TS, stir until the tablet is completely disintegrated, stir for a further 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly V' mL so that each mL contains about 10 μg of clotiazepam ($C_{16}H_{15}ClN_2OS$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of clotiazepam ($C_{16}H_{15}ClN_2OS$)
$= M_S \times A_T/A_S \times V'/V \times 1/50$

M_S: Amount (mg) of clotiazepam for assay taken

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 1st fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of Clotiazepam Tablets is not less than 80%.

Start the test with 1 tablet of Clotiazepam 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 or more 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 clotiazepam ($C_{16}H_{15}ClN_2OS$), and use this solution as the sample solution. Separately, weigh accurately about 28 mg of clotiazepam for assay, previously dried at 80°C for 3 hours, and dissolve in ethanol (95) to make exactly 25 mL. Pipet 5 mL of this solution, and add the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of clotiazepam ($C_{16}H_{15}ClN_2OS$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 18$

M_S: Amount (mg) of clotiazepam for assay taken
C: Labeled amount (mg) of clotiazepam ($C_{16}H_{15}ClN_2OS$) in 1 tablet

Assay To 20 Clotiazepam Tablets add 350 mL of 0.1 mol/L hydrochloric acid TS, stir until the tablets are completely disintegrated, stir for a further 10 minutes, and add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly V' mL so that each mL contains about 10 μg of clotiazepam ($C_{16}H_{15}ClN_2OS$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of clotiazepam for assay, previously dried at 80°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 2 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, of the sample solution and standard solution at 261 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of clotiazepam ($C_{16}H_{15}ClN_2OS$) in 1 tablet
$= M_S \times A_T/A_S \times V'/V \times 1/100$

M_S: Amount (mg) of clotiazepam for assay taken

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cloxacillin Sodium Hydrate

クロキサシリンナトリウム水和物

Change the Purity (4) as follows:

Purity

(4) Related substances—Dissolve 50 mg of Cloxacillin Sodium 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 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 other than cloxacillin obtained from the sample solution is not larger than the peak area of cloxacillin from the standard solution, and the total area of the peaks other than chloxacillin from the sample solution is not larger than 3 times the peak area of cloxacillin 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 cloxacillin.

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 cloxacillin 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 cloxacillin are not less than 5000 and not more than 1.3, respectively.

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

Colistin Sodium Methanesulfonate

コリスチンメタンスルホン酸ナトリウム

Change the origin/limits of content as follows:

Colistin Sodium Methanesulfonate is the sodium salt of colistin derivatives.

It is a mixture of colistin A sodium methanesulfonate and colistin B sodium methanesulfonate.

It, when dried, contains not less than 11,500 Units and not more than 15,500 Units per mg. The unit of

Colistin Sodium Methanesulfonate is expressed as mass of colistin A (R = 6-methyloctanic acid, R′ = H; $C_{53}H_{100}N_{16}O_{13}$: 1169.46).

Demethylchlortetracycline Hydrochloride

デメチルクロルテトラサイクリン塩酸塩

Change the Purity as follows:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Demethylchlortetracycline Hydrochloride 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 25 mg of Demethylchlortetracycline Hydrochloride in 50 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak other than demethylchlortetracycline obtained from the sample solution is not larger than 1.2 times the peak area of demethylchlortetracycline from the standard solution, and the total area of the peaks other than demethylchlortetracycline from the sample solution is not larger than 2 times the peak area of demethylchlortetracycline 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 demethylchlortetracycline, beginning after the solvent peak.

System suitability—

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

Test for required detectability: Measure 10 mL of the standard solution, add 0.01 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of demethylchlortetracycline 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 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 demethylchlortetracycline is not more than 1.0%.

Dextran 40

デキストラン**40**

Add the following next to the Origin/limits of content:

Manufacture Dextran 40 is produced by the manufacturing method to eliminate or minimize impurities having a possible antigenicity. The manufacturing method is verified to meet the antigenicity test.

Antigenicity Dissolve 10.0 g of Dextran 40 in isotonic sodium chloride solution to make 100 mL, sterilize, and use this solution as the sample solution. Inject 1.0 mL of the sample solution on 3 occasions at intervals of 2 days intraperitoneally to each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Inject 0.10 mL of horse serum intraperitoneally to each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously to each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit no signs mentioned above.

All the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

Delete the Antigenicity:

Delete the following Monographs:

Diclofenamide

ジクロフェナミド

Diclofenamide Tablets

ジクロフェナミド錠

Digitoxin

ジギトキシン

Digitoxin Tablets

ジギトキシン錠

Digoxin

ジゴキシン

Change the Purity (2) as follows:

Purity

(2) Related substances—Dissolve 25.0 mg of Digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add 10 mL of water and dilute ethanol to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve exactly 5.0 mg of Gitoxin RS for Purity, previously dried under reduced pressure at 105°C for 1 hour, in a mixture of acetonitrile and water (7:3) to make exactly 200 mL. Pipet 2 mL of this solution, add dilute ethanol 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 gitoxin: A_T is not larger than A_S, and the total area of the peaks other than digoxin and gitoxin from the sample solution, obtained by the area percentage method, is not more than 3%.

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 digoxin, beginning after the solvent peak.

System suitability—

Test for required detectability: Dissolve 25 mg of Digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add dilute ethanol to make exactly 100 mL. Pipet 5 mL of this solution, and add dilute ethanol to make exactly 100 mL. Confirm that the peak area of digoxin obtained with 10 μL of this solution is equivalent to 0.07 to 0.13% of that with 10 μL of the solution for system suitability test.

System performance: Dissolve 25 mg of Digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make 100 mL. To 10 mL of this solution add 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, digoxin and propyl parahydroxybenzoate 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 solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of digoxin is not more than 2.5%.

Doxorubicin Hydrochloride

ドキソルビシン塩酸塩

Change the Purity (2) and Assay as follows:

Purity

(2) Related substances—Dissolve 25 mg of Doxorubicin Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than doxorubicin obtained from the sample solution is not larger than 1/4 times the peak area of doxorubicin from the standard solution, and the total area of the peaks other than doxorubicin from the sample solution is not larger than the peak area of doxorubicin 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 doxorubicin.

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 doxorubicin 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: Dissolve 5 mg of Doxorubicin Hydrochloride in 20 mL of water, add 1.5 mL of phosphoric acid, and allow to stand at room temperature for 30 minutes. Adjust the pH of this solution to 2.5 with 2 mol/L sodium hydroxide TS. When the procedure is run with 20 μL of this solution under the above operating conditions, doxorubicinone, having the relative retention time of about 0.6 to doxorubicin, and doxorubicin are eluted in this order with the resolution between these peaks being not less than 5.

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

Assay Weigh accurately amounts of Doxorubicin Hydrochloride and Doxorubicin Hydrochloride RS, equivalent to about 10 mg (potency), add exactly 5 mL of the internal standard solution to each, dissolve each in the mobile phase 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 doxorubicin to that of the internal standard.

Amount [μg (potency)] of doxorubicin hydrochloride ($C_{27}H_{29}NO_{11}$.HCl)
$= M_S \times Q_T/Q_S \times 1000$

M_S: Amount [mg (potency)] of Doxorubicin Hydrochloride RS taken

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (1 in 1000).

Operating conditions—

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

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

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

Mobile phase: Dissolve 3 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (7 in 5000), and add 1000 mL of acetonitrile.

Flow rate: Adjust so that the retention time of doxorubicin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, doxorubicin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5, and the symmetry factor of the peak of doxorubicin is 0.8 to 1.2.

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 doxorubicin to that of the internal standard is not more than 1.0%.

Doxycycline Hydrochloride Hydrate

ドキシサイクリン塩酸塩水和物

Change the Identification and Purity (2) as follows:

Identification **(1)** Determine the absorption spectrum of a solution of Doxycycline Hydrochloride Hydrate in 0.01 mol/L hydrochloric acid-methanol TS (1 in 74,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Doxycycline 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 Doxycycline 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 Doxycycline Hydrochloride RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 10 mg of Doxycycline Hydrochloride Hydrate in 10 mL of water, and add silver nitrate TS: a white turbidity is produced.

Purity

(2) Related substance—Dissolve 20 mg of Doxycycline Hydrochloride Hydrate in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 6-epidoxycycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the 6-epidoxycycline hydrochloride stock solution. Separately, dissolve 20 mg of metacycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the metacycline hydrochloride stock solution. Pipet 2 mL each of the 6-epidoxycycline hydrochloride stock solution and the metacycline hydrochloride stock solution, add 0.01 mol/L hydrochloric acid TS 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 areas of metacycline and 6-epidoxycycline obtained from the sample solution are not larger than the peak areas of them from the standard solution, respectively, and the areas of the two peaks, appeared between the solvent peak and metacycline and behind of doxycycline, from the sample solution are not larger than 1/4 times the peak area of 6-epidoxycycline from the standard solution, and the total area of the peaks other than doxycycline from the sample solution is not larger than 1.5 times the peak area of 6-epidoxycycline from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: Take 125 mL of 0.2 mol/L potassium dihydrogen phosphate TS and 117 mL of 0.2 mol/L sodium hydroxide TS, and add water to make 500 mL. To 400 mL of this solution add 50 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 25), 60 g of *t*-butyl alcohol and 200 mL of water, adjust to pH 8.0 with 2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Flow rate: Adjust so that the retention time of doxycycline is about 19 minutes.

Time span of measurement: About 2.4 times as long as the retention time of doxycycline, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak areas of 6-epidoxycycline and metacycline obtained with 20 μL of this solution are equivalent to 3.5 to 6.5% of them with 20 μL of the standard solution, respectively.

System performance: To 8 mL of the sample solution, 3 mL of the 6-epidoxycycline hydrochloride stock solution and 2 mL of the metacycline hydrochloride stock solution add 0.01 mol/L hydrochloric acid TS to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, metacycline, 6-epidoxycycline and doxycycline are eluted in this order with the resolutions between the peaks, metacycline and 6-epidoxycycline, and 6-epidoxycycline and doxycycline, being not less than 1.3 and not less than 2.0, respectively, and the symmetry factor of the peak of doxycycline is not more than 1.3.

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 areas of metacycline and 6-epidoxycycline are not more than 3.0% and not more than 2.0%, respectively.

Edaravone Injection

エダラボン注射液

Change the Purity, Assay, and Containers and storage as follows:

Purity Related substance—(i) Perform the following test 1). For preparations to which the test 2) can be applied, the test 2) may be performed instead of 1).

1) To a suitable amount of Edaravone Injection add the mobile phase so that each mL contains 0.3 mg of edaravone ($C_{10}H_{10}N_2O$), 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 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 other than edaravone obtained from the sample solution is not larger than 2 times the peak area of edaravone obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the 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 50 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 50 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%.

2) 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 obtained from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the 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) Perform the following test 1). For preparations to which the test 2) can be applied, the test 2) may be performed instead of 1).

1) To a suitable amount of Edaravone Injection add the mobile phase so that each mL contains 0.3 mg of edaravone ($C_{10}H_{10}N_2O$), 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 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, 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 obtained from the standard solution, the area of the peak, having the relative

retention time of about 0.4 to edaravone obtained from the sample solution, 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 from the sample solution 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 1).

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

Flow rate: Adjust 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 50 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 50 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%.

2) 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 obtained from the standard solution, the area of the peak, having the relative retention time of about 0.4 to edaravone obtained from the sample solution , 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 from the sample solution 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 1).

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

Flow rate: Adjust 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%.

Assay Perform the following test 1). For preparations to which the test 2) can be applied, the test 2) may be performed instead of 1).

1) To exactly V mL of Edaravone Injection add methanol to make exactly V' mL so that each mL contains about 0.3 mg of edaravone ($C_{10}H_{10}N_2O$). Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, then add methanol to make 20 ml, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of edaravone for assay, previously dried in vacuum using phosphorus (V) oxide as a desiccant for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, then add methanol to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of edaravone to that of the internal standard.

$$\text{Amount (mg) of edaravone } (C_{10}H_{10}N_2O) = M_S \times Q_T/Q_S \times V'/V \times 1/100$$

M_S: Amount (mg) of edaravone for assay taken

Internal standard solution—A solution of ethyl aminobenzoate in methanol (1 in 2500).

Operating conditions—

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

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

Column temperature: A constant temperature of about 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 so that the retention time of edaravone is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, 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 10 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 stand-

ard is not more than 1.0%.

2) 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 using phosphorus (V) oxide as a desiccant for 3 hours, 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 taken

Internal standard solution—A solution of ethyl aminobenzoate in methanol (1 in 500).

Operating conditions—

Proceed as directed in the operating conditions in the Assay 1).

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.

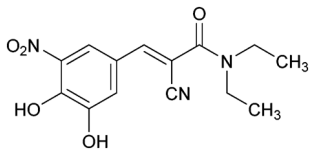
System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of edaravone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Add the following:

Entacapone

エンタカポン



$C_{14}H_{15}N_3O_5$: 305.29

(2*E*)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-*N*,*N*-diethylprop-2-enamide

[*130929-57-6*]

Entacapone contains not less than 98.0% and not more than 102.0% of entacapone ($C_{14}H_{15}N_3O_5$), calculated on the dried basis.

Description Entacapone occurs as a yellow to greenish yellow crystalline powder.

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

It shows crystal polymorphism.

Identification **(1)** Dissolve 35 mg of Entacapone in 200 mL of methanol. To 7 mL of this solution 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*>, using a solution prepared by adding 0.1 mol/L hydrochloric acid TS to 7 mL of methanol to make 100 mL as the blank, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Entacapone 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 Entacapone 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 Entacapone RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals—Dissolve 1.0 g of Entacapone in 20 mL of a mixture of methanol and *N*,*N*-dimethylformamide (3:1), and use this solution as the sample solution. Separately, weigh exactly 0.400 g of lead (II) nitrate, dissolve in water to make exactly 250 mL. Before use, dilute this solution with water to make exactly 10 times the initial volume, then dilute this solution with water to make exactly 10 times the initial volume. Pipet 1 mL of this solution, add a mixture of methanol and *N*,*N*-dimethylformamide (3:1) to make exactly 20 mL, and use this solution as the standard solution. To each of the sample solution and standard solution add 2 mL of acetate buffer solution (pH 3.5), mix, add 1.2 mL of thioacetamide TS, and mix immediately. Allow them to stand for 2 minutes, filter separately all the amount of each solution through a membrane filter with a pore size of 0.45 μm, wash the membrane filters with not less than 20 mL of methanol, and compare the colors on the membrane filters: the color obtained from the sample solution is not darker than that obtained from the standard solution (not more than 10 ppm).

(2) Halide—Being specified separately when the drug is granted approval based on the Law.

(3) Related substances—Dissolve 50 mg of Entacapone in 50 mL of a mixture of methanol and tetrahydrofuran (7:3), and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add a mixture of methanol and tetrahydrofuran (7:3) to make exactly 50 mL. Pipet 5 mL of this solution, and add a mixture of methanol and tetrahydrofuran (7:3) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of methanol and tetrahydrofuran (7:3) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each

of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the peak area of the related substance A, having the relative retention time of about 0.8 to entacapone, obtained from the sample solution is not larger than 1.5 times the peak area of entacapone obtained from the standard solution, the area of the peak other than entacapone and the peak mentioned above from the sample solution is not larger than the peak area of entacapone from the standard solution, and the total area of the peaks other than entacapone and the related substance A, having the relative retention time of about 0.8 to entacapone, from the sample solution is not larger than 2 times the peak area of entacapone from the standard solution. For the areas of the peaks of related substances B and C, having the relative retention times of about 0.6 and about 1.4 to entacapone, multiply their relative response factors, 1.7 and 2.5, 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 2.5 times as long as the retention time of entacapone, beginning after the solvent peak.

System suitability—

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

Test for required detectability: Pipet 5 mL of the standard solution, add a mixture of methanol and tetrahydrofuran (7:3) to make exactly 10 mL. Confirm that the peak area of entacapone obtained with 10 μL of this solution is equivalent to 35 to 65% of that obtained with 10 μL of the standard solution.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of entacapone is not more than 5%.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

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

Assay Weigh accurately about 50 mg each of Entacapone and Entacapone RS (separately determine the loss on drying <2.41> under the same conditions as Entacapone), dissolve each in a mixture of methanol and tetrahydrofuran (7:3) to make exactly 50 mL. Pipet 5 mL each of these solutions, add a mixture of methanol and tetrahydrofuran (7:3) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of entacapone in each solution.

Amount (mg) of entacapone ($C_{14}H_{15}N_3O_5$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Entacapone RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 300 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 25°C.

Mobile phase: Dissolve 2.34 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL, and adjust to pH 2.1 with phosphoric acid. To 540 mL of this solution add 440 mL of methanol and 20 mL of tetrahydrofuran.

Flow rate: 1 mL per minute.

System suitability—

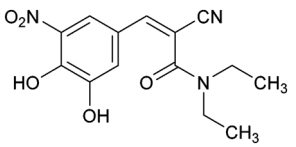
System performance: Dissolve 5 mg of Entacapone Related Substance A RS for System Suitability in a mixture of methanol and tetrahydrofuran (7:3) to make 25 mL. To 1 mL of this solution add a mixture of methanol and tetrahydrofuran (7:3) to make 20 mL, and use this solution as the solution for system suitability test. Separately, to 5 mL of the standard solution add a mixture of methanol and tetrahydrofuran (7:3) to make 50 mL. To 1 mL of this solution and 1 mL of the solution for system suitability test add a mixture of methanol and tetrahydrofuran (7:3) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the related substance A and entacapone 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 entacapone is not more than 1.0%.

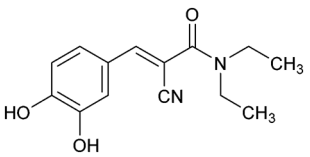
Containers and storage Containers—Well-closed containers.

Others

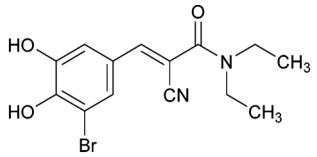
Related substance A:
(2*Z*)-2-Cyano-3-(3,4-dihydroxy-5-nitrophenyl)-*N*,*N*-diethylprop-2-enamide



Related substance B:
(2*E*)-2-Cyano-3-(3,4-dihydroxyphenyl)-*N*,*N*-diethylprop-2-enamide



Related substance C:
(2*E*)-3-(3-Bromo-4,5-dihydroxyphenyl)-2-cyano-*N*,*N*-diethylprop-2-enamide



Add the following:

Entacapone Tablets

エンタカポン錠

Entacapone Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of entacapone ($C_{14}H_{15}N_3O_5$: 305.29).

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

Identification To 1 mL of the sample solution obtained in the Assay add methanol to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 301 nm and 305 nm.

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

Conduct this procedure using light-resistant vessels. To 1 tablet of Entacapone Tablets add 70 mL of methanol, shake for 5 minutes, and add 60 mL of tetrahydrofuran. Sonicate for 3 minutes, shake for 5 minutes, and add methanol to make exactly 200 mL. Centrifuge this solution, pipet V mL of the supernatant liquid, add methanol to make exactly V' mL so that each mL contains about 0.5 mg of entacapone ($C_{14}H_{15}N_3O_5$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of entacapone ($C_{14}H_{15}N_3O_5$)
$= M_S \times A_T/A_S \times V'/V \times 2$

M_S: Amount (mg) of Entacapone RS taken, 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 0.05 mol/L potassium dihydrogen phosphate TS, adjusted to pH 5.5 with sodium hydroxide TS, as the dissolution medium, the dissolution rate in 30 minutes of Entacapone Tablets is not less than 80%.

Start the test with 1 tablet of Entacapone Tablets, withdraw not less than 15 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 5 mL or more 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 entacapone ($C_{14}H_{15}N_3O_5$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Entacapone RS (separately determine the loss on drying <*2.41*> under the same conditions as Entacapone), add 4 mL of methanol, dissolve by sonicating, and add the dissolution medium to make exactly 200 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 313 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, using the dissolution medium as the blank.

Dissolution rate (%) with respect to the labeled amount of entacapone ($C_{14}H_{15}N_3O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of Entacapone RS taken, calculated on the dried basis
C: Labeled amount (mg) of entacapone ($C_{14}H_{15}N_3O_5$) in 1 tablet

Assay Conduct this procedure using light-resistant vessels. Weigh accurately the mass of not less than 20 Entacapone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of entacapone ($C_{14}H_{15}N_3O_5$), add 60 mL of tetrahydrofuran, and sonicate for 3 minutes. Add 60 mL of methanol, shake for 5 minutes, and add methanol to make exactly 200 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of Entacapone RS (separately determine the loss on drying <*2.41*> under the same conditions as Entacapone), dissolve in 30 mL of tetrahydrofuran, 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 entacapone in each solution.

Amount (mg) of entacapone ($C_{14}H_{15}N_3O_5$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of Entacapone RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 300 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 25°C.

Mobile phase: Dissolve 2.34 g of sodium dihydrogen phosphate dihydrate in water, add 2 mL of phosphoric acid, and add water to make 1000 mL. To 540 mL of this solution add 440 mL of methanol and 20 mL of tetrahydrofuran.

Flow rate: Adjust so that the retention time of entaca-

pone is about 12 minutes.

System suitability—

System performance: To 20 mL of the standard solution add a mixture of methanol and tetrahydrofuran (7:3) to make 50 mL, and use this solution as the solution for system suitability test. Separately, dissolve 5 mg of Entacapone Related Substance A RS for System Suitability in a mixture of methanol and tetrahydrofuran (7:3) to make 25 mL. To 15 mL of this solution and 15 mL of the solution for system suitability test add a mixture of methanol and tetrahydrofuran (7:3) to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the related substance A, having the relative retention time of about 0.8 to entacapone, and entacapone 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 entacapone is not more than 1.0%.

Containers and storage Containers—Tight containers.

Others

Related substance A: refer to it described in Entacapone.

Epalrestat

エパルレスタット

Change the origin/limits of content as follows:

Epalrestat, when dried, contains not less than 98.0% and not more than 102.0% of epalrestat ($C_{15}H_{13}NO_3S_2$).

Erythromycin

エリスロマイシン

Change the Description and Purity as follows:

Description Erythromycin occurs as a white to light yellow-white powder.

It is freely soluble in methanol and in ethanol (95), and very slightly soluble in water.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Erythromycin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 40 mg of Erythromycin in 2 mL of methanol, add a mixture of phosphate buffer solution (pH 7.0) and methanol (15:1) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 16 mg of Erythromycin RS in 2 mL of methanol, add a mixture of phosphate buffer solution (pH 7.0) and methanol (15:1) to make exactly 10 mL, and use this solution as the standard stock solution. Dissolve 5 mg each of erythromycin B and erythromycin C in 2 mL of methanol, add exactly 2 mL of the standard stock solution, add a mixture of phosphate buffer solution (pH 7.0) and methanol (15:1) to make exactly 25 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 each peak area by the automatic integration method: the peak areas of erythromycin B and erythromycin C from the sample solution are not larger than those of erythromycin B and erythromycin C from the standard solution, respectively, and the area of the peaks other than erythromycin, erythromycin B and erythromycin C from the sample solution is not larger than the peak area of erythromycin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 70°C.

Mobile phase: Dissolve 3.5 g of dipotassium hydrogen phosphate in water to make 100 mL, and adjust the pH to 9.0 with diluted phosphoric acid (1 in 10). To 50 mL of this solution add 190 mL of *t*-butyl alcohol, 30 mL of acetonitrile and water to make 1000 mL.

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

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

System suitability—

System performance: Dissolve 2 mg of *N*-demethylerythromycin in 10 mL of the standard solution. When the procedure is run with 100 μL of this solution under the above operating conditions, *N*-demethylerythromycin, erythromycin C, erythromycin and erythromycin B are eluted in this order, with the resolution between the peaks of *N*-demethylerythromycin and erythromycin C being not less than 0.8, and with the resolution between the peaks of *N*-demethylerythromycin and erythromycin being not less than 5.5.

System repeatability: When the test is repeated 3 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of erythromycin is not more than 3.0%.

Ethanol

エタノール

Change the Purity (3) as follows:

Purity

(3) Volatile impurities—Pipet 500 mL of Ethanol, add 150 μL of 4-methylpentan-2-ol, and use this solution as the sample solution. Separately, to 100 μL of anhydrous methanol add Ethanol to make exactly 50 mL. Pipet 5 mL of this solution, add Ethanol to make exactly 50 mL, and use this solution as the standard solution (1). Separately, to 50 μL each of anhydrous methanol and acetaldehyde add Ethanol to make exactly 50 mL. To 100 μL of this solution add Ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to 150 μL of acetal add Ethanol to make exactly 50 mL. To 100 μL of this solution add Ethanol to make exactly 10 mL, and use this solution as the standard solution (3). Separately, to 100 μL of benzene add Ethanol to make exactly 100 mL. To 100 μL of this solution add Ethanol to make exactly 50 mL, and use this solution as the standard solution (4). Perform the test with exactly 1 μL each of Ethanol, the sample solution and standard solutions (1), (2), (3) and (4) as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas of acetaldehyde, A_E, benzene, B_E and acetal, C_E obtained with Ethanol, and the peak area of methanol with the standard solution (1), the peak area of acetaldehyde, A_T with the standard solution (2), the peak area of acetal, C_T with the standard solution (3) and the peak area of benzene, B_T with the standard solution (4) by the automatic integration method: the peak area of methanol obtained with Ethanol is not larger than 1/2 times the peak area of methanol with the standard solution (1). When calculate the amounts of the volatile impurities by the following equation, the total amount of acetaldehyde and acetal is not more than 10 vol ppm as acetaldehyde, and the amount of benzene is not more than 2 vol ppm. The total area of the peaks other than the peak mentioned above with the sample solution is not larger than the peak area of 4-methylpentan-2-ol. For this calculation the peak having the area not more than 3% that of 4-methylpentan-2-ol is excluded.

$$\text{Total amount (vol ppm) of acetaldehyde and acetal} = (10 \times A_E)/(A_T - A_E) + (30 \times C_E \times 44.05)/\{(C_T - C_E) \times 118.2\}$$

$$\text{Amount (vol ppm) of benzene} = 2B_E/(B_T - B_E)$$

If necessary, identify the peak of benzene by using a different stationary liquid phase and suitable chromatographic conditions.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated with 6% cyanopropyl phenyl-94% dimethyl silicone polymer for gas chromatography in 1.8 μm thickness.

Column temperature: Inject at a constant temperature of about 40°C, maintain the temperature for 12 minutes, then raise to 240°C at a rate of 10°C per minute, and maintain at a constant temperature of about 240°C for 10 minutes.

Injection port temperature: 200°C.

Detector temperature: 280°C.

Carrier gas: Helium.

Flow rate: 35 cm per second.

Split ratio: 1:20.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution (2) under the above operating conditions, acetaldehyde and methanol are eluted in this order with the resolution between these peaks being not less than 1.5.

Anhydrous Ethanol

無水エタノール

Change the Purity (3) as follows:

Purity

(3) Volatile impurities—Pipet 500 mL of Anhydrous Ethanol, add 150 μL of 4-methylpentan-2-ol, and use this solution as the sample solution. Separately, to 100 μL of anhydrous methanol add Anhydrous Ethanol to make exactly 50 mL. Pipet 5 mL of this solution, add Anhydrous Ethanol to make exactly 50 mL, and use this solution as the standard solution (1). Separately, to 50 μL each of anhydrous methanol and acetaldehyde add Anhydrous Ethanol to make exactly 50 mL. To 100 μL of this solution add Anhydrous Ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to 150 μL of acetal add Anhydrous Ethanol to make exactly 50 mL. To 100 μL of this solution add Anhydrous Ethanol to make exactly 10 mL, and use this solution as the standard solution (3). Separately, to 100 μL of benzene add Anhydrous Ethanol to make exactly 100 mL. To 100 μL of this solution add Anhydrous Ethanol to make exactly 50 mL, and use this solution as the standard solution (4). Perform the test with exactly 1 μL each of Anhydrous Ethanol, the sample solution and standard solutions (1), (2), (3) and (4) as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas of acetaldehyde, A_E, benzene, B_E and acetal, C_E obtained with Anhydrous Ethanol, and the peak area of methanol with the standard solution (1), the peak area of acetaldehyde, A_T with the standard solution (2), the peak area of acetal, C_T with the standard solution (3) and the peak area of benzene, B_T with the standard solution (4) by the automatic integration method: the peak area of methanol obtained with Anhydrous Ethanol is not larger than 1/2 times the peak area of methanol with the standard solution (1). When calculate the amounts of the volatile impurities by the following equation, the total amount of acetaldehyde and acetal is not

more than 10 vol ppm as acetaldehyde, and the amount of benzene is not more than 2 vol ppm. The total area of the peaks other than the peak mentioned above with the sample solution is not larger than the peak area of 4-methylpentan-2-ol. For this calculation the peak having the area not more than 3% that of 4-methylpentan-2-ol is excluded.

$$\text{Total amount (vol ppm) of acetaldehyde and acetal} = (10 \times A_E)/(A_T - A_E) + (30 \times C_E \times 44.05)/\{(C_T - C_E) \times 118.2\}$$

$$\text{Amount (vol ppm) of benzene} = 2B_E/(B_T - B_E)$$

If necessary, identify the peak of benzene by using a different stationary liquid phase and suitable chromatographic conditions.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated with 6% cyanopropyl phenyl-94% dimethyl silicone polymer for gas chromatography in 1.8 μm thickness.

Column temperature: Inject at a constant temperature of about 40°C, maintain the temperature for 12 minutes, then raise to 240°C at a rate of 10°C per minute, and maintain at a constant temperature of about 240°C for 10 minutes.

Injection port temperature: 200°C.

Detector temperature: 280°C.

Carrier gas: Helium.

Flow rate: 35 cm per second.

Split ratio: 1:20.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution (2) under the above operating conditions, acetaldehyde and methanol are eluted in this order with the resolution between these peaks being not less than 1.5.

Delete the following Monograph:

Fluoxymesterone

フルオキシメステロン

Folic Acid

葉酸

Change the Purity (2) as follows:

Purity

(2) Free amines—Pipet 30 mL of the sample solution obtained in the Assay, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of *p*-Aminobenzoyl Glutamic Acid RS for Purity, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, dissolve in diluted ethanol (2 in 5) to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 1000 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and standard solution, proceed as directed in the Assay, and perform the test as directed under Ultraviolet-visible Spectrophotometry <*2.24*>. Determine the absorbances, A_T and A_S, of subsequent solutions of the sample solution and standard solution at 550 nm: the content of free amines is not more than 1.0%.

$$\text{Content (\%) of free amines} = M_S/M_T \times A_T/A_S$$

M_S: Amount (mg) of *p*-Aminobenzoyl Glutamic Acid RS for Purity taken

M_T: Amount (mg) of Folic Acid taken, calculated on the anhydrous basis

Fosfomycin Calcium Hydrate

ホスホマイシンカルシウム水和物

Add the following next to the Purity (2):

Purity

(3) Glycol substance—Weigh accurately about 0.2 g of Fosfomycin Calcium Hydrate, transfer into a 250-mL iodine flask, add 100 mL of water, and dissolve by sonicating while cooling in ice. Add exactly 50 mL of phthalate buffer solution (pH 5.8) and exactly 5 mL of sodium periodate solution (107 in 100,000), stopper, stir, and add 1 mL of water in the receiving part. Avoid exposure to light, allow to stand in a water bath at 30°C for 60 minutes, add exactly 10 mL of a solution of potassium iodide (2 in 5) without haste, and titrate <*2.50*> with 0.01 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction: amount of glycol substance ($C_3H_7CaO_5P$) is not more than 1.5%.

$$\text{Each mL of 0.01 mol/L sodium thiosulfate VS} = 0.4854 \text{ mg of } C_3H_7CaO_5P$$

Fosfomycin Sodium

ホスホマイシンナトリウム

Add the following next to the Purity (3):

Purity

(4) Glycol substance—Weigh accurately about 0.2 g of Fosfomycin Sodium, and dissolve in 100 mL of water in a 250-mL iodine flask. Add exactly 50 mL of phthalate buffer solution (pH 5.8) and exactly 5 mL of sodium periodate solution (107 in 100,000), stopper, stir, and add 1 mL of water in the receiving part. Allow to stand in a dark place for 90 minutes, add exactly 10 mL of a solution of potassium iodide (2 in 5) without haste, and titrate <*2.50*> with 0.01 mol/L sodium thiosulfate VS (indicator: 2 mL of starch

TS). Perform a blank determination in the same manner, and make any necessary correction: amount of glycol substance ($C_3H_7Na_2O_5P$) is not more than 0.5%.

Each mL of 0.01 mol/L sodium thiosulfate VS
= 0.5001 mg of $C_3H_7Na_2O_5P$

Gentamicin Sulfate

ゲンタマイシン硫酸塩

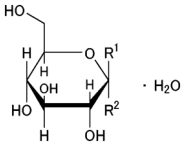
Change the Purity (1) as follows:

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Gentamicin Sulfate 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 0.08.

Add the following:

Glucose Hydrate

ブドウ糖水和物



α-D-glucopyranose monohydrate: R^1 = H, R^2 = OH
β-D-glucopyranose monohydrate: R^1 = OH, R^2 = H

$C_6H_{12}O_6.H_2O$: 198.17
D-Glucopyranose monohydrate
[*77938-63-7*]

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

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Glucose Hydrate is the monohydrate of D-glucopyranose derived from starch.

It contains not less than 97.5% and not more than 102.0% of glucose [D-glucopyranose ($C_6H_{12}O_6$: 180.16)], calculated on the anhydrous basis.

◆**Description** Glucose Hydrate occurs as white, crystals or crystalline powder, and has a sweet taste.

It is freely soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).◆

Identification ◇**(1)** Add 2 to 3 drops of a solution of Glucose Hydrate (1 in 20) to 5 mL of boiling Fehling's TS: a red precipitate is produced.◇

(2) Perform the test with 20 μL each of the sample solution and standard solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions: the principal peak in the chromatogram obtained from the sample solution is similar in retention time and size to the principal peak in the chromatogram from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

Purity **(1)** Clarity and color of solution—Dissolve 10.0 g of Glucose Hydrate in 15 mL of water, and use this solution as the test solution. Perform the test with the test solution as directed under Turbidity Measurement <*2.61*>: the solution is clear. Perform the test with the test solution according to Method 2 under Methods for Color Matching <*2.65*>: the solution is not more colored than Matching Fluid BY7.

◆**(2)** Heavy metals <*1.07*>—Proceed with 5.0 g of Glucose Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).◆

(3) Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 250 mL, and use this solution as the standard solution (1). Pipet 25 mL of the standard solution (1), add water to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution, the standard solution (1) and the standard solution (2) 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 of maltose and isomaltose, having the relative retention time of about 0.8 to glucose, obtained from the sample solution, is not larger than the peak area of glucose from the standard solution (1) (not more than 0.4%), and the peak area of maltotriose, having the relative retention time of about 0.7 from the sample solution, is not larger than 1/2 times the peak area of glucose from the standard solution (1) (not more than 0.2%), and the peak area of fructose, having the relative retention time of about 1.3 from the sample solution, is not larger than 3 times the peak area of glucose from the standard solution (2) (not more than 0.15%), and the area of the peak other than glucose and the peaks mentioned above from the sample solution is not larger than 2 times the peak area of glucose from the standard solution (2) (not more than 0.10%). Furthermore, the total area of the peaks other than glucose from the sample solution is not larger than 1.25 times the peak area of glucose from the standard solution (1) (not more than 0.5%). For these calculations the peak areas not larger than the peak area of glucose from the standard solution (2) are excluded (disregard limit: 0.05%).

Operating conditions—

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

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

System suitability—

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

◇Test for required detectability: Confirm that the peak area of glucose obtained with 20 μL of the standard solution (2) is equivalent to 8.8 to 16.3% of that with 20 μL of the standard solution (1).

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of glucose is not more than 1.0%.◇

(4) Dextrin—To 1.0 g of powdered Glucose Hydrate add 20 mL of ethanol (95), and boil under a reflux condenser: the solution is clear.

(5) Soluble starch and sulfite—To 7.4 g of Glucose Hydrate add 15 mL of water, dissolve by heating on a water bath, cool, and add 25 μL of 0.05 mol/L iodine VS: a yellow color develops (not more than 15 ppm as SO_3).

Conductivity ⟨*2.51*⟩ Dissolve 20.0 g of Glucose Hydrate in a fleshly boiled and cooled distilled water to make 100 mL, and use this solution as the sample solution. Measure the conductivity of the sample solution at 25 ± 0.1°C while gently stirring with a magnetic stirrer: not more than 20 $\mu S \cdot cm^{-1}$.

Water ⟨*2.48*⟩ 7.5 – 9.5% (0.25 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.33 g of Glucose Hydrate and 0.3 g of ◆Glucose RS◆ (separately determine the water ⟨*2.48*⟩ in the same manner as Purified Glucose), dissolve separately in water to make exactly 10 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of glucose in each solution.

$$\text{Amount (g) of glucose } (C_6H_{12}O_6) = M_S \times A_T/A_S$$

M_S: Amount (g) of Glucose RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: A differential refractometer maintained at a constant temperature (40°C for example).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (Ca type) composed with a sulfonated polystyrene cross-linked with divinylbenzene (degree of cross-linkage: 8%) (9 μm in particle diameter).

Column temperature: A constant temperature of about 85°C.

Mobile phase: Water.

Flow rate: 0.3 mL per minute (the retention time of glucose is about 21 minutes).

System suitability—

System performance: Dissolve 5 mg of maltose, 5 mg of maltotriose and 5 mg of fructose in 50 mL of water, and use this solution as the solution for system suitability test. When the procedure is run with 20 μL each of the solution for system suitability test and the standard solution (2) in Purity (3) under the above operating conditions, maltotriose, maltose, glucose and fructose are eluted in this order, the relative retention times of maltotriose, maltose, isomaltose and fructose to glucose are about 0.7, about 0.8, about 0.8 and about 1.3, respectively, and the resolution between the peaks of maltotriose and maltose is not less than 1.3.

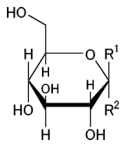
◇System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glucose is not more than 1.0%.◇

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

Add the following:

Purified Glucose

精製ブドウ糖



α-D-glucopyranose: $R^1 = H$, $R^2 = OH$
β-D-glucopyranose: $R^1 = OH$, $R^2 = H$

$C_6H_{12}O_6$: 180.16
D-Glucopyranose
[*50-99-7*]

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

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Purified Glucose is D-glucopyranose derived from starch.

It contains not less than 97.5% and not more than 102.0% of glucose [D-glucopyranose ($C_6H_{12}O_6$)], calculated on the anhydrous basis.

◆**Description** Purified Glucose occurs as white, crystals or crystalline powder, and has a sweet taste.

It is freely soluble in water, and slightly soluble in methanol and in ethanol (95).◆

Identification ◇**(1)** Add 2 to 3 drops of a solution of Purified Glucose (1 in 20) to 5 mL of boiling Fehling's TS: a red precipitate is produced.◇

(2) Perform the test with 20 μL each of the sample solution and standard solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions: the principal peak in the chromatogram obtained from the sample solution is similar in retention time and size to the principal peak in the chromatogram from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

Purity **(1)** Clarity and color of solution—Dissolve 10.0 g of Purified Glucose in 15 mL of water by heating on a water bath, and allow to cool to room temperature, and use this solution as the test solution. Perform the test with the test solution as directed under Turbidity Measurement <*2.61*>: the solution is clear. Perform the test with the test solution according to Method 2 under Methods for Color Matching <*2.65*>: the solution is not more colored than Matching Fluid BY7.

◆**(2)** Heavy metals <*1.07*>—Proceed with 5.0 g of Purified Glucose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).◆

(3) Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 250 mL, and use this solution as the standard solution (1). Pipet 25 mL of the standard solution (1), add water to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution, the standard solution (1) and the standard solution (2) 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 of maltose and isomaltose, having the relative retention time of about 0.8 to glucose, obtained from the sample solution, is not larger than the peak area of glucose from the standard solution (1) (not more than 0.4%), and the peak area of maltotriose, having the relative retention time of about 0.7 from the sample solution, is not larger than 1/2 times the peak area of glucose from the standard solution (1) (not more than 0.2%), and the peak area of fructose, having the relative retention time of about 1.3 from the sample solution, is not larger than 3 times the peak area of glucose from the standard solution (2) (not more than 0.15%), and the area of the peak other than glucose and the peaks mentioned above from the sample solution is not larger than 2 times the peak area of glucose from the standard solution (2) (not more than 0.10%). Furthermore, the total area of the peaks other than glucose from the sample solution is not larger than 1.25 times the peak area of glucose from the standard solution (1) (not more than 0.5%). For these calculations the peak areas not larger than the peak area of glucose from the standard solution (2) are excluded (disregard limit: 0.05%).

Operating conditions—

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

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

System suitability—

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

◇Test for required detectability: Confirm that the peak area of glucose obtained with 20 μL of the standard solution (2) is equivalent to 8.8 to 16.3% of that with 20 μL of the standard solution (1).

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of glucose is not more than 1.0%.◇

(4) Dextrin—To 1.0 g of powdered Purified Glucose add 20 mL of ethanol (95), and boil under a reflux condenser: the solution is clear.

(5) Soluble starch and sulfite—To 6.7 g of Purified Glucose add 15 mL of water, dissolve by heating on a water bath, cool, and add 25 μL of 0.05 mol/L iodine VS: a yellow color develops (not more than 15 ppm as SO_3).

Conductivity <*2.51*> Dissolve 20.0 g of Purified Glucose in a fleshly boiled and cooled distilled water to make 100 mL, and use this solution as the sample solution. Measure the conductivity of the sample solution at 25 ± 0.1°C while gently stirring with a magnetic stirrer: not more than 20 $\mu S \cdot cm^{-1}$.

Water <*2.48*> Not more than 1.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.3 g each of Purified Glucose and ◆Glucose RS◆ (separately determine the water <*2.48*> in the same manner as Purified Glucose), dissolve separately in water to make exactly 10 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of glucose in each solution.

$$\text{Amount (g) of glucose } (C_6H_{12}O_6) = M_S \times A_T/A_S$$

M_S: Amount (g) of Glucose RS taken, calculated on the anhydrous basis

Operating conditions—

Detector: A differential refractometer maintained at a constant temperature (40°C for example).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with strongly acidic ion-ex-

change resin for liquid chromatography (Ca type) composed with a sulfonated polystyrene cross-linked with divinylbenzene (degree of cross-linkage: 8%) (9 μm in particle diameter).

Column temperature: A constant temperature of about 85°C.

Mobile phase: Water.

Flow rate: 0.3 mL per minute (the retention time of glucose is about 21 minutes).

System suitability—

System performance: Dissolve 5 mg of maltose, 5 mg of maltotriose and 5 mg of fructose in 50 mL of water, and use this solution as the solution for system suitability test. When the procedure is run with 20 μL each of the solution for system suitability test and the standard solution (2) in Purity (3) under the above operating conditions, maltotriose, maltose, glucose and fructose are eluted in this order, the relative retention times of maltotriose, maltose, isomaltose and fructose to glucose are about 0.7, about 0.8, about 0.8 and about 1.3, respectively, and the resolution between the peaks of maltotriose and maltose is not less than 1.3.

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

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

Glucose Injection

ブドウ糖注射液

Change the Method of preparation, Identification and Purity as follows:

Method of preparation Prepare as directed under Injections, with Purified Glucose.

No preservative is added.

Identification Measure a volume of Glucose Injection, equivalent to 0.1 g of Purified Glucose, and, if necessary, add water or evaporate on a water bath to make 2 mL. Add 2 to 3 drops of the solution to 5 mL of boiling Fehling' s TS: a red precipitate is produced.

Purity 5-Hydroxymethylfurfural and related substances—Measure exactly a volume of Glucose Injection, equivalent to 2.5 g of Purified Glucose, and add water to make exactly 100 mL. Determine the absorbance of this solution at 284 nm as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it is not more than 0.80.

Delete the following Monograph:

Gramicidin

グラミシジン

Heparin Calcium

ヘパリンカルシウム

Change the Identification (2), the Purity (8) and (9) as follows:

Identification

(2) Dissolve 1 mg each of Heparin Calcium and Heparin Sodium RS for Identification in 1 mL of water, and use these solutions as the sample solution and the standard solution, respectively. 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: the retention time for the major peak from the sample solution and standard solution is identical.

Operating conditions—

Detector, column, column temperature, mobile phases A and B, flowing of mobile phase and flow rate: Proceed as directed under the operating conditions in Purity (9).

System suitability—

System performance: Dissolve 1.0 mg of Heparin Sodium RS for Identification in 0.60 mL of water. Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS for System Suitability in 0.20 mL of water. Dissolve 1.0 mg of dermatan sulfate in 2.0 mL of water. To 90 μL of the solution of Heparin Sodium RS for Identification add 30 μL each of the solutions of Over-sulfated Chondroitin Sulfate RS for System Suitability and dermatan sulfate, and mix. When the procedure is run with 20 μL of the mixture under the above operating conditions, dermatan sulfate, heparin and over-sulfated chondroitin sulfate are eluted in this order with the resolution between the peaks of dermatan sulfate and heparin being not less than 1.0 and that between the peaks of heparin and over-sulfated chondroitin sulfate being not less than 1.5.

Purity

(8) 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, or the signal disappears when determining the spectrum of the sample solutions as directed under ^{1}H with ^{13}C-decoupling.

Operating conditions—

Spectrometer: 1.1. 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: SN ratio of the signal of *N*-acetyl proton 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 for System Suitability 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 for System Suitability. 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.

(9) 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 synthetic polymer for liquid chromatography to which diethylaminoethyl group binds (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 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 RS for Identification 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 for System Suitability 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 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 oversulfated 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 Identification, the Purity (6) and (7) as follows:

Identification Dissolve 1 mg each of Heparin Sodium and Heparin Sodium RS for Identification in 1 mL of water, and use these solutions as the sample solution and the standard solution, respectively. 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: the retention time for the major peak from the sample solution and standard solution is identical.

Operating conditions—

Detector, column, column temperature, mobile phases A and B, flowing of mobile phase and flow rate: Proceed as directed under the operating conditions in Purity (7).

System suitability—

System performance: Dissolve 1.0 mg of Heparin Sodium RS for Identification in 0.60 mL of water. Dissolve 0.10 mg of Over-sulfated Chondroitin Sulfate RS for System Suitability in 0.20 mL of water. Dissolve 1.0 mg of dermatan sulfate in 2.0 mL of water. To 90 μL of the solution of Heparin Sodium RS for Identification add 30 μL each of the solutions of Over-sulfated Chondroitin Sulfate RS for System Suitability and dermatan sulfate, and mix. When the procedure is run with 20 μL of the mixture under the above operating conditions, dermatan sulfate, heparin and over-sulfated chondroitin sulfate are eluted in this order with the resolution between the peaks of dermatan sulfate and heparin being not less than 1.0 and that between the peaks of heparin and over-sulfated chondroitin sulfate being not less than 1.5.

Purity

(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: 1.1. 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: SN ratio of the signal of *N*-acetyl proton 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 RS for Identification 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 for System Suitability 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 RS for Identification add 0.20 mL of the solution of Over-sulfated Chondroitin Sulfate RS for System Suitability. 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 synthetic polymer for liquid chromatography to which diethylaminoethyl group binds (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 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 RS for Identification 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 for System Suitability 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%.

Hydrocortisone Acetate

ヒドロコルチゾン酢酸エステル

Change the Optical rotation as follows:

Optical rotation <*2.49*> $[\alpha]_D^{20}$: +158 – +167° (after drying, 50 mg, 1,4-dioxane, 10 mL, 100 mm).

Hydrocortisone Butyrate

ヒドロコルチゾン酪酸エステル

Change the Description and Purity as follows:

Description Hydrocortisone Butyrate occurs as a white powder. It is odorless.

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

Melting point: about 200°C (with decomposition).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Hydrocortisone Butyrate 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 Hydrocortisone Butyrate in 50 mL of a mixture of acetonitrile and the mobile phase A (4:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and the mobile phase A (4:1) 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 other than hydrocortisone butyrate obtained from the sample solution is not larger than the peak area of hydrocortisone butyrate from the standard solution, and the total area of the peaks other than hydrocortisone butyrate from the sample solution is not larger than 2 times the peak area of hydrocortisone butyrate from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

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

Mobile phase A: Dissolve 1 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 5.5 with potassium hydroxide TS.

Mobile phase B: 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 – 12.5	80 → 35	20 → 65
12.5 – 15.5	35	65

Flow rate: 2.0 mL per minute.

Time span of measurement: For 15.5 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 acetonitrile and the mobile phase A (4:1) to make exactly 20 mL. Confirm that the peak area of hydrocortisone butyrate 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 hydrocortisone butyrate are not less than 10,000 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 hydrocortisone butyrate is not more than 2.0%.

Hydroxocobalamin Acetate

ヒドロキソコバラミン酢酸塩

Change the Origin/limits of content, Description and Purity as follows:

Hydroxocobalamin Acetate contains not less than 96.0% and not more than 101.0% of hydroxocobalamin acetate ($C_{62}H_{89}CoN_{13}O_{15}P.C_2H_4O_2$), calculated on the anhydrous and residual solvent-free basis.

Description Hydroxocobalamin Acetate occurs as dark red, crystals or powder. It is odorless.

It is freely soluble in water and in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Purity Conduct this procedure using light-resistant vessels. Dissolve 75 mg of Hydroxocobalamin Acetate in 100 mL of the dissolving solution, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the dissolving solution to make exactly 20 mL, and use this so-

lution 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 hydroxocobalamin from the sample solution is not larger than the peak area of hydroxocobalamin from the standard solution.

Dissolving solution: A mixture of water, mobile phase C and methanol (41:5:4).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 351 nm).

Column: Connect two columns which are 4.6 mm in inside diameter and 10 cm in length, composed of octadecylsilanized monolithic silica for liquid chromatography, having a bimodal pore structure with 2 μm macropore and 13 nm mesopore, coated with polyether ether ketone.

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

Mobile phase A: Water.

Mobile phase B: Methanol.

Mobile phase C: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust to pH 3 with diluted phosphoric acid (1 in 100).

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

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)
0 – 20	82	8	10
20 – 40	82 → 50	8 → 40	10

Flow rate: 2 mL per minute.

Time span of measurement: For 40 minutes after injection of the sample solution.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add the dissolving solution to make exactly 50 mL. Confirm that the peak area of hydroxocobalamin obtained with 20 μL of this solution is equivalent to 1.4 to 2.6% 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 hydroxocobalamin are not less than 4000 and not more than 2.4, 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 hydroxocobalamin is not more than 2.0%.

Delete the Loss on drying:

Add the following next to the purity:

Water <2.48> 8.0 – 12.0% (50 mg, volumetric titration, direct titration).

Change the Assay as follows:

Assay Weigh accurately about 0.1 g of Hydroxocobalamin Acetate, and dissolve in water to make exactly 500 mL. Pipet 5 mL of this solution, and add acetic acid-sodium acetate buffer solution (pH 4.5) to make exactly 25 mL. Determine the absorbance, A, of this solution at 351 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of hydroxocobalamin acetate ($C_{62}H_{89}CoN_{13}O_{15}P.C_2H_4O_2$)
$= A/187 \times 25{,}000$

Hydroxypropylcellulose

ヒドロキシプロピルセルロース

Change the Purity (2) as follows:

Purity

(2) Silicon dioxide—Apply to Hydroxypropylcellulose, if the addition of silicon dioxide is stated on the label and if more than 0.2% residue is found in the Residue on ignition. Weigh accurately the crucible containing the residue tested in the Residue on ignition of Hydroxypropylcellulose (*a* (g)). Moisten the residue with water, and add 5 mL of hydrofluoric acid, in small portions. Evaporate it on a steam bath to dryness and cool. Add 5 mL of hydrofluoric acid and 0.5 mL of sulfuric acid, and evaporate to dryness. Slowly increase the temperature until all the acids have been volatilized, and ignite at 1000 ± 25°C. Cool the crucible in a desiccator, and weigh (*b* (g)). Calculate the amount of silicon dioxide by the following equation: not more than 0.6%.

Amount (%) of silicon dioxide (SiO_2)
$= (a - b)/M \times 100$

M: Amount (g) of Hydroxypropylcellulose used for Residue on ignition

Low Substituted Hydroxypropylcellulose

低置換度ヒドロキシプロピルセルロース

Change as follows:

[*9004-64-2*, Hydroxypropylcellulose]

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

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Low Substituted Hydroxypropylcellulose is a low substituted hydroxypropyl ether of cellulose.

It contains not less than 5.0% and not more than 16.0% of hydroxypropoxy group ($-OC_3H_6OH$: 75.09), calculated on the dried basis.

◆**Description** Low Substituted Hydroxypropylcellulose occurs as a white to yellowish white, powder or granules.

It is practically insoluble in ethanol (99.5).

It dissolves in a solution of sodium hydroxide (1 in 10), and produces a viscous solution.

It swells in water, in sodium carbonate TS and in 2 mol/L hydrochloric acid TS.◆

Identification

(1) Shake thoroughly 0.1 g of Low Substituted Hydroxypropylcellulose with 10 mL of water: it does not dissolve.

(2) To the dispersed solution obtained in (1) add 1 g of sodium hydroxide, and shake until the solution becomes uniform. Transfer 5 mL of this solution to a suitable vessel, add 10 mL of a mixture of acetone and methanol (4:1), and shake: a white, flocculent precipitate is formed.

(3) Determine the infrared absorption spectrum of Low Substituted Hydroxypropylcellulose 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*> To 1.0 g of Low Substituted Hydroxypropylcellulose add 100 mL of freshly boiled and cooled water, and shake: the pH of the solution is between 5.0 and 7.5.

Purity ◇Heavy metals <*1.07*>—Proceed with 2.0 g of Low Substituted Hydroxypropylcellulose 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).◇

Loss on drying <*2.41*> Not more than 5.0% (1 g, 105°C, 1 hour).

Residue on ignition <*2.44*> Not more than 0.8% (1 g).

Assay (i) Apparatus—Reaction vial: A 5-mL pressure-tight serum vial, 20 mm in outside diameter and 50 mm in height, 20 mm in outside diameter and 13 mm in inside diameter at the neck, equipped with a septum made of butyl-rubber whose surface is processed with fluoroplastics, which can be fixed to the vial and stopper tightly with an aluminum cap or another system providing an equivalent air-tightness.

Heater: A square-shaped aluminum block, having holes 20 mm in diameter and 32 mm in depth, adopted to the reaction vial. Capable of stirring the content of the reaction vial by means of a magnetic stirrer or of a reciprocal shaker about 100 times per minute.

(ii) Procedure—Weigh accurately about 65 mg of Low Substituted Hydroxypropylcellulose, transfer to the reaction vial, 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, immediately stopper the vial tightly, and weigh accurately. Using a magnetic stirrer equipped in the heating module, or using a shaker, mix for 60 minutes while heating so that the temperature of the vial content is 130 ± 2°C. In the case when a magnetic stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5-minute intervals by hand, and continue heating for an additional 30 minutes. Allow the vial to cool, and again weigh accurately. If the mass loss is less than 26 mg and there is no evidence of a leak of the content, 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 vial, immediately stopper the vial tightly, and weigh accurately. Add 15 to 22 μL of isopropyl iodide for assay through the septum using a micro-syringe, and weigh accurately. Shake the reaction vial thoroughly, and use the upper layer of the content 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, Q_T and Q_S, of the peak area of isopropyl iodide to that of the internal standard.

$$\text{Amount (\%) of hydroxypropoxy group } (C_3H_7O_2) = M_S/M_T \times Q_T/Q_S \times 44.17$$

M_S: Amount (mg) of isopropyl iodide for assay taken

M_T: Amount (mg) of Low Substituted Hydroxypropylcellulose taken, 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 fused silica column 0.53 mm in inside diameter and 30 m in length, coated with dimethylpolysiloxane for gas chromatography 3 μm in thickness. Use a guard column if necessary.

Column temperature: Maintain the temperature at 50°C for 3 minutes after injection, raise to 100°C at a rate of

10°C per minute, then to 250°C at a rate of 35°C per minute and maintain at 250°C for 8 minutes.

Injection port temperature: 250°C.

Detector temperature: 280°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of the internal standard is about 10 minutes (4.3 mL per minute).

Split ratio: 1:40.

System suitability—

System performance: When the procedure is run with 1 to 2 μL of the standard solution under the above operating conditions, isopropyl iodide and *n*-octane are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 1 to 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of isopropyl iodide to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Hypromellose

ヒプロメロース

Change the Viscosity and Assay (ii) as follows:

Viscosity ⟨*2.53*⟩ (i) Method I: Apply to Hypromellose having a labeled viscosity of less than 600 mPa·s. Put an exact amount of Hypromellose, equivalent to 4.000 g calculated on the dried basis, in a tared, wide-mouth bottle, add hot water (between 90°C and 99°C) to make 200 g, stopper the bottle, stir by mechanical means at 350 to 450 revolutions per minute for 10 to 20 minutes to get a homogeneous dispersion. If necessary, take off the sample attached on the walls of the bottle, put them in the dispersed solution, and dissolve by continuing the stirring in a water bath at not exceeding 10°C for 20 to 40 minutes. Add cold water, if necessary, to make 200 g, and use this solution as the sample solution. Centrifuge the solution if necessary to expel any entrapped air bubbles. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method I under Viscosity Determination: not less than 80% and not more than 120% of the labeled viscosity.

(ii) Method II: Apply to Hypromellose having a labeled viscosity of not less than 600 mPa·s. Put an exact amount of Hypromellose, equivalent to 10.00 g calculated on the dried basis, in a tared, wide-mouth bottle, add hot water (between 90°C and 99°C) to make 500 g, and prepare the sample solution in the same manner as directed in Method I. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method II under Viscosity Determination, using a single cylinder-type rotational viscometer, according to the following operating conditions: not less than 75% and not more than 140% of the labeled viscosity.

Operating conditions—

Apparatus: Brookfield type viscometer LV model or an equivalent apparatus.

Rotor No., rotation frequency, and calculation multiplier: According to the following table, depending on the labeled viscosity.

Labeled viscosity (mPa·s)				Rotor No.	Rotation frequency /min	Calculation multiplier
Not less than 600 and less than			1400	3	60	20
〃	1400	〃	3500	3	12	100
〃	3500	〃	9500	4	60	100
〃	9500	〃	99,500	4	6	1000
〃	99,500			4	3	2000

Procedure of apparatus: Read the value after 2 minutes of rotation, and stop the rotation for at least 2 minutes. Repeat this procedure more two times, and average the three observed values.

Assay

(ii) Procedure—Weigh accurately about 65 mg of Hypromellose, transfer to the reaction vial, 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, immediately stopper the vial tightly, and weigh accurately. Using a magnetic stirrer equipped in the heating module, or using a shaker, stir for 60 minutes while heating so that the temperature of the vial content is 130 ± 2°C. In the case when a magnetic stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5-minute intervals by hand, and continue heating for an additional 30 minutes. Allow the vial to cool, and again weigh accurately. If the mass loss is less than 0.50% and there is no evidence of a leak of the content, 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 vial, immediately stopper the vial tightly, 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 micro-syringe with weighing accurately every time, shake thoroughly, and use the upper layer of the content 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, Q_{Ta} and Q_{Tb}, of the peak area of iodomethane and isopropyl iodide to that of the internal standard obtained from the sample solution, and Q_{Sa} and Q_{Sb}, of the peak area of iodomethane and isopropyl iodide to that of the internal standard from the standard solution.

$$\text{Content (\%) of methoxy group } (CH_3O) = Q_{Ta}/Q_{Sa} \times M_{Sa}/M \times 21.86$$

$$\text{Content (\%) of hydroxypropoxy group } (C_3H_7O_2) = Q_{Tb}/Q_{Sb} \times M_{Sb}/M \times 44.17$$

M_{Sa}: Amount (mg) of iodomethane for assay taken

M_{Sb}: Amount (mg) of isopropyl iodide for assay taken

M: Amount (mg) of Hypromellose taken, 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 column 3 – 4 mm in inside diameter and 1.8 – 3 m in length, packed with siliceous earth for gas chromatography, 125 to 150 μm in diameter, coated with methyl silicone polymer for gas chromatography at the ratio of 10 – 20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium for thermal conductivity detector, or Helium or Nitrogen for hydrogen flame-ionization detector.

Flow rate: Adjust 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 complete separation of these peaks.

Add the following:

Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension

イソフェンインスリン　ヒト(遺伝子組換え)水性懸濁注射液

Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension is an aqueous suspension for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled Insulin Unit of insulin human (genetical recombination) ($C_{257}H_{383}N_{65}O_{77}S_6$: 5807.57). It contains not less than 10 μg and not more than 40 μg of zinc (Zn: 65.38) per the labeled 100 Insulin Units.

Method of preparation Prepare as directed under Injections, with Insulin Human (Genetical Recombination) and Protamine Sulfate.

Description Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension is a white aqueous suspension. When allowed to stand, it separates into a white precipitate and colorless supernatant liquid, and the precipitate returns to the suspension state on gentle shaking.

When it is examined microscopically, the precipitate mostly consists of fine, oblong crystals of 1 to 30 μm in major axis, and does not contain amorphous substances or large aggregates.

Identification Adjust Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension to pH between 2.5 and 3.0 with dilute hydrochloric acid: the precipitate dissolves, and the solution is clear and colorless.

pH Being specified separately when the drug is granted approval based on the Law.

Purity **(1)** Desamido substance—Perform the test with 20 μL of the sample solution obtained in the Assay (1) as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of the peak, having the relative retention time of about 1.3 to insulin human, is not more than 1.5%.

Operating conditions—

Proceed as directed in the operating conditions in the Assay (1).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay (1).

Test for required detectability: Pipet 1 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of insulin human obtained with 20 μL of this solution is equivalent to 1.4 to 2.6% of that with 20 μL of the sample solution.

System repeatability: Dissolve Insulin Human RS in 0.01 mol/L hydrochloric acid TS so that each mL contains about 4 Insulin Units. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of insulin human is not more than 2.0%.

(2) Dissolved insulin human—Centrifuge Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, and use the supernatant liquid as the sample solution. Separately, dissolve exactly Insulin Human RS in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains about 1.0 Insulin Units, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_T and A_S, of insulin human by the automatic integration method, and calculate the amount of dissolved insulin human by the following equation: not more than 0.5 Insulin Units per mL.

$$\text{Amount (mg) of dissolved insulin human (Insulin Unit /mL)} = (M_S \times F)/D \times A_T/A_S$$

M_S: Amount (mg) of Insulin Human RS taken

F: Labeled unit (Insulin Unit /mg) of Insulin Human RS

D: Volume (mL) of 0.01 mol/L hydrochloric acid TS used to dissolve Insulin Human RS

Operating conditions—

Proceed as directed in the operating conditions in the Assay (1).

System suitability—

System performance: When the procedure is run with 20 μL of insulin human desamido substance-containing TS under the above operating conditions, insulin human and insulin human desamido substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of insulin human is not more than 1.6.

System repeatability: When the test is repeated 4 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of insulin human is not more than 6.0%.

(3) High-molecular mass protein—Take a suitable volume of gently shaken Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, add 4 μL of 6 mol/L hydrochloric acid TS for each mL of the suspension, and mix until the solution becomes clear. Perform the test with 100 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 the amount of them by the area percentage method: the total amount of the peaks other than insulin human is not more than 2.5%.

Operating conditions—

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

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography.

Time span of measurement: From the retention time corresponding to the exclusion volume of the size-exclusion column to the completion of the elution of insulin human.

System suitability—

System performance: Proceed as directed in the system suitability in the Purity (2) under Insulin Human (Genetical Recombination).

Test for required detectability: Pipet 1 mL of the sample solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of insulin human obtained with 100 μL of this solution is equivalent to 1.4 to 2.6% of that with 100 μL of the sample solution.

Extractable volume ⟨*6.05*⟩ It meets the requirement.

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 1: it meets the requirement.

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

Assay **(1)** Insulin human—Pipet 10 mL of gently shaken Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, and add exactly 40 μL of 6 mol/L hydrochloric acid TS. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay under Insulin Human (Genetical Recombination).

Amount (Insulin Unit) of insulin human ($C_{257}H_{383}N_{65}O_{77}S_6$) in 1 mL

$= (M_S \times F)/D \times (A_{TI} + A_{TD})/(A_{SI} + A_{SD}) \times 1.004 \times 5/2$

M_S: Amount (mg) of Insulin Human RS taken
F: Labeled unit (Insulin Unit /mg) of Insulin Human RS
D: Volume (mL) of 0.01 mol/L hydrochloric acid TS used to dissolve Insulin Human RS

(2) Zinc—Pipet a volume of gently shaken Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, equivalent to 300 Insulin Units, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. If necessary, further add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, pipet a suitable volume of Standard Zinc Solution for Atomic Absorption Spectroscopy, dilute with 0.01 mol/L hydrochloric acid TS to make three solutions containing 0.20 μg, 0.60 μg and 1.20 μg of zinc (Zn: 65.38) in each mL, respectively, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectroscopy ⟨*2.23*⟩ according to the following conditions, using 0.01 mol/L hydrochloric acid TS as the blank, and calculate the content of zinc in the sample solution by using the calibration curve obtained from the absorbances of the standard solutions.

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

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, at a temperature between 2°C and 8°C avoiding freezing.

Add the following:

Biphasic Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension

二相性イソフェンインスリン ヒト(遺伝子組換え)水性懸濁注射液

Biphasic Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension is an aqueous suspension for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled Insulin Unit of insulin human (genetical recombination) ($C_{257}H_{383}N_{65}O_{77}S_6$: 5807.57). It contains not less than 10 μg and not more than 40 μg of zinc (Zn: 65.38) per the labeled 100 Insulin Units.

Method of preparation Prepare as directed under Injections, with Insulin Human (Genetical Recombination) In-

jection and Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension.

Description Biphasic Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension is a white aqueous suspension. When allowed to stand, it separates into a white precipitate and colorless supernatant liquid, and the precipitate returns to the suspension state on gentle shaking.

When it is examined microscopically, the precipitate mostly consists of fine, oblong crystals of 1 to 30 μm in major axis, and does not contain amorphous substances or large aggregates.

Identification Adjust Biphasic Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension to pH between 2.5 and 3.0 with dilute hydrochloric acid: the precipitate dissolves, and the solution is clear and colorless.

pH Being specified separately when the drug is granted approval based on the Law.

Purity (1) Desamido substance—Perform the test with 20 μL of the sample solution obtained in the Assay (1) as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of the peak, having the relative retention time of about 1.3 to insulin human, is not more than 1.5%.

Operating conditions—

Proceed as directed in the operating conditions in the Assay (1).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay (1).

Test for required detectability: Pipet 1 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of insulin human obtained with 20 μL of this solution is equivalent to 1.4 to 2.6% of that with 20 μL of the sample solution.

System repeatability: Dissolve Insulin Human RS in 0.01 mol/L hydrochloric acid TS so that each mL contains about 4 Insulin Units. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of insulin human is not more than 2.0%.

(2) High-molecular mass protein—Take a suitable volume of gently shaken Biphasic Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, add 4 μL of 6 mol/L hydrochloric acid TS for each mL of the suspension, and mix until the solution becomes clear. Perform the test with 100 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 the amount of them by the area percentage method: the total amount of the peaks other than insulin human is not more than 2.0%.

Operating conditions—

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

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography.

Time span of measurement: From the retention time corresponding to the exclusion volume of the size-exclusion column to the completion of the elution of insulin human.

System suitability—

System performance: Proceed as directed in the system suitability in the Purity (2) under Insulin Human (Genetical Recombination).

Test for required detectability: Pipet 1 mL of the sample solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of insulin human obtained with 100 μL of this solution is equivalent to 1.4 to 2.6% of that with 100 μL of the sample solution.

Extractable volume <*6.05*> It meets the requirement.

Foreign insoluble matter <*6.06*> Perform the test according to Method 1: it meets the requirement.

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

Soluble Insulin Human Being specified separately when the drug is granted approval based on the Law.

Assay (1) Insulin human—Pipet 10 mL of gently shaken Biphasic Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, and add exactly 40 μL of 6 mol/L hydrochloric acid TS. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay under Insulin Human (Genetical Recombination).

Amount (Insulin Unit) of insulin human ($C_{257}H_{383}N_{65}O_{77}S_6$) in 1 mL

$$= (M_S \times F)/D \times (A_{TI} + A_{TD})/(A_{SI} + A_{SD}) \times 1.004 \times 5/2$$

M_S: Amount (mg) of Insulin Human RS taken
F: Labeled unit (Insulin Unit /mg) of Insulin Human RS
D: Volume (mL) of 0.01 mol/L hydrochloric acid TS used to dissolve Insulin Human RS

(2) Zinc—Pipet a volume of gently shaken Biphasic Isophane Insulin Human (Genetical Recombination) Injectable Aqueous Suspension, equivalent to 300 Insulin Units, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. If necessary, further add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, pipet a suitable volume of Standard Zinc Solution for Atomic Absorption Spectroscopy, dilute with 0.01 mol/L hydrochloric acid TS to make three solutions containing 0.20 μg, 0.60 μg and 1.20 μg of zinc (Zn: 65.38) in each mL, respectively, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectroscopy <*2.23*> according to

the following conditions, using 0.01 mol/L hydrochloric acid TS as the blank, and calculate the content of zinc in the sample solution by using the calibration curve obtained from the absorbances of the standard solutions.

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

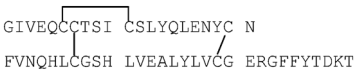
Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, at a temperature between 2°C and 8°C avoiding freezing.

Add the following:

Insulin Aspart (Genetical Recombination)

インスリン　アスパルト(遺伝子組換え)



$C_{256}H_{381}N_{65}O_{79}S_6$: 5825.54
[*116094-23-6*]

Insulin Aspart (Genetical Recombination) is an analogue of human insulin (genetical recombination), in which proline residue at 28th of B chain is substituted with aspartic acid. It is a peptide composed of A chain consisting of 21 amino acid residues and B chain consisting of 30 amino acid residues.

It contains not less than 92.6% and not more than 109.5% of insulin aspart (genetical recombination) ($C_{256}H_{381}N_{65}O_{79}S_6$), calculated on the dried and residue on ignition-free basis.

0.0350 mg of Insulin Aspart (Genetical Recombination) is equivalent to 1 Insulin Unit.

Description Insulin Aspart (Genetical Recombination) occurs as a white powder.

It is practically insoluble in water and in ethanol (95).

It dissolves in 0.01 mol/L hydrochloric acid TS.

It is hygroscopic.

Identification Weigh a suitable amount of Insulin Aspart (Genetical Recombination), and dissolve in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains 2.0 mg. Separately, dissolve Insulin Aspart RS in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains 2.0 mg. Transfer 25 μL each of these solutions into clean test tubes, add 100 μL of HEPES buffer solution (pH 7.5) and 20 μL of V8-protease TS, and allow to react at 25°C for 6 hours. Then add 145 μL of ammonium sulfate buffer solution to stop the reaction, 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 compare the peak (peak 1) eluted just after the peak of the solvent and the succeeding three peaks (peaks 2, 3 and 4) with apparently higher peak height in the chromatograms obtained from these solutions: the similar peaks are observed at the same retention times.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 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 (not exceeding 5 μm in particle diameter).

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

Mobile phase A: A mixture of water, ammonium sulfate buffer solution and acetonitrile for liquid chromatography (7:2:1).

Mobile phase B: A mixture of water, acetonitrile for liquid chromatography and ammonium sulfate buffer solution (2:2:1).

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	90 → 30	10 → 70
60 – 65	30 → 0	70 → 100
65 – 70	0	100

Flow rate: 1 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the symmetry factors of the peaks 2 and 3 are not more than 1.5, respectively, and the resolution between these peaks is not less than 8.

Purity **(1)** Related substances—Perform the test with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the amount of the peak of B28isoAsp insulin aspart, having the relative retention time of about 0.9 to insulin aspart, is not more than 0.3%, the total amount of the peak of A21Asp insulin aspart and B3Asp insulin aspart, having the relative retention time of about 1.3, and the peak of B3isoAsp insulin aspart, having the relative retention time of about 1.5, is not more than 1.0%, and the total amount of the peaks other than insulin aspart and the peaks mentioned above is not more than 0.5%.

Operating conditions—

Detector, column, column temperature, mobile phase A, mobile phase B, flowing of mobile phase and flow rate: Proceed as directed in the operating conditions in the

Assay.

Time span of measurement: From 4 minutes to 50 minutes after injection of the sample solution.

System suitability—

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

Test for required detectability: Pipet 5 mL of the solution for system suitability test obtained in the Assay, add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL. Confirm that the area percentage of the peak of B28isoAsp insulin aspart obtained with 10 μL of this solution is equivalent to 80 to 120% of that with 10 μL of the solution for system suitability test.

(2) High-molecular proteins—Store the sample solution at a temperature between 2°C and 8°C, and use within 48 hours after preparation. Dissolve 4 mg of Insulin Aspart (Genetical Recombination) in 1 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test with 100 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than insulin aspart is not more than 0.3%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 276 nm).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of a solution of L-arginine (1 in 1000), acetonitrile for liquid chromatography and acetic acid (100) (13:4:3).

Flow rate: 0.5 mL per minute.

Time span of measurement: From the retention time corresponding to the exclusion volume of the size-exclusion column to the completion of the elution of insulin aspart.

System suitability—

Test for required detectability: Allow Insulin Aspart (Genetic Recombination) to stand at ordinary temperature for about 10 days, which results in containing about 0.4% of high-molecular proteins, dissolve in 0.01 mol/L hydrochloride TS so that each mL contains about 4 mg of insulin aspart, and use this solution as the solution for system suitability test. Store the solution for system suitability test at a temperature between 2°C and 8°C, and use within 7 days. Pipet 5 mL of the solution for system suitability test, and add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL. Confirm that the area percentage of the peak of insulin aspart dimer obtained with 100 μL of this solution is equivalent to 80 to 120% of that with 100 μL of the solution for system suitability test.

System performance: When the procedure is run with 100 μL of the solution for system suitability test under the above operating conditions, insulin aspart polymer (retention time: 13 to 17 minutes), insulin aspart dimer (retention time: about 17.5 minutes) and insulin aspart (retention time: 18 to 20 minutes) are eluted in this order, and determine the peak height of the dimer and the height of the bottom between the peaks of the dimer and the monomer: the peak-valley ratio is not less than 2.0.

System repeatability: When the test is repeated 6 times with 100 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of insulin aspart is not more than 2.0%.

(3) Host cell proteins—Being specified separately when the drug is granted approval based on the Law.

(4) DNA—Being specified separately when the drug is granted approval based on the Law.

Loss on drying <*2.41*> Not more than 10.0% (0.2 g, 105°C, 24 hours).

Residue on ignition <*2.44*> Not more than 6.0% (0.2 g).

Assay Store the sample solution and the standard solution at a temperature between 2°C and 8°C, use the sample solution within 24 hours after preparation, and use the standard solution within 48 hours after preparation. Weigh accurately a suitable amount of Insulin Aspart (Genetical Recombination), dissolve in 0.01 mol/L hydrochloric acid TS so that each mL contains 4.0 mg, and use this solution as the sample solution. Separately, dissolve Insulin Aspart RS in 0.01 mol/L hydrochloric acid TS so that each mL contains 4.0 mg, 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 total areas, A_T and A_S, of the peak of B28isoAsp insulin aspart (relative retention time to insulin aspart: about 0.9), the peak of insulin aspart (retention time: 20 to 24 minutes), the peak of A21Asp insulin aspart and B3Asp insulin aspart (usually eluted together having the relative retention time of about 1.3) and the peak of B3isoAsp insulin aspart (relative retention time: about 1.5) in each solution.

$$\text{Amount (mg) of insulin aspart } (C_{256}H_{381}N_{65}O_{79}S_6) = M_S \times A_T/A_S$$

M_S: Total amount (mg) of insulin aspart, B28isoAsp insulin aspart, A21Asp insulin aspart and B3Asp insulin aspart, and B3isoAsp insulin aspart in 1 mL of the standard solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 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 (not exceeding 5 μm in particle diameter).

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

Mobile phase A: Dissolve 142.0 g of anhydrous sodium sulfate in water, add 13.5 mL of phosphoric acid, and add

water to make 5 L. Adjust to pH 3.6 with sodium hydroxide TS. To 4500 mL of this solution add 500 mL of acetonitrile for liquid chromatography.

Mobile phase B: A mixture of water and acetonitrile for liquid chromatography (1:1).

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 – 35	58	42
35 – 40	58 → 20	42 → 80
40 – 45	20	80
45 – 46	20 → 58	80 → 42
46 – 60	58	42

Flow rate: 1 mL per minute.

System suitability—

System performance: Dissolve Insulin Aspart (Genetical Recombination) in 0.01 mol/L sodium dihydrogen phosphate TS (pH 7.5) so that each mL contains 8 mg, and allow to stand at ordinary temperature for 10 to 15 days. To 1 mL of this solution add 1 mL of 0.01 mol/L hydrochloric acid TS, allow to stand at ordinary temperature for 1 to 3 days, and use this solution as the solution for system suitability test. The solution for system suitability test contains 0.1 to 2.2% of B28isoAsp insulin aspart, and not less than 1% of B3Asp insulin aspart and A21Asp insulin aspart. Store the solution for system suitability test at a temperature between 2°C and 8°C, and use within 72 hours. When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, B28isoAsp insulin aspart, insulin aspart, A21Asp insulin aspart and B3Asp insulin aspart, and B3isoAsp insulin aspart are eluted in this order with the resolution between the peak of insulin aspart and the peak of A21Asp insulin aspart and B3Asp insulin aspart being not less than 2.0.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of A_S is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Not exceeding −18°C.

Iohexol Injection

イオヘキソール注射液

Change the Containers and storage as follows:

Containers and storage Containers—Hermetic containers. Colored containers and plastic containers for aqueous injections may be used.

Add the following:

Irbesartan Tablets

イルベサルタン錠

Irbesartan Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of irbesartan ($C_{25}H_{28}N_6O$: 428.53).

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

Identification To a quantity of powdered Irbesartan Tablets, equivalent to about 25 mg of Irbesartan, add 2 mL of acetone, shake, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Evaporate the filtrate to dryness, and determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩: it exhibits absorptions at the wave numbers of about 1733 cm^{-1}, 1617 cm^{-1}, 1435 cm^{-1} and 758 cm^{-1}.

Uniformity of dosage unit ⟨*6.02*⟩ Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Irbesartan Tablets add 1.5 mL of water, shake vigorously to disintegrate, and add 15 mL of methanol. Shake vigorously for 15 minutes, add methanol to make exactly 20 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 20 mg of irbesartan ($C_{25}H_{28}N_6O$), and add a mixture of water and acetonitrile for liquid chromatography (3:2) to make exactly 20 mL. Pipet 2.5 mL of this solution, add a mixture of water and acetonitrile for liquid chromatography (3:2) to make exactly 20 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of irbesartan } (C_{25}H_{28}N_6O) = M_S \times A_T/A_S \times 16/V$$

M_S: Amount (mg) of irbesartan for assay taken, 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 2nd fluid for dissolution test as the dissolution medium, the dissolution rate in 45 minutes of 50-mg and 100-mg tablets is not less than 85%, respectively, and that in 60 minutes of 200-mg tablet is not less than 70%.

Start the test with 1 tablet of Irbesartan 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 22 μg of irbesartan ($C_{25}H_{28}N_6O$), and use this solution as the sample solution. Separately, weigh accurately about 44 mg of irbesartan for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Irbesartan), and dissolve in methanol to make

exactly 20 mL. Pipet 2 mL of this solution, add the dissolution medium to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 244 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the dissolution medium as the control.

Dissolution rate (%) with respect to the labeled amount of irbesartan ($C_{25}H_{28}N_6O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45$

M_S: Amount (mg) of irbesartan for assay taken, calculated on the anhydrous basis
C: Labeled amount (mg) of irbesartan ($C_{25}H_{28}N_6O$) in 1 tablet

Assay To 10 Irbesartan Tablets add 15 mL of water, shake vigorously to disintegrate, and add 150 mL of methanol. Shake vigorously for 15 minutes, add methanol to make exactly 200 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 20 mg of irbesartan ($C_{25}H_{28}N_6O$), and add a mixture of water and acetonitrile for liquid chromatography (3:2) to make exactly 20 mL. Pipet 2.5 mL of this solution, add a mixture of water and acetonitrile for liquid chromatography (3:2) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of irbesartan for assay (separately determine the water <2.48> in the same manner as Irbesartan), and dissolve in methanol to make exactly 10 mL. Pipet 2.5 mL of this solution, add a mixture of water and acetonitrile for liquid chromatography (3:2) to make exactly 50 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 irbesartan in each solution.

Amount (mg) of irbesartan ($C_{25}H_{28}N_6O$) in 1 tablet
$= M_S \times A_T/A_S \times 16/V$

M_S: Amount (mg) of irbesartan for assay taken, 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 octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: To 5.5 mL of phosphoric acid add 950 mL of water, adjust to pH 3.0 with triethylamine, and add water to make 1000 mL. To 3 volume of this solution add 2 volume of acetonitrile for liquid chromatography.

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

System suitability—

System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of irbesartan are not less than 10,000 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 irbesartan is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Irbesartan and Amlodipine Besilate Tablets

イルベサルタン·アムロジピンベシル酸塩錠

Irbesartan and Amlodipine Besilate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of irbesartan ($C_{25}H_{28}N_6O$: 428.53) and amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$: 567.05).

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

Identification (1) Perform the test with 5 μL each of the sample solution and standard solution obtained in the Assay (1) as directed under Liquid Chromatography <2.01> according to the following conditions: the retention time of the peak of irbesartan in the chromatogram from the sample solution is the same with that of the principal peak in the chromatogram from the standard solution, and both absorption 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 (1).

Detector: A photodiode array detector (wavelength: 237 nm, spectrum range of measurement: 210 – 400 nm).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay (1).

(2) Perform the test with 5 μL each of the sample solution and standard solution obtained in the Assay (2) as directed under Liquid Chromatography <2.01> according to the following conditions: the retention time of the peak of amlodipine in the chromatogram from the sample solution is the same with that of the principal peak in the chromatogram from the standard solution, and both absorption 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 (1).

Detector: A photodiode array detector (wavelength: 237 nm, spectrum range of measurement: 210 – 400 nm).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay (2).

Uniformity of dosage unit ⟨*6.02*⟩ **(1)** Irbesartan—Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Irbesartan and Amlodipine Besilate Tablets add 4 mL of 0.02 mol/L phosphate buffer solution (pH 3.0), and sonicate. Add 16 mL of methanol, shake vigorously until the tablet is disintegrated completely, and add the mobile phase to make exactly 100 mL. Pipet V mL of this solution, add the mobile phase to make exactly V' mL so that each mL contains about 1 mg of irbesartan ($C_{25}H_{28}N_6O$), 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. Then, proceed as directed under the Assay (1).

Amount (mg) of irbesartan ($C_{25}H_{28}N_6O$)
$= M_S \times A_T/A_S \times V'/V \times 2$

M_S: Amount (mg) of irbesartan for assay taken, calculated on the anhydrous basis

(2) Amlodipine besilate—Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Irbesartan and Amlodipine Besilate Tablets add 4 mL of 0.02 mol/L phosphate buffer solution (pH 3.0), and sonicate. Add 16 mL of methanol, shake vigorously until the tablet is disintegrated completely, and add the mobile phase to make exactly 100 mL. Pipet V mL of this solution, add the mobile phase to make exactly V' mL so that each mL contains about 69 μg of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), 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. Then, proceed as directed under the Assay (2).

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

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

Dissolution ⟨*6.10*⟩ **(1)** Irbesartan—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 Irbesartan and Amlodipine Besilate Tablets is not less than 70%.

Start the test with 1 tablet of Irbesartan and Amlodipine Besilate Tablets, withdraw not less than 15 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL or more of the filtrate, pipet V mL of the subsequent filtrate, and add the mobile phase to make exactly V' mL so that each mL contains about 0.11 mg of irbesartan ($C_{25}H_{28}N_6O$). Pipet 2 mL of this solution, add exactly 2 mL of the mobile phase, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of irbesartan for assay (separately determine the water ⟨*2.48*⟩ in the same manner as Irbesartan), dissolve in the mobile phase to make exactly 25 mL, and use this solution as the irbesartan standard stock solution. Pipet 7 mL of the irbesartan standard stock solution, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the dissolution medium, 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 irbesartan in each solution.

Dissolution rate (%) with respect to the labeled amount of irbesartan ($C_{25}H_{28}N_6O$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 504$

M_S: Amount (mg) of irbesartan for assay taken, calculated on the anhydrous basis

C: Labeled amount (mg) of irbesartan ($C_{25}H_{28}N_6O$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay (1).

System suitability—

System performance: To 7 mL of the irbesartan standard stock solution and 5 mL of the amlodipine besilate standard stock solution obtained in (2) add the mobile phase to make 50 mL. To 5 mL of this solution add 5 mL of the dissolution medium. When the procedure is run with 10 μL of this solution under the above operating conditions, amlodipine and irbesartan are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of irbesartan is not more than 2.0%.

(2) Amlodipine besilate—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 Irbesartan and Amlodipine Besilate Tablets is not less than 75%.

Start the test with 1 tablet of Irbesartan and Amlodipine Besilate Tablets, withdraw not less than 15 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add the mobile phase to make exactly V' mL so that each mL contains about 7.7 μg of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$). Pipet 2 mL of this solution, add exactly 2 mL of the mobile phase, and use this solution as the sample solution. Separately,

weigh accurately about 26 mg of Amlodipine Besilate RS (separately determine the water <2.48> in the same manner as Amlodipine Besilate), and dissolve in the mobile phase to make exactly 50 mL. Pipet 15 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the amlodipine besilate standard stock solution. Pipet 5 mL of the amlodipine besilate standard stock solution, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the dissolution medium, 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 amlodipine in each solution.

Dissolution rate (%) with respect to the labeled amount of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 27$

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

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

Operating conditions—

Proceed as directed in the operating conditions in the Assay (1).

System suitability—

System performance: To 7 mL of the irbesartan standard stock solution obtained in (1) and 5 mL of the amlodipine besilate standard stock solution add the mobile phase to make 50 mL. To 5 mL of this solution add 5 mL of the dissolution medium. When the procedure is run with 10 μL of this solution under the above operating conditions, amlodipine and irbesartan are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amlodipine is not more than 2.0%.

Assay (1) Irbesartan—To 10 tablets of Irbesartan and Amlodipine Besilate Tablets add 20 mL of 0.02 mol/L phosphate buffer solution (pH 3.0), and sonicate. Add 120 mL of methanol, shake vigorously until the tablets are disintegrated completely, and add the mobile phase to make exactly 200 mL. Pipet V mL of this solution, add the mobile phase to make exactly V' mL so that each mL contains about 1 mg of irbesartan ($C_{25}H_{28}N_6O$), 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 irbesartan for assay (separately determine the water <2.48> in the same manner as Irbesartan), dissolve in methanol to make exactly 25 mL, and use this solution as the irbesartan standard stock solution. Pipet 10 mL of the irbesartan standard stock solution, add 2 mL of methanol, add 0.02 mol/L phosphate buffer solution (pH 3.0) 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, and determine the peak areas, A_T and A_S, of irbesartan in each solution.

Amount (mg) of irbesartan ($C_{25}H_{28}N_6O$) in 1 tablet
$= M_S \times A_T/A_S \times V'/V \times 2/5$

M_S: Amount (mg) of irbesartan for assay taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 75 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (2.2 μm in particle diameter).

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

Mobile phase: A mixture of methanol and 0.02 mol/L phosphate buffer solution (pH 3.0) (3:2).

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

System suitability—

System performance: To 10 mL of the irbesartan standard stock solution and 2 mL of the amlodipine besilate standard stock solution obtained in (2) add 0.02 mol/L phosphate buffer solution (pH 3.0) to make 20 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, amlodipine and irbesartan 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 peak area of irbesartan is not more than 1.0%.

(2) Amlodipine besilate—To 10 tablets of Irbesartan and Amlodipine Besilate Tablets add 20 mL of 0.02 mol/L phosphate buffer solution (pH 3.0), and sonicate. Add 120 mL of methanol, shake vigorously until the tablets are disintegrated completely, and add the mobile phase to make exactly 200 mL. Pipet V mL of this solution, add the mobile phase to make exactly V' mL so that each mL contains about 69 μg of amlodipine besilate ($C_{20}H_{25}ClN_2O_5.C_6H_6O_3S$), 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 35 mg of Amlodipine Besilate RS (separately determine the water <2.48> in the same manner as Amlodipine Besilate), dissolve in methanol to make exactly 50 mL, and use this solution as the amlodipine besilate standard stock solution. Pipet 2 mL of the amlodipine besilate standard stock solution, add 10 mL of methanol, add 0.02 mol/L phosphate buffer solution (pH 3.0) 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, and determine the peak areas, A_T and A_S, of amlodipine in each solution.

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

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

Operating conditions—

Proceed as directed in the operating conditions in (1).

System suitability—

System performance: To 10 mL of the irbesartan standard stock solution obtained in (1) and 2 mL of the amlodipine besilate standard stock solution add 0.02 mol/L phosphate buffer solution (pH 3.0) to make 20 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, amlodipine and irbesartan 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 peak area of amlodipine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Isosorbide Mononitrate 70%/ Lactose 30%

70%一硝酸イソソルビド乳糖末

Change the Identification (2) as follows:

Identification

(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 for Identification: both spectra exhibit similar intensities of absorption at the same wave numbers.

Anhydrous Lactose

無水乳糖

Change the Identification and Isomer ratio as follows:

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 for Identification: both spectra exhibit similar intensities of absorption at the same wave numbers.

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 sonicate 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 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: Maintain the temperature at 80°C for 1 minute after injection, raise to 150°C at a rate of 35°C per minute, then raise to 300°C at a rate of 12°C per minute, and maintain at 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 to 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 5.0%.◆

Lactose Hydrate

乳糖水和物

Change the Identification as follows:

Identification Determine the infrared absorption spectrum of Lactose Hydrate, 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 Lactose RS for Identification: both spectra exhibit similar intensities of absorption at the same wave numbers.

Delete the following Monographs:

Lanatoside C

ラナトシドC

Lanatoside C Tablets

ラナトシドC錠

Add the following:

Magnesium Aluminosilicate

ケイ酸アルミン酸マグネシウム

Magnesium Aluminosilicate contains not less than 27.0% and not more than 34.3% of aluminum oxide (Al_2O_3: 101.96), not less than 20.5% and not more than 27.7% of magnesium oxide (MgO: 40.30), and not less than 14.4% and not more than 21.7% of silicon dioxide (SiO_2: 60.08), calculated on the dried basis.

Description Magnesium Aluminosilicate occurs as a white, powder or grain.

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

When heat 1 g of Magnesium Aluminosilicate with 10 mL of dilute hydrochloric acid, most of it dissolves.

Identification **(1)** To 0.5 g of Magnesium Aluminosilicate add 5 mL of diluted sulfuric acid (1 in 3), heat until white fumes are evolved, cool, add 20 mL of water, and filtrate. Neutralize the filtrate with ammonia TS, and filter the precipitate produced. Dissolve the residue in dilute hydrochloric acid: the solution responds to Qualitative Tests <*1.09*> for aluminum salt.

(2) The filtrate obtained in (1) responds to Qualitative Tests <*1.09*> (2) for magnesium salt.

(3) Wash the residue obtained in (1) with 30 mL of water, add 2 mL of a solution of methylene blue trihydrate (1 in 10,000), and wash with 30 mL of water: the precipitate has a blue color.

Purity **(1)** Soluble salts—To 10.0 g of Magnesium Aluminosilicate add 150 mL of water, boil gently for 15 minutes while shaking thoroughly. After cooling, add water to make 150 mL, and centrifuge. To 75 mL of the supernatant liquid add water to make 100 mL, and use this solution as the sample solution. Evaporate 25 mL of the sample solution on a water bath to dryness, then ignite the residue at 700°C for 2 hours: the mass of the residue is not more than 20 mg.

(2) Alkalinity—To 20 mL of the sample solution obtained in (1), add 2 drops of phenolphthalein TS, and add 0.1 mol/L hydrochloric acid VS until the solution becomes colorless: the consumed volume is not more than 0.50 mL.

(3) Chloride <*1.03*>—To 10 mL of the sample solution obtained in (1), 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 with 0.75 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053%).

(4) Sulfate <*1.14*>—To 2 mL of the sample solution obtained in (1), 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 with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(5) Heavy metals <*1.07*>—To 2.67 g of Magnesium Aluminosilicate add 20 mL of water and 8 mL of hydrochloric acid, evaporate to dryness on a water bath. To the residue add 5 mL of dilute acetic acid and 20 mL of water, boil for 2 minutes, add 0.4 g of hydroxylammonium chloride, and heat to boiling. After cooling, add water to make exactly 100 mL, and filter. Pipet 25 mL of the filtrate, adjust to pH 3.0 with dilute acetic acid or ammonia TS, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 2 mL of hydrochloric acid to dryness on a water bath, add 2.0 mL of Standard Lead Solution, 0.1 g of hydroxylammonium chloride and water to make 25 mL, adjust to pH 3.0 with dilute acetic acid or ammonia TS, and add water to make 50 mL (not more than 30 ppm).

(6) Iron—To 0.11 g of Magnesium Aluminosilicate add 8 mL of 2 mol/L nitric acid TS, boil for 1 minute, cool, add water to make exactly 100 mL, and centrifuge. Pipet 30 mL of the supernatant liquid, add water to make 45 mL, add 2 mL of hydrochloric acid, and shake. Add 50 mg of ammonium peroxodisulfate and 3 mL of a solution of ammonium thiocyanate (3 in 10), and shake: the solution is not more colored than the following control solution (not more than 0.03%).

Control solution: Pipet 1 mL of Standard Iron Solution, add water to make 45 mL, add 2 mL of hydrochloric acid, shake, and proceed in the same manner.

(7) Arsenic <*1.11*>—To 1.0 g of Magnesium Aluminosilicate add 10 mL of water and 1 mL of sulfuric acid, and shake thoroughly. After cooling, perform the test using this solution as the test solution (not more than 2

ppm).

Loss on drying ⟨*2.41*⟩ Not more than 20.0% (1 g, 110°C, 7 hours).

Acid-consuming capacity ⟨*6.04*⟩ Weigh accurately about 0.2 g of Magnesium Aluminosilicate, transfer to a glass-stoppered flask, add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper the flask tightly, shake at 37 ± 2°C for 1 hour, and filter. Pipet 50 mL of the filtrate, and titrate ⟨*2.50*⟩ the excess hydrochloric acid, while stirring thoroughly, with 0.1 mol/L sodium hydroxide VS until the pH of the solution becomes 3.5. Perform a blank determination in the same manner. The consumed volume of 0.1 mol/L hydrochloric acid VS is not less than 250 mL per g of Magnesium Aluminosilicate calculated on the dried basis.

Assay (1) Aluminum oxide—Weigh accurately about 1.25 g of Magnesium Aluminosilicate, transfer to a conical flask, add 10 mL of 3 mol/L hydrochloric acid TS and 50 mL of water, and heat on a water bath for 15 minutes. To the solution add 8 mL of hydrochloric acid, heat on a water bath for 10 minutes. After cooling, transfer to a 250-mL volumetric flask, rinse the flask with water, and add water to make 250 mL. Centrifuge the solution, and use the supernatant liquid as the sample solution. Pipet 20 mL of the sample solution, add exactly 20 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS. To this solution add 15 mL of acetic acid–ammonium acetate buffer solution (pH 4.8) and 20 mL of water, and boil for 5 minutes. After cooling, add 50 mL of ethanol (95), and titrate ⟨*2.50*⟩ with 0.05 mol/L zinc sulfate VS until the color of the solution changes from light dark green to light red (indicator: 2 mL of dithizone TS). Perform a blank determination in the same manner.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.549 mg of Al_2O_3

(2) Magnesium oxide—Pipet 50 mL of the sample solution obtained in (1), add 50 mL of water and 25 mL of a solution of 2,2′,2″-nitrilotriethanol (1 in 2), shake thoroughly, then add 25 mL of ammonia-ammonium chloride buffer solution (pH 10.7), and titrate ⟨*2.50*⟩ with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue lasting for 30 seconds (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.015 mg of MgO

(3) Silicon dioxide—Weigh accurately about 1 g of Magnesium Aluminosilicate, add 30 mL of dilute hydrochloric acid, and evaporate to dryness on a water bath. Moisten the residue with hydrochloric acid, evaporate to dryness on a water bath. To the residue add 8 mL of hydrochloric acid, stir, then add 25 mL of hot water, and stir again. After allowing to stand, filter the supernatant liquid through a filter paper for quantitative analysis, add 10 mL of hot water to the residue, stir, and decant the supernatant liquid on a filter paper to filter. Then wash the residue with three 10-mL portions of hot water, add 50 mL of water to the residue, and heat on a water bath for 15 minutes. Transfer the residue onto the filter paper, wash the residue with hot water until the last 5 mL of washing yields no precipitate on addition of 1 mL of silver nitrate TS, place the residue and the filter paper in a platinum crucible, ignite to ash, and then ignite at 800 ± 25°C for 1 hour. After cooling, weigh the crucible, and designate the mass as *a* (g). Then add 6 mL of hydrofluoric acid, evaporate to dryness, ignite for 5 minutes, weigh the crucible after cooling, and designate the mass as *b* (g).

Amount (g) of silicon dioxide (SiO_2) = $a - b$

Containers and storage Containers—Well-closed containers.

Add the following:

Magnesium Aluminometasilicate

メタケイ酸アルミン酸マグネシウム

Magnesium Aluminometasilicate contains not less than 29.1% and not more than 35.5% of aluminum oxide (Al_2O_3: 101.96), not less than 11.4% and not more than 14.0% of magnesium oxide (MgO: 40.30), and not less than 29.2% and not more than 35.6% of silicon dioxide (SiO_2: 60.08), calculated on the dried basis.

Description Magnesium Aluminometasilicate occurs as a white, powder or grain.

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

When heat 1 g of Magnesium Aluminometasilicate with 10 mL of dilute hydrochloric acid, most of it dissolves.

Identification (1) To 0.5 g of Magnesium Aluminometasilicate add 5 mL of diluted sulfuric acid (1 in 3), heat until white fumes are evolved, cool, add 20 mL of water, and filtrate. Neutralize the filtrate with ammonia TS, and filter the precipitate produced. Dissolve the residue in dilute hydrochloric acid: the solution responds to Qualitative Tests ⟨*1.09*⟩ for aluminum salt.

(2) The filtrate obtained in (1) responds to Qualitative Tests ⟨*1.09*⟩ (2) for magnesium salt.

(3) Wash the residue obtained in (1) with 30 mL of water, add 2 mL of a solution of methylene blue trihydrate (1 in 10,000), and wash with 30 mL of water: the precipitate has a blue color.

Purity (1) Soluble salts—To 10.0 g of Magnesium Aluminometasilicate add 150 mL of water, boil gently for 15 minutes while shaking thoroughly. After cooling, add water to make 150 mL, and centrifuge. To 75 mL of the supernatant liquid add water to make 100 mL, and use this solution

as the sample solution. Evaporate 25 mL of the sample solution on a water bath to dryness, then ignite the residue at 700°C for 2 hours: the mass of the residue is not more than 20 mg.

(2) Alkalinity—To 20 mL of the sample solution obtained in (1), add 2 drops of phenolphthalein TS, and add 0.1 mol/L hydrochloric acid VS until the solution becomes colorless: the consumed volume is not more than 0.50 mL.

(3) Chloride <*1.03*>—To 10 mL of the sample solution obtained in (1), 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 with 0.75 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053%).

(4) Sulfate <*1.14*>—To 2 mL of the sample solution obtained in (1), 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 with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(5) Heavy metals <*1.07*>—To 2.67 g of Magnesium Aluminometasilicate add 20 mL of water and 8 mL of hydrochloric acid, evaporate to dryness on a water bath. To the residue add 5 mL of dilute acetic acid and 20 mL of water, boil for 2 minutes, add 0.4 g of hydroxylammonium chloride, and heat to boiling. After cooling, add water to make exactly 100 mL, and filter. Pipet 25 mL of the filtrate, adjust to pH 3.0 with dilute acetic acid or ammonia TS, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 2 mL of hydrochloric acid to dryness on a water bath, add 2.0 mL of Standard Lead Solution, 0.1 g of hydroxylammonium chloride and water to make 25 mL, adjust to pH 3.0 with dilute acetic acid or ammonia TS, and add water to make 50 mL (not more than 30 ppm).

(6) Iron—To 0.11 g of Magnesium Aluminometasilicate add 8 mL of 2 mol/L nitric acid TS, boil for 1 minute, cool, add water to make exactly 100 mL, and centrifuge. Pipet 30 mL of the supernatant liquid, add water to make 45 mL, add 2 mL of hydrochloric acid, and shake. Add 50 mg of ammonium peroxodisulfate and 3 mL of a solution of ammonium thiocyanate (3 in 10), and shake: the solution is not more colored than the following control solution (not more than 0.03%).

Control solution: Pipet 1 mL of Standard Iron Solution, add water to make 45 mL, add 2 mL of hydrochloric acid, shake, and proceed in the same manner.

(7) Arsenic <*1.11*>—To 1.0 g of Magnesium Aluminometasilicate add 10 mL of water and 1 mL of sulfuric acid, and shake thoroughly. After cooling, perform the test using this solution as the test solution (not more than 2 ppm).

Loss on drying <*2.41*> Not more than 20.0% (1 g, 110°C, 7 hours).

Acid-consuming capacity <*6.04*> Weigh accurately about 0.2 g of Magnesium Aluminometasilicate, transfer to a glass-stoppered flask, add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper the flask tightly, shake at 37 ± 2°C for 1 hour, and filter. Pipet 50 mL of the filtrate, and titrate <*2.50*> the excess hydrochloric acid, while stirring thoroughly, with 0.1 mol/L sodium hydroxide VS until the pH of the solution becomes 3.5. Perform a blank determination in the same manner. The consumed volume of 0.1 mol/L hydrochloric acid VS is not less than 210 mL per g of Magnesium Aluminometasilicate calculated on the dried basis.

Assay (1) Aluminum oxide—Weigh accurately about 1.25 g of Magnesium Aluminometasilicate, transfer to a conical flask, add 10 mL of 3 mol/L hydrochloric acid TS and 50 mL of water, and heat on a water bath for 15 minutes. To the solution add 8 mL of hydrochloric acid, heat on a water bath for 10 minutes. After cooling, transfer to a 250-mL volumetric flask, rinse the flask with water, and add water to make 250 mL. Centrifuge the solution, and use the supernatant liquid as the sample solution. Pipet 20 mL of the sample solution, add exactly 20 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS. To this solution add 15 mL of acetic acid–ammonium acetate buffer solution (pH 4.8) and 20 mL of water, and boil for 5 minutes. After cooling, add 50 mL of ethanol (95), and titrate <*2.50*> with 0.05 mol/L zinc sulfate VS until the color of the solution changes from light dark green to light red (indicator: 2 mL of dithizone TS). Perform a blank determination in the same manner.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.549 mg of Al_2O_3

(2) Magnesium oxide—Pipet 50 mL of the sample solution obtained in (1), add 50 mL of water and 25 mL of a solution of 2,2′,2″-nitrilotriethanol (1 in 2), shake thoroughly, then add 25 mL of ammonia-ammonium chloride buffer solution (pH 10.7), and titrate <*2.50*> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue lasting for 30 seconds (indicator: 40 mg of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.015 mg of MgO

(3) Silicon dioxide—Weigh accurately about 1 g of Magnesium Aluminometasilicate, add 30 mL of dilute hydrochloric acid, and evaporate to dryness on a water bath. Moisten the residue with hydrochloric acid, evaporate to dryness on a water bath. To the residue add 8 mL of hydrochloric acid, stir, then add 25 mL of hot water, and stir again. After allowing to stand, filter the supernatant liquid through a filter paper for quantitative analysis, add 10 mL of hot water to the residue, stir, and decant the supernatant liquid on a filter paper to filter. Then wash the residue with three 10-mL portions of hot water, add 50 mL of water to the residue, and heat on a water bath for 15 minutes. Transfer the residue onto the filter paper, wash the residue with hot water until the last 5 mL of washing yields no precipitate on addition of 1 mL of silver nitrate TS, place the

residue and the filter paper in a platinum crucible, ignite to ash, and then ignite at 800 ± 25°C for 1 hour. After cooling, weigh the crucible, and designate the mass as *a* (g). Then add 6 mL of hydrofluoric acid, evaporate to dryness, ignite for 5 minutes, weigh the crucible after cooling, and designate the mass as *b* (g).

Amount (g) of silicon dioxide (SiO_2) = $a - b$

Containers and storage Containers—Well-closed containers.

D-Mannitol

D-マンニトール

Change the Purity (1) as follows:

Purity **(1)** Clarity and color of solution—Dissolve 5.0 g of D-Mannitol in water to make 50 mL, and use this solution as the test solution. Perform the test with the test solution as directed under Turbidity Measurement <*2.61*>: the solution is clear. Perform the test with the test solution according to Method 2 under Methods for Color Matching <*2.65*>: the solution is colorless.

Delete the following Monographs:

Mercurochrome

マーキュロクロム

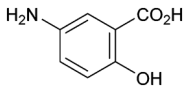
Mercurochrome Solution

マーキュロクロム液

Add the following:

Mesalazine

メサラジン



$C_7H_7NO_3$: 153.14
5-Amino-2-hydroxybenzoic acid
[*89-57-6*]

Mesalazine, when dried, contains not less than 98.5% and not more than 101.0% of mesalazine ($C_7H_7NO_3$).

Description Mesalazine occurs as white, light gray or reddish-white, crystals or crystalline powder.

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

It dissolves in dilute hydrochloric acid.

Identification **(1)** Determine the absorption spectrum of a solution of Mesalazine in 0.1 mol/L hydrochloric acid TS (1 in 80,000) 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 Mesalazine, 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)** Clarity and color of solution—Perform this procedure while keeping the solution at 40°C. A solution obtained by dissolving 0.5 g of Mesalazine in 20 mL of 1 mol/L hydrochloric acid TS is clear, and its absorbance at 440 nm and 650 nm, determined immediately as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, is not more than 0.15 and not more than 0.10, respectively.

(2) Chloride <*1.03*>—Dissolve 0.30 g of Mesalazine in 6 mL of dilute nitric acid and 40 mL of water, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.095%).

(3) Sulfate—To 1.0 g of Mesalazine add 20 mL of water, shake for 1 minute, and filter. To 15 mL of the filtrate add 0.5 mL of acetic acid (31), then add 2.5 mL of the following solution A, and use this solution as the test solution. Solution A: To 3 mL of barium chloride TS add 4.5 mL of a solution of potassium sulfate in diluted ethanol (3 in 10) (181 in 10,000,000), shake, and allow to stand for 1 minute. Prepare the control solution by adding 14.7 mL of water and 0.5 mL of acetic acid (31) to 0.31 mL of 0.005 mol/L sulfuric acid VS, and then proceeding in the same manner for the test solution. Compare the test solution and the control solution after allowing to stand for 5 minutes: the turbidity of the test solution is not more intense than that of the control solution (not more than 0.02%).

(4) Heavy metals <*1.07*>—Proceed with 0.5 g of Mesalazine according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Reducing substances—Dissolve 0.10 g of Mesalazine in dilute hydrochloric acid to make 25 mL, add 0.2 mL of starch TS and 0.25 mL of dilute iodine TS, and allow to stand for 2 minutes: a blue or purple-brown color is produced.

(6) 2-Aminophenol and 4-aminophenol—Weigh exactly 50 mg of Mesalazine, dissolve in the mobile phase A to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 5.0 mg of 2-aminophenol, and dissolve in the mobile phase A to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase A to make exactly 100 mL, and use this solution as the 2-aminophenol standard stock solution. Weigh exactly 5.0 mg

of 4-aminophenol, dissolve in the mobile phase A to make exactly 250 mL, and use this solution as the 4-aminophenol standard stock solution. Pipet 1 mL each of the 2-aminophenol standard stock solution and 4-aminophenol standard stock 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 peak areas of 4-aminophenol and 2-aminophenol: the peak area of 4-aminophenol obtained from the sample solution is not larger than that of 4-aminophenol from the standard solution (not more than 0.02%), and the peak area of 2-aminophenol from the sample solution is not larger than 4 times that of 2-aminophenol from the standard solution (not more than 0.02%).

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 (3 μm in particle diameter).

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

Mobile phase A: Mix 2.2 g of perchloric acid and 1.0 g of phosphoric acid with water to make 1000 mL.

Mobile phase B: Mix 1.7 g of perchloric acid and 1.0 g of phosphoric acid with acetonitrile for liquid chromatography to make 1000 mL.

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 – 10	100	0
10 – 25	100 → 40	0 → 60

Flow rate: About 0.8 mL per minute (the retention time of mesalazine is about 16 minutes).

System suitability—

System performance: To 1 mL of the sample solution add the mobile phase A to make 200 mL. To 5 mL of this solution add 5 mL of the 2-aminophenol standard stock solution. When the procedure is run with 20 μL of this solution under the above operating conditions, 2-aminophenol and mesalazine 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 deviation of the peak area of 2-aminophenol is not more than 2.5%.

(7) Aniline—Dissolve exactly 0.10 g of Mesalazine in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve exactly 30.5 mg of aniline sulfate in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 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 aniline in each solution: the peak area of aniline obtained from the sample solution is not larger than that of aniline from the standard solution (not more than 10 ppm).

Operating conditions—

Detector: A fluorophotometer (excitation wavelength: 280 nm, fluorescence wavelength: 340 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 9.52 g of sodium acetate trihydrate in a suitable amount of water, add 1.72 mL of acetic acid (100), then add water to make 1000 mL, and adjust to pH 5.0 with acetic acid (100) or dilute sodium hydroxide TS. To 500 mL of this solution add 500 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of aniline is about 5 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 aniline 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 aniline is not more than 2.0%.

(8) 3-Aminophenol, 3-aminobenzoic acid, gentisic acid, salicylic acid and other related substances—Weigh exactly 50 mg of Mesalazine, dissolve in the mobile phase A to make exactly 50 mL, 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. Separately, weigh exactly 10 mg of 3-aminophenol, and dissolve in the mobile phase A to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase A to make exactly 50 mL, and use this solution as the 3-aminophenol standard solution. Weigh exactly 5.0 mg of 3-aminobenzoic acid, dissolve in the mobile phase A to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase A to make exactly 50 mL, and use this solution as the 3-aminobezoic acid standard solution. Weigh exactly 5.0 mg of gentisic acid, dissolve in the mobile phase A to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase A to make exactly 50 mL, and use this solution as the gentisic acid standard solution. Weigh exactly 15 mg of salicylic acid, dissolve in the mobile phase A to make exactly 100 mL. Pipet 1 mL of this solution, add the

mobile phase A to make exactly 50 mL, and use this solution as the salicylic acid standard solution. Perform the test with exactly 10 μL each of the sample solution, standard solution, 3-aminophenol standard solution, 3-aminobezoic acid standard solution, gentisic acid standard solution and salicylic acid 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 3-aminophenol obtained from the sample solution is not larger than that from of 3-aminophenol standard solution (not more than 0.2%), the peak area of 3-aminobenzoic acid from the sample solution is not larger than that from 3-aminobenzoic acid standard solution (not more than 0.1%), the peak area of gentisic acid from the sample solution is not larger than that from gentisic acid standard solution (not more than 0.1%), and the peak area of salicylic acid from the sample solution is not larger than that from salicylic acid standard solution (not more than 0.3%). The area of the peak other than 3-aminophenol, mesalazine, 3-aminobenzoic acid, gentisic acid and salicylic acid from the sample solution is not larger than 1/10 times the peak area of mesalazine from the standard solution (not more than 0.1%), and the total area of the peaks other than mesalazine from the sample solution is not larger than the peak area of mesalazine from the standard solution (not more than 1.0%).

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 octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase A: Mix 2.2 g of perchloric acid and 1.0 g of phosphoric acid with water to make 1000 mL.

Mobile phase B: Mix 1.7 g of perchloric acid and 1.0 g of phosphoric acid with acetonitrile for liquid chromatography to make 1000 mL.

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 – 7	100	0
7 – 25	100 → 40	0 → 60

Flow rate: About 1.8 mL per minute (the retention time of mesalazine is about 5 minutes).

Time span of measurement: For 25 minutes after injection of the sample solution.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase A to make exactly 20 mL. Confirm that the peak area of mesalazine 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: To 1 mL of the sample solution and 2 mL of a solution of 3-aminobenzoic acid in the mobile phase A (1 in 20,000) add the mobile phase A to make 100 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, mesalazine and 3-aminobenzoic 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 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mesalazine is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 50 mg of Mesalazine, previously dried, dissolve in 100 mL of hot water, cool to room temperature quickly, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 15.31 mg of $C_7H_7NO_3$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Add the following:

Mesalazine Extended-release Tablets

メサラジン徐放錠

Mesalazine Extended-release Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of mesalazine ($C_7H_7NO_3$: 153.14).

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

Identification Powder Mesalazine Extended-release Tablets. To a portion of the powder, equivalent to 20 mg of Mesalazine, add 100 mL of diluted phosphoric acid (1 in 1000) and shake vigorously. To 5 mL of this solution add diluted phosphoric acid (1 in 1000) to make 50 mL, filter, and determine the absorbance spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 227 nm and 231 nm, and between 298 nm and 302 nm.

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

To 1 tablet of Mesalazine Extended-release Tablets add

$6V/25$ mL of diluted phosphoric acid (1 in 1000), shake until the tablet is disintegrated, then add $3V/5$ mL of methanol, and sonicate for 30 minutes. Add diluted phosphoric acid (1 in 1000) to make exactly V mL so that each mL contains about 1 mg of mesalazine ($C_7H_7NO_3$), and centrifuge. Pipet 8 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution and 13 mL of methanol, then add diluted phosphoric acid (1 in 1000) to make 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

$$\text{Amount (mg) of mesalazine } (C_7H_7NO_3) = M_S \times Q_T/Q_S \times V/40$$

M_S: Amount (mg) of mesalazine for assay taken

Internal standard solution—A solution of ethyl aminobenzoate in methanol (1 in 800).

Dissolution ⟨*6.10*⟩ When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium, the dissolution rates in 3 hours, in 6 hours and in 24 hours of Mesalazine Extended-release Tablets are 10 to 40%, 30 to 60%, and not less than 80%, respectively.

Start the test with 1 tablet of Mesalazine Extended-release Tablets, withdraw exactly 20 mL of the medium at the specified minutes after starting the test and supply exactly 20 mL of dissolution medium warmed to 37 ± 0.5°C immediately after withdrawing of the medium every time. Filter the withdrawn media through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL or more 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 56 μg of mesalazine ($C_7H_7NO_3$), and use these solutions as the sample solutions. Separately, weigh accurately about 28 mg of mesalazine for assay, previously dried at 105°C for 2 hours, and dissolve in the dissolution medium to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, $A_{T(n)}$ and A_S, of the sample solutions and standard solution at 330 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of mesalazine ($C_7H_7NO_3$) on the nth medium withdrawing (n = 1, 2, 3)

$$= M_S \times \left\{ \frac{A_{T(n)}}{A_S} + \sum_{i=1}^{n-1} \left(\frac{A_{T(i)}}{A_S} \times \frac{1}{45} \right) \right\} \times \frac{V'}{V} \times \frac{1}{C} \times 180$$

M_S: Amount (mg) of mesalazine for assay taken
C: Labeled amount (mg) of mesalazine ($C_7H_7NO_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Mesalazine Extended-release Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 40 mg of mesalazine ($C_7H_7NO_3$), add 100 mL of diluted phosphoric acid (1 in 1000), shake vigorously, and sonicate for 5 minutes. Add exactly 10 mL of the internal standard solution, then add 90 mL of methanol and diluted phosphoric acid (1 in 1000) to make 250 mL. Filter this solution through a membrane filter with a pore size 0.45 μm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of mesalazine for assay, previously dried at 105°C for 2 hours, add 100 mL of diluted phosphoric acid (1 in 1000), shake vigorously, and sonicate for 5 minutes to dissolve. Add exactly 10 mL of the internal standard solution, then add 90 mL of methanol and diluted phosphoric acid (1 in 1000) to make 250 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 mesalazine to that of the internal standard.

$$\text{Amount (mg) of mesalazine } (C_7H_7NO_3) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of mesalazine for assay taken

Internal standard solution—A solution of ethyl aminobenzoate in methanol (1 in 800).

Operating conditions—

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

Column: A stainless steel column 4.0 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 40°C.

Mobile phase: Dissolve 400 mL of methanol, 1 mL of phosphoric acid, 0.865 g of sodium lauryl sulfate and 0.679 g of tetrabutylammonium hydrogensulfate in water to make 1000 mL.

Flow rate: Adjust so that the retention time of mesalazine 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, mesalazine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

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

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Add the following:

Methotrexate Tablets

メトトレキサート錠

Methotrexate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$: 454.44). (This monograph is applied to only 2.5-mg tablets.)

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

Identification To a quantity of powdered Methotrexate Tablets, equivalent to 2.5 mg of Methotrexate, add 100 mL of diluted hydrochloric acid (1 in 100), shake, and filter or centrifuge. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 241 nm and 245 nm and between 305 nm and 309 nm.

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

To 1 tablet of Methotrexate Tablets add the mobile phase, stir, and add the mobile phase to make exactly V mL so that each mL contains about 0.1 mg of methotrexate ($C_{20}H_{22}N_8O_5$). Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of methotrexate ($C_{20}H_{22}N_8O_5$)
$= M_S \times A_T/A_S \times V/250$

M_S: Amount (mg) of Methotrexate RS taken, 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 45 minutes of Methotrexate Tablets is not less than 85%.

Start the test with 1 tablet of Methotrexate 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 or more of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 2.8 μg of methotrexate ($C_{20}H_{22}N_8O_5$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Methotrexate RS (separately determine the water ⟨*2.48*⟩ in the same manner as Methotrexate), and dissolve in the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩, and determine the peak areas, A_T and A_S, of methotrexate in each solution.

Dissolution rate (%) with respect to the labeled amount of methotrexate ($C_{20}H_{22}N_8O_5$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9$

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

C: Labeled amount (mg) of methotrexate ($C_{20}H_{22}N_8O_5$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 302 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: To 250 mL of 0.2 mol/L of potassium dihydrogen phosphate TS add 29 mL of 0.2 mol/L sodium hydroxide TS and water to make 1000 mL. To 890 mL of this solution add 110 mL of acetonitrile.

Flow rate: Adjust so that the retention time of methotrexate 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 methotrexate 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 operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 1.0%.

Assay Weigh accurately the mass of not less than 20 Methotrexate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 10 mg of methotrexate ($C_{20}H_{22}N_8O_5$), add 50 mL of the mobile phase, shake, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of Methotrexate RS (separately determine the water ⟨*2.48*⟩ in the same manner as Methotrexate), dissolve in 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 methotrexate in each solution.

Amount (mg) of methotrexate ($C_{20}H_{22}N_8O_5$)
$= M_S \times A_T/A_S \times 2/5$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 302 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 mixture of disodium hydrogen phosphate-citric acid buffer solution (pH 6.0) and acetonitrile (89:11).

Flow rate: Adjust so that the retention time of methotrexate is about 8 minutes.

System suitability—

System performance: Dissolve 10 mg each of methotrexate and folic acid in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, folic acid and methotrexate 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 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Methylcellulose

メチルセルロース

Change the Viscosity and Assay (ii) as follows:

Viscosity <*2.53*>

(i) Method I: Apply to Methylcellulose having a labeled viscosity of less than 600 mPa·s. Put an exact amount of Methylcellulose, equivalent to 4.000 g on the dried basis, in a tared, wide-mouth bottle, add hot water (between 90°C and 99°C) to make 200 g, stopper the bottle, stir by mechanical means at 350 to 450 revolutions per minute for 10 to 20 minutes to get a homogeneous dispersion. If necessary, take off the sample attached on the walls of the bottle, put them in the dispersed solution, and dissolve by continuing the stirring in a water bath at not exceeding 5°C for 20 to 40 minutes. Add cold water, if necessary, to make 200 g, and use this solution as the sample solution. Centrifuge the solution if necessary to expel any entrapped air bubbles. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method I under Viscosity Determination: not less than 80% and not more than 120% of the labeled viscosity.

(ii) Method II: Apply to Methylcellulose having a labeled viscosity of not less than 600 mPa·s. Put an exact amount of Methylcellulose, equivalent to 10.00 g on the dried basis, in a tared, wide-mouth bottle, add hot water (between 90°C and 99°C) to make 500 g, and prepare the sample solution in the same manner as directed in Method I. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method II under Viscosity Determination, using a single cylinder-type rotational viscometer, according to the following operating conditions: not less than 75% and not more than 140% of the labeled viscosity.

Operating conditions—

Apparatus: Brookfield type viscometer LV model or an equivalent apparatus.

Rotor No., rotation frequency, and calculation multiplier: According to the following table, depending on the labeled viscosity.

Labeled viscosity (mPa·s)				Rotor No.	Rotation frequency /min	Calculation multiplier
Not less than 600 and less than 1400				3	60	20
〃	1400	〃	3500	3	12	100
〃	3500	〃	9500	4	60	100
〃	9500	〃	99,500	4	6	1000
〃	99,500			4	3	2000

Procedure of apparatus: Read the value after 2 minutes of rotation, and stop the rotation for at least 2 minutes. Repeat this procedure more two times, and average the three observed values.

Assay

(ii) Procedure—Weigh accurately about 65 mg of Methylcellulose, transfer to the reaction vial, 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, immediately stopper the vial tightly, and weigh accurately. Using a magnetic stirrer equipped in the heating module, or using a shaker, stir for 60 minutes while heating so that the temperature of the vial content is 130 ± 2°C. In the case when a magnetic stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5-minute intervals by hand, and continue heating for an additional 30 minutes. Allow the vial to cool, and again weigh accurately. If the mass loss is less than 0.50% and there is no evidence of a leak of the content, 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 vial, stopper the vial immediately, and weigh accurately. Add 45 μL of iodomethane for assay through the septum using a micro-syringe, weigh accurately, shake, 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, Q_T and Q_S, of the peak area of iodomethane to that of the internal standard.

$$\text{Content (\%) of methoxy group } (CH_3O) = M_S/M \times Q_T/Q_S \times 21.86$$

M_S: Amount (mg) of iodomethane for assay taken

M: Amount (mg) of Methylcellulose taken, 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 column 3 – 4 mm in inside diameter and 1.8 – 3 m in length, packed with siliceous earth for gas chromatography, 125 to 150 μm in diameter, coated with methyl silicone polymer for gas chromatography at the ratio of 10 – 20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium for thermal conductivity detector, or Helium or Nitrogen for hydrogen flame-ionization detector.

Flow rate: Adjust 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 and the internal standard are eluted in this order, with complete separation of these peaks.

Add the following:

Montelukast Sodium Granules

モンテルカストナトリウム顆粒

Montelukast Sodium Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$: 586.18).

Method of preparation Prepare as directed under Granules, with Montelukast Sodium.

Identification To an amount of Montelukast Sodium Granules, equivalent to 5 mg of montelukast ($C_{35}H_{36}ClNO_3S$), add 500 mL of a mixture of methanol and water (3:1), shake, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 281 nm and 285 nm, between 325 nm and 329 nm, between 343 nm and 347 nm, and between 357 nm and 361 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 water (3:1) 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, and determine each peak area by the automatic integration method: the total area of the two peaks of related substance A, having the relative retention time of about 0.45 to montelukast, obtained from the sample solution is not larger than the peak area of montelukast from the standard solution, and the peak area of related substance B, having the relative retention time of about 0.92, from the sample solution is not larger than 3/20 times the peak area of montelukast from the standard solution, and the area of the peak other than montelukast and the peaks mentioned above from the sample solution is not larger than 1/10 times the peak area of montelukast from the standard solution. Furthermore, the total area of the peaks other than montelukast from the sample solution is not larger than 1.2 times the peak area of montelukast from the standard solution. However, the peaks of the related substances derived from Montelukast Sodium [having the relative retention time of about 1.04 (related substance E), about 1.16 (related substance C), about 1.18 (related substance D), about 1.24 and about 1.55 (related substance F) to montelukast] are excluded. For the area of the peak, having the relative retention time of about 0.71 to montelukast, multiply the relative response factor 0.6.

Operating conditions—

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

Time span of measurement: About 1.5 times as long as the retention time of montelukast, beginning after the solvent peak.

System suitability—

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

Test for required detectability: Pipet 10 mL of the standard solution, and add a mixture of methanol and water (3:1) to make exactly 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the SN ratio of the peak of montelukast is not less than 10.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of montelukast is not more than 2.0%.

Uniformity of dosage unit ⟨*6.02*⟩ Perform the test according to the following method: Montelukast Sodium Granules in single-dose packages meet the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To the total content of 1 package of Montelukast Sodium Granules add 130 mL of methanol, disperse the fine particles by sonicating, and add methanol to make exactly V mL so that each mL contains about 20 μg of montelukast ($C_{35}H_{36}ClNO_3S$). Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 33 mg of Montelukast Dicyclohexylamine RS, and dissolve in methanol to make exactly 100 mL. Pipet 8 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of montelukast in each solution.

$$\text{Amount (mg) of montelukast } (C_{35}H_{36}ClNO_3S) = M_S \times A_T/A_S \times V/1250 \times 0.764$$

M_S: Amount (mg) of Montelukast Dicyclohexylamine RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 389 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 10 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A solution of trifluoroacetic acid in a mixture of water and acetonitrile (1:1) (1 in 500).

Flow rate: Adjust so that the retention time of montelukast is about 2 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of montelukast are not less than 1500 and not more than 1.5, respectively.

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 montelukast 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 a solution of sodium lauryl sulfate (1 in 200) as the dissolution medium, the dissolution rate in 15 minutes of Montelukast Sodium Granules is not less than 85%.

Conduct this procedure using light-resistant vessels. Start the test with an accurately weighed amount of Montelukast Sodium Granules, equivalent to about 4 mg of montelukast ($C_{35}H_{36}ClNO_3S$), withdraw not less than 15 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL or more of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 27 mg of Montelukast Dicyclohexylamine RS, and dissolve in methanol 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 montelukast in each solution.

Dissolution rate (%) with respect to the labeled amount of montelukast ($C_{35}H_{36}ClNO_3S$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 18 \times 0.764$

M_S: Amount (mg) of Montelukast Dicyclohexylamine RS taken

M_T: Amount (g) of Montelukast Sodium Granules taken

C: Labeled amount (mg) of montelukast ($C_{35}H_{36}ClNO_3S$) in 1 g

Operating conditions—

Proceed as directed in the operating conditions in the Uniformity of dosage units.

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 montelukast are not less than 2000 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 montelukast is not more than 1.0%.

Assay Conduct this procedure using light-resistant vessels. Weigh accurately an amount of Montelukast Sodium Granules, equivalent to about 48 mg of montelukast ($C_{35}H_{36}ClNO_3S$), and add exactly 200 mL of a mixture of methanol and water (3:1). Disperse the fine particles by sonicating, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 33 mg of Montelukast Dicyclohexylamine RS, and 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 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 montelukast in each solution.

Amount (mg) of montelukast ($C_{35}H_{36}ClNO_3S$)
$= M_S \times A_T/A_S \times 2 \times 0.764$

M_S: Amount (mg) of Montelukast Dicyclohexylamine RS taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 255 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with phenylhexylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

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

Mobile phase A: A solution of trifluoroacetic acid (1 in 500).

Mobile phase B: A mixture of methanol and acetonitrile (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 – 5	48 → 45	52 → 55
5 – 12	45	55
12 – 22	45 → 25	55 → 75
22 – 23	25	75

Flow rate: 1.5 mL per minute (the retention time of montelukast is about 14 minutes).

System suitability—

System performance: Take 10 mL of the standard solution in a transparent vessel, add 4 μL of hydrogen peroxide (30), and allow to stand under 4000 lx white light for 10 minutes. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peak of related substance B, having the relative retention time of about 0.92 to montelukast, and the peak of montelukast is not less than 1.5. And proceed with 20 μL of the standard solution under the above operating conditions, the number of the theoretical plates and the symmetry factor of the peak of montelukast are not less than 5000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of montelukast is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Others

Related substances A, B, C, D, E and F: Refer to them described in Montelukast Sodium.

Noradrenaline

ノルアドレナリン

Change the Purity (3) as follows:

Purity

(3) Adrenaline—Dissolve 10.0 mg of Noradrenaline in 2.0 mL of diluted acetic acid (100) (1 in 2). Pipet 1 mL of this solution, add water to make 10 mL, then mix with 0.3 mL of a solution of sodium nitrite (1 in 100), and observe after 1 minute: the solution has no more color than the following control solution.

Control solution: Dissolve 2.0 mg of Adrenaline Bitartrate RS for Purity and 90 mg of Noradrenaline Bitartrate RS in water to make exactly 10 mL. Measure exactly 1 mL of this solution, add 1.0 mL of diluted acetic acid (100) (1 in 2) and water to make 10 mL, and proceed in the same manner.

Oxytetracycline Hydrochloride

オキシテトラサイクリン塩酸塩

Change the Description and Identification as follows:

Description Oxytetracycline Hydrochloride occurs as yellow, crystals or crystalline powder.

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

It shows crystal polymorphism.

Identification (1) Determine the absorption spectrum of a solution of Oxytetracycline Hydrochloride in 0.1 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 Oxytetracycline Hydrochloride RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

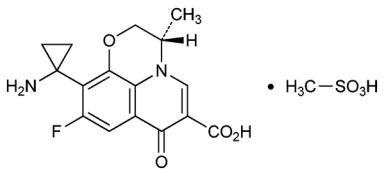
(2) Determine the infrared absorption spectrum of Oxytetracycline Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Oxytetracycline Hydrochloride RS: both 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 methanol, evaporate the solvent, and perform the test with the residues.

(3) Dissolve 20 mg of Oxytetracycline Hydrochloride in 3 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

Add the following:

Pazufloxacin Mesilate

パズフロキサシンメシル酸塩



$C_{16}H_{15}FN_2O_4.CH_4O_3S$: 414.41

(3*S*)-10-(1-Aminocyclopropyl)-9-fluoro-3-methyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid monomethanesulfonate

[*163680-77-1*]

Pazufloxacin Mesilate, when dried, contains not less than 98.0% and not more than 102.0% of pazufloxacin mesilate ($C_{16}H_{15}FN_2O_4.CH_4O_3S$).

Description Pazufloxacin Mesilate occurs as a white to light yellow crystalline powder.

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

It dissolves in sodium hydroxide TS.

The pH of a solution prepared by dissolving 0.4 g of Pazufloxacin Mesilate in 10 mL of water is between 3.0 and 4.0.

Melting point: about 258°C (with decomposition).

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Pazufloxacin Mesilate in a mixture of methanol and 1 mol/L hydrochloric acid TS (49:1) (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 Pazufloxacin Mesilate 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 Pazufloxacin Mesilate, previously dried, as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Pazufloxacin Mesilate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Pazufloxacin Mesilate responds to Qualitative Tests <*1.09*> for mesilate.

Optical rotation <*2.49*> $[\alpha]_D^{20}$: -61 – $-65°$ (after drying, 0.2 g, sodium hydroxide TS, 20 mL, 100 mm).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Pazufloxacin Mesilate 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 26 mg of Pazufloxacin Mesilate in 100 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 the amounts of them by the area percentage method: the amount of the peak other than pazufloxacin is not more than 0.10%. For the area of the peak, having the relative retention time of about 2.7 to pazufloxacin, multiply the relative response factor, 1.6.

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: Dissolve 1.08 g of sodium 1-octanesulfonate in 1000 mL of a mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (39:11).

Flow rate: Adjust so that the retention time of pazufloxacin is about 8 minutes.

Time span of measurement: About 6 times as long as the retention time of pazufloxacin, 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 10 mL. Confirm that the peak area of pazufloxacin 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 pazufloxacin are not less than 2500 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 pazufloxacin is not more than 2.0%.

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% (0.2 g, platinum crucible).

Assay Weigh accurately about 26 mg each of Pazufloxacin Mesilate and Pazufloxacin Mesilate RS, both previously dried, dissolve each in water to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 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 pazufloxacin to that of the internal standard.

$$\text{Amount (mg) of pazufloxacin mesilate } (C_{16}H_{15}FN_2O_4.CH_4O_3S) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Pazufloxacin Mesilate RS taken

Internal standard solution—A solution of acetanilide in the mobile phase (3 in 10,000).

Operating conditions—

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

Column: A stainless steel column 4 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 25°C.

Mobile phase: To 200 mL of water add gradually 30 mL

of methanesulfonic acid while ice-cooling, then add 30 mL of triethylamine in the same manner, and add water to make 300 mL. To 50 mL of this solution add 150 mL of acetonitrile, 35 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution and water to make 1000 mL.

Flow rate: Adjust so that the retention time of pazufloxacin 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, pazufloxacin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

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

Containers and storage Containers—Tight containers.

Add the following:

Pazufloxacin Mesilate Injection

パズフロキサシンメシル酸塩注射液

Pazufloxacin Mesilate Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pazufloxacin mesilate ($C_{16}H_{15}FN_2O_4.CH_4O_3S$: 414.41).

Method of preparation Prepare as directed under Injections, with Pazufloxacin Mesilate.

Description Pazufloxacin Mesilate Injection is a clear, colorless liquid.

Identification To a volume of Pazufloxacin Mesilate Injection, equivalent to 20 mg of Pazufloxacin Mesilate, add a mixture of methanol and 1 mol/L hydrochloric acid TS (49:1) to make 100 mL. To 5 mL of this solution add a mixture of methanol and 1 mol/L hydrochloric acid TS (49:1) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 237 nm and 241 nm, between 314 nm and 324 nm, between 328 nm and 332 nm, and between 343 nm and 347 nm.

pH Being specified separately when the drug is granted approval based on the Law.

Bacterial endotoxins <*4.01*> Less than 0.30 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 Pipet a volume of Pazufloxacin Mesilate Injection, equivalent to about 12 mg of pazufloxacin mesilate ($C_{16}H_{15}FN_2O_4.CH_4O_3S$), and add water to make exactly 50 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 about 23 mg of Pazufloxacin Mesilate RS, previously dried at 105°C for 3 hours, and add 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 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 pazufloxacin to that of the internal standard.

$$\text{Amount (mg) of pazufloxacin mesilate } (C_{16}H_{15}FN_2O_4.CH_4O_3S) = M_S \times Q_T/Q_S \times 1/2$$

M_S: Amount (mg) of Pazufloxacin Mesilate RS taken

Internal standard solution—A solution of acetanilide in the mobile phase (3 in 10,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Pazufloxacin Mesilate.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, pazufloxacin and acetanilide are eluted in this order with the resolution between these peaks being not less than 3.

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

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Storage—Light-resistant.

Pentobarbital Calcium

ペントバルビタールカルシウム

Change the Assay as follows:

Assay Weigh accurately about 20 mg of Pentobarbital Calcium, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution and water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately

about 18 mg of Pentobarbital RS, previously dried at 105°C for 2 hours, dissolve in 10 mL of acetonitrile for liquid chromatography, add exactly 5 mL of the internal standard solution, and add water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 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 pentobarbital to that of the internal standard.

Amount (mg) of pentobarbital calcium ($C_{22}H_{34}CaN_4O_6$) $= M_S \times Q_T/Q_S \times 1.084$

M_S: Amount (mg) of Pentobarbital RS taken

Internal standard solution—Dissolve 0.2 g of isopropyl parahydroxybenzoate in 20 mL of acetonitrile for liquid chromatography, and add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

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

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

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 4.0 with diluted phosphoric acid (1 in 10). To 650 mL of this solution add 350 mL of acetonitrile for liquid chromatography.

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

System suitability—

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

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

Add the following:

Pentobarbital Calcium Tablets

ペントバルビタールカルシウム錠

Pentobarbital Calcium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of pentobarbital calcium ($C_{22}H_{34}CaN_4O_6$: 490.61).

Method of preparation Prepare as directed under Tablets, with Pentobarbital Calcium.

Identification To a quantity of powdered Pentobarbital Calcium Tablets, equivalent to 5.6 mg of Pentobarbital Calcium, add 60 mL of water, shake thoroughly, then add water to make 100 mL, and filter. To 6 mL of the filtrate add dilute sodium hydroxide TS to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 240 nm and 244 nm.

Uniformity of dosage unit <6.02> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Pentobarbital Calcium Tablets add exactly $V/10$ mL of the internal standard solution, add 60 mL of water, shake vigorously until the tablet is completely disintegrated, then add water to make 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, to 2 mL of the subsequent filtrate add water to make V mL so that each mL contains about 10 μg of pentobarbital calcium ($C_{22}H_{34}CaN_4O_6$), and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of pentobarbital calcium ($C_{22}H_{34}CaN_4O_6$) $= M_S \times Q_T/Q_S \times V/50 \times 1.084$

M_S: Amount (mg) of Pentobarbital Calcium RS taken

Internal standard solution—Dissolve 0.5 g of isopropyl parahydroxybenzoate in 20 mL of acetonitrile for liquid chromatography, and add water to make 200 mL.

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 Pentobarbital Calcium Tablets is not less than 80%.

Start the test with 1 tablet of Pentobarbital Calcium 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 or more 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 56 μg of pentobarbital calcium ($C_{22}H_{34}CaN_4O_6$). Pipet 3 mL of this solution, add dilute sodium hydroxide TS to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 26 mg of Pentobarbital RS, previously dried at 105°C for 2 hours, dissolve in 2 mL of ethanol (99.5), and add water to make exactly 100 mL. Pipet 4 mL of this solution, and add the dissolution medium to make exactly 20 mL. Pipet 3 mL of this solution, add dilute sodium hydroxide TS to make exactly 10 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 solution, prepared by adding dilute sodium hydroxide TS to 3 mL of the dissolution medium to make 10 mL, as the blank.

Dissolution rate (%) with respect to the labeled amount of pentobarbital calcium ($C_{22}H_{34}CaN_4O_6$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 180 \times 1.084$

M_S: Amount (mg) of Pentobarbital RS taken
C: Labeled amount (mg) of pentobarbital calcium ($C_{22}H_{34}CaN_4O_6$) in 1 tablet

Assay To 20 Pentobarbital Calcium Tablets add 120 mL of water, shake vigorously for 10 minutes, then add water 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 5 mL of the subsequent filtrate, add exactly $V/10$ mL of the internal standard solution, and add water to make V mL so that each mL contains about 0.5 mg of pentobarbital calcium ($C_{22}H_{34}CaN_4O_6$). To 2 mL of this solution, add water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 23 mg of Pentobarbital RS, previously dried at 105°C for 2 hours, dissolve in 10 mL of acetonitrile for liquid chromatography, add exactly 5 mL of the internal standard solution, and add water to make 50 mL. To 2 mL of this solution add water to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of pentobarbital to that of the internal standard.

Amount (mg) of pentobarbital calcium ($C_{22}H_{34}CaN_4O_6$) in 1 tablet
$= M_S \times Q_T/Q_S \times V/25 \times 1.084$

M_S: Amount (mg) of Pentobarbital RS taken

Internal standard solution—Dissolve 0.5 g of isopropyl parahydroxybenzoate in 20 mL of acetonitrile for liquid chromatography, and add water to make 200 mL.
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 210 nm).
Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).
Column temperature: A constant temperature of about 40°C.
Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 4.0 with diluted phosphoric acid (1 in 10). To 650 mL of this solution add 350 mL of acetonitrile for liquid chromatography.
Flow rate: Adjust so that the retention time of pentobarbital is about 7 minutes.
System suitability—
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, pentobarbital and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pentobarbital to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Polymixin B Sulfate

ポリミキシン B 硫酸塩

Change the Origin/limits of content and Description as follows:

Polymixin B Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus polymyxa*.

It contains not less than 6500 units and not more than 10,500 units per mg, calculated on the dried basis. The potency of Polymixin B Sulfate is expressed as mass unit of polymixin B ($C_{55-56}H_{96-98}N_{16}O_{13}$). One unit of Polymixin B Sulfate is equivalent to 0.129 μg of polymixin B sulfate ($C_{55-56}H_{96-98}N_{16}O_{13}.1\text{–}2H_2SO_4$).

Description Polymixin B Sulfate occurs as a white powder.

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

Polysorbate 80

ポリソルベート 80

Change the Composition of fatty acids as follows:

Composition of fatty acids Dissolve 0.10 g of Polysorbate 80 in 2 mL of a solution of sodium hydroxide in methanol (1 in 50) in a 25-mL conical flask, and boil under a reflux condenser for 30 minutes. Add 2.0 mL of boron trifluoride-methanol TS through the condenser, and boil for 30 minutes. Add 4 mL of heptane through the condenser, and boil for 5 minutes. After cooling, add 10.0 mL of saturated sodium chloride solution, shake for about 15 seconds, and add a quantity of saturated sodium chloride solution such that the upper layer is brought into the neck of the flask. Collect 2 mL of the upper layer, wash with three 2-mL portions of water, dry with anhydrous sodium sulfate, and use this solution as the sample solution. Perform the test with 1 μL each of the sample solution and fatty acid methyl esters mixture TS as directed under Gas Chromatography <*2.02*> according to the following conditions. Identify each peak obtained with the sample solution using the chromatogram with fatty acid methyl esters mixture TS. Determine each peak area with the sample solution by the automatic in-

tegration method, and calculate the composition of fatty acids by the area percentage method: myristic acid is not more than 5.0%, palmitic acid is not more than 16.0%, palmitoleic acid is not more than 8.0%, stearic acid is not more than 6.0%, oleic acid is not less than 58.0%, linoleic acid is not more than 18.0% and linolenic acid is not more than 4.0%.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated with polyethylene glycol 20 M for gas chromatography 0.5 μm in thickness.

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

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

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

Carrier gas: Helium.

Flow rate: 50 cm per second.

Split ratio: 1: 50.

System suitability—

Test for required detectability: Dissolve 0.50 g of the mixture of fatty acid methyl esters described in the following table in heptane to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test add heptane to make exactly 10 mL. When the procedure is run with 1 μL of this solution under the above operating conditions, the SN ratio of methyl myristate is not less than 5.

Mixture of fatty acid methyl esters	Composition (%)
Methyl myristate for gas chromatography	5
Methyl palmitate for gas chromatography	10
Methyl stearate for gas chromatography	15
Methyl arachidate for gas chromatography	20
Methyl oleate for gas chromatography	20
Methyl eicosenoate for gas chromatography	10
Methyl behenate	10
Methyl behenate	10

System performance: When the procedure is run with 1 μL of the solution for system suitability test under the above operating conditions, ◆methyl stearate and methyl oleate are eluted in this order,◆ the resolution between these peaks is not less than 1.8, and the number of theoretical plates of the peak of methyl stearate is not less than 30,000.

Povidone

ポビドン

Change the Identification (2) as follows:

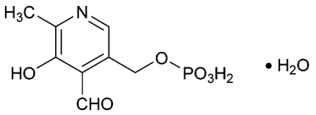
Identification

(2) Determine the infrared absorption spectrum of Povidone, 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 or the spectrum of Povidone RS for Identification (previously dried at 105°C for 6 hours): both spectra exhibit similar intensities of absorption at the same wave numbers.

Add the following:

Pyridoxal Phosphate Hydrate

ピリドキサールリン酸エステル水和物



$C_8H_{10}NO_6P.H_2O$: 265.16

(4-Formyl-5-hydroxy-6-methylpyridin-3-yl)methyl dihydrogenphosphate monohydrate

[*41468-25-1*]

Pyridoxal Phosphate Hydrate contains not less than 98.0% and not more than 101.0% of pyridoxal phosphate ($C_8H_{10}NO_6P$: 247.14), calculated on the anhydrous basis.

Description Pyridoxal Phosphate Hydrate occurs as a pale yellow-white to light yellow crystalline powder.

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

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

The pH of a solution prepared by dissolving 0.1 g of Pyridoxal Phosphate Hydrate in 200 mL of water is between 3.0 and 3.5.

Pyridoxal Phosphate Hydrate is colored to light red by light.

Identification **(1)** Determine the absorption spectrum of a solution of Pyridoxal Phosphate Hydrate in phosphate buffer solution (pH 6.8) (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 Pyridoxal Phosphate 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 Pyridoxal Phosphate 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 Pyridoxal Phosphate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 4.0 g of Pyridoxal Phosphate Hydrate 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).

(2) Arsenic ⟨*1.11*⟩—Dissolve 1.0 g of Pyridoxal Phosphate Hydrate in 5 mL of dilute hydrochloric acid. Use this solution as the test solution, and perform the test (not more than 2 ppm).

(3) Free phosphoric acid—Weigh accurately about 0.1 g of Pyridoxal Phosphate Hydrate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL each of the sample solution and Standard Phosphoric Acid Solution, to each add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, and shake. Add water to make exactly 25 mL, and allow to stand at 20 ± 1°C for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, using a solution prepared with 5 mL of water in the same manner as the blank. Determine the absorbances, A_T and A_S, of each solution from the sample solution and Standard Phosphoric Acid Solution at 740 nm: the amount of free phosphoric acid is not more than 0.5%.

$$\text{Content (\%) of free phosphoric acid } (H_3PO_4) = 1/M \times A_T/A_S \times 258.0$$

M: Amount (mg) of Pyridoxal Phosphate Hydrate taken, calculated on the anhydrous basis

(4) Related substances—Dissolve 50 mg of Pyridoxal Phosphate Hydrate in 20 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 other than pyridoxal phosphate obtained from the sample solution is not larger than the peak area of pyridoxal phosphate from the standard solution, and the total area of the peaks other than pyridoxal phosphate from the sample solution is not larger than 2 times the peak area of pyridoxal phosphate from the standard solution.

Operating conditions—

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

Column: A stainless steel column 4.6 mm in inside diameter and 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: Dissolve 3.63 g of potassium dihydrogen phosphate and 5.68 g of anhydrous disodium hydrogen phosphate in water to make 1000 mL.

Flow rate: Adjust so that the retention time of pyridoxal phosphate is about 6 minutes.

Time span of measurement: About 2.5 times as long as the retention time of pyridoxal phosphate, 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 20 mL. Confirm that the peak area of pyridoxal phosphate obtained with 5 μL of this solution is equivalent to 7 to 13% of that of pyridoxal phosphate 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 pyridoxal phosphate are not less than 3000 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 pyridoxal phosphate is not more than 2.0%.

Water ⟨*2.48*⟩ 6.0 – 9.0% (0.1 g, volumetric titration, direct titration. Use a solution prepared by dissolving 50 g of imidazole for water determination in 100 mL of the dissolving solution instead of methanol for water determination).

Dissolving solution: A solution containing 80% of 1-methoxy-2-propanol, 18% of ethanol (99.5), 1% of imidazole and 1% of imidazole hydrobromide.

Assay Weigh accurately about 45 mg each of Pyridoxal Phosphate Hydrate and Pyridoxal Phosphate RS (separately determine the water ⟨*2.48*⟩ in the same manner as Pyridoxal Phosphate Hydrate), and dissolve each in phosphate buffer solution (pH 6.8) to make exactly 250 mL. Pipet 10 mL each of these solutions, add phosphate buffer solution (pH 6.8) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 388 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ using phosphate buffer solution (pH 6.8) as the blank.

$$\text{Amount (mg) of pyridoxal phosphate } (C_8H_{10}NO_6P) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Pyridoxal Phosphate RS taken, calculated on the anhydrous basis

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Delete the following Monographs:

Rokitamycin

ロキタマイシン

Rokitamycin Tablets

ロキタマイシン錠

Add the following:

Roxithromycin Tablets

ロキシスロマイシン錠

Roxithromycin Tablets contain not less than 95.0% and not more than 110.0% of the labeled potency of roxithromycin ($C_{41}H_{76}N_2O_{15}$: 837.05).

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

Identification To a quantity of powdered Roxithromycin Tablets, equivalent to 0.3 g (potency) of Roxithromycin, add 10 mL of acetonitrile, shake, and centrifuge. Evaporate the supernatant liquid on a water bath under reduced pressure, dry the residue at 60°C under reduced pressure for 1 hour, and 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 3460 cm^{-1}, 2940 cm^{-1}, 1728 cm^{-1}, 1633 cm^{-1} and 1464 cm^{-1}.

Uniformity of dosage unit <*6.02*> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Roxithromycin Tablets add $7V/10$ mL of the mobile phase, sonicate to disintegrate the tablet, shake, add exactly $V/25$ mL of the internal standard solution, and add the mobile phase to make V mL so that each mL contains about 1.5 mg (potency) of roxithromycin ($C_{41}H_{76}N_2O_{15}$). 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. Then, proceed as directed in the Assay.

Amount [mg (potency)] of roxithromycin ($C_{41}H_{76}N_2O_{15}$)
$= M_S \times Q_T/Q_S \times V/25$

M_S: Amount [mg (potency)] of Roxithromycin RS taken

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 800).

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 Roxithromycin Tablets is not less than 80%.

Start the test with 1 tablet of Roxithromycin 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 or more 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 0.17 mg (potency) of roxithromycin ($C_{41}H_{76}N_2O_{15}$), and use this solution as the sample solution. Separately, weigh accurately about 33 mg (potency) of Roxithromycin RS, dissolve in 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 roxithromycin in each solution.

Dissolution rate (%) with respect to the labeled amount [mg (potency)] of roxithromycin ($C_{41}H_{76}N_2O_{15}$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 450$

M_S: Amount [mg (potency)] of Roxithromycin RS taken
C: Labeled amount [mg (potency)] of roxithromycin ($C_{41}H_{76}N_2O_{15}$) in 1 tablet

Operating conditions—

Detector, column temperature and mobile phase: 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).

Flow rate: Adjust so that the retention time of roxithromycin is about 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 roxithromycin are not less than 2300 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 roxithromycin is not more than 1.0%.

Assay Weigh accurately the mass of not less than 20 Roxithromycin Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 38 mg (potency) of roxithromycin ($C_{41}H_{76}N_2O_{15}$), add 20 mL of the mobile phase, shake vigorously, add exactly 1 mL of the internal standard solution, and then add the mobile phase to make 25 mL. Filter the 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 38 mg (potency) of Roxithromycin RS, dissolve in the mobile phase, add exactly 1 mL of the internal standard solution, then add the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the

sample solution and standard solution as directed under Liquid Chromatography <2.01>: according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of roxithromycin to that of the internal standard.

Amount [mg (potency)] of roxithromycin ($C_{41}H_{76}N_2O_{15}$) $= M_S \times Q_T/Q_S$

M_S: Amount [mg (potency)] of Roxithromycin RS taken

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (1 in 800).

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 octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: Dissolve 49.1 g of ammonium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 5.3 with 2 mol/L sodium hydroxide TS. To 690 mL of this solution add 310 mL of acetonitrile.

Flow rate: Adjust so that the retention time of roxithromycin 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, roxithromycin 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 roxithromycin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Saccharin Sodium Hydrate

サッカリンナトリウム水和物

Change the Identification (1) and Purity (1):

Identification (1) Determine the infrared absorption spectrum of Saccharin Sodium Hydrate, previously dried at 105°C to constant mass, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the spectrum of Saccharin Sodium RS for Identification dried in the same manner as Saccharin Sodium Hydrate: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 2.0 g of Saccharin Sodium Hydrate in water to make 10 mL, and use this solution as the test solution. Perform the test with the test solution as directed under Turbidity Measurement <2.61>: the solution is clear. Perform the test with the test solution according to Method 2 under Methods for Color Matching <2.65>: the solution is colorless.

Delete the following Monograph:

Serrapeptase

セラペプターゼ

Sodium Lauryl Sulfate

ラウリル硫酸ナトリウム

Change to read:

$C_{12}H_{25}NaO_4S$: 288.38
Monosodium monododecyl sulfate
[*151-21-3*]

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

The corresponding part of the attributes/provisions which are agreed as non-harmonized within the scope of the harmonization is marked with symbols (◆ ◆), and the corresponding parts which are agreed as the JP local requirement other than the scope of the harmonization are marked with symbols (◇ ◇).

Sodium Lauryl Sulfate is a mixture of sodium alkyl sulfate consisting chiefly of sodium lauryl sulfate.

It contains not less than 85.0% of sodium alkyl sulfate [as sodium lauryl sulfate ($C_{12}H_{25}NaO_4S$)].

◆**Description** Sodium Lauryl Sulfate occurs as white to light yellow, crystals or powder. It has a slightly characteristic odor.

It is sparingly soluble in ethanol (95).

A solution of 1 g of Sodium Lauryl Sulfate in 10 mL of water is a clear or an opalescent solution.◆

Identification (1) Put 2.5 g of Sodium Lauryl Sulfate in a platinum or quartz crucible, and add 2 mL of 5 mol/L surfuric acid TS. Heat on a water bath, cautiously raise the temperature gradually with a burner, and ignite. Ignite, preferably in an electric furnace, at 600 ± 25°C and incinerate the residue completely. After cooling, add a few drops of 1 mol/L sulfuric acid TS, and heat and ignite as above. After cooling, add a few drops of ammonium carbonate TS, evaporate to dryness, and further ignite as above. After cooling, dissolve the residue in 50 mL of water, and stir. To 2 mL of this solution add 4 mL of potassium hexahydroxoantimonate (V) TS. If necessary, rub the inside wall of the vessel with a glass rod: a white, crystalline precipitate is formed.

(2) Acidify a solution of Sodium Lauryl Sulfate (1 in 10) with hydrochloric acid, and boil for 20 minutes: no precipi-

tate is formed. To this solution add barium chloride TS: a white precipitate is formed.

(3) Dissolve 0.1 g of Sodium Lauryl Sulfate in 10 mL of water, and shake: the solution foams strongly.

(4) To 0.1 mL of the solution obtained in (3) add 0.1 mL of methylene blue TS and 2 mL of dilute sulfuric acid, then add 2 mL of dichloromethane, and shake: a deep blue color develops in the dichloromethane layer.

Purity **(1)** Alkalinity—Dissolve 1.0 g of Sodium Lauryl Sulfate in 100 mL of water, add 0.1 mL of phenol red TS, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS: the consumed volume is not more than 0.5 mL.

(2) Sodium chloride—Dissolve about 5 g of Sodium Lauryl Sulfate, accurately weighed, in 50 mL of water, neutralize the solution with dilute nitric acid, if necessary, add exactly 5 mL of 0.1 mol/L sodium chloride TS, and titrate <2.50> with 0.1 mol/L silver nitrate VS until the color of the solution changes from yellow-green through yellow to orange (indicator: 2 drops of fluorescein sodium TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

The combined content of sodium chloride (NaCl: 58.44) and sodium sulfate (Na_2SO_4: 142.04) obtained in (3) is not more than 8.0%.

(3) Sodium sulfate—Dissolve about 1 g of Sodium Lauryl Sulfate, accurately weighed, in 10 mL of water, add 100 mL of ethanol (95), and heat at a temperature just below the boiling point for 2 hours. Filter through a glass filter (G4) while hot, and wash with 100 mL of boiling ethanol (95). Dissolve the residue on the glass filter by washing with 150 mL of water, collecting the washings in a beaker. Add 10 mL of dilute hydrochloric acid, heat to boiling, add 25 mL of barium chloride TS, and allow to stand overnight. Collect the precipitate, and wash with water until the last washing produces no opalescence with silver nitrate TS. Dry the precipitate together with the filter paper, ignite to a constant mass between 500°C and 600°C by raising the temperature gradually, and weigh as barium sulfate ($BaSO_4$: 233.39).

Amount (mg) of sodium sulfate (Na_2SO_4)
= amount (mg) of barium sulfate ($BaSO_4$) × 0.6086

(4) Unsulfated alcohols—Dissolve about 10 g of Sodium Lauryl Sulfate, accurately weighed, in 100 mL of water, add 100 mL of ethanol (95), and transfer to a separator. Extract the solution with three 50-mL portions of pentane. If an emulsion forms, sodium chloride may be added to promote separation of the two layers. Combine the pentane extracts, wash with three 50-mL portions of water, dehydrate with anhydrous sodium sulfate, and filter. Put the filtrate to a tared beaker, and evaporate the pentane on a water bath. Dry the residue at 105°C for 30 minutes, cool, and weigh: the mass of the residue is not more than 4.0%.

◇**Water** <2.48> Not more than 5.0% (0.5 g, volumetric titration, direct titration).◇

◇**Total alcohol content** Dissolve about 5 g of Sodium Lauryl Sulfate, accurately weighed, in 150 mL of water and 50 mL of hydrochloric acid, and boil under a reflux condenser for 4 hours. Cool, extract with two 75-mL portions of diethyl ether, and evaporate the combined diethyl ether extracts on a water bath. Dry the residue at 105°C for 30 minutes, and weigh: the mass of the residue is not less than 59.0%.◇

Assay Weigh accurately about 1.15 g of Sodium Lauryl Sulfate, and dissolve in water to make exactly 1000 mL, by warming if necessary. Transfer exactly 20 mL of this solution to a 100-mL stoppered graduated cylinder, add 15 mL of dichloromethane and 10 mL of dimidium bromide-patent blue TS, and shake. Titrate <2.50> with 0.004 mol/L benzethonium chloride VS until the color of the dichloromethane layer changes from light red to grayish blue, while shaking vigorously. Allow the layers to separate before each titration.

Each mL of 0.004 mol/L benzethonium chloride VS
= 1.154 mg of $C_{12}H_{25}NaO_4S$

◆**Containers and storage** Containers—Well-closed containers.◆

Spiramycin Acetate

スピラマイシン酢酸エステル

Change the Purity as follows:

Purity Heavy metals <1.07>—Proceed with 1.0 g of Spiramycin Acetate 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).

Sulbactam Sodium

スルバクタムナトリウム

Change the Purity and Assay as follows:

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Sulbactam Sodium in 20 mL of water: the solution is clear, and its absorbance at 430 nm determined as directed under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.10.

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

(3) Sulbactam penicillamine—Weigh accurately about 0.2 g of Sulbactam Sodium, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of sulbactam sodium for sulbactam penicillamine, dissolve in 2 mL

of water, and add 0.5 mL of sodium hydroxide TS. Allow to stand at room temperature for 10 minutes, add 0.5 mL of 1 mol/L hydrochloric acid TS, and then add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 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 sulbactam penicillamine by the automatic integration method: the amount of sulbactam penicillamine is not more than 1.0%.

$$\text{Amount (\%) of sulbactam penicillamine} = M_S/M_T \times A_T/A_S \times 5$$

M_S: Amount (mg) of sulbactam sodium for sulbactam penicillamine taken

M_T: Amount (mg) of Sulbactam Sodium taken

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: 230 nm).

System suitability—

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 sulbactam penicillamine is not more than 2.0%.

Assay Weigh accurately amounts of Sulbactam Sodium and Sulbactam RS, equivalent to about 50 mg (potency), dissolve each in a suitable amount of the mobile phase, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 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 sulbactam to that of the internal standard.

$$\text{Amount [}\mu\text{g (potency)] of sulbactam } (C_8H_{11}NO_5S) = M_S \times Q_T/Q_S \times 1000$$

M_S: Amount [mg (potency)] of Sulbactam RS taken

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (7 in 1000).

Operating conditions—

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

Column: A stainless steel column 3.9 mm in inside diameter and 30 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: To 750 mL of 0.005 mol/L tetrabutylammonium hydroxide TS add 250 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of sulbactam 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, sulbactam and the internal standard 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 sulbactam is not more than 1.0%.

Sultamicillin Tosilate Hydrate

スルタミシリントシル酸塩水和物

Change the Identification and Purity as follows:

Identification **(1)** Determine the absorption spectrum of a solution of Sultamicillin Tosilate Hydrate in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Sultamicillin Tosilate 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 Sultamicillin Tosilate Hydrate as directed in the paste method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum or the spectrum of Sultamicillin Tosilate RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Sultamicillin Tosilate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL Standard Lead Solution (not more than 20 ppm).

(2) Ampicillin—Perform the procedure rapidly. Weigh accurately about 20 mg of Sultamicillin Tosilate Hydrate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Ampicillin RS, equivalent to about 20 mg (potency), dissolve in the mobile phase to make exactly 100 mL. Pipet 6 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 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 area of ampicillin by the automatic integration method: the peak area from the sample solution is not larger than that from the standard solution.

Operating conditions—

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

Mobile phase: Dissolve 3.12 g of sodium dihydrogen phosphate dihydrate in about 750 mL of water, adjust to pH 3.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL. To 80 mL of acetonitrile for liquid chromatography add this solution to make 1000 mL.

Flow rate: Adjust so that the retention time of ampicillin is about 14 minutes.

System suitability—

System performance: Dissolve 12 mg of Ampicillin RS, 4 mg of Sulbactam RS and 4 mg of *p*-toluenesulfonic acid monohydrate in 1000 mL of the mobile phase. When the procedure is run with 25 μL of this solution under the above operating conditions, sulbactam, *p*-toluenesulfonic acid and ampicillin are eluted in this order, and the resolutions between these peaks are not less than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ampicillin is not more than 2.0%.

(3) Sulbactam—Perform the procedure rapidly. Weigh accurately about 20 mg of Sultamicillin Tosilate Hydrate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Sulbactam RS, equivalent to about 20 mg (potency), dissolve in the mobile phase to make exactly 100 mL. Pipet 2 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 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 area of sulbactam by the automatic integration method: the peak area from the sample solution is not larger than that from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (2).

System suitability—

Proceed as directed in the system suitability in the Purity (2).

(4) Penicilloic acids—Weigh accurately about 25 mg of Sultamicillin Tosilate Hydrate, dissolve in 1 mL of acetonitrile, and add 25 mL of 0.02 mol/L phosphate buffer solution (pH 3.0) in a 100-mL glass-stopperd flask. Add exactly 5 mL of 0.005 mol/L iodine VS, and allow to stand the stoppered flask for 5 minutes. Titrate <*2.50*> with 0.005 mol/L sodium thiosulfate VS (indicator: 1.0 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction. Calculate the amount of penicilloic acid ($C_{25}H_{34}N_4O_{11}S_2$: 630.69) by using the following equation: it is not more than 3.0%.

Each mL of 0.005 mol/L sodium thiosulfate VS
= 0.2585 mg of $C_{25}H_{34}N_4O_{11}S_2$

(5) Residual solvent <*2.46*>—Weigh accurately about 0.1 g of Sultamicillin Tosilate Hydrate, dissolve in 2 mL of methanol, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of ethyl acetate, and mix with water to make exactly 200 mL. Pipet 2 mL of this solution, add 10 mL of methanol, then add water 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 the peak areas, A_T and A_S, of ethyl acetate in each solution. Calculate the amount of ethyl acetate by the following equation: not more than 2.0%.

$$\text{Amount (\%) of ethyl acetate} = M_S/M_T \times A_T/A_S \times 1/5$$

M_S: Amount (mg) of ethyl acetate taken
M_T: Amount (mg) of the Sultamicillin Tosilate Hydrate taken

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 1 m in length, packed with porous styrene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0085 μm, 300 – 400 m^2/g) (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 155°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the retention time of ethyl acetate is about 6 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ethyl acetate are not less than 500 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 ethyl acetate is not more than 5%.

Suxamethonium Chloride Injection

スキサメトニウム塩化物注射液

Change the Expiration date as follows:

Shelf life 12 months after preparation.

Teicoplanin

テイコプラニン

Change the Purity (1) and (3) as follows:

Purity **(1)** Clarity and color of solution—Dissolve 0.8 g of Teicoplanin in 10 mL of water: the solution is clear. Per-

form the test with this solution according to Method 1 under Methods for Color Matching <*2.65*>: the color is not more colored than Matching Fluids BY3 and B4.

(3) Heavy metals <*1.07*>—Place 2.0 g of Teicoplanin in a quartz or porcelain crucible, cover loosely with a lid, and heat gently to carbonize. After cooling, add 2 mL of nitric acid and 5 drops of sulfuric acid, heat cautiously until white fumes are no longer evolved, and incinerate by ignition between 500°C and 600°C. If a carbonized substance remains, add 2 mL of nitric acid and 5 drops of sulfuric acid, heat in the same manner as above, and incinerate by ignition between 500°C and 600°C. Cool, then proceed according to Method 2, and perform the test. The control solution is prepared as follows: Evaporate a mixture of 4 mL of nitric acid, 10 drops of sulfuric acid and 2 mL of hydrochloric acid on a water bath, further evaporate to dryness on a sand bath, and moisten the residue with 3 drops of hydrochloric acid. Then proceed in the same manner as the test solution, and add 1.0 mL of Standard Lead Solution and water to make 50 mL (not more than 5 ppm).

Delete the Bacterial endotoxins and Blood pressure depressant:

Change the Containers and storage as follows:

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature between 2°C and 8°C.

Tetracycline Hydrochloride

テトラサイクリン塩酸塩

Change the Purity as follows:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 1.0 g of Tetracycline Hydrochloride 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 25 mg of Tetracycline Hydrochloride in 50 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tetracycline from the sample solution is not larger than the peak area of tetracycline from the standard solution, and the total area of the peaks other than tetracycline from the sample solution is not larger than 3 times the peak area of tetracycline 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 7 times as long as the retention time of tetracycline, beginning after the solvent peak.

System suitability—

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

Test for required detectability: Pipet 3 mL of the standard solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and confirm that the peak area of tetracycline obtained with 20 μL of this solution is equivalent to 1 to 5% of that with 20 μL of the standard solution.

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 tetracycline is not more than 1.0%.

Thrombin

トロンビン

Change the Expiration date as follows:

Shelf life 36 months after preparation.

Tobramycin

トブラマイシン

Change the Purity (1) as follows:

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Tobramycin 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 0.05.

Delete the following Monograph:

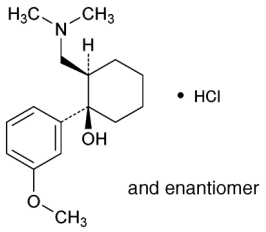
Tolazamide

トラザミド

Add the following:

Tramadol Hydrochloride

トラマドール塩酸塩



$C_{16}H_{25}NO_2$.HCl: 299.84
(1*RS*,2*RS*)-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol monohydrochloride
[*36282-47-0*]

Tramadol Hydrochloride contains not less than 99.0% and not more than 101.0% of tramadol hydrochloride ($C_{16}H_{25}NO_2$.HCl), calculated on the anhydrous basis.

Description Tramadol Hydrochloride occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in methanol, in ethanol (95) and in acetic acid (100).

A solution of Tramadol Hydrochloride (1 in 20) shows no optical rotation.

Melting point: 180 – 184°C

Tramadol Hydrochloride shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Tramadol Hydrochloride in ethanol (95) (1 in 10,000) 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 Tramadol Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <*2.25*>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tramadol Hydrochloride (1 in 100) responds to Qualitative Tests <*1.09*> (2) for chloride.

Purity **(1)** Acidity or alkalinity—Dissolve 1.0 g of Tramadol Hydrochloride in water to make 20 mL. To 10 mL of this solution add 0.2 mL of methyl red TS for acidity or alkalinity test and 0.2 mL of 0.01 mol/L hydrochloric acid VS: a red color develops. To this solution add 0.01 mol/L sodium hydroxide VS until the color of the solution changes from red to yellow: the consumed volume is not more than 0.4 mL.

(2) Heavy metals <*1.07*>—Proceed with 1.0 g of Tramadol Hydrochloride 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—(i) Dissolve 0.10 g of Tramadol Hydrochloride 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 500 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. Allow the plate to stand in ammonia vapor for 20 minutes, develop with a mixture of toluene, isopropanol and ammonia solution (28) (80:19:1) to a distance of about 15 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 1 hour, and examine under ultraviolet light (main wavelength: 254 nm): the spot at the *R*f value of about 0.5 obtained from the sample solution is not more intense than the spot from the standard solution.

(ii) Dissolve 0.15 g of Tramadol Hydrochloride 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 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 0.9 to tramadol obtained from the sample solution is not larger than 1/5 times the peak area of tramadol from the standard solution, the area of the peak other than tramadol and the peak mentioned above from the sample solution is not larger than 1/10 times the peak area of tramadol from the standard solution, and the total area of the peaks other than tramadol from the sample solution is not larger than 2/5 times the peak area of tramadol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.0 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 diluted trifluoroacetic acid (1 in 500) and acetonitrile (141:59).

Flow rate: Adjust so that the retention time of tramadol is about 5 minutes.

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

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add the mobile phase to make exactly 20 mL. Confirm that the peak area of tramadol 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: When the procedure is run with 20 μL of the standard solution under the above operating con-

ditions, the number of theoretical plates and the symmetry factor of the peak of tramadol 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 tramadol is not more than 2.0%.

Water ⟨*2.48*⟩ Not more than 0.5% (1 g, volumetric titration, direct titration).

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

Assay Weigh accurately about 0.18 g of Tramadol Hydrochloride, dissolve in 25 mL of acetic acid(100), add 10 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.

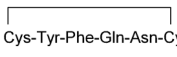
Each mL of 0.1 mol/L perchloric acid VS
= 29.98 mg of $C_{16}H_{25}NO_2.HCl$

Containers and storage Containers—Tight containers.

Vasopressin Injection

バソプレシン注射液

Change as follows:



$C_{46}H_{65}N_{15}O_{12}S_2$: 1084.23
[*113-79-1*]

Vasopressin Injection is an aqueous injection.

It is a synthetic vasopressin consisting of 9 amino acid residues.

It contains not less than 90.0% and not more than 120.0% of the labeled Units of vasopressin ($C_{46}H_{65}N_{15}O_{12}S_2$).

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

Description Vasopressin Injection is a clear and colorless liquid.

pH ⟨*2.54*⟩ 3.0 – 4.0

Purity Related substances—To a suitable amount of Vasopressin Injection add diluted acetic acid (100) (1 in 400) so that each mL contains 20 Units of vasopressin ($C_{46}H_{65}N_{15}O_{12}S_2$), and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of the peak eluted before the vasopressin is not more than 2.0%, and the total amount of the peaks other than vasopressin is not more than 10.0%.

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 (3 μm in particle diameter).

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

Mobile phase A: Dissolve 6.6 g of diammonium hydrogen phosphate in 950 mL of water, adjust to pH 3.0 with phosphoric acid, and add water to make 1000 mL. To 950 mL of this solution add 50 mL of acetonitrile.

Mobile phase B: Dissolve 6.6 g of diammonium hydrogen phosphate in 950 mL of water, adjust to pH 3.0 with phosphoric acid, and add water to make 1000 mL. To 450 mL of this solution add 550 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 – 45	90	10
45 – 90	90 → 30	10 → 70
90 – 100	30	70

Flow rate: About 0.6 mL per minute.

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

System suitability—

Test for required detectability: To 1 mL of the sample solution add diluted acetic acid (100) (1 in 400) 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 diluted acetic acid (100) (1 in 400) to make exactly 10 mL. Confirm that the peak area of vasopressin 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 vasopressin are not less than 17,500 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 vasopressin is not more than 2.0%.

Bacterial endotoxins ⟨*4.01*⟩ Less than 15 EU/ Unit.

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 Pipet V mL of Vasopressin Injection, equivalent to about 40 Units of vasopressin, add diluted acetic acid (100) (1 in 400) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 4 mg of Vasopressin RS, and dissolve in diluted acetic acid (100) (1 in 400) to make exactly 20 mL. Pipet 4 mL of this solution, and add diluted acetic acid (100) (1 in 400) to make exactly 50 mL. Pipet 10 mL of this solution, add diluted acetic acid (100) (1 in 400) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of vasopressin in each solution.

Amount (Vasopressin Unit) of vasopressin in 1 mL
$= M_S \times A_T/A_S \times F \times 1/V \times 2$

M_S: Amount (mg) of Vasopressin RS taken
F: Content (Unit/mg) of Vasopressin 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 40°C.

Mobile phase: Dissolve 6.6 g of diammonium hydrogen phosphate in 950 mL of water, adjust to pH 3.0 with phosphoric acid, and add water to make 1000 mL. To 870 mL of this solution add 130 mL of acetonitrile.

Flow rate: About 1 mL per minute.

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 vasopressin are not less than 9500 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 vasopressin is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Verapamil Hydrochloride

ベラパミル塩酸塩

Change the Origin/limits of content, Description and Purity (4) as follows:

Verapamil Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl).

Description Verapamil Hydrochloride occurs as a white crystalline powder.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95) and in acetic anhydride, and sparingly soluble in water.

Purity

(4) Related substances—Dissolve 0.50 g of Verapamil Hydrochloride 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 100 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Separately, pipet 5 mL of the standard stock solution, add methanol to make exactly 50 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 standard solutions (1) and (2) on two plates of silica gel for thin-layer chromatography. With the one plate, develop the plate with a mixture of cyclohexane and diethylamine (17:3) to a distance of about 15 cm, air-dry the plate, heat at 110°C for 1 hour, and cool. Examine immediately after spraying evenly iron (III) chloride-iodine TS on the plate: the spots other than the principal spot and the spot on the original point from the sample solution, are not more intense than the spot from the standard solution (2), and the number of them which are more intense than the spot from the standard solution (1) is not more than 3. With another plate, develop the plate with a mixture of toluene, methanol, acetone and acetic acid (100) (14:4:1:1), and perform the test in the same manner.

Verapamil Hydrochloride Tablets

ベラパミル塩酸塩錠

Change the Identification, Uniformity of dosage unit and Assay, and add the Disintegration as follows:

Identification To 2.5 mL of the sample solution obtained in the Assay add the mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make 100 mL, and determine the absorption spectrum of this solution as directed under

Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 228 nm and 232 nm, and between 277 nm and 281 nm.

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

To 1 tablet of Verapamil Hydrochloride Tablets add $7V/10$ mL of a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1), and sonicate until the tablet is disintegrated. After cooling, add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly V mL so that each mL contains about 0.8 mg of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl). Centrifuge this solution, and use the supernatant liquid as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl)
$= M_S \times A_T/A_S \times V/50$

M_S: Amount (mg) of verapamil hydrochloride for assay taken

Disintegration ⟨*6.09*⟩ It meets the requirement.

Assay To 25 Verapamil Hydrochloride Tablets, add $7V/10$ mL a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1), and sonicate until the tablets are disintegrated. Further, sonicate for about 5 minutes. After cooling, add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly V mL so that each mL contains about 2 mg of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl). Centrifuge this solution, pipet 10 mL of the supernatant liquid, add a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of verapamil hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in a mixture of methanol and 0.1 mol/L hydrochloric acid TS (3:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of verapamil in each solution.

Amount (mg) of verapamil hydrochloride ($C_{27}H_{38}N_2O_4$.HCl) in 1 tablet
$= M_S \times A_T/A_S \times V/500$

M_S: Amount (mg) of verapamil hydrochloride for assay taken

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: A mixture of methanol, water and perchloric acid (550:450:1).

Flow rate: Adjust so that the retention time of verapamil is about 5 minutes.

System suitability—

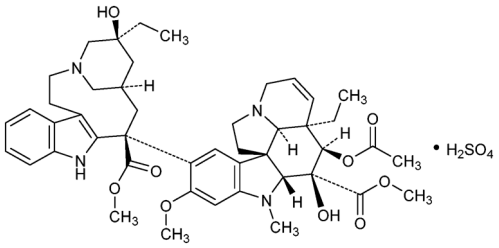
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of verapamil are not less than 2000 and not more than 2.0, respectively.

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 verapamil is not more than 1.0%.

Vinblastine Sulfate

ビンブラスチン硫酸塩

Change the Structural formula as follows:

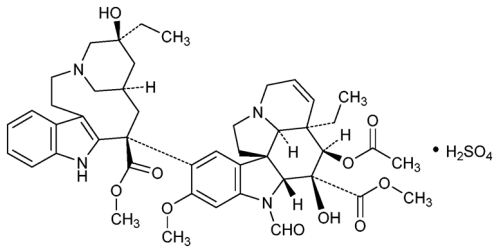


Methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-acetoxy-3a-ethyl-9-[(5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9-methoxycarbonyl-1,4,5,6,7,8,9,10-octahydro-3,7-methano-3-azacycloundecino[5,4-*b*]indol-9-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate monosulfate

Vincristine Sulfate

ビンクリスチン硫酸塩

Change the Structural formula as follows:



Methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-acetoxy-3a-ethyl-9-[(5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9-methoxycarbonyl-1,4,5,6,7,8,9,10-octahydro-3,7-methano-3-azacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-5-hydroxy-8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate monosulfate

Add the following:

Voriconazole for Injection

注射用ボリコナゾール

Voriconazole for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 105.0% of the labeled amount of voriconazole ($C_{16}H_{14}F_3N_5O$: 349.31). Correct the amount obtained in the Assay with *T* value.

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

Description Voriconazole for Injection is white, masses or powder.

Identification To 5 mL of the sample solution obtained in the Assay add the mobile phase in the Assay to make 25 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits a maximum between 254 nm and 258 nm.

pH Being specified separately when the drug is granted approval based on the Law.

Purity **(1)** Related substances—Dissolve the content of 1 container of Voriconazole for Injection in water so that each mL contains about 10 mg of voriconazole ($C_{16}H_{14}F_3N_5O$). To 5 mL of this solution add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.26 to voriconazole, obtained from the sample solution is not larger than 2.5 times the peak area of voriconazole from the standard solution, the area of the peak, having the relative retention time of about 0.32, from the sample solution is not larger than the peak area of voriconazole from the standard solution, the area of the peak, having the relative retention time of about 0.5, from the sample solution is not larger than 2 times the peak area of voriconazole from the standard solution, and the area of peak other than voriconazole, the peak having the relative retention time of about 0.61 and the peaks mentioned above from the sample solution is not larger than the peak area of voriconazole from the standard solution. Furthermore, the total area of the peaks other than voriconazole and the peak having the relative retention time of about 0.61 from the sample solution is not larger than 7 times the peak area of voriconazole from the standard solution. For the areas of the peaks, having the relative retention times of about 0.26, about 0.32 and about 0.5, multiply their relative response factors, 0.7, 0.7 and 1.2, 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 1.3 times as long as the retention time of voriconazole.

System suitability—

System performance: Suspend 0.1 g of voriconazole in 10 mL of a solution of sodium hydroxide (1 in 25), add the mobile phase to make 20 mL, and allow to stand for 30 minutes. To 1 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peaks, having the relative retention times about 0.26 and about 0.32 to voriconazole, is not less than 1.5.

System repeatability: To 5 mL of the standard solution add the mobile phase to make 10 mL. When the test is repeated 6 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of voriconazole is not more than 5.0%.

(2) Optical isomer—Dissolve the content of 1 container of Voriconazole for Injection in the mobile phase so that each mL contains about 1 mg of voriconazole ($C_{16}H_{14}F_3N_5O$). To 5 mL of this solution add 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 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 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 1.3 to voriconazole, obtained from the sample solution is not larger than 4 times the peak area of voriconazole from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Voriconazole.

System suitability—

Proceed as directed in the system suitability in the Purity (3) under Voriconazole.

Bacterial endotoxins ⟨*4.01*⟩ Less than 1.5 EU/mg.

Uniformity of dosage units ⟨*6.02*⟩ It meets the requirement of the Mass variation test (*T*: 106.0%).

Foreign insoluble matter ⟨*6.06*⟩ Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter ⟨*6.07*⟩ It meets the requirement.

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

Assay Take 10 containers of Voriconazole for Injection, dissolve the contents of each in the mobile phase, combine the solutions, and add the mobile phase to make exactly 1000 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Voriconazole RS (separately determine the water <2.48> in the same manner as Voriconazole), and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of voriconazole in each solution.

Amount (mg) of voriconazole ($C_{16}H_{14}F_3N_5O$) in 1 container of Voriconazole for Injection
$= M_S \times A_T/A_S \times 4$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 256 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.9 g of ammonium formate in 1000 mL of water, and adjust to pH 4.0 with formic acid. To 550 mL of this solution add 300 mL of methanol and 150 mL of acetonitrile.

Flow rate: Adjust so that the retention time of voriconazole is about 9 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 voriconazole are not less than 5000 and not more than 1.7, 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 voriconazole is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Delete the following Monograph:

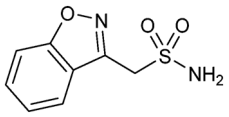
Zinostatin Stimalamer

ジノスタチン　スチマラマー

Add the following:

Zonisamide

ゾニサミド



$C_8H_8N_2O_3S$: 212.23
1,2-Benzisoxazol-3-ylmethanesulfonamide
[*68291-97-4*]

Zonisamide, when dried, contains not less than 98.0% and not more than 101.0% of zonisamide ($C_8H_8N_2O_3S$).

Description Zonisamide occurs as white to pale yellow, crystals or crystalline powder.

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

Identification **(1)** Determine the absorption spectrum of a solution of Zonisamide in methanol (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 Zonisamide 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 Zonisamide, 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 dried Zonisamide RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 164 – 168°C

Purity **(1)** Chloride <1.03>—Dissolve 1.0 g of Zonisamide in 30 mL of acetone, and 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 1.0 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.036%).

(2) Sulfate <1.14>—Dissolve 1.0 g of Zonisamide in 30 mL of acetone, and 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 1.0 mL of 0.005 mol/L sulfuric acid VS add 30 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Zonisamide 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).

(4) Related substances—Dissolve 25 mg of Zonisamide in 8 mL of tetrahydrofuran, add water to make 50 mL, 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. Determine each peak area by the automatic integration method: the area of the peak other than zonisamide obtained from the sample solution is not larger than 1/5 times the peak area of zonisamide 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 zonisamide, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 3 mL of the standard solution, add the mobile phase to make exactly 50 mL. Confirm that the peak area of zonisamide obtained with 10 μL of this solution is equivalent to 4.2 to 7.8% 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 zonisamide are not less than 8000 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 zonisamide is not more than 2.0%.

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 0.1 g of Zonisamide, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Zonisamide RS, previously dried, and dissolve in methanol 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 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 zonisamide to that of the internal standard.

$$\text{Amount (mg) of zonisamide } (C_8H_8N_2O_3S) = M_S \times Q_T/Q_S \times 2$$

M_S: Amount (mg) of Zonisamide RS taken

Internal standard solution—A solution of 4-aminoacetophenone in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 239 nm).

Column: A stainless steel column 5 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 tetrahydrofuran (5:1).

Flow rate: Adjust so that the retention time of zonisamide is about 11 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 zonisamide 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 zonisamide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Add the following:

Zonisamide Tablets

ゾニサミド錠

Zonisamide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of zonisamide ($C_8H_8N_2O_3S$: 212.23).

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

Identification To 5 mL of the sample solution obtained in the Assay add 5 mL of methanol. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 237 nm and 241 nm, between 243 nm and 247 nm, and between 282 nm and 286 nm.

Uniformity of dosage unit <6.02> Perform the Mass variation test, or the Content uniformity test according to the following method: it meets the requirement.

To 1 tablet of Zonisamide Tablets add V/25 mL of water, disintegrate completely by sonicating, add 7V/10 mL of methanol, and shake for 15 minutes. Add methanol to make exactly V mL so that each mL contains about 0.5 mg of zonisamide ($C_8H_8N_2O_3S$). Centrifuge this solution, pipet 3 mL of the supernatant liquid, add methanol to make exactly 50 mL, and use this solution as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of zonisamide ($C_8H_8N_2O_3S$)
$= M_S \times A_T/A_S \times V/75$

M_S: Amount (mg) of Zonisamide RS taken

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 25-mg tablet is not less than 75%, and those in 10 minutes and 45 minutes of 100-mg tablet are not more than 65% and not less than 70%, respectively.

Start the test with 1 tablet of Zonisamide Tablets. In the case of 25-mg tablets, withdraw not less than 20 mL of the medium at the specified minutes after starting the test. In the case of 100-mg tablets, withdraw exactly 20 mL of the medium at the specified minutes after starting the test, and supply exactly 20 mL of water warmed to 37 ± 0.5°C immediately after withdrawing of the medium every time. Filter these media through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL or more 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 zonisamide ($C_8H_8N_2O_3S$), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Zonisamide RS, 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. Determine the absorbances, $A_{T(n)}$ and A_S, of the sample solution and standard solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of zonisamide ($C_8H_8N_2O_3S$) on the nth medium withdrawing (n = 1, 2)

$$= M_S \times \left\{ \frac{A_{T(n)}}{A_S} + \sum_{i=1}^{n-1} \left(\frac{A_{T(i)}}{A_S} \times \frac{1}{45} \right) \right\} \times \frac{V'}{V} \times \frac{1}{C} \times 90$$

M_S: Amount (mg) of Zonisamide RS taken
C: Labeled amount (mg) of zonisamide ($C_8H_8N_2O_3S$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Zonisamide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg of zonisamide ($C_8H_8N_2O_3S$), and moisten with 2 mL of water. Add 70 mL of methanol, shake for 15 minutes, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 38 mg of Zonisamide RS, previously dried at 105°C for 3 hours, dissolve in 1 mL of water and methanol to make exactly 50 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, of the sample solution and standard solution at 284 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of zonisamide ($C_8H_8N_2O_3S$)
$= M_S \times A_T/A_S \times 2$

M_S: Amount (mg) of Zonisamide RS taken

Containers and storage Containers—Tight containers.

Crude Drugs and Related Drugs

Powdered Alisma Tuber

タクシャ末

Add the following next to the Description:

Identification To 1.0 g of Powdered Alisma Tuber add 10 mL of diethyl ether, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Use alisma tuber triterpenes TS for identification 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 1 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) (10:10:3) 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: one of the spot among the several spots obtained from the sample solution has the same color tone and *R*f value with a spot among the three spots obtained from the standard solution.

Artemisia Capillaris Flower

インチンコウ

Change the alias in Japanese as follows:

茵蔯蒿

Bakumondoto Extract

麦門冬湯エキス

Change the Origin/limits of content, Identification and Assay (2) as follows:

Bakumondoto Extract contains not less than 1.2 mg of ginesenoside Rb_1 ($C_{54}H_{92}O_{23}$: 1109.29), and not less than 14 mg and not more than 42 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 5 mL of 1-butanol, shake, centrifuge, remove the 1-butanol layer, and use the aqueous layer as the sample solution. Separately, heat 3.0 g of pulverized ophiopogon root in 50 mL of water under a reflux condenser for 1 hour. After cooling, shake 20 mL of the extract with 5 mL of 1-butanol, centrifuge, remove the 1-butanol layer, and use the aqueous layer as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL of the sample solution and 5 μL of the standard solution as bands on the original line on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), water and acetic acid (100) (120:80:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the dark blue-green spot (*R*f value: about 0.3) from the standard solution (Ophiopogon Root).

(2) Shake 5.0 g of the dry extract (or 15 g of the viscous extract) with 15 mL of water, add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of cycloartenyl ferulate for thin-layer chromatography in 1 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 30 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, acetone and acetic acid (100) (50:20:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution. Or examine under ultraviolet light (main wavelength: 365 nm) after spraying evenly a mixture of sulfuric acid and ethanol (99.5) (1:1) on the plate, and heating the plate at 105°C for 5 minutes: one of the several spots from the sample solution has the same color tone and *R*f value with the yellow fluorescent spot from the standard solution (Brown Rice).

(3) 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-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_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 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 the plate at 105°C for 5 minutes, and allow to cool: one of the several

spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Ginseng).

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(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 <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Bofutsushosan Extract

防風通聖散エキス

Change the Origin/limits of content as follows:

Bofutsushosan Extract contains not less than 9 mg and not more than 36 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 4 mg and not more than 12 mg of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 54 mg and not more than 162 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 13 mg and not more than 39 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Change the Identification (7), (8), (9) and (15) as follows:

Identification

(7) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of 0.1 mol/L hydrochloric acid TS, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 1 mL of methanol to the residue, and use the solution as the sample solution. Separately, dissolve 1 mg of rosmarinic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 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 acetic acid (100) (60:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the greenish brown spot from the standard solution (Schizonepeta Spike; Mentha Herb).

(8) For preparation prescribed Saposhnikovia Root and Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of 4′-*O*-glycosyl-5-*O*-methylvisamminol for thin-layer chromatography in 1 mL of methanol, and use this so-

lution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 2 minutes, then examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution (Saposhnikovia Root and Rhizome).

(9) For preparation prescribed Glehnia Root and Rhizome—To 0.5 g of the dry extract (or 1.5 g of the viscous extract) add 5 mL of ethyl acetate, and heat on a water bath under a reflux condenser for 30 minutes. After cooling, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of scopoletin 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 20 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 and hexane (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution (Glehnia Root and Rhizome).

(15) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. 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 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Add the following next to the Identification (17) as follows:

Identification

(18) Place 2.0 g of the dry extract (or 6.0 g of the viscous extract) in a crucible, and ignite at 550°C for 5 hours to incinerate. To the residue add 3 mL of diluted sulfuric acid (1 in 3), and heat until white fumes are evolved. After cooling, add 20 mL of water, shake, and filter. To 5 mL of the filtrate add ammonia TS until a white gelatinous precipitate is formed, centrifuge, and remove the supernatant liquid. To the residue add 5 mL of water, shake, centrifuge, and remove the supernatant liquid. Then, to the residue add 5 mL of water, shake, centrifuge, and remove the supernatant liquid. To the obtained residue add 5 drops of alizarin red S TS, and shake occasionally in lukewarm water: the residue is red to red-brown in color (Kasseki).

Change the Assay (4) as follows:

Assay

(4) 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 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. When the procedure is run with 10 μL of this solu-

tion under the above operating conditions, the resolution between the peak having the relative retention time of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Boiogito Extract

防已黄耆湯エキス

Change the Origin/limits of content, Identification (4), (6) and Assay (2) as follows:

Boiogito Extract contains not less than 4 mg and not more than 16 mg of shinomenine, and not less than 10 mg and not more than 30 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification

(4) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, and shake. Separate the hexane layer, evaporate the solvent under reduced pressure, then 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 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 hexane and acetone (7:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(6) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. 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 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(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 <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Chotosan Extract

釣藤散エキス

Change the Origin/limits of content, Identification (1) to (8) and Assay (2) as follows:

Chotosan Extract contains not less than 24 mg and not more than 72 mg of hesperidin, not less than 6 mg and not more than 18 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), and not less than 0.3 mg of the total alkaloid (rhyncophylline and hirsutine), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 20 mL of water and 2 mL of ammonia TS, add 20 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg each of rhyncophylline for thin-layer chromatography and hirsutine 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 with fluorescent indicator 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. Examine under ultraviolet light (main wavelength: 254 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with one of the two dark purple spots from the standard solution (Uncaria Hook).

(2) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of hesperidin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:6:3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in an ammonia gas: one of 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 (Citrus Unshiu Peel).

(3) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 5 mL of 1-butanol, shake, centrifuge, remove the 1-butanol layer, and use the aqueous layer as the sample solution. Separately, heat 3.0 g of pulverized ophiopogon root in 50 mL of water under a reflux condenser for 1 hour. After cooling, shake 20 mL of the extract with 5 mL of 1-butanol, centrifuge, remove the 1-butanol layer, and use the aqueous layer as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL of the sample solution and 5 μL of the standard solution as bands on the original line on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), water and acetic acid (100) (120:80:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the dark blue-green spot (around *R*f value 0.3) from the standard solution (Ophiopogon Root).

(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-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_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 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 the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Ginseng).

(5) 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-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of 4′-*O*-glycosyl-5-*O*-methylvisamminol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 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 several spots obtained from the sample solution has the same color tone and *R*f value with the blue spot from the standard solution (Saposhnikovia Root and Rhizome).

(6) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 20 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of luteolin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as di-

rected under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 3 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 formic acid (5:5:1) 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 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 (Chrysanthemum Flower).

(7) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(8) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the 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 vanillin-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple spot from the standard solution (Ginger).

Assay

(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 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Cornus Fruit

サンシュユ

Change the Assay as follows:

Assay Weigh accurately about 1 g of fine cuttings of Cornus Fruit (separately determine the loss on drying <5.01>), put in a glass-stoppered centrifuge tube, add 30 mL of diluted methanol (1 in 2), shake for 20 minutes, centrifuge, and take the supernatant liquid. To the residue add 30 mL of diluted methanol (1 in 2), and repeat the above process twice more. Combine all the supernatant liquids, add diluted methanol (1 in 2) to make exactly 100 mL, and use

this solution as the sample solution. Separately, weigh accurately about 10 mg of loganin for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of loganin in each solution.

$$\text{Amount (mg) of loganin} = M_S \times A_T/A_S$$

M_S: Amount (mg) of loganin for assay taken

Operating conditions—

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

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

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

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: Adjust so that the retention time of loganin is about 25 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 loganin 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 loganin is not more than 1.5%.

Zedoary

ガジュツ

Change the Title of the monograph, Latin name, Origin/limits of content and Description as follows:

Curcuma Rhizome

Curcumae Rhizoma

ガジュツ

Curcuma Rhizome is the rhizome of 1) *Curcuma zedoaria* Roscoe, 2) *Curcuma phaeocaulis* Valeton or 3) *Curcuma kwangsiensis* S. G. Lee et C. F. Liang (*Zingiberaceae*), usually after being passed through hot water.

Description Nearly ovoid to oblong-ovoid or conical rhizome, 2 – 8 cm in length, 1.5 – 4 cm in diameter; externally grayish yellow-brown to grayish brown; nodes protruded as rings; internode of 0.3 – 0.8 cm, with scars of roots, and small protrusions consisting of scars of branched rhizomes; hard in texture; a transverse section reveals cortex and stele distinctly; cortex 2 – 5 mm in thickness; a transverse section, grayish brown in rhizome of 1) *Curcuma zedoria* origin, light yellow to grayish yellow or light yellow-green to grayish yellow-green in 2) *Curcuma phaeocaulis* origin and purplish brown to dark purple-brown in 3) *Curcuma kwangsiensis* origin, and sometimes lustrous.

Odor, characteristic; taste, pungent, bitter and cool feeling on chewing.

Under a microscope <*5.01*>, a transverse section of central part reveals the outermost layer usually consisting of a cork layer 4 – 10 cells thick; cortex and stele divided by endodermis, composed of parenchyma cells, vascular bundles scattered; small sized vascular bundles line up beneath the endodermis; oil cells contain yellow-brown to dark brown oily substances, scattered in parenchyma; parenchyma contains gelatinized starch and rarely crystals of calcium oxalate.

Cyperus Rhizome

コウブシ

Add the following next to the Description:

Identification To 2.0 g of pulverized Cyperus Rhizome add 10 mL of diethyl ether, 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 diethyl ether, cyclohexane and formic acid (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot appears at an *R*f value of about 0.35.

Powdered Cyperus Rhizome

コウブシ末

Add the following next to the Description:

Identification To 2.0 g of Powdered Cyperus Rhizome add 10 mL of diethyl ether, 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 diethyl ether, cyclohexane and formic acid (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes: a red-purple spot appears at an *R*f value of about 0.35.

Daiokanzoto Extract

大黄甘草湯エキス

Change the Origin/limits of content, Identification and Assay (2) as follows:

Daiokanzoto Extract contains not less than 3.5 mg of sennoside A ($C_{42}H_{38}O_{20}$: 862.74), and not less than 7 mg and not more than 21 mg (for preparation prescribed 1 g of Glycyrrhiza) or not less than 14 mg and not more than 42 mg (for preparation prescribed 2 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** To 1.0 g of Daiokanzoto Extract add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the orange fluorescent spot from the standard solution (Rhubarb).

(2) To 0.5 g of Daiokanzoto Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. 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 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(2) Glycyrrhizic acid—Weigh accurately about 0.2 g of Daiokanzoto Extract, add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Daisaikoto Extract

大柴胡湯エキス

Change the Identification (5) as follows:

Identification

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to

the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

Euodia Fruit

ゴシュユ

Change the Identification as follows:

Identification To 1.0 g of pulverized Euodia Fruit 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 acetone, 2-propanol, water and formic acid (7:7:1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a blue-white fluorescent spot is observed at an *R*f value of about 0.6. The spot shows a yellow-red color after being sprayed evenly Dragendorff's TS for spraying.

Gardenia Fruit

サンシシ

Change the origin/limits of content as follows:

Gardenia Fruit is the fruit of *Gardenia jasminoides* Ellis (*Rubiaceae*), sometimes after being passed through hot water or steamed.

It contains not less than 3.0% of geniposide, calculated on the basis of dried material.

Glycyrrhiza Extract

カンゾウエキス

Change the Origin/limits of content and Assay as follows:

Glycyrrhiza Extract contains not less than 3.6% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93).

Assay Weigh accurately about 0.15 g of Glycyrrhiza Extract, place in a glass-stoppered centrifuge tube, add 25 mL of dilute ethanol, and heat at 50°C for 30 minutes with occasional shaking. Cool, centrifuge, and take the supernatant liquid. To the residue add 20 mL of dilute ethanol, and proceed in the same manner. Combine the supernatant liquids, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in dilute ethanol 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.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: Adjust so that the retention time of glycyrrhizic acid is about 15 minutes.

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak with the relative retention time of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Crude Glycyrrhiza Extract

カンゾウ粗エキス

Change the Origin/limits of content and Assay as follows:

Crude Glycyrrhiza Extract contains not less than 4.8% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93).

Assay Weigh accurately about 0.15 g of Crude Glycyrrhiza Extract, place in a glass-stoppered centrifuge tube, add 25 mL of dilute ethanol, and heat at 50°C for 30 minutes with occasional shaking. Cool, centrifuge, and take the supernatant liquid. To the residue add 20 mL of dilute ethanol, and proceed in the same manner. Combine the supernatant liquids, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), dissolve in dilute ethanol 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.

$$\text{Amount (mg) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Glycyrrhizic Acid RS taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: Adjust so that the retention time of glycyrrhizic acid is about 15 minutes.

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak with the relative retention time of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Add the following:

Goreisan Extract

五苓散エキス

Goreisan Extract contains not less than 0.3 mg and not more than 1.2 mg (for preparation prescribed 1.5 g of Cinnamon Bark) or not less than 0.4 mg and not more than 1.6 mg (for preparation prescribed 2 g of Cinnamon Bark) or not less than 0.5 mg and not more than 2.0 mg (for preparation prescribed 2.5 g of Cinnamon Bark) or not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 3 g of Cinnamon Bark) of (*E*)-cinnamic acid, per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)	3)	4)	5)
Alisma Tuber	5 g	6 g	6 g	4 g	6 g
Polyporus Sclerotium	3 g	4.5 g	4.5 g	3 g	4.5 g
Poria Sclerotium	3 g	4.5 g	4.5 g	3 g	4.5 g
Atractylodes Rhizome	3 g	4.5 g	4.5 g	—	—
Atractylodes Lancea Rhizome	—	—	—	3 g	4.5 g
Cinnamon Bark	2 g	2.5 g	3 g	1.5 g	3 g

Prepare a dry extract or viscous extract as directed under Extracts, according to the prescription 1) to 5), using the crude drugs shown above.

Description Goreisan Extract occurs as a light red-brown to light brown powder, or a black-brown viscous extract. It has a characteristic odor, and a slightly sweet first, bitter, then acrid taste.

Identification (1) Weigh exactly 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 20 mL of water and 2 mL of ammonia solution (28), and shake. Add 20 mL of a mixture of hexane and ethyl acetate (20:1), shake, centrifuge, and separate the supernatant liquid. Add 20 mL of a mixture of hexane and ethyl acetate (20:1) to the residue, shake, centrifuge, and separate the supernatant liquid. Combine these supernatant liquids, evaporate the solvent under reduced pressure, add exactly 2 mL of methanol to the residue, and use this solution as the sample solution. Separately, weigh exactly 10 mg of alisol A for thin-layer chromatography, and dissolve in exactly 10 mL of methanol. Pipet 1 mL of this 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>. 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 formate, water and formic acid (30:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid TS on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and examine under ultraviolet light (main wavelength: 365 nm): one of 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, and it is larger and more intense than the spot from the standard solution (Alisma Tuber).

(2) 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, and shake. Separate the diethyl ether layer, evaporate the solvent under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of 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 hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red to red-purple spot from the standard solution (Atractylodes Rhizome).

(3) 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. Separate the hexane layer, and evaporate the solvent under reduce 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 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(4) Perform the test according to the following i) or ii) (Cinnamon Bark).

i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect an apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydradine TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot from the standard solution.

ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 5 mL of hexane, and shake. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution.

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 10.0% (1 g, 105°C, 5 hours).

The viscous extract: Not more than 66.7% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 10.0%, calculated on the dried basis.

Assay Conduct this procedure 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*)-cinnamic acid for assay, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 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 Chromatog-

raphy <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of (*E*)-cinnamic acid in each solution.

$$\text{Amount (mg) of (}E\text{)-cinnamic acid} = M_S \times A_T/A_S \times 1/20$$

M_S: Amount (mg) of (*E*)-cinnamic acid for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 273 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 (750:250:1).

Flow rate: 1.0 mL per minute (the retention time of (*E*)-cinnamic 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 (*E*)-cinnamic acid are not less than 5000 and not more than1.5, 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*)-cinnamic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Goshajinkigan Extract

牛車腎気丸エキス

Change the Identification (1), (3) to (7) as follows:

Identification **(1)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract), add 10 mL of water, shake, then add 30 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, methanol and 1-butanol (1:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool; a dark-green spot is observed at an *R*f value of about 0.6 (Rehmannia Root).

(3) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid 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 20 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, hexane and acetic acid (100) (10:10:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool, and examine under ultraviolet light (main wavelength: 365 nm): one of 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 (Alisma Tuber).

(4) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of paeonol 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 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the orange spot from the standard solution (Moutan Bark).

(5) Perform the test according to the following i) or ii) (Cinnamon Bark).

i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect an apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate 1 mL of the hexane layer, add 0.5 mL of sodium hydroxide TS, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot from the standard solution.

ii) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of

hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution.

(6) To 3.0 g of the dry extract (or 9.0 g of the viscous extract), add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 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 for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of 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 (Powdered Processed Aconite Root).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, to 0.3 g of pulverized plantago seed for thin-layer chromatography, add 1 mL of methanol, warm on a water bath for 3 minutes, centrifuge after cooling, 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 acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the deep blue spot (*R*f value: about 0.3) from the standard solution (Plantago Seed).

Change the Assay (1) as follows:

Assay (1) Loganin—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 loganin for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of loganin in each solution.

$$\text{Amount (mg) of loganin} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of loganin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (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 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 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 symmetry factor of the peak of loganin 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 loganin is not more than 1.5%.

Hachimijiogan Extract

八味地黄丸エキス

Change the Identification (1), (3) to (6) as follows:

Identification (1) To 1.0 g of the dry extract (or 3.0 g of the viscous extract), add 10 mL of water, shake, then add 30 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, methanol and 1-butanol (1:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool; a dark green spot is observed at an *R*f value of about 0.6 (Rehmannia Root).

(3) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid 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 20 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, hexane and acetic acid (100) (10:10:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid TS on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and examine under ultraviolet light (main wavelength: 365 nm): one of 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 (Alisma Tuber).

(4) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of paeonol 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 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the orange spot from the standard solution (Moutan Bark).

(5) Perform the test according to the following i) or ii) (Cinnamon Bark).

i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect an apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate 1 mL of the hexane layer, add 0.5 mL of sodium hydroxide TS, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot from the standard solution.

ii) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution.

(6) To 3.0 g of the dry extract (or 9.0 g of the viscous extract), add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 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 for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of 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 (Processed Aconite Root or Powdered Processed Aconite Root).

Change the Assay (1) as follows:

Assay (1) Loganin—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 loganin for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of loganin in each solution.

$$\text{Amount (mg) of loganin} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of loganin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 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 50°C.

Mobile phase: A mixture of water, acetonitrile and methanol (55:4:1).

Flow rate: 1.2 mL per minute (the retention time of loganin is about 25 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 symmetry factor of the peak of loganin 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 loganin is not more than 1.5%.

Hangekobokuto Extract

半夏厚朴湯エキス

Change the Identification as follows:

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. Separate 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 magnolol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot from the standard solution (Magnolia Bark).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of 0.1 mol/L hydrochloric acid TS, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of rosmarinic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 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 (60:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot from the standard solution (Perilla Herb).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the 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 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

Hangeshashinto Extract

半夏瀉心湯エキス

Change the Origin/limits of content, Identification (2), (3), (5) and Assay (2) as follows:

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 18 mg and not more than 54 mg (for preparation prescribed 2.5 g of Glycyrrhiza) or not less than 20 mg and not more than 60 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 [as berberine chloride ($C_{20}H_{18}ClNO_4$: 371.81)], per extract prepared with the amount specified in the Method of preparation.

Identification

(2) For preparation prescribed Processed 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. Separate 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 chroma-

tography 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot 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. Separate 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

(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-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(2) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) 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 <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5. Dissolve 1 mg of baicalein for resolution check in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of glycyrrhizic acid and baicalein 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 glycyrrhizic acid is not more than 1.5%.

ii) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use

this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), 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 taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in i).

System suitability—

System repeatability: Proceed as directed in the system suitability in i).

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

Hochuekkito Extract

補中益気湯エキス

Change the Origin/limits of content, Identification and Assay (3) as follows:

Hochuekkito Extract contains not less than 16 mg and not more than 64 mg of hesperidin, not less than 0.3 mg and not more than 1.2 mg (for preparation prescribed 1 g of Bupleurum Root) or not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 2 g of Bupleurum Root) of saikosaponin b_2, and not less than 10 mg and not more than 30 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, and shake. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_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 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, 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 the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Ginseng).

(2) For preparation prescribed Atractylodes Rhizome—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and separate 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 atractylenolide III 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 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, shake, and separate the hexane layer. Evaporate the layer under reduced pressure, add 2 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 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(4) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 40 mL of a solution of potassium hydroxide in methanol (1 in 50), shake for 15 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 30 mL of water and 20 mL of diethyl ether to the residue, shake, remove the diethyl ether layer, and separate the aqueous layer. To the aqueous layer add 20 mL of 1-butanol, shake, and separate the 1-butanol layer. To the 1-butanol layer add 20 mL of water, shake, separate the 1-butanol layer, evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of astragaloside 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 octadecylsilanized silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, water, 1-butanol and acetic acid (100) (60:30:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red-brown spot from the standard solution (Astragalus Root).

(5) To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and separate 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 (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution (Japanese Angelica Root).

(6) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and separate the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of hesperidin 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 2 μL of the sample solution and 20 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:6:3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and expose to ammonia vapor: one of 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 (Citrus Unshiu Peel).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, and shake. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of 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 (Bupleurum Root).

(8) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and separate the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of 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 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(9) For preparation prescribed Ginger—To 3.0 g of the dry extract (or 9.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and separate 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 [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 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 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

(10) For preparation prescribed Processed Ginger—Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect an apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour,

separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of [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 60 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Processed Ginger).

(11) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and separate the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Use (*E*)-isoferulic acid-(*E*)-ferulic acid TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (20:12:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the light yellow-white fluorescent spot from the standard solution (Cimicifuga Rhizome).

Assay

(3) 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 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), 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.

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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Jujube

タイソウ

Change the latin name and origin/limits of content as follows:

Ziziphi Fructus

Jujube is the fruit of *Ziziphus jujuba* Miller var. *inermis* Rehder (*Rhamnaceae*).

Jujube Seed

サンソウニン

Change the latin name and origin/limits of content as follows:

Ziziphi Semen

Jujube Seed is the seed of *Ziziphus jujuba* Miller var. *spinosa* Hu ex H. F. Chou (*Rhamnaceae*).

Juzentaihoto Extract

十全大補湯エキス

Change the Origin/limits of content, Identification and Assay (3) as follows:

Juzentaihoto Extract contains not less than 1.5 mg (for preparation prescribed 2.5 g of Ginseng) or not less than 1.8 mg (for preparation prescribed 3 g of Ginseng) of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$: 1109.29), not less than 26 mg and not more than 78 mg of paeonifrolin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 6 mg and not more than 18 mg (for preparation prescribed 1 g of Glycyrrhiza) or not less than 10 mg and not more than 30 mg (for preparation prescribed 1.5 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 15 mL of sodium hydroxide TS, centrifuge, and take the supernatant liquid. To the liquid add 10 mL of 1-butanol, shake, centrifuge, and separate the 1-butanol layer. To the 1-butanol layer add 10 mL of water, shake, centrifuge, and separate the 1-butanol layer. Evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_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 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 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the dark brown spot from the standard solution (Ginseng).

(2) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 15 mL of sodium hydroxide TS, centrifuge, and take the supernatant liquid. To the liquid add 10 mL of 1-butanol, shake, centrifuge, and separate the 1-butanol layer. To the 1-butanol layer add 10 mL of water, shake, centrifuge, and separate the 1-butanol layer. Evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of astragaloside 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 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 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red-brown spot from the standard solution (Astragalus 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 5 mL of diethyl ether, shake, and centrifuge. Use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of atractylenolide III 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 and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red spot from the standard solution (Atractylodes Rhizome).

(4) For preparation prescribed Atractylodes Lancea Rhizome—Shake 5.0 g of the dry extract (or 15.0 g of the viscous extract) with 10 mL of water, add 25 mL of hexane , and shake. Separate the hexane layer, evaporate the layer under reduced pressure, add 2 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 40 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 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(5) 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, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, then add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of

the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution (Cnidium Rhizome; Japanese Angelica Root).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorine for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of 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 (Peony Root).

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 30 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, methanol and 1-butanol (1:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: a dark green spot is observed at an *R*f value of about 0.6 (Rehmannia Root).

(8) Perform the test according to the following i) or ii) (Cinnamon Bark).

i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot from the standard solution.

ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution.

(9) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(3) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) 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 <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5. Dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of glycyrrhizic acid and (*E*)-cinnamaldehyde 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 glycyrrhizic acid is not more than 1.5%.

ii) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), 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 taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in i).

System suitability—

System repeatability: Proceed as directed in the system suitability in i).

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

Kakkonto Extract

葛根湯エキス

Change the Origin/limits of content, Identification (1), (3) to (6) and Assay as follows:

Kakkonto Extract contains not less than 7 mg and not more than 21 mg (for preparation prescribed 3 g of Ephedra Herb) or not less than 10 mg and not more than 30 mg (for preparation prescribed 4 g of Ephedra Herb) of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 14 mg and not more than 56 mg (for preparation prescribed 2 g of Peony Root) or not less than 21 mg and not more than 84 mg (for preparation prescribed 3 g of Peony Root) of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 15 mg and not more than 45 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification (1) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Puerarin RS or puerarin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution (Pueraria Root).

(3) Perform the test according to the following i) or ii) (Cinnamon Bark).

i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect an apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of

hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot from the standard solution.

ii) To 2.0 g of the dry extract (or 6.0 g of the viscous extract), add 10 mL of water, shake, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution.

(4) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of 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 (Peony Root).

(5) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. 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*>. Spot1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(6) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, shake, and separate 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

Assay (1) Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the aqueous layer add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and separate the supernatant liquid. In addition, repeat twice in the same manner for the aqueous layer using 1.0 mL of ammonia TS and 20 mL of diethyl ether. Combine all the supernatant liquids, evaporate the solvent under reduced pressure, dissolve the residue in diluted methanol (1 in 2) to make exactly 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay of crude drugs, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine obtained with the sample solution, and the peak area, A_S, of ephedrine with the standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]
$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drugs taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

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

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

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, and add 650 mL of water and 1 mL of phosphoric acid to dissolve sodium lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay of crude drugs and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

(2) Paeoniflorin—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, elute with 20 mL of water, add 1 mL of acetic acid (100) and water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of paeoniflorin in each solution.

Amount (mg) of paeoniflorin ($C_{23}H_{28}O_{11}$)
$= M_S \times A_T/A_S \times 5/8$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

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

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: 1.0 mL per minute (the retention time of paeoniflorin is about 9 minutes).

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin RS and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

(3) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) 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 <*2.48*> by coulometric titration, using 10 mg), 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.

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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of

glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5. Dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of glycyrrhizic acid and (*E*)-cinnamaldehyde 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 glycyrrhizic acid is not more than 1.5%.

ii) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), 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 taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in i).

System suitability—

System repeatability: Proceed as directed in the system suitability in i).

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

Kakkontokasenkyushin'i Extract

葛根湯加川芎辛夷エキス

Change the Origin/limits of content, Identification (5), (6) and Assay (3) as follows:

Kakkontokasenkyushin'i Extract contains not less than 9.5 mg and not more than 28.5 mg (for preparation prescribed 3 g of Ephedra Herb) or not less than 13 mg and not more than 39 mg (for preparation prescribed 4 g of Ephedra Herb) of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 17 mg and not more than 51 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 14 mg and not more than 42 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), and not less than 1.5 mg and not more than 6 mg (for preparation prescribed 2 g of Magnolia Flower) or not less than 2 mg and not more than 8 mg (for preparation prescribed 3 g of Magnolia Flower) of magnoflorine [as magnoflorine iodide ($C_{20}H_{24}INO_4$: 469.31)], per extract prepared with the amount specified in the Method of preparation.

Identification

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

Assay

(3) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) 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 <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5. Dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of glycyrrhizic acid and (*E*)-cinnamaldehyde 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 glycyrrhizic acid is not more than 1.5%.

ii) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), 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 taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in i).

System suitability—

System repeatability: Proceed as directed in the system suitability in i).

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

Kamikihito Extract

加味帰脾湯エキス

Change the Origin/limits of content, Identification (9) and Assay (3) as follows:

Kamikihito Extract contains not less than 0.8 mg and not more than 3.2 mg of saikosaponin b_2, not less than 27 mg and not more than 81 mg of geniposide, and not less than 6 mg and not more than 18 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of

preparation.

Identification

(9) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(3) 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 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), 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.

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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Kamishoyosan Extract

加味逍遙散エキス

Change the Origin/limits of content, Identification and Assay (3) as follows:

Kamishoyosan Extract contains not less than 28 mg and not more than 84 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), not less than 25 mg and not more than 75 mg of geniposide, and not less than 10 mg and not more than 30 mg (for preparation prescribed 1.5 g of Glycyrrhiza) or not less than 13 mg and not more than 39 mg (for preparation prescribed 2 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution (Japanese Angelica Root).

(2) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of albiflorin in 1 mL of methanol, and use this solution as the stand-

ard 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 ammonia solution (28) (6:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, allow to cool for more than 30 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the orange fluorescent spot from the standard solution (Peony Root).

(3) For preparation prescribed Atractylodes Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of atractylenolide III 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 and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red spot from the standard solution (Atractylodes Rhizome).

(4) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, and shake. Separate the hexane layer, evaporate the layer under reduced pressure, add 2 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 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(5) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of 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 (Bupleurum Root).

(6) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 15 mL of diethyl ether, and shake. Separate 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 paeonol 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 hexane and diethyl ether (5:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the orange spot from the standard solution (Moutan Bark).

(7) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 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 ammonia solution (28) (6:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of 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 (Gardenia Fruit).

(8) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one

of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(9) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid 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 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

(10) To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of diluted phosphoric acid (1 in 30), shake, then add 15 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, shake 0.2 g of pulverized mentha herb with 10 mL of diluted phosphoric acid (1 in 30), add 15 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 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 acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red-brown spot (around *R*f value 0.4) from the standard solution (Mentha Herb).

Assay

(3) 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 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Keishibukuryogan Extract

桂枝茯苓丸エキス

Change the Identification as follows:

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. Separate the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 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 hexane, ethyl acetate, formic acid and water (60:40:4:1) to a distance of about 7 cm, and air-

dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Cinnamon Bark).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the layer under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of paeonol 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 hexane and diethyl ether (5:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the orange spot from the standard solution (Moutan Bark).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of methanol, 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 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate and water (4:4:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 10 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the green-brown spot from the standard solution (Peach Kernel).

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of albiflorin in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat the plate at 105°C for 5 minutes, allow to cool for more than 30 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the orange fluorescent spot from the standard solution (Peony Root).

Maoto Extract

麻黄湯エキス

Change the Origin/limits of content, Identification (4) and Assay (3) as follows:

Maoto Extract contains not less than 15 mg and not more than 45 mg of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 48 mg and not more than 192 mg of amygdalin, and not less than 11 mg and not more than 33 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(3) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) 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 <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5. Dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of glycyrrhizic acid and (*E*)-cinnamaldehyde 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 glycyrrhizic acid is not more than 1.5%.

ii) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), 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 taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in i).

System suitability—

System repeatability: Proceed as directed in the system suitability in i).

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

Mukoi-Daikenchuto Extract

無コウイ大建中湯エキス

Change the Identification as follows:

Identification **(1)** Shake 2.0 g of Mukoi-Daikenchuto Extract with 10 mL of water, add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, shake 2.0 g of pulverized japanese zanthoxylum peel with 10 mL of water, add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, 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): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot (*R*f value: about 0.3) from the standard solution (Japanese Zanthoxylum Peel).

(2) Shake 2.0 g of Mukoi-Daikenchuto Extract with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_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 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 the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Ginseng).

(3) Shake 2.0 g of Mukoi-Daikenchuto Extract with 10 mL of water, add 10 mL of diethyl ether, shake, centrifuge,

and use the supernatant liquid 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 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 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Processed ginger).

Orengedokuto Extract

黄連解毒湯エキス

Change the Identification as follows:

Identification **(1)** 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 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).

(2) Shake 0.5 g of the dry extract (or 1.5 g of the viscous extract) with 5 mL of water, then add 25 mL of ethyl acetate, and shake. Separate the ethyl acetate layer, evaporate the solvent under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of limonin 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 (5:1) 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 the plate at 105°C for 5 minutes, and allow to cool: one of 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 (Phellodendron Bark).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the 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).

(4) 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 geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot from the standard solution (Gardenia Fruit).

Oriental Bezoar

ゴオウ

Change the Identification (1) as follows:

Identification **(1)** To 25 mg of pulverized Oriental Bezoar add 10 mL of methanol, shake for 5 minutes, and centrifuge. Take the supernatant liquid, evaporate the solvent under reduced pressure, dissolve the residue in 0.5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 5 mg each of cholic acid for thin-layer chromatography and deoxycholic acid for thin-layer chromatography in 5 mL of methanol, respectively, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution, standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, formic acid and methanol (30:1:1) 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 10 minutes: two of the several spots obtained from the sample solution

have the same color tone and *R*f value with each spot from the standard solutions (1) and (2).

Otsujito Extract

乙字湯エキス

Change the Origin/limits of content, Identification (4) and Assay (3) as follows:

Otsujito Extract contains not less than 1.2 mg and not more than 4.8 mg of saikosaponin b_2, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), not less than 14 mg and not more than 42 mg (for preparation prescribed 2 g of Glycyrrhiza) or not less than 20 mg and not more than 60 mg (for preparation prescribed 3 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), and not less than 0.5 mg of sennoside A ($C_{42}H_{38}O_{20}$: 862.74) or not less than 1.5 mg of rhein (for preparation prescribed 0.5 g of Rhubarb) or not less than 1 mg of sennoside A ($C_{42}H_{38}O_{20}$: 862.74) or not less than 3 mg of rhein (for preparation prescribed 1 g of Rhubarb), per extract prepared with the amount specified in the Method of preparation.

Identification

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(3) 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 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Platycodon Root

キキョウ

Change the origin/limits of content as follows:

Platycodon Root is the root of *Platycodon grandiflorus* A. De Candolle (*Campanulaceae*).

Rape Seed Oil

ナタネ油

Change the origin/limits of content as follows:

Rape Seed Oil is the fixed oil obtained from the seed of *Brassica napus* Linné or *Brassica rapa* Linné var. *oleifera* De Candolle (*Cruciferae*).

Rikkunshito Extract

六君子湯エキス

Change the Origin/limits of content, Identification and Assay (3) as follows:

Rikkunshito Extract contains not less than 2.4 mg of ginsenoside Rb_1 ($C_{54}H_{92}O_{23}$: 1109.29), not less than 16 mg and not more than 48 mg of hesperidin, and not less than 6 mg and not more than 18 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_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 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 the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Ginseng).

(2) 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, and shake. Separate 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 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution (Atractylodes Rhizome).

(3) 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. Separate the hexane layer, evaporate the layer under reduced pressure, add 2 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 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(4) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of hesperidin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:6:3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in an ammonia gas: one of 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 (Citrus Unshiu Peel).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the 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 30 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

Assay

(3) 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 <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Ryokeijutsukanto Extract

苓桂朮甘湯エキス

Change the Origin/limits of content, Identification and Assay (2) as follows:

Ryokeijutsukanto Extract contains not less than 1 mg and not more than 4 mg of (*E*)-cinnamic acid, and not less than 17 mg and not more than 51 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate 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 (*E*)-cinnamic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 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 hexane, ethyl acetate, formic acid and water (60:40:4:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Cinnamon Bark).

(2) For preparation prescribed Atractylodes Rhizome—To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate 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 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the

plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, and shake. Separate the hexane layer, evaporate the layer under reduced pressure, add 2 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 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(4) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, and 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 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(2) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) 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 <*2.48*> by coulometric titration, using 10 mg), 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.

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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5. Dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of glycyrrhizic acid and (*E*)-cinnamaldehyde 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 glycyrrhizic acid is not more than 1.5%.

ii) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), 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 taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in i).

System suitability—

System repeatability: Proceed as directed in the system suitability in i).

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

Saibokuto Extract

柴朴湯エキス

Change the Origin/limits of content, Identification and Assay (3) as follows:

Saibokuto Extract contains not less than 2 mg and not more than 8 mg of saikosaponin b_2, not less than 90 mg and not more than 270 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 14 mg and not more than 42 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of 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 (Bupleurum Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, shake, and separate 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 wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 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, 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 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).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, shake, and separate 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 magnolol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot from the standard solution (Magnolia Bark).

(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-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_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 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 the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-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 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chro-

matography. 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, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of 0.1 mol/L hydrochloric acid TS, add 25 mL of diethyl ether, shake, and separate the diethyl ether layer. Evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of rosmarinic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 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 (60:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot from the standard solution (Perilla Herb).

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

Assay

(3) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) 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 <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5. Dissolve 1 mg of baicalein for resolution check in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of glycyrrhizic acid and baicalein 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 glycyrrhizic acid is not more than 1.5%.

ii) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Per-

form 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.

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 taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in i).

System suitability—

System repeatability: Proceed as directed in the system suitability in i).

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

Saikokeishito Extract

柴胡桂枝湯エキス

Change the Origin/limits of content, Identification and Assay (4) as follows:

Saikokeishito Extract contains not less than 1.5 mg and not more than 6 mg of saikosaponin b_2, not less than 60 mg and not more than 180 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), not less than 17 mg and not more than 51 mg (for preparation prescribed 2 g of Peony Root) or not less than 21 mg and not more than 63 mg (for preparation prescribed 2.5 g of Peony Root) of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 10 mg and not more than 30 mg (for preparation prescribed 1.5 g of Glycyrrhiza) or not less than 14 mg and not more than 42 mg (for preparation prescribed 2 g of Glycyrrhiza) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of 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 (Bupleurum Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, shake, and separate 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 wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution and 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, 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 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).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of 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 (Peony Root).

(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-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_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 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 the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot from the stand-

ard solution (Ginseng).

(5) Perform the test according to the following i) or ii) (Cinnamon Bark).

i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect an apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 50 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, diethyl ether and methanol (15:5:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydradine TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot from the standard solution.

ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution.

(6) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

Assay

(4) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) 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 <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. When the procedure is run with 10 μL of this solu-

tion under the above operating conditions, the resolution between the peak having the relative retention time of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5. Dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography and 1 mg of baicalein for resolution check in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, two peaks other than glycyrrhizic acid are observed with the resolutions between the peak of glycyrrhizic acid and each of the two 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 glycyrrhizic acid is not more than 1.5%.

ii) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), 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 taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in i).

System suitability—

System repeatability: Proceed as directed in the system suitability in i).

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

Saireito Extract

柴苓湯エキス

Change the Origin/limits of content, Identification and Assay (3) as follows:

Saireito Extract contains not less than 2 mg and not more than 8 mg of saikosaponin b_2, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 14 mg and not more than 42 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** To 2.0 g of Saireito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of 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 (Bupleurum Root).

(2) To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate 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 15 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

(3) To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate 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 wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 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, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of 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).

(4) To 2.0 g of Saireito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_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 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 the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Ginseng).

(5) To 2.0 g of Saireito Extract add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(6) To 2.0 g of Saireito Extract add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid 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 20 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, hexane and acetic acid (100) (10:10:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid TS on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and examine under ultraviolet light (main wavelength: 365 nm): one of 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 (Alisma Tuber).

(7) For preparation prescribed Atractylodes Rhizome—To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate 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 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution (Atractylodes Rhizome).

(8) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of hexane, and shake. Separate the hexane layer, evaporate the layer under reduced pressure, add 2 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 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(9) To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate 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 (*E*)-cinnamic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 40 μL of the sample solution and 2 μL of the 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, formic acid and water (60:40:4:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultravio-

let light (main wavelength: 254 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the dark purple spot from the standard solution (Cinnamon Bark).

Assay

(3) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) Weigh accurately about 0.5 g of Saireito Extract, 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 <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5. Dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography and 1 mg of baicalein for resolution check in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, two peaks other than glycyrrhizic acid are observed with the resolutions between the peak of glycyrrhizic acid and each of the two 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 glycyrrhizic acid is not more than 1.5%.

ii) Weigh accurately about 0.5 g of Saireito Extract, add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), 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 taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in i).

System suitability—

System repeatability: Proceed as directed in the system suitability in i).

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

Saposhnikovia Root and Rhizome

ボウフウ

Change the Identification as follows:

Identification To 1.0 g of pulverized Saposhnikovia Root and Rhizome, add 5 mL of methanol, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of 4′-*O*-glucosyl-5-*O*-methylvisamminol 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 4 μL of the sample solution and 1 μL of the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl formate, formic acid, 2-butanone, and water (20:5:5:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wave-

length: 254 nm): one of the several spots from the sample solution has the same color tone and *R*f value with the spot from the standard solution.

Delete the following monograph:

Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder

ロートエキス·パパベリン·アネスタミン散

Shakuyakukanzoto Extract

芍薬甘草湯エキス

Change the Origin/limits of content, Identification and Assay (2) as follows:

Shakuyakukanzoto Extract contains not less than 50 mg and not more than 150 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 40 mg and not more than 120 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** Shake 0.5 g of the dry extract (or 1.5 g of the viscous extract) with 10 mL of water, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of 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 (Peony Root).

(2) Shake 0.5 g of the dry extract (or 1.5 g of the viscous extract) with 10 mL of water, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(2) Glycyrrhizic acid—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 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 <*2.48*> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Shimbuto Extract

真武湯エキス

Change the Identification as follows:

Identification **(1)** To 2.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of 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 (Peony Root).

(2) For preparation prescribed Atractylodes Rhizome—To 1.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate 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 7 cm, and air-dry the plate. Spray evenly diluted sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution (Atractylodes Rhizome).

(3) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of hexane, and shake. Separate the hexane layer, evaporate the layer under reduced pressure, add 2 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 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(4) To 1.0 g of Shimbuto Extract, add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

(5) To 3.0 g of Shimbuto Extract, add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and take the supernatant liquid. Evaporate the supernatant liquid under reduced pressure, add 1 mL of acetonitrile to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 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 for spraying on the plate, and air-dry the plate. Then spray evenly sodium nitrite TS on the plate: one of 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 (Processed Aconite Root or Powdered Processed Aconite Root).

Shosaikoto Extract

小柴胡湯エキス

Change the Origin/limits of content, Identification and Assay (3) as follows:

Shosaikoto Extract contains not less than 2 mg and not more than 8 mg of saikosaponin b_2, not less than 80 mg and not more than 240 mg of baicalin ($C_{21}H_{18}O_{11}$: 446.36), and not less than 14 mg and not more than 42 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification **(1)** Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of 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 (Bupleurum Root).

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 25 mL of diethyl ether, and shake. Separate 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 15 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

(3) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 25 mL of diethyl ether, and shake. Separate 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 wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 20 μL of the sample solution and 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, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of 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).

(4) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb_1 RS or ginsenoside Rb_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 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 the plate at 105°C for 5 minutes, and allow to cool: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Ginseng).

(5) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography *<2.03>*. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(3) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water *<2.48>* by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5. Dissolve 1 mg of baicalein for resolution check in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of glycyrrhizic acid and baicalein 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 glycyrrhizic acid is not more than 1.5%.

ii) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), 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 taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in i).

System suitability—

System repeatability: Proceed as directed in the system suitability in i).

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

Shoseiryuto Extract

小青竜湯エキス

Change the Origin/limits of content, Identification (2)-(8) and Assay (1), (3) as follows:

Shoseiryuto Extract contains not less than 8 mg and not more than 24 mg of the total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 26 mg and not more than 78 mg of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 14 mg and not more than 42 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification

(2) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of 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 (Peony Root).

(3) For preparation prescribed Processed 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. Separate 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. De-

velop 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Processed Ginger).

(4) 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. Separate 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 the plate at 105°C for 5 minutes, allow to cool, and spray water: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-green to grayish green spot from the standard solution (Ginger).

(5) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

(6) Perform the test according to the following i) or ii) (Cinnamon Bark).

i) Put 10 g of the dry extract (or 30 g of the viscous extract) in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for 1 hour, separate the hexane layer, and use the layer as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-orange spot from the standard solution.

ii) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 10 mL of water, then add 5 mL of hexane, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (*E*)-2-methoxycinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 2 μL the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution.

(7) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of water, then add 25 mL of diethyl ether, and shake. Separate 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 asarinin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) 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 105°C for 5 minutes: one of 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 (Asiasarum Root).

(8) Shake 1.0 g of the dry extract (or 3.0 g of the viscous extract) with 10 mL of sodium hydroxide TS, then add 25 mL of diethyl ether, and shake. Separate 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 schisandrin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry

the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-purple spot from the standard solution (Schisandra Fruit).

Assay

(1) Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of dried substance), add 20 mL of diethyl ether, shake, then add 3.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the aqueous layer add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, centrifuge, and separate the supernatant liquid. In addition, repeat twice in the same manner for the aqueous layer using 1.0 mL of ammonia TS and 20 mL of diethyl ether. Combine all the supernatant liquids, evaporate the solvent under reduced pressure, dissolve the residue in diluted methanol (1 in 2) to make exactly 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay of crude drugs, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_{TE} and A_{TP}, of ephedrine and pseudoephedrine obtained from the sample solution, and the peak area, A_S, of ephedrine from the standard solution.

Amount (mg) of total alkaloids [ephedrine ($C_{10}H_{15}NO$) and pseudoephedrine ($C_{10}H_{15}NO$)]
$= M_S \times (A_{TE} + A_{TP})/A_S \times 1/10 \times 0.819$

M_S: Amount (mg) of ephedrine hydrochloride for assay of crude drugs taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

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

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

Mobile phase: To 5 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, and add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of ephedrine is about 27 minutes).

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay of crude drugs and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

(3) Glycyrrhizic acid—Perform the test according to the following i) or ii).

i) 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 <*2.48*> by coulometric titration, using 10 mg), 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.

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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5. Dissolve 1 mg of (*E*)-cinnamaldehyde for thin-layer chromatography in 50 mL of methanol. To 2 mL of this solution add 2 mL of the standard solution. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peaks of glycyrrhizic acid and (*E*)-cinnamaldehyde 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 glycyrrhizic acid is not more than 1.5%.

ii) Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), 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.

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 taken, calculated on the anhydrous basis

Operating conditions—

Proceed as directed in the operating conditions in i).

System suitability—

System repeatability: Proceed as directed in the system suitability in i).

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid is not less than 1.5.

Powdered Sweet Hydrangea Leaf

アマチャ末

Change the Identification as follows:

Identification To 1.0 g of Powdered Sweet Hydrangea Leaf add 10 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of sweet hydrangea leaf dihydroisocoumarin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, hexane and formic acid (5:5:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two of the several spots obtained from the sample solution have the same color tone and *R*f value with the spots from the standard solution.

Tokakujokito Extract

桃核承気湯エキス

Change the Origin/limits of content, Identification (4) and Assay (5) as follows:

Tokakujokito Extract contains not less than 38 mg and not more than 152 mg of amygdalin, not less than 1 mg and not more than 4 mg of (*E*)-cinnamic acid, not less than 3 mg of sennosides A ($C_{42}H_{38}O_{20}$: 862.74) or not less than 9 mg of rhein, and not less than 10 mg and not more than 30 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification

(4) To 1.0 g of Tokakujokito Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. 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 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(5) Glycyrrhizic acid—Weigh accurately about 0.5 g of Tokakujokito Extract, add 20 mL of ethyl acetate and 10 mL of water, and shake for 10 minutes. After centrifugation remove the upper layer, add 20 mL of ethyl acetate, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh

accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Tokishakuyakusan Extract

当帰芍薬散エキス

Change the Identification as follows:

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, add 25 mL of diethyl ether, and shake. Separate 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 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-white fluorescent spot from the standard solution (Japanese Angelica Root and 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-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Paeoniflorin RS or paeoniflorin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat the plate at 105°C for 5 minutes: one of 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 (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, and shake. Separate 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 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the blue-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. Separate the hexane layer, evaporate the layer 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 7 cm, and air-dry the plate. Examine under ultraviolet light

(main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(5) Shake 2.0 g of the dry extract (or 6.0 g of the viscous extract) with 20 mL of water and 2 mL of ammonia solution (28), add 20 mL of a mixture of hexane and ethyl acetate (20:1), shake, and centrifuge. Separate the upper layer, evaporate the layer under reduced pressure, add 2 mL of methanol 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 formate, water and formic acid (30:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid-acetic acid TS on the plate, heat the plate at 105°C for 5 minutes, allow to cool, and examine under ultraviolet light (main wavelength: 365 nm): one of 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 (Alisma Tuber).

Trichosanthes Root

カロコン

Add the following next to the Description:

Identification To 2.0 g of pulverized Trichosanthes Root add 5 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 hexane, ethyl acetate 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 10 minutes, and examine under ultraviolet light (main wavelength: 365 nm): a light yellow to light yellow-green fluorescent spot appears at an *R*f value of about 0.4.

Turmeric

ウコン

Change the latin name as follows:

Curcumae Longae Rhizoma

Powdered Turmeric

ウコン末

Change the latin name as follows:

Curcumae Longae Rhizoma Purveratum

Yokukansan Extract

抑肝散エキス

Change the Origin/limits of content, Identification (4) and (6), and Assay (3) as follows:

Yokukansan Extract contains not less than 0.15 mg of total alkaloids (rhyncophylline and hirsutine), not less than 0.6 mg and not more than 2.4 mg of saikosaponin b_2, and not less than 10 mg and not more than 30 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), per extract prepared with the amount specified in the Method of preparation.

Identification

(4) For preparation prescribed Atractylodes Lancea Rhizome—To 2.0 g of the dry extract (or 6.0 g of the viscous extract) add 10 mL of water, shake, then add 25 mL of hexane, and shake. Separate the hexane layer, evaporate the solvent under reduced pressure, add 2 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 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at an *R*f value of about 0.5. The spot shows a greenish brown color after being sprayed evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heated at 105°C for 5 minutes, and allowed to cool (Atractylodes Lancea Rhizome).

(6) To 1.0 g of the dry extract (or 3.0 g of the viscous extract) add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. 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 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the several spots obtained from the sample solution has

the same color tone and *R*f value with the yellow-green fluorescent spot from the standard solution (Glycyrrhiza).

Assay

(3) 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 20 mL of diethyl ether and 10 mL of water, and shake for 10 minutes. After centrifugation, remove the upper layer, add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the upper layer. To the resultant aqueous layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid RS (separately determine the water <2.48> by coulometric titration, using 10 mg), 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 taken, 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: Dissolve 3.85 g of ammonium acetate in 720 mL of water, and add 5 mL of acetic acid (100) and 280 mL of acetonitrile.

Flow rate: 1.0 mL per minute (the retention time of glycyrrhizic acid is about 15 minutes).

System suitability—

System performance: Dissolve 5 mg of monoammonium glycyrrhizinate for resolution check in 20 mL of dilute ethanol. 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 of about 0.9 to glycyrrhizic acid and the peak of glycyrrhizic acid 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 glycyrrhizic acid is not more than 1.5%.

Infrared
Reference Spectra

Delete the following spectra:

Diclofenamide

Fluoxymesterone

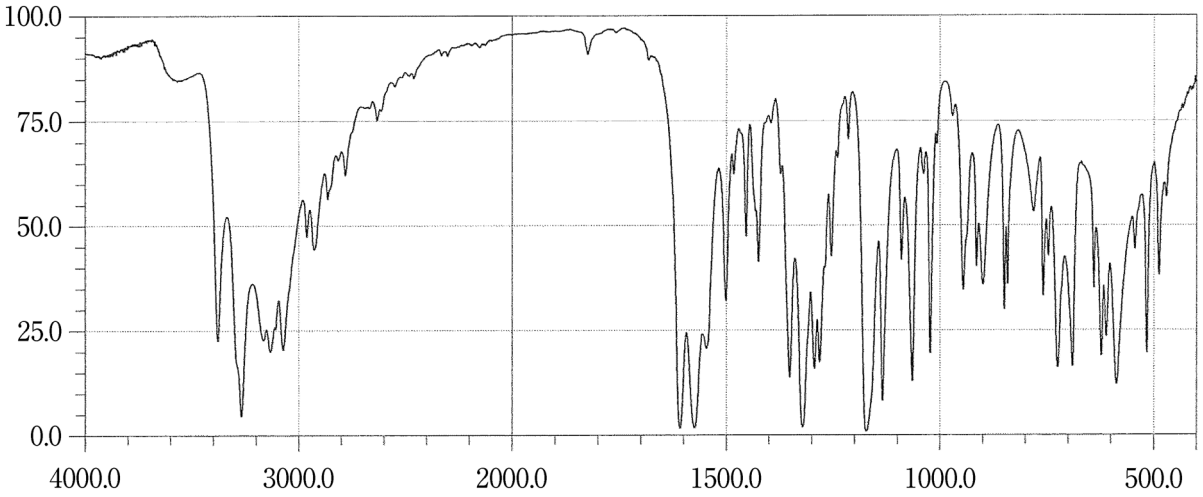
Rokitamycin

Saccharin Sodium Hydrate

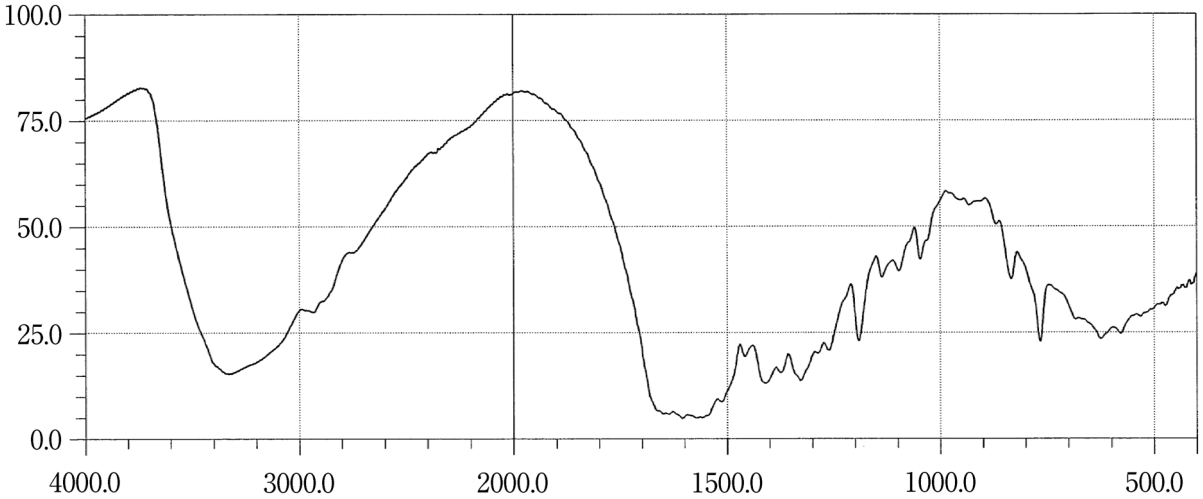
Tolazamide

Add the following spectra:

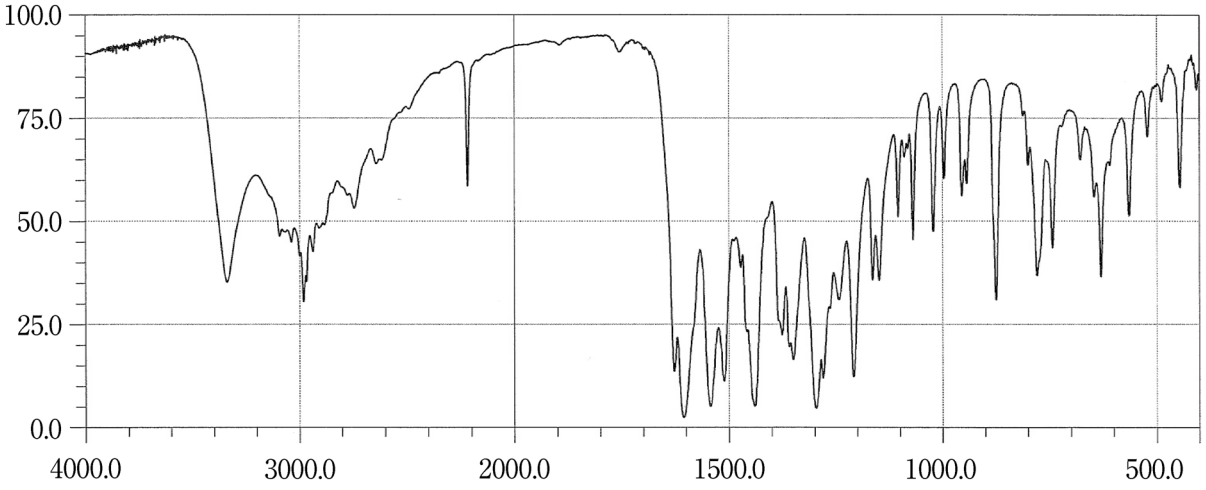
Azosemide



Calcium Levofolinate Hydrate

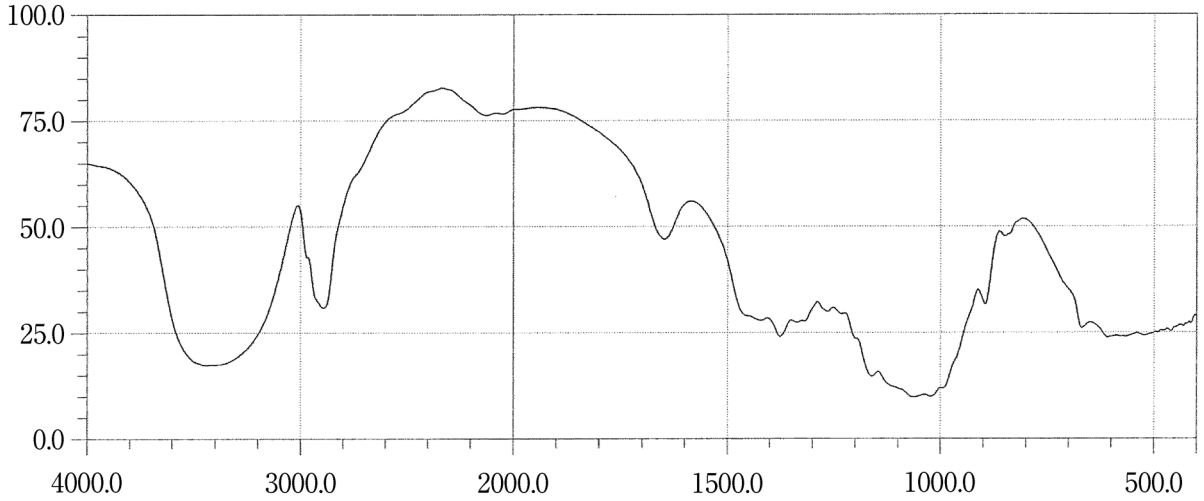


Entacapone

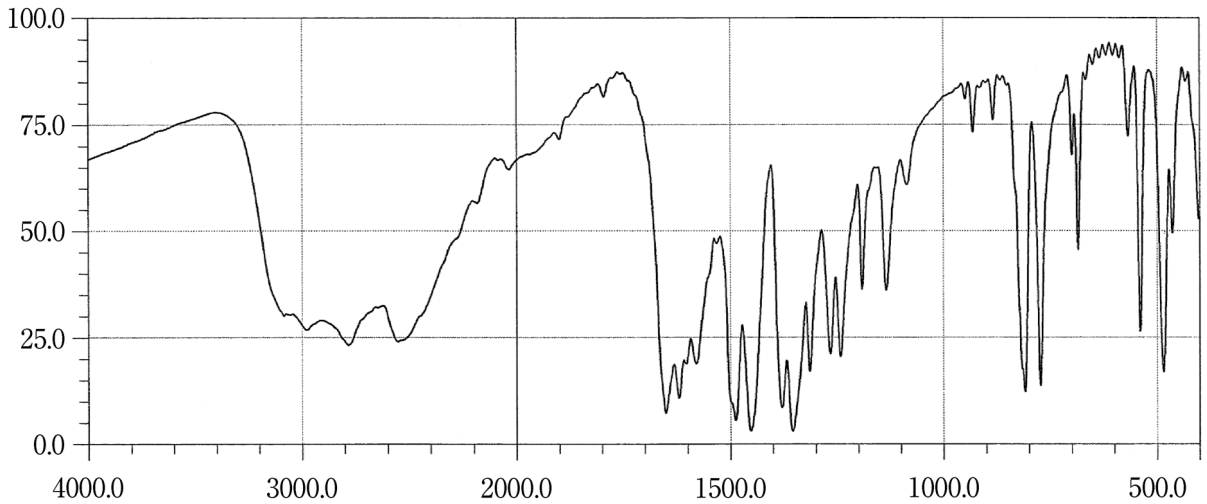


The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

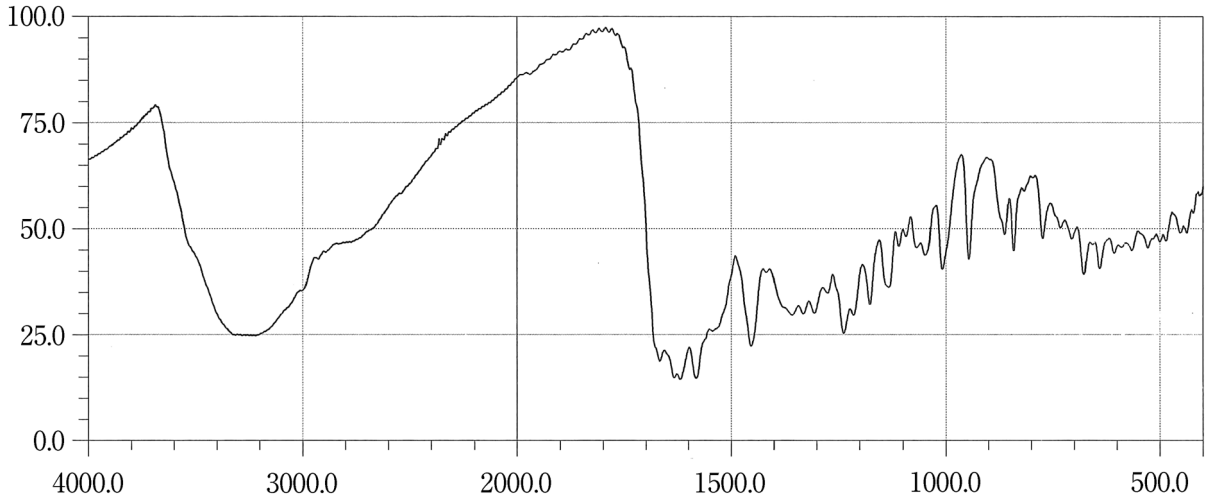
Low Substituted Hydroxypropylcellulose



Mesalazine

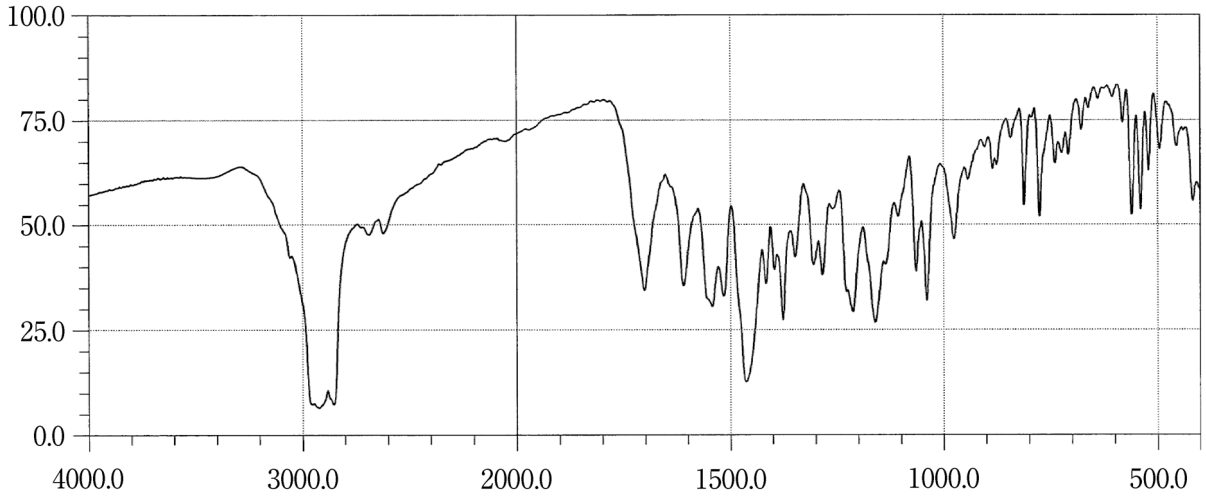


Oxytetracycline Hydrochloride

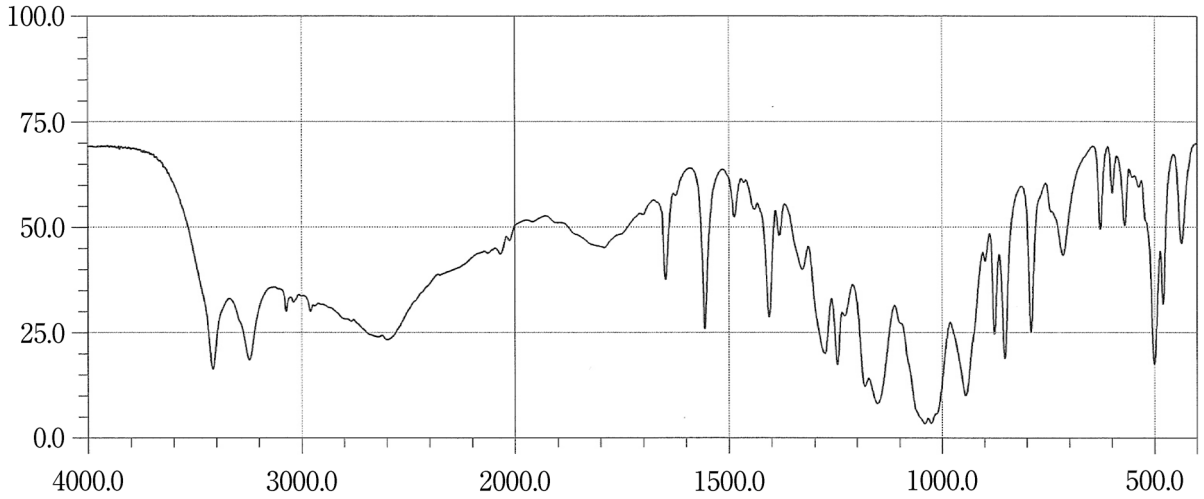


The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

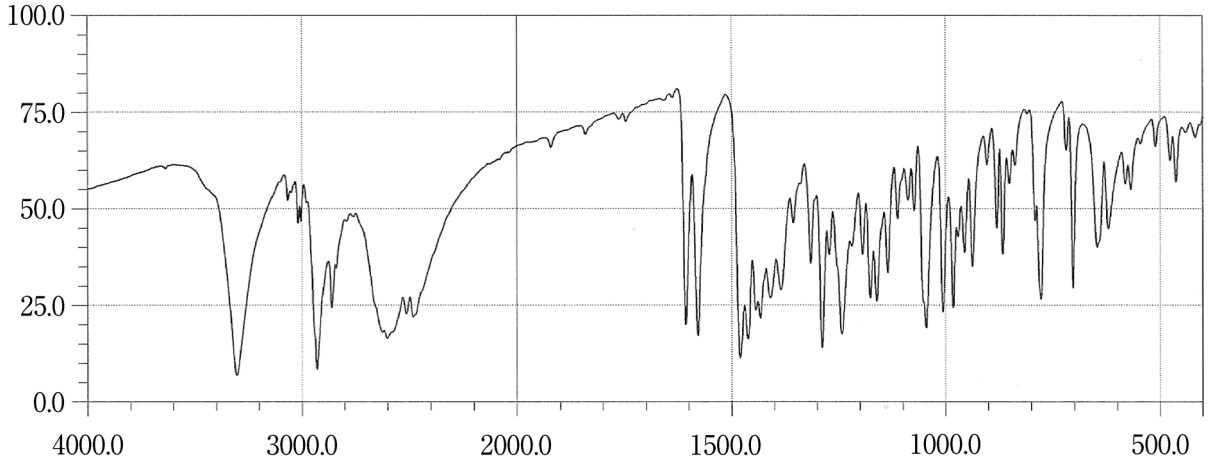
Pazufloxacin Mesilate



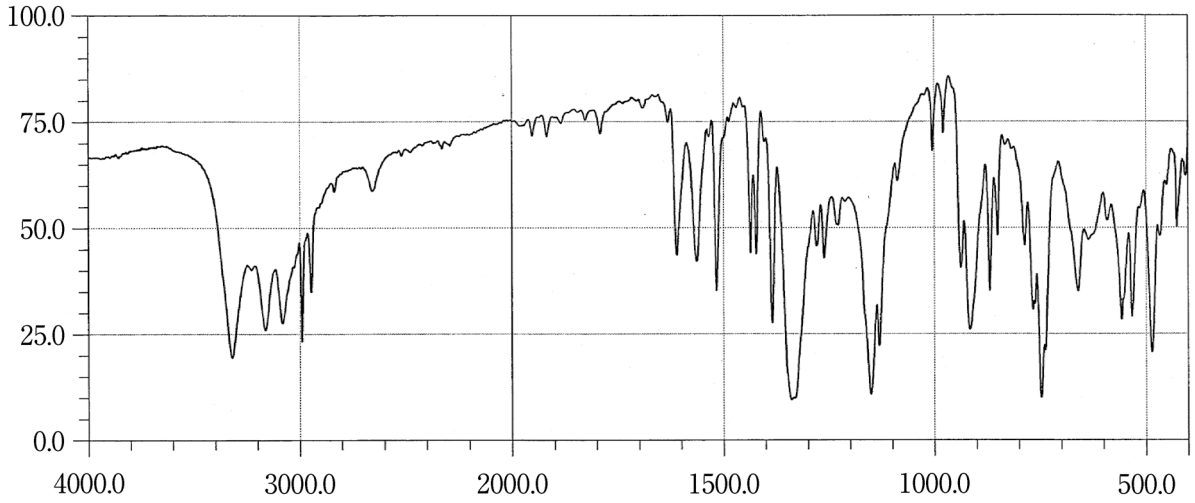
Pyridoxal Phosphate Hydrate



Tramadol Hydrochloride



Zonisamide



Ultraviolet-visible Reference Spectra

Delete the following spectra:

Diclofenamide

Fluoxymesterone

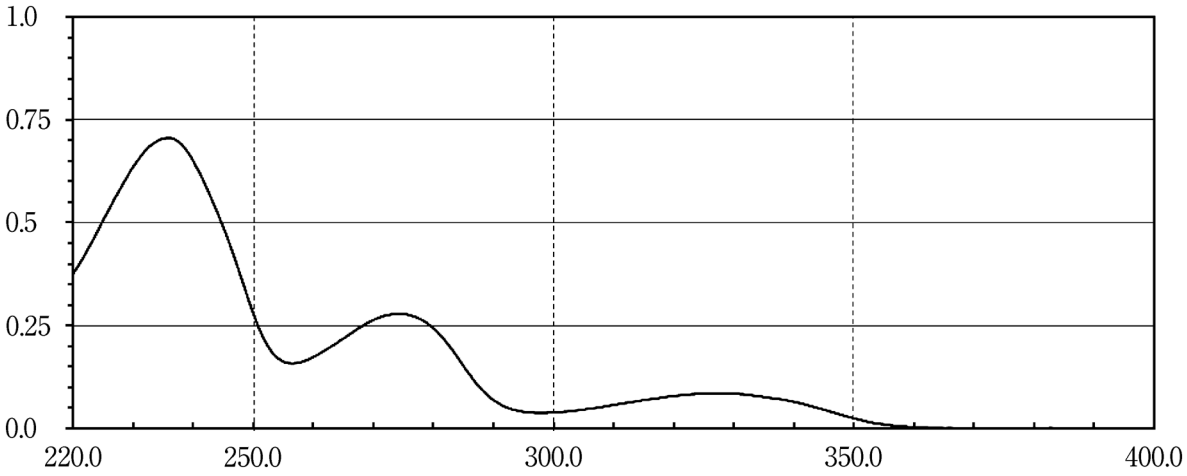
Gramicidin

Rokitamycin

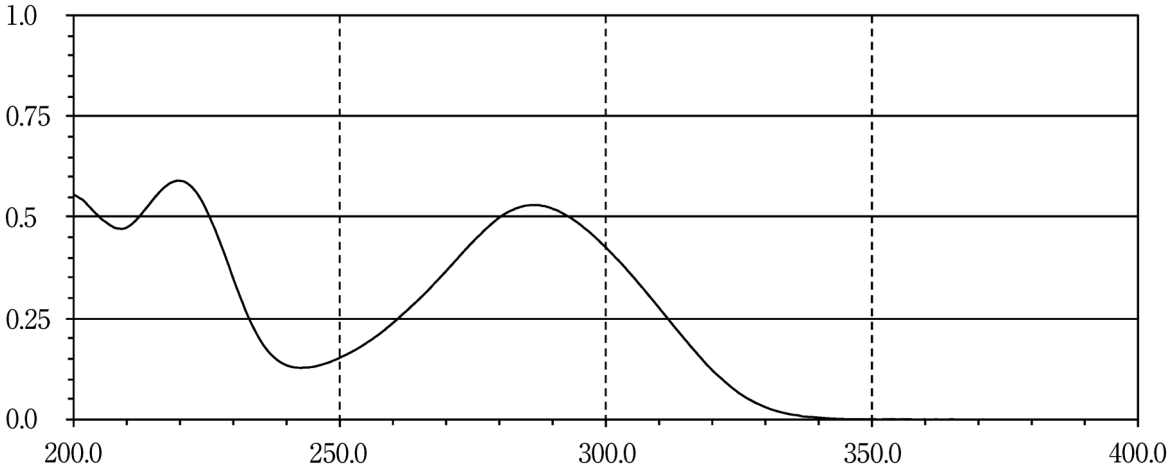
Tolazamide

Add the following spectra:

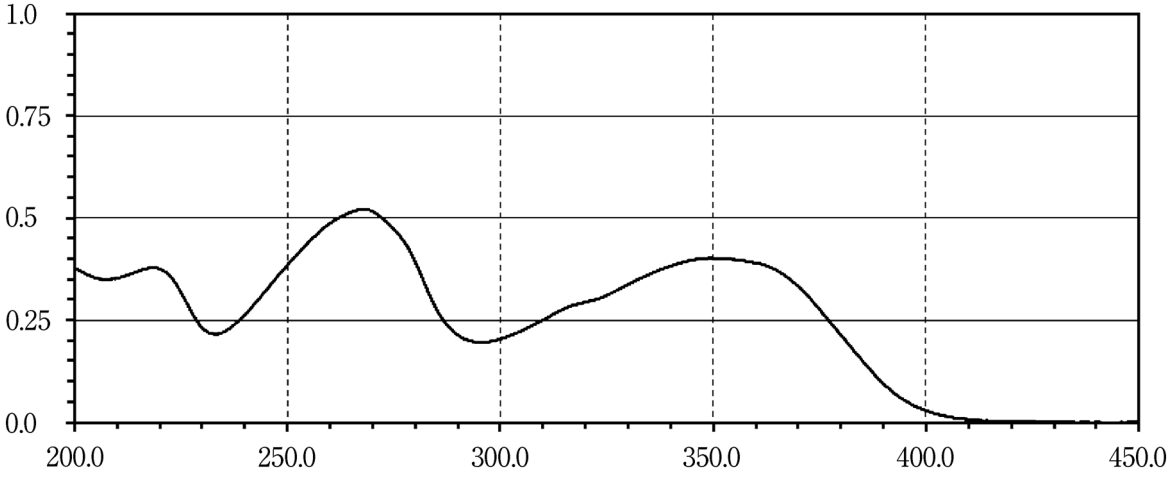
Azosemide



Calcium Levofolinate Hydrate

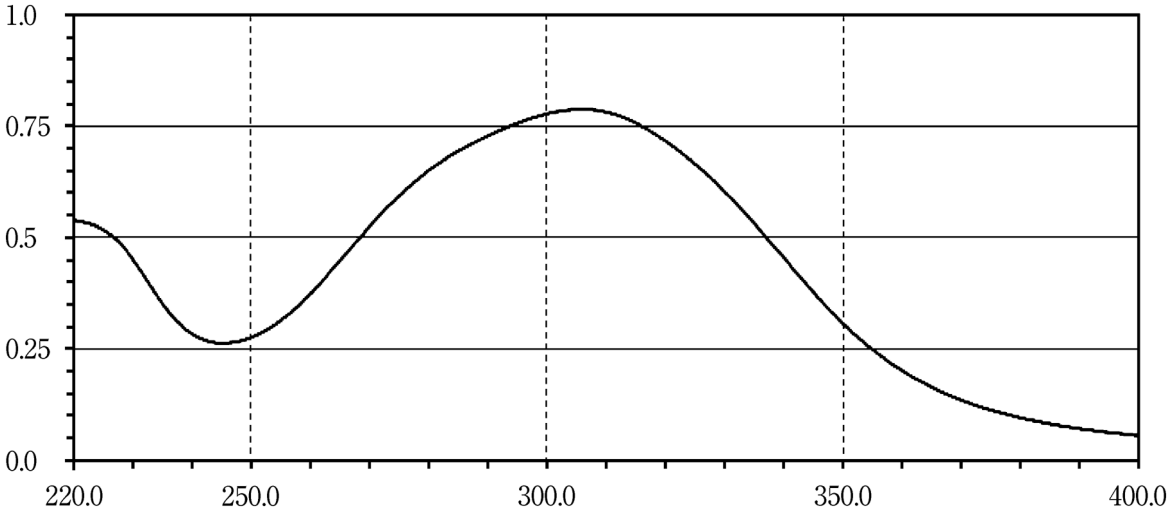


Doxycycline Hydrochloride Hydrate

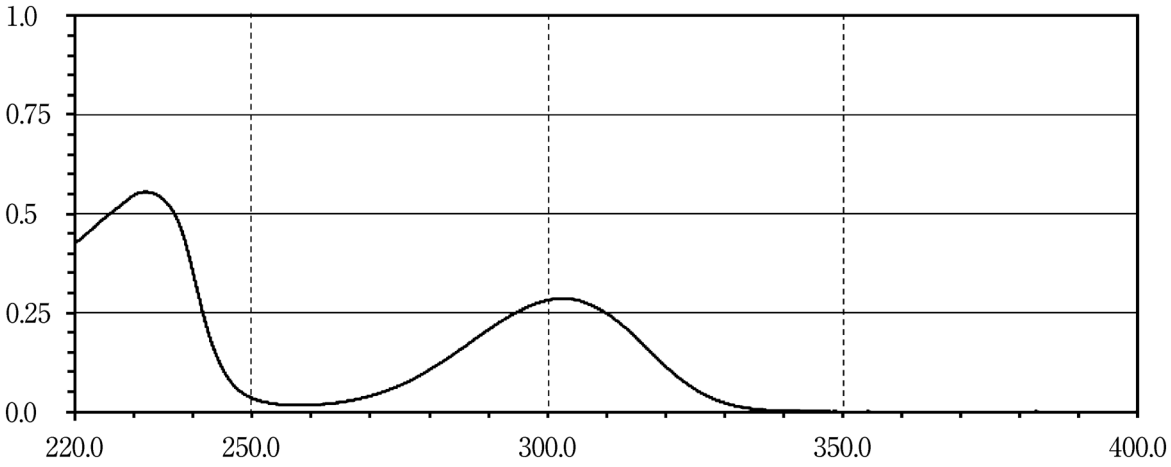


The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

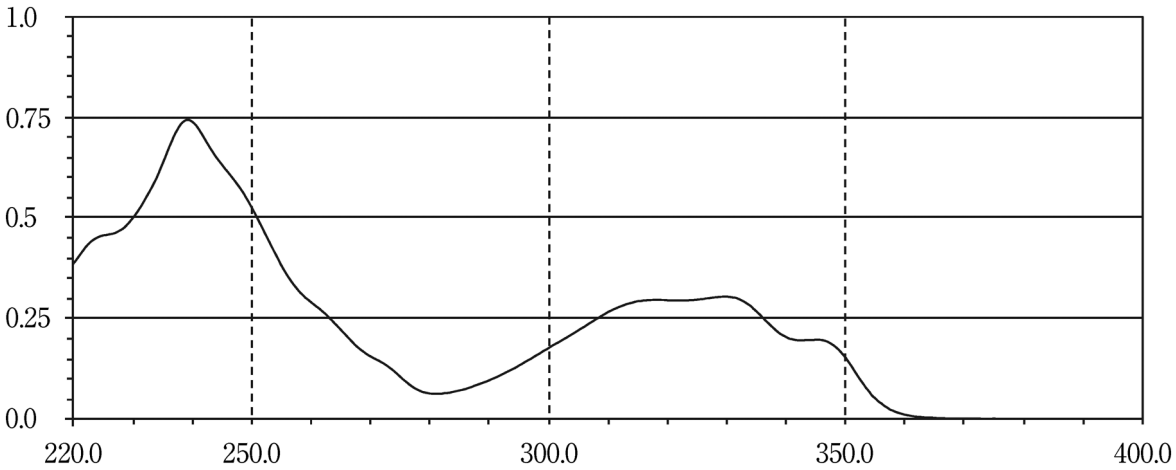
Entacapone



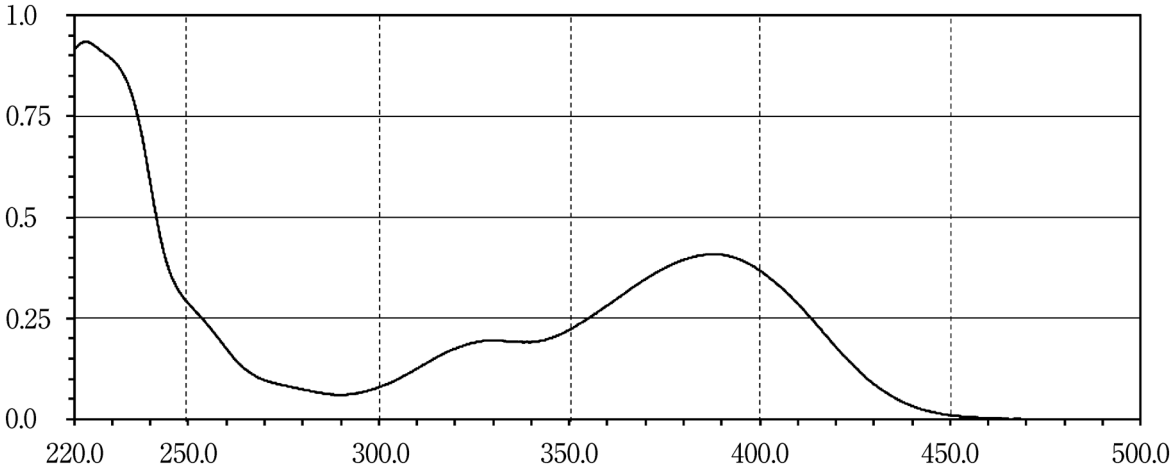
Mesalazine



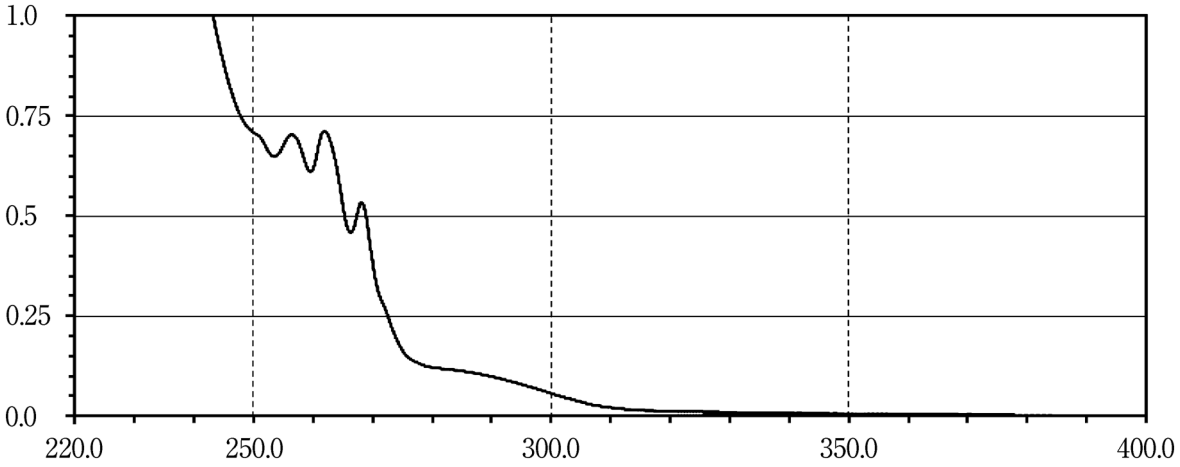
Pazufloxacin Mesilate



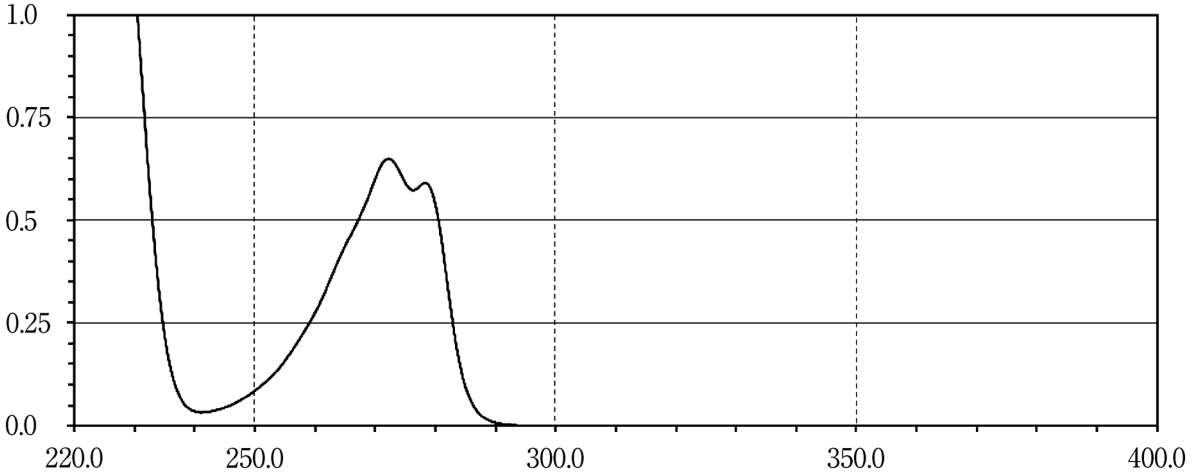
Pyridoxal Phosphate Hydrate



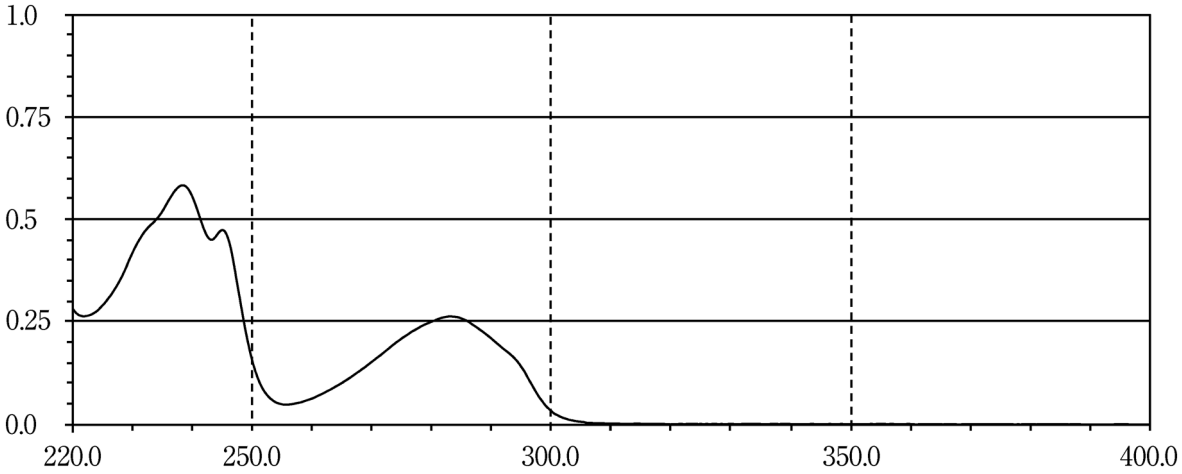
Sultamicillin Tosilate Hydrate



Tramadol Hydrochloride



Zonisamide



General Information

GENERAL INFORMATION

G2 Solid-state Properties

Delete the following item:

Laser Diffraction Measurement of Particle Size

Powder Fineness

Change the following:

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

A simple descriptive classification of powder fineness is provided in this chapter. Sieving is most suitable where a majority of the particles are larger than about 75 μm, although it can be used for some powders having smaller particle sizes where the method can be validated. Light diffraction is also a widely used technique for measuring the size of a wide range of particles. Where the cumulative distribution has been determined by analytical sieving or by application of other methods, particle size may be characterized in the following manner:

x_{90}: Particle size corresponding to 90% of the cumulative undersize distribution
x_{50}: Median particle size (i.e. 50% of the particles are smaller and 50% of the particles are larger)
x_{10}: Particle size corresponding to 10% of the cumulative undersize distribution

It is recognized that the symbol d is also widely used to designate these values. Therefore, the symbols d_{90}, d_{50} and d_{10} may be used.

The following parameters may be defined based on the cumulative distribution.

$Q_r(x)$: cumulative distribution of particles with a dimension less than or equal to x where the subscript r reflects the distribution type

r	Distribution type
0	Number
1	Length
2	Area
3	Volume

Therefore, by definition:

$Q_r(x) = 0.90$ when $x = x_{90}$
$Q_r(x) = 0.50$ when $x = x_{50}$
$Q_r(x) = 0.10$ when $x = x_{10}$

An alternative but less informative method of classifying powder fineness is by use of the descriptive terms in the following table.

Classification of powders by fineness

Descriptive term	x_{50} (μm)	Cumulative distribution by volume basis, $Q_3(x)$
Coarse	> 355	$Q_3(355) < 0.50$
Moderately fine	180 – 355	$Q_3(180) < 0.50$, $Q_3(355) \geqq 0.50$
Fine	125 – 180	$Q_3(125) < 0.50$, $Q_3(180) \geqq 0.50$
Very fine	$\leqq 125$	$Q_3(125) \geqq 0.50$

Powder Flow

Change the 4.1. Basic methods for shear cell as follows:

4.1. Basic methods for shear cell

One type of shear cell is the cylindrical shear cell which is split horizontally, forming a shear plane between the lower stationary base and the upper movable portion of the shear cell ring. After powder bed consolidation in the shear cell (using a well-defined procedure), the force necessary to shear the powder bed by moving the upper ring is determined. Annular shear cell designs offer some advantages over the cylindrical shear cell design, including the need for less material. A disadvantage, however, is that because of its design, the powder bed is not sheared as uniformly because material on the outside of the annulus is sheared more than material in the inner region. A third type of shear cell (parallel-plate type) consists of a thin sandwich of powder between a lower stationary rough surface and an upper rough surface that is moveable.

All of the shear cell methods have their advantages and disadvantages, but a detailed review is beyond the scope of this chapter. As with the other methods for characterizing powder flow, many variations are described in the literature. A significant advantage of shear cell methodology in general is a greater degree of experimental control. The methodology generally is rather time-consuming and requires significant amounts of material and a well-trained operator.

Solid and Particle Densities

Change the Particle Density as follows:

Particle Density

The particle density takes account both the crystal density and the intraparticulate porosity (sealed and/or experimentally non-accessible open pores) as a part of the particle volume. The particle density depends on the value of the volume determined, and the volume in turn depends on the method of measurement. Concerning the determination of particle density , the Japanese Pharmacopoeia specifies the pycnometry as the ''Powder Particle Density Determination''.

The pycnometric density is obtained by assuming that the volume of the gas displaced, which is measured with the gas displacement pycnometer, is equivalent to that of a known mass of the powder. In pycnometric density measurements, any volume with the open pores accessible to the gas is not included as a part of volume of the powder, but the sealed pores or pores inaccessible to the gas is included as a part of the volume of the powder. Due to the high diffusivity of helium which can penetrate to most open pores, it is recommendable as the measurement gas of particle density. Therefore, the pycnometric particle density of a finely milled powder is generally not very different from the crystal density. Hence, the particle density by this method is the best estimate of the true density of an amorphous or partially crystalline sample, and can be widely used for manufacturing control of the processed pharmaceutical powder samples.

G3 Biotechnological/Biological Products

Amino Acid Analysis

Change the Method 6 and 7 in Protein Hydrolysis as follows:

Method 6

Cysteine-cystine oxidation is accomplished with dimethyl sulfoxide (DMSO).

Hydrolysis Solution 6 mol/L hydrochloric acid containing 0.1% to 1.0% of phenol, to which DMSO is added to obtain a final concentration of 2% (v/v).

Vapor Phase Hydrolysis The protein/peptide hydrolysis is conducted at about 110°C for 24 hours. During the hydrolysis, the cysteine-cystine present in the sample is converted to cysteic acid by the DMSO present in the *Hydrolysis Solution*. As an approach to limit variability and compensate for partial destruction, it is recommended to evaluate the cysteic acid recovery from oxidative hydrolyses of standard proteins containing 1 to 8 mol of cysteine per mol of protein. The response factors from protein/peptide hydrolysates are typically about 30% lower than those for nonhydrolyzed cysteic acid standards. Because histidine, methionine, tyrosine, and tryptophan are also modified, a complete compositional analysis is not obtained with this technique.

Method 7

Cysteine-cystine reduction and alkylation is accomplished by a vapor phase pyridylethylation reaction.

Reducing Solution Transfer 83.3 μL of pyridine, 16.7 μL of 4-vinylpyridine, 16.7 μL of tributylphosphine, and 83.3 μL of water to a suitable container, and mix.

Procedure Add the protein/peptide (between 1 μg and 100 μg) to a hydrolysis tube, and place in a larger tube. Transfer the *Reducing Solution* to the large tube, seal in vacuum (about 50 μm of mercury or 6.7 Pa), and incubate at about 100°C for 5 minutes. Then remove the inner hydrolysis tube, and dry it in a vacuum desiccator for 15 minutes to remove residual reagents. The pyridylethylated protein/peptide can then be acid hydrolyzed using previously described procedures. The pyridylethylation reaction is performed simultaneously with a protein standard sample containing 1 to 8 mol of cysteine per mol of protein to improve accuracy in the pyridylethylcysteine recovery. Longer incubation times for the pyridylethylation reaction can cause modifications to the α-amino terminal group and the ε-amino group of lysine in the protein.

Add the following:

Enzyme-linked Immunosorbent Assay (ELISA)

ELISA (Enzyme-linked immunosorbent assay) is one of the immunological assay methods to detect analytes by antigen-antibody reaction, in which an enzyme-labeled reagent is used as a detection reagent. In general, 96-well-plates and such are used, on which capture molecules specifically bound to the analyte are immobilized. A test sample, an enzyme-labeled reagent and other required reagents are sequentially added and washed to have the enzyme-labeled reagent bind onto a plate. After reaction by adding a substrate for the labeled enzyme, the response (e.g., absorbance) by the enzymatic reaction is measured to determine the concentration of the analyte or binding activity in the test sample. ELISA is also used as a qualitative test to detect the presence or absence of binding of the analyte with specific molecules.

In tests for biotechnological/biological products, ELISA is mainly used for two different purposes. One is to quantitate the target product or process-related impurities usually by measuring the concentration of the analyte with antibodies which specifically bind to the analyte. Another is to evaluate the biological activity of products such as therapeutic antibodies. For the latter purpose, ELISA is used to evaluate the binding activity of the target product with

molecules related to its pharmacological action, or to evaluate the cell response based on the amount of the endogenous protein secreted from the cells treated with the test samples containing the target product.

1. Analytical methods

ELISA is broadly classified into competitive and noncompetitive methods, and also classified into direct and indirect detection methods based on the detection procedures (Fig. 1). In addition, ELISA is also classified into direct and indirect immobilization methods by the method for immobilizing capture molecules (Fig. 2).

An analyte bound to a solid phase is detected by the antibody against the analyte or other reagents (Fig. 1). In the direct detection method, an enzyme-labelled antibody against the analyte is used. In the indirect detection method, a molecule indirectly bound to the analyte such as an antibody (secondary antibody) against the antibody binding to the analyte (primary antibody), is used. The procedure of the direct detection method is simple, but the enzyme-labeled antibody against the analyte is required for each analyte. Compared to the direct detection method, the procedure of the indirect detection method is more complex, however, it allows for using a common secondary antibody such as an anti-IgG antibody even if the analyte is different.

When ELISA is used for measuring analyte concentration, an antibody against the analyte is typically used as a capture molecule. When ELISA is used to evaluate biological activity by measuring binding activity, the target molecule of a drug involved in its pharmacologic action is used as a capture molecule.

1.1. Noncompetitive method

In the noncompetitive method, an analyte is bound to a capture molecule without competing with other molecules (Fig. 1). This method can be used when the analyte possesses rather high molecular mass and has binding sites for the capture molecule as well as for the molecule used for detection.

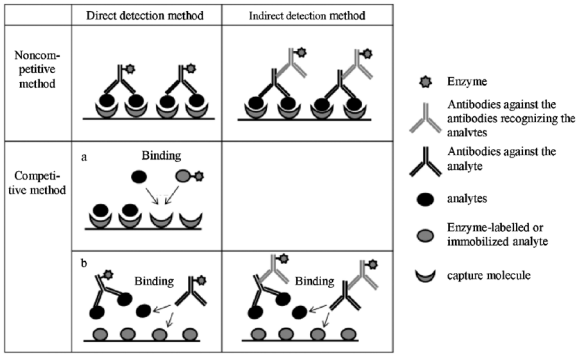


Fig. 1 Classification of ELISA by analytical method

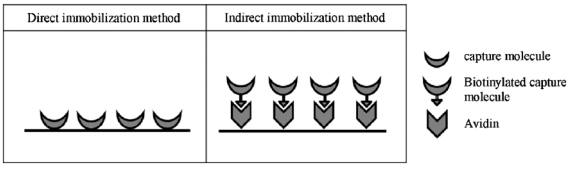


Fig. 2 Examples of direct immobilization method and indirect immobilization method

1.2. Competitive method

The competitive method has two approaches: the first is to immobilize a capture molecule, then has an analyte and an enzyme-labeled antibody compete to each other for binding with the capture molecule (Fig. 1a), and the second is to use the analyte prepared as the reagent which is immobilized onto a plate, then have the immobilized analyte and the analyte in test samples compete with each other for binding with an enzyme-labeled antibody (Fig. 1b). The competitive method is used when the molecular mass of the analyte is rather low, and it is difficult to prepare two molecules which bind to the analyte specifically.

2. Analytical procedures

2.1. Procedure

General procedures for both noncompetitive and competitive methods are shown below. As for a quantitative test, prepare reference material solutions diluted serially in order to obtain a dose-response curve or a calibration curve.

2.1.1. Noncompetitive method

1) Add a solution containing capture molecules onto a plate, and incubate to immobilize the capture molecules on a solid phase, then wash off the unbound capture molecules.

2) Add a blocking reagent, and have the reagent bind on the surface not occupied by the capture molecules. Wash off the unbound blocking reagent.

3) Add a reference material or a test sample onto each well of the plate, and have the analyte bind on the solid phase. Wash off the unbound analyte.

4) When the direct detection method is used, add an enzyme-labeled antibody to bind to the analyte. When the indirect detection method is used, add an antibody against the analyte, then wash and add the enzyme-labeled antibody which binds to the antibody against the analyte in order to bind it to the solid phase. Wash off the unbound enzyme-labeled antibody.

5) Add a substrate solution, incubate and add a stopping solution if required. Then measure the absorbance, luminescent intensity, or fluorescent intensity, which reflects the amount of substrate converted by the enzyme reaction.

6) Determine the binding activity or concentration of the analyte with reference to the dose-response curve (calibration curve) of the reference material.

2.1.2. Competitive method

1) Competitive method (a): Add a solution containing capture molecules onto a plate, then incubate so that the capture molecules bind to a solid phase. Wash off the unbound capture molecules.

Competitive method (b): Add an analyte prepared for immobilizing onto a plate, and incubate so that the analyte bind to the solid phase. Wash off the unbound analyte.

2) Add a blocking reagent to bind on the solid phase sur-

face that is not occupied by the operation of 1). Wash off the unbound blocking reagent.

3) Competitive method (a): Add a solution containing a reference material and an enzyme-labeled analyte, or a test sample and an enzyme-labeled analyte onto each well of the plate. Then have the analyte and the enzyme-labeled analyte bind on the solid phase. Wash off the unbound molecules. Competitive method (b): In the direct detection method, add a solution containing a reference material and an enzyme-labeled antibody, or a test sample and an enzyme-labeled antibody onto each well of the plate, and then have the enzyme-labeled antibody bind to the solid phase. Wash off the unbound molecules. In the indirect detection method, add a solution containing a reference material and an antibody against the analyte, or a test sample and an antibody against the analyte onto each well of the plate. After washing, add the enzyme-labeled antibody which binds to the antibody against the analyte. Wash off the unbound enzyme-labeled antibody.

4) Add the substrate of the enzyme, incubate and then add a stopping solution if required. Measure the amount of the substrate converted by the enzyme reaction by measuring absorbance, luminescent intensity or fluorescent intensity.

5) Calculate the binding activity or the concentration of the analyte from the dose-response curve (calibration curve) of the reference material.

2.2. Data analysis

2.2.1. Quantitation

When ELISA is applied to determine the concentration of analyte, use an appropriately diluted test sample and calculate the concentration of the analyte in the test sample from the calibration curve obtained from the reference material. Usually, the calibration curve is prepared by using an equation of such as 4-parameter logistic regression, setting the log concentrations of the target molecule on the x-axis and responses obtained on the y-axis.

4-parameter logistic model

$$y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^{B}}$$

A: Lower asymptote
B: Slope parameter
C: EC_{50} (IC_{50})
D: Upper asymptote
x: Concentration of test sample
y: Response

When the calibration curve is not bilateral symmetric as a sigmoid curve, applying 5-parameter logistic regression may improve the analytical result. As for the noncompetitive method, a calibration curve may be obtained by linear regression by limiting the concentration at the lower range.

2.2.2. Biological activity

For evaluating biological activity, the methods such as 1) to 3) described below are used.

1) Use the test sample diluted with an appropriate dilution ratio. Determine the relative activity against the reference material by calculating the relative concentration based on the dose-response curve (the calibration curve) of the reference material.

2) Obtain the dose-response curves of the reference material and the test sample, respectively. Calculate the relative activity of the test sample against the reference material from the ratio of the concentration corresponding to 50% of the maximum response (EC_{50} for the noncompetitive method, and IC_{50} for the competitive method).

3) Use the range that can be approximated linear regression in the dose-response curve. Calculate the relative activity of the test sample against the reference material based on the ratio of the dose that arise the same response.

1) uses the same method as 2.2.1. and calculate the relative concentration against the reference material. 2) uses the same method as 2.2.1. to lead the regression equation on the reference material and the test sample. Better regression can be obtained by weighting to equalize the contribution of each concentration response in leading the regression equation. The methods of weighting are to use $1/y^2$, $1/y$ and $1/x$. Upon the establishment of the test method, choose a regression method to obtain a better result based on the accuracy and precision. 3) uses the concentration region near EC_{50} or IC_{50} that can be approximated as a straight line for analysis.

2.3. Reagents, test Solutions

2.3.1. Capture reagents

Use molecules (antigen, antibody, etc.) which can specifically bind to the analyte. Physical adsorption is frequently used for immobilizing a capture reagent on a plate, and covalent binding is also possible to use for its binding to the plate which is covered by materials having the binding activity with an amino or sulfhydryl functional group. Note that there is a case that binding activity with an analyte may be changed due to the conformational change by the binding onto the plate.

The capture reagent is a critical reagent that affects assay performance, and therefore, its quality should be controlled by setting necessary specifications. Establish the procedure for lot renewal as well.

2.3.2. Blocking reagents

A buffer solution containing protein such as albumin, gelatin, or casein, which is supplemented with surfactants such as polysorbate 20 as required, is used as a blocking reagent.

2.3.3. Detection reagents

As enzymes for detection, peroxidase, alkaline phosphatase, and β-galactosidase are typically used. As a labeling method for an enzyme, covalent binding with a target protein is used; A *N*-hydroxysuccinimide ester group introduced into an enzyme binds to the amino group of the labeled protein, and a maleimide group introduced into the enzyme binds to the sulfhydryl group of the labeled protein. As a method used for enzyme labeling of antibodies, covalent binding with which the maleimide group introduced into the enzyme is bound to the sulfhydryl group of the antibody is often used.

Detection Reagents are the critical reagents affecting assay performance, and therefore, their quality should be

Table 1 Examples of substrates

Enzyme	Chromogenic substrate	Chemiluminescent substrates	Fluorescent substrates
peroxidase	TMB OPD ABTS	Luminol	
alkaline phosphatase	pNPP		
β-galactosidase			MG NG

TMB: 3,3′,5,5′-Tetramethylbenzidine
OPD: *o*-Phenylenediamine
ABTS: 2,2′-Azino-bis[3-ethylbenzothiazoline-6-sulfonate]
pNPP: *p*-Nitrophenyl phosphate
MG: 4-Methylumbelliferyl galactoside
NG: Nitrophenyl galactoside

controlled by setting necessary specifications. Establish the procedures for lot renewal as well. As for the indirect detection method, unlabeled antibodies against an analyte are also used as a detection reagent, and therefore, it is necessary to control by setting necessary specifications.

2.3.4. Substrates

Use substrates which are appropriate for each enzyme. There are chromogenic, chemiluminescent and fluorescent substrates. Chemiluminescent substrates or fluorescent substrates are suitable when high sensitivity is required.

2.4. Points to consider

Since types of plates, amount of immobilized capture molecule, and incubation time as well as incubation temperature may affect test results, determine these procedures including materials and reagents of use. Also determine the test conditions and the sample placement in plates to prevent that the sample placement on the plates (the position of the well where the test is performed) affects the test results.

3. Application on specifications

3.1. Identification

In monographs of biotechnological/biological products, ELISA is used as an identification test which uses specific antibodies against the target product to evaluate the binding with the antibodies. As to therapeutic antibodies ELISA is also used as an identification test which evaluates the binding of the antibodies with antigen. Usually it is used as a qualitative test. In the meantime, acceptance criteria can also be set regarding the binding activity compared with a reference material when used as an identification test which evaluates the binding of therapeutic antibody with antigen.

3.2. Purity test

ELISA is used mainly as a purity test for process-related impurities such as host cell proteins, impurities derived from culture media and ligands eluted from affinity column resin. When ELISA is used as a test to determine the amount of impurities, calculate the concentrations in a test sample by using calibration curves. When it is used as a limit test, confirm the test sample response is not higher than that of the control containing the impurities equal to the upper limits of the acceptance criteria.

In general, samples include much more amount of a target product than impurities, and therefore, the target product may disturb the detection of the impurities. Especially when ligands of affinity column are analytes, pay attention to the disturbance by the target product as the target product binds to the ligands. Consider a recovery rate when sample pretreatment is performed.

3.3. Biological assay

ELISA is used as a test to determine the binding activity of a therapeutic antibody as the target product with its target molecule, and used to quantitate bioactive proteins secreted from the cells treated with test samples containing the target product in cell-based assay.

Determine relative activity by the method indicated in 2.2.2. 1) to 3).

3.4. Assay

ELISA is used for measuring the amount of target products. Obtain the calibration curve of reference materials and calculate the concentration of the target products.

4. Validity of test

In general, the validity of the test can be set as follows; use those in combination as necessary.

4.1. Identification

Confirm the results of reference materials and a negative control pass the acceptance criteria specified in the monograph.

4.2. Purity test

As for a quantitative test, confirm the reliability of calibration curves. Accuracy and/or precision of each concentration of material solutions for the calibration curve and the coefficient of determination (R^2 Value) calculated from a regression equation are used to confirm the reliability. Precision of test samples or accuracy of control samples prepared from the known concentration of a reference material (Quality Control Sample: QC sample) could be set as the test suitability. As for a limit test, confirm the response of the control sample containing the analyte at a concentration equal to the upper limit of the acceptance criteria satisfies the criterion specified in the monograph.

4.3. Biological assay

When determining biological activity by using the method of 1) of 2.2.2., confirm the reliability of the dose-response curve (the calibration curve) of the reference material . To confirm the reliability of the dose-response curve, accuracy and/or precision of each concentration of the reference material and R^2 value calculated from the regression equation or each parameter value of the regression equation obtained from the dose-response curve of the reference material can be used. Magnitude of the response of test samples, precision of the relative activity calculated from the response or accuracy of the concentration of QC samples can also be used to confirm the validity of the test.

When determining biological activity by using the method

of 2) of 2.2.2., confirm the parallelism of the two regression curves obtained from a reference material and a test sample. As for the parallelism confirmation, following methods are the examples. Obtain the ration of the difference between the upper asymptote and the lower asymptote ($D - A$ of the 4-parameter regression equation in 2.2.1.) of the test sample to that of reference material or the ratio of the slope parameter (B of the 4-parameter regression equation in 2.2.1.), then confirm that those ratios are within the predetermined range. R^2 value of the dose-response curves of the reference material and the test sample, and accuracy of the QC samples are also used to confirm the validity of the test.

When determining biological activity by using the method of 3) of 2.2.2., confirm the linearity of the dose-response lines of a reference material and a test sample as well as the parallelism of these lines.

As for 2) and 3) of 2.2.2., there is a method to confirm the parallelism by comparing the residual variances of two regression curves, using the constrained model for control and sample data and using unconstrained models for the control and sample data, and determining the parallelism of the two regression curves by the method of analysis of variance. However, it should be noted that if the precision of the data is low, then the determination can be unrigorous.

4.4. Assay

Confirm the reliability of calibration curves obtained from the dose-response curves of reference materials. To confirm the reliability of the calibration curve, accuracy and/or precision of each concentration of the reference material calculated from the regression equation, each parameter value of the regression equation and R^2 value can be used. Precision of the measured results of test samples or accuracy of QC samples is also used to confirm the validity of the test.

G5 Crude Drugs

Purity Tests on Crude Drugs using Genetic Information

Change the introduction as follows:

The first step in the quality assurance of natural products is the use of raw materials from the right part of the right origin. Therefore, it is clearly stated in Article 4 of the General Rules For Crude Drugs that the source of a crude drug is an approval or rejection criterion. There are various methods for differentiating the sources of crude drugs, such as morphological methods, organoleptic tests, and chemical methods, and appropriate methods for each are described in the individual monographs. Morphological methods, organoleptic tests, and chemical methods are discrimination methods for species that are based on the phenotypic characteristics of the crude drugs. On the other hand, together with recent progress in molecular biology techniques and the accumulation of genetic information on plants, differentiating methods of crude drugs based on genotypes is being established. Unlike morphological and other methods that are based on phenotypic characteristics, the genotypic methods are not affected by environmental factors. Also, the methods have several advantages, such as specialized expertise and skill for classification are not needed, and objective results are easily obtained.

The evolution of living organisms is accomplished by genetic mutation, and differences among the nucleotide sequences of genes of closely related species reflect the strain relationships between the species. Based on this theory, methods that classify species phylogenetically using the nucleotide sequence of rDNA that codes for ribosomal RNA (rRNA) on the nuclear genome have recently been adopted for the classification of microorganisms. In the same way, the sequence of this rDNA is also most often used in the classification of higher plants based on the genotype. In particular, it is very easy to classify closely related species using the internal transcribed spacer (ITS) region of the rDNA region, since nucleotide substitution is more often undertaken by comparison with the coded gene region. Furthermore, since the genes on the nuclear genome originate from the parents' genom, there is an advantage that interspecies hybrids can be detected. Higher plants also have mitochondrial genes and chloroplastic genes. Although the genes on these genomes are also often used for classification, interspecies hybrids cannot be confirmed because the genes are normally uniparental inheritance.

The two methods presented here have been developed based on the reported identification methods of Atractylodes Lancea Rhizome and Atractylodes Rhizome[1,2] utilizing the gene sequence of the ITS region of rDNA. Inter-laboratory validation study for the purity test of Atractylodes Rhizome targeted for Atractylodes Lancea Rhizome have been completed. The plant sources for Atractylodes Lancea Rhizome stipulated in the individual monographs are *Atractylodes lancea* De Candolle and *A. chinensis* Koidzumi (*Compositae*), while those for Atractylodes Rhizome are *A. japonica* Koidzumi ex Kitamura and *A. macrocephala* Koidzumi (*Compositae*). The approval or rejection of the both sources is, in principle, determined by the description of each crude drug, including microscopy, together with thin-layer chromatography in identification test. In the above scientific paper, it was shown that these 4 plant species can be clearly classified by comparing the nucleotide sequences of the ITS region mentioned above, and that the species can be easily classified without performing sequence analysis by performing PCR using a species-specific primer set or by using a restriction enzyme which recognizes species-specific sequence.

In collaborative studies, the simplicity of the test is given maximum consideration. We examined methods that observe PCR amplification bands using species-specific primer sets (Mutant Allele Specific Amplification: Method 1) and methods that observe DNA fragments produced by restriction enzyme treatment of the PCR products, which are pre-

pared using a primer set common to each plant source (PCR-Restriction Fragment Length Polymorphism: Method 2), without nucleotide sequence analyses. In these methods based on PCR, an extremely small amount of template DNA is amplified to billions to hundreds of billions times. Therefore, when using them as identification tests for powdered crude drugs, the target DNA fragment can be observed even if the vast majority of the crude drug for analysis is not appropriate plant species and there is only a minute amount of powder from a crude drug derived from a suitable plant. (Consequently, in identification tests, either a cut or a whole crude drug must be used, as long as one is careful to avoid contamination by powder originating from other crude drugs.) On the other hand, when used as a purity test, the form of the crude drug is irrelevant as long as the gene amplification is performed properly and the target gene is not polymorphic, so if DNA fragments of an inappropriate plant to be examined are confirmed in the purity test, regardless of the form of the crude drug, it becomes clear there is contamination by an inappropriate crude drug to be examined.

The methods shown here are general information and at the present stage results obtained using the methods do not affect the approval or rejection of the crude drug in each monograph. Furthermore, by performing the sequence analysis outlined in the previous paper, it goes without saying that more accurate decision concerning the source species can be made.

On the Scientific Names of Crude Drugs listed in the JP

Change the following as follows:

Scientific names used in the JP and those being used taxonomically

Crude Drug	Scientific names used in the JP = Scientific names being used taxonomically (Combined notation, Standard form for author or authors)	Family
	Scientific names that are different from those written in JP but identical to them taxonomically or being regarded as identical, and typical subclassified groups belonging to their species. The names marked with "*" are those being written together in JP.	
Curcuma Rhizome ガジュツ	*Curcuma zedoaria* Roscoe	*Zingiberaceae*
	Curcuma phaeocaulis Valeton	
	Curcuma kwangsiensis S. G. Lee et C. F. Liang	
Jujube Seed サンソウニン	*Ziziphus jujuba* Miller var. *spinosa* Hu ex H. F. Chou = *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chou	*Rhamnaceae*
Jujube タイソウ	*Ziziphus jujuba* Miller var. *inermis* Rehder = *Ziziphus jujuba* Mill. var. *inermis* (Bunge) Rehder	*Rhamnaceae*
Platycodon Root キキョウ	*Platycodon grandiflorus* A. De Candolle = *Platycodon grandiflorus* (Jacq.) A. DC.	*Campanulaceae*
Rape Seed Oil ナタネ油	*Brassica napus* Linné = *Brassica napus* L.	*Cruciferae*
	Brassica rapa Linné var. *oleifera* De Candolle = *Brassica rapa* L. var. *oleifera* DC.	

G6 Drug Formulation

Add the following:

Aerodynamic Particle Size Measurement for Inhalations by Glass Impingers

This test is used to evaluate the fine particle characteristics of the aerosol clouds generated by preparations for inhalation, and is performed using the following apparatus and test procedures. If justified, modified apparatus or test procedure may be used.

1. Stage mensuration

The most reliable calibration for the separation characteristics of each impaction stage is performed in terms of the relationship between the aerodynamic diameter of particles and droplets passing through it and the stage collection efficiency as an aerosol.

Calibration is usually performed by examination of a property of the jet dimensions, the spatial arrangement of the jet and its collection surface, and the airflow rate passing through it.

Because jets can corrode and wear over time, the critical dimensions of each stage must be measured on a regular basis to confirm them being within required ranges.

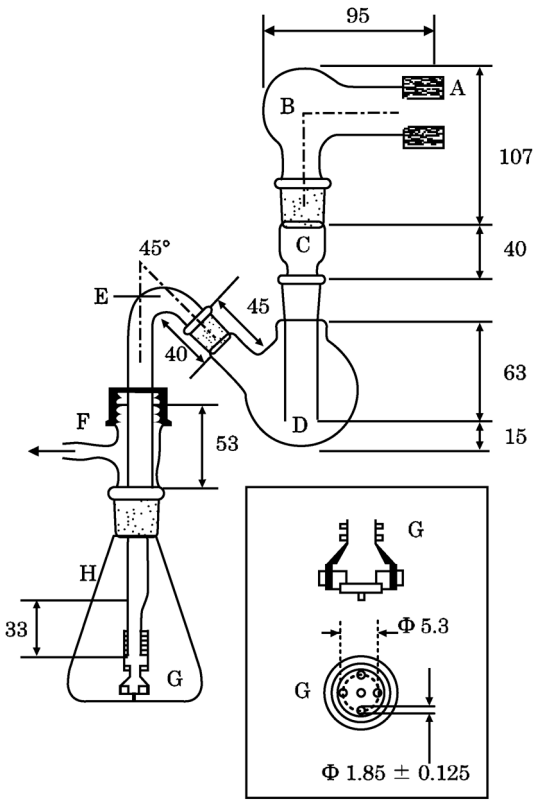
Only apparatuses that conform to specifications are used for the aerodynamic particle size measurement for inhalations by glass impingers. An alternate validated and justified method of mensuration may be used.

2. Inter-stage drug losses (wall losses)

Wall losses should be considered in method development and validation. If the wall losses affect the recovery rate (mass balance) of drugs, they should be controlled. Wall losses will be dependent upon a number of factors including the impactor type, operating conditions, formulation type and discharged amount to an impactor. How the wall loss is reflected within the calculation of the aerodynamic diameter of particles should be judged based up on the level and variability of the wall loss. For example, in the cases where wall losses that are low or have a low level of variability, the aerodynamic particle size is calculated by the assay of the drug collected on the stage. In cases where wall losses are high or variable, it may be necessary to collect the wall loss drug separately and take it into account for the calculation of the aerodynamic particle size.

3. Recovery rate of drugs (mass balance)

In addition to the size distribution, good analytical practice dictates that a mass balance be performed in order to confirm that the amount of the drug discharged from the inhaler, which is collected in the mouthpiece adapter and the apparatus, is within an acceptable range around the expected value. The total mass of drug collected in all of the components of the mouthpiece adapter and the apparatus divided by the minimum recommended dose described in the dosage and administration is not less than 75% and not more than 125% of the average delivered dose determined under Uniformity of Delivered Dose for Inhalations ⟨*6.14*⟩. This mass balance is necessary to ensure that the test results of particle size distributions of inhalations are valid.



Capital letters of alphabet refer to Table 1
The figures are in mm. (Tolerances are ±1 mm unless otherwise stated.)

Fig. 1 Glass impinger

4. Glass impinger method

The apparatus used for the glass impinger method is shown in Fig. 1. The apparatus consists of glass parts from the throat (B) to the lower impingement chamber (H) and plastic clips to hold them.

This apparatus is operated based on a collision to a liquid surface and separate the drug discharged from the inhaler to an inhalation part and a non-inhalation part. The drug in the non-inhalation part, which collides with an oral cavity and a pharyngeal region to result in being swallowed, is recovered in the rear of the throat and the upper impingement chamber (collectively stage 1). The drug in the inhalation part, which reaches lungs, is recovered in the lower impingement chamber (stage 2). Because the upper impingement chamber is designed so that the cut-off diameter is 6.4 μm when the test flow rate is 60 L per minute, particles with a diameter of 6.4 μm or less flow down to the lower impingement chamber.

Table 1 Component specification for apparatus shown in Fig. 1

Code	Item	Description	Dimensions*
A	Mouthpiece adapter	Moulded rubber adapter for actuator mouthpiece.	
B	Throat	Modified round-bottomed flask: —ground-glass inlet socket —ground-glass outlet cone	50 mL 29/32 24/29
C	Neck	Modified glass adapter: —ground-glass inlet socket —ground-glass outlet cone Lower outlet section of precision-bore glass tubing: —bore diameter Selected bore light-wall glass tubing: —external diameter	 24/29 24/29 14 17
D	Upper impingement chamber	Modified round-bottomed flask: —ground-glass inlet socket —ground-glass outlet cone	100 mL 24/29 14/23
E	Coupling tube	Medium-wall glass tubing: —ground-glass cone Bent section and upper vertical section: —external diameter Lower vertical section: —external diameter	 14/23 13 8
F	Screwthread, side-arm, adapter	Plastic screw cap Silicone rubber ring Polytetrafluoroethylene (PTFE) washer Glass screwthread: —thread size Side-arm outlet to vacuum pump: —minimum bore diameter	28/13 28/11 28/11 28 5
G	Lower jet assembly	Modified polypropylene filter holder connected to lower vertical section of coupling tube by PTFE tubing Acetal circular disc with the centres of four jets arranged on a projected circle of diameter 5.3 mm with an integral jet spacer peg: —peg diameter —peg protrusion	see Fig. 1 10 2 2
H	Lower impingement chamber	Conical flask —ground-glass inlet socket	250 mL 24/29

* Dimensions in mm, unless otherwise stated.

4.1. Procedure for nebulizers

Introduce 7 mL and 30 mL of a suitable solvent into the upper and lower impingement chambers, respectively.

Connect all the component parts. Ensure that the assembly is vertical and adequately supported and that the jet spacer peg of the lower jet assembly just touches the bottom of the lower impingement chamber. Connect a suitable pump fitted with a filter (of suitable pore size) to the outlet of the apparatus. Adjust the air flow through the apparatus, as measured at the inlet to the throat, to 60 ± 5 L per minute.

Introduce the inhalation liquids and solutions into the reservoir of the nebulizer. Fit the mouthpiece and connect it by means of a mouthpiece adapter to the device.

Switch on the pump of the apparatus and after 10 seconds switch on the nebulizer.

After 60 seconds, unless otherwise justified, switch off the nebulizer, wait for 5 seconds and then switch off the pump of the apparatus.

Dismantle the apparatus and wash the inner wall surface of the upper impingement chamber collecting the washings in a volumetric flask. Wash the inner wall surface of the lower impingement chamber collecting the washings in a second volumetric flask. Finally, wash the filter preceding the pump and its connections to the lower impingement chamber and combine the washings with those obtained

from the lower impingement chamber. Determine the amount of active substance collected in each of the two flasks. Express the results for each of the two parts of the apparatus as a percentage of the total amount of active substance.

4.2. Procedure for metered-dose inhalers

Install a suitable mouthpiece adapter in position at the end of the throat. When the mouthpiece end of the inhaler is inserted in the mouthpiece adapter to a depth of about 10 mm, the mouthpiece end of the inhaler lines up along the horizontal axis of the throat. The open end of the inhaler, which accepts the pressurized container, is uppermost and in the same vertical plane as the rest of the apparatus.

Introduce 7 mL and 30 mL of a suitable solvent into the upper and lower impingement chambers, respectively.

Connect all the component parts. Ensure that the assembly is vertical and adequately supported and that the jet spacer peg of the lower jet assembly just touches the bottom of the lower impingement chamber. Connect a suitable pump to the outlet of the apparatus. Adjust the air flow through the apparatus, as measured at the inlet to the throat, to 60 ± 5 L per minute.

Unless otherwise prescribed in the patient instruction, shake for 5 seconds and discharge once to waste. After not less than 5 seconds, shake and discharge again to waste. Repeat the procedure a further three times.

Shake for about 5 seconds, switch on the pump to the apparatus and locate the mouthpiece end of the inhaler in the adapter, discharge once immediately in the apparatus. Remove the assembled inhaler from the adapter, shake for not less than 5 seconds, relocate the mouthpiece end of the inhaler in the adapter and discharge again. Repeat the discharge sequence. The number of discharges should be minimized and typically would not be greater than 10. After the final discharge wait for not less than 5 seconds and then switch off the pump.

Dismantle the apparatus. Wash the inner wall surface of the coupling tube to the lower impingement chamber and its outer wall surface that projects into the chamber with a suitable solvent, collecting the washings in the lower impingement chamber. Determine the content of active substance in this solution. Calculate the amount of active substance collected in the lower impingement chamber per discharge and express the results as a percentage of the active substance stated on the label.

4.3. Procedure for dry powder inhalers

Introduce 7 mL and 30 mL of a suitable solvent into the upper and lower impingement chambers, respectively.

Connect all the component parts. Ensure that the assembly is vertical and adequately supported and that the jet spacer peg of the lower jet assembly just touches the bottom of the lower impingement chamber. Connect a suitable pump to the outlet of the apparatus. Adjust the air flow through the apparatus, as measured at the inlet to the throat, to 60 ± 5 L per minute.

Prepare an inhaler and connect it to the throat using a suitable adapter. Switch on the pump of the apparatus, after 5 seconds switch off the pump of the apparatus, and repeat the discharge sequence. The number of discharges should be minimized and typically would not be greater than 10.

Dismantle the apparatus. Wash the inner wall surface of the coupling tube to the lower impingement chamber and its outer wall surface that projects into the chamber with a suitable solvent, collecting the washings in the lower impingement chamber. Determine the content of active substance in this solution. Calculate the amount of active substance collected in the lower impingement chamber per discharge and express the results as a percentage of the active substance stated on the label.

G7 Containers and Package

Add the following:

Glass Containers for Pharmaceutical Products

Glass containers for pharmaceutical products are widely used. Glass bottles are used for tight and well-closed containers for bulk packaging of solid preparations for oral administration such as tablets and capsules etc., and ampules, vials or glass syringes are for hermetic containers of injections etc.

Glass containers used as a primary packaging have characteristics of high chemical durability etc. in addition to high strength, high transparency, no air permeability and no moisture permeability. On the other hand, they are heavy, bulky, fragile and easy to be broken by a physical shock during manufacturing or transportation, so they require attention on handling.

This chapter provides basic information about glass containers, items to be confirmed for the selection of glass containers and for the proper performance of a quality evaluation that comes along with the selection, and information about the quality control at the manufacturing stage of preparations.

1. Basic information about glass containers for pharmaceutical products

Glass containers for pharmaceutical products do not interact physically or chemically with the contained medicaments to alter any property or quality. Glass containers for injections can protect the contained medicaments from the invasion of microbes by means of perfect sealing or other suitable process.

To ensure the quality of contained medicaments over the shelf life, it is necessary to select a suitable glass container. In the selection of container, it is necessary to consider the physicochemical condition of the contained medicaments, i.e., solid or liquid and the adoption of a well-closed container, a tight container, a hermetic container or a colored container to ensure the chemical stability of the contained medicaments. Furthermore, it is necessary to consider sur-

face treatment on the inner surface of containers in the case where it is assumed that foreign substances occur by interactions with the preparation ingredients.

1.1 Glass composition and molding

Composition of the glass used for primary packaging of pharmaceutical products is either borosilicate glass or sodalime glass.

Borosilicate glass has a reticulated network made of silicon dioxide (silica: SiO_2) and diboron trioxide (B_2O_3). Borosilicate glass has a small coefficient of thermal expansion, relatively high hardness and high hydrolytic resistance[1]. Containers made of this chemical composition are classified as Type I glass in the USP and the EP.

Cylinder-shaped and long material glass tubes made of borosilicate glass are cut and undergo secondary processing to mold ampules, vials or syringes, which are mostly used for containers of small amount of injections or lyophilized preparations.

Sodalime glass is composed of silicon dioxide (silica: SiO_2), sodium oxide (Na_2O) and calcium oxide (CaO) as the principal components. It has low hydrolytic resistance as a drawback, but it is easy to manufacture and process[1]. Containers made of this chemical composition are classified as Type II or III glass in the USP and the EP.

A glass container made of sodalime glass is called a blown bottle or a molded bottle because it is molded by pouring melted glass into a mold and blowing air. Also it is called a standard bottle or an automatic bottle because of its mass production at low cost. It is widely used not only for glass bottles of solid preparations for oral administration but also as containers for injections such as large volume vials of parenteral infusions or vials of powder injections for antibiotics etc.

1.2 Surface treatment of inner surface of glass containers for pharmaceutical products

Surface treatments are performed to modify the nature of the inner surface of glass containers. The treatments are such as dealkalization treatment and coating, etc.

The dealkalization treatment is a method to neutralize the surface layer of the glass by selectively extracting and removing alkali components using sulfur compounds at high temperature above the glass-transition, which results in exposure of the surface rich silica. This treatment reduce the elution of alkali components. The coating includes methods using silica (SiO_2), silicon resin and fluorine resin, etc.

Silica processing is a method to form a thin film on an inner surface by melt coating of silica (SiO_2) on the inner surface of the glass at a high temperature. It is expected to suppress the elution of glass components and the occurrence of flakes, because the thin film is high purity silica with no water-soluble component such as alkali, weld to the inner surface of the glass container and the drug solution does not contact directly with the inner surface of the glass.

Silicone processing is a method to form a thin film of silicone resin on a glass surface by immersing the glass in dimethylpolysiloxane solution and baking. This treatment enhance water repellency and prevent a drug solution from remaining to the inner surface of the glass. Also it is expected to suppress the elution of glass components and the occurrence of flakes because the drug solution does not contact directly with the inner surface of the glass.

Fluorine resin processing is a method to form a thin film of fluorine resin on an inner surface by coating fluorine resin using coupling agents and baking. This treatment enhance water repellency and prevent a drug solution from remaining to the inner surface of the glass. Also it is expected to suppress the elution of glass components and the occurrence of flakes because the drug solution does not contact directly with the inner surface of the glass.

2. Quality evaluation of glass containers for pharmaceutical products at the design stage of preparations

At the design stage of preparations it is necessary to perform the quality evaluation of a glass container used and the compatibility of it with the contained medicaments.

Since each glass container for pharmaceutical products has characteristic properties and properties of pharmaceutical products packed in the glass containers are diverse, the compatibility of glass containers with pharmaceutical products should be judged by considering the combination of the both.

When evaluated, refer to General Rules for Preparations [2] General Notices for Packaging of Preparations, ''Basic Requirements and Terms for the Packaging of Pharmaceutical Products'' and ''Basic Requirements for Plastic Containers for Pharmaceutical Use and Rubber Closures for Containers for Aqueous Infusions'' under General Information, and verify that the glass container used for preparations conform to the basic requirements, i.e., the design specifications, based on tests and literatures.[2,3] The compatibility must be maintained based on an appropriate quality assurance plan.

2.1 Glass containers for pharmaceutical products equipped with closures

In the case of solid preparations for oral administration, glass containers with closures consist of a glass bottle and a resin cap with a packing or a metal cap with a compound, and in the case of lyophilized injections they consist of a vial and a rubber closure. In the case of syringe preparations they consist of a glass outer (barrel, some has a needle), a gasket and a top cap.

In the case of pharmaceutical products susceptible to be oxidized, it is unsuitable to select the closure material that permeate oxygen easily. In the case of aqueous pharmaceutical products and hygroscopic pharmaceutical products, it is unsuitable to select the closure material that permeate water vapor easily. Closures must not be deformed, deteriorated and degenerated by contained medicaments. Unacceptable loss of function of containers must not be caused by a possible high temperature or low temperature or their repetitions during storage and transportation and vibrations during transportation. Glass containers for multiple-dose pharmaceutical products equipped with closures are required to have an appropriate stability after opening.

The compatibility (fitting compatibility) of closures with glass containers for pharmaceutical products must be main-

tained based on an appropriate quality assurance plan.

2.2 Transparency of glass containers for pharmaceutical products and colored glass containers

In the case of pharmaceutical products such as injections where foreign matters and turbidity must be examined visually, glass containers for pharmaceutical products should have the required level of transparency that enables inspection.

On the other hand, the quality of contained medicaments unstable to light must not be lowered during storage because of a high transparency of glass containers for pharmaceutical products. A sufficient level of light shielding is required to ensure light stability, and the select of colored glass containers must be considered.

When colored glass containers are used for injections, it must meet the requirements of the light transmission test for light-resistant containers under Test for Glass Containers for Injections <*7.01*>.

2.3 Glass containers for pharmaceutical products required to be sterile

In selecting suitable glass containers (ampules) or glass containers with closures (vials, syringes) as a primary packaging for injections, it is desirable to obtain information on the manufacturing processes of the glass container including substances added.

For pharmaceutical products that require terminal sterilization, it is required for glass containers to satisfy the basic requirements even after the sterilization. There must be no residue or generation of new toxic substances of more than a certain quantity after the sterilization. Structures and materials of glass containers must cause no microbial contamination to contained medicaments during storage and transportation after the sterilization.

2.4 Foreign matters derived from glass containers for pharmaceutical products for injections

In the case of glass containers for injections, glass fragments generated at cutting ampules, flakes generated by peeling of inner surfaces of glass and insoluble foreign matters generated by elution of glass components or by stains on inner surfaces of glass should be examined.

Eluates and flakes etc. derived from glass containers must be sufficiently small from the viewpoint of safety. They must not damage the efficacy and safety of the contained medicaments.

Foreign matters derived from glass containers must be sufficiently evaluated at the design stage of preparations. It must be also evaluated, when the molding process or supplier is changed.

Scanning Electron Microscopy–Energy Dispersive X-ray Spectroscopy (SEM-EDX) is useful to analyze flakes derived from glass containers and inorganic foreign matters, for example aerosol of reaction products etc.

3. Test results to be recorded for each management unit

At the manufacturing stage of glass containers for pharmaceutical products the specification of the following test items should be set, and the test results should be recorded for each management unit of glass containers for pharmaceutical products.

1) Glass bottles used for solid preparations for oral administration etc.:
   (i) Appearance[4]: Shape and dimensions are correct, and there must not be failures of wall thickness, failures of color tone, breakage, lacks, cracks, internal cracks, scratches, bubbles, foreign matters, striae, streaks, rough surfaces, burrs, stains and insoluble matters, which cause a hindrance in usage.
   (ii) Quality tests: Soluble alkali test for a container, heat resistance and distortion.
   (iii) Others: Items to be necessary.
2) Ampules or vials used for injections etc.:
   (i) Appearance[4]: Shape and dimensions are correct, and there must not be failures of wall thickness, failures of color tone, breakage, lacks, cracks, internal cracks, scratches, foreign matters, striae, streaks, stains and insoluble matters, which cause a hindrance in usage.
   (ii) Quality tests: Tests prescribed under Test for Glass Containers for Injections <*7.01*>, heat resistance (only for sodalime glass) and distortion.
   (iii) Others: Items to be necessary.

4. References

1) Glossary of terms relating to fine ceramics, JIS R 1600 (2011), Japanese Industrial Standards
2) Containers-Glass, US Pharmacopeia 40 (2017) <660>
3) Evaluation of the inner surface durability of glass containers, US Pharmacopeia 40 (2017) <1660>
4) Glass bottles for drug, JIS R 3522 (1955), Japanese Industrial Standards

Add the following:

Moisture Permeability Test for Blister Packaging of Solid Preparations

The test is the method to measure the moisture transmission rate of the blister packaging represented by PTP packaging. It can be used for the following studies to evaluate moisture transmission through a packaging of drug preparation.

(i) Screening of the material and/or thickness for plastic sheets, forming conditions and/or size of pockets, etc. in the development phase.

(ii) Comparison of the moisture transmission rate of a plastic sheet before and after the change in material, thickness, forming conditions, and/or size of pockets, etc. in the development or production phase.

Note that when a sufficient amount of desiccant cannot be filled up in the pockets due to the minute pockets a reliable result might be not obtained. The test is intended to determine the moisture transmission rate of successfully prepared blister packaging, but not to detect the leakage due to pinholes and the like.

1. Terms

(i) Molding materials: Materials forming pockets and sealing areas. Usually, a single or double layer plastic sheet or that laminated with aluminum foil is used.

(ii) Sealing materials: Materials to seal tightly pockets packed with drug preparations. Usually, an aluminum foil is used.

(iii) Pockets: Places where the molding material is inflated in a convex shape to put drug preparations.

(iv) Moisture transmission rate: An amount of water transmitted into the pockets of a blister packaging per unit time (mg/day/pocket).

2. Apparatus

(i) Constant temperature and humidity chamber: An apparatus which can maintain a temperature and humidity storage condition.

(ii) Balance: A chemical balance.

3. Desiccants

It may be chosen from the following.

(i) Calcium chloride for water determination

Pretreatment before use: Put the desiccant taking out of fine powder in a depthless vessel, dry at 110°C for 1 hour, then allow to cool in a desiccator [phosphorus (V) oxide].

(ii) Synthetic zeolite for drying

Moisture adsorption ability: Not less than 15%. Weigh accurately about 10 g of the desiccant, allow to stand at 40°C and 75% relative humidity for 24 hours, then weigh the mass, and calculate the gain in weight.

Pretreatment before use: Put the desiccant in a depthless vessel, dry at 350 − 600°C for 2 hours, then allow to cool in a desiccator [phosphorus (V) oxide].

4. Samples

4.1. Preparation of sample

Amount of the desiccant filled in a pocket is determined appropriately depending on the form or size of the pocket, however, it should be about 80% of the capacity of the pocket for avoiding the deforming or impairing of the covering material. For preparation of the sample, carefully prepare the sample avoiding moisture adsorption of the desiccant. Fill the desiccant in all the pockets as evenly as possible, seal with a sealing material, and cut out to a suitable size. Separately, prepare a control in the same manner by packing with the similar mass of glass beads. The form and size of the sample and the control should be as identical as possible.

Examine the appearances of the prepared sample and control with the naked eye or by using a stereomicroscope, and use them whose pockets maintain their shapes as prescribed, and without any forming faults, aberrant wrinkles on the sealing material, pinholes or any sealing faults.

4.2. Number of samples

Five to ten sheets are used for the sample with not less than 10 pockets per sheet. An appropriate amount of sheets equivalent to 20 to 100 pockets (not less than 10 sheets), depending on the number of pockets per sheet are used for the sample with less than 10 pockets per sheet. The number of the control is at least 2 sheets, however, desirable to be the same as the sample number.

5. Method

5.1. Storage conditions

The following conditions are desirable, though other conditions may be used.

(i) 25 ± 2°C/60 ± 5%RH

(ii) 40 ± 2°C/75 ± 5%RH

5.2. Storage

Place the samples and controls in a constant temperature and humidity chamber without overlapping each other of the sheets, not in standing position, as the pocket facing upwards, as not intercepting the air circulation and avoiding exposure to the air flow from the outlet.

5.3. Mass measuring

Take out the samples and controls from the chamber, allow cooling to room temperature, measure the mass of each sheet quickly, and place them back to the chamber. Weigh exactly the masses of them to a degree of 0.1 mg.

5.4. Measuring intervals

Intervals of the measurement are adjusted depending on the moisture transmission rate and avoiding large change in the temperature and humidity inside of the chamber (for example, 0, 1, 3, 7, 14, 21 and 28 days).

5.5. Termination of measurements

Measure the mass of each sheet of the sample and control at each measuring point, and calculate the differences in their average values (the increasing amount of the sample mass). Prepare a linear regression equation by the least-squares method by plotting the increasing amount (mg) of the sample mass on the vertical axis against the time (day) on the horizontal axis. The measurement should be finished when the increase in the mass shows linearity in at least three sequenced points (expect for the starting point) and before the desiccant absorbs moisture of 10% amount of the mass of packed desiccant. The correlation coefficient of the linearity is desirable to be not less than 0.98.

5.6. Others

Data of samples showing extremely larger mass increase as compared to the others should be excluded since the package may have some leakage due to pinholes or the like. Appropriate statistical tests are performed, as needed.

6. Calculation of moisture transmission rate

The moisture transmission rate (mg/day/pocket) is calculated by dividing the slope, i.e. the mass increasing amount (mg/day), obtained by the least-squares method, by the number of pocket per sheet. Record the moisture transmission rate together with the storage conditions and the name of the desiccant used.

7. Information

7.1. Factors affecting the moisture transmission rate

There are as follows:

(i) Qualities (molecular structure, density, degree of crystallinity, etc.), composition and/or thickness of the molding materials

(ii) Methods and conditions to form the pocket

(iii) Size and/ or uniformity of wall thickness of the pocket

(iv) Storage conditions, water activity inside the pocket

7.2. Measurement of pocket wall thickness

Measure the wall thickness of at least one position of upper or side face or R part of not less than 10 pockets of the sampling sheet to a unit of 1 μm, using a micrometer or dial gage with an accuracy of better than 1 μm or an equivalent measuring instrument, as necessary. Measuring position is selected in consideration of the shape of the pocket or difficulty of the measurement. It is desirable to identify the site that may become thinner in the phase of study for pocket forming conditions, and to measure the thickness of the site while paying attention to the pressure.

8. Reference

1) Tsuneo Okubo, *et al.*: *PMDRS*, 45(2), 155 − 165, (2014)

G10 Others

Basic Concepts for Quality Assurance of Drug Substances and Drug Products

Change the Basic Concept and section 4. as follows:

Basic Concept

In recent years, the mainstream concept for quality of drugs is that their quality is assured by management of manufacturing process, including management of raw material and other materials, and quality tests of final products (drug substances and products) that are conducted mutually complementary.

4. Real time release testing (RTRT) and parametric release

Determination of the suitability for release can be based on the result of RTRT instead of final product testing when approved by the regulatory authority. RTRT is a type of tests to evaluate the quality of in-process and/or final products based on process data (including results of in-process testing and data on process parameters). The usage of RTRT does not mean unnecessity of setting final product tests directly. Even if the decision of release is made by RTRT, the tests for final products need to be set as specifications. It is because final product testing may be requested for some reasons such as failure of data acquisition due to troubles of equipments used for RTRT and evaluation of stability of final products. The final products, of course, need to meet their specifications, when tested. If RTRT results fail or trending toward failure, RTRT should not easily be substituted by final product testing. In this case, it is important to investigate the cause properly and need to take corrective action. Also, if RTRT results fail, the products can not be released unless they were caused by equipment failure. If RTRT results are trending toward failure, the products release should be made carefully based on the results of the investigation.

Parametric release can be considered a type of real-time release. One example of parametric release is to determine the suitability for release of terminally sterilized drug products based on the data on sterilizing process instead of the results of sterility testing. In this case, the release of each batch is based on satisfactory results from monitoring specific parameters, e.g., temperature, pressure, and time during the terminal sterilization phase(s) of drug product manufacturing. Parametric release based on above parameters is more reliable in predicting sterility assurance than determination of suitability for release based on sterility testing using limited number of final products. Besides, even if parametric release is applied, the final product testing need to be set because the testing is necessary in stability testing and survey after release. The parametric release differs from RTRT in that the usage of the final product testing as alternative should not be applicable in principle in case of failure. If the data of monitoring specific parameters in terminally sterilized process is failed to obtain by a certain reason, the sterilization process is not able to be assured.

Add the following:

Criteria for Content Uniformity in Real Time Release Testing by Process Analytical Technology

1. Introduction

In recent years, the new criteria for Content Uniformity Test using a large sample size for Real Time Release Testing (RTRT) have become necessary with the rapid development of Process Analytical Technology (PAT). PAT using a non-destructive method such as Near Infrared (NIR) spectrometry enable to measure a large number of samples in real time, resulting in the generation of large amounts of data in a short time, and PAT can improve process control and process capability. However, the current pharmacopoeial criteria for Uniformity of Dosage Unit (the sample size is 10 and 30 for first and second stage respectively) may not be used adequately for large sample sizes over a hundred. For example, zero tolerance criteria has been used for outliers (no unit showing over the 25% deviation from label claim must be observed in the sample tested). However, the probability of occurrence of outliers cannot be ignored when sample size was well over a hundred. This document display the consideration about criteria applicable for the large sample size over a hundred in RTRT.

2. Theoretical basis of the criteria

The Content Uniformity Test of pharmacopoeia is a kind of sampling tests, using small picked sample(s) from a large population (batch, lot), used for release of products. Therefore, the quality of estimations (test performance) depends on the sample size. In general, estimate the better the larger

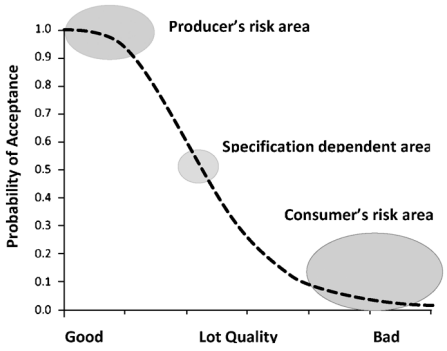
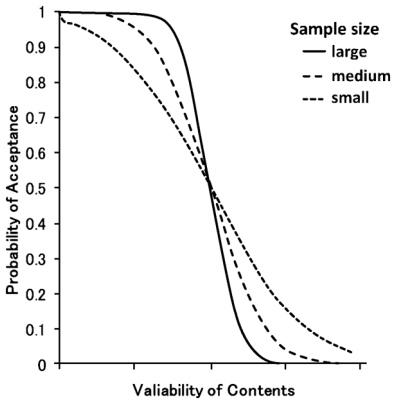


Fig. 1 Consumer's risk and producer's risk areas in an OC curve

A. Effects of sample sizes



B. Effects of specification limits

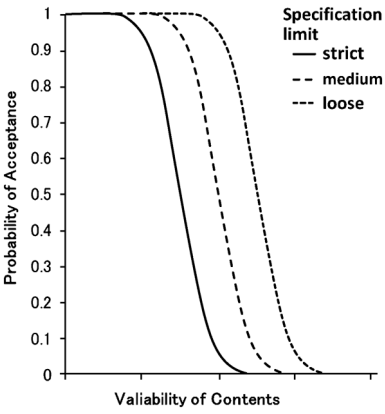
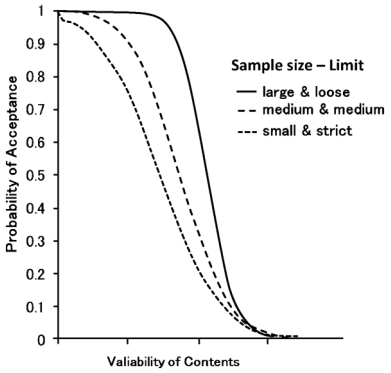


Fig. 2 OC Curves of Content Uniformity Tests — Effects of sample sizes and specification limits

A. Constant risk of consumers



B. Constant risk of producers

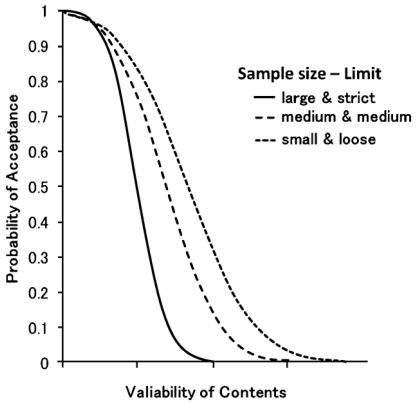


Fig. 3 OC Curves of Content Uniformity Tests — Risks of consumers or producers are constant

the sample size, and it is considered that a large sample size makes it possible to determine the quality of lots certainly. On the other hand, usage of a large sample size causes consumption of resources. For this reason, compendial tests like pharmacopoeias tests use a minimum and optimal sample size accompanying with strict criteria in order not to release bad products. Now a day, as a large sample size (Large-N) has become popular with development of PAT, it needs to set the appropriate criteria for RTRT using Large-N.

In setting of a specification limit, the limit value is determined by the balance between a guaranteed quality limit (acceptable limit) and the severity of a realistically capable test. When the specification limit is too strict, the acceptable quality becomes better, however a stock shortage caused by a low rate of passing the test of actual products occurs and the cost may become abnormally high. In order to maintain an acceptable quality, it is the most reasonable to compare the consumer's risk (risk of poor quality passing the test) and the producer's risk (risk of good quality failing the test) and to determine the severity of the most suitable test. Fig. 1 shows the OC (operating characteristic) curve describes the relationship as above.

The consumer's risk level, an acceptable quality corresponding to pass rate of 5% in release tests, is important to guarantee the quality of the product to be released. This means that possibility of releasing low quality products is considered low ($<5\%$). On the other hand, the producer's risk is important for producers. They should consider how good quality is needed to pass (usually 90 – 95%) the test sufficiently. In spite of the sample size, the lot quality corresponding to 50% of pass rate is almost same the quality on

the specification limit. If the sample size is increased without change of the specification limit, the OC curves change as in Fig. 2-A, where the quality (x-axis showing variability in unit content) of the 50% acceptance level is unchanged in all the OC curves while the slope of the OC curves become steeper with the larger the sample size. In contrast, if the limit value is changed to more strict without changing the sample size, the OC curves shift to the left at a constant slope (Fig. 2-B). To be constant the consumer's risk level regardless of change of sample size, it is necessary to set the limit value in response to changes in sample size as in Fig. 3-A. In general, the large sample size can have the consumer's risk level maintain to be constant even if the limit value becomes loose.

When products is tested by PAT in a large sample size and then released, they will be subjected to stability tests and survey tests using the usual small sample sizes after releasing. In this case, though the consumer's risk as in Fig. 3-A is at a constant, the producer's risk increase. In order not to increase the producer's risk after releasing, it is necessary to set the test limits so as not to differ very much in producer's risk between the test by PAT and the conventional test. In this case, it is necessary to tighten the test limit in larger sample size, as shown in Fig. 3-B.

Our recommended criteria were determined in consideration of such a point as described above[1]. It should be noted that our criteria are simple and non-parametric criteria that do not depend on the type of distribution of unit content, and also has the same attitude with the Alternate 2[2] of the European Pharmacopoeia (EP) being a standard corresponding to the above mentioned Large-N. In the case of using the Alternate 1 of EP, there could be no problem from the point of view about quality assurance.

3. Criteria for Content Uniformity in sample size equal to or more than 100

The criteria recommended are consisted of two tests by attribute (limits are *C1* and *C2*). The sample sizes and acceptance numbers are shown in Table 1.

Criteria

Select n units representing a lot submitted, and assay the units individually using an appropriate analytical method and calculate individual contents expressed by the percentage of label claim. The requirements are met if the number of dosage units outside 15.0% is less than or equal to *C1*, and the number of dosage units outside 25.0% is less than or equal to *C2*. The central point of content bias can be alter to an appropriate value from the label claim if it is needed by quality control issue.

Table 1 Criteria for Content Uniformity

Sample size (n)	Acceptance number*	
	*C1*** (± 15.0%)	*C2*** (± 25.0%)
$n < 100$	Criteria of 6.02 Uniformity of Dosage Units	
$100 \leq n < 150$	3	0
$150 \leq n < 200$	4	0
$200 \leq n < 300$	6	1
$300 \leq n < 500$	8	2
$500 \leq n < 1000$	13	4
$1000 \leq n < 2000$	25	8
$2000 \leq n < 5000$	47	18
$5000 \leq n < 10000$	112	47
$10000 \leq n$	217	94

* The requirements are met if the number of outliers is less than or equal to acceptance number.

** Critical acceptance number.

4. References

1) Noriko Katori and Haruhiro Okuda, Sakura Bloom Tablets P2 Mock by MHLW sponsored QbD Drug Product Study Group (Mar. 2015).

2) European Pharmacopoeia 7.7 DEMONSTRATION OF UNIFORMITY OF DOSAGE UNITS USING LARGE SAMPLE SIZES

International Harmonization Implemented in the Japanese Pharmacopoeia Seventeenth Edition

Add the following:

Nov. 2014/July 2015 (Corr. 1)

Harmonized items	JP 17 (Supplement I)	Remarks
Hydroxypropylcellulose, Low Substituted	**Hydroxypropylcellulose, Low Substituted**	
Definition	limits of content	
	Description	Non-harmonized item
Packing and storage	Containers and storage	
Identification A	Identification (3)	
Identification B	Identification (1)	
Identification C	Identification (2)	
	Purity Heavy metals	
pH	pH	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Assay for hydroxypropoxy groups	Assay	
JP's particular description: Purity Heavy metals.		

June 2014

Harmonized items	JP 17 (Supplement I)	Remarks
Glucose Anhydrous	**Purified Glucose**	
Definition	limits of content	
	Description	Non-harmonized item
Identification A	Identification (1)	
Identification B	Identification (2)	
Appearance of solution	Purity (1) Clarity and color of solution	
Conductivity	Conductivity	
	Purity (2) Heavy metals	Non-harmonized item
Related substances	Purity (3) Related substances	
Dextrin	Purity (4) Dextrin	
Soluble starch and sulfite	Purity (5) Soluble starch and sulfite	
Water	Water	
Assay	Assay	
	Containers and storage	Non-harmonized item
JP's particular description: Identification (1); Purity (3) Related substances Test for required detectability, System repeatability; Assay System repeatability.		

June 2014/Jan. 2015 (Corr. 1)

Harmonized items	JP 17 (Supplement I)	Remarks
Glucose Monohydrate	**Glucose Hydrate**	
Definition	limits of content	
	Description	Non-harmonized item
Identification A	Identification (1)	
Identification B	Identification (2)	
Appearance of solution	Purity (1) Clarity and color of solution	
Conductivity	Conductivity	
	Purity (2) Heavy metals	Non-harmonized item
Related substances	Purity (3) Related substances	
Dextrin	Purity (4) Dextrin	
Soluble starch and sulfite	Purity (5) Soluble starch and sulfite	
Water	Water	
Assay	Assay	
	Containers and storage	Non-harmonized item
JP's particular description: Identification (1); Purity (3) Related substances Test for required detectability, System repeatability; Assay System repeatability.		

Nov. 2014

Harmonized items	JP 17 (Supplement I)	Remarks
Sodium Lauryl Sulfate	**Sodium Lauryl Sulfate**	
Definition	limits of content	
	Description	Non-harmonized item
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
Identification D	Identification (4)	
Alkalinity	Purity (1) Alkalinity	
Sodium chloride	Purity (2) Sodium chloride	
Sodium sulfate	Purity (3) Sodium sulfate	
Unsulfated alcohol	Purity (4) Unsulfated alcohols	
	Water	
	Total alcohol content	
Assay—Content of sodium alkyl sulfate	Assay	
	Containers and storage	Non-harmonized item
JP's particular description: Water; Total alcohol content.		

Change the following as follows:

Nov. 2008

Harmonized items	JP 17 (Supplement I)	Remarks
Particle-size Analysis by Laser Light Diffraction	**3.06 Laser Diffraction Scattering Measurement of Particle size**	
Introduction	Introduction	
Principle	3. Measurement	
Instrument	1. Instrument	
Development of the method	2. Development of the method	
Sampling	2.1. Sampling	
Evaluation of the dispersion procedure	2.2. Evaluation of the dispersion procedure	
Optimisation of the liquid dispersion	2.3. Optimization of the liquid dispersion	
Optimisation of the gas dispersion	2.4. Optimization of the gas dispersion	
Determination of the concentration range	2.5. Determination of the concentration range	
Determination of the measuring time	2.6. Determination of the measuring time	
Selection of an appropriate optical model	2.7. Selection of an appropriate optical model	
Validation	2.8. Validation	
Measurement	3. Measurement	
Precautions	3.1. Precautions	
Measurement of the light scattering of dispersed sample(s)	3.2. Measurement of the light scattering of dispersed sample(s)	
Conversion of scattering pattern into particle-size distribution	3.3. Conversion of scattering pattern into particle-size distribution	
Replicates	3.4. Replicates	
Reporting of results	4. Reporting of results	
Control of the instrument performance	5. Control of the instrument performance	
Calibration	5.1. Calibration	
Qualification of the system	5.2. Qualification of the system	
Note		
Figure 1 Example of a set-up of laser light diffraction instrument	Fig. 3.06-1 Example of a set-up of laser light diffraction instrument	

Nov. 2015 (Rev. 2)

Harmonized items	JP 17 (Supplement I)	Remarks
Uniformity of Dosage Units	**6.02 Uniformity of Dosage Units**	
(Introduction)	(Introduction)	
Content uniformity	1. Content uniformity	
Solid dosage forms	(i) Solid dosage forms	
Liquid or semi-solid dosage forms	(ii) Liquid or semi-solid dosage forms	
Calculation of acceptance value	1.1. Calculation of acceptance value	
Mass variation	2. Mass variation	
Uncoated or film-coated tablets	(i) Uncoated or film-coated tablets	
Hard capsules	(ii) Hard capsules	
Soft capsules	(iii) Soft capsules	
Solid dosage forms other than tablets and capsules	(iv) Solid dosage forms other than tablets and capsules	
Liquid dosage forms	(v) Liquid dosage forms	
Calculation of acceptance value	2.1. Calculation of acceptance value	
Criteria	3. Criteria	
Solid, semi-solid and liquid dosage forms	(i) Solid, semi-solid and liquid dosage forms	
Table 1 Application of content uniformity (CU) and mass variation (MV) test for dosage forms	Table 6.02-1 Application of content uniformity (CU) and mass variation (MV) test for dosage forms	
Table 2	Table 6.02-2	
JP's particular description: (Introduction) Additional explanation on Liquids, Additional explanation for the part not containing drug substance; 2. Mass variation Additional explanation that the test is performed based on the assumption of the concentration of drug substances being uniform; Table 6.02-1 Additional explanation on ''solids in single-dose packages'' and ''solutions enclosed in unit-dose containers''.		

May 2016 (Rev. 2, Corr. 1)

Harmonized items	JP 17 (Supplement I)	Remarks
Saccharin Sodium	**Saccharin Sodium Hydrate**	
Definition	limits of content	
Identification A	Identification (1)	
Identification B	Identification (2)	
Clarity of solution	Purity (1) Clarity and color of solution	
Color of solution		
Acidity or alkalinity	Purity (2) Acidity or alkalinity	
Water	Water	
Readily carbonizable substances	Purity (6) Readily carbonizable substances	
Limit of benzoate and salicylate	Purity (4) Benzoate and salicylate	
Assay	Assay	

July 2015 (Rev. 1, Corr. 2)

Harmonized items	JP 17 (Supplement I)	Remarks
Hypromellose	**Hypromellose**	
Definition	limits of content of methoxy group and hydroxypropoxy group	
Labeling	labeling of viscosity	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Identification (3)	Identification (3)	
Identification (4)	Identification (4)	
Identification (5)	Identification (5)	
Viscosity	Viscosity	
Method 1	Method I	
Method 2	Method II	
pH	pH	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Assay	Assay	

July 2016 (Corr. 3)

Harmonized items	JP 17 (Supplement I)	Remarks
Polysorbate 80	**Polysorbate 80**	
Definition	origin	
Identification (Composition of fatty acids)	Identification	
Acid value	Acid value	JP's particular description: Applying Fats and Fatty Oils Test 1.13, using ethanol (95) as the solvent.
Hydroxyl value	Hydroxyl value	
Peroxide value	Purity (3) Peroxide value	
Saponification value	Saponification value	
Composition of fatty acids	Composition of fatty acid	
Ethylene oxide and dioxan	Purity (2) Ethylene oxide and 1,4-dioxane	
Water	Water	
Total ash	Residue on ignition	
Storage	Containers and storage	

Oct. 2016 (Rev. 1)

Harmonized items	JP 17 (Supplement I)	Remarks
	General Information	
Amino Acid Analysis	**Amino Acid Analysis**	
Apparatus	Apparatus	
General precautions	General precautions	
Reference standard material	Reference standard material	
Calibration of instrumentation	Calibration of instrumentation	
Repeatability	Repeatability	
Sample preparation	Sample preparation	
Internal standards	Internal standards	
Protein hydrolysis	Protein hydrolysis	
Method 1	Method 1	
Hydrolysis solution	Hydrolysis solution	
Procedure	Procedure	
Liquid phase hydrolysis	Liquid phase hydrolysis	
Vapor phase hydrolysis	Vapor phase hydrolysis	
Method 2	Method 2	
Hydrolysis solution	Hydrolysis solution	
Vapor phase hydrolysis	Vapor phase hydrolysis	
Method 3	Method 3	
Hydrolysis solution	Hydrolysis solution	
Vapor phase hydrolysis	Vapor phase hydrolysis	
Method 4	Method 4	
Oxidation solution	Oxidation solution	
Procedure	Procedure	
Method 5	Method 5	
Hydrolysis solution	Hydrolysis solution	
Liquid phase hydrolysis	Liquid phase hydrolysis	
Method 6	Method 6	
Hydrolysis solution	Hydrolysis solution	
Vapor phase hydrolysis	Vapor phase hydrolysis	
Method 7	Method 7	
Reducing solution	Reducing solution	
Procedure	Procedure	
Method 8	Method 8	
Stock solutions	Stock solutions	
Reducing solution	Reducing solution	
Procedure	Procedure	
Method 9	Method 9	
Stock solutions	Stock solutions	
Carboxymethylation solution	Carboxymethylation solution	
Buffer solution	Buffer solution	

Procedure	Procedure	
Method 10	Method 10	
Reducing solution	Reducing solution	
Procedure	Procedure	
Method 11	Method 11	
Reducing solutions	Reducing solutions	
Procedure	Procedure	
Methodologies of amino acid analysis general principles	Methodologies of amino acid analysis general principles	
Method 1-Postcolumn ninhydrin detection general principle	Method 1-Postcolumn ninhydrin detection general principle	
Method 2-Postcolumn OPA fluorometric detection general principle	Method 2-Postcolumn OPA fluorometric detection general principle	
Method 3-Precolumn PITC derivatization general principle	Method 3-Precolumn PITC derivatization general principle	
Method 4-Precolumn AQC derivatization general principle	Method 4-Precolumn AQC derivatization general principle	
Method 5-Precolumn OPA derivatization general principle	Method 5-Precolumn OPA derivatization general principle	
Method 6-Precolumn DABS-Cl derivatization general principle	Method 6-Precolumn DABS-Cl derivatization general principle	
Method 7-Precolumn FMOC-Cl derivatization general principle	Method 7-Precolumn FMOC-Cl derivatization general principle	
Method 8-Precolumn NBD-F derivatization general principle	Method 8-Precolumn NBD-F derivatization general principle	
Data calculation and analysis	Data calculation and analysis	
Calculations	Calculations	
Amino acid mole percent	Amino acid mole percent	
Unknown protein samples	Unknown protein samples	
Known protein samples	Known protein samples	

Add the following:

Stability Testing of Drug Substances and Drug Products

1. Introduction

It is essential that the quality of a drug is maintained during the period from being manufactured to being administered in a patient. Stability testing is performed in order to ensure that the quality is maintained during the period. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, and to establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions.

The re-test period of a drug substance is the period of time during which the drug substance is expected to remain within its specification and, therefore, can be used in the manufacture of a given drug product, provided that the drug substance has been stored under the defined conditions. After this period, a batch of drug substance destined for use in the manufacture of a drug product should be re-tested for compliance with the specification and then used immediately. A batch of drug substance can be re-tested multiple times. For certain antibiotics known to be labile, it is more appropriate to establish a shelf life than a re-test period. The shelf life of a drug product is the period in which a batch of the product is expected to remain within the approved shelf life specification if stored under defined conditions.

This general information mainly illustrates a standard implementation that can be set when we perform stability tests of a chemical drug substance and the associated drug product, and it is also helpful in stability tests of pharmaceuticals other than chemical drugs. Also, this leaves

sufficient flexibility to encompass the variety of different practical situations that may be encountered due to specific scientific considerations and characteristics of the materials being evaluated. Alternative approaches can be used when there are scientifically justifiable reasons.

2. Conditions of stability testing

Stress testing, long term testing, accelerated testing and if necessary intermediate testing are performed as stability testing for drugs.

2.1 Stress testing

Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. Stress testing should include the effect of temperatures (in 10°C increments (e.g., 50°C, 60°C, etc.) above that for accelerated testing), humidity (e.g., 75% RH or greater) where appropriate, oxidation, and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension.

Stress testing of the drug product is undertaken to assess the effect of severe conditions on the drug product. Such studies include photostability testing and specific testing on certain products, (e.g., metered dose inhalers, creams, emulsions, refrigerated aqueous liquid products).

2.2 Long term testing, accelerated testing and intermediate testing

Long term testing is undertaken on batches of a drug substance or drug product according to a prescribed stability protocol to establish the re-test period of the drug substance or the shelf life of the drug product.

Accelerated testing is a stability study designed to increase the rate of chemical degradation or physical change of a drug substance or drug product by using exaggerated storage conditions. Data from these studies, in addition to long term stability studies, can be used to assess longer term chemical effects at non-accelerated conditions and to evaluate the effect of short term excursions outside the label storage conditions such as might occur during shipping.

Intermediate testing is conducted at 30°C/65% RH and designed to moderately increase the rate of chemical degradation or physical changes for a drug substance or drug product intended to be stored long term at 25°C. Intermediate testing is implemented only when a significant change occurs in the accelerated testing.

Long term and accelerated testing, also if needed intermediate testing should be performed on at least three primary batches. The primary batches of the drug substance should be manufactured to a minimum of pilot scale by the same synthetic route as, and using a method of manufacture and procedure that simulates the final process to be used for, production batches. The overall quality of the batches of drug substance placed on the stability studies should be representative of the quality of the material to be made on a production scale. The stability studies should be conducted on the drug substance packaged in a container closure system that is the same as or simulates the packaging proposed for storage and distribution. The primary batches of the drug product should be of the same formulation and packaged in the same container closure system as proposed for marketing (including, as appropriate, any secondary packaging and container label). The manufacturing process used for primary batches should simulate that to be applied to production batches and should provide the product of the same quality and meeting the same specification as that intended for marketing. Two of the three batches should be at least pilot scale batches and the third one can be smaller, if justified. The primary batch may be a production batch. Where possible, batches of the drug product should be manufactured by using different batches of the drug substance. The pilot scale batch is a batch of a drug substance or drug product manufactured by a procedure fully representative of and simulating that to be applied to a full production scale batch. For solid oral dosage forms, a pilot scale is generally, at a minimum, one-tenth that of a full production scale or 100,000 tablets or capsules, whichever is the larger.

The storage conditions used for stability testing are shown as follows.

Table 1 Storage condition

Storage condition and package	Long term	Accelerated	Intermediate
General case (drug substance and product)	25 ± 2°C/ 60 ± 5%RH or 30 ± 2°C/ 65 ± 5%RH[1)]	40 ± 2°C/ 75 ± 5%RH	30 ± 2°C/ 65 ± 5%RH[2)]
Storage in a refrigerator (drug substance and product)[3)]	5 ± 3°C	25 ± 2°C/ 60 ± 5%RH	—
Storage in a freezer (drug substance and product)[4)]	− 20 ± 5°C	—	—
Storage below − 20°C (drug substance and product)	case-by-case basis		
Drug products packaged in impermeable containers	Study can be conducted under any controlled or ambient humidity condition		
Drug products packaged in semi-permeable containers[5)]	25 ± 2°C/ 40 ± 5%RH or 30 ± 2°C/ 35 ± 5%RH[6)]	40 ± 2°C/ not more than (NMT) 25%RH	30 ± 2°C/ 65 ± 5%RH[7)]

[1)] It is up to the applicant to decide whether long term stability studies are performed at 25 ± 2°C/60 ± 5%RH or 30 ± 2°C/65 ± 5%RH.

[2] If ''significant change'' occurs at the accelerated storage condition, additional testing at the intermediate storage condition should be conducted. However, if 30 ± 2°C/65 ± 5%RH is the long term condition, there is no intermediate condition. ''Significant change'' for a drug substance is defined as failure to meet its specification. In general, ''significant change'' for a drug product is defined as:

1. A 5% change in assay from its initial value; or failure to meet the acceptance criteria for potency when using biological or immunological procedures;

2. Any degradation product's exceeding its acceptance criterion;

3. Failure to meet the acceptance criteria for appearance, physical attributes, and functionality test (e.g., color, phase separation, resuspendibility, caking, hardness, dose delivery per actuation); however, some changes in physical attributes (e.g., softening of suppositories, melting of creams) may be expected under accelerated conditions;

and, as appropriate for the dosage form:

4. Failure to meet the acceptance criterion for pH; or

5. Failure to meet the acceptance criteria for dissolution for 12 dosage units.

6. Physical changes shown in the following may be observed in accelerated testing, but the changes are not considered as ''significant change'' which needs intermediate testing, when there is no ''significant change'' in other attributes.

• Softening of suppositories designed to melt at 37°C, when its melting point is shown clearly.

• When it is clear that ''significant change'' is due to crosslinking, the dissolution of gelatin capsules and gel coating tablets do not conform to the acceptance criteria for 12 dosage units.

When confirming that there is no ''significant change'' in other attributes, consider the possibility that these physical changes affect the other attributes.

[3] The drug product is packaged in a semi-permeable container, appropriate information should be provided to assess the extent of water loss. In the accelerated testing of drug substances or products intended for storage in a refrigerator, if significant change occurs within the first 3 months, it is considered unnecessary to continue to test a product through 6 months.

[4] Testing on a single batch at an elevated temperature (e.g., 5 ± 3°C or 25 ± 2°C) for an appropriate time period should be conducted to address the effect of short term excursions outside the label storage condition, e.g., during shipment and handling.

[5] Aqueous-based products packaged in semi-permeable containers should be evaluated for potential water loss under conditions of low relative humidity. Other comparable approaches can be developed and used for non-aqueous, solvent-based products.

[6] It is up to the applicant to decide whether long term stability studies are performed at 25 ± 2°C/40 ± 5% RH or 30 ± 2°C/35 ± 5%RH.

[7] If ''significant change'' other than water loss occurs during the 6 months' testing at the accelerated storage condition. Additional testing at the intermediate storage condition should be performed. A significant change in water loss alone at the accelerated storage condition does not necessitate testing at the intermediate storage condition. However, data should be provided to demonstrate that the drug product will not have significant water loss throughout the proposed shelf life if stored at 25°C and the reference relative humidity of 40% RH. If 30 ± 2°C/35 ± 5% RH is the long term condition, there is no intermediate condition. A 5% loss in water from its initial value is considered a significant change for a product packaged in a semi-permeable container after an equivalent of 3 months' storage at 40°C/NMT 25% RH. However, for small containers (1 mL or less) or unit-dose products, a water loss of 5% or more after an equivalent of 3 months' storage at 40°C/NMT 25% RH may be appropriate, if justified.

3. Testing attributes and testing frequency

Stability studies should include testing of those attributes of the drug substance or the product that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. Validated stability-indicating analytical procedures should be applied. Whether and to what extent replication should be performed will depend on the results from validation studies.

For long term studies, frequency of testing should be sufficient to establish the stability profile of the drug substance and product. For drug substances or products with a proposed re-test period or shelf life of at least 12 months, the frequency of testing at the long term storage condition should normally be every 3 months over the first year, every 6 months over the second year, and annually thereafter through the proposed re-test period or shelf life. At the accelerated storage condition, a minimum of three time points, including the initial and final time points (e.g., 0, 3, and 6 months), from a 6-month study is recommended. When testing at the intermediate storage condition is called for as a result of significant change at the accelerated storage condition, a minimum of four time points, including the initial and final time points (e.g., 0, 6, 9, 12 months), from a 12-month study is recommended.

A reduced design, i.e., matrixing or bracketing, where the testing frequency is reduced or certain factor combinations are not tested at all, can be applied, if justified, for the testing of combination of drug products having multiple design factors (e.g., strength, container size and/or fill). A bracketing design assumes that the stability of any intermediate levels is represented by the stability of the extremes tested. This is the design of a stability schedule such that only samples on the extremes of certain design factors (e.g., strength, container size and/or fill). Bracketing can be applied to studies with multiple strengths of identical or closely related formulations. Examples include but are not limited to (1) capsules of different strengths made with different fill plug sizes from the same powder blend, (2) tablets of different strengths manufactured by compressing varying amounts of the same granulation, and (3) oral solutions of different strengths with formulations that differ only in minor excipients (e.g., colorants, flavorings). Bracketing can be applied to studies of the same container closure system where either container size or fill varies while the other remains constant. The use of a bracketing design would not be applicable if it cannot be demonstrated that the strengths or container sizes and/or fills selected for testing are indeed the extremes. An example of a bracketing design is given in Table 2. This design is provided for illustrative purpose, and should not be considered the only, or the most appropriate, design in all cases.

A matrixing design assumes that the stability of each subset of samples tested represents the stability of all samples at a given time point. This is the design of a stability schedule such that a selected subset of the total number of possible samples for all factor combinations would be tested at a specified time point. At a subsequent time point, another subset of samples for all factor combinations would be

Table 2 Example of a Bracketing Design

Strength		50 mg			75 mg			100 mg		
Batch		1	2	3	1	2	3	1	2	3
Container size	15 mL bottle	T	T	T				T	T	T
	100 mL bottle									
	500 mL bottle	T	T	T				T	T	T

T = Sample tested

Table 3 Example of a Matrixing Design on Time Points for a Product with Two Strengths
''One-Half Reduction''

Time point (months)			0	3	6	9	12	18	24	36
Strength	S1	Batch 1	T	T		T	T		T	T
		Batch 2	T	T		T	T	T		T
		Batch 3	T		T		T	T		T
	S2	Batch 1	T		T		T		T	T
		Batch 2	T	T		T	T	T		T
		Batch 3	T		T		T		T	T

T = Sample tested

tested. Matrixing designs can be applied to strengths with identical or closely related formulations. Examples include but are not limited to (1) capsules of different strengths made with different fill plug sizes from the same powder blend, (2) tablets of different strengths manufactured by compressing varying amounts of the same granulation, and (3) oral solutions of different strengths with formulations that differ only in minor excipients (e.g., colorants or flavorings). Other examples of design factors that can be matrixed include batches made by using the same process and equipment, and container sizes and/or fills in the same container closure system.

An example of a matrixing design is given in Table 3. This design is provided for illustrative purpose, and should not be considered the only, or the most appropriate, design in all cases.

4. Photostability testing

Photostability testing is a part of stress testing evaluating the photostability characteristics of drug substances and products.

4.1. Light sources

The light sources described below may be used for photostability testing.

(i) Option 1 Any light source that is designed to produce an output similar to the D_{65}/ID_{65} emission standard such as an artificial daylight fluorescent lamp combining visible and ultraviolet (UV) outputs, xenon, or metal halide lamp.

(ii) Option 2 For option 2 the same sample should be exposed to both the cool white fluorescent and near ultraviolet fluorescent lamp.

1. A cool white fluorescent lamp designed to produce an output similar to that specified in ISO10977(1993); and

2. A near ultraviolet fluorescent lamp having a spectral distribution from 320 nm to 400 nm with a maximum energy emission between 350 nm and 370 nm; a significant proportion of energy emission should be in both bands of 320 to 360 nm and 360 to 400 nm.

4.2. Light exposure level and testing condition

For drug substances, photostability testing should consist of two parts: forced degradation testing and confirmatory testing. The purpose of forced degradation testing studies is to evaluate the overall photosensitivity of the material for method development purposes and/or degradation pathway elucidation. This testing may involve the drug substance alone and/or in simple solutions/suspensions to validate the analytical procedures. In these forced degradation testing studies, a variety of exposure conditions may be used, depending on the photosensitivity of the drug substance involved and the intensity of the light sources used. For development and validation purposes it is appropriate to limit exposure and end the studies if extensive decomposition occurs. For photostable materials, studies may be terminated after an appropriate exposure level has been used. The design of these experiments is left to the applicant's discretion although the exposure levels used should be justified. Confirmatory studies of drug substance should then be undertaken to provide the information necessary for handling, packaging, and labeling. For confirmatory studies, samples should be exposed to light providing an overall illumination of not less than 1.2 million lx·h and an integrated near ultraviolet energy of not less than 200 W·h/m^2 to allow direct comparisons to be made between the drug substance and drug product. Care should be taken to ensure that the physical characteristics of the samples under test are taken into account and efforts should be made, such as cooling and/or placing the samples in sealed containers, to ensure that the effects of the changes in physical states such as sublimation, evaporation or melting are minimized. All such precautions should be chosen to provide minimal interference with the exposure of samples under test. Possible interactions between the samples and any material used for containers or for general protection of the sample should also be considered and eliminated wherever not relevant to the test being carried out. As a direct challenge for samples of solid drug substances, an appropriate amount of sample should be taken and placed in a suitable glass or plastic dish and protected with a suitable transparent cover if considered necessary. Solid drug substances should be spread across the container to give a thickness of typically not more than 3 mm. Drug substances that are liquids should be exposed in chemically inert and transparent containers. Where practicable when testing samples of the drug product outside of the primary pack, these should be presented in a way similar to the conditions mentioned for the drug substance. The samples should be positioned to provide maximum area

of exposure to the light source. For example, tablets, capsules, etc., should be spread in a single layer. If direct exposure is not practical (e.g., due to oxidation of a product), the sample should be placed in a suitable protective inert transparent container (e.g., quartz). If testing of the drug product in the immediate container or as marketed is needed, the samples should be placed horizontally or transversely with respect to the light source, whichever provides for the most uniform exposure of the samples. Some adjustment of testing conditions may have to be made when testing large volume containers (e.g., dispensing packs).

5. Evaluation of stability data

In the stability data evaluation, data from long term and accelerated testing, also if needed from intermediate testing and, as appropriate, supporting data (data of stability testing using drug substances and products in developing stage) should be evaluated to determine the critical quality attributes likely to influence the quality and performance of the drug substance or product. Each attribute should be assessed separately, and an overall assessment should be made of the findings for the purpose of proposing a re-test period or shelf life. An approach for analyzing data of a quantitative attribute that is expected to change with time is to determine the time at which the 95% one-sided confidence limit for the mean curve intersects the acceptance criterion. The re-test period or shelf life proposed should not exceed that predicted for any single attribute.

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