SUPPLEMENT II
TO
THE JAPANESE PHARMACOPOEIA
EIGHTEENTH EDITION

Official from June 28, 2024

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. 238

Pursuant to Paragraph 1, Article 41 of Act 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. 220, 2021) as follows*.

TAKEMI Keizo
The Minister of Health, Labour and Welfare

June 28, 2024

Japanese Pharmacopoeia

(The text referred to by the term ''as follows'' are omitted here. All of the revised Japanese Pharmacopoeia in accordance with this notification 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, and made public by publishing it on the website of the Ministry of Health, Labour and Welfare.)

Supplementary Provisions

(Effective Date)

Article 1 This Notification is applied from the date of the notification. (referred to as the ''notification date'' in the next and third articles)

(Transitional measures)

Article 2 In the case of drugs which are listed in the Japanese Pharmacopoeia (hereinafter referred to as ''previous Pharmacopoeia'') [limited to those listed in the Japanese Pharmacopoeia whose standards are changed in accordance with this notification (hereinafter referred to as ''new Pharmacopoeia'')] and drugs which have been approved as of the notification date 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 the day before the notification date 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 December 31, 2025.

Article 3 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 the notification date 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 December 31, 2025.

Article 4 In the case of drugs which are listed in the new Pharmacopoeia, the previous provisions are applied before and on June 30, 2027, regardless of the Article 2.66 Elemental Impurities of General Tests, Processes and Apparatus in the new Pharmacopoeia.

*The term ''as follows'' here indicates the content of Supplement II to the Japanese Pharmacopoeia Eighteenth Edition from General Tests, Processes and Apparatus to Ultraviolet-visible Reference Spectra (pp. 2991 – 3074).

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PREFACE

The 18th Edition of the Japanese Pharmacopoeia (JP) was promulgated by Ministerial Notification No. 220 of the Ministry of Health, Labour and Welfare (MHLW) on June 7, 2021.

In July 2021, the Committee on JP established the basic principles for the preparation of the JP 19th Edition, setting out the roles and characteristics of the JP, the definite measures for the revision, and the date of the revision.

It was agreed that the JP should be an official document that defines the specifications, criteria and standard test methods necessary to properly assure the quality of medicines in Japan in response to the progress of science and technology and medical demands, in order to contribute to ensuring public health. 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 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 in the international community, it should play an appropriate role and contribute to the utilization of advanced technology and the promotion of international consistency in order to ensure the quality of drugs beyond the national level.

As the policy of the JP, the five basic principles, which we refer to as the ''five pillars'', were established as follows: 1) Enhancing listed articles by prioritizing 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 domestically and internationally. 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 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 19th Edition (the Japanese edition) was set as April 2026.

JP drafts are discussed in the following committees that were established in the Pharmaceuticals and Medical Devices Agency: Expert Committee; Committee on Chemicals; Committee on Antibiotics; Committee on Biologicals; Committee on Crude Drugs; Committee on Pharmaceutical Excipients; Committees 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 Expert Committee, Committee on Biologicals, Committee on Pharmaceutical Excipients and Committee on Drug Formulation.

The committees initiated deliberations on the several revisions. Draft revisions covering subjects in General Tests and Monographs, Ultraviolet-visible Reference Spectra, Infrared Reference Spectra, for which discussions were finished between July 2022 and November 2023, were prepared for a supplement to the JP 18.

Numbers of discussions in the committees to prepare the supplement drafts were as follows: Expert Committee (14, including working group); Committee on Chemicals (17); Committee on Antibiotics (5); Committee on Biologicals (12, including working group); Committee on Crude Drugs (11); Committee on Pharmaceutical Excipients (9, including working group); Committee on Physico-Chemical Methods (6); Committee on Drug Formulation (15, including working group); Committee on Physical Methods (4); Committee on Biological Methods (5); Committee on Nomenclature for Pharmaceuticals (4); Committee on International Harmonization (4); 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 Kansai Pharmaceutical Manufacturers Association, the Asian Society of Innovative Packaging Technology, the Pharmacopeia and CMC Committee of the Pharmaceutical Manufacturers' 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 Natural Medicines Association, 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 Analytical Instruments Manufacturers' Association, the Japan Association of Vaccine Industries and the Association of Membrane Separation Technology of Japan.

The draft revisions were examined by the Committee on JP in January 2024, followed by the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) in March 2024, and then submitted to the Minister of Health, Labour and Welfare. In the committee on JP, HASHIDA Mitsuru took the role of the chairman from January 2011 to December 2020, OHTA Shigeru from January 2021 to December 2022, and GODA Yukihiro from January 2023 to June 2024.

In consequence of this revision, the Supplement II to the JP 18th Edition carries 2048 articles, owing to the addition of 13 articles and the deletion of 7 articles.

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

1. The Supplement II to the JP 18th Edition comprises the following items, in order: Notification of MHLW; Contents; Preface; 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, Supplement I and Supplement II.

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

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

(1) English title
(2) Commonly used name(s)
(3) Latin title (only for crude drugs)
(4) Title in Japanese
(5) Structural formula or empirical formula
(6) Molecular formula and molecular mass
(7) Chemical name
(8) 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, Loss on 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 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) Cyanide
(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) Enantiomer
(40) Diastereomer
(41) Polymer
(42) Residual solvent
(43) Other impurities
(44) Residue on evaporation
(45) Readily carbonizable substances

7. The following item was newly added to General Tests, Processes and Apparatus:
(1) 3.07 Measurement of the Diameter of Particles Dispersed in Liquid by Dynamic Light Scattering

8. The following items in General Tests, Processes and Apparatus were revised:
(1) 2.03 Thin-layer Chromatography
(2) 2.46 Residual Solvents
(3) 2.66 Elemental Impurities
(4) 3.01 Determination of Bulk Density
(5) 4.02 Microbial Assay for Antibiotics
(6) 5.01 Crude Drugs Test
(7) 9.01 Reference Standards
(8) 9.41 Reagents, Test Solutions
(9) 9.42 Solid Supports/Column Packings for Chromatography
(10) 9.62 Measuring Instruments, Appliances

9. The following Reference Standards were newly added:
(1) Aripiprazole RS
(2) Aripiprazole *N*- Oxide for System Suitability RS
(3) Febuxostat RS
(4) Febuxostat Related Substance A for System Suitability RS
(5) Febuxostat Related Substance B for System Suitability RS
(6) Goserelin Acetate RS
(7) Goserelin Acetate Related Substance for System Suitability RS
(8) Lornoxicam RS
(9) Oxaliplatin RS
(10) Oxaliplatin Related Substance B Dinitrate for Purity RS
(11) Residual Solvents Class 2D RS
(12) Residual Solvents Class 2E RS

(13) Tolvaptan RS

10. The following Reference Standards were deleted.
(1) Amlexanox RS
(2) Cefadroxil RS
(3) Tolbutamide RS

11. The following Reference Standards were deleted from the list of ''9.01 (2) The reference standards which are prepared by National Institute of Infectious Diseases'' and added to the list of ''9.01 (1) The reference standards which are prepared by those who have been registered to prepare them by the Minister of Health, Labour and Welfare, according to the Ministerial ordinance established by the Minister separately'':
(1) Cefcapene Pivoxil Hydrochloride RS
(2) Cefditoren Pivoxil RS
(3) Cefoperazone RS
(4) Cefozopran Hydrochloride RS
(5) Cefpodoxime Proxetil RS
(6) Ceftazidime RS

12. The following substances were newly added to the Official Monographs:
(1) Aripiprazole
(2) Febuxostat
(3) Febuxostat Tablets
(4) Gefitinib Tablets
(5) Goserelin Acetate
(6) Lithium Carbonate Tablets
(7) Lornoxicam
(8) Lornoxicam Tablets
(9) Oxaliplatin
(10) Oxaliplatin Injection
(11) Tolvaptan
(12) Tolvaptan Tablets
(13) Shin'iseihaito Extract

13. The following monographs were revised:
(1) Aluminum Monostearate
(2) Beclometasone Dipropionate
(3) Calcium Stearate
(4) Carmellose Calcium
(5) Citicoline
(6) Clindamycin Phosphate
(7) Clonidine Hydrochloride
(8) Cyclophosphamide Hydrate
(9) Dextran 70
(10) Disodium Edetate Hydrate
(11) Glucose
(12) Glycerin
(13) Concentrated Glycerin
(14) Low Substituted Hydroxypropylcellulose
(15) Hypromellose
(16) Loxoprofen Sodium Hydrate
(17) Magnesium Silicate
(18) Magnesium Stearate
(19) Meglumine
(20) Methylcellulose
(21) Paraffin
(22) Liquid Paraffin
(23) Light Liquid Paraffin
(24) Polyoxyl 40 Stearate
(25) Propylene Glycol
(26) Light Anhydrous Silicic Acid
(27) Sodium Bisulfite
(28) Dried Sodium Carbonate
(29) Sodium Carbonate Hydrate
(30) Sodium Iodide
(31) Sodium Polystyrene Sulfonate
(32) Sodium Pyrosulfite
(33) Dried Sodium Sulfite
(34) Sorbitan Sesquioleate
(35) Talc
(36) Teceleukin (Genetical Recombination)
(37) White Soft Sugar
(38) Akebia Stem
(39) Artemisia Capillaris Flower
(40) Artemisia Leaf
(41) Asparagus Root
(42) Bearberry Leaf
(43) Belladonna Extract
(44) Boiogito Extract
(45) Cardamon
(46) Chrysanthemum Flower
(47) Citrus Unshiu Peel
(48) Codonopsis Root
(49) Coix Seed
(50) Powdered Coix Seed
(51) Cornus Fruit
(52) Digenea
(53) Epimedium Herb
(54) Gentian
(55) Powdered Gentian
(56) Goshajinkigan Extract
(57) Hachimijiogan Extract
(58) Hedysarum Root
(59) Jujube
(60) Leonurus Herb
(61) Lindera Root
(62) Lonicera Leaf and Stem
(63) Loquat Leaf
(64) Lycium Fruit
(65) Mentha Herb

(66) Nelumbo Seed
(67) Nutmeg
(68) Nux Vomica Extract
(69) Nux Vomica Extract Powder
(70) Nux Vomica Tincture
(71) Ophiopogon Root
(72) Perilla Herb
(73) Pogostemi Herb
(74) Polygonatum Rhizome
(75) Processed Aconite Root
(76) Pueraria Root
(77) Quercus Bark
(78) Rehmannia Root
(79) Rhubarb
(80) Powdered Rhubarb
(81) Royal Jelly
(82) Salvia Miltiorrhiza Root
(83) Sappan Wood
(84) Schisandra Fruit
(85) Scopolia Extract
(86) Scopolia Extract Powder
(87) Scopolia Extract and Carbon Powder
(88) Compound Scopolia Extract and Diastase Powder
(89) Scopolia Extract and Ethyl Aminobenzoate Powder
(90) Senna Leaf
(91) Shimbuto Extract
(92) Sweet Hydrangea Leaf
(93) Tokishakuyakusan Extract
(94) Uncaria Hook
(95) Yokukansankachimpihange Extract

14. The following monographs were deleted:
(1) Amlexanox
(2) Amlexanox Tablets
(3) Cefadroxil
(4) Cefadroxil Capsules
(5) Cefadroxil for Syrup
(6) Tolbutamide
(7) Tolbutamide Tablets

15. The following articles were newly added to Ultraviolet-visible Reference Spectra:
(1) Aripiprazole
(2) Febuxostat
(3) Lornoxicam
(4) Oxaliplatin
(5) Tolvaptan

16. The following articles were newly added to Infrared Reference Spectra:
(1) Aripiprazole
(2) Cyclophosphamide Hydrate
(3) Disodium Edetate Hydrate
(4) Febuxostat
(5) Oxaliplatin
(6) Tolvaptan
(7) Lornoxicam

17. The following article in Infrared Reference Spectra was deleted:
(1) Clindamycin Phosphate

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Supplement II to The Japanese Pharmacopoeia Eighteenth Edition

GENERAL TESTS, PROCESSES AND APPARATUS

2.03 Thin-layer Chromatography

Change the 1. Instruments and equipment and below as follows:

1. Instruments and equipment

Generally, the following instruments and equipment are used.

(i) Thin-layer plate: A smooth, uniformly thick glass plate coated in advance with the powder of a solid support for thin-layer chromatography specified in General Tests *<9.42>*. In the case where requirements specified in a monograph are met, a thin-layer plate with a preadsorbent zone or a thin-layer plate using a rigid aluminum polyester sheet or the like as a support can be used instead of a glass plate. Preserve thin-layer plates protecting from moisture. If necessary, dry them by heating at a constant temperature between 105°C and 120°C for 30 to 60 minutes before use.

(ii) Developing chamber: Generally, a flat-bottomed chamber or a twin trough chamber made of inert, transparent material and having a lid is used. A developing chamber should be of a size appropriate for the size of a thin-layer plate.

(iii) Device for coloring: A glass atomizer, an electric atomizer, etc. are used for spraying a coloring reagent. In order to visualize components to be tested, thin-layer plates may be heated with a heating device after spraying a coloring reagent. Generally, using a hot plate set at a constant temperature or a thermostatic oven, heat a thin-layer plate evenly. A developing chamber, a desiccator, etc. are used for coloring by immersion and visualization by exposure to evaporated reagent vapor (fumigation).

(iv) Detection device: A light source capable of irradiating visible light, ultraviolet light with main wavelengths of 254 nm and 365 nm and equipped with corresponding filters, and a dark box. Alternatively, a dark room equipped with these functions. The light source should meet requirements for tests prescribed in monographs. The suitability of the light source should be confirmed with respect to radiation intensity when the light source is changed or as necessary. Generally, in the case of irradiation with a main wavelength of 254 nm to a thin-layer plate containing a fluorescent indicator, confirm whether the thin-layer plate produce a green fluorescence. Also, in the case of irradiation with a main wavelength of 365 nm, for example, confirm whether blue-white fluorescence is emitted by spotting 2 μL of a methanol solution of 5 μg/mL scopoletin for thin-layer chromatography on a thin-layer plate, for example. A high-intensity light source with stable radiation intensity at around 365 nm within the ultraviolet wavelength range includes lamps having a narrow line spectrum at 365 nm and lamps having a line spectrum at 366 nm (within a range from 364 to 367 nm) with a more intense emission signal. Even though a light source and a wavelength described in specification differs depending on a lamp used, a light source lamp with a wavelength of 366 nm can be treated as a light source for ultraviolet (main wavelength: 365 nm) irradiation.

(v) Chromatogram recording device: A photographing device to be added to the detection device is used for taking photographs to be recorded, and requires adequate sensitivity, resolution and reproducibility to perform tests. Photographs are taken by a camera and recorded/stored in a format of film or electronic image. In the case of recording color tones of chromatograms detected under a visible light, it is preferable to take the pictures of color samples for reference concurrently, and an image scanner with sufficient resolution can also be used. Further, it should be noted that color tones identified visually and those recorded are different in some cases when recording fluorescent spots caused by irradiation with a wavelength of 365 nm. A TLC scanning device using densitometry measures absorption due to ultraviolet light, absorption due to visible light, or fluorescence due to excitation light on a developed thin-layer plate, converts the obtained chromatogram into peak information, and records/stores it. Data converted to peak information is analyzed quantitatively.

2. Procedure

Unless otherwise specified, generally proceed by the following methods.

(i) Spotting of sample solutions: Prepare a sample solution and a standard solution specified in a monograph, and spot specified volumes on the starting line of a thin-layer plate. Designate a line about 20 mm distant from the bottom of the thin-layer plate as the starting line, and designate the positions where the sample solution and standard solution are spotted at least 10 mm away from both sides of the plate as the original points. Spot a solution in circular shape of 2 to 6 mm in diameter or in belt-like shape of 4 to 10 mm in width at points separated by more than 10 mm using a capillary tube of a constant volume, a microsyringe or a micropipet, and air-dry. If the requirements specified in the monograph are met, the position of the starting line and the distance between the original points may be changed.

(ii) Development with developing solvent: Generally, components are separated in a developing chamber saturated with a vaporized developing solvent according to the following method.

Place a filter paper along with the inside wall of a container containing a small amount of the developing solvent

beforehand, and wet the filter paper with the developing solvent. Then, the developing solvent is placed up to about 10 mm in height from the bottom. Seal the container closely, allow it to stand for 1 hour at ordinary temperature, and saturate the developing chamber with the vaporized developing solvent. If a saturated developing chamber prepared under conditions other than those described here is used, it is specified separately. Place the thin-layer plate in the container, avoiding contact with the inside wall except for the top of the plate, confirm that spots are not immersed in the developing solvent, seal the container tightly, and develop them at ordinary temperature. Allow to stand until the developing solvent has risen to a required developing distance, remove the plate, and air-dry. In addition, the starting line (original point) before development and the developing solvent front after development are marked.

(iii) Visualization and detection: After development, visualize the spots of components to be tested on a plate, and confirm their color tones and *R*f values. Generally, pull out a thin-layer plate after development, air-dry, and the detection of spots separated on the plate is visually confirmed directly or by uniformly spraying a coloring reagent on the components to be tested on the plate to react. In the case of components to be tested having ultraviolet absorptivity, detection is performed using a thin-layer plate containing a fluorescent agent (fluorescent indicator) by ultraviolet irradiation with a main wavelength of 254 nm. A fluorescent indicator contained in the thin-layer plate emits greenish fluorescence excited by irradiation with a main wavelength of 254 nm. The spots of components to be tested reduce radiation light emission by absorbing irradiation light to reduce the excitation of the fluorescent indicator resulting in observation as black color (dark purple) spots. The spot of components to be tested, which is excited under ultraviolet irradiation and emit fluorescence on itself, is excited and emit fluorescence on the thin-layer plate by ultraviolet irradiation with a main wavelength of 365 nm even without using a fluorescent indicator. Spraying, immersion and fumigation of an appropriate coloring reagent can visualize the spots of components to be tested. In the case of some coloring reagents, such visualization may be performed by subsequent heating after spraying reagents. In some cases, characteristic fluorescence may be produced by irradiation with a main wavelength of 365 nm after spraying or after spraying and heating as well. Development operation and visualization by a coloring reagent shall be performed in an apparatus such as a draft chamber in which solvent vapor is efficiently removed with sufficient air ventilation.

3. Identification and purity tests

When using this test method for an identification test, it is confirmed in general that the color tone and *R*f value of components to be tested in a sample solution is equal to those in a standard solution. Alternatively, it is also possible to identify by the patterns of spots. Semi-quantitative identification of components to be tested can also be made by visually comparing the size and intensity of spots with the same color tone and *R*f value in a chromatogram obtained by applying the same volume of the sample solution and standard solution.

In the case of using this test method for a purity test, a standard solution with a concentration corresponding to the limit of impurities in a sample solution is used in general, and purity is confirmed by whether no spots of components to be tested derived from the sample solution is detected or whether the intensity of the spot of impurities is not more intense than that of the standard solution.

4. Points to consider in the changing test conditions for identification tests

Among tests prescribed in monographs, developing distance, saturation time, development solvent composition, coloring reagent composition, amount of spotting (limited to reduction), heating temperature and duration of a thin-layer plate can be partially changed in identification tests using a standard solution containing components to be tested, provided that the specificity of spots is equal to or better than the prescribed method by the appropriate verification of the analytical performance. However, such semi-quantitative identification tests that use the sizes and intensities of spots as the criteria for judgement are excluded. In the identification tests of crude drugs, etc., which do not use a standard solution containing components to be tested, developing distance, amount of spotting (limited to reduction), heating temperature and duration of a thin-layer plate can be partially changed, provided that the specificity of spots is demonstrated to be equal to or better than the prescribed method by the appropriate verification of the analytical performance and at the same time the *R*f value and color tone prescribed in a monograph is observed.

5. Terminology

The terminology used conforms to the definition in Chromatography <*2.00*>.

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 manufacture 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 manufacture. 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 manufacture 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 manufacture 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 the limit, no testing of the drug product for residual solvents needs to be considered. If, however, the calculated level is above the limit, 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 as a pharmaceutically acceptable daily intake of residual solvents. Residual solvents regulated by this test method are placed into one of three classes as follows based on their possible risk to human health:

(i) Class 1 solvents (Solvents to be avoided in the 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 human; 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 drug substances, excipients, or drug 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 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 the 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 summing 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. If only Class 3 solvents are present, a nonspecific method such as loss on drying may be used. The analytical method should be validated adequately.

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 the assessment of the safety data.

Table 2.46-1 Class 1 solvents (solvents that should be avoided in the manufacture of pharmaceuticals).

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

5.2. Solvents to be regulated in pharmaceuticals

Solvents in Table 2.46-2 should be regulated in pharmaceuticals 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.

Table 2.46-2 Class 2 Solvents (residual amount should be limited in pharmaceuticals)

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
Cyclopentyl methyl ether	15.0	1500
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
Methyl isobutyl ketone	45	4500
N-Methylpyrrolidone	5.3	530
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
t-Butyl alcohol	35	3500
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

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.

Table 2.46-3 Class 3 solvents (limited by GMP or other quality- based requirements in pharmaceuticals)

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	2-Methyl-1-propanol
Dimethylsulfoxide	2-Methyltetrahydrofuran
Ethanol	Pentane
Ethyl acetate	1-Pentanol
Diethyl ether	1-Propanol
Ethyl formate	2-Propanol
Formic acid	Propyl acetate
	Triethylamine

5.4 Solvents for which no adequate toxicological data was found

The following solvents (Table 2.46-4) may also be related to the manufacture of drug substances, excipients, 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 2.46-4 Solvents for which no adequate toxicological data was found.

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

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 drug products 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 sample is not available. When the information about the presence of specific residual solvents is 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.

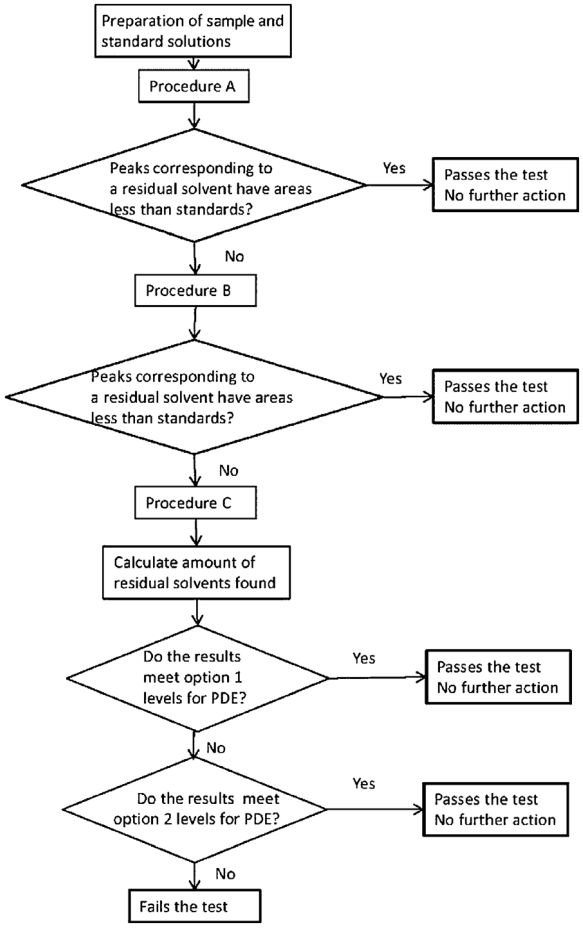


Fig. 2.46-1 Flow chart for the identification of residual solvents and the application of limit and qualification tests

1.1. Water-soluble samples

1.1.1. Procedure A

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

Class 1 standard stock solution: To about 9 mL of dimethylsulfoxide add exactly 1 mL of Residual Solvents Class 1 RS, 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 the Class 1

standard stock solution in an appropriate head space vial containing exactly 5 mL of water, apply the stopper, cap, and shake.

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

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

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

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

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

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

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

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

Sample stock solution: Dissolve 0.25 g of the sample in water, and add water to make exactly 25 mL.

Sample solution: Pipet 5 mL of the sample stock solution in an appropriate headspace vial, add exactly 1 mL of water, apply the stopper, cap, and shake.

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

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 with 6% cyanopropylphenyl-94% dimethyl silicone polymer for gas chromatography in 1.8 μm (or 3.0 μm) thickness.

Column temperature: Maintain the temperature at 40°C for 20 minutes after injection, raise 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 the Class 1 standard solution and Class 1 system suitability test solution under the above operating conditions, the SN ratio of the peak of 1,1,1-trichloroethane obtained with the Class 1 standard solution is not less than 5, and the SN ratio of each peak obtained with the Class 1 system suitability test solution is not less than 3 respectively.

System performance: When the procedure is run with the Class 2 standard solution A or the solution for system suitability test 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 for System Suitability RS (1 in 100) in an appropriate headspace vial, add exactly 5 mL of water, apply the stopper, cap, mix, and use this solution as the solution for system suitability test.

System repeatability: When the test is repeated 6 times with the 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 the Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, Class 2 standard solution C, Class 2 standard solution D, Class 2 standard solution E and sample 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 sample solution is greater than or equal to a corresponding peak in either the Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, Class 2 standard solution C, Class 2 standard solution D or Class 2 standard solution E, 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 the Class 1 standard solution, proceed to Procedure B to verify the identity of the peak; otherwise the sample 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 test solution, Class 2 standard stock solutions A, B, C, D and E, Class 2 standard solutions A, B, C, D and E, sample stock solution and sample 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 with polyethylene glycol for gas chromatography in 0.25 μm thickness.

Column temperature: Maintain the temperature at 50°C for 20 minutes after injection, raise 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 the Class 1 standard solution and Class 1 system suitability test solution under the above operating conditions, the SN ratio of the peak of benzene obtained with the Class 1 standard solution is not less than 5, and the SN ratio of each peak obtained with the Class 1 system suitability test solution is not less than 3, respectively.

System performance: When the procedure is run with the Class 2 standard solution A or the solution for system suitability test 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 for System Suitability RS (1 in 100) in an appropriate headspace vial, add exactly 5 mL of water, apply the stopper, cap, mix, and use this solution as the solution for system suitability test.

System repeatability: When the test is repeated 6 times with the 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 the Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, Class 2 standard solution C, Class 2 standard solution D, Class 2 standard solution E and sample solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If the peak response(s) of the peak(s) in the sample solution is/are greater than or equal to a corresponding peak(s) in either the Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, Class 2 standard solution C, Class 2 standard solution D or Class 2 standard solution E, proceed to Procedure C to quantify the peak(s); otherwise the sample 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.

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 the 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 the standard stock solution in an appropriate headspace vial, add exactly 5 mL of water, apply the stopper, cap, and shake.

Sample stock solution: Weigh accurately about 0.25 g of the sample, dissolve in water, and add water to make exactly 25 mL.

Sample solution: Pipet 5 mL of the sample stock solution in an appropriate headspace vial, add exactly 1 mL of water, and apply the stopper, cap, and shake.

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

Operating conditions and system suitability fundamentally follow the procedure A. Test for required detectability is unnecessary, and use the standard solution instead of Class 1 standard solution for system repeatability. 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.

Perform the test (following one of the headspace operating parameters described in Table 2.46-5) with equal volumes of about 1.0 mL each of the standard solution, sample solution, and spiked sample solution, and measure the peak areas for the major residual solvents. Calculate the amount of each residual solvent by the formula:

$$\text{Amount of residual solvent (ppm)} = 5\,(C/M)\{A_T/(A_S - A_T)\}$$

C: Concentration (μg/mL) of the reference standard in the standard stock solution

M: Amount (g) of the sample taken to prepare the sample stock solution

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

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

1.2. Water-insoluble samples

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: To about 80 mL of *N*,*N*-dimethylformamide, add 1 mL of Residual Solvents Class 1 RS, 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 diluent prepared from Residual Solvents Class 1 RS and use it for preparation of the Class 1 system suitability test 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 the Class 1 standard stock solution in an appropriate head space vial containing exactly 5 mL of water, apply the stopper, cap, and shake.

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

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

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

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

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

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

Sample stock solution: Dissolve 0.5 g of the sample in *N*,*N*-dimethylformamide, and add *N*,*N*-dimethylformamide to make exactly 10 mL.

Sample solution: Pipet 1 mL of the sample stock solution in an appropriate headspace vial, add exactly 5 mL of water, apply the stopper, cap, and shake.

Class 1 system suitability test solution: Pipet 5 mL of the sample 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, apply the stopper, cap, and shake.

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 with 6% cyanopropylphenyl-94% dimethyl silicone polymer for gas chromatography in 3.0 μm thickness.

Column temperature: Maintain the temperature at 40°C for 20 minutes after injection, raise 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 the Class 1 standard solution and Class 1 system suitability test solution under the above operating conditions, the SN ratio of the peak of 1,1,1-trichloroethane obtained with the Class 1 standard solution is not less than 5, and the SN ratio of each peak obtained with the Class 1 system suitability test solution is not less than 3, respectively.

System performance: When the procedure is run with the Class 2 standard solution A or the solution for system suitability test 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 for System Suitability RS (1 in 100) in an appropriate headspace vial, add exactly 5 mL of water, apply the stopper, cap, and shake, and use this solution as the solution for system suitability test.

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

Separately inject (following headspace operating parameter sets described in column 3 of Table 2.46-5) equal volumes of headspace (about 1.0 mL) of the Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, Class 2 standard solution C, Class 2 standard solution D, Class 2 standard solution E and the sample 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 sample solution is greater than or equal to a corresponding peak in either the Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, Class 2 standard solution C, Class 2 standard solution D or Class 2 standard solution E, 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 sample 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. Dimethylsulfoxide may be substituted as an alternative solvent to *N*,*N*-dimethylformamide.

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

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

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 the Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, Class 2 standard solution C, Class 2 standard solution D, Class 2 standard solution E and sample solution into the chromatograph, record the chromatograms, and measure the responses for the major peaks. If the peak response(s) of the peak(s) in the sample solution is/are greater than or equal to a corresponding peak(s) in either the Class 1 standard solution, Class 2 standard solution A, Class 2 standard solution B, Class 2 standard solution C, Class 2 standard solution D or Class 2 standard solution E, proceed to Procedure C to quantify the peak; otherwise, the sample 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. Dimethylsulfoxide may be substituted as an alternative solvent to *N*,*N*-dimethylformamide.

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 the 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, apply the stopper, cap, and shake.

Sample stock solution: Weigh accurately about 0.5 g of the sample, and add *N*,*N*-dimethylformamide to make exactly 10 mL.

Sample solution: Pipet 1 mL of the sample stock solution in an appropriate headspace vial, add exactly 5 mL of water, apply the stopper, cap, and shake.

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

Operating conditions and system suitability fundamentally follow the procedure A. Test for required detectability is unnecessary, and use the standard solution instead of the Class 1 standard solution for system repeatability. 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.

Perform the test (following one of the headspace operating parameter sets described in Table 2.46-5) with about 1.0 mL each of the standard solution, sample solution, and spiked sample solution, and measure the responses for the major residual solvents. Calculate the amount of each residual solvent by the formula:

$$\text{Amount of residual solvent (ppm)} = 10\,(C/M)\,\{A_T/(A_S - A_T)\}$$

C: Concentration (μg/mL) of the reference standard in the standard stock solution

M: Amount (g) of the sample taken to prepare the sample stock solution

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

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

1.3. Headspace operating parameters and other considerations

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

This test method describes the analytical methods using the headspace gas chromatography. The following Class 2 residual solvents are not readily detected by the headspace injection conditions because of the low sensitivity: *N*,*N*-dimethylacetamide, 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 this test method, *N*,*N*-dimethylformamide is used as a solvent. As not only 7 solvents described above but the solvent is not included in the Residual Solvents Class 2A RS, Residual Solvents Class 2B RS, Residual Solvents Class 2C RS, Residual solvents class 2D RS and/or Residual solvents class 2E 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 1 mL 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 sample 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 (ethylbenzene, *m*-xylene, *p*-xylene, *o*-xylene))

(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 Class 2C RS (Methyl isobutyl ketone)

(v) Residual Solvents Class 2D RS (*t*-butyl alcohol)

(vi) Residual Solvents Class 2E RS (cyclopentyl methyl ether)

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

2.66 Elemental Impurities

Change 3. The PDEs for Elemental Impurities for Oral, Parenteral and Inhalation Routes of Administration, and Element Classification, 4. Risk Assessment and Control of Elemental Impurities, and 5. Converting between PDEs and Concentration Limits in I. Control of Elemental Impurities in Drug Products as follows:

3. The PDEs for Elemental Impurities for Oral, Parenteral, Inhalation and Cutaneous Routes of Administration, and Element Classification Based on the Risk

The PDEs of elemental impurities established for preparations for oral, parenteral, inhalation and cutaneous routes of administration are shown in Table 2.66-1. In the case of elements which have the PDE values for preparations for cutaneous application and the Cutaneous and Transcutaneous Concentration Limit (CTCL), it is necessary to conform to the both limits. If the PDEs for the other administration route are necessary, generally consider the oral PDE as a starting point in the establishment, and assess if the elemental impurity is expected to have local effects when administered by the intended route of administration.

Parenteral drug products with maximum daily volumes up to 2 L may use the maximum daily volume to calculate permissible concentrations from PDEs. For products whose daily volumes or general clinical practice may exceed 2 L (e.g., Isotonic Sodium Chloride Solution, Glucose Injection, total parenteral nutrition, solutions for irrigation), a 2-L volume may be used to calculate permissible concentrations from PDEs.

Because the maximum total daily dose of a preparation for cutaneous application is not always clearly claimed, a prerequisite for the product risk assessment is a justified estimation of a worst-case exposure to the elemental impurities that can form the basis for the assessment. Since the CTCL is calculated based on a once-daily application, the acceptable concentration may need to be modified according to the maximum number of applications per day and following an assessment of various factors such as retention time of the drug product. Although the risk of sensitization does not depend on the dose per application, it may increase with multiple daily applications to the same area.

As shown in Table 2.66-1, elemental impurities are divided into three classes based on their toxicity (PDE) and likelihood of occurrence in the drug product. The likelihood of occurrence is derived from several factors, such as probability of use in pharmaceutical processes, elemental impurities in materials used in pharmaceutical processes, the observed natural abundance and environmental distribution of the element.

Class 1: The elements, As, Cd, Hg, and Pb, are classified as this category and are human toxicant elements. As these elements are limited in the manufacture of pharmaceuticals, they are rarely used. Their presence in drug products typically comes from commonly used materials such as mined excipients. These four elements require evaluation during the risk assessment, across all sources and routes of administration having possibility of contamination. It is not expected that all components will require testing for Class 1 elemental impurities; testing should only be applied when the risk assessment identifies it as the appropriate control to ensure that the PDE will be met.

Class 2: Elemental impurities classified as Class 2 have lower toxicity than the elements in Class 1, and are route-dependent human toxicants. These elements are further divided in 2A and 2B based on their relative likelihood of occurrence in drug products. The class 2A elements are Co, Ni and V, which are known to exist naturally. These elements have relatively high probability of occurrence in drug products, and thus require evaluation during the risk assessment, across all sources and routes of administration for potential elemental impurities. The Class 2B elements are Ag, Au, Ir, Os, Pd, Pt, Rh, Ru, Se and Tl. Because the Class 2B elements have low probability of their existence in natural, they may be excluded from the risk assessment unless they are intentionally added during the manufacture of drug substances, excipients or other components of the drug product.

Table 2.66-1 PDEs and CTCLs for Elemental Impurities

Element	Class	Oral PDE (μg/day)	Parenteral PDE (μg/day)	Inhalation PDE (μg/day)	Cutaneous PDE (μg/day)	Cutaneous CTCL in case of sensitization (μg/day)
Cd	1	5	2	3	20	—
Pb	1	5	5	5	50	—
As	1	15	15	2	30	—
Hg	1	30	3	1	30	—
Co	2A	50	5	3	50	35
V	2A	100	10	1	100	—
Ni	2A	200	20	6	200	35
TI	2B	8	8	8	8	—
Au	2B	300	300	3	3000	—
Pd	2B	100	10	1	100	—
Ir	2B	100	10	1	*	—
Os	2B	100	10	1	*	—
Rh	2B	100	10	1	*	—
Ru	2B	100	10	1	*	—
Se	2B	150	80	130	800	—
Ag	2B	150	15	7	150	—
Pt	2B	100	10	1	100	—
Li	3	550	250	25	2500	—
Sb	3	1200	90	20	900	—
Ba	3	1400	700	300	7000	—
Mo	3	3000	1500	10	15000	—
Cu	3	3000	300	30	3000	—
Sn	3	6000	600	60	6000	—
Cr	3	11000	1100	3	11000	—

* In the case of Ir, Os, Rh, and Ru, data are insufficient for setting PDE values for preparations for cutaneous application. For these elements, apply the PDE values of Pd for the relevant routes.

Class 3: The elements in this class have relatively low toxicities by the oral route of administration, and their oral PDEs are more than 500 μg/day. The Class 3 elements are Ba, Cr, Cu, Li, Mo, Sb and Sn. For oral routes of administration, unless these elements are intentionally added, they do not need to be considered during the risk assessment. For parenteral and inhalation products, the potential for inclusion of these elemental impurities should be evaluated even in the case where they are not intentionally added, unless the route specific PDE is above 500 μg/day.

4. Risk Assessment and Control of Elemental Impurities

The principle of quality risk management should be considered in controls for elemental impurities in drug products, and the risk assessment should be based on scientific knowledge and principles. The risk assessment would be focused on assessing the levels of elemental impurities in a drug product in relation to the PDEs. Useful information for this risk assessment includes measured data of drug products and components, measured data and the risk assessment result supplied by manufactures of drug substance and excipient, and data available in published literature, but is not limited to them.

The risk assessment should be performed depending on the level of risk, and do not always require a formal risk management process. The use of informal risk management processes may also be considered acceptable.

4.1. General Principles

The risk assessment process consists of the following three steps.

1) Identify sources of elemental impurities during manufacturing process of the drug product.

2) Evaluate the presence of a particular elemental impurity in the drug product by determining the observed or predicted level of the impurity and comparing with the established PDE.

3) Summarize the risk assessment, and confirm whether controls built into the manufacturing process are sufficient or not. Identify additional controls to be considered to limit elemental impurities in the drug product.

In many cases, the steps are considered simultaneously. The risk assessment may be iterated to develop a final approach to ensure the elemental impurities do not exceed the PDE certainly.

4.2. Sources of Elemental Impurities

In considering the production of a drug product, there are broad categories of potential sources of elemental impurities.

• Residual impurities resulting from elements intentionally added (e.g., metal catalysts) in the manufacture of the drug substance, excipients or other components. In the risk assessment of the drug substance, the potential for inclusion of elemental impurities in the drug product should be considered.

• Elemental impurities that are not intentionally added but are potentially present in the drug substance, water or excipients used in the manufacture of the drug product.

• Elemental impurities that are potentially introduced into the drug substance and drug product from manufacturing equipment.

• Elemental impurities that have the potential to be leached into the drug substance and drug product from container closure systems.

During the risk assessment, the potential contributions from each of these sources should be considered to determine the overall contribution of elemental impurities to the drug product.

4.3. Identification of Potential Elemental Impurities

Elemental impurities that may derived from intentionally added catalysts and inorganic reagents: If any element is intentionally added, it should be considered in the risk assessment.

Elemental impurities that may be present in drug substances or excipients: While not intentionally added, some elemental impurities may be present in some drug substances or excipients. The possibility for inclusion of these elements in the drug product should be reflected in the risk assessment.

Potential elemental impurities derived from manufacturing equipment: The contribution of elemental impurities from this source may be limited and the subset of elemental impurities that should be considered in the risk assessment

depends on the manufacturing equipment used in the production of the drug product. The specific elemental impurities of concern should be assessed based on the knowledge of the composition of the components of the manufacturing equipment that come in contact with components of the drug product. The risk assessment of this source of elemental impurities can be utilized for many drug products using similar process trains or processes.

In general, the processes used to prepare a given drug substance are considerably more aggressive than processes used in preparing the drug product when assessed relative to the potential to leach or remove elemental impurities from manufacturing equipment. Contributions of elemental impurities from drug product manufacturing equipment would be expected to be lower than that for the drug substance. However, when this is not the case based on knowledge or understanding of the processes, the potential for incorporation of elemental impurities from the drug product manufacturing equipment should be considered in the risk assessment (e.g., hot melt extrusion).

Elemental impurities leached from container closure systems: The identification of potential elemental impurities that may be introduced from container closure systems should be based on a scientific understanding of likely interactions between a particular dosage form and its packaging. When a review of the materials of the container closure system demonstrates that it does not contain elemental impurities, no additional risk assessment needs to be performed. Because the probability of elemental leaching into solid dosage forms is minimal, further risk assessment is not necessary. For liquid and semi-solid dosage forms there is a higher probability that elemental impurities could leach from the container closure system during the shelf-life of the drug product. Studies to understand potential elemental impurities leached from the container closure system (after washing, sterilization, irradiation, etc.) should be performed.

Factors that should be considered for liquid and semi-solid dosage forms are shown as follows, but are not limited to them.

• Hydrophilicity/hydrophobicity, Ionic content, pH, Temperature (cold chain vs room temperature and processing conditions), Contact surface area, Container/material composition, Terminal sterilization, Packaging process, Material sterilization, Duration of storage

Table 2.66-2 provides recommendations for inclusion of elemental impurities in the risk assessment. This table can be applied to all sources of elemental impurities in the drug product.

4.4. Evaluation

As the potential elemental impurity identification process is concluded, there are following two possible outcomes.

1) The risk assessment process does not identify any potential elemental impurities.

2) The risk assessment process identifies one or more potential elemental impurities. For any elemental impurities identified in the process, the risk assessment should consider possibility of any source of the identified elemental impurities.

Table 2.66-2 Elements to be Considered in the Risk Assessment

Element	Class	If intentionally added (all routes)	If not intentionally added			
			Oral	Parenteral	Inhalation	Cutaneous
Cd	1	○	○	○	○	○
Pb	1	○	○	○	○	○
As	1	○	○	○	○	○
Hg	1	○	○	○	○	○
Co	2A	○	○	○	○	○
V	2A	○	○	○	○	○
Ni	2A	○	○	○	○	○
Tl	2B	○	×	×	×	×
Au	2B	○	×	×	×	×
Pd	2B	○	×	×	×	×
Ir	2B	○	×	×	×	×
Os	2B	○	×	×	×	×
Rh	2B	○	×	×	×	×
Ru	2B	○	×	×	×	×
Se	2B	○	×	×	×	×
Ag	2B	○	×	×	×	×
Pt	2B	○	×	×	×	×
Li	3	○	×	○	○	×
Sb	3	○	×	○	○	×
Ba	3	○	×	×	○	×
Mo	3	○	×	×	○	×
Cu	3	○	×	○	○	×
Sn	3	○	×	×	○	×
Cr	3	○	×	×	○	×

○: necessary ×: unnecessary

During the risk assessment, a number of factors that can influence the level of the potential elemental impurity in the drug product should be considered.

4.5. Summary of Risk Assessment Process

The risk assessment is summarized by reviewing relevant products or component specific data combined with information and knowledge gained across products or processes to identify the significant probable elemental impurities that may be observed in the drug product.

The significance of the observed or predicted level of the elemental impurity should be considered in relation to the PDE of the elemental impurity. As a measure of the significance of the observed elemental impurity level, a control threshold is defined as a level that is 30% of the established PDE (and CTCLs for Co and Ni). The control threshold may be used to determine if additional controls may be required.

If the total elemental impurity level from all sources in the drug product is expected to be consistently less than 30% of the PDE, additional controls are not required, provided adequate controls on elemental impurities are demonstrated by the appropriate assessment of the data.

If the risk assessment fails to demonstrate that an elemental impurity level is consistently less than the control threshold, controls should be established to ensure that the elemental impurity level does not exceed the PDE in the

drug product.

The variability of the level of an elemental impurity should be factored into the application of the control threshold to drug products. Sources of variability include the following.

• Variability of the analytical method
• Variability of the elemental impurity level in the specific sources
• Variability of the elemental impurity level in the drug product

For some components that have inherent variability (e.g., mined excipients), additional data may be needed to apply the control threshold.

5. Converting between PDEs and Concentration Limits

The PDEs reported in μg per day (μg/day) give the maximum permitted quantity of each element that may be contained in the maximum daily dose of a drug product. Because the PDE reflects total exposure from the drug product, it is useful to convert the PDE into concentrations as a tool in evaluating elemental impurities in drug products or their components. Any of the following options may be selectable as long as the resulting permitted concentrations assure that elemental impurities in the drug product does not exceed the PDEs. In the choice of a specific option the daily dose of the drug product needs to be determined or assumed.

Option 1: Common permitted concentration limits of elemental impurities across drug product components for drug products with daily doses of not more than 10 g: This option is not intended to imply that all elements are present at the same concentration, but rather provides a simplified approach to the calculations of permitted concentration limits. The option assumes the daily dose of the drug product is 10 g or less, and that elemental impurities identified in the risk assessment (the target elements) are present in all components of the drug product. Using Equation (1) below and assuming a daily dose of 10 g of drug product, this option calculates a targeted permissible elemental concentration common for each component in the drug product.

$$\text{Concentration } (\mu g/g) = \frac{PDE\ (\mu g/\text{day})}{\text{daily dose of drug product (g/day)}} \qquad (1)$$

This approach, for each target element, allows determination of a fixed common maximum concentration in μg per g in each component.

The permitted concentrations are provided in Table 2.66-3.

If all the components in a drug product do not exceed the Option 1 permitted concentrations for all target elements identified in the risk assessment, then all these components may be used in any proportion in the drug product. In the case of elements which have the PDE values for preparations for cutaneous application and CTCL, it is necessary to conform to the both limits. If the permitted concentrations in Table 2.66-3 are not applied, Options 2a, 2b, or 3 should be applied.

Table 2.66-3 Permitted Concentrations of Elemental Impurities for Option 1

Element	Class	Oral	Parenteral	Inhalation	Cutaneous	
		Concentration (μg/g)	Concentration (μg/g)	Concentration (μg/g)	Concentration (μg/g)	CTCL in case of sensitization (μg/g)
Cd	1	0.5	0.2	0.3	2	—
Pb	1	0.5	0.5	0.5	5	—
As	1	1.5	1.5	0.2	3	—
Hg	1	3	0.3	0.1	3	—
Co	2A	5	0.5	0.3	5	35
V	2A	10	1	0.1	10	—
Ni	2A	20	2	0.6	20	35
Tl	2B	0.8	0.8	0.8	0.8	—
Au	2B	30	30	0.3	300	—
Pd	2B	10	1	0.1	10	—
Ir	2B	10	1	0.1	*	—
Os	2B	10	1	0.1	*	—
Rh	2B	10	1	0.1	*	—
Ru	2B	10	1	0.1	*	—
Se	2B	15	8	13	80	—
Ag	2B	15	1.5	0.7	15	—
Pt	2B	10	1	0.1	10	—
Li	3	55	25	2.5	250	—
Sb	3	120	9	2	90	—
Ba	3	140	70	30	700	—
Mo	3	300	150	1	1500	—
Cu	3	300	30	3	300	—
Sn	3	600	60	6	600	—
Cr	3	1100	110	0.3	1100	—

* In the case of Ir, Os, Rh, and Ru, data are insufficient for setting PDE values for preparations for cutaneous application. For these elements, apply the PDE values of Pd for the relevant routes.

Option 2a: Common permitted concentration limits of elemental impurities across drug product components for a drug product with a specified daily dose: This option is similar to Option 1, except that the drug daily dose is not assumed to be 10 g. The common permitted concentration of each element is determined using Equation (1) and the actual maximum daily dose. This approach, for each target element, allows determination of a fixed common maximum concentration in μg per g in each component based on the actual daily dose provided. If all the components in a drug product do not exceed the Option 2a permitted concentrations for all target elements identified in the risk assessment, then all these components may be used in any proportion in the drug product.

Option 2b: Permitted concentration limits of elements in individual components of a drug product with a specified daily dose: Permitted concentrations based on the distribution of elements among components (e.g., higher permitted concentrations in components containing the element in question) may be set. For each element identified as potentially present in the components of the drug product, the maximum expected mass of the elemental impurity in the final drug product can be calculated by multiplying the mass

of each component times the permitted concentration preestablished in each material and summing over all components in the drug product, as described in Equation (2). The total mass of the elemental impurity in the drug product should comply with the PDEs unless justified according to other relevant sections of this test method. If the risk assessment has determined that a specific element is not a potential impurity in a specific component, there is no need to establish a quantitative result for that element in that component. This approach allows that the maximum permitted concentration of an element in a specific component of the drug product is higher than the Option 1 or Option 2a limit, but this should then be compensated by lower permitted concentrations in the other components of the drug product. Equation (2) may be used to demonstrate that component-specific limits for each element in each component of a drug product assure the PDE will be met.

$$PDE\ (\mu g/day) \geqq \sum_{k=1}^{N} C_k \cdot M_k \qquad (2)$$

k = an index for each of N components in the drug product

C_k = permitted concentration of the elemental impurity in component k (mg/g)

M_k = mass of component k in the maximum daily dose of the drug product (g)

Option 3: Finished Product Analysis: The concentration of each element may be measured in the final drug product. Equation (1) may be used to calculate a maximum permitted concentration of the elemental impurity from the maximum total daily dose of the drug product.

Change the following as follows:

3.01 Determination of Bulk Density

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

The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

◆Determination of Bulk Density is a method to determine the bulk densities of powdered drugs under untapped and tapped packing conditions. Untapped packing is defined as the state obtained by a powder sample into a vessel without any consolidation, and tapped packing is defined as the state obtained when the vessel containing the powder sample is to be repeatedly dropped a specified distance at a constant drop rate until the apparent volume of sample in the vessel becomes almost constant.◆

1. Bulk density

The bulk density of a powder is the ratio of the mass of a powder sample to its volume, including the contribution of the interparticulate void volume. Hence, the bulk density depends on the material density, and the packing arrangement in the powder bed. The bulk density is commonly expressed in g/mL (1 g/mL = 1 g/cm^3 = 1000 kg/m^3).

The bulk properties of a powder are dependent upon the preparation, treatment and storage of the sample, i.e. how it has been handled. The particles can be packed to have a range of bulk densities. Therefore, it is necessary to differentiate the untapped bulk density and tapped bulk density.

The tapped and untapped bulk densities are used to evaluate powder flow. A comparison of the tapped bulk and untapped bulk densities can give an indirect measure of the relative importance of the interparticulate interactions influencing the bulk properties of a powder.

2. Untapped bulk density

The untapped bulk density of a powder is determined by measuring the volume of a known mass of powder sample, which may have been passed through a sieve, in a graduated cylinder (Method 1), or by measuring the mass of a known volume of powder that has been passed through a volumeter into a cup (Method 2) or has been introduced in to a measuring vessel (Method 3).

The slightest disturbance of the powder bed may result in a changed untapped bulk density, especially for cohesive powders. In these cases, the untapped bulk density is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was made.

2.1. Method 1: Measurement in a graduated cylinder

2.1.1. Procedure

Pass a quantity of powder sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the powder. Gently pour approximately 100 g (M) of the test sample, weighed with 0.1 per cent accuracy, into a dry graduated cylinder of 250 mL (readable to 2 mL). Any significant compacting stress should be avoided, for example, by using a funnel or by tilting the graduated cylinder. If necessary, carefully level the powder without compacting, and read the untapped bulk volume (V_0) to the nearest graduated unit. Calculate the untapped bulk density in g/mL using the formula M/V_0. Replicate determinations performed on separate powder samples are desirable.

If the powder density is too low or too high, such that the test sample has an untapped bulk volume of more than 250 mL or less than 150 mL, it is not possible to use 100 g of powder sample. In this case, a different amount of powder is selected as the test sample, such that its untapped bulk volume is between 150 mL and 250 mL (i.e. untapped bulk volume greater than or equal to 60 per cent of the total volume of the graduated cylinder); the mass of the test sam-

ple is specified in the expression of results.

For test samples having an untapped bulk volume between 50 mL and 100 mL, a 100 mL graduated cylinder readable to 1 mL can be used; the volume of the graduated cylinder is specified in the expression of results.

2.2. Method 2: Measurement in a volumeter

2.2.1. Apparatus

The apparatus (Fig. 3.01-1) consists of a top funnel fitted with a 1.0 mm sieve, mounted over a baffle box containing four glass baffles over which the powder slides and bounces as it passes. At the bottom of the baffle box is a funnel that collects the powder and allows it to pour into a cup mounted directly below it. The cup may be cylindrical (25.00 ± 0.05 mL volume with an internal diameter of 29.50 ± 2.50 mm) or cubical (16.39 ± 0.05 mL volume).

2.2.2. Procedure

Allow an excess of powder to flow through the apparatus into the sample receiving cup until it overflows, using a minimum of 25 cm^3 of powder with the cubical cup and 35 cm^3 of powder with the cylindrical cup. Carefully, scrape excess powder from the top of the cup by smoothly moving the edge of a reclined spatula blade across the top surface of the cup, taking care to keep the spatula tilted backwards to prevent packing or removal of powder from the cup. Remove any powder from the side of the cup and determine the mass (M) of the powder to the nearest 0.1 per cent. Calculate the untapped bulk density in g/mL using the formula M/V_0 (where V_0 is the volume of the cup). Replicate determination performed on separate powder samples are desirable.

2.3. Method 3: Measurement in a vessel

2.3.1. Apparatus

The apparatus consists of a 100 mL cylindrical vessel of stainless steel with dimensions as specified in Fig. 3.01-2.

2.3.2. Procedure

Pass a quantity of powder sufficient to complete the test through a 1.0 mm sieve, if necessary, to break up agglomerates that may have formed during storage, and allow the obtained sample to flow freely into the measuring vessel until it overflows. Carefully scrape the excess powder from the top of the vessel as described for Method 2. Determine the mass (M_0) of the powder to the nearest 0.1 per cent by subtracting the previously determined mass of the empty measuring vessel. Calculate the untapped bulk density (g/mL) using the formula $M_0/100$. Replicate determinations performed on separate powder samples are desirable.

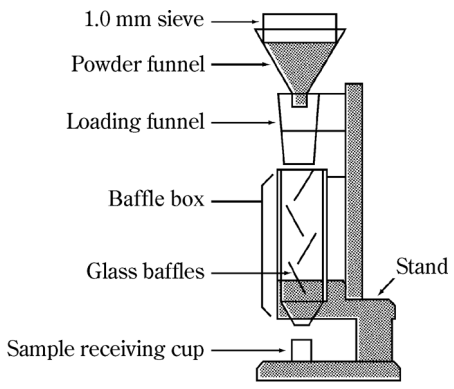


Fig. 3.01-1 Volumeter

3. Tapped bulk density

The tapped bulk density is an increased bulk density attained after mechanically tapping a receptacle containing the powder sample.

The tapped bulk density is obtained by mechanically tapping a graduated cylinder or vessel containing the powder sample. After recording the initial untapped bulk volume (V_0) and mass (M_0) of the powder sample, the graduated cylinder or vessel is mechanically tapped, and volume or mass readings are taken until little further volume or mass change is observed as described in the method. The mechanical tapping is achieved by raising the graduated cylinder or vessel and allowing it to drop a specified distance, under its own mass, by one of three methods as described below. Devices that rotate the graduated cylinder or vessel during tapping may be preferred to give a more levelled surface after tapping.

3.1. Method 1: Measurement in a graduated cylinder (high drop)

3.1.1. Apparatus

The apparatus (Fig. 3.01-3) consists of the following:

(i) a 250 mL graduated cylinder (readable to 2 mL) with a mass of 220 ± 44 g,

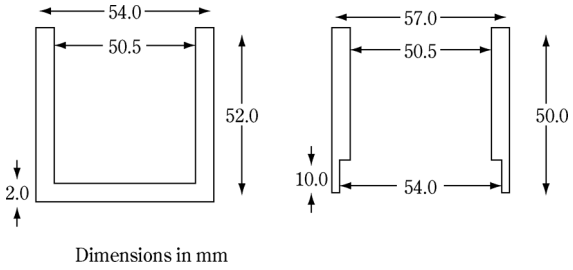


Fig. 3.01-2 Measuring vessel (left) and cap (right)

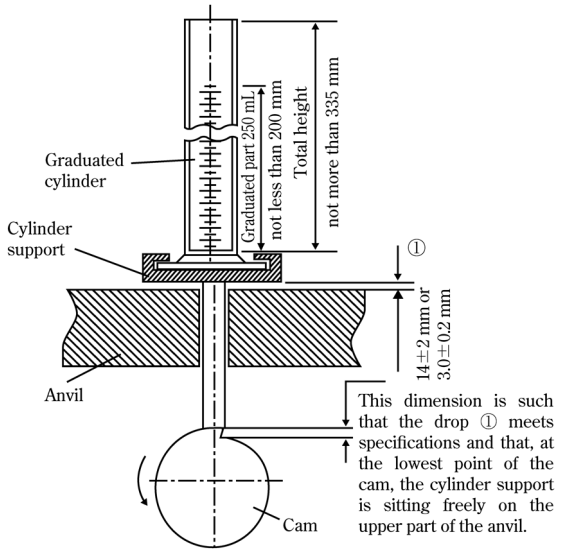


Fig. 3.01-3 Tapping apparatus

(ii) a tapping apparatus capable of producing, per minute, nominally 300 ± 15 taps from a height of 14 ± 2 mm. The support for the graduated cylinder, with its holder, has a mass of 450 ± 10 g.

3.1.2. Procedure

Proceed as described above for the determination of the untapped bulk volume (V_0). Secure the graduated cylinder in the support. Carry out 10, 500 and 1250 taps on the same powder sample and read the corresponding volumes V_{10}, V_{500} and V_{1250} to the nearest graduated unit. If the difference between V_{500} and V_{1250} is less than or equal to 2 mL, V_{1250} is the tapped bulk volume. If the difference between V_{500} and V_{1250} exceeds 2 mL, repeat in increments of, for example, 1250 taps, until the difference between successive measurements is less than or equal to 2 mL. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped bulk density (g/mL) using the formula M/V_f (where V_f is the final tapped bulk volume). Replicate determinations are desirable for the determination of this property. Specify the drop height with the results.

If available sample amount is insufficient for an tapped volume of 150 mL, use a reduced amount and a suitable 100 mL graduated cylinder (readable to 1 mL) weighing 130 ± 16 g and mounted on a support weighing 240 ± 12 g. The untapped volume of the sample should be between 50 mL and 100 mL. If the difference between V_{500} and V_{1250} is less than or equal to 1 mL, V_{1250} is the tapped bulk volume. If the difference between V_{500} and V_{1250} exceeds 1 mL, repeat in increments of, for example, 1250 taps, until the difference between successive measurements is less than or equal to 1 mL. The modified test conditions are specified in the expression of the results.

3.2. Method 2: Measurement in a graduated cylinder (low drop)

3.2.1. Procedure

Proceed as directed under Method 1 except that the mechanical tester provides a fixed drop of 3.0 ± 0.2 mm at a nominal rate of 250 ± 15 taps per minute.

3.3. Method 3: Measurement in a vessel

3.3.1. Procedure

Proceed as described under Method 3 for measuring the untapped bulk density, using the measuring vessel equipped with the cap shown in Fig. 3.01-2. The measuring vessel with the cap is lifted 50 – 60 times per minute by the use of a suitable tapped density tester. Carry out 200 taps, remove the cap and carefully scrape excess powder from the top of the measuring vessel by smoothly moving the edge of a reclined spatula blade across the top surface of the cup, taking care to keep the spatula tilted backwards to prevent packing or removal of powder from the vessel. Determine the mass (M) of the powder to the nearest 0.1 per cent by subtracting the previously determined mass of the empty measuring vessel. Repeat the procedure using 400 taps. If the difference between the 2 masses obtained after 200 and 400 taps exceeds 2 per cent, repeat the test using 200 additional taps until the difference between successive measurements is less than 2 per cent. Calculate the tapped bulk density (g/mL) using the formula $M_f/100$ (where M_f is the final tapped mass of powder in the measuring vessel). Replicate determinations performed on separate powder samples are desirable. The test conditions including tapping height are specified in the expression of the results.

4. Measures of powder compressibility

Because the interparticulate interactions influencing the bulk properties of a powder also interfere with powder flow, a comparison of the untapped bulk and tapped bulk densities can give an indirect measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow, for example the compressibility index or the Hausner ratio.

The compressibility index and Hausner ratio are measures of the propensity of a powder to be compressed as described above.

Calculate the compressibility index and Hausner ratio by the following equations.

$$\text{Compressibility index} = (V_0 - V_f)/V_0 \times 100$$

V_0: Untapped bulk volume
V_f: Final tapped bulk volume

$$\text{Hauser ratio} = V_0/V_f$$

Depending on the powder, the compressibility index can be determined using V_{10} instead of V_0. If V_{10} is used, it is clearly stated with the results.

Add the following:

3.07 Measurement of the Diameter of Particles Dispersed in Liquid by Dynamic Light Scattering

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

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Dynamic light scattering (DLS) can be used to determine the average hydrodynamic particle size and the broadness of the size distribution of submicron particles dispersed in a liquid. Particle size distribution is an important characteristic of dispersed systems such as emulsions, suspensions and liposome formulations. DLS can be used to determine hydrodynamic size of particles in the submicron range and is therefore particularly suitable for the particle size analysis of dispersed systems that are composed of randomly moving particles measuring up to approximately 1 μm.

1. Principle

Submicron particles dispersed in a liquid, and that are free from sedimentation, are subject to a perpetual random movement, known as Brownian motion. When these parti-

cles are irradiated with a laser, scattered light intensity from the moving particles fluctuates depending on their diffusion coefficients. The intensity of the scattered light from larger particles fluctuates more slowly, because larger particles move more slowly and conversely the intensity of the scattered light from smaller particles fluctuates more rapidly. In dynamic light scattering measurements the diffusion dependent fluctuations of the scattered light intensity are measured and analyzed. The translational diffusion coefficient and the particle equivalent spherical diameter are related by the Stokes-Einstein equation.

$$x = \frac{kT}{3\pi\eta D}$$

x: Hydrodynamic diameter of an equivalent spherical particle (m)
k: Boltzmann constant (1.38×10^{-23} J·K^{-1})
T: Absolute temperature (K)
η: Viscosity of the dispersing medium (Pa·s)
D: Translational diffusion coefficient (m^2·s^{-1})

The intensity fluctuations of the scattered light can be evaluated either as a time-dependent phase shift or as a spectral frequency shift.

Based on these concepts, the time-dependent intensity of the scattered light is processed either by photon correlation spectroscopy (PCS) or by frequency analysis.

In PCS, the time-dependent intensity of the scattered light is correlated with a time-delayed copy of itself (autocorrelation function) or with the signal from a second detector (cross-correlation function). Both the auto- and cross-correlation function of a disperse particle system decreases with increasing correlation time. This can be described by an exponential decay. The decay rate depends on the fluctuation of the scattered light as a function of particle size (slower for large particles and faster for small particles).

In frequency analysis, the frequency-based power spectrum of the scattered light is analysed. For a disperse particle system, the power spectrum can be described by a Lorentzian type function.

These two methods are mathematically equivalent. The time-based autocorrelation function in PCS is equal to the Fourier transform of the frequency-based power spectrum in frequency analysis. Therefore, the average diameter ($\bar{x}_{DLS}$) and the polydispersity index (PI), which indicates the broadness of the particle size distribution, can be evaluated with each method.

Different mathematical approaches are applied for data evaluation, including a Laplace inversion for particle size distribution or the cumulants method to evaluate the time-based autocorrelation function.

Three types of optical detection are used with DLS instruments: homodyne detection, in which only the scattered light is measured and heterodyne detection, in which the scattered light and a portion of the incident light are combined for interference and cross-correlation setup which corresponds to two simultaneous homodyne experiments.

2. Instrument

The measuring system typically consists of:

(i) A laser: a monochromatic and coherent laser beam polarized with its electric field component perpendicular to the plane formed by the incident light beam and light-receiving optical axes (vertical polarization), illuminating the sampler in the measuring cell.

(ii) A sample holder: the sample holder must maintain the temperature of the sample within the appropriate range (for example, ± 0.3°C).

(iii) Optics and a detector: a beam splitter as used for heterodyne detection or cross-correlation setup, a light detector positioned at a fixed angle relative to the incident laser beam measuring (usually at only one scattering angle) the apparent scattered light intensity (i.e. the sum of the scattered light from all the particles in the scattering volume) at appropriate intervals. When a polarization analyser is included, it is positioned so that the transmittance of the vertically polarized light is maximized.

(iv) A correlator (photon correlation spectroscopy) or spectrum analyser (frequency analysis).

(v) A computation unit and data processing software (some computation units also function as correlators or spectrum analysers).

3. Control of instrument performance/qualification

As the particle sizes obtained by DLS are not relative values calculated using standard particles but absolute values based on the first principle, calibration cannot be performed.

However, the performance of the instrument must be checked after it is first installed or if abnormal performance is suspected using particles with a certified diameter; it is recommended to repeat this check at least once a year thereafter. The use of certified reference materials with appropriate average particle size verified by DLS is recommended or electron microscopy if applicable.

Dispersions of polystyrene latex with narrow size distribution with certified particle diameter of about 100 nm or other suitable size can be used.

The measured average particle size must be within the stated range of the certified reference material expanded by 2% on each side. Using cumulant analysis, the polydispersity index must be not more than 0.1 and the relative standard deviation of at least five repeated measurements on a sample must be not more than 2%.

4. Procedure

4.1. Sample preparation

(i) Test samples consist of the article well-dispersed in a liquid. The dispersion medium must:

a. be non-absorbing at the wavelength of the laser;
b. be compatible with the materials used in the instrument;
c. not induce particle dissolution, swelling or agglomeration/aggregation;
d. have a known refractive index different from that of the test substance;

e. have known value of viscosity within ± 2 per cent at the measuring temperature;
f. be clean and free of particulate contamination e.g., dust, for low background scattering.

(ii) To eliminate the influence of multiple light scattering, their concentration must be within an appropriate range. When appropriate, the particle concentration range is determined prior the analysis based on the measurements of systematically diluted samples to ensure that the results of the measurements do not vary significantly. The lower limit of the particle concentration range is determined mainly so that scattered light from the dispersion medium and foreign particles will not affect the measurement. Typically, scattered light signals from the dispersion medium used for sample dilution must be undetectable or very weak.

It is also important to remove dust since it may affect the measurement, and to prevent its re-introduction during preparation. If large fluctuations in the scattered light signals accompanied by abnormally strong signals are recorded or if light spots appear in the path of the laser light in the sample, foreign or other intrinsic large particles are likely to be present in the sample. In such cases, further purification of the dispersion medium is necessary (by filtration, distillation, etc.) before use.

When water is chosen as the dispersion medium, use of fresh distilled water or desalted and filtered (nominal pore size 0.2 μm) water is recommended.

Long-range electrostatic interactions arising between highly charged particles may affect the measurement result. In such cases, a small amount of salt (for example, about 10^{-2} mol/L sodium chloride) may be added to the dispersion medium to reduce the effect. Air bubbles may also appear in the test sample, particularly when measuring an initially refrigerated sample at room temperature, and are to be avoided.

If measured values are dependent on the particle concentration, ensure that the concentration range is appropriate for the sample of interest.

4.2. Test procedure

Switch the instrument on and allow it to warm up.

Clean the measurement cell if necessary. The degree of cell washing required depends on the conditions of the measurement. When an individually packaged clean disposable cell is used, cleaning is not necessary. When a cell is intended to be washed, it is rinsed with water or an organic solvent. If required, a non-abrasive detergent may be used.

Place the measurement cell containing the sample in the sample holder, and wait until temperature equilibrium is reached between the sample and the sample holder. It is recommended to measure and maintain the temperature to within ± 0.3°C.

Perform a preliminary measurement of the sample, and set the particle concentration within the appropriate range (see 4.1. Sample preparation).

Perform the measurement with the appropriate measuring time and number of acquisitions.

Record the average particle diameter and the PI for each measurement.

Confirm that no significant settling has occurred in the sample at the end of the measurement. The presence of a sediment indicates that the sample may have agglomerated/aggregated or precipitated, or that it may not be a suitable candidate for DLS.

4.3. Repeatability

The achievable repeatability of the method mainly depends on the characteristics of the test substance (emulsion/suspension; robust/fragile; broadness 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 repeatability (different sample preparations) may vary appreciably from one substance to another. However, it is good practice to aim for repeatability at a relative standard deviation of not more than 10 per cent [$n \geq 3$] for $\bar{x}_{DLS}$.

5. Results

The test report must include the average particle diameter and PI.

It must state the dispersion medium used, the refractive index, viscosity of the dispersion medium and temperature of the test sample, and give sufficient information about the measurement system, including the principle of measurement (PCS or frequency analysis), optical configuration (homodyne or heterodyne), laser wavelength and observation angle. The measuring time or number of acquisitions, the sample (nature, concentration and preparation method), the dispersion conditions, the instrument settings, and the measurement cell type must also be described. As the results depend also on the data analysis program, these details must be provided as well.

6. Glossary

(i) Average particle diameter, $\bar{x}_{DLS}$: Scattered light intensity-weighted harmonic mean particle diameter expressed in meters. X_{DLS} is also commonly referred to as the z-average diameter or Cumulants diameter.

(ii) Polydispersity index, PI: dimensionless measure of the broadness of the particle size distribution.

(iii) Scattering volume: section of the incident laser beam viewed by the detector optics. Its order of magnitude is typically 10^{-12} m^3.

(iv) Scattered intensity, count rate: intensity of the light scattered by the particles in the scattering volume as measured by a detector. In PCS, the number of photon pulses per unit time expressed in counts per second. In frequency analysis, the photodetector current which is proportional to the scattered light intensity.

(v) Viscosity, η: viscosity of the dispersion medium in Pa·s.

(vi) Refractive index, n: dimensionless refractive index of the dispersion medium at the wavelength of the laser.

4.02 Microbial Assay for Antibiotics

Change the 1.10. Procedure, 2.1. Preparation of perforated agar plates and 2.2. Procedure as follows:

1. Cylinder-plate method

1.10. Procedure

Unless otherwise specified, use 5 cylinder-agar plates as one assay set when Petri dishes are employed. When large dishes are employed, the number of cylinders for one assay set should be equal to that defined when using Petri dishes. Apply the standard solution of high concentration and that of low concentration to a pair of cylinders set opposite each other on each plate. Apply the high and low concentration sample solutions to the remaining 2 cylinders. The same volume of these solutions must be added to each cylinder. Incubate the plates at 32 to 37°C for 16 to 20 hours. Measure the diameters of formed circular inhibition zones using a measuring instrument with a precision that can discriminate differences of at least 0.25 mm, or calculate the diameters from the areas of the formed circular inhibition zones. Each procedure should be performed quickly under clean laboratory conditions.

2. Perforated plate method

2.1. Preparation of perforated agar plates

Dispense 4 to 6 mL of the seeded agar layer specified in the individual monograph on each agar base layer plate of the Petri dish. In the case of large dishes, dispense a quantity of the agar medium to form a uniform layer 1.5 to 2.5 mm thick, and spread evenly over the surface before hardening. After coagulating the agar, allow the plate to stand under a clean atmosphere to exhale moisture vapor of the inside of Petri or large dishes and water on the agar surface. Using a tool, prepare 4 circular cavities having a diameter of 7.9 to 8.1 mm on a Petri dish agar plate so that the individual cavities are equidistant from the center of the plate. The cavities spaced equally from one another on the circumference of a circle with radius 25 to 28 mm, and are deep enough to reach the bottom of dish. When large dish plates are used, prepare the circular cavities on each plate according to the method of preparation for Petri dish agar plates. A set of 4 cavities on each large dish plate is considered to be equivalent to one Petri dish plate. Prepare the perforated agar plates before use.

2.2. Procedure

Unless otherwise specified, use 5 perforated agar plates as one assay set when Petri dishes are employed. When large dishes are employed, the number of cavities for one assay set should be equal to that defined when using Petri dishes. Apply the high and low concentration standard solutions to a pair of cavities prepared opposite each other on each plate, and apply the high and low concentration sample solutions to the remaining 2 cavities. The same volume of these solutions must be added to each cavity. Incubate the plates at 32 to 37°C for 16 to 20 hours. Measure the diameters of formed circular inhibition zones using a measuring instrument with a precision that can discriminate differences of at least 0.25 mm, or calculate the diameters from the areas of the formed circular inhibition zones. Each procedure should be performed quickly under clean laboratory conditions.

5.01 Crude Drugs Test

Change the 3. Microscopic examination as follows:

3. Microscopic examination

3.1. Apparatus

Use an optical microscope with objectives of 10 and 40 magnifications, and an ocular of 10 magnifications.

3.2. Preparation for microscopic examination

(i) Section: Prepare a transverse section or sections in any direction where the morphological features and elements described in each monograph can be identified. To a section on a slide glass add 1 to 2 drops of a mounting agent, and put a cover glass on it, taking precaution against inclusion of bubbles. Usually use a section 10 to 20 μm in thickness.

(ii) Powder: Place about 1 mg of powdered sample on a slide glass, apply 1 to 2 drops of a swelling agent, stir well with a small rod preventing inclusion of bubbles, and allow to stand for a while to swell the sample. Apply 1 drop of the mounting agent, and put a cover glass on it so that the tissue sections spread evenly without overlapping each other, taking precaution against inclusion of bubbles. In the case where the tissue sections are opaque, place about 1 mg of powdered sample on a slide glass, apply 1 to 2 drops of chloral hydrate TS, heat to make the tissues clear while stirring with a small glass rod to prevent boiling. After cooling, apply 1 drop of mounting agent, and put a cover glass on it in the same manner as above.

Unless otherwise specified, use a mixture of water and glycerin (1:1) or a mixture of water, ethanol (95) and glycerin (1:1:1) as the mounting agent and swelling agent.

3.3. Observation of components in the Description

In the microscopic examination of description of crude drugs, description of a transverse section is usually given of the outer portion and the inner portion of a section in this order, followed by a specification of cell contents, and observation should be made in the same order. In the case of a powdered sample, description is given of a characteristic component or a matter present in large amount, rarely existing matter, and cell contents in this order. Observation should be made in the same order.

9.01 Reference Standards

Add the following to Section (1):

Aripiprazole RS
Aripiprazole *N*-Oxide for System Suitability RS
Febuxostat RS
Febuxostat Related Substance A for System Suitability RS
Febuxostat Related Substance B for System Suitability RS
Goserelin Acetate RS
Goserelin Acetate Related Substance for System Suitability RS
Lornoxicam RS
Oxaliplatin RS
Oxaliplatin Related Substance B Dinitrate for Purity RS
Residual Solvents Class 2D RS
Residual Solvents Class 2E RS
Tolvaptan RS

Delete the following from section (1):

Amlexanox RS
Tolbutamide RS

Delete the following from section (2):

Cefadroxil RS

Delete the following from section (2), and add them to section (1):

Cefcapene Pivoxil Hydrochloride RS
Cefditoren Pivoxil RS
Cefoperazone RS
Cefozopran Hydrochloride RS
Cefpodoxime Proxetil RS
Ceftazidime RS

9.41 Reagents, Test Solutions

Add the following:

Aconitum monoester alkaloids standard TS for resolution check Prepare as described in the following 1) or 2).

1) Dissolve 2 mg of benzoylmesaconine hydrochloride for thin-layer chromatography, 1 mg of benzoylhypaconine hydrochloride and 2 mg of 14-anisoylaconine hydrochloride in dichloromethane to make exactly 1000 mL. Pipet 5 mL of this solution, and evaporate the solvent under low pressure (in vacuo). Before using, add exactly 5 mL of a mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17) to the residue, and use this solution as aconitum monoester alkaloids standard TS for resolution check. When the procedure is run with 20 μL of aconitum monoester alkaloids TS for resolution check as directed under Liquid Chromatography <*2.01*> according to the following conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4.

Operating conditions

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

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

2) Dissolve 2 mg of benzoylmesaconine hydrochloride for thin-layer chromatography, 1 mg of benzoylhypaconine hydrochloride and 2 mg of 14-anisoylaconine hydrochloride in a mixture of phosphate buffer for processed aconite root and tetrahydrofuran (183:17) to make exactly 1000 mL, and use this solution as aconitum monoester alkaloids standard TS for resolution check. When the procedure is run with 20 μL of aconitum monoester alkaloids TS for resolution check as directed under Liquid Chromatography <*2.01*> according to the following conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order with the resolution between these peaks being not less than 4.

Operating conditions

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

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

2-Aminopyridine $C_5H_6N_2$ White to light yellow, or light brown, crystals, powder or masses.

Melting point <*2.60*>: 56 – 62°C

Identification—Determine the absorption spectrum of a solution of 2-aminopyridine in ethanol (95) (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits maxima between 232 nm and 236 nm, and between 294 nm and 298 nm.

Content: not less than 98.0%. *Assay*—Dissolve 1 g of 2-aminopyridine in 10 mL of acetone. Perform the test with 1 μL of this solution as directed under Gas Chromatography <*2.02*> according to the following conditions, and determine the peak area of each component by the automatic integration method.

$$\text{Content (\%)} = \frac{\text{peak area of 2-aminopyridine}}{\text{total area of all peaks}} \times 100$$

Operating conditions

Detector: A hydrogen flame-ionization detector.

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

Column temperature: A constant temperature of about

170°C.

Injection port temperature: A constant temperature of about 260°C.

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

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of 2-aminopyridine is about 4 minutes.

Split ratio: 1:100.

Time span of measurement: About 5 times as long as the retention time of 2-aminopyridine beginning after the solvent peak.

Ammonia solution (25) NH_3 [K 8085, Ammonia Water, Special class, Density: about 0.91 g/mL, Content: 25.0 – 27.9%]

40 mmol/L ammonium acetate TS Dissolve 3.08 g of ammonium acetate in water to make 1000 mL.

Amphoteric electrolyte solution for pH 7 to 9 Light yellow to yellow liquid. Mixture consisting of multiple types of molecules. Forms a pH gradient over a pH range of 7 to 9 when mixed with polyacrylamide gel and placed in an electric field.

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

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

Benzoic acid for assay C_6H_5COOH White, crystalline powder or powder. Freely soluble in ethanol (95) and in acetone, and slightly soluble in water. Correct the content based on the amount (%) obtained in the Assay.

Identification—Proceed as directed in the Assay: it exhibits a multiplet signal equivalent to two protons around δ 7.26 ppm, triple triplet-like signals equivalent to one proton around δ 7.38 ppm and a multiplet signal equivalent to two protons around δ 7.80 ppm.

Unity of peak—Dissolve 1 mg of benzoic acid for assay in 100 mL of a mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17), 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, and compare the absorption spectra of at least 3 points including the top of benzoic acid 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 Goshajinkigan Extract.

Detector: A photodiode array detector (wavelength: 231 nm; spectrum range of measurement: 220 – 400 nm).

System suitability

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard TS for resolution check under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order, and the number of theoretical plates and the symmetry factor of benzoylmesaconine are not less than 5000 and not more than 1.5, respectively.

However, the unity of peak is unnecessary if the content (%) of benzoic acid (C_6H_5COOH) is between 99.5% and 100.5%.

Assay—Weigh accurately 30 mg of benzoic acid for assay and 5 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 5 mL of deuterated acetone 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 reference standard for qNMR. Calculate the resonance intensities, A_1 (equivalent to 3 hydrogens) and A_2 (equivalent to 2 hydrogens), of the signals around δ 7.24 – 7.40 ppm and δ 7.79 – 7.80 ppm, assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of benzoic acid } (C_6H_5COOH) = M_S \times I \times P/(M \times N) \times 0.5392$$

M: Amount (mg) of benzoic acid for assay taken

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

I: Sum of the signal resonance intensities, A_1 and A_2, based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000

N: Sum of numbers of the hydrogen derived from A_1 and A_2

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 nuclei: ^{1}H.

Digital resolution: 0.25 Hz or lower.

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

Spinning: off.

Pulse angle: 90°.

^{13}C decoupling: on.

Delay time: Repeating pulse waiting time 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 condi-

tions, the SN ratio of each signal around δ 7.24 – 7.28 ppm, δ 7.36 – 7.40 and δ 7.79 – 7.80 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signals around δ 7.24 – 7.40 ppm and δ 7.79 – 7.80 ppm are not overlapped with any signal of obvious foreign substances, and the ratio of the resonance intensity of each signal, $(A_1/3)/(A_2/2)$, 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_1 or A_2 to that of the reference standard for qNMR is not more than 1.0%.

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

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

Buffer solution for teceleukin It contains 0.67 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride, 0.68 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 0.80 g of lithium lauryl sulfate, 6 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate and 4 g of glycerin per 10 mL.

Buffer solution for teceleukin SDS polyacrylamide electrophoresis Dissolve 97.6 g of 2-(*N*-morpholino) ethanesulfonic acid, 60.6 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 10.0 g of sodium lauryl sulfate and 3.0 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 500 mL. To 50 mL of this solution add water to make 1000 mL.

Deuterated acetic acid for nuclear magnetic resonance spectroscopy CD_3CO_2D Prepared for nuclear magnetic resonance spectroscopy.

Lithium Carbonate for assay Li_2CO_3 [Same as the monograph Lithium Carbonate]

Lithium lauryl sulfate $C_{12}H_{25}LiO_4S$ White, crystals or crystalline powder.

Purity Determine the absorbances at 260 nm and at 280 nm of 0.1 mol/L solution of lithium lauryl sulfate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: each absorbance is not more than 0.05.

Lysyl endopeptidase for teceleukin Mass spectrometry grade.

Methylophiopogonanone A for thin-layer chromatography $C_{19}H_{18}O_6$ White to pale yellow, crystals or powder. Sparingly soluble in ethanol (99.5), slightly soluble in methanol, and practically insoluble in water.

Identification—Determine the infrared absorption spectrum of methylophiopogonanone A 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 3430 cm^{-1}, 1619 cm^{-1} and 1251 cm^{-1}.

Purity Related substances—Dissolve 2 mg of methylophiopogonanone A for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions 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) (30:10:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS to the plate: no spot other than the principal spot at an *R*f value of about 0.3 and the spot at the original point appears. Furthermore, spot 10 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 and water (9:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS to the plate: the spot other than the principal spot at an *R*f value of about 0.4 obtained from the sample solution is not more intense than the spot from the standard solution.

2-(*N*-Morpholino)ethanesulfonic acid $C_6H_{13}NO_4S$ White, crystals or powder.

Oxaliplatin $C_8H_{14}N_2O_4Pt$ [Same as the namesake monograph]

Polyacrylamide gel for teceleukin A polyacrylamide gel composed with resolving gel 12% in acrylamide concentration and stacking gel 4% in acrylamide concentration.

0.3 mol/L potassium permanganate TS Dissolve 5 g of potassium permanganate in water to make 100 mL.

Reduction TS A solution containing 0.5 mol/L dithiothreitol.

0.02 mol/L Sodium hydroxide TS To 20 mL of sodium hydroxide TS add water to make 1000 mL. Prepare before use.

Teceleukin for identification $C_{698}H_{1127}N_{179}O_{204}S_8$: 15547.01 [Same as the monograph Teceleukin (Genetical Recombination). However, it conforms to the following identification test.]

Identification—Prepare the sample solution according to Identification (2) under Teceleukin (Genetical Recombination). Perform the analysis with the sample solution using liquid chromatography-mass spectrometer: the peaks with *m/z* values supporting the structure of teceleukin are obtained.

Tetramethylbenzidine $C_{16}H_{20}N_2$ White to light grayish brown, crystals or powder. Melting point: 165 – 172°C.

Tetramethylbenzidine TS Dissolve 0.25 g of tetramethylbenzidine in 50 mL of ethanol (95), and add cyclohexane to make 250 mL.

1 mol/L Tris buffer solution (pH 9.0) Dissolve 12.11 g

of 2-amino-2-hydroxymethyl-1,3-propanediol in 50 mL of water, adjust to pH 9.0 with 1 mol/L hydrochloric acid TS, then add water to make 100 mL.

Change the following as follows:

Atractylenolide III for assay $C_{15}H_{20}O_3$ Use atractylenolide III for thin-layer chromatography. It meets the requirements of the following 1) atractylenolide III for assay 1 or 2) atractylenolide III for assay 2 (Purity value by quantitative NMR). The former is used after drying in a desiccator (silica gel) for not less than 24 hours, and the latter is used with correction for its amount based on the result obtained in the Assay 2.

1) Atractylenolide III for assay 1

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

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

Operating conditions

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

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

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

System suitability

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

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atractylenolide III are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atractylenolide III is not more than 1.5%.

2) Atractylenolide III for assay 2 (Purity value by quantitative NMR)

Unity of peak—Dissolve 5 mg of atractylenolide III for assay 2 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, and compare the absorption spectra of at least 3 points including the top of atractylenolide III 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 Tokishakuyakusan Extract.

Detector: A photodiode array detector (wavelength: 220 nm, spectrum range of measurement: 200 – 400 nm).

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 atractylenolide III are not less than 5000 and not more than 1.5, respectively.

Assay—Weigh accurately 5 mg of atractylenolide III 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 reference standard for qNMR. Calculate the resonance intensities, A_1 (equivalent to 1 hydrogen) and A_2 (equivalent to 1 hydrogen), of the signals around δ 1.97 ppm and δ 2.42 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of atractylenolide III } (C_{15}H_{20}O_3) = M_S \times I \times P/(M \times N) \times 1.0963$$

M: Amount (mg) of atractylenolide III 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, A_1 and A_2, based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000

N: Sum of numbers of the hydrogen derived from A_1 and A_2

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 1.97 ppm and δ 2.42 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signals around δ 1.97 ppm and δ 2.42 ppm are not overlapped with any signal of obvious foreign substances, and the ratio of the resonance intensities, A_1/A_2, of each signal around δ 1.97 ppm and δ 2.42 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_1 or A_2, to that of the reference standard for qNMR is not more than 1.0%.

Atractylodin for assay $C_{13}H_{10}O$ White to pale yellow crystals. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 54°C. It meets the requirements of the following Atractylodin for assay 1 or atractylodin 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 2.

1) Atractylodin for assay 1

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

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

Purity Related substances—

(i) Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 2 mg of atractylodin for assay 1 in 2 mL of methanol, and use this solution as the sample solution. To exactly 1 mL of the sample solution add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography and immediately develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid-ethanol TS for spraying on the plate, and heat the plate at 105°C for 5 minutes: the spot other than the principle spot which appears at an *R*f value of about 0.4 obtained from the sample solution is not more intense than the spot from the standard solution.

(ii) Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 5 mg of atractylodin for assay 1 in 250 mL of methanol, and use this solution as the sample solution. To exactly 1 mL of the sample solution add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than atractylodin obtained from the sample solution is not larger than the peak area of atractylodin from the standard solution.

Operating conditions

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

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

System suitability

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

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

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atractylodin is not more than 1.5%.

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

Identification—Proceed as directed in the Assay: it exhibits a double doublet-like signal equivalent to three protons around δ 1.58 ppm, a double quartet-like signal equivalent to one proton around δ 5.40 ppm, a doublet signal equivalent to one proton around δ 5.86 ppm, a double quartet-like signal equivalent to one proton around δ 6.08 ppm, a multiplet signal equivalent to two protons around δ 6.22 – 6.25 ppm, a doublet signal equivalent to one proton around δ 6.60 ppm and a doublet-like signal equivalent to one proton around δ 7.25 ppm.

Unity of peak—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 1 mg of atractylodin for assay 2 in 50 mL of methanol, 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,

and compare the absorption spectra of at least 3 points including the top of atractylodin 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 (4) under Tokishakuyakusan Extract.

Detector: A photodiode array detector (wavelength: 340 nm; spectrum range of measurement: 220 – 400 nm).

System suitability

System performance: To 1 mL of the sample solution add methanol to make 100 mL. Put this solution in a colorless vessel, and expose to ultraviolet light (main wavelength: 365 nm) for about 1 minute. When the procedure is run with 20 μL of this solution under the above operating conditions, a peak of an isomer is found in addition to the peak of atractylodin, and the isomer and atractylodin are eluted in this order with the resolution between these peaks being not less than 1.5.

Assay—Conduct this procedure without exposure to light. Weigh accurately 5 mg of atractylodin 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 reference standard for qNMR. Calculate the resonance intensity A (equivalent to 1 hydrogen) of the signal around δ 6.60 ppm, assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of atractylodin } (C_{13}H_{10}O) = M_S \times I \times P/(M \times N) \times 0.8045$$

M: Amount (mg) of atractylodin 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 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 the signal around δ 6.60 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal around δ 6.60 ppm is not overlapped with any signal of obvious foreign substances. Furthermore, when determined the resonance intensities, A (equivalent to 1 hydrogen) and A_1 (equivalent to 1 hydrogen) of each signal around δ 6.60 ppm and δ 7.25 ppm, the ratio of the resonance intensity, A/A_1, 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 reference standard for qNMR is not more than 1.0%.

Atractylodin TS for assay Prepare as described in the following 1) or 2).

1) Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 5 mg of atractylodin for assay (for assay 1), and dissolve in methanol to make exactly 1000 mL.

2) Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 5 mg of atrctylodin for assay (for assay 2), dissolve in methanol to make exactly 1000 mL. This TS is corrected by the content obtained in the Assay for atractylodin for assay (for assay 2).

Calcium hydroxide for pH determination See calcium hydroxide.

(*E*)-Ferulic acid for assay $C_{10}H_{10}O_4$ Use (*E*)-ferulic acid meeting the following additional specifications. Correct the content based on the amount (%) obtained in the Assay.

Unity of peak—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 5 mg of (*E*)-ferulic acid for assay in 10 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. 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 (*E*)-ferulic acid 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 Tokishakuyakusan Extract.

Detector: A photodiode array detector (wavelength: 320 nm, spectrum range of measurement: 220 – 400 nm).

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 (*E*)-ferulic acid are not less than 5000 and not more than 1.5, respectively.

Assay—Weigh accurately 5 mg of (*E*)-ferulic acid for assay 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 reference standard for qNMR. Calculate the resonance intensity A (equivalent to 1 hydrogen) of the signal around δ 6.06 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of (}E\text{)-ferulic acid } (C_{10}H_{10}O_4) = M_S \times I \times P/(M \times N) \times 0.8573$$

M: Amount (mg) of (*E*)-ferulic acid for assay 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 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 the signal around δ 6.06 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal around δ 6.06 ppm is not overlapped with any signal of obvious foreign substances.

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 reference standard for qNMR is not more than 1.0%.

10-Hydroxy-2-(*E*)-decenoic acid for assay $C_{10}H_{18}O_3$

Use 10-hydroxy-2-(*E*)-decenoic acid for thin-layer chromatography meeting the following additional specifications. Correct the content based on the amount (%) obtained in the Assay.

Unity of peak—Dissolve 1 mg of 10-hydroxy-2-(*E*)-decenoic acid for assay 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, and compare the absorption spectra of at least 3 points including the top of 10-hydroxy-2-(*E*)-decenoic acid 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 under Royal Jelly.

Detector: A photodiode array detector (wavelength: 215 nm: spectrum range of measurement: 200 – 400 nm).

System suitability

System performance: Dissolve 1 mg each of 10-hydroxy-2-(*E*)-decenoic acid for assay and propyl parahydroxybenzoate for resolution check in methanol to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, 10-hydroxy-2-(*E*)-decenoic acid and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.5.

Assay—Weigh accurately 5 mg of 10-hydroxy-2-(*E*)-decenoic acid for assay 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 reference standard for qNMR. Calculate the resonance intensities, A_1 (equivalent to 1 hydrogen) and A_2 (equivalent to 1 hydrogen), of the signals around δ 5.54 ppm and δ 6.70 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of 10-hydroxy-2-(}E\text{)-decenoic acid } (C_{10}H_{18}O_3) = M_S \times I \times P/(M \times N) \times 0.8223$$

M: Amount (mg) of 10-hydroxy-2-(*E*)-decenoic acid for

assay taken

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

I: Sum of the signal resonance intensities, A_1 and A_2, 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 A_1 and A_2

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 5.54 ppm and δ 6.70 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signals around δ 5.54 ppm and δ 6.70 ppm are not overlapped with any signal of obvious foreign substances, and the ratio of the resonance intensities, A_1/A_2, of each signal around δ 5.54 ppm and δ 6.70 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 deviations of the ratios of the resonance intensity, A_1 or A_2, to that of the reference standard for qNMR are not more than 1.0%.

Methylthymol blue-potassium nitrate indicator Mix 0.1 g of methylthymol blue with 9.9 g of potassium nitrate, and triturate until the mixture becomes homogeneous.

Sensitivity—When 20 mg of methylthymol blue-potassium nitrate indicator is dissolved in 100 mL of 0.02 mol/L sodium hydroxide TS, the solution is slightly blue in color. On adding 0.05 mL of 0.01 mol/L barium chloride VS to this solution, the solution shows a blue color, then on the subsequent addition of 0.1 mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, it becomes colorless.

Molecular mass marker for teceleukin Molecular mass known marker proteins, which are adjusted for molecular mass determination [Molecular mass: 1.0×10^4, 1.5×10^4, 2.0×10^4, 2.5×10^4, 3.7×10^4, 5.0×10^4, 7.5×10^4, 1.0×10^5, 1.5×10^5, 2.5×10^5].

Sinomenine for assay $C_{19}H_{23}NO_4$ Use sinomenine for thin-layer chromatography meeting the following additional specifications. Correct the content based on the amount (%) obtained in the Assay.

Unity of peak—Dissolve 5 mg of sinomenine for assay in 10 mL of a mixture of water and acetonitrile (7:3), 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 sinomenine 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 Boiogito Extract.

Detector: A photodiode array detector (wavelength: 261 nm, spectrum range of measurement: 220 – 400 nm).

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

Assay—Weigh accurately 5 mg of sinomenine for assay and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated acetone 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 reference standard for qNMR. Calculate the resonance intensity A (equivalent to 1 hydrogen) of the signal around δ 5.42 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

$$\text{Amount (\%) of sinomenine } (C_{19}H_{23}NO_4) = M_S \times I \times P/(M \times N) \times 1.4543$$

M: Amount (mg) of sinomenine for assay 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 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 the signal around δ 5.42 ppm is not less than 100.

System performance: When the procedure is run with the sample solution under the above operating conditions, the signal around δ 5.42 ppm is not overlapped with any signal of obvious foreign substances.

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 reference standard for qNMR is not more than 1.0%.

9.42 Solid Supports/Column Packings for Chromatography

Add the following:

Silica gel coated with phenylcarbamoylated cellulose for liquid chromatography Prepared for liquid chromatography.

9.62 Measuring Instruments, Appliances

Change the following paragraph as follows:

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

(2) Semimicrobalances—Balances readable to the digit of 10 μg.

(3) Microbalances—Balances readable to the digit of 1 μg.

(4) Ultramicrobalances—Balances readable to the digit of 0.1 μg.

(5) Balances shall be calibrated to ensure traceability to the International System of Units (SI). In addition, they shall have performance that meets the following requirements.

Requirements for repeatability (precision)

Using the standard deviation s of the indicated value of a balance obtained by loading and unloading a weight 10 or more times, confirm the estimated minimum weight using equation (1). Also, using the standard deviation s, confirm that the precision of the smallest net weight obtained from equation (2) is 0.10% or less. The smallest net weight is the actual lower limit of the weighing which can ensure repeatability (precision) in consideration of the minimum weight.

$$m_{\mathrm{min}} = 2000 \times s \quad (1)$$

$$\frac{2 \times s}{m_{\mathrm{snw}}} \times 100 \leqq 0.10 \quad (2)$$

m_{min}: Estimated minimum weight

s: Standard deviation of the indicated value of the balance in 10 or more repeated weighings

m_{snw}: Smallest net weight

However, if $s < 0.41 \times d$, where d is the readability (scale interval) of the balance, s is replaced by $0.41 \times d$.

The minimum weight is confirmed as the temporary instrumental performance value of a balance, and should be checked periodically since it varies depending on the conditions when weighing is performed. When checking, the mass of the weight should be about 5% of the balance's capacity and 100 mg or more. The balance's capacity is the maximum mass that can be weighed on the balance.

Requirements for accuracy (trueness)

Accuracy (trueness) includes sensitivity errors, linearity errors, and eccentricity errors. Among them, regarding the accuracy of sensitivity, the error obtained by the following equation from the indicated value of a balance obtained by loading and unloading a weight once and the mass value of the weight shall be less than or equal to 0.05%.

$$\frac{|I - m|}{m} \times 100 \leqq 0.05$$

I: The indication of the balance for one weighing of the weight

m: Mass value of the weight (nominal value or conventional mass value)

Use the weight with the mass which is about the upper limit of the weighing range or from 5% to 100% of the balance's capacity.

(6) Exception for confirmation of eccentricity errors, weights used to confirm the accuracy (trueness) of a balance shall be calibrated to ensure traceability to the International System of Units (SI). In addition, they shall have the accuracy class that meets the requirements for use.

Official Monographs

Aluminum Monostearate

モノステアリン酸アルミニウム

Delete the arsenic item in the Purity.

Delete the following Monographs:

Amlexanox

アンレキサノクス

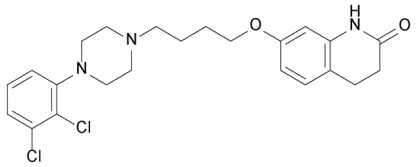
Amlexanox Tablets

アンレキサノクス錠

Add the following:

Aripiprazole

アリピプラゾール



$C_{23}H_{27}Cl_2N_3O_2$: 448.39
7-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy}-3,4-dihydroquinolin-2(1*H*)-one
[*129722-12-9*]

Aripiprazole, when dried, contains not less than 98.0% and not more than 102.0% of aripiprazole ($C_{23}H_{27}Cl_2N_3O_2$).

Description Aripiprazole occurs as white, crystals or crystalline powder.

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

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Aripiprazole in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Aripiprazole 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 Aripiprazole 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 Aripiprazole RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Aripiprazole and Aripiprazole RS separately in dichloromethane, then evaporate the dichloromethane to dryness, and perform the test using these residues.

Purity Related substances—Conduct this procedure using light-resistant vessels. Use the sample solution obtained in the Assay as the sample solution. Pipet 1 mL of the sample solution, add the dissolving solution to make exactly 100 mL. Pipet 5 mL of this solution, add the dissolving solution to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than aripiprazole obtained from the sample solution is not larger than the peak area of aripiprazole from the standard solution, and the total area of the peaks other than aripiprazole from the sample solution is not larger than 3 times the peak area of aripiprazole from the standard solution. For the areas of the related substance A having the retention time of about 0.2 to aripiprazole and the related substance B having the retention time of about 0.8, multiply the correction factor 0.7, respectively.

Dissolving solution—A mixture of water, acetonitrile, methanol and acetic acid (100) (60:30:10:1).

Operating conditions—

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

Time span of measurement: For 25 minutes after injection, beginning after the solvent peak.

System suitability—

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

Test for required detectability: To 1 mL of the sample solution add the dissolving solution to make 20 mL. To 2 mL of this solution add the dissolving solution to make 20 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add the dissolving solution to make exactly 20 mL. Confirm that the peak area of aripiprazole 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 aripiprazole is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.1% (1 g, 105°C, 3 hours).

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

Assay Conduct this procedure using light-resistant vessels. Weigh accurately about 50 mg each of Aripiprazole and Aripiprazole RS, both dried previously, dissolve each in the dissolving solution to make exactly 50 mL. Pipet 5 mL each of these solutions, add dissolving solution to make exactly 50 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 aripiprazole in each solution.

$$\text{Amount (mg) of aripiprazole } (C_{23}H_{27}Cl_2N_3O_2) = M_S \times A_T/A_S$$

M_S: Amount (mg) of Aripiprazole RS taken

Dissolving solution—A mixture of water, acetonitrile, methanol and acetic acid (100) (60:30:10:1).

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: A mixture of diluted trifluoroacetic acid (1 in 2000) and acetonitrile for liquid chromatography (9:1).

Mobile phase B: A mixture of acetonitrile for liquid chromatography and diluted trifluoroacetic acid (1 in 2000) (9:1).

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 – 2	80	20
2 – 10	80 → 65	20 → 35
10 – 20	65 → 10	35 → 90
20 – 25	10	90

Flow rate: 1.2 mL per minute.

System suitability—

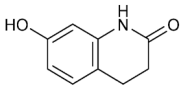
System performance: Dissolve 5 mg each of Aripiprazole RS and Aripiprazole *N*-oxide for System Suitability RS in 100 mL of the dissolving solution. To 1 mL of this solution add the dissolving solution to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, aripiprazole and aripiprazole *N*-oxide 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 aripiprazole is not more than 1.5.

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

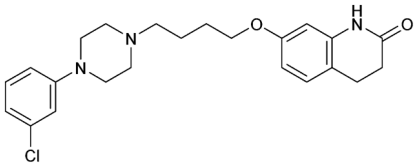
Containers and storage Containers—Tight containers.

Others

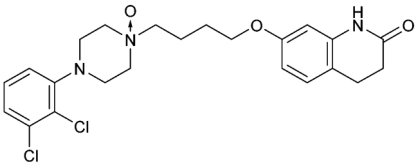
Related substance A:
7-Hydroxy-3,4-dihydroquinolin-2(1*H*)-one



Related substance B:
7-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butoxy}-3,4-dihydroquinolin-2(1*H*)-one



Aripiprazole *N*-oxide:
4-(2,3-Dichlorophenyl)-1-{4-[(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)oxy]butyl}piperazine 1-oxide



Beclometasone Dipropionate

ベクロメタゾンプロピオン酸エステル

Change the Description and Purity (2) as follows:

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

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

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

It shows crystal polymorphism.

Purity

(2) Related substances—Dissolve 20 mg of Beclometasone Dipropionate in 5 mL of ethyl acetate, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethyl acetate to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatogra-

phy ⟨*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 pentane (3:2) to a distance of about 15 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution.

Calcium Stearate

ステアリン酸カルシウム

Delete the arsenic item in the Purity.

Carmellose Calcium

カルメロースカルシウム

Change the beginning of the text, Identification (4), Purity (3) and Residue on ignition as follows:

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 (◆ ◆).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Device Agency.

Identification

(4) Ignite 1 g of Carmellose Calcium to ash, dissolve the residue in 10 mL of water and 6 mL of acetic acid (31), and filter, if necessary. Boil the filtrate, cool, and neutralize with ammonia TS: the solution responds to Qualitative Tests ⟨*1.09*⟩ (3) for calcium salt.

Purity

(3) Sulfate ⟨*1.14*⟩—This is required if sulfuric acid is used in the manufacturing process. Heat 10 mL of the sample solution obtained in (2) with 1 mL of hydrochloric acid in a water bath until a flocculent precipitate is produced. Cool, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant and the washings, and add water to make 100 mL. To 25 mL of this solution add 1 mL of 3 mol/L hydrochloric acid TS and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 25 mL of water add 0.42 mL of 0.005 mol/L sulfuric acid VS, then add 1 mL of 3 mol/L hydrochloric acid TS and water to make 50 mL. To the test solution and the control solution add 3 mL of barium chloride TS (not more than 1.0%).

Residue on ignition ⟨*2.44*⟩ 10.0 – 20.0% (after drying, 1 g).

Delete the following Monographs:

Cefadroxil

セファドロキシル

Cefadroxil Capsules

セファドロキシルカプセル

Cefadroxil for Syrup

シロップ用セファドロキシル

Citicoline

シチコリン

Change the Purity (3) as follows:

Purity

(3) Related substances—Dissolve 0.10 g of Citicoline 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 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks other than citicoline obtained from the sample solution is not larger than 3/5 times the peak area of citicoline from the standard solution, and the total area of the peaks other than citicoline from the sample solution is not larger than the peak area of citicoline from the standard solution. For the area of the peaks, the related substances A, B and C, having the relative retention times of about 0.62, about 0.64 and about 1.3 to citicoline, multiply the correction factors, 1.2, 0.7 and 0.5, 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 times as long as the retention time of citicoline.

System suitability—

Test for required detectability: Pipet 4 mL of the standard solution, and add water to make exactly 50 mL. Confirm that the peak area of citicoline obtained with 10 μL of this solution is equivalent to 5.6 to 10.4% 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 con-

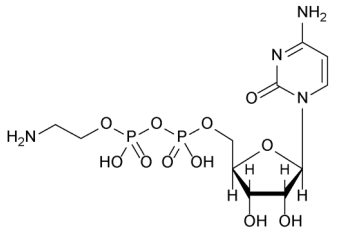
ditions, the number of theoretical plates and the symmetry factor of the peak of citicoline are not less than 2000 and 0.9 to 1.6, respectively.

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 citicoline is not more than 2.0%.

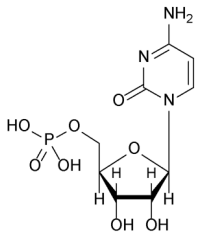
Add the following next to the Containers and storage:

Others

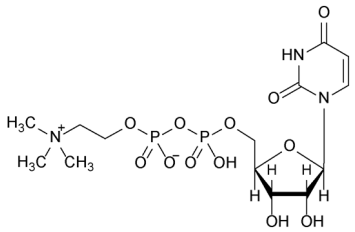
Related substance A:
P″-(2-Aminoethyl) cytidine 5′-(dihydrogen diphosphate)



Related substance B:
Cytidine 5′-(dihydrogen phosphate)



Related substance C:
P″-[2-(Trimethylammonio)ethyl] uridine 5′-(monohydrogen diphosphate)



Clindamycin Phosphate

クリンダマイシンリン酸エステル

Change the Description and Identification as follows:

Description Clindamycin Phosphate occurs as a white to pale yellow-white crystalline powder.

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

It shows crystal polymorphism.

Identification Determine the infrared absorption spectrum of Clindamycin Phosphate, previously dried at 100°C for 2 hours, as directed in the paste method or the ATR method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the spectrum of Clindamycin Phosphate RS previously dried at 100°C for 2 hours: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, weigh 50 mg each of clindamycin phosphate and clindamycin phosphate RS, to each add 0.2 mL of water to dissolve by heating, evaporate to dryness, then repeat the test on the residues dried at 100 to 105°C for 2 hours.

Clonidine Hydrochloride

クロニジン塩酸塩

Change the Description and Purity (4) as follows:

Description Clonidine Hydrochloride occurs as white, crystals or crystalline powder.

It is soluble in water, sparingly soluble in ethanol (99.5), slightly soluble in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

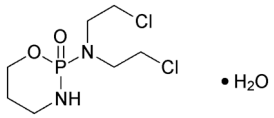
Purity

(4) Related substances—Dissolve 0.20 g of Clonidine Hydrochloride in 2 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (99.5) to make exactly 100 mL. Pipet 1 mL and 2 mL of this solution, to each add ethanol (99.5) to make exactly 20 mL, and use these solutions as the standard solutions (1) and (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 2 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (17:2:1) to a distance of about 12 cm, air-dry the plate, and then dry at 100°C for 1 hour. Spray evenly sodium hypochlorite TS on the plate, air-dry the plate for 15 minutes, and then spray evenly potassium iodide starch TS on the plate: the spots other than the principal spot and the spot of the starting point obtained from the sample solution are not more intense than the spot from the standard solution (2), and the number of spots other than the principal spot and the spot of the starting point, which are more intense than the spot from the standard solution (1), is not more than 3.

Change the following as follows:

Cyclophosphamide Hydrate

シクロホスファミド水和物



$C_7H_{15}Cl_2N_2O_2P.H_2O$: 279.10
N,*N*-Bis(2-chloroethyl)-3,4,5,6-tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide monohydrate
[*6055-19-2*]

Cyclophosphamide Hydrate contains not less than 97.0% and not more than 101.0% of cyclophosphamide hydrate ($C_7H_{15}Cl_2N_2O_2P.H_2O$).

Description Cyclophosphamide Hydrate occurs as white, crystals or crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (95), and soluble in water.

Melting point: 45 – 53°C

Identification Determine the infrared absorption spectrum of Cyclophosphamide Hydrate as directed in the paste method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Clarity and color of solution—Dissolve 0.20 g of Cyclophosphamide Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Chloride ⟨*1.03*⟩—Perform the test with 0.40 g of Cyclophosphamide Hydrate at a temperature not exceeding 20°C. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Related substances—Dissolve 0.20 g of Cyclophosphamide Hydrate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (95) to make 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 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, acetic acid (100), water and methanol (50:25:17:13) to a distance of about 10 cm, dry the plate with warm wind, and heat the plate at 100°C for 10 minutes. Place an evaporating dish containing 0.3 mol/L potassium permanganate TS on the bottom of a developing container, add an equivalent volume of hydrochloric acid, place the heated plate in the developing container, cover the container, and allow to stand for 2 minutes. Take out the plate, remove the excess chlorine with cold wind, and spray evenly tetramethylbenzidine TS on the plate: any spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Water ⟨*2.48*⟩ 5.5 – 7.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Cyclophosphamide Hydrate, add 50 mL of a solution of potassium hydroxide in ethylene glycol (1 in 1000), and heat in an oil bath under a reflux condenser for 30 minutes. After cooling, wash the reflux condenser with 25 mL of water, and combine the washing with the solution. To this solution add 75 mL of 2-propanol and 15 mL of 2 mol/L nitric acid TS, and add exactly 10 mL of 0.1 mol/L silver nitrate VS. Titrate ⟨*2.50*⟩ with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L silver nitrate VS
= 13.96 mg of $C_7H_{15}Cl_2N_2O_2P.H_2O$

Containers and storage Containers—Tight containers.

Dextran 70

デキストラン**70**

Add the following next to the origin/limits of content:

Manufacture Dextran 70 is produced by the manufacturing method that eliminates or minimizes impurities having a possible antigenicity. The manufacturing method is verified to meet the following antigenicity test.

Antigenicity—Dissolve 6.0 g of Dextran 70 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. Separately, 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.

Add the following next to the Residue on ignition:

Bacterial endotoxins ⟨*4.01*⟩ Less than 4.2 EU/g.

Delete the Antigenicity and pyrogen.

Disodium Edetate Hydrate

エデト酸ナトリウム水和物

Change the Identification as follows:

Identification **(1)** Dissolve 0.5 g of Disodium Edetate Hydrate in 20 mL of water, and add 1 mL of dilute hydrochloric acid: a white precipitate is produced. Filter the precipitate, wash with 50 mL of water, and dry at 105°C for 1 hour: the precipitate melts ⟨*2.60*⟩ between 240°C and 244°C (with decomposition).

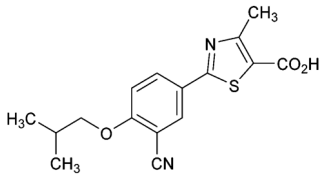
(2) Determine the infrared absorption spectrum of Disodium Edetate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry ⟨*2.25*⟩, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Disodium Edetate Hydrate (1 in 20) responds to Qualitative Tests ⟨*1.09*⟩ (1) for sodium salt.

Add the following:

Febuxostat

フェブキソスタット



$C_{16}H_{16}N_2O_3S$: 316.37

2-[3-Cyano-4-(2-methylpropoxy)phenyl]-4-methyl-1,3-thiazole-5-carboxylic acid

[*144060-53-7*]

Febuxostat contains not less than 98.0% and not more than 102.0% of febuxostat ($C_{16}H_{16}N_2O_3S$).

Description Febuxostat occurs as white, crystals or crystalline powder.

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

Melting point: about 209°C (with decomposition, after drying).

It shows crystal polymorphism.

Identification **(1)** Determine the absorption spectrum of a solution of Febuxostat in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Febuxostat 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 Febuxostat 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 Febuxostat RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the Reference Standard according to the method otherwise specified, filter and dry the crystals, and perform the test with the crystals.

Purity Related substances—(i) Weigh accurately about 50 mg of Febuxostat, dissolve in acetonitrile to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Febuxostat RS, dissolve in acetonitrile to make exactly 50 mL. Pipet 10 mL of this solution, add acetonitrile to make exactly 100 mL, and use this solution as the febuxostat stock solution. Pipet 10 mL of the febuxostat stock solution, add acetonitrile to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Determine each peak area, A_T, of related substances in the sample solution and the peak area, A_S, of febuxostat in the standard solution by the automatic integration method, and calculate the amount of each related substance by the following equation. For the peak area of the related substance A having the relative retention time of about 1.2 to febuxostat, multiply the correction factor 1.8.

$$\text{Amount (\%) of each related substance} = M_S/M_T \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Febuxostat RS taken

M_T: Amount (mg) of Febuxostat taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 217 nm).

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

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

Mobile phase A: Diluted acetic acid (100) (1 in 5000).

Mobile phase B: A solution of acetic acid (100) in acetonitrile for liquid chromatography (1 in 5000).

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 – 40	60 → 0	40 → 100

Flow rate: 0.7 mL per minute.

Time span of measurement: For 40 minutes after injec-

tion.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of febuxostat obtained with 40 μL of this solution is equivalent to 7 to 13% of that with 40 μL of the standard solution.

System performance: Dissolve 1 mg of Febuxostat Related Substance A for System Suitability RS in acetonitrile to make 100 mL. Pipet 2 mL of this solution and 1 mL of the febuxostat stock solution, and add acetonitrile to make exactly 20 mL. When the procedure is run with 40 μL of this solution under the above operating conditions, febuxostat and the related substance A 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 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of febuxostat is not more than 2.0%.

(ii) Weigh accurately about 50 mg of Febuxostat, dissolve in acetonitrile to make exactly 50 mL. Pipet 10 mL of this solution, add 40 mmol/L ammonium acetate TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Febuxostat RS, add acetonitrile to make exactly 50 mL. Pipet 10 mL of this solution, add acetonitrile to make exactly 100 mL, and use this solution as the febuxostat stock solution. Pipet 10 mL of the febuxostat stock solution, and add acetonitrile to make exactly 200 mL. Then, pipet 10 mL of this solution, add 40 mmol/L ammonium acetate 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 the peak area, A_T, of the related substance B having the relative retention time of about 1.1 to febuxostat in the sample solution and the peak area, A_S, of febuxostat in the standard solution by the automatic integration method, and calculate the amount of the related substance B by the following equation.

$$\text{Amount (\%) of the related substance B} = M_S/M_T \times A_T/A_S \times 1/2$$

M_S: Amount of Febuxostat RS taken

M_T: Amount of Febuxostat taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 317 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with triacontylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 15°C.

Mobile phase: A mixture of diluted trifluoroacetic acid (1 in 2000) and a solution of trifluoroacetic acid in acetonitrile for liquid chromatography (1 in 2000) (11:9).

Flow rate: Adjust so that the retention time of febuxostat is about 47 minutes.

System suitability—

Test for required detectability: Weigh exactly 1 mg of Febuxostat Related Substance B for System Suitability RS, dissolve in acetonitrile to make exactly 100 mL, and use this solution as the related substance B solution. Pipet 2 mL of the febuxostat stock solution, add acetonitrile to make exactly 20 mL, and use this solution as the febuxostat 10 times dilution solution. Pipet 1 mL each of the febuxostat 10 times dilution solution and related substance B solution, and add acetonitrile to make exactly 20 mL. Pipet 2 mL of this solution, and add 40 mmol/L ammonium acetate TS to make exactly 20 mL. Confirm that the peak areas of febuxostat and the related substance B obtained with 20 μL of this solution are equivalent to 7 to 13% of those with 20 μL of the solution for system suitability test prepared in the System performance section.

System performance: Pipet 2.5 mL each of the febuxostat 10 times dilution solution and related substance B solution, add 40 mmol/l ammonium acetate TS to make exactly 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, febuxostat and the related substance B 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 febuxostat is not more than 2.0%.

(iii) Each amount of the related substances determined in (i) and (ii) is not more than 0.10%, and the total amount of the related substances is not more than 0.5%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

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

Assay Weigh accurately about 50 mg of Febuxostat, and dissolve in acetonitrile to make exactly 50 mL. Pipet 10 mL of this solution, and add acetonitrile to make exactly 100 mL. Pipet 25 mL of this solution and 10 mL of the internal standard solution, add acetonitrile to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Febuxostat RS, and dissolve in acetonitrile to make exactly 50 mL. Then, proceed as in the same manner as the sample solution, and use the solution so obtained as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of febuxostat to that of the internal standard.

$$\text{Amount (mg) of febuxostat } (C_{16}H_{16}N_2O_3S) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Febuxostat RS taken

Internal standard solution—A solution of diphenyl in aceto-

nitrile (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 217 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 a solution of acetic acid (100) in acetonitrile for liquid chromatography (1 in 500) and diluted acetic acid (100) (1 in 500) (3:2).

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

System suitability—

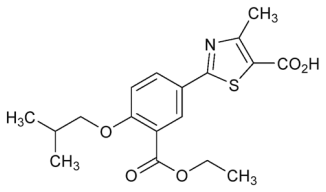
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, febuxostat and the internal standard are eluted in this order with the resolution being not less than 10.

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

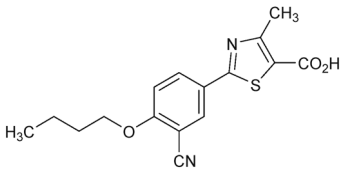
Containers and storage Containers—Tight containers.

Others

Related substance A:
2-[3-Ethoxycarbonyl-4-(2-methylpropoxy)phenyl]-4-methyl-1,3-thiazole-5-carboxylic acid



Related substance B:
2-(4-Butoxy-3-cyanophenyl)-4-methyl-1,3-thiazole-5-carboxylic acid



Add the following:

Febuxostat Tablets

フェブキソスタット錠

Febuxostat Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of febuxostat ($C_{16}H_{16}N_2O_3S$: 316.37).

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

Identification 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 retention times of the principal peaks in the chromatograms obtained from the sample solution and standard solution are the same, 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.

Detector: A photodiode array detector (wavelength: 317 nm, spectrum range of measurement: 210 – 350 nm).

System suitability—

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

Purity Related substances—To 5 tablets of Febuxostat Tablets add 3*V*/4 mL of a mixture of acetonitrile and water (3:2), shake vigorously for 30 minutes until the tablets completely disintegrated, then add a mixture of acetonitrile and water (3:2) to make exactly *V* mL so that each mL contains about 1 mg of febuxostat ($C_{16}H_{16}N_2O_3S$). Centrifuge this solution, filter the supernatant liquid, and use the filtrate as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile and water (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions: the area of the peak other than the related substance TA, having the relative retention time of about 0.4 to the related substance A observed in the solution for system suitability test, and febuxostat obtained from the sample solution is not larger than 1/5 times the peak area of febuxostat from the standard solution. Furthermore, the total area of the peaks other than febuxostat is not larger than 1/2 times the peak area of febuxostat from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 217 nm).

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

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

Mobile phase A: Diluted acetic acid (100) (1 in 5000).

Mobile phase B: A solution of acetic acid (100) in acetonitrile (1 in 5000).

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 – 40	60 → 0	40 → 100
40 – 60	0	100

Flow rate: 0.7 mL per minute.

Time span of measurement: For 60 minutes after injection.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of acetonitrile and water (3:2) to make exactly 10 mL. Confirm that the peak area of febuxostat obtained with 40 μL of this solution is equivalent to 14 to 26% of that with 40 μL of the standard solution.

System performance: Dissolve 10 mg of Febuxostat RS in a mixture of acetonitrile and water (3:2) to make 100 mL, and use this solution as the febuxostat solution. Separately, dissolve 1 mg of Febuxostat Related Substance A for System Suitability RS in a mixture of acetonitrile and water (3:2) to make 100 mL. Pipet 2 mL of this solution and 1 mL of the febuxostat solution, add a mixture of acetonitrile and water (3:2) to make exactly 20 mL, and use this solution as the solution for system suitability test. When the procedure is run with 40 μL of the solution for system suitability test under the above operating conditions, febuxostat and the related substance A 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 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of febuxostat is not more than 2.0%.

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

To 1 tablet of Febuxostat Tablets add $3V/4$ mL of a mixture of acetonitrile and water (3:2), shake vigorously for 30 minutes until the tablets are completely disintegrated, then add a mixture of acetonitrile and water (3:2) to make exactly V mL. Centrifuge this solution, pipet a volume of the supernatant liquid, equivalent to about 4 mg of febuxostat ($C_{16}H_{16}N_2O_3S$), add a mixture of acetonitrile and water (3:2) to make exactly 50 mL. Then pipet 2.5 mL of this solution, add a mixture of acetonitrile and water (3:2) to make exactly 20 mL, filter this solution, and use the filtrate as the sample solution. Then, proceed as directed in the Assay.

Amount (mg) of febuxostat ($C_{16}H_{16}N_2O_3S$)
$= M_S \times A_T/A_S \times C/10$

M_S: Amount (mg) of Febuxostat RS taken

C: Labeled amount (mg) of febuxostat ($C_{16}H_{16}N_2O_3S$) in 1 tablet.

Dissolution *<6.10>* When the test is performed at 50 revolutions per minute according to the Paddle method, using 900 mL of disodium hydrogen phosphate-citric acid buffer solution (pH 5.5) as the dissolution medium for 10-mg and 20-mg tablets and 900 mL of 0.05 mol/L disodium hydrogen phosphate-citric acid buffer solution (pH 6.0) as the dissolution medium for 40-mg tablet, the dissolution rates in 30 minutes of 10-mg and 40-mg tablets are not less than 80%, and that in 60 minutes of 20-mg tablet is not less than 75%.

Start the test with 1 tablet of Febuxostat Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add 2nd fluid for disintegration test to make exactly V' mL so that each mL contains about 11 μg of febuxostat ($C_{16}H_{16}N_2O_3S$), and use this solution as the sample solution. Separately, weigh accurately about 11 mg of Febuxostat RS, and dissolve in 2nd fluid for disintegration test to make exactly 50 mL. Pipet 5 mL of this solution, add 2nd fluid for disintegration test 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 317 nm as directed under Ultraviolet-visible Spectrophotometry *<2.24>*.

Dissolution rate (%) with respect to the labeled amount of febuxostat ($C_{16}H_{16}N_2O_3S$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 90$

M_S: Amount (mg) of Febuxostat RS taken

C: Labeled amount (mg) of febuxostat ($C_{16}H_{16}N_2O_3S$) in 1 tablet.

Assay To 10 tablets of Febuxostat Tablets add $3V/4$ mL of a mixture of acetonitrile and water (3:2), shake vigorously for 30 minutes until the tablets are completely disintegrated, then add a mixture of acetonitrile and water (3:2) to make exactly V mL. Centrifuge this solution, pipet a volume of the supernatant liquid, equivalent to about 4 mg of febuxostat ($C_{16}H_{16}N_2O_3S$), add a mixture of acetonitrile and water (3:2) to make exactly 50 mL. Then pipet 2.5 mL of this solution, add a mixture of acetonitrile and water (3:2) to make exactly 20 mL, filter this solution, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Febuxostat RS, dissolve in a mixture of a solution of acetonitrile and water (3:2) to make exactly 200 mL. Pipet 5 mL of this solution, add a mixture of acetonitrile and water (3:2) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of febuxostat in each solution.

Amount (mg) of febuxostat ($C_{16}H_{16}N_2O_3S$)
$= M_S \times A_T/A_S \times C/10$

M_S: Amount (mg) of Febuxostat RS taken
C: Labeled amount (mg) of febuxostat ($C_{16}H_{16}N_2O_3S$) in 1 tablet.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 317 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 a solution of acetic acid (100) in acetonitrile for liquid chromatography (1 in 500) and diluted acetic acid (100) (1 in 500) (3:2).

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

System suitability—

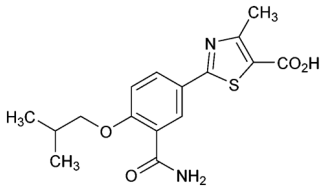
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of the theoretical plates and the symmetry factor of the peak of febuxostat are not less than 1500 and 0.9 to 1.4, respectively.

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 ratio of the peak area of febuxostat is not more than 1.0%.

Containers and storage Containers—Tight containers.

Others

Related substance TA:
2-[3-Carbamoyl-4-(2-methylpropoxy)phenyl]-4-methyl-1,3-thiazole-5-carboxylic acid



Add the following:

Gefitinib Tablets

ゲフィチニブ錠

Gefitinib Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of gefitinib ($C_{22}H_{24}ClFN_4O_3$: 446.90).

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

Identification To a quantity of powdered Gefitinib Tablets, equivalent to 0.25 g of Gefitinib, add 175 mL of a mixture of water, acetonitrile and trifluoroacetic acid (59:40:1), shake, and add a mixture of water, acetonitrile and trifluoroacetic acid (59:40:1) to make 500 mL. To 2 mL of this solution add a mixture of water, acetonitrile and trifluoroacetic acid (59:40:1) to make 100 mL, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩: it exhibits maxima between 252 nm and 256 nm, and between 342 nm and 346 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 Gefitinib Tablets add 175 mL of a mixture of water, acetonitrile and trifluoroacetic acid (59:40:1), sonicate until the tablets are completely disintegrated, shake, then add a mixture of water, acetonitrile and trifluoroacetic acid (59:40:1) to make exactly 500 mL. Allow to stand for more than 30 minutes, pipet 2 mL of the supernatant liquid, and add a mixture of water, acetonitrile and trifluoroacetic acid (59:40:1) to make exactly V mL so that each mL contains about 10 μg of gefitinib ($C_{22}H_{24}ClFN_4O_3$). Filter this solution through a membrane filter with a pore size not exceeding 0.45 μm. Discard 3 mL of the first filtrate, and use the subsequent solution as the sample solution. Separately, weigh accurately about 40 mg of Gefitinib RS (separately determine the water ⟨*2.48*⟩ in the same manner as Gefitinib), add 150 mL of a mixture of water, acetonitrile and trifluoroacetic acid (59:40:1), sonicate to dissolve. To this solution add a mixture of water, acetonitrile and trifluoroacetic acid (59:40:1) to make exactly 200 mL. Pipet 5 mL of this solution, add a mixture of water, acetonitrile and trifluoroacetic acid (59:40:1) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 344 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Amount (mg) of gefitinib ($C_{22}H_{24}ClFN_4O_3$)
$= M_S \times A_T/A_S \times V/16$

M_S: Amount (mg) of Gefitinib 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 1000 mL of a solution of polysorbate 80 (1 in 20) as the dissolution medium, the dissolution rate in 45 minutes of Gefitinib Tablets is not less than 75%.

Start the test with 1 tablet of Gefitinib Tablets, withdraw not less than 10 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard not less than 2 mL of the first filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 25 μg of gefitinib ($C_{22}H_{24}ClFN_4O_3$), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Gefitinib

RS (separately determine the water <2.48> in the same manner as Gefitinib), add about 70 mL of the dissolution medium, sonicate to dissolve, then add the dissolution medium to make exactly 100 mL. Pipet 10 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 334 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Dissolution rate (%) with respect to the labeled amount of gefitinib ($C_{22}H_{24}ClFN_4O_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 100$

M_S: Amount (mg) of Gefitinib RS taken, calculated on the anhydrous basis
C: Labeled amount (mg) of gefitinib ($C_{22}H_{24}ClFN_4O_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 10 tablets of Gefitinib Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 35 mg of gefitinib ($C_{22}H_{24}ClFN_4O_3$), add 85 mL of a mixture of a solution of trifluoroacetic acid (1 in 500) and acetonitrile (3:2), sonicate, and add a mixture of a solution of trifluoroacetic acid (1 in 500) and acetonitrile (3:2) to make exactly 100 mL. Allow to stand for more than 30 minutes, filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard not less than 3 mL of the first filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 35 mg of Gefitinib RS (separately determine the water <2.48> in the same manner as Gefitinib), and add 85 mL of a mixture of a solution of trifluoroacetic acid (1 in 500) and acetonitrile (3:2), and sonicate to dissolve. To this solution add a mixture of a solution of trifluoroacetic acid (1 in 500) and acetonitrile (3:2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed in the Assay under Gefitinib.

Amount (mg) of gefitinib ($C_{22}H_{24}ClFN_4O_3$) $= M_S \times A_T/A_S$

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

Containers and storage Containers—Tight containers.

Glucose

ブドウ糖

Delete the arsenic item in the Purity.

Glycerin

グリセリン

Delete the arsenic item in the Purity.

Concentrated Glycerin

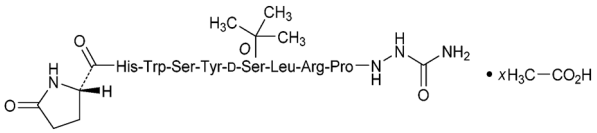
濃グリセリン

Delete the arsenic item in the Purity.

Add the following:

Goserelin Acetate

ゴセレリン酢酸塩



$C_{59}H_{84}N_{18}O_{14}.xC_2H_4O_2$
2-(5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-*O*-*tert*-butyl-D-seryl-L-leucyl-L-arginyl-L-prolyl)hydrazine-1-carboxamide acetate
[*145781-92-6*]

Goserelin Acetate contains not less than 94.5% and not more than 103.0% of goserelin ($C_{59}H_{84}N_{18}O_{14}$: 1269.41), calculated on the anhydrous and residual acetic acid-free basis.

Description Goserelin Acetate occurs as a white powder.

It is freely soluble in acetic acid (100), soluble in water, and slightly soluble in ethanol (95).

It is hygroscopic.

Identification (1) Adjust the pHs of solutions of Goserelin Acetate and Goserelin Acetate RS in deuterated water for nuclear magnetic resonance spectroscopy (1 in 10) to 4.0 with deuterated acetic acid for nuclear magnetic resonance spectroscopy, and use these solutions as the sample solution and the standard solution. Determine the ^{13}C spectra of these solutions as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> with ^1H-decoupling, and compare the spectra: both spectra exhibit signals with similar integrated intensities at the same chemical shifts. Furthermore, determine the ^{13}C spectra of these solutions under the following conditions, measure the integrated intensities of the signals around 23.5 ppm, 26.0 ppm, 26.3 ppm, 41.8 ppm, 55.7 ppm, 62.2 ppm, 62.5 ppm, 116.7 ppm, 118.4 ppm, and 162.2 ppm corresponding to leucine, proline, pyroglutamic acid, arginine, tryptophan, *tert*-butylserine, serine, tyrosine, histidine, and azoglycine

in the sample solution and standard solution, and define the ratio of the integrated intensity of each signal in the sample solution to that of the individual signal in the standard solution as the amino acid ratio: the amino acid ratios of leucine, proline, pyroglutamic acid, arginine, tryptophan, *tert*-butylserine, serine, tyrosine, and histidine are 0.9 to 1.1, and that of azoglycine is 0.8 to 1.2.

Operating conditions—

Apparatus: A nuclear magnetic resonance spectrometer having ^{13}C resonance frequency of not less than 100 MHz.

Measuring spectrum range: 0 – 200 ppm.

Temperature: A constant temperature of about 25°C.

(2) Perform the test with 10 μL each of the sample solution and the standard solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the conditions described in the Assay: the retention times of the principal peaks obtained from the sample solution and standard solution are the same.

Optical rotation ⟨*2.49*⟩ $[\alpha]_D^{20}$: −52 – −56° (20 mg calculated on the anhydrous and residual acetic acid-free basis, water, 10 mL, 100 mm).

Acetic acid Weigh accurately about 15 mg of Goserelin Acetate calculated on the anhydrous basis, add water to make exactly 5 mL, and use this solution as the sample solution. Separately, dissolve potassium acetate (CH_3COOK: 98.15) in water to make solutions so that each mL contains 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg and 0.5 mg as acetic acid, and use these solutions as the standard solutions (1), (2), (3), (4) and (5). Perform the test with exactly 20 μL each of the sample solution and standard solutions (1), (2), (3), (4) and (5) as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions. Calculate the concentration of acetic acid (mg/mL) in the sample solution using the calibration curve obtained from the peak area of the standard solutions, and determine the content of acetic acid in Goserelin Acetate by the following equation: 4.5 – 10.0%.

Content (%) of acetic acid (CH_3COOH)
= $1/M_T$ × concentration (mg/mL) of acetic acid of sample solution × 5 × 100

M_T: Amount (mg) of Goserelin Acetate taken, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

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

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

Mobile phase: A mixture of water, methanol, phosphoric acid and ammonium water (25) (968:20:7:5).

Flow rate: 1.5 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of acetic acid are not less than 3500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of acetic acid is not more than 3.0%.

Purity Related substances—Use the sample solution in the Assay as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine each peak area in each solution by the automatic integration method: the peak area of the related substance E, having the relative retention time of about 0.89 to goserelin, obtained from the sample solution is not larger than the peak area of goserelin from the standard solution, and each peak area of other related substances from the sample solution is not larger than 1/2 times the peak area of goserelin from the standard solution. Furthermore, the total area of the peaks other than goserelin from the sample solution is not larger than 2.5 times the peak area of goserelin 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 goserelin.

System suitability—

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

Test for required detectability: Pipet 1 mL of the standard solution obtained in the Assay, add water to make exactly 200 mL, and use this solution as the solution for system suitability test. Pipet 10 mL of the solution for system suitability test, and add water to make exactly 100 mL. Confirm that the peak area of goserelin 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 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 goserelin is not more than 3%.

Water ⟨*2.48*⟩ Not more than 10.0% (20 mg, coulometric titration).

Assay Weigh accurately about 25 mg each of Goserelin Acetate and Goserelin Acetate RS (previously determine the water ⟨*2.48*⟩ and acetic acid in the same manner as Goserelin), dissolve each in water to make exactly 25 mL, respectively, and use these solutions as the sample solution and 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 goserelin in each solution.

Amount (mg) of goserelin ($C_{59}H_{84}N_{18}O_{14}$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Goserelin Acetate RS taken, calculated on the anhydrous and residual acetic acid-free basis

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 (3.5 μm in particle diameter).

Column temperature: A constant temperature of about 53°C.

Mobile phase: A mixture of water, acetonitrile for liquid chromatography and trifluoroacetic acid (1600:400:1).

Flow rate: Adjust so that the retention time of goserelin is between 40 and 50 minutes.

System suitability—

System performance: Mix equal volumes of the diluted sample solution (1 in 10) and a solution of Goserelin Acetate Related Substance for System Suitability RS (1 in 10,000). When the procedure is run with 10 μL of this solution under the above operating conditions, [4-D-serine]goserelin and goserelin are eluted in this order with the resolution between these peaks being not less than 7, and the symmetry factor of the peak of goserelin is between 0.8 and 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 goserelin is not more than 2.0%.

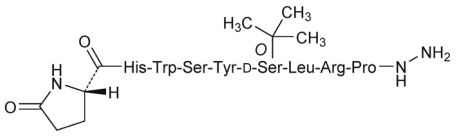
Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature between 2°C and 8°C.

Others

Related substance E:

5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-*O*-*tert*-butyl-D-seryl-L-leucyl-L-arginyl-L-prolinohydrazide



Low Substituted Hydroxypropylcellulose

低置換度ヒドロキシプロピルセルロース

Change the Assay as follows:

Assay (i) Apparatus—Reaction vial: A 5-mL pressure-tight serum vial equipped with a septum made of butyl rubber whose surface is processed with fluoroplastics, which can be fixed to the vial and stoppered tightly with an aluminum cap or another system providing an equivalent air-tightness.

Heater: A square-shaped aluminum block, having holes, 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 a 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-minutes 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 microsyringe, 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.

Amount (%) of hydroxypropoxy group ($C_3H_7O_2$)
= $M_S/M \times Q_T/Q_S \times 44.17$

M_S: Amount (mg) of isopropyl iodide for assay taken

M: Amount (mg) of Low Substituted Hydroxypropylcellulose taken, calculated on the dried basis

44.17: Formula weight of hydroxypropoxy group/Molecular mass of isopropyl iodide × 100

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 in 3 μm 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: 4.3 mL per minute (the retention time of the internal standard is about 10 minutes).

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 the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 1 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%.

Hypromellose

ヒプロメロース

Change the Assay as follows:

Assay (i) Apparatus—Reaction vial: A 5-mL pressure-tight serum vial equipped with a septum made of butyl rubber whose surface is processed with fluoroplastics, which can be fixed to the vial and stoppered tightly with an aluminum cap or another system providing an equivalent air-tightness.

Heater: A square-shaped aluminum block, having holes, 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 Hypromellose, transfer to a reaction vial, add 60 to 100 mg 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 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 60 to 100 mg 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.

Content (%) of methoxy group (CH_3O)
$= M_{Sa}/M \times Q_{Ta}/Q_{Sa} \times 21.86$

Content (%) of hydroxypropoxy group ($C_3H_7O_2$)
$= M_{Sb}/M \times Q_{Tb}/Q_{Sb} \times 44.17$

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
21.86: Formula weight of methoxy group/Molecular mass of iodomethane × 100
44.17: Formula weight of hydroxypropoxy group/Molecular mass of isopropyl iodide × 100

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 in 3 μm thickness. Use a guard column, if necessary.

Column temperature: Maintain the temperature at 50°C for 3 minutes, raise to 100°C at a rate of 10°C per minute, then raise to 250°C at a rate of 35°C per minute, and maintain at 250°C for 8 minutes.

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

Detector temperature: A constant temperature of about 280°C.

Carrier gas: Helium.

Flow rate: 4.3 mL per minute (the retention time of the internal standard is about 10 minutes).

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, iodomethane, isopropyl iodide and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 1 to 2 μL of the standard solution under the above op-

erating conditions, the relative standard deviations of the ratio of the peak area of iodomethane and isopropyl iodide to that of the internal standard are not more than 2.0%, respectively.

Add the following:

Lithium Carbonate Tablets

炭酸リチウム錠

Lithium Carbonate Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of lithium carbonate (Li_2CO_3: 73.89).

Method of preparation Prepare as directed under Tablets, with Lithium Carbonate.

Identification **(1)** Perform the test with powdered Lithium Carbonate Tablets as directed under Flame Coloration Test <*1.04*> (1): a persistent red color appears.

(2) To a quantity of powdered Lithium Carbonate Tablets, equivalent to 0.2 g of Lithium Carbonate, add 3 mL of dilute hydrochloric acid, shake thoroughly, add water to make 20 mL, and filter. To 5 mL of the filtrate add 2 mL each of sodium hydroxide TS and disodium hydrogen phosphate TS, warm, and cool: a white precipitate is produced. To the precipitate add 2 mL of dilute hydrochloric acid: it dissolves.

(3) Weigh a quantity of powdered Lithium Carbonate Tablets, equivalent to 0.5 g of Lithium Carbonate, add 50 mL of water, shake thoroughly, and filter: the filtrate responds to Qualitative Tests <*1.09*> for carbonate.

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

Dissolution <*6.10*> When the test is performed at 100 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rates in 15 minutes and in 180 minutes of a 100-mg tablet are not more than 45% and not less than 80%, respectively, and those in 30 minutes and in 180 minutes of a 200-mg tablet are not more than 50% and not less than 80%, respectively.

Start the test with 1 tablet of Lithium Carbonate Tablets, withdraw exactly 20 mL of the medium at the specified minute 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 the media through a membrane filter with a pore size not exceeding 0.45 μm. Discard not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add exactly 5 mL of dilute hydrochloric acid, add water to make exactly V' mL so that each mL contains about 4.4 μg of lithium carbonate (Li_2CO_3), and use this solution as the sample solution. Separately, weigh accurately about 22 mg of lithium carbonate for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 0.5 mL, 2 mL, 3 mL, 4 mL and 5 mL of this solution, add water to make them exactly 20 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of dilute hydrochloric acid, add water to make them exactly 50 mL, and use these solutions as the standard solutions (1), (2), (3), (4) and (5), respectively. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <*2.23*> according to the following conditions. Determine the absorbances, $A_{T(n)}$, A_{S1}, A_{S2}, A_{S3}, A_{S4} and A_{S5}, and calculate the dissolution rates (%) using a calibration curve obtained from the absorbances of the standard solutions.

Dissolution rate (%) with respect to the labeled amount of lithium carbonate (Li_2CO_3) on the nth medium withdrawing (n = 1, 2)

$$= \left\{ (A_{T(n)} - \text{ordinate intercept of calibration curve}) + \sum_{i=1}^{n-1} (A_{T(i)} - \text{ordinate intercept of calibration curve}) \times \frac{1}{45} \right\} \times \frac{1}{\text{slope of calibration curve}} \times \frac{V'}{V} \times \frac{1}{C} \times 90$$

C: Labeled amount (mg) of lithium carbonate (Li_2CO_3) in 1 tablet

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: Lithium hollow-cathode lamp.
Wavelength: 670.8 nm.

Assay Weigh accurately the mass of not less than 20 tablets of Lithium Carbonate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of lithium carbonate (Li_2CO_3), add exactly 100 mL of water and 50 mL of 0.5 mol/L sulfuric acid VS, remove carbon dioxide by boiling gently, cool, and titrate <*2.50*> the excess sulfuric acid with 1 mol/L sodium hydroxide VS until the color of the solution changes from red to yellow (indicator: 3 drops of methyl red TS). Perform a blank determination in the same manner.

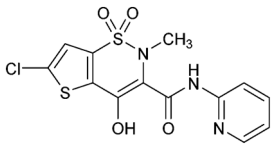
Each mL of 0.5 mol/L sulfuric acid VS
= 36.95 mg of Li_2CO_3

Containers and storage Containers—Well-closed containers.

Add the following:

Lornoxicam

ロルノキシカム



$C_{13}H_{10}ClN_3O_4S_2$: 371.82
6-Chloro-4-hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-thieno[2,3-*e*][1,2]thiazine-3-carboxamide 1,1-dioxide
[*70374-39-9*]

Lornoxicam, when dried, contains not less than 98.0% and not more than 102.0% of lornoxicam ($C_{13}H_{10}ClN_3O_4S_2$).

Description Lornoxicam occurs as a yellow crystalline powder.

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

Melting point: about 207°C (with decomposition).

It shows crystal polymorphism.

Identification **(1)** Dissolve 5 mg of Lornoxicam in 1000 mL of a solution of hydrochloric acid in methanol (9 in 10,000). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Lornoxicam 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 Lornoxicam, 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 Lornoxicam RS: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, to 0.2 g of Lornoxicam add 2 mL of methanol, and stir at 55 – 60°C for 1 hour. Cool to room temperature while stirring, then collect the crystals formed, dry at 120°C for 2 hours, and perform the test with the crystals.

Purity Related substances—Dissolve 20 mg of Lornoxicam in 100 mL of a mixture of acetonitrile and methanol (1:1), and use this solution as the sample solution. Pipet 2 mL of the sample solution, add a mixture of acetonitrile and methanol (1:1) to make exactly 20 mL. Then pipet 1 mL of this solution, add a mixture of acetonitrile and methanol (1:1) to make exactly 20 mL and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of the related substance A, having the relative retention time of about 0.3 to lornoxicam, obtained from the sample solution is not larger than the peak area of lornoxicam from the standard solution, the peak area of the related substance B having the relative retention time of about 0.8 from the sample solution is not larger than 2/25 times the peak area of lornoxicam from the standard solution, the peak area of the related substance C having the relative retention time of about 1.1 from the sample solution is not larger than 19/50 times the peak area of lornoxicam from the standard solution, the peak area of the related substance D having the relative retention time of about 1.4 from the sample solution is not larger than 3/10 times the peak area of lornoxicam from the standard solution, and the area of the peak other than the peak of lornoxicam and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of lornoxicam from the standard solution. Furthermore, the total area of the peaks other than the peak of lornoxicam and the peaks mentioned above from the sample solution is not larger than the peak area of lornoxicam from the standard solution. For the peak areas of the related substances B, C and D, multiply their correction factors, 0.4, 1.9 and 1.5, respectively.

Operating conditions—

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

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

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

Mobile phase A: A mixture of a solution of sodium lauryl sulfate (1 in 2500) and phosphoric acid (1000:1).

Mobile phase B: A mixture of a solution of sodium lauryl sulfate in methanol (1 in 2500) and phosphoric acid (1000:1).

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 – 15	59	41
15 – 30	59 → 30	41 → 70
30 – 35	30	70

Flow rate: 1.0 mL per minute (the retention time of lornoxicam is about 20 minutes).

Time span of measurement: For 35 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution, and add a mixture of acetonitrile and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of lornoxicam obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard

solution.

System performance: To 2 mL of the sample solution add 1 mL of a solution of 2-aminopyridine in a solution of a mixture of acetonitrile and methanol (1:1) (1 in 12,500), then add a mixture of acetonitrile and methanol (1:1) to make 20 mL. To 1 mL of this solution add a mixture of acetonitrile and methanol (1:1) to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, 2-aminopyridine and lornoxicam 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 lornoxicam is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 0.5% (1 g, 105°C, 4 hours).

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

Assay Weigh accurately about 20 mg each of Lornoxicam and Lornoxicam RS, both previously dried, add exactly 1 mL each of the internal standard solution, then dissolve in acetonitrile to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of lornoxicam to that of the internal standard.

$$\text{Amount (mg) of lornoxicam } (C_{13}H_{10}ClN_3O_4S_2) = M_S \times Q_T/Q_S$$

M_S: Amount (mg) of Lornoxicam RS taken

Internal standard solution—A solution of diphenylamine in acetonitrile (1 in 160).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 295 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 50°C.

Mobile phase: A mixture of methanol, a solution of sodium lauryl sulfate (2 in 175) and phosphoric acid (650:350:1).

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

System suitability—

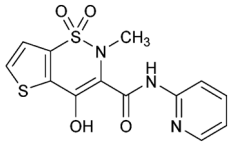
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, lornoxicam and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lornoxicam is not more than 1.0%.

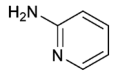
Containers and storage Containers—Well-closed containers.

Others

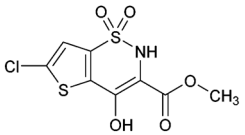
Related substance A:
4-Hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-thieno[2,3-*e*][1,2]thiazine-3-carboxamide 1,1-dioxide



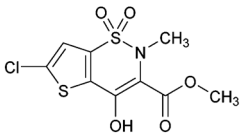
Related substance B:
Pyridin-2-amine



Related substance C:
Methyl 6-chloro-4-hydroxy-2*H*-thieno[2,3-*e*][1,2]thiazine-3-carboxylate 1,1-dioxide



Related substance D:
Methyl 6-chloro-4-hydroxy-2-methyl-2*H*-thieno[2,3-*e*][1,2]thiazine-3-carboxylate 1,1-dioxide



Add the following:

Lornoxicam Tablets

ロルノキシカム錠

Lornoxicam Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of lornoxicam ($C_{13}H_{10}ClN_3O_4S_2$: 371.82).

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

Identification Take an amount of powdered Lornoxicam Tablets, equivalent to 4 mg of Lornoxicam, add 70 mL of a solution of hydrochloric acid in methanol (9 in 10,000), sonicate, and add a solution of hydrochloric acid in methanol (9 in 10,000) to make 100 mL. Centrifuge this solution, to 5 mL of the supernatant liquid add a solution of hydrochloric acid in methanol (9 in 10,000) to make 20 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩ using a

solution of hydrochloric acid in methanol (9 in 10,000) as the blank: it exhibits maximum between 359 nm and 363 nm.

Purity Related substances—Take a number of Lornoxicam Tablets, equivalent to 4 mg of Lornoxicam, add exactly 20 mL of the mobile phase, and sonicate. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 40 mg of Lornoxicam RS, previously dried at 105°C for 4 hours, dissolve in acetonitrile to make exactly 200 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of the related substances by the following equation: the amount of the related substance B having the relative retention time of about 0.13 to lornoxicam is not more than 2.0%, the amount of the related substance TA having the relative retention time of about 0.15 is not more than 1.2%, the amount of the related substance TB having the relative retention time of about 0.21 is not more than 2.0%, the amount of the related substance TC having the relative retention time of about 0.25 is not more than 3.0%, the amount of the related substance TD having the relative retention time of about 0.36 is not more than 2.0%, and the amount of the related substances other than lornoxicam, the related substances A having the relative retention time of about 0.4 to lornoxicam and the related substances mentioned above is not more than 2.0%. Furthermore, the total amount of the related substances is not more than 5.0%. For the peak areas of the related substances TA and TC, multiply their correction factors 0.6 and 1.5, respectively.

$$\text{Amount (\%) of related substance (\%)} = M_S \times A_T/A_S \times 1/40$$

M_S: Amount (mg) of Lornoxicam RS taken
A_T: Peak area of each related substance obtained from the sample solution
A_S: Peak area of lornoxicam obtained from the standard solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

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

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

Mobile phase: Dissolve 4.2 g of tetra-*n*-butylammonium bromide, 4.6 g of disodium hydrogen phosphate dodecahydrate and 4.4 g of potassium dihydrate phosphate in 1300 mL of water, and add 700 mL of acetonitrile for liquid chromatography.

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

Time span of measurement: About 1.5 times as long as the retention time of lornoxicam, 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 symmetry factor of the peak of lornoxicam are not less than 10,000 and not more than 1.5, respectively.

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

Loss on drying <*2.41*> Not more than 2.0% (in vacuum, phosphorus (V) oxide, 24 hours). Take a number of Lornoxicam Tablets, equivalent to 24 mg of Lornoxicam, powder immediately, and perform the test.

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

To 1 tablet of Lornoxicam Tablets add $V/10$ mL of water, and sonicate. Add $3V/5$ mL of a mixture of acetonitrile and methanol (1:1), sonicate, then add a mixture of acetonitrile and methanol (1:1) to make exactly V mL so that each mL contains about 80 μg of lornoxicam ($C_{13}H_{10}ClN_3O_4S_2$), and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 1 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Lornoxicam RS, previously dried 105°C for 4 hours, dissolve in a mixture of acetonitrile and methanol (1:1) to make exactly 200 mL. Pipet 20 mL of this solution, add 5 mL of water, and add a mixture of acetonitrile and methanol (1:1) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 1 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of lornoxicam to that of the internal standard.

$$\text{Amount (mg) of lornoxicam } (C_{13}H_{10}ClN_3O_4S_2) = M_S \times Q_T/Q_S \times V/500$$

M_S: Amount (mg) of Lornoxicam RS taken

Internal standard solution—A solution of diphenylamine in the mobile phase (1 in 4000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, lornoxicam and the internal standard are eluted in this order with the resolution being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of lornoxicam to that of the internal standard is not more than 1.5%.

Dissolution ⟨*6.10*⟩ When the test is performed at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, the dissolution rate in 10 minutes of Lornoxicam Tablets is not less than 80%.

Prepare the sample solution within 1 hour. Start the test with 1 tablet of Lornoxicam Tablets, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 1.1 μg of lornoxicam ($C_{13}H_{10}ClN_3O_4S_2$), and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Lornoxicam RS, previously dried 105°C for 4 hours, dissolve in acetonitrile to make exactly 200 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions, and determine the peak areas, A_T and A_S, of lornoxicam in each solution.

Dissolution rate (%) with respect to the labeled amount of lornoxicam ($C_{13}H_{10}ClN_3O_4S_2$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 9/4$

M_S: Amount (mg) of Lornoxicam RS taken
C: Labeled amount (mg) of lornoxicam ($C_{13}H_{10}ClN_3O_4S_2$) in 1 tablet

Operating conditions—
Proceed as directed in the operating conditions in the Assay.
System suitability—
System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak area of lornoxicam are not less than 1500 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 lornoxicam is not more than 1.5%.

Assay To 15 tablets of Lornoxicam Tablets, add $V/10$ mL of water, and sonicate. Add $7V/10$ of a mixture of acetonitrile and methanol (1:1), sonicate, then add a mixture of acetonitrile and methanol (1:1) to make exactly V mL so that each mL contains about 0.12 mg of lornoxicam ($C_{13}H_{10}ClN_3O_4S_2$), and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 1 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Lornoxicam RS, previously dried at 105°C or 4 hours, and dissolve in a mixture of acetonitrile and methanol (1:1) to make exactly 200 mL. Pipet 20 mL of this solution, add 5 mL of water, and add a mixture of acetonitrile and methanol (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid chromatography ⟨*2.01*⟩ according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of lornoxicam to that of the internal standard.

Amount (mg) of lornoxicam ($C_{13}H_{10}ClN_3O_4S_2$) in 1 tablet
$= M_S \times Q_T/Q_S \times V/7500$

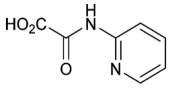
M_S: Amount (mg) of Lornoxicam RS taken

Internal standard solution—A solution of diphenylamine in the mobile phase (1 in 5000).
Operating conditions—
Detector: An ultraviolet absorption photometer (wavelength: 295 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 methanol, a solution of sodium lauryl sulfate (1 in 90) and phosphoric acid (550:450:1).
Flow rate: Adjust so that the retention time of lornoxicam is about 4 minutes.
System suitability—
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, lornoxicam and the internal standard are eluted in this order with the resolution being not less than 6.
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of lornoxicam to that of the internal standard is not more than 1.5%.

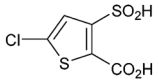
Containers and storage Containers—Tight containers.

Others
Related substances A and B: Refer to them described in Lornoxicam.

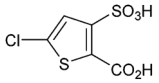
Related substance TA:
(Pyridin-2-yl)oxamic acid



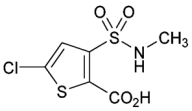
Related substance TB:
5-Chloro-3-sulfinothiophene-2-carboxylic acid



Related substance TC:
5-Chloro-3-sulfothiophene-2-carboxylic acid



Related substance TD:
5-Chloro-3-(*N*-methylsulfamoyl)thiophene-2-carboxylic acid



Loxoprofen Sodium Hydrate

ロキソプロフェンナトリウム水和物

Change the Description and Purity (3) as follows:

Description Loxoprofen Sodium Hydrate occurs as white to yellowish white, crystals or crystalline powder.

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

A solution of Loxoprofen Sodium Hydrate (1 in 20) does not show optical rotation.

The pH of a solution of 1.0 g of Loxoprofen Sodium Hydrate in 20 mL of freshly boiled and cooled water is between 6.5 and 8.5.

Purity

(3) Related substances—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of pentane, ethyl acetate and acetic acid (100) (10:9:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution.

Magnesium Silicate

ケイ酸マグネシウム

Delete the arsenic item in the Purity.

Magnesium Stearate

ステアリン酸マグネシウム

Change the Purity (2) as follows:

Purity

(2) Chloride <*1.03*>—To 10.0 mL of the sample solution obtained in Identification add 1 mL of 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 1.4 mL of 0.02 mol/L hydrochloric acid VS add 1 mL of nitric acid and water to make 50 mL (not more than 0.1%).

Meglumine

メグルミン

Delete the arsenic item in the Purity.

Methylcellulose

メチルセルロース

Change the Assay as follows:

Assay (i) Apparatus—Reaction vial: A 5-mL pressure-tight serum vial equipped with a septum made of butyl rubber whose surface is processed with fluoroplastics, which can be fixed to the vial and stoppered tightly with an aluminum cap or another system providing an equivalent air-tightness.

Heater: A square-shaped aluminum block, having holes, 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 Methylcellulose, transfer to a reaction vial, add 60 to 100 mg 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 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 60 to 100 mg 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 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
21.86: Formula weight of methoxy group/Molecular mass of iodomethane × 100

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 in 3 μm thickness. Use a guard column, if necessary.

Column temperature: Maintain the temperature at 50°C for 3 minutes, raise to 100°C at a rate of 10°C per minute, then raise to 250°C at a rate of 35°C per minute, and maintain at 250°C for 8 minutes.

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

Detector temperature: A constant temperature of about 280°C.

Carrier gas: Helium.

Flow rate: 4.3 mL per minute (the retention time of the internal standard is about 10 minutes).

Split ratio: 1:40.

System suitability—

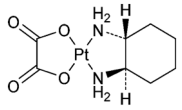
System performance: When the procedure is run with 1 to 2 μL of the standard solution under the above operating conditions, iodomethane and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 1 to 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of iodomethane to that of the internal standard is not more than 2.0%.

Add the following:

Oxaliplatin

オキサリプラチン



$C_8H_{14}N_2O_4Pt$: 397.29

(*SP*-4-2)-[(1*R*,2*R*)-Cyclohexane-1,2-diamine-$\kappa N,\kappa N'$][ethanedioato(2-)-$\kappa O^1,\kappa O^2$]platinum

[*61825-94-3*]

Oxaliplatin contains not less than 98.0% and not more than 102.0% of oxaliplatin ($C_8H_{14}N_2O_4Pt$), calculated on the dried basis.

Description Oxaliplatin occurs as a white crystalline powder.

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

Optical rotation $[\alpha]_D^{20}$: + 74.5 – + 78.0° (0.25 g calculated on the dried basis, water, 50 mL, 100 mm).

Identification **(1)** To 2 mL of a solution of Oxaliplatin (1 in 500) add 2 to 3 drops of diluted tin (II) chloride TS (1 in 15), and allow to stand for 30 minutes: a yellow to orange-yellow precipitate is formed.

(2) Determine the absorption spectrum of a solution of Oxaliplatin (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Oxaliplatin RS prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Oxaliplatin 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 Oxaliplatin RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity **(1)** Acidity or Alkalinity—Dissolve 0.20 g of Oxaliplatin in freshly boiled and cooled water to make 100 mL. To 50 mL of this solution add 0.5 mL of phenolphthalein TS: no color develops. To this solution add 0.6 mL of 0.01 mol/L sodium hydroxide VS: a pale red color develops.

(2) Related substance B—Conduct this procedure within 20 minutes after preparation of the sample solution. Weigh accurately about 0.1 g of Oxaliplatin, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 12.5 mg of Oxaliplatin Related Substance B Dinitrate for Purity RS, dissolve in 63 mL of methanol, and add water to make exactly 250 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solu-

tion and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_{T1} and A_S, of related substance B in each solution by the automatic integration method, and calculate the amount of related substance B by the following equation: the amount of related substance B is not more than 0.1%.

$$\text{Amount of related substance B (\%)} = M_S/M_T \times A_{T1}/A_S \times 0.797$$

M_S: Amount (mg) of Oxaliplatin Related Substance B Dinitrate for Purity RS taken

M_T: Amount (mg) of Oxaliplatin taken

0.797: Conversion factor for related substance B dinitrate to related substance B

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 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 and 1 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust to pH 3.0 with phosphoric acid. To 800 mL of this solution add 200 mL of acetonitrile for liquid chromatography.

Flow rate: 2.0 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of related substance B, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add water to make exactly 10 mL. Confirm that the peak area of related substance B obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: Heat a solution of Oxaliplatin in diluted dilute sodium hydroxide TS (1 in 20) (1 in 500) at 60°C for 2 hours, and allow to cool. To 1 mL of this solution add water to make exactly 10 mL. 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 and peak having the relative retention time of about 1.4 to related substance B is not less than 4, and the symmetry factor of the peak of related substance B is not more than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of related substance B is not more than 3.0%.

(3) Other related substances—Conduct this procedure within 20 minutes after preparation of the sample solution. Dissolve 0.10 g of Oxaliplatin in water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 100 mL. Then, pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of related substance C, having the relative retention time of about 0.6 to oxaliplatin, obtained from the sample solution is not larger than 4.4 times the peak area of oxaliplatin from the standard solution. Furthermore, the total area of the peaks other than oxaliplatin and the peak mentioned above from the sample solution is not larger than the peak area of oxaliplatin 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 oxaliplatin, beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of oxaliplatin obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: To 1 mL of the sample solution and 1 mL of 1 mol/L sodium chloride TS add water to make 10 mL. Separately, to 1 mL of the sample solution and 1 mL of diluted hydrogen peroxide (30) (1 in 3000) add water to make 10 mL. Heat these solutions at 60°C for 2 hours, and allow to cool. Mix 1 mL each of these solutions, and add water to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, the resolution between the peak having the relative retention time of about 0.9 to oxaliplatin and the peak of oxaliplatin is not less than 2.0, and the symmetry factor of the peak of oxaliplatin is not more 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 oxaliplatin is not more than 3.0%.

(4) Enantiomer—Dissolve 30 mg of Oxaliplatin in methanol to make 50 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak height by the automatic peak height method: the height of the peak having the relative retention time of about 1.2 to oxaliplatin obtained from the sample solution is not higher than the peak height of oxaliplatin 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 silica gel coated with phenylcarbamoylated cellulose for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of methanol and ethanol (99.5) (7:3).

Flow rate: 0.3 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 oxaliplatin are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak height of oxaliplatin is not more than 3.0%.

(5) Oxalic acid—Conduct this procedure within 20 minutes after preparation of the sample solution. Dissolve exactly 0.100 g of Oxaliplatin in water to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve exactly 14 mg of oxalic acid dihydrate in water to make exactly 250 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 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 area of oxalic acid in each solution by the automatic integration method: the peak area of oxalic acid obtained from the sample solution is not larger than the peak area of oxalic acid from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

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

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

Mobile phase: Dissolve 2.6 mL of 40% tetrabutylammonium hydroxide TS and 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 6.0 with phosphoric acid. To 800 mL of this solution add 200 mL of acetonitrile for liquid chromatography.

Flow rate: 2.0 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 oxalic acid are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxalic acid is not more than 3.0%.

Loss on drying <*2.41*> Not more than 0.5% (1 g, 105°C, 2 hours).

Assay Weigh accurately about 20 mg each of Oxaliplatin and Oxaliplatin RS (separately determine the loss on drying <*2.41*> under the same conditions as Oxaliplatin), dissolve each in water to make exactly 200 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 oxaliplatin in each solution.

Amount (mg) of oxaliplatin ($C_8H_{14}N_2O_4Pt$) = $M_S \times A_T/A_S$

M_S: Amount (mg) of Oxaliplatin RS taken, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

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

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

Mobile phase: Adjust the pH of 1000 mL of water to 3.0 with phosphoric acid. To 990 mL of this solution add 10 mL of acetonitrile for liquid chromatography.

Flow rate: 1.2 mL per minute.

System suitability—

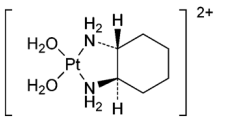
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of oxaliplatin are not less than 3000 and not more than 2.0, respectively.

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

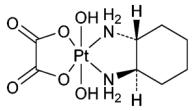
Containers and storage Containers—Tight containers.

Others

Related substance B:
(*SP*-4-2)-Diaqua[(1*R*,2*R*)-cyclohexane-1,2-diamine-κN,κN^1]platinum



Related substance C:
(*OC*-6-33)-[(1*R*,2*R*)-Cyclohexane-1,2-diamine-κN,κN^1][ethanedioato(2-)-κO^1,κO^2]dihydroxyplatinum



Add the following:

Oxaliplatin Injection

オキサリプラチン注射液

Oxaliplatin Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of oxaliplatin ($C_8H_{14}N_2O_4Pt$: 397.29).

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

Description Oxaliplatin Injection is a clear, colorless liquid.

Identification To a volume of Oxaliplatin Injection, equivalent to 5 mg of Oxaliplatin, add water to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <*2.24*>: it exhibits a maximum between 247 nm and 251 nm.

pH Being specified separately when the drug is granted approval based on the Law.

Purity **(1)** Related substances—To an extract volume of Oxaliplatin Injection, equivalent to 50 mg of Oxaliplatin, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 12.5 mg of Oxaliplatin Related Substance B Dinitrate for Purity RS, add 25 mL of methanol, shake thoroughly, and add diluted 2 mol/L nitric acid TS (1 in 200) to make exactly 100 mL. Pipet 25 mL of this solution, add diluted 2 mol/L nitric acid TS (1 in 200) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_{T1} and A_S, of related substance B in each solution, the peak area, A_{T2}, of related substance IA having the relative retention time of about 1.4 to related substance B, and the peak area, A_{Tn}, of each other related substance in the sample solution by the automatic integration method. Calculate their amounts by the following equations: the amounts of related substances B and IA are not more than 0.65% and not more than 0.50%, respectively, the amount of the other related substances is not more than 0.20%, and the total amount of the other related substances is not more than 1.00%. For the peak areas of related substance IA and the other related substances, multiply their correction factors, 0.40 and 0.25, respectively.

Amount of related substance B (%)
$= M_S \times A_{T1}/A_S \times 0.797 \times 1/20$

Amount of related substance IA (%)
$= M_S \times A_{T2}/A_S \times 0.797 \times 1/20$

Amount of each of the other related substances (%)
$= M_S \times A_{Tn}/A_S \times 0.797 \times 1/20$

M_S: Amount (mg) of Oxaliplatin Related Substance B Dinitrate for Purity RS taken

0.797: Conversion factor for related substance B dinitrate to related substance B

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 75 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 10°C.

Mobile phase A: Dissolve 0.55 g of sodium 1-heptane sulfonate and 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 3.0 with phosphoric acid. To 810 mL of this solution add 190 mL of methanol for liquid chromatography.

Mobile phase B: Dissolve 0.55 g of sodium 1-heptane sulfonate and 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 3.0 with phosphoric acid. To 495 mL of this solution add 505 mL of methanol for liquid chromatography.

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 – 0.1	100	0
0.1 – 45.1	100 → 0	0 → 100

Flow rate: 1.0 mL per minute.

Time span of measurement: For 45 minutes after injection.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add water to make exactly 10 mL. Confirm that the peak area of related substance B obtained with 20

μL of this solution is equivalent to 8 to 12% of that with 20 μL of the standard solution.

System performance: Heat a solution of oxaliplatin in diluted dilute sodium hydroxide TS (1 in 20) (1 in 500) at 60 °C for 2 hours, and allow to cool. To 1 mL of this solution add water to make 10 mL, and use this solution as the solution for system suitability test. When the procedure is run with 20 μL of the solution for system suitability test under the above operating conditions, related substance B and related substance IA are eluted in this order with the resolution between these peaks being not less than 8, and the symmetry factor of the peak of related substance B is not more than 2.0.

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

(2) Oxalic acid—Pipet a volume of Oxaliplatin Injection, equivalent to 50 mg of Oxaliplatin, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 44 mg of oxalic acid dihydrate, and add water to make exactly 250 mL. Pipet 20 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak area of oxalic acid in each solution by the automatic integration method: the peak area of oxalic acid obtained from the sample solution is not larger than 3/5 times the peak area of oxalic acid from the standard solution.

Operating conditions—

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

Mobile phase: Dissolve 2.6 mL of 40% tetrabutylammonium hydroxide TS and 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 6.0 with phosphoric acid. To 800 mL of this solution add 200 mL of acetonitrile for liquid chromatography.

Flow rate: 2.0 mL per minute.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, add water to make exactly 10 mL. Confirm that the peak area of oxalic acid obtained with 10 μL of this solution is equivalent to 8 to 12% 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 oxalic acid are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxalic acid is not more than 2.0%.

Bacterial endotoxins <*4.01*> Less than 2.67 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 Oxaliplatin Injection, equivalent to about 10 mg of oxaliplatin ($C_8H_{14}N_2O_4Pt$), and add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Oxaliplatin RS (separately determine the loss on drying <*2.41*> under the same conditions as Oxaliplatin), dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S of oxaliplatin in each solution.

$$\text{Amount (mg) of oxaliplatin } (C_8H_{14}N_2O_4Pt) = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of Oxaliplatin RS taken, calculated on the dried basis

Operating conditions—

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

System suitability—

System performance: To 1 mL of a solution of oxaliplatin (1 in 500) and 1 mL of 1 mol/L sodium chloride TS add water to make 10 mL. Heat this solution at 60°C for 2 hours, and allow to cool. When the procedure is run with 20 μL of this solution under the above operating conditions, the resolution between the peak having the relative retention time of about 0.9 to oxaliplatin and the peak of oxaliplatin is not less than 2.0, and the symmetry factor of the peak of oxaliplatin is not more than 2.0.

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

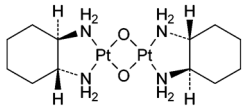
Containers and storage Containers—Hermetic containers.

Others

Related substance B: refer to it described in Oxaliplatin.

Related substance IA:

(*SP*-4-2)-Di-μ-oxobis[(1*R*,2*R*)-cyclohexane-1,2-diamine-κ*N*,κ*N*']diplatinum



Paraffin

パラフィン

Delete the arsenic item in the Purity.

Liquid Paraffin

流動パラフィン

Delete the arsenic item in the Purity.

Light Liquid Paraffin

軽質流動パラフィン

Delete the arsenic item in the Purity.

Polyoxyl 40 Stearate

ステアリン酸ポリオキシル**40**

Delete the arsenic item in the Purity.

Propylene Glycol

プロピレングリコール

Delete the arsenic item in the Purity.

Light Anhydrous Silicic Acid

軽質無水ケイ酸

Delete the arsenic item in the Purity.

Sodium Bisulfite

亜硫酸水素ナトリウム

Delete the arsenic item in the Purity.

Dried Sodium Carbonate

乾燥炭酸ナトリウム

Delete the arsenic item in the Purity.

Sodium Carbonate Hydrate

炭酸ナトリウム水和物

Delete the arsenic item in the Purity.

Sodium Iodide

ヨウ化ナトリウム

Delete the arsenic item in the Purity.

Sodium Polystyrene Sulfonate

ポリスチレンスルホン酸ナトリウム

Change the origin/limits of content, Description and Assay as follows:

Sodium Polystyrene Sulfonate is a cation exchange resin prepared as the sodium form of the sulfonated styrene divinylbenzene copolymer.

It contains not less than 9.4% and not more than 11.5% of sodium (Na: 22.99), calculated on the anhydrous basis.

Each g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, exchanges with not less than 0.110 g and not more than 0.135 g of potassium (K: 39.10).

Description Sodium Polystyrene Sulfonate occurs as a yellow-brown powder. It is odorless and tasteless.

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

It is practically insoluble in dilute hydrochloric acid and in sodium hydroxide TS.

Assay (1) Sodium—Weigh accurately about 0.75 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, add exactly 50 mL of 3 mol/L hydrochloric acid TS, shake for 60 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, and add water to make exactly 300 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 2.542 g of sodium chloride (standard reagent), previously dried at 130°C for 2 hours, dissolve in 0.02 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the standard stock solution. Pipet a suitable quantity of the standard stock solution, dilute exactly with 0.02 mol/L hydrochloric acid TS so that each mL of the solution contains 1 to 3 μg of sodium (Na: 22.99), and use these solutions as the standard solutions. Perform the test with the sample solution and the standard solutions as directed under Atomic Absorption

Spectrophotometry <2.23> according to the following conditions, and calculate the amount of sodium in the sample solution using the calibration curve obtained from the standard solutions.

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: A sodium hollow-cathode lamp.
Wavelength: 589.0 nm.

(2) Potassium exchange capacity—Weigh accurately about 1.5 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, add exactly 100 mL of Standard Potassium Stock Solution, shake for 15 minutes, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, and add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 2 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the sample solution. Separately, pipet a suitable quantity of Standard Potassium Stock Solution, dilute exactly with 0.02 mol/L hydrochloric acid TS so that each mL of the solution contains 1 to 5 μg of potassium (K: 39.10), and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and calculate the amount Y (mg) of potassium in 1000 mL of the sample solution using the calibration curve obtained from the standard solutions. The quantity of potassium absorbed on each g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, is calculated from the following equation: it is between 0.110 g and 0.135 g.

Quantity (mg) of potassium (K) absorbed on 1 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis
$= (X - 100Y)/M$

X: Amount (mg) of potassium in 100 mL of Standard Potassium Stock Solution before exchange
M: Mass (g) of Sodium Polystyrene Sulfonate taken, calculated on the anhydrous basis

Gas: Combustible gas—Acetylene.
Supporting gas—Air.
Lamp: A potassium hollow-cathode lamp.
Wavelength: 766.5 nm.

Sodium Pyrosulfite

ピロ亜硫酸ナトリウム

Delete the arsenic item in the Purity.

Dried Sodium Sulfite

乾燥亜硫酸ナトリウム

Delete the arsenic item in the Purity.

Sorbitan Sesquioleate

ソルビタンセスキオレイン酸エステル

Delete the arsenic item in the Purity.

White Soft Sugar

白糖

Delete the arsenic item in the Purity.

Talc

タルク

Change the beginning of the text, Purity (2) and delete (8) as follows:

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 (◇ ◇).

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

Purity

◇**(2)** Acid-soluble substances—Weigh accurately about 1 g of Talc, heat with 20 mL of dilute hydrochloric acid at 50°C for 15 minutes with stirring. Cool, add water to make exactly 50 mL, and filter. Centrifuge, if necessary, until the filtrate becomes clear. To 25 mL of the filtrate add 1 mL of dilute sulfuric acid, evaporate to dryness, and ignite to constant mass at 800 ± 25°C: the amount of the residue is not more than 2.0%.◇

Teceleukin (Genetical Recombination)

テセロイキン(遺伝子組換え)

Change the Identification, Molecular mass, Purity (1) (2) and (4), Acetic acid as follows:

Identification

(2) Dilute Teceleukin (Genetical Recombination) and teceleukin for identification with water so that each mL contains about 0.6 mg of protein, respectively. To 320 μL

each of these solutions add 40 μL each of 1 mol/L tris buffer solution (pH 9.0) and diluted lysyl endopeptidase for teceleukin (1 in 10,000), react at 37°C for 2 hours, then add 40 μL of 1 mol/L hydrochloric acid TS to stop the reaction, and use these solutions as the sample solution and the standard solution. Perform the test with 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the chromatograms obtained from these solutions: 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 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

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

Mobile phase A: Trifluoroacetic acid TS.

Mobile phase B: A mixture of acetonitrile for liquid chromatography, water and trifluoroacetic acid (950:50:1).

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 – 3	98	2
3 – 15	98 → 55	2 → 45
15 – 25	55 → 30	45 → 70
25 – 35	30	70

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 40 μL of the standard solution under the above operating conditions, the solvent peak is observed around the retention time of 3 minutes, and 9 principal peaks of peptides constituting teceleukin are observed between the retention times of 4 minutes and 20 minutes. Furthermore, the resolution between the 6th peak and the 7th peak is not less than 1.5.

Molecular mass To 10 μL of Teceleukin (Genetical Recombination) add 45 μL of water, 20 μL of reduction TS and 25 μL of buffer for teceleukin, heat at 65°C for 10 minutes, and use this solution as the sample solution. Perform the test with 10 μL each of the sample solution and molecular mass marker for teceleukin by electrophoresis, using buffer solution for teceleukin SDS polyacrylamide gel electrophoresis and polyacrylamide gel for teceleukin. After the electrophoresis, stain by immersing the gel in a solution containing Coomassie brilliant blue G-250. Then, decolorize the gel, and detect the bands. Determine the migration distances of the stained bands of molecular mass markers for teceleukin, and prepare a calibration curve by linear regression against the logarithm of the molecular masses in the range of 1.0×10^4 to 2.5×10^4. Determine the relative mobility of the center of the main band obtained from the sample solution, and calculate the molecular mass of Teceleukin (Genetical Recombination) from the calibration curve: it is between 1.40×10^4 and 1.60×10^4.

Purity **(1)** Desmethionyl form—To 1 mL of Teceleukin (Genetical Recombination) add water so that each mL contains about 0.5 mg of protein, and use this solution as the sample solution. Perform the test with 1.2 mL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak area, A_2, of teceleukin and the peak area, A_1, of the desmethionyl form having the relative retention time of about 0.8 to teceleukin by the automatic integration method. The amount of the desmethionyl form is not more than 1.0% when determined using the following formula.

$$\text{Amount (\%) of desmethionyl form} = A_1/(A_1 + A_2) \times 100$$

Operating conditions—

Detector: Ultraviolet absorption photometer (wavelength: 280 nm).

Columns: Two stainless steel columns with inside diameters of 7.5 mm and lengths of 7.5 cm connected in sequence and packed with diethylaminoethyl group bound to synthetic polymer for liquid chromatography (10 μm in particle diameter).

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

Mobile phase A: Mix 0.66 g of diethanolamine in 400 mL of water, adjust the pH to 9.0 with 1 mol/L hydrochloric acid TS, and then add water to make 500 mL.

Mobile phase B: To 2 mL of amphoteric electrolyte solution for pH 7 to 9 and 5 mL of amphoteric electrolyte solution for pH 8 to 10.5, add 1500 mL of water, adjust to pH 7.0 with 1 mol/L hydrochloric acid TS, and then add water to make 2000 mL.

Switching mobile phases and sample injection: Inject the sample solution while running the mobile phase A. Repeatedly inject 12 times a sample solution volume of 100 μL. After injecting the entire volume and running mobile phase A for 60 minutes, switch to mobile phase B. After measuring the sample solution and after running 1 mol/L sodium chloride TS for 10 minutes for posttreatment and cleaning of the columns, inject 100 μL of sodium hydroxide TS while running the mobile phase A and then 55 minutes later start injection of the next sample solution. Measure the retention time from the point at which the mobile phase is switched to the mobile phase B.

Flow rate: 0.8 mL per minute.

System suitability—

System performance: Dissolve a mixture of two kinds of equine heart-derived myoglobin whose isoelectric points are 6.76 and 7.16 in water to make a concentration of approximately 0.5 mg/mL. Mix together 200 μL of this solution, 200 μL of Teceleukin (Genetical Recombination) and 2.74 mL of water. When the procedure is run with 1.2 mL of this

solution under the above operating conditions, myoglobin and teceleukin are eluted in this order with the resolution between these peaks being not less than 1.5.

(2) Dimer—Prepare a sample solution by adding 1 volume of 0.2% sodium lauryl sulfate TS to 1 volume of Teceleukin (Genetical Recombination). Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the teceleukin peak area, A_2, and the peak area, A_1, of the dimer having the relative retention time of 0.8 to 0.9 to teceleukin, by the automatic integration method. The amount of the dimer is not more than 1.0% when calculated by the following equation.

$$\text{Amount (\%) of dimer} = A_1/(A_1 + A_2) \times 100$$

Operating conditions—

Detector: Ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with glycol etherified silica gel for liquid chromatography (10 μm in particle diameter).

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

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 0.1 mol/L sodium phosphate buffer (pH 7.0) to make 1000 mL.

Flow rate: Adjust so that the retention time of teceleukin is 30 – 40 minutes.

System suitability—

System performance: Add 1 volume of 0.2% sodium lauryl sulfate TS to 1 volume of a solution consisting of 1 mg of carbonic anhydrase and 1 mg of α-lactoalbumin dissolved in 20 mL of water. When the procedure is run with 20 μL of this solution under the above operating conditions, carbonic anhydrase and α-lactoalbumin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: Pipet an appropriate amount of the sample solution, add the mobile phase to dilute 200 times. When the test is repeated 3 times with 20 μL of this solution under the above operating conditions, the relative standard deviation of the teceleukin peak area is not more than 7%.

(4) Other related proteins—Perform the test with 5 μL of Teceleukin (Genetical Recombination) as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine each peak area by the automatic integration method. When the amounts of the peaks are calculated by the area percent method, the total amount of peaks other than teceleukin and solvent peaks is not more than 1.0%.

Operating conditions—

Detector: Ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column with an inside diameter of 4.6 mm 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: Trifluoroacetic acid TS.

Mobile phase B: A solution of trifluoroacetic acid in acetonitrile for liquid chromatography (1 in 1000).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as follows.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 2	55	45
2 – 28	55 → 0	45 → 100
28 – 32	0	100

Flow rate: 0.5 mL per minute.

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

System suitability—

Test for required detectability: To 990 μL of diluted acetic acid (100) (3 in 1000) add exactly 10 μL of Teceleukin (Genetical Recombination), and use this solution as the stock solution for system suitability test. Pipet 800 μL of diluted acetic acid (100) (3 in 1000), add exactly 200 μL of the stock solution for system suitability test, and use this solution as the solution for system suitability test. Confirm that the peak area of teceleukin obtained with 5 μL of the solution for system suitability test is equivalent to 10 to 30% of that with 5 μL of the stock solution for system suitability test.

System performance: To 167.2 μL of Teceleukin (Genetical Recombination) add 7.6 μL of water, then add 33.2 μL of a solution prepared by adding water to 1 g of polysorbate 80 to make 100 mL, and allow to stand for more than 1 hour. When the procedure is run with 5 μL of this solution under the above operating conditions, the resolution between the peak having the relative retention time of about 0.96 to teceleukin and the peak of teceleukin is not less than 1.5.

Acetic acid Pipet an appropriate amount of Teceleukin (Genetical Recombination), dilute 20 times with water, and use this solution as the sample solution. Separately, pipet 1 mL of acetic acid (100), add water to make exactly 100 mL. Pipet 3 mL of this 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 the peak areas, A_T and A_S, of acetic acid in each solution, and calculate the amount of acetic acid ($C_2H_4O_2$) in 1 mL of Teceleukin (Genetical Recombination) by the following equation: 2.85 – 3.15 mg.

$$\text{Amount (mg) of acetic acid } (C_2H_4O_2) \text{ in 1 mL of Teceleukin (Genetical Recombination)} = A_T/A_S \times 0.15 \times 1.049 \times 20$$

0.15: Concentration (μL/mL) of acetic acid (100) in the standard solution

1.049: Density (mg/μL) of acetic acid (100) at 25°C

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 0.7 mL of phosphoric acid add 900 mL of water, and adjust to pH 3.0 with 8 mol/L sodium hydroxide TS, then add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol for liquid chromatography.

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

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of acetic acid are not less than 3000 and not more than 2.0, respectively.

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

Delete the following Monographs:

Tolbutamide

トルブタミド

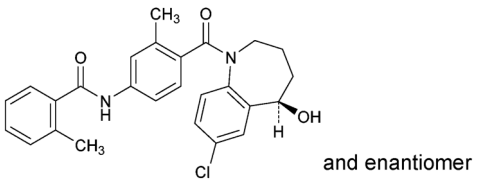
Tolbutamide Tablets

トルブタミド錠

Add the following:

Tolvaptan

トルバプタン



and enantiomer

$C_{26}H_{25}ClN_2O_3$: 448.94

N-{4-[(5*RS*)-7-Chloro-5-hydroxy-2,3,4,5-tetrahydro-1*H*-1-benzazepine-1-carbonyl]-3-methylphenyl}-2-methylbenzamide [*150683-30-0*]

Tolvaptan, when dried, contains not less than 98.5% and not more than 101.5% of tolvaptan ($C_{26}H_{25}ClN_2O_3$).

Description Tolvaptan occurs as white, crystals or crystalline powder.

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

A solution of Tolvaptan in methanol (1 in 50) shows no optical rotation.

Identification **(1)** Determine the absorption spectrum of a solution of Tolvaptan in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <*2.24*>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tolvaptan 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 Tolvaptan 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 Tolvaptan RS: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Related substances—Dissolve 40 mg of Tolvaptan in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area by the automatic integration method, and calculate their amounts by the area percentage method: the amount of the peaks other than tolvaptan is not more than 0.10%. Furthermore, the total amount of the peaks other than tolvaptan is not more than 0.20%.

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: A mixture of water and phosphoric acid (1000:1).

Mobile phase B: A mixture of acetonitrile for liquid chromatography and phosphoric acid (1000:1).

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 – 20	60 → 20	40 → 80
20 – 25	20	80

Flow rate: 1.0 mL per minute.

Time span of measurement: For 25 minutes after injection, beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add methanol 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 methanol to make exactly 20 mL. Confirm that the peak area of tolvaptan obtained with 5 μL of this solution is equivalent to 3.5 to 6.5% of that with 5 μL of the solution for system suitability test.

System performance: Dissolve 15 mg of isoamyl parahydroxybenzoate in 50 mL of methanol. To 2 mL of this solution and 2 mL of the sample solution add methanol to make 20 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, tolvaptan and isoamyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of tolvaptan is not more than 2.0%.

Loss on drying ⟨*2.41*⟩ Not more than 1.0% (1 g, 105°C, 2 hours).

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

Assay Weigh accurately about 50 mg each of Tolvaptan and Tolvaptan RS, both previously dried, add exactly 5 mL each of the internal standard solution, and add methanol to make exactly 50 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 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 tolvaptan to that of the internal standard.

Amount (mg) of tolvaptan ($C_{26}H_{25}ClN_2O_3$) = $M_S \times Q_T/Q_S$

M_S: Amount (mg) of Tolvaptan RS taken

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (3 in 500).

Operating conditions—

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

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

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

Mobile phase: A mixture of acetonitrile for liquid chromatography, water and phosphoric acid (600:400:1).

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

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, tolvaptan and the internal standard are eluted in this order with the resolution between these peaks being not less than 15.

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

Containers and storage Containers—Well-closed containers.

Add the following:

Tolvaptan Tablets

トルバプタン錠

Tolvaptan Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tolvaptan ($C_{26}H_{25}ClN_2O_3$: 448.94).

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

Identification Perform the test with 10 μL each of the sample solution and standard solution obtained in the Assay as directed under Liquid Chromatography ⟨*2.01*⟩ according to the following conditions: the retention times of the principal peaks in the chromatograms obtained from the sample solution and standard solution are the same, 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.

Detector: A photodiode array detector (wavelength: 254 nm, spectrum range of measurement: 210 – 350 nm).

System suitability—

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

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

To 1 tablet of Tolvaptan Tablets add exactly $V/6$ mL of the internal standard solution, add methanol to make V mL so that each mL contains about 0.5 mg of tolvaptan ($C_{26}H_{25}ClN_2O_3$), sonicate while shaking to disintegrate, then shake thoroughly for 10 minutes. To 2 mL of this solution add methanol to make 10 mL, and filter this solution through a membrane filter with a pore size not exceeding 0.5 μm. Discard 1 mL of the first filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 30 mg of Tolvaptan RS, previously dried at 105°C for 2 hours, add exactly 10 mL of the internal standard solution, and add methanol to make 60 mL. To 2 mL of this solution add methanol to make 10 mL, and use this solution as the standard solution. Then, proceed as directed in

the Assay.

Amount (mg) of tolvaptan ($C_{26}H_{25}ClN_2O_3$)
$= M_S \times Q_T/Q_S \times V/60$

M_S: Amount (mg) of Tolvaptan RS taken

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (9 in 5000).

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 (11 in 5000) as the dissolution medium, the Q value in 30 minutes of Tolvaptan Tablets is 80%.

Start the test with 1 tablet of Tolvaptan 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 not less than 10 mL of the first filtrate, pipet V mL of the subsequent filtrate, add the dissolution medium to make exactly V' mL so that each mL contains about 8.3 μg of tolvaptan ($C_{26}H_{25}ClN_2O_3$), and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Tolvaptan RS, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 100 mL. Pipet 2.5 mL of this solution, add the dissolution medium to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of the sample solution and standard solution at 268 nm as directed under Ultraviolet-visible Spectrophotometry ⟨*2.24*⟩.

Dissolution rate (%) with respect to the labeled amount of tolvaptan ($C_{26}H_{25}ClN_2O_3$)
$= M_S \times A_T/A_S \times V'/V \times 1/C \times 45/2$

M_S: Amount (mg) of Tolvaptan RS taken
C: Labeled amount (mg) of tolvaptan ($C_{26}H_{25}ClN_2O_3$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 tablets of Tolvaptan Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 15 mg of tolvaptan ($C_{26}H_{25}ClN_2O_3$), add exactly 9 mL of the internal standard solution, add methanol to make 30 mL, sonicate to disperse, then shake thoroughly for 10 minutes. To 2 mL of this solution add methanol to make 10 mL, and filter this solution through a membrane filter with a pore size not exceeding 0.5 μm. Discard 1 mL of the first filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Tolvaptan RS, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 15 mL of this solution, add exactly 9 mL of the internal standard solution, and add methanol to make 30 mL. To 2 mL of this solution add methanol to make 10 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 tolvaptan to that of the internal standard.

Amount (mg) of tolvaptan ($C_{26}H_{25}ClN_2O_3$)
$= M_S \times Q_T/Q_S \times 3/10$

M_S: Amount (mg) of Tolvaptan RS taken

Internal standard solution—A solution of hexyl parahydroxybenzoate in methanol (1 in 1000).

Operating conditions—

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

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, tolvaptan and the internal standard are eluted in this order with the resolution between these peaks being not less than 15.

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

Containers and storage Containers—Tight containers.

Crude Drugs and Related Drugs

Akebia Stem

モクツウ

Change the Description as follows:

Description Circular or ellipsoidal sections 0.2 – 0.3 cm in thickness, and 1 – 3 cm in diameter; phloem on both fractured surfaces is dark grayish brown; xylem reveals light brown vessel portions and grayish white medullary rays lined alternately and radially; pith light grayish yellow, and distinct; flank grayish brown, and with circular or transversely elongated elliptical lenticels.

Almost odorless; slightly acrid taste.

Under a microscope ⟨*5.01*⟩, a transverse section reveals ring layers mainly consisting of fiber bundles with crystal cells and stone cell groups and surrounding the outside of the phloem in arc shape. Medullary rays of the secondary cortex consisting of sclerenchyma cells containing solitary crystals; portion near cambium is distinct; cells around the pith remarkably thick-walled; xylem medullary rays and parenchyma cells around the pith contain solitary crystals of calcium oxalate and starch grains less than 8 μm in diameter. Under a microscope ⟨*5.01*⟩, a longitudinal section reveals crystal cell rows around fiber bundles.

Artemisia Capillaris Flower

インチンコウ

Change the Description as follows:

Description Capitulum, of ovoid to spherical, about 1.5 – 2 mm in length, about 2 mm in diameter, with the stalk and linear leaves. Outer surface of capitulum, light green to light yellow-brown in color; outer surface of stalk, green-brown to dark brown; outer surface of leaf, green to green-brown. Under a magnifying glass, at the capitulum, involucral scale in 3 – 4 succubous rows; outer involucral scale, of ovate with obtuse apex; inner involucral scale, of elliptical, 1.5 mm in length, longer than outer one, with keel midrib and thin membranous margin. Floret, tubular; marginal flower, of female; disk flower, of hermaphrodite. Achene, of obovoid, 0.8 mm in length. Light in texture.

Odor, characteristic, slight; taste, slightly acrid, which gives slightly numbing sensation to the tongue.

Artemisia Leaf

ガイヨウ

Change the Description as follows:

Description Wrinkled leaves and their fragments, frequently with thin stems. The adaxial surface of leaf dark green, the abaxial surface covered densely with grayish white cotton-like hairs. When smoothed by immersion in water, unfolded laminas 4 – 15 cm in length, 4 – 12 cm in width, 1- to 2- pinnately cleft or pinnately parted. Segments in 2 to 4 pairs, oblong-lanceolate to oblong, apex acuminate sometimes obtuse, margins irregularly lobed or entire. Small sized leaves tri-cleft or entire, lanceolate.

Order, characteristic; taste, slightly bitter.

Under a microscope ⟨*5.01*⟩, a transverse section of leaf reveals several-cells-layered collenchyma beneath epidermis on adaxial and abaxial sides of midvein; vascular bundles at the central portion of midvein, occasionally fiber bundles adjacent to phloem and xylem; laminas composed of adaxial side epidermis, palisade tissue, spongy tissue and abaxial side epidermis, long soft hairs, T-shaped hairs and glandular hairs on epidermis of laminas; epidermal cells contain tannin-like substances, parenchyma cells contain oil-like substances and tannin-like substances.

Asparagus Root

テンモンドウ

Change the Purity as follows:

Purity **(1)** Heavy metals ⟨*1.07*⟩—Proceed with 3.0 g of coarse cuttings of Asparagus Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic ⟨*1.11*⟩—Prepare the test solution with 1.0 g of coarse cuttings of Asparagus Root according to Method 4, and perform the test. Use 5.0 mL of Standard Arsenic Solution for the preparation of the standard color (not more than 5 ppm).

Bearberry Leaf

ウワウルシ

Change the Description as follows:

Description Obovate to spatulate leaves, 1 – 3 cm in length, 0.5 – 1.5 cm in width; adaxial surface yellow-green to dark green; abaxial surface light yellow-green; margin entire; apex obtuse or round, sometimes retuse; base cuneate; petiole very short; lamina thick with characteristic reticulate venation on adaxial surface; Easily broken.

Odor, slight; taste, slightly bitter and astringent.

Under a microscope <*5.01*>, the transverse section reveals thick cuticle in epidermis on adaxial and abaxial sides; parenchyma cells of palisade tissue and sponge tissue being similar in form; in the vascular bundle, medullary ray consisting of 2 to 7 rows of one-cell line, appearing as bones of Japanese fan; polygonal solitary crystals and clustered crystals of calcium oxalate present sparsely in cells on both adaxial and abaxial sides of the vascular bundle, but no crystals in mesophyll.

Belladonna Extract

ベラドンナエキス

Change the Description as follows:

Description Belladonna Extract has a dark brown color and a characteristic odor.

Boiogito Extract

防已黄耆湯エキス

Change the Assay (1) as follows:

Assay (1) Sinomenine—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 5.0 mL of 0.1 mol/L hydrochloric acid TS, and shake for 10 minutes, centrifuge, and remove the diethyl ether layer. To the aqueous layer add 20 mL of diethyl ether, and proceed in the same manner as described above. To the aqueous layer add 5.0 mL of diluted sodium hydroxide TS (1 in 10) and 10 mL of methanol, shake for 15 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and take the supernatant liquid. Combine all the 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 5 mg of sinomenine 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 sinomenine in each solution.

$$\text{Amount (mg) of sinomenine} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of sinomenine for assay taken, calculated on the basis of the content obtained by qNMR

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 30°C.

Mobile phase: To 3 g of sodium lauryl sulfate add 350 mL of acetonitrile, shake, then add 650 mL of water and 1 mL of phosphoric acid to dissolve lauryl sulfate.

Flow rate: 1.0 mL per minute (the retention time of sinomenine is about 18 minutes).

System suitability—

System performance: When the procedure is run with 10 μL each of the sample solution, the sinomenine standard solution and the glycyrrhizic acid standard solution obtained in Assay (2) under the above operating conditions, peaks of sinomenine and glycyrrhizic acid are observed in the sample solution, glycyrrhizic acid and sinomenine are eluted in this order with the resolution between these peaks being not less than 4.5. Furthermore, except for the peak of glycyrrhizic acid, distinct peaks are observed before and after the peak of sinomenine, and the resolutions between sinomenine and these peaks are respectively 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 sinomenine is not more than 1.5%.

Cardamon

ショウズク

Change the Japanese commonly used name as follows (No effect to English text):

Chrysanthemum Flower

キクカ

Change the Description as follows:

Description 1) *Chrysanthemum indicum* origin— Capitulum, 3 – 10 mm in diameter, often with stalk; involucre, consisting of 3 to 5 rows of involucral scales; the outer involucral scale, linear to lanceolate; inner involucral scale,

narrow ovate to ovate; outer surface of involucre, yellow-brown to brown; ligulate flower, in a single circle, yellow to light yellow-brown in color; tubular flowers, numerous, light yellow-brown; light in texture and easy to break.

Odor, characteristic; taste, slightly bitter.

2) *Chrysanthemum morifolium* origin—Capitulum, 15 – 40 mm in diameter, often with stalk; involucre, consisting of 3 to 4 rows of involucral scales; the outer involucral scale, linear to lanceolate; inner involucral scale, narrow ovate to ovate; outer surface of involucre, green-brown to brown; ligulate flowers, numerous, white to yellow in color; tubular flowers, small in number, light yellow-brown, occasionally degenerate; light in texture and easy to break.

Odor, characteristic; taste, slightly bitter.

Citrus Unshiu Peel

チンピ

Change the Assay as follows:

Assay Weigh accurately about 0.1 g of pulverized Citrus Unshiu Peel, add 30 mL of methanol, heat under a reflux condenser for 15 minutes, centrifuge after cooling, and separate the supernatant liquid. To the residue add 20 mL of methanol, and proceed in the same manner. Combine the extracts, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of hesperidin for assay, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of hesperidin in each solution.

$$\text{Amount (mg) of hesperidin} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of hesperidin for assay taken

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

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

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

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (82:18:1).

Flow rate: 1.0 mL per minute (the retention time of hesperidin is about 15 minutes).

System suitability—

System performance: Dissolve 1 mg each of hesperidin for assay and naringin for thin-layer chromatography in 10 mL of methanol, and add water to make 20 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, naringin and hesperidin 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 hesperidin is not more than 1.5%.

Codonopsis Root

トウジン

Change the Identification and Purity as follows:

Identification To 2.0 g of coarse cuttings of Codonopsis Root add 50 mL of water, and heat in a water bath for 1 hour. After cooling, filter, and wash the filtrate with two 20-mL portions of ethyl acetate. Separate the aqueous layer, extract with two 30-mL portions of water saturated 1-butanol. Combine the 1-butanol layers, and evaporate the solvent in a water bath under low pressure (in vacuo). Dissolve the residue in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, water and ethyl acetate (6:5:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly naphthoresorcin-phosphoric acid TS on the plate, and heat the plate at 105°C for 10 minutes: an orange to red-purple spot at an *R*f value of about 0.5 is observed.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of coarse cuttings of Codonopsis Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of coarse cuttings of Codonopsis Root according to Method 4, and perform the test. Use 5.0 mL of Standard Arsenic Solution for the preparation of the standard color (not more than 5 ppm).

Coix Seed

ヨクイニン

Change the Identification as follows:

Identification Cut Coix Seed transversely, macerate in diluted iodine TS (1 in 10) for 5 seconds, remove, wipe excessive TS, and observe the cut surface: a dark red-brown color develops in the endosperm.

Powdered Coix Seed

ヨクイニン末

Change the Identification and Purity as follows:

Identification Place a small amount of Powdered Coix Seed on a slide glass, add dropwise diluted iodine TS (1 in 10), and examine under a microscope ⟨*5.01*⟩: nearly equidiameter and obtuse polygonal simple and compound starch grains, usually 10 – 20 μm in diameter, have a reddish brown color. Small spheroidal starch grains, coexisting with fixed oil and with aleuron grains in parenchymatous cells, have a blue-purple color.

Purity Foreign matter—Under a microscope ⟨*5.01*⟩, Powdered Coix Seed reveals no fragments of tissue having silicified cell wall, no stone cells, no fragments of other thick-walled and lignified cells, no fragments of reticulate, scalariform and pitted vessels, no fragments of fibers and hairs. No, if any, a few large starch grains, more than 20 μm in diameter, appearing blue-purple upon addition of diluted iodine TS (1 in 10).

Cornus Fruit

サンシュユ

Change the Purity (2) as follows:

Purity

(2) Total BHC's and total DDT's ⟨*5.01*⟩—Not more than 0.2 ppm, respectively (A test sample for analysis is fine cuttings).

Digenea

マクリ

Change the Identification as follows:

Identification To 2 g of coarse cuttings of Digenea add 10 mL of dilute ethanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of kainic acid in 10 mL of dilute ethanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography ⟨*2.03*⟩. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl formate, water and formic acid (5:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly ninhydrin-ethanol TS for spraying on the plate, and heat 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 spot from the standard solution.

Epimedium Herb

インヨウカク

Change the Description as follows:

Description Epimedium Herb is composed of a stem and a ternate to triternate compound leaf; leaflet ovate to broadly ovate or ovate-lanceolate, 3 – 20 cm in length, 2 – 8 cm in width, petiolule 1.5 – 7 cm in length, apex of leaflet acuminate, needle hair on margin 0.1 – 0.2 cm in length, base of leaflet cordate to deeply cordate, lateral leaflet asymmetry; adaxial surface green to green-brown, sometimes lustrous, abaxial surface light green to light grayish green-brown, often pilose, especially on vein densely pilose, papery or coriaceous; petiole and stem cylindrical, light yellowish brown to slightly purplish and light green-brown, easily broken.

Odor, slight; taste, slightly bitter.

Under a microscope ⟨*5.01*⟩, a transverse section of the leaf reveals 3 – 6 vascular bundles in midvein; mesophyll composed of adaxial side epidermis, single-layered palisade, spongy tissue and abaxial side epidermis; leaf margins orbicular or oblong, sclerenchymatous; multi-cellular hairs on epidermis; 8 – 20 vascular bundles in petiole and 6 – 15 vascular bundles in petiolule. Under a microscope ⟨*5.01*⟩, a transverse section of the stem reveals a single to several-layered hypodermis, cortex of 4 – 10 cellular layers of sclerenchyma layer, vascular bundle 13 – 30 in number, oblong to obovate.

Gentian

ゲンチアナ

Change the Identification (1) as follows:

Identification **(1)** Place 0.1 g of pulverized Gentian on a slide glass, put a glass ring 10 mm in both inside diameter and in height on it, then cover with another slide glass, and heat gently and gradually: pale yellow crystals are sublimed on the upper slide glass. The crystals are insoluble in water and in ethanol (95), and soluble in potassium hydroxide TS.

Powdered Gentian

ゲンチアナ末

Change the Identification (1) as follows:

Identification **(1)** Place 0.1 g of Powdered Gentian on a slide glass, put a glass ring 10 mm in both inside diameter and in height on it, then cover with another slide glass, and heat gently and gradually: light yellow crystals are sublimed on the upper glass. The crystals are insoluble in water and in ethanol (95), and soluble in potassium hydroxide TS.

Goshajinkigan Extract

牛車腎気丸エキス

Change the Assay (3) Total alkaloids as follows:

Assay

(3) Total alkaloids (Benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride)—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 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. Centrifuge this solution, remove the diethyl ether layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the diethyl ether layer. To the aqueous layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, then centrifuge, and take the diethyl ether layer. To the aqueous layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the extracts, and evaporate the solvent under low pressure (in vacuo). Dissolve the residue in a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of benzoic acid for assay, and dissolve in a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, A_M, A_H and A_A, in the sample solution and the peak area of benzoic acid, A_S, in the standard solution.

Amount (mg) of benzoylmesaconine hydrochloride
$= M_S \times A_M/A_S \times 1/100 \times 4.19$

Amount (mg) of benzoylhypaconine hydrochloride
$= M_S \times A_H/A_S \times 1/100 \times 4.06$

Amount (mg) of 14-anisoylaconine hydrochloride
$= M_S \times A_A/A_S \times 1/100 \times 3.69$

M_S: Amount (mg) of benzoic acid for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylmesaconine and benzoylhypaconine; 254 nm for 14-anisoylaconine).

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 phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard solution TS for resolution check under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order, and the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine 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 benzoic acid is not more than 1.5%.

Hachimijiogan Extract

八味地黄丸エキス

Change the Assay (3) Total alkaloids as follows:

Assay

(3) Total alkaloids (Benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride)—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 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. Centrifuge this solution, remove the diethyl ether layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the diethyl ether layer. To the aqueous layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, then centrifuge, and take the diethyl ether layer. To the aqueous layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the extracts, and evaporate the solvent under low pressure (in vacuo). Dissolve the residue in a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of benzoic acid for assay, and dissolve in a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak

areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, A_M, A_H and A_A, in the sample solution and the peak area of benzoic acid, A_S, in the standard solution.

Amount (mg) of benzoylmesaconine hydrochloride
$= M_S \times A_M/A_S \times 1/100 \times 4.19$

Amount (mg) of benzoylhypaconine hydrochloride
$= M_S \times A_H/A_S \times 1/100 \times 4.06$

Amount (mg) of 14-anisoylaconine hydrochloride
$= M_S \times A_A/A_S \times 1/100 \times 3.69$

M_S: Amount (mg) of benzoic acid for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylmesaconine and benzoylhypaconine; 254 nm for 14-anisoylaconine).

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 phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard solution TS for resolution check under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order, and the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine 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 benzoic acid is not more than 1.5%.

Hedysarum Root

シンギ

Change the Description as follows:

Description Hedysarum Root is nearly cylindrical, 20 – 100 cm in length, 0.5 – 2.5 cm in diameter; outer surface yellow-brown to red-brown, with irregular longitudinal wrinkles; often horizontal lenticels and scars of lateral roots; periderm peeled easily, internally light yellow-brown to light red-brown; soft in texture, flexible and difficult to break; fractured surface fibrous, powdery; in transverse section nearly white in cortex, brownish around cambium, light yellow-brown in xylem; medullary ray obvious.

Odor, slightly characteristic; taste, slightly sweet.

Under a microscope <*5.01*>, a transverse section reveals cork layer 6 – 8 cells layered, 2 – 4 cells layered parenchyma cells with sparingly thick wall inside the cork layer; medullary ray obvious in secondary cortex and often appearing cracked tissue in outer portion of secondary cortex; phloem fiber bungles arranged stepwise in phloem; medullary ray obvious in xylem; xylem fibers around vessels; thin walled crystal cells containing solitary crystals of calcium oxalate in peripheral region of phloem fibers and xylem fibers; solitary crystals of calcium oxalate 7 – 20 μm in diameter; simple starch grains and 2- to 8-compound starch grains in parenchyma. Under a microscope <*5.01*>, a longitudinal section reveals reticulate, scalariform, pitted, and spiral vessels; crystal cell rows around phloem fibers and xylem fibers.

Jujube

タイソウ

Change the Purity (2) as follows:

Purity

(2) Total BHC's and total DDT's <*5.01*>—Not more than 0.2 ppm, respectively (A test sample for analysis is fine cuttings).

Leonurus Herb

ヤクモソウ

Change the Description as follows:

Description Stem, leaves, and flowers usually cross sectioned, stems square, 0.2 – 3 cm in diameter, yellow-green to green-brown in color, covered densely with white short hairs; the pith white, a great parts of central of cut surface. Light in texture. Leaves opposite, petiolated, 3-dissected to 3-incised, each lobe splits pinnately, and end lobes reveals linear-lanceolate, acute or acuminate, the adaxial surface light green, the abaxial surface covered densely with white short hairs, grayish green. Flower, verticillate; sepal, tubular, and the upper end acerate with five lobes; light green to light green-brown in color, corolla labiate, light red-purple to light brown.

Odor, slightly; taste, slightly bitter, astringent.

Under a microscope <*5.01*>, a transverse section of stem reveals four ridges, a part of the ridge of *Leonurus sibiricus* protruding knobby. Epidermis, observed non-glandular hairs from 1 to 3 cells, glandular hairs with head of 1 to 4 celled or glandular scale with 8 cells. Each ridge parts, beneath epidermis, collenchyma developed, development of xylem fibers remarkably. Cortex composed of several cellular layers parenchymatous cells. Collateral vascular bundle arranged in a circle. Phloem fibers observed at the outer portion of phloem. Parenchymatous cells of cortex and pith observe needle crystals or plate-like crystals of calcium oxalate.

Lindera Root

ウヤク

Change the Description as follows:

Description Fusiform or rosary-like root, 10 – 15 cm in length, 1 – 2.5 cm in diameter; externally yellow-brown to brown, with a few scars of rootlets; a transversely cut surface reveals cortex brown, xylem light yellow-brown, concentric circles and radially arranged lines brown; dense and hard in texture.

Odor, camphor-like; taste, bitter.

Under a microscope <*5.01*>, a transverse section of the root with secondary cortex reveals the outermost layer to be composed of a cork layer several cells thick, cork cells partially consisting of cork stone cells; secondary cortex sometimes contains oil cells and fibers; the root with secondary cortex removed, the outermost layer composed of cambium or secondary xylem; in xylem, vessels-xylem fibers and medullary rays are arranged alternately; parenchymatous cells of secondary cortex and xylem contain simple starch grains 1 – 15 μm in diameter, and 2- to 4- compound starch grains. Crystals of calcium oxalate are not observed or very few, if any.

Lonicera Leaf and Stem

ニンドウ

Change the Description as follows:

Description Stem with opposite leaves; leaf, ovate and entire, with short petiole, 3 – 7 cm in length, 1 – 3 cm in width; adaxial surface green-brown, abaxial surface light grayish green; under a magnifying glass, both surfaces pubescent. Stem, 1 – 4 mm in diameter; externally grayish yellow-brown to purplish brown, a transversely cut surface of stem, round and hollow.

Almost odorless; taste, slightly astringent, followed by a slight bitterness.

Under a microscope <*5.01*>, a transverse section of leaf reveals the outermost layer of adaxial and abaxial sides to be composed of a single-layered epidermis, uni-cellular non-glandular hairs and multi-cellular glandular hairs on epidermis; in midvein, several-cellular-layered collenchyma present beneath the epidermis and vascular bundles in the center; in mesophyll, palisade layer adjacent to adaxial side epidermis, spongy tissue adjacent to abaxial side epidermis; glandular hairs contain brown secretion, parenchymatous cells contain aggregate crystals of calcium oxalate, and occasionally starch grains.

Loquat Leaf

ビワヨウ

Change the Description as follows:

Description Loquat Leaf is an oblong to wide lanceolate leaf, 12 – 30 cm in length, 4 – 9 cm in width; pointed at the apex and wedge-shaped at the base; roughly serrate leaf with short petiole; occasionally being cut into strips 0.5 – 1 cm in shorter diameter and several cm in longer diameter; adaxial surface green to green-brown in color, abaxial surface light green-brown with light brown woolly hairs; vein, light yellow-brown in color, raised out on the abaxial surface of the leaf.

Odor, slight; practically tasteless.

Under a microscope <*5.01*>, a transverse section of Loquat Leaf reveals thick cuticle in epidermis on adaxial and abaxial sides; palisade tissue, mostly 4 to 5 cellular layers with several large cells without chloroplast; at main vein, ring of collateral bundle partly cut by intruding fundamental tissue at xylem side, and group of fiber attaching to phloem; solitary and clustered crystals of calcium oxalate in mesophyll tissue; woolly hair, unicellular and curved, about 25 μm in thickness, and up to 1.5 mm in length.

Lycium Fruit

クコシ

Change the Identification as follows:

Identification To 1.0 g of coarse cuttings of Lycium Fruit add 5 mL of ethyl acetate, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (10:1) to a distance of about 7 cm, and air-dry the plate: a yellow principal spot appears at an *R*f value of about 0.6.

Mentha Herb

ハッカ

Change the Description as follows:

Description Stem with opposite leaves; stem, square, light brown to red-purple in color, and with fine hairs; when smoothed by immersing in water, leaf, ovate to oblong, with acute apex and base, 2 – 8 cm in length, 1 – 2.5 cm in width, margin irregularly serrated; the adaxial surface, light brown-yellow to light green-yellow, and the abaxial surface, light green to light green-yellow in color; petiole 0.3 – 1 cm in length. Under a magnifying glass, leaf reveals hairs, glan-

dular hairs and scales.

It has a characteristic aroma and gives a cool feeling on keeping in the mouth.

Nelumbo Seed

レンニク

Change the Description as follows:

Description Ovoid to ellipsoidal seed, at the base a papillate protuberance surrounded with shallow depression, 1.0 – 1.7 cm in length, 0.5 – 1.2 cm in width; externally light red-brown to light yellow-brown; projection part dark reddish brown; endocarp not lustrous and hardly peeled off; cotyledon yellow-white, a green embryo in the center.

Almost odorless; taste, slightly sweet and oily, embryo is extremely bitter.

Under a microscope <*5.01*>, a transverse section of the seed at central portion reveals endocarp composed of parenchyma or endocarp occasionally left out; seed coat composed of epidermis and parenchyma of compressed cells; vascular bundles scattered in parenchyma; cotyledon observed inside seed coats; aggregate crystals of calcium oxalate and tannin-like substances contained in endocarp remained; parenchymatous cells of seed coat contain tannin-like substances; parenchyma of cotyledon contain starch grains.

Nutmeg

ニクズク

Change the Japanese commonly used name as follows (No effect to English text):

Nux Vomica Extract

ホミカエキス

Change the Description as follows:

Description Nux Vomica Extract occurs as yellow-brown to brown powder. It has a slight characteristic odor.

Nux Vomica Extract Powder

ホミカエキス散

Change the Description as follows:

Description Nux Vomica Extract Powder occurs as a yellow-brown to grayish brown powder. It has a slight, characteristic odor.

Nux Vomica Tincture

ホミカチンキ

Change the Description as follows:

Description Nux Vomica Tincture is a yellow-brown liquid.

Specific gravity d_{20}^{20}: about 0.90

Ophiopogon Root

バクモンドウ

Add the following next to the Description:

Identification To 5 g of moderately fine cuttings of Ophiopogon Root add 15 mL of water and 25 mL of ethyl acetate, shake for 10 minutes, centrifuge, and separate the ethyl acetate layer. Take 10 mL of this solution, evaporate the solvent under low pressure (in vacuo), dissolve the residue in 0.5 mL of acetone, and use this solution as the sample solution. Separately, dissolve 1 mg of methylophiopogonanone 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 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (30:10:1) 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 spot from the standard solution.

Change the Purity as follows:

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of moderately fine cuttings of Ophiopogon Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of moderately fine cuttings of Ophiopogon Root according to Method 4, and perform the test. Use 5.0 mL of Standard Arsenic Solution for the preparation of the standard color (not more than 5 ppm).

Perilla Herb

ソヨウ

Change the Description as follows:

Description Usually, contracted and wrinkled leaves, often with thin stems. Adaxial and abaxial surfaces of the

leaf are brownish purple in color, or the adaxial surface is grayish green to brownish green and the abaxial surface is brownish purple in color. When smoothed by immersion in water, the lamina is ovate to obcordate, 5 – 12 cm in length, 5 – 8 cm in width; the apex, acuminate; the margin, serrate; the base, broadly cuneate; petiole, 3 – 5 cm in length; cross sections of stem and petiole, square. Under a magnifying glass, hairs are observed on adaxial and abaxial surfaces of the leaf, but abundantly on the vein and sparsely on other parts; small glandular hairs are observed on the abaxial surface.

Odor, characteristic; taste, slightly bitter.

Pogostemi Herb

カッコウ

Change the Description as follows:

Description Stems with opposite leaves, leaves wrinkled and shriveled. When smoothed by immersion in water, leaves are obovate to ovate-oblong, 2.5 – 10 cm in length, 2.5 – 7 cm in width, with obtusely serrate margins and petioles at the cuneate bases; the adaxial surface of leaves dark brown, the abaxial surface grayish brown, both sides covered densely with hairs. Stems are square, solid, grayish green, covered with grayish white to yellow-white hairs; the pith broad, whitish, spongy. Under a magnifying glass, leaf reveals hairs, glandular hairs and glandular scales.

Odor, distinct; taste, slightly bitter.

Under a microscope <*5.01*>, a transverse section of petiole reveals central portion of the adaxial side protruding remarkably, with collenchyma cells beneath epidermis; vascular bundles at the center divided into two groups. Under a microscope <*5.01*>, a transverse section of the midvein of lamina reveals the adaxial side protruding remarkably, with collenchyma cells beneath epidermis; vascular bundles at the center arranged in fan-shape. Under a microscope <*5.01*>, a transverse section of stem reveals several-cells-layered collenchyma beneath epidermis, occasionally with cork layer developed; beneath cortex, collateral vascular bundles arranged in a circle, phloem fibers in groups observed at the outer portion of phloem; oil droplets observed in parenchymatous cells of cortex, needle, solitary or columnar crystals of calcium oxalate in parenchyma cells of pith.

Polygonatum Rhizome

オウセイ

Change the Identification and Purity as follows:

Identification **(1)** To 0.5 g of coarse cuttings of Polygonatum Rhizome add 2 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add gently 0.5 mL of sulfuric acid: a red-brown color appears at the zone of contact.

(2) To 1.0 g of coarse cuttings of Polygonatum Rhizome add 10 mL of dilute hydrochloric acid, boil gently for 2 minutes, and filter. Neutralize the filtrate with sodium hydroxide TS. To 3 mL of this solution add 1 mL of Fehling's TS, and warm: red precipitates appear.

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of coarse cuttings of Polygonatum Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 1.0 g of coarse cuttings of Polygonatum Rhizome according to Method 4, and perform the test. Use 5.0 mL of Standard Arsenic Solution for the preparation of the standard color (not more than 5 ppm).

Processed Aconite Root

ブシ

Change the Description as follows:

Description **1)** Processed Aconite Root 1: Cut pieces irregularly polygonal, less than 10 mm in diameter; externally dark grayish brown to black-brown; hard in texture; cut surface flat, light brown to dark brown, usually horny and lustrous.

Odor, weak and characteristic.

Under a microscope <*5.01*>, section reveals pitted, scaraliform, reticulate and spiral vessels; starch grains in parenchymatous cells usually gelatinized but sometimes not gelatinized; starch grains, spherical or ellipsoid, 2 – 25 μm in diameter, simple or 2- to a dozen or so- compound, hilum of starch grain distinct.

2) Processed Aconite Root 2: Nearly obconical root, 15 – 30 mm in length, 12 – 16 mm in diameter, slices cut longitudinally or transversely, 20 – 60 mm in length, 15 – 40 mm in width, and 0.2 – 0.7 mm in thickness, or cut pieces irregularly polygonal, less than 12 mm in diameter; externally light brown to dark brown or yellow-brown; in the case of Processed Aconite Root without metaderm, externally yellow-white to yellow-brown; hard in texture, usually without wrinkles; cut surface flat, light brown to dark brown or yellow-white to light yellow-brown, usually horny, semi-transparent and lustrous.

Odor, weak and characteristic.

Under a microscope <*5.01*>, transverse sections reveal metaderm, primary cortex, endodermis, secondary cortex, cambium, and xylem; in the case of Processed Aconite Root without metaderm, sometimes lacks parts of primary cortex and endodermis in addition to metaderm; primary cortex contains oblong to oblong-square sclerenchymatous cells, 30 – 75 μm in short axis, 60 – 150 μm in long axis; endodermis single layered cell, endodermal cells elongated in tangential direction; cambium, star shaped or irregular polygons to orbicular; a group of vessel in xylem v-shaped;

sometimes isolated ring of cambium appears in secondary cortex or in pith; starch grains in parenchymatous cells gelatinized. Under a microscope <*5.01*>, longitudinal section reveals pitted, scaraliform, reticulate and spiral vessels.

3) Processed Aconite Root 3: Cut pieces irregularly polygonal, less than 5 mm in diameter; externally grayish brown; hard in texture; cut surface flat, light grayish brown to grayish white, not lustrous.

Odor, weak and characteristic.

Under a microscope <*5.01*>, section reveals pitted, scaraliform, reticulate and spiral vessels; starch grains, spherical or ellipsoid, 2 – 25 μm in diameter, simple or 2- to a dozen or so-compound, hilum of starch grain distinct.

Pueraria Root

カッコン

Change the Description as follows:

Description Usually cut into small pieces of irregular hexagons of about 0.5 cm cube, or cut into longitudinally plate-like pieces 20 – 30 cm in length, 5 – 10 cm in width, and about 1 cm in thickness; externally light grayish yellow to grayish white; transverse section showing concentric annulate ring or part of it formed by abnormal growth of cambium. Under a magnifying glass, phloem light grayish yellow in color; in xylem, numerous vessels appearing as small dots; medullary rays slightly dented; vertical section showing longitudinal patterns formed alternately by fibrous xylem and parenchyma; easily breakable lengthwise, and its section extremely fibrous.

Almost odorless; taste, at first slightly sweet, followed by a slight bitterness.

Under a microscope <*5.01*>, a transverse section reveals fiber bundles accompanied by crystal cells in phloem; well-developed vessels and xylem fibers in xylem; starch grains numerous in parenchyma, mainly composed of polygonal simple grains, rarely 2- to 3-compound grains, 2 – 18 μm, mostly 8 – 12 μm, in size, with hilum or cleft in the center, and also with striae. Under a microscope <*5.01*>, a longitudinal section reveals crystal cell rows around phloem fibers.

Quercus Bark

ボクソク

Change the Description as follows:

Description Plate-like or semi-tubular pieces of bark, 5 – 15 mm in thickness; externally grayish brown to dark brown, with thick periderm and longitudinal coarse splits; internally brown to light brown, with longitudinal ridges, the transverse section brown to light brown, white small spots composed of stone cells in groups observed sporadically.

Almost odorless, tasteless.

Under a microscope <*5.01*>, a transverse section reveals a cork layer with scattered cork stone cells; in secondary cortex phloem fiber bundles lined almost stepwise, large groups of stone cells arranged irregularly; in parenchyma aggregated crystals of calcium oxalate scattered; adjacent to stone cells and phloem fiber, crystal cells containing solitary crystals of calcium oxalate observed. Under a microscope <*5.01*>, a longitudinal section reveals crystal cell rows attaching to fiber cells.

Rehmannia Root

ジオウ

Change the Identification and Purity as follows:

Identification **1)** Kan-jio—Sake 0.5 g of coarse cuttings of Rehmannia Root with 5 mL of water, add 20 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of stachyose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, water and methanol (3:2:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS on the plate, heat 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 spot from the standard solution. When further heat for more than 5 minutes, a blue spot is not observed at just lower than the spot mentioned above, or even appears it is only few.

2) Juku-jio—Sake 0.5 g of coarse cuttings of Rehmannia Root with 5 mL of water, add 20 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 2 mg of fructose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the standard solution (1). Separately, dissolve 3 mg of manninotriose for thin-layer chromatography in 1 mL of a mixture of water and methanol (1:1), and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 2 μL each of the sample solution and the standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, water and methanol (3:2:2) to a distance of about 7 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS on the plate, heat the plate at 105°C for 10 minutes: the principal spot obtained from the sample solution has the same color tone and *R*f value with the spot from the standard solution (1). Furthermore, one of the several spots from the sample solution has the same color tone and *R*f value with the blue spot from the standard so-

lution (2).

Purity **(1)** Heavy metals <*1.07*>—Proceed with 3.0 g of coarse cuttings of Rehmannia Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <*1.11*>— Prepare the test solution with 1.0 g of coarse cuttings of Rehmannia Root according to Method 4, and perform the test. Use 5.0 mL of Standard Arsenic Solution for the preparation of the standard color (not more than 5 ppm).

Rhubarb

ダイオウ

Change the Identification as follows:

Identification To 1.0 g of pulverized Rhubarb add 10 mL of water, shake, then add 10 mL of diethyl ether, shake for 10 minutes, centrifuge, and use the diethyl ether layer as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the spot from the standard solution, and the spot develops a red color on spraying sodium carbonate TS.

Powdered Rhubarb

ダイオウ末

Change the Identification as follows:

Identification To 1.0 g of Powdered Rhubarb add 10 mL of water, shake, then add 10 mL of diethyl ether, shake for 10 minutes, centrifuge, and use the diethyl ether layer as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 7 cm, and air-dry the plate: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the spot from the standard solution, and the spot develops a red color on spraying sodium carbonate TS.

Royal Jelly

ローヤルゼリー

Change the Assay as follows:

Assay Weigh accurately a portion of Royal Jelly, equivalent to 0.2 g of the dried substance, add 20 mL of methanol, sonicate for 30 minutes, and add methanol to make exactly 50 mL. Centrifuge this solution, pipet 2 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, then add 25 mL of water and methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of 10-hydroxy-2-(*E*)-decenoic acid for assay, dissolve in methanol to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 2 mL of the internal standard solution, then add 25 mL of water and methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of 10-hydroxy-2-(*E*)-decenoic acid to that of the internal standard.

$$\text{Amount (mg) of 10-hydroxy-2-(}E\text{)-decenoic acid} = M_S \times Q_T/Q_S \times 3/4$$

M_S: Amount (mg) of 10-hydroxy-2-(*E*)-decenoic acid for assay taken, calculated on the basis of the content obtained by qNMR

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

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

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

Mobile phase: A mixture of water, methanol for liquid chromatography and phosphoric acid (550:450:1).

Flow rate: Adjust so that the retention time of 10-hydroxy-2-(*E*)-decenoic acid is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, 10-hydroxy-2-(*E*)-decenoic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of 10-hydroxy-2-(*E*)-decenoic acid to that of the internal standard is not more than 1.0%.

Salvia Miltiorrhiza Root

タンジン

Change the Description as follows:

Description Nearly cylindrical root, 5 – 25 cm in length, 0.3 – 1.5 cm in diameter; slightly curved, often with lateral roots; outer surface red-brown, dark red-brown or black-brown; with irregular rough wrinkles; hard in texture, and easily broken; fracture surface fine or rough with clefts; cortex grayish yellow-white or red-brown, xylem light yellow-white or black-brown.

Odor, slight; taste, sweet at first and followed by slight bitterness and astringency.

Under a microscope <*5.01*>, a transverse section reveals usually cork layer in the outermost part, or rarely parenchyma or endodermis at the outside of the cork layer; several sclerenchyma cells observed or not in secondary cortex; cambium obvious; vessels radially arranged in secondary xylem, sometimes radial lines of vessels unite in the center of root; xylem fibers surrounding vessels; primary xylem divided into 2 – 3. Under a microscope <*5.01*>, a longitudinal section reveals vessels of secondary xylem mainly pitted and reticulate vessels.

Sappan Wood

ソボク

Change the Identification as follows:

Identification To 1 g of fine cuttings of Sappan Wood add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water, formic acid and 2-propanol (20:1:1:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly sodium carbonate TS on the plate, and air-dry the plate: a red-purple spot appears at an *R*f value of about 0.7.

Schisandra Fruit

ゴミシ

Change the Identification as follows:

Identification To 1.0 g of coarse cuttings of Schisandra Fruit add 10 mL of methanol, warm on a water bath for 3 minutes with shaking, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of schisandrin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 7 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the several spots obtained from the sample solution has the same color tone and *R*f value with the spot from the standard solution.

Scopolia Extract

ロートエキス

Change the Description as follows:

Description Scopolia Extract is brown to dark brown in color. It has a characteristic odor.

It dissolves in water with a slight turbidity.

Scopolia Extract Powder

ロートエキス散

Change the Description as follows:

Description Scopolia Extract Powder is a brownish yellow to grayish yellow-brown powder. It has a faint, characteristic odor.

Scopolia Extract and Carbon Powder

ロートエキス·カーボン散

Change the Description as follows:

Description Scopolia Extract and Carbon Powder is easily dustable and black in color.

Compound Scopolia Extract and Diastase Powder

複方ロートエキス·ジアスターゼ散

Change the Description as follows:

Description Compound Scopolia Extract and Diastase Powder is light yellow in color.

Scopolia Extract and Ethyl Aminobenzoate Powder

ロートエキス·アネスタミン散

Change the Description as follows:

Description Scopolia Extract and Ethyl Aminobenzoate Powder is slightly brownish white in color.

Senna Leaf

センナ

Change the Description as follows:

Description Lanceolate to narrow lanceolate leaflets, 1.5 – 5 cm in length, 0.5 – 1.5 cm in width, light grayish yellow to light grayish yellow-green in color; margin entire, apex acute, base asymmetric, petiole short; under a magnifying glass, vein marked, primary lateral veins running toward the apex along the margin and joining the lateral vein above; abaxial surface having slight hairs.

Odor, slight; taste, bitter.

Under a microscope <*5.01*>, a transverse section of Senna Leaf reveals thick cuticle in epidermis on adaxial and abaxial sides with numerous stomata, and with thick-walled, warty unicellular hairs; epidermal cells are often separated into two loculi by a septum which is in parallel with the surface of the leaf, and contain mucilage in the inner loculus; in mesophyll, palisade tissue of a single cellular layer under epidermis on adaxial and abaxial sides, spongy tissue consisting of 3 to 4 cellular layers between palisade tissues; clustered crystals of calcium oxalate in each tissue; cells adjacent to vascular bundles, crystal cells containing solitary crystals of calcium oxalate in vein. Under a microscope <*5.01*>, a longitudinal section reveals crystal cell rows around vascular bundles.

Shimbuto Extract

真武湯エキス

Change the Assay (3) Total alkaloids as follows:

Assay

(3) Total alkaloids (Benzoylmesaconine hydrochloride and 14-anisoylaconine hydrochloride, or benzoylmesaconine hydrochloride and benzoylhypaconine hydrochloride)—Weigh accurately about 1 g of Shimbuto Extract, 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. Centrifuge this solution, remove the diethyl ether layer, then add 20 mL of diethyl ether, proceed in the same manner as described above, and remove the diethyl ether layer. To the aqueous layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, shake for 30 minutes, then centrifuge, and take the diethyl ether layer. To the aqueous layer, add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all the extracts, and evaporate the solvent under low pressure (in vacuo). Dissolve the residue in a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 10 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of benzoic acid for assay, and dissolve in a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 100 mL. Pipet 10 mL of this solution, add a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas of benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine, A_M, A_H and A_A, in the sample solution and the peak area of benzoic acid, A_S, in the standard solution.

Amount (mg) of benzoylmesaconine hydrochloride
$= M_S \times A_M/A_S \times 1/100 \times 4.19$

Amount (mg) of benzoylhypaconine hydrochloride
$= M_S \times A_H/A_S \times 1/100 \times 4.06$

Amount (mg) of 14-anisoylaconine hydrochloride
$= M_S \times A_A/A_S \times 1/100 \times 3.69$

M_S: Amount (mg) of benzoic acid for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for benzoylmesaconine and benzoylhypaconine; 254 nm for 14-anisoylaconine).

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 phosphate buffer solution for processed aconite root and tetrahydrofuran (183:17).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of aconitum monoester alkaloids standard solution TS for resolution check under the above operating conditions, benzoylmesaconine, benzoylhypaconine and 14-anisoylaconine are eluted in this order, and the number of theoretical plates and the symmetry factor of the peak of benzoylmesaconine 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 benzoic acid is not more than 1.5%.

Add the following:

Shin'iseihaito Extract

辛夷清肺湯エキス

Shin'iseihaito Extract contains not less than 5 mg or not more than 20 mg of mangiferin, 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 23 mg and not more than 69 mg (for preparation prescribed 1.5 g of Gardenia Fruit) or not less than 45 mg and not more than 135 mg (for preparation prescribed 3 g of Gardenia Fruit) of geniposide, per extract prepared with the amount specified in the Method of preparation.

Method of preparation

	1)	2)
Magnolia Flower	3 g	2 g
Anemarrhena Rhizome	3 g	3 g
Lilium Bulb	3 g	3 g
Scutellaria Root	3 g	3 g
Gardenia Fruit	1.5 g	3 g
Ophiopogon Root	6 g	5 g
Gypsum	6 g	5 g
Cimicifuga Rhizome	1.5 g	1 g
Loquat Leaf	1 g	2 g

Prepare a dry extract as directed under Extracts, according to the prescription 1) or 2), using the crude drugs shown above.

Description Shin'iseihaito Extract occurs as a reddish yellow to yellow-red powder. It has a slight odor, and a slightly bitter, slightly acid and slightly sweet taste.

Identification **(1)** To 1.0 g of Shin'iseihaito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, to 1 g of powdered Magnolia Flower add 10 mL of methanol, shake, then centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <*2.03*>. Spot 5 μL of the sample solution and 10 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (3:1) to a distance of about 7 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat 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 red-brown to brown spot (*R*f value: about 0.4) from the standard solution (Magnolia Flower).

(2) To 2.0 g of Shin'iseihaito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer as the sample solution. Separately, to 1 g of pulverized Anemarrhena Rhizome add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer 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, 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, and heat the plate at 105°C for 2 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 yellowish red to dark red spot (*R*f value: about 0.3) from the standard solution (Anemarrhena Rhizome).

(3) To 1.0 g of Shin'iseihaito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Separate the diethyl ether layer, evaporate the solvent under low pressure (in vacuo), 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 hexane and acetone (7:5) 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 to grayish brown spot from the standard solution (Scutellaria Root).

(4) To 1.0 g of Shin'iseihaito Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer 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 of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) 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 1 minute: one of the several spots obtained from the sample solution has the same color tone and *R*f value with the red-purple to dark purple spot from the standard solution (Gardenia Fruit).

(5) Place 2.0 g of Shin'iseihaito Extract in a porcelain crucible, and ignite to incinerate at 500 – 550°C. To the residue add 60 mL of water, shake, centrifuge, and use the supernatant liquid as the sample solution. Add ammonium oxalate TS to the sample solution: a white precipitate is

formed. The precipitate does not dissolve in dilute acetic acid, but dissolves on the addition of dilute hydrochloric acid (Gypsum).

(6) To 1.0 g of Shin'iseihaito Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the 1-butanol layer 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 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (20:12:3) to a distance of about 7 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, 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 to yellow-green fluorescent spot from the standard solution (Cimicifuga Rhizome).

Purity **(1)** Heavy metals <*1.07*>—Prepare the test solution with 1.0 g of Shin'iseihaito Extract as directed under the Extracts (4), and perform the test (not more than 30 ppm).

(2) Arsenic <*1.11*>—Prepare the test solution with 0.67 g of Shin'iseihaito Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <*2.41*> Not more than 9.0% (1 g, 105°C, 5 hours).

Total ash <*5.01*> Not more than 14.0%.

Assay **(1)** Mangiferin—Weigh accurately about 0.5 g of Shin'iseihaito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of mangiferin for assay, dissolve in diluted methanol (1 in 2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of mangiferin in each solution.

$$\text{Amount (mg) of mangiferin} = M_S \times A_T/A_S \times 1/4$$

M_S: Amount (mg) of mangiferin for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 367 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 (1780:220:1).

Flow rate: 1.0 mL per minute.

System suitability—

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

(2) Baicalin—Weigh accurately about 0.1 g of Shin'iseihaito Extract, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin RS (separately determine the water <*2.48*> by coulometric titration, using 10 mg), and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of baicalin in each solution.

$$\text{Amount (mg) of baicalin } (C_{21}H_{18}O_{11}) = M_S \times A_T/A_S \times 1/4$$

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

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

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

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

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Geniposide—Weigh accurately about 0.5 g of Shin'iseihaito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of geniposide for assay, dissolve in

diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of geniposide in each solution.

$$\text{Amount (mg) of geniposide} = M_S \times A_T/A_S \times 1/2$$

M_S: Amount (mg) of geniposide for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

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

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

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

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (900:100:1).

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of geniposide are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

Containers and storage Containers—Tight containers.

Sweet Hydrangea Leaf

アマチャ

Change the Description as follows:

Description Usually wrinkled and contracted leaf, dark green to dark yellow-green in color. When soaked in water and smoothed out, it is lanceolate to acuminately ovate, 5 – 15 cm in length, 2 – 10 cm in width; margin serrated, base slightly wedged; coarse hair on adaxial and abaxial surfaces, especially on the veins; lateral veins not reaching the margin but curving upwards and connecting with each other; petiole short and less than one-fifth of the length of lamina.

Odor, slight; taste, characteristically sweet.

Tokishakuyakusan Extract

当帰芍薬散エキス

Change the Assay (1), (3) as follows:

Assay

(1) (*E*)-Ferulic acid—Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of (*E*)-ferulic acid for assay, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 2 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of (*E*)-ferulic acid in each solution.

$$\text{Amount (mg) of (}E\text{)-ferulic acid} = M_S \times A_T/A_S \times 1/50$$

M_S: Amount (mg) of (*E*)-ferulic acid for assay taken, calculated on the basis of the content obtained by qNMR

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 320 nm).

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

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

Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and add 2 mL of phosphoric acid. To 850 mL of this solution add 150 mL of acetonitrile.

Flow rate: 1.0 mL per minute [the retention time of (*E*)-ferulic acid is about 10 minutes].

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (*E*)-ferulic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of (*E*)-ferulic acid is not more than 1.5%.

(3) Atractylenolide III—Weigh accurately about 0.5 g of the dry extract (or an amount of the viscous extract, equivalent to about 0.5 g of the dried substance), add exactly 50 mL of diluted methanol (1 in 2), shake for 15

minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of atractylenolide III for assay, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of atractylenolide III in each solution.

Amount (mg) of atractylenolide III = $M_S \times A_T/A_S \times 1/40$

M_S: Amount (mg) of atractylenolide III for assay 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: A mixture of water, acetonitrile and phosphoric acid (550:450:1).

Flow rate: 1.0 mL per minute (the retention time of atractylenolide III is about 10 minutes).

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of atractylenolide III are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atractylenolide III is not more than 1.5%.

Uncaria Hook

チョウトウコウ

Change the Assay as follows:

Assay Weigh accurately about 0.2 g of moderately fine powder of Uncaria Hook, transfer into a glass-stoppered centrifuge tube, add 30 mL of a mixture of methanol and dilute acetic acid (7:3), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add two 10-mL portions of a mixture of methanol and dilute acetic acid (7:3), proceed in the same manner, and combine all of the supernatant liquid. To the combined liquid add a mixture of methanol and dilute acetic acid (7:3) to make exactly 50 mL, and use this as the sample solution. Separately, weigh accurately about 5 mg of rhynchophylline for assay, and dissolve in a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL. Pipet 1 mL of this solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 10 mL, and use this solution as the standard solution (1). Separately, dissolve 1 mg of hirsutine in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the standard solution (2). Perform the test with exactly 20 μL each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine the peak areas, A_{Ta} and A_{Tb}, of rhynchophylline and hirsutine obtained from the sample solution, and the peak area, A_S, of rhynchophylline from the standard solution (1).

Amount (mg) of total alkaloids (rhynchophylline and hirsutine)
= $M_S \times (A_{Ta} + 1.23A_{Tb})/A_S \times 1/20$

M_S: Amount (mg) of rhynchophylline for assay taken

Operating conditions—

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

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

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

Mobile phase: Dissolve 3.85 g of ammonium acetate in 200 mL of water, add 10 mL of acetic acid (100) and water to make 1000 mL, and add 350 mL of acetonitrile.

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

System suitability—

System performance: Dissolve 5 mg of rhynchophylline for assay in 100 mL of a mixture of methanol and dilute acetic acid (7:3). To 5 mL of this solution add 1 mL of ammonia solution (28), and heat under a reflux condenser for 10 minutes or warm at about 50°C for 2 hours. After cooling, to 1 mL of the solution so obtained add a mixture of methanol and dilute acetic acid (7:3) to make 5 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, the peak of isorhynchophylline appears in addition to the peak of rhynchophylline, and the resolution between these peaks is not less than 1.5.

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 areas of rhynchophylline is not more than 1.5%.

Yokukansankachimpihange Extract

抑肝散加陳皮半夏エキス

Change the origin/limits of content as follows:

Yokukansankachimpihange Extract contains not less than 0.6 mg and not more than 2.4 mg of saikosaponin b_2, not less than 10 mg and not more than

30 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), not less than 18 mg and not more than 72 mg of hesperidin, and not less than 0.15 mg of total alkaloids (rhynchophylline and hirsutine), per extract prepared with the amount specified in the Method of preparation.

Add the following next to the Assay (3):

Assay

(4) Total alkaloids (rhyncophylline and hirsutine)—Weigh accurately about 1 g of the dry extract (or an amount of the viscous extract, equivalent to about 1 g of the dried substance), add 20 mL of diethyl ether, shake, then add 3 mL of 1 mol/L hydrochloric acid TS and 7 mL of water, and shake for 10 minutes, centrifuge, and remove the diethyl ether layer. To the aqueous layer add 20 mL of diethyl ether, and proceed in the same manner as above. To the aqueous layer add 10 mL of sodium hydroxide TS and 20 mL of diethyl ether, shake for 10 minutes, centrifuge, and separate the diethyl ether layer. To the aqueous layer add 20 mL of diethyl ether, proceed in the same manner, and repeat this procedure twice. Combine all the extracts, evaporate the solvent under low pressure (in vacuo) at not more than 40°C, dissolve the residue in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg each of rhyncophylline for assay and hirsutine for assay, and dissolve in a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL. Pipet 10 mL of this solution, add the mixture of methanol and dilute acetic acid (7:3) 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_{TR} and A_{TH}, and A_{SR} and A_{SH}, of rhyncophylline and hirsutine, in each solution.

Amount (mg) of total alkaloids (rhyncophylline and hirsutine)

$$= (M_{SR} \times A_{TR}/A_{SR} + M_{SH} \times A_{TH}/A_{SH}) \times 1/50$$

M_{SR}: Amount (mg) of rhynchophylline for assay taken

M_{SH}: Amount (mg) of hirsutine for assay taken

Operation conditions—

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

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

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

Mobile phase: To 1 g of sodium lauryl sulfate add 600 mL of methanol, shake, then add 400 mL of water and 5 mL of acetic acid (100) to dissolve sodium lauryl sulfate.

Flow rate: 1.0 mL per minute.

Systemic 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 peaks of rhyncophylline and hirsutine 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 deviations of the peak area of rhyncophylline and hirsutine is not more than 1.5%, respectively.

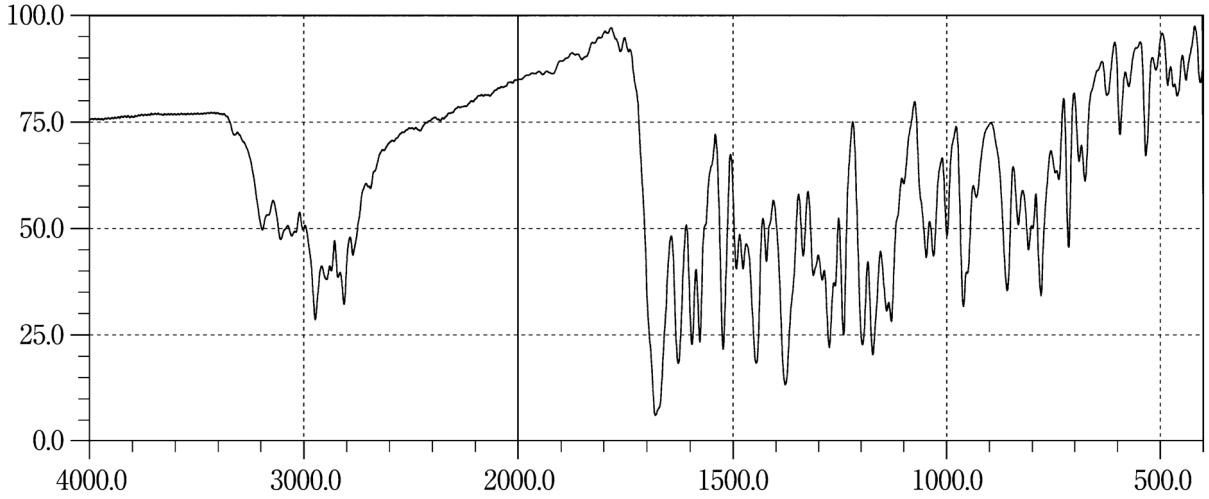
Infrared
Reference Spectra

Delete the following spectra:

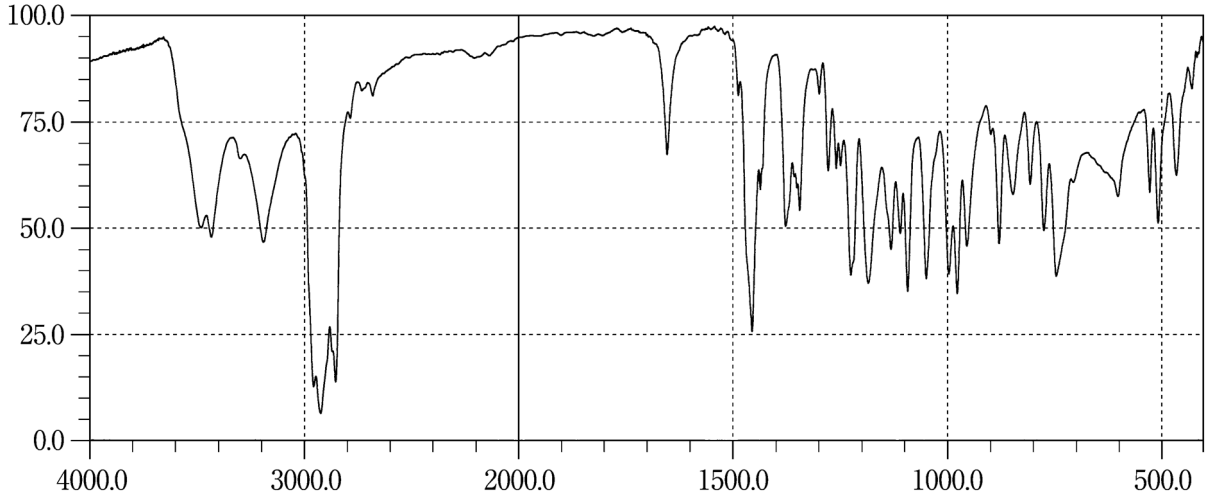
Clindamycin Phosphate

Add the following spectra:

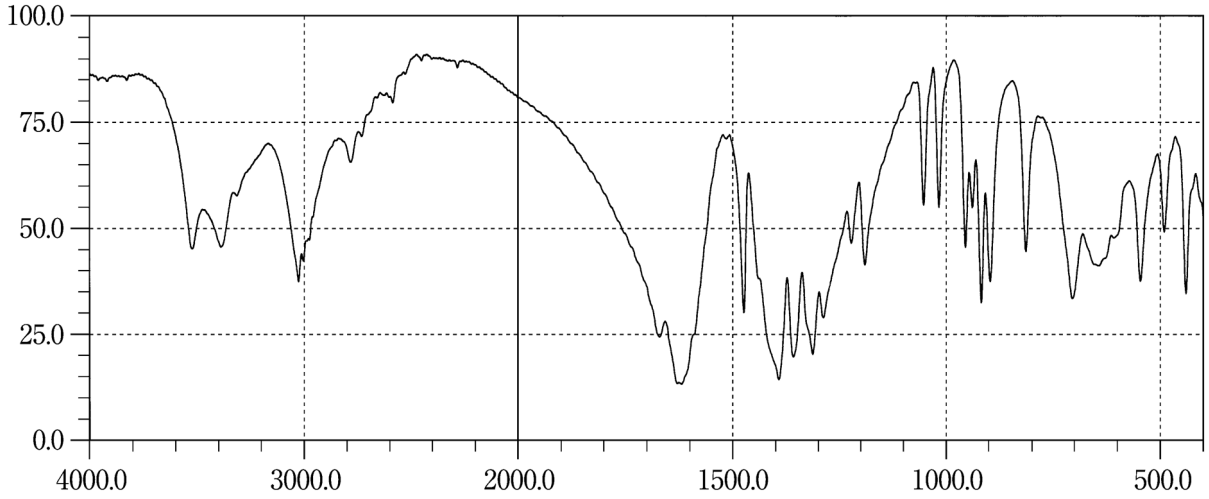
Aripiprazole



Cyclophosphamide Hydrate

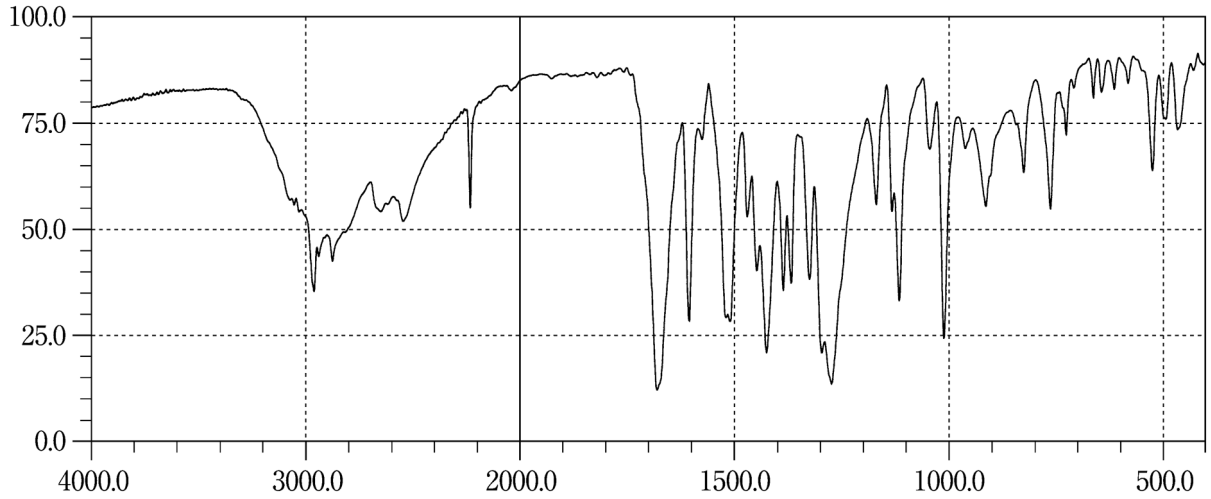


Disodium Edetate Hydrate

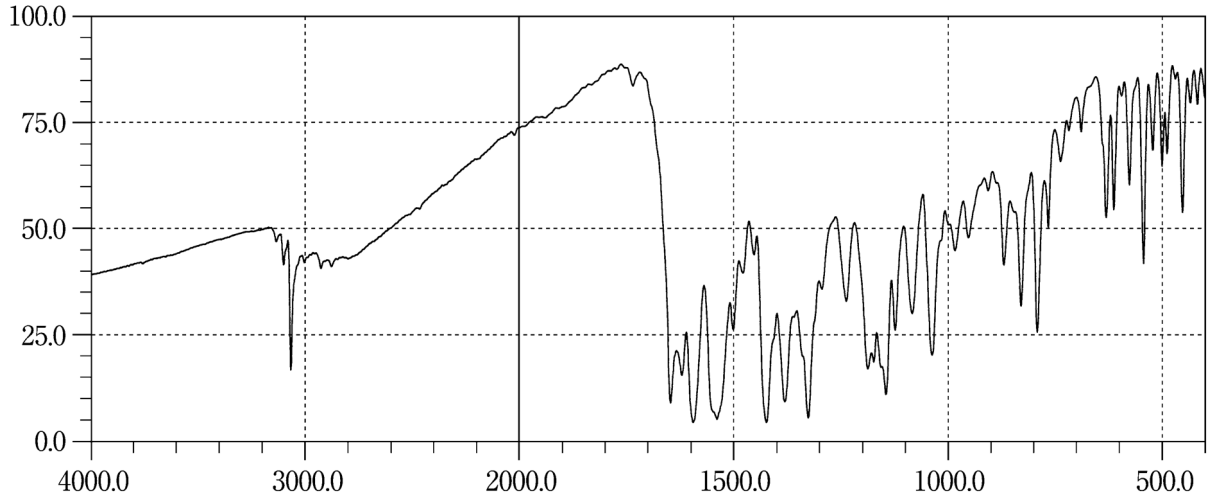


The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

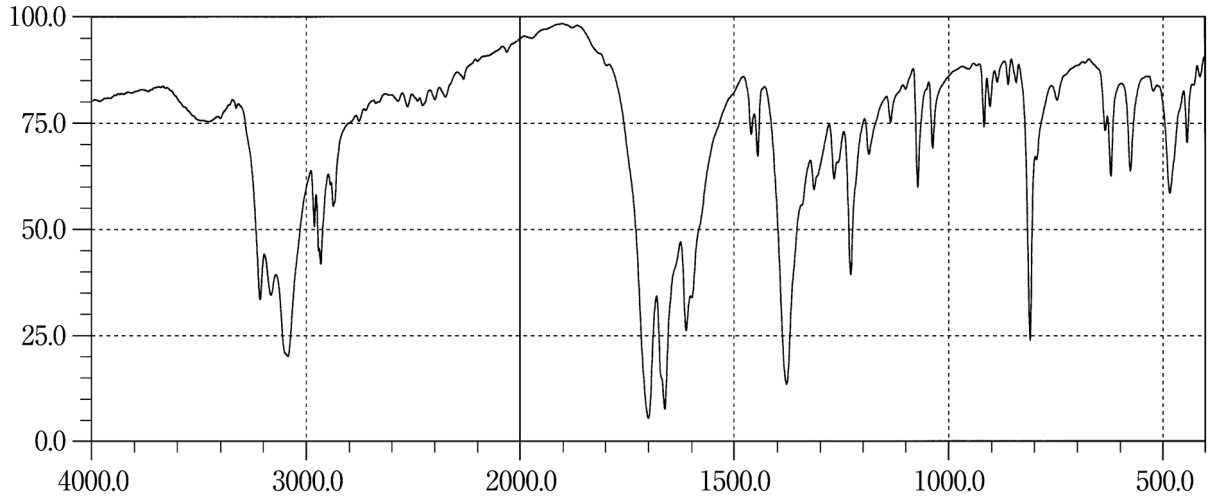
Febuxostat



Lornoxicam

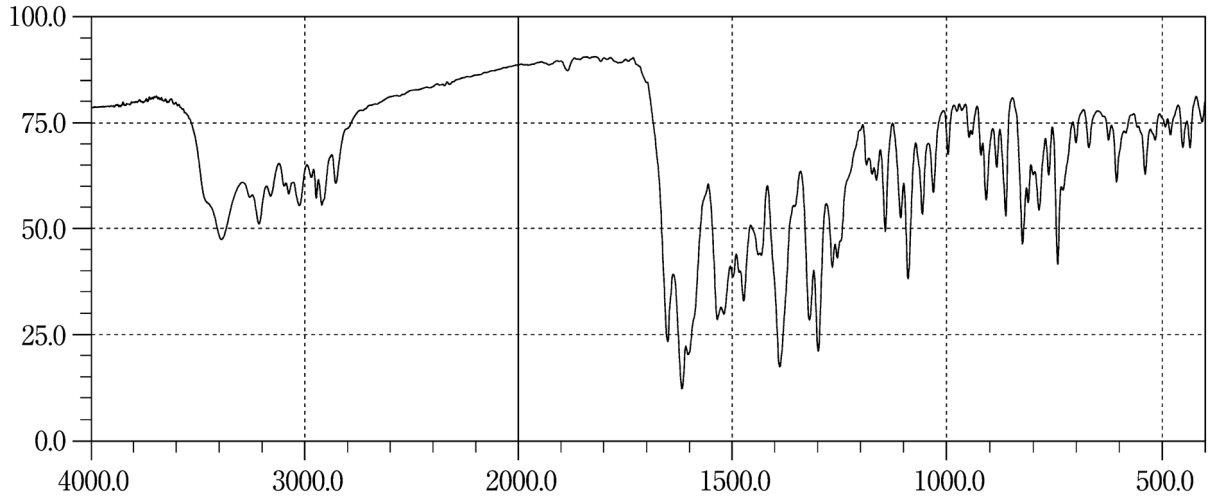


Oxaliplatin



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.*)

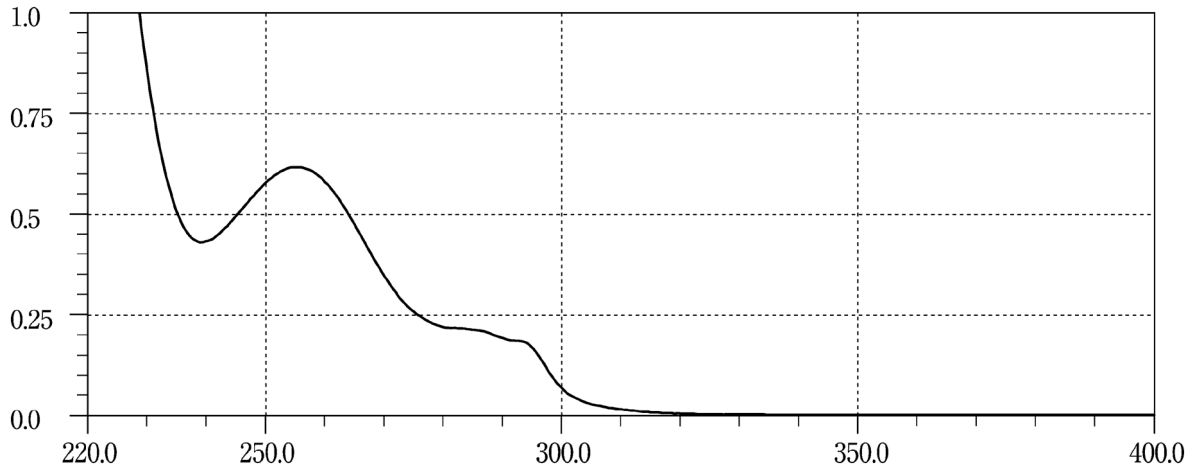
Tolvaptan



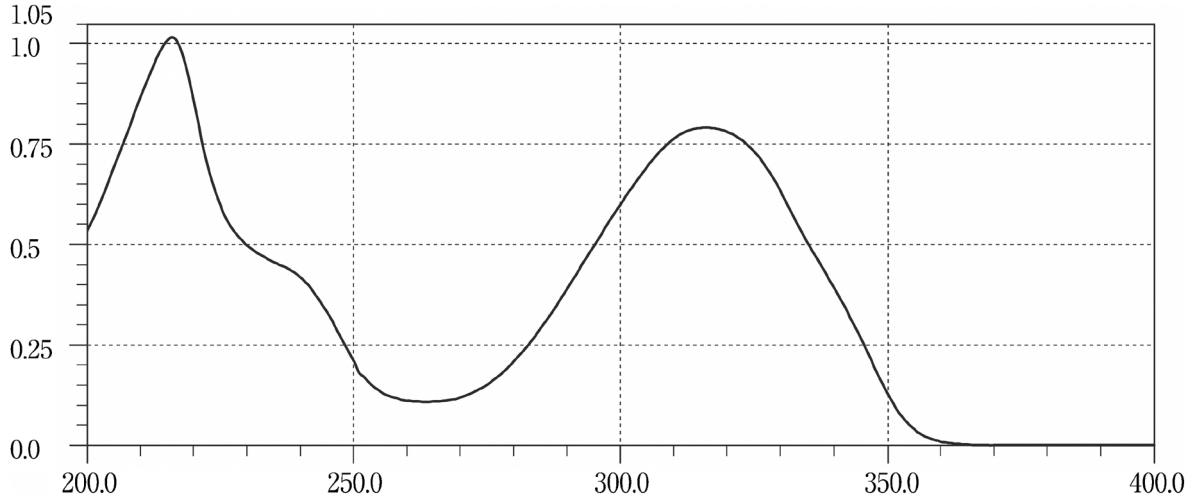
Ultraviolet-visible Reference Spectra

Add the following spectra:

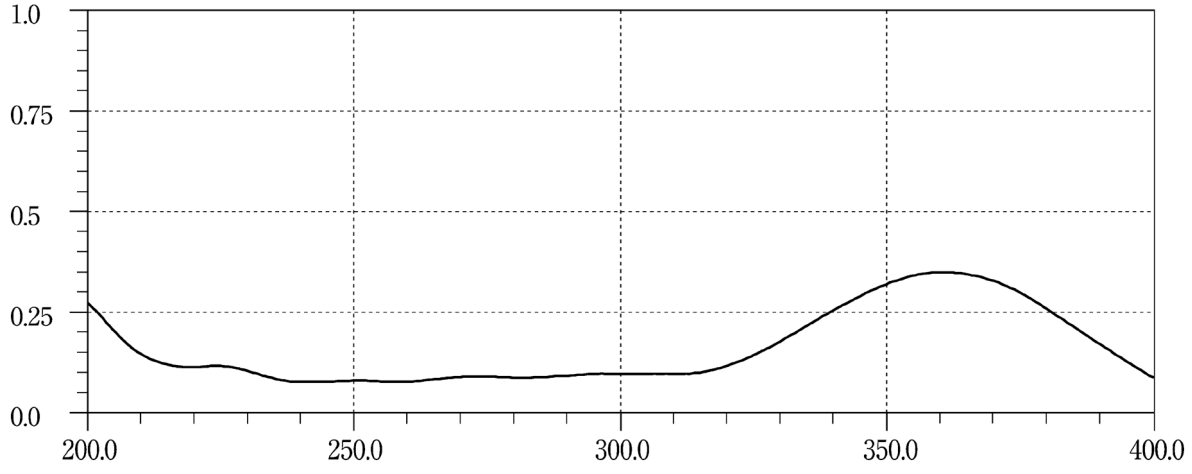
Aripiprazole



Febuxostat

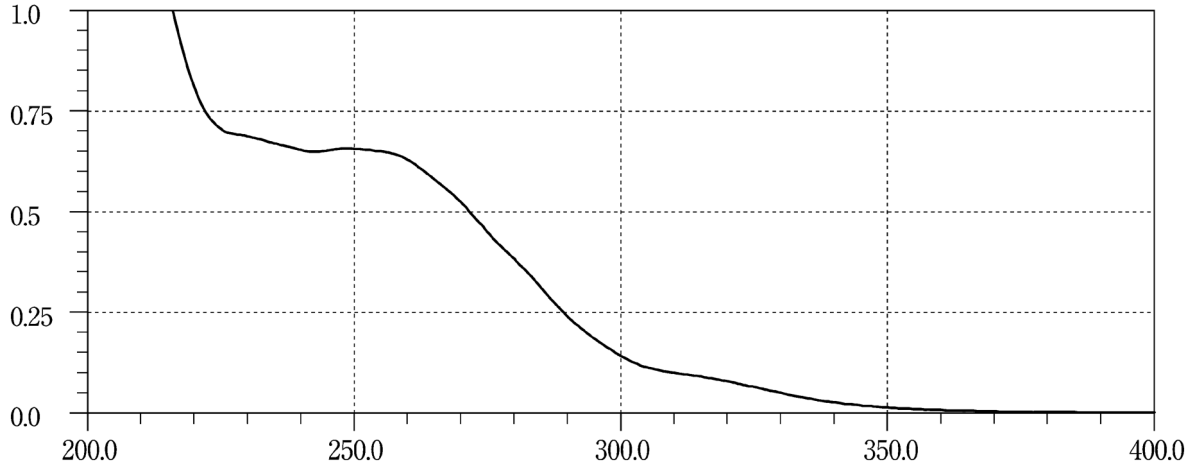


Lornoxicam

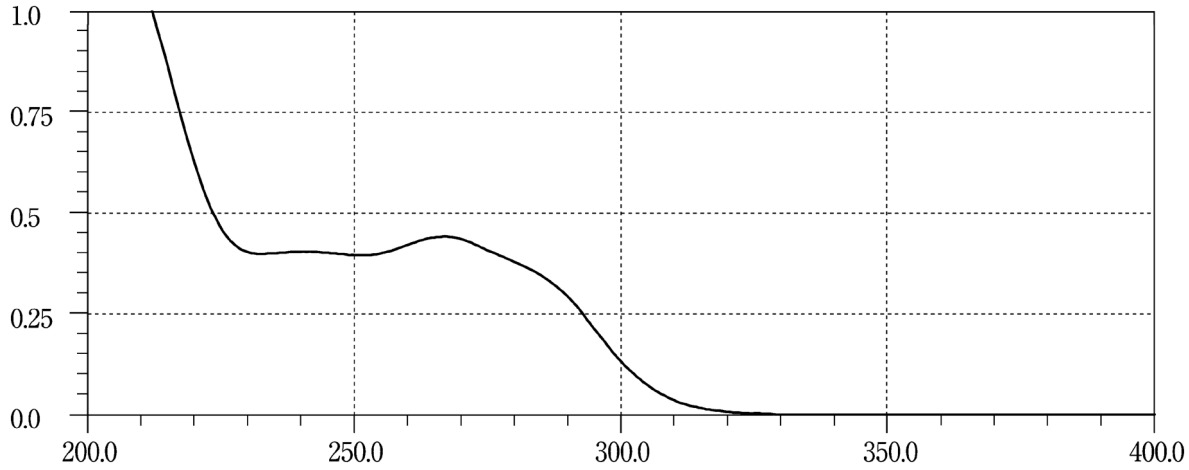


The JP Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. (*See the General Notices 5.*)

Oxaliplatin



Tolvaptan



General Information

GENERAL INFORMATION

General Information attached to the JP describes reference information and reference test methods which are necessary to ensure the quality of medicines. Therefore, General Information is positioned as important information supplementing the JP although it shall not be taken as indicating standards for conformity of drugs, except in the case specified when a drug is granted approval based on the Law on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices. Combination use of General Information and the JP can contribute to improving quality of the JP and user's convenience.

The general information is classified into either of the following categories according to its contents, and is individually numbered.

An individual number consists of three blocks. The left block indicates the category number, and the central block indicates the number within the category. The figures in the right block consist of the first two digits from the left indicating the JP at the recent revision (or new preparation, if never revised) and the third digit indicating as follows: 0 for major revision of the JP, 1 for supplement I, 2 for supplement II, and 3 for partial revision. For citation between the general information, the number corresponding to the general information is indicated in angle brackets < >.

G0. Basic Concepts on Pharmaceutical Quality
G1. Physics and Chemistry
G2. Solid-state Properties
G3. Biotechnological/Biological Products
G4. Microorganisms
G5. Crude Drugs
G6. Drug Formulation
G7. Containers and Package
G8. Reference Standards
G9. Pharmaceutical Excipients
GZ. Others

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

1. The following were newly prepared.

(1) Analyses of Sizes and Morphology of Nanoparticles by Atomic Force Microscope <*G1-9-182*>

(2) Concept of Weighing in the Japanese Pharmacopoeia <*G1-6-182*>

(3) Calibration and Performance Check of a Balance, and Weights <*G1-7-182*>

(4) Installation Environment and Basic Handling Method of a Balance, and Precautions for Weighing <*G1-8-182*>

(5) Flow Cytometry <*G3-16-182*>

(6) Evaluation Method of Insoluble Particulate Matter in Biotechnological Products (Biopharmaceuticals) Drug Substances/Drug Products by Flow Imaging Method <*G3-17-182*>

2. The following were revised.

(1) Solid and Particle Densities <*G2-1-182*>

(2) Powder Flow <*G2-3-182*>

(3) Peptide Mapping <*G3-3-182*>

(4) On the Scientific Names of Crude Drugs listed in the JP <*G5-1-182*>

(5) Thin-layer Chromatography for Crude Drugs and Crude Drug Preparations <*G5-3-182*>

3. The following was deleted.

(1) Measurement of the Diameter of Particles Dispersed in Liquid by Dynamic Light Scattering <*G2-4-161*>

G1 Physics and Chemistry

Add the following:

Analyses of Size and Morphology of Nanoparticles by Atomic Force Microscope <*G1-9-182*>

Atomic Force Microscopy (AFM) is a technique to analyze the size, morphology, and surface shape of nanoparticles with the images captured by detecting the atomic force between a sample surface and a tiny probe tip with a curvature radius in the order of nanometers (Fig. 1) mounted on a cantilever. It can be performed in air or liquid. Mechanical attributes of nanoparticles such as stiffness can also be determined as well. AFM has been used for the characterization of pharmaceuticals based on nanotechnology.

1. Equipment and operating principle

1.1. AFM system

The AFM system consists of a semiconductor laser, an AFM head (the component part of the instrument that a cantilever is mounted on), a cantilever with a probe tip, a sample stage, and a split photodiode, etc. and is equipped with an optical microscope and a charged coupled device (CCD) camera to properly align the laser beam irradiated at the cantilever (Fig. 1). The AFM system is placed on a vibration-isolation table to prevent any vibration that may affect the measurement.

1.2. AFM operating principle

The operating principle of AFM is generally described as follows (Fig. 1).

1) A semiconductor laser is irradiated to the back of the cantilever and the reflected laser beam is constantly monitored at the split photodiode.
2) As the cantilever approaches the vicinity of a sample surface, the cantilever deflects in response to the bend-

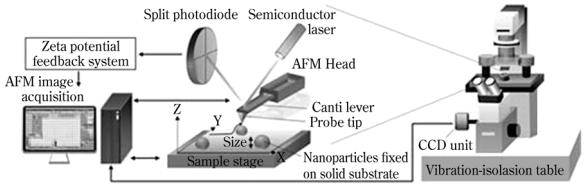


Fig. 1. Schematic diagram of a typical atomic force microscope system and PC for image acquisition[1)]

ing moment generated by the intersurface force (attractive or repulsive). The deflection is measured as upward or downward displacement of the laser detection position at the split photodiode.

3) For the deflection of the cantilever to remain constant, while the distance in the *z*-axis direction between the cantilever and the sample surface is controlled by a piezoelectric drive attached to the sample stage or AFM head, the cantilever is scanned in the directions of *x* and *y* directions of the sample.

Based on the operating principle as 1)～3) above, an AFM image is captured with the height information recorded per pixel. In actual image acquisition, the nanoparticles to be measured are fixed onto a flat solid substrate and the height of the particles is measured from the substrate surface. In measuring the size of nanoparticles, assuming that the object nanoparticles are spherical, the height measured by AFM is equivalent to the particle diameter. By further using a standard sample for calibration, the height in the *z*-axis direction in AFM images can be highly accurate and precise. On the other hand, information on the lateral (*x*, *y*) dimension obtained with AFM images needs to consider the difficulty of calibration and the influence of the geometry of the probe tip.

1.3. Other equipment

Acoustic enclosure: An acoustic wall box may be used to house the AFM system, to protect from vibration that may be caused by external sound.

UV cleaning system: This is used to clean the cantilever.

Temperature control system: This is used when samples need to be measured at a constant temperature.

2. Measurement

Measurement of the size of nanoparticles using AFM is generally performed in the following procedure.

2.1. Preparation of samples

Samples are prepared by dispersing the object nanoparticles in a suitable solvent at an appropriate concentration. The solvent and concentration are selected so as to maintain the stable dispersion of the nanoparticles.

2.2. Preparation of substrate for fixing nanoparticles

Fixing the sample to be observed onto a solid substrate is essential for acquiring AFM images. To select an appropriate substrate based on physicochemical properties of the sample to be observed is an important element in studying for the optimal conditions such as the number of the observed particles and the morphology.

To make sure a stable baseline for measuring the height, the substrate surface roughness needs to be sufficiently flat compared to the particles to be measured. The surface roughness (arithmetic mean roughness, which is the average of the absolute values of deviations from the center line for bumps and dips of the surface) is desirably not more than 5% of the size of the particles to be measured. It is also important that the physical properties of the substrate surface are relatively uniform for fixing nanoparticles easily.

In general, the surfaces of nanoparticles in a stable dispersion state are either positively or negatively charged, and the fixation of the nanoparticles onto a solid substrate is often made by electrostatic interaction. Negatively charged polystyrene standard nanoparticles, for example, can be easily fixed onto a positively charged solid substrate surface. A number of studies are required for selecting an appropriate solid substrate, particularly when the interactions are complex such that surface forces between a particles and a substrate depend on van der Waals or hydrophobic interactions, or such that deformation or disintegration of the soft particles to be fixed occurs due to the interaction with a substrate. A high-quality mica (muscovite mica), gold (111)-vapor fixed mica and single crystal silicon are commercially available representative substrates for AFM measurement. These substrates have atomically flat surfaces and can be surface-treated for controlling the electronic charge state of the substrate surface. For instance, to fix negatively charged nanoparticles, 0.3 vol% 3-aminopropyltriethoxysilane (APTES) solution can be used to treat the surface for a positive charge. Relatively flat cover glass with surface roughness of approximately not more than 5 nm is commercially available, which may be used as a substrate for particles with the size of approximately not less than 100 nm. It is desirable to acquire AFM images of the substrate in advance to get the information on the surface roughness of the substrate to be used.

2.3. Nanoparticle fixation on a solid substrate

Apply a liquid sample of nanoparticles onto a suitable substrate dropwise and incubate for a sufficient time to allow the particles fixed onto a substrate. When AFM images are acquired in air, after the incubation, rinse the substrate with ultrapure water to remove excess components such as salts and dry.

2.4. Acquisition of AFM images

2.4.1 Selection of measurement mode

Nanoparticles are fixed to a substrate by weak intermolecular interactions such as electrostatic interaction and van der Waals interaction. It is therefore important to minimize the force applied to the lateral dimension by an AFM measurement mode. One of the measurement modes to meet this requirement is the intermittent contact mode (also called dynamic mode, tapping mode, dynamic force mode, or amplitude-modulated mode), which is available for most commercially available AFM equipment. In recent years, however, the nonresonant mode (force curve mapping) that does not vibrate a cantilever may be used in observing a particularly soft sample or in the measurement of mechanical properties (e.g., stiffness).

In the intermittent contact mode, the cantilever is oscillated up and down at a frequency close to the resonance frequency by a small piezoelectric element fit in the cantilever holder. The amplitude of the oscillation is very sensitive to the distance between the probe tip and the sample, and the amplitude quickly becomes small upon the probe tip contacting the sample surface by dissipating the kinetic energy of the cantilever toward the sample. The particle surface in the sample is scanned by oscillating the cantilever up and down constantly with the distance between the probe tip and the sample being feed-back controlled to keep the oscillation amplitude constant, resulting in an advantage of almost nil force toward the lateral dimension. For this reason, this is a valid measurement mode also for those samples that move easily, have rough surface, are soft or with adsorption to the surface. Size measurement of nanoparticles can be made in air or in liquid environments by the intermittent contact mode. How to acquire images by the intermittent contact mode is described in the following.

2.4.2. Selection of cantilever

The characteristics and geometry of the cantilever and the probe tip attached to its end are important factors that determine the sensitivity and resolution of AFM. Points to consider are given in the following.

Images obtained by AFM include factors derived from both the probe tip shape and the shape of sample particles. In other words, the shape of the probe tip has effect in displaying the shape in the *x* and *y* directions of the nanoparticle, while it has no effect in measuring the height of a particle . Therefore, caution is required to deal with the information on the sizes of nanoparticles in the *x* and *y* directions. To minimize artifacts derived from the probe tip shape, use of a probe tip having the tip radius of not more than 10 nm is recommended.

Stable excitation oscillation of a cantilever is an important factor in imaging sample surfaces by the intermittent contact mode, and the use of a cantilever with large stiffness (a high spring constant) is desirable to overcome adhesive forces between the probe tip and sample particles (e.g., capillary, van der Waals, and electrostatic forces). On the other hand, since particles may be deformed by the force upon contact with the cantilever, it is thus desirable to use a cantilever with smaller stiffness (a lower spring constant) than that of the particles to be measured. A cantilever with a high resonance frequency may shorten the measurement time due to better scanning sensitivity, but caution is required for the damage to the particles to be measured due to the large stiffness (spring constant) in general. In addition, the selection of a cantilever with different stiffness may be necessary for observation in air or in liquid. The cantilever to be used should be determined in consideration of these points and should be optimized as required.

2.4.3. Acquiring AFM images

A prepared sample is placed on the sample stage, and an AFM image is acquired. The AFM image has information on the *x-y* plane coordinates and the vertical *z* coordinates. The number of the data points in the *x-y* plane, or the pixel number, must be considered, when acquiring and analyzing the image. For example, a 10 μm × 10 μm image with 200 pixels on a side acquired gives the size per pixel of 50 nm × 50 nm. With this setting condition, particles smaller than 50 nm are not discriminable. The scan size should therefore be set for the size of the particles to be measured. Generally, it is desirable to set a scan size to be 10 pixels or more per particle in the measurement. In the analysis of the average particle size and particle size distribution by AFM, assuring a random sampling of representative particles plays an important role. Generally, it is recommended to measure the size of at least 100 or so nanoparticles and to acquire images from different fields to avoid artificiality in measurements due to a single field of view. If the image quality becomes suddenly deteriorated during the acquisition, the cantilever should be cleaned or replaced as is often the cause that the cantilever gets dirt or worn.

An AFM image should be acquired under the same operation conditions using a substrate without the test nanoparticles fixed. This is to make sure that artifacts or foreign matters that may be misconstrued as the target nanoparticles are not contaminated from the operating process or the substrate itself.

3. Image analysis and size (height) measurement of nanoparticles

Acquired AFM images are analyzed after correcting the tilt of height on the images derived from the placement of the sample or the thermal drift of the equipment, using the software provided by the AFM equipment manufacturer (software by other developers for AFM image analysis can be also used as well). Essential operating procedure for data analysis in the size measurement of nanoparticles is described hereunder.

3.1 Size measurement by cross-sectional shape analysis

A cross-sectional shape profile in a vertical direction along a line drawn across any part of an image is obtained using the cross-sectional shape analysis tool of the software, thereby making measurement of distance in horizontal and vertical directions possible. With the cross-sectional shape profile, it is possible to know the agglomerating property of nanoparticles, as well as the height. The information can be obtained on the appropriateness as to correcting the tilt at the substrate around the nanoparticles. Cross-sectional shape analysis is performed with respect to individual nanoparticles in the image to measure the height. The benchmark for the height measurement may be the lowest point of all the data or the point where geometry of the particle begins to rise in the scanning direction, or the benchmark may be set by the operator's discretion. A series of measurement should be performed under the same conditions regardless of the benchmark chosen. To avoid influence of artifacts derived from the sample preparation, obvious foreign particles and large agglomerates that are not individually distinguishable should be excluded in calculating the average particle size.

3.2. Size measurement by automated particle analysis

Software can be used to automatically identify particles and enable the batch mode measurement of the particle size

in a short time. Particles are identified based on the threshold of height set by users. That is, particles with a height equal to or higher than the set threshold value are included in the analysis while those with a height lower than the set threshold value are excluded. In addition, obvious foreign particles or large agglomerates of indistinguishable particles are picked up by the software and can be excluded from the analysis. After the above operations, the maximum height of individual particles relative to the height of the substrate as the benchmark is automatically measured. In the automated particle analysis, caution is required for artificial influence in the results unless the tilt of the image to be analyzed is appropriately corrected. When performing automated particle analysis, the output of the results should be checked against the results obtained by the cross-sectional shape analysis to confirm the validity of the results. The average height of nanoparticles by the automated analysis software tends to be greater than the average height by the cross-sectional shape analysis. In addition, some software analyzes particle size based on the area occupied by individual particles in images. In this case, the particle size is analyzed as an area-equivalent diameter.

3.3. Analysis of nanoparticles having shapes other than true sphere

When evaluating particle size, if particles are deformed upon fixation to a substrate or non-spheric particles are to be analyzed, it is important to consider that parameters other than the height are additionally analyzed using particle analysis software. Where particles become deformed upon fixation to a substrate, for example, the volume-equivalent diameter may be used as a size evaluation parameter (Fig. 2A), assuming that the volume is constant before and after fixation to the substrate. In addition, it is also possible to obtain information on the deformed shape of particles under analysis based on the area-equivalent diameter, or the height/area-equivalent diameter ratio (Fig. 2A). When the particle under analysis is elliptically shaped, it is possible to measure the length of the major and minor axes, assuming that the particle corresponds to an ellipse, and is further possible to evaluate the shape based on the oblateness of the particle derived from the minor axis/major axis ratio (Fig. 2B). In the analysis of non-sphere particles with additional information on the lateral (x y) dimension, evaluate the tip shape of the cantilever using a calibration grating to pay attention because of the significant influence of the tip curvature.

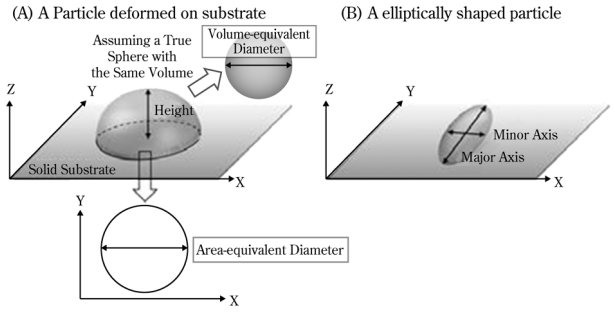


Fig. 2. Geometry evaluation of a particle deformed on a substrate (A) and an elliptically shaped particle (B)[1)]

3.4. Reporting size data

The size (height) distribution and the average with the standard deviation of nanoparticles measured should be reported. The information on the method used to fix the particles, the cantilever, the measurement mode, whether the measurement was made in air or in liquid, the number of the nanoparticles measured, and the analysis method for size should be described, as these factors involved in the measurement have impact on the results of size measurement of nanoparticles.

4. Verifying AFM performance

In AFM, the z-position of the cantilever is controlled for the distance by the expansion and contraction of the piezoelectric element. The expansion and contraction have properties such as non-linearity and hysteresis to the applied voltage. The z-height is determined based on the voltage applied to the piezoelectric element in conventional AFM. However, due to the properties mentioned above, 'height correction' based on a calibration curve prepared by measuring actual samples with certified height should be required. For example, selecting a calibration grating with a step height close to the height of nanoparticles to be measured, the average of step heights measured in at least three different locations using a sharp probe tip should be compared to the certified step height of the calibration grating used.

If the average value measured is significantly different from the certified value, it is necessary to consider the recalibration of the z-displacement of the piezo drive device by the manufacturer or others.

Some of recent AFM equipment is, on the other hand, equipped with a length-measuring sensor attached to the piezoelectric element and can precisely measure the degree of expansion and contraction, thereby allowing constant measurement of z-height. In other words, there exists the equipment with a control method that correct the height or the displacement.

References

1) Sakai-Kato, K, et al., Pharmaceutical and Medical Device Regulatory Science, 50, 634-640 (2019).
2) ASTM E2859-11: 2017 Standard Guide for Size Measurement of Nanoparticles using Atomic Force Microscopy.

Add the following:

Concept of Weighing in the Japanese Pharmacopoeia

<G1-6-182>

In the section of balances and weights in ''Measuring instrument, Appliances *<9.62>*'' in General Tests of the JP, it is required that balances and weights in the JP shall be

calibrated ensuring traceability to the International System of Units (SI).

Traceability in metrological measurement is defined as follows: ''property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.''[1] The most significant sources of metrological traceability are the following basic units of the International System of Units (SI): meter (length), kilogram (mass), second (time), ampere (electric current), kelvin (thermodynamic temperature), candela (light intensity), and mole (amount of substance). In the case of a balance, calibration that ensures traceability for mass shall be performed. Factors of traceability include a) an unbroken chain of comparisons, b), measurement uncertainty, c) documentation, d) technical ability, e) reference to the International System of Units (SI), and f) calibration, and f) is required in this chapter. In addition, for a balance used in the JP, the requirements for repeatability (precision) and accuracy (trueness) are specified, as well as being specified to perform calibration that ensures traceability to the International System of Units (SI). By meeting the requirements, weighing results can be traceable to the International System of Units (SI).

On the other hand, for metrology in the JP, results traceable to the International System of Units (SI) are not always required. This is clear because most reference standards and reference materials used in the JP are determined by the mass balance method, which is not traceable to the International System of Units (SI). Analysis in the JP are performed in accordance with the predetermined regulations to judge whether the specifications (values) are met.

In other words, if the specification for assay in each monograph is not less than 99.0%, when an analytical value is not less than 98.95% considering the number of significant digits and an analysis is performed according to the assay method specified in each monograph, it meets the JP. Therefore, it is important that accurate weighing up to the fourth significant digit is possible. It is known that a semimicrobalance, which can indicate up to the digit of 10 μg, generally has an error of not less than 130% (an error of not less than $\pm$ 13 μg) at the digit of the reading limit, even if it was calibrated in accordance with the above rules.[2] Therefore, for example, if the semimicrobalance displays 50.65432 g, including the tare weight, when about 0.1 g of a sample or a reference standard is weighed for the assay method, ''3'' at the digit of 100 μg is considered to be almost accurate, and therefore, it can be well used for weighing of samples and reference standards for the assay method. In most assay methods in the JP, the number of significant digits required is four at maximum. For example, the number of significant digits required for the calculation is three for a water content of 0.10% and loss on drying of 4.0%, and it is two for residue on ignition of 0.1%. Therefore, it is necessary to use a balance that satisfies these numbers of significant digits for analysis. In other words, in the JP, it is important to perform weighing in line with the concept that fits for the purpose. Therefore, when weighing 0.2 g of a drug used for the color reaction as an identification test or a purity test, two significant digits are enough for the balance used. On the other hand, when weighing about 5 mg of a reagent used for purity determination with quantitative NMR using an ultramicrobalance, for example, if 25.2345 mg, which includes the tare weight, is displayed, ''4'' at the digit of 1 μg is considered to be almost accurate. Because the number of significant digits used for the calculation of purity is three, the fourth digit is almost accurate as the weighed value of a reagent, and the balance can be well used even if the tare weight is about 20 mg. In addition, even if only a microbalance is available, when weighing not less than 10 mg of a reagent, up to the fourth significant digit is considered to be almost accurate.

In addition, when weighing, it is important to understand what kinds of error occur. Factors that cause errors during weighing with an appropriately calibrated balance include change in sensitivity, repeatability, linearity, and eccentricity, etc. Changes in sensitivity are caused by changes in gravitational acceleration applied to the place, temperature drift, and other factors. When a place where a balance is used is changed, sensitivity adjustment may be required because the gravitational acceleration applied to the place is different. In particular, an electronic balance displays mass being corrected according to the balance between the electromagnetic force and free fall acceleration (gravitational force). Therefore, the electronic balance, whose sensitivity was adjusted at the place before moving, displays mass that is different from the actual mass if the environment of the place after moving is different. In addition, displayed values change because of changes in the environment; therefore, the sensitivity shall be adjusted using the balance's built-in weight or external weight(s).

Repeatability is the closeness of agreement between values displayed when the same sample is weighed on the weighing pan of a balance multiple times and is an essential characteristic for the performance evaluation of a balance that has a high display resolution allowing a reading up to the digit of 10 μg or less.

Based on the results obtained from the requirements for repeatability shown in the section of balances and weights in the general test ''Measuring instruments, Appliances <*9.62*>'' of the JP, the minimum weight of the balance at that time is estimated. To make the weighing traceable to the International System of Units (SI), it is guided to weigh a mass larger than the minimum weight of the balance.

Because the minimum weight is affected by the installation environment of the balance (presence or absence of vibration at the installation location, etc.), temperature changes during weighing, and other factors, it is important for accurate weighing to record the minimum weight value routinely. The minimum weight is an estimated value that shows the lower limit of weighing to ensure the accuracy of the balance, not including the tare, and it is necessary that repeatability to ensure the precision of the smallest net weight using the standard deviation obtained by the requirements of repeatability (precision) is not more than 0.10%. In other words, it is necessary to weigh not less than the

minimum weight when performing weighing traceable to the International System of Units (SI). Factors that may affect repeatability (precision) of a balance are as follows:

1) The minimum weight indicates the performance of a balance and may change depending on the change of environment or the elapse of time.
2) Methods for weighing may differ among analysts. In other words, the minimum weight determined may vary among analysts.
3) Note that the standard deviation for a limited number of replicates is an estimated value of the true standard deviation and cannot actually be identified.
4) Determination of the minimum weight with a test weight may not be completely representative for the weighing application.
5) If the tare-container used affects mass depending on environment, it may affect the minimum weight.

Based on these factors, weighing shall be performed above the minimum weight in most cases. In other words, the smallest net weight using a balance actually shall be set larger than the minimum weight to some extent.

The error of linearity is the degree of deviation from the ideal straight line at each point, which divides the interval from the zero point to the balance's capacity almost evenly. The error of sensitivity is the degree of inclination of a straight line from the zero point, taking the error of linearity into account. Generally speaking, an error becomes larger from the zero point to the balance's capacity, and becomes significant in conjunction with environmental changes. Therefore, for the requirements of accuracy (trueness), use a test weight with mass near the upper limit of the weighing range, or slightly lower than the balance's capacity in order to confirm the allowable error of sensitivity. The error of eccentricity is the degree of change in the value displayed when a load is applied to a position distant from the center of the balance, and is less necessary to be taken into consideration unless a sample or sampling container has a special shape. Evaluation of accuracy (trueness) in a normal environment includes the three errors of sensitivity, linearity, and eccentricity, and the acceptance criterion, 0.10%, according to the error propagation rule (square root value of the sum of squares) satisfies the following equation.[2)]

$$0.10\% \fallingdotseq \sqrt{0.05\%^2[\text{err. of sen.}] + 0.05\%^2[\text{err. of lin.}] + 0.05\%^2[\text{err. of ecc.}]}$$

(err. = error, sen. = sensitivity, lin. = linearity, ecc. = eccentricity)

Therefore, in the requirements for accuracy (trueness), not more than 0.05% is required as the difference between the displayed value of a balance obtained by loading and unloading a weight once and the mass value of the weight. In other words, 0.05% each is allocated to the error of sensitivity and the error of linearity.

When the above-mentioned errors are considered, in the test of a balance, it is necessary to implement the requirements for repeatability (precision) and the error of sensitivity [accuracy (trueness)] for the purpose of confirming at least the precision for a point near 5% of the balance's capacity and the accuracy (trueness) for a point near the balance's capacity (or near the upper operating range). For confirmation of repeatability (precision), a weight with no change in mass is used, and for confirmation of accuracy (trueness), a weight with a calibration certificate traceable to the International System of Units (SI) is used. If the requirements for accuracy (trueness) are not met, the balance shall be adjusted and re-calibrated ensuring traceability with the value of uncertainty[3)].

References

1) ISO/IEC Guide 99: 2007, International vocabulary of metrology–Basic and general concepts and associated terms (VIM).
2) Reichmuth. A and Fritsch. K, Pharmaceutical Engineering 29(6), 46-58 (2009).
3) ISO/IEC Guide 98-3: 2008, Uncertainty of measurement Part 3: Guide to the expression of uncertainty in measurement (GUM:1995).

Add the following:

Calibration and Performance Check of a Balance, and Weights
<G1-7-182>

In the periodic calibration (including calibration when a device is introduced/installed) to qualify that a balance used satisfies the required performance, it is necessary to obtain calibration results that include uncertainties for weights used as the standard of mass and for a balance used as a measuring device for the purpose of ensuring traceability to the International System of Units (SI). To ensure the validity of calibration results, it is necessary that the calibration is performed in compliance with the international technical guidelines (ISO/IEC 17025, etc.), and it is recommended to obtain a properly documented calibration certificate accordingly.

The weight that conforms to the Japan Industrial Standards (JIS B 7609)[1)] and has nominal value or accuracy class that meets the requirements for a balance shall be selected. In some cases, it is enough to use only the nominal value of the test weight for performance check, but in the cases, the maximum permissible error determined by the indicated value and accuracy class of the weight shall not exceed one-third of the acceptance criterion for accuracy (trueness). Or, when the conventional mass value of the test weight for performance check (mass value when the density of the weight at 20°C is regarded as 8000 kg/m^3, and the density of air is regarded as 1.2 kg/m^3) is considered, the expanded uncertainty of the calibration shall not exceed one-third of the acceptance criterion. When multiple weights are used for performance check, it is necessary to sum up the uncertainties of calibration of the weights, and the sum shall not

exceed one-third of the acceptance criterion. In the performance check of eccentricity and repeatability, it is not specified to use a calibrated weight, but it shall be ensured that the mass of the test weight does not change during performance check.

Performance check using an external weight ensures that a balance meets the specifications required. Performance check of a balance shall be performed based on each standard operating procedure, and the frequency and interval of performance checks differ depending on the risks associated with the assaying method and weighing application. Performance check with an external weight can be replaced partially using automatic or manually triggered adjustment by means of built-in weight.

The table below shows check items, methods for determination, and acceptance criteria for each device characteristic for a balance.

Property	Definition	Method	Acceptance Criteria
Sensitivity error	Deviation between mass value and indicated value of a test weight	A value obtained by dividing the difference between the mass value of weight around the balance's capacity and the indicated value by the mass value of the weight	Not more than 0.05%
Linearity error	Deviation between the mass value and indicated value over the entire weighing range	The maximum value of deviation between the mass value and indicated value of a weight at each point, which was obtained by dividing the weighing range (from zero point to the balance's capacity) into 3 to 6 points	Not more than 0.05%
Eccentricity error	Deviation between the mass value and indicated value when weighing is performed at a position deviating from the center of the weighing pan	The maximum value of deviation among the indicated values of weights when they are placed at the center and the four corners of the weighing pan. At that time, the mass values of the weights shall be not less than 30% of the balance's capacity.	Not more than 0.05%
Repeatability	Closeness of agreement among indicated values when a same sample was repeatedly weighed in a short time under the same conditions (procedures, operator, place, etc.)	Calculate from the standard deviation of the indicated values obtained by loading and unloading a weight, whose weight is not less than 100 mg and about 5% of the balance's capacity, not less than 10 times.	Not more than 0.10%

The standard weights used for the verification and testing of a specified weighing instrument used for commercial transaction cannot be used for the confirmation of accuracy (trueness) of the balance used by the JP because they are not weights traceable to the International System of Units (SI) and are not calibrated with evaluation of uncertainties of measurement.

References

1) International Recommendation OIML R111-1:2004; The Japanese Standards Association, JIS B 7609: 2008, Weights

Add the following:

Installation Environment and Basic Handling Method of a Balance, and Precautions for Weighing

<G1-8-182>

The mass to be weighed should be usually larger than the minimum weight. Before weighing, tools used for weighing should be prepared and kept tidy and in order (cleaning), and the sensitivity of a balance should be adjusted. The installation environment and basic handling method of a balance, and precautions for weighing are shown as follows.

1. Installation environment of a balance

It is desirable to install a balance at a place whose surrounding environment is unchanged at any time in a room not too spacious, avoiding vibration sources, ventilation ports, and wall surfaces that receive heat radiation from the room light and direct sunlight. It is desirable to use a balance in the corner of a room or near a large pillar where the impact of vibrations is considered to be small. It is also desirable that a weighing table (anti-vibration table, vibration isolated table, etc.) on which a balance is installed has enough mass by itself and is so rigid that it is not distorted vertically even if the load of a heavy object is applied, and magnetism and static electricity are taken into consideration. Especially for a balance with the scale interval or readability of not more than 0.1 mg, precautions must be taken for installation or relocation because displayed values become unstable from the transmission of minor vibrations that cannot be sensed by humans to a sample itself or the reaction of the weighing sensor of the balance to minor vibrations. For the aspect of maintenance and control, an environment without sudden temperature changes, which may cause condensation, is required to avoid the deterioration of the components of a balance. The installation environment for a balance as an electronic device should be within a temperature range from 5°C to 40°C and relative humidity range from 20% to 80%, and a relative humidity of not less than 45% is desirable when considering the effect of static electricity.

2. Operation check before the use of a balance

The following items should be checked before using a balance.

2.1. Securement of a warming-up time

After turning on the power, secure a warming-up time to stabilize the internal temperature of the detector. It is desirable to secure a following warming-up time of not less than 30 minutes when the scale interval or readability is 10 mg or more, not less than 1 hour when it is 1 mg, not less than 2 hours when it is 0.1 mg, and not less than half a day when it is not more than 0.01 mg.

2.2. Check of installation condition

Check that a balance is leveled using the level gauge installed, for example, the air bubble in the level gauge is positioned at the center. For the level adjustment, it is desirable to visually check whether the balance is not unstable and whether there is no gap between the legs of the balance, which contact the weighing table, and the surface of the table.

2.3. Implementation of sensitivity adjustment

In the case of a balance equipped with a sensitivity adjustment function (equipped with an internal weight for adjustment), it is possible to perform appropriate sensitivity adjustments depending on the surrounding temperature condition at the zero point and around the balance's capacity. The higher the resolution, the greater the effect of changes in sensitivity, and measurement errors due to changes in sensitivity generally become relatively large from the zero point to around the mass weighed. As for a device that is not equipped with a sensitivity adjustment function, it is desirable to manually adjust sensitivity using the weight of around the balance's capacity.

3. Cleaning

To avoid weighing an object other than the target object, cleaning should be performed periodically. If the structure of a balance is understood, and the balance can be simply dismantled and cleaned, clean each part using a glass cleaner and lint free cloth frequently and keep the weighing pan and the inside of the weighing room clean.

4. Elimination of external factors that affect weighing results

External factors that affect weighing results must be eliminated as much as possible. In the case of samples that are prone to moisture absorption, adsorption, volatilization, or evaporation, measures must be taken according to the characteristics of the sample to prevent the deviation of weighed values. For example, when a hygroscopic sample is weighed, weighing with good repeatability is possible if a balance is installed in a constant temperature and humidity box, and the sample is weighed after it is acclimated to the specific temperature and humidity conditions in advance. External factors that affect weighing results other than the characteristics of a sample itself are shown below.

4.1. Temperature differences between area around the weighing pan and a sample (including sampling container)

Temperature differences between area around the weighing pan and a sample occur because of the refrigerated storage of a sample, bringing a sample from outside of the room with a different temperature, heat treatment, heat conduction by body temperature, and other factors. If the temperature of a sample and sampling container is higher than the temperature in the weighing room, slight upward wind (convection) generates around the weighing pan, and the sample and sampling container are pushed up by the phenomenon, resulting in a decrease or instability of displayed values. If the relationship of the temperature is contrary, an opposite trend appears. These phenomena are physical phenomena that occur around the weighing pan and therefore cannot be avoided even if a balance is equipped with a windshield device. Therefore, weighing should be performed under the condition where the temperature in the weighing room of a balance and the temperatures of a sample and a sampling container are as equivalent as possible.

4.2. Wind due to air conditioners, etc.

When the weighing pan is affected by an airflow generated by an air conditioner, access to the weighing room by people, and the weighing operation of a person who performs measurement, the displayed values become unstable. To suppress the impact of such wind, a windshield device should be installed to prevent direct wind onto the weighing pan. Or, relocate it to a place where there is no wind. When a balance equipped with an open/close door is used under the condition where it is affected by direct wind, it is important not to open the open/close door wider than necessary.

4.3. Static electricity

When using a sample such as powders and a sampling container, which are likely to be electrically charged by friction, or the inside of a weighing room is in a low humidity condition of relative humidity of 40% or lower, weighing results are affected by displayed values that fluctuate upward or downward due to the action of the force of electrical charges with a balance. The following items are considered to prevent such static electricity: keep humidity in the weighing room at 45% or higher, wait for dissipation of accumulated static electricity, and change the sampling container to an antistatic container. If these measures cannot be taken, it is recommended to perform the measurement after performing discharging as much as possible using an instrument, such as an ionizer, which neutralizes charged electrical charges or promotes the dissipation of the charges. However, avoid using an instrument that directly blows wind, which makes displayed values unstable, to the weighing pan during discharging.

G2 Solid-state Properties

Change the following as follows:

Solid and Particle Densities
<G2-1-182>

Density of a solid or a powder as a state of aggregation has different definitions depending on the way of including of the interparticulate and intraparticulate voids that exist between the particles and inside the particle. Different figures are obtained in each case, and there are different practical meanings. Generally, there are three levels of definitions of the solid or powder density.

(1) Crystal density: It is assumed that the system is homogeneous with no intraparticulate void. Crystal density is also called true density.

(2) Particle density: The sealed pores or the experimentally non-accessible open pores are also included as a part of the volumes of the solid or the powder.

(3) Bulk density: The interparticulate void formed in the powder bed is also included as a part of the volumes of the solid or the powder. Bulk density is also called apparent density. Generally, the powder densities at loose packing and at tapping are defined as the untapped bulk density and the tapped bulk density, respectively.

Generally, the densities of liquid or gas are affected only by temperature and pressure, but the solid or powder density is affected by the state of aggregation of the molecules or the particles. Therefore, the solid or powder densities naturally vary depending on crystal structure or crystallinity of the substance concerned, and also varies depending on the method of preparation or handling if the sample is amorphous form or partially amorphous. Consequently, even in a case that two solids or powders are chemically identical, it may be possible that the different figures of density are obtained if their crystal structures are different. As the solid or powder particle densities are important physical properties for the powdered pharmaceutical drugs or the powdered raw materials of drugs, the Japanese Pharmacopoeia specifies each density determination as ''3.03 Powder Particle Density Determination'' for the particle density and as ''3.01 Determination of Bulk Density'' for the bulk density.

The solid or powder densities are expressed in mass per unit volume (kg/m^3), and generally expressed in g/cm^3 ($1\ g/cm^3 = 1000\ kg/m^3$).

Crystal Density

The crystal density of a substance is the average mass per unit volume, exclusive of all voids that are not a fundamental part of the molecular packing arrangement. It is an intrinsic property concerning the specific crystal structure of the substance, and is not affected by the method of determination. The crystal density can be determined either by calculation or by simple measurement.

A. The calculated crystal density is obtained, for example, from the crystallographic data (volume and composition of the unit cell) obtained by the perfect crystal X-ray diffraction data from single crystal or indexing the powder X-ray diffraction data.
B. The measured crystal density is obtained as the mass to volume ratio after measuring the single crystal mass and volume.

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 ''3.03 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.

Bulk Density

The bulk density of a powder includes the contribution of interparticulate void volume as a part of the volume of the powder. Therefore, the bulk density depends on both the powder particle density and the space arrangement of particles in the power bed. Further, since the slightest disturbance of the bed may result in variation of the space arrangement, it is often very difficult to determine the bulk density with good reproducibility. Therefore, it is essential to specify how the determination was made upon reporting the bulk density.

The Japanese Pharmacopoeia specifies ''3.01 Determination of Bulk Density''.

A. The untapped bulk density is determined by measuring the apparent volume (untapped bulk volume) of a known mass of powder sample that has been passed through a screen in a graduated cylinder (constant mass method). Separately, the Japanese Pharmacopoeia specifies the method of determining bulk density by measuring the mass of powder in a vessel having a known volume (untapped bulk volume) (constant volume method).

B. The tapped bulk density is obtained by mechanically tapping a cylinder containing a powder sample. After determining the initial untapped bulk volume, carry out tapping a specified number of times under a fixed measurement condition (tapping rate and drop height), and the measurement is carried out repeatedly until the bulk volume variation obtained at consecutive two measurements is within an acceptable range (constant mass method). Separately, the Japanese Pharmacopoeia specifies the method of determining the tapped bulk density by measuring the mass of a fixed volume of the tapped powder (constant volume method).

Change the following as follows:

Powder Flow <*G2-3-182*>

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

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

The widespread use of powders in pharmaceutics has generated a variety of methods for characterizing powder flow. Not surprisingly, scores of references appear in the pharmaceutical literature, attempting to correlate the various measures of powder flow to manufacturing properties. The development of such a variety of test methods was inevitable; powder behavior is multifaceted and thus complicates the effort to characterize powder flow. The purpose of this chapter is to describe the methods for characterizing powder flow that are most frequently used in pharmaceutical applications. In addition, while it is clear that no single and simple test method can adequately characterize the flow properties of pharmaceutical powders, this chapter proposes the standardization of these test methods. For testing the powder flow, the four most commonly used methods are described below. Important experimental considerations are identified and recommendations are made regarding standardization of the methods: ''(1) angle of repose'', ''(2) compressibility index or Hausner ratio'', ''(3) flow through an orifice'', and ''(4) shear cell''.

In general, any method of measuring powder flow must be practical, useful, reproducible and sensitive, and must yield meaningful results. Replicate determinations are desirable for the determination using any of these techniques. It bears repeating that no simple powder flow method will adequately or completely characterize the wide range of flow properties experienced in pharmaceutical applications. An appropriate strategy may well be the use of multiple standardized test methods to characterize the various aspects of powder flow as needed by the pharmaceutical scientist.

1. Angle of repose

The angle of repose has been used in several branches of science to characterize the flow properties of solids. Angle of repose is a characteristic related to interparticulate friction, or resistance to movement between particles. Angle of repose test results are reported to be very dependent upon the method used. Experimental difficulties arise due to segregation and consolidation or aeration of the powder as the cone is formed. Despite its difficulties, the method continues to be used in the pharmaceutical industry, and a number of examples demonstrating its value in predicting manufacturing problems appear in the literature.

The angle of repose is the constant, three-dimensional angle (relative to the horizontal base) assumed by a cone-like pile of powder formed by any of several different methods, described briefly below.

1.1. Methods for angle of repose

A variety of angle of repose test methods are reported in the literature. The most common methods for determining the static angle of repose can be classified based on two important experimental variables:

(i) The height of the ''funnel'' through which the powder passes may be fixed relative to the base, or the height may be varied as the pile forms.

(ii) The base upon which the pile forms may be of fixed diameter or the diameter of the powder cone may be allowed to vary as the pile forms.

Variations of the above methods have also been used to some extent in the pharmaceutical applications.

(i) Drained angle of repose: This is determined by allowing an excess quantity of powder positioned above a fixed diameter base to drain from the container. Formation of a cone of powder on the fixed diameter base allows determination of the drained angle of repose.

(ii) Dynamic angle of repose: This is determined by filling a cylinder (with a clear, flat cover on one end) and rotating it at a specified speed. The dynamic angle of repose is the angle (relative to the horizontal) formed by the flowing powder. The internal angle of kinetic friction is defined by the plane separating those particles sliding down the top layer of the powder and those particles that are rotating with the drum (with roughened surface).

1.2. Relative ranking of flow for angle of repose

While there is some variation in the qualitative description of powder flow using the angle of repose, much of the pharmaceutical literature appears to be consistent with the classification by Carr[1)], which is shown in Table 1. There are examples in the literature of formulations with an angle of repose in the range of 40 to 50 degrees that manufactured satisfactorily. When the angle of repose exceeds 50 degrees, the flow is rarely acceptable for manufacturing purposes.

Table 1 Relative ranking of flow by angle of repose[1)]

Flow property	Angle of repose (degrees)
Excellent	25 – 30
Good	31 – 35
Fair (aid not needed)	36 – 40
Passable (may hang up)	41 – 45
Poor (must agitate, vibrate)	46 – 55
Very poor	56 – 65
Very, very poor	> 66

1.3. Experimental considerations for angle of repose

Angle of repose is not an intrinsic property of the powder, that is to say, it is very much dependent upon the method used to form the cone of powder. On this subject, the existing literature raises these important considerations:

(i) The peak of the cone of powder can be distorted by the impact of powder from above. By carefully building the powder cone, the distortion caused by impact can be minimized.

(ii) The nature of the base upon which the powder cone is formed influences the angle of repose. It is recommended that the powder cone be formed on a ''common base'', which can be achieved by forming the cone of powder on a layer of powder. This can be done by using a base of fixed diameter with a protruding outer edge to retain a layer of powder upon which the cone is formed.

1.4. Recommended procedure for angle of repose

Form the angle of repose on a fixed base with a retaining lip to retain a layer of powder on the base. The base must be free of vibration. Vary the height of the funnel to carefully build up a symmetrical cone of powder. Care must be taken to prevent vibration as the funnel is moved. The funnel height is maintained at approximately 2–4 cm from the top of the powder pile as it is being formed in order to minimize the impact of falling powder on the tip of the cone. If a symmetrical cone of powder cannot be successfully or reproducibly prepared, this method is not appropriate. Determine the angle of repose by measuring the height of the cone of powder and calculating the angle of repose, α, from the following equation:

$$\tan\alpha = \text{height} / (\text{diameter of base} \times 0.5)$$

2. Compressibility index and Hausner ratio

The compressibility index and the closely related Hausner ratio may predict powder flow characteristics as being affected by e.g., size and shape, material density, surface area, moisture content, and cohesiveness of powder. The compressibility index and the Hausner ratio are calculated from the untapped and tapped bulk density or untapped and tapped bulk volume of a powder. For additional information see Determination of Bulk Density <*3.01*>.

2.1. Methods for compressibility index and Hausner ratio

While there are some differences in the method of determining the compressibility index and Hausner ratio, the basic procedure is to measure the untapped bulk volume, V_0, and the final tapped bulk volume, V_f, of the same powder sample after tapping the powder until no further volume changes occur. The compressibility index and the Hausner ratio are calculated as follows:

$$\text{Compressibility index} = (V_0 - V_f) / V_0 \times 100$$
$$\text{Hausner ratio} = V_0 / V_f$$

Alternatively, the compressibility index and Hausner ratio may be calculated using measured values of untapped bulk density ($\rho_{untapped}$) and tapped bulk density (ρ_{tapped}) as follows:

$$\text{Compressibility index} = (\rho_{tapped} - \rho_{untapped}) / \rho_{tapped} \times 100$$
$$\text{Hausner ratio} = \rho_{tapped} / \rho_{untapped}$$

In a variation of these methods, the rate of consolidation is sometimes measured rather than, or in addition to, the change in volume that occurs on tapping. For the compressibility index and the Hausner ratio, a commonly reported relative ranking of flow is given in Table 2.

Table 2 Relative ranking of flow by compressibility index and Hausner ratio

Compressibility index (%)	Flow character	Hausner ratio
1 – 10	Excellent	1.00 – 1.11
11 – 15	Good	1.12 – 1.18
16 – 20	Fair	1.19 – 1.25
21 – 25	Passable	1.26 – 1.34
26 – 31	Poor	1.35 – 1.45
32 – 37	Very poor	1.46 – 1.59
> 38	Very, very poor	> 1.60

Compressibility index and Hausner ratio are not intrinsic properties of the powder, that is to say, they are dependent upon the methodology used. Several important considerations affecting the determination of the untapped bulk volume, V_0, the final tapped bulk volume, V_f, the untapped bulk density, $\rho_{untapped}$, and the tapped bulk density, ρ_{tapped}, are the following:

(i) The diameter and the mass of the graduated cylinder used with its holder

(ii) The number of times the powder is tapped to achieve the tapped bulk density

(iii) The apparatus drop height

(iv) The mass of powder used in the test

(v) Rotation of the sample during tapping

3. Flow through an orifice

The flow of a powder depends upon many factors, some of which are particle-related and some related to the process. Monitoring its ability to flow through an orifice (by assessing the ''arching diameter,'' the orifice diameter at which the powder arches and is no longer able to discharge) and its flow rate have been used to measure powder flow. Of particular significance is the utility of monitoring flow continuously, since pulsating flow patterns have been observed even for free-flowing powders. Changes in flow rate

as the container empties can also be observed. Empirical equations relating flow rate to the diameter of the opening, particle size, and particle density have been determined. Whereas assessing the arching diameter of a powder may be used for cohesive and free-flowing powders, determining the flow rate through an orifice is useful only with free-flowing powders.

The flow rate through an orifice is generally measured as the mass per time flowing from any of a number of types of containers (cylinders, funnels, hoppers). Measurement of the flow rate can be in discrete increments or continuous.

3.1. Methods for flow through an orifice

There are a variety of methods described in the literature. The most common for determining the flow through an orifice can be classified based on three important experimental variables:

(1) The type of container used to contain the powder. Common containers are cylinders, funnels and hoppers from production equipment.

(2) The size and shape of the orifice used. The orifice diameter and shape are critical factors in determining powder flow.

(3) The method of measuring powder flow rate. Flow rate can be measured continuously using an electronic balance and with some sort of recording device (strip chart recorder, computer). It can also be measured in discrete samples (for example, the time it takes for 100 g of powder to pass through the orifice to the nearest tenth of a second or the amount of powder passing through the orifice in 10 seconds to the nearest tenth of a gram).

3.2. Variations in methods for flow through an orifice

Either mass flow rate or volume flow rate can be determined. Mass flow rate is the easier of the methods, but it biases the results in favor of high-density powders. Since die fill is volumetric, determining volume flow rate may be preferable. A vibrator is occasionally attached to facilitate flow from the container, however, this appears to complicate interpretation of results. A moving orifice device has been proposed to more closely simulate rotary press conditions. The minimum diameter orifice through which powder flows can also be identified.

No general scale is available because flow rate is critically dependent on the method used to measure it. Comparison between published results is difficult.

3.3. Experimental considerations for flow through an orifice

Flow through an orifice is not an intrinsic property of the powder. It is very much dependent upon the methodology used. The existing literature points out several important considerations affecting these methods:

(i) The diameter and shape of the orifice

(ii) The type of container material (metal, glass, plastic)

(iii) The diameter and height of the powder bed.

3.4. Recommended procedure for flow through an orifice

Flow rate through an orifice can be used only for powders that have some capacity to flow. It is not useful for cohesive powders. Provided that the height of the powder bed (the 'head' of powder) is much greater than the diameter of the orifice, the flow rate is virtually independent of the powder head. It is advisable to use a cylinder as the container, because the walls of the container must have little effect on flow. This configuration results in flow rate being determined by the movement of powder over powder, rather than powder along the wall of the container. Powder flow rate often increases when the height of the powder column is less than two times the diameter of the column. The orifice must be circular and the cylinder must be free of vibration. General guidelines for dimensions of the cylinder are as follows:

(i) Diameter of the opening greater than 6 times the diameter of the particles

(ii) Diameter of the cylinder greater than twice the diameter of the opening

Use of a hopper as the container may be appropriate and representative of flow in a production situation. It is not advisable to use a funnel, particularly one with a stem, because flow rate will be determined by the size and length of the stem as well as the friction between the stem and the powder. A truncated cone may be appropriate, but flow will be influenced by the powder-wall friction coefficient, thus, selection of an appropriate construction material is important.

For the opening in the cylinder, use a flat-faced bottom plate with the option to vary orifice diameter to provide maximum flexibility and better ensure a powder-over-powder flow pattern. Rate measurement can be either discrete or continuous. Continuous measurement using an electronic balance can more effectively detect momentary flow rate variations.

4. Shear cell methods

In an effort to put powder flow studies and hopper design on a more fundamental basis, a variety of powder shear testers and methods that permit more thorough and precisely defined assessment of powder flow properties have been developed. Shear cell methodology has been used extensively in the study of pharmaceutical powders. From these methods, a wide variety of parameters can be obtained, including the yield locus representing the shear-stress to normal-stress relationship at incipient flow, the angle of internal friction, the unconfined yield strength, powder cohesion, and a variety of related parameters such as the flow function coefficient. Because of the ability to control experimental parameters more precisely, flow properties can also be determined as a function of consolidation load, time, and other environmental conditions. These methods have been successfully used to determine critical hopper and bin dimensions.

4.1. Methods for shear cell

One type of shear cells corresponds to translational shear cells which are split horizontally, forming a shear plane between the stationary and the movable portion of the shear cell. After powder bed consolidation in the shear cell (using a well-defined procedure), the force necessary to shear the powder bed is determined. Translational shear cells may have a cylindrical shape or a rectangular box shape.

A second type of shear cell corresponds to rotational shear cells. These include cylindrical shape and annular shape cells. Their design offers some advantages over the translational shear cell design, including the need for less material. A disadvantage, however, is that because of their design, the powder bed is not sheared as uniformly because material on the outside of the rotational shear cell is sheared more than material in the inner region.

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.

4.2. Recommendations for shear cell

The many existing shear cell configurations and test methods provide a wealth of data and can be used very effectively to characterize powder flow. They are also helpful in the design of equipment such as hoppers and bins. Because of the diversity of available equipment and experimental procedures, no specific recommendations regarding methodology are presented in this chapter. It is recommended that the results of powder flow characterization using shear cell methodology include a complete description of equipment and methodology used.

5. Reference

1) Carr R.L.: Evaluating flow properties of solids. *Chem. Eng*. 1965; 72: 163-168.

Delete the following:

Measurement of the Diameter of Particles Dispersed in Liquid by Dynamic Light Scattering *<G2-4-161>*

G3 Biotechnological/Biological Products

Peptide Mapping *<G3-3-182>*

Change the following as follows:

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

Information on the harmonization with the European Pharmacopoeia and the U.S. Pharmacopeia is available on the website of the Pharmaceuticals and Medical Devices Agency.

1. Introduction

Proteins can exist as large complex structures, with some molecules in the population displaying heterogeneity in their amino acid sequence due to improper assembly, degradation or post-translational modification. The high molecular mass of proteins combined with their complexity makes it particularly challenging to chemically identify an intact protein product using a single analytical method. It is possible to cleave the test protein into smaller fragments which can be identified with sufficient mass resolution to determine the amino acid sequence of the protein. This process is the basis of the protein identification technique commonly known as peptide mapping. The peptide mapping technique involves a digestion step in which the protein is selectively cleaved at amide bonds between specific amino acid residues to yield a predictable set of peptides. Analytical chromatographic separation, detection, and identification of the peptide mixture reveal information on the amino acid sequence of the protein which can be used to identify the protein. Peptide mapping is a comparative procedure; the results from the test protein are contrasted with the results of the reference standard or material similarly treated to determine the identity of the test protein. This comparative identification confirms that the primary structure of the test protein matches that of the reference protein.

Peptide mapping's ability to detect gross alterations in the primary structure has resulted in many applications for the determination of protein quality which are outside the scope of this chapter. The purity of the test protein with regard to amino acid misincorporation or other misassembly such as disulfide bond scrambling, post-translational modifications, and degradation can be determined using a quantitative peptide map. Peptide mapping comparison during scale up or manufacturing changes can support studies of process consistency. Additionally, peptide mapping can be used to determine the degree and specific amino acid location of modifications such as glycosylation and conjugation (e.g., degree of pegylation). The focus of this chapter will be on the use of peptide mapping for the chemical identification of a protein product where specificity is the primary attribute of the analytical method.

2. Development of a peptide mapping identity test procedure—Points to consider

Prior to development of an identity test method procedure it is important to understand the application and level of specificity required to differentiate the identity of the test protein from other products processed in the same facility. In some instances orthogonal methods may be required to differentiate samples of structurally related proteins. Each protein presents unique characteristics that must be well understood so that the scientific approach used during development of the peptide map procedure will result in an analytical method that can be validated with sufficient specificity. The amino acid sequence of the test protein should be evaluated in order to select pretreatment and cleavage conditions resulting in optimal peptide length for analysis. Depending on application, complete or nearly complete sequence coverage is important, because there may be no prior knowledge of the alterations to the protein during development. The following points should be

considered during development of a peptide mapping analytical technique. These elements are also presented graphically in Figure 1.

3. Pretreatment

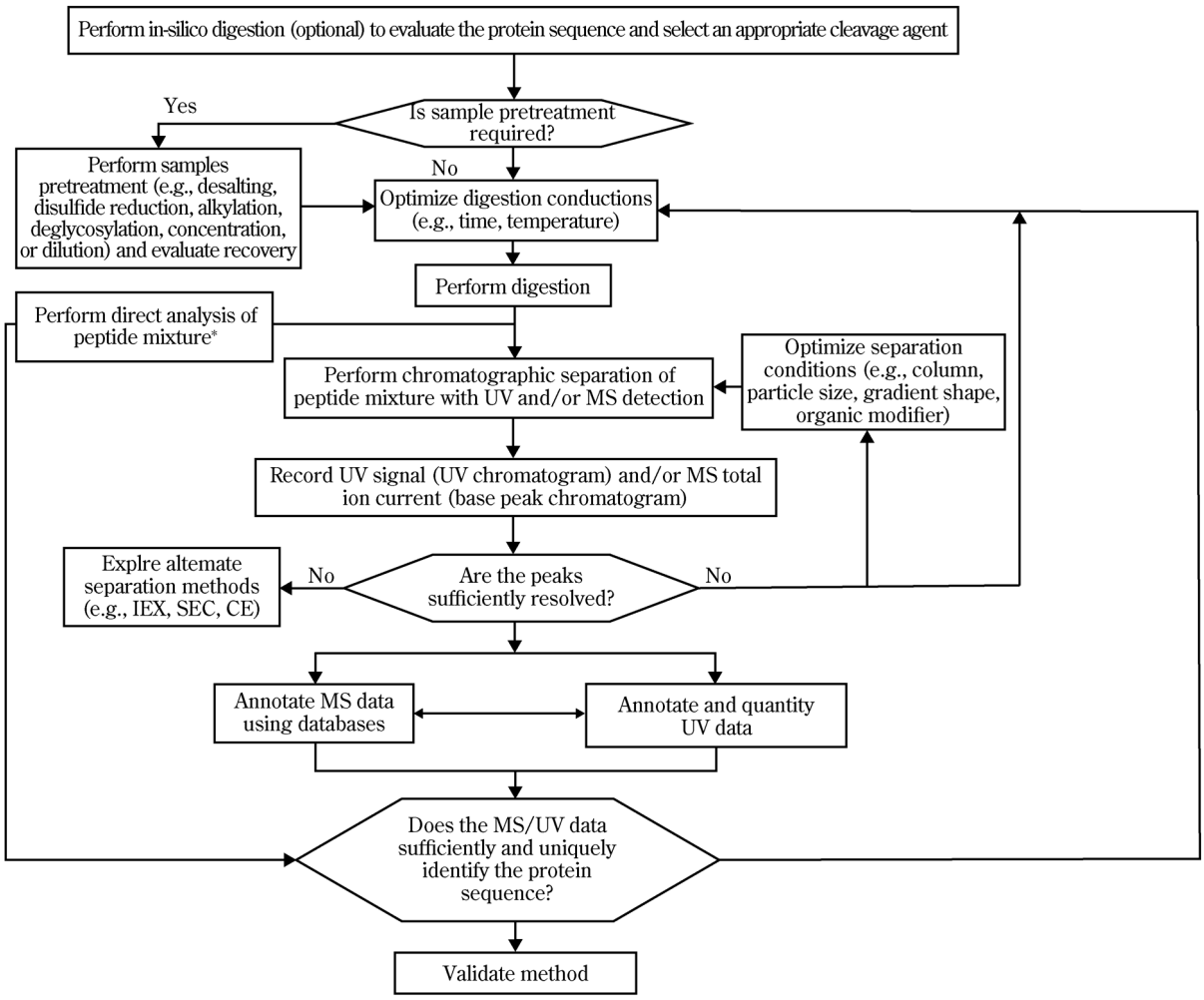
Isolation and purification may be necessary for analysis of bulk drugs, dosage forms, or reference standards or materials containing interfering excipients or carrier proteins. Residual interfering substances may impact enzymatic cleavage efficiency and appearance of the peptide map. The impact of residual substances or the sample purification process on the final test peptide map should be assessed during the development process.

The tertiary structure of proteins may hinder full access of the cleavage enzyme to all cleavage sites resulting in unacceptable sequence coverage. The treatment of proteins with chaotropic agents (e.g., guanidinium chloride, urea) and surfactants (e.g., sodium dodecyl sulfate) can be used to unfold the protein prior to digestion. Denaturing agents can affect enzyme activity and additional purification (e.g., diafiltration) or dilution steps may be needed prior to digestion. It may be necessary to reduce and alkylate the disulfide bonds prior to digestion in order to allow the enzyme to have full access to cleavage sites; however, the cysteine-to-cysteine linkage information is then lost. Common reagents for disulfide reduction include dithiothreitol and trialkylphosphine compounds such as tris(2-carboxyethyl)phosphine. Reagents for alkylating reduced cysteines include iodoacetamide, iodoacetic acid, and 4-vinylpyridine. The use of alkylating agents may create adducts which will impact the chromatographic separation and alter the molecular weight of the affected peptide.

Since peptide mapping is a comparative procedure, any purification or pretreatment steps performed on the test protein must also be performed on the product reference standard or material. The impact of residual substances, purification procedures, or pretreatment of the protein on method specificity and precision should be investigated during development and considered for inclusion in robustness studies conducted for method validation.

4. Digestion

The choice of a cleavage technique is protein dependent. Some of the more common cleavage agents, both enzymatic



*Peptide mass fingerprint

Figure 1. Identify Peptide Map Method and Target Performance Parameters

and chemical, and their specificity are shown in Table 1. There may be specific reasons for using other cleavage agents or combinations of methods.

Table 1 Examples of Cleavage Agents

Type	Agent	Specificity
Enzymatic	Trypsin, EC 3.4.21.4	C-terminal side of Arg and Lys
	Chymotrypsin, EC 3.4.21.1	C-terminal side of hydrophobic residues (e.g., Leu, Met, Ala, aromatics)
	Pepsin A (Pepsin), EC 3.4.23.1	Low-specificity digest
	Lysyl endopeptidase (Lys-C endopeptidase), EC 3.4.21.50	C-terminal side of Lys
	Glutamyl endopeptidase (Glu-C endoproteinase; V8 protease); (from *S. aureus* strain V8), EC 3.4.21.19	C-terminal side of Glu and Asp
	Peptidyl-Asp metalloendopeptidase (Asp-N endoproteinase), EC 3.4.24.33	N-terminal side of Asp
	Clostripain (Arg-C endopeptidase), EC 3.4.22.8	C-terminal side of Arg
Chemical	Cyanogen bromide	C-terminal side of Met
	2-Nitro-5-thiocyanobenzoic acid	N-terminal side of Cys
	o-Iodosobenzoic acid	C-terminal side of Trp and Tyr
	Dilute acid	Asp and Pro
	3-Bromo-3-methyl-2-(2-nitrophenylthio-3*H*-indole (BNPS-skatole)	Trp

Factors that impact the effectiveness and reproducibility of protein digestion include pH, digestion buffer, temperature, time, and ratio of digest enzyme/reagent to protein. The optimal digestion mixture pH is generally determined by the enzyme or reagent. Chemical stability of the peptides including amino acid side chains and protein modifications at the selected pH must be considered. For example, a highly acidic environment (e.g., pH 2, formic acid) is necessary when using cyanogen bromide as a cleavage agent; however, a slightly alkaline environment (pH 8) is optimal when using trypsin as a cleavage agent.

The optimal temperature is dependent on the cleavage reagent; for example, most enzymes have optimum activity in a range of 25–37°C. The temperature can define the specificity of the enzyme to some extent. In these cases the adjustment of the temperature can be used to optimize the digestion conditions for certain proteins. Ideally, the digestion temperature will minimize sample-related chemical side reactions, such as deamidation, and protein aggregation while maximizing the susceptibility of the test protein to digestion while maintaining the activity of the cleavage agent.

It is necessary to ensure the digestion time is sufficient for intended use to avoid variable digests. A simple time-course study should be performed to ensure sufficient digestion with minimal peptide fragments resulting from partial digestion. Time of digestion varies from minutes to days and aliquots of a single reaction may be appropriately stabilized for analysis to determine the time required for complete digestion of the protein.

A sufficient cleavage agent should be used to attain the desired level of digestion within a practical time period (i.e., 2–20 hours), while the amount of the cleavage agent is minimized to avoid its contribution to the peptide map. For an enzymatic digest, the protein-to-protease mass ratio between 20:1 and 200:1 is generally used. In cases where the cleavage agent is unstable, the cleavage efficiency may be improved by making multiple additions of the cleavage agent. Enzymes may be bound to a solid support to allow the use of higher relative amounts of protease while avoiding enzyme autolysis contamination and contribution of enzyme fragments to the peptide map. Chemical cleavage reagents are usually used in significant molar excess, and may need to be removed at the end of the digestion.

The optimal concentration of the test protein in the digestion should be empirically determined. The concentration should be low enough to minimize the potential aggregation of intact and partially digested proteins but must be sufficient to result in acceptable limit of detection of peptides following chromatographic separation with the selected detection method. Sample dilution or sample concentration by techniques such as centrifugal filtration may be required. Any dilution or concentration steps performed on the test protein must also be performed on the product reference standard or material. Protein recovery should be evaluated for any concentration step and the impact of dilution or concentration on method specificity and precision should be investigated during development and considered for inclusion in robustness studies conducted for method validation.

The digestion step can introduce ambiguities in the peptide map as a result of side reactions, such as nonspecific cleavage, deamidation, disulfide isomerization, oxidation of methionine residues, carbamylation of lysine residues, or formation of pyroglutamic groups created from the deamidation of glutamine at the N-terminus of a peptide. Autolysis may introduce extraneous peaks produced by the proteolytic enzyme digesting itself. The intensities of autolysis pep-

tide peaks are dependent on the enzyme to substrate ratio and the modifications and quality of the enzyme used. To avoid autolysis, reagent solutions of proteolytic enzymes should be prepared at a pH which inhibits enzyme activity or the reagent solutions should be prepared immediately before use. Modified enzymes, where changes are made to the protease to prevent autolysis, may be used. Commercial preparations of trypsin (often called ''proteomics grade'') are available in which the lysine residues of the enzyme have been methylated or acetylated to reduce the number of autolytic cleavage sites. To identify digestion artifacts, a blank determination is performed using a digestion control with all the reagents except the test protein.

5. Separation

Chromatographic separation of the peptide mixture resulting from the digestion step is meant to resolve its complexity so that a valid interpretation of the data is meaningful and reproducible. The complexity of the peptide map will ultimately dictate the optimal set of chromatography conditions, column, and mobile phases. Method optimization experiments will be required to obtain the highest quality reproducible chromatogram. The molecular weight of the test protein will also influence the complexity of the map and the optimal separation.

Many techniques (e.g., ion-exchange high performance liquid chromatography [HPLC], hydrophobic interaction HPLC, and capillary electrophoresis) have been used to separate peptides for peptide map analysis. However, reversed phase HPLC (RP-HPLC) is the most common method for the peptide mapping separation step and will be the focus of this chapter.

The selection of a chromatographic column is empirically determined for each protein. Columns with different pore sizes (8-100 nm) or nonporous based on silica, polymeric, or hybrid supports have been shown to give adequate separation. Columns with particle sizes $< 2\,\mu$m are available and are typically more efficient than those with 3–5 μm particle sizes. Generally, octyl or octadecylsilyl bonded phases are ideal for peptides. Octadecylsilane (C18) with 30 nm or smaller pores is the most commonly employed bonded phase for the peptide mapping separation step.

The most common mobile phase for the RP-HPLC separation of peptides is water with acetonitrile as the organic modifier; however other organic modifiers such as methanol, 2-propanol, or 1-propanol can be employed. Solvents such as the propanols in the mobile phase may be useful for separating samples that contain many highly hydrophobic peptides; however, it should be noted that hydrophilic or small peptides may possibly elute in a column void volume. Mobile phase additives such as acids, bases, buffer salts, and ion-pairing reagents are generally needed to produce high quality chromatographic separations of peptides. The most common mobile phase additive has been trifluoroacetic acid (TFA) with typical concentrations of 0.05%–0.2% being employed. The use of phosphate as an additive is less common but can be useful in cases where ultraviolet (UV) detection is used. Volatile acids and salts can be used in the mobile phase to improve compatibility with mass spectrometer detection. While TFA has a significant positive impact on the quality of peptide separation, sensitivity with mass spectrometer detection can suffer with TFA due to ion suppression. Formic acid, acetic acid, or combinations of these with TFA increase mass spectrometer sensitivity by reducing ion suppression. Temperature control of the chromatographic column is necessary to achieve good reproducibility. The column temperature may be used to optimize peptide separation or improve the retention or elution of certain peptides since the resolution typically increases with temperature for a reversed-phase column.

6. Detection

While RP-HPLC is the most common separation method employed with peptide mapping for identity testing, the most common detection method is UV light absorption at 214 nm. The peptides resulting from protein digestion may not contain amino acids with aromatic side chains that absorb light at higher wavelengths (e.g., 280 nm) so detection at 214 nm (i.e., wavelength where peptide bonds absorb light) is essential to ensure sequence coverage of the protein while taking care to minimize background due to the mobile phase. Other detection methods may also be suitable.

The limitation of UV detection is that it provides no peptide structural information. Mass spectrometry is a useful detection method which provides mass information to aid in identification of peptides, as well as selectivity in cases when peptides co-elute. In most applications, the RP-HPLC effluent can be directly introduced into the mass spectrometer, provided that the mobile phase is compatible. Specific mobile phase considerations are dependent on the ionization method selected. Electrospray ionization (ESI) is the most common method for the introduction of proteins and peptides into the mass analyzer, and volatile, water-solvent mixtures provide the greatest ionization efficiency. Peptide mapping by ESI-MS is most often performed in positive ion mode. Formic acid or acetic acid are commonly added to the mobile phase to reduce pH and thereby enhance protonation of the peptides. Buffers and salts should be minimized since they can reduce signal, and nonvolatile salts can deposit in the source. As mentioned previously, TFA should be avoided because it can result in ion suppression, a type of matrix interference, which may reduce the signal of some peptides, particularly when ESI is used. Ion suppression may also reduce the ionization efficiency of glycosylated peptides, resulting in reduced sensitivity. It is thus important to optimize conditions in order to achieve optimal results for both UV and MS detection.

7. Data analysis

Peptide mapping is a comparative procedure. To determine if the test protein is the desired protein of interest, the test protein's peptide map must be compared to the peptide map of the reference standard or material generated using identical pre-treatment, separation and detection procedures. Visual comparison of the retention times, the peak responses (the peak area or the peak height), the number of peaks, and the overall elution pattern is the first step of the

procedure. It is a best practice to conduct a further non-subjective analysis of the peak response ratios of the critical peaks and the peak retention times. If all critical peaks in the test protein digest and in the reference standard or material digest have the same retention times and peak response ratios, then the identity of the test protein is confirmed. For example, peptide mapping tests for monoclonal antibody samples often include a common Fc peptide that is used as a reference peak. The reference peptide can be spiked into the sample digest and then peak response ratios of the critical peaks and retention times can be examined in comparison with the predefined acceptance criteria. The method of comparison selected should depend on the complexity of the resulting peptide map and the specificity required for the particular identity test application (e.g. differentiation between different protein products manufactured at the same facility or differentiation of variants of the same protein product).

When high specificity is required, a mass spectrometer can be used for routine analyses to provide insight into peptide modifications, truncations, missed cleavages, impurities, and unresolved co-eluting peak(s) under a single peak.

8. Points to consider prior to validation

During the development of the peptide mapping procedure, knowledge and experience are gained that lead to selection of system suitability criteria and analytical method validation acceptance criteria. A final review of the procedure prior to validation can ensure that the procedure is ready for validation, reducing risk of failure to meet criteria. As a general procedure, peptide mapping may encompass a significant range of experimental designs, applications, and requirements for performance. As a consequence, in a general text, it is not possible to set out specific system suitability or validation criteria. The following elements are suggested for evaluation prior to starting the validation.

It should be noted that the scope of this chapter does not include routine application of mass spectrometry (MS)–based peptide mapping applications; however, the application of mass spectrometry for structural identification of peptides during the development of peptide mapping methods is a best practice. Mass spectrometric detection can be utilized to evaluate the following performance parameters.

8.1. Coverage

Coverage refers to the percentage of the amino acid sequence identified in the peptide map to the target protein sequence. Although no specific figure can be identified for all applications, in many cases, coverage approaching 95% has been found to be an acceptable performance target for a peptide mapping procedure.

8.2. Specific Bond Cleavages

The specific bonds cleaved by the chosen enzyme or chemical digestion procedure should be identified and listed.

8.3. Major Peaks

The major peptides recovered from the specific bond cleavages should be identified and listed.

8.4. Partial Cleavages

Peptide bonds susceptible to partial or incomplete cleavage and their associated chromatographic peaks or signals should be identified.

8.5. Minor/Non-specific Cleavages

The extent of cleavage at non-specific bonds should be identified and limited or controlled.

8.6. Protease-derived Peaks

If a protease is used for the test protein digestion then any peaks above background derived from the protease should be identified and, where appropriate, limited.

8.7. Undigested ''Core'' Protein

Undigested or partially digested protein (often called ''core'') should be identified and limited.

8.8. Mean Peptide Length

It describes the peptide set produced by the combination of the chosen protease and/or chemical cleavage reagent and the test protein. This is a trade-off between smaller peptides, which show a higher level of structural selectivity with peptide mapping but produce a more complex map with more peaks, and longer peptides which produce simpler maps but with less resolving capacity for structural variants. No specific peptide length is suitable for all applications, but a mean peptide length of 10–20 residues is often considered appropriate.

8.9. Resolution Capacity

Resolution capacity refers to the capacity of the separation system to resolve the peptide set generated by the protease or chemical cleavage reagent. For example, a digest may produce 30 peptides but only 20 peaks due to co-elutions or nonrecoveries. Problematic separations should be identified and resolved by appropriate chromatographic procedures and, if necessary, controlled by the use of peptide reference standard or material or system performance criteria.

8.10. System Suitability Criteria Selection

System suitability criteria should be developed to ensure that the elements of the procedure for protein digestion, separation, and detection have successfully provided a structural identification of the test protein at the level of unambiguity required for the application. System suitability criteria evaluated during routine analysis for identity tests will typically include an assessment of the reference protein digest chromatogram and may include such performance characteristics as:

- Qualitative similarity to reference chromatogram
- Extent of digestion
- Partial cleavages
- Non-specific cleavages
- Peak heights/signal-to-noise ratio
- Peak shape
- Peak retention time
- Resolution of specific peaks

For test method procedures that require sample isolation, purification, or concentration, a sample recovery criteria should be determined and included as part of the system suitability assessment. In cases where digestion artifacts

may be present, assessment of a blank digestion control may be needed to demonstrate a lack of interference.

9. Validation

Before validating a peptide mapping procedure, the procedure should have been developed to its final form and documented with system suitability criteria. Each time the procedure is performed the results are evaluated against the system suitability criteria to determine if the procedure has successfully provided reproducible results consistent with previous testing instances. Pre-approved acceptance criteria often evolve based on the system suitability criteria of the procedure. The elements of the analytical validation protocol are as follows:

9.1. Specificity

Method performance requirements will vary depending on the application of the identity test method and may require a risk assessment to understand what degree of specificity is needed to differentiate the identity of the test protein from other products processed in the same facility. Peptide mapping is a comparative technique confirming that the primary structure of the test protein matches that of the reference protein. Specificity is established by the comparison of the peptide maps of a suitable reference standard or material and samples of structurally related proteins. The selection of comparator samples should be selected based on a risk assessment of other products processed in the same facility and should be documented in the validation protocol. In order to minimize the inherent variability of the test, the procedure is executed on reference standard or material and test protein during the same testing instance. A peptide mapping test design that analyzes the test protein digest, reference standard or material digest, and a 1:1 (v/v) co-mixture of the test protein and reference standard or material after digestion is a useful specificity validation experiment. Occasionally a peak can appear in a test protein's peptide map that elutes at a slightly different retention time than the corresponding peak in the reference standard or material peptide map, leading the analyst to judge the peaks as nonidentical. Testing a co-mixture sample during the specificity validation experiment can demonstrate that two peaks are identical if they co-elute in the co-mixture peptide map and confirm the identity. Chemically modified forms of the reference standard or material can be produced by exposure to conditions of pH, temperature, or chemical agents known to cause alteration of the primary structure. These alterations typically include deamidation of asparagine and glutamine residues, oxidation of methionine, histidine, or tryptophan residues, and acid catalyzed cleavage of peptide bonds. Peptide maps of a chemically modified reference standard or material and the reference standard or material can be compared based on predetermined acceptance criteria to demonstrate if the specificity of the peptide mapping procedure is affected by amino acid side chain modifications.

9.2 Precision

To facilitate the determination of the precision (repeatability and intermediate precision) of the peptide mapping procedure, an empirical method of quantifying peak responses (peak areas or peak heights) and peak retention factor should be part of the procedure. One approach is to make peak response and peak retention time comparisons that are expressed relative to a highly reproducible reference peak within the same chromatogram. The precision results obtained during the analytical procedure validation are reported and should meet the acceptance criteria of the validation. Failure of the precision results to meet the acceptance criteria can lead the analyst to reassess the digestion and/or separation steps in the procedure.

9.3 Robustness

Robustness may be evaluated during the development of analytical procedures. It is not necessarily repeated, but it may be included as a part of method validation. Factors such as composition of the mobile phase, protease quality or chemical reagent purity, column variation and age, digestion temperature, and digest stability are likely to affect the overall performance of the test and its reproducibility. Tolerances for each of the key parameters are evaluated and baseline limits established in case the test is used for routine lot release purposes.

Variations in purification, pretreatment, dilution, or concentration procedures of the protein sample can have an impact on recovery test system, and the chromatogram. The variations and their impacts should be identified during the development process and controlled. Impact of residual substances remaining after sample preparation on method specificity and precision should be considered. Critical parameters identified during development should be included in robustness studies conducted for method validation.

Many protein fragmentation strategies employ the use of proteolytic enzymes. As a result, the digestion portion of the peptide mapping procedure is inherently more sensitive to minor variation of test parameters. These parameters may include all or a subset of the following: digestion pH, buffer, buffer concentration, ionic strength, digestion temperature, digestion kinetics, test protein concentration, protease quantity, protease quality, and the stability of the digest. Using a design-of-experiments approach, the identified critical parameters are systematically studied to understand their impact on method variability. Those digestion parameters where small variations have been shown to impact the precision of the peptide mapping procedure should be carefully controlled within the test procedure using operating ranges established and validated by these studies.

To evaluate the protease quality or chemical reagent purity, a sample of the reference standard or material is prepared and digested with different lots of cleavage agent. The chromatograms for each digest are compared in terms of peak areas, peak shape, and number. The same procedure can be applied to other critical chemicals or pretreatment procedures used during sample preparation, such as reducing and *S*-carboxymethylation reagents.

The length of time a digest can be held before proceeding to the separation step of the procedure, as well as the condi-

tions under which the digest is stored before separation, are assessed. Several aliquots from a single digest are stored under different storage conditions and resolved by the chromatographic method. These maps are then evaluated for significant differences.

During the separation step, column-to-column variability, even within a single column lot, can affect the performance of the peptide mapping procedure. To evaluate column lot differences, the reference standard or material of the protein of interest is digested and the digest is subjected to separation using different column lots from a single manufacturer. The resulting peptide maps are then evaluated in terms of the overall elution profile, retention times, and resolution according to predetermined acceptance criteria.

To evaluate the lifetime of a column in terms of robustness, a single digest of the reference standard or material can be analyzed using the peptide mapping procedure with columns that vary by the injection number history (e.g., 10–250 injections per column). The resulting peptide maps can then be compared for significant differences in peak broadening and overall resolution. As a column ages, an increase in back pressure might be observed that can affect the peptide map. System suitability or assay validity criteria can be designed to be diagnostic of column aging or other events that may affect the peptide mapping results.

10. Summary

The peptide mapping procedure consists of multiple steps possibly including protein isolation, denaturation, chemical modification (e.g., blocking sulfhydryl groups) if necessary, protein digestion, peptide separation and detection, and data analysis. Each step should be optimized during development to result in a well-qualified analytical procedure for the peptide mapping identity test. In combination with the use of a suitable reference standard or material, system suitability criteria should be chosen that evaluate if all the steps in the procedure worked together properly to produce a successful peptide map of that reference standard or material that is consistent with the validation of the analytical procedure. When properly developed, validated, and performed, the analytical peptide mapping procedure can be used to verify the identity of the test protein which is a critical quality attribute of the product.

Add the following:

Flow Cytometry <*G3-16-182*>

Flow cytometry is a measurement technique for analyzing the optical properties of individual cells or particles dispersed in liquid and aligned by a fluidics system. In addition to obtaining morphological parameters such as the size and complexity of the internal structure of cells using scattered light, it is also possible to quantitatively obtain information about protein expression on cell surface and in cells and nucleic acid contents at a single cell level by staining cells with fluorescent-labeled antibodies or fluorescent dyes, etc. Also, by combining different fluorescent probes, information on multiple parameters can be obtained simultaneously. In the characterization and specifications of biotechnological/biological products, flow cytometry is used to evaluate the binding activity of a desired product to target cells, cell response, and the qualification of cultured cells used for bioassays.

1. Instrument and principle of measurement

An instrument used for flow cytometry (flow cytometer) generally consists of a fluidics system, a light source, an optical detection system, an electronic processing system (electrical pulse processing system), and a data processing system (Fig. 1).

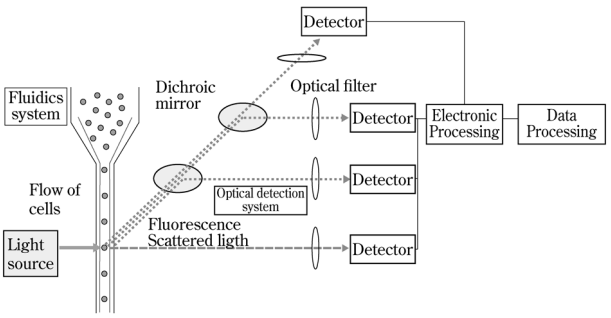


Fig. 1. Configuration of flow cytometer

In many flow cytometers, cell suspensions are transported by a fluidics system to a flow cell, where hydrodynamic focusing through a sheath fluid forms a thin stream of cells in a row, and the cells pass an observation point (laser interrogation point) one by one. An argon laser (488 nm), a helium-neon laser (633 nm), and diode lasers having various wavelengths are commonly mounted in combination, and appropriate light sources for the fluorescence to be detected are selected. When cells pass through the laser interrogation point, light scattered in various directions is generated by the physical structure of the cells, and fluorescent dyes are excited to emit intrinsic fluorescence.

Scattering forward (usually within 20°) of the optical axis of a laser is called Forward Scatter (FSC), and the larger the cell, the stronger the FSC. Therefore, the relative size of cells can be estimated by measuring FSC. Scattering at 90° to the optical axis of a laser is called Side Scatter (SSC). The intensity of SSC is an indicator of the complexity of cell structure (the higher the complexity of the internal structure of the cell, the higher the SSC intensity), since the intensity of SSC is affected by the amount and type of intracellular granules, the morphology of nucleus and cell membranes, etc.

Depending on the type of a light source, fluorescent signals are produced by fluorescent substances contained in cells or by fluorescent probes (fluorescent dyes, fluorescent-labeled proteins, fluorescent proteins, etc.) used for specific analyses. Fluorescence emitted from cells is separated by an optical system and detected in individual channels. Optical filters include long-pass filters that allow fluorescence above

a specific wavelength to pass through, short-pass filters that allow fluorescence below a specific wavelength to pass through, and band-pass filters that allow fluorescence in a specific narrow wavelength range to pass through. By combining these filters with a dichroic mirror placed at a certain angle to an incident light, fluorescence with a specific wavelength can be distributed to a target channel. The specificity of detection depends on the setting of an optical system, so the combination must be appropriate for the fluorescence to be detected.

The scattered light and fluorescence distributed by the optical filter are detected by a photomultiplier tube (PMT) or photodiode, and converted into voltage pulses. The voltage pulses detected by PMT can be amplified by applying voltage to the detector. There are two types of amplification methods, linear and logarithmic. In general, the linear amplification is used to measure the scattered light (FSC, SSC) of cells, while the logarithmic amplification is often used to measure fluorescence. A threshold is usually set for FSC to prevent the acquisition of data unrelated to experimental data, such as signals derived from particulate matters (contaminants such as cell fragments) contained in a sample. Signals that do not exceed the threshold are ignored by all detectors. The voltage pulse is an analog value, and in most flow cytometers currently in use it is converted to a digital value that can be processed on a computer by analog-to-digital conversion.

When two or more fluorescent dyes are used simultaneously to stain cells, a portion of the fluorescence spectrum of each dye may overlap, in which case each fluorescence detector detects the fluorescence emitted by another dye in addition to the specific fluorescence from the intended fluorescent dye. To solve this problem of spillover, fluorescence compensation is performed. By using samples such as being stained independently with each fluorescent dye used in tests, the spillover of each fluorescent dye to other detectors can be calculated, and the data from which interfering signals are selectively subtracted can be obtained. Amplified and compensated data for each parameter (FSC, SSC, fluorescence) obtained for individual cells through the above process are used for analysis.

2. Data Analysis

2.1 Data Display

Data obtained by flow cytometry can be displayed and analyzed in various ways (Fig. 2). One common display method is a histogram, which shows the signal intensity of one measurement parameter on the x-axis and the number of cells on the y-axis. Histograms are useful for evaluating the expression level and expression ratio of a specific marker molecule. In addition, a dot plot, which plots the signal intensities of different parameters on the x- and y-axes, is used to identify cell populations combined with two type of cell surface markers and evaluate the proportion.

2.2 Gating

The acquired data may contain contaminants such as dead cells and cell fragments that are unnecessary for analysis, and signals derived from cell populations that are not

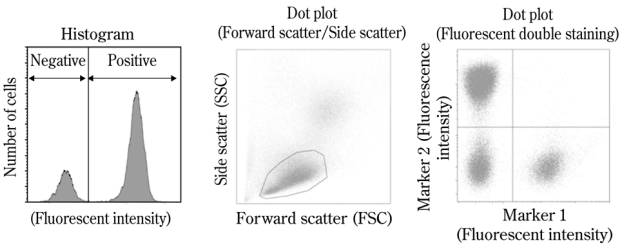


Fig. 2. Examples of data display

the target of analysis, so gating is performed to analyze only target cell populations. Gating based on morphological characteristics of cells estimated by FSC and SSC is usually performed first. For example, dead cells or cell fragments with smaller FSC and larger SSC than live cells can be excluded from analysis by gating on an FSC/SSC plot. In addition, for the analysis of blood samples, lymphocytes and granulocytes can be distinguished and gated using FSC/SSC plots based on differences in cell size and complexity. In experiments using fluorescent-labeled antibodies against cell surface markers, cell populations expressing a specific marker molecule (e.g., CD3 in T cells, CD19 in B cells) can be gated and analyzed. Stepwise multiple gating can be set up using analysis software. For cell populations narrowed down for analysis by gating, the ratio of cells to which a fluorescent-labeled substance used in the test binds (e.g., the ratio of cells expressing a marker molecule recognized by a fluorescent-labeled antibody), the mean fluorescence intensity as an indicator of the amount of binding, and other parameters are calculated.

3. Points to note when measuring

3.1 Calibration of instrument

In order to obtain data with high reliability and reproducibility, instruments should be calibrated periodically. Many flow cytometers are provided with calibration software and reagents (usually fluorescent beads) by the instrument manufacturers, which are used to calibrate the instrument, and record the monitoring state of the instrument performance (variation in fluorescence intensity obtained from standard beads, setting of detection sensitivity, etc.).

3.2 Use of control samples

Control samples are used to identify background or nonspecific signals and to establish appropriate measurement conditions. Control samples are also used for routine test qualification (e.g., judgement of system suitability).

Unstained control: An unstained sample is used for gating cell populations to be analyzed, adjusting a detector based on a background due to cell autofluorescence, and setting a negative fraction.

Isotype control: When fluorescent-labeled antibodies are used, a control stained with an antibody that targets an antigen not present in cells being analyzed and is the same immunoglobulin subclass as the antibody used in the test is used to confirm that the staining observed is due to specific binding to the target antigen. Antibodies used for isotype

controls should be labeled with the same fluorescent dye in the same ratio as the antibody used for testing. An isotype control is used to evaluate a background such as nonspecific bindings of antibodies and fluorescent dyes to cells and antibody bindings to Fc receptors on immune cells such as monocytes and macrophages.

Single-stained control: In the case of performing tests using multiple types of fluorescent dyes, a single-stained control is used for each fluorescent dye to evaluate spillover between the different fluorescent dyes and perform fluorescence compensation.

Fluorescence minus one (FMO) control: The FMO control is a control in which only one fluorescent dye is excluded from all fluorescent dyes used for staining. Using this control, it is confirmed by examining the spillover of other fluorescent dyes into the channel of the missing fluorescent dyes that fluorescence compensation is made correctly. The control can also be used to set up gating to determine negative/positive fractions.

Biological control (assay control): In addition to the controls for staining described above, prepare positive and negative control samples corresponding to a test to be performed. For example, in a test to measure changes in the expression of marker molecules associated with cell responses, untreated/unstimulated samples or samples with treatment known to certainly induce cell responses are used as controls. Measured data from these assay controls can be used to judge system suitability.

3.3 Setting measurement conditions

When measuring samples, select an optical system appropriate for fluorescence to be detected, and set detector sensitivity, gating, and fluorescence correction using control samples. Usually, the sensitivity of FSC and SSC are first adjusted so that cell populations to be analyzed is appropriately displayed in the FSC/SSC plot, and the cell populations to be analyzed is gated. Next, a histogram or dot plot is developed for fluorescence parameters to be detected, and the sensitivity of the detector is adjusted so that fluorescence detected in unstained controls and positive/negative controls is within a measurement range. The detected fluorescence intensity is the relative value that varies depending on the output of a laser, etc., and it is useful to set the sensitivity of the detector so that the fluorescence intensity of the control samples is within a predefined range to ensure reproducibility. When analyzing multiple-stained samples using multiple fluorescent dyes, use a single-stained control or FMO control to evaluate the spillover of each fluorescence to other detectors, and set fluorescence compensation so that the spillover does not affect the analysis result. When calculating the ratio of positive fractions (expression ratio of marker molecules, etc.), gating is set so that positive and negative fractions can be distinguished using the fluorescence intensity of a control sample as an indicator. Set up the system suitability using an assay control, etc., and confirm that the measurement conditions are appropriate for routine testing.

3.4 Control of Cells and Reagents

Since cells and fluorescent-labeled antibodies used for staining are important reagents that affect the performance and results of tests, they should be managed in an appropriate manner by defining the criteria for evaluating their qualification. Since there is the possibility that characteristics of cells may change over the process of culture, a cell bank system should be established, and a culture method, the maximum number of passages and criteria for the condition of cells at the time of testing (cell viability, etc.) should be defined. When used in tests targeting a specific receptor, etc., the expression level of the target receptor should be defined and controlled as the specification. When performing the tests, it is also important to confirm that cells used in each test show expected cell responses using assay controls. Fluorescent labeled antibodies used for staining and cytokines used for cell stimulation should be used after confirming their suitability for the intended use. Since the specific activity of protein reagents may differ from lot to lot even if they are commercially available, when a lot is renewed, the old and new lots should be compared, and if necessary, the concentration of the reagent added should be adjusted for use in the test.

4. Examples using flow cytometry in tests of biotechnological/biological products

4.1 Evaluation of the binding activity of target substance to target cells

When a desired product exerts its pharmacological activity by its binding to a target protein on a cell surface (antibodies targeting cell membrane proteins, hormones/cytokines, etc.), the binding activity of the product to cells expressing the target molecule can be evaluated by flow cytometry. Cell-based binding assays have the advantage of evaluating the binding activity to target proteins on cell membranes under more physiological conditions, and are also useful for binding assays to multiple transmembrane proteins, which are difficult to purify recombinant proteins. On the other hand, nonspecific binding to non-target molecules present in cells used for the assay may occur, and the specificity of the observed binding should be considered.

As with binding assays based on other principles, either a non-competitive or competitive method can be used. In the non-competitive method, a fluorescent-labeled antibody against a desired product (e.g., fluorescent-labeled anti-human IgG antibodies against antibody drugs) is used to detect the binding of the product to target cells. In the competitive method, a sample is mixed with a fluorescent-labeled standard material or the equivalent, and added to target cells. The inhibitory activity of the sample on the binding of the fluorescent-labeled material to the target cells is measured. A dose-response curve is constructed from signals (mean fluorescence intensity) obtained by testing the dilution series of samples prepared at an appropriate dilution factor, and the dose that gives a signal equivalent to 50% of the maximum response (EC_{50} for the non-competitive method and IC_{50} for the competitive method) is calculated. To determine the relative activity to the standard material, a dose-response curve is prepared for the standard material and the sample, respectively, and the ratio of the

EC_{50} or IC_{50} is calculated.

4.2 Evaluation of cell response

When an increase or decrease in the expression of cell surface marker molecules is observed as cell response to cell stimulation, the expression change can be quantitatively analyzed by flow cytometry. In addition to hormones and cytokines that induce cell responses through their receptors, flow cytometry is also used to evaluate the biological activity of humoral factors that induce cell responses and neutralizing antibodies that target their receptors. Cells that have been treated by adding a sample and culturing for a certain period of time are stained with a fluorescent-labeled antibody against a marker molecule to determine the ratio of cells expressing the marker molecule and the expression level of the marker molecule.

4.3 Qualification of cultured cells for bioassay

Flow cytometry is one of useful methods to confirm the expression of target proteins such as receptors in cells used for bioassay. Cultured cells may show heterogeneous gene expression patterns even in cloned cell lines, and their characteristics may change over the process of a culture period. In addition, in cell lines generated by transfection to express target proteins, it is necessary to consider the possibility of loss or reduction of the target protein expression due to deletion or silencing of transgenes. The expression rate and level of a target protein should be measured by staining with a fluorescent-labeled antibody against the target protein to confirm that the expression rate and level of the target protein meet the predetermined criteria.

Add the following:

Evaluation Method of Insoluble Particulate Matter in Biotechnological Products (Biopharmaceuticals) Drug Substances/Drug Products by Flow Imaging Method <*G3-17-182*>

Biotechnological pharmaceutical products (hereinafter referred to as ''biopharmaceuticals'') may contain, insoluble particulate matter such as protein aggregates generated by proteins aggregating themselves, in addition to exogeneous materials, manufacturing-process-derived materials and extractable substances from the formulation composition or the primary container. Evaluation and control of particulate matter contained in injections play an important role in assuring the quality of final products. For the protein aggregates, more rigorous evaluation and control is required because of immunogenicity concern over protein drug products.

The flow imaging method is a technique to count particulates contained in a solution and measure their size distribution, and evaluate their morphological and optical properties, by analyzing the numerical information converted from the digital images which are captured continuously on the sample solution flowing into a flow cell. The light obscuration particle count test may not detect protein aggregates at all or underestimate their particle size due to the difference in refractive index from water being so small. This is because the particle size is calculated by a particle size response curve based on polystyrene standard particles with a high refractive index. The flow imaging method has been, on the other hand, shown to be less sensitive to refractive index difference between the particles and the dispersion solvent than the light obscuration particle count test. Furthermore, by evaluating the morphological and optical properties it is also possible in some cases to classify protein-aggregates, silicone oil, air bubbles and other insoluble particulates. Quantitative evaluation of the number of particles and characterization of the particles by the flow imaging method is a useful evaluation method for insoluble particulates in protein drugs. In this general information, evaluation methods for insoluble particulates contained in biopharmaceuticals including therapeutic protein injections are mainly described.

1. Principles of Measurement

The apparatus generally consists of a sample port, a flow cell which is the area for capturing images, flow path tubes for connection, a pump (a tube pump or a syringe pump), an optical system including a light source, a camera as an imaging instrument, and an image analyzer for the captured images. A sample solution flowing into the flow-cell is irradiated by light from the source and is captured by the imaging instrument. A measurable particle size depends on the thickness of the flow-cell, the magnification of the objective lens and the performance of the camera, and in most cases the measurement range is approximately 2 to 100 μm. The particle image data is processed by the image analyzer and evaluated for the shape and optical properties of each particle by recognizing the boundary of each particle in the image based on, for example, the contrast of the particles against the image background. The particle concentration is obtained by dividing the particulate count by the measured volume.

2. Measurement

2.1. Instrument

General procedure for the measurement is as follows. Employ the magnification of the objective lens according to the size of the particles to be measured, which is usually 4 to 20-fold. Clean the flow cell in advance and ensure that there are no particle remaining in the flow cell. For cleaning the flow cell use particle-free water or use, as necessary, detergent, diluted sodium hydroxide aqueous solution or ethanol, etc. Thereafter, focus the instrument appropriately following its operation procedure. Set required measurement parameters (flow rate, sample volume, image acquisition frequency, particle identification threshold against the background, etc.) for each instrument. An image acquisition efficiency is defined as the rate of the portion of the solution for which the images are analyzed to

the total introduced into the flow cell. For instruments that allow the setting of image acquisition efficiency, the efficiency is calculated from the sample volume, flow rate, and image acquisition frequency [image acquisition efficiency = image acquisition frequency (frames/s) × measured volume per image (mL/frame)／flow rate (mL/s) × 100 (%)]. The settings should be properly performed so that same particles are not counted in multiple times and that the sample volume for actual measurement is adequate. Where the area to be measured can be set, accuracy in counting particles can be verified by measuring a particle count reference standard. Due to the principle of measurement, a particle image with missing part may be captured due to part of particles being out of the measurement area. Handling of partially captured images of particles should be stipulated in advance.

2.2. Operating method

The measurement should be carried out under conditions limiting particulate contamination, preferably, in a clean cabinet with laminar flows, etc. Gently shake a sample thoroughly, swirling the container slowly for example, so that the particles in the sample are uniformly dispersed. When opening the container, clean the outer surface of the container opening with *particle-free water* if necessary and remove the closure cautiously to avoid contamination of the contents. When measuring particulates in solution, caution is required not to generate bubbles or new aggregates during the operation. If necessary, allow the container to stand under ambient pressure or reduced pressure for the moment to eliminate air bubbles. Sonication is not appropriate as it may cause aggregation or denaturation of proteins. The volume of sample to be introduced into the instrument is determined considering the sample volume and the tare volume. The sample volume is determined in an adequate volume considering the properties of the sample, the image acquisition efficiency, and the precision required for the analysis. If necessary, such as when the sample has high viscosity or a large number of particles, it would be possible to dilute the sample confirming a dilutional linearity. The number of the measurements should be determined appropriately based on the performance of the instrument and the properties of the sample.

When using an instrument that can set the threshold value individually, confirm in advance that the particle borders are properly recognized, as the threshold value impacts the results of analysis significantly. It is also advisable to verify that the particle shapes are correctly evaluated, and that noise is not misconstrued as a particle by using an actual sample, a degraded actual sample, or particle standards prepared to imitate protein aggregates. When comparing the data acquired at different threshold values, the impact of the difference in threshold values on the results of measurements should be duly considered.

3. Image analysis

The sizes of detected particles are often represented by an equivalent circle diameter (the diameter of a circle having an area equivalent to the projected area of the particle). Other than the circular equivalent diameter, a sphere equivalent diameter or a Feret's diameter can also be used. Comparison of the particle sizes represented by such different particle definitions needs some caution.

While the counting of particulates by the flow imaging method is the main subject in this general information, the particle image may provide an estimate as to the origin, or the particles may be classified according to the features of the image. The main parameters to feature the particle properties, obtained as a result of the image analysis, in addition to particle size, include morphological parameters such as area, particle perimeter, aspect ratio, circularity, etc., as well as optical parameters such as brightness, standard deviation of brightness within the particles. Using these parameters, it is possible to classify the particles in the sample by the origin, such as, for example, silicone oil droplets derived from the container. Aspect ratio, roundness, perimeter, length, average and standard deviation of brightness are used for differentiation from silicone oil droplets. Combine some of these parameters, set an optimal threshold of each parameter, and sieve step by step. A classification model can be established using sufficient image data accumulated, and used to classify the detected particles by their origin, by applying to image data acquired by the same instrument. As these parameters, however, depend on the definition formula embedded in the imaging instrument and analysis software, as well as on the image analyzer system and the measurement conditions, the measured values may differ depending on the resolution, pixel number, and focusing method. Further to identify the origin, it should be necessary to use other appropriate technology such as micro-Raman spectroscopy which provides information on molecular structure and composition.

4. Validation of analytical method

Validation of an analytical method is to demonstrate the validity of the method by demonstrating conformity to the pre-defined criteria for performance characteristics, such as accuracy, precision, specificity (selectivity) in general. The performance characteristics to be evaluated depend on the purpose of the test which uses the analytical method concerned. When a test method is to perform counting of insoluble particulates in pharmaceuticals, it would be difficult to conduct method validation in a similar manner as for usual quantitative assays, because there is no control sample with known accuracy that reflects an actual sample, making accuracy evaluation difficult, and the particulates contained in the actual samples of a drug product or a drug substance are distributed widely and heterogeneously in respect of particle sizes. The performance characteristics explained in the following example of validation procedure are evaluated to demonstrate the validity of the method, using, for example, polystyrene particle standard with certified average particle size or polystyrene particle count reference standard with certified average particle size and particle concentration. The particle sizes and concentrations of the particle standard and particle count reference standard to be used should be appropriately stipulated considering the particle concen-

tration and particle size distribution in the actual samples and specification values. Multiple particle standards having different particle sizes may also benefit the evaluation of analytical method performance. The particle size distribution or the number of particles of particle standards to be used should be certified and quality-assured by an appropriate organization. In addition, silica particles or polymethylmethacrylate particles, both having a low refractive index, may serve as suitable model particles for protein-aggregates. These particles may therefore be useful in confirming if the particle size to be measured varies due to a small difference in refractive index between the model particle and the solvent, by utilizing the sample prepared by adding them to the solution with the same formulation composition as that of the actual sample solution to be tested.

Example of validation procedure for counting the number of particles by flow imaging method

Accuracy: Measure 5, 10, and 25 μm polystyrene particle count reference standards and verify that the results obtained are within the certified particle size and particle concentration ranges.

Precision: Evaluate repeatability and intermediate precision. Add 5, 10 or 25 μm standard particles to particle-free water or a solution consisting of the same formulation composition as that of the sample to be tested, to prepare samples for 3 levels of the particle concentrations for each standard particle. Measure each sample 3 times for repeatability. Using similarly prepared samples, measure the samples at least on different days and by different operators under the same conditions to calculate intermediate precision.

Linearity: Add 5, 10 or 25 μm standard particles to particle-free water or a solution consisting of the same formulation composition as that of the sample to be tested, and evaluate the linearity, for example, at 5 levels of particle concentration.

Specificity: When the particles are to be classified using a classification model or as otherwise required, verify that the classification is properly performed, using a degraded sample or an actual sample with a target analyte added.

5. Assuring instrument performance

5.1. Calibration

The particle sizes and the number of particles calculated by the flow imaging method are absolute values based on the principle of the measurement instead of relative values calculated from the measured values of particle standards. Therefore, confirm using particle count reference standards that the instrument is operating correctly, and adjust the settings if necessary. It is essential to confirm that the optical system operates appropriately in respect of focusing, brightness of the light source, etc. In addition, since the performance of the pump can also affect the measurement results, the flow rate should be adjusted and checked. For calibration of the instruments, use a polystyrene particle count reference standard and a polystyrene particle standard, with the particle size distribution and number of particles assured based on the absolute methods and certified by an appropriate organization.

5.2. System Suitability

To confirm in advance of the measurement that the instrument is in appropriate operation condition and has been adequately cleaned, it is recommended to set the following system suitability.

Confirm that the measured values (particle size and number of particles) obtained for an appropriate particle standard are within the pre-defined range. Confirm that the number of particles in filtered water (prepared immediately before use) is not more than the specified value. Set an appropriate particle size range according to the purpose. When the number of particles in the filtered water falls outside the appropriate range, repeat preparation of water to be used and cleaning of the instrument, and re-measure.

G5 Crude Drugs

Change the following as follows:

On the Scientific Names of Crude Drugs listed in the JP <*G5-1-182*>

The notation system of the scientific names for the original plants, algae, fungi and animals of crude drugs listed in the JP is not necessary the same as the taxonomic system used in the literature. The reason for this is that the JP is not an academic text, but an ordinance. The relationship between the scientific names used in the JP and those generally used taxonomically is indicated in the following table, to avoid misunderstanding by JP users owing to differences in the notation system. In addition, the family names of plants listed in the JP are based on the modified Engler's classification system. The APG classification system based on DNA sequence information was published in 1998, revised several times and is mainly used in the systematic botany currently. Therefore the relationship of family names in both the modified Engler's system and the APG system is also described.

For gymnosperms, animals and fungi, which are not included in the APG classification system, the family names follow Yonekura[1)] and GBIF[2)].

Scientific Names used in the JP and Those used Taxonomically

Crude Drug	Scientific names used in the JP = Scientific names being used taxonomically[3,4] / Scientific names that are different from those written in the JP but identical to them taxonomically or being regarded as identical, and typical sub-classified groups belonging to their species. The names marked with "*" are those being written together in the JP.	Family Description in the JP	Family APG IV etc.[1,2,5,6]
Acacia アラビアゴム	*Acacia senegal* Willdenow = *Acacia senegal* (L.) Willd.	*Leguminosae*	*Leguminosae/ Fabaceae*
	Other species of the same genus		
Achyranthes Root ゴシツ	*Achyranthes bidentata* Blume	*Amaranthaceae*	*Amaranthaceae*
	Achyranthes fauriei H. Léveillé et Vaniot = *Achyranthes fauriei* H. Lev. & Vaniot		
Agar カンテン	*Gelidium elegans* Kuetzing	*Gelidiaceae*	*Gelidiaceae*[#]
	Other species of the same genus		
	Several red algae		
Akebia Stem モクツウ	*Akebia quinata* Decaisne = *Akebia quinata* (Thunb. ex Houtt.) Decne.	*Lardizabalaceae*	*Lardizabalaceae*
	Akebia trifoliata Koidzumi = *Akebia trifoliata* (Thunb.) Koidz.		
	Interspecific hybrid between above species		
Alisma Tuber タクシャ	*Alisma orientale* Juzepczuk = *Alisma orientale* (Sam.) Juz.	*Alismataceae*	*Alismataceae*
	Alisma plantago-aquatica L. var. *orientale* Sam.		
Aloe アロエ	*Aloe ferox* Miller = *Aloe ferox* Mill.	*Liliaceae*	*Asphodelaceae*
	Interspecific hybrid between *Aloe ferox* Miller and *Aloe africana* Miller *Aloe africana* Miller = *Aloe africana* Mill.		
	Interspecific hybrid between *Aloe ferox* Miller and *Aloe spicata* Baker		
Alpinia Officinarum Rhizome リョウキョウ	*Alpinia officinarum* Hance	*Zingiberaceae*	*Zingiberaceae*

Amomum Seed シュクシャ	*Amomum villosum* Loureiro var. *xanthioides* T. L. Wu et S. J. Chen = *Amomum villosum* Lour. var. *xanthioides* (Wall. ex Baker) T. L. Wu & S. J. Chen	*Zingiberaceae*	*Zingiberaceae*
	Amomum xanthioides Wallich = *Amomum xanthioides* Wall. ex Baker		
	Amomum villosum Lour. var. *nanum* H. T. Tsai & S. W. Zhao		
	Amomum villosum Loureiro var. *villosum* = *Amomum villosum* Lour. var. *villosum*		
	Amomum villosum Lour.		
	Amomum longiligulare T. L. Wu		
Anemarrhena Rhizome チモ	*Anemarrhena asphodeloides* Bunge	*Liliaceae*	*Asparagaceae*
Angelica Dahurica Root ビャクシ	*Angelica dahurica* Bentham et Hooker filius ex Franchet et Savatier = *Angelica dahurica* (Hoffm.) Benth. & Hook. f. ex Franch. & Sav.	*Umbelliferae*	*Umbelliferae/ Apiaceae*
Apricot Kernel キョウニン	*Prunus armeniaca* Linné = *Prunus armeniaca* L.	*Rosaceae*	*Rosaceae*
	Prunus armeniaca Linné var. *ansu* Maximowicz = *Prunus armeniaca* L. var. *ansu* Maxim.		
	Prunus sibirica Linné = *Prunus sibirica* L.		
Aralia Rhizome ドクカツ	*Aralia cordata* Thunberg = *Aralia cordata* Thunb.	*Araliaceae*	*Araliaceae*
Areca ビンロウジ	*Areca catechu* Linné = *Areca catechu* L.	*Palmae*	*Palmae/ Arecaceae*
Artemisia Capillaris Flower インチンコウ	*Artemisia capillaris* Thunberg = *Artemisia capillaris* Thunb.	*Compositae*	*Compositae/ Asteraceae*
Artemisia Leaf ガイヨウ	*Artemisia princeps* Pampanini = *Artemisia princeps* Pamp.	*Compositae*	*Compositae/ Asteraceae*
	Artemisia montana Pampanini = *Artemisia montana* (Nakai) Pamp.		
Asiasarum Root サイシン	*Asiasarum heterotropoides* F. Maekawa var. *mandshuricum* F. Maekawa = *Asiasarum heterotropoides* (F. Schmidt) F. Maek. var. *mandshuricum* (Maxim.) F. Maek.	*Aristolochiaceae*	*Aristolochiaceae*
	Asarum heterotropoides F. Schmidt var. *mandshuricum* (Maxim.) Kitag.		
	Asiasarum sieboldii F. Maekawa = *Asiasarum sieboldii* (Miq.) F. Maek.		
	Asarum sieboldii Miq. *Asarum sieboldii* Miq. var. *seoulense* Nakai		
Asparagus Root テンモンドウ	*Asparagus cochinchinensis* Merrill = *Asparagus cochinchinensis* (Lour.) Merr.	*Liliaceae*	*Asparagaceae*

Astragalus Root オウギ	*Astragalus mongholicus* Bunge	*Leguminosae*	*Leguminosae/ Fabaceae*
	Astragalus membranaceus (Fisch.) Bunge var. *mongholicus* (Bunge) Hsiao		
	Astragalus membranaceus Bunge = *Astragalus membranaceus* (Fisch.) Bunge		
Atractylodes Lancea Rhizome ソウジュツ	*Atractylodes lancea* De Candolle = *Atractylodes lancea* (Thunb.) DC.	*Compositae*	*Compositae/ Asteraceae*
	Atractylodes chinensis Koidzumi = *Atractylodes chinensis* (Bunge) Koidz.		
	Interspecific hybrid between above species		
Atractylodes Rhizome ビャクジュツ	*Atractylodes japonica* Koidzumi ex Kitamura = *Atractylodes japonica* Koidz. ex Kitam.	*Compositae*	*Compositae/ Asteraceae*
	Atractylodes macrocephala Koidzumi = *Atractylodes macrocephala* Koidz.		
	* *Atractylodes ovata* De Candolle = *Atractylodes ovata* (Thunb.) DC.		
Bear Bile ユウタン	*Ursus arctos* Linné = *Ursus arctos* L.	*Ursidae*	*Ursidae*[#]
	Closely related species		
Bearberry Leaf ウワウルシ	*Arctostaphylos uva-ursi* Sprengel = *Arctostaphylos uva-ursi* (L.) Spreng.	*Ericaceae*	*Ericaceae*
Beef Tallow 牛脂	*Bos taurus* Linné var. *domesticus* Gmelin = *Bos taurus* L. var. *domesticus* Gmelin	*Bovidae*	*Bovidae*[#]
Yellow Beeswax ミツロウ	*Apis mellifera* Linné = *Apis mellifera* L.	*Apidae*	*Apidae*[#]
	Apis cerana Fabricius		
Belladonna Root ベラドンナコン	*Atropa belladonna* Linné = *Atropa belladonna* L.	*Solanaceae*	*Solanaceae*
Benincasa Seed トウガシ	*Benincasa cerifera* Savi	*Cucurbitaceae*	*Cucurbitaceae*
	Benincasa hispida (Thunb.) Cogn.		
	Benincasa cerifera Savi forma *emarginata* K. Kimura et Sugiyama = *Benincasa cerifera* Savi f. *emarginata* K. Kimura & Sugiyama		
Benzoin アンソッコウ	*Styrax benzoin* Dryander = *Styrax benzoin* Dryand.	*Styracaceae*	*Styracaceae*
	Other species of the same genus		
Bitter Cardamon ヤクチ	*Alpinia oxyphylla* Miquel = *Alpinia oxyphylla* Miq.	*Zingiberaceae*	*Zingiberaceae*
Bitter Orange Peel トウヒ	*Citrus aurantium* Linné = *Citrus aurantium* L.	*Rutaceae*	*Rutaceae*
	Citrus aurantium Linné var. *daidai* Makino = *Citrus aurantium* L. var. *daidai* Makino		
	Citrus aurantium L. 'Daidai'		

Brown Rice コウベイ	*Oryza sativa* Linné = *Oryza sativa* L.	*Gramineae*	*Gramineae/* *Poaceae*
Bupleurum Root サイコ	*Bupleurum falcatum* Linné = *Bupleurum falcatum* L.	*Umbelliferae*	*Umbelliferae/* *Apiaceae*
	Bupleurum chinense DC. *Bupleurum scorzonerifolium* Willd.		
Burdock Fruit ゴボウシ	*Arctium lappa* Linné = *Arctium lappa* L.	*Compositae*	*Compositae/* *Asteraceae*
Cacao Butter カカオ脂	*Theobroma cacao* Linné = *Theobroma cacao* L.	*Sterculiaceae*	*Malvaceae*
Calumba コロンボ	*Jateorhiza columba* Miers	*Menispermaceae*	*Menispermaceae*
Camellia Oil ツバキ油	*Camellia japonica* Linné = *Camellia japonica* L.	*Theaceae*	*Theaceae*
Capsicum トウガラシ	*Capsicum annuum* Linné = *Capsicum annuum* L.	*Solanaceae*	*Solanaceae*
Cardamon ショウズク	*Elettaria cardamomum* Maton	*Zingiberaceae*	*Zingiberaceae*
Carnauba Wax カルナウバロウ	*Copernicia cerifera* Martius = *Copernicia cerifera* Mart.	*Palmae*	*Palmae/* *Arecaeae*
Cassia Seed ケツメイシ	*Cassia obtusifolia* Linné = *Cassia obtusifolia* L.	*Leguminosae*	*Leguminosae/* *Fabaceae*
	Cassia tora Linné = *Cassia tora* L.		
Castor Oil ヒマシ油	*Ricinus communis* Linné = *Ricinus communis* L.	*Euphorbiaceae*	*Euphorbiaceae*
Catalpa Fruit キササゲ	*Catalpa ovata* G. Don	*Bignoniaceae*	*Bignoniaceae*
	Catalpa bungei C. A. Meyer = *Catalpa bungei* C. A. Mey.		
Cherry Bark オウヒ	*Prunus jamasakura* Siebold ex Koidzumi = *Prunus jamasakura* Siebold ex Koidz.	*Rosaceae*	*Rosaceae*
	Prunus verecunda Koehne = *Prunus verecunda* (Koidz.) Koehne		
Chrysanthemum Flower キクカ	*Chrysanthemum indicum* Linné = *Chrysanthemum indicum* L.	*Compositae*	*Compositae/* *Asteraceae*
	Chrysanthemum morifolium Ramatuelle = *Chrysanthemum morifolium* Ramat.		
Cimicifuga Rhizome ショウマ	*Cimicifuga dahurica* Maximowicz = *Cimicifuga dahurica* (Turcz.) Maxim.	*Ranunculaceae*	*Ranunculaceae*
	Cimisifuga heracleifolia Komarov = *Cimisifuga heracleifolia* Kom.		
	Cimicifuga foetida Linné = *Cimicifuga foetida* L.		
	Cimicifuga simplex Turczaninow = *Cimicifuga simplex* (DC.) Turcz.		

Cinnamon Bark ケイヒ	*Cinnamomum cassia* J. Presl = *Cinnamomum cassia* (L.) J. Presl	*Lauraceae*	*Lauraceae*
Cinnamon Oil ケイヒ油	*Cinnamomum cassia* J. Presl = *Cinnamomum cassia* (L.) J. Presl	*Lauraceae*	*Lauraceae*
	Cinnamomum zeylanicum Nees		
Cistanche Herb ニクジュヨウ	*Cistanche salsa* G. Beck = *Cistanche salsa* (C.A.Mey.) Beck	*Orobanchaceae*	*Orobanchaceae*
	Cistanche deserticola Y. C. Ma = *Cistanche deserticola* Ma		
	Cistanche tubulosa Wight		
Citrus Unshiu Peel チンピ	*Citrus unshiu* Marcowicz = *Citrus unshiu* (Swingle) Marcow.	*Rutaceae*	*Rutaceae*
	Citrus reticulata Blanco 'Unshiu'		
	Citrus reticulata Blanco		
Clematis Root イレイセン	*Clematis mandshurica* Ruprecht = *Clematis mandshurica* Rupr.	*Ranunculaceae*	*Ranunculaceae*
	Clematis chinensis Osbeck		
	Clematis hexapetala Pallas = *Clematis hexapetala* Pall.		
Clove チョウジ Clove Oil チョウジ油	*Syzygium aromaticum* Merrill et L. M. Perry = *Syzygium aromaticum* (L.) Merr. & L. M. Perry	*Myrtaceae*	*Myrtaceae*
	* *Eugenia caryophyllata* Thunberg = *Eugenia caryophyllata* Thunb. *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison		
Cnidium Monnieri Fruit ジャショウシ	*Cnidium monnieri* Cusson = *Cnidium monnieri* (L.) Cusson	*Umbelliferae*	*Umbelliferae/ Apiaceae*
Cnidium Rhizome センキュウ	*Cnidium officinale* Makino	*Umbelliferae*	*Umbelliferae/ Apiaceae*
Coconut Oil ヤシ油	*Cocos nucifera* Linné = *Cocos nucifera* L.	*Palmae*	*Palmeae/ Arecaceae*
Codonopsis Root トウジン	*Codonopsis pilosula* Nannfeldt = *Codonopsis pilosula* Nannf.	*Campanulaceae*	*Campanulaceae*
	Codonopsis tangshen Oliver = *Codonopsis tangshen* Oliv.		
Coix Seed ヨクイニン	*Coix lacryma-jobi* Linné var. *mayuen* Stapf = *Coix lacryma-jobi* L. var. *mayuen* (Rom. Caill.) Stapf	*Gramineae*	*Gramineae/ Poaceae*
Condurango コンズランゴ	*Marsdenia cundurango* Reichenbach filius = *Marsdenia cundurango* Rchb. f.	*Asclepiadaceae*	*Apocynaceae*

Coptis Rhizome オウレン	*Coptis japonica* Makino = *Coptis japonica* (Thunb.) Makino	*Ranunculaceae*	*Ranunculaceae*
	Coptis japonica (Thunb.) Makino var. *dissecta* (Yatabe) Nakai *Coptis japonica* (Thunb.) Makino var. *japonica* *Coptis japonica* (Thunb.) Makino var. *major* (Miq.) Satake		
	Coptis chinensis Franchet = *Coptis chinensis* Franch.		
	Coptis deltoidea C. Y. Cheng et Hsiao		
	Coptis teeta Wallich = *Coptis teeta* Wall.		
Corn Oil トウモロコシ油	*Zea mays* Linné = *Zea mays* L.	*Gramineae*	*Gramineae/ Poaceae*
Cornus Fruit サンシュユ	*Cornus officinalis* Siebold et Zuccarini = *Cornus officinalis* Siebold & Zucc.	*Cornaceae*	*Cornaceae*
Corydalis Tuber エンゴサク	*Corydalis turtschaninovii* Besser forma *yanhusuo* Y. H. Chou et C. C. Hsu = *Corydalis turtschaninovii* Besser f. *yanhusuo* (W. T. Wang) Y. H. Chou & C. C. Hsu	*Papaveraceae*	*Papaveraceae*
	Corydalis yanhusuo W. T. Wang		
Crataegus Fruit サンザシ	*Crataegus cuneata* Siebold et Zuccarini = *Crataegus cuneata* Siebold & Zucc.	*Rosaceae*	*Rosaceae*
	Crataegus pinnatifida Bunge var. *major* N. E. Brown = *Crataegus pinnatifida* Bunge var. *major* N. E. Br.		
Curcuma Rhizome ガジュツ	*Curcuma zedoaria* Roscoe	*Zingiberaceae*	*Zingiberaceae*
	Curcuma phaeocaulis Valeton		
	Curcuma kwangsiensis S. G. Lee et C. F. Liang		
Cyperus Rhizome コウブシ	*Cyperus rotundus* Linné = *Cyperus rotundus* L.	*Cyperaceae*	*Cyperaceae*
Digenea マクリ	*Digenea simplex* C. Agardh = *Digenea simplex* (Wulfen) C. Agardh	*Rhodomelaceae*	*Rhodomelaceae*[#]
Dioscorea Rhizome サンヤク	*Dioscorea japonica* Thunberg = *Dioscorea japonica* Thunb.	*Dioscoreaceae*	*Dioscoreaceae*
	Dioscorea batatas Decaisne = *Dioscorea batatas* Decne.		
	Dioscorea opposita Thunb.		
Dolichos Seed ヘンズ	*Dolichos lablab* Linné = *Dolichos lablab* L.	*Leguminosae*	*Leguminosae/ Fabaceae*
Eleutherococcus Senticosus Rhizome シゴカ	*Eleutherococcus senticosus* Maximowicz = *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim.	*Araliaceae*	*Araliaceae*
	* *Acanthopanax senticosus* Harms = *Acanthopanax senticosus* (Rupr. & Maxim.) Harms		

Ephedra Herb マオウ	*Ephedra sinica* Stapf	*Ephedraceae*	*Ephedraceae*[#]
	Ephedra intermedia Schrenk et C. A. Meyer = *Ephedra intermedia* Schrenk & C. A. Mey.		
	Ephedra equisetina Bunge		
Epimedium Herb インヨウカク	*Epimedium koreanum* Nakai	*Berberidaceae*	*Berberidaceae*
	Epimedium grandiflorum Morren var. *thunbergianum* Nakai = *Epimedium grandiflorum* Morr. var. *thunbergianum* (Miq.) Nakai		
	Epimedium pubescens Maximowicz = *Epimedium pubescens* Maxim.		
	Epimedium brevicornu Maximowicz = *Epimedium brevicornu* Maxim.		
	Epimedium wushanense T. S. Ying		
	Epimedium sagittatum Maximowicz = *Epimedium sagittatum* (Siebold & Zucc.) Maxim.		
	Epimedium sempervirens Nakai		
Eucalyptus Oil ユーカリ油	*Eucalyptus globulus* Labillardiere = *Eucalyptus globulus* Labill.	*Myrtaceae*	*Myrtaceae*
	Closely related species		
Eucommia Bark トチュウ	*Eucommia ulmoides* Oliver = *Eucommia ulmoides* Oliv.	*Eucommiaceae*	*Eucommiaceae*
Euodia Fruit ゴシュユ	*Euodia officinalis* Dode	*Rutaceae*	*Rutaceae*
	* *Evodia officinalis* Dode *Evodia rutaecarpa* (A. juss.) Benth. var. *officinalis* (Dode) Huang		
	Euodia bodinieri Dode		
	* *Evodia bodinieri* Dode *Evodia rutaecarpa* (A. Juss.) Benth. var. *bodinieri* (Dode) Huang		
	Euodia ruticarpa Hooker filius et Thomson = *Euodia ruticarpa* (A. Juss.) Hook. f. & Thomson		
	* *Evodia rutaecarpa* Bentham = *Evodia rutaecarpa* (A. Juss.) Benth. *Tetradium ruticarpum* (A. Juss.) Hartley		
Fennel ウイキョウ	*Foeniculum vulgare* Miller = *Foeniculum vulgare* Mill.	*Umbelliferae*	*Umbelliferae/ Apiaceae*
Fennel Oil ウイキョウ油	*Foeniculum vulgare* Miller = *Foeniculum vulgare* Mill.	*Umbelliferae*	*Umbelliferae/ Apiaceae*
	Illicium verum Hooker filius = *Illicium verum* Hook. f.	*Illiciaceae*	*Schisandraceae*
Forsythia Fruit レンギョウ	*Forsythia suspensa* Vahl = *Forsythia suspensa* (Thunb.) Vahl	*Oleaceae*	*Oleaceae*

Fritillaria Bulb バイモ	*Fritillaria verticillata* Willdenow var. *thunbergii* Baker = *Fritillaria verticillata* Willd. var. *thunbergii* (Miq.) Baker	*Liliaceae*	*Liliaceae*
	Fritillaria thunbergii Miq.		
Gambir アセンヤク	*Uncaria gambir* Roxburgh = *Uncaria gambir* (Hunter) Roxb.	*Rubiaceae*	*Rubiaceae*
Gardenia Fruit サンシシ	*Gardenia jasminoides* J. Ellis	*Rubiaceae*	*Rubiaceae*
	Gardenia jasminoides J. Ellis f. *longicarpa* Z. W. Xie & M. Okada		
Gastrodia Tuber テンマ	*Gastrodia elata* Blume	*Orchidaceae*	*Orchidaceae*
Gentian ゲンチアナ	*Gentiana lutea* Linné = *Gentiana lutea* L.	*Gentianaceae*	*Gentianaceae*
Geranium Herb ゲンノショウコ	*Geranium thunbergii* Siebold et Zuccarini = *Geranium thunbergii* Siebold & Zucc.	*Geraniaceae*	*Geraniaceae*
Ginger ショウキョウ	*Zingiber officinale* Roscoe	*Zingiberaceae*	*Zingiberaceae*
Ginseng ニンジン	*Panax ginseng* C. A. Meyer = *Panax ginseng* C. A. Mey.	*Araliaceae*	*Araliaceae*
	* *Panax schinseng* Nees		
Glehnia Root and Rhizome ハマボウフウ	*Glehnia littoralis* F. Schmidt ex Miquel = *Glehnia littoralis* F. Schmidt ex Miq.	*Umbelliferae*	*Umbelliferae/ Apiaceae*
Glycyrrhiza カンゾウ	*Glycyrrhiza uralensis* Fischer = *Glycyrrhiza uralensis* Fisch.	*Leguminosae*	*Leguminosae/ Fabaceae*
	Glycyrrhiza glabra Linné = *Glycyrrhiza glabra* L.		
Hedysarum Root シンギ	*Hedysarum polybotrys* Handel-Mazzetti = *Hedysarum polybotrys* Hand.-Mazz.	*Leguminosae*	*Leguminosae/ Fabaceae*
Hemp Fruit マシニン	*Cannabis sativa* Linné = *Cannabis sativa* L.	*Moracea*	*Cannabaceae*
Honey ハチミツ	*Apis mellifera* Linné = *Apis mellifera* L.	*Apidae*	*Apidae*#
	Apis cerana Fabricius		
Houttuynia Herb ジュウヤク	*Houttuynia cordata* Thunberg = *Houttuynia cordata* Thunb.	*Saururaceae*	*Saururaceae*
Immature Orange キジツ	*Citrus aurantium* Linné var. *daidai* Makino = *Citrus aurantium* L. var. *daidai* Makino	*Rutaceae*	*Rutaceae*
	Citrus aurantium L. 'Daidai'		
	Citrus natsudaidai Hayata		
	Citrus aurantium Linné = *Citrus aurantium* L.		
	Citrus aurantium L. subsp. *hassaku* (Tanaka) Hiroe = *Citrus hassaku* hort. ex Tanaka		

Imperata Rhizome ボウコン	*Imperata cylindrica* Beauvois = *Imperata cylindrica* (L.) P. Beauv.	*Gramineae*	*Gramineae/ Poaceae*
	Imperata cylindrica (L.) P. Beauv. var. *major* (Nees) C. E. Hubb.		
Ipecac トコン	*Cephaelis ipecacuanha* A. Richard = *Cephaelis ipecacuanha* (Brot.) A. Rich.	*Rubiaceae*	*Rubiaceae*
	Cephaelis acuminata Karsten = *Cephaelis acuminata* H. Karst.		
Japanese Angelica Root トウキ	*Angelica acutiloba* Kitagawa = *Angelica acutiloba* (Siebold & Zucc.) Kitag.	*Umbelliferae*	*Umbelliferae/ Apiaceae*
	Angelica acutiloba Kitagawa var. *sugiyamae* Hikino = *Angelica acutiloba* (Siebold & Zucc.) Kitag. var. *sugiyamae* Hikino		
Japanese Gentian リュウタン	*Gentiana scabra* Bunge	*Gentianaceae*	*Gentianaceae*
	Gentiana scabra Bunge var. *buergeri* (Miq.) Maxim.		
	Gentiana manshurica Kitagawa = *Gentiana manshurica* Kitag.		
	Gentiana triflora Pallas = *Gentiana triflora* Pall.		
	Gentiana triflora Pall. var. *japonica* Hara		
Japanese Valerian カノコソウ	*Valeriana fauriei* Briquet = *Valeriana fauriei* Briq.	*Valerianaceae*	*Caprifoliaceae*
	Valeriana fauriei Briq. f. *yezoensis* Hara		
Japanese Zanthoxylum Peel サンショウ	*Zanthoxylum piperitum* De Candolle = *Zanthoxylum piperitum* (L.) DC.	*Rutaceae*	*Rutaceae*
	Zanthoxylum piperitum (L.) DC. f. *inerme* Makino		
Jujube タイソウ	*Ziziphus jujuba* Miller var. *inermis* Rehder = *Ziziphus jujuba* Mill. var. *inermis* (Bunge) Rehder	*Rhamnaceae*	*Rhamnaceae*
Jujube Seed サンソウニン	*Ziziphus jujuba* Miller var. *spinosa* Hu ex H. F. Chow = *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chow	*Rhamnaceae*	*Rhamnaceae*
Koi コウイ	*Zea mays* Linné = *Zea mays* L.	*Gramineae*	*Gramineae/ Poaceae*
	Manihot esculenta Crantz	*Euphorbiaceae*	*Euphorbiaceae*
	Solanum tuberosum Linné = *Solanum tuberosum* L.	*Solanaceae*	*Solanaceae*
	Ipomoea batatas Poiret = *Ipomoea batatas* (L.) Poir.	*Convolvulaceae*	*Convolvulaceae*
	Ipomoea batatas (L.) Lam.		
	Oryza sativa Linné = *Oryza sativa* L.	*Gramineae*	*Gramineae/ Poaceae*
Purified Lanolin 精製ラノリン	*Ovis aries* Linné = *Ovis aries* L.	*Bovidae*	*Bovidae*[#]

Lard 豚脂	*Sus scrofa* Linné var. *domesticus* Gray = *Sus scrofa* L. var. *domesticus* Gray	*Suidae*	*Suidae*[#]
Leonurus Herb ヤクモソウ	*Leonurus japonicus* Houttuyn = *Leonurus japonicus* Houtt.	*Labiatae*	*Labiatae/* *Lamiaceae*
	Leonurus sibiricus Linné = *Leonurus sibiricus* L.		
Lilium Bulb ビャクゴウ	*Lilium lancifolium* Thunberg = *Lilium lancifolium* Thunb.	*Liliaceae*	*Liliaceae*
	Lilium brownii F. E. Brown var. *colchesteri* Wilosn = *Lilium brownii* F. E. Br. var. *colchesteri* (Van Houtte) E. H. Wilson ex Elwes		
	Lilium brownii F. E. Brown var. *viridulum* Baker		
	Lilium brownii F. E. Brown = *Lilium brownii* F. E. Br.		
	Lilium pumilum De Candolle = *Lilium pumilum* DC.		
Lindera Root ウヤク	*Lindera strychnifolia* Fernandez-Villar = *Lindera strychnifolia* (Siebold & Zucc.) Fern.-Vill.	*Lauraceae*	*Lauraceae*
	Lindera aggregata (Sims) Kosterm.		
Lithospermum Root シコン	*Lithospermum erythrorhizon* Siebold et Zuccarini = *Lithospermum erythrorhizon* Siebold & Zucc.	*Boraginaceae*	*Boraginaceae*
Longan Aril リュウガンニク	*Euphoria longana* Lamarck = *Euphoria longana* Lam.	*Sapindaceae*	*Sapindaceae*
	Dimocarpus longan Lour.		
Lonicera Leaf and Stem ニンドウ	*Lonicera japonica* Thunberg = *Lonicera japonica* Thunb.	*Caprifoliaceae*	*Caprifoliaceae*
Loquat Leaf ビワヨウ	*Eriobotrya japonica* Lindley = *Eriobotrya japonica* (Thunb.) Lindl.	*Rosaceae*	*Rosaceae*
Lycium Bark ジコッピ	*Lycium chinense* Miller = *Lycium chinense* Mill.	*Solanaceae*	*Solanaceae*
	Lycium barbarum Linné = *Lycium barbarum* L.		
Lycium Fruit クコシ	*Lycium chinense* Miller = *Lycium chinense* Mill.	*Solanaceae*	*Solanaceae*
	Lycium barbarum Linné = *Lycium barbarum* L.		
Magnolia Bark コウボク	*Magnolia obovata* Thunberg = *Magnolia obovata* Thunb.	*Magnoliaceae*	*Magnoliaceae*
	* *Magnolia hypoleuca* Siebold et Zuccarini = *Magnolia hypoleuca* Siebold & Zucc.		
	Magnolia officinalis Rehder et E. H. Wilson		
	Magnolia officinalis Rehder et E. H. Wilson var. *biloba* Rehder et E. H. Wilson		

Magnolia Flower シンイ	*Magnolia biondii* Pampanini = *Magnolia biondii* Pamp.	*Magnoliaceae*	*Magnoliaceae*
	Magnolia heptapeta Dandy = *Magnolia heptapeta* (Buchoz) Dandy		
	* *Magnolia denudata* Desrousseaux = *Magnolia denudata* Desr.		
	Magnolia sprengeri Pampanini = *Magnolia sprengeri* Pamp.		
	Magnolia salicifolia Maximowicz = *Magnolia salicifolia* (Siebold & Zucc.) Maxim.		
	Magnolia kobus De Candolle = *Magnolia kobus* DC.		
Mallotus Bark アカメガシワ	*Mallotus japonicus* Müler Argoviensis = *Mallotus japonicus* (Thunb.) Müll. Arg.	*Euphorbiaceae*	*Euphorbiaceae*
Malt バクガ	*Hordeum vulgare* Linné = *Hordeum vulgare* L.	*Gramineae*	*Gramineae/* *Poaceae*
Mentha Herb ハッカ Mentha Oil ハッカ油	*Mentha arvensis* Linné var. *piperascens* Malinvaud = *Mentha arvensis* L. var. *piperascens* Malinv.	*Labiatae*	*Labiatae/* *Lamiaceae*
	Mentha haplocalyx Briq.		
	Hybrid originated from *Mentha arvensis* L. var. *piperascens* Malinv. as the mother species		
Moutan Bark ボタンピ	*Paeonia suffruticosa* Andrews	*Paeoniaceae*	*Paeoniaceae*
	* *Paeonia moutan* Sims		
Mulberry Bark ソウハクヒ	*Morus alba* Linné = *Morus alba* L.	*Moraceae*	*Moraceae*
Nelumbo Seed レンニク	*Nelumbo nucifera* Gaertner = *Nelumbo nucifera* Gaertn.	*Nymphaeaceae*	*Nelumbonaceae*
Notopterygium キョウカツ	*Notopterygium incisum* Ting ex H. T. Chang	*Umbelliferae*	*Umbelliferae/* *Apiaceae*
	Notopterygium forbesii Boissieu		
Nuphar Rhizome センコツ	*Nuphar japonica* De Candolle = *Nuphar japonica* DC.	*Nymphaeaceae*	*Nymphaeaceae*
	Nuphar pumila De Candolle = *Nuphar pumila* (Timm) DC.		
	Interspecific hybrid between above species		
Nutmeg ニクズク	*Myristica fragrans* Houttuyn = *Myristica fragrans* Houtt.	*Myristicaceae*	*Myristicaceae*
Nux Vomica ホミカ	*Strychnos nux-vomica* Linné = *Strychnos nux-vomica* L.	*Loganiaceae*	*Loganiaceae*
Olive Oil オリブ油	*Olea europaea* Linné = *Olea europaea* L.	*Oleaceae*	*Oleaceae*
Ophiopogon Root バクモンドウ	*Ophiopogon japonicus* Ker-Gawler = *Ophiopogon japonicus* (L. f.) Ker Gawl.	*Liliaceae*	*Asparagaceae*
Orange Oil オレンジ油	Several *Citrus* species	*Rutaceae*	*Rutaceae*

Oriental Bezoar ゴオウ	*Bos taurus* Linné var. *domesticus* Gmelin = *Bos taurus* L. var. *domesticus* Gmelin	*Bovidae*	*Bovidae*[#]
Oyster Shell ボレイ	*Ostrea gigas* Thunberg = *Ostrea gigas* Thunb.	*Ostreidae*	*Ostreidae*[#]
Panax Japonicus Rhizome チクセツニンジン	*Panax japonicus* C. A. Meyer = *Panax japonicus* C. A. Mey.	*Araliaceae*	*Araliaceae*
Peach Kernel トウニン	*Prunus persica* Batsch = *Prunus persica* (L.) Batsch	*Rosaceae*	*Rosaceae*
	Prunus persica Batsch var. *davidiana* Maximowicz = *Prunus persica* (L.) Batsch var. *davidiana* (Carrière) Maxim.		
	Prunus davidiana (Carrière) Franch.		
Peanut Oil ラッカセイ油	*Arachis hypogaea* Linné = *Arachis hypogaea* L.	*Leguminosae*	*Leguminosae/ Fabaceae*
Peony Root シャクヤク	*Paeonia lactiflora* Pallas = *Paeonia lactiflora* Pall.	*Paeoniaceae*	*Paeoniaceae*
Perilla Herb ソヨウ	*Perilla frutescens* Britton var. *crispa* W. Deane = *Perilla frutescens* (L.) Britton var. *crispa* (Thunb.) W. Deane	*Labiatae*	*Labiatae/ Lamiaceae*
Peucedanum Root ゼンコ	*Peucedanum praeruptorum* Dunn	*Umbelliferae*	*Umbelliferae/ Apiaceae*
	Angelica decursiva Franchet et Savatier = *Angelica decursiva* (Miq.) Franch. & Sav.		
	* *Peucedanum decursivum* Maximowicz = *Peucedanum decursivum* (Miq.) Maxim.		
Pharbitis Seed ケンゴシ	*Pharbitis nil* Choisy = *Pharbitis nil* (L.) Choisy	*Convolvulaceae*	*Convolvulaceae*
Phellodendron Bark オウバク	*Phellodendron amurense* Ruprecht = *Phellodendron amurense* Rupr.	*Rutaceae*	*Rutaceae*
	Phellodendron amurense Rupr. var. *sachalinense* F. Schmidt *Phellodendron amurense* Rupr. var. *japonicum* (Maxim.) Ohwi *Phellodendron amurense* Rupr. var. *lavallei* (Dode) Sprague		
	Phellodendron chinense Schneider = *Phellodendron chinense* C. K. Schneid.		
Picrasma Wood ニガキ	*Picrasma quassioides* Bennet = *Picrasma quassioides* (D. Don) Benn.	*Simaroubaceae*	*Simaroubaceae*
Pinellia Tuber ハンゲ	*Pinellia ternata* Breitenbach = *Pinellia ternata* (Thunb.) Breitenb.	*Araceae*	*Araceae*
Plantago Herb シャゼンソウ	*Plantago asiatica* Linné = *Plantago asiatica* L.	*Plantaginaceae*	*Plantaginaceae*
Plantago Seed シャゼンシ	*Plantago asiatica* Linné = *Plantago asiatica* L.	*Plantaginaceae*	*Plantaginaceae*
Platycodon Root キキョウ	*Platycodon grandiflorus* A. De Candolle = *Platycodon grandiflorus* (Jacq.) A. DC.	*Campanulaceae*	*Campanulaceae*

Pogostemon Herb カッコウ	*Pogostemon cablin* Bentham = *Pogostemon cablin* (Blanco) Benth.	*Labiatae*	*Labiatae/ Lamiaceae*
Polygala Root オンジ	*Polygala tenuifolia* Willdenow = *Polygala tenuifolia* Willd.	*Polygalaceae*	*Polygalaceae*
Polygonatum Rhizome オウセイ	*Polygonatum kingianum* Collett et Hemsley = *Polygonatum kingianum* Collett & Hemsl.	*Liliaceae*	*Asparagaceae*
	Polygonatum sibiricum Redouté		
	Polygonatum cyrtonema Hua		
	Polygonatum falcatum A. Gray		
Polygonum Root カシュウ	*Polygonum multiflorum* Thunberg = *Polygonum multiflorum* Thunb.	*Polygonaceae*	*Polygonaceae*
Polyporus Sclerotium チョレイ	*Polyporus umbellatus* Fries = *Polyporus umbellatus* (Pers.) Fries	*Polyporaceae*	*Polyporaceae*[#]
Poria Sclerotium ブクリョウ	*Wolfiporia cocos* Ryvarden et Gilbertson = *Wolfiporia cocos* (Schw.) Ryv. & Gilbn.	*Polyporaceae*	*Polyporaceae*[#]
	* *Poria cocos* Wolf = *Poria cocos* (Schw.) Wolf		
Powdered Opium アヘン末	*Papaver somniferum* Linné = *Papaver somniferum* L.	*Papaveraceae*	*Papaveraceae*
Prepared Glycyrrhiza シャカンゾウ	*Glycyrrhiza uralensis* Fischer = *Glycyrrhiza uralensis* Fisch.	*Leguminosae*	*Leguminosae/ Fabaceae*
	Glycyrrhiza glabra Linné = *Glycyrrhiza glabra* L.		
Processed Aconite Root ブシ	*Aconitum carmichaeli* Debeaux	*Ranunculaceae*	*Ranunculaceae*
	Aconitum japonicum Thunberg = *Aconitum japonicum* Thunb.		
Processed Ginger カンキョウ	*Zingiber officinale* Roscoe	*Zingiberaceae*	*Zingiberaceae*
Prunella Spike カゴソウ	*Prunella vulgaris* Linné var. *lilacina* Nakai = *Prunella vulgaris* L. var. *lilacina* Nakai	*Labiatae*	*Labiatae/ Lamiaceae*
Pueraria Root カッコン	*Pueraria lobata* Ohwi = *Pueraria lobata* (Willd.) Ohwi	*Leguminosae*	*Leguminosae/ Fabaceae*
Quercus Bark ボクソク	*Quercus acutissima* Carruthers = *Quercus acutissima* Carruth.	*Fagaceae*	*Fagaceae*
	Quercus serrata Murray		
	Quercus mongholica Fischer ex Ledebour var. *crispula* Ohashi = *Quercus mongholica* Fisch. ex Ledeb. var. *crispula* (Blume) Ohashi		
	Quercus variabilis Blume		
Rape Seed Oil ナタネ油	*Brassica napus* Linné = *Brassica napus* L.	*Cruciferae*	*Cruciferae/ Brassicaceae*
	Brassica rapa Linné var. *oleifera* De Candolle = *Brassica rapa* L. var. *oleifera* DC.		

Red Ginseng コウジン	*Panax ginseng* C. A. Meyer = *Panax ginseng* C. A. Mey.	*Araliaceae*	*Araliaceae*
	* *Panax schinseng* Nees		
Rehmannia Root ジオウ	*Rehmannia glutinosa* Liboschitz var. *purpurea* Makino = *Rehmannia glutinosa* Libosch. var. *purpurea* Makino	*Scrophulariaceae*	*Orobanchaceae*
	Rehmannia glutinosa Liboschitz = *Rehmannia glutinosa* Libosch.		
Rhubarb ダイオウ	*Rheum palmatum* Linné = *Rheum palmatum* L.	*Polygonaceae*	*Polygonaceae*
	Rheum tanguticum Maximowicz = *Rheum tanguticum* Maxim.		
	Rheum officinale Baillon = *Rheum officinale* Baill.		
	Rheum coreanum Nakai		
	Interspecific hybrid between above species		
Rose Fruit エイジツ	*Rosa multiflora* Thunberg = *Rosa multiflora* Thunb.	*Rosaceae*	*Rosaceae*
Rosin ロジン	Several *Pinus* species	*Pinaceae*	*Pinaceae*[♯]
Royal Jelly ローヤルゼリー	*Apis mellifera* Linné = *Apis mellifera* L.	*Apidae*	*Apidae*[♯]
	Apis cerana Fabricius		
Safflower コウカ	*Carthamus tinctorius* Linné = *Carthamus tinctorius* L.	*Compositae*	*Compositae/ Asteraceae*
Saffron サフラン	*Crocus sativus* Linné = *Crocus sativus* L.	*Iridaceae*	*Iridaceae*
Salvia Miltiorrhiza Root タンジン	*Salvia miltiorrhiza* Bunge	*Labiatae*	*Labiatae/ Lamiaceae*
Saposhnikovia Root and Rhizome ボウフウ	*Saposhnikovia divaricata* Schischkin = *Saposhnikovia divaricata* (Turcz.) Schischk.	*Umbelliferae*	*Umbelliferae/ Apiaceae*
Sappan Wood ソボク	*Caesalpinia sappan* Linné = *Caesalpinia sappan* L.	*Leguminosae*	*Leguminosae/ Fabaceae*
Saussurea Root モッコウ	*Saussurea lappa* Clarke = *Saussurea lappa* (Decne.) C. B. Clarke	*Compositae*	*Compositae/ Asteraceae*
	Aucklandia lappa Decne.		
Schisandra Fruit ゴミシ	*Schisandra chinensis* Baillon = *Schisandra chinensis* (Turcz.) Baill.	*Schisandraceae*	*Schisandraceae*
Schizonepeta Spike ケイガイ	*Schizonepeta tenuifolia* Briquet = *Schizonepeta tenuifolia* Briq.	*Labiatae*	*Labiatae/ Lamiaceae*

Scopolia Rhizome ロートコン	*Scopolia japonica* Maximowicz = *Scopolia japonica* Maxim.	*Solanaceae*	*Solanaceae*
	Scopolia carniolica Jacquin = *Scopolia carniolica* Jacq.		
	Scopolia parviflora Nakai = *Scopolia parviflora* (Dunn) Nakai		
Scutellaria Root オウゴン	*Scutellaria baicalensis* Georgi	*Labiatae*	*Labiatae/ Lamiaceae*
Senega セネガ	*Polygala senega* Linné = *Polygala senega* L.	*Polygalaceae*	*Polygalaceae*
	Polygala senega Linné var. *latifolia* Torrey et Gray = *Polygala senega* L. var. *latifolia* Torr. & A. Gray		
Senna Leaf センナ	*Cassia angustifolia* Vahl	*Leguminosae*	*Leguminosae/ Fabaceae*
	Cassia acutifolia Delile		
Sesame ゴマ Sesame Oil ゴマ油	*Sesamum indicum* Linné = *Sesamum indicum* L.	*Pedaliaceae*	*Pedaliaceae*
Sinomenium Stem ボウイ	*Sinomenium acutum* Rehder et E. H. Wilson = *Sinomenium acutum* (Thunb.) Rehder & E. H. Wilson	*Menispermaceae*	*Menispermaceae*
Smilax Rhizome サンキライ	*Smilax glabra* Roxburgh = *Smilax glabra* Roxb.	*Liliaceae*	*Smilacaceae*
Sophora Root クジン	*Sophora flavescens* Aiton	*Leguminosae*	*Leguminosae/ Fabaceae*
Soybean Oil ダイズ油	*Glycine max* Merrill = *Glycine max* (L.) Merr.	*Leguminosae*	*Leguminosae/ Fabaceae*
Sweet Hydrangea Leaf アマチャ	*Hydrangea macrophylla* Seringe var. *thunbergii* Makino = *Hydrangea macrophylla* (Thunb.) Ser. var. *thunbergii* (Siebold) Makino	*Saxifragaceae*	*Hydrangeaceae*
Swertia Herb センブリ	*Swertia japonica* Makino = *Swertia japonica* (Shult.) Makino	*Gentianaceae*	*Gentianaceae*
Toad Cake センソ	*Bufo gargarizans* Cantor	*Bufonidae*	*Bufonidae*[#]
	= *Bufo bufo gargarizans* Cantor		
	Bufo melanostictus Schneider = *Duttaphrynus melanostictus* Schneider		
Tragacanth トラガント	*Astragalus gummifer* Labillardiére = *Astragalus gummifer* Labill.	*Leguminosae*	*Leguminosae/ Fabaceae*
Tribulus Fruit シツリシ	*Tribulus terrestris* Linné = *Tribulus terrestris* L.	*Zygophyllaceae*	*Zygophyllaceae*

Trichosanthes Root カロコン	*Trichosanthes kirilowii* Maximowicz = *Trichosanthes kirilowii* Maxim.	*Cucurbitaceae*	*Cucurbitaceae*
	Trichosanthes kirilowii Maximowicz var. *japonica* Kitamura = *Trichosanthes kirilowii* Maxim. var. *japonica* (Miq.) Kitam.		
	Trichosanthes bracteata Voigt = *Trichosanthes bracteata* (Lam.) Voigt		
Turmeric ウコン	*Curcuma longa* Linné = *Curcuma longa* L.	*Zingiberaceae*	*Zingiberaceae*
Turpentine Oil テレビン油	Several *Pinus* species	*Pinaceae*	*Pinaceae*♯
Uncaria Hook チョウトウコウ	*Uncaria rhynchophylla* Miquel = *Uncaria rhynchophylla* (Miq.) Miq.	*Rubiaceae*	*Rubiaceae*
	Uncaria sinensis Haviland = *Uncaria sinensis* (Oliv.) Havil.		
	Uncaria macrophylla Wallich = *Uncaria macrophylla* Wall.		
Wood Creosote 木クレオソート	Several *Pinus* species	*Pinaceae*	*Pinaceae*♯
	Several *Cryptomeria* species	*Taxodiaceae*	*Cupressaceae*♯
	Several *Fagus* species	*Fagaceae*	*Fagaceae*
	Afzelia (*Intsia*) species	*Leguminosae*	*Leguminosae*/ *Fabaceae*
	Shorea species	*Dipterocarpaceae*	*Dipterocarpaceae*
	Tectona species	*Verbenaceae*	*Labiatae*/ *Lamiaceae*

Reference

1) Yonekura K.: Updated Syllabus of Vascular Plant Families, Hokuryukan, Tokyo, 2019, ISBN 978-4-8326-1008-8.
2) Global Biodiversity Information Facility, https://www.gbif.org. (Accessed April 15, 2022).
3) Terabayashi S. et al.: Pharmaceutical and Medical Device Regulatory Science, 41, 407-418 (2010).
4) When ''Other species of the same genus'' is included as its original plants the scientific name is not written in Monograph, however, it is written in this table.
5) Takano A. et al.: Pharmaceutical and Medical Device Regulatory Science, 52, 291-302 (2021).
6) Gymnosperms, algae, fungi and animals which are not included in the APG IV are marked with ♯.

Thin-layer Chromatography for Crude Drugs and Crude Drug Preparations <*G5-3-182*>

Change the following read as follows:

Thin-layer chromatography for crude drugs and preparations containing crude drugs as main ingredient (crude drug preparations) is used for identifying whether characteristic components or groups of constituents in crude drugs and extracts based on Kampo formulae are included or not. In this general information, the following items are described to supplement Thin-layer Chromatography <*2.03*> when performing a test of thin-layer chromatography for crude drugs and crude drug preparations.

1. Instruments and equipment

Thin-layer chromatography <*2.03*> is applied. However, for thin-layer plates, because more precise separation of components may be required for multi-component crude drugs and crude drug preparations, high-performance thin-

layer plates (HPTLC plate) coated with silica gel for chromatography (5 to 7 μm) that has a smaller particle size than that of silica gel for thin-layer chromatography prescribed in Solid Support/Column Packings for Chromatography <*9.42*> can be used. The suitability of the light source of a detection device should be confirmed when the specification of a lamp or irradiation system is changed, or when a specified spot is not recognized by irradiation with a wavelength of a line light source specified in a monograph.

2. Operating method

Thin-layer chromatography <*2.03*> is applied.

3. Identification and purity tests

Thin-layer Chromatography <*2.03*> is applied. For the identification and purity tests of crude drugs and crude drug preparations by thin-layer chromatography, a reference standard, a component to be tested, a crude drug as a reagent, or a crude drug prescribed in the individual monograph is generally used as the reference material. In the case of a multi-component sample solution, it is possible to set a test method that evaluates by the color tone and *R*f value of spots without using a reference material when components to be tested are recognized as single spots clearly showing characteristic fluorescence and coloration. Since crude drugs and crude drug preparations are natural products and have complex component patterns, the combination of Thin-layer Chromatography <*2.03*> with a spectroscopic method (such as Ultraviolet-visible Spectrophotometry <*2.24*>, Nuclear Magnetic Resonance Spectroscopy <*2.21*>) or Mass Spectrometry <*2.62*> is expected for further improvement in the reliability of identification or purity tests.

4. Points to consider in changing test conditions for identification tests

Thin layer chromatography <*2.03*> is applied. The test method without using a reference material can be changed to the method that confirm the identification by comparing the color tone and *R*f values with a reference material.

5. Terminology

The definitions described under Chromatography <*2.00*> are applied.

6. Others

When quantification is performed by thin-layer chromatography, quantitative measurements are possible by using an automated sample spotting device and densitometry. For the system suitability of these scanning devices for thin-layer chromatography, the specification of the system suitability under Liquid Chromatography <*2.01*> is applied as necessary.

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