Consultation documents

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The International Pharmacopoeia

Ganciclovir (Ganciclovirum)

This is a draft proposal of a monograph for The International Pharmacopoeia (Working document QAS/16.652/Rev.1, July 2017). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

Molecular formula. C₉H₁₃N₅O₄
Relative molecular mass. 255.23
Graphic formula

Chemical name. 2-Amino-9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-1,9-dihydro-6H-purin-6-one. CAS Reg. No. 82410-32-0.
Description. White or almost white, crystalline powder.
Solubility. Slightly soluble in water or glacial acetic acid, very slightly soluble in dehydrated ethanol, practically insoluble in methanol and dichloromethane. It dissolves in dilute solutions of mineral acids and alkali hydroxides.
Category. Antiviral (Purine nucleoside analogue).
Storage. Preserve in well-closed containers. Protect from light and moisture.
Additional information. Ganciclovir is hygroscopic and may exhibit polymorphism. Caution: Ganciclovir is a potent cytotoxic agent and suspected carcinogen. It must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements

Definition. Ganciclovir contains not less than 99.0% and not more than 101.0% of C₉H₁₃N₅O₄, calculated with reference to the anhydrous substance.

Identity tests

Either test A alone, or tests B and D, or tests C and D may be applied.

A. Carry out the test as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ganciclovir RS or with the reference spectrum of ganciclovir.

If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and ganciclovir RS in a small amount of hot water R (80°C), allowing to cool in an ice-bath, filtering and drying the precipitate at 105°C for 3 hours. The infrared absorption spectrum is concordant with the spectrum obtained from ganciclovir RS.

B. Carry out test B.1 or, where UV detection is not available, test B.2.

B.1 Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R6 as the coating substance and a mixture of 4 volumes of ammonia (260 g/L) TS, 40 volumes of methanol R and 60 volumes of dichloromethane R as the mobile phase. Apply separately to the plate 5 μL of each of the following three solutions. For solution (A) dissolve 10 mg of the substance to be examined in 2 mL of sodium hydroxide (~0.8 g/L) TS and dilute to 10 mL with methanol R. For solution (B) dissolve 10 mg of ganciclovir RS in 2 mL of sodium hydroxide (~0.8 g/L) TS and dilute to 10 mL with methanol R. For solution (C) dissolve 10 mg of ganciclovir RS and 10 mg of aciclovir R in 2 mL of sodium hydroxide (~0.8 g/L) TS and dilute to 10 mL with methanol R. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air and examine the chromatogram under ultraviolet light (254 nm). The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots. The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to ganciclovir in the chromatogram obtained with solution (B).

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described above under test B.1 but using silica gel R5 as the coating substance. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or heat the plate for five minutes at 120°C. Spray the plate with Dragendorff reagent TS and allow it to dry exhaustively in air. Then spray the plate with a mixture of sulfuric acid (~1760 g/L) TS and dehydrated ethanol R (1:1). Examine the chromatogram in daylight. The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots. The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and
intensity with the spot due to ganciclovir in the chromatogram obtained with solution (B).

C. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Related substances”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the ganciclovir peak in the chromatogram obtained with solution (3).

D. Dissolve about 5 mg of the sample in 500 mL of water R. The absorption spectrum (1.6) of this solution, when observed between 200 nm and 300 nm, exhibits a minimum at about 222 nm and a maximum at about 252 nm with a shoulder at about 275 nm.

**Clarity and colour of solution.** Dissolve 1.25 g in sodium hydroxide (~40 g/L) TS and dilute to 25 mL. This solution is clear and not more intensely coloured than reference solution Y5, when compared as described under 1.11.2 Degree of coloration of liquids, Method II.

[Note from the Secretariat. The chapter 1.11 Colour of liquids is currently under revision. Reference is already made to a new test procedure to be added under the section 1.11.2 Degree of coloration of liquids in the 7th Edition of The International Pharmacopoeia.]

**Heavy metals.** Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the content of heavy metals according to Method A; not more than 10 μg/g.

**Sulfated ash** (2.3). Not more than 1.0 mg/g.

**Water.** Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.300 g of the substance and methanol as solvent. The substance to be examined has a limited solubility in methanol and will appear as a slurry. Replace the solvent after each titration. The water content is not more than 40 mg/g.

**Related substances.** Carry out the test as described under 1.14.4 High performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded strong acidic cation-exchange groups (3–10 μm).¹

Use the following mobile phase: Dilute 0.5 mL of trifluoroacetic acid R to 1000 mL with water R. Mix 500 volumes of this solution with 500 volumes of acetonitrile R.

Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column at 40°C.

Prepare the following solutions using mobile phase as a diluent. For solution (1) dissolve about 30 mg of the test substance using sonication and dilute to 50.0 mL. For solution (2) dilute 1 volume of solution (1) to 1000 volumes. For solution (3) dissolve 3.0 mg of ganciclovir RS using sonication and dilute to 5.0 mL. For solution (4) dissolve the content of a vial of ganciclovir for system suitability RS (containing the impurities A, B, C, D, E and F) in 1.0 mL of solution (3).

Inject alternately 20 μL each of solutions (1), (2), (3) and (4). Record the chromatograms for about 2.5 times the retention time of ganciclovir (retention time about 14 minutes).

¹ A Thermo BioBasic SCX column (4.6 mm × 250 mm, 5 μm) has been found suitable.
Ganciclovir (Ph. Int.)

Use the chromatogram supplied with ganciclovir for system suitability RS and the chromatograms obtained with reference solution (3) and (4) to identify the peaks due to ganciclovir and the impurities A, B, C, D, E and F. The following peaks are eluted at the following relative retention with reference to the peak of ganciclovir: impurity A about 0.6; impurity B about 0.67; impurity C about 0.71; impurity D about 0.8; impurity E about 0.9; impurity F about 2.0.

The test is not valid unless in the chromatogram obtained with solution (4) the peak-to-valley ratio ($H_p/H_v$) is at least 5, where $H_p$ is the height above the baseline of the peak due to impurity E and $H_v$ is the height above the baseline of the lowest point of the curve separating this peak from the peak due to ganciclovir.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A, C, D or E is not greater than 1.5 times the area of the peak due to ganciclovir in the chromatogram obtained with solution (2) (0.15%);
- the area of any peak corresponding to impurity B, when multiplied by a correction factor of 1.3, is not greater than twice the area of the peak due to ganciclovir in the chromatogram obtained with solution (2) (0.2%);
- the area of any peak corresponding to impurity F, when multiplied by a correction factor of 0.7, is not greater than 4 times the area of the peak due to ganciclovir in the chromatogram obtained with solution (2) (0.4%);
- the area of any other impurity peak is not greater than 0.5 times the area of the peak due to ganciclovir in the chromatogram obtained with solution (2) (0.05%);
- the sum of the corrected areas of the peaks corresponding to impurity B and impurity F and the areas of all other impurity peaks is not greater than 6 times the area of the peak due to ganciclovir in the chromatogram obtained with solution (2) (0.6%). Disregard any peak with an area less than 0.3 times the area of the peak due to ganciclovir obtained with solution (2) (0.03%).

**Assay.** Dissolve about 0.200 g, accurately weighed, in 10 mL of anhydrous formic acid R and dilute to 60 mL with anhydrous glacial acetic acid R. Titrate with perchloric acid (0.1 mol/L) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titration. Carry out a blank titration. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 25.52 mg of ganciclovir ($C_9H_{13}N_5O_4$).

**Additional requirements for Ganciclovir for parenteral use**

Complies with the monograph for Parenteral preparations.

**Bacterial endotoxins.** If intended for use in the manufacture of a parenteral dosage form without a further appropriate procedure for the removal of bacterial endotoxins, carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.84 IU of endotoxin RS per mg of ganciclovir.
Impurities

[Note from the Secretariat. The impurities will be brought into alphabetical order at a later stage of the monograph development.]

A. $R = \text{CH}_2\text{O-CH}_2\text{-CCl=CH}_2$: 2-amino-9-[[2-chloroprop-2-en-1-yl]oxy] methyl]-1,9-di hydro-6$H$-purin-6-one (synthesis-related impurity),

D. $R = \text{CH}_2\text{O-CH}_2\text{-O-CH(CH}_2\text{OH)}_2$: 2-amino-9-[[2-hydroxy-1-(hydroxymethyl) ethoxy] methoxy]methyl]-1,9-dihydro-6$H$-purin-6-one (synthesis-related impurity),

F. $R = \text{H}$: 2-amino-1,9-dihydro-6$H$-purin-6-one (guanine) (synthesis-related impurity, degradation product),

B. $R = \text{O-CO-CH}_2\text{CH}_3$: (2$RS$)-2-[(2-amino-6-oxo-1,6-dihydro-9$H$-purin-9-yl) methoxy]-3-hydroxypropyl propionate (synthesis-related impurity),

C. $R = \text{Cl}$: 2-amino-9-[[1$RS$]-2-chloro-1-(hydroxymethyl)ethoxy]methyl]-1,9- dihydro-6$H$-purin-6-one (synthesis-related impurity),

E. 2-amino-9-[[2$RS$]-2,3-dihydroxypropoxy]methyl]-1,9-dihydro-6$H$-purin-6-one (synthesis-related impurity),
**New reference substances**

**Ganciclovir RS**

**Ganciclovir for system suitability RS** (containing the impurities A, B, C, D, E and F)

**New reagent**

**Aciclovir R**

Aciclovir of a suitable quality should be used.

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Ganciclovir for injection
(Gancicloviri ad injectionem)

This is a draft proposal of a monograph for The International Pharmacopoeia (Working document QAS/16.653, July 2017). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

**Description.** A white powder or loose lumps.

**Category.** Antiviral (Purine nucleoside analogue).

**Storage.** Ganciclovir for injection should be kept in a tightly closed container, protected from moisture and light.

**Additional information.** Ganciclovir for injection 500 mg is listed on the 12th invitation to manufacturers of medicinal products for HIV infection and related diseases to submit an Expression of Interest (EOI) for product evaluation to the WHO Prequalification of Medicines Team. Handle Ganciclovir for injection with great care because it is a potent cytotoxic agent and suspected carcinogen.

Ganciclovir for injection is hygroscopic.

**Requirements**

The powder for injection and the reconstituted solution for injection complies with the monograph for Parenteral preparations.

**Definition.** Ganciclovir for injection is a freeze-dried powder prepared by the neutralization of Ganciclovir with the aid of sodium hydroxide. Ganciclovir for injection contains not less than 90.0% and not more than 110.0% of the labelled amount of ganciclovir (C₉H₁₃N₅O₄).

**Identity tests**

Either test A alone, or tests B and D, or tests C and D may be applied.

A. Dilute a quantity of the test substance, containing the equivalent of about 0.2 g of Ganciclovir with 10 mL water R. Adjust the suspension to pH 6–7 with hydrochloric acid (0.1 mol/L) TS and allow to stand for 30 minutes. Filter the suspension, wash the filtrate with 20 mL water R and dry it at 105°C for 3 hours. Carry out the test as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the reference spectrum of ganciclovir or with the spectrum obtained from ganciclovir RS treated similarly.

If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the dried filtrate and ganciclovir RS in a small amount of hot water R (80°C), allowing to cool in an ice-bath, filtering and drying the precipitate at 105°C for 3 hours. The infrared absorption spectrum is concordant with the spectrum obtained from ganciclovir RS.
Ganciclovir for injection (Ph. Int.)

B. Carry out test B.1 or, where UV detection is not available, test B.2.

B.1 Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R6 as the coating substance and a mixture of 4 volumes of ammonia (260 g/L) TS, 40 volumes of methanol R and 60 volumes of dichloromethane R as the mobile phase. Apply separately to the plate 5 μL of each of the following three solutions. For solution (A) dissolve a quantity of the test substance, containing the equivalent of about 10 mg of ganciclovir in 2 mL water R and dilute to 10 mL with methanol R. For solution (B) dissolve 10 mg of ganciclovir RS in 2 mL of sodium hydroxide (0.8 g/L) TS and dilute to 10 mL with methanol R. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air and examine the chromatogram under ultraviolet light (254 nm). The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to ganciclovir in the chromatogram obtained with solution (B).

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described above under test B.1 but using silica gel R5 as the coating substance. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or heat the plate for five minutes at 120°C. Spray the plate with Dragendorff reagent TS and allow it to dry exhaustively in air. Then spray the plate with a mixture of sulfuric acid (~1760 g/L) TS and dehydrated ethanol R (1:1). Examine the chromatogram in daylight. The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots. The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to ganciclovir in the chromatogram obtained with solution (B).

C. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to ganciclovir in the chromatogram obtained with solution (2).

D. Dissolve a quantity of the powder for injection equivalent to 20 mg of ganciclovir in 2 mL hydrochloric acid (~420 g/L) TS, evaporate the solution to dryness on a hot water-bath, add 1 mL hydrochloric acid (~420 g/L) TS and about 30 mg potassium chlorate R. Then evaporate the solution to dryness on a hot water-bath and add drops of ammonia (~100 g/L) TS to the residues; a violet-red colour is produced. Add drops of sodium hydroxide (~40 g/L) TS and the violet-red colour disappears.

**pH value** (1.13). pH of a solution containing the equivalent to 12.5 mg of ganciclovir per mL of water R, 10.5–11.5.

**Clarity and colour of solution.** A solution, containing the equivalent to 0.10 g of ganciclovir in 10 mL of water R, is clear and not more intensely coloured than reference solution Y5, when compared as described under 1.11.2 Degree of coloration of liquids, Method II.

*[Note from the Secretariat. The chapter 1.11 Colour of liquids is currently under revision. Reference is already made to a new test procedure to be added under the section 1.11.2 Degree of coloration of liquids.]*
Ganciclovir for injection (Ph. Int.)

**Water.** Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.300 g of the substance and methanol as solvent. The substance to be examined has a limited solubility in methanol and will appear as a slurry. Replace the solvent after each titration. The water content is not more than 30 mg/g.

**Related substances.** Carry out the test as described under 1.14.4 High performance liquid chromatography using the conditions given under “Assay”.

Prepare the following solutions using mobile phase as a diluent. For test solution (1) dissolve a quantity of the powder for injection, containing the equivalent of about 30 mg ganciclovir, using sonication, and dilute to 50.0 mL. For solution (2) dilute 1 volume of solution (1) to 1000 volumes. For solution (3) dissolve 3.0 mg of ganciclovir RS and dilute to 5.0 mL. For solution (4) dissolve the content of a vial of ganciclovir for system suitability RS (containing the impurities A, B, C, D, E and F) in 1.0 mL of solution (3).

Inject alternately 20 μL each of solutions (1), (2), (3) and (4). Record the chromatograms for 2.5 times of the retention time of ganciclovir (retention time about 14 minutes).

Use the chromatogram supplied with ganciclovir for system suitability RS and the chromatogram obtained with reference solution (4) to identify the peaks due to ganciclovir and the impurities A, B, C, D, E and F. The following peaks are eluted at the following relative retention with reference to the peak of ganciclovir: impurity A = about 0.6; impurity B = about 0.67; impurity C = about 0.71; impurity D = about 0.8; impurity E = about 0.9; impurity F = about 2.0.

The test is not valid unless in the chromatogram obtained with solution (4) the peak-to-valley ratio ($H_p/H_v$) is at least 5, where $H_p$ is the height above the baseline of the peak due to impurity E and $H_v$ is the height above the baseline of the lowest point of the curve separating this peak from the peak due to ganciclovir.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity F, when multiplied by a correction factor of 0.7, is not greater than 4 times the area of the peak due to ganciclovir in the chromatogram obtained with solution (2) (0.4%);

**Assay.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded strong acidic cation-exchange groups (3–10 μm).\(^1\)

Use the following mobile phase: Dilute 0.5 mL of trifluoroacetic acid R to 1000 mL with water R. Mix 500 volumes of this solution with 500 volumes of acetonitrile R.

Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column at 40°C.

Weigh and mix the contents of 5 containers. Prepare the following solutions in mobile phase. For solution (1) dissolve a quantity of the powder of injection, equivalent to about 30.0 mg of ganciclovir, accurately weighed, and dilute to 50.0 mL. Dilute 10.0 mL of this solution to

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\(^1\) The Thermo BioBasic SCX column (4.6 mm × 250 mm, 5 μm) has been found suitable.
Ganciclovir for injection (Ph. Int.)

100.0 mL. For solution (2) dissolve 15.0 mg of ganciclovir RS, and dilute to 25.0 mL. Dilute 10.0 mL of this solution to 100.0 mL.

Inject alternately 20 µL each of solution (1) and (2).

Measure the areas of the peaks corresponding to ganciclovir in the chromatograms of solution (1) and (2) and calculate the percentage content of ganciclovir \((C_9H_{13}N_5O_4)\) per container, using the declared content of \(C_9H_{13}N_5O_4\) in ganciclovir RS.

**Bacterial endotoxins.** Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.84 IU of endotoxin per mg of ganciclovir.

**Impurities**

- The impurities limited by the requirements of this monograph include impurity F listed in the monograph on Ganciclovir.
Protionamide
(Protionamidum)

This is a draft proposal of a monograph for The International Pharmacopoeia (Working document QAS/17.722/Rev.2, July 2017). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

Molecular formula. $C_9H_{12}N_2S$

Relative molecular mass. 180.3

Graphic formula

Chemical name. 2-Propylthioisonicotinamide; 2-propyl-4-pyridinecarbothioamide; CAS Reg. No. 14222-60-7.

Description. Yellow crystals or a crystalline powder.

Solubility. Practically insoluble in water; soluble in dehydrated ethanol R and methanol R; slightly soluble in ether R.

Category. Tuberculostatic.

Storage. Protionamide should be kept in a well-closed container, protected from light.

Additional information. Protionamide may exhibit polymorphism.

Requirements

Definition. Protionamide contains not less than 99.0% and not more than 101.0% of $C_9H_{12}N_2S$, calculated with reference to the dried substance.

Identity tests

• Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from protionamide RS or with the reference spectrum of protionamide.

If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the test substance and protionamide RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from protionamide RS.
Protonamide (Ph. Int.)

B. The absorption spectrum (1.6) of a 10 μg/mL solution of the test substance in ethanol (~750 g/L) TS, when observed between 230 nm and 350 nm, exhibits a maximum at about 291 nm and a minimum at 256 nm.

C. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Related substances”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to protonamide in the chromatogram obtained with solution (3).

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3. Determine the heavy metals content according to Method A; not more than 20 μg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry 1.000 g of the test substance to constant weight at 105°C; it loses not more than 5.0 mg/g.

Acidity. Dissolve 2.0 g in 20 mL of methanol R by warming. Add 20 mL of water R, shake and cool to precipitate protonamide. Add 2 drops of cresol red/ethanol TS and titrate with sodium hydroxide (0.1 mol/L) VS. Not more than 0.20 mL is required to change the colour of the indicator.

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel for chromatography R (5 μm).¹

As the mobile phase use a mixture of 72 volumes of a buffer solution, prepared by mixing 2.0 mL of triethylamine R with 1000 mL water and adjusting the pH to 6.0 with phosphoric acid (~105 g/L) TS, and 28 volumes of acetonitrile R. Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 290 nm.

Prepare the following solutions in mobile phase. For solution (1) dissolve about 50 mg of the test substance in 100.0 mL. For solution (2) dilute 1 volume of solution (1) to 200 volumes. For solution (3) use a solution containing 0.05 mg of protonamide RS and 0.01 mg of ethionamide R per mL.

Inject 20 μL of solution (3). Ethionamide is eluted at a relative retention of about 0.6 with reference to protonamide (retention time about 10 minutes). The test is not valid unless the resolution between the peaks due to ethionamide and protonamide is at least 5.0.

Inject alternately 20 μL each of solution (1) and (2). Record the chromatograms for 2 times the retention time of protonamide.

In the chromatogram obtained with solution (1):

¹ Inertsil ODS was found suitable.
• the area of any peak corresponding to impurity A (ethionamide), is not greater than the area of the peak due to protionamide in the chromatogram obtained with solution (2) (0.5%);
• the area of any impurity peak is not greater than 0.4 times the area of the peak due to protionamide in the chromatogram obtained with solution (2) (0.2%);
• the sum of the areas of all impurity peaks is not greater than 2 times the area of the peak due to protionamide in the chromatogram obtained with solution (2) (1.0%).

Disregard any peak with an area less than 0.2 times the area of the principal peak obtained with solution (2) (0.10%).

**Assay.** Dissolve about 0.45 g, accurately weighed, in 30 mL of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 18.03 mg of C$_9$H$_{12}$N$_2$S.

**Impurity**

![Structure of 2-Ethylthioisonicotinamide](image)

A. 2-Ethylthioisonicotinamide; 2-ethyl-4-pyridinecarbothioamide (ethionamide).

**Reference substance to be established**

Protionamide RS

**Reagent to be established**

Ethionamide R

Ethionamide of a suitable quality should be used.

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Protionamide tablets
(Protionamidi compressi)

This is a draft proposal of a monograph for The International Pharmacopoeia (Working document QAS/17.723/Rev.2, July 2017). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

Category. Tuberculostatic.

Storage. Protionamide tablets should be kept in a well-closed container, protected from light.

Additional information. Strength in the current WHO Model List of Essential Medicines (EML): 125 mg; 250 mg. Strength in the current WHO EML for children: 125 mg; 250 mg.

Requirements

Comply with the monograph for Tablets.

Definition. Protionamide tablets contain not less than 90.0% and not more than 110.0% of the amount of protionamide (C₉H₁₂N₂S) stated on the label.

Identity tests

- Either test A alone or tests B and C may be applied.

A. Extract a quantity of the powered tablets containing about 25 mg of protionamide with 5 mL of methanol R, filtrate and evaporate the filtrate to dryness. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from protionamide RS or with the reference spectrum of protionamide.

If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the test substance and protionamide RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from protionamide RS.

B. To a quantity of powdered tablets containing the equivalent of about 2.5 mg of protionamide add 25 mL ethanol (~750 g/L) TS, shake and filter. Dilute 1 mL of the filtrate to 10 mL with the same solvent. The absorption spectrum (1.6) of the resulting solution, when observed between 230 nm and 350 nm, exhibits a maximum at about 291 nm and a minimum at 256 nm.

C. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”. The retention time of the principle peak in the chromatogram obtained from solution (1) corresponds to the retention time of the peak due to protionamide in the chromatogram obtained with solution (2).
Dissolution. Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium 900 mL of hydrochloric acid (~4 g/L) TS and rotating the paddle at 100 revolutions per minute. At 30 minutes withdraw a sample of 10 mL of the medium through an in-line filter and allow the filtered sample to cool to room temperature. Measure the absorbance (1.6) of a 1 cm layer of the resulting solution, suitably diluted if necessary, at a wavelength of 277 nm using the dissolution medium as the blank. Measure at the same time and under the same conditions the absorbance of a suitable solution of protionamide RS in the dissolution medium.

For each of the tablets tested, calculate the total amount of protionamide (C₉H₁₂N₂S) in the dissolution medium from the absorbances obtained. Evaluate the results as described under 5.5 Dissolution test for solid dosage forms. The amount of protionamide in solution for each tablet is not less than 75% (Q) of the amount stated on the label.

[Note from the Secretariat. It is intended to determine the absorptivity value of protionamide during the establishment of protionamide RS. The value will then be included in the test description.]

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”.

For solution (1) transfer a quantity of the powdered tablets equivalent to about 250 mg of protionamide, accurately weighed, into a 250 mL volumetric flask, disperse in 100 mL, shake vigorously and dilute to volume. Filter the resulting solution and dilute 25.0 mL of this solution to 50.0 mL. For solution (2) dilute 1 volume of solution (1) to 100 volumes with mobile phase. For solution (3) use a solution containing 0.05 mg of protionamide RS and 0.01 mg of ethionamide R per mL mobile phase.

Inject 20 µL of solution (3). Ethionamide is eluted at a relative retention of about 0.6 with reference to protionamide (retention time about 10 minutes). The test is not valid unless the resolution between the peaks due to ethionamide and protionamide is at least 5.0.

Inject alternately 20 µL each of solution (1) and (2). Record the chromatograms for 2 times the retention time of protionamide.

In the chromatogram obtained with solution (1):

- the area of any impurity peak is not greater than the area of the peak due to protionamide in the chromatogram obtained with solution (2) (0.5%).

Assay. Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm ×4.6 mm) packed with particles of silica gel for chromatography R (5 µm).¹

As the mobile phase use a mixture of 72 volumes of a buffer solution prepared by mixing 2.0 mL of triethylamine R with 1000 mL water and adjusting the pH to 6.0 with phosphoric acid (~105 g/L) TS and 28 volumes of acetonitrile R.

Operate with a flow rate of 1 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 290 nm.

¹ Inertsil ODS was found suitable.
Prepare the following solutions in mobile phase. For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing about 250 mg of protionamide, accurately weighed, into a 250 mL volumetric flask, disperse in 100 mL, shake vigorously and dilute to volume. Filter the resulting solution and dilute 10.0 mL of this solution to 200.0 mL. For solution (2) dilute 50.0 mg of protionamide RS and 10.0 mg of ethionamide R in 100.0 mL. Dilute 10.0 mL of this solution to 100.0 mL.

Inject 20 μL of solution (2). Ethionamide is eluted at a relative retention of about 0.6 with reference to protionamide (retention time about 10 minutes). The test is not valid unless the resolution between the peaks due to ethionamide and protionamide is at least 5.0.

Inject alternately 20 μL each of solution (1) and (2). Record the chromatogram.

Measure the areas of the peaks corresponding to protionamide obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of protionamide (C₉H₁₂N₂S) in the tablets, using the declared content of protionamide (C₉H₁₂N₂S) in protionamide RS.

**Impurities**

The impurities limited by the requirements of this monograph include the impurity listed in the monograph on Protionamide.

**Reference substance to be established**

Protionamide RS
Reagent to be established

Ethionamide R
Ethionamide of a suitable quality should be used.
This is a draft proposal of a monograph for *The International Pharmacopoeia* (Working document QAS/17.724/Rev.2, July 2017). The working document with line numbers is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects](http://www.who.int/medicines/areas/quality_safety/quality_assurance/projects).

**Molecular formula.** $C_{27}H_{38}O_3$

**Relative molecular mass.** 410.6

**Chemical names.** 17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one heptanoate; 17-[(1-oxoheptyloxy]-19-nor-17α-pregn-4-en-20-yn-3-one; CAS Reg. No. 3836-23-5.

**Other name.** Norethindrone enantate.

**Description.** A white to yellowish white, crystalline powder.

**Solubility.** Practically insoluble in water R; freely soluble in acetone R, methanol R, dehydrated ethanol R and dioxan R.

**Category.** Contraceptive.

**Storage.** Norethisterone enantate should be kept in a tightly closed container, protected from light.

**Requirements**

Norethisterone enantate contains not less than 98.0% and not more than 102.0% (“Assay”, Method A) or not less than 97.0% and not more than 102.0% (“Assay”, Method B) of $C_{27}H_{38}O_3$, calculated with reference to the dried substance.

**Identity tests**

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from norethisterone enantate RS or with the reference spectrum of norethisterone enantate.
Norethisterone enantate (Ph. Int.)

The absorption spectrum \((1.6)\) of a solution of about 15 μg of the test substance per mL in methanol R, when observed between 210 nm and 290 nm, exhibits a maximum at about 240 nm.

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”, Method B. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to norethisterone enantate in the chromatogram obtained with solution (2).

Specific optical rotation. Use a 20 mg/mL solution in dichloromethan R; \(\alpha_D^{20^\circ} = -10.0^\circ\) to \(-15.0^\circ\).

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry over desiccant silica gel R at ambient temperature for 4 hours; it loses not more than 5.0 mg/g.

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the chromatographic conditions as described under “Assay”, Method B.

Prepare the following solutions in methanol R. For solution (1) dilute a suitable amount of sample to obtain a concentration of 1.0 mg of Norethisterone enantate per mL. For solution (2) dilute 1 volume of solution (1) to 100 volumes. For solution (3) prepare a solution containing 1.0 mg per mL of norethisterone enantate RS and 0.1 mg per mL of norethisterone caproate R.

Inject 20 μL of solution (3). The test is not valid unless the resolution between the peak due to norethisterone caproate (with a relative retention of about 0.95) and the peak due to norethisterone enantate (retention time about 27 minutes) is at least 4.0.

Inject alternatively 20 μL of solutions (1) and (2).

In the chromatogram obtained with solution (1):

- the area of any impurity peak is not greater than 0.3 times the area of the peak due to norethisterone enantate in the chromatogram obtained with solution (2) (0.3%);
- the sum of the areas of all impurities is not greater than the area of the peak due to norethisterone enantate in the chromatogram obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.05 times the area of the peak due to norethisterone enantate in the chromatogram obtained with solution (2) (0.05%).

Free enantic acid. Dissolve 0.3 g in 10 mL of neutralized ethanol (~750 g/L) TS. Titrate the solution quickly with sodium hydroxide (0.01 mol/L) VS to a light blue end-point using bromothymol blue/ethanol TS as indicator; not more than 0.3 mL (corresponding to 1.3 mg/g of enantic acid).

Assay

- Either method A or B may be applied.

A. Dissolve about 15 mg, accurately weighed, in sufficient methanol R and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of this solution to 100.0 mL with methanol R.
Measure the absorbance of the diluted solution in a 1 cm layer at the maximum at about 240 nm and calculate the content of \( \text{C}_{27}\text{H}_{38}\text{O}_3 \) using the absorptivity value of 42.8 \((A_{\text{1cm}} = 428)\).

B. Carry out the test under **1.14.4 High-performance liquid chromatography** using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated particles of silica gel, the surface of which has been modified with chemically-bonded octadecysilyl groups (5 μm).\(^1\)

Use the following conditions for gradient elution:
- mobile phase A: water R;
- mobile phase B: acetonitrile R.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–17</td>
<td>40</td>
<td>60</td>
<td>Isocratic</td>
</tr>
<tr>
<td>17–20</td>
<td>40 to 10</td>
<td>60 to 90</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>20–45</td>
<td>10</td>
<td>90</td>
<td>Isocratic</td>
</tr>
<tr>
<td>45–46</td>
<td>10 to 40</td>
<td>90 to 60</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>46–60</td>
<td>40</td>
<td>60</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column temperature at 40°C.

Prepare the following solutions in methanol R. For solution (1) dissolve 20.0 mg of the test substance and dilute to 100.0 mL. For solution (2) dissolve 20.0 mg of norethisterone enantate RS and dilute to 100.0 mL.

Inject alternately 20 μL each of solution (1) and (2) and record the chromatograms.

Measure the areas of the peaks corresponding to norethisterone enantate obtained in the chromatograms of solution (1) and (2) and calculate the percentage content of \( \text{C}_{27}\text{H}_{38}\text{O}_3 \) using the declared content of \( \text{C}_{27}\text{H}_{38}\text{O}_3 \) in norethisterone enantate RS.

**Additional requirement for Norethisterone enantate for parenteral use**

Complies with the monograph for Parenteral preparations.

**Reagent to be established**

**Norethisterone caproate R**

Norethisterone caproate of a suitable quality should be used.

---

\(^1\) BDS HYPERSIL \( C_{18} \) is suitable.
Norethisterone enantate injection
(Norethisteroni enantas injectio)

This is a draft proposal of a monograph for *The International Pharmacopoeia* (Working document QAS/17.725/Rev.2, July 2017). The working document with line numbers is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects](http://www.who.int/medicines/areas/quality_safety/quality_assurance/projects).

**Description.** A clear, colourless or almost colourless, oily solution.

**Category.** Contraceptive.

**Storage.** Norethisterone enantate injection should be kept in a tightly closed container, protected from light.

**Labelling.** The oil used in the formulation should be indicated.

**Additional information.** Strength in the current WHO Model List of Essential Medicines (EML): 200 mg/mL in 1 mL ampoule.

**Requirements**

Complies with the monograph for Parenteral preparations.

**Definition.** Norethisterone enantate injection contains not less than 90.0% and not more than 110.0% of the amount of Norethisterone enantate (C_{27}H_{38}O_{3}) stated on the label.

**Identity tests**

- Either test A or test B may be applied.

**A.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions as given under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to norethisterone enantate in the chromatogram obtained with solution (2).

**B.** Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R6 as the coating substance and a mixture of 2 volumes of cyclohexane R and 1 volume of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µL of each of the following two solutions in dichloromethane R. For solution (A) use a dilution of the test solution containing the equivalent of 1.0 mg of Norethisterone enantate per mL. For solution (B) use a solution containing 1.0 mg of norethisterone enantate RS per mL. After removing the plate from the chromatographic chamber allow it to dry in air and examine the chromatogram in ultraviolet light (254 nm). Spray the plate with antimony trichloride TS, heat at 110°C for 15 minutes and examine the chromatogram in ultraviolet light (365 nm). The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).
Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 1.5 IU of endotoxin RS per mg.

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the chromatographic conditions as described under “Assay”.

Prepare the following solutions in methanol R. For solution (1) dilute a suitable volume of the sample to obtain a concentration of 1.0 mg of Norethisterone enantate per mL. For solution (2) dilute 1 volume of solution (1) to 100 volumes. For solution (3) use a solution containing 0.1 mg of benzyl benzoate R per mL. For solution (4) use a solution containing 1.0 mg per mL of norethisterone enantate RS and 0.1 mg per mL of norethisterone caproate R.

Inject 20 μL of solution (4). The test is not valid unless the resolution between the peak due to norethisterone caproate (with a relative retention of about 0.95) and the peak due to norethisterone enantate (retention time about 27 minutes) is at least 4.0.

Inject alternatively 20 μL of solutions (1), (2) and (3) and record the chromatograms. Use the chromatogram obtained with solution (3) to identify any peak due to benzyl benzoate, if present.

In the chromatogram obtained with solution (1):

- the area of any impurity peak is not greater than 0.5 times the area of the peak due to norethisterone enantate in the chromatogram obtained with solution (2) (0.5%);
- the sum of the areas of all impurities is not greater than the area of the peak due to norethisterone enantate in the chromatogram obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak obtained with solution (2) (0.1%) and disregard any peak due to benzyl benzoate.

Assay. Carry out the test under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 μm).¹

Use the following conditions for gradient elution:

- mobile phase A: water;
- mobile phase B: acetonitrile R.

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<td>60</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column temperature at 40°C.

¹ BDS HYPERSIL C₁₈ is suitable
Norethisterone enantate injection (Ph. Int.)

Prepare the following solution in methanol R. For solution (1) dilute 1.0 mL of the injection to 100.0 mL. Dilute 10.0 mL of this solution to 100.0 mL. For solution (2) dissolve 20.0 mg of norethisterone enantate RS and dilute to 100.0 mL.

Inject 20 μL of each solution (1) and (2) and record the chromatograms.

Measure the areas of the peaks corresponding to norethisterone enantate obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of $C_{27}H_{38}O_3$ using the declared content of $C_{27}H_{38}O_3$ in norethisterone enantate RS.

***
Ciclosporin
(Ciclosporinum)

This is a draft proposed revision of a monograph for The International Pharmacopoeia (Working document QAS/17.701, August 2017). The working document with line numbers and tracked changes is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

[Note from the Secretariat. It is proposed to revise the monograph based on information found in the European Pharmacopoeia and the United States Pharmacopeia.]

Molecular formula. $C_{62}H_{111}N_{11}O_{12}$

Relative molecular mass. 1203

Chemical name

Other name. Cyclosporin.

Description. A white or almost white powder.

Solubility. Practically insoluble in water; freely soluble in ethanol (~750 g/L) TS and dichloromethane R.

Category. Immunosuppressant.

Storage. Ciclosporin should be kept in a well-closed container, protected from light.

Additional information. Ciclosporin is a product derived from a fermentation process or obtained by other ways.

Requirements
Definition. Ciclosporin contains not less than 97.0% and not more than 102.0% of $C_{62}H_{111}N_{11}O_{12}$, calculated with reference to the dried substance.
Identity tests

- Either test A alone or tests B and C may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from ciclosporin RS or with the reference spectrum of ciclosporin.

B. Carry out as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to ciclosporin in the chromatogram obtained with solution (2).

C. Dissolve 5 mg in 5 mL of methanol R, and 1 drop of potassium permanganate (10 g/L) TS and allow to stand; the blue-red colour is gradually discharged.

Specific optical rotation. Use a 5.0 mg/mL solution in methanol R and calculate with reference to the dried substance; $\alpha_p^{20^\circ} = -193^\circ$ to $-185^\circ$.

Heavy metals. Use 1.0 g of the test substance for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 20 μg/g.

Clarity and colour of solution in ethanol. A solution of 1.5 g in 15 mL of ethanol (~750 g/L) TS is clear and not more intensely coloured than standard colour solution Y₅, BY₅ or R₇ when compared as described under 1.11.2 Colour of liquids.

[Note from the Secretariat. The chapter 1.11 Colour of liquids is currently under revision. Reference is already made to a new test procedure to be added under the section 1.11.2 Degree of coloration of liquids.]

Sulfated ash (2.3). Not more than 1.0 mg/g.

Loss on drying. Dry 1.000 g of the test substance at 60°C under reduced pressure (not exceeding 15 Pa) for 3 hours; it loses not more than 20 mg/g.

Related substances. Carry out the test as described below under “Assay”.

Prepare the following solutions in a mixture of equal volumes of acetonitrile R and water R. For solution (1) dissolve 30.0 mg of the test substance and dilute to 25.0 mL. For solution (2) dilute 2.0 mL of solution (1) to 200 mL. For solution (3) prepare a solution containing 1.0 mg of ciclosporin for system suitability RS (containing a 100:1 (w/w) mixture of ciclosporin and ciclosporin U) per mL.

Inject 20 μL of solution (3). The test is not valid unless the peak-to-valley ratio (Hp/Hv) is at least 1.4, where Hp is the height above the baseline of the peak due to ciclosporin U and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to ciclosporin (retention time 25 to 30 minutes).

Inject alternately 20 μL each of solutions (1) and (2). Record the chromatograms for 1.7 times the retention time of the principal peak.
In the chromatogram obtained with solution (1):

- the area of any impurity peak, is not greater than 0.7 times the area of the peak due to ciclosporin in the chromatogram obtained with solution (2) (0.7%),
- the sum of the areas of all impurities is not greater than 1.5 times the area of the peak due to cyclosporine in the chromatogram obtained with solution (2) (1.5%). Disregard any peak with an area less than 0.05 times the area of the peak due to ciclosporin in the chromatogram obtained with solution (2) (0.05%).

**Assay.** Determine as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (3–5 μm). The column is connected to the injection port by a steel capillary tube about 1 m long with an internal diameter of 0.25 mm. Maintain the temperature of the column and of the steel capillary at 80°C. As the mobile phase use a mixture of 52 volumes of water, 43 volumes of acetonitrile R, 5 volumes of tert-butyl methyl ether R and 0.1 volume of phosphoric acid (~1440 g/L) TS.

Prepare the following solutions in a mixture of equal volumes of acetonitrile R and water R. For solution (1) dissolve 30.0 mg of the test substance and dilute to 25.0 mL. For solution (2) dissolve 30.0 mg of cyclosporine RS and dilute to 25.0 mL. For solution (3) dilute 2.0 mL of solution (2) to 200 mL. For solution (4) prepare a solution containing 1.0 mg of ciclosporin for system suitability RS (containing a 100:1 (w/w) mixture of ciclosporin and ciclosporin U) per mL.

Operate with a flow rate of about 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 210 nm.

Inject 20 μL of solution (4). The assay is not valid unless the peak-to-valley ratio (Hp/Hv) is at least 1.4, where Hp is the height above the baseline of the peak due to ciclosporin U and Hv is the height above the baseline of the lowest point of the curve separating this peak from the peak due to ciclosporin (retention time 25 to 30 minutes). Inject alternately 20 μL each of solutions (1) and (2). Record the chromatograms for 1.7 times the retention time of the principal peak.

Measure the areas of the peaks corresponding to ciclosporin obtained in the chromatograms and calculate the percentage content of C_{62}H_{111}N_{11}O_{12}, using the declared content of C_{62}H_{111}N_{11}O_{12} in ciclosporin RS.
**Impurities**

A. Different ciclosporins [difference from ciclosporin (R = CH₃; ciclosporin A)]: ciclosporin B [7-l-Ala]; ciclosporin C [7-l-Thr]; ciclosporin D [7-l-Val]; ciclosporin E [5-l-Val]; ciclosporin G [7-(l-2-aminopentanoyl)]; ciclosporin H [5-d-MeVal]; ciclosporin L [R = H]; ciclosporin T [4-l-Leu]; ciclosporin U [11-l-Leu]; ciclosporin V [1-l-Abu]

B. [6-((2S,3R,4R)-3-hydroxy-4-methyl-2-(methylamino)octanoic acid)]ciclosporin A,

C. Isociclosporin A.

**Reference substances to be established**

Ciclosporine RS
Ciclosporine for system suitability RS

***
Dacarbazine
(Dacarbazinum)

This is a draft proposed revision of a monograph for The International Pharmacopoeia (Working document QAS/17.711, August 2017). The working document with line numbers and tracked changes is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

[Note from the Secretariat. It is proposed to revise the monograph on Dacarbazine based on information found in the United States Pharmacopoeia, in the European Pharmacopoeia and in the scientific literature.]

Molecular formula. C₆H₁₀N₆O
Relative molecular mass. 182.2
Graphic formula

Chemical name. 5-(3,3-dimethyltriaz-1-en-1-yl)-1H-imidazole-4-carboxamide; CAS Reg. No. 4342-03-4.
Description. A colourless or pale yellow, crystalline powder.
Solubility. Slightly soluble in water and ethanol (~750 g/L) TS, practically insoluble in Dichloromethane R.
Category. Cytotoxic drug.
Storage. Dacarbazine should be kept in a tightly closed container, protected from light, and stored at a temperature not exceeding 8°C.
Additional information. CAUTION: Dacarbazine must be handled with care, avoiding contact with the skin and inhalation of airborne particles.

Requirements
Dacarbazine contains not less than 98.5% and not more than 101.0% of C₆H₁₀N₆O, calculated with reference to the anhydrous substance.

Identity tests
• Either test A alone or tests B and C may be applied.
A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from dacarbazine RS or with the reference spectrum of dacarbazine.

B. The absorption spectrum of a 6 μg/mL solution in hydrochloric acid (~4 g/L) TS, when observed between 200 nm and 400 nm, exhibits a maximum at about 323 nm and a pronounced shoulder at 275 nm.

C. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R6 as the coating substance and a mixture of glacial acetic acid R, water R and butanol R (1:2:5 V/V/V) as the mobile phase. Apply separately to the plate 10 μL of each of the following 2 solutions in methanol R: containing (A) 0.4 mg of the test substance per mL and (B) 0.4 mg of dacarbazine RS per mL. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber allow it to dry in air or in a current of air. Examine the chromatogram under ultraviolet light (254 nm). The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to dacarbazine in the chromatogram obtained with solution (B).

Clarity and colour of solution. Dissolve 0.25 g of the test substance in a 210 g/L solution of citric acid R and dilute to 25.0 mL with the same solution. The solution is clear and not more intensely coloured than reference solution BY6, when analysed as described under 1.11.2 Degree of coloration of liquids, Method II.

[Note from the Secretariat. Chapter 1.11 Colour of liquids is currently under revision. Reference is already made to a new test procedure to be added under the section 1.11.2 Degree of coloration of liquids.]

Sulfated ash (2.3). Not more than 1.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, method A. Use 1.00 g of the test substance. The water content is not more than 5 mg/g.

Impurity D

Carry out the test as described under 1.14.5 Gas chromatography with the apparatus equipped with an injection system for the performance of static head-space chromatography. Use a fused-silica capillary column 30 m long and 0.53 mm in internal diameter coated with base-deactivated polyethyleneglycol R (film thickness: 1.0 μm).

As a detector use a flame ionization detector.

Use helium for chromatography R as the carrier gas with a flow rate of 13 mL/min.

Use a split ratio of 1:1.

The following head-space injection conditions may be used:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equilibration temperature (°C)</td>
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</tr>
<tr>
<td>Equilibration time (min)</td>
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<tr>
<td>Transfer line temperature (°C)</td>
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<tr>
<td>Pressurization time (s)</td>
<td>30</td>
</tr>
<tr>
<td>Injection volume (mL)</td>
<td>1</td>
</tr>
</tbody>
</table>
Maintain the temperature of the column at 35°C for 3 minutes, then raise the temperature within 8 minutes to 165°C, maintaining the temperature of the injection port at 180°C and that of the flame ionization detector at 220°C.

Prepare the following solutions. For solution (1) transfer 0.200 g of the test substance into a 20 mL headspace vial and firmly attach the septum and cap. Using a 10 µL syringe, inject 5 µL of water R into the vial. For solution (2) dilute 1.00 g of dimethylamine R (impurity D) to 100.0 mL with water R. Firmly attach the septum and cap to a 20 mL vial. Using a 10 µL syringe, inject 10 µL of solution (2) into the vial. For solution (3) dilute 1.00 g of trimethylamine R to 100.0 mL with water R. Firmly attach the septum and cap to a 20 mL vial. Using a 10 µL syringe, inject 10 µL of solution (2) and 10 µL of solution (3) into the vial.

Analyse solution (3). The test is not valid unless the resolution between the peaks due to impurity D and trimethylamine is at least 2.5.

Analyse solution (1) and (2).

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity D is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (0.05%).

Related substances

Use freshly prepared solutions and protect them from light.

- Perform test A and B.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).

Prepare the mobile phase by dissolving 15.63 g of docusate sodium R in a solution containing 2.33 g of glacial acetic acid R per L of water R and dilute to 1000 mL with the same solution. Prepare the mobile phase freshly every day and flush the column with a mixture of equal volumes of methanol R and water R after all tests have been completed or at the end of the day, for at least 2 hours.

Operate with a flow rate of 1.2 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Prepare the following solutions in distilled water R. For solution (1) dissolve 50.0 mg of the test substance and 75 mg of citric acid R and dilute to 5.0 mL. For solution (2) dissolve 5.0 mg of dacarbazine impurity A RS and dilute to 50.0 mL. Dilute 5.0 mL of this solution to 25.0 mL.

Inject alternately 25 µL each of solution (1) and (2). Record the chromatograms for about 3 times the retention time of impurity A (retention time about 3 minutes).

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A is not greater than the area of the corresponding peak in the chromatogram obtained with solution (2) (0.2%);
Dacarbazine (Ph. Int.)

- the area of any other impurity peak eluting after impurity A is not greater than 0.5 times the area of the peak due to impurity A in the chromatogram obtained with solution (2) (0.10%).

B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given below under test A with the following modifications.

Prepare the mobile phase by mixing 45 volumes of the mobile phase described under test A with 55 volumes of methanol R.

Prepare the following additional solution in distilled water R. For solution (3) dissolve 5.0 mg of dacarbazine impurity B RS, add 0.5 mL of solution (1) and dilute to 10.0 mL. Dilute 1.0 mL of this solution to 50.0 mL.

Inject alternately 10 μL each of solution (1) and (3). Record the chromatograms for about twice the retention time of dacarbazine (retention time about 12 minutes). The test is not valid unless the resolution between the peaks due to impurity B (with a relative retention of about 0.7) and dacarbazine is at least 1.5.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity B is not greater than the area of the corresponding peak in the chromatogram obtained with solution (3) (0.1%);
- the area of any other impurity peak is not greater than the area of the peak due to dacarbazine in the chromatogram obtained with solution (3) (0.10%);
- the sum of the areas of all impurities peaks is not greater than 5 times the area of the peak due to dacarbazine in the chromatogram obtained with solution (3) (0.5%). Disregard any peak with an area less than 0.5 times the area of the peak due to dacarbazine in the chromatogram obtained with solution (3) (0.05%).

Assay

- Protect the solutions from light throughout the assay.

Dissolve about 0.150 g, accurately weighed, in 30 mL of anhydrous acetic acid R. Titrate with perchloric acid (0.1 mol/L) VS, determining the end-point potentiometrically. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 18.22 mg of $C_6H_{10}N_6O$.

Impurities

A. 1,5-dihydro-4H-imidazo[4,5-P]-1,2,3-triazin-4-one (2-azahypoxanthine) (degradation product)
B. 5-amino-1\textit{H}-imidazole-4-carboxamide (synthesis-related impurity)

\textit{Note from the Secretariat. Chemical structure to be added.}

C. 5-diazenyl-1\textit{H}-imidazole-4-carboxamide

\textit{Note from the Secretariat. Chemical structure to be added.}

D. N-methylmethanamine

\textbf{Reagents to be established}

\textbf{Water, distilled R}

Water R prepared by distillation.

\textbf{Polyethyleneglycol, base-deactivated R}

Cross-linked, base-deactivated polyethyleneglycol, specially designed to be used as a stationary phase for gas chromatographic analysis of amine.

***
Replacement of mercury salts in non-aqueous titration

This is a draft proposal of a monograph for The International Pharmacopoeia (Working document QAS/17.708, July 2017). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

[Note from the Secretariat. As part of the activities to update The International Pharmacopoeia, mercury salts and other toxic reagents shall be replaced in order to reduce the risk to analysts and the environment. In the past, the addition of mercuric acetate has been necessary to permit the titration of halide salts of weak bases. These titrations can now be replaced by alternative procedures, notably the direct titration of the halide salts of weak bases with perchloric acid in anhydrous acetic acid or the titration of the halide salts of bases in alcoholic media with sodium hydroxide. The general chapter 2.6. Non-aqueous titration was already revised following a decision at the 51st meeting the Expert Committee on Specifications for Pharmaceutical Preparations. It is now proposed to revise individual monographs that prescribe the use of mercuric acetate in volumetric titration. The proposed alternative procedures are predominantly based on provisions found in other pharmacopoeias; some of them are based on laboratory investigations.]

<table>
<thead>
<tr>
<th>Current procedure</th>
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<tr>
<td><strong>Amiloride</strong></td>
<td><strong>Amiloride</strong></td>
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<tr>
<td><strong>Assay.</strong> Dissolve about 0.45 g, accurately weighed, in a mixture of 100 mL of glacial acetic acid R1, 15 mL of dioxan R and 10 mL of mercuric acetate/acetic acid TS, and titrate with perchloric acid (0.1 mol/L) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 26.61 mg of $C_6H_8ClN_7O,HCl.$</td>
<td><strong>Assay.</strong> Dissolve 0.20 g, accurately weighed, in a mixture of 5.0 mL of hydrochloric acid (0.01 mol/L) VS and 50 mL of dehydrated ethanol R. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. Read the volume added between the two points of inflexion. 1 mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 26.61 mg of $C_4H_4ClN,O,HCl.$ [based on a method published in Ph.Eur. 9th Edition 2017]</td>
</tr>
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Continued
### Amitriptyline hydrochloride

**Assay.** Dissolve about 0.3 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of dioxan R and 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 31.39 mg of C\textsubscript{20}H\textsubscript{23}N\textsubscript{H}Cl.

**Assay.** Dissolve 0.25 g, accurately weighed, in 30 mL of dehydrated ethanol R. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. 1 mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 31.39 mg of C\textsubscript{20}H\textsubscript{23}N\textsubscript{H}Cl.

*Based on a method published in Ph.Eur. 9th Edition 2017*

### Biperiden hydrochloride

**Assay.** Dissolve about 0.4 g, accurately weighed, in 30 mL of glacial acetic acid R1, warming slightly to effect solution, add 10 mL of mercuric acetate/acetic acid TS and 0.15 mL of 1-naphtholbenzein/ acetic acid TS as indicator. Titrate with perchloric acid (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 34.79 mg of C\textsubscript{21}H\textsubscript{29}NO\textsubscript{H}Cl.

**Assay.** Dissolve 0.20 g, accurately weighed, in 60 mL of dehydrated ethanol R. Carry out a potentiometric titration using potassium hydroxide/ethanol (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. 1 mL of potassium hydroxide/ethanol (0.1 mol/L) VS is equivalent to 34.79 mg of C\textsubscript{21}H\textsubscript{29}NO\textsubscript{H}Cl.

*Based on a method published in Ph.Eur. 9th Edition 2017*

### Chlorhexidine dihydrochloride

**Assay.** Dissolve about 0.4 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/ acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 14.46 mg of C\textsubscript{22}H\textsubscript{30}Cl\textsubscript{2}N\textsubscript{10}2HCl.

**Assay.** In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached. Dissolve 0.10 g, accurately weighed, in 5 mL of anhydrous formic acid R and add 70 mL of acetic anhydride R. Carry out a potentiometric titration using perchloric acid (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 14.46 mg of C\textsubscript{22}H\textsubscript{30}Cl\textsubscript{2}N\textsubscript{10}2HCl.

*Based on a method published in Ph.Eur. 9th Edition 2017*
Continued

### Chlorpromazine hydrochloride

**Assay.** Dissolve about 0.7 g, accurately weighed, in 200 mL of acetone R, add 10 mL of mercuric acetate/acetic acid TS and 3 mL of methyl orange/acetone TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 35.53 mg of C_{17}H_{19}ClN_{2}S,HCl.

**Assay.** Dissolve 0.25 g, accurately weighed, in a mixture of 5.0 mL of hydrochloric acid (0.01 mol/L) VS and 50 mL of dehydrated ethanol R. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration, Method A. Read the volume added between the two points of inflexion. 1 mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 35.53 mg of C_{17}H_{19}ClN_{2}S,HCl.

*[based on a method published in Ph.Eur. 9th Edition 2017]*

### Dopamine hydrochloride

**Assay.** Dissolve about 0.4 g, accurately weighed, in 140 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 18.96 mg of C_{8}H_{11}NO_{2},HCl.

**Assay.** In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached. Dissolve 0.15 g, accurately weighed, in 10 mL of anhydrous formic acid R and add 50 mL of acetic anhydride R. Carry out a potentiometric titration using perchloric acid (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. 1 mL of 0.1 M perchloric acid is equivalent to 18.96 mg of C_{8}H_{11}NO_{2},HCl.

*[based on a method published in Ph.Eur. 9th Edition 2017]*
Edrophonium chloride

**Assay.** Dissolve about 0.20 g, accurately weighed, in 20 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and 0.25 mL of quinaldine red/ethanol TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 20.17 mg of C$_{10}$H$_{16}$ClNO.

**Assay.** In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached. Dissolve 0.15 g, accurately weighed, in 60 mL of a mixture of equal volumes of acetic anhydride R and anhydrous acetic acid R. Carry out a potentiometric titration using perchloric acid (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. 1 mL of perchloric acid (0.1 mol/L) VS is equivalent to 20.17 mg of C$_{10}$H$_{16}$ClNO. [based on a method published in Ph.Eur. 9th Edition 2017]

Ephedrine hydrochloride

**Assay.** Dissolve about 0.2 g, accurately weighed, in 10 mL of warm mercuric acetate/acetic acid TS, add 50 mL of acetone R and 1 mL of methyl orange/acetone TS as indicator and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 20.17 mg of C$_{10}$H$_{15}$NO,HCl.

**Assay.** Dissolve 0.15 g, accurately weighed, in 50 mL of dehydrated ethanol R and add 5.0 mL of hydrochloric acid (0.01 mol/L) VS. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. Read the volume added between the two points of inflexion. 1 mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 20.17 mg of C$_{10}$H$_{15}$NO,HCl. [based on a method published in Ph.Eur. 9th Edition 2017]

Ethambutol hydrochloride

**Assay.** Dissolve about 0.3 g, accurately weighed, in 100 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 13.86 mg of C$_{10}$H$_{24}$N$_2$O$_2$.2HCl.

**Assay.** Dissolve 0.20 g, accurately weighed, in 50 mL of water R and add 1.0 mL of hydrochloric acid (0.1 mol/L) VS. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS. Read the volume added between the two points of inflexion. 1 mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 27.72 mg of C$_{10}$H$_{24}$N$_2$O$_2$.2HCl. [based on a method published in Ph.Eur. 9th Edition 2017]
## Current procedure

### Fluphenazine hydrochloride

**Assay.** Dissolve about 0.5 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 25.52 mg of C₂₂H₂₆F₃N₃OS,2HCl.

**Assay.** In order to avoid overheating during the titration, mix thoroughly throughout and stop the titration immediately after the end-point has been reached. Dissolve 0.22 g, accurately weighed, in a mixture of 10 mL of anhydrous formic acid R and 40 mL of acetic anhydride R. Carry out a potentiometric titration using perchloric acid (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. 1 mL of 0.1 M perchloric acid is equivalent to 25.52 mg of C₂₂H₂₆F₃N₃OS,2HCl.


### Homatropine hydrobromide

**Assay.** Dissolve about 0.3 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 35.63 mg of C₁₆H₂₁NO₃,HBr.

**Assay.** Dissolve 0.30 g, accurately weighed, in a mixture of 5.0 mL of hydrochloric acid (0.01 mol/L) VS and 50 mL of dehydrated ethanol R. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. Read the volume added between the two points of inflexion. 1 mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 35.63 mg of C₁₆H₂₁NO₃,HBr.


### Homatropine methylbromide

**Assay.** Dissolve about 0.7 g, accurately weighed, in 50 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 37.03 mg of C₁₇H₂₄BrNO₃.

**Assay.** Dissolve 0.30 g, accurately weighed, in 10 mL of water R. Carry out a potentiometric titration using silver nitrate (0.1 mol/L) VS and a silver indicator electrode and a silver-silver chloride reference electrode. 1 mL of silver nitrate (0.1 mol/L) is equivalent to 37.03 mg of C₁₇H₂₄BrNO₃.

Ketamine hydrochloride

**Assay.** Dissolve about 0.5 g, accurately weighed, in 1 mL of formic acid (~1080 g/L) TS and add 70 mL of a mixture of 6 volumes of acetic anhydride R and 1 volume of glacial acetic acid R1. Add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 27.42 mg of $\text{C}_{13}\text{H}_{16}\text{ClN}\text{O}_{2}\text{HCl}$.

Lidocaine hydrochloride

**Assay.** Dissolve about 0.55 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 27.08 mg of $\text{C}_{14}\text{H}_{22}\text{N}_{2}\text{O}_{2}\text{HCl}$.

Loperamide hydrochloride

**Assay.** Dissolve about 0.38 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and 0.15 mL of 1-naphtholbenzein/acetic acid TS as indicator and titrate with perchloric acid (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 51.35 mg of $\text{C}_{29}\text{H}_{33}\text{ClN}_{2}\text{O}_{2}\text{HCl}$.
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<td>Dissolve about 0.3 g, accurately weighed, in 80 mL of acetic anhydride R, add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS, determining the end-point potentiometrically as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 33.63 mg of C₁₄H₂₂ClN₃O₂.HCl.</td>
<td>Dissolve 0.25 g, accurately weighed, in a mixture of 5.0 mL of hydrochloric acid (0.01 mol/L) VS and 50 mL of dehydrated ethanol R. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. Read the volume added between the two points of inflexion. 1 mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 33.63 mg of C₁₄H₂₂ClN₃O₂.HCl. [based on a method published in Ph.Eur. 9th Edition 2017]</td>
<td>Assay. Dissolve 0.25 g, accurately weighed, in a mixture of 5.0 mL of hydrochloric acid (0.01 mol/L) VS and 50 mL of dehydrated ethanol R. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. Read the volume added between the two points of inflexion. 1 mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 33.63 mg of C₁₄H₂₂ClN₃O₂.HCl. [based on a method published in Ph.Eur. 9th Edition 2017]</td>
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Morphine hydrochloride

Assay. Dissolve about 0.3 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 32.18 mg of C₁₇H₁₉NO₃.HCl. | Assay. Dissolve 0.30 g, accurately weighed, in a mixture of 5 mL of hydrochloric acid (0.01 mol/L) VS and 30 mL of dehydrated ethanol R. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. Read the volume added between the two points of inflexion. 1 mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 32.18 mg of C₁₇H₁₉NO₃.HCl. [based on a method published in Ph.Eur. 9th Edition 2017] | Assay. Dissolve 0.30 g, accurately weighed, in a mixture of 5 mL of hydrochloric acid (0.01 mol/L) VS and 30 mL of dehydrated ethanol R. Carry out a potentiometric titration using sodium hydroxide/ethanol (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. Read the volume added between the two points of inflexion. 1 mL of sodium hydroxide/ethanol (0.1 mol/L) VS is equivalent to 32.18 mg of C₁₇H₁₉NO₃.HCl. [based on a method published in Ph.Eur. 9th Edition 2017] |

Naloxone hydrochloride

Assay. Dissolve about 0.3 g, accurately weighed, in 40 mL of glacial acetic acid R1, 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 36.38 mg of C₁₉H₂₁NO₄.HCl. | Assay. Dissolve 0.30 g, accurately weighed, in 50 mL of dehydrated ethanol R and add 5.0 mL of hydrochloric acid (0.01 mol/L) VS. Carry out a potentiometric titration using sodium hydroxide/ethanol (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. Read the volume added between the two points of inflexion. 1 mL of sodium hydroxide/ethanol (0.1 mol/L) VS is equivalent to 36.38 mg of C₁₉H₂₁NO₄.HCl. [based on a method published in Ph.Eur. 9th Edition 2017] | Assay. Dissolve 0.30 g, accurately weighed, in 50 mL of dehydrated ethanol R and add 5.0 mL of hydrochloric acid (0.01 mol/L) VS. Carry out a potentiometric titration using sodium hydroxide/ethanol (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. Read the volume added between the two points of inflexion. 1 mL of sodium hydroxide/ethanol (0.1 mol/L) VS is equivalent to 36.38 mg of C₁₉H₂₁NO₄.HCl. [based on a method published in Ph.Eur. 9th Edition 2017] |

[Continued]
Neostigmine bromide

**Assay.** Dissolve about 0.25 g, accurately weighed, in 20 mL of glacial acetic acid R1, add 5 mL of acetic anhydride R and 10 mL of mercuric acetate/acetic acid TS. Titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 30.32 mg of C$_{12}$H$_{19}$BrN$_2$O$_2$.

**Assay.** In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached. Dissolve 0.23 g, accurately weighed, in 2 mL of anhydrous formic acid R and add 50 mL of acetic anhydride R. Carry out a potentiometric titration using perchloric acid (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. 1 mL of 0.1 M perchloric acid (0.1 mol/L) is equivalent to 30.32 mg of C$_{12}$H$_{19}$BrN$_2$O$_2$.

_pilocarpine hydrochloride

**Assay.** Dissolve about 0.5 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 24.47 mg of C$_{11}$H$_{16}$N$_2$O$_2$.HCl.

**Assay.** Dissolve 0.20 g, accurately weighed, in 50 mL of dehydrated ethanol R and add 5 mL of hydrochloric acid (0.01 mol/L) VS. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. Read the volume added between the two points of inflexion. 1 mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 24.47 mg of C$_{11}$H$_{16}$N$_2$O$_2$.HCl.

_Procarbazine hydrochloride

**Assay.** Dissolve about 0.125 g, accurately weighed, in a mixture of 5 mL of formic acid (~1080 g/L) TS and 20 mL of glacial acetic acid R1, add 5 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 25.78 mg of C$_{12}$H$_{19}$N$_3$O$_2$.HCl.

**Assay.** Dissolve 0.2 g, accurately weighed, in 100 mL of water R. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS. 1 mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 25.78 mg of C$_{12}$H$_{19}$N$_3$O$_2$.HCl.

[Based on a method published in Indian Pharmacopoeia, 2014]
Replacement of mercury salts in non-aqueous titration (Ph. Int.)

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**Proguanil hydrochloride**

**Assay.** Dissolve about 0.3 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/l) VS as described under 2.6 Non-aqueous titration, Method A, determining the end-point potentiometrically. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 14.51 mg of C₁₁H₁₆ClN₅,HCl.

**Assay.** Suspend 0.10 g, accurately weighed, in 20 mL of anhydrous acetic acid R, shake and heat at 50°C for 5 minutes. Cool to room temperature and add 40 mL of acetic anhydride R. In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached. Carry out a potentiometric titration using perchloric acid (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. 1 mL of perchloric acid (0.1 mol/L) is equivalent to 14.51 mg of C₁₁H₁₆ClN₅,HCl.


**Propranolol hydrochloride**

**Assay.** Dissolve about 0.6 g, accurately weighed, in 50 mL of glacial acetic acid R1 and add 10 mL of mercuric acetate/acetic acid TS, warming slightly if necessary to effect solution. Cool and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 29.58 mg of C₁₆H₂₁NO₂,HCl.

**Assay.** Dissolve 0.25 g, accurately weighed, in 25 mL of dehydrated ethanol R. Carry out a potentiometric titration using 0.1 M sodium hydroxide (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. 1 mL of 0.1 M sodium hydroxide (0.1 mol/L) is equivalent to 29.58 mg of C₁₆H₂₁NO₂,HCl.

Continued

| Pyridostigmine bromide | Assay. | Dissolve about 0.5 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and 2 drops of quinaldine red/ethanol TS as indicator and titrate with perchloric acid (0.1 mol/L) VS as described under \textit{2.6 Non-aqueous titration}, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 26.11 mg of C$_9$H$_{13}$BrN$_2$O$_2$.

| Pyridoxine hydrochloride | Assay. | Dissolve about 0.4 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and 2 drops of quinaldine red/ethanol TS as indicator and titrate with perchloric acid (0.1 mol/L) VS as described under \textit{2.6 Non-aqueous titration}, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 26.11 mg of C$_9$H$_{13}$BrN$_2$O$_2$.

| Quinine dihydrochloride | Assay. | Dissolve about 0.3 g, accurately weighed, in 50 mL of glacial acetic acid R1, add 20 mL of acetic anhydride R and 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under \textit{2.6 Non-aqueous titration}, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 26.11 mg of C$_9$H$_{13}$BrN$_2$O$_2$.

| Assay. | In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached. Dissolve 0.23 g, accurately weighed, in 10 mL of anhydrous acetic acid R and add 40 mL of acetic anhydride R. Carry out a potentiometric titration using perchloric acid (0.1 mol/L) VS, as described under \textit{2.6 Non-aqueous titration}. 1 mL of perchloric acid (0.1 mol/L) is equivalent to 26.11 mg of C$_9$H$_{13}$BrN$_2$O$_2$.

[\textit{based on a method published in Ph.Eur. 9th Edition 2017}]

| Assay. | In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the end-point has been reached. Dissolve 0.15 g, accurately weighed, in 5 mL of anhydrous formic acid R. Add 50 mL of acetic anhydride R. Carry out a potentiometric titration using perchloric acid (0.1 mol/L) VS, as described under \textit{2.6 Non-aqueous titration}. 1 mL of perchloric acid (0.1 mol/L) VS is equivalent to 26.11 mg of C$_9$H$_{13}$BrN$_2$O$_2$.

[\textit{based on a method published in Ph.Eur. 9th Edition 2017}]

| Assay. | Dissolve 0.15 g, accurately weighed, in 50 mL of dehydrated ethanol R and add 5.0 mL of 0.01 M hydrochloric acid. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS, as described under \textit{2.6 Non-aqueous titration}. Read the volume added between the two inflexion points. 1 mL of 0.1 M sodium hydroxide (0.1 mol/L) VS is equivalent to 39.73 mg of C$_{20}$H$_{24}$N$_2$O$_2$.2HCl.

[\textit{based on a proposal by a collaborating laboratory}]

\textit{Continued}
### Replacement of mercury salts in non-aqueous titration (Ph. Int.)

**Continued**

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<th><strong>Current procedure</strong></th>
<th><strong>Alternative procedure</strong></th>
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</table>

### Quinine hydrochloride

**Assay.** Dissolve about 0.35 g, accurately weighed, in 50 mL of glacial acetic acid R1, add 20 mL of acetic anhydride R and 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 18.04 mg of C₂₀H₂₄N₂O₂·HCl.

**Assay.** Dissolve 0.250 g, accurately weighed, in 50 mL of dehydrated ethanol R and add 5.0 mL of hydrochloric acid (0.01 mol/L) VS. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS. Read the volume added between the two inflexion points. 1 mL of 0.1 M sodium hydroxide is equivalent to 36.09 mg of C₂₀H₂₄N₂O₂·HCl. [based on a method published in Ph.Eur. 9th Edition 2017]

### Suxamethonium chloride

**Assay.** Dissolve about 0.3 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 30 mL of acetic anhydride R and 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 18.07 mg of C₁₄H₃₀Cl₂N₂O₄.

**Assay.** In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached. Dissolve 0.15 g, accurately weighed, in 50 mL of acetic anhydride R. Carry out a potentiometric titration using perchloric acid (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. 1 mL of 0.1 M perchloric acid is equivalent to 18.07 mg of C₁₄H₃₀Cl₂N₂O₄. [based on a method published in Ph.Eur. 9th Edition 2017]

### Tetracycline hydrochloride

**Assay A.** Dissolve about 0.25 g, accurately weighed and previously dried at 60°C under reduced pressure, in 5 mL of formic acid (~1080 g/L) TS and 10 mL of glacial acetic acid R1, add 10 mL of dioxan R, 5 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 48.09 mg of C₂₂H₂₄N₂O₈·HCl.

[Revision of the monograph will be dealt with in a separate document.]
Replacement of mercury salts in non-aqueous titration (Ph. Int.)

Continued

<table>
<thead>
<tr>
<th>Current procedure</th>
<th>Alternative procedure</th>
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</table>

**Thiamine hydrobromide**

**Assay.** Dissolve about 0.30 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 21.31 mg of \( C_{12}H_{17}BrN_4OS,HBr \).

**Assay.** In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached. Dissolve 0.11 g, accurately weighed, in 5 mL of anhydrous formic acid R and add 50 mL of acetic anhydride R. Carry out a potentiometric titration using perchloric acid (0.1 M) VS, as described under 2.6 Non-aqueous titration. Perform the titration within 2 minutes and carry out a blank titration. 1 mL of perchloric acid (0.1 M) VS is equivalent to 16.86 mg of \( C_{12}H_{17}ClN_4OS,\text{HCl} \).

**Thiamine hydrochloride**

**Assay.** Dissolve about 0.25 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 16.86 mg of \( C_{12}H_{17}ClN_4OS,\text{HCl} \).

**Assay.** In order to avoid overheating in the reaction medium, mix thoroughly throughout the titration and stop the titration immediately after the end-point has been reached. Dissolve 0.11 g, accurately weighed, in 5 mL of anhydrous formic acid R and add 50 mL of acetic anhydride R. Carry out a potentiometric titration using perchloric acid (0.1 M) VS, as described under 2.6 Non-aqueous titration. Perform the titration within 2 minutes and carry out a blank titration. 1 mL of perchloric acid (0.1 M) VS is equivalent to 16.86 mg of \( C_{12}H_{17}ClN_4OS,\text{HCl} \).

**Verapamil hydrochloride**

**Assay.** Dissolve about 0.5 g, accurately weighed, in 30 mL of glacial acetic acid R1, add 10 mL of mercuric acetate/acetic acid TS followed by 0.15 mL of 1-naphtholbenzein/acetic acid TS as indicator and titrate with perchloric acid (0.1 mol/L) VS as described under 2.6 Non-aqueous titration, Method A. Each mL of perchloric acid (0.1 mol/L) VS is equivalent to 49.11 mg of \( C_{27}H_{38}N_2O_4,\text{HCl} \).

**Assay.** Dissolve 0.40 g, accurately weighed, in 50 mL of dehydrated ethanol R and add 5.0 mL of hydrochloric acid (0.01 mol/L) VS. Carry out a potentiometric titration using sodium hydroxide (0.1 mol/L) VS, as described under 2.6 Non-aqueous titration. Measure the volume added between the two points of inflexion. 1 mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 49.11 mg of \( C_{27}H_{38}N_2O_4,\text{HCl} \).

Reagents to be established

**Potassium hydroxide/ethanol (0.1 mol/L) VS**

Potassium hydroxide R dissolved in ethanol (~710 g/L) TS to contain 5.61 g of KOH in 1000 mL.

**Method of standardization.** Ascertain the exact concentration of the solution following the method described under potassium hydroxide/ethanol (0.5 mol/L) VS.

**Sodium hydroxide/ethanol (0.1 mol/L) VS**

Sodium hydroxide R dissolved in ethanol (~710 g/L) TS to contain 4.00 g of NaOH in 1000 mL.

**Method of standardization.** Ascertain the exact concentration of the solution in the following manner:

Dissolve 0.10 g of benzoic acid R, accurately weighted, in 10 mL of water R and 40 mL of dehydrated ethanol R. Titrate with the sodium hydroxide/ethanol solution, determining the end-point potentiometrically or using 0.2 mL of thymolphthalein solution R as indicator. Standardize immediately before use.

1 mL of 0.1 M sodium hydroxide/ethanol (0.1 mol/L) VS is equivalent to 12.21 mg of $C_7H_6O_2^-$. 

***
Polymorphism

This is a draft proposal of a monograph for The International Pharmacopoeia (Working document QAS/17.716, July 2017). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

[Note from the Secretariat. It is proposed to publish the following chapter on Polymorphism in the Supplementary Information section under “Notes for guidance”.

Active pharmaceutical ingredients (APIs) and excipients, in the solid phase, can be classified as either crystalline or amorphous solids or a mixture thereof. A crystalline structure implies that the structural units (i.e. unit cells) are repeated regularly, indefinitely and three-dimensionally in space. The unit cells of an amorphous solid, however, are arranged in a non-ordered, random system, related to the liquid state.

Polymorphism is the phenomenon where crystalline forms of the same chemical compound exist in different crystalline phases. The difference in internal crystal structure could be attributed to differences in crystal packing arrangements and/or different molecular conformations. When a chemical element exists in different crystalline forms, it is referred to as allotropy, not polymorphism (1). The phenomenon where crystals with the same internal structure exhibit different external shapes is referred to as differences in crystal habit.

Other variations in the crystal structures of the same chemical compound are encountered where these unit cells differ in elemental composition through the inclusion of one or more solvent molecules, known as solvates. Solvent inclusion can be in stoichiometric or non-stoichiometric order. In the past solvent inclusion has been considered to be a mechanism of polymorphism (due to changes/differences in the unit cell of a solid) but was dubbed pseudopolymorphism, due to the fact that the composition of the pseudopolymorph differs chemically (due to the presence of solvent molecules) from the unsolvated form. The terms solvatomorphs and solvatomorphism are also used to avoid issues associated with inconsistent nomenclature (2).

When water is incorporated in stoichiometric proportions into the crystal lattice of the compound, the molecular adduct(s) formed is referred to as a hydrate.

Occasionally a compound of a given hydration/solvation state may crystallize into more than one crystalline form, thus producing hydrates/solvates that exhibit polymorphism themselves, which is known as polymorphic pseudopolymorphs. An example of this phenomenon is nitrofurantoin (3). Nitrofurantoin can be crystallized as two monohydrated forms (Forms I and II) and two anhydrous species (designated polymorphs α and β) (3). Solvated forms (from different solvents) that do not exhibit significant differences in XRPD patterns and crystal packing (e.g. hydrate and isopropanolate of hexakis(2,3,6-tri-O-acetyl)-α-cyclodextrin) are called isostructural pseudopolymorphs (4).
The term desolvated solvates has been used to classify a compound that was originally crystallized as a solvate but when the incorporated solvent is removed the crystal lattice of the solvated and desolvated crystal lattices do not show any or relatively small differences, for example, desolvated monohydrate of terazosin HCl (5).

Amorphous forms of APIs and excipients are of substantial interest because they are usually more soluble than their crystalline counterparts but are usually considered to be thermodynamically less stable. Solid-state properties of amorphous forms of the same chemical compound (i.e. melting behaviour, solubility profile, etc.) may differ; this phenomenon is referred to as polymorphism (6).

Another phenomenon of crystal engineering is that of pharmaceutical co-crystals. Pharmaceutical co-crystals can be defined as crystalline materials comprised of an API and one or more unique co-crystal formers (e.g. fluoxetine HCl/succinic acid co-crystal), which are solids at room temperature (7), thus it is suggested that co-crystal formation could be considered a subdivision of pseudopolymorphism.

Variation in the crystallization conditions (temperature, pressure, solvent, concentration, rate of crystallization, seeding of the crystallization medium, presence and concentration of impurities, etc.) may cause the formation of different crystalline forms and/or solvates.

In general, the more stable the form (including polymorphs, amorphous forms, etc.), the less soluble the form is in water.

Figure 1 provides a summary of the groups wherein solids can be classified.

Crystalline forms are characterized based on the differences of their physical properties. Table 1 lists some examples of the properties that may differ among different morphic forms (9).

**Table 1. Examples of physical properties that may differ among different morphic forms (9)**

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Table 2 summarizes some of the most commonly used techniques to study and/or classify different morphic forms of substances. These techniques are often complementary and it is indispensable to use several of them.

Any method(s) chosen to confirm the identity of the specific form(s) must be validated to show the method is suitably specific for the identification of the desired form(s).
Polymorphism (Ph. Int.)

Table 2. Examples of some techniques that may be used to study and/or classify different crystalline forms of substances

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* Methods employed by The International Pharmacopoeia

When a substance shows polymorphism the form with the lowest enthalpy at a given temperature and pressure is the most thermodynamically stable. The other forms are said to be in a metastable state. At normal temperature and pressure a metastable form may remain unchanged or may change to a thermodynamically more stable form.

Polymorphism, amorphism and pseudopolymorphism (or solvatomorphism) of APIs and excipients are of interest, as they may affect the bioavailability, suitability for manufacturing of solid dosage forms and thermodynamic stability of the polymorphic form included in the solid dosage form. Control of the morphic form by the manufacturer is necessary during the processing of APIs and excipients and during the manufacturing of a dosage form to ensure the correct physical characteristics thereof. The control of a specific morph is especially critical in the areas where the bioavailability and stability are directly impacted.

The morphic form of a readily soluble starting material that is incorporated into a solution, for example, an injection, an oral solution or eye drops, is normally non-critical (an exception to this statement might be if the concentration of the solution is such that it is close to the limit of solubility of one of the possible polymorphs). Similarly, if the API is processed during the manufacturing process to obtain an amorphous form (e.g. hot melt extrusion), the original form is considered non-critical.

The morphic form may be critical when the material is included in a solid dosage form or as a suspension in a liquid dosage form when the characteristics of the different polymorphs are such as to affect the bioavailability or dissolution of the material (10). The polymorphic form of a biopharmaceutical classification system (BCS) class I or III API in a solid oral dosage form is normally non-critical in terms of dissolution rate or bioavailability.

The inclusion of potentially harmful solvents in the crystal lattice which may render APIs or excipients to be toxic or harmful to patients (i.e. solvates) should also be suitably regulated and monitored by the manufacturer.
Where a monograph indicates that a substance shows polymorphism this may be true crystal polymorphism, occurrence of solvates, allotropy or occurrence of the amorphous form. Due to the identical chemical composition of the polymorphic substance it will have the same chemical behaviour in solution, irrespective in the form in which it is presented.

The *International Pharmacopoeia* controls the morphic forms of a limited number of substances by restricting it to either:

- a single form, for example, Carbamazepine API (Anhydrous Form III), Mebendazole API (Form C); or
- by limiting the presence of unwanted morphic forms, for example, Chloramphenicol palmitate API (should contain at least 90% of polymorph B).

The control of morphic forms may be achieved by:

- permitting no deviation from the infrared absorption spectrum of the reference substance prescribed (or reference spectrum supplied) – when the infrared absorption spectrum has been validated to be specific to the preferred form and able to distinguish the undesired form(s), for example, Indomethacin API;
- restricting the melting point range, for example, Phenobarbital API;
- recommending the use of any other suitable methods such as X-ray powder diffractometry, for example, Carbamazepine tablets;
- limiting the incorporated solvent (in the case of solvatomorphs) with a specific limit test, for example, Nevirapine hemihydrate API.

In the instance where polymorphism may be present the user will be able to deduce this from the infrared identification test (if infrared spectrophotometry is suitable for the detection of differences in morphic forms of the specific compound) where the user may be instructed to:

- recrystallize both the test substance and the specified reference substance, in the event where the infrared spectra are found to be not concordant, for example, Fluconazole API; and/or
- dry the API and/or specified reference substance to ensure that both forms are in the anhydrous or dehydrated state, for example, Nevirapine hemihydrate API.

In the event where the choice of a specific morphic form is critical with regard to bioavailability and/or stability, the method of the manufacture of the product should ensure the presence of desired polymorph in the final product. The Secretariat will include a statement under the heading “Manufacturing” to draw attention to the control of a specified morphic form during manufacture where control is known to be critical, for example, Carbamazepine oral suspension.

It is the intention of *The International Pharmacopoeia* to extend the inclusion of explicit statements in monographs, where appropriate, as information on the occurrence of polymorphism becomes available. The Secretariat thus cordially invites the users of *The International Pharmacopoeia* and manufacturers to share such information that could be included in the monographs if considered being appropriate.
Bibliography


Proposal for updating the definition of “stringent regulatory authority”

This is a proposed concept for identifying a new term to replace the current term “stringent regulatory authority” and its abbreviation “SRA”, and for proposing a definition for the new term. The text shown below is reproduced from the WHO Working document QAS/17.728/Rev.1, August 2017. The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

1. Introduction and background

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has been undergoing structural changes and its membership has changed. For details please see the following website: http://www.ich.org/about/organisational-changes.html

In view of these changes a need was identified for the definition for a “stringent regulatory authority” (SRA) to be reviewed since it is directly linked to ICH membership.

The definition used in World Health Organization (WHO) guidance texts prior to the organization changes in ICH reads as follows:

“A regulatory authority which is:

a. a member of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (as specified on www.ich.org);

or

b. an ICH observer, being the European Free Trade Association (EFTA), as represented by Swissmedic and Health Canada (as may be updated from time to time); or

c. a regulatory authority associated with an ICH member through a legally-binding, mutual recognition agreement including Australia, Iceland, Liechtenstein and Norway (as may be updated from time to time). “


The term (and definition) for SRA is currently used in the following guidelines in the context of the WHO Expert Committee on Specifications for Pharmaceutical Preparations (ECSPP):
Proposal for updating the definition of “stringent regulatory authority”

- Procedure for prequalification of pharmaceutical products.
  (Annex 10, 45th ECSPP report, TRS 941, 2011)
- Guidelines on submission of documentation for a multisource (generic) finished pharmaceutical product for the WHO Prequalification of Medicines Programme: quality part
  (Annex 4, 46th ECSPP report, TRS 970, 2012)
- Pharmaceutical development of multisource (generic) finished pharmaceutical products - points to consider
  (Annex 3, 46th ECSPP report, TRS 970, 2012)
- WHO guidelines on variations to a prequalified product
  (Annex 3, 47th ECSPP report, TRS 981, 2013)
- Model quality assurance system for procurement agencies
  (Annex 3, 48th ECSPP report, TRS 986, 2014)
- Guidelines on submission of documentation for prequalification of finished pharmaceutical products approved by stringent regulatory authorities
  (Annex 5, 48th ECSPP report, TRS 986, 2014)
- Guidelines on submission of documentation for a multisource (generic) finished pharmaceutical product: quality part
  (Annex 6, 48th ECSPP report, TRS 986, 2014)
- Guidance on the selection of comparator pharmaceutical products for equivalence assessment of interchangeable multisource (generic) products
  (Annex 8, 49th ECSPP report, TRS 992, 2015)
- List of International Comparator Pharmaceutical Products and related Notes
  (Update 2016)

(References:
  (link: http://www.who.int/medicines/services/expertcommittees/pharmprep/20160302_QASterminologyDB.pdf?ua=1);
and
(2) WHO ECSPP guidelines and guidance, website (link: http://www.who.int/medicines/areas/quality_safety/quality_assurance/guidelines/en/.)

2. Interim definition

Based on the latest definition published at the time of the meeting, the members of the 51st ECSPP discussed the need for revision and the resulting deliberations in their report read as follows (TRS 1003):


“Definition of stringent regulatory authority

The WHO prequalification procedure and several other WHO guidance documents provide for mechanisms to rely on SRAs, defining an SRA as a regulatory authority which is a member or an observer of ICH, or is associated with an ICH member through a legally-binding mutual recognition agreement. The definition originated from the Global Fund and it is reflected in the quality assurance policies of most major international organizations involved in procuring medicines.

ICH has undergone structural changes and has expanded its reach to include organizations and associations at the global level. In view of these developments the WHO Secretariat proposed an interim definition of an SRA. The interim definition of an SRA will include the same elements as the current definition, each qualified by the wording ‘as before 23 October 2015’, as follows:

A regulatory authority which is:

• a member of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), being the European Commission, the US Food and Drug Administration and the Ministry of Health, Labour and Welfare of Japan also represented by the Pharmaceuticals and Medical Devices Agency (as before 23 October 2015); or

• an ICH observer, being the European Free Trade Association, as represented by Swissmedic, and Health Canada (as before 23 October 2015); or

• a regulatory authority associated with an ICH member through a legally-binding, mutual recognition agreement, including Australia, Iceland, Liechtenstein and Norway (as before 23 October 2015).’

The Expert Committee adopted the interim definition and noted the work being done towards developing a new approach to the assessment of national regulatory authorities, based on the various existing systems currently in place such as that used by the Pan American Health Organization and that applied by WHO with respect to vaccines. The Committee requested that an update on this work be provided at its fifty-second meeting.

3. Proposal

As a follow-up action, internal discussions have taken place within the WHO Regulation of Medicines and other Health Technologies unit towards the development of a new proposal in reply to the ECSPP recommendations. Please find herewith the elements for a new concept that have come out of these discussions.

One governing principle discussed was that the definition and criteria should be acceptable to Member States, agencies that use this definition, such as international procurement agencies, and WHO.
Proposal for updating the definition of “stringent regulatory authority”

Moreover, the criteria/principle to be used for establishing effective performance, confidence/trust (and the process to build these) are part of the WHO Global Benchmarking Tool (GBT) maturity level (ML) 4 assessments of national regulatory authorities (NRAs). The GBT, including ML 4 requirements and assessment process, are being discussed for endorsement by Member States. The GBT will be used to assess medicines, vaccines, blood and blood products and medical devices, including in vitro diagnostics.

Based on the above, it is proposed that the definition for stringent regulatory authority (SRA) could be replaced by the following concept for NRAs to be “on the list”, i.e. qualify as “XXX”, including:

1. grandfathering NRAs identified as “SRAs” in accordance with the current interim definition;
2. need for transparent process for expansion to additional NRAs: the results and basis should be publicly available to be utilizable;
3. use of the GBT ML 4 assessment and risk-based re-assessment as criteria for adding and maintaining NRAs “on the list”;
4. modular approach to enable NRAs to be “on the list” for a specific function and/or product group.

A new term and abbreviation for “SRAs”, “XXX”, will need to be determined. Therefore, we seek your input on proposals for the new term to replace the current “stringent regulatory authority” term and the abbreviation “SRA”, and proposed definition for the new term taking into account the main principles and concepts noted in the previous paragraphs in this document.

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Other draft guidelines

The following medicines quality-related guidelines have been posted for public comment on the WHO website. The working documents with line numbers are available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

▪ Guidelines on heating, ventilation and air-conditioning systems for non-sterile pharmaceutical products
  Working document QAS/15.639/Rev.2 (July 2017)
  These guidelines focus primarily on good manufacturing practice (GMP) for the design, qualification, management and maintenance of heating, ventilation and air-conditioning (HVAC) systems in facilities manufacturing non-sterile dosage forms. The text should be read together with the parent WHO guideline on GMP (Annex 2 to WHO Technical Report Series 986, 2014). Additional requirements for air-handling systems of pharmaceutical hazardous, sterile and biological products are covered in separate WHO guidelines.
  The proposed text is a revision of the 2011 guidelines (Annex 5 to WHO Technical Report Series 961) and sets out recommended standards in line with current technical and regulatory approaches. The illustrative examples included in the 2011 text have been removed from the revised text and will be provided in a separate document in the future.

▪ Stability testing of active pharmaceutical ingredients and finished pharmaceutical products
  These guidelines seek to exemplify the core stability data package required for registration of active pharmaceutical ingredients (APIs) and finished pharmaceutical products (FPPs), replacing the previous WHO guidelines in this area. However, alternative approaches can be used when they are scientifically justified. Further guidance can be found in relevant ICH and WHO guidelines. It is recommended that these guidelines should also be applied to products that are already being marketed, e.g. upon re-registration or re-evaluation. The guidelines do not apply to biological products.

▪ Facilitated registration of stringently approved medicines and vaccines
  (Full title:) Facilitated procedure in the assessment and accelerated national registration of pharmaceutical products and vaccines approved by stringent regulatory authorities (SRAs)
  This is a revision of the draft text proposing a scheme to facilitate accelerated national registration of medicines and vaccines approved by stringent regulatory authorities.
WHO draft guidance on testing of “suspect” falsified medicines

Working document QAS/15.634/Rev.3 (August 2017)

This document provides technical guidance on laboratory testing of samples of suspected falsified products detected on the markets of WHO Member States and related aspects of sampling and reporting.

Good practices for desk assessment

(Full title:) Guidance on good practices for desk assessment of compliance with good manufacturing practices, good laboratory practices and good clinical practices for medical products regulatory decisions


Inspection of manufacturing, testing, clinical trial and distribution sites poses an increasing burden on regulatory authorities. It is therefore good practice to rely on inspection information from other trusted authorities as part of risk-based inspection planning, so that there is no on-site inspection without well-founded cause. This text aims to provide general guidance on performing desk assessments in lieu of onsite inspections.

Draft notes on the conduct of solubility studies


The objective of this document is to provide some guidance on the design and conduct of solubility studies undertaken for the purpose of active pharmaceutical ingredient (API) classification within the Biopharmaceutics Classification System (BCS).

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