Consultation documents

To receive draft monographs by email please contact Mrs Wendy Bonny (bonnyw@who.int), specifying that you wish to be added to the electronic mailing list.

The International Pharmacopoeia

Cycloserine
(Cycloserinum)

This is a draft proposal for The International Pharmacopoeia (Working document QAS/15.638, August 2015).

The working document with line numbers and tracked changes is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/.

Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

[Note from the Secretariat. Following up on information received from a customer of The International Pharmacopoeia it is proposed to revise the monograph on Cycloserine.]

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\[
\text{C}_3\text{H}_6\text{N}_2\text{O}_2
\]

Relative molecular mass. 102.1.

Chemical name. (4R)-4-aminooxazolidin-3-one; (4R)-4-amino-1,2-oxazolidin-3-one; (+)-4-amino-3-isoxazolidinone; CAS Reg. No. 68-41-7.

Description. A white or pale yellow, crystalline, powder.

Solubility. Freely soluble in water; slightly soluble in methanol R and propylene glycol R; very slightly soluble in ethanol (~750 g/L) TS; practically insoluble in dichloromethane R.

Category. Antibacterial drug; antituberculosis drug.

Storage. Cycloserine should be kept in a tightly closed container.
Additional information. Cycloserine is slightly hygroscopic and degrades upon exposure to a humid atmosphere, decomposition being faster at higher temperatures.

Requirements

Definition. Cycloserine is an analogue of the amino acid D-alanine with broad-spectrum antibiotic and glycineric activities produced by *Streptomyces* *garyphalus* and *Streptomyces orchidaceus* or obtained by synthesis.

Cycloserine contains not less than 98.5% and not more than 101.5% of cycloserine \((C_3H_6N_2O_2)\), calculated with reference to the dried substance.

Identity tests

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

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

A.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 1-butanol R, 1 volume of glacial acetic acid R and 2 volumes of water R as the mobile phase. Apply separately to the plate 10 µL of each of the following two solutions. For solution (A) dissolve 20 mg of the test substance in 0.5 mL of water R, add 4.5 mL of methanol R and shake. For solution (B) use 20 mg of cycloserine RS prepared in the same manner. After removing the plate from the chromatographic chamber allow it to dry exhaustively in a current of air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described above under test A.1, but using silica gel R5 as the coating substance. After removing the plate from the chromatographic chamber allow it to dry in a current of air and place the plate in a chamber with iodine vapours. Examine the chromatogram in daylight.

The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

B. Dissolve about 1 mg in 10 mL of sodium hydroxide (0.1 mol/L) VS. To 1 mL of the resulting solution add 3 mL of acetic acid (~60 g/L) TS and 1 mL of a recently prepared mixture of equal volumes of a 40 mg/mL solution of sodium nitroprusside R and sodium hydroxide (~200 g/L) TS; a blue colour gradually develops.

C. The absorption spectrum (1.6) of a freshly prepared 25 µg/mL solution in hydrochloric acid (0.1 mol/L) VS, when observed between 215 nm and 360 nm, exhibits a maximum at about 219 nm; the specific absorbance \((A_{1%}^{10})\) is between 327 and 361.

D. 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 cycloserine RS or with the reference spectrum of cycloserine.

Specific optical rotation (1.4). Use a 50 mg/mL solution in sodium hydroxide (~80 g/l) TS and calculate with reference to the dried substance; \(\alpha_D^{20^\circ} = +108^\circ\) to +114°.
Heavy metals. Use 2.0 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 10 µg/g.

Sulfated ash (2.3). Not more than 5.0 mg/g.

Loss on drying. Dry at 60°C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) for 3 hours; it loses not more than 10 mg/g.

pH value (1.13). pH of a 100 mg/mL solution in carbon-dioxide-free water R, 5.5 to 6.5.

Related substances

Prepare fresh solutions and perform the tests without delay.

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 base deactivated particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).

The mobile phases for gradient elution consist of a mixture of mobile phase A and mobile phase B using the following conditions:

Mobile phase A: 4 volumes of acetonitrile R, 70 volumes of 0.02 mol/L sodium octanesulfonate R solution, 10 volumes of phosphate buffer pH 2.8 and 16 volumes of water R.

Mobile phase B: 17 volumes of acetonitrile R, 70 volumes of 0.02 mol/L sodium octanesulfonate R solution, 10 volumes of phosphate buffer pH 2.8 and 3 volumes of water R.

Prepare the sodium octanesulfonate solution by dissolving 4.7 g of sodium octanesulfonate R in 1000 mL of water R.

Prepare the phosphate buffer pH 2.8 by dissolving 27.2 g of potassium dihydrogen phosphate R in 800 mL of water R, adjust the pH to 2.8 by adding phosphoric acid (~20 g/L) TS and dilute to 1000 mL with water R.

<table>
<thead>
<tr>
<th>Time (min)</th>
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<td>22–24</td>
<td>0 to 100</td>
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</tr>
<tr>
<td>24–30</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Prepare the following solutions in mobile phase A. For solution (1) use a solution containing 1.0 mg of the test substance per mL. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 5.0 µg of cycloserine per mL. For solution (3) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 25 µg of cycloserine per mL. Heat carefully in a boiling water-bath for 30 minutes. For solution (4) use a solution containing 2.0 µg of D-serine R per mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 195 nm.

Maintain the column temperature at 45°C.
Inject 50 μL of solution (3). The test is not valid unless the resolution between the principal peak corresponding to cycloserine (retention time about 14 min) and the large degradation peak with a relative retention time of about 0.23 is not less than 20.

Inject alternately 50 μL each of solutions (1), (2) and (4).

In the chromatogram obtained with solution (1) the following impurities, if present, are eluted at the following relative retention with reference to cycloserine (retention time about 14 minutes): impurity B (D-serine) about 0.23, impurity C about 0.35 and impurity A about 1.8.

In the chromatogram obtained with solution (1):
• the area of any peak corresponding to impurity B (D-serine) is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.2%);
• the area of any other peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);
• the sum of the areas of all peaks, other than the principal peak and any peak corresponding to impurity B (D-serine), is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

**Assay**

Dissolve about 0.1 g, accurately weighed, in 5 mL of water R. Add 75 mL of 2-propanol R and titrate with carbonate-free sodium hydroxide (0.1 mol/L) VS using thymolphthalein/ethanol TS as indicator. Perform a blank determination and make any necessary correction.

Each mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 10.21 mg of C₃H₆N₂O₂.

**Impurities**

A. (3R,6R)-3,6-bis[(aminooxy)methyl]piperazine-2,5-dione (cycloserine dimer),

B. (2R)-2-amino-3-hydroxypropanoic acid (D-serine),

C. condensation product with unknown structure.

***
Cycloserine capsules
(Cycloserini capsulae)

This is a draft proposal for The International Pharmacopoeia (Working document QAS/15.637, August 2015).

The working document with line numbers and tracked changes is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/.
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Category. Antibacterial drug; antituberculosis drug.

Storage. Cycloserine capsules should be kept in a tightly closed container.


Requirements
Comply with the monograph for Capsules.

Definition. Cycloserine capsules contain Cycloserine. They contain not less than 90.0% and not more than 110.0% of the amount of cycloserine (C₇H₆N₂O₂) stated on the label.

Manufacture. The manufacturing process and the product packaging are designed and controlled so as to minimize the moisture content of the capsules. They ensure that, if tested, the contents of the capsules would comply with a loss on drying limit of not more than 20 mg/g when determined by drying a suitable quantity of the contents of the capsules for 3 hours under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury) at 60°C.

Identity tests
- Either tests A and B or tests B and C may be applied.
  A. Carry out test A.1 or, where UV detection is not available, test A.2.
    A.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 1-butanol R, 1 volume of glacial acetic acid R and 2 volumes of water R as the mobile phase. Apply separately to the plate 10 µL of each of the following two solutions. For solution (A) shake a quantity of the contents of the capsules equivalent to 40 mg of cycloserine with 1 mL of water R, add 9 mL of methanol R, shake again, filter and use the filtrate. For solution (B) dissolve 20 mg of cycloserine RS in 0.5 mL of water R, add 4.5 mL of methanol R and shake. After removing the plate from the chromatographic chamber allow it to dry exhaustively in a current of air. Examine the chromatogram in ultraviolet light (254 nm).
The principal spot obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B.

A.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described above under test A.1, but using silica gel R5 as the coating substance. After removing the plate from the chromatographic chamber allow it to dry in a current of air and place the plate in a chamber with iodine vapours. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B.

B. Shake a quantity of the contents of the capsules containing 10 mg of cycloserine with 100 mL of sodium hydroxide (~40 g/L) TS and filter. To 1 mL of the filtrate add 3 mL of acetic acid (~60 g/L) TS and 1 mL of a recently prepared mixture of equal volumes of a 40 mg/mL solution of sodium nitroprusside R and sodium hydroxide (~200 g/L) TS; a blue colour gradually develops.

C. See the test described under “Assay”, Method A. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).

Related substances

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

Prepare the following solutions in mobile phase A. For solution (1) transfer a quantity of the contents of the capsules containing 100 mg of cycloserine into a 100 mL volumetric flask. Add about 70 mL, shake for 5 minutes, make up to volume and filter. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration containing 5.0 µg of cycloserine per mL. For solution (3) dilute a suitable volume of solution (1) to obtain a concentration of 25 µg of cycloserine per mL. Heat carefully in a boiling water-bath for 30 minutes. For solution (4) use a solution containing 2.0 µg of D-serine R per mL.

Inject 50 μL of solution (3). The test is not valid unless the resolution between the principal peak corresponding to cycloserine (retention time about 14 minutes) and the large degradation peak with a relative retention time of about 0.23 is not less than 20.

Inject alternately 50 μL each of solutions (1), (2) and (4).

In the chromatogram obtained with solution (1) the following impurities, if present, are eluted at the following relative retention with reference to cycloserine (retention time about 14 minutes): impurity B (D-serine) about 0.23, impurity C about 0.35 and impurity A about 1.8.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity B (D-serine) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (4) (0.4%);
- the area of any other peak, other than the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%);
- the sum of the areas of all peaks, other than the principal peak and any peak corresponding to impurity B (D-serine), is not greater than six times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%). Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).
Assay

- Either method A or method B may be applied.

A. Carry out the assay as described 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).

The mobile phases for gradient elution consist of a mixture of Mobile phase A and Mobile phase B, using the following conditions:

Mobile phase A: 4 volumes of acetonitrile R, 70 volumes of a 0.02 mol/L sodium octanesulfonate R solution, 10 volumes of phosphate buffer pH 2.8 and 16 volumes of water R.

Mobile phase B: 17 volumes of acetonitrile R, 70 volumes of a 0.02 mol/L sodium octanesulfonate R solution, 10 volumes of phosphate buffer pH 2.8 and 3 volumes of water R.

Prepare the sodium octanesulfonate solution by dissolving 4.7 g of sodium octanesulfonate R in 1000 mL of water R.

Prepare the phosphate buffer pH 2.8 by dissolving 27.2 g of potassium dihydrogen phosphate R in 800 mL of water R, adjust the pH to 2.8 by adding phosphoric acid (~20 g/L) TS and dilute to 1000 mL with water R.

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<td>0</td>
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Prepare the following three solutions in mobile phase A. For solution (1) weigh and mix the contents of 20 capsules and transfer a quantity of the contents containing about 10 mg of cycloserine, accurately weighed, into a 100 mL volumetric flask. Add about 70 mL, shake for 5 minutes, make up to volume and filter. For solution (2) use 0.1 mg of cycloserine RS per mL. For solution (3) dilute a suitable volume of solution (1) to obtain a concentration of 25 μg of cycloserine per mL. Heat carefully in a boiling water-bath for 30 minutes.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 195 nm.

Maintain the column temperature at 45°C.

Inject 50 μL of solution (3). The test is not valid unless the resolution between the principal peak corresponding to cycloserine (retention time about 14 minutes) and the large degradation peak with a relative retention time of about 0.23 is not less than 20.

Inject alternately 50 μL each of solutions (1) and (2).
Measure the areas of the peaks responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of cycloserine (C$_3$H$_6$N$_2$O$_2$) in the capsules, using the declared content of C$_3$H$_6$N$_2$O$_2$ in cycloserine RS.

B. Weigh and mix the contents of 20 capsules and transfer a quantity of the contents containing 0.250 g of cycloserine, accurately weighed, into a 200 mL volumetric flask. Add hydrochloric acid (0.1mol/L) VS to volume, shake for 10 minutes and filter. Dilute 2 mL of the filtrate to 100 mL with hydrochloric acid (0.1mol/L) VS.

Measure the absorbance (1.6) of this solution in a 1 cm layer at the maximum at about 219 nm against a solvent cell containing hydrochloric acid (0.1mol/L) VS.

Calculate the content of cycloserine (C$_3$H$_6$N$_2$O$_2$) in the capsules, using an absorptivity value of 34.3 (A$_{1\%}$ = 343).

**Impurities**

The impurities limited by the requirements of this monograph include those listed in the monograph for Cycloserine.

***
Ceftriaxone sodium  
*(Ceftriaxonum natricum)*

This is a draft proposal for The International Pharmacopoeia (Working document QAS/15.644, November 2015).

The working document with line numbers is available for comment at [www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/](http://www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/). Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

**C**<sub>18</sub>H<sub>16</sub>N<sub>8</sub>Na<sub>2</sub>O<sub>7</sub>S<sub>3</sub>·3½H<sub>2</sub>O

**Relative molecular mass.** 661.60

**Chemical name.** Disodium (6R,7R)-7-[(2Z)-(2-aminothiazol-4-yl)(methoxyimino) acetyl]amino]-3-[[2-methyl-6-oxido-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)sulfanyl]meth- yl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 3.5 hydrate.

**Description.** Almost white or yellowish, slightly hygroscopic, crystalline powder.

**Solubility.** Freely soluble in water, sparingly soluble in methanol, very slightly soluble in anhydrous ethanol.

**Labelling.** The label states, where applicable:
- that the substance is free of bacterial endotoxins;
- that the substance is sterile.

**Category.** Antibacterial

**Storage.** Ceftriaxone sodium should be kept in an air-tight container protected from light. If the substance is sterile, store in a sterile and air-tight container protected from light.

**Requirements**

Ceftriaxone sodium contains not less than 96.0% and not more than 102.0% of the labelled amount of ceftriaxone sodium (C<sub>18</sub>H<sub>16</sub>N<sub>8</sub>Na<sub>2</sub>O<sub>7</sub>S<sub>3</sub>), calculated with reference to the anhydrous substance.

**Identity tests**

- Either tests A and C 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 ceftriaxone sodium RS or with the reference spectrum of ceftriaxone sodium.
B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under the Assay test. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak corresponding to ceftriaxone in the chromatogram obtained with solution (2).

C. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reaction.

**Specific optical rotation** (1.4). Dissolve 0.250 g in water R and dilute to 25.0 mL with the same solvent. Calculate with reference to the anhydrous substance; $\chi_0^{20^\circ} = -155^\circ \text{ to } -170^\circ$.

**Clarity and colour of solution.** Dissolve 2.40 g in carbon-dioxide-free water R and dilute to 20.0 mL with the same solvent (Solution A). Dilute 2 mL of Solution A to 20 mL carbon-dioxide-free water R. The solution is clear and not more intensely coloured than reference solution YW2 when compared as described under 1.11 Colour of liquids. (Keep the remaining solution (Solution A) for the “pH value”.)

**pH value** (1.13). pH of the solution prepared for the “Clarity and colour of solution” (Solution A), 6.0 to 8.0.

**Water.** Determine as described under 2.8 Determination of water by the Karl Fischer method, method A, using about 0.1 g of the test substance. The water content is not less than 80 mg per g and not more than 110 mg per g.

**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 0.20 IU of endotoxin per mg of ceftriaxone sodium.

**Related substances**

Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given below under assay method.

Prepare the following solutions in mobile phase: for solution (1) dissolve about 30 mg of the test substance and dilute to 100.0 mL. For solution (2) dilute 1 volume of solution (1) to 100 volumes. For solution (3) dissolve about 5 mg ceftriaxone sodium RS and 5 mg of ceftriaxone impurity G to 100.0 mL.

Inject 20 µL of solution (3). The test is not valid unless the resolution factor between the peaks due to ceftriaxone and ceftriaxone impurity G is at least 3.0. Ceftriaxone impurity G is eluted at a relative retention of 1.4 with reference to ceftriaxone (retention time about 9 min).

Inject alternately 20 µL each of solution (1) and (2). Record the chromatograms for about 2 times the retention time of ceftriaxone. The chromatogram obtained with solution (1) may show the following impurities at the following relative retention with reference to ceftriaxone: impurity A: about 0.2; impurity B: about 0.34; impurity C: about 0.62; impurity D: about 0.72; impurity E: about 0.78; impurity F: about 1.3 and impurity G: about 1.4.

In the chromatogram obtained with solution (1):

- the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0 %);
- the sum of the areas of all peaks, other than the principal peak, is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5 %).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).
B. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under the Assay test. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak corresponding to ceftriaxone in the chromatogram obtained with solution (2).

C. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reaction.

**Specific optical rotation** (1.4). Dissolve 0.250 g in water R and dilute to 25.0 mL with the same solvent. Calculate with reference to the anhydrous substance; \( [\alpha]_{D}^{20^\circ} = -155^\circ \text{ to } -170^\circ \).

**Clarity and colour of solution.** Dissolve 2.40 g in carbon-dioxide-free water R and dilute to 20.0 mL with the same solvent (Solution A). Dilute 2 mL of Solution A to 20 mL carbon-dioxide-free water R. The solution is clear and not more intensely coloured than reference solution YW2 when compared as described under 1.11 Colour of liquids. (Keep the remaining solution (Solution A) for the “pH value”)

**pH value** (1.13). pH of the solution prepared for the “Clarity and colour of solution” (Solution A), 6.0 to 8.0.

**Water.** Determine as described under 2.8 Determination of water by the Karl Fischer method, method A, using about 0.1 g of the test substance. The water content is not less than 80 mg per g and not more than 110 mg per g.

**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 0.20 IU of endotoxin per mg of ceftriaxone sodium.

**Related substances**

Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given below under assay method.

Prepare the following solutions in mobile phase: for solution (1) dissolve about 30 mg of the test substance and dilute to 100.0 mL. For solution (2) dilute 1 volume of solution (1) to 100 volumes. For solution (3) dissolve about 5 mg ceftriaxone sodium RS and 5 mg of ceftriaxone impurity G to 100.0 mL.

Inject 20 µL of solution (3). The test is not valid unless the resolution factor between the peaks due to ceftriaxone and ceftriaxone impurity G is at least 3.0. Ceftriaxone impurity G is eluted at a relative retention of 1.4 with reference to ceftriaxone (retention time about 9 min).

Inject alternately 20 µL each of solution (1) and (2). Record the chromatograms for about 2 times the retention time of ceftriaxone. The chromatogram obtained with solution (1) may show the following impurities at the following relative retention with reference to ceftriaxone: impurity A: about 0.2; impurity B: about 0.34; impurity C: about 0.62; impurity D: about 0.72; impurity E: about 0.78; impurity F: about 1.3 and impurity G: about 1.4.

In the chromatogram obtained with solution (1):

- the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0 %);
- the sum of the areas of all peaks, other than the principal peak, is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5 %). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).
Assay

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 base-deactivated silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm) 1.

As the mobile phase use a solution prepared as follows: dissolve 2.0 g of tetradecylammonium bromide R and 2.0 g of tetradeccylammonium bromide R in a mixture of 440 mL of water R, 55 mL of phosphate buffer pH 7.0 (0.067 mol/L) TS, 5.0 mL of citrate buffer pH 5.0 TS and 500 mL of acetonitrile R and filter.

Prepare the following solutions in mobile phase. For solution (1) dissolve 30 mg of the test substance, accurately weighed and dilute to 100.0 mL. For solution (2) dissolve about 30 mg of ceftriaxone sodium RS, accurately weighed and dilute to 100.0 mL. For solution (3) dissolve about 5 mg ceftriaxone sodium RS and about 5 mg of ceftriaxone impurity G and dilute to 100.0 mL.

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.

Inject 20 µL of solution (3). The test is not valid unless the resolution factor between the peaks due to ceftriaxone and ceftriaxone impurity G is at least 3.0.

Inject alternately 20 µL each of solution (1) and (2). Measure the areas of the peaks corresponding to ceftriaxone and calculate the percentage content of ceftriaxone sodium (C_{18}H_{16}N_{8}Na_{2}O_{7}S_{3}) with reference to the anhydrous substance.

Sterility. If intended for use in the manufacture of a parenteral dosage form without a further appropriate sterilization procedure, complies with 3.2 Test for sterility.

Impurities

[Note from the Secretariat. The structures of the impurities will be added at a later stage.]

A. (Z)-2-(2-Aminothiazol-4-yl)-N-{(5aR,6R)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl}-2-(methoxyimino)acetamide. (Deacetylcefotaxime lactone)
B. (6R,7R)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. (7-Aminocephalosporanic acid)
C. 3-Mercapto-2-methyl-1,2-dihydro-1,2,4-triazine-5,6-dione. (Ceftriaxone triazine analog)
D. (Z)-S-Benzothiazol-2-yl 2-(2-aminothiazol-4-yl)-2-(methoxyimino)thioacetate (Ceftriaxone benzothiazolyl oxide).
E. (6R,7R)-7-Amino-3-{{(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio}methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. (Decacyl ceftriaxone).
F. (6R,7R)-7-{{(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido}-3-{{(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio}methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid (Ceftriaxone 3-ene isomer).
G. (6R,7R)-7-{{(E)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido}-3-{{(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio}methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Ceftriaxone E-isomer).

Reagent to be included:

Citrate buffer, pH 5 TS
Procedure. Dissolve 20.17 g of citric acid R in 800 ml of water R, adjust to pH 5.0 with sodium hydroxide (~400 g/L) TS and dilute to 1000 mL with water R.

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1 Hypersil BDS C18 has been found suitable.
Ceftriaxone for injection
(Ceftriaxoni ad injectionem)

This is a draft proposal for The International Pharmacopoeia (Working document QAS/15.645, November 2015).

The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects/en/. Please address any comments to: World Health Organization, Quality Assurance and Safety: Medicines, Dr Herbert Schmidt, 1211 Geneva 27, Switzerland; fax: +41 22 791 4730; email: schmidth@who.int.

Description. A white and almost white powder.

Category. Antibacterial.

Storage. Ceftriaxone for injection should be stored in a tightly closed container. The reconstituted solution should be used immediately after preparation.

Additional information. Strengths in the current WHO Model List of Essential Medicines (EML): 250 mg, 1 g (as sodium salt) in vial. Strength in the current WHO EML for children: 250 mg, 1 g (as sodium salt) in vial.

Requirements

The powder for injection and the reconstituted solution for injections comply with the monograph on Parenteral preparations.

Definition. Ceftriaxone for injection is a sterile powder containing Ceftriaxone sodium with or without excipients.

Ceftriaxone for injection contains not less than 90.0% and not more than 110.0% of the labelled amount of ceftriaxone (C_{18}H_{18}N_{8}O_{7}S_{3}).

Identity tests

• Either tests A and C 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 ceftriaxone sodium RS or with the reference spectrum of ceftriaxone sodium.

B. 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 ceftriaxone peak in the chromatogram obtained with solution (2).

C. When tested for sodium as described under 2.1 General identification tests, yields the characteristic reaction.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, method A, using about 0.1 g of the powder. The water content is not more than 110 mg per g.
Clarity and colour of solution. Dissolve 2.40 g in carbon-dioxide-free water R and dilute to 20.0 mL with the same solvent (Solution A). Dilute 2 mL of Solution A to 20 mL carbon-dioxide-free water R. The solution is clear and not more intensely coloured than reference solution YW2 when compared as described under 1.11 Colour of liquids. (Keep the remaining solution (Solution A) for the “pH value”.)

pH value (1.13). pH of the solution prepared for the “Clarity and colour of solution” (Solution A), 6.0 to 8.0.

Bacterial endotoxins. Carry out the test described under 3.4 Test for bacterial endotoxins, contains not more than 0.19 IU of endotoxin per mg of ceftriaxone.

Related substances
Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given below under assay method.

Prepare the following solutions in mobile phase: for solution (1) dissolve about 30 mg of the powder and dilute to 100.0 mL. For solution (2) dilute 1 volume of solution (1) to 100 volumes. For solution (3) dissolve about 5 mg ceftriaxone sodium RS and 5 mg of ceftriaxone impurity G to 100.0 mL.

Inject 20 µL of solution (3). The test is not valid unless the resolution factor between the peaks due to ceftriaxone and ceftriaxone impurity G is at least 3.0. Ceftriaxone impurity G is eluted at a relative retention of 1.4 with reference to ceftriaxone (retention time about 9 min).

Inject alternately 20 µL each of solutions (1) and (2). Record the chromatograms for about 2 times the retention time of ceftriaxone. The chromatogram obtained with solution (1) may show the following impurities at the following relative retention with reference to ceftriaxone: impurity A: about 0.2; impurity C: about 0.62; impurity D: about 0.72; impurity E: about 0.78; impurity F: about 1.3 and impurity G: 1.4.

In the chromatogram obtained with solution (1)

• the area of any peak, other than the principal peak, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0 %);

• the sum of the areas of all peaks, other than the principal peak, is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with solution (2) (2.5%).

Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Assay
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 base-deactivated silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm) \(^2\).

As the mobile phase use a solution prepared as follows: dissolve 2.0 g of tetradecylammonium bromide R and 2.0 g of tetraheptylammonium bromide R in a mixture of 440 mL of water R, 55 ml of phosphate buffer, pH 7.0 (0.067 mol/L) TS, 5.0 mL of a citrate buffer pH 5.0 TS and 500 mL of acetonitrile R and filter.

\(^2\) Hypersil BDS C18 has been found suitable.
Prepare the following solutions in mobile phase: for solution (1) determine the weight of the contents of 10 containers. Transfer a quantity of the mixed contents containing about 30 mg of ceftriaxone, accurately weighed, to a 100 mL volumetric flask, dissolve and dilute to volume. For solution (2) dissolve about 35 mg of ceftriaxone sodium RS, accurately weighed and dilute to 100.0 mL. For solution (3) dissolve about 5 mg ceftriaxone sodium RS and 5 mg of ceftriaxone impurity G to 100.0 mL.

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.

Inject 20 µL of solution (3). The test is not valid unless the resolution factor between the peaks due to ceftriaxone and ceftriaxone impurity G is at least 3.0.

Inject 20 µL of solution (1) and (2) Measure the areas of the peaks corresponding to ceftriaxone and calculate the content of ceftriaxone \((C_{18}H_{18}N_8O_7S_3)\) per container. Each mg of \(C_{18}H_{16}N_8NaO_7S_3\) is equivalent to 0.9274 mg of \(C_{18}H_{18}N_8O_7S_3\).

**Impurities**

The impurities limited by the requirements of this monograph include those listed in the monograph for Ceftriaxone sodium.