CYCLOSERINE CAPSULES
Proposal for revision of The International Pharmacopoeia
(August 2012)

Draft for comment

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Please send any request for permission to:

Dr Sabine Kopp, Medicines Quality Assurance Programme, Quality Assurance and Safety: Medicines, Department of Essential Medicines and Health Products, World Health Organization, CH-1211 Geneva 27, Switzerland. Fax: (41-22) 791 4730; e-mail: kopp@who.int.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/12.464

*Draft proposal for revision of a published monograph in the Fourth Edition of The International Pharmacopoeia*

**CYCLOSERINE CAPSULES**

<table>
<thead>
<tr>
<th>Description</th>
<th>Date</th>
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<tbody>
<tr>
<td>Discussion of preliminary draft revision at consultation on specifications for medicines and quality control laboratory issues</td>
<td>29-31 May 2012</td>
</tr>
<tr>
<td>Draft sent out for comments following discussion at consultation on specifications for medicines and quality control laboratory issues</td>
<td>August 2012</td>
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<tr>
<td>Collation of comments received</td>
<td>August-September 2012</td>
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<tr>
<td>Discussion at forty-seventh meeting of the WHO Expert Committee on Specifications for Pharmaceutical Preparations</td>
<td>9-12 October 2012</td>
</tr>
<tr>
<td>Further follow-up action as required</td>
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</tbody>
</table>
CYCLOSERINE CAPSULES

[Note from the Secretariat. The following change to the system suitability criterion of the test for related substances is proposed.
Changes from the current monograph are indicated in the text by insert or delete.]

Category. Antibacterial drug; antituberculosis drug.

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

Additional information. Strength in the current WHO Model list of essential medicines:
250 mg. Strength in the current WHO Model list of essential medicines for children: 250 mg.

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 (C₃H₆N₂O₂) 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 about 50 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 0.5 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.

Inject 50 µl of solution (3). The test is not valid unless the resolution between the principal peak and the large degradation peak with a retention time of about 3 minutes is not less than 35 20. If necessary adjust the amount of acetonitrile in mobile phase A.

Inject alternatively 50 µl each of solutions (1) and (2).

In the chromatogram obtained with solution (1), the area of any peak, other than the principal peak, is not greater than four times the area of the principal peak in the chromatogram obtained with solution (2) (0.4%). The sum of the areas of all peaks, other than the principal peak, is not greater than ten times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). 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 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 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.

<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 – 16</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>16 – 18</td>
<td>100 to 0</td>
<td>0 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>18 – 22</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>22 – 24</td>
<td>0 to 100</td>
<td>100 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>24 – 30</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

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 219 nm.

Maintain the column temperature at 45 °C.

Inject 50 µl of solution (3). The assay test is not valid unless the resolution between the principal peak and the large degradation peak with a retention time of about 3 minutes is not less than ≥20. If necessary adjust the amount of acetonitrile in mobile phase A.

Inject alternatively 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 (C₃H₆N₂O₂) in the capsules.
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 \((\text{C}_3\text{H}_6\text{N}_2\text{O}_2)\) in the capsules, using an absorptivity value of 34.3 \((A_{\text{1cm}}^{1\%} = 343)\).

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