CYCLOSERINE CAPSULES

Revised draft proposal for *The International Pharmacopoeia*  
(August 2008)

**REVISED DRAFT FOR ADOPTION**

This document was provided by a quality control expert. Previous comments received have been incorporated into this revised draft. Should you have any further comments, please send these to Dr S. Kopp with copies to Ms M.-L. Rabouhans and Ms C. Mendy, Medicines Quality Assurance Programme, Quality and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or e-mail: kopp@who.int, rabouhansm@who.int and mendyc@who.int by 20 October 2008.

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### SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/07.250

*International Pharmacopoeia monograph on Cycloserine capsules*

<table>
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<th>Event</th>
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<tr>
<td>Preliminary draft monograph prepared by laboratory</td>
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<tr>
<td>Preliminary draft monograph mailed out for comments</td>
<td>November 2007</td>
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<tr>
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<td>January 2008</td>
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<tr>
<td>Discussion of draft monograph at informal consultation, Geneva</td>
<td>17-19 June 2008</td>
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<tr>
<td>Revised draft monograph sent out for comments</td>
<td>September 2008</td>
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<tr>
<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations for possible adoption</td>
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<tr>
<td>Further follow-up action as required</td>
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(August 2008)

**CYCLOSERINII CAPSULAE**  
**CYCLOSERINE CAPSULES**

**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 or tests B and C may be applied.

  A. 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).
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 B. The absorption spectrum (1.6) of the solution, when observed between 215 nm and 360 nm, exhibits a maximum at about 219 nm.

[Note from the Secretariat: possibility of the replacement of the current identity test C by a TLC method is under investigation.]

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. 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>

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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 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 35. 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 \( \text{(C}_3\text{H}_6\text{N}_2\text{O}_2) \) 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 \( (A_{1cm}^{1\%} = 343) \).

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