ATAZANAVIR SULFATE

Draft proposal for The International Pharmacopoeia

(June 2014)

REVISED DRAFT FOR COMMENT

Should you have any comments on the attached text, please send these to Dr Herbert Schmidt, Medicines Quality Assurance, Technologies, Standards and Norms, World Health Organization, 1211 Geneva 27, Switzerland; email: schmidt@who.int; fax: (+41 22) 791 4730 by 1 August 2014.

In order to speed up the process for receiving draft monographs and for sending comments, please let us have your email address (to bonnyw@who.int) and we will add it to our electronic mailing list. Please specify if you wish to receive monographs.
<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
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<tr>
<td>Draft monograph submitted by a WHO collaborating laboratory</td>
<td>October 2013</td>
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<tr>
<td>Draft monograph mailed out for comment</td>
<td>5 December 2013</td>
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<tr>
<td>Collation of comments</td>
<td>March 2014</td>
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<tr>
<td>Discussion at informal consultation on specifications for new medicines</td>
<td>3–4 April 2014</td>
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<tr>
<td>Revised draft monograph mailed out for comments</td>
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<tr>
<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations for discussion</td>
<td>13–17 October 2014</td>
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<td>Further follow-up action as required</td>
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</tbody>
</table>
**ATAZANAVIR SULFAS**

**ATAZANAVIR SULFATE**

![Chemical structure of Atazanavir Sulfate](image)

**Molecular formula.** C\textsubscript{38}H\textsubscript{52}N\textsubscript{6}O\textsubscript{7}. H\textsubscript{2}O\textsubscript{4}S

**Relative molecular mass.** 802.9

**Chemical name**

**Dimethyl** \((3S,8S,9S,12S)\)-9-benzyl-3,12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-6-[[4-(pyridin-2-yl)phenyl]methyl]-2,5,6,10,13-pentaazatetradecanedioate monosulfate

\((3S,8S,9S,12S)-3,12-Bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl)phenyl]methyl]-2,5,6,10,13-pentaazatetradecanedioic acid 1,14-dimethyl ester, sulfate (1:1); CAS 229975-97-7

**Description.** A white to a pale yellow crystalline powder.

**Solubility.** Freely soluble in methanol, practically insoluble in water.

**Category.** Antiretroviral (protease inhibitor).

**Storage.** Atazanavir sulfate should be kept in a tightly closed container.

**Additional information.** Atazanavir sulfate is slightly hygroscopic and may exhibit polymorphism.
Requirements

Atazanavir sulfate contains not less than 99.0% and not more than 101.0% of C₃₈H₅₂N₆O₇•H₂SO₄ calculated on the dried basis.

[Note from the Secretariat. Comments are being sought in particular on the suitability of the proposed content limits.]

Identity tests

Either test A and D, or test B, C and D should be performed

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 atazanavir sulfate RS or with the reference spectrum of atazanavir sulfate. If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the test substance and atazanavir sulfate RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from atazanavir sulfate 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 9.5 volumes of dichloromethane R and 0.5 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 10 μL of each of 2 solutions in methanol R containing (A) 1 mg of the test substance per mL and (B) 1 mg of atazanavir sulfate RS per mL. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or 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 to that obtained with solution (B).
B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography using the conditions described under test B.1, but using a plate containing silica gel R5 as the coating substance.

Spray the plate with potassium permanganate, basic (~5 g/L) TS. Examine the chromatogram in daylight. The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).

C. The absorption spectrum of a 10 µg/mL solution in methanol R, when observed between 230 nm and 340 nm, exhibits two maxima at about 250 nm and 280 nm.

D. A 20 mg/mL solution yields Reaction A described under 2.4 General identification tests as characteristic of sulfates.

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

Loss on drying. Dry for 3 hours at 105 °C; it loses not more than 10.0 mg/g.

Specific optical rotation. Use a 10 mg/mL solution in equal volumes of methanol R and water R at 22 °C calculated with reference to the anhydrous substance; the optical rotation is between -40° and -44°.

Related substances

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a column (150 mm x 4.6 mm) packed with end-capped, base-deactivated particles of silica gel the surface of which has been modified with chemically-bonded octylsilyl groups (5 µm).¹ Use the following conditions for gradient elution:

Mobile phase A: 0.02 M phosphate buffer pH 3.5, acetonitrile R (70:30 v/v)

¹ An Inertsil C8 column has been found suitable.
Working document QAS/13.566/Rev.1

Mobile phase B: 0.02 M phosphate buffer pH 3.5, acetonitrile R (30:70 v/v).

Prepare the phosphate buffer pH 3.5 by dissolving 2.72 g of anhydrous potassium dihydrogen phosphate R in 800 mL of water R, adjust the pH to 3.5 by adding phosphoric acid (~105 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>0–2</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>2–10</td>
<td>100–75</td>
<td>0–25</td>
<td>Linear gradient</td>
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<td>10–30</td>
<td>75–50</td>
<td>25–50</td>
<td>Linear gradient</td>
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<tr>
<td>30–45</td>
<td>50–0</td>
<td>50–100</td>
<td>Linear gradient</td>
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<tr>
<td>45–50</td>
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<tr>
<td>50–52</td>
<td>0–100</td>
<td>100–0</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>52–60</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
</tbody>
</table>

Prepare the following solutions using as diluent a mixture of equal volumes of water R and acetonitrile R. For solution (1) use 1 mg of the test substance per mL. For solution (2) dilute a suitable volume of solution (1) with the diluent to obtain a concentration equivalent to 5 µg of Atazanavir sulfate per mL. For solution (3) mix 1 mL of solution (1) with 4.5 mL of water R and 0.5 mL of sodium hydroxide (10 g/l) TS and heat the mixture in a water-bath at 85 °C for 15 minutes.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 250 nm. Maintain the column at a temperature of 30 °C.

Inject 20 µL of solution (3). The test is not valid unless the resolution between the peak due to atazanavir (retention time about 22 minutes) and the peak with a relative retention of about 1.2 is at least 4.
Inject alternatively 20 µL each of solutions (1) and (2).

In the chromatograms obtained with test 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) (0.5%). The sum of the areas of all peaks, 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%). 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 0.300 g, accurately weighed, in 30 mL of methanol R and by sonication for 10 minutes. Then add 30 mL of water and titrate with sodium hydroxide (0.1 mol/L), VS, determining the end-point potentiometrically. Each mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 40.145 mg of C$_{38}$H$_{52}$N$_6$O$_{7}$•H$_2$SO$_4$.  

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