ATAZANAVIR SULFATE

Draft proposal for *The International Pharmacopoeia*

(December 2013)

**DRAFT FOR COMMENT**

Should you have any comments on this draft, please send these to Dr Herbert Schmidt, Medecines Quality Assurance Programme, Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or email: schmidt@who.int by 5 February 2014.

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Please send any request for permission to:

Dr Sabine Kopp, Manager, Medicines Quality Assurance Programme, Technologies, Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, CH-1211 Geneva 27, Switzerland. Fax: (+41 22) 791 4730; email: kopps@who.int.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/13.566: ATAZANAVIR SULFATE

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Draft monograph submitted by a WHO collaborating laboratory</td>
<td>October 2013</td>
</tr>
<tr>
<td>Draft monograph mailed out for comments</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 (if necessary)</td>
<td>May 2014</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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<tr>
<td>Further follow-up action as required</td>
<td></td>
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</tbody>
</table>
**ATAZANAVIRI SULFAS**

**ATAZANAVIR SULFATE**

Molecular formula. \( C_{38}H_{52}N_{6}O_{7} \cdot H_2SO_4 \)

Relative molecular mass. 802.9

Chemical name. \((3S,8S,9S,12S)-3,12\text{-Bis}(1,1\text{-dimethylethyl})-8\text{-hydroxy}-4,11\text{-dioxo}-9-(\text{phenylmethyl})-6\text{-}[[4-(2-pyridinyl)\text{phenyl}methyl]-2,5,6,10,13\text{-pentaazatetradecanedioic acid dimethyl ester, sulfate (1:1).}}\)

Description. A white or almost white 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 at a temperature not exceeding 30 °C.

Additional information. Atazanavir sulfate is slightly hygroscopic.

Requirements. Atazanavir sulfate contains not less than 98.0 % and not more than 102.0 % of \( C_{38}H_{52}N_{6}O_{7} \cdot H_2SO_4 \) calculated on the dried basis.
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.

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) 1.0 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, respectively.
D. A 20 mg/ml solution yields Reaction A described under 2.1 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.

**pH value.** Apparent pH of a 10 mg/ml solution in carbon-dioxide-free water R and acetonitrile R (50:50, v/v), 2.0–2.5.\(^1\)

**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).\(^2\) Use the following conditions for gradient elution:

<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>70</td>
<td>30</td>
<td>Isocratic</td>
</tr>
<tr>
<td>2–10</td>
<td>70–60</td>
<td>30–40</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>10–30</td>
<td>60–50</td>
<td>40–50</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>30–45</td>
<td>50–30</td>
<td>50–70</td>
<td>Linear gradient</td>
</tr>
</tbody>
</table>

\(^1\) Value subject to confirmation.

\(^2\) An Inertsil C8 column has been found suitable.
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) 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 min.

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 0.150 g, accurately weighed, in 30 ml of methanol R and sonicate for 10 minutes. Then add 30 ml of water and titrate with sodium hydroxide (0.1 mol/l), carbonate-free, VS. Determine the end-point potentiometrically as described under 2.6 Non-aqueous titration Method A. 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$$\cdot$H$_2$SO$_4$. 

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