Saquinavir mesilate (Saquinaviri mesilas)

\[C_{38}H_{50}N_6O_{15}CH_4O_3S]\n
**Relative molecular mass.** 767.0

**Chemical name.** (2S)-N\(^1\)-(1S,2R)-1-benzyl-3-[(3S,4aS,8aS)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1H)-yl]-2-hydroxypropyl]-2-[(quinolin-2-ylcarbonyl)amino]butanediamide methanesulfonate; CAS Reg. No. 149845-06-7.

**Description.** A white or almost white powder.

**Solubility.** Very slightly soluble in water and sparingly soluble in methanol R.

**Category.** Antiretroviral (Protease Inhibitor).

**Storage.** Saquinavir mesilate should be kept in a tightly-closed container, protected from light.

**Additional information.** Saquinavir mesilate is slightly hygroscopic.

**Requirements**

**Definition.** Saquinavir mesilate contains not less than 98.5 % and not more than 101.0 % of \(C_{38}H_{50}N_6O_{15}CH_4O_3S\) calculated with reference to the dried substance.

**Manufacture.** The production method must be evaluated to determine the potential for formation of alkyl mesilates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesilates are not detectable in the final product.

**Identity tests**

• Either tests A and B or test 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 8 volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 \(\mu\)l of each of the following 2 solutions in methanol R (A) 5 mg of the test substance per mL and (B) 5 mg of saquinavir mesilate RS per mL. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool 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 silica gel R5 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 \(\mu\)l of each of the following 2 solutions in methanol R (A) 5 mg of the test substance per mL and (B) 5 mg of saquinavir mesilate RS per mL. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Dip the plate in basic potassium permanganate (~1 g/l) TS. 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. The absorption spectrum of a 10 μg/mL solution in methanol R, when observed between 220 nm and 280 nm, exhibits one maximum at about 239 nm; the specific absorbance \( A^1_{10\text{cm}} \) is 580 to 640.

C. 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 saquinavir mesilate RS or with the reference spectrum of saquinavir mesilate.

**Specific optical rotation.** Use a 5.0 mg/mL solution in methanol R and calculate with reference to the dried substance: \( [\alpha]_{20^\circ}^D = -33^\circ \) to \(-39^\circ\).

**Heavy metals.** Use 0.5 g in 30 mL of methanol R for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 2; determine the heavy metals content according to Method A; not more than 20 μg/g.

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry for 5 hours at 105 °C; it loses not more than 10 mg/g.

**Related substances.** 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 octadecylsilyl silica gel for chromatography R (5 μm).

Use the following conditions for gradient elution:

- Mobile phase A: 50 volumes of a mixture of 5 parts of acetonitrile R and 2 parts of methanol R, 15 volumes of phosphate buffer pH 3.4 and 35 volumes of purified water.
- Mobile phase B: 70 volumes of acetonitrile R, 15 volumes of phosphate buffer pH 3.4 and 15 volumes of purified water.

Prepare the phosphate buffer pH 3.4 by dissolving 4.88 g of anhydrous sodium dihydrogen phosphate R in 800 mL of purified water, adjust the pH to 3.4 by adding phosphoric acid (~105 g/l) TS and dilute to 1000 mL with purified water.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (%)</th>
<th>Mobile phase B (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-25</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>25-45</td>
<td>100 to 45</td>
<td>0 to 55</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>45-55</td>
<td>45</td>
<td>55</td>
<td>Isocratic</td>
</tr>
<tr>
<td>55-60</td>
<td>45 to 100</td>
<td>55 to 0</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>60-70</td>
<td>100</td>
<td>0</td>
<td>Isocratic re-equilibration</td>
</tr>
</tbody>
</table>

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 0.5 mg of the test substance per mL. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 0.5 μg per mL.

For the system suitability test: prepare solution (3) using 2 mL of solution (1) and 5 mL of sulfuric acid (475 g/l), 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 220 nm. Maintain the column temperature at 30 °C.

Inject 20 μl of solution (3). The test is not valid unless the resolution between the peak due to saquinavir (retention time about 21 minutes) and the peak of similar size with a relative retention of about 0.45 is not less than 14. The test is also not valid unless the resolution between two smaller peaks of similar size, eluted after the saquinavir peak and which increase during decomposition, is not less than 2.0. The relative retention of these two peaks is about 1.8 and 1.9, respectively. If necessary, adjust the amount of acetonitrile in both mobile phases A and B, or adjust the gradient programme.

Inject alternatively 20 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 twice the area of the principal peak obtained with solution (2) (0.2 %) and the area of not more than one such peak is greater than the area of the principal peak obtained with solution 2 (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than five times the area of the principal peak obtained with solution (2) (0.5 %). 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. Dissolve about 0.500 g, accurately weighed, in 70 mL of methanol R and titrate with sodium hydroxide (0.1 mol/l) VS determining the end-point potentiometrically. Perform a blank determination and make the necessary correction. Each mL of sodium hydroxide (0.1 mol/l) VS is equivalent to 76.70 mg of C_{38}H_{50}N_{6}O_{5}CH_{4}O_{3}S; calculate with reference to the dried substance.