TENOFOVIR TABLETS:
Final text for addition to The International Pharmacopoeia
(June 2010)

This monograph was adopted at the Forty-fourth WHO Expert Committee on Specifications for Pharmaceutical Preparations in October 2009 for addition to the 4th Edition of the International Pharmacopoeia

Category. Antiretroviral (Nucleotide Reverse Transcriptase Inhibitor).

Storage. Tenofovir tablets should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 300 mg.

300 mg of tenofovir disoproxil fumarate is equivalent to approximately 245 mg of tenofovir disoproxil and to approximately 136 mg of tenofovir.

Requirements

Comply with the monograph for “Tablets”.

Definition. Tenofovir tablets contain Tenofovir disoproxil fumarate. They contain not less than 90.0% and not more than 110.0% of the amount of tenofovir disoproxil fumarate (C_{19}H_{30}N_{5}O_{10}P_{4}C_{4}H_{4}O_{4}) 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 tablets. They ensure that, if tested, the tablets would comply with a water content limit of not more than 50 mg/g when determined as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.5 g of the powdered tablets.
Identity tests

- Either tests A, B and D 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 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 μl of each of the following 2 solutions. For solution (A) disperse a quantity of powdered tablets in methanol R to obtain 10 mg of Tenofovir disoproxil fumarate per ml, filter and use the filtrate and for solution (B) use 10 mg of tenofovir disoproxil fumarate RS per ml in methanol R. 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 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. Stain the plate with iodine vapour and 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. 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 50 volumes of heptane R, 30 volumes of glacial acetic acid R and 20 volumes of dichloromethane R as the mobile phase. Apply separately to the plate 5 μl of each of the following 2 solutions. For solution (A) disperse a quantity of powdered tablets in ethanol R to obtain 10 mg of Tenofovir disoproxil fumarate per ml, filter and use the filtrate and for solution (B) use 2 mg of fumaric acid R per ml in ethanol R. Develop the plate in an unsaturated tank over a path of 10 cm. 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).

One of the principal spots obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B.2 Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions described above under test B.1 but using silica gel R5 as the coating...
substance. Spray lightly with a 16 g/l solution of potassium permanganate R and examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

C. See the test described under Assay, Method A. The retention times of the principal peaks in the chromatogram obtained with the test solution are similar to those due to tenofovir disoproxil and to fumarate in the chromatogram obtained with the reference solution.

D. To a quantity of the powdered tablets containing 25 mg of tenofovir disoproxil fumarate, add 50 ml of methanol R, shake and filter. Dilute 1.0 ml of the filtrate to 20 ml with the same solvent. The absorption spectrum (1.6) of the resulting solution, when observed between 220 nm and 320 nm, exhibits one maximum at about 260 nm.

**Dissolution.** Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 900 ml of hydrochloric acid (0.1 mol/l) VS, and rotating the paddle at 50 revolutions per minute. At 45 minutes withdraw a sample of 10 ml of the medium through an in-line filter. Allow the filtered sample to cool to room temperature. Measure the absorbance (1.6) of a 1-cm layer of the resulting solution, suitably diluted if necessary, at the maximum at about 260 nm. Determine the content of tenofovir disoproxil fumarate (C$_{19}$H$_{30}$N$_5$O$_{10}$P,C$_4$H$_4$O$_4$) in the medium from the absorbance obtained from a solution of known concentration of tenofovir disoproxil fumarate RS.

For each of the six tablets tested, calculate the total amount of tenofovir disoproxil fumarate (C$_{19}$H$_{30}$N$_5$O$_{10}$P,C$_4$H$_4$O$_4$) in the medium from the results obtained. The amount in solution for each tablet is not less than 80% of the amount stated on the label. If the amount obtained for one of the six tablets is less than 80%, repeat the test using a further six tablets; the average amount for all 12 tablets tested is not less than 75% and the amount obtained for no tablet is less than 60%.

**Related substances.** Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given under Assay, Method A.

After preparation, keep the solutions at about 6°C, or use an injector with cooling. Prepare the following solutions. For solution (1) weigh and powder 20 tablets. Disperse a quantity of the powdered tablets containing 100 mg of Tenofovir disoproxil fumarate, accurately weighed, in 100 ml of mobile phase B, filter and use the filtrate. For solution (2) dilute in water R a suitable volume of solution (1) to obtain a concentration of 5 µg of
Tenofovir disoproxil fumarate per ml. For solution (3) use 0.2 mg of fumaric acid R per ml of water R.

For the system suitability test: prepare solution (4) by dispersing a quantity of the powdered tablets containing 10 mg of Tenofovir disoproxil fumarate in 10 ml of water R. Filter and use the filtrate. Heat carefully in a boiling water-bath for 20 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 260 nm.

Maintain the column temperature at 30°C.

Inject 20 µl of solution (4). The test is not valid unless the resolution between the principal peak due to tenofovir disoproxil (retention time about 40 minutes) and the peak due to the tenofovir monoester (with a relative retention of about 0.5) is at least 25.

Inject alternatively 20 µl each of solutions (1), (2) and (3). In the chromatogram obtained with solution (1), the following peak is eluted at the following relative retention, with reference to tenofovir (retention time about 40 minutes): fumarate about 0.15.

In the chromatogram obtained with solution (1), the area of any peak due to the tenofovir monoester (impurity A), is not greater than 7 times the area of the principal peak obtained with solution (2) (3.5%); the area of any other impurity peak is not greater than the area of the principal peak obtained with solution (2) (0.5%) and the areas of not more than two such peaks are greater than 0.4 times the area of the principal peak obtained with solution (2) (0.2%). The sum of the areas of all peaks, other than the principal peak, is not greater than 12 times the area of the principal peak obtained with solution (2) (6.0%). Disregard any peak corresponding to the peak obtained in the chromatogram with solution (3) and any peak with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

**Assay**

Either test A or test B may be applied.

A. 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 particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm).¹

The mobile phases for gradient elution consist of a mixture of Mobile phase A and Mobile phase B, using the following conditions.

<table>
<thead>
<tr>
<th>Mobile phase A</th>
<th>Mobile phase B</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 volumes of acetonitrile R</td>
<td>20 volumes of phosphate buffer pH 6.0</td>
</tr>
<tr>
<td>20 volumes of phosphate buffer pH 6.0</td>
<td>78 volumes of water R</td>
</tr>
</tbody>
</table>

¹ Hypersil BDS column was found suitable.
Mobile phase B: 65 volumes of acetonitrile R, 20 volumes of phosphate buffer pH 6.0 and 15 volumes of water R.

Prepare the phosphate buffer pH 6.0 by dissolving 3.50 g of potassium dihydrogen phosphate R and 1.70 g of tetrabutyl ammonium hydrogen sulfate R in 800 ml of water R, adjust the pH to 6.0 by adding sodium hydroxide (1 mol/l) VS 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>0-5</td>
<td>81</td>
<td>19</td>
<td>Isocratic</td>
</tr>
<tr>
<td>5-40</td>
<td>81 to 49</td>
<td>19 to 51</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>40-60</td>
<td>49 to 0</td>
<td>51 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>60-65</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>65-70</td>
<td>0 to 81</td>
<td>100 to 19</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>70-80</td>
<td>81</td>
<td>19</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

After preparation, keep the solutions at about 6°C, or use an injector with cooling.

Prepare the following solutions. For solution (1), weigh and powder 20 tablets. Disperse a quantity of the powdered tablets containing 100 mg of Tenofovir disoproxil fumarate, accurately weighed, in 100 ml of mobile phase B, filter and use the filtrate. Dilute 2.0 ml of the filtrate to 20.0 ml with the same solvent. For solution (2) dissolve a quantity of tenofovir disoproxil fumarate RS in the mobile phase to obtain a concentration of 0.1 mg per ml. For solution (3) use 0.2 mg of fumaric acid R per ml of water R.

For the system suitability test: prepare solution (4) by dispersing a quantity of the powdered tablets containing 10 mg of Tenofovir disoproxil fumarate in 10 ml of water R. Filter and use the filtrate. Heat carefully in a boiling water-bath for 20 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 260 nm.

Maintain the column temperature at 30°C.

Inject 20 µl of solution (4). The assay is not valid unless the resolution between the principal peak due to tenofovir disoproxil (retention time about 40 minutes) and the peak due to the tenofovir monoester (with a relative retention of about 0.5) is at least 25.

Inject alternatively 20 µl each of solutions (1), (2) and (3). In the chromatogram obtained with solution (1), the following peak is eluted at the following relative retention, with reference to tenofovir (retention time about 40 minutes): fumarate about 0.15.
Calculate the content of tenofovir disoproxil fumarate \( (C_{19}H_{30}N_{5}O_{10}P,C_{4}H_{4}O_{4}) \) in the tablets from the declared content of tenofovir disoproxil fumarate RS.

B. Disperse a quantity of the powdered tablets containing 25 mg of Tenofovir disoproxil fumarate, accurately weighed, in 50.0 ml of methanol R, filter and use the filtrate. Dilute 1.0 ml of the filtrate to 20.0 ml with the same solvent. Measure the absorbance of the resulting solution in a 1-cm layer at the maximum at about 260 nm.

Calculate the content of tenofovir disoproxil fumarate \( (C_{19}H_{30}N_{5}O_{10}P,C_{4}H_{4}O_{4}) \), using the absorptivity value of 24.0 \( (A_{\text{1cm}^{1\%}} = 240) \).

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