INTERNATIONAL PHARMACOPOEIA MONOGRAPH ON
ZIDOVUDINE AND LAMIVUDINE TABLETS

DRAFT FOR COMMENT

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ZIDOVUDINE AND LAMIVUDINE TABLETS: Draft proposal for *The International Pharmacopoeia* (May 2006)

**Category.** Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

**Storage.** Zidovudine and Lamivudine tablets should be kept in a tightly closed container, protected from light.

**Additional information.** Strengths in the current WHO Model List of Essential Drugs: 300 mg Zidovudine and 150 mg Lamivudine. The tablets may be uncoated or coated.

**Requirements**

Comply with the monograph for “Tablets”.

**Definition.** Zidovudine and Lamivudine tablets contain Zidovudine and Lamivudine. They contain not less than 90.0% and not more than 110.0% of the amounts of zidovudine (C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>) and lamivudine (C<sub>8</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>S) stated on the label.

**Identity tests**

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 90 volumes of dichloromethane R, 10 volumes of methanol R and 3 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of the following 2 solutions. For solution (A), shake a quantity of the powdered tablets equivalent to about 50 mg of Lamivudine (about 100 mg of Zidovudine) with 50 ml of methanol R, filter, and use the filtrate. For solution (B), use 2.0 mg of zidovudine RS and 1.0 mg of lamivudine RS per ml of methanol. After removing the plate from the chromatographic chamber, allow it to dry in a current of cool air, and examine the chromatogram in ultraviolet light (254 nm).

The two principal spots obtained with solution A correspond in position, appearance, and intensity with those 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 90 volumes of dichloromethane R, 10 volumes of methanol R and 3 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 10 µl of each of the following 2 solutions. For solution (A), shake a quantity of the powdered tablets equivalent to about 50 mg of Lamivudine (about 100 mg of Zidovudine) with 50 ml of methanol R, filter, and use the filtrate. For solution (B), use
2.0 mg of zidovudine RS and 1.0 mg of lamivudine RS per ml of methanol. After removing the plate from the chromatographic chamber, allow it to dry in a current of cool air. Dip the plate in dilute basic potassium permanganate (1 g/l) TS. Examine the chromatogram in daylight.

The two principal spots obtained with solution A correspond in position, appearance, and intensity with those obtained with solution B.

B. See the test described below under assay. The retention times of the principal peaks in the chromatogram obtained from solution (1) of assay are similar to those obtained from solution (2) of assay.

[Note from Secretariat: The possibility of additional tests for Identity is under investigation.]

Related Substances

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm × 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). Use a mixture of 5 volumes of methanol R and 95 volumes of buffer pH 3.8 (a 1.9 g/l solution of ammonium acetate R, previously adjusted to pH 3.8 with glacial acetic acid R) as the mobile phase A. Use 100% methanol as mobile phase B.

For solution (1), weigh and powder 20 tablets. Transfer a quantity of the powder containing about 100 mg of Zidovudine (about 50 mg of Lamivudine) into a 100 ml volumetric flask. Add about 50 ml of mobile phase A and dissolve by sonicating for 15 minutes. Dilute to volume with the same solvent and mix. Filter through a 0.45 µm filter, discarding the first few ml of the filtered solution. For solution (2), dissolve 2 mg of thymine R in 10 ml of methanol R. Then dilute 2 ml to 20 ml with the mobile phase A. For solution (3), dissolve 1 mg of zidovudine impurity B RS (3’-chloro-3’-deoxythymidine) in 10 ml of methanol R. Then dilute 2 ml to 20 ml with the mobile phase A. For solution (4), dilute 1 ml of solution (1) to 100 ml with mobile phase A.

For the system suitability test: prepare solution (5) in mobile phase A containing about 10 µg per ml of lamivudine impurity B RS, 200 µg per ml of lamivudine RS, 400 µg per ml of zidovudine RS and 10 µg per ml of zidovudine impurity B RS.

Use the following gradient elution:

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>% A</th>
<th>% B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>30 – 40 (linear gradient)</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>40 – 45 (hold)</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>45 – 55 (linear gradient)</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Hypersil ® BDS C18 is suitable.
Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 270 nm.

Inject separately 20 µl each of solutions (1), (2), (3), (4) and (5).

The test is not valid unless in the chromatogram obtained with solution (5), the resolution between lamivudine (retention time about 9 minutes) and lamivudine impurity B (relative retention time is about 0.92 with reference to lamivudine) is greater than 1.5 and the resolution between zidovudine (retention time about 42 minutes) and zidovudine impurity B (relative retention time is about 1.03 with reference to zidovudine) is greater than 2.0.

In the chromatogram obtained with solution (1), the area of any peak corresponding to the impurity with a relative retention time of about 0.40 with respect to lamivudine is not greater than 0.3 times the area of the lamivudine peak in the chromatogram obtained with solution (4) (0.3%). The area of any peak corresponding to the impurity with a relative retention time of about 0.92 with respect to lamivudine is not greater than 0.2 times the area of the lamivudine peak in the chromatogram obtained with solution (4) (0.2%). The area of any peak corresponding to thymine is not greater than the area of the peak in the chromatogram obtained with solution (2) (2% with respect to zidovudine). The area of any peak corresponding to 3’-chloro-3’-deoxythymidine is not greater than the area of the peak in the chromatogram obtained with solution (3) (1% with respect to zidovudine).

Assay

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given above under Related Substances. For solution (1), weigh and powder 20 tablets. Transfer a quantity of the powder containing about 300 mg of Zidovudine (about 150 mg of Lamivudine) into a 100 ml volumetric flask. Add about 50 ml of mobile phase A and dissolve by sonicating for 15 minutes. Dilute to volume with the same solvent and mix. Filter through a 0.45 µm filter, discarding the first few ml of the filtered solution. Dilute 5 ml of the filtrate to 50 ml with the same solvent. For solution (2), prepare a 0.3 mg/ml solution of zidovudine RS and 0.15 mg/ml of lamivudine RS in mobile phase A.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 270 nm.

Inject separately 20 µl of solution (2) in six replicate injections in the chromatographic system. The assay is not valid unless the relative standard deviation for the peak area of both zidovudine and lamivudine is less than 2.0%.

Inject alternately 20 µl each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the percentage content of zidovudine (C$_{10}$H$_{13}$N$_{5}$O$_{4}$) and lamivudine (C$_{8}$H$_{11}$N$_{3}$O$_{3}$S).