



ZIDOVUDINE, LAMIVUDINE AND ABACAVIR TABLETS

Draft proposal for *The International Pharmacopoeia* (September 2006)

This document was provided by a contracted quality control laboratory. Comments have been provided by collaborating laboratories following discussion at an informal meeting and at a consultation held in Geneva on 4 May and on 25-27 July 2006, respectively. Comments have also been provided by the WHO Secretariat.

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*International Pharmacopoeia monograph on Zidovudine, Lamivudine
and Abacavir tablets*

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ZIDOVUDINE, LAMIVUDINE AND ABACAVIR TABLETS: **Draft proposal for *The International Pharmacopoeia*** **(September 2006)**

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

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

Additional information. Strengths in the current WHO Model List of Essential Medicines: 300 mg Zidovudine , 150 mg Lamivudine and 300 mg of abacavir (as sulphate).
The tablets are usually film-coated.

Labelling. The designation on the container of zidovudine, lamivudine and abacavir tablets should state that abacavir is in the sulfate form and the quantity should be indicated in terms of the equivalent amount of abacavir.

Requirements

Comply with the monograph for "Tablets".

Definition. Zidovudine, Lamivudine and Abacavir tablets contain Zidovudine, Lamivudine and Abacavir sulfate. They contain not less than 90.0% and not more than 110.0% of the amounts of zidovudine, $C_{10}H_{13}N_5O_4$, lamivudine, $C_8H_{11}N_3O_3S$ and abacavir, $C_{14}H_{18}N_6O$ stated on the label.

Identity tests

- Either test A or B 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 90 volumes of dichloromethane R, 10 volumes of methanol R and 3 volumes of acetic acid R as the mobile phase. Apply separately to the plate 10 μ l of each of the following 4 solutions in methanol R: (A) 3 mg zidovudine RS per ml, (B) 1.5 mg lamivudine RS per ml, (C) 3.5 mg abacavir sulphate RS per ml.

For solution (D), shake a quantity of the powdered tablets containing about 15 mg of lamivudine (about 30 mg of zidovudine, and the equivalent of about 30 mg of abacavir) with 10 ml of methanol R, filter, and use the filtrate. After removing the plate from the chromatographic chamber, allow it to dry in a current of air, and examine the chromatogram in ultraviolet light (254 nm).

The three principal spots obtained with solutions A, B and C correspond in position, appearance, and intensity to those obtained with solution D.

A.2. Carry out the test as described under 1.14.1 Thin-layer chromatography, using the conditions given above under test A.1 but using silica gel R5 as the coating substance.

After removing the plate from the chromatographic chamber and allowing it to dry in a current of air, spray with vanillin/sulfuric acid TS1. Heat the plate for a few minutes at 120°C. Examine the chromatogram in daylight.

The three principal spots obtained with solution D correspond in position, appearance, and intensity to those obtained with solutions A, B and C.

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

Related Substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (15 cm × 4.6 mm) packed with particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (3.5 µm)¹. As the mobile phase use the following solutions:

Mobile phase A: ammonium acetate buffer pH 3.9

Mobile phase B: methanol R

Mobile phase C: acetonitrile R

Prepare the ammonium buffer pH 3.9 by dissolving 1.9 g of ammonium acetate in 900 ml of purified water. Adjust to pH 3.9 by addition of glacial acetic acid. Dilute to 1000 ml with water.

Use the following gradient elution:

Time (min.)	Mobile phase A	Mobile phase B	Mobile phase C	Comments
0-8	97	3	0	isocratic
8-19	97 to 76	3 to 24	0	linear gradient
19-27	76	24	0	isocratic
27-33	76 to 40	24 to 60	0	linear gradient
33-35	40	60	0	isocratic
35-35.1	40 to 0	60 to 0	0 to 100	
35.1-45	0	0	100	washing of the column
45-45.1	0 to 97	0 to 3	100 to 0	return to the initial conditions
45.1 to 60	97	3	0	isocratic re-equilibration

Prepare the following solutions in mobile phase A.

For solution (1), transfer a quantity of the powdered tablets containing about 15 mg of lamivudine (30 mg of zidovudine, and the equivalent of about 30 mg of abacavir) to a 100 ml volumetric flask, add 80 ml of mobile phase A, sonicate for 5 min and make up to volume with the same solvent. Filter through a 0.45 µm filter, discarding the first few ml of the filtered solution. For solution (2) dilute 1.0 ml of solution (1) to 100.0 ml with mobile phase A. For solution (3) dissolve a small amount (about 2 mg) each of cytosine R, uracil R, thymine R, thymidine R and zidovudine impurity B RS in 10 ml of mobile phase A. Pipette 1.0 ml of this solution into a 100 ml volumetric flask and make up to volume with solution (1).

Operate with a flow rate of 0.7 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 270 nm. Inject 10 µl of each solution.

¹ Agilent Zorbax Eclipse XDB-C18, 15 cm x 4.6 mm, 3.5 µm is suitable.

In the chromatogram obtained with solution (3) the three principal peaks elute in the order: lamivudine (retention time about 12.5 minutes), zidovudine (retention time about 25 minutes) and abacavir (retention time about 31 minutes), and the following peaks are eluted at the following retention time ratios with reference to lamivudine: lamivudine impurity E (cytosine) about 0.19, lamivudine impurity F (uracil) about 0.23, and with reference to zidovudine: zidovudine impurity C (thymine) about 0.23, thymidine about 53 and zidovudine impurity B about 1.06.

The test is not valid unless in the chromatogram obtained with solution (3) the resolution between cytosine and uracil is greater than 4, the resolution between zidovudine and zidovudine impurity B is greater than 3 and the peak to valley ratio between lamivudine and thymidine is greater than 25.

In the chromatogram obtained with solution (1) the area of any peak corresponding to thymine, when multiplied by a correction factor of 0.6, is not more than twice the area of the principal peak due to zidovudine in the chromatogram obtained with solution (2) (2% with reference to zidovudine); the area of any peak eluting between those, if any, corresponding to uracil and thymine is not more than 0.3 times the area of the principal peak due to lamivudine in the chromatogram obtained with solution (2) (0.3 % with reference to lamivudine) and the area of any peak eluting between those corresponding to lamivudine and zidovudine, with the exception of the peak, if any, corresponding to thymidine is not more than 0.3 times the area of the principal peak due to abacavir in the chromatogram obtained with solution (2) (0.3 % with reference to abacavir).

Assay

Weigh and powder 20 tablets. Carry out the test under 1.14.4 High-performance liquid Chromatography using the chromatographic conditions as described under the test for Related Substances.

Prepare the following solutions in mobile phase A. For solution (1), transfer a quantity of the powdered tablets containing about 15 mg of lamivudine (30 mg of zidovudine, and the equivalent of about 30 mg of abacavir), accurately weighed, to a 100 ml volumetric flask, add 80 ml of mobile phase A, sonicate for 5 min and make up to volume with the same solvent. Filter through a 0.45 µm filter, discarding the first few ml of the filtered solution. For solution (2), weigh 30 mg zidovudine RS, 15 mg lamivudine RS and 35 mg abacavir sulphate RS in a 100 ml volumetric flask. Add approximately 80 ml of mobile phase A, sonicate until dissolved and dilute to volume. Solution (3) contains about 0.02 mg per ml of each of cytosine R, uracil R, thymine R, thymidine R and zidovudine impurity B RS in solution (2).

Inject 10 µl of each solution.

The assay is not valid unless in the chromatogram obtained with solution (3) the resolution between cytosine and uracil is greater than 4, the resolution between zidovudine and zidovudine impurity B is greater than 3 and the peak to valley ratio between lamivudine and thymidine is greater than 25.

Measure the areas of the peak responses in the chromatograms obtained with solutions (1) and (2) and calculate the content of zidovudine ($C_{10}H_{13}N_5O_4$), lamivudine ($C_8H_{11}N_3O_3S$) and abacavir ($C_{14}H_{18}N_6O$) in the tablets, using the declared content of abacavir in abacavir sulfate RS.

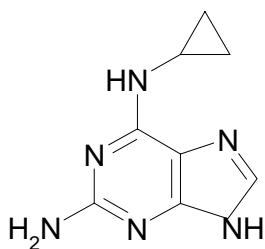
Impurities

The impurities limited by the requirements of this monograph include:

impurity C (thymine) listed in the monograph for Zidovudine and the following zidovudine-related impurity

E. [Structure and name to be provided since not listed in the drug substance monograph (as impurity B with NH₂ in place of Cl)];

impurities E (cytosine) F (uracil), G and H listed in the monograph for Lamivudine; impurity C listed in the monograph for Abacavir sulfate and the following abacavir-related impurity



G. 2-Amino-6-(cyclopropylamino)-purine

(structure and name provided since not listed in the drug substance monograph)
