Zidovudine and lamivudine tablets (Zidovudini et lamivudini compressi)

Category. Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

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

Additional information. Strength in the current WHO Model list of essential medicines: 60 mg Zidovudine and 30 mg Lamivudine, 300 mg Zidovudine and 150 mg Lamivudine. Strength in the current WHO Model list of essential medicines for children: 60 mg Zidovudine and 30 mg Lamivudine, 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 ($C_{10}H_{13}N_5O_4$) and lamivudine ($C_8H_{11}N_3O_3S$) stated on the label.

Identity tests

-Either tests A and C or tests B and 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 Chromatography*, 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 containing 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 to those obtained with solution B.

A.2 Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography using the conditions described above under test A.1, but using silica gel R5 as the coating substance Spray the plate with 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 to those obtained with solution B.

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

C. To a quantity of the powder containing 300 mg of Zidovudine (150 mg of Lamivudine) add 100 mL of acetone TS, sonicate for 10 minutes and filter. Use the filtrate for test C.1 and the residue for Test C.2 below.

C.1 Evaporate the filtrate to dryness in a water-bath. Dissolve the residue in 50 mL of water R (solution A). Dilute 1 mL of solution A to 100 mL with methanol R, mix and filter. Further dilute 5 mL of the diluted solution to 25 mL with methanol R. Measure the *absorbance (1.6)* of a 1 cm layer of the final solution at the maximum at about 266 nm against a solvent cell containing the blank. For the blank use 1 mL of water R in place of solution A and dilute in the same manner.

The absorption spectrum of the final solution, when observed between 210 nm and 300 nm, exhibits one maximum at about 266 nm (zidovudine).

C.2 Dry the residue in an oven at 100 °C for 15 minutes. Weigh about 0.1 g of the dried residue in 100 mL of water R. Dilute 3 mL of the resulting solution to 25 mL with 0.2 N sulfuric acid VS, mix and filter. For the blank use 3 mL water R diluted to 25 mL with 0.2 N sulfuric acid VS. Measure the *absorbance (1.6)* of a 1 cm layer of the final solution at the maximum at about 280 nm against a solvent cell containing the blank.

The absorption spectrum of the final solution, when observed between 210 nm and 300 nm, exhibits one maximum at about 280 nm (lamivudine).

Related substances

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using the conditions given below under Assay method A.

Prepare the following solutions. For solution (1) transfer a quantity of the powdered tablets 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 filtrate. For solution (2) dilute 1 mL of solution (1) to 100 mL with mobile phase A. For solution (3) dissolve a small amount (about 2 mg) each of cytosine R, uracil R, thymine R, lamivudine for system suitability 1 RS (containing lamivudine and lamivudine impurities A and B) and zidovudine impurity B RS in 10 mL of mobile phase A. Transfer 1.0 mL of this solution into a 100 mL volumetric flask and make up to volume with solution (1).

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

In the chromatogram obtained with solution (3) the two principal peaks elute in the order lamivudine (retention time about 9 minutes) and zidovudine (retention time about 42 minutes) and the following peaks are eluted at the following relative retention: *with reference to lamivudine*, lamivudine impurity E (cytosine) about 0.32; lamivudine impurity F (uracil) about 0.37; lamivudine impurity B about 0.9: *with reference to zidovudine*, zidovudine impurity C (thymine) about 0.13; zidovudine impurity B about 1.03. The test is not valid unless in the chromatogram obtained with solution (3) the resolution between lamivudine and lamivudine impurity B is at least 1.5 and the resolution between zidovudine and zidovudine impurity B is at least 2.0.

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 corresponding to zidovudine impurity B is not greater than the area of the principal peak due to zidovudine in the chromatogram obtained with solution (2) (1% with reference to zidovudine) and the area of any peak eluting before that due to lamivudine, with the exception of that, if any, corresponding to 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).

Assay

Carry out the test as described under *1.14.1 Chromatography*, 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 R as mobile phase B.

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), accurately weighed, into a 100 mL volumetric flask. Add about 50 mL of mobile phase A and sonicate 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 filtrate. 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.

Use the following conditions for gradient elution:

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–30	100	0	Isocratic
30–40	100 to 80	0–20	Linear gradient
40–45	80	20	Isocratic
45–55	80 to 100	20–0	Linear gradient

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 20 µl of solution (3).

In the chromatogram obtained the two principal peaks elute in the order lamivudine (retention time about 9 minutes) and zidovudine (retention time about 42 minutes) and the following peaks are eluted at the following relative retention: *with reference to lamivudine*, lamivudine impurity E (cytosine) about 0.32; lamivudine impurity F (uracil) about 0.37; lamivudine impurity B about 0.9: *with reference to zidovudine*, zidovudine impurity C (thymine) about 0.13; zidovudine impurity B about 1.03. The assay is not

valid unless in the chromatogram obtained with solution (3) the resolution between lamivudine and lamivudine impurity B is at least 1.5 and the resolution between zidovudine and zidovudine impurity B is at least 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 ($C_{10}H_{13}N_5O_4$) and lamivudine ($C_8H_{11}N_3O_3S$) in the tablets.

Impurities

The impurities limited by the requirements of this monograph include impurities B, E and F listed in the monograph for Lamivudine and impurities A to C listed in the monograph for Zidovudine.