

Zidovudine, lamivudine and nevirapine tablets (Zidovudini, lamivudini et nevirapini compressi)

Category. Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

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

Additional information. Strengths in the current WHO Model list of essential medicines:

60 mg Zidovudine, 30 mg Lamivudine and 50 mg Nevirapine
300 mg Zidovudine, 150 mg Lamivudine and 200 mg Nevirapine

Strengths in the current WHO Model list of essential medicines for children:

60 mg Zidovudine, 30 mg Lamivudine and 50 mg Nevirapine
300 mg Zidovudine, 150 mg Lamivudine and 200 mg Nevirapine

The tablets may be uncoated or coated.

Requirements

Comply with the monograph for [Tablets](#).

Definition. Zidovudine, lamivudine and nevirapine tablets contain Zidovudine, Lamivudine and Nevirapine. 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 nevirapine ($C_{15}H_{14}N_4O$) 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 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 two solutions. For solution (A) shake a quantity of the powdered tablets containing about 50 mg of Lamivudine (about 100 mg of Zidovudine and 66 mg of Nevirapine) with 50 mL of methanol R, filter and use the filtrate. For solution (B) use 2.0 mg of zidovudine RS, 1.0 mg of lamivudine RS and 1.3 mg of nevirapine RS per mL of methanol R. 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 in the chromatogram obtained with solution A correspond in position, appearance and intensity with those in the chromatogram 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 identity test A.1 but using silica gel R5 as the coating substance.

After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or in a current of cool air. Spray the plate with basic potassium permanganate (~1g/l) TS. Examine the chromatogram in daylight.

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

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 in the chromatogram obtained with solution (2).

Related substances

Prepare fresh solutions and perform the tests without delay. 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 high-purity, base-deactivated particles of silica gel the surface of which has been modified with chemically-bonded octyl- and octadecylsilanes groups (5 µm).

For the mobile phase use the following solutions:

Mobile phase A: ammonium acetate buffer pH 4.5

Mobile phase B: methanol R

Prepare the ammonium acetate buffer pH 4.5 by dissolving 7.708 g ammonium acetate R in 900 mL of water R. Adjust to pH 4.5 by addition of glacial acetic acid R. Dilute to 1000 mL with water R.

For the dissolution solvent, prepare a mixture of 15 volumes of methanol R and 85 volumes of ammonium acetate buffer pH 4.5.

Use the following gradient elution:

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–15	96	4	Isocratic
15–20	96 to 70	4–30	Linear gradient
20–25	70	30	Isocratic
25–30	70–60	30–40	Linear gradient
30–35	60	40	Isocratic
35–45	60–20	40–80	Linear gradient
45–52	20	80	Isocratic
52–60	20–96	80–4	Return to initial composition

Prepare the following solutions. For solution (1) transfer a quantity of the powdered tablets containing 50 mg of Lamivudine (100 mg of Zidovudine and 66 mg of Nevirapine) to a 100 mL volumetric flask, add 15 mL of methanol R, sonicate for 15 minutes and make up to volume with mobile phase A. Centrifuge and filter through a 0.45 µm filter, discarding the first few mL of the filtrate. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL with the dissolution solvent. For solution (3) transfer 1 mg each of thymidine R, zidovudine impurity B RS and nevirapine impurity B RS to a 10 mL volumetric flask, add 5 mL of the dissolution solvent, sonicate to dissolve and make up to volume with the same solvent. Transfer 3.0 mL of the resulting solution into a 50 mL volumetric flask and make up to volume with solution (1).

Operate with a flow rate of 1.3 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) and of the dissolution solvent.

In the chromatogram obtained with solution (3) the three principal peaks elute in the order: lamivudine (retention time about 15 minutes); zidovudine (retention time about 25 minutes) and nevirapine (retention time about 35 minutes) and the following impurity peaks, if present, are eluted at the following relative retention: *with reference to lamivudine*, lamivudine impurity E (cytosine) about 0.18; *with reference to zidovudine*, zidovudine impurity C (thymine) about 0.25; thymidine about 0.52; zidovudine impurity B about 1.04; *with reference to nevirapine*, nevirapine impurity B about 0.95.

The test is not valid unless in the chromatogram obtained with solution (3) the resolution between the peaks due to thymidine and lamivudine is greater than 4.5, the resolution between the peaks due to zidovudine and zidovudine impurity B is greater than 3.5 and the resolution between the peaks due to nevirapine impurity B and nevirapine is greater than 5.

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); the area of any peak corresponding to nevirapine impurity B, when multiplied by a correction factor of 0.77, is not more than 0.2 times the area of the principal peak due to nevirapine in the chromatogram obtained with solution (2) (0.2% with reference to nevirapine); the area of any peak eluting after the peak due to nevirapine, excluding the peaks due to the blank, is not greater than 0.2 times the area of the principal peak due to nevirapine in the chromatogram obtained with solution (2) (0.2% with reference to nevirapine); the area of any peak eluting before the peak due to lamivudine, with the exception of the peaks, if any, corresponding to thymine and thymidine, is not greater 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 high-purity, base-deactivated particles of silica gel the surface of which has been modified with chemically-bonded octyl- and octadecylsilanes groups (5 µm).

For the mobile phase use the following solutions:

Mobile phase A: ammonium acetate buffer pH 4.5

Mobile phase B: methanol R.

Prepare the ammonium acetate buffer pH 4.5 by dissolving 7.708 g ammonium acetate R in 900 mL of water R. Adjust to pH 4.5 by addition of glacial acetic acid R. Dilute to 1000 mL with water R.

For the dissolution solvent, prepare a mixture of 15 volumes of methanol R and 85 volumes of ammonium acetate buffer pH 4.5.

Use the following gradient elution:

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0–15	96	4	Isocratic
15–20	96–70	4–30	Linear gradient
20–25	70	30	Isocratic
25–30	70–60	30–40	Linear gradient
30–35	60	40	Isocratic
35–38	60–96	40–4	Return to initial composition

Weigh and powder 20 tablets. For solution (1) transfer a quantity of the powdered tablets containing 150 mg of Lamivudine (300 mg of Zidovudine and about 200 mg of Nevirapine) to a 200 mL volumetric flask, add 30 mL of methanol R, sonicate for 15 minutes and make up to volume with mobile phase A. Centrifuge a portion of the solution and dilute 5.0 mL of the supernatant liquid to 100.0 mL with the dissolution solvent. Filter through a 0.45 µm filter, discarding the first few mL of the filtrate. For solution (2) prepare a solution containing 0.0375 mg/mL of lamivudine RS, 0.075 mg/mL of zidovudine RS and 0.05 mg/mL of nevirapine RS in the dissolution solvent. For solution (3) transfer 1 mg each of thymidine R, zidovudine impurity B RS and nevirapine impurity B RS to a 10 mL volumetric flask, add 5 mL of the dissolution solvent, sonicate to dissolve and make up to volume with the same solvent. Transfer 3.0 mL of this solution into a 50 mL volumetric flask and make up to volume with solution (1).

Operate with a flow rate of 1.3 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) and (3).

The assay is not valid unless in the chromatogram obtained with solution (3) the resolution between the peaks due to thymidine and lamivudine is greater than 4.5, the resolution between the peaks due to zidovudine and zidovudine impurity B is greater than 3.5 and the resolution between the peaks due to nevirapine impurity B and nevirapine is greater than 5.0.

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 nevirapine ($C_{15}H_{14}N_4O$) in the tablets.

Impurities

The impurities limited by the requirements of this monograph include:

impurities B and C (thymine) listed in the monograph for *Zidovudine*;

impurities A and E (cytosine) listed in the monograph for *Lamivudine*;

impurities A, B and C listed in the monograph for *Nevirapine*.