Zidovudine capsules (Zidovudini capsulae)

Category. Antiretroviral drug (Nucleoside Reverse Transcriptase Inhibitor)

Storage. Zidovudine capsules should be kept in a tightly closed container, protected from light.

Additional information. Strengths in the WHO Model list of essential medicines: 100 mg, 250 mg. Strengths in the WHO Model list of essential medicines for children: 100 mg, 250 mg.

Requirements

Comply with the monograph for *Capsules*.

Definition. Zidovudine capsules contain Zidovudine. They contain not less than 90.0% and not more than 110.0% of the amount of zidovudine ($C_{10}H_{13}N_5O_4$) 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 5 µl of each of the following two solutions. For solution (A) sonicate a quantity of the contents of the capsules in methanol R to produce a solution containing 1 mg/mL of Zidovudine. Filter and use the clear filtrate. For solution (B) prepare a 1 mg/mL solution of zidovudine RS in methanol R. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or in a current of cool air and examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance and intensity to that 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. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or in a current of cool air. Dip the plate in basic potassium permanganate (~1 g/l) TS. Examine the chromatogram in daylight.

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

B. See the test described under Assay method A. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

C. The *absorption spectrum (1.6)* of the final solution prepared for Assay method B, when observed between 210 nm and 300 nm, exhibits one maximum at about 267 nm.

Related substances

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

Prepare the following solutions. For solution (1) transfer a quantity of the contents of the capsules containing 100 mg of Zidovudine into a 100 mL volumetric flask. Add about 80 mL of the mobile phase and sonicate for 20 minutes. Dilute to volume with the same solvent and mix. Dilute 5.0 mL of the resulting solution to 50.0 mL with the same solvent, mix and filter. For solution (2) dilute 1.0 mL of solution (1) to 200.0 mL with the mobile phase. For solution (3) dissolve a small amount (about 2 mg) each of thymine R (impurity C) and zidovudine impurity B RS in 10 mL of methanol R. Transfer 1.0 mL of this solution into a 100 mL volumetric flask and make up to volume with solution (1).

Inject separately 10 µl each of solutions (1), (2) and (3). In the chromatogram obtained with solution (3) the following peaks are eluted at the following relative retention with reference to zidovudine (retention time about 12 to 13 minutes): impurity C (thymine) about 0.3; impurity B about 1.2. The test is not valid unless in the chromatogram obtained with solution (3) the resolution between the peaks due to zidovudine and impurity C is at least 5.0, the resolution between the peaks due to zidovudine and impurity B is at least 2.0 and the tailing factor of zidovudine is less than 2.0.

In the chromatogram obtained with solution (1) the area of any peak corresponding to impurity C, when multiplied by a correction

factor of 0.6, is not greater than 6 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%). The area of any other peak, apart from the principal peak, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). The sum of the corrected area of any peak corresponding to impurity C and of the areas of all other peaks, apart from the principal peak, is not greater than 8 times the area of the principal peak in the chromatogram obtained with solution (2) (4.0%). Disregard 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 method A or B may be applied.

A. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with octadecysilyl groups (5 µm). As the mobile phase use a mixture of 20 volumes of methanol R and 80 volumes of water R.

Prepare the following solutions. For solution (1) mix the contents of 20 capsules and transfer a quantity containing about 100 mg of Zidovudine, accurately weighed, into a 100 mL volumetric flask. Add about 80 mL of the mobile phase and sonicate for 20 minutes. Dilute to volume with the same solvent and mix. Dilute 5.0 mL of the resulting solution to 50.0 mL with the same solvent, mix and filter. For solution (2) prepare a 0.1 mg/mL solution of zidovudine RS in the mobile phase. For solution (3) dissolve a small amount (about 2 mg) each of thymine R (impurity C) and zidovudine impurity B RS in 10.0 mL methanol R. Pipette 1.0 mL of this solution into a 100 mL volumetric flask and dilute to volume with solution (2).

Operate with a flow rate of 1.2 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 265 nm.

Inject separately 10 µl of each of solutions (1), (2) and (3). In the chromatogram obtained with solution (3) the following peaks are eluted at the following relative retention with reference to zidovudine (retention time about 12 to 13 minutes): impurity C (thymine) about 0.3; impurity B about 1.2. The assay is not valid unless the resolution between the peaks due to zidovudine and impurity C is at least 5.0, the resolution between the peaks due to zidovudine and impurity B is at least 2.0 and the tailing factor of zidovudine is less than 2.0.

Measure the areas of the peak responses corresponding to zidovudine obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of zidovudine ($C_{10}H_{13}N_5O_4$).

B. Mix the contents of 20 capsules and transfer a quantity containing about 100 mg of Zidovudine, accurately weighed, into a 100 mL volumetric flask. Add about 80 mL of a mixture consisting of 20 volumes of methanol R and 80 volumes of water R and sonicate for 20 minutes. Dilute to volume with the same solvent and mix. Dilute 5.0 mL of the resulting solution to 25.0 mL with the same solvent, mix and filter. Further dilute 5.0 mL of the diluted solution to 50.0 mL with sulfuric acid (0.1 mol/l) VS and mix. For the blank use 5.0 mL of the mixture consisting of 20 volumes of methanol R and 80 volumes of water R diluted to 50.0 mL with sulfuric acid (0.1 mol/l) VS.

Measure the *absorbance (1.6)* of a 1 cm layer of the final solution at the maximum at about 267 nm against a solvent cell containing the blank. Calculate the content of zidovudine ($C_{10}H_{13}N_5O_4$) in the capsules using the absorptivity value of 38.0 ($A^{1\%}_{1cm}$ = 380).

Impurities

The impurities limited by the requirements of this monograph include impurities A to C listed in the monograph for Zidovudine.