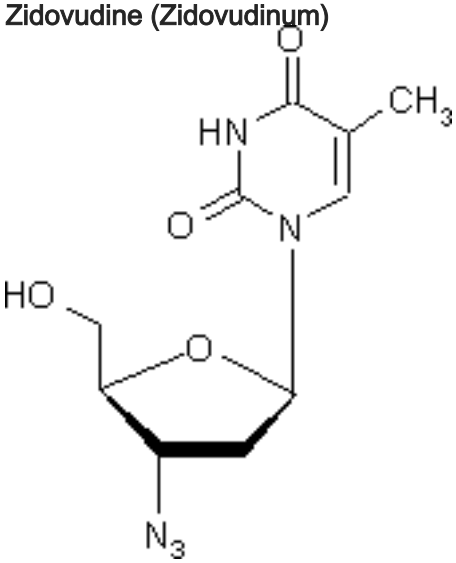
Zidovudine (Zidovudinum)



$C_{10}H_{13}N_5O_4$

Relative molecular mass. 267.2

Chemical name. 1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)tetrahydrofuran-2-yl]-5-methyl-pyrimidine-2,4(1*H*,3*H*)-dione; 1-(3-azido-2,3-dideoxy-β-d-*erythro*-pentofuranosyl)-5-methyl-pyrimidine-2,4(1*H*,3*H*)-dione; 3'-azido-3'-deoxythimidine (AZT); CAS Reg. No. 30516-87-1.

Description. A white or brownish powder.

Solubility. Soluble in ethanol (~750 g/l) TS, sparingly soluble in water.

Category. Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

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

Additional information. Zidovudine may exhibit polymorphism.

Requirements

Definition. Zidovudine contains not less than 97.0% and not more than 103.0% of $C_{10}H_{13}N_5O_4$, calculated with reference to the dried substance.

Identity tests

• Either tests A, B and D or tests C and D 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 2 solutions in methanol R containing (A) 1 mg of the test substance per mL and (B) 1 mg of zidovudine RS per mL. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. 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. Dip the plate in dilute 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. The absorption spectrum (1.6) of the final solution prepared for the Assay, when observed between 210 nm and 300 nm,

exhibits one maximum at about 267 nm; the specific absorbance ($A^{1\%}_{1cm}$) is between 361 to 399.

C. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from zidovudine RS or with the *reference spectrum* of zidovudine.

If the spectra thus obtained are not concordant, repeat the test using the residues obtained by separately dissolving the test substance and zidovudine RS in a small amount of ethanol (~750 g/l) TS and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from zidovudine RS.

D. Determine the specific optical rotation (1.4) using a 10 mg/mL solution in ethanol (~750 g/l) TS and calculate with reference to the dried substance; $[\alpha]_D^{25\,°C}$ = +60° to +63°.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 4. Determine the heavy metals content according to Method A; not more than 20 µg/g.

Sulfated ash (*2.3*). Not more than 2.5 mg/g.

Loss on drying. Dry for 3 hours at 105°C; it loses not more than 10 mg/g.

Related substances

A. Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography, using silica gel R4 as the coating substance and a mixture of 90 volumes of dichloromethane R and 10 volumes of methanol R as the mobile phase. Apply separately to the plate 10µl of each of the 2 solutions in methanol R containing (A) a mixture of 0.1 mg per mL each of zidovudine RS and triphenylmethanol R and (B) 20 mg per mL of the test substance. Develop over a path of 12 cm. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air. Examine the chromatogram in ultraviolet light (254 nm).

Any spot in the chromatogram obtained with solution B, other than the principal spot, is not more intense and not larger than the principal spot in the chromatogram obtained with solution A (0.5%).

Spray the plate with a mixture of 0.5 g of carbazole R in 95 volumes of ethanol (~750 g/l) TS and 5 volumes of sulfuric acid R, heat for 10 minutes at 120°C.

In the chromatogram obtained with solution B any spot corresponding to triphenylmethanol R (R_f value about 1.6 with reference to zidovudine) is not more intense than the corresponding spot in the chromatogram obtained with solution A (0.5%), any other spot, other than the principal spot, is not more intense and not larger than the principal spot corresponding to zidovudine obtained with solution A (0.5%).

B. 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 by chemically bonded octadecylsilyl 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 in the mobile phase. Solution (1) is a 1 mg/mL solution of the test substance. For solution (2) dilute 1.0 mL of solution (1) to 200 mL. For solution (3) dissolve about 5 mg of zidovudine RS in solution (TSB), prepared as described below, and dilute to 10 mL with the same solution. For solution (TSB) dissolve 1 mg of each of thymine R, stavudine RS and zidovudine impurity B RS in the mobile phase and dilute to 10 mL with the mobile phase; dilute 1 mL of the resulting solution to 10 mL with the same solvent.

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 each of solutions (1), (2) and (3). Record the chromatogram for 4 times the retention time of zidovudine in solution (1). Use the chromatogram supplied with zidovudine impurity B RS and the chromatogram obtained with solution (3) to identify the peaks due to impurities A, B and C. The impurity peaks are eluted at the following relative retention times with reference to zidovudine (retention time about 12 to 13 minutes): impurity C (thymine) about 0.3; impurity A (stavudine) about 0.4; impurity B about 1.2. The test is not valid unless the resolution factor between the peaks due to zidovudine and impurity B is at least 2.

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 four times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%),

- the area of any peak corresponding to impurity B, is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (1.0%),

- the area of any peak corresponding to impurity A, when multiplied by a correction factor of 0.9, is not greater

than the area of the principal peak in the chromatogram obtained with solution (2) (0.5%),

- the area of any other peak, apart from the principal peak, is not greater than the area of the peak in the chromatogram obtained with solution (2) (0.5%) and

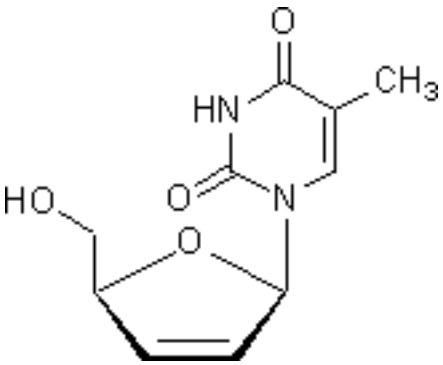
- the sum of the areas (corrected, where necessary) of all the peaks, other than the principal peak, is not greater than 6 times the area of the principal peak in the chromatogram obtained with solution (2) (3.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Assay. Transfer about 0.05 g, accurately weighed, into a 250-mL volumetric flask. Add about 200 mL of a mixture of 20 volumes of methanol R and 80 volumes of water R and dissolve by using an ultrasonic bath. Dilute to volume with the same solvent and mix. Dilute 5 mL of this solution to 50 mL with sulfuric acid (0.1 mol/l) VS and mix. For the blank, use 5 mL of a mixture consisting of 20 volumes of methanol R and 80 volumes of water R diluted to 50 mL with sulfuric acid (0.1 mol/l) VS.

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

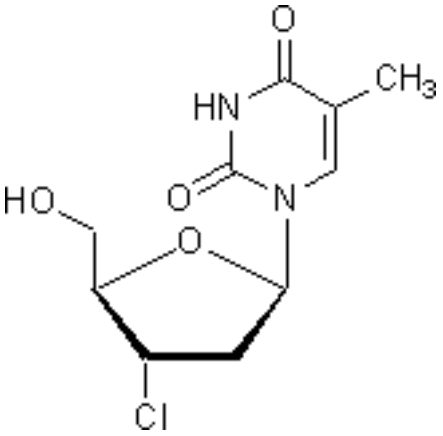
Impurities

A.



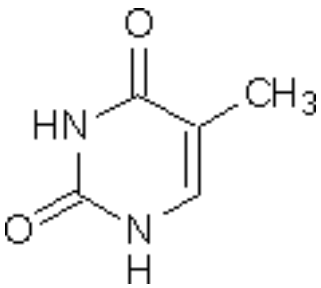
1-[(2*R*,5*S*)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (stavudine),

B.



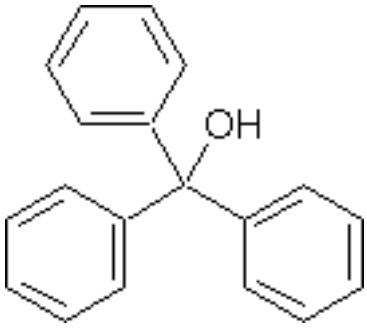
1-(3-chloro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (3'-chloro-3'-deoxythimidine),

C.



5-methylpyrimidine-2,4(1*H*,3*H*)-dione (thymine),

D.



triphenylmethanol.