World Health Organization

ZIDOVUDINE

(ZIDOVUDINUM)

Draft proposal for revision in *The International Pharmacopoeia*

(08 December 2022)

DRAFT FOR DISCUSSION

Please send any comments you may have on this draft working document to **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (email: schmidth@who.int), with a copy to **Mrs Bezawit Kibret** (kibretb@who.int; nsp@who.int) before **17 February 2023**.

Our working documents are sent out electronically and they will be placed on the WHO Medicines website (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/pharmaceuticals/current-projects) for comments under the "*Working documents in public consultation*" link. If you wish to receive our draft guidelines, please send your e-mail address to nsp@who.int and your name will be added to our electronic mailing list.

SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/22.918

ZIDOVUDINE (Zidovudinum)

Description	Date
Drafting of the revised monograph based on information received from manufacturers and information available in public domain	November 2022
Draft revision sent out for public consultation.	December 2022 – February 2023
Further follow-up action as required	

*[**Note from the Secretariat.** The revised monograph on Zidovudine is proposed for inclusion in* The International Pharmacopoeia. *The revision is based on information received from manufacturers and current research literature available in the public domain.*

The revised monograph is expected to play an important role in ensuring access to safe, effective and quality assured zidovudine containing medicines. Manufacturers, regulatory authorities, procurement agencies and other stakeholders are therefore invited to provide their feedback to the Secretariat of The International Pharmacopoeia.

In particular, comments are sought as to whether the impurities listed in the section "Impurities" are synthesis-related impurities, degradation products are both.

If not already done, manufacturers are also invited to submit information and samples of their products. Manufacturers will thereby help to ensure that the proposed monograph adequately controls the quality of the product(s) they manufacture.

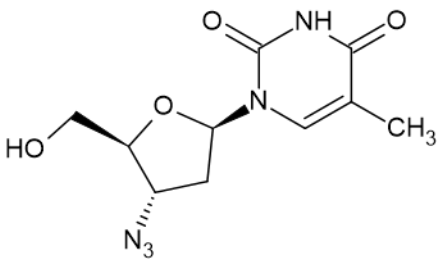
For further information, please contact Dr Herbert Schmidt at schmidth@who.int .]

ZIDOVUDINE (ZIDOVUDINUM)

Molecular formula. $C_{10}H_{13}N_5O_4$

Relative molecular mass. 267.2

Graphic formula.



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. 30516-87-1.

Description. A white or slightly brownish powder.

Solubility. Sparingly soluble in water R, soluble in ethanol (~ 750 g/l) TS practically insoluble in n-heptane R.

Category. Antiretroviral (Nucleoside reverse transcriptase inhibitor).

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

Additional information. Zidovudine is hygroscopic and exhibits polymorphism .

Requirements

Manufacture. The production method is validated to demonstrate that the presence of potential genotoxic impurities (such as the methyl ester of methane sulfonic acid) and /or potential nitrosamine impurities are adequately controlled in the final product.

Definition. Zidovudine contains not less than 97.0% and not more than 102.0% of $C_{10}H_{13}N_5O_4$, calculated with reference to the dried substance.

Identity tests

- Either tests A or tests D and F or any two of tests B, C or E together with test F may be applied.

A. Carry out the test 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.

B. Carry out test B.1 or, where UV detection is not available, test B.2.

B.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.

B.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.

C. Transfer about 0.050 g 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 5.0 mL of this solution to 50.0 mL with sulfuric acid (0.1 mol/l) TS 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) TS. The absorption spectrum (*1.6*) of this solution when observed between 210 nm and 300 nm, exhibits one maximum at about 267 nm; the specific absorbance ($A_{1cm}^{1\%}$) ranges between 361 to 399.

D. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using the conditions given under "Assay", but using, as the detector, a diode array detector to record the UV spectrum of the principal peak in each chromatogram in the range of 200 nm to 400 nm. The retention time and the UV spectrum of the principal peak in the chromatogram obtained with solution (2) correspond to the retention time and the UV spectrum of the peak due to zidovudine in the chromatogram obtained with solution (6).

E. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using the conditions given under "Assay". The retention time of the principal peak in the chromatogram obtained with solution (2)

correspond to the retention time of the peak due to zidovudine in the chromatogram obtained with solution (6).

F. 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;$[\alpha]_D^{25}$= +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*, High-performance liquid chromatography, using a stainless steel column (4.6 mm x 25 cm) packed with particles of silica gel, the surface of which has been modified with base-deactivated end-capped octadecylsilyl silica gel (5 µm)[1].

Use the following conditions for gradient elution:

- mobile phase A: 2 g/L solution of ammonium acetate R adjusted to pH 6.8 with acetic acid (~120 g/L) TS.
- mobile phase B: acetonitrile R.

Use the following conditions for gradient elution:

Time (Min)	Mobile phase A (% v/v)	Mobile Phase B (% v/v)	Comments

[1] A Phenomenex Luna 5µm C18(2) 100 Å has been found to be suitable

0 – 3	95	5	Isocratic
3 – 18	95 to 85	5 to 15	Linear gradient
18 – 28	85 to 30	15 to 70	Linear gradient
28 – 43	30	70	Isocratic

Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 265 nm.

Prepare the following solvent mixtures:

Solvent mixture A: Mix 4 volumes of acetonitrile R, 20 volumes of methanol R and 76 volumes of a 2 g/L solution of ammonium acetate R previously adjusted to pH 6.8 with acetic acid (~120 g/L) TS.

Solvent mixture B: Mix 4 volumes of acetonitrile R, 40 volumes of methanol R and 56 volumes of a 2 g/L solution of ammonium acetate R previously adjusted to pH 6.8 with acetic acid (~120 g/L) TS.

Prepare the following solutions.

For solution (1), dissolve 20.0 mg of the test substance in solvent mixture A and dilute to 20.0 mL with solvent mixture A.

For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL with solvent mixture A.

For solution (3), dilute 1.0 mL of solution (2) to 20.0 mL with solvent mixture A.

For solution (4), dissolve 2 mg of zidovudine impurity B RS in solvent mixture A and dilute to 50 mL with solvent mixture A. Dilute 1 mL of this solution to 20 mL with solvent mixture A.

For solution (5), dissolve 5 mg of zidovudine for system suitability A RS (containing zidovudine and impurity G) in solution (4) and dilute to 5 mL with solution (4).

For solution (6), dissolve 1 mg of zidovudine impurity D RS in solvent mixture B and dilute to 50 mL with solvent mixture B. Dilute 5.0 mL of this solution to 10 mL with solvent mixture B.

Inject 20 µL each of solutions (1), (2), (3), (5) and (6).

Use the chromatogram supplied with zidovudine for system suitability A CRS and the chromatogram obtained with solution (5) to identify the peaks due to impurities B and G. Use the chromatogram obtained with solution (6) to identify the peak due to impurity D.

The following peaks are eluted at the following relative retention with reference to the peak of zidovudine (retention time about 16 min): impurity B about 1.05; impurity G about 1.5; impurity D about 2.0.

The test is not valid unless in the chromatogram obtained with solution (5) the resolution factor between the peak due to zidovudine and the peak due to impurity B is at least 2.0. Also, the test is not valid unless in the chromatogram obtained with solution (3) the signal-to-noise ratio of the peak due to zidovudine is at least 10.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A is not greater than 0.3 times the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (0.3 %);

- the area of any peak corresponding to impurity B is not greater than the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (1.0 %);
- the area of any peak corresponding to impurity C is not greater than the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (1.0 %);
- the area of any peak corresponding to impurity G is not greater than 0.2 times the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (0.2 %);
- the area of any other impurity peak is not greater than 0.1 times the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (0.10 %).
- Determine the sum of the areas of all impurity peaks disregarding any peak with an area less than 0.5 times the area of due to zidovudine in the chromatogram obtained with solution (5). Calculate the percentage content of all impurities using the area of the peak due to zidovudine in the chromatogram obtained with solution (2) as a reference.

B. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using a stainless steel column (4.6 mm x 15 cm) packed with particles of silica gel, the surface of which has been modified with base-deactivated end-capped octadecylsilyl silica gel (5 µm)[2].

As the mobile phase use a filtered and degassed mixture of 30 volumes water R and 70 volumes acetonitrile for chromatography R.

Operate with a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 210 nm.

[2] A Phenomenex Luna 5µm C18(2) 100 Å has been found to be suitable

Prepare the following solutions. For solution (1), dissolve 0.5 g of the test substance in 10 mL of acetonitrile R and dilute to 100.0 mL with mobile phase. For solution (2), dissolve 5.0 mg of zidovudine impurity D CRS in acetonitrile R and dilute to 10.0 mL with acetonitrile R. For solution (3), dilute 1.0 mL of solution (2) to 100.0 mL with mobile phase. For solution (4), dilute 5.0 mL of solution (3) to 10.0 mL with mobile phase. For solution (5) dilute to 1.0 mL of solution (2) to 50 mL with solution (1).

Inject 20 µL each of solutions (1), (3), (4) and (5). Run the chromatogram for 10 times the retention time of zidovudine.

Use the chromatogram obtained with solution (3) to identify the peak due to impurity D.

The peak due to impurity D is eluted at a relative retention with reference to the peak of zidovudine of about 2.5.

The test is not valid unless in the chromatogram obtained with solution (5) the resolution factor between the peak due to zidovudine and the peak due to impurity D is at least 5.0. Also, the test is not valid unless in the chromatogram obtained with solution (5) the signal-to-noise ratio of the peak due to impurity D is at least 10..

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity D is not greater than 2.5 times the area of the peak due to impurity D in the chromatogram obtained with solution (3) (0.25 %);
- the area of any impurity peak eluting after impurity D is not greater than the area of the peak due to impurity D in the chromatogram obtained with solution (3) (0.10 %);

- Determine the sum of the area of any peak corresponding to impurity D and the areas of all impurity peaks eluting after impurity D. Disregard any peak with an area of less than the area of the peak due to impurity D in the chromatogram obtained with solution (4). Calculate the percentage content of impurity D and all impurities eluting after impurity D using the area of the peak due to impurity D in the chromatogram obtained with solution (3) as a reference.
- The sum of the impurities determined with method A and B is not greater than 3.0%.

Assay

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using the conditions given under "Related substances test A".

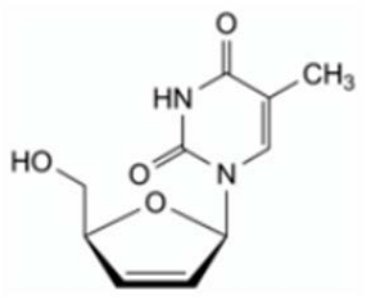
Prepare the following solutions in solvent mixture A:

For solution (1), dissolve 40.0 mg of the test substance and dilute to 200.0 mL. For solution (2), dissolve 40.0 mg of zidovudine RS and dilute to 200.0 mL.

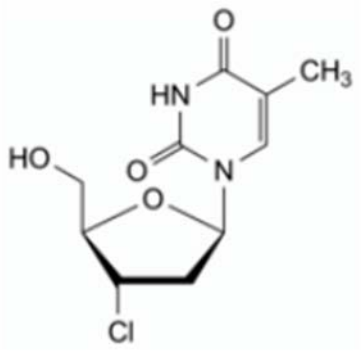
Inject 20 µL of solutions (1) and (2).

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

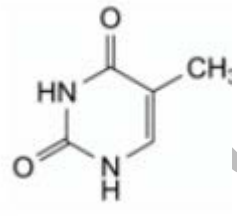
Impurities



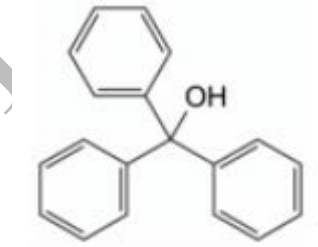
A. 3′-Deoxy-2′,3′-didehydrothymidine (Stavudine)



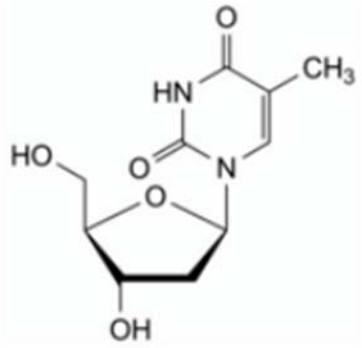
B. 3′-Chloro-3′-deoxythymidine



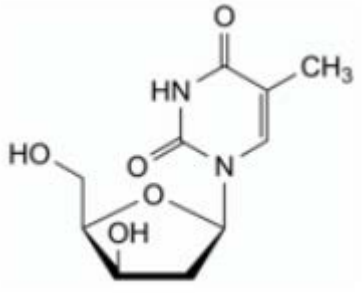
C. 5-Methylpyrimidine-2,4(1*H*,3*H*)-dione (thymine)



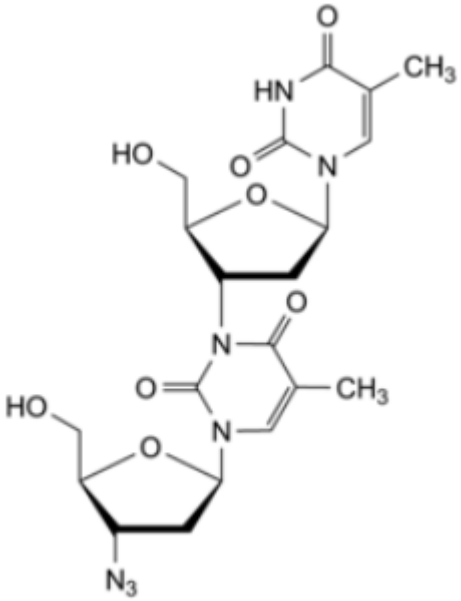
D. Triphenylmethanol



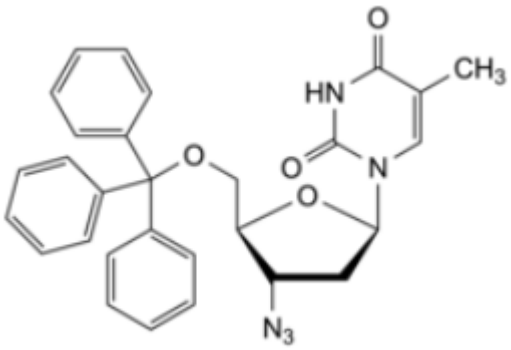
E. Thymidine



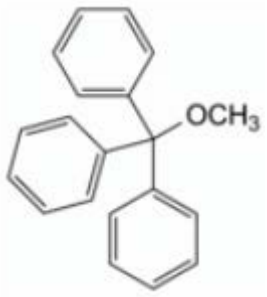
F. 3′-*epi*-Thymidine



G. 3′-(3′-Azido-3′-deoxythymidin-3-yl)-3′-deoxythymidine, 1-{3-[3-(3-Azido-2,3-dideoxy-β-d-pentofuranosyl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1-yl]-2,3-dideoxy-β-d-pentofuranosyl]-5-methylpyrimidine-2,4-dione.



J. 3′-Azido-3′-deoxy-5′-O-(triphenylmethyl)thymidine (trityl-zidovudine)



K. 1,1′,1″-(Methoxymethanetriyl)tribenzene (methyl trityl ether)

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