World Health Organization

ZIDOVUDINE

(ZIDOVUDINUM)

Draft proposal for revision in *The International Pharmacopoeia*

(30 July 2024)

DRAFT FOR DISCUSSION

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For any technical questions, you may contact **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (schmidth@who.int), with a copy to Ms Bezawit Kibret (kibretb@who.int)
Comments should be submitted through the online platform by **24 September 2024**. Please note that only comments received by this deadline will be considered for the preparation of this document.
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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
Discussion at the Consultation on Quality Control and Pharmacopoeial Specifications for Medicines	May 2024
Draft revision sent out for public consultation.	August 2024 – September 2024
Presentation at the 58th meeting of the Expert Committee on Specifications for Pharmaceutical Preparations	October 2024
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 found in current research literature available in the public domain.*

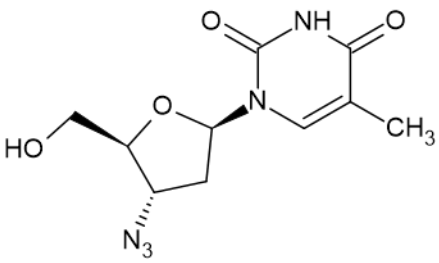
The revised monograph is expected to play an important role in ensuring access to safe, effective and quality assured zidovudine containing medicines. Manufacturers, regulatory authorities, procurement agencies and other stakeholders are therefore invited to provide their feedback to the Secretariat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

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 Registry Number. 30516-87-1.

Description. A white or slightly brownish powder.

Solubility. Sparingly soluble in water R, soluble in ethanol (~ 750 g/L) TS and 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) is 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 and F or tests B and F or any two of tests C, D 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 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 (1) correspond to the retention time and the UV spectrum of the peak due to zidovudine in the chromatogram obtained with solution (2).

C. 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 (1) correspond to the retention time of the peak due to zidovudine in the chromatogram obtained with solution (2).

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

D.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 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).

D.2 Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography, using the conditions described above under test D.1 but using silica gel R5 as the coating substance and dipping 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)

E. Transfer 0.05 g of the test substance, which has previously been dried for 3 hours at 105 ˚C, 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 using sonication. 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.

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; $[\alpha]_D^{25}$ = +60.5 to +63.0.

Colour of solution. Dissolve 0.5 g of the test substance in 50 mL of water R by heating, if necessary. This solution not more intensely coloured than reference solution BY_5, when compared as described under *1.11.2 Degree of coloration of liquids*, Method II.

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.

[1] A Phenomenex Luna 5µm C18(2) 100 Å or a Spherisorb ODS-2 column have been found suitable.

- 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**
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
43-44	95	5	Return to initial composition
44-54	95	5	Re-equilibration

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 the same solvent. 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 RS 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 L about 0.26; impurity C about 0.28; impurity J about 0.30; impurity A about 0.54; impurity M about 0.61; impurity H about 0.96; impurity B about 1.05; impurity G about 1.44; impurity D about 1.98.

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 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, excluding any peak due to impurity D and any peak eluting after this impurity and disregarding any peak with an area less than the area due to zidovudine in the chromatogram obtained with solution (3)(0.05%). 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 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)[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 1.7 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 210 nm.

[2] A Hypersil BDS-C18 250 x 4.6 mm (5µm) 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 RS 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 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 following peaks are eluted at the following relative retention with reference to the peak of impurity D (retention time about 4 min): impurity J about 2.9; impurity K about 6.2.

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 (4) the signal-to-noise ratio of the peak due to impurity D is at least 20.

Measure the areas of the peaks due to impurity D and all impurities eluting after impurity D, obtained in the chromatograms of solutions (1), and calculate their percentage contents using the area of the peak due to impurity D in the

chromatogram obtained with solution (3) and its concentration. The percentage content of each impurity is not more than 0.10%.

Calculate the sum of the percentage contents of impurity D and all impurities eluting after impurity D, disregarding any peak with a percentage content of less than 0.05%.

The sum of the percentage contents of the impurities determined with method A and B is not greater than 1.0%.

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".

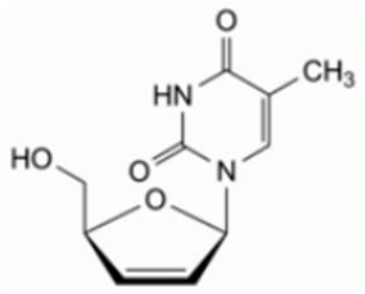
Prepare the following solutions in solvent mixture A:

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.

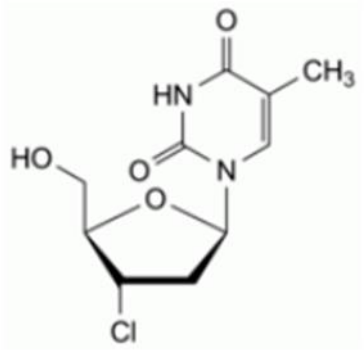
Inject 20 µL of solutions (1) and (2).

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 RS.

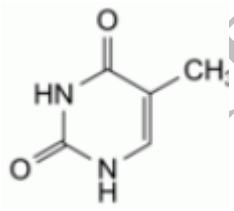
Impurities



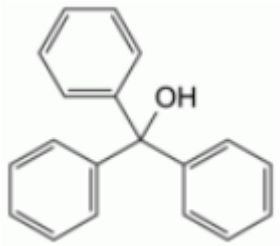
A. 3′-Deoxy-2′,3′-didehydrothymidine; 1-[(2*R*,5*S*)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione, (stavudine) (synthesis related impurity),



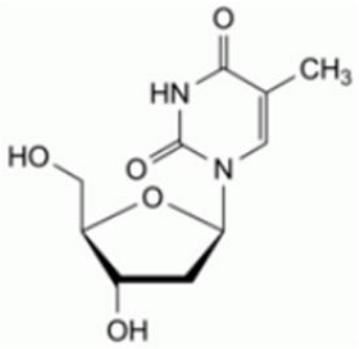
B. 3′-Chloro-3′-deoxythymidine (synthesis related impurity),



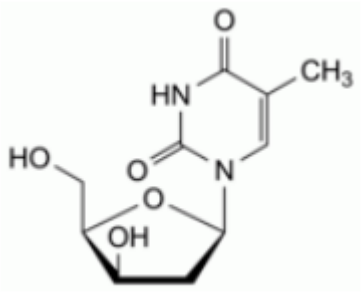
C. 5-Methylpyrimidine-2,4(1*H*,3*H*)-dione (thymine) (synthesis or degradation product),



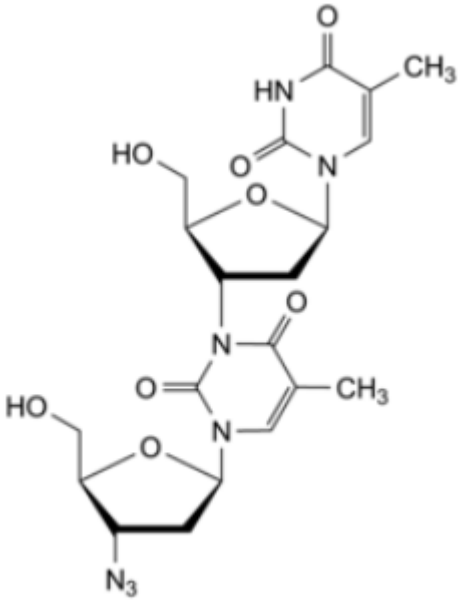
D. Triphenylmethanol (synthesis related impurity),



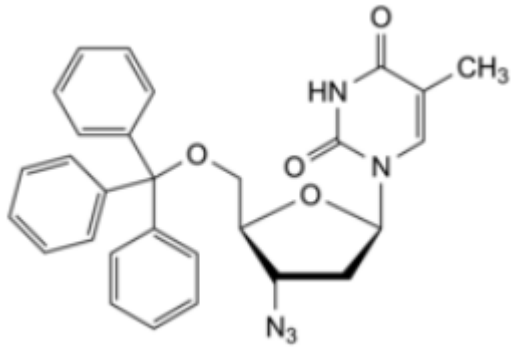
E. Thymidine (synthesis related impurity),



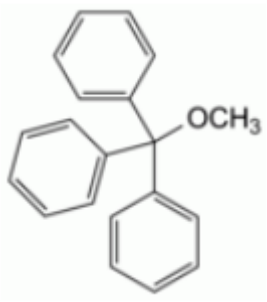
F. 3′-*epi*-Thymidine (synthesis related impurity),



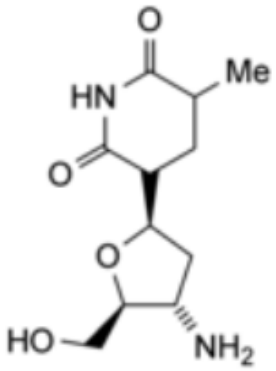
G. 3′-(3′-Azido-3′-deoxythymidin-3-yl)-3′-deoxythymidine; 1-{3-[3-(3-Azido-2,3-dideoxy-β-d-pentofuranosyl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1-yl]-2,3-dideoxy-β-d-pentofuranosyl]-5-methylpyrimidine-2,4-dione. (Degradation impurity),



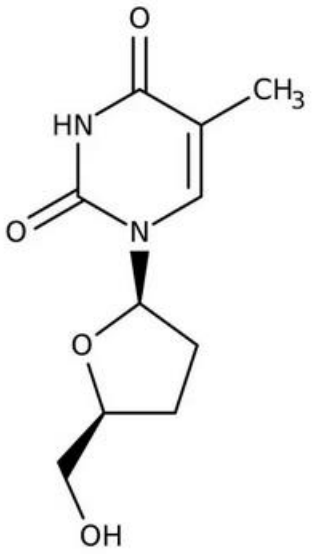
J. 3′-Azido-3′-deoxy-5′-O-(triphenylmethyl)thymidine (trityl-zidovudine) (synthesis related impurity),



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



L. 3’-Amino-3’-deoxythymidine; 1-[(2*R*,4*S*,5*S*)-4-amino-5-(hydroxymethyl)oxalan-2-yl]-5-methylpyrimidin-2,4(1*H*,3*H*)dione (degradation product,



M. 1-[(2R,5S)-5-(Hydroxymethyl)oxolan-2-yl]-5-methylpyrimidin-2,4-dion (degradation product.

Reference substances evoked

Zidovudine RS

ICRS already established.

Zidovudine impurity B RS

ICRS already established.

Zidovudine impurity D RS

It is intended to refer to the corresponding CRS established by the European Pharmacopoeia

Zidovudine for system suitability A RS (containing zidovudine and impurity G)

It is intended to refer to the corresponding CRS established by the European Pharmacopoeia

Test solutions/ reagents to be included in the Ph.Int.

Sulfuric acid (0.1 mol/l) TS

Sulfuric acid (~1760 g/L) TS diluted with water to contain 9.808 g of H_2SO_4 in 1000 mL.

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