World Health Organization

ZIDOVUDINE CAPSULES

(ZIDOVUDINI CAPSULAE)

Draft proposal for revision in *The International Pharmacopoeia*

(30 July 2024)

DRAFT FOR DISCUSSION

Please submit your comments through the online platform, PleaseReview™ (https://who.pleasereview.net/Main/Default.aspx?action=loaddocument&reviewid=272) If not registered or included in our mailing list, kindly submit your request with your full name, email address and organization/affiliation to nsp@who.int.
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.
Our working documents are sent out electronically and uploaded into PleaseReview™. The working documents are also placed on the WHO Medicines website (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/pharmaceuticals/working-documents-public-consultation) under "Working documents in public consultation".
If you wish to receive all our draft guidelines during the course of the year, please send your full name, organization/affiliation and email address to jonessi@who.int, nsp@who.intand your name will be added to our electronic mailing list and review platform.

SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/22.933

ZIDOVUDINE CAPSULES (Zidovudini capsulae)

Description	Date
Drafting of the revised monograph based on information received from manufacturers and information available in public domain	June 2023
Discussion at the Consultation on Quality Control and Pharmacopoeial Specifications for Medicines	April 2024
Draft revision sent out for public consultation.	July - August 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 capsules 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.*]*

ZIDOVUDINE CAPSULES (ZIDOVUDINI CAPSULAE)

Category. Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

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

Additional information. Strength in the current WHO Model list of essential medicines: 250 mg. Zidovudine capsules are also available as a 100 mg capsule on the market.

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 test A or test B or any two of tests C, D or E may be applied.

A. Shake a quantity of the mixed contents of the capsules, equivalent to 0.2 g of Zidovudine with 50 mL of ethanol (~ 750 g/l) TS, and filter. Evaporate the filtrate to dryness. Carry out the examination with the residue 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 each of the following two solutions: For solution (A) sonicate a quantity of the mixed 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. 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. Prepare as a solvent a mixture of 20 volumes of methanol R and 80 volumes of water R. Transfer a quantity of the mixed contents of the capsules, containing 0.05 g of Zidovudine into a 250 mL volumetric flask, add 200 mL of the solvent. and dissolve using sonication. Dilute to volume with the 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, dilute 5.0 mL of the solvent 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.

Dissolution. Carry out the test as described under *5.5 Dissolution test for oral dosage forms*, using 900 mL water R as the dissolution medium. Rotate the paddle at 50 revolutions per minute. At 45 minutes, withdraw a sample of 10 mL of the medium through an in-line filter. Allow the filtered solution to cool down to room temperature and dilute as follows: For 100 mg capsules, dilute 3.0 mL of the filtered solution to 20.0 mL with dissolution medium. For 250 mg capsules: dilute 3.0 mL of the filtered solution to 50.0 mL with dissolution medium.

Measure the absorbance of the resulting solutions as described under *1.6 Spectrophotometry in the visible and ultraviolet regions* in a cuvette with an optical pathlength of 10 mm at the maximum at about 266 nm, using the dissolution medium as the blank. At the same time measure the absorbance of a suitable solution of zidovudine RS in dissolution media.

For each of the capsules tested, calculate the amount of zidovudine ($C_{10}H_{13}N_5O_4$) in the medium.

Evaluate the results as described under *5.5 Dissolution test for oral dosage forms*, Acceptance criteria. The amount of zidovudine in solution for each capsule is not less than 75% (Q) of the amount declared on the label.

[Note from the Secretariat. It is intended to determine the absorptivity value of zidovudine during the establishment of zidovudine RS and to use this value for the calculation of the test result.]

Related substances. 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
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

[1] A Hypersil BDS C18 – 250 x 4.6 mm - 5µm column has been found to be suitable.

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), shake a quantity of the mixed contents of the capsules, containing 0.3 g of Zidovudine with 5 mL of water R in a 100 mL volumetric flask, add 30 mL of methanol R, sonicate for 10 minutes, dilute to volume with water R and filter. Dilute 2 volumes to 5 volumes with water R.

For solution (2), dilute 1 volume of solution (1) to 100 volumes with mobile phase.

For solution (3), dilute 1 volume of solution (2) to 5 volumes with mobile phase.

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 20.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity C is not greater than 1.5 times the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (1.5 %);

- the area of any other impurity peak is not greater than the area of the peak due to zidovudine in the chromatogram obtained with solution (3) (0.2 %).
- The sum of the areas of all impurity peaks is not greater than 2 times the area of the peak due to zidovudine in the chromatogram obtained with solution (2) (2.0%). Disregard any peak with an area less than 0.5 times the area of the peak due to zidovudine in the chromatogram obtained with solution (3) (0.1%).

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), weigh and mix the contents of 20 capsules and transfer a quantity containing the equivalent of 40.0 mg of Zidovudine into a 200 mL volumetric flask, fill to volume and mix. Filter a portion of this solution, discarding the first few mL of the filtrate.

For solution (2), dissolve 40.0 mg of zidovudine RS and dilute to 200 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.

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 ($C_{10}H_{13}N_5O_4$) in the capsules, using the declared content of zidovudine ($C_{10}H_{13}N_5O_4$) in zidovudine CRS.

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.

***