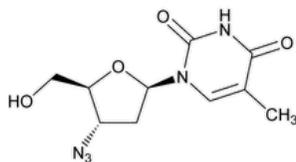


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# Zidovudine



$C_{10}H_{13}N_5O_4$  267.24

Thymidine, 3'-azido-3'-deoxy-

3'-Azido-3'-deoxythymidine CAS RN®: 30516-87-1.

## DEFINITION

Zidovudine contains NLT 97.0% and NMT 102.0% of zidovudine ( $C_{10}H_{13}N_5O_4$ ), calculated on the anhydrous basis.

## IDENTIFICATION

- **A.** [SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), *Infrared Spectroscopy*: 197K
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

## ASSAY

**Change to read:**

### PROCEDURE

▲**Buffer:** 2 g/L of [ammonium acetate](#) in [water](#). Adjust with [dilute acetic acid](#) or dilute [ammonium hydroxide](#) (1 in 10) to a pH of 6.8.

**Solution A:** [Acetonitrile](#)

**Mobile phase:** See [Table 1](#).

**Table 1**

Time (min)	Buffer (%)	Solution A (%)
0	95	5
3	95	5
18	85	15
28	30	70
43	30	70
45	95	5

**Diluent:** [Acetonitrile](#), [methanol](#), and *Buffer* (4:20:76)

**Standard solution:** 0.2 mg/mL of [USP Zidovudine RS](#) in *Diluent*

**Sample solution:** 0.2 mg/mL of Zidovudine in *Diluent*

### Chromatographic system

(See [Chromatography \(621\)](#), *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%▲ (USP 1-May-2022)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of zidovudine ( $C_{10}H_{13}N_5O_4$ ) in the portion of Zidovudine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of zidovudine from the *Sample solution*

$r_S$  = peak response of zidovudine from the *Standard solution*

$C_S$  = concentration of [USP Zidovudine RS](#) in the *Standard solution* (mg/mL)

$C_U$  = concentration of Zidovudine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the anhydrous basis

**IMPURITIES**

• [RESIDUE ON IGNITION \(281\)](#): NMT 0.25%

**Delete the following:**

▲ **LIMIT OF TRIPHENYLMETHANOL AND OTHER IMPURITIES**

**Standard solution:** 0.1 mg/mL each of [USP Zidovudine RS](#) and triphenylmethanol in methanol

**Sample solution:** 20 mg/mL of Zidovudine in methanol

**Chromatographic system**

(See [Chromatography \(621\)](#), [Thin-Layer Chromatography](#).)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture containing a fluorescent indicator having an optimal intensity at 254 nm

**Application volume:** 10 μL

**Developing solvent system:** Chloroform and methanol (9:1)

**Spray reagent:** 5 mg/mL of carbazole in alcohol and sulfuric acid (95:5)

**Analysis:** Develop the chromatogram until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution* with those of the principal spot in the chromatogram of the *Standard solution*.

**Acceptance criteria 1:** No secondary spot from the chromatogram of the *Sample solution* is larger or more intense than the principal spot from the *Standard solution*, and the sum of the intensities of the secondary spots obtained from the *Sample solution* corresponds to NMT 3.0%.

Spray the plate with *Spray reagent*, heat for 10 min at 120°, and compare the intensities of any secondary spots observed in the chromatogram of the *Sample solution* with those of the principal spots in the chromatogram of the *Standard solution*.

**Acceptance criteria 2:** No spot corresponding to triphenylmethanol ( $R_F$  about 2.3 relative to the  $R_F$  of zidovudine) is more intense than the corresponding spot from the *Standard solution*. No secondary spot from the chromatogram of the *Sample solution* is larger or more intense than the principal spot obtained from the *Standard solution*. The sum of the intensities of the secondary spots from the *Sample solution* corresponds to NMT 3.0%. ▲ (USP 1-May-2022)

**Delete the following:**

▲ **LIMIT OF ZIDOVUDINE RELATED COMPOUNDS B AND C**

**Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of zidovudine related compound B and zidovudine related compound C in the portion of Zidovudine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for zidovudine related compound B or zidovudine related compound C

$r_T$  = sum of the responses of all of the peaks

**Acceptance criteria**

**Individual impurities:** NMT 1.0% for zidovudine related compound B and NMT 2.0% for zidovudine related compound C

**Total impurities:** NMT 3.0% for all impurities from the tests for *Limit of Triphenylmethanol and Other Impurities* and *Limit of Zidovudine Related Compounds B and C*. ▲ (USP 1-May-2022)

**Add the following:**

▲ • **ORGANIC IMPURITIES**

**Buffer, Solution A, Mobile phase, Diluent, and Chromatographic system:** Proceed as directed in the Assay.

**System suitability solution:** 1 mg/mL of [USP Zidovudine RS](#) and 2 µg/mL of [USP Zidovudine Related Compound B RS](#) in *Diluent*

**Standard solution:** 1 µg/mL of [USP Zidovudine RS](#) in *Diluent*

**Sample solution:** 1 mg/mL of Zidovudine in *Diluent*

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See [Table 2](#) for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 2.0 between zidovudine and zidovudine related compound B, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Zidovudine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of zidovudine from the *Standard solution*

$C_S$  = concentration of [USP Zidovudine RS](#) in the *Standard solution* (mg/mL)

$C_U$  = concentration of Zidovudine in the *Sample solution* (mg/mL)

$F$  = relative response factor (see [Table 2](#))

**Acceptance criteria:** See [Table 2](#). Disregard the triphenylmethanol peak, any peak eluting after triphenylmethanol impurity, and any peak less than 0.05%.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zidovudine related compound C <sup>a</sup>	0.2	1.6	1.0
Stavudine <sup>b</sup>	0.5	1.0	0.3
Zidovudine	1.0	—	—
Zidovudine related compound B <sup>c</sup>	1.05	1.0	1.0

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zidovudine related compound G (dimer) <sup>d</sup>	1.5	1.0	0.15
Triphenylmethanol	2.0	—	— <sup>e</sup>
Any individual unspecified impurity	—	—	0.10
Total impurities <sup>f</sup>	—	—	3.0

<sup>a</sup> Thymine; also known as 5-Methylpyrimidine-2,4(1H,3H)-dione.

<sup>b</sup> 1-[(2R,5S)-5-(Hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methylpyrimidine-2,4(1H,3H)-dione.

<sup>c</sup> 3'-Chloro-3'-deoxythymidine.

<sup>d</sup> 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.

<sup>e</sup> See the test for *Limit of Triphenylmethanol*.

<sup>f</sup> Includes impurities from the tests for *Organic Impurities* and *Limit of Triphenylmethanol*.

▲ (USP 1-May-2022)

**Add the following:**

▲ • **LIMIT OF TRIPHENYLMETHANOL**

**Mobile phase:** [Acetonitrile](#) and [water](#) (70:30)

**System suitability solution:** 0.01 mg/mL of [USP Zidovudine RS](#) and 5 µg/mL of [USP Triphenylmethanol RS](#) prepared as follows. Transfer a suitable amount of [USP Zidovudine RS](#) and [USP Triphenylmethanol RS](#) to a suitable volumetric flask and add 10% of the final flask volume of [acetonitrile](#). Dilute with *Mobile phase* to volume.

**Standard stock solution:** 0.5 mg/mL of [USP Triphenylmethanol RS](#) in [acetonitrile](#)

**Standard solution:** 5 µg/mL of [USP Triphenylmethanol RS](#) in *Mobile phase* from *Standard stock solution*

**Sample solution:** 5 mg/mL of Zidovudine prepared as follows. Transfer a suitable amount of Zidovudine to a suitable volumetric flask and add 10% of the final flask volume of [acetonitrile](#). Dilute with *Mobile phase* to volume.

**Chromatographic system**

(See [Chromatography \(621\), System Suitability.](#))

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**Run time:** NLT 10 times the retention time of zidovudine

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 5.0 between zidovudine and triphenylmethanol, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of triphenylmethanol in the portion of Zidovudine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of triphenylmethanol from the *Sample solution*

$r_S$  = peak response of triphenylmethanol from the *Standard solution*

$C_s$  = concentration of [USP Triphenylmethanol RS](#) in the *Standard solution* (mg/mL)

$C_u$  = concentration of Zidovudine in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.25%▲ (USP 1-May-2022)

#### SPECIFIC TESTS

- [WATER DETERMINATION \(921\)](#), *Method I*: NMT 1.0%
- [OPTICAL ROTATION \(781S\)](#), *Procedures, Specific Rotation*

**Sample solution:** 10 mg/mL, in alcohol

**Acceptance criteria:** +60.5° to +63°

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

#### Change to read:

- [USP REFERENCE STANDARDS \(11\)](#).

▲ [USP Triphenylmethanol RS](#)

Triphenylmethanol.

$C_{19}H_{16}O$  260.33

▲ (USP 1-May-2022)

[USP Zidovudine RS](#)

[USP Zidovudine Related Compound B RS](#)

3'-Chloro-3'-deoxythymidine.

$C_{10}H_{13}ClN_2O_4$  260.68

▲▲ (USP 1-May-2022)

**Auxiliary Information** - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
ZIDOVDINE	<a href="#">Documentary Standards Support</a>	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services <a href="mailto:RSTECH@usp.org">RSTECH@usp.org</a>	SM12020 Small Molecules 1

**Chromatographic Database Information:** [Chromatographic Database](#)

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