

BRIEFING

Lamivudine and Zidovudine Tablets. This monograph has been posted on the USP Pending Monograph Web page for review and public comments for at least 90 days. No comments were received. The SM1 Expert Committee has approved the monograph as an Authorized USP Pending Monograph. The HPLC validation procedures in the *Assay, Dissolution, and Organic Impurities, Procedure 1* were performed with the Inertsil ODS-3 brand of L1 column. The typical retention times for the lamivudine peak and the zidovudine peak are about 9 and 36 min, respectively, for the gradient *Organic Impurities, Procedure 1*. The typical retention times for the lamivudine peak and the zidovudine peak are about 3 and 5 min, respectively, for the isocratic *Assay* method. The HPLC procedure in *Organic Impurities, Procedure 2* is performed with the Ace C18 brand of L1 column. The typical retention times for the lamivudine peak and the zidovudine peak are about 11 and 26 min, respectively, for the gradient *Organic Impurities, Procedure 2*.

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Lamivudine and Zidovudine Tablets

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DEFINITION

Lamivudine and Zidovudine Tablets contains NLT 90.0% and NMT 110.0% of the labeled amount of lamivudine (C₈H₁₁N₃O₃S) and zidovudine (C₁₀H₁₃N₅O₄).

IDENTIFICATION

- A.** The retention times of the zidovudine and lamivudine peaks in the *Sample solution* correspond to those in the *Standard solution*, as obtained in the *Assay*.

ASSAY

LAMIVUDINE AND ZIDOVUDINE

Dilute acetic acid: 0.1% (v/v) of glacial acetic acid
Buffer: 7.7 g/L of ammonium acetate in *Dilute acetic acid*
Mobile phase: Methanol and *Buffer* (1:1)
Standard solution: 30 µg/mL of USP Zidovudine RS and 15 µg/mL of USP Lamivudine RS in *Mobile phase*
Sample solution: 30 µg/mL of zidovudine and 15 µg/mL of lamivudine in *Mobile phase* from intact Tablets (NLT 5).
[NOTE—Sonicate for 30 min to dissolve, and pass the solution through a suitable filter.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 270 nm

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

Column temperature: 30°

Flow rate: 1 mL/min

Run time: Twice the retention time of zidovudine

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for lamivudine and zidovudine are 0.6 and 1.0, respectively.]

Suitability requirements

Tailing factor: NMT 2.0 for zidovudine and lamivudine

Relative standard deviation: NMT 2.0% for zidovudine and lamivudine

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of zidovudine (C₁₀H₁₃N₅O₄) and lamivudine (C₈H₁₁N₃O₃S) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- r_u = peak response of zidovudine or lamivudine from the *Sample solution*
- r_s = peak response of zidovudine or lamivudine from the *Standard solution*
- C_s = concentration of USP Zidovudine RS or USP Lamivudine RS in the *Standard solution* (µg/mL)
- C_u = nominal concentration of zidovudine or lamivudine in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 75 rpm

Time: 30 min

Standard solution: 0.325 mg/mL of USP Zidovudine RS and 0.15 mg/mL of USP Lamivudine RS dissolved with methanol initially at 10% of final volume. Dilute with *Medium* to final volume. [NOTE—Sonicate as necessary.]

Sample solution: Pass a portion of the solution through a suitable filter.

Mobile phase and Chromatographic system: Proceed as directed in the *Assay*.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity dissolved as percentage of the labeled amount of zidovudine or lamivudine in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/L) \times V \times 100$$

- r_u = peak response of the zidovudine or lamivudine peak from the *Sample solution*
- r_s = peak response of the zidovudine or lamivudine peak from the *Standard solution*
- C_s = concentration of USP Zidovudine RS or USP Lamivudine RS in the *Standard solution* (mg/mL)
- L = label claim (mg/Tablet)
- V = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of zidovudine and lamivudine are dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements for lamivudine and zidovudine

IMPURITIES

[NOTE—Use *Organic Impurities, Procedure 1* when the impurity profile includes salicylic acid. Use *Organic Impurities, Procedure 2* when the impurity profile includes zidovudine related compound D.]

- ORGANIC IMPURITIES, Procedure 1**

Solution A: 1.94 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 3.8.

Solution B: Methanol

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	90	10
25	80	20
40	90	10
90	90	10

Diluent: *Solution B* and *Solution A* (1:9)

Standard stock solution: 15 µg/mL of USP Salicylic Acid RS, 150 µg/mL of USP Zidovudine Related Compound B RS, and 150 µg/mL of USP Zidovudine Related Compound C RS in methanol

Standard solution: 1.5 µg/mL of USP Salicylic Acid RS, 15 µg/mL of USP Zidovudine Related Compound B RS, and 15 µg/mL of USP Zidovudine Related Compound C RS from the *Standard stock solution*; 6 µg/mL of USP Zidovudine RS and 3 µg/mL of USP Lamivudine RS in *Diluent*

System suitability solution: 1.5 µg/mL of USP Salicylic Acid RS, 15 µg/mL of USP Zidovudine Related Compound B RS,

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and 15 µg/mL of USP Zidovudine Related Compound C RS from the *Standard stock solution*; 3 mg/mL of USP Zidovudine RS and 1.5 mg/mL of USP Lamivudine RS in *Diluent*

Sample solution: 3 mg/mL of zidovudine and 1.5 mg/mL of lamivudine in *Diluent* from finely powdered Tablets (NLT 20). [NOTE—Sonicate to dissolve, and pass the solution through a suitable filter.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 277 nm

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

Column temperature: 35°

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

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

[NOTE—The relative retention times are listed in *Table 2*.]

Suitability requirements

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

Tailing factor: NMT 2.0 for zidovudine and lamivudine, *Standard solution*

Relative standard deviation: NMT 5.0% for zidovudine and lamivudine, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zidovudine related compound B or zidovudine related compound C in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of zidovudine related compound B or zidovudine related compound C from the *Sample solution*

r_s = peak response of zidovudine related compound B or zidovudine related compound C from the *Standard solution*

C_s = concentration of USP Zidovudine Related Compound B RS or USP Zidovudine Related Compound C RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of zidovudine in the *Sample solution* (mg/mL)

Calculate the percentage of salicylic acid or lamivudine-carboxylic acid impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of salicylic acid or the lamivudine-carboxylic acid impurity from the *Sample solution*

r_s = peak response of salicylic acid or lamivudine-carboxylic acid from the *Standard solution*

C_s = concentration of USP Salicylic Acid RS or USP Lamivudine RS in the *Standard solution* (mg/mL)

C_u = nominal concentration of lamivudine in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_r) \times 100$$

r_u = peak response of each unspecified impurity from the *Sample solution*

r_r = sum of peak responses from the *Sample solution*

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lamivudine-carboxylic acid ^a	0.12	0.30
Zidovudine related compound C ^b	0.18	1.00
Lamivudine	0.25	—
Salicylic acid ^c	0.82	0.10
Zidovudine	1.0	—
Zidovudine related compound B ^d	1.2	0.30
Any unspecified impurity	—	0.20
Total impurities	—	2.5

^a (2R,5S)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.

^b 5-Methylpyrimidine-2,4(1H,3H)-dione.

^c 2-Hydroxybenzoic acid.

^d 3'-Chloro-3'-deoxythymidine.

• **ORGANIC IMPURITIES, Procedure 2**

Solution A: 1.93 g/L of ammonium acetate in water. Adjust with trifluoroacetic acid to a pH of 3.0. Pass the solution through a suitable filter.

Solution B: Methanol

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	95	5
18	85	15
30	55	45
40	40	60
48	40	60
51	95	5
60	95	5

Diluent: Methanol and 0.2% (v/v) phosphoric acid (3:7)

Standard solution: 30 µg/mL of USP Zidovudine RS and 7.5 µg/mL of USP Lamivudine RS in *Diluent*

System suitability solution: 1.5 mg/mL of USP Zidovudine RS, 0.75 mg/mL of USP Lamivudine RS, and 3 µg/mL of USP Zidovudine Related Compound D RS in *Diluent*. Pass the solution through a suitable filter.

Sample solution: 0.75 mg/mL of lamivudine from finely powdered Tablets (NLT 20) in *Diluent*. [NOTE—Sonicate for 10 min to dissolve, and pass the solution through a suitable filter.]

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 266 nm

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

Flow rate: 1.2 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.5 between lamivudine and zidovudine related compound D

Tailing factor: NMT 1.5 for lamivudine and zidovudine

Relative standard deviation: NMT 5.0% for lamivudine and zidovudine

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Disregard any peak area less than 0.05% and peaks from blank and excipients.]

Calculate the percentage of each lamivudine related impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

r_u = peak response of each lamivudine related impurity from the *Sample solution*

- r_s = peak response of lamivudine from the *Standard solution*
 C_s = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)
 C_u = nominal concentration of lamivudine in the *Sample solution* (mg/mL)

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lamivudine-carboxylic acid ^a	0.21	0.3
Lamivudine-trans RR ^{b,c}	0.37	—
Lamivudine	0.42	—
Zidovudine	1.00	—

- ^a (2*R*,5*S*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.
^b 1-[(2*R*,5*R*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.
^c Process impurity with no individual limit but needs to be included in the calculation of total impurities.

Calculate the percentage of each zidovudine related impurity or any unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- r_u = peak response of each zidovudine related impurity or any unspecified 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 = nominal concentration of zidovudine in the *Sample solution* (mg/mL)

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Zidovudine related compound C ^a	0.23	0.5
Zidovudine related compound D ^{b,g}	0.39	—

- ^a 5-Methylpyrimidine-2,4(1*H*,3*H*)-dione.
^b [1-(2-Deoxy- β -D-ribofuranosyl)]thymine.
^c Thymidine, 2',3'-didehydro-3'-deoxy-.
^d 1-(3-Azido-2,3-dideoxy- β -D-threopentafuranosyl)-5-methylpyrimidine-2,4-dione.
^e 3'-Chloro-3'-deoxythymidine.
^f 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.
^g Process impurity with no individual limit but needs to be included in the calculation of total impurities.

Table 5 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lamivudine	0.42	—
Stavudine ^{e,g}	0.58	—
threo-Zidovudine ^{d,g}	0.98	—
Zidovudine	1.00	—
Zidovudine related compound B ^e	1.04	1.0
Zidovudine-dimer ^{f,g}	1.30	—
Any unspecified impurity	—	0.2

- ^a 5-Methylpyrimidine-2,4(1*H*,3*H*)-dione.
^b [1-(2-Deoxy- β -D-ribofuranosyl)]thymine.
^c Thymidine, 2',3'-didehydro-3'-deoxy-.
^d 1-(3-Azido-2,3-dideoxy- β -D-threopentafuranosyl)-5-methylpyrimidine-2,4-dione.
^e 3'-Chloro-3'-deoxythymidine.
^f 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.
^g Process impurity with no individual limit but needs to be included in the calculation of total impurities.

Acceptance criteria

Total impurities: NMT 2.6%. Sum of the individual impurities in *Table 4* and *Table 5*.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light, and store at room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.

• **USP REFERENCE STANDARDS (11)**

- USP Lamivudine RS
 (-)-1-[(2*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.
 $C_8H_{11}N_3O_3S$ 229.26
 USP Salicylic Acid RS
 Benzoic acid, 2-hydroxy-.
 $C_7H_6O_3$ 138.12
 USP Zidovudine RS
 3'-Azido-3'-deoxythymidine.
 $C_{10}H_{13}N_5O_4$ 267.24
 USP Zidovudine Related Compound B RS
 3'-Chloro-3'-deoxythymidine.
 $C_{10}H_{13}ClN_2O_4$ 260.68
 USP Zidovudine Related Compound C RS
 2,4(1*H*,3*H*)-Pyrimidinedione, 5-methyl-.
 $C_5H_6N_2O_2$ 126.12
 USP Zidovudine Related Compound D RS
 [1-(2-Deoxy- β -D-ribofuranosyl)]thymine.
 $C_{10}H_{14}N_2O_5$ 242.23