

BRIEFING

**Lamivudine and Stavudine 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 procedures in the *Assay*, *Dissolution*, and *Organic Impurities, Procedure 1* were performed with the Inertsil C18 brand of 5- $\mu$ m L1 column. [NOTE—The Inertsil ODS 3V column has been shown to be a suitable alternative.] The typical retention times for the lamivudine peak and the stavudine peak are about 7.6 and 2.3 min, respectively, for the isocratic *Assay* method. The typical retention times for the lamivudine peak and the stavudine peak are about 15.4 and 22.8 min, respectively, for *Organic Impurities, Procedure 1*. The HPLC procedure in *Organic Impurities, Procedure 2* was performed with the Prontosil C18 H brand of 5- $\mu$ m L1 column. The typical retention times for the lamivudine peak and the stavudine peak are about 11.8 and 21.1 min, respectively, for the gradient *Organic Impurities, Procedure 2*.

(SM1: B. Davani, M. Marques.)  
Correspondence Number—C54651;  
C63870

## Lamivudine and Stavudine Tablets

v.1 Authorized March 1, 2011

### DEFINITION

Lamivudine and Stavudine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of lamivudine (C<sub>8</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>S) and stavudine (C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>).

### IDENTIFICATION

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

### ASSAY

#### LAMIVUDINE AND STAVUDINE

**Buffer:** 2.16 g/L of sodium octane sulfonate in water. Adjust with phosphoric acid to a pH of 2.60  $\pm$  0.05.

**Mobile phase:** Methanol and *Buffer* (2:3)

**Diluent:** Methanol and water (2:3)

[NOTE—Prepare all solutions fresh before use.]

**Standard stock solution:** 0.75 mg/mL of USP Lamivudine RS and 0.20 mg/mL of USP Stavudine RS in methanol

**Standard solution:** 75  $\mu$ g/mL of USP Lamivudine RS and 20  $\mu$ g/mL of USP Stavudine RS in *Diluent* from *Standard stock solution*

**Sample stock solution:** Transfer an equivalent to 1500 mg of lamivudine and 400 mg of stavudine from intact Tablets (NLT 10) into a 1000-mL volumetric flask. Add 20 mL of water, and shake until the Tablets have completely dispersed. Add 700 mL of methanol, and sonicate for 30 min with intermittent swirling. Allow the solution to cool, and dilute with methanol to volume. Pass the solution through a suitable filter.

**Sample solution:** 75  $\mu$ g/mL of Lamivudine and 20  $\mu$ g/mL of Stavudine in *Diluent* from *Sample stock solution*

#### Chromatographic system

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

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 8 between the stavudine and lamivudine peaks

**Tailing factor:** NMT 2.0 for lamivudine and stavudine

**Relative standard deviation:** NMT 2.0% for lamivudine and stavudine

#### Analysis

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

Calculate the percentage of the labeled amount of lamivudine (C<sub>8</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>S) and stavudine (C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>) in the portion of Tablets taken:

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

$r_u$  = peak response of lamivudine or stavudine from the *Sample solution*

$r_s$  = peak response of lamivudine or stavudine from the *Standard solution*

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

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

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION (711)

**Medium:** 0.1 N hydrochloric acid, 900 mL

**Apparatus 2:** 75 rpm

**Time:** 45 min

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

[NOTE—Prepare all solutions fresh before use.]

**Standard stock solution:** 0.75 mg/mL of USP Lamivudine RS and 0.20 mg/mL of USP Stavudine RS, initially dissolved in 70% of final volume with methanol. Sonicate for 5 min, cool to room temperature, and dilute with methanol to final volume.

**Standard solution A** (for Tablets labeled as containing 150 mg of lamivudine and 30 mg of stavudine): 0.15 mg/mL of USP Lamivudine RS and 0.04 mg/mL of USP Stavudine RS in *Medium*

**Standard solution B** (for Tablets labeled as containing 150 mg of lamivudine and 40 mg of stavudine): 0.19 mg/mL of USP Lamivudine RS and 0.05 mg/mL of USP Stavudine RS in *Medium*

**Sample solution:** Pass the solution through a suitable filter.

#### Analysis

**Samples:** *Standard solution A* or *B* and *Sample solution*

Calculate the percentage of the labeled amount of lamivudine (C<sub>8</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>S) and stavudine (C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>) dissolved 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 lamivudine or stavudine from the *Sample solution*

$r_s$  = peak response of lamivudine or stavudine from the *Standard solution*

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

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 75% (Q) of the labeled amounts of lamivudine and stavudine are dissolved.

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

## 2 / Lamivudine

### IMPURITIES

[NOTE—Use *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2* when the impurity profile includes the lamivudine diastereomer or the thymidine epimer.]

#### • ORGANIC IMPURITIES, Procedure 1

**Dilute acetic acid:** 0.1% (v/v) glacial acetic acid in water

**Buffer:** 15.4 g/L of ammonium acetate in *Dilute acetic acid*

**Mobile phase:** Methanol and *Buffer* (9:91)

[NOTE—Prepare all solutions fresh before use.]

**System suitability solution:** 3 mg/mL of USP Lamivudine RS, 0.8 mg/mL of USP Stavudine RS, 8 µg/mL of USP Zidovudine Related Compound C RS, and 0.8 µg/mL of USP Zidovudine Related Compound D RS in *Mobile phase*

**Standard solution:** 3.2 µg/mL of USP Lamivudine RS, 0.8 µg/mL of USP Stavudine RS, 8 µg/mL of USP Zidovudine Related Compound C RS, and 0.8 µg/mL of USP Zidovudine Related Compound D RS in *Mobile phase*

**Sample solution:** Transfer an equivalent to 150 mg of lamivudine from finely powdered Tablets (NLT 10) to a 50-mL volumetric flask. Add 30 mL of *Mobile phase*, and sonicate for 10 min. Cool to room temperature, and dilute with *Mobile phase* to volume. 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

**Injection size:** 10 µL

**Run time:** Two times the retention time of lamivudine

#### System suitability

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

#### Suitability requirements

**Resolution:** NLT 1.5 between the peaks for zidovudine related compound D and lamivudine, *System suitability solution*

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

**Relative standard deviation:** NMT 5.0% for lamivudine, stavudine, zidovudine related compound C, and zidovudine related compound D, *Standard solution*

#### Analysis

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

Calculate the percentage of lamivudine specified unidentified impurity 1 in the portion of Tablets taken:

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

$r_U$  = peak response of lamivudine specified unidentified impurity 1 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)

Calculate the percentage of 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 C from the *Sample solution*

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

$C_S$  = concentration of USP Zidovudine Related Compound C RS in the *Standard solution* (mg/mL)

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

Calculate the percentage of zidovudine related compound D 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 D from the *Sample solution*

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

$C_S$  = concentration of USP Zidovudine Related Compound D RS in the *Standard solution* (mg/mL)

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

Calculate the percentage of each 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 unspecified impurity from the *Sample solution*

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

$C_S$  = concentration of USP Stavudine RS in the *Standard solution* (mg/mL)

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

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Specified unidentified impurity 1	0.30	0.30
Zidovudine related compound C <sup>a</sup>	0.48	1.00
Zidovudine related compound D <sup>b</sup>	0.90	0.10
Lamivudine	1.00	—
Stavudine	1.48	—
Any unspecified impurity	—	0.10
Total impurities	—	3.0

<sup>a</sup> 5-Methylpyrimidine-2,4(1*H*,3*H*)-dione.

<sup>b</sup> [1-(2-Deoxy-β-D-ribofuranosyl)]thymine.

#### • ORGANIC IMPURITIES, Procedure 2

**Solution A:** 2.3 g/L of ammonium dihydrogen phosphate in water. Adjust with phosphoric acid to a pH of 3.50 ± 0.05. Pass the solution through a suitable filter of 0.45-µm pore size.

**Solution B:** Acetonitrile and methanol (19:1)

**Mobile phase:** See *Table 2*.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	97	3
10	97	3
18	90	10
30	60	40
35	60	40
40	97	3
50	97	3

**Diluent:** Methanol and water (1:4)

**System suitability solution:** 0.5 mg/mL of USP Stavudine System Suitability Mixture RS in *Diluent*

**Standard solution:** 2.25 µg/mL of USP Lamivudine RS, 0.4 µg/mL of USP Stavudine RS, and 6 µg/mL of USP Zidovudine Related Compound C RS in *Diluent*. Pass the solution through a suitable filter. [NOTE—Sonicate to dissolve if necessary. Keep the solution at 5°.]

**Sample solution:** 0.75 mg/mL of lamivudine in *Diluent*. Transfer an equivalent to 75 mg of lamivudine from finely powdered Tablets (NLT 10) to a 100-mL volumetric flask. Add 70 mL of *Diluent*, and sonicate for 10 min. Cool to room temperature, and dilute with *Diluent* to volume. Pass the solution through a suitable filter. [NOTE—Keep the solution at 5°.]

#### Chromatographic system

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

**Mode:** LC  
**Detector:** UV 266 nm  
**Column:** 4.6-mm × 25-cm; 5-μm packing L1  
**Column temperature:** 30°  
**Sample temperature:** 5°  
**Flow rate:** 1 mL/min  
**Injection size:** 20 μL

**System suitability**

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

**Suitability requirements**

**Resolution:** NLT 1.2 between the peaks for thymidine epimer and zidovudine related compound D; NLT 1.2 between the peaks for stavudine and α-stavudine, *System suitability solution*

**Tailing factor:** NMT 1.5 for lamivudine, stavudine, and zidovudine related compound C, *Standard solution*

**Relative standard deviation:** NMT 5.0% for lamivudine, stavudine, and zidovudine related compound C, *Standard solution*

**Analysis**

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

Calculate the percentage of each lamivudine related impurity (see *Table 3*) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of the 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)

$F$  = relative response factor (see *Table 3*)

**Table 3**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lamivudine-carboxylic acid <sup>a</sup>	0.22	0.58	0.3
Specified unidentified impurity 1	0.23	1.00	0.2
Lamivudine diastereomer <sup>b</sup>	0.50	0.61	0.2
Lamivudine	0.59	—	—
Specified unidentified impurity 2	0.94	1.00	0.2
Stavudine	1.00	—	—

<sup>a</sup> (2R,5S)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.

<sup>b</sup> 1-[(2R,5R)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

Calculate the percentage of 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 C from the *Sample solution*

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

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

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

Calculate the percentage of each stavudine related impurity or any unspecified impurity (see *Table 4*) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of each stavudine related impurity and each unspecified impurity from the *Sample solution*

$r_s$  = peak response of stavudine from the *Standard solution*

$C_s$  = concentration of USP Stavudine RS in the *Standard solution* (mg/mL)

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

$F$  = relative response factor (see *Table 4*)

**Table 4**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Zidovudine related compound C <sup>a</sup>	0.37	—	2.0
Lamivudine	0.59	—	—
Thymidine epimer <sup>b,f</sup>	0.77	0.84	—
Zidovudine related compound D <sup>c,f</sup>	0.79	0.87	—
Stavudine	1.00	—	—
α-Stavudine <sup>d,f</sup>	1.02	1.00	—
3,5'-Anhydro-thymidine <sup>e,f</sup>	1.11	0.97	—
Any unspecified impurity	—	1.00	0.2

<sup>a</sup> 5-Methylpyrimidine-2,4(1H,3H)-dione.

<sup>b</sup> [1-(2-Deoxy-β-D-xylofuranosyl)]thymine.

<sup>c</sup> [1-(2-Deoxy-β-D-ribofuranosyl)]thymine.

<sup>d</sup> 1-(2,3-Dideoxy-β-D-glycero-pent-2-enofuranosyl)thymine.

<sup>e</sup> [1-(3,5-anhydro-2-Deoxy-β-D-xylofuranosyl)]thymine.

<sup>f</sup> Process impurity that has no individual limit, but that must be included in the calculation of total impurities.

**Acceptance criteria**

**Total impurities:** NMT 3.0%. Sum of the individual impurities in *Table 3* and *Table 4*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states with which *Organic Impurities* test the article complies.
- **USP REFERENCE STANDARDS (11)**  
USP Lamivudine RS  
(-)-1-[(2R,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.  
C<sub>8</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>S 229.26  
USP Stavudine RS  
1-(2,3-Dideoxy-β-D-glycero-pent-2-enofuranosyl)thymine.  
C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub> 224.21  
USP Stavudine System Suitability Mixture RS  
Mixture of stavudine and the following related compounds:  
thymidine, thymine, alpha-stavudine, and xylo-thymidine.  
USP Zidovudine Related Compound C RS  
5-Methylpyrimidine-2,4(1H,3H)-dione.  
C<sub>5</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub> 126.12  
USP Zidovudine Related Compound D RS  
[1-(2-Deoxy-β-D-ribofuranosyl)]thymine.  
C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>O<sub>5</sub> 242.23