

BRIEFING

Stavudine, Lamivudine, and Nevirapine Tablets A new USP Pending Standard monograph based on validated methods of analysis is being proposed. The liquid chromatographic methods in the *Assay* and the tests for *Dissolution* and *Organic Impurities, Procedure 1* are based on analyses performed using the Inertsil ODS 3V brand of L1 columns. The typical retention times for stavudine, lamivudine, and nevirapine are 2.3, 8.2, and 10.8 min, respectively. The liquid chromatographic method in the test for *Organic Impurities, Procedure 2* is based on analyses performed using a Supelcosil LC-ABZ brand of L60 column. The typical retention time for nevirapine is 7.6 min.

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Add the following:

► **Stavudine, Lamivudine, and Nevirapine Tablets**

Draft 1

DEFINITION

Stavudine, Lamivudine, and Nevirapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of stavudine ($C_{10}H_{12}N_2O_4$), lamivudine ($C_8H_{11}N_3O_3S$), and nevirapine ($C_{15}H_{14}N_4O$), respectively.

IDENTIFICATION

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

ASSAY

• **PROCEDURE**

Solution A: 2.34 mg/mL of octanesulfonic acid sodium salt adjusted with dilute phosphoric acid to a pH of 2.6 ± 0.05

Mobile phase: Methanol and *Solution A* (2:3)

Diluent: Methanol and water (2:3)

System suitability solution: 0.02 mg/mL of USP Stavudine RS and 0.2 µg/mL of USP Zidovudine Related Compound C RS in *Diluent*

Standard stock solution: 0.2 mg/mL of USP Stavudine RS, 0.75 mg/mL of USP Lamivudine RS and 1 mg/mL of USP Nevirapine Anhydrous RS in methanol

Standard solution: 0.02 mg/mL of stavudine, 0.075 mg/mL of lamivudine and 0.1 mg/mL of nevirapine in *Diluent* from *Standard stock solution*. [NOTE—Sonicate if necessary to dissolve.]

Sample stock solution: Equivalent to 0.4 mg/mL of stavudine, 1 mg/mL of lamivudine, and 2 mg/mL of nevirapine in methanol

Sample solution: 0.02 mg/mL of stavudine, 0.075 mg/mL of lamivudine and 0.1 mg/mL of nevirapine in *Diluent* from the *Sample stock solution*

Chromatographic system

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

Mode: LC

Detector: UV 270 nm

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

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

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

[NOTE—The relative retention times for stavudine, lamivudine, and nevirapine are 0.3, 1.0, and 1.3, respectively.]

Suitability requirements

Resolution: NLT 1.0 between zidovudine related compound C and stavudine, *System suitability solution*; NLT 2.0 between lamivudine and nevirapine, *Standard solution*

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

Relative standard deviation: NMT 2.0% for stavudine, lamivudine, and nevirapine, *Standard solution*

Analysis

Samples:

Calculate the percentage of $C_{10}H_{12}N_2O_4$, $C_8H_{11}N_3O_3S$, or $C_{15}H_{14}N_4O$ in the portion of Tablets taken:

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

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

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

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

C_u = nominal concentration of stavudine, lamivudine, or nevirapine 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

Standard stock solution: 0.2 mg/mL of USP Stavudine RS, 0.75 mg/mL of USP Lamivudine RS, and 1 mg/mL of USP Nevirapine Anhydrous RS in methanol

Standard solution: 0.05 mg/mL of stavudine, 0.20 mg/mL of lamivudine, and 0.25 mg/mL of nevirapine in *Medium* from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable 0.45-µm filter.

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

Analysis

Samples

Calculate the percentage of $C_{10}H_{12}N_2O_4$, $C_8H_{11}N_3O_3S$, or $C_{15}H_{14}N_4O$ dissolved:

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

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

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

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

L = tablet label claim for stavudine, lamivudine, or nevirapine (mg/tablet)

V = volume of *Medium* (mL), 900

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

- **UNIFORMITY OF DOSAGE UNITS** <905>: Meet the requirements

IMPURITIES

Organic Impurities

• **PROCEDURE 1**

Solution A: Prepare a 0.1% acetic acid solution by diluting a suitable aliquot of glacial acetic acid with water

Solution B: 15.4 mg/mL of ammonium acetate in *Solution A*

Mobile phase: Methanol and *Solution B* (9:91)

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

Sample solution: Equivalent to 3 mg/mL of lamivudine in *Mobile phase*. [NOTE—Sonicate if necessary to dissolve. Use this solution immediately after it is prepared.]

Chromatographic system

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

Mode: LC
 Detector: UV 270 nm
 Column: 4.6-mm × 25-cm; 5-μm packing L1
 Temperature: 30°
 Flow rate: 1 mL/min
 Injection size: 10 μL
 Run time: At least 3 times the retention time of stavudine

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0, lamivudine and stavudine
Relative standard deviation: NMT 5.0%, lamivudine, thymine, and stavudine

Analysis

Samples: Standard solution and Sample solution

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 lamivudine-carboxylic acid 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-carboxylic acid 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 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

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 3.0%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lamivudine-carboxylic acid ^a	0.29	0.3
Zidovudine related compound C (thymine)	0.49	1.0
Isomer of β-thymidine ^b	0.85	—
β-thymidine ^b	0.89	—
Lamivudine	1.0	—
Salicylic acid ^b	1.3	—

^a (2*R*,5*S*)-5-(4-Amino-2-oxypyrimidin-1(2*H*)-yl)-1,3-oxathiolane-2-carboxylic acid.

^b These are process impurities that are controlled in the drug substance; they are listed here for information only.

Impurity Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Stavudine	1.5	—
Individual unspecified impurity	—	0.1

^a (2*R*,5*S*)-5-(4-Amino-2-oxypyrimidin-1(2*H*)-yl)-1,3-oxathiolane-2-carboxylic acid.

^b These are process impurities that are controlled in the drug substance; they are listed here for information only.

PROCEDURE 2 (LIMIT OF NEVIRAPINE-RELATED COMPOUNDS)

Solution A: 2.88 mg/mL of monobasic ammonium phosphate adjusted with 1*N* sodium hydroxide to a pH of 5.0 ± 0.05

Mobile phase: Acetonitrile and *Solution A* (1:4)

System suitability solution: 1 mg/mL of USP Nevirapine Anhydrous RS and 1 μg/mL of USP Nevirapine Related Compound B RS in acetonitrile and *Mobile phase* (1:9). [NOTE—Dissolve first in acetonitrile, using 10% of the final volume. Sonicate if necessary to dissolve. Dilute with *Mobile phase* to volume.]

Standard stock solution: 1 mg/mL of USP Nevirapine Anhydrous RS in acetonitrile and *Mobile phase* (1:9). [NOTE—Dissolve first in acetonitrile, using 10% of the final volume. Sonicate if necessary to dissolve. Dilute with *Mobile phase* to volume.]

Standard solution: 1 μg/mL of nevirapine in *Mobile phase*

Sample solution: Equivalent to 1 mg/mL nevirapine in acetonitrile and *Mobile phase* (1:9). [NOTE—Dissolve first in acetonitrile, using 10% of the final volume. Sonicate if necessary to dissolve. Dilute with *Mobile phase* to volume.]

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-μm packing L60

Temperature: 35°

Flow rate: 1 mL/min

Injection size: 10 μL

Run time: At least 10 times the retention time of nevirapine

System suitability

Sample: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for nevirapine related compound B and nevirapine are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between nevirapine related compound B and nevirapine, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each nevirapine-related compound 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 impurity from the *Sample solution*

r_S = peak response of nevirapine from the *Standard solution*

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

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

Acceptance criteria

Individual impurities: NMT 0.10%

Total impurities: NMT 1.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** <11>

- USP Lamivudine RS
- USP Nevirapine Anhydrous RS
- USP Nevirapine Related Compound B RS
- USP Stavudine RS

USP Zidovudine Related Compound C RS ◀ (1-Mar-2010)