Labeling—The label states that this article is not intended for direct administration to humans or animals.

USP Reference standards ⟨11⟩**—**
USP Epilactose RS
USP Fructose RS
USP Galactose RS
USP Anhydrous Lactose RS
USP Lactulose RS

Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: Add a few drops of a solution (1 in 20) to 5 mL of hot alkaline cupric tartrate TS: a red precipitate of cuprous oxide is formed.

Refractive index ⟨831⟩: not less than 1.451, at 20°.

Residue on ignition ⟨281⟩: not more than 0.1%.

Related compounds—

Phosphate buffer solution and *Mobile phase*—Proceed as directed in the *Assay*.

Standard solution—Transfer accurately weighed quantities of USP Galactose RS, USP Anhydrous Lactose RS, USP Epilactose RS, and USP Fructose RS to a 10-mL volumetric flask, and dissolve in and dilute with a mixture of water and acetonitrile (1:1) to volume to obtain a solution having known concentrations of about 6.4 mg per mL, 4.8 mg per mL, 3.2 mg per mL and 0.4 mg per mL, respectively.

Test solution—Prepare as directed for the *Assay preparation* in the *Assay*.

Chromatographic system—Proceed as directed in the *Assay*. To evaluate the system suitability requirements, use the *Standard preparation* prepared as directed in the *Assay*.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Identify the peaks based on their relative retention times given in *Table 1*.

Table 1

Name	Relative Retention Time
Fructose	0.30
Galactose	0.42
Epilactose	0.85
Lactulose	1.0
Lactose	1.1

Calculate the percentages of galactose, lactose, epilactose, and fructose, if found, in the portion of Concentrate taken by the formula:

$$100(CV/W)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of the relevant USP Reference Standard in the *Standard solution; V* is the volume, in mL, of the *Test solution; W* is the weight, in mg, of lactulose in the *Test solution;* and r_U and r_S are the peak responses for the relevant related compounds obtained from the *Test solution* and the *Standard solution,* respectively: relative to lactulose, not more than 16% of galactose is found, not more than 12% of lactose is found, not more than 8% of epilactose is found, and not more than 1% of fructose is found.

Assay—

Phosphate buffer solution—Dissolve 1.15 g of monobasic sodium phosphate in 1000 mL of water.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *Phosphate buffer solution* (82:18). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). [NOTE—Ensure that the concentration of acetonitrile in the *Mobile phase* is between 78% and 85% to obtain appropriate retention times.]

Standard preparation—Transfer accurately weighed quantities of USP Lactulose RS, USP Anhydrous Lactose RS, and USP Epilactose RS to a 10-mL volumetric flask, and dissolve in and dilute with a mixture of water and acetonitrile (1:1) to volume, to obtain a solution having known concentrations of 40 mg per mL, 4.8 mg per mL, and 3.2 mg per mL, respectively.

Assay preparation—Transfer an accurately weighed quantity of Concentrate containing about 2.0 g of lactulose to a 50-mL volumetric flask, and dissolve in 20 mL of water. Add 25.0 mL of acetonitrile, mix, allow the solution to reach ambient temperature, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a refractive index detector maintained at a temperature of 40 ± 1° and a 4.6-mm × 15-cm column that contains 3-µm packing L8. The column temperature is maintained at 40 ± 1°. The flow rate is about 1.3 mL per minute. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure.* Identify the components based on their relative retention times given in *Table 1:* the resolution, *R,* between lactulose and lactose is not less than 1.5, and that between lactulose and epilactose is not less than 0.9; and the relative standard deviation for replicate injections determined from the main peak is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in mg, of lactulose ($C_{12}H_{22}O_{11}$) in the portion of Concentrate taken by the formula:

$$CV(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Lactulose RS in the *Standard preparation; V* is the volume, in mL, of the *Assay preparation;* and r_U and r_S are the peak responses for lactulose obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Lactulose Solution

» Lactulose Solution is a solution in water prepared from Lactulose Concentrate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lactulose ($C_{12}H_{22}O_{11}$).

Packaging and storage—Preserve in tight containers, preferably at a temperature between 2° and 30°. Avoid subfreezing temperatures.

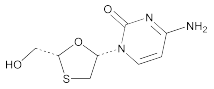
USP Reference standards ⟨11⟩**—**
USP Epilactose RS
USP Fructose RS
USP Galactose RS
USP Anhydrous Lactose RS
USP Lactulose RS

Microbial enumeration tests ⟨61⟩ **and Tests for specified microorganisms** ⟨62⟩**—**The total bacterial count does not exceed 100 cfu per g of lactulose, and the tests for *Salmonella* species and *Escherichia coli* are negative.

pH ⟨791⟩: between 2.5 and 6.5, after 15 minutes of contact with the electrodes.

Other requirements—It meets the requirements for the *Identification* tests, the *Related compounds* test, and the *Assay* under *Lactulose Concentrate*.

Lamivudine



$C_8H_{11}N_3O_3S$ 229.26

2(1*H*)-Pyrimidinone, 4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-, (2*R-cis*)-;
(–)-1-[(2*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine [134678-17-4].

DEFINITION

Lamivudine contains NLT 98.0% and NMT 102.0% of $C_8H_{11}N_3O_3S$, calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨197M⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution,* as obtained in the test for *Lamivudine Enantiomer*.

ASSAY

- **Procedure**

Buffer: 1.9 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 3.8 ± 0.2 before final dilution.
Mobile phase: Methanol and *Buffer* (5:95)
System suitability solution: 0.25 mg/mL of USP Lamivudine Resolution Mixture B RS in *Mobile phase*
Standard solution: 0.25 mg/mL of USP Lamivudine RS in *Mobile phase*
Sample solution: 0.25 mg/mL of Lamivudine in *Mobile phase*
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 277 nm
Column: 4.6-mm × 25-cm; packing L1
Column temperature: 35°
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
[NOTE—The relative retention times for lamivudine diastereomer and lamivudine are 0.9 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 1.5 between lamivudine and lamivudine diastereomer, *System suitability solution*
Relative standard deviation: NMT 2.0% for lamivudine, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of lamivudine ($C_8H_{11}N_3O_3S$) in the portion of Lamivudine taken:

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

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)
C_U = concentration of Lamivudine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous, solvent-free basis

IMPURITIES

- **Organic Impurities, Lamivudine Enantiomer**

Buffer: 7.7 g/L of ammonium acetate in water
Mobile phase: Methanol and *Buffer* (5:95)
System suitability solution: 0.25 mg/mL of USP Lamivudine Resolution Mixture A RS in water
Sample solution: 0.25 mg/mL of Lamivudine in water
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 270 nm
Column: 4.6-mm × 25-cm; packing L45
Column temperature: 15°–30° (constant temperature)
Flow rate: 1 mL/min
Injection size: 10 µL
System suitability
Sample: *System suitability solution*
[NOTE—The relative retention times for lamivudine and lamivudine enantiomer are 1.0 and 1.2, respectively.]
Suitability requirements
Resolution: NLT 1.5 between lamivudine and lamivudine enantiomer
Analysis
Sample: *Sample solution*
Calculate the percentage of the lamivudine enantiomer in the portion of Lamivudine taken:

$$\text{Result} = [r_U/(r_U + r_S)] \times 100$$

r_U = peak response of the lamivudine enantiomer
r_S = peak response of lamivudine

Acceptance criteria: NMT 0.3%

- **Organic Impurities, Other Related Compounds**

Buffer, Mobile phase, Standard solution (Lamivudine), System suitability solution, Suitability requirements, and **Chromatographic system:** Proceed as directed in the *Assay*.
Standard solution: 0.625 µg/mL of salicylic acid in *Mobile phase*
Sample solution: 0.25 mg/mL of Lamivudine in *Mobile phase*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of salicylic acid in the portion of Lamivudine taken:

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

r_U = peak response of salicylic acid from the *Sample solution*
r_S = peak response of salicylic acid from the *Standard solution*
C_S = concentration of salicylic acid in the *Standard solution* (mg/mL)
C_U = concentration of Lamivudine in the *Sample solution* (mg/mL)

Calculate the percentage of other individual impurities in the portion of Lamivudine taken:

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

r_U = peak response of each impurity other than salicylic acid from the *Sample solution*
r_T = sum of the responses of all the peaks

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Specified impurity 1	0.4	0.3
Specified impurity 2	0.9	0.2
Salicylic acid	1.0	0.1
Any other individual impurity	—	0.1
Total impurities	—	0.6

- **Organic Impurities, Residual Solvents**

Internal standard solution: Dilute 1 mL of 2-pentanone with dimethyl sulfoxide and water (1:1) to 100.0 mL.