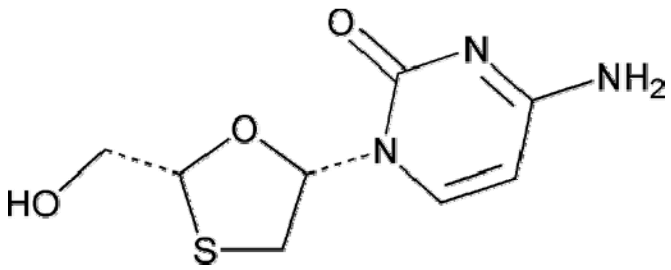
Lamivudine

USP Method Lamivudine RS



Original Manufacturer:	GlaxoSmithKline (GSK) patent expiry: 2010 (US) and 2011 (Europe)
Brand Name:	Zeffix, Heptovir, Epivir, and Epivir-HBV
Generics:	Lamivir HBV (Cipla)
Combination Drugs:	Combivir (with zidovudine); Epzicom/Kivexa (with abacavir); Trizivir (with zidovudine and abacavir)

Lamivudine (2',3'-dideoxy-3'-thiacytidine, commonly called 3TC) is a potent nucleoside analog reverse transcriptase inhibitor (nRTI).

Lamivudine has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV. It improves the seroconversion of e-antigen positive hepatitis B and also improves histology staging of the liver.

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Lamivudine

USP34 – NF29 S1

USP Columns:

Hypersil BDS C-18 Assay and Chromatographic purity 4.6-mm x 25-cm., Thermo

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 250x4.6 mm (1.51456.0001)

Recommended Solvents and Reagents:

Methanol for liquid chromatography LiChrosolv® (1.06018)

Water Water for chromatography LiChrosolv® (1.15333)
or freshly purified water from Milli-Q water purification system

Ammonium Acetate Use ACS reagent grade.

Acetic Acid Acetic acid (glacial) 100%. Use ACS reagent grade.

USP Standards

Lamivudine (200 mg)	USP Product Number:1356836
Lamivudine Resolution Mixture A (10 mg)	USP Product Number:1356847
Lamivudine Resolution Mixture B (10 mg)	USP Product Number: 1356858

USP Method Lamivudine Assay

Buffer - 0.025 M Ammonium acetate solution:

Transfer about 1.9 g of ammonium acetate to a 1000-mL volumetric flask, dissolve in about 900 mL of water, adjust with acetic acid to a pH of 3.8 ± 0.2, dilute with water to volume, and mix.

Mobile phase

Prepare a filtered and degassed mixture of 0.025 M Ammonium acetate solution and methanol (95:5). Make adjustments if necessary (see System Suitability under Chromatography 621).

System suitability solution

Dissolve an accurately weighed quantity of USP Lamivudine Resolution Mixture B RS in Mobile phase to obtain a solution having a known concentration of about 0.25 mg per mL.

Standard preparation

Dissolve an accurately weighed quantity of USP Lamivudine RS in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 0.25 mg per mL.

Assay preparation

Transfer about 25 mg of Lamivudine, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with Mobile phase to volume, and mix.

Chromatographic system (*see Chromatography 621*)

The liquid chromatograph is equipped with a 277-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 35° C.

Chromatograph the System suitability solution, and record the peak responses as directed for Procedure:

Resolution, R, between lamivudine and lamivudine diastereomer is not less than 1.5.
Relative retention times are about 1.0 for lamivudine and 0.9 for lamivudine diastereomer.

Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 2.0%.

USP Method Lamivudine RS

Procedure

Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the lamivudine peaks. Calculate the quantity, in mg, of $C_8H_{11}N_3O_3S$ in the portion of Lamivudine taken by the formula:

$100C(rU / rS)$

in which C is the concentration, in mg per mL, of USP Lamivudine RS in the Standard preparation; and rU and rS are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

Chromatographic purity

0.025 M Ammonium acetate solution, Mobile phase, System suitability solution, and Chromatographic system, proceed as directed in the Assay.

Salicylic acid solution

Dissolve an accurately weighed quantity of salicylic acid in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a concentration of about 0.625 µg/mL.

Standard solution

Use the Standard preparation, prepared as directed in the Assay.

Test solution

Use the Assay preparation.

Procedure

Separately inject equal volumes (about 10 µL) of Salicylic acid solution and the Test solution into the chromatograph, record the chromatograms, and measure all the peak responses. Calculate the percentage of salicylic acid in the portion of Lamivudine taken by the formula:

$(10C/W)(r_U / r_S)$

in which C is the concentration, in µg per mL, of salicylic acid in the Salicylic acid solution; W is the weight, in mg, of Lamivudine taken for the Test solution; and r_U and r_S are the salicylic acid peak responses obtained from the Test solution and the Salicylic acid solution, respectively. Calculate the percentage of other individual impurities in the portion of Lamivudine taken by the formula:

$100(r_i / r_s)$

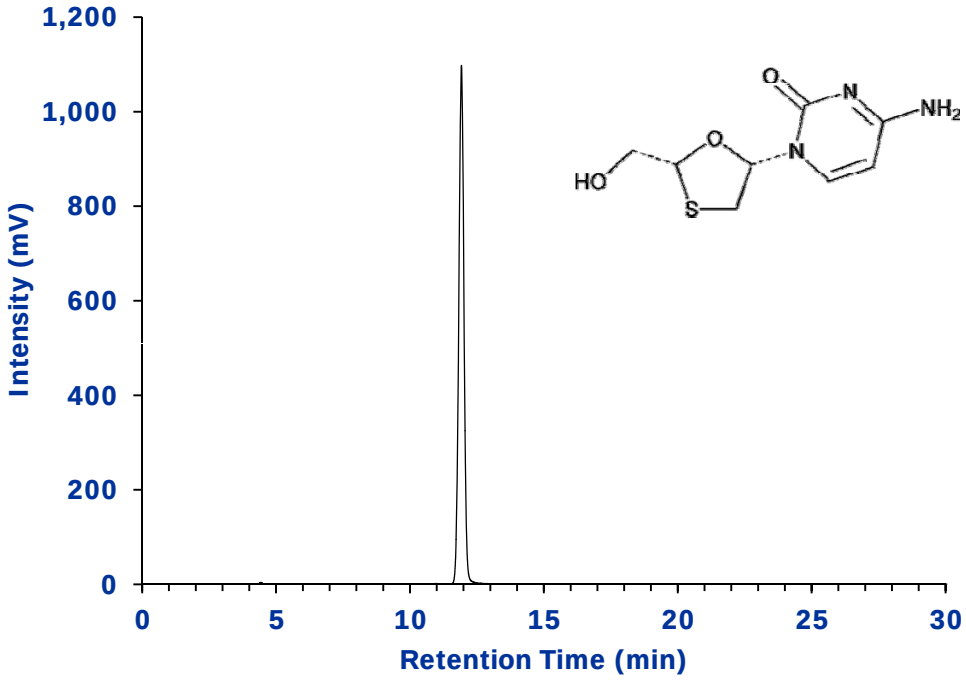
in which r_i is the peak response for each impurity other than salicylic acid obtained from the Test solution; and r_s is the sum of the responses for all the peaks: not more than 0.3% for any peak at a relative retention time of about 0.4 is found; not more than 0.2% for any peak at a relative retention time of about 0.9 is found; not more than 0.1% of salicylic acid is found; not more than 0.1% of any other individual impurity is found; and not more than 0.6% of total impurities is found.

USP Method for Lamivudine Assay

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence 2010, UV@277 nm	
Cell:	10 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Buffer: 0.025 M Ammonium acetate solution, with pH adjusted to 3.8 ± 0.2 with acetic acid Mix buffer and methanol 95:5.	
Temperature:	35° Celsius	
Diluent	mobile phase	
Sample:	250 ppm (0.25 mg/mL) Lamivudine	
Pressure Drop:	134 Bar (1943 psi)	



Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (TUSP)	Relative Retention Time (RRT)	Resolution (Rs)
1	Lamivudine	11.9	1.0	-	-

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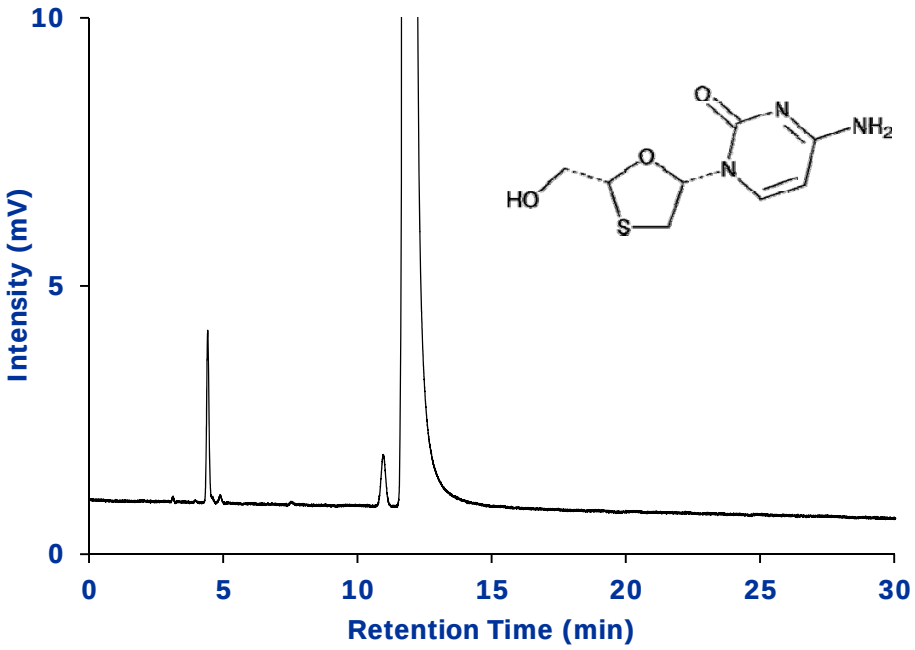
USP Method for Lamivudine RS

Purospher®STAR RP-18 endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18 endcapped (5 µm) 250x4.6 mm	1.51456.0001
Injection:	10 µL	
Detection:	Shimadzu Prominence 2010, UV@277 nm	
Cell:	8 µL	
Flow Rate:	1.0 mL/min	
Mobile Phase (v/v):	Buffer: 0.025 M Ammonium acetate solution, with pH adjusted to 3.8 ± 0.2 with acetic acid Mix buffer and methanol 95:5.	
Temperature:	35° Celsius	
Diluent	mobile phase	
Sample:	250 ppm (0.25 mg/mL) Lamivudine and traces of lamivudine diastereomer	
Pressure Drop:	134 Bar (1943 psi)	



Chromatographic Data

No.	Compound	Time (min)	Tailing Factor (TUSP)	Relative Retention Time (RRT)	Resolution (Rs)
1	Impurity	4.4	1.1	0.4	
2	Lamivudine diastereomer	11.0	1.0	0.9	
3	Lamivudine	11.9	1.0	1.0	2.9

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