CONTROLLED COPY

MASTER COPY

Mylan	FINISHED PRODUCT (REGULATORY AND SHELF LIFE) STANDARD TEST PROCEDURE	Mylan, Nashik

Name of the Product: Abacavir and Lamivudine Tablets USP (600 mg/300 mg)	
Product code: ABL000T66X1A	**Supersedes:** FPPABL507R-00
STP No.: FPPABL507R-01	**Effective Date:** 08 DEC 2020
Reference/s: USP+IH	**Market:** PERU

	Department	Name	Sign & Date
Prepared by	QA	Ram Phad	22/10/20
Reviewed by	QA	Mukesh Maliya	23/10/20
Approved by	QC	Ami S. Undejaweera	26.10.2020
	QA	Subhash Phad	29/10/20

Note:

1. Unless otherwise specified, maintain centrifugation chamber temperature and sonicator temperature about 25° C, wherever applicable.
2. Use Millipore make filters and 33 mm diameter wherever applicable.
3. Use glass syringes for filtration wherever applicable.

1.0 DESCRIPTION:

Procedure:

Put 10 tablets on butter paper observe the size, shape and color. Compare the sample appearance to specification description. The tablets should be free of foreign matter or surface defects, including sticking, discoloration or disfiguration.

2.0 IDENTIFICATION (By HPLC):

Procedure:

Proceed as directed under the test, "Assay by HPLC". Compare the retention times of the Abacavir and Lamivudine peaks from the test chromatogram with that from the standard chromatogram. The retention time of the Abacavir and Lamivudine peaks in the chromatogram of the test preparation corresponds to that in the chromatogram of the standard preparation.

3.0 DISSOLUTION (By HPLC):

Reagents:

a.	Ammonium acetate	:	AR grade or equivalent
b.	Methanol	:	HPLC grade
c.	Hydrochloric acid	:	AR grade or equivalent
d.	Acetonitrile	:	HPLC Grade
e.	Water	:	DM water for dissolution medium and HPLC grade for Mobile phase

Dissolution parameters:

a.	Medium	:	0.1 N Hydrochloric acid
b.	Volume	:	900 mL
c.	Apparatus	:	USP-II (Paddle)
d.	RPM	:	75
e.	Temperature	:	37 ± 0.5°C
f.	Time point	:	As specified in the specification

Chromatographic condition:

Column	:	ACE C8, 150 mm × 4.6 mm, 5 µm
Flow rate	:	1.5 mL / minute
Wavelength	:	272 nm
Column temperature	:	40°C
Injection Volume	:	5 µL
Run time	:	16 minutes

Dissolution medium (0.1 N hydrochloric acid):

Mix 8.5 mL of concentrated hydrochloric acid in 1000 mL of water in a suitable container and mix.

Buffer:

Dissolve 1.54 g of Ammonium acetate in 1000 mL of water. Filter through 0.45 µm or a finer porosity membrane filter.

Mobile Phase: Mix buffer and acetonitrile in the ratio of 900:100 v/v respectively and degas.

Note: Mobile phase is stable for 5 days on bench top.

Standard stock preparation:

1. Weigh accurately and transfer about 98 mg of Abacavir sulfate working standard and 42 mg Lamivudine working standard into a 25 mL volumetric flask.

2. Add about 5 mL of Methanol and 15 mL of dissolution medium, sonicate to dissolve and dilute to volume with dissolution medium.

Standard solution

1. Dilute 5.0 mL of the standard stock preparation to 25 mL with dissolution medium.
2. Filter the solution through 0.45 µm nylon or 0.45 µm PVDF membrane filter.

Note:

1. Appropriate standard solution shall be injected.
2. Standard solution is stable for 24 hours at room temperature.

Test solution:

1. Transfer 900 mL of the dissolution medium into each of the six dissolution vessels.
2. After reaching required temperature, drop one tablet into each of the dissolution vessels and immediately start the instrument run.
3. At the end of specified time, withdraw 10 mL of the sample from each of the dissolution vessels.
4. Filter the solution through 0.45 µm nylon or 0.45 µm PVDF membrane filter or 10 µm full flow filter of fraction collector of the Vankel instrument.

Note: Test solution is stable for 24 hours at room temperature.

Procedure:

Inject standard solution (5 injections) and test solution into the chromatograph, record the chromatograms and measure the peak responses. (Refer Typical Chromatograms).

Note: The retention time of Lamivudine peak and Abacavir peak is about 1.7 minutes and 7-14 minutes respectively.

System suitability:

From standard solution:

1. The USP Tailing factor for Abacavir and Lamivudine peaks should be not more than 1.5.
2. The column efficiency for Abacavir and Lamivudine peaks should be not less than 2000 USP theoretical plates.
3. The Relative standard deviation of Abacavir and Lamivudine peak areas for five replicate injections should be not more than 2.0%.

Calculation:

$$\text{Quantity of Abacavir dissolved (as \% w/w of labeled amount)} = \frac{A_{TA}}{A_{SA}} \times \frac{W_{SA}}{25} \times \frac{5}{25} \times \frac{900}{LC_A} \times \frac{P_A}{100} \times \frac{572.66}{670.74} \times 100$$

Where,

A_{TA} – Peak area of Abacavir from test solution.

A_{SA} = Average peak area of Abacavir from standard solution.

W_{SA} = Weight of Abacavir sulfate standard taken, in mg, for standard solution.

LC_A = Labeled amount of Abacavir per tablet, in mg.

P_A = % Potency of Abacavir sulfate standard, on as is basis.

572.66 = Molecular weight of Abacavir multiplied by 2.

670.74 = Molecular weight of Abacavir Sulfate.

$$\text{Quantity of Lamivudine dissolved (as \% w/w of labeled amount)} = \frac{A_{TL}}{A_{SL}} \times \frac{W_{SL}}{25} \times \frac{5}{25} \times \frac{900}{LC_L} \times \frac{P_L}{100} \times 100$$

Where,

A_{TL} = Peak area of Lamivudine from test solution.

A_{SL} = Average peak area of Lamivudine from standard solution.

W_{SL} = Weight of Lamivudine standard taken, in mg, for standard solution.

LC_L = Labeled amount of Lamivudine per tablet, in mg.

P_L = % Potency of Lamivudine standard, on as is basis.

4.0 UNIFORMITY OF DOSAGE UNITS: (By Content Uniformity)

Proceed as directed under the General Test Procedure "Uniformity of Dosage Units – USP", GTP No.: GTP017#.

Instrumentation:

HPLC	:	Waters Alliance HPLC equipped with 2695 Separation module or equivalent
Detector	:	UV or PDA Detector
Software	:	Empower or equivalent

Reagents:

Trifluoroacetic acid	:	AR grade or equivalent
Hydrochloric acid	:	AR grade or equivalent
Methanol	:	HPLC Grade
Acetonitrile	:	HPLC Grade
Water	:	HPLC grade

Chromatographic conditions:

Column	: Xterra MS C18, 150 mm x 4.6 mm, 3.5 µm
Flow rate	: 1.5 mL /minute
Wavelength	: 270 nm
Column oven temperature	: 40°C
Sample cooler temperature	: Room temperature
Sampling rate	: 2 points / second
Injection volume	: 10 µL

Gradient program:

Time (in minutes)	Solution A (%v/v)	Solution B (%v/v)	Curve Type$
0	100	0	6
4	100	0	6
12	70	30	6
12.1	40	60	6
13.1	40	60	6
13.2	100	0	6
20.2	100	0	6

$ As per Waters Empower Software.

Note: Use volumetric flasks and Pipettes for solution A and Solution B preparation.

Solution A: Prepare a mixture of Water and Trifluoroacetic acid in the ratio 2000:1 v/v respectively. Filter the solution through 0.45 µm or finer porosity membrane filter.

Solution B: Prepare a mixture of Acetonitrile, Methanol and Trifluoroacetic acid in the ratio 1000:1000:1 v/v/v respectively. Filter the solution through 0.45 µm or finer porosity membrane filter.

Note: The mobile phases are stable on bench top for 4 days.

Diluent (0.1N Hydrochloric acid):

Dilute 8.5 mL of Hydrochloric acid to 1000 mL with water and mix.

System Suitability preparation:

Dissolve the contents of one vial of USP Lamivudine resolution mixture C RS in 2.5 mL of diluent and mix. [**Note:** One vial of USP Lamivudine resolution mixture C contains 0.8 mg of USP Lamivudine resolution mixture C RS].

Standard preparation (Lamivudine and Abacavir Sulfate):

1. Weigh accurately and transfer about 37.5 mg of Lamivudine form-1 RS/ working standard and 87.5 mg of Abacavir Sulfate RS/ working standard into a 50 mL volumetric flask.
2. Add about 25 mL of diluent, sonicate to dissolve, dilute to volume with diluent and mix.
3. Dilute 4.0 mL of the solution to 20 mL with diluent and mix.
4. Filter the solution through 0.45 µm PVDF or 0.45 µm Nylon syringe filter.

Test solution:

1. Transfer 1 tablet into a 200 mL volumetric flask.
2. Add about 100 mL of diluent, shake for 30 minutes to disperse the tablets, dilute to volume with diluent and mix (stock solution).
3. Dilute 5.0 mL of the solution to 50 mL with diluent and mix.
4. Filter the solution through 0.45 µm PVDF or 0.45 µm Nylon syringe filter.

Note: Similarly perform the UOD on other 9 tablets.

Procedure:

Inject diluent as blank, system suitability preparation, diluent as blank, standard preparation (five times) and test preparation into the chromatograph. Record the chromatogram and measure the peak responses (Refer Typical Chromatograms).

Note:

1. The relative retention times for Lamivudine- S-sulfoxide and Lamivudine-R-sulfoxide, in relation to the lamivudine peak, are 0.31 and 0.36, respectively; the relative retention times for lamivudine diastereomer and lamivudine are 0.88 and 1.0, respectively.

2. The retention time of Lamivudine and Abacavir peaks are about 6.7 and 10.8 minutes respectively. (For information only).

System suitability:

From system suitability preparation:

1. The USP resolution between Lamivudine Diastereomer and Lamivudine peaks should be not less than 1.0.

2. The USP resolution between Lamivudine-S-sulfoxide and Lamivudine-R-sulfoxide peaks should be not less than 1.0

From standard preparation:

3. The relative standard deviation for Lamivudine peak and Abacavir peak areas from five replicate injections should be not more than 1.5%.

Calculation:

$$\text{Assay of Abacavir (\% of label claim)} = \frac{A_A}{S_A} \times \frac{W_{SA}}{50} \times \frac{4}{20} \times \frac{200}{LC_A} \times \frac{50}{5} \times \frac{P_A}{100} \times \frac{572.66}{670.74} \times 100$$

$$\text{Assay of Lamivudine (\% of label claim)} = \frac{A_L}{S_L} \times \frac{W_{SL}}{50} \times \frac{4}{20} \times \frac{200}{LC_L} \times \frac{50}{5} \times \frac{P_L}{100} \times 100$$

Where,

A_A = Peak area of Abacavir from test preparation.

S_A = Average peak area of Abacavir from standard preparation.

W_{SA} = Weight of Abacavir Sulfate standard taken, in mg, for standard preparation.

LC_A = Labeled claim of Abacavir in mg, per unit dose.

P_A = Potency of Abacavir Sulfate standard in %, on as is basis.

572.66 = Molecular weight of Abacavir multiplied by 2.

670.74 = Molecular weight of Abacavir Sulfate.

A_L = Peak area of Lamivudine from test preparation.

S_L = Average peak area of Lamivudine from standard preparation.

W_{SL} = Weight of Lamivudine standard taken, in mg, for standard preparation.

LC_L = Labeled claim of Lamivudine in mg, per unit dose.

P_L = Potency of Lamivudine standard in %, on as is basis.

5.0 ASSAY (By HPLC):

Instrumentation:

HPLC : Waters Alliance HPLC equipped with 2695 Separation module or equivalent

Detector : UV or PDA Detector

Software : Empower or equivalent

Reagents:

Trifluoroacetic acid	:	AR grade or equivalent
Hydrochloric acid	:	AR grade or equivalent
Methanol	:	HPLC Grade
Acetonitrile	:	HPLC Grade
Water	:	HPLC grade

Chromatographic conditions:

Column	:	Xterra MS C18, 150 mm x 4.6 mm, 3.5 µm
Flow rate	:	1.5 mL /minute
Wavelength	:	270 nm
Column oven temperature	:	40°C
Sample cooler temperature	:	Room temperature
Sampling rate	:	2 points / second
Injection Volume	:	10 µL

Gradient program:

Time (in minutes)	Solution A (%v/v)	Solution B (%v/v)	Curve Type[$]
0	100	0	6
4	100	0	6
12	70	30	6
12.1	40	60	6
13.1	40	60	6
13.2	100	0	6
20.2	100	0	6

[$] As per Waters Empower Software.

Note: Use volumetric flasks and Pipettes for solution A and Solution B preparation.

Solution A:

Prepare a mixture of Water and Trifluoroacetic acid in the ratio 2000:1 v/v respectively. Filter the solution through 0.45 µm or finer porosity membrane filter.

Solution B:

Prepare a mixture of Acetonitrile, Methanol and Trifluoroacetic acid in the ratio 1000:1000:1 v/v/v respectively. Filter the solution through 0.45 µm or finer porosity membrane filter.

Note: The mobile phases are stable on bench top for 4 days.

Diluent (0.1N Hydrochloric acid):

Dilute 8.5 mL of Hydrochloric acid to 1000 mL with water and mix.

System suitability preparation:

Dissolve the contents of one vial of USP Lamivudine resolution mixture C RS in 2.5 mL of diluent and mix. [**Note:** One vial of USP Lamivudine resolution mixture C contains 0.8 mg of USP Lamivudine resolution mixture C RS].

Standard preparation (Lamivudine and Abacavir Sulfate):

1. Weigh accurately and transfer about 37.5 mg of Lamivudine form-1 RS/ working standard and 87.5 mg of Abacavir Sulfate RS/ working standard into a 50 mL volumetric flask.
2. Add about 25 mL of diluent, sonicate to dissolve, dilute to volume with diluent and mix.
3. Dilute 4.0 mL of the solution to 20 mL with diluent and mix.
4. Filter the solution through 0.45 µm PVDF or 0.45 µm Nylon syringe filter.

Test solution:

1. Transfer 5 tablets into a 1000 mL volumetric flask.
2. Add about 500 mL of diluent, shake for 30 minutes to disperse the tablets, dilute to volume with diluent and mix (stock solution).
3. Dilute 5.0 mL of the solution to 50 mL with diluent and mix.
4. Filter the solution through 0.45 µm PVDF or 0.45 µm Nylon syringe filter.

Procedure:

Inject diluent as blank, system suitability preparation, diluent as blank, standard preparation (five times) and test preparation (in duplicate) into the chromatograph. Record the chromatogram and measure the peak responses. (Refer Typical Chromatograms)

Note:

1. The relative retention times for Lamivudine- S-sulfoxide and Lamivudine-R-sulfoxide, in relation to the lamivudine peak, are 0.31 and 0.36, respectively; the relative retention times for lamivudine diastereomer and lamivudine are 0.88 and 1.0, respectively.

2. The retention time of Lamivudine peak and Abacavir peak are about 6.7 and 10.8 minutes respectively. (For information only).

System suitability:

From system suitability preparation:

1. The USP resolution between Lamivudine Diastereomer peak and Lamivudine peak should be not less than 1.0.

2. The USP resolution between Lamivudine-S-sulfoxide peak and Lamivudine-R-sulfoxide peak should be not less than 1.0

From standard preparation:

3. The relative standard deviation for the five replicate standard injections of Lamivudine peak and Abacavir peak should not be more than 1.5%.

Calculation:

$$\text{Assay of Abacavir (mg / unit dose)} = \frac{A_A}{S_A} \times \frac{W_{SA}}{50} \times \frac{4}{20} \times \frac{1000}{5} \times \frac{50}{5} \times \frac{P_A}{100} \times \frac{572.66}{670.74}$$

$$\text{Assay of Abacavir (\% w/w of Label claim)} = \frac{\text{Reported Assay value in mg/tablet (Rounded off value)}}{LC_A} \times 100$$

$$\text{Assay of Lamivudine (mg / unit dose)} = \frac{A_L}{S_L} \times \frac{W_{SL}}{50} \times \frac{4}{20} \times \frac{1000}{5} \times \frac{50}{5} \times \frac{P_L}{100}$$

$$\text{Assay of Lamivudine (\% w/w of Label claim)} = \frac{\text{Reported Assay value in mg/tablet (Rounded off value)}}{LC_L} \times 100$$

Where,

A_A	=	Average peak area of Abacavir from test preparation.
S_A	=	Average peak area of Abacavir from standard preparation.
W_{SA}	=	Weight of Abacavir Sulfate standard taken, in mg, for standard preparation.
LC_A	=	Label claim of Abacavir in mg, per unit dose
P_A	=	Potency of Abacavir Sulfate standard in %, on as is basis.
572.66	=	Molecular weight of Abacavir multiplied by 2.
670.74	=	Molecular weight of Abacavir Sulfate.

	Prepared by (QA)	Reviewed by (QA)
Sign & Date	RMP 22/10/20	23/10/20

A_L	=	Average peak area of Lamivudine from test preparation.
S_L	=	Average peak area of Lamivudine from standard preparation.
W_{SL}	=	Weight of Lamivudine standard taken in mg, for standard preparation.
LC_L	=	Label claim of Lamivudine in mg, per unit dose
P_L	=	Potency of Lamivudine standard in %, on as is basis.

6.0 ORGANIC IMPURITIES (By HPLC):

Instrumentation:

HPLC : Waters Alliance HPLC equipped with 2695 Separation module or equivalent

Detector : UV or PDA Detector

Software : Empower or equivalent

Reagents:

Trifluoroacetic acid	:	AR grade or equivalent
Hydrochloric acid	:	AR grade or equivalent
Methanol	:	HPLC Grade
Acetonitrile	:	HPLC Grade
Water	:	HPLC grade

Chromatographic conditions:

Column	:	Xterra MS C18, 150 mm x 4.6 mm, 3.5 µm
Flow rate	:	1.5 mL /minute
Wavelength	:	270 nm
Column oven temperature	:	40°C
Sample cooler temperature	:	Room temperature
Sampling rate	:	2 points / second
Injection volume	:	10 µL

Gradient program:

Time (in minutes)	Solution A (%v/v)	Solution B (%v/v)	Curve Type[$]
0	100	0	6
4	100	0	6
12	70	30	6
12.1	40	60	6
13.1	40	60	6
13.2	100	0	6
20.2	100	0	6

[$] As per Waters Empower Software.

Note: Use volumetric flasks and Pipettes for solution A and Solution B preparation.

Solution A:

Prepare a mixture of Water and Trifluoroacetic acid in the ratio 2000:1 v/v respectively. Filter the solution through 0.45 µm or finer porosity membrane filter.

Solution B:

Prepare a mixture of Acetonitrile, Methanol and Trifluoroacetic acid in the ratio 1000:1000:1 v/v/v respectively. Filter the solution through 0.45 µm or finer porosity membrane filter.

Note: The mobile phases are stable on bench top for 4 days.

Diluent (0.1N Hydrochloric acid):

Dilute 8.5 mL of Hydrochloric acid to 1000 mL with water and mix.

System Suitability preparation:

Dissolve the contents of one vial of USP Lamivudine resolution mixture C RS in 2.5 mL of diluent and mix. [**Note:** One vial of USP Lamivudine resolution mixture C contains 0.8 mg of USP Lamivudine resolution mixture C RS].

Standard preparation (Lamivudine and Abacavir Sulfate):

1. Weigh accurately and transfer about 37.5 mg of Lamivudine form-1 RS/ working standard and 87.5 mg of Abacavir Sulfate RS/ working standard into a 50 mL volumetric flask.
2. Add about 25 mL of diluent, sonicate to dissolve, dilute to volume with diluent and mix.
3. Dilute 4.0 mL of the solution to 20 mL with diluent and mix.
4. Filter the solution through 0.45 µm PVDF or 0.45 µm Nylon syringe filter.

Test preparation:

1. Transfer 5 tablets into a 1000 mL volumetric flask. Add about 500 mL of diluent, shake for 30 minutes to disperse the tablets, dilute to volume with diluent and mix (stock solution).
2. Dilute 5.0 mL of the solution to 50 mL with diluent and mix.
3. Filter the solution through 0.45 µm PVDF or 0.45 µm Nylon syringe filter.

Placebo preparation:

[Placebo without any drug substances or Placebo for Abacavir sulfate and Lamivudine Tablets]:

1. Weigh accurately and transfer placebo powder equivalent to 3000 mg of Abacavir into a 1000 mL volumetric flask.
2. Add about 500 mL of diluent, shake for 30 minutes, dilute to volume with diluent and mix.
3. Dilute 5.0 mL of the solution to 50 mL with diluent and mix.
4. Filter the solution through 0.45 µm PVDF or 0.45 µm Nylon syringe filter.

Placebo with Lamivudine or Placebo of Abacavir sulfate:

1. Weigh accurately and transfer placebo powder equivalent to 1500 mg of Lamivudine into a 1000 mL volumetric flask.
2. Add about 500 mL of diluent, shake for 30 minutes, dilute to volume with diluent and mix.
3. Dilute 5.0 mL of the solution to 50 mL with diluent and mix.
4. Filter the solution through 0.45 µm PVDF or 0.45 µm Nylon syringe filter.

Placebo with Abacavir Sulfate or Placebo of Lamivudine:

1. Weigh accurately and transfer placebo powder equivalent to 3000 mg of Abacavir into a 1000 mL volumetric flask.
2. Add about 500 mL of diluent, shake for 30 minutes and make up to volume with diluent and mix.
3. Dilute 5.0 mL of the solution to 50 mL with diluent and mix.
4. Filter the solution through 0.45 µm PVDF or 0.45 µm Nylon syringe filter.

Procedure:

Inject diluent as blank, system suitability preparation, diluent as blank, standard preparation (five times), placebo preparation and test preparation into the chromatograph. Record the chromatogram and measure the peak responses. (Refer Typical Chromatogram)

Note:

1. The relative retention times for Lamivudine- S-sulfoxide and Lamivudine-R-sulfoxide, in relation to the lamivudine peak, are 0.31 and 0.36, respectively; the relative retention times for Lamivudine Diastereomer and lamivudine are 0.88 and 1.0, respectively.
2. Disregard the peak in the sample chromatogram which is there at the same retention time in the chromatogram of placebo preparation without any drug substances.
3. The retention time of Lamivudine peak and Abacavir peak are about 6.7 and 10.8 minutes respectively. (For information only).
4. Disregard the impurity peaks which are below 0.05%.
5. If necessary, subtract the area counts obtained from the Blank injected just before the standard preparation, peak corresponding to the Retention time of Lamivudine and Abacavir in standard preparation for calculating the amount of impurities present in the test preparation.
6. The Relative Retention Time (RRTs) w.r.t. Abacavir peak are given below;

S.No.	Name of the impurity	RRT(about)
1	Cytosine	0.12
2	Lamivudine S-Sulfoxide	0.19

	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	23/10/20

S.No.	Name of the impurity	RRT(about)
3	Lamivudine R-Sulfoxide	0.21
4	Lamivudine Carboxylic acid [$]	0.49
5	Lamivudine Diastereomer (Lamivudine-trans) [@]	0.52
6	Lamivudine	0.60
7	Lamivudine Uracil derivative	0.78
8	Cyclopropyldiaminopurine Abacvir	0.80
9	Descyclopropyl Abacavir or Amino Impurity	0.85
10	3-Hydroxy Abacavir [@]	0.89

[$] Monitor impurity upto 0.3%, if crosses 0.3%, it should be further investigated. These impurities are process related impurities and shall not be considered in total impurities calculation.

[@] Monitor impurity upto 0.2%, if crosses 0.2%, it should be further investigated. These impurities are process related impurities and shall not be considered in total impurities calculation.

System suitability:

From system suitability preparation:

1. The USP resolution between Lamivudine Diastereomer peak and Lamivudine peak should be not less than 1.0.
2. The USP resolution between Lamivudine-S-sulfoxide peak and Lamivudine-R-sulfoxide peak should be not less than 1.0.

From standard preparation:

3. The relative standard deviation for Lamivudine peak and Abacavir peak from five replicate injections should be not more than 1.5%.

Calculation:

Note: Calculate the amount of Abacavir related impurities and each unspecified impurity in % using the formula;

$$\text{Abacavir related impurities and any unspecified impurity (\%w/w)} = \frac{r_u}{r_t} \times 100$$

Where,

r_u = Peak area response of each Abacavir related impurity or unspecified impurity.

rt = Sum of peak responses of Abacavir, all Abacavir related impurities and all unspecified impurities.

Note: Calculate the amount of Lamivudine related impurities in % using the formula;

$$\text{Lamivudine related impurities (\%w/w)} = \frac{r_u}{r_t} \times 100$$

Where,

r_u = peak response of each Lamivudine related impurity.

rt = Sum of peak responses of Lamivudine and all Lamivudine related impurities.

Total Abacavir related impurities (% w/w) = Sum of all the impurities of Abacavir.

Total Lamivudine related impurities (% w/w) = Sum of all the impurities of Lamivudine.

Chemical names of the impurities:

Cyclopropyldiaminopurine Abacavir	: N^6-Cyclopropyl-9*H*-purine-2,6-diamine.
Descyclopropyl abacavir	:[(1*S*,4*R*)-4-(2,6-Diamino-9*H*-purin-9-yl)cyclopent-2-enyl]methanol
Cytosine	: 4-Aminopyrimidin-2(1*H*)-one
Lamivudine-*S*-sulfoxide	: 1-[(2*R*,3*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide
Lamivudine-*R*-sulfoxide	:1-[(2*R*,3*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide
Lamivudine-carboxylic acid	: (2*RS*,5*SR*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid
Lamivudine Diastereomer (Lamivudine-trans)	: 1-[(2*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine
Lamivudine-uracil derivative	: 1-[(2*RS*,5*SR*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil
3-Hydroxy Abacavir	:(1*R*,2*R*,4*S*)-2-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-4-(hydroxymethyl)cyclopentan-1-ol.

ADDITIONAL TESTS:

7.0 Identification (By Thin-Layer Chromatography):

Reagents:

Dichloromethane	:	AR grade or equivalent
Methanol	:	AR grade or equivalent
Acetic acid	:	AR grade or equivalent

Requirements:

Thin layer chromatographic (TLC) chamber (volume is about 5 liter), silica gel 60 F_{254} Aluminum TLC plates (20cm x 20cm) and UV chamber.

Mobile phase:

Mix Dichloromethane, methanol and acetic acid in the ratio of 90:10:3 v/v/v.

Abacavir standard solution:

Accurately weigh and transfer about 35mg of Abacavir sulfate working standard into a 10 mL volumetric flask. Add 5mL of methanol and sonicate to dissolve, dilute to volume with methanol and mix.

Lamivudine standard solution:

Accurately weigh and transfer about 15mg of Lamivudine working standard into a 10 mL volumetric flask. Add 5mL of methanol and sonicate to dissolve, dilute to volume with methanol and mix.

Test solution:

Abacavir and Lamivudine Tablets:

Crush not less than 20 tablets into a fine powder with mortar and pestle. Accurately weigh and transfer the powdered tablets equivalent to about 15 mg of Lamivudine into a 10 mL volumetric flask.

Add 7 mL of methanol and sonicate for 15 minutes, dilute to volume with methanol and mix.

Filter the solution through 0.45µ PVDF filter.

Procedure:

Add about 250mL of mobile phase to the TLC chamber, cover it and leave the chamber for equilibration.

Take a TLC plates and draw a thin line at a distance of about 2.5 cm from the bottom with a pencil. Care should be taken that the line is not below the level of the solvent in the chamber. Separately apply 10 µL of each of the Abacavir standard solution, Lamivudine solution and test solution on the line drawn, as spots.

Place the TLC plate having sample spots carefully in the TLC chamber which is equilibrated with solvent vapours. Cover the chamber and leave undisturbed for about one hour. After ensuring that the solvent is no more moving, take out the plate carefully from the TLC chamber and allow to dry under air current. After drying the plate, examine under UV light at 254nm.

Acceptance criteria:

The two principal spots obtained with sample chromatogram should be corresponding to that of standard chromatogram of Abacavir and Lamivudine.

Retention factor of Abacavir and Lamivudine

Sr. No	Name	*Retention factor (about)
1.	Abacavir	0.73
2.	Lamivudine	0.33

* This is for information only.

8.0 COLOR IDENTIFICATIONS:

A. For Iron oxide:

Reagents:

Ammonium Thiocyanate solution:

Dissolve 8 g of ammonium thiocyanate in water to make 100 mL.

Dilute mineral acid (Dilute hydrochloric acid):

Dilute 17mL of concentrated hydrochloric acid to 100mL with water and mix well.

Procedure:

1. To about 4.0 g of the tablets' powder, add 5 mL of concentrated sulfuric acid, and heat gently.
2. Heat till the white fumes no longer appear. Ignite at 600 ± 50°C till the black mass is removed completely.

3. To the residue add 5 mL of concentrated sulphuric acid and digest for 15 minutes. Cool, and dilute to 50 mL with water.
4. Filter, and to 5 mL of the clear filtrate add a few mL of Ammonium Thiocyanate solution: a deep red color develops which does not disappear on addition of dilute hydrochloric acid.

B. For Titanium Dioxide:

Procedure:

1. To about 4.0 g of the tablets' powder, add 3 mL of concentrated sulfuric acid, and heat gently.
2. Heat till the white fumes no longer appear. Ignite at 600 ± 50°C till the black mass is removed completely.
3. To the residue add 5 mL of sulphuric acid and digest for 15 minutes. Cool, and dilute to 50 mL with water.
4. Filter, and to 5 mL of the clear filtrate add a few drops of hydrogen peroxide (30%): a yellow-red to orange-red color develops immediately.

9.0 AVERAGE WEIGHT:

Procedure:

Perform the test on 20 tablets. Measure the average weight by using following formula.

$$\text{Average weight (mg)} = \frac{\text{Weight of 20 tablets (g)}}{20} \times 1000$$

10.0 LOSS ON DRYING:

Procedure:

Crush not less than 4 tablets to get sufficient tablets' powder for analysis.

Proceed as directed under the General Test Procedure "Loss on drying – USP" GTP No.: GTP005# at 105°C for 3 hours.

11.0 MICROBIOLOGICAL TEST:

Procedure:

Proceed as directed under the General Test Procedure, "Microbiological testing of Non Sterile Products (Microbial Enumeration tests and Tests for Specified Microorganisms)" GTP No.: GTP004#.

12.0 THICKNESS:

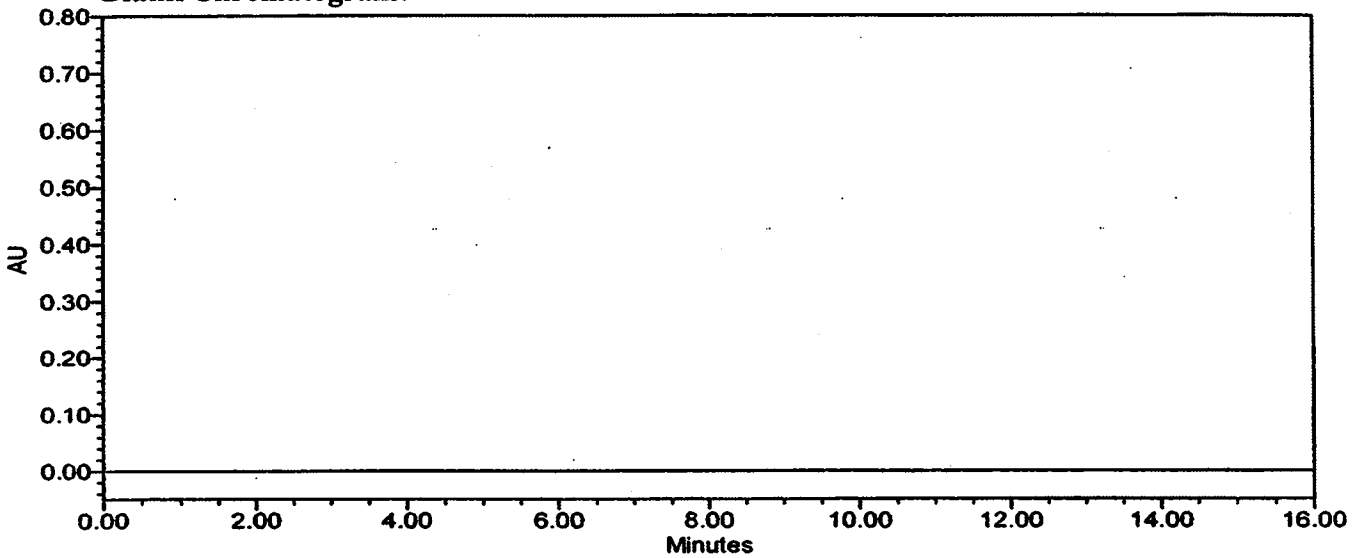
Procedure:

Perform the test on 10 tablets. Measure the thickness of each tablet using Vernier Calipers.

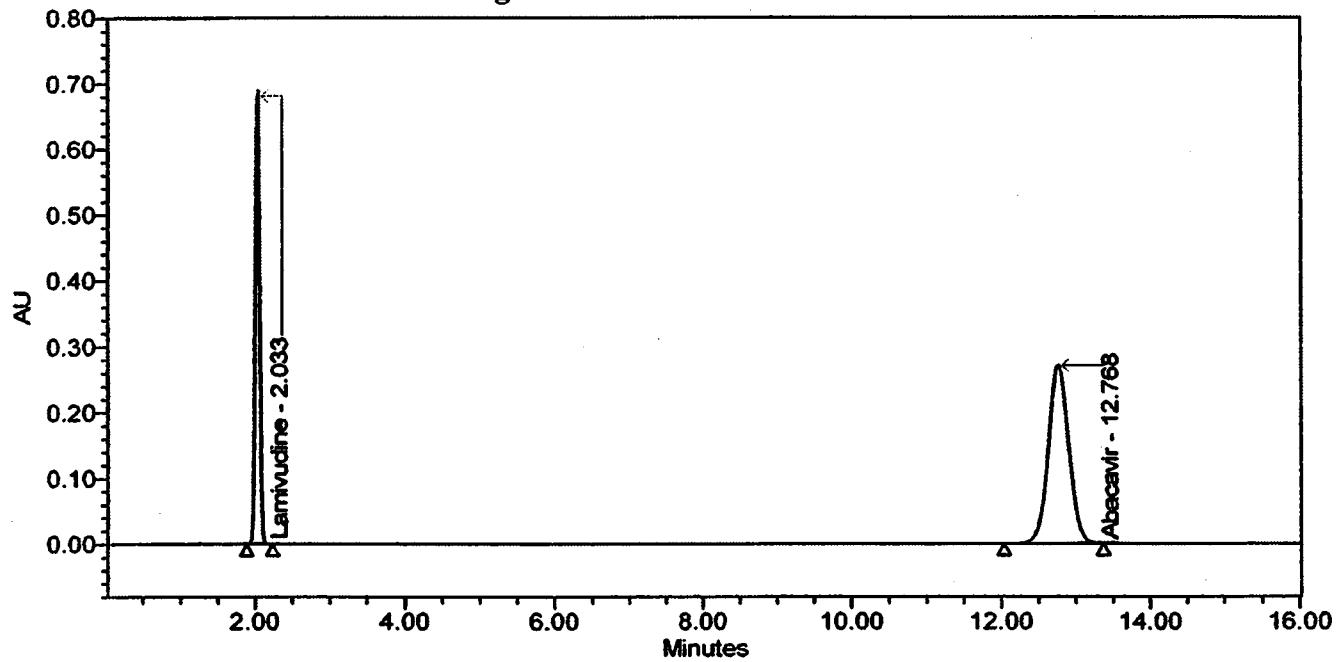
Follow the current version of the GTP

Typical Chromatograms for Dissolution (By HPLC):

Blank Chromatogram:

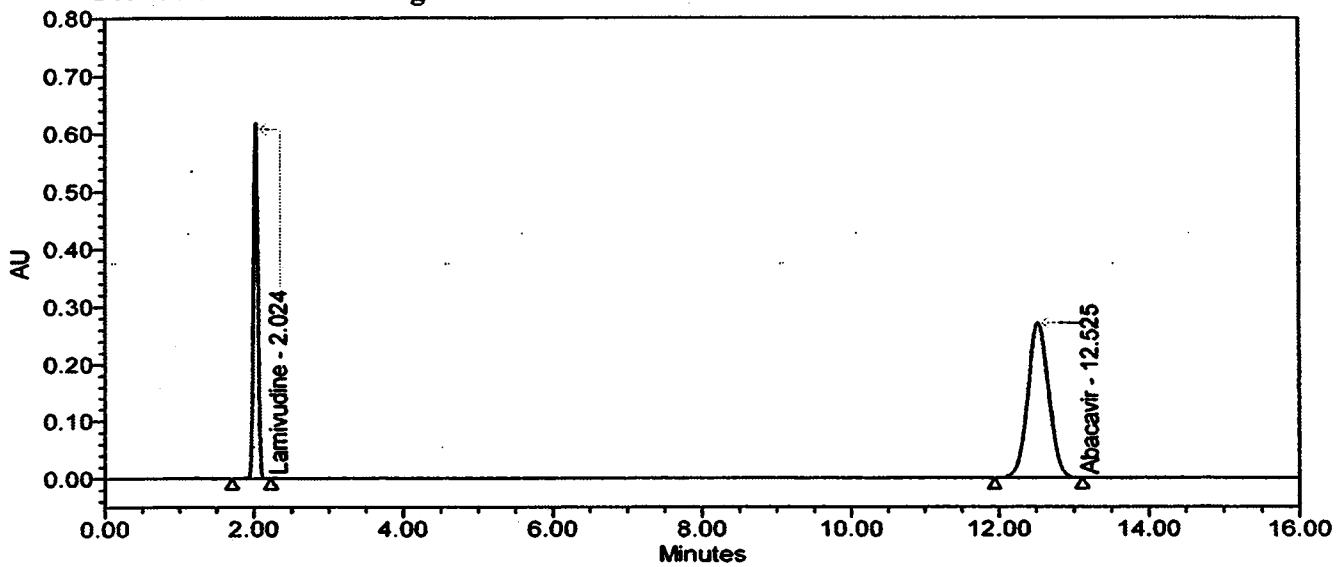


Standard Solution Chromatogram:



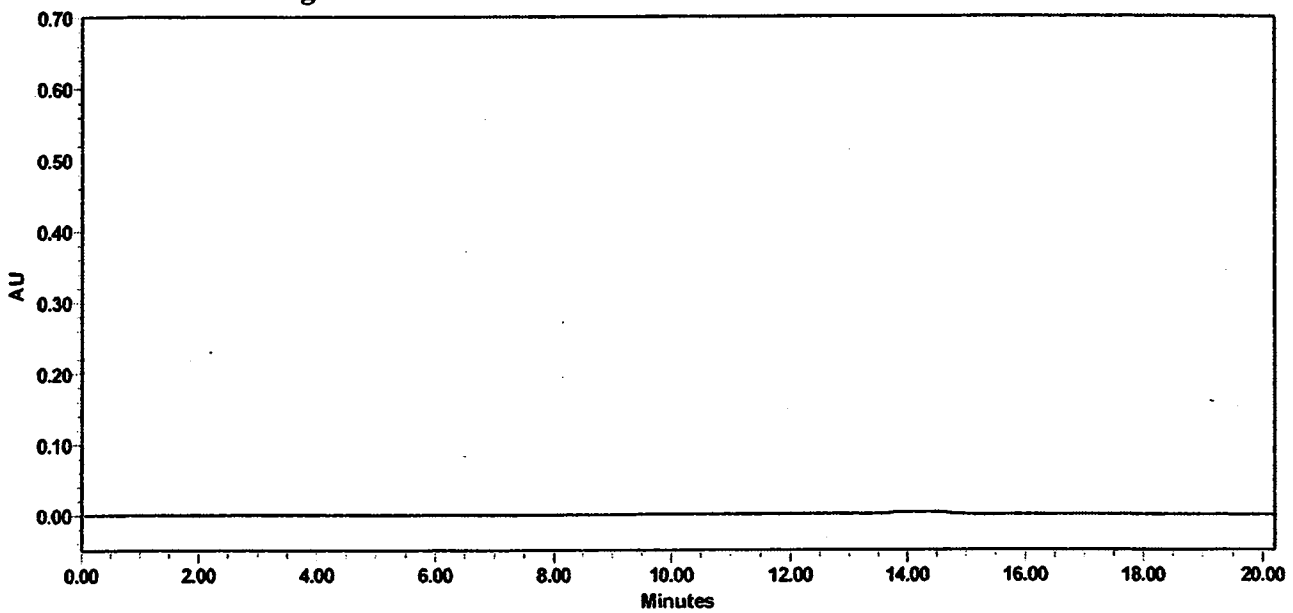
	Prepared by (QA)	Reviewed by (QA)
Sign & Date	RMP 22/10/20	23/10/20

Test Solution Chromatogram:



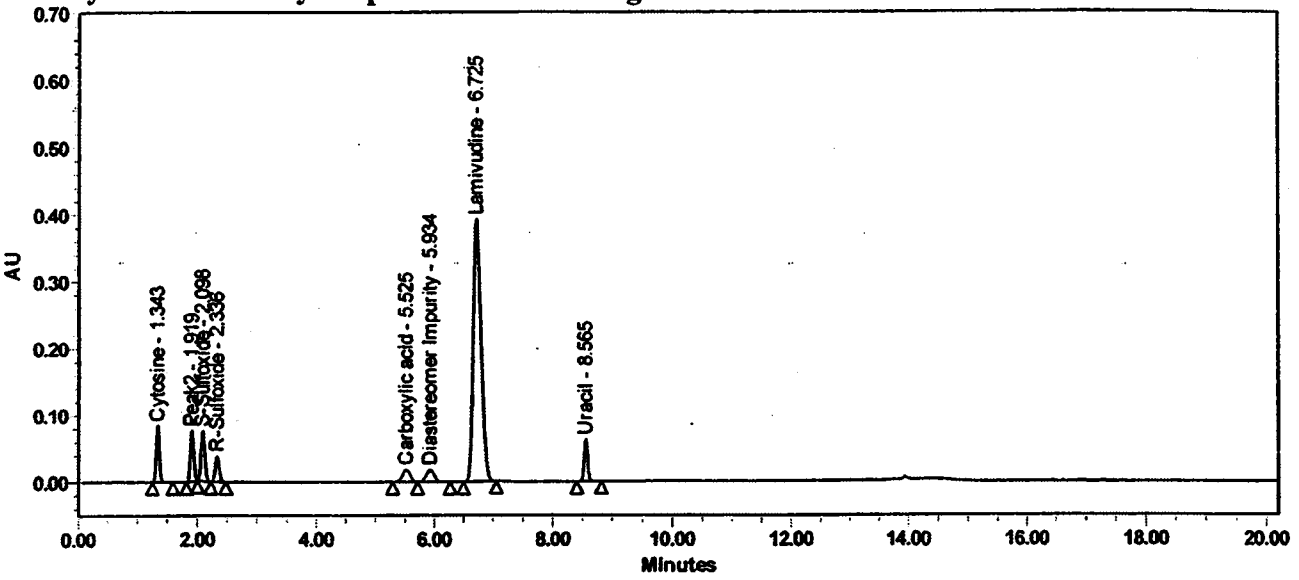
Typical Chromatograms for Uniformity of Dosage Units (By Content Uniformity) and Assay (By HPLC):

Blank Chromatogram:

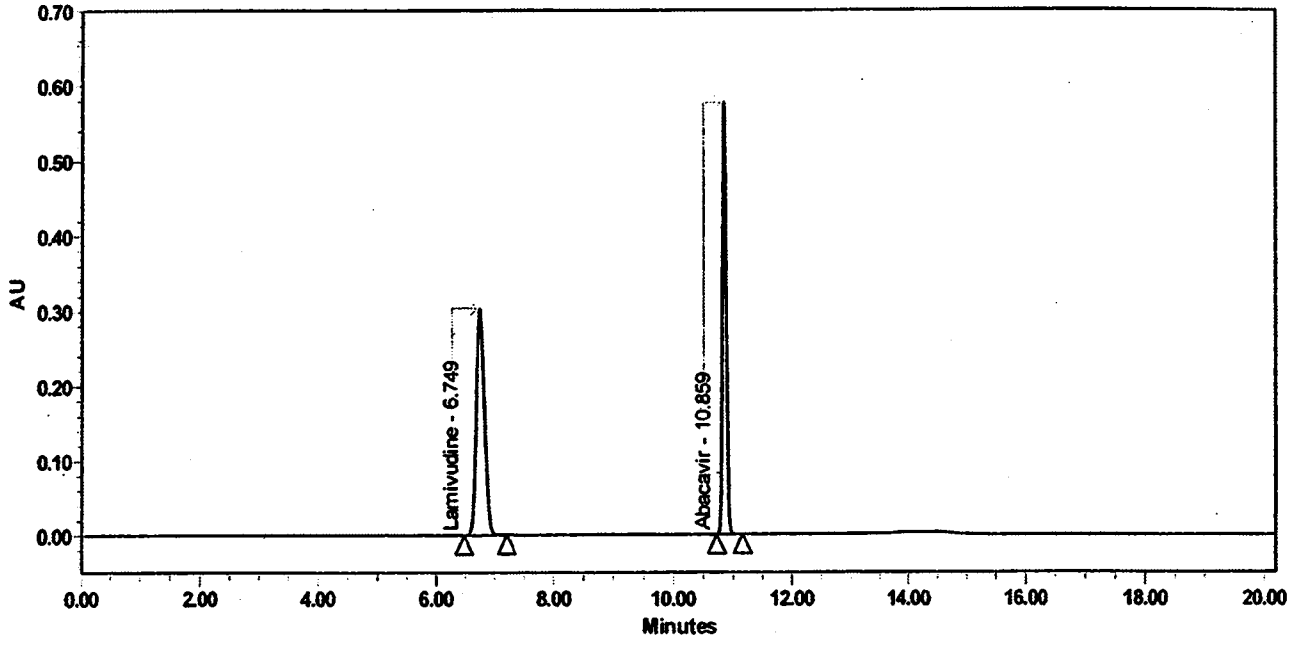


	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	23/10/20

System Suitability Preparation Chromatogram:

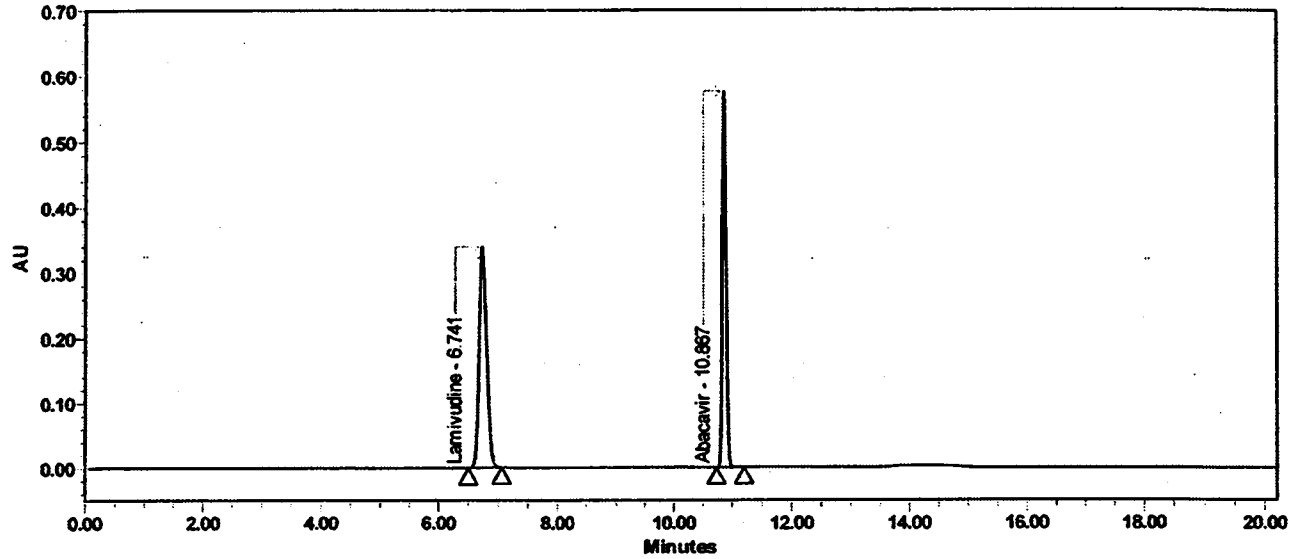


Standard Preparation Chromatogram:



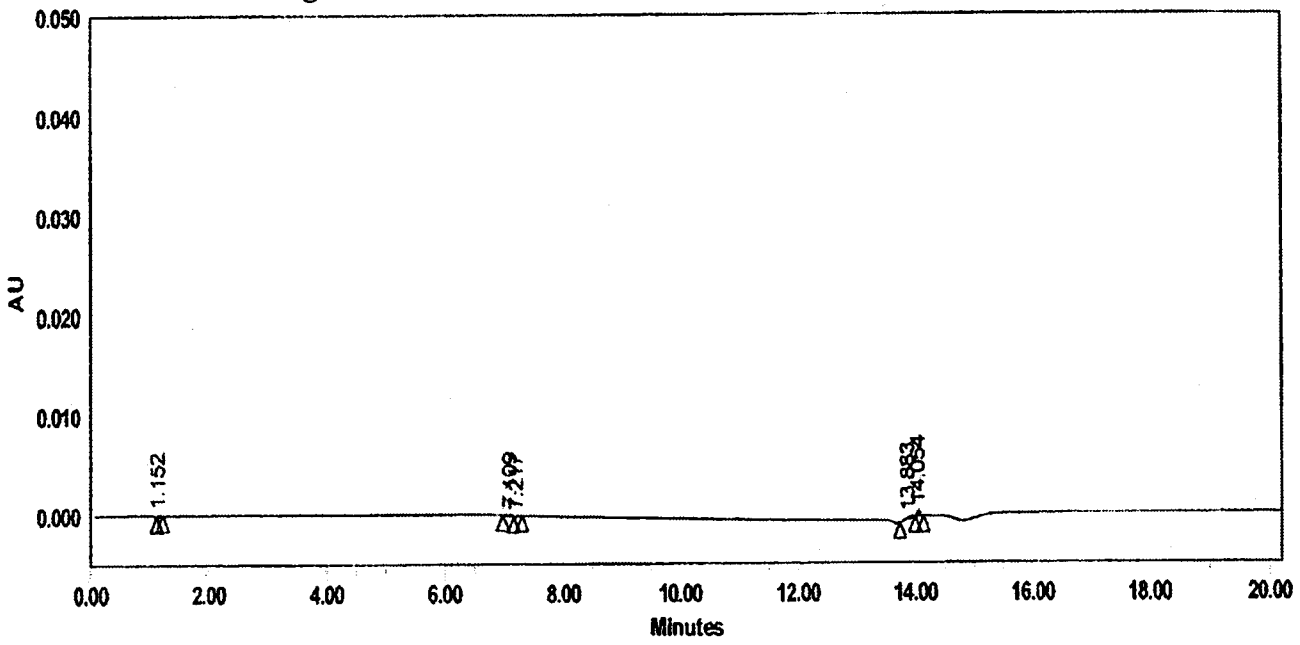
	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	23/10/20

Test Preparation Chromatogram:

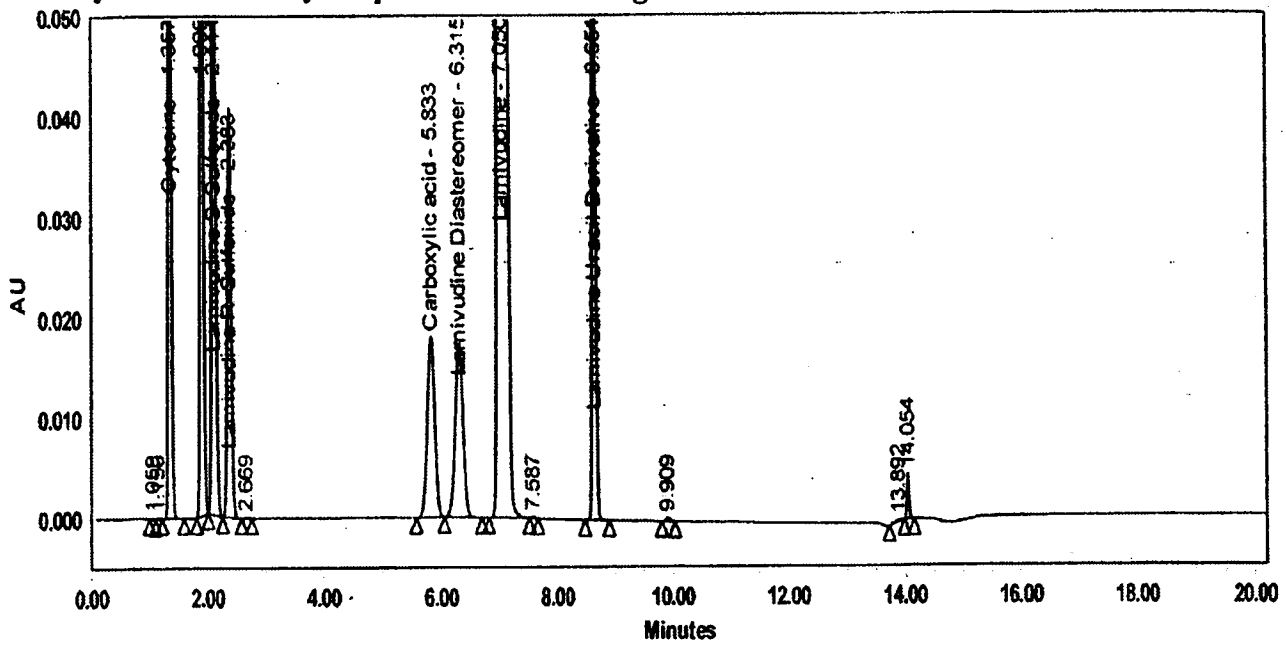


Typical Chromatograms for Organic Impurities (By HPLC):

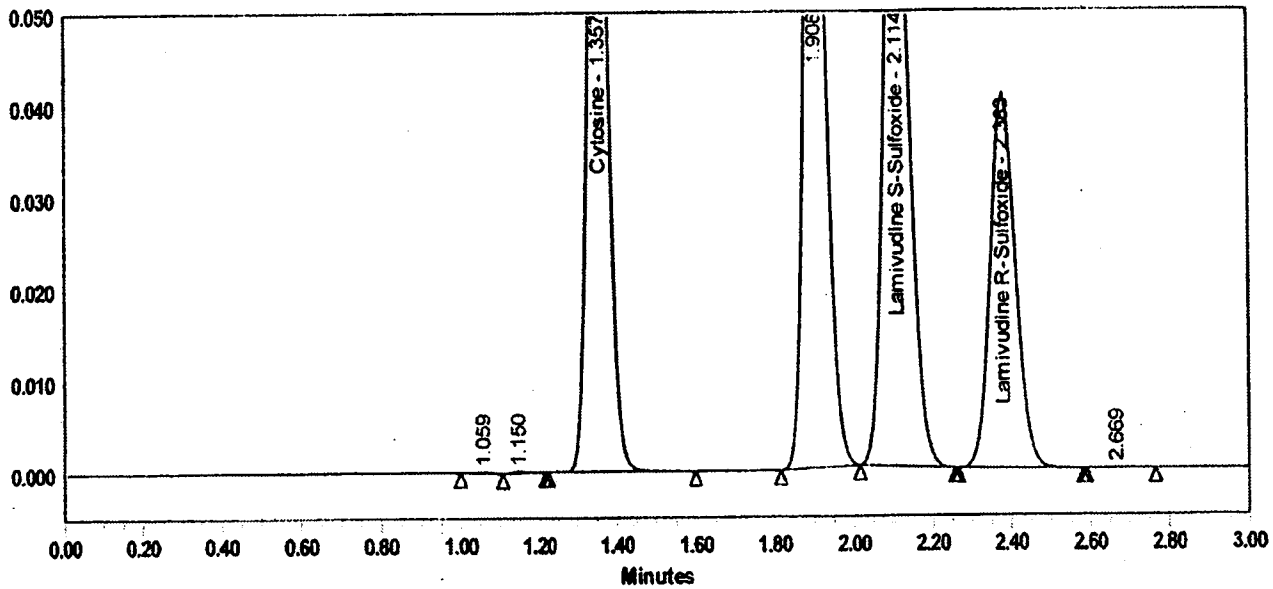
Blank Chromatogram:



Systcm Suitability Prcparation Chromatogram:

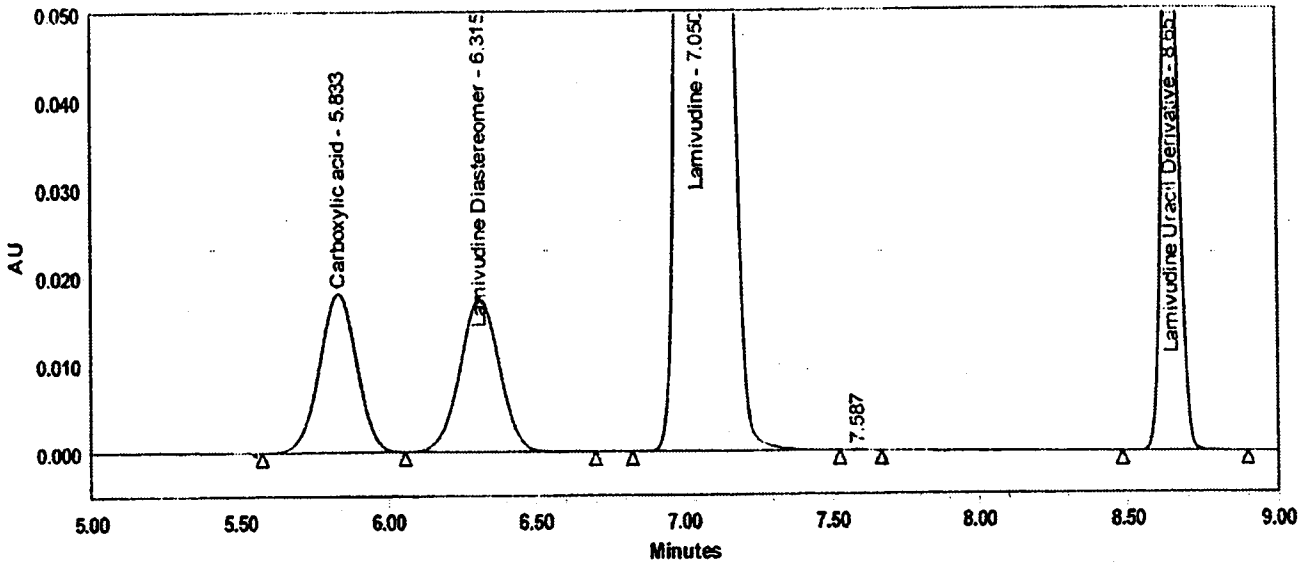


Zoomed Chromatogram 1:

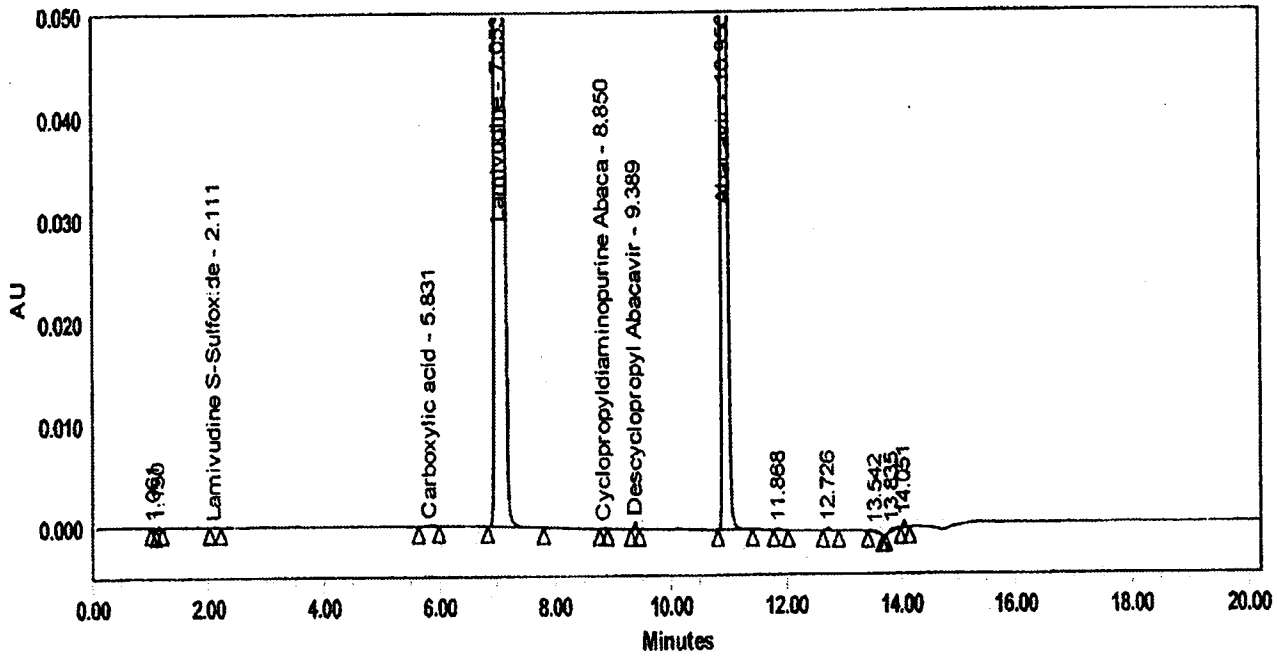


	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	23/10/20

Zoomed Chromatogram 2:

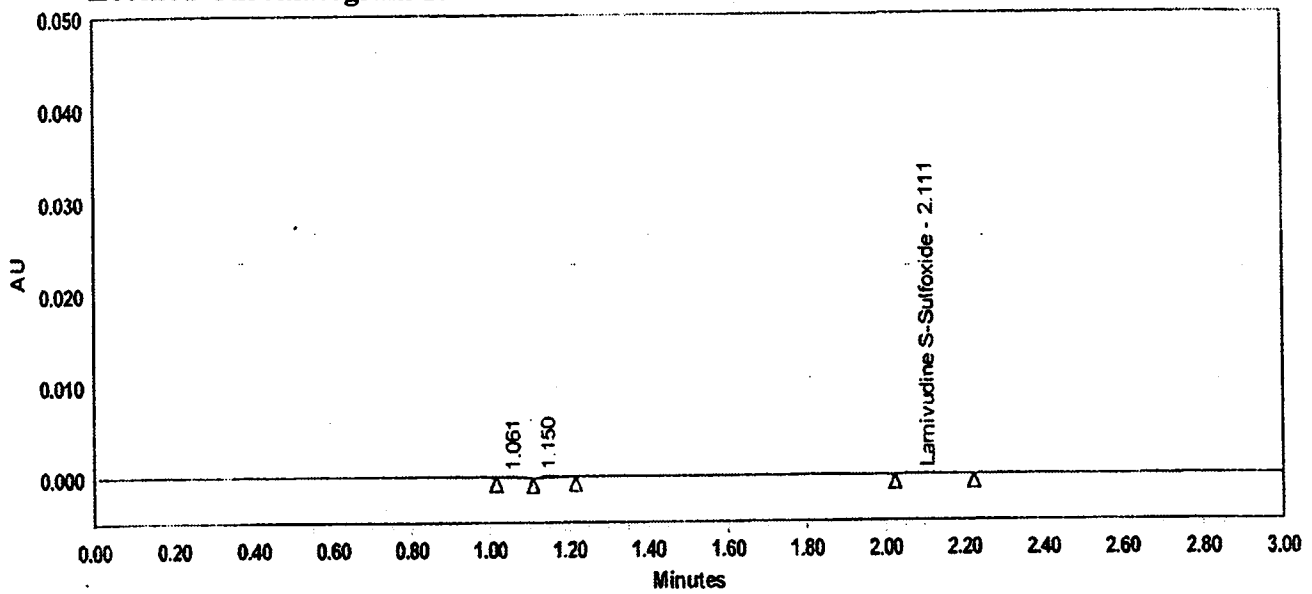


Standard Preparation Chromatogram:

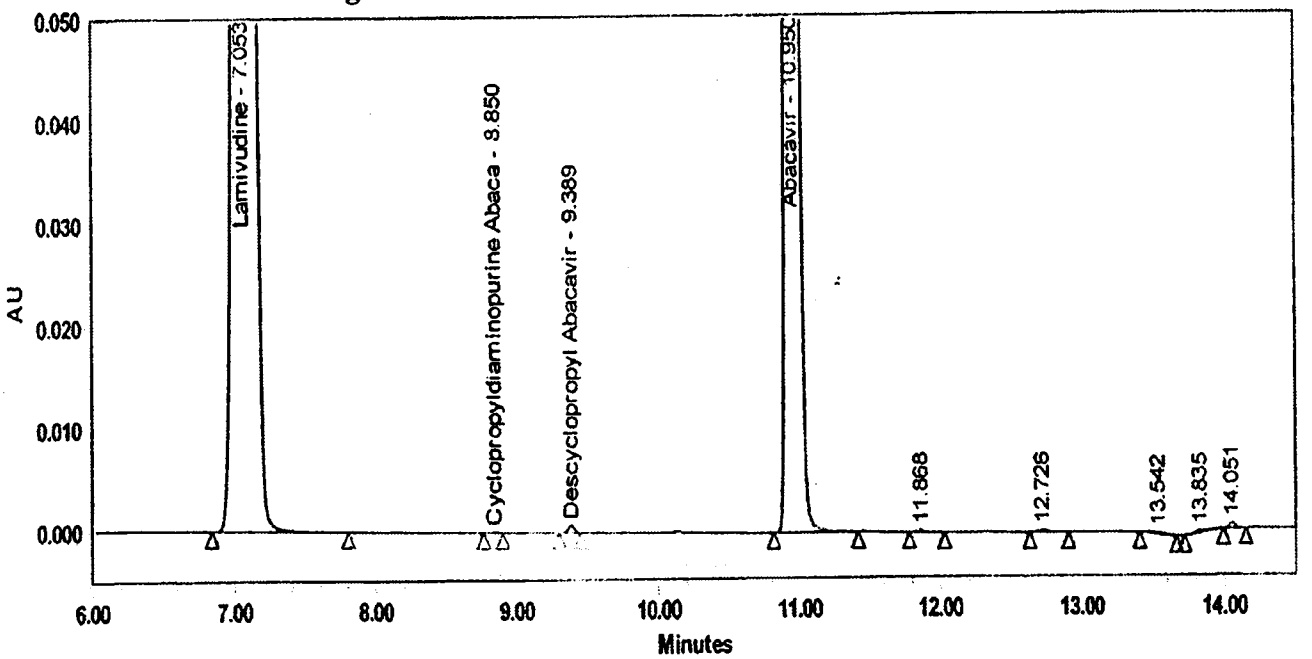


	Prepared by (QA)	Reviewed by (QA)
Sign & Date	RMP 22/10/20	23/10/20

Zoomed Chromatogram 1:

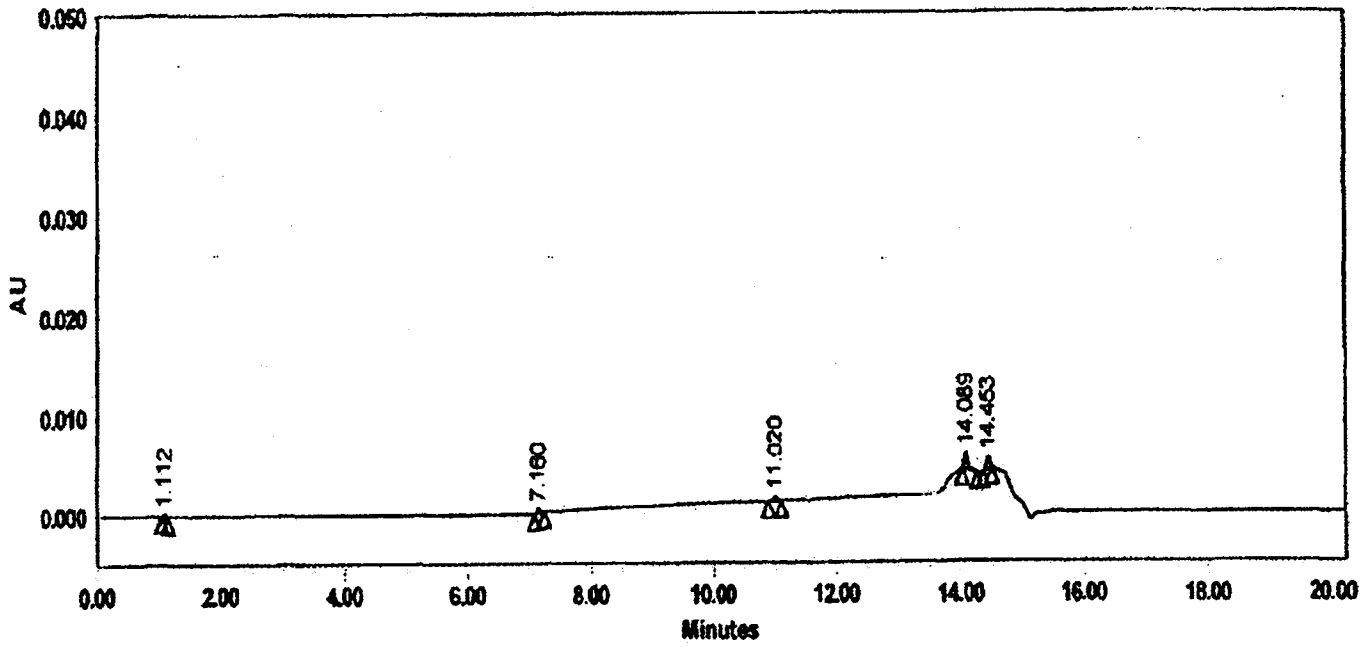


Zoomed Chromatogram 2:

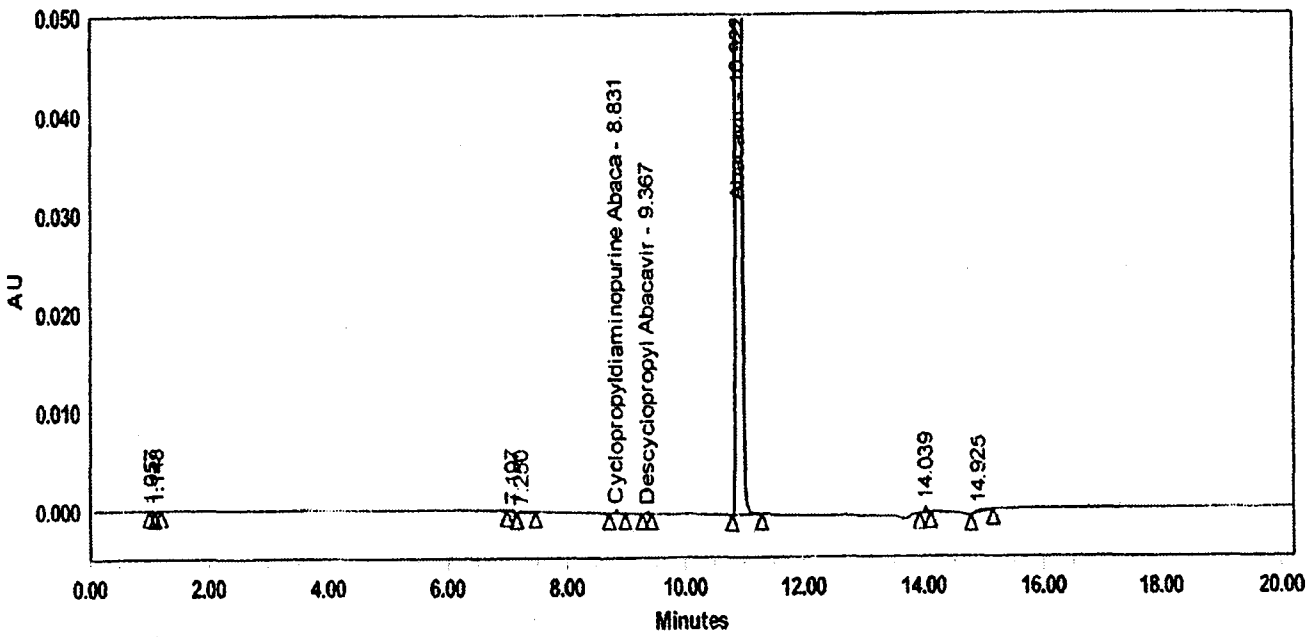


	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	23/10/20

Plain Placebo Preparation Chromatogram:

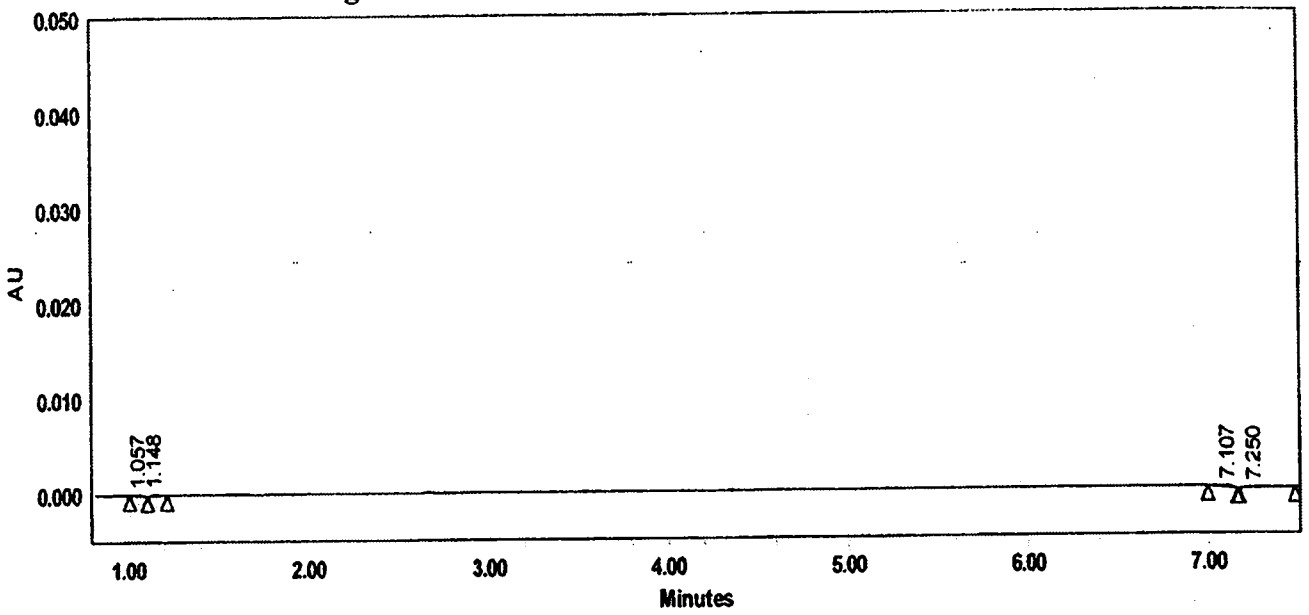


Placebo with Abacavir Sulfate Chromatogram:

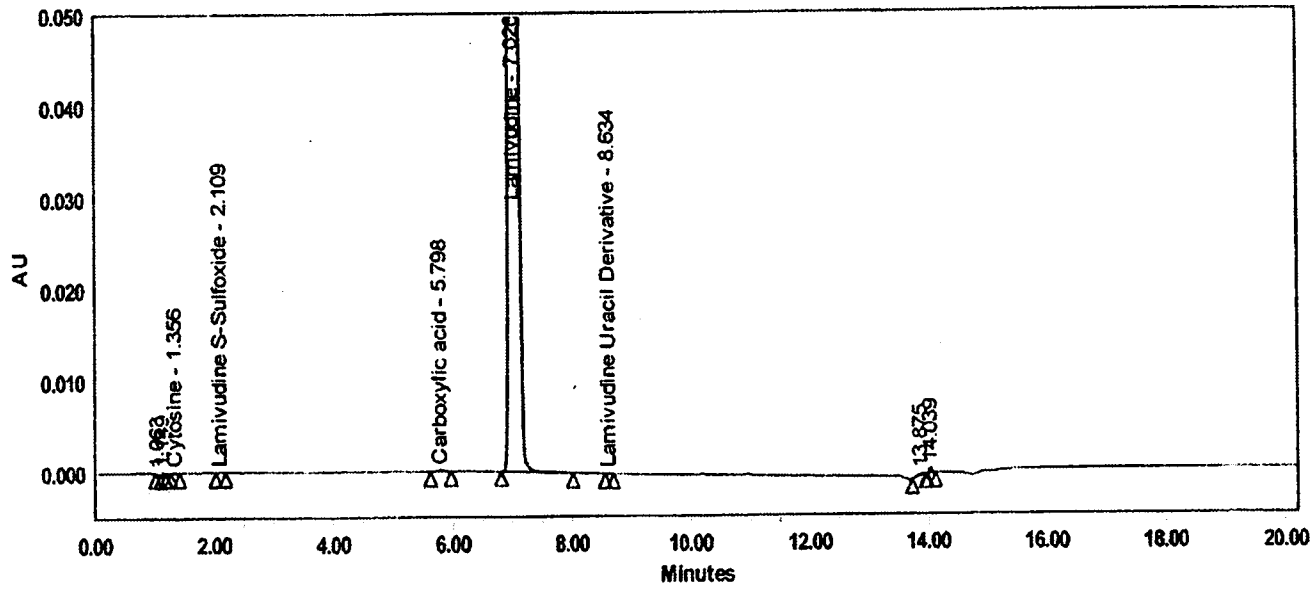


	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	23/10/20

Zoomed Chromatogram:

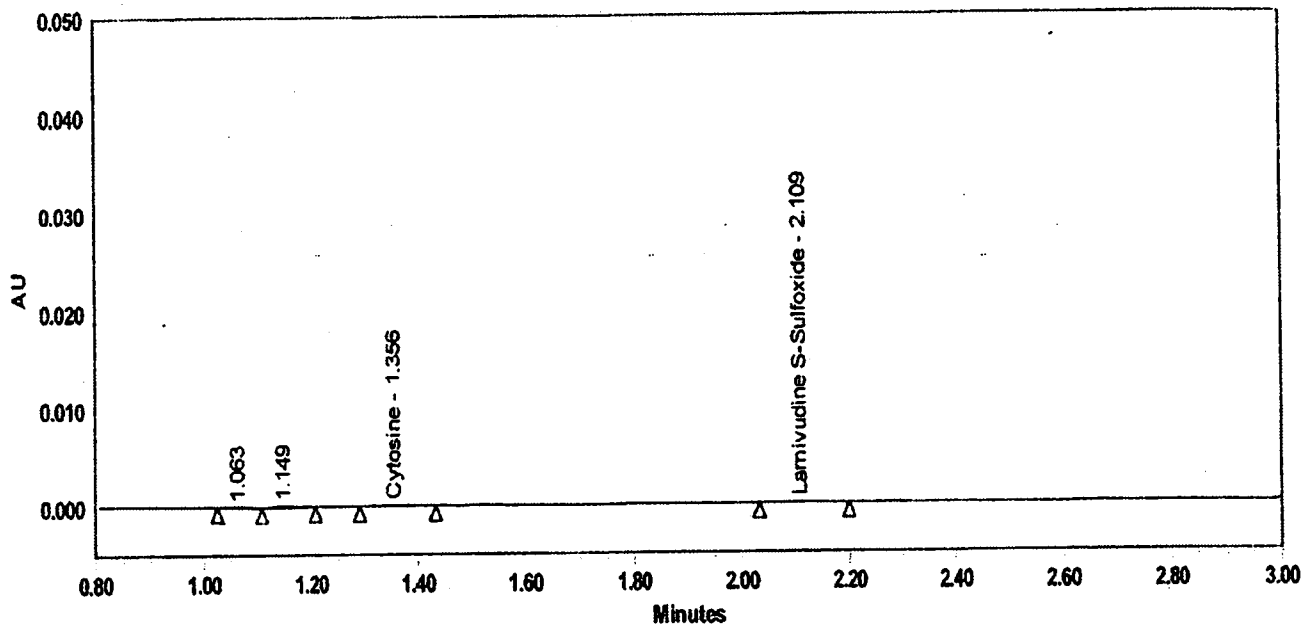


Placebo with Lamivudine Chromatogram:

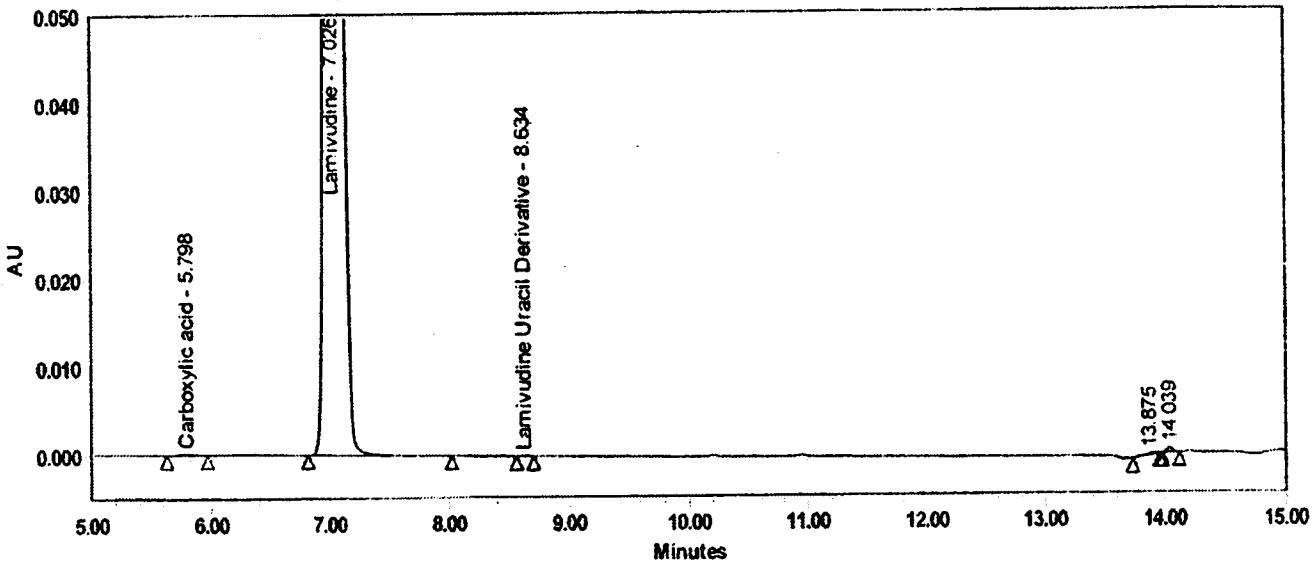


	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	23/10/20

Zoomed Chromatogram 1:

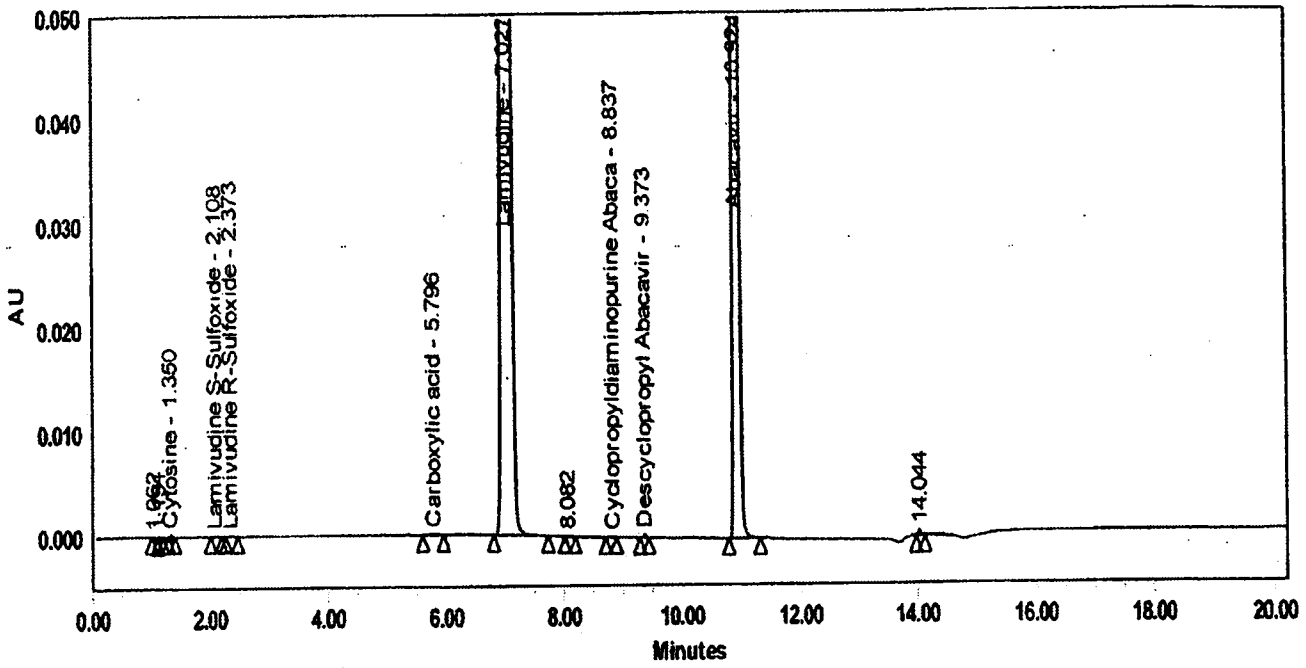


Zoomed chromatogram 2:

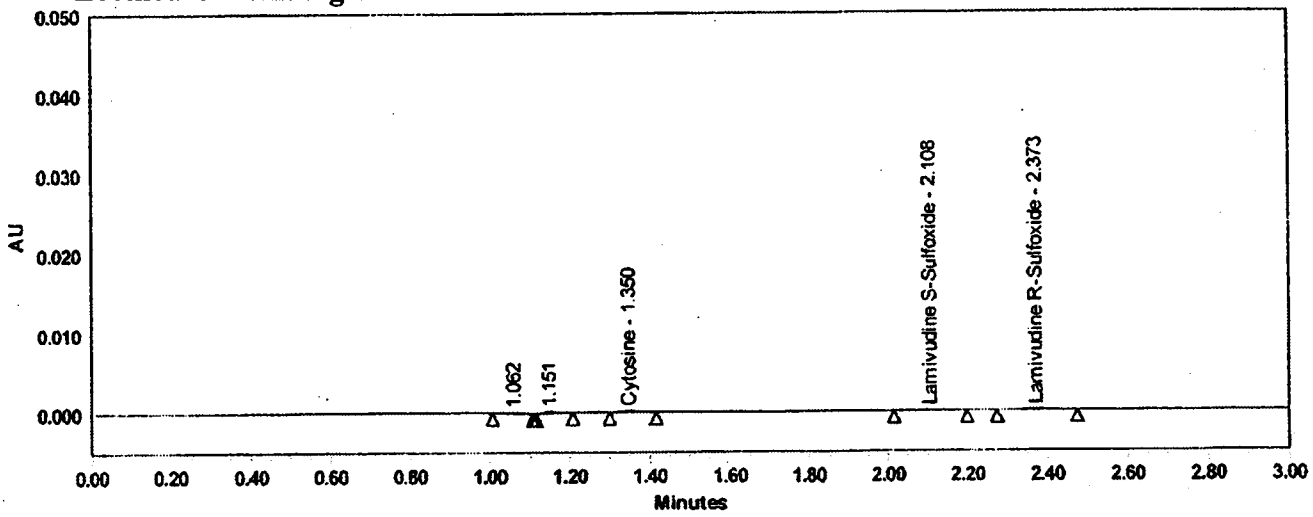


	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	23/10/20

Test Preparation chromatogram:



Zoomed Chromatogram:



	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	23/10/20

Change History:

Effective Date	STP No.	Supersedes	Change Details
24 DEC 2012	FPPABL507R-00	Nil	New STP
08 DEC 2020	FPPABL507R-01	FPPABL507R-00	As per Change Control PR Number 2293240 1. Format has been revised as per revised SOP. 2. At the beginning of the STP, 'Notes' have been newly incorporated. 3. Following changes have been made to make inline with USP:- 3.1 Name of the product has been revised to 'Abacavir and Lamivudine Tablets USP' from 'Abacavir Sulfate and Lamivudine Tablets'. 3.2 Under the column 'Reference(s)', 'IH' has been revised to 'USP + IH'. 3.3 Under the tests 'Uniformity of Dosage Units (By Content uniformity)' and 'Assay (By HPLC)' methods have been changed. 3.4 The test 'Related substances (By HPLC)' has been renamed as 'Organic Impurities (By HPLC)' and its method has been changed. 3.5 Under the test 'Loss on drying' General Chapter reference has been revised to 'USP' from 'Ph.Eur.' 3.6 The molecular weight of Abacavir Sulfate has been corrected to '670.74' from '670.76' and a statement 'multiplied by 2' has been added to the molecular weight of 'Abacavir', wherever it is applicable. 3.7 The test for 'Identification (By Thin-layer chromatography)', 'Color Identifications', 'Average weight', 'Loss on drying', 'Microbiological Test' and 'Thickness' have been covered under 'Additional Tests'. 3.8 Under the test procedure "Microbiological Test" the GTP reference has been renamed from

	Prepared by (QA)	Reviewed by (QA)
Sign & Date	22/10/20	23/10/20

Effective Date	STP No.	Supersedes	Change Details
			"Microbiological Test" to "Microbiological testing of Non Sterile Products (Microbial Enumeration tests and Tests for Specified Microorganisms)". 3.9 Under the tests Dissolution (By HPLC), Uniformity Of Dosage Units (By Content Uniformity, 'Assay (By HPLC)' & 'Organic Impurities (By HPLC)' in "Procedure", the term "(Refer Typical chromatograms)" has been newly incorporated. Accordingly, typical chromatograms have been incorporated at the end of the STP.