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Development and Validation of Stability Indicating RP-UPLC Method for Quantitative Estimation of Lamivudine in Tablet Dosage Form

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase ultra performance liquid Chromatography assay method has been developed for estimation of Lamivudine in tablet formulations. The separation was achieved by using column Acquity UPLC BEH Phenyl (100×2.1mm, 1.7µm), in mobile phase consisted of pH 3.8 ammonium acetate buffer and methanol. The flow rate was 0.5mL.min-1 and the separated Lamivudine was detected using UV detector at the wavelength of 277 nm. The retention time of Lamivudine was noted to be 2.50 min respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: Liquid chromatography; Lamivudine, Validation and Reproducible.

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INTRODUCTION

Lamivudine, is chemically 4-amino-1-[(2R, 5S)-2-(hydroxymethyl) - 1, 3-oxathiolan-5-yl]-1,2-dihydropyrimidinone-2-one [1-4]. E K Kano et al, evaluated the Lamivudine in human plasma by HPLC, finally they concluded that the two Lamivudine formulations are bioequivalent in their rate and extent of absorption, and thus, may be used interchangeably [5]. Bengi Uslu etal, determined the binary mixture of Lamivudine and Zidovudine by first derivative spectrophotometric, first derivative of the ratio-spectra and high performance liquid chromatography–UV methods [6]. Namita Kapoor et al, performed simultaneous determination of Lamivudine and Stavudine in antiretroviral fixed dose combinations by first derivative spectrophotometry and high performance liquid chromatography [7]. But there is no simple method for the analysis of Lamivudine. Hence, it is necessary to develop a rapid, accurate and validated RP-UPLC method for the determination of Lamivudine in tablet dosage form. The method proved to be simple model since it does not contain a buffer system. The present study illustrates development and validation of a simple, accurate and precise procedure for determination of Lamivudine by RP-UPLC.

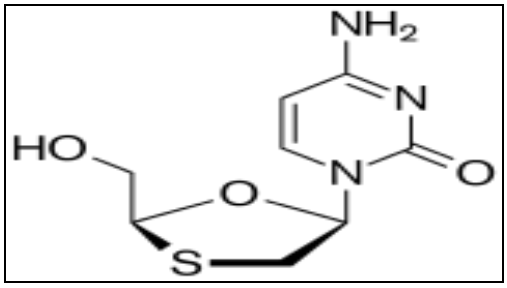


Figure 1: Structure of Lamivudine

Hence, a new sensitive, economical, stability indicating RP-UPLC method was developed and validated in accordance with ICH guidelines by the author.

MATERIALS AND METHOD

Chemicals and reagents:

Analytical-grade Ammonium acetate, Glacial acetic acid, was from Merck chemicals Mumbai, India. Methanol, Acetonitrile and water, both HPLC-grades, were from Merck chemicals. Mumbai, India. Millex syringe filters (0.45 µm) were from Millex-HN, Millipore Mumbai, India.

Instrumentation:

Waters-Acquity H Class equipped with Empower 2 software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) were use in the present assay.

Preparation of 0.025M Ammonium Acetate Buffer (Mobile Phase A):

1.9gms of Ammonium acetate was accurately weighed and transferred in 1000ml beaker, add 900ml of HPLC grade water was added and dissolved, then the pH of the solution was adjusted to 3.8±0.05 with Glacial acetic acid and made up to volume with water. The solution was filtered through 0.22µm membrane filter.

Mobile phase B:

100% Methanol filtered through 0.22µm membrane filter.

Diluent preparation:

Use Milli-Q water.

Standard preparation:

Accurately weighed 33.4 mg of Lamivudine working standard or reference standard was transferred into a 200 ml volumetric flask. 180 ml of diluent was added and sonicated to dissolve. The solution was diluted to volume with diluent and mixed well. (Concentration of Lamivudine is about 167 ppm).

Sample preparation:

Accurately weighed portion of a powder equivalent about 33.4 mg of Lamivudine is transferred to a 200 ml volumetric flask. 180 ml of diluent was added and sonicated to dissolve for 10 minutes with intermediate shaking. Cool to room temperature, dilute up to volume with diluent and mixed well. Filter the above solution through 0.45µ PVDF syringe filter. (Concentration of Lamivudine is about 167 ppm).

Chromatographic conditions:

Chromatographic analysis was performed on Acquity UPLC BEH Phenyl 100×2.1mm, 1.7µm column. The mobile phase consisted of pH 3.8 ammonium acetate buffer and methanol. The flow rate was 0.5mL/min, column oven temperature 40°C, the injection volume was 2µL, and detection was performed at 277 nm using a photodiode array detector (PDA).

RESULTS AND DISCUSSION

Method development:

Spectroscopic analysis of compound Lamivudine showed that maximum UV absorbance (λmax) at 277 nm respectively. To develop a suitable and robust LC method for the determination of Lamivudine, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Acquity UPLC BEH Phenyl 100×2.1mm, 1.7µm with the following different mobile phase compositions like that 1% OPA pH 1.8 and methanol in the ratio of 95:5 v/v. 0.1% OPA pH adjusted to 3.0 with triethylamine and methanol in the ratio of

90:10 v/v . Phosphate Buffer pH 4.5 and acetonitrile in the ratio of 95:5 v/v. Acetate Buffer pH 3.2 and methanol in the ratio of 98:2 v/v. It was observed that when Lamivudine was injected, higher retention time, Peak Tailing, not satisfactory.

For next trial the mobile phase composition was change same but pH of the acetate buffer slightly increased 3.2 to 3.8. The mobile phase composition was acetate buffer pH 3.8 and methanol in the ratio of 98:2 v/v. respectively as eluent at flow rate 0.5mL/min. UV detection as performed at 277nm. The retention time of Lamivudine is 2.50 minutes and the peak shape was good. The chromatogram of Lamivudine standard using the proposed method is shown in (Fig: 2) system suitability results of the method are presented in Table 1.

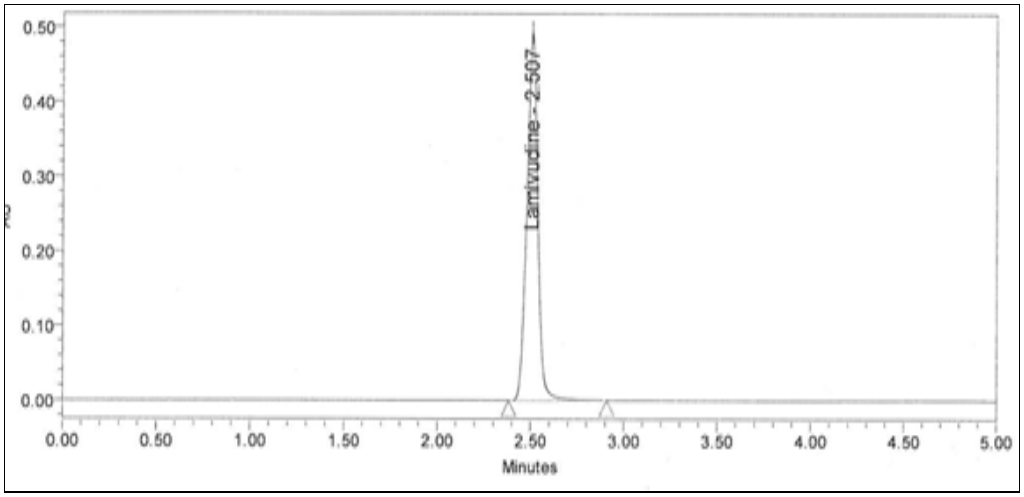


Figure 2: Chromatogram showing the peak of Lamivudine

Method validation:

The developed RP-UPLC method extensively validated for assay of Lamivudine using the following parameters.

Specificity:

Preparation of blank solution: Use Milli-Q water as a blank solution.

Preparation of Placebo solution: Accurately weighed portion of a placebo, equivalent about 33.4 mg of Lamivudine is transferred to a 200 ml volumetric flask. 180 ml of diluent was added and sonicated to dissolve for 10 minutes with intermediate shaking. Cool to room temperature, dilute up to volume with diluent and mixed well. Filter the solution through 0.45µ PVDF syringe filter.

Blank and Placebo interference: A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution (Fig: 3) showed no peak at the retention time of Lamivudine

peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Lamivudine in Lamivudine tablets. Similarly chromatogram of placebo solution (Fig: 4) showed no peaks at the retention time of Lamivudine peak. This indicates that the placebo used in sample preparation do not interfere in estimation of Lamivudine in Lamivudine tablets.

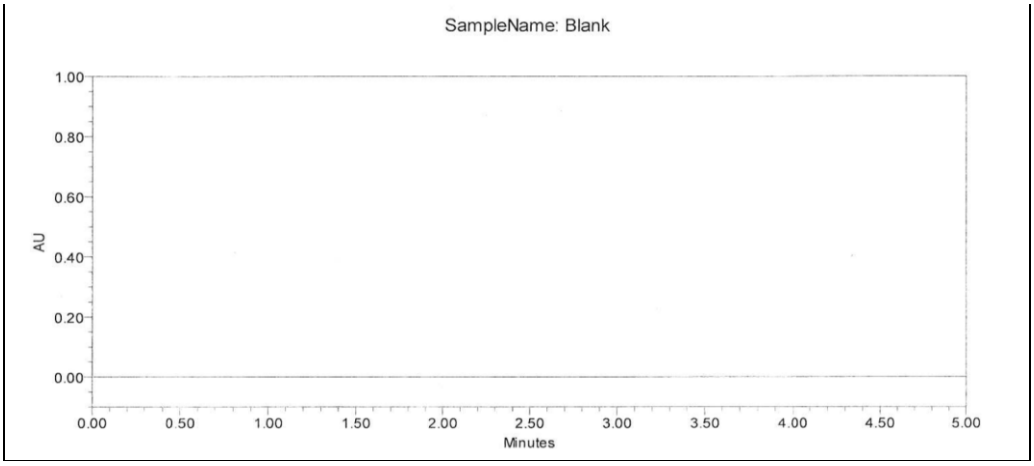


Figure: 3 Chromatogram showing the no interference of diluent for Lamivudine.

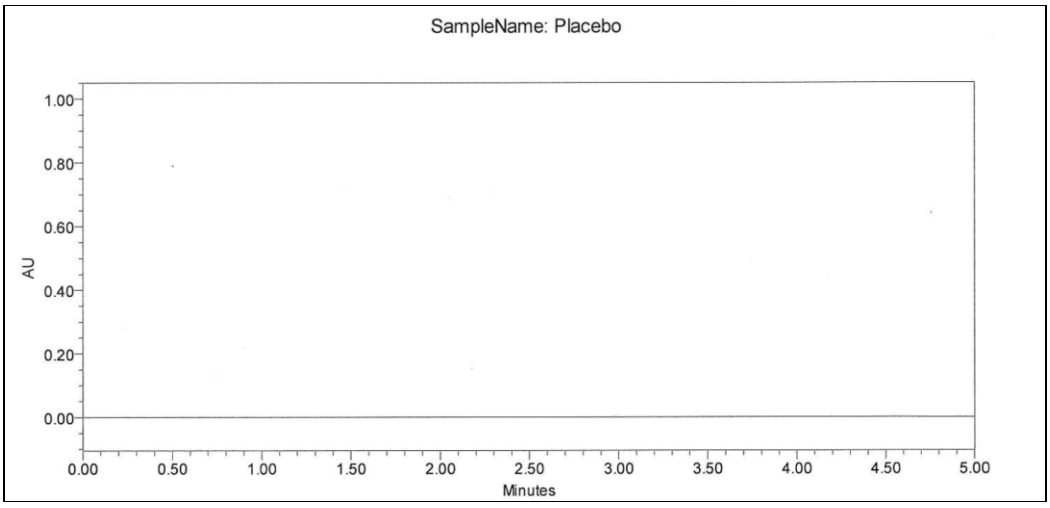


Figure: 4 Chromatogram showing the no interference of placebo for Lamivudine.

Table 1: System suitability parameters for Lamivudine by proposed method

S.No	Name	Retention time	Mean Area	USP Tailing	USP Plate Count
1	Lamivudine	2.570	1946365	1.4	3883

Method precision:

The precision of test method was evaluated by doing assay for six samples of Lamivudine tablet as per test method. The content in mg and % label claim for Lamivudine for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The chromatogram was shown in Figure: 5 and data were shown in Table: 3.

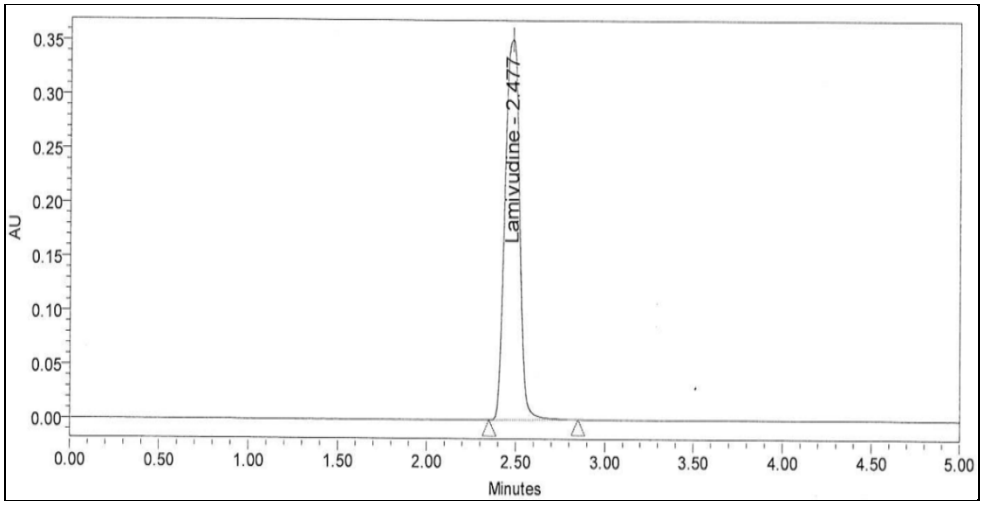


Figure: 5 Method precision sample chromatogram

Table: 3 Method precision data for Lamivudine

No. of injections	Lamivudine % assay
1	97.9
2	97.4
3	97.4
4	97.3
5	97.8
6	96.8
Average	97.4
%RSD	0.4

Linearity of detector response:

The standard curve was obtained in the concentration range of 16.7-250.5µg/ml for Lamivudine. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in Figure: 5 to demonstrate the linearity of the proposed method. From the data obtained which given in Table: 4 the method was found to be linear within the proposed range.

Table: 4 Linearity studies for Lamivudine by proposed method

S.No	Linearity Level (%)	Concentration of Lamivudine (µg/ml)	Area of Lamivudine		
			Injection 1	Injection 2	Mean Area
1	10	16.7	196914	197198	197056
2	20	33.4	404385	405882	405134
3	50	83.5	1023594	1022582	1023088
4	80	133.6	1641733	1642043	1641888
5	100	167.0	2014949	2023285	2019117
6	120	200.4	2429322	2424485	2426904
7	150	250.5	3050553	3048126	3049340

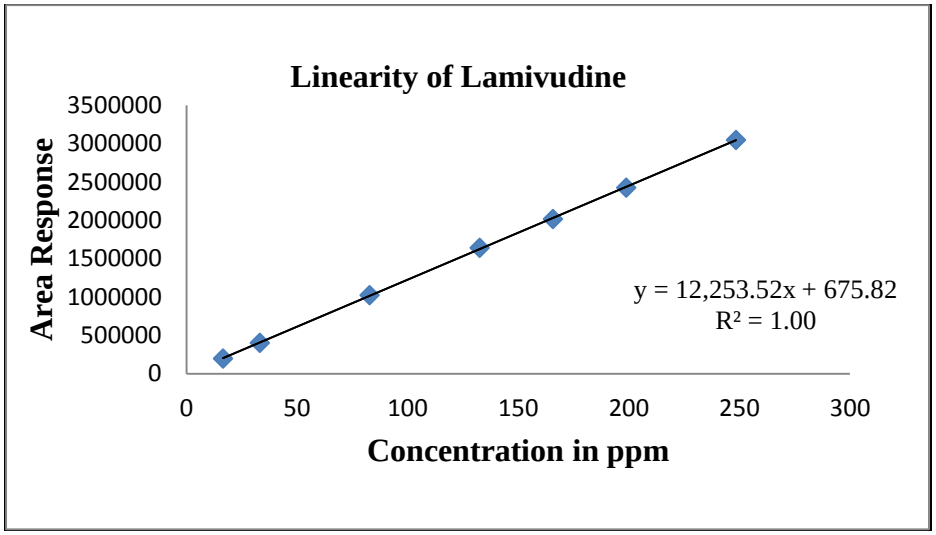


Figure: 5 Calibration curve for Lamivudine

Accuracy:

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Lamivudine, analyzed as per the proposed method. The percentage recoveries with found in the range of 99.0 to 99.9 for Lamivudine. The chromatogram was shown in Figure: 6 to 8 the data obtained which given in Table: 5 the method was found to be accurate.

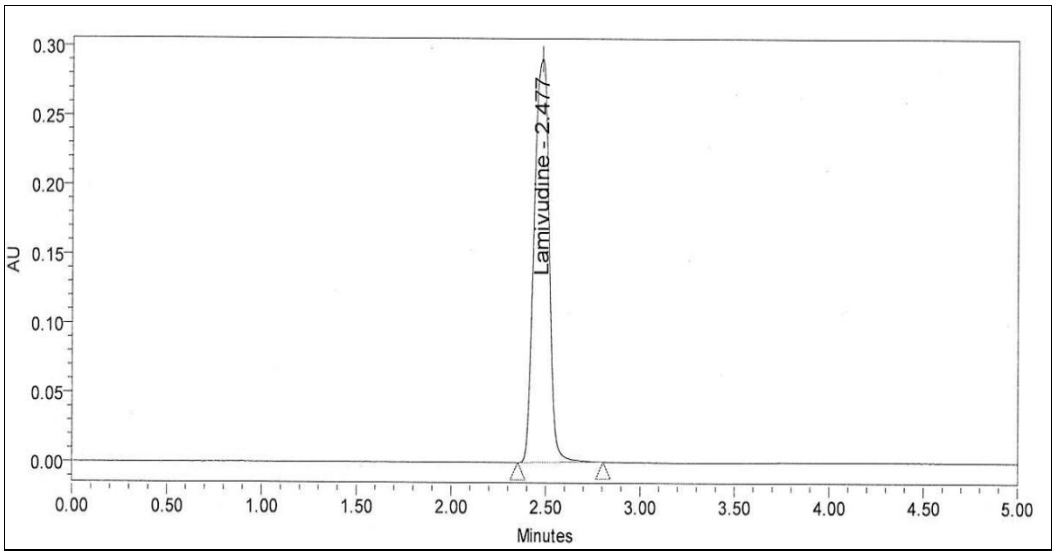


Figure: 7 Accuracy (Spike level 80%) chromatogram

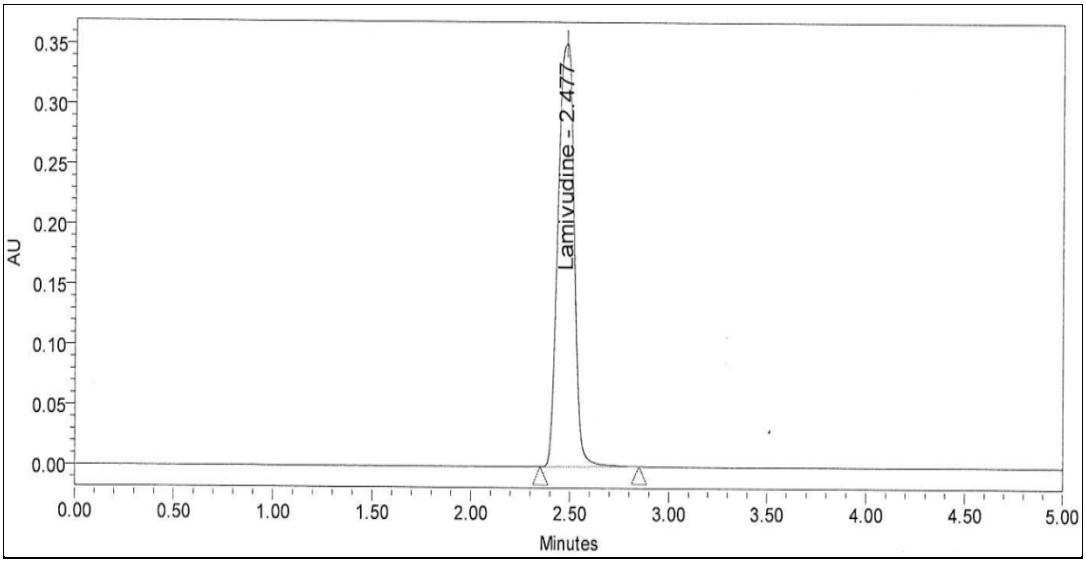


Figure 8 Accuracy (Spike level 100%) chromatogram

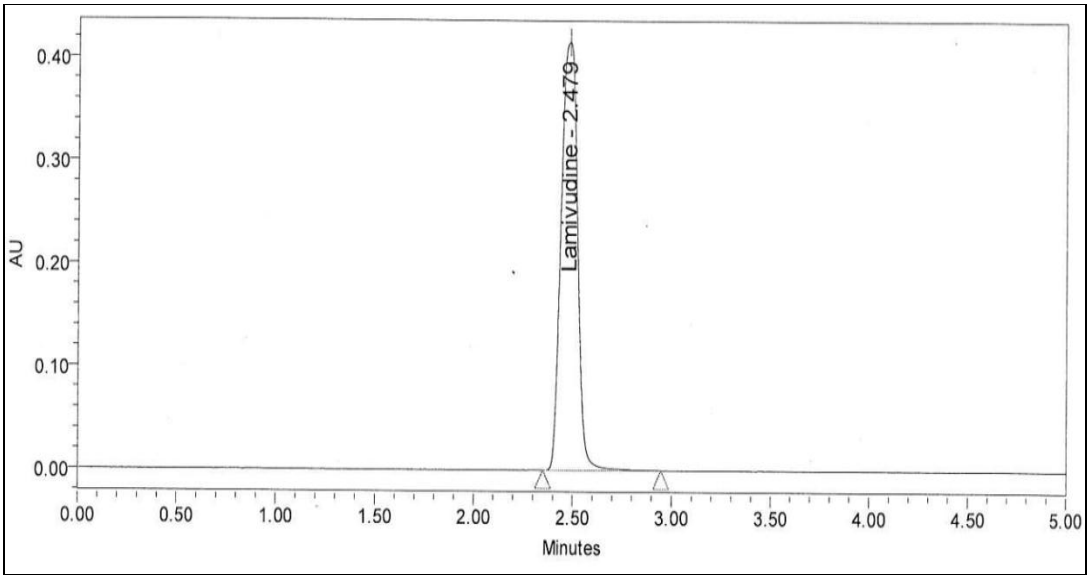


Figure: 9 Accuracy (Spike level 120%) chromatogram

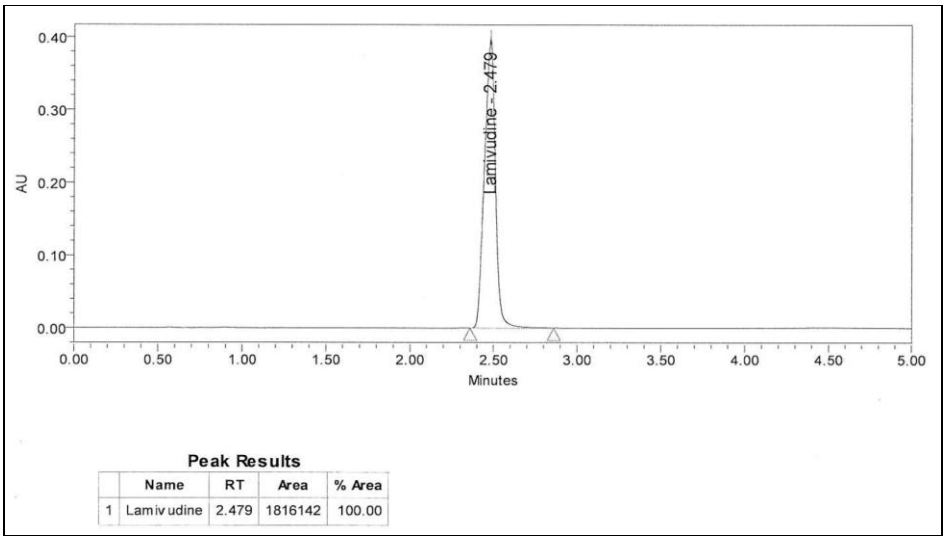
Table: 5 Recovery studies for Lamivudine by proposed method

Recovery Level	Lamivudine				
	Amount Added(mg)	**Amount recovered (mg)**	**Percentage recovery**	**Average Recovery (%)**	**% RSD**
80%	26.88	26.34	99.5		0.3
	26.91	26.49	99.9	99.7	
	26.91	26.40	99.6		
100%	33.60	32.96	99.6		0.4
	33.64	33.06	99.8	99.6	
	33.64	32.98	99.5		
120%	40.32	39.57	99.6		0.4
	40.37	39.48.	99.3	99.3	
	40.37	39.37	99.0		

Forced Degradation:

Control Sample:

Accurately weighed portion of a powder equivalent about 33.4 mg of Lamivudine is transferred to a 200 ml volumetric flask. 180 ml of diluent was added and sonicated to dissolve for 10 minutes with intermediate shaking. Cool to room temperature, dilute up to volume with diluent and mixed well. Filter the above solution through 0.45µ PVDF syringe filter.

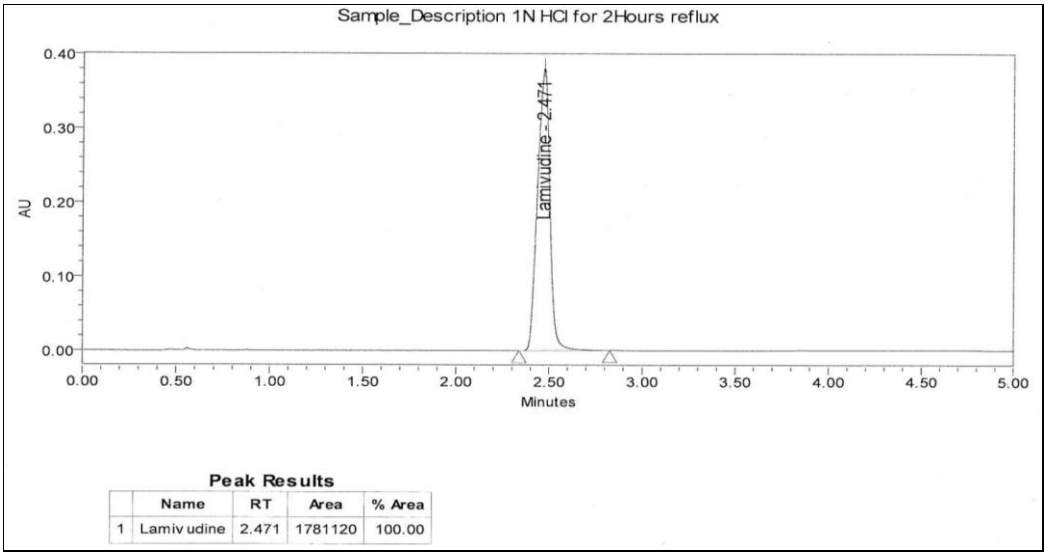


Peak Results

	Name	RT	Area	% Area
1	Lamivudine	2.479	1816142	100.00

Acid Degradation Sample:

Accurately weighed portion of a powder equivalent about 33.4 mg of Lamivudine is transferred to a 200 ml volumetric flask. 180 ml of diluent was added and sonicated to dissolve for 10 minutes with intermediate shaking. Cool to room temperature. Then add 20mL of 1N acid, refluxed for 2hrs at 60°C, then cooled to room temperature, neutralize with 1N NaOH and dilute to volume with diluent and mix. Filter the above solution through 0.45µ PVDF syringe filter.

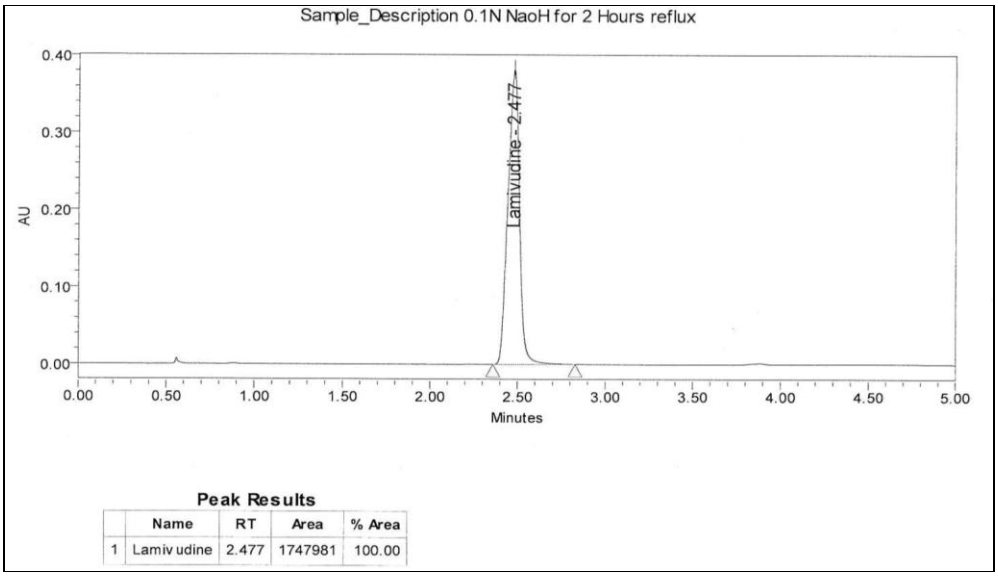


Peak Results

	Name	RT	Area	% Area
1	Lamivudine	2.471	1781120	100.00

Base Degradation Sample:

Accurately weighed portion of a powder equivalent about 33.4 mg of Lamivudine is transferred to a 200 ml volumetric flask. 180 ml of diluent was added and sonicated to dissolve for 10 minutes with intermediate shaking. Cool to room temperature. Then add 20mL of 0.1N NaOH, refluxed for 1hr at 60°C, then cooled to room temperature, neutralize with 0.1N acid and dilute to volume with diluent and mix. Filter the above solution through 0.45µ PVDF syringe filter.

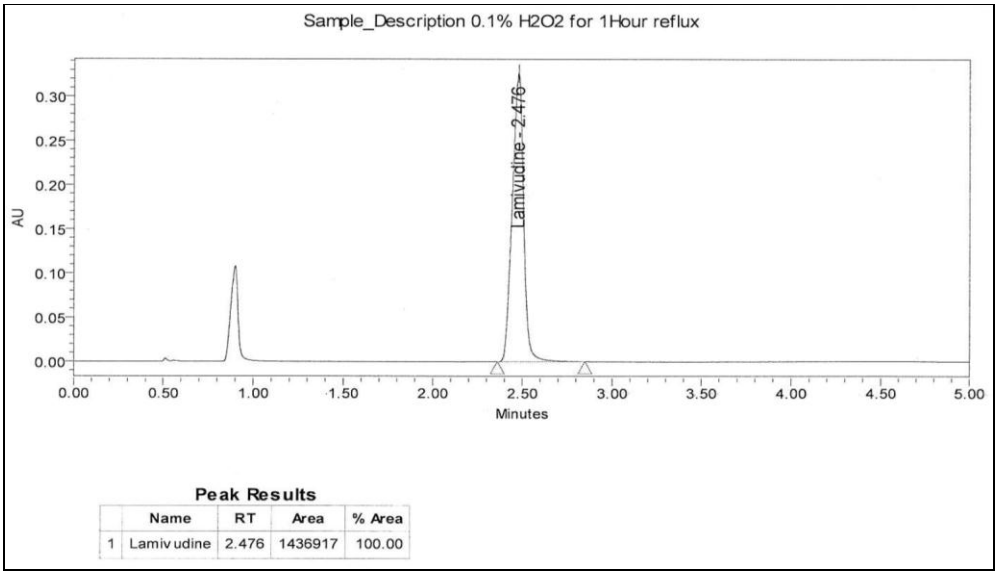


Peak Results

	Name	RT	Area	% Area
1	Lamiv udine	2.477	1747981	100.00

Peroxide Degradation Sample:

Accurately weighed portion of a powder equivalent about 33.4 mg of Lamivudine is transferred to a 200 ml volumetric flask. 180 ml of diluent was added and sonicated to dissolve for 10 minutes with intermediate shaking. Cool to room temperature. Then add 20mL of 0.1%Hydrogen Peroxide, refluxed for 30min at 60°C, then cooled to room temperature, and dilute to volume with diluent and mix. Filter the above solution through 0.45µ PVDF syringe filter.

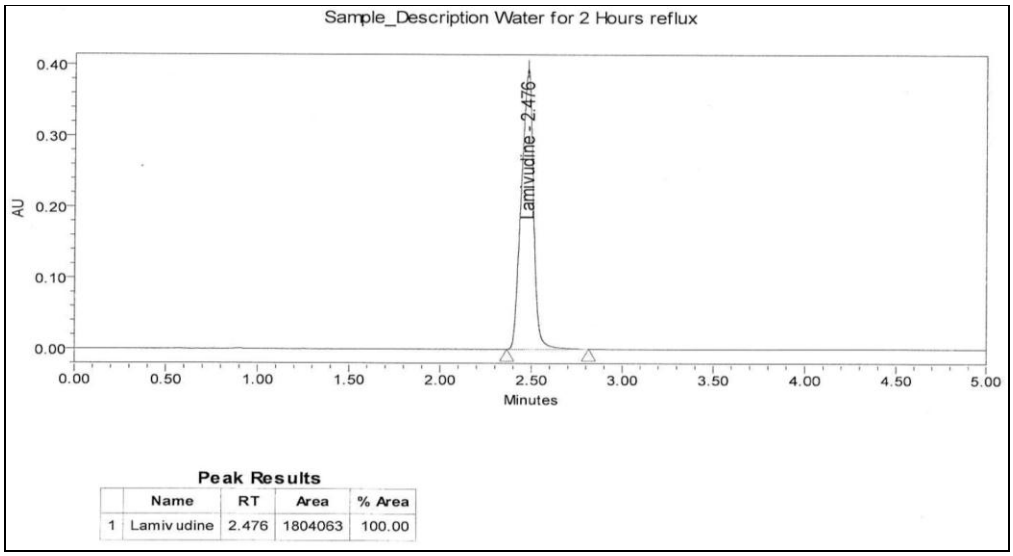


Peak Results

	Name	RT	Area	% Area
1	Lamivudine	2.476	1436917	100.00

Water Degradation Sample:

Accurately weighed portion of a powder equivalent about 33.4 mg of Lamivudine is transferred to a 200 ml volumetric flask. 180 ml of diluent was added and sonicated to dissolve for 10 minutes with intermediate shaking. Cool to room temperature. Then add 20mL of water, refluxed for 2hrs at 60°C, then cooled to room temperature, and dilute to volume with diluent and mix. Filter the above solution through 0.45µ PVDF syringe filter.

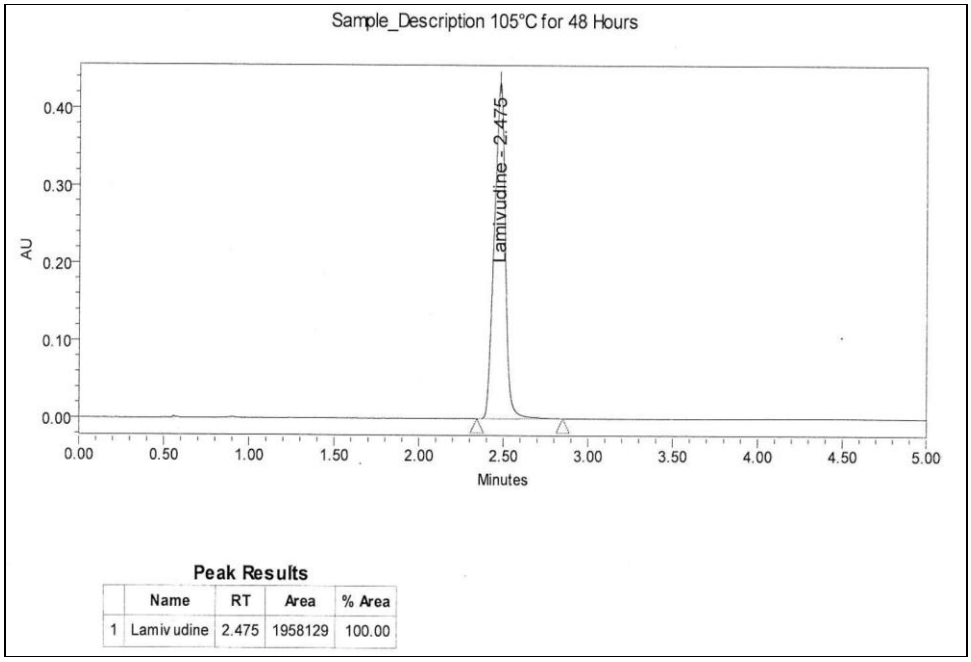


Peak Results

	Name	RT	Area	% Area
1	Lamivudine	2.476	1804063	100.00

Thermal Degradation Sample:

Powder collected from 20 tablets is exposed to heat at 105°C for about 2days. Accurately weighed portion of a powder equivalent about 33.4 mg of Lamivudine is transferred to a 200 ml volumetric flask. 180 ml of diluent was added and sonicated to dissolve for 10 minutes with intermediate shaking. Cool to room temperature, dilute up to volume with diluent and mixed well. Filter the above solution through 0.45µ PVDF syringe filter.



UV-Light Degradation Sample:

Powder collected from 20 tablets is exposed to UV-Light at 254nm for about 168hrs. Accurately weighed portion of a powder equivalent about 33.4 mg of Lamivudine is transferred to a 200 ml volumetric flask. 180 ml of diluent was added and sonicated to dissolve for 10 minutes with intermediate shaking. Cool to room temperature, dilute up to volume with diluent and mixed well. Filter the above solution through 0.45µ PVDF syringe filter.

Humidity Degradation Sample:

Powder collected from 20 tablets is exposed to Humidity at 90% RH at 25°C for about 168hrs. Accurately weighed portion of a powder equivalent about 33.4 mg of Lamivudine is transferred to a 200 ml volumetric flask. 180 ml of diluent was added and sonicated to dissolve for 10 minutes with intermediate shaking. Cool to room temperature, dilute up to volume with diluent and mixed well. Filter the above solution through 0.45µ PVDF syringe filter.

Table: 6 Forced degradation results

Degradation mechanism condition	Lamivudine			
	% Assay	% Degradation Assay	Purity angle	Purity threshold
Unstressed Sample	98.5	0	0.185	0.258
1.0 N HCl / 60°C for 2 hours	95.4	3.1	0.184	0.257
0.1N NaOH / 60°C for 1 hours	93.9	4.6	0.194	0.258
Thermal 105°C for 48 hours	94.7	3.8	0.143	0.259
Water for 2hours	95.8	2.7	0.188	0.256
0.1% H_2O_2/ 60°C for 30 min	93.1	5.4	0.128	0.270
UV Light at 254nm for 7 days	95.7	2.8	0.088	0.265
Humidity for 7 days	86.4	12.1	0.100	0.265

Robustness studies:

Table: 7 Robustness studies Results

Chromatographic Conditions		Lamivudine		
Parameter	**Changes**	**% recovered**	**% variation**	**%RSD**
Original method (Precision)	None	97.8	N/A	0.2
Wavelength(± 2nm)	275 nm	98.0	-0.2	0.2
	279 nm	98.0	-0.2	0.2
pH Variation	3.6 pH Buffer	96.1	1.7	0.1
	4.0 pH Buffer	96.2	1.6	0.2
Flow Rate	0.490 ml/ min	95.7	2.1	0.3
	0.510 ml/ min	96.1	1.7	0.1
Temperature	38 °C	95.8	2.2	0.2
	42 °C	95.9	1.9	0.1

CONCLUSION

An RP-UPLC method for estimation of Lamivudine was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed over a concentration range of 16.7-250.5µg/ml. The method has been successfully applied for the analysis of marketed tablets. It can be used for the routine analysis of formulations containing any one of the drug or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations and their individual drug substances are analyzed. We have developed a fast, simple and reliable analytical method for determination of Lamivudine in pharmaceutical preparation using RP-LC. As there is no interference of blank and placebo at the retention time of Lamivudine. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and Linearity. It allows reliably the analysis of Lamivudine in its different pharmaceutical dosage forms.

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