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[Research article]

RP-HPLC Assay Method Validation for the estimation of new Anti-retroviral drug Lamivudine in Bulk and Tablet dosage form

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ABSTRACT

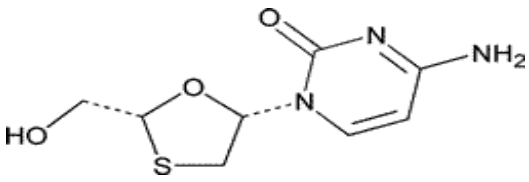
A Reverse phase HPLC method was developed for estimation of the Lamivudine in bulk and tablet formulation by using ODS column (250mm×4.6mm, 5µm) and Acetate buffer: acetonitrile (50:50) as mobile phase, at a flow rate of 1.5ml/min. The detection was carried at the 272nm the retention time of the Lamivudine is 1.850. The developed method was validated for the various parameters as per the ICH guidelines like accuracy precision, linearity and range, Robustnes. Linearity was obtained in the concentration range of 10µg/ml to 50µg/ml with correlation coefficient of 0.999. The accuracy of the method was assessed by recovery studies at three different concentration levels. The percentage recovery of Lamivudine was found to be in the range of 98% -102%. The method was found to be precise as indicated by the repeatability, inter-day, intra-day analysis, showing %RSD less than 2.

Key words: RP-HPLC, Lamivudine, Pharmaceutical dosage form.

INTRODUCTION

Lamivudine is chemically 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] pyrimidin-2-one [(1)]. Which is a pyrimidine analogue reverse transcriptase inhibitor active against HIV-1, HIV-2 and Hepatitis B virus [(2)] The therapeutic importance justifies research to establish some simple analytical method is planned to develop with sensitivity, accuracy, precision and economical .So the present work is aimed at development of RP-HPLC method for the estimation of New Antiretroviral drug Lamivudine in the bulk and tablet dosage form.

Fig-1: Structure of Lamivudine [(3)].



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MATERIALS AND METHODS

Instruments

Agilent made HPLC 1120 LC Compact System consists of VWD detector with Agilent TC C-18 ODS column and equipped with an Auto sampler 20µl sample loop capacity. The Output signal was monitored and integrated using Lab monitor diagnosis and EZ-Chrome software. Lambda max checking was done by UV-visible Spectrophotometer (UV-2310 Techomp)

Chemicals

Aceonitrile (HPLC grade), Glacial acetic acid, Sodium acetate (analytical grade), Millipore DQ3 water

Raw Material

Lamivudine pure drug was supplied by local Pharmaceutical industry, India as gift sample and used as such, Lamivudine Tablets were purchased from the local market: Lamivir HBV 100mg (Cipla laboratories Ltd., India)

EXPERIMENTAL

Chromatographic conditions

Column

4.6mm×250mm ODS column, 5µ particle size with Injection volume-20µl and Run time-5min. The Detection wave length is 272nm and the Column temperature is Ambient.

Mobile Phase

Mix Acetate buffer solution pH4.0, Acetonitrile in the ratio of 50:50 respectively and mix the solution. Degas in sonicator for about 10 mins. Use mobile phase as diluent.

Standard Preparation

Stock solution of Lamivudine was prepared by dissolving accurately weighed 25 mg of drugs in 25 ml of mobile phase (final concentration, 1000 µg / ml). The prepared stock solutions were stored away from light. From the stock standard solutions 100 µg / ml was freshly prepared during the day of analysis. Pipette 1 ml of above solution in to a 10 ml of volumetric flask, and dilute to volume with mobile phase and then filtered through Whattmann filterpaper No.1 (final concentration, 10 µg / ml).

Sample Preparation

Weigh accurately 5 tablets and then calculate the average weight, powder it in mortar. Stock solution of Lamivudine was prepared by dissolving the powder equivalent to 25 mg of drug in 25 ml of mobile phase Sonicate to dissolve and the filter through Whattmann filter paper No.1 (final concentration, 1000 µg / ml). The prepared stock solutions were stored away from light. From the stock standard solutions 100 µg / ml was freshly prepared during the day of analysis. Pipette 1 ml of above solution in to a 10 ml of volumetric flask, and dilute to volume with mobile phase and then filtered through Whattmann filter paper No.1 (final concentration, 10 µg / ml).

Calculation

The amount and % purity present in each Lamivudine Tablet formulation was calculated by comparing the peak area of the Sample and standard.

$$\textbf{Amount of Lamivudine} = \frac{\text{Sample peak area x standard dilution}}{\text{Standard peak area x sample dilution}} \times \text{average weight}$$

$$\textbf{\% purity of Lamivudine} = \frac{\text{Amount of Lamivudine present in the Tablet}}{\text{Label claim}} \times 100$$

Validation of the developed analytical method

Specificity

Specificity was evaluated by injecting lamivudine and placebo solutions individually and it was observed that there was no interference from placebo.

System Suitability

A Standard solution was prepared by using, Lamivudine working standards as per test method and was injected ten times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from ten replicate injections for Lamivudine retention times and peak areas. (Table-1)

Linearity of Test Method

A Series of solutions are prepared using Lamivudine working standard at concentration levels from (50%-250%) of target concentration (5-50µg/ml). Linear response was observed with correlation coefficient of 0.999 and Slope of regression line is 506932.6 with Y-intercept value 0.0162(Table-2), (fig-2)

Accuracy

Drug Assay was performed in triplicate as per test method with equivalent amount of Lamivudine into each volumetric flask for each spike level to get the concentration of Lamivudine equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Lamivudine was calculated. The result of the accuracy study is reported in table-3.

Precision

The tablet formulation was analyzed by injecting six times for the content and finally % purity of lamivudine to determine Intra day precision, results were given in table-4 .Inter day precision was carried in three different days with different analysts and the results were given in table-5.

Limit of detection & Limit of Quantisation

The parameters LOD and LOQ were determined on the basis of peak area and slope of the regression equation. The results were given in table-6

Robustness

Standard and sample solution 10µg/ml was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.The system suitability parameters were evaluated and found to be within the limits for mobile phase having 48% of less organic and 52% of highest organic phase (nominal 50%). Flow rate 1.30ml/min 1.70ml/min (nominal 1.5ml/min).pH 3.8 pH 4.2(nominal 4 pH). The results are reported in table-7.

RESULTS AND DISCUSSION

Lamivudine is an antiretroviral official only in I.P [3]. and B.P and the literature review indicates there are several methods have been reported for the estimation of New Antiretroviral drug Lamivudine by LC-MS, HPLC-MS/MS, HPTLC, and UV-Spectroscopy in Pharmaceutical formulations and in biological fluids [4, 5]. So it is worthwhile to develop simple rapid and accurate methods for estimation of Lamivudine by reverse phase high performance liquid chromatography. A Reverse phase HPLC method was developed for estimation of the Lamivudine in bulk and tablet formulation was achieved by ODS column (250mm×4.6mm, 5µm) and Acetate buffer: acetonitrile (50:50) as mobile phase, at a flow rate of 1.5ml/min. The detection was carried at the 272nm the retention time of the and Lamivudine is 1.850.The developed method was validated for the various parameters as per the ICH guidelines like accuracy precision, linearity and range, Robustness. Linearity was obtained in the concentration range of 10µg/ml to 50µg/ml with correlation coefficient of 0.999. The percentage recovery of Lamivudine was found to be in the range of 98% -102%. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in flow rate, Mobile phase composition separately and analysis being performed by different day with different columns respectively.

The results are found to be complying with the acceptance criteria for each parameter. Moreover the methods are quite sensitive to determine microgram quantities. Therefore it was concluded that the proposed method can be used for routine analysis of Lamivudine in its tablet dosage form.

Table-1 System Suitability

S.No	Peak name	RT	Area	Height(volts)	USP plate count	USP tailing
1	Lamivudine standard	1.870	5049556	1473296	5018.52	1.05
2	Lamivudine sample	1.857	5046403	1457456	5116.77	1.13

Table-2 Linearity

S.No	Linearity level	Final standard concentration(µg/ml)	Response
1	50%	5	2563623
2	100%	10	5049556
3	150%	15	7546325
4	200%	20	1012149
5	250%	25	12701091
Correlation coefficient(r)			0.999
Slope of regression line			506932.6
Y-intercept			0.0162
Residual sum of squares			3.53032+14

Fig-2 Linearity Plot (Concentration (µg/ml) Vs area)

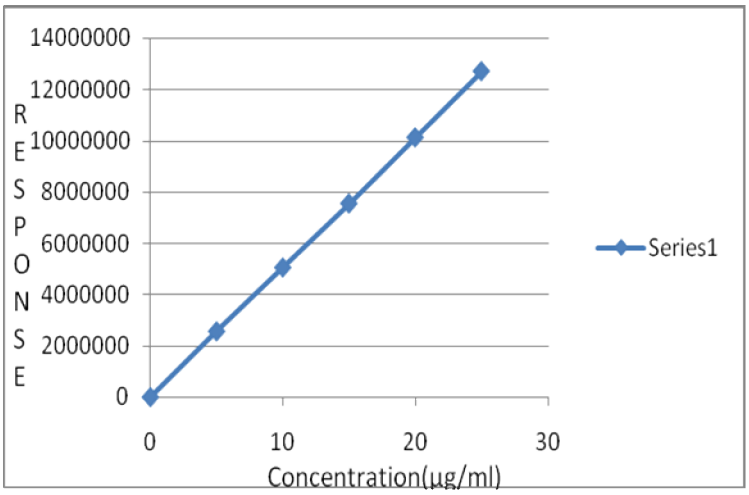

Fig-3 Chromatogram of Lamivudine in sample solution with retention time.

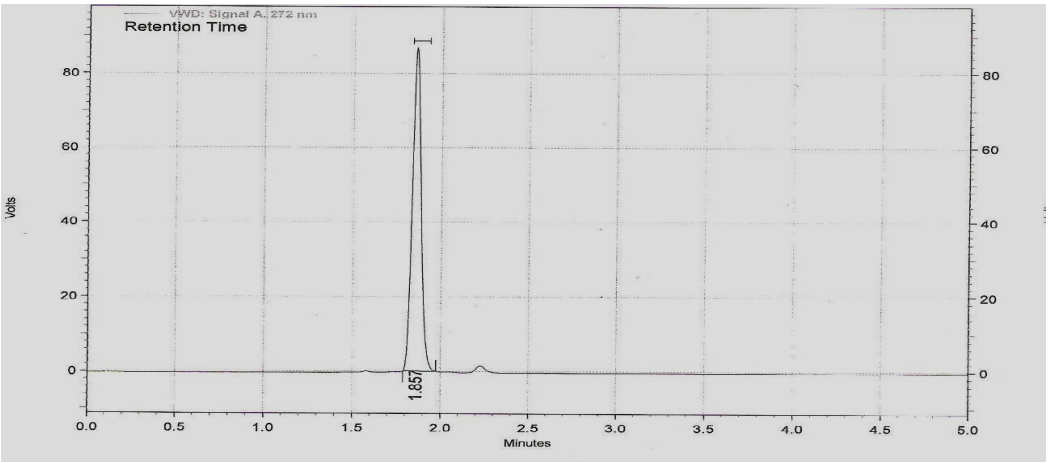

Table -3 Accuracy (% Recovery)

Level	µg/ml added	µg/ml recovered	%Recovery	Mean%	%RSD
Level-1	5	5.027	100.54	99.73	0.25
50%	5	4.94	98.98		
	5	4.98	99.68		
Level-2 100%	10	10.06	100.60	100.70	0.62
	10	9.99	99.93		
	10	10.15	101.59		
Level-3	15	14.74	98.29	99.61	1.33
150%					
	15	14.99	99.98		
	15	15.07	100.56		

Table -4 Intra day Precision

Sample preparation No	(Lamivudine Assay %)
1	99.72
2	98.78
3	100.38
4	101.53
5	98.77
6	100.94
Mean	100.02
%RSD	1.03

Table 5 Inter day Precision

S.NO	Assay % of Lamivudine Day-1	Assay % of Lamivudine Day-2	Assay % of Lamivudine Analyst-1	Assay % of Lamivudine Analyst-2
1	100.08	98.09	100.25	100.59
2	98.43	99.86	100.35	98.79
3	99.67	99.72	98.91	100.17
mean	99.39	99.22	99.83	99.85
%RSD	0.70	0.80	0.65	0.77

Table: 6 Robustness

Test	Flow rate 1.70ml/min	Flow rate 1.30ml/min	Mobile phase composition ACN:Buffer (52:48)	Mobile phase composition ACN:Buffer (48:52)	pH 4.2	pH 3.8
Mean standard area	4422874	5708310	4281695	4411876	5155110	5172627
%RSD	1.03	0.24	1.48	0.75	0.24	0.13

Table-7 Limit of Detection & Limit of Quantitation

	Limit of Detection (0.1µg/ml)	Limit of Quantitation (0.3µg/ml)
Retention Time	1.820	1.823
Area	151938	241018
Height (volts)	19380	33525

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