Validated Stability Indicating RP-HPLC Method for Determination of Lamivudine in Bulk and Pharmaceutical Formulations

T N V S S Satyadev[1], Dr Madhu[1], Dr T V Reddy[2], V. Sailaja[1], G. Hephzibah[1]

[1]Department of Chemistry, P B Siddhartha College of Arts& Science, Siddhartha Nagar, Vijayawada

[2]Professor & Vice- Principal, Mallareddy College of Engineering, Hyderabad.

sdtpati@gmail.com

Abstract - The determination of Lamivudine in dosage form was achieved by a validated stability indicating RP – HPLC method. The developed method was highly sensitive, precise and accurate. The Luna (C18 250mmx4.6mm, 5µ particle size) column was used in isocratic mode with mobile phase of composition 0.1% OPA : Acetonitrile (30:70 v/v) at a flow rate of 1.0 mL/min with UV detection at 227 nm for Lamivudine. The retention time of Lamivudine was 3.7 minutes. The developed method was validated as per ICH guidelines for specificity, linearity, precision, accuracy and robustness. Linearity was found in the range of 10.0 – 150.0 µg/ml. The percentage recoveries of the drug ranged from 100.5 – 100.8 %. This method can be used for routine analysis of Lamivudine.

Keywords: **Liquid Chromatography; Lamivudine, dosage forms, determination, Validation**

I. INTRODUCTION

Lamivudine {($C_8H_{11}N_3O_3S$) IUPAC name: 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one} is used to prevent and treat HIV/AIDS along with other medication. Effectively, it can be used in the treatment of HIV-1 and HIV-2. Hepatitis B can also be cured using Lamivudine when no other options are available. Lamivudine[1-6] belongs to the class of nucleoside reverse transcriptase inhibitors (NRTIs). It is used in combination with other antiretrovirals such as zidovudine and abacavir. Lamivudine can be included as a part of post-exposure prevention in those who have been potentially exposed to HIV and Lamivudine can be taken orally as a liquid or tablet.

Spectrophotometry[7-17], HPLC[18-23] etc., were the reported analytical methods for the determination of Lamivudine. A thorough review of literature has revealed that there is a need of new analytical method for the determination of Lamivudine in pharmaceutical dosage form. The present investigation is aimed to develop a new, efficient and reproducible RP-HPLC method for the analysis of Lamivudine. The method was developed as per ICH guidelines [24].

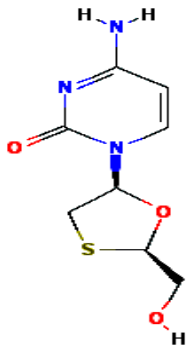

Fig 1:Structure of Lamivudine

II. MATERIALS AND METHODS

Materials

HPLC grade Merck made orthophosphoric (H_3PO_4), acetonitrile, hydrochloric acid were used. Dilutions were performed in standard class-A, volumetric glassware. Lamivudine was received from Glenmark Pharmaceuticals Ltd. In the estimation of commercial formulation, Epivir® tablets having 100 mg of Lamivudine were procured from the local market. Milli-Q HPLC grade water was used throughout the analysis.

Instrumentation

Waters make (model 2695) LC chromatographic system, with UV-Visible detector and a fixed injector equipped with 10µL loop was used in the present work. The chromatograms were recorded at suitable temperature and peaks were quantified by means of Empower software. The separation was carried out on a C18 column [Luna C18 250 mmx4.6 mm 5µ]. Electronic digital balance of Ohaus make was used for weighing the samples. For degassing and mixing of the mobile phase ultra-sonic bath sonicator was used.

Chromatographic conditions

The effective separation of Lamivudinet was achieved on a C18 column. The composition of the mobile phase was 0.1 % ortho phosphoric acid and acetonitrile in the ratio of 30:70 v/v. The mobile was filtered through a 0.45 µ membrane filter and degassed for 15 minutes. The flow rate was maintained at 1.0 ml/min. The UV detector was fixed at 227 nm.

Method development Standard Stock Solution

The required amount of Lamivudine was weighed and dissolved in mobile to have final concentration of 100

µg/ml of Lamivudine. This solution is used for recording chromatogram. Further dilutions were made by taking 5mL, 10mL, 20mL etc., from the stock and were diluted to 50 mL in a standard flask with the diluent to obtain different concentrations.

Preparation of Sample solutions

20 tablets of Lamivudine (each containing 100 mg) were taken into a mortar and crushed to fine powder. Stock solutions of Lamivudine tablet (µg/ml) were prepared by dissolving weight equivalent to 5 mg of Lamivudine in sufficient volume of mobile phase. The solution was filtered using 0.45-micron syringe filter and sonicated for 5 min and dilute to 50ml with mobile phase. further dilutions are prepared in 5 replicates of 100 µg/ml of Lamivudine.

Method validation

The RP-HPLC method developed for the determination of Lamivudine was validated as per the ICH guidelines.

System suitability and System Precision

In order to show that the performance of the system met the standards required by the method, the system suitability for chromatographic separation was checked on each day to evaluate the components of the analytical system. The parameters of system suitability for the developed method include number of theoretical plates (efficiency), tailing factor. The equilibration of HPLC system was attained using the initial mobile phase composition, followed by 5 injections of the standard solution of 100% concentration containing 100 µg/mL Lamivudine. To evaluate the system suitability on each day of method validation these 5 consecutive injections were used. The results were given in the Table 1.

III. SPECIFICITY

Blank interference

The specificity studies of the proposed method include application of blank, placebo solution, sample solution (control sample), standard solution. To establish the interference of blank a study was conducted. In the above defined chromatographic conditions, diluent was injected into the chromatograph and the blank chromatogram was recorded. No peaks were observed at the retention time of Lamivudine peak in the chromatogram of Blank solution **(**Fig. no.-2**)**. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Lamivudine in Epivir tablets. The typical representative chromatogram of sample and standard were recorded similarly and were shown in figure -3 & 4.

Forced Degradation

Deliberate degradation of the tablet sample by exposure to stress conditions were included in the specificity studies. Forced Degradation study was carried out by treating the sample under the acidic, alkaline, thermal, peroxide and photo conditions. Ten tablets of Lamivudine were weighed and powdered uniformly in a mortar. Equivalent to 15.5 mg of powdered portion was accurately weighed and transferred into 50 mL volumetric flask. For complete solubility of the drug, the contents of the flask were sonicated for about 15 min and the volume was made up to 50 mL with mobile phase. Then the mixture was filtered through a 0.45µ membrane filter. The degradation study results were given in table – 2.

Linearity and range

The linearity of Lamivudine was observed in the concentration range of 10.0 – 150.0 µg/ml and resulted in a standard curve. To evaluate the linearity of the curve a statistical method known as linear regression analysis was used. The linearity of the proposed method was accessed from the slope, intercept and correlation coefficient [r^2] of standard curve and the standard curve was represented in Figure-11. The results pertaining to these were given in the Table- 3. From the data obtained, the method was found to be linear within the proposed range. The linearity chromatograms were given in figure- 5-10

Accuracy

Accuracy is defined as the closeness of results obtained by that method to the true value for the sample. In terms of percentage recovery accuracy is expressed. The percentage Recovery is determined by the standard addition method. Recovery studies were carried out at 50%, 100% and 150% spiked levels in the present investigation. The percentage Recovery results were given in Table – 5.

Precision

The precision of the method is defined as the closeness of replicate results obtained from analysis of the same homogeneous sample. The precision of the method and intermediate precision were assessed by six replicate injections of 100% test concentration. Standard deviation and %RSD were used to express the precision. The results of precision were given in Table 6 and 7.

Robustness

Robustness is defined as the ability of the developed method to remain unaffected by the small deliberate changes in the parameters. To assess the robustness of the method the parameters such as percent organic content, pH of the mobile phase, buffer concentration, temperature, injection volume and flow rate are varied. A variation of ± 0.1 mL/min in the flow rate, change in organic content of mobile phase were adopted to study Robustness in the present study. The results of robustness were given in Table -8.

IV. RESULTS AND DISCUSSION

Luna C18, 250 X 4.6, 5µm was used in this new reversed phase HPLC method for the determination of Lamivudine tablets in combined dosage form with a flow rate of

1.0ml/min at a wavelength 227 nm and column temperature was maintained 22-25 °C. 0.1 % Ortho phosphoric acid and Acetonitrile in the ratio of 30:70 v/v was used as mobile in the present study. The analysis was run for 8 minutes and the retention time of Lamivudine was 3.7 minutes. In the determination of lamivudine this developed method was specific and the same was known from the blank, placebo and forced degradation studies, as no other peak was found at the retention time of Lamivudine, during these studies. The developed method for the determination of Lamivudine in pharmaceutical dosage forms was validated as per ICH guidelines. The new HPLC method assured satisfactory precision and accuracy and also useful determining lower concentration of drug in its solid combined dosage form. The linearity range for Lamivudine is 10.0-150.0 µg/ml the co-relation co-efficient was found to be 0.9996. 0.2456 and 0.158 were the percentage RSDs obtained for intermediate precision and method precision of Lamivudine. In each condition the system suitability was evaluated and compared the results with method precision results and the method is robust for change in flow rate and mobile phase composition. At the retention time of Lamivudine no peak was observed and the developed method was found to be specific. The sample solution was injected and the amount of Lamivudine present in the formulation was calculated from the calibration curve. The amount of Lamivudine found in the commercial sample as per the developed method and the assay of Lamivudine was found to be 100.4%.

Table No 1: Results for System Suitability of Lamivudine

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor
1	3.715	2683214	5826	1.14
2	3.720	2648521	5811	1.41
3	3.710	2698456	5814	1.52
4	3.718	2654789	5824	1.35
5	3.715	2698312	5838	1.46
6	3.717	2654789	5836	1.52
Mean	3.719	2687963	--	--
SD	0.142	5816.22	--	--
%RSD	0.44	0.5852	--	--

Table No 2: Degradation Studies Data

S. No	Degradation Parameters	Time	Peak Area	%Recovery	%Degradation
1	Acid	15 min	2126543	75.65	26.52
2	Alkali	15 min	2147853	77.36	28.44
3	Peroxide	15 min	2178531	72.46	25.36
4	Reduction	15 min	2156321	76.44	29.32
5	Thermal	15 min	2178563	77.52	26.37
6	Photolytic	15 min	2185631	76.14	24.63
7	Hydrolysis	15 min	2145631	77.36	23.24

Table No 3: Linearity of Detector Response for Lamivudine

S.No.	Conc.(µg/ml) of Lamivudine	Area	Acceptance criteria
		Lamivudine	
1	10 µg/ml	261453	Squared co relation coefficient should be not less than0.999.
2	25 µg/ml	661308	
3	50 µg/ml	1322616	
4	100 µg/ml	2645232	
5	125 µg/ml	3306540	
6	150 µg/ml	3967848	

Table No 4: Results of linearity response graphs of Lamivudine

S.No	Linearity Parameters	Lamivudine
1.	Linearity range	10-150 µg/ml
2.	Correlation coefficient	0.999
3.	Y intercept	26576x - 17143

Table No 5: - Accuracy data of Lamivudine

Recovery level	Accuracy Lamivudine						%RSD
	Amount taken (µg/ml)	Area	%Assay	Average area	Amount recovered (µg /ml)	%Recovery	
50%	50.0	1352563	100.2	1345688	100.52	100.5	0.52
	50.0	132546	100.				
	50.0	135263	100.				
100 %	100.0	2645823	100.4	2665874	100.87	100.8	0.48
	100.	265247	100.				
	100.	267893	100.				
150 %	150.0	3923229	100.8	3925214	100.86	100.8	0.52
	150.	392585	100.				
	150.	392845	100.				

Table No 6: - Method precision data for Lamivudine (100 mg)

Lamivudine		
S.No.	Rt	Area
1	3.712	2662583
2	3.725	2621103
3	3.758	2634253
4	3.723	2632541
5	3.711	2642354
6	3.742	2653241
Avg	3.785	2654231
St dev	0.1421	1025.28
%RSD	0.11	0.2456

Table No 7: Intermediate precision data of Lamivudine

S. No	Analyst-1	
	Peak area	% assay
1.	2648563	100.6
2	2652486	100.5
3	2628546	100.8
4	2658453	100.4
5	2685432	100.5
6	2687453	100.3
mean	2685746	100.2
SD	1524.25	1.126
%RSD	0.158	0.18

Table No 8: Flow rate and Organic phase variations

Parameters	Lamivudine		
Flow rate	Retention Time	Tailing factor	%RSD
0.8ml/min	3.841	1.04	1.11
1.0ml/min	3.715	1.01	1.14
1.2ml/min	3.025	1.11	0.22
Organic phase			
55:45	3.925	1.16	1.08
60:40	3.705	1.18	0.48
65:35	3.041	1.11	0.56

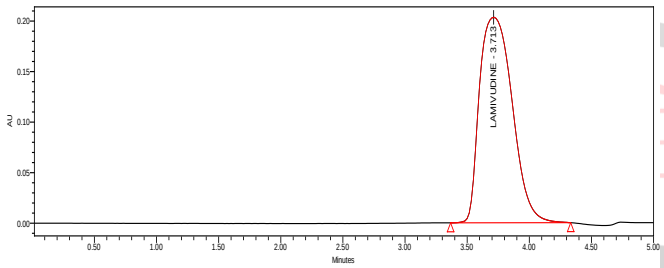

Fig 2: - Chromatogram of Lamivudine Blank

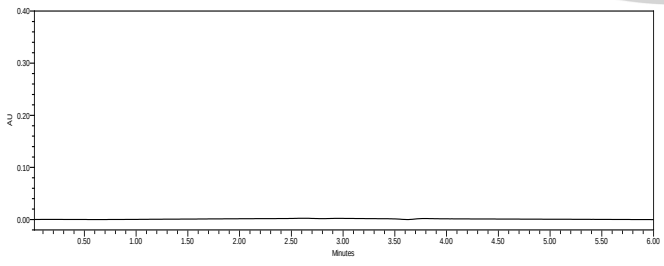

Fig 3: Typical chromatogram of sample

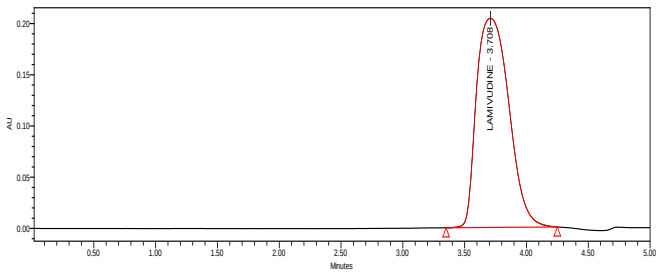

Fig 4: Standard Chromatogram of Lamivudine

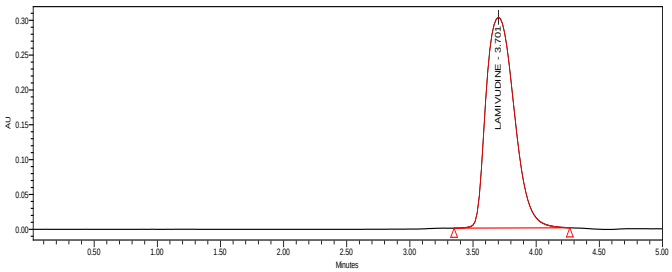

Fig 5: Linearity chromatogram of Lamivudine (10µg/mL)

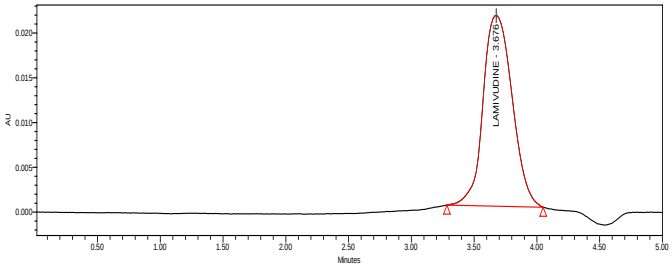

Fig 6: Linearity chromatogram of Lamivudine (25µg/mL)

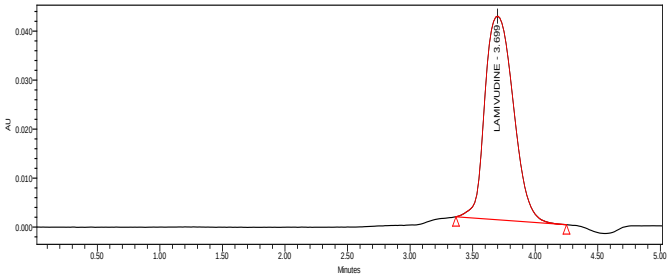

Fig 7: Linearity chromatogram of Lamivudine (50µg/mL)

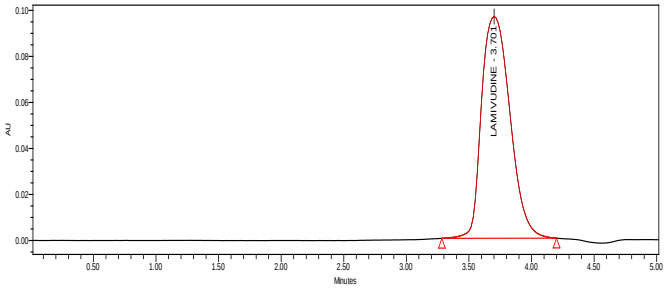

Fig 8:Linearity chromatogram of Lamivudine (100 µg/mL)

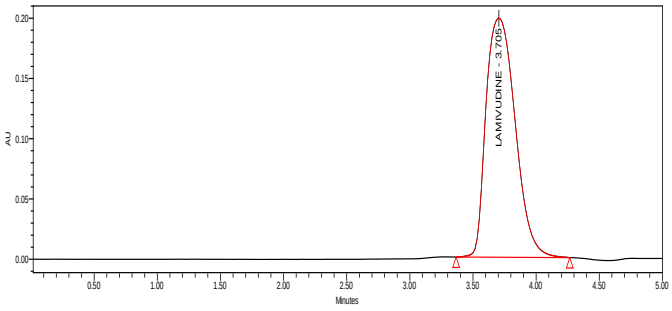

Fig 9: Linearity chromatogram of Lamivudine (125 µg/mL)

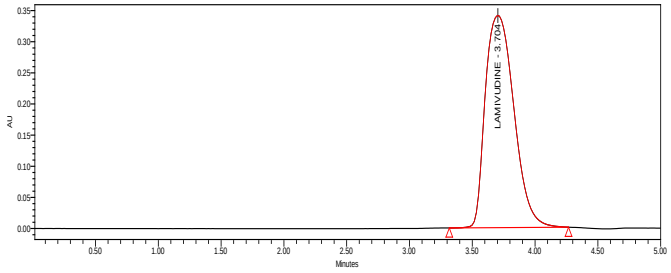

Fig 10: Linearity chromatogram of Lamivudine (150µg/mL)

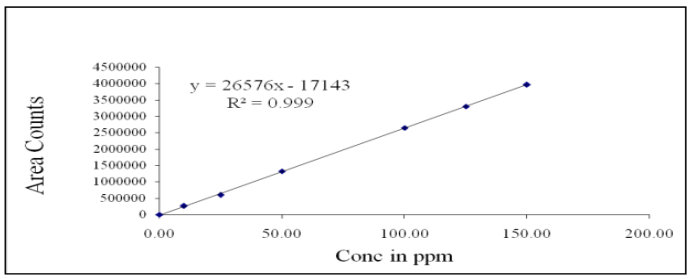

Fig 11: calibration curve for Lamivudine at 227 nm

REFERENCES

[1] Goodman, Gileman . The Pharmacological Basis of Therapeutics. In: Joel GH, editor. 11th ed. Mc-Graw Hill: Medical publishers Div; 2006. p. 1288.

[2] HMSO. Vol. 2. Cambridge: International edition; 2007. Stationery Office (Great Britain). British Pharmacopoeia; p. 1216

[3] Sean CS. The complete drug reference. In: Sean CS, editor. 35th ed. London: Pharmaceutical Press; 2009.

[4] Maryadel JO. The Merck Index. In: Maryadel JO, editor. 14th ed. New Jersey: Merck Research Laboratories; 2006.

[5] US Pharmacopoeia. Asian Edition 30. United States Pharmacopoeial Convention, Inc: 2007. p. 2447.

[6] Indian Pharmacopoeia. Vol. 2. Government of India: Controller of Publications; 2. Government of India Ministry of Health & Family Welfare; p. 1276.

[7] Aymard G,.Legrand M, Trichereau N and Diquet B J, Chromatogr B: Biomed Sci Appl., 2000,744, 227-240.

[8] Sharma C S N, Kamala Sastry C and Sastry C S P, Acta Ciencia Indiaca Chem., 2002, 28, 221-225.

[9] Wankede S B, Gupta K R and Wadodkar S G, Indian J Pharm Sci., 2005,67,96-97.

[10] Sarma G N, Sastry C K and Sastry C S P, Asian J Chem., 2002,14,683-690.

[11] Appalaraju S, Karadi A B,.Kamalapurkar G S and Sarasambia P S, Asian J Chem 2002,14,475-478.

[12] Pallad M S, Rajesh P M N, Chatter M and .Bhat A R, Indian J Pharm Sci., 2005, 67, 110-112.

[13] Hoetelmanur R M W, Profit M, Mecnhorst P I, Mulder J W and Beijnen J H J, Chromatogr B: Biomed Sci Appl., 1998,713,387-394.

[14] Moore J D, Valette G, Dorque A and.Zhon J J, Am Soc Mass Spectrum., 2000, 11, 1134-1143.

[15] Pereira A S, Kenney K B, Cohen M S, Hall J E, Eron J J, Tidwell R R and Dunn J A, J Chromatogr B: Biomed Sci Appl., 2000,742, 173-183.

[16] Ozkan S A and Uslu B, J Liq Chromatogr Rel Technol., 2002, 25, 1447-1456.

[17] Fan B and Stewart, J Pharm Biomed Annal., 2002, 28, 903-908.

[18] D.K.Mandloi, P.K.Tyagi, V.K.Rai, S. Dey, R.K Ashada1,and P.Mohanraj "Method development and validation of RP- HPLC in the application of invitro dissolution study of Lamivudine in bulk drug and tablet formulation" Journal of chemical and Pharmaceutical Research, 2009, 1(1) 286-296

[19] Ashenafi Dunge, Nishi Sharda, Baljinder Singh and Saranjit Singh "Validated specific HPLC method for determination of zidovudine during stability studies"Journal of Pharmaceutical and Biomedical Analysis 2005 37, (5), 1109-1114

[20] Vibhuti Kabra, Vivek Agrahari, Chandrabose Karthikeyan and Piyush Trivedi "Simultaneous quantitative determination of zidovudine and nevirapine in Humanplasma using isocratic,reverse phase high performance liquid chromatography"Tropical Journal of Pharmaceutical Research, 2009, 8, (1), 79-86

[21] Santoro MI, Fazio TT, Singh AK, and Kedor-Hackmann ER, "Quantitative determination of Lamivudine and zidovudine residues for cleaning validation in a production area." International journal of applied organic chemistry. 2007, 90(3), 71

[22] S.Jayaseelan, S.Suresh, G.Sathishkumar,V.Sekarand P.Perumal "Bioanalyticalmethod development and validation lamivudine by Rp-Hplc method International journal of chemtech research 2001 ,2(1) 163- 167.

[23] Kathryn B. Kenney, Stephen A. WringRichard M. Carr Guy N. Wells and John A. Dunn, "Simultaneous determination of zidovudine and lamivudine in human serum using HPLC with tandem mass spectrometry" Journal of Pharmaceutical and Biomedical Analysis 2000, 22, (6) 967-983.

[24] ICH. Guideline, Validation of analytical procedures : Text and Methodology Q2 (R1);November. J Pharm Biomed Anal 2005.