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Analytical Method Development and Validation of Lamivudine by RP-HPLC Method

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Abstract: A reliable, sensitive reversed phase high performance liquid chromatography (RP-HPLC) method was developed and validated for lamivudine. Lamivudine has been widely used as antiretroviral drug in the treatment of Human Immunodeficiency Virus (HIV) and AIDS. The method was developed on HiQSil C-18 column (250 mm×4.6 mm, 5µm) using a mobile phase of Acetonitrile: Phosphate buffer pH 4 (85:15 % v/v). The flow rate was 1.0 mL/min with injection volume 20µl. The efficient was monitored by UV detector at 271 nm. The retention time of the lamivudine was 3.16 min. The linearity range of lamivudine were found to be range 5-30µg/ml. of lamivudine Linear regression coefficient value was 0.999.

Keyword: Lamivudine, RP-HPLC, Analytical Method Development, Validation

I. INTRODUCTION

LAMIVUDINE (LAM) (4-AMINO-1-[(2R,5S)-2-(HYDROXYMETHYL)-1,3-OXATHIOLAN-5-YL]-1,2-DIHYDROPYRIMIDIN-2-ONE)[1] IS NUCLEOSIDE ANALOGUES WITH A STRUCTURE THAT CONSISTS OF A PYRIMIDINE BASE, WHICH IS N-SUBSTITUTED AT THE 1-POSITION WITH A 3'-THIA DERIVATIVE (1,3-OXAZOLIDINE) OF THE RIBOSE MOIETY THAT IS CHARACTERISTIC OF NUCLEOSIDES. IT IS REVERSE TRANSCRIPTASE INHIBITOR AND ZALCITABINE ANALOG IN WHICH A SULPHUR ATOM REPLACES THE 3' CARBON OF THE PENTOSE RING. LAMIVUDINE IS USED TO TREAT HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) AND IT IS ALSO USED IN LOWER DOSES TO TREAT PATIENTS WITH CHRONIC HEPATITIS B IN WHOM THE VIRUS HAS REPLICATED AND CAUSED LIVER INFLAMMATION. [2-6] THIS COMPOUND BELONGS TO THE CLASS OF ORGANIC COMPOUNDS KNOWN AS 3'-THIA PYRIMIDINE NUCLEOSIDES.[7]

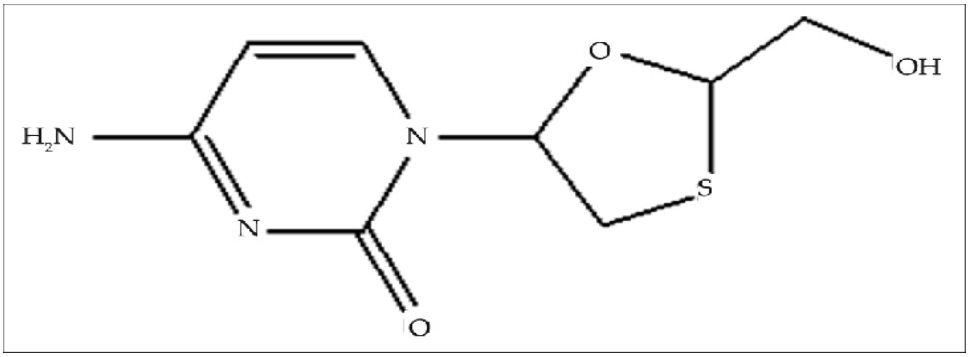

Fig 1: CHEMICAL STRUCTURE OF LAMIVUDINE

Instrumentation and Chromatographic Conditions:

HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump, Rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and ChromNAV CFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm × 4.6 mm, 5 µm) column using Acetonitrile: Phosphate buffer pH 4 (85:15 v/v) as mobile phase at flow rate of 1.0 mL/min. Samples were injected using Rheodyne injector with 20 µL loop, Detection was carried out at 271nm. All weighing were done on Shimadzu balance (Model AY-120)

MATERIALS AND METHODS [8-9]

Material

All Chemicals utilized for method development are of HPLC grade.

Preparation of mobile phase

The preparation of mobile phase was done by mixing Acetonitrile: Phosphate buffer pH 4 (85:15 v/v). Removal of gases was carried out in ultrasonic water bath for 30 minutes. Filtered the solution through 0.45µ filter.

Diluent preparation

Mobile phase used as diluents.

Preparation of standard stock solution

50mg of Lamivudine was transferred into 50ml volumetric flask, dissolved & make up to volume with mobile phase to get 1000µg/ml. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Preparation of test solution

50 mg equivalent of Lamivudine API standard was transferred into 50ml volumetric flask, dissolved & make up to volume with mobile phase 1000µg/ml. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase and performed the subsequent dilutions.

Selection of analytical wavelength

It is the characteristic of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of Lamivudine was carried out under UV ranging from 200-400nm using the standard solution

Preparation of stock solution of lamivudine

An accurately weighed 10 mg of lamivudine was dissolved in 10 mL of methanol and transferred into a 50 mL volumetric flask. The stock solution (1000µg/mL) was stored in a refrigerator (2–4°C). From the stock solution working solutions were prepared by serial dilution.

Analytical method development

Lamivudine API

Determination of Lambda maximum

The criteria employed for assessing the suitability of a particular solvent system for the analysis was cost, time required for analysis, sensitivity of the assay and solvent noise for the analysis of Lamivudine. The mobile phase consisted of Acetonitrile: Phosphate buffer pH 4 (85:15 v/v).

Acetonitrile: Phosphate buffer pH 4 were previously filtered under vacuum through 0.22 µm membrane filters and degassed by using sonicator before injection into the HPLC apparatus.

Preparation of stock solution of Lamivudine

Standard stock solution (1000 µg/ml) of Lamivudine was prepared by dissolving 50 mg in 20 ml of mobile phase with shaking and then volume was made up to the mark of 50 ml with the mobile phase. The stock solutions were degassed by using sonicator and filtered through a 0.22 µm membrane filter.

The lambda maximum for Lamivudine was found to be 271nm.

Method Validation

Linearity:

The linearity of the developed method was studied over the concentration ranges between 5- 30µg/ml. The aliquots of 5, 10, 15, 20, 25 and 30µg/ml were prepared by diluting standard stock solution of 0.5, 1, 1.5, 2.0, 2.5 and 3.0 ml with mobile phase. The obtained concentrations were injected into the chromatographic system. Calibration curve of Lamivudine was constructed by plotting peak area versus used concentration of Lamivudine. To assure the concentration range studied is linear the regression equation and correlation coefficient were evaluated.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analysed sample solution of Lamivudine, a known amount of standard drug powder of Lamivudine was added to 80, 100, 120% level.

Precision method

By studying the changes in the inter-day and intra-day determined the precision of the method. In the intra-day studies, six repeated injections of standard solution were made and % RSD were calculated. In the inter-day variation studies, six repeated injections of standard solution were made for six consecutive days and %RSD were calculated.

Limit of Detection and Limit of Quantitation

Based on the standard deviation of response of the calibration curve the LOD and LOQ of the drug was determined separately.

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and wavelength.

Result and discussion:

Selection of wavelength maxima

The solution of Lamivudine was scanned between ranges 200- 400nm. UV spectra of the drug show maximum absorbance at 271nm.

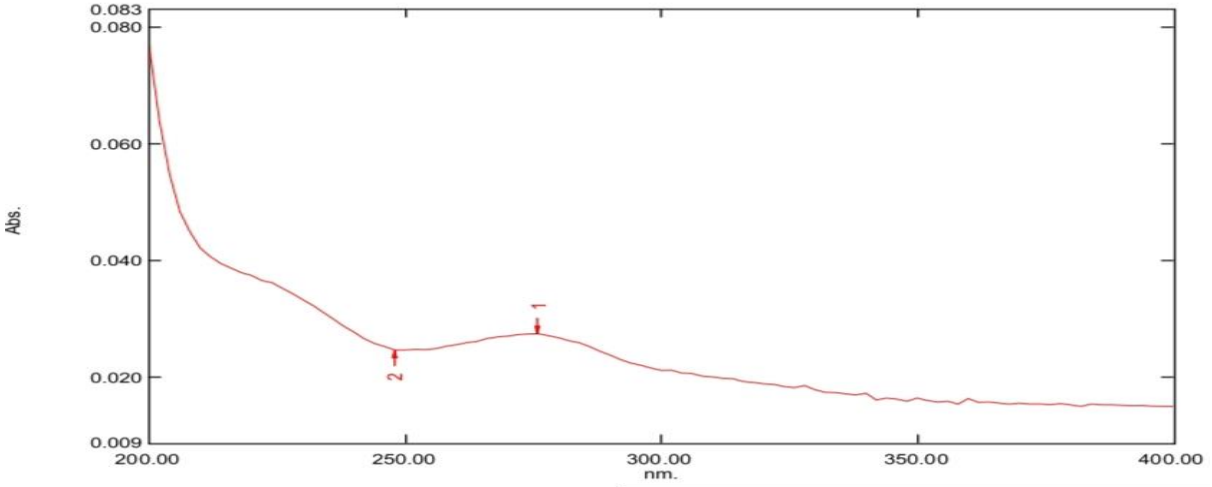

Fig 2: UV Spectrum of Lamivudine

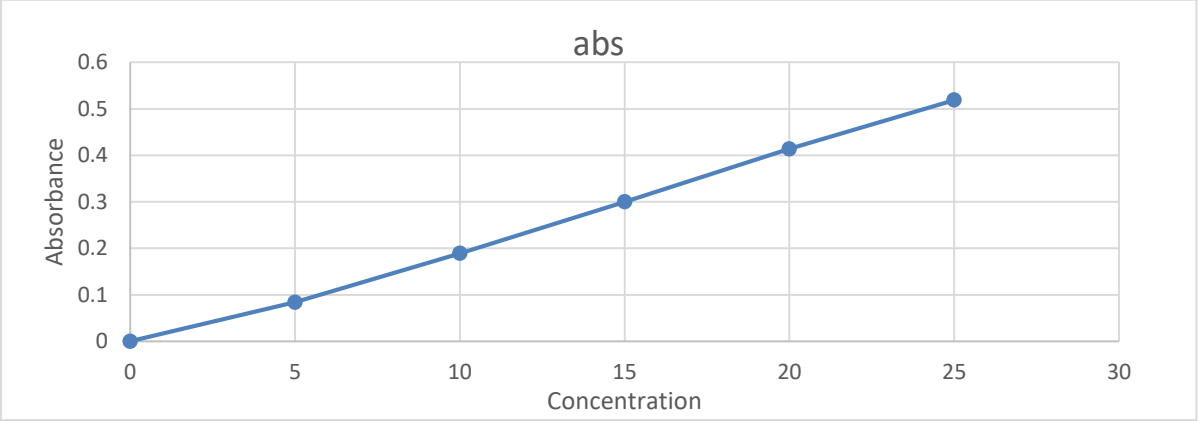

Fig 3: Calibration curve of Lamivudine

Standard curve of Lamivudine was determined by plotting absorbance Vs concentration at 271 nm. Using solution prepared in Ethanol at 271 nm; It follow Beer's law. The R2 value found to be 0.998.

Method development

The proposed chromatographic method was found to be suitable for effective separation of Lamivudine with good resolution, peak shape given in the figure 2.

The mobile phase composed of Acetonitrile: Phosphate buffer pH 4 (85:15 v/v), at a flow rate of 1.0 ml/min was selected as it gave well resolved peaks of standard Lamivudine. The optimum wavelength 271nm selected for detection and quantitation.

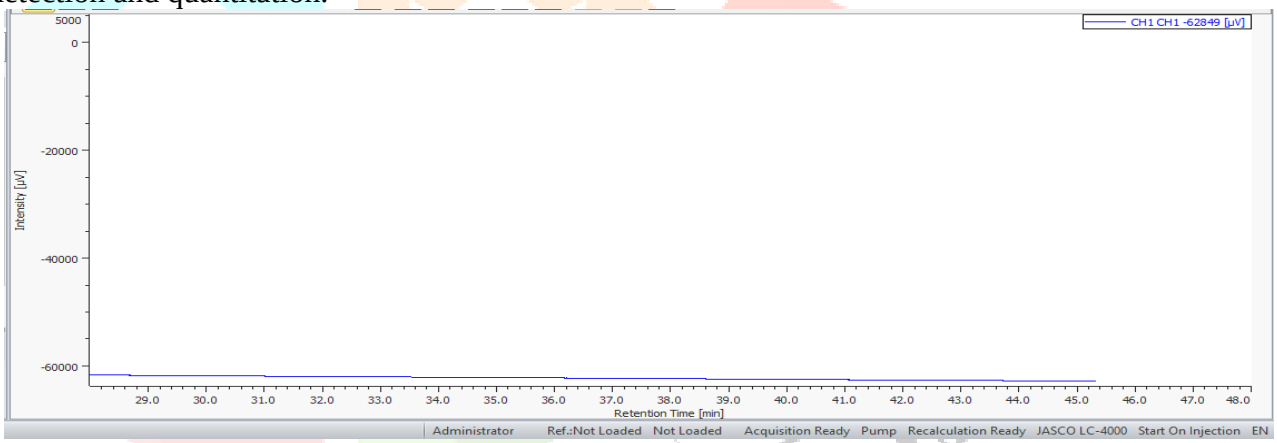

Fig 4: HPLC chromatogram of blank.

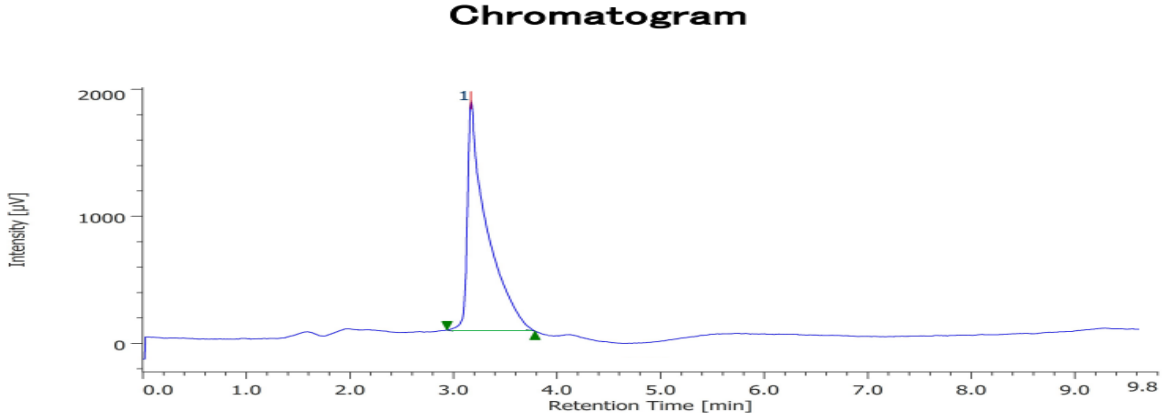

Fig 5. HPLC chromatogram of standard Lamivudine.

The retention time was found to be 3.16 with distinct peak

Method validation

Linearity

The calibration curves were found be linear for the concentration range of 5-30ppm. The standard working curve equation for drug was found to be y = 23484x + 12436 with correlation coefficient value r^2 = 0.9998. The results of linearity are given in Table -2 and Figure -6 below.

Fig-6: Linearity curve of standard Lamivudine

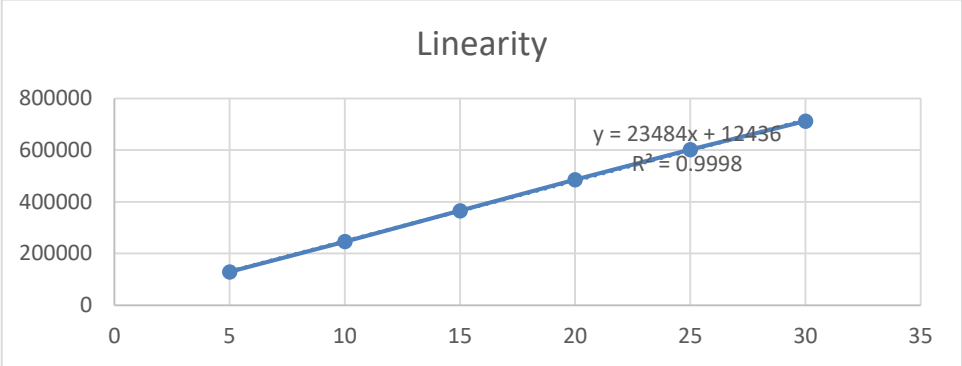

Table- 2: Linearity data of Lamivudine

Concentration µg/mL	Area
5	128615
10	245756
15	365405
20	485875
25	602223
30	712589

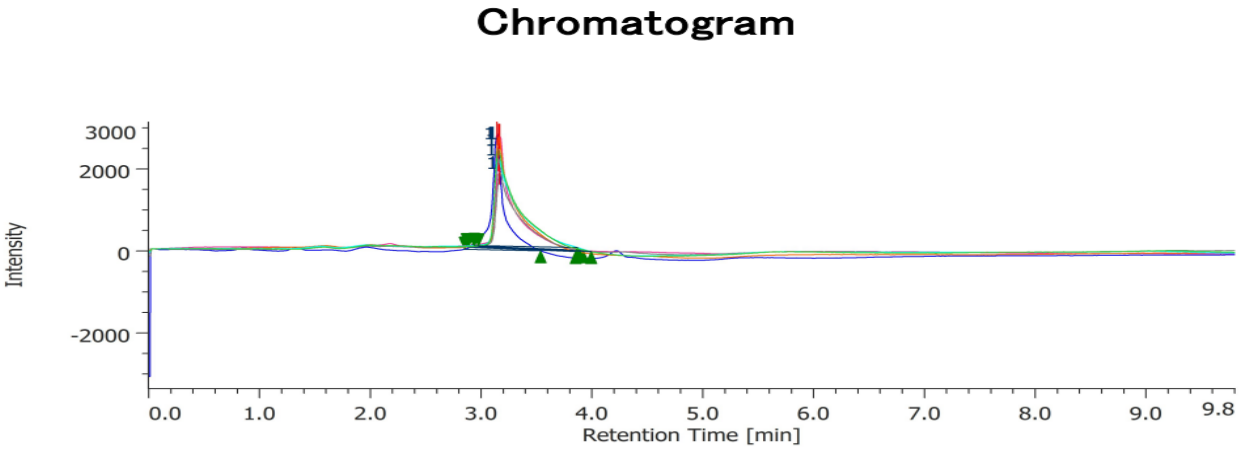

Fig 7. HPLC chromatogram of standard Lamivudine (Overlay)

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for drug Lamivudine given in Table.

Tab-3: Recovery data of Lamivudine

Level (%)	Drug Conc. (mg)	Amt. recovered (mg)	% Recovery
80	8	7.99	99.875
100	10	10.11	101.1
120	12	12.09	100.75

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2.0%. The results of precision studies are shown in Table.

Table- 4: Precision study (intra- day) of Lamivudine

Conc. µg/mL	**Area**	**AVG**	**%RSD**
10	245756	246052.33	0.18923195
	245812		
	246589		
15	365405	364824	0.1384571
	364578		
	364489		
20	485875	486347.33	0.22491377
	487598		
	485569		

Conc., Concentration; AVG, average; RSD, Relative standard deviation

Table-5: Precision study (inter-day) of Lamivudine

Conc µg/mL	**Area**	**AVG**	**%RSD**
10	235647	241475.67	2.11225115
	245122		
	243658		
15	365879	367320.67	0.53120642
	366542		
	369541		
20	485236	486237	0.23907605
	487512		
	485963		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Limit of Detection (LOD) and Limit of Quantification (LOQ)

This data showed that the sensitivity of method to determine the drug Lamivudine. The Minimum concentration level at which the analyte can be reliable detected (LOD) &quantified (LOQ) were found to be 2.02 & 3.15 µg/m respectively.

Robustness

Robustness of method was measured by multiple injections of a homogenous sample containing Lamivudine by changing flow rate 1.2 mL/min and 1.6 mL/min, mobile phase composition Acetonitrile: water ratio 79:21 and 81:19, wavelength i.e. 289nm and 291nm. The method was found to be robust in the range of deliberate changes made.

Table-6: Robustness study with change in flow rate of Lamivudine

Flow rate mL/min	Conc. µg/mL	Area	AVG	%RSD
0.9	20	120451	120496	0.04909
0.9		120563		
0.9		120474		
1.1	20	120458	120452.7	0.0084
1.1		120459		
1.1		120441		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Table-7: Robustness study with change in concentration of mobile phase of Lamivudine

Mobile phase	Conc µg/mL	Area	AVG	%RSD
84:16	20	485745	487969.7	0.78008
84:16		492365		
84:16		485799		
86:14	20	482351	481031.3	1.11686
86:14		475122		
86:14		485621		

Conc, Concentration; AVG, average; RSD, Relative standard deviation

Table-8: Robustness study with change in Wavelength of Lamivudine**.**

Wavelength nm	Conc. µg/mL	Area	AVG	%RSD
270	20	495623	495049.7	0.33108
270		493201		
270		496325		
272	20	497512	496575	0.52079
272		498562		
272		493651		

Conclusion:

The analytical method development of lamivudine was done by RP-HPLC. The Phosphate buffer was pH 4 and the mobile phase was optimized with consists of acetonitrile: Phosphate buffer mixed in the ratio of 85:15 % v/ v. A C18 column contains octadecylsilane chemically linked to porous silica particles was used as stationary phase. UV detector was used at 271 nm. The solutions were chromatographed at a constant flow rate of 1 ml/min. The linearity range of lamivudine were found to be range 5-30µg/ml. of lamivudine Linear regression coefficient value was 0.999.

Acknowledgement

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References:

1. Indian Pharmacopoeia, Government of India, Ministry of health and Family welfare, the controller of publication, New Delhi, Lamivudine, 2007; II:1278-1279.
2. S. Abdul Jameela, 2009, Ravichandrana Application of UV-Spectrophotometric methods for estimation of lamivudine in tablets digest journal of nanomaterials and biostructure, 4, (2), 357 – 360
3. Hitesh.H.Bulchandani "Simultaneous quantitative determination of zidovudine and nevirapine in human plasma using isocratic, RP-HPLC" Tropical journal pharmaceutical research,2009,8
4. D.K.Mandloi, 2009, "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, 1(1) 286-296
5. Vibhuti Kabra, 2009,"Simultaneous quantitative determination of zidovudine and nevirapine in Human plasma using isocratic, reverse phase high performance liquid chromatography" Tropical Journal of Pharmaceutical Research, 8, (1), 79-86
6. K. Basavaiah and U.R.anilkumar, 2007, "Spectrophotometric Determination of zidovudine in Pharmaceuticals Based on Charge-Transfer Complexation Involving N- Bromosuccinimide, Metol and Sulphanilic Acid as Reagents" E- Journal of Chemistry 4, (2), 173-179,
7. https://go.drugbank.com/drugs/DB01132
8. http:/www.chromatography.com
9. Chatwal G. R., Anand S. K.; 2005, Instrumental Method of chemical analysis; Himalaya Publishing House, 11th edition, 2.634-2.638.