IJBPAS, August, 2024, 13(8): 4137-4145

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF LAMIVUDINE FOR ESTIMATION OF ACTIVE PHARMACEUTICAL DOSAGE FORMS

RAJA B[1*], DEDEEPYA P[2], DISH O[2], RAUFY DS[2] AND K RAVEENDRA BABU[2]

1: Department of Department of pharmaceutical Analysis, Anurag Pharmacy College, Ananthagiri (V&M), Suryapet (Dt.), T.S-508206

2: Anurag Pharmacy College, Ananthagiri (V & M), Suryapet (Dt.), T.S-508206

***Corresponding Author: Dr. B. Raja: E Mail: rajabadavathu@gmail.com**

Received 18th May 2023; Revised 20th Aug. 2023; Accepted 20th Nov. 2023; Available online 1st Aug. 2024

https://doi.org/10.31032/IJBPAS/2024/13.8.8274

ABSTRACT

The aim of this work was to develop and validate a simple estimation method for Lamivudine in Active pharmaceutical dosage form using UV spectroscopic method. The method was developed using distilled water as a solvent and absorbance was measured at 270 nm. Beers law was obeyed the concentration range of 5 – 25 µg/ ml. Calibration curve shows a linear relationship between the absorbance and concentration. The line equation y =0.375x+0.004 with correlation coefficient (r) of 0.9998 was obtained. The method was validated as per ICH guidelines. The method was validated statistically and by recovery studies. The percentage recovery was found to be in the range between 98.09 and 101.46%. The %RSD value was found to be less than 1. A simple, accurate and cost-efficient spectroscopic method has been developed for the estimation of Lamivudine in active pharmaceutical dosage form.

Keywords: Lamivudine; Distilled water; Validation; ICH Guidelines, UV Spectroscopy

1. INTRODUCTION:

Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs. Analytical chemistry involves separating determining the relative amounts

of components in a sample matrix. Pharmaceutical analysis is a specialized branch of analytical chemistry. Pharmaceutical analysis derives its principles from various branches of sciences like physics, microbiology, nuclear science, and electronics etc. Qualitative analysis reveals the chemical identity of the sample. Quantitative analysis establishes the relative amount of one or more of these species or analyses in numerical terms **[1-3]**. Qualitative analysis is required before a quantitative analysis can be undertaken. A separation step is usually a necessary part of both a qualitative and quantitative analysis. The results of typical quantitative analysis can be computed from two measurements. One is the mass or volume of sample to be analyzed and second is the measurement of some quantity that is proportional to the amount of analyze in that sample and normally completes the analysis **[3]**.

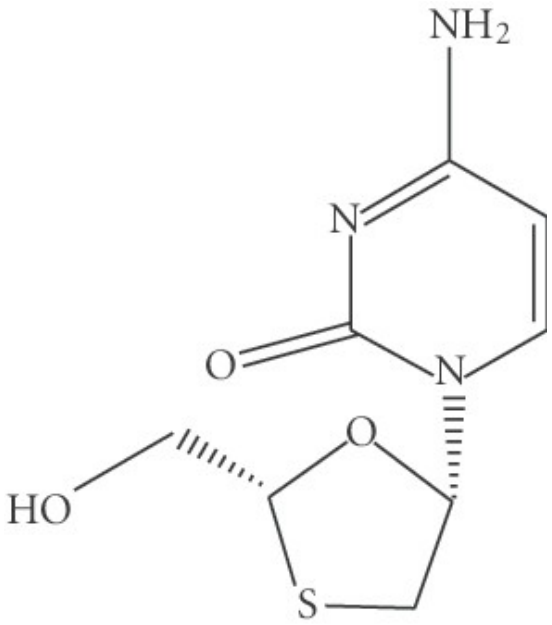

Figure 1: Structure of lamivudine

Lamivudine is (2R, 5S)-4-amino-1[2(hydroxyl methyl)-1, 3-oxathiolan-5yl]-2(1H)-pyrimidinone were shown in **(Figure 1)**. Lamivudine is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B virus. It is used as an Anti retroviral agent and Nucleoside reverse transcriptase inhibitor. Lamivudine is used for the prophylaxis and chronic treatment of HIV in adults and children of 3 months to 12 years. Lamivudine is rapidly absorbed after oral doses and peak plasma concentration occur about in 1 hour. Lamivudine crosses the blood

brain barrier with the ratio of cerebrospinal fluid to serum concentration of about 0.12. It crosses the placenta and is distributed into the breast milk. It is metabolized intracellular to the active antiviral triphosphate. Hepatic metabolism is low eliminated through renal excretion. Extensive literature survey revealed that only UV spectroscopy and RP-HPLC methods were reported for the estimation of Lamivudine in combination with other drug but there is no method was reported for the estimation of Lamivudine alone in bulk and in formulation by UV Spectrophotometry. A few HPLC and HPTLC methods were also developed for the determination of Lamivudine. So, an attempt was made to develop simple, cost effective and accurate UV spectrophotometric method for the estimation of Lamivudine in bulk and in active pharmaceutical dosage forms **[7-9]**

MATERIALS AND METHODS

Lamivudine raw material was procured from yarrow pharma limited. Hyderabad, India with 98% purity. Tablet formulation LAMIVIR HBV (Cipla Ltd. Sikkim, India) containing Lamivudine IP 100 mg was purchased from local market. All reagents and solvents used were analytical grade. Double distilled water was obtained from the Millipore unit. UV spectrophotometric method was performed on PERKIN ELMER Double Beam UV-Visible Spectrophotometer with pair of 10 mm matched quartz cell.

Selection of solvent

Different solvents such as distilled water, methanol, ethanol, toluene, acetic acid, isopropyl alcohol, N-butanol carbon tetrachloride, benzene, hexane, ethyl alcohol, acetonitrile, chloroform, diethyl ether and acetone were tried for the estimation of Lamivudine in tablet dosage form **[10]**. Because of easy availability and cost effectiveness distilled water was selected as the solvent for the analysis of Lamivudine.

Preparation of standard stock solution

100 mg of Lamivudine raw material was accurately weighed and transferred into the 100 ml volumetric flask. Dissolved in minimum quantity of distilled water and made up to 100 ml with the same. 10 ml of the stock solution was transferred into 100 ml and dilute with distilled water. The dilution was observed to contain 10 µg/ ml **[11, 12]**.

Selection of wavelength for estimation and stability studies

The concentration solution of 10 µg/ ml was scanned between the ranges of 200 - 400 nm using distilled water as blank. From the UV spectra, λmax was found to be 270 nm and was selected as analytical wavelength. The UV spectrum of Lamivudine was shown in **Figure 2**.

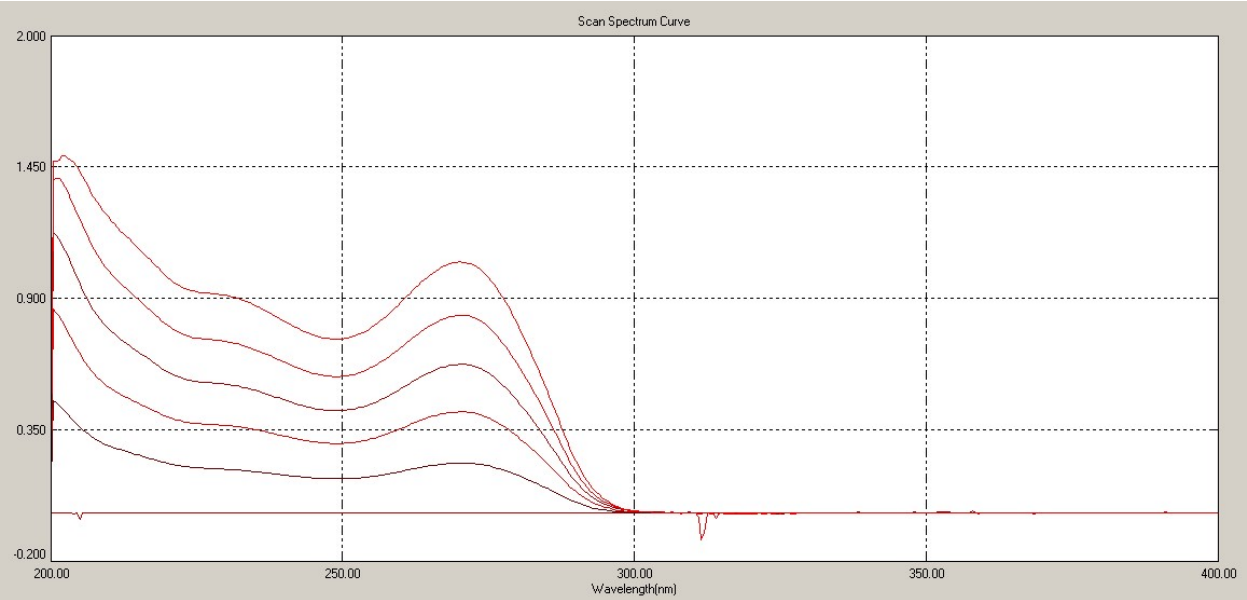

Figure 2: spectrum of lamivudine

Determination of calibration curve

A liquots (0.5,1,1.5,2,2.5 µg/ml) of prepared standard solution were transferred into series of 10 ml volumetric flasks and diluted by distilled water to give the concentration range of 0.5 -2.5µg/ml. The above solutions were scanned over the range of 200 nm to 400 nm against reagent blank. The absorbance of each solution at 270 nm against distilled ater as blank. A calibration curve was prepared by plotting absorbance versus concentration.

Method Validation

The following parameter was evaluated for method validation

Linearity:

Fresh aliquots were prepared from standard stock solution ranging from 0.5-2.5 µg/ml and the absorbance values of each concentration was recorded at 270nm for this method using Methanol as blank **[13]**. The drug shows linearity between 0.5-2.5µg/ml for this method. Results were shown below in **Figure 3**.

Linearity results of Lamivudine

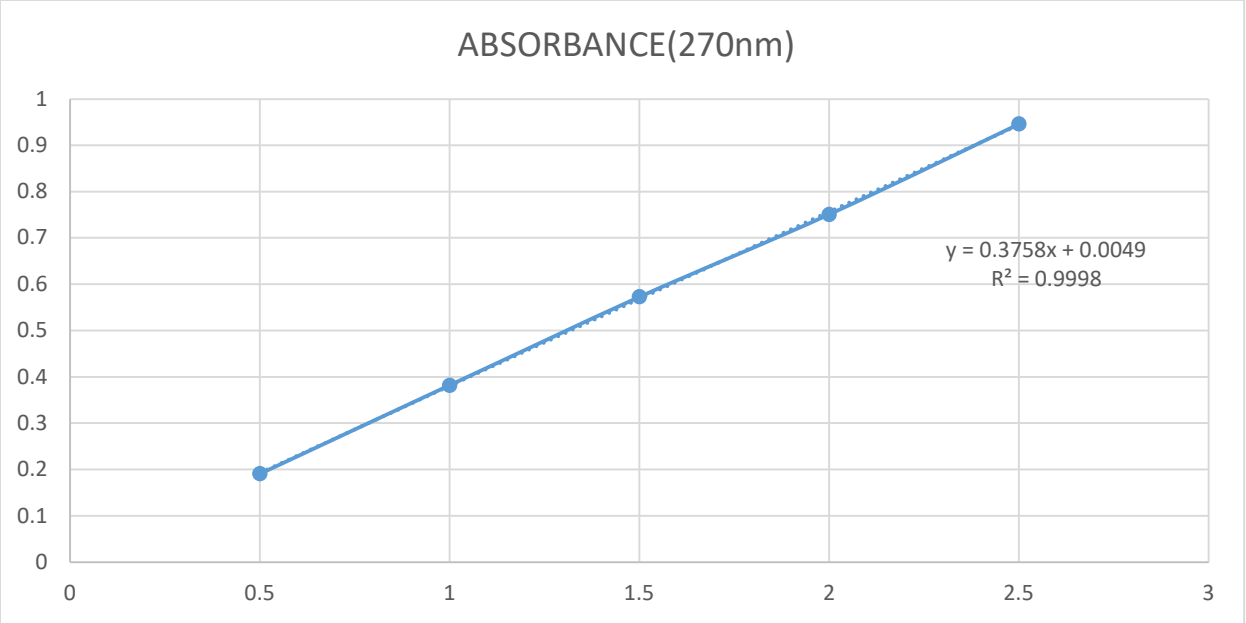

Figure 3: Linearity Curve

Precision:

In intraday study, concentration of replicates of drug was calculated on the same day for two times. In inter-day study the concentration of drug were calculated on two successive days which expresses the laboratory variation in different days. In both intra and inter day precision study for the methods %RSD was calculated and results are shown below:

Intra-day precision (Repeatability) data of proposed method

S. No.	CONCENTRATION (µg/ml)	ABSORBANCE (270nm)
1	0.5	0.191
2	1	0.382
3	1.5	0.573
4	2	0.751

S. No.	CONCENTRATION (µg/ml)	ABSORBANCE	AT (270nm)
		morning	Evening
1	20	0.108	0.107
2	20	0.109	0.108
3	20	0.107	0.107
4	20	0.108	0.107
5	20	0.107	0.106
6	20	0.106	0.107
Average		0.107	0.107
Standard deviation		0.000816	0.00057
%RSD		0.769%	0.532%

Inter-day precision (Reproducibility) data of proposed method

S. No.	CONCENTRATION(µg/ml)	ABSORBANCE	AT(270nm)
		Morning	evening
1	20	0.106	0.107
2	20	0.108	0.106
3	20	0.107	0.108
4	20	0.106	0.108
5	20	0.108	0.107
6	20	0.108	0.108
Average		0.107	0.107
Standard deviation		0.000912	0.000816
%RSD		0.852%	0.762%

Robustness:

Robustness of the method was determined by carrying out the analysis at three different wavelengths (±2nm). The respective absorbance was noted and the result was indicated by % RSD and results were shown below Robustness studies data of proposed method.

S. No.	Concentration (µg/ml)	Absorbance at (270nm)		
		269nm	270nm	271nm
1	20	0.107	0.108	0.108
2	20	0.106	0.109	0.107
3	20	0.107	0.107	0.108
4	20	0.106	0.109	0.109
5	20	0.107	0.108	0.109
6	20	0.107	0.108	0.107
Average		0.106	0.108	0.108
Standard Deviation		0.000816	0.000707	0.000816
%RSD		0.769%	0.654%	0.755%

Ruggedness:

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbance was noted. The result was indicated by % RSD and results were shown below:

Table 5: Ruggedness studies data of proposed method

S. No.	CONCENTRATION (µg/ml)	ABSORBANCE	At 270nm
		Analyst 1	Analyst 2
1	30	0.118	0.119
2	30	0.120	0.118
3	30	0.119	0.119
4	30	0.116	0.117
5	30	0.120	0.118
6	30	0.119	0.120
average		0.118	0.118
Standard Deviation		0.00048	0.00108
%RSD		0.406%	0.915%

Accuracy:

Accuracy of the developed method was confirmed by performing recovery studies at three different concentration ranges 50%, 100%, 150% each one in triplicate. From the recovery studies it was clear that the method is very accurate for quantitative estimation of tablet as the statistical results were within the acceptance range. Results were shown below.

Accuracy studies data of proposed method

S. No.	SPIKE LEVEL	CONCENTRTION ADDED (µg/ml)	CONCENTRATION FOUND(µg/ml)	%RECOVERY	MEAN% RECOVERY
1	50%	10	10.9	100.9	100.9%
2	50%	10	10.9	100.9	
3	50%	10	10.9	100.9	
1	100%	20	20.6	100.6	100.6%
2	100%	20	20.5	100.5	
3	100%	20	20.6	100.6	
1	150%	30	29.6	98.6	98.7%
2	150%	30	29.7	98.7	
3	150%	30	29.8	98.8	

RESULTS

1. Linearity

Linearity was observed within 0.5-2.5µg/ml and 10mg/ml concentration the acceptable limit is, it should be linear in the specified range and the correlation coefficient, 0.99. The correlation coefficient, R=0.999. Hence the relationship between the concentrations and the absorbance of lamivudine showed linearity.

2. Determination of active ingredients in Tablets

According to the IP, lamivudine tablets should not contain less than 90% and not more than 100% of the stated amount of lamivudine. Hence the average percentage recovery of 98 % was found to be within the acceptance limit. %RSD values did not exceed the acceptance limit of 1 %.

3. Accuracy

According to IP Lamivudine contains not less than 99.0 %and not more than 101.0 percent of lamivudine. The results showed that drug content was within specified limits and the %RSD values did not exceed the accepted limit of 1%. Hence the method can be said to be accurate.

4. Precision

The %RSD values for repeatability, intraday and inter day precision data were well below the specified limit of 1% and 2%, respectively. Hence, the method was found to be precise in the specified range

5. Robustness

The assay was done under different temperature and wave length conditions . The results showed %RSD values to be with in the acceptance criteria of 1%. Hence, the method was found to be robust in the given conditions.

CONCLUSION

The proposed method was simple, sensitive, and cost-effective. Method was validated in terms of precision, linearity and accuracy. The results are reproducible, and can be used successfully for the estimation of lamivudine in active pharmaceutical formulations.

REFERENCE:

[1] Tripahti KD. Essential of medical pharmacology. 6thEdn. Jaypee Brother Medical publisher, New Delhi; 2003. p. 809-11, 815, 816.

[2] Satoskar RS, Rage NN. Pharmacology and pharmacotherapeutics. 23 rd Edn. Popular Prakashan, Mumbai; 2013. p. 818.

[3] Rang HB, Dale MM, Rither JM. Pharmacology. 4th Edition: Churchill Livingstone; 1999. p. 725-31.

[4] SK Berar. Essentials of pharmaceutics. 6thedn. Chand and Company Ltd, New Delhi; 2000. p. 458-9.

[5] Nachname, Vorname. Derivative-differential UV spectrophotometry and compensation technique for the simultaneous determination of zidovudine and lamivudine in human serum. Die Pharmazie Int J Pharm Sci 2004; 59: 106-11.

[6] Savaşer A. Determination of abacavir, lamivudine and zidovudine in pharmaceutical tablets, human serum and in drug dissolution studies by HPLC. Chromatographia 2007; 65: 259-65.

[7] Jayaseelan S. A new analytical method development and validation for the simultaneous estimation of lamivudine and stavudine in tablet dosage form by RP-HPLC method. Int J Pharm Tech Res 2010; 2: 1539-42.

[8] Manikanta Kumar A, B Naga, Abstract Sandhya, Mahesh Nasare, VVLN Prasad, Prakash V Diwan. Development and validation of UV spectrophotometric method for simultaneous estimation of lamivudine and efavirenz in the pharmaceutical dosage form. J Adv Pharm Education Res 2012; 2: 210-4.

[9] Nevens, Frederik. Lamivudine therapy for chronic hepatitis B: a six-month randomized dose-ranging study. Gastro-enterology 1997; 113: 1258-63.

[10] PV Rajesh, CP Karunasree, G Dharmamoorthy, K Padmini, CH Sudeer. Development and partial validation of the lamivudine drug in bulk and solid dosage form by uv spectroscopy. IJPDT 2012; 2: 15-9.

[11] Steinmüller, Thomas. Increasing applicability of liver transplantation for patients with hepatitis B–related liver disease. Hepatology 2002; 35: 1528-35.

[12] ICH draft Guidelines on Validation of Analytical Procedures: Definitions and Terminology, Federal Register, 60, IFPMA, Switzerland; 1995. p. 1260.

[13] Becket AH, Stenlak JB. Practical pharmaceutical chemistry. 4thEdn. CBS Publisher and Distribution, New Delhi; 2004. p. 275-337.

[14] Mendham J, Denney RC. Vogel's Textbook of Quantative Chemical Analysis. 6th Edn. Dorling Kindersley Pvt. Ltd New Delhi; 2006. p. 704-15.

[15] Willard H Hobart, Merritt L. Lynne; Instrumental method of analysis. 1st edn CBS Publishers and Distribution, New Delhi; 1986. p. 164-84.

[16] Lahane SB, Deokate UA. Development and validated UV spectrophotometric method for estimation of Albendazole in tablet dosage form. WJPR 2014;3:1461-7.

[17] British Pharmacopoeia. Vol. I. Published by the stationary office on behalf of the Medicine and Healthcare Products Regulatory Agencies, London; 2008. p. 76-7.

[18] United States Pharmacopoeia. In Validation of Compendial Methods. 26th edn: Pharmacopoeial Convention Inc., Rockville; 2003. p. 2439–42.

[19] Indian Pharmacopoeia. Vol. II. Ministry of Health and Family Welfare Government of India: Published by Indian Pharmacopoeia Commission, Ghaziabad; 2007. p. 692-3.