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DEVELOPMENT AND PARTIAL VALIDATION OF THE LAMIVUDINE DRUG IN BULK AND SOLID DOSAGE FORM BY UV-SPECTROSCOPY

*** P.V. Rajesh, C.P. Karunasree, G. Dharmamoorthy, K. Padmini, CH. Sudeer**

Seven Hills College of Pharmacy, Tirupati, Andhra Pradesh, India.

Abstract

A new, simple and sensitive spectrophotometric method in ultraviolet region has been developed for the determination of Lamivudine in bulk and in pharmaceutical formulations. Lamivudine exhibits absorption maxima at 270 nm. Developed method obeyed the Beer's law in the concentration range of 5 - 25 µg/mL. The method is accurate, precise and economical. The proposed method has been applied successfully for the analysis of the drug in pure and in its tablet dosage forms. In this method, there is no interference from any common pharmaceutical additives and diluents. The % recovery is greater than 98 to101%. %, this shows that the method was free from the interference of excepients. The results of the tablet analysis were validated with respect to accuracy (recovery), linearity, limit of detection and limit of quantization were found to be satisfactory.

Keywords: UV Spectrophotometry, Lamivudine, Lamivudine Tablet.

INTRODUCTION

Lamivudine is a potent nucleoside analog reverse transcriptase inhibitor (nRTI) used as an antiretroviral agent that inhibits replication of some retroviruses in combination with zidovudine in the management of HIV (human immunodeficiency virus).Lamivudine (2',3'-dideoxy-3'-thiacytidine, commonly called 3TC) is a potent nucleoside analog reverse transcriptase inhibitor (nRTI).It is marketed by GlaxoSmithKline with the brand names Zeffix, Heptovir, Epivir, and Epivir-HBV.Lamivudine has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV. It improves the seroconversion of e-antigen positive hepatitis B and also improves histology staging of the liver. Long term use of lamivudine unfortunately leads to emergence of a resistant hepatitis B virus (YMDD) mutant. Despite this, lamivudine is still used widely as it is well tolerated. Literature survey reveals only few analytical methods that have been developed for its determination of Lamivudine in human plasma has been mainly determined using liquid or gas chromatography with mass spectrometry, following a liquid – liquid extraction Hence it was thought Worthwhile to develop simple spectrophotometric method for the same [1-3].

MECHANISM OF ACTION

Lamivudine is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, Lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination [8].

MATERIALS AND METHODS

Experimental

A Systronics UV-Vis double beam spectrophotometer (model 2201) with 1 cm matched quartz cells was used for all spectral measurements. All chemicals used were of A.R. grade from S.D. Fine-chem, Merck, Fischer scientific, and Spectrochem, Mumbai. Authentic drug sample of Lamivudine was given as a gift sample by Hetero drugs limited, Hyderabad. Tablets of Lamivudine are procured from local market.

Corresponding Author :- **P.V. Rajesh** Email:- rajesh.paranjivasu@gmail.com

Working Standard Solution of Lamivudine

Standard stock solution was prepared by dissolving accurately weighed, 100mg of Lamivudine in distilled water and the volume was made upto 100ml with distilled water (stock solution-I, 1000 mcg/ml).From this, a working standard solution containing 100 mcg/ml was prepared with distilled water and the same was used for UV method using distilled water .

Sample Preparation of Lamivudine

20 tablets of two different brands of Lamivudine were weighed and powdered in glass mortar and the powder equivalent to 25 mg of Lamivudine was weighed accurately and transferred into a 25 ml standard volumetric flask. The contents were dissolved in distilled water and sonicated for five minutes. This solution was filtered through 0.45 µ Whatmann filter paper. 5 ml of the filtrate was diluted to 50 ml with distilled water to get the solution of 100 mcg/ml and the same was used for UV method using distilled water.

UV Spectrophotometric Determination of Lamivudine using Distilled water

This method has been developed for the quantitative estimation of Lamivudine in bulk drug and pharmaceutical dosage forms. Lamivudine has an absorption maximum at 270 nm in distilled water (Fig. 02); Beer's law is obeyed in the concentration range of 5-25 mcg/ml (Fig. 03).

RESULTS & DISCUSSION

The absorption spectra were recorded in the wavelength region of 200-400 nm in UV method. The absorption maxima (λ max) were observed at 270nm for Lamivudine. Obeys the beer's law was confirmed by the linearity of the calibration curve of Lamivudine, which is represented in Graph. Lamivudine showed linearity in the concentration range of 5-25µg/ml. The data regarding the calibration curve are given in table-2.

The quantitative estimation was carried out on formulation by taking a concentration range of 5-25µg/ml for Lamivudine. The quantitative results obtained were subjected to statistical analysis to find out standard deviation and standard error values. The relative standard deviation values are given below 2% indicating the precision of the methodology and low standard error values shown the accuracy of the method. The validation of the proposed method was further conformed by recovery studies.

The percentage recovery values vary from 98 to101%. For Lamivudine formulation. This serves as a good index of accuracy and reproducibility of the studies. The results obtained in repeatability test expresses the precision of the method. The percentage recovery greater than 98% shows that the method was free from the interference of exicipients used in the formulation.

OPTICAL CHARACTERISTICS, PRECISION, ACCURACY

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity, regression analysis using the method of least squares was made for the slope (b), intercept (x) and correlation (r) obtained from different concentrations, percent relative standard deviation. LOD and LOQ calculated from the five measurements, 3/4th of the amount of upper Beer's law limits in each method are presented in Tables: 1 and 7 the results showed that the methods have reasonable precision. Results obtained with the visible spectrophotometric methods are compared with the results obtained with UV Spectrophotometric method.

RECOVERY STUDIES

Results obtained with proposed methods confirm the suitability of these methods for pharmaceutical dosage forms. The other active ingredients and excipients usually present in the pharmaceutical dosage forms did not interfere in the estimation when some commercial dosage forms were analyzed by these methods. The accuracy of the methods is confirmed by the recovery studies.

INTERFERENCE STUDIES

The other active ingredients and excipients present in the dosage forms of Lamivudine in did not interfere, when added in the above concentration range to the drug and estimated by the proposed method.

Table 1. DRUG PROFILE [4-7]

S.NO.	CHARACTER	DETAILS
1.	CHEMICAL FORMULA	$C_8H_{11}N_3O_3S$
2.	PHYSICAL APPEARANCE	It is a white to off-white crystalline powder
3.	MOLECULAR WEIGHT	229.26 g/mol
4.	IUPAC NAME	4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1H-pyrimidin-2-one
5.	SOLUBILITY	Soluble in water
6.	pKa	10.2
7.	MELTING POINT	186-188 ^{o}C
8.	STORAGE CONDITION	Stable under normal conditions. Store in air tight container at room temperature

Table 2. Optical characteristics and precision data Parameters

Parameter	UV Method
λ_{max} (nm)	270
Beer's law limits (mcg/ml)	5-25
Molar extinction coefficient (mol^{-1} cm^{-1})	0.0465 $X10^4$
Sandell's sensitivity(mcg/cm^2-0.001 absorbance units)	0.021
Regression equation (Y*)	Y=0.046C + 0.0014
Slope (b)	0.046
Intercept (a)	0.0014
Correlation coefficient(r^2)	0.9999
% RSD**	0.648
Limit of detection (mcg/ml)	0.130
Limit of quantitation (mcg/ml)	0.422

*Y= bC + a where C is the concentration of Lamivudine in mcg/ml and Y is the absorbance at the respective $\lambda_{max.}$
**Average of five determinations.

Table: 3. Assay and recovery of lamivudine in tablet dosage form

Brand used	Label claimed(mg)	Amount found by proposed method(mg)	% label claim	% RSD*
Tab-a	100	99.77	99.77	0.86
Tab-b	100	99.56	99.56	1.18

*Average of five determinations

Table 4. Results of recovery studies of Lamivudine

Brand used	Label claimed(mg)	Mean assay value	Known amount of Nevirapine added	Mean % recovery ±%RSD*
Tab-a	100	99.77	10mg	99.80±1.130
			20mg	99.95±1.586
Tab-b	100	99.56	10mg	99.88±1.158
			20mg	99.74±1.479

*Average of five determinations.

Table 5. Results of intraday precision studies of Lamivudine estimation by UV method

Brand used	Label claimed(mg)	Amount found by proposed method(mg)	% label claim	% RSD*
Tab-a	100	99.70	99.70	1.261
Tab-b	100	99.52	99.52	1.188

*Average of five determinations.

Table 6. Results of inter day precision studies of Lamivudine estimation by UV method

Brand used	Label claimed(mg)	Amount found by proposed method(mg)	% label claim	% RSD*
Tab-a	100	99.17	99.17	1.237
Tab-b	100	99.04	99.04	1.121

*Average of five determinations.

Table 7. Results of ruggedness studies of Lamivudine estimation by UV method

Brand used	Label claimed(mg)	Normal condition (Mean assay value)	Changed condition (Mean assay value) ±%RSD*
Tab-a	100	99.77	99.38±1.235
Tab-b	100	99.56	99.21±1.193

*Average of five determinations.

Table 8. Results of robustness studies of Lamivudine estimation by UV method

Brand used	Label claimed(mg)	Normal condition (Mean assay value)	Changed condition (Mean assay value) ±%RSD*
Tab-a	100	99.77	99.18±0.465
Tab-b	100	99.56	99.08±0.373

*Average of five determinations.

Fig 1. STRUCTURE OF LAMIVUDINE

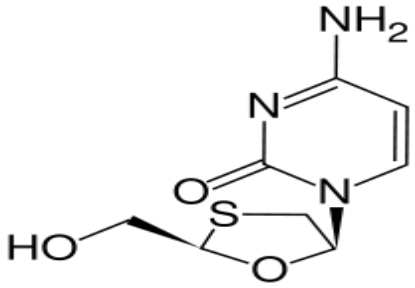


Fig. 2. Absorption spectrum of Lamivudine with Distilled water

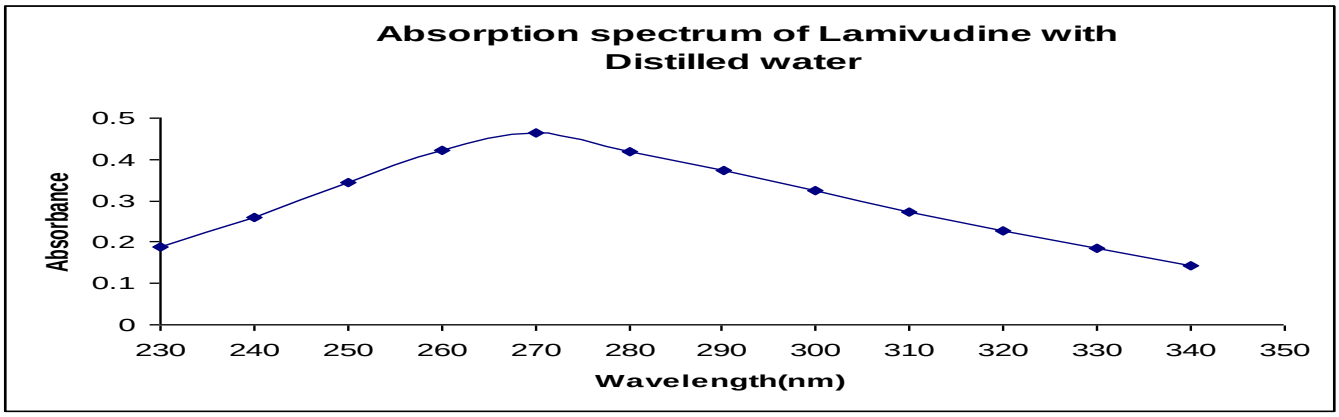
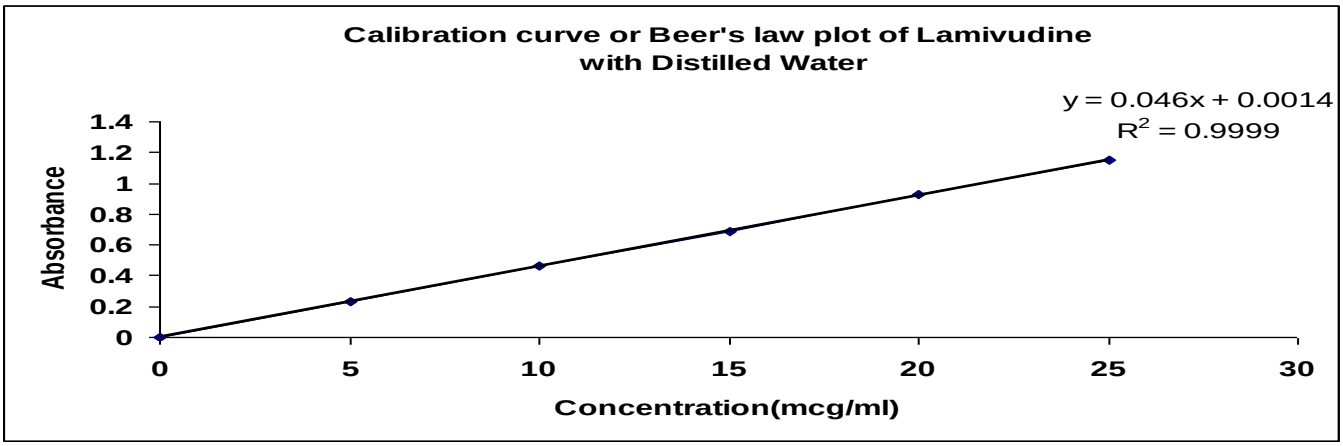


Fig. 3. Calibration curve of Lamivudine with Distilled water



CONCLUSION

The proposed UV method validation for estimation of Lamivudine in bulk dosage form is carried out as per ICH and USP Guidelines. The method found to be specific for validation of estimation of Lamivudine in bulk dosage form. The method found to be linear in the specified range. Hence, this method stands validated and can be used for routine analysis.

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