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**DEVELOPMENT AND PARTIAL VALIDATION OF THE
LAMIVUDINE DRUG IN BULK AND SOLID DOSAGE FORM BY
COLORIMETRY**

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Abstract

A new, simple and sensitive spectrophotometric method in visible region has been developed for the determination of Lamivudine in bulk and in pharmaceutical formulations. Lamivudine exhibits absorption maxima at 425 nm. Developed method obeyed the Beer's law in the concentration range of 5 - 20 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 99 to 101%. This shows that the method was free from the interference of excipients. 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, Colorimetric Estimation, 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.

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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 m 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 Visible method using distilled water.

Colorimetric Estimation of Lamivudine using Hydroxyl amine

Method is based on condensation of Lamivudine with hydroxyl amine under acidic conditions to form an oxime that is a yellow coloured chromogen which shows maximum absorption at 425 nm (Fig. 2) and obeys Beer's law in the concentration range of 5-20 mcg/ml (Fig. 3)

Preparation of reagents

Hydroxyl amine hydrochloride solution (0.5% w/v): It was prepared by dissolving 0.5 g of hydroxyl amine hydrochloride in 50 ml of water. Then 50 ml of ethanol (95%) and 1 ml of bromo phenol blue solution was added .Then 0.1M NaOH was added until the solution becomes green.

Bromophenol blue solution: It was prepared by dissolving 0.1 g of bromophenol blue with gentle heating in 1.5 ml of 0.1M NaOH and 20 ml of ethanol (95%). Then sufficient water was added to produce 100 ml.

Development of colour and study of spectra in visible region

Aliquots of standard solution of Lamivudine ranging from 0.5 to 2.0 ml (1 ml = 100 mcg) were transferred into a series of 10 ml volumetric flask. To each flask, 2.0 ml of hydroxyl amine solution (0.5% w/v) and 1 ml of Con.HCl was added and heated on boiling water bath for 30 min at a temperature of 100° C. The flasks were cooled and the volume in each flask was made up to 10 ml with distilled water. The absorbances of yellow colored chromogen were measured at 425 nm against the reagent blank. The colored chromogen was stable for 2 hours. The amount of Lamivudine present in the sample solution was computed from the respective calibration curve.

RESULTS & DISCUSSION

The objectives of the proposed work was to develop some new and sensitive analytical methods for the determination of Lamivudine and to validate the methods according to USP and ICH guidelines and applying the same for its estimation in pharmaceutical formulations. There is no official method for the estimation of Lamivudine.

The absorption spectra were recorded in the wavelength 350-800 nm in visible spectrometry. The UV spectra of Lamivudine are presented in fig-2 and 3. The absorption maxima (λ max) were observed at 425nm for Lamivudine. Obeys the beer's law was confirmed by the linearity of the calibration curve of Lamivudine, which is represented in fig-3. Lamivudine showed linearity in the concentration range of 5-20 μ g/ml. The data regarding the calibration curve are given in table-2.

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 recovery data is given in table 2 to 7 the percentage recovery values vary from 99 to 101% 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. UV/Vis Spectrophotometric methods developed were found to be rapid, simple, precise, accurate and economic for routine estimation of Lamivudine commercial dosage forms.

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.

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, by adding known amount of the pure drug to the formulation already analyzed by this method.

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. The methods reported here are found to be simple, sensitive, accurate, precise, and economical and can be used in the determination of Lamivudine from pharmaceutical dosage forms in a routine manner.

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 °C
8.	STORAGE CONDITION	Stable under normal conditions. Store in air tight container at room temperature

Table 2. Optimum conditions in colorimetric method using Hydroxyl amine

Parameter	Colorimetric method using Hydroxyl amine
λ_{max} (nm)	425
Beer's law limits (mcg/ml)	5-20
Molar extinction coefficient ($mol^{-1} cm^{-1}$)	0.0141×10^4
Sandell's sensitivity (mcg/cm^2 -0.001 absorbance units)	0.070
Regression equation (Y*)	$Y=0.0141C + 0.0002$
Slope (b)	0.0141
Intercept (a)	0.0002
Correlation coefficient(r^2)	0.9998
% RSD**	1.126
Limit of detection (mcg/ml)	0.161
Limit of quantitation (mcg/ml)	0.489

*Y= bC + a where C is the concentration of Lamivudine in mcg/ml and Y is the absorbance at the respective λ_{max} .

**Average of five determinations.

Table 3. Lamivudine in pharmaceutical formulations by using Hydroxyl amine

Brand used	Label claimed(mg)	Amount found by proposed method(mg)	% label claim	% RSD*
Tab-a	100	99.62	99.62	0.334
Tab-b	100	99.40	99.40	0.545

*Average of five determinations.

Table 4. recovery studies of Lamivudine

Brand used	Label claimed(mg)	Mean assay value	Known amount of Lamivudine added	Mean % recovery \pm %RSD*
Tab-a	100	99.62	10mg	99.90 \pm 1.09
			20mg	99.93 \pm 1.88
Tab-b	100	99.40	10mg	99.48 \pm 0.89
			20mg	99.73 \pm 0.65

*Average of five determinations.

Table 5. Results of intraday precision studies of Lamivudine

Brand used	Label claimed(mg)	Amount found by proposed method(mg)	% label claim	% RSD*
Tab-a	100	99.58	99.58	0.334
Tab-b	100	99.34	99.34	0.745

*Average of five determinations.

Table 6. Results of inter day precision studies of Lamivudine

Brand used	Label claimed(mg)	Amount found by proposed method(mg)	% label claim	% RSD*
Tab-a	100	99.51	99.51	0.306
Tab-b	100	99.31	99.31	0.72

*Average of five determinations.

Table 7. Results of ruggedness studies of Lamivudine

Brand used	Label claimed(mg)	Normal condition (Mean assay value)	Changed condition (Mean assay value) \pm %RSD*
Tab-a	100	99.62	99.34 \pm 0.76
Tab-b	100	99.40	99.17 \pm 0.36

*Average of five determinations.

Table 8. Results of robustness studies of Lamivudine estimation

Brand used	Label claimed(mg)	Normal condition (Mean assay value)	Changed condition (Mean assay value) \pm %RSD*
Tab-a	100	99.62	99.24 \pm 0.736
Tab-b	100	99.40	99.10 \pm 0.96

*Average of five determinations.

Table 9. Optimum conditions established for colorimetric method using Hydroxyl amine

Parameter	λ_{max} (nm)	Volume of Hydroxyl amine (0.5%) (ml)	Volume of Con.HCl (ml)	Solvent and Temperature	Stability Period After Final Dilution
Optimum Range	380-460	1.0-3.0	0.5–1.5	Water 100°C for 30 min	Immediate- 2 h
Condition in Procedure	425	2.0	1.0	Water 100°C for 30 min	30 min
Remarks	-	2.0 ml required for maximum color development	Variation of volume of Con.HCl <0.5 or >1.5 ml, Beer's law is not obeying at upper limit	Specific conditions were necessary for maximum color development	Stable up to 2 h

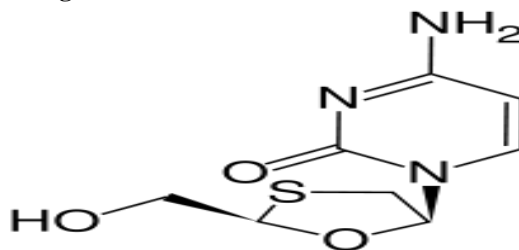
Fig 1. STRUCTURE OF LAMIVUDINE

Fig. 2. Absorption spectrum of Lamivudine with Hydroxyl amine

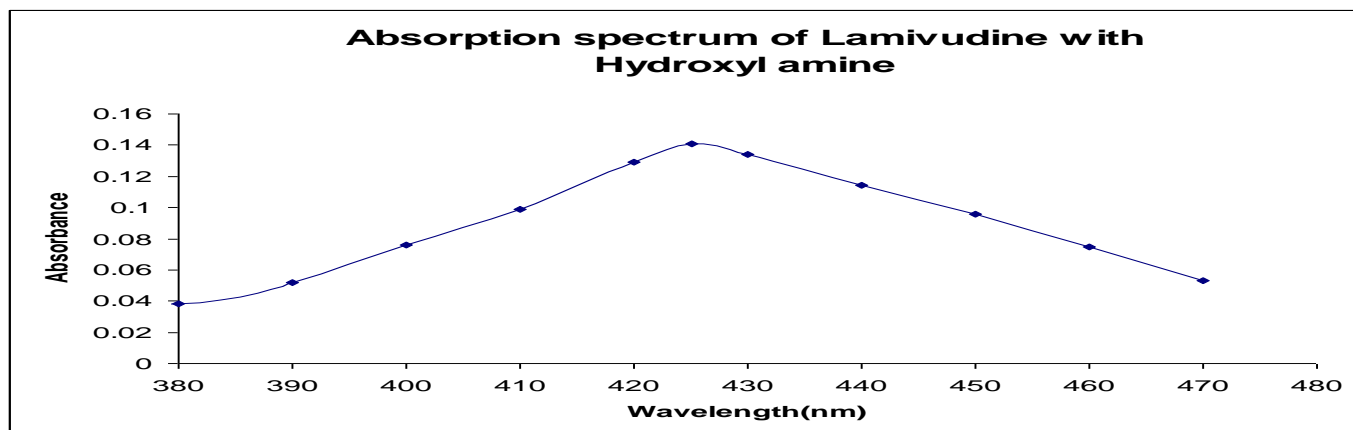
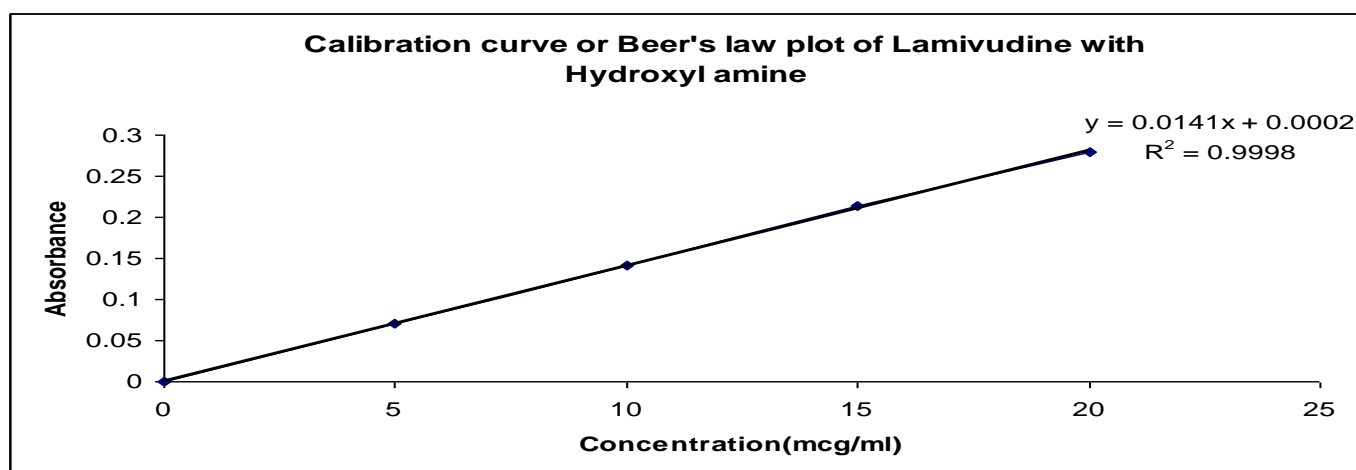


Fig. 3. Calibration curve of Lamivudine with Hydroxyl amine



CONCLUSION

The proposed visible 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. The new procedure for the spectrophotometric determination of Lamivudine described in this work is simple, rapid and cost-effective with high accuracy and precision when compared with previously reported procedures. It could find application as a convenient technique for the in-process control of Lamivudine.

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