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Research Article

Development and Validation of Visible Spectrophotometric Method for the Estimation of Lamivudine in Bulk and Pharmaceutical Dosage Form

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Abstract: A Simple, accurate, precise and economical procedure was developed for UV Spectroscopic estimation of Lamivudine in pure state and in its pharmaceutical formulation. The developed method is based upon complex formation of the drug with methyl red reagent. Linearity was observed in the concentration range of 0.5-5 µg/ml. Calibration curve was constructed by plotting absorbance values against concentrations, which gave good regression values. The method was validated statistically and recovery study was performed to confirm the accuracy of the method.
Keywords: Lamivudine, Spectrophotometric, complex, methyl red.

INTRODUCTION

Lamivudine is antiretroviral drug and acts by blocking reverse transcriptase [1]. Chemically Lamivudine is 4- amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2 dihydropyrimidin-2-one [2]. It has a molecular formula of C8H11N3O3S and a molecular weight of 229.26 g / mol and its structure was given in Fig: 1.

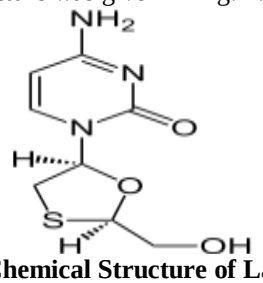

Fig. 1: Chemical Structure of Lamivudine

The dose of Lamivudine is 300 mg per day. Several combinations of Lamivudine with other antiretroviral drugs are available in the market for treatment of HIV infected patients [3]. Literature survey revealed, few analytical methods which include simultaneous determination of Lamivudine and Stavudine in human serum using HPLC with tandem mass spectrometry [4], development and validation of normal phase HPTLC method for analysis of Lamivudine, Stavudine and Nevirapine in fixed dosed combination tablet[5], simultaneous determination of HIV nucleoside analogues of reverse transcriptase inhibitors Lamivudine , Didanosine, Stavudine, Zidovudine and Abacavir in human plasma using reverse phase high performance liquid chromatography [6].Simultaneous determination of Lamivudine, Stavudine and Nevirapine in antiretroviral fixed dose combination by high performance liquid chromatography [7], Validation of high-performance liquid chromatography methods for determination of Zidovudine, Stavudine, Lamivudine and Indinavir in human plasma[8]. Lamivudine is not official in IP, BP and USP. The present work deals with estimation of Lamivudine in tablets by UV-Spectrophotometry and first order derivative [9].

MATERIALS AND METHODS

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 Ltd., Hyderabad. Tablets of Lamivudine were procured from local market. Distilled water was used for the preparation of solutions.

Instrument

Labindia – 3000+ UV / Vis double beam Spectrophotometer with a fixed slit width (2 nm) and 10 millimeter quartz cell was used to obtain spectrum and absorbance measurement.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving accurately weighed 100 mg of Lamivudine in water and the volume was made up to 100 ml with water in 100 ml volumetric flask (1000 mcg / ml). 10 ml of the above solution was diluted to 100 ml with water (100 mcg / ml). 1 ml of the solution was taken, 1ml of methyl red solution was added in 10 ml standard flask diluted to 10 ml with water to get the concentration 10 mcg / ml. The absorbance of resulting solution was measured against respective blank solution in the UV region of 400-800 nm, which shows maximum absorbance at 570 nm and was given in Fig: 1.

Preparation of standard curve

Aliquots of standard solution of Lamivudine ranging from 0.5 - 5.0 ml (1 ml = 100 mcg) were transferred into a series of 10 ml volumetric flasks. 1ml of methyl red solution was added and the volume in each flask was made up to 10 ml with water and the absorbances were measured at 570 nm against solvent blank and the absorbance values were shown in Table: 2. The obtained absorbance values are plotted against the concentration of Lamivudine to get the calibration graph and were represented as Fig: 2. The concentration of the unknown sample was determined from the calibration graph. The regression equation and correlation coefficient were determined and are given in Table: 3.

Sample preparation of Lamivudine

20 tablets of Lamivudine were weighed, powdered in glass mortar and the powder equivalent to 100 mg of Lamivudine was weighed accurately and transferred into a 100 ml standard volumetric flask. The contents were dissolved in water. This solution was filtered through (0.45µ) Whatmann filter paper. 1 ml of the filtrate was diluted to 10 ml with water to get the solution of 100 mcg / ml. To aliquot of 1 ml of test solution, 1ml of methyl red solution was added was diluted to 10 ml with water in 10 ml standard volumetric flask to produce the concentration 10 mcg / ml.

Validation of Spectrophotometric method

Accuracy was determined by recovery studies. The recovery studies were carried out by adding the known amount of Standard Lamivudine drug to the sample solution of the capsules. Precision for assay were determined by repeatability, interday, intraday precision for drug (each in three replicate). Ruggedness studies were carried out by changing the analysts.

Limits of detection (LOD) and limits of quantitation (LOQ)

The LOD and LOQ of Lamivudine are determined by using calibration standards. Value of LOD is determined by using the formula: 3.3σ/S And the value of LOQ is determined by: 10σ/S , where 'σ ' is the standard deviation of the y intercept of the regression equation and 'S' is the slope of calibration curve.

RESULTS

Table 1: Results of calibration curve at 570 nm for Lamivudine by UV Spectroscopy

Sl. No.	Conc. (mcg / ml)	Absorbance at 540 nm
1	0.5	0.289
2	1	0.313
3	1.5	0.328
4	2	0.350
5	2.5	0.368
6	3	0.389
7	3.5	0.408
8	4	0.428

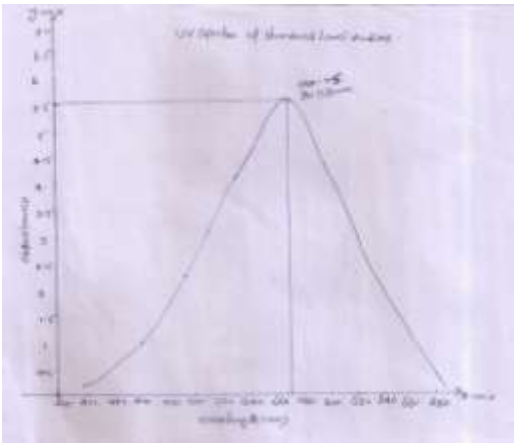

Fig: 2 Spectra of Lamivudine at 570 nm

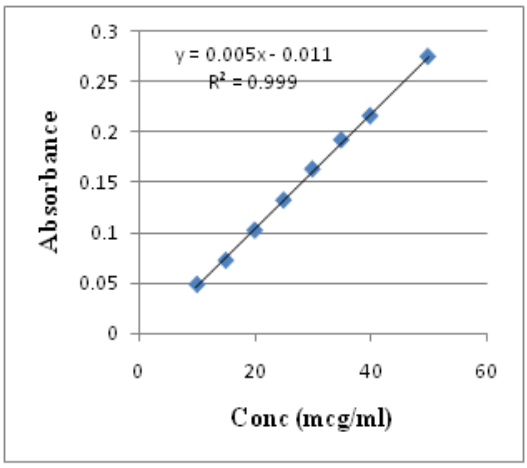

Fig. 3: Calibration curve of Lamivudine

Table 2: Shows Optical Characteristics of Lamivudine

Parameter	UV method
λ_{max} (nm)	570
Beer's law limits (mcg / ml)	0.5-5.0
Sandell's sensitivity (mcg / cm^2-0.001 absorbance units)	0.0182
Regression equation (Y*)	Y = 0.0388X - 0.2717
Slope (b)	0.0388
Intercept (a)	+0.2717
Correlation coefficient(r^2)	0.9992
% RSD**	< 2%
Limit of detection (mcg / ml)	0.120
Limit of quantitation (mcg / ml)	0.364

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

Table 3: Assay of Lamivudine formulation

Sl. No	Formulation	Label claim (mg/tab)	Amount found (mg) (n=3) Mean ± SD	Assay	%RSD
1	**Epivir**	300	30.003±0.055	99.51%	1.088

Table 4: Determination of Accuracy results for Lamivudine at 570 nm

Brand name	Amount of sample (mcg / ml)	Amount of drug added (mcg / ml)	Amount Recovered	% Recovery ± SD**
Epivir	10	4.5	0.447	99.32 ± 0.001414
Epivir	10	5	0.463	99.56 ± 0.001414
Epivir	10	5.5	0.471	99.78 ± 0.001414

Table: 5 Determination of Precision results for Lamivudine at 570 nm

Conc. (mcg / ml)	Inter-day Absorbance Mean ± SD**	% RSD	Intra-day Absorbance Mean ± SD**	% RSD
LQC (1mcg/ml)	0.311 ± 0.001414	0.454	0.313 ± 0.001414	0.451
MQC (2.5mcg/ml)	0.367 ± 0.001414	0.385	0.368 ± 0.001414	0.384
HQC (4.5mcg/ml)	0.446 ± 0.001414	0.317	0.447 ± 0.001414	0.316

**Average of six determinations.

DISCUSSION

The absorption spectral analysis shows the λ_{max} of Lamivudine at 570 nm. The calibration curve was obtained for a series of concentration in the range of 0.5 - 5 mcg/ml. It was found to be linear and hence, suitable for the estimation of the drug. The slope, intercept, correlation coefficient and optical characteristics are summarized in Table 2. Regression analysis of Beer's law plot revealed a good correlation. The effects of various excipients generally present in the tablet dosage form of Lamivudine were investigated. The results indicated that they did not interfere in the assay in amounts far in excess of their normal occurrence in it. The proposed method was validated as per the ICH guidelines. The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample. The % RSD was found to be within the limits. This showed that the precision of the method is satisfactory. The recovery technique was performed to study the accuracy and reproducibility of the proposed method. For this, known quantities of the Lamivudine solution was mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed. The total amount of Lamivudine was determined by using the proposed methods and the amount of added drug was calculated by the difference. The % RSD was less than ± 2.0. This showed that the recoveries of Lamivudine by the proposed methods are satisfactory and the results are shown in Table 3 & 4. Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by the proposed method. Thus it can be concluded that the methods developed in the present investigation are simple, sensitive, accurate, rapid and precise. Hence, the above said method can be successfully applied for the estimation of Lamivudine in pharmaceutical dosage form.

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