Original Article

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METHOD DEVELOPMENT AND VALIDATION OF LAMIVUDINE IN TABLET DOSAGE FORM BY UV-SPECTROPHOTOMETRY

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Abstract

Analytical method development and validation play important roles in the discovery, development and manufacture of Pharmaceuticals. A simple and reproducible UV- spectrophotometric method for the quantitative determination of Lamivudine in Tablet formulation was developed and validated in the present work. The parameters linearity, precision, accuracy was studied according to ICH guidelines. Lamivudine has the maximum wavelength at 275 nm. Analytical Calibration curves were linear within a concentration range from 4.8 to 7.2µg/ml. The developed method was applied directly and easily to the analysis of the pharmaceutical tablet preparations. %R.S.D was found to be 0.475 (Lamivir HBV 100 mg Tablet) respectively. The result of analysis has been validated statistically. Hence the proposed method can be used for the reliable quantification of Lamivudine in Tablet formulation.

Keywords: UV-Spectrophotometry, Lamivudine, Pharmaceutical dosage form.

Introduction

Lamivudine is a reverse transcriptase inhibitor and zalcitabine analog in which a sulfur atom replaces the 3' carbon of the pentose ring. It is used to treat Hepatitis B and HIV disease. Lamivudine contains not less than 98.0 percent and not more than 102.0 percent of C8H11N3O3S, calculated on the anhydrous and solvent-free basis. Lamivudine is chemically 1[(2R, 5S)-2-(Hydroxymethyl)-1-3 oxathiolan-5yl] cytosine and used as an antiretroviral activity.The literature survey (Mandloi DK etal., 2009; Krishnareddy NV et al., 2011; Patro SK et al., 2010) reveals that there is some UV methods have been reported. The aim of the present study was to develop andvalidate a simple, UV Spectroscopic method for the determination of lamivudine tablets. The developed method was validated using ICH guidelines for validation (ICH, 1995).

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Materials and Methods

Absorption spectral measurements were carried out with a UV – Visible spectrophotometer (Analytical technologies model, spectro 2060 plus version 5) was employed with spectral bandwidth of 5 nm and wavelength accuracy of 0.3nm (with automatic wavelength correction with a pair of 5 cm matched quartz cells).

Lamivudine pure drug was supplied by local Pharmaceutical industry, India as gift sample and used as such Spectroscopy graded ethanol, tetrahydrofuran, Water and analytical reagent grade formic acid was used.

Preliminary solubility studies of drug

A small quantity of standard drug was dissolved in different solvents like distilled water, methanol, ethanol, acetonitrile, isopropyl alcohol, tetrahydro furan, 60mM formic acid and in various buffer solutions. By the solubility studies we determined that the drug was dissolved in tetrahydrofuran, 60mM formic acid, 95%ethanol, hence this combination was used in the present study.

Preparation of standard stock solution

Standard solution of Lamivudine was prepared by dissolving 10mg of Lamivudine. In 10ml of mobile phase (tetrahydrofuran : 60mM formic acid in 1:1) to get concentration 1000µg/ml from this stock 1 ml solution was taken and transferred into 10ml volumetric flask to get the concentration 100µg/ml. Different aliquots of above solution in the range 4.8 to 7.2ml were transferred into series of 10ml volumetric flask and volume made upto the mark with 95%ethanol to obtain the concentrations 4.8 to7.2µg/ml. scanning ranges was finalized for study and solutions were scanned on spectrophotometer in the uv range of 200-400nm.

Determination of λ max

From the stock solutions, a working standard was prepared. The absorption spectrum for Lamivudine, was recorded using 6µg/ml solution and the maximum absorption was found to be 275nm The Calibration curves is prepared for Lamivudine in the concentration range of 4.8-7.2 µg/ml at selected wave length by diluting aliquot portions of stock solution of drug. The plots of Beer's law limit are shown in Fig.1and their absorbance values for respective concentrations were given in table 01.

Table 01: Calibration Curve

Con(µg/ml)	Response
0	0.000
4.8	0.273
5.4	0.318
6.0	0.361
6.6	0.400
7.2	0.447

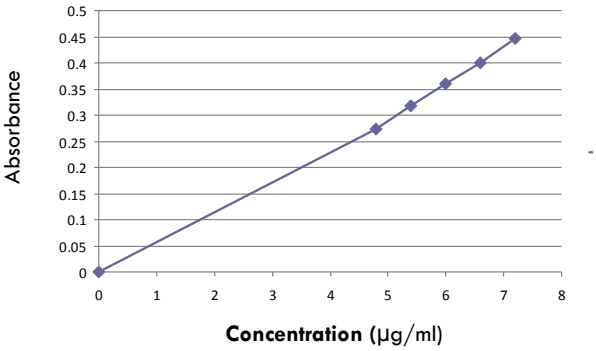

Figure 01: Calibration curve of Lamivudine.

Preparation of Sample solution:

Sample label claim 100 mg. The average weight was determined with 20 tablets, which were grounded in a mortar until fine powder. Accurately weighed amount of powder equivalent to 10mg of Lamivudine was quantitatively transferred to a 10 ml calibrated volumetric flask with the mobile phase (tetrahydrofuran: 60mM formic acid in 1:1). The volume was made up to mark, shake for 10 min. From above solution 1ml was transferred to 10ml calibrated volumetric flask and made up to mark with the aid of (tetrahydrofuran: 60mM formic acid in 1:1) to obtain the concentration 100 µg /ml. .From above solution 0.6ml was transferred to 10ml calibrated volumetric flask and made up to mark with the aid of (95%ethanol) to obtain the concentration 6 µg/ml. And filtered through whatman Filter paper no.1.Then the solution was scanned at 275nm.

Method Validation

The method was validated with reference to linearity, accuracy, precision, and specificity.

Linearity

Linearity was performed by taking aliquots of 4.8,5.4,6.0,6.6, and 7.2 mL from(100 µg/ml) in 10ml volumetric flasks and diluted up to the mark with 95% ethanol such that the final concentration of Lamivudine in the range of 4.8 to 7.2 µg/ml. Under the experimental conditions described the graphs obtained by plotting concentration (µg/ml) Vs absorbance. The observations and calibration curve is shown in Table 2 and Figure 1.

Table 02: Optical characteristic and linearity data

Parameters	Lamividine
λmax for Lamivudine (in nm)	275
Beer's law limits	4.8-7.2 µg/ ml
Correlation coefficient	0.99820
Regression equation (Y)	Y=0.07+0.05x
Intercept(a)	0.06562
Slope(b)	0.05019
Molar absorptivity	1379.3L/mol/cm
Sandell sensitivity	0.0166205

Accuracy

The accuracy was assessed as the percentage relative error and the accuracy of the proposed method was confirmed by recovery studies by standard addition

method at 100% drug concentration. The resulting solutions were then reanalyzed in triplicate by proposed method; the results are shown in table 3.

Table 03: Recovery studies

% Conc.	Amount taken from formulation	Amount of pure drug added (µg/ ml)	Amount found	% Recovery	%RSD
100	6	6	5.90	98.3	0.637

Precision

Precision of the methods was studied as intraday, interday and repeatability. Intra-day study was performed by analyzing, the triplicate measurements of 100%concentration of drug in the same day. Inter-day precision was performed by analyzing three different concentration of the drug (80%, 100%, and 120%) for three days in a week. Repeatability was performed by analyzing the 100% concentration of drug for six times the results are shown in table 4.

Table 04: Results from precision

S.no	Concentration (µg/ml)	Inter day (%RSD) (Day-1)	(Day-2)	(Day-3)	Intraday (%RSD)	Repeatability (%RSD)
1	8	0.772	0.320	0.274	------	-----
2	10	0.274	0.320	0.841	1.01	0.331
3	12	0.388	0.393	0.532	------	-----

Results and Discussion

The wavelength 275nm (λmax for Lamivudine) was selected for analysis of the drugs in and Linearity was observed in the range 4.8-7.2 µg/ml (r =0.99820) for the amount of drugs estimated by the proposed method was in good agreement with the label claim. The proposed methods were validated with reference to linearity, accuracy, precision, and specificity.. The accuracy of the methods was assessed by recovery studies at 100% concentration level. Molar absorptivity (e), low values of Sandell sensitivity indicated the high sensitivity of the proposed method.

The method was found to be precise as indicated by the repeatability, intra-day, inter-day analysis, showing %RSD less than 2. The results of the study indicate that the proposed method of analysis can be used in quality control department with respect routine analysis for assay of the tablets containing Lamivudine.

Table 05: Analysis data of Tablet formulation

Drug	Label claim (mg/tab)	Assay(% of label claim) ± %RSD
Lamivir HBV	100	99.12±0.274

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