Original Article

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

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Abstract

A simple, fast and reliable Spectrophotometric method was developed for determination of Lamivudine in bulk and pharmaceutical formulation. Spectrophotometrically, Lamivudine was determined by measuring the maximum absorption at 280nm. Analytical Calibration curves were linear within a concentration range from 2 to 10µg/ml. The developed method was applied to directly and easily to the analysis of the pharmaceutical tablet preparations. %R.S.D was found to be 1.20(Lamivir HBV 100 mg Tablet) respectively. The percentage recoveries were100- 104% for given method. The method was completely validated. The results showed that this method can be used for rapid determination of Lamivudine in pharmaceutical tablet formulation with linearity, precision, accuracy specificity.

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

Introduction

Lamivudine is chemically 1[(2R, 5S)-2-(Hydroxymethyl)-1-3 oxathiolan-5yl] cytosine and used as an antiretroviral activity. 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. It needs to be phosphorylated to its triphosphate form before it is active. 3TC-triphosphate also inhibits cellular DNA polymerase. The mechanism of action of 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.The literature survey (Mandloi DK et al., 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 and validate a simple, UV Spectroscopic method for the determination of lamivudine in bulk and tablets. The developed method was validated using ICH guidelines for validation (ICH, 1995).

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Materials and Methods

Instrument

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).

Chemicals

Lamivudine pure drug was supplied by local Pharmaceutical industry, India as gift sample and used as such. Spectroscopy graded Acetonitrile, Water and analytical reagent grade Kcl, Hcl were used.

Preparation of standard stock solution:

Standard solution of Lamivudine was prepared by dissolving 10mg of Lamivudine in 10ml of mobile phase (Acetonitrile: Hydrochloric acid buffer Ph 1.8 in 3:7) to get concentration 1000µg/ml. Different aliquots of above solution in the range 0.2 to 1.0ml were transferred into series of 10ml volumetric flask and volume made up to the mark with water to obtain the concentrations 2 to 10µg/ml. scanning ranges was finalized for study and solutions were scanned on spectrophotometer in the UV range of 200-400nm.

Preliminary solubility studies of drugs

A small quantity of standard drug Was dissolved in different solvents like distilled water, methanol, ethanol, acetonitrile, isopropyl alcohol, and in various buffer solutions. By the solubility studies we determined that the drug was dissolved in Water, Acetonitrile, and Hydrochloric acid buffer P^H 1.8, hence this combination was used in the present study.

Determination of λ max

From the stock solutions, a working standard was prepared. The absorption spectrum for Lamivudine, the absorption spectrum was recorded using 10µg/ml solution and the maximum absorption was found to be 280nm. The Calibration curves were prepared for Lamivudine in the concentration range of 2-10 µg/ml at selected wave lengths by diluting aliquot portions of stock solution of each drug. The plots of Beer's law limit are shown in Fig.1.

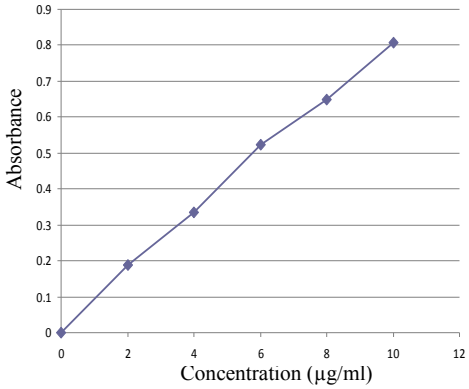

Fig.1: Calibration curve of Lamivudine

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 (Acetonitrile: Hydrochloric acid buffer P^H 1.8 in 3:7). 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

(Acetonitrile: Hydrochloric acid buffer Ph 1.8 in 3:7) to obtain the concentration 100 µg /ml.From above solution 1ml was transferred to 10ml calibrated volumetric flask and made up to mark with the aid of (Acetonitrile: Hydrochloric acid buffer P^H 1.8 in 3:7) to obtain the concentration 10 µg/ml and filtered through Whatman Filter paper no.1.Then the solution was scanned at 280nm.

Method Validation

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

Linearity

Linearity was performed by taking aliquots of 0.2, 0.4, 0.6, 0.8 and 1.0 mL from stock solution (1mg/ml) in 10ml volumetric flasks and diluted upto the mark with the (Acetonitrile: Hydrochloric acid buffer P^H 1.8 in 3:7) such that the final concentration of Lamivudine in the range of 2 to10 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 1 and Fig.1.

Table 1: Optical characteristic and linearity data

Parameters	Lamividine
λmax for Lamivudine (in nm)	280
Beer's law limits	2-10 µg/ ml
Correlation coefficient	1.0011
Regression equation (Y=mx+c)	Y=0.08x +0.02
Intercept(a)	0.02452
Slope(b)	0.07923
Molar absorptivity	15199.4L/Mole/Cm
Sandell sensitivity	0.015

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 three different levels of drug concentrations i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution at three deferent levels. The resulting solutions were then reanalyzed by proposed method; the results are shown in table 2.

Table 2: Recovery studies

% Conc.	Amount taken	Amount added (µg/ ml)	Amount found	%Recovery	%RSD
80	10	8	7.92	99.0	1.57
100	10	10	9.85	98.5	1.81
120	10	12	11.89	99.0	1.57
				Mean 98.8	

Precision

Precision of the methods was studied as intra-day, interday and repeatability. Intra-day study was performed by analyzing, the three different concentrations of drug (80%, 100%, 120%) for three times 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 three different concentration of drug (80%, 100%, and 120%) for three times the results are shown in table 3.

Results and Discussion

Development and validation of spectro-photometric method for the estimation of Lamivudine could be used as a valuable analytical tool in routine analysis, to check the batch to batch variations After the drug is approved, pharmaceutical validation and development of finger printing are necessary to

ensure that the drug product will meet/set pharmaceutical standards for identity, strength, quality,purity, safety and efficacy. The wavelength 280nm (λmax for Lamivudine) was selected for analysis of the drugs in Acetonitrile: Hydrochloric acid buffer Ph 1.8 in 3:7 and Linearity was observed in the range 2-10 µg/ml (r =1.0011) for the amount of drugs estimated by the proposed methods 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 three different concentration levels. Molar absorptivity (e), low values of Sandell sensitivity indicated the high sensitivity of the proposed method.

Table 3: Results from precision

S.No	Concentration (µg/ml)	Interday (%RSD) (Day-1)	(Day-2)	(Day-3)	Intraday (%RSD)	Repeatability (%RSD)
1	8	0.45	0.28	0.27	------	0.43
2	10	0.37	0.60	0.87	0.26	0.44
3	12	0.68	0.99	0.38	------	0.70

The method was found to be precise as indicated by the repeatability, intra-day, inter-day analysis, showing %RSD less than 2. The results did not show any statistical difference between analysts suggesting that the method which is developed is rugged. The results of precision shown in table 3. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical formulation.

Table 4: Analysis data of Tablet formulation

Drug	Label claim (mg/tab)	Assay(% of label claim) ± %RSD
Lamivir HBV	100	98.9±0.67

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