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Original Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF LAMIVUDINE IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Lamivudine in tablet dosage form.

Methods: The drug is freely soluble in analytical grade water. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in analytical grade water. The drug obeyed the Beer's law and showed a good correlation of concentration with absorption which reflects in linearity. The UV spectroscopic method was developed for estimation of lamivudine in tablet dosage form and also validated as per ICH guidelines.

Results: The drug is freely soluble in analytical grade water, slightly soluble in methanol and practically insoluble in acetone. So, the analytical grade water is used as a diluent in the method. The melting point of lamivudine was found to be 160-161°C (uncorrected). It showed absorption maxima 268 nm in analytical grade water. On the basis of the absorption spectrum, the working concentration was set on 10µg/ml (PPM). The linearity was observed between 6-14 µg/ml (PPM). The results of the analysis were validated by recovery studies. The recovery was found to be 98.7, 101 and 99.2% for three levels respectively. The % RSD for precision was found to be 0.62%.

Conclusion: A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Lamivudine in tablet dosage form. The method could be considered for the determination of Lamivudine in quality control laboratories.

Keywords: Lamivudine, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision

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INTRODUCTION

Lamivudine (3TC; 2, 3-dideoxy-3-thiacytidine) (-) enantiomer of a dideoxy analogue of cytidine can inhibit both types of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B virus [1-2]. It is phosphorylated intracellular 1to it's active 5'-thiophosphate metabolite, lamivudine thiophosphate (L-TP) [3-4]. This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination [5]. Lamivudine is taken by mouth as liquid or tablet. Lamivudine has very low cytotoxicity. It is rapidly absorbed with a bioavailability of approximately 80% [6]. It is used in combination with other antiretroviral such as ziovudine and abacavir [7].

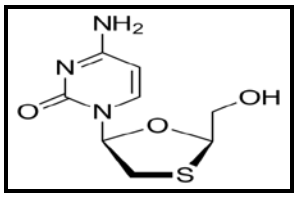

Fig. 1: Chemical structure of lamivudine

Literature review reveals that lamivudine inhibits hepatitis B virus replication, as well as the lamivudine treatment, is beneficial in patients with severely decompensated cirrhosis and actively replicating hepatitis B infection awaiting liver transplantation.

From the literature review, it's found that one method was reported on derivative spectrophotometry for simultaneous estimation of lamivudine their combined dosage form. Also, the method was reported on human serum and drug dissolution studies of lamivudine with another drug on HPLC [8, 9]. Lot of work was done on UV method development for lamivudine in combination with other drugs [10-13]. But very few methods were reported on the estimation of lamivudine in tablet dosage form for UV spectroscopic method. This indicates that so far no UV method exists for the estimation and determination of Lamivudine in tablet dosage forms. The aim of the study was to develop a simple, precise, linear, economic and accurate UV method for determination of Lamivudine in tablet dosage forms [14].

MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair

Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.

Melting point apparatus of Equiptronics were used.

- **Materials**

Lamivudine was obtained as a gift sample. Lamivudine tablets were procured from a local pharmacy. Water used was of analytical grade. Glass double distilled analytical grade water was used throughout the experiment. Freshly prepared solutions were employed.

Method development

Determination of λ max (15 PPM)

100 mg weighed the amount of lamivudine was dissolved into 100 ml of the volumetric flask with analytical grade water. Pipette out

1.5 ml and added in 100 ml of volumetric flask dissolved and diluted up to the mark with analytical grade water. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined [15-16].

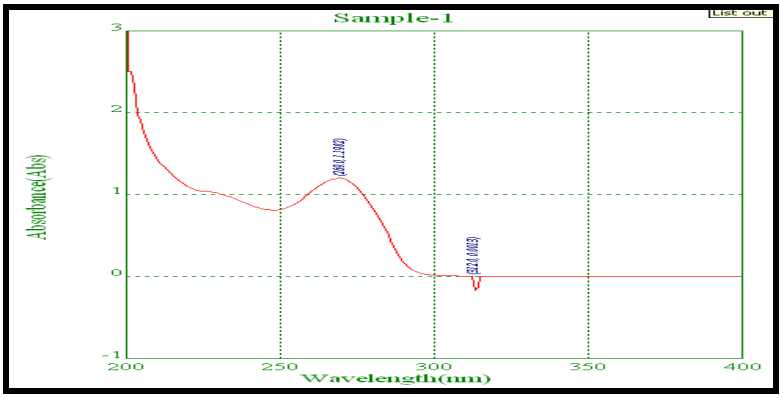

Fig. 2: Calibration curve

A. Preparation of working concentration

Preparation of standard stock solution

Standard stock was prepared by dissolving 100 mg of lamivudine in 100 ml of analytical grade water to get a concentration of 1000 µg/ml (PPM).

Preparation of standard solution

Pipette out 1 ml from standard stock solution and diluted up to 100 ml with analytical grade water to get a concentration of 10 µg/ml (PPM).

B. Preparation of working concentration

Preparation of standard stock solution

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Preparation of standard solution

Pipette out 1 ml from standard stock solution and diluted up to 100 ml with analytical grade water to get a concentration of 10 µg/ml (PPM).

C. Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with analytical grade water. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with the sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 100 mg of lamivudine was accurately weighed and transferred into the 100 ml of volumetric flask, added 60 ml analytical grade water, the solution was sonicated for 20 min. After sonication cool the flask and diluted up to 100 ml with analytical grade water. Filtered the solution through whatmann filter paper. Pipette out 1 ml of the above solution and diluted up to 100 ml with analytical grade water. The absorbance was measured at 268 nm [17-21]. The absorbance was recorded:

Table 1: Absorbance of dosage form

Cipla pharmaceutical limited (100 mg)		
S. No.	**Sample**	**Absorbance**
1	Blank	0.0001
2	Standard	0.6209
3	Sample	0.6207

Table 2: Dosage form specifications

Type	**Company**	**M. D.**	**E. D.**	**Batch No.**	**Average weight (g)**	**Assay (%)**
1	Cipla Pharma LTD (100 mg)	05/2016	07/2019	GPH 02145	0.2027	99.7

E. Method of validation

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 6-14 PPM at 268 nm. The calibration data showed a linear relationship between concentrations.

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100 ml of the volumetric flask with analytical grade water and measuring the absorbance at 268 nm.

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the % RSD was calculated.

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 3: Linearity studies

S. No.	Sample concentration	Absorbance
1	6 PPM	0.3867
2	8 PPM	0.5198
3	10 PPM	0.6295
4	12 PPM	0.7392
5	14 PPM	0.864
Correlation coefficient		0.998

Table 4: Accuracy studies

Accuracy (%)	Qty weighed (mg)	Qty found (mg)	Recovery (98-102%)
80	0.8	0.81	100.92
100	1	1.02	101.86
120	1.2	1.18	98.55

Table 5: Precision studies

S. No.	Sample solution	Absorbance
1	Sample Solution 1	0.6206
2	Sample Solution 2	0.6207
3	Sample Solution 3	0.6208
4	Sample Solution 4	0.6205
5	Sample Solution 5	0.6209
MEAN		0.6207
SD		0.0002
% RSD		0.0255

Table 6: Results for ruggedness studies

S. No.	Analyst	Results	Mean	% assay	% RSD
1	Analyst 1	0.6210 0.6215	0.6213	100.08	0.2821
2	Analyst 2	0.6207 0.6212	0.6210	100.48	

Table 7: Results for solubility studies

S. No.	Title	Result
1	Analytical grade water	Soluble
2	Methanol	Slightly soluble
3	Acetone	Practically insoluble

RESULTS

Solubility of lamivudine

Solubility test was passed as per criteria.

Melting point of lamivudine

The melting point of lamivudine was found to be 160-161˚C (uncorrected).

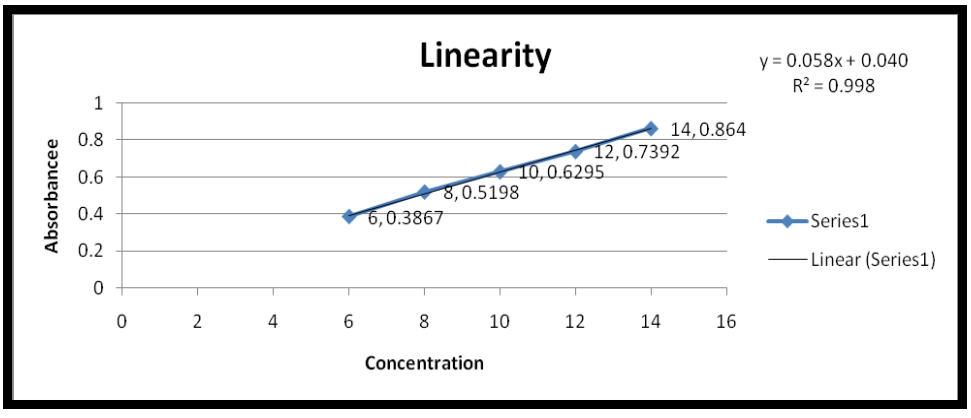

Fig. 3: Lamivudine standard curve

Results for linearity for assay method of lamivudine

The linearity of method was determined at concentration level ranging from 6 to 14 µg/ml (PPM). The correlation coefficient value was found to be (R^2) 0.998.

Results for accuracy for assay method of lamivudine

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in table-4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

Results for precision for assay method of lamivudine

The % RSD for different sample of precision was found to be 0.0255 and it is within acceptance criteria represented in table 5.

Results for ruggedness for assay method of lamivudine

The %RSD for different sample of ruggedness was found to be 0.2329 and it is within acceptance criteria represented in table 6.

CONCLUSION

A method for the estimation of lamivudine in tablet form has been developed. From the spectrum of Lamivudine, it was found that the maximum absorbance was 268 nm in analytical grade water. A good linear relationship was observed in the concentration range of 6-14 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of lamivudine in solid dosage forms. Hence, the method could be considered for the determination of lamivudine in quality control laboratories.

ABBREVARTION

PPM-Parts per Million, nm–Nanometer, HPLC-High Performance Liquid Chromatography, UV-Ultra violet, HBV-Hepatitis B virus, DNA-Deoxyribonucleic acid, HIV-Human Immunodeficiency Virus, ICH-International Council for Harmonization, RSD-Relative Standard Deviation, SD-Standard Deviation, Qty–Quantity, C–Celsius, M. D.-Manufacturing Date, E. D.-Expiry Date

CONFLICT OF INTERESTS

Declared none

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