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Two simple UV-spectrophotometry - Area under curve technique for quantitative estimation of lamivudine in bulk and in tablets

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ABSTRACT

Two economical, simple and precise UV-Spectrophotometric methods have been established for the quantification of Lamivudine (LMV) in tablets. In 'Method I', Area under Curve (AUC) was measured in the wavelength range of 255.20–281.40 nm. While, in 'Method II', zero order spectrum of LMV was derivatized into first order ($\Delta\lambda = 4$) using software UV-probe and AUC was calculated in wavelength range between 273.40 - 296.80 nm. In 'Method I' and 'Method II', LMV obeyed linearity in the concentration range of 5 - 30 µg/mL with $r^2 > 0.99$. Calibration curves were constructed using instrument response between choosen wavelengths and concentrations of analyte in the solution. The proposed methods were effectively executed out for the qualitative estimation of LMV in marketed tablets and % amounts of LMV estimated by 'Method I' and 'Method II' were established to be 99.52 % and 100.55%, respectively. The proposed methods were validated for precision, accuracy and ruggedness as per ICH Guidelines.

KEYWORDS

Lamivudine;
UV-spectrophotometric method;
Area under curve;
Tablets.

INTRODUCTION

Lamivudine (LMV), 2,3-dideoxy-3-thiacytidine is a nucleoside reverse transcriptase inhibitor[1]. LMV is used in combination with other medications to treat human immunodeficiency virus (HIV) infection in patients with Acquired Immunodeficiency Syndrome (AIDS) and also Hepatitis B infection[2].

Literature survey revealed that LMV is estimated by UV-spectrophotometric[3,4], HPLC[5-8] and HPTLC[9] methods in bulk material and in pharmaceutical formulations.

The present paper deals with establishment of 'zero order UV-Spectrophotometric' and 'first order Derivative UV-Spectrophotometric Method' using AUC technique for quantitative determination of LMV in bulk and in tablets. Further, validation of both these methods as per ICH guidelines[10,11].

EXPERIMENTAL WORK

Materials and methods

Lamivudine (LMV) working standard was obtained as gift sample from Cipla Pharmaceuticals, India.

Double distilled water was used throughout the study.

Instruments

UV-Visible double beam spectrophotometer (UV-2450, SHIMADZU Limited, and Japan) with1cm matched quartz cells and electronic balance (Model Shimadzu AUX 120)

Preparationof stock standard solution and preparation of calibration curve

Stock standard solution was prepared by dissolving 10 mg of LMV in 100 mL distilled water to obtain concentration 100 µg/mL.

Method I: Zero order UV-spectrophotometric method using AUC technique

Different volumes of stock solution was further diluted to obtain concentrations in the range of 5– 30 µg/mL of LMV and scanned in the range of 400 –200 nm. Zero order spectrum of LMV obtained and two wavelengths 255.20–281.40 nm were integrated for calculating AUC. The selections of both these wavelengths were done on the basis of reproducibility of results.

Method II: First order derivative UV-spectrophotometric method using AUC technique

The zero order spectra obtained for concentration in the range of 5-30 µg/mL (LMV) in Method I were derivatized into first order ($\Delta\lambda = 4$, scaling factor = 8) and the area between two wavelengths 273.40 - 296.80 nm were chosen for determination of AUC.

The linearity curves were plotted in both these methods using instrument responses and concentrations obtained.

Analysis of tablet formulation

To estimate LMV in tablet formulation; twenty tablets were accurately weighed; average weight was determined and ground into fine powder. A quantity of powder drug equivalent to 100 mg of LMV was transferred to 100 mL volumetric flask containing 40 ml of distilled Water, sonicated for 15 min; volume was adjusted to mark with same solvent and filtered through Whatmann filter paper No. 41. From it, 0.1 mL was withdrawn and diluted to 10 mL to obtained required concentration. The solution was scanned as mentioned in 'Method I' and 'Method II'. The concentration of LMV determined in Method I and II using respective linearity curves.

VALIDATION

Accuracy

The accuracy of the proposed methods was studied by mean % recovery studies, checked at three different levels i.e. 80%, 100% and 120%. To the pre-analyzed sample solution a known amount of LMV bulk drug was added and re-analyzed the LMV by proposed methods.

Precision

The precision of the methods was determined as repeatability, intra-day and inter-day studies. The repeatability of both these methods were performed using concentration 10 µg/mL. For intra-day and inter-day variation studies an appropriate concentration 10 µg/mL, 15 µg/mL and 20 µg/mL were selected in both these methods and instrument responses were determined.

Ruggedness

Ruggedness of the both proposed methods were determined by analyzing fixed concentration 10 µg/mL of sample solution by two different analysts keeping operational and environmental conditions similar. The results are reported in terms of % RSD.

RESULTS AND DISCUSSION

For dissolving LMV double distilled water was choosen as solvent. In Method I and II, LMV followed linearity in the concentration range of 5 - 30 µg/mL. The details of optical characteristics are given in TABLE 1. The % amount of LMV in tablet estimated by Method I and II was found to be 99.52 % and 100.55 %, respectively. Results indicated that there was no interference from the excipients generally occurs in tablet formulation. The accuracy of LMV which was evaluated by percent recovery studies performed at concentration levels of 80, 100, and 120 % and found to be in the acceptable limits (≤ 2%). Results are shown in TABLE 2.

The intra-day and inter-day precision values (%

RSD) were calculated (TABLE 3) and falling in the acceptable limits (≤ 2%) for LMV. The results from rugged-

TABLE 1 : Optical characteristics of LMV

Parameters	Method I	Method II
Beer-Lambert's range(µg/mL)	5-30	5–30
λ max(nm)/ wave length range (nm)	255.20 - 281.40	273.40 - 296.80
Regression Equation	Y=0.187X- 0.0417	Y=0.024X+ 0.061
Slope	0.187	0.024
Intercept	0.0417	0.061
Correlation coefficient (r^2)	0.999	0.998
Limit of detection (µg)	0.49	0.51
Limit of quantitation (µg)	1.43	1.53

TABLE 2: Accuracy*

	Initial amount (µg/mL)	% Amount of drug Added	% Recovery	% RSD
Method I	10	80	98.93	0.52
	10	100	99.87	0.40
	10	120	101.08	0.74
Method II	10	80	99.30	0.34
	10	100	100.37	0.30
	10	120	100.45	0.22

*** mean of three estimations at each level**

ness studies of methods are represented in TABLE 4.

Results showed that, both the methods are suitable for the analysis of LMV without interference from sample matrices. Figure 1 and Figure 2 shows, 'zero order area under curve absorption spectrum' and 'first order derivative absorption spectrum' of LMV in double distilled water.

The developed methods were validated for accuracy, precision and ruggedness as per ICH guidelines.

TABLE 3 : Precision*

Conc. (µg/ml)	% RSD			
	Intra-day		Inter-day	
	Method I	Method II	Method I	Method II
10	0.19	0.75	0.20	1.2
15	0.18	0.45	0.13	0.50
20	0.65	0.12	0.78	0.17

*** mean of three estimations at each level**

TABLE 4: Ruggedness*

Conc. (µg/mL)	Method I		Method II	
	Analyst I	Analyst II	Analyst I	Analyst II
	10	10	10	10
% Mean Amount found	99.02	99.89	100.02	99.89
± SD	0.08	0.05	0.05	0.06
%RSD	1.03	0.80	1.10	1.53

*** mean of six estimations**

CONCLUSION

Both these developed UV-Spectrophotometric

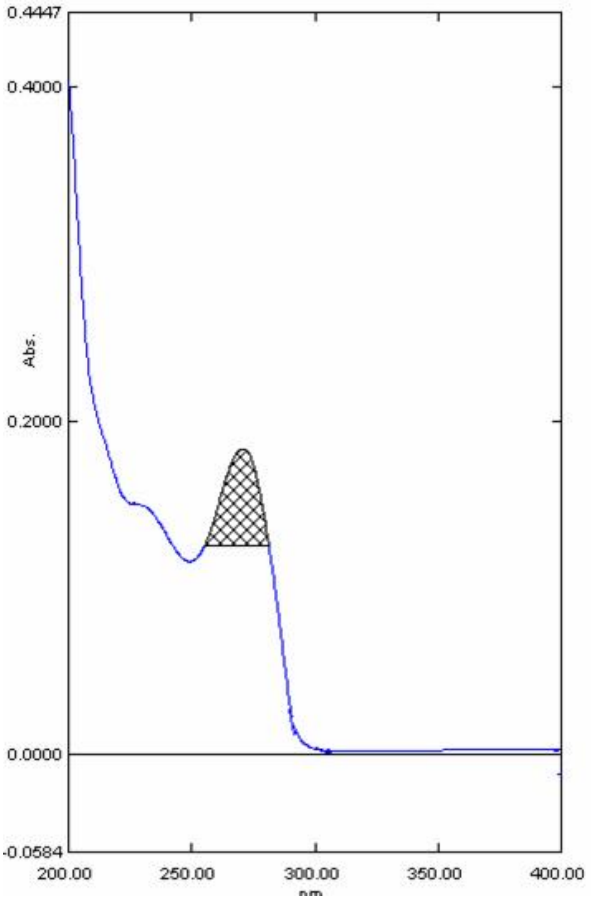

Figure 1 : Zero order AUC spectrum of LMV in double distilled water

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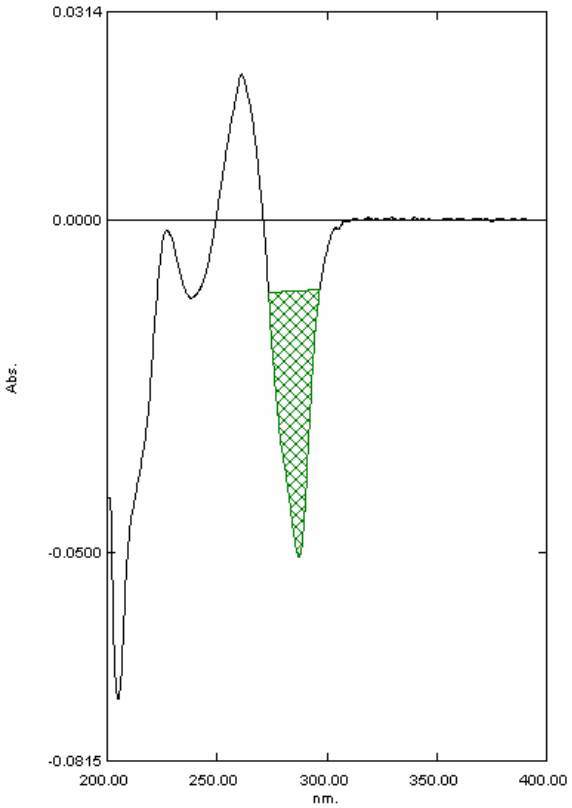

Figure 2 : First order derivative AUC spectrum of LMV in double distilled water

methods are simple, accurate and precise and can be used for regular analysis of LMV in tablet formulation.

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