

VALIDATED SPECTROPHOTOMETRIC ESTIMATION OF LAMIVUDINE IN PURE AND TABLET DOSAGE FORM

C. JOSE GNANA BABU*, G. VIJAYA KUMAR.

Department of Pharmaceutical Analysis, Bharathi College of Pharmacy,
Bharathi Nagar, K.M. Doddi, Maddur Taluk, Mandya District,
Karnataka, India – 571 422

*Corres.author: josejino2@gmail.com

ABSTRACT: Two simple and sensitive spectrophotometric methods have been developed for the estimation of Lamivudine in both pure and tablet dosage form. Methods A and B are based on the condensation reaction of Lamivudine with carbonyl reagents such as p-dimethylaminobenzaldehyde (PDAB) and vanillin in acidic condition to form yellow colored chromogen with absorption maxima at 476 nm and 474 nm respectively. Beer's law is valid in the concentration range of 2-10 µg/ml. This method was validated for precision, accuracy, ruggedness and robustness. Statistical analysis proves that the method is reproducible and selective for the estimation of the said drug.

Key words: Lamivudine, spectrophotometer, p-dimethylaminobenzaldehyde, vanillin, validation.

INTRODUCTION AND EXPERIMENTAL

Lamivudine is a synthetic nucleoside analogue with activity against HIV-1 and HBV^{1, 2}. The chemical name of lamivudine is (2R, cis)-4- amino-1-(2-hydroxymethyl)- 3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one. Lamivudine is the (-) enantiomer of a dideoxy analogue of cytidine. Lamivudine has, also been referred to as (-) 2', 3'- dideoxy, 3'-thiacytidine. It has a molecular formula of C₈H₁₁N₃O₃S and a molecular weight of 229.3. It has the structural formula (Figure 1).

Lamivudine is a white to off-white crystalline solid with a solubility of approximately 70 mg/ml in water at 20°C³. The drug is officially listed in Martindale, the Extra Pharmacopoeia⁴. Several analytical methods that have been reported for the estimation of Lamivudine in biological fluids or pharmaceutical formulations include high performance liquid chromatography, Titrimetry and UV-visible spectrophotometry⁵⁻¹⁵. In view of the above fact, a simple analytical method is in need for its quantitative estimation. The objective of the work is to develop new spectrophotometric methods for its estimation in bulk and tablet dosage form with good accuracy, simplicity, precision and economy. The proposed method is based on the formation of yellow colored Schiff's bases with PDAB and vanillin respectively¹⁶.

A Shimadzu UV/VIS spectrophotometer (model 1201, shimadzu, japan) was employed for all the spectral measurements. All the chemicals used in the investigation were of analytical grade. The ethanolic solution of PDAB was prepared by dissolving 1 gm in 30 ml of 95 % ethanol, 180 ml of butanol and 30 ml of concentrated hydrochloric acid and made up to volume with water in a 250 ml volumetric flask. Ethanolic vanillin of 0.5 % and 5 N nitric acid were prepared. Standard solution of lamivudine was prepared by dissolving 100 mg in 100 ml and diluting 10 ml of this solution to 100 ml with distilled water (100 µg/ml). The method was extended for determination of lamivudine in tablet dosage form. The tablet containing 100 and 150 mg strength were taken.

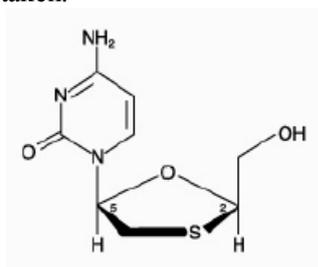


Fig. 1: Chemical Structure of Lamivudine

Twenty tablets were weighed and powdered. The tablet powder equivalent to 100 mg of lamivudine was transferred into 100 ml volumetric flask containing 50 ml of distilled water and flask was kept for ultrasonication for 5 min, then it was diluted up to the mark with distilled water and the solution was filtered through Whatman filter paper No. 41. From the above solution 10 ml was pipetted out into a 100 ml volumetric flask and the volume was made up to the mark with distilled water. The final concentration of lamivudine was brought to 100 mcg/ml with distilled water and used for the analysis. In method A aliquots of lamivudine ranging from 0.2-1.0 ml of standard solution were transferred into a series of 10 ml volumetric flasks. To each flask 1 ml of ethanolic PDAB and 2 ml of 5 N nitric acid were added, the solution was heated on a boiling water bath for 25 min., cooled to room temperature and made up to 10 ml with distilled water. The absorbance were measured at 476 nm against the reagent blank prepared simultaneously. The amount of the drug in a sample was calculated from the calibration graph. In method B aliquots of lamivudine ranging from 0.2-1.0 ml were transferred into a series of 10 ml volumetric flasks. To each 1.5 ml of ethanolic vanillin and 1 ml of 5 N nitric acid were added, the solution was heated on a boiling water bath for 25 min., cooled to room temperature and made up to 10 ml with distilled water. The absorbance of the yellow colored chromogen was measured at 474 nm against the reagent blank. The amount of lamivudine present in the sample was computed from the calibration curve.

RESULTS AND DISCUSSION

The absorption spectral analysis shows the λ max of Lamivudine was found to be 476 nm for method A and 474 nm for method B. The calibration curve was obtained for a series of concentration in the

range of 2-10 mcg/ml for both the methods (Fig. 2 and Fig. 3). They were 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 1. 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 methods were validated as per the ICH guidelines¹⁷⁻¹⁹. The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample. The % RSD was found and lying with in the range of 1.053-1.093. This showed that the precision of the methods are satisfactory. The recovery technique was performed to study the accuracy and reproducibility of the proposed methods. For this, known quantities of the Lamivudine solution were 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 2. Ruggedness and Robustness were determined and the % RSD values were calculated from precision study was less than ± 2.0 . Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by the proposed methods. Thus it can be concluded that the methods developed in the present investigation are simple, sensitive, accurate, rapid and precise. Hence, the above said methods can be successfully applied for the estimation of Lamivudine in tablet dosage form.

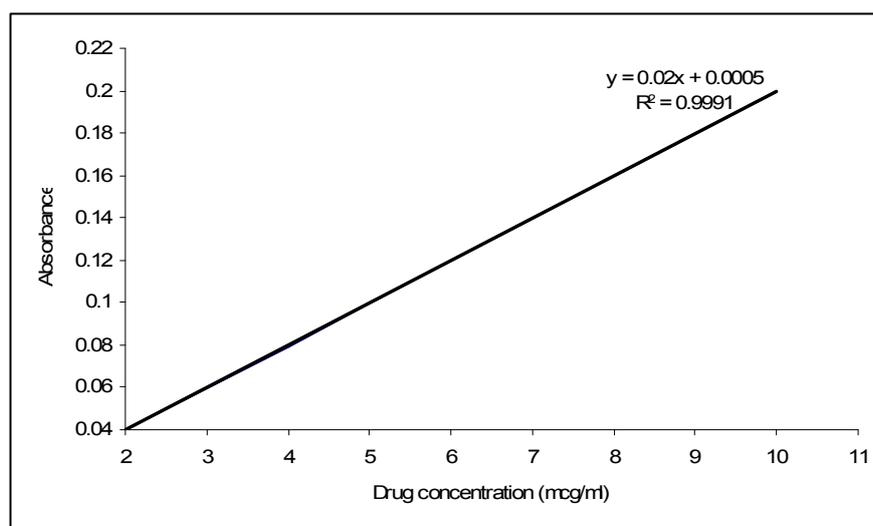
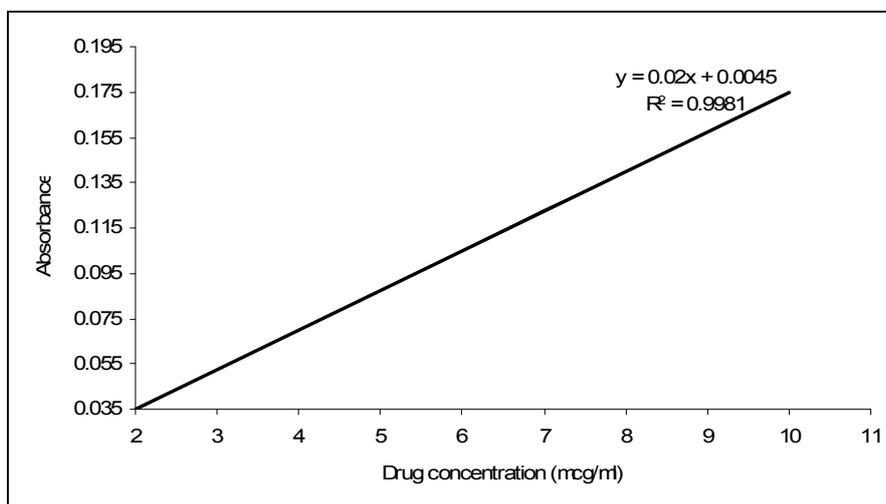
Table 1: Regression analysis of the calibration curve for the proposed method

Parameters	Values	
	Method A	Method B
Absorbance maximum (nm)	476	474
Linearity range (mcg/ml)	2-10	2-10
Correlation coefficient (r^2)	0.9991	0.9981
Regression equation	$Y = 0.02 X + 0.0005$	$Y = 0.02 X + 0.0045$
Slope	0.02	0.02
Intercept	0.0005	0.0045
Limit of detection (mcg/ml)	0.64	0.66
Limit of quantitation (mcg/ml)	1.92	1.98

Table 2: Summary of validation parameters

Parameters	Method A		Method B	
Label claim (tablet- mg)	100	150	100	150
Amount found \pm SEM ^a	100.1 \pm 0.24	150.2 \pm 0.23	100.3 \pm 0.22	150.4 \pm 0.21
Precision (% RSD)	1.061	1.053	1.093	1.074
% Recovery \pm SEM ^a	100.4 \pm 0.74	100.5 \pm 0.63	100.7 \pm 0.50	99.6 \pm 0.84
Recovery (% RSD)	0.78	0.96	1.06	0.89

^aMean of six determinations, SEM indicates standard error mean, RSD indicates relative standard deviation

**Fig. 2: Calibration curve of Lamivudine by method A****Fig. 2: Calibration curve of Lamivudine by method B****REFERENCES**

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