VALIDATED SPECTROPHOTOMETRIC ESTIMATION OF LAMIVUDINE IN PURE AND TABLET DOSAGE FORM

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ABSTRACT

A simple and sensitive spectrophotometric method has been developed for the estimation of Lamivudine in both pure and tablet dosage form. This was based on the condensation reaction of Lamivudine with carbonyl reagent such as p-dimethylaminocinnamaldehyde (PDCA) in acidic condition to orange yellow colored chromogen with absorption maxima at 496 nm. 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: Sprectrophotometric Evaluation, Lamivudine.

INTRODUCTION

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-l-(2-hydroxymethyl-l, 3-oxathiolan-5-yl)-(lH)-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

$C_8H_{11}N_3O_3S$ 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 $20°C$[3]. The drug is officially listed in Martindale, the Extra Pharmacopoeia[4]. 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 spectrophotometry[5-15]. 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 method 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 orange yellow colored Schiff's base with PDCA[16].

MATERIALS AND METHODS

A Schimadzu UV/VIS spectrophotometer (model 1201, schimadzu, japan) was employed for all the spectral measurements. All the chemicals used in the investigation were of analytical grade. The ethanolic solution of PDCA 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. 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.

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

pipette 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 this method 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 PDCA 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 496 nm against the reagent blank prepared simultaneously. The amount of the drug in a sample was calculated from the calibration graph.

RESULTS AND DISCUSSION

The absorption spectral analysis shows the λ max of Lamivudine was found to be 496 nm. The calibration curve was obtained for a series of concentration in the range of 2-10 mcg/ml (Fig. 2). It was 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. The proposed method was validated as per the ICH guidelines[12 -14]. 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 ± 2.0. This showed that the precision of the method is satisfactory. The recovery technique was performed to study the accuracy and reproducibility of the proposed method. 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 method and the amount of added drug was calculated by the difference. The % RSD was less than ±

2.0. This showed that the recovery of Lamivudine by the proposed method is 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 for the proposed method. Thus it can be concluded that the methods developed in the present investigation are simple, sensitive, accurate, rapid and precise. Hence, the above said method can be successfully applied for the estimation of Lamivudine in tablet dosage form.
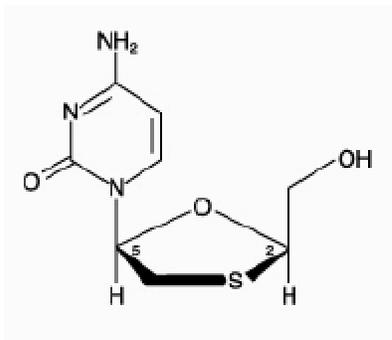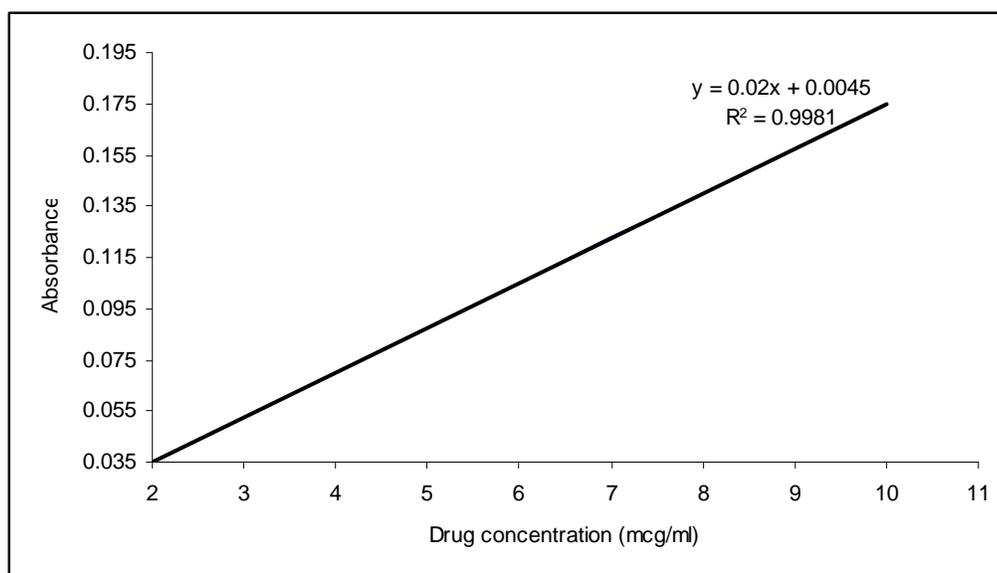

Fig. 1: Chemical Structure of Lamivudine.

$y = 0.02x + 0.0045$
$R^2 = 0.9981$

Fig. 2: Calibration curve of Lamivudine by the proposed method

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

Parameters	Values
Absorbance maximum (nm)	496
Linearity range (mcg/ml)	2-10
Correlation coefficient (r^2)	0.9981
Regression equation	Y=0.02 X + 0.0045
Slope	0.02
Intercept	0.0045
Limit of detection (mcg/ml)	0.69
Limit of quantitation (mcg/ml)	1.88

Table 2: Summary of validation parameters.

Parameters	Values	
Label claim (tablet- mg)	100	150
Amount found ± SEM[a]	100.1±0.24	150.2±0.23
Precision (% RSD)	1.061	1.053
% Recovery ± SEM[a]	100.4±0.74	100.5±0.63
Recovery (% RSD)	0.78	0.96

[a]*Mean of six determinations, SEM indicates standard error mean, RSD indicates relative Standard deviation*

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