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VALIDATED RP- HPLC METHOD FOR THE QUANTITATION OF LAMIVUDINE IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT: A simple, specific, accurate, precise and sensitive reverse phase high performance liquid chromatographic method has been developed for the quantitation of Lamivudine in both pure and tablet dosage forms. A Phenomenex Gemini C- 18, 5 µm column having 250×4.6 mm i.d. in isocratic mode with mobile phase containing 0.02 M potassium dihydrogen phosphate: methanol (40:60) adjusted to pH 3.8 using ortho phosphoric acid. The flow rate was 1.0 ml/min and the effluents were monitored at 272 nm. The retention time was 3.2 min. The linearity was in the range of 10-50 mcg/ml. This method was validated for linearity, precision, specificity, limit of detection, limit of quantitation, accuracy, ruggedness and robustness. Statistical analysis proves that the method is reproducible and selective for the estimation of the said drug.
Key words: RP-HPLC, Lamivudine, 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-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).

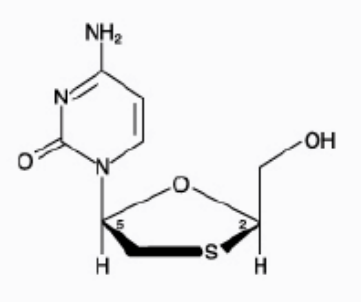

Fig. 1: Chemical Structure of Lamivudine

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]. The objective of the work is to develop simple, accurate, precise and economic RP-HPLC method with lesser run time to estimate the Lamivudine in bulk and tablet dosage forms.

A Schimadzu HPLC model containing LC-10 AT pump, variable wavelength programmable UV/VIS detector and Rheodyne injector was employed for the investigation. All the chemicals used in the investigation were of HPLC grade. The chromatographic analysis was performed on a Phenomenex Gemini C18 column. The mobile phase consisting of 0.02 M potassium dihydrogen phosphate and methanol in the ratio of 40:60 v/v was selected; the pH was adjusted to 3.8 by using 0.1M ortho phosphoric acid. The optimized chromatographic conditions are summarized in table.1. The standard

solution of lamivudine was prepared by dissolving 10 mg in 100 ml of mobile phase to give the concentration 100 µg/ml. The mobile phase and the solution were sonicated for 10 min. and filtered using whatman filter paper No.1 and used. The various dilutions of lamivudine in the concentration of 10, 20, 30, 40 and 50 µg/ml were prepared. The solutions were injected using a 20 µl fixed loop in to the chromatographic system at the flow rate of 1.0 ml/min and the effluents were monitored at 272 nm, chromatograms were recorded. The lamivudine was eluted at 3.2 min as shown in fig.2. The calibration curves were constructed by plotting average peak area versus concentrations (fig. 3) and regression equations were computed. The method was extended for determination of lamivudine in tablet dosage form. The tablet containing 100 mg strength was taken.

Twenty tablets were weighed and powdered. The tablet powder equivalent to 10 mg of lamivudine was transferred into 100 ml volumetric flask containing 50 ml of mobile phase and flask was kept for ultrasonication for 15 min, then it was diluted up to the mark with mobile phase and the solution was filtered through Whatman filter paper No. 1. From the above solution various dilutions were made with the mobile phase, which were analysed. The concentration of the drug in tablet sample solution was calculated by comparing the peak area of standard chromatogram of lamivudine.

RESULTS AND DISCUSSION

A suitability test was applied to representative chromatograms for various parameters. The results obtained were within acceptable limits (Table 2). Thus, the system meets suitable criteria. The calibration curve was obtained for a series of concentration in the range of 10-50 mcg/ml and it was found to be linear. The data of regression analysis of the calibration curves are shown in Table 3. Selectivity and specificity were studied for the examination of various excipients generally present in the tablet dosage form of lamivudine. The results indicated that they did not interfere in the assay. The proposed methods were validated as per the ICH guidelines[16 -18]. 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. 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. 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. The results of validation parameters are summarized in Table 4. The results of tablet analysis and recovery studies obtained by the proposed method were validated by statistical evaluation (Table 5).

Thus it can be concluded that the method developed in the present investigation are simple, sensitive, accurate, rugged, robust, rapid and precise. Hence, the above said method can be successfully applied for the estimation of lamivudine in tablet dosage forms.

Table 1: Optimized Chromatographic conditions for the proposed method

Parameters	Optimized condition
Column	Phenomenex Gemini C-18 (5µ)
Mobile phase	0.02 M potassium dihydrogen phosphate: methanol (40:60)
pH	3.8
Flow rate	1.0 ml/min
Injection volume	20 µl
Detection	272 nm in uv detector
Temperature	Ambient
Retention time	3.2
Run time	6 min

Table 2: System Suitability Test Parameters for the proposed method

Parameters	Values
Theoretical plates	3456
Asymmetric factor	1.192
Tailing factor	1.23

Table 3: Regression analysis of the Calibration curve for the proposed method

Parameters	Values
Linearity range (µg/ml)	10-50
Correlation coefficient (r^2)	0.9983
Regression equation	Y= 49143 X + 4761.9
Slope	49143
Intercept	4761.9

Table 4: Summary of Validation Parameters for the proposed method

Parameters	Values
Limit of detection (µg/ml)	0.542
Limit of quantitation (µg/ml)	1.62
[a]Precision (% RSD)	
Intraday	1.02
Interday	1.44
[a]Ruggedness (% RSD)	
Analyst I	1.02
Analyst II	1.09
[a]Robustness (% RSD)	
Normal condition	1.54
Changed condition I (detector wavelength)	1.33
Changed condition II (pH of mobile phase)	1.11
Changed condition III (ratio of mobile phase)	1.07
Changed condition IV (flow rate of mobile phase)	1.12
% Recovery[a] ± SEM	100.4± 0.74
Recovery (% RSD)	0.78

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

Table 5: Assay Results of Lamivudine Tablets using proposed method

Tablets	Labelled amount (mg)	[b]Amount found (mg)	% Recovery[b]
A	100	99.89±0.28	99.59±0.78
A	150	150.39±0.48	99.79±1.08

[b]*Mean value ± standard deviation of six determinations*

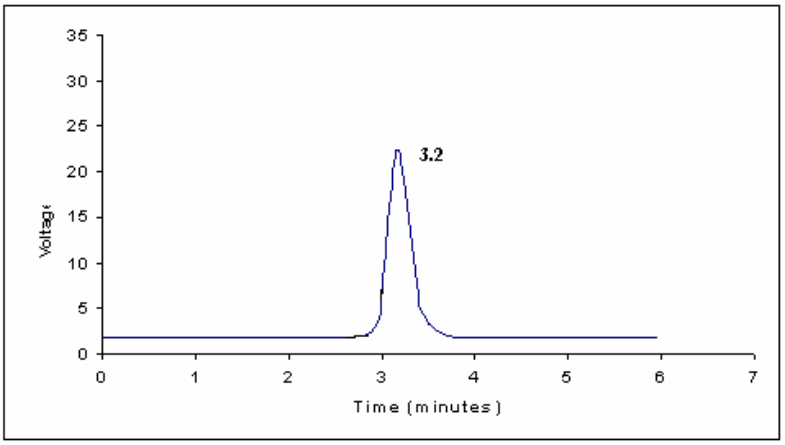

Fig. 2: Typical RP-HPLC Chromatogram of Lamivudine by the proposed method.

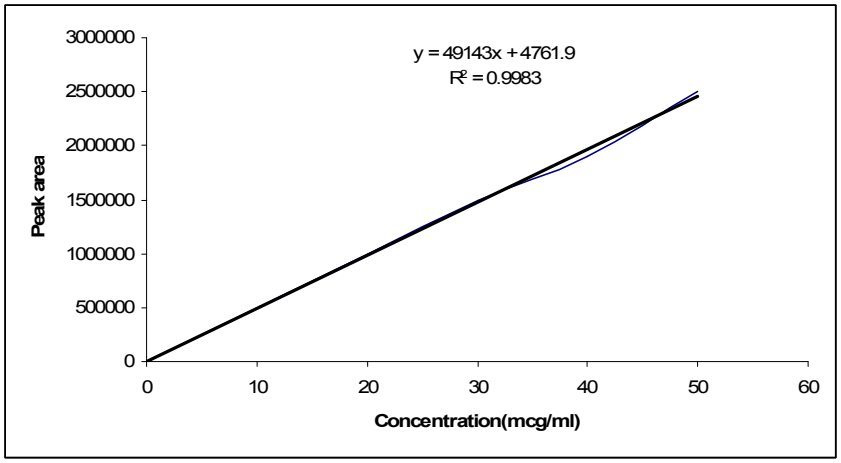

Fig. 3: Calibration curve of Lamivudine by the proposed method.

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