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Estimation of Emtricitabine in Tablet Dosage Form by RP-HPLC

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A simple, precise, rapid and accurate reverse phase HPLC method in isocratic mode has been developed for the estimation of emtricitabine in tablet dosage form. A Hypersil BDS C18, 250 × 4.6 mm, 5 µm particle size, with mobile phase consisting of acetonitrile and 0.03 M KH_2PO_4 water (pH adjusted to 3.2 with orthophosphoric acid) in the ratio of 60:40 v/v was used. The flow rate was 0.8 mL/min and the effluents were monitored at 260 nm. The retention time was 3.105 min for emtricitabine. The detector response was linear for emtricitabine 4-48 mcg/mL. The respective linear regression equation being, Y = 35167.413x + 22780.1317 for emtricitabine. The limit of detection (LOD) for emtricitabine was found to be 0.04 µg/mL. The limit of quantification (LOQ) for emtricitabine was 0.12 µg/mL. The percentage assay of emtricitabine was 98/57 %. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of emtricitabine in bulk drug and in its pharmaceutical dosage form.

Key Words: Emtricitabine, RP-HPLC, Estimation and Tablets.

INTRODUCTION

Emtricitabine[1-3]. is a novel drug used in combining fixed doses of the nucleoside reverse transcriptase inhibitor tenofovir disoproxil fumarate with the non-nucleoside reverse transcriptase inhibitor efavirenz represents the first once-daily, one-tablet antiretroviral regimen. Emtricitabine is chemically 5-fluoro-1-(2R, 5S)-[2-hydroxymethyl)-1,3-oxathiolan-5-ylcytosine[2]. Literature survey reveals few chromatographic methods[4-9] for the determination of emtricitabine in biological fluids along with other antiretroviral drugs like tenofovir disoproxil and efavirenz. So far, only one HPLC procedure has been reported in gradient mode for the estimation of tenofovir disoproxil, emtricitabine and efavirenz from pharmaceutical dosage form. The availability of an HPLC method with high sensitivity and selectivity will be useful for

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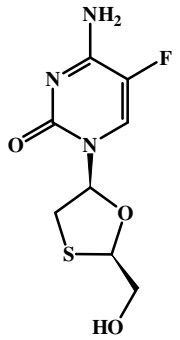
the determination of emtricitabine in pharmaceutical formulations. The aim of the study is to develop a simple, precise and accurate reversed-phase HPLC method in isocratic mode for the estimation of emtricitabine in bulk drug samples and in pharmaceutical dosage form.



Structure of emtricitabine

EXPERIMENTAL

Emtricitabine was obtained as a gift sample from Hetero Drugs Ltd, Hyderabad. Potassium dihydrogen orthophosphate was of analytical grade and supplied by M/s S.D. Fine Chem. Limited, Mumbai. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available Emtricitabine tablets (Emtriva 200 mg) were procured from local market.

Quantitative HPLC was performed on liquid chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 20 µL and 2693 pump. A RP C-18 Hypersil BDS column (250 × 4.6 mm i.d; particle size 5 µm) was used. The HPLC system was equipped with Empower Software.

HPLC Conditions: The contents of the mobile phase were acetonitrile and 0.03M KH_2PO_4 in water (pH adjusted to 3.2 with orthophosphoric acid) in the ratio of 60:40 v/v. They were filtered before use through a 0.45 µm membrane filter and pumped from the respective solvent reservoirs to the column at a flow rate of 0.8 mL/min. The run time was set at 15.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 0.5 h with the mobile phase flowing through the system. The eluents were monitored at 260 nm.

Preparation of standard stock solution: A standard stock solution of the drug was prepared by dissolving 20 mg of emtricitabine in 100 mL volumetric flask containing 30 mL of diluent (60:40 acetonitrile:water), sonicated for *ca.* 0.5 h and then made up to 100 mL with diluent to get the primary standard stock solution containing 200 mcg/mL of emtricitabine.

Working standard solution: 10 mL of the above stock solution was taken in 50 mL volumetric flask and thereafter made up to 50 mL with diluent to get the working standard solution containing 40 mcg/mL of emtricitabine.

Preparation of sample solution: Twenty tablets (Emtriva 200 mg) were weighed and then powdered. A sample of the powdered tablets, equivalent to 20 mg of emtricitabine was mixed with 50 mL of diluent (60:40 acetonitrile:water) in 100 mL of volumetric flask. The mixture was allowed to stand for 1 h with intermittent sonication to ensure complete solubility of the drug and then filtered through a 0.45 μm membrane filter, followed by adding diluent to obtain a stock solution containing 200 mcg/mL of emtricitabine. 10 mL of the above stock solution was taken in 50 mL volumetric flask and thereafter made up to 50 mL with diluent to get the working standard solution containing 40 mcg/mL of emtricitabine.

Linearity: Aliquots of primary standard emtricitabine stock solution were taken in different 10 mL volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of emtricitabine is in the range of 4-48 mcg/mL. Each of these drug solutions (20 μL) was injected three times into the column and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 260 nm and a calibration graph was obtained by plotting peak area *versus* concentration of emtricitabine (Fig. 1). The plot of peak area of each sample against respective concentration of emtricitabine was found to be linear in the range of 4-48 mcg/mL with correlation coefficient of 1.0. Linear regression least square fit data obtained from the measurements are given in Table-1. The respective linear regression equation being Y = 35167.413x + 22780.1317 for emtricitabine. The regression characteristics, such as slope, intercept and % RSD were calculated for this method and given in Table-1.

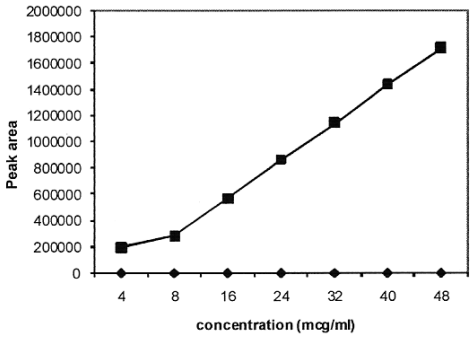


Fig. 1. Calibration curve of emtricitabine by RP-HPLC

TABLE-1
LINEAR REGRESSION DATA FOR CALIBRATION CURVE

Parameter	Emtricitbine
Concentration range (μg/mL)	4-48
Slope (m)	35167.413
Intercept (b)	22780.1317
Correlation coefficient	0.999
% RSD	0.19
Standard error of estimate	19107.2859

Assay: 20 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was 3.105 min for emtricitabine. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table-2.

ABLE-2
RESULTS OF HPLC ASSAY AND RECOVERY STUDIES

Sample	Amount claim (mg/tablet)	Amount obtained in (mg)* by proposed method	**% Recovery by the proposed method
1	200	199.4	98.87
2	200	199.8	98.28
3	200	199.4	98.57

*Average of three different concentration levels.
**After spiking the sample.

Recovery studies: Accuracy was determined by recovery studies of emtricitabine by known amount of standard was added to the preanalyzed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table-2. The study was done at three different concentration levels.

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solution of emtricitabine. Parameters that were studied to evaluate the suitability of the system are given in Table-3.

TABLE-3
VALIDATION SUMMARY

Parameter	Emtricitbine
System suitability	
Theoretical plates (N)	7594.57
Tailing factor	1.37
Retention time (min)	3.107
Resolution	1.78
K'	0.423
LOD (µg/mL)	0.04
LOQ (µg/mL)	0.12

Limit of detection (LOD) and limit of quantification (LOQ): The limit of detection (LOD) for emtricitabine was found to be 0.04 µg/mL. The limit of quantification (LOQ) for emtricitabine was found to be 0.12 µg/mL. The signal to noise ratio is 3 for LOD and 10 for LOQ.

From the typical chromatogram of emtricitabine as shown in Fig. 2, it was found that the retention times were 3.105 min for emtricitabine. A mixture of acetonitrile and 0.03 M KH_2PO_4 in water (pH adjusted to 3.2 with orthophosphoric acid) in the ratio of 60:40 v/v was found to be most suitable to obtain a peak well defined

and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r = 1.0) was observed between the concentration range of linear in the range of 4-48 mcg/mL emtricitabine. Low values of standard deviation are indicative of the high precision of the method. The assay of emtricitabine tablets was found to be 98.57 %. Based on the recovery studies, it was found that about 98.57 % of emtricitabine was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible.

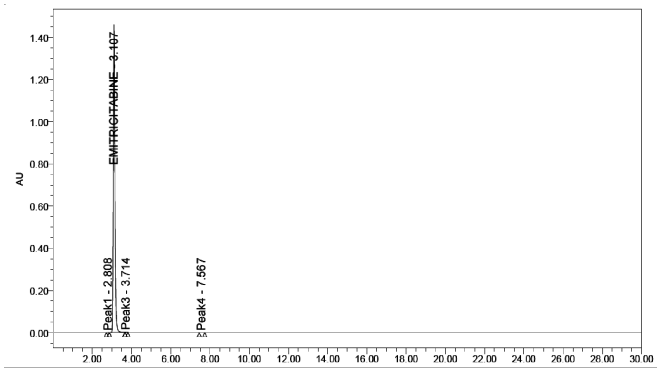


Fig. 2. Typical chromatogram of emtricitabine by RP-HPLC

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