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A simple HPLC method for quantitation of Emtricitabine in capsule dosage form

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Abstract

A simple, economic, accurate reverse phase isocratic RPHPLC method was developed for the quantitation of emtricitabine in capsule dosage form. The quantification was carried out using Gracesmart RP18, 5μ (100mm x 4.6mm). The mobile phase comprised of mixture of buffer, adjusted to pH 2.2 with dilute orthophosphoric acid and acetonitrile in the ratio of 88:12. The flow rate was 1.0ml/min with UV detection at 280nm. The method has been validated and proved to be robust. The calibration curve was linear in the concentration range of 50 to 500mcg/ml with coefficient of correlation 0.9981. The percentage recovery for emtricitabine was found to be 101.0%. The method is useful in the quality for the estimation of emtricitabine in capsule dosage form.

Keywords: Emtricitabine, Capsules, HPLC, Validation.

INTRODUCTION

Emtricitabine is chemically known as 4-amino-5-fluoro-1-[(2R, 5S)-2-(hydroxymthyl)-1, 3-oxathiolan-5-yl] pyrimidin-2-one [1]. It is used as antiviral drug. Emtricitabine is nucleoside reverse transcriptase inhibitors [2]. Literature survey reveals few chromatographic methods in biological fluids reported along with other antiretroviral drugs [3]. The focus of present study was to develop and validate a rapid, stable and economic, high performance liquid chromatographic method for the quality control of emtricitabine in capsule dosage form.

RESULTS AND DISCUSSION

Method development

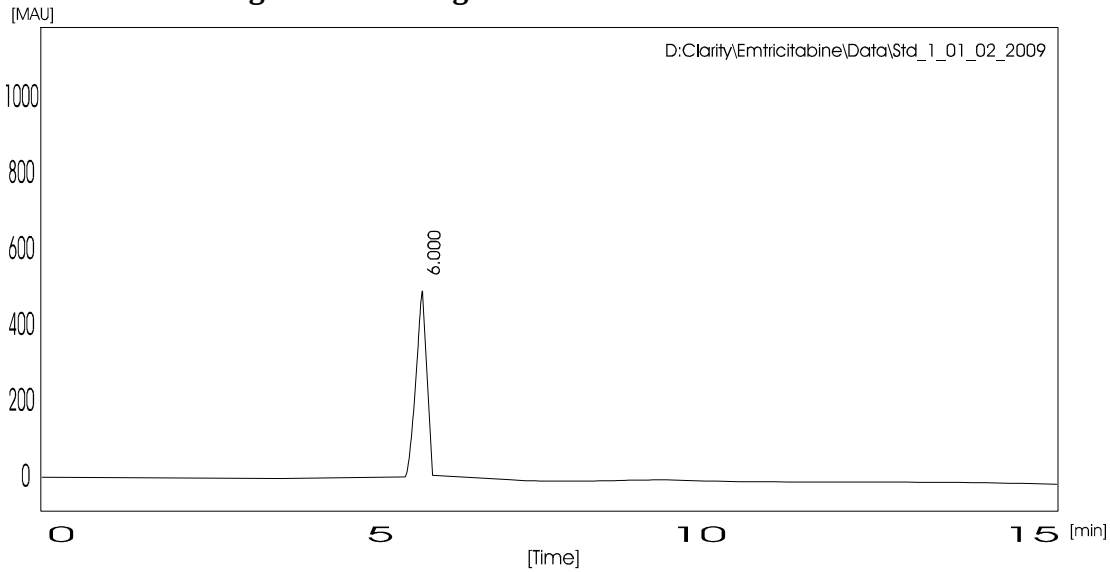
The system suitability test was carried out a freshly prepared standard stock solution of emtricitabine to check various parameters. System suitability results are as follows [4].

Retention time	:	6.000minutes
Theoretical plate no	:	not less than 3000
Calibration range	:	50-500mcg/ml

Method Validation

The described method has been validated for the assay of emtricitabine using following parameters [5]

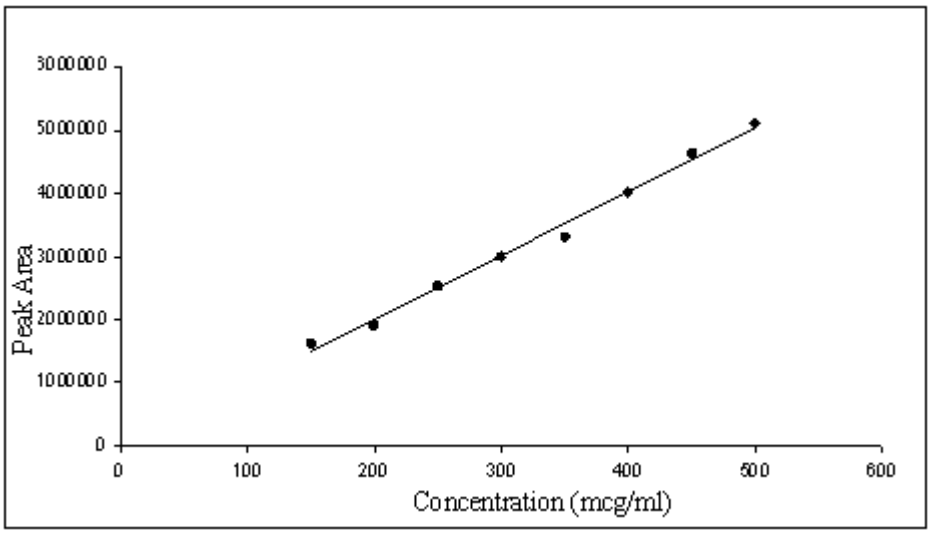
Fig. I: Chromatogram of Emtricitabine standard



Result Table(D:Clarity\Emtricitabine\Data\Std_1_01_02_2009)

S.No	Reten Time(min)	Response	Amount(%)	Peak Type	Compound Name
1	6.000	6402536.02	100		Emtricitabine
	Total	6402536.02	100		

Figure II: Calibration curve for linearity data



Precision

Precision was studied to find out intra and inter day variations in the test methods of emtricitabine at 3 different concentration levels, 50, 100 and 150mcg/ml three times same day and different day respectively. The %RSD was calculated which should be less than 2%.

Intra day precision
This was done on the same day and the %RSD was calculated, data's was present in Table: I

Table I: Intra day precision

Conc. (mcg/ml)	Peak area	Injection volume (mcl)	Mean	Standard deviation	RSD%
50	525268.14	10	534157.1	9724.979	1.820621
	532658.98				
	544544.23				
100	1000024.14	10	1001401	2470.167	0.246671
	1004252.35				
	999925.36				
150	1599688.25	10	1637026	32498.86	1.985239
	1652435.25				
	1658953.14				

Inter day precision
This was done on the different day and %RSD was calculated. Data's was present in Table: II

Table II: Inter day precision

Concentration (mcg/ml)	Peak area	Injection volume (mcl)	Mean	Standard deviation	RSD%
50	524525.69	10	528624.9	5954.294	1.126374
	525894.25				
	535454.78				
100	1009856.23	10	1012514	11640.84	1.149696
	1025254.2				
	1002432.25				
150	1566854.34	10	1583793	16003.06	1.010426
	1585865.47				
	1598658.48				

Accuracy
Accuracy was determined by recovery study of emtricitabine known amount of working standard emtricitabine was added into pre-analysed sample and subject them to the proposed HPLC method. The study was carried out at three different concentration levels like 90, 100 and 110. The percentage recoveries were found to be 101.03%, 102.38% and 101.60% respectively.

Specificity
By subjecting the drug solution to different stress conditions like acid, base and peroxide, test for specificity was done. With the acid stress(0.1M HCl), Retention time of drug was found to be in the order of 6.002, 5.984 and 5.759 with respect to 0 hour, 8 hour and 24 hours at a concentration

of 100mcg/ml. With the same concentration and time intervals the Retention time of drug for base stress (0.1M NaOH) were 6.012, 5.978 and 5.958. In peroxide stress (5% H_2O_2), Retention time of drug were 6.011, 5.981 and 5.968.

Robustness

This was done by small deliberate changes in the chromatographic conditions at 3 different levels -1, 0, +1 and retention time of the drug was noted. The factors selected were flow rate, pH and percentage of Acetonitrile in the mobile phase. The results were indicated that the selected factors remained unaffected by small variations of these parameters.

Stability studies

Stability of reagents, mobile phase, standard and sample solutions were studied for 48hrs and compared with freshly prepared solutions and was found to be stable.

Limit of Detection and limit of Quantification

Limit of detection and limit of quantitation were calculated by the method which was based common approach which is to compare measured signals from samples with known low concentrations of analyte with those of blank samples, the minimum concentration at which the analyte can be reliably detected is established. Limit of detection and limit of quantitation were found to be 0.153mcg/mL and 0.459mcg/mL respectively.

Assay Determination of Emtricitabine from its capsule

The assay results of sample at three different independent concentrations were comparable with claimed value. The obtained results are presented in Table III and retention time was found to be 6.000 min.

Table III: Assay of Emtricitabine

Experiment	Claim(mg)	Formulation found (mg)	% of Assay
1	200	196.2	98.1%
2	200	194.1	97.05%
3	200	199.5	99.75%

MATERIALS AND METHODS

Chemicals and reagents

Emtricitabine working standard was purchased from market. The emtricitabine capsule was purchased from the local market. The HPLC grade solvents used were of E-merk(India) Ltd., Mumbai, HPLC grade water was prepared using Millipore purification system.

Equipment

The instrument was a Analytical Technologies Ltd. Liquid chromatograph equipped with a pump, UV detector in isocratic mode. The system was connected with the help of Crystal software in a computer system for data collection and processing. The analytical column used was Gracesmart RP18, 5µ (100mm x 4.6mm).

Chromatographic conditions

The mobile phase consists of Acetonitrile (12 volumes) and a mixture (88 volumes) of buffer (dissolved 2.72g of potassium dihydrogen orthophosphate and 4.32g of 1-Octane sulfonic acid sodium salt in 1000ml of water, adjusted the pH to 2.2±0.05 with dilute orthophosphoric acid and filtered through 0.45 µ or filter porosity membrane filter), was filtered through 0.45 µ or filter porosity membrane filter before use. The column used was Gracesmart RP18, 5µ (100mm x 4.6mm). The injection volume was 10 µl with a flow rate 1.0ml/min and detection wavelength 280nm having ambient condition and run time 15 minutes.

Preparation of diluent

The diluent consists of mixture of buffer (70 volumes) and methanol (30 volume) was filtered through 0.45 µ or filter porosity membrane filter before use.

Preparation of standard

About 50mg of emtricitabine working standard was accurately weighed and dissolved in 50ml of diluent in the volumetric flask to get a concentration of 1000mcg/ml from this stock solution suitable dilutions were made to get the concentrations of 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500mcg/ml and filtered through 0.45 µ or filter porosity membrane filter before use. 10 µl of each of the solution was injected.

Estimation of Emtricitabine in capsule dosage form

Five intact capsules were transferred in to a 1000ml volumetric flask containing 100ml of water and kept for sonication for 15min with intermittent vigorous shaking or until the capsule shells get disperse. Then, the solution was added with 600ml of diluents and sonicate for 25 minutes with frequent intermittent shaking. The volume made upto the mark with diluent and centrifuged the solution at 10000RPM for 10 minutes and 5ml of supernatant solution was again diluted to 50ml with diluent and mixed and filtered through 0.45 µ or filter porosity membrane filter before use.

CONCLUSION

Analytical RP Liquid Chromatographic method was developed and validated for the determination of Emtricitabine in capsule dosage form. The developed method was found to be specific, accurate, precise and robust for its intended use.

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