AN ECONOMIC RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF EMTRICITABINE IN BULK AND FORMULATION

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A new rapid, economic and sensitive RP-HPLC method was developed for the estimation of Emtricitabine in bulk and commercial pharmaceutical formulation. Emtricitabine was well eluted on an isocratic c18 column (ODS UG 5 column, 250 mm × 4.5 nm) utilizing a mobile phase composition of methanol : water (70 : 30 v/v) at a flow rate of 1.0 ml/min with UV detection at 277 nm. The retention time was 2.287 ± 0.004 mins. The developed method was validated for specificity, linearity, precision, accuracy, LOD, LOQ and robustness. The developed reverse phase liquid chromatography method can be applied in routine analysis in bulk and pharmaceutical formulations. The determined validation parameters were within the specified ICH guideline limits.

KEYWORDS : Emtricitabine, RP-HPLC, validation.

INTRODUCTION

Emtricitabine is an antiviral drug used to treat HIV infections. It is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults and children. Chemically, Emtricitabine is 4-amino-5-fluoro-1-[(2*R*, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one. A very few analytical methods have been reported for its estimation in pharmaceutical formulations and biological samples especially in human plasma, serum, which includes HPLC with UV detection, HPTLC, HPLC with Tandem mass spectrometry and spectrophotometry [2-8].

The purpose of the present work is to develop simple, precise and economic RP-HPLC method for the estimation of Emtricitabine in raw material and in its marketed dosage form and perform validation of the method as per International Conference of Harmonisation (ICH) guidelines [1]. The developed method has been validated by evaluation of system suitability, linearity, limit of detection and limit of quantification, precision and accuracy. The validated method was applied to the commercially available pharmaceutical formulation of Emtricitabine (tablets).

Literature survey revealed that very few methods were reported for the estimation of Emtricitabinealone in tablets by RP-HPLC and efficient economic methods were less. Hence an attempt has been made to develop an accurate, precise and economically viable RP-HPLC method for the estimation in the current research.

MATERIALS AND METHODS

Materials : Emtricitabine was obtained as a gift sample from MYLAN Labs Pvt. Ltd. HPLC grade methanol and water were procured from Merck India. For the estimation of commercial formulations, Emtricitabine tablets (EMTRICITABINE) manufactured by CIPLA Ltd, were procured from the local pharmacy.

Instrumentation and analytical conditions

HPLC : Agilent 1120 compact LC chromatographic system, with variable wavelength UV detector and Rheodyne injector with 20µl fixed loop was used for the chromatographic separation. Ezchrome software was used for data analysis. Chromatographic separation was carried out on a c18 column (250nm×4.5mm).

AXIS AGN204- PO electronic balance was used for weighing purpose.

Ultra –sonic bath sonicator was used for degassing purpose.

Chromatographic conditions

Column	**:** Agilent 100-5C_{18} Column [250mm × 4.6mm]
Detector Wave length :	277nm
Mobile Phase	**:**Methanol:Water (70:30 v/v).
Flow	: 1.0 ml/min
Injection Volume	**:** 20µL
Diluent	**:** Methanol: Water (70:30 v/v)
Run Time	: 10minutes
Temperature	**:** Ambient

Method development

Spectroscopic determination of Emtricitabine indicated that the drug absorbs maximum at 277nm, hence 277 nm was selected as the detection wavelength. Several different mobile phases were used for the initial trials in the estimation of Emtricitabine, but optimum results were attained with methanol: water in the ratio of 70 : 30 v/v. The peak was shown in figure 1.

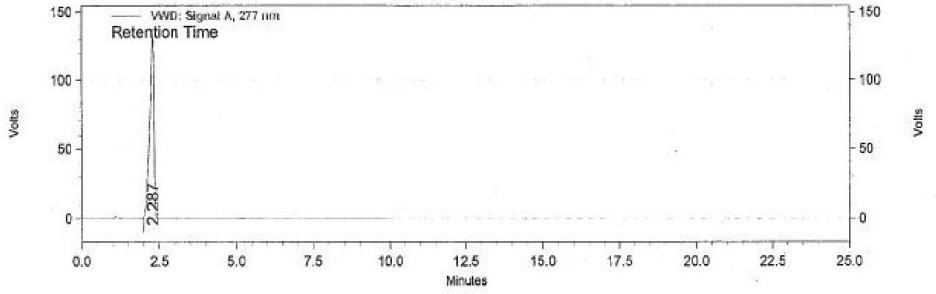

Fig. 1. Optimised Chromatogram of Emtricitabine

Mobile phase composition

Methanol and water were mixed (ratio 70 : 30 v/v) filtered through a 0.45 µ membrane filter and sonicated for 30 min.

Preparation of standard stock solution

25 mg of Emtricitabine was weighed accurately and transferred in to 25 ml volumetric flask and dissolved in 10 ml of mobile phase and sonicated it for 10min and made up to the mark with the mobile phase to obtain a final concentration of 1000 µg/ml.

PREPARATION OF SAMPLE STOCK SOLUTION

The contents of twenty marketed tablets were taken and finely powdered. Weighed accurate tablet powder equivalent to 100 mg of Emtricitabine and transferred to 100ml volumetric flask and added diluent (mobile phase). The solution is sonicated for 20 minutes. The volume is made up with the same diluent and mixed well. The solution was filtered through a membrane filter of 0.45µm and discarded the first 2 ml. This is used for the assay by following the procedure described.

Method Validation

System suitability

System suitability was carried out by injecting 6 replicate injections of 100µg/ml concentration. It is checked on each day of validation to evaluate the performance of the system. The parameters like theoretical plates, retention time, tailing factor and its % RSD were determined. The results were given in the table 1.

Table 1. System suitability parameters of Emtricitabine (*n* = 6)

S. No	Parameters	Results
1	Theoretical plates	11354
2	Tailing factor	0.7
3	Retention time	2.287±0.02
4	% RSD	0.081

Linearity and range

The peak areas corresponding to the concentration range of Emtricitabine 20-100µg/ml prepared in triplicate and were plotted against the respective concentrations. The calibrated curves were linear in the range studied for Emtricitabine with mean correlation coefficient of 0.998 and the representative calibration curve was shown in figure 3. Regression analysis was given in the table 2.

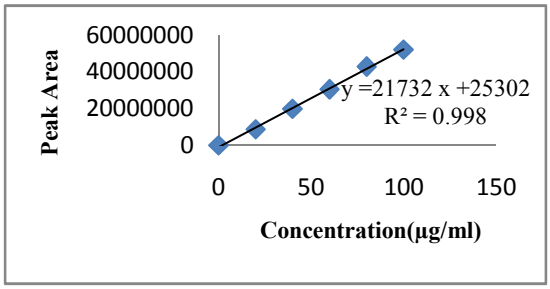

Fig. 2. Calibration curve of Emtricitabine

Table 2. Linearity of Emtricitabine (*n* = 3)

Parameters	Valves
Linearity range	20-100µg/ml
Slope	21732
Intercept	25302
Correlation coefficient	0.998
Regression equation	y =21732 x +25302

Accuracy

Accuracy of the method was examined by performing recovery studies by standard addition method for drug product. This parameter is studied to identify the interaction of excipients on the response of drug. The recovery was calculated at three spike levels i.e., 80%, 100% and 120%. The results of recovery were given in the table 3.

Table 3. Results for Accuracy (*n* = 3)

Recovery level	Amount Added (mg)		Amount Found (mg)	%Recovery (%w/w)
	Std.	Test		
80%	60	20	76.84	96.05
100%	80	20	98.46	98.46
120%	100	20	117.42	97.82
Mean %	97.44-98.0			

Precision

The precision of the method was determined by the injection of six replicates of 100µg/mL concentration. Precision was performed intraday (same day) with three intervals as well as inter day (three successive days). The results of intraday were given in the table 4.

Table 4. Precision of Emtricitabine

Precision		% RSD
Intraday		1.35
Interday	Day 1	1.36
	Day 2	1.42
	Day 3	1.62

Limit of Detection and Limit of Quantitation

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10 σ/s. The values were tabulated in table 5.

Table 5. Results for LOD and LOQ

Parameters	Values (µg/mL)
LOD	1.03
LOQ	1.83

Robustness

Robustness was carried out by change in the flow rate (± 0.2ml/min) and variation wavelength (±2nm). Solution of 100µg/ml concentration was prepared and injected in triplicate for each varied operational condition and %RSD was calculated. The results were given in the table 6.

Table 6. Results for Robustness (*n* = 3)

Parameters		**%RSD**
Wavelength	275	0.89
± 2nm	279	0.34
Flow rate	0.8	0.87
±0.2ml/min	1.2	1.20

Assay

A 20µl injection of concentration 80µg/ml. Emtricitabine solution was injected in triplicate to the chromatographic system and the peak response was measured. The content of each component in the formulation was estimated by comparing the peak area of the test sample with that of the peak area of the standard. The results of estimation were given in the Table 7.

Table 7. Results for Assay of Emtricitabine marketed formulation

Drug	**Label claim (mg)**	**Amount recovered (mg)**	**% Amount found**
Emtricitabine	200	196.92	98.46

Results and discussion

The absorption maximum for the emtricitabine drug was found to be at 277 nm in the diluent used. Hence 277 nm was selected as the detection wavelength. Different chromatographic conditions were used for the method development like variations in mobile phases and compositions and variations in RP HPLC columns. From the initial trials it is observed that the optimum results were attained with methanol: water in the ratio (70 : 30 v/v) and Agilent 100-5C_{18} Column [250 mm × 4.6 mm]. The system suitability was carried using 6 injections and the number of theoretical plates was more than 2000. The retention time for the drug is at 2.287 ± 0.02. Tailing factor was less than 1.5.

The peaks obtained were symmetric. The linearity for the emtricitabine was observed in the range of 20-100 µg/mL. The specificity of the chromatographic method was determined by injecting the sample concentration prepared from marketed formulation. The response was compared with that of the standard drug. The chromatogram confirms the emtricitabine presence without any interference from the excipients. The accuracy of the method was studied by doing recovery studies by standard addition method for the drug. The recovery was calculated and found to be 97.44-98.0% w/w. The % RSD was less than 2 which indicates a good accuracy of the method. The method developed was precise from the data of interday and intraday precision. The % RSD was less than 2 for precision. LOD and LOQ of emtricitabine were 1.03 µg/mL and 1.83µg/mL respectively. The robustness of the method was determined by changing the wavelength (± 2 nm) and flow rate (± 2 mL/min) of the optimised method. The results were found to be within the limits of ICH guidelines.

Conclusion

The proposed RP-HPLC method was developed and validated as per the International Conference of Harmonisation (ICH) guidelines, and was found to be economic and simple than previously reported methods, due to use of readily available mobile phase and lack of extraction procedures. The results of linearity, precision, accuracy were proved to be within the limits. The proposed method was reproducible, reliable, rapid and robust. Therefore, this method can be employed in quality control to estimate the amount of Emtricitabine in bulk and pharmaceutical formulation.

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