A NOVEL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF EMTRICITABINE IN BULK AND PHARMACEUTICAL FORMULATIONS

Kavya. Mannem[1], D.Varun, Rishitha bhupathi[2], Samyuktha reddy[3], VIjay Nimmala[4], Shivani Gongalla[5], Shashi Orusu[6]

Associate Professor
Department of Pharmaceutical Analysis
Sri indu institute of pharmacy,
Sheriguda, Ibrahimpatnam
Ranga Reddy
Telangana, 501510

Abstract: A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Emtricitabine bulk and pharmaceutical formulations. Separation of Emtricitabine was successfully achieved Dona:YMC PACK PRO 150X4.6mm, 5µm, C18 or equivalenting an isocratic mode utilizing KH_2PO_4: Methanol(65:35) at a flowrate of 1.0mL/mins and eluate was monitored at 265nm, with a retention time of 3.150 minutes for Emtricitabine respectively. Assay Results 98.82%The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Assay.

Keywords: Emtricitabine, RP-HPLC, Method development, Validation

INTRODUCTION:

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) indicated for the treatment of HIV infection in adults or combined with tenofovir alafenamide for the prevention of HIV-1 infection in high-risk adolescents and adults.[1] Emtricitabine is a cytidine analogue.[2] The drug works by inhibiting HIV reverse transcriptase, preventing transcription of HIV RNA to DNA. IUPAC Name: 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. Chemical Formula is C8H10FN3O3S. Molecular Weight is 247.247 g·mol−1. Emtricitabine is a white to off-white powder with a solubility of approximately 112 mg/mL in water at 25 °C. The log P for emtricitabine is -0.43 and the pKa is 2.65.

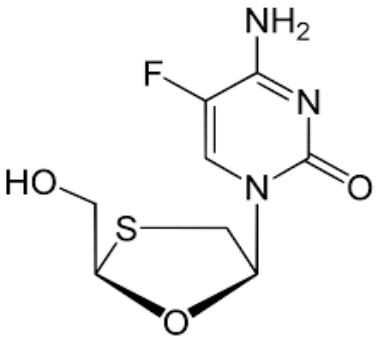

Figure 1: Structure of Emtricitabine

Extreme literature survey proved that very few methods were reported for the determination of Emtricitabine and combination with other drugs by RP-HPLC. Hence, the current work aims to develop an accurate, specific method for the estimation of Emtricitabine in bulk and pharmaceutical dosage form. The objective of the present work is to development and validates a HPLC method development and validation Emtricitabine of tablets. To be employed in routine analysis. In the method development of Emtricitabine, we have decided to carry out our project work by incorporating the Reverse phase High performance Liquid chromatography (HPLC). Then the developed method will be validated according to ICH guidelines for its various parameters.

MATERIALS AND METHODS:

Chemicals and Reagents: Emtricitabine Gift samples obtained from Hetero pharmaceuticals. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 265 nm with column: YMC PACK PRO, C18, 150X4.6mm, 5µm, dimensions at Ambient temperature. The optimized mobile phase consists of KH_2PO_4: Methanol (65:35). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

PREPARATION OF MOBILE PHASE:

Transfer 1000ml of HPLC water into 1000ml of beaker and KH_2PO_4 adjust pH 4.5.

Transfer the above solution 600ml KH_2PO_4 of, 400ml of Methanol is used as mobile phase. They are mixed and sonicate for 20min.

Diluent Preparation:
Mobile phase is used as Diluent**.**

PREPARATION OF THE EMTRICITABINE STANDARD AND SAMPLE SOLUTION:

PREPARATION OF STANDARD SOLUTION:

Accurately weigh and transfer 200mg Emtricitabine into 100ml of volumetric flask and add 10ml of Methanol and sonicate 10min (or) shake 5min and make with water.

Transfers the above solution into 1ml into 10ml volumetric flask dilute to volume with water.

PREPARATION OF SAMPLE STOCK SOLUTION:

Accurately weigh and transfer 200mg of Emtricitabine) of active ingredients were transfer into a 100ml of volumetric flask and add 10ml of Methanol and sonicate 20min (or) shake 10min and makeup with water.

Transfers above solution 1ml into 10ml of the volumetric flask dilute the volume with Methanol. And the solution was filtered through 0.45µm filter before injecting into HPLC system.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 30 minutes to equilibrate the column at ambient temperature. The overlay spectrum of Emtricitabine was obtained and the Emtricitabine showed absorbance's maxima at 265 nm. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into YMC PACK PRO, C18, 150X4.6mm, 5µm, the mobile phase of composition KH_2PO_4: Methanol (65:35) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Emtricitabine in bulk and pharmaceutical dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

RESULTS AND DISCUSSION

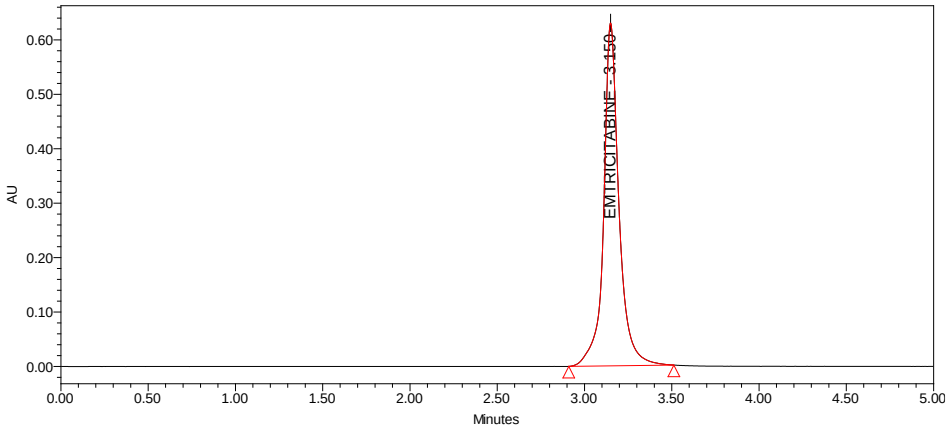

Figure 2: Standard chromatogram

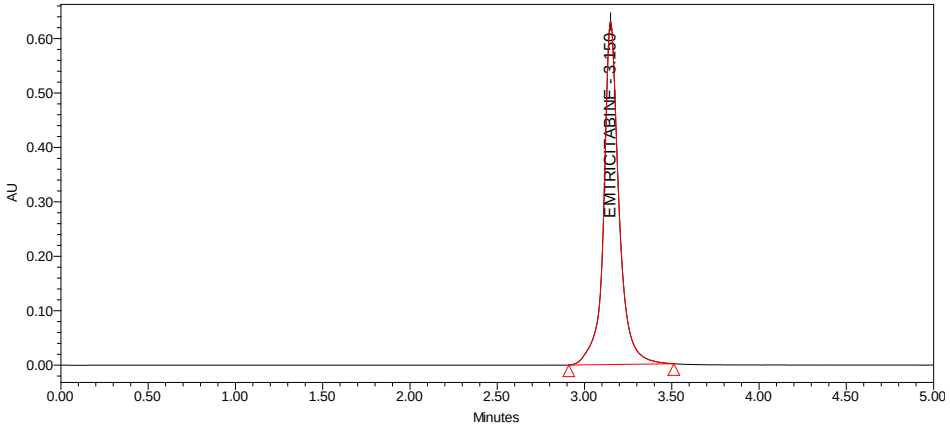

Figure 3: Sample chromatogram

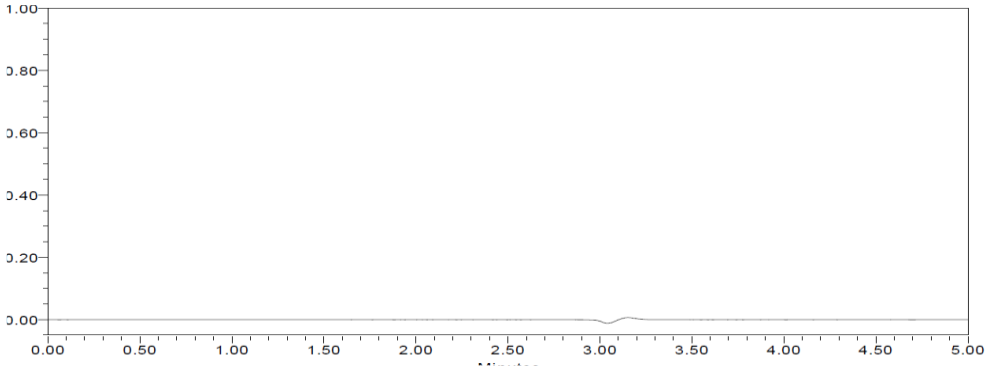

Figure 4: Blank chromatogram

Table 1: System suitability parameters

Parameter	Emtricitabine	Acceptance Criteria
Retention time	3.150	+-10
Theoretical plates	7916.8	>2500
Tailing factor	1.0	<2.00
% RSD	0.4	<2.00

Results of system suitability study are summarized in the above table. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

Table 2: Assay results for Emtricitabine

Drug	Purity
Emtricitabine	99.8%

Table 3: Specificity

S .no	Sample name	Emtricitabine area	Rt
1	Standard-1	3839189	3.150
2	Sample injection-1	3890145	3.142
3	Sample injection-2	3889140	3.141
4	Blank	-	-

Chromatograms explain that retention time for standard, sample and commercial product of Emtricitabine are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So, the method is highly selective.

CONCLUSION:

The study is focused to develop and validate HPLC methods for estimation of Emtricitabine in tablet dosage form. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Emtricitabine.

REFERENCES:

1. FDA Approved Drug Products: Emtriva (Emtricitabine) Oral Capsules

2. FDA Press Announcements: FDA approves second drug to prevent HIV infection as part of ongoing efforts to end the HIV epidemic.

3. Sayyed Z M, Sheikh A, Shaikh Z A, Shinde S A, Chaware V J and Biyani K R. Development and Validation of Analytical Method for Simultaneous Estimation of Tenofovir and Emtricitabine in Pharmaceutical Dosage Forms by HPLC, International Journal of Pharmaceutics & Drug Analysis, 2016; 4: 24 –29.

4. Naresh C J, Pradeep Kumar and Rakesh Kumar. Development and validation of RP-HPLC method for the estimation of antiretroviral drugs and their Pharmaceutical Formulations, An International Research Journal, 2016; 7: 152-165.

5. Ajay D M and Uttam B. RP-HPLC Method for Simultaneous estimation of Impurities from Emtricitabine and Tenofovir disoproxil fumarate Tablet, International Journal of Pharmaceutical Sciences and Research, 2016; 7: 1662-1669.

6. Manasi A, Rohit Ch and Vaishali S. A Rapid Stability Indicating RP-HPLC Method and the Degradation Kinetics Data for the Simultaneous Estimation of Tenofovir disoproxil fumarate and Emtricitabine, International Journal of Pharmacy and Pharmaceutical Research, 2015; 3: 145-159.

7. Abdelhay MH, Gazy AA, Shaalan RA, and Ashour HK. Selective RP-HPLC DAD Method for Determination of Tenofovir fumarate and Emtricitabine in Bulk Powder and in Tablets, Acta Chromatographica, 2015; 27: 41–54.

8. Akbar B, Sireesha D, Anil D, Rajini T, Akifulhaque M, Harshini S and Vasudha B. Method development and validation for simultaneous estimation of Tenofovir disoproxil fumarate and Emtricitabine in pharmaceutical dosage form by RP-HPLC method, International Journal of Innovative Pharmaceutical Sciences and Research, 2015; 3: 1537-1545.

9. Bala R R, Mutta Reddy Y S and Bala Sekhara R Ch. Simultaneous Estimation of Emtricitabine and Tenofovir disoproxil fumarate in tablet dosage form by Reverse Phase High-performance Liquid Chromatography, Chromatographic Science, 2015; 1: 1-6.

10. Sujatha K, Sheela Rani T, AnandBabu K, Kavitha K and Chitra K. RP-HPLC Method Development and Validation of Emtricitabine Synthetic Mixture, World Journal of Pharmaceutical Research, 2014; 3: 499-505.

11. Srinivasan R, Lurdhu Mary K, Lakshmana G Rajesh K D and Rajini B. Method Development and Validation of Tenofovir disoproxil fumarate and Emtricitabine in combined tablet dosage form by UV-Spectrophotometry and RP-HPLC, International Journal of Pharmacy and Analytical Research, 2014; 3: 414-421.

12. Vanitha C, PradeepKumar P, Rajanisekhar V, Swarnalatha G and Sekar V. Simultaneous Estimation of Emtricitabine and Tenofovir disoproxil fumarate in a tablet dosage form by RP-HPLC Method, International Journal of Pharmaceutical Development & Technology, 2014; 4: 279-283.

13. Venkatesh, Sumanth A L M N and Venkateswa Rao P. Analytical Method development and validation of simultaneous estimation of Tenofovir and Emtricitabine in bulk and Pharmaceutical dosage forms By Using RPHPLC, Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry, 2013; 1: 60-69.

14. Sreenivasa Rao B, Nagaraju, Siva and Kiran BV. Development, Validation and Stress Degradation Studies of Emtricitabine and Tenofovir disoproxil fumarate by High Performance Liquid Chromatography, Asian Journal of Research in Chemistry, 2013; 6: 936.

15. Viswanath V, Shanmuga Sundaram P and Ravichandiran. RP-HPLC Method for the Simultaneous Estimation of Tenofovir disoproxil fumarate and Emtricitabine in Combined tablet dosage form, International Journal of Pharm Tech Research, 2013; 5: 1186-1195.

16. Deepthin K, Nagarjuna Reddy G and Dhanalakshmi K. Method Development and Validation for Simultaneous Estimation of Emtricitabine and Tenofovir disoproxil fumarate in Pure and Tablet Dosage Form by using RPHPLC, International Journal of Pharma Research & Review, 2013; 2: 1-11.