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Analytical Method Development and Validation for the Determination of Emtricitabine and Tenofovir Disoproxil Fumarate Using Reverse Phase HPLC Method in Bulk and Tablet Dosage Form

MD Abdul Sattar* and Suneetha Achanta
Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi Road, Guntur- 522002, Andhra Pradesh, India.

Abstract:

A new method was established for simultaneous estimation of Emtricitabine and Tenofovir Disoproxil Fumarate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Emtricitabine and Tenofovir Disoproxil Fumarate by using Inspire C18 column (150×4.6mm) 5.0µm, flow rate was 1.0ml/min, mobile phase ratio was (30:70 v/v) Ortho-phosphoric acid Buffer (adjust the pH 2.5 with NaOH solution): Methanol. The detection of wavelength was 272nm.The developed and validated method was successfully used for the quantitative analysis of commercially available dosage forms. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, UV detector 2487, Empower-software version-2. The retention times of Emtricitabine 2.802mins and Tenofovir Disoproxil Fumarate were found to be 3.677 mins. The % purity of Emtricitabine and Tenofovir Disoproxil Fumarate and was found to be 99.77% and 99.04 % respectively. The system suitability parameters for Emtricitabine and Tenofovir Disoproxil Fumarate such as theoretical plates and tailing factor were found to be 2744.20 and 1.56, 3375, 1.19 and the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study Emtricitabine and Tenofovir Disoproxil Fumaratewas found in concentration range of 20µg-100 µg and 30µg-150µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 100.35% and 100.24%, %RSD for repeatability was 0.22 and 0.5, % RSD for intermediate precision was 0.6 and 0.69 respectively. The precision study was precise, robust, and repeatable.LOD value was 2.98 and 2.96 , and LOQ value was 9.98 and 9.96 respectively.

Keywords: Inspire C18 column,Emtricitabine, Tenofovir Disoproxil Fumarate and RP-HPLC

INTRODUCTION

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. Emtricitabine is an analogue of cytidine. The drug works by inhibiting reverse transcriptase, the enzyme that copies HIVRNA into new viral DNA.IUPAC NAME: 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one.Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Emtricitabine helps to block HIV reverse transcriptase, a chemical in your body (enzyme) that is needed for HIV to multiply. Emtricitabine is always used with other anti-HIV medicines to treat people with HIV infection. Emtricitabine works by inhibiting reverse transcriptase[1], the enzyme that copies HIV RNA into new viral DNA. Emtricitabine is a synthetic nucleoside analogue of cytidine. It is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate[2], which is responsible for the inhibition of HIV-1 reverse transcriptase.

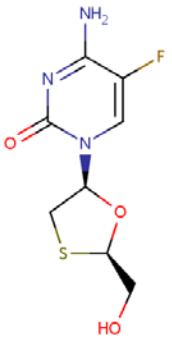

Figure 1: Structure of Emtricitabine

Tenofovir disoproxil fumarate (a prodrug of tenofovir), marketed by Gilead Sciences under the trade name Viread, belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors[3] (nRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. In vivo tenofovirdisoproxilfumarate is converted to tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate.IUPAC:is({[(2R)-1-(6-amino-9H-purin-9-yl)propan-yl]oxy}methyl)phosphonic acid. Tenofovir belongs to a class of antiretroviral drugs known as nucleotide[4] analogue reverse transcriptase inhibitors (NtRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. Tenofovir is currently in late-stage clinical trials for the treatment of hepatitis B. Tenofovir disoproxil fumarate is an acyclic nucleoside phosphonatediester analog of adenosine monophosphate.Tenofovir inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination. Specifically, the drugs are analogues of the naturally occurring deoxynucleotides needed to synthesize the viral DNA and they compete with the natural deoxynucleotides[5] for incorporation into the growing viral DNA chain. Hence in the present communication we would like to report a simple ,economic, feasible, rapid,sensitive and validated[6-12] specific RP-HPLC method for the simultaneous estimation of Emtricitabine and Tenofovir disoproxil fumarate in Bulk and formulation.

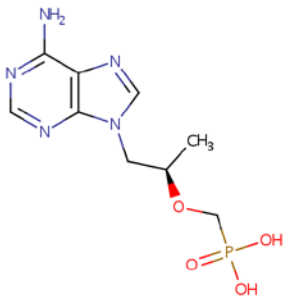

Figure 2:.Structure of Tenofovir

MATERIALS AND METHODS

Instruments used

WATERS HPLC Auto Sampler, Separation module 2695, UV detector 2487, Empower-software version-2, pump (LC-10AT) and (LC-10ATVP),EV-100 UV-Visible spectrophotometer .Electronic balance and Ultra sonicator, Inspire C18 RP column, 250mm*5mm.PH analyzer (ELICO).HPLC injecting syringe (25ug) HAMILTON.

Chemicals and reagents

Emtricitabine and Tenofovir DF were supplied from Hetero Laboratories, Hyderabad and Potassium dihydrogen o-phosphate and Methanol (MOLY CHEM,HPLC GRADE),Double distilled water and o-phosphoric acid(MERCK) were employed in the present work.

Selection Of Wavelength

UV spectrum of 10 µg / ml Emtricitabine and Tenofovir DF in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 272. At this wavelength both the drugs show good absorbance.

Preparation of buffer and mobile phase:

Preparation of 0.1% Ortho phosphoric acid buffer:

Pipetted 1 ml of ortho phosphoric acid in 1000 ml HPLC water and adjust the pH 2.5 with NaOH solution.

Preparation of mobile phase:

Mix a mixture of above buffer 300 ml (30%) and 700 ml MethonolHPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 045 µ filter under vacuum filtration.

Diluent Preparation: Use the Mobile phase as Diluents.

Optimized chromatographic conditions:

Instrument used : High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector
Temperature : Ambient
Column : Inspire C_{18} (4.6 x 150mm, 5.0µm)
Buffer : Ortho phosphoric acid pH 2.5
Mobile phase : 30% buffer: 70% Methanol
Flow rate : 1.0 ml per min
Wavelength : 272 nm
Injection volume : 20 µl
Run time : 7min.

Standard Solution Preparation:

Accurately weigh and transfer 20mg of Emtricitabine& 30mg of Tenofovir DF working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1ml of Emtricitabine&Tenofovir DF of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 3ml of Emtricitabine&Tenofovir DF of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 20mg of Emtricitabine& 30mg Tenofovir DF equivalent weight of the sample into a 10ml clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Emtricitabine&Tenofovir DF of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Further pipette 3ml of Emtricitabine&Tenofovir DF of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Emtricitabine&Tenofovir DF peaks and calculate the %Assay by using the formulae.

RESULTS AND DISCUSSION

1. Linearity:

Preparation of stock solution:

Accurately weigh and transfer 20mg of Emtricitabine& 30mg of Tenofovir DF working standard into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Emtricitabine&Tenofovir DF the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Preparation of Level – I (20ppm & 30ppm of Emtricitabine&Tenofovir DF):

1ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – II (40ppm & 60ppm of Emtricitabine&Tenofovir DF):

2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – III (60ppm & 90ppm of Emtricitabine&Tenofovir DF):

3ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – IV (80ppm & 120ppm of Emtricitabine&Tenofovir DF):

4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – V (100ppm & 150ppm of Emtricitabine&Tenofovir DF):

5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

2. Precision:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

3. Intermediate precision/ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory.The standard solution was injected for five times and measured the area for all Five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

4. Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10mg of Emtricitabine and 15mg of Tenofovir DF working standard into a 10ml clean dry

volumetric flask add about 7 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1ml of Emtricitabine&Tenofovir DF the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents
Further pipette 3ml of Emtricitabine&Tenofovir DF the above stock solution into a10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 20mg of Emtricitabine and 30mg of Tenofovir DFworking standard into a into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1ml of Emtricitabine&Tenofovir DF the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
Further pipette 3ml of Emtricitabine&Tenofovir DF the above stock solution into a10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 30mg of Emtricitabine and 45mg of Tenofovir DF equivalent weight of tablet powder into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1ml of Emtricitabine&Tenofovir DF the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
Further pipette 3ml of Emtricitabine&Tenofovir DF the above stock solution into a10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Emtricitabine&Tenofovir DF and calculate the individual recovery and mean recovery values.

5. Limit of detection:

Preparation of Emtricitabine solution: Accurately weigh and transfer 20mg of Emtricitabine working standard into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1ml of Emtricitabine the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.
Further pipette 3ml Emtricitabine the above stock solution into a10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of 0.077µg/ml solution:

Further pipette 0.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.
Further pipette 0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Tenofovir DF solution:

Accurately weigh and transfer 30mg of Tenofovir DF working standard into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1ml of Tenofovir DF the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.
Further pipette 3ml of Tenofovir DF the above stock solution into a10ml volumetric flask and dilute up to the mark with Diluent.

Preparation 0.163µg/ml solution:

Further pipette 0.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents
Further pipette 0.9ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents

6. Limit of quantification:

Preparation of Emtricitabine solution:

Preparation of 0.257µg/ml solution:

Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.
Further pipette 0.8ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Tenofovir DF solution:

Preparation of 0.549µg/ml solution:

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.
Further pipette 0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

7. Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a) The flow rate was varied at 0.9 ml/min to 1.1ml/min.Standard solution 60 & 90 µg/ml of Emtricitabine & Tenofovir DF prepared and analysed using the varied flow rates along with method flow rate.
b) The Organic composition in the Mobile phase was varied from 63% to 77%.Standard solution 60 & 90 µg/ml of Emtricitabine&Tenofovir DF was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

Table 1: Results of system suitability parameters

S. No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Emtricitabine	2.802	1187582	114245		1.56	2744.20
2	Tenofovir DF	3.677	992186	81814	2.95	1.19	3375.11

Table 2: Results of Assay for Emtricitabine and Tenofovir DF

	Label Claim (mg)	% Assay
Emtricitabine	200	99.77
Tenofovir DF	300	99.04

Table 3: Area of different concentration of Emtricitabine and Tenofovir DF

S. No	Emtricitabine		Tenofovir DF	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	20	406525	30	345813
2	40	830261	60	690673
3	60	1178855	90	1090011
4	80	1558003	120	1413252
5	100	1951429	150	1757423

Table 4: Analytical performance parameters of Emtricitabine and Tenofovir DF

Parameters	Emtricitabine	Tenofovir DF
Slope (m)	19088	11819
Intercept (c)	39750	4305
Correlation coefficient (R^2)	0.999	0.999

Table 5: Results of Precision for Emtricitabine

Injection	Area
Injection-1	1154085
Injection-2	1155893
Injection-3	1160220
Injection-4	1157541
Injection-5	1159687
Average	1157485
Standard Deviation	2570.3
%RSD	0.22

Table 6: Results of Precision for Tenofovir DF

Injection	Area
Injection-1	949525
Injection-2	941958
Injection-3	947875
Injection-4	952978
Injection-5	951911
Average	948849.4
Standard Deviation	4339.2
%RSD	0.5

Table 7: Results of Intermediate precision for Emtricitabine

Injection	Area
Injection-1	1166019
Injection-2	1167161
Injection-3	1171511
Injection-4	1180276
Injection-5	1183918
Injection-6	1177468
Average	1174392
Standard Deviation	7287.3
%RSD	0.6

Table 8: Results of Intermediate precision for Tenofovir DF

Injection	Area
Injection-1	956847
Injection-2	960447
Injection-3	956523
Injection-4	944789
Injection-5	957643
Injection-6	964689
Average	956823
Standard Deviation	6642.1
%RSD	0.69

Table 9: Accuracy (recovery) data for Emtricitabine

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	598249	10	10.06	100.41	100.35
100%	1186057	20	19.95	99.77	
150%	1798511	30	30.26	100.86	

Table 10: Accuracy (recovery) data for Tenofovir DF

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	501791	15	15.05	100.36	100.24
100%	992126	30	29.76	99.21	
150%	1517427	45	45.52	101.16	

Table 11: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Emtricitabine	48	143	2.98
Tenofovir DF	48	142	2.96

Table 12: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Emtricitabine	48	479	9.98
Tenofovir DF	48	478	9.96

Table 13: Results for variation in flow for Emtricitabine

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	2647.56	1.62
2	1.0	2744.20	1.56
3	1.2	2729.27	1.59

Table 14: Results for variation in flow for Tenofovir DF

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	3290.41	1.15
2	1.0	3375.11	1.19
3	1.2	3378.66	1.12

Table 15: Results for variation in mobile phase composition for Emtricitabine

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2505.98	1.54
2	*Actual	2744.20	1.56
3	10% more	2495.48	1.62

Table 16: Results for variation in mobile phase composition for Tenofovir DF

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3117.88	1.07
2	*Actual	3375.11	1.19
3	10% more	3014.82	1.31

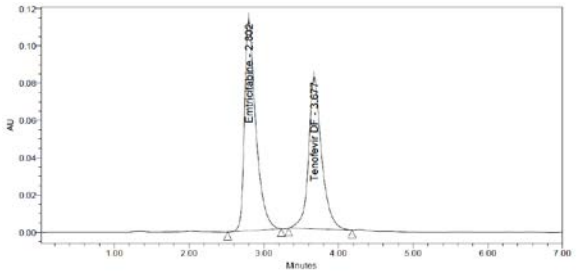

Figure 3: Chromatogram for system suitability

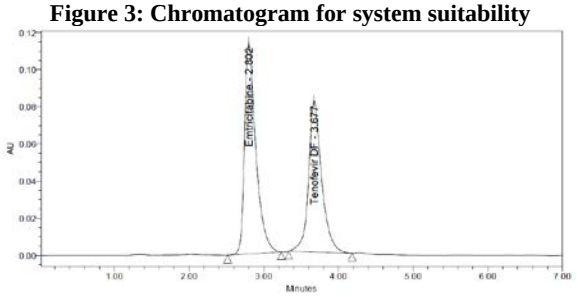

Figure 4: Chromatogram for Standard

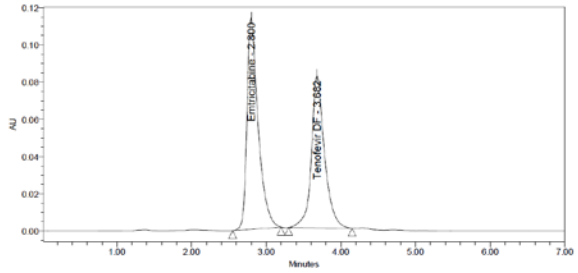

Figure 5: Chromatogram for Sample

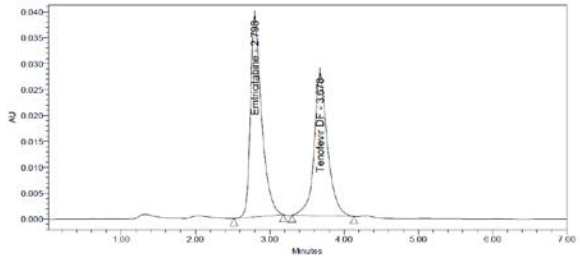

Figure 6: Chromatogram for linearity-1

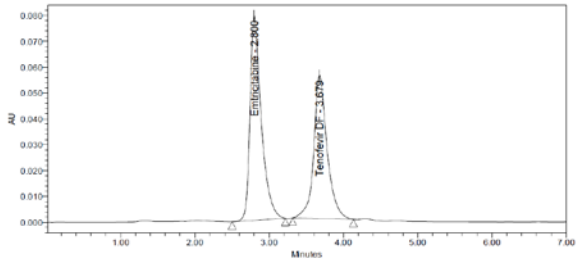

Figure 7: Chromatogram for linearity-2

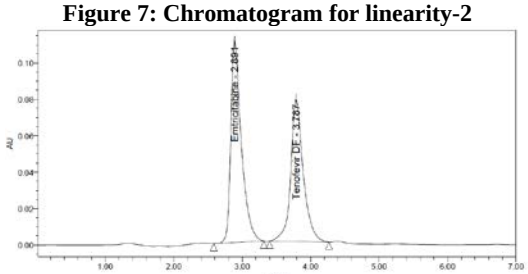

Figure 8: Chromatogram for linearity-3

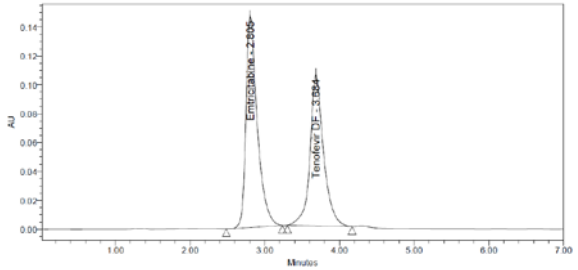

Figure 9: Chromatogram for linearity-4

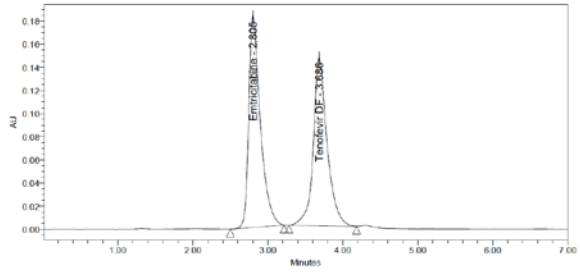

Figure 10: Chromatogram for linearity-5

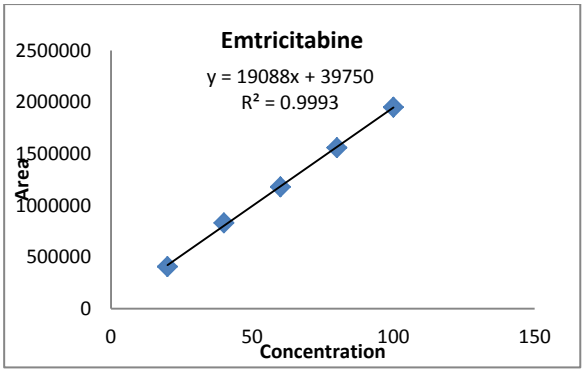

Figure 11: Calibration graph for Emtricitabine

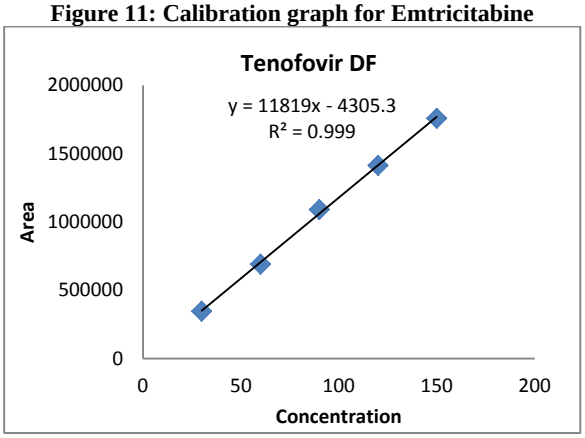

Figure 12: Calibration graph for Tenofovir DF

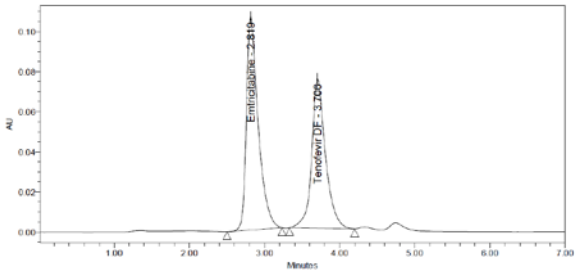

Figure 13: Chromatogram for Precision -1

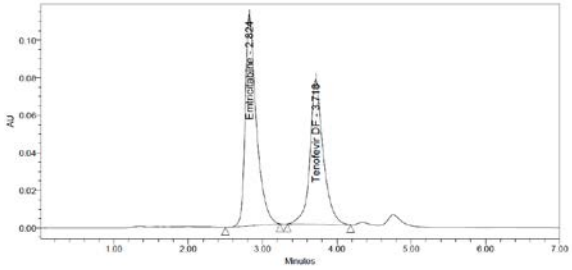

Figure 14: Chromatogram for Intermediate Precision -1(Ruggedness)

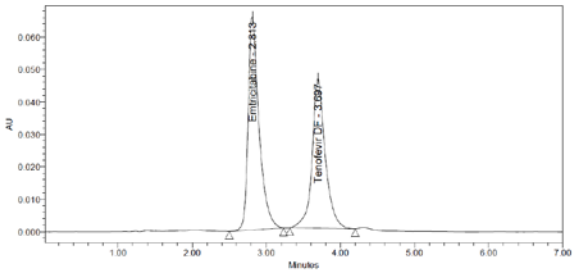

Figure 15: Chromatogram for Accuracy 50%-1

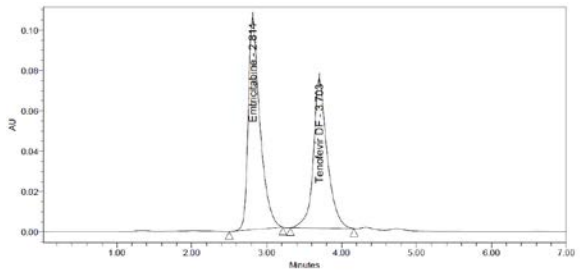

Figure 16: Chromatogram for Accuracy 100%-1

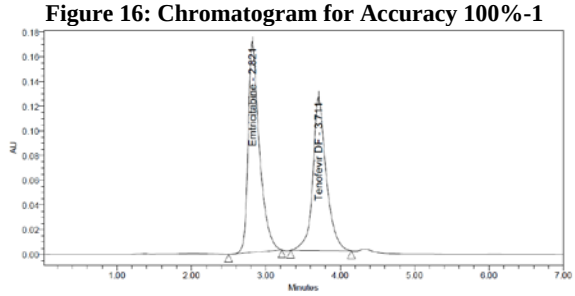

Figure 17: Chromatogram for Accuracy 150%-1

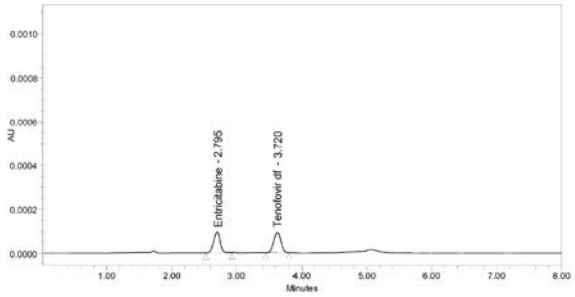

Figure 18: Chromatogram of Emtricitabine, Tenofovir DF showing LOD

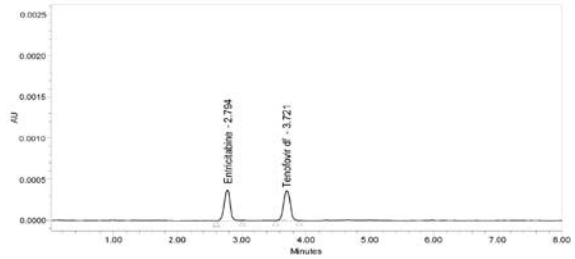

Figure 19: Chromatogram of Emtricitabine, TenofovirDF showing LOQ

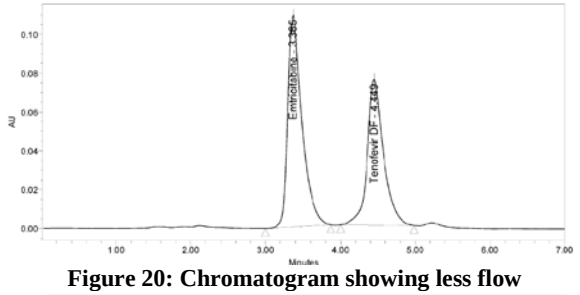

Figure 20: Chromatogram showing less flow

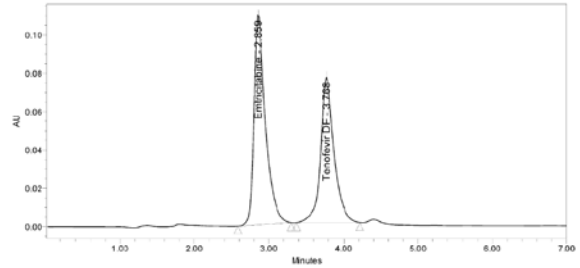

Figure 21: Chromatogram showing more flow

SUMMARY AND CONCLUSION

The estimation of Emtricitabine and Tenofovir DF was done by RP-HPLC. The assay of Emtricitabine and Tenofovir DF was performed with tablets and the % assay was found to be 99.77 and 99.04 which shows that the method is useful for routine analysis.

The linearity of Emtricitabine and Tenofovir DF was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity.

The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.22 and 0.5 for Emtricitabine and Tenofovir DF which shows that the method is precise.

The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.69 for Emtricitabine and Tenofovir DF which shows that the method is repeatable when performed in different days also.

The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.35% and 100.24% for Emtricitabine and Tenofovir DF. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Emtricitabine was found to be 2.98 and 9.98 and LOD and LOQ for Tenofovir DF was found to be 2.96 and 9.96.

The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

REFERENCES

1- Budagamlavanya,PerumallaHariprasad,AllumelluVenkatapraveen,DudipalaPrasannalakshmi and MaramRamireddy, Simultaneous estimation of emtricitabine and tenofovirdisproxilfumerate by HPLC method, Der Pharmacia Lettre, 2012, 4 (6):1855-1862.

2- Syed SajjadHussen, PremnathShenoy, Udupa, Muddu Krishna, Development And Validation Of Stability Indicating Rp-Hplc Method For Tenofovir Nanoparticle Formulation, International Journal of Pharmacy and Pharmaceutical Sciences, Vol 5, Suppl 2, 2013, 245-248.

3- Rajesh Sharma1 and Pooja Gupta, A Validated RP - HPLC Method for Simulataneous Estimation of Emtricitabine and TenofovirDisoproxilFumarate in a Tablet Dosage Form, Eurasian J. Anal. Chem. 4(3): 276-284, 2009.

4- Viswanath V, Shanmugasundaram P and Ravichandiran V, RP-HPLC Method For The Simultaneous Estimation Of TenofovirDisoproxilFumerate And Emtricitabine In Combined Tablet Dosage Form, International Journal of PharmTech Research, Vol.5, No.3, pp1186-1195, July-Sept2013.

5- N AppalaRaju and Shabana Begum, Simultaneous RP-HPLC Method for the Estimation of the Emtricitabine, TenofovirDisoproxilFumerate and Efavirenz in Tablet Dosage Forms, Research J. Pharm. and Tech. 1(4): Oct.-Dec. 2008, 522-525.

6- International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures: definitions and terminology, Geneva (1996).

7- International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: methodology, adopted in (1996) Geneva.

8- US FDA Technical Review Guide: Validation of Chromatographic Methods, Center for Drug Evaluation and Research (CDER), Rockville, MD, (1993).

9- US FDA, Guidelines for submitting samples and analytical data for method validation, Rockville, MD, Center for Drugs and Biologics Department of Health and Human Services (1987).

10- General Chapter <1225>, Validation of compendial methods, United States Pharmacopeia XXIII, National Formulary, XVIII, Rockville, MD, The United States Pharmacopeial Convention, Inc, (1995) 1982-1984.

11- US FDA HSS, 21 CFR Part 211, Current Good Manufacturing Practice of Certain Requirements for Finished Pharmaceuticals; Rockville (1997).