Research Article

Method Development and Validation for Simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in Pure and Tablet Dosage Form by using RP-HPLC

***Deepthi Komaroju, G. Nagarjuna Reddy, K. Dhanalakshmi**

KLR pharmacy college, New Paloncha-507115, Khammam, Andhra Pradesh, India.

ABSTRACT

A simple, precise, accurate and rapid RP-HPLC method with PDA detector has been developed and subsequently validated for the simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate in pure and tablet dosage form. The estimation was carried out on a Phenomenax Luna C18 (250mm x 4.6mm i.d; particle size 5µm) column with mixture of methanol: phosphate buffer pH-3 (70:30 v/v) as mobile phase. The flow rate was 1ml/min. UV detection was performed at 258 nm. The method was validated for linearity, accuracy, precision, robustness, LOD, LOQ as per ICH guidelines. The developed and validated method was successfully used for the quantitative analysis of commercial available dosage form. The retention time was 2.605 min and 3.781 min for emtricitabine and tenofovir disoproxil fumarate, respectively. The calibration curve was linear over concentration range of 45-105µg/ml for tenofovir disoproxil fumarate and 30-70µg/ml for emtricitabine. The correlation coefficient (r^2) was found to be 0.999.Amount of emtricitabine and tenofovir disoproxil fumarate was found to be 199.4mg/tab and 298.6mg/tab respectively. The %RSD values were less than 2 for method precision. LOD and LOQ were found to be limits for emtricitabine and tenofovir disoproxil fumarate. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method for the simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate in pure and tablet dosage form.

Keywords: Emtricitabine, RP-HPLC determination, tenofovir disoproxil fumarate, UV detection, validation

Received 29 July 2013 Received in revised form 03 Sept 2013 Accepted 05 Sept 2013

***Address for correspondence:**
Deepthi Komaroju
Department of Pharmaceutical Analysis, K. L.R Pharmacy College, Paloncha-– 507 115, Khammam, Andhra Pradesh, India.
E- mail: deepthi.087@gmail.com

INTRODUCTION

Emtricitabine: The chemical name of emtricitabine is 5-fluoro-1-(2R, 5 S)-[2-(hydroxymethyl)-1, 3-oxathiolan-5-yl] cytosine. Emtricitabine is the (-) enatiomer of a thio analog of cytidine. It has a molecular formula of $C_8H_{10}FN_303$ and a molecular weight of 247.24 EMT is an antiviral agent used for the prevention of perinatal HIV-1 reverse transcriptase [1]. It is also acts activly against Hepatitis B virus [2,3].

Emtricitabine works by inhibiting reverse transcriptase, 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, which is responsible for the inhibition of HIV-1 reverse transcriptase. It competes with the natural substrate deoxycytidine 5'-triphosphate and incorporates into nascent viral DNA, resulting in early chain termination. Therefore emtricitabine inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate deoxycytidine 5'-triphosphate and by its incorporation into viral DNA. By inhibiting HIV-1 reverse transcriptase, emtricitabine can help to lower the amount of HIV, or "viral load", in a patient's body and can indirectly increase the number of immune system cells (called

T cells or CD4+ T-cells). Both of these changes are associated with healthier immune systems and decreased likelihood of serious illness. [4-7]

Structural formula [8]:

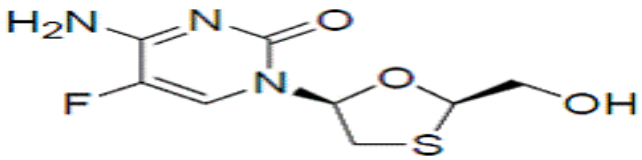


Figure 1: Structural Formula of Emtricitabine

Emtricitabine a synthetic nucleoside analog of cytidine is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 reverse transcriptase (RT) by being incorporated into nascent viral DNA which results in chain termination. Emtricitabine 5'-triphosphate is a weak inhibitor of mammalian DNA polymerases α, β, ε and mitochondrial DNA polymerase

Tenofovir Disoproxil Fumarate:
Tenofovir disoproxil fumarate is a fumaric acid salt of the bis isopropoxycarbony-loxymethyl ester derivative of tenofovir. The chemical name of tenofo-vir disoproxil fumarate is 9 [(R) 2[[bis[[(iso propoxycarbonyl)oxy] methoxy]phosphinyl]methoxy]propyl]adenine fumarate (1:1).
It has a molecular formula of $C_{19}H_{30}N_5O_{10}P \bullet C_4H_4O_4$ and a molecular weight of 635.52.

Structural formula [8]:

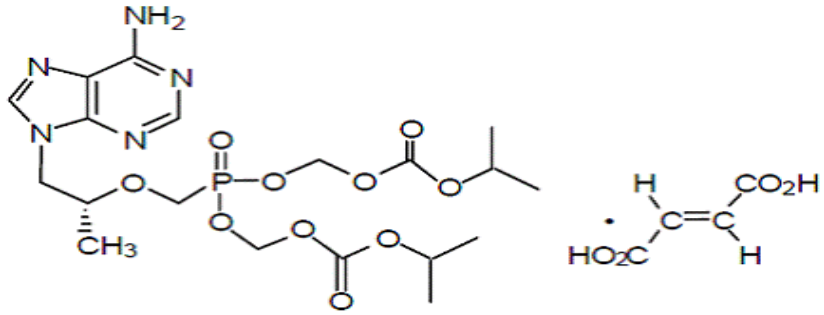


Figure 2: Structural Formula of Tenofovir Disoproxil Fumarate

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. The drugs are analogues of the naturally occurring deoxynucleotides needed to synthesize the viral DNA and they compete with the natural deoxynucleotides for incorporation into the growing viral DNA chain. Tenofovir belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NtRTIs). It blocks reverse transcriptase, an enzyme which is 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 phosphonate diester analog of adenosine monophosphate. Tenofovir requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylation by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases α, β, and mitochondrial DNA polymerase γ. [11, 14].
In combination studies evaluating the in Vitro antiviral activity of emtricitabine and tenofovir together, synergistic antiviral effects were observed. A Literature review revealed that very few analytical methods like UV spectroscopy, liquid chromat-ography and tandem mass spectroscopy appeared in the literature for the determination of simultaneous estimation

of Emtricitabine and Tenofovir Disoproxil Fumarate and Official assay of Emtricitabine and Tenofovir Disoproxil Fumarate is not described in any pharmacopoeias. In view of the need for a suitable RP-HPLC method for routine analysis of Tenofovir Disoproxil Fumarate in formulation, an attempt was made to develop simple, precise and accurate analytical method for estimation of Emtricitabine and Tenofovir Disoproxil Fumarate and find out its applicability to its determination in formulation. [9, 10]

MATERIALS AND METHOD

Chemicals and Reagents used:

Tenofovir and Emtricitabine as pure standard reference drugs were obtained from cipla pharmaceuticals, Mumbai, India. Methanol and orthophosphoricacid, Sodium Di hydrogen Ortho Phosphate, Milli-Q water were supplied by KLR Pharmacy College. Milli-Q Water obtained from all glass double distillation apparatus. Truvada, (200mg of Emtricitabine and 300mg of tenofovirdisoproxilfumerate combination tablets) tablets was purchased from local market.

Apparatus:

HPLC Analysis was performed on chromatographic system of water 2695 separation module with empower software liquid chromatography comprising water 996 photo Diode array detector (PDA Detector), column waters (250 mm x 4.6 mm, 5µ) was used and a equipped with Auto Sampler. Derivative Spectral and photometric absorbance measurements are done on UV Spectrophotometer with software UV Win, lab India make 3092. 10mm path length quartz cells were used. Digital analytical balance Shimadzu make AUX 220 was used for weighing drug

Experimental Condition:

Flow rate of the mobile phase was changed from0.5 –1 ml/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that1.0 ml/min flow rate was ideal for the successful elution of the analyte. The HPLC system was hence operated using an isocratic mode at a flow rate of 1.0 ml/min at 25 ± 2º**c**. For analysis the most suitable mobile phase was found to be Methanol, buffer in the ratio of 70:30.Detection was carried out at wavelength of 258nm.

Solutions of EMT and TDF in diluents were also injected directly for HPLC analysis and the responses (peak area) were recorded. It was observed that there was no interference from the mobile phase or baseline disturbances and both the analyze absorbed well at 258 nm.

Preparation of Phosphate buffer (P^H: 3.0):

Weighed 7.0 grams of Sodium Di hydrogen Ortho Phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjust Ph 3.0with Orthophosphoric acid

Preparation of mobile phase:

Mix a mixture of above Buffer 300 ml (30%) 700 ml of Methanol HPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Use the Mobile phase as Diluent

Standard Solution Preparation:

Accurately weigh and transfer 15 mg of Emtricitabine and 10mg of Tenofovir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution) Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 694.2 mg of Emtricitabine and Tenofovir sample into a 100mL clean dry volumetric flask add about 70mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.25ml of Emtricitabine and Tenofovir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents

Under the optimum chromatographic conditions, the retention time obtained for EMT and TDF were 2.601 and 3.781 min, respectively for sample preparation and is shown in Fig 3.The results of USP plate count, USP tailing and USP resolution are reported in **Table 1**.

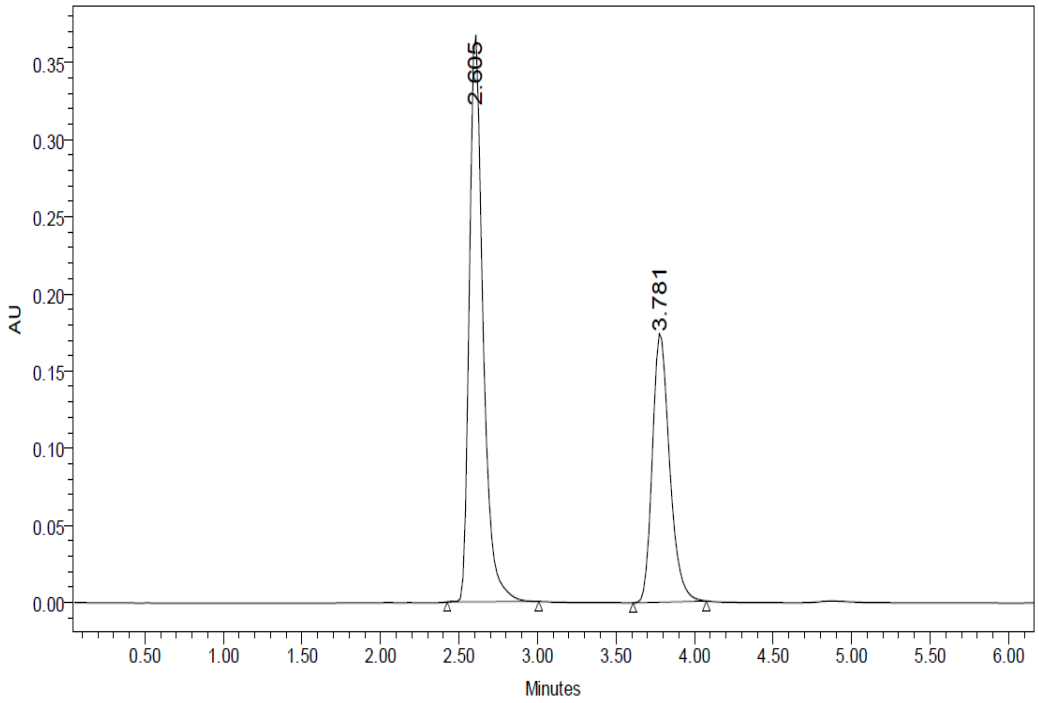


Figure 3: Chromatogram of Mixed Standard Solutions

Table 1: Chromatogram Data

	Peak name	RT	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Emtricitabine	2.605	2233704	365596	4456	1.4	
2	Tenofovir	3.781	1328106	174637	5823	1.3	6.5

The values obtained for these properties which are indicated in **Table 1** shows that, the chromatographic conditions are appropriate for separation and determination of compounds.

RESULTS AND DISCUSSION

LINEARITY:

Preparation of stock solution:

Accurately weigh and transfer 15 mg of Emtricitabine and 10mg of Tenofovir working standard into a 10ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Preparation of Level – I (45ppm Tenofovir of &30ppm of Emtricitabine):

0.3ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

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

0.4ml of stock solution has taken in 10ml of Volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (75ppm Tenofovir of &50ppm of Emtricitabine):

0.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

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

0.6ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (105ppm Tenofovir of &70ppm of Emtricitabine):

0.7ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

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.

Table No: 2 Linearity Results: (for Emtricitabine)

Table 2: Linearity Data

S.No	Linearity Level	Concentration	Area	Height(µVsec)	RT
1	I	30ppm	1224140	208904	2.592
2	II	40ppm	1595681	275199	2.590
3	III	50ppm	1992966	338901	2.594
4	IV	60ppm	2356546	401936	2.593
5	V	70ppm	2797214	479784	2.590
Correlation Coefficient			0.999		

Acceptance Criteria: Correlation coefficient should be not less than 0.999 and it found to be in limits.

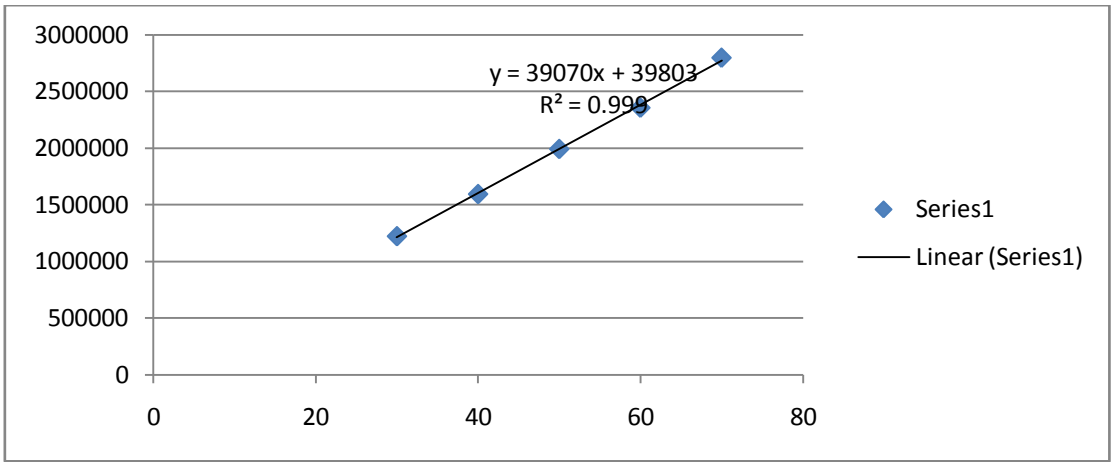
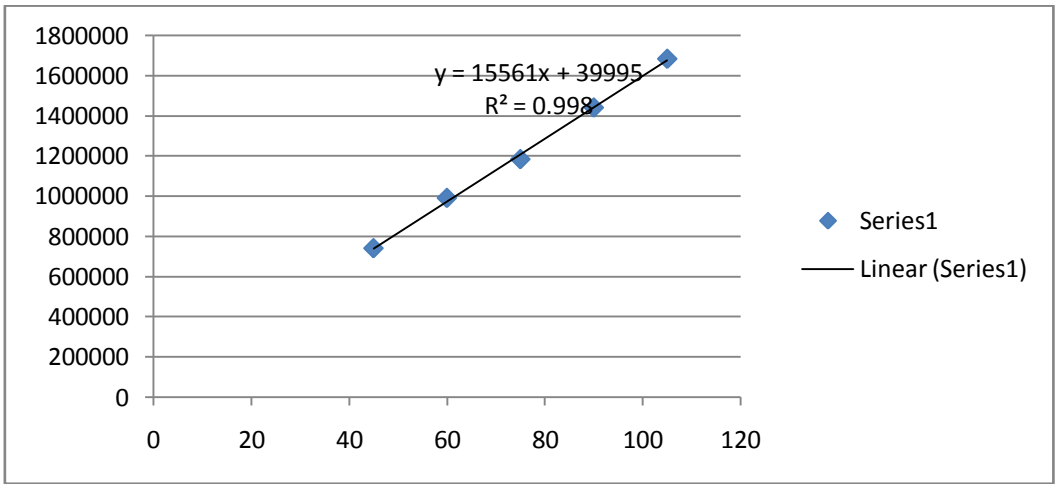


Table No: 2.1 Linearity Results: (for Tenofovir)

S.No	Linearity Level	Concentration	Area	Height(µVsec)	RT
1	I	45ppm	740046	100436	3.733
2	II	60ppm	990204	135739	3.728
3	III	75ppm	1183023	160635	3.757
4	IV	90ppm	1439886	198488	3.748
5	V	105ppm	1682302	237013	3.722
Correlation Coefficient			0.9983		



Acceptance Criteria: Correlation coefficient should be not less than 0.999

Conclusion: Response for the analyte peak were found to be linear over the concentration range of 45ppm to 105ppm for tenofovir disoproxil fumarate and 30 to 70 ppm for emtricitabine.

PRECISION:

Preparation of stock solution:

Accurately weigh and transfer 15 mg of Emtricitabine and 10mg of Tenofovir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

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.

The results are summarized Emtricitabine.

Table 3: Precision Data for Emtricitabine

Injection	Area
Injection-1	2010800
Injection-2	2002956
Injection-3	2012800
Injection-4	2005243
Injection-5	2011092
Average	2008578.1
Standard Deviation	4237.0
%RSD	0.2

The results are summarized Tenofovir

Table 4: Precision Data for Tenofovir

Injection	Area
Injection-1	1184689
Injection-2	1188199
Injection-3	1195842
Injection-4	1184210
Injection-5	1198327
Average	1190253.2
Standard Deviation	6483.1
%RSD	0.5

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%. From table 3 and 4 tell us %RSD was found to be in limits.

INTERMEDIATE PRECISION/RUGGEDNESS:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Preparation of stock solution:

Accurately weigh and transfer 15 mg of Emtricitabine and 10mg of Tenofovir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

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

Procedure:

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.

The results are summarized Emtricitabine

Table 5: Intermediate Precision or Ruggedness Data for Emtricitabine

Injection	Area
Injection-1	2005053
Injection-2	2007362
Injection-3	2007473
Injection-4	2009153
Injection-5	2012800
Average	2008368.1
Standard Deviation	2874.8
%RSD	0.1

The results are summarized Tenofovir

Table 6: Intermediate Precision or Ruggedness Data for Tenofovir

Injection	Area
Injection-1	1183951
Injection-2	1184689
Injection-3	1186232
Injection-4	1186406
Injection-5	1188564
Average	1185968.3
Standard Deviation	1782.3
%RSD	0.2

Acceptance Criteria: The % RSD for the area of five standard injections results should not be more than 2%. From table 5 and 6 we can consider it was found to be in limits

Accuracy:

Preparation of Standard stock solution:

Accurately weigh and transfer 15 mg of Emtricitabine and 10mg of Tenofovir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution)

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

Preparation Sample solutions:

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

Accurately weigh and transfer 5.05mg of Emtricitabine and 8.1mg of Tenofovir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock Solution).

Further pipette 0.5ml of Emtricitabine& Tenofovir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

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

Accurately weigh and transfer 10 mg of Emtricitabine and 15mg of Tenofovir **working** standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 0.5ml of Emtricitabine& Tenofovir **of** the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

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

Accurately weigh and transfer 15mg of Emtricitabine and 23.3mg of Tenofovir working standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 0.5ml of Emtricitabine& Tenofovir **of** the above stock solution into a

10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

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

The accuracy results for Emtricitabine

Table 7: Accuracy Data for Emtricitabine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1017499	5	5.12	101.3%	
100%	1987385	10	10.0	100.0%	100.5%
150%	2992493	15	15.0	100.3%	

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%.

The accuracy results for Tenofovir

Table 8: Accuracy Data of Tenofovir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	648293.3	8.1	8.25	101.9%	
100%	1174011	15.0	14.9	99.6%	101.2%
150%	1868236	23.3	23.7	102.0%	

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%. From table 7 and 8 we can consider it was found to be in limits.

LIMIT OF DETECTION: (for Tenofovir)

Preparation of 50µg/ml solution:

Accurately weigh and transfer 10mg of Tenofovir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. **(Stock solution)**

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

Preparation of 0.048µg/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 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Pipette 0.64mL of solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 54 µV

Signal Obtained from LOD solution: 158 µV

S/N =158/54 =2.92

Acceptance Criteria: S/N Ratio value shall be 3 for LOD solution.

LIMIT OF DETECTION: (for Emtricitabine)

Preparation of 75µg/ml solution:

Accurately weigh and transfer 15 mg of Emtricitabine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.03µg/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 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent

Pipette 0.6mL of solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 54µV

Signal Obtained from LOD solution: 165 µV

S/N =165/54 =3.05

Acceptance Criteria: S/N Ratio value should be 3 for LOD solution and found to be in limits.

Table 9: LOD Data for Emtricitabine and Tenofovir

	Peak name	RT	Area	Height
1	emtricitabine	2.592	967	165
2	tenofovir	3.733	1164	158

LIMIT OF QUANTIFICATION: tenofovir

Preparation of 50µg/ml solution:

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

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

Preparation of 0.16µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and

Dilute up to the mark with diluent.

Pipette 0.22mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 54 µV

Signal Obtained from LOQ solution: 536µV

S/N =536/54 = 9.92

LIMIT OF QUANTIFICATION: emtricitabine

Preparation of 75µg/ml solution:

Accurately weigh and transfer 15 mg of Emtricitabine working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.12µg/ml solution):

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Pipette 1.0mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Pipette 2.4 mL of above solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank: 54 µV

Signal Obtained from LOQ solution: 544µV

S/N = 544/54 = 10.0

Acceptance Criteria: S/N Ratio value shall be 10 for LOQ solution and found to be in limits.

Table 10: LOQ Data for Emtricitabine and Tenofovir

	Peak name	RT	Area	Height
1	emtricitabine	2.561	3188	544
2	tenofovir	3.725	3949	536

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. The flow rate was varied at 0.8 ml/min to 1.2ml/min.

Change in the Flow rate: Standard solution 75ppm of Emtricitabine and 50ppm of Tenofovir was prepared and analysed using the varied flow rates along with method flow rate.

System suitability results for Emtricitabine:

Table 11: Change in Flow Rate for Emtricitabine

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	5752.0	1.4
2	1.0	5026.5	1.3
3	1.2	4207.0	1.3

System suitability results for Tenofovir:

Table 12: Change in Flow Rate Data for Tenofovir

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.5	7187.0	1.2
2	0.6	6381.5	1.2
3	0.7	5419.0	1.2

The results are summarized on evaluation of the above results from table 11 and 12, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%

Change in mobile phase: Standard solution 75 µg/ml of Emtricita-bine&50µg/ml of Tenofovir was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

System suitability results for Emtricitabine

Table 13: Change in Organic Composition Data for Emtricitabine

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4577.0	1.3
2	*Actual	5026.5	1.3
3	10% more	4476.0	1.3

System suitability results for Tenofovir:

Table 14: Change in Organic Composition for Tenofovir

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6498.0	1.2
2	*Actual	6381.5	1.2
3	10% more	6471.0	1.2

The results are summarized:

On evaluation of the above results from table 13 and 14 it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10

CONCLUSION

A new, reversed-phase HPLC method has been developed for simultaneous analysis of Emtricitabine and Tenofovir Disoproxil Fumarate in a tablet formulation. It was

shown that, the method was linear, accurate, reproducible, repeatable, precise, selective and specific proving the reliability of the method.

The run time is relatively short, i.e.1min, which enable rapid determination of any samples in routine and quality control analysis of tablet formulations. The same solvent was used throughout the experimental work and no interference from any excipient was observed. Hence, the proposed method was successfully applied to analyze the tablet formulation containing Emtricitabine and Tenofovir Disoproxil Fumarate. In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost HPLC method that can be successfully applied for the determination of emtricitabine and tenofovir disoproxil fumarate pharmaceutical preparations without the interferences of other constituents in the formulations.

All the above methods do not suffer from any interference due to common excipients. Therefore it was shown that the proposed methods could be successfully applied to estimate commercial pharmaceutical products containing emtricitabine and tenofovir disoproxil fumarate. Thus the above studies and findings will enable the quantification of the drug for future investigation in the field of analytical chemistry.

ACKNOWLEDGEMENTS

In the first place we would like to record our gratitude to Dr. G. Nagarjuna Reddy, M. Pharm, Ph. D., Principal, KLR Pharmacy College, for extending his support. My sincere thank goes to K. Dhanalakshmi, HOD for Department of pharmaceutical analysis, KLR Pharmacy College, for his help in completion of my work.

REFERENCES

1. Budawari S, the Merck Index; 13th Edition, Merck and Co. Inc. Whitehouse Station. NJ, 2001.
2. Martindale: The Complete Drug Reference; 33 rd Edition, Pharmaceutical Press, London, 2002.
3. Gish RG, Trinh H, Leung N, Chan FKL, Fried ML, Wright TL, Wang C, Anderson J, Mondou E, Snow A, Sorbel J, Rousseau F, Corey L, HepatolJ. 2005; 43, 60.
4. Masho SW, Wang CL, Nixon DE: Review of tenofovir-emtricitabine. Ther Clin Risk Manag. 2007; 3(6):1097-104. Pubmed
5. Long MC, King JR, Acosta EP: Pharmacologic aspects of new antiretroviral drugs. Curr HIV/AIDS Rep. 2009; 6(1):43-50. Pubmed
6. Emtricitabine/tenofovir disoproxil fumarate. Drugs R D. 2004; 5(3):160-1. Pubmed
7. Goicoechea M, Best B: Efavirenz/emtricitabine/tenofovir disoproxil fumarate fixed-dose combination: first-line therapy for all? Expert Opin Pharmacother. 2007; 8(3):371-82. Pubmed.
8. Truvada® (Emtricitabine/Tenofovir Disoproxil Fumarate Tablets) Product monograph http://www.gilead.com/~/media/Files/pdfs/medicines/hiv/truvada/truvada_pi.pdf pg.27, 28.
9. Gnanarajan G, Gupta AK, Juyal V. A validated method for development of tenofovir as API and tablet dosage forms by UV Spectroscopy. 2009; 1(4):351-353.
10. Noel AG, Vikas, Vaidyaa V. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous determination of tenofovir and emtricitabine in human plasma and its application to a bioequivalence study. Journal of pharmaceutical and biomedical analysis. 2008; 489:918-926.
11. http://www.drugs.com/cdi/tenofovir.html
12. http://www.rxlist.com/cgi/generic/viread.html.
13. http://www.pdrhealth.com/drug_info/rxdrugprofiles/drugs/vir1605.shtml.
14. http://en.wikipedia.org/wiki/Tenofovir