IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

SIMULATNEOUS EVALUATION OF TENOFOVIR DISPROXIL FUMARATE AND EMTRICITABINE BY RP-HPLC METHOD IN PHARMACETICAL DOSAGE FORMS

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Abstract: **A simple, precise and accurate chromatographic method was developed for the estimation of Emtricitabine and Tenofovir Disoproxil Fumarate and validated as per ICH guidelines in bulk and formulation. The chromatographic parameters were selected as per the optimization are X bridge C18 (4.6×150mm) 5µ used as stationary phase, Methanol: Phosphate Buffer pH3 (60:40 % v/v) implied as eluent with the spurge flow of 1.0 mL/min and quantification was done at 252nm respectively. The elution times were found to be 2.664 and 3.801 min respectively for Emtricitabine and Tenofovir. The method was validated in the linear concentration range of 5-25 µg/mL of Emtricitabine and 20-100 µg/mL of Tenofovir correspondingly. At the end of analysis the method developed was analysed for accuracy and assay and were found to be with in the compendial limits stating the method can be further used for quality control analysis for both bulk and formulation using chromatographic methods.**

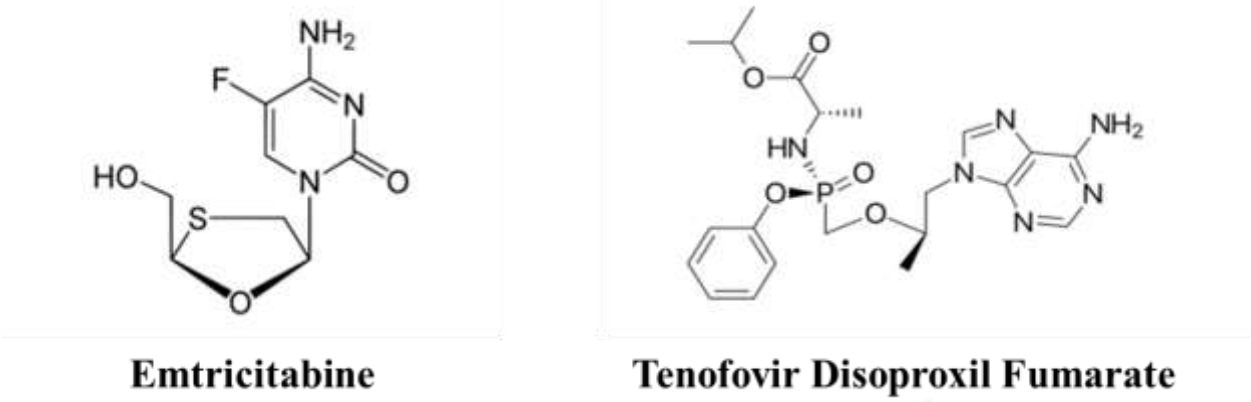
Key Words: Emtricitabine; Tenofovir Disoproxil Fumarate; RP-HPLC; Method validation

INTRODUCTION

Emtricitabine (FTC) is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. Emtricitabine is a cytidine analogue. The drug works by inhibiting HIV reverse transcriptase, preventing transcription of HIV RNA to DNA. Emtricitabine is a white to off-white powder[1-3]. Emtricitabine was found to be DMSO and Dimethyl Formamide, Freely soluble in methanol R and water R, Dichloromethane and Acetonitrile. Emtricitabine works by inhibiting reverse transcriptasem preventing transcription of HIV RNA to 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.

Tenofovir Disoproxil Fumarate (TDF) is a novel Tenofovir prodrug developed in order to improve renal safety when compared to the counterpart Tenofovir disoproxil.1 Both of these prodrugs were first created to cover the polar phosphonic acid group on Tenofovir by using a novel oxycarbonyloxymethyl linkers to improve the oral bioavailability and intestinal diffusion.8 Tenofovir Disoproxil Fumarate is an alanine ester form characterized for presenting low systemic levels but high intracellular concentration. It has been reported to produce a large antiviral efficacy at

doses ten times lower than Tenofovir Disoproxil. Tenofovir Disoproxil Fumarate is a white to off-white powder. Tenofovir Disoproxil Fumarate is freely soluble in methanol, soluble in ethanol, slightly soluble in DMSO, Acetonitrile. Tenofovir Disoproxil Fumarate has been shown to be a potent inhibitor of the hepatitis B virus replication [4-, 5].



Emtricitabine **Tenofovir Disoproxil Fumarate**

1.1 Structure of Emtricitabine and Tenofovir Disoproxil Fumarate

MATERIALS AND METHODS:

2.1 Materials:
Emtricitabine and Tenofovir Disoproxil Fumarate pure drugs (API), Combination purchased as Synthetic Formulation Tenof-EM of Hetero Healthcare, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem Pharmaceuticals.

2.2 Instruments:
Electronics Balance-Denver, pH meter -BVK enterprises, India, Ultrasonicator - BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Autosampler integrated with Empower 2 Software, UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10 mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Nivolumab and Cabozantinib solutions.

2.3 Methods:
2.3.1. Buffer Preparation: Phosphate buffer (pH-3)-Dissolve 0.9g of anhydrous di hydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000mL. Adjust the pH 3 by using ortho phosphoric acid**.**

2.3.2 Chromatographic conditions:
Chromatographic analysis was done using isocratic elution, the Stationary phase used was X bridge C18 (4.6×150mm) 5 µ, Mobile phase containing Methanol: Phosphate Buffer pH3 (60:40v/v), elutant was pumped through the column at a spurge flow of 1.0 mL/min. The temperature was upheld at 30°C. The optimized wavelength selected was 252.0 nm.

2.3.3 Selection of wavelength:
By Utilizing a PDA locator, the retention spectra of the arrangement of two drugs were examined in the UV light region 200-400nm spectra, the overlay of the two spectra consolidated at 260nm was chosen as the detection wavelength for the HPLC chromatographic technique.

2.3.4 Standard solution:
Accurately weigh and transfer 10 mg of Emtricitabine and 10mg of Tenofovir Disoproxil Fumarate working standard into a 10 mL clean dry volumetric flasks, add about 10 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6 mL of Emtricitabine and Tenofovir Disoproxil Fumarate from stock solution into a 10mL volumetric flask and dilute up to the mark with diluents.

2.3.5 Sample solution:
Accurately weigh 10 combination tablets crush in mortar and pestle and transfer equivalent to 10 mg of Emtricitabine, Tenofovir Disoproxil Fumarate (marketed formulation-dose of Emtricitabine is 15mg, Dose of Tenofovir Disoproxil Fumarate is 100mg in combination tablet) sample s

Method Validation

3.1 Linearity:
3.1.1 Preparation of Standard stock solutions: Accurately weigh and transfer 10 mg of Emtricitabine and 10mg of Tenofovir Disoproxil Fumarate working standard into a 10 mL clean dry volumetric flasks, add about 10 mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6 mL of Emtricitabine and Tenofovir Disoproxil Fumarate from stock solution into a 10mL volumetric flask and dilute up to the mark with diluents.

Linearity– I (5ppm of TDF & 20ppm of FTC): Pipette out 0.05mL of FTC and 0.2mL of TDF stock solutions was take in a 10mL of volumetric flask dilute up to the mark with diluent**.**

Linearity – II (10ppm of TDF & 40ppm of FTC): Pipette out 0.1mL of FTC and 0.4mL of TDF stock solutions was take in a 10mL of volumetric flask dilute up to the mark with diluent**.**

Linearity – III (15ppm of TDF & 60ppm of FTC): Pipette out 0.15mL of FTC and 0.6mL of TDF stock solutions was take in a 10mL of volumetric flask dilute up to the mark with diluent**.**

Linearity – IV (20ppm of TDF & 80ppm of FTC): Pipette out 0.2mL of FTC and 0.8mL of TDF stock solutions was take in a 10mL of volumetric flask dilute up to the mark with diluent.
Linearity – V (25ppm of TDF & 100ppm of FTC): Pipette out 0.25mL of FTC and 1.0mL of TDF stock solutions was take in a 10mL of volumetric flask dilute up to the mark with diluent.)

3.2 Accuracy:

3.2.1 Preparation of Sample stock solutions: 10 tablets of synthetic formulation were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet (200mg) was transferred into a 500 mL volumetric flask, and 50 mL of diluents were added, and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (480µg/mL of FTC and 80 µg/mL of TDF).

3.2.2 Preparation of Spiked Solution: 0.5mL, 1.0mL and 1.5mL of sample stock solution was transferred into a 10mL volumetric flask individually, to which each 1.0mL of the standard solution was spiked to make up the accuracy level of 50, 100 and 150% respectively and made up the solution with diluent.

Introduce the recovery samples at varying levels, determine the amount of Emtricitabine and Tenofovir added, and then compute the individual and average recovery results. The analysis was done in triplicates. The solutions were filtered using a 0.45µm membrane. Recorded the chromatograms and evaluated the peak responses.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102 %

3.3 Robustness: Modest intentional changes in a procedure, such as flow rate, mobile phase ratio, and temperature, are made, but there are no recognized changes in the results, and they are within range as per ICH Guidelines.. Robustness conditions like Flow rate ± 0.1 mL/min i.e., 0.9mL/min and 1.1mL/min, mobile phase 40B:60A, 30B:70A, temperature ± 5° C i.e., 25°Cand 35°C was upheld and samples were injected in duplicate manner. System suitability characteristics were not significantly impacted, and all parameters were passed. %RSD was within the limit.

3.4 LOD and LOQ sample Preparation: Individually 0.25mL of the standard solution was carefully transferred into a 10Ml standard flask and made up the mark with the respective diluent, from which 0.3mL of the solution was further diluted to the same 10mL and estimated for the detection and quantification limits

RESULTS AND DISCUSSION

4.1 System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines. FTC and TDF were eluted at 2.243 min and 2.953 min respectively with good resolution. The plate count and tailing factor were very satisfactory, so this method was optimized and validated. All the system-suitable parameters were passed and were within the limits.

Table 1: System suitability parameters for Emtricitabine and Tenofovir Disoproxil Fumarate

S.NO	*Emtricitabine*			*Tenofovir Disoproxil Fumarate*			
Injection	*RT (min)*	*USP Plate count*	*Tailing*	*RT (min)*	*USP Plate count*	*Tailing*	*Resolution*
1	2.669	4925	1.21	3.855	8541	0.92	3.6
2	2.661	4927	1.27	3.841	8617	0.97	3.6
3	2.668	4215	1.24	3.846	8124	0.92	3.5
4	2.612	4931	1.29	3.859	8624	0.95	3.7
5	2.671	4231	1.25	3.855	7967	0.94	3.6
6	2.629	4706	1.24	3.857	8046	0.92	3.6

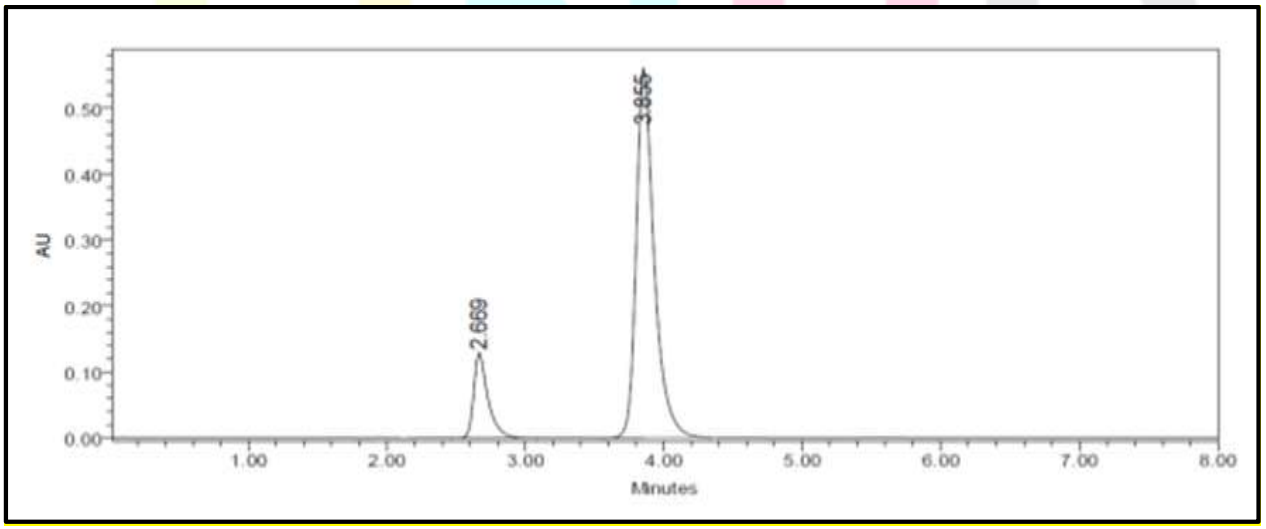
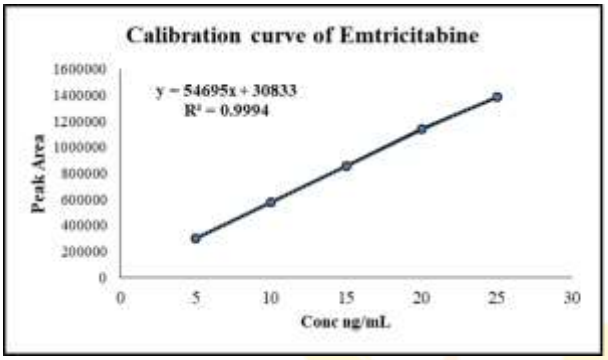


Fig. 1. System suitability Chromatogram Emtricitabine and Tenofovir Disoproxil Fumarate

4.2 Specificity:
In this test procedure, the placebo, sample, and standard solution were examined separately to identify undesired interferences. There was no placebo effect on the standard peak. Hence, the strategy is provided based on the specificity research observation.

4.3 Linearity:
Six linear concentrations of Emtricitabine (5-25 µg/mL) and **Tenofovir Disoproxil Fumarate** (20-100 µg/mL) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for FTC was y = 54695x + 30833 and for TDF was y = 81975x + 301068. The correlation coefficient obtained was 0.999 for both drugs.



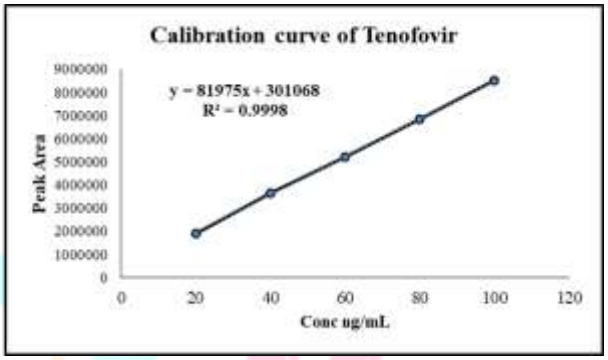


Fig. 2. Standard curve of FTC **Fig. 3. Standard curve of TDF**

4.4 Precision:
System precision: Six injections were made from the same volumetric flask of working standard solution, and the results are shown below. The average area, standard deviation, and percentage RSD were computed for two medicines. Emtricitabine and Tenofovir had % RSD values of 0.4% and 0.8%, respectively. This procedure passed the system precision limit since % RSD was less than 2.
Repeatability and Inter-day Precision: The Mean, Standard deviation, and %RSD were calculated and mentioned in the below table.
4.5 Accuracy: Three levels of Accuracy samples were prepared by the standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 98.0 %, 99.2 %and 99.3 % for FTC and 98.50%, 99.09%, and 100.36% for TDF respectively.
4.6 Sensitivity: The method was evaluated according to the US FDA guidelines, and the LOD and LOQ concentrations for Emtricitabine are 0.45 and 1.67 and the LOD and LOQ concentrations for Tenofovir are 0.92 and 3.24.

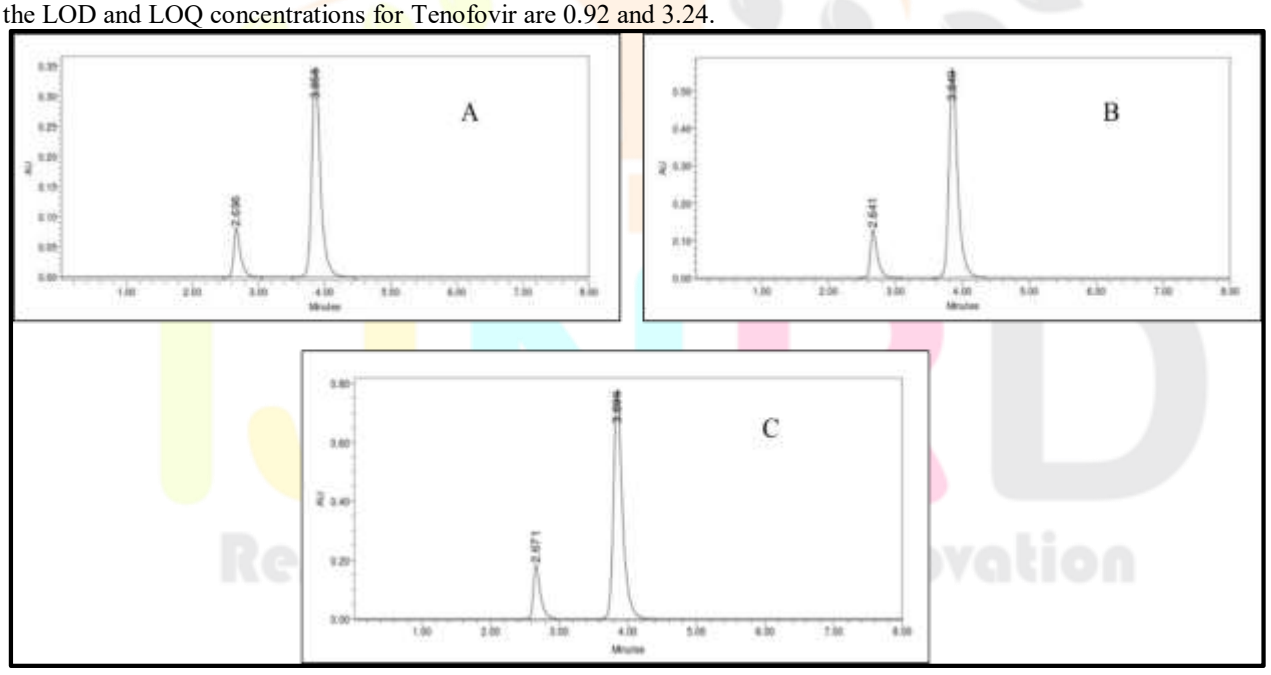


Fig 5: Accuracy Chromatograms of Emtricitabine and Tenofovir Disoproxil Fumarate at 50 %, 100 % and 150 % respectively

4.7 Robustness: Robustness conditions like Flow rate ± 0.1 mL/min i.e., 0.9mL/min and 1.1mL/min, mobile phase 40B:60A, 30B:70A, temperature ± 5° C i.e., 25°Cand 35°C was upheld and samples were injected in duplicate manner. System suitability characteristics were not significantly impacted, and all parameters were passed. %RSD was within the limit.
4.8 Assay: Pool and weigh 10 tablets, smash in a mortar and pestle, and transfer equivalent to 10 mg of Emtricitabine and Tenofovir Disoproxil Fumarate. The percentage purity of Emtricitabine and Tenofovir Disoproxil Fumarate in pharmaceutical dosage form was discovered to be 100.01% and 100.33%, respectively.

Table 2: System Precision, Repeatability, and Inter-day Precision Table of FTC and TDF

S.NO	System Precision (Area)		Method Precision (Area)	
(N=6)	FTC	TDF	FTC	TDF
I	918296	4940174	918296	5040174
II	908296	4951174	918356	5046151
III	907194	4942175	918247	5053141
IV	909291	4840174	918636	5076521
V	908296	4950176	919578	5063147
VI	908458	4942312	918456	5071563
Mean	909971.8	4927698	918622.6	50558270
S.D	4132.316	43117.6	554.9295	14384.71
% RSD	0.45	0.87	0.60	0.28

Table 3: LOD and LOQ estimation of FTC and TDF

Molecule	*LOD (µg/mL)*	*LOQ (µg/mL)*
Emtricitabine	0.45	1.67
Tenofovir	0.92	3.24

Table 4: Robustness data for Emtricitabine and Tenofovir Disoproxil Fumarate

S.No	*Condition*	*% RSD of FTC*	*% RSD of TDF*
1	Flow rate (-) 0.9mL/min	0.71	0.62
2	Flow rate (+) 1.1mL/min	0.93	0.88
3	Mobile phase (-) 40B:60A	1.15	1.42
4	Mobile phase (+) 30B:70A	0.82	1.09
5	Temperature (-) 25°C	0.45	0.59
6	Temperature (+) 35°C	0.60	0.37

Table 4: Assay Data of Emtricitabine and Tenofovir Disoproxil Fumarate

S.No	*FTC*			*TDF*		
	Std. Area	*Spl. Area*	*% Purity*	*Std. Area*	*Spl. Area*	*% Purity*
I	918296	918296	100.00	5041296	50401746	99.98
II	918482	919583	100.11	5040174	5091294	101.01
III	918215	917426	99.91	5040154	5040215	100.00
Mean	918331	918435	**100.01**	5040541	5057228	**100.33**
S.D	136.90	1085.20	0.10	653.64	29502.32	0.59
% RSD	0.01	0.12	0.10	0.01	0.58	0.59

CONCLUSION:

Chromatographic estimations have been wide spread as it has been very refined tool in recent times for the estimation of pharmaceutical preparations. The final outcome was a new robust and accurate method developed for the estimation of Emtricitabine and Tenofovir. The method was strictly validated as per the ICH guidelines and found to be precise, accurate and the recovery studies shows were within limits. The detection and quantification limits also prove the sensitivity of the method without any trace of interference. Hence, the developed estimate can be widely used for the estimation of Emtricitabine and Tenofovir in routine analysis.

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