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Research Article

## Development and Validation of RP-HPLC Method for Simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in Bulk and Tablet Dosage Form

Nachiket S Dighe\*, Ganesh S Shinde, Sagar. D. Magar, Ankita V. Deodhe

Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravaranagar. Tal-Rahata, Dist.- Ahmednagar, 413736, India (MS)

### ABSTRACT

An analytical method was developed for estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in drug product by using high performance liquid chromatography. The chromatographic separation was performed on column Cosmosil C18 (250mm x 4.6ID, Particle size: 5micron) using methanol: water (70:30 v/v) as mobile phase with flow rate of 0.8 ml/min. The detection was carried by using UV-300-M detector at 266 nm wavelength. The retention times of Emtricitabine and Tenofovir Disoproxil Fumarate were found to be 3.561 and 5.478 min respectively. Run time was observed as 5.54 min and 7.05 min respectively for Emtricitabine and Tenofovir Disoproxil Fumarate. Validation was performed as per ICH guidelines which include accuracy, precision, linearity, specificity, LOD, LOQ, ruggedness and robustness all of these were found within standard range as per ICH norms. Preparation of mobile phase is very simple & does not require lengthy procedure. Proposed method was found to be accurate and specific for routine quality control of pharmaceutical dosage form like bulk and tablet dosage form.

**Keywords:** Emtricitabine, Tenofovir Disoproxil Fumarate, HPLC, Validation.

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### \*Address for Correspondence:

Dr. Nachiket S. Dighe, Associate Professor & HOD, Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravaranagar, A/P- Loni Bk. Taluka -Rahata, Dist-Ahmednagar 413736, India (MS).

### INTRODUCTION

One of the deadliest and unmanageable chronic health catastrophes is HIV/AIDS. Fixed dose combinations(FDCs) form the main stay in clinical management of HIV-1 infection as they offer several advantages over single products with respect to storage, prescribing, dispensing, patient use, consumption and disease management.<sup>1</sup> Emtricitabine (EMT) is the most important nucleoside reverse transcriptase inhibitor(NRTI)used for the treatment of HIV infection in adults. Emtricitabine acts as an analogue of cytidine. By using this drug to inhibiting the reverse transcriptase and the enzyme that copies human immune deficiency virus RNA into novel viral Deoxyribo nucleic acid.<sup>2</sup> Emtricitabine may divide down measure of HIV in blood (viral weight). Emtricitabine may in like way escalate measure of T cells called CD4 cells. Hacking down measure of HIV in blood severs down probability of death that happen when your protected structure is slight (entrepreneurial defilements).

Emtricitabine can cut down measure of HIV or viral weight, in patient's body and can indirectly increase cd4 cells. both of these headways are connected with more important safe structure and decrease probability of guaranteed contamination.<sup>3</sup> chemical name of Emtricitabine is 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one

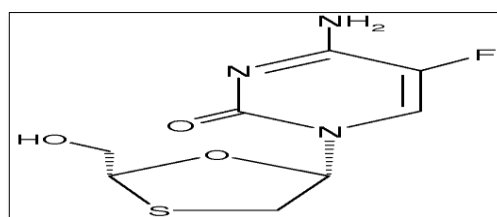


Figure No.1: structure of Emtricitabine

Molecular formula  $C_8H_{10}FN_3O_3S$  with Molecular weight-247.24 g/mol. Emtricitabine Soluble in Acetonitrile & methanol and slightly soluble in water and Appearance is White to off white powder<sup>4</sup>. Tenofovir Disoproxil Fumarate(TDF) belongs to class of antiretroviral drugs known as nucleotide reverse transcriptase inhibitor, analogue of adenosine <sup>5</sup>. Tenofovir Disoproxil Fumarate is fumaric acid salt of the bisisopropoxycarbonyl-oxymethyl ester derivative, Tenofovir. Tenofovir Disoproxil fumarate is a fumarate salt prepared from equimolar amount of Tenofovir Disoproxil and fumaric acid.<sup>6</sup>Chemical Name is - (2E)-but-2-enedioic acid; bis{[(propan-2-yloxy) carbonyl] oxy} methyl} {[[2R]-1-(6-amino-9H-purin-9-yl) propan-2-yl] oxy} methane phosphonate.

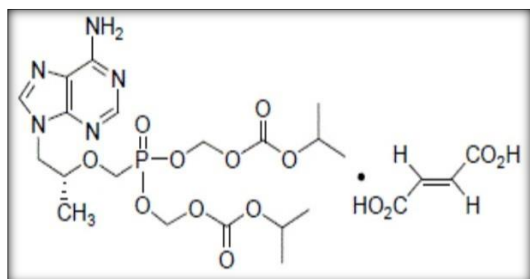


Figure No.2: Tenofovir Disoproxil fumarate

Molecular formula  $C_{23}H_{34}N_5O_{14}P$  with Molecular weight-635.52g / mol. Tenofovir Disoproxil fumarate Soluble in methanol, slightly soluble in water, very slightly soluble in dichloromethane & sparingly soluble in acetone and Appearance is White to off-white crystalline powder.<sup>7</sup> The method developed is precise, simple and precise RP-HPLC method to determine Emtricitabine and Tenofovir Disoproxil Fumarate in pharmaceutical dosage forms.

## MATERIAL AND METHOD

### Chemical and solvent

Emtricitabine and Tenofovir Disoproxil Fumarate were Obtained as gift samples from LUPIN Pharmaceutical Ltd, Pune, India. Solvents will be made available commercially from Pharmaceutical Lab in Pune, India

### Selection of Mobile phase

For effective separation and in order to determine optimum Chromatographic Conditions mixture of different solvent were tried initially to find the ideal mobile phase. Finally,

mobile phase was selected as Methanol: Water (70:30 v/v). This mobile phase gave high resolution and minimal tailing. Methanol has better solubility in combination with water and gave lesser column backpressure compared to acetonitrile.

### Preparation of Mobile phase

Mobile phase was prepared by mixing 70ml of methanol and 30ml water. mobile phase was filtered through 0.45 $\mu$  Membrane filter and then it was ultra-sonicated for 30 minutes

### Preparation of standard stock solution

An accurately weighted 10mg of pure drug of Emtricitabine and Tenofovir Disoproxil Fumarate and separately transferred to 10 ml volumetric flask. both the drug was dissolved in 10 ml of solvent used as mobile phase. With shaking and then volume Was made up to the mark to get 1000ppm of standard Stock solution of individual drug. It was ultra-sonicated and filtered through 0.20  $\mu$  membrane filter

### Selection of analytical wavelength

Dilutions are prepared from standard stock solutions of Emtricitabine and Tenofovir Disoproxil Fumarate, were scanned separately used for wavelength detection as well as simultaneous determination of drugs before RP-HPLC Method, it is also for solubility studies. The isobastic point was found to be 266 nm

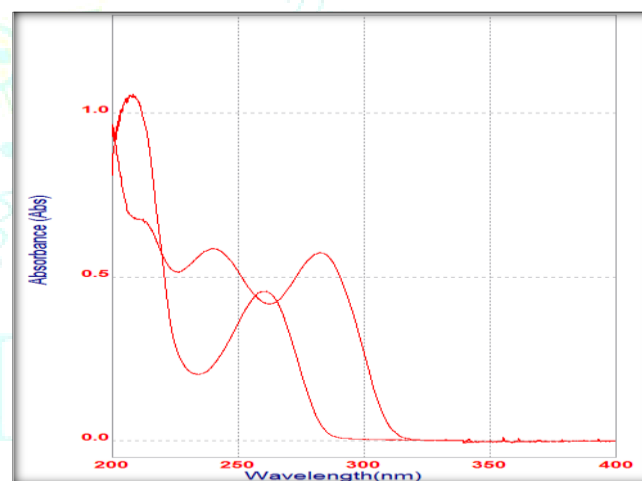


Figure No.3: Overlain spectrum of Emtricitabine and Tenofovir Disoproxil Fumarate.

## System Suitability Parameters

Table No. 1 – Parameters of System Suitability

Sr. No	Parameters	Emtricitabine	Tenofovir Disoproxil Fumarate
1	Retention time (min)	3.561	5.478
2	Resolution (R)	6.47	
3	No of theoretical plate (N)	8219	7514
4	Tailing factor	1.04	

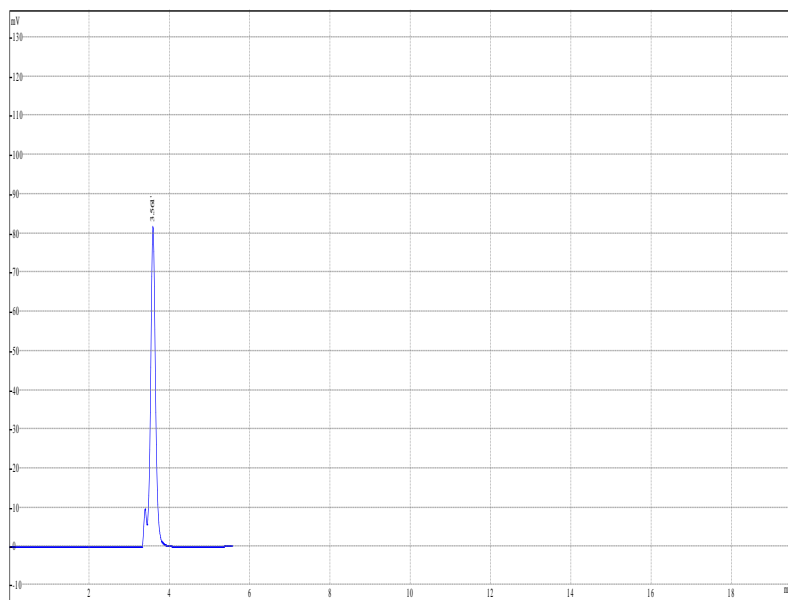


Figure No.4 - Chromatogram of Emtricitabine with their retention time

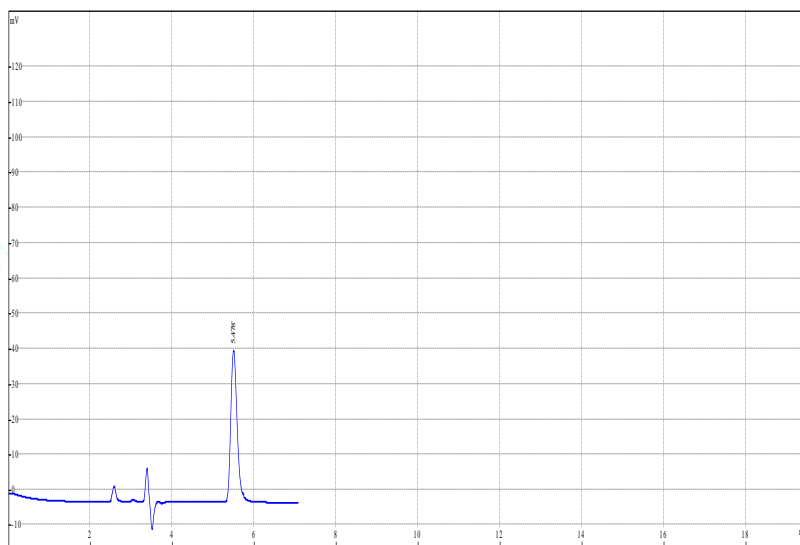


Figure No. 5 - Chromatogram of Tenofovir Disoproxil Fumarate with their retention time

**Assay preparation of Tablet formulation:**

20 tablets were accurately weighed and average weight was determined. Powdered tablet equivalent to 200 mg of Emtricitabine and was transferred into a 200 ml volumetric flask. 10 ml of water was added and sonicated for 10 minutes to disperse the tablet powder. Then 130 ml of methanol was added and sonicated for 25 minutes with

intermittent shaking. The volume was made upto mark with methanol after cooling and mixed well, filtered through 0.45 μ nylon filter. First 2 ml of the filtrate was discarded and 5 ml of this filtrate was diluted to 50 ml with diluent and mixed well to get concentration of Emtricitabine as about 200μg/ml, Tenofovir Disoproxil Fumarate 300μg/ml, The blank, standard and sample solutions were injected and result are given in table no 1

Table No. 2– Assay Data of Emtricitabine and Tenofovir Disoproxil Fumarate.

Sr no	parameters	Drug	
		Emtricitabine	Tenofovir Disoproxil Fumarate
1	Label claim (%/tab)	200 mg	300 mg
2	Drug content(%)	200.14	300.8
3	% RSD	0.14	0.20

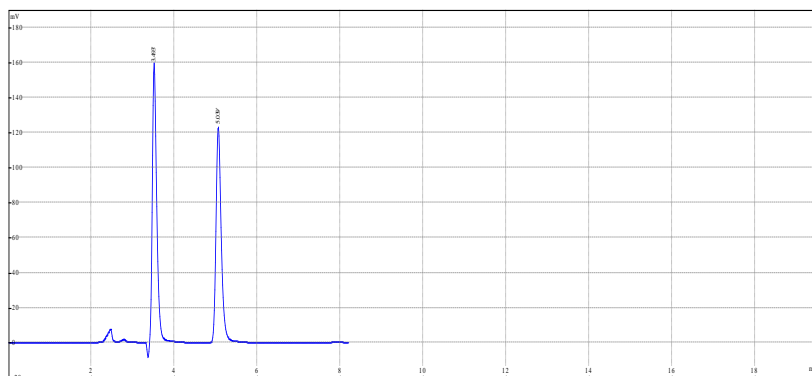


Fig.No.6- Chromatogram of Emtricitabine and Tenofovir Disoproxil Fumarate.

**Validation.**

The method was validated for Linearity, precision, accuracy, Robustness, Ruggedness, limit of detection, limit of quantification.

A calibration curve was obtained by plotting peak area on Y-axis and concentration on X-axis. linearity OF Emtricitabine and Tenofovir Disoproxil Fumarate was found to be in the range of 10-50µg/ml and 15-75µg/ml. correlation coefficient value was found to be 0.9994 and 0.9995 for Emtricitabine and Tenofovir Disoproxil Fumarate.

**A. Linearity**

Table 3: Linearity data of Emtricitabine and Tenofovir Disoproxil Fumarate

Concentration of Emtricitabine (µg/ml)	Peak area	Concentration of Tenofovir Disoproxil Fumarate (µg/ml)	Peak area
10	359283	15	283852
20	878147	30	766242
30	1416658	45	1296985
40	1979742	60	1838271
50	2567232	75	2373994

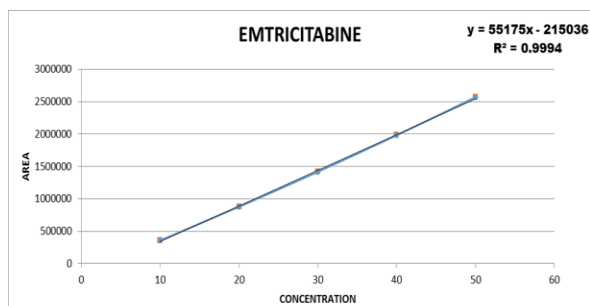


Figure No.7 - linearity graph for Emtricitabine

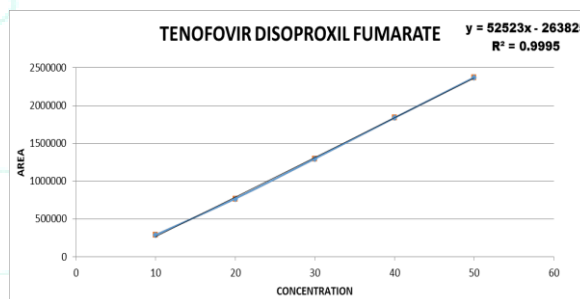


Figure No.8- linearity graph for Tenofovir Disoproxil Fumarate

**B. Accuracy**

The accuracy expresses the closeness of agreement between the true value which is accepted either as a conventional true value or an accepted reference value and the value found. Recovery study was performed by standard addition method by adding the known amount of Emtricitabine, Tenofovir , Disoproxil Fumarate (Reference standard) to the placebo at

three different concentration levels i.e 50%, 100% and 150% of assay concentration and % recovery for all these drug were calculated.% Recoveries Emtricitabine, Tenofovir Disoproxil Fumarate were found in the range of 100.9-101.4 %, and 100.0-100.99% respectively(RSD < 2) .

Table No. 4 - Accuracy data of Emtricitabine

Sr no	Level of % Recovery	Weight Taken (mg)	Mg Recovered	% recovery	% mean recovery
Emtricitabine	50	100	101.74	101.8	101.4
	100	200	204.79	101.05	
	150	300	300.07	100.9	
Tenofovir Disoproxil Fumarate	50	150	151.18	101.7	100.7
	100	300	300.81	100.9	
	150	450	450.54	100.1	

### C. Precision

#### Inter-day Precision

The relative standard deviation for inter-day was found to be less than 2%. The %RSD of inter-day was 0.18% for Emtricitabine and 0.20 for Tenofovir Disoproxil Fumarate

Table No. 5 – Inter-day Variability for Emtricitabine and Tenofovir Disoproxil Fumarate

Emtricitabine		Tenofovir Disoproxil Fumarate.	
Day 1	Area	Day 1	Area
	1416658		1296985
	1420559		1297677
	1422385		1298510
Day 2	1419698	Day 2	1294590
	1418601		1295669
	1415587		1291530
Mean	1418915	Mean	1295827
% RSD	0.18%	% RSD	0.20%

#### Intraday Precision

The relative standard deviation for intraday was found to be less than 2%. The %RSD of intraday was 0.26% for Emtricitabine and 0.42% for Tenofovir Disoproxil Fumarate.

Table No. 6 – Intraday Variability for Emtricitabine and Tenofovir Disoproxil Fumarate

Emtricitabine		Tenofovir Disoproxil Fumarate.	
Morning	Area	Morning	Area
	141665		1296985
	1420559		1297677
	1422385		1298510
Evening	1412145	Evening	1297137
	1415669		1308683
	1418339		1291939
Mean	1417625.8	Mean	1298488.5
% RSD	0.26%	% RSD	0.42%

### D. Robustness

Robustness is the measure of a method by deliberate changes in chromatographic condition such as change in PH, flow rate, wavelength. but result remain unaffected.

Robustness was found to be satisfactory within the range. % RSD at different pH was found to be 0.268647394 for Emtricitabine and 0.282261806 for Tenofovir Disoproxil Fumarate. % RSD at different Wavelengths was found to be 0.158228626 for Emtricitabine and 0.151856765 for Tenofovir Disoproxil Fumarate.

#### a. At different pH

Table No. 7: Robustness data of Emtricitabine & Tenofovir Disoproxil Fumarate at different Ph

Drug Sample	PH	Area	Mean	SD	% RSD
Emtricitabine	Not adjust pH	878147			
	6.4	879413	877469	2357.298	0.268647394
	7.0	874847			
Tenofovir Disoproxil Fumarate	Not adjust pH	766242			
	6.4	763461	765807	2161.581	0.282261806
	7.0	767718			

#### b. At Different Wavelengths

Table No. 9 – Robustness data of Emtricitabine and Tenofovir Disoproxil Fumarate at different Wavelength

Drug Sample	Wavelength (nm)	Area	Mean	SD	% RSD
Emtricitabine	266	878147	878790.3333	1390.497872	0.158228626
	264	877838			
	268	880386			
Tenofovir Disoproxil Fumarate	266	766242	767467	1165.450557	0.151856765
	264	767597			
	268	768562			

### E. Ruggedness -

It is the degree of reproducibility of test results obtained by analysing the same sample under variety of normal test conditions such as different analysts, days, Sinstruments, reagents and column i.e. lack of influence of environmental variables on the method.

Table No. 10 – Ruggedness data of Emtricitabine and Tenofovir Disoproxil Fumarate

Concentration Of Emtricitabine (µg/ml)	Area	Concentration Tenofovir Disoproxil Fumarate. (µg/ml)	Area
10	360371	15	285208
20	881213	30	772702
30	1423333	45	1308968
40	1981950	60	1843778
50	2565314	75	2379853

### F. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection was found to be 0.182 µg/ml for Emtricitabine and 0.132 µg/ml for Tenofovir Disoproxil Fumarate. Limit of Quantitation was found to be 0.551 µg/ml for Emtricitabine and 0.401 µg/ml for Tenofovir Disoproxil Fumarate. Result are summarized in below table

Table No. 11 – Limit of Detection (LOD) for Emtricitabine and Tenofovir Disoproxil Fumarate

Sr. No	Name	Standard deviation	Slope	LOD
1	Emtricitabine	3038.45	55175	0.182 µg/ml
2	Tenofovir Disoproxil Fumarate	2104.17	52523	0.132 µg/ml

Table No. 12 – Limit of Quantitation (LOQ) for Emtricitabine and Tenofovir Disoproxil Fumarate

Sr. No	Name	Standard deviation	Slope	LOQ
1	Emtricitabine	3038.45	55175	0.551 µg/ml
2	Tenofovir Disoproxil Fumarate	2104.17	52523	0.401 µg/ml

### CONCLUSION

The present study describes a simple and accurate RP HPLC-method for the simultaneous determination of Emtricitabine & Tenofovir Disoproxil Fumarate in pharmaceutical dosage form. As per ICH guidelines all the outcomes of validations parameters are falling within the standard limits for individual parameters. No interference of the excipients was observed in formulation. It is concluded that this method exhibits high resolution with minimum tailing.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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