

A Validated RP - HPLC Method for Simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in a Tablet Dosage Form

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Abstract

A simple, rapid reversed-phase high performance liquid chromatographic method had been developed and validated for estimation of emtricitabine and tenofovir disoproxil fumarate in tablet dosage form. The estimation was carried out on Luna C₁₈ (25cm x 4.60 mm, particle size 5µm) column with a mixture of acetonitrile: potassium dihydrogen phosphate buffer (pH 3.0 ± 0.05 adjusted with orthophosphoric acid): triethylamine in the ratio of 70:30:0.5(v/v) as mobile phase. UV detection was performed at 260 nm. The method was validated for linearity, accuracy, precision, specificity and sensitivity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form. The retention time was 1.78 and 2.27 min. for emtricitabine and tenofovir disoproxil fumarate respectively and total run time was 4 min. at a flow rate of 1.5 mL min⁻¹. The calibration curve was linear over the concentration range of 5-50 µg mL⁻¹ for emtricitabine and 5-50 µg mL⁻¹ for tenofovir disoproxil fumarate. The LOD and LOQ values were found to be 0.015 and 0.045 µg mL⁻¹ for emtricitabine and 0.039 and 0.117 µg mL⁻¹ for tenofovir disoproxil fumarate respectively. 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 tablet dosage form.

Keywords:

Emtricitabine; Tenofovir disoproxil fumarate; RP-HPLC; Validation

1. Introduction

Emtricitabine (FTC) is a nucleoside reverse transcriptase inhibitor (NRTIs). Chemically it is 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (Fig.1). FTC is the (-) enantiomer of thio analog of cytidine which differs from other cytidine analogs, in that it has a fluorine in 5th position. FTC is an antiviral agent used for the prevention of perinatal HIV-1 reverse transcriptase [1]. It is also active against Hepatitis B virus [2, 3].

Tenofovir disoproxil Fumarate (TDF) is fumaric acid salt of the bisisopropoxycarbonyloxymethyl ester derivative of tenofovir. Chemically it is 9-[(R)-2-[[[isopropoxycarbonyl]oxy]methoxy]phosphiny] methoxy]propyl]adeninefumarate [1]. Fig.1 show the nucleotide reverse transcriptase inhibitor (NtRTIs) used in combination with other antiretrovirals for the treatment of HIV infection [2].

Both the drugs are not official in any of the pharmacopoeias. These are listed in the Merck Index and Martindale: The complete drug reference. Literature survey reveals that few

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RP-HPLC [4,5,6] methods are reported for estimation of FTC, TDF and efavirenz in pharmaceutical formulation. TDF is estimated individually by UV [7], derivative-HPLC [8], Plasma RP-HPLC [9,10] and Plasma LC/MS/MS [11,12,13] methods. Similarly for FTC, HPLC with Fluorometric detection [14] in human plasma and Stability indicating liquid chromatographic [15] methods are reported. RP-HPLC [16] and LC-MS/MS [17] method is reported for simultaneous estimation of FTC and TDF in human plasma. HPTLC [18] is reported for simultaneous estimation of FTC and TDF in pharmaceutical formulation. The purpose of this study was to develop simple, rapid, precise and accurate RP-HPLC method for the simultaneous estimation of both the drugs in combined tablet dosage form.

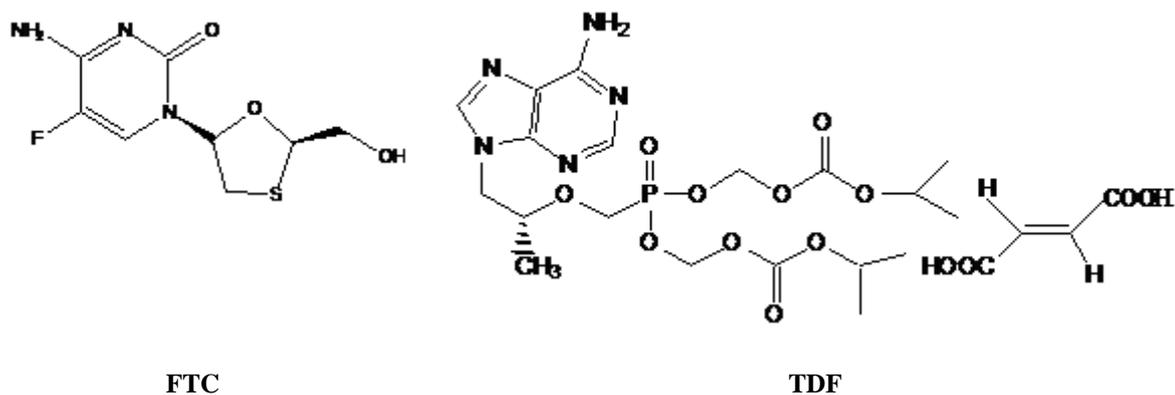


Fig1. The chemical structures of FTC and TDF

2. Experimental

2.1. Apparatus

RP-HPLC was performed with a Shimadzu LC-10 AT VP solvent-delivery system, a Shimadzu SPD-10 AVP UV-visible photodiode-array detector, DGA-12A degasser and a Rheodyne 7725i universal loop injector of injection capacity 20 μ L. The monitoring software was CLASS-LC 10 version 1.6. The equipment was controlled by a PC workstation. Compounds were separated on a 25 cm \times 4.6 mm i.d, 5- μ m particle, Phenomenex Luna C₁₈ column under reversed-phase partition chromatographic conditions. Ultrasonicator Model USB 30 was used. The work was carried out in an air-conditioned room maintained at temperature 25 \pm 2 $^{\circ}$ C. The flow rate was 1.5 mL min⁻¹, analytes were monitored at 260 nm and run time was 4 min.

2.2. Chemicals and Reagents

Working Standards of pharmaceutical grade FTC and TDF were obtained as gift samples from Ranbaxy Laboratories. Ltd, Dist.-Sirmour, Himachal Pradesh. The tablet dosage form, TAVIN-EM, manufactured by Hetero Drugs Limited, Hyderabad, India (Label claim: FTC 200 mg and TDF 300 mg), was procured from the local pharmacy. All the chemicals and reagents used were of HPLC grade and purchased from Spectrochem, Mumbai, India.

2.3. Mobile phase

The mobile phase selected was acetonitrile: potassium dihydrogen phosphate buffer (pH 3.0 \pm 0.05 adjusted with orthophosphoric acid) in the ratio of 70:30(v/v), 0.5 mL triethylamine was added in buffer to sharpen the peak and before analysis mobile phase was degassed.

2.4. Standard stock solution and Construction of Calibration curve

Standard stock solution of FTC and TDF (10 mg) each were prepared separately in 100 mL of mobile phase to get the final concentration of $100 \mu\text{g mL}^{-1}$.

From the standard stock solution of drugs, different dilutions were prepared, injected and their peak area was measured. After that, calibration curves were drawn between concentration against their respective area for FTC and TDF respectively. Unknown samples were determined by reference to these calibration curves.

2.5. Standard mixture solution

Mixed standard analysis was performed to validate the procedure. From the standard stock solutions of the drugs, different mixed standard solutions of 5:30, 10:25, 15:20, 20:15, 25:10, 30:5 of FTC and TDF respectively were prepared and analyzed, statistical results were within the range of acceptance i.e. %COV<2.0 and S.D.<1.0.

2.6. Sample preparation

For analysis of the tablet dosage form, twenty tablets (TAVIN-EM) were weighed individually and their average weight was determined. The tablets were then crushed to a fine powder and powder equivalent to the weight of 10 mg of TDF was transferred to a 100 mL volumetric flask and dissolved in about 30 mL of mobile phase. The solution was shaken for 5 min. and then ultrasonicated for 15-20 min. and filtered through Whatman # 41, and the residue was washed with mobile phase, and the combined filtrate was made up to the mark with the same solvent to get the final concentration of $100 \mu\text{g mL}^{-1}$. The solutions (20 μL) were then injected for quantitative analysis. The identities of both the compounds were established by comparing retention time of the sample solution with those of standard mixed solution. The amount of FTC and TDF per tablet was calculated by extrapolating the peak area from the calibration curve. The results are reported in Table 1.

Table 1. Assay of Tablet Formulation

Drug	TAVIN- EM Label claim (mg tab ⁻¹) (n=6)	Amount found		S.D.	% COV	S.E.
		mg	%			
FTC	200	200.18	100.09	0.1245	0.1244	0.0508
TDF	300	300.12	100.04	0.0632	0.0632	0.0258

S.D.: standard deviation, COV: coefficient of variance, S.E.: standard error.

3. Result and Discussion

3.1. HPLC method development and optimization

Column chemistry, solvent type, solvent strength, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so that the components were not interfered from the solvent and excipients.

After trying column C₈ and C₁₈, the final choice of stationary phase giving satisfactory resolution and run time was the reversed phase column Luna C₁₈. Mobile phase and flow rate selection was based on peak parameters (height, area, tailing, theoretical plates, capacity

factor and resolution) and run time. The best result was obtained by use of 70:30 (v/v) ratio of acetonitrile and potassium dihydrogen phosphate (pH 3.0 ± 0.05 adjusted with orthophosphoric acid) with 1.5 mL min^{-1} . From the overlain UV spectra (Shimadzu-1700), suitable wavelength considered for monitoring the drugs was 260 nm (Fig 2). Solutions of FTC and TDF in diluent 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 analytes absorbed well at 260 nm. The chromatogram of placebo and standard mixture is shown in Fig 3 and 4 respectively.

Under the optimum chromatographic conditions, the retention time obtained for FTC and TDF were 1.78 and 2.27 min. respectively for sample preparation shown in Fig 5. The result of capacity factor, tailing factor, theoretical plate number and resolution are reported in Table 2.

The values obtained for these properties ($1 < k < 10$, $R_s > 2$) shows that, the chromatographic conditions are appropriate for separation and determination of compounds.

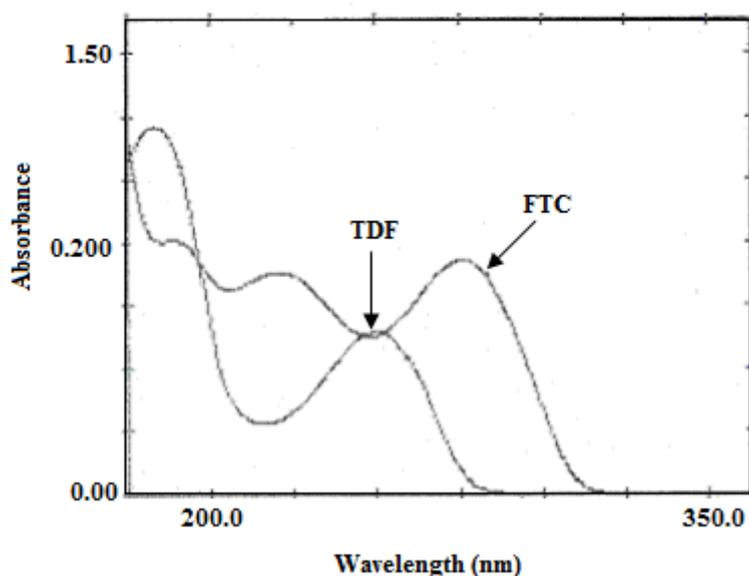


Fig 2. Overlain Spectra of FTC and TDF

Table 2. System suitability parameters

Property	FTC	TDF
R_t	1.78	2.27
T_f	1.33	1.38
K'	1.45	1.80
N	5116	4481
R_s	3.33	

R_t -retention time; T_f - tailing factor; k' - capacity factor; N - number of theoretical plates; R_s . resolution

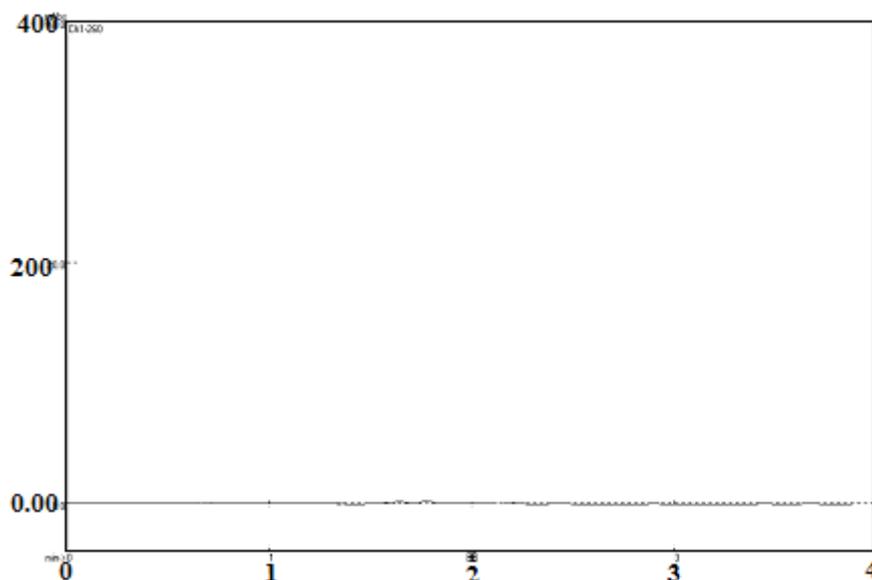


Fig 3. Chromatogram of Placebo

3.2. Validation of the developed method

The method was validated for linearity, accuracy, precision, repeatability, selectivity and specificity study as per ICH norms [19]. All the validation studies were carried out by replicate injection of the sample and standard solutions.

3.3. Linearity

Linearity was found to be $5-50 \mu\text{g mL}^{-1}$ for FTC and $5-50 \mu\text{g mL}^{-1}$ for TDF. The linear regression equations for FTC and TDF were-

$$\text{FTC} \quad y = 17492x + 2429.8 \quad (n=6, r^2 = 0.9995)$$

$$\text{TDF} \quad y = 14277x + 13551 \quad (n=6, r^2 = 0.9986)$$

Where y is response (peak area) and x is the concentration.

3.4. Accuracy

Accuracy of developed method was confirmed by doing recovery study as per ICH norms at three different concentration levels 80%, 100% and 120% by replicate analysis ($n=3$). The result of accuracy study was reported in Table 3. From the recovery study it was clear that the method is very accurate for quantitative estimation of FTC and TDF in tablet dosage form as all the statistical results were within the range of acceptance i.e. %COV < 2.0 and S.D. < 1.0.

3.5. Precision, Limit of Detection, and Limit of Quantitation

The concentrations of both the drugs were measured three times on the same day at intervals of 1 h and on three different days for intra and interday study respectively. The limits of detection and quantitation, LOD and LOQ, were calculated by use of the equations $\text{LOD} = 3.3\sigma/S$ and $\text{LOQ} = 10\sigma/S$, where σ is the standard deviation of the blank and S is the slope of the calibration curve. The results are reported in Table 4.

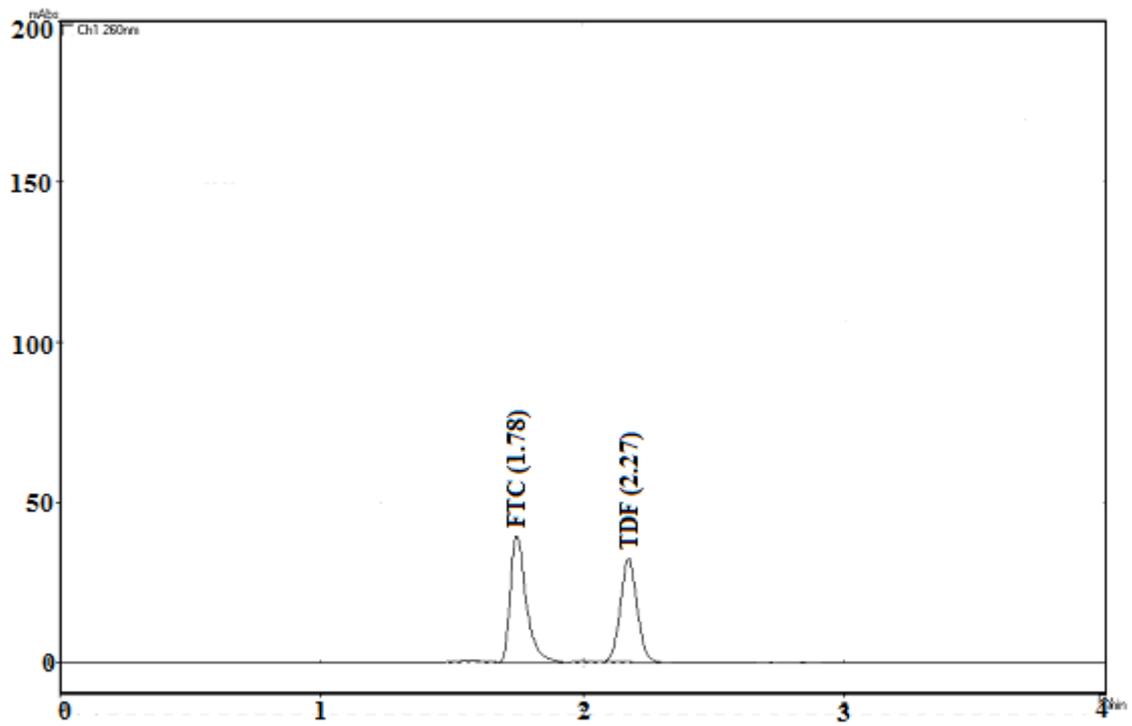


Fig 4. Chromatogram of mixed standard solution

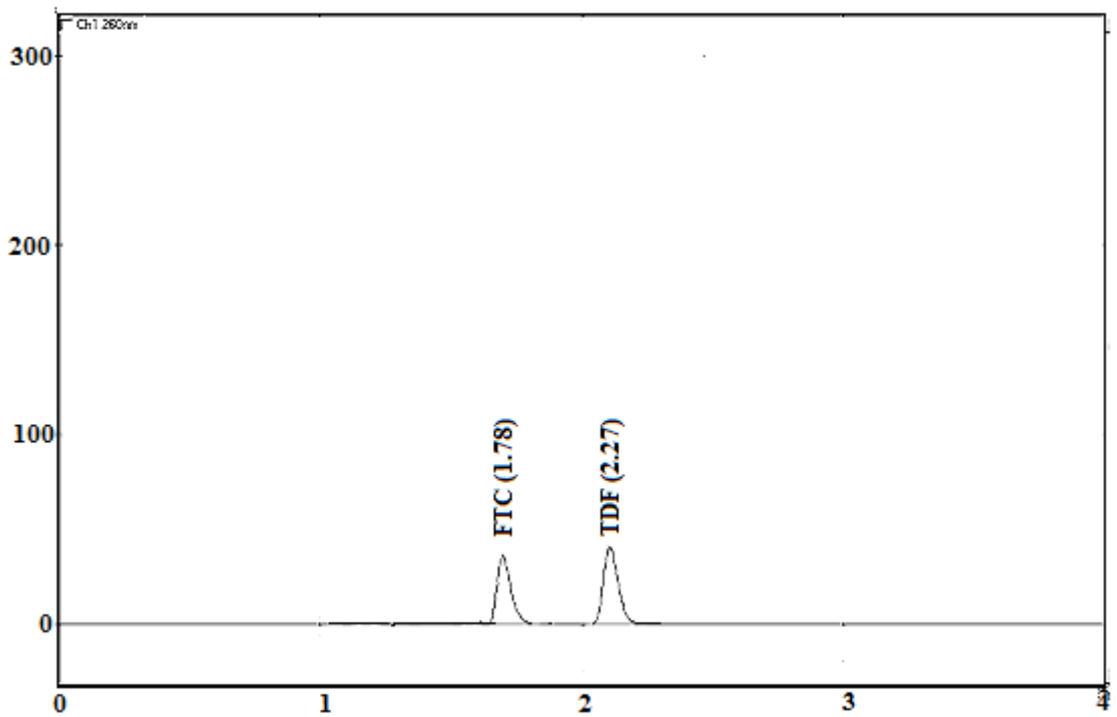


Fig 5. Chromatogram of FTC and TDF in sample solution with their retention time.

Table 3. Recovery Studies

Drug	Amount taken ($\mu\text{g mL}^{-1}$)	Amount added		% Recovery	%COV
		%	($\mu\text{g mL}^{-1}$)		
FTC	20	80	16	100.06	0.0776
		100	20	100.11	0.1221
		120	24	100.09	0.0888
TDF	20	80	16	100.03	0.0721
		100	20	100.02	0.0849
		120	24	100.08	0.0888

COV: coefficient of variance

Table 4. Intra Day and Inter Day Precision, LOD and LOQ Studies

Drug	Intra day precision (n=6) %COV	Inter day precision % COV			LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)
		Day 1	Day 2	Day 3		
FTC	0.1247	0.1529	0.1300	0.2166	0.015	0.045
TDF	0.1191	0.1160	0.4514	0.4159	0.039	0.117

Mean of six determinations, COV: coefficient of variance, LOD: limit of detection, LOQ: limit of quantitation.

3.6. Selectivity and Specificity

The selectivity was checked by injecting the solution of both the drugs into the system and it was observed that two sharp peaks of FTC and TDF having resolution of 3.33 were obtained at retention time of 1.78 and 2.27 min respectively in reference to placebo solution. Specificity of the method was assessed by comparing the chromatograms obtained from standard drugs, with the chromatogram obtained from tablet solutions. As the retention time of standard drugs and the retention time of the drugs in sample solution was same, so the method was specific. The developed method was found specific and selective, as there was no interference of excipients found.

4. Conclusion

A new, reversed-phase HPLC method has been developed for simultaneous analysis of FTC and TDF in a tablet formulation. It was shown above 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. 4 min, which enable rapid determination of many 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 preparation containing FTC and TDF.

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