

Simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in Tablet Dosage Form by Reverse Phase High-performance Liquid Chromatography

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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 Phenomenex-Luna C18 (25 cm x 4.60 mm, particle size 5 µm) column with a mixture of 10mM phosphate buffer (pH 6.8): Acetonitrile; 40: 60 (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 2.81 and 7.42 min for emtricitabine and tenofovir disoproxil fumarate respectively and total run time was 10 min. at a flow rate of 1.0 mL/min. The calibration curve was linear over the concentration range of 40.00 - 240.00 µg/ mL for emtricitabine and 60.00 - 360.00 µg/ mL for tenofovir disoproxil fumarate. The LOD and LOQ values were found to be 1.54 and 4.54 µg/ mL for emtricitabine and 4.60 and 13.65 µg/ mL 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

Introduction

Emtricitabine (ETB) is a nucleoside reverse transcriptase inhibitor (NRTIs). Chemically it is 5-fluoro-1-(2R, 5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (Figure 1). FTC is the (-) enantiomer of thio analog of cytidine which differs from other cytidine analogs, in that it has 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

bis(isopropoxycarbonyl)- oxymethyl ester derivative of tenofovir. Chemically it is 9-[(R)-2-[[[isopropoxycarbonyl]oxy]-methoxy]phosphiny]methoxy]propyl]adenine fumarate (Figure 1). It is used in combination with other antiretroviral for the treatment of HIV infection [2,3]

Literature survey reveals that few RP-HPLC [4-6] methods are reported for estimation of ETB, TDF and efavirenz in pharmaceutical formulation. TDF is estimated individually by UV [7], derivative-HPLC [8], HPTLC [9] in pharmaceutical formulation. ETB, TDF were estimated in biological matrices by HPLC [11,12] and LC/ MS/ MS [13-25], methods. The reported methods [4-6] for estimation of ETB, TDF in pharmaceutical formulations have some drawbacks in terms of sensitivity, ruggedness and robustness. 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.

Experimental

Apparatus

RP-HPLC was performed with an Agilent chromatographic system equipped with 1200 series isocratic pump UV-visible and a Rheodyne universal loop injector of injection capacity 50 µL. The monitoring software was Ezichrome Elite. The equipment was controlled by a PC workstation. Compounds were separated

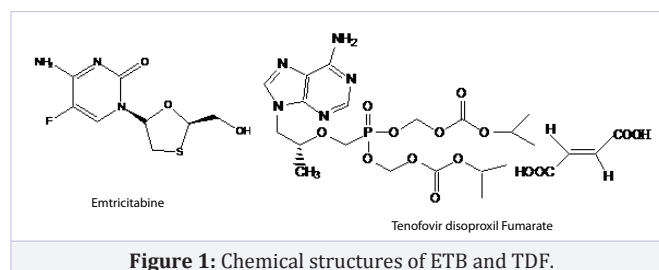


Figure 1: Chemical structures of ETB and TDF.

on a 25 cm x 4.6 mm i.d, 5- μ m particle, Phenomenex–Luna C₁₈ column under reversed-phase partition chromatographic conditions. The flow rate was 1.0 mL/ min and injection volume was 20 μ L, analyte were monitored at 260 nm and run time was 10 min.

Chemicals and reagents

Working Standards of pharmaceutical grade ETB and TDF were obtained as gift samples from Micro labs, Bangalore. The tablet dosage form, manufactured by Hetero Drugs Limited, Hyderabad, India (Label claim: ETB 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 Merck, Mumbai, India.

Mobile phase

The mobile phase selected was acetonitrile: 10 mM phosphate buffer (pH 6.8) (60:40, v/ v), and filtered through 0.45 μ m pore size membrane filter. Before analysis mobile phase was degassed.

Preparation of standard stock solution

Standard stock solution of Emtricitabine and Tenofovir pure drug prepared by accurately weighing about 100 mg drugs and transferring in to 100 mL volumetric flask and dissolved in acetonitrile.

Construction of calibration curve

A series of standard concentrations were prepared from 50 % to 150 % of the target concentration of ETB and TDF. Linearity was assessed by performing single measurement at several analyte concentration varying quantities of stock standard solution diluted with the mobile phase to get final concentrations of 40, 80, 120, 160, 200, 240 μ g/ mL of ETB and 60, 120, 180, 240, 300, 360 μ g/ mL of TDF.

Sample preparation

A total of 20 tablets were accurately weighed and powdered in a mortar. An amount equivalent to 66.5 mg (tablet Containing 20 mg of ETB and 30 mg of TDF) was transferred to 50 mL volumetric flask and added 10ml of mobile phase and sonicated for 10 min and make up to 50 mL with mobile phase. The solution was filtered 0.45 μ m pore size membrane filter. The filtered sample solution 5mL was diluted to 10 mL with mobile phase to get the solution containing ETB and TDF in 200 & 300 μ g/ mL proportions respectively. The test solution was injected in to HPLC and % assay was calculated. The results are depicted in Table 1.

Result and Discussion

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 series of trials with various C₈ and C₁₈ columns, the final choice of stationary phase giving satisfactory resolution and run time was the reversed phase Phenomenex-Luna C₁₈ column. 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 acetonitrile: 10 mM phosphate buffer (pH 6.8) (60:40, v/ v), with 1.0 mL/ min. From the overlain UV spectra, suitable wavelength considered for monitoring the drugs was 260 nm (Figure 2). Solutions of ETB 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 analytes absorbed well at 260 nm. Under the optimum chromatographic conditions, the retention time obtained for ETB and TDF were 2.81 and 7.42 min respectively. The chromatogram of placebo, standard and test formulation was depicted Figure 2-4.

Method Validation

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

Selectivity and Specificity

The selectivity was checked by injecting placebo solution and compared with standard chromatogram of ETB and TDF. 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.

Linearity: Linearity was found to be 40-240 μ g/ mL for ETB and 60-360 μ g/ mL for TDF. The linear regression for ETC and TDF were ($r^2 = 0.9934$) and ($r^2 = 0.9999$). The results were depicted in Table 2 and Figure 5,6.

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 ETB and TDF in tablet dosage form as all the statistical results were within the range of acceptance. The results were depicted in Table 3.

Table 1: Assay of Tablet Formulation.

Tablet	Label claimed (mg)		Conc. found (mg)		% Assay	
	ETB	TDF	ETB	TDF	ETB	TDF
ETOF Tablets	20.0	30.0	20.05	29.98	100.21	98.97

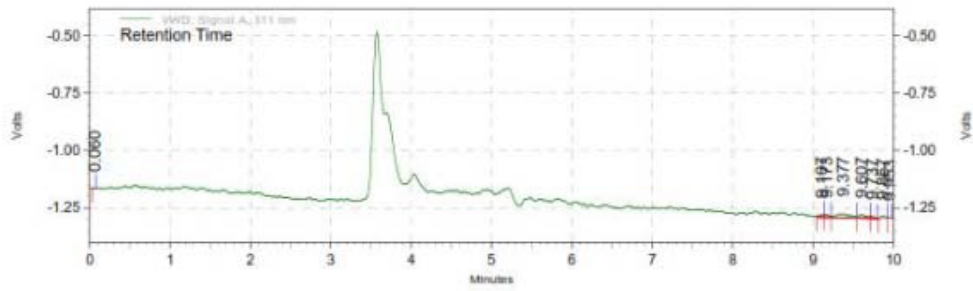


Figure 2: Chromatogram of Placebo.

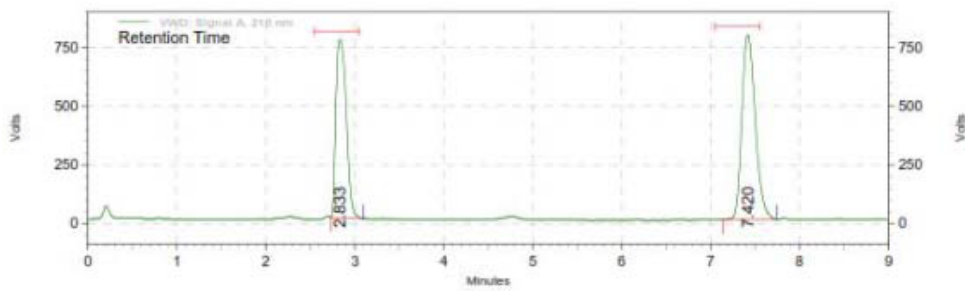


Figure 3: Chromatogram of standard ETB and TDF.

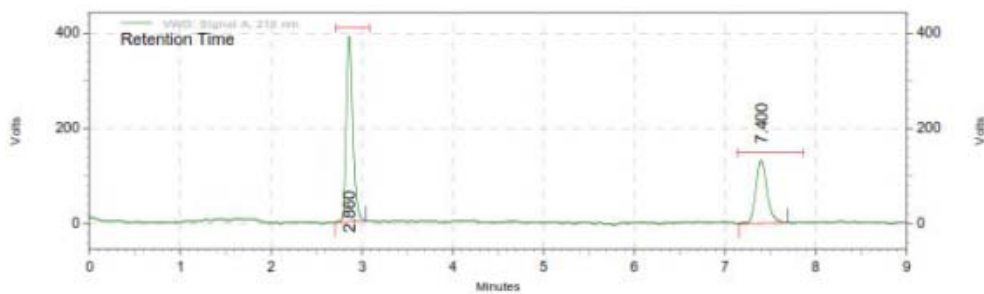


Figure 4: Chromatogram of ETB and TDF in test formulation.

Table 2: Linearity and range of ETB and TDF.

S.No.	Concentration µg/ml	Area of Emtricitabine	Concentration µg/ml	Area of Tenofovir
1	40	22988.567	60	16998.424
2	80	47605.237	120	33602.702
3	120	68028.039	180	50459.14
4	160	90704.169	240	67137.714
5	200	102120.617	300	84014.837
6	240	130654.429	360	101710.108
Concentration range µg/ml	40-240		60-360	
Slope (m)	543.85		281.04	
Correlation co-efficient (r ²)	0.993		0.999	

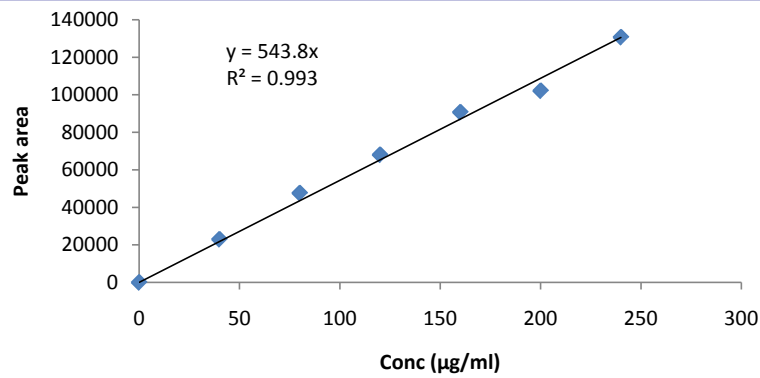


Figure 5: Linearity of Emtricitabine.

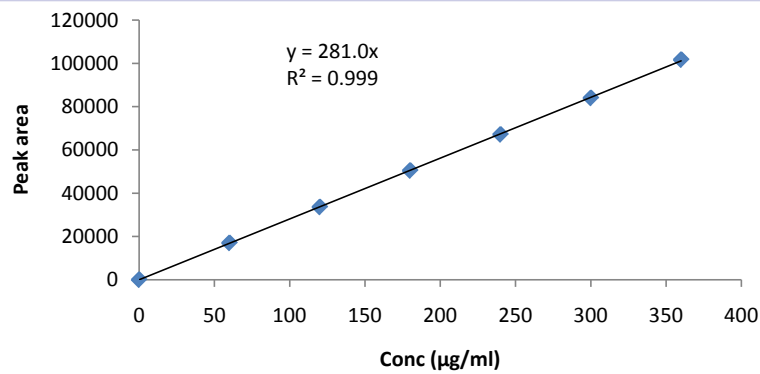


Figure 6: Linearity of Tenofovir.

Table 3: Recovery Studies.

Level of % recovery	Target Conc. (µg/ml)	Amount of drug spiked (µg/ml)	Drug recovered (µg/ml)	%Recovery	Mean	SD	%RSD
80	100	80	181.13	100.62	99.95	1.42	1.27
			180.01	100.22			
			179.85	99.98			
100	100	100	200.10	100.21	100.22	1.23	1.12
			202.21	100.23			
			200.11	100.21			
120	100	120	221.21	101.15	100.32	1.15	1.46
			220.34	100.33			
			220.41	100.42			

Table 4: Recovery Studies.

Sample. No	Intra day		Inter day	
	%Label Claim		%Label Claim	
	ETB	TDF	ETB	TDF
1	100.31	100.21	99.67	99.541
2	99.41	99.61	99.12	99.93
3	99.34	100.02	100.42	100.35
4	99.22	100.05	99.98	99.76
5	100.33	100.08	100.1	100.43
6	100.14	99.99	100.22	99.99
Mean	99.75	100.21	99.26	99.54
S.D	0.2262	0.226247	0.3742	0.3742
% R.S.D	0.2264	0.2264	0.3742	0.3742

Precision: 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 results are depicted in Table 4.

Limit of detection and Limit of Quantification: LOD is found to be 1.5456 µg/ mL for Emtricitabine and 0.0712 µg/ mL for Tenofovir and LOQ are found to be 4.5924 µg/ mL for Emtricitabine and 13.931 µg/ mL for Tenofovir.

Conclusion

A new, reversed-phase HPLC method has been developed for simultaneous analysis of ETB and TDF 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 (10 min), which enables rapid determination of many samples in routine and quality control analysis of tablet formulations. Hence, the proposed method was successfully applied to analyze preparation containing ETB and TDF.

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