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Simultaneous estimation of emtricitabine and tenofovir disproxil fumerate by HPLC method

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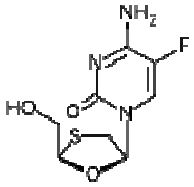
ABSTRACT

A validated HPLC method for simultaneous estimation of Emtricitabine and Tenofovir Disproxil Fumerate in pharmaceutical dosage forms. Chromatography was carried out on a C18 column [250mm, 4.6m, 5µm] using a mixture of methanol: phosphate Buffer (65:35 v/v) as the mobile phase at a flow rate of 1 ml/min. Detection was carried out by using PDA detector. The retention time of the drugs was 2.461 and 6.231 min for Emtricitabine and Tenofovir Disproxil Fumerate respectively. The method produced linear responses in the concentration range of 10 to 50µg/ml for both drugs. The LOD and LOQ values were found to be 0.00752, 0.00218ug/ml for Emtricitabine and The LOD and LOQ values were found to be 0.00851,0.0315ug/ml for Tenofovir Disproxil Fumerate. The method was validated for linearity, precision, accuracy, LOD &LOQ in accordance with ICH guidelines. The proposed method was found to be applicable for determination of the drug in tablet dosage forms.

Keywords: Emtricitabine, Tenofovor disproxil fumerate, EMT, TDF, HPLC.

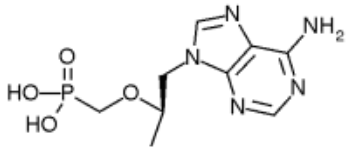
INTRODUCTION

Emtricitabine is chemically 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl)cytosine[1-3].It is an antiretroviral agent belonging to the class of nucleotide reverse transcriptase inhibitors. It has a molecular formula of C8H10FN3O3S and a molecular weight of 247.2467. It is soluble in water, acetonitrile and methanol.
Molecular structure:



Tenofovir disproxil fumerate: TDF is chemically, 9-[(R)-2-[[bis[(Isopropoxycarbonyl) oxy]methoxy] phosphinyl]methoxy]propyl]adenine fumarate (1:1)[1-3]. Is an antiretroviral agent belonging to the class of nucleotide reverse transcriptase inhibitors [4-7]. It has a molecular formula of C19H30N5O10P • C4H4O4 and a molecular weight of 635.52. TDF is the first nucleotide analog approved for HIV-1 treatment and remains in cells for longer periods of time than many other antiretroviral drugs.[8-9] TDF is a prodrug of Tenofovir and converted to an acyclic nucleoside phosphate in vivo by competing with the natural DNA substrates to inhibit reverse transcriptase and subsequently decreasing or preventing HIV replication in infected cells with a view to block HIV replication.[10-11]

Molecular structure:



These are the prescribed drugs for Antiretroviral, an attempt was made to report a simple, reliable and reproducible RP-HPLC method which was duly validated by statistical parameters precision, accuracy, linearity, LOD & LOQ.

Previous Studies:
The simultaneous assay of Tenofovir and Emtricitabine in plasma using LC/MS/MS and isotopically labelled internal standards. Journal of Chromatographyb, [12] simultaneous Ultraviolet Spectrophotometric estimation of Tenofovir disproxil fumerate and Emtricitabine in bulk and in tablet Dosage form. International Journal of Biopharmaceutics[13], a validated RP - HPLC Method for Simultaneous Estimation of Emtricitabine and Tenofovir Disproxil Fumarate in a tablet dosage Form**.** Eurasian J. Anal. Chem[14] , Steady-State Pharmacokinetics of Emtricitabine and Tenofovir Disproxil Fumarate Administered Alone and in Combination in Healthy Volunteers**.** J Clinical Pharmacol[15], Simultaneous quantification of emtricitabine and tenofovir in human plasma using high-performance liquid chromatography after solid phase extraction Journal of Chromatography B[16], HPTLC Method for the Simultaneous Estimation of Emtricitabine and Tenofovir in Tablet Dosage Form. Indian J Pharm Sci[17], 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,[18] Simultaneous determination of emtricitabine tenofovir by area under curve and dual wavelength Spectrophotometricmethod. J.Chill.Chem... Soc **[**19**],** Development and validation of first derivative spectrophotometric method for the simultaneous estimation of lamivudine and tenofovir disproxil fumerate in pure and in tablet formulation. Scholars research library[22], A simple HPLC method for quantitation of emtricitabine in capsule dosage form. Scholars research library[23].

MATERIALS AND METHODS

Chemicals and solvents
Potassium di hydrogen phosphate (AR grade, Qualigens) was used for preparing the buffer. HPLC grade methanol was used. Pure sample of Emtricitabine and Tenofovir Disproxil Fumerate was a gift sample from a local pharmaceutical industry. Commercial samples of tablets containing the drug Emtricitabine and Tenofovir Disproxil Fumerate was purchased from the local pharmacy.

Chromatographic Conditions
A High pressure liquid chromatography (Shimadzu LC-2010HT) with variable wavelength programmable UV-Visible detector and phenomena C-18 column [250mm, 4.6m, 5µm] was used. The HPLC system was equipped with the soft ware Class VP series version 5.03 (Shimadzu). A freshly prepared mixture of methanol: phosphate buffer (pH-3.5) (65:35v/v) used as the mobile phase. Buffer solution was prepared by dissolving 6.8gms of potassium dihydrogen phosphate in 1000ml of water. Adjust the pH to 3.5 with Acetic acid. Mobile phase was filtered through a 0.45µm membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1ml/min. The detection was carried out by PDA detector.

Preparation of the standard solution:
Accurately weighed 100 mg of both drugs (Emtricitabine and Tenofovir Disproxil Fumerate).Weighed powder of both drugs were accurately transferred to two different volumetric flask of 10ml and made volume up to the mark with diluents (Buffer: Methanol) to obtain a standard stock solution (solution A) of Emtricitabine (1mg/1ml) of the concentration 1000µg/ml and standard stock solution (solution B) Tenofovir Disproxil Fumerate (1mg/1ml) of the concentration 1000µg/ml. Accurately measured solution A of 1 ml was transferred to volumetric flask of 10ml and made volume up to the mark with diluent to obtain the concentration 100µg/ml for Emtricitabine . Accurately measured solution B of 1 ml was transferred to volumetric flask of 10ml and made volume up to the mark with diluent to obtain the concentration 100µg/ml for Tenofovir Disproxil Fumerate.

Preparation of Sample solution:
Twenty tablets were weighed and finely powdered. Powder equivalent to 10mg of sample was transferred to 10ml volumetric flask and made volume up to the mark with diluent of i.e.1mg/1ml to obtain the concentration of 1000µg/ml .From this solution 1ml was transferred to 10ml volumetric flask and made volume up to the mark with diluent to obtain solution of 100ug/ml.

Method validation
The proposed method was validated as per ICH guidelines. The drug solutions were prepared as[20-21] per the earlier adopted procedure given in the experiment.

Linearity study
Linearity was performed by taking from stock solution aliquots of , 1, 2, 3,4and 5ml were taken in 10ml volumetric flasks and diluted up to the mark with diluent (Buffer: Methanol) from the stock solution of Emtricitabine and Tenofovir Disproxil Fumerate (concentration 100µg/ml) Such that the final concentration in the range of 10 to 50µg/ml. Volume of 10µl of each sample was injected in five times for each concentration level and calibration curve was constructed by plotting the peak area versus the drug concentration. The observations and calibration curve is shown in Table 1, Fig.1, 2.

Assay:
Accurately weighed the powder equivalent to 10mg of Emtricitabine and Tenofovir Disproxil Fumerate was transferred to 10ml volumetric flask and made volume up to the mark with diluent to obtain solution of Emtricitabine (10mg/ml) and Tenofovir (10mg/ml). From this solution 1ml was transferred to 10ml volumetric flask and made volume up to the mark with diluents to obtain solution of 100ug/ml for Emtricitabine and 100ug/ml for Tenofovir Disproxil Fumerate . The chromatogram was shown in Figure-3.

Accuracy as recovery:
It was done by recovery study. Sample solutions were prepared by spiking at about 50 %, 100% and 150 % of specification limit to Placebo and analyzed by the proposed HPLC method. Results are shown in Table 3.

System precision
Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. Standard solution of (10µg/ml) were prepared as per test method and injected for 3 times. Results are shown in Table 4.

Method precision
Three samples were Prepared and analyzed as per the test method on same day and three different days and calculated the % RSD for Assay of five preparations. Results are shown in Table 5.

Limit of detection and limit of quantitation:
The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. Results are shown in Table 6.

RESULTS AND DISCUSSION

Emtricitabine and Tenofovir Disproxil Fumerate, indicated for the treatment of HIV. Fig 3, 4 and 5 shows typical chromatograms of Emtricitabine and Tenofovir Disproxil Fumerate. The retention time of Emtricitabine and Tenofovir Disproxil Fumerate was 2.461, 6.231 min. The calibration curve was linear over the range 10 - 50 µg/ml for the determination of Emtricitabine and Tenofovir Disproxil Fumerate. The linearity of method was statistically confirmed. The correlation coefficients (r^2) for calibration curves were not less than 0.999. The LOD and LOQ values of Emtricitabine were found to be 0.00752 , 0.0218 and Tenofovir Disproxil Fumerate were found to be 0.00851, 0.0315 ug /ml respectively. The Precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). Precision was expressed as the RSD of the results. The values obtained for the precision studies presented (Table-4, 5), indicates good repeatability and low inter day variability. The analytical recovery at five different concentrations of Emtricitabine and Tenofovir Disproxil Fumerate was determined and the recovery results were in the range for Emtricitabine and Tenofovir Disproxil Fumerate 99-101%. Therefore proposed validated method was successfully applied to determine Emtricitabine and Tenofovir Disproxil Fumerate in tablet dosage forms.

Linearity:

Table-1: Emtricitabine and Tenofovir Disproxil Fumerate Assay

Concentration	Emtricitabine Area	Tenofovir Area
10	787686	798966
20	1656750	1619897
30	2475780	2463789
40	3251678	3251678
50	4132180	4021188

Table- 2

Drug	Label claim mg/tab	Amount found mg/tab	Label claim (%)	S.D.*	S.E*
Emtricitabine	200	198.9	98.9	0.162737	0.007685
Tenofovir	300	299.3	99.3	0.168906	0.007685

Accuracy:

Table-3: Accuracy of Emtricitabine and Tenofovir Disproxil Fumerate

Drug	Label claim in mg	Sample conc in mg/ml	Amount added in µg	Amount added in µg	% Recovery	Average recovery(%)
Emtricitabine	200	10	10 20 30	9.99±1.32 20.03±1.7 30.02±1.1	99.5 100.83 100.36	100.23
Tenofovir Disproxil Fumerate	300	10	10 20 30	9.98±0.01 19.99±0.152 29.99±0.025	99 99.75 99.83	99.52

System precision:

Table -4

Drug	Injections	Peak Area	Mean	S.D	%R.S.D
Emtricitabine	1 2 3 4 5	336547 336328 335987 337826 335397	336417	899.3612	0.26
Tenofovir Disproxil Fumerate	1 2 3 4 5	396057 397100 395873 396106 396792	396385.6	530.198	0.1

Method precision

Table-5

Concentrations	Inter-day precision		Intra-day precision	
	Mean ± S.D	%R.S.D	Mean ± S.D	%R.S.D
10	99.81±0.616	0.617	99.73±0.123	0.123
20	99.53±0.225	0.226	99.44±0.572	0.575
30	99.31±0.415	0.417	99.72±0.323	0.323

Figure-1 Emtricitabine

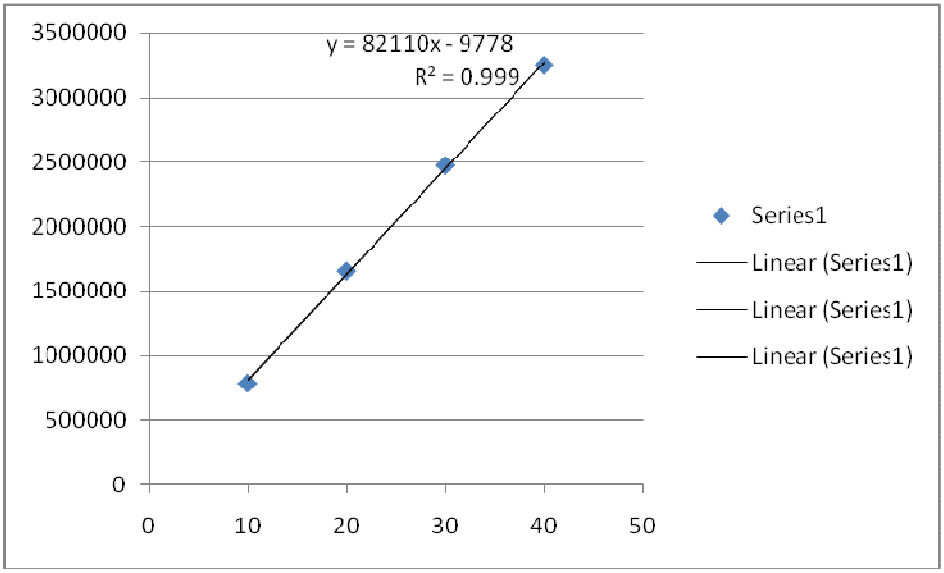


Figure-2 Tenofovir Disproxil Fumerate

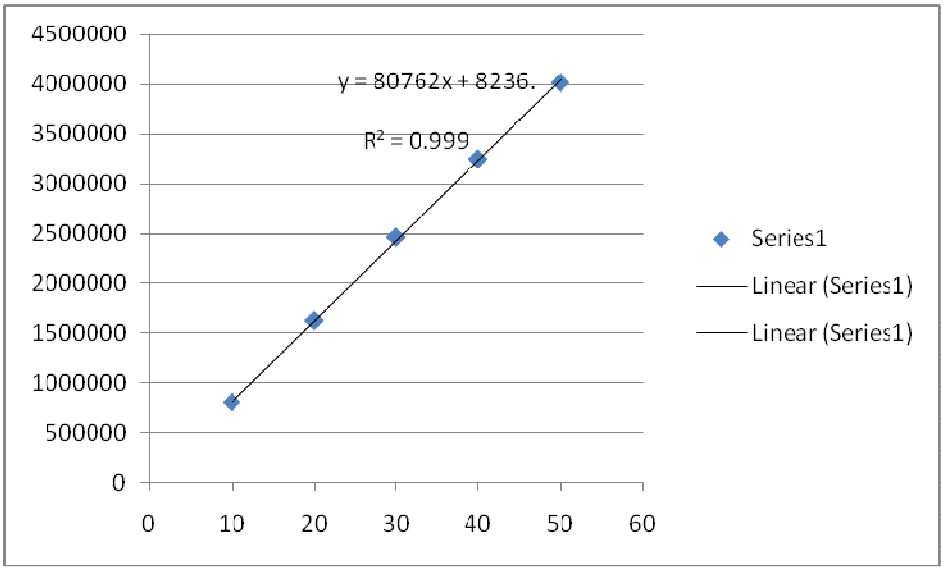


Figure-3 Standard chromatogram of Emtricitabine

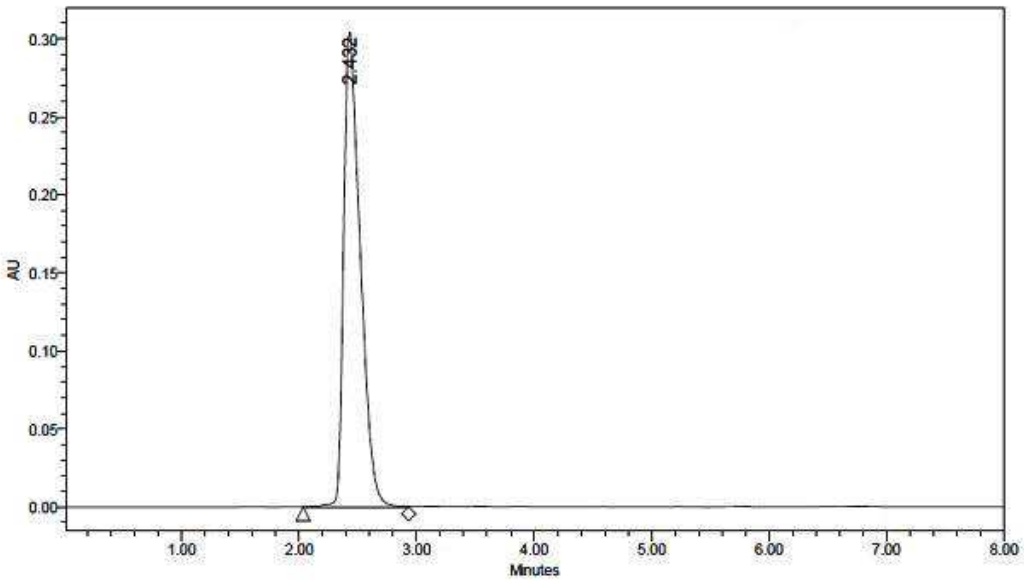


Figure-4 Standard chromatogram of Tenofovir Disproxil Fumerate

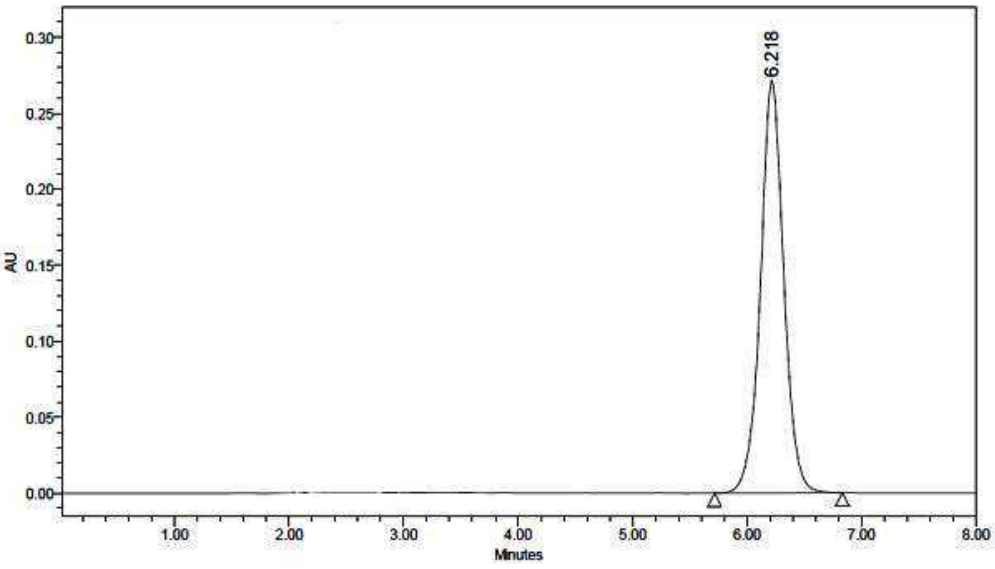
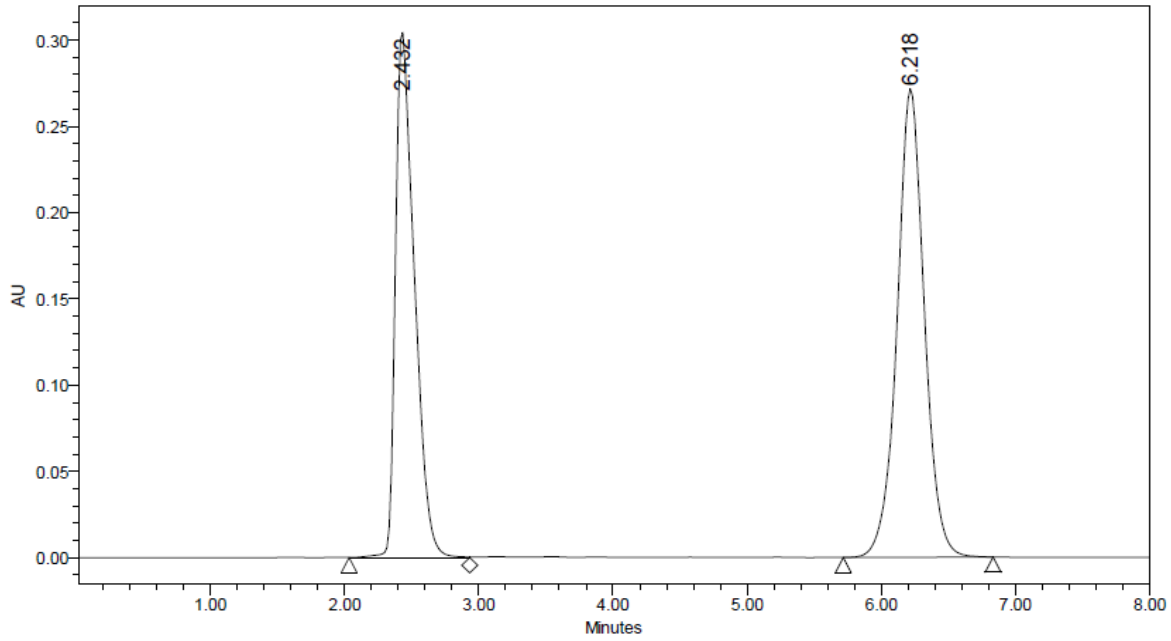


Table-6: Characteristics of HPLC method

Drug	Parameters Determined	Obtained Value
Emtricitabine	Linearity range (µg/ml)	10-50
	Slope	82110
	Intercept	9778
	Regression Coefficient(r^2)	0.999
	LOD(ug/ml)	0.00752
	LOQ(ug/ml)	0.0218
Tenofovir Disproxil Fumerate	Linearity range(µg/ml)	10-50
	Slope	80762
	Intercept	8236
	Regression Coefficient(r^2)	0.999
	LOD(ug/ml)	0.00851
	LOQ(ug/ml)	0.0315

Figure-5 Sample chromatogram of Emtricitabine and Tenofovir Disproxil Fumerate



CONCLUSION

For the determination of Emtricitabine and Tenofovir Disproxil Fumerate , the proposed HPLC method was found to be superior due to high percentage recovery which shows that the method was free from interference of excipients used in the formulations. The results of the study indicate that the proposed HPLC method of analysis can be used in quality control department with respect to routine analysis for the assay of the tablets containing Emtricitabine and Tenofovir Disproxil Fumerate.

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