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SIMULATANEOUS ESTIMATION OF EMTRICITABINE AND TENOFOVIR DISOPROXIL FUMARATE IN A TABLET DOSAGE FORM BY RP-HPLC METHOD

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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 C_{18} (25cm x 4.60 mm, particle size 5µm) column with a mixture of acetonitrile: phosphate buffer (pH 6.8) in the ratio of 60:40 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 2.883 and 3.89 min. for emtricitabine and tenofovir disoproxil fumarate respectively and total run time was 8 min. at a flow rate of 1.0mL min-1. The calibration curve was linear over the concentration range of 4 - 24 µgmL-1 for emtricitabine and 6-36 µg mL-1 for tenofovir disoproxil fumarate. The LOD and LOQ values were found to be 0.05318 and 0.16115 µg mL-1 for emtricitabine and 0.06782 and 0.2553 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.

INTRODUCTION

Emtricitabine (EMT) 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. EMT 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 bisisopropoxycarbonyl- oxymethyl ester derivative of tenofovir. Chemically it is 9-[(R)-2-[[(isopropoxcarbonyl)- oxy] methoxy] phosphiny] methoxy] propyl] adeninefumarate [1] (Fig.2). TDF itself is an acyclic nucleoside phosphonate (nucleotide) analogue of adenosine 5'-monophosphate. The negative charge on Tenofovir at neutral pH limits its oral bioavailability, hence its provision as a pro-drug that is rapidly converted to Tenofovir after absorption. Literature survey reveals that few RP-HPLC [3-4] methods are reported for estimation of EMT and TDF in pharmaceutical formulation, RP-HPLC [5-6] methods are reported for estimation of EMT, 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] methods. Similarly for EMT, HPLC with Fluorometric detection [13] in human plasma and Stability indicating liquid chromatographic [14] methods are reported. RP-HPLC [15] and LC-MS/MS [16] method is reported for simultaneous estimation of EMT and TDF in human plasma. HPTLC [17-18] is reported for simultaneous estimation of EMT 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.

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MATERIALS AND METHODS

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 µL. The monitoring software was SPINCHROME. The equipment was controlled by a PC workstation. Compounds were separated on a 25 cm × 4.6 mm i.d, 5-µm particle, Phenomenex Luna C_18 column under reversed-phase partition chromatographic conditions. Ultrasonicator –BANDELIN was used. The work was carried out in an air-conditioned room maintained at temperature 25±2 °C. The flow rate was 1.0 mL min^{-1}, analytes were monitored at 260 nm and run time was 8 min.

Mobile phase

The mobile phase selected was acetonitrile: phosphate buffer (Ph 6.8) and before analysis mobile phase was degassed.

Standard stock solution and Construction of Calibration curve

Standard stock solution of EMT(20mg) and TDF (30 mg) each were prepared separately in 50 mL of mobile phase to get the final concentration of 100 µg 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 EMT and TDF respectively. Unknown samples were determined by reference to these calibration curves.

Diluted Standard solution

Mixed standard analysis was performed to validate the procedure. From the standard stock solutions of the drugs, 1,2,3,4,5,6 ml were taken and diluted it in 10 ml mobile phase were prepared and analyzed, statistical results were within the range of acceptance i.e. %COV<2.0 and S.D.<1.0.

Sample preparation

For analysis of the tablet dosage form, twenty tablets (TAVIN-EM) were weighed. From the powdered tablets, weigh accurately about 65.6 mg of powdered tablets (equivalent to 20 mg of *Emtricitabine* and 30 mg of *Tenofovir disoproxil fumarate*) into a 50 ml volumetric standard flask, and add 25 ml, and make up to 50 ml with mobile phase. The mixture was subjected to sonications for 10 min.Take 10ml of the solution and dilute it up to 100ml with mobile phase. Then from this solution take 5ml diluted it in 10ml.The sample solution was centrifuged in tight enclosure for 10 min at 300 RPM.

METHOD

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.

VALDATION

Selectivity / specificity

A method is said to be specific when it produces a responses only for a single analyte. Selectivity is the ability of the method produces a response for the analyte in the presence of other interferences, in order to prove that the method chosen was specific and selective.

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 amounts of standard recovered were calculated in the terms of mean recovery with the upper and lower limits of % relative standard deviation.

Precision

The concentrations of both the drugs were measured three times on the same day at intervals of 1 h and on two different days for intra and interday study respectively. It is expressed as the percentage coefficient of variation (% CV) which is calculated as per the following expression

% CV = (standard deviation / mean) x 100

Linearity and range

The limits of detection and quantitation, LOD and LOQ, were calculated by use of the equations LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the blank and S is the slope of the calibration curve. Linearity of the concentrations was taken in the range of 4-24µg/ml for Emtricitabine and -364µg/ml Tenofovir.

RESULT AND DISCUSSION

HPLC method development and optimization

After trying column C_8 and C_{18}, the final choice of stationary phase giving satisfactory resolution and run time was the reversed phase column Luna C_{18}. The best result was obtained by use of 60:40 (v/v) ratio of Acetonitrile and phosphate buffer (pH 6.8) with 1mL min^{-1}. From the overlain UV spectra (Shimadzu-1700), suitable wavelength considered for monitoring the drugs was 260 nm (Fig 3). The chromatogram standard mixture is shown in Fig 4 respectively.

Under the optimum chromatographic conditions, the result of the retention time capacity factor, tailing factor, theoretical plate number and resolution are reported in Table 1(Fig 5.)

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

Validation of the developed method

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.

Linearity

The linear regression equations for FTC and TDF were EMT $y = 56.47x + 3.867$ ($r^2 = 0.999$)

TDF $y = 47.21x + 8.114$ ($r^2 = 0.999$)

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

Accuracy

The result of accuracy study was reported in Table 2. From the recovery study it was clear that the method is very accurate for quantitative estimation of EMT 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.

Precision, Limit of Detection, and Limit of Quantitation

Precision, limits of detection and limit of quantitation were calculated and the results are reported in Table 3.

Specificity

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.

Assay

The sample 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 EMT and TDF per tablet was calculated by extrapolating the peak area from the calibration curve. The results are reported in Table 4.

Table 1. System suitability parameters

Property	EMT	TDF
Rt	2.883	3.89
T f N	1.731	1.697
R s	2591	2901
	3.906	

Table 2. Recovery studies

S. No	Inj.Sample	Spike level	Amount Present (µg/ml)	Amount Recovered (µg/ml)	% Recovered
1	**Emtricitabine**	80 %	16	15.957	99.73
2		100 %	20	19.969	99.84
3		120 %	24	23.9389	99.74
4.	**Tenofovir**	80 %	24	23.9549	98.64
5		100 %	30	29.9275	99.75
6		120 %	36	35.9046	99.73

Table 3. Intra Day and Inter Day Precision, LOD and LOQ Studies

Drug	Intra day Precision % COV (n = 6)	Interday Precision % COV		LOD (µgmL$^{-1)}$	LOQ (µgmL^{1})
		Day 1	Day 2		
EMT	0.2519	0.2776	0.1936	0.05318	0.016115
TDF	0.377	0.3741	0.5649	0.06782	0.2553

Table 4. Assay of Tablet Formulation

Drug	TAVIN – EM Label Claim mg/ tab (n=6)	Amount Found		SD	%COV
		mg	%		
EMT	200	198.73	99.368	0.898	0.450
TDF	300	298.71	99.57	0.9121	0.3047

Figure 1. The chemical structures of EMT

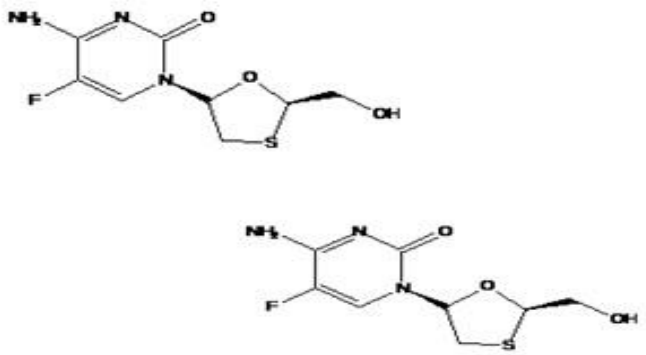


Figure 2. The chemical structures of TDF

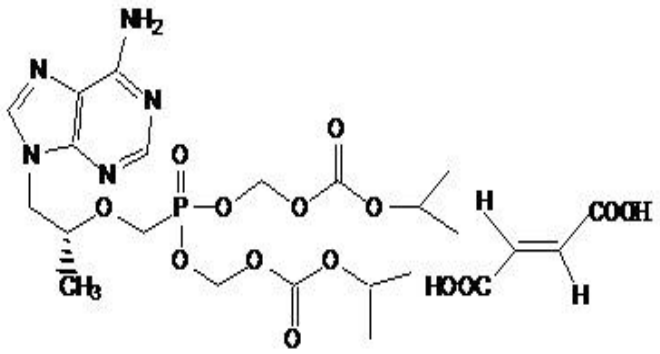


Figure 3. Overlain Spectra of EMT and TDF

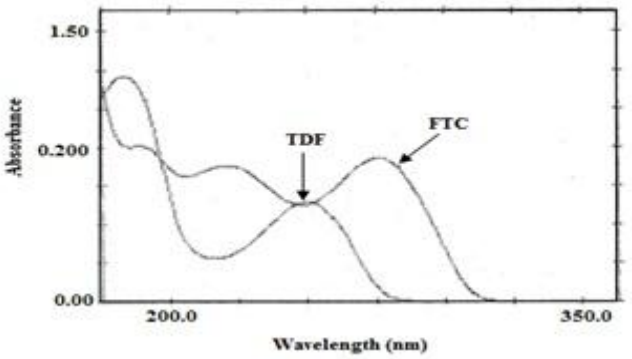


Figure 4. Chromatogram of mixed standard solution

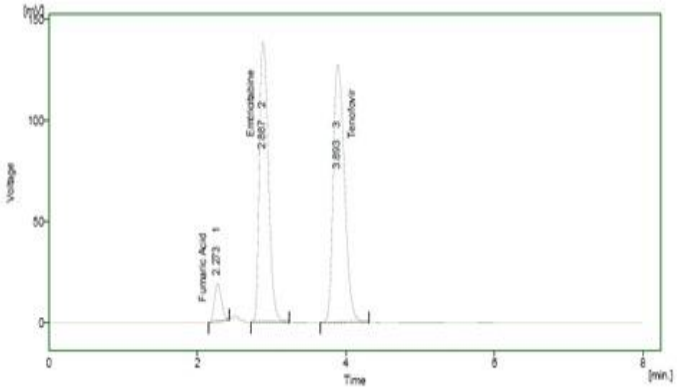
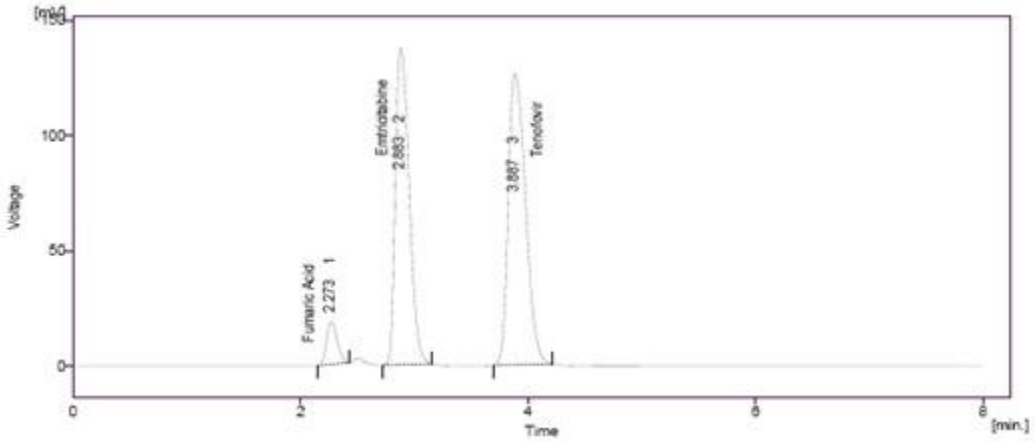


Figure 5. Chromatogram of EMT and TDF in sample solution with their retention time



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. 8 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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