SIMULTANEOUS DETERMINATION OF EMTRICITABINE AND TENOFOVIR BY AREA UNDER CURVE AND DUAL WAVELENGTH SPECTROPHOTOMETRIC METHOD

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ABSTRACT

Two methods for the simultaneous determination of Emtricitabine and Tenofovir by spectroscopy have been developed. These two simple, accurate and precise methods include Area Under the Curve (AUC) method and Dual Wavelength Method. From a solvent effect studies and the spectral behaviours of Emtricitabine and Tenofovir, methanol was selected as solvent. Emtricitabine shows maximum absorbance at 281 nm and Tenofovir shows maximum absorbance at 259 nm. For the AUC method, the wavelength ranges between 242-248 nm and 269-275 nm were selected with reference to the absorbance curves plotted between the wavelengths of 200-400 nm. In the second method i.e. Dual method in which two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for another drug. Emtricitabine shows equal absorbance at 230.696 nm and 250 nm, where the differences in absorbance were measured for the determination of Tenofovir. Similarly, differences in absorbances at 250 nm and 268.670 nm were measured for determination of Emtricitabine. These methods allows rapid analysis of two drug combination. The results of analysis were validated statistically and by recovery studies. This tablet containing both drugs was assayed using the methods developed, showing a good accuracy and precision.

Keywords: Spectroscopy; Emtricitabine; Tenofovir; Area Under Curve Method ; Dual Wavelength; Pharmaceutical Formulation.

INTRODUCTION

Tenofovir (TE; 9-[(*R*)-2-[[bis[[(isopropoxycarbonyl) oxy] methoxy] phosphinyl] methoxy] propyl) and Emtricitabine (EM; 5-fluro-1-(2*R*, 5*S*)-[2-9hydroxymethyl]- 1,3-oxathiolan-5-y, Figure 1) both are the antiviral agents. Acts as the Nucleoside Reverse Transcriptase enzyme Inhibitors. These are the Nucleoside analogues which are phosphorylated by host cell enzyme to give 5`-triphosphate derivative. This moiety competes with the equivalent host cellular triphosphate substrates for proviral DNA synthesis by viral reverse transcriptase which is viral RNA-dependent DNA polymerase. Eventually, the incorporation of the 5`-triphosphate moiety into the growing viral DNA chain results in chain termination. Mammalian α-DNA polymerase is relatively resistant to the effect. EM is potent and selective against HIV types I and II and hepatitis B virus. TE is active against a variety of drug resistant HIV-I strains. Recently, the combination of EM and TE has demonstrated significantly greater HIV RNA suppression compared to the combination of zidovudine and lamivudine[1-3]

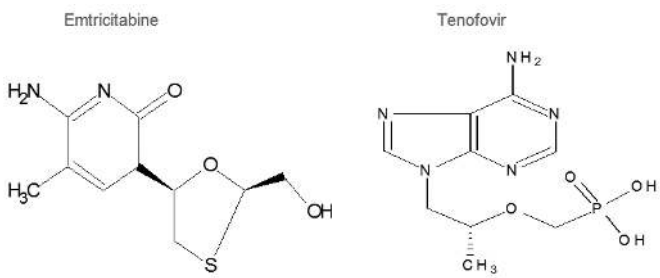

Fig. 1. The structures of Emtricitabine and Tenofovir.

Several analytical methods that have been reported for the individual determination of TE in biological fluids and pharmaceutical formulations which include liquid chromatography coupled with spectrofluorimetric, UV, and mass spectroscopy detection [4-10]. For EM several analytical methods have been reported for its individual analysis which includes chiral liquid chromatography, liquid chromatography with UV detection[11-13]. Few Bioanalytical methods are reported for combination of TE and EM which includes liquid chromatography with PDA and UV detection[14,15] . There is no spectroscopic method available in the literature for the simultaneous estimation of EM and TE in combined dosage form. The aim of this study was to develop and validate simple, rapid, selective and low cost Area under Curve and Dual Wavelength Spectrophotometer Methods for the determination of TE and EM in tablet dosage. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines [16].

EXPERIMENTAL:

Instruments

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells were used for spectrophotometric methods. All weighing were done on electronic balance (ModelShimadzu AUW-220D).

MATERIALS AND METHODS

Reagents :

Spectroscopy grade methanol was used throughout the study. Pure drug sample of TE, % purity 99.86 and EM, % purity 99.82 were kindly supplied as a gift sample by Emcure Pharmaceuticals Pvt. Ltd. Pune, India. It was used without further purification. Tablets were purchased from local market, each containing Tenofovir disoproxil fumarate 300 mg and Emtricitabine 200 mg. Tablet used for analysis were TENVIR-EM (Batch No. X81241) manufactured by Cipla Ltd., Goa.

Preparation of standard Stock Solutions and Sample solution:

Standard stock solution of 1000 µg/mL of both the drugs was prepared separately in methanol. For verification of Beer's Law, a series of diluted solutions of Tenofovir and Emtricitabine ranging from 6-48 µg/mL (series A) and 4-32 µg/mL(series B), respectively were prepared and mixture of both the drugs in (series C) of same concentration range were prepared in methanol.[17]

For preparation of sample stock solution, twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 100 mg of TE (66.66 mg of EM) was weighed and dissolved in the 80 mL of methanol with the aid of ultrasonication for 5 min and solution was filtered through Whatman filter paper No. 41 into a 100 mL volumetric flask. Filter paper was washed with methanol, adding washings to the volumetric flask and volume was made up to the mark with methanol. The sample stock solution was suitably diluted further to get required final concentration of TE (24 µg / mL) and EM (16 µg / mL).

METHODS

Method A: AREA UNDER CURVE METHOD

For the simultaneous determination using the area under the curve method, suitable dilutions of the standard stock solutions (1000 µg/ml) of TE and EM were prepared separately. The solution of drugs were scanned in the range of 200-400 nm. For Area Under Curve method, the sampling wavelength ranges selected for estimation of EM and TE are 242-248 nm (λ_1-λ_2) and 269-275 nm (λ_3-λ_4). Mixed standard were prepared and their Area Under the Curve were measured at the selected wavelength ranges[18,19]. Concentration of two drugs in mixed standard and the sample solution were calculated using equation (1) and (2).

$A_1 = 868.25\ C_{TE} + 1985\ C_{EM}$ (1) at 242-248 nm.

$A_2 = 819.54 C_{TE} + 1710.06\ C_{EM}$ (2) at 269-275 nm.

Where,

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868.25 And 819.54 are absortivities of TE at (λ_1-λ_2) and (λ_3-λ_4) respectively.
1985 and 1710.06 are absortivities of EM at (λ_1-λ_2) and (λ_3-λ_4) respectively.
A_1 and A_2 are absorbances of mixed standard at (λ_1-λ_2) and (λ_3-λ_4) respectively.C_{TE} and C_{EM} are the concentrations in g/100mL.

Method B: DUAL WAVELENGTH METHOD

The spectrum of TE Figure 2 show that absorbance of TE is identical at 250 nm (λ1) and 268.679 nm (λ2) therefore these two wavelength were selected for the analysis of EM. All the solutions of **series A** were scanned to ensure that the difference of absorbance between λ_1 and λ_2 is zero. Similarly, the EM solutions were scanned to determine the two wavelengths, where absorbance is same. These two wavelengths were found to be 230.696 nm (λ_3) and 250 nm (λ_4). All the solution of **series B** were scanned to ensure that difference of absorbance between (λ_3) and (λ_4) is zero. Thereafter, the solution of **series C** were scanned to ensure that varying concentration of TE and EM are not affecting the absorbance at selected wavelength. Difference in absorbances between 250 nm (λ1) and 268.679 nm (λ2) of series C solutions was used for preparation of calibration curve for EM. Similarly difference in absorbance between 230.696 nm (λ_3) and 250 nm (λ_4) of mixed standard solutions was used for preparation of calibration curve for TE.

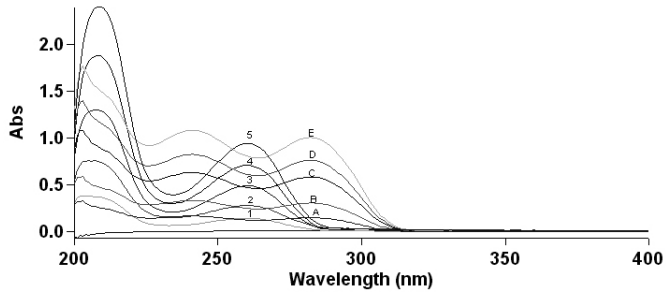

Fig. (2). Overlain spectra of Tenofovir in methanol: (1) 6µg/mL; (2) 12 µg/mL; (3) 24 µg/mL; (4) 36 µg/mL; (5) 48 µg/mL and Emtricitabin (A) 4 µg/mL; (B) 8 µg/mL; (C) 16 µg/mL; (D) 24 µg/mL; (E) 32 µg/mL.

RESULTS AND DISCUSSION

RECOVERY METHOD

The accuracy of the proposed method was checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (50 %, 100 % and 150 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 12 µg /mL of TE and 8 µg /mL of EM. Results of the recovery are presented in Table 1 and 2.

Table 1 : Recovery study of Tenofovir and Emtricitabine for **Method A**

Level of % Recovery	Amount Spiked (µg /mL)		Amount recovered (µg /mL)		% Mean recovery		Relative standard deviation % (R.S.D., n= 6)	
	TE	EM	TE	EM	TE	EM	TE	EM
50	6.06	4.04	6.08	3.98	100.33	98.51	0.53	0.52
100	12.12	8.08	11.97	8.09	98.76	100.12	1.92	0.79
150	18.18	12.12	18.10	12.13	99.55	100.08	1.04	0.21

Table 2 : Recovery study of Tenofovir and Emtricitabine by **Method B**

Level of % Recovery	Amount Spiked (µg /mL)		Amount. recovered (µg /mL)		% Mean Recovery		Relative standard deviation % (R.S.D., n= 6)	
	TE	EM	TE	EM	TE	EM	TE	EM
50	6.08	4.05	6.10	4.03	100.32	99.50	0.96	0.97
100	12.09	8.06	12.14	8.04	100.43	100.37	0.89	0.38
150	17.96	11.97	17.94	12.12	99.88	101.25	0.72	0..51

Analytical features:

Simple, precise and accurate Area under curve and Dual wavelength methods were developed for the simultaneous estimation of tenofovir and emtricitabine in combined dosage form. Optical characteristics and statistical data for the proposed method in table 3.

For **METHOD A** Beer's law obeyed in the concentration range 6-48 µg/mL and 4-32 µg/mL TE and EM, respectively. Results of recovery studies are shown in Table 2. For TE, the recovery study results ranged from 99.01% to 101.46 % with % RSD values ranging from 0.52% to 1.72 %. For EM the recovery results ranged from 99.06 % to 99.96 %, with % RSD values ranging from 0.5 % to 0.99 %. The accuracy and reproducibility is evident from the data as results are close to 100 % and standard deviation is low.

For **METHOD B** Beer's law obeyed in the concentration range 6-48 µg/mL and 4-32 µg/mL TE and EM, respectively. Results of recovery studies for Dual Wavelength Method are shown in Table 3. For TE, the recovery study results ranged from 99.69 % to 101.48 % with % RSD values ranging from 0.89% to 0.96 %. For EM the recovery results ranged from 99.15 % to 101.45%, with % RSD values ranging from 0.51 % to 1.38 %. The accuracy and reproducibility is evident from the data as results are close to 100 % and standard deviation is low

PRECISION

To study intraday precision method was repeated 5 times in a day and average % RSD was found to be 1.16 and 0.59; 0.34 and 0.18 for TE and EM respectively, for method A and B respectively. Similarly to study interday precision, the method was repeated on five different days and the average % RSD was found to be 1.16 and 0.59; 0.34 and 0.18 for TE and EM respectively, for method A and B, respectively. These values confirm the intra and inter day precision.

Table 3: Optical characteristics and statistical data for the proposed method

Parameter		Tenofovir		Emtricitabine	
		METHOD A	METHOD B	METHOD A	METHOD B
λ (nm)		Area between 242-248	230.69 and 250	Area between 269-275	250 and 268.67
Beer's law limit (µg/mL)		6-48	6-48	4-32	4-32
Regression Equation (y = mx + c)	Intercept (c)	-	0.00904	-	0.00369
	Slope (m)	-	0.002	-	-0.0007
Correlation Coefficient		-	0.9982	-	0.9903
Accuracy (%Recovery)		100.53	101.41	99.57	101.12
Precision	Intraday	1.16	0.59	0.34	0.18
	Interday	1.09	0.98	1.40	1.19

FORMULATION ANALYSIS:

The proposed methods were used for analysis of the marketed tablet formulation as described in Instruments and reagents section. Drugs were extracted from the formulation as described in Preparation of Stock and Sample solutions section and subjected to the proposed methods, overlain spectrum of standard mixture and formulation are shown in figure(3) and results of formulation analysis are presented in Table 4.

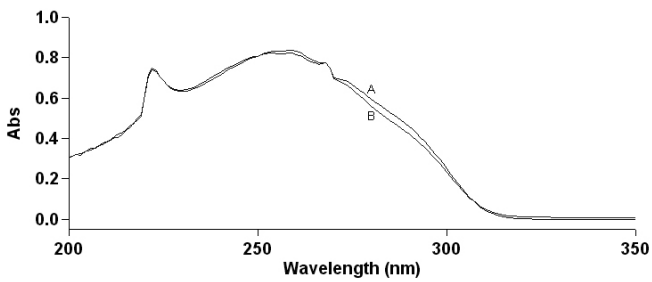

Fig (3). Overlain Spectra of A :-Std. mixture of TE.(24µg/mL) and EM.(16µg/mL) and B :-Sample mixture of TE.(24µg/mL) and EM.(16µg/mL).

Table 4: Results of commercial formulation analysis

Method	Drug	Label Claim (mg/ tablet)	% of Label Claim Estimated	% R.S.D.
Area Under Curve Method	Tenofovir	300	99.70	0.98
	Emtricitabine	200	99.92	1.23
Dual Wavelength Method	Tenofovir	300	99.75	0.92
	Emtricitabine	200	99.96	1.34

CONCLUSION

The validated spectrophotometric method employed here proved to be simple, economical, precise and accurate. Thus it can be used as IPQC test and for routine simultaneous determination of TE and EM in tablet dosage form.

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