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SPECTROPHOTOMETRIC ESTIMATION OF EMTRICITABINE AND TENOFOVIR DISOPROXIL FUMARATE IN TABLET DOSAGE FORM BY SIMULTANEOUS EQUATION AND ABSORBANCE RATIO METHODS

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ABSTRACT
Two methods for simultaneous determination of Emtricitabine and Tenofovir Disoproxil Fumarate by spectroscopy have been developed. First method is Simultaneous equation method and second method is Absorbance ratio Method. From a solvent effect studies and the spectral behaviours of Emtricitabine and Tenofovir Disoproxil Fumarate, methanol was selected as solvent. Emtricitabine shows maximum absorbance at 281 nm and Tenofovir Disoproxil Fumarate shows maximum absorbance at 259 nm. Linearity in concentration range of 6-48 µg/mL and 4-32 µg/ mL was shown for Tenofovir Disoproxil Fumarate and Emtricitabine, respectively. In the first method, the concentration of the drugs were determined by using simultaneous equation and in second method the concentration of drugs were determined by using ratio of absorbances at isoabsorptive point and at the ëmax of one of the drug. The results of analysis were validated statistically and by recovery studies. Tablet containing both drugs was assayed using the methods developed, showing a good accuracy and precision.

Keywords: *Emtricitabine; Tenofovir Disoproxil Fumarate; Simultaneous equation Method; Absorbance ratio Method; Pharmaceutical Formulation.*

INTRODUCTION

Tenofovir Disoproxil Fumarate (TDF); 9-{(*R*)-2-[(Bis{[(isopropoxycarbonyl) oxy]methoxy}phosphinyl) methoxy]propyl}adenine fumarate and Emtricitabine (EMT); 5-Fluro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine, both are antiviral agents. They act as the Nucleoside Reverse Transcriptase Inhibitor. EMT is potent and selective against HIV types I and II and hepatitis B virus. TDF is active against a variety of drug resistant HIV-I strains. Recently the combination of TDF and EMT has demonstrated significantly greater HIV RNA suppression compared to the combination of zidovudine and lamivudine.[1-3]

Several analytical methods have been reported for the individual determination of TDF in biological fluids and pharmaceutical formulations [4-7]. For EMT several analytical methods have been reported for its individual analysis which includes chiral liquid chromatography, liquid chromatography with UV detection[8-9]. Few Bioanalytical methods are reported for combination of TDF and EMT which includes liquid chromatography with PDA and UV detection[10].

Since there is no specific spectroscopic method available in the literature for the simultaneous estimation of EMT and TDF in tablet dosage form, the aim of this work is to develop a simple, rapid, economic method for the simultaneous determination of TDF and EMT by Simultaneous equation Method and Absorbance ratio Method. The proposed methods were applied to pharmaceutical formulations and were optimized and validated as per the International Conference on Harmonization (ICH) guidelines [11].

MATERIALS & METHODS

Instruments and reagents

A UV-Visible double beam spectrophotometer (Varian Cary 100) with 10mm matched quartz cells was used for spectrophotometric method. All weighing were done on electronic balance (Model Shimadzu AUW-220D). Spectroscopic grade methanol was used throughout the study. Pure drug sample of TDF (99.86 % purity) and EMT (99.92 % purity) were kindly supplied as a gift sample by Emcure Pharmaceutical Pvt., Ltd., Pune, India and were used without further purification. Tablets were purchased from local market, containing TDF (300mg) and EMT (200mg). Tablets used for analysis were TENVIR-EM (Batch No. X81241) manufactured by Cipla Ltd. Goa, India.

Preparation of Standard Stock and Sample Solutions

Stock Solutions (1000 µg/ml) of TDF and EMT were prepared separately in methanol for verification of Beer's Law, a series of diluted solutions of TDF and EMT ranging from 6-48 µg/ml and 4-32 µg/ml, respectively, were prepared.

For formulation analysis, twenty tablets were weighed accurately, powdered and a quantity of tablet powder equivalent to 100mg of TDF (66.66mg of EMT) was

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weighed and dissolved in 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 solution was further diluted to get required final concentration of TDF (24 µg/ml) and EMT (16 µg/ml).

METHODS

Method A - Simultaneous equation Method

For the simultaneous determination using the Simultaneous equation method, standard stock solutions were suitably diluted to have concentrations in the range of 6-48µg/ml TDF and 4-32µg/ml EMT. These solutions were scanned in the range of 200-400 nm and overlain spectra were obtained (Fig.1). From the observation of the overlain spectra the sampling wavelengths were selected for estimation of TDF and EMT as 250 nm ($ë_1$) and 274 nm ($ë_2$). Absorptivity values of both drugs at both the wavelengths were calculated form absorbance values for the drugs at the selected wavelengths. Simultaneous equations were constructed from the calculated absorptivity values.[12] Concentrations of two drugs in sample solution was calculated using equation (1) and (2).

$A_1 = 180.58\ C_{TDF} + 301.125\ C_{EMT}$ …(1) at 250 nm

$A_2 = 110.70 C_{TDF} + 292.54\ C_{EMT}$.....(2) at 274 nm.

Where,

180.58 and 110.70 are absorptivities of TDF at ($ë_1$) and ($ë_2$) respectively.

301.125 and 292.54 are absorptivities of EMT at ($ë_1$) and ($ë_2$) respectively.

A_1 and A_2 are absorbances of mixed standard at ($ë_1$) and ($ë_2$) respectively.

C_{TDF} and C_{EMT} are the concentrations in g/100mL.

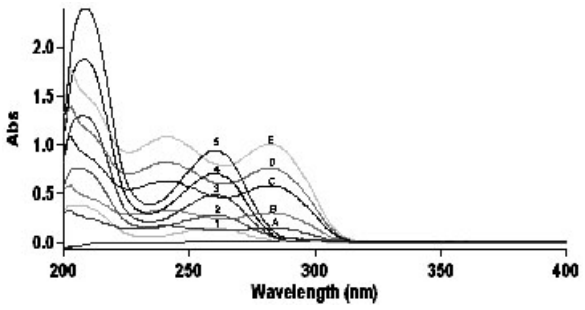

Fig : 1 *Overlain spectra of TDF: (1) 6µg/ml;(2) 12 µg/ml; (3) 24 µg/ml;(4) 36 µg/ml; (5) 48 µg/ml; and EMT (A) 4 µg/ml;(B) 8 µg/ml; (C) 16 µg/ml;(D)24 µg/ml;(E)32 µg/ml in methanol*

Method B-Absorbance ratio Method or Q Analysis Method

From the overlain spectra of TDF and EMT, two wavelengths were selected one at 255 nm, isoabsorptive point for both the drugs and the other at 274 nm, ëmax of Emtricitabine. The solutions of the standard and sample were prepared and measured in the same manner as in the previous method. The absorptivity value for both drugs at the selected wavelength were calculated and used for calculation. The concentration of two drugs in the mixture was calculated using following equations[13].

$$C_X=(Q_M-Q_Y/Q_X-Q_Y) \times A_1/ax_1 \quad \text{..... (3)}$$

$$C_Y= A_1/ax_1 - C_X \quad \text{..... (4)}$$

Where, A_1 and A_2 are absorbances of mixture at 255nm and 274nm, and ax_1 (215.95) and ay_1 (323.93) are absorptivity of TDF and EMT, respectively at 255 nm, ax_2 (110.70) and ay_2 (292.05) are the absorptivity of TDF and EMT, respectively at 274 nm and $Q_M= A_2 / A_1$, $Q_X = ax_2 / ax_1$, and $Q_Y= ay_2 / ay_1$.

RECOVERY STUDIES

The accuracy of both the proposed methods 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 ìg / ml of TDF and 8 ìg /ml of EMT.

RESULTS AND DISCUSSION

Simple, precise and accurate Simultaneous equation method and Absorbance ratio method were developed for the simultaneous estimation of TDF and EMT in combined dosage form.

METHOD A obeyed Beer's law in the concentration range of 6-48 ìg/ml and 4-32 ìg/ml for TDF and EMT, respectively. For TDF, the recovery study results ranged from 99.01% to 101.46 % with % RSD values ranging from 0.52% to 1.72 %. For EMT the recovery results ranged from 99.06 % to 99.97 %, with % RSD values ranging from 0.52 % to 0.99 %. The accuracy and reproducibility is evident from the data as results are close to 100 % and standard deviation is low.

METHOD B obeyed Beer's law in the concentration range of 6-48 ìg/ml and 4-32 µg/ml for TDF and EMT respectively. And For TDF, the recovery study results ranged from 99.69 % to 101.48 % with % RSD values ranging from 0.89% to 0.96 %. For EMT 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 the average % RSD was found to be 1.16, 0.59 and 0.34, 0.18 by method A and B, for TDF and EMT, respectively. Similarly the method was repeated on five different days and average % RSD was found to be 1.09 and 0.98; 1.40 and 1.19 for method A and B, for TDF and EMT, respectively. These

values confirm the intra and inter day precision of the methods.

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. Results of formulation analysis are presented in Table 1.

Table 1: *Results of formulation analysis*

Method	Drug	Label Claim (mg/tablet)	% of Label Estimated Claim	% R.S.D.
Simultaneous equation method	TDF	300	99.70	0.98
	EMT	200	99.92	1.23
Absorbance ratio Method	TDF	300	99.75	0.92
	EMT	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 In Process Quality Control Test and for routine simultaneous determination of TDF and EMT in tablet dosage form.

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