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DEVELOPMENT AND VALIDATION OF EMTRICITABINE AND TENOFOVIR DISOPROXIL FUMERATE IN PURE AND IN FIXED DOSE COMBINATION BY UV SPECTROPHOTOMETRY

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A simple, efficient, precise and accurate simultaneous equation method have been developed for the estimation of emtricitabine and tenofovir disoproxil fumerate in pure and in fixed dose combination. The method is based on the ultraviolet absorbance maxima of the above two drugs at 281 nm and 210 nm, respectively. Both the drugs obeyed Beer's law in the concentration range of 4 – 24 µg/ ml. The validity of the proposed method was assessed by applying the standard addition technique where the percentage recovery of the added standard was found to be 99.15 ± 0.2840 and 99.11 ± 0.2732 for emtricitabine and tenofovir disoproxil fumerate, respectively. The proposed procedure is rapid, simple, require no preliminary separation steps and can be used for routine analysis of both drugs in quality control laboratories. The results of analysis have also been validated statistically.

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Keywords: Emtricitabine, Tenofovir Disoproxil Fumerate, Simultaneous equation method, Method Validation

1. 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. EMT is the (-) enantiomer of thio analog of cytidine which differs from other cytidine analogs, in that it has a fluorine in 5 th 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] ethoxy] propyl] adenine fumarate [1]. It is also the nucleotidereverse transcriptase inhibitor (NRTIs) used in combination with other antiretrovirals for the treatment of HIV infection [2]. Both the drugs are not official in any of the pharmacopoeias. These are listed in the Merck Index and Martindale: The complete drug reference.

Literature survey reveals that few RP-HPLC [4, 5, 6] methods are reported for estimation of EMT, TEN and efavirenz in pharmaceutical formulation. TEN is estimated individually by UV [7], derivative - HPLC [8], Plasma RP-HPLC [9,10] and Plasma LC/MS/MS [11,12,13] methods. Similarly for EMT, HPLC with Fluorometric detection [14] in human plasma and Stability indicating liquid chromatographic [15] methods were reported. RP-HPLC [16] and LC-MS/MS [17] method is reported for simultaneous estimation of EMT and TEN in human plasma. HPTLC [18] method is also reported for simultaneous estimation of EMT and TEN in pharmaceutical formulation. But there is no method was reported for the simultaneous estimation of EMT and

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TEN in pure and in combined fixed dose combination. Hence, the purpose of this study was to develop simple, rapid, precise and accurate spectrophotometric (Vierodt's) [19] method for the simultaneous estimation of both the drugs in combined tablet dosage form.

2. Experimental

2.1. Materials and Equipments

EMT and TEN were gift samples from Strides Arcolab Ltd., Bangalore, India. The commercial fixed dose combination product Tavin - EM containing 200 mg of EMT and 300 mg of TEN (Hetero Drugs Limited, Hyderabad, India) was procured from the local market. Double distilled water was used as solvent in this study.

Shimadzu UV- 1700 UV-Visible spectrophotometer with 1cm matched quartz cells was used for the measurement of absorbance. Shimadzu-AX-200 electronic balance was used for weighing the samples. Class 'A' volumetric glassware were used.

2.1 Preparation of standard stock solution

Accurately, 100 mg of both EMT and TEN were weighed separately and transferred in to two different 50 ml volumetric flasks. Each drug was dissolved in double distilled water and made up to the mark with the same. The standard stock solutions contain 2000 μg/ ml of EMT and TEN. The working standard solution was prepared by diluting 5 ml in to 50 ml with distilled water for both the drugs. These solutions were further diluted separately to obtain (10 μg/ ml) of each drug individually.

2.2 Study of spectra and selection of wavelengths

Each standard drug solution was scanned between the range 200 – 400 nm in 1 cm cell against blank. After examining the overlain spectra, two drugs have different λ max and both the drugs showed the absorbance at each other's λmax. The wavelengths selected for the analysis of EMT was 281 nm where TEN has absorbance and the wavelength selected for the analysis of TEN was 210 nm where the EMT has absorbance.

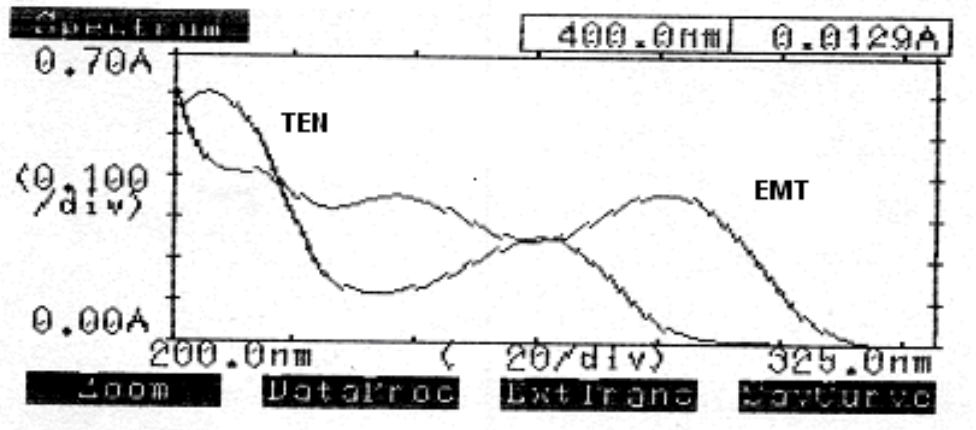

Fig 1. Overlain spectra for metoprolol tartrate and ramipril.

2.3. Preparation of calibration graph

1.0 – 6.0 ml of working standard solution of EMT and TEN were transferred into a series of six 50 ml volumetric flasks separately and made up to mark with distilled water. The absorbance of different concentration solutions was measured at 281 nm and 210 nm against blank. The calibration curve was plotted using concentration against absorbance. The solutions were found to be linear with the concentration range of 4 – 24 μg/ ml for both the drugs. The

optical characteristics such as correlation coefficient, slope, intercept, LOD, LOQ, Molar absorpitivity and Sandells sensitivity were calculated and are shown in Table 1.

2.4. Application of the proposed procedures for the simultaneous determination of EMT and TEN in laboratory prepared mixtures

Different mixtures of the two drugs were prepared by diluting different volumes of EMT and TEN with distilled water. The concentrations of both EMT and TEN were determined by measuring the absorbance of the prepared mixtures at 281 nm and 210 nm. From these absorbance values, the concentrations of EMT and TEN were determined using Simultaneous equation method.

2.5 Application of the proposed procedure for the determination of dosage form

Twenty tablets were weighed accurately and average weight was calculated. The tablets were triturated to a fine powder. An accurately weighed quantity of tablet powder equivalent to 100 mg of EMT was weighed and transferred into 50 ml volumetric flask and added a minimum quantity of distilled water to dissolve the substance and made up to the volume with the same. The solution was sonicated for 15

Table 1. Optical Characteristics of EMT and TEN.

Parameters	EMT (n = 6)		TEN (n = 6)	
	At 281 nm	At 210 nm	At 281 nm	At 210 nm
Beer's Law Limit (µg/ ml)	4 – 24	4 – 24	4 – 24	4 – 24
Molar absorptivity ($L\ mol^{-1}\ cm^{-1)}$	9300.46	10677.12	2512.18	37232.17
Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U	0.02663	0.02296	0.2538	0.01731
Correlation coefficient (r)				
Slope	0.99991	0.99978	0.99977	0.9997
Intercept				
LOD (µg/ ml)	0.03768	0.04369	0.00394	0.05790
LOQ (µg/ ml)	-0.00065	-0.00505	0.00009	0.00682
	0.13637	0.29449	0.34034	0.77371
	0.41326	0.89240	1.0313	2.3446

15 minutes and centrifuged for 15 minutes at 100 rpm. The supernatant liquid was separated and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 5 ml to 50 ml and 2.5 ml in to 50 ml with distilled water to obtain 10 µg/ ml solution of EMT which also contains 15 µg/ ml of TEN theoretically.

The samples containing two absorbing species EMT and TEN (X & Y) each of which absorbs at the λ max of the other. So the absorbance of each drugs were measured at both wavelengths λ_1 & λ_2 respectively. Both the drugs were determined by simultaneous method (Vierodt's method). The absorptivity of EMT (X) at λ_1 (281) and λ_2 (210) is ax_1 and ax_2, respectively. The absorptivity of TEN (Y) at λ_1 (281) and λ_2 (210) is $ay_1$and ay_2, respectively.

Absorptivity = absorbance / concentration

The absorbance of the sample (formulation) at λ_1 (281) and λ_2 (210) is A_1 and A_2 respectively. The total absorbance of the mixture is equal to the sum of individual absorbance of X and Y.

$$A_1 = ax_1bcx + ay_1bcy$$
$$A_2 = ax_2bcx + ay_2bcy$$

Cx – concentration of EMT
Cy – concentration of TEN

$$c_x = \frac{A_2 a_{y_1} - A_1 a_{y_2}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}}$$

$$c_y = \frac{A_2 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}}$$

By using this formula both the drugs EMT and TEN were estimated (Table 2). The procedure was repeated for six times.

Table2. Results of Analysis of Formulation Containing EMT and TEN.

Drug	Label Claim (mg/ tablet) n= 6	Amount Found		SD	% RSD	S.E
		mg/ tablet	mg/ tablet			
EMT	200	200.85	100.42	0.5912	0.5887	0.0417
TEN	300	302.62	100.87	0.3151	0.3123	0.0181

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2.6 Recovery studies

The accuracy of the proposed method was confirmed by recovery studies. To the pre analyzed formulation a known amount of raw material was added and it can be analyzed by proposed methods. To an accurately weighed quantity of the tablet powder equivalent to 100 mg of EMT, 20 mg, 40 mg and 60 mg of EMT and 15 mg, 30 mg and 45 mg of TEN raw materials were added into a series of 50 ml volumetric flasks. Then the procedure was followed as per the analysis of formulation. The amount of each drug recovered was calculated. The procedure was repeated for three times for each concentration. The results for recovery analysis are shown in table 3.

Table 3. Results for recovery analysis of EMT and TEN.

Drug	Amount taken (µg/ ml)	Amount added		% Recovery ± SD	% RSD
		%	(µg/ ml)		
EMT	10.04	40 80 120	4 8 12	98. 70 ± 0.4747 98.2 ± 0.2592 100.14 ±	0.4810 0.2628
TEN	15.13	20 40 60	3 6 9	0.1181 99.64 ± 0.4719 98.67 ± 0.0386 99.00 ± 0.3092	0.1180 0.4736 0.0391 0.3123

3. Results and discussion

3.1 Selection of solvent for analysis

The solubility of EMT and TEN were studied with different solvents like methanol, 2 - propanol, distilled water, 0.1 M hydrochloric acid and 0.1 M sodium hydroxide, etc. The drugs were soluble in distilled water, 0.1 M sodium hydroxide, methanol, ethanol, 2 - propanol and in 0.1 M hydrochloric acid. In methanol, ethanol and 2 – propanol, the stability of EMT is less. In distilled water, EMT is freely soluble and TEN is soluble. The stability of both the drugs was found to be 8 hours and six hours for EMT and TEN, respectively. At the end of these studies, distilled water was chosen, because of the time gain while preparing solutions and cost saving by eliminating the purchase and disposal of organic solvents.

3.2. Vierodt's method of simultaneous equation

The overlain spectra of EMT and TEN shows overlap, that prevents the use of direct absorbance measurements for determination of both the drugs in their mixtures. The λ max for EMT at 281 nm and for TEN at 210 nm were used for the analysis of these drugs by simultaneous equation method. They were linear in concentration range of 4 - 24μg/ ml for both the drugs. The r^2 values were found to be 0.99978 and 0.99991 for EMT and 0.99979 and 0.99977 for TEN at 281 nm and 210 nm, respectively. To study the mutual interference, if any, in the simultaneous estimation of EMT and TEN, synthetic mixtures containing various proportions of EMT and TEN were prepared and the contents were estimated by the proposed method. The percentage recovery varied from 99.74% to 101.28% for EMT and 99.79% to 101.11% for TEN indicating that no mutual interference for both the drugs. Commercial formulation containing EMT and TEN were analysed by proposed method. Six replicate analysis of formulation were carried out and the mean EMT content was 200.85 mg/ tablet and the mean content of TEN was 302.62 mg/tablet. The corresponding standard deviation was found to be 0.5912 for EMT and 0.3150 for TEN indicating that the method has required precision [20,21].

Further, the precision was confirmed by intermediate precision. The analysis of formulation was carried out for three times in the same day and on three successive days. The % RSD values for inter day and intraday analysis of formulation was found to be less than 2% The results for intermediate precision are shown in table 4.

Table 4. Intraday and Inter day Precision

Parameter	Amount found (%)		SD		% RSD	
	EMT	TEN	EMT	TEN	EMT	TEN
Intra day (n = 3)	100.29	100.83	0.7102	0.5684	0.70841	0.5637
Interday (n = 3)	100.5587	100.92	1.0968	0.3900	1.0910	0.3867

The accuracy of method was confirmed by recovery studies. To the pre analyzed formulation a known quantity of raw material was added in different concentrations. The amount of drug recovered was calculated and the percentage recovery was found to be in the range of 98.61% - 100.14% for EMT, 98.67 – 99.64% for TEN. The procedure was repeated for three times for each concentration and the % RSD values were calculated. The low %RSD values ensure that the excipients used in formulation are not interfering in the analysis of EMT and TEN.

4. Conclusion

Based on the results obtained, the proposed method is precise, accurate, and simple to perform. Also, no separation step is required. It is rapid and does not require any expensive or sophisticated apparatus in contrast with chromatographic technique. Hence, the proposed UV spectrophotometric method can be effectively used for the routine analysis of EMT and TEN in bulk and in combined tablet dosage form.

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