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Simultaneous Determination of Emtricitabine and Tenofovir Disoproxil Fumerate in Tablet Dosage Form by UV-Spectrophotometry

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Three accurate, precise, sensitive and economical procedures for the simultaneous estimation of emtricitabine and tenofovir disoproxil fumerate in tablet dosage form have been developed. The methods employed were absorbance ratio method (I), absorbance correction method (II) and first order derivative spectroscopic method (III). The first method employs 262 nm as λ_1 (isobestic point) and 281 nm as λ_2 (λ_{max} of emtricitabine) for formation of equations. The second method employs estimation of a drug concentration by selecting λ_{max} where the absorbance of these drugs is maximum. But in one of the drug's λ_{max}, the other drug has zero absorbance. So absorbance was corrected for interference. The λ_{max} for emtricitabine and tenofovir disoproxil fumerate is 296 and 281 nm, respectively. The third method is based on first order derivative spectroscopic method. Wavelengths 298.5 nm and 226.5 nm were selected for the estimation of the emtricitabine and tenofovir disoproxil fumerate, respectively. Both the drugs obey Beer's law in the concentration range 4-24 µg/mL. The results of analysis have been validated statistically and by recovery studies.

Key Words: Emtricitabine, Tenofovir disoproxil fumerate, Absorbance ratio method, Absorbance correction method, First order derivative spectroscopy.

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. Emtricitabine is the (-)enantiomer of thio analogue of cytidine which differs from other cytidine analogues, in that it has a fluorine in 5th position. Emtricitabine 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 bisisopropoxycarbonyloxymethyl 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[4-6] methods are reported for estimation of EMT, TDF and efavirenz in pharmaceutical formulation. Tenofovir is estimated individually by UV[7], derivative-HPLC[8], plasma RP-HPLC[9,10] and plasma LC/MS/MS[11-13] 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 TDF in human plasma. HPTLC[18] method is also reported for simultaneous estimation of EMT and TDF in pharmaceutical formulation. But there is no method was reported for the simultaneous estimation of EMT and TDF in pure and in combined fixed dose combination by UV spectrophotometry. Hence, the purpose of this study is to develop simple, rapid, precise and accurate spectrophotometric methods for the simultaneous estimation of both the drugs in combined tablet dosage form.

EXPERIMENTAL

The instrument used in the present study was Shimadzu double beam UV/visible spectrophotometer (Model UV-1700) with 1 cm matched quartz cells and slit width fixed at 2 nm. All weighing were done on electronic balance (Model Shimadzu AX -220).

Analytically pure samples of EMT and TDF were kindly supplied by Strides Arcolabs Ltd., Bangalore, India and used as such without further purification. The commercial fixed

dose combination product Tavin-EM containing 200 mg of EMT and 300 mg of TDF (Hetero Drugs Limited, Hyderabad, India) was procured from the local market. Class 'A' volumetric glassware were used.

Theory

Absorbance ratio method (method I): In this method, the isoabsorptive point for both the drugs was determined from the spectra of standard drug solutions, which was found to be 262 nm. The wavelengths selected were 262 nm as λ_1 (isoabsoptive point) and 281 nm (λ_{max} for EMT) as λ_2 for formation of equations as shown in Fig. 1. The Q-values for both the drugs were calculated and were found to be 0.1575 for TDF and 1.4978 for EMT. Absorptivity (a) for both the drugs at isoabsorptive point was found to be 25.1090. The equations obtained for the estimation of concentration are

$$C_{EMT} = \frac{Q_0 - 0.1575}{1.4978 - 0.1575} \times \frac{A}{25.1090}$$

$$C_{TEN} = \frac{Q_0 - 1.4978}{0.1575 - 1.4978} \times \frac{A}{25.1090}$$

where, A is the absorbance of sample at isoabsorptive point (λ_1).

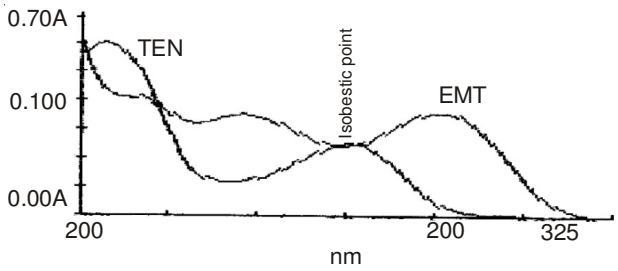

Fig. 1. Zero order overlain spectra of EMT (10 µg/ mL) and TDF (10 µg/ mL) and indicating Isoabsorptive point

Absorbance correction method (method II): Emtricitabine and tenofovir disoproxil fumarate showed λ_{max} at 296 and 261 nm, respectively. As their λ_{max} differ more then 20 nm, absorption correction method was tried for their simultaneous estimation in formulation. Emtricitabine also showed absorbance at 261 nm and give interference in determination of TDF. Quantitative estimation of TDF was carried out by subtracting interference of EMT using experimentally calculated absorption factor.

First order derivative spectroscopic method (method III): The third method is based on first order derivative spectroscopy to overcome spectral interference from other drug. First order derivative spectra of both the drugs were recorded (Fig. 2). It was observed that EMT showed dA/dλ zero at 226.5 nm in contrast to TDF that has considerable dA/dλ at this wavelength. Further, TDF has zero dA/dλ at 298.5 nm while at this wavelength EMT has significant dA/dλ. Therefore these two wavelengths were employed for the estimation of EMT and TDF without any interference. The calibration curves were plotted at these two wavelengths of concentrations against dA/dλ within the above mentioned range. The equations of line obtained to determine concentrations of EMT and TDF are as follows:

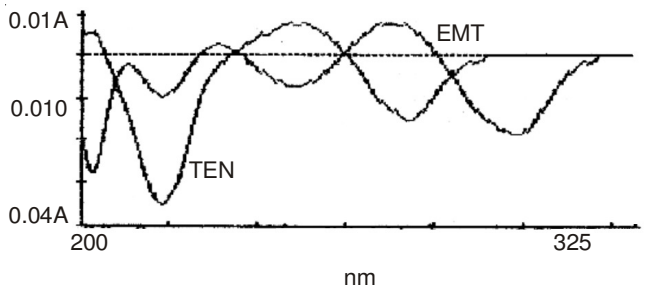

Fig. 2. First order derivative overlain spectra of EMT(10 µg/mL) and TDF (10 µg/ mL)

$$C_{EMT} = dA/d\lambda_{298.5} - 0.00006/0.00078$$

$$C_{TDF} = dA/d\lambda_{226.5} - (-0.00018)/0.00086$$

Preparation of standard stock solutions: Standard stock solutions were prepared by dissolving separately 20 mg of each drug in 100 mL of double distilled water to get concentration of 0.2 mg/mL. 2.5 mL of the stock solution was further diluted to 50 mL with distilled water to get a working standard solution of 10 µg/mL of both EMT and TDF and scanned in the wavelength range of 200-400 nm.

1.0-6.0 mL of working standard solution of EMT and TDF 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 their selected wavelengths 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.

Application of the proposed procedures for the simultaneous determination of EMT and TDF in laboratory prepared mixtures: Different mixtures of the two drugs were prepared by diluting different volumes of EMT and TDF with distilled water. The concentrations of both EMT and TDF were determined by measuring the absorbance of the prepared mixtures at their selected wavelengths for all the methods. From these absorbance values, the concentrations of EMT and TDF were determined.

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 50 mg of EMT was weighed and transferred into 100 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 min and centrifuged for 15 min 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 1 mL to 50 mL with distilled water to obtain 10 µg/mL solution of EMT which also contains 15 µg/mL of TDF theoretically. The absorbance was measured at the selected wavelengths and the amount was calculated based on their corresponding method.

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 50 mg of EMT, 20 mg, 40 mg and 60 mg of EMT and 15 mg, 30 mg and 45 mg of TDF 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.

RESULTS AND DISCUSSION

Three simple, precise and accurate methods were developed for the simultaneous estimation of EMT and TDF in pure and in combined dosage forms. The methods applied for the analysis are Absorbance ratio method, Absorbance correction method and first derivative spectrophotometric method. Under experimental conditions described the linearity was found in the concentration range of 4-24 µg/ mL for all the methods. The optical characteristics like correlation coefficient, slope, intercept, LOD and LOQ were calculated and all the values were found to be within the limit. The proposed methods was evaluated by the assay (n = 6) of commercially available tablets containing EMT and TDF. The results of assay are presented in Table-1. Results of recovery studies are shown in Table-2. The accuracy and reproducibility is evident from the data as results are close to 100 % and low standard deviation. The proposed methods are simple, economical, rapid, precise and accurate. Hence these can be used for routine analysis of EMT and TDF in tablet formulation.

TABLE-1
RESULTS OF COMMERCIAL FORMULATION ANALYSIS

Method	Label claim (mg/tabet)	% Label claim estimated* (mean ± SD)	RSD (%)
I	LAM-200	100.84 ± 0.4770	0.4730
	TEN-300	100.30 ± 0.4738	0.4378
II	LAM-200	99.92 ± 0.6595	0.6691
	TEN-300	99.86 ± 0.6190	0.6199
III	LAM-200	100.18 ± 1.5496	1.5467
	TEN-300	100.29 ± 0.6326	0.6308

*Mean of six determinations, RSD is relative standard deviation.

TABLE-2
RECOVERY STUDIES OF EMT AND TEN

Drug	Conc. of drug added (µg/mL)	% Recovery (mean ± SD)		
		Method I	Method II	Method III
EMT	4	101.15±0.7576	101.30±0.1601	101.12±0.0444
	8	101.85±0.1121	101.84±0.1716	100.85±0.1285
	12	98.82±0.1313	101.24±0.1849	101.95±0.1513
TEN	3	102.60±0.6711	100.80±0.0286	99.87±0.0296
	6	102.61±0.6405	102.88±0.1746	101.16±0.0680
	9	101.95±0.1017	100.62±0.1560	101.80±0.0335

Conclusion

The validated spectrophotometric methods employed here proved to be simple, economical, rapid, precise and accurate. Thus these can be used for routine simultaneous determination of EMT and TDF in tablet dosage form instead of processing and analyzing each drug separately.

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