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UV-Spectrophotometric Absorbance Correction Method For The Simultaneous Estimation Of Tenofovir Disoproxil Fumerate And Emtricitabine In Combined Tablet Dosage Form

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Abstract: A new, simple, accurate and sensitive U.V-Spectrophotometric absorbance correction method has been developed and validated for simultaneous estimation of Tenofovir disoproxil fumerate (TDF) & Emtricitabine (EMT) in a combined tablet dosage form. 50% v/v Methanol was used as solvent. The wavelengths selected for the absorption correction method were 260 nm & 290 nm. The method was found to be linear between the range of 5-25 µg/ml for TDF and7-35 µg/ml for EMT. The mean percentage recovery was found in the range of 99.24%-100.42% and 100.03-101.04% for TDF and EMT respectively at three different levels of standard additions. The precision (intra-day, inter-day) of method were found within limits (RSD <2%). Thus the proposed method was simple, precise, economic, rapid and accurate and can be successfully applied for simultaneous determination of TDF and EMT in combined tablet dosage form.
Keywords : Absorbance correction method, Tenofovir disoproxil fumerate, Emtricitabine.

INTRODUCTION AND EXPERIMENTAL

Tenofovir disoproxil fumerate is a white to off white crystalline powder. It is a salt of bis(isopropyloxycarbonyloxymethyl ester of (*R*)-9-(2 phosphonomethoxypropyl)adenine with fumaric acid. It is soluble in water : methanol (1:1) with empirical formula $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$ having molecular weight of 635.5. currently it is used as an anti-HIV agent.it comes under the category of Nucleoside and Nucleotide Reverse Transcriptase Inhibitors. The structure of TDF was shown in the figure-1[1-4].

Emtricitabine is a white to off-white crystalline powder. Chemically it is 4-amino-5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1*H*)-pyrimidone with empirical formula $C_8H_{10}FN_3O_3S$ having molecular weight of 247.3 it is Soluble in water and sparingly soluble in methanol. It comes under the category of Anti-HIV Agent. The structure of emt was shown in the figure-2[1-4].

Literature survey reveals that few methods like HPLC, UV-Spectrophotometric, HPTLC were available for the simultaneous estimation of Tenofovir disoproxil fumerate and Emtricitabine in combined tablet dosage form[5-10]. So far no method was reported by absorbance correction method of quantitative estimation of TDF and EMT by UV-Spectrophotometric method as there is a spectral interference. So, in the current study it is designed to develop a new, simple, accurate, less time consuming method of analysis for the simultaneous estimation of TDF and EMT in combined tablet dosage form by absorbance correction method (UV-Spectrophotometry)

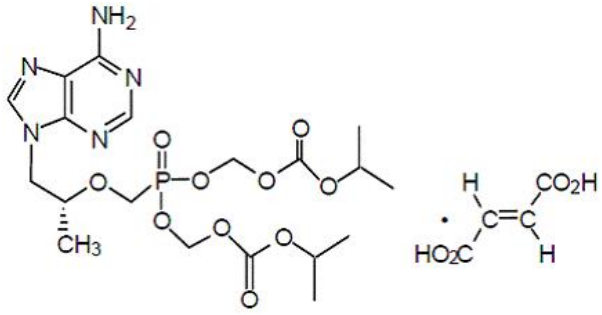


Figure-1 (Structure of tenofovir disoproxil fumerate)

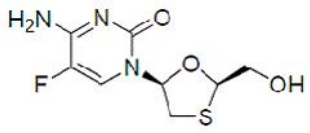


Figure-2 (Structure of emtricitabine

MATERIALS & METHODS

Materials: Tenofovir disoproxil fumerate (working standard), Emtricitabine (working standard), Methanol, Distilled water, TENVIR-EM tablets (formulation)

Instruments used: Digital balance – shimadzu**,** UV-Visible spectrophotometer – UV-1700 shimadzu

Method:

1. Solvent : methanol (50% v/v)

2. Identification of spectrum :

Accurately weighed quantities (100 mg) of TDF and EMT were taken in 100ml standard flasks, dissolved separately by adding 50 ml methanol and volumes were made up with distilled water (1000 µg/ml). These solutions were used as working standards. Aliquot portions of stock solutions of TDF and EMT were diluted appropriately with distilled water to obtain concentration 30 µg/ml of TDF and 20 µg/ml of EMT. The working standard solutions were scanned from 200 to 400 nm to select the wavelengths for estimation. From the overlain spectrum shown in Fig.1, the wavelength selected for estimation of TDF was 260 nm, where EMT has no significant absorbance and for EMT it was 290 nm, where absorbance of EMT is corrected. Different binary mixture solutions of TNF and EMT were then run in entire range from 200 to 400 nm. The drugs obey Beer's law in the concentration range of 5 to 30 µg/ml and 7 to 35 µg/ml for TDF & EMT respectively. All the optical characteristics were tabulated in Table-1.

Table-1 (Optical characteristics of TDF & EMT)

	TDF	**EMT**
λ max(nm)	260	281
Beer's law limit (µg/ml)	5-25	7-35
Molar absorption (liter,mole-1 cm-1)	11845.72	4809.98
Sandell's sensitivity	0.053879	0.068966
Slope	0.0185	0.0145
Intercept	0.0008	0.0495
Regression equation (y=mx+c)	Y=0.0185x + 0.0008	Y = 0.0145x + 0.0495
Correlation coefficient (r^2)	0.999	0.999
Standard error	0.0027	0.0027
LOD (µg/ml)	0.485	0.367
LOQ (µg/ml)	0.147	1.930

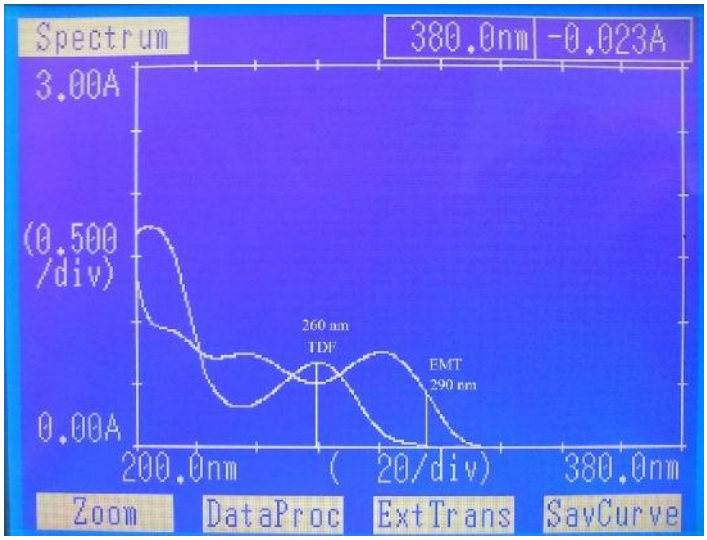
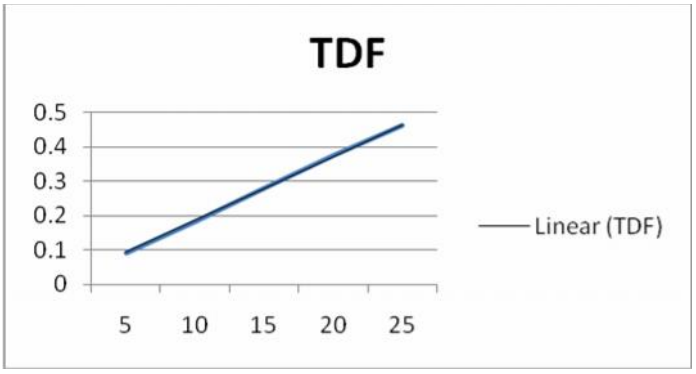


Figure-3 (UV-Spectrum Of TDF & EMT)



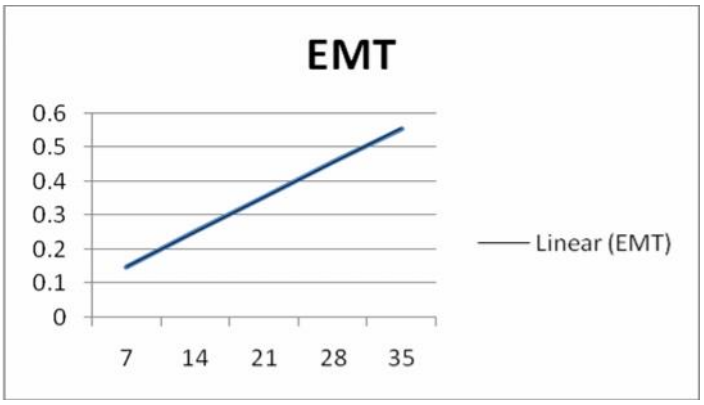


Figure-4 (Linearities of TDF & EMT)

Analysis of TDF (300mg) and EMT (200mg) mixture:

The A 1%,1cm values of TDF & EMT were calculated at 260 and 290 according to the formula, A=abc.
TDF (260 nm) = 185
TDF (290 nm) = 0
EMT (260 nm) = 182
EMT (290 nm) = 240

Mixture of TDF (300mg) & EMT (200mg) was taken in 100ml volumetric flask and then diluted to get final concentration of 3µg/ml and 2µg/ml of TDF & EMT respectively in the solution. The resulting solution was scanned at 260 and 290 nm. The absorbances were found to be 0.096 at 260 nm and 0.048 at 290 nm. Similar ratio mixture concentrations were prepared and their respective absorbances were tabulated (table-3). Results for the assay were tabulated (Table 4 & 5).

Quantitative estimation of TDF & EMT in the mixture was carried out by using following formulae:

1) Concentration of EMT at 290nm

$c = A/ab$

Where, A = absorbance of mixture at 290
a = A(1%,1cm) of EMT at 290nm
b = path length (= 1)
c = concentration
= 0.048/240
= 0.0002 gms/100ml = 2 µg/ml

2) Absorbance of EMT at 260

$A = abc$

Where, A= absorbance of EMT alone at 260 nm
a = A(1%,1cm) of EMT at 260nm
b = path length
c = concentration of EMT at 290nm

A = 182 X 0.0002
= 0.0364

3) Calculation of concentration of TNF from the corrected absorbance at 260nm

Corrected absorbance $= A_{260}$ (mixture) – A_{260} (EMT)

= 0.096 – 0.0364

= 0.0596

Concentration of TDF from corrected absorbance ,

c = A/ab (a= A1%,1cm of TDF at 260 nm)
= 0.0596/185 = 0.0003 gm/100ml
= 3 µg/ml

Table-2 (Linearity)

TDF		**EMT**	
Conc. (µg/ml)	**Absorbance**	**Conc. (µg/ml)**	**Absorbance**
5	0.093	7	0.149
10	0.185	14	0.253
15	0.281	21	0.356
20	0.375	28	0.458
25	0.462	35	0.554

Table-3

Conc. of mixture TDF/EMT (µg/ml)	Absorbance of mixture at λ 260 nm	Absorbance of mixture at λ 290 nm
3/2	0.096	0.048
5/4	0.178	0.096
7/6	0.262	0.144
9/8	0.347	0.194
11/10	0.437	0.248

Table-4 (Quantification of TENVIR-EM tablets by UV-absorbance correction method (TDF))

Formulation	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
Tenvir-em	1	300	301.7209	100.43				
	2	300	292.9806	98.25				
	3	300	296.4314	99.11	99.07	0.7815	0.7888	0.0217
	4	300	296.6860	99.17				
	5	300	296.4224	99.11				
	6	300	293.4053	98.35				

Table-5 (Quantification of TENVIR-EM tablets by uv-absorbance correction method (EMT))

Formulation	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%) ± SD	% R.S.D	S.E
Tenvir-em	1	200	402.4878	100.62			
	2	200	402.3744	100.59			
	3	200	399.9746	99.99	100.41 ±	0.7015	0.0196
	4	200	406.4196	101.61	0.70		
	5	200	398.3380	99.59			
	6	200	400.2978	100.07			

METHOD VALIDATION[11]

The method was validated according to ICH Q2B guidelines validation of analytical procedures for the determination of the following validation parameters. Results for linearity were tabulated in Table 2.

Accuracy

To check the accuracy of the developed methods and to study the interference of the excipients in the formulation, analytical recovery experiments were carried out using the standard addition method at 80, 100, 120% levels. The percentage recovery was calculated from the total and the amount of drug yields. The results revealed no interference of excipients.(Table 6 & 7).

Precision

The precision of the methods could be evaluated by the determination of the following parameters such as repeatability, intermediate precision. Accordingly, six dilutions in three replicates were analyzed in the same day, in two different days and by two analysts for day to day and analyst to analyst variation. The low value of standard deviation showed that the methods were precise (Table 8 & 9).

Sensitivity

The limit of detection (LOD) was calculated using the following equation LOD=3.3σ/s where σ is standard deviation of y intercept of the calibration curve (n=6) and s is the slope of regression equation.

Table-6 (Accuracy (recovery studies of TDF))

Drug (level of % recovery)	Sample No	Amount present (µg/ml)	Amount added (µg/ml)	Amount found* (µg/ml)	Amount recovered (µg/ml)	% Recovered	S.D
TDF (80%)	1 2 3	10.00 10.00 10.00	8.00 8.00 8.00	17.989 18.011 18.103	7.989 8.011 8.103	99.86 100.13 101.28	0.754
					Mean	100.42	
TDF (100%)	1 2 3	10.00 10.00 10.00	10.00 10.00 10.00	19.909 20.031 19.911	9.909 10.031 9.911	99.09 100.31 99.11	0.698
					Mean	99.50	
TDF (120%)	1 2 3	10.00 10.00 10.00	12.00 12.00 12.00	21.816 22.118 21.903	11.816 12.118 11.903	98.46 100.09 99.19	0.816
					Mean	99.24	

Table-7 (Accuracy (recovery studies of TDF))

Drug (level of % recovery)	Sample No	Amount present (µg/ml)	Amount added (µg/ml)	Amount found* (µg/ml)	Amount recovered (µg/ml)	% Recovered	S.D
EMT (80%)	1 2 3	7.00 7.00 7.00	5.60 5.60 5.60	12.701 12.611 12.503	5.701 5.611 5.503	100.79 100.08 99.23	0.781
					Mean	100.03	
EMT (100%)	1 2 3	7.00 7.00 7.00	7.00 7.00 7.00	14.091 13.991 14.132	7.091 6.991 7.132	101.30 99.87 101.80	1.001
					Mean	100.99	
EMT (120%)	1 2 3	7.00 7.00 7.00	8.40 8.40 8.40	15.516 15.399 15.549	8.516 8.399 8.549	101.38 99.98 101.77	0.941
					Mean	101.04	

Table-8 (Precision studies: Intraday analysis of formulation (TENVIR-EM))

Drug	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
TDF	1 2 3	300 300 300	297.9455 306.0884 299.4248	99.49 101.52 99.86	100.29	1.0811	1.0780	0.1201
EMT	1 2 3	200 200 200	206.4196 199.3380 200.2978	101.61 99.59 100.07	100.42	1.0554	1.0509	0.1173

Table-9 (Precision studies: Interday analysis of formulation (TENVIR-EM))

Drug	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
TDF	1 2 3	300 300 300	295.4918 293.8593 297.7405	98.87 98.45 99.44	98.92	0.4968	0.5023	0.0552
EMT	1 2 3	200 200 200	202.4878 202.3744 199.9746	100.62 100.59 99.99	100.40	0.3554	0.3540	0.0395

SUMMARY & CONCLUSION

The method was found to be linear between the range of 5-25 µg/ml for TDF and7-35 µg/ml for EMT. The mean percentage recovery was found in the range of 99.24%-100.42% and 100.03-101.04% for TDF and EMT at three different levels of standard additions. The precision (intra-day, inter-day) of methods were found within limits (RSD <2%).

It could be concluded from the results obtained in the present investigation that the two methods for the simultaneous estimation of TDF & EMT in tablet dosage form are simple, rapid, accurate, precise and economical and can be used, successfully in the quality control of pharmaceutical formulations and other routine laboratory analysis.

REFERENCES

1. Indian Pharmacopoeia 2007.
2. www.rxlist.com
3. www.drugbank.com
4. http://www.druglib.com/druginfo/truvada/
5. Vishnu P. Choudhari et. al., Spectrophotometric simultaneous determination of Tenofovir disoproxil fumarate and Emtricitabine in combined tablet dosage form by ratio derivative, first order derivative and absorbance corrected methods and its application to dissolution study, Pharmaceutical Methods, 2011, 2(1),48-52.
6. K. Anand kumar et. al., Development and validation of emtricitabine and tenofovir disoproxil fumerate in pure and in fixed dose combination by uv Spectrophotometry, Digest Journal of Nanomaterials and Biostructures, 2011,6(3),1085-1090.
7. Rajesh Sharma and Pooja Gupta, A Validated RP - HPLC Method for Simulataneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in a Tablet Dosage Form, Eurasian J. Anal. Chem, 2009, 4(3),276-284.
8. Bapatla J N N Sai., et. al., Simultaneous RP-HPLC method for the estimation of Emitricitabine and Tenofovir Disoproxil Fumarate in Pharmaceutical dosage forms, Pelagia Research Library, 2011, 2(5),163-168.
9. Patel Suhel, et. al., Spectrophotometric Method Development and Validation for Simultaneous Estimation of Tenofoir disoproxil fumarate and Emtricitabine in Bulk Drug and Tablet Dosage form, International Journal of Pharmaceutical and Clinical Research, 2009; 1(1),28-30.
10. N Appala Raju and Shabana Begum, Simultaneous RP-HPLC Method for the Estimation of the Emtricitabine, Tenofovir Disoproxil Fumerate and Efavirenz in Tablet Dosage Forms, Research J. Pharm. and Tec, 2008,1(4).
11. Validation of analytical procedures: Methodology, ICH harmonized tritatrite guidelines 1996.

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