CHEMOMETRIC ASSISTED UV SPECTROPHOTOMETRIC METHOD FOR QUANTIFICATION OF EMTRICITABINE AND TENOFOVIR DISOPROXIL FUMARATE

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ABSTRACT

The aim of this study was to verify the ability of the UV spectrophotometric method for the simultaneous determination of emtricitabine and tenofovir disoproxil fumarate in tablet dosage form using the Principal Component Regression (PCR) and Partial Least Squares (PLS) multivariate calibration methods. Double beam UV-Vis spectrophotometer (Jasco V-730) with 1 cm quartz cell with 1 nm data interval and scanning speed of 400 nm min^{-1} was used in the study. The optimized wavelength range selected was 225-275 nm. The data obtained was processed using Unscrambler X (10.5) (64bit) software. The developed models showed good results over the concentration range of 6-36 µg mL^{-1} for tenofovir disoproxil fumarate and 4-24 µg mL^{-1} for emtricitabine with co-relation coefficient greater than 0.995 and % RSD less than 2%. The accuracy studies show % recovery within limits. The method was validated as per ICH Q2(R1) guideline.

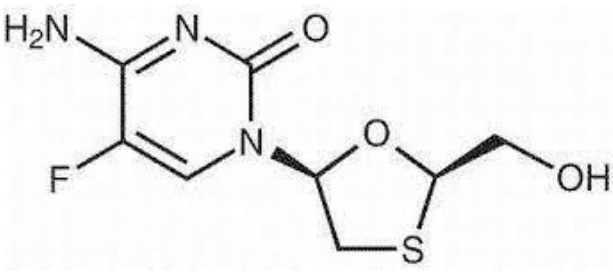
Keywords: Chemometric, emtricitabine, tenofovir disoproxil fumarate, PLS, PCR

INTRODUCTION

Tenofovir disoproxil fumarate (TDF) is a nucleotide analog reverse transcriptase inhibitor used in the treatment of Hepatitis B infection and in the management of HIV infection[1,2]. Emtricitabine (EMT) is a nucleoside reverse transcriptase inhibitor used for the treatment and prophylaxis of HIV[3,4]. The structures of both the drugs are given in Fig. 1.

Literature survey revealed few spectrophotometric methods reported for simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate for resolving mixtures of compounds with overlapping spectra, such as simultaneous equation method[5], baseline manipulation method[6], ratio spectra derivative spectroscopy[7], area under curve[8] and Vierodt's method[9]. Several RP-HPLC methods were found in the literature for the simultaneous estimation of both drugs in bulk and tablet dosage form[10-16]. HPTLC methods were also applied for the determination of these drugs in binary mixture[17,18].

No multivariate calibration methods for simultaneous estimation of these drugs were found in the literature,



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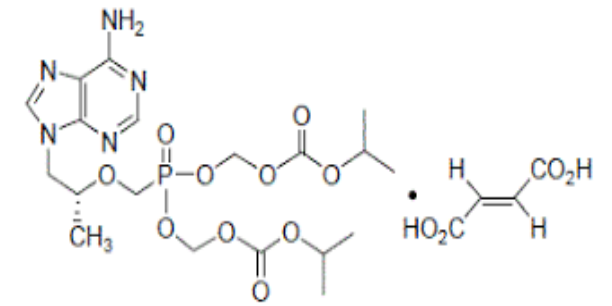

b)

Fig. 1: Structures of a) Emtricitabine b) Tenofovir disoproxil fumarate

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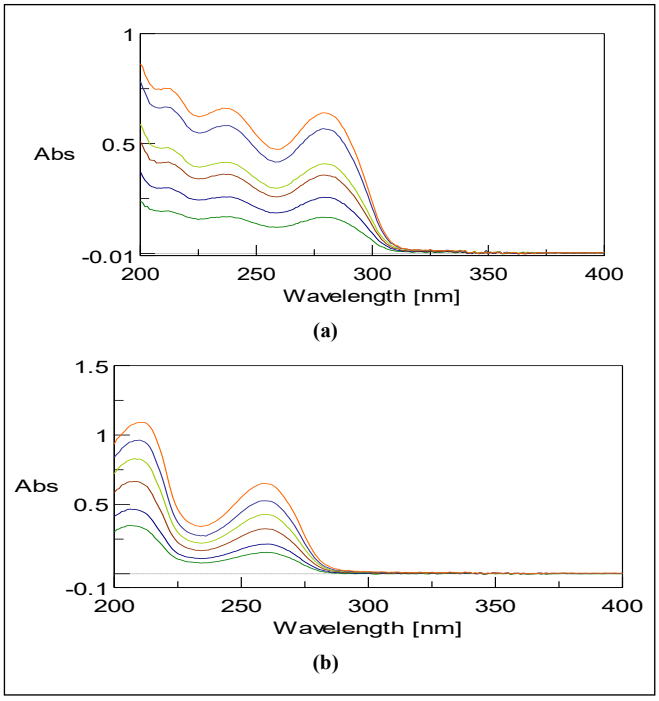

Fig. 2: Overlay spectra of linearity data for a) emtricitabine (4-24 µg mL^{-1}) b) tenofovir disoproxil fumarate (6-36 µg mL^{-1})

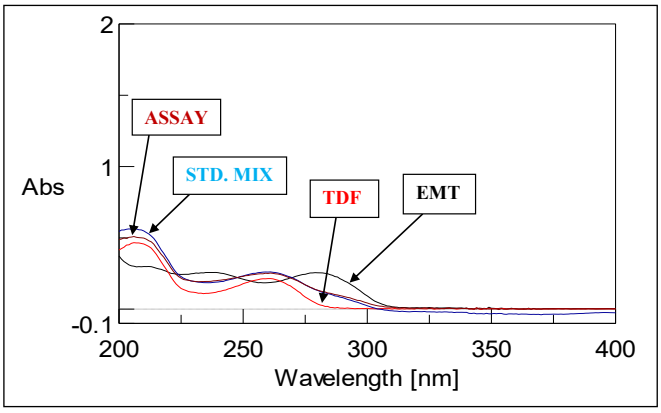

Fig. 3: Overlay spectra of EMT (8 µg mL^{-1}), TDF (12 µg mL^{-1}) + Standard mixture (8+12 µg mL^{-1}) + Assay solution (8+12 µg mL^{-1})

so an attempt was made to develop fast and accurate chemometric methods of analysis.

MATERIALS AND METHODS

Instrumentation

A double beam UV-Vis spectrophotometer (Jasco V-730) with 1 cm quartz cell was used for measurements.

Table I: Composition of calibration and validation set

Mixture*	Tenofovir disoproxil fumarate (µg mL^{-1})	Emtricitabine (µg mL^{-1})	Mixture*	Tenofovir disoproxil fumarate (µg mL^{-1})	Emtricitabine (µg mL^{-1})
1	6	4	19	24	4
2	6	8	20	24	8
3	6	12	21	24	12
4	6	16	22	24	16
5	6	20	23	24	20
6	6	24	24	24	24
7	12	4	25	30	4
8	12	8	26	30	8
9	12	12	27	30	12
10	12	16	28	30	16
11	12	20	29	30	20
12	12	24	30	30	24
13	18	4	31	36	4
14	18	8	32	36	8
15	18	12	33	36	12
16	18	16	34	36	16
17	18	20	35	36	20
18	18	24	36	36	24

**1-26 used as calibration set and 27-36 used as validation set*

The spectra were recorded in the range of 200-400 nm with 1 nm data interval and scanning speed of 400 nm min^{-1}. The optimized data in the range 225-275 nm was processed using Unscrambler X software (10.5 version) (64bit).

One component calibration

Linear ranges were selected over the 6-36 µg mL^{-1} and 4-24 µg mL^{-1} concentration range for TDF and EMT; respectively. Overlay spectra of linearity data is shown in Fig. 2. Fig. 3 shows overlay spectra of EMT (8 µg mL^{-1}),

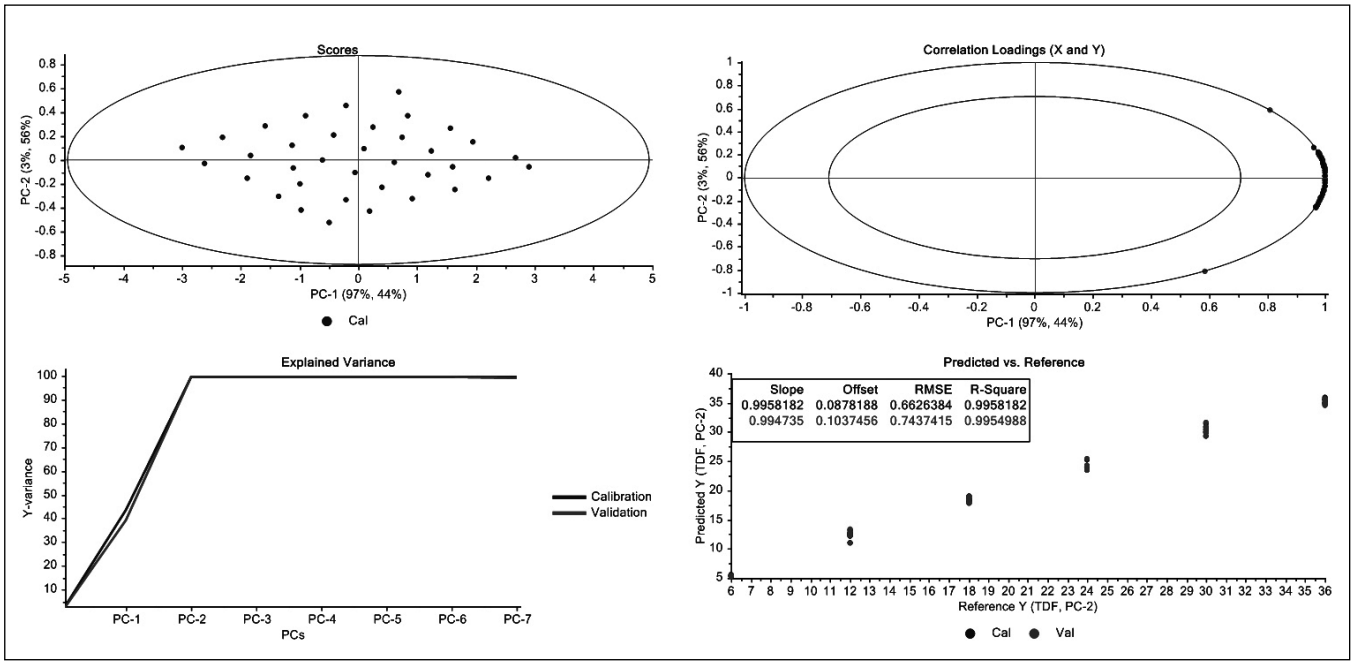

Fig. 4: PCR model for all 36 mixtures

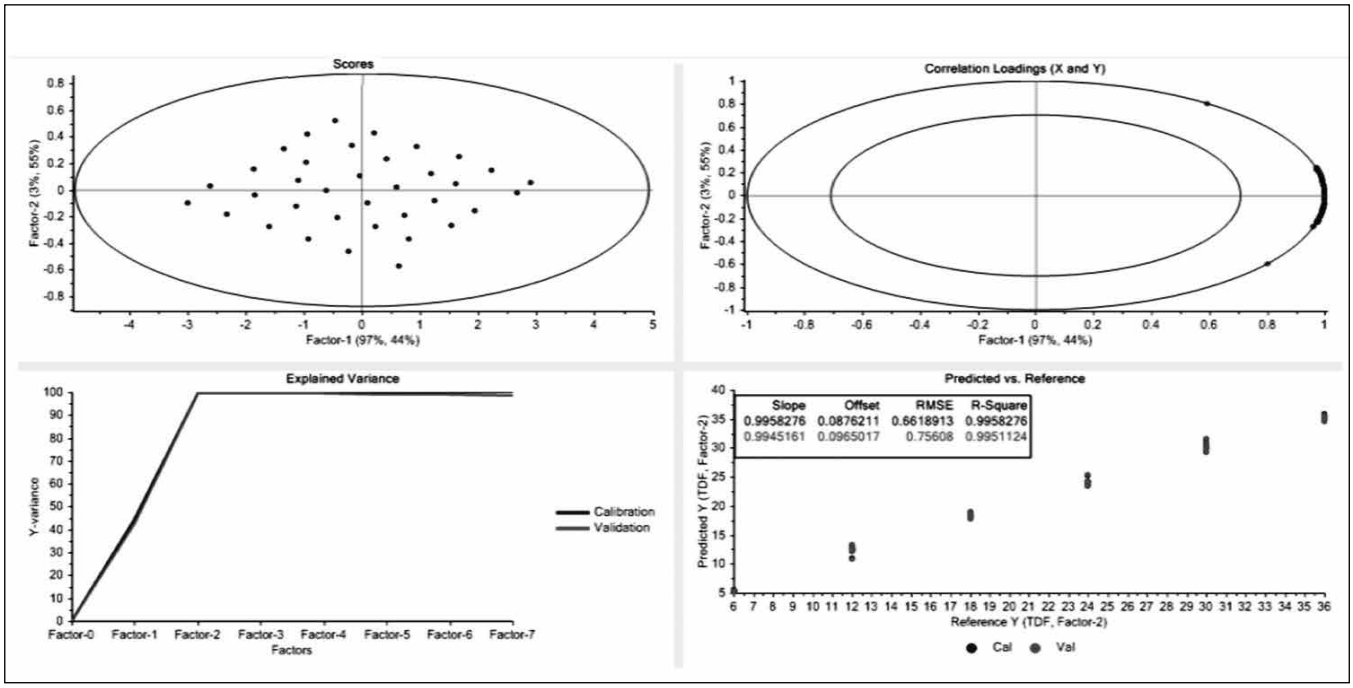

Fig. 5: PLS model for all 36 mixtures

Table II: PCR and PLS model data with predicted results for assay study of EMT and TDF

Sr. No.	EMT actual (µg mL^{-1})	EMT predicted (µg mL^{-1})	% R	TDF actual (µg mL^{-1})	TDF predicted (µg mL^{-1})	% R
1	8	8.16	102.03	12	12.24	102.03
2	8	8.16	102.00	12	12.24	102.00
3	8	8.15	101.98	12	12.23	101.98
4	8	8.16	102.02	12	12.24	102.02
5	8	8.16	102.03	12	12.24	102.03
6	8	8.16	102.04	12	12.24	102.04
		Mean	102.01	Mean		102.02
		SD	0.02	SD		0.02
		%RSD	0.02	%RSD		0.01

Table III: PCR and PLS model data with predicted results for accuracy study of EMT and TDF

Level	Standard concentration (µg mL^{-1})	Amount added (µg mL^{-1})	Total concentration (µg mL^{-1})	Predicted concentration (µg mL^{-1})	%Recovery	%RSD
EMT (Accuracy)						
	8	4	12	12.00	100.04	
50%	8	4	12	12.00	100.02	0.008
	8	4	12	12.00	100.03	
	8	8	16	15.95	99.72	
100%	8	8	16	15.95	99.73	0.11
	8	8	16	15.98	99.92	
	8	12	20	20.07	100.35	
150%	8	12	20	20.36	100.81	0.75
	8	12	20	20.29	101.47	
TDF (Accuracy)						
	12	6	18	18.00	100.01	
50%	12	6	18	17.99	99.95	0.034
	12	6	18	18.00	100.01	
	12	12	24	23.96	99.83	
100%	12	12	24	24.10	100.45	0.379
	12	12	24	23.94	99.96	
	12	18	30	30.00	100.01	
150%	12	18	30	30.13	100.46	0.491
	12	18	30	29.84	99.47	

Table IV: PCR and PLS model data with predicted results for intra-day precision and inter-day precision study of EMT

Sr. No.	Reference (µg mL^{-1})	Predicted (µg mL^{-1})	% Recovery	Average	%RSD
EMT (Intra-day Precision)					
1	8	7.73	96.65		
2	8	7.72	96.56	97.01	0.73
3	8	7.82	97.83		
4	12	12.05	100.43		
5	12	12.18	101.54	100.74	0.69
6	12	12.02	100.24		
7	16	15.81	98.82		
8	16	15.89	99.33	99.63	1.00
9	16	16.12	100.75		
EMT (Inter-day Precision)					
1	8	7.92	99.03		
2	8	7.95	99.46	99.22	0.21
3	8	7.93	99.19		
4	12	12.26	102.20		
5	12	12.27	102.30	102.14	0.81
6	12	12.23	101.93		
7	16	15.99	99.94		
8	16	15.87	99.23	99.47	0.40
9	16	15.88	99.26		

TDF (12 µg mL^{-1}) , mixture (8+12 µg mL^{-1}) and, assay solution.

Standard stock solution preparation

25 mg of drug was dissolved in 25 mL of distilled water to get a solution of concentration 1000 µg mL^{-1} for both drugs. 2.5 mL of this solution was diluted upto 25 mL with distilled water to give a standard stock solution of 100 µg mL^{-1}.

Working solution preparation

Standard stock solution of 100 µg mL^{-1} was diluted with distilled water to obtain final concentrations of 6-36 µg mL^{-1} for tenofovir disoproxil fumarate and 4-24 µg mL^{-1} for emtricitabine as working standard solutions.

Construction of calibration and validation set

The calibration set used 26 mixtures with a linear concentration range of 6–36 µg mL^{-1} for TDF and 4–24 µg mL^{-1} for EMT and 10 mixtures were used for the validation series as shown in Table I. Spectra were recorded between 225-275 nm with 1 nm data intervals. The spectra were saved in ASCII (.txt) format and then entered in an Excel spreadsheet to create the model. After that, principal component regressions (PCR) and partial least squares (PLS) models were developed using Unscrambler X software. Figs. 4 and 5 show the PCR and PLS models for all 36 models' mixtures.

Assay

20 tablets (TAVIN-EM) were weighed and crushed. Powder corresponding to 10 mg of EMT (15 mg of TDF) was weighed accurately and diluted to 10 mL with distilled water. This solution was sonicated and then filtered. From the filtrate, 2.5 mL was pipetted out and diluted to 25mL with distilled water. 0.8 mL was further diluted to 10 mL to have of 8 µg mL^{-1} of emtricitabine and 12 µg mL^{-1} of tenofovir disoproxil fumarate as final

Table V: PCR and PLS model data with predicted results for intra-day precision and inter-day study of TDF

Sr. No.	Reference (µg mL^{-1})	Predicted (µg mL^{-1})	% Recovery	Average	%RSD
TDF (Intra-day Precision)					
1	12	11.93	99.45		
2	12	11.93	99.45	99.45	0.01
3	12	11.93	99.47		
4	18	18.13	100.72		
5	18	18.15	100.85	100.74	0.09
6	18	18.11	100.66		
7	24	23.92	99.70		
8	24	23.92	99.68	99.71	0.03
9	24	23.93	99.74		
TDF (Inter-day Precision)					
1	12	11.88	99.00		
2	12	11.96	99.67	99.45	0.38
3	12	11.96	99.67		
4	18	18.09	100.52		
5	18	18.14	100.81	100.75	0.21
6	18	18.16	100.94		
7	24	23.92	99.67		
8	24	23.93	99.73	99.70	0.02
9	24	23.92	99.70		

concentration. The procedure was repeated 6 times. The spectral measurement obtained was put in software to get results. Table II shows predicted results of assay by developed model.

RESULTS AND DISCUSSION

Specificity

Specificity of method was assessed by overlay of spectra of standard mixture and spectra of assay solution of same concentrations (Fig. 3). As the spectra get exactly overlaid in the selected wavelength range, this indicates no interference from excipients or any other component.

Accuracy study

Accuracy was studied at 3 levels of assay concentration. EMT and TDF from standard solution were spiked into sample solution and the spectra were recorded. Developed PCR and PLS models were used for prediction of concentrations. Table III shows predicted results for accuracy study of EMT and TDF.

Precision

It was established by intraday and interday variability studies. In intraday studies, 3 replicates were analyzed at 3 different concentrations. For interday precision studies, 3 different concentrations were analyzed on 3 different days. Percentage RSD was calculated for both intraday and interday studies. The results of the intraday and interday studies are shown in Tables IV and V, respectively.

LOD (detection limit) and LOQ (quantitation limit)

Although LOD and LOQ value determination is not mandatory for assay procedures as per ICH guidelines[19],

they are calculated from linearity data to check sensitivity of method. LOD and LOQ were calculated using the formula LOD = 3.3σ/S and LOQ = 10 σ/S where, σ = Standard deviation of y-intercept of the linearity equation and S = slope of the analyzed calibration curve. LOD and LOQ were found to be 0.22 µg mL^{-1} and 0.69 µg mL^{-1}; respectively for EMT. LOD and LOQ were found to be 0.07 µg mL^{-1} and 0.23 µg mL^{-1}, respectively, for TDF.

CONCLUSION

A study was conducted on the use of a UV spectrophotometer in combination with PCR and PLS for the simultaneous determination of emtricitabine and tenofovir disoproxil fumarate in a binary mixture. The obtained result confirmed the appropriateness of the described method for the easy, accurate and precise analysis of emtricitabine and tenofovir disoproxil fumarate in pharmaceutical preparations.

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