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Development and Validation of UV-Visible Spectrophotometric Method for Estimation of Emtricitabine and Tenofovir in Bulk and Dosage Form

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ABSTRACT

Simple, rapid, sensitive, precise and specific UV spectrophotometric method for the determination of Emtricitabine (EMB) and Tenofovir (TEN) in bulk drug and pharmaceutical dosage form was developed and validated**.** A simple double beam UV spectrophotometric method has been developed and validated with different parameters such as linearity, precision, repeatability, limit of detection (LOD), Limit of Quantification (LOQ), accuracy as per ICH guidelines. UV-visible spectrophotometric method, measurement of absorption at the maximum wavelength in 10 ml acetonitrile and volume make with water solvent system as reference EMB and TEN were found to be at 277 nm and 259 nm respectively. The drug obeyed the Beer's law and showed good correlation. Beer's law was obeyed in concentration range 1-5µg/ml for Emtricitabine and 1.5-7.5µg/ml for Tenofovir respectively with correlation coefficient was 0.999. The LOD and LOQ of EMB were found to be 0.598 (µg/ml) and 8.1630 (µg/ml), TEN were found to be 1.79 (µg/ml) and 1.814 (µg/ml), respectively. Percentage assay of EMB and TEN in tablets. The proposed method is precise, accurate and reproducible and can be used for routine analysis of EMB and TEN in bulk and tablet dosage form.

INTRODUCTION

Emtricitabine and Tenofovir (Figure 1) are antiretroviral drugs used for the treatment of HIV. [1] Forstavir - EM is the combination of the two drugs containing 150mg of EMB and 300mg of TEN. Emtricitabine is chemically 4-Amino-5-fluoro-1-[2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-pyrimidin-2-one [2, 3]. It is a nucleoside reverse transcriptase inhibitor. Chemically Tenofovir is 1-(6-Aminopurin-9-yl)-prapan-2-yl-oxymethylphosphonic acid. [3, 4] It is a nucleotide analog reverse transcriptase inhibitor. Extensive literature survey revealed that only LC-MS/MS [5] and RP-HPL [6] methods for the determination of EMB and TEN in human plasma, RP-HPLC [7] for determination of TEN in plasma, LC/MS/MS for determination of plasma TEN concentrations, [8] LC-MS method for determination of plasma TEN concentrations [9] and HPLC with fluorimetric detection for determination of EMB in human plasma [10] have been reported so far. There is no evidence of the determination of the drug combination by UV Spectrophotometry. Thus the present study is to develop simple, precise and accurate UV Spectrophotometric methods for the quantification of EMB and TEN in combined dosage form.

MATERIALS AND METHODS

Reagents and Materials

A Shimadzu UV/Visible double beam spectrophotometer (Model 1700) with 1cm matched quartz cells was used in present study for multi-component analysis. EMB and TEN in the form of gift samples were kindly supplied by R. S. I .T. C, Jalgaon respectively. AR grade methanol used for UV method and Methanol: Water (0.1% OPA), prepared in solvent double distilled water was used as solvent throughout the study. A combination of EMB (20 mg) and TEN (30 mg) in tablet formulation was procured from the local pharmacy (Tavin- EM, Emcure Pvt. Ltd.

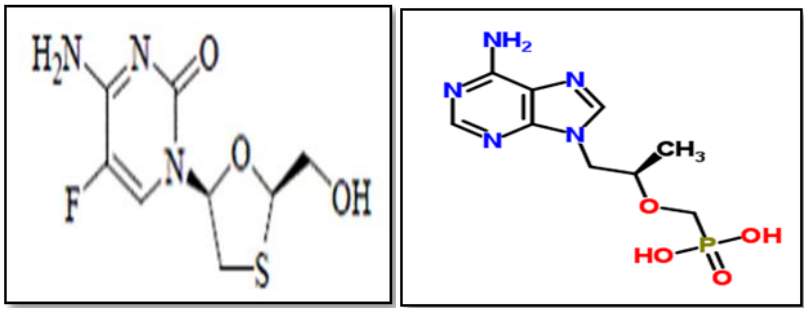
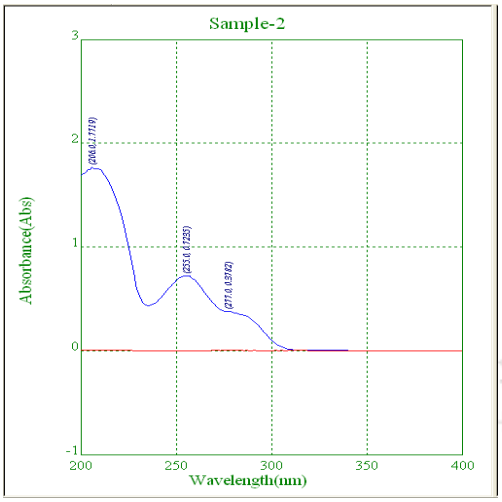


Fig. 1: Structure of Emtricitabine and Tenofovir

Preparation of standard stock solution

Accurately weight and transfer 20mg Emtricitabine and Tenofovir 30mg working standard into 10 ml volumetric flask as about diluents Methanol completely and make volume up to the mark with the same solvent to get 1000µg/ml standard (stock solution) and 15 min sonicate to dissolve it and remove the unwanted gas, further an aliquots portion of EMB and TEN stock solution in ratio of 70:30 were mixed in volumetric flask in 10 ml and volume was adjusted up to mark with mobile phase from the resulting solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with methanol: water (0.1% OPA), prepared in solvent .Result as shown as; (Figure 2, 3 and 4).



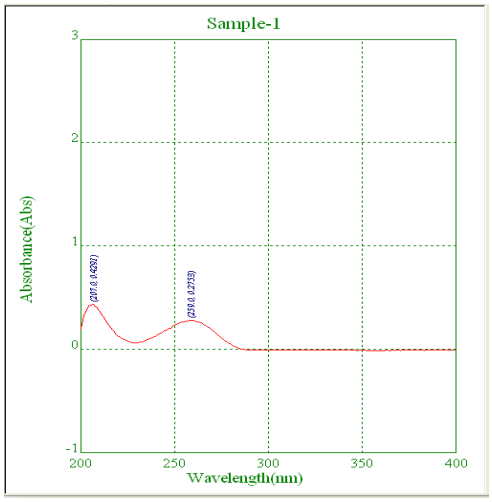


Figure 2: UV Spectrum of Emtricitabine **Figure 3: UV Spectrum of Tenofovir**

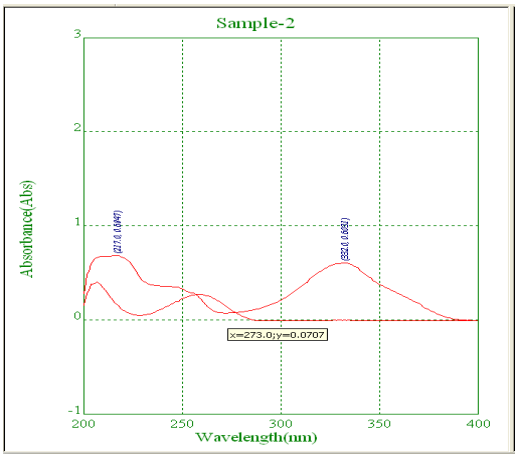


Figure 4: Iso-absorptive point of EMB and TEN

Procedure for calibration curve of Emtricitabine and Tenofovir:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. From the freshly prepared standard stock solution, pipette out 10mg Emtricitabine and 10mg Tenofovir in 10ml of volumetric flask and diluted with mobile phase. From it 0.1, 0.2, 0.3, 0.4 and 0.5ml of solution were pipetted out in 10 ml volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 10, 20, 30, 40, 50 µg/ml of Emtricitabine and 15, 30, 45, 60, 75µg/ml of Tenofovir (Table 1 and 2). The respective linear equation for Emtricitabine was y= 0.155x - 0.019 and Tenofovir equation y = 0.085x + 0.07 where x is the concentration and y is area of peak. The correlation coefficient was 0.997. The calibration curve of Emtricitabine and Tenofovir is depicted in (Figure 5 and 6) respectively. [11-12].

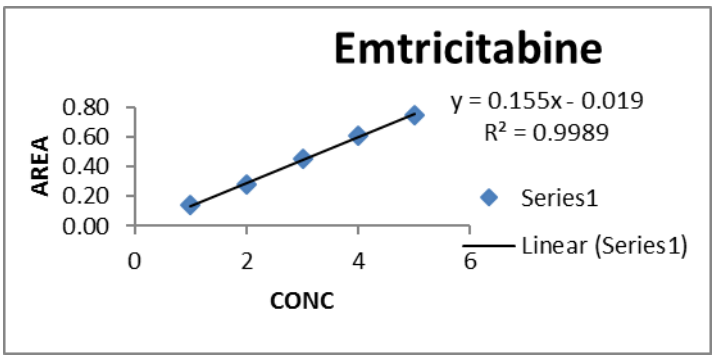


Fig. 5: Calibration curve of Emtricitabine

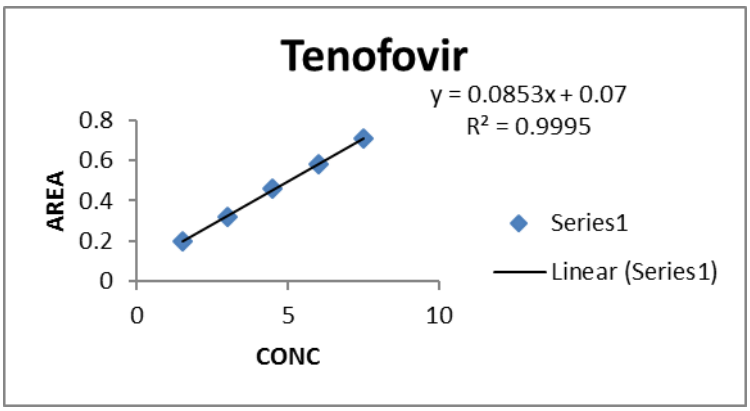


Fig. 6: Calibration curve of Tenofovir

Table 1: Linearity data for Emtricitabine

Method	Conc. µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
UV Method	1	0.1445	0.1454	0.14	0.00	0.44
	2	0.2878	0.2901	0.28	0.00	0.58
	3	0.4521	0.4532	0.45	0.00	0.17
	4	0.6134	0.6192	0.62	0.00	0.69
	5	0.7534	0.7554	0.25	0.00	0.19
	Equation		y= 0.155x-0.019			
	R^2		0.997			

Table 2: Linearity data for Tenofovir

Method	Conc. µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
UV Method	1	0.2019	0.2011	0.20	0.00	0.28
	2	0.3213	0.3248	0.32	0.00	0.77
	3	0.4616	0.4685	0.47	0.00	1.05
	4	0.5854	0.5814	0.58	0.00	0.49
	5	0.7111	0.7134	0.71	0.00	0.23
	Equation		y = 0.085+0.07			
	R^2		0.999			

Selection of detection wavelength:

Standard solutions were scanned in the range of 200-400 nm, against 10 ml methanol and volume make with water solvent system as reference EMB (Figure 2) and TEN (Figure 3) were showed absorbance maxima (lambda max) at 277 nm and 259 nm respectively (Fig. 4). If Two EMB and TEN sample Interact with this point are called isosbestic point. The detection of wavelength in isosbestic point in 273 nm.

Procedure for analysis of tablet formulation

Weigh 20 Emtricitabine and Tenofovir combination tablets weight 14.98gms and calculated the average weight of powder 0.749gm, accurately weigh and transfer the sample equivalent to 49.93 mg EMB and TEN into 10 ml volumetric flask. Add about 10ml MEOH of diluents and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45 µm filter. Further pipette 0.1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents.(10 µg/ml). The simple chromatogram of test EMB and TEN showed in (Figure 7). The amounts of EMB and TEN per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet assay for % label claim for % RSD calculated Result was shown in (Table 3).

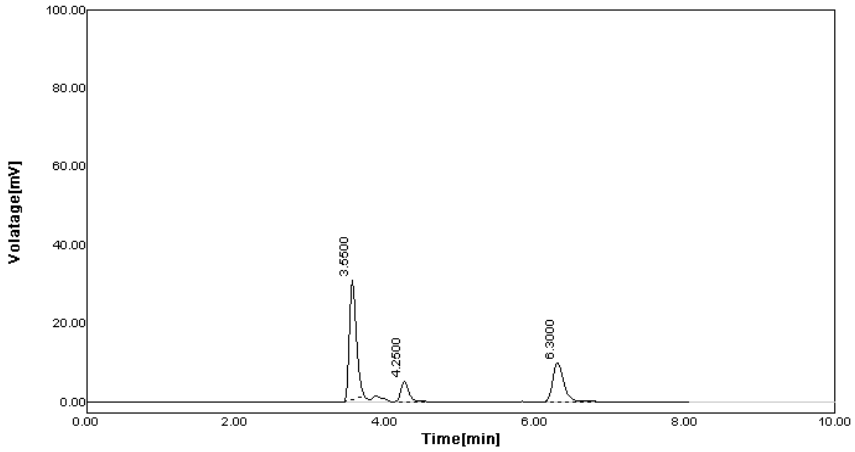


Fig. 7: Chromatogram for Marketed Formulation

Table 3: Analysis of marketed formulation

Assay	Drug	Label claimed	amt. found	% label claim	SD	% RSD
UV Method	EMB	4	4.01	101.82	0.01	0.05
	TEN	6	60.06	101.00	0.01	0.64
	EMB	4	4.06	101.62	0.35	0.37
	TEN	6	6.08	101.28	0.06	0.61

Method validation

The proposed methods were validated accordance to ICHQ2 (R1) guidelines for linearity, precision, accuracy, limit of detection, limit of quantification.

RESULTS

Linearity and Range:

The linearity of proposed methods was evaluated by linear regression analysis, which was calculated by least square method. Calibration standards were prepared by spiking required volume of working standard solution10mg EMB and 10mg TEN in 10ml of volumetric flask and diluted with mobile phase. From it 0.1, 0.2, 0.3, 0.4 and 0.5ml of solution were pipetted out in 10 ml volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 10, 20, 30, 40, 50 µg/ml of Emtricitabine and 15, 30, 45, 60, 75µg/ml of Tenofovir (Table 1 and 2). The absorbance of the drugs was measured. A calibration curve was plotted between absorbance of the drug against the concentration of the drug. These results were shown there was an excellent correlation between absorbance and analyte concentration of drug versus peak area is depicted in (Figure 5 and 6) respectively.

Accuracy:

The accuracy of the methods was determined at three different concentration levels i.e. 80%, 100% and 120% in triplicate for each drug as per ICH guidelines. From the total amount of drug found, the percentage recovery was fond in a range of 99-101% (Table 4 and 5).

Table 4: Result of recovery data for Emtricitabine and Tenofovir

Method	Drug	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Absorbance Mean*± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
UV Method	EMB	80%	2	1.6	5.42 ±2.56	2.42± 1.15	101.0± 0.25
		100%	2	2	3.96 ±0.01	2.47 ±0.70	98.49± 0.01
		120%	2	2.4	4.44± 0.00	2.44± 0.00	101.8± 0.06
	TEN	80%	3	2.4	5.38 ±0.01	2.38 ±0.01	99.26± 0.28
		100%	3	3	3.96 ±0.01	2.47 ±0.70	98.49± 0.36
		120%	3	3.6	6.60 ±0.01	3.60 ±0.01	101.07± 0.36

***mean of each 3 reading for UV method**

Table 5: Statistical validation of recovery studies Emtricitabine and Tenofovir

Method	Level of recovery (%)	Drug	Mean % recovery	S. D.*	% RSD
UV Method	80%	EMB	2.42	1.15	0.30
		TEN	5.38	0.01	0.13
	100%	EMB	2.47	0.70	0.28
		TEN	6.03	0.02	0.32
	120%	EMB	2.44	0.00	0.09
		TEN	6.60	0.01	0.12

***Denotes average of three determinations for UV method**

Precision:

Precision was studied to find out intra and inter-day variations in the test method of EMB and TEN. Intra-day precision was determined by analyzing three concentration in three replicate measurements of within linearity range of drugs on three different times in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days. Intraday and Inter-day Precision studies on UV method for EMB and TEN which shows the high precision % amount in between 98% to 100% indicates to the analytical method that concluded (Table 6).

Table 6: Result of Intra-day and inter-day precision studies on UV method for Emtricitabine and Tenofovir

Method	Drug	Conc. (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	% amt. found	Mean± SD	% amt found
UV Method	EMB	2	0.29± 0.00	99.67	0.30 ±0.00	99.01
		3	0.45 ±0.00	100.86	0.50 ±0.00	102.10
		4	0.61 ±0.00	101.45	0.02± 0.01	101.00
	TEN	3	0.32 ±0.00	100.00	0.33 ±0.00	101.66
		4.5	0.46 ±0.00	101.00	0.45 ±0.00	101.96
		6	0.60 ±0.00	102.02	0.58±0.00	100.00

***Mean of each 3 reading for UV method**

Limit of detection (LOD) and Limit of Quantification (LOQ):

LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions. LOQ is the lowest concentration of the analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions.

Repeatability:

Repeatability studies on UV method for EMB and TEN were found to be, the % RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded **(**Table 7).

Table 7: Repeatability studies on UV method for Emtricitabine and Tenofovir

Method	Conc. of EMB and TEN (mg/ml)	Peak area	Amount found (mg)	% Amount found
UV Method for EMB	4	0.6145	4.08	102.19
	4	0.6124	4.07	101.83
	4	0.6118	4.06	101.74
	4	0.6159	4.10	102.40
	4	0.6185	4.10	102.50
		Mean	**4.08**	
		SD	**0.02**	
		%RSD	**0.42**	
UV Method for TEN	6	0.5817	6.02	100.33
	6	0.8511	5.93	98.88
	6	0.5721	6.02	100.41
	6	0.5823	6.03	100.45
	6	0.5834	6.04	10.66
		Mean	**6.01**	**100.15**
		SD	**0.04**	**0.72**
		%RSD	**0.74**	**0.72**

DISCUSSION

The proposed methods for simultaneous estimation of EMB and TEN in tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration curves for EMB and TEN were linear with correlation coefficients (r^2) values in the range of 98.49–101.7 at all the selected wavelengths and the values were the average of three readings. The values of %RSD are within the prescribed limit of 2 %, showing the high precision of methods and recovery was close to 100% for both the drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of EMB and TEN in formulations.

The proposed method utilize two medium i.e. Methanol + Water [Acetic acid 0.1% (OPA)] (70 :30%) v/v) 273nm,1.0ml, pH 3.0 gave adequate retention time at 3.166 min and 7.500min. with good peak shape (Theoretical plates of 4085.3of EMB & 11229.0 of TEN. The comparison of method with already published two methods shows that the developed method is more accurate and economic as compared to other two methods further the method complies with detection of drugs as per their label claim also no further derivatization or modification in spectra is required so the proposed method can be said as simple accurate and economic as compared to other published method.

CONCLUSION

The developed UV methods were found to be more accurate, precise and reproducible. The analysis of tablets containing two drugs gave the satisfactory results. The statistical parameter of these methods showed good results. The recovery studies revealed excellent accuracy and high precision of the method. The methods were found to be simple & time-saving. All proposed methods could be applied for routine analysis in quality control laboratories.

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