Research Article

A Stability Indicating Method for Estimating Emtricitabine and Tenofovir Disoproxil Fumarate Simultaneously in Bulk and Combined Dosage Form by UV Spectrophotometery

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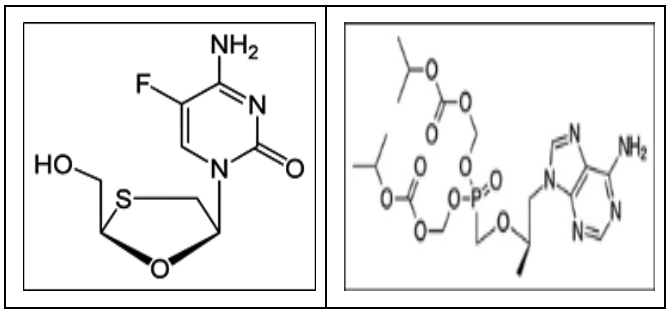
ABSTRACT

A Stability indicating method for estimating Emtricitabine (EMT) and Tenofovir disoproxil fumarate (TDF) simultaneously in bulk and combined dosage form using UV Spectrophotometry has been developed and validated. The method is based on simultaneous equation method where λmax was 284nm and 260nm were selected to determine Emtricitabine and Tenofovir disoproxil fumarate, respectively and methanol was used as solvent. Both the drugs obey Beer's law in the concentration range of 10-30µg/ml. The proposed method was validated as per the ICH Q1A (R2) guidelines. The recovery studies also carried out and found 98% and 99%. The method was found to be precise, the relative standard deviation for inter-day and intra-day precision was found to be less than 2%. From the experiment we conclude that Emtricitabine degrades most when subjected to heat, acidic and basic medium and slightly degrades when subjected to UV light. Tenofovir disoproxil fumarate mostly degraded when exposed to heat and in acidic medium and but slightly degraded in the basic medium and when exposed to UV light.

Keywords: UV Spectrophotometry, Simultaneous equation method, Stability studies, Validation.

INTRODUCTION

Emtricitabine (EMT) and Tenofovir disoproxil fumarate (TDF) belongs to a class of antiretroviral drug. It is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. EMT is an analogue of cytidine. The drug works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. This drug is prescribed in combination with other drugs in the management of HIV infection as well as in Hepatitis B therapy[1,2]. Structure of EMT and TDF was shown in figure 1.



(A) **(B)**

Figure 1: (A) Structure of Emtricitabine (B) Structure of Tenofovir Disoproxil Fumarate

A literature survey revealed that few chromatographic and spectrophotometric methods are reported for determination of Emtricitabine and Tenofovir Disoproxil Fumarate individually and in combination[3-7]. So, attempt has been made to develop stability indicating method for estimating Emtricitabine and Tenofovir Disoproxil Fumarate simultaneously. The objective of work was to develop a method with precise, accurate and sensitive stability indicating for estimation EMT and TDF.

MATERIALS AND METHODS

Material

Gift sample of TDF and EMT were procured from Hetero Healthcare Ltd., India. The commercial fixed dose combination product (Tenof-EM containing 300 mg TDF and 200 mg EMT) was procured from the local Pharmacy.

Equipment

PG Pharmaceutical, UV spectrophotometer with 1 cm matched quartz cells was used for the measurement of the absorbance.

Chemicals

Methanol, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide.

Preparation of standard stock solution

Standard solution of EMT and TDF was prepared separately by dissolving 10 mg of each drug in 10 ml of methanol (1000mcg/ml). Further dilution was made by adding 1 ml of the stock solution to 10 ml standard flask and making up the volume with the methanol (100mcg/ml).

Preparation of 10µg/ml solution

From above stock solution 10 µg/ml is prepared by diluting 1ml to 10ml with solvent. The wavelength of maximum absorption (λmax) of the drugs in methanol was scanned using UV-Visible spectrophotometer within the wavelength region of 200-400 nm against methanol as blank.

Development of The Method

***Simultaneous Equation Method*[8-9]**

From the stock solution of 100mcg/ml, working standard solutions of drugs were prepared by appropriate dilution and were scanned in entire UV range to determine the max. EMT has λ max of 284 nm while TDF has λ max at 260nm respectively shown in Figure 2, 3.

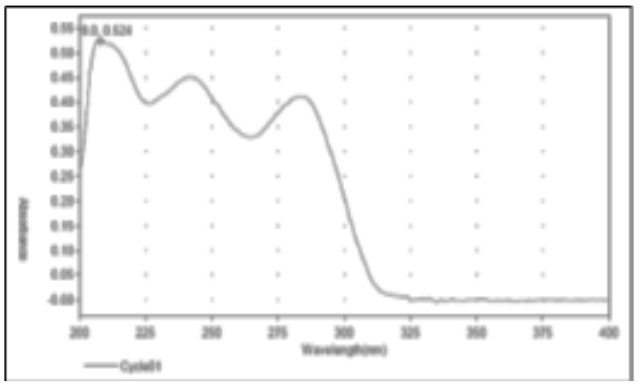


Figure 2: UV spectrum of Emtricitabine in methanol at 284nm

Standard solutions of concentration10-30 µg/mlwere prepared for EMT and TDF. The absorbanceof these standard solutions was measured at 284 nm and 260 nm and calibration curves were plotted at these wavelengths. Two simultaneous equations (in two variables C1 and C2) were formed using these absorptivity coefficient values.Absorptivity value was given table 1, 2.

At λ1 (284nm) $A1 = a_{X1}\, b\, C_Y + a_{Y1}\, b\, C_Y$ (1)

At λ2 (260nm) $A2 = a_{X2}\, b\, C_Y + a_{Y2}\, b\, C_Y$ (2)

A1 = (0.0173) C1 + (0.0276) C2 (3)

A2 = (0.0197) C1 + (0.00322) C2 (4)

Where, C1 and C2 are the concentrations of EMT and TDF measured in µg/ml, in sample solutions. A1 and A2 are the absorbance of mixture at selected wavelengths 284 nm and 260 nm respectively. By applying the Cramer's rule to equation 1 and 2, the concentration C_{EMT} and C_{TDF}, can be obtained as follows,

$C_x = A2\, a_{Y1} - A1\, a_{Y2} / a_{X2}\, a_{Y1} - a_{X1}\, a_{Y2}$

$C_Y = A1\, a_{X2} - A2\, a_{X1} / a_{X2}\, a_{Y1} - a_{X1}\, a_{Y2}$

C_{EMT}= A2 (0.0276) - A1 (0.0032)/0.00066

C_{TDF}=A1 (0.01970) - A2 (0.0173)/0.00066

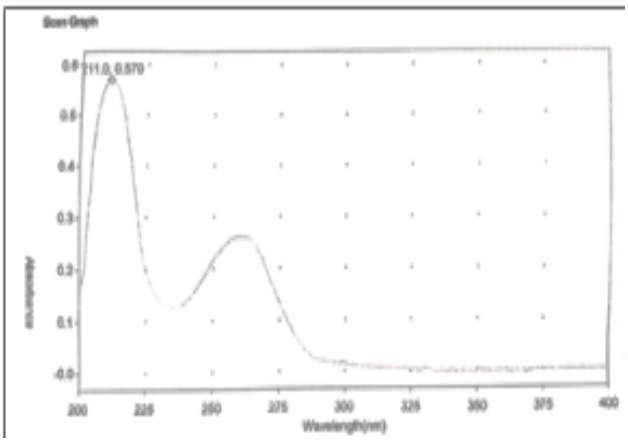


Figure 3: UV spectrum of Tenofovir Disoproxil Fumarate at 260nm

Table 1: Absorptivity values for Emtricitabine

Concentration (mcg/ml)	Absorbance		Absorptivity	
	284nm	260nm	284nm	260nm
10	0.239	0.224	0.0239	0.0224
15	0.33	0.31	0.0022	0.0206
20	0.415	0.405	0.02075	0.02025
25	0.504	0.465	0.02016	0.0186
30	0.595	0.510	0.0198	0.017
Mean			0.017368 (a_{x1})	0.01977 (a_{x2})

Table 2: Absorptivity values for Tenofovir disoproxil fumarate

Concentration (mcg/ml)	Absorbance		Absorptivity	
	284nm	260nm	284nm	260nm
10	0.026	0.271	0.0028	0.027
15	0.048	0.404	0.0032	0.0269
20	0.064	0.558	0.0032	0.0279
25	0..089	0.683	0.0035	0.0273
30	0.108	0.838	0.0036	0.0289
Mean			0.00322(a_{y2})	0.0276(a_{y1})

STABILITY STUDIES

Preparation of 0.1N Hydrochloric Acid

Take 20ml of distilled water and then add 8.3 ml of analytical grade hydrochloric acid and make upto the volume to 1000 ml with distilled water.

Preparation of 0.1N Sodium Hydroxide

Take 4 gm of Sodium hydroxide and transfer it in 100ml volumetric flask and dissolved it in small quantity of water and finally make up to the mark of the flask with distilled water.

Stress degradation studies[10]

Preparation of solution

Take 10mg of EMT and TDF in 10ml of different volumetric flask then add small quantity of methanol and dissolve it. Finally make the volume up to the mark with methanol.

Photolytic degradation

Specific amount of EMT and TDF was weighed accurately &kept into the UV chamber for three days. After three days 10mg drug was weighed and made 1000µg/ml solution with specified solvent i.e. methanol. 5ml of solution of EMT and TDF in different test tube and add 5ml of methanol, left it for 30 minutes. Determine the absorbance of the solution by UV spectrophotometer at wavelengths of 284 nm and 260 nm.

Thermal degradation

A specific amount of drug was taken in a Petridish which was previously cleaned and dried, then the Petridish along with drug was kept inside the oven for 24 hours, then it was taken out and with weighed quantity required concentration was prepared to check the absorbance using UV spectrophotometer.

Acid degradation

Transfer 5ml of 100g/ml solution of EMT and TDF in different test tubes and add 0.5ml of 0.1N HCl and leave it for 1 hour. The resultant solution was neutralized by NaOH before determining the absorbance of the solution by UV spectrophotometer at wavelengths of 284 nm and 260 nm.

Alkali Degradation

To determine the effect of base on EMT and TDF, transfer 5ml of each solution in different test tubes and add 5ml of 0.1N NaOH and leave it for an hour. The resultant solution was neutralized by HCl before determining the absorbance of the solution by UV spectrophotometer at wavelengths of max. 284 nm and 260 nm.

Oxidation degradation

Transfer 5ml of EMT and TDF solution to different test tubes and add 5 ml hydrogen peroxide solution and leave it for an hour. Determine the absorbance of the solution by UV spectrophotometer at wavelengths of 284 nm and 260 nm.

Method Validation[11-13]

Linearity

Preparation of stock solution

10 mg of EMT and 10 mg of TDF was accurately weighed and transferred separately into a 10ml clean dry volumetric flask, add about 2 ml of diluent and sonicate it to dissolve it completely and make volume up to the mark with the same solvent.

0.1, 0.15, 0.20, 0.25, 0.30ml of stock solution transferred into a series of 10ml clean dry volumetric flask and dilute up to the mark with diluent to get a concentration of 10-30µg/ml of EMT and TDF.

EMT and TDF at different concentrations level were prepared. Calibration curve was constructed by plotting the concentration level of drug versus corresponding absorbance at 284nm and 260nm.

Recovery

Accuracy of the method was determined by recovery studies. To the formulation (pre analysed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80%, 100%, 120%.

Precision

The inter-day and intra-day precision studies of EMT and TDFwere carried out by estimating same concentration six times on same day as intra-day precision and six times on the six different days as inter-day precision. The %relative standard deviation (RSD) was calculated.

Limit of detection (*LOD)* and limit of quantitation *(LOQ)*

The LOD and LOQ of EMT and TDF were estimated from standard deviation of the response and slope of the calibration curve, by using following equation

$LOD=3.3\times\sigma/s$, $LOQ=10\times\sigma/s$

Whereas σ is standard deviation of the response and S is slope of calibration curve.

Robustness

To demonstrate the robustness of the method, prepare standard solution as per test method and record absorbance of five replicate at different wavelength. It is concluded that the method is robust as it is found that the % RSD is less than 2.

RESULTS AND DISCUSSION

Analytical method has been developed for simultaneous estimation of EMT and TDF in pure and combined dosage form using simultaneous equation method. The wavelength of maximum absorption (λ_{max}) of the drug, 10

µg/ml solution of the drugs in methanol were scanned using UV spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The absorption curve shows characteristic absorption maxima at 260 nm for TDF and 284 nm for EMT.

When EMT and TDF subjected to 0.1N HCl showed decreased availability (66.8%, 66.3%). In the same way EMT and TDF subjected to 0.1N NaoH, TDF do not show significant change in term of availability (85%), EMT showed availability 67.1%. When EMT and TDF exposed to heat for24 hours, decreased availability observed (55.40%, 77.5%) and when exposed to UV light slightly decreased in availability (72.8%, 89.5%). Result are shown in figure 4, 5 and table 3, 4.

Linearity was observed in range of 10-30µg/ml. Linearity graph of EMT and TDF were shown in Figure 6, 7 respectively. Linearity data was shown in table 5. Accuracy of the method was checked by the recovery studies at three different levels, that is, 80%, 100%, 120%. The mean percentage recovery for EMT and TDF was found to be 98.5% and 99% respectively. Result was shown in table 6.

Table 3: Absorbance of Emtricitabine and Tenofovir disoproxil fumarate

Degradation Parameter	EMT	TDF
	Average	Average
Untreated sample	0.374	0.550
After acid	0.152	0.368
After base	0.257	0.478
After heat	0.208	0.431
After UV	0.274	0.498
After H_2O_2	0.268	0.489

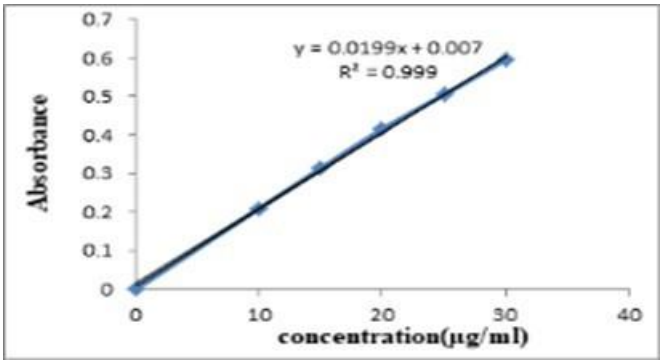


Figure 6: Calibration curve for Emtricitabine

Table 4: Degradation pattern in term of percentage for Emtricitabine and Tenofovir disoproxil fumarate

	Degradation Parameter	Untreated sample	After acid	After base	After heat	After UV	After H_2O_2
EMT	Average	99.7	66.8	67.1	55.4	72.8	71.3
	% Degraded	-	32.9	32.6	44.3	26.9	28.4
TDF	Average	99.6	66.3	85	77.5	89.5	88.83
	% Degraded	-	33.3	14.6	22.1	10.1	10.7

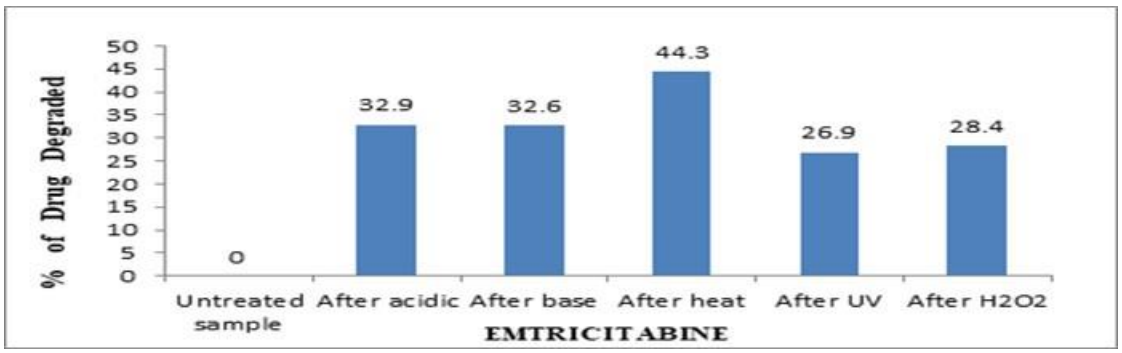


Figure 4: Degradation Pattern for Emtricitabine

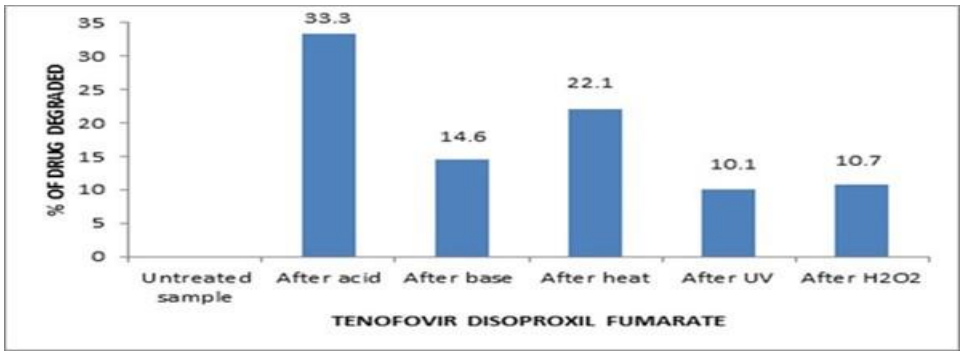


Figure 5: Degradation Pattern for Tenofovir disoproxil fumarate

Table 5: Linearity range for Emtricitabine and Tenofovir disoproxil fumarate

Concentration (µg/ml)	EMT	TDF
	Absorbance	Absorbance
0	0	0
10	0.209	0.271
15	0.313	0.404
20	0.415	0.548
25	0.504	0.683
30	0.595	0.838

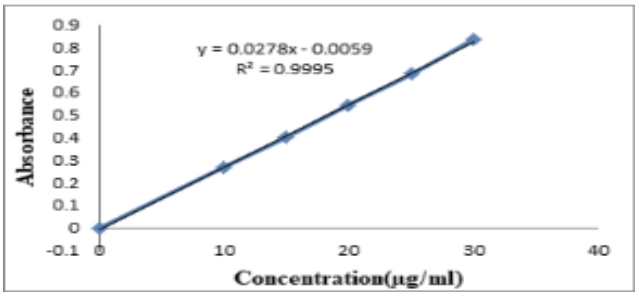


Figure 7: Calibration curve for Tenofovir Disoproxil Fumarate

Table 6: Recovery studies for Emtricitabine and Tenofovir disoproxil fumarate

		EMT				TDF			
Level of Recovery	Amount Added (Mcg/ml)	Amount Found	Average Amount Found	% Recovery	Average Recovery	Amount Found	Average Amount Found	% Recovery	Average Recovery
80%	10	9.8	9.8	98%	98.5%	9.8	9.8	98%	98.5%
80%	10	9.9				9.9			
80%	10	9.8				9.8			
100%	20	19.9	19.7	98.6%		19.9	19.7	98.6%	
100%	20	19.6				19.6			
100%	20	19.7				19.7			
120%	30	28.4	29	99%		28.4	29	99%	
120%	30	29				29			
120%	30	29.5				29.5			

Table 7: Inter-day and Intra-day precision for Emtricitabine and Tenofovir disoproxil fumarate

SNO	EMT		TDF	
	Absorbance		Absorbance	
	Interday	Intraday	Interday	Intraday
1	0.375	O.367	0.548	0.528
2	0.367	0.361	0.542	0.521
3	0.365	0.376	0.538	0.534
4	0.374	0.37	0.548	0.52
5	0.361	0.36	0.536	0.519
6	0.361	0.371	0.524	0.524
Mean	0.3671667	0.3676	0.539333	0.524333
STD Deviation	0.00561	0.006151	0.008219	0.005249
%RSD	1.52	1.67	1.523	1.00

The method was found to be precise as indicated by interday and intraday analysis showed that %RSD is less than 2 for EMT and TDF. The result was shown in table 7. LOD and LOQ were found to be 19.7 and 40.6 µg/ml. For TDF, LOD and LOQ were found to be 13.4 and 59.7 µg/ml for EMT, Result was shown in table 8.

Table 8: Summary for Analytical method validation

Validation Parameter	Emtricitabine	Tenofovir Disoproxil Fumarate
Linearity Range	10-30µg/ml	10-30µg/ml
R^2 Value for Linearity	0.999	0.9995
Recovery	98.5%	99%
Precision (Interday)%RSD	1.5	1.6
Intraday (%RSD)	1.5	1.0
LOD	19.7µg/ml	13.4 µg/ml
LOQ	40.6 µg/ml	59.7 µg/ml
Robustness(%RSD)	1.01	1

CONCLUSION

From the above experimental results and parameters it can be concluded that, this newly developed methods for estimation of EMT and TDF was found to be simple, rapid, precise, accurate and reproducible. The official assay limit of the content should not more than 90%and not more than 120% of the labelled amount. From the experiment we can conclude that EMT degrades mostly when subjected to acidic and basic medium, heat and degrades slightly when subjected to UV light. TDF mostly degraded when exposed to heat and in acidic medium but slightly degraded in the basic medium and when exposed to U.V light. Forced degradation studies carried out as these are helpful to determine stability of the drug in combination. The analytical techniques showed reliable method hence it can be effectively applied for routine analysis in research institution and quality control department in industries.

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