

Determination of Tenofovir and Emtricitabine by Simultaneous Equation Spectrophotometric Method

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Abstract: A simple, precise and selective method was developed for the determination of Tenofovir fumarate and Emtricitabine in tablet formulation. This method involves the application of simultaneous equation spectrophotometry method where the lambda max (λ_{max}) measured at 296nm and 271nm for determination of Tenofovir and Emtricitabine. The Spectrophotometric method using diluents comprising methanol and acetonitrile in ratio(70:30). Different variables affecting the method were carefully investigated and optimized. Reliability and analytical performance of the proposed methods, including linearity, precision, range, accuracy, detection and quantitation limits, were statistically validated. The methods were successfully applied for the determination of Tenofovir and Emtricitabine on combined tablet formulations. The Linearity was performed in the range of 5 $\mu\text{g/ml}$ to 30 $\mu\text{g/ml}$ for Tenofovir at two wavelengths 296nm and 271nm and for Emtricitabine in the range of 2 $\mu\text{g/ml}$ to 20 $\mu\text{g/ml}$ at 271nm and 296 nm. The coefficient of correlation was found to be less than 0.990 at both wavelengths. Precision and recovery studies also found to be within the range. Hence the suggested simultaneous equation Spectroscopic method can be used for routine analysis of Emtricitabine and Tenofovir in API and Pharmaceutical dosage form.

Keywords: UV Spectrophotometry, Emtricitabine, Tenofovir, simultaneous equation method, validation.

I. Introduction

Tenofovir (9[(R)-2-[[bis[[isopropoxycarbonyl) oxy] methoxy] phosphinyl] methoxy] propyl) and Emtricitabine (5-fluoro-1-(2if, 5S)-[2-9hydroxymethyl]- 1,3-oxathiolan-5-y) both are the antiviral agents. They act as the Nucleoside Reverse Transcriptase enzyme Inhibitors. These are the Nucleoside analogues which are phosphorylated by host cell enzyme to give 5'-triphosphate derivative. This moiety competes with the equivalent host cellular triphosphate substrates for proviral DNA synthesis by viral reverse transcriptase which is viral RNA-dependent DNA polymerase. Eventually, the incorporation of the 5'-triphosphate moiety into the growing viral DNA chain results in chain termination. Emtricitabine is potent and selective against HIV types I and II and hepatitis B virus. Tenofovir is active against a variety of drug resistant HIV-I strains. Recently, the combination of Tenofovir and Emtricitabine has demonstrated significantly greater HIV RNA suppression compared to the combination of zidovudine and lamivudine¹⁻³

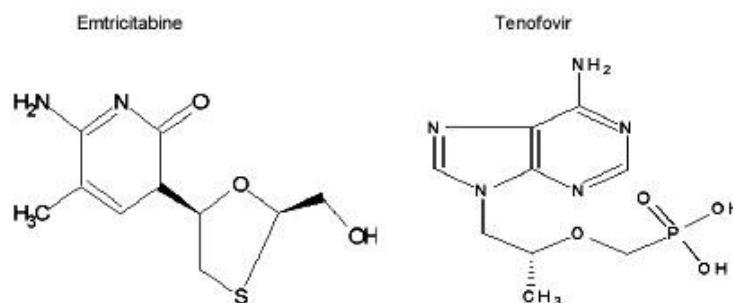


Fig. 1. The structures of Emtricitabine and Tenofovir.

Several analytical methods that have been reported for the individual determination of Tenofovir in biological fluids and in pharmaceutical formulations which include liquid chromatography coupled with spectrofluorimetric and mass spectroscopy detection⁴⁻¹⁰. For Emtricitabine several analytical methods have been reported for its individual analysis which includes chiral liquid chromatography, liquid chromatography with UV detection¹¹⁻¹³. Few Bioanalytical methods are reported for combination of Tenofovir and Emtricitabine which includes liquid chromatography with PDA and UV detection^{14,15}. There is no spectroscopic method available in the literature for the estimation of Emtricitabine and Tenofovir by simultaneous equation method in

combined dosage form. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines¹⁶.

Experimental

Instruments

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells were used for spectrophotometric methods. All weighing were done on electronic balance (Model Shimadzu AUW-220D).

II. Materials And Methods

Reagents

Spectroscopy grade methanol and acetonitrile was used throughout the study. Pure drug sample of Tenofovir fumarate (99.86% pure) and Emtricitabine (99.82%) pure were kindly supplied as a gift sample by Emcure Pharmaceuticals Pvt. Ltd. Pune, India. It was used without further purification. Tablets were purchased from local market, each containing Tenofovir disoproxil fumarate 300 mg and Emtricitabine 200 mg. Tablet used for analysis were TENVIR-EM manufactured by Cipla Ltd., Goa. Method development for the estimation of Emtricitabine and Tenofovir by using UV simultaneous equation method.

Selection of wavelength: 10 mg of Emtricitabine and 10mg of Tenofovir was dissolved separately in mobile phase. The solutions were scanned from 200-400 nm and the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Emtricitabine and Tenofovir. The isobestic point was taken as detection wavelength.

Procedure

Preparation of mobile phase

Mix a mixture of above Acetonitrile 30 ml (30%) and 70 ml of methanol and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 μ filter under vacuum filtration. Diluents preparation Mobile phase was used as the diluent.

Preparation of the individual Emtricitabine standard preparation

10 mg of Emtricitabine working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual Tenofovir standard preparation

10 mg of Tenofovir working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Sample solution preparation:

10 mg of Emtricitabine and 2 mg Tenofovir tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation:

10 mg Emtricitabine and 2 mg Tenofovir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

III. Results And Discussion

Selection of solvent

Solutions of Emtricitabine and Tenofovir were prepared in different solvents like methanol, ethanol, acetonitrile and UV spectrum of each were recorded by scanning between 200-400 nm.

An overlain spectrum of Emtricitabine and Tenofovir were prepared in different solvents like methanol, acetonitrile etc. Better absorbances were observed for both the drugs when Methanol and acetonitrile (70:30) (mobile phase) used as a solvent as shown in the figure 2. Hence, Mobile phase was selected as diluent for present study. At 296nm and 271nm good linearity was observed and hence these wavelengths were fixed for the study.

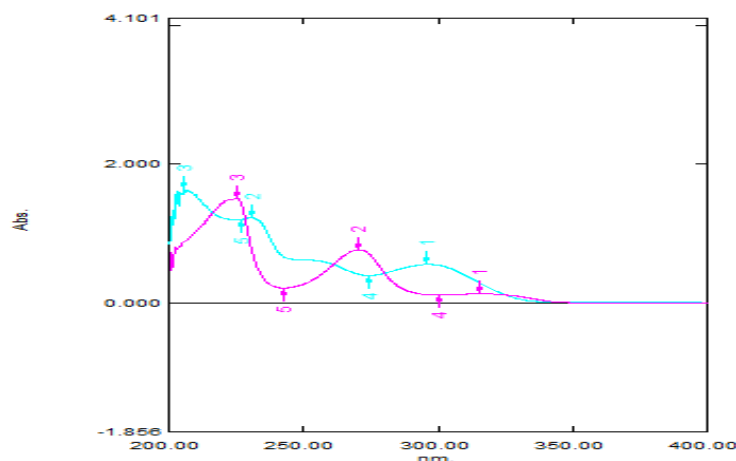


Fig: 2 -Overlain Normal spectra of Emtricitabine and Tenofovir in Mobile phase.

IV. Validation Of The Method

Linearity

Emtricitabine was found to be linear in a concentration range of 5-30 μ g/ml. The absorbances of these solutions were noted at wavelengths 296 and 271 nm, respectively. Calibration curves were plotted using concentration Vs absorbance at wavelength of 296 nm and the slope, intercept and correlation coefficient values were found to be 0.008, 0.007 and 0.997 respectively. At wavelength 271nm, slope, intercept and correlation coefficient values were found to be 0.007, 0.015 and 0.997, respectively.

S.NO	Concentration(μ g/ml)	Absorbance at 296nm	Absorbance at271nm
1	5	0.452	0.537
2	10	0.942	0.852
3	15	1.364	1.168
4	20	1.788	1.583
5	25	2.179	1.923
6	30	2.598	2.343

Table: 1- Calibration data of Emtricitabine at 296nm and 271 nm

Tenofovir

Tenofovir was found to be linear at a concentration range of 2-12 μ g/ml. The absorbances of these solutions were noted at 271 and 296 nm, respectively. Calibration curves were plotted using concentration Vs absorbance. At a wavelength of 271 nm, the slope, intercept and correlation coefficient values were found to be 0.080, 0.001 and 0.999, respectively. At wavelength 275 nm, the slope, intercept and correlation coefficient values were found to be 0.005, 0.006 and 0.999, respectively.

S. No	Concentration (μ g/ml)	Absorbance at 271 nm	Absorbance at 296 nm
1	2	0.079	0.011
2	4	0.230	0.021
3	6	0.385	0.032
4	8	0.548	0.044
5	10	0.700	0.056
6	12	0.873	0.069

Table: 2- Calibration data of Tenofovir at 271 and 296nm

Precision

Precision studies were performed by preparing the standards six times and measuring the absorbances of drugs at 296 nm and 271 nm. Low RSD values indicate that the method is precise. The values are shown in table 3 and table 4.

Concentration	Absorbance		%RSD	
	Emtricitabine 296nm	Tenofovir 271nm	296nm	271nm
10ug/ml	1.675	0.077	0.51%	0.53%
	1.677	0.078		
	1.678	0.079		

	1.689	0.079		
	1.677	0.077		

Table: 3- Intra Day Precision Studies

Concentration	Absorbance		%RSD	
	Emtricitabine	Tenofovir	296 nm	271 nm
10ug/ml			1.65%	1.56%
	0.603	0.727		
	0.623	0.748		
	0.625	0.754		
	0.627	0.755		
	0.636	0.759		
	0.625	0.754		

Table:4- Inter day Precision studies

Recovery studies

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation powder (10mg), a known quantity of standard Emtricitabine and Tenofovir were added at 80%, 100% and 120% level and the contents were re-analysed by the proposed method. The % recovery and %RSD were calculated as shown below in table 5 and table 6.

Level	%Recovery		%RSD
	Emtricitabine	Tenofovir	
80%	103%	99.56%	0.18%
100%	101%	102.5%	0.99%
120%	99.28%	100.2%	0.80%

Table: 5- Recovery studies

Analysis of formulation

Preparation of standard solutions

Standard stock solution of Emtricitabine was prepared by dissolving 10 mg of the drug in diluent and the volume was made up to 10ml in a standard flask. From the stock solution, concentrations ranging from 5-30 µg/ml was prepared for Emtricitabine. and 10mg of drug is dissolved in diluents and volume was made up to 10ml from the stock solution concentration ranging from 2-12µg/ml was prepared for Tenofovir and scanned in the UV region.

Preparation of sample solution

Twenty tablets are powdered and the average weight was calculated. A quantity equivalent to 40 mg of drug was dissolved in diluent. Finally the volume was made up to get a working concentration of 10µg/ml each of Emtricitabine and Tenofovir and absorbances were noted at 296nm(λ₁) and 271nm(λ₂) respectively. The amounts of Emtricitabine and Tenofovir were calculated using the simultaneous equation given below. UV spectra of formulation is seen in figure 3.

At λ₁ A₁ = ax₁bc_x + ay₁bc_y

λ₂ A₂ = ax₂bc_x + ay₂bc_y

$$C_{Em} = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2}$$

$$C_{Te} = \frac{A_1 a x_2 - A_2 a x_1}{a x_2 a y_1 - a x_1 a y_2}$$

- A₁ = absorbance of formulation at 296nm.
- A₂ = absorbance of formulation at 271 nm.
- ax₁ = Absorptivity of Emtricitabine at 296nm.
- ax₂ = Absorptivity of Tenofovir at 271nm.
- ay₁ = Absorptivity of Emtricitabine at 296nm.
- ay₂ = Absorptivity of Tenofovir at 271nm.
- C_{Em} = Concentration of Emtricitabine.
- C_{Te} = Concentration of Tenofovir.

Drug	Amount (mg/tab)		Amount recovered in percentage	% RSD*
	Labeled	Found		
Emtricitabine	200mg	123.5	98.66%	0.95

Tenofovir	300mg	158.3	98.4%	0.80
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*RSD of three observations

Table: 6- Analysis of formulation

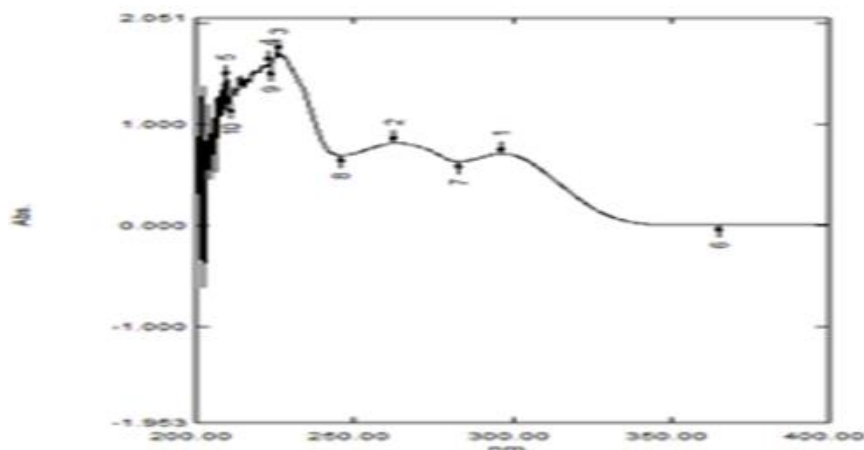


Fig: 3 UV spectra of formulation

V. Conclusion

The validated spectrophotometric method employed here proved to be simple, economical, precise and accurate. Thus it can be used as quality control test and for routine simultaneous determination of Tenofovir and Emtricitabine in tablet dosage form.

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