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UV-Spectrophotometric Estimation and Forced Degradation Studies of Tenofovir Alafenamide Fumarate (TAF) in its Bulk and Tablet Dosage Form

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Tenofovir Alafenamide Fumarate; UV-Visible spectrometer; Forced degradation studies; Stress conditions.

ABSTRACT: A simple, sensitive and reproducible Spectrophotometric method is developed for estimation of Tenofovir Alafenamide Fumarate (TAF) in bulk and its tablet dosage form. Forced degradation studies are also conducted on standard TAF and % degradation of drug under various stress conditions is reported. Different concentrations of TAF are prepared using distilled water: acetonitrile as diluent. The absorption maximum of TAF is found to be at 261nm. Linearity was established in the range of 6.25-37.5 µg/ml with regression coefficient of 0.999. The drug was subjected to acid and alkali hydrolysis, oxidation degradation, thermal and photo degradation conditions.

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INTRODUCTION

Tenofovir Alafenamide Fumarate (TAF), (E)-but-2-enedioic acid; propan-2-yl (2S)-2-[[(2R)-1-(6-aminopurin-9-yl) propan-2-yl] oxymethyl phenoxyphosphoryl] amino] propionate is an antiretroviral drug which is used in treatment of chronic Hepatitis B and HIV/AIDS infection. [1-2]. It is nucleotide reverse transcriptase inhibitor. Tenofovir Alafenamide Fumarate (**Fig. 1**) is a Fumarate salt prepared from Tenofovir Alafenamide by reaction of one molecule of fumaric acid for every two molecules of Tenofovir Alafenamide, a prodrug for Tenofovir; it is used in combination therapy for the treatment of HIV-1 infection. It has a role as an antiviral drug, a HIV-1 reverse transcriptase inhibitor and a prodrug.

A literature survey has revealed that only two articles on UV spectroscopic assay of TAF are reported. A few methods in literature review are found for determination of TAF in combined dosage forms and include High Pressure Liquid Chromatography (HPLC)[1-3].A method based on the measurement absorbance of the drug in water at 260nm has been reported and the method obeys Beer's law in the 2-10 µg/ml concentration range [7].

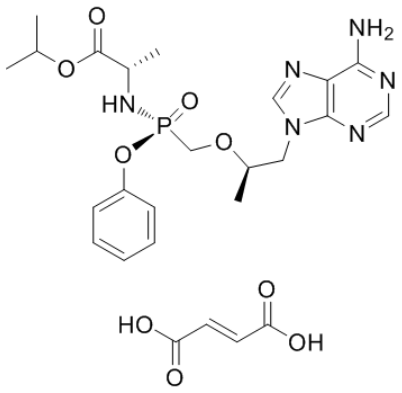


Fig. 1 Structure of TAF

Simultaneous estimation of TAF and Emtricitabine by UV spectroscopic method is found in a literature. The method involving determination of TAF and Emtricitabine at 260nm and 280nm over the concentration ranges of 5-30µg/ml for both drugs has been described [6].

Most of the reported methods are often time consuming, expensive, use multi or expensive reagents, cumbersome and required expertise operational personnel. UV spectrophotometry, because of simplicity, reproducibility and speed and also it requires minimum solvent/reagent system and less analysis time, is widely used for the assay of the therapeutic compounds used as medications.

MATERIALS AND METHODS

Apparatus

The Spectrophotometric measurements were carried out using Elico SL 210 UV/Visible spectrophotometer.

Materials

All chemicals used were of reagent grade. Distilled water was used to prepare solutions wherever required. Acetonitrile, hydrogen peroxide (H2O2), hydrochloric acid and sodium hydroxide were purchased from Merck (Mumbai, India). Tenofovir Alafenamide Fumarate sample (purity99.5%) was kindly supplied by Mylan laboratories, Hyderabad, India. Commercial brand of tablets namely HepBest (Mylan Pharmaceuticals Ltd., Indore, India) were purchased from local commercial sources.

Reagents

Hydrochloric acid (1 M) was prepared by appropriate dilution of concentrated acid with water. A 5% solution of H2O2 was prepared by diluting suitable volume of the commercially available reagent to 100 ml with water in a volumetric flask. Sodium hydroxide solution (1M) was prepared by dissolving required amount of the pellets in water.

Selection of Diluent

The solvent was selected by determining the solubility of Tenofovir Alafenamide Fumarate in various solvents namely Distilled water, Hydrochloric Acid, Sodium Hydroxide Solution, Methanol. Finally, acetonitrile: Distilled water (20:80) was chosen as the solvent for Tenofovir Alafenamide Fumarate depending on absorption at its analytical wavelength.

Standard Drug Solution

Preparation of stock solution (250µg/ml): weigh about 25mg of Tenofovir Alafenamide Fumarate and transfer to 25ml volumetric flask, dissolve it in diluent and make up the final volume to 25 ml with diluent.

Procedures

Preparation of calibration curve

Into a series of 10 ml calibration flasks, aliquots of standard drug solution (0.25–1.25 ml of 250µg/ml)equivalent to 6.25 - 37.5 µg/ml TAF were accurately transferred and the volume was made up to the mark with the diluent. The absorbance of each solution was then measured at 261 nm against the respective diluent. Calibration curve was prepared by plotting the absorbance versus concentration of drug. The concentration of the unknown was read from the respective calibration curve or computed from the regression equation derived using the Beer's law data.

Analysis of tablets

Twenty tablets from commercial brand (HepBest) were weighed and crushed into a fine powder using a Pestle and Mortar. An amount of tablet powder equivalent to 10 mg of TAF was transferred into a 100 ml volumetric flask. The content was shaken well with about 50 ml of the respective diluent for 20 min.The mixture was diluted to the mark with the same diluent. It was filtered using Whatmann No 42 filter paper. First 10 ml portion of the filtrate was discarded and a subsequent portion was diluted to get a working concentration and subjected to analysis following the procedures described earlier.

Forced degradation study

A 1 ml aliquot of the standard 25µg/ml TAF was taken (in triplicate) in a 10 ml volumetric flask and mixed with 5 ml of 1 M HCl (acid hydrolysis) or 1 M NaOH (alkaline hydrolysis) and boiled for 2 h at 400C on a hot water bath or 3% H2O2 (oxidative degradation) at room temperature for 2h. The solution was cooled to room temperature, neutralized and diluted to the mark with diluent. In thermal degradation, solid drug was kept in Petri dish in an oven at 400Cfor 2h. After cooling to room temperature, 10 mg of TAF was weighed and transferred to a 100 ml volumetric flask, dissolved in and diluted up to the mark with the respective diluent. For UV degradation study, suitable aliquot of the stock solution (250µg/ml) was exposed to UV radiation for 4h in a UV chamber. Finally, the absorbance of all the resulting solutions (25µg/ml) obtained from acid and alkaline hydrolysis, oxidative degradation, thermal and UV degradation of TAF was measured at 261nm against the respective solvent as blank in each case. [16 – 20].

RESULTS AND DISCUSSION

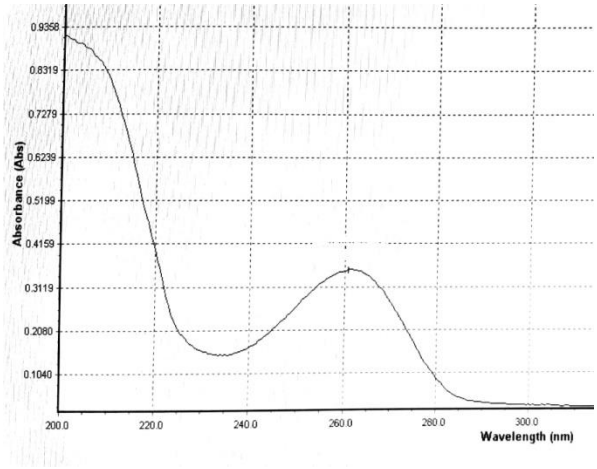
Spectral characteristics

The standard test solution (25µg/ml) in acetonitrile: distilled water (20:80) showed absorption maximum at 261nm. The spectrum thus obtained is shown in **Fig. 2**.

Table 1. Regression and analytical parameters

Parameter	Method
λ_{max}, nm	261
Beer's law limit	6.25-37.5 µg/ml
Molar absorptivity (L/ mol/cm)	2.80 X 10^7
Limit of detection, µg/ml	0.331
Limit of quantification, µg/ml	1.002
Regression equation, Y*	
Intercept (a)	-0.008
Slope (b)	0.026
Correlation coefficient (r)	0.999
**Y = a + bX, where Y is the absorbance, a is the intercept, b is the slope and X is the concentration in µg/ml*	

Fig. 2 Spectrum of TAF in diluent



Method validation

Linearity, sensitivity, limits of detection and quantification

A linear correlation was found between absorbance at λmax and concentration of TAF. The graphs are described by the regression equation: Y = a + bX (where Y = absorbance of drug solution; a = intercept; b = slope and X = concentration of drug in 25µg/ml). The slope (b), intercept (a) and correlation coefficient (r) for each system were evaluated by using the method of least squares Optical characteristics such as Beer's law limits, molar absorptivity (ICH-Q1A (R2)) of the method are calculated. The limit of detection (LOD) and limit of quantitation (LOQ) are also calculated and all these data are presented in **Table 1**. High values of molar absorptivity (e), low values LOD revealed that, the proposed method are highly sensitive. A plot was constructed by concentration on x-axis and absorbance on y-axis. The correlation coefficient (r) was found to be 0.999 and the summary of study is given in **Table 2** and graph is represented as **Fig. 3**.

Precision and accuracy

To check the repeatability and reproducibility of the proposed methods, the standard drug solution of three different concentrations were scanned, each concentration six times within the day (intra-day precision, n=6) and six times on two different days (inter-day precision, n=6). System precision and method precision were also performed using 25µg/ml standard drug solution (n=6).The percentage relative standard deviation (%RSD) values were within limit indicating high precision of the method, summarized in **Table 3**. The accuracy of the methods was evaluated by standard addition method. A known concentration standard drug solution was spiked to known concentration of sample solution in three levels and %recovery is calculated. The results of this study are summarized in **Table 4**.

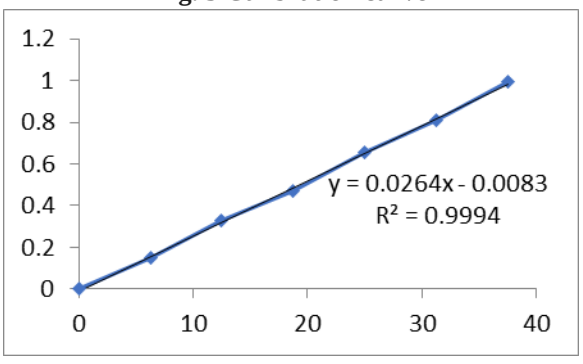
Ruggedness and robustness

The robustness of the methods was evaluated by measuring the absorbance at different wavelengths whereas the method ruggedness was performed by two different analysts. Intermediate precision values (% RSD) are presented in **Table 3**.

Table 2. Calibration data

Concentration(µg/ml)	Absorbance
0	0
6.25	0.151
12.5	0.327
18.75	0.473
25	0.654
31.25	0.809
37.5	0.992

Fig. 3 Calibration curve



Analysis of tablets

Commercial TAF tablets were analyzed using developed method and % assay was calculated which was found to be to 100.10% using following equation:

$$\%\ assay = \frac{A(sample)}{A(std)} \times \frac{C(std)}{C(sample)} \times 100$$

Where, A is absorbance and C is concentration

Table 3. Results of precision, robustness and ruggedness

Parameter	%RSD
PRECISION	
System precision	0.397%
Method precision	0.508%
Intra-day (n=6)	0.71%(12.5 µg/ml)
	0.40%(25 µg/ml)
	0.52%(37.5 µg/ml)
Inter-day (n=6)	0.87%(12.5 µg/ml)
	0.68%(25 µg/ml)
	0.44%(37.5 µg/ml)
ROBUSTNESS	
At 259nm	0.92%
At 263nm	0.86%
RUGGEDNESS	
Analyst-1	0.39%
Analyst-2	0.43%

Force degradation study

The stress studies of the drug were carried out by subjecting TAF to acid and alkali hydrolysis, dry heat treatment, UV-degradation and hydrogen peroxide oxidation and later absorption spectra were recorded. The comparison of the absorbance of stressed TAF samples with that of the standard TAF solution showed that TAF has undergone degradation under all stress condition and a summary is given in **Table 5**.

Table 4. Accuracy results and % recovery

Level	Standard concentration(µg/ml)	Sample concentration(µg/ml)	%recovery	%RSD
50 %	5	10	101.88	0.53 %
	5	10	97.95	
	5	10	99.52	
100 %	10	10	99.35	0.42 %
	10	10	98.73	
	10	10	100.68	
150 %	15	10	98.19	0.19 %
	15	10	99.78	
	15	10	99.27	

Table 5. Summary of force degradation study.

Degradation condition	Time	Absorbance	% degradation
Acid (1M HCl) at 40^0C	2h	0.566	14.88
Base (1M NaOH) at 40^0C	2h	0.583	12.06
3% H_2O_2 at RT*	2h	0.493	25.86
Thermal at 40 ^{0}C	2h	0.560	15.27
In UV chamber	4h	0.529	19.97
**RT means room temperature*			

CONCLUSION

In this study, the degradation behavior of TAF was studied by subjecting the drug to various stress conditions recommended by ICH. The additional findings in this study show that the drug undergoes an extensive degradation under oxidative (peroxide) condition. The method was validated for parameters like linearity, precision, accuracy, ruggedness and robustness. Application of this method for the analysis of TAF tablet dosage forms showed that there was no interference of excipients in the determination. The method is advantageous over most of the reported methods in-terms of sensitivity, simplicity, cost-effectiveness and experimental conditions. The method does not involve any tedious procedural steps; do not require any extra reagents or longer analysis time and a very simple instrument is required. The method can be used to determine the purity of the drug available from various sources.

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DATA AVAILABILITY

Not declared.

CONFLICTS OF INTEREST

The authors declare no conflict of interest in this research article.

FUNDING SOURCE

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