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Derivative UV- Spectrophotometric Estimation of Tenofovir alafenamide fumarate in Bulk and solid Dosage Form

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Abstract:
Development and Validation of an analytical UV derivative Spectrophotometric method to quantify Tenofovir alafenamide fumarateas a single active principle in pharmaceutical formulation was done. Based on the Spectrophotometric characteristics of Tenofovir alafenamide, a signal of zero (260nm), first (285nm, 250nm, 230nm) order derivative spectra were found to be adequate for quantification. The method obeyed Beers law in the concentration range of (5-30 µg/ml) with square correlation coefficient (r^2) of 0.9991. The mean percentage recovery was found to be 99.56 ± 0.7179. As per ICH guidelines the results of the analysis were validated in terms of linearity, precision, accuracy, limit of detection and limit of quantification and were found to be in good accordance with the prescribed values.

Keywords: Tenofovir alafenamide, Derivative spectrophotometry, ICH guidelines, Beer's Law

I. Introduction:

Analytical chemistry is often described as the area of chemistry responsible for characterizing the composition of matter, both qualitatively (Is there any lead in this sample?) and quantitatively (How much lead is in this sample?)[1].

Tenofovir alafenamide fumarate (TAF) is a nucleotide reverse transcriptase inhibitor, Propan-2-yl (2s)-2-{[(S)-({[2R)-1-(6-amino -9H-purin -9-yl) propan-2oxyl (methyl) (phenoxy) phosphoryl] amino} propanoate[2]. TAF is used in treatment of viral diseases.Several methods are available in the literature for the determination of TAF. Most of these methods are for the determination of TAF separately or in combination with other drug[3]. Analytical methods reported for quantitative determination of TAF individually in pharmaceutical formulations or biological fluids are HPLC and UV. Analytical methods reported for quantitative determination of TAF using methanol and water in combination[4]. Derivative methods were introduced by using menthol and water in the ratio of 80:20[4, 5].

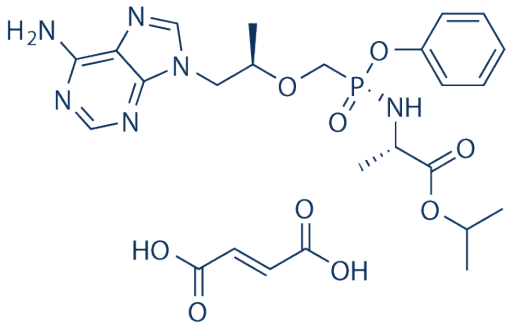

Figure 1: Chemical structure of Tenofovir alafenamide fumarate

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II. Material And Methods:

Chemicals and reagents:Tenofovir alafenamide was procured from the Mylan Laboratories Ltd. (hyd). Commercial pharmaceutical preparation TAFERO tablets, manufactured by HETERO Pharma. Ltd., containing 25mg of TAF mg was collected from local market. Analytical grade water was used.

Instrumentation:
The proposed work was carried out on a TG Ultra violet spectrophotometer, T-60 Model, which is a double beam double detector configuration with a1 cm quartz matched cell. All weighing was done on weighing balance: PGB-200 Model.

Selection of Solvents:
On the basis of solubility study water was selected as the solvent for dissolving TAF.

Preparation of Standard Stock Solutions of TAF:
Stock Solution A (1mg/ml): Accurately weighed 0.01g of Tenofovir alafenamide fumarate was transferred into 10ml volumetric flask and dissolve in a minute quantity of distilled water by using sonicator for 2mins and volume was made up to 10ml with distilled water.
Stock Solution B (100µg/ml): 2.5ml of stock solution A was made up to 25ml with distilled water**.**
Dilutions: Dilutions were done by taking 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 ml of stock B and makeup the volume with distilled water up to 10ml.

Determination of λ_{max} of Individual Drug:
An appropriate aliquot portion of TAF (0.2ml) was transferred to 10 mL volumetric flasks; the volume was made up to the mark using water. Drug solutions were scanned separately between 200 nm to 400 nm. TAF showedλ max at 260nm. (Figure: 2)

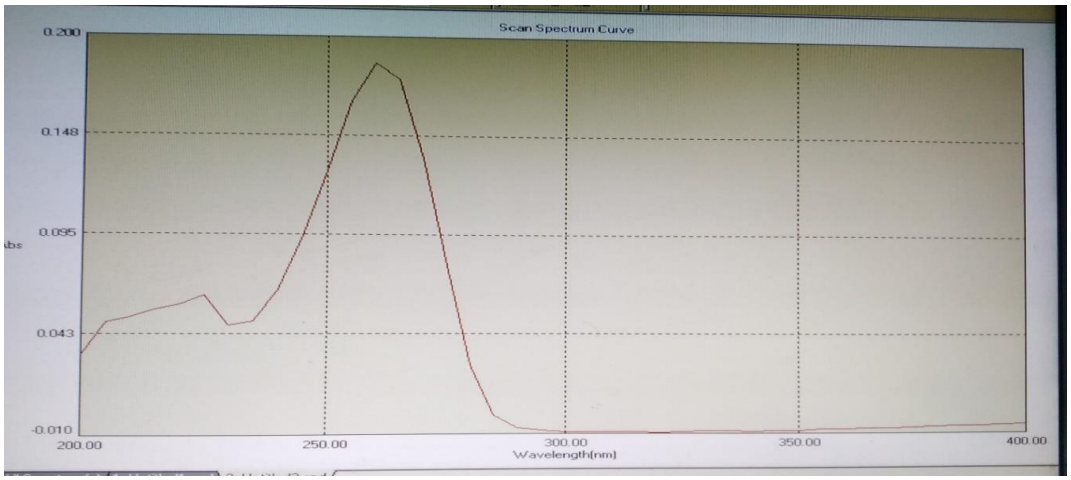

Figure 2: Spectrum of TAF

Linearity Study for TAF:
An accurately measured aliquot portion of working standard solution of TAF was transferred to five separate 10 mL volumetric flasks. The volume was made up to the mark using water to obtain concentrations (5-30 µg/mL). Absorbance of these solutions was measured at 260 nm. Calibration curve was plotted against absorbance vsconcentration as shown in (Figure: 3).

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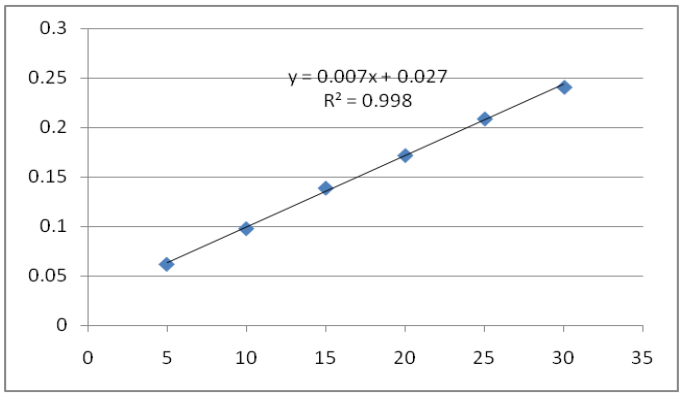
Parameters	Value
λ max (nm)	260nm
Beer's law limit (µg /ml)	5-30
Regression equation (Y=a +b x)	y=0.007x+0.027
Slope (b)	0.0279
Intercept (a)	0.0993
Correlation coefficient (r)	0.9984
LOD (µg/ml)	0.326
LOD (µg/ml)	0.99
% Concentration estimated (Mean±S.D)	0.5466± 0.00216
%R.S.D.	0.34

Table 1: Regression and Optical characteristics of TAF

Figure 3: Calibration Curve of TAF at 260nm



Application of the Proposed Method for Estimation of Drugs in Tablets:

Ten TAFERO Tablets containing TAF (25mg) were weighed and ground to fine powder. A quantity of sample equivalent to 25mg of TAF was transferred into 100 mL volumetric flask containing water, sonicated for 10 min and the volume was made up to the mark and filtered through Whatman filter paper (No. 45). This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved and volume was adjusted to the mark. The absorbance of the solutions was measured at 260nm against blank. The results are reported in the (Table: 2).

Formulation	Label claim mg/tab	Amount found Mean±SD	Assay	%RSD
Tablet	25	24.86±0.063	99.49	0.063

Table 2: Results of Estimation of TAF in Tablets.

Validation of Proposed Method:

The Proposed method was validated as per the ICH guidelines.

Accuracy [Recovery Study]:

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions were added to the tablet powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula. % Recovery = [A - B/ C] X 100

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Where,
A = Total amount of drug estimated
B = Amount of drug found on pre analyzed basis
C = Amount of Pure drug added
The results are reported in (Table 3).

Ruggedness:
Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two

Precision:
Precision was determined as intra-day and inter-day variations. Intra-day precision was determined by analyzing TAF (5, 20, 30µg/mL) for three times on the same day. Inter-day precision was determined by analyzing the same concentration of solutions for three different days over a period of week.The results are reported in (Table 3). different analyst using same operational and environmental conditions. The results are reported in (Table: 3).

Sl. No	Parameters	concentration	Mean ± SD	%RSD
1	**Accuracy**			
	% sample spiked			
	80%	20 µg/ml	98.05±0.00675	0.0068
	100%	20 µg/ml	102.12±0.00121	0.0011
	120%	20 µg/ml	100.01±0.0034	0.0033
2	**Precision**			
	Intra-Day			
		5 µg/ml	0.203±0.0014	0.6
		20 µg/ml	0.5156±0.00392	0.7
		30 µg/ml	0.7426±0.002422	0.3
	Intra-Day			
		5 µg/ml	0.103±0.0044	0.9
		20 µg/ml	0.5856±0.00492	1.8
		30 µg/ml	0.6843±0.005422	0.9
3	**Robustness**			
	Absorbance			
	259nm	20 µg/ml	0.215±0.00275	1.7
	261nm	20 µg/ml	0.225±0.00295	1.3
4	**Ruggedness**			
	Analyst-1	20 µg/ml	0.2061±0.00278	1.3
	Analyst-2	20 µg/ml	0.203±0.00164	0.8

Table 3: Analytical Parametersand Validation Results of Tenofovir Alafenamide Fumarate

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Derivative spectra of TAF at Zero and First order:

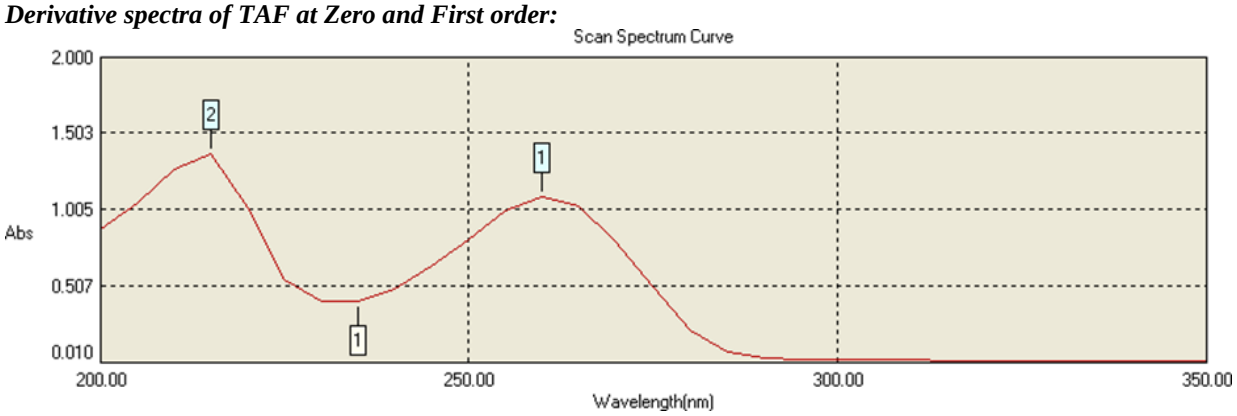

Figure: 4 Zero order (25mg/ml)

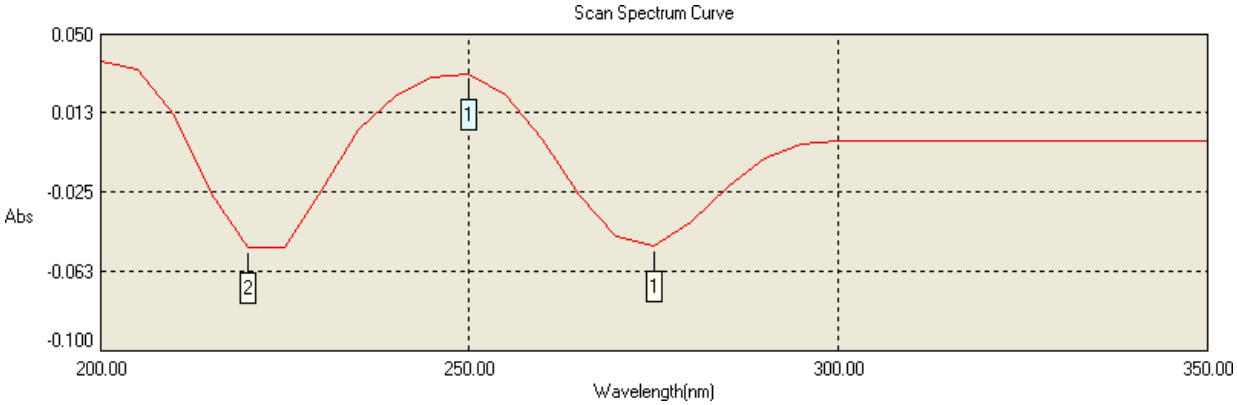

Figure: 5 first order (25mg/ml)

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Conflict of interest:
The authors declare no conflict of interest.

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