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Development and Validation of First Order Derivative Method for Tenofovir alafenamide in Bulk using UV Visible Spectroscopy

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Abstract : A simple, rapid, accurate, and economical UV-spectrophotometric method has been developed for the estimation of tenofoviralafenamide from bulk drug. The developed method is validated as per ICH guidelines. The method uses a shimadzu UV-Visible with matched quartz cells (1 cm) for the estimation of drug from bulk. The λmax of tenofoviralafenamide in methanol was found to be 259 nm. The drug follows linearity in the concentration range 5-35µg/mL with a correlation coefficient value of 0.9968. Themethod applied was area under curve (AUC) in which area was integrated in the wavelength of range250.12- 261.26 nm.The proposed method was found to be precise as % RSD values for intraday aswell as interday precision was satisfactory. The drug at each of the 80 %, 100 % and 120 % levels showed goodrecoveries that is in the range of 98.00 to 99.00%, hence itcould be said that the method was accurate.The LODand LOQ were calculated as 0.3819 µg/ml and 1.5917µg/ml.Thus, the developed method is found to be robust and rugged which can be applied as a rapid tool for routine analysis of tenofoviralafenamide in the bulk and in the pharmaceutical dosage form.

Keywords : UV, validation, Assay, Precision, % Recovery, Tenofoviralafenamide, area under curve.

Introduction:

Tenofoviralafenamide(Fig. 1) is chemically a (S)-isopropyl 3-(R-((((R)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)(phenoxy)phosphoryl)-2-methylpropanoate. It is one of rational drug development in the treatment of retroviral diseases.Tenofoviralafenamidefumarate (TAF) is a nucleotide reverse transcriptase inhibitor (NRTI) and a novel ester prodrug of the antiretroviral tenofovir. Tenofovir causes early chain termination and prevents proviral DNA transcription. Tenofovir has a good safety profile and efficacy, and is currently a cornerstone of HIV antiviral treatment.There is an older drug available in market similar to

Ten of oviralafenamidefumaratei.e., Ten of ovirdisoproxilfumarate (TAF). TAF has a similar tolerability, safety, and effectiveness to TDF and probably less adverse events related to renal and bone density outcomes in the treatment of naive and experienced patients with HIV-1.[7]

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Literature survey reveals that a few spectrophotometric[1,2,3], RP-HPLC[4,5,6]methods are reported for the estimation of Tenofoviralafenamide in combination with other drugs.

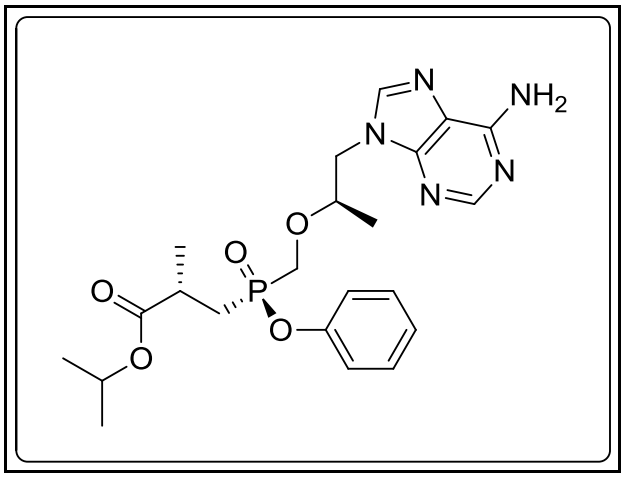

Fig 1: Chemical Structure of TenofovirAlafenamide

Materials and Methods:

The Tenofovir alafenamide was kindly supplied as a gift sample by mylan laboratories pvt. ltd.,Hyderabad (India). All rest of chemicals used were of Analytical grade.

Adouble-beamUV-Visiblespectrophotometer,(UV-1800, shimadzu limited, japan)having two matched cells with1cm lightpath. A Citizen analytical balance (Sartorius) was used forweighingthe samples.

Preparation of standard stock solutions:

Standard solution of tenofovir alafenamide was prepared by transferring accurately weighed 10 mg of drug into a 100ml volumetric flask and the volume was made up to 100ml using methanol as a solvent to get the concentration of 100µg/ml.

Selection of wavelength for analysis of tenofovir alafenamide:

Accurately pipetted 1.0 mL volume of standard stock solution of tenofovir alafenamide was transferred into a 10 mL volumetric flask, diluted to a mark with methanol to give concentration of 10 µg/mL. The resulting solution was scanned in the UV range (200–400 nm) using shimadzu UV- VIS spectrophotometer instrument. The maximum absorbance of solution was measured at the wavelength 259 nm (Figure 2).

Preparation of calibration curve:

From the standard stock solution fresh aliquots were pipette out and suitably diluted with methanol to get final concentration in the range of 5-35 (µg/ml). The solutions were scanned under 200-400 nm wavelength range and a sharp peak was obtained at 259nm (figure 2). Calibration curve was plotted by taking absorbance on y-axis and concentration of solution on x-axis (figure 3). The drug follows linearity in the concentration range 5-35µg/mL with a correlation coefficient value of 0.9968

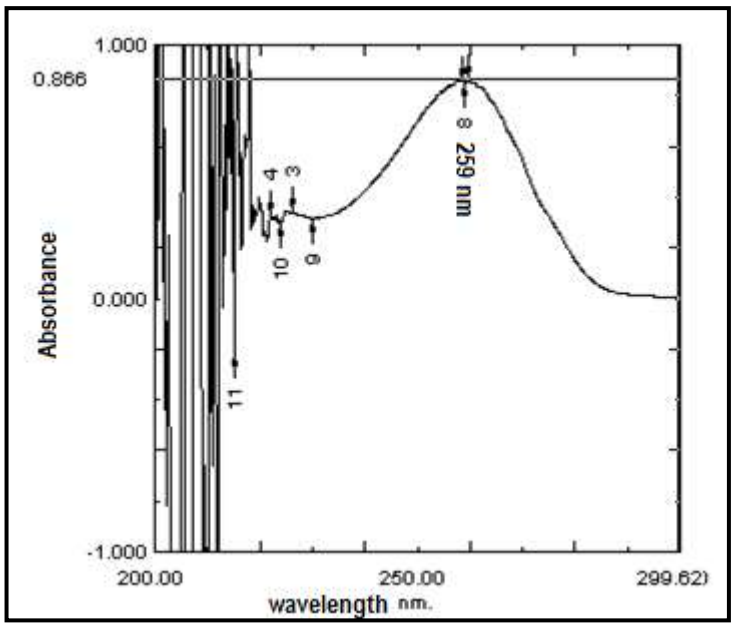

Fig 2: Determination of $C_{max}$of Tenofovir alafenamide std. stock solution

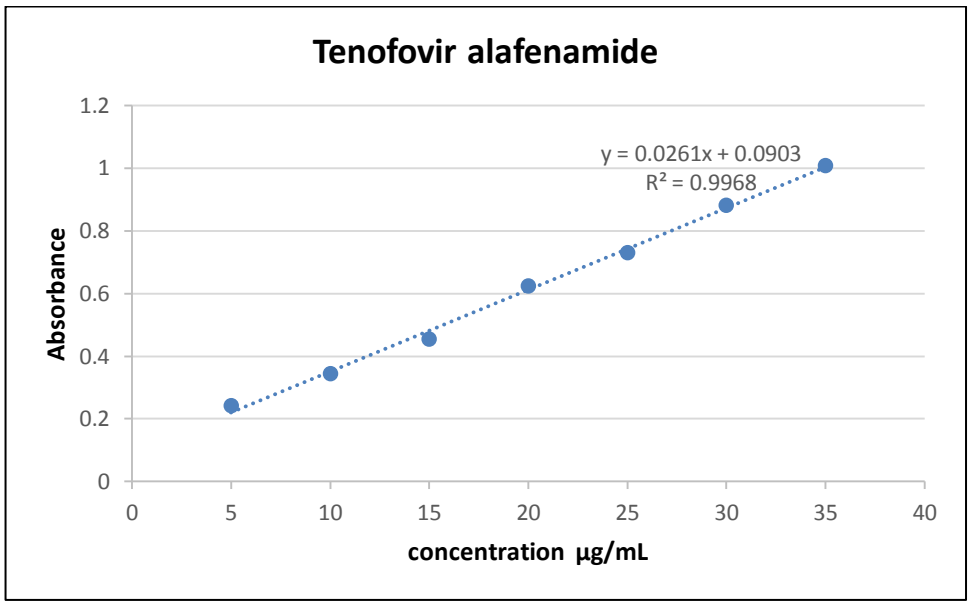

Fig 3:Calibration curveAUC of Tenofovir alafenamide

Area under curve (Area calculation):

This method involves calculation ofintegrated value of absorbance with respect to wavelengthinindicatedrange.Areacalculationprocessingitemcalculatestheareaboundedbythecurveandhorizontal axis. Here horizontal axis represents baseline.

$$Areacalculation(\alpha+\beta)=\frac{\lambda_1}{\lambda_2}Ad\lambda$$

Whereas, αisarea of portion bounded by curve data and a straight line connecting the start and end point, βisarea of portion bounded by a straight line connecting the start and end point on curve data and horizontalaxis, $\lambda_1$andλ_2 are wave lengthsre presenting start and end point of curve region. In this study area was integrated between wavelength ranges from250.12- 261.26nm [Figure 4].The calibration curves for tenofovir alafenamide was prepared in the concentration range of 5-35 µg/mL at their respective AUC range[8,14].

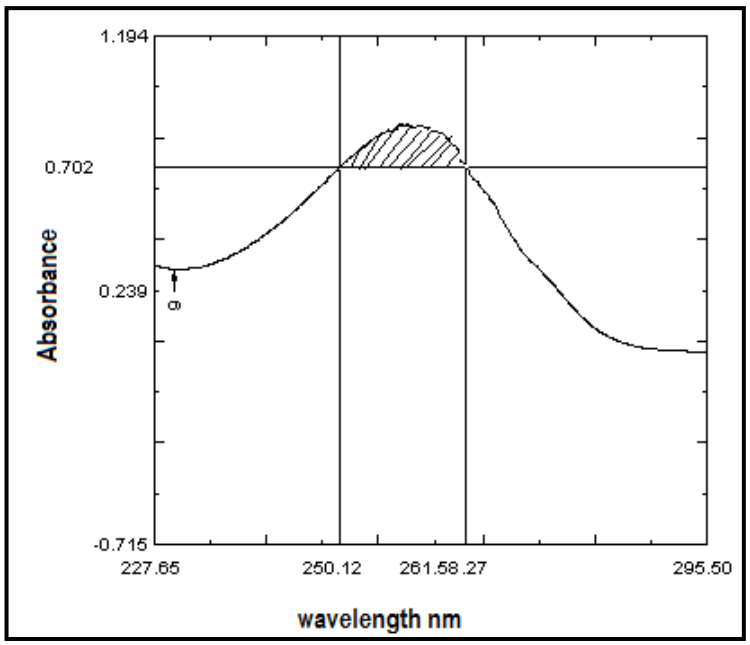

Fig 4: Area under curve graph of 30 µg/mL tenofovir alafenamide

Validation of the developed method:

The objective of validation of ananalytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline.

Table 1: Linearity results of tenofovir alafenamide in methanol

Concentration (µg/ml)	**Absorbance nm**
5	0.241
10	0.344
15	0.455
20	0.623
25	0.731
30	0.881
35	1.008

Table 2: Results of Precision

Precision	**Method AUC(%RSD)**
Repeatability	0.6654
Intraday	0.7664
Interday	0.8835

Linearity:

Fresh aliquots were prepared from the stock solution (100µg/ml) in different concentrations. The samples were scanned in UV–visible spectrophotometer against reagent blank. It was found that the selected drug shows linearity between the 5-35µg/ml (Table 2& 3).

Repeatability:

The precision of the method was checked by repeatedly injecting (n=6) standard solutions of tenofovir alafenamide (30 µg/mL). Area under curve of each of these solutions was measured in the range of 250.12-261.26 nm. Percentage relative standard deviation (%RSD) was calculated (Table 2).

Intermediate Precision (Reproducibility):

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of tenofovir alafenamide (5, 10 and15µg/mL).The results were reported in terms of relative standard deviation (%RSD).The results were tabulated in (Table 2).

Accuracy (Recovery studies):

The accuracy for the analytical procedure was determined at 80%, 100% and120% levels of standard solution. Area under curve was measured in the range of 250.12- 261.26 nm and results were expressed in terms of % recoveries. Three determinations at each level were performed and% RSD was calculated. The results were tabulated in (Table 3).

Table 3: Recovery Study of tenofovir alafenamide

Accuracy level	Mean % recovery	%RSD
80%	99.95	0.856
100%	99.58	0.743
120%	98.02	0.688

Table 4:LOD and LOQ of Cycloserine

Method	Method AUC
LOD	0.3819
LOQ	1.5917

Limit of detection and Limit of quantitation:

The objective of validation of ananalytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline. (Table 4).

Results and Discussion:

An attempt was made to develop a simple and specific method forthedetermination of tenofovir alafenamide in bulk form. The generated regression equations were,

Method A – $\int_{261.26}^{250.12} A\, d'\lambda$0.0261x+0.0903 R^2=0.9968

Where $\int_{261.26}^{250.12} A\, d'\lambda$ is area under curve between 250.12- 261.26 nm, $\frac{d'A}{d'\lambda}$ is amplitude difference, x is concentration and R^2 is correlation coefficient. The R2 values was 0.9968for AUC method indicated thatdeveloped methodwere linear. The proposed method was found to be precise as % RSD values for intraday aswell as interday precision was satisfactory. The drug at each of the 80 %, 100 % and 120 % levels showed goodrecoveries that is in the range of 98.00 to 99.00%, hence itcould be said that the method was accurate.The LODand LOQ were calculated as 0.3819 µg/ml and 1.5917µg/ml.Thus, the developed method is found to be robust and rugged which can be applied as a rapid tool for routine analysis of tenofovir alafenamide in the bulk and in the pharmaceutical dosage form. The validation parameters for method is summarized in Table 5.

Table No. 5: Optical Parameters/ Summary of tenofovir alafenamide

Parameter	Result
Range	250.12- 261.26 nm
Absorption maxima	259 nm
Linearity range	5-35 (ug/mL)
Standard regression equation	0.0261x+0.0903
Correlation coefficient	0.9968
Repeatability	0.6654
Intraday	0.7664
Interday	0.8835
Accuracy (Mean % Recovery)	99.18
LOD	0.3819
LOQ	1.5917

Conclusion:

From the results and discussion the method described in this paper for the determination of tenofovir alafenamide in bulk is simple, sensitive and reproducible. The proposed methods can be successfully applied for tenofovir alafenamide without any interference in quality control.

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