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A Validated Method for Development of Tenofovir as API and Tablet Dosage Forms by UV Spectroscopy

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ABSTRACT

A simple new spectrophotometric method has been developed for estimation of Tenofovir disoproxil fumarate in bulk and tablet dosage form. Tenofovir disoproxil fumarate is estimated to be 261 nm in triple distilled water. The Beer's law is obeyed in the concentration range of 5 – 90 µg/mL of the drug. The slope and intercept values are 0.0109 and 0.1075, respectively. Results of analysis of this method have been validated statically and by recovery studies. The method is applied to the marketed tablet formulation. A result of the analysis of tablet formulation, given as a percentage of label claim ± standard deviation is 98.15 ± 0.76. The precision and accuracy has been examined by performing recovery studies and found to be 100.06 ± 1.24. The developed method is simple, sensitive, and reproducible, and can be used for the routine analysis of Tenofovir disoproxil fumarate in bulk and tablet dosage form.

Key words: Tenofovir disoproxil fumarate, triple distilled water, UV Spectrophotometric method

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INTRODUCTION

Tenofovir disoproxil fumarate (a prodrug of Tenofovir), which is a fumaric acid salt of bis-isopropoxycarbonyloxymethyl ester derivative of Tenofovir. In vivo Tenofovir disoproxil fumarate is converted to Tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate. Tenofovir exhibits activity against HIV-1 reverse transcriptase,[1] chemically, TDFis9-[(R)-2-[[bis[[(isopropoxycarbonyl) oxy]methoxy] phosphinyl] methoxy] propyl]adenine fumarate(1:1). A literature survey reveals that very few high performance liquid chromatography (HPLC) and Liquid chromatography-mass spectrometry (LC-MS) methods are available for estimation of Tenofovir.[2-5] Hence, the proposal to estimate Tenofovir disoproxil fumarate by the UV spectroscopic method.

MATERIALS AND METHODS

Instrument used

UV-visible spectrophotometer (Elico 210) 10 mm quartz cell and spectral bandwidth 1 nm.

Reagent used

Triple distilled water.

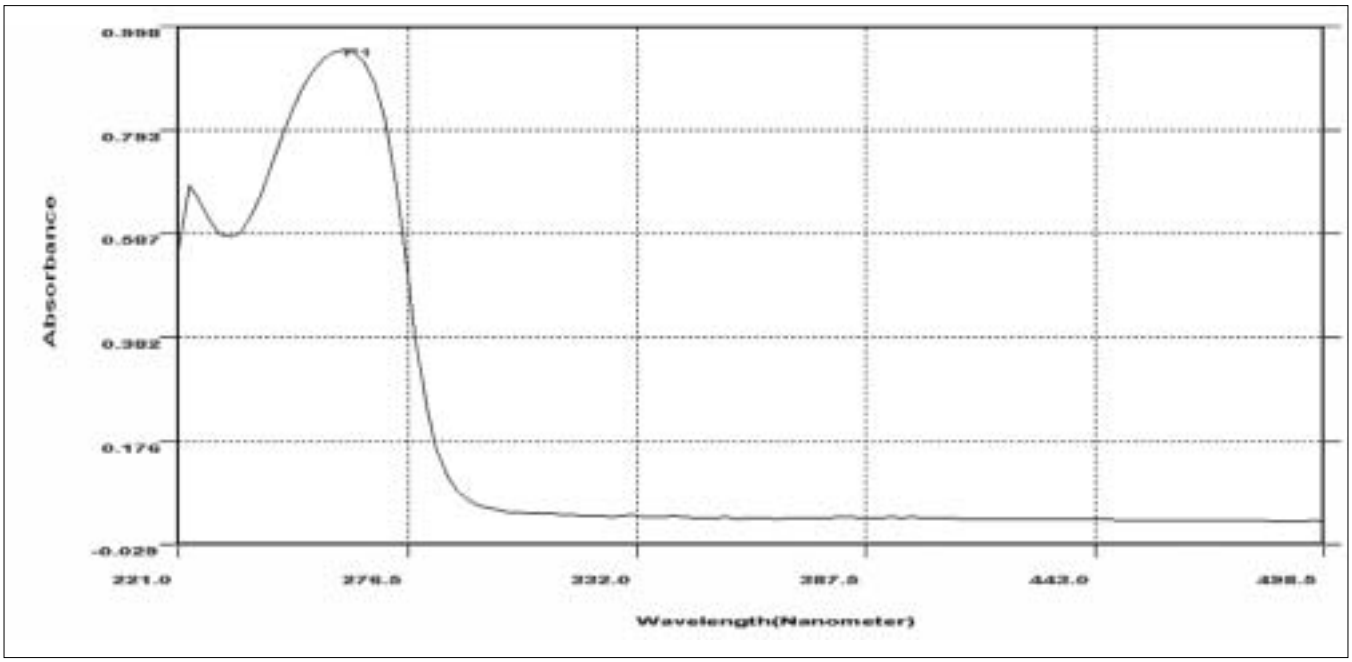

Figure 1: UV-Spectrum of Tenofovir disoproxil fumarate

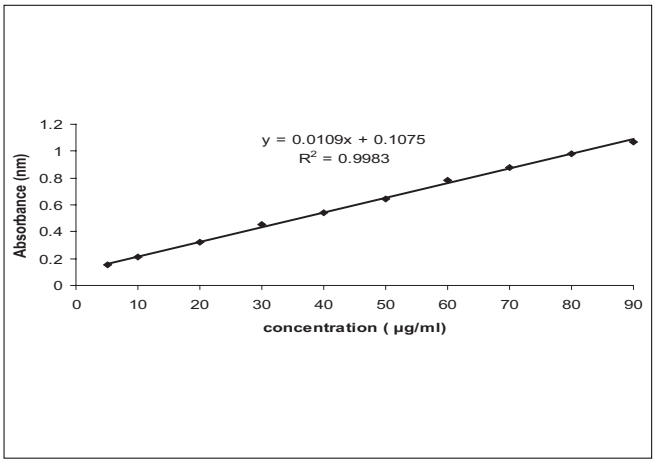

Figure 2: Calibration curve of Tenofovir at 261 nm

Preparation of Standard stock solution

About 100 mg of the drug was accurately weighed and transferred to a 100 mL volumetric flask and dissolved in about 15 mL of distilled water. The volume was then made up to the mark with distilled water. Ten milliliters of this drug solution was transferred to a 100 mL volumetric flask and further diluted up to the mark with distilled water. This solution contained 100 µg of drug per milliliter of the solution.

Determination of wavelength of maximum absorbance

Five milliliters of stock solution of Tenofovir was transferred to a 10 ml volumetric flask. It was diluted up to the mark with water. The absorbance of the final solution was scanned in the range of 200 – 400 nm, against Triple distilled water as the blank. Tenofovir showed absorbance maxima at 261 nm [Figure 1]. The drug followed linearity in the concentration range of 5 – 90 µg/mL (Y = 0.0109 x + 0.1075, r^2 = 0.9983). [Figure 2].

Preparation of calibration curve for Tenofovir disoproxil fumarate

Stock solution of tenofovir disoproxil fumarate (0.5 – 9 ml) were pipetted out in to a series of Ten volumetric flask of 10 ml. The volume in each volumetric flask was made up to the mark with distilled water and the mixer was shacked. That produced the concentration range of 5-90 µg/ml of Tenofovir disoproxil fumarate. The absorbances of solutions were measured at 261 nm against water as blank.

Table 1: Optical characteristics, regression equation, and coefficient of the method

Data	Results
Maximum wavelength (λmax)	261 nm
Beer's law limit	5 – 90 µg/mL
Molar's absorbtivity (1 $mole^{-1}$ cm^{-1})	10.08647 x 10^3
Sandell's Sensitivity (mg/cm^2/.001abs unit)	2.49 x 10^{-2}
Regression equation	Y = 0.0109x + 0.1075
Slope	0.0109
Intercept	0.1075
Correlation coefficient (r)	0.9981
Accuracy (% Recovery) (n=6)	100.062
Precision (% RSD)	
Intra day (n=3)	1.14
Inter day (n=3)	1.19
% Recovery	100.06

Table 2: Recovery method from placebo solution

% of solution in placebo	Amount recovered (µg/ml)	Actual amount added (µg/ml)	Percent recovery	Mean recovery ±Standard Deviation
80	23.934	23.904	100.12	99.40 ± 0.659
100	29.532	29.880	98.84	
120	35.580	35.856	99.23	

Table 3: Results of estimation of Tenofovir disoproxil fumarate (Tentide)

Tablet	Labeled Amount (mg/mL)	Amount found	% amount ± SD RSD
Tentide	300 mg	299.24	99.78 ±.037

The linearity, slope, intercept, correlation coefficient and optical characteristics are summarized in Table 1.

Recovery studies and validation of the method according to International conference on Harmonization Guidelines[6-9]

To study the accuracy of the above proposed method, recovery studies were carried out by the addition of the standard drug solution to the placebo, and recovery of the drug was calculated. The result of the recovery studies are summarized in Table 2. The precision of the method was studied by carrying out Interday and intraday analysis and was expressed as a relative standard deviation. Specificity was checked by spiking the references standard by placebo. The results were found to be satisfactory and are reported in Table 2.

Estimation of tenofovir in tablet dosage form

For analysis of commercial formulation 20 tablets were weighed accurately and triturated to a fine powder. Powder equivalent to 100 mg of Tenofovir was weighed and transferred to a 100 mL volumetric flask. To this 25 mL of triple distilled water was added and shaken manually for 15 minutes. The volume was made up to mark with the same solvent and filtered through Whatmann filter paper No. 42. Ten milliliters of this solution was transferred to a 100 mL volumetric flask for further dilution and contained 100 µg/mL of the solution. An appropriate aliquot was transferred to a 10 mL volumetric flask. The volume was adjusted to the mark and absorbance was recorded at 261 nm. The results were found to be satisfactory and are reported in Table 3.

RESULTS AND DISCUSSION

The method for the estimation of Tenofovir disoproxil fumarate in the tablet dosage form was developed. The drug shows absorption maxima at 261 nm. Spectrophotometric method linear response was obtained in the concentration range of 5 – 90 µg/mL, with a correlation coefficient of 0.9981. The method was statistically validated according to the ICH guidelines. The developed validated method was simple, rapid, precise, and accurate. The newly developed method could be used for routine analysis of Tenofovir disoproxil fumarate in tablet dosage forms.

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