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Simple Validated UV method for Tenofovirin Bulk and It's Tablets Dosage Form

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A B S T R A C T

Tenofovir is prescribed to HIV patients. The present research work is to develop and validate Tenofovir by UV spectrometric method. Simple, accurate, precise and cost efficient spectrophotometric method has been developed for the estimation of Tenofovir in bulk and its tablets dosage form. The optimum condition for the analysis of the drug was established. The maximum wavelength (λmax) was found to be 260nm in water. The mean percentage recovery of Tenofovir was found to be in range of 99.40-101.64%. Beers law was obeyed in the concentration range of 10-200µg/ml. Calibration curve shows a linear relationship between the absorbance and concentration. The line equation y = 0.026X+0.252 with R^2 = 0.998 was obtained. Validation was performed as ICH guidelines for linearity, accuracy, precision, LOD & LOQ. The proposed method may be suitable for analysis of Tenofovir in bulk and tablets formulation for routine quality control purposes.

Keywords: Tenofovir tablets, UV spectrophotometric, beers law

A R T I C L E I N F O

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1. Introduction

Tenofovir ({[(2R)-1-(6-amino-9H-purin-9-yl) propan-2-yl] oxy} methyl) phosphonic acid. Tenofovir disoproxil fumarate (TDF) is one of the most commonly used antiretroviral drugs for the treatment and prevention of HIV infection. Currently, TDF is a component in several one-pill once-a-day combination antiretroviral therapy (ART)

formulations and is recommended for first line ART regimens by international guidelines. Tenofovir inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination [1, 2].

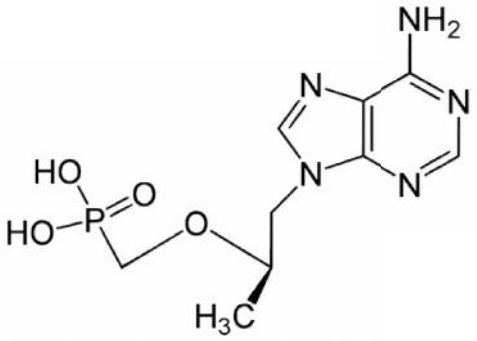

Figure 1: Chemical Structure of Tenofovir

2. Materials and method

Experimental methods:

An UV–Visible double beam spectrophotometer with 1cm matched quartz cells were used for the spectral and absorbance measurements. All the chemicals and reagents used were of analytical grade and the aqueous solutions were freshly prepared with triple distilled water.

Preparation of Stock solution

Accurately weighed 10 mg of Tenofovir disoproxil fumaratewas transferred into 100 ml volumetric flask, dissolved in 10ml of Distilled water. Take 0.1 ml from above solution and make up to 10ml. Take 0.5 ml from the above solution and make up to 10 ml to get a concentration of 0.5µg/ml. working standard solution of 0.5µg/ml for10ml with Distilled water. The working standard solutions were daily prepared by diluting stock solution in water [3].

Preparation of test sample

Ten tablets of Tenofovir disoproxil fumarate were weighed and powdered. The quantity of the powder equivalent to 10 mg (22.6mg) of Tenofovir disoproxil fumarate was transferred in to 10ml of volumetric flask. Distilled water was added up to 10ml and mixed for 5-10 min, filtered the solution and first few ml was discarded. 0.1 ml from the above stock solution was transferred to 10 ml volumetric flask along with Distilled water. From the above solution 0.5ml was transferred to 10 ml volumetric flask along with Distilled water to get 0.5µg/ml concentration [3].

Solubility Studies

Tenofovir was tested for solubility in various solvents at room temperature [4].

λ_{max} Determination:

The absorbance of the solutions containing Tenofovir at 10µg/ml was determined in the UV range 200-400nm using an appropriate blank [5].

Accuracy

The accuracy of the method was checked by recovery determinations and percentage bias. The determination was done over three concentration levels in triplicate according to the ICH guidelines. The concentration levels selected as QC samples were 5µg/ml, 10µg/ml and 15µg/ml of Tenofovir [6].

Precision

The precision was evaluated on the basis of repeatability and intermediate precision. On at least three occasions, six replicates of each QC sample pool at low, three replicates middle, and six replicates at high concentrations were assayed. Percentage relative standard deviation (%RSD) was calculated [6].

Linearity

The linearity of the described spectrophotometric method was studied in the concentration range 10-100µg/ml for Tenofovir. The calibration curves were constructed by plotting concentration versus intensities. In the overall concentration range examined, the linearity was evaluated by linear regression analysis that was calculated by the least square regression method [6].

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value [6].

Limit of Quantification

The Quantization limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy [6].

Degradation studies

The degradation studies were performed for the Tenofovir in water [7].

3. Results and Discussion

Solubility of Tenofovir disoproxil fumarate performed in various solvents like water, dilute hydrochloric acid (HCl), glacial acetic acid, acetone, lactic acid, sodium hydroxide. The λ max was found to be 260nm. The λ_{max} of Tenofovir pure drug and in tablets were shown in fig 2 and 3 respectively. The Molar Absorptivity was found to be 7.5 x 10^{-6} by using the formula (e= A / c l), (A= absorbance, c = sample concentration in moles/liter &l = length of light path through the sample in cm). The Sandell's sensitivity (Sandell's sensitivity = mol. wt / molar absorvitity) was found to be 2.96 X 10^{7}µg cm^{-2}.The Accuracy results were shown in table 1.The precision was carried out for 10µg/ml. The intraday precision for 10µg/ml was found to be 1.48% RSD, while it gave a value of 1.579% RSD at interday (intermediate precision). The accuracy of the method was calculated as percent bias. The Intraday Precision and Inter-day precision of Tenofovir tablets were shown in table 2 and 3 respectively. The spectrum of standard solution of Tenofovir in water was given in Fig. 2 and 3.The λ_{max} was found to be 260nm. The calibration curve was prepared by plotting concentration (in µg/ml) on the abscissa and absorbance in ordinate axis in the range of 10-100µg/ml. Good linearity was obtained in the concentration range considered. The linearity equation was developed by using least square regression analysis. The Limit of detection and Limit of Quantization was calculated according to the ICH guidelines. The Beer-Lamberts limit was found to be10-100µg/ml (table 4).Calibration curve of Tenofovir was plotted (shown in fig. 4) and the linearity, regression, LOD and LOQ were shown in table 5.The LOD was found to be

0.9160µg/ml and the LOQ was found to be 3.053µg/ml. The acid, base, heat and peroxide degradation spectrums of Tenofovir tablets were shown in fig 5, 6, 7 and 8 respectively. The summary of these degradation results were shown in table 6.

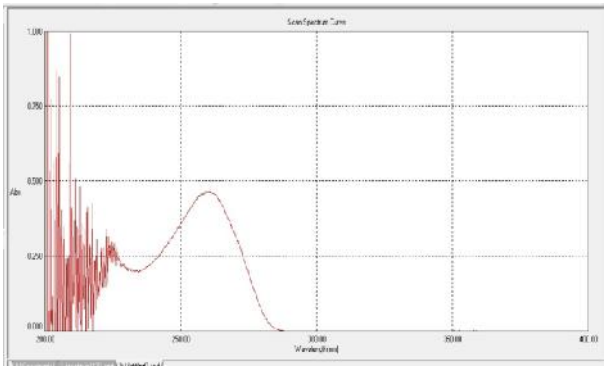

Figure 2: λ_{max} of Tenofovirpure drug

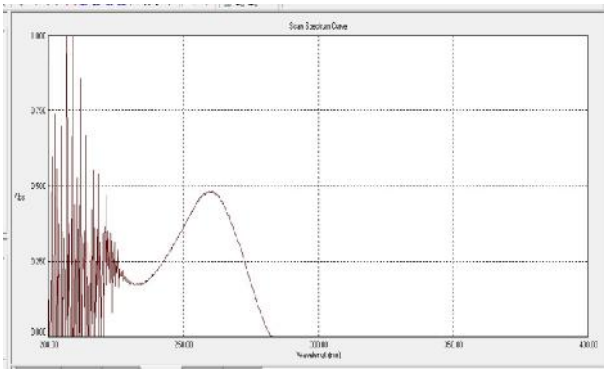

Figure 3: λ_{max} of Tenofovir tablet powder

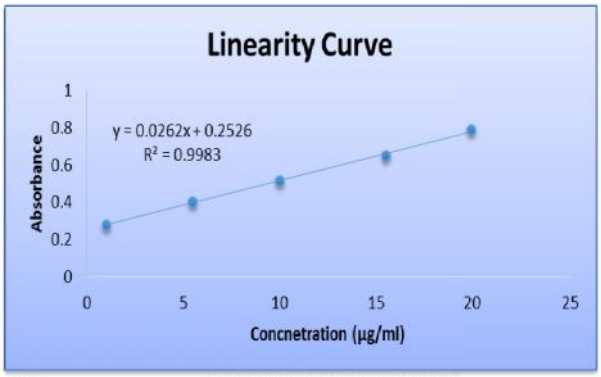

Figure 4: Calibration curve of Tenofovir

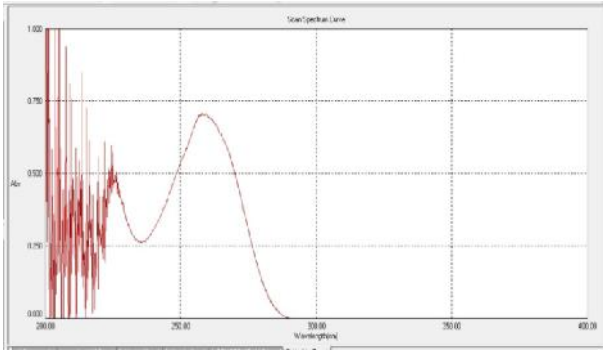

Figure 5: Acid degradation Spectrum of Tenofovir tablets

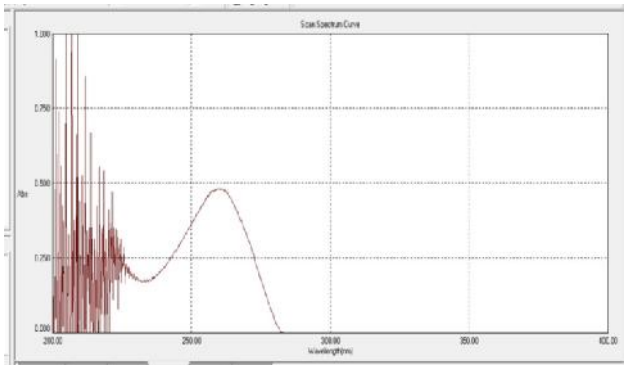

Figure 6: Base degradation Spectrum of Tenofovir tablets

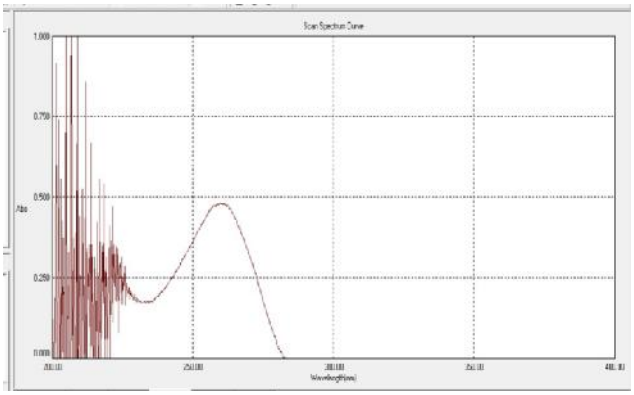

Figure 7: Heat degradation Spectrum of Tenofovir tablets

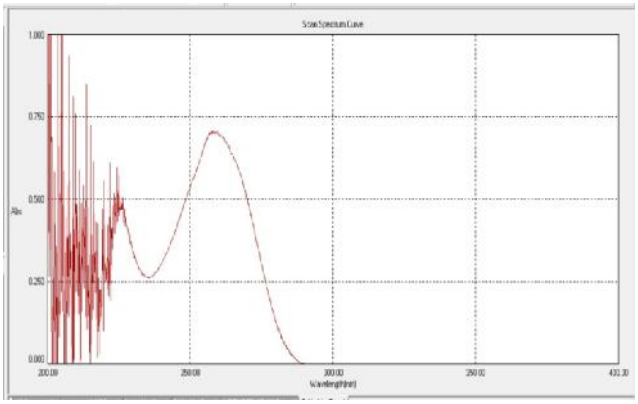

Figure 8: Peroxide degradation Spectrum of Tenofovir tablets

Table 4: Calibration (Linearity) of Tenofovir tablets

Conc. (µg/ml)	Absorbance
1	0.278
5.5	0.403
10	0.513
15.5	0.647
20	0.786

Table 6: Degradation studies of Tenofovir tablets

Parameter	Result
Acid degradation	0.547
Base degradation	0.512
Heat degradation	0.512
Peroxide degradation	0.549

Table 1: Accuracy results of Tenofovir tablets

Conc. Level	Absorbance	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean % Recovery
50	0.276	4.98	4.96	99.49	99.91
	0.277	4.98	4.97	99.85	
	0.278	4.98	4.99	100.21	
	0.278	4.98	4.99	100.21	
	0.276	4.98	4.96	99.49	
	0.278	4.98	4.99	100.21	
100	0.552	9.96	9.91	99.49	99.84
	0.556	9.96	9.98	100.21	
	0.554	9.96	9.95	99.85	
150	0.834	14.94	14.97	100.21	99.91
	0.822	14.94	14.76	98.77	
	0.832	14.94	14.94	99.97	
	0.836	14.94	15.01	100.45	
	0.833	14.94	14.96	100.09	
	0.832	14.94	14.94	99.97	

Table 2: Intraday Precision of Tenofovir tablets

S. No	Intraday		
	Absorbance		
	9:00 AM	1:00 PM	5:00 PM
1	0.522	0.541	0.54
2	0.525	0.54	0.546
3	0.527	0.543	0.553
4	0.507	0.548	0.555
5	0.510	0.534	0.542
6	0.522	0.521	0.545
Average	0.519	0.538	0.547
SD	0.008	0.009	0.006
% RSD	1.596	1.750	1.094

Table 3: Inter-day precision of Tenofovir tablets

Interday		
Absorbance		
1st Day	2nd Day	3rd Day
0.552	0.486	0.552
0.526	0.488	0.569
0.529	0.472	0.554
0.525	0.478	0.548
0.525	0.476	0.564
0.53	0.479	0.55
0.531	0.480	0.556
0.010	0.006	0.008
1.962	1.267	1.510

Table 5: Slope, Linearity, LOD and LOQ of Tenofovir tablets

Slope	Linearity (R^2)	LOD(µg/ml)	LOQ(µg/ml)
0.026x+0.252	0.998	0.9160	3.053

4. Conclusion

In this study simple, fast and reliable UV spectrophotometric method was developed and validated for the determination of Tenofovir in tablet formulation. The method was applied directly to the analysis of pharmaceutical dosage forms without the need for separation such as extraction steps prior to the drug analysis. As these proposed methods have the lowest LOD value and wider linear range so these methods were more sensitive methods. From the results obtained, we concluded that the suggested methods showed high sensitivity, accuracy, reproducibility and specificity. Moreover, these

methods were simple and inexpensive and they can be employed for the routine quality control analysis of Tenofovir in pharmaceutical formulations.

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