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Original Article

VALIDATED ZERO ORDER AND FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHODS FOR INVITRO ANALYSIS OF TENOFOVIR DISOPROXIL FUMARATE TABLETS USING AZEOTROPIC MIXTURE

M HIMAJA*[1], J KALPANA[1], C ANBARASU[2]

[1]Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore, 632014, Tamilnadu, [2]Strides Arcolab, Bangalore, 560076, Karnataka.
Email: drmhimaja@gmail.com

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ABSTRACT

Objective: To develop a simple and reliable derivative spectro photometric methods for the determination of Tenofovir disoproxil fumarate in bulk and pharmaceutical dosage forms.

Methods: The solutions of standard and the sample were prepared in azeotropic mixture (methanol: water, 40:60 v/v). The quantitative determination of the drug was carried out with zero order derivative values measured at 260 nm and first order derivative values measured at 273 nm (n=6).

Results: Calibration graphs constructed at their wavelengths of determination, were linear within the concentration range of 4-24 µg/mL (r^2 = 0.999 and r^2 = 0.998) for zero order and first order derivative spectro photometric method.

Conclusion: No significant difference between the performance of the proposed methods regarding the mean values and standard deviations and is suitable for the routine quality control application of Tenofovir disoproxil fumarate in pharmaceutical formulations.

Keywords: Tenofovir disoproxil fumarate, Derivative spectro photometric, Zero order derivative spectrum, First order derivative spectrum.

INTRODUCTION

Tenofovir disoproxil fumarate (shown in fig.1) is converted intercellularly to the diphosphate. This diphosphate halts the DNA synthesis of HIV through competative inhibition of reverse transcriptase and incorporation into viral DNA [1]. Chemically, bis(isopropyloxy-carbonyloxymethylester of (R)-9-(2-phosphonomethoxy-propyl) adenine with fumaric acid [2]. Literature survey reveals that very few HPTLC [3], UV [4,8], high performance liquid chromatography (HPLC) [5,8,9], and Liquid chromatography-mass spectrometry (LC-MS)[6,7] methods are available for estimation of Tenofovir individually or in combination with other drugs.

Various spectrophotometric measurements like simultaneous equation, absorbance ratio methods are widely used for the analysis of bulk and pharmaceutical dosage forms [10-11]. Tenofovir disoproxil fumarate is slightly soluble in water. In the existing methods, the organic solvents used for the estimation were costly and not environment-friendly. So we have applied concept of azeotropes and combined an organic solvent (methanol) with water. The developed method using azeotropic mixture was simple, precise, specific and accurate. Hence it can be used for the routine quality control analysis of Tenofovir disoproxil fumarate in bulk drug and tablet formulations.

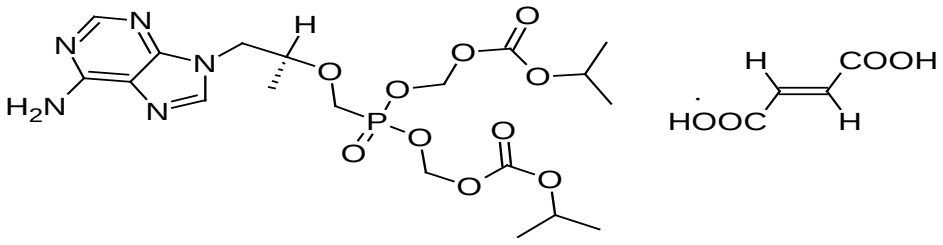

Fig. 1: Structure of Tenofovir disoproxil fumarate

MATERIALS AND METHODS

Materials and chemicals

Tenofovir disoproxil fumarate standard was a gift sample by Strides Arcolab Ltd. Bangalore, Karnataka, India and was used without further purification. All chemicals and reagents used were of analytical grade. All stock solutions were prepared using double distilled water.

Instrumentation

Spectrophotometric measurements were performed using a Jasco V 670 UV/VIS/NIR diode array spectrophotometer (scan speed 400 nm/min and wavelength interval 1 nm), associated with Spectra manager software (Jasco, Japan).

Preparation of standard and sample solutions

Stock solution of 800 µg/mL of Tenofovir disoproxil fumarate was prepared in azeotropic mixture (Methanol: Water, 40:60 v/v), for zero order and first order derivative spectrophotometric analysis. The standard solutions were prepared by dilution of the stock solution with azeotropic mixture in a concentration range of 4, 8, 12, 16, 20 and 24µg/mL for zero order and first order derivative spectrophotometric measurements. Methanol: Water, 40:60 v/v was used as a blank solution.

Assay procedure

A total of 20 tablets of Tenofovir disoproxil fumarate were accurately weighed and powdered. Powder equivalent to 10 mg was accurately weighed and transferred to volumetric flask of 25 mL capacity. 15 mL of the mixture of methanol and water (40:60, v/v) was transferred to volumetric flask and sonicated for 5 min. The flask was shaken and volume was made up to the mark with the mixture of methanol and water (40:60, v/v). The above solution was filtered through Whatman filter paper (0.45 mm). From this solution, 5 mL was transferred to volumetric flask of 25 mL capacity. The volume was made up to the mark to get a concentration 80 µg/mL (Solution A). From the solution A, 1.5 mL was transferred to volumetric flask of 10 mL capacity. The volume was made up to the mark with the mixture of methanol and water (40:60, v/v) to give a solution containing 12 µg/mL for both zero order and first order derivative spectrophotometric methods.

RESULTS AND DISCUSSION

The developed method was validated for linearity, range, precision, accuracy, specificity, LOD and LOQ as per ICH Q2 (R1) guidelines [12]. The zero order and first order derivative spectra for Tenofovir disoproxil fumarate were recorded at the wavelength of 260 nm and 273 nm respectively [fig. 2-3].

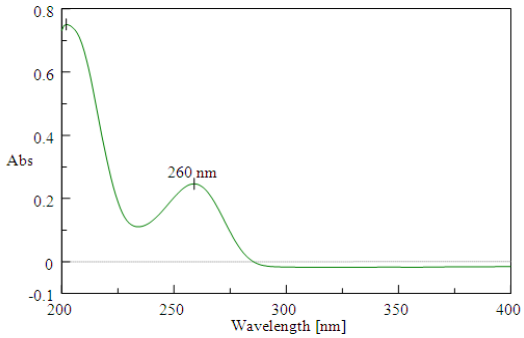

Fig. 2: Zero order derivative spectrum of 20µg/mL Tenofovir disoproxil fumarate in Azeotropic Mixture

Linearity and range

Under the experimental conditions described, the graph obtained for zero order and first order derivative spectra showed linear relationship. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were y = 0.04x - 0.0297 (r2 = 0.999) for zero order derivative spectrophotometry and y = 0.002x + 0.003 (r2 = 0.998) for first order derivative spectrophotometry. The range was found to be 4-24 µg/mL for both zero order and first order derivative spectrophotometric methods (Table no. 1).

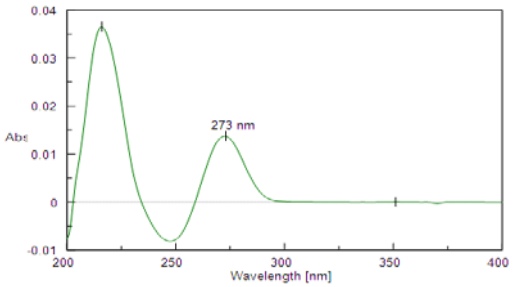

Fig. 3: First order derivative spectrum of 20 µg/mL Tenofovir disoproxil fumarate in Azeotropic Mixture

Precision

To determine the precision of the method, Tenofovir disoproxil fumarate solutions at a concentration of 20µg/mL was analyzed six times for both zero order and first order derivative spectrophotometric methods. Solutions for the standard curves were prepared fresh everyday (Table no. 2).

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3 x σ / S and LOQ = 10 x σ /S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.0409 µg/mL and 0.1241 µg/mL respectively for zero order derivative and The LOD and LOQ were found to be 0.792 µg/mL and 1.942 µg/mL for first order derivative methods respectively.

Recovery

To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amount of Tenofovir disoproxil fumarate to reanalyzed solutions of commercial tablets (Table no. 3).

Table 1: Stastical data for the calibration graphs for determination of Tenofovir disoproxil fumarate by proposed methods

Parameters	Zero order derivative	First order derivative
Linearity range (µg/mL)	4-24	4-24
r^2 ± S.D.	0.999 ± 0.012	0.998 ± 0.023

[a]n=6

Table 2: Results of Intra and Inter Day Precision

Parameters	Intra Day Precision		Inter Day Precision	
	S.D	% RSD	S.D	% RSD
Zero order derivative	0.00044	0.136	0.0011	0.351
First order derivative	0.0000837	1.37	0.0000548	0.90

[a]n=6 [b] Average of one concentrations 20 µg/ml

Table 3: Data of recovery studies

Amount Added (mg)	Amount Found (mg)	Recovery (%)	Mean Recovery
Zero order derivative spectrophotometric method			
5	4.96	99.2	98.7
10	9.96	99.6	
15	14.6	97.3	
First order derivative spectrophotometric method			
5	4.99	99.8	99.4
10	9.98	99.8	
15	14.8	98.6	

Table 4: Assay results for the determination of Tenofovir Disoproxil Fumarate in pharmaceutical formulation

Parameters	Amount of Tablet label claim	Drug content (%)	% RSD
Zero order derivative	300 mg	99.6	0.179
First order derivative	300 mg	99.3	0.951

[a]n=6, Average of three concentrations 9 µg/Ml

Table 5: Summary of validation parameters

Parameter	Zero derivative method	First derivative method
Wavelength (nm)	260	273
Linearity range (µg/mL)	4-24	4-24
Regression equation y=mx+c	y = 0.04x - 0.0297	y = 0.002x + 0.003
Correlation coefficient	0.999	0.998
Limit of detection (µg/mL)	0.0409	0.7920
Limit of quantitation	0.1241	1.9420
Recovery %	97.3-99.6	98.6-99.8

Analysis of the marketed formulation

There was no interference from the excipients commonly present in the tablets. The drug content was found to be 100.01% with a % R.S.D. of 0.14 and 99.93% with a % R.S.D. of 0.37 for zero order and first order derivative spectrophotometric methods respectively. It may therefore be inferred that degradation of Tenofovir disoproxil fumarate had not occurred in the marketed formulations. The low % R.S.D. value indicated the suitability of this method for routine analysis of Tenofovir disoproxil fumarate in pharmaceutical dosage form (Table no. 4). The summary of the validation parameters is depicted in (Table no. 5).

CONCLUSION

No UV or derivative spectrophotometric methods have been reported yet for the determination of Tenofovir disoproxil fumarate using azeotropic mixture. Therefore simple, fast and reliable derivative spectrophotometric methods were developed for the routine determination of Tenofovir disoproxil fumarate. The developed methods can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

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