Journal of Pharmaceutical and Biomedical Analysis Letters

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Research Article Open Access

Analytical Method Development and Validation for the Determination of Tenofovir Disoproxil Fumarate in bulk and tablet using UV Spectrophotometer

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A B S T R A C T

Tenofovir disoproxil Fumarate (TDF), acyclic phosphonate nucleotide analogue, used as antiretroviral agents in the treatment of HIV-1 infection. An UV spectrophotometric method for the quantitative determination of Tenofovir in bulk and tablets was developed in present work. Distilled water was used as solvent. The wavelengths selected for the absorption correction method was 260 nm. The method was found to be linear between the ranges of 1-20 µg/ml. The mean percentage recovery was found in the range of 99.84%-99.91% at three different levels of standard additions. The precision (intra-day, inter-day) of method were found within limits (RSD <2%). Thus the proposed method was simple, precise, economic, rapid and accurate and can be successfully applied for the determination of Tenofovir in bulk and its tablet dosage form.

Keywords: Tenofovir, HIV, UV spectrophotometric, tablet.

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Article History: Received 29 January 2016, **Accepted** 10 March 2016, **Available Online** 18 July 2016

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Manuscript ID: JPBMAL2917

PAPER-QR CODE

Citation: B. Mohammed Ishaq, *et al.* Analytical Method Development and Validation for the Determination of Tenofovir Disoproxil Fumarate in bulk and tablet using UV Spectrophotometer. *J. Pharm, Biomed. A. Lett*., 2016, 4(2): 70-73.

1. Introduction

Antiviral drugs development has become a very active area in the last decade, especially with the challenges of AIDS, hepatitis, and avian and swine flu epidemics. The antiviral drugs are used in the treatment of viral infections. They

may also be used to provide protection, usually for a brief period only, against infection. There is little evidence that these compounds affect latent or non-replicating virus. Nonspecific symptomatic and supportive treatment is also important in the management of viral infections.

Tenofovir disoproxil Fumarate (TDF), acyclic phosphonate nucleotide analogue, is a fumaric acid salt of the bis iso propoxy carbonyl oxy methyl ester derivative of tenofovir. Chemically it is [[(1R)-2-(6- Amino-9H-purin-9-yl)-1-methylethoxy] methyl] phosphonic acid [1] (Figure 1). It is the first nucleotide reverse transcriptase inhibitor (NtRTIs) approved for use in combination with other antiretroviral agents in the treatment of HIV-1 infection in the United States. Unlike the nucleoside reverse transcriptase inhibitors, which must undergo three intracellular phosphorylation steps for activation, nucleotide analogues such as Tenofovir require only two such steps. This reduction in the phosphorylation requirement has the potential to produce more rapid and complete conversion of the drug to its pharmacologically active metabolite [2].

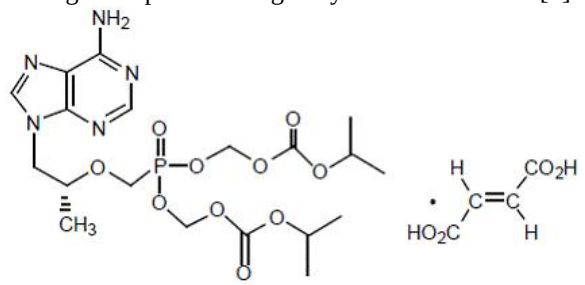

Figure 1: Chemical structure of Tenofovir disoproxil Fumarate

The literature reveals that few spectrophotometric techniques have been published involving determination of TDF by direct methods [3,4], derivative calculation [5-7]. Other spectrophotometric methods published include colorimetric assay using various derivatizing reagents [8-11]. Few HPLC methods were found in the literature for the assay of TDF [12-15]. But no simple UV spectroscopic method was reported by using distilled water as solvent. So, the aim of the current research work was to develop a simple UV spectroscopic method for the determination of TDF in bulk and its tablet dosage forms and validation [16] of the method according to ICH guidelines [17].

2. Materials and Methods

Instrumentation:

Systronics double beam UV–Visible spectrophotometer, 1800, with spectral band width of 1.0 nm and wavelength accuracy ± 0.5 nm with a pair of 1 cm matched quartz cells was used for measuring the absorbance of the resulting solution.

Chemicals and Reagents:

A standard drug of TDF was procured from Matrix Laboratories (Hyderabad, India). Tablet dosage form TDF (Tenvir; containing 300 mg TDF, manufactured by Cipla Pvt. Ltd,) was purchased from the local market. Double distilled water was prepared in-house, used as the solvent.

Preparation of standard stock solution:

Standard stock solution containing 10 µg/ml of TDF was prepared in distilled water. From the stock, different aliquots were taken and diluted to 10 ml mark with same solvent to obtain series of concentrations. The solutions were scanned on spectrophotometer in the UV range 200-400 nm. TDF showed absorbance maxima at 260 nm (figure 2).

Preparation of sample solution

For analysis of commercial formulation; twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 10 mg (22.62 mg) of tenofovir was transferred into 10 ml volumetric flask dissolved in 5 ml of distilled water, shaken manually for 10 min, volume was made up to mark with same solvent and filtered through Whatmann filter paper No.41. An appropriate aliquot was transferred to 10 ml volumetric flask, volume was adjusted to the mark and absorbance was recorded at 260 nm.

Method Validation:

The developed method was validated as per the ICH guidelines [17] for accuracy, precision, linearity, specificity, LOD, LOQ and assay.

3. Results and Discussion

Development and optimization of the sectorophotometric method:

Proper wave length selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of tenofovir, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration, and other conditions. Our preliminary trials were by using different compositions of diluents consisting of water with buffer and methanol. By using diluent consisted of methanol - water (50:50, v/v) best solubility result was obtained in distilled water.

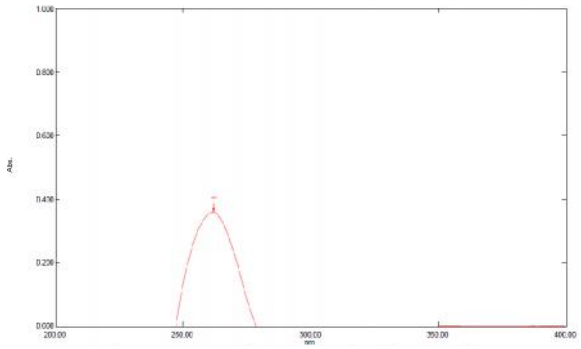

Figure 2: λ_{max} curve of Tenofovir disoproxil Fumarate API

Selection of wavelength

Scanned standard solution in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank. Tenofovir shows λ_{max} at 260 nm. The proposed analytical method is simple, accurate and reproducible. Figure 2 & 3 shows the λ_{max} of Tenofovir API and tablet spectra.

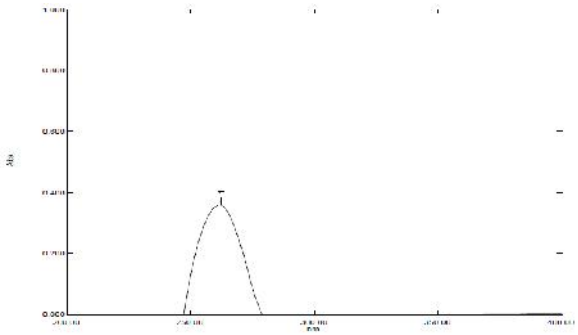

Figure 3: λ_{max} curve of Tenofovir disoproxil Fumarate Tablet dosage form

Method validation

Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 1.0–20.0 µg/mL was linear with a correlation coefficient (R^2) greater than 0.998. The LOD and LOQ were calculated as 0.9160 µg/mL and 3.053 µg/mL respectively. Figure 4 shows the linearity curve of Tenofovir.

Intra-day and inter-day precision

The intra-day and inter-day precision study of the developed method confirmed adequate sample stability and method reliability where all the RSDs were <2%. Results of precision were shown in table 1.

Accuracy/recovery

Results within the range of 99.84–99.91% ensure an accurate method as well as indicate non-interference with the excipients of formulation. Table 2 shows the accuracy results.

Specificity

The specificity of the analytical method was proved by comparing the spectra of placebo, Active pharmaceutical ingredient with tablet sample solution. The tablet spectra show no interference with excipients. Figure 2 & 3 shows the results of specificity.

Assay: Tenofovir content of the marketed product determined by the proposed method and was in good agreement with the label claims and was in the range of 98.38–100.53% with the RSD value was 0.841.

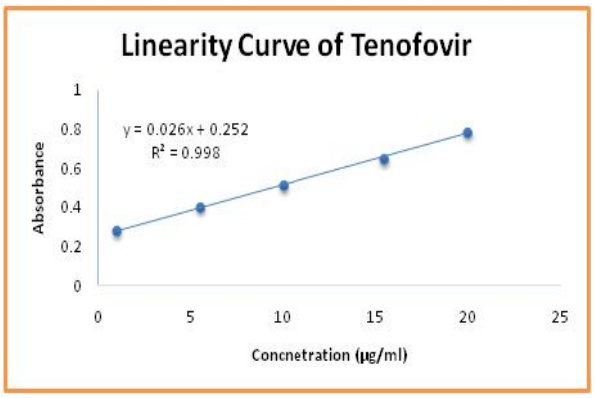

Figure 4: Linearity curve of Tenofovir

Table 3: Assay Results

S. No	Absorbance	% Assay
1	0.555	99.641
2	0.552	99.102
3	0.56	100.539
4	0.56	100.539
5	0.548	98.384
6	0.554	99.461
Average		99.611
STDEV		0.838
% RSD		0.841

Table 1: Intra-day and inter-day precision results

S. No	Intraday			Inter-day		
	Absorbance			Absorbance		
	9:00 AM	1:00 PM	5:00 PM	1st Day	2nd Day	3rd Day
1	0.522	0.541	0.54	0.552	0.486	0.552
2	0.525	0.54	0.546	0.526	0.488	0.569
3	0.527	0.543	0.553	0.529	0.472	0.554
4	0.507	0.548	0.555	0.525	0.478	0.548
5	0.51	0.534	0.542	0.525	0.476	0.564
6	0.522	0.521	0.545	0.53	0.479	0.55
Average	0.519	0.538	0.547	0.531	0.480	0.556
SD	0.008	0.009	0.006	0.010	0.006	0.008
% RSD	1.596	1.750	1.094	1.962	1.267	1.510

Table 2: Accuracy results

S. No	Conc. Level	Absorbance	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery
1	50 %	0.276	4.98	4.96	99.49
2	100 %	0.552	9.96	9.91	99.49
3	150 %	0.834	14.94	14.97	100.21

4. Conclusion

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, rapid, specific, accurate and precise. Therefore, this method can be used for the determination of Tenofovir either in bulk or in the dosage formulations without interference with commonly used excipients and related substances.

5. Acknowledgment

Authors wish to thank Matrix Laboratories, Hyderabad, India for supplying the reference standard, Tenofovir. We also extend our thanks to the Principal and Management of Narayana Pharmacy College, Nellore for providing all the facilities to carry out our project work.

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