Journal of Pharmaceutical and Biomedical Analysis Letters

Journal Home Page: www.pharmaresearchlibrary.com/jpbmal

Research Article **Open Access**

Development and Validation of a Stability Indicating UV Spectrophotometric Assay Method for Tenofovir Disoproxil Fumarate by Using Different Stress Conditions

K. Dharmendra, R. Surendra, C. Tejasri, D. Jagadeesh, B. Mohammed Ishaq*, D. China Babu, Sreenivasulu Munna

Department of Pharmaceutical Analysis, Narayana Pharmacy College, Chinthareddypalem, Nellore – 524002, A.P. India

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. A stability indicating 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%). The drug was subjected to acid, alkali, peroxide, UV and Heat degradation. Forced degradation studies of drug reveal good stability under the chosen experimental conditions. 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, acid, alkali, peroxide, heat, tablet.

A R T I C L E I N F O

CONTENTS

Article History: Received 19 February 2016, **Accepted** 31 March 2016, **Available Online** 18 July 2016

***Corresponding Author**
Dr. B. Mohammed Ishaq
Associate Professor, Dept. Pharmaceutical Analysis, Narayana Pharmacy College, Chinthareddypalem, Nellore, India
Manuscript ID: JPBMAL2918

PAPER-QR CODE

Citation: B. Mohammed Ishaq, *et al.* Development and Validation of a Stability Indicating UV Spectrophotometric Assay Method for Tenofovir Disoproxil Fumarate by Using Different Stress Conditions. *J. Pharm, Biomed. A. Lett*., 2016, 4(2): 74-78.

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].

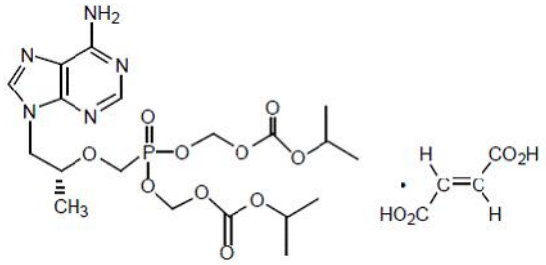

Figure 1: Chemical structure of Tenofovir disoproxil Fumarate

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 methd was reported by using distilled water as solvent. Forced degradation studies were carried out using aqueous solution of TDF. Test solution was exposed to various types of ICH prescribed stress conditions, like acid, alkali, peroxide, UV and Heat degradation, so as to check its stability. 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 1.0nm and wavelength accuracy ± 0.5nm with a pair of 1cm 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 300mg 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 guidelines17 for accuracy, precission, linearity, specificity, LOD, LOQ and assay.

Forced Degradation Studies:

ICH guidelines entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of active substance. Stability studies of TDF was carried out under extreme conditions acidic, alkaline, oxidative, thermolytic and photolytic as per stability indicating assays. In present study, TDF was exposed to 0.1N acid, (0.1 to 5N) NaOH, 3 % H2O2, heat and UV light at different time intervals till sufficient degradation of TDF was not achieved.

Standard stock solution of Tenofovir:

10 mg of pure TDF was transferred in 10 ml volumetric flask and dissolved in 6 ml of water, sonicated for 10 minutes and then final volume made upto mark with water to form std. stock solution of 10 µg/ml which was then filtered through 0.45 µ Whatman filter paper before used for forced degradation study.

Preparation of blank solution:

In separate 10 ml volumetric flask, each containing 5 ml of solvents used for degradation such as 0.1 N HCl, 0.1 N NaOH, 3 % H2O2 and Distilled water was added and heated at 60º C. From this, 2 ml of aliquot taken in separate 10 ml of volumetric flask at different time intervals, which was then neutralized with proper solvent and final volume made upto mark with water and absorbance was recorded at 260 nm on UV spectrophotometer.

Acid induced degradation:

In separate 10 ml volumetric flask containing 5 ml 0.1 N HCl, 5 ml TDF std. stock solution was added and heated at 60º C. From this, 2 ml of aliquot taken at different time intervals in separate 10 ml of volumetric flasks till sufficient degradation of TDF was not achieved, which was then neutralized with proper solvent and final volume made upto mark with water to form solution containing 10 µg/ml of TDF and absorbance was recorded at 260 nm on UV spectrophotometer against blank.

Alkali induced degradation:

In separate 10 ml volumetric flask containing 5 ml (0.1 to 5 N) of NaOH, 5 ml TDF std. stock solution was added and heated at 60º C. From this, 2 ml of aliquot taken at different time intervals taken in separate 10 ml of volumetric flasks till sufficient degradation of TDF was not achieved, which was then neutralized with proper solvent and final volume made upto mark with water to form solution containing 10 µg/ml of TDF and absorbance was recorded at 260 nm on UV spectrophotometer against blank.

Oxidation induced degradation:

In separate 10 ml volumetric flask containing 5 ml 3% H2O2, 5 ml TDF std. stock solution was added and heated at 60º C. From this, 2 ml of aliquot taken at different time intervals in separate 10 ml of volumetric flasks till sufficient degradation of TDF was not achieved, which was then neutralized with proper solvent and final volume made upto mark with water to form solution containing 10 µg/ml of TDF and absorbance was recorded at 260 nm on UV spectrophotometer against blank.

Thermal degradation:

10 mg of pure TDF was exposed in an oven at 80ºC till sufficient degradation of TDF was not achieved. From this, 10 mg of exposed TDF was transferred in 10 ml of volumetric flask and final volume made with water to form solution containing 10 µg/ml of TDF and absorbance was recorded at 260 nm on UV spectrophotometer against water as blank.

Photo degradation:

10 mg of pure TDF was exposed in a sunlight till sufficient degradation of TDF was not achieved. From this, 0.1 mg of exposed TDF was transferred in 10 ml of volumetric flask and final volume made with water to form solution containing 10 µg/ml of TDF and absorbance was recorded at 260 nm on UV spectrophotometer against water as blank.

3. Results and Discussion

Development and optimization of the spectrophotometric 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.

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 λmax 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.

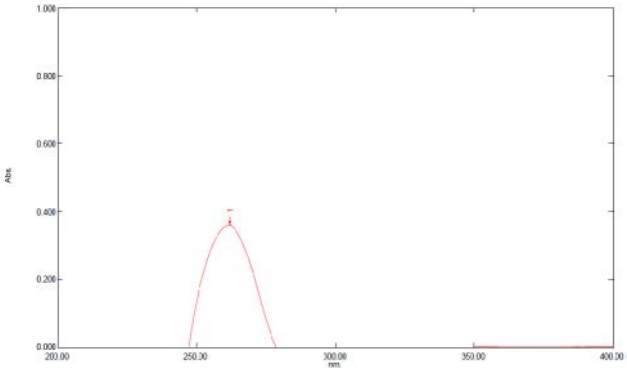

Figure 2: λ_{max} curve of Tenofovir disoproxil Fumarate API

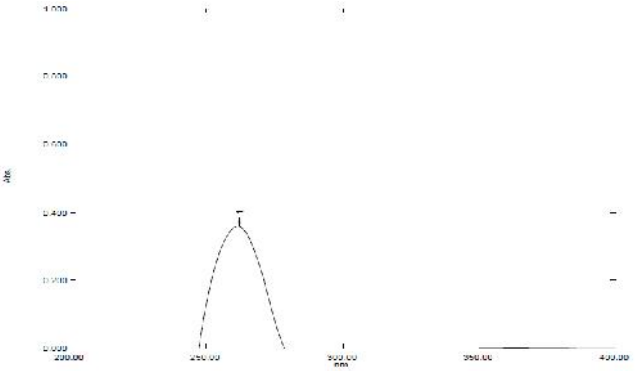

Figure 3: λ_{max} curve of Tenofovir disoproxil Fumarate Tablet dosage form

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.

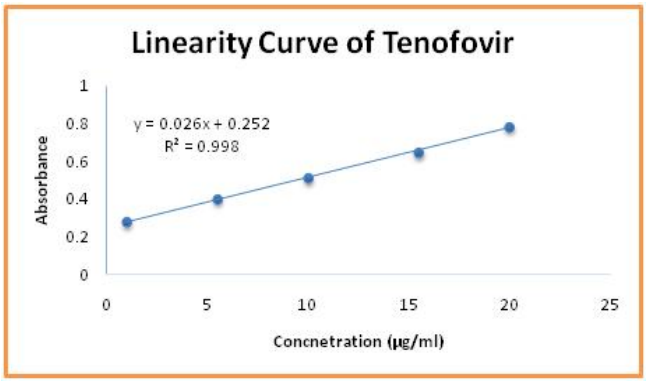

Figure 4: Linearity curve of Tenofovir

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.

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

Forced degradation study:

The results of forced degradation study indicated that If considering the time span and stress condition, TDF was found to be stable in acid (5 N HCl), peroxide (H_2O_2) photolytic conditions (In UV light) as compared to other conditions while drug was found to unstable in basic, and thermal conditions which shown sufficient degradation of TDF (upto 10 %). The results was shown in table 4. Figure 5 shows the spectra of stress studies of TDF.

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

Table 4: Results of Degradation study of Tenofovir at different stress conditions

S. No	Condition	Absorbance	% Assay	% Degradation
1	Acid Hydrolysis (0.1N HCl)	0.547	98.20	1.80
2	Alkaline Hydrolysis (0.1N NaOH)	0.512	91.92	8.08
3	Oxidation (3% H2O2)	0.549	98.56	1.44
4	Photolytic degradation	0.553	99.28	0.72
5	Thermolytic degradation (600C)	0.512	91.92	8.08

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.

6. References

[1] Budawari S., The Merck Index, Merck and Co. Inc., New Jersey, **2001**, pp 3568, 9151.

[2] Fung H.B., Stone E.A. and Piacenti F.J. Tenofovir Disoproxil Fumarate: A nucleotide reverse transcriptase inhibitor for the treatment of HIV infection. Clin. Ther., **2002**, 24(10) 1515–1538.

[3] M. C. Sharma, S. Sharma, and A. D. Sharma, "Hydrotropic solubilization phenomenon spectrophotometric estimation of Tenofovir disoproxil fumarate tablet," Journal of Chemical and Pharmaceutical Research, vol. 2, no. 2, pp. 411–415, **2010**.

[4] G. Gnanarajan, A. K. Gupta, V. Juyal, P. Kumar, P. K. Yadav, and P. Kailash, "A validated method for development of tenofovir as API and tablet dosage forms by UV spectroscopy," Journal of Young Pharmacists, vol. 1, no. 4, pp. 351–353, **2009**.

[5] A. Shirkhedkar Atul, H. Bhirud Charushila, and J. Surana Sanjay, "Application of UV-spectrophotometric methods for estimation of tenofovir disoproxil fumarate in tablets," Pakistan Journal of Pharmaceutical Sciences, vol. 22, no. 1, pp. 27–29, **2009**.

[6] A. Shirkhedkar Atul, H. Bhirud Charushila, and J. Surana Sanjay, "Determination of tenofovir in pharmaceutical formulation by zero order and first order derivative UV-spectrophotometry methods,"Research Journal of Chemistry and Environment, vol. 12, no. 1, pp. 49–50, **2008**.

[7] A. A. Shirkhedkar, C. H. Bhirud, and S. J. Surana, "Determination of tenofovir in tablets by UV spectrophotometric and derivative spectrophotometric methods," Oriental Journal of Chemistry, vol. 23, no. 3, pp. 1115–1118, **2007**.

[8] K. V. Prakash, M. Padmalatha, and E. Dopadally, "Extractive spectrophotometric determination of tenofovir," Biosciences Biotechnology Research Asia, vol. 7, no. 1, pp. 445–448, **2010**.

[9] S. M. Mallipatil, S. Noola, M. A. Nandedkar, P. S. Sarsambi, and A. Sonawane, "Spectrophotometric determination of tenofovir disoproxil fumarate," International Journal of Chemical Sciences, vol. 8, no. 2, pp. 977–982, **2010**.

[10] M. Majumder, B. Gopinath, G. Koni, and S. K. Singh, "New spectrophotometric determination of tinofovir in bulk and pharmaceutical dosage form," E-Journal of Chemistry, vol. 6, no. 2, pp. 537–540, **2009**.

[11] N. Appala Raju, J. Venkateswara Rao, K. Vanitha Prakash, and K. Mukkanti, "Spectrophotometric estimation of tenofovir in pharmaceutical formulations," Biosciences Biotechnology Research Asia, vol. 5, no. 1, pp. 439–442, **2008**.

[12] N. L. Rezk, R. D. Crutchley, and A. D. M. Kashuba, "Simultaneous quantification of emtricitabine and tenofovir in human plasma using high-performance liquid chromatography after solid phase extraction," Journal of Chromatography B, **2005,** 822(1-2), 201–208.

[13] N. A. Gomes, V. V. Vaidya, A. Pudage, S. S. Joshi, and S. A. Parekh, "Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous determination of tenofovir and emtricitabine in human plasma and its application to a bioequivalence study," Journal of Pharmaceutical and Biomedical Analysis, 48, no. 3, pp. 918–926, **2008**.

[14] A. Karunakaran, K. Kamarajan, and V. Thangarasu, "A validated RP-HPLC method for simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate in pure and in tablet dosage form,"Pharmacia Sinica, vol. 1, no. 2, pp. 52–60, **2010**.

[15] R. Sharma and P. Gupta, "A validated RP-HPLC method for simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate in a tablet dosage form," Eurasian Journal of Analytical Chemistry, vol. 4, no. 3, pp. 276–284, **2009**.

[16] Ishaq B.M, Praksh K.V, Manjula B., Kumar C.H, Rani G.U: New aurum coupling reaction for visible spectrophotometric determination of tolterodine tartrate in pharmaceutical preparations. Int J Chemical and Analytical Sci. 1: **2010**; 165-167.

[17] ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures, Text and Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva, Switzerland, **2005**.