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SPECTROPHOTOMETRIC METHOD FOR DEGRADATION STUDY OF TENOFOVIR DISOPOXIL FUMARATES

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ABSTRACT

Spectrophotometric method for degradation study of Tenofovir disoproxil fumarate was described. The ultra violet spectrum of both acidic and basic degraded product was found to substantial difference from pure drug. The extent of degradation can be calculated by comparing the decrease in absorbance at selective wavelength. The zero order UV spectrums of TDF showed maximum absorbance at 260nm and at 220nm. The solution in NaOH did not show any absorbance at 220 nm in zero order spectrums and showed decrease in absorbance at 260nm. So the decrease in absorbance at 260 nm was used as measure of extent of degradation in NaOH. In case of HCl degradation also decrease of absorbance is seen due to degradation but in comparison to NaOH degradation is lesser. Absorbance is seen at 260nm in zero order spectrums and there is no peak observed at 220nm. In case of HCl degradation also decrease of absorbance is seen due to degradation but in comparison to NaOH degradation is lesser. Absorbance is seen at 260nm in zero order spectrums and there is no peak observed at 220nm. In the basic degradation study it is found that Tenofovir disoproxil fumarate is more sensitive to alkaline hydrolysis. The 20.49% of drug is degraded if treated with 0.1N sodium hydroxide. The degradation is somewhat slower in acidic conditions as 13.17% of drug is degraded if treated with 0.1N hydrochloric acid. The relative standard deviation (RSD) of both alkaline and acidic degradation was found to be less than 2% which indicate that method is reproducible. The LOD and LOQ for TDF was found to be 0.104, 0.319 and 0.211, 0.639 µg ml^{-1} at 260nm and 220nm respectively indicates the method is sensitive.

INTRODUCTION: Tenofovir Disoproxil Fumarate belongs to the class of antiretroviral drugs as nucleotide reverse transcriptase inhibitors which block reverse transcriptase an enzyme crucial to viral production in HIV infected people. After oral absorption, Tenofovir Disoproxil Fumarate is rapidly converted to Tenofovir and then undergoes subsequent phosphorylation by cellular enzymes to the active Tenofovir Diphosphate, which inhibits the activity of HIV-1 reverse transcriptase.

Chemically it is 9-[(R)-2-[[bis[[(isopropoxycarbonyl) oxy] methoxy] phosphinyl] methoxy] propyl] adenine fumarate (1:1).It is white to light-yellow crystalline powder and it is soluble in water and in Dimethyl Sulfooxide (DMSO). There are very few reported methods for analysis of degradation products and impurities of Tenofovir Disoproxil Fumarate have been reported including Liquid chromatography–tandem mass spectrometric, Validated liquid chromatographic, reversed-phase liquid chromatography, LC/MS/MS, LC-

positive ESI-MS/MS and RP - HPLC Method [1-6]. Study of extent of drug degradation in the acidic and alkaline medium using suitable analytical method is the part of work.

Hence, the main purpose of this investigation was to develop a precise, accurate, simple, reliable and less time consuming UV-Spectrophotometric method for estimation of drug in bulk form.

MATERIAL AND METHOD:

Drug and Chemicals: Tenofovir Disoproxil Fumarate as a gift sample from Cipla ltd. Patalganga. HCl (AR grade), Sodium Hydroxide (NaOH), was purchased from Loba chemie Pvt. Ltd. Mumbai and Finar Ltd. Ahemedabad.

Instrument Used: The instrument used for the present study was PC based Jasco V-630 UV-Visible double beam Spectrophotometer with 1cm matched pair quartz cell and spectral bandwidth of 2nm.

Preparation of Standard Drug Solution: Standard stock solution containing Tenofovir disoproxil fumarate was prepared by dissolving 10 mg of Tenofovir disoproxil fumarate in few ml of distilled water, sonicated for 10 minutes and then final volume of the solutions was made up to 10 ml with distilled water. 1ml of this solution was further diluted up to 10ml to get stock solution of 100 µg ml^{-1}.

Forced Degradation Study:

Alkaline Degradation Study: The alkaline degradation was done against 0.1N sodium hydroxide.

Procedure: Accurately weighed 50mg of Tenofovir disoproxil fumarate was dissolved in 5ml of 0.1N sodium hydroxide in 100ml of volumetric flask. The solution was allowed to stand for 2min then it was neutralized with HCl and volume made up to 100ml with ACN: Water (70:30v/v). The 0.08 to 0.32ml solution was withdrawn and diluted up to 10ml with distilled water and used for study.

Acidic Degradation Study: The acidic degradation was done against 0.1N hydrochloric acid.

Procedure: Accurately weighed 50mg of Tenofovir disoproxil fumarate was dissolved in 5ml of 0.1N Hydrochloric acid in 100ml of volumetric flask. The solution was allowed to stand for 2min then it was neutralized with NaOH and volume made up to 100ml with ACN: Water (70:30 v/v). The 0.08ml to 0.32 ml solution was withdrawn and diluted up to 10ml with distilled water and used for study.

Selection of method and Wavelength for Degradation Study: The zero order UV spectrums of TDF showed maximum absorbance at 260nm and at 220nm. The solution in NaOH did not show any absorbance at 220 nm in zero order spectrums and showed decrease in absorbance at 260nm. So the decrease in absorbance at 260nm was used as a measure of extent of degradation in NaOH.

In case of HCl degradation, also decrease of absorbance is seen due to degradation but in comparison to NaOH degradation is lesser. Absorbance is seen at 260nm in zero order spectrums and there is no peak observed at 220nm. The overlain zero order spectrum of TDF, NaOH degraded and HCl degraded respectively showed in **Fig. 1. to Fig. 3**.

Linearity Study of Drug at Selected Wavelength: In to a series of 10 ml volumetric flasks, 0.4 to 1.4 ml of drug stock solution (100 µg/ml) was pipette and final volume of the solutions was made up to 10 ml with water. These solutions were scanned at 200-400nm and the absorbance at 260nm and 220nm was measured and plotted against concentration which is shown in **Fig. 4. & Fig. 5 and Table 1 & 2.**

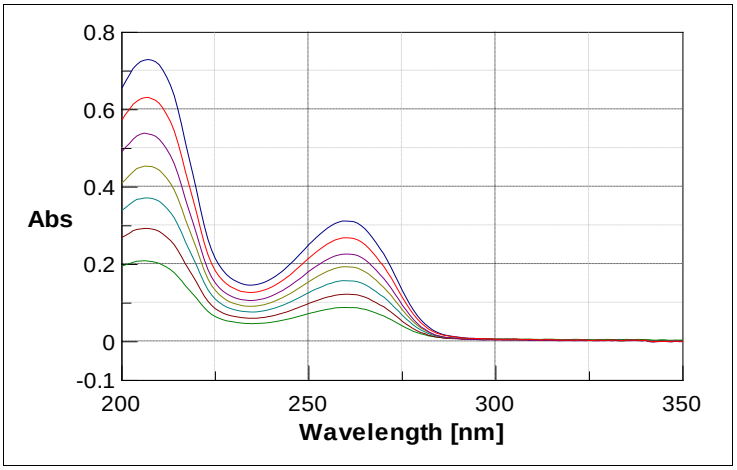

FIG. 1: OVERLAIN ZERO ORDER SPECTRA OF TDF

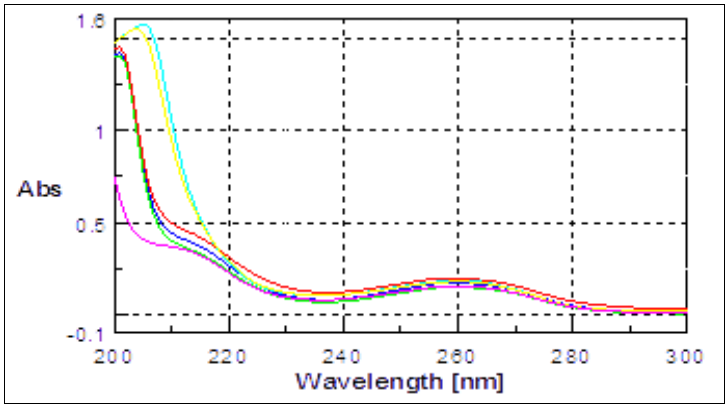

FIG. 2: OVERLAIN ZERO ORDER SPECTRA OF ALKALINE DEGRADATION

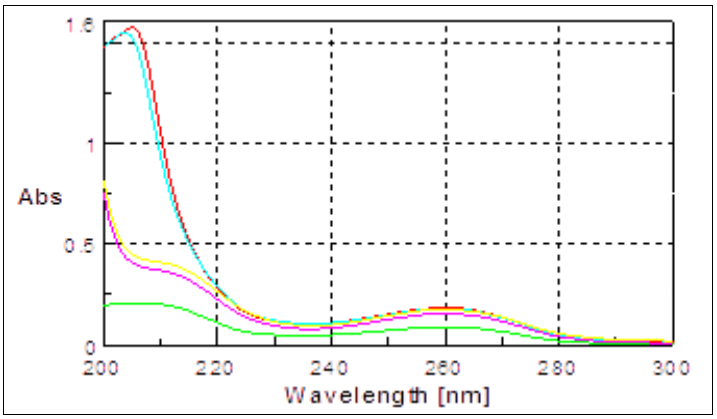

FIG. 3: OVERLAIN ZERO ORDER SPECTRA OF ACID DEGRADATION

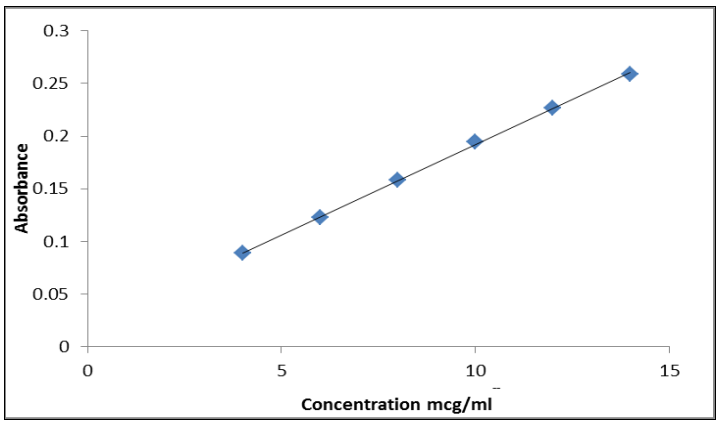

FIG. 4: CALIBRATION PLOT OF TDF AT 260nm

TABLE NO. 1: LINEARITY OF TDF AT 260nm

Sr. No.	Concentration (µg ml^{-1})	Absorbance
1.	4	0.0887
2.	6	0.1231
3.	8	0.1580
4.	10	0.1941
5.	12	0.2268
6.	14	0.2588

Regression Equation Data For Tenofovir,	Y = A + B*C
Slope (B)	0.01710
Intercept (A)	0.02096
Correlation coefficient	0.999804

Where C is the concentration in µg ml^{-1} and Y is the absorbance

TABLE 2: LINEARITY OF TDF AT 220nm

Sr. No.	Concentration (µg ml^{-1})	Absorbance
1.	4	0.2096
2.	6	0.2932
3.	8	0.372
4.	10	0.4542
5.	12	0.5396
6.	14	0.6321

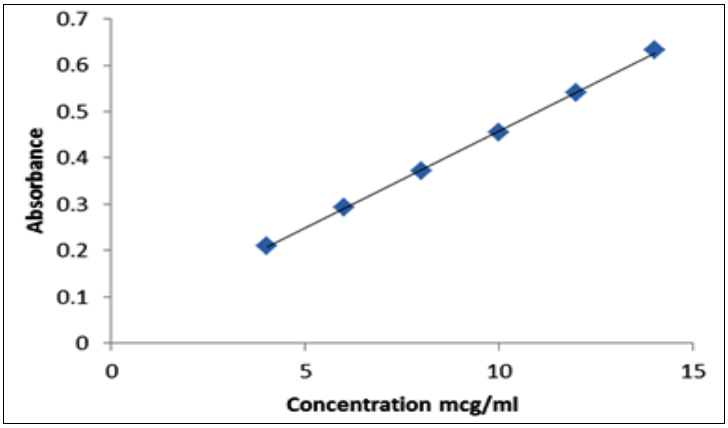

FIG. 5: CALIBRATION PLOT OF TDF AT 220 nm

Regression Equation Data For Tenofovir,	Y = A + B*C
Slope (B)	0.041913
Intercept (A)	0.039568
Correlation coefficient	0.999673

Where C is the concentration in µg ml^{-1} and Y is the absorbance

TABLE 3: DEGRADATION OF TDF IN NaOH

Concentration (µg ml^{-1})	Degradation in 0.1N NaOH	
	%*	R.S.D.
4	9.840	0.176
6	13.093	0.116
8	23.380	0.085
10	23.483	0.162
12	27.183	0.076
14	26.191	0.100

*Average of three determinations

TABLE 4: DEGRADATION OF TDF IN HCl

Concentration (µg ml^{-1})	Degradation in 0.1N HCl	
	%*	R.S.D.
4	9.793	0.412
6	12.813	0.392
8	10.815	0.297
10	10.160	0.548
12	15.783	0.222
14	19.546	0.212

*Average of three determination

TABLE 5: SENSITIVITY OF METHOD

Parameter	At 260nm	At 220nm
LOD µg ml^{-1}	0.104	0.211
LOQ µg ml^{-1}	0.319	0.639

TABLE 6: RESULTS OF ANALYSIS OF LABORATORY SAMPLES

Analyte	% Concentration estimated* (Mean ± S.D.)	R.S.D.
TDF	99.81 ± 0.005464	0.00547

*Average of six determinations; S.D., standard deviation; R.S.D., relative standard deviation

TABLE 7: RESULTS OF RECOVERY STUDY

Analyte	% Concentration estimated* (Mean ± S.D.)	R.S.D.
TDF	99.58±1.031	1.033

*Average of nine determinations; S.D., standard deviation; R.S.D., relative standard deviation

RESULTS AND DISCUSSION: The ultra violet spectrum of both acidic and basic degraded product was found to substantial different from pure drug. The extent of degradation can be calculated by comparing the decrease in absorbance at selective wavelength. The solution which was degraded in NaOH and HCl did not show any absorbance at 220nm in zero order spectrums and decrease in absorbance at 260nm was measure of extent of degradation in NaOH and HCl. The percentage degradation for each concentration was calculated by comparing the decrease in absorbance with untreated drug solution. .

In the basic degradation study, it is found that Tenofovir disoproxil fumarate is more sensitive to alkaline hydrolysis. The 20.49% of drug is degraded if treated with 0.1N sodium hydroxide. The degradation is somewhat slower in acidic conditions as 13.17% of drug is degraded if treated with 0.1N hydrochloric acid **(Tablen 3 & 4)**.

The relative standard deviation (RSD) of both alkaline and acidic degradation was found to be less than 2% which indicate that method is reproducible [7-10]. The LOD and LOQ for TDF was found to be 0.104, 0.319 and 0.211, 0.639 µg ml^{-1} at 260nm and 220nm respectively indicates the method is sensitive (**Table 5,6 & 7**).

CONCLUSION: A convenient and rapid spectro-photometric method has been developed for estimation of Tenofovir Disoproxil Fumarate in bulk form. The proposed method is simple, fast, accurate and precise for estimation of Tenofovir Disoproxil Fumarate and its degradation. The proposed method could be applied for routine analysis in quality control laboratories.

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