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Original Article

New Analytical Method Development and Validation of Tenofovir disoproxil fumarate in Bulk and Tablet by Using UV- Spectrophotometric Method

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ABSTRACT

A novel, simple, accurate and precise Zero order derivative spectroscopic method was developed and validated for the estimation of Tenofovir Disoproxil Fumarate in bulk and Pharmaceutical dosage forms and has an absorption maximum at 258 nm in 0.1 N H_2SO_4. The Linearity was found to be in the concentration range of 5-40µg/ml and the correlation coefficient was found to be 0.9995 and it has showed good linearity, reproducibility, precision in this concentration range. The regression equation was found to be Y = 0.0243 X + 0.0134. The % recovery values were found to be within 99.82 -100.14 % showed that the method was accurate. The LOD and LOQ were found to be 0.3266 and 0.9800 mcg / ml, respectively. The % RSD values were less than 2. The method has been validated according to ICH guidelines for linearity, accuracy, precision, robustness, ruggedness. Limit of detection and limit of quantitation. Proposed method was successfully applied for the quantitative estimation of Tenofovir Disoproxil Fumarate in bulk and pharmaceutical dosage form.

Key words: Tenofovir disoproxil fumarate, Zero order derivative Spectroscopy, 0.1N H_2SO_4, Linearity, Precision, Reproducibility, and Accuracy.

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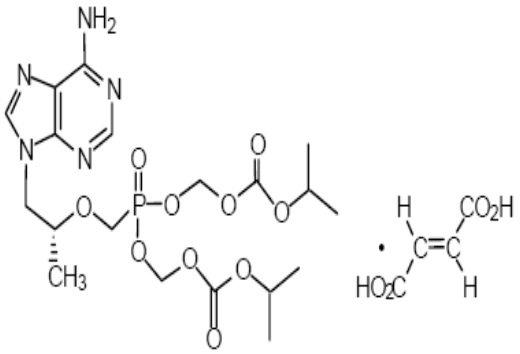
INTRODUCTION

Tenofovir disoproxil fumarate is an orally bioavailable ester prodrug of Tenofovir (also known as PMPA), an acyclic nucleotide analog with activity invitro against retroviruses, including HIV-1, HIV-2, and hepatitis B virus (HBV). Due to the presence of a phosphonate group, Tenofovir is negatively charged at neutral pH, which limits its oral bioavailability, following absorption, Tenofovir Disoproxil Fumarate is rapidly converted to Tenofovir which is metabolized intracellularly to the active metabolite Tenofovir diphosphate, a competitive inhibitor of HIV-1 reverse transcriptase (RT) that terminates the growing DNA chain[1].



Chemical structure of Tenofovir Disoproxil Fumarate.

Tenofovir Disoproxil Fumarate is chemically 9 [[R] 2 [[bis [[isopropoxycarbonyl] oxy] methoxy] phosphiny Methoxy] Propyl] fumarate. It has a molecular formula of $C_{23}H_{34}N_5O_{14}P$ and molecular weight of 635.515g/mol. It has the structural formula. Tenofovir Disoproxil Fumarate is a white crystalline powder which is freely soluble in distilled water, 0.1 N HCl, methanol, slightly soluble in dichloromethane[2].

Literature Survey revealed that the drug has been estimated by UV-Spectrophotometric, HPTLC, and RP- HPLC method has been reported so far. The aim of present work was to develop and validate a novel, rapid, simple, precise, and specific Zero order derivative UV-Spectrophotometric method for estimation of Tenofovir Disoproxil Fumarate in its bulk and tablet dosage form.

MATERIALS AND METHOD

Instrument: UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken on analytical balance.

Chemicals: TDF pure form was obtained as gifted sample from pharma industry and its pharmaceutical dosage form viread 30 Tablets labelled claim 300 mg were purchased from local pharmacy manufactured by STRIDES SHASUN LTD.

Solvent: 0.1N H_2SO_4 (prepared by dissolving 2.75ml in 1000ml of distilled water).

Selection of analytical wavelength: Appropriate dilutions were prepared for drug from the standard stock solution and the solution was scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained were derivatized from Zero order method. It shows maximum absorbance at 258 nm was shown in Fig.1 and Zero order overlain spectra of Tenofovir Disoproxil Fumarate at 258 nm were shown in Fig.2.

Preparation of Standard stock solution: Accurately weigh 100mg of Tenofovir Disoproxil Fumarate was transferred into 100ml volumetric flask and diluted with 0.1N H_2SO_4up to the mark. From this pipette out 10ml into 100ml volumetric flask and diluted with 0.1N H_2SO_4 up to the mark, from this solution pipette out 0.5, 1, 1.5, 2, 2.5, 3 ,3.5 and 4.0ml into 10ml individual volumetric flask and add 0.1N H_2SO_4 up to the mark , this gives 5, 10, 15, 20, 25, 30,35 and 40 µg/ml concentrations.

Preparation of Sample solution: Twenty tablets were weighed and powdered, the tablet powder equivalent to 100mg of Tenofovir Disoproxil Fumarate was transferred into100ml volumetric flask then it was diluted with 0.1N H_2SO_4 and made up to mark and the solution was filtered through Whatmans filter paper no.41. From this pipette out 10 ml in a 100ml volumetric flask and make up the volume up to the mark with 0.1N H_2SO_4. From this solution pipette out 2 ml into 10ml volumetric flask and make up the volume with 0.1N H_2SO_4, this gives 20µg/ml concentrations.

Method validation: The method is validated according to the ICH guidelines.

RESULTS AND DISCUSSION

Method: Zero order derivative spectroscopy.

Linearity: The working standard solution were diluted serially with 0.1N H_2SO_4 to obtain the range of 5-40 µg/ml. a calibration curve for Tenofovir Disoproxil Fumarate was obtained by measuring the absorbance at the λmax of 258nm and absorbance values are shown in Table.1 and Calibration graph were presented in Fig.3. Statistical parameters like slope, intercept,

coefficient of correlation, and Sandel's sensitivity were determined and presented in Table.2.

Precision: Precision of the method was studied as intra-day and inter-day precision. Intra-day precision was determined by analyzing the 5, 10, 15, 20, 25, 30, 35 and 40µg/ml concentration for three times in same day. Inter-day precision was determined by analyzing the same concentration of solution daily for three days. Precision results are shown in Table.3.

Accuracy: To assess the accuracy of the proposed method, recovery studies were carried out at three different levels i. e, 80%, 100% and 120%. In which the formulation concentration was kept constant and varied pure drug concentration. Accuracy results were shown in Table.4.

Ruggedness: Ruggedness was determined between different analysts. The value of %RSD was found to be less than 2 were shown in Table.5.

Limit of detection and Limit of Quantitation: The LOD and LOQ of the present method were calculated based on standard deviation of the Response and slope of linearity curve. LOD and LOQ values of Tenofovir Disoproxil Fumarate were found to be 0.3266µg/ml and 0.9800µg/ml.

Conclusion: From the above it can be concluded that all validation parameters (precision, accuracy, linearity, LOD, LOQ and Ruggedness) met the predetermined acceptance criteria as mentioned in ICH guidelines. The developed spectrophotometric method is simple, rapid, accurate, precise and can be applied for routine analysis of Tenofovir disoproxil fumarate in bulk and its dosage forms.

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Table 1: Results of calibration curve at 258 nm by zero order Spectroscopy.

SL. NO	Concentration in µg/ml.	Absorbance± Standard deviation
1	5	0.139 ± 0.000816
2	10	0.261 ± 0.001247
3	15	0.379 ± 0.00170
4	20	0.510 ± 0.001633
5	25	0.626 ± 0.002055
6	30	0.737 ± 0.002160
7	35	0.868 ± 0.002494
8	40	0.977 ± 0.002944

Table 2: Regression parameters for Tenofovir Disoproxil Fumarate by zero order spectroscopy

Regression Parameters	Tenofovir disoproxil fumarate
Range	5-40µg/ml
λ Max	258nm
Regression Equation	Y=0.0243x+0.0134
Slope (b)	0.0243
Intercept(a)	0.0134
Correlation coefficient (r2)	0.9995
Sandell's Sensitivity	0.0392

Table 3: Determination of precision results for Tenofovir Disoproxil Fumarate at 258 nm by zero order derivative spectroscopy.

Concentration (µg/ml)	Intra-day Absorbance ±SD**	%RSD	Inter-day Absorbance ±SD**	%RSD
5	0.121 ± 0.001247	1.03	0.122 ± 0.001633	1.338
10	0.252 ± 0.0017	0.674	0.249 ± 0.000816	0.327
15	0.379 ± 0.001247	0.329	0.372 ± 0.00216	0.580
20	0.508 ± 0.000816	0.160	0.496 ± 0.004643	0.936
25	0.634 ± 0.002055	0.324	0.622 ± 0.002625	0.422
30	0.761 ± 0.002944	0.386	0.752 ± 0.001247	0.165
35	0.876 ± 0.0017	0.194	0.868 ± 0.002867	0.330
40	0.965 ± 0.001633	0.169	0.970 ± 0.001247	0.128

Table.4: Determination of accuracy results for Tenofovir Disoproxil Fumarate at 258nm by Zero order derivative spectroscopy.

Spiked levels	Amount of sample (µg/ml)	Amount of Standard (µg/ml)	Amount recovered	%Recovery ±SD**	%RSD
80	10	8	17.97	99.82±0.2522	0.252
100	10	10	19.98	99.91±0.3171	0.317
120	10	22	22.03	100.14±0.5152	0.514

**Average of six determinations

Table.5: Ruggedness results of Tenofovir Disoproxil Fumarate at 258 nm by Zero order Spectroscopy

Analysts	Analyst-1	Analyst-2
Mean absorbance	0.252	0.249
Standard deviation	0.0017	0.000816
%RSD	0.674	0.327

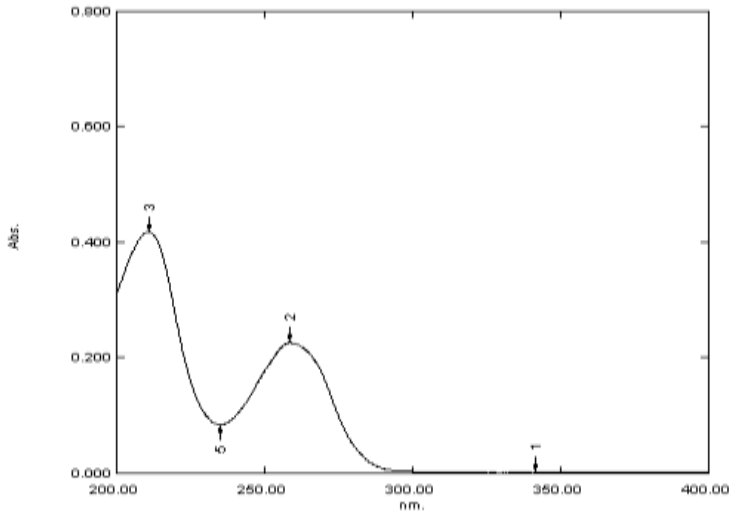

Fig.1: Zero order spectra of Tenofovir Disoproxil Fumarate showing the absorbance at 258 nm.

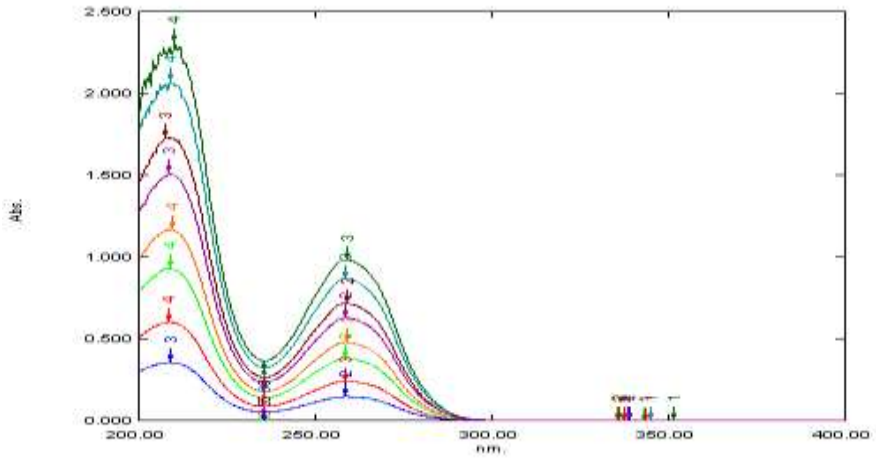

Fig.2: Zero order overlain spectra of Tenofovir Disoproxil Fumarate showing absorbance at 258 nm

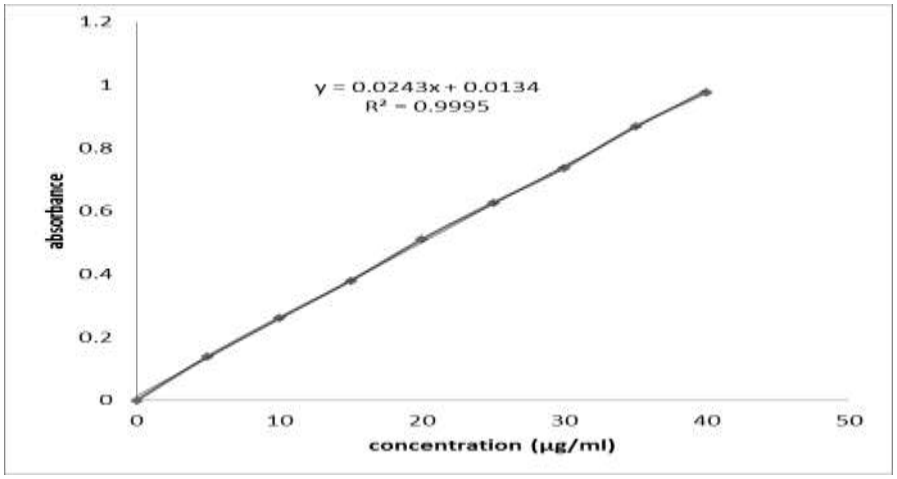

Fig.3: Linearity curves for Tenofovir Disoproxil Fumarate at 258 nm by zero order Spectroscopy

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