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Validated Spectrophotometric Quantitation of Tenofovir disoproxil fumarate in Bulk and Tablet Dosage Form

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ABSTRACT

A novel, simple, accurate and precise Zero order derivative spectroscopic method was developed and validated for the estimation of TDF in bulk and Pharmaceutical dosage forms and has an absorption maximum at 260 nm in Ethanol. The Linearity was found to be in the concentration range of 5-30µg/ml and the correlation coefficient was found to be 0.9994 and it has showed good linearity, reproducibility, precision in this concentration range. The regression equation was found to be Y = 0.0305 X + 0.0009. The % recovery values were found to be within 99.70 -100.62 % showed that the method was accurate. The LOD and LOQ were found to be 0.120 and 0.362 µg/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 TDF in bulk and pharmaceutical dosage form.

Keywords: Tenofovir disoproxil fumarate, Zero order derivative Spectroscopy, Ethanol, Accuracy.

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].

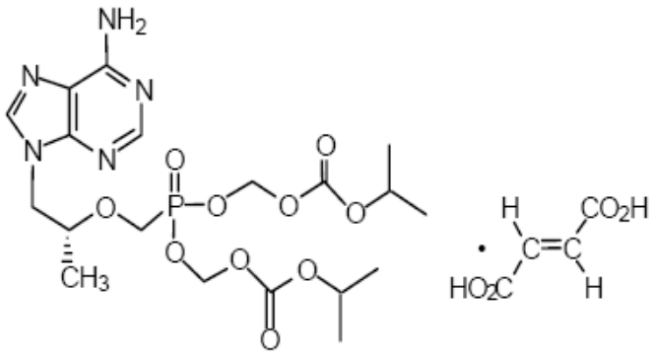

Fig 1: Chemical structure of TDF

TDF 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 (Fig.1).TDF is a white crystalline powder which is freely soluble in distilled water, 0.1N HCl, slightly soluble in dichloromethane[2].
Literature Survey revealed that the drug has been estimated by UV spectrophotometric [3-8], RP- HPLC method [9-15] and HPTLC[16]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 TDF 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 100 mg were purchased from local pharmacy manufactured by STRIDES SHASUN LTD.

Ethanol available in the laboratory of Bharathi college of Pharmacy, Bharthinagara.

Solvent

Ethanol.

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 260 nm and Zero order overlain spectra of TDF at 260 nm were shown in Fig.2.

Preparation of Standard Stock Solution

Accurately weigh 10mg of TDF was transferred into 10ml volumetric flask and diluted with Ethanol up to the mark. From this pipette out 2.5ml into 25ml volumetric flask and diluted with Ethanol up to the mark, from this solution pipette out 0.5, 1.0,1.5,2.0,2.5 and 3.0ml into 10ml individual volumetric flask and add Ethanol up to the mark , this gives 5, 10, 15, 20, 25 and 30 µg/ml concentrations.

Preparation of Sample Solution

Twenty tablets were weighed and powdered, the tablet powder equivalent to 10mg of TDF was transferred into10ml volumetric flask then it was diluted with Ethanol and made up to mark and the solution was filtered through Whatmans filter paper no.41. From this pipette out 1 ml in a 10ml volumetric flask and make up the volume up to the mark with Ethanol. From this solution pipette out 2.0 ml into 10ml volumetric flask and make up the volume with Ethanol, this gives 20µg/ml concentrations.

Method Validation

The method is validated according to the ICH guidelines[17-19].

Validation methods like Linearity, Precision, Accuracy, Ruggedness, LOD and LOQ.

RESULTS AND DISCUSSION

Table 1: Results of calibration curve at 260nm by zero order Spectroscopy

SL. NO	Concentration in µg/ml.	Absorbance± Standard deviation
1	5	0.155 ± 0.002317
2	10	0.314 ± 0.003082
3	15	0.456 ± 0.003601
4	20	0.598 ± 0.004637
5	25	0.757 ± 0.002714
6	30	0.926 ± 0.001751

Table 2: Regression parameters for TDF by zero order spectroscopy

Regression Parameters	Tenofovir disoproxil fumarate
Range	5-30µg/ml
Max	260nm
Regression Equation	Y=0.0305x+0.0009
Slope (b)	0.0305
Intercept(a)	0.0009
Correlation coefficient (r2)	0.9994
Sandell's Sensitivity	0.0334

Table 3: Determination of precision results for TDF at 260 nm by zero order derivative spectroscopy

Concentration (µg/ml)	Intra-day Absorbance ±SD**	%RSD	Inter-day Absorbance ±SD**	%RSD
5	0.154 ± 0.001528	0.992	0.157 ± 0.002646	1.685
10	0.315 ± 0.003055	0.969	0.313 ± 0.003512	1.122
15	0.454 ± 0.003055	0.672	0.459 ± 0.003	0.653
20	0.598 ± 0.003055	0.510	0.598 ± 0.006658	1.113
25	0.756 ± 0.001155	0.152	0.759 ± 0.003606	0.475
30	0.927 ± 0.001528	0.164	0.926 ± 0.002	0.215

Table 4: Determination of accuracy results for TDF by Zero order derivative spectroscopy

Spiked Levels	Amount of sample (µg/ml)	Amount of standard (µg/ml)	Amount recovered	%Recovery ±SD**	%RSD
50	10	5	15.09	100.62±0.683	0.678
100	10	10	19.94	99.71±0.259	0.260
150	10	15	24.92	99.70±0.406	0.408

Table 5: Ruggedness results at 260 nm by Zero order Spectroscopy

Analysts	Analyst-1	Analyst-2
Mean absorbance	0.599	0.602
Standard deviation	0.004082	0.005164
%RSD	0.681	0.857

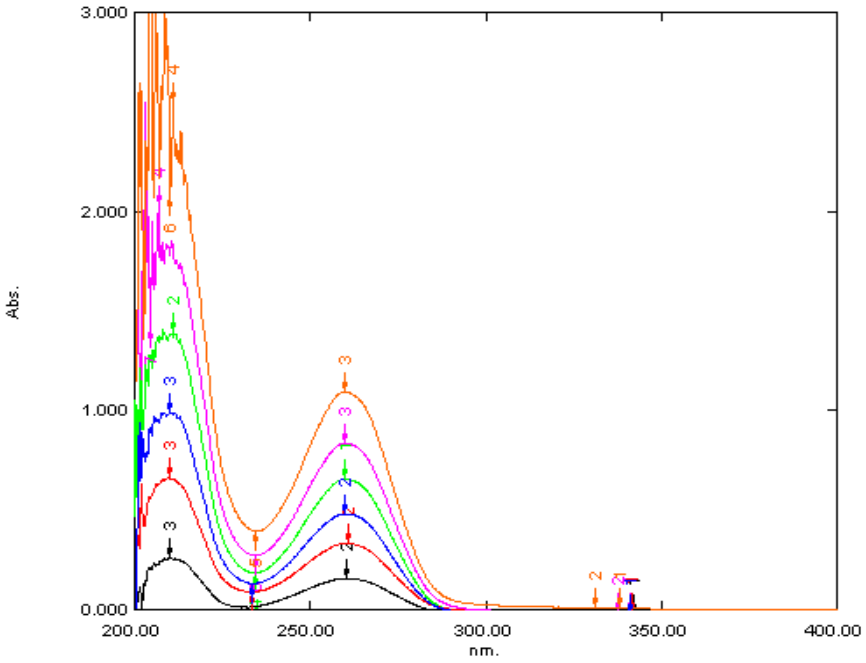

Fig 2: Zero order overlain spectra of TDF showing absorbance at 260 nm

Method: Zero Order Derivative Spectroscopy.

Linearity

The working standard solution were diluted serially with Ethanol to obtain the range of 5-30 µg/ml. a calibration curve for TDF was obtained by measuring the absorbance at the λmax of 260nm and absorbance values are shown in Table.1 and 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 and 30µg/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., 50%, 100% and 150%. 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 TDF were found to be 0.120µg/ml and 0.362µg/ml.

CONCLUSION

Thus, the main advantage of the proposed method is its suitability for routine estimation of TDF in bulk and pharmaceutical dosage form, the developed Spectrophotometric method was found to be easy, simple, accurate, precise, selective, economical and shows the good linearity.

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