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SPECTROPHOTOMETRIC DETERMINATION OF TENOFOVIR DISOPROXIL FUMARATE

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ABSTRACT

Three simple, sensitive, rapid and accurate spectrophotometric methods (A, B and C) in the visible region have been developed for the estimation of tenofovir disoproxil fumarate (TDF) in bulk drug and pharmaceutical formulations. Methods A and B are based on reduction of ferric ions to ferrous ion by TDF, which in presence of 1,10-phenanthroline and 2, 2-bipyridyl form orange red and blood red colored chromogens with absorption maxima at 500.2 and 511.2 nm, respectively. The Beer's law was obeyed in the concentration range of 2-10 and 5-25 µg/mL. Method C is based on the oxidation followed by coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) with TDF in presence of ferric chloride to form green color chromogen with absorption maxima at 640 nm. The Beer's law was obeyed in the concentration range of 5-25 µg/mL. The results of analysis for these methods have been validated statistically and by recovery studies. The results are compared with those obtained by using UV spectrophotometric methods developed in our laboratory with double distilled water at 260 nm.

Key words: Spectrophotometric, Tenofovir disoproxil fumarate, 1, 10-Phenanthroline, 2, 2'-Bipyridyl, MBTH.

INTRODUCTION

Tenofovir[1-2] disoproxil fumarate is a fumaric acid salt of bis-isopropoxycarbonyloxy methyl ester derivative of tenofovir. Chemically, it is 9-[(R)-2-[[bis[[isopropoxycarbonyl) oxy] methyl] phosphinyl]methoxy]propyl] adenine fumarate. TDF is the first nucleotide analog approved for HIV-1 treatment. Tenofovir is a nucleotide reverse transcriptase inhibitor[3] used in combination with other antiretrovirals for the treatment of HIV infection[4]. TDF remains in cells for longer periods of time than many other antiretroviral drugs; thereby, allowing for once-daily dosing.

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The aim of the present work is to develop some simple, accurate and precise analytical methods for the quantitative estimation of TDF from bulk drug and pharmaceutical formulations. Literature survey reveals that there are several reports describing the determination of tenofovir in plasma using HPLC coupled with fluorescence and UV detection[5,6]. Liquid chromatography coupled with tandem mass spectrometry were also reported[7-9]. In view of the above facts; some UV-visible methods are developed, which are highly sensitive, accurate and precise.

EXPERIMENTAL

Instrument

All spectral measurements were made on Systronics 119 UV/visible spectrophotometer. 1 cm matched quartz cell was used for the absorbance measurements.

Reagents

All the chemicals and reagents used were of analytical reagent grade.

(i) Alcoholic 1, 10-phenanthroline (0.03 M)

(ii) Aqueous ferric chloride solution (0.08M, 0.03 M)

(iii) Alcoholic 2, 2'-Bipyridine (0.1 M)

(iv) Alcoholic 3-methyl-2-benzothiazolinone hydrozone (MBTH) (0.03 M)

(v) Distilled alcohol

(vi) Double distilled water

Preparation of standard and sample drug solutions

About 100 mg of tenofovir disoproxil fumarate (pure or formulation) was accurately weighed and dissolved in 50-60 mL double distilled water. It was allowed to stand for some time to ensure complete solubilisation. The solution was filtered. The residue was washed 3 times with 10 mL portions of double distilled water and the total volume of the filtrate made upto 100 mL with double distilled water. The final concentration was made to 1 mL = 1000 μg/mL (Stock solution-I). Further dilution was made with double distilled water to get the concentration of 100 μg/mL (Stock solution-II).

Assay procedure

Method A: Fresh aliquots of TDF ranging from 0.2 to 1.0 mL (1 mL = 100 μg) were transferred to a series of 10 mL volumetric flask. To each of above aliquots, 0.08 M ferric chloride solution (0.5 mL) and alcoholic solution 0.03 M 1, 10 phenanthroline (0.5 mL) were added and heated at 50-60ºC for 10 minutes. After cooling, the volume was brought up to the mark with double distilled water and the absorbance of orange red colored species was measured at 500.2 nm against reagent blank. The colored species was stable for more than 2 hours. The amount of drug was computed from the calibration curve.

Method B: Fresh aliquots of TDF ranging from 0.5 to 2.5 mL (1 mL = 100 μg) were transferred to a series of 10 mL volumetric flask. To each of above aliquots, 0.03 M ferric chloride solution (0.6 mL), alcoholic solution of 0.1 M 2'2-bipyridyl (1.0 mL) were added and heated at 50-60ºC for 10 minutes. After cooling, the volume was brought up to the mark with double distilled water and the absorbance of blood red colored species was measured at 511.2 nm against reagent blank. The colored species was stable for more than 2 hours. The amount of drug was computed from the calibration curve.

Method C: Fresh aliquots of TDF ranging from 0.5 to 2.5 mL (1 mL = 100 μg) were transferred to a series of 10 mL volumetric flask. To each of above, 0.03 M ferric chloride solution (2.0 mL), alcoholic solution of 0.03M MBTH (1.0 mL) were added and kept aside for 10 minutes to complete the reaction. The volume was brought up to the mark with double distilled water and the absorbance of green colored species was measured at 640 nm against reagent blank. The colored species was stable for more than 2 hours. The amount of drug was computed from the calibration curve.

RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity and regression analysis using the method of least squares was made. The slope (b), intercepts (a) and correlation coefficient (r) were obtained from different concentrations and the results are summarised in Table 1. The percent relative standard deviation and percent range of error (0.05 and 0.01 level of confidence limits) were calculated from the eight measurements. $3/4^{th}$ of the amount of upper Beer's law limits in each method are summarised in Table 1. The results showed that the methods have reasonable precision. Results obtained with the proposed methods were compared with the results obtained with other UV-spectrophotometric method.

Results obtained with the proposed methods confirm the suitability of these methods

for pharmaceutical dosage forms. The other active ingredients and excipients usually present in pharmaceutical dosage forms did not interfere in the estimation, when some commercial dosage forms. (T_1, T_2) were analyzed by this method. The accuracy of the method was confirmed by the recovery studies, by adding a known amount of the pure drug to the formulation already analyzed by this method and the analytical data are presented in Tables 2.

In all the above methods, the optimum concentration for the estimation of TDF was established by varying one parameter at a time and keeping the other fixed and observing the effect of product on the absorbance of the colored species and was incorporated in the procedures. The optimum concentration for the estimation of TDF was established by varying drug concentration by keeping the reagent concentration fixed. After establishing the optimum concentration for drug, the reagent concentration was varied. The above ranges of drug and reagent concentrations were chosen because the colored species formed gave better absorbance and obeyed Beer's law satisfactorily.

Table 1: Optical characteristics and precision

Parameters	Method A	Method B	Method C
λ_{max} (nm)	500.2	511.2	640
Beer's law limits (μg/mL) (C)	2-10	5-25	5-25
Molar absorptivity (L moles^{-1} cm^{-1})	7.02 x 10^4	2.84 x 10^4	5.92 x 10^4
Sandell's sensitivity (μg/cm^2 – 0.001 absorption units)	0.018	0.051	0.035
Regression equation Y* = (bC+a)			
Slope (b)	0.728	0.627	0.731
Intercept (a)	0.763	0.427	0.520
Correlation coefficient (r)	0.9992	0.9997	0.9999
% RSD	0.392	0.450	0.425
Range of errors**			
Confidence limits 0.05 level	± 0.00070	± 0.00251	± 0.00065
Confidence limits 0.01 level	± 0.00104	± 0.00193	± 0.00128

*Y is absorbance and C is the concentration in μg/mL.
**For eight measurements.

Table 2: Assay and recovery of TDF in pharmaceutical dosage form

Sample	Labelled Amount	Amount found by proposed methods (mg)			Reference method (UV in double distilled water)	Percentage recovery*		
		A	B	C		A	B	C
T_1	100	99.72	99.28	99.10	99.60	99.42	99.50	99.74
T_2	100	99.60	99.50	98.95	99.50	98.50	99.70	99.54

*Mean and standard deviation of eight determinations
T_1 and T_2 are tablets from Cipla and Emcure Pharmaceuticals

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