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Assay of tenofovir disproxil fumarate in bulk and its marketed formulations by visible spectrophotometry

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ABSTRACT

A simple and sensitive visible spectrophotometric method for the determination of tenofovir disproxil fumarate using 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) reagent has been developed in bulk and tablet dosage forms. It is based on the formation of intense blue colored species by treating the drug with MBTH reagent in the presence of ferric chloride with an absorption maximum of 631nm. The Regression analysis of Beer's Law plot showed good correlation in a general concentration range of 10-30µg/ml. The proposed method is validated with respect to accuracy, precision, linearity and limit of detection. The suggested procedure is successfully applied to the determination of the drug in pharmaceutical preparation, with high percentage of recovery, good accuracy and precision. The results of analysis have been validated statistically by repeatability and recovery studies. The results are found satisfactory and reproducible. The method is applied successfully for the estimation of tenofovir disproxil fumarate in tablet dosage form without the interference of excipients.

KEYWORDS

Beer's law;
Chemotherauptic agent;
Ferric chloride;
MBTH;
Oxidative coupling reaction;
Regression equation.

INTRODUCTION

Tenofovir Disoproxil Fumarate (TDF) (Figure 1) is an antiretroviral agent belonging to the class of nucleotide reverse transcriptase inhibitors (NRTI) used in the management of HIV infection in adults. It is an orally bio available prodrug of tenofovir and the first nucleotide analogue approved for HIV-1 treatment[1,2]. In vivo, TDF is converted to tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate. Tenofovir exhibits activity against HIV-1, HIV-2 and hepatitis-B virus. Chemically it is the 1:1 salt of the bis-isopropyloxy carbonyl oxy methyl ester of tenofovir and fumaric acid [9-[(R)-2-[[bis [(isopropoxycarbonyl) oxy] methyl] phosphinyl] methoxy] propyl] adenine fumarate]. Its empirical for-

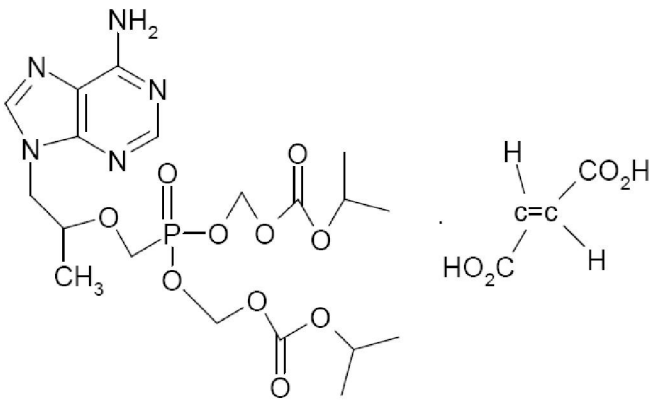

Figure 1 : Chemical structure of tenofovir disproxil fumarate

mula is $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$ representing molecular weight of 635.52. It is a white to off-white crystalline powder with a solubility of 13.4mg/ml in distilled water and freely soluble in methanol and in DMF. The drug is available in tablet dosage forms only. TDF remains in cells for longer periods of time than many other antiretroviral drugs, thereby allowing for once-daily dosing. The drug is official in IP[3].

Before phosphorylation, TDF is converted to tenofovir in the intestinal lumen and plasma by diester hydrolysis. Tenfovir then internalized into cells, possibly by endocytosis, and subsequently phosphorylated in sequential steps to tenfovir monophosphate and to the active metabolite, tenfovir diphosphate. In a mechanism similar to that of NRTI's, tenfovir diphosphate competes with its natural nucleotide counterpart deoxyadenosine5'-triphosphate, for incorporation into newly forming HIV DNA. Once successfully incorporated, termination of the elongating DNA chain ensues, and DNA synthesis is interrupted.

TDF has been well tolerated in clinical trials with duration of follow-up to 96 weeks. It is associated with more favorable lipid profiles than stavudine and has not been associated with the mitochondrial toxicity attributed to other nucleoside analogues.

Extensive literature review reveals that several spctrophotometric (UV &Visible)[4-15], HPLC[16-24], HPTLC[25,26] and LC/LC-MS[27-33] methods have been reported so far for determination of tenofovir alone and its combination with other drugs. Even though some visible spectrophotometric assay procedures have been reported for the determination of TDF, many of them concern with biological fluid samples and very few in pharmaceutical formulations. Hence it is felt necessary to develop a suitable visible spectrophotometric method for the assay of TDF in both bulk drug and pharmaceutical formulations. Honing and Fritsch[34] described oxidative coupling of MBTH with aromatic amines or phenols in the presence of an oxidant under acidic conditions to form an intense colored oxidative coupling products. So the authors have made some attempts in this direction and succeeded in developing a method based on the reaction between the drug and MBTH-Fe (III)[35].

The proposed method for TDF determination has many advantages over other analytical methods due to its rapidity, normal cost and environmental safety. Unlike HPLC, HPTLC procedures, the instrument is simple and is not costly. Economically, all the analytical reagents are inexpensive and available in any analytical laboratory. The method can be extended for the routine quality control analysis of pharmaceutical products containing TDF.

MATERIALS & METHODS (EXPERIMENTAL)

Apparatus and chemicals

A Shimadzu UV-Visible spectrophotometer 1601 with10mm matched quartz cells was used for all spectral measurements. A Systronics digital pH meter mode-361 was used for pH measurements. All the chemicals used were of analytical grade. MBTH (Fluka, 0.2%, $8.56x10^{-3}$M, solution prepared by dissolving 200mg of MBTH in 100ml distilled water), Ferric chloride (Qualigens, 0.5%, $1.65x10^{-2}$M solution prepared by dissolving 500mg of ferric chloride hexahydrate in 100ml of 0.1N HCl) were prepared.

Preparation of bulk and sample solution

About 100mg of TDF [pure or formulation] was accurately weighed and dissolved in 100ml of 3M Hydrochloric acid in a volumetric flask to form the stock solution of 1mg/ml. The solution was refluxed gently for 60 minutes to hydrolyze the phosphate groups from the drug. The hydrolyzed drug was partitioned with chloroform (25ml x4). The chloroform extract was evaporated to dryness and the residue so obtained was dissolved in 100 ml with distilled water. The working standard solution of TDF (100 µg/ml) was obtained by appropriately diluting the standard stock solution with the same solvent. The prepared stock solution was stored at 4p C protected from light. From this stock solution, a series of standards were freshly prepared during the analysis day.

Determination of wavelength maximum (λ_{max})

The 3.0 ml of working standard solution of TDF (100µg/ml) was taken in 10ml calibrated tube. To this, 1.5ml MBTH and 1.0ml of ferric chloride was added successively, kept for 10min.at room temperature for complete color development. The volume was made

up to the mark with distilled water. In order to investigate the wavelength maximum, the above colored solution was scanned in the range of 400-760 nm UV-Visible spectrophotometers against a reagent blank. From the absorption spectra (Figure 2), it was concluded that 631nm is the most appropriate wavelength for analyzing TDF with suitable sensitivity.

Preparation of calibration graph

Aliquots of working standard TDF solution (100µg/ml) such as 1.0, 1.5, 2.0, 2.5, 3.0 ml were taken separately in a series of 10ml graduated test tubes, to get a concentration of 10, 15, 20, 25 and 30 µg/ml respectively. A 1.5 ml portion of MBTH ($8.56x10^{-3}$M) solution was added to each test tube and allowed to stand for 2 minutes at room temperature. Then 1.0ml of ferric chloride ($1.65x10^{-2}$M) solution was added, kept for 10 minutes and diluted to the mark with distilled water. The absorbance was measured at 631 nm against a similar reagent blank within 30 min. The calibration graph was constructed by plotting the drug concentration versus absorbance (Figure 3).

RESULTS AND DISCUSSION

In the present investigation the reactive electrophillic intermediate formed insitu from MBTH upon treatment with an oxidant Fe (III), was found

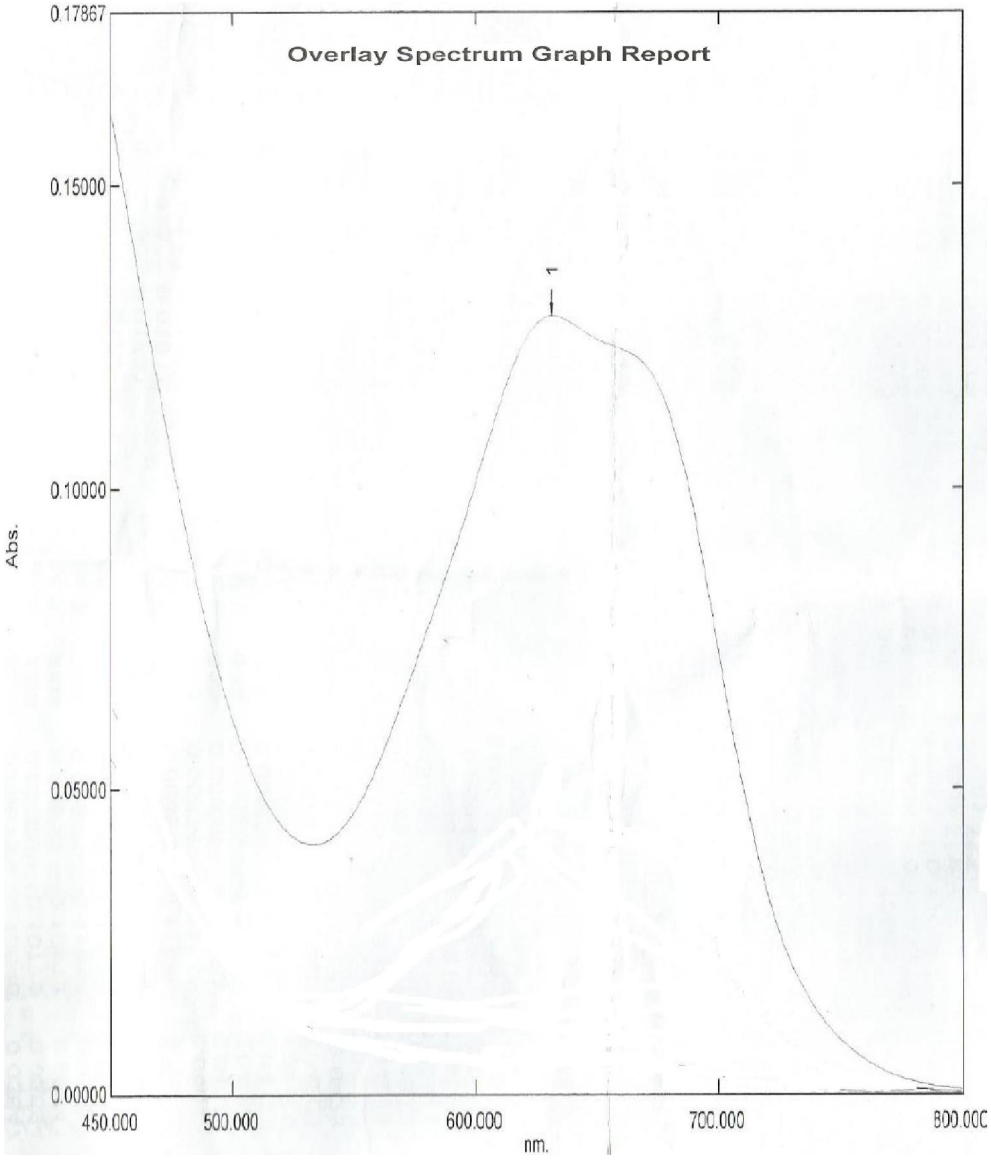

Figure 2 : Absorption spectra of TT-MBTH-Fe (III) system

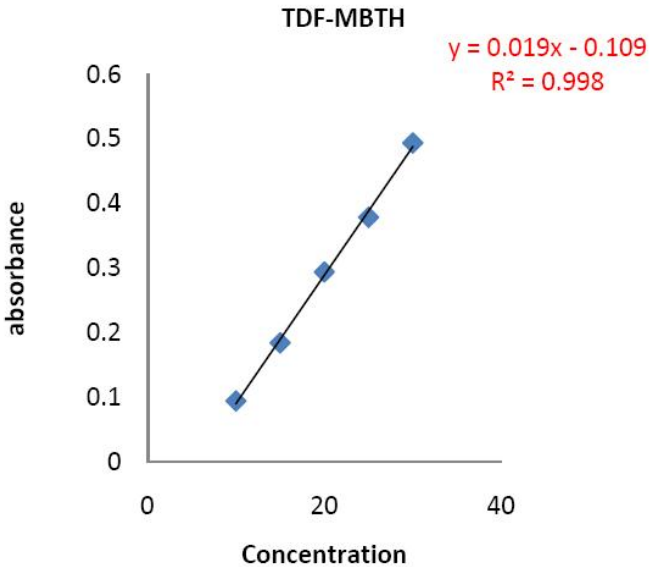

Figure 3 : Beer's law plot of TT-MBTH-Fe (III) system

to oxidative couple with TDF which possesses hetero cyclic amino group. Based on the analogy, the probable sequence of reactions is presented in scheme (Figure 4).

In developing a method, systematic studies of the effects of various parameters were undertaken by varying one parameter at a time and controlling all others fixed. The effect of various parameters such as time, temperature, nature and concentration of oxidant, volume and strength of MBTH reagent, order of addition of reagents on color development and solvent for final dilution on the intensity and stability of the colored species were studied and the optimum conditions were established. Among the various oxidants ($NaIO_4$, $K_2Cr_2O_7$, Chloramine-T, potassium hexacyanaferrate (III), Ce (IV) and Fe (III) tried in combination with MBTH for oxidative coupling reaction. Ce (IV) and Fe (III) were responded for color development with MBTH. But MBTH-Fe (III) was found to be the best by virtue of high $^{a}_{max}$ values and stability considerations. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile were found to provide no additional advantage. So distilled water is selected as a solvent for final dilution of the colored species. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from the six measurements

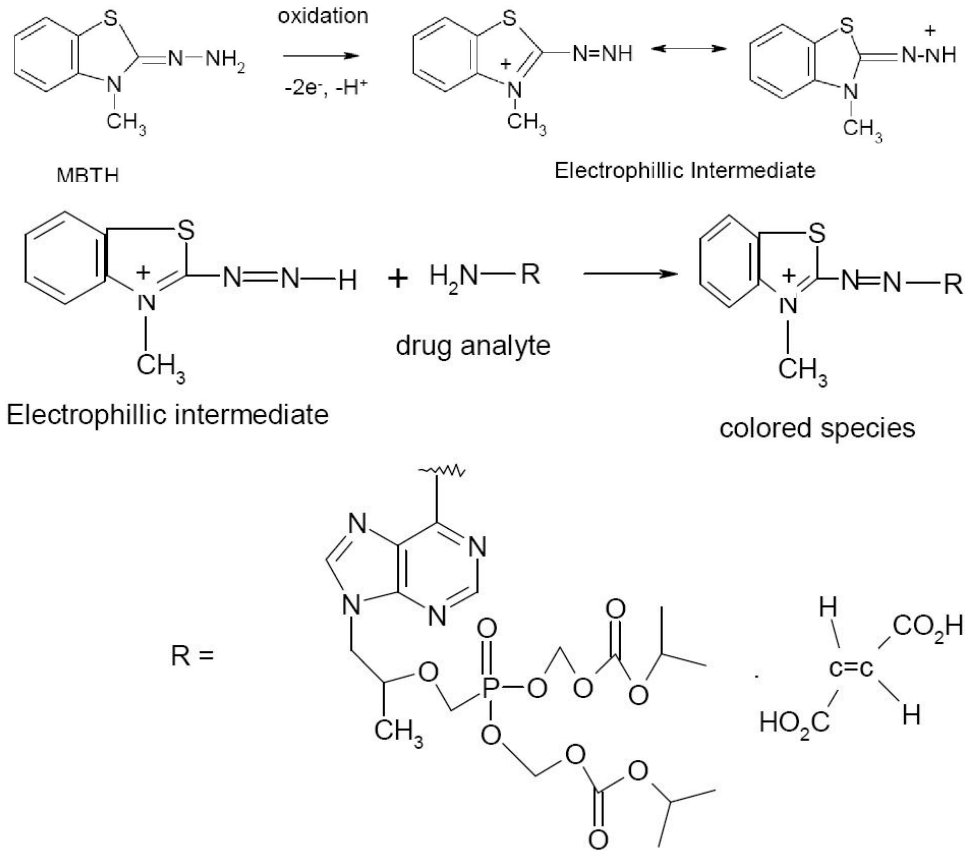

Figure 4 : Probable scheme for the colored reaction of the TDF with MBTH

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TABLE 1: Optical characteristics, precision and accuracy of proposed method.

Parameter	Values
λ_{max} (nm)	631
Beer's law limit(µg/ml)	10-30
Sandell's sensitivity (µg/cm^2/0.001 abs. unit	0.006802721
Molar absorptivity (Litre/mole/cm)	93421.44
Regression equation (Y)*	
Intercept (a)	-0.109
Slope(b)	0.019
%RSD	2.06
% Range of errors(95% Confidence limits)	
0.05 significance level	2.16
significance level	3.39

***Y = a+ b x, where Y is the absorbance and x is the concentration of TDF in µg/ml**

containing 3/4th of the amount of the upper Beer's law limits), Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and are shown in TABLE 1.

Commercial formulations containing TDF were successfully analyzed by the proposed method. The values obtained by the proposed and reference method (reported UV method in distilled water λ_{max} 260nm) for formulations were compared statistically by the t-and F-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. These results are summarized in TABLE 2. The ingredients

TABLE 2 : Analysis of tenofovir disproxil fumarate in pharmaceutical formulations

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method ± SD	#% Recovery by Proposed Method ± SD
			**Amount found ± SD	t	F		
TDF-MBTH-Fe(III)	Batch-1	300	297.65 ±0.74	2.4	3.7	297.49 ±0.38	99.22± 0.25
	Batch -2	300	296.04 ± 2.12	0.37	1.42	296.85 ± 1.78	98.68± 0.71

*** Batch 1 and Batch 2 are tablets of different pharmaceutical companies of Tenof (Hetro) and Tavin (Emcure); **Average ± Standard deviation of six determinations, the t- and F-values refer to comparison of the proposed method with reference method. (UV). Theoretical values at 95% confidence limits t =2.57 and F = 5.05; # Recovery of 10mg added to the pre analyzed sample (average of three determinations); Reference method (reported UV method) using distilled water after dissolving in 1ml methanol ($2\hat{u}_{max}$=260nm).**

usually present in formulations of TDF did not interfere with the proposed analytical method.

CONCLUSIONS

The reagents utilized in the proposed method are readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed analytical method is validated as per ICH guide lines and possess reasonable precision, accuracy. The method offers the advantages of rapidity, simplicity, sensitivity and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents and can be used as an alternative method to the reported ones for the routine determination of TDF depending on the need and situation.

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