

QUANTITATIVE DETERMINATION OF TENOFOVIRDISPROXIL FUMARATE AS AN ACTIVE PHARMACEUTICAL INGREDIENT IN TABLET FORMULATIONS BY VISIBLE SPECTROPHOTOMETRY

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ABSTRACT

In the present investigation, a direct, simple and sensitive visible spectrophotometric method without using any buffer solution is described for the assay of tenofovir disoproxil fumarate in pure and tablet dosage forms. The method is based on the formation of yellowish brown colored species by the drug with NQS reagent and exhibits absorption maxima at 451 nm. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges (8-40) µg/ml. The proposed method is applied to commercial available tablets and the results are statistically compared with those obtained by the reported UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. The method is applied successfully for the estimation of the tenofovir disoproxil fumarate in the presence of other ingredients that are usually present in dosage forms. The method offers the advantages of rapidity, simplicity and sensitivity and normal cost and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.

Key words: Assay, Beer's Law, Chemotherapeutic agent, NQS reagent, Regression equation, Statistical analysis.

INTRODUCTION:

Tenofovir Disoproxil Fumarate (TDF) (Fig.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 pro drug of tenofovir and the first nucleotide analogue approved for HIV-1 treatment¹⁻². 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-isopropoxy carbonyl oxy methyl ester of tenofovir and fumaric acid [9-[(R)-2-[[bis [(isopropoxycarbonyl) oxy] methyl] phosphinyl] methoxy] propyl] adenine fumarate]. Its empirical formula is C₁₉H₃₀N₅O₁₀P.C₄H₄O₄ 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 IP³.

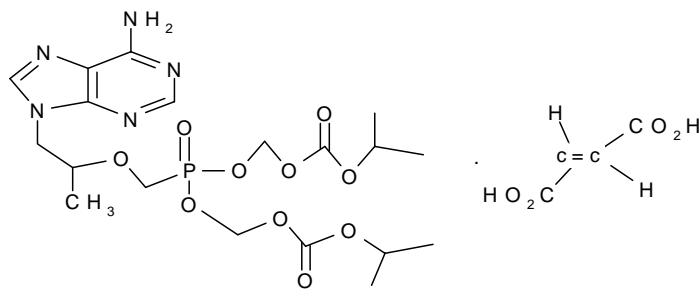


Figure 1: Chemical structure of tenofovir disoproxil fumarate

Before phosphorylation, TDF is converted to tenofovir in the intestinal lumen and plasma by diester hydrolysis. Tenofovir then internalized into cells, possibly by endocytosis, and subsequently phosphorylated in sequential steps to tenofovir monophosphate and to the active metabolite, tenofovir diphosphate. In a mechanism similar to that of NRTI's, tenofovir 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.

Extensive literature review reveals that several spectrophotometric UV⁴⁻¹¹ & Visible¹²⁻¹⁵, HPLC¹⁶⁻²⁴, HPTLC²⁵⁻²⁶ and LC/LC-MS²⁷⁻³³ 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 and functional groups present in the drug not fully exploited. So the author has made some attempts in this direction and succeeded in developing the method based on the reaction between the drug and folin reagent (NQS)³⁴ in presence of NaOH instead of phosphate buffer pH8.0 under specified experimental conditions. 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 1800 with 10mm 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.

Folin reagent (NQS) solution (Loba, 0.5%, $1.93 \times 10^{-2} M$ prepared by dissolving 500mg of NQS in 100 ml of distilled water), NaOH solution (E. Merck, 20%, 5M prepared by dissolving 20 g of NaOH in 100 ml of distilled water) 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 10 ml 0.1M sodium hydroxide and the volume was brought to 100 ml with distilled water. The working standard solution of TDF (200 µg/ml) was obtained by appropriately diluting the standard stock solution with the same solvent. The prepared stock solution was stored at 4° 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 5.0 ml of working standard solution of TDF (200µg/ml) was taken in 25ml standard flask. To this, 0.5ml of folin reagent ($1.092 \times 10^{-2} M$), 1.0ml NaOH and 1.5ml of distilled water were added and kept aside for 15 min for complete color development. Then the volume was made up to 25 ml using distilled water and sonicated for 1 minute to get a concentration of 40µg/ml. In order to investigate the wavelength maximum, the above standard stock solution was scanned in the range of 360-560nm by UV-Visible spectrophotometer. From the spectra (Fig.2), it was concluded that 451nm is the most appropriate wavelength for analyzing TDF with suitable sensitivity.

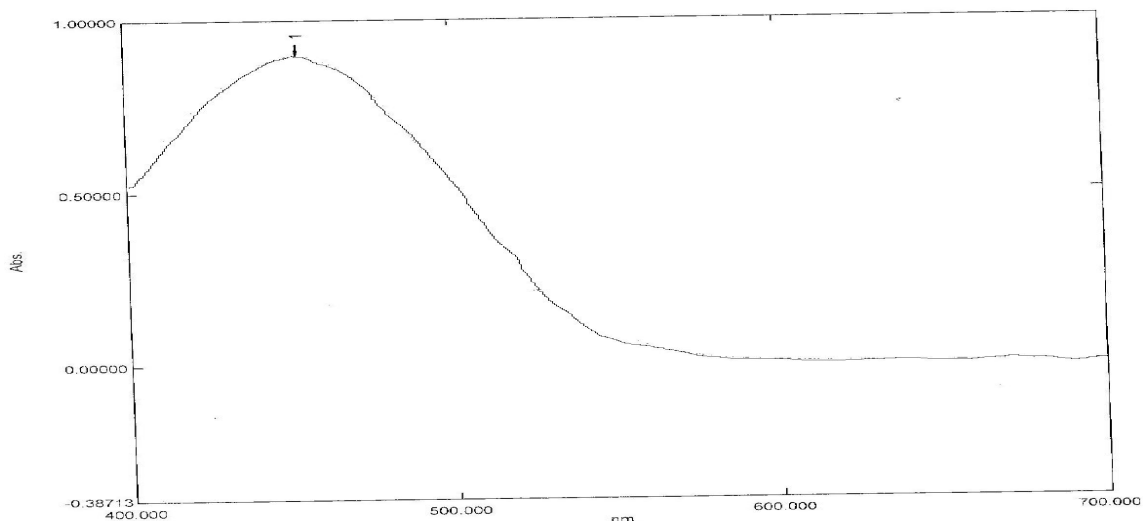


Figure 2: Absorption spectra of TDF-NQS system

Preparation of calibration curve:

Aliquots of the standard TDF solution [1.0-5.0ml, 200µg/ml] were placed in a series of 25ml standard flask. Then 0.5ml of folin reagent ($1.092 \times 10^{-2}M$), 1.0ml NaOH and 1.5ml of distilled water were added and kept aside for 15 min for complete color development. Then the volume was made up to 25 ml using distilled water and sonicated for 1 minute. The absorbance was measured at 451 nm against a reagent blank within the stability period (5minutes to 30min). The calibration graph was constructed by plotting the drug concentration versus absorbance. The amount of drug was computed from its calibration graph (Fig.3).

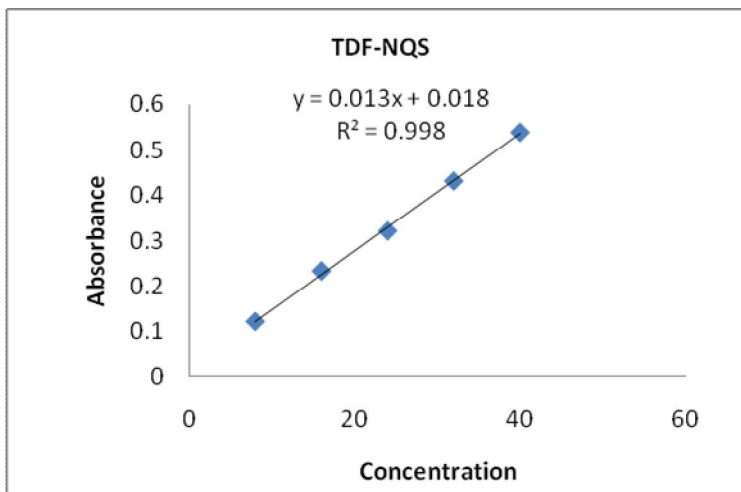


Figure 3: Calibration graph of TDF-NQS system

RESULTS AND DISCUSSION

Optimum operating conditions used in the procedure were established by adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of reagents, the order of addition of reagents and solvent for final dilution of the colored species were studied. Distilled water was found to be best solvent for final dilution. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile have no additional advantage in increasing the intensity of the color. The optical characteristics such as Beer's law

limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements 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 the results are summarized 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 using distilled water after dissolving in 1ml methanol, $\lambda_{max}=260nm$) 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.

Chemistry of colored species:

In the present investigation, the presence of heterocyclic amino moiety of TDF permits the development of visible spectrophotometric method for its determination. Yellowish brown colored species (N-alkyl amino naphthaquinone) was formed by replacement of the sulphonate group of the naphthaquinone sulphonic acid by a primary amino group of drug. The formation of colored species with the reagent may be assigned through above analogy as shown in scheme (Fig.4).

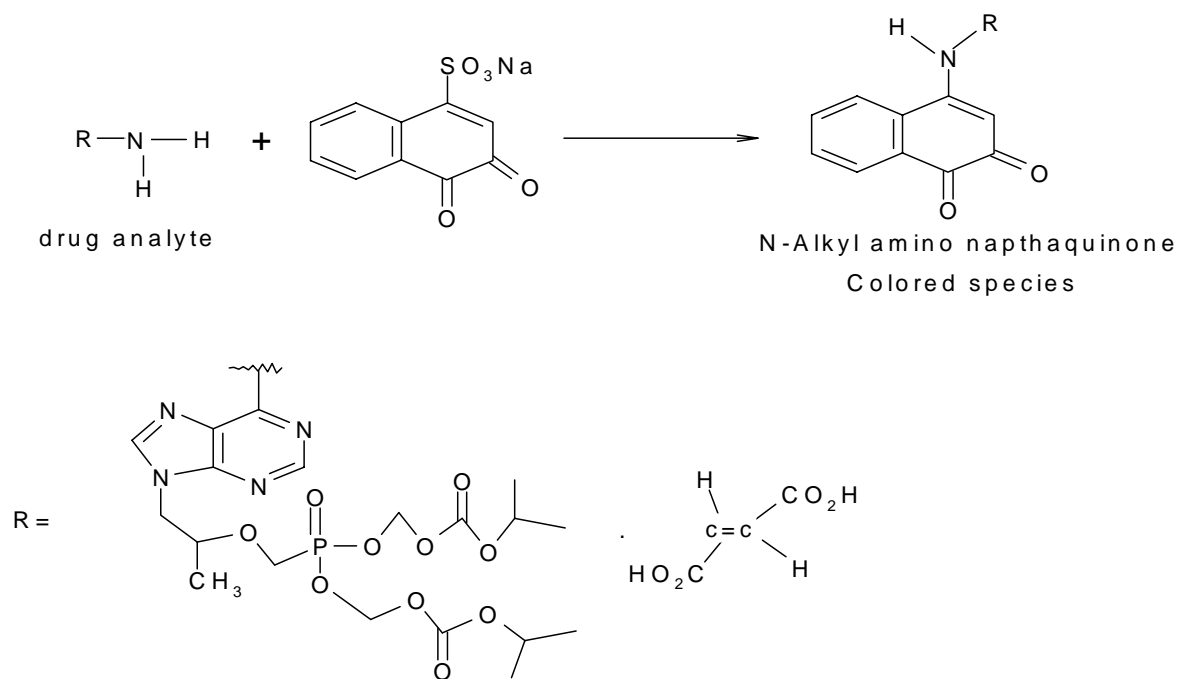


Fig.4: Probable scheme of the reaction

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF PROPOSED METHOD.

Parameter	Values
λ_{\max} (nm)	451
Beer's law limit($\mu\text{g/ml}$)	8-40
Sandell's sensitivity ($\mu\text{g/cm}^2/0.001$ abs. unit)	0.002990654
Molar absorptivity (Litre/mole/cm)	212502
Regression equation (Y)*	
Intercept (a)	0.018
Slope(b)	0.013
%RSD	1.58
% Range of errors(95% Confidence limits)	
0.05 significance level	1.66
0.01 significance level	2.59

* $Y = a + b x$, where Y is the absorbance and x is the concentration of TDF in $\mu\text{g/ml}$

TABLE 2: ANALYSIS OF TENOFOVIR DISPROXIL FUMARATE BY PROPOSED AND REFERENCE METHODS.

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Method			Found by Reference Method \pm SD	#% Recovery by Proposed Method \pm SD
			**Amount found \pm SD	t	F		
NQS	Batch-1	300	298.18 \pm 0.68	1.97	3.2	297.49 \pm 0.38	99.39 \pm 0.23
	Batch-2	300	297.11 \pm 2.01	0.14	1.28	296.85 \pm 1.78	99.04 \pm 0.67

* Batch 1 and Batch 2 are different pharmaceutical companies of Tenof (Hetro) and Tavin (Emcure).

**Average \pm 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 (λ_{\max} =260nm).

CONCLUSION

The reagents utilized in the proposed method are normal cost, readily available in all chemical laboratories and small scale industries and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed colorimetric method possesses reasonable precision, accuracy, and simple, sensitive and can be used as alternative method to the reported ones for the routine determination of TDF depending on the need and situation.

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