Research Article

Novel Spectrophotometric Methods for the Assay of Tenofovir Disoproxil Fumarate in Bulk and Dosage Forms

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ABSTRACT

The present paper describes two simple and sensitive methods A and B for the spectrophotometric determination of Tenofovir disoproxil fumarate. The proposed methods are based on the formation of purple color and red- violet colored chromogens obtained when the drug was diazotized with nitrous acid followed by coupling with Phloroglucinol and Resorcinol, exhibiting absorption maximum (λmax) at 520 and 600nm respectively. These methods obey Beer's law in the concentration range of 2-10 µg/mL. Statistical data reveals that the methods developed are highly reproducible and have been applied to a wide variety of pharmaceutical preparations.

Keywords: Tenofovir disoproxil fumarate, chromogen, absorption maximum, spectrophotometry.

INTRODUCTION

Tenofovir disoproxil fumarate[TDF] is chemically designated as ({[(2*R*)-1-(6-amino-9*H*-purin-9-yl) propan-2-yl] oxy} methyl) phosphoric acid. It is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults[1,2]. It is a prodrug and so it is metabolized de novo to Tenofovir[3], an acyclic nucleoside phosphonate (nucleotide) analogue of Adenosine 5.-monophosphate. It is available in tablet dosage form only. Although no assay procedure has been presented in any of the Official Pharmacopoeias, literature survey reveals a very few analytical methods which include liquid chromatography with tandem mass spectrometry[4,5], HPLC with solid phase extraction[6], Reversed phase HPLC[7,8], HPLC with spectrophotometric detection[9] were reported. The present paper describes two simple and sensitive visible spectrophotometric methods for the assay of Tenofovir disoproxil fumarate in its formulations through diazo coupling reactions.

MATERIALS AND METHODS

Instrumentation

All spectral and absorbance measurements were made on ELICO SL-159, UV-visible spectrophotometer with 1cm quartz cells was used.

Preparation of reagents

All chemicals used were of analytical grade.

Phloroglucinol solution: (Loba; 0.1%, 8.26×10^{-3}M): Prepared by dissolving 100 mg of Phloroglucinol in 100 ml distilled water.

Resorcinol Solution (Sd.fine; 0.1%, 9.08×10^{-3}M): Prepared by dissolving 100 mg of Resorcinol in 100 ml of distilled water.

NaOH solution (Loba; 4.0%, 1.0M): Prepared by dissolving 400 mg of NaOH in 100 ml of distilled water and standardized.

HCl solution (Sd.fine; 0.25M): Prepared by dissolving 2.15ml of Conc.HCl in 100 ml of distilled water and standardized.

Sodium nitrite solution (Loba; 0.1%, 1.45×10^{-2}M): Prepared by dissolving 100 mg of Sodium nitrite in 100 ml distilled water.

Standard stock solution

Tenofovir disoproxil fumarate (100mg) was accurately weighed and dissolved in 20ml of distilled water, transferred to a standard 100ml volumetric flask. The final volume was made up to the mark with distilled water. The final concentration was brought to 100µg/mL with distilled water.

Assay of TDF in pharmaceutical formulations

Twenty tablets containing Tenofovir disoproxil fumarate were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 25mg of Tenofovir disoproxil fumarate was dissolved in a 25ml of methanol and mixed for about 5 minutes and then filtered. The methanol was evaporated to dryness. The remaining portion of solution was diluted in a 25ml volumetric flask to the volume with distilled water. The general procedure was then followed in the concentration ranges mentioned above.

Recommended Procedures for the assay of TDF

Method A & B

Aliquots of (0.5-2.5ml) Tenofovir disoproxil fumarate (0.5ml=50µg for method-A and B) were transferred into a series of 25ml volumetric flasks. To each of the above aliquots, hydrochloric acid (0.25M) (1.0ml) and 1.0ml cold

aqueous solution of sodium nitrite (0.1% w/v) were added and set aside for 10 min at 0-5°C temperature. Later 1.0ml of Phloroglucinol (0.1% w/v)/Resorcinol(0.1% w/v) and 1.5ml of aqueous Sodium hydroxide (4% w/v) were added successively, and then the volume in each tube was made up to 25ml with distilled water. The absorbance was measured at 520nm/600nm against reagent blank for the method-A and Method-B respectively. The color was found to be stable for more than 2 hours at room temperature. The concentration of Tenofovir disoproxil fumarate was calculated either from calibration curve or from regression equation.

RESULTS AND DISCUSSION

The presence of amino group in Tenofovir disoproxil fumarate enabled the use of diazotization of the drug with nitrous acid and coupling the resulting diazonium salt with phloroglucinol, to form purple colored chromogen in method A exhibiting λmax at 520nm. In method B diozotization reaction was followed by coupling with resorcinol in presence of sodium hydroxide solution resulting in the formation of red-violet chromogen exhibiting λmax at 600nm. The Beer's law was obeyed by these two methods in the concentration range of 2-10 µg/mL. The optical characteristics such as Beer's law limits, absorption maxima, molar absorptivity, Sandell's sensitivity, percent relative standard deviation (%RSD) calculated from six measurements containing ¾ th of the amount of the upper Beer's law limits of Tenofovir disoproxil fumarate and percent range of errors (0.05 level and 0.01 confidence limits) were calculated for the two methods are reported in Table-1.

Table 1: Optical Characteristics and Precision

Optical Characteristics	Method A	Method B
λ max (run)	520	600
Beer's law limits(µg/mL)(C)	2-10	2-10
Molar absorptivity (lit.mol^{-1}cm^{-1})	2.99×10^4	1.90×10^4
Sandell's sensitivity (µg/cm^2)-0.001 abs units	0.0210	0.02941
Slope (b)	0.0468	0.0303
Intercept (a)	0.0015	0.0047
Correlation coefficient (r)	0.9999	0.9996
% RSD	0.7929	0.00622
Confidence limits with 0.05 level	0.1586	0.1556
Confidence limits with 0.01 level	0.2630	0.2581

The optimum conditions for the color development for method A and B were established by varying the parameters one at a time and keeping the other parameters fixed while observing the effects on the absorbance of colored species. The optimum concentration for the estimation of Tenofovir disoproxil fumarate was established by varying the drug concentration, keeping reagent concentration fixed. After establishing the optimum concentration for the 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. The values obtained for the determination of Tenofovir disoproxil fumarate in its brand sample (Viread) by the developed methods are compared with the reference method and are listed in Table-2. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical preparation and the mixtures were analyzed by the developed methods. The percent recoveries are given in Table-2.

Table 2: Assay of Tenofovir disoproxil fumarate in dosage forms.

Sample	Labelled amount (mg)	Amount obtained by proposed methods*(mg)		Reference method	% Recovery of proposed methods**	
		Method A	Method B		Method A	Method B
VIREAD	300	298.6	297.9	299.4	99.73	99.49

*Average of six determinations.
** Mean and standard deviation of six determinations.

CONCLUSION

The methods reported here are found to be simple, sensitive, accurate and precise. The reaction occurs at 0 - 5°C temperature and no extractions procedure is involved as compared with other established methods. Further, spectrophotometric methods involve simple instrumentation which is cost effective compared with other instrumental techniques, which ordinary laboratories cannot afford to have. The proposed methods involved in the formation of highly stable colored species, which makes it easier for the estimation of Tenofovir disoproxil fumarate from its dosage forms in a routine manner. These studies revealed that the common excipients usually present in the tablet form, did not interfere at their regularly added levels.

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