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Extractive spectrophotometric determination of tenofovir

K.VANITHA PRAKASH[1], M. PADMALATHA[2] and ERANNA DOPADALLY[3]

[1]SSJ College of Pharmacy, V.N. Pally, Gandipet, Hyderabad (India).
[2]Vijaya College of Pharmacy, Munganoor, Hayathnagar, Hyderabad (India).
[3]Sultan-Ul-Uloom College of Pharmacy, Banjara Hills, Hyderabad (India).

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ABSTRACT

Two simple, economical, precise, reliable and reproducible visible spectrophotometric methods (A and B) have been developed for the estimation of Tenofovir (TNF) in bulk as well as in Tablet formulations. The developed methods A and B are based on the formation of chloroform extractable complex of Tenofovir with Bromocresol green (Method A) and Erichrome (Method B), which shows absorbance maxima at 420 nm and 510 nm respectively. The absorbance-concentration plot is linear over the range of 25-250 mcg/ml for Method A, and 25-200 mcg/ml for Method B. The different experimental parameters affecting the development and stability were studied carefully and optimized. Results of analysis for all the methods were validated statistically and by recovery studies.

Key words: Tenofovir, Bromocresol green, Erichrome, Ultraviolet-Visible double beam spectrophotometer.

INTRODUCTION

Tenofovir[1] is novel HIV-1 reverse transcriptase inhibitor, with a chemical name [[(1R)-2- (6-amino-9H-purin-9-yl)-1-methylethoxy]methyl] phosphonic acid. It is official in Martindale[2]-The Extra pharmacopoeia. It is an antiretroviral drug that acts by converting intracellularly to the diphosphate. This diphosphate halts the DNA synthesis of HIV through competitive inhibition of reverse transcriptase and incorporation in to viral DNA. Literature survey reveals many Chromatographic methods[3-10] for the determination of Tenofovir in biological fluids and in combination with other antivirals and very few Spectrophotometric methods [11-14] only. Therefore the need for fast, low cost and selective method is obvious especially for routine Quality Control analysis of pharmaceutical formulation.

EXPERIMENTAL

Instrument

Elico double beam Ultraviolet-Visible double beam spectrophotometer SL-244 with 1 cm matched quartz cells was used for all spectral measurements.

Reagents

All the chemicals used were of analytical reagent grade.

- Bromocresol green (0.1% w/v)-100 mg is weighed accurately and dissolved in *0.74 ml of 0.1N Sodium Hydroxide and 20 ml of methanol.* After the solution is mixed thoroughly, it is made up to 100 ml with distilled water.
- Preparation of Buffer pH 2.4: 50 ml of 0.2 M potassium hydrogen phthalate, 49.5 ml of 0.2 N Hydrochloric acid were added and solution

is made up to 200 ml with distilled water.

- Erichrome solution: 10 mg of Erichrome is dissolved in distilled water and made the volume up to 10 ml.
- Chloroform AR grade

Procedure

Standard stock solution

A standard stock solution containing 1 mg/ml was prepared by dissolving 100 mg of Tenofovir in 100 ml of distilled water. From this, a working standard solution containing 500 mcg/ml for Method A and Method B were prepared with distilled water.

Assay procedure

Method A

Aliquots of the drug solution of TNF 0.5-5.0 ml (500 mcg/ml) are taken and transferred into a series of 125 ml of separating funnel. To each funnel 2 ml of buffer and 2 ml of 0.1% w/v Bromocresol green reagent is added. Reaction mixture was shaken gently for 5 min. Then 5 ml of chloroform was added to each of them. The contents are shaken thoroughly for 5 min and allowed to stand for 15 minutes, so as to separate the aqueous and chloroform layer. Colored chloroform layer was separated out and absorbance was measured at 420 nm against reagent blank. Calibration curve was prepared from absorbance values so obtained

Method B

Aliquots of the drug solution of TNF 0.5-5.0 ml (500 mcg/ml) are taken and transferred into a series of 125 ml of separating funnel. To each funnel 2 ml of buffer and 2 ml of 0.1% w/v Erichrome reagent are added. Reaction mixture was shaken gently for 5 min. Then 5 ml of chloroform was added to each of them. The contents are shaken thoroughly for 5 min and allowed to stand for 15 min, so as to separate the aqueous and chloroform layer. Colored chloroform layer was separated out and absorbance was measured at 510 nm against reagent blank. Calibration curve was prepared from absorbance values so obtained.

Preparation of sample solution

Tablets containing Tenofovir were successfully analyzed by the proposed methods. Ten tablets of Tenofovir (TENOF, 300 mg, Genix Pharma) were accurately weighed and powdered. Tablet powder equivalent to 100 mg of Tenofovir was dissolved in 100 ml of distilled water and filtered and washed with distilled water, the filtrate and washings were combined and the final volume was made to 100 ml with distilled water. The solution was suitably diluted and analyzed as given under the assay procedure for bulk samples. The results are represented in Table 2. None of the excipients usually employed in the formulation of tablets interfered in the analysis of Tenofovir, by the proposed methods.

Recovery Studies

To ensure the accuracy and reproducibility of the results obtained, adding known amounts of pure drug to the previously analysed formulated samples and these samples were reanalyzed by the proposed method performed recovery experiments. The percentage recoveries thus obtained were given in Table 2.

RESULTS AND DISCUSSIONS

In the present work two methods have been developed for the estimation of Tenofovir from Tablet formulation. The developed Methods A and B are based on formation of chloroform extractable colored complexes with Bromocresol green and Erichrome respectively. The conditions required for the formation of colored complexes were optimized. Statistical analysis was carried out and the results of which were satisfactory. Relative Standard Deviation values were low that indicates the reproducibility of the proposed methods. Recovery studies were close to 100 % that indicates the accuracy and precision of the proposed methods. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sand ell's sensitivity are presented in Table 1. The regression analysis using the method of least squares was made for slope (m), intercept (b) and correlation obtained from different concentrations and the results are summarized in Table 1.

In conclusion, the proposed methods are simple, economical, sensitive, precise reliable and reproducible for the routine estimation of Tenofovir in bulk as well as in tablet formulation.

Table 1: Optical characteristics and precision data

Parameters	Method A	Method B
λ_{max} (nm)	420	510
Beer's law limits	25-250 mcg/ml	25-200 mcg/ml
Molar absorptivity (l/mol.cm)	1.67×10^3	1.46×10^3
Sandell's sensitivity (micrograms/cm^2/0.001 absorbance unit)	0.1041	0. 0476
Regression Equation* (Y)		
Slope (m)	0.012	0.021
Intercept (c)	0.1284	0.017
Correlation Coefficient(r)	0.996	0.998
Precision (%RSD)	0.228	0.366
Standard error of mean	0.0428	0.0401

*Y=mx+c, where X is the concentration in micrograms/ml and Y is absorbance unit.

Table 2: Assay of tenofovir in tablets

Sample Tablet	Labelled Amount	Amount Obtained (mg)* by proposed method		** % Recovery by the proposed method	
	(mg)	Method A.	Method B	Method A.	Method B
1	300	299.5	299.8	98.5%	99.7%
2	300	298.5	300.2	99.4%	99.3%
3	300	301.3	301.6	100.9%	99.5%

*Average of three determinations.

** After spiking the sample

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