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New Spectrophotometric Determination of Tinofovir in Bulk and Pharmaceutical Dosage Form

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Abstract: Two new, selective and sensitive visible spectrophotometric methods (method A and B) have been developed for the estimation of tinofovir in bulk and in pharmaceutical preparations. Tinofovir was subjected to acid hydrolysis and this acid hydrolyzed drug was used for the estimation. Method A is based on the reaction with 3-methyl-2-benzothiazolinone hydrazone in the presence of ferric chloride, to form a colored species with a λ_{max} at 628.5 nm. Method B is based on the reaction with Folin-ciocalteu phenol's reagent under alkaline condition with a λ_{max} at 768 nm. Beer's law is obeyed in the concentration range of 5-40 µg/mL for method A and 2-30 µg/mL for method B, respectively. The methods were extended to pharmaceutical formulations and there was no interference from any common pharmaceutical excepients and diluents. The result of analysis has been validated statistically and by recovery studies.

Keywords: Spectrophotometric determination, 3-Methyl-2-benzothiazolinone hydrazone, Folin-ciocalteu phenol's reagent, Tinofovir.

Introduction

Tinofovir is a nucleotide analog reverse transcriptase inhibitor (nRTI) antiviral drug used to treat HIV/AIDS and is in clinical trials for treatment of hepatitis B infection. The triphosphate form of the drug competes with the natural DNA nucleotide deoxyadenosine triphosphate (dATP) during DNA formation and it acts as a DNA chain terminator once incorporated because it lacks the normal deoxyribose sugar needed for connecting to the next DNA base.

It is a non pharmacopial drug. The IUPAC chemical name for tinofovir is [(2R)-1-(6-aminopurin-9-yl) propan-2-yl]oxymethylphosphonic acid and it has chemical formula $C_9H_{14}N_5O_4P$, giving it a molecular mass of 287.2123 g/mole. It is chemically similar to the natural nucleotide adenosine but lacks a ribose sugar unit.

There is no work reported about visible spectrophotometric methods for the quantification of tinofovir. In literature the survey reveals that very few analytical methods for this drug are available in human plasma and biological fluid. These include different HPLC and other chromatographic methods[1-5]. The present investigation has been undertaken to develop two simple visible spectrophotometric methods using 3-methyl-2-benzothiazolinone hydrazone[6-7] and Folin-ciocalteu phenol's reagent[8-9], which are essential for routine quality control analysis of pharmaceutical products containing tinofovir as active constituent.

Experimental

A shimadzu model 1700 double beam UV-Visible spectrophotometer with a pair of 1 cm matched quartz cells was used to measure absorbance of the resulting solutions. Tinofovir was gifted by Ranbaxy Pharmaceutical. All the chemicals used were of analytical grade.

Reagents

3-Methyl-2-benzothiazolinone hydrazone's (0.2%)

200 mg of MBTH dissolved in 100 mL of distilled water.

Folin-ciocalteu phenol's reagent (10%)

5 mL of FCP reagent with 50 mL distilled water.

Ferric chloride (0.3%)

Freshly prepared was prepared by dissolving 300 mg of ferric chloride in 100 mL of distilled water.

Sodium carbonate (10%)

10 g of sodium carbonate dissolved in 100 mL of distilled water.

Preparation of standard solution

Accurately weighed 100 mg of tinofovir was dissolved in 70 mL of water; it was added with 10 mL of conc. HCl, reflux for two and half hours at 85 ^{0}C and the solution was diluted to 100 mL with distilled water to obtain 1000 µg/mL stock solution. This stock solution was further diluted with water to obtain the working standard of 100 µg/mL for method A and method B.

Preparation of sample solution

Accurately weighed tablet powder equivalent to 100 mg of drug was dissolved in 50 mL of water; it was added 10 mL of conc. HCl, refluxed at 85 ^{0}C for two and half hour and the solution was diluted to 100 mL with distilled water to obtain 1000 µg/mL stock solution. This stock solution was further diluted with water to obtain the working standard of 100 µg/mL for method A and method B.

Optimization of parameter

The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance. Different concentrations and different volumes were tried for all the reagents, by varying the parameters at a time. For method A it was found that optimum concentration of $FeCl_3$ was 0.3% *w/v* and optimum concentration of MBTH was 0.2% w/v. The optimum volume was found to be 1.5 mL for $FeCl_3$ and that of MBTH was 1.3 mL. For method B it was found that optimum concentration of FCP reagent was 10% v/v and optimum concentration of Sodium bicarbonate was 10% w/v. The optimum volume was found to be 0.5 mL for FCP and that of Sodium bicarbonate was 1.5 mL.

Assay procedure

Method A

Aliquots of standard drug solution ranging from 0.5-2.5 mL (1000 µg/mL) were transferred to a series of 10 mL volumetric flasks. To each, 1.3 mL of MBTH, 1.5 mL of ferric chloride was added and the volume was made up to mark with distilled water and allowed to stand for 20 min. The absorbance was measured at 628.5 nm against a reagent blank. The colored species was stable for 2 h and the amount of drug in the sample was computed from its calibration curve.

Method B

Aliquots of standard drug solution ranging from 0.5-2.5 mL (1000 µg/mL) were transferred to a series of 10 mL volumetric flasks. To each, 0.5 mL of FCP, 1.5 mL of Sodium bicarbonate was added and the volume was made up to mark with distilled water and absorbance was measured at 768 nm against a reagent blank. The colored species was stable for 1.3 h and the amount of drug in the sample was computed from its calibration curve.

Results and Discussion

The optical characteristics such as Beer's law limits, Sandell's sensitivity and molar extinction coefficient, percent relative standard deviation, (calculated from the eight measurements containing $3/4^{th}$ of the amount of the upper Beer's law limits) were calculated and the results are summarized in Table 1. Regration characteristics like slope, intercept, correlation coefficient and % range of error (0.05 and 0.01 confidence limits) were calculated and are shown in Table 1.

Table 1. Optical and regression characteristic, precision and accuracy of the proposed method for tinofovir.

Parameter	Method A	Method B
λ_{max} , nm	628.5	768
Beer's law limits, μgmL^{-1}	5-40	2-30
Molar absorptivity, $Lmol^{-1}cm^{-1}$	0.139×10^2	0.406×10^2
Sandell's sensitivity $\mu g/cm^2$/0.001 absorbance unit	0.0738	0.0246
Regression equation(Y=a+bc)		
Slope (b)	0.0136	0.0470
Intercept (a)	0.0036	0.0047
Correlation coefficient (r^2)	0.9989	0.9994
Relative standard deviation (% RSD)*	0.8531	0.8289
%Range of errors**		
Confidence limits with 0.05 levels	1.944×10^{-3}	1.531×10^{-3}
Confidence limits with 0.01 levels	2.877×10^{-3}	2.266×10^{-3}
LOD, µg/mL	0.0281	0.0273
LOQ, µg/mL	0.0853	0.0820

**Average of eight determination **Average of three determination. In Y=a+bc, Y is the absorbance and c is concentration.*

Commercial formulations of tinofovir tablets were successfully analyzed by the proposed methods and the values obtained by the proposed methods are presented in Table 2. To evaluate validity and reproducibility of the methods, a fixed amount of pure drug was added to the

pre-analyzed formulation. The results are summarized in Table 3. There is no interference in the proposed analytical methods. In conclusion the proposed method spectrophotometric methods for the estimation of tinofovir are simple, sensitive, and accurate and can be used for the routine quality control of the drug in bulk as well as in pharmaceutical formulation

Table 2. Evaluation of tinofovir in pharmaceutical dosage form.

Samples*	Labeled claimed, mg	Amount obtained, mg		% Assay	
		Proposed methods		Proposed methods	
		A	B	A	B
1	10	9.95	9.87	99.50	98.70
2	10	9.94	9.93	99.40	99.30
3	10	9.91	9.96	99.10	99.60

**Average of three determination*

Table 3. Recovery studies.

Tablet*	Pre-analyzed Amount, mg	Amount Added, mg	Amount Obtained, mg		Percentage recovery	
			Method-A	Method-B	Method-A	Method-B
1	10	8	17.95	17.96	99.72	99.22
2	10	10	19.97	19.95	99.85	99.75
3	10	12	22.08	21.98	100.36	99.90

***** *Average of three determination*

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