A SIMPLE SPECTROPHOTOMETRIC ASSAY OF TENOFOVIR IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, sensitive, rapid and accurate spectrophotometric method has been developed for the estimation of Tenofovir in pharmaceutical dosage forms. Tenofovir undergoes diazotisation when treated with sodium nitrite and hydrochloric acid. The excess nitrous acid during the diazotisation is removed by the addition of urea solution. The solution was shaken frequently to allow the nitrogen gas to escape. The diazonium cation reacts with the coupling reagent, resorcinol to produce a orange red azo product. This orange red colour product shows maximum absorbance at 440 nm.

KEYWORDS: Spectrophotometry, resorcinol, Tenofovir, Pharmaceutical and Formulation.

INTRODUCTION

Tenofovir¹ is a acyclic phosphonate nucleotide analogue, chemically (1-R)-2-(6-amino-9H-purin-9-yl)-1-methoxy)methyl phosphonic acid monohydrate. It is official in Indian pharmacopoeia. It’s molecular weight is 287.21 with molecular formula C₉H₁₄N₅O₄P. Tenofovir is a nucleotide reverse transcriptase inhibitor used in combination with other antiretrovirals for the treatment HIV infection. The literature suggested and reported which includes, spectrophotometric method¹⁻³, RP-HPLC method⁴⁻⁶, HPTLC Method⁷⁻⁸, high-performance liquid chromatography with spectrofluorimetric detection⁹, Liquid chromatography-tandem mass spectrometry (LC/MS/MS) method¹⁰⁻¹¹, and HPLC method¹², in bulk, formulations and in biological samples. The present investigation aims to develop simple, sensitive, accurate, rapid and cost effective spectrophotometric method for the estimation of Tenofovir in its tablet formulations.
MATERIALS AND METHODS

Instrument: All measurement were done on Milton Roy 1001 spectrophotometer by using 10 mm matched quartz cuvettes.

Materials: All chemicals used are of A.R. grade and were purchased from S.D. fine chemicals and LOBA-Chemi, Mumbai. Doubled distilled water were used for preparation of solutions.

Hydrochloric acid (0.1N): Hydrochloric acid solution (0.1N) is prepared by diluting the requisite volume of concentrated AR hydrochloric acid with distilled water and standardized by usual procedure.

Sodium nitrite (0.1N): 0.69 g of Sodium nitrite is dissolved in distilled water and the resulting solution is made up to the mark in 100 ml standard flask with distilled water. This solution is standardized by the usual analytical procedure.

Resorcinol (1%): Accurately weighed 1.0 g of resorcinol is dissolved in methanol and the volume adjusted to 100 ml with methanol.

Urea solution (1%): Accurately weighed 1 gm of Urea is dissolved in double distilled water and the volume made up to 100 ml with double distilled water.

Preparation of standard stock solution: The standard stock solution (1mg/ml) of tenofovir was prepared by dissolving 100mg of Tenofovir in 100 ml distilled water. The working standard solutions of Tenofovir were obtained by appropriately diluting the standard stock solution with the same solvent.

Preparation of Calibration curve
Various aliquots of the standard tenofovir solution ranging from 0.2-1.0 ml are transferred into a series of 10 ml volumetric flasks. To each flask, 1.0 ml of 0.1N hydrochloric acid solution and 1.5 ml of cold 0.1N sodium nitrite solution are added. The resultant solution in each flask is well shaken and allowed to stand for five minutes at 0-5°C temperature for diazotization to complete. 1.0 ml of 1% urea solution is added to each flask and the solution is shaken frequently to allow nitrogen gas to escape. Then 1.0 ml of 0.1N sodium hydroxide solution and 1.0 ml of 1% resorcinol solution are added and the volume in each flask is made upto 10 ml with methanol. The absorbance of the orange red colour solution is measured at
440 nm against the reagent blank. Calibration graph is obtained by plotting absorbance values against the concentration of tenofovir solution. The calibration curve is found to be linear over a concentration range of 20 to 100 μg/ml of tenofovir. The amount of tenofovir present in the sample is estimated from the calibration graph. The results are presented in table 2.

**Assay of pharmaceutical Formulations**

Powdered tablet equivalent to 50 mg of the drug is weighed accurately and transferred into a 50 ml beaker and mixed well with 30 ml of methanol. The solution is filtered and transferred into a 50 ml volumetric flask and the volume is made up to 50 ml with methanol. The concentration of the drug solutions is now 1mg/ml. This stock solution is further diluted to obtain the working concentration of 100 μg/ml and analysed as given under the assay procedure for bulk samples. The results are represented in table 2.

**RESULTS AND DISCUSSION**

Tenofovir undergoes diazotisation when treated with sodium nitrite and hydrochloric acid. The excess nitrous acid during the diazotisation is removed by the addition of urea solution. The solution was shaken frequently to allow the nitrogen gas to escape. The diazonium cation reacts with the coupling reagent, resorcinol by electrophilic substitution at the o-position of the coupling agent to produce a orange red azo product. This orange red colour product shows maximum absorbance at 440 nm. The colour of the product is stable for more than 24 hours. The calibration curve (concentration vs. absorbance) is linear over the range of 20-100 μg/ml of tenofovir. The optical characteristics of the proposed method such as absorption maxima, Beer’s law limits, molar absorptivity and Sandell’s sensitivity are presented in Table 1. The molar absorptivity and Sandell’s sensitivity values shows sensitivity of the method. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and results are summarized in the Table 1. The value of correlation coefficient was 0.999, which indicated the good linearity of calibration lines. The percent relative standard deviation calculated from the five measurements of tenofovir shown in Table 2. The % RSD is less than 2, which indicates that the method has good reproducibility. The values of standard deviation, coefficient of variation values are low, indicates high accuracy and reproducibility of the method. The ‘t’ calculated values are compares well with the theoretical value of 2.78 there by indicating that the precision of the method is good. There no effect of additives and
excipients such starch, calcium lactose and glucose in the concentrations those present in general pharmaceutical preparations.

**Table. 1: Optical Characteristics of the Proposed Method.**

<table>
<thead>
<tr>
<th>parameters</th>
<th>Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>440</td>
</tr>
<tr>
<td>Beer’s limits, mcg/ml</td>
<td>20-100</td>
</tr>
<tr>
<td>Sandell’s, sensitivity, (μg cm²)</td>
<td>0.1427</td>
</tr>
<tr>
<td>Molar absorptivity, (L mol⁻¹ cm⁻¹)</td>
<td>1.96x10³</td>
</tr>
<tr>
<td>Regression equation, Y</td>
<td>Y = 0.0044x+0.0011</td>
</tr>
<tr>
<td>Correlation coefficient, (r)</td>
<td>0.999</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0011</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0044</td>
</tr>
</tbody>
</table>

**Table. 2: assay and recovery of tenofovir In tablet formulations.**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample (mg)</th>
<th>Amount Found (mg)±S.D</th>
<th>% Label claim</th>
<th>%RSD</th>
<th>t_cal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>200.1±0.60</td>
<td>100.5</td>
<td>0.3038</td>
<td>0.7667</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>199.97±0.27</td>
<td>99.98</td>
<td>0.1366</td>
<td>0.2457</td>
</tr>
</tbody>
</table>

**Fig. 1: Calibration curve of tenofovir.**

**CONCLUSION**

The proposed method is found to be simple, precise, accurate and time saving, reproducible and can be conveniently adopted for routine analysis of estimation of tenofovir in bulk drugs samples and pharmaceutical formulations.

**REFERENCES**


