INTRODUCTION

Tenofovir disoproxil fumarate (TDF) is chemically designated as (((2R)-1-(6-amino-9H-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. A prodrug and so it is metabolized de novo to Tenofovir, 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, HPLC with spectrophotometric detection 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 1 cm quartz cells was used.

Preparation of reagents

All chemicals used were of analytical grade.

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

Resorcinol Solution (Sd.fine; 0.1%, 9.08×10⁻³M): 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%. 1.45×10⁻²M): 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
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 diazotization 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>
<thead>
<tr>
<th>Sample</th>
<th>Labelled amount (mg)</th>
<th>Amount obtained by proposed methods* (mg)</th>
<th>Reference method</th>
<th>% Recovery of proposed methods**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method A</td>
<td>Method B</td>
<td></td>
</tr>
<tr>
<td>VIREAD</td>
<td>300</td>
<td>298.6</td>
<td>297.9</td>
<td>299.4</td>
</tr>
</tbody>
</table>

*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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REFERENCES


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