

New Spectrophotometric Determination of Tenofovir Disoproxil Fumarate 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 Tenofovir disoproxil fumarate in bulk and in pharmaceutical preparations. Method 'A' is based on the reaction with wool fast blue, in the presence of buffer at P^H 1.5 to form a colored species with a λ_{max} 590nm. Method B is based on the reaction with tropaeoline -00 dye under acidic condition with a λ_{max} 410nm. Beer's law is obeyed in the concentration range of 50 to 250 μ g/ml for both the methods. The methods were extended to pharmaceutical formulations and there was no interference from any common pharmaceutical excipients and diluents. The result of analysis has been validated statistically and by recovery studies.

Keywords: Spectrophotometric determination Wool fast blue, tropaeoline-00, Tenofovir disoproxil fumarate

I. INTRODUCTION

Tenofovir disoproxil fumarate is an ulcer-healing drug, that inhibits gastric acid by blocking the proton-pump (Hydrogen-Potassium ATPase enzyme system) of the gastric parietal cells. Tenofovir disoproxil fumarate is used for the short-term treatment of gastric and duodenal ulcers and in gastro-oesophageal reflux disease with severe symptoms. Tenofovir disoproxil fumarate is also used in combination with antibacterials for the eradication of *Helicobacter pylori*.

Tenofovir disoproxil fumarate magnesium is a bis(5-methoxy-2-[(S)-[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl]sulfinyl]-1H-benzimidazole-1-yl) magnesium trihydrate. Tenofovir disoproxil fumarate is the S-isomer of omeprazole, which is a mixture of the S- and R- isomers. The magnesium salt is a white to slightly colored crystalline powder. It contains 3 moles of water of solvation and is slightly soluble in water. The stability of Tenofovir disoproxil fumarate magnesium is a function of pH; it rapidly degrades in acidic media, but it has acceptable stability under alkaline conditions. At pH 6.8 (buffer), the half-life of the magnesium salt is about 19 hours at 25°C and about 8 hours at 37°C. Its molecular formula is (C₁₇H₁₈N₃O₃S)₂Mg x 3 H₂O with molecular weight of 767.2 as a trihydrate and 713.1 on an anhydrous basis. The method is based on the reaction of Tenofovir disoproxil fumarate magnesium drug as a secondary amine with wool fast blue, the formed complex extracted into chloroform at pH 1.5. The chloroform extractable layer is used to determine the Tenofovir disoproxil fumarate magnesium spectrophotometrically.

II. EXPERIMENTAL

All spectral measurements were made on MAPADA double beam UV-Visible 1600 spectrophotometer. An ELICO LI-120 Digital pH meter was used for pH measurements.

Method-A

The method is based on the reaction of each drug having secondary or tertiary or primary amino (aliphatic) group with wool fast blue, the formed complex extracted into chloroform at pH 1.5. The absorbance of ion pair complex form in extractable chloroform layer is measured at the wavelength of maximum absorbance for each drug against the reagent blank and the amount of drug is determined from the calibration curve made between the absorbance and the amount of drug.

III. Spectrum of Tenofovir disoproxil fumarate treated with wool fast blue

The wavelength of maximum absorbance of the Tenofovir disoproxil fumarate drug treated with wool fast blue solution is ascertained by the following procedure.

Into a 10 ml volumetric flask, the standard drug solution (1.0 mg/ml) in chloroform were transferred and diluted with same so as to obtain 100 μ g/ml of Tenofovir disoproxil fumarate. 0.5 ml of Tenofovir disoproxil fumarate solution is transferred into a separating funnel. To this solution 0.5 ml of wool fast blue reagent 2 ml buffer solution are added. Reaction mixture was shaken gently for 5 min and allowed to stand for 5 min so as to separate aqueous and chloroform layer. The chloroform layer is separated out and absorbance is

measured in the wavelength range of 450 to 650 nm, against the reagent blank. The spectrum is given in fig.4(a).2.1.

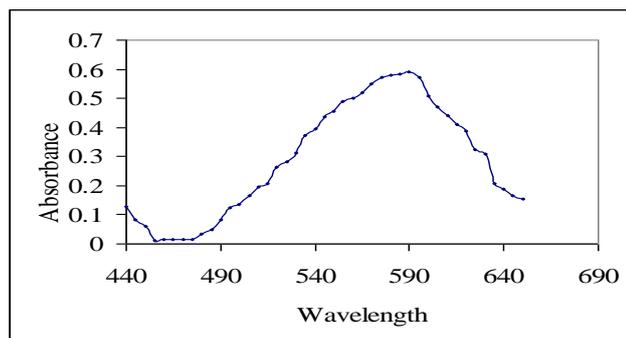


Fig: 4(a).3.1: Spectrum of tenofovir disoproxil fumarate

IV. Assay Procedure

Various aliquots of the standard Tenofovir disoproxil fumarate solution ranging from 0.5-2.5 ml are transferred into a series of separating funnel. To each flask, 1.5 ml of wool fast blue solution, 2.0 ml of buffer solution and 5 ml of chloroform are added. Reaction mixture in each funnel is shaken gently for 5 min and allowed to stand for 5 min so as to separate aqueous and chloroform layer. The chloroform layer is separated out and absorbance is measured at 590 nm, against the reagent blank prepared in similar manner omitting drug solution. Calibration graph is obtained by plotting absorbance values against the concentration of Tenofovir disoproxil fumarate solution. The calibration curve is found to be linear over a concentration range of 50 to 250 µg/ml of Tenofovir disoproxil fumarate. The amount of Tenofovir disoproxil fumarate present in the sample is estimated from the calibration graph. The results are represented in fig.4(a).2.2

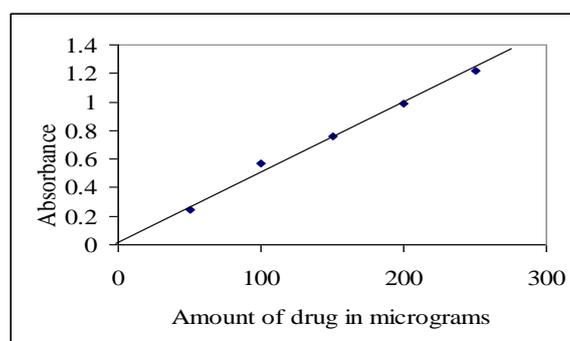


Fig.4(a).3.2: Calibration curve of tenofovir disoproxil fumarate

Pharmaceutical formulation of Tenofovir disoproxil fumarate

For analysis of tablet formulation, twenty tablets of Tenofovir disoproxil fumarate are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of Tenofovir disoproxil fumarate was taken in a 50 ml volumetric flask containing 25 ml of chloroform, sonicated for 20 minutes. The resultant solution is filtered through Whatman filter paper No. 41 into another 50 ml volumetric flask. The filter paper was washed several times with chloroform. The washings were added to the filtrate and the final volume was made up to the mark with methanol. Further sample solution is diluted and treated as per the procedure of the calibration curve. Amount of the drug present in sample was computed from respective calibration curve. The results are present in table. 4(a).2.3.

**Table. 4(a).3.3:
Assay of tenofovir disoproxil fumarate**

Pure sample Taken (mg)	*Amount Found(mg) S.D*	Percentage of Label claim	*C.V	* t _{cal}
100	100.06±0.21	100.06	0.2188	0.6128

V. Results and discussion

In this method the Tenofovir disoproxil fumarate treated with wool fast blue dye at 1.5 pH. The resultant solution is extracted with chloroform. The ion pair complex is formed in extractable chloroform layer. The absorbance of the extractable ion pair complex is measured at 590 nm against the reagent blank (prepared in a similar manner devoid of drug solution). The calibration curve (concentration vs absorbance) is linear over the range of 50-250 µg/ml of Tenofovir disoproxil fumarate. The values of standard deviation are low, indicates high accuracy and reproducibility of the method. The 't' calculated values are compared well with the theoretical value of 2.78 thereby indicating that the precision of the method. There is no effect of additives and excipients such as starch, calcium lactose and glucose in the concentrations of those present in general pharmaceutical preparations.

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 disoproxil fumarate in bulk drugs samples and pharmaceutical formulations as seen from the agreement of the amount of Tenofovir disoproxil fumarate in the present method and the labeled amount of the pharmaceutical preparation.

Method-B

The method is based on the reaction of Tenofovir disoproxil fumarate having secondary amino (aliphatic) group with tropaeoline-OO, the formed complex extracted into chloroform in acidic medium. The chloroform extractable layer is used to determine the Tenofovir disoproxil fumarate spectrophotometrically. The amount of drug is computed from the calibration curve.

Spectrum of Tenofovir disoproxil fumarate treated with tropaeoline-OO

The wavelength of maximum absorbance of the Tenofovir disoproxil fumarate drug treated with tropaeoline-OO solution is ascertained by the following procedure.

Into a 10 ml volumetric flasks, the standard drug solution (1.0 mg/ml) in chloroform were transferred and diluted with same so as to obtain 100 µg/ml of Tenofovir disoproxil fumarate. 1 ml of Tenofovir disoproxil fumarate solution is transferred into a separating funnel. To this solution 1.0 ml of tropaeoline-OO reagent 2.0 ml hydrochloric acid solution are added. Reaction mixture was shaken gently for 5 min and allowed to stand for 5 min so as to separate aqueous and chloroform layer. The chloroform layer is separated out and absorbance is measured in the wavelength range of 340 to 540 nm, against the reagent blank. The spectrum is given in fig.4 (b).2.1.

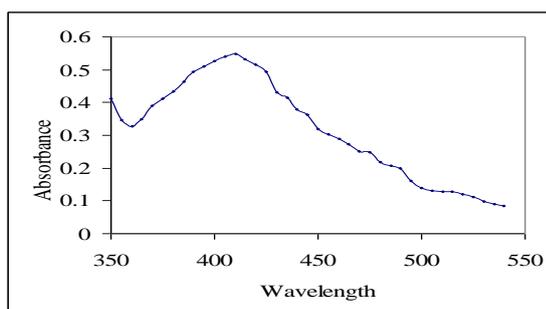


Fig: 4(b).3.1: Spectrum of tenofovir disoproxil fumarate

Assay Procedure

To study the effect of drug concentration on the absorbance of the ion pair complex under optimal conditions now arrived is studied by the following method to know the suitability of the method for the assay of Tenofovir disoproxil fumarate.

Various aliquots of the standard Tenofovir disoproxil fumarate solution ranging from 0.5-2.5 ml are transferred into a series of separating funnels. To each flask, 1.0 ml of tropaeoline-OO solution, 1.5 ml of hydrochloric acid solution and 5 ml of chloroform are added. Reaction mixture in each funnel is shaken gently for 5 min and allowed to stand for 5 min so as to separate aqueous and chloroform layer. The chloroform layer is separated out and absorbance is measured at 410 nm, against the reagent blank prepared in similar manner omitting drug solution. Calibration graph is obtained by plotting absorbance values against the concentration of Tenofovir disoproxil fumarate solution. The calibration curve is found to be linear over a concentration range of 50 to 250 µg/ml of Tenofovir disoproxil fumarate. The amount of Tenofovir disoproxil fumarate present in the sample is estimated from the calibration graph. The results are presented in fig.4(b).2.2

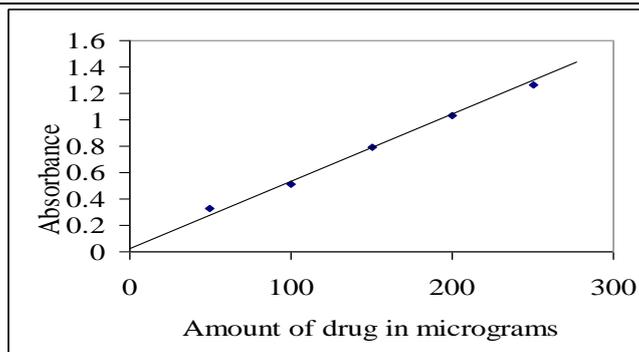


Fig.4(b).3.2: Calibration curve of tenofovir disoproxil fumarate

For analysis of tablet formulation, twenty tablets of Tenofovir disoproxil fumarate are weighed accurately and finely powdered. An accurately weighed portion of powdered sample, equivalent to 50 mg of Tenofovir disoproxil fumarate was taken in a 50 ml volumetric flask containing 25 ml of chloroform, sonicated for 20 minutes. The resultant solution is filtered through Whatman filter paper No. 41 into another 50 ml volumetric flask. The filter paper was washed several times with chloroform. The washings were added to the filtrate and the final volume was made up to the mark with chloroform. 5 ml filtrate of the sample solution was diluted to 10 ml with chloroform and treated as per the procedure of the calibration curve. Amount of the drug present in sample was computed from respective calibration curve. The results are present in table. 4(b).2.3.

Table. 4(b).2.3:

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Assay of tenofovir disoproxil fumarate

Pure sample Taken (mg)	*Amount Found(mg) S.D*	Percentage of Label claim	*C.V	*t _{cal}
100	100.1±0.33	100.1	0.3387	0.6596

VI. Results and discussion

In this method the Tenofovir disoproxil fumarate treated with tropaeoline-OO dye in acidic medium. The resultant solution is extracted with chloroform. The ion pair complex is formed in extractable chloroform layer. The absorbance of the extractable ion pair complex is measured at 410 nm against the reagent blank (prepared in a similar manner devoid of drug solution). The calibration curve (concentration vs absorbance) is linear over the range of 50-250 µg/ml of Tenofovir disoproxil fumarate. The values of standard deviation 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 thereby indicating the precision of the method. There is no effect of additives and excipients such as starch, calcium lactose and glucose in the concentrations of those present in general

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 disoproxil fumarate in bulk drugs samples and pharmaceutical formulations as seen from the agreement of the amount of Tenofovir disoproxil fumarate in the present method and the labeled amount of the pharmaceutical preparation.

VII. ACKNOWLEDGEMENTS

The authors are thankful to M/S Hetero Drugs Limited, Hyderabad for gift sample and. Department of Chemistry, Rayalaseema University Kurnool. For providing additional laboratory facilities

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