APPLICATION OF UV-SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF TENOFOVIR DISOPROXIL FUMARATE IN TABLETS

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ABSTRACT

Two new, simple and cost effective UV-spectrophotometric and first order derivative methods were developed for estimation of tenofovir disoproxil fumarate in bulk and tablets. Tenofovir disoproxil fumarate was estimated at 260 nm in 0.1N HCl. In first order derivative, it showed amplitude at 273 nm. In both the methods linearity was found to be in the range of 5 - 40 µg/ml; for UV-spectrophotometric method (Y=0.02586 X+0.0083; r^2=0.9999) and for first order derivative spectrophotometric method (Y=0.00132 X+0.00035; r^2=0.9995), respectively. These methods were tested and validated for various parameters according to USP guidelines. The quantitation limits were found to be 1.546 and 1.986 µg/ml, for both the methods. The proposed methods were successfully applied for the determination of tenofovir disoproxil fumarate in pharmaceutical formulations. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation <2%), while being simple, cheap and less time consuming and can be suitably applied for the estimation of tenofovir disoproxil fumarate in different dosage forms.

Keywords: Tenofovir disoproxil fumarate, UV-spectrophotometric method, first order derivative spectrophotometry.

INTRODUCTION

Tenofovir disoproxil fumarate (TDF) is antiretroviral drug and acts by blocking reverse transcriptase (Miller *et al*., 2004). Chemically TDF is 9-[(R)-2-[[bis[[(isopropoxy-carbonyl)oxy]methoxy]phosphinyl]methoxy]propyl]adeni ne fumarate (1:1) (Sweetman SC, 2007). The dose of TDF is 300 mg per day (Gilead Sciences Inc, 2001).Several combinations of tenofovir with other antiretroviral drugs are available in the market for treatment of HIV infected patients (Gallant *et al*., 2006).

Literature survey revealed, few analytical methods which include liquid chromatography with tandem mass spectrometry (Tracy *et al*., 2006), simultaneous quantification of emtricitabine and tenofovir in human plasma using high- performance liquid chromatography after solid phase extraction (Naser *et al*., 2005). Sensitive determination of tenofovir in human plasma samples using reversed-phase liquid chromatography (Sentenac *et al*., 2003). TDF is not official in IP, BP and USP. The present work deals with estimation of TDF in tablets by UV-spectrophotometry and first order derivative spectrophotometry (Beckett and Stenlake, 1998).

MATERIALS AND METHODS

Instruments

a) UV-visible spectrophotometer (2450 Shimadzu with UV probe 2.21 software), 10 mm quartz cell and spectral bandwidth 1nm
b) Micropipette, Variable volume 20-200 µL Biosystem classic

Reagents

a) 0.1N HCl
b) Double Reverse Osmosis (R.O.) water

Preparation of standard stock solution

Standard stock solution containing 100 µg/ml of TDF was prepared in 0.1N HCl. From the stock, different aliquots were taken and diluted to 10 ml mark with same solvent to obtain series of concentrations. The solutions were scanned on spectrophotometer in the UV range 200-400 nm. TDF showed absorbance maxima at 260 nm (fig. 1). The same solutions were subjected to first order derivative, using UV probe software of instrument, where $\Delta\lambda$ = 2 (fig. 2). The amplitudes of the corresponding troughs were measured at 273 nm. In both the methods, drug follows linearity in the concentration range of 5-40 µg/ml (Y=0.02586 X+0.0083, r^2=0.9999 and Y=0.00132 X+0.00035, r^2=0.9995), respectively.

Preparation of sample solution

For analysis of commercial formulation; twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 150 mg of tenofovir was transferred into 100 ml volumetric flask containing 30 ml, 0.1N HCl, shaken manually for 10 min., volume was made up to mark with same solvent and filtered through Whatmann filter paper No.41. An appropriate aliquot was transferred to 10 ml volumetric flask, volume was adjusted to the mark and absorbance was recorded at 260 nm. The same solution was subjected for first order derivative using UV-

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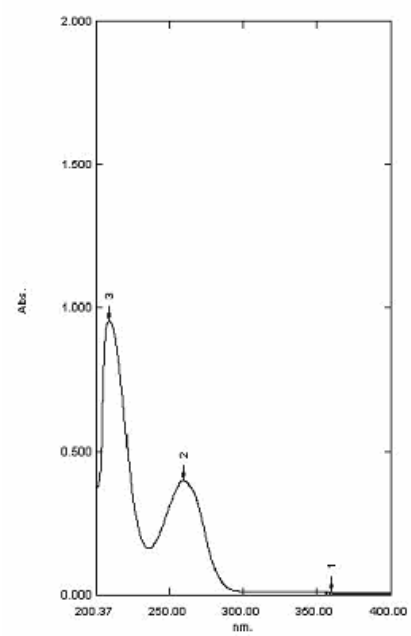

Fig. 1: UV-Spectrum of TDF in 0.1N HCl.

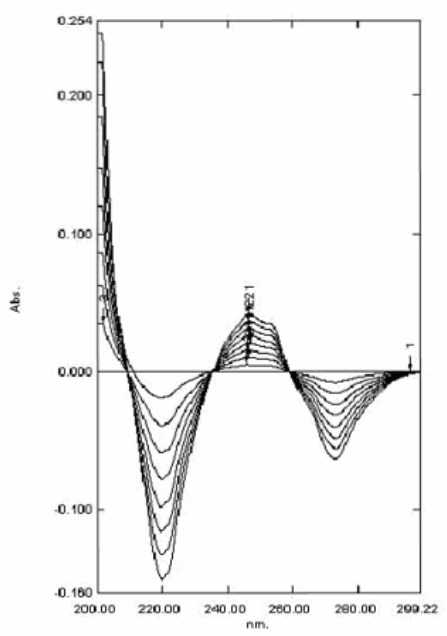

Fig. 2: An overlain first order derivative spectra of TDF in 0.1N HCl

Table 1: Results of assay

Label claim	*Amount found (%)	
	UV-Spectrophotometric ± SD	First order derivative spectrophotometry ±SD
Tenofovir (300mg/tablet)	100.75 ± 0.528	100.04 ± 0.349

*mean of six determinations

Table 2: Summary of validation parameters

Parameters	UV-Spectrophotometric	First order derivative Spectrophotometry
Linearity and range (µg/ml)	5.00-40.00	5.00-40.00
LOD	0.510	0.655
LOQ	1.546	1.986
Accuracy (% Recovery) (n = 9)	99.62 %	100.15%
%RSD	0.297	0.118
Precision (%RSD)		
Intra-day (n = 3)	0.139-0.466	0.297-0.581
Inter-day (n = 3)	0.247-0.475	0.101-0.466
Repeatability(%RSD) (n = 6)	0.701	0.349
Ruggedness (%RSD) (n = 3)		
Analyst I (% label claim)	0.247	0.139
Analyst II(% label claim)	0.475	0.466
Robustness(% RSD) (n = 3)		
Laboratory-I	0.442	0.459
Laboratory-II	0.364	0.466

probe software and amplitude of the trough was recorded at 273 nm. The concentration of the drug was calculated from linear regression equation; results are shown in table 2.

RESULT AND DISCUSSION

The zero order UV spectrum of TDF in 0.1N HCl has showed maximum absorbance at 260 nm. The first

derivative spectrum of TDF has sharper and well- defined peak. The structural features are sharpened to give improved resolution of overlapping peaks. In first order derivative spectrum, the amplitude of the trough was recorded at 273 nm. The amount of drug determined was in the good agreement with the label claim as shown in table 1. The methods were validated for accuracy, precision, ruggedness and robustness. The accuracy of the methods was assessed by recovery studies at three different levels i.e. at 80%, 100% and 120%. The precision of the methods were studied as intra-day, inter-day and repeatability. The % RSD values less than 2 indicate the methods are accurate and precise. Ruggedness of the proposed methods was studied with the help of two analysts. Robustness of the methods was studied in two different laboratories using UV-visible spectrophotometer. The results did not show any statistical difference between operators and environmental conditions, suggesting that methods developed were rugged and robust. The results from validation studies are shown in table 2.

CONCLUSION

Both these methods are simple, rapid and accurate and precise and can be used for routine analysis of tenofovir from tablet formulations

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