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## SHORT COMMUNICATION

## DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR THE QUANTIFICATION OF EFAVIRENZ IN TABLETS

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## ABSTRACT

Two simple and precise spectrophotometric methods ( A and B ) were developed for the estimation of efavirenz ( EFZ ) in bulk drug as well as in pharmaceutical dosage form( tablets). Methods A is based on the formation of pink coloured chromogen by the diazotization and coupling reaction of EFZ with  $\beta$ -Naphthol. The  $\lambda_{\max}$  of the pink coloured chromogen was found to be 494nm. Method B is First derivative spectroscopy method. The first derivative spectrum is a plot of the rate of change of absorbance with wavelength against wavelength (dA /d $\lambda$  versus  $\lambda$ ). It is characterized by a maximum, minimum and a cross- over point at the  $\lambda_{\max}$  of the absorption band. Beer's law was obeyed in the concentration range of 10-60 $\mu$ g/ml and 4-24  $\mu$ g/ml for methods A and B respectively. The proposed methods were statistically validated and found to be useful for the routine determination of EFZ in tablets.

**Key words:** Efavirenz,  $\beta$ - Naphthol, First derivative spectroscopy, Tablets, Validation

## INTRODUCTION

Efavirenz is an antiviral medication that prevents human immuno deficiency virus (HIV) cells from multiplying in our body. Chemically it is (+)6-chloro-4-cyclopropylethynyl-1,4-dihydro-4(trifluoromethyl)2H-3,1 benzoxazin- 2- one<sup>1</sup>. Literature review revealed very few analytical methods including RP-HPLC, HPTLC and UV-spectrophotometry for determination of EFZ in plasma, bulk drug and pharmaceutical formulations<sup>2-8</sup>. In the present work, two simple and sensitive spectrophotometric methods ( A and B ) have been developed for the estimation of EFZ in bulk drug and pharmaceutical dosage form. In Method A, EFZ is first diazotized with an aqueous solution of nitrous acid followed by coupling with  $\beta$ -Naphthol to form an azo derivative<sup>9,10</sup> which absorbs intensely at 494nm. Method B is First derivative spectroscopic method. Spectrophotometric parameters are established for standardization of the methods including statistical analysis of data.

## EXPERIMENTAL

**Instrument:** All spectral and absorbance measurements were made on Shimadzu UV-Vis Spectrophotometer-1650.

**Standard solution of EFZ:** A 1mg/ml stock solution of EFZ was prepared by dissolving 100mg of drug in 100ml of ethanol.

**Sample preparation:**

Twenty tablets were weighed. A quantity equivalent to 100mg of EFZ was weighed accurately, transferred to a beaker, dissolved in ethanol, filtered through Whatmann filter paper No.1 into a 100ml volumetric flask and made up to volume with ethanol to get a concentration of 1mg/ml.

**ASSAY:****Method A:**

Aliquots of EFZ ranging from 0.25 – 1.5 ml ( 1.0 ml = 1000  $\mu$ g) were pipette out into a series of 25ml volumetric flasks. To each flask, 1ml of sodium nitrite (0.1M), 2ml of hydrochloric acid (2M) and 2ml of  $\beta$ -Naphthol (0.5% w/v) were added, mixed thoroughly and made upto volume with distilled water. The  $\lambda_{\max}$  of the pink coloured chromogen was found to be 494nm as shown in Fig.1. The absorbance of the pink coloured chromogen was measured at 494nm against the reagent blank. The pink chromogen was stable for more than 3 hours. The analytical curve was constructed by plotting concentration versus absorbance.

**Method B:**

The stock solution was diluted suitably with ethanol to give a series of concentration ranging from 4-24 $\mu$ g/ml of EFZ. The above solutions were scanned in the range of 200-400nm and the resultant spectra were derivatised to get the first order spectra as shown in Fig.2. The amplitude of the corresponding concentrations were measured in mm. The calibration curve was constructed by plotting amplitude versus concentration. The amount of EFZ was computed from the calibration curve.

**Sample Analysis:**

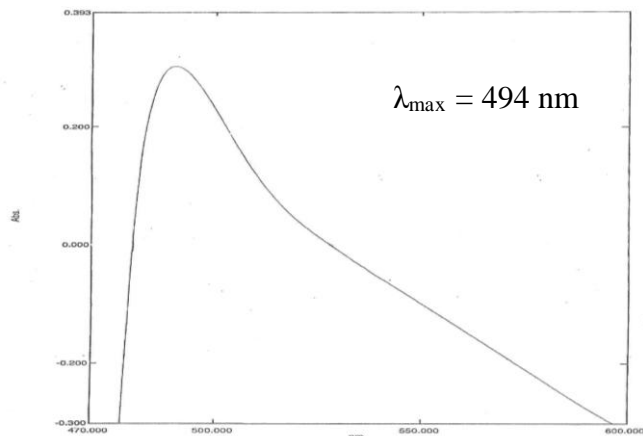
Pharmaceutical formulation of EFZ was successfully analysed by the proposed methods. Appropriate aliquots were subjected to the above methods and the amount of EFZ was determined from the calibration curves.

**RESULTS AND DISCUSSION:**

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are furnished in Table-1. The regression characteristics like slope(m), intercept(c), correlation coefficient(r), percent relative standard deviation(% RSD) and standard error(SE) were calculated and the results are summarized in Table-1. The results of sample analysis

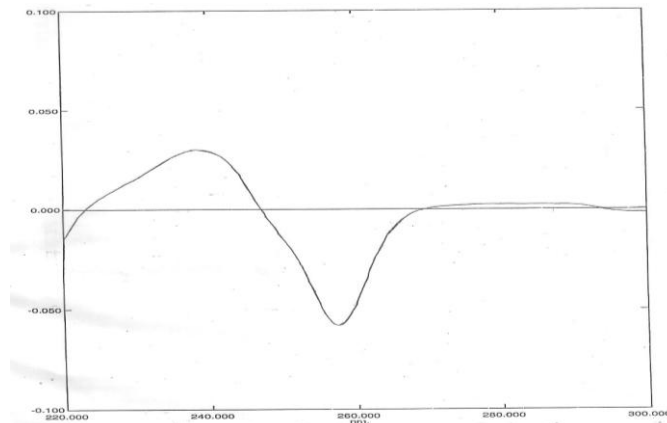
showed that the drug determined by the proposed methods was in good agreement with the label claim proving the accuracy of the proposed methods. The results of sample analysis are furnished in Table-2.

To study the accuracy and reproducibility of the proposed methods, recovery experiments were carried out by adding a known amount of drug to preanalysed sample and the percentage recovery was calculated.



**Figure 1:  $\lambda_{\max}$  of pink chromogen of EFZ by Method A**

The results are furnished in Table-2. The results indicate that there is no interference of other ingredients present in the formulations. Thus, the proposed methods are simple, sensitive, economical, accurate and reproducible and useful for the routine determination of EFZ in bulk drug and its pharmaceutical dosage forms.



**Figure 2: First derivative spectrum of EFZ by Method B**

**Table 1: Optical and Statistical parameters by Methods A and B**

Parameters	Method A	Method B
Absorption maximum/ Wavelength range(nm)	494	200-400
Linearity Range( $\mu\text{g/ml}$ )	10-60	4-24
Correlation coefficient(r)	0.999	0.998
% RSD	0.0001	0.0005
Standard Error(SE)	0.0424	0.0122
Regression Equation $y=mx+c$	$0.179x+0.0113$	$0.0066x+0.1102$
Intercept (c)	0.0113	0.1102
Slope (m)	0.179	0.0066
Sandell's Sensitivity ( $\mu\text{g}/\text{cm}^2/0.001\text{A unit}$ )	0.0008	-
Molar absorptivity ( $\text{Lmol}^{-1}\text{cm}^{-1}$ )	$3.6 \times 10^5$	-

**Table 2: Assay and recovery of EFZ in dosage form**

Method	Labelled amount(mg)	Amount obtained(mg)*	Percentage recovery**
A	600	600.01	100.1%
B	600	600.02	99.99%

\*Average of six determinations

\*\*Average of three determinations

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