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Development and Validation of Spectrophotometric Methods for the Determination 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. Method A is based on the formation of purple coloured chromogen by the diazotization and coupling reaction of EFZ with Bratton-Marshall reagent [N-(1-naphthyl)-ethylene diamine dihydrochloride] The λ_{max} of the purple coloured chromogen was found to be 523nm. Method B is Area Under Curve (AUC) method which involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths. Beer's law was obeyed in the concentration range of 10-60µg/ml and 4-24 µg/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.

Keywords: Efavirenz, Spectrophotometry, Tablets, Validation

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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[1]. 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[2-8]. 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 Bratton- Marshall reagent to form an azo derivative[9,10] which absorbs intensely at 523nm. Method B is Area Under Curve Method. Spectrophotometric parameters are established for standardization of the methods including statistical analysis of data.

MATERIALS AND METHODS

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 µg) 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 Bratton- Marshall reagent (0.5% w/v) were added, mixed thoroughly and made upto volume with distilled water. The absorbance of the purple coloured chromogen was measured at 523nm against the reagent blank. The purple chromogen was stable for more than 3 hours. The analytical curve was constructed by plotting concentration versus absorbance.

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Method B

Area Under Curve (AUC) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths 225nm and 260nm. The area under curve between 225nm and 260nm were calculated by inbuilt software. Aliquots of stock solution of EFZ were suitably diluted with ethanol to give varying concentrations ranging from 4-24 µg/ml. The solutions were scanned in the spectrum mode in the wavelength range of 400-200nm. The calibration curve was obtained by plotting concentration versus area. 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(b), intercept(c), correlation co-efficient(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 are furnished in Table-2. 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.

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. 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.

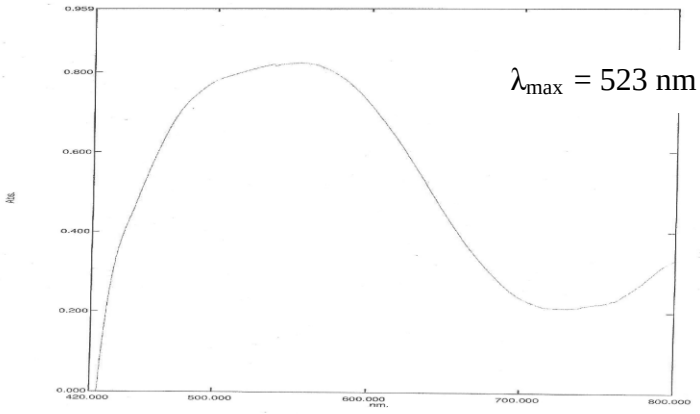

Fig.1: λ_{max} of purple chromogen of EFZ by Method A

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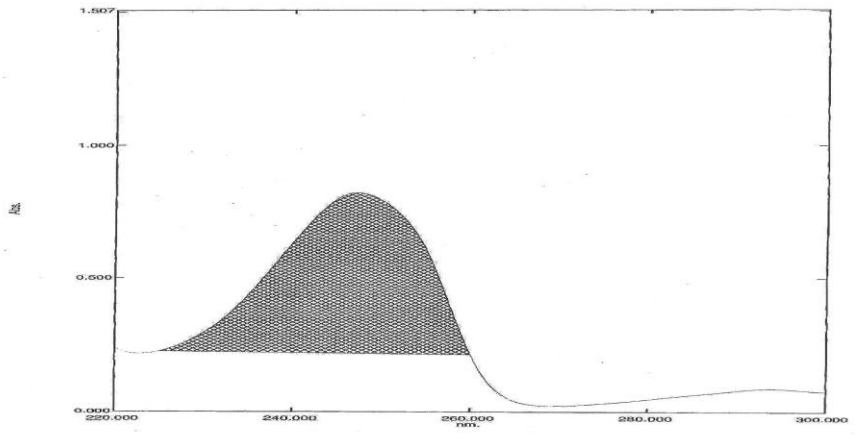

Fig.2: AUC of Efavirenz by Method B

Table 1. Optical and Statistical parameters of methods A and B

Parameters	METHOD A	METHOD B
Absorption maximum/ Wavelength range(nm)	523	225-260
Linearity Range(µg/ml)	10-60	4-24
Correlation coefficient	0.996	0.998
% RSD	0.0084	0.0028
Standard Error(SE)	0.03439	0.0114
Regression Equation y=mx+c	0.068x+ 0.047	0.8054x+ 0.6389
Intercept (c)	0.047	0.6389
Slope (m)	0.068	0.8054
Sandell's Sensitivity (µg/cm^2/0.001A unit)	0.0099	-
Molar absorptivity (Lmol^{-1} cm^{-1})	3.295×10^3	-

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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REFERENCES

[1] Martindale, The complete drug reference, Pharmaceutical Press, London, 35th Edition Vol-1, 2007, pp. 785.
[2] Seloi Mogatle, Isadore Kanfer. J Pharm Bio Med Anal 2009; 5: 130.
[3] Hamrapurkar P, Phale M, Shah N. J Young Pharmacists 2009; 4: 359.
[4] Gita R. J Chromatogr 2008; 1: 55-61.
[5] Agnes I. J Chromatogr 2008; 1: 55-61.
[6] Naser L Rezk, Richard R Tidwell, Angela DM Kashuba. J Chromatogr 2002; 1: 79-88.
[7] Matthews CZ , Woolf EJ, Mazenko RS , Haddix H Wiener, Chavez-Eng CM , Constanzer ML. J Pharm Biochem Anal 2002; 5: 925-934.
[8] Montgomery ER. J Pharm Bio Med Analysis 2001; 267-28
[9] Willard, Instrumental methods of analysis, 6th Edition, CBS Publishers, pp. 82-83.
[10] Beckett A.H, Stenlake J.B,Practical Pharmaceutical Chemistry, Vol II, 4th edition, CBS Publishers, 1997, pp.301.