AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Development and Validation of UV Spectrophotometric Method for Estimation of Efavirenz in Bulk and Pharmaceutical Tablet Dosage Form

Mahipalsingh M. Bayas[1]*, Sugandha V. Mulgund[1]

1. Department Quality Assurance Techniques, STES's, Sinhgad College of Pharmacy, Vadgaon (Bk.), Sinhgad Road, Pune - 411041, Maharashtra, India.

ABSTRACT

A simple, accurate and precise UV spectrophotometric method has been developed for the quantitative estimation of Efavirenz in bulk and tablet dosage form. The λmax was found to be 239 nm. Beer's law was obeyed in the concentration range of 10-20µg/ml. The regression equation was Y=0.052x-0.113 with value of R^2 as 0.996. The method showed good linearity, accuracy and reproducibility. Accuracy as expressed mean percent recovery ± standard deviation was found to be 95.29% ± 0.0429. Percent relative standard deviation values for the intra-day and inter-day precision studies were found to be 0.23 and 0.31, respectively. The limit of detection and limit of quantitation values were found to be 1.39 and 4.22, respectively. Assay of Efavirenz in tablet formulation was performed and percent purity of tablet was found to be 98.65% ± 0.0078.

Keywords: Efavirenz, UV spectrophotometry, Validation.

*Corresponding Author Email: Mayur.bayas@outlook.com

Received 09 April 2015, Accepted 17 April 2015

Please cite this article as: Bayas MM *et al*., Development and Validation of UV Spectrophotometric Method for Estimation of Efavirenz in Bulk and Pharmaceutical Tablet Dosage Form. American Journal of PharmTech Research 2015.

INTRODUCTION

Efavirenz is chemically (4S)-6-ch1oro-4-(cyclopropylethynyl)-l,4dihydro-4 (trifluoromethy1)-*2B*-3,1-benzoxazin-2-one (Figure 1)[1]. It belongs to a class of antiretroviral drugs known as non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used as part of highly active antiretroviral therapy (HAART) for the treatment of a human immunodeficiency virus (HIV) type-1[2].

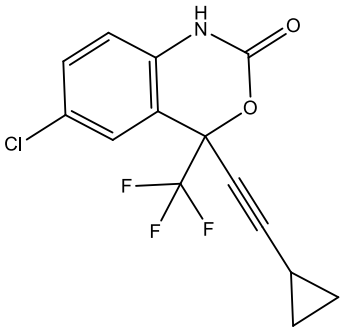

Figure 1: Chemical structure of Efavirenz

Efavirenz is official in Indian pharmacopoeia[1]. Literature survey reveals that few UV[3-4], HPLC,[5-13] HPTLC,[14] LC-MS[15] and LC-MS/MS[16] methods are available for the estimation of efavirenz alone or in combination with other drugs. The present investigation has been undertaken to develop simple UV spectrophotometric method for the estimation of efavirenz in pure form and its formulation.

MATERIALS AND METHOD

A Shimadzu UV/VIS double beam spectrophotometer (model 1800) with 1 cm matched quartz cells was used for all spectral measurements. Methanol used was of analytical grade. The pharmaceutical grade Efavirenz was gifted by Mylan Laboratories. Pvt. Ltd., Hyderabad, India. The tablet samples of Efavirenz (Estiva 600mg Genix Pharma Ltd.) were purchased from local market.

Preparation of Standard solution

100 mg of pure drug was weighed accurately and dissolved in 100 ml of methanol (1000µg/ml) with the help of sonication. This solution was further diluted with the same solvent to obtain a solution of concentration 100 µg/ml. This solution was scanned in the range of 400-200 nm against solvent methanol as blank.

Preparation of sample solution for assay

10 tablets containing label claim of 600 mg were weighed and average weight was determined. The tablets were triturated and the weight of the powder equivalent to 100 mg of Efavirenz was

dissolved in 70 ml of methanol and sonicated for about 20 minutes. Volume was then made up to 100 ml using methanol and filtered with the help of Whatman filter paper no. 41 to separate the insoluble matter. This solution was further diluted with the same solvent to obtain a solution of 16 µg/ml concentration. The results are given in Table 1.

Table 1: Assay

Drug	Label claim	Amount taken (µg/ml)	Amount found (µg/ml)	Mean % Assay ± S.D*
Estiva	600mg	16	15.11	98.65% ± 0.0078

(*n=3)

Validation of the Developed Method

The developed method was validated as per ICH Q2(R1) guideline[17] for the following parameters.

Linearity and range

The standard solutions were prepared by dilution of the stock solution with methanol in the range of 10-20 µg/ml. The Absorbance's were measured at 239 nm (Figure 2) and plotted against the corresponding concentration to obtain the calibration Curve.

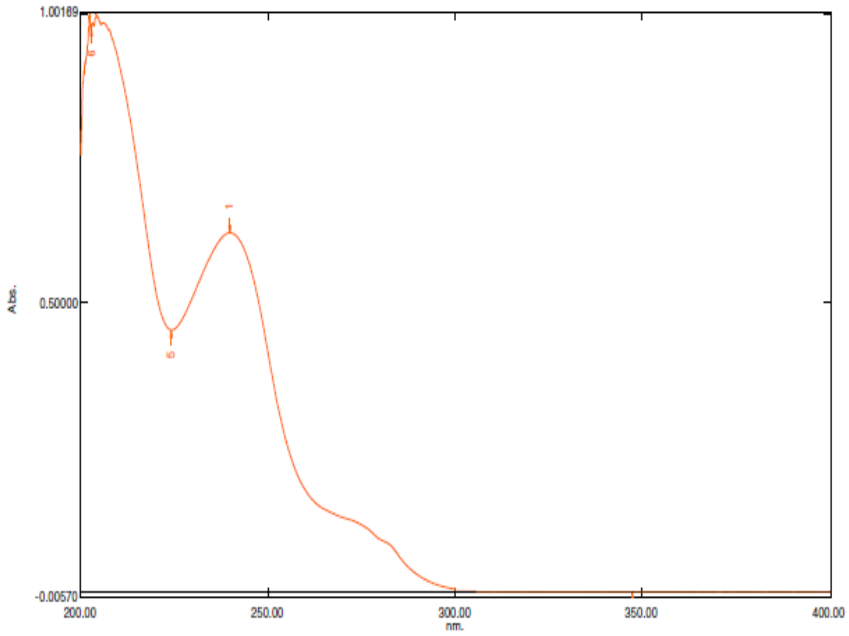

Figure 2: UV spectrum of 10(µg/ml) Efavirenz in methanol

Precision studies

Intra and inter-day precision studies were performed by measuring the absorbance of standard solution of 16 µg/ml at three different times during the single day and on three subsequent days respectively. The percentage relative standard deviation (% RSD) values for interday & intraday precision studies were calculated.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Calibration curves were plotted for six sets. LOD and LOQ were calculated using the formulae as LOD= 3.3 (SD)/S and LOQ= 10 (SD)/S, where S is average value of slopes of calibration plots and SD is calculated using values of y intercepts of regression equations.

Accuracy

Recovery studies were carried out by taking absorbance of standard efavirenz corresponding to 80, 100 and 120%. At each level of the amount three determinations were performed. The results are given in Table 3.

RESULTS AND DISCUSSION

The UV spectrum of Efavirenz in methanol has maximum absorption (λmax), at 239 nm. The absorbance of excipients in tablet solution did not interfere with Efavirenz at 239 nm. As a result, the wavelength was selected for quantitative analysis. The developed method was applied for estimation of Efavirenz in tablet formulation. The results obtained are shown in Table 1.

The drug showed linearity in the concentration range of 10–20 µg/mL. Linear regression data is shown in Table 2 and Figure 3. The developed method was found to be accurate, indicated by mean % recoveries ranging from 96.78 to 99.54% in table 3. The method was also found to be precise as the %RSD values for intraday and interday precision were found to be less than 2%.

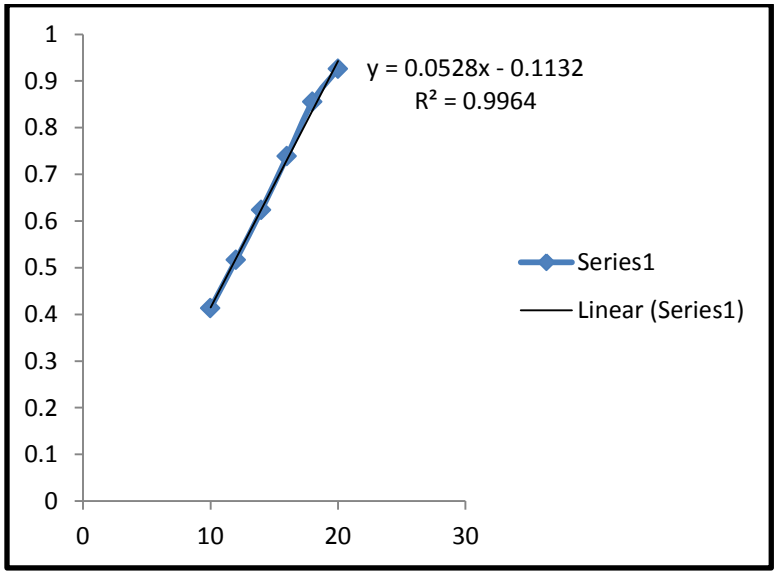

Figure 3: Calibration Curve of Efavirenz in methanol

Table 2: Linear regression data

Sr.No.	Parameters	Results
1	λmax (nm)	239
2	Beer's law limit (µg/mL)	10-20
3	Correlation coefficient	0.9964
4	Regression equation	y = 0.0528x - 0.1132
5	Slope (m)	0.0528
6	Intercept (c)	-0.1132
7	Detection limit (µg/mL)	1.3
8	Quantification limit (µg/mL)	4.2

Table 3: Accuracy studies

Drug	Recovery levels	Amount taken (µg/ml)	Amount recovered(µg/ml)	Mean % Recovery ± S.D*
Efavirenz	80%	14.4	13.58	94.30% ± 0.052
	100%	16	15.11	94.43% ± 0.0097
	120%	17.6	17.10	97.15% ± 0.067

(*n=3)

Table 4: Result of Intraday, and Interday precision studies

Drug	Concentration (µg/ml)	Intraday Precision (n=3) %RSD	Inter day Precision (n=3) % RSD
Efavirenz	16	0.2330	0.3117

CONCLUSION

A simple, accurate and precise UV Spectrophotometric method for estimation of efavirenz is developed. The method was validated as per the guidelines laid by ICH. The method is suitable for routine estimation of efavirenz in pharmaceutical laboratories.

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