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Research Article

Spectrophotometric Estimation of Efavirenz in Formulation and Biological Fluid

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ABSTRACT

A simple, sensitive and accurate spectrophotometric method was developed in ultraviolet region for the estimation of efavirenz (EFA) in pure drug, pharmaceutical formulation. Linear response obtained was in the concentration range of 5-40 µg/ml with correlation coefficient of 0.9993, 0.9989 in solvent and plasma respectively. Excellent recovery proved that the method was sufficiently accurate. There is no interference from any common pharmaceutical additives and diluents. Results of the analysis were validated by recovery studies according to ICH Q2B guidelines and the same method is applied for estimation of efavirenz from plasma and these results were validated according to USFDA guidelines for bioanalytical method.

Keywords: Efavirenz; Spectrophotometry; Validation; Pharmaceutical formulations; Biological fluid.

INTRODUCTION

Chemically, Efavirenz (EFA) is (S)-6-chloro-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one. Its empirical formula is $C_{14}H_9ClF_3NO_2$. Efavirenz is a white to slightly pink crystalline powder with a molecular mass of 315.68 g/mol. It is practically insoluble in water. EFA is non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used as a part of highly active antiretroviral therapy for the treatment of human immunodeficiency virus (Fig. 1). The drug is used in combination with other anti retroviral agents for the treatment of HIV-1 infection in children and adults. The usual dose of EFA is 600 mg per day and its plasma half life is ~50 h. (Nikalje, 2009; Rang, 2007). Both nucleoside and non-nucleoside RTIs inhibit the same target, the reverse transcriptase enzyme, an essential viral enzyme which transcribes viral RNA into DNA. Unlike nucleoside RTIs, which bind at the enzyme's active site, NNRTIs act allosterically by binding to a distinct site away from the active site known as the NNRTI pocket. EFA is not effective against HIV-2, as the pocket of the HIV-2 reverse transcriptase has a different structure, which confers intrinsic resistance to the NNRTI class (Ren, 2002). However, more than 50% of patients starting EFA treatment experience its related neuropsychiatric adverse events (NPAEs), such as dizziness, feeling of drunkeness and sleep disorders and even severe psychiatric symptoms have been reported with EFA (Allcia, 2009) .

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Several analytical methods of EFA were reported including LCMS, HPLC (Gadkari, 2010; Garg, 2009) .The information about spectrophotometric method used to analyze the EFA concentration was rather scanty. In the present study an attempt has been made to develop simple, sensitive, and economical method in UV region with greater precision and accuracy for the estimation of EFA in pure drug, tablet formulation and biological fluid.

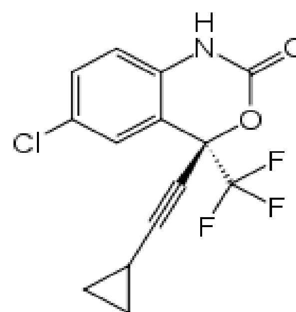

Figure 1: Chemical structure of Efavirenz

MATERIALS AND METHODS

EFA sample was supplied by Sandoz Ltd., Mumbai, as a gift sample and used as such. Methanol used was spectro grade purchased from S. D. Fine Chemicals ltd., India. All other reagents used were of analytical reagent grade supplied by Research Lab., India. Spectral and absorbance measurements were made on a UV-Visible spectrophotometer Jasco V 530 model with 10mm matched pair of quartz cell and spectral band width of ±2nm.

Selection of solvent

The ideal property of a solvent should be that the drug should be completely soluble in the solvent used. The drug should be stable in the solvent used and should be economical and volatile. After suitable literature survey, practical experience and taking above factors into consideration the suitable solvents selected was methanol.

Selection of Method and Wavelength

For estimation of EFA single-wavelength spectrophotometric method employing 247nm analytical wavelengths was used, spectra shown in Fig. 2

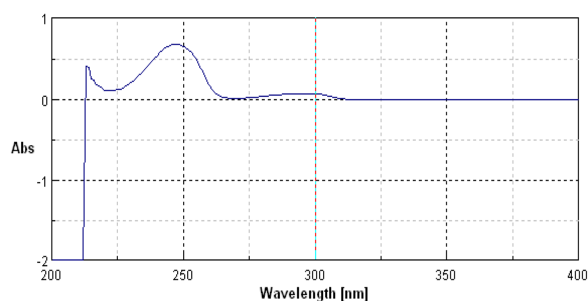

Figure 2: Spectrum of Efavirenz at wavelength 200 to 400 nm

Standard solutions and calibration curve

Accurately weighed 10mg of EFA is transferred into a 100ml volumetric flask and dissolved in 30ml of methanol. It was then sonicated for 10 minutes, and made up to the mark with methanol to give a stock solution having 100 µg/ml concentration. For calibration curve, serial dilutions were made for EFA in the range of 5, 10, 15, 20, 25, 30, 35 and 40µg/ml concentrations were prepared by diluting the stock solution with methanol. The absorbance values of above solutions were measured in the wavelength at λ_{max} 247nm against methanol as blank and calibration curve was prepared. It obeyed beer's law in these concentration ranges (Skoog, 1992; Beckett, 1998)

Sample preparation for tablet analysis

To determine the content of EFA in conventional tablets (label claim: 600mg EFA per tablet), twenty tablets were weighed, their mean weight was determined and they were finely powdered and powder equivalent to 10 mg of EFA was weighed and transferred into a 10 ml volumetric flask containing 10 ml methanol, sonicated for 10 min and the resulting sample solution was then filtered through Whatmann filter paper (No. 41). The filtrate was further diluted to obtain the final concentration of 100µg/ml. Appropriate dilutions of EFA were scanned over the range of 400-200 nm and the absorbance at wavelength 247nm was measured. From calibration curve the final drug concentration in tablet was calculated.

Standard drug solution in plasma and calibration curve

To the 2.5 ml of blood sample 0.5 ml of 0.1 M acetic acid was added. RBC allowed settling down. Sample was centrifuged at 5000 rpm for 35 min. The supernatant was collected as plasma. To the 20 ml solution of weight sample of drug dissolved in methanol, 2 ml of plasma solution was added. Obtained 22 ml solution was filtered through syringe filter of size 45 µm size. Final volume was adjusted to 100 ml with methanol to get stock solutions containing 100 µg/ml of EFA. A series of dilutions were made with methanol to get the concentrations of 5, 10, 15, 20, 25, 30, 35 and 40µg/ml. The absorbance values of above solutions were measured in the wavelength at λ_{max} 247nm against methanol as blank and calibration curve was prepared (Kappelhoff, 2003; Smith, 2009).

Analysis of tablet formulation in plasma:

Twenty tablets were accurately weighed; ground to powder and an equivalent of 10mg of active ingredient dissolved in 10 ml methanol was taken into a 100 ml volumetric flask, sonicated for about 10 min. This solution was filtered through Whatmann filter paper No.41; previously separated 2 ml plasma solution was added. The solution was filtered through hydrophilic PVDF 45µm size syringe filter. Final volume was made up to 100 ml with solvent. Aliquots in suitable concentration were prepared analysed using proposed method.

Method Validation

Method validation was performed in terms of specificity and selectivity, precision and accuracy, linearity and stability (ICH Q2B,1996; Joachim, 2003).

Precision and Accuracy

Accuracy of analysis was determined by performing recovery studies by spiking different concentrations of pure drug in the pre analyzed tablet samples within the analytical concentration range of the proposed method at three different set at level of 80%, 100% and 120%.

Precision was calculated for inter day and for intraday. The data obtained shows that method is sufficiently precise. Precision is calculated as % Relative Standard Deviation.

Linearity and Stability

The response for EFA was linear in the concentration range of 5 - 40µg/ml. with coefficient of correlation r^2 = 0.9993 for pure drug and r^2 = 0.9989 for drug in plasma.

Problems of stability are usually encountered with these compounds, mainly affecting plasma concentration at room temperature, storage in freezer eliminates decomposition.

RESULTS AND DISCUSSION

Standard calibration curve for EFA, covering the range 5-40µg/ml, prepared by serial dilution with methanol for pure drug, tablet formulation and drug in plasma were developed and validated. The procedure was adopted as per designed protocol, based on ICH Q2B guidelines. The calibration curve was obtained by plotting absorbance vs analyte concentration. The slope and intercept of the calibration line was determined by linear regression.

Selectivity and specificity

The drug EFA in the formulation and the plasma was well identified under this condition. No interference observed in nine different plasma samples of EFA. Fig.3 and 4 showed a linear relationship between the absorbance and the concentration, with correlation coefficient and percentage estimated with standard deviation of 0.9993, 99.74 ± 1.22,respectively. The results are shown in Table 1 and 2.

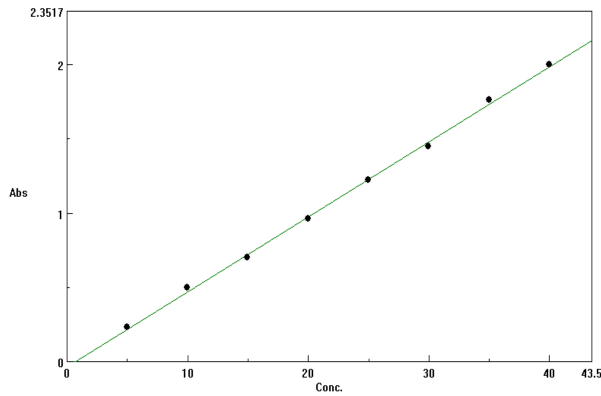

Figure 3: Calibration curve of Efavirenz showing linearity relationship

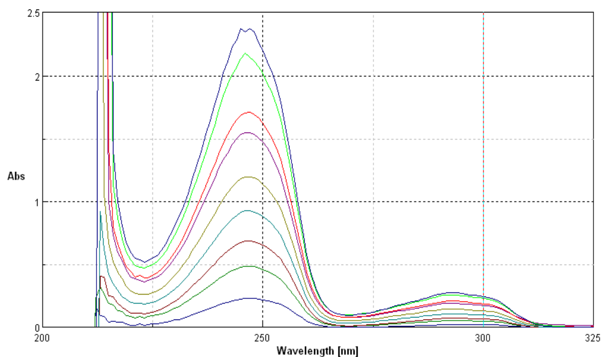

Figure 4: Overlain spectra of Efavirenz in methanol

Table 1: Linearity regression data for EFA

Parameters	Value for EFA
Beer's law limit (µg/ml)	5-40 µg /ml
Correlation coefficient	0.9993
Regression equation (Y*)	Abc=A+B*C
Slope (B)	0.0504
Intercept (A)	-0.0304

Table 2: Results of analysis of laboratory samples

Label claim (mg/tab)	% Concentration estimated* (Mean ± % R.S.D.)
600 mg	99.74 ± 1.22

* Average of nine determinations; R.S.D., Relative Standard Deviation

In Fig 5 and 6 the regression analysis of the calibration curve for efavirenz in plasma showed a linear relationship between the absorbance and the concentration, with correlation coefficient and percentage estimated with standard deviation of 0.9989, 99.69 ±1.22, respectively. The results are shown in table 3 and 4.

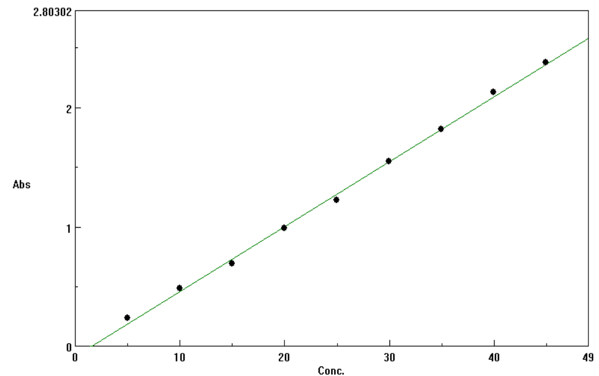

Figure 5: Calibration curve of Efavirenz in plasma showing linearity relationship

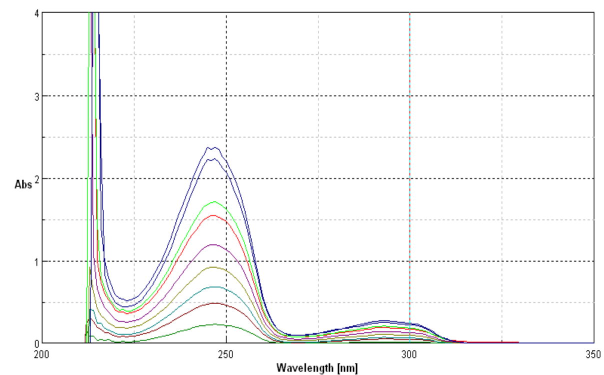

Figure 6: Overlain spectra of Efavirenz in plasma

Table 3: Linearity regression data for calibration curve of Efavirenz in plasma

Parameters	Value for EFA
Correlation coefficient	0.9989
Regression equation (Y*)	Abs = A+B x C
Slope (B)	0.0542
Intercept (A)	-0.0800

Table 4: Results of analysis of laboratory samples

Label claim (mg/tab)	% Concentration estimated* (Mean ± % R.S.D.)
600 mg/tab	99.68 ± 1.22

Recovery

As shown in Table 5 and 6 excellent recoveries were made at each added concentration.

Table 5: Recovery data for EFA

Level added (%)	Recovery (%)*	SD
80	98.88	0.7426
100	99.75	0.7823
120	97.77	0.5674

* Mean of three determinations

Table 6: Recovery data for EFA in plasma

Level added (%)	Recovery (%)*	SD
80	97.98	0.0844
100	98.39	0.4792
120	98.45	0.4888

* Mean of three determinations

Precision

Precision evaluated through intraday and inter day of the pure drug from solvent and plasma are presented in Tables 7, 8, 9 and 10 respectively.

Limit of detection (LOD) and Limit of quantification (LOQ)

The LOD determined as the amount of drug and LOQ was determined as the lowest concentration for drug shown in Table 11.

Table 11: Limit of detection and limit of quantitation for drug in solvent

LOD (µg/ml)*	LOQ (µg/ml)*
0.3135	0.9502

* Data obtained by nine determinations

Table 12: Lowest Limit of detection and lowest limit of quantitation for drug in plasma

LLOD (µg/ml)*	LLOQ (µg/ml)*
0.3615	1.2502

* Data obtained by nine determinations

Table 7: Results of intraday precision of efavirenz

Parameter	% Drug estimated* (Mean ± %R.S.D.)		
	5 µg/ml	**20 µg/ml**	**40 µg/ml**
Morning	98.30±0.68	99.90±0.48	99.45±0.17
Afternoon	99.92±0.83	99.62±0.23	101.02±0.61
Evening	99.62±1.20	100.02±1.32	99.22±1.03

*Average of nine determinations; R. S. D., Relative Standard Deviation

Table 8: Results of interday precision of EFA

Parameter	% Drug estimated* (Mean ± %R.S.D.)		
	5 µg/ml	**20 µg/ml**	**40 µg/ml**
Day 1	100.30±1.08	99.90±1.18	99.45±0.99
Day 2	101.02±1.33	101.02±0.96	98.02±0.98
Day 3	100.72±0.62	99.72±0.92	99.62±0.73

*Average of nine determinations; R. S. D., Relative Standard Deviation

Table 9: Results of intraday precision of efavirenz in plasma

Parameter	% drug estimated* (Mean ± %R.S.D.)		
	5 µg/ml	**20 µg/ml**	**40 µg/ml**
Morning	99.30±1.48	99.30±1.48	98.45±1.17
Afternoon	99.02±1.23	98.02±1.23	99.02±0.91
Evening	98.02±1.62	98.02±1.62	98.02±1.23

*Average of nine determinations; R. S. D., Relative Standard Deviation

Table 10: Results of inter day precision of efavirenz in plasma

Parameter	% drug estimated*(Mean ± %R.S.D.)		
	5 µg/ml	**20 µg/ml**	**40 µg/ml**
Day 1	98.87±1.78	99.38±1.18	101.45±1.17
Day 2	101.02±0.83	99.62±1.03	101.02±0.67
Day 3	99.72±1.02	99.09±0.92	99.02±1.73

*Average of nine determinations; R. S. D., Relative Standard Deviation

Lowest Limit of detection (LLOD) and Lowest Limit of quantification (LLOQ)

The LLOD determined as the lowest amount of drug and LLOQ was determined as the lowest concentration for drug in plasma shown in Table 12.

CONCLUSION

A spectrophotometric method for quantifying EFA in tablet and plasma has been developed and validated. The method is selective, precise, accurate and linear over the concentration range studied. The method is simple and suitable for the determination of EFA in formulation and plasma without interference from excipients or from common degradation products, suggesting its application in IPQC and pharmacokinetic studies.

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