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Quantitative determination of Efavirenz in bulk drug and formulation by colorimetry

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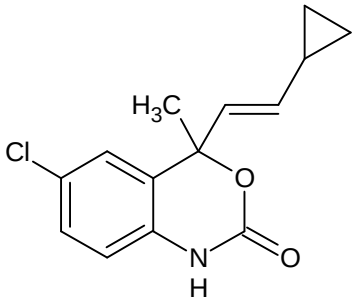
ABSTRACT

A simple fast and reproducible spectrophotometric method has been developed for the determination of Efavirenz in pure and tablet dosage forms using β - naphthol reagent. The method is based on the reaction of Efavirenz with sodium nitrite and HCl, form N – nitroso compound, which react with β – naphthol and measuring the resulting low colour complex at 561nm under the prepared optimum conditions. Beer's law was obeyed at the concentration range of 10-20µg/ml. The method was validated according to ICH guidelines by performing linearity, accuracy, precision, limit of quantification, limit of detection and selectivity. The recovery study was carried out by standard method and good results were obtained for Efavir and Sustiva.

Key words: Efavirenz [EFA], Spectrophotometric, β - naphthol

INTRODUCTION

Efavirenz belongs to the class of the non-nucleoside reverse transcriptase inhibitors. Efavirenz exerts its action by non-competitive inhibition of the human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. HIV-2 reverse transcriptase and human cellular DNA polymerases are not inhibited by Efavirenz.



Molecular structure of Efavirenz

Efavirenz is metabolized by the cytochrome (CYP) P450 isoenzymes 3A4 and 2B6 and induces metabolizing enzymes, resulting in induction of its own metabolism [1].

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. 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. 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 in-trinsic resistance to the NNRTI class [2].

Extensive literature survey reveals that several analytical methods have been reported for the quantitative determination of efavirenz which includes, Spectrophotometry[1],HPLC-UV[2], HPL[3], HPTLC[4], HPLC/MASS Spectrometry[5],UPLC-MS/MS[6] and HPLC with post-column photochemical derivatization and fluorescence detection[7,8].

However, no colorimetric method is reported yet. In the present study an attempt has been made to develop simple, sensitive and economical method in visible region with greater precision and accuracy for the estimation of EFA in pure drug and tablet formulation. This method can be adopted for routine analysis in pharmaceutical industry.

MATERIALS AND METHODS

Chemicals and reagents

Ethanol was used for the quantification of efavirenz which was procured from Sigma Aldrich. EFA film coated tablet of strength 600 mg Sustiva (Bristo-Myers Squibb-Germany) and Efavir (Cipla Ltd-India) were procured from the market. The pure sample of efavirenz for the research work was procured from Shasun Pharmaceuticals Ltd, Pondicherry. Hydrochloric acid, sodium hydroxide, and β – naphthol were purchased from Sigma Aldrich. All the chemicals and reagents for the development of new analytical method to estimate efavirenz was of analytical grade.

Preparation of β – naphthol reagent

Accurately 1g of β – naphthol was weighed and transferred to 100ml volumetric flask. To it 30ml of 0.1N NaOH was added and shacked such that β – naphthol gets completely dissolved in NaOH. Make up the volume to 100ml with 0.1N NaOH.

Preparation of Efavirenz standard solutions

100mg of the drug was weighed and transferred in to 100ml volumetric flask, to it ethanol was added and volume was made up to 100ml with ethanol. From this 10ml was transferred to 100ml volumetric flask and volume was made up to 100ml with ethanol such that the solution gives the concentration of $100\mu g/ml$.

Preparation of reagent blank

The reagent blank was prepared in the same manner as discussed, omitting the standard drug solution.

Procedure for the analysis of tablet formulation

Twenty tablets of Efavirenz (600mg) and Sustiva were purchased from local market. Accurately weighed and finely powdered. The weight of the tablet equivalent to 100mg from each brand was accurately weighed and extracted with ethanol. The yellow coloured complex (**Scheme-1)** having final concentration of 100mg/ml of each brand was prepared. The absorbance of the solutions was measured at 561nm using reagent blank.

RESULTS AND DISCUSSION

Selection of analytical wavelength:

Appropriate dilutions were prepared for drug from the standard stock solution and the solutions were scanned in the wavelength range of 400 - 800 nm. Absorption maxima were found to be 561nm which was selected as wavelength of analytical measurement for this method.

Calibration curve

Appropriate volume of aliquots from standard Efavirenz stock solution II were transferred to different volumetric flasks of 10 ml capacity. To all the volumetric flasks 1ml of 1% sodium nitrite, 1ml of HCl and finally β $naphthol$ was added. The volume was adjusted to the mark with ethanol to obtain concentrations of 10, 12, 14, 16, 18 and $20\mu g/ml$. Absorbance of each solution blank was measured at 561 nm and the graph of absorbance against concentration was plotted and is shown in **Figure 1.** The regression equation and correlation coefficient were determined.

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures [9,10,11].

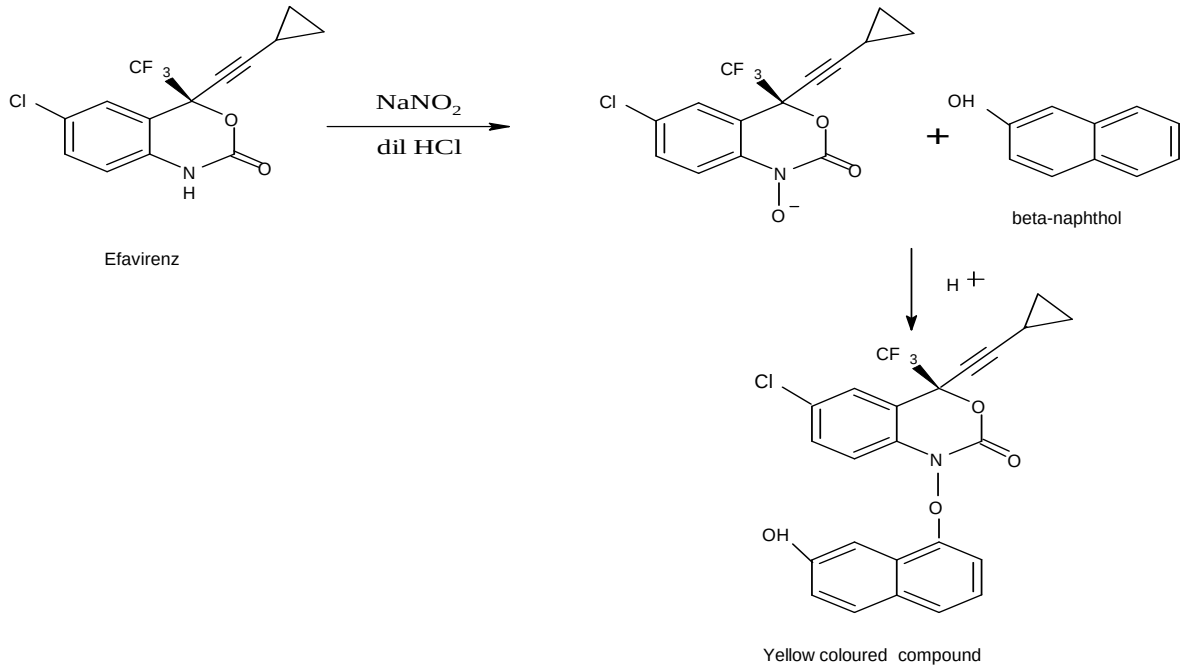
Efavirenz has the absorbance maxima at 561 nm for the first order derivative visible-Spectrosphotometric method. The optical characteristics such as Beer's limits, Limit of detection and Limit of quantification etc., in each method were calculated and the results were presented in **Table 1** respectively. The regression characteristics like slope (b), intercept (a), and correlation coefficient (R_2) using the method of least squares were calculated and presented in **Table 1** respectively. The results showed that the methods have reasonable precise.

The validation parameters like Precision and Accuracy results were presented in **Table 2** and **3** for Visible Spectrophotometric method. There was no interference of excipents in recovery study of the method. From results, the method found applicable for both bulk and pharmaceutical dosage for the estimation of Efavirenz.

Recovery studies

The recovery studies were carried out at three different level i.e. 80,100 and 120%. It was performed by adding known amount of standard drug solutions of Efavirenz to preanalysed tablets solutions. The resulting solutions were then reanalyzed by proposed methods. The results of recovery studies are shown in **Table 4** and **5.**

Scheme-1:



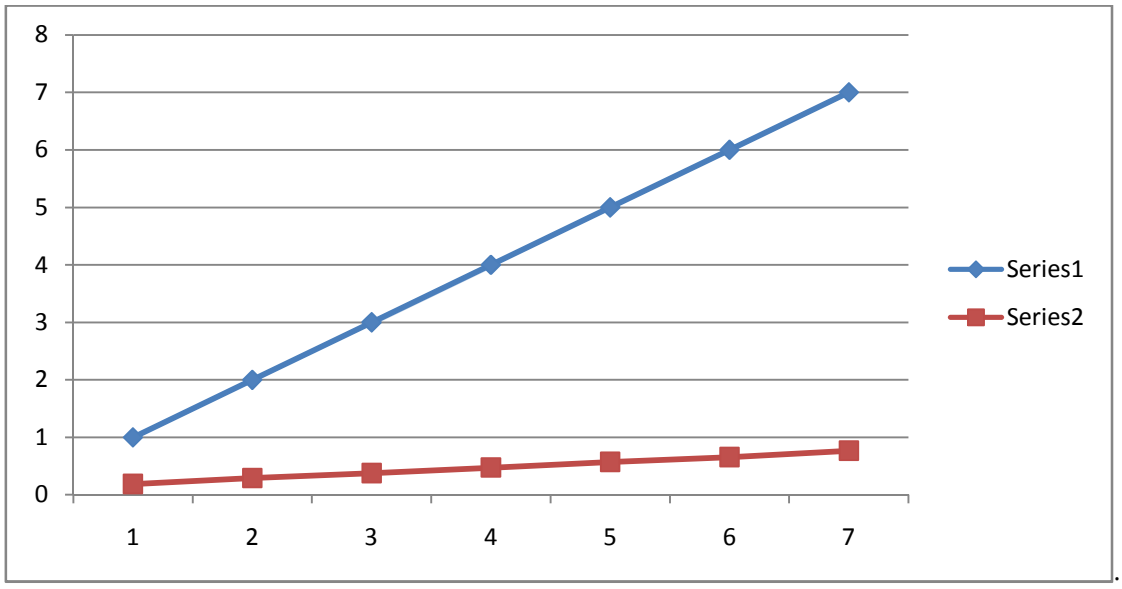


Figure no. 1- Calibration curve

Table No .1 - Optical characteristic

1	Detection wavelength (nm)	561
2	Beer's law limits (µg/ml)	10-20
3	Regression equation (y*)	y = 0.038 x - 0.001
4	Slope (b)	0.038
5	Intercept (a)	0.001
6	Correlation coefficient(R2)	0.999
7	Limit of Detection (µg/ml)	0.078
8	Limit of Quantification	0.236

Table No. 2 - Determination of Accuracy results for Efavirenz

Amount of sample (µg/ml)	Amount of drug added (µg/ml)	Amount Recovered(µg/ml)	% Recovery ± SD
16	12	11.98	99.78±0.46
16	16	15.89	99.08±0.95
16	20	19.99	99.97±0.11

Table No.3 - Determination of precision results for Efavirenz

Concentration (µg/ml)	Intra-day absorbance Mean ± SD	%RSD	Inter-day absorbance Mean ± SD	%RSD
12	**0.227±0.0008**	0.394	0.227±0.0010	0.461
16	**0.458±0.0008**	0.178	0.459±0.0008	0.194
20	**0.697±0.0037**	0.541	0.698±0.0012	0.173

Table No.4- Results of recovery studies for Efavirenz

Amount Of Sample(*µ*g/ml)	Amount of drug added (*µ*g/ml)	% Recovery ± RSD	%RSD
16	8	99.10±0.41	0.41
16	10	99.58±0.60	0.64
16	12	98.80±0.88	0.89

Table No. 5- Results of recovery studies for Sustiva

Amount Of Sample(*µ*g/ml)	Amount of drug added(*µ*g/ml)	% Recovery ± RSD	%RSD
16	8	98.88±0.70	0.74
16	10	99.75±0.74	0.78
16	12	98.80±0.51	0.56

CONCLUSION

A colorimetric method for quantifying Efavirenz in bulk drug and formulation 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 Efavirenz in bulk drug and in formulation without interference from excipients.

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