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Development of analytical method for efavirenz by UV spectrophotometry using sodium hydroxide as solvent

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ABSTRACT

The main aim of the present study is to develop a simple, sensitive, specific, spectrophotometric method developed for the detection of Efavirenz in bulk drug and pharmaceutical formulation. The optimum condition for the analysis of the drug was established. The wavelength (λmax) of the Efavirenz was found to be 267nm. The proposed method can be performed by using UV-Visible spectrophotometry using 0.5N NaOH as solvent. The regression coefficient of the linearity study was found to be 0.9918, and the slope of the linear is y=0.0738x. This method shows the linearity of 2 to 12µg/ml. Limit of detection was found to be 0.454µg/ml and the Limit of quantification was determined as the lowest concentration was found to be 1.499µg/ml. The proposed method will be suitable for the analysis of Efavirenz in bulk and pharmaceutical formulation.

Key words: Efavirenz, Spectrophotometry, Estimation.

INTRODUCTION

Efavirenz (EFA) is belonging to the chemical class of non-nucleoside reverse transcriptase inhibitor (NNRTI) and chemically it is (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one,with molecular formula $C_{14}H_9ClF_3NO_2$ was presented in figure 1. Efavirenz is a white to slightly pink crystalline powder with a molecular mass of 315.675g/mol. It is practically insoluble in water. EFA is used as a part of highly active anti-retroviral (anti-viral) therapeutically for the treatment of human immunodeficiency virus (HIV). The drug is used in combination with other anti-retroviral agents for the treatment of HIV-1 infection in children and adults. The trade name of EFA is Efavir (CIPLA), and the usual dose of EFA in film coated tablet is 600 mg/day and capsules is 200mg. Its plasma half-life is ~50 h [1,2]. Some example for combinations of EFA is lazid-E (EMCURE), Zidovudine 300mg, Lamivudine 150mg, Efavirenz 600mg. 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 [3]. However, more than 50% of patients starting EFA treatment experience its related neuropsychiatric adverse events (NPAEs), such as dizziness, feeling of drunkenness and sleep disorders and even severe psychiatric symptoms have been reported by EFA [4]. Several analytical methods of EFA were reported including UV spectrometry [5] LCMS, HPLC [6, 7] .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 and in tablet formulation.

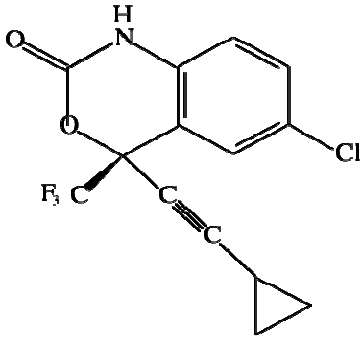

Figure 1: Structure of Efavirenz (EFA)

MATERIALS AND METHODS

Reagent & Materials:
Efavirenz working standard was kindly supplied by APEX Pharmaceutical Private Ltd. Efavir ® of EFA drug was procured from local pharmacies and all other chemicals used in the analysis were AR grade.

Apparatus:
Ultra Violet-Visible Spectrophotometer, of Perkin Elmer (USA) was used for detection of absorbance, Electronic balance (precisa 92sm-202A) and Sonicater (Branson 2510) were used for the study.

METHOD
Preparation of Stock solution:
About 500 mg of pure drug was weighted accurately and transferred into 250ml standard volumetric flask. 50ml of NaOH was added to solubilize the drug and then it was sonicated for 5min to ensure the drug was completely dissolved in the solvent. Then required volume of NaOH is added to dilute to up to 250ml.

Selection of solvent:
The ideal property of a solvent should be that the drug is completely soluble in it. The drug should be stable in the solvent used and must be economical and non-volatile. After literature survey, practical experience and taking above factors into consideration sodium hydroxide (NaOH) was selected as suitable solvent. The range of strength for NaOH selected was from 0.1N to 0.8N. Because of its low absorbance value with 0.1N to 0.4N NaOH, method was carried out with 0.5N NaOH which is cost effective and best at its response.

In our previous study for the detection of EFA by Ultra Violet-Visible spectrometry [5] methanol has been used as a solvent which is costlier than aqueous sodium hydroxide. Since the initial stage of development we have used methanol and later studies precede that including stability studies with sodium hydroxide (0.5N) has been used for the study and confirmed. For the estimation of EFA, single wavelength spectrophotometric method at the wavelength of 267nm was used.

Linearity studies:
About 25mg of pure drug was weighed accurately and transferred into 25ml standard volumetric flask. To that 10ml of 0.5N NaOH was added and sonicated for 5min. Then the volume was made with 0.5N NaOH and mixed well. The above solution in referring as a standard stock solution. Further dilution was made to get the concentration of 2, 4, 6, 8, 10, 12µg/ml. Then the sample solution was demonstrated out by UV-Vis spectrophotometer at 267nm.

Precision:
Thirty tablets were weighed and its average weight was calculated. The tablets were triturated into a fine powder using a glass mortar. Weight of powder equivalent to 25mg of EFA was taken and transferred into a 25ml standard volumetric flask and about 15ml of 0.5N NaOH was added and subjected to sonication for 5min. The solution was diluted with 0.5N NaOH to reach the standard mark. The above solution was filtrated using whatmann filter paper

number 4. From the above filtrate, 5ml was pipetted out and transferred into the 50ml standard volumetric flask, volume was made up with 0.5N NaOH. 1ml of the above solution was taken and transferred to 25ml standard volumetric flask and diluted with 0.5N NaOH up to 25ml of total volume, so that the concentration of the final solution will be 4µg/ml. The absorbance was measured by using UV-Vis spectrophotometer at 267nm.

LOD and LOQ:
The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations- LOD = 3.3/Sσ; LOQ = 10/Sσ
Where s, the noise of estimate, is the standard deviation of the absorbance of the sample and m is the slope of the related calibration graphs. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability [8-10].

Stability test:
The stability of the sample solution was carried out for 24 h to ensure whether the solvent and drug interaction, which lead to deterioration subsequently affect, the absorbance spectra (λmax) has been determined.

Recovery studies
Recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy or the bias of the method. The same range of concentrations, as employed in the linearity study was used. To study the accuracy or reproducibility of the proposed method, the dosage form, and recovery experiment were carried out using the standard addition method. These studies were performed by the addition of known amounts of pure EFV to the pre-analyzed tabled formulation and the mixtures were analyzed using the proposed techniques. After parallel analyses, the recovery results were calculated using the related calibration equations. Recovery study was carried out at three different concentration levels 50%, 100%, and 150% overages. Absorbance is measured maximum at the wavelength 267nm.

RESULTS AND DISCUSSION

The wavelength of the drug EFA in sodium hydroxide has been detected by using the single wavelength spectrophotometric method employing 248nm as wavelength. A schematic representation of the spectrum has been presented in figure 2.

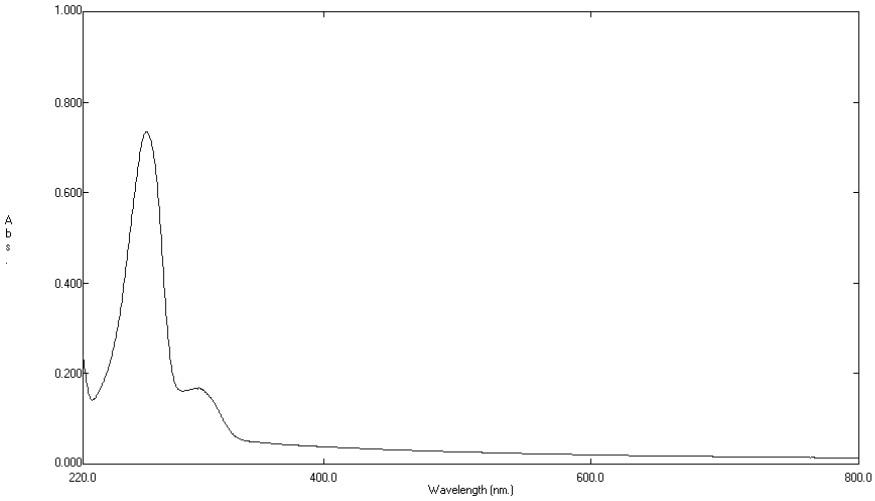

Figure 2: Spectrum of EFA in sodium hydroxide

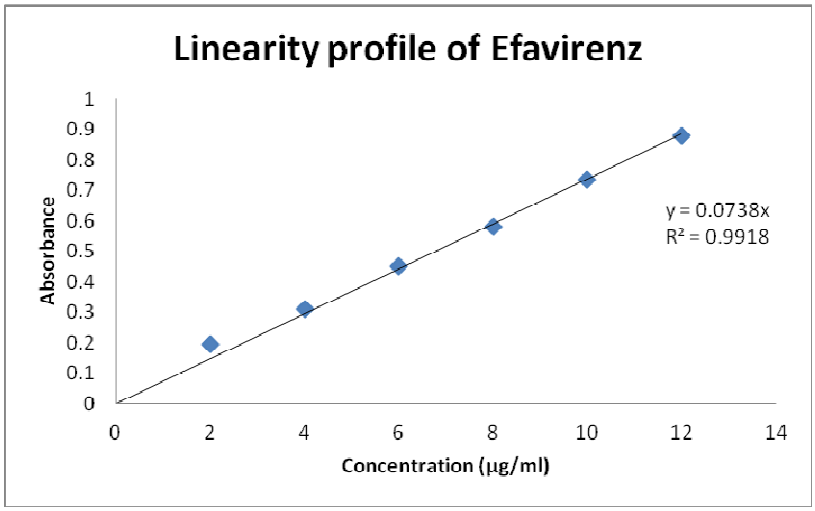

Figure 3: Linearity profile of EFA

Linearity studies:
The linearity study was carried out from 2 to 12µg/ml and the observed values were presented in Table 1.

Table 1: Linearity profile of Efavirenz

S.No	Concentration (µg/ml)	Absorbance
1	2	0.196
2	4	0.309
3	6	0.450
4	8	0.580
5	10	0.734
6	12	0.880

The linearity graph was plotted against the absorbance and the concentration as shown in the (figure 3). The slope was determined by linear regression using the least squares method. The values in table 1 and (figure 3) showed a linear relationship between the absorbance of Efavirenz and the concentrations. The graph clearly showed that slope line passes through origin and touches almost all points (different concentration) and the absorbance was measured at particular wavelength 267nm (λmax) in 0.5N NaOH. The regression coefficient of the linearity study was found to be 0.9918. The slope of the linearity is y=0.0738x.

Precision studies:
The precision study was carried out and the values of individual determination was calculated and presented in Table 2. The precision (repeatability) study shows that there is no significant difference in the precision values; hence the developed method can be used to analyze the EFA in tablet formulation. It was found to be that there is no evidence of interference of excipients with EFA. The analysis of the study lies between 98.40% and 102.51%. It shows that only 2% deviation and that is allowed in the any of tablet dosage form. The mean value of the precision study is 100.27%.

Table 2: Analysis of Tablet Formulation

S. No	Weight of powder(mg)	Absorbance	Weight of drug content(mg)	Amount detected (mg)	% Content
1	46.9	0.326	25.05	24.7	98.60%
2	46.8	0.324	25.0	24.6	98.40%
3	48.8	0.338	25.05	25.7	102.51%
4	48.5	0.336	24.94	25.5	102.24%
5	47.2	0.327	25.15	24.8	98.61%
6	47.9	0.332	24.89	25.2	101.25%
	Mean		25.01	25.08	100.27%
	SD		0.083808	0.413993	0.017748

Recovery Studies:

Recovery study for Efavirenz was carried out with 0.5N NaOH at 50%, 100% and 150% concentration. The results were shown in the Table 3. From the above data drug-drug interaction, drug-excipients interaction, and drug solvent interactions has not been noticed or identified. Hence, there is no interference of any component with the drug has been proved.

Table 3: Recovery for Efavirenz (50,100,150%)

S. No	Level Added (%)	Drug content in tablet powder (mg)	Pure drug added (mg)	Total content (mg)	Absorbance	Amount of drug recovered (mg)	% of drug recovered	Average
1	50%	25.35	12.5	37.85	0.252	36.93	98.5%	
2	50%	25.15	12.5	37.65	0.259	38.21	101.5%	99.73%
3	50%	25.20	12.5	37.70	0.253	37.39	99.2%	
4	100%	25.20	25.0	50.20	0.325	49.69	99%	
5	100%	25.15	25.0	50.15	0.332	50.65	101%	98.33%
6	100%	25.20	25.0	50.20	0.322	49.196	98%	
7	150%	25.65	37.5	63.15	0.364	64.41	102%	
8	150%	24.45	37.5	61.50	0.351	60.51	98.4%	100.56%
9	150%	25.15	37.5	62.65	0.361	63.46	101.3%	

Stability study:

Stability study of the sample solution was carried out for 24h. The results pronounces that Efavirenz test sample solution with sodium hydroxide used for method development is mostly un deteriorated and was stable even after 24h. It can be inferred that the sample solution can be analyzed within 24h, even any other interference happened in the due time of work.

CONCLUSION

A spectrophotometry method for quantifying Efavirenz in formulation has been developed and validated as per ICH guidelines. The developed method is selective, precise, accurate, economically cheap and linear over the concentration range from 2µg/ml to 12µg/ml in the solvent of 0.5N NaOH .The LOD and LOQ profile for the EFA in NaOH were found to be 0.454µg/ml and 1.494µg/ml. The developed method is simple, suitable and cost effective for the determination of Efavirenz in bulk and as well as pharmaceutical formulation.

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