DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE ESTIMATION OF EFAVIRENZ IN BULK AND PHRMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, specific, accurate and precise First order derivative UV Spectrophptometry method was developed and validated for the estimation of Efavirenz in bulk and pharmaceutical dosage forms. The stock solution was prepared by weighing 100 mg of standard EFV in 100 ml volumetric flask with methanol and water (50:50) (Stock solution I). The final stock solution was made to produce 100μg/ml with methanol and water (50:50).Further dilutions were prepared as per procedure. The first derivative amplitude at 238.50 nm was selected for the assay. The linearity was found in the concentration range of 3-18 µg/ml. The Correlation coefficient was 0.999. The regression equation was found to be y = 0.038 x - 0.001. The method was validated for linearity, sensitivity, precision, accuracy and ruggedness. The limit of detection and limit of quantification for estimation of EFV was found to be 0.078μg/ml and 0.236 µg/ml, respectively. Recovery of EFV was found to be in the range of 99.08-99.97 %. Proposed method was successfully applied for the quantitative determination of EFV in bulk and pharmaceutical dosage forms. These methods were tested and validated for various parameters according to ICH guidelines. The proposed methods were successfully applied for the determination of EFV in capsule formulations. This method was successfully applied to the pharmaceutical dosage form and there no interference of capsule excipients was found in recovery study.

Key words: Efavirenz (EFV)**,** ICH (International Conference on Harmonization), and UV-Spectrophotometric method.

INTRODUCTION:

Efavirenz [1, 2] (dideoxyinosine, DDI) is an oral nucleoside reverse transcriptase inhibitor (NRTI). It is a synthetic purine derivative and, similar to zidovudine, zalcitabine, and stavudine. Efavirenz was originally approved specifically for the treatment of HIV infections in patients who failed therapy with zidovudine. Currently, the CDC (Centers of Disease Control) recommends that Efavirenz be given as part of a three-drug regimen that includes another nucleoside reverse transcriptase inhibitor (e.g., lamivudine, stavudine, and zidovudine) and a protease inhibitor or efavirenz when treating HIV infection. It is used as part of highly active antiretroviral therapy (HAART) for the treatment of a human immunodeficiency virus (HIV) type 1. The chemical name ofEfavirenz(4S)-6-chloro- 4-(2-cyclopropylethynyl) -4-(trifluoromethyl) -1H-3,1benzoxazin-2-one. It has the structural formula shown in (Figure 1).
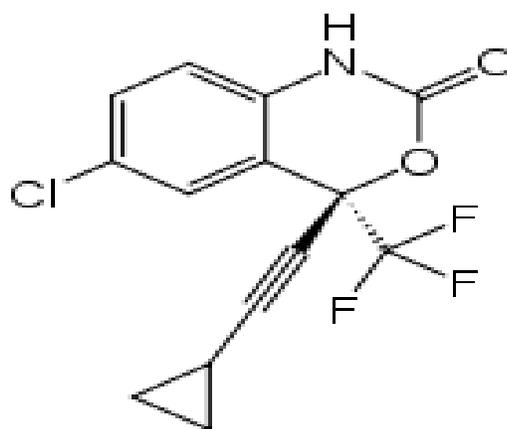
Figure 1: Chemical Structure of Efavirenz.

Efavirenz is white or almost white powder. It is practically insoluble in water; it is soluble in methanol, ethanol, isopropyl alcohol, acetonitrile, phosphate buffer, acetate buffer etc. It is commercially available in the form of oral tablets and capsules of different strengths. From the literature survey, it was found that Efavirenz estimated by analytical methods such as reversed-phase high-performance liquid chromatographic (RP-HPLC) method [3], UV-Spectrophotometric method for EFV in plasma and urine [4], and liquid chromatographic-mass spectrometric (LC-MS) method [5]. Apart from the above no other methods such as UV- Spectroscopic method was reported for the quantitative determination of Efavirenz in pharmaceutical dosage forms. The developed method was simple, precise, specific and accurate. The statistical analysis proved that method is reproducible and selective for the analysis of Efavirenz in bulk drug and capsule formulations.

MATERIALS AND METHODS

Instruments and reagents:

An analytically pure sample of Efavirenz was procured as for the research work will be gifted by Cipla Pharmaceutical, Goa. Analytical grade methanol and water was used as solvent for dilution. A Shimadzu UV-1800 UV/VIS spectrophotometer was used with 1 cm matched quartz cell.

Preparation of working standard drug solution:

100 mg standard Efavirenz [EFV] was weighed and transferred to a 100 ml volumetric flask and dissolved in methanol and water (50:50). The flask was shaken and volume was made up to the mark with methanol and water (50:50) to give a solution of 1000 µg/ml (Stock solution I). From this stock solution I, 10 ml solution was pipetted out and placed into 100 ml volumetric flask. The volume was made up to mark with methanol and water to give a solution containing 100 µg/ml (Stock solution II).

Analysis of marketed formulations:

One brand of capsule (Efavir *Cipla* 200mg) was used for all analytical study. Twenty capsules each containing 200 mg of EFV was weighed. The powder equivalent to 100 mg of EFV was accurately weighed and transferred to volumetric flask of 100 ml capacity containing 50 ml of the methanol and water (50:50) and sonicated for 10 min. The flask was shaken and volume was made up to the mark with methanol and water (50:50) to give a solution of 1000 µg/ml (Stock solution I). The above solution was carefully filtered through Whatmann filter paper (No. 41). From this solution, 10 ml was taken and diluted to 100 ml with methanol and water (50:50) to give a solution of 100 µg/ml(Stock solution II). Appropriate volume of aliquots from stock solution II were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with methanol and water (50:50) to obtain concentrations of 3, 6, 9, 12, 15, and 18µg/ml.

Validation of Spectrophotometric method:

For the first order derivative UV-Spectroscopy method the following validation parameters were studied.

1) Linearity,

2) Precision,

3) Accuracy,

4) Ruggedness.

RESULT 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 200 - 400 nm. Absorption spectra obtained were derivatives to first order derivative spectra with $\Delta\lambda=8$. First order derivative spectra were showing the absorption maxima [λ_{max}] and minima [λ_{min}] at 238.50 nm and 211.5 nm, respectively. Out of these two wavelengths, 238.50 nm was selected as wavelength of analytical measurement for this method.
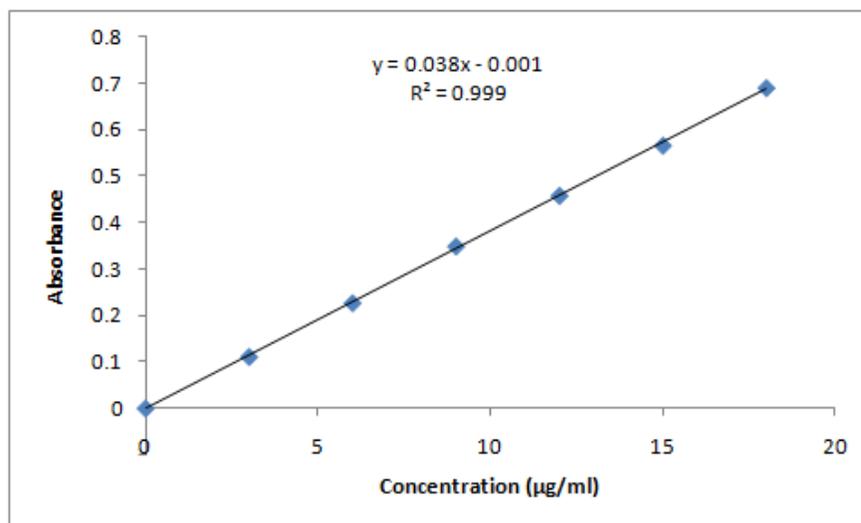
Calibration curve:

First order derivative UV-Spectrophotometric method:

Appropriate volume of aliquots from standard EFV stock solution II were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with methanol and water (50:50) to obtain concentrations of 3, 6, 9, 12, 15, and 18 µg/ml. Absorbance of each solution against methanol and water (50:50) as blank were measured at 238.50 nm and the graph of absorbance against concentration was plotted and is shown in **Figure 2, 3.**The regression equation and correlation coefficient were determined which are presented in **Table: 1.**
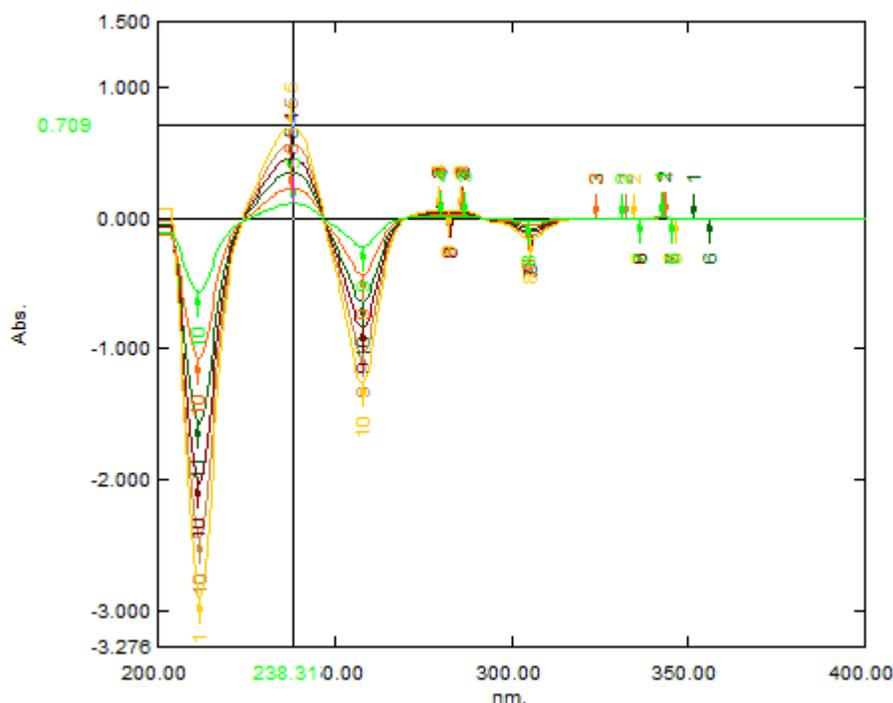
Figure 2: First order Spectra of EFV at 238.50 nm.

Figure 3: Calibration curve of EFV at 238.50 nm by First order Derivative Spectroscopy.

Table: 1 Optimum conditions, Optical characteristics and Statistical data of the Regression equation in First order Derivative Spectroscopy.

Parameters	First order Derivative Spectroscopy
Detection wavelength (nm)	238.50
Beer's law limits (µg/ml)	3 – 18
Molar extinction coefficient (L mol^{-1} cm^{-1})	0.038×10^3
Sandell's sensitivity (µgcm^{-2} / 0.001 absorbance unit)	0.0262
Regression equation (y*)	y = 0.038 x - 0.001
Slope (b)	0.038
Intercept (a)	0.001
Correlation coefficient(R^2)	0.999
Limit of Detection (µg/ml)	0.078
Limit of Quantification (µg/ml)	0.236

* y = b x + a where x is the concentration of EFV in µg/ml and y is the absorbance at the

respective wavelength.

The first order derivative UV-Spectroscopy method is advantageous as it is applicable to the drug which shows the broad spectra without a sharp peak.

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures [6, 7, 8].

Efavirenz has the absorbance maxima at 238.50 nm for the first order derivative UV-Spectroscopy method. The optical characteristics such as Beer's law limits, Molar absorptivity, Sandell's sensitivity, Limit of detection and Limit of quantification etc., in each method were calculated and the results were presented in **Table: 1** respectively. Also the regression

characteristics like slope (b), intercept (a), and correlation coefficient (R^2) using the method of least squares were calculated and were presented in **Table: 1** respectively. The results showed that the methods have reasonable precise.

The validation parameters like Precision, Accuracy, and Ruggedness results were presented in **Table: 2, 3, 4** and **5** for first order derivative UV-Spectroscopy method. There was no any interference of excipents in recovery study of the method. From the results these methods was applicable for both bulk and pharmaceutical dosage for the estimation of Efavirenz.

Table: 2 Determination of Accuracy results for EFV by First order Derivative Spectroscopy.

Amount of sample (µg/ml)	Amount of drug added (µg/ml)	Amount Recovered* (µg/ml)	% Recovery ± SD*
Capsule 1 (EFAVIR)			
12	6	5.98	99.78±0.46
12	12	11.89	99.08±0.95
12	18	17.99	99.97±0.11

*Average of six determinations.

Table: 3 Determination of System Precision results for EFV at 238.50 nm by First order Derivative Spectroscopy.

Concentration (µg/ml)	Intra-day absorbance Mean ± SD*	%RSD	Inter-day absorbance Mean ± SD*	%RSD
6	0.227 ± 0.0008	0.394	0.227 ± 0.0010	0.461
12	0.458 ± 0.0008	0.178	0.459 ± 0.0008	0.194
18	0.697 ± 0.0037	0.541	0.698 ± 0.0012	0.173

*Average of six determination.

Table: 4 Determination of Method Precision results for EFV at 238.50 nm by First order Derivative Spectroscopy.

Label claim(mg) Cap.1	Intra-day			Inter day		
	Amount found*	% Label claim*	% RSD	Amount found*	% Label claim*	% RSD
200	199.70	99.85	0.600	199.56	99.78	0.748

* Average of six determinations. Capsule.1: Efavir

Table: 5 Ruggedness results for EFV at 238.50 nm by First order Derivative Spectroscopy.

Sample	Label claim (mg)	Analyst I		Analyst II	
		Amount found*(mg)	(%) Recovery ± SD*	Amount found* (mg)	(%) Recovery ±SD*
Cap. 1	200	199.70	99.85 ± 0.59	197.80	98.90 ± 0.31

* Average of six determinations. Capsule 1: Efavir

CONCLUSION:

The developed and validated First order derivative UV-Specctrophotometry method reported here is rapid, simple, accurate, sensitive, and specific. This method was validated as per ICH guidelines and results of accuracy, precision, ruggedness was in the limit. There was no any interference of excipients in the recovery study. The method was also successfully used for quantitative estimation and analysis of efavirenz from formulation. Thus, the reported method is of considerable importance and has great industrial applicability for quality control and analysis of efavirenz from bulk drug and formulations.

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