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Spectroscopic Determination of Efavirenz in Bulkand Pharmaceutical Dosage Form

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Abstract: A simple, selective, linear, precise and accurate spectroscopic method was developed and validated for rapid assay ofEfavirenzin Pharmaceutical Dosage form. The method has been developed and validated for the assay of Efavirenz using 0.1N NoaH as diluent. A sample of API was dissolved in 0.1N NaoH to produce a solution containing 40 µg/mL of Efavirenz. Similarly, a sample of ground tablets were extracted with 0.1N NaoH centrifuged and diluted with the same solvent. The absorbance of the sample preparation was measured at 218 nm against the solvent blank and the assay was determined by comparing with the absorbance of a similarly prepared 40 µg/mL standard solution of Efavirenz. The calibration graph was rectilinear from 14 µg/mL to 70 µg/mL for Efavirenz with the correlation coefficient being more than 0.998. The percent recovery was within the range of 98%–102%, indicating insignificant interference from the other ingredients in the formulation. The method can be applied for the routine QC quantitation of Efavirenz in API and tablet formulation.

Key words: Efavirenz · 0.1N NaoH · UV detection · Recovery

INTRODUCTION

Spectroscopy Methods [1, 2]: It is the branch of science dealing with the study of interaction between Electromagnetic radiation and matter. It is a most powerful tool available for the study of atomic and molecular structures and is used in the analysis of wide range of samples. Optical spectroscopy includes the region on electromagnetic spectrum between 100 Å and 400 nm. The regions of electromagnetic spectrum are shown in Table 1.

Ultraviolet-Visible Spectrophotometry [3]: UV-Visible spectrophotometry is one of the most frequently employed technique in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers.

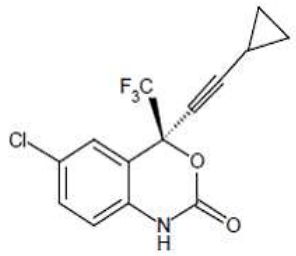
Introduction to Efavirenz: Efavirenz belongs to a class (group) of HIV drugs called non-nucleoside reverse transcriptase inhibitors (NNRTIs). NNRTIs attach to and block an HIV enzyme called reverse transcriptase.

Table 1: Linearity

S No.	Concentration(ppm)	Absorbance
1.	14	0.69
2.	28	1.12
3.	42	1.67
4.	56	2.13
5.	70	2.68
Slope		0.499
Intercept		0.161
Correlation Coefficient		0.998

(An enzyme is a protein that starts or increases the speed of a chemical reaction.) By blocking reverse transcriptase, NNRTIs prevent HIV from multiplying and can reduce the amount of HIV in the body(4, 5).

Efavirenz is chemically described as (S)-6-chloro-4(cyclopropylethynyl)-1, 4-dihydro-4-(trifluoromethyl)-2H-3, 1-benzoxazin-2-one. Its empirical formula is $C_{14}H_9ClF_3NO_2$ and its structural formula is:



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Description: Efavirenz is a white to slightly pink crystalline powder with a molecular mass of 315.68. It is practically insoluble in water.Efavirenz (brand names Sustiva® and Stocrin®) is a 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.

Efavirenz is also used in combination with other antiretroviral agents as part of an expanded post exposure prophylaxis regimen to prevent HIV transmission for those exposed to materials associated with a high risk for HIV transmission (6).

Mechanism of Action: Efavirenz inhibits the activity of viral RNA-directed DNA polymerase (i.e., reverse transcriptase). Antiviral activity of efavirenz is dependent on intracellular conversion to the Activetriphosphorylated form. The rate of efavirenz phosphorylation varies, depending on Cell type. It is believed that inhibition of reverse transcriptase interferes with the generation of DNA copies of viral RNA, which, in turn, are necessary for synthesis of new virions. Intracellular enzymes subsequently eliminate the HIV particle that previously had been uncoated and left unprotected, during entry into the host cell. Thus, reverse transcriptase inhibitors are virustatic and do not eliminate HIV from the body. Even though human DNA polymerase is less susceptible to the pharmacologic effects of triphosphorylatedefavirenz, this action may nevertheless account for some of the drug's toxicity (7). Literatures review reveals that the various analytical method like HPLC-UV method for the simultaneous quantitation Liquid chromatographic [8], solid-phase extraction and liquid chromatographic method [9], liquid-liquid extraction using LC-MS/MS with electrospray ionization [10]. The present groups of authors have already reported UV Method development different pharmaceutical dosage form [11-16].

ICH Guidelines (ICH Q2R1) for Analytical Procedure and Validation [17]: The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formula for the calculation, etc.

Types of Analytical Procedures to Be Validated: The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests
- Quantitative tests for impurities' content;
- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

- Accuracy
- Precision
- Repeatability
- Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range

Furthermore revalidation may be necessary in the following circumstances:

- Changes in the synthesis of the drug substance;
- Changes in the composition of the finished product;
- Changes in the analytical procedure.

Aim of Present Work: This work deals with the validation of the developed method for the assay of Efavirenz from its dosage form (tablets). Hence, the method can be used for routine quality control analysis and also stability.

The aim and scope of the proposed work are as under:

- To develop suitable spectrophotometric method for assay of Efavirenz tablet.
- Perform the validation for the method.

MATERIAL AND METHODS

Instrumentation and Reagents: Perkin Elmer UV-visible spectrophotometer equipped with a matched quartz

cells ultrasonic bath was used to carry out the assay. The solvent used for the assay was spectroscopic-grade methanol. Efavirenz working standard was supplied by Aurabindopharma Ltd. Marketed sample for the analysis which bought from local pharmacies sustiva (600mg/tablet) was manufactured by Bristol-Myers Squibb Pharmaceutical Ltd. All other chemicals used in the analysis were AR grade.

Evaluation of Wavelength: About 40 µg/mL of Efavirenz drug substance was accurately prepared in 0.1N NaoH as solvent. This preparation was then scanned in the 200-350 nm UV region The wavelength maximum (ëmax) was observed at 218 nm and this wavelength was adopted for absorbance measurement Figure 1.

Standard Preparation: Accurately weighed 100 mg of Efavirenz test standard was transferred to a volumetric flask containing 10 mL of 0.1N NaoH solvent. This was sonicated for about 10 min to dissolve it and the resultant solution was diluted to 100 mL with0.N NaoHsolvent. Ten milliliters of this standard preparation was transferred to another volumetric flask and then diluted to 100 mL with same solvent.

Sample Preparation: Ten tablets from the marketed sample were weighed and crushed uniformly with the help of a mortar and pestle. An accurately weighed powder sample equivalent to 100 mg of Efavirenzwas transferred into a volumetric flask containing 10 mL of 0.1N NaoH.The contents were sonicated for about 10 min so that the dissolution is enhanced and is completed in 15 min. Tenmillititers of the supernatant solution was then taken and diluted to 100 mL with samesolvent

Method Validation: The method was validated for specificity, linearity, accuracy, ruggedness and solution stability.

Specificity: The specificity of the method was established by measuring the interference, if any, observed due to the 0.1N NaoH solvent at the wavelength maxima of Efavirenz. No significant absorbance due to 0.1N NaoH was observed at 218 nm

Linearity: The linearity of the method was established by determining the absorbance of different concentrations of Efavirenz drug substance over a range of 50% (14 µg/mL) to 150% (70 µg/mL) of the normal sample preparation. Each level was measured in triplicate.

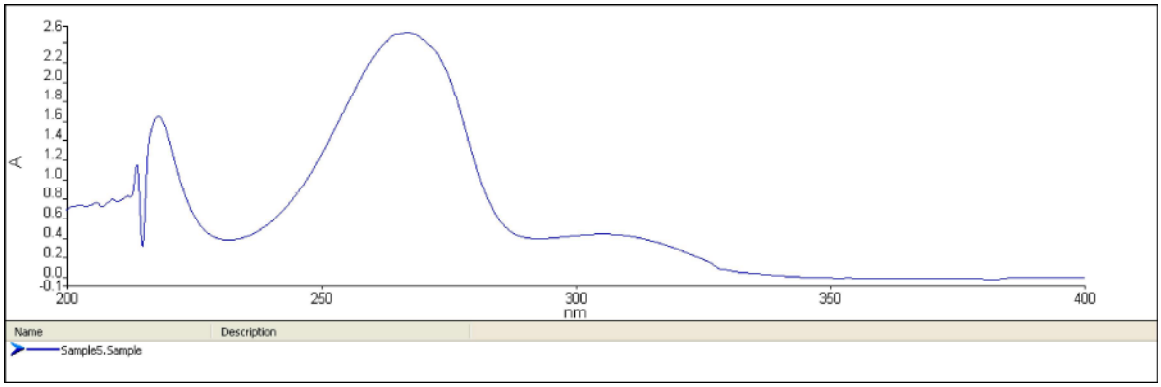


Fig. 1: UV spectrum of Efavirenz in standard solution

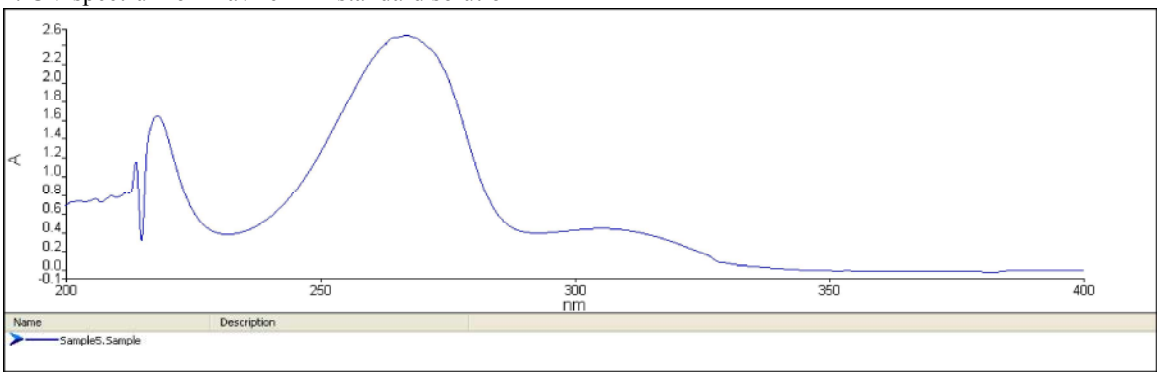


Fig. 2: UV spectrum of Efavirenz in standard solution

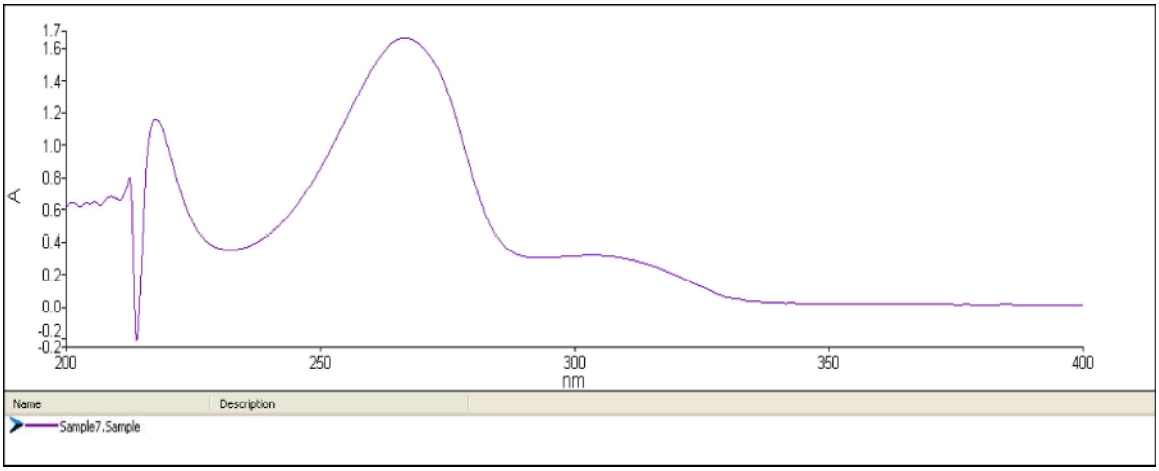
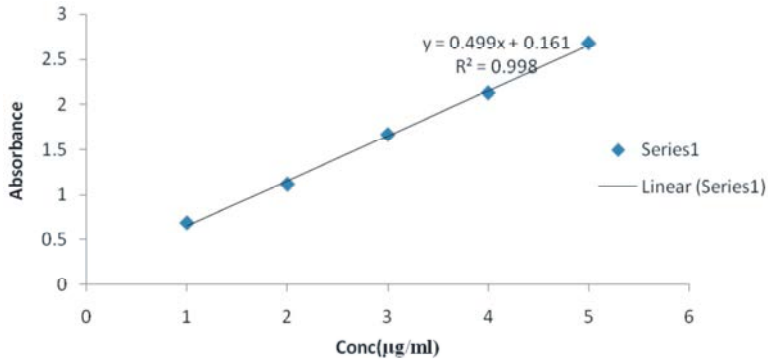


Fig. 3: UV spectrum of Efavirenz in sample solution



1-14µg/ml, 2-28 µg/ml, 3-42 µg/ml, 4-56 µg/ml, 5-70 µg/ml

Fig. 3: Lineartity curve for Efavirenz

Table 2: Precision

Sample No.	Abs	%Assay
1.	0.690	98.6
2.	0.692	98.8
3.	0.688	98.4
4.	0.690	98.6
5.	0.692	98.8
6.	0.688	98.4
Average		98.6
Standard Deviation		0.167
% RSD		0.16

The calibration curve, as plot of absorbance vs concentration in µg/mL of Efavirenz, was found to be rectilinear for 14, 28, 42, 56 and 70 µg/mL concentrations of Efavirenz.. The correlation coefficient was found to be more than 0.998 [Figure 4].

Precision: The assay of the same batch was performed in six replicates and the percentage relative standard deviation (%RSD) measured. The %RSD was found to be not more than 0.2%.

Accuracy: The accuracy of the method was established by adding the Efavirenz test standard solution of the pre-analyzed tablet formulation. The analysis at each level was performed in triplicate and the mean recovery of Efavirenzwas measured. The percent recovery at each level was found to be well within the range of 98.0%-102.0%, indicating insignificant interference from the excipients.

Ruggedness: The ruggedness of the method was established by having the precision study performed on another instrument by another analyst. The cumulative %RSD for content of Efavirenz for the samples of precision and ruggedness study were found to be not more than 1.0%.

Solution Stability: The absorbance of the same sample solution at the initial stage and intervals of 4 hours, 8 hours, 12 hours and 24 hours were measured and the cumulative %RSD determined. The %RSD was found to be not more than 2.0%

Table 3: Accuracy

Spike level (%)	Absorbance	Average mg added (API)	mg Found	Avg. mg Found	% Recovery
80	0.919	1.09	1.1	1.1	99
	0.920		1.0		
	0.922		1.3		
100	1.118	2.14	2.1	2.1	101
	1.120		2.2		
	1.122		2.1		
120	1.327	3.05	3.2	3.0	98.4
	1.325		3.0		
	1.327		3.1		

Table 4: Analytical and Regression Parameters

Parameters	Efavirenz
Linearity	14-70 µg/ml
Coefficient correlation	0.998
Slope	0.499
Intercept	0.161
Regression equation	0.499x+0.161
Detection wavelength	218nm

RESULTS AND DISCUSSSION

The proposed methods were validated as per ICH Guidelines and the absorbance vs concentration of Efavirenz was plotted.

The linearity for spectrophotometric methods was established in the concentration 14-70µg/ml for the drug absorbance at 218nm for Efavirenz standard and tablets. Calibration curve were plotted using concentration vs absorbance. Slope, intercept and correlation coefficient values were found to be 0.499, 0.161 and 0.998 respectively.

Precision studies were performed by preparing the standard 5 concentrations and measuring the absorbance of drugs at 218nm. Low % RSD shows that the method has good precision.

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation powders a known quantity of standard Efavirenz was added and contents were analysed by the proposed method.

The developed spectrophotometric method was validated by using linearity, range, accuracy and precision and the estimation was done by direct comparison method. The RSD for all parameters were found to less than 2, which indicates validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of Efavirenz in multi component analysis.

The proposed methods were found to be simple, precision and sensitive for the routine determination in tablet formulation. To study the validity and reproducibility of proposed methods, recovery studies were carried out. The methods were validated in terms of linearity accuracy, precision specificity and reproducibility. The proposed method can be successfully used for estimation of Efavirenz.

Interference studies, accurate, precision, linearity interference studies reveled that the common excipients used in the dosage form do not interference with the estimation of Efavirenz using the proposal method.

CONCLUSION

The present analytical method was validated as per ICH Q2 (R1) guideline and it meets to specific acceptance criteria. It is concluded that the analytical method was specific, precise, linear, accurate, robust and having stability indicating characteristics. The present analytical method can be used for its intended purpose.

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