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Research Article

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Method Development and Validation of Efavirenz in Bulk and Pharmaceutical Capsule Dosage Form by Using UV–Visible Spectrophometric Method

Somsubhra Ghosh*, B. Rajni, M. Alagar Raja, Dr. David Banji

Department of Pharmaceutical Analysis and Quality Assurance, Nalanda College of pharmacy, Cherlapally, Nalgonda, Andhra Pradesh -500081, India.

*E-mail: som_subhra_ghosh@yahoo.co.in

Abstract

The present research work discusses the development of UV Spectrophotometric method for the determination of Efavirenz in bulk and pharmaceutical capsule dosage form. The present method is simple, rapid, accurate, precise and economical when compared to other methods. The absorption maxima of the drug were found to be 264nm for Efavirenz in DMSO solvent system. The method is applied to capsule dosage form it gives best results of accuracy, precision & linearity over a range of 2-18µg/ml for Efavirenz. The percentage recovery was found to be 99.63- 101.48% for Efavirenz. Results were analysed and validated for various parameters as per ICH guidelines.

Key words: Efavirenz , Capsule dosage form, Method, Development, Validation.

Introduction

Analytical chemistry is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparation.[(1)] It is the subject of science, which deals with interaction of radiation and matter. All atoms and molecules are capable of absorbing energy in accordance with certain restrictions, these limitations depending upon the structure of the substance. Spectroscopic analytical methods are based on measuring the amount of radiation produced and absorbed by molecular or atomic species. The kind and amount of radiation absorbed depends upon the number of molecules interacting with the radiation. The study of these dependencies is called absorption spectroscopy. Absorption spectroscopy is one of the most valuable analytical techniques; its advantages include simplicity, speed, specificity and sensitivity [(2)]. The parts of the molecule, that is, the atoms or groups of atoms, may

move with respect to each other called as vibration and energy called as vibrational energy. The molecule may rotate about an axis; such rotation is characterized by the rotational energy. This mode of movement molecules possesses an electronic energy.[3]
E= E trans+ Evib+ Erot+ Eelect

It 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 1and chemically it is (4*S*)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1*H* 3,1-benzoxazin-2-one with molecular formula $C_{14}H_9ClF_3NO_2$ [4] is presented in **Figure No: 1.** For HIV infection that has not previously been treated, the United States Department of Health and Human Services Panel on Antiretroviral Guidelines currently recommends the use of efavirenz in combination with lamivudine/zidovudine (Combivir) or tenofovir/emtricitabine (Truvada) as the preferred NNRTI-based regimens in adults and adolescents. [5]

For HIV infection that has not previously been treated, efavirenz and lamivudine in combination with zidovudine or tenofovir is the preferred NNRTI-based regimen.Efavirenz is also used in combination with other antiretroviral agents as part of an expanded postexposure prophylaxis regimen to prevent HIV transmission for those exposed to materials associated with a high risk for HIV transmission.

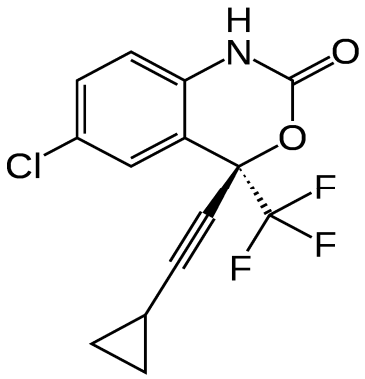

Figure No: 1. Shows structure of Efavirenz

Literature review [6-18] for Efavirenz analysis revealed there is very few methods based on different techniques. The aim of present work is to find out a simple, sensitive, specific, spectrophotometric method developed and validation for the detection of Efavirenz in bulk drug and pharmaceutical formulation.

Materials and Methods

Efavirenz working standard was supplied by Hetero Labs Ltd, Hyderabad. Efavirenz (Label claim: 200mg tablet) was manufactured by Cipla. All other chemicals used in the analysis were AR grade. A double–beam spectrophotometer Simazdu 1600 was used for the detection of absorbance, Afcoset ER-200A (weighing balance) and Borosil glassware were used for experimental purpose.

Method:

Preparation of standard:

Accurately weigh and transfer 25 mg of Efavirenz working standard into a 25 mL volumetric flask add about 10 mL of Diluent and dissolve it completely and make volume up to the mark with the same solvent. From this solution again take 1 ml & dilute to 10 ml (Stock solution). Further pipette 2ml of the Efavirenz stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of sample:

Accurately weigh and transfer equivalent to56.75 mg of (Avg. Of 20 tablet) powder of Capsule Efavirenz into a 25 mL volumetric flask add about 10 mL of diluent and dissolve it completely and make volume up to the mark with the same solvent. From this solution again take 1 ml & dilute to 10 ml (Stock solution). Further pipette 2ml of the Efavirenz stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

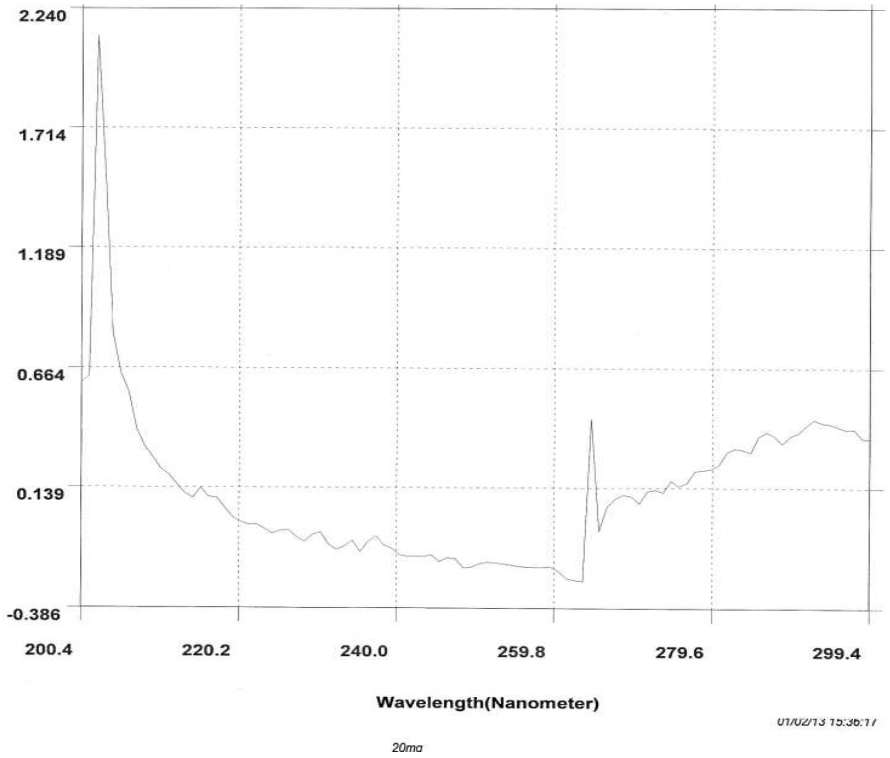

Figure No: 2. Shows spectrum for standard of Efavirenz

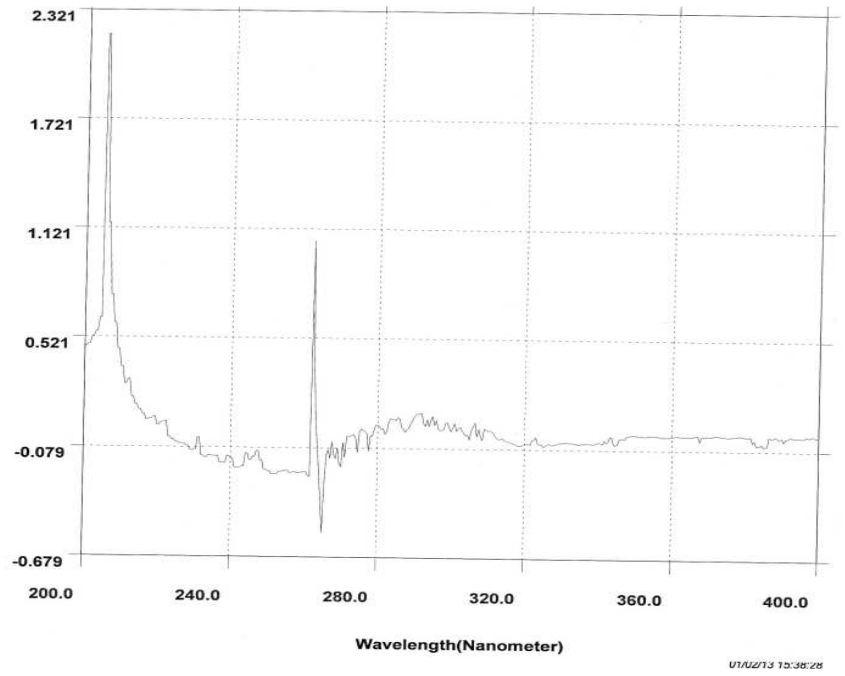

Figure No: 3. Shows spectrum for sample of Efavirenz

Method Validation:

Various methods for analysis of Efavirenz in bulk and pharmaceutical formulation were carried out as per ICH guideline.

Linearity:
The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy of the analyte. For Efavirenz , five point calibration curves were generated with the appropriate volumes of the working standard solutions for UV methods.

Precision and accuracy:
Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicate at different concentration levels covering the entire linearity range. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as RSD % for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assays on three different days and the results are documented as the standard deviation and RSD %. Accuracy is the percent of analyte recovered by assay from a known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained.

Limit of detection:
The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD is calculated by signal to noise ratio method.

Recovery study:
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 studies, was used. To study the accuracy, precision and reproducibility of the proposed method and dosage forms, recovery experiments were carried out using the standard addition method. These studies were performed by the addition of known amounts of pure Effaverinz to the pre-analyzed capsule formulation and the mixtures were analyzed using the proposed techniques. After parallel analyses, the recovery results were calculated using the related calibration equations.

Results and Discussion

The development of a simple, rapid, sensitive and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary tedious sample preparations and the cost of materials and labour. Efavirenz is a UV-absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method. The λ Max of the drug for analysis was determined by taking scans of the drug sample solutions in the entire UV region. It was found to be that only one peak was observed in this method at the wavelength of 264 nm (λ Max).Calibration curve data were constructed in the range of the expected concentrations of 2µg/ mL to 18µg/ mL. Beer's law was obeyed over this concentration range. The correlation coefficient (r) of the standard curve was found to be greater than 0.997. The stock solutions and working standards were prepared in DMSO. Calibration curve & Linearity table is presented in **Figure No: 4** & **Table No: 1** respectively.

Table No: 1. Linearity study of Efavirenz

S.No	Linearity Level	Concentration	Absorbance
1	**I**	2µg/ml	0.065
2	**II**	6µg/ml	0.177
3	**III**	10µg/ml	0.276
4	**IV**	14µg/ml	0.412
5	**V**	18µg/ml	0.512
Correlation Coefficient			0.997

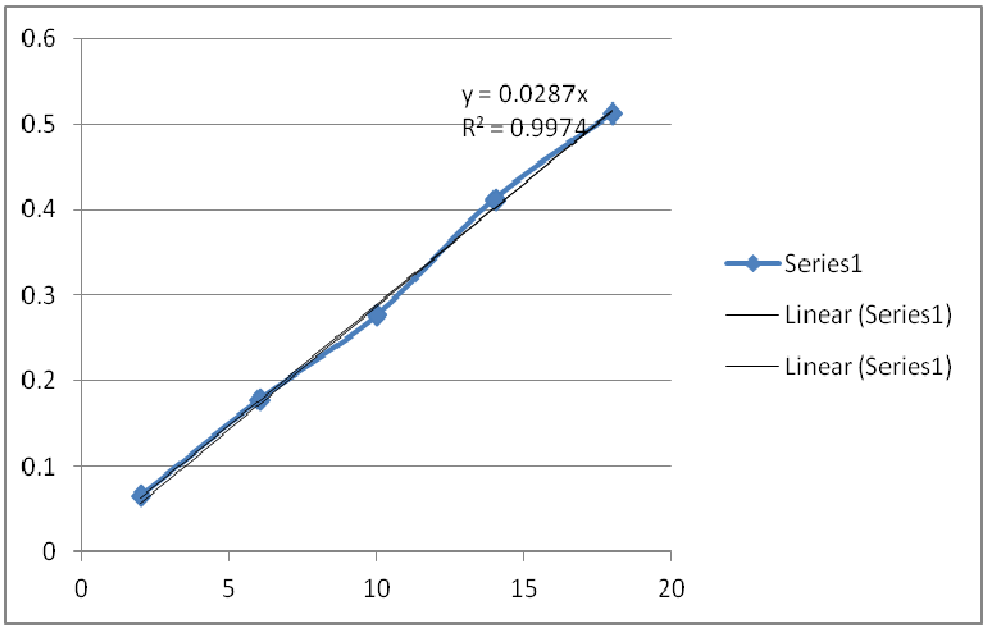

Figure No: 4 Shows calibration curve for linearity of Efavirenz .

Performing replicate analyses of the standard solutions was used to assess the accuracy, precision & reproducibility of the proposed methods. The selected concentration within the calibration range was prepared in DMSO.

The accuracy of the method was shown by analyzing the model mixtures contained 50, 100 and 150% of samples of Efavirenz within the linearity ranges were taken. After injected the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions, the Amount found, Amount added for Efavirenz , individual recovery and mean recovery values were calculated. The recovery of Efavirenz was evaluated from 50 to 150% of the labeled tablet. The mean percentage recoveries were found to be 101.48%, 100.18% and 99.63% for 50%, 100% and 150% respectively. Accuracy data were present in **Table No: 3**.

The intra- and inter day precision were determined as the RSD %. Intraday precision (repeatability) study was done by measuring absorbance of 5 sample of same concentration, % RSD value was 1.3 which is less than 2% & well accepted according to guideline data is given in **Table No: 2.** Other parameter of assay of Efavirenz by UV-Vis Spectroscopy is given in **Table No: 4.**

Table No: 2. Data for Precision

Reading	Absorbance
Reading-1	0.551
Reading-2	0.540
Reading-3	0.548
Reading-4	0.546
Reading-5	0.560
Average	0.550
Standard Deviation	0.007
%RSD	1.3

Table No: 3. Accuracy data of Effaverinz

%Concentration (at specification Level)	Absorbance	Con. Added (µg)	Con. Found (µg)	% Recovery	Mean Recovery
50%	0.274	10.0	10.15	101.48%	100.43%
100%	0.552	20.0	20.03	100.18%	
150%	0.821	30.0	29.88	99.63%	

Table No: 4. Summary for assay of Efavirenz UV spectroscopy

1.	**Linearity range (µg/ml)**	-	2-18
2.	**Correlation coefficient**	NLT 0.999	0.997
3.	**Precision**	% RSD (NMT 2%)	1.3
4.	**Ruggedness**	% RSD (NMT 2%)	0.002
7.	**% Recovery**	98% to 102%	100.43

The proposed methods can be successfully applied for Efavirenz assay in capsule dosage forms without any interference. The assay showed the drug content of this product to be in accordance with the labelled claim 25mg. The recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy of the method. In order to check the accuracy and precision of the developed method and to prove the absence of interference by excipients, recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulations of all drugs. The application of this procedure is explained in the experimental section. The obtained results demonstrate the validity and accuracy of the proposed method for the determination of all drugs in tablets. These results reveal that the developed method have an adequate precision and accuracy and consequently, can be applied to the determination of Efavirenz capsule in pharmaceuticals without any interference from the excipients.

Conclusion

The developed Spectrophotometric method was simple, sensitive, and specific, for the detection of Efavirenz in bulk & pharmaceutical formulation. It could be precisely quantify and LOD was found to be 0.05 µg . All the calibration curves shows a linear relationship between the absorbance and concentration and coefficient correlation was higher than 0.997. Precision of the method was found to be 1.3% against the label claim of 200mg. The percentage recovery was found to be 99.63-101.48%. The proposed method will be suitable for the analysis of Efavirenz in bulk and pharmaceutical formulation.

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