Development of Rapid UV Spectrophotometric Method for the Estimation of Efavirenz in Formulations

Y.ANAND KUMAR* and N.RAMA RAO

*Department of Pharmaceutics, V.L.College of Pharmacy, Raichur-584103, India.
Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, India.

neeru241586@yahoo.co.in

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Abstract: Simple, sensitive, accurate, precise and rapid ultraviolet (UV) spectrophotometric method was developed for the estimation of efavirenz in pure form, its formulations and stability samples. For the estimation of efavirenz, solvent system employed was 1% w/v sodium lauryl sulphate (SLS) and wavelength of detection (λ_{det}) was 247 nm. The developed method was used to estimate the total drug content in two commercially available oral formulations of efavirenz and recovery studies were also carried out. Sample recovery in both the formulations using the above method was in good agreement with their respective labeled claims, thus suggesting the validity of the method and non-interference of formulation excipients in the estimation. The developed method was found to be stability specific and were validated as per ICH guidelines-2005, USP-2000 and statistical methods.

Keywords: Spectrophotometric determination, Sodium lauryl sulphate, Efavirenz

Introduction

Efavirenz belongs to the class of non-nucleoside reverse transcriptase inhibitors and is indicated in the treatment of HIV infection¹⁻⁴. Efavirenz is chemically known as (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one and molecular weight is 315.7. It is white to slightly pink powder, practically insoluble in water and freely soluble in methanol. It should be kept in a well closed container, protected from light.

Literature survey reveals that only HPLC methods are available for the estimation of efavirenz alone, in combination with other drugs, in its dosage form and in plasma⁵⁻¹⁰ however, no UV spectrophotometric method was found in literature. The present investigation has been undertaken to develop simple UV spectrophotometric method for the estimation of efavirenz in pure form and its formulations.

Experimental

A Hitachi-U2000 spectrophotometer with a pair of matched quartz cells was used to measure absorbance of the resulting solutions. Efavirenz was gifted by Strides Pharma. All the other reagents used were of analytical grade.

Preparation of standard curve

A 100 µg/mL stock solution of efavirenz was prepared in 1%w/v SLS by first dissolving 10 mg of the drug in 10 mL of methanol and then, made up to the final volume with 1%w/v SLS.

The λ_{max} of efavirenz was determined by scanning suitable dilutions with high correlation coefficient. From the stock solution, various standard dilutions were made to obtain solutions of 1,2,3,4 and 5 µg/mL. Their respective absorbance values were measured at fixed λ_{max} with parameter set at 0.5 nm for bandwidth as well as data pitch. Average absorbance values, standard deviation and % coefficient of variance at each concentration were calculated. One way ANOVA test for linearity was carried out by picking five sets of calibration curves on random basis.

Sample preparation (Bulk drug)

Efavirenz 50 mg was accurately weighed and taken into 100 mL volumetric flask containing 10 mL of methanol and further it was made up to 100 mL with 1.5% w/v sodium lauryl sulphate solution. This solution was further serially diluted with 1.5% w/v sodium lauryl sulphate solution to get 10, 20, 40, 50, 100 µg/mL solution. The efavirenz content was further determined by measuring the absorbance at 247 nm.

Sample preparation (Dosage forms)

Twenty tablets were weighed and powdered; the powder equivalent to 50 mg of efavirenz was weighed accurately and taken in 100 mL volumetric flask containing 50 mL of methanol. The contents were shaken well for 30 minutes and made up to the volume 100 mL with methanol. This solution was further suitably diluted with 1.5% w/v sodium lauryl sulphate solution and determined the efavirenz content by measuring the absorbance at 247 nm.

Recovery experiments

To keep an additional check on the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were performed by adding known amount of pure drug to the previously analyzed pharmaceutical preparation and analyzed by the developed method. The concentration levels used were 10 µg/mL.

Results and Discussion

Method development

To develop accurate, precise and sensitive UV spectrophotometric method for efavirenz, various solvent systems such as water, methanol *etc.* were tried alone and in combinations or in the presence of surfactants at different proportions. The final decision of using 1% w/v SLS in water was based on sensitivity, minimal interference, ease of preparation, suitability for drug content estimation, stability, analysis time and cost. The λ_{max} for efavirenz in 1% w/v SLS (Figure 1) showed linear relationship (with correlation coefficient of 0.9988) in the concentration range of 1-5 µg/mL (Table 1 & 2).

Sample solution stability studies

Overlay scans obtained at zero time, 12, 24, and 48 h revealed no degradation up to 48 h, in the selected solvent at controlled (25±2°C; 65±5 %RH) and accelerated (40±2°C; 75±5 %RH). Drug was stable for more than 48 hours, thus there can be time between collection of the sample and analysis of the same.

Figure 1. Spectra of 5µg/mL solution of efavirenz in 1%w/v sodium lauryl sulphate.

Table 1. Calibration curve points of the proposed method for the estimation of efavirenz.

Parameters	Values
Absorption maxima, nm	247
Beer's law limit, µg/mL	1-20
Molar absorptivity, 1 mole ⁻¹ /cm ⁻¹	2.21×10 ⁻⁴
Coefficient of correlation	0.9998
Regression equation*A	
Slope(b)	0.05306 ± 0.0004014
Intercept(a)	0.0005238 ± 0.001215
1/slope	-18.85
Correlation coefficient(f)	0.9999

Table 2. Results of least square regression analysis of UV methods for the estimation of efavirenz.

Concentration, µg/mL	Mean ±SD(n=6)	CV (%)
1	0.055 ±0.00634	1.20
2	0.107 ±0.00624	1.43
3	0.157 ±0.00610	1.58
4	0.214 ±0.00650	1.62
5	0.266 ±0.00818	1.71

Recovery studies

The method developed for the estimation of efavirenz in bulk and in its dosage forms was found to be simple, accurate, economical and rapid. Table 3-5 clearly indicates the drug content was uniform ranging from 98 to 99.55% and SD, CV values were found to be satisfactorily low. Recovery studies were also carried out and found to be 98.89 to 98.89% for the both batches of tablets. The method requires only measuring the absorbance of sample solution at the selected wavelength followed by simple calculations. Hence, it was further employed for our study.

Table 3. Efavirenz estimation in bulk by proposed method.

S.No	Efavirenz taken $\mu\text{g/mL}$	Efavirenz found (CV)	% of efavirenz found(CV)
1	10	9.85(0.935)	98.5(0.76)
2	20	19.87(0.884)	99.4(0.85)
3	40	39.56(0.845)	98.9(1.12)
4	50	49.55(0.765)	99.1(0.99)
5	100	98.95(0.928)	98.9(0.10)

Table 4. Efavirenz estimation in dosage form by developed method.

Tablet sample	Label Claim, mg/tablet	Actual content found, $\text{mg}\pm\text{S.D}$	Percent Actual content found, $\pm\text{S.D}$	CV
Sample-1 Efavirenz Tab.(Generic)	100	98.89 $\pm$ 0.153	98.89 $\pm$ 0.153	0.959
Sample-2 Efavirenz Tab.(Generic)	600	590 $\pm$ 2.04	98.33 $\pm$ 0.74	0.679

Table 5. Efavirenz estimation in dosage form in recovery studies by proposed method.

Tablet sample	Concentration of added amount of drug in the final dilution, $\mu\text{g/mL} \pm\text{S.D}$	Recovery, $\mu\text{g/mL} \pm\text{S.D}$	Percent recovery, $\pm\text{S.D}$	CV
Sample-1 Efavirenz Tab.(Generic)	10	9.85 $\pm$ 0.0123	98.5 $\pm$ 0.989	0.959
Sample-2 Efavirenz Tab.(Generic)	10	9.89 $\pm$ 0.0142	98.9 $\pm$ 0.975	0.958

Method validation

The developed estimation method proved to be accurate (accuracy varies between 10.2-5.5%) and precise (Intra day precisions were less than 4.5%). The method has been validated for the range 1-5 $\mu\text{g/mL}$ using 1% w/v sodium lauryl sulphate solution. The method is linear over this concentration range as indicated by the *F*-test for lack of fit. Analyte recovery was better than 90% at all points on the standard curve, Intraday precision was better than 5% CV, while accuracy was between 98-100% of nominal over this range of the estimation.

Conclusions

The developed UV spectrophotometric method for the estimation of efavirenz was found to be simple and useful with high accuracy, precision, repeatability. Sample recoveries in all formulations using the above method was in good agreement with their respective label claim or theoretical drug content, thus suggesting the validity of the method and non interference of formulation excipients in the estimation. In the selected solvent system, drugs were stable for more than 48 hours, thus suggesting that samples need not be estimated immediately after collection. The developed method was found to be stability specific and were validated as per ICH guidelines (2005) and statistical method.

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