

## DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF EFAVIRENZ IN BULK AND IN TABLET DOSAGE FORMS.

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### ABSTRACT

A simple, precise, rapid, accurate and economic high performance liquid chromatographic (HPLC) method has been developed for the estimation of Efavirenz in bulk and its tablet dosage forms. A sunfire C<sub>18</sub> column was used for the determination of efavirenz using methanol and acetonitrile as mobile phase in the ratio of 7:3 v/v, at ambient temperature and the detector was set at 249 nm. The Linearity of efavirenz was found in the range of 10-400 µg/ml with acceptable value of correlation coefficient 0.9997. The method was validated for accuracy, precision, robustness and recovery studies. The recovery of the drug was found to be 99.89%, relative standard deviation was found to be less than 2% for precession studies and method was found to be robust with the varying condition of flow rate (±10%), wavelength of detection (±5nm), column oven temperature (±5°C). The suitability of the developed HPLC method for quantitative determination of efavirenz was proved by validation in accordance with the ICH guidelines. The developed method can be successfully applied in the routine analysis of commercial pharmaceutical tablets.

**Keywords:** Efavirenz; HPLC; Method development and validation.

### INTRODUCTION

Efavirenz (EFZ) (Fig. 1) is chemically known as (4S)-6-Chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one<sup>1</sup> 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 reduce the risk of HIV infection in people exposed to a significant risk (e.g. needle stick injuries, certain types of unprotected sex etc.). The usual adult dose is 600 mg once a day.

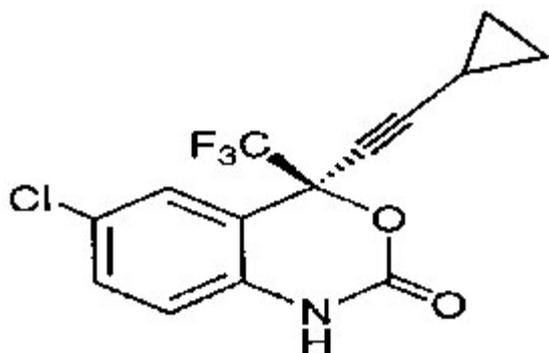


Fig. 1 : Structure of efavirenz

Literature survey reveals that several HPLC methods with UV detection and HPTLC methods have been reported to quantify efavirenz<sup>2-11</sup>, with Tandem Mass Spectrometry<sup>12</sup> alongwith other anti-HIV drugs in human biological fluids. Several GC/MS methods<sup>13</sup> for simultaneous quantitation of efavirenz with other HIV suppressing drugs have also been reported. The objective of present work was to develop a simple and precise HPLC method for estimation of efavirenz in bulk and in tablet dosage forms.

### EXPERIMENTAL

#### Materials and Methods

Standard material of efavirenz was provided by Cipla Ltd, Mumbai. Efavirenz (200 mg) tablets were provided by Aurobindo Pharma, Hyderabad, India. All chemicals and reagents used were of analytical grade. Methanol and acetonitrile were purchased from E. Merck Chemicals, Mumbai, India.

#### Instrumentation

The instrument used for the study was a Waters High Performance Liquid Chromatography, Millennium Software system equipped with pump 600, inline degasser, 717 plus Autosampler, 486 Tunable Absorbance Detector.

#### Chromatographic conditions

The selected and optimized mobile phase was methanol: acetonitrile (70:30 v/v). The mobile phase was filtered through 0.45 µm filter paper, mixed properly

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and degassed by sonication. The column used was Sunfire C<sub>18</sub> (25 cm × 4.6 mm) with 5 µm particle size, operated at ambient temperature. The detector was set at 249 nm. The injection volume was 20 µl. The elution was carried out in isocratic mode with the flow rate of 2.0 ml per min.

### Standard Preparations

Approximately 100 mg of efavirenz reference standard was accurately weighed and transferred to a 100 ml volumetric flask. The volume was made up to the mark with methanol to obtain a concentration of 1000 µg/ml of efavirenz. The solution was further diluted with methanol to obtain different concentrations in the range of 10- 400 µg/ml of efavirenz.

### Sample Preparation

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed portion of the powder equivalent to about 10 mg of efavirenz was transferred to a 100 ml volumetric flask. This content was then mixed with methanol, sonicated for 20 minutes and diluted with the same solvent. The resultant mixture was filtered through 0.45 µm nylon filter.

### Method validation

The developed HPLC method was validated with respect to the following parameters given below as per ICH guidelines<sup>14</sup>.

#### Determination of retention time of efavirenz

The composition of the mobile phase for development of chromatographic method was optimized by testing different solvent mixtures of varying polarity. The best results were obtained using methanol:acetonitrile (70:30 v/v). This mobile phase showed good resolution of efavirenz peak. The wavelength of detection selected was 249 nm, as the drug showed optimum absorbance at this wavelength. Result indicates that the retention time of efavirenz peak is about 1.9 minute and none of the impurities were interfering in its assay (Fig. 2).

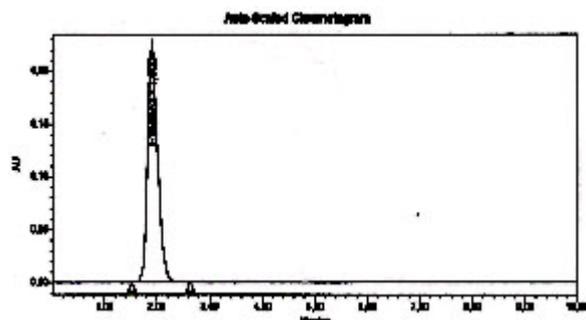


Fig. 2 : A typical chromatogram of efavirenz standard Linearity

#### Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range was found to be 10-400 µg/ml of efavirenz. A linear

correlation was found between the peak areas and the concentrations of efavirenz and the regression coefficient ( $r^2$ ) obtained was 0.999.

#### Precision

The intra-day precision was carried out at three different concentration levels of 50, 100 and 200 µg/ml three times on the same day. Inter and intra-day accuracy (expressed as %RSD) ranged from 1.40 to 1.64 for efavirenz.

#### Accuracy

It was investigated by means of addition of efavirenz standard to the solution of tablet. To study the accuracy of the proposed method, the recovery studies were carried out by addition of 80%, 100% and 120% of standard drug solution to the sample solution and the result of the recovery studies are summarized in Table 1.

Table 1: Recovery study of efavirenz

S.No.	Initial Amount (mg) [A]	Addition of known quantity (mg) [B]	A+B	Amount recovered (mg)	% Recovery	Average Recovery n=3
1	10	8	18	18.01	100.05	99.31
2				17.89	98.88	
3				17.82	99.00	
4	10	10	20	20.06	100.30	100.18
5				20.01	100.05	
6				20.04	100.20	
7	10	12	22	22.12	100.54	100.40
8				22.09	100.40	
9				22.06	100.27	

#### Robustness and Ruggedness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of efavirenz was noted. The factors selected were flow rate, temperature and % Acetonitrile in the mobile phase. The results remained unaffected by small variations in these parameters. Ruggedness of the method was checked by using different analysts and instruments. The relative standard deviation of the results obtained from different analysts and instruments was < 1.0 %.

#### Assay

Standard and sample solutions (20 ml) were separately injected on HPLC system. From the peak area of efavirenz the amount of drug in the bulk and in tablets were computed. The amount of efavirenz was found to be 99.62% ±0.5% in bulk drug and 97.41% ±0.5% in tablets.

### RESULT AND DISCUSSION

RP-HPLC method for estimation of efavirenz in bulk and tablet dosage forms was developed. The elution was carried out with the mobile phase consisting of methanol: acetonitrile (7: 3 v/v), at flow rate of 2.0 ml/min, detecting at the wavelength of 249 nm, at ambient temperature. The retention time was obtained 1.9 min with run time of 10 min. The linear response obtained

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in the concentration range of 10-400 µg/ml with correlation coefficient 0.9997. The relative standard deviation found to be less than 2 % for precision studies and the method was found to be robust with varying condition of flow rate ( $\pm 2\%$ ), wavelength of detection ( $\pm 2$  nm), column temperature ( $\pm 1^\circ$  C). The results were found to be satisfactory. The amount of efavirenz present in bulk and tablet dosage forms were determined and found to be 99.62 %, 97.41 % respectively.

### CONCLUSION

The developed RP-HPLC method was found suitable for the determination of efavirenz in bulk and marketed solid dosage formulation. Statistical analysis proves that the method is repeatable and selective for the analysis of efavirenz. This method may be employed for quality control analysis. Its advantages are low cost of reagents, speed and simplicity of sample treatment.

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