Original Research Article

VALIDATED STABILITY INDICATING ANALYTICAL METHOD AND *IN-VITRO* DISSOLUTION STUDIES OF EFAVIRENZ FORMULATION BY RP-HPLC

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ABSTRACT

A reverse phase stability indicating HPLC method was developed for the analysis of efavirenz bulk and pharmaceutical formulations. The developed method was also utilized for *in-vitro* dissolution studies of efavirenz formulations. Acetonitrile and acetate buffer pH 3.4 was the mobile phase (75:25% v/v), with retention time of 4.007 min at a flow rate of 1.5 mL/min detected at 292 nm wavelength. Linear regression analysis calibration plot showed an excellent linearity between response and concentration in the range of 50-300 μgmL^{-1}. The regression co-efficient was 0.999 and the linear regression equation was y = 7780x+11159. Limits of detection (LOD) and quantification (LOQ) were 0.238 and 0.793 μgmL^{-1} respectively. The method was validated for accuracy, precision, specificity, robustness, detection and quantification limits, in accordance with ICH guidelines. The specificity of the method was ascertained by forced degradation studies by acid, alkali hydrolysis, oxidation and thermal degradation methods. The degraded products were well resolved from the analysis peak with significant differences at their retention time values. Wide linearity range, sensitivity, accuracy, short retention time and simple mobile phase indicate the method is suitable for routine quantification of efavirenz with high precision and accuracy.

Keywords: RP-HPLC, stability indicating, forced degradation.

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1. INTRODUCTION

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used as a part of highly active anti-retroviral therapy (HAART) for the treatment of human immunodeficiency virus (HIV-1). It is chemically (4*S*)-6-chloro -4- (cyclopropylethynyl)-1, 4-dihydro-4-(trifluoromethyl) - 2*H*-3, 1-benzoxazin-2-one (Figure 1). Efavirenz activity is mediated predominantly by non-competitive inhibition of HIV-1 RT.[1-3]

The drug stability test guidelines Q1A (R2) issued by International Conference on Harmonization (ICH) requires that analytical test procedures for stability samples should be fully validated and the assay should be stability indicating. Dissolution is considered as one of the most important quality control tests performed on pharmaceutical dosage forms and is now developing into a tool for predicting bioavailability. The profiles obtained from dissolution rate

studies, have also been used in an attempt to characterize *in vitro* behavior of drugs with success. It must be performed under precisely specified conditions.[4]

Literature survey revealed that few spectrophotometric, RP-HPLC and LC-MS methods available for stability indicating and quantification of efavireng in pharmaceutical formulations and biological samples.[3-10] Moreover, Efavirenz has not been reported by method development and validation including dissolution studies and related kinetic parameters of tablet dosage form analysis individually. Therefore, the goal of research work was to develop a rapid, simple, sensitive, cost-effective and validated method for direct estimation of efavirenz in bulk and also aimed at the drug dissolution studies.

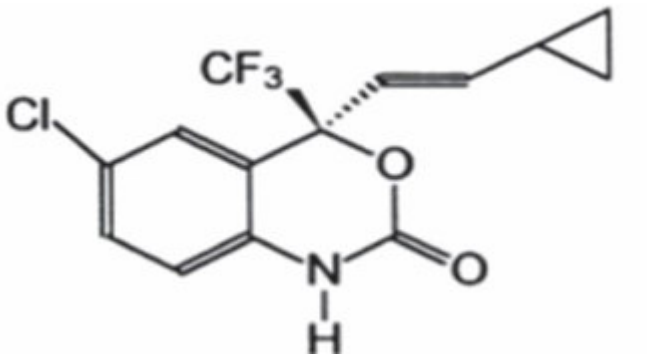

Figure 1. Structure of efavirenz

2. MATERIALS AND METHODS

2.1. Instruments Used: A Shimadzu HPLC-LC-20AD series binary gradient pump with Shimadzu SPD-20A UV detector, LC-solution version software, intersex C_{18} column (250 X 4.6 mm, 5 am particle size) as stationary phase, a calibrated electronic span balance Shimadzu (AUX-220), a pH meter of Ellice (LI-120) were used during the analysis.

2.2. Reagents and Chemicals: Analytically pure efavirenz has been obtained as a gift sample from Hetero Pharmaceuticals Ltd. (Hyderabad, India). Tablets were purchased from the local market. Sodium acetate, acetic acid of AR grade and HPLC-grade acetonitrile were used.

2.3. Preparation of mobile phase and standard stock solution: Mobile phase was prepared by mixing 125mL of 0.1 M acetate buffer (pH adjusted to 3.4 ±0.05 with sodium acetate or acetic acid) with 375 mL of acetonitrile. The mobile phase was filtered through a 0.45 µ membrane filter paper.

Accurately weighed 50 mg of drug was transferred to 50 mL volumetric flask and was dissolved in acetonitrile. The volume was made with acetonitrile to give 1000 μgmL^{-1}.

2.4. Optimized chromatographic conditions

RP-HPLC analysis was performed by Iso-cratic elution method with a flow rate of 1.5 mL/min. The mobile phase containing 0.1 M acetate buffer (pH 3.4 was adjusted using acetic acid/sodium acetate) and acetonitrile in the ratio 25:75 (% v/v) to obtain well resolved peak of efavirenz (Rt =4.007 min) as shown in figure 2.1 Wavelength of maximum absorption was selected by UV-detector. The drug shows good response at 292 nm.

Table 2.1. System Suitability Parameters

Parameters	Value
Regression equation	Y=7780x +11159
Correlation Coefficient	$r^2 = 0.999$
Slope	7780
Intercept	11159
Retention Time	4.007
Theoretical plates	8639
Tailing factor	1.258
LOD (µg/mL)	0.238
LOQ (µg)	0.793

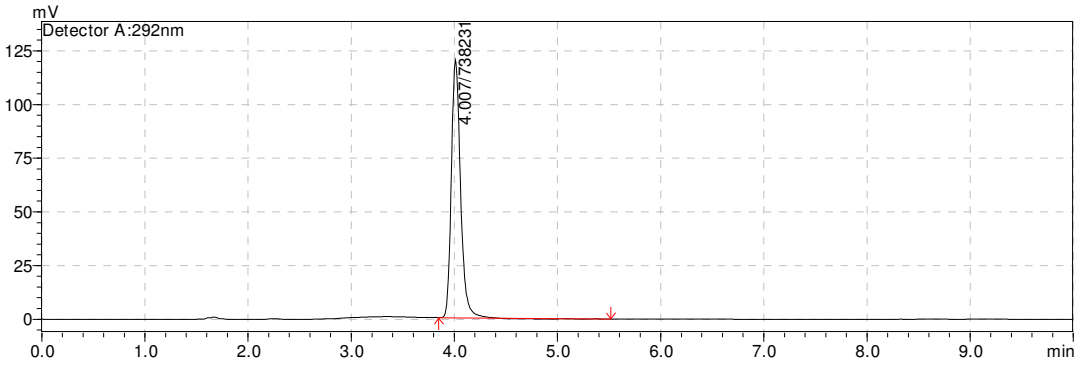

Figure 2.1. Chromatogram of efavirenz 100 µgmL^{-1}

2.5: Validation of the method

2.5.1. Linearity: Linearity was found for the concentration range of 50-300 µgmL^{-1}.

2.5.2. Precision: Intra-day and inter-day precision of the assay samples containing efavirenz (50 and 100 µgmL^{-1}) were analyzed three times.

2.5.3. Accuracy: Accuracy was found out by recovery study using standard addition method.

2.5.4. Robustness: By introducing small but deliberate changes in the mobile phase pH (± 0.2 %), mobile phase composition (± 2.0 %) and flow rate (± 10%) robustness of the described method were studied.

2.5.5. Sensitivity: The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined based on the standard calibration curve.[11]

2.6: Degradation studies

In an attempt to develop a stability indicating assay method, the samples of tablet powder were subjected separately for different degradation conditions. Acidic degradation using 10 mL 5N HCl, basic degradation using 10 mL 1N NaOH, oxidative degradation using 10 mL 6% hydrogen peroxide solution was carried out. The samples were subjected to degradation for 24 hours at ambient temperature and thermal degradation for 24 hours at 105°C. Photo-degradation

was performed for the same samples by exposing them to UV light by using photo stability chamber.[4]

2.7: ***In-vitro*** **dissolution studies**

The method developed was used to analyze samples after *in-vitro* dissolution of efavirenz under standard conditions specified in the Indian pharmacopoeia. The *in-vitro* dissolution was performed in 1% sodium lauryl sulphate for 30 min at a temperature of 37±5°C by USP apparatus paddle type with 50 rpm.[1,12-17]

3. RESULTS AND DISCUSSION

3.1. Calibration curve for efavirenz: Appropriate aliquots of standard stock solutions were taken in different 10 mL volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 50, 100, 150, 200, 250 and 300 μgmL^{-1} of efavirenz respectively. The solutions were injected using a 20 μgmL^{-1} fixed loop system and chromatograms were recorded. Calibration curve was drawn by plotting average peak area versus concentration as shown in figure 3.1.

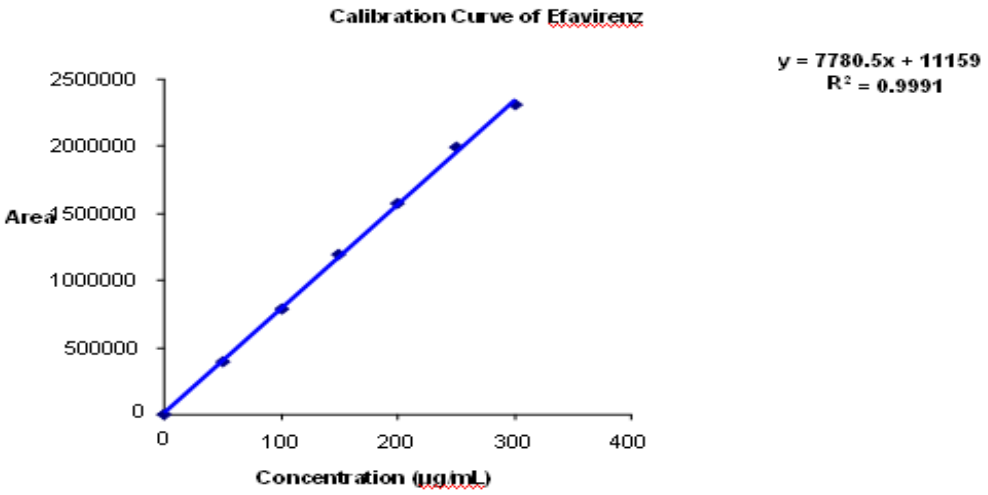

Figure 3.1. Calibration curve of efavirenz

3.2. Analysis of the marketed formulations: Ten tablets (Estiva 600 mg) were weighed accurately and crushed to form fine powder. Accurately weighed quantity of powder equivalent to 50 mg of efavirenz was dissolved in a 50 mL volumetric flask with acetonitrile. The solution was filtered using Whattmann filter paper.

Appropriate volumes of the aliquots were transferred into six different 10 mL volumetric flasks and then volume was made up to the mark with mobile phase to obtain 100 $\mu gmL^{-1}$of efavirenz. The chromatographic conditions and peak areas were measured. The tablet analysis results are shown in Table 3.1.

Table 3.1. Analysis of commercial tablets (Estiva-600 mg)

Analyte	Label Claim (mg)	Average amount found (mg±SD)	% RSD
Efavirenz	600	603.0 ± 1.61	0.267

3.3. Validation of the method

The developed method was validated in terms of linearity, accuracy, precision, specificity, limit of detection and limit of quantification as per ICH guidelines.

3.3.1. Linearity: The linearity range was found 50-300 μgmL^{-1}. The regression equation for efavirenz was found to be y=7780x+11159 and correlation co-efficient (r^2 = 0.999). Table 3.3.1 shows linearity range data.

Table 3.3.1. Linearity

Concentration(μgmL^{-1})	Area
0	0
50	398388
100	783911.7
150	1194696
200	1571911
250	1994767
300	2303997

3.3.2. Precision: Intra-day and inter-day precision of the assay samples containing efavirenz (50 and 100 μgmL^{-1}) were analyzed three times in the same day (intra day) and for three consecutive days by different analytical methods. The results are shown in the Table 3.3, revealed that the % RSD was less than 2.

Table 3.3.2. Precision

	Intraday Precision		Interday Precision	
Concentration (μgmL^{-1})	Avg conc. ± SD (n=3)	RSD (%)	Avg conc. ±SD (n=3)	RSD (%)
50	49.76 ± 0.100	0.20	49.81 ± 0.07	0.14
100	99.32 ± 1.67	1.68	98.55 ± 1.08	1.09

3.3.3. Accuracy: Accuracy was found out by recovery study using standard addition method. Known amount of efavirenz was added to pre-analyzed samples at a level from 80% up to 120% and then subjected to the proposed HPLC method. The % recovery was 98-102 and % RSD was found to be less than 2. Results of recovery studies are shown in Table 3.3.3.

Table 3.3.3. Data on accuracy of the method (n=3)

Excess drug added to analyte (%)	Theoretical Content (mg)	Conc. Found (mg ± SD)	Recovery (%)	% RSD
0	20	19.96 ± 0.223	99.8	1.117
80	36	36.31 ± 0.46	100.8	1.23
100	40	41.04 ± 0.25	102.6	0.609
120	44	43.61 ± 0.05	99.11	0.114

3.3.4. Robustness

By introducing small but deliberate changes in the mobile phase pH (± 0.2 %), mobile phase composition (± 2.0 %) and flow rate (± 10%) robustness of the described method were studied. The robustness of the method was assessed for 2 different amounts of calibration plot (50 & 100 µg). The results of the robustness study also indicated that the method is robust and is unaffected by small variations in the chromatographic conditions. Table 3.3.4. shows results of robustness.

Table 3.3.4. Robustness data

Parameter	Variation (%) level	System suitability		
		Theoretical plates	Peak Tailing Factor	% RSD
S.T.P	-	8709	1.25	1.22
Flow rate	- 10 %	9559	1.26	1.29
	+ 10 %	8167	1.27	1.12
% Organic in mobile phase	- 2 %	8816	1.27	0.27
	+ 2 %	8404	1.28	0.45
pH of mobile phase	- 0.2 %	8289	1.29	1.12
	+ 0.2 %	8501	1.27	0.51
Wavelength	- 5.0 nm	8179	1.28	0.46
	+ 5.0 nm	8183	1.28	0.79

3.3.5. Sensitivity: The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined based on the standard calibration curve and were found to be 0.238 μgmL^{-1} and 0.793 μgmL^{-1} respectively.

3.4. Degradation studies

In an attempt to develop a stability indicating assay method, the samples of tablet powder were subjected separately for different degradation conditions. All the degraded drug solutions after appropriate dilutions (100 μgmL^{-1}) with mobile phase (neutralization for the acidic and basic degradation) were injected in the chromatographic system. Typical chromatograms of all degraded samples are shown in Figures 3.4.1-3.4.3. The degraded samples were compared with a standard efavirenz. The developed method was capable of determining efavirenz in presence of its degradation products.

3.5. *In- vitro* dissolution studies from tablet dosage form

The developed method was successfully applied to determine the release rate pattern of the drug form the tablet dosage form in dissolution rate studies. The cumulative percentage of drug released versus time profile showed that more than 90% of efavirenz was dissolved in 1% w/v sodium lauryl sulphate within 30 min, at 50 rpm, 37±5°C by USP apparatus paddle type. The release rate constants for zero order, first order, Higuchi equation and Hixon-Crosswell cube root were calculated. From the *in-vitro* drug release studies, it was observed that Higuchi Equation was best fitted for the drug release kinetics in comparison to other models (Table-3.5.1.).

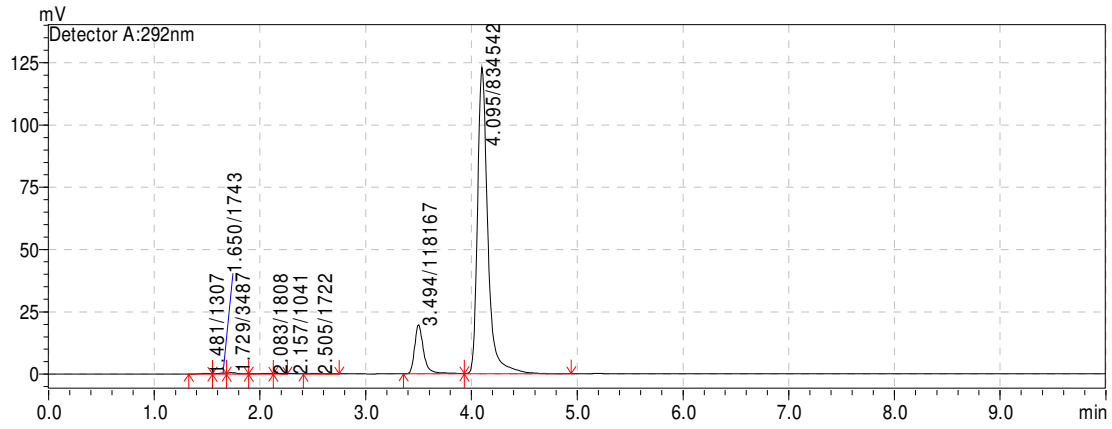

Figure 3.4.1. Acid degraded analytical peak

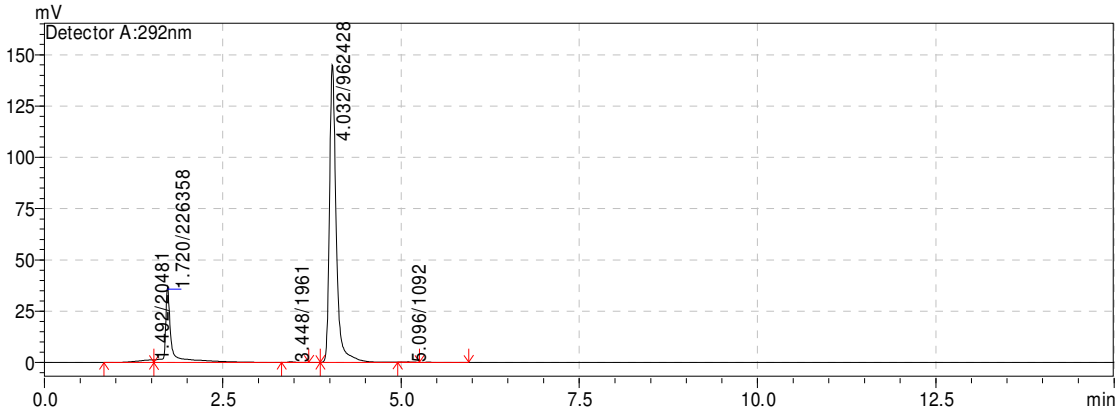

Figure 3.4.2. Basic degraded analytical peak

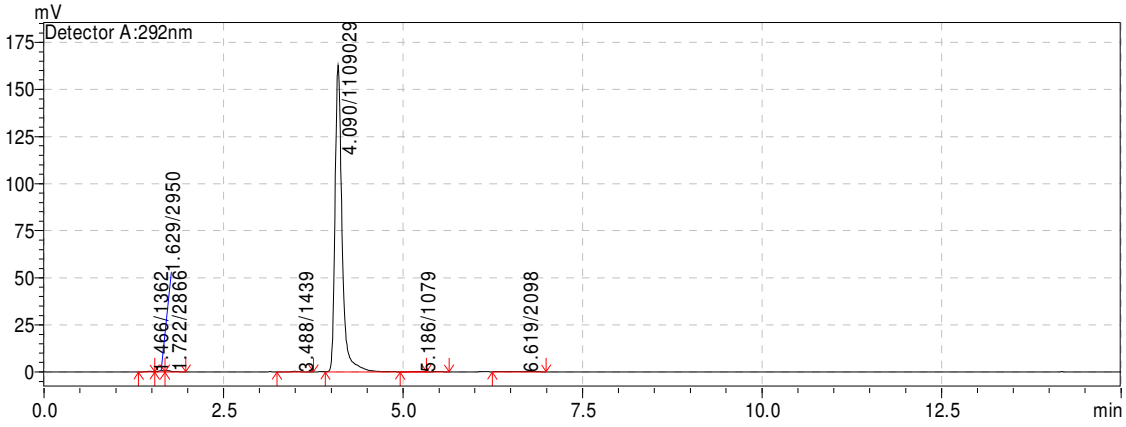

Figure 3.4.3. Oxidative degraded analytical peak

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Table 3.4.1. Degradation data

Stress conditions	Degradation (%)	% RSD
Acidic (5N Hcl), 24 hrs	49.08	0.89
Basic (1N NaOH), 24 hrs	85.83	0.25
6% v/v H_2O_2, 24 hrs	20.23	1.21
Thermal degradation at 105° C, 24 hrs	0	
Photo-degradation at 254 nm, 24 hrs	decoloration	

Table 3.5.1. Data of *In-vitro* dissolution studies

Kinetics	Equation	r^2 value
Zero order	Y=2.317x+26.304	0.8063
First order	Y=0.0299+1.0415	0.5077
Higuchi equation	Y=11.564+14.74	0.8063
Hixon-Crosswell	Y=0.0752x+2.2335	0.5757

4. CONCLUSION

Developed RP- HPLC method is precise, reliable and reproducible for the analysis of efavirenz formulation and its dissolution studies. This method has been found to be better because of its wide range linearity and readily available mobile phase, UV detection, lack of extraction procedures, low Rt, and no internal standard. The method can be successfully used for routine analysis of efavirenz in bulk drug, dosage forms and its dissolution studies without interference.

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