Method development and validation for the estimation of Efavirenz by RP-HPLC method

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Abstract

A new method was developed and validated for the estimation of Efavirenz by RP-HPLC method. The estimation was carried out on HITACHI L2130 with D Elite 2000 Software with Develosil ODS-HG 150mm*4.6mm 5µ column with a mixture of Acetonitrile:phosphate buffer in a ratio of 40:60 (v/v) as mobile phase. UV detection was performed at 246 nm. The method was validated for linearity, accuracy, precision, specificity and sensitivity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form. The retention time of Efavirenz was found to be 3.14 min and total run time was 10 min. at a flow rate of 1 mL/ min. The analytical method was validated according to ICH guidelines (ICH Q2b). The correlation coefficient (r2) was found to be 0.999. The percentage RSD was NMT 2% which proved the precision of the developed method. The LOD and LOQ values was found to be 1.7 and 5.1 respectively. This method represents a fast analytical procedure for the single quantization of Efavirenz.. The method is a menable to the routine analysis of large numbers of samples with good precision and accuracy.

KEYWORDS: Efavirenz, RP-HPLC Method, Simultaneous estimation Validation as per ICH guidelines.

Introduction:

Efavirenz:

Efavirenz (ABC) is a powerful nucleoside analog reverse transcriptase inhibitor (NRTI) used to treat HIV and AIDS. ABC is an analog of guanosine (a purine). Its target is the viral reverse transcriptase enzyme.

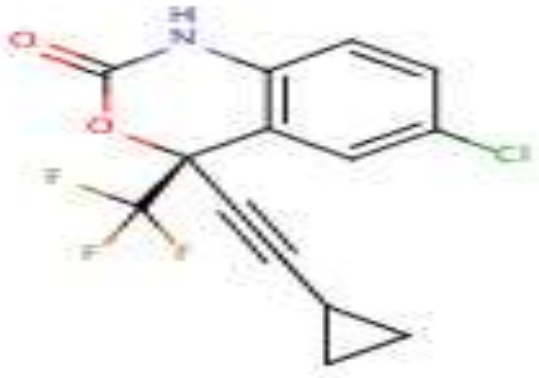


Fig 1 : Chemical structure of Efavirenz

Methods & materials:

Instrumentation:

The HPLC system employed was HITACHI L2130 with D Elite 2000 Software with Isocratic with UV-Visible Detector (L-2400) with Develosil ODS-HG 150mm*4.6mm 5µ column. ELICO SL-159 UV – Vis spectrophotometer. Ultra Sonicator (Wensar wuc-2L). P^H Analyzer (ELICO).

Reagents and chemicals:

The reference sample of was Efavirenz provided as gift samples from Aurobindo Pharma, Hyderabad, India. HPLC grade water and all other chemicals were purchased from Sd fine-Chem ltd, Mumbai, India.

Preparation of solutions:

Standard & sample preparation for UV-spectrophotometer analysis:

10 mg of Efavirenz standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.2 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Efavirenz, so that the same wave number can be utilized in HPLC UV detector for estimating the Efavirenz. While scanning the Efavirenz solution we observed the maxima at 246 nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectophotometer model UV-2450. The scanned UV spectrum is attached in The following page.

Mobile phase preparation:

The mobile phase used in this analysis consists of a mixture of water and Acetronitrile in a ratio of 70:30. 700 ml of this water was added and properly mixed with 300 ml of acetronitrile and a homogenous solution is achieved. This mobile phase was filled and sonicated for 15 minutes before using in the experiment.

10 mg of Efavirenz standard was transferred into 50 ml volumetric flask, dissolved & make up to volume with mobile phase.Further dilution was done by transferring 0.5 ml of the above solution into a 50 ml volumetric flask and make up to volume with mobile phase.

Preparation of sample solutions for accuracy studies:

Preparation of 80% solution:

0.8 ml of the sample stock solution was pipette out and transferred to 10 ml volumetric flask and made up to the volume with water. The solution was filtered through 0.45µm membrane filter.

Preparation of 100% solution:

1 ml of the sample stock solution was pipette out and transferred to 10 ml volumetric flask and make up the volume with water.

Preparation of 120% solution:

1.2 ml of the sample stock solution was pipette out and transferred to 10 ml volumetric flask and make up the volume with water. The solution was filtered through 0.45µm membrane filter.

Preparation of solution for precision :

10mg of sample was weighed accurately and transferred into 10 ml volumetric flask, about 5 ml of diluent was added and final makeup with 10ml with water, after sonicated for 5 minutes to dissolve it. The solution was filtered through 0.45µm membrane filter (Stock solution).

From this 5 ml of solution was pipetted out and transferred into 50 ml of volumetric flask and the volume was made up with water. The solution was filtered through 0.45µm membrane filter. The concentration of solution is 100µg/ml of Efavirenz.

From this 0.8ml of solution was pipette out and transferred into 10 ml of volumetric flask and the volume was made up with water. The solution was filtered through 0.45µm membrane filter. The concentration of solution is 8µg/ml of Efavirenz.

Preparation of solution for linearity (10-60ug/ml)

Weigh Accurately 10mg of sample is weighed and transferred into 100 ml volumetric flask, about 5 ml of diluent was added and final makeup with the volume with water ,After sonicated for 5 minutes to dissolve it. The solution was filtered through 0.45µm membrane filter (Stock solution).

From this 10 ml of solution was pipetted out and transferred into 100 ml of volumetric flask and the volume was made up with water. The solution was filtered through 0.45µm membrane filter. The concentration of solution is 100µg/ml of Efavirenz.

Results and discussion:

Selection of wavelength by uv-spectroscopy:

From the UV-visible spectrophotometric results, a detection wavelength of 246nm was selected. Because at this wavelength they showed maximum absorbance with good peak intensity, good peak shape and height was observed**.**

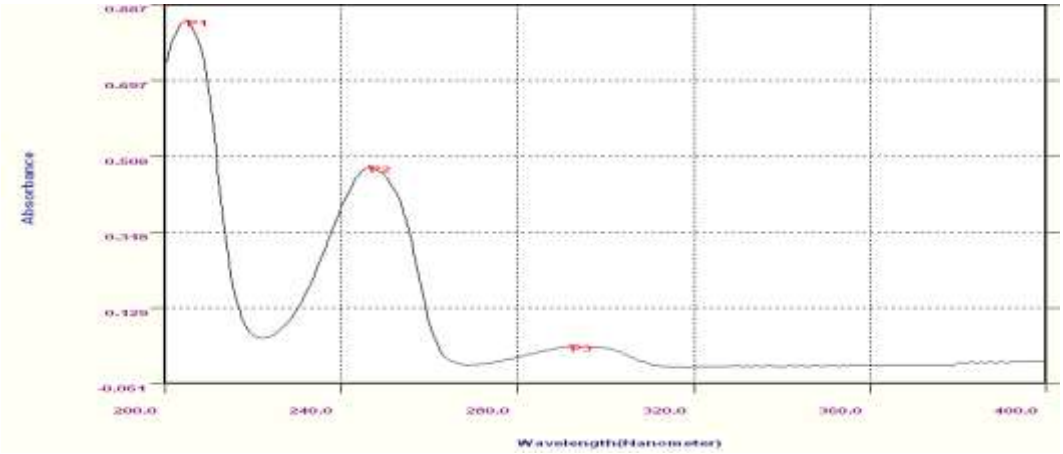


Fig no.2:UV Spectrum of the Efaverinz

Selection of Chromatographic Method:

Proper selection of method depends upon the nature of the sample(ionic/ionisable/neutral molecules), its molecular weight and solubility. The drug selected in the present study is polar in nature and hence Reverse phase method may be used. The RP-HPLC selected for the initial separation Because of its simplicity and suitable.

Table-1: HPLC parameters for Optimized method:

MoilePhase	Acetonitrile:phosphatebuffer40:60 (Ph2.7)Adjusted with OPA
Column	Develosil ODS-HG 150mm*4.6mm 5µ
Flowrate	1ml/min
DetectionWaelethng	246nm
Injectionolume	20µl
columnoentempreature	Ambient
Runtime	10min

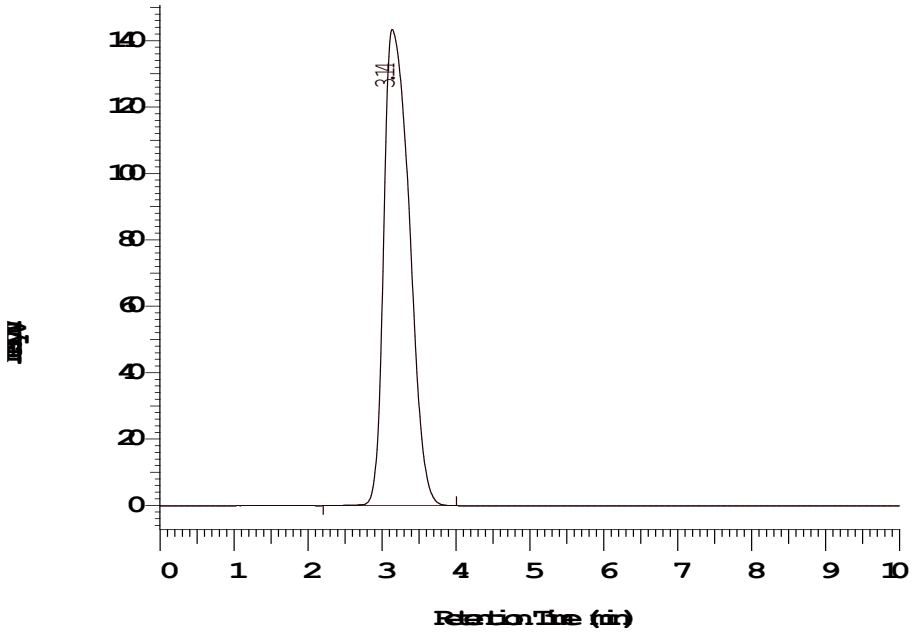


Fig. 3: Optimized Chromatogram

Table-2: Results of Optimized Chromatogram

Peak name	Rt	Peak area	Usp tailing
Efavirenz	3.14	3449091	0.77

Validation:

Linearity:

The linearity was checked over the concentration ranges of 10-100 µg/ml, for efavirenz.The calibration curves s were linear in the studied range and equation f the regression analysis wera obtained y = 82386x + 566 for efavirenz

Table-3: Results of Linearity for

s.no	Peak name	CONC	Rt	Peak area	% Area
1	EFAVIRENZ	16	3.11	1415536	100
2	EFAVIRENZ	24	3.11	2083592	100
3	EFAVIRENZ	32	3.11	2808838	100
4	EFAVIRENZ	40	3.14	3338936	100
5	EFAVIRENZ	48	3.11	4043959	100
6	EFAVIRENZ	56	3.11	4695400	100

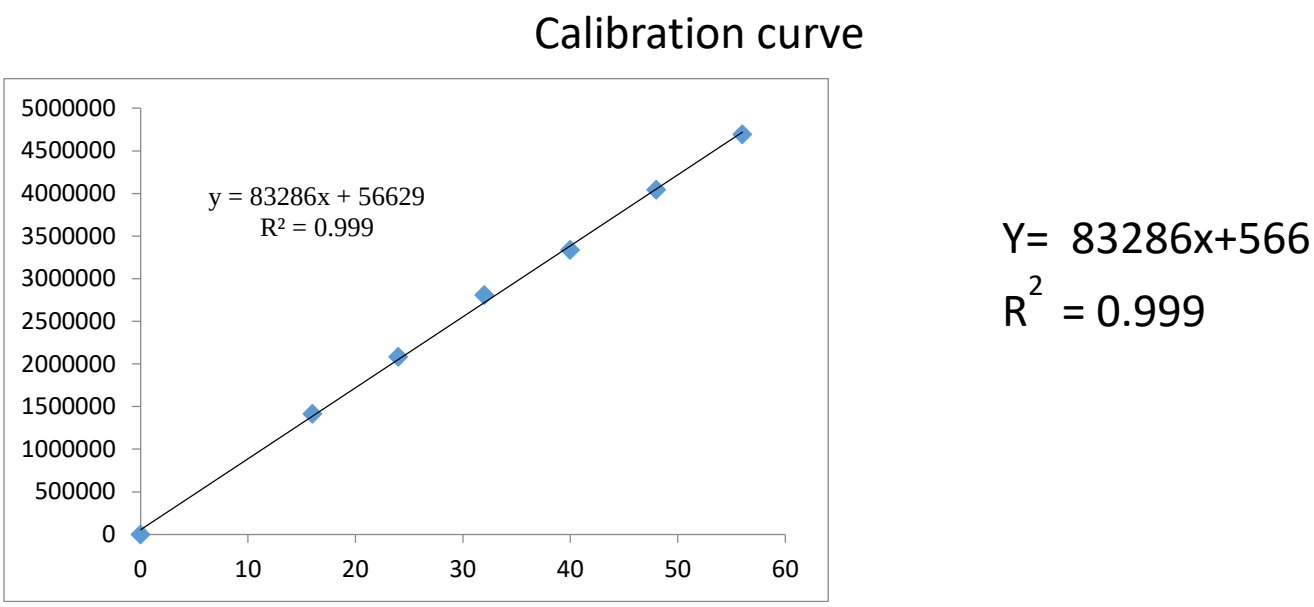


Fig.4:Calibration plot of Efavirenz

Accuracy:

Accuracy of the method was determined by Recovery studies. To the formulation (pre analysed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug.

Table-4: Results of Accuracy (80 %) for Efaverinz

S.NO	PEAK NAME	CONC	RT	PEAK AREA	%AREA
1	EFAVIRENZ	32	3.11	2708838	100
2	EFAVIRENZ	32	3.11	2716534	100
3	EFAVIRENZ	32	3.11	2772443	100
AVG			3.11	2732605	
SD			0	34714.65	
RSD			0	1.270387	

Table-5: Results of Accuracy (100 %) for Efaverinz

S.NO	PEAK NAME	CONC	RT	PEAK AREA	%AREA
1	EFAVIRENZ	40	3.13	3451068	100
2	EFAVIRENZ	40	3.14	3381353	100
3	EFAVIRENZ	40	3.13	3415266	100
AVG			3.133333	3415896	
SD			0.005774	34861.77	
RSD			0.184261	1.020575	

Table-6: Results of Accuracy (120 %) for Efaverinz

S.NO	PEAK NAME	CONC	RT	PEAK AREA	%AREA
1	EFAVIRENZ	48	3.11	4014923	100
2	EFAVIRENZ	48	3.11	4043959	100
3	EFAVIRENZ	48	3.11	4083229	100
AVG			3.11	4047370	
SD			0	34280.54	
RSD			0	0.846983	

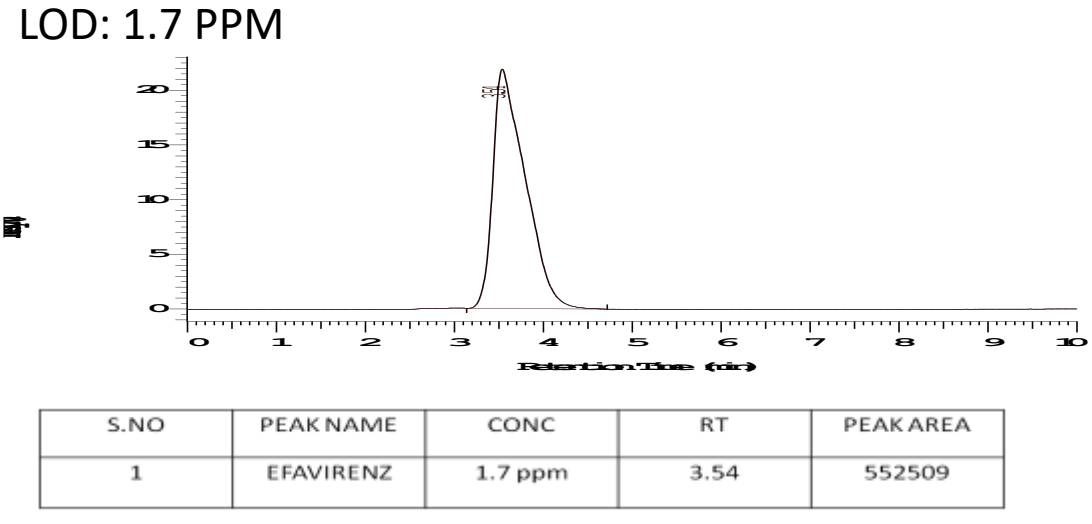
PRESSION:

The precision study was performed for six injections of Efavirenz. Each standard was injected into chromatographic system. The area of each standard injection was used for calculation of %RSD.

Table 7: precision results for Efavirenz

S.NO	PEAK NAME	CONC	RT	PEAK AREA	%AREA
1	EFAVIRENZ	40PPM	3.14	3449091	100
2	EFAVIRENZ	40PPM	3.13	3338936	100
3	EFAVIRENZ	40PPM	3.13	3451068	100
4	EFAVIRENZ	40PPM	3.14	3381353	100
5	EFAVIRENZ	40PPM	3.13	3415266	100
6	EFAVIRENZ	40PPM	3.14	3420374	100
AVG			3.135	3409348	
SD			0.005477	42937.7	
%RSD			0.174712	1.259411	

Limit of detection: LOD was calculated by using standard deviation and slope values obtained from calibration curve. LOD of Efavirenz was found to be 1.7.

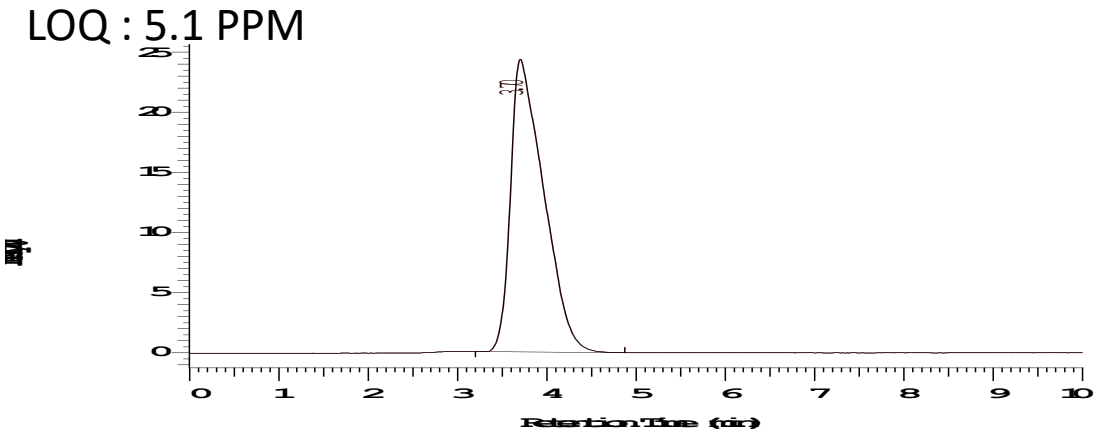


S.NO	PEAK NAME	CONC	RT	PEAK AREA
1	EFAVIRENZ	1.7 ppm	3.54	552509

Fig.5: LOD of Efavirenz

Limit of Quantification:

LOQ of Efavirenz was found to be5.1.



S.NO	PEAK NAME	CONC	RT	PEAK AREA
1	EFAVIRENZ	5.1 ppm	3.7	626511

Fig.6:LOQ of Efavirenz

Assay of Efavirenz tablets:

Brand name of tablets	Labeled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Efavir (Cipla Ltd)	100	100.34 (±0.06)	100.114 (±0.48)

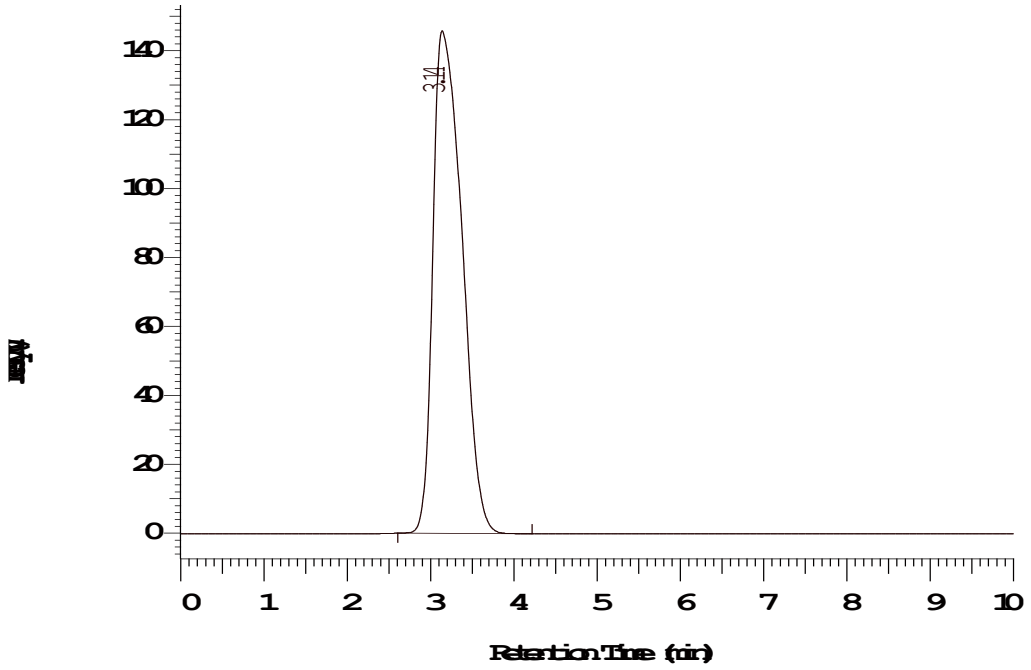


Fig.7:Assay chromatogram of Efavirenz formulation

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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