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Original Article

Method Development and Validation of Efavirenz by RP-HPLC Method

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ABSTRACT

A selective and sensitive stability-indicating high-performance liquid chromatographic method was developed and validated for the determination of Efavirenz. 10 mg of Efavirenz was dissolved in mobile phase. 25 mg of Efavirenz standard was transferred into 25ml volumetric flask, dissolved in mobile phase & make up the volume with mobile phase. Accurately weighed around 25mg of Efavirenz working standard, taken into a 25ml volumetric flask, then dissolved in mobile phase and diluted to volume with the mobile phase to obtain a solution having a known concentration of about 1000 mcg/ml. To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of EFAVIRANZ were taken and added to the pre-analyzed formulation of concentration 10g/ml. From that percentage recovery values were calculated.The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug. Efavirenz (API). Influence of small changes in chromatographic conditions such as change in flow rate (0.1ml/min), Temperature (20C), Wavelength of detection (2nm) & acetonitrile content in mobile phase (2%) studied to determine the robustness of the method are also in favour of (Table-4, % RSD < 2%) the developed RP-HPLC method for the analysis of Efavirenz. Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up the volume with mobile phase.

Keywords: Efavirenz e., RP-HPLC, Acetonitrile (30:70). & Retention time.

1. INTRODUCTION

A large number of chemically bonded stationary phases based on silica are available commercially. Silica based stationary phases are still most popular in reversed phase chromatography however other absorbents based on polymer (styrene-di-vinyl benzene copolymer) are slowly gaining ground.The retention decreases in the following order: aliphatics > induced

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dipoles (i.e. CCl4) > permanent dipoles (e.g.CHCl3) > weak Lewis bases (ethers, aldehydes, ketones) > strong Lewis bases (amines) > weak Lewis acids (alcohols, phenols) > strong Lewis acids (carboxylic acids). [1-3] Also the retention increases as the number of carbon atoms increases. As a general rule the retention increases with increasing contact area between sample molecule and stationary phase i.e. with increasing number of water molecules, which are released during the adsorption of a compound. Branched chain compounds are eluted more rapidly than their corresponding normal isomers. In reversed phase systems the strong attractive forces between water molecules arising from the 3-dimentional inter molecular hydrogen bonded network, from a structure of water that must be distorted or disrupted when a solute is dissolved. Only higher polar or ionic solutes can interact with the water structure. Non- polar solutes are squeezed out of the mobile phase and are relatively insoluble in it but with the hydrocarbon moieties of the stationary phase. [4, 5]Chemically bonded octadecylsilane (ODS) an alkaline with 18 carbon atoms is the most popular stationary phase used in pharmaceutical industry. Since most pharmaceutical compounds are polar and water soluble, the majority of HPLC methods used for quality assurance, decomposition studies, quantitative analysis of both bulk drugs and their formulations use ODS HPLC columns. The solvent strength in reversed phase chromatography is reversed from that of adsorption chromatography (silica gel) as stated earlier. Water interacts strongly with silanol groups, so that, adsorption of sample molecules become highly restricted and they are rapidly eluted as a result. Exactly opposite applies in reversed phase system; water cannot wet the non-polar (hydrophobic) alkyl groups such as C18 of ODS phase and therefore does not interact with the bonded moiety. Hence water is the weakest solvent of all and gives slowest elution rate. The elution time (retention time) in reversed phase chromatography increases with increasing amount of water in the mobile phase. In normal phase chromatography, the stationary phase is a polar adsorbent and the mobile phase is generally a mixture of non-aqueous solvents. The silica structure is saturated with silanol groups at the end. These OH groups are statistically disturbed over the whole of the surface. The silanol groups represent the active sites (very polar) in the stationary phase. This forms a weak type of bond with any molecule in the vicinity when any of the following interactions are present. [6, 7]

Efavirenz is an HIV-1 specific, non-nucleoside, reverse transcriptase inhibitor (NNRTI).

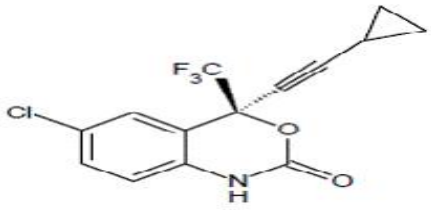

Fig 1: Structure of Efavirenz

2. EXPERIMENTAL WORK

2.1 Materials and Methods

Selection of wavelength: 10 mg of Efavirenz was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Efavirenz. The isobestic point was taken as detection wavelength. [8]

2.2 Chromatographic trials for simultaneous estimation of Efavirenz by RP- HPLC.

Chromatographic conditions

Column	:	Agilent (4.6×150mm) 5µ
Mobile phase ratio	:	water: ACN (40:60% v/v)
Detection wavelength	:	274 nm
Flow rate	:	0.7 ml/min
Injection volume	:	10µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient

Run time : 10min

Retention time : 2.425min

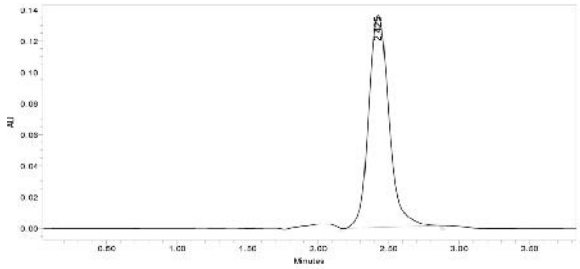

Fig 2: Chromatogram showing injection

The separation was good, peak shape was good, so we conclude that there is no required for decrese the retention times of peak, so it is taken as final method.

Preparation of mobile phase.

Mix a mixture of 40 ml water (40%) and 60 ml of Acetonitrile (60%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Preparation of the individual Efavirenz standard preparation

10 mg of Efavirenz working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the Efavirenz standard and sample solution

2.3 Sample solution preparation

10 mg of Efavirenz tablet powder was accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

2.4 Standard solution preparation

10 mg Efavirenz working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. [9, 10]

Repeatability

2.5 Preparation of stock solution

10 mg of Efavirenz working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. [11, 12]

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Table 1: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	4.15
2	Asymmetry	T ≤ 2	Efavirenz=0.59
3	Theoretical plate	N > 2000	Efavirenz=3698

Table.2: Accuracy Readings

	Concentration (µg/ml)		%Recovery of	
Sample ID	**Pure drug**	**Formulation**	**Pure drug**	**Statistical Analysis**
S_1 : 80 %	8	10	99.63	Mean= 99.67667%
S_2 : 80 %	8	10	99.92	S.D. = 0.223681
S_3 : 80 %	8	10	99.48	% R.S.D.= 0.224407
S_4 : 100 %	10	10	99.19	Mean= 99.19%
S_5 : 100 %	10	10	99.25	S.D. = 0.06

S_6 : 100 %	10	10	99.13	% R.S.D.= 0.06049
S_7 : 120 %	12	10	99.25	Mean= 99.49%
S_8 : 120 %	12	10	99.54	S.D. = 0.219317
S_9 : 120 %	12	10	99.68	% R.S.D. = 0.220441

3. RESULTS AND DISCUSSION

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table 39.

The performance test of the method has been conducted on market sample. As per the label claim, each tablet contains 600mg of Effavirenz. To estimate this powder of the tablet equivalent to 25mg of Effavirenz has been dissolved in 25ml of the mobile phase. Further dilution was done by taking 1ml of this solution in 10ml volumetric flask, dissolve and making up the volume upto the mark with mobile phase by which 100ppm solution was prepared. Again same process is repeated to make 10ppm from 100ppm solution. To extract the drug in the solution, it has been sonicated for 5 minutes followed by cyclo-mixing for 5 minutes. Resulting solution was filtered by using Millipore syringe filter (0.45 micron). Resulting clear solution was injected in HPLC in duplicate as per the above mentioned HPLC method. Chromatogram obtained for the injection is shown below with Rt of 2.90 mins.

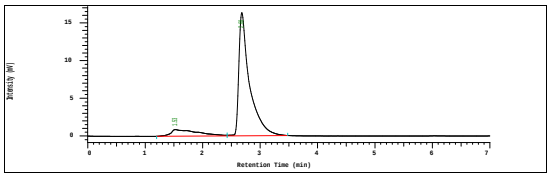

Fig 2: Chromatogram for specificity sample

Recovery study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of EFAVIRANZ were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated.

Repeatability- The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of five replicates of a fixed amount of drug. Efavirenz (API). The percent relative standard deviation were calculated for Efavirenz are presented.

Table 3: Result of retention time

HPLC Injection Replicates of Efavirenz std	Retention Time	Area
Replicate – 1	2.69	789939
Replicate – 2	2.69	790996
Replicate – 3	2.69	809774
Replicate – 4	2.69	796107
Replicate – 5	2.69	821313
Average	2.69	801625.8
Standard Deviation	0.0	13546.31
% RSD	0.00	1.689855

Linearity & Range:

The calibration curve showed good linearity in the range of 0-35 µg/ml, for Efavirenz (API) with correlation coefficient (r^2) of 0.996 (Fig. 4). A typical calibration curve has the regression equation of y = 76594x – 24947 for Efavirenz.

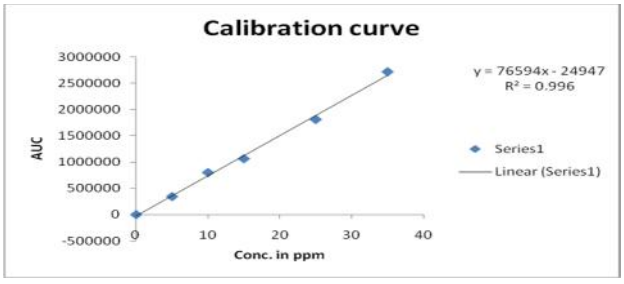

Fig. 3: Calibration curve of Efavirenz (API).

Table 4: Result of AUC

Conc.	AUC (n -=6)
0	0
5	343726
10	801625

15	1064970
25	1811846
35	2721573

Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-4, % RSD < 2%) the developed RP-HPLC method for the analysis of Efavirenz(API).

Table 5: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.25
Flow (0.9 ml/min)	0.86
Temperature (27^{0}C)	0.24
Temperature (23^{0}C)	0.75
Wavelength of Detection (206 nm)	0.31
Wavelength of detection (204 nm)	0.15

4. CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Efavirenz, different chromatographic conditions were applied & the results observed are presented in previous chapters.Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.In case of RP-HPLC various columns are available, but here Develosil C18, 5µm, 150 x 4.6 mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good.Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). The drug was found to be highly soluble in acetonitrile, soluble in methanol. Drug was insoluble in water. Using these solvents with appropriate composition newer methods can be developed and validated. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Efavirenz it is evident that most of the HPLC work can be accomplished in the wavelength range of 240-320 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay and which can help in the analysis of Efavirenz in different formulations.

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