Research Article

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VALIDATION OF AN ISOCRATIC HPLC ASSAY OF EFAVIRENZ IN PHARMACEUTICAL FORMULATIONS OF DRUG SUBSTANCE

G.Satyavathi, M.Sidhakar, V.Rajesh, K.Satyanarayana, D.Rama Krishna, G.Prathap, J.Suvarchala, Rambabu .Kuchi,Dept of P.G chemistry, D.N.R.College, Bhimavaram, W.G

Name: Rambabu.Kuchi

Address: Bhimavaram

Email: rambabu.kuchi@gmail.com

Phone: 09440538478

Corresponding Author

ABSTRACT

This paper describes the validation of an isocratic HPLC method for the assay of Efavirenz . This work is concerned with application of simple, precise, accurate and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Efavirenz. The method employ a Zodiac C18 column with Methanol and acetonitrile 80:20 (v/v) as the mobile phase and ultraviolet (UV) detection at 280 nm. A linear response (r>0.998) was observed over the concentration range of 15-45 μg/ml. The results showed good recoveries, ranging from 98.77 to 101.45% and the relative standard deviation (R.S.D.) intra-day and inter-day were ≤ 0.80%.. As the method could effectively separate the drug from its degradation products, it can be used in stability studies for drug substance.

Key words: Efavirenz , Method development, Validation, 280 nm

INTRODUCTION

Efavirenz (brand names Sustiva and Stocrin) 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 postexposure prophylaxis regimen to reduce the risk of HIV infection in people exposed to a significant risk (e.g. needlestick injuries, certain types of unprotected sex etc.).
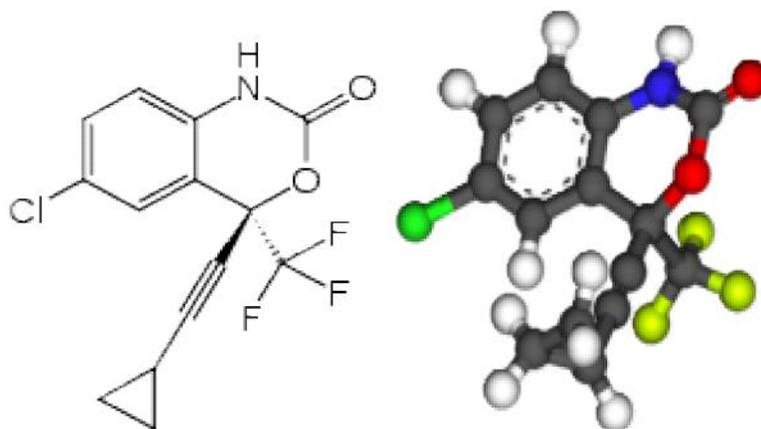
Fig -1

Chemically, efavirenz is (S)-6-chloro (cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-,1-benzoxazin-2-one. The usual adult dose is 600 mg once a day. It is usually taken on an empty stomach at bedtime to reduce neurological and psychiatric adverse effects. Efavirenz was combined with the popular HIV medication Truvada, which consists of tenofovir and emtricitabine, all of which are reverse transcriptase inhibitors.

Several methods have been reported for determination of efavirenz. Careri et al. (1993) achieved separation of alkynes by reversed phase HPLC using ruthenium complexes. Gita et al. (2008) and Agnes et al. (2008) reported separation of efavirenz in human plasma byusing reversed phase HPLC technique using C18column. There was very few HPLC methods have been reported35 for simultaneous estimation of Efavirenz in pharmaceutical dosage form, which prompted to pursue the present work. The objective of the present work is to develop and validate new analytical methods for simultaneous determination of Efavirenz

EXPERIMENTAL

Materials

Working standard of Efavirenz was obtained from well reputed research laboratories. HPLC grade water, methanol, Acetonitrile was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251),Analytical column Zodiac100-5 C18. 250×4.6mm, Electronic balance-DENVER (SI234), A manual Rheodyne injector with a 20 µl loop was used for the injection of sample., PEAK LC software were used. UV 2301 SPECOPHOTOMETER was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of Efavirenz were scanned in the range of 200 -400 nm against mobile phase as a blank. Efavirenz showed maximum absorbance at 280 nm. So the wavelength selected for the determination of Efavirenz was 280 nm.

Chromatographic equipment and conditions

The development and validation of the assay was performed on A Series 200 HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251),Analytical column Zodiac100-5 C18. 250×4.6mm, , manual injector rheodyne valve) with 20µL fixed loop, PEAK LC software were used.

The mobile phase consisted of a Methanol and acetonitrile 80:20 (v/v). Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1.0 ml/min. Detection was performed at 280 nm with 8min runtime.

Standard and sample solutions

A 10 mg amount of Efavirenz reference substance was accurately weighed, dissolved in mobile phase and diluted to volume in a 100 ml volumetric flask. Standard solution was obtained by diluting the above solution with mobile phase to a concentration of 30 µg/ml. A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of, Efavirenz was accurately weighted and quantitatively transferred into a 100 ml volumetric flask. Approximately 30 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 10 µg/ml.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness

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RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000 13. In all

cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. Systemsuitability parameters were shown in Table:1.

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 1, 0.5, 0.25, 0.12, 0.06, 0.03 mg/ml for Efavirenz. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = -1299 + 89210x (r= 0.9986). The R.S.D. values of the slope were 2811646 (n=3) and the R.S.D. of y-intercept was 81392.71 (n=3). Linearity values can shown in Table :2

Precision

Method precision was demonstrated by the assay of a series of six samples, prepared as described above, on three consecutive days. The inter- (RSD-1.742)and intra-(RSD-1.236)day means and relative standard deviation (R.S.D.) were calculated (Table 1). The assay method precision acceptance criteria set in the validation were a R.S.D. ≤ 2.0% for each data s. The data of Table 2 meet these acceptance criteria.

Accuracy

The accuracy of the method was evaluated by determination of the recovery of Efavirenz on two days at six levels concentration. Tablets and capsules sample solutions were spiked with Efavirenz standard solution, corresponding to 75 to 125% of the nominal analytical concentration (30 µg/ml). The results showed good recoveries ranging from 98.77 to 101.45%. The mean recovery data obtained for each level as well as for all levels combined (Table 2) were within 2.0% of the label claim for the active substance with an R.S.D. < 2.0%, which satisfied the acceptance criteria set for the study.recovery values can shown in Table:3.

LIMIT OF DETECTION TEST (LOD)

The sample was dissolved by using Mobile Phase and injected until peak was diapered. After 0.0019µg/ml dilution Peak was not clearly observed. So it confirms that 0.0019µg/ml limit of Detection. And Limit of Quantification is 0.00643 µg/ml.

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chro matographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

CONCLUSION

The proposed method for the assay of Efavirenz in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its degradation products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.
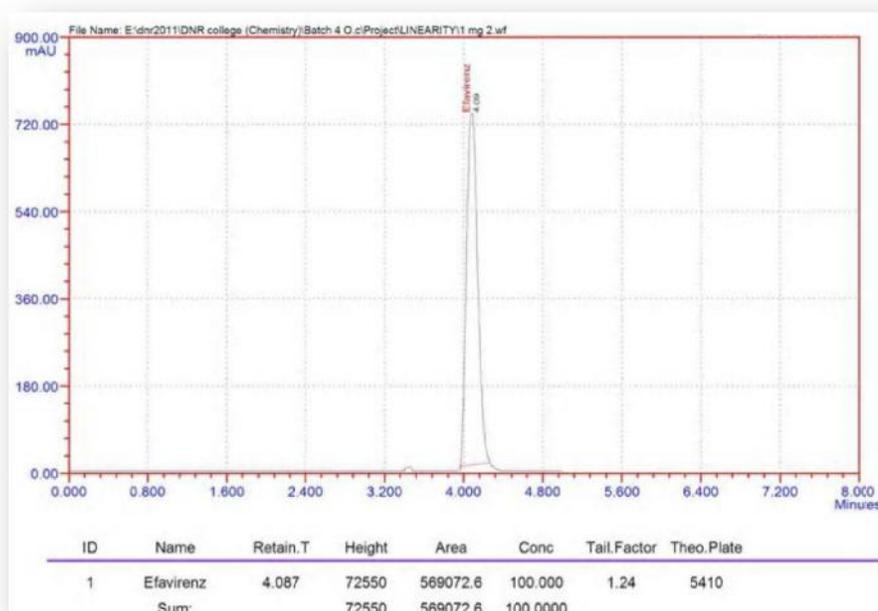

Fig: 2 Chromatogram of Efavirenz

Table 1: Statistical analysis of parameters required for system suitability testing of the HPLC method

System Suitability Parameter	Efavirenz
Retention Time	4.08min
Tailing factor	1.24
Theoretical plate	5410

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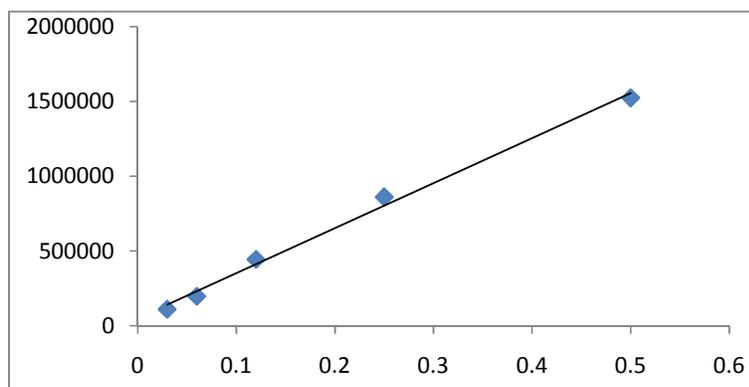
Fig :3

Table 3: Data of recovery study for Efavirenz by HPLC method

Amount taken (μg/ml	Amount added (μg/ml)	Amount found (μg/ml)	% Recovery ± S.D(n=3)
10	2	12.85	99.0
10	4	13.52	98.3
10	6	15.12	102.5

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