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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF EFAVIRENZ IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A specific, accurate, precise and reproducible stability indicating HPLC method has been developed and subsequently validated for Efavirenz in commercial tablets. The proposed HPLC method utilizes Agilent eclipse XDB C18 column (150 mm - 4.6 mm i.e., 5 µm) and mobile phase consisting of Acetonitrile: Water (60:40 v/v) at a flow rate of 1.2 mL/min. Quantitation was achieved with UV detection at 240 nm based on peak area with linear calibration curves at concentration range 12.5-200.0µg/mL for Efavirenz (R^2> 0.999). The method was validated in terms of accuracy (% recovery 99.7%), precision (%RSD 1.1), linearity, limits of detection (3.6 ng/ml), limits of quantitation (11 ng/ml), assay (100.4%), and robustness. This method has been successively applied to pharmaceutical formulation and no interference from the tablet excipients was found when Efavirinz drug product was exposed to acid, base, oxidation, thermal and photolytic stress conditions, the stressed samples was analyzed by the proposed method. As the proposed method could effectively separate the drug from its degradation products, it can be employed as stability-indicating method for the determination of instability of these drugs in bulk and commercial pharmaceutical formulations. [(1)]

KEY WORDS

HPLC method, Efavirenz.

INTRODUCTION

Efavirenz is chemically (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-1H-3, 1-benzoxazin-2-one (Figure. 1). It is a white powder form and used as antiretroviral agent, for the treatment of HIV infection. It has an empirical formula of $C_{14}H_9ClF_3NO_2$ and molecular weight of 315.675. Efavirenz belongs to a class of antiretroviral drugs known as 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-1) [(1)].

Literature survey reveals that very few analytical methods have been established for the determination of Efavirenz [(3-6)]. Development of Rapid UV Spectrophotometric Method for the Estimation of Efavirenz in Formulations, High-performance liquid chromatographic method for the determination of HIV-1 non-nucleoside reverse transcriptase inhibitor, Efavirenz in plasma of patients during highly active antiretroviral therapy [(3-6)], Development of a competitive immunoassay for Efavirenz.

The stability of a drug substance or drug product is defined as its capacity to remain within established specifications, i.e. to maintain its identity, strength, quality, and purity until the retest or expiry date. Stability testing of an active substance or finished product provides evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example

temperature, humidity, and light. Knowledge from stability studies is used in the development of manufacturing processes, selection of proper packaging and storage conditions, and determination of product shelf-life.

The objective of this work was to develop a new, simple, economic, rapid, precise, and accurate stability-indicating HPLC method for quantitative analysis of Efavirenz, and to validate the method in accordance with ICH guidelines.

EXPERIMENTAL MATERIALS AND METHODS

CHEMICALS AND REGENTS:

Pure standard of Efavirenz (Assigned purity 99.98%) was obtained as a gift sample from Mylan Pvt. Ltd, Hyderabad, India. The gift sample was used as standard without further purification. HPLC grade water, Acetonitrile and methanol, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide, (S.D.Fine chemicals, Mumbai, India), was used throughout the experiment. Commercial pharmaceutical preparation (Efavir) which was claimed to contain 200mg of Efavirenz was used in analysis.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

High performance liquid chromatography, Shimadzu auto sampler with PDA detector was used. The Lab solution software was used for acquisition, evaluation and storage of chromatographic data. Isocratic elution of mobile phase comprising of Acetonitrile: Water in the ratio of 60:40 % (v/v) with flow rate of 1.2mL min was performed on C18 column Agilent Eclipse XDB (150x 4.6 mm, 5µm). The effluent was detected at 240 nm. The retention time of Efavirenz was 4.937 minutes. The column temperature was maintained at ambient and the volume of injection was 20µl. Prior to injection of analyte, the column was equilibrated for 30- 40 min with mobile phase.

Different kinds of equipment's viz Analytical weighing balance (Shimadzu AX 200), Sonicator (model SONICA 2200MH), Water purification system, Vacuum pump (model XI 5522050 of Millipore), Millipore filtration kit for solvents and sample filtration was used throughout the experiment.

PREPARATION OF MOBILE PHASE

The HPLC grade solvents of Acetonitrile and Water in the ratio of 60:40 % (v/v) was used for the preparation of Mobile phase, contents of the mobile phase was filtered through a 0.45 µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1.2 mL/min baseline equilibration.

PREPARATION OF STANDARD SOLUTION

A stock solution of drug was prepared by dissolving 100 mg of Pure Efavirenz in a 100 ml volumetric flask containing 10 mL of methanol (HPLC grade) to dissolve the drug, sonicated for about 15 min and then made up to volume with mobile phase. Pipette out 10ml of above solution and made up to the volume to 100ml with diluent (mobile phase) to produce 100mcg/mL.

PROCEDURE FOR SAMPLE SOLUTION (FROM FORMULATION)

Twenty tablets were weighed accurately and powdered. An amount of the powder equivalent to 100 mg of Efavirenz was dissolved in 100 ml volumetric flasks containing 10 mL of methanol (HPLC grade) to dissolve the drug, sonicated for about 15 min and then made up to volume with mobile phase. Pipette out 10ml of above solution and made up the volume to 100ml with diluent (mobile phase) to produce 100mcg/mL.

OPTIMIZATION OF METHOD

The goal of this study was to develop a single isocratic phase HPLC method for the determination of Efavirenz. During optimizing some important parameters like pH of the mobile phase, concentration of the acid or buffer solution, percentage and type of the organic modifier, etc., was tested for a good chromatographic separation.

Trials showed that with reverse phase aAgilent Eclips XDBC18 column gives symmetric and sharp peaks. Mobile phase composition of Acetonitrile and water (60:40v/v) at flow rate of 1.2mL/min showed good separation. The typical optimized chromatogram was shown in Figure 2.

STABILITY STUDIES [7]

HYDROLYSIS (ACID AND ALKALI)

Initially for hydrolytic degradation the Efavirenz was dissolved in known amount of methanol and diluted with 0.1N HCl or 0.1N NaOH to obtain a concentration of 100mcg/mL. After completion of degradation process, both the solutions were neutralized with acid or base, as necessary and diluted with the mobile phase to achieve a concentration of 10mcg/mL. The solution for hydrolysis was prepared in methanol and 0.1 N HCl and 0.1N NaOH (60:40 v/v). The prepared solutions in

acid were injected to the chromatographic system at 0 h (immediately after preparing the solution) and after reflux at 60^{0}C about 2h and the solutions prepared in alkali

was injected at 0 h and after reflux at 60^{0}c about 2h. The respective chromatogram was recorded for the study of extent of degradation.

PEROXIDE DEGRADATION

The solution for peroxide degradation was prepared in methanol and 3% hydrogen peroxide (60:40 v/v). The prepared solution was refluxed at 60^{0}c about 2h and injected into chromatographic system after 2 h. The respective chromatogram was recorded for the study of extent of degradation.

THERMAL DEGRADATION AT DIFFERENT TEMPERATURE AND DIFFERENT TIME INTERVAL

Sufficient amount of Efavirenz was placed in a petri dish and kept in the hot air oven at 80^{o}C for 24 hours. Aliquots was withdrawn initially (0 hrs.) and at different time points (30mins, 1, 2, 4 and 8hr); diluted to 10 µg/ml with mobile phase and injected into the HPLC system. The standard solution of 10µg/ml was considered as 100 % and the percentage degradation of drug was calculated by area normalization method.

PHOTOCHEMICAL DEGRADATION

The photochemical stability of the Efavirenz was studied by exposing them ethanolic stock solution to direct sunlight for 8h (from 9 AM to 5 PM, at 20^{o}C).

METHOD VALIDATION

The chromatographic conditions was validated by evaluating linearity, recovery, method and system precision, accuracy, system suitability, solution stability, limit of detection(LOD), Limit of Quantification (LOQ), robustness, ruggedness studies in accordance with ICH guideline Q2(R1). [8]

RESULTS AND DISCUSSIONS

OPTIMIZED CONDITIONS

Developed method was optimized for different parameters. Optimized chromatogram was given in Figure 2.

STABILITY STUDIES

Efavirenz contains 9 impurities (A, B, C, D, E,F,G,H,I), in that some impurities are enantiomers, hence when drug is exposed to degradation conditions they may converted to impurities.

Drug was highly degraded in base hydrolysis study due the instability of functional enantiomers. In light stress and thermal stress conditions moderate degradation was observed, partially degraded in acid and oxidation stress conditions. The degradation results were shown in table: 1. Comparison of degradation results were given in Figure 3.

Table: 1 Results of degradation studies under different stress conditions

Stress Condition	Drug substance % degradation	Drug product % degradation
Acidic	8.2	8.09
Alkaline	60.1	54.1
Oxidative	19.5	16
Thermal	53	51.3
Photo stability	35	29

Table: 2 Linearity results of Efavirenz

Concentration	Area
12.5	176231
25	349212
50	697032
100	1413256
125	1723452
150	2132121
200	2832561

Table: 3 Accuracy results of Efavirenz

Spiked Levels	Area	Drug Added	Drug Recovered	%Recovered
50	707325	50	49.6	99.2
100	1414671	100	99.8	99.8
150	2122050	150	150.1	100
Average				99.7

Table: 4 Intraday precision of Efavirenz

RT	Area
4.962	1412134
4.832	1390212
4.897	1414672
4.932	1393213
4.901	1437297
4.928	1413289
4.962	1412134
Avg	1410136.2
SD	15575.527
% RSD	1.1

Table: 5 Results of robustness (Change in the mobile phase)

S.No	Change in Mobile Phase (Acetonitrile:Water)	System Suitability Results	
		USP Plate Count	USP Tailing
1	70:40	10534	0.91
2	60:40	10250	0.94
3	60:50	10572	0.91

Table: 6 Results of robustness (Change in the Flow rate)

S.No	Change in Flow Rate	System Suitability Results	
		USP Plate Count	USP Tailing
1	1.0	9875	0.93
2	1.2	10250	0.94
3	1.4	10756	0.95

Table: 7 Assay of Efavirinz

Sample Area	Standard Area
1413256	
1426729	1414671
1420932	
Average	1420305
% Assay	100.4

Fig 1: Structure of Efavirenz

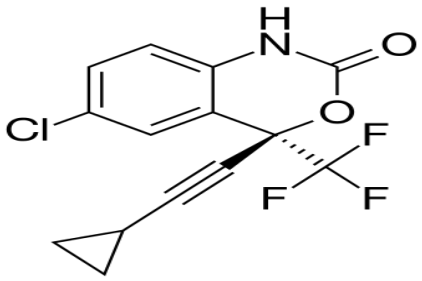


Fig 2: Optimized Chromatogram of Efavirinz

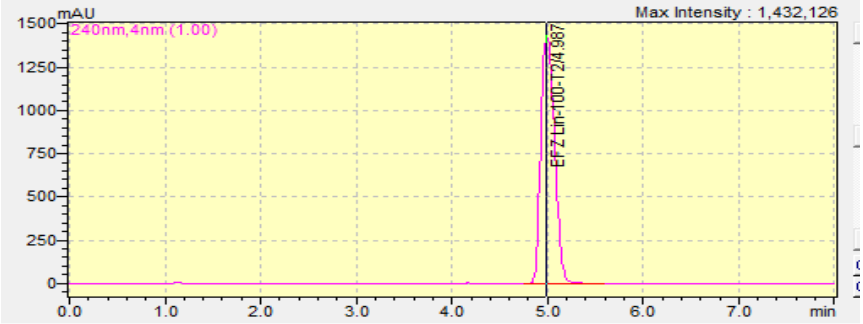


Fig3: Results of degradation studies under different stress conditions

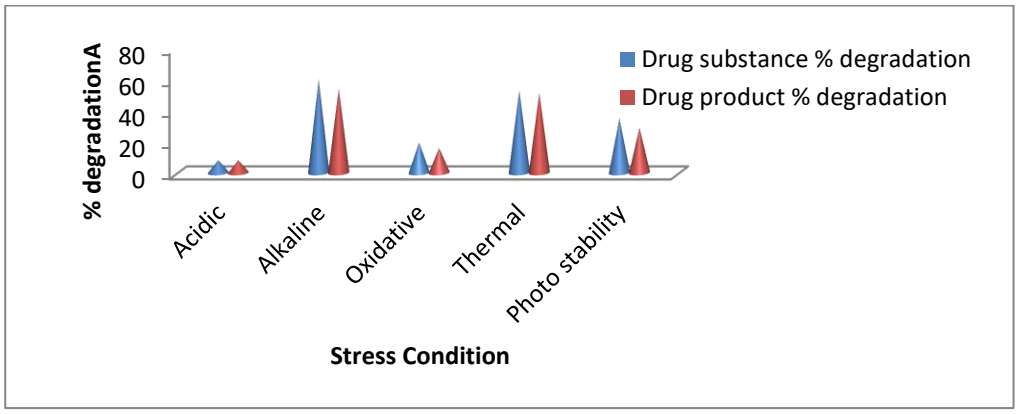
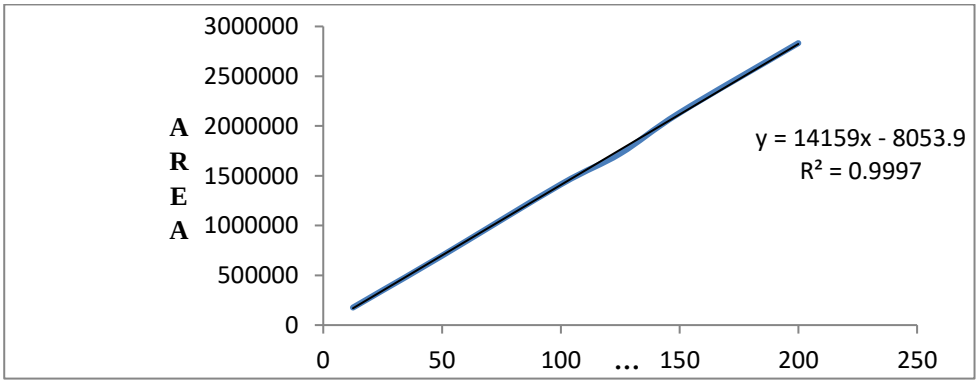


Fig4: Linearity results of Efavirenz



VALIDATION OF ANALYTICAL METHOD

LINEARITY

Acceptance criteria: Coefficient of correlation (r^2) should be greater than 0.999

PROCEDURE: Six sets of the drug solution were prepared in the mobile phase containing Efavirenz at a concentration of 12.5 to 200 mcg/ml. Each of these drug solutions was injected in six concentrations in three replicates times into the column, the peak area and retention times was determined. Results were shown in Figure 4 and Table: 2.

RESULT

Correlation coefficient (r^2) of Efavirenz was found to be 0.999, indicating the linearity and the method is linear between the concentrations of 12.5-200 mcg/mL, and y+14159x-8053.9.

ACCURACY

The accuracy is the closeness of the measured value to the true value for the sample. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. A series of solutions were prepared in triplicate by spiking the known standard concentrations of Efavirenz in the range of 50-150% on the tablet solution and analyzed. The accuracy of method was provided at three different concentration levels at 50, 100, and 150 µg/ml of Efavirenz standard. Each concentration triplicate samples were injected and average % recovered was calculated. The average % recovery was found to be 99.7%. Results was confirming that the method is accurate and free from any positive or negative interference of the excipients. The recovery data was generated for Efavirenz are presented in the Table No.3.

RESULT

The percentage recovery by the proposed method was ranging from 99.2 to 100.0 % indicating no interference of the tablet excipients with drug under analysis.

INTRADAY PRECISION

Acceptance criteria*:* RSD<2.0% for peak area and retention time10.

Precision is measure of repeatability or reproducibility and it was determined by injecting 6 times the expected operating range concentration. The chromatograms were recorded to determine mean standard deviation and relative standard deviation. Results were given in Table:4.

RESULT

From the above analytical data, it is observed that RSD for the assay is 1.1 which indicates that the method is precise and reproducible.

LIMITS OF DETECTION AND QUANTIFICATION

The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. The lower limit of detection for Efavirenz is 3.6ng/ml in reference material and formulation. Limit of Quantification (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The LOQ values were found to be 11ng/ml for raw material and formulations.

ROBUSTNESS & RUGGEDNESS

CHANGE IN THE MOBILE PHASE: On evaluation of the results, it can be concluded that the variation in mobile phase affected the method significantly. Hence it indicates that method was not affected even by change in the mobile phase. The system suitability parameters were within the limit. The results were given in Table: 5.

CHANGE IN THE FLOW RATE: Results for actual flow (1.2 ml/min) have been considered from Assay standard. System suitability parameters were studied and the results were within the limit. These results were shown in the Table:6.

ASSAY

Twenty tablets of Domperidone were taken and powdered, weigh accurately about 10 mg of equivalent weight of drug and transferred into 100 ml volumetric flask to it added 30 ml of diluent, sonicated 5 minutes and finally made up the volume with diluent. Pipette out 1 mL above solution into 10 mL volumetric flask to it made up to volume with diluent. Obtained standard concentration is 100µg/ml solution. These results were shown in the Table: 7.

$$\% \text{ Assay} = \frac{(\text{Area of unknown} \times \text{Conc Of standard})}{(\text{Area of standard} \times \text{Conc of unknown})} \times 100$$

CONCLUSION

The proposed RP-HPLC method is found to be accurate, precise, linear, stable, specific, and simple, for quantitative estimation of Efavirenz in raw material and pharmaceutical formulations. Hence the present RP-HPLC method is suitable for routine assay of Efavirenz in raw materials and in pharmaceutical formulations in the quality control laboratories.

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