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Development and Validation of HPTLC Method for the Determination of Efavirenz as Bulk Drug and in Tablet Dosage form

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ABSTRACT

A simple, accurate, precise, and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of Efavirenz in tablet dosage forms. The method employed TLC aluminium plates precoated with silica gel 60 F 254 as the stationary phase. The mobile phase used was a mixture of Toluene: Ethyl acetate: Formic acid (10: 3: 1 v/v). The detection of spot was carried out at 254nm. The calibration curve was found to be linear between 300 to 1800 ng mL^{-1} with regression coefficient of 0.9991. The proposed method can be successfully used to determine the drug content of marketed formulation. The accuracy of the proposed method was determined by recovery studies and found to be 99.38 to 99.68 %. The proposed method is applicable to routine analysis of Efavirenz in bulk and pharmaceutical formulations. The proposed method was validated according to various ICH parameters like linearity, accuracy, precision, specificity, limits of detection, limits of quantification, range and solution stability.

Keywords: Efavirenz, Validation, ICH guidelines, HPTLC

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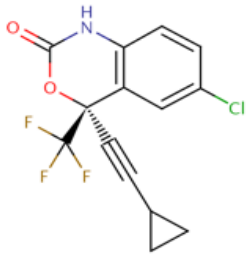
INTRODUCTION

Efavirenz is chemically (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-1H-3, 1-benzoxazin-2-one (Fig. 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 315.6750. 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) type-1 [1]. Literature survey reveals that very few analytical methods has been established for the determination of Efavirenz viz. Development of Rapid UV Spectrophotometric Method for the Estimation of Efavirenz in Formulations [2], 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], Development of a competitive immunoassay for efavirenz: Hapten design and validation studies [4], Simultaneous quantification of a non-nucleoside reverse transcriptase inhibitor efavirenz, a nucleoside reverse transcriptase inhibitor emtricitabine and a nucleotide reverse transcriptase inhibitor tenofovir in plasma by liquid chromatography positive ion electrospray tandem mass spectrometry [5], Determination of efavirenz, a selective non-nucleoside reverse transcriptase inhibitor, in human plasma using HPLC with post-column photochemical derivatization and fluorescence detection [6],Quantitative Estimation of Efavirenz by High Performance Thin Layer Chromatography [7], Development and validation of stability indicating HPTLC method for determination of efavirenz as bulk drug and in pharmaceutical formulation [8].

Fig. 1: Chemical structure of Efavirenz



The objective of this work was to develop a new, simple, economic, rapid, precise, and accurate HPTLC method for quantitative analysis of Efavirenz, and to validate the method in accordance with ICH guidelines [9].

MATERIALS AND METHODS

Materials

Pure standard of Efavirenz (Assigned purity 99.98%) was obtained as a gift sample from Ranbaxy labs Pvt. Ltd, Gurgaon, India. The gift sample was used as standard without further

purification. Silica gel 60 F 254 TLC plates (20x10cm) were used as stationary phase. All chemicals and reagents used were of analytical grade and obtained from Qualigens. Commercial pharmaceutical preparation (Sustiva) which was claimed to contain 600mg of Efavirenz was used in analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, Melting point studies.

Equipment

The instrument used in the present study was Camag Linnomat V- automatic sample applicator, Hamilton syringe (100µl), Camag TLC scanner 3, Camag Twin through chamber of appropriate size, Analytical weighing balance (Shimadzu AX 200), Sonicator (model SONICA 2200MH) were used throughout the experiment. Camag Wincats software was used for acquisition, evaluation and storage of chromatographic data.

Preparation of Standard Solution

A stock solution of drug was prepared by dissolving 100 mg of Pure Efavirenz in a 100 ml volumetric flasks containing sufficient amount of methanol to dissolve the drug, sonicated for about 15 min and then made up to volume with methanol (1 mg/ml). A standard solution was prepared by dilution of the stock solution with methanol to give in concentration of 100µg/ml. Further dilutions were made with methanol to give a solution in concentration range of 300-1800ng/ml.

Procedure for Sample Solution (From Formulation)

To determine the content of the drug in a solid dosage form, 20 tablets of Efavirenz (600mg) were accurately weighed, their average weight was calculated. Powder equivalent to 600 mg of the drug (content of one tablet) was dissolved in sufficient amount of methanol to dissolve the drug, sonicated for about 15 min. and then filtered into a 100 ml volumetric flask through 0.45 µm membrane filter. The residue was washed 3 times with 10 ml of methanol, and then the volume was completed to 100 ml with the same solvent. Make further dilutions with methanol to obtain a stock solution of 10µg/ml. An aliquot of this solution (1 ml) was transferred to a 10 ml volumetric flask and made up sufficient volume with the methanol to give an expected concentration of 1 µg/ml.

Prewashing of TLC plates

HPTLC was performed on 20 cm × 10 cm precoated silica gel 60 F 254 TLC plates. The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack has been opened. The non-volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid possible interference from such impurities in quantitative analysis, plates were prewashed with methanol, dried, and activated for 30 min. at 110 C, with the plates being placed between two

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sheets of glass to prevent deformation of the aluminum during heating.

RESULTS AND DISCUSSION

Procedure

A methanolic solution of Efavirenz (1 mg/ml) was prepared. This solution was further diluted with methanol to yield a solution containing 1µg/ml. Different concentrations of Efavirenz in a concentration range of 300-1800ng/ml were applied on plates as 8 mm bands, 8 mm apart and 1 cm from edge of the plate, by means of Camag Linomat V automatic sample applicator fitted with 100 µl Hamilton syringe. A methanol blank was applied to parallel track. The mobile phase, Toluene: Ethyl acetate: Formic acid (10: 3: 1 v/v) was poured into the twin trough glass chamber and the glass chamber left to equilibrate for 10 min at 25 ± 2^0 C. After that the plate was placed in Camag twin trough glass chamber. After development, the plate was removed from the chamber, dried in current of hot air, and scanned at 254 nm, using a deuterium lamp, by means of Camag TLC scanner III densitometer. Densitograms were obtained by HPTLC of Efavirenz at various concentrations. This method was followed for all quantitative analysis. The Wincats software was used for data acquisition and processing of the plate. Peak height and peak area were integrated for the entire track. The calibration curve was established by plotting the obtained peak area on ordinate against corresponding concentration on abscissa.

VALIDATION OF ANALYTICAL METHOD

Validation of an analytical method is process to establish by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

Typical analytical parameters used in validation area:

1. Linearity
2. Accuracy
3. Precision
4. Specificity
5. Limit of detection
6. Limit of quantification
7. Range
8. Solution stability

LINEARITY

Acceptance criteria: Coefficient of correlation (r2) should be greater than 0.998

Procedure: A stock solution of drug was prepared by dissolving 100 mg of Pure Efavirenz in a

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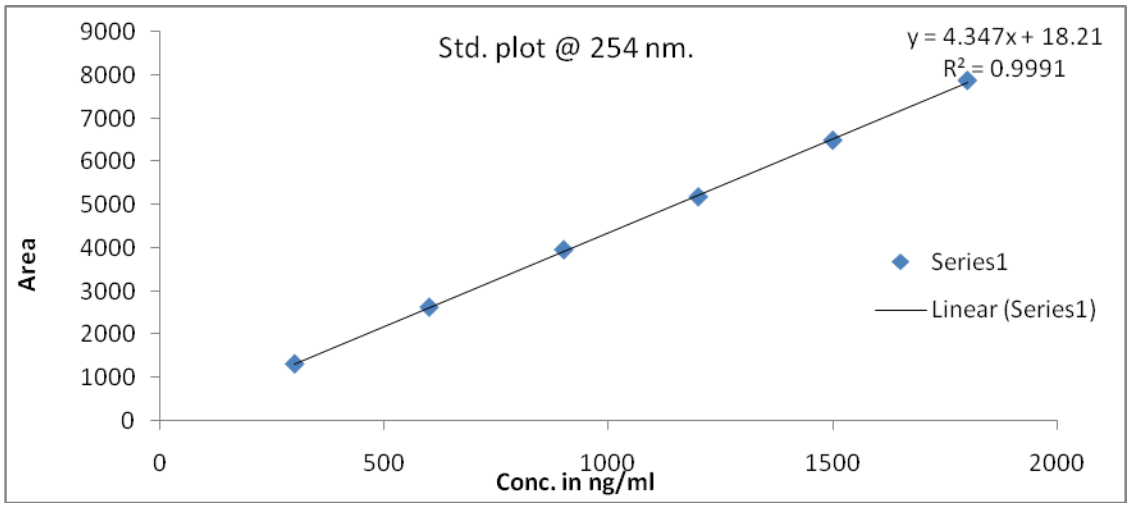
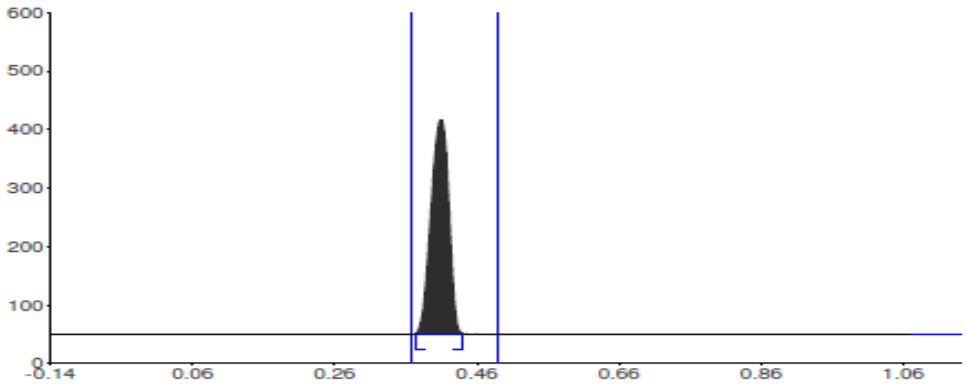
100 ml volumetric flasks containing sufficient amount of methanol to dissolve the drug, sonicated for about 15 min and then made up to volume with methanol (1 mg/ml). A standard solution was prepared by dilution of the stock solution with methanol to give in concentration of 100µg/ml. Further dilutions were made with methanol to give a solution in concentration range of 300-1800ng/ml. The calibration curve was established by plotting the obtained peak area on ordinate against corresponding concentration on abscissa.

Graph No. 1 and chromatogram No. 1





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.37	0.1	0.41	368.5	100.00	0.44	1.6	7885.7	100.00

Result: Correlation coefficient (r^2) for Efavirenz was found to be 0.9991, indicating the linearity and the method is linear between the concentrations of 300-1800ng/ml with Rf value 0.41±0.01.

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ACCURACY

The accuracy is the closeness of the measured value to the true value of the sample. To evaluate the accuracy of the method, known amount of pure drug was added to the previously analyzed solution containing pharmaceutical formulation and the mixture was analyzed by the proposed method and the recoveries were calculated. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 0.2 ml, 0.5ml and 0.8 ml of sample drug (Efavirenz) solution of 10µg/ml were pipetted into each of three volumetric flasks. To this 0.4 ml of standard drug (Efavirenz) solution of 10µg/ml was added to each volumetric flask respectively. The volume was made up to 10 ml with methanol. The range of recovery studies were found between 99.38 to 99.68 %. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients. (Table No. 1)

Conc. taken in ng/ml (A)	Std addition in ng/ml (B)	Total drug conc. in ng/ml (A+B)	Peak Area*	% Recovery	Average	% RSD
200	400	600	2597.07	99.38	99.50	0.159543
500	400	900	3949.96	99.68		
800	400	1200	5129.48	99.44		

*Average of three readings

Table No. 1: Results of Recovery Studies of Drug

Result: The percentage recovery by the proposed method was ranging from 99.38 to 99.68 % indicating no interference of the tablet excipients with drug under analysis.

PRECISION

Precision is measure of repeatability or reproducibility and it was determined by injecting 5 times the expected operating range concentration. The chromatograms were recorded to determine mean standard deviation and relative standard deviation. (Table No. 2)

Acceptance criteria: RSD<2.0% for peak area.

Result: From the above analytical data it is observed that RSD for the assay is 0.4841 which indicates that the method is precise and reproducible.

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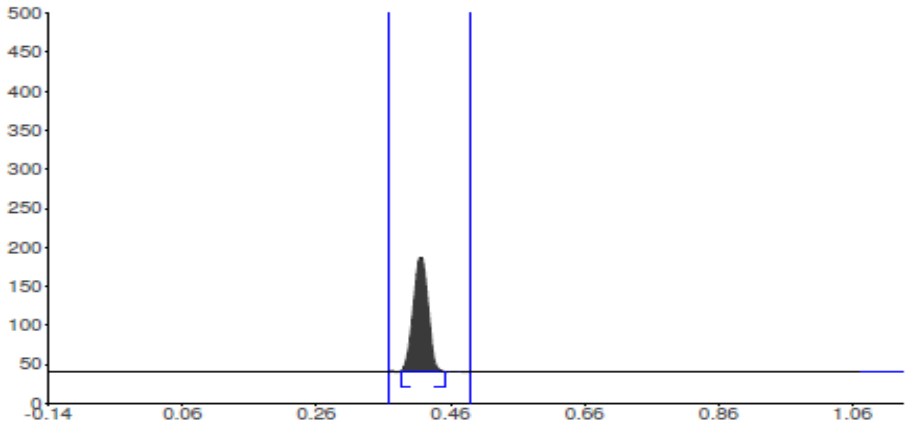
Table No.2: Precision of Efavirenz

S.No.	Area Response*
1	7880.4
2	7972.7
3	7902.2
4	7878.5
5	7912.8
Average	7909.32
S.D	38.29513
R.S.D	0.484177

*Average of three readings

SPECIFICITY

Specificity is the ability to assess the analyte in the presence of components that may be expected to be present in the sample matrix (USP 2004). For demonstrating the specificity of the method for drug formulation the drugs was spiked and observe the chromatogram (chromatogram No.2)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.38	1.3	0.41	148.6	100.00	0.45	0.2	2612.7	100.00

HPTLC Chromatogram showing Specificity (chromatogram No. 2)

Result: The excipients used in different formulation products did not interfere with the drug peak and thus, the method is specific for Efavirenz.

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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. It may be expressed as a concentration that gives a signal-to-noise ratio of 2:1 or 3:1. The lower limit of detection for Efavirenz is 8.190ng/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. A signal-to-noise ratio of 10:1 can be taken as LOQ of the method. The LOQ values were found to be 24.819ng/ml for raw material and formulations.

RANGE

The specific range derived from the linearity studies. The range was calculated from the linearity graph. From the lower to higher concentration between which the response is linear, accurate and precise.
Acceptance criteria: RSD < 2.0
The range for Efavirenz was found to be 300-1800 ng/ml.

SOLUTION STABILITY

The solution stability of the standard and sample prepared in methanol was studied for 5 days at bench top. The solution under study was compared with freshly prepared standard solution, the samples were found to be stable for period of more than 48 hours.

CONCLUSION

The proposed HPTLC 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 HPTLC method is suitable for routine assay of Efavirenz in raw materials and in pharmaceutical formulations in the quality control laboratories.

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