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## Quantitative Estimation of Efavirenz by High Performance Thin Layer Chromatography

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### ABSTRACT

A simple, accurate, and precise HPTLC method has been developed and validated for the estimation of efavirenz from bulk drug and capsule formulations. The separation was achieved on TLC plates using an appropriate solvent system. The spots so developed were densometrically scanned at 247 nm. The linearity of the method was found to be within the concentration range of 1-40 µg/ml. The validation parameters, tested in accordance with the requirements of ICH guidelines, prove the suitability of this method. The method was successfully applied for determination of drug in capsules, wherein no interference from capsule excipients was observed, indicating the specificity of the developed method. Thus, the proposed method can be used successfully for routine analysis of efavirenz from capsule and bulk formulations.

**Key words:** Accurate, efavirenz, HPTLC, validation, precise

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### INTRODUCTION

Efavirenz [Figure 1], (4S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one, is a non-nucleoside reverse transcriptase (RT) inhibitor of human immunodeficiency virus type 1 (HIV-1).<sup>[1,2]</sup> Efavirenz activity is mediated predominantly by non-competitive inhibition of HIV-1 RT. HIV-2 RT and human cellular DNA polymerases alpha, beta, gamma, and delta are not inhibited by efavirenz.<sup>[3]</sup>

The literature survey reveals that there are analytical methods available for determination of efavirenz from biological matrices,<sup>[4-9]</sup> bulk drug, and dosage forms,<sup>[10,11]</sup> and analytical methods for determination of efavirenz with combination of other antiviral drugs.<sup>[12-35]</sup> However, there is no reported HPTLC method for the analysis of efavirenz. The literature survey further revealed that the

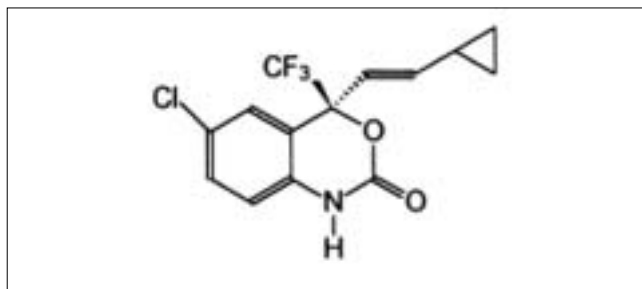


Figure 1: Chemical structure of efavirenz

drug efavirenz is still not official in USP or BP, and there are no official HPTLC method reported for analysis of efavirenz from bulk drugs. Thus, an appropriate analytical procedure for the quantitative determination of efavirenz from bulk drugs is of considerable importance.

Keeping this objective in mind an attempt has been made

to develop and validate the HPTLC method for the analysis of efavirenz which would be highly sensitive, having good resolution and reproducible. Various validation aspects of the analysis, accuracy, precision, recovery, and the limits of detection and quantification etc. have been measured.

## EXPERIMENTAL DETAILS

### Chemicals and Reagents

The working standard of efavirenz was procured from Cipla Ltd, India. HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Deionized and ultra-pure water used in all experiments was obtained from Milli-Q System (Millipore). Glacial acetic acid of AR (Analytical Reagent) grade was procured from S. D. Fine Chemicals (India).

### Equipment

The HPTLC system employed in the method development and validation was Camag Linomat V applicator (Muttentz, Switzerland), a Camag twin trough chamber of appropriate size, Camag TLC scanner III, Wincats (version 1.2.2) software as data integrator and a Hamilton syringe (Switzerland) of 100 µl capacity.

### Preparation of standard solution

A stock solution of efavirenz (1mg/ml) was prepared in methanol. A standard solution was prepared by dilution of the stock solution with methanol to give solution in of 100 µg/ml. Further dilutions were made with methanol to give a solution in concentration range of 1–40 µg/ml.

### HPTLC

#### Prewashing of plates

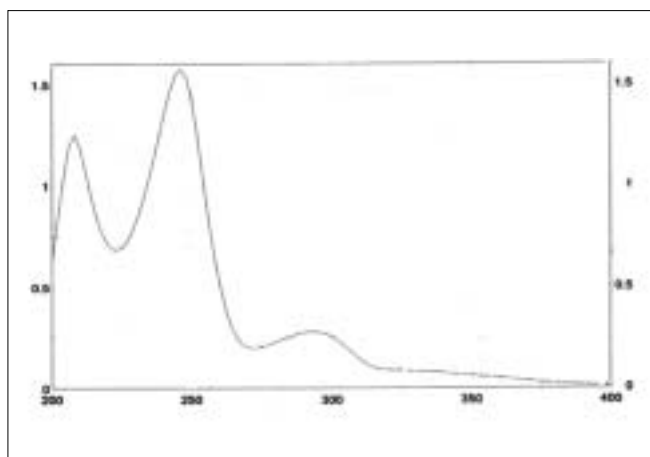
HPTLC was performed on 10 cm × 20 cm pre-coated silica gel GF<sub>254</sub> pre-coated HPTLC plates from E. Merck (Darmstadt, Germany). The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack has been opened. 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 sheets of glass to prevent deformation of the aluminum during heating.

### Procedure

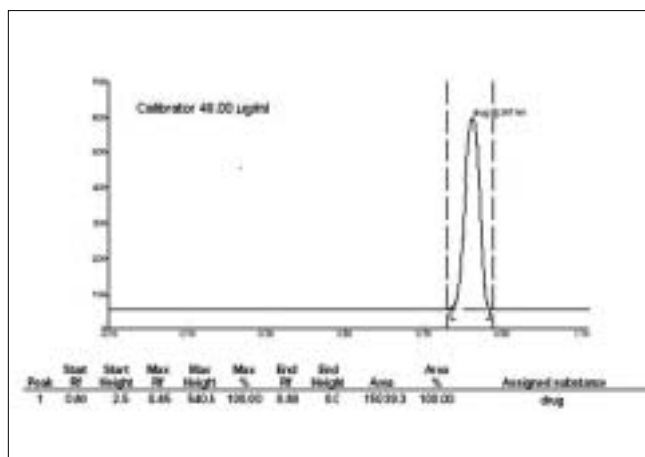
A methanolic solution of efavirenz (1 mg/ml) was prepared. This solution was further diluted with methanol to yield a solution containing 100 µg/ml. Different concentrations of efavirenz in a concentration range of 1- 40µg/ml were applied on plates as 6 mm bands, 6 mm apart and 1 cm from edge of the plate, by means of Camag Linomat V automatic sample applicator fitted with 100 µl Hamilton syringe. A methanol blank was applied to parallel track. After drying of bands, the plate was placed in one of the troughs of Camag twin trough glass chamber. The mobile phase, acetonitrile: water: glacial acetic acid (6:4:0.5 %v/v) was poured into the second trough and the plates was left to equilibrate in the chamber for 10 min at 25 ± 2°C. The plate was then moved to the second trough containing the mobile phase and developed to a distance 90 mm. After development, the plate was removed from the chamber, dried in current of hot air, and scanned at 247 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 (version 1.2.2) was used for data acquisition and processing of the plate. The scanning speed was 20 mm/s, the offset was 10%, and the sensitivity (SPAN) was optimized to 20 min. 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. The reproducibility of this method was ascertained by repeating the experiment six times.

### Method validation

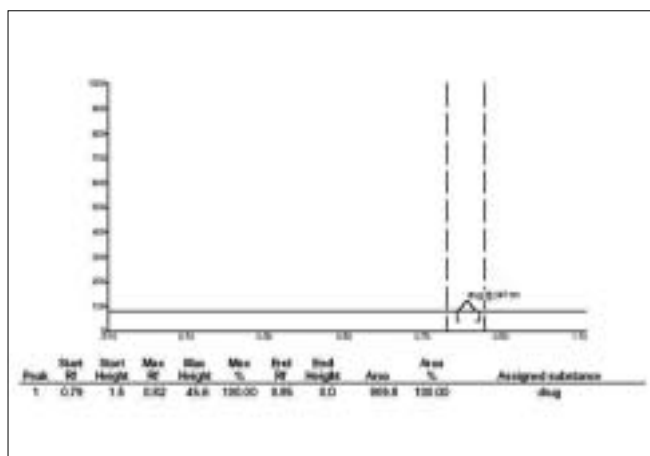
The method was validated for specificity, accuracy, and precision by use of calibration standards of efavirenz. Limit of detection and limit of quantitation were determined by the visual method, by spotting different concentration of efavirenz. The lowest concentration that could be detected for three replicate spots was regarded as the limit of detection. The lowest concentration for which the RSD [%] of six replicate spots less than 5 % was regarded as the limit of quantitation. LOD was calculated using formula  $LOD = 3.3 \times \sigma/S$ , where  $\sigma$  is the residual standard deviation of regression line and  $S$  is the slope of corresponding line. Accuracy was determined by measurement of the recovery of efavirenz standard added at three different levels, each being analyzed as described for the assay. Intraday and interday precisions were determined by applying 3 µg/ml, 20 µg/ml, and 40 µg/ml standard efavirenz. After the



**Figure 2:** A typical absorption spectrum of efavirenz scanned at 200-400 nm



**Figure 3:** Representative chromatogram of standard 40 µg/ml efavirenz at 247 nm



**Figure 4:** Representative chromatogram of formulation of efavirenz at 247 nm

development and densitometric scanning of the plates the peak-area response was measured and precision was calculated as RSD (%). The method was validated as per ICH guidelines.<sup>[36]</sup>

### Application of the validated method

To determine the content of the drug in a solid dosage form, 20 capsules of efavirenz (200 mg) were accurately weighed, their average weight was calculated. Powder equivalent to 200 mg of the drug was dissolved in 100 ml methanol in a volumetric flask and sonicated for 20 min. (Solution A ~ 2000 µg/ml). Further dilutions were made with mobile phase as follows: 1 ml of Solution A was diluted to 100 ml in a volumetric flask (Solution B ~ 20 µg/ml). One milliliter of Solution B was diluted to 10 ml in a volumetric flask (Solution C ~ 2 µg/ml). This Solution C was spotted (40 µl) on to the HPTLC plate. The

average area of six such spots was taken for calculation. The concentration of efavirenz was calculated from the standard graph, which was plotted using the area of known concentration (1-40 µg/ml) of efavirenz standard. The results are summarized in Table 1.

## RESULTS AND DISCUSSION

While choosing a solvent for HPTLC, the chemical nature and polarity of the drug molecule must taken into account. Efavirenz is practically insoluble in water and in the pH range of 3-9, freely soluble in methanol, soluble in strong base. Acetonitrile: ethyl acetate (1:1) was used as the solvent system initially, but it was observed that the spot moved along with the solvent front and thus was not retained on the plate. When ethyl acetate was replaced with water, the RF decreased. Finally, the solvent system comprising acetonitrile: water (6:4) gave good separation and there was no interference around the drug RF. Slight tailing was observed which was removed when 0.1% glacial acetic acid was added in the solvent system. Since the drug is weakly acidic in nature, it remains unionized at acidic pH, which

**Table 1: Assay of efavirenz in capsule**

Label claim	200 mg
Amount found ±SD (n=6)	200.00 ± 0.592 mg
% Label claim	100.30%
% RSD	0.0055

n= no. of repetitions

**Table 2: Linearity and range**

Linearity and range	Efavirenz
Range (µg/ml)	1 - 40
r <sup>2</sup>	0.9968
Slope	366.00

**Table 3: Precision and recovery data**

Actual concentration (µg/ml)	Precision		% Recovery	
	Measured concentration (µg/ml) ± S.D.; % R.S.D.		Intra-day	Inter-day
	Intra-day	Inter-day		
3.00	3.11 ± 0.11; 3.46	3.10 ± 0.40; 3.16	103.64	103.25
20.00	19.95 ± 0.39; 1.94	19.95 ± 0.10; 1.99	99.74	99.73
40.00	39.73 ± 0.98; 2.47	39.65 ± 1.00; 2.51	99.33	99.12

in turn reduces the tailing of the peak. Thus, after much experiments, the optimized system was developed which consisted of acetonitrile: water: glacial acetic acid (6:4:0.5 %v/v). The optimized system gave a good separation and resolution of efavirenz.

The plate material employed was silica gel 60 GF<sub>254</sub>. Scanning of efavirenz on HPTLC plate showed UV max at 247 nm [Figure 2]; hence, quantitation was carried out at 247 nm using Camag TLC scanner III.

The method developed was validated for limit of detection (LOD) and limit of quantitation (LOQ) in order to determine and ensure sensitivity of the developed method. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.7 and 1 µg per spot, respectively. The method was found to be linear over the range 1-40 µg per spot with coefficient of regression 0.9968 [Table 2]. Intra-day and inter-day precision studies showed that a %RSD was less than 5.00%, indicating the method was precise. The accuracy values obtained in the range 99.12-103.64% for drug are indicative of excellent accuracy and recovery. There was no evidence of peaks or any other interfering co eluting peaks at the RF of standard (0.85). This indicates that the method is specific. Stability studies were carried out for standard. It was found to be stable in sample solution, prior to development and after development.

The developed method was then validated and successfully applied for quantitation of efavirenz from the formulation. To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100%, and 120% levels, to the pre-analyzed samples and the subsequent solutions were re-analyzed. At each level, three determinations were performed and the results obtained are shown in Table 3. The results of recovery studies were within the specified limits of ICH guidelines. Lower values of %RSD reflect the accuracy of the method. Precision, expressed in terms of %RSD was determined in terms of intra-day and inter-day precisions, analyzing the drug at three different concentrations, determining each concentration thrice. The sample solutions were analyzed

using the method for three consecutive days, repeating the process twice a day at different periods. The results obtained are summarized in Table 3 and reflect high degree of precision. Two different analysts performed assay on marketed capsules of the drug, in similar operational and environmental conditions, using the developed method to determine its ruggedness.

The optimized solvent system yielded a symmetrical peak for the drug with RF0.85 [Figure 3]. A typical absorbance spectrum of the drug is shown in Figure 2. The peak of the drug from capsules [Figure 4] was identified by comparing the RF and also comparing its absorbance spectrum with that obtained with the standard drug.

## CONCLUSION

The developed and validated HPTLC method reported here is rapid, simple, accurate, sensitive, and specific. The method was also successfully used for quantitative estimation and analysis of efavirenz from formulation. Thus, the reported method is of considerable importance and has great industrial applicability for quality control and analysis of efavirenz from bulk drug and formulations.

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