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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR DETERMINATION OF EFAVIRENZ AS BULK AND IN CAPSULE DOSAGE FORM

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ABSTRACT

A new simple, accurate and precise stability-indicating high performance thin layer chromatography (HPTLC) method has been developed and validated for determination of Efavirenz as bulk drug and in capsule dosage form. The drug was exposed to hydrolytic, oxidative, thermal and photolytic stress conditions to check the stability nature of drug. Chromatographic resolution of Ebastine was accomplished by use of precoated silica gel 60 F_{254} aluminium plates as stationary phase and solvent mixture comprising of Toluene: Ethyl acetate: Methanol (8:5: 1: 0.5, v/v/v) as mobile phase. Densitometric detection was carried out at 247 nm. The retention factor was found to be 0.45 ±0.01. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. Results were found to be linear in the range of 200-1200 ng band^{-1}. The developed method has been successfully applied for the estimation of drug in capsule dosage form.

KEYWORDS: Efavirenz, HPTLC, Stability Indicating Method, Validation, ICH.

INTRODUCTION

Efavirenz, chemically, (4S)-6-Chloro-4-(2-Cyclopropylethyl)-4-(trifluromethyl)-2, 4-dihydro-1H-3, 1-benzoxazin-2-one is a non-nucleoside reverse transcriptase inhibitor which blocks DNA polymerase activities including HIV replication.[1]

Extensive literature review with respect to analytical methods revealed that Spectrophotometric methods[2-3] has been reported for the estimation of Efavirenz in tablet dosage form either as single drug or in combination with other drugs. Analytical reports also revealed availability of High Performance Liquid Chromatographic (HPLC)[4-11] methods for estimation of Efavirenz either as single drug or in combination with other drugs in pharmaceutical dosage form and in human plasma.

To best of our knowledge, no reports were available in the literature for determination of Efavirenz in capsule formulation by stability-indicating high performance thin layer chromatography (HPTLC) method. This paper describes development and validation of simple, precise and accurate stability indicating HPTLC method for determination of Efavirenz in capsule dosage form in accordance with International Conference on Harmonisation Guidelines.[12,13]

MATERIALS AND METHODS

Chemicals and Reagents

Efavirenz working standard was obtained as a gift sample. The pharmaceutical dosage form used in this study was Efavir labelled to contain 200 mg of Efavirenz was procured from local pharmacy. Toluene, ethyl acetate, methanol (all AR grade) were purchased from LOBA Chemie Pvt. Ltd. Mumbai, India.

Instrumentation and chromatographic conditions

Chromatographic separation of the drug was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm × 10 cm) from E. MERCK, Darmstadt, Germany, using a CAMAG Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band under nitrogen stream with a 6 mm of band width using Camag 100 µL sample syringe (Hamilton, Switzerland). A constant application rate of 0.1 µL sec^{-1} was employed.

Linear ascending development was carried out in 10 ×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Toluene: Ethyl acetate: Methanol (8.5: 1: 0.5, v/v/v) as mobile phase. The mobile phase was saturated in the (CAMAG) twin trough TLC chamber for 20 min before chromatogram development at room temperature. After development, TLC plates were removed and dried. A Camag TLC scanner III with winCATS software version 1.4.2 was used for densitometric evaluation.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving 10 mg drug in 10 mL methanol to get concentration of 1000 µg mL^{-1} which was further diluted with mobile phase to get final concentration 100 ng μL^{-1}.

Estimation of drug in tablet dosage form

Twenty capsules (Efavir labelled to contain 200 mg) were weighed accurately and powdered. A quantity of powder equivalent to 20 mg was weighed and transferred to 100 mL volumetric flask containing about 60 mL of methanol and ultrasonicated for 15 min and filtered through Whatman paper No. 41 and volume was made upto the mark with methanol to obtain the final concentration of 200 ng $band^{-1}$. Two µL volume of this solution was applied on TLC plate to obtain final sample concentration of 400 ng $band^{-1}$. After chromatographic development peak areas of the bands were measured at 247 nm and the amount of drug present in sample was estimated from the calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Forced degradation studies

Forced degradation studies were carried out to check the stability by exposing the bulk drug to the physical stress conditions. The study was carried out at concentration of 1000 ng μL^{-1}. The hydrolytic studies were carried out by treatment of stock drug solution separately with 0.1 N HCl and 0.1 N NaOH at room temperature for 2 h. The acid and alkali stressed samples were neutralized with NaOH and HCl, respectively to furnish the final concentration of 800 ng $band^{-1}$. The drug was treated with water at room temperature for 2 h for neutral hydrolysis. Standard drug solution was treated with 3 % H_2O_2 at room temperature for 2 h to perform the oxidative degradation and was diluted with methanol to obtain 800 ng $band^{-1}$ solution. Thermal stress degradation was performed by keeping drug in oven at 60ºC for period of 24 h. The solid drug powder was exposed UV light up to 200 watt h $square\ meter^{-1}$ to check photolytic degradation. Thermal and photolytic samples were diluted with methanol to get concentration of 800 ng $band^{-1}$.

RESULTS AND DISCUSSION

Method development and optimization

The objective of present research work was to develop suitable stability indicating HPLTC method which would be proficient to give the satisfactory resolution of Efavirenz. For this, various solvent systems comprising different ratios of benzene, chloroform, toluene,

methanol, ethyl acetate, triethylamine were examined (data not shown) to separate and resolve spot of Efavirenz from its impurities and other excipients present in formulation. Finally, the mobile phase comprising of toluene: ethyl acetate: methanol (8.5: 1: 0.5, v/v/v) was chosen as optimum for attaining well defined and resolved peak. Densitometric detection was carried out at 247 nm. The retention factor was found to be 0.45 ± 0.01. Representative densitogram of standard solution of Efavirenz is represented in Fig. 1.

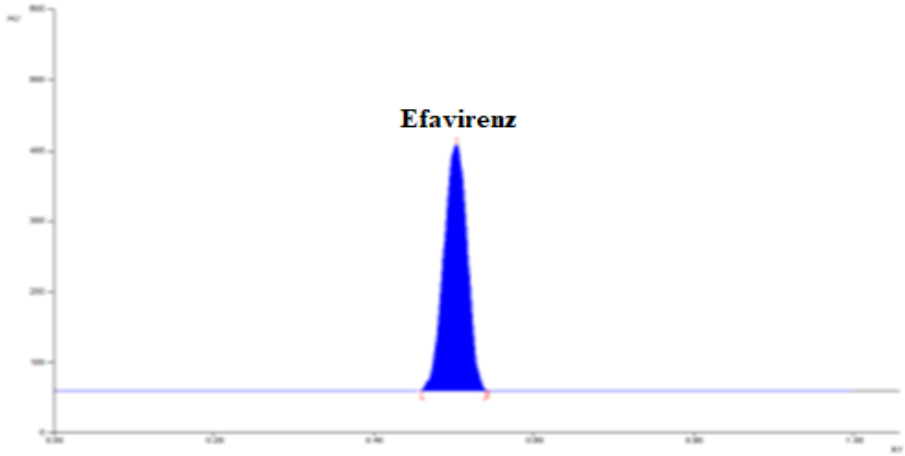

Fig: 1: Representative densitogram of standard solution of Efavirenz. (400 ng band^{-1}, Rf= 0.45 ± 0.01)

The results of forced degradation revealed susceptibility of drug to hydrolytic, oxidative, thermal as well as photolytic stress conditions. Marked degradation was observed in base, oxidative and thermal stress conditions. Fig. 2 and 3 denotes the densitograms of acid and alkali hydrolytic degradation, while Fig. 4 and 5 illustrates the densitograms of oxidative and thermal degradation, respectively. The findings of degradation studies along with % degradation and % of drug recovered are summarized in Table 1.

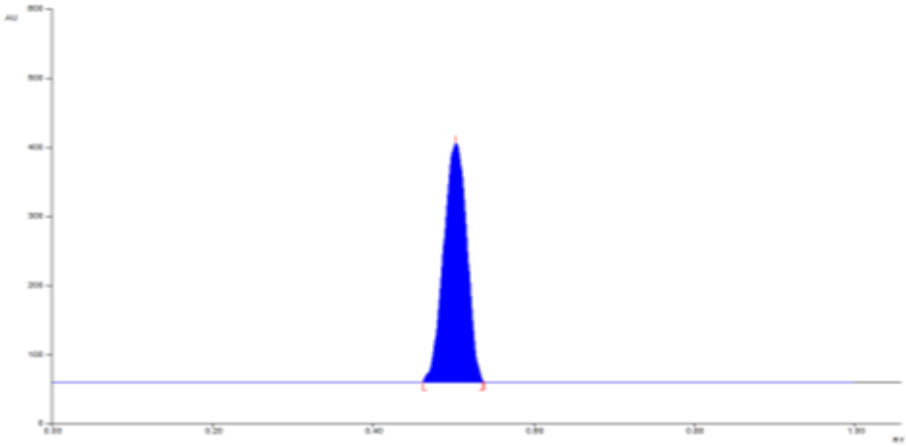

Fig. 2: Densitogram of acid treated sample (0.1 N HCl kept at RT for 2 h).

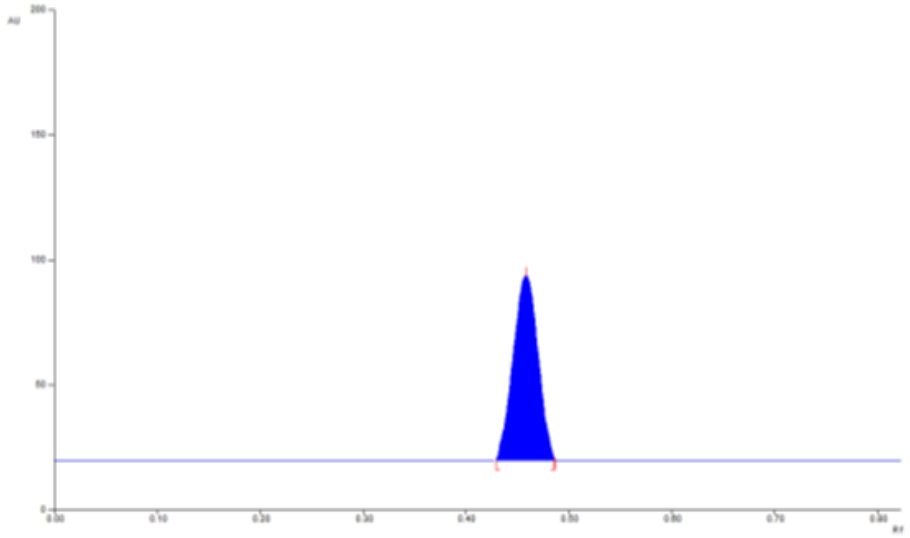

Fig. 3: Densitogram of base treated sample (0.1 N NaOH kept at RT for 2 h).

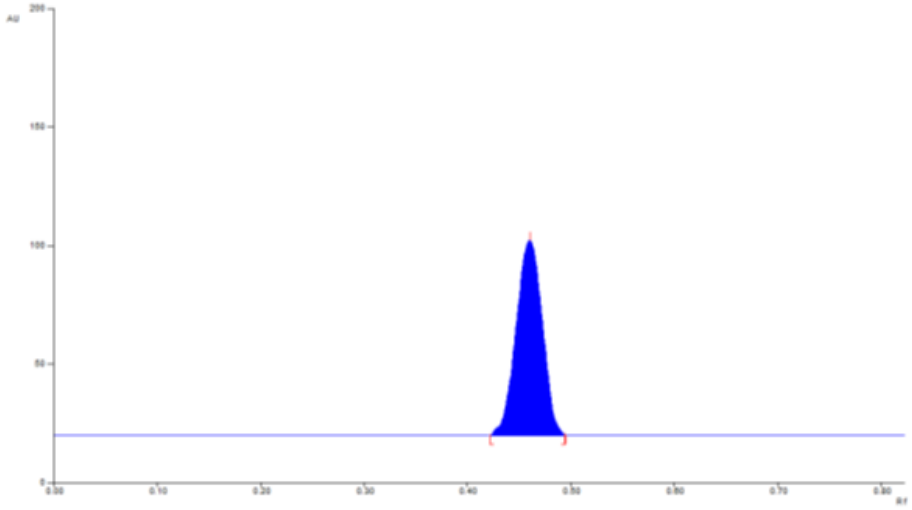

Fig. 4: Densitogram of oxidative degradation (3% H_2O_2 kept at RT for 2 h).

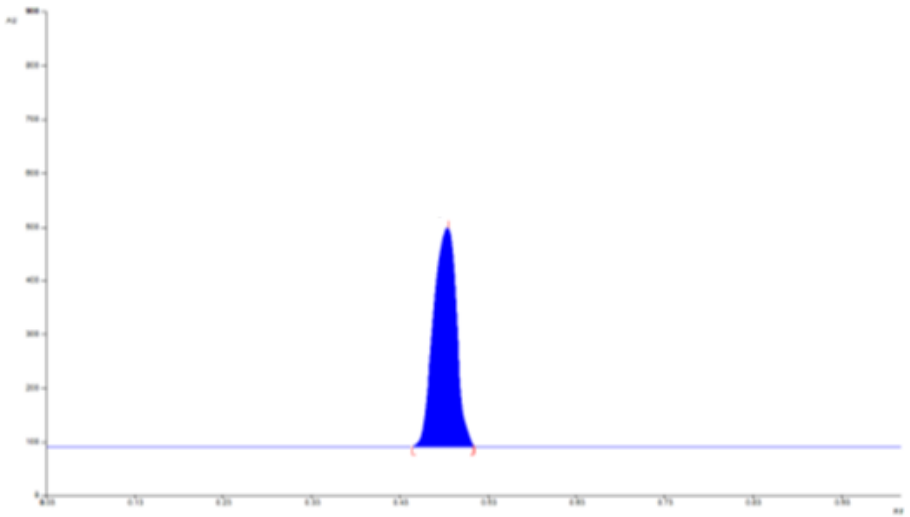

Fig. 5: Densitogram after thermal degradation at 60°C for 24 h.

Table 1: Summary of forced degradation studies.

Sr. No.	Stress conditions	% Recovery	% Degradation
1.	Acid/ 0.1 N HCL/ Kept at RT for 2 h	89.92	10.08
2.	Alkali/ 0.1 N NaOH/ Kept at RT for 2 h	78.18	21.82
3.	Neutral/ H_2O/ Kept at RT for 2 h	80.62	19.32
4.	Oxidative/ 3% H_2O_2/ Kept at RT for 2 h	76.01	23.99
5.	Thermal degradation/ 60ºC for 24 h	77.60	22.40
6.	Photolytic degradation	85.90	14.88

Method validation

The method has been validated according to the guidelines of ICH Q2 (R1) for parameters such as linearity, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness.

Linearity

Volumes 2, 4, 6, 8, 10 and 12 µL from standard solution of Efavirenz (100 ng μL^{-1}) were spotted onto the TLC plates, developed and scanned under optimized chromatographic conditions. The established method was found to be linear in the concentration range 200-1200 ng $band^{-1}$ with high correlation coefficient. The linear regression equation was found to be y = 6.3982x + 1186.9 with correlation coefficient (R^2) value of 0.990. The calibration curve achieved by plot of concentration vs peak area is depicted in Fig. 6. A 3D densitogram of linearity is shown in Fig. 7.

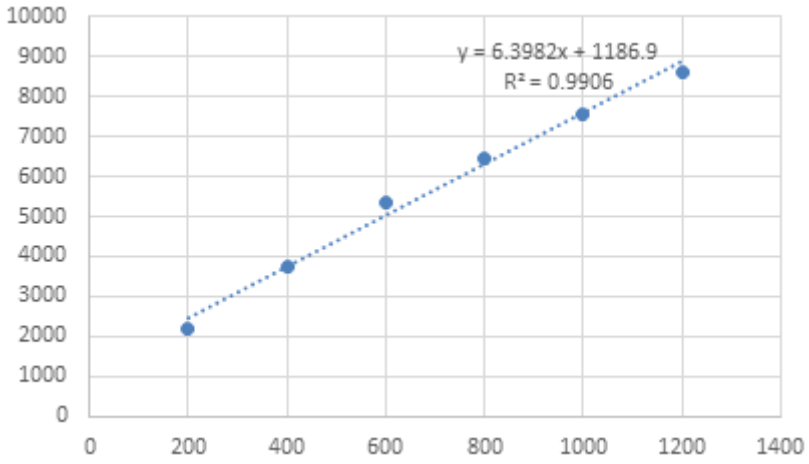

Fig. 6: Calibration curve for Efavirenz.

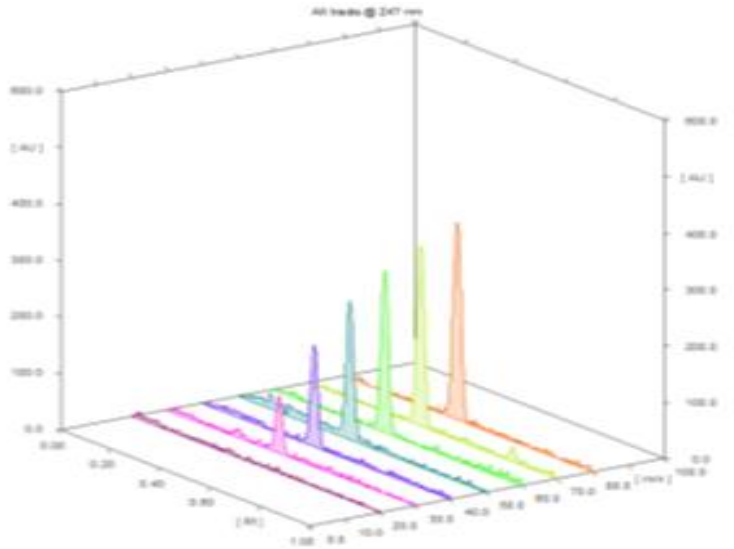

Fig. 7: 3D densitogram of linearity in concentration range 200-1200 ng band^{-1}.

Precision

The method was subjected to intra-day and inter-day precision studies. Precision was evaluated by applying three replicates of three different concentrations (400, 600 and 800 ng band^{-1}) within linearity range on same day and on three consecutive days. Intra-day variation, as R.S.D. (%), was found to be in the range of 0.59 to 0.94. Interday variation, as R.S.D. (%) was found to be in the range of 0.25 to 0.58. The method was found to be precise as % R.S.D. was less than 2 %.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ values were found to be 7.46 ng band^{-1} and 21.36 ng band^{-1}, respectively.

Accuracy

Accuracy of developed method was checked by performing recovery studies by standard addition method. It involved addition standard drug solution to pre-analysed sample solution at three different levels 80%, 100% and 120%. Basic concentration of sample chosen was 400 ng band^{-1} from sample solution. The drug concentrations were calculated from linear regression equation. The results of the recovery studies indicated accurateness of developed method for estimation of drug in capsule formulation.

Table 3: Recovery studies.

Drug	Amount taken (ng/band)	Amount added (ng/band)	Amount found (ng/band)	% Recovery	% R.S.D.*
Efavirenz	400	320	716.85	99.56	0.72
	400	400	801.08	100.13	0.79
	400	480	885.42	100.61	0.97

*Average of three determinations

Robustness

The robustness of method was checked by making deliberate variations in method parameters. The parameters varied were wavelength (± 1 nm), mobile phase composition (± 1 % ethyl acetate) and the effect on the area of drug was noted. No marked changes in the densitograms and peak areas of drug demonstrated the robustness of the method.

CONCLUSION

Stability indicating HPTLC method without interference from excipients has been developed and validated for determination of Efavirenz as bulk drug and in capsule formulation. Validation results proved that the developed method performs well with specificity, linearity, range, accuracy, precision. The suggested method was found to be less time consuming and cost effective and may be more advantageous for routine analysis of drug in marketed formulation.

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