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Validated Stability Indicating High-Performance Thin-Layer Chromatographic Method for Determination of Rifabutin in Capsule Dosage Form

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ABSTRACT

A new simple, accurate and precise stability-indicating high-performance hin-layer chromatographic (HPTLC) method for determination of rifabutin as a bulk drug and in capsule formulation has been developed and validated. As stability testing is a major step in the development of the new drug as well as formulation, stress degradation studies were carried out according to ICH guidelines. Rifabutin was found susceptible to all the analyzed stress conditions. HPTLC plates precoated with silica gel 60 F_{254} were used as the stationary phase and chromatographic separation was achieved by using Chloroform: Methanol: n-hexane (5: 1: 4, v/v/v) as the mobile phase. Densitometric detection was carried out at 256 nm. The retention factor was found to be 0.39 ± 0.03. The developed method was validated concerning linearity, accuracy, precision, the limit of detection, the limit of quantitation, and robustness as per ICH guidelines. The developed method was found to be linear in the concentration range of 150-750 ng band^{-1}. The LOD and LOQ for Rifabutin were found to be 25.34 ng band^{-1} and 76.81 ng band^{-1} respectively. The developed method is stability-indicating and can be successfully employed for quantification of the drug in the capsule dosage form.

1.0 INTRODUCTION

Rifabutin, chemically, 1', 4-didehydro-1-deoxy-1, 4-dihydro-5'-(2-methylpropyl)-1 oxorifamycin is an antibiotic used to treat tuberculosis and prevent and treat *Mycobacterium avium* complex. It is typically only used in those who cannot tolerate rifampin such as people with HIV/AIDS on antiretrovirals. [1]

An extensive literature survey revealed that different analytical methods have been reported for quantitative analysis of Rifabutin. UV spectrophotometric methods for the determination of Rifabutin either as a single or in combination with other drugs in bulk and pharmaceutical formulations have been reported. [2-5] RP-HPLC methods for estimation of Rifabutin either as single or in combination with other drugs as bulk and pharmaceutical formulations are also reported. [6-10] High-performance liquid chromatography with ultraviolet detection for the determination of rifabutin in human plasma is also found in the literature. [11] The capillary electrophoresis method has been also reported for simultaneous determination of rifabutin and human serum albumin in pharmaceutical formulations. [12] One HPTLC method involving method development and validation for estimation of rifabutin in bulk and formulation is also reported. [13]

To the best of our knowledge, no reports were found in the literature for the determination of Rifabutin in pharmaceutical capsule dosage form by stability-indicating high-performance thin-layer chromatographic (HPTLC) method. Hence the purpose of the present work was to develop and validate simple, sensitive, precise, and accurate stability-indicating HPTLC procedure for the determination of Rifabutin as bulk drug and in capsule dosage form by the International Conference on Harmonization Guidelines. [14, 15]

2.0 MATERIALS AND METHODS

2.1 Chemicals and reagents

Analytically pure working standard Rifabutin was obtained as a gift sample from Lupin Pharmaceuticals Ltd., Mumbai, India. A pharmaceutical capsule dosage form Ributin labeled to contain 150 mg (Lupin Pharmaceuticals Ltd.) was procured from the local pharmacy. Methanol, Chloroform, and n-hexane (all AR grade) were purchased from Loba Chemicals Pvt Ltd., India.

2.2 Instrumentation and chromatographic conditions

Chromatographic resolution of the drug was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm × 10 cm with 250 µm layer thickness) from E. MERCK, Darmstadt, Germany, using a CAMAG Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with a 6 mm 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 a 10 ×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Chloroform: Methanol: n-hexane (5: 1: 4 v/v/v) as the mobile phase. The saturation of the mobile phase was done for 20 min in the chamber at room temperature. The length of the chromatogram run was 82 mm. Densitometric scanning was performed on a CAMAG TLC scanner III at 256 nm for all developments operated by winCATS software version 1.4.3. A deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm was used as the radiation source.

2.3 Preparation of working standard solution

Accurately weighed 15 mg of pure drug was dissolved in 10 mL of methanol to get the final concentration of 1500 ng μL^{-1} which was further diluted with methanol to acquire the final working standard solution having a concentration of 150 ng μL^{-1}.

2.4 Analysis of capsule formulation

The content of Rifabutin was estimated by use of a commercial brand of capsule namely Ributin. A quantity of powder equivalent to 15 mg of Rifabutin was weighed and transferred to a 10 mL volumetric flask. The mixture was sonicated for 5 min and diluted to volume with methanol. The solution was filtered and one milliliter of the above solution was further diluted with methanol to obtain the concentration of 150 ng μL^{-1}. Two µL volume of this solution was applied on a TLC plate to obtain a final sample concentration of 300 ng $band^{-1}$. After chromatographic development, the peak areas of the bands were measured at 256 nm and the amount of drug in the sample was determined from the calibration plot. The analytical procedure was repeated six times for the homogenous powder sample.

2.5 Forced degradation studies

Stress degradation studies were carried out to confirm the stability by exposing the bulk drug to different physical stress conditions recommended by ICH. The study was carried out at a

concentration of 1500 ng μL^{-1}. The acid and base hydrolytic studies were performed by treating stock drug solution separately with 0.01 N HCl and 0.01 N NaOH at room temperature for 1 h and 30min, respectively. The acid and alkali stressed samples were neutralized with NaOH and HCl, respectively to provide the final concentration of 750 ng $band^{-1}$. The standard drug solution was treated with 3 % H_2O_2 at room temperature 1 h to perform the oxidative degradation and was diluted with methanol to obtain a 750 ng $band^{-1}$ solution. Thermal stress degradation was performed by keeping the drug in the oven at 60ºC for a period of 1 h. The solid drug powder was exposed to UV light up to 200-watt hour $square\ meter^{-1}$ to check photolytic degradation. Thermal and photolytic samples were diluted with methanol to get the concentration of 150 ng $band^{-1}$.

3.0 RESULTS AND DISCUSSION

3.1 Method optimization

Current research work aimed to develop a stability-indicating HPLTC method that would be proficient to give a satisfactory resolution between rifabutin and its degradation products. Varied solvent systems comprising different ratios of n-hexane, benzene, chloroform, toluene, methanol, ethyl acetate, and glacial acetic acid were examined (data not shown) to achieve better separation and to resolve spot of Rifabuin. Finally, the mobile phase comprising of chloroform: methanol: n-hexane (5: 1: 4, v/v/v) was chosen as optimum which gave an acceptable resolution of drug with a symmetrical peak shape. Densitometry detection was carried out at 256 nm. The retention factor (Rf) was found to be 0.39±0.03 (Figure 1).

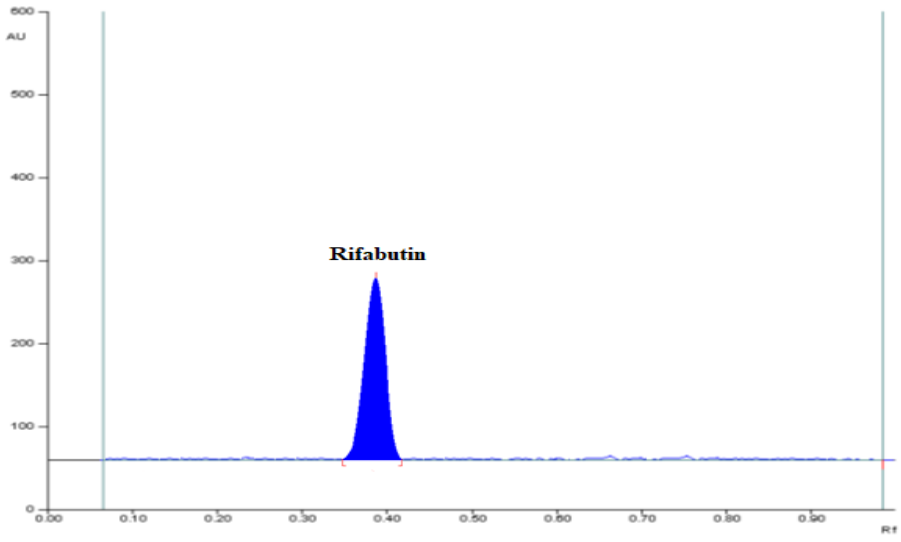

Figure 1: Representative densitogram of standard solution of Rifabutin

(600 ng band^{-1}, Rf= 0.39±0.03)

3.2 Result of forced degradation studies

The forced degradation studies indicated the susceptibility of the drug to all the analyzed stress conditions. Rifabutin concentrations decreased with the formation of degradation products under acid, alkaline and oxidative stress conditions (Figure 2-4). The degradation products were well resolved from the active drug indicating the specificity of the method. However, Rifabutin concentrations also decreased under thermal and photolytic stress conditions without the formation of any degradation product. The percentage degradation of Rifabutin under different forced stress conditions is depicted in Table 1.

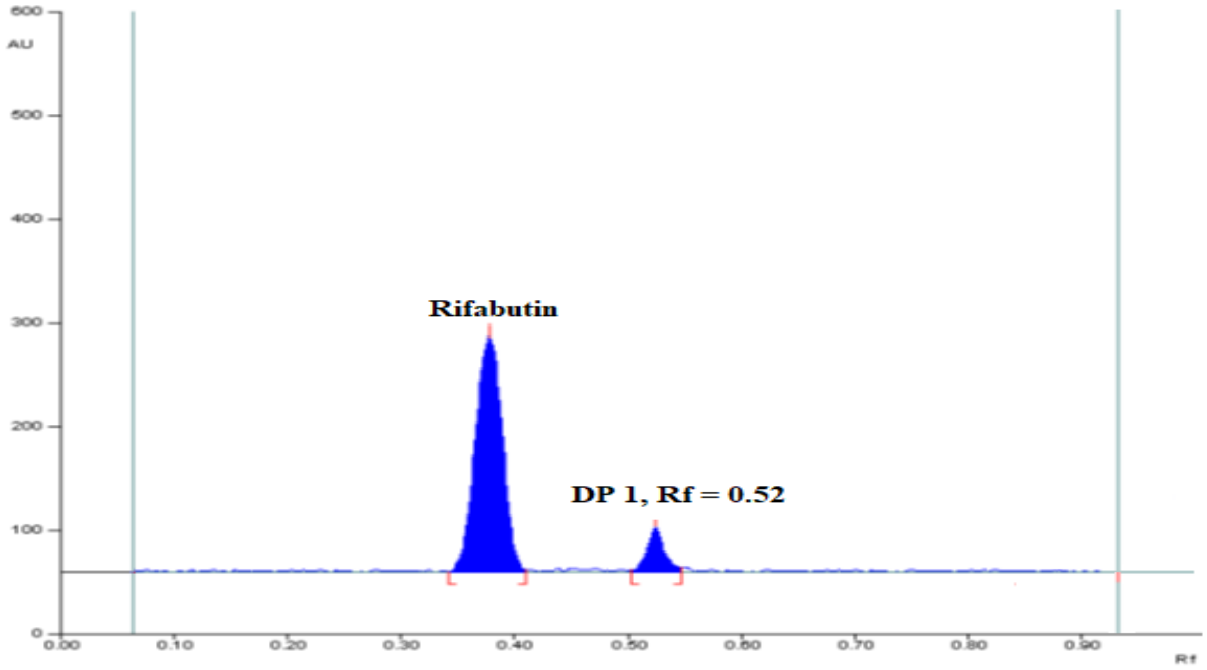

Figure 2: Densitogram after treatment with 0.01 N HCl with degradation product

(Rf= 0.52)

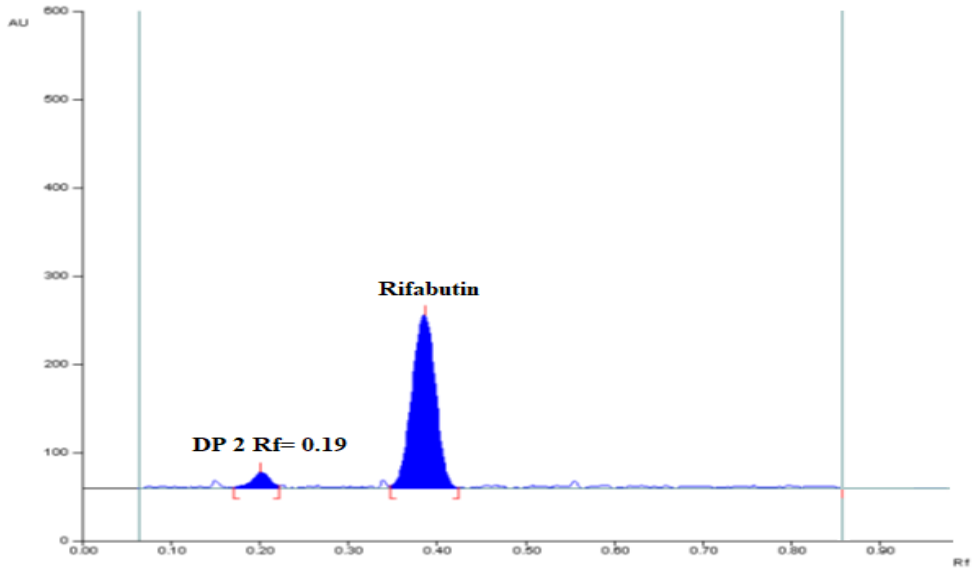

Figure 3: Densitogram after treatment with 0.01 N NaOH with degradation product (Rf= 0.19)

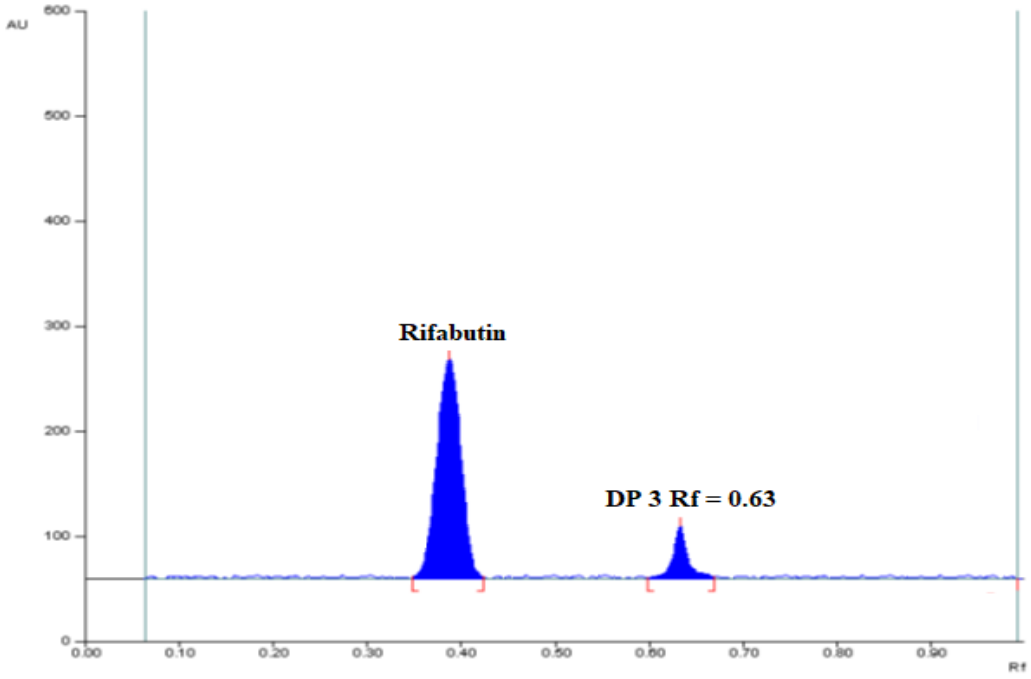

Figure 4: Densitogram after treatment with 3% H_2O_2 with degradation product (Rf= 0.63)

Table 1: Summary of forced degradation studies

Stress conditions	% Recovery	% Degradation
Acid hydrolysis (0.01 N HCl, Kept at RT for 1 h)	84.36	15.64
Base hydrolysis (0.01 N NaOH, Kept at RT for 30 min)	79.77	20.23
Oxidative degradation (3 % H_2O_2, Kept at RT for 1h)	77.40	22.60
Thermal degradation (60º C for 1 h)	81.53	18.47
Photolytic degradation (UV light, 200 watt hr square meter^{-1})	79.12	20.88

3.3 Method Validation

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, the limit of detection, the limit of quantitation, and robustness, by ICH Q2 (R1) guidelines.

3.3.1 Linearity

For the preparation of a calibration plot, volumes 1, 2, 3, 4, and 5 µL from the standard solution of Rifabutin (150 ng µL^{-1}) were withdrawn and diluted with methanol to get the range150-750 ng band^{-1} and spotted onto the TLC plates, developed and scanned under optimized chromatographic conditions. The method was found to be linear in the concentration range 150-750 ng band^{-1} with a high correlation coefficient. The linear regression equation was found to be y = 4.3593x + 568.3 with a correlation coefficient (R^2) value of 0.991 (Figure 5). The calibration curve was obtained by the plot of concentration vs peak area of the drug. A 3D densitogram of obtained in the concentration range 150-750 ng band^{-1} is shown in Figure 6.

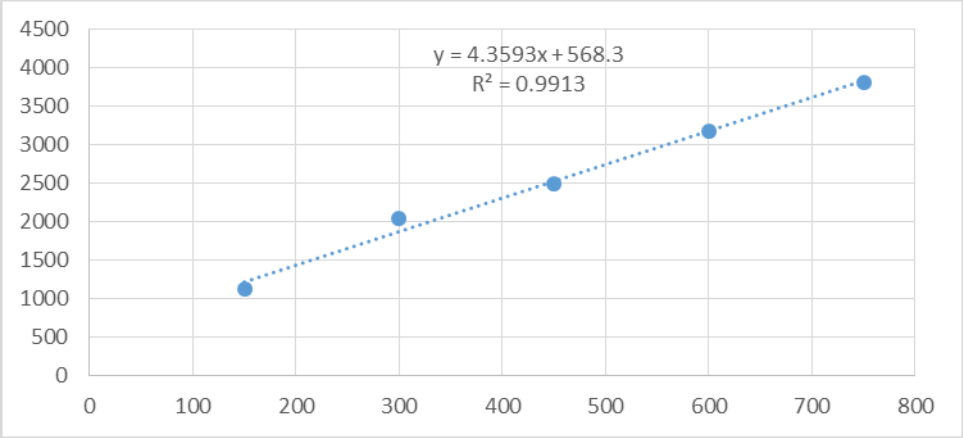

Figure 5: Calibration curve for Rifabutin

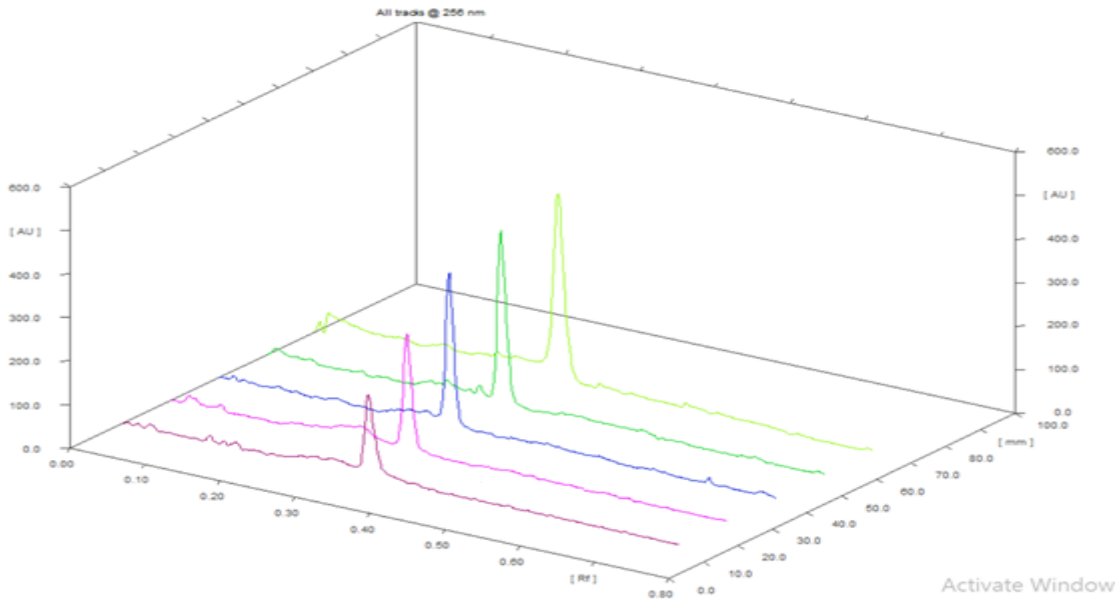

Figure 6: 3D densitogram in concentration range 150-750 ng band^{-1}

3.3.2 Precision

The precision of the method was demonstrated by intraday and interday variation studies in which three replicates of three concentrations within the linearity range were analyzed on the same day and three consecutive days, respectively, and percentage R.S.D. was calculated. The % R.S.D. values obtained for intraday and interday variations were found to be < 2 which indicated that the method is precise. The results obtained for intraday and inter-day precision studies are shown in Tables 2 and 3, respectively.

Table 2: Intraday precision studies

Spotted concentration (ng band^{-1})	Average Area	Recovered concentration (ng band^{-1})	% RSD*
300	1875	298.37	1.10
450	2536	451.30	1.20
600	3171	597.11	0.69

*Average of three determinations

Table 3: Interday precision studies

Spotted concentration (ng band^{-1})	Average Area	Recovered concentration (ng band^{-1})	% RSD*
300	1860	296.30	1.03
450	2527	449.31	1.07
600	3149	591.99	0.82

*Average of three determinations

3.3.3 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 25.34 ng band^{-1} and 76.81 respectively.

3.3.4 Recovery studies

The accuracy of the developed method was checked by performing recovery studies by the standard addition method. It involved the addition of standard drug solution to pre-analyzed sample solutions at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 300 ng band^{-1}. The results of the recovery studies indicated the accuracy of the method for estimation of drug in the tablet dosage form. The results obtained are represented in Table 4.

Table 4: Recovery studies

Drug	Concentration taken (ng band^{-1})	Concentration added (ng band^{-1})	Concentration found (ng band^{-1})	% Recovery	% R.S.D.*
Rifabutin	300	240	537.09	99.45	1.23
	300	300	600.48	100.07	0.97
	300	360	656.53	99.46	0.94

*Average of three determinations

3.3.5 Robustness

By introducing deliberate variation in the method parameters, the effects on the results were examined to check the robustness of the method. The parameters varied were mobile phase composition (± 1 % methanol), wavelength (± 1 nm), and the effect on the area of drug was noted. The areas of peaks of interest remained unaffected by small changes in the operational parameters which indicated the robustness of the method.

4.0 CONCLUSION

Stability-indicating HPTLC-densitometric method without interference from degradation products has been developed and validated for the estimation of Rifabutin as bulk drug and in capsule formulation. The drug was found to be susceptible all analyzed stress conditions including heat and light. The degradation products formed during forced degradation were well resolved from the active drug indicating the specificity of the developed method. The developed method is simple, sensitive, precise, accurate, and reproducible. The developed method can be used for quantitative analysis of drugs in the pharmaceutical dosage form.

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