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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR DETERMINATION OF RIFABUTINE IN API AND CAPSULE DOSAGE FORM

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ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed and validated for determination of Rifabutine in bulk and capsule dosage form. The Rifabutine samples were applied on TLC aluminium plate pre coated with Silica gel60 GF254 and developed using acetone as a mobile phase. The bands were scanned at λ=282 nm using Camag TLC scanner 3 and detection and quantification were carried out densitometrically using an UV detector. The Rf value was found to be 0.58. The linearity of the method was found to be within the concentration range of 100-700 ng/spot and its percentage recovery was found to 97.89 %. The limit of detection and the limit of quantification were found to be 36.83 ng/spot and 111.6 ng/spot respectively. The Correlation of determination (r2) was 0.9995. The regression equation was found to be y = 11.906x+856.83. The method was also validated for precision, specificity and recovery. This developed method was used to analyze marketed formulation.

Keywords: HPTLC, Rifabutine, Acetone, Rf Value

1. INTRODUCTION

Rifabutin (Rfb) (fig. 1) is a bactericidal antibiotic drug primarily used in the treatment of tuberculosis. The drug is a semi-synthetic derivative of rifamycin . Its effect is based on blocking the DNA-dependent RNA-polymerase of the bacteria. It is effective against Gram-positive and some Gram-negative bacteria, but also against the highly resistant Mycobacteria, e.g. *Mycobacterium tuberculosis*, *M. leprae*, and *M. avium intracellulare*. Literature survey reveals that one combination method has been developed for their determination by HPTLC. In this study, HPTLC method for the analysis of Rifabutine using a solvent system of acetone has been developed.

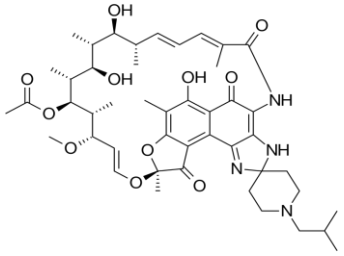

Fig.1: Chemical Structure of Rifabutine

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Pure Rifabutine was obtained as gift sample from Lupin pharmaceuticals, Aurangabad.India. Commercially available capsule manufactured by Lupin Rifabutine containing 150 mg of Rifabutine was procured from local pharmacy. Methanols, Chloroform, Acetone used were of analytical grade (E. Merck, Mumbai, India). All the other chemicals used were also of analytical grade. (E. Merck, India).

2.2. Instrumentation and conditions

HPTLC plates (Merck) pre-coated with silica gel GF aluminium TLC plate; (10cm×10cm) were used. Densitometry was carried out with a CAMAG TLC Scanner 3, fitted with win-CATS 1.4.0 planar chromatography manager software. Sample were applied to the HPTLC plates using the spray on technique of CAMAG LINOMAT V under nitrogen gas, and developed in a CAMAG 10 cm×10 cm twin trough chambers.

2.3. Standard preparation

A standard stock solution of Rifabutine was prepared by dissolving 10 mg of standard API in 10 ml of acetone to get concentration of 1000 µg/ml. This solution was further diluted to get 100 µg/ml solution of Rifabutine as working standard.

2.4. Preparation of Sample solution

Twenty capsules of brand Rifabutine (Lupin Pharmaceuticals Ltd.) containing 150 mg of Rifabutine were weighed, average weight determined. Appropriate quantity of powder equivalent to 100 mg of Rifabutine was accurately weighed and transferred to a 100 ml volumetric flask and dissolved with acetone and shaken vigorously for 5 minutes. The solution was then sonicated for 20 minutes and volume was made up to 100 ml and filtered through the Whatman filter paper no.41. Necessary dilutions of filtrate were made

with acetone to get final concentration 10 µg/ml of Rifabutine.

2.5. Selection of mobile phase

A trial and error method was used to select the optimised mobile phase. The solvent system of acetone was the most appropriate mobile phase for the HPTLC analysis of Rifabutine in acetone as solvent.

2.6. Application of standard solutions

Separate HPTLC pre-coated plate of silica gel G 60 F254 (10x10) was employed for the spotting of standard solutions. 10 µl of standard solutions of concentration 100, 200, 300, 400, 500 and 600 ng/spot were applied in the six tracks respectively in one plate.

2.7. Application of sample solution

Ten mg of rifabutine is weighed and dissolved in 10ml of acetone and final volume is make up with acetone 10 µl of sample solution of 10 µg/ml for Rifabutine was applied. The same procedure was repeated with the sample solution prepared from capsule dosage form. After application the position of spots were visualized and confirmed under UV cabinet at 282 nm.

2.8. Development of spot

Twin Trough chamber containing 10 ml of mobile phase acetone system was used for developing the spotted plates and saturated for 15 minutes. The plates were dried after development and viewed under UV lamp to evaluate the spot obtained. The spots were uniform and there was no tailing.

2.9. Selection of wavelength for Detection

The working standard of Rifabutine in acetone was scanned by Camag TLC scanner 4 with UV visible detector over wavelength range 200 to 400 nm. Wavelength 282 nm was selected for detection of obtained spectrum (Fig. 2 &3).

2.10. Chromatographic conditions

The analysis was performed on Camag HPTLC system (Switzerland). It is equipped with a Linomat-5 applicator, 100 µl sample syringe (Hamilton, Switzerland) and Camag TLC scanner-4. On the basis of trial and error method using different solvent system, following chromatographic conditions were chosen for analysis. Pre-coated silica gel 60 F_{254} TLC (E-Merck, Germany) plates (10x10 cm) were used as stationary phase. TLC plates were pre-washed with methanol and activated at 110°C for 10 min prior to application. The standard samples of Rifabutine were spotted on pre-coated TLC plates in the form of bands of length 4 mm using 100 µl sample syringe with a Linomat-5 applicator. The chromatographic development was carried using Acetone as mobile phase with chamber saturation time of 20 minutes and the migration distance of 80 mm. Densitometric scanning was performed using Camag TLC scanners at 282 nm, operated by win CATS Software (Version 1.4.3, Camag).

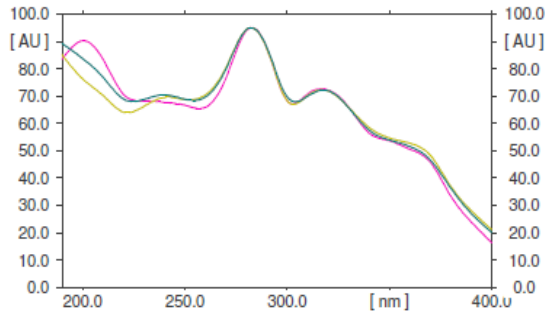

Fig. 2: The overlain UV spectra of Rifabutine (API and sample) between 200 and 400 nm

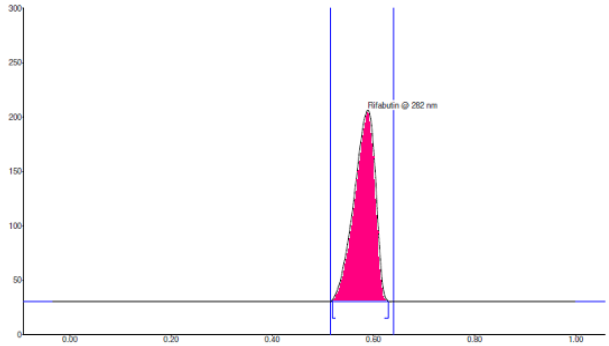

Fig. 3: Typical chromatograms obtained for Rifabutine

2.11. Assay for marketed preparation

Twenty capsules were weighed and average weight was calculated. The capsule powder equivalent to mg of average weight of Rifabutine was accurately weighed, transferred to a 100 ml of volumetric flask dissolved in acetone then solution was ultrasonicated for 20 min and diluted up to mark with acetone then filtered with Whatmann filter paper No. 41 and the first 5 ml of filtrate was discarded. This solution was further diluted with same solvent and subjected for HPTLC study (Table1).

The plate was developed under previously described chromatographic conditions.

Table 1. Assay of marketed formulation of Rifabutine

Sample solution concentration (ng/spot)	Sample solution area	Mean Sample solution area	% Drug content
300	4502.3		
300	4462.6	4495.23	102.04
300	4520.8		

3. METHOD VALIDATION

The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity & Range, Precision, Limit of Detection (LOD) & Limit of Quantitation (LOQ) and Accuracy according to ICH Q2 (R1) guidelines [10].

3.1. Linearity and Range

The linearity was determined by using working standard solutions between 100-700ng/spot was recorded. The spectrums of these solutions were recorded and area in wavelength 282 nm. Calibration curve of peak area *v/s* concentration was plotted after suitable calculation and simple linear regression was performed [Table 2]. Regression equation and correlation coefficient were obtained. The regression equations for Rifabutine was y=11.906x + 856.83, where, y is response and x the concentration of drug. The correlation coefficients were 0.9995 [Fig. 4].

Table 2. Concentration, Rf and Area of Rifabutine

Amount ng/spot	Rf	Area
100	0.61	1989.9
200	0.61	3239.9
300	0.61	4504.2
400	0.61	5651.3
500	0.61	6798.5
600	0.61	7959.4

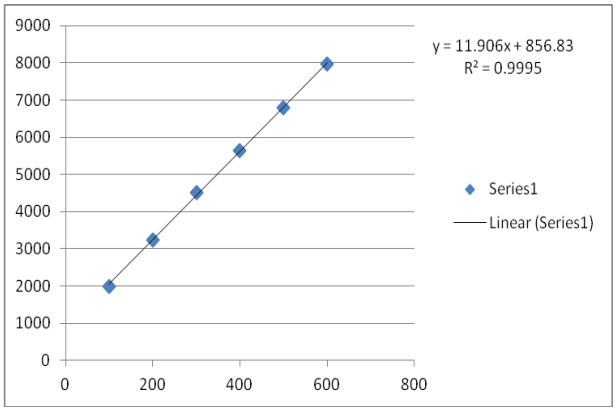

Fig. 4: Calibration Curve of Rifabutine

3.2. Precision Studies

The precision of the method was checked by repeatedly injecting (n= 8) standard solutions of Rifabutine (500 ng/spot). Area of each curve of these solutions was measured at the 282 nm. The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days of same concentrations of 500 ng/spot of Rifabutine. The results were reported in terms of percentage relative standard deviation (%RSD). The RSD values were found to be below 2% which indicate that the proposed methods are precise. The results were tabulated in (Table 3).

Table 3: Intermediate Precision

Drug	Conc. (ng/band)	Intra-day precision (%RSD)	Inter-day precision (%RSD)
Rifabutine	500	0.8025	1.0980
	500	0.9278	1.1411

3.3. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and the LOQ of the drug were calculated using the equations 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 limit of detection was found to be 36.83 ng/spot. The limit of quantification was found to be 111.6 ng/spot.

3.4. Accuracy

To check the accuracy of the method, recovery studies were carried out by over spotting standard drug solution to pre-analyzed sample solution at three different levels 80 %, 100 % and 120 %. Basic concentration of sample chosen was 400 µg/ml. The areas were noted after development of plate. The drug concentration was calculated by using regression equation [Table 4].

Table 4. Recovery Studies

Level Recovery %	Amount taken (ng/spot)	Amount added (ng/spot)	% Recovery	% Mean Recovery	% RSD
80	400	320	96.03		
100	400	400	98.45	97.89	1.67
120	400	480	99.12		

3.5. Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for drug in sample was confirmed by comparing the Rf and spectra of the spot with that of standard drug spot. The specificity of the method was also ascertained by peak purity profiling studies by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on Win CATS software 5 [Fig. 5].

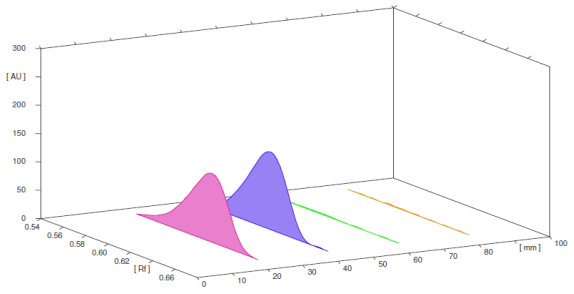

Fig. 5: Specificity curve of Rifabutine

4. RESULTS AND DISCUSSION

The calibration curve was plotted of Rifabutine peak area v/s Concentration. The generated regression equation was y=11.906x+856.83 (r^2=0.9995). The r^2 value as 0.9996 indicates that developed method was linear. The calibration curve was obtained in the range of 100-700 ng/spot. The proposed method was found to be precise as % R.S.D values for intraday as well inter-day precision were satisfactory. The average percentage recovery at 80 %, 100 % and 120 % was found to be 97.89% which showed good recoveries. Hence, it can be said that this method was accurate. The LOD and LOQ were calculated as 36.83 ng/spot and 111.6 ng/spot respectively. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for the routine analysis of the rifabutine in capsule dosage form. The summary of validation parameters of proposed HPTLC method is given in Table 5.

Table 5: Summary of validation parameters

Parameters	Results
Linearity range (ng/spot)	100-700
Correlation co-efficient	0.9995
Slope (m)	11.906
Intercept (C)	856.83
Precision (intraday) %RSD	1.0980
Precision (interday) %RSD	1.1411
Accurracy (mean)	97.89
LOD (ng/spot)	36.83
LOQ (ng/spot)	111.6

5. CONCLUSION

The developed and validated HPTLC method is found to be rapid, accurate, precise and economical, thus can be used for routine analysis of Rifabutine in capsule dosage form.

6. ACKNOWLEDGEMENT

The authors are grateful to Lupin pharmaceuticals, Aurangabad, India for providing API of Rifabutine as gift sample. Authors also thank to Anchrom Enterprises Pvt. Ltd. Mumbai, India and Smt. Kashibai Navale College of Pharmacy, Pune, India for providing necessary facilities to complete this project.

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