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Analytical Assay Method for Rifabutin in Bulk and Dosage Form by Using Rifabutin Capsule Dosage Form

Seema B Kharwade* ;Dr. Narendra G Patre; Dr. Ajay D Kshirsagar ; Sudam G Mule

D.K. Patil Institute Of Pharmacy, SRTMU University,MH, India

**Corresponding Author: Seema B Kharwade, D.K. Patil Institute Of Pharmacy, SRTMU University, MH, India*

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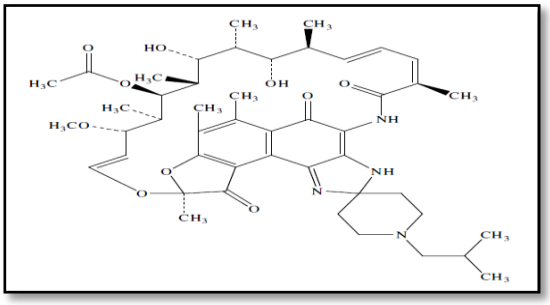
ABSTRACT: A selective, accurate, HPLC method was developed by this study for the determination of rifabutin in capsule and bulk dosage form. This method was developed Shimadzu SCL-10AVP using C-18 column (Zodiac-100) was used as stationary phase mixture of 0.5% trifluoroacetic acid and acetonitrile (30:70 v/v) gives good resolution of peaks with acceptable peak symmetry. At 1.2 ml/min flow rate the mobile phase was pumped and the sample was detected at 273 nm. The method was validated for analytical standards such as linearity and range, limit of detection, limit of quantitation, accuracy, repeatability and ruggedness. In a wide range of 10-50 µg/ml the linearity was observed. The method was validated, and a recovery study indicates accuracy of this method. The proposed method is simple, suitable, accurate and stable in accordance with the ICH guidelines.

KEYWORDS: RP-HPLC, Rifabutin, validation method development, Trifluoroacetic Acid And Acetonitrile, LOD, LOQ

Fig. 01: Structure of RIFABUTN



I. INTRODUCTION

Rifabutin is chemically (9S,12E,14S,15R,16S,17R,18R,19R,20S,21S,22E,24Z)-6,16,18,20-Tetrahydroxy-1'-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethylspiro[9,4(epoxypentadeca[1,11,13]trienimino)-2H-furo-[2',3':7,8]-naphth[1,2-d]imidazol-2,4'-piperidin]-5,10,26-(3H,9H)-trione[1].Fig.01 molecular weight is 847.019 g/mol.[2] Rifabutin acts via the inhibition of DNA-dependent RNA polymerase in gram-positive and some gram-negative bacteria, leading to a suppression of RNA synthesis and cell death.[3] Adults dose daily dose of 150 mg (one Rifabutin capsule) so that used as an Anti bacterial agent.[4] Antibiotic and Antitubercular Category.[5] with Plasma Protein Binding 85% and Half-life 45 hours (range: 16–69 hours.[6]

II. MATERIAL AND METHOD

Pure sample of Rifabutin was received as a gift sample from Auriga Research Pvt. Ltd.Gujrat . Marketed formulation containing Rifabutin 150 mg (Ributine Capsule 150 mg, Lupin Pharmaceuticals, Aurangabad, Maharashtra).

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INSTRUMENTATION

The HPLC system consisted with detector DAD operated at a wavelength range is 200 nm-800 nm. The software used was Empower 2.0. The column used was Shimadzu SCL-10AVP using C-18 column (Zodiac-100). Balance was used and Wenser Ultra Sonicator Model: WUC- 4L was used.

METHOD

Selection of wavelength for HPLC analysis was determined by recording UV spectrum in range 190-400nm for rifabutin. The scanned result showed that reasonable maximum absorbance was recorded at 273nm.

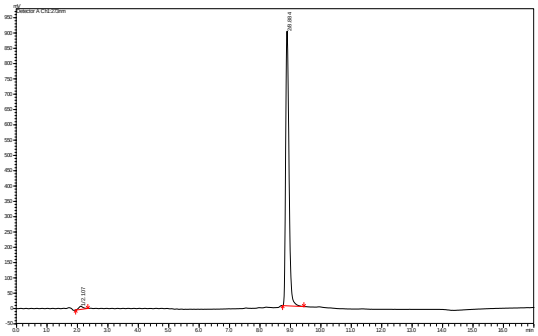

Fig.2 : Rifabutin at Wavelength 273nm

PREPARATION OF STANDARD STOCK SOLUTION

Standard stock solution of Rifabutin was prepared by dissolving 10 mg of drug in 0.5ml of 0.1N HCl and upto 10 ml of Distilled water to get standard stock solution of 1000 µg/ml by sonicating for 15 min. and further dilutions were made by using distilled water.

ANALYSIS OF THE MARKETED FORMULATIONS

Twenty capsules (Ributine Capsule 150 mg, Lupin Pharmaceuticals, Aurangabad, Maharashtra) were weighed accurately and capsules weight equivalent to 10 mg of Rifabutin was weighed accurately and transferred to 10 ml volumetric flask and add 0.5 ml 0.1N HCl and diluted up to mark with water. The solution was filtered through filter paper no. 41; 10 ml of this filtrate was further diluted to 10 ml distilled water. From this solution, further dilutions were made using distilled water to get the final concentration of 10 µg/ml of Rifabutin. The solution was scanned in the range of 200-400 nm against blank. Absorbance's were recorded at wavelength 273 nm.[7] The concentration of drug was then calculated. The amount of the drugs were calculated and tabulated in Table : 01

Sr. No.	Name of drug	Label claim (mg)	Concentration of drug µg/ml	% Drug content	% RSD
1	Rifabutin	150	30	99.22	0.037

Table 01 :Results of marketed formulation

The results Rifabutin of marketed formulation analysis and recovery studies for UV and RP-HPLC has been summaries. % mean recovery and %RSD values are found within limits. Hence UV and RP-HPLC was found accurate.

SAMPLE PREPARATION FOR ASSAY

To determine the content of drugs in capsule formulation, twenty capsules were weighed, their average weight was determined and finely powder was weighed equivalent to 10 mg Rifabutin was weighed. Then, equivalent weight of the drug was transferred into a 10 ml volumetric flask containing 0.5 ml 0.1N HCl, sonicate for 15 minutes and diluted to 10 ml with Distilled water. The resulting solution was then filtered using 0.45µ Whatmann filter paper. The original stock solution was further diluted to get sample solution of drug concentration of 30 µg/ml Rifabutin.

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METHOD VALIDATION

The method was validated for linearity and range, limit of detection, limit of quantitation, accuracy, repetability and ruggedness, robustness, and system suitability parameter as per ICH guideline. Validation of HPLC method was done with respect to following parameters.

LINEARITY AND RANGE

Linearity of Rifabutin, the method was studied by five concentrations of the drug prepared in the distilled water in the range of 10-50 µg/ml Fig.2.[8] Absorbance of these solutions were recorded at 278 nm wavelength, Calibration curve was plotted, absorbance versus concentration. Results are shown in Table 2. The correlation coefficient for Rifabutin was found to be 0.999. Therefore the method was found to be linear.

sr	Concentration ($\mu g.mL^{-1}$)	Area	Average (Mean)
1	250	8041789	8041789
2	125	4097241	4097241
3	62.5	2049768	2049768
4	31.25	1022862	1022862
5	15.62	528640	528640
Regression Equation		y = 32099x + 38538	
Correlation coefficient (R^2)		0.9999	
Std. error of intercept		21809.8407	
Std. Dev. Of intercept		48768.28638	
LOQ		15.19	
LOD		5.01	

Table 02 : Linearity data of Rifabutin

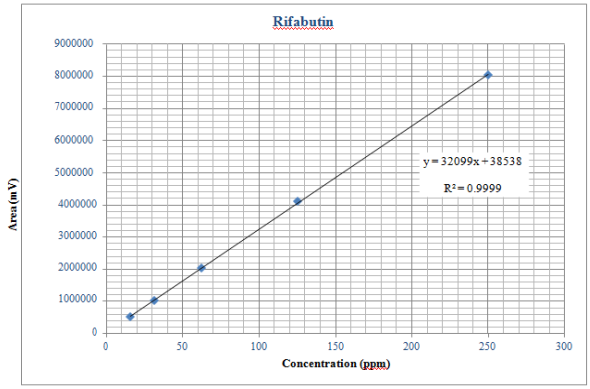

Fig.3 : Linearity of Rifabutin

Limit of detection (LOD) and limit of Quantitation (LOQ)

The standard deviation of Y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ for both the drugs using the following formulae.[9] Results are shown in Table 3 ,LOD and LOQ was found to be 5.01 µg/ml and 15.19 µg/ml resp.

$$LOD = 3.3(Sd)/s$$

$$LOQ = 10\ (Sd)/s$$

Where,

Sd = Standard Deviation

S = Slope of the line

Drug	LOD (µg/ml)	LOQ (µg/ml)
Rifabutin	5.01	15.19

Table 03 : LOD and LOQ of Rifabutin

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ACCURACY

For UV method recovery studies was carried out by known amount of standard drug corresponding to 80, 100 and 120% w/w of label claim had been added to marketed drug sample (Standard addition method). At each level of the amount three determinations were performed and the results obtained were compared with expected results. The solutions were analyzed by proposed method and the results of % recovery of Rifabutin were found to be reported in Table 4. Therefore the method was found to be accurate.

Conc. (%)	Ref. Std. (mg)	Mkt. Drug (mg)	Recovery (mg)	% Recovery	Peak Area (500 ppm)	Mean Recovery (%)	STD. Deviation	% RSD
80%	150	120	268.78	99.55	6077092	99.63	0.37	0.37
	150	120	270.09	100.03	6106711			
	150	120	268.12	99.30	6062169			
100%	150	150	303.1	101.03	6853064	100.86	0.40	0.39
	150	150	301.23	100.41	6810784			
	150	150	303.45	101.15	6860978			
120%	150	180	328.23	99.46	7421251	100.32	0.44	0.44
	150	180	329.02	99.70	7439113			
	150	180	331.04	100.32	7484785			

Table 04: Accuracy Studies of Rifabutin

SYSTEM SUITABILITY TESTING PARAMETERS

System suitability testing parameters are a test to determine the suitability and effectiveness of chromatographic system prior to use. The performance of any chromatographic system may continuously change during their regular use, which can affect the reliability of the analytical results. Some are show in Table 05

Sr. No.	Parameter	Rifabutin	Formula	Limits
1.	Capacity factor (k')	4.162	$k'=t_R-t_0/t_0$	$k' > 2.0$
2.	Repeatability	1.54	____	≤ 2%
3.	Retention time (mins)	8.9	____	<K<20
4.	No. of theoretical plates (N)	25835.56	$N=16(t/W)^2$	>2000
5.	Tailing factor	1.332	$T_f = W_{0.05\%}/2f$	$_f < 2$

Table 05 : System Suitability Parameters

REPEATABILITY OF RIFABUTIN

The analysis of drug was carried out three times on the same day to find the repeatability of the sample by using 15 µg/ml and the % RSD was calculated for the resultant peak area The peak area of 250 ppm drug solution was analysed six times on the same day. The % RSD was calculated for the resultant peak area.[10]

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S. No.	Peak Area; Conc. 250 ppm
1	6616642
2	6583252
3	6570151
4	6602481
5	6594464
6	6841789
Mean	6634796.5
STD. DEV.	102647.3177
RSD (%)	1.54

Table 06 : Repeatability of Rifabutin

Ruggedness of Rifabutin

Ruggedness of Rifabutin, the proposed method was determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions. The proposed method was found to be rugged as indicated by % RSD of Rifabutin was 0.330 and results was shown in Table 07.

Sr. no	Conc.	Absorbance		Avg	SD	%RSD
		Analyst I	Analyst II			
1	10	0.397	0.399	0.398	0.001414	0.3552
2	20	0.587	0.581	0.584	0.004243	0.7265
3	30	0.772	0.776	0.774	0.002828	0.3653
4	40	0.945	0.947	0.946	0.001414	0.1494
5	50	1.15	1.151	0.1505	0.000707	0.0614

Table 07: Ruggedness studies for Rifabutin

Method	Drug	% Mean Recovery	S.D.	%RSD
HPLC Method	Rifabutin	99.22	0.25	0.037

Table 08 : Results of marketed formulation

III. RESULT AND DISCUSSION

The present study was aimed to developing accurate, precise and linear RP-HPLC method for estimation of Rifabutin. There was found to be linear in range of 10-50µg/ml with correlation coefficient (R^2) 0.9999. The % assay was found to be 99.22 (±%RSD). The % recovery was found to be in the range of 99.22 for Rifabutin, LOD and LOQ method calculated to be 15.19µg/ml 5.01ug/ml and respectively. The summary of validation parameters of proposed RP-HPLC method is given in Table 8.

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Parameters	Rifabutin
Linearity ranges(µg/ml)	10-50 µg/ml
Std. Error of Intercept	**48768.28638**
Coefficient Correlation R^2	**0.9999**
% Recovery	99.22
LOD	**5.01**
LOQ	**15.19**

Table 09 : Summary of validation parameters of proposed RP-HPLC method.

IV. CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of Rifabutin in bulk and tablet dosage form. The said method was developed using economical percentage of organic phase in aqueous media as solvent. Said validated UV- visible method can be efficiently used for the estimation of Rifabutin in bulk as well as formulation

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CONFLICT OF INTEREST

There is no conflict of interest.

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