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RP-HPLC method for the estimation of Rifabutin in bulk dosage form

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Abstract

A simple, economic, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the determination of rifabutin. A phenomenex C-8 Luna (250 × 4.6 mm, 5 µm) in isocratic mode with mobile phase containing Methanol and Water (75:25 v/v) was used. The flow rate was 1 ml/min and effluents were monitored at 240 nm and run time was 10 min. The retention time for Rifabutin was 5.5 min. The RP-HPLC method was extensively validated for linearity, accuracy, precision (Interday precision, Intraday precision), repeatability and robustness. The validation studies were carried out fulfilling ICH requirements. The procedure was found to be linear, precise, accurate and robust.

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Key words:
Rifabutin, RP-HPLC, Phenomenex, Methanol

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1. Introduction:

Rifabutin is semi-synthetic derivative of rifamycin S, a bactericidal antibiotic which is primarily used in the treatment of tuberculosis [1].It is effective against Gram-positive and some Gram-negative bacteria by blocking the DNA-dependant RNA-polymerase of the bacteria, it is also effective against the highly resistance Mycobacteria, e.g. Mycobacterium tuberculosis, M.laprae and M.avium intracellulare. Rifabutin shown the activity against Mycobacterium aviumintracellular isolated from the patients with AIDS [1-2]. Rifabutin is official in United States Pharmacopoeia [3] and European Pharmacopoeia [4]. Determination of rifabutin in bulk drugs and formulations by spectrometric method and chromatography method [5] were reported. Rifabutin determination in biological samples like Human plasma [6], rat urine [7] was subjected to extensive

study by high performance liquid chromatographic method. A new method for the HPLC determination of rifabutin is described in this paper. The method is substantially simpler, faster and more economic.

IUPAC name of rifabutin is (9*S*, 12*E*, 14*S*, 15*R*, 16*S*, 17*R*, 18*R*, 19*R*, 20*S*, 21*S*, 22*E*, 24*Z*) 6, 16, 18, 20-tetra hydroxyl 1'isobutyl 14 methoxy 7, 9, 15, 17, 19, 21, 2 5 heptamethylspiro [9, 4 (epoxypentadeca [1, 11, 13] trienimino) -2*H*-furo-[2', 3' : 7, 8] – naphtha [1,2-*d*] imidazol-2,4'-piperidin]-5,10,26-(3*H*,9*H*)-trione-16-acetate, (Fig.No.1: Chemical structure Rifabutin) with empirical formula $C_{46}H_{62}N_4O_{11}$.

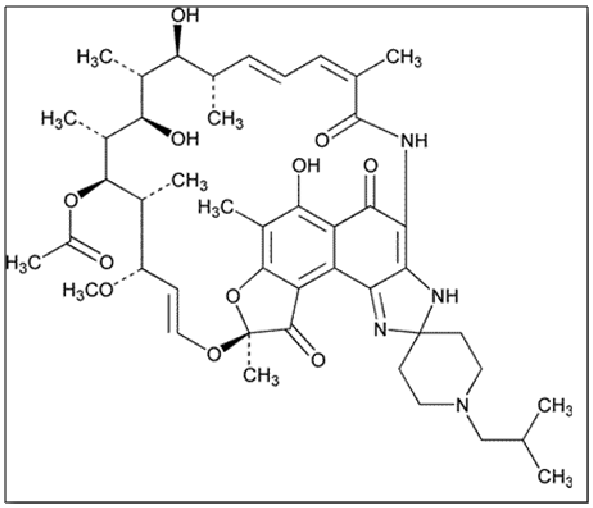

Fig.1: Chemical structure of Rifabutin

2. Experimental:

2.1 Apparatus

A HPLC (Perkin Elmer Lambda 25) equipped with Perkin Elmer Binary LC Pump 200B/ 250, solvent degasser, Series 200 UV/VIS detector and phenomenex C-8 Luna (250 × 4.6 mm, 5 µm) column was used with Total Chrome Navigator Software (version 6.3.1).

2.2 Reagent and Materials:

Analytical reagent grade Methanol (Merck Specialities Private Limited), Acetonitrile (Merck Specialities Private Limited) and distilled water filtered through a 0.45 µm Millipore PVDF (polyvinyl difluoride) filter were used.

2.3 Mobile Phase:

The mobile phase for chromatography consisted by Methanol : Water (75:25).

2.4 Rifabutin and its preparation

Rifabutin API was provided as a gift sample by Lupin ltd, Aurangabad and its dosage form is purchase from the market.

2.5 Standard solution

Accurately weighed quantity of rifabutin 10 mg was transferred to 100 ml volumetric flask and made up to volume with diluent. Samples were dilute to concentration of 10, 20, 40, 60, 80 and 100 µg ml^{-1} and used for validation study.

2.6 Chromatographic conditions:

Chromatographic separation was performed at ambient temperature on a reversed-phase phenomenex C-8 Luna (250 × 4.6 mm, 5 µm) column using a mobile phase consisting of Methanol: water (75:25) at a flow rate 1 ml min^{-1}. The detector wavelength was set at 240 nm as determined by Perkin Elmer Lambda 25 UV/VIS spectrometer.

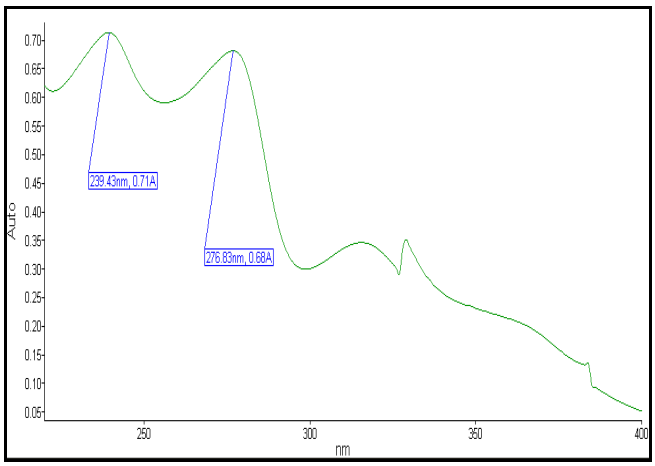

Fig. 2: λ_{max} of Rifabutin

3. Result and Discussion

3.1 Method Development

The method utilizing Methanol: Water as mobile phase yielded broad peak, whereas with MeOH: Water tailing was observed with methanol as diluent. Procedure utilizing Methanol : Water as mobile phase with water as diluents also yielded tailing

where as with MeOH: Water mobile phase and acetonitrile as diluent sharp peak was obtained.

During method development, a number of variations were tested like MeOH concentration and flow rate to give a symmetric peak. With a mobile phase MeOH: Water (75:25) at flow rate 1 ml min^{-1} and wavelength is 240 nm, symmetric peak was obtained [Fig. 3].

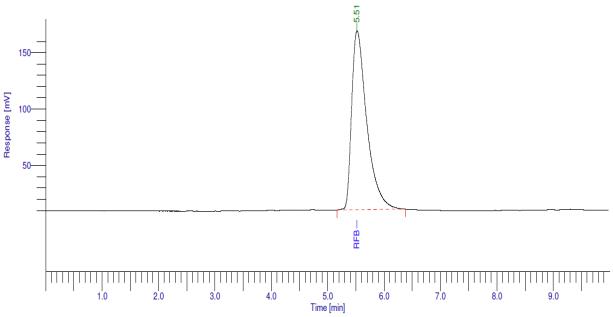

Fig.3: Chromatogram of Rifabutin

3.2 Validation

3.2.1 Linearity

Six serial dilutions were prepared in concentration range from 10 to 100 µg/ml . A volume of 20 µl from each concentration of the solution was injected and chromatograms were recorded; three independent determinations were performed at each concentration.

A linear calibration graph (y = 62492x + 9786; where y and x are peak area and concentration, respectively) was obtained over six concentrations 10, 20, 40, 60, 80,100 µg/ml. Correlation coefficient was found to be 0.997.

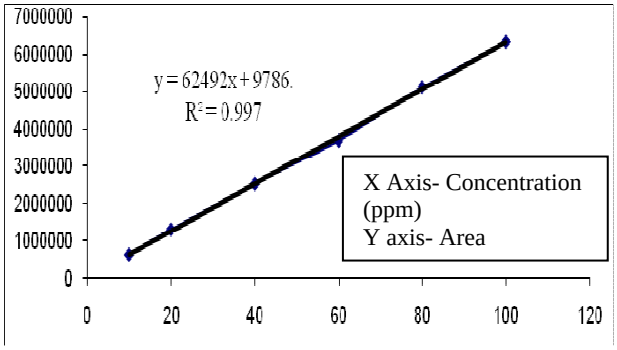

Fig.4: Linearity curve

3.2.2 Accuracy

To ensure the accuracy of the analytical method, the recovery studies were carried out. Known amount of rifabutin was added to a pre quantified sample solution of its dosage form and the amounts of rifabutin were estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range of rifabutin. Accuracy was evaluated at three different concentrations equivalent to 80, 100 and 120% of the active ingredient by calculating the recovery of rifabutin with %RSD.

Table 1: Recovery study

	Concentration of STD (ppm)	Concentration of Sample (ppm)	% Recovery found	% RSD
80%	100	80	99.77	0.18
100%	100	100	99.52	0.41
120%	100	120	99.4	0.49

3.2.3 Precision

Intra-day precision of the method was determined by repeat analysis (three identical injections) at three concentration levels. Inter-day precision was established by performing the analysis next day on a freshly prepared solution. The low RSD values of Table 2 indicate the ruggedness of the method. The low RSD values indicate the ruggedness of the method.

Table 2: Precision study

Conc.	Mean Peak area	± SD	%RSD
Interday			
20 µg ml^{-1}	1345278.99	3204.57	0.238
40 µg ml^{-1}	2513897.90	19419.48	0.772
60 µg ml^{-1}	3537530.55	30353.72	0.858
Intraday			
20 µg ml^{-1}	1335222.67	13204.09	0.989
40 µg ml^{-1}	2505210.047	21941.55	0.876
60 µg ml^{-1}	3537806.17	30132.94	0.851

3.2.4 Repeatability

The peak area of 40 ppm drug solution was analyzed six times on the same day. The %RSD was calculated for the resultant peak area.

Table 3: Repeatability study (n = 6)

Concentration	% RSD
40 µg ml^{-1}	0.59

3.2.5 Robustness

The robustness was assessed by altering the following experimental conditions such as, by changing the flow rate from 0.8 to 1.2 ml/min, the mobile phase composition with Methanol : Water (77:23, 73:27) and analyzed in triplicate. In all varied Chromatographic conditions, there was no significant change in chromatographic parameters. There was no effect of mobile phase composition on retention time as seen in Table 4.

Table 4: Robustness study (n = 3)

Concentration	Conditions changed	% RSD	Mean RT
100 µg ml^{-1}	**Mobile phase composition**		
	77:23	0.53	5.5
	73:27	0.37	5.6
	Flow rate		
	0.8 ml min^{-1}	1.35	5.5
	1.2 ml min^{-1}	1.36	5.6

4. Conclusion

A RP-HPLC method has been developed for the determination of Rifabutin. The proposed method is simple, rapid, accurate and precise. Its chromatographic run time of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Rifabutin. The results of the study reveal that the proposed RP-HPLC method for the estimation of Rifabutin is simple and accurate in bulk and pharmaceutical dosage forms.

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