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HIGH - PERFORMANCE LIQUID CHROMATOGRAPHY METHOD VALIDATION AND DEVELOPMENT STRATEGY FOR RIFABUTIN

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Keywords:

Rifabutin, HPLC (High - Performance Liquid Chromatography), Methanol, Method Development and Validation, International Conference of Harmonization (ICH) guidelines

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ABSTRACT: A simple, economical, accurate and precise high performance liquid chromatographic method was developed and validated for the determination of rifabutin. A Shimadzu LC-2010C HT version 3.01 system using a C-18 column with dimensions of 250 × 4.6 mm and silica particle size of 5 µm. Isocratic elution was employed with methanol, acetonitrile and water (75:25 v/v) were used. The flow rate was 1 ml/min, and effluents were monitored at 242 nm. The HPLC method was extensively validated for linearity, accuracy, precision (Interday precision, Intraday precision), repeatability and robustness. The exalted results receive of a drug were procured all significance values were established to be under the acceptable range in International Conference of Harmonization (ICH) guidelines requirements. The results demonstrate that the developed HPLC modus could be successfully used up for identification and quantification of Rifabutin in any form of the drug, with high resolution, accuracy, and precision.

INTRODUCTION: Rifabutin is a semi-designed subordinate of Rifamycin S, it's an against bactericidal contamination which is used as a part of the treatment of Tuberculosis. It is effective against Gram-positive and some Gram-negative microscopic organisms by hindering the DNA-dependant RNA - polymerase of the microorganisms; it is moreover feasible against the high protein Mycobacteria, *e.g. Mycobacterium tuberculosis*, *M. laprae* and *M. avium* intracellular [1 - 3]. Rifabutin has exhibited the development against Mycobacterium avium intracellular restricted from the patients with AIDS.

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Rifabutin is true blue in United States Pharmacopeia and European Pharmacopeia [4]. Affirmation of rifabutin in mass medicines and points of interest by spectrometric procedure and chromatography method were represented Rifabutin confirmation in natural cases like Human plasma, rat Urine was subjected to wide examination by unrivalled liquid chromatographic system [5 - 9].

Another procedure for the HPLC confirmation of rifabutin is delineated in this paper. The technique is generously less complex, speedier and more monetary. IUPAC name of rifabutin is (9S, 12E, 14S, 15R, 16S, 17R, 18R, 19R, 20S, 21S, 22E, 24Z) 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) -2H-furo-[2', 3' : 7, 8] – naphtha [1, 2-d] imidazol-2, 4'-piperidin]-5, 10, 26 - (3H, 9H) - trione - 16 - acetate [10].

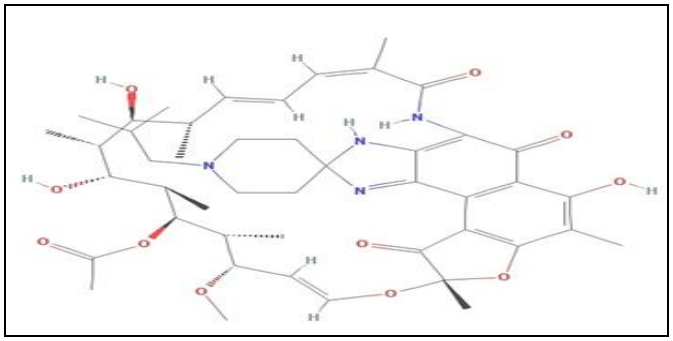

FIG. 1: CHEMICAL STRUCTURE RIFABUTIN WITH EMPIRICAL FORMULA $C_{46}H_{62}N_4O_{11}$

Experimental:

Apparatus: The liquid chromatographic method development was performed on a Shimadzu LC-2010C HT version 3.01 system (M/s Shimadzu Inc., Tokyo, Japan) equipped with a variable capacity quaternary pump, mobile phase degasser, column thermostat controller, UV-Vis detector [11]. Chromatographic separation was attained using a C-18 column (M/s Thermo Scientific, Massachusetts, USA) with dimensions of 250 × 4.6 mm and silica particle size of 5 µm.

Isocratic elution was employed with methanol, acetonitrile and water with pH adjusted by using orthophosphoric acid used as mobile phase with UV detection at 242 nm [12 - 14]. Before injection of the drug solution, the column was equilibrated with mobile phase for attaining saturation of the stationary phase. The data acquisition, analysis were performed with Shimadzu LC solution version 1.23 software [15 - 17].

Reagent and Materials: Rifabutin was provided as a gift sample from M/s Simpex Pharma Pvt. Ltd., Kotdwar, Uttarakhand, India, used as the working standard. HPLC-grade methanol and acetonitrile were obtained from M/s Merck Ltd., Mumbai, India. An orthophosphoric acid of analytical reagent grade purchased from M/s SD Fine Chemicals, Mumbai, India, was used for the study. Distilled water filtered through a 0.45 µm Millipore PVDF (polyvinyl difluoride) filter were used. All other chemicals and reagents used in this study were of analytical grade.

Mobile Phase: The mobile phase for chromatography consisted of acetonitrile + Methanol (1:1): Water (75:25) [18].

Sample Preparation of Rifabutin: Rifabutin API was provided as a gift sample by M/s Simpex Pharma Pvt. Ltd., Kotdwar, Uttarakhand, India and its dosage form is purchase from the market. An accurately weighed quantity of rifabutin 10 mg was transferred to 100 ml volumetric flask and made up to volume with diluent. Samples were diluted to a concentration of 10, 20, 40, 60, 80 and 100 µg ml^{-1} and used for method development and validation study [19 - 20].

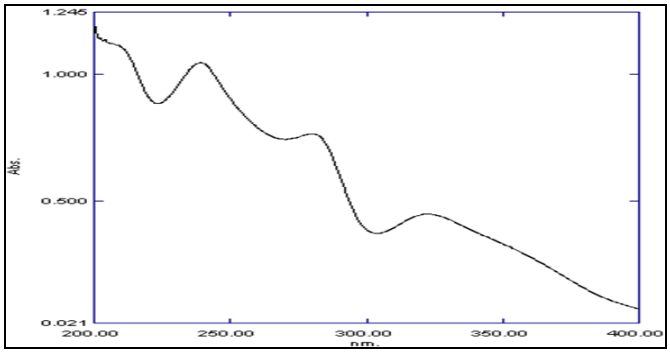

FIG. 2: Λ_{max} OF RIFABUTIN

Chromatographic Conditions: Chromatographic separation was attained using a C-18 column (M/s Thermo Scientific, Massachusetts, USA) with dimensions of 250 × 4.6 mm and silica particle size of 5 µm. Isocratic elution was employed with methanol, acetonitrile and water with pH adjusted by using orthophosphoric acid [21 - 23].

RESULT AND DISCUSSION:

Method Development: The method utilizing Acetonitrile + Methanol: Water as mobile phase yielded broad peak, whereas with Acetonitrile + Methanol: Water tailing was observed with acetonitrile and methanol as diluent. During method development, a number of variations were tested like Acetonitrile + Methanol concentration and flow rate to give a symmetric peak [24]. With a mobile phase Acetonitrile + Methanol: Water (75:25) at flow rate 1 ml min^{-1} and wavelength is 242 nm, the symmetric peak was obtained **Fig. 3**.

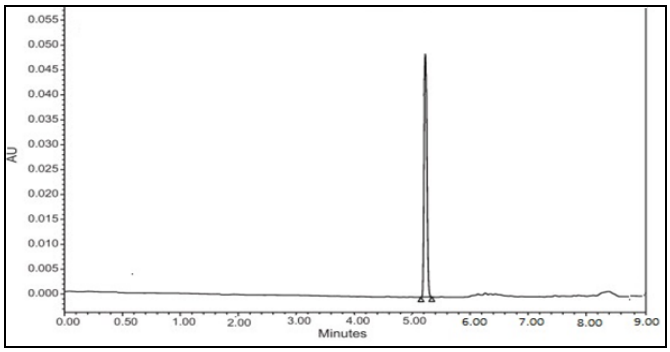

FIG. 3: CHROMATOGRAM OF RIFABUTIN

Validation:

Linearity: Six serial dilutions were prepared in a concentration range from 10 to 100 µg/ ml.

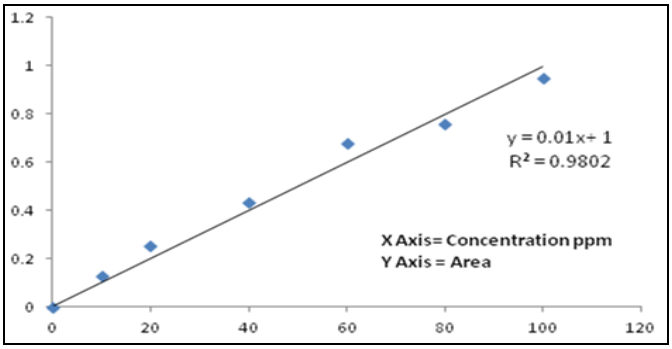

FIG. 4: LINEARITY CURVE OF RIFABUTIN

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 = 0.01x + 1; where y and x are peak area and concentration, respectively) was obtained over six concentrations 10, 20, 40, 60, 80, 100 µg/ml. A correlation coefficient was found to be 0.9802 [25 - 27].

Accuracy: To ensure the accuracy of the analytical method, the recovery studies were carried out. The 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 [28]. The recovery studies were carried out three times over the specified concentration range of rifabutin. The Accuracy was calculated at three different concentrations of drug which is equivalent to 80, 100 and 120% of the active ingredient by calculating the recovery of rifabutin with % RSD [29 - 30].

TABLE 1: RECOVERY STUDY OF RIFABUTIN

S. no.	Concentration Percentage (%)	Concentration of STD (ppm)	Concentration of Sample (ppm)	Percentage (%) recovery found	Percentage (%) RSD
1	80	100	80	99.71	0.16
2	100	100	100	99.63	0.38
3	120	100	120	99.48	0.47

Precision: The 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 [31, 32]. The low RSD values indicate the ruggedness of the method.

TABLE 2: PRECISION STUDY OF RIFABUTIN

S. no.	Concentration	Mean Peak Area	(±) Standard Deviation	Percentage (%) RSD
		Inter Day		
1	20 µg ml^{-1}	1452572.81	2801.58	0.218
2	40 µg ml^{-1}	2688315.80	18723.31	0.639
3	60 µg ml^{-1}	3733491.49	29835.63	0.739
		Intra Day		
1	20 µg ml^{-1}	1451482.59	13603.45	0.979
2	40 µg ml^{-1}	2615819.89	20361.59	0.861
3	60 µg ml^{-1}	3436306.11	29302.51	0.839

Repeatability: The peak area of 40 ppm drug solution was analysed six times on the same day. The % RSD was calculated for the resultant peak area [33 - 35].

TABLE 3: REPEATABILITY STUDY

S. no.	Concentration	Percentage (%) RSD
1	40 µg ml^{-1}	0.62

Robustness: The robustness was assessed by altering the following experimental conditions such as, by changing the flow rate from 0.5 to 1.5 ml/min, the mobile phase composition with Acetonitrile + Methanol: Water (76:24, 74:26) and analysed in triplicate.

In all Chromatographic varied conditions, there was no significant change in chromatographic parameters [36]. There was no effect of mobile phase composition on retention time as seen in **Table 4**.

TABLE 4: ROBUSTNESS STUDY (n = 3)

Concentration 100 µg ml^{-1}	Conditions changed	Percentage (%) RSD	Mean RT
	Mobile Phase Composition		
	76:24	0.51	5.3
	74:26	0.35	5.1
		Flow Rate	
	0.5 ml min^{-1}	1.32	5.2
	1.5 ml min^{-1}	1.47	5.4

CONCLUSION: A 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 HPLC method for the estimation of Rifabutin is simple and accurate in bulk and pharmaceutical dosage forms.

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CONFLICT OF INTEREST: There is no conflict of interest.

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