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HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF RIFABUTIN IN BULK AND CAPSULE DOSAGE FORM

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ABSTRACT: A selective, accurate, HPLC method was developed by this study for the determination of rifabutin in bulk and capsule dosage form. This method was developed by SHIMADZU LC-2010 HT using C_{18} column in solvents methanol: acetonitrile: ammonium acetate buffer (50: 45: 05) as mobile phase. At 1.0 ml/min flow rate the mobile phase was pumped, and the sample was detected at 278 nm. For standard rifabutin the retention time was 4.8 min. The method was validated for analytical standards such as linearity, accuracy, precision, and robustness. In a wide range of 5-25 (µg/ml) the linearity was observed. The method was validated, and a recovery study indicates accuracy of this method.

INTRODUCTION: Rifabutin is a broad-spectrum semisynthetic antibiotic derived from Rifamycin S [1]. It acts as anti-aid drug used for treating immunocompromised patients. Rifabutin is used in the first-line treatment for tuberculosis [2]. 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, 25 hepta methyl spiro[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[3]. Rifabutin is commonly used as an antitubercular drug. It has bactericidal activity particularly active against mycobacterium species [4]. The structure of Rifabutin is depicted in **Fig. 1**.[5]

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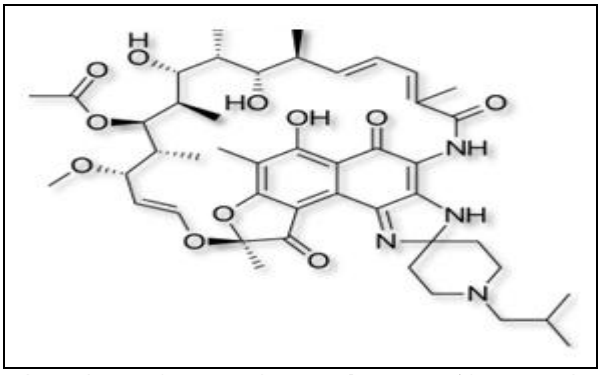

FIG. 1: STRUCTURE OF RIFABUTIN ($C_{46}H_{62}N_4O_{11}$)

MATERIALS AND METHOD: The instrument used for developing a method for HPLC was Shimadzu LC-2010HT equipped with a degassed unit, low-pressure gradient unit, pump unit, ultra-fast autosampler, UV-Vis detector [6, 7]. By using a C-18 column Chromatographic separation was attained Isocratic elution was carried out with mobile phase of methanol, acetonitrile and ammonium acetate buffer used as mobile phase. Before injecting the drug, to acquire the saturation the column was equilibrated with mobile phase of stationary phase [8, 9].

Reagent and Materials: Standard rifabutin received from Vivan life sciences Pvt. Ltd., as a gift sample. Capsule dosage form was purchased from (Lupin Pharmaceuticals) containing 150 mg of Rifabutin from local pharmacy in Chennai. All solvents are in HPLC grade methanol and acetonitrile were procured from M/s Merck Ltd., Mumbai, India. Ammonium acetate was obtained from Sisco research laboratories Pvt. Ltd. Maharashtra, India.

Mobile Phase Preparation: Based on the solubility of the rifabutin, the mobile phase was selected. The mobile phase for chromatography is methanol + acetonitrile + ammonium acetate buffer (50:45:05)

Ammonium Acetate Buffer (pH 4.6) Preparation: 38.5 g of ammonium acetate and 35 ml of glacial acetic acid was taken in 500 ml standard flask, then made up the volume with 500 ml of distilled water and adjusted to pH 4.6.

Selection of Analytical UV Wavelength (λ_{max}): To fix wavelength for analysis the prepared stock solution was scanned in ultraviolet spectroscopy over the range of 200-800 nm from resultant spectrum wavelength at 278 nm was chosen as in this range maximum absorption of drug occurs. So, this range is taken to analyze the sample [10].

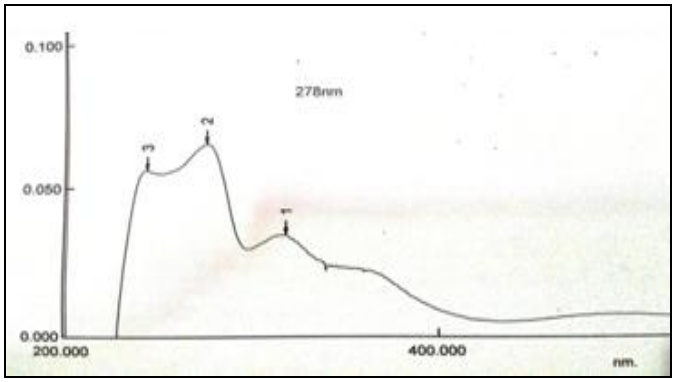

FIG. 2: λ_{max} OF RIFABUTIN AT 278 nm

Preparation of Standard Stock Solution of Rifabutin: About 10 mg of precisely weighed quantity of the standard drug was added to 100 ml volumetric flask and dissolved with little quantity of distilled water. The resultant solution made up to mark with diluents. Further Samples were prepared in various concentration of 5, 10, 15, 20, 25 µg/ml and used for method development and validation study.

Preparation of Sample: The contents of 20 capsules are removed weighed accurately powder equivalent to 10 mg of rifabutin was diluted with 100 ml mobile phase (methanol: acetonitrile: ammonium acetate buffer [50:45:05]. About 1.5 ml solution was taken in 10 ml volumetric flask, and it was made with 10 ml of mobile phase (1.5 µg/ml). Filtered through microsyringe and used for further studies**.**

RESULTS AND DISCUSSION:

Method Development: The method was developed by methanol: acetonitrile: ammonium acetate buffer: buffer as a high concentration in mobile phase yield tailing in the peak due to the presence of water in the buffer. During method development, a number of variations have been done with mobile phase in different concentration and 0.8 to 1.2 ml/ml flow rate to give asymmetric peak.

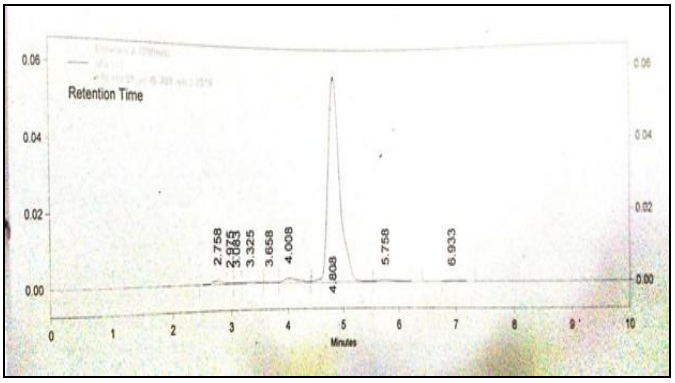

FIG. 3: CHROMATOGRAM OF STANDARD RIFABUTIN (15 µg/ml)

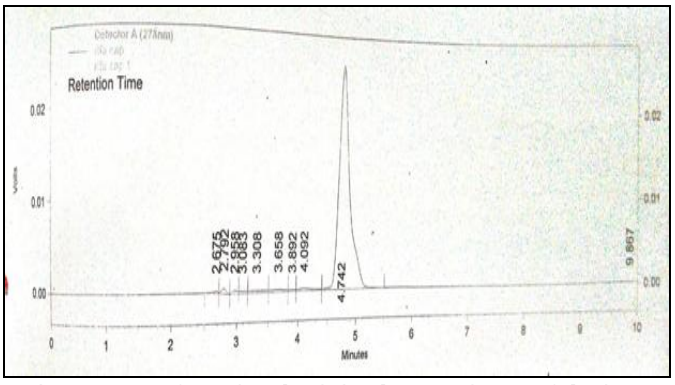

FIG. 4: CHROMATOGRAM OF CAPSULE FORMULATED RIFABUTIN (15 µg/ml)

Validation:

Linearity: The different concentration varies from 5 to 25 µg/ml were prepared [8, 9]. Chromatograms were recorded by injecting 20 µl from each concentration of the solution. All estimation were carried out at triplicate for each concentration [11].

A linear calibration graph (y = 38772x+1747; representing x as peak area and y as concentration respectively) was obtained. The correlation coefficient was found to be 0.983.

TABLE 1: LINEARITY STUDIES OF RIFABUTIN BY HPLC METHOD

Concentration (µg/ml)	Peak area
5	194356
10	401188
15	659621
20	839108
25	918488

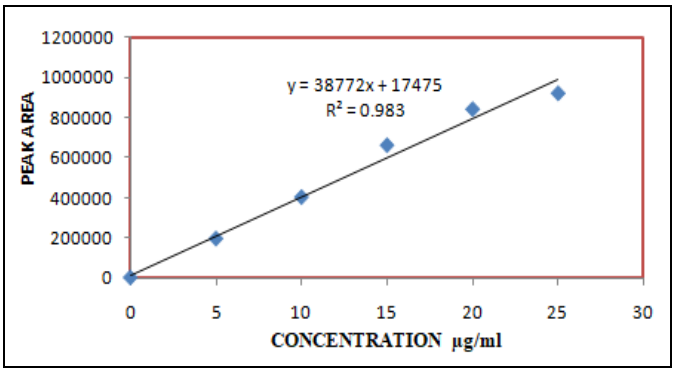

FIG. 5: CALIBRATION CURVE OF RIFABUTIN BY HPLC METHOD

Accuracy: Accuracy was estimated by using a known amount of standard rifabutin added to measured quantity of sample. By calculating peak area ratios the amount of rifabutin were estimated. By the use of three different concentrations equivalent to 80, 100 and 120% of the active ingredient accuracy was evaluated by calculating the recovery of rifabutin with % RSD [12]. The percentage of drug present in formulation is reported in **Table 2**. The result showed that the amount present in capsule dosage form equivalent to label claimed of formulation.

Precision: Both Intraday and Inter day analysis was carried out in the triplicate analysis. Inter day precision did in consequential days by the use of freshly prepared sample. The lowest RSD values prove that ruggedness of the method [13].

Repeatability: 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 [14].

TABLE 2: RECOVERY STUDIES OF RIFABUTIN BY HPLC METHOD

	Concentration of STD (ppm)	Concentration of Sample (ppm)	% recovery found	% RSD
80%	100	80	97.87	0.675
100%	100	100	99.05	0.574
120%	100	120	98.97	0.49

TABLE 3: INTERDAY AND INTRADAY PRECISION STUDIES OF RIFABUTIN BY HPLC

Concentration	Mean peak area	±SD	% RSD
Interday			
5 µg ml^{-1}	153512.87	829.912	0.54
15 µg ml^{-1}	565256.90	15383.58	2.72
25 µg ml^{-1}	886511.11	10169.77	1.15
Intraday			
5 µg ml^{-1}	153622.60	22373.88	1.45
15 µg ml^{-1}	585915.33	15601.08	2.66
25 µg ml^{-1}	877082.33	22072.52	2.52

TABLE 4: REPEATABILITY STUDY OF RIFABUTIN BY HPLC

Run	Area under the peak
1	597289
2	568130
3	592327
Mean	585915.33
RSD%	2.66

Robustness: The robustness of the method was evaluated by deliberately varying the chromatographic conditions such as the flow rate was changed to ± 2 ml min^{-1} and wavelength to about ± 2 nm[15].

TABLE 5: ROBUSTNESS STUDY OF RIFABUTIN BY HPLC

Conc.	Conditions changed	% RSD	Mean retention time
15µg ml^{-1}	**Wavelength**		
	276 nm	0.27	4.7
	280nm	0.69	4.7
	Flow rate		
	0.8 ml	0.47	5.8
	1.2 ml	0.68	3.9

CONCLUSION: This present work is precise and validated for the estimation of rifabutin using HPLC with C_{18} column with UV detection at 278

nm. This method is accurate, can be employed in determination of rifabutin in various dosage forms. Hence, this study is an adaptable method of analysis due to its rapidity and repeatability.

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CONFLICTS OF INTEREST: Nil

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