DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ANALYSIS OF RIFABUTIN IN BULK FORM AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT: ***The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the measurement of active pharmaceutical ingredient and Marketed Pharmaceutical Dosage form of Rifabutin.A simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Rifabutin. The chromatographic strategy utilized Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm, using isocratic elution with a mobile phase of Phosphate Buffer (0.02M) and Acetonitrile were consists of 48:52% v/v (pH-2.80). A flow rate of 1.0 ml/min and a detector wavelength of 248 nm utilizing the UV detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines.LOD and LOQ for the two active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R2>0.999, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range.The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drug.***

Key Words: Rifabutin, RP-HPLC, Method Development, Validation, Accuracy, Robustness.

I. INTRODUCTION

Rifabutin is a member of rifamycins. It has a role as an antitubercular agent. A broad-spectrum antibiotic that is being used as prophylaxis against disseminated Mycobacterium Avium complex infection in HIV-positive patients. Rifabutin is an antibiotic that inhibits DNA-dependent RNA polymerase activity in susceptible cells. Specifically, it interacts with bacterial RNA polymerase but does not inhibit the mammalian enzyme[1]. It is bactericidal and has a very broad spectrum of activity against most gram-positive and gram-negative organisms (including Pseudomonas aeruginosa) and specifically Mycobacterium tuberculosis. Because of rapid emergence of resistant bacteria, use is restricted to treatment of mycobacterial infections and a few other indications[2]. Rifabutin is well absorbed when taken orally and is distributed widely in body tissues and fluids, including the CSF. It is metabolized in the liver and eliminated in bile and, to a much lesser extent, in urine, but dose adjustments are unnecessary with renal insufficiency.Rifabutin is an antibiotic that inhibits DNA-dependent RNA polymerase activity in susceptible cells. Specifically, it interacts with bacterial RNA polymerase but does not inhibit the mammalian enzyme. It is bactericidal and has a very broad spectrum of activity against most gram-positive and gram-negative organisms (including Pseudomonas aeruginosa) and specifically Mycobacterium tuberculosis[3]. Because of rapid emergence of resistant bacteria, use is restricted to treatment of mycobacterial infections and a few other indications. Rifabutin is well absorbed when taken orally and is distributed widely in body tissues and fluids, including the CSF. It is metabolized in the liver and eliminated in bile and, to a much lesser extent, in urine, but dose adjustments are unnecessary with renal insufficiency[4]. The IUPAC name of Rifabutin is

[(7S,9E,11S,12R,13S,14R,15R,16R,17S,18S,19E,21Z)-2,15,17,32-tetrahydroxy-11-methoxy-3,7,12,14,16,18,22-heptamethyl-1'-(2-methylpropyl)-6,23-dioxospiro[8,33-dioxa-24,27,29-triazapentacyclo[23.6.1.14,7.05,31.026,30]tritriaconta-1(32),2,4,9,19,21,24,26,30-nonaene-28,4'-piperidine]-13-yl] acetate. The Chemical Structure of Rifabutin is shown in following fig-1.

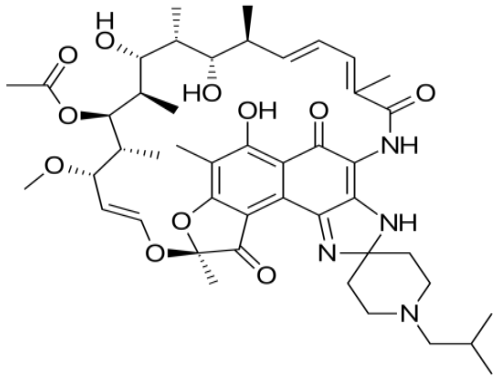

Fig.1. Chemical Structure of Rifabutin

II. EXPERIMENTAL

Table-1:List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	**Waters HPLC**with Empower2 Software with UV-Visible Detector.
2.	**ELICO SL-159** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	Thermal Oven
6.	Symmetry RP C_{18}, 5µm, 250mmx4.6mm i.d.
7.	P^H Analyzer **(ELICO)**
8.	Vacuum filtration kit**(BOROSIL)**

Table-2:List of Chemicals used

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Dipotassiumhydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9%	L.R.	Sd fine-Chem ltd; Mumbai

6.	Sodium hydroxide	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
7.	Hydrochloric acid	96%	A.R.	Sd fine-Chem ltd; Mumbai
8.	3% Hydrogen Peroxide	96%	A.R.	Sd fine-Chem ltd; Mumbai

Method Development and its Validation forRifabutinby RP-HPLC

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. Itscanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Rifabutin, so that the same wave number can be utilized in HPLC UV detector[5] for estimating the Rifabutin. While scanning the Rifabutin solution we observed the maxima at 248 nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450. The scanned UV spectrum is attached in the following page,

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Rifabutin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Acetonitrile and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Acetonitrile.

Further pipette 0.5ml of the above Rifabutin stock solutions into a 10ml volumetric flask and dilute up to the mark with Acetonitrile.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions[6] of proper peak elution for performing validation parameters as per ICH guidelines[26,31].

Preparation of SampleSolution:

Twenty capsules were taken and the average weight was calculated as per the method prescribed in I.P. The weighed capsules were finally powdered and triturated well. A quantity of powder of Rifabutin equivalent to 10mg were transferred to clean and dry 10 ml volumetric flask and 7 ml of HPLC grade Acetonitrile was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade Acetonitrile. One ml (0.5 ml) of the prepared stock solution[7] diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

Optimization of Chromatographic Conditions:

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

5.4.5. Preparation of 0.02M Potassium Dihydrogen Orthophosphate Solution:

About 2.72172grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 2.80 with diluted orthophosphoric acid Solution.

Preparation of Mobile Phase:

480mL (48%) of above Phosphate buffer solution and 520mL of HPLC Grade Acetonitrile (52%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The resulted solution was filtered through 0.45 µm filter under vacuum filtration.

Validation of Analytical Method

The following list includes validation parameters[8-11]to be studied for an HPLC quantitative analytical assay, specifically chromatographic procedures. Not all parameters are necessary when other types of analytical technologies are used.

Specificity

Specificity[12] is the ability to assess the analyte unequivocally in the presence of components which may be expected to be present, and it plays a critical role in the method validation.2 The following citations from warning letters are typical for lack of specificity requirement:

Precision

The precision of a method is the degree of repeated measurements under the same conditions over the operating range of concentrations. The method precision[13] can take place at different levels. For a typical HPLC for assay and impurities measurement, several levels of precision should be studied.

Detection Limit/Quantitation Limit (DL/QL)

These parameters are studied when quantitation of a low level of a component of interest is needed - for example,

an analytical method for impurities or degradation products. The DL/QL levels can be determined based on the signal-to-noise ratio or based on the standard deviation of the response and the slope[14]. These values should also be verified with the concentration determined. For methods measuring the impurities or degradation product, a verification of the QL should be included in the system suitability testing section for method performance.

Ruggedness and Robustness

The ruggedness also referred to as intermediate precision, measures the method performance under normal variability, while the robustness measures the method performance with small but deliberate changes. The ruggedness[15] is usually addressed in the precision study (intermediate precision).

Robustness

While precision assesses the typical variations in the normal operation of a procedure, robustness measures deliberate variations (e.g., flow rate +/- 20%, column length, mobile phase composition, pH detector wavelength, sonication time). These changes can be studied individually or with an experimental design. The full set of system suitability parameters are evaluated for robustness[16]. Several references discussed these parameters in detail. The changes in sample preparation schemes must also be studied, such as shaking time, extraction time, filtering.

III. RESULTS AND DISCUSSION

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. Itscanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Rifabutin, so that the same wave number can be utilized in HPLC UV detector for estimating the Rifabutin. While scanning the Rifabutin solution we observed the maxima at 248 nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450. The scanned UV spectrum is attached in the following page,

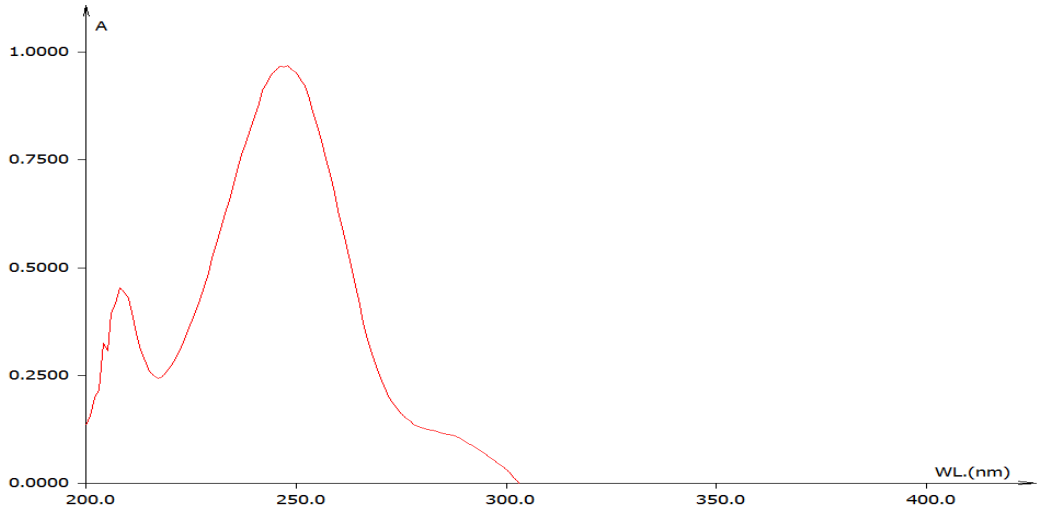

Fig.2. UV Spectrum for Rifabutin

Optimization of Analytical Method

Summary of Optimized Chromatographic Conditions:

The Optimum conditions[17] obtained from experiments can be summarized as below:

Table-3:Summary of Optimised Chromatographic Conditions

Mobile phase	Phosphate Buffer (0.02M): Acetonitrile = 48:52 (pH-2.80)
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	248 nm
Flow rate	1.0 ml/ min.
Run time	08 min.
Temperature of Auto sampler	Ambient

Diluent	Mobile Phase
Injection Volume	20µl
Mode of Elution	Isocratic
Retention time	3.867 minutes

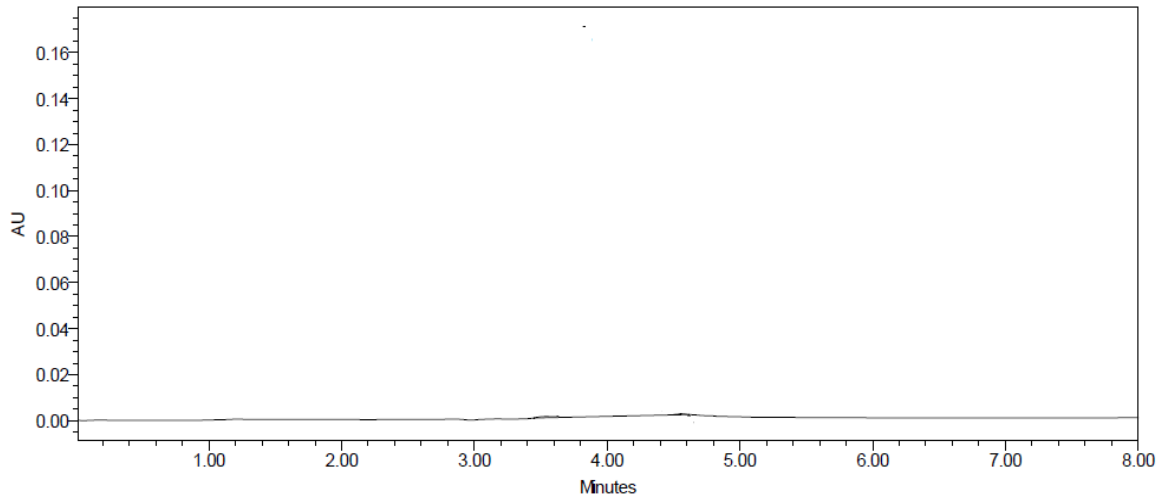

Fig.3. HPLC Chromatogram of Rifabutin (Blank Solution)

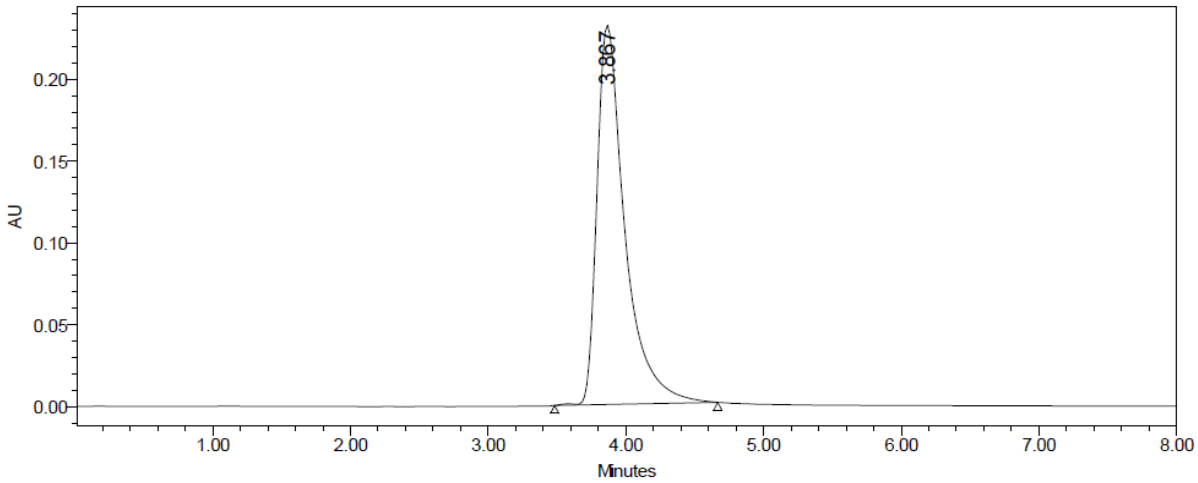

Fig.4. Chromatogram of Rifabutin in Optimized Chromatographic Condition

Analytical Method Validation

The objective of validation[18-20]of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included. Other analytical procedures may be considered in future additions to this document.

1. Accuracy:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Rifabutin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5ml of the above Rifabutin stock solutions into a 10ml volumetric flask and dilute up to the mark with Acetonitrile.

For Preparation of 80% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Rifabutin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

For Preparation of 100% Standard Stock Solution:
Accurately weigh and transfer 10 mg of Rifabutin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
For Preparation of 120% Standard Stock Solution:
Accurately weigh and transfer 10 mg of Rifabutin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
Recovery Study:
To determine the accuracy of the planned technique, recovery studies were distributed by adds completely different amounts (80%, 100%, and 120%) of pure drug of Rifabutin were taken and extra to the pre-analyzed formulation of concentration 50µg/ml. From that proportion recovery values[21] were calculated. The results were shown in table-4.

Table-4: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	40	40.141	502647	100.352	Mean= 100.3947% S.D. = 0.071319 % R.S.D.=0.071038
S_2 : 80 %	40	40.191	503214	100.477	
S_3 : 80 %	40	40.142	502656	100.355	
S_4 : 100 %	50	50.044	614215	100.088	Mean= 99.98533% S.D. = 0.183045 % R.S.D.=0.183071
S_5 : 100 %	50	49.887	612451	99.774	
S_6 : 100 %	50	50.047	614254	100.094	
S_7 : 120 %	60	60.192	728547	100.32	Mean= 100.311% S.D. = 0.408574 % R.S.D.=0.407308
S_8 : 120 %	60	59.939	725698	99.898	
S_9 : 120 %	60	60.429	731211	100.715	

2.Precision:
2.1. Repeatability
Preparation of Rifabutin Product Solution for Precision:
Accurately weigh and transfer 10 mg of Rifabutin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
Procedure:
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
The exactitude of every technique was determined one by one from the height areas & retention times obtained by actual determination of six replicates of a set quantity of drug. Rifabutin (API). The % relative variance[22] was calculated for Rifabutin square measure bestowed within the table-5.

Table-5: Repeatability Readings

HPLC Injection Replicates of Rifabutin	Retention Time (Minutes)	Peak Area
Replicate – 1	3.649	5674158
Replicate – 2	3.684	5654715
Replicate – 3	3.687	5665841
Replicate – 4	3.688	5654578
Replicate – 5	3.688	5652284
Replicate – 6	3.687	5641487
Average		**5657177**
Standard Deviation		**11369.72**
% RSD		**0.200979**

2.2. Intermediate Precision/Ruggedness:

2.2.1. Intra-Day & Inter-Day:

The intra & inter day variation[23] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Rifabutin revealed that the proposed method is precise.

Procedure:

Analyst 1: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Table-6: Results of Ruggedness for Rifabutin Analyst 1

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rifabutin	3.687	584968	65982	5985	1.65
2	Rifabutin	3.688	582479	66354	5876	1.69
3	Rifabutin	3.688	586236	67425	5896	1.63
4	Rifabutin	3.687	586985	65982	5986	1.65
5	Rifabutin	3.684	582679	65932	5216	1.67
6	Rifabutin	3.649	583989	65874	5987	1.68
Mean			**584556**			
Std.Dev.			**1846.658**			
%RSD			**0.315908**			

Table-7: Results of Intermediate Precision Analyst 2 for Rifabutin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rifabutin	3.649	598698	66985	5265	1.62
2	Rifabutin	3.684	596847	67458	5168	1.68

3	Rifabutin	3.687	596354	66985	5436	1.69
4	Rifabutin	3.688	598676	67854	5369	1.64
5	Rifabutin	3.688	596874	68521	5247	1.67
6	Rifabutin	3.687	598989	67898	5375	1.63
Mean			**597739.7**			
Std.Dev.			**1168.098**			
%RSD			**0.195419**			

3. Linearity & Range:

Preparation of DrugSolutions for Linearity:

Accurately weigh and transfer 10 mg of Rifabutin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5ml of the above Rifabutin stock solutions into a 10ml volumetric flask and dilute up to the mark with Mobile Phase.

Preparation of Level – I (30ppm of Rifabutin):

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – II (40ppm of Rifabutin):

Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – III (50ppm of Rifabutin):

Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – IV (60ppm of Rifabutin):

Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – V (70ppm of Rifabutin):

Take 0.7ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

The calibration curve showed good linearity[24] in the range of 0-70µg/ml, for Rifabutin (API) with correlation coefficient (r^2) of 0.999 (Fig-5). A typical calibration curve has the regression equation of y = 11266.x + 50416 for Rifabutin.

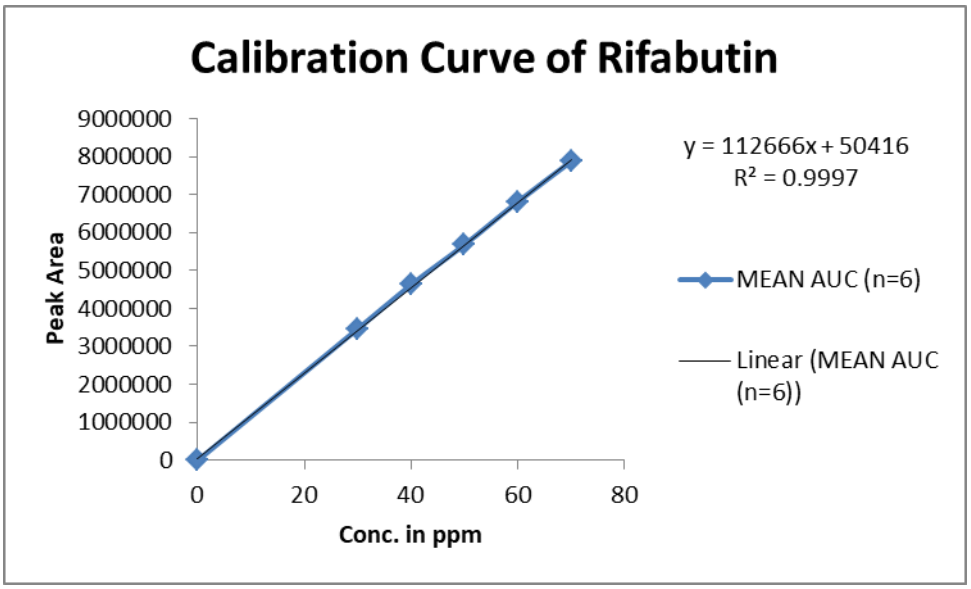

Fig.5. Calibration Curve of Rifabutin (API)

Table-8: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
30	3465974
40	4626478
50	5682284
60	6815478
70	7878721

Linearity Plot:

The plot of Concentration (x) versus the Average Peak Area (y) data of Rifabutin is a straight line.

Y = mx + c

Slope (m) = 112666

Intercept (c) = 50416

Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 50416. These values meet the validation criteria.

4. Method Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Rifabutin. The method is robust[25] only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Rifabutin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Rifabutin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent[27]. (Stock solution)

Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase[28] i.e. Acetonitrile: Phosphate Buffer was taken in the ratio and 40:60, 30:70 instead of 35:65, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Table-9: Results for Robustness

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	584624	3.649	5765	1.42
Less Flow rate of 0.9 mL/min	598676	3.687	5856	1.49

More Flow rate of 1.1 mL/min	612543	3.649	5965	1.46
Less organic phase	578642	3.688	5758	1.49
More organic phase	569896	3.684	5962	1.47

5. LOD & LOQ:

LOD: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

LOQ: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$\mathbf{LOQ = 10 \times \sigma/S}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Table-10: Results of LOD & LOQ

SE of Intercept	48846.22527
SD of Intercept	109223.4801
LOD	3.199168
LOQ	9.694449

Observation:

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 3.19 & 9.69 µg/ml respectively.

6. System Suitability Parameter:

System quality testing[29] is associate degree integral a part of several analytical procedures. The tests square measure supported the idea that the instrumentation, physics, associate degree analytical operations and samples to be analyzed represent an integral system that may be evaluated intrinsically. Following system quality check parameters were established. The information square measured shown in Table-11 & 12.

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Rifabutin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Acetonitrile and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Acetonitrile.

Further pipette 0.5ml of the above Rifabutin stock solutions into a 10ml volumetric flask and dilute up to the mark with Acetonitrile.

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Table-11: Knowledge of System quality Parameter

S.No.	Parameter	Limit	Result
1	Asymmetry	T ≤ 2	Rifabutin =0.98
2	Theoretical plate	N > 2000	Rifabutin = 5782
3	Tailing Factor	T<2	Rifabutin = 1.59

Table-12: Results of System Suitability for Rifabutin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rifabutin	3.644	584635	65847	5857	1.63
2	Rifabutin	3.645	582695	65421	5955	1.69
3	Rifabutin	3.644	587432	65369	5875	1.67
4	Rifabutin	3.662	589687	65748	5796	1.65
5	Rifabutin	3.660	582547	65398	5952	1.69
6	Rifabutin	3.660	589656	652418	5896	1.64
Mean			**586108.7**			
Std.Dev.			**3275.654**			
%RSD			**0.558882**			

7. Specificity:

Specificity can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared individually and the sample containing one drug was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method was specific.

The chromatograms representing the peaks of blank, Rifabutin and the sample containing the one drug was shown in following figures respectively.

Observation: In this test method blank, standard solutions were analyzed individually to examine the interference. The abovechromatograms show that the active ingredient was well separated from blank and their excipients and there was no interference of blank with the principal peak. Hence the method is specific.

8. Estimation of Rifabutin in Pharmaceutical Dosage Form

Twenty pharmaceutical dosage forms[30] were taken and the I.P. method was followed to work out the typical weight. On top of weighed capsules were finally pulverized and triturated well. A amount of powder cherish twenty five mg of medicine were transferred to twenty five cc meter flask, build and resolution was sonicated for quarter-hour, there once volume was created up to twenty five cc with same solvent. Then ten cc of the on top of resolution was diluted to a hundred cc with mobile part. The answer was filtered through a membrane filter (0.45 µm) and sonicated to remove. The answer ready was injected in 5 replicates into the HPLC system and therefore the observations were recorded.

A duplicate injection of the quality resolution was conjointly injected into the HPLC system and therefore the peak areas were recorded. The info square measure shown in Table-13.

ASSAY:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak space of drug obtained with check preparation
AS = Peak space of drug obtained with normal preparation
WS = Weight of operating normal taken in mg
WT = Weight of sample taken in mg
DS = Dilution of normal resolution

DT = Dilution of sample resolution
P = proportion purity of operating normal

Table-13: Recovery Data for Estimation Rifabutinin Ributin Capsule

Brand Name of Rifabutin	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the Proposed Method (n=6)	Assay % (± SD)
Ributin Capsule (150mg) (Lupin Ltd)	150mg	149.367 (± 0.587)	99.749 (±0.325)

Result & Discussion: The amount of drugs in Ributin Capsule was found to be 149.367 (± 0.587) mg/tab for Rifabutin& % assay was 99.749 (± 0.325).

Forced Degradation Studies

Results of Degradation Studies:

The results of the stress studies[31] indicated the Specificity of the method that has been developed. Rifabutin was stable in photolytic and peroxide stress conditions. The result of forced degradation studies are given in the following table-14.

Table-14:Results of Forced Degradation Studies of Rifabutin API

Stress Condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	98.76	1.24	100.0
Basic Hydrolysis (0.I M NaOH)	24Hrs.	98.63	1.37	100.0
Thermal Degradation (50 ^{0}C)	24Hrs.	93.98	6.02	100.0
UV (248nm)	24Hrs.	98.84	1.16	100.0
3 % Hydrogen Peroxide	24Hrs.	94.61	5.39	100.0

IV. SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters.First of all, maximum absorbance was found to be at 248nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm particle size because it was giving good peak.Ambient temperatures were found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Phosphate Buffer (0.02M) and Acetonitrile were taken in the ratio of 48:52 % v/v (pH-2.80)was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 8.0 min because analyze gave peak around 3.867min and also to reduce the total run time.The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 30-70ppm of the Rifabutin target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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