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Research Article

METHOD DEVELOPMENT AND VALIDATION OF RIFAMPICIN BY USING RP-HPLC METHOD IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A rapid and sensitive RP-HPLC method with UV detection (219nm) for routine analysis of Rifampicin in a pharmaceutical formulation was developed. Chromatography was performed with a mobile phase containing a methanol of assay (99.8%) with flow rate of 0.9ml/min. Quantitation was accomplished with an internal standard method. The procedure was validated for linearity (correlation coefficient = 0.9957), accuracy and limit of detection (LOD) intraday precision. To test validation of the Rifampicin three factors were considered as linearity, precision, LOD where mobile phase, flowrate and pressure are respectively selected as methanol, 0.9ml/min, 1200 pascals. To ensure precise intraday measurements, the variables taken into account were the analyst and the equipment. The RSD value (0.20%) indicated a good precision of the analytical method. The proposed method was simple; highly sensitive, precise, accurate and retention time less than 3 min indicating that the method is useful for routine quality control.

INTRODUCTION

Aim of the Work: Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Rifampicin. The developed method will be validated according to ICH guidelines.

Objective of the work: The analytical method for the simultaneous estimation of Rifampicin will be developed by RP-HPLC method by optimizing the chromatographic conditions. The developed method is validated according to ICH guidelines Q2.

Rifampicin: Rifampicin is a member of the class of rifamycins that is a semisynthetic antibiotic derived from Amycolatopsisrifamycinica (previously known as Amycolatopsismediterranei and Streptomyces mediterranei).

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It has a role as an DNA synthesis inhibitor, an antitubercular agent, a leprostatic drug, an Escherichia coli metabolite, a protein synthesis inhibitor, a neuroprotective agent, an angiogenesis inhibitor, a pregnaneX receptor agonist, an antineoplastic agent, an antiamoebic agent and a geroprotector. Use of rifampin can prevent the spread of meningitis and other meningococcal diseases caused by Neisseria meningititis bacteria.

Structure

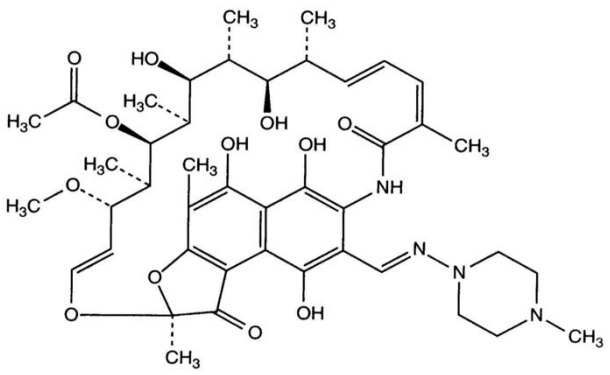


Figure-1

IUPACName
[(7S,9E,11S,12R,13S,14R,15R,16R,17S,18S,19E,21Z)-2,15,17,27,29-pentahydroxy-11-methoxy-3,7,12,14,16,18,22-heptamethyl-26-[(E)-(4-

methylpiperazin-1-yl)iminomethyl]-6,23 dioxo-8, 30-dioxa-24-azatetracyclo[23.3.1.1^{4,7}.0^{5,28}]triaconta-1(29),2,4,9,19,21,25,27-octaen-13-yl] acetate.

Molecular formula: $C_{43}H_{58}N_4O_{12}$

Molecular weight: 822.9gm/mole

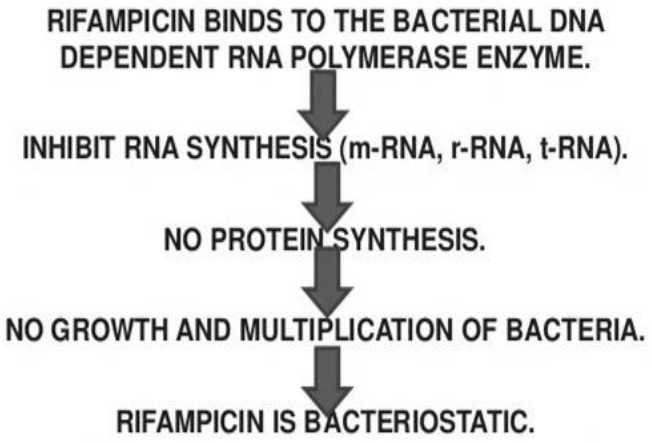
Melting Point: 183°C

Synonyms: Benemycin, Rifadin, Rifampicin, Rifampin, Rimactan, Rimactane, Tubocin.

Category: RNA polymerase inhibitors- Rifamycins (Macrolactams).

Solubility: 1400 mg/L (at 25°C).

Mechanism of Action



MATERIALS AND METHODS

Instrumentation: HPLC (Water, Isocratic method, ADM), Software (Autochro 3000), Detector (UV Water-model 487), Column [C18 (ZODIAC COMPANY)], weighing machine (Afcoset ER-1000A), Pipettes, Burettes and Beakers (Borosil).

Chemicals used: Rifampicin (Lupinltd), Methanol (Thermo Fisher Scientific India Pvt LTD).

Table 1: Optimized conditions

Parameter	Condition
Mobile phase	Methanol and Acetonitrile (50:50)
Pump mode	Isocratic method
PH	4.8
Diluents	Hplc grade Methanol
Column	C_{18} (5 µm pore size)
Column Temp	27^{o}C
Wavelength	219nm
Injection Volume	20 µl
Flow rate	0.9ml/min
Run time	5 minutes
Retention Time	0.8 minutes

Method Development

Preparation of Solutions

1. **Preparation of standard stock solution:** Accurately weigh and transfer 10µg standard drug of Rifampicin into volumetric flask and add 10 ml of methanol and dissolve by sonication process for 3 minutes and label it as standard stock solution of 1000µg/ml. From the 1000µg/ml prepare 100µg/ml concentration by taking the volumes as 1 ml of stock solution and 9 ml of methanol into a test tube and label it as 100µg/ml. From 100µg/ml prepare 10µg/ml concentration by taking the volumes as 1 ml of stock solution and 9 ml of methanol into an empty test tube and label it as 10µg/ml.
2. **Preparation of sample solution:** To detect the Rifampicin tablet concentration take any branded tablet like R-cin 600 of Rifampicin drug of powered dosage 10mg of equivalent weight and dissolve it in 10ml of methanol of equivalent weight and sonicate it for 5 minutes, label it as sample solution.
3. **Preparation of Standard Dilutions:** Mobile phase (methanol) is used as a diluent. From the stock solution of concentration 1000µg/ml pipette out the required volumes of concentration as 100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, 500µg/ml, 600 µg/ml.
4. **Preparation of Sample Dilutions:** From the sample stock solution of 1000µg/ml prepare 100µg/ml by taking volumes as 1 ml of stock solution and 9 ml of methanol for 10 ml of solution and label it as sample dilution.

Procedure

After injecting each level into the chromatographic system, the peak area should be measured to quantify the amount of the compound present in the sample. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis peak area) and calculate the correlation co-efficient.

Acceptance Criteria

Correlation coefficient should be not less than 0.990 and not more than 0.999.

Optimised Chromatogram

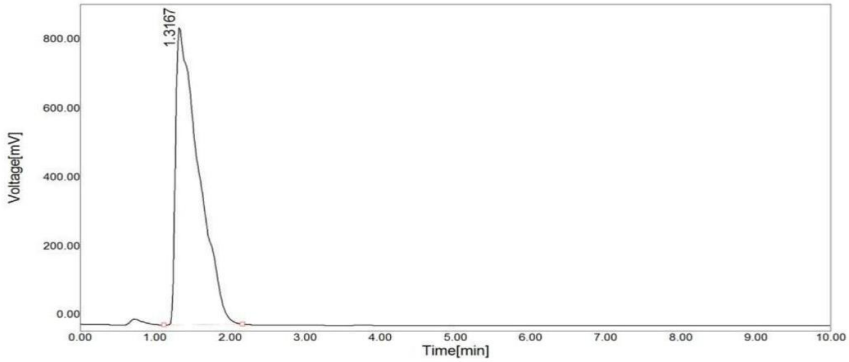


Figure 2: **Optimised chromatogram for standard**

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1		1.2500	5851.5804	100.00	549.7	0.7892	0.0000
Sum			5851.5804				

Formulation: The sample solution prepared at a concentration of 100µg/ml was analyzed in the developed method conditions. The method can successfully separate and identify the Rifampicin. Hence the method was found to be suitable for routine analysis of Rifampicin and formulations.

Chromatogram

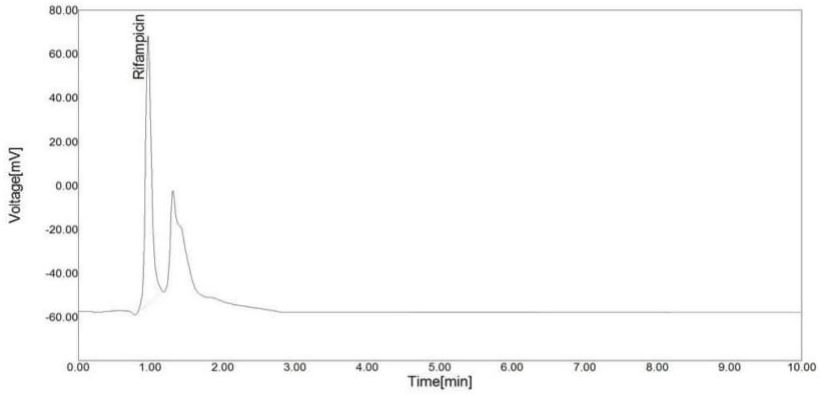
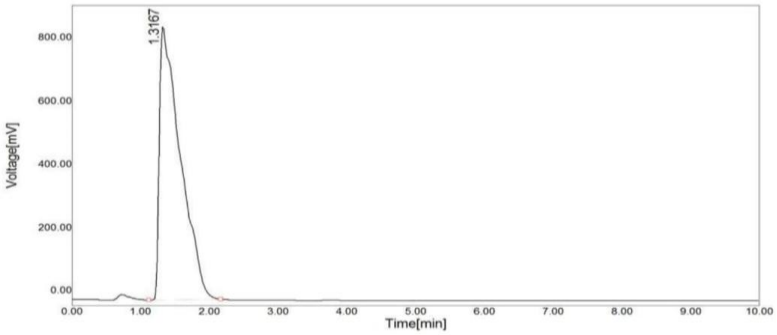


Figure 3: Chromatogram for Formulation

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Rifampicin	0.8167	1854.6331	0.00	527.2	0.9741	0.0000
Sum			1854.6331				



Assay: Standard and sample solution injected as described under experimental work.

Table 2: Formulation assay

Formulation	Dosage	Assay%
Rifampicin capsules	600mg	95.6%

Method Validation

Linearity: From the prepared stock solution, a series of calibration standards were prepared at concentrations of 100, 200, 300, 400, 500 and 600µg/ml using mobile phase as solvent. The column was injected with each of these drug solutions (20µl), and the peak area and retention times were subsequently recorded. The calibration curve for Rifampicin was constructed by plotting the mean peak area against the drug concentration. Regression equation was found to be y = 2.2071x + 2028. (r^2 = 0.9957). Table 3 presented the results of linearity.

Level	Concentration(µg/ml)	peak area
Level – 1	100 µg/ml	2239
Level – 2	200µg/ml	2476
Level – 3	300µg/ml	2885
Level – 4	400µg/ml	2951
Level – 5	500µg/ml	3089
Level - 6	600µg/ml	3363

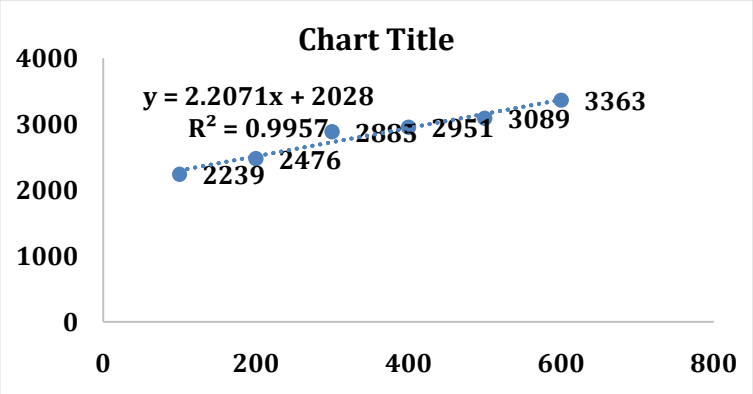


Table 3: Linearity results Figure 4: Calibration Curve for Rifampicin

Precision: Four replicate analysis of 20µg/ml stock solution of Rifampicin was analyzed. The % RSD was found to be 0.20 for intraday precision. The % RSD was found to be less than 2 hence the method was found to be precised.

Chromatogram

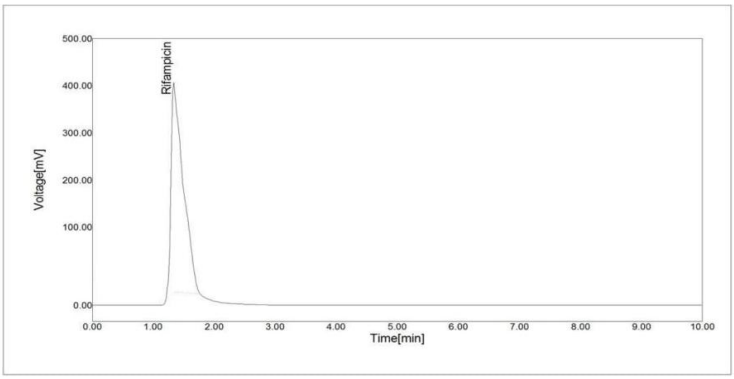


Figure 5: Chromatogram for precision

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Rifampicin	1.3333	6123.6094	0.00	117.4	2.5040	0.0000
Sum			6123.6094				

Table 4: Precision Results

S.No.	Injection	Area value
1	Injection 1	3410
2	Injection 2	3991
3	Injection 3	3977
4	Injection 4	4306

AVERAGE	3727
% RSD	0.20

Limit of Detection (LOD): It is done as the concentration is taken from the lowest concentration and furthermore it is diluted to 100µg/ml concentration, retention time and area is taken as 0.9 and 2239 respectively. The peak has been detected.

Chromatogram

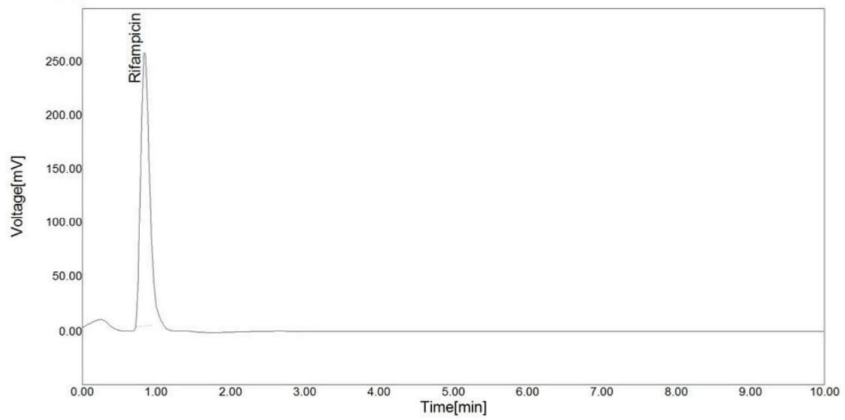


Figure 6: Chromatogram for Limit of Detection

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Rifampicin	0.8333	2127.3250	0.00	260.3	1.3696	0.0000
Sum			2127.3250				

Table 5: Summary of Results

Parameters	Rifampicin	Limit
Linearity Range (µg/ml)	100-600µg/ml	R< 1
Correlation coefficient	0.9957	
Slope (m)	2.2071	
Intercept (c)	2028	
Regression equation (Y=mx+c)	y = 2.2071x + 2028	
Assay (% mean assay)	91.6%	90% - 103.0%
LOD	0.8	NMT 3
System precision %RSD	0.20	NMT 2.0%

CONCLUSION

The estimation of Rifampicin was done by RP-HPLC. The assay of Rifampicin was performed with tablets and the % assay was found to be 95.6% which shows that the method is useful for routine analysis. The method demonstrated good sensitivity as evidenced by the linearity of Rifampicin, which exhibited correlation coefficients of 0.990 and 0.999. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.20, Rifampicin which shows that the method is precise. The acceptable range for percentage recovery, indicating accuracy, is between 90% and 103.0%.The total recovery was found to be 91.6% for Rifampicin. The validation of the developed method demonstrates that the accuracy falls comfortably within the established limit, thereby indicating the method's capability to exhibit good accuracy and reproducibility. The acceptance criteria for LOD is 3. The LOD for Rifampicin was found to be 0.833. The method demonstrates good system suitability and precision under the given set of conditions, as the robustness limits for mobile phase variation and flow rate variation are well within acceptable limits.

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