



RESEARCH ARTICLE

**A Validated RP-HPLC Method for Estimation of Rifaximin in its Bulk and
Pharmaceutical Dosage Forms**

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ABSTRACT

An attempt has been made to develop and validate a simple, rapid, linear, precise, reproducible and economical method for Rifaximin in its bulk and pharmaceutical dosage forms by using Reverse Phase-HPLC method. The method was developed by using Reverse Phase-HPLC, the maximum wavelength was found to be at 276 nm. The linear regression coefficient was not more than 0.999. The estimation of Rifaximin was done by Reverse Phase-HPLC. The mobile phase optimised which consists of Acetonitrile: Triethylamine (TEA) of pH-4.6 mixed in a ratio of 70:30% v/v. A Phenomenex® Luna-C18 column (4.6×150cm, 5µm, Maker: waters) or equivalent chemically bonded porous silica particles as the stationary phase. The results obtained on the validation of parameters met the ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with a high degree of accuracy and precision.

KEYWORDS

Rifaximin, Acetonitrile, Triethylamine, Phenomenex® Luna-C18, Reverse Phase-HPLC, Orthophosphoric acid (OPA)

INTRODUCTION

Rifaximin(7S,9E,11S,12R,13S,14R,15R,16R,17,19E,21Z)-2,15,17,36-tetrahydroxy-11-methoxy-3,7,12,14,16,18,22,30-octamethyl-6,23-dioxo-8,37-dioxo-24,27,33-triazahexacycloheptatriaconta 1,3,5(35),9,19,21,25(36),26(34),28,30,32-undecaen-13-yl acetate. It is a Red-Orange, Crystalline Powder, soluble in DMSO (47 mg/ml), water (<1 mg/ml), ethanol (157 mg/ml), alcohol, and chloroform. It is a semi synthetic antibiotic based on Rifamycin. It has poor oral bioavailability.

It is used in the treatment of traveller's diarrhoea and hepatic encephalopathy¹⁻².

Literature review reveals that there are less analytical methods reported for the analysis of Rifaximin by RP-HPLC. There is a need of new analytical method development for the estimation of Rifaximin in pharmaceutical dosage form. Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the analysis of Rifaximin. The analytical method for the estimation of Rifaximin will be developed by RP-HPLC method by optimizing the chromatographic conditions. The developed method is validated according to ICH guidelines for various parameters specified in ICH guidelines, Q2 (R1)³.

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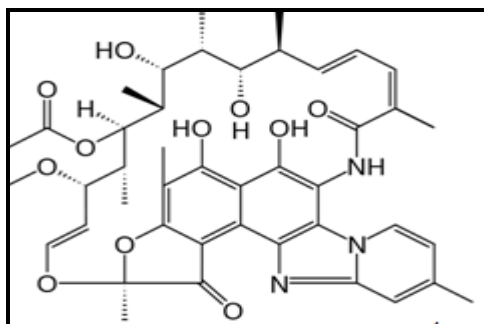


Figure 1: Structure of Rifaximin⁴

MATERIALS AND METHODS

Chemicals and Reagents

Rifaximin was provided as gift sample from Sura Labs, Hyderabad. Water, Triethylamine and Methanol for HPLC was purchased from Lichrosolv (Merck). Acetonitrile of HPLC Grade was purchased from Merck. Anhydrous disodium hydrogen phosphate and Citric Acid was purchased from Finar Chemicals. All other chemical reagents used were of analytical grade.

Instrumentation

The High Performance Liquid Chromatography (HPLC) system was of Waters[®] with 2695 Separation Module and 996 PDA Detector which consisted of following components: a gradient pump, variable wavelength programmable UV/Vis detector, a manual injection facility with 20 μ l fixed loop. The chromatographic analysis was performed using the Empower 2[®] software on a Phenomenex[®] Luna-C18 (4.6 \times 150cm, 5 μ m). In addition, an electronic balance (Sartorius), a pH meter (Lab India), a Digital Ultra Sonicator (Enertech) were used in this study.

Preparation of Solutions

Preparation of Buffer

Dissolve accurately 1.5 ml of TEA in 150 ml of HPLC water and make up the volume up to 250 ml with HPLC water. Adjust the pH to 4.6 by adding Orthophosphoric acid (OPA).

Preparation of Mobile Phase

Accurately measure 30ml (30%) of TEA buffer and 70 ml of Acetonitrile (70%) were mixed and degassed in a Digital Ultra Sonicator for 10

minutes and then filtered through 0.45 μ filter under vacuum filtration.

The Diluents

The Mobile phase was used as the diluents.

Preparation of Standard Stock Solution

Accurately weigh and transfer 10 mg of Rifaximin working standard into a 10mL of clean dry volumetric flasks add about 7ml of Diluents and subjected to Ultra-sonication to dissolve it completely and make volume up to the mark with the same solvent (Stock solution-A) (1000 μ g/ml). Further pipette out 1 ml of the above Rifaximin stock solution-A into a 10mL volumetric flask and dilute up to the mark with diluents. (Stock solution-B) (100 μ g/ml)

Stock solution of 100 μ g/ml was further diluted to get the concentration of 10 μ g/ml. The wavelength was selected by scanning the above standard drug solution between 200 nm to 400 nm. The maximum absorbance was recorded at 276 nm. Therefore 276 nm was selected as the detection wavelength for the RP-HPLC investigation.

Construction of Calibration Curve

Aliquots of different concentrations of standard solution were prepared and their chromatograms were recorded at the optimized chromatographic conditions. The mean peak areas at different concentration levels were calculated from the chromatograms. Then the linearity plot was constructed using the mean peak areas at their respective concentrations.

HPLC Method Development

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Water and Methanol: Phosphate buffer with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Triethylamine buffer (pH 4.6) in proportion 70:30 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, X-bridge column, Xterra, and

C8 column. Phenomenex Luna C-18 was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Method Validation

The developed method was validated for Linearity, accuracy, precision, Limit of Detection, Limit of Quantitation, Robustness and System suitability parameters as described in ICH guidelines.

Linearity

From the Stock solution 5, 10, 15, 20, 25 µg/ml solutions were made and their chromatograms were recorded. From the recorded chromatograms, their respective mean peak areas were calculated and the linearity plot was constructed using the mean peak areas at their respective concentrations. 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 correlation coefficient was found to be 0.999.

System Suitability Parameter

System Suitability was analyzed by giving six replicates and evaluated the chromatographic parameters like retention time, tailing factor, theoretical plates and peak area.

Accuracy

Accuracy of the method was determined by calculating the % recovery of Rifaximin by the method of standard edition (standard stock solution spiked in to the placebo).

Preparation of Standard Stock Solution

Accurately weigh and transfer 10 mg of working Rifaximin 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 1ml of the above Rifaximin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation Sample Solutions

A. For preparation of 50% solution (With respect to target Assay concentration)

Accurately weigh and transfer 5mg of Rifaximin working standard into a 10ml of clean dry volumetric flask 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 1ml of the above Rifaximin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

B. For preparation of 100% solution (With respect to target Assay concentration)

Accurately weigh and transfer 10 mg of Rifaximin 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 1 ml Rifaximin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents

C. For preparation of 150% solution (With respect to target Assay concentration)

Accurately weigh and transfer 15 mg of Rifaximin 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 1ml of Rifaximin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Calculate the Amount found and Amount added for Rifaximin and calculate the individual recovery and mean recovery values. These solutions were filtered through 0.45µ membrane and then each concentration; three replicate injections were made under the optimized conditions. Recorded the chromatograms and measured the peak responses.

Precision

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Rifaximin 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-I) (1000 µg/ml). Further pipette out 1 ml of the above Rifaximin stock solution-1 into a 10mL volumetric flask and dilute up to the mark with diluents.(Stock solution-II) (100 µg/ml).

Preparation of Sample Solution

Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Rifaximin (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution-I). Further pipette 1 ml of above Rifaximin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. (Stock solution-II) The standard and sample solution of 15ppm (µg/ml) of was injected for five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

Ruggedness

To evaluate the Ruggedness of the method, Precision was performed on different day by using different make column of same dimensions.

Preparation of Stock Solution

Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Rifaximin (marketed formulation) sample into a 10mL clean dry volumetric flask 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 1 ml of the above Rifaximin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents

Procedure

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

Robustness

Robustness of the method was determined by slightly changing the flow rate, temperature and mobile phase composition from the optimized chromatographic conditions.

Preparation of Sample Solution (15µg/ml of Rifaximin)

About 10mg of Rifaximin was weighed and transferred to 10ml volumetric flask, it was dissolved with diluents and the volume was made up to the mark with same solvent. Further 1.5 ml of above solution was diluted to 10ml with the diluents to get 15µg/ml of Rifaximin

Effect of Variation of flow

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

Effect of Variation of Mobile Phase Organic Composition

The sample was analyzed by variation of mobile phase i.e. Acetonitrile: TEA buffer 4.6pH was taken in the ratio and 80:20, 60:40 instead of 70:30 remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

RESULTS

Linearity

Table 1: Linearity Data for Rifaximin

S.No.	Concentration (µg/ml)	Area
1	5	429288
2	10	828423
3	15	1189473
4	20	1594546
5	25	1992367

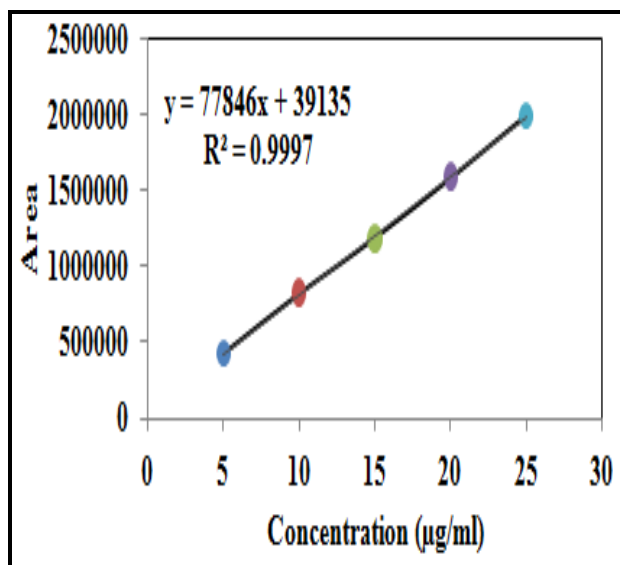


Figure 2: Calibration Curve of Rifaximin

System Suitability Parameter

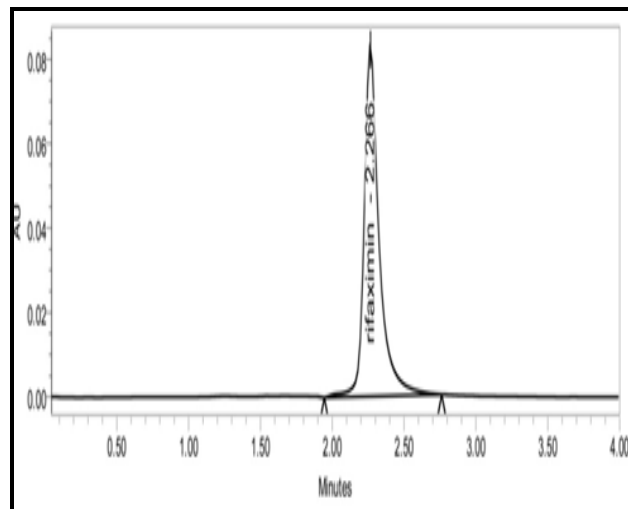


Figure 3: System Suitability Parameters for Rifaximin

Table 2: System suitability parameters for Rifaximin

Name	Retention time (min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
Rifaximin	2.266	606289	83035	1.4	2735.7

Accuracy

Table 3: Accuracy results for Rifaximin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	605972.6	5	4.9	98%	100%
100%	1195448.5	10	10.1	101%	
150%	1822550.8	15	15.1	100.6%	

Precision

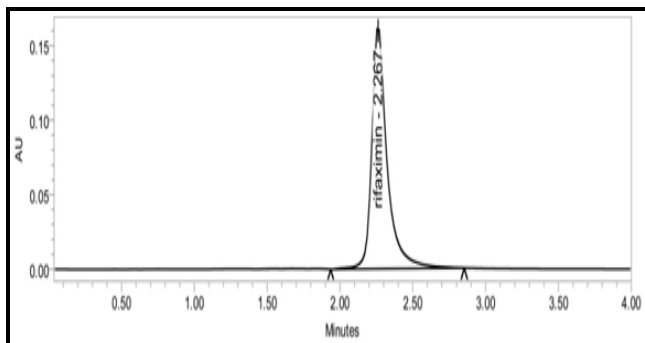


Figure 4: Chromatogram showing precision injection-1

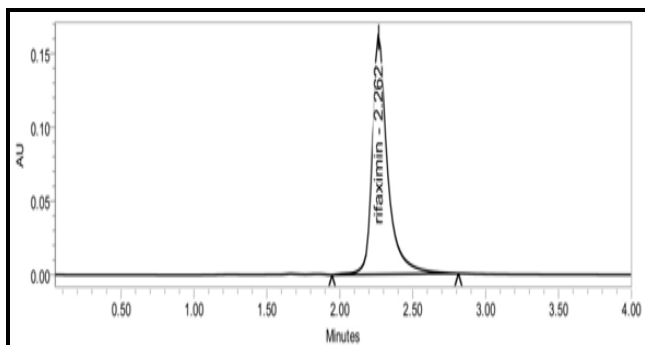


Figure 5: Chromatogram showing precision injection-2

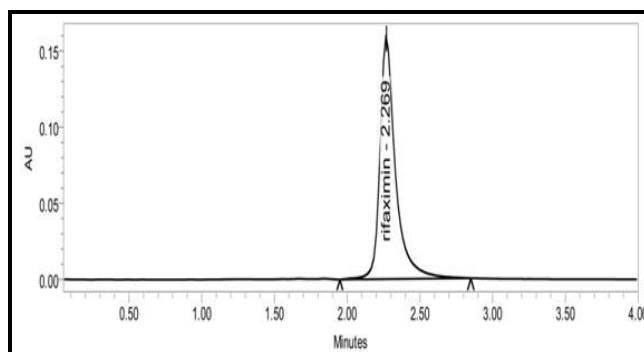


Figure 6: Chromatogram showing precision injection-3

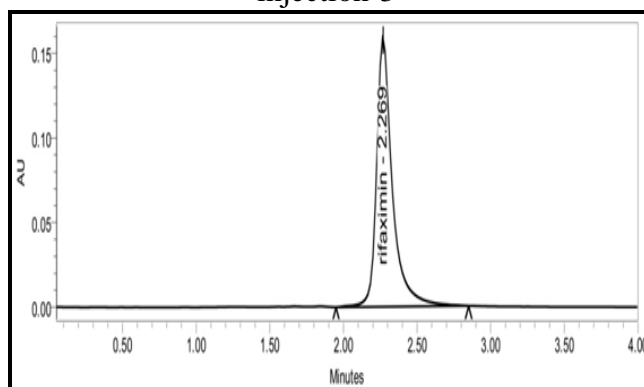


Figure 7: Chromatogram showing precision injection-4

Table 4: Results of method precision for Rifaximin

S. No	Peak name	Retention time	Area ($\mu\text{V}\cdot\text{sec}$)	Height (μV)	USP Plate Count	USP Tailing
1	Rifaximin	2.269	1187187	159416	2622.7	1.4
2	Rifaximin	2.264	1188125	161793	2758.1	1.5
3	Rifaximin	2.267	1189202	161854	2700.8	1.4
4	Rifaximin	2.270	1191196	159246	2619.9	1.5
5	Rifaximin	2.262	1192867	162665	2652.7	1.4
Mean			1189715.3			
Standard.dev			2308.1			
%RSD			0.2			

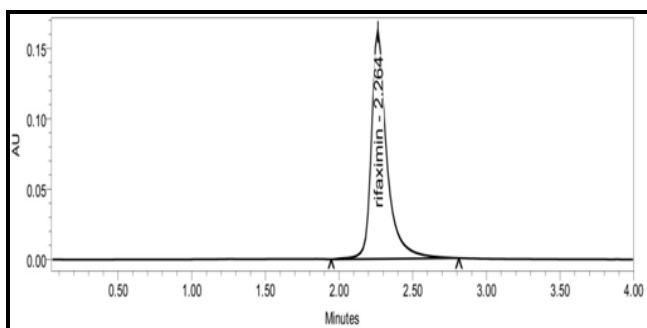


Figure 8: Chromatogram showing precision injection-5

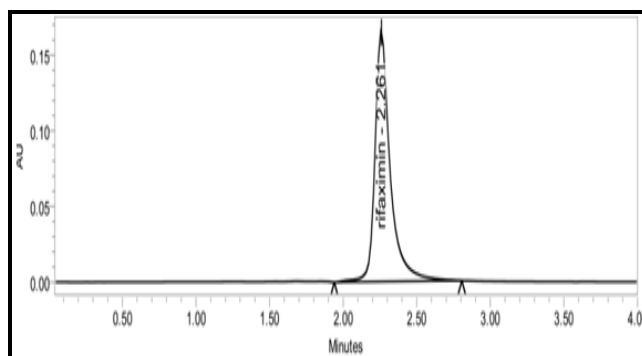


Figure 10: Chromatogram showing intermediate precision injection-2

Ruggedness

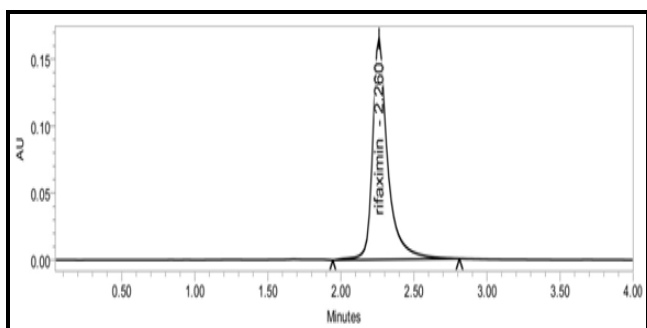


Figure 9: Chromatogram showing intermediate precision injection-1

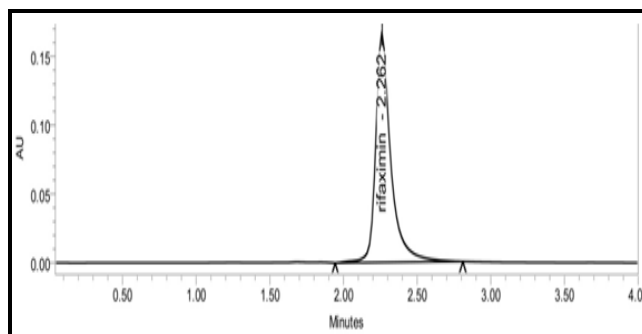


Figure 11: Chromatogram showing intermediate precision injection-3

Table 5: Results of Ruggedness for Rifaximin

Sl. No	Peak Name	RT	Area ($\mu\text{V}\cdot\text{sec}$)	Height (μV)	USP Plate count	USP Tailing
1	Rifaximin	2.262	1192194	165295	2731.9	1.4
2	Rifaximin	2.262	1192990	166061	2790.9	1.4
3	Rifaximin	2.262	1193772	166385	2877.3	1.4
4	Rifaximin	2.260	1194120	165951	2846.7	1.4
5	Rifaximin	2.261	1197708	169456	2821.3	1.5
Mean			1194748.6		2814.9	1.43
Standard. Dev.			2386.7			
% RSD			0.19			

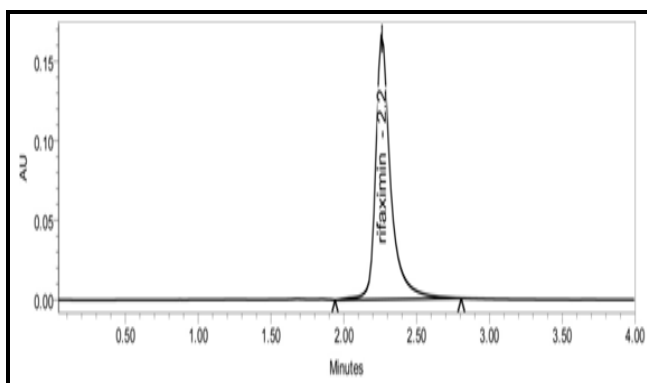


Figure 12: Chromatogram showing intermediate precision injection-4

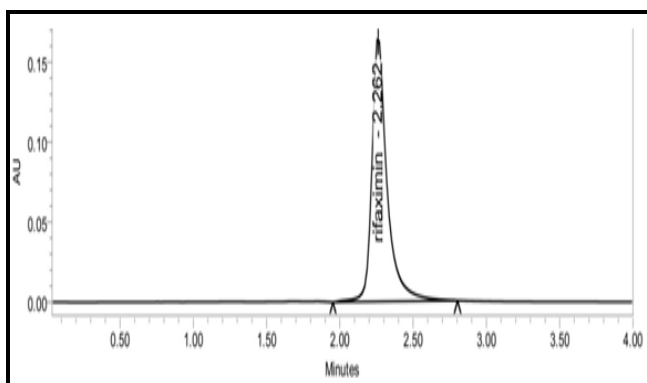


Figure 13: Chromatogram showing intermediate precision injection-5

Robustness

Variation in Flow

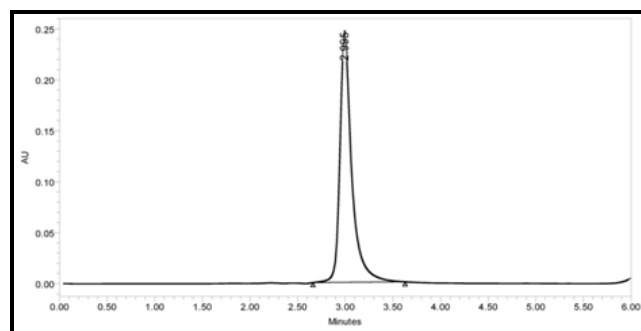


Figure 14: Chromatogram showing less flow of 0.9ml/min

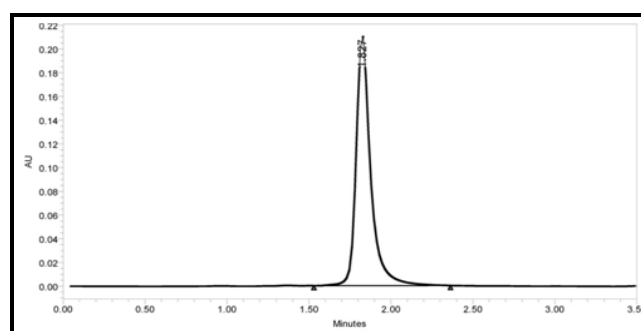


Figure 15: Chromatogram showing more flow of 1.1 ml/min

Table 6: Results for variation in flow

S.No	Drug name	Flow (ml/min)	Area	Height	USP plate count	USP Tailing
1	Rifaximin	Less (0.9)	2130397	245660	3353	1.5
		Actual(1)	1195448.5	163552	2733.4	1.4
		More (1.1)	1301504	208313	2384	1.4

Table 7: Results for variation in mobile phase composition

S.No	Drug name	Organic (ml/min)	Area	Height	USP plate count	USP Tailing
1.	Rifaximin	Less	1606935	93158	2396	1.3
		Actual	1195448.5	163552	2733.4	1.4
		More	1301125	207985	2218	1.4

Variation of Mobile Phase Organic Composition

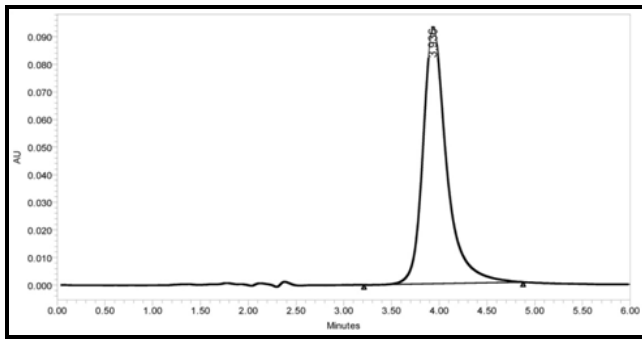


Figure 16: Chromatogram showing less organic composition

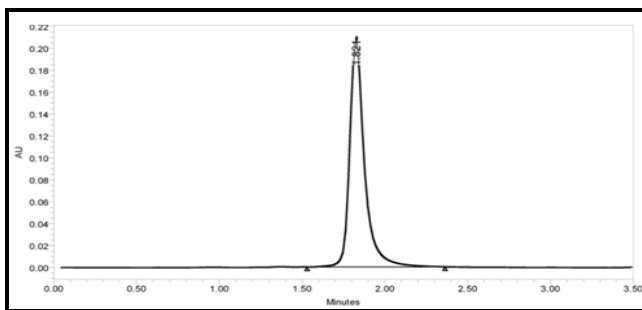


Figure 17: Chromatogram showing more organic composition

Optimized Chromatographic Conditions

Instrument used : Waters® HPLC with Auto sampler and PDA Detector 996 model.

Elution : Gradient

Temperature : Ambient

Column : Phonemenex® Luna C-18.

Buffer : Triethylamine buffer (pH-4.6) Dissolve 1.5ml of Triethylamine in 150ml water and make up the volume to 250ml with HPLC water and adjust the pH to 4.6

pH : 4.6

Mobile phase : Acetonitrile: Triethylamine (TEA) buffer (70:30)

Flow rate : 1 ml/min

Wavelength : 276 nm

Injection volume : 10 µL

Run time : 4 min

Retention Time : 2.262

Area : 1199561

LOD : 0.65µg/ml

LOQ : 1.99µg/ml

Tailing Factor : 1.4

Theoretical Plates : 2848.8

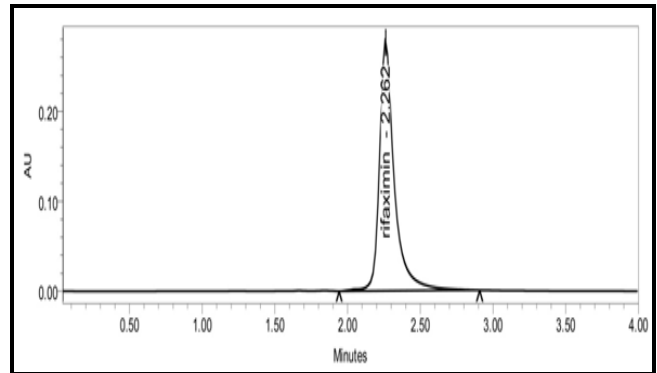


Figure 18: Standard Chromatogram of Rifaximin

DISCUSSION

The present study was aimed at developing an accurate, precise and linear RP-HPLC method for estimation of Rifaximin in its bulk and pharmaceutical dosage forms as per ICG guidelines. The method was found to be linear with 5-25µg/ml with a correlation coefficient (R^2) of 0.999. The LOD and LOQ of the method were calculated to be 0.65µg/ml and 1.99 µg/ml respectively. The precision was estimated by employing repeatability; intra-day and inter-day studies and the results were calculated as % RSD values and were found to be within the limits. Recovery of Rifaximin was found to be in the range of 98-101% which confirms the accuracy of the method. The system suitability was studied with six replicates standard solution of Rifaximin and results were found to be acceptance criteria.

CONCLUSION

In the present study, we have developed a New, Rapid RP-HPLC method and validated for different parameters Linearity, Accuracy, Precision, and LOD, LOQ, Robustness and System suitability. By studying all these validation parameters we have concluded that the method was linear, accurate, precise, robust and rapid for the determination of Rifaximin in Bulk

and Pharmaceutical dosage forms. Hence the method can be successfully applied for the estimation of Rifaximin in Bulk and Pharmaceutical Dosage forms.

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