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# RP-UFLC Method Development and Validation of Rifaximinin Bulk and Tablet Dosage Form

Srinidhi B. S[#1], Jose GnanaBabu C[#2,] and Senthil Kumar G. P[#3].
*Department of Pharmaceutical Analysis,
Bharathi College of Pharmacy, Bharathinagara, MaddurTaluk, Mandya District, Karnataka, India – 571 422

***Abstract***
*A simple, novel, accurate, precise, linear, rapid, and economic RP-UFLC method was developed for the estimation of Rifaximin in bulk and tablet dosage form. The chromatographic separation was achieved using a PhenomenexLuna C18 (250 x 4.6 mm, 5 µ) column and binary gradient elution, a mobile phase comprising of Methanol: Water in the ratio (95:5). The flow rate was 1.0ml/min with detection at 235nm using a UV detector and drug eluted with a retention time of 3.44 min. The calibration curves were linear ($r^2$=0.999) in the concentration range of 2-10 µg/ml. The limit of detection and limit of quantitation were found to be 2.5148195 and 7.6206642 µg/ml, respectively. The developed method was validated as per ICH guidelines. Hence it is successfully applied for the quantitative estimation of Rifaximin in tablet dosage form*

**Keywords** - *Rifaximin, ICH guidelines, RP-UFLC.*

## I.INTRODUCTION

Rifaximin is used as an antibiotic for the treatment of traveler's diarrhea and hepatic encephalopathy but not used for systemic bacterial infection and for children below the age of 12 years. Rifaximin binds to the beta-subunit of bacterial DNA-dependent RNA polymerase and prevents catalysis of polymerization of deoxyribonucleotides into a DNA strand, thereby inhibiting bacterial RNA synthesis. In vitro studies of rifaximin have demonstrated broad-spectrum coverage, including gram-positive, gram-negative, and anaerobic bacteria, as well as a limited risk of bacterial resistance[2].

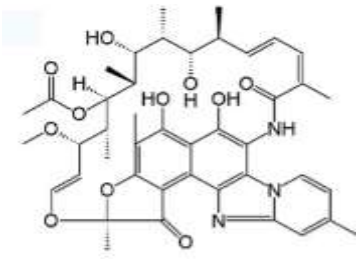


**Fig.1: Chemical structure of Rifaximin.**

Rifaximin is chemically (2S,16Z,18E,20S,21S,22R,23R,24R,25S,26S,27S,28E)-5,6,21,23,25-Pentahydroxy-27-methoxy-2,4,11,16,20,22,24,26-Octamethyl-2,7-(epoxypentadeca-[1,11,13]trienimino)benzofuro[4,5-e]pyrido[1,2-a]-benzimida-zole-1,15(2H)-dione,25-acetate.

It has a molecular formula of $C_{43}H_{51}N_3O_{11}$and a molecular weight of 785.879 g/mol. It has the structural formula (Fig.1). Rifaximin is an orange-brown to red-brown powder. It is soluble in dimethylsulfoxide, Methanol, chloroform, ethanol, ethylacetate, and acetone, slightly soluble in water.

Literature Survey revealed that the drug had been estimated by UV Spectrophotometricand RP-HPLC[3-12].

The aim of the present work was to develop and validate a novel, rapid, simple, precise, and specific RP-UFLC method for the estimation of Rifaximin in bulk and tablet dosage form.

## II. MATERIALS AND METHODS

### *A. Instrumentation*

Chromatographic separation was performed on a Shimadzu LC-20AD system comprising a SIL-20 ACHT UV-Vis detector, Shimadzu LC-20AD pump, and enable Phenomenex Luna C18 (250 x 4.6 mm, 5µ). A manually operating SIL-20 ACTH injector 20µl injection valve was used for injecting samples and standard solutions. Baseline chromatography Data system N2000 software was used to collect and process the data.

### *B. Chemicals and reagents*

Rifaximin is formed obtained as a gifted sample from the pharma industry and its pharmaceutical dosage form Rifaximin 10 tablets labeled claim 400 mg were purchased from a local pharmacy, manufactured by INTAL Pharmaceuticals Ltd. were purchased from local pharmacy Methanol: Water (95:5) were obtained from JSS college of Pharmacy Ltd. Mysore. All the chemicals used in this investigation are HPLC grade.

### *C. Selection of Mobile phase*

Based on sample solubility, stability, and suitability, various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases.

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From the various mobile phases, Methanol: Water (95:5) was chosen with detection wavelength 235nm since it gave a sharp peak with good symmetry within limits.

### *D. Mobile phase preparation*

The mobile phase was prepared by mixing Methanol: Water (95:5) by RP-UFLC grade. This solution was filtered using a Microfilter and was sonicated for 10mins. The total volume of the mobile phase prepared was 1000ml.

### *E. Chromatographic conditions*

The optimized parameters were used as a final method for the estimation of Rifaximin Represented in Table 1.

### *F. Standard preparation*

100 mg of Rifaximin was taken in a 100 ml volumetric flask and make up the volume to 100 ml with Methanol (the concentration of this solution is **1**mg/ml). From this above solution working solution, 1ml was pipetted into 10ml volumetric flask, and volume was made up to the mark with Methanol (the concentration of this solution is 100µg/ml). This is a working solution from this different concentration ranging from 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, and 10µg/ml was prepared by transferring required aliquotes of the solution to 10ml volumetric flask and make up the volume up to the mark by Methanol. This was sonicated for 10mins then the solution was filtered using Microfilter.

### *G. Sample preparation*

0.7526gm of the tablet was taken in a 100ml volumetric flask and was dissolved using RP-UFLC grade methanol and made up to volume. The solution was sonicated for 10mins. The concentration of this solution is (10mg/ml). From this, 1.00ml of aliquot was transferred to a 10ml volumetric flask, and volume was made up to the mark. The concentration of this solution is 1mg/ml. From this, 1.00ml is transferred to 10ml, and volume was made up with Methanol, the concentration of this being 100µg/ml. This solution was filtered using a Microfilter and was used for analysis.

### *H. Flow rate selection*

Different flow rates between 0.50 to 1.50 ml/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time.

### *I. Method Validation*

The method is validated according to the ICH guidelines.

Validation methods like Linearity, Precision, Accuracy, Ruggedness, Robustness, LOD, and LOQ.

## III. RESULTS AND DISCUSSION

### *A. System suitability*

20 µl of the standard solution was injected under optimized chromatographic conditions to evaluate the suitability of the system. Parameters such as the number of theoretical plates (N), tailing factor (T), Retention time ($T_R$), Asymmetry (K), and area were determined. The obtained values indicate a good performance of the system. The values of system suitability parameters were shown in Table- 2.

### *B. Specificity*

The specificity of the RP-UFLC method was checked for the interference of impurities, degradants, or excipients in the analysis of sample solution and was determined by injecting a volume of 20µl of the sample solution, and the chromatogram was recorded. There is no interference of impurities, excipient peak on the peak of Rifaximin, indicating the high specificity of the method.

### *C. Linearity and Range*

The calibration curve was plotted for different concentrations of working standards prepared from standard drug solution of pure drug, shown in Fig-3, and showed linearity over a concentration range of 2-10µg/ml shown in Table-3, along with regression parameters in Table-4. Each calibration was injected six times. The calibration curve was performed in five replicates.

**Table 1: Optimized chromatographic conditions:**

| Parameters | Values |
|---|---|
| Column | phenomenex Luna C18 |
| Mobile phase | Methanol: Water (95:5) |
| Flow rate | 1ml/min |
| Injection volume | 20µl |
| Wavelength | 235nm |
| Temperature | Ambient |
| Retention time | 3.44min |
| Run time | 10min |
| Mode of operation | Binary gradient elution |

**Table 2: Results of System suitability studies**

| System suitability Parameter | Acceptance Criteria | Result RP-UFLC |
|---|---|---|
| Injection precision for Retention time (min) | - | 3.44 |
| Injection precision for peak area (n = 6) | - | 94300 |
| USP tailing factor (T) | T = ≤ 2.0 | 1.19 |
| Asymmetry | K = ≤ 2.0 | 0.99 |
| Theoretical Plates (N) | N = ≥ 2000 | 2939 |

**Table 3: Linearity data**

| Concentration (µg/ml) | Retention time | Peak area* |
|---|---|---|
| 2 | 3.44 | 266926 |
| 4 | 3.44 | 481169 |
| 6 | 3.44 | 701205 |
| 8 | 3.44 | 891802 |
| 10 | 3.44 | 108635 |

***Average of six determinations**

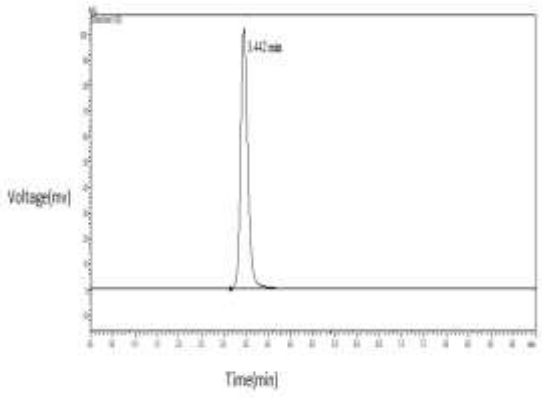


**Fig 2: Chromatogram of Rifaximin**

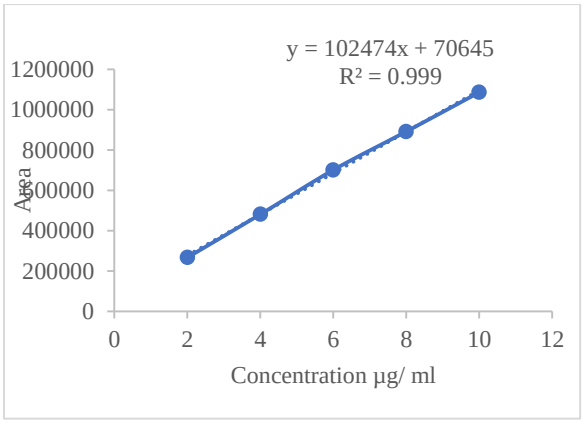


**Fig.3: Linearity graph of Rifaximin.**

**Table 4: Regression parameters**

| Regression Parameters | Values |
|---|---|
| Linearity range(µg/ml) | 2-10µg/ml |
| Regression equation | Y=102474×+70645 |
| Correlation Coefficient r2 | 0.999 |
| Slope | 102474 |
| Intercept | 70645 |

***D. Precision***

The precision of the analytical method was determined by intraday and inter-day precision. The sample solution was prepared as per the test method. In intraday precision, the same concentration of sample solution was injected 6 times on the same day, and in inter-day precision, injecting five solutions of the same concentration for six different days in a week. The results of precision were tabulated in table-5. The average and standard deviation of the mean area were taken, and %RSD was calculated and reported. %RSD values were within the limits and the method was found to be precise.

**Table 5: Results of Precision studies**

| Sl.No | Conc | Intraday Precision | Interday precision |
|---|---|---|---|
| 1 | 6 | 744437 | 764834 |
| 2 | 6 | 746135 | 766502 |
| 3 | 6 | 747930 | 767881 |
| 4 | 6 | 749678 | 769899 |
| 5 | 6 | 752407 | 771837 |
| 6 | 6 | 755485 | 773057 |

| | | | |
|---|---|---|---|
| Mean | | 749345.333 | 769001.666 |
| Std Dev* | | 4088.547 | 3167.450 |
| % RSD | | 0.545615 | 0.411891 |

*indicates an average of six determinations, RSD indicates the relative standard deviation

The accuracy of the method was determined by recovery studies by the determination of % mean recovery of the drug at three different levels (80%, 100%, and 120%). At each level, three determinations were performed. A known amount of standard pure drug was added to pre-analyzed tablet powder, and the sample was then analyzed by the developed method. Results of recovery studies were reported in table-6. The observed data were within the range, which
indicates good recovery values.

***E. Accuracy***

**Table 6: Results of Accuracy studies**

| Level of recovery | Amount of formulation | Amount of Pure drug | The total amount of drug | Peak Area | Difference | % Recovery | Mean |
|---|---|---|---|---|---|---|---|
| 50 | 4 | 2 | 6 | 754889 | 487963 | 101.41197 | 100.0929 |
| | 4 | 2 | 6 | 744511 | 477585 | 99.25514 | |
| | 4 | 2 | 6 | 746226 | 479300 | 99.61157 | |
| 100 | 4 | 4 | 8 | 901929 | 419516 | 87.18683 | 90.0797 |
| | 4 | 4 | 8 | 915804 | 433391 | 90.07043 | |
| | 4 | 4 | 8 | 929813 | 447400 | 92.98188 | |
| 150 | 4 | 4 | 10 | 1206325 | 461888 | 95.99288 | 98.1339 |
| | 4 | 4 | 10 | 1216196 | 471759 | 98.04434 | |
| | 4 | 4 | 10 | 1227361 | 482924 | 100.36473 | |

**SD indicates standard deviation, and RSD indicates the relative standard deviation

***F. Robustness***

The robustness of the analytical method was carried by varying the parameters deliberately from the optimized chromatographic conditions like different Wavelength. The observed results were within the limit. The results were shown in table-7.

**Table 7: Results of Robustness studies**

| Wavelength | Conc | Peak Area | Average | St. Dev | %RSD |
|---|---|---|---|---|---|
| 232 | 6 | 782163 | 779573.667 | 6271.59 | 0.8044 |
| | 6 | 772422 | | | |
| | 6 | 784136 | | | |
| 234 | 6 | 778856 | 775644 | 2787.04 | 0.3593 |
| | 6 | 774211 | | | |
| | 6 | 773865 | | | |
| 237 | 6 | 754447 | 753474 | 877.95 | 0.1165 |

| | | | | | |
|---|---|---|---|---|---|
| | 6 | 752741 | | | |
| | 6 | 753234 | | | |
| | | | | | |

*indicates an average of six determinations, RSD indicates the relative standard deviation

***G.Ruggedness***

Ruggedness was determined between different analysts. The value of %RSD was found to be <2, which showed the ruggedness of the developed analytical method. The values were shown in Table-8.

**Table 8: Results of Ruggedness studies**

| Parameters | Analyst-1 | Analyst-2 |
|---|---|---|
| Mean absorbance | 769001.6667 | 749345.33 |
| Standard deviation** | 3167.450815 | 4088.54794 |
| %RSD | 0.4118912 | 0.5456159 |

**Mean of six determinations, RSD indicates relative standard deviations

**Limit of detection and Limit of Quantitation:**

The LOD and LOQ of the present method were calculated based on the standard deviation of the response and slope of the linearity curve. LOD and LOQ values of Rifaximin were shown in Table-9.

**Table 9: Results of LOD and LOQ**.

| Parameters | Results |
|---|---|
| Limit of Detection | 2.514µg/ml |
| Limit of Quantitation | 7.620µg/ml |

## IV. CONCLUSION

From the above, it can be concluded that all validation parameters (precision, accuracy, linearity, LOD, LOQ,Ruggedness, and Robustness) met the predetermined acceptance criteria, as mentioned in ICH guidelines. The developed RP-UFLC method is simple, rapid, accurate, precise, and shown good linearity. Hence it can be applied for routine analysis of Rifaximin in Pharmaceutical dosage form.

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