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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ANALYISIS OF RIFAXIMIN IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of RIFAXIMIN in tablet dosage form. Isocratic elution at a flow rate of 1ml min^{-1} was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Acetonitrile: Ammonium Acetate 85:15 (v/v). The UV detection wavelength was at 236nm.Linearity was observed in concentration range of 5-50ppm. The retention time for RIFAXIMIN was 4.3 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of RIFAXIMIN in pharmaceutical dosage forms.

Key words: Rifaximin, HPLC, Development, 236nm.

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INTRODUCTION

Rifaximin is a semisynthetic, rifamycin-based non-systemic antibiotic, meaning that very little of the drug will pass the gastrointestinal wall into the circulation as is common for other types of orally administered antibiotics. It is used in the treatment of traveler's diarrhea and hepatic encephalopathy, for which it received orphan drug status from the U.S. Food and Drug Administration in 1998.

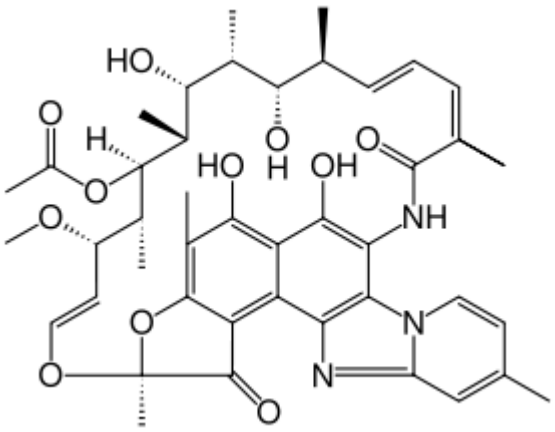

Figure 1: Stricture of Rifaximin

Rifaximin is licensed by the U.S. Food and Drug Administration to treat traveler's diarrhea caused by E. coli.[1] Clinical trials have shown that rifaximin is highly effective at preventing and treating traveler's diarrhea among travelers to Mexico, with few side effects and low risk of developing antibiotic resistance. [2] It is not effective against Campylobacter jejuni, and there is no evidence of efficacy against Shigella or Salmonella species.It may be efficacious in relieving chronic functional symptoms of bloating and flatulence that are common in irritable bowel syndrome.[3] There was recently a pilot-study done on the efficacy of rifaximin as a means of treatment for rosacea, according to the study, induced by the co-presence of small intestinal bacterial overgrowth.[4]

In the United States, rifaximin has orphan drug status for the treatment of hepatic encephalopathy.[5] Although high-quality evidence is still lacking, rifaximin appears to be as effective as or more effective than other available treatments for hepatic encephalopathy (such as lactulose), is better tolerated, and may work faster. The drawbacks to rifaximin are increased cost and lack of robust clinical trials for HE without combination lactulose therapy.A recent study suggests that treatment with rifaximin relieves symptoms for some sufferers of irritable bowel syndrome.

Usual Adult Dose for Traveler's Diarrhea is 200 mg orally 3 times a day for 3 days , for Hepatic Encephalopathy is 550 mg orally twice a day and Usual Pediatric Dose for Traveler's Diarrhea for 12 years or older is 200 mg orally 3 times a day for 3 days.

Clostridium difficile associated diarrhea (CDAD) has been reported with almost all antibiotics and may potentially be life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea following rifaximin therapy. Mild cases generally improve with discontinuation of the drug, while severe cases may require supportive therapy and treatment with an antimicrobial agent effective against C difficile. Hypertoxin producing strains of C difficile cause increased morbidity and mortality; these infections can be resistant to antimicrobial treatment and may necessitate colectomy.Safety and effectiveness for travelers' diarrhea have not been established in pediatric patients less than 12 years of age. Safety and effectiveness for hepatic encephalopathy have not been established in pediatric patients less than 18 years of age.

EXPERIMENTAL

Chemicals and reagents

All HPLC SOLVENTS used like Acetonitrile, ammonium acetate which are of HPLC grade were purchased from E.Merck,

Instrumentation and analytical conditions

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Gemini C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with ,Acetonitrile,Ammonium Acetate 85:15 (v/v) was selected with a flow rate of 0.8 ml min^{-1}.The detection wavelength was set at 236 nm with a runtime of 8 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of Stock, working standard solutions and Sample solutions

100mg of RIFAXIMIN was weighed and transferred (working standard) into a 100ml volumetric flask. The diluent methanol was added and sonicated to dissolve it completely and made up to the mark with the same solvent. Further 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent. The contents were mixed well and filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. The calibration curve was plotted with the concentrations of the 50 to 5 ppm working standard solutions. Calibration solutions were prepared and analyzed immediately after preparation.

The formulation tablets of RIFAXIMIN were crushed to give finely powdered material. Powder equivalent to10 mg of drug was taken in 10 ml of volumetric flask containing 5 ml of mobile phase and was shaken to dissolve the

drug and then filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 30 ppm.

Method Validation procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.

LINEARITY

The developed method has been validated as per ICH guidelines (Zucman D, 2007). Working standard solutions of RIFAXIMIN in the mass concentration range of 5 ppm to 50 ppm was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of RIFAXIMIN was obtained by plotting the peak area ratio versus the applied concentrations of RIFAXIMIN. The linear correlation coefficient was found to be 0.998

S.NO	CONC	AREA
1	5ppm	4258
2	10ppm	8017
3	25ppm	11347
4	20ppm	14258
5	25ppm	17896
6	30ppm	21253
7	35ppm	24547
8	40ppm	28792
9	45ppm	32046
10	50ppm	36516

Table 1: Linearity of RIFAXIMIN

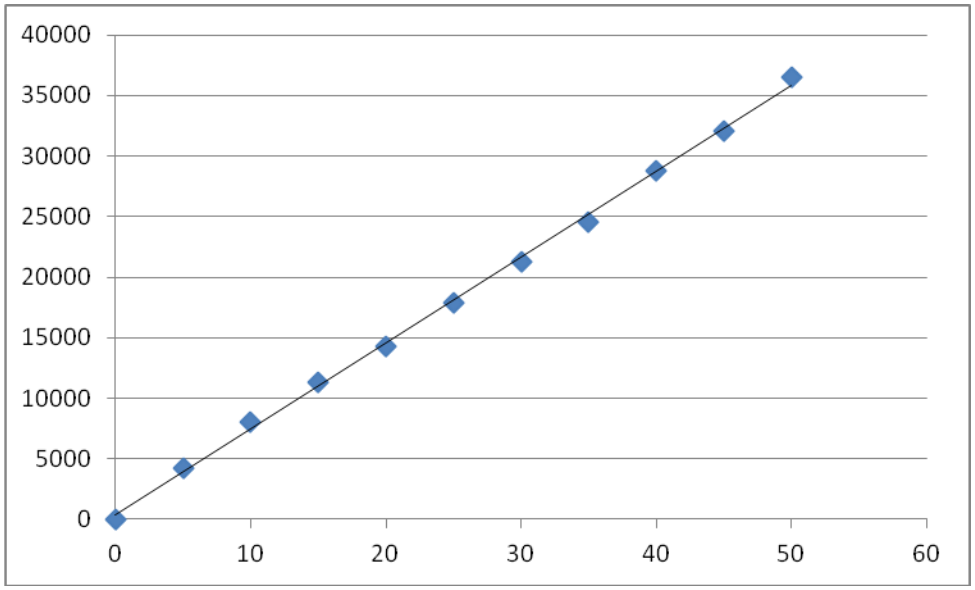

Figure 2: Calibration curve of RIFAXIMIN

Drug	RIFAXIMIN
Concentration range	50-5ppm
Slope (m)	694.8286
Intercept (b)	581.7143
Correlation coefficient	0.998
% RSD	0.15

Table.2 Linear Regression Data for Calibration curve

PRECISION

Repeatability of the method was checked by injecting replicate injections of 30 ppm of the solution for six times on the same day as intraday precision study of RIFAXIMIN and the RSD was found to be 0.04 for intraday and 0.25 for interday

INJECTION	CONCENTRATION	INTRADAY	INTERDAY
1	30ppm	21253	21138
2	30ppm	21260	21159
3	30ppm	21258	21235
4	30ppm	21242	21275
5	30ppm	21239	21197
6	30ppm	21249	21252
	RSD	0.04	0.255

Table 3: Precision parameters of RIFAXIMIN

Accuracy

The accuracy of the method was determined by calculating recovery of RIFAXIMIN by the method of standard addition. Known amount of RIFAXIMIN (10ppm, 20ppm and 30ppm) was added to a pre quantified sample solution and the amount of RIFAXIMIN was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of RIFAXIMIN was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Recovery	Conc. of sample	Recovery	% of recovery
50%	10ppm	9.97	99.7
100%	20ppm	19.82	99.1
150 %	30ppm	30.15	100.5
Average recovery			99.77

Table 4: Accuracy results of RIFAXIMIN

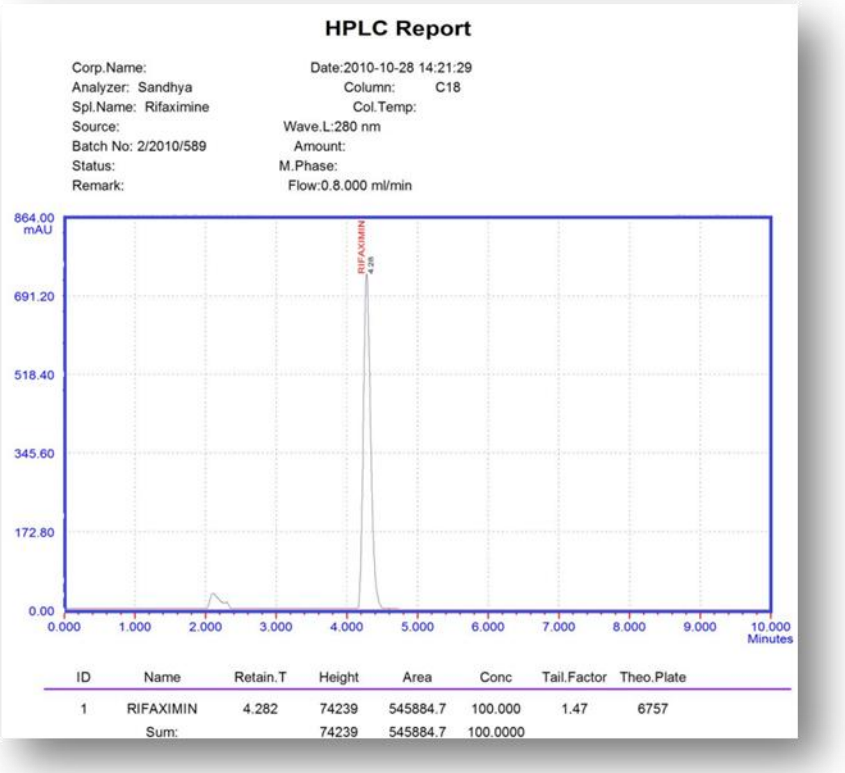

Figure 3: Typical chromatogram of RIFAXIMIN standard.

SPECIFICITY

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 0.2ppm and 0.5ppm respectively as per ICH guide-lines.Results are shown in table 5.

Parameter	Measured
LOD	0.2ppm
LOQ	0.5ppm

Table 5: Results of LOD and LOQ

RUGGEDNESS

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation

INJECTION	CONCENTRATION	INTRA DAY
1	30ppm	21205
2	30ppm	21237
3	30ppm	21153
4	30ppm	21098
5	30ppm	21137
6	30ppm	21375
	RSD	0.47

Table 1: Linearity of RIFAXIMIN

ROBUSTNESS

To determine the robustness of the method, two parameters from the optimized chromatographic conditions were varied. Results of Robustness are shown in table 7.

Parameter	Modification	Peak Area	% of change
Standard	No change	21253	…….
M.PHASE	Acetonitrile : ammoniumacetate 80:20	21198	0.26
PH	5.6	20995	1.214
WAVELENGTH	242nm	21235	0.085

Table 7: Robustness results

SYSTEM SUITABILITY PARAMETER

System suitability tests were carried out on freshly prepared standard stock solutions of RIFAXIMIN and it was calculated by determining the standard deviation of RIFAXIMIN standards by injecting standards in six replicates at 6 minutes interval and the values were recorded inTable8.

λ max (nm)	236nm
Beer's law limit (µg/ml)	50 – 5ppm
Correlation coefficient	0.998
Retention time	4.3 min
Theoretical plates	6757
Tailing factor	1.47
Limit of detection	0.2ppm
Limit of quantification	0.5ppm

Table8: System suitability parameters of RIFAXIMIN

RESULT AND DISCUSSION

Optimization of the chromatographic conditions

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug RIFAXIMIN being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. The concentration of the methanol and Acetonitrile were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Acetonitrile,ammonium acetate 85:15 (V/V). The retention time of RIFAXIMIN was found to be 4.3 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability and validation parameters are given in Table 4. The high percentage of recovery of RIFAXIMIN was found to be 99.77 indicating that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of RIFAXIMIN in tablet formulation. The result for RIFAXIMIN was comparable with a corresponding labelled amount (Table 9). The absence of additional peaks indicates no interference of the excipients used in the tablets.

Formulation	Dosage	Sample concentration	%Estimation	%Recovery
XIFAXAN	550mg	30ppm	29.85ppm	99.5%

Table 9: Formulation results of RIFAXIMIN

CONCLUSION

A validated RP-HPLC method has been developed for the determination of RIFAXIMIN in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 8 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of RIFAXIMIN in pharmaceutical dosage form.

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