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RP-HPLC DETERMINATION OF RIFAXIMIN IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, rapid, sensitive, accurate and precise HPLC method has been developed and validated for the estimation of Rifaximin in bulk and its pharmaceutical dosage forms. The method was carried out using Chromosil Symmetry C18 (150 x 4.6 mm I.D., 5 µm particle size) column and mobile phase comprised of phosphate buffer pH 4.0 and acetonitrile in proportion of ratio 40:60 v/v and degassed in ultrasonic water bath. The flow rate was 1.0 mL/min and the detection wavelength was at 292 nm. The linearity was observed in the range of 10-60 µg/mL with a correlation coefficient of 0.999. The retention time of Rifaximin was 2.963 min. The method was validated as per the ICH guidelines for its linearity, precision, accuracy, specificity, limit of detection, limit of quantitation and by performing recovery studies. The percentage recovery of the drug Rifaximin was 100.6% to 101.4% from the tablet formulation. The proposed method is suitable for the routine quality control analysis for the estimation of Rifaximin in bulk and pharmaceutical dosage form.

Keywords: Rifaximin; Estimation; RP-HPLC; Validation.

INTRODUCTION

Rifaximin (Fig. 1) is a non-aminoglycoside semi-synthetic, nonsystemic antibiotic derived from rifamycin SV [1]. Rifaximin is a structural analog of rifampin. Chemically it is (2S,16Z,18E,20S,21S, 22R,23R,24R,25S,26S,27S,28E)5,6,21,23,25-penta hydroxy-27-methoxy-2,4,11,16,20,22,24,26-octa methyl-2,7(epoxypentadeca-[1,11,13]trienimino) benzofuro[4,5-e]pyrido[1,2-a]-benzimidazole-1,15 (2H)dione,25-acetate[2]. Rifaximin is an antibacterial drug. Rifaximin is indicated for the treatment of patients with travelers diarrhea caused by noninvasive strains of *Escherichia coli* and hepatic encephalopathy. Rifaximin interferes with transcription by binding to the β-subunit of bacterial RNA polymerase. This results in the blockage of the translocation step that normally follows the formation of the first phosphodiester bond, which occurs in the transcription process [3].

A few Spectrophotometric [4-7], HPLC [8-12] and LC-MS [13-15] methods were reported earlier for the estimation of Rifaximin in bulk and pharmaceutical dosage forms. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of Rifaximin in bulk drug and in tablet dosage forms.

EXPERIMENTAL

Chromatographic conditions: The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Chromosil Symmetry C18 (150 x 4.6 mm I.D., 5 µm particle size), a 2695 binary pump, a 20 µL injection loop, auto sampler and a 2487 dual absorbance DAD or UV detector and running on Waters Empower software.

Chemicals and solvents: The reference sample of Rifaximin was provided as gift sample from Sumages Pharma Pvt. Ltd., Bhimavaram, India. Rifaximin tablets (Rifagut-200 mg) were purchased from local market. HPLC grade acetonitrile was purchased from E. Merck (India) Ltd., Mumbai, India. Potassium dihydrogen ortho phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai, India. HPLC grade water obtained from Milli-Q water purification system was used throughout the study.

Preparation of phosphate buffer pH 4.0: 2.72 grams of potassium dihydrogen ortho phosphate was weighed into a 1000 mL beaker, dissolved in 400 mL HPLC water. Diluted to 1000 mL with HPLC water and pH adjusted to 4.0 with orthophosphoric acid.

Preparation of mobile phase and diluents: 400 ml of the phosphate buffer was mixed with 600 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

Preparation of standard stock solution: Accurately weigh and transfer 10 mg of Rifaximin working standard into a 10 mL volumetric flask, add about 7 mL of diluent, sonicate to dissolve it completely and make volume up to the mark with the same diluent. Further pipette 0.4 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Preparation of sample solution: Weigh 20 Rifaximin tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Rifaximin into a 10 mL volumetric flask. Add about 7 mL of diluent, sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.4 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Calibration plot: About 10 mg of Rifaximin was weighed accurately, transferred into a 10 mL volumetric flask and dissolved in 7 mL of a 40:60 v/v mixture of phosphate buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a stock solution. From this, a working standard solution of the drug (40 µg/mL) was prepared by diluting 0.4 mL of stock solution to 10 mL of diluent in a volumetric flask. Further dilutions ranging from 10-60 µg/mL were prepared from the solution in 10 mL volumetric flasks using the above diluent. 20 µL of each dilution was injected six times into the column at a flow rate of 1.0 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area (Fig. 2) was found to be linear in the concentration range of 10-60 µg/mL of the drug. The relevant data are furnished in Table 1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of Rifaximin in tablet dosage forms.

Procedure: A mixture of phosphate buffer pH 4.0 and acetonitrile in the ratio of 40:60 v/v was found to be the most suitable mobile phase for ideal separation of Rifaximin. The solvent mixture was filtered through 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. Inject 20 µL of the standard, sample solutions into the chromatographic system and measure the area for the Rifaximin peak. The detection of the drug was monitored at 292 nm. The run time was set at 5 min. Under these optimized chromatographic conditions the retention time obtained for the drug Rifaximin was 2.963 min. A typical chromatogram showing the separation of the drug is given in Fig. 3.

Validation of the proposed method: The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method as per the ICH guidelines for the estimation of Rifaximin [16-17]. Solution containing 40 µg/mL solution of Rifaximin was subjected to the proposed HPLC analysis to check precision of the method and the results are furnished in Table 2. The accuracy of the HPLC method was assessed by analyzing solutions of Rifaximin at 50%, 100% and 150% concentration levels by the proposed method. The results are furnished in Table 3. The system suitability parameters are given in Table 4.

Estimation of Rifaximin in tablet dosage forms: Commercial formulations of Rifaximin tablets were chosen for testing the suitability of the proposed

method to estimate Rifaximin in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 10 mg of Rifaximin was transferred into a 10 mL volumetric flask and dissolved in 5 mL of a 40:60 v/v mixture of phosphate buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 3 mL of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µm membrane filter. This solution of Rifaximin was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table 5.

RESULTS AND DISCUSSION

In the proposed method, the retention time of Rifaximin was found to be 2.963 min. Quantification was linear in the concentration range of 10-60 µg/mL. The regression equation of the linearity plot of concentration of e over its peak area was found to be y=88.333+35997.486x (r^2=0.999), where x is the concentration of Rifaximin (µg/mL) and y is the corresponding peak area. The number of theoretical plates calculated was 3414, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.02 µg/mL and 0.10 µg/mL respectively, which indicate the sensitivity of the method. The use of phosphate buffer pH 4.0 and acetonitrile in the ratio of 40:60 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug Rifaximin by the proposed HPLC method.

CONCLUSION

The proposed HPLC method is rapid, sensitive, accurate and precise for the determination of Rifaximin and can be reliably adopted for routine quality control analysis of Rifaximin in its tablet dosage forms.

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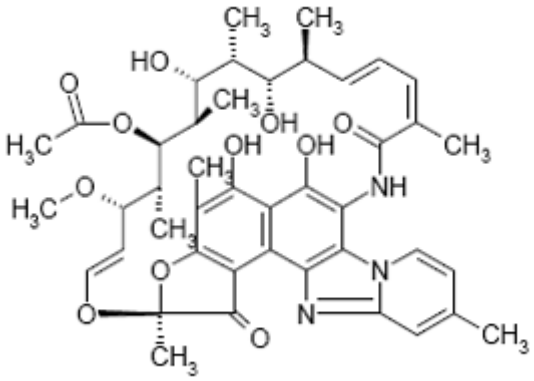

Fig. 1: Chemical structure of Rifaximin

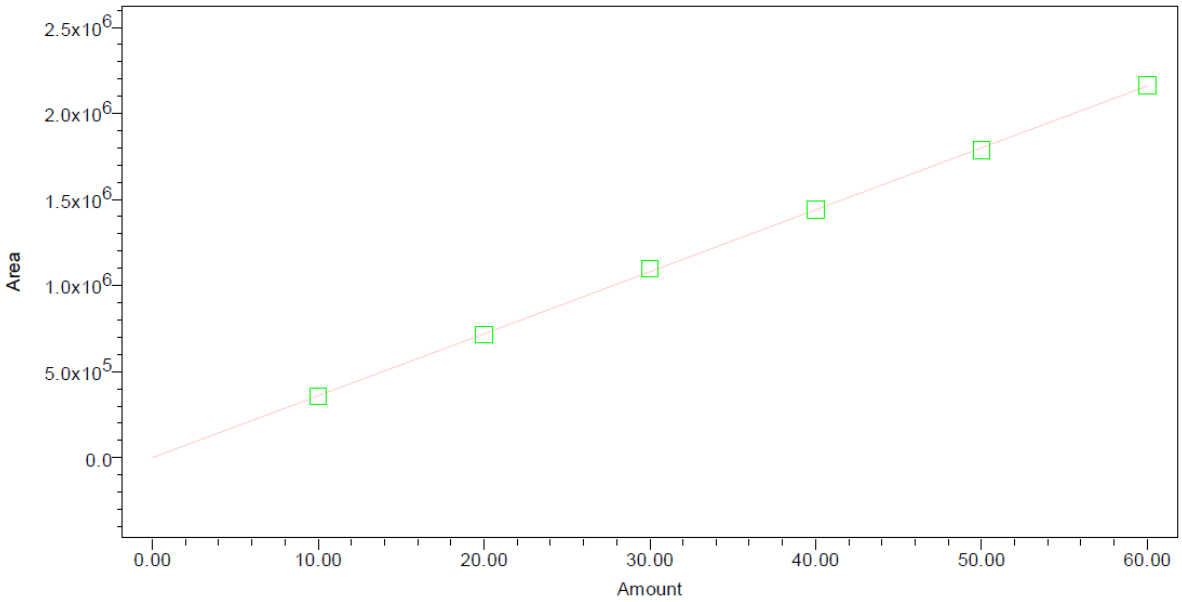

Fig. 2: Calibration curve of Rifaximin

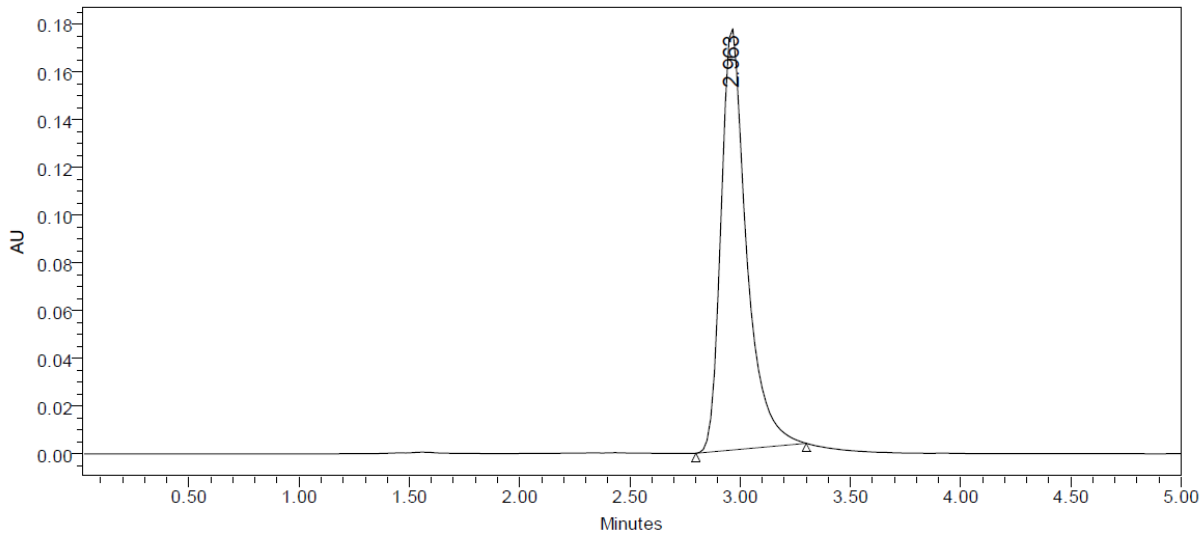

Fig. 3: Typical chromatogram of Rifaximin

Table 1: Calibration data of the method

Concentration (μg/mL)	Mean peak area (n=6)
10	356924
20	713849
30	1097589
40	1440046
50	1787570
60	2164024

Table 2: Precision data of the proposed HPLC method

Concentration of Rifaximin (40 μg/mL)	Peak area
Injection-1	1380840
Injection-2	1399132
Injection-3	1390002
Injection-4	1393830
Injection-5	1388258
Average	1390412
Standard Deviation	6785.13
%RSD	0.48

Table 3: Accuracy studies

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1525887	5.0	5.0	100.6%	
100%	3076104	10.0	10.1	101.4%	101.0%
150%	4630487	15.1	15.2	101.1%	

Table 4: System suitability parameters

Parameter	Result
Linearity ((μg/mL)	10-60
Correlation coefficient	0.999
Theoretical plates (N)	3414
Tailing factor	1.41
LOD (μg/mL)	0.02
LOQ (μg/mL)	0.10

Table 5: Assay studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Rifagut	10	10.17	98.3

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