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Research Article

A Stability Indicating Method for the Estimation of Rifaximin in its Bulk and Pharmaceutical Dosage Form by RP-HPLC Method

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ABSTRACT

A new stability-indicating method was established for the estimation of Rifaximin by RP-HPLC The chromatographic conditions were successfully developed for the estimation of Rifaximin by using symmetryC18 column (150×4.6mm,5µ), flow rate of 1ml/min, mobile phase ratio used was (65:35 v/v) methanol: phosphate buffer pH 3 (pH was adjusted with orthophosphoric acid),detection was carried at 454nm. The Retention time was found to be 2.262mins. The Proposed stability indicating HPLC method was validated as per ICH guidelines and can be applied for the determination of Rifaximin in Pharmaceutical dosage forms. The method was found to be accurate, precise, robust and specific. As the drug peak elution did not interfere with any degradants during the forced degradation studies and therefore the proposed method can be successfully applied to perform long-term and accelerated stability studies of Rifaximin formulation.

Keywords: Rifaximin; stability indicating;HPLC; ICH; Validation.

1. INTRODUCTION

Rifaximin is a newer antibiotic used for the treatment of patients (more than 12 years of age) with travellers diarrhoea caused by non-invasive strains of Escherichia coli. Rifaximin (RFX) 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]- benzimidazole-1,15(2H)-dione,25 acetate[1]. Rifaximin is a product of synthesis of Rifamycin, an antibiotic with low gastrointestinal absorption and good antibacterial activity. Rifaximin binds to the beta-subunit of bacterial DNA-dependent RNA polymerase and prevents catalysis of polymerization of deoxyribonucleotides into a DNA strand. As a result, bacterial RNA synthesis is inhibited. 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. Methods reported for the determination of Rifaximin in pharmaceutical dosage forms and biological fluids include RP-HPLC, LC-MS and spectrophotometric methods have been developed for the determination of RFX in pharmaceutical formulations and biological fluids.In the present work the simple, rapid, precise and accurate robust liquid chromatographic method was developed for the determination of Rifaximin in tablets.

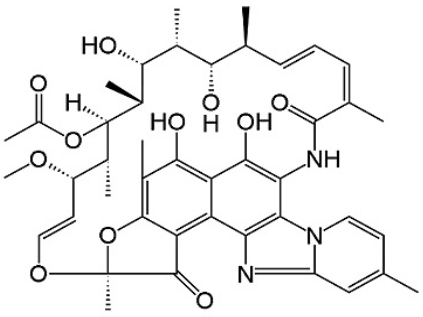

Fig 1: Chemical structure of Rifaximin

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2. MATERIALS AND METHODS

2.1 Instruments

Table no.1: Apparatus / Instruments used

S.NO	Instruments	Manufacturer
1	HPLC	Schimazdu- SPD
2	UV- Spectrophotometer	Spectro 2060 plus
3	Analytical balance	Citizen
4	p^H meter	Sisco
5	Sonicator	Sisco

2.2 Methodology[2-5]

chromatographic separation was achieved by using C-18 Symmetry (150 x4.6mm ,5µm)column equipped with PDA detector maintained at ambient temperature. isocratic elution was performed using Methanol :Phosphate buffer pH3(65:35%v/v) as mobile phase.the mobile phase was prepared by Mixing a mixture of phosphate buffer 350 ml and 650 ml of methanol (HPLC grade-65%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration. Rifaximin stock solution was prepared by weighing10 mg of Rifaximin and transferring into a 10 ml clean dry volumetric flask and 2 ml of diluent was added and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution-1000µg/ml).Further pipette out 1.0 ml from the above stock solution into a 10 mlvolumetric flask and was made up to the mark.

2.3 Analysis of marketed formulation:

17.95mg of Rifaximin powdered drug was accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and make the volume up to the mark with the same solvent(Stock solution-1000µg/ml). Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluents (100µg/ml or ppm). Mix well and filter through 0.45µm filter 10µL of the blank, standard and sample were injected into the chromatographic system and recorded the chromatograms areas for the Rifaximin the peaks were used for calculating the % assay by using the formulae.

2.4 Assay Calculation:

$$\text{Assay} = \frac{\text{sample area}}{\text{standard area}} \times \frac{\text{wt of standard}}{\text{wt of sample}} \times \frac{\text{dilution of sample}}{\text{dilution of standard}} \times \frac{p}{100} \times \frac{\text{Avgwt}}{LC} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Method Development:

After the number of trials we arrived at the optimized parameter for the estimation of Rifaximin, which are given in Table.1.

3.2 Preparation of System Suitability solution

10 mg of Rifaximinworking standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution-1000ppm).Further pipette out 0.6ml of Rifaximin from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluents (60ppm-which is the target assay concentration). The resulting standard solution was injected for six times and measured the area for all six injections in HPLC.The results are tabulated in table 2

3.3 Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e 50%, 100% and 150%. The standard solutions of accuracy 50%, 100% and 150% were injected into chromatographic system. The results are tabulated in table 3

3.4 Precision

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. Results are tabulated in table 4

3.5 Linearity

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels (20 - 100µg/ml) of the assay analyte concentration and 10µl of each solution was injected in to the HPLC system and the peak area of the chromatogram obtained was noted.

Results are tabulated in Table 5

3.6 Limit of Detection and Quantitation

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the

standard deviation of y-intercepts of regression lines. Results are tabulated in Table 6&7

Formula:

LOD = 3.3 X σ/S

Where σ - Standard deviation (SD), S - Slope

LOD = 10 X σ/S

Where σ - Standard deviation, S - Slope

3.7 Robustness

As part of the robustness, deliberate change in the flow rate was made to evaluate the impact on the method. The flow rate was varied at 0.9ml/min to 1.1ml/min. Results are tabulated in Table 8.

3.8 Forced Degradation Studies

The study was intended to ensure the effective separation of Rifaximin and its degradation peaks of formulation ingredients at the retention time of rifaximin. The samples were exposed to the following stress conditions to induce degradation. Forced degradation studies were performed to evaluate the stability indicating properties and the specificity of the method. All solutions for use in stress studies were prepared at an initial concentration of 1mg/mland filtered before injection.

3.8.1 Acid degradation:

18.06mg of Powered drug of Rifaximin tablets was accurately weighed and was transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely. Acid decomposition was carried out for the above solution in 3ml of 1M HCL and neutralized with 3ml of 1M NaOH and make up the volume up to the mark (Stock solution-1000µg/mL). The concentration of 10µg/mL RFX solution was prepared from the above stock solution. The solution is kept aside for 3hrs filtered through 0.45µ filter and analyzed.

3.8.2 Alkali degradation:

Similarly stress studies in alkaline conditions were conducted using a concentration of 1000µg/mL of Rifaximin in 3ml of 1M NaOH and neutralized with 3ml of 1M HCL. The concentration of 10µg/mL RFX was prepared from the above stock solution. The solution is kept aside for 3hrs, filtered through 0.45µ filter and analyzed.

3.8.3 Oxidative degradation:

Solutions for oxidative stress studies were prepared using 3ml of H_2O_2 at a concentration of 1000µg/mL of RFX. The concentration of 10µg/mL RFX was prepared from the above stock solution. The solution is kept aside for 3hrs, filtered through 0.45µ filter and analyzed.

3.8.4 Photo Stability

The drug solution 10µg/mL for photo stability testing was exposed to UV light for 4 hours in UV light (365 nm) chamber and analyzed.

3.8.5 Thermal Degradation:

For thermal stress testing, the drug solution 10 µg/mL was heated in thermostat or hot air oven at 100 ºC for 1hr, cooled and used.

Table 1: Optimized Chromatographic Conditions

Column	C_{18} Symmetry (150 x4.6mm ,5µm)
Mobile phase ratio	Methanol :Phosphate buffer pH3(65:35%v/v)
Flow rate	1ml/min
Injection volume	10µl
Column temperature	Ambient
Auto sampler temperature	Ambient
Run time	8min
Retention time	2.262min

Table 2: Results of System suitability

S. No	Injection no	R_t	Area	Height	USP Plate count	USP Tailing
1	Injection 1	2.269	1187187	159416	2622.7	1.4
2	Injection 2	2.264	1188125	161793	2758.1	1.5
3	Injection 3	2.267	1189202	161854	2700.8	1.4
4	Injection 4	2.270	1191196	159246	2619.9	1.5
5	Injection 5	2.262	1192867	162665	2652.7	1.4
6	Injection 6	2.262	1192194	165295	2731.9	1.4
Mean			**1190129**		**2681**	**1.4**
S.D			**2299.13**			
%RSD			**0.2**			

Table 3: Over all Accuracy results (50%, 100%150%) for Rifaximin

% Concentration (at specification level)	Average Area	Amount added (mg)	%Recovery	Mean Recovery
50%(30ppm)	605972.6	5	100.68	100.30%
100%(60ppm)	1195448.5	10	99.31	
150%(90ppm)	1822550.8	15	100.93	

Table 4: Showing results of precision

S. No	Injection no	R_t	Area	Height	USP Plate count	USP Tailing
1	Injection 1	2.269	1187187	159416	2622.7	1.4
2	Injection 2	2.264	1188125	161793	2758.1	1.5
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4	Injection 4	2.270	1191196	159246	2619.9	1.5
5	Injection 5	2.262	1192867	162665	2652.7	1.4
6	Injection 6	2.262	1192194	165295	2731.9	1.4
Mean			**1190129**		**2681**	**1.4**
S.D			**2299.13**			
%RSD			**0.2**			

Table 5: Showing results of Linearity

S. No	Peak name & concn	RT	Area	Height	USP plate count	USP Tailing
1	Rifaximin (20ppm)	2.273	499288	67860	2741.8	1.4
2	Rifaximin (40ppm)	2.268	899423	119430	2712.8	1.4
3	Rifaximin (60ppm)	2.264	1289473	1162712	2630.6	1.4
4	Rifaximin (80ppm)	2.268	1654546	223361	2828.0	1.5
5	Rifaximin (100ppm)	2.262	1999367	278423	2896.2	1.5
Mean			**1268419.4**		**2761.9**	**1.4**
Std.dev			**594041.8**			
Co relation coefficient			**0.9991**			

Table 6: Showing results for Limit of Detection

Drug name	Base Line Noise	Signal Obtained	S/N Ratio
Rifaximin	40	123	3.07

Table 7: Showing results for Limit of Quantification

Drug name	Base Line Noise	Signal Obtained	S/N Ratio
Rifaximin	40	418	10.45

Table 8: Showing Robustness results of Rifaximin forchange in flow rate

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.9	3353	1.5
2	1.0	2848.8	1.4
3	1.1	2384	1.4

Table 9: Showing Summarized results of Forced degradation studies of Rifaximin

Stress conditions	Peak area	%Drug recovered	%Drug decomposed
Standard drug	1199561	100	-
Acid degradation	203852	83.01	16.99
Alkali degradation	458064	61.82	38.18
Oxidative degradation	313682	73.86	26.14
Photo Stability	143852	88.01	11.99
Thermal degradation	258064	78.49	21.51

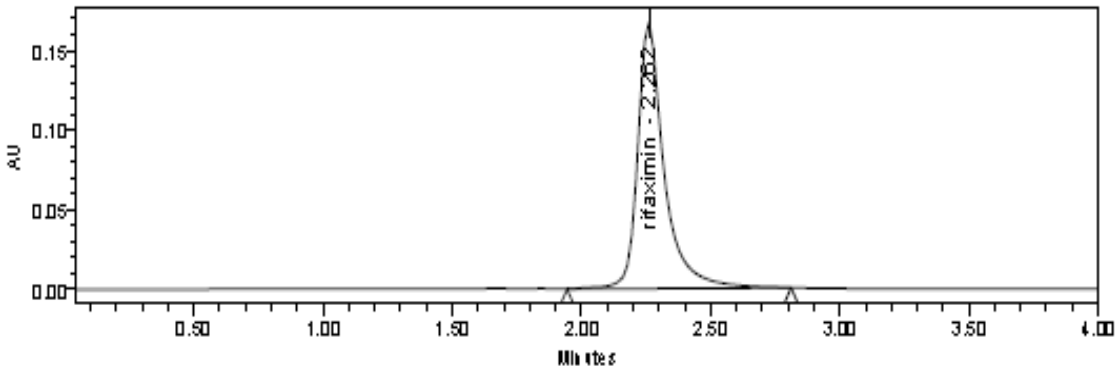

Figure 1: Chromatogram showing Assay of Standard injection

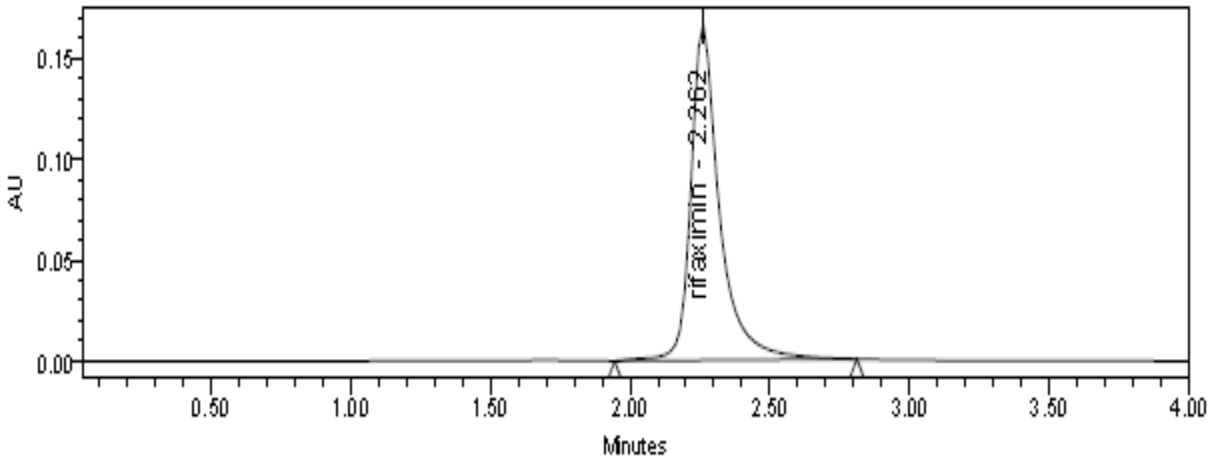

Figure 2: Chromatogram showing Assay of sample injection

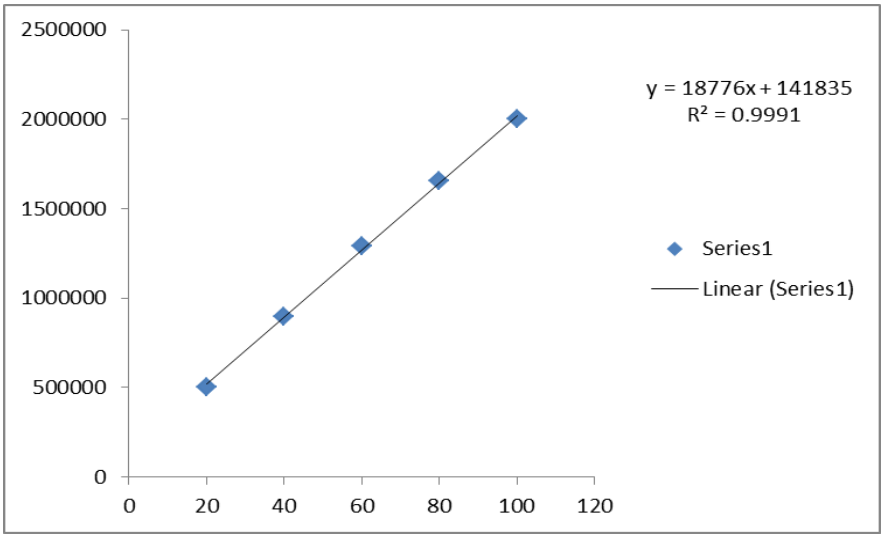

Fig 3: Showing Linearity curve for Rifaximin

4. CONCLUSION

A new stability-indicating method was established for the estimation of Rifaximin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Rifaximin by using SYMMETRY C18 column (150×4.6mm,5µ), flow rate was 1ml/min, mobile phase ratio was (65:35 v/v) methanol: phosphate buffer pH 3 (pH was adjusted with orthophosphoric acid),detection wave length was 454nm. The retention time was found to be 2.262 mins. The % purity of Rifaximin was found to be 99.27%.The system suitability parameters for Rifaximin such as theoretical plates and tailing factor were found to be 2848.8, 1.4. The linearity study for Rifaximinwas found in concentration range of 20-100µg/ml and correlation coefficient (r^2) was found to be 0.9991 as the drug peak elution did not interfere with any degradants during the forced degradation studies and therefore the proposed method can be successfully applied to perform long-term and accelerated stability studies of Rifaximin formulation.

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REFERENCES

1. Maryadele Neil JO.The Merck Index, An Encyclopedia of Chemicals Drug and Biologicals N Eds, 14th edition, Merck Research Laboratories, Division of Merck and Co. Inc., White house Station, NJ, 2006.
2. Gillis JC, BrogdenRN .Rifaximin: A review of its antibacterial activity, pharmacokinetic properties and therapeutic potential in conditions mediated by gastrointestinal bacteria, Drugs, 49 (3), 1995, 467-484.
3. Huang DB, Dupont HL. Rifaximin a novel antimicrobial for enteric infections, J. Infect., 2005, 50, 97–106.

4. Rao RN, Shinde DD, Agawane SB. Rapid determination of rifaximin in rat serum and urine by direct injection on to a shielded hydrophobic stationary phase by HPLC, Biomed. Chromatogram. 2009, 23 (6), 563-567.
5. Nageswara Rao R, Mastan Vali R, Dhananjay DS. On-line 2D-LC-ESI/MS/MS Determination ofrifaximin in rat serum, Biomed. Chromatogr, 2009, 23 (11), 1145-1150.
6. MaixiLiuchao, Wan Wangchaoa, Chunpeng. Impurity profile of rifaximin produced in China, Die Pharmazie, 2012, 67 (4), 283-287.
7. Bidyut Nanda, Amrutansu Panda, Mathrusri Annapurna M. Spectrophotometric estimation of rifaximin in pure and tablet dosage form, Int. J. Pharmacy & Tech. 2010,2 (4), 1098-1104.