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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF RIFAXIMIN IN BULK AND FORMULATION.

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Key words:-

Rifaximin, RP-HPLC, Validation, Forced Degradation studies and ICH guidelines.

Abstract

A reverse-phase high-performance liquid chromatographic method was developed and validated as per ICH guidelines for the determination of Rifaximin in bulk and formulation. The samples were analyzed by high-performance liquid chromatography (HPLC). Chromatographic separation was achieved on an inertsil C₁₈ (250 x 4.6 mm, packed with 5 μ) column using an aqueous sodium acetate buffer:Acetonitrile (60:40 v/v pH adjusted to 5.0 by using NaOH) with flow rate 1.0ml/min at ambient temperature and the retention time was about 3.489 minutes. UV detection was found at 293nm. Linearity was observed over the concentration range of 20-80 μ g/ml ($R^2 = 0.9997$). The LOD and LOQ were found to be 0.7129 μ g/ml and 2.160 μ g/ml respectively. Rifaximin was subjected to stress conditions of degradation in aqueous solutions including acidic, alkaline, oxidation and thermal degradation. The forced degradation studies were performed by using 0.1 M HCl, 0.1 M NaOH, 30% H₂O₂ and thermal at 100^oC. Rifaximin is more sensitive towards acidic conditions in comparison to oxidation and alkaline degradation. The method was validated as per ICH guidelines. The proposed method is simple, specific, selective, precise, linear, cost effective and rapid and is applicable to routine analysis of Rifaximin in bulk and pharmaceutical dosage forms.

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Introduction:-

Rifaximin is a product of synthesis of Rifamycin, an antibiotic with low gastrointestinal absorption and good antibacterial activity. Rifaximin is a semi synthetic non-systemic antibiotic works differently from other antibiotics because it passes through stomach and into intestines without being absorbed into blood stream (Sudha T and Saminathan J, 2013). Rifaximin is used to treat travellers' diarrhea caused by non-invasive strains of E. coli in adults and children (Sudha T et al., 2009). Rifaximin is also used to treat irritable bowel syndrome and to lower the risk of worsened brain function in adults with liver failure (Hepatic Encephalopathy) (Sandhya B et al., 2011). 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 (Kalpana GP et al., 2013). Rifaximin is benzimidazole derivative and chemically it is 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.

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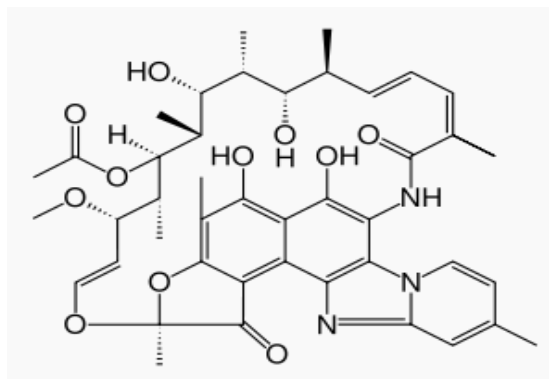


Fig 1:- Chemical Structure of Rifaximin.

Literature survey reveals few analytical methods were reported for the determination of Rifaximin in bulk and pharmaceutical preparations by RP-HPLC (Narendra A et al., 2013, Mathrusri Annapurna M et al., 2014 and Sunitha P et al., 2015). However the available methods have limitations such as a long runtimes, low sensitivity, uneconomical and have poor symmetry and poor resolution. Keeping in view of these, an attempt has been made to develop a simple, accurate, precise, linear, cost effective and reliable RP-HPLC method for the estimation of Rifaximin in bulk and pharmaceutical dosage forms. The established method was validated with respect to specificity, linearity, precision, accuracy, robustness, LOD and LOQ according to ICH guidelines.

Materials and methods:-

Chemicals and solutions:-

Rifaximin standard (purity $\geq 99.0\%$) and RCIFAX (Label claim: 200 mg), commercially available tablet dosage form was obtained as free sample from Lupin Pharmaceuticals Limited, Mumbai, India. Sodium acetate, Glacial acetic acid, HPLC grade water, Acetonitrile (HPLC grade), Sodium hydroxide (NaOH), Hydrochloric acid (HCl) and Hydrogen peroxide (H_2O_2) were obtained from Merck (India). All chemicals were of analytical grade and used as received.

HPLC instrumentation and conditions:-

Chromatographic separation was achieved by using Inertsil C_{18} (250 mm \times 4.6 mm, 5 μ) column of Waters Model-2690 with PDA detector. An automatic injector with 20 μ l of sample loop was equipped with the HPLC system. The HPLC system was equipped with "Empower 2" data acquisition software. The method was performed using Sodium acetate buffer and Acetonitrile (60:40 v/v) as a mobile phase. The overall run time was 7min, temperature was ambient and the flow rate was 1.0 ml/min was injected into the HPLC system.

Preparation of mobile phase:-

Accurately weighed 32.812 grams of Sodium acetate was taken in 2 liters volumetric flask then 12 ml of Glacial acetic acid was added to dissolve the substance. The volume was made up to mark with HPLC grade water. The pH of resulting solution was adjusted to 5.0 with NaOH. Then filtered through 0.45 μ membrane filter and then sonicated for degassed for 20 min. Acetonitrile was filtered through 0.45 μ membrane filter and then sonicated for degassed for 20 min. Finally, Sodium acetate buffer: Acetonitrile in the ratio of 60:40 v/v (pH 5.0, adjusted with NaOH) was used as mobile phase.

Preparation of stock and working standard solutions

Rifaximin stock solution (1000 μ g/ml) was prepared by accurately weighed quantity of 10 mg of Rifaximin in 10 ml volumetric flask and dissolved in DMSO and the solution made up to volume with mobile phase. Working solutions for HPLC injections were prepared from the stock solution. Solutions were filtered through a 0.45 μ membrane filter prior to injection.

Analysis of marketed formulation (Tablets):-

Twenty tablets from brand of RCIFAX marketed formulation were weighed individually and powdered to get a fine powder. Powder equivalent to 10 mg Rifaximin was accurately weighed and transferred in to a 10 ml volumetric flask and dissolved in small quantity of DMSO and then the solution is made up the volume with mobile phase.

Then the solution was sonicated for 20 mins and filtered through 0.45 μ membrane filter before injection. The filtrate obtained from the extracted marketed formulation was diluted as per the requirement and 20 μ l was injected in to the HPLC system for conducting the assay. Standard and sample solutions were injected into the chromatographic system and recorded the chromatograms, peak areas for the Rifaximin and the peak areas were used for calculating the % assay of Rifaximin.

Selection of detection wavelength:-

Weighed quantity of Rifaximin was dissolved in DMSO and the resulting solution was scanned in UV spectroscopy in the range of 200-400 nm. The absorption maximum of Rifaximin was found to be 293 nm.

Method validation (ICH guidelines, 2005):-

The method was validated according to ICH guidelines for the following parameters:

Precision:-**Repeatability:-**

Method precision: Six sample preparations were prepared individually as per test method and injected in to HPLC.

Intermediate precision (analyst to analyst variability):-

This study was conducted by two analysts as per test method. The test solution was prepared and injected into the HPLC by two different analysts and the precision study was performed. Results are tabulated in Table 2.

Accuracy (Recovery):-

A study of Accuracy was performed in triplicate as per test method with equivalent amount of Rifaximin into each volumetric flask for each spike level to get the concentration of Rifaximin equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Rifaximin was calculated. Results are tabulated in table 2.

Specificity:-

Solutions of standard and sample were prepared as per the test method and are injected into the chromatographic system. Then the retention times of two chromatograms were compared with each other.

Linearity of test Method:-

A Series of solutions were prepared using Rifaximin working standard at concentration range from 20 μ g/ml to 80 μ g/ml of target concentration. The prepared concentrations were injected into the HPLC and peak areas were measured. Then the calibration curve was plotted by taking concentration on X-axis and peak area on Y-axis shown in Fig 3. From the linearity data the limit of detection (LOD) and limit of quantitation (LOQ) was calculated.

Robustness:-

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method and was injected into the HPLC system. Results are tabulated in table 4.

Ruggedness of Test Method:-

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and injected into the HPLC and each was analyzed as per test method.

System Suitability:-

A Standard solution was prepared by using Rifaximin working standard as per test method and was injected five times into the HPLC system.

Forced degradation studies (ICH guidelines, 2003):-

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the developed method. All solutions used in stress conditions were prepared at an initial concentration of 1 mg/ml of Rifaximin and refluxed at 60 $^{\circ}$ C for 4 hrs. Then all sample solutions were diluted with mobile phase to get a final concentration of 10 μ g/ml and filtered before injection (Shete S et al., 2014).

Acid and alkali degradation studies:-

Acid decomposition was carried out in 0.1 M HCl at a concentration of 10 μ g/ml of Rifaximin and after refluxation at 60 °C for 4 hrs in thermostat the stressed sample was cooled at room temperature, neutralized and diluted with mobile phase. Similarly stress studies in alkaline conditions were conducted using a concentration of 10 μ g/ml in 0.1 M NaOH and refluxed at 60°C for 4 hrs in thermostat and cooled at room temperature. After cooling the solution was neutralized and diluted with mobile phase.

Oxidative degradation studies:-

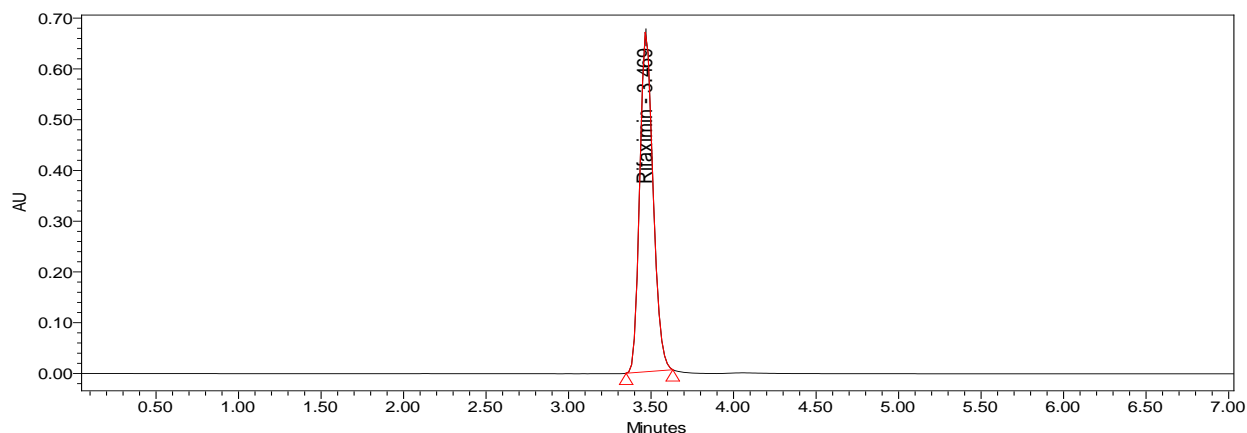
Forced degradation studies in oxidative conditions was performed by the addition of an aliquot of stock solution (1 mg/ml) of Rifaximin was added to 10 ml of 30% H₂O₂ solution and the mixture was refluxed at 60°C for approximately 4 hours. The solution was kept aside for few min to reach the room temperature and diluted to 100 ml with the mobile phase, so as to get a final concentration of 10 μ g/ml.

Thermal degradation:-

Accurately weighed 50 mg of Rifaximin drug and was stored at 100°C in a hot air oven for 24 hours. Then it was dissolved in small quantity of DMSO and transferred into a 50 ml volumetric flask and the volume was adjusted to 50 ml with the mobile phase. The above solution was further diluted with the mobile phase, to get a solution of final concentration equivalent to 10 μ g/ml of Rifaximin. Results are tabulated in Table 4.

Results and Discussion:-**Table 1:-** The optimised chromatographic conditions.

Parameters	Method
Stationary phase (column)	Inertsil -ODS C ₁₈ (250 x 4.6 mm, packed with 5 micron)
Mobile Phase	Sodium Acetate Buffer : Acetonitrile (60:40 v/v)
Ionic strength of buffer	0.2M
pH	5
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	7
Column temperature (°C)	Ambient
Volume of injection loop (μ l)	20
Detection wavelength (nm)	293nm
Drug RT (min)	3.469

**Fig 2:-** Representative chromatogram of Rifaximin peak in optimized mobile phase

Intermediate Precision:-**Table 2:-** Data of Intermediate precision (b/w Analyst-1 and Analyst-2) for Rifaximin.

No. of Injections	Concentration ($\mu\text{g/ml}$)	Peak area of Rifaximin	
		Analyst-1	Analyst-2
1	40	1257189	1236712
2		1241965	1265245
3		1253298	1259876
4		1266242	1258124
5		1249726	1241638
6		1251933	1244419
Mean peak area		1253392	1251002
Mean		1252197	
S.D		1689.985	
% RSD		0.1349616	

Intermediate precision was performed by two analysts. Mean, SD and % RSD values were calculated. The % RSD was found to be within the limits and the developed method was found to be precise Table 2.

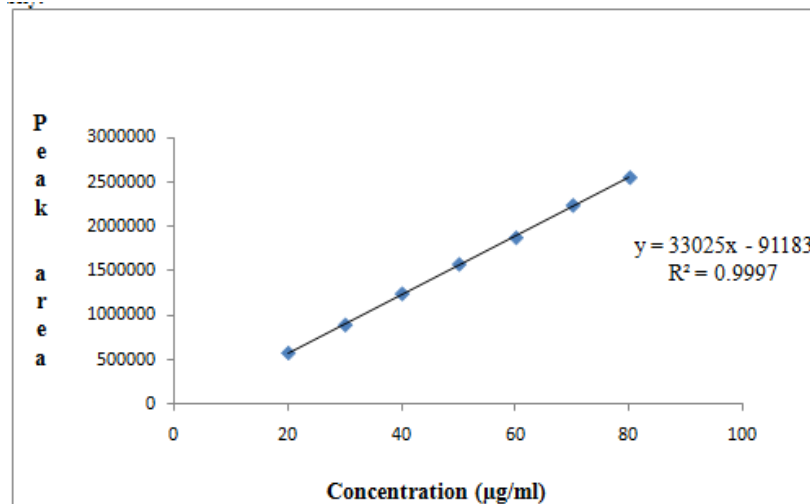
Accuracy (Recovery)**Table 3:-** Data of Accuracy studies for Rifaximin.

Concentration % of spiked level	Amount added ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$) n=3			Statistical Analysis of % Recovery		
		1	2	3	Mean of % Recovery	SD of % recovery	%RSD
50%	20	19.95	19.86	20.08	99.81	0.5530	0.5540
100%	40	40.14	39.96	39.80	99.91	0.4252	0.4255
150%	60	59.89	60.04	60.09	100.007	0.1761	0.1760

The mean % recovery of the Rifaximin at each spike level should be not less than 98.0% and not more than 102.0% as per the acceptable limit, hence the obtained datas at 50%, 100% and 150% levels were within the acceptable limit.

Specificity:-

The specificity study for Rifaximin was performed. The chromatograms of Standard and Sample were found to be identical with near retention time and the developed method was found to be specific.

Linearity:-**Fig 3:-** Linearity curve of Rifaximin (Concentration Vs Peak area).

Linearity studies were performed. A calibration curve was plotted. The calibration curve was linear with slope, y-intercept and correlation coefficient were found to be 33025, 91183 and 0.999 respectively. From the linearity curve the LOD and LOQ are calculated as 0.7129 $\mu\text{g/ml}$ and 2.160 $\mu\text{g/ml}$ respectively.

Robustness:-

Table 4:- Data for Effect of variation in flow rate for Rifaximin.

No. of Injections	Flow rate 0.8 ml/min		Flow rate 1.0 ml/min		Flow rate 1.2 ml/min	
	Std area	Tailing Factor	Std area	Tailing Factor	Std area	Tailing Factor
1	1239361	1.133372	1239678	1.146235	1249381	1.129133
2	1243411	1.164125	1243389	1.129133	1254986	1.159150
3	1237979	1.123397	1264984	1.159150	1247372	1.141469
4	1246482	1.125612	1248352	1.141469	1256493	1.130372
5	1241537	1.123857	1248352	1.130372	1239664	1.133372
Mean peak area	1241754	1.134073	1248951	1.141272	1249579	1.138699
Mean	B/w flow rate of 0.8ml/min and 1ml/min			B/w flow rate of 1ml/min and 1.2ml/min		
	1245353			1249265		
SD	5089.048			444.2045		
% RSD	0.4086			0.03555		

The Tailing Factor and % RSD of Rifaximin standards should be NMT 2.0 for Variation in Flow rate. Robustness studies were performed for effect of variation in flow rate for Rifaximin drug at different flow rates like 0.8ml/min, 1ml/min and 1.2ml/min. The tailing factor for Rifaximin and % RSD were found to be within the limits.

Ruggedness:-

The ruggedness (system to system variability) studies were performed for Rifaximin drug by two systems. Six replicates were prepared as per test method and measured in HPLC. Then the mean, SD and % RSD were calculated. The % RSD value of Rifaximin was found to be 0.2461 which is within the limits.

System Suitability Studies:-

The system suitability parameters were evaluated from standard chromatograms. The number of theoretical plates (NTM 3000), tailing factor (NMT 2) and % RSD of retention times, peak areas, theoretical plate count and tailing factor were found to be 0.0632, 0.5251, 1.6275 and 1.5231 respectively which are within the limits.

Assay:-

The % assay of the Rifaximin drug was found to be 100.72 % which was found to be within the acceptable limits (98-102%).

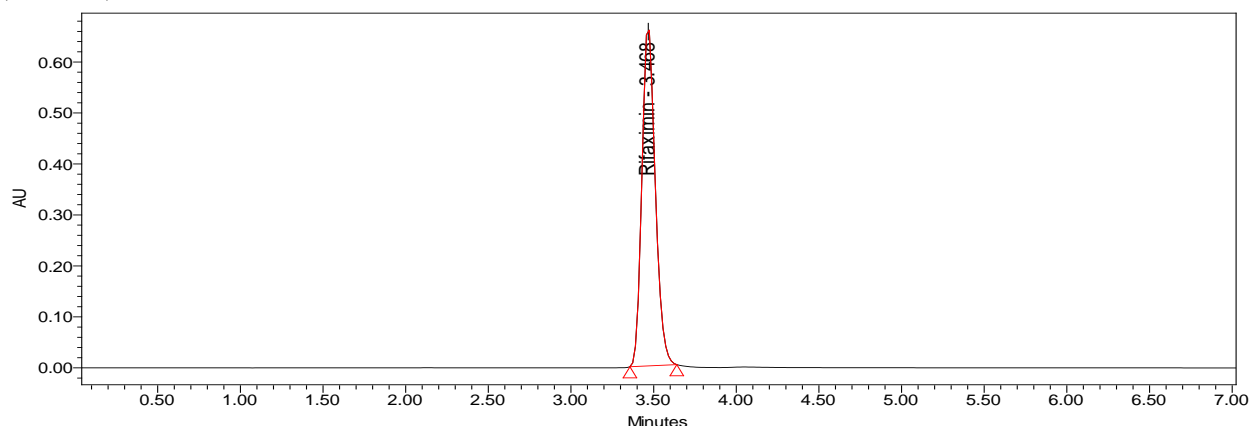
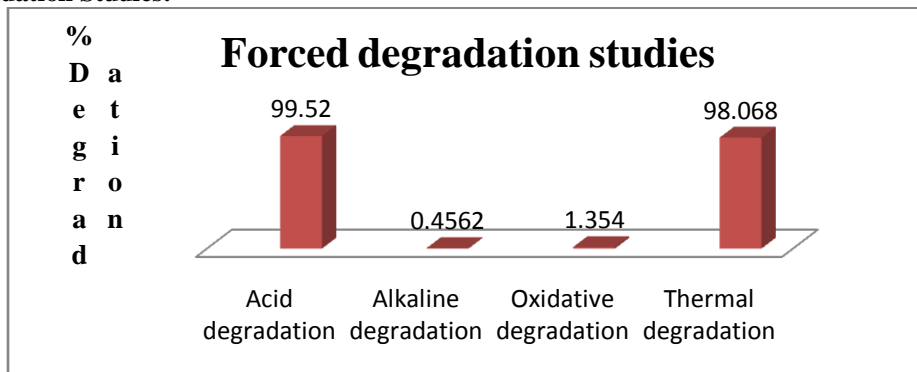


Fig 4:- Representative chromatogram of Rifaximin for assay.

Forced Degradation Studies:-**Fig 5:-** Forced Degradation studies for Rifaximin.

Forced degradation studies were performed for the Rifaximin drug under various stress conditions. Degradation of Rifaximin drug in acidic condition is 99.52 %. Degradation of Rifaximin drug in alkaline condition is 0.4562 %. Degradation of Rifaximin drug in oxidative condition is 1.3540 %. Degradation of Rifaximin drug in thermal condition is 98.068 %. The amount of drug degradation was found to be more in acidic condition. The drug was stable in alkaline condition.

Conclusion:-

A new stability indicating method was established for the estimation of Rifaximin by RP-HPLC method. The developed and validated RP-HPLC method was found to be simple, specific, precise, accurate, linear and cost effective for estimation of Rifaximin in bulk and formulations. The developed method was validated as per the ICH guidelines and the results obtained were well within the limits. Forced degradation study of Rifaximin shown that the amount of drug degradation was found to be more in acidic medium (99.52%) and the drug was found to be more stable in alkaline medium (0.4562%). The statistical analysis of the developed method confirms minimal deviation and all the validation parameter was well within the specified range. Hence the developed method can be successfully applied for analysis of Rifaximin in bulk and formulation.

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Conflict of interest statement:-

The authors hereby declare that there are no conflicts of interest in the proposed article.

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