Available Online at

http://www.ijcpa.in

April-June 2017

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

DOI : http://dx.doi.org/10.21276/ijcpa

Research Article | Volume-4 | Issue-3 | Article ID: 1208

FORCED DEGRADATION STUDY OF RIFAXIMIN FORMULATED TABLETS TO DETERMINE STABILITY INDICATING NATURE OF HPLC METHOD

Abid Fida Masih[1]

[1]Brookes Pharma (Private) Limited, Karachi, Pakistan
[1]Department of Chemistry, University of Karachi, Pakistan

*Corresponding Author: Email: brookespharma.com

Received: 23 December 2016 / Revised: 31 May 2017 / Accepted: 25 June 2017 / Available online : 29 June 2017

ABSTRACT

Forced degradation study of Rifaximin was conducted to determine the stability indicating potential of HPLC testing method for detection of Rifaximin in formulated tablets to br employed for quality control and stability testing. The questioned method applied with mobile phase methanol: water (70:30), 5µ, 250 x 4.6mm, C18 column, wavelength 293nm and flow rate of 1.0 ml/min. Forced degradation of study was performed under oxidative, acidic, basic, thermal and photolytic conditions. The applied method successfully determined the degradation products after acidic and basic degradation without interfering with Rifaximin detection. Therefore, the method was said to be stability indicating and can be applied for quality control and stability testing of Rifaxmin tablets during its shelf life.

Keywords – Rifaximin, Forced Degradation, Method Validation, High Performance Liquid Chromatography (HPLC).

1. INTRODUCTION

This work presents the forced degradation study of Rifaximin Tablets to determine the stability indicating nature of proposed analytical testing method under more stress conditions than accelerated stability conditions namely acid/base hydrolysis, heat, oxidation and light. Stability indicating method is a necessary requirement for both quality control and stability testing of pharmaceutical products.

According to various pharmaceutical guidelines related to stability of pharmaceutical product, a stability indicating method is one that has the potential to determine the changes in the related properties of a drug substance or drug product over the period of time. A stability indicating method can determine the main component without any interference from different kind of impurities or degradation products.

To develop stability indicating method a drug substance or drug product is exposed to various environmental conditions more severe than normal stability conditions. This process is called forced degradation or stress testing. Generally, stress conditions are light, humidity, heat and acid/base hydrolysis. After stress conditions, the drug substance or drug product used to validate the stability indicating property of proposed analytical method. If method is able to detect any loss in content of drug substance or drug product

and increase in degradation products, the method is said to be stability indicating method and can use for stability study of drug samples[1-5].

2. MATERIALS AND METHODS

Rifaximin working standard and formulated Rifaximin tablets were supplied by Brookes Pharma (Private) Limited. Methanol from Fischer Scientific was used in this study. Sodium hydroxide and hydrogen peroxide were used from Merck. Hydrochloric acid and potassium di hydrogen phosphate were used from Riedel- de Haen. All chemical were of analytical grade except methanol which was of HPLC grade. Purified water was used to prepare all solutions and samples.

2.1. Instrumental Conditions

HPLC chromatograph: Perkin Elmer Series 200 with auto sampler and UV/VIS detector

Software: Total Chrom Navigator

Column: Phenomenex, Gemini 5µ, 250 x 4.6mm, C18

Wavelength: 293nm

Mobile phase: Methanol: phosphate buffer previously adjusted pH 3.0 (70:30)

Flow rate: 1.0 ml/min

Temperature: Ambient

Injection volume: 20µl

Filter paper: 0.45µm

2.2 Preparation of Stock and Standard Solution

Stock solution of Rifaximin was prepared by dissolving accurately weighed 30 mg of Rifaximin in to a 100ml volumetric flask with small quantity of HPLC grade methanol and then diluted up to the mark with same solvent. This solution further diluted by pipette 10 ml stock solutions to 100 ml volumetric flask and diluted up to the mark with HPLC grade methanol having final concentration of 30 µg/ml of Rifaximin.

2.3. Calibration of Standards

Calibration curve was obtained by Rifaximin standard solutions in the range of 13.75µg/ml to 41.2 5µg/ml. Calibration curve was shown in Fig.1. Different concentrations in the range of 13.75 µg/ml to 41.25 µg/ml of Rifaximin were prepared by taking different volumes of stock solutions to different final volumes.

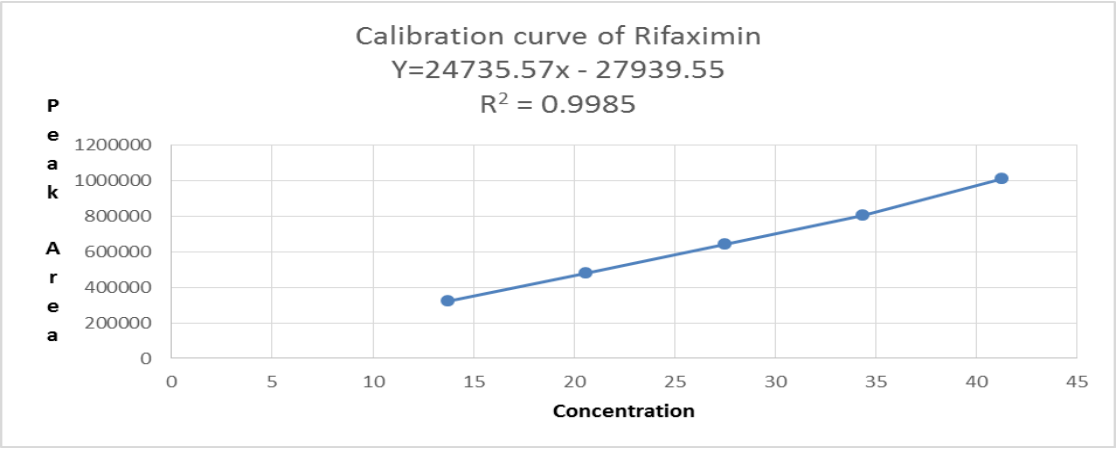

Fig. 1. Calibration curve of Rifaximin

3. METHOD VALIDATION

3.1. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of the analyte in samples within a given range. The calibration curve constructed for Rifaximin were linear over the concentration range of 13.75 - 41.25 µg/ml. Peak areas of Rifaximin were plotted versus concentration and linear regression analysis performed on the resultant curve. The correlation coefficient of 0.9598 was obtained following linear regression analysis as shown in table 1.

Table 1. Linearity of Rifaximin

Conc. (µg/ml)	Area Mean (n=2)	Linearity (µg/ml)	Slope	Intercept	Linearity Equation	Correlation Coefficient
13.750	323367.00	13.75-41.25	24735.57	-27939.55	y = 24735.57x – 27939.55	0.9985
20.625	480370.00					
27.500	640577.00					
34.375	806584.75					
41.250	1010545.00					

3.2. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy was investigated by injecting three replicate samples of each of the 13.75, 27.5 and 41.25 µg/ml at 50%, 100% and 150% respectively. The accuracy of method was shown in table 2.

Table 2: Accuracy of Rifaximin

Accuracy Level	Theoretical Conc. (µg/ml)	Practical Conc. (µg/ml)	Observed Conc. (µg/ml)	% Accuracy	Average	SD	%RSD
50%	13.75	13.88	14.05	101.22	101.58	0.4005	0.3942
		13.97	14.18	101.5			
		13.9	14.18	102.01			
100%	27.5	27.7	28.12	101.52	101.13	0.3353	0.3316
		27.76	28.02	100.94			
		27.83	28.09	100.93			
150%	41.25	43.48	43.58	100.23	100.49	0.863	0.8587
		43.21	43.84	101.46			
		43.62	43.53	99.79			

3.3. Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samples of a homogeneous sample. The repeatability was investigated by injecting 6 replicates of 27.5 µg/ml concentration of tablet samples. Similarly intraday precession was assessed by injecting three injections of tablet sample of 27.5 µg/ml of Rifaximin at three different times of the day. Interday precession was observed by injecting 5 replicates of 27.5 µg/ml of Rifaximin on different days. The precesion results were shown in table 3.

Table 3. Precision of Rifaximin

Sample No.	Conc. (µg/ml)	Peak Area of Sample	Average of Area	SD	% RSD
Repeatability					
1	27.5	749405.00	751107.42	1850.29	0.2463
2	27.5	750599.00			
3	27.5	749205.00			
4	27.5	750772.00			
5	27.5	752923.00			
6	27.5	753740.50			
Intraday Precision					
7	27.5	732428.5	733669.50	1189.61	0.1621
8	27.5	733780			
9	27.5	734800			
Interday Precision					
1	27.5	671058.00	666478.50	4664.54	0.6999
2	27.5	671243.00			
3	27.5	671098.00			
4	27.5	669893.00			
5	27.5	662498.00			
6	27.5	662425.00			

3.4. LOD and LOQ

LOD nad LOQ of testing method were obtained by straight line equation using standard solution having Rifaximin concentrations of 13.75, 27.5 and 41.25 µg/ml at 50%, 100% and 150% respectively**.** The LOD and LOQ were shown in Fig. 2 and table 4.

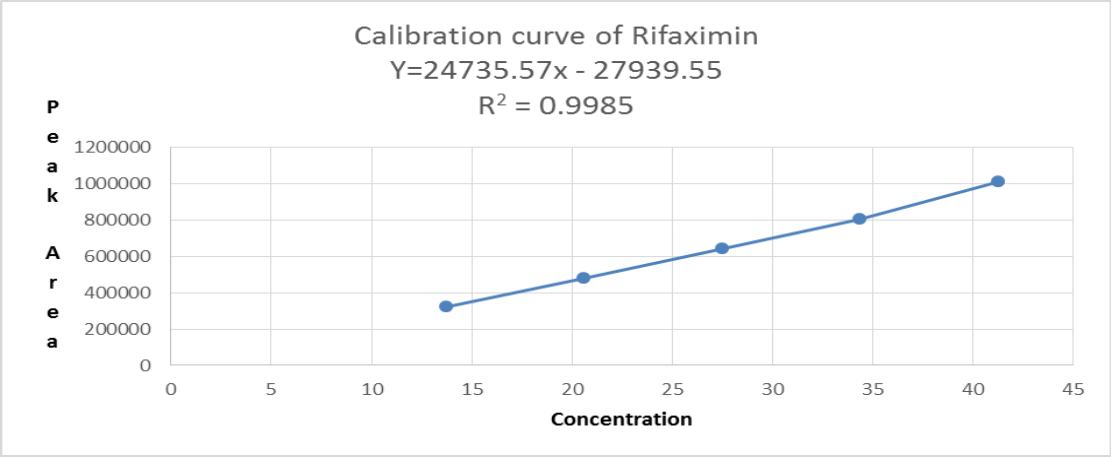

Fig. 2. LOD and LOQ by Linear Equation

Table 4: LOD and LOQ of Rifaximin

Conc. (µg/ml)	Peak Area of Sample	Average of Area	SD	% RSD	Variance	Slope	Intercept	LOD (µg/ml)	LOQ (µg/ml)
13.75	320868.00	323713.00	2569.85	0.7939	460076.69	25001.62	-28723.56	0.339	1.028
	325866.00								
	324405.00								
27.5	641075.00	641492.67	1662.33	0.2591					
	640079.00								
	643324.00								
41.25	1010690.00	1011257.67	1242.86	0.1229					
	1010400.00								
	1012683.00								

Robustness of the method was determined by deliberately changing the flow rate from 1 ml to 1.3 ml using tablet sample of 27.5 µg/ml of Rifaximin. The robustness of method was shown in table 5

Table 5. Robustness of Rifaximin

Conc. (µg/ml)	Parameters	Peak Area of Sample	Average of Area	SD	% RSD
30 µg/ml	Flow Rate: 1.0ml/min	793699.00	796935.40	2346.88	0.2945
		795172.50			
		798705.00			
		798776.50			
		798324.00			
30µg/ml	Flow Rate: 1.3ml/min	658557.00	664394.40	3436.69	0.5173
		664816.50			
		667610.50			
		665832.00			
		665156.00			

3.6. Selectivity

The specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. As degradation products after forced degradation study not interfered with Rifaximin peak t,he method was said to be selective to Rifaximin even in the presence of degradation products.

3.7. Assay of Formulated Rifaximin Tablets

Transferred accurately weighed quantity of pulverized formulated Rifaximin tablets equivalent to one tablet (550mg of Rifaximin) in to 100ml volumetric flask, dissolved and diluted with HPLC grade methanol. Filtered the sample with whattman filter paper, discarded first 10 ml and then diluted 5ml of filtrate to 20ml with HPLC grade methanol. Further Diluted 2ml of this solution to 100ml with HPLC grade methanol having final concentration of 27.5 µg/ml. This solution was filtered using 0.45µm and 20µl was injected in to chromatograph using instrumental conditions mentioned above and chromatogram was recorded and peak area was measured. Chromatogram is shown in Fig. 3.

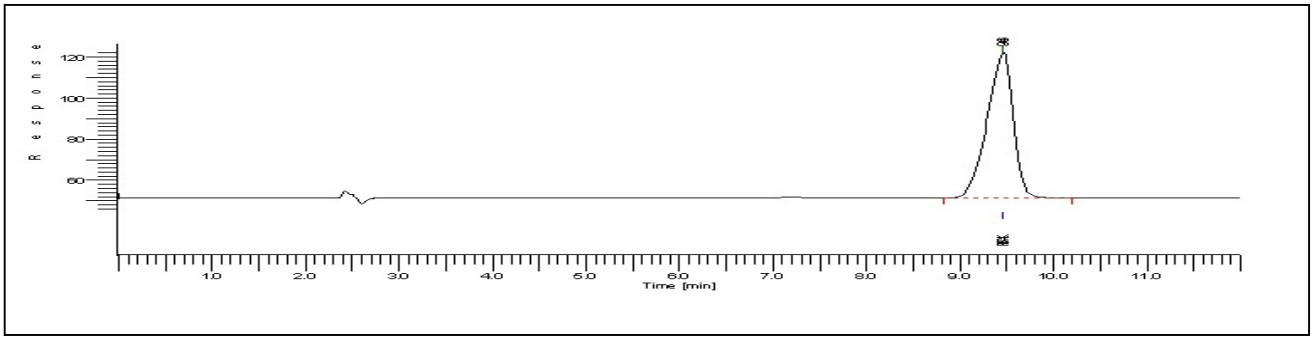

Fig. 3. Chromatogram of Rifaximin standard

4. FORCED DEGRADATION

4.1. Oxidative Degradation

Solutions for oxidative degradation were prepared by dissolving pulverized tablets sample first in HPLC grade methanol and then dilute this solution in 2% H_2O_2 at neutral pH and stored at 25°C for 24 hours. To avoid photo degradation amber glass vials were used for sample preparation. To determine initial concentration of Rifaximin this solution was further diluted in HPLC grade methanol for HPLC analysis as per instrumental conditions having final concentration of Rifaximin of approximately 27.5 µg/ml. After twenty four hour

same procedure was repeated to determine any degradation of Rifaximin. Chromatograms of oxidative degradation were shown in Fig. 4 and 5.

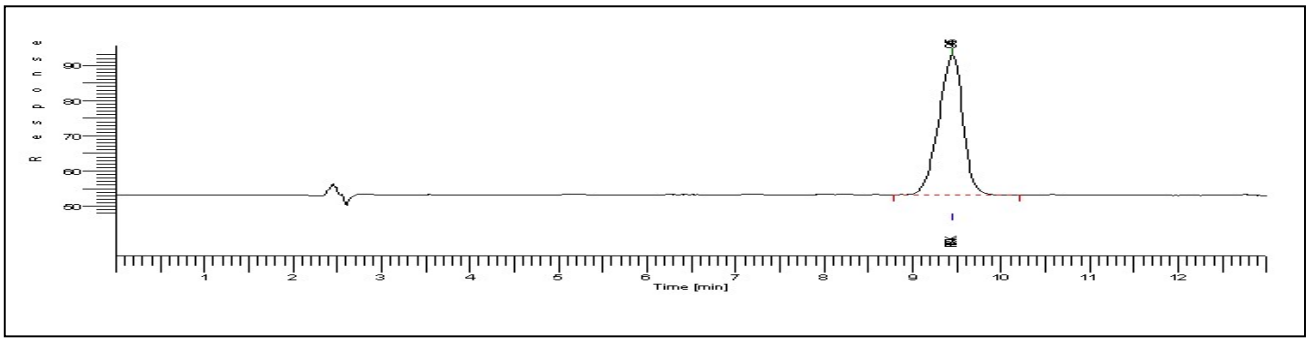

Fig. 4. Chromatogram of Rifaximin tablet (27.5µg/ml) oxidative degradation (initial)

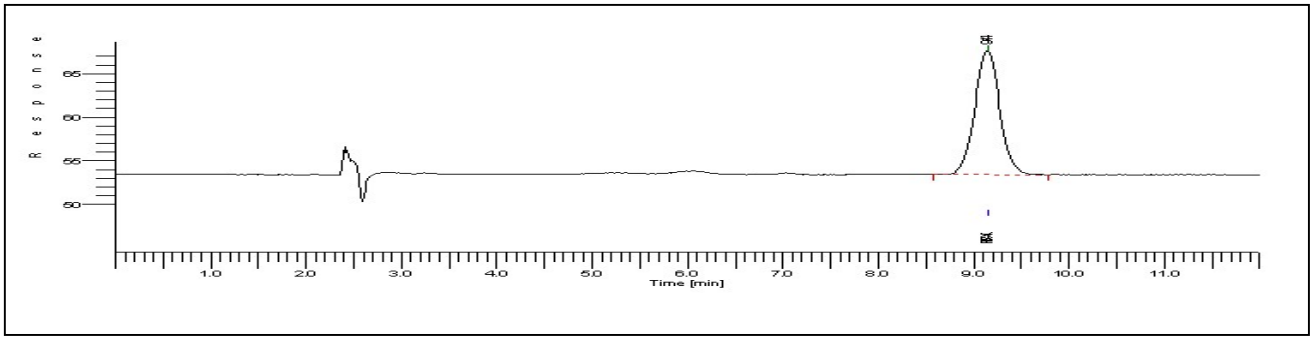

Fig. 5. Chromatogram of Rifaximin tablet (27.5µg/ml) oxidative degradation after 24 hours

4.2. Acid Degradation

Solutions for acid degradation were prepared by dissolving pulverized tablets sample first in methanol and then dilute this solution in HCl solution with pH of 1.3. Stored resulting solution at 25°C in amber glass flask for two weeks. After predefined testing interval this solution was further diluted in HPLC grade methanol for HPLC analysis as per instrumental conditions having final concentration of Rifaximin of approximately 27.5nµg/ml. Chromatograms of oxidative degradation were shown in Fig.6, 7 and 8.

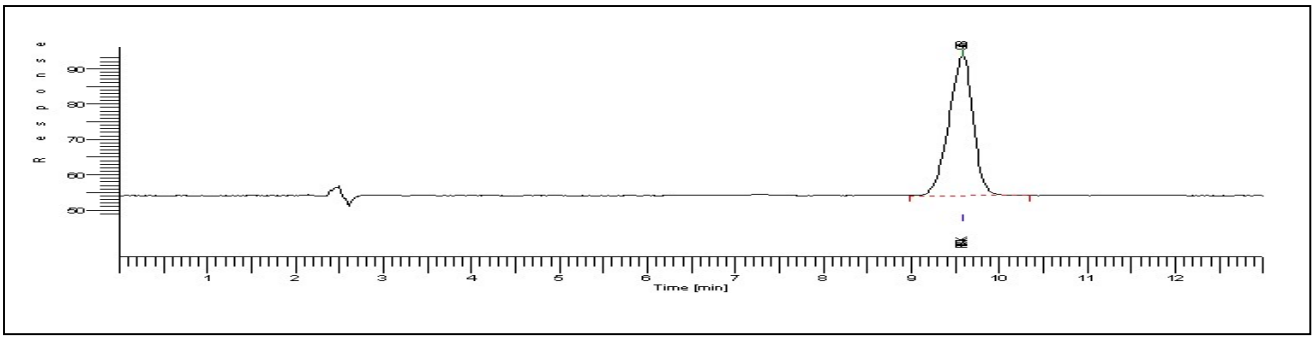

Fig. 6. Chromatogram of Rifaximin tablet (27.5µg/ml) acid degradation at 0 time

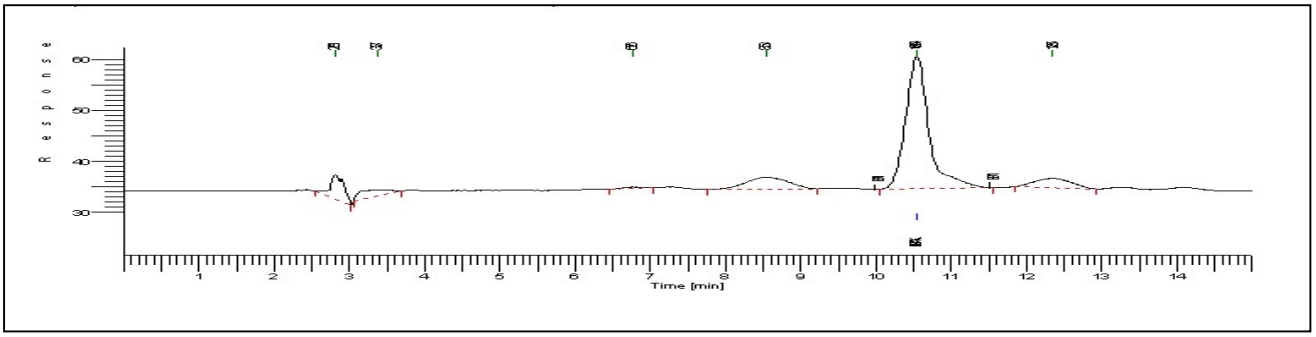

Fig. 7. Chromatogram of Rifaximin tablet (27.5µg/ml) acid degradation after

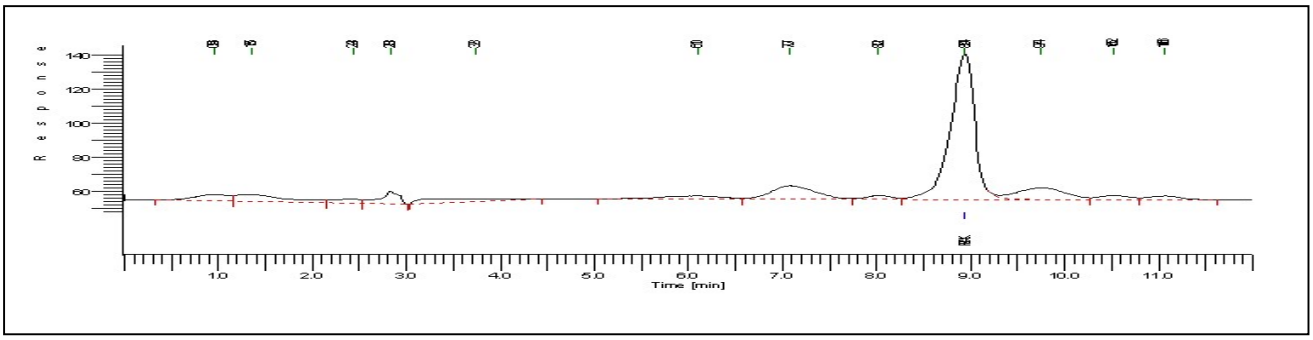

Fig. 8. Chromatogram of Rifaximin tablet (27.5µg/ml) acid degradation after

4.3. Basic Degradation

Solutions for basic degradation were prepared by dissolving pulverized tablets sample first in HPLC grade methanol and then diludte this solution in NaOH solution with pH of 12. Resulting solution was stored at 25°C in amber glass flask for two weeks. After predefined testing interval this solution was further diluted in HPLC grade methanol for HPLC analysis as per instrumental conditions having final concentration of Rifaximin of approximately 27.5 µg/ml. Chromatograms of oxidative degradation were shown in Fig. 9-12.

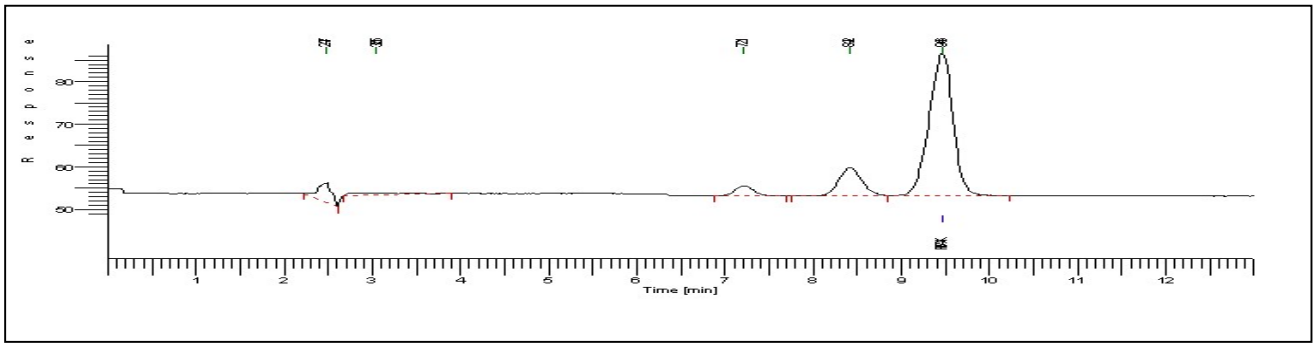

Fig.9. Chromatogram of Rifaximin tablet (27.5µg/ml) basic degradation after half hours

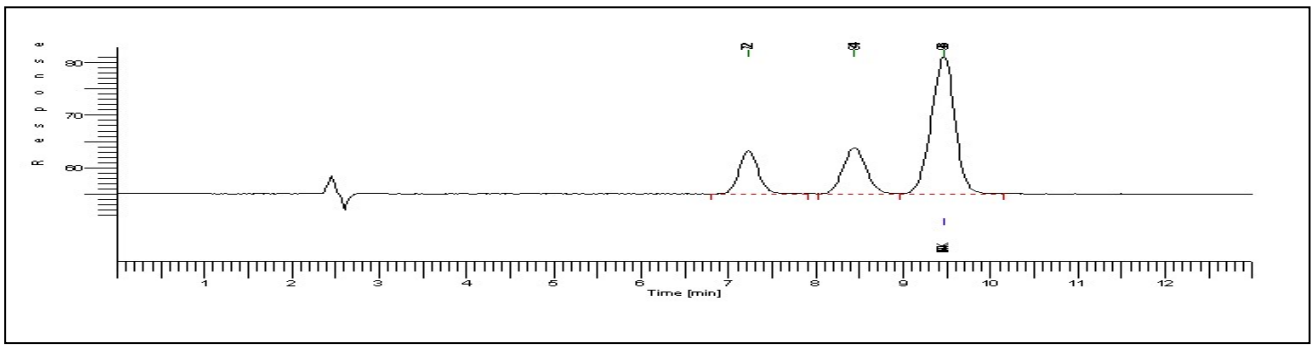

Fig. 10. Chromatogram of Rifaximin tablet (27.5µg/ml) basic degradation after one hours

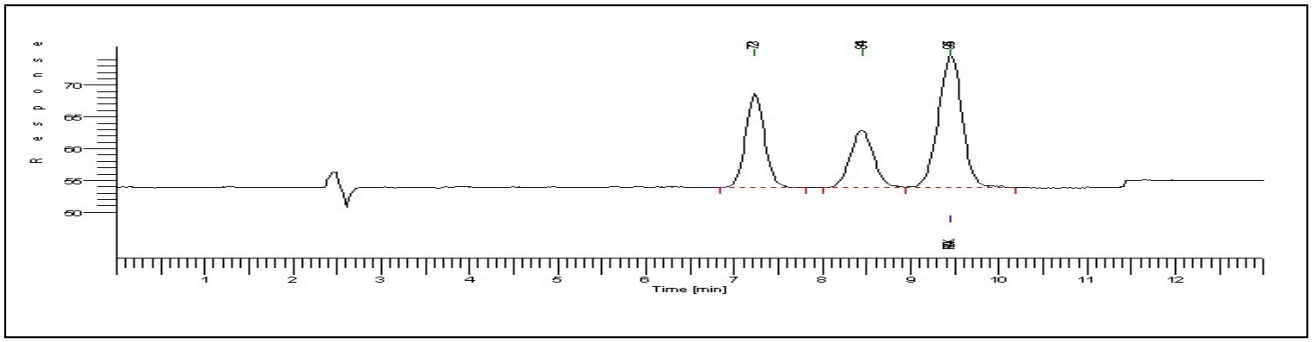

Fig. 11. Chromatogram of Rifaximin tablet (27.5µg/ml) basic degradation after 1.5 hours

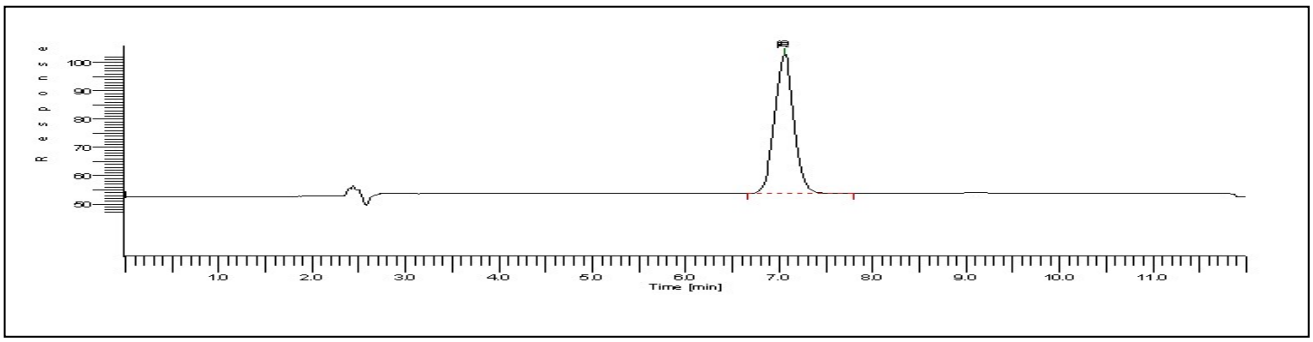

Fig. 12. Chromatogram of Rifaximin tablet (27.5µg/ml) basic degradation after five hours

4.4. Thermal Degradation

Tablets were exposed to dry heat (50 ^{0}C) in an oven for 3 months. The tablets were removed from after specified time interval till 3 months from the oven and treated them as per testing procedure defined in assay of formulated tablets.

4.5. Photo Degradation

Rifaximin tablets were exposed directly, in blisters and in pack to different light sources, to determine the effect of light irradiation on the stability of Rifaximin in the tablets. The tablet samples were exposed to cool light, laboratory light and direct sunlight. Following removal from the light source, all samples were prepared for analysis as testing procedure of assay of formulated tablets.

5. RESULTS AND DISCUSSION

The primary objective of present work is to conduct forced degradation study of Rifaximin in formulated product to evaluate the stability indicating nature of testing method for determination of Rifaximin in formulated products. The HPLC separation was performed using mobile phase methanol: phosphate buffer (70:30) with adjusted pH of 3.0. The detection on chromatograph was carried out at 293 nm. Before performing analysis of degradation samples, the method was validated as per ICH guidelines. Method was linear with respect to concentration and shown by regression as well. Linearity graph and regression equation were shown in Fig. 1 and table 1 respectively. Method was accurate within the range of 99.79% to 102.01%. Method was also précised with respect to repeatability, intraday and inter day precision with 0.2463, 0.1621 and 0.6999 %R.S.D as shown in table 3. LOD and LOQ of Rifaximin were 0.339 µg/ml and 1.028 µg/ml respectively. Robustness of method was obtained by deliberately altering flow rate from 1 ml/min. to 1.3 ml/min. Results of robustness was shown in table 5. Method was selective as well as there was no interference of excepient and degradation products in the determination of Rifaximin. Forced degradation study was conducted under different conditions and method successfully separated all degradation products without affecting Rifaximin peak. The most significant and interesting degradation was observed in basic conditions. Degradation of Rifaximin was initiated immediately with the formation of two degradation products. Rifaximin was first degraded to degradation product which led to the formation of product B. Hence, degradation of Rifaximin, formation of degradation product A and degradation product B took place simultaneously. This process continued until all the Rifaximin degraded in to A which further degraded in to B. After about five hours all Rifaximin and degradation product A degraded in to degradation product B. This degradation process was clearly depicted in Fig. 10-13. Degradation products also detected in acidic degradation but they were not as significant as in basic degradation. Rifaximin also underwent degradation under oxidative conditions. Rifaximin was stable under thermal and photolytic degradation.

Table 6. Summary of Method

Parameter	Values for Rifaximin	Remarks
Linearity (µg/ml)	13.75 - 41.25	Linear
% Accuracy (%)	99.79 - 102.01	Accurate (97% - 103%
Precision (%RSD)		Precise (%RSD less than 2%)
Repeatability	0.2463	
Intraday	0.1621	
Interday	0.6999	
LOD (µg/ml)	0.339	-
LOQ (µg/ml)	1.028	-
Robustness	Robust	Robust (No difference in results)

Table 7. Stability of Rifaximin

Condition	Optimized forced degradation	%Degradation	No. of Degradation products
Acidic	HCl pH 1.3 at 25^{0}C for two weeks	38.44	4
Alkaline	NaOH pH 12 at 25^{0}C for two weeks	) %100within six hours(	2
Oxidative	%2H_2O_2 at 25^{O}C for 24 hours	62.6	_
Thermal	At 50^{0}C for 3 months	_	_
Photolytic	Cool light	_	_
	Lab. Light	_	_
	Sunlight	_	_

6. CONCLUSION

Forced degradation study of Rifaximin formed degradation products under basic and acidic conditions which were successfully separated by employed testing method without interfering with Rifaximin determination. Therefore, this method was said to be stability indicating method and can be used for stability study of Rifaximin tablets for quality control purpose and during shelf life of product.

7. ACKNOWLEDGEMENT

I am really thankful to Brookes Pharma (Private) Limited to provide standard and tablet sample of Rifaxmin as gift. I also want to say thank to management of Brookes Pharma (Private) Limited to provide their facility to conduct this work.

8. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. ICH Q1B, Photostability testing of new drug substances and products, 1996.
2. ICH Q1C, Stability testing of new dosage forms, 2003.
3. ICH Q2(R1) , Validation of analytical procedures, 2005.
4. Vamsi Krishna Marothun, Rajendra N.Dash, SarithaVemula, Shravani Donkena, RameshDevi, Madhavi Gorrepati, et al, Kinetics study of metaxalone degradation under hydrolytic, oxidative and thermal stress conditions using stability- indicating HPLC method, Journal of Pharmaceutical Analysis, (2012); 2(6): 431–436.

5. Effat Souri, Ali Hatami, Nazanin Shabani Ravari, Farhad Alvandifar and Maliheh Barazandeh Tehrani, et al, Validating a stability indicating HPLC method for kinetic Study of cetirizine degradation in acidic and oxidative conditions, Iranian Journal of Pharmaceutical Research, 2013; 12 (2): 287-294.
6. Shaik Harun Rasheed, M Ramakotaiah, P Sandhya Vani, Mulla Arief, Silpa Rani Gajavalli, et al, A stability indicating LC method for the simultaneous determination of metoprolol, atorvastatin and ramipril in combined pharmaceutical dosage form, Research Journal of Pharmaceutical, Biological and Chemical Sciences, (2010); 1(4): 816-829.
7. Blessy Mn, Ruchi D.Patel, Prajesh N.Prajapati, Y.K.Agrawa, et al, Development of forced degradation and stability indicating studies of drugs—A review, Journal of Pharmaceutical Analysis, 2014; 4(3): 159–165.
8. Bhoomi P. Shah, Suresh Jain, Krishna K. Prajapati and Nasimabanu Y. Mansuri. Stability indicating HPLC method development: A Review, *et al,* IJPSR, 2012; 3(9): 2978-2988.
9. M. Mathrusri Annapurnan, S.V.S. Goutam, S. Anusha, L. Srinivas, et al, Development and validation of the stability-indicating LC–UV method for the determination of cefditoren pivoxil, Journal of Pharmaceutical Analysis, 2012; 2(6):466–469
10. Kiran Khandelwal, Shakti Deep Pachauri, Sofia Zaidi, Pankajwivedi, Ashok Kumar Sharma, Chandan Singh, Anil Kumar Dwivedi, et al, Assay method for quality control and stability studies of a new antimalarial agent (CDRI99/411), Journal of Pharmaceutical Analysis, 2013; 3(5): 335–340.
11. Md. F. Hossain, Subrata Bhadra, Uttom Kumar and Abu S. S. Rouf, et al, The ICH guidance in practice, Stress degradation studies on aceclofenac and development of a validated stability-indicating reversed-phase HPLC assay in tablet dosage form, Der Pharma Chemica, 2013; 5(4): 131-146.
12. M.L. Lal Prasanth and Sridhar Siddiraju, et al, Stability indicating HPLC method for the determination of Dacarbazine in pharmaceutical dosage form.Lal Prasanth, Int J Pharm, 2014; 4(3): 5-12.
13. K. Raghavi, M. Sindhura, R. Prashanthi, Buchi N. Nalluri, et al, Studies on forced degradation of oxcarbazepine using LC-MS compatible stability indicating RP-HPLC method, Journal of Chemical and Pharmaceutical Research, 2012; 4(8) :3885-3893.
14. Tejal Rathod, Anandkumari D. Captain, Development and validation of stability indicating RP-HPLC method for simultaneous estimation of ethinyl estradiol and levonorgestrel, International Journal of Pharmaceutical Research and Analysis, 2014; 4(3): 178-185.