Available online on 15.09.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Check for updates

Research Article

Comparative UV Spectroscopic Method Analysis and Validation for Estimation of Rifaximin in Pharmaceutical Preparation

Dr. Aney Joice[1]* and Farheen Mohammed Zubair Sange[2]

[1] *Assistant Professor Department of Pharmaceutics, M.C.E Society's Allana College of Pharmacy, Pune-411001, Maharashtra, India.*

[2] *Postgraduate Student M. Pharm Department of Pharmaceutical Quality Assurance, M.C.E Society's Allana College of Pharmacy, Pune-411001, Maharashtra, India*

Article Info:

Article History:

Received 19 June 2023
Reviewed 04 Aug 2023
Accepted 18 Aug 2023
Published 15 Sep 2023

Cite this article as:

Joice A, Sange FMZ, Comparative UV Spectroscopic Method Analysis and Validation for Estimation of Rifaximin in Pharmaceutical Preparation, Journal of Drug Delivery and Therapeutics. 2023; 13(9):33-40

DOI: http://dx.doi.org/10.22270/jddt.v13i9.6193

*Address for Correspondence:

Dr. Aney Joice, Assistant Professor Department of Pharmaceutics, M.C.E Society's Allana College of Pharmacy, Pune-411001, Maharashtra, India.

Abstract

In the present research work the absorption characteristics of rifaximin was studied and validated in different solvents in visible region for its quantitative estimation in bulk and dosage forms by UV spectrophotometric method. The solution of Rifaximin were prepared in 0.1N HCL (pH 1.2), Phosphate buffer (pH 6.8) & Phosphate buffer (pH 7.4) and showed maximum absorption at wavelength 439 nm, 440 nm & 433 nm in respective solvents. The drug obeyed Beer–Lambert's law in the concentration range of 2, 4, 6, 8, 10 µg/mL with coefficient of correlation (r^2) of 0.998. The estimation method was validated as per the ICH guidelines. Statistical analysis proved that the methods are repeatable and specific for the estimation of the said drug. These methods can be adopted in routine analysis of Rifaximin in bulk or tablet dosage form.

Keywords: Rifaximin, 0.1N HCL, phosphate buffer, UV spectrophotometer.

INTRODUCTION

Rifaximin

Rifaximin is an oral antibiotic with broad spectrum of action that acts locally in the gastrointestinal tract with minimal systemic adverse effects. It is used for the treatment of traveler's diarrhea caused by non-invasive strains of E. coli. It is benzimidazole derivative. Rifaximin is a product of synthesis of Rifamycin, an antibiotic with low gastro-intestinal absorption and good anti-bacterial activity. It acts on the ß-subunit of the deoxyribonucleic acid (DNA) dependent ribonucleic acid (RNA) polymerase enzyme of the microorganism to inhibit RNA synthesis. Rifaximin ($C_{43}H_{51}N_3O_{11}$ and molecular weight 785.9 g mol^{-1}) is a derivative of rifamycin and is a structural analogue of rifampicin. [1-5]

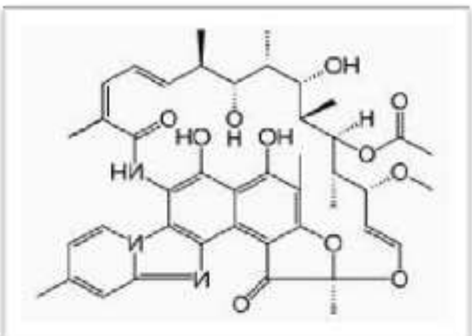

Figure 1: Chemical Structure of Rifaximin

UV Spectroscopy

A UV-visible spectrophotometer records a UV or visible spectrum as a plot of wavelengths of absorbed radiations versus the intensity of absorption in terms of absorbance (optical density) A or molar absorptivity (molar extinction coefficient) e as defined by the Lambert-Beer law. According to Lambert's law, the fraction of incident. Monochromatic radiation absorbed by a homogeneous medium is independent of the intensity of the incident radiation while Beer's law states that the absorption of a monochromatic radiation by a homogeneous medium is proportional to the number of absorbing molecules [6,7].

The absorption of electromagnetic radiations in the UV and visible regions induces the excitation of an electron from a lower to higher molecular orbital (electronic energy Level). UV Visible spectroscopy is also called as electronic spectroscopy in which the light is absorbed at each wavelength of UV and Visible region of electromagnetic spectrum. Organic chemists use ultraviolet and visible spectroscopy mainly for detecting the presence and elucidating the nature of the conjugated multiple bonds or aromatic rings [7].

UV-Visible spectrophotometry is one of the most important technique used in analytical chemistry in the pharmaceutical analysis which is being used in the quantitative analysis of a specific analyte. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measures the ratio, or function of ratio, of the intensity of two beams of light in the U.V. visible region is called Ultraviolet-Visible Spectrophotometer [8].

In qualitative analysis, the analysis of conjugated organic compounds and transition metal ions can be identified by use of spectrophotometer, if any recorded data is available, and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds [9].

Ultraviolet and visible (UV) spectroscopy records the absorption of radiations in ultraviolet and visible regions of the electromagnetic spectrum. The ultraviolet radiation extends from 10nm to 400nm and the visible radiation extends from 400nm to 800nm.

MATERIAL AND METHODS

1. Materials

Shimadzu UV - 1700 UV/VISIBLE spectrophotometer with UV probe 2.10 software and 1 cm matched quartz cells were used for absorbance measurements. Make- Mettler Toledo, Model-X was used as analytical balance for weighing standard and sample.

2. Methods

2.1. Preparation of Standard Stock Solution

Standard Stock Solution was prepared by dissolving 10mg of the drug in 10ml of 0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4 to get concentration of 1000 µg/ml. From the above Standard Stock Solution, working standard solution was prepared containing 100 µg/ml of Rifaximin.

2.2. Selection of Wavelength for Analysis

From the Standard Stock Solution (1000ug/ml) further dilutions were made using 0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4 and scanned over the range of 200-800 nm and the spectra was obtained using 0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4 as a blank.

2.3. Preparation for Calibration Curve

Aliquots of standard stock solution were further diluted with 0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4 to get the solutions of concentration within range 2, 4, 6, 8, 10 µg/mL. The absorbance was measured using 0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4 as blank. All measurements were repeated three times for each concentration.

2.4. Assay of Rifaximin in Tablet

Twenty tablets were weighed; their average weight was determined and finely powdered. Powder equivalent to 50mg Rifaximin of was accurately weighed and dissolved in small amount of methanol in 50 mL volumetric flask and then the volume was adjusted with methanol to obtain the final concentration 1000 µg/mL. From this, 10 mL solution was taken and diluted up to 100 mL with the same solvent in a volumetric flask to obtain the solution of concentration 100 µg/mL. From this solution, aliquot of 2 mL was diluted to 10 mL using methanol. The drug content was measured using UV spectrophotometer.

2.5. METHOD VALIDATION [10,11,12]

The analytical method was validated as per ICH guidelines for following parameters

❖ **Linearity**

Aliquots of standard stock solution were further diluted with 0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4 to get the solutions of concentration within range from 2, 4, 6, 8, 10 µg/mL. The absorbance was measured at wavelength 440 nm, 439 nm & 440nm. Linear calibration graph was obtained by plotting the absorbance value versus concentration of Rifaximin.

❖ **Accuracy**

To ensure accuracy of the method, recovery studies were performed by standard addition method at 80%, 100% and 120% level to pre-analyzed samples and subsequent solutions were reanalyzed. At each level, three determinations were performed. Accuracy is reported as % recovery.

❖ **Precision**

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intra-day and inter-day precisions.

❖ **Limit of Detection (LOD) & Limit of Quantitation (LOQ)**

In UV method development LOD & LOQ was determined by utilizing the following equation

$$LOD = 3.3 \times SD/S$$

$$LOQ = 10 \times SD/S$$

Where, S= Slope, SD= Standard deviation of Y-intercepts.

❖ **Robustness:**

Robustness of the method was determined by carrying out the analysis under additions during which scanning wavelength was altered. Time was also changed from spotting to development to scanning and the effect on the area were noted.

3. RESULTS AND DISCUSSION

3.1 Selection of Wavelength for Analysis

The UV spectrum of Rifaximin showed the maximum absorbance at the wavelength 439 nm,440 nm & 433 nm respectively for 0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4 [Figure 2-4]. It was selected for the analysis of Rifaximin in bulk and tablet formulation.

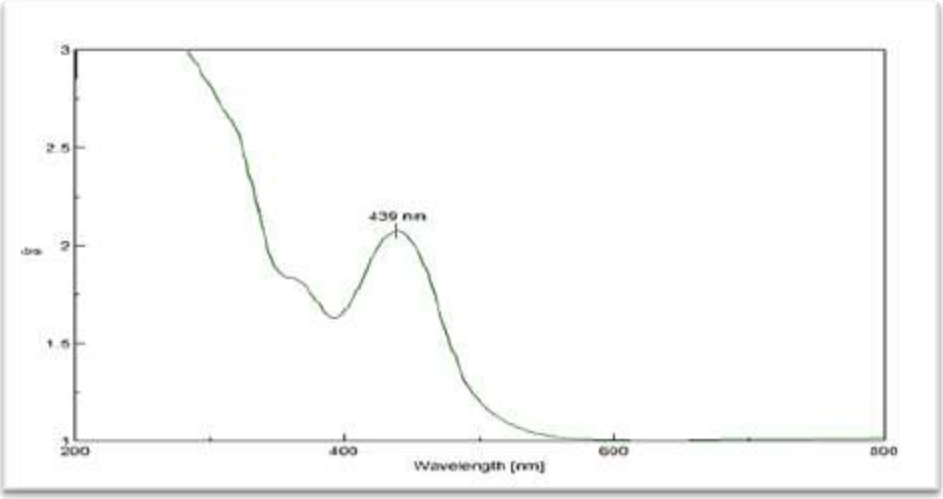

Figure 2: UV Spectrum of Rifaximin in 0.1N HCL (pH 1.2)

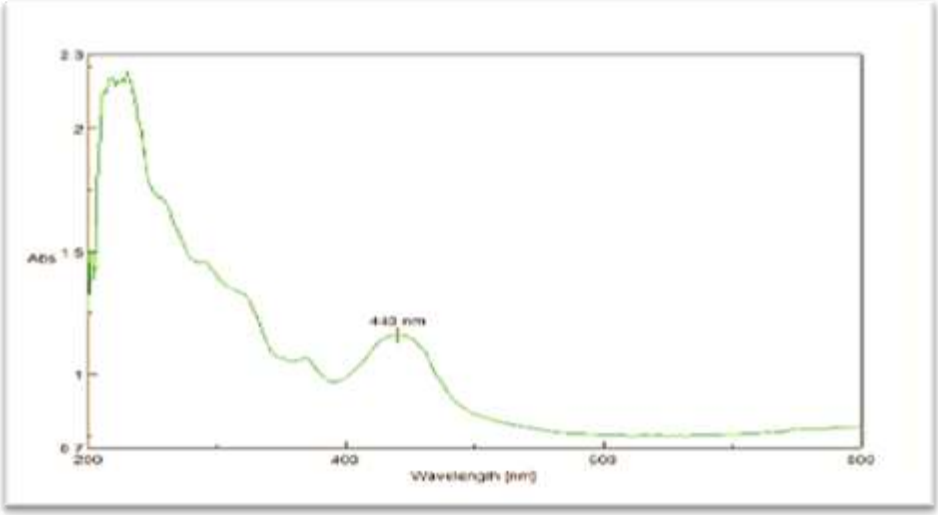

Figure 3: UV Spectrum of Rifaximin in Phosphate buffer (pH 6.8)

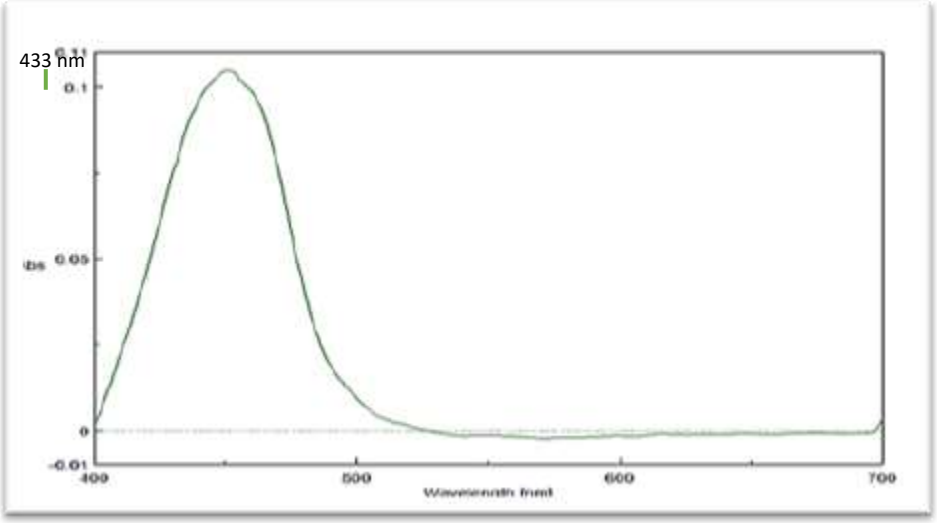

Figure 4: UV Spectrum of Rifaximin in Phosphate buffer (pH 7.4)

3.2 Preparation of the Calibration Curve

The calibration curve was constructed by plotting absorbance against corresponding concentration as shown in [Figure 5, 6 & 7] The calibration curve for Rifaximin. The drug obeyed Beer–Lambert's law in the concentration range of 2, 4, 6, 8, 10 µg/mL with coefficient of correlation (r^2) of 0.998. [Table 1]

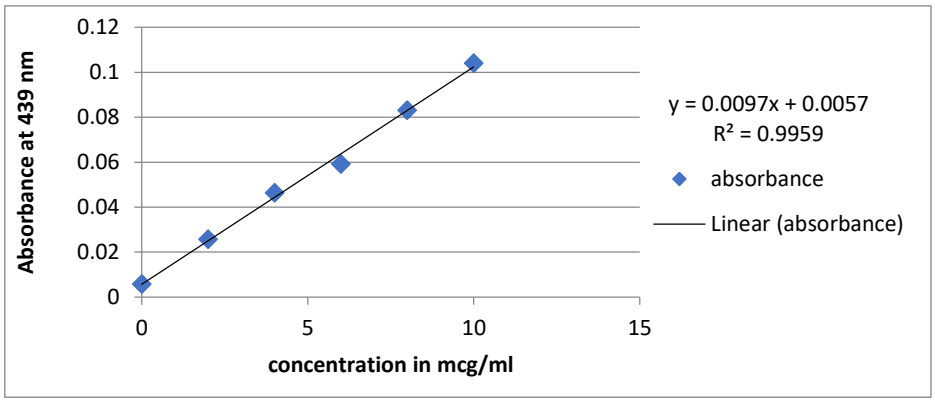

Figure 5: Calibration Curve of Rifaximin in 0.1N HCL (pH 1.2)

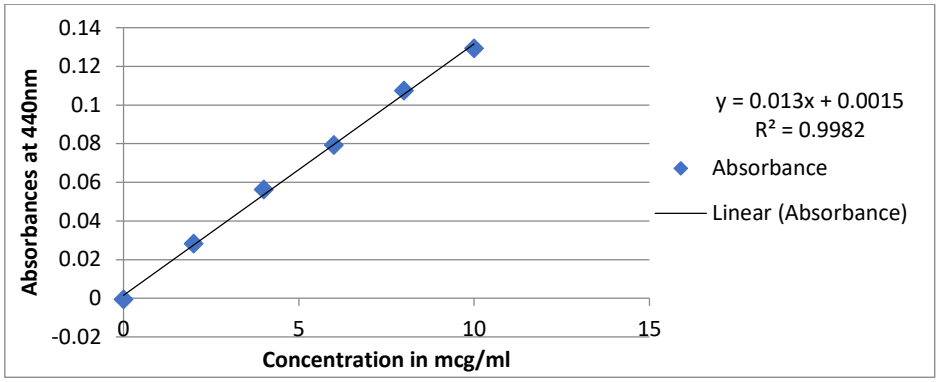

Figure 6: Calibration Curve of Rifaximin in Phosphate buffer (pH 6.8)

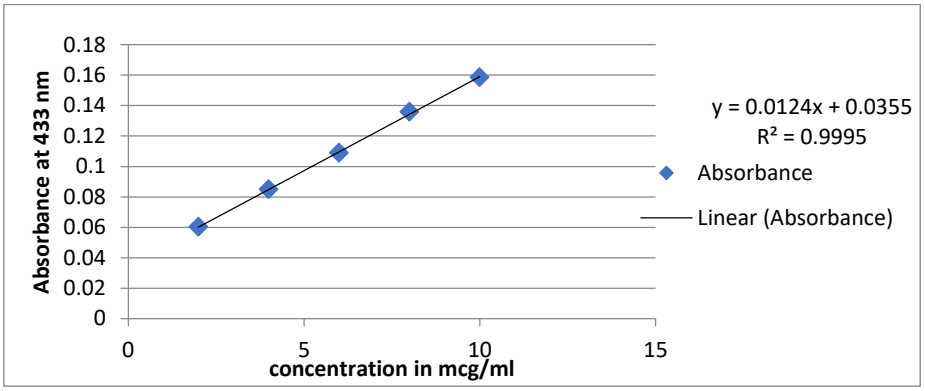

Figure 7: Calibration Curve of Rifaximin in Phosphate buffer (pH 7.4)

Table 1: Linearity data of Rifaximin in 0.1N HCL of 0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4.

Parameters	Results		
	0.1N HCL pH 1.2	Phosphate buffer pH 6.8	Phosphate buffer pH 7.4
Linearity range	2-10µg/ml	2-10µg/ml	2-10µg/ml
Regression line equation	y = 0.0097x + 0.0057	y = 0.013x + 0.0015	y = 0.0124x + 0.0355
Slope	0.0097	0.0131	0.0124
Y – intercept	0.0057	0.0015	0.0355
Correlation coefficient	R^2 = 0.9959	R^2 = 0.9982	R^2 = 0.9995

3.3 Assay of Rifaximin in Tablet

The amount of Rifaximin present in formulation was calculated by comparing the absorbance of sample with standard absorbance. Content of Rifaximin in tablet formulation determined by developed method was in good agreement with the label claim. [Table 2]

Table 2: Assay of Tablet Formulation by UV method

Assay	
Labelled claim (mg)	200mg
Drug content ± SD (mg)	200.24 ± 0.0031
% Assay	103.11
% RSD	0.54

3.4 Method Validation

3.4.1. Accuracy

The responses were reanalyzed using the suggested method, and the accuracy results are shown in [Table 3-5], which demonstrate that the percentage amount recovered was between 98.60%-99.96%, 95.12% - 95.59% & 98.17%-98.87% with % RSD less than 2.

Table 3: Results of Accuracy for Rifaximin in 0.1N HCL (pH 1.2)

Observation table for accuracy (0.1N HCL pH 1.2)						
Levels	**Conc. In ppm**	**Absorbance**	**Conc. Found**	**Mean**	**SD**	**% Recovery**
80	18	0.1765	17.6082	17.7491	0.1774	98.6063
		0.1773	17.6907			
		0.1798	17.9484			
100	20	0.1976	19.7835	19.8453	0.0676	99.2268
		0.1981	19.8350			
		0.1989	19.9175			
120	22	0.2187	21.9587	21.9931	0.0429	99.9687
		0.2189	21.9793			
		0.2195	22.0412			

Table 4: Results of Accuracy for Rifaximin in Phosphate buffer (pH 6.8)

Observation table for accuracy (Phosphate buffer pH 6.8)						
Levels	**Conc. In ppm**	**Absorbance**	**Conc. Found**	**Mean**	**SD**	**%Recovery**
80	18	0.2251	17.213	17.2076	0.03525	95.5982
		0.2248	17.1769			
		0.2257	17.2461			
100	20	0.2313	19.6769	19.6717	0.0235	93.3589
		0.2315	19.6923			
		0.2309	19.6461			
120	22	0.2736	20.9307	20.9282	0.0270	95.1282
		0.2732	20.9061			
		0.2739	20.9538			

Table 5: Results of Accuracy for Rifaximin in Phosphate buffer (pH 7.4)

Observation table for accuracy (Phosphate buffer pH 7.4)						
Levels	**Conc. In ppm**	**Absorbance**	**Conc. Found**	**Mean**	**SD**	**%Recovery**
		0.2562	17.7983			
80	18	0.2569	17.8548	17.7983	0.2229	98.1793
		0.2613	18.2096			
		0.2751	19.3225			
100	20	0.2759	19.3870	19.3717	0.0235	96.8682
		0.2762	19.4112			
		0.2976	21.1370			
120	22	0.2998	21.3145	21.5994	0.6532	98.8799
		0.3126	22.3467			

3.4.2. Precision

The developed method's precision was reported as a % RSD. These findings demonstrate the assay's repeatability. % RSD values less than 2 shows that the method for determining rifaximin is precise. [Table 6-8]

Table 6: Results of Precision for Rifaximin in 0.1N HCL (pH 1.2)

Observation Table for Precision (0.1N HCL pH 1.2)				
Conc. (ppm)	**Intra-day precision**		**Inter-day precision**	
	Conc. Found ± SD (µg/ml)	**%RSD**	**Conc. Found ± SD (µg/ml)**	**%RSD**
10	9.95 ± 0.006	1.47	9.99 ± 0.003	1.43
20	19.95 ± 0.004	0.54	19.92 ± 0.002	0.61
30	29.64 ± 0.002	1.24	29.94 ± 0.001	1.13

Table 7: Results of Precision for Rifaximin in Phosphate buffer (pH 6.8)

Observation Table for Precision (Phosphate buffer pH 6.8)				
Conc. (ppm)	**Intra-day precision**		**Inter-day precision**	
	Conc. Found ± SD (µg/ml)	**%RSD**	**Conc. Found ± SD (µg/ml)**	**%RSD**
10	9.01 ± 0.005	0.02	8.83 ± 0.004	1.5
20	19.53 ± 0.003	0.63	19.54 ± 0.002	0.39
30	28.82 ± 0.001	0.28	28.72 ± 0.005	0.58

Table 8: Results of Precision for Rifaximin in Phosphate buffer (pH 7.4)

Observation Table for Precision (Phosphate buffer pH 7.4)				
Conc. (ppm)	**Intra-day precision**		**Inter-day precision**	
	Conc. Found ± SD (µg/ml)	**%RSD**	**Conc. Found ± SD (µg/ml)**	**%RSD**
10	9.76 ± 0.001	0.61	9.75 ± 0.005	0.96
20	19.85 ± 0.003	1.26	19.83 ± 0.001	1.2
30	29.80 ± 0.005	1.82	29.89 ± 0.001	1.84

3.4.3. LOD & LOQ

By using the given formula, the LOD & LOQ were calculated for rifaximin in 0.1N HCL pH 1.2, Phosphate buffer pH 6.8 & Phosphate buffer pH 7.4 respectively in [Table 9]

Table 9: Results of LOD & LOQ

Observation Table for LOD & LOQ			
Conc (ppm)	**Absorbance (0.1N HCL pH 1.2)**	**Absorbance (Phosphate buffer pH 6.8)**	**Absorbance (Phosphate buffer pH 7.4)**
0.1	0.0234	0.0911	0.1330
0.2	0.0252	0.0912	0.2689
0.3	0.0271	0.0919	0.3897
0.4	0.0326	0.0921	0.1299
0.5	0.0421	0.0925	0.2691
0.6	0.0435	0.0956	0.3884
SD	0.0086	0.0016	0.1152
Slope	0.0447	0.0076	0.2908
LOD	**0.6410**	**0.7192**	**1.3083**
LOQ	**1.9426**	**2.1796**	**3.9647**

3.4.4. Robustness

This method's robustness was tested using variations in wavelength change. The experimental results demonstrated that the suggested UV technique is robust, with the change since% RSD being less than 0.9%. [Table 10-12]

Table 10: Results of Robustness for Rifaximin in 0.1N HCL (pH 1.2)

Wavelength	**Chamber Saturation Time(Min)**			**Time form application to development (min)**		
439	**14**	**15**	**16**	**0**	**30**	**60**
0.258	1.076	1.021	0.965	0.754	0.971	1.326

Table 11: Results of Robustness for Rifaximin in Phosphate buffer (pH 6.8)

Wavelength	**Chamber Saturation Time(Min)**			**Time form application to development (min)**		
440	**14**	**15**	**16**	**0**	**30**	**60**
0.432	1.219	1.237	1.223	1.021	0.651	1.351

Table 12: Results of Robustness for Rifaximin in Phosphate buffer (pH 7.4)

Wavelength	**Chamber Saturation Time(Min)**			**Time form application to development (min)**		
433	**14**	**15**	**16**	**0**	**30**	**60**
0.213	0.328	0.265	1.012	0.976	0.322	0.432

3.5 THE SUMMARY OF VALIDATION PARAMETERS BY UV METHOD

Table 13: Summary of Results of Validation Parameters by UV Method

Results of validation parameters by UV method				
Sr.No.	**Parameters**	**Results**		
		0.1N HCL pH 1.2	**Phosphate buffer pH 6.8**	**Phosphate buffer pH 7.4**
1	Absorption maxima(nm)	439nm	440nm	433nm
2	Beers range (µg/ml)	02-10µg/ml	2-10µg/ml	2-10µg/ml
3	Standard Regression Equation	y = 0.0097x + 0.0057	y = 0.013x + 0.0015	y = 0.0124x + 0.0355
4	Correlation Coefficient (r^2)	R^2 = 0.9959	R^2 = 0.998	R^2 = 0.9995
5	Precision	% RSD= Below 2%	% RSD= Below 2%	% RSD= Below 2%
6	Accuracy	98.60%-99.96%	95.12% - 95.59%	98.17%-98.87%
7	Robustness	0.258	0.432	0.213

4. CONCLUSION

The present study reports a comparative validations data of UV spectrophotometric analysis for qualitative determination of Rifaximin in bulk drug and formulation. Validation of UV method was conducted at different pH (1.2, 6.8 & 7.4) solutions with the wavelengths of 439nm, 440nm & 433 nm respectively. The results of the study suggest that the analytical approach described is relatively simple, accurate, precise & reproducible. Hence, the UV method is suitable for routine determination of Rifaximin in pharmaceutical formulations.

REFERENCES

1. https://link.springer.com/chapter/10.1007/978-1-4020-2575-4_2

2. Rivkin A., Gim S. Rifaximin: new therapeutic indication and future directions. Clinical Therapeutics. 2011; 33(7):812-827. https://doi.org/10.1016/j.clinthera.2011.06.007 PMid:21741091

3. Sudha T., Anandakumar K., Hemalatha P. V., Ravikumar V. R., Radhakrishnan Spectrophotometric estimation methods for Rifaximin in tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences. 2010; 2(1):43-46.

4. Mullen K. D., Sanyal A. J., Bass N. M., et al. Rifaximin is safe and well tolerated for long-term maintenance of remission from overt hepatic encephalopathy. Clinical Gastroenterology and Hepatology. 2014; 12(8):1390-1397. https://doi.org/10.1016/j.cgh.2013.12.021 PMid:24365449

5. Yoshida, T. Bioorg & Med Chem., 2012; 20:5705-5719.

6. Scott, A.I., Interpretation of Ultraviolet Spectra of Natural Products, Pergamon Press, New York, 1964.

7. Skoog, D.A. Holler, F.J., Nieman, D.A., Introduction to UV Spectroscopy in, principle of instrumental analysis, 5thed., Cole publication, 2004.

8. Chatwal G. R, Anand S. K, "Instrumental Methods of Chemical Analysis", 5 th Edn., Himalaya Publishing House, New Delhi, 2002; 566-587, 624-639. 3. Beckett. A. H., Stenlake J. B. Practical Pharmaceutical chemistry CBS Publishers and distributors, New Delhi., 1997, Ultraviolet visible absorption spectrophotometric. 2002; 275-278.

9. Rivkin A., Gim S. Rifaximin: new therapeutic indication and future directions. ClinicalTherapeutics. 2011; 33(7):812-827. https://doi.org/10.1016/j.clinthera.2011.06.007 PMid:21741091

10. International Conference on Harmonization. ICH. (2005). ICH - Q2 (R1): Guideline on Validation of Analytical Procedure: Text and Methodology.

11. International Conference on Harmonization, ICH (2003). Q2 (R1): Validation of analytical procedures: test and methodology.

12. Martindale. The Extra Pharmacopoeia. Published by direction of the Council of Royal Pharmaceutical Society of Great Britain. 34th ed, Vol. 11. London Royal Pharmaceutical Society, 2005; 653-656.