



## VALIDATED AUC METHOD FOR THE SPECTROPHOTOMETRIC ESTIMATION OF RIFAXIMIN TABLET

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Article Received on 05/12/2018

Article Revised on 26/12/2018

Article Accepted on 16/01/2019

### ABSTRACT

A novel, simple, accurate and precise Area under curve spectroscopic method was developed and validated for the estimation of Rifaximin in bulk and Pharmaceutical dosage forms and AUC was measured at 230-240nm in Methanol : Acetonitrile (60:40). The Linearity was found to be in the concentration range of 5-30 µg/ml and the correlation coefficient was found to be 0.9992 and it has showed good linearity, reproducibility, precision in this concentration range. The regression equation was found to be  $Y = 0.0245 X + 0.007$ . The % recovery values were found to be within 98.74-100.8 % showed that the method was accurate. The LOD and LOQ were found to be 0.2138 and 0.6414 µg/ml, respectively. The % RSD values were less than 2. The present methods is accomplish the validation parameters according to ICH guidelines. The developed method was successfully applied for the quantitative estimation of Rifaximin in bulk and pharmaceutical dosage forms.

**KEYWORDS:** Rifaximin, Area Under Curve Spectroscopy, Methanol: Acetonitrile (60:40), Linearity, Precision, Ruggedness, Accuracy.

### INTRODUCTION

Rifaximin is used as an antibiotic for the treatment of traveler's diarrhea and hepatic encephalopathy but not used for the systemic bacterial infection and for children below age of 12 years. 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. 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<sup>1</sup>.

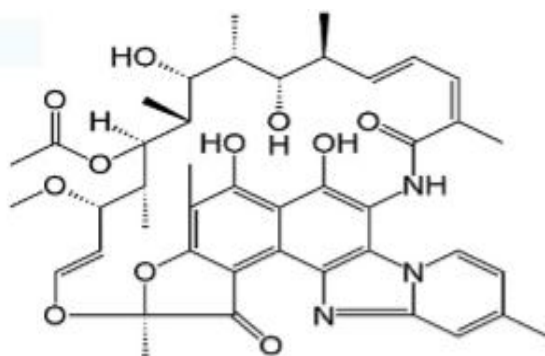


Figure.1: Chemical structure of Rifaximin.

Rifaximin is Chemically (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. It has a molecular formula of  $C_{43}H_{51}N_3O_{11}$  and molecular weight of 785.879 g/mol. It has the structural formula (Fig.1). Rifaximin is a orange-brown to red-brown powder. It is soluble in dimethylsulfoxide, methanol, chloroform, ethanol, ethylacetate and acetone, slightly soluble in water.

Literature Survey revealed that the drug has been estimated by UV Spectrophotometric, HPLC, and HPTLC method has been reported so far.

The aim of present work was to develop and validate a novel, rapid, simple, precise, and specific Area Under Curve UV-Spectrophotometric method for estimation of Rifaximin in its bulk and tablet dosage form.

### MATERIALS AND METHOD INSTRUMENT

UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software.

**CHEMICALS**

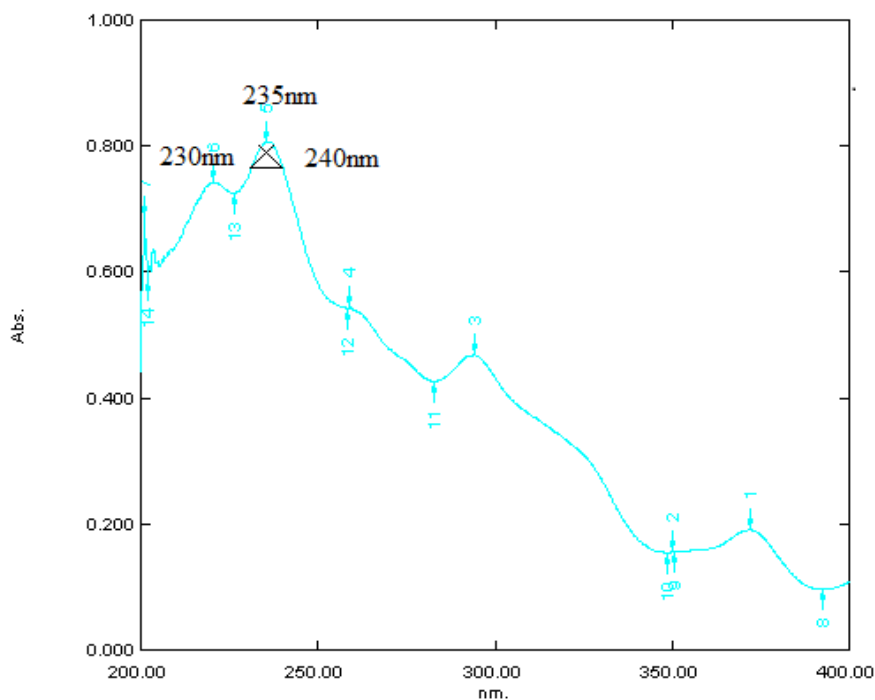
- Rifaximin pure form was obtained as gifted sample from pharma industry INTAS Ltd located at Bangalore. Its pharmaceutical dosage form Rifaximin 10 Tablets labelled claim 200mg (manufactured by INTAS PHARMACEUTICALS LTD) were purchased from local pharmacy.
- Methanol: Acetonitrile available in the laboratory of Bharathi college of Pharmacy, Bharathinagara.

**SOLVENT**

Methanol: Acetonitrile (60:40).

**SELECTION OF ANALYTICAL WAVELENGTH**

Appropriate dilutions were prepared for drug from the standard stock solution and the solution was scanned in the wavelength range of 200-400 nm. The absorption spectra thus obtained were derivatized from Area under curve method which illustrated in fig 1.



**Fig.2: Typical Zero order spectra of Rifaximin showing Area under curve [AUC] from 230nm to 240nm.**

**PREPARATION OF STANDARD STOCK SOLUTION**

Accurately weigh 100mg of Rifaximin was transferred into 100ml volumetric flask and diluted with Methanol: Acetonitrile (60:40) up to the mark. From this pipette out 10ml into 100ml volumetric flask and diluted with Methanol: Acetonitrile (60:40) up to the mark, from this solution pipette out 0.5,1.0,1.5,2.0,2.5 and 3.0ml into 10ml individual volumetric flask and add methanol: Acetonitrile (60:40) up to the mark, this gives 5,10,15,20,25 and 30  $\mu\text{g}/\text{ml}$  concentrations.

**PREPARATION OF SAMPLE SOLUTION**

Twenty tablets were weighed and powdered, the tablet powder equivalent to 100mg of Rifaximin was transferred into 100ml volumetric flask then it was diluted with Methanol : Acetonitrile (60:40) and made up to mark and the solution was filtered through Whatmans filter paper no.41. From this pipette out 10 ml in a 100ml volumetric flask and make up the volume up to the mark with Methanol : Acetonitrile (60:40). From this solution pipette out 1.5ml into 10ml volumetric flask and make

up the volume with Methanol : Acetonitrile (60:40), this gives 15 $\mu\text{g}/\text{ml}$  concentrations.

**METHOD VALIDATION**

The method is validated according to the ICH guidelines. Validation methods like Linearity, Precision, Accuracy, Ruggedness, LOD and LOQ.

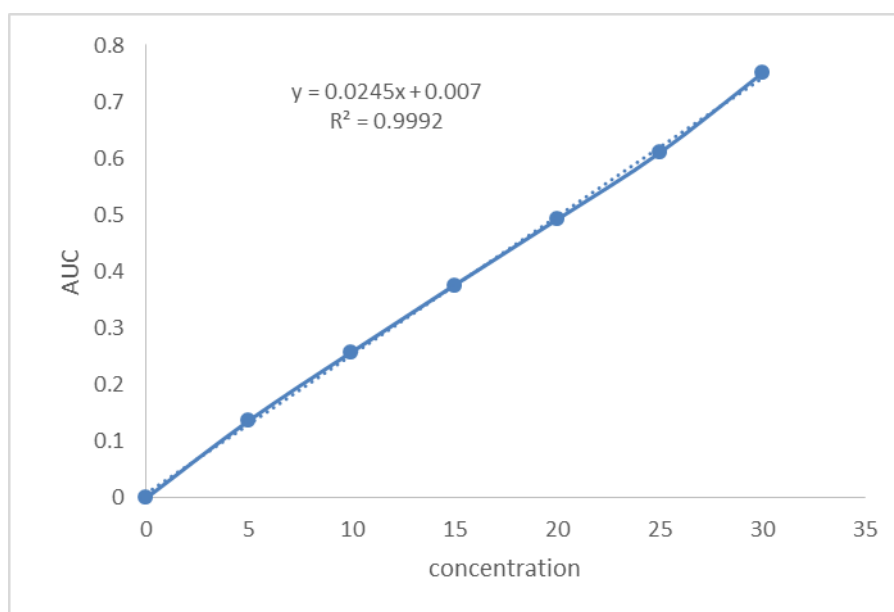
**RESULTS AND DISCUSSION****METHOD: AREA UNDER CURVE [AUC] METHOD.****LINEARITY**

The working standard solution were diluted serially with Methanol : Acetonitrile(60:40) to obtain the range of 5-30 $\mu\text{g}/\text{ml}$ . a calibration curve for Rifaximin was obtained by measuring the absorbance between 230-240nm and absorbance values are shown in Table.1 and Calibration graph were presented in Fig.3. Statistical parameters like slope, intercept, coefficient of correlation, and Sandell's sensitivity were determined and presented in Table.2.

**Table.1: Results of calibration curve at 230-240nm by Area Under Curve.**

SL. NO	Concentration in $\mu\text{g/ml}$ .	Area under curve $\pm$ Standard deviation
1	5	0.137 $\pm$ 0.002137
2	10	0.258 $\pm$ 0.002608
3	15	0.376 $\pm$ 0.002757
4	20	0.492 $\pm$ 0.005076
5	25	0.610 $\pm$ 0.004021
6	30	0.751 $\pm$ 0.002066

\*\*Average of six determinations

**Fig.3: Calibration curves for Rifaximin at by AUC method.****Table 2: Regression parameters for Rifaximin by Area Under curve method.**

Regression parameters	Rifaximin
Range	5-30
AUC wave lengths	230-240nm
Regression equation	$Y=0.0245x+0.007$
Slope (b)	0.0245
Intercept(a)	0.007
Correlation coefficient (r2)	0.9992
Sandell's sensitivity	0.4065

**PRECISION**

Precision of the method was studied as intra-day and inter-day precision. Intra-day precision was determined by analyzing the 5, 10, 15, 20, 25 and 30 $\mu\text{g/ml}$

concentration for three times in same day. Inter-day precision was determined by analyzing the same concentration of solution daily for three days. Precision results are shown in Table.3.

**Table.3: Determination of precision results for Rifaximin at 230-240 nm by Area Under Curve.**

Concentration ( $\mu\text{g/ml}$ )	Intra-day Area under curve $\pm\text{SD}^{**}$	%RSD	Inter-day Area under curve $\pm\text{SD}^{**}$	%RSD
5	0.137 $\pm$ 0.002081	1.518	0.138 $\pm$ 0.002517	1.823
10	0.257 $\pm$ 0.002646	1.029	0.258 $\pm$ 0.002	0.775
15	0.374 $\pm$ 0.002082	0.556	0.377 $\pm$ 0.002082	0.552
20	0.494 $\pm$ 0.002	0.404	0.493 $\pm$ 0.004583	0.929
25	0.612 $\pm$ 0.004	0.653	0.613 $\pm$ 0.003055	0.498
30	0.752 $\pm$ 0.002309	0.307	0.749 $\pm$ 0.001528	0.204

\*\*Average of six determinations

**ACCURACY**

To assess the accuracy of the proposed method, recovery studies were carried out at three different levels i.e.,

50%, 100% and 150%. In which the formulation concentration was kept constant and varied pure drug concentration. Accuracy results were shown in Table.4.

**Table.4: Determination of accuracy results for Rifaximin by Area Under Curve.**

Spiked Levels	Amount of sample ( $\mu\text{g/ml}$ )	Amount of Standard ( $\mu\text{g/ml}$ )	Amount recovered	%Recovery $\pm\text{SD}^{**}$	%RSD
50	10	5	14.81	98.74 $\pm$ 1.8864	1.9105
100	10	10	20.16	100.8 $\pm$ 0.9268	0.9194
150	10	15	25.08	100.37 $\pm$ 0.5666	0.5645

\*\*Average of six determinations

**RUGGEDNESS:**

Ruggedness was determined between different analysts. The value of %RSD was found to be less than 2 were shown in Table.5.

**Table.5: Ruggedness results at 230-240 nm by Area Under Curve.**

Analysts	Analyst-1	Analyst-2
Mean AUC	0.376	0.381
*Standard deviation	0.002757	0.002805
*%RSD	0.733	0.736

\*Average of six determinations

**LIMIT OF DETECTION AND LIMIT OF QUANTITATION:**

The LOD and LOQ of the present method were calculated based on standard deviation of the Response

and slope of linearity curve. LOD and LOQ values of Rifaximin were found to be 0.2138 $\mu\text{g/ml}$  and 0.6414 $\mu\text{g/ml}$ .

**Table.6: Assay of Rifaximin in tablet formulation Zimigut for area under curve method.**

Tablet	Label Claim (mg)	% Content	Mean	Standard Deviation	% RSD
Rifaximin	200	98.17	99.691	1.100825	1.1042
	200	98.78			
	200	99.39			
	200	100.2			
	200	101.01			
	200	100.6			

**CONCLUSION**

The developed method is suitable for the routine estimation Rifaximin in pure and tablet dosage form, It was found to be simple, accurate, precise, selective, economical and shows good linearity.

**ACKNOWLEDGEMENT**

We like to thanks Management, Director, Principal, Teaching and non-teaching staff of Bharathi College of pharmacy and my dear friends for their continues co-operation and support.

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