P. Ravi Kumar et al /J. Pharm. Sci. & Res. Vol. 11(5), 2019, 2007-2011

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Study of Effect of Solvents on Absorption Characteristics of Rifaximin in Visible Region and Its Estimation in Bulk and Dosage Forms

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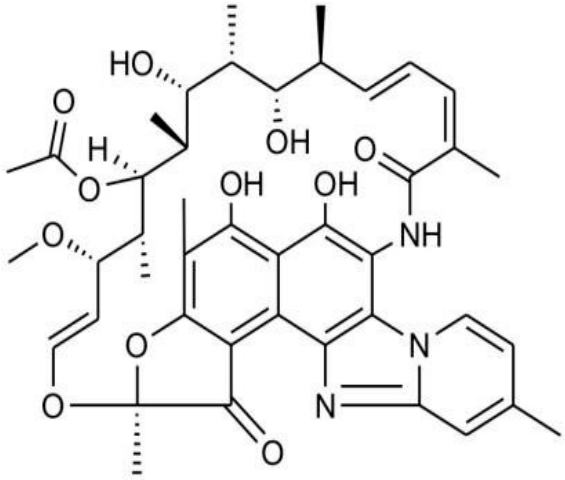
Abstract:

Rifaximin is an oral antibiotic with broad spectrum of action that acts locally in the gastrointestinal tract with minimal systemic adverse effects. In the present research work the absorption characteristics of rifaximin studied in different solvents in visible region and a method developed and validated for its quantitative estimation in bulk and dosage forms. The different solvents selected for study includes 0.1N NaOH, methanol, phosphate buffer pH 7, mixture of phosphate buffer pH 7 and methanol(50:50) and phosphate buffer pH 4. Beer's law concentration range in all the solvents except 0.1 N NaOH is 5-25µg/ml whereas in 0.1N NaOH it was 1-5µg/ml. The absorption maxima in 0.1N NaOH was 440 nm, in methanol was 442nm, in phosphate buffer pH 7 was 441nm, mixture of phosphate buffer pH 7 and methanol (50:50) was 450nm and the drug was insoluble in phosphate buffer pH 4. So the method developed using 0.1N NaOH is more sensitive when compared with other solvents and hence it was optimized and validated. The LOD and LOQ were 0.145 and 0.4418 µg/ml respectively with the coefficient of correlation 0.997. The method has been validated for linearity, accuracy and precision as per ICHQ2(R1) guidelines.

Keywords: Rifaximin, 0.1N NaOH, Methanol, Phosphate buffer pH 7, Phosphate buffer pH 4, ICHQ2(R1) guidelines.

INTRODUCTION:

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 traveller's diarrhoea 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.



Structure of Rifaximin

UV-Visible spectrophotometry is used for qualitative and quantitative analysis which involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution.

MATERIALS AND METHOD:

Materials:

Sodium hydroxide, Methanol, Phosphate buffer, Rifaximin Hydrochloride(Bulk Drug),Rifaximin tablets(Rifinim-550mg,Rifagut-400mg,Rifaxia-400mg,Rifsure-200mg)

Method A (0.1N NaOH as solvent):

Preparation of standard stock-I solution and stock-II solution (1000 and 100µg/ml):

Standard rifaximin solution was prepared by dissolving 10 mg of rifaximin working standard in 10ml of 0.1 N NaOH. Standard rifaximin of 100µg/ml solution was prepared by taking 2.5ml of rifaximin standard stock-I solution into 10ml of 0.1 N NaOH taken in a 25ml volumetric flask.

Determination of absorption maximum and construction of calibration curve:

0.1ml of stock-II solution is taken in 10ml graduated tube and volume is made upto 10ml with 0.1 N NaOH. It is kept aside for 5 minutes; pale yellow colored solution is formed. This gives 1µg/ml solution of rifaximin and was scanned in the range of 400-800nm to determine the absorption maximum for the drug. The absorption maximum was found to be 440 nm. (Fig.no.:01). Similarly, solutions of concentrations ranging from 1-5µg/ml were prepared and the absorbance of these solutions were measured at 440nm. (table no.:01, Fig.no.:01 and 02,Graph no:01)

Method B,C and D:

Preparation of standard stock-I solution and stock-II solution (1000 and 100µg/ml):

Three 10ml of volumetric flasks were taken. 10mg of rifaximin transferred to each flask. Then dissolved in methanol(method B), phosphate buffer pH 7 (methodC) and phosphate buffer pH 7 and methanol 50:50 (method D)

to get 1000µg/ml solution. From this 2.5ml solution was taken and made up to 25ml with respective solvent to get 100 µg/ml

Determination of absorption maximum and construction of calibration curve:

From stock II solution of methanol, phosphate buffer pH 7 and phosphate buffer pH 7 and methanol (50:50), concentrations ranging from 5-25µg/ml were prepared and scanned at 400 – 800nm and absorbances were determined. (method B - table no.:02, Fig.no.:03,Graph no:02, method C- table no.:03, Fig.no.:04,Graph no.:03, method - D table no.:04,Fig.no.:05,Graph no.:04) .

Validation of developed method

The developed methods were validated according to the ICH Guidelines Q2 (R1)

Linearity

Linearity was observed for drugs in all solvents from calibration curve.

Limit of Detection and Limit of Quantification

Both were calculated using standard deviation method.

Precision

Precision may be considered at three levels repeatability, intermediate precision and reproducibility.

Repeatability

The repeatability was checked by using standard rifaximin at a concentration of 3µg/ml to ensure that the analytical system was precise.The absorbance of six determinations was measured and % RSD is calculated.(table no.:06)

Intra-day precision and Inter – day precision

The intra-day precision of the proposed method was determined on the samples of drug at various concentration levels (2.4µg/ml, 3µg/ml and 3.6µg/ml) by analyzing three replicates. The inter-day precision determined at same concentration levels on three consecutive days. The % relative standard deviation was calculated at each level. (table no.:07 and table no.:08)

Accuracy:

Accuracy for drug substance was determined on samples of drug solutions at varying concentration levels in the range of 80%-120% (3.2µg/mL, 4µg/mL and 4.8µg/mL) by analyzing three replicates of each sample as a batch in a single assay. The %RSD was calculated and reported at each level.(table no.:09 & 10)

Assay of rifaximin tablets:

The average absorbance of the three dilutions of concentration (15µg/ml) of rifaximin tablets was estimated and the percentage purity was calculated. (table no.:11)

RESULTS:

Limit of Detection and Limit of Quantification

LOD = 3.3 X Standard deviation / slope

= 3.3 X 0.00707/0.016

0.145 µg/ml

LOQ = 10 X Standard deviation / slope

= 10 X 0.00707/0.016

= 0.4418 µg/ml

Table - 01: linearity of rifaximin using 0.1N NaOH

Concentration (µg/ml)	Absorbance
1	0.022
2	0.037
3	0.054
4	0.069
5	0.089

Table - 02: linearity of rifaximin using methanol

Concentration(µg/ml)	Absorbance
5	0.078
10	0.146
15	0.232
20	0.317
25	0.402

Table – 3: linearity of rifaximin using phosphate buffer pH 7

Concentration(µg/ml)	Absorbance
5	0.018
10	0.074
15	0.113
20	0.183
25	0.230

Table – 4: linearity of rifaximin using mixture of phosphate buffer pH 7 and methanol (50:50)

Concentration(µg/ml)	Absorbance
5	0.116
10	0.241
15	0.318
20	0.424
25	0.510

Table – 5: Optimized spectrophotometry conditions in 0.1N NaOH

Parametrs	Results
λ_{max}	240nm
Beers law range	1 -5µg/ml
Molar absorptivity	1.5 X 10^4 lit mol $^{-1}$cm^{-1}
Limit of detection	0.145µg/ml
Limit of quantification	0.4418µg/ml
Regression equation	y = 0.016x + 0.004
Slope	0.016
Co-efficient of correlation	0.997

Table – 6: Repeatability data of pure rifaximin in 0.1N NaOH

S.NO	Concentration(µg/ml)	Absorbance
1	3	0.05
2	3	0.05
3	3	0.06
4	3	0.05
5	3	0.05
6	3	0.05
Average		0.05
Standard deviation		0.01414
% RSD		1.4

Table – 7: Intra-day precision data for rifaximin in 0.1N NaOH

Concentration (µg/ml)	Mean absorbance*			Mean ± SD	% RSD
	Morning	Afternoon	Evening		
2.4(80%)	0.040	0.040	0.041	0.040 ± 0.000707	1.7
3(100%)	0.052	0.053	0.052	0.052 ± 0.00071	1.3
3.6(120%)	0.064	0.065	0.66	0.065 ± 0.001	1.5

Table – 8: Inter -day precision data for rifaximin in 0.1N NaOH

Concentration (µg/ml)	Mean absorbance*			Mean ± SD	% RSD
	Morning	Afternoon	Evening		
2.4(80%)	0.039	0.040	0.030	0.039 ± 0.00070	1.8
3(100%)	0.050	0.050	0.051	0.0503 ± 0.001	1.4
3.6(120%)	0.059	0.058	0.060	0.059 ± 0.001	1.6

Table – 9: Accuracy data for rifaximin drug substance in 0.1N NaOH

Concentration (µg/ml)	Mean absorbance*			Mean ± SD	% RSD
	Morning	Afternoon	Evening		
2.4(80%)	0.054	0.056	0.056	0.055 ± 0.001	1.8
3(100%)	0.069	0.068	0.067	0.068 ± 0.001	1.4
3.6(120%)	0.077	0.077	0.078	0.077 ± 0.0007	0.9

Table – 10: accuracy data for rifaximin drug product in 0.1N NaOH

Amount of standard (µg/ml)	Amount of the sample (µg/ml)	Total concentration(µg/ml)	Total concentration found(µg/ml)	% recovered
4	3.2	7.2	7.1	98.6
4	4	8	7.9	98.75
4	4.8	8.8	8.78	99.77

Table – 11: Assay value of rifaximin in 0.1N NaOH

Formulation	Labelled amount(mg)	Amount obtained(mg)	% recovered
Rifagut (tablet)	400	390	97.50

Limit: 95-105%

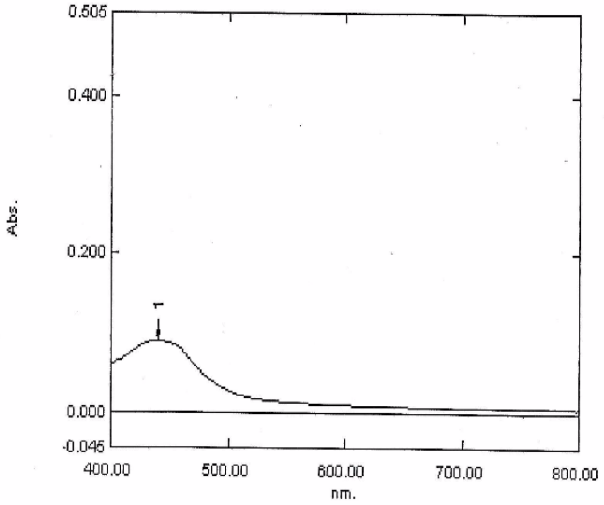


Figure-1: Absorption Spectrum of Rifaximin 1µg/mL with NaOH

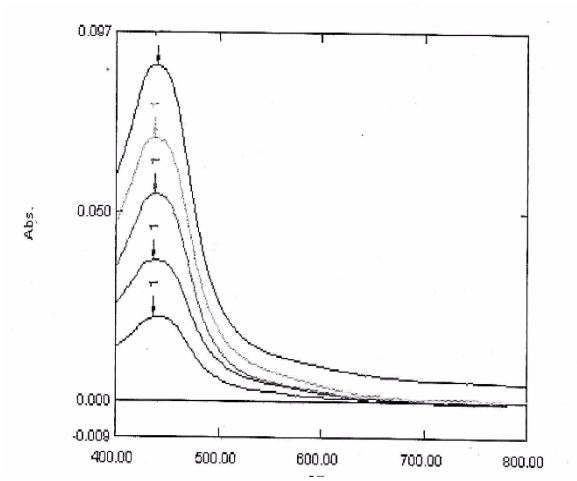


Figure- 2: Overlay spectrum of rifaximin using NaOH as solvent 1-5µg/mL (λmax 440nm)

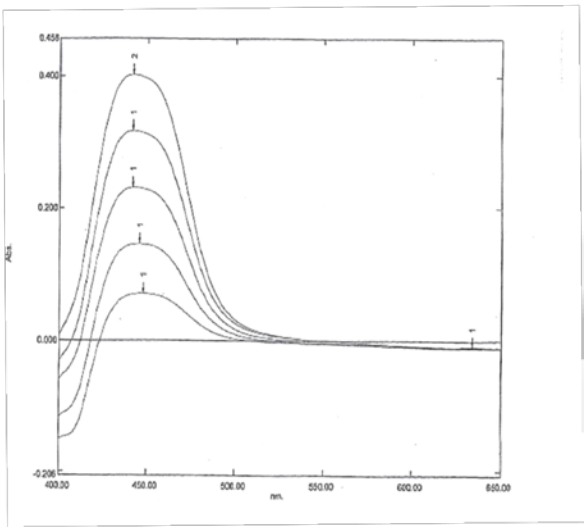


Figure- 3: Overlay spectrum of rifaximin using methanol as solvent 5-25 µg/mL(λmax=442 nm)

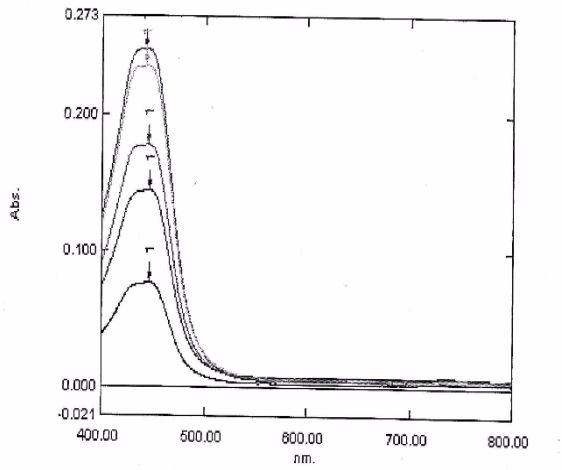


Figure-4: Overlay spectrum of rifaximin using phosphate buffer pH 7 as solvent 5-25µg/mL (λmax 441nm)

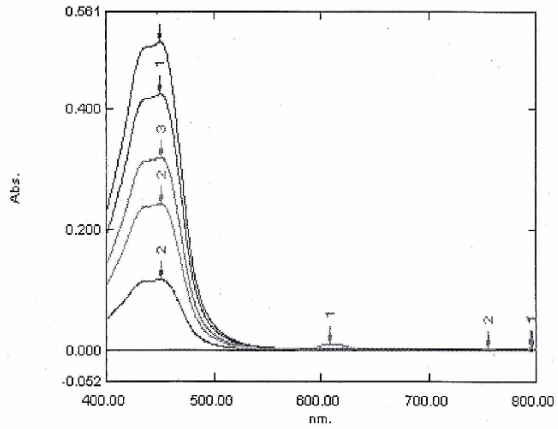
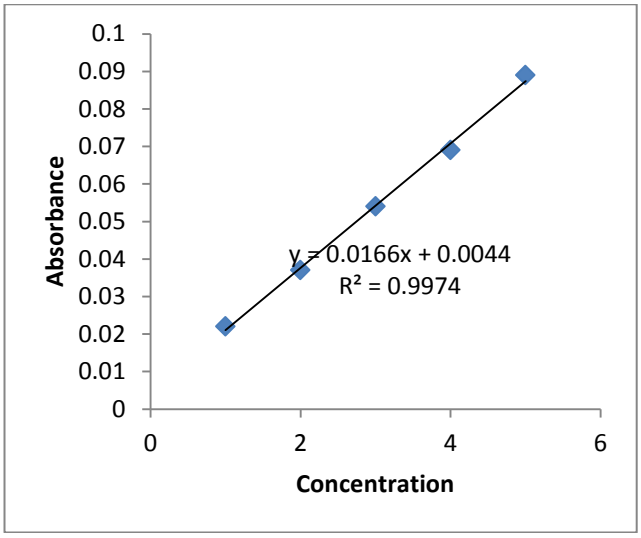
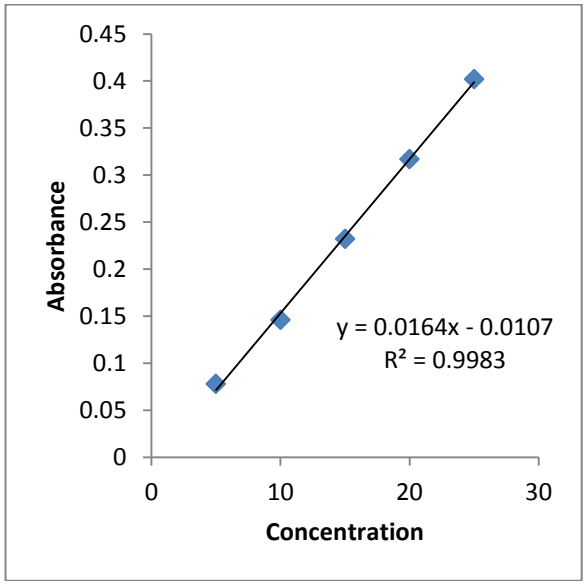


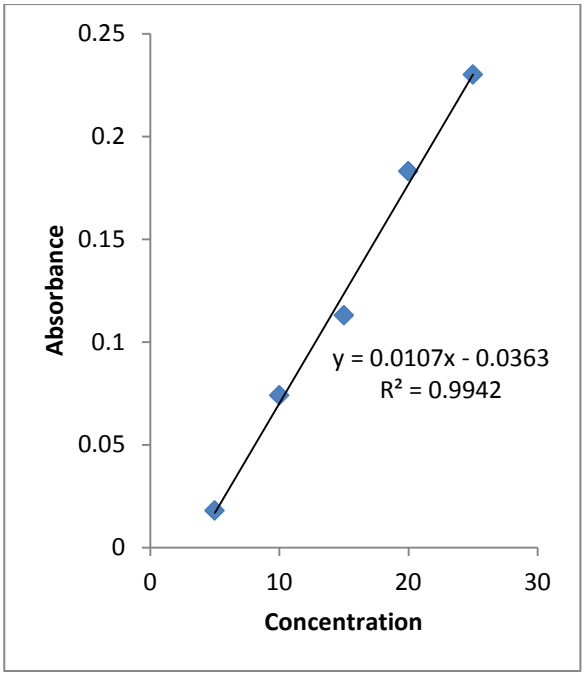
Figure-5: Overlay spectrum of rifaximin using phosphate buffer pH 7 and methanol (50:50 ratio) as solvent 5-25µg/mL (λmax 450nm)



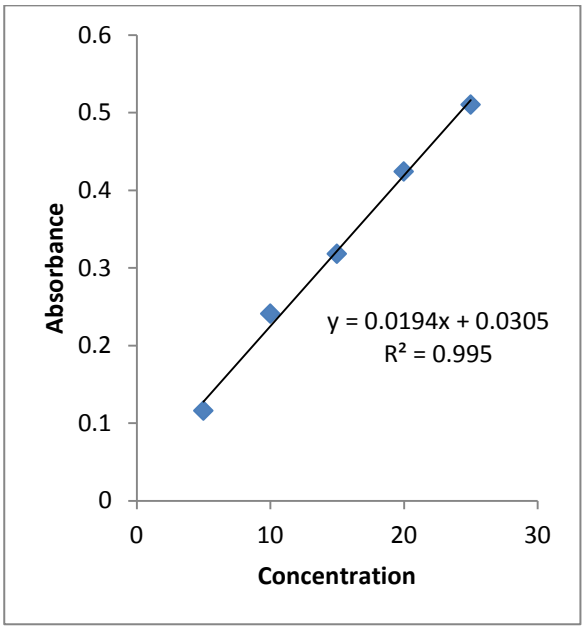
Graph – 1: Standard calibration curve of rifaximin using NaOH



Graph – 2: Standard calibration curve of rifaximin using methanol



Graph – 3: Standard calibration curve of rifaximin using phosphate buffer pH 7



Graph – 4: Standard calibration curve of rifaximin using phosphate buffer pH 7 and methanol

DISCUSSION:

The structure of rifaximin reveals the presence of hetero atoms, alkyl groups, alkoxy groups and aromaticity because of which the compound absorbs in visible region between 400 – 450nm and color of the compound reveal in the same. The λmax in 0.1 NaOH, methanol and phosphate buffer pH 7 is found to be 440nm where as in mixture of phosphate buffer pH 7 and methanol was found to be 450nm that is bathochromic shift was observed when compare to other solvents alone. Generally, increase in the polarity off solvents shifts n → σ^* , n → π^* transitions to

shorter wavelengths and $\pi \rightarrow \pi^*$ bands to longer wavelengths. In rifaximin above three transitions are possible. But there is no change λmax in 0.1 NaOH , in methanol and phosphate buffer pH 7. When compared to 0.1N NaOH and methanol the absorbance value in phosphate buffer pH 7 experienced an hypochromic effect, but in the mixture of methanol and phosphate buffer pH 7 an hyperchromic effect is observed. This may be because of $\pi \rightarrow \pi^*$ bands towards longer wavelengths, because of methanol and decreased hypsochromic shift on $n \rightarrow \sigma^*$ and $n \rightarrow \pi^*$ transitions because of phosphate buffer pH 7. The drug is insoluble in acidic conditions but soluble in pH 7 , which indicates there may be increased conjugation effect because of phosphate buffer pH 7 which ultimately showing bathochromic shift and hyperchromic shift .

CONCLUSION:

The effects of different solvents such as 0.1N NaOH, methanol, phosphate buffer ph 7 , mixture of phosphate buffer ph 7 and methanol on absorption characteristics of rifaxamin was studied. In 0.1N NaOH best linearity is observed at concentration range of 1-5µg/ml where as in other solvents linearity is observed at concentration range of 5-25 µg/ml .Hence the method developed in 0.1N NaOH is more sensitive and hence it is optimized, validated and applied for the estimation.

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