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Research Article

SPECTROPHOTOMETRIC ESTIMATION METHODS FOR RIFAXIMIN IN TABLET DOSAGE FORM

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Abstract

Two simple, sensitive, accurate and rapid spectrophotometric methods were developed for the estimation of Rifaximin in pure and tablet dosage forms. Method A is a colorimetric method based on the oxidative coupling reaction of Rifaximin with ferric chloride and 3-methyl 1, 2 benzothiazoline hydrazone hydrochloride (MBTH) reagent giving green colored chromogen which shows maximum absorbance at 637 nm against blank. Method B is Ultra Violet method which uses Alkaline Borate Buffer (pH-12) and the maximum absorbance was found to be 296 nm. Rifaximin Beer – Lambert's law in the concentration range of 5-25µg/ml in both method A & B. Sandell's sensitivity, molar extinction coefficient, slope, intercept, LOD and LOQ were determined for both the methods. The percentage recovery was found to be 100.03 ± 1.0608 and 100.10 ± 1.2928 in Method A & B respectively. The proposed methods were accurate, precise, reproducible and economical for the routine analysis of Rifaximin in bulk drug and in formulation.

Keywords: Rifaximin, Ferric chloride, 3- methyl 1, 2- benzothiazoline hydrazone hydrochloride, Alkaline borate buffer (pH-12), UV- visible spectrophotometry.

INTRODUCTION

Rifaximin[1 is] a newer antibiotic used for the treatment of patients (more than 12 years of age) with travelers diarrhea caused by non – invasive strains of *E.coli*. Rifaximin (RFX) is a benzimdazole derivative. RFX is a product of synthesis of Rifamycin, an antibiotic with low gastro- intestinal absorption and good antibacterial activity. It acts on the β – sub unit of the deoxyribonucleic acid (DNA) dependent ribonucleic acid (RNA) polymerase enzyme of the microorganism to inhibit RNA synthesis. RFX is not included in officially in any pharmacopoeia. Literature survey revealed that only application of derivative resolution of UV spectra to the quality control of Rifaximin and the possible impurities[2] and Rifaximin in human plasma by LC-MS[3] methods have been reported. UV-Visible methods has not been so far reported for the quantitation of RFX in bulk and in tablet dosage form. The present research work describes two simple, precise and accurate UV and Visible Spectrophotometric method for the estimation of RFX in bulk and tablet dosage form.

MATERIALS AND METHODS

A Shimadzu – 1700 Double Beam UV- Visible Spectrophotometer with 1 cm matched Quartz cells were used for all absorbance measurements.Rifaximin pure drug samples and tablet formulation was generously gifted by Zhizang Pharmaceuticals, China.The tablet formulation Rifaximin contains 100 mg of RFX. All the chemicals and reagents used were of analytical grade and procured from Qualigens India Ltd., Loba Chemicals Ltd.

Experiments

Preparation of standard stock solution

In method (A), 25 mg of Rifaximin was dissolved in 10 ml of methanol and made up to 100 ml with distilled water. In method (B), 25 mg of Rifaximin was dissolved in methanol and made up to 100 ml with methanol. Both the solutions contain 250 µg/ml of Rifaximin.

Calibration graph

Aliquots of the stock solution of RFX (1-5 ml of 250 µg/ml) were transferred into 50 ml standard flasks. To each flask, 5 ml of 4%ferric chloride and 4 ml of 0.4% MBTH reagents were added. The absorbance of the green colored chromogen formed was measured at 637 nm. Linearity was observed between 5-25 µg/ml for method (A). In method (B), aliquots of the stock solution of Rifaximin (1-5 ml of 250 µg/ml) were transferred into 50 ml standard flasks and made up to volume with alkaline borate buffer pH (12). The absorbances of the solutions of different concentrations were measured at 296 nm against blank. Linearity was observed between 5-25 µg/ml.

Analysis of formulation

In method (A) Twenty tablets of RFX containing 100 mg of Rifaximin was weighed accurately. Tablet powder equivalent to 25 mg of RFX was transferred to a 100 ml volumetric flask, dissolved in 10 ml of methanol and made up to volume with distilled water. The resulting solution was sonicated for 10 minutes and then filtered through Whatman filter paper no. 41.Transferred 3 ml of the filtrate into six [i]separate

100 ml volumetric flask. Then added 5 ml of 4% ferric chloride and 4 ml of 0.4% MBTH reagent and final dilution was made with distilled water. The absorbance of the resulting solution was measured at 637 nm. The procedure was repeated for six times. In method (B) powdered tablet equivalent to 25 mg of RFX was transferred to 100 ml volumetric flask, dissolved and made up to volume with methanol. The solution was sonicated for 10 minutes and filtered through Whatman filter paper no. 41. Transferred 3 ml of the filtrate to six 100 ml volumetric flasks and made up to volume with alkaline borate buffer pH 12.The absorbance was measured at 296 nm. The procedure was repeated for six times.

Recovery studies

In method (A), the recovery experiment was performed by adding a known concentration of RFX raw material to pre-analyzed formulation. The tablet powder equivalent to 25 mg of Rifaximin was weighed into a series of 3 x 100 ml standard flasks. To this 5, 10 and 15 mg of RFX raw material (20%, 40% and 60 %) was added and dissolved in 10 ml of methanol and finally made up to volume with distilled water. The solution was sonicated for 10 minutes and filtered through Whatmann filter paper no.41.Transferred 3 ml of each test solution and made into six replicates in 50 ml standard flask. Added 5 ml of 4%ferric chloride and 4 ml of 0.4% MBTH reagent and the final dilution is made up with distilled water. The absorbances of these solutions were measured at 637 nm against reagent blank. The same procedure was followed for method (B), up to the addition of raw material. From each standard solution, 3 ml of the clear filtrate was transferred into a series of six 50 ml volumetric flasks and made up to volume with alkaline borate buffer (pH 12). The absorbances of the resulting solutions were measured at 296 nm against blank. The procedure was repeated three times for each concentration.

Table 1: Optical Characteristics of Rifaximin

Parameters	Methods	
	A	B
Λ max (nm)	637	296
Beer's Law limit (µg/ml)	5-25	5-25
Sandell's sensitivity (µg/cm)	0.05299	0.03154
Molar absorptivity (L $mol^{-1}cm^{-1}$)	6.9388 x 10^{3}	1.16611 x 10^{3}
Correlation coefficient (r)	0.9999	0.9998
Regression equation (y=mx=c)	Y = 0.0189x + 0.0003	y = 0.03172x + 0.00086
Slope (m)	0.0189	0.03172
Intercept (C)	0.0003	0.00086
LOD (µg/ml)	0.4691	0.5588
LOQ (µg/ml)	1.4217	1.6934
Standard error of mean of Regression line	0.0030	0.0065

Repeatability

Repeatability is given by interday and intraday precision. The assay and recovery procedures were repeated for three times, on the same day and once for three successive days for both the methods.

Ruggedness

The degree of reproducibility of the test results obtained in method (A) and method (B) of RFX was detected by analyzing the drug sample under the variety of test conditions like different analyst and different instruments is ruggedness. The procedure was repeated under the above conditions.

RESULTS AND DISCUSSION

Two simple, precise and accurate ultraviolet and visible spectrophotometric methods were reported for the estimation of RFX in bulk and in tablet formulation. Method (A) is based on oxidative coupling reaction with ferric chloride and MBTH reagent, which gives green colored complex and it was measured at 637 nm. Method (B) is a simple UV method in which the drug, Rifaximin shows a maximum absorbance at 296 nm in alkaline borate buffer (pH 12). The optical characteristics such as Beer's law limit, molar extinction coefficient, Sandell's sensitivity, correlation coefficient, slope and intercept values for method (A) and method (B) were calculated and shown in Table-1. The spectrum obtained for method (A) and method (B) are shown in figure 1 and 2, respectively. Rifaximin was found to obeyed Beer's law in the concentration range of 5-25 µg/ml for both the methods. The correlation coefficient for method (A) and method (B) was found to be 0.9999 and 0.9998, respectively. The formulation Rifaximin was selected for analysis and the percentage purity was found to be 100.37 ± 0.9991 and 100.31 ± 0.4638 for method (A) and method (B), respectively. The procedure was repeated for six times to validate the methods. The developed methods were validated according to ICH [4,5] Guidelines.

Table 2: Results of analysis of Rifaximin in tablet formulation – Rifaximin (Label Claim 100 mg/tab)

S. No.	Amount Found*		% Label Claim*		S.D		%R.S.D		S.E	
	A	B	A	B	A	B	A	B	A	B
1	101.42	101.06	101.42	101.06						
2	100.21	100.71	100.21	100.71						
3	99.33	100.26	99.33	100.26	0.9991	0.4638	0.9954	0.4623	0.0278	0.0129
4	100.69	99.97	100.69	99.97						
5	101.45	100.04	101.45	100.04						
6	99.14	99.92	99.14	99.92						
		Mean	100.37	100.33						

The percentage RSD [6] was found to be less than 2%, which indicates that method (A) and (B) had good precision. The results of the analysis are shown in Table 2.Further the precision of the developed methods is confirmed by interday and intraday analysis. The results show good agreement with the label claim of the formulation which is shown in Table 3.The accuracy of the method was confirmed by the recovery studies. To the pre-analysed formulation, a different concentration of the raw material was added and the amount of the drug recovered was calculated. The percentage recovery was found to be 100.03 ± 1.0608 for method (A) and 100.10 ± 1.2928 for method (B). The procedure was repeated for three times. The % RSD value was calculated to be 1.0604 and 1.2915 for method (A) and method (B), respectively. The % RSD value indicates that there is no interference due to the excipients used in the formulation. Thus both the developed methods are found to be accurate, which is shown in Table 4. Both the methods were validated for ruggedness. The result confirmed the ruggedness of the developed methods. This is shown in table 3.

Table 3: Ruggedness and repeatability studies of Rifaximin

RUGGEDNESS									
S. No	Type of analysis	% Estimated*		S.D		%R.S.D		S.E	
		A	B	A	B	A	B	A	B
1.	Analyst – I	99.29	99.33	0.4026	1.0524	0.4055	1.0595	0.0111	0.0292
2.	Analyst – II	100.10	99.29	1.2095	1.3345	1.2119	1.3439	0.0335	0.1482
3.	Instrument – I	100.07	100.73	0.6415	0.8281	0.6411	0.8221	0.0178	0.0230
4.	Instrument – II	101.06	99.75	1.2513	0.7961	1.2380	0.7981	0.1390	0.0884
REPEATABILITY									
1.	Intraday	100.43	99.83	0.5629	2.0052	0.5605	2.0085	0.0156	0.2228
2.	Interday	100.10	100.22	0.3300	1.0480	0.3297	1.0457	0.0090	0.1164

Table 4: Recovery analysis of Rifaximin

Formulation	Label Claim	% Recovery*		S.D.		% R.S.D		S.E.	
		A	B	A	B	A	B	A	B
Rifaximin	**100mg/tab**	100.00	99.35						
		101.63	102.70						
		100.00	98.93						
		100.00	99.51						
		98.12	99.67	1.0608	1.2928	1.0604	1.2915	0.0294	0.0359
		101.12	99.66						
		99.88	98.87						
		100.56	101.53						
		100.00	100.77						
	Mean	100.03	100.10						

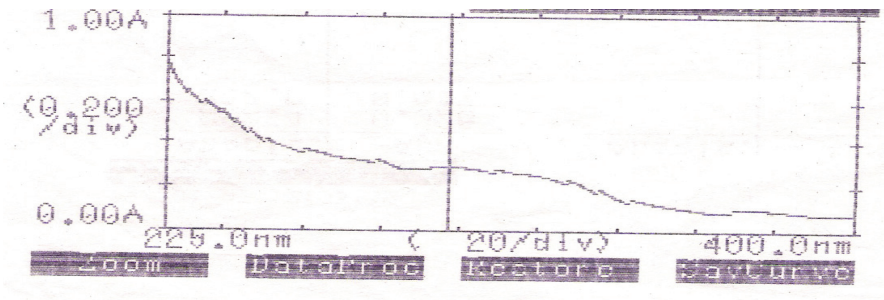

Fig. 1: UV Spectrum of Rifaximin in alkaline borate buffer pH12 (10µg/ml)

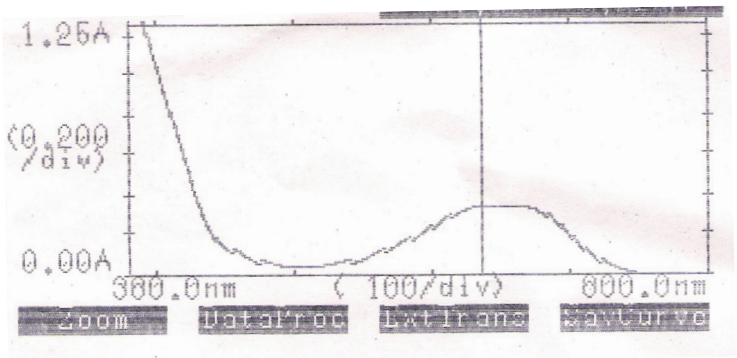

Fig. 2: spectrum of Rifaximin by Colorimetric method

Conclusion

The UV-Visible Spectrophotometric methods developed for RFX shows good precision and accuracy. The low percentage RSD values in the recovery studies for both the methods, indicates that there is no interference due excipients used in the formulation. Hence it is concluded that the developed methods are simple, precise, accurate and rapid for the analysis of Rifaximin in pure and in tablet dosage form. Thus the developed methods can be adopted for the routine analysis of Rifaximin in bulk and in tablet dosage form.

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