Asian Journal of Pharmaceutical and Clinical Research
Vol.2 Issue 4, October-December 2009 ISSN 0974-2441

Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF RIFAXIMIN IN BULK AND IN TABLET DOSAGE FORM

* T.SUDHA, Department of Pharmaceutical Analysis, The Erode College of Pharmacy, ERODE, 638112, E-mail: jvchrsty@yahoo.co.in
P.V.HEMALATHA, V.R.RAVIKUMAR, R.JOTHI, Department of Pharmacognosy, The Erode College of Pharmacy, ERODE 638112
M.RADHAKRISHNAN., Department of Pharmaceutics,The Erode College of Pharmacy, ERODE 638112

ABSTRACT
A simple, selective and rapid reverse phase high performance liquid chromatographic (RP-HPLC) method for the analysis of Rifaximin in bulk and in tablet dosage form has been developed and validated. Sample was resolved on a Luna Phenomenax, C_{18} (150mm X 4.6 mm i.d., particle size 5µ) column. The mobile phase consisted of methanol l:10 mM phosphate buffer (70:30 v/v pH adjusted to 3.0 by using orthrophosphoric acid) was delivered at a flow rate of 1.2 ml/min at ambient temperature and the retention time was about 5.12 minutes. Studies were performed on an HPLC system equipped with a UV/Visible detector at 293nm. The method is specific to Rifaximin and able to resolve the drug peak from formulation excipients. The calibration curve was linear over the concentration range of 5-30 µg/ml (R=0.9996).The results of analysis of formulation was found to be 100.31±0.5737. The lower limits detection for Rifaximin was found to be 0.0417 µg/ml and the quantification limit was about 0.1266 µg/ml. The proposed method is applicable to routine analysis of Rifaximin in bulk and in tablet dosage form.

KEY WORDS Rifaximin, RP-HPLC, Traveller's diarrhoea, validation.

INTRODUCTION

Rifaximin[1] (RFX) is a benzimdazole derivative. Chemically it is 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,25acetate. Rifaximin is a newer antibiotic, used for the treatment of patients (more than 12 years of age) with travellers diarrhoea caused by noninvasive strains of Escherichia coli. RFX is a product of synthesis of Rifamycin, an antibiotic with low gastrointestinal absorption and good antibacterial activity. It acts on the beta –subunit of the deoxyribonucleic acid (DNA) dependent ribonucleic acid (RNA) polymerase enzyme of micro organisms to inhibit RNS synthesis. RFX is not official in any Pharmacopoeia. Literature survey revealed that only the application of derivative resolution of UV spectra to the quality control of RFX and its possible impurities[2] and RFX in human plasma by LC-MS[3] methods was reported. Therefore, this study focused on the development of simple and rapid isocratic RP-HPLC method which can be employed for the routine analysis of RFX in bulk and in tablet dosage form.

MATERIALS AND METHOD

A Shimadzu HPLC system equipped with LC-10AVp UV visible detector, a Luna Phenomenax, C_{18}(150 mm X 4.6 mm i.d., particle size 5µ) column was used. The chromatographic and integrated data were recorded using Winchrom software system. The mobile phase consisted of methanol: phosphate buffer (70:30 v/v pH adjusted to 3.0 using orthophosphoric acid). The flow rate was 1.2 ml/min, the wavelength was monitored at 293nm and the injection volume was 20µl. Analytically pure RFX and tablet formulation was gifted by ZhiZang Sixian Pharmaceuticals, China. HPLC grade methanol and water

were procured from Qualigens India Limited, Mumbai. Analytical grade orthophosphoric acid and potassium dihydrogen phosphate were obtained from Loba Chemicals Ltd., Mumbai.Stock solution of RFX was prepared by dissolving 25 mg of RFX in 25 ml of methanol. Further dilution was made by diluting 2.5ml with mobile phase to obtain 50 µg/ml solution. Working standard solutions were prepared by diluting the stock solution with mobile phase to obtain final concentrations of 5, 10, 15, 20, 25 and 30 µg/ml of RFX. 20 µl of the solutions were injected and the chromatograms were recorded. The calibration was done by external standard calibration. The calibration graph was plotted by using peak area against concentration. The procedure was repeated for three times to determine the limit of detection and limit of quantification.

Table -1: System Suitability Studies

Parameters	Experimental value	Limit as per USP
Tailing factor	1.06	Less than 2
Asymmetric factor	1.07	Less than 2
Number of Theoretical plates	4291	More than 2000
Capacity factor	2.38	2 to 10
HETP	0.035	-
Theoretical plate Per unit length	286.2	-

Table- 2 Regression Statistics for analysis of Rifaximin

S.No	Range	r^2	Slope	Intercept	LOD	LOQ
1.	5-30	0.9997	289151.05	33637.85		
2.	5-30	0.9999	282300.75	23785.17	0.04178	0.1266
3.	5-30	0.9992	287789.17	41481.92		
	Mean	0.9996	286413.70	5313.70		

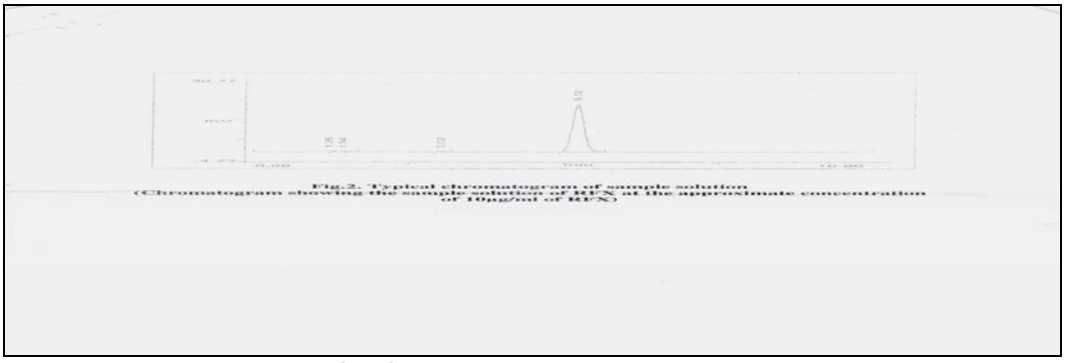

FIG. 1. RP-HPLC Data of Rifaximin

The marketed tablet formulation Rifaximin contains 100 mg of RFX (Zhizang Sixian Pharmaceuticals China). Twenty tablets were weighed accurately, finely powered and mixed. The average mass per tablet was determined. The tablet powder equivalent to 25 mg of Rifaximin was accurately weighed and added a minimum quantity of methanol to dissolve the substance; the total volume was brought to 25ml with more methanol (1000µg/ml) in six volumetric flasks. The solutions were sonicated for 10minutes and then filtered through Whatmann filter paper No.41, to separate out the insoluble excipients. Collected the filtrate after rejecting the first portion of the filtrate. From the clear solution, further dilutions were made by diluting 2.5 ml in to 50 ml with mobile phase to obtain 50 µg/ml solutions. Further dilution was made by diluting 2 ml in to 10 ml with mobile phase. 20 µl of each solution was injected and the chromatograms were recorded. The peak area was determined. The amount of RFX in the tablet formulation was calculated from the calibration graph. The procedure was repeated for six times. The accuracy of the method was confirmed by recovery studies. A known quantity of the raw material was added to the previously analysed test solution and the amount of drug recovered was calculated. The tablet powder equivalent to 25 mg of RFX was accurately weighed in to 25 ml volumetric flask , dissolved in methanol and made up to volume with methanol. The solution was then sonicated for 10 minutes, filtered through Whatmann filter paper No.41. Then 2 ml of the filtrate was pipetted out into 50 ml volumetric flask and made up to the volume with mobile phase (50 µg/ml). To each 2 ml of the preanalysed formulation solution (50 µg/ml) was added 1,2,3,4,5 and 6 ml of raw material stock solution (20 µg/ml) in to 10 ml volumetric flasks. Each flask was made up to 10 ml with mobile phase. The procedure was then repeated as per the analysis of formulation.

Table- 3 Results of analysis of Rifaximin in pharmaceutical formulation

S.No	Formulation	Labeled Amount (mg/tab)	Amount Found* (mg/tab)	% Label claim*	S.D	%R.S.D	S.E
1			101.69	101.69			
2			99.18	99.18			
3	Rifaximin Tablet dosage form	100	99.76	99.76	1.3606	1.3541	0.0377
4			100.10	100.10			
5			102.61	102.61			
6			99.53	99.53			
		Mean	100.37	100.47			

Table 4- Recovery Studies for formulation

S.NO	Amount Present (µg)	Amount Added (µg)	Amount Estimated (µg)	Amount Recovered (µg)	% Recovery	Average	S.D	%RSD	S.E
1.	10.04	1.98	12.02	1.98	100.00				
2.	10.04	3.96	13.98	3.94	99.45				
3.	10.04	5.94	16.02	5.98	100.67	100.31	0.5737	0.5719	0.0159
4.	10.04	7.92	17.99	7.95	100.38				
5.	10.04	9.90	20.07	10.07	101.13				
6.	10.04	11.88	21.95	11.91	100.25				

RESULTS AND DISCUSSION

A simple, precise, accurate RP-HPLC meyhod has been developed for the estimation of RFX in bulk and in tablet formulation. A shimadzu HPLC system with Luna Phenomenax C_{18} column was used for analysis.The mobile phase optimized with methanol 10mM phosphate buffer in the proportion of 70:30 v/v column was used for analysis with the above mentioned composition of mobile phase. Sharp peak was obtained with the retention of 5.12 minutes. The UV detection was carried out at 293 nm, as RFX showed very good absorbance at this wavelength. An opimized chromatogram of RFX is shown in figure 1. The system suitability parameters like tailing factor, asymmetric factor, number of theoretical plates and capacity factor were calculated and these values were compared with the standard limit as per USP.[4] It was found that the values were within the limits (TABLE 1). The linearity of an analytical method is its ability to elicit a test result that are directly, or by a well defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity of the method was observed with in the expected concentration range demonstrating its suitability for analysis. The linearity of RFX was obeyed Beer's law in the range of 5-30 µg/ml. The correlation coefficient 'r^2'value (n=3) for RFX was found to be 0.9996 and the value of intercept was less than 2% of the response of 100% of the test concentration in all the cases indicating functional linear relationship between the concentration of analyte and area under the peak area. The proposed method was validated as per ICH guidelines.[5,6] The detection limit or LOD is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as the concentration that gives a signal to noise ratio of 2:1 or 3:1. Based on the standard deviation of the response and slope,the detection limit (DL) may be expressed as $DL=3.3\ \sigma/S$. The lower limit of detection for Rifaximin was found to be 0.0417 µg/ml. Quantitation Limit or LOQ is the lowest amount of analyte in a sample

that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal to noise ratio 10:1 can be taken as LOQ of the method. Based on the standard deviation of the response and slope,the Quantitation Limit(QL) may be expressed as QL = 10 σ/S. The LOQ was found to be 0.1266 µg/ml (TABLE 2). Precision is the degree of reproducibility or repeatability of the method under normal operating conditions. The method passed the test for repeatability as determined by %RSD[7] of the peak area of six replicate injections of the test concentrations. The percentage concentration of RFX was found to be 100.47 ± 1.3606. The low % RSD value indicated that the method has good precision. This is shown in Table 3. To evaluate the accuracy of the method, known amount of pure drug was added to the previously analyzed solution containing pharmaceutical formulation and the mixture was analyzed by the proposed method and the recoveries were calculated. The percentage recovery of RFX was found to be in the range of 99.45 ± 101.13. The %RSD value was found to be 0.5719. The low %RSD value indicated that there is no interference due to the excipients used in formulation. Hence the accuracy of the method was confirmed (Table 4). Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix. The chromatogram of formulation is shown in FIG., which illustrates that the excipients used in the formulation did not interfere with the drug peak, so, the method is specific for Rifaximin.

The reproducibility and accuracy of the methods were found to be good which is evidenced by low standard deviation. The percentage recovery values obtained indicate no interference from excipients used in formulations. Hence proposed method is new, simple, rapid, sensitive, accurate and precise and can be successfully applied for the estimation of Rifaximin in bulk and in tablet dosage form.

REFERENCES

1. www.Drugbank.com.
2. Zhang X, Duan J, LiK Zhou L, Zhai S. Sensitive Quantification of Rifaximin in Human Plasma by Liquid Chromatoraphy-Tandem Mass Spectrometry. Journal of Chromatography Bio Analytical Technology Biomed Life Sciences, 2007; 850; 348-355.
3. Cort P, Savini L, Celesti G, Monotecchi L. Application of derivative resolution of UV spectra to the quality control of Rifaximin and it is possible impurities. Pharma Acta Helv, 1992; 67; 76-79.
4. The United States of Pharmacopoeia, National formulary USP 23. Asian Edition. Rockville MD; United States Pharmacopoeial Convention, Inc; 1995.
5. International Conference on Harmonisation, Guidance for Industry In; Q2A Text on Validation of Analytical Methods, Switzerland; IFPMA, 1994; 1-4.
6. International Conference on Harmonisation, Guidance for Industry In; Q2B Validation of Analytical Procedures, Methodology, Switzerland; IFPMA 1996; 1-8.
7. Gupta SC, Kapoor VK. Fundamentals of Mathematical Statistics. 9th ed. Sultan Chand and Sons, New Delhi; 1994; 26; 3.2-3.8.