Annals of Phytomedicine 12(1): 595-600, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.1.66

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Original Article : Open Access

Analytical method development and validation of rifaximin and ornidazole in bulk and combined tablet dosage form as per ICH guidelines

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Article Info

Article history
Received 4 April 2023
Revised 20 May 2023
Accepted 21 May 2023
Published Online 30 June-2023

Keywords
Rifaximin
Ornidazole
HPLC analysis
Pharmaceutical formulation
Validation

Abstract

A precise and accurate HPLC method was developed for the detection of ornidazole and rifaximin in bulk and combination dosage form. Chromatography was done using acetonitrile and ammonium acetate buffer as the mobile phase, at a 65:35 ratio, on a 250 x 4.6 mm, 5 µm hypersil C18 column. Based on the features superimposed on it, a detection wavelength of 280 nm was selected for the study. According to ICH criteria, the procedure was verified for rifaximin. Linearity was determined to be 100 to 300 g/ml for ornidazole 125 to 375 g/ml for rifaximin. Ornidazole and rifaximin both had correlation coefficients of 0.9999. The formulation analysis findings and the label claim were in good accord the accuracy of the approach was demonstrated by the percentage RSD, which was determined to be 0.15 per cent for ornidazole and 0.23 per cent for rifaximin, respectively, recovery studies validated the method precision. Ornidazole's percentage recovery was found to be between 100.54 per cent and 100.33 per cent and rifaximin percentage recovery was found to be between 99.90 per cent and 100.34 per cent, demonstrating the method accuracy. The findings of the validation parameters demonstrate the method adequacy for the simultaneous estimate of the chosen medicines. The suggested RP-HPLC processes are accurate, precise, robust, repeatable, and suitable for routine quality control analysis of rifaximin and ornidazole in bulk and in its combination medicinal dosage form.

1. Introduction

Analytical chemistry is one of the most important tools in the pharmaceutical sector. In the modern period, when more pharmaceuticals are being developed, the significance of analytical chemistry has expanded in the production of various pharmaceutical items (Saranjit Singh *et al.,* 2001). The quantitative and qualitative analysis of several raw materials, completed goods, and intermediate items benefit greatly from drug analysis (Nunes Salgado *et al.,* 2016). In actual practise, extreme care should be taken to ensure that pharmaceutical analysis yields reliable and precise results (Maryadele *et al.,* 2006). The official pharmacopoeias include a number of specifications (Kendre *et al.,* 2009; Mounika *et al.,* 2021). These pharmacopoeias do not; however, provide information on newer medications. Therefore, it is crucial to create better, more accurate analytical techniques that are linear, quick, easy, and simple (Punit R *et al.*, 2019; Amisha *et al.,* 2021). The more recent analytical methods should be affordable to in pharmaceuticals and biological fluids; a number of investigations on ornidazole and rifaximin have been documented, according to the literature review (Tripathi *et al.,* 2008; Divya Singh *et al.,* 2021). Due to numerous extraction processes and laborious sample preparations, such as liquid-liquid extraction, centrifugation, and electrophoresis techniques, certain approaches are complicated and time-consuming (Yamina Bouatrous *et al.,* 2019). In the current effort, a straightforward, precise, and exact RP-HPLC technique is being developed and validated for the simultaneous measurement of ornidazole and rifaximin in bulk and combination tablet dosage form (Himanshu Gupta *et al*., 2020).

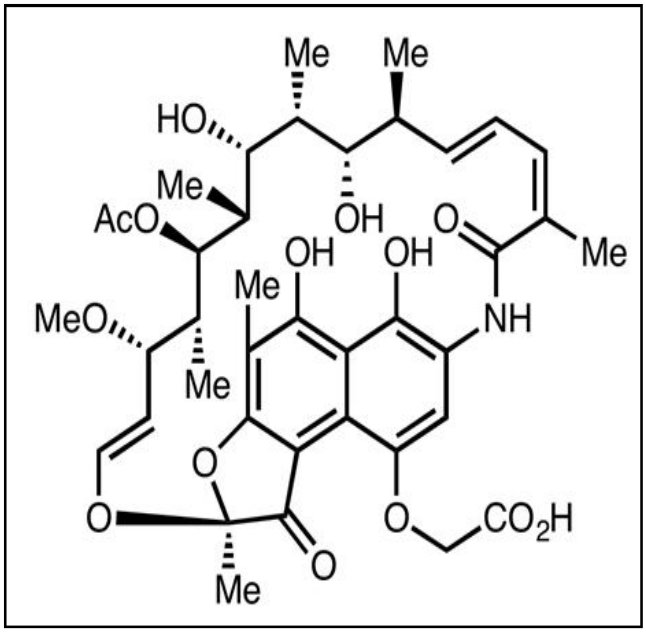

Figure 1: Structure of rifaximin.

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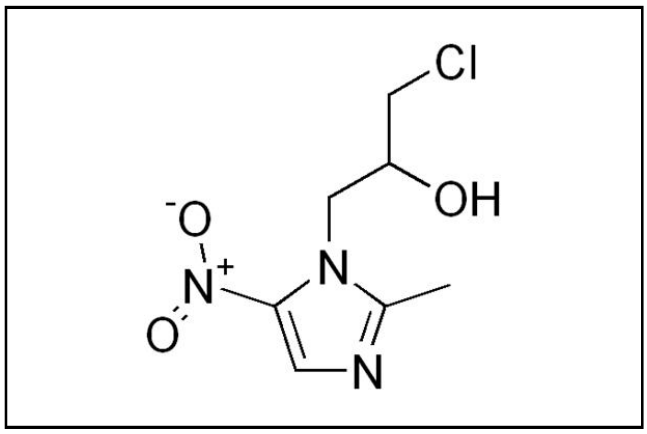

Figure 2: Structure of ornidazole.

2. Materials and Methods

2.1 Chemicals and reagent

Waters 2996 PDA detector waters 2487 dual absorbance detector software empower 3 column hypersil C_{18} (250 x 4.5 mm, 5 µm) pump - LC - 20 ATVP series injector autosampler detector, PDA detector, LC - 20, 2989 and 2487 syringe filter - PTFE, 0.4 µm pore LC injection vials - 2 ml with teflon coated caps.

2.2 Instrumentation and apparatus required

Uni bloc - analytical balance shimadzu LC - 2010 AHT, HPLC system, lab solution waters 2487, photodiode array detector waters 2996 Boost software shimadzu with an IR solution, IR affinity 1 model LI-120 pH metre from ELICO centrifuge device REMI micropipette, CYBERLAB.

2.3 Chromatographic conditions

Relative retention periods for the peaks of ornidazole and rifaximin are approximately 3.6 and 8.6, respectively.

2.4 Buffer preparation

1000 ml of distilled water was used to dissolve 4.5 g of ammonium acetate, and the pH was then corrected to 2.5 using diluted orthophosphoric acid. It was degassed after being filtered using a 0.45 µm membrane filter.

2.5 Preparation of mobile phase

Buffer: acetonitrile 65:35 v/v was used as the mobile phase, which was then removed, sonicated, degassed for 10 minutes, and filtered through a 0.45 µm membrane filter.

2.6 Preparation of diluent

Water: acetonitrile 60:40 v/v diluent was collected, sonicated, and degassed for 10 minutes before being filtered through a 0.45 µm membrane filter.

2.7 Standard preparation

Standard preparation involved accurately weighing and transferring 50 mg of ornidazole and 40 mg of rifaximin into a 200 ml volumetric flask.

2.8 Sample preparation

Sample preparation involved accurately weighing and transferring 50 mg of ornidazole and 40 mg of rifaximin into a 200 ml volumetric flask.

3. Results

3.1 Method validation

3.1.1 Specificity

Blank solution, placebo solution, sample solution, and standard solutions were used to assess specificity. At the retention time of the chromatographic peak for rifaximin and ornidazole, neither the placebo nor the blank solution caused any interference under ideal HPLC conditions.

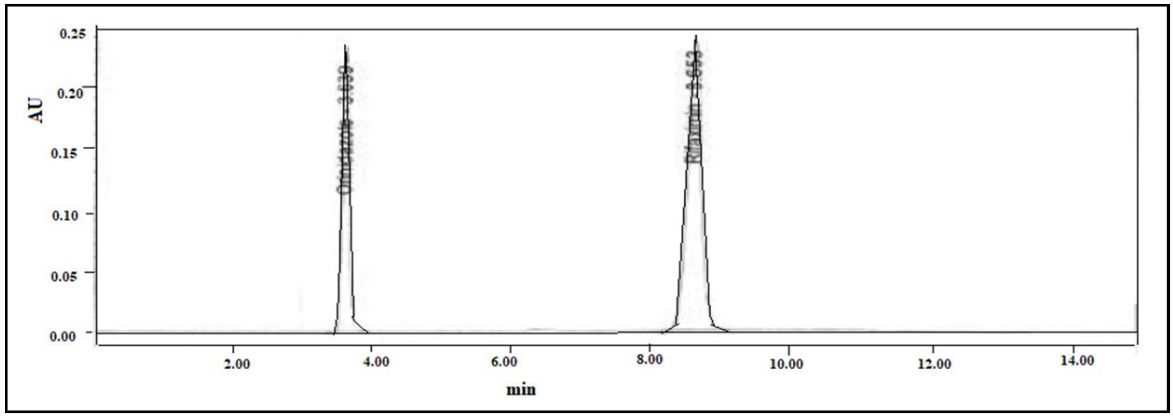

Figure 3: Standard peak of ornidazole and rifaximin.

Table 1: Specificity studies for ornidazole and rifaximin standard

Peaks	Name	Retention time	Area
01	Ornidazole	3.639	2512561
02	Rifaximin	8.653	5125451

3.1.2 Accuracy

At known concentrations of ornidazole and rifaximin that were 50%, 100%, and 150% of the test concentration of the sample solution, the analytical method accuracy was assessed. Rifaximin and ornidazole's per cent accuracy at the individual level and overall average of per cent recovery at every level were found to be in the range of 100.30 and 100.24 per cent, 100.10 and 100.38 per cent, 100.29 and 100.06 per cent, respectively, and their respective RSD were found to be 0.35 and 0.45 per cent, 0.23 and 0.15 per cent, 0.12 and 0.19 per cent, as shown in Table 2.

Table 2: Accuracy studies for ornidazole and rifaximin

Accuracy levels	Triplicate	% Recovery		Average % recovery (n = 3)		% RSD (n=3)	
		Rifaximin	Ornidazole	Rifaximin	Ornidazole	Rifaximin	Ornidazole
50%	Sample-1	99.93	100,15	100.30	100.24	0.35	0.45
	Sample-2	100.61	99.84				
	Sample-3	100.35	100.73				
100 %	Sample-1	100.34	100.54	100.10	100.38	0.23	0.15
	Sample-2	100.07	100.26				
	Sample-3	99.90	100.33				
150%	Sample-1	100.43	99.85	100.29	100.06	0.12	0.19
	Sample-2	100.21	100.20				
	Sample-3	100.25	100.14				

3.1.3 Method precision

For the evaluation of method precision of the analytical method, six samples from homogenous mixture of single batch were prepared as per the test procedure of methodology and analyzed on HPLC system. % RSD for % assay of ornidazole and rifaximin of six samples found to be 0.33% and 0.34% as tabulated in Table 3.

Table 3: Method precision studies for ornidazole and rifaximin

No. of sample	% Assay (ornidazole)	% Assay (rifaximin)
01	100.09	100.43
02	99.65	99.62
03	99.75	99.65
04	100.25	100.15
05	100.03	99.87
06	100.42	99.60
Mean	100.09	99.89
% RSD	0.33	0.34

3.1.4 System precision

The standard solution was injected into the HPLC apparatus six times in duplicate, and the chromatograms and area ratio of ornidazole and rifaximin were recorded. For ornidazole and rifaximin, it was found that the theoretical plates and the tailing factor were 12980, 14870 and 1.0,1.1, respectively. The 1.0 and 1.2 per cent RSD for the area ratio of ornidazole and rifaximin from six replicate injections of standard solution demonstrate the accuracy of the technique.

3.1.5 Ruggedness

Six samples from a homogeneous mixture of single batches were analyzed by several analysts using various columns, various systems, and various days in order to determine the ruggedness. Ornidazole and rifaximin's % RSD for the test of ruggedness samples were determined to be 0.33 and 0.34%, respectively, as shown in Table 5.

Table 4: System precision studies for ornidazole and rifaximin

Name of the parameter	Ornidazole	Rifaximin
Retention time (RT)	3.6	8.6
Area (% RSD not more than 2.0)	1.0	1.2
Tailing factor (Not more than 1.8)	0.9	1.1
Theoretical plates (Not less than 2000)	12980	14870

Table 5: Method ruggedness studies for ornidazole and rifaximin

No. of sample	% Assay (ornidazole)	% Assay (rifaximin)
01	99.65	99.62
02	100.25	100.15
03	100.42	99.60
04	100.03	99.87
05	99.75	99.65
06	100.09	100.43
Mean	100.09	99.89
% RSD	0.33	0.34

3.1.6 Linearity

Rifaximin and ornidazole were present in concentrations ranging from 100 to 300 and 125 to 375 g/ml, respectively, and the analyte response was linear (r^2=0.9999). Table presented the findings, and figure depicts the calibration curve. The graph demonstrates that over a broad concentration range, the chosen concentration provides sufficient accuracy and precision. The findings show a strong association between the concentration of rifaximin and ornidazole medicinal ingredient and its absorbance.

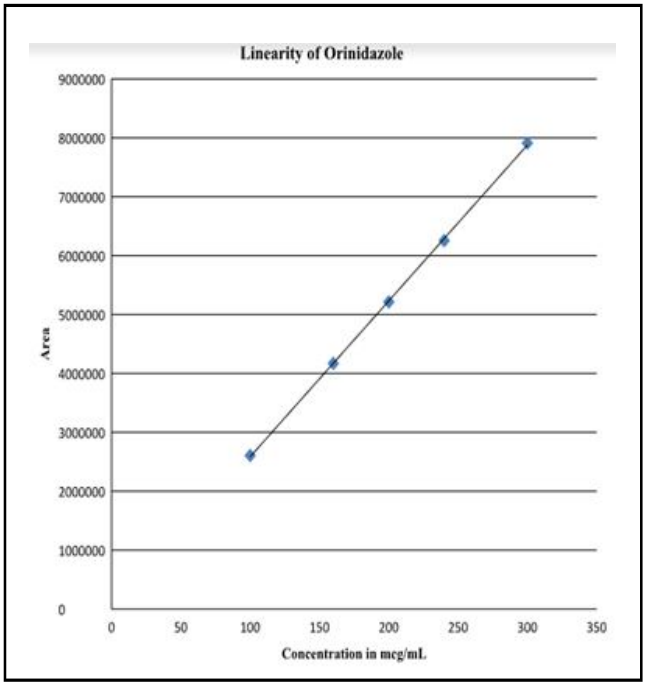

Figure 4: Ornidazole linearnity.

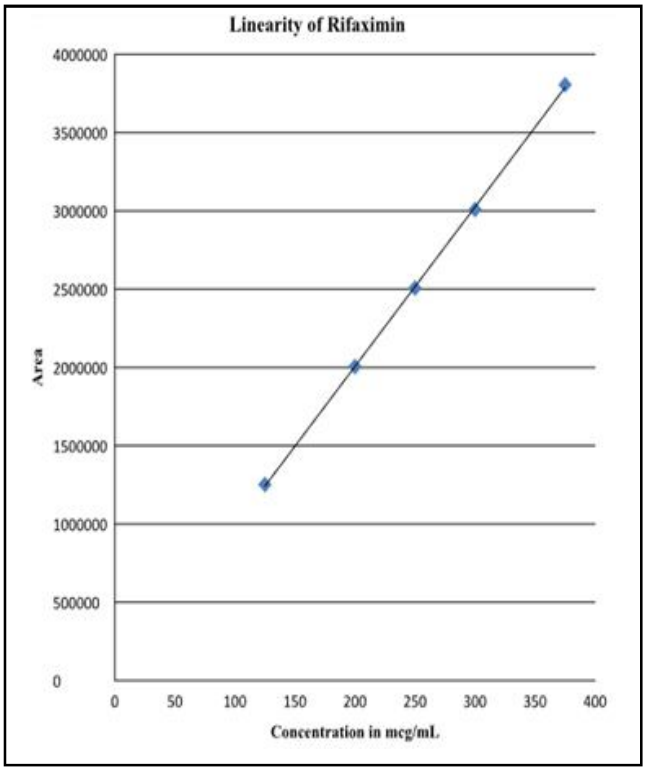

Figure 5: Rifaximin linearnity.

3.1.7 Robustness

The robustness of the approach was shown by making small, purposeful changes to the flow rate, wavelength, column temperature, and mobile phase composition. The sample was injected into six replicates, and the % RSD was calculated.

3.1.8 Filter validation

It was discovered that the outcomes and percentage difference between centrifuged samples, 0.45-micron PVDF filters, and 0.45-micron nylon filter samples all fell within the acceptable range.

Table 6: Filter validation studies for ornidazole and rifaximin

Filter details	% Results	% Difference
Centrifuged (unfiltered)	98.00	-
0.45µ nylon Filter 1 ml discarded	98.00	0
0.45µ nylon Filter 2 ml discarded	96.00	-2.5
0.45µ nylon Filter 4 ml discarded	96.00	-2.5

3.1.9 Solution stability of analytical solutions

A variety of rifaximin and ornidazole conditions, including bench top at ambient temperature and in a refrigerator between 2 and 8 degrees Celsius, were used to establish the stability of analytical solutions. The stability of standard and sample solutions was established by comparing initially prepared and sample solutions with recently prepared standard solutions. Table 7 presents results for standard solution stability displays the information obtained.

Table 7: Solution stability studies for ornidazole and rifaximin

Time period	Similarity factor	
	Ambient temperature	Refrigerator
Initial	N.A	N.A

Table 8: Solution stability studies for standard of ornidazole and rifaximin

Time point	% Recovery refrigerator
0 h	NAP
6 h	100.4
20h	99.9
24 h	99.7
36 h	100.6
48 h	99.4

Table 9: Solution stability studies for sample of ornidazole and rifaximin

Time point	% Recovery refrigerator
0 h	NAP
6 h	100.7
20 h	99.6
24 h	99.7
36 h	100.9
48 h	99.4

4. Discussion

The current work focuses on the development of an analytical method for the simultaneous measurement of rifaximin and ornidazole in tablet dosage form and bulk form. The techniques created include the RP-HPLC with PDA method and the RP-HPLC method.

A precise reverse phase chromatography was developed and used to estimate rifaximin and ornidazole concurrently. Rifaximin and ornidazole were combined to make a solution that contained 200 mg/ml of each. This solution was then scanned in the UV spectrum between 200 and 400 nm. At 280 nm, both drugs showed appreciable absorption. Therefore, 280 nm was selected as the detecting wave length. The chromatographic conditions were enhanced through a series of studies in which the column, flow rate, and mobile phase composition were varied. During the tests, it was discovered that there was a splitting effect, a tailing effect, longer retention duration, asymmetry, and decreased resolution. Following optimisation of the chromatographic conditions, a standard solution of rifaximin and ornidazole at concentrations of 200 g/ml of rifaximin and 250 g/ml of ornidazole was prepared using a mobile phase mixture of ammonium acetate buffer and acetonitrile in a composition of 65:35 with a pH of 2.5 and orthophosphoric acid. Stock solutions with concentration ranges of 100-300 mg/ml for rifaximin and 125-375 mg/ml for ornidazole were used to manufacture the drugs. Then, chromatograms were captured. The correlation coefficient, intercept, and slope were computed. The correlation coefficient for ornidazole was found to be 0.9999, while the correlation value for rifaximin was also 0.9999. A calibration curve was made by plotting concentration versus peak area. The calibration curve shows that a linear response was obtained for the applied concentration range. information on linearity and range. To evaluate the specificity of the approach, the chromatograms of the placebo and blank samples were recorded. It has been established that the placebo and blank have no impact on the retention time of the selected drugs. The pills containing 200 mg of rifaximin and 250 mg of ornidazole were chosen for the analysis. Using the prepared nominal concentration of rifaximin and ornidazole derived from the linearity, the area of the solution was measured at the selected wavelengths. The percentage label claim of the tablet formulation for ornidazole was found to be 249.84 mg and RSD 0.47%, whereas the percentage claim for rifaximin was 199.13 mg and RSD 0.54%. The amount in the tablet formulation closely matched the amount stated on the label. The percentage relative standard deviation (% RSD) values for ornidazole and rifaximin were discovered to be 0.47% and 0.54%, respectively, the low% RSD value is proof that the method has good precision. The standard stock solutions were used to create solutions of ornidazole and rifaximin with concentrations of 200 g/ml and 250 g/ml, respectively, which were then injected into the chromatographic system in six replicate injections. The tailing factor, theoretical plates, and % RSD were calculated as system suitability traits. The acceptability limit was determined to be met for the theoretical plates, tailing factor, resolution, and percent RSD of ornidazole and rifaximin. The results show that the approach was created with good precision.

5. Conclusion

The robustness of the developed approach was validated. Rifaximin and ornidazole both had percentage RSD values of 0.34% and 0.33% respectively. The method's robustness is demonstrated by the low% RSD results. Recovery analysis demonstrates the method's accuracy. Three various concentrations, such as 50%, 100%, and 150% concentration, of the reference pharmaceuticals were added in known amounts to the pre-analyzed formulation. The solutions' surface area was measured, and recovery % was computed. Ornidazole and rifaximin recovery percentages were determined to be in the range of 100.54 to 100.33% and 99.90 to 100.34%, respectively, demonstrating good technique accuracy. By purposefully making minor adjustments to the chromatographic conditions, robustness was examined. The mobile phase ratio was modified to 63:37 v/v and 67:33 v/v, the flow rate to 1.9 ml/ min and 2.1 ml/ min, the wavelength to 278 nm and 282 nm, the pH of the buffer to 2.3 and 2.7, and the column temperature to 23°C and 27°C. It was noted that there were no noticeable changes in the technique parameters, proving the approach's robustness. After 24 h, 36 h, and 48 h, the solution stability of the initial ornidazole and rifaximin standard solution and test solution was examined. The standard and test solutions are stable for up to 48 h. Centrifuge, PVDF 0.45 m, nylon 0.45 m, and PTFE 0.45 m filter integrity of ornidazole and rifaximin standard solution and test solution were examined. Test solution's filter integrity indicated a variation of less than 2.0%.

Acknowledgements

The author thank the management and staffs, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science , Technology and Advanced Studies (VISTAS), Green Tree Testing Labs Pvt.Ltd, Chennai for providing us the raw material to carry out this work,

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **K. Subhamalar and M. Vijey Aanandhi and Afroz patan (2023). Analytical method development and validation of rifaximin and ornidazole in bulk and combined tablet dosage form as per ICH guidelines. Ann. Phytomed., 12(1):595-600. http://dx.doi.org/10.54085/ap.2023.12.1.66.**