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Development and Validation of RP-HPLC Method for estimation of Ornidazole in Ornidazole injection 5mg/mL

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for estimation of Ornidazole in injection formulations. The separation was achieved by using column Inertsil ODS-3V (150×4.6 mm, 5µ), in mobile phase consisted of acetonitrile and pH 3.0 phosphate buffer, adjusted to pH 3.0 with the help of dilute orthophosphoric acid in the ratio of (10:90, v/v). The flow rate was 2.0 mL/min-1 and the separated Ornidazole was detected using UV detector at the wavelength of 300 nm. Column temperature 25°C and sample temperature ambient and injection volume 20µL. The retention time of Ornidazole, was noted to be 12.05 min respectively. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: Liquid Chromatography, Ornidazole, Force degradation and Validation.

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INTRODUCTION

Ornidazole, a 5-nitroimidazole is used in the treatment of protozoal infections and also in the treatment and prophylaxis of anaerobic infections. It has been investigated for use in Crohn's disease after bowel resection. Chemically, it is 1-chloro-3-(2-methyl-5-nitro-1 H-imidazol-1-yl propan-2-ol. It is used either as monotherapy or in combination with cephalosporins and in fluoroquinolone antibiotics [1-6]. Molecular formula is $C_7H_{10}ClN_3O_3$ and Molecular weight 219.63 g/mol. The chemical structure of Ornidazole shown in (Figure 1).

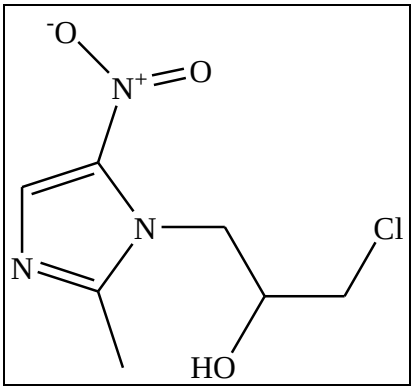

Figure 1: Chemical Structure of Ornidazole

A few analytical methods have been reported for the determination of Ornidazole in pure drug, pharmaceutical dosage forms and biological samples using spectrophotometry [7-8], Ornidazole in its various drug combinations have been estimated using UV-Spectrophotometry [9-16] high performance liquid chromatography [17-18], high performance thin layer chromatography [18-19].

The objective of the present work is to develop a stability indicating HPLC method and validated as per ICH guidelines [20] for the estimation of Ornidazole in applied for routine analysis in laboratories and is suitable for the quality control of the raw materials.

MATERIALS AND METHOD

Chemicals and Reagents

Analytical-grade Potassium dihydrogen phosphate, ortho phosphoric acid, were from Merck Chemicals Mumbai, India. Acetonitrile, Methanol and Water, both HPLC-grades, were from Merck Chemicals. Mumbai, India. Millex syringe filters (0.45 µm) were from Millex-HN, Millipore Mumbai, and India.

Instrumentation

Agilent-1200, Open-lab software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) Centrifuge Eppendorf 5810 were use in the present assay.

Preparation phosphate Buffer pH 3.0:

Accurately weighed and transferred 1.36 g of potassium dihydrogen phosphate in 1000 mL of water and mixed well and pH adjusted to 3.0 with ortho phosphoric acid. The solution was filtered through 0.45µ filter paper and degassed.

Mobile phase preparation

900 volumes of pH 3.0 phosphate buffer and 100 volumes of acetonitrile in the ratio of 90:10 volume/volume and sonicated to degas.

Diluent preparation

Mixed 500mL water and 500mL methanol in the ratio of 50:50 volume/volume and sonicated to degas.

Preparation standard preparation:

Weighed accurately and transferred 25.0 mg of Ornidazole working standard into 100 mL volumetric flask. Added 50 mL of diluent and sonicated to dissolve for 5 minutes. Dilute to the volume with diluent and mix well.

Further diluted 5.0 mL of the above solution into 50 mL volumetric flask with diluent and mixed well (25µg/mL)

Placebo solution:

Accurately transferred 1 ml of placebo into 200 ml of volumetric flask, added 50 mL of diluent and sonicated to dissolve for 5 minutes. Dilute to the volume with diluent and mix well.

Preparation of test solution:

Accurately transferred 1 ml of Ornidazole test solution into 200 ml of volumetric flask, added 50 mL of diluent and sonicated to dissolve for 5 minutes. Dilute to the volume with diluent and mix well.

Chromatographic conditions

Chromatographic analysis was performed on Inertsil ODS-3V (150x4.6 mm, 5µ) column. The mobile phase consisted of pH 3.0 phosphate buffer and Acetonitrile in the ratio of 90:10%v/v. The flow rate was 2.0 mL/min, column oven temperature 25°C, the injection volume was 20 µL, and detection was performed at 300 nm using a photodiode array detector (PDA).

Method development

Spectroscopic analysis of compound Ornidazole showed that maximum UV absorbance (λmax) at 300 nm respectively. To develop a suitable and robust LC method for the determination of Ornidazole, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Agilent Zorbax AQ with the following different mobile

phase compositions like that Buffer and acetonitrile in the ratio of 40:50 v/v 50:50 v/v & 55:45. It was observed that when Ornidazole was injected, Peak Tailing, not satisfactory.

For next trial Inertsil ODS-3V (150x4.6 mm, 5µ) column used and the mobile phase composition were changed slightly. The mobile phase composition was buffer and acetonitrile in the ratio of 90:10 v/v. respectively as eluent at flow rate 2.0 mL/min. UV detection was performed at 300nm. The retention time of Ornidazole is 12.02 minutes and the peak shape was good. The chromatogram of Ornidazole standard using the proposed method is shown in (Figure 2) system suitability results of the method are presented in Table 1.

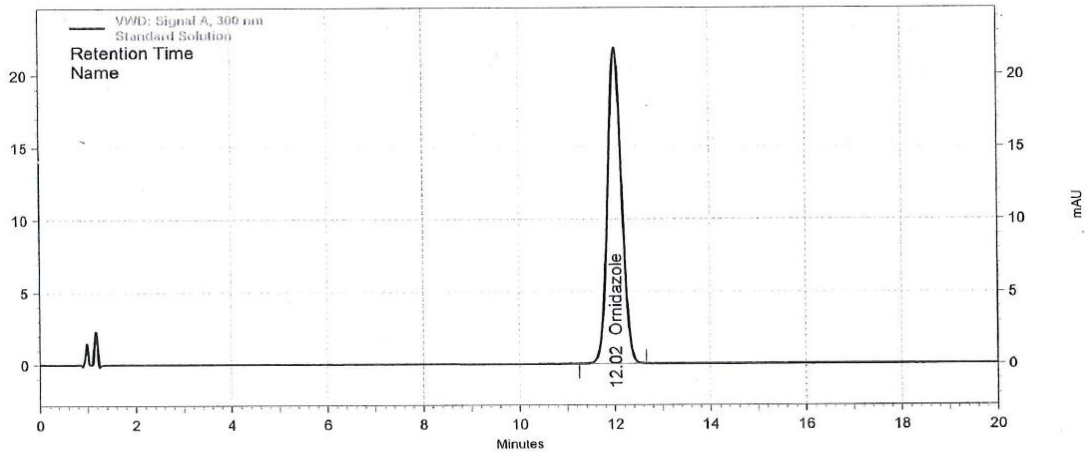

Figure 2: Chromatogram showing the peak of Ornidazole

Method validation

The developed RP-LC method extensively validated for assay of Ornidazole using the following parameters.

Specificity

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Blank and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution (Figure 3) showed no peak at the retention time of ornidazole peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of ornidazole in ornidazole injection formulations. Similarly chromatogram of placebo solution (Figure 4) showed no peaks at the retention time of ornidazole peak. This indicates that the placebo used in sample preparation do not interfere in estimation of ornidazole in ornidazole injection formulations.

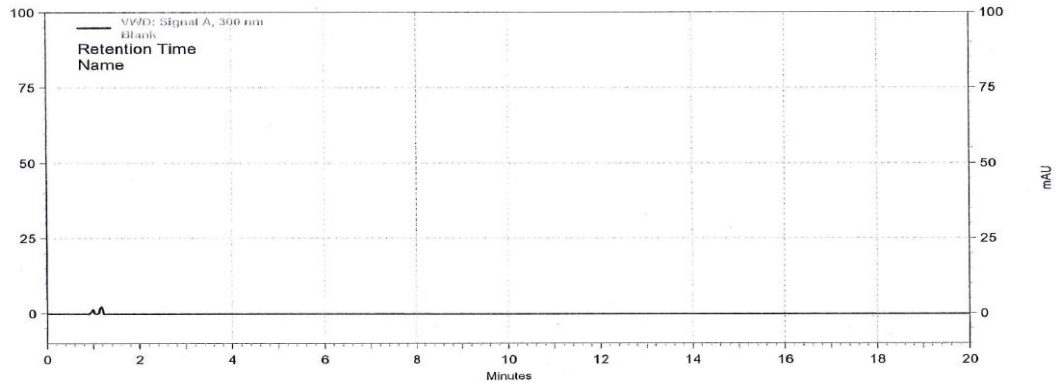

Figure 3: Chromatogram showing the no interference of diluent

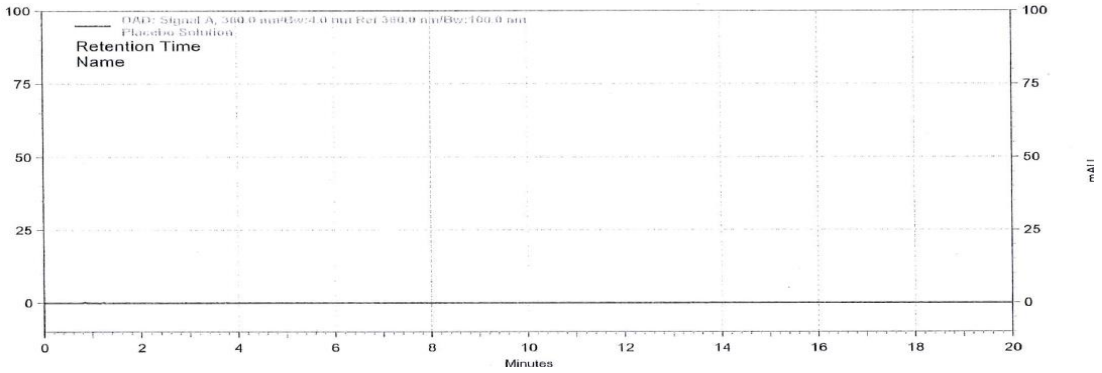

Figure 4: Chromatogram showing the no interference of placebo

Table 1: System suitability parameters for Ornidazole by proposed method

Name of the Compound	Retention Time	Theoretical plates	Tailing factor
Ornidazole	12.02	7675	1

System precision:

The standard solution was prepared as per the test method, injected into the HPLC system for six times and evaluated the % RSD for the area responses. The data were shown in Table 2.

Table 2: System precision data for Ornidazole

S.No.	No. of injections	Area of Ornidazole
1	Inj-1	7519980
2	Inj-2	7517708
3	Inj-3	7517759
4	Inj-4	7516134
5	Inj-5	7510598
6	Inj-6	7523828
	Average	7517668
	SD	4373.5313
	%RSD	0.06

Method precision:

The precision of test method was evaluated by doing assay for six samples of Ornidazole tablet as per test method. The content in mg and % label claim for Ornidazole for each of the test

preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The data were shown in Table 3.

Table 3: Method precision data for Ornidazole

No. of injections	Ornidazole % assay
Preparation 1	97.9
Preparation 2	97.9
Preparation 3	97.7
Preparation 4	97.6
Preparation 5	98
Preparation 6	97.4
Average	97.8
SD	0.215
%RSD	0.22

Linearity of detector response

The linearity of an analytical method is its ability to obtain test results which has a definite mathematical relation to the concentration of analyte. The linearity of response for Ornidazole was determined in the range of 25 to 150 % (6.55- 38.30 µg/ml for Ornidazole). The calibration curve of analytical method was assessed by plotting concentration versus peak area and represented graphically. The correlation coefficient [r2] was found to be 0.9998.Therefore the HPLC method was found to be linear standard curve were calculated and given in Figure 5: to demonstrate the linearity of the proposed method. From the data obtained which given in Table 4: the method was found to be linear within the proposed range.

Table 4: Linearity studies for Ornidazole by proposed method

%Level	Concentration (ppm)	Ornidazole Area
25	6.55	2005051
50	12.6	3807858
75	18.9	5505002
100	25.2	7269509
125	31.25	9091848
150	38.3	11182298
Correlation		0.9998
Slope		287383.4444
intercept		115982.3407
%Y-intercept		1.6

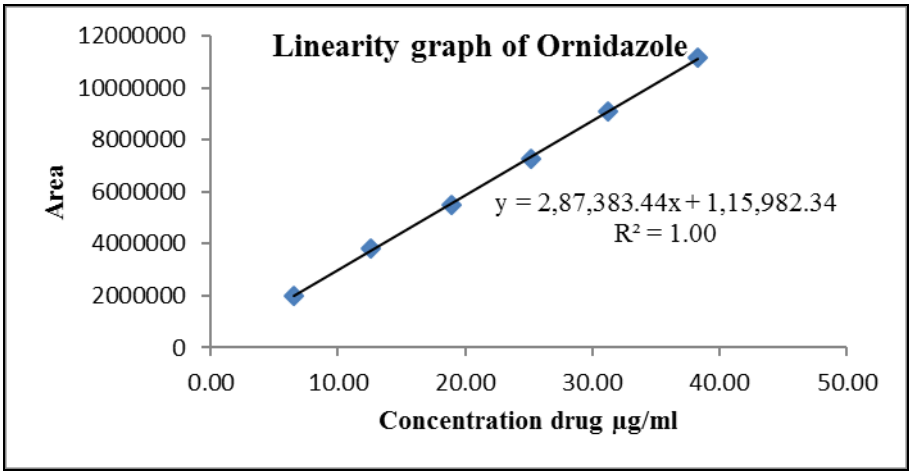

Figure 5: Calibration curve for Ornidazole

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on pooled sample collected from 10 vials of Ornidazole, analyzed as per the proposed method. The mean percentage recovery for 50%, 100%, 150% level. was found to be 100.93, 101.1 and 100.67. %RSD was found to be 0.25, 1.29 and 0.15 respectively. They are within the acceptance limits. Therefore, the HPLC method for the determination of assay of Ornidazole in formulation was found to be accurate. The data obtained which given in Table: 5 the method was found to be accurate.

Table: 5 Recovery studies for Ornidazole by proposed method

Levels	%Recovery	Mean % recovery	%RSD
50%	100.9	100.93	0.25
	101.2		
	100.7		
100%	99.6	101.10	1.29
	101.7		
	102		
150%	100.5	100.67	0.15
	100.7		
	100.8		

Solution stability of analytical solutions:

Standard and sample solutions were kept for about 24 hrs at room temperature in transparent bottles in auto sampler and in refrigerator 2-8°C. The stability of standard and sample solutions was determined by comparison of "old" prepared standard solutions with freshly prepared standard solutions.

Table 6: Results for solution stability of standard

Time Interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
12hrs	1.01	1.01
24hrs	1.00	1.02

Robustness

To validate the method robustness the chromatographic performance at changed conditions was evaluated compared to nominal conditions of the method. System suitability solution was injected at each of the following changed conditions:

Table 7: Results of Robustness

Parameter		Theoretical plates	Tailing factor	%RSD of peak area
Flow variation ±10%	10%	6959	1.0	0.11
	-10%	7896	1.0	0.1
Temperature variation ±5^{o}C	+5^{o}C	7240	1.0	0.09
	-5^{o}C	6178	1.0	1.39
pH variation ±0.2	0.2	6831	1.0	0.07
	-0.2	6820	1.0	0.16
Mobile phase Variation± 10%	10	7597	1.0	0.06
	-10	7373	1.0	0.04

RESULTS AND DISCUSSION

An RP-HPLC method for estimation of Ornidazole was developed and validated as per ICH guidelines. A simple, accurate and reproducible reverse phase HPLC method was developed for the estimation of Ornidazole in bulk drugs and formulations. The optimized method consists of mobile phase pH 3.0 phosphate buffer and Acetonitrile in the ratio of 90:10%v/v with Inertsil ODS-3V (150 × 4.6mm, 5µ) column. The retention time of Ornidazole was found to be 12.02 min. The developed method was validated as per ICH Q2A (R1) guideline. The proposed HPLC method was linear over the range of 6.55-38.30 µg/ml, the correlation coefficient was found to be 0.9998. Relative standard deviation for method precision was found to be 0.22%.

The accuracy studies were shown as % recovery for Ornidazole 50%, 100% and 150% level. The limit of % recovered shown is in the range of 98 and 102% and the results obtained were found to be within the limits. Hence the method was found to be accurate

The robustness of the method was checked by varying flow rate, mobile phase composition and temperature and pH found that the system suitability parameters were within the limit at all variable conditions, hence the method was robust.

CONCLUSION

We have developed a fast, simple and reliable analytical method for determination of Ornidazole in pharmaceutical preparation using RP-LC. As there is no interference of blank and placebo at the retention time of Ornidazole. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and Linearity. It allows reliably the analysis of Ornidazole in its different pharmaceutical dosage forms.

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