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Method development and validation of ornidazole by using RP-HPLC

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Abstract

Analytical way is more suitable, highly precise, safe and selective. Developing analytical method for newly introduced pharmaceutical formulation is a matter of most importance. The technique which is widely used to check the quality of drug is known as 'CHROMATOGRAPHY'. Our present plan is to develop a new, simple, precise or accurate method for analysis of ORNIDAZOLE formulation after a detailed study a new RP-HPLC method was decided to be developed and validated. Ornidazole is a popular anti-protozoal agent. Different concentrations of drug solutions are prepared for method validation. Injected each level into the chromatographic system and measure the peak area. The accuracy limit is the percentage recovery should be in the range of 98.0% - 102.0%. The total recovery was found to be 100.34% for Ornidazole. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

Keywords: Ornidazole; RP-HPLC; Accuracy; Validation; Efficacy

1. Introduction

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, strength and quality, for the quantification of the drug substances and drug products. Method validation has received considerable attention in the literature and from industrial committees and regulatory agencies. The U.S. FDA has also proposed industry guidance for Analytical Procedures and Methods Validation [1]. ISO/IEC 17025 includes a chapter on the validation of methods with list validation parameters. The ICH [18] has developed a consensus text on the validation of analytical procedures. ICH also developed guidance with detailed methodology [2]. The U.S. EPA prepared guidance for method's development and validation for the Resource Conservation and Recovery Act (RCRA). The influence of operating parameters on the performance of the method can be assessed at the validation stage which was not done during development/optimization stage of the method. The defined validation parameters by the ICH and other regulatory bodies are summarized as under: a) Specificity study b) Linearity and range study c) Limit of detection and Limit of quantization study d) Precision study e) Accuracy study f) Robustness study g) Solution stability study h) System suitability. The present plan is to develop a new, simple, precise or accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated.[3] Bhavesh R. Sharma et al, To Study and develop an accurate, precise and linear Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for Ornidazole in tablets the developed method results in Ornidazole eluting at 2.75min. and it exhibit the linearity at the range of 15-50ug/ml, the precision exemplified by relative standard deviation of 1.53%. Shafrose Syed et al ,the retention time and percentage assay was found to be at 3min-103.4% and at 4.8min -104.16%.A. Shravan Kumar et al., The Method employs Waters HPLC system on XTerra RP18 Column (4.6 x 150 mm and 5 µm) and flow rate of 0.5 ml/min with a load of 20µl. The Detection was carried out at 315 nm. Retention time was 3.096 and 4.097 min for ornidazole. Puranik, et al , The objective of this work was to develop and validate simple, rapid and accurate chromatographic methods for simultaneous determination of ofloxacin and ornidazole in solid dosage form. The detection was done at 293nm and 311nm and the retention time for Ofloxacin

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and Ornidazole was 4.278 min and 6.750 m respectively.[4] L. Veena et al , The compounds were eluted at a flow rate of 1.0 ml/min. The retention times of CEF and ORD were found to be 2.565min and 3.557min respectively method validation parameters are linearity, precision, limit of detection.

2. Material and methods

2.1. Chemicals and reagents

Ornidazole(Orni-500, Ornida , Dazolic.), Methanol for HPLC(Therma Fisher Scientific India Pvt Ltd), Acetonitrile for HPLC (Therma Fisher Scientific India).

2.2. Instruments

High performance liquid chromatography carried out on waters Peak HPLC, operating with Isocratic method with ADM flow meter with Detector UV (Water-model 487) experimental conditions were optimized on C18 column (zodiac company). Chromatogram were integrated by EMPOWER software . all the standard and sample drugs were weighed on electronic balance, PH and ultrasonicator were used for study. Proper conditioned Borosilicate glassware were used.

2.3. Selection of solvent

Solubility studies of drugs were performed it was found that drug is mostly soluble in Acetonitrile: methanol (70:30) and was selected as the solvent for study.

2.3.1. Preparation of mobile phase

Accurately measured 70 ml of Methanol (70%) and 30 ml of Acetonitrile (30%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.[5]

Standard Solution Preparation: Accurately weigh and transfer 50 mg of Ornidazole working standard into a 5 ml clean dry volumetric flask add about 5 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. 10000µg/m/ml Further pipette 0.1ml of the above stock solutions into a 0.9 ml volumetric flask and dilute up to 1ml with diluent.(1000µg/ml) (Stock solution) .Further pipette 0. 1 ml of the above stock solutions into a 0.9 ml volumetric flask and add 9ml diluent. (100µg/ml) [6]

Sample Solution Preparation: Accurately weigh 0.05gm of tablet from 3 brands of Ornidazole crush in motor and pestle and transfer sample into a 50 ml clean dry volumetric flask. Add about 10mL of diluent and sonicate it up to 3 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron injection filter. (Stock solution). Procedure: L of the standard, sample into the chromatographic system and measure the areasµInject 20for Ornidazole peaks and calculate the %Assay by using the formulae.[7]

Wave length selection: UV spectrum of 10 µg/ml Ornidazole in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 220 nm. At this wavelength both the drugs show good absorbance. Mobile Phase Optimization: Initially the mobile phase tried was Methanol : Acetonitrile , Methanol: 0.1% OPA, Acetonitrile: Phosphate buffer and Methanol: Phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to Methanol: Water (pH 3.0) in proportion 70:30 v/v respectively.[8]

2.4. Assay

Standard Solution Preparation: Accurately weighed and transfer 50 mg of Ornidazole working standard into a 5 ml clean dry volumetric flask add about 5 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. 10000µg/m/ml Further pipette 0.1ml of the above stock solutions into a 0.9 ml volumetric flask and dilute up to 1ml with diluents.(1000µg/ml) (Stock sol.)[9]

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2.5. Method validation

Linearity: the linearity of the method was obtained by preparing suitable aliquots of standard stock solution in different concentrations for (50-300µg/ml).the calibration was obtained by plotting concentration vs peak area.

2.6. Precision

Intraday precision: the method Intra Day precision was determined by injecting six standard working solutions. The areas of all the injections were taken, and standard deviations, % relative standard deviation (RSD) were calculated.

2.6.1. Limit of Detection (LOD)

It is done as the sample is taken from the lowest concentration. Lowest concentration was found to be 50µg/Ml. Furthermore it is diluted by taking 25% from this concentration and added4ml of diluent to it. Injected thrice. Determined the Retention time and area [10]

3. Results

Method development: the standard stock solution was run through a series of mobile phase combinations with interchanging the columns and method parameters from time to time where proper selection of compounds takes place.

A clear, distinct and symmetric peak shape was observed with Acetonitrile: methanol (70:30)waters Peak Hplc, operating with Isocratic method with ADM flow meter with Detector UV (Water-model 487) experimental conditions were optimized on C18 column(zodiac company). Chromatogram were integrated by EMPOWER software.

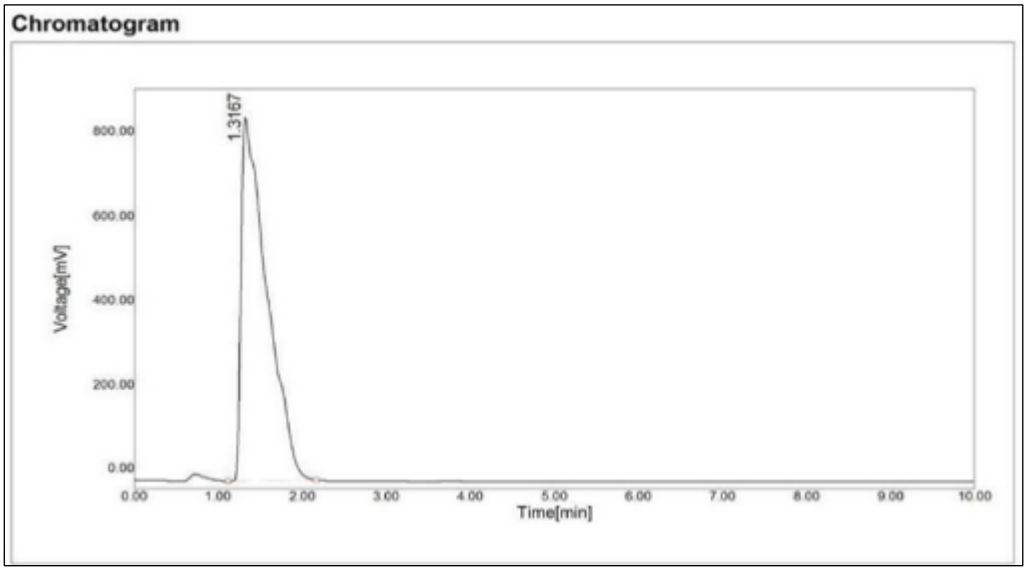

Figure 1 Chromatogram for Ornidazole

Table 1 Results of system suitability parameters

Parameters	Ornidazole
Peak area	100.0%
Retention time	1.3167
Tailing factor	3.6428

3.1. ASSAY

Table 2 Results of Assay for Ornidazole

Drug	Label Claim(mg)	%Assay
Ornidazole	500 mg	95.65

3.2. Validation parameters

3.2.1. Linearity

The linearity range was found to lie from 50µg/ml to 300µg/ml of Ornidazole with regression coefficients of 0.9964.

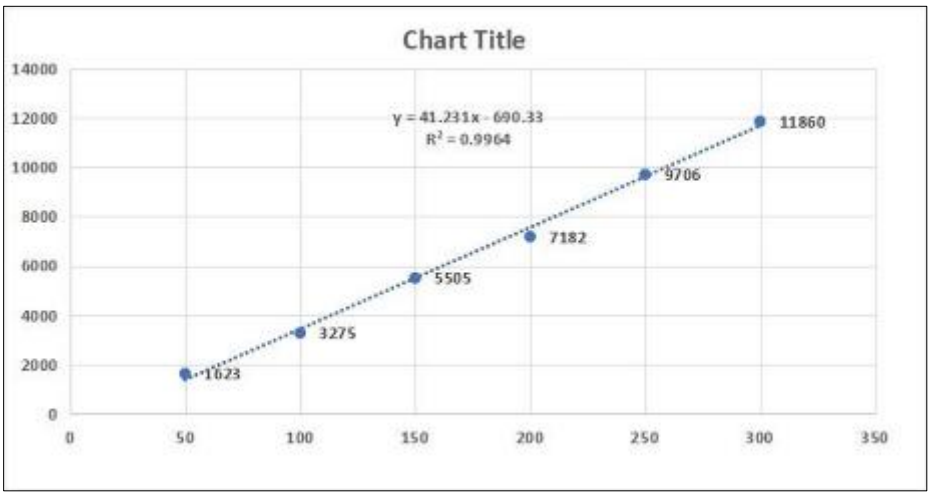

Figure 2 Linearity graph of Ornidazole

3.2.2. Precision

Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatograms and results are shown below.[11]

3.3. Chromatograms for Precision

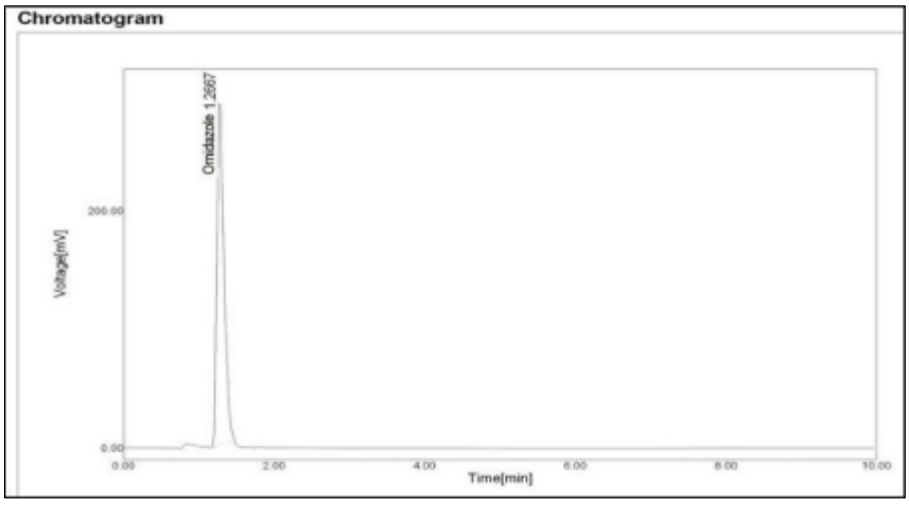

Figure 3 Chromatogram for Precision -1

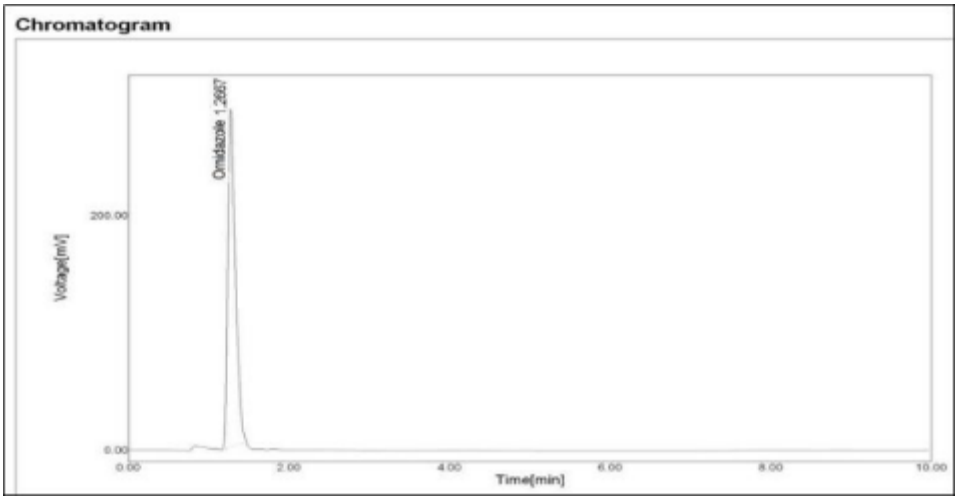

Figure 4 Chromatogram For Precision -2

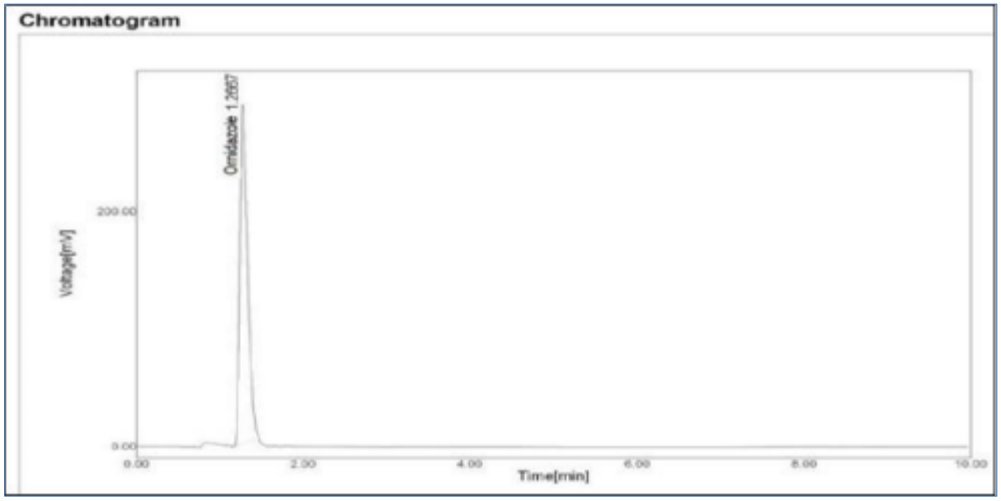

Figure 5 Chromatogram for Precision -3

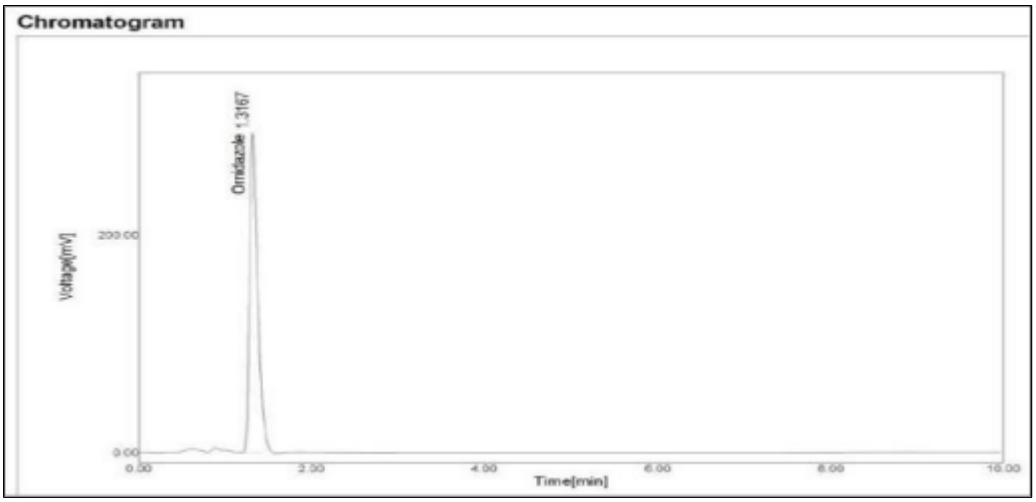

Figure 6 Chromatogram for Precision -4

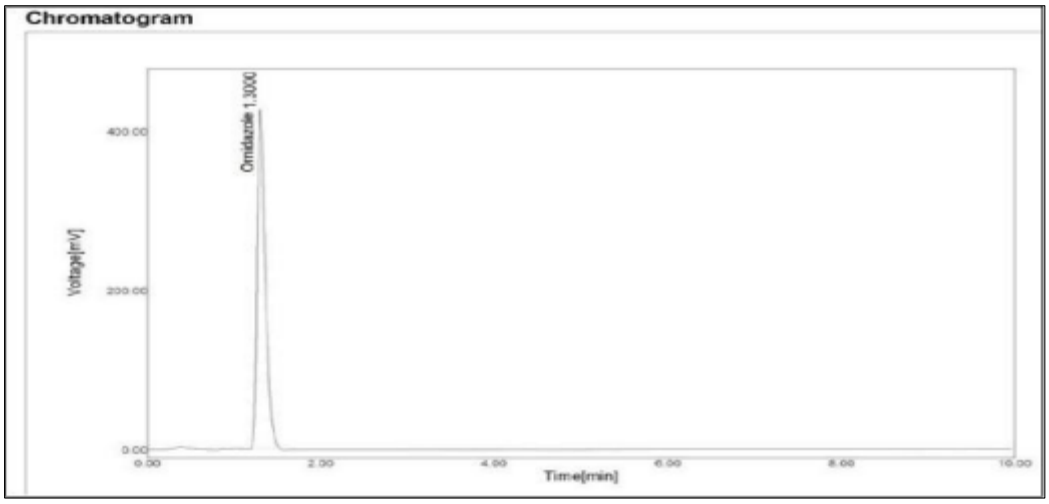

Figure 7 Chromatogram for Precision -5

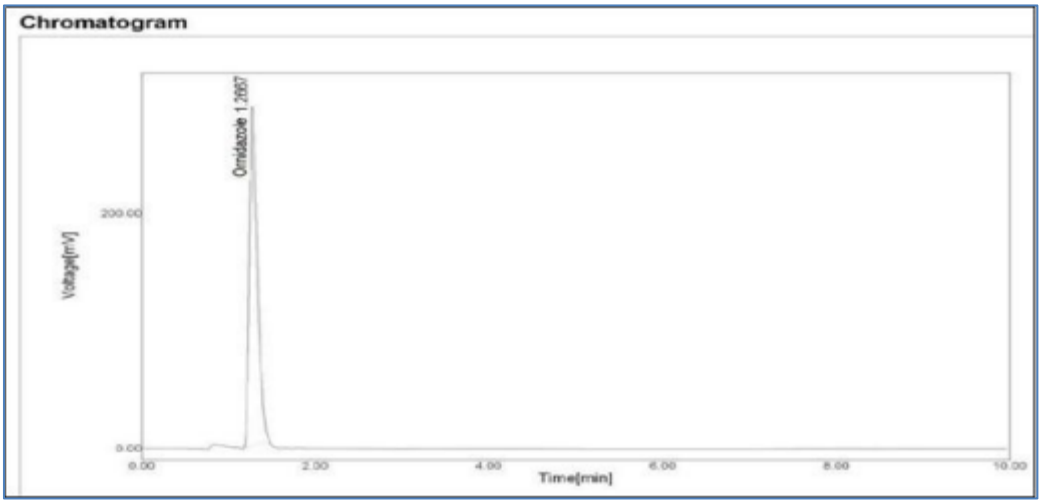

Figure 8 Chromatogram for Precision -6

Table 3 Results of Precision for Ornidazole

Injection	Area
Injection-1	6591
Injection-2	6639
Injection-3	6570
Injection-4	8839
Injection-5	7412
Injection-6	7182

Table 4 Total Results of precision

Average	**7205.5**
Standard Deviation	43233
%RSD	0.1666

3.4. Limit of Detection (LOD)

Limit of Detection was carried out as described under experimental work. The corresponding chromatograms and results are shown below[12].

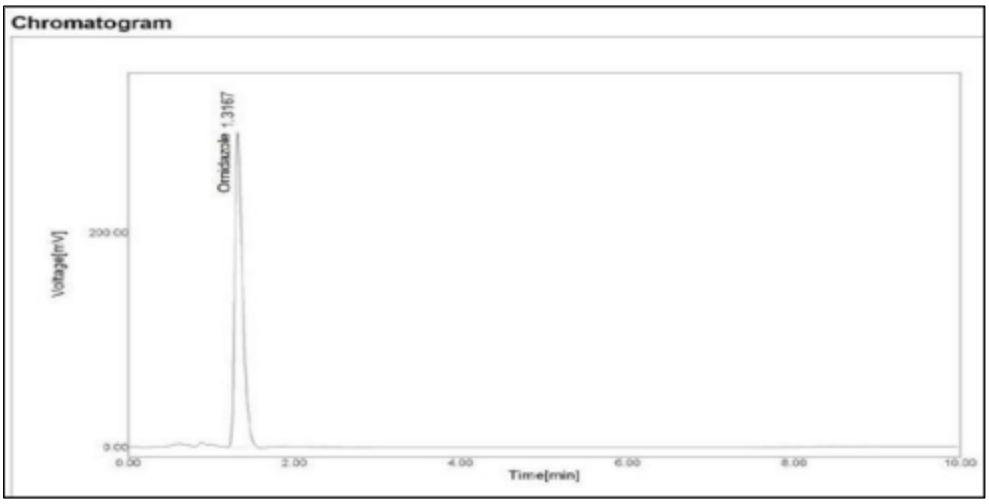

Figure 9 Chromatogram for LOD

Table 5 Results for Limit Of Detection

Limit Of Detection	**Area**
LOD 1	1334
LOD 2	1250
LOD 3	1420

4. Discussion

Selection of diluents is based on polarity index. Based on the polarity results methanol and acetonitrile is used as mobile phase with considerable proportions. Using the optimized method condition Ornidazole is analyzed. No interferences detected at respective retention time. Linearity was found near to 1 i.e., 0.99 which shows good regression. Percentage RSD for this method was less than 2

5. Conclusion

The estimation of Ornidazole was done by RP-HPLC. The assay of Ornidazole was performed with tablets and the % assay was found to be 90.99% which show that the method is useful for routine analysis. The linearity of Ornidazole was found to be linear with a correlation coefficient of 0.9996, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.1666 for Ornidazole which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.1666 for Ornidazole which shows that the method is repeatable when performed in different days also. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed

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