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Method Development and Validation for Ciprofloxacin and Ornidazole in its Bulk and Combined Dosage Forms by RP-HPLC

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Abstract

A new method was established for simultaneous estimation of Ciprofloxacin and Ornidazole by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ciprofloxacin and Ornidazole by using Inertsil C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The retention times were found to be 2.399 mins and 3.907 mins. The % purity of Ciprofloxacin and Ornidazole was found to be 100.7% and 101.4% respectively. The system suitability parameters for Ciprofloxacin and Ornidazole such as theoretical plates and tailing factor were found to be 1.3, 5117.5 and 1.4, 3877.3 the resolution was found to be 8.0%. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Ciprofloxacin and Ornidazole was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Ciprofloxacin and Ornidazole in API and Pharmaceutical dosage form.

Keywords
Inertsil C18, Ciprofloxacin and Ornidazole, RP-HPLC

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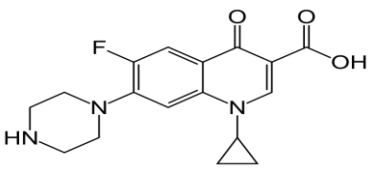
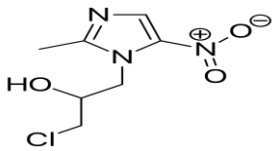
INTRODUCTION:

Ciprofloxacin is a fluoroquinolone antibiotic used to treat a number of bacterial infections. This includes bone and joint infections, intra-abdominal infections, certain type of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever and urinary tract infections, among others.



Ciprofloxacin

Ornidazole is an antibiotic used to treat protozoan infections. A synthetic nitroimidazole, it is commercially obtained from an acid-catalyzed reaction between 2-methyl-5-nitroimidazole and epichlorohydrin. Antimicrobial spectrum is similar to that of metronidazole and is more well tolerated.



Ornidazole

MATERIALS AND METHOD:

Instrumentation

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C-18 RP-column, Sonicator Analytical Technologies Limited-Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000^{+}pH meter, Weighing machine

Chemicals

Ciprofloxacin and Ornidazole, Potassium dihydrogen orthophosphate, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

Trial-5 (Optimized)

Chromatographic conditions:

Column: Inertsil C18 5µm (4.6*250mm)
Mobile phase ratio: Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v)
Detection wavelength: 255nm
Flow rate: 1ml/min
Injection volume: 20µl Column temperature: Ambient

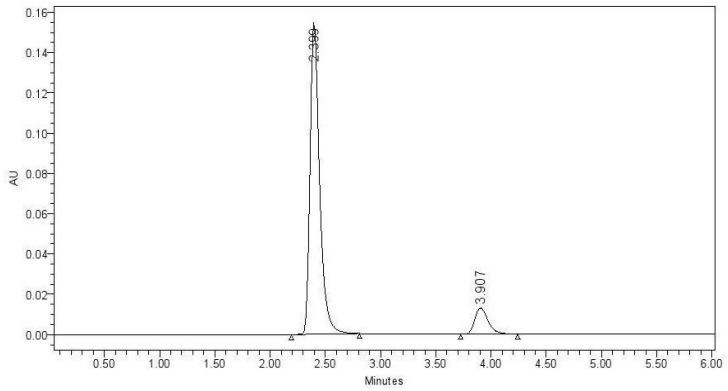


Fig. 1. Chromatogram of Trail-5

Table 1 Details of Trail-5

S.No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Ciprofloxacin	2.399	946124	155429	5105	1.3	8.1
2	Ornidazole	3.907	111541	13239	3788	1.4	

Preparation of Sample Solution: **(Tablet)**

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Ornidazole and Ciprofloxacin (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluant.

METHOD VALIDATION:

- System Suitability
- Linearity
- Specificity
- Precision
- Intermediate Precision
- Accuracy
- Limit of Detection and Limit of Quantification
- Robustness

RESULTS AND DISCUSSION:

Accuracy

Table.2. Accuracy results of Ornidazole

%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovey	Mean Recovery
50%	2332744	5	5.10	101.8%	100.5%
100%	3132697	10	9.99	99.9%	
150%	3918997	15	14.9	99.1%	

Table. 3. Accuracy results of Ciprofloxacin

%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovey	Mean Recovery
50%	353867	5	5.0	101.3%	100.0%
100%	4735088	10	9.94	99.4%	
150%	5911798	15	14.8	99.2%	

Precision

Table 4. Repeatability results of Ciprofloxacin and Ornidazole.

Injection	Area
Injection-1	1501417
Injection-2	1486940
Injection-3	1490656
Injection-4	1487329
Injection-5	1490384
Average	1491345
Standard Deviation	5881.4
%RSD	0.39

Injection	Area
Injection-1	2235319
Injection-2	2240678
Injection-3	2249490
Injection-4	2245822
Injection-5	2251694
Average	2244601
Standard Deviation	6656.8
%RSD	0.32

Intermediate precision/Ruggedness

Table 5. Ruggedness results of Ornidazole & Ciprofloxacin

Injection	Area
Injection-1	2194758
Injection-2	2195700
Injection-3	2196191
Injection-4	2195326
Injection-5	2200951
Average	2196585
Standard Deviation	2496.0
%RSD	0.11

Injection	Area
Injection-1	1456296
Injection-2	1457422
Injection-3	1456513
Injection-4	1454579
Injection-5	1451483
Average	1455259
Standard Deviation	2347.6
%RSD	0.16

Linearity

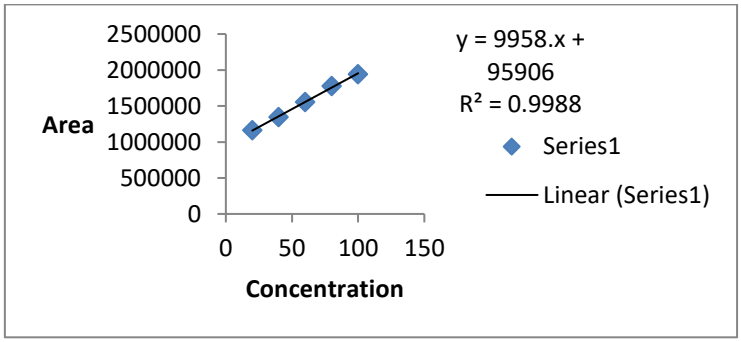


Fig. 2 Calibration curve of Ornidazole

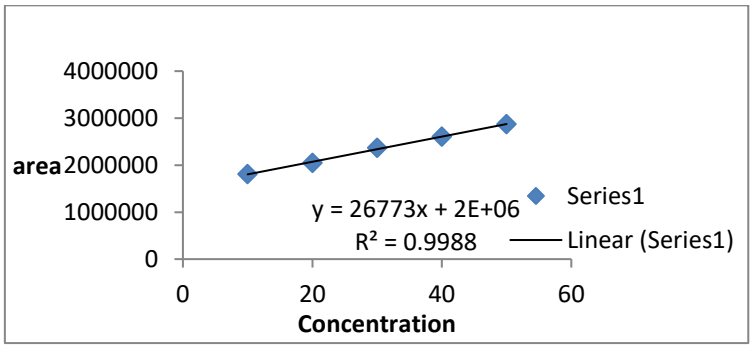


Fig.3 Calibration curve of Ciprofloxacin

Robustness

Table 6. System suitability results For Ornidazole (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	1748.5	1.22
2	1.0	1548.2	1.2
3	1.2	1948.0	1.2

Table 7. System suitability results for Ciprofloxacin (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	883.3	1.56
2	1.0	1234.0	1.1
3	1.2	969.2	1.6

Table 8. System suitability results for Ornidazole (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results

		USP Plate count	USP Tailing
1	10% Less	1748.5	1.22
2	Actual	1548.2	1.2
3	10% More	1948.0	1.2

Table 9. System suitability results for Ciprofloxacin (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	883.3	1.56
2	Actual	1234.0	1.1
3	10% More	969.2	1.6

SUMMARY AND CONCLUSION:

Proposed study describes a new RP-HPLC method for estimation of Ciprofloxacin and Ornidazole combination in mixture using simple mobile phase. The method gives good resolution between both the compounds with a short analysis time. The method was validated and found to be simple, sensitive, accurate and precise. Hence it can be easily and conveniently adopted for routine quality control analysis.

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