



**A NEW VALIDATED RP-HPLC METHOD FOR DETERMINATION OF
CIPROFLOXACIN AND ORNIDAZOLE IN BULK PHARMACEUTICAL DOSAGE
FORMS**

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ABSTRACT

It is a simple and selective RP-HPLC method for the determination of ciprofloxacin and Ornidazole bulk pharmaceutical dosage form. Chromatographic separation was achieved on a c-18 column using mobile phase consisting a mixture of methanol, Acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1 N Hcl with detection of 295nm. Linearity was observed in the range of 50 g/ml for ciprofloxacin and 40 g/ml for Ornidazole. R² = ciprofloxacin (0.0160) and Ornidazole (0.871) for the amount of drugs estimated by the proposed method was in good agreement with the label claim. The proposed method was validated. The accuracy of the methods was assessed by recovery studies at two different levels. Recovery experiments indicated the absence of interference from commonly encounter pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis showing ≥ 2 . All statistical data proves validity of the method and can be used for routine analysis of pharmaceutical dosage form.

KEYWORD: Liquid Chromatography (LC) RSD (Relative Standard Deviation), correlation co-efficient (r^2).

INTRODUCTION

Pharmaceutical analysis basically identify for analysis of active pharmaceuticals. Based on the active pharmaceutical ingredient (API) or active ingredient the prepared formulated drug is distinguished upon its therapeutic activity. It is very important, to bring an advanced analytical techniques for development of drugs. This is like a challenge for an analytical chemist for the discovery and validation of analytical methods.^[1-4]

Pharmaceutical analysis deals with the invention of newer medicaments related with its purity, and quantity.^[5] Combination therapy or poly therapy is therapy that uses more than one medication. Typically, these terms refer to using multiple therapies to treat a single disease. Poly therapy is a related term, referring to the use of multiple medications.^[6,7] Ciprofloxacin is a 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid. It is a synthetic fluoroquinolone, it has the bactericidal act of CPX grades from preventing the enzymes to poisoermease II (DNA gyrase) and topoisomerase IV, which is needed for bacterial DNA replication, transcription, repair, strand super coiling repair, and recombination.^[8,9] Ornidazole is 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-Nitroimidazole.

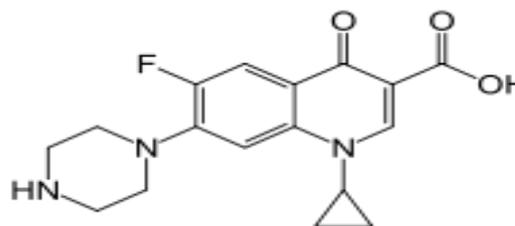
Ornidazole is a drug that cures some protozoan infections. It has been investigated for use in Crohn's

disease after bowel resection.^[14,15] The method was validated according to ICH guidelines for specificity, LOD, LOQ, Precision, Accuracy, and Linearity. The method showed good reproducibility and recovery with %RSD less than 2. Hence we had made an attempt to develop a simple accurate and precise RP HPLC method for the simultaneous estimation of Ciprofloxacin and Ornidazole in bulk and in tablet dosage form.

Drug profile of 2 drugs

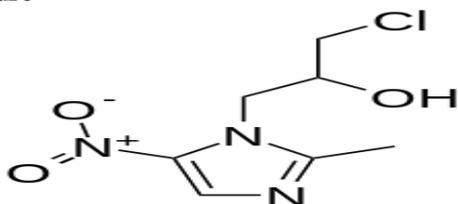
1. Ciprofloxacin^[10,11]

Structure



IUPAC Name

1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid.

2. Ornidazole^[12,13]**Structure****IUPAC Name**

1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole.

MATERIALS AND METHODS**Chemicals and Reagents**

Ciprofloxacin and Ornidazole were obtained as gift samples from Spectrum Labs, Hyderabad. Commercial tablet of Ciprofloxacin and Ornidazole (100 mg) was procured from the local drug market. Acetonitrile and water, Methanol, Ortho phosphoric acid, KH_2PO_4 , K_2HPO_4 were HPLC from Merck.

Instruments

Analytical HPLC-auto sampler –UV detector Separation module 2695, UV detector 2487 Empower-software version-2 Waters, U.V double beam spectrometer UV 3000+, Digital weighing balance (sensitivity 5mg), pH meter and Sonicator.

Preparation of the Ornidazole and Ciprofloxacin standard and sample solution**Preparation****Preparation of standard solution**

Weigh accurately 10 mg of each drug into 2 different 10ml volumetric flask, in each flask add about 4ml of HPLC grade water and allow to keep under a sonicator for complete dissolution. Then 0.5 ml of Ciprofloxacin and Ornidazole 0.4 ml of were transferred in a 10 ml volumetric flask & diluted to 10 ml with the solvent system. It is filtered through a 0.45 μm membrane filter and placed under ultrasonicator bath for degassing. From that standard sample various working standard solutions are allowed to prepare serial dilution.

Sample solution preparation

Accurately weigh and transfer 59.8 mg of Ciprofloxacin and Ornidazole tablet powder into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the

mark with the same solvent. (Stock solution). Further pipette 0.6ml of Ciprofloxacin and Ornidazole the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure

20 μL of the blank, standard and sample were injected into the chromatographic system and areas for the Ciprofloxacin and Ornidazole the peaks were used for calculating the % assay by using the formula.

Optimized chromatographic conditions

By performing the trials the chromatographic conditions were optimized as follows

Column: Develosil ODS HG-5 RP C_{18} , 5 μm , 15cmx4.6mm i.d.

Mobile phase: H_2O (pH adjusted to 2.5 with Ortho H_3PO_4): Acetonitrile (35:65v/v)

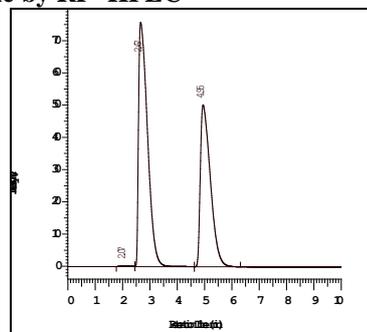
Wavelength: 295nm

Flow rate: 1.0ml/min

Injection volume: 20 μl

Column Temperature: Ambient

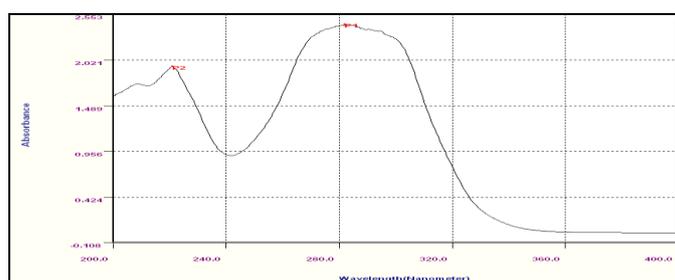
Run time: 10 min.

Chromatographic trial for simultaneous estimation of Ciprofloxacin and Ornidazole by RP- HPLC

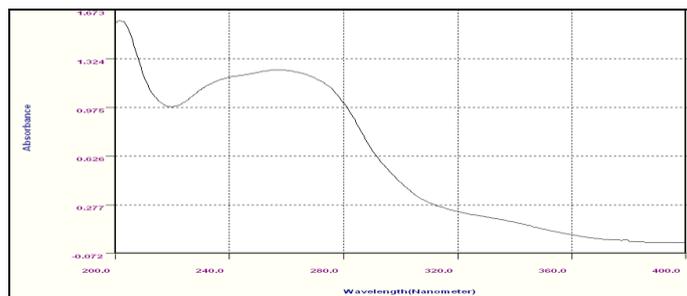
Optimized chromatographic conditions are shown in Fig. No. 1.

RESULTS AND DISCUSSIONS**Method Development**

The λ_{max} of the two ingredients i.e. Ciprofloxacin and Ornidazole was found to be 320nm and 275 nm respectively in methanol as solvent system. The isobestic point for the drugs was found at 295 nm.



The UV Spectrum for Ciprofloxacin is shown in Fig. No. 2.



The UV Spectrum for Ornidazole is shown in Fig. No. 3.

Preparation of mobile phase

It was prepared by taking water (pH adjusted to 2.5 with Orthophosphoric acid): Acetonitrile (35:65v/v), and

filtered through 0.45 µm filter membrane and placed under ultra Sonicator for degassing. And pumped throughout the column at a flow rate of 1.0 ml/min.

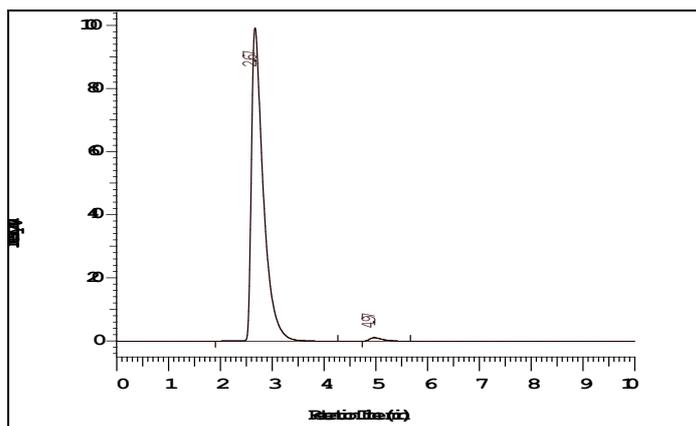


Fig. No. 4: Chromatogram showing standard Ciprofloxacin injection.

Retention time was found to be 3.67 min.

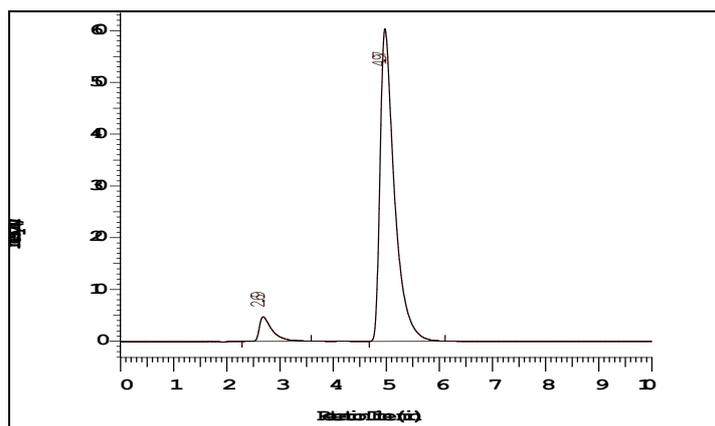


Fig. No. 5: Chromatogram showing standard Ornidazole injection.

Retention time was found to be 3.97 min.

The HPLC system was set with the optimized chromatographic conditions to run the standard solution of Ciprofloxacin and Ornidazole for 10 min. The retention time were found to be 3.67 min and 3.95 min respectively.

Linearity and Range

Different concentrations of like 0-60 & 0-40 ppm of Ciprofloxacin and Ornidazole were prepared respectively for linearity.

Table No. 1: Linearity Results for Ciprofloxacin.

Con(µg/ml)	AUC
0	0
10	148528
20	272580
30	387574
40	493125
50	601256
60	722010

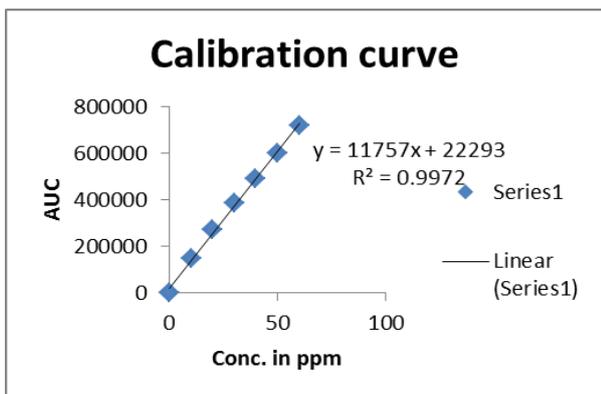


Fig. No. 6: Showing calibration graph for Ciprofloxacin.

Table No. 2: Linearity Results for Ornidazole.

Con(µg/ml)	AUC
0	0
5	72586
10	158963
15	248722
20	308830
30	476594
40	616852

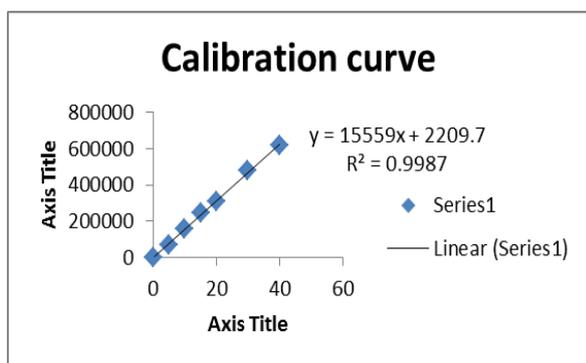


Fig. No. 7: Showing calibration graph for Ornidazole.

Table 3: Accuracy readings for ciprofloxacin.

Sample ID	Concentration (µg/ml)		% Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S ₁ : 80%	40	50	99.13	Mean= 98.94656% S.D. = 0.171671 % R.S.D.= 0.1723
S ₂ : 80%	40	50	98.79	
S ₃ : 80%	40	50	98.92	
S ₄ : 100%	50	50	99.72	Mean= 99.77% S.D. = 0.045936 % R.S.D.= 0.0469
S ₅ : 100%	50	50	99.81	
S ₆ : 100%	50	50	99.75	
S ₇ : 120%	60	50	99.36	Mean= 99.37667% S.D. = 0.105897 % R.S.D. = 0.1067
S ₈ : 120%	60	50	99.28	
S ₉ : 120%	60	50	99.49	

Recovery study: Ornidazole

For the determination of accuracy the performed technique, recovery studies were done by addition of various degrees (80%, 100%, and 120%) of pure drug of Ornidazole were further determined by the pre-analyzed prepared of concentration 40µg/ml. From that % age

Linearity range was found to be 0-60 µg/ml for Ciprofloxacin. The correlation coefficient was found to be 0.997, the slope was found to be 11757 and intercept was found to be 22293 for Ciprofloxacin. Linearity range was found to be 0-40 µg/ml for Ornidazole. The correlation coefficient was found to be 0.998, the slope was found to be 15559 and intercept was found to be 2209 for Ornidazole.

Accuracy

Recovery study: Ciprofloxacin:

For the determination of accuracy the accuracy the performed technique, recovery studies were done by adding various amounts (80%, 100%, and 120%) of pure drug of Ciprofloxacin was collected and add to the pre-analyzed preparations of concentration 50µg/ml. From that %age recovery results were determined. The results were shown in table-3.

recovery results were determined. The results were shown in table-4.

Table 4: Accuracy Results for Ornidazole.

Sample ID	Concentration ($\mu\text{g/ml}$)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S ₁ : 80%	32	40	99.18	Mean=98.97667% S.D. = 0.200083% R.S.D.=0.202152
S ₂ : 80%	32	40	98.78	
S ₃ : 80%	32	40	98.97	
S ₄ : 100%	40	40	99.87	Mean= 99.54% S.D. = 0.33% R.S.D.=0.331525
S ₅ : 100%	40	40	99.54	
S ₆ : 100%	40	40	99.21	
S ₇ : 120%	48	40	99.32	Mean= 99.567% S.D. = 0.33% R.S.D.=0.331159
S ₈ : 120%	48	40	99.65	
S ₉ : 120%	48	40	99.98	

The mean recoveries be initiated as 98.976, 99.54, 99.57% for Ornidazole and 98.94, 99.76, 99.31% for Ciprofloxacin. The limit for mean % recovery is 95-105% and as both the values are within the limit, hence it can be said that the performed technique was accurate.

of a fixed dose of Ornidazole & Ciprofloxacin. The % RSD was determined for Ornidazole & Ciprofloxacin are mentioned in the table-5.

Precision

1. Repeatability

It was estimated by individually determination from the peak areas obtained by actual estimation of 5 replicates

Table 5: Data showing repeatability analysis for Ciprofloxacin & Ornidazole.

HPLC Injection Replicates	AUC for Ciprofloxacin	AUC for Ornidazole
Replicate – 1	3871256	308414
Replicate – 2	3870248	308451
Replicate – 3	3871563	302415
Replicate – 4	3871248	308569
Replicate – 5	3870236	308120
Average	3870910	307193.8
Standard Deviation	623.0748	2676.537
% RSD	0.016096	0.871286

The repeatability study which was conducted on the solution having the concentration of about 50 $\mu\text{g/ml}$ for Ciprofloxacin and 40 $\mu\text{g/ml}$ for Ornidazole (n =5) showed a RSD of 0.016096% for Ciprofloxacin and 0.871286% for Ornidazole. It was concluded that the analytical technique showed good repeatability.

value (n = 3), was introduced in triplicate into the HPLC for 3days. Results were subjected to statistical treatment for the calculation of SD and RSD. The values are revealed in Table No 6 and 7.

2. Intermediate precision

For intra and inter day results the drug having concentration value 80%, 100% & 120% of the target

Table 6: Data for Ornidazole analysis.

Conc. Of Ornidazole (API) ($\mu\text{g/ml}$)	Observed Conc. Of Ornidazole ($\mu\text{g/ml}$) by the performed analysis			
	Intra-Day		Inter-Day	
	Mean (n=3)	% RSD	Mean (n=3)	% RSD
30	29.95	1.05	30.01	0.24
40	40.08	0.55	40.051	0.41
50	49.84	0.18	49.95	0.18

Table 7: Data for Ciprofloxacin analysis.

Conc. Of Ciprofloxacin (API) ($\mu\text{g/ml}$)	Observed Conc. Of Ciprofloxacin ($\mu\text{g/ml}$) by the performed analysis			
	Intra-Day		Inter-Day	
	Mean (n=3)	%RSD	Mean (n=3)	% RSD
40	40.01	0.86	40.03	0.87
50	50.02	0.30	50.03	0.32
60	59.97	0.13	59.95	0.11

Intraday and inter day studies show that the mean RSD (%) was found to be contained by acceptance limit ($\leq 2\%$), so it was accomplished that there be no major dissimilarity for the assay, which was tested within day and between days. therefore, performed analysis at selected wavelength was found to be precise.

Detection limit

The detection limit (LOD) and Quantitation limit (LOQ) may be expressed as:

$$\text{L.O.D.} = 3.3(\text{SD/S})$$

$$\text{L.O.Q.} = 10(\text{SD/S})$$

SD = Standard deviation of the response

S = Slope of the calibration curve

$$\text{L.O.Q.} = 10(\text{SD/S})$$

SD = Standard deviation of the response

S = Slope of the calibration curve

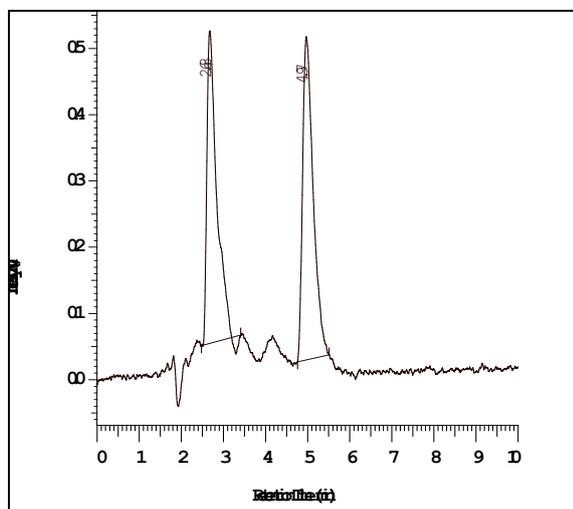


Fig. No. 8: Showing Chromatogram for LOD.

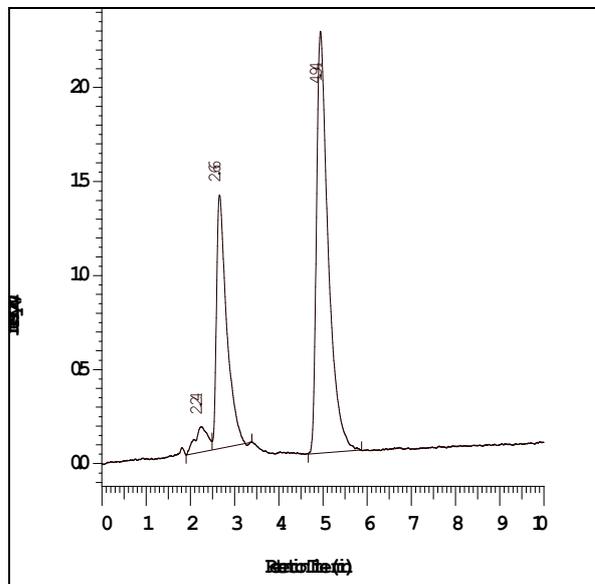


Fig. No. 9: Showing Chromatogram for LOQ.

The LOD were found to be 1.02 $\mu\text{g/ml}$ and 2.44 $\mu\text{g/ml}$ and LOQ was found to be 1.96 $\mu\text{g/ml}$ and 7.32 $\mu\text{g/ml}$ for Ciprofloxacin & Ornidazole respectively which represents that sensitivity of the method is high.

Assay calculation for Ciprofloxacin & Ornidazole

The assay study was performed for the Ciprofloxacin & Ornidazole. The chromatograms area unit shown in Fig.No.10 and results area unit tabulated in Table.No.8.

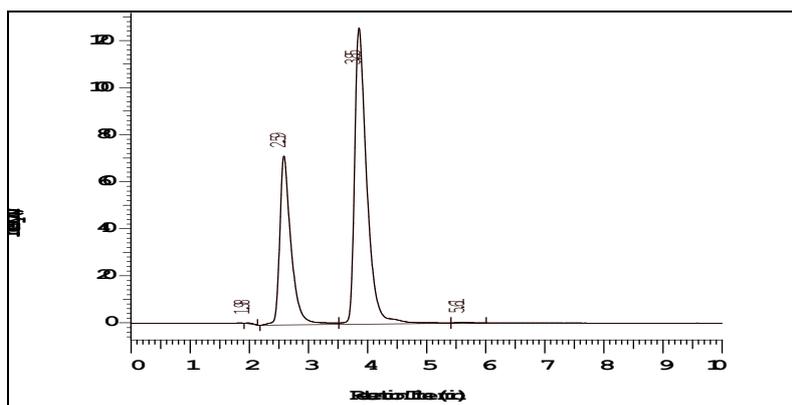


Fig. No. 10. Chromatogram showing assay of sample injection-1, 2.

Table No. 8: Showing assay results.

Labelled amount of Drug (mg) ciprofloxacin & Ornidazole	Mean (\pm SD) amount (mg) found by the proposed method (n=6)	Mean (\pm SD) Assay (n = 6)
500 & 1	500.05 (\pm 0.05), 0.998 (\pm 0.08)	100.007% (\pm 0.59), 99.8% (\pm 0.81)

The assay of 500.05 mg of Ciprofloxacin and Ornidazole 0.998 mg of was found to be 100.007%. and 99.80% respectively.

SUMMARY AND CONCLUSION

The developed method was successfully applied for simultaneous estimation of Ciprofloxacin and Ornidazole in compound tablet formulation. The proposed method was found to be simple, accurate and precise. There is no analytical method available to determine the same combination of drugs. Therefore, this method may be useful for routine analysis of Ciprofloxacin and Ornidazole in API drugs and pharmaceutical dosage forms.

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