Original Article

Method Development and Validation of Ciprofloxacin HCl and Ornidazole by UFLC in Combined Dosage Form

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ABSTRACT

Background: The combination of Ciprofloxacin and Ornidazole is used for the effective treatment of intra-abdominal infections. Many branded and generic combined dosage formulations of Ciprofloxacin and Ornidazole are available in market. **Objective:** To develop and validate rapid, sensitive, simple and accurate reverse phase ultra-fast liquid chromatographic method for the determination of Ciprofloxacin and Ornidazole in combined dosage forms. **Methods:** The separation was carried out by using Phenomenex Luna C_{18} (250 × 4.6 mm, 5 μm) column as stationary phase and acetonitrile: methanol: 0.1% formic acid as a mobile phase in ratio of 35:35:30 % v/v/v. The analysis was performed at flow rate of 0.8 ml/min using PDA detector at 300 nm. The column temperature was maintained at 20°C for better separation and resolution. **Results:** The retention time for Ciprofloxacin HCl and Ornidazole was found to be 2.1 min and 4.2 min respectively. The developed method was validated with respect to specificity, linearity and range, precision, robustness and accuracy according to ICH guidelines. The method was found to be linear between the concentration ranges of 2-10 μg/ml with correlation coefficient of 0.999. The % relative standard deviation of precision and robustness was found to be less than 2%. The percent accuracy was found to be 98-103% and 99-102% for Ciprofloxacin HCl and Ornidazole respectively. **Conclusion**: The method is applicable for the routine analysis and quality control of Ciprofloxacin HCl and Ornidazole in combination.

Key words: Ciprofloxacin HCl, Ornidazole, Intra-abdominal infections, Ultra-fast, PDA detector.

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INTRODUCTION

Ciprofloxacin HCl (Figure 1), chemically 1- cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid[1] is used as broad-spectrum antibacterial agent. It is a fluoroquinolone having potent antibacterial activity against gram positive and gram-negative bacteria. It acts by inhibiting the enzymes DNA gyrase and topoisomerases which are essential for bacterial replication.[2] It is mainly used in the infections of urinary tract, GI tract and skin tissues by bacteria.[3,4] Ornidazole, chemically (Figure 2) 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol is used as an anti-protozoal agent and in combination with Ciprofloxacin HCl it is used to treat intraabdominal infections.[5,6]

Literature survey reveals several analytical methods have been developed and validated for estimation of Ciprofloxacin HCl and Ornidazole in bulk, pharmaceutical dosage forms and biological fluids. Ciprofloxacin HCl were analysed by few spectroflourimetric,[7] spectrophotometric[7-9] and HPLC[10-21] and HPTLC methods.[22] Ornidazole were estimated by few HPLC[13,14,16,23]

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and HPTLC[22] methods. But no Ultra-Fast Liquid Chromatographic (UFLC) method was reported for estimation of Ciprofloxacin HCl and Ornidazole in combined bulk dosage forms. Hence in the present research work an attempt has been made to develop and validate simple, rapid, sensitive, precise, robust and accurate UFLC method determination of Ciprofloxacin HCl and Ornidazole.

MATERIALS AND METHODS

Reagents and Chemicals

All the chemicals and reagents used for the analysis were pure and analytical grade was obtained from the KLE College of Pharmacy, Belgaum. Ciprofloxacin HCl was procured from Godavari Drugs Limited with 99.7 % purity. Ornidazole was procured from Endoc Life care Pvt Ltd with 95 % purity. Acetonitrile, methanol and formic acid of Fisher Scientific was used. Milli-Q Water was used for the UFLC analysis.

Instruments and apparatus

Shimadzu prominence UFLC system (Kyoto, Japan) equipped with LC-20AD pump, SPD-M20A PDA detector, DGU-20 AS online degasser, SIL-20AC HT auto sampler, Rheodyne injector with 20 µl capacity and CTO-10ASVP column oven was used. For data acquisition and integration of chromatograms Shimadzu LC Solution software (Version 1.25) was used. For the weighing analytes analytical balance of Shimadzu make was used. Branson 1800 water bath Sonicator and Syringe Filters (Spin-Pure): PTFE 0.2 µm, Nylon 0.2 µm were used.

Method Development

In order to develop suitable chromatographic method few trials were made by using different mobile phase composition utilising various polar and non-polar solvents also by utilising different columns as stationary phase.

Chromatographic Conditions

Stationary phase

Phenomenex Luna C_{18} column (250×4.6 mm x 5 µm) connected with Phenomenex ODS C-18 (4×3.0 mm x 5 µm) as a guard column was used as stationary phase for better separation.

Mobile phase solvent system

Solvent system composed with mixture of Acetonitrile: Methanol: 0.1 % Formic acid in the ratio of 35:35:30v/v/v were filtered through PTFE 0.2 µm and Nylon 0.2 µm membrane filters and sonicated for 10 min were used for the analysis.

Preparation of Standard

10 mg of Ciprofloxacin and Ornidazole were weighed accurately using analytical balance and transferred into 10 mL of volumetric flask individually and volume was made upto the mark using mobile phase to obtained 1000 µg/ml of each analyte. 2.5 mL of above solution from each flask was pipetted out and transferred into 25 mL of volumetric flask individually and volume was made upto the mark using mobile phase. From the above stock solution 20 mL of solution was transferred together into 50 mL of volumetric flask and volume was made upto the mark using mobile phase to obtained40 µg/ml of each analyte.

Determination of retention time of analytes

10 µl of 40 µg/ml of Ciprofloxacin and Ornidazole solution were injected into UFLC connected with Phenomenex C_{18} column. The separation of two analytes was carried out by using Acetonitrile: Methanol: 0.1 % Formic acid in the ratio of 35:35:30v/v/v as mobile phase with flow rate of 0.8 mL/min with UV detection at 300 nm. Isocratic modes with run time of 10 min

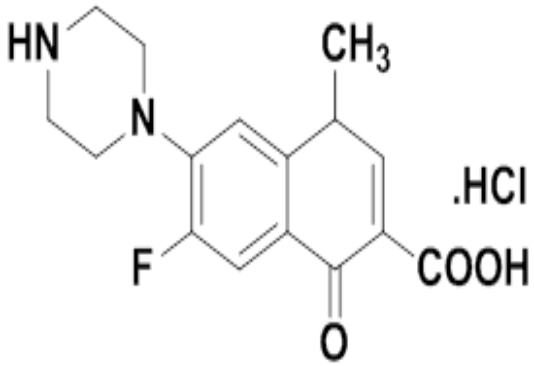

Figure 1: Structure of Ciprofloxacin HCl.

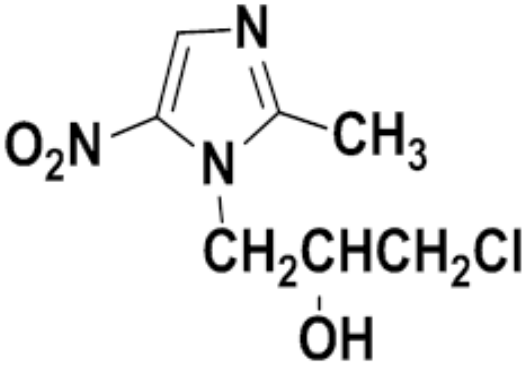

Figure 2: Structure of Ornidazole.

were used. The column oven temperature was maintained at 20°C and pump pressure was 82 kgf/c.

Method Validation

Developed method was validated as per the guidelines of ICH by performing system suitability, linearity and range, specificity, precision, robustness, limit of detection, limit of quantification and accuracy.[24-26]

System suitability

In order to check the suitability of instrument system suitability was performed on daily basis by injecting 10 µl of 40 µg/ml of Ciprofloxacin and Ornidazole solution in six replicates. Chromatograms were obtained and % RSD of peak area and Retention time was calculated.

Linearity and range

Linearity and range for both drugs was obtained by injecting 2,4,6,8 and 10 µg/ml solutions in triplicate. Peak area was obtained from the chromatograms and calibration curve was plotted.

Specificity and Selectivity

10 µl of mobile phase and 2 µg/ml solutions of each drug solutions were injected into UFLC in six replicates and chromatograms was obtained.

Precision

Precision study was carried out by performing repeatability, intraday and interday precision. Repeatability was performed by injecting six replicates of each drug solutions. Intraday and interday precision was performed by injecting three replicates of each drug solution into UFLC on same day at three different times and on three different days. Chromatograms was obtained and % RSD of retention time and peak area was calculated.

Robustness

Robustness was performed by changing the flow rate and column oven temperature. The optimized flow rate was 0.8 mL/min and temperature was 20ºC. For the robustness study flow rate was varied to 0.6 and 1.0 mL/min and temperature condition was varied to 15ºC and 25ºC. By changing the above variables in developed method parameters six replicates of each drug solution was injected and chromatograms was obtained, retention time and peak area was obtained and % RSD was calculated.

Accuracy

Accuracy was performed by standard addition method at three different levels mainly 50%, 100% and 150% levels by adding standard and sample at different proportions. Three replicates of each drug solution at three different levels was prepared and injected into UFLC. Chromatograms was obtained and retention time and peak area was obtained and % RSD was calculated.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Initially Acetonitrile and water in the proportion 50:50 v/v were tried however only Ciprofloxacin HCl showed elution and Ornidazole was poorly eluted. Therefore, acetonitrile was replaced with methanol, when methanol and water in the ratio 50:50 v/v were tried it resulted in the elution of Ornidazole and Ciprofloxacin HCl did not eluted. Further trials were made by using mobile phase composition acetonitrile: methanol: water in the ratio 40:40:20 v/v/v resulted in the elution of both the peaks, however tailing was observed and resolution between the two peaks was less. Therefore, to reduce peak tailing and increase resolution acetonitrile: methanol: 0.1% formic acid was used in the ratio 35:35:30 v/v/v. Column temperature and flow rate had significant impact on peak sharpness and retention time hence various trials were carried out to select optimum temperature between 20-30ºC and flow rate between 0.8 to 1ml/min. The developed RP-UFLC method was optimized and separation was achieved on Luna C_{18} column using mobile phase acetonitrile: methanol: 0.1% formic acid (35:35:30 v/v/v), maintaining the column temperature at 20ºC and flow rate at 0.8 ml/min and detection was achieved at 300 nm using PDA detector. The standard chromatograms of Ciprofloxacin HCl and Ornidazole in combination were showed in Figure 3.

Method validation

System Suitability

The system suitability for both drugs was found to be within the acceptance limit as the % RSD of Retention time and peak area was less than 2% with theoretical plates of each injection more than 2000. Hence the

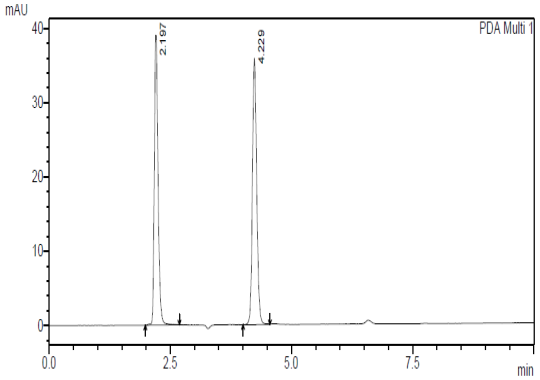

Figure 3: Chromatogram of Ciprofloxacin and Ornidazole in combination.

tested parameters were within the limit which ensures the suitability of the instrument Table 1.

Specificity

The developed method was found to be specific as the mobile phase showed there is no interference of peak at retention time of two analytes.

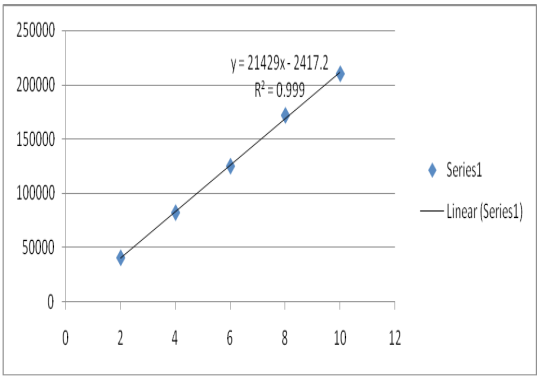

Figure 4: Standard calibration plot of Ciprofloxacin HCl.

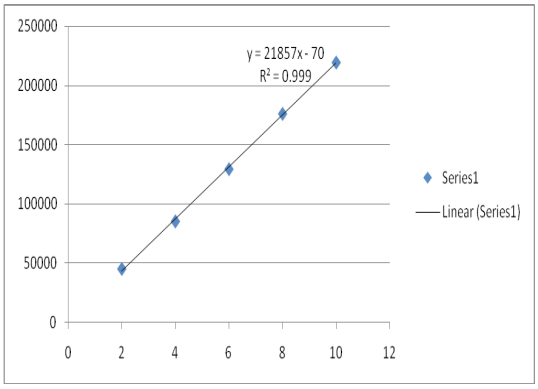

Figure 5: Standard calibration plot of Ornidazole.

Table 1: System suitability data of Ciprofloxacin HCl and Ornidazole.

Analytes	Retention Time	Theoretical plates	Tailing Factor	Area
Ciprofloxacin HCl	2.1 Min	3095	1.413	40813
Ornidazole	4.2 Min	8981	1.209	45606

Table 2: Linearity and range data report.

Ciprofloxacin HCl		Ornidazole	
Concentration	Peak Area	Concentration	Peak Area
2 µg/ml	40813	2 µg/ml	45606
4 µg/ml	82441	4 µg/ml	85508
6 µg/ml	125190	6 µg/ml	129385
8 µg/ml	172017	8 µg/ml	175874
10 µg/ml	210311	10 µg/ml	218996

Linearity

The peak areas of the Ciprofloxacin HCl and Ornidazole were plotted against their corresponding concentration. The calibration curve for Ciprofloxacin HCl and Ornidazole were found to be linear in the concentration range of 2-10 µg/ml respectively. The correlation coefficient of both drugs was found to be 0.999. The linearity data was presented in Table 2 and standard calibration curve was showed in Figure 4 and Figure 5.

Precision

Developed method was found to be precise as the results of intraday, interday and precision repeatability was found to be within the acceptance. The results were summarised in the Table 3.

Robustness

The method was found to be robust as all the results obtained in the robustness study were within the acceptance of limit. The results was summarised in the Table 4.

Accuracy

The % recovery was found in the range 98-103% and 99-102 % for Ciprofloxacin HCl and Ornidazole respectively. The mean % recoveries along with their % RSD are summarised in Table 5 and 6.

Table 3: Precision data of Ciprofloxacin HCl and Ornidazole.

Variable parameters	Ciprofloxacin HCl		Ornidazole	
	Peak area	% RSD	Peak area	% RSD
Precision Repeatability	160540	0.144	181926.8	0.227
Intraday Precision	171363	0.40	175638	0.17
Interday Precision	175087	0.45	178574	0.39

Table 4: Robustness report of Ciprofloxacin HCl and Ornidazole.

Variable parameters	Ciprofloxacin HCl		Ornidazole	
	Peak area	% RSD	Peak area	% RSD
Flow rate 0.6 ml/min	226707	0.4	238774	0.1
Flow rate 1 ml/min	138286	0.4	145355	0.2
Temp. 15°C	171613	0.3	180983	0.06
Temp. 15°C	171082	0.07	180592	0.3

Table 5: Accuracy data of Ciprofloxacin HCl.

Levels	Conc.	Quantity Added	Recovered Amount	Recovery %	% RSD
50 %	8 µg/ml	4 µg/ml	6.2 µg/ml	103.3 %	0.076
100 %	8 µg/ml	8 µg/ml	8.1 µg/ml	101.2 %	0.027
150 %	8 µg/ml	12 µg/ml	9.9 µg/ml	99 %	0.058

Table 6: Accuracy data of Ornidazole.

Levels	Conc.	Quantity Added	Recovered Amount	Recovery %	% RSD
50 %	8 µg/ml	4 µg/ml	6.1 µg/ml	101.6 %	0.002
100 %	8 µg/ml	8 µg/ml	8.2 µg/ml	102.5 %	0.29
150 %	8 µg/ml	12 µg/ml	9.9 µg/ml	99 %	0.26

CONCLUSION

Statistical analysis proved that the developed method was simple, robust, specific, accurate and precise Ciprofloxacin HCl and Ornidazole in combined form. The use of C_{18} column showed better column efficiency and elution of the analytes with good resolution, tailing factor and theoretical plate count. The quantification of both the drugs using UFLC has been achieved with shorter analysis time. Hence it was proved that the developed UFLC method was simple, robust, specific, accurate and precise for the analysis of Ciprofloxacin HCl and Ornidazole in combination and can be adopted during routine quality control analysis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

UFLC: Ultra-Fast Liquid Chromatography; **PDA:** Photo Diode Array; **ICH:** International Conference on Harmonization; **HPLC:** High Performance Liquid Chromatography; **HPTLC:** High Performance Thin Layer Chromatography; **UV:** Ultra Violet; **RSD:** Relative Standard Deviation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification.

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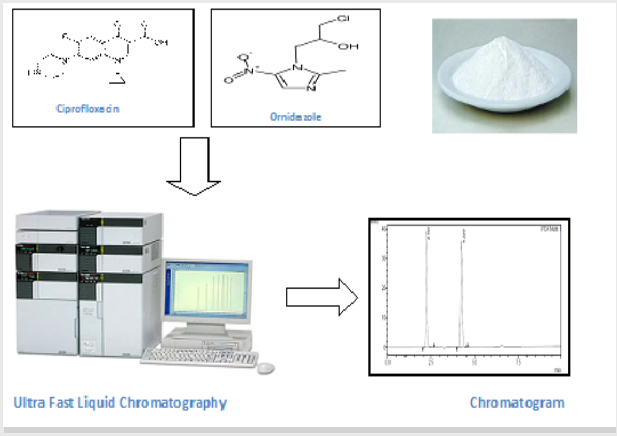
PICTORIAL ABSTRACT



SUMMARY

Ultra Fast Liquid Chromatographic method was developed and validated for estimation of Ciprofloxacin HCl and Ornidazole in combined bulk dosage forms. UFLC method was developed and validated as per ICH Q2 guidelines. Phenomenex Luna C_{18} column (250 × 4.6 mm x 5 µm) was used as stationary phase. Solvent system composed with mixture of acetonitrile: methanol: 0.1% formic acid in the ratio of 35:35:30v/v/v was used as mobile phase. The flow rate was 0.8 ml/minutes. Detection of analytes was carried out at 300 nm using PDA detector. The column temperature was maintained at 20ºC. Retention time of Ciprofloxacin HCl and Ornidazole 2.1 and 4.2 minutes respectively. All the values of validation parameters were found to be within the acceptance limit as per the ICH guidelines. Hence the developed and validated UFLC method can be used for routine quality control of Ciprofloxacin HCl and Ornidazole.

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