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Research Paper

RP-HPLC Method Development and Validation for The Estimation of Metronidazole and Ciprofloxacin in Tablets

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ABSTRACT

The present study aimed to develop and validate a simple, precise, accurate, and robust RP-HPLC method for the simultaneous estimation of Metronidazole and Ciprofloxacin in tablet dosage forms. Chromatographic separation was achieved using an Inertsil ODS C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of Acetonitrile:Methanol:Water (50:30:20, v/v/v) at a flow rate of 1.0 mL/min under isocratic conditions. Detection was carried out at 250 nm. The method exhibited excellent linearity over concentration ranges of 20–60 µg/mL for Ciprofloxacin and 50–150 µg/mL for Metronidazole, with correlation coefficients greater than 0.999. The developed method was validated according to ICH guidelines for specificity, linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ. Recovery studies demonstrated excellent accuracy with recoveries ranging from 99% to 101%, while precision studies showed %RSD values below 2%. Accelerated stability studies confirmed the stability-indicating nature of the method, with no significant degradation observed under storage conditions of 40 ± 2°C/75 ± 5% RH for three months. The validated method was successfully applied to the assay of marketed Ciprodiazole tablets, yielding assay values of 97.31% for Ciprofloxacin and 98.43% for Metronidazole. The results demonstrated that the developed RP-HPLC method is reliable, sensitive, and suitable for routine quality control and stability analysis of combined tablet formulations containing Metronidazole and Ciprofloxacin.

INTRODUCTION

Metronidazole and Ciprofloxacin are widely used antimicrobial agents employed either alone or in combination for the treatment of various bacterial and protozoal infections. Metronidazole, a nitroimidazole derivative, exhibits potent activity against anaerobic bacteria and protozoa by disrupting microbial DNA synthesis. Ciprofloxacin, a second-generation

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fluoroquinolone antibiotic, exerts its antibacterial effect through inhibition of bacterial DNA gyrase and topoisomerase IV, resulting in the suppression of DNA replication and cell division. The combination of these drugs is frequently prescribed for the management of mixed aerobic and anaerobic infections, gastrointestinal infections, intra-abdominal infections, and gynecological infections.[1-3]

The increasing use of combination pharmaceutical formulations necessitates the development of reliable analytical methods for their routine quality control and regulatory compliance. Accurate quantification of active pharmaceutical ingredients in combined dosage forms is essential to ensure product efficacy, safety, and consistency. Various analytical techniques, including spectrophotometry, HPLC, HPTLC, and LC-MS, have been reported for the estimation of Metronidazole and Ciprofloxacin individually or in combination with other drugs. However, there remains a need for simple, rapid, precise, and cost-effective methods suitable for routine analysis in quality control laboratories.[4-5]

Reverse-phase high-performance liquid chromatography (RP-HPLC) is one of the most widely employed analytical techniques in pharmaceutical analysis due to its high sensitivity, selectivity, accuracy, and reproducibility. Method validation according to the International Council for Harmonisation (ICH) guidelines is essential to demonstrate the suitability of the developed analytical procedure for its intended purpose.[6-9]

Therefore, the present study was undertaken to develop and validate a simple, accurate, precise, robust, and economical RP-HPLC method for the simultaneous estimation of Metronidazole and Ciprofloxacin in tablet dosage forms. The developed method was validated in accordance with ICH guidelines and successfully applied to the analysis of marketed pharmaceutical formulations.

MATERIALS AND METHODS:

MATERIALS:

Metronidazole and Ciprofloxacin reference standards were procured from Yarrow Chem Products and Trimurti Drug Supplier, Surat, Gujarat, respectively. HPLC-grade methanol and acetonitrile used as mobile phase components were obtained from Advent. Milli-Q water of HPLC grade was used throughout the study. Hydrochloric acid (HCl) and sodium hydroxide (NaOH), employed for pH adjustment and stress degradation studies, were procured from Dipa Chemical Industry and were of EP grade. All chemicals and reagents utilized in the present investigation were of analytical or HPLC grade and were used without further purification.

Characterization of Drugs

Organoleptic Evaluation

The obtained samples of Metronidazole and Ciprofloxacin were subjected to organoleptic evaluation. The drugs were examined visually for their color, odor, appearance, and other physical characteristics to confirm their identity and purity.[10]

Physical Characterization

The melting points of Metronidazole and Ciprofloxacin were determined using the open capillary method and recorded as uncorrected values. Solubility studies were performed in various solvents, including water, phosphate buffer, ethanol, methanol, and acetonitrile, to identify suitable solvents for analytical method development and ensure drug stability.[11-12]

Fourier Transform Infrared (FT-IR) Spectroscopy

The identity and purity of Metronidazole and Ciprofloxacin were confirmed using Fourier Transform Infrared (FT-IR) spectroscopy. The

infrared spectra of both drugs were recorded using an FTIR-4600 spectrophotometer (Jasco, Japan), and the characteristic absorption peaks were compared with reported reference values.[13-15]

Differential Scanning Calorimetry (DSC)

Thermal analysis of Metronidazole and Ciprofloxacin was carried out using Differential Scanning Calorimetry (DSC) (PerkinElmer, San Jose, CA, USA). Samples were scanned over an appropriate temperature range under a nitrogen atmosphere at a controlled heating rate to evaluate their thermal behavior and melting characteristics.[16-17]

Spectroscopic Analysis

Determination of λmax

Prior to RP-HPLC method development, UV spectroscopic studies were performed to determine the maximum absorbance wavelength (λmax) of Metronidazole and Ciprofloxacin. Standard solutions of both drugs were scanned in the wavelength range of 200–400 nm using a UV-Visible spectrophotometer, and the wavelengths showing maximum absorbance were selected for further analysis.[18-20]

Preparation of Calibration Curve

Standard stock solutions of Metronidazole and Ciprofloxacin were prepared by accurately weighing 10 mg of each drug and dissolving separately in Methanol:Acetonitrile (70:30 v/v). The solutions were sonicated, filtered through a 0.45 µm membrane filter, and further diluted to obtain concentration ranges of 5–25 µg/mL for Metronidazole and 4–20 µg/mL for Ciprofloxacin. The absorbance of these solutions was measured, and calibration curves were constructed by plotting absorbance against concentration.[21-22]

Instrumentation

UV spectroscopic measurements were carried out using a Lasany LI-2702 double-beam UV-Visible spectrophotometer equipped with matched 1 cm quartz cells. Drug samples were accurately weighed using an Elder digital analytical balance, and solution preparation was facilitated using a Prama ultrasonic sonicator.[23]

UV Method Development and Validation

A suitable solvent system for UV spectrophotometric analysis was selected based on the solubility of Metronidazole and Ciprofloxacin in various solvents, including distilled water, ethanol, methanol, acetonitrile, phosphate buffer, and their combinations. Among the tested systems, Methanol:Acetonitrile (70:30 v/v) provided satisfactory solubility and stability for both drugs and was therefore selected as the common solvent for further studies.

The developed UV method was validated according to ICH guidelines with respect to linearity, accuracy, precision, sensitivity, robustness, and ruggedness. Linearity was evaluated by preparing standard solutions in the concentration range of 5–25 µg/mL for Metronidazole and 4–20 µg/mL for Ciprofloxacin, and calibration curves were constructed by plotting absorbance against concentration.

The sensitivity of the method was determined by calculating the limit of detection (LOD) and limit of quantification (LOQ) using the standard deviation of the response and the slope of the calibration curve. Accuracy was assessed by recovery studies using the standard addition method at 80%, 100%, and 120% levels, and the percentage recovery was calculated.

Precision was evaluated in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision) by analyzing selected concentrations of both drugs on the same day and on different days. The results were expressed as percentage relative standard deviation (%RSD).

Robustness of the method was examined by introducing small deliberate variations in analytical conditions, while ruggedness was assessed by evaluating the reproducibility of the method under different operating conditions. The obtained results demonstrated the reliability and suitability of the developed UV spectrophotometric method for the simultaneous estimation of Metronidazole and Ciprofloxacin.[23-25]

RP-HPLC Method Development

RP-HPLC analysis was carried out using a Shimadzu LC-2010 series HPLC system equipped with an autosampler and UV-Visible detector. Chromatographic separation was performed on an Inertsil ODS-3V C18 column (250 × 4.6 mm, 5 µm particle size). Standard and sample solutions were prepared using HPLC-grade solvents and filtered before analysis.

The detection wavelength was selected by scanning individual solutions of Metronidazole and Ciprofloxacin in the UV range of 200–400 nm. Based on the overlay spectra, 250 nm was chosen as the optimum wavelength for simultaneous estimation of both drugs. Several mobile phase compositions comprising methanol, acetonitrile, and water were evaluated to achieve satisfactory peak resolution and system suitability.

After optimization, the chromatographic separation was achieved using a mobile phase consisting of Methanol:Acetonitrile:Water (50:30:20, v/v/v) at a flow rate of 1.0 mL/min under isocratic conditions. The mobile phase was filtered through a 0.45 µm membrane filter and degassed by ultrasonication before use. Prior to sample analysis, the HPLC system was primed and the column was conditioned to ensure stable chromatographic performance and a consistent baseline.[26-28]

Standard stock solutions

of Metronidazole and Ciprofloxacin were prepared in the mobile phase and subsequently diluted to the required concentrations. The prepared solutions were filtered through a 0.20 µm nylon membrane filter, sonicated, and injected into the HPLC system. Chromatograms were recorded under the optimized chromatographic conditions and evaluated for peak characteristics, retention time, and system suitability parameters.[29-30]

RP-HPLC Method Validation[31-38]

System Suitability and Specificity

The suitability of the RP-HPLC system was evaluated by analyzing replicate injections of standard solutions and assessing chromatographic parameters including retention time, peak area, theoretical plates, tailing factor, and resolution. Specificity of the method was established by demonstrating complete separation of Metronidazole and Ciprofloxacin without interference from tablet excipients or other components present in the sample matrix.

Linearity and Sensitivity

The linearity of the method was assessed over concentration ranges of 50–150 µg/mL for Metronidazole and 20–60 µg/mL for Ciprofloxacin. Calibration curves were constructed by plotting peak area against concentration, and the corresponding regression equations and correlation coefficients were determined. The sensitivity of the method was evaluated by calculating the limit of detection (LOD) and limit of quantification (LOQ) using the standard deviation of the response and the slope of the calibration curve.

Accuracy and Precision

The accuracy of the developed method was determined by recovery studies using the standard addition technique at 80%, 100%, and 120% concentration levels. Precision was evaluated in

terms of repeatability (intra-day precision) and intermediate precision (inter-day precision) by analyzing replicate samples and expressing the results as percentage relative standard deviation (%RSD). The obtained results demonstrated the reliability and reproducibility of the method.

Robustness and Ruggedness

Robustness was assessed by introducing small deliberate variations in chromatographic conditions, such as flow rate, detection wavelength, and mobile phase composition, and evaluating their effect on chromatographic performance. Ruggedness was determined by examining the reproducibility of the analytical results under different operating conditions. The validation results confirmed that the developed RP-HPLC method was accurate, precise, sensitive, robust, and suitable for the routine quantitative estimation of Metronidazole and Ciprofloxacin in tablet dosage forms.

Stability Studies

Accelerated stability studies were conducted in accordance with ICH Q1A(R2) guidelines to evaluate the stability of Metronidazole and Ciprofloxacin tablets and to establish the stability-indicating nature of the developed RP-HPLC method. The marketed tablet formulation was stored under accelerated conditions of 40 ± 2°C and 75 ± 5% relative humidity for a period of three months. Samples were withdrawn at predetermined intervals and analyzed for physical appearance and drug content.

The collected samples were prepared using the optimized analytical procedure and analyzed under the established chromatographic conditions. Assay values and chromatographic characteristics were monitored throughout the study period. No significant changes in drug content or physical appearance were observed, indicating adequate stability of the formulation. The results demonstrated the suitability of the developed RP-HPLC method for routine stability testing and quality control applications.

Analysis of Marketed Tablet Formulation

The validated RP-HPLC method was successfully applied to the quantitative estimation of Metronidazole and Ciprofloxacin in a marketed tablet formulation containing 500 mg of each drug. Tablet samples were accurately weighed, powdered, and extracted using the optimized mobile phase. The resulting solution was sonicated, filtered, and suitably diluted to obtain concentrations within the analytical range of the method.

The prepared sample solutions were injected into the RP-HPLC system under the optimized chromatographic conditions. Peak areas of Metronidazole and Ciprofloxacin were measured and compared with those obtained from corresponding standard solutions. The assay results confirmed satisfactory drug content and demonstrated the applicability of the developed method for routine quality control analysis of combined tablet dosage forms containing Metronidazole and Ciprofloxacin.

RESULTS AND DISCUSSION:

Organoleptic Evaluation

The organoleptic properties of Metronidazole and Ciprofloxacin were evaluated for preliminary identification and quality assessment. Both drugs appeared as pale yellow, fine crystalline powders, were odorless, and exhibited a characteristic bitter taste. The observed properties were found to be in agreement with the reported specifications, confirming the authenticity and purity of the drug samples.

Table 1. Organoleptic Properties of Metronidazole and Ciprofloxacin

Parameter	Metronidazole	Ciprofloxacin
Colour	Pale yellow	Pale yellow
Odour	Odourless	Odourless
Taste	Bitter	Bitter
Appearance	Fine crystalline powder	Fine crystalline powder

Physical Characterization

Melting Point Determination

The observed melting points of Metronidazole and Ciprofloxacin were found to be in close agreement with the reported values, indicating the purity and identity of the drug samples.

Table 2. Melting Point of Metronidazole and Ciprofloxacin

Drug	Reported Melting Point (°C)	Observed Melting Point (°C)
Metronidazole	150–154	153.75
Ciprofloxacin	196–200	196.52

Solubility Studies

Solubility studies were performed in various solvents to identify a suitable solvent system for analytical method development. Both drugs exhibited good solubility in methanol and poor solubility in water, supporting the selection of organic solvents for further RP-HPLC studies.

Table 3. Solubility Profile of Metronidazole and Ciprofloxacin

Solubility	Metronidazole	Ciprofloxacin
Soluble	Methanol	Methanol
Slightly Soluble	Buffer	Acetonitrile
Insoluble	Water	Water

The results of organoleptic evaluation, melting point determination, and solubility studies confirmed the identity, purity, and suitability of Metronidazole and Ciprofloxacin for subsequent method development and validation studies.

FT-IR spectroscopy study:

Identification of Metronidazole and Ciprofloxacin was confirmed by FTIR Spectra. All peaks was found in Metronidazole and Ciprofloxacin drugs.

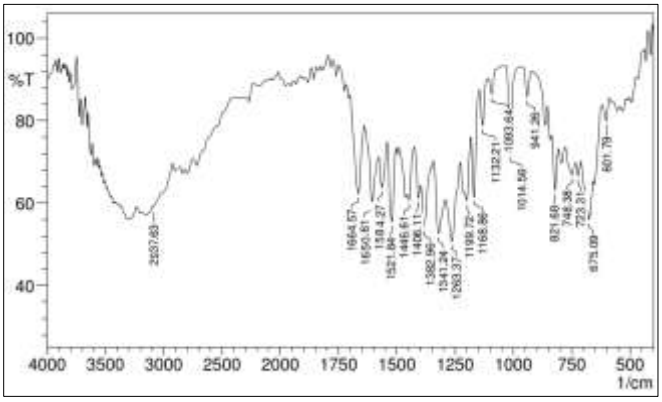

Figure 1: FTIR spectrum of Metronidazole

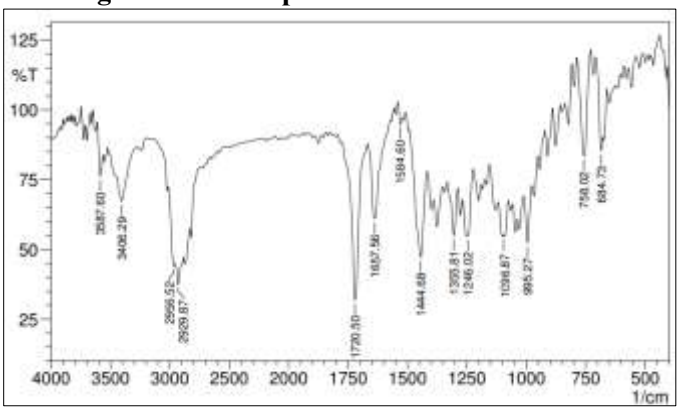

Figure 2: FTIR spectrum of Ciprofloxacin

DSC study of pure drug:

DSC profile of Metronidazole and Ciprofloxacin. Metronidazole showed a sharp endothermic peak at 196.00^{0}C whereas, Ciprofloxacin showed a peak at 163.00^{0}C corresponding to the melting transition temperature and decomposition of these drugs. Such sharp endothermic peak signifies that MNZ and CFC used were in pure crystalline state.

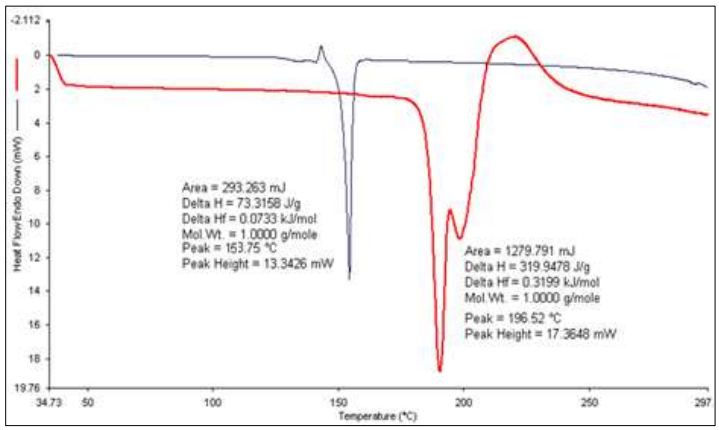

Figure 3: DSC Thermogram of MNZ and CFC

UV analysis:

Determination of λmax:

The std. solution of concentration 10µg/ml exhibited max absorbance at 295 nm for Metronidazole and 240 nm for Ciprofloxacin.

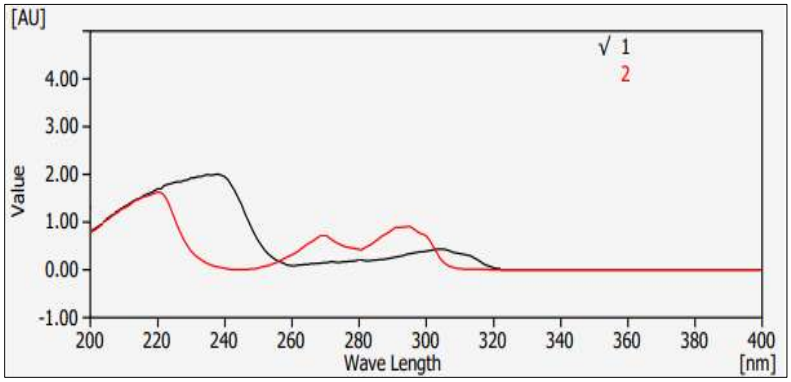

Figure 4: λmax of drug MNZ and CFC

UV Method Validation

Linearity

The developed UV spectrophotometric method exhibited good linearity over the concentration range of 5–25 µg/mL for Metronidazole (MNZ) and 4–20 µg/mL for Ciprofloxacin (CFC). Calibration curves showed excellent linear relationships between concentration and absorbance, with correlation coefficients (R^2) of 0.999 for both drugs.

Table 4. Linearity Data of Metronidazole and Ciprofloxacin

Parameter	Metronidazole	Ciprofloxacin
Linearity Range (µg/mL)	5–25	4–20
Slope	0.0374	0.0481
Intercept	0.0698	0.0981
Correlation Coefficient (R^2)	0.999	0.999

Sensitivity (LOD and LOQ)

The sensitivity of the method was evaluated in terms of the limit of detection (LOD) and limit of quantification (LOQ). The low values obtained

indicated adequate sensitivity for the estimation of both drugs.

Table 5. LOD and LOQ of Metronidazole and Ciprofloxacin

Parameter	Metronidazole (µg/mL)	Ciprofloxacin (µg/mL)
LOD	0.271	0.574
LOQ	0.563	0.768

Accuracy

Accuracy was assessed by recovery studies at 80%, 100%, and 120% levels using the standard addition method. The percentage recoveries ranged from 99.22% to 101.09%, indicating excellent accuracy of the proposed method.

Table 6. Accuracy Study of Metronidazole and Ciprofloxacin

Drug	Level (%)	Mean Recovery (%)	%RSD
Metronidazole	80	101.09	0.617
	100	100.46	1.203
	120	99.63	0.154
Ciprofloxacin	80	99.41	1.071
	100	100.25	0.562
	120	99.22	0.288

Precision

The precision of the method was evaluated through intra-day and inter-day studies. The %RSD values for both drugs were below 2%, demonstrating satisfactory repeatability and reproducibility of the method.

Table 7. Precision Study of Metronidazole and Ciprofloxacin

Drug	Parameter	%RSD
Metronidazole	Intra-day	0.619
Metronidazole	Inter-day	0.458
Ciprofloxacin	Intra-day	0.653
Ciprofloxacin	Inter-day	0.645

Ruggedness

Ruggedness was evaluated by performing the analysis under different analyst conditions. The low %RSD values obtained confirmed the reproducibility of the method.

Table 8. Ruggedness Study of Metronidazole and Ciprofloxacin

Drug	Condition	%RSD
Metronidazole	Analyst 1	0.632
Metronidazole	Analyst 2	0.489
Ciprofloxacin	Analyst 1	0.486
Ciprofloxacin	Analyst 2	0.367

Robustness

The robustness study demonstrated that minor deliberate variations in analytical conditions did not significantly affect the analytical performance. The %RSD values were well below the acceptable limit of 2%, confirming the robustness of the method.

Table 9. Robustness Study of Metronidazole and Ciprofloxacin

Drug	%RSD
Metronidazole	0.332
Ciprofloxacin	0.452

Overall, the validation results confirmed that the developed UV spectrophotometric method is linear, accurate, precise, sensitive, rugged, and robust for the simultaneous estimation of Metronidazole and Ciprofloxacin.

RP-HPLC Method Development and System Suitability

Selection of Analytical Wavelength

The UV spectra of Metronidazole and Ciprofloxacin were recorded, and 250 nm was selected as the analytical wavelength as both drugs exhibited significant absorbance at this wavelength, making it suitable for simultaneous estimation.

Optimization of Chromatographic Conditions

Several mobile phase compositions were evaluated to achieve satisfactory separation, peak symmetry, and resolution of Metronidazole and Ciprofloxacin. Initial trials using binary solvent systems of acetonitrile-water and methanol-water produced broad peaks with poor resolution.

Further optimization using ternary mixtures of acetonitrile, methanol, and water improved chromatographic performance.

Among all the tested conditions, the mobile phase consisting of Acetonitrile:Methanol:Water (50:30:20, v/v/v) at a flow rate of 1.0 mL/min provided well-resolved peaks with acceptable peak symmetry and theoretical plate counts. Therefore, this chromatographic condition was selected for further validation studies.

Table 10. Optimization of RP-HPLC Conditions

Trial	Mobile Phase Composition (v/v)	Observation	Status
1	Acetonitrile:Water (80:20)	Broad peaks with poor separation	Rejected
2	Methanol:Water (80:20)	Improved separation but broad peaks	Rejected
3	Acetonitrile:Methanol:Water (50:40:10)	Better peak shape but broad peaks observed	Rejected
4	Acetonitrile:Methanol:Water (50:30:20)	Good peak shape, resolution, and intensity	Accepted

Table 11. Chromatographic Parameters Obtained During Optimization

Trial	Drug	Retention Time (min)	Theoretical Plates	Asymmetry
1	Ciprofloxacin	2.523	2413	1.34
	Metronidazole	5.341	2568	1.12
2	Ciprofloxacin	3.148	3176	1.14
	Metronidazole	7.512	2861	1.26
3	Ciprofloxacin	3.861	4267	1.02
	Metronidazole	9.836	3867	1.10
4 (Optimized)	Ciprofloxacin	4.509	4637	0.886
	Metronidazole	11.738	4236	0.924

The optimized chromatographic conditions consisted of an Inertsil C18 column (250 × 4.6 mm, 5 µm), Acetonitrile:Methanol:Water (50:30:20, v/v/v) as the mobile phase, a flow rate of 1.0 mL/min, and UV detection at 250 nm under isocratic elution.

Trial No: 1

1. Acetonitrile: Water (80:20 v/v)

Flow rate: 1 ml/min

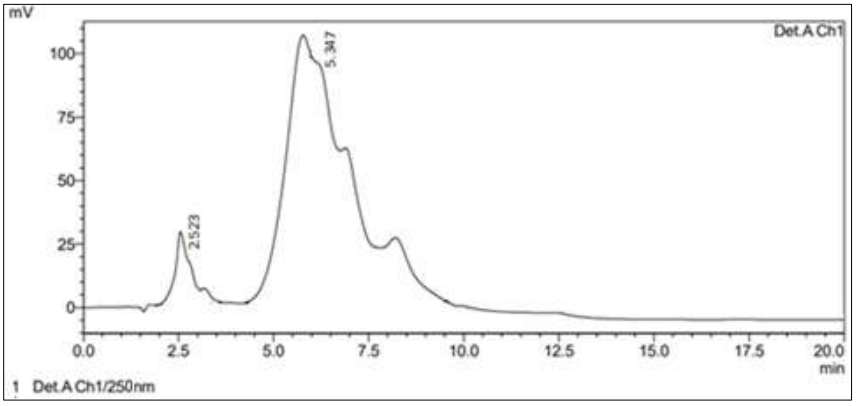

Figure 5: Mobile phase optimization (trial 1) - Acetonitrile: Water (80:20 v/v)

Optimized Method:

Mobile phase composition- Acetonitrile: Methanol: Water (50:30:20 v/v)

Flow rate: 1ml/min

Wavelength: 250nm

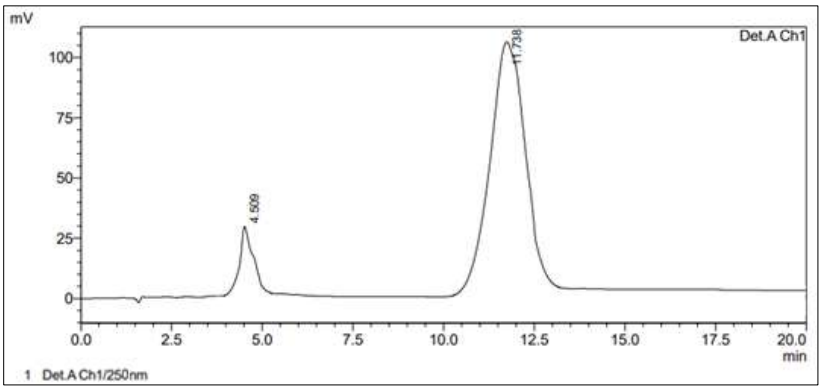

Figure 6: Mobile phase optimization (optimized trial: Acetonitrile: Methanol: Water (50:30:20 v/v)

System Suitability

System suitability testing was performed to verify the adequacy of the chromatographic system before analysis. The chromatographic parameters, including retention time, peak area, tailing factor, and theoretical plate count, were found to be within acceptable limits, indicating satisfactory system performance. The developed method provided adequate resolution and reproducible chromatographic responses for both Metronidazole and Ciprofloxacin.

Table 12. System Suitability Parameters

Drug	Retention Time (min)	Peak Area	USP Tailing Factor	USP Plate Count
Metronidazole	4.512	852146	1.68	2761
Ciprofloxacin	11.725	12173226	1.41	2367

The chromatograms obtained under the optimized conditions exhibited well-resolved and symmetrical peaks for both analytes without any interference, demonstrating the suitability of the developed RP-HPLC method for simultaneous estimation of Metronidazole and Ciprofloxacin in tablet dosage forms.

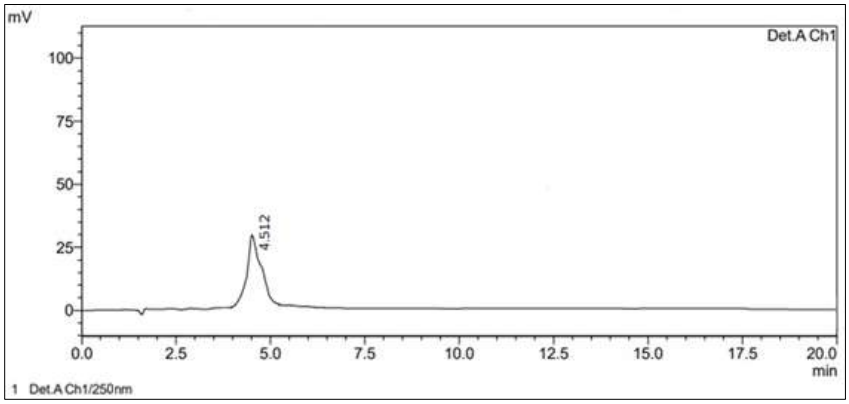

Figure 7: Standard Chromatogram of Ciprofloxacin

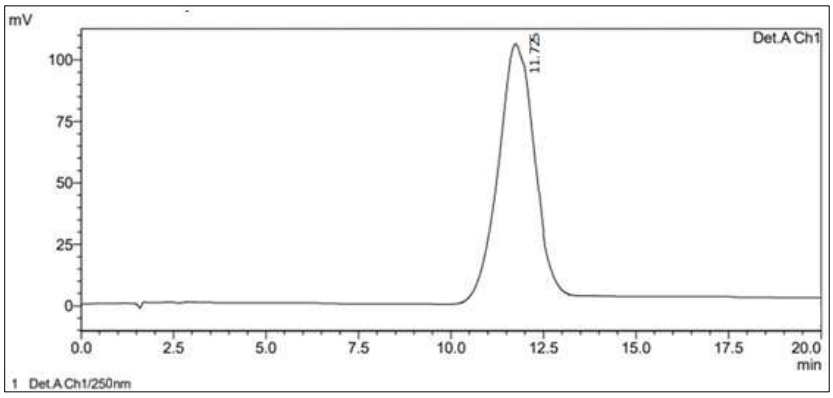

Figure 8: Standard Chromatogram of Metronidazole

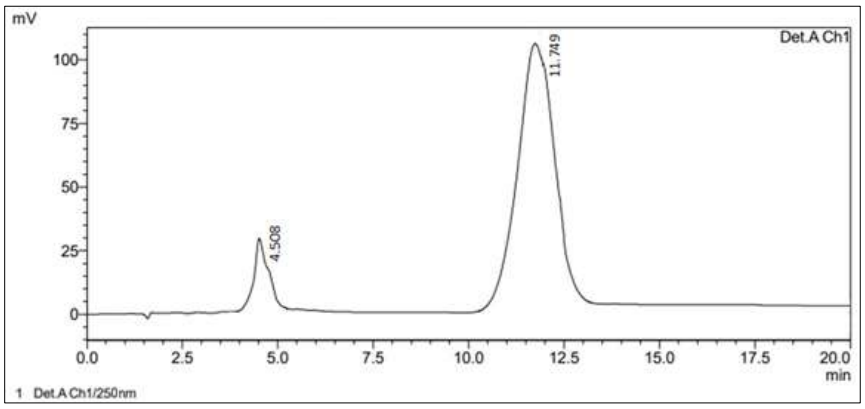

Figure 9: Standard Chromatogram of Mixture of Ciprofloxacin and Metronidazole

RP-HPLC Method Validation

Specificity

The specificity of the developed RP-HPLC method was evaluated by comparing blank, standard, and sample chromatograms. No interfering peaks were observed at the retention times of Metronidazole and Ciprofloxacin, indicating the absence of interference from excipients or other formulation components. Peak purity analysis confirmed the homogeneity of both analyte peaks, demonstrating the specificity of the method.

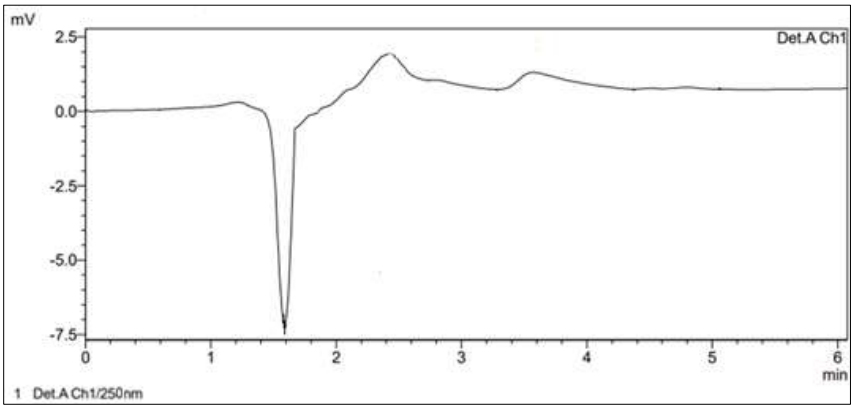

Figure 10 : Blank Chromatogram for Specificity

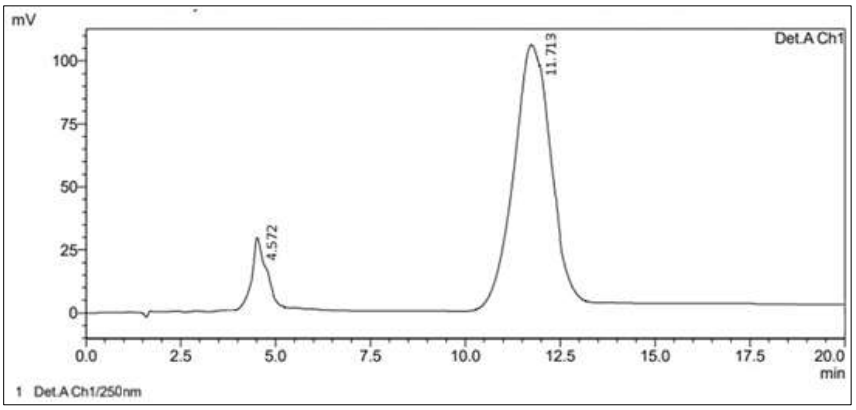

Figure 11: Purity chromatogram of Standard CFC and MNZ

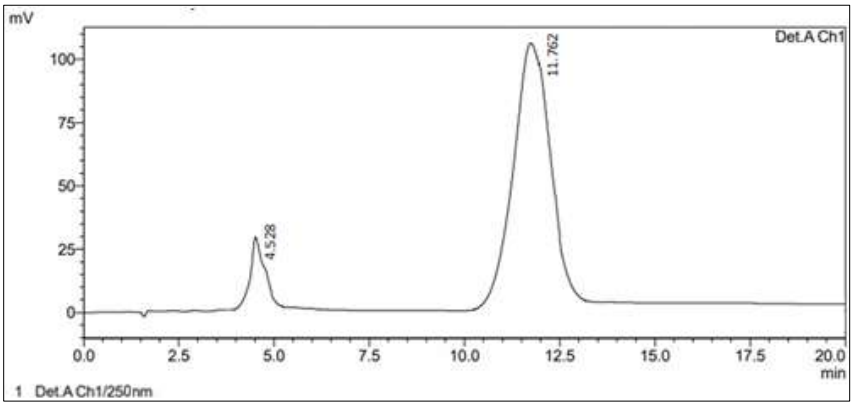

Figure 12: Purity chromatogram of Sample of CFC and MNZ

Precision

The precision of the method was assessed in terms of system precision, method precision, intra-day precision, and inter-day precision. System precision evaluated from six replicate injections showed %RSD values of 0.0440% for Ciprofloxacin and 0.0005% for Metronidazole. Method precision studies yielded mean assay values of 100.21% and 100.56% for Ciprofloxacin and Metronidazole, respectively, with %RSD values below 1.0%.

The intra-day and inter-day precision studies at different concentration levels also demonstrated excellent repeatability and intermediate precision, with all %RSD values well below the acceptance criterion of 2%, confirming the reproducibility of the developed method.

Table 13. Summary of Precision Studies

Parameter	Ciprofloxacin (%RSD)	Metronidazole (%RSD)
System Precision	0.0440	0.0005
Method Precision	0.914	0.593
Intra-day Precision	0.0045–0.0295	0.0012–0.0250
Inter-day Precision	0.014–0.082	0.001–0.039

Linearity

The linearity of the RP-HPLC method was evaluated over concentration ranges of 20–60 µg/mL for Ciprofloxacin and 50–150 µg/mL for Metronidazole. A linear relationship between peak area and concentration was observed for both analytes. The regression coefficients obtained were 0.9997 for Ciprofloxacin and 0.9992 for Metronidazole, indicating excellent linearity within the studied ranges.

Table 14. Linearity and Regression Data

Parameter	Ciprofloxacin	Metronidazole
Linearity Range (µg/mL)	20–60	50–150
Slope	45701	246911
Intercept	73433	96879
Correlation Coefficient (R^2)	0.9997	0.9992

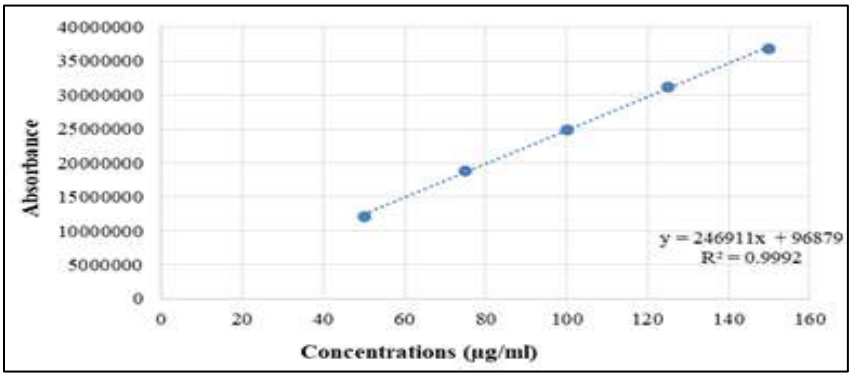

Figure 13: Plot of linearity study for Metronidazole

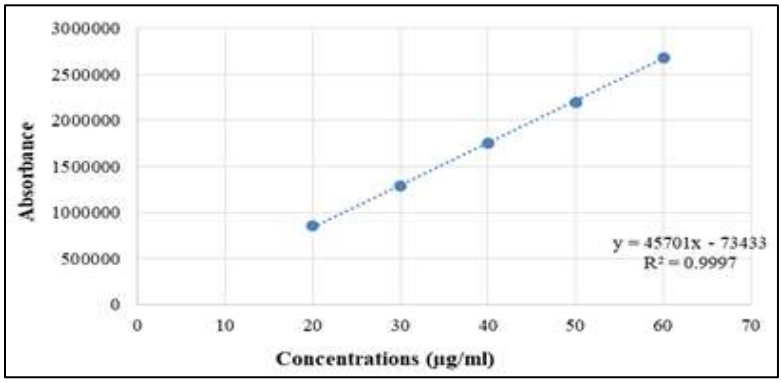

Figure 14: Plot of linearity study for Ciprofloxacin

Sensitivity (LOD and LOQ)

The sensitivity of the developed method was determined by calculating the limit of detection (LOD) and limit of quantification (LOQ). The low values obtained indicated that the method was sufficiently sensitive for the simultaneous estimation of both drugs.

Accuracy

The accuracy of the method was evaluated by recovery studies using the standard addition technique at 80%, 100%, and 120% levels. Mean recoveries ranged from 99.84% to 100.24% for Ciprofloxacin and from 100.17% to 100.46% for Metronidazole. The low %RSD values (<2%) demonstrated the accuracy of the developed method.

Table 15. Summary of Accuracy Studies

Drug	Recovery Level (%)	Mean Recovery (%)	%RSD
Ciprofloxacin	80	100.24	0.437
	100	99.84	0.226
	120	99.86	0.189
Metronidazole	80	100.46	0.459
	100	100.22	0.390
	120	100.17	0.152

Robustness

Robustness was evaluated by introducing small deliberate changes in chromatographic parameters, including flow rate and detection wavelength. These variations did not significantly

affect retention times or chromatographic performance, indicating the robustness of the developed method.

Table 16. Robustness Study

Parameter Variation	Ciprofloxacin RT (min)	Metronidazole RT (min)
Flow Rate (0.8–1.2 mL/min)	4.462–4.754	11.521–12.353
Wavelength (223–227 nm)	No significant change	11.468–11.782

Ruggedness

Ruggedness was assessed by performing the analysis at different temperatures (25°C, 37°C, and 60°C). The %RSD values for both drugs remained below 0.1%, indicating that the method is reproducible under varied operating conditions.

Overall, the validation results demonstrated that the developed RP-HPLC method is specific, linear, accurate, precise, sensitive, robust, and rugged, making it suitable for routine quality control analysis of Metronidazole and Ciprofloxacin in tablet dosage forms.

Stability Studies

Accelerated stability studies were conducted to evaluate the stability of Metronidazole and Ciprofloxacin tablets and to establish the stability-indicating capability of the developed RP-HPLC method. The tablets were stored under accelerated conditions (40 ± 2°C/75 ± 5% RH) for a period of three months and analyzed at predetermined intervals for physical appearance and assay content.

No significant changes in color, odor, texture, or tablet integrity were observed throughout the study period, indicating satisfactory physical stability. Assay values of both drugs remained within the acceptable pharmacopoeial limits (90–110%) with less than 1% reduction from the initial assay values. Furthermore, no additional peaks due to degradation products were observed, and chromatographic parameters such as retention time, peak symmetry, and resolution remained unchanged.

Table 18. Accelerated Stability Study Results

Time Point	Metronidazole (% Assay)	% Change	Ciprofloxacin (% Assay)	% Change
Initial (0 Month)	98.43	—	97.31	—
1 Month	98.21	-0.22	97.08	-0.23
2 Months	97.96	-0.47	96.84	-0.47
3 Months	97.62	-0.81	96.55	-0.76

The results confirmed that the tablet formulation remained stable under accelerated storage conditions and demonstrated that the developed RP-HPLC method is stability-indicating and suitable for routine stability testing.

Analysis of Marketed Formulation

The validated RP-HPLC method was successfully applied to the assay of a marketed tablet formulation, Ciprodiazole tablets (Minapharm Pharmaceuticals), containing Metronidazole and Ciprofloxacin. The chromatographic analysis produced well-resolved and symmetrical peaks for both drugs without interference from formulation excipients, confirming the applicability of the method for routine quality control analysis.

The chromatographic parameters obtained during the assay were within acceptable limits. Ciprofloxacin exhibited a retention time of 4.621 min, whereas Metronidazole eluted at 11.649 min. The percentage assay values were found to be 97.31% for Ciprofloxacin and 98.43% for Metronidazole, indicating compliance with the labeled claim and pharmacopoeial specifications.

Table 19. Assay Results of Marketed Formulation

Parameter	Ciprofloxacin	Metronidazole
Retention Time (min)	4.621	11.649
Peak Area (µV·sec)	838661	11845682
Tailing Factor	1.121	1.284
Theoretical Plate Count	2452.03	2367.76
% Assay	97.31	98.43

The assay results confirmed the accuracy, precision, and suitability of the developed RP-HPLC method for the simultaneous estimation of Metronidazole and Ciprofloxacin in combined tablet dosage forms and its application in routine pharmaceutical quality control.

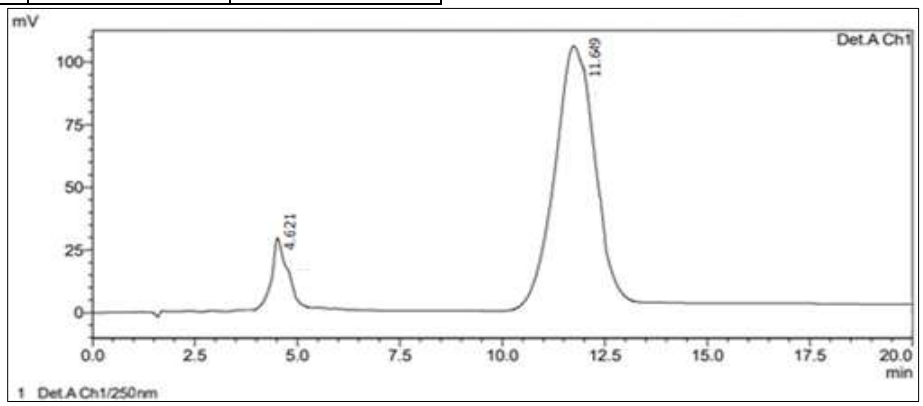

Figure 15: Analysis of Marketed formulation (Ciprodiazole tablets, Minapharm Pharmaceuticals)

CONCLUSION

A simple, rapid, accurate, and stability-indicating RP-HPLC method was successfully developed and validated for the simultaneous estimation of Metronidazole and Ciprofloxacin in tablet dosage forms. The optimized chromatographic conditions provided satisfactory separation with good peak symmetry, resolution, and reproducibility. Validation studies performed according to ICH guidelines confirmed that the method is specific, linear, precise, accurate, sensitive, robust, and rugged. The developed method exhibited excellent recovery values and low %RSD values, demonstrating its reliability for quantitative analysis.

The method was effectively applied to the analysis of marketed Ciprodiazole tablets, and the assay results were found to be within acceptable pharmacopoeial limits. Accelerated stability studies further established the stability-indicating capability of the method, as no significant degradation or changes in chromatographic performance were observed during the study period. Therefore, the proposed RP-HPLC method can be successfully employed for routine quality control, assay determination, and stability testing of pharmaceutical formulations containing Metronidazole and Ciprofloxacin.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest regarding the publication of this research work.

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