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SPECTROPHOTOMETRIC APPROACH FOR METRONIDAZOLE ASSAY: METHOD DEVELOPMENT AND VALIDATION IN SOLID DOSAGE FORMS

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Abstract

Objective: This study aims to develop and validate a simple, cost-effective, and rapid UV-spectrophotometric method for the quantitative determination of Metronidazole in both its pure form and pharmaceutical tablet formulations.

Methods: The analysis was conducted using 0.1 N NaOH as a solvent. Spectral measurements were performed at a maximum wavelength (λmax) of 320 nm using a Shimadzu UV-1800 spectrophotometer with 1 cm quartz cells. The method was strictly validated according to ICH guidelines. High-purity Metronidazole (>99%, Sigma-Aldrich) served as the standard, while commercial tablets (Cipla) were used for the formulation assay to evaluate the method's practical applicability.

Results: The method exhibited excellent linearity over the tested concentration range, with a regression equation of Y=0.0215x+0.0743 and a correlation coefficient (R2) of 0.9935. The percentage recovery ranged from

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99.33% to 99.88%, demonstrating high accuracy and no significant interference from common excipients. Precision was confirmed by low relative error values (0.1129% to 1.1226%) for concentrations of 10–25 mg/mL. Both intra-day and inter-day precision remained within acceptable limits (Relative Error < 2%). The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined to be 0.3069 µg/mL and 0.6302 µg/mL, respectively, indicating high sensitivity.

Conclusion: The developed UV-spectrophotometric technique is accurate, precise, and robust. Its simplicity and reliability make it highly suitable for routine quality control and standard assays of Metronidazole in bulk and pharmaceutical dosage forms.

Keywords: Metronidazole, UV-Visible spectrophotometry, Method validation, ICH guidelines, Pharmaceutical analysis.

1. Introduction

UV-Visible spectrophotometry remains one of the most widely adopted techniques in pharmaceutical analysis due to its precision, simplicity, and cost-effectiveness. This analytical method relies on measuring the intensity of ultraviolet or visible radiation absorbed by a substance in solution, which is directly proportional to its concentration according to the Beer-Lambert Law [1].

Metronidazole, chemically identified as 2-(2-Methyl-5-nitro-1H-imidazol-1-yl) ethanol, is a potent synthetic nitroimidazole derivative with broad-spectrum antimicrobial activity. It serves as a cornerstone in the therapeutic management of infections caused by anaerobic bacteria, microaerophilic organisms, and protozoa. Its mechanism of action involves the reduction of

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the nitro group within the microbial cell, creating cytotoxic intermediates that disrupt DNA synthesis [2].

Clinically, Metronidazole is indispensable for treating a variety of conditions, including:

- Amebiasis: Both intestinal and hepatic forms.
- Systemic Infections: Intra-abdominal, lower respiratory tract, and skin structure infections.
- Central Nervous System: Meningitis and brain abscesses.
- Gynecological & Surgical: Bacterial vaginosis, endometritis, and as a prophylactic agent in colorectal surgeries.

Physicochemical Properties of Metronidazole: The analytical determination of Metronidazole requires a comprehensive understanding of its chemical and physical profile to ensure stability and accuracy during the spectrophotometric assay.

Chemical Profile

- IUPAC Name: 2-(2-hydroxyethyl)-2-methyl-5-nitro-1H-imidazole.
- Molecular Formula: $C_6H_9N_3O_3$
- Molecular Weight: 171.15 g/mol.
- Acidity (pKa): Approximately 7.5, reflecting its weakly acidic nature, which influences its solubility and absorption spectra in different pH environments.
- Solubility: Highly soluble in water, ethanol, and methanol; however, it remains insoluble in ether [3].

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Physical Profile

- Appearance: Characteristic white to pale yellow crystalline powder.
- Melting Point: Ranges between 160–162 °C.
- Stability: Generally stable under standard laboratory conditions; however, it is sensitive to environmental factors and must be protected from light and moisture to prevent degradation [3].

2. Materials and Methods

2.1. Materials and Reagents

- Pharmaceutical Formulation: Metronidazole (MTD) tablets (800 mg) were procured from Care Formulation Labs Pvt Ltd (Mumbai, India).
- Standard Drug: Pure Metronidazole working standard was obtained from Sigma-Aldrich Ltd (Purity > 99%).
- Chemicals: All chemicals and reagents used, including Sodium Hydroxide (NaOH), were of analytical grade (AR) and were purchased from Sigma-Aldrich.

2.2. Instrumentation

Spectral and absorbance measurements were conducted using a Shimadzu UV-1800 double-beam UV-Vis spectrophotometer. The system was equipped with a 1 cm matched quartz cell pair. Data acquisition and analysis were performed at a fixed wavelength of 320 nm.

2.3. Preparation of Solutions

2.3.1. Standard Stock Solution (10 µg/mL): Accurately weighed 1 mg of pure Metronidazole was transferred into a 100 mL volumetric flask and dissolved in 0.1 N NaOH. The mixture was sonicated for 10 minutes to ensure

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complete dissolution, and the volume was made up to the mark with 0.1 N NaOH to obtain a final concentration of 10 µg/mL.

2.3.2. Sample Preparation (Tablet Dosage Form): An appropriate amount of tablet powder (equivalent to 1 mg of MTD) was weighed and transferred into a 100 mL volumetric flask. The drug was extracted using 0.1 N NaOH followed by sonication. The solution was filtered to remove excipients, and various aliquots were taken to prepare test samples with concentrations of 10, 15, 20, and 25 µg/mL.

2.4. Method Development and Calibration

A series of standard solutions were prepared by diluting the stock solution to obtain a concentration range of 8–40 µg/mL. The absorbance of each solution was measured at 320 nm against 0.1 N NaOH as a reagent blank. A calibration curve was constructed by plotting absorbance versus concentration to determine the linearity and regression equation.

2.5. Analytical Method Validation

The developed method was validated according to the International Council for Harmonisation (ICH) guidelines [4]. The validation parameters included:

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2.5.1. Linearity and Range: The linearity was evaluated by analyzing six different concentrations of MTD (8, 16, 24, 32, and 40 µg/mL). Each concentration was analyzed in triplicate (n=3) to confirm adherence to the Beer-Lambert Law.

2.5.2. Accuracy (Recovery Studies): Accuracy was assessed using the standard addition method. Known amounts of pure MTD were added to pre-analyzed tablet formulations at three levels: 80%, 100%, and 120%. The percentage recovery was calculated to evaluate the influence of excipients and the reliability of the method.

2.5.3. Precision: Intraday and interday precision were determined by analyzing three different concentrations of MTD within the same day and on consecutive days, respectively. The results were expressed as percentage relative error (%RE) and relative standard deviation (RSD).

2.5.4. Precision (Intraday and Inter-day): The precision of the developed UV-spectrophotometric method was evaluated by determining the repeatability (intraday) and intermediate precision (inter-day).

- Intraday Precision: This was assessed by analyzing two different concentrations of MTD (10 µg/mL and 20 µg/mL) in triplicate (n=3) at different time intervals within the same day.
- Inter-day Precision: This was determined by analyzing the same concentrations (10 µg/mL and 20 µg/mL) over three consecutive days. The results were expressed as the percentage relative standard deviation (%RSD) and percentage relative error to confirm the consistency of the method [5].

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2.6. Estimation of Drug Content in Commercial Formulations

To evaluate the practical applicability of the method, the concentration of MTD was estimated in commercial tablet formulations (200 mg and 400 mg).

1. Sample Preparation: Five tablets were weighed to determine the average weight and then finely powdered using a mortar and pestle.
2. Extraction: An accurately weighed portion of the powder (equivalent to 200 mg or 400 mg of MTD) was transferred into a 100 mL volumetric flask containing 0.1 N NaOH. The mixture was sonicated for 10 minutes to ensure complete drug extraction.
3. Filtration: The resulting solution was filtered through a 0.45 µm membrane filter to obtain a clear filtrate.
4. Dilution (200 mg Formulation): 1 mL of the filtrate was diluted to 100 mL with 0.1 N NaOH to achieve a theoretical concentration of 20 µg/mL.
5. Dilution (400 mg Formulation): 0.5 mL of the filtrate was diluted to 100 mL with 0.1 N NaOH to achieve a theoretical concentration of 20 µg/mL.
6. Analysis: The absorbance of the prepared samples was measured at 320 nm, and the drug content was calculated using the pre-established calibration curve.

2.7. Analytical Recovery Studies

To further validate the accuracy and assess the influence of excipients, recovery studies were performed using the standard addition technique. Known quantities of pure MTD standard (2, 10, 20, and 25 µg/mL) were spiked into pre-analyzed tablet samples. The total concentration of the spiked samples was measured, and the percentage analytical recovery was calculated by comparing the experimental concentration against the theoretical added amount across the range of 10–25 µg/mL.

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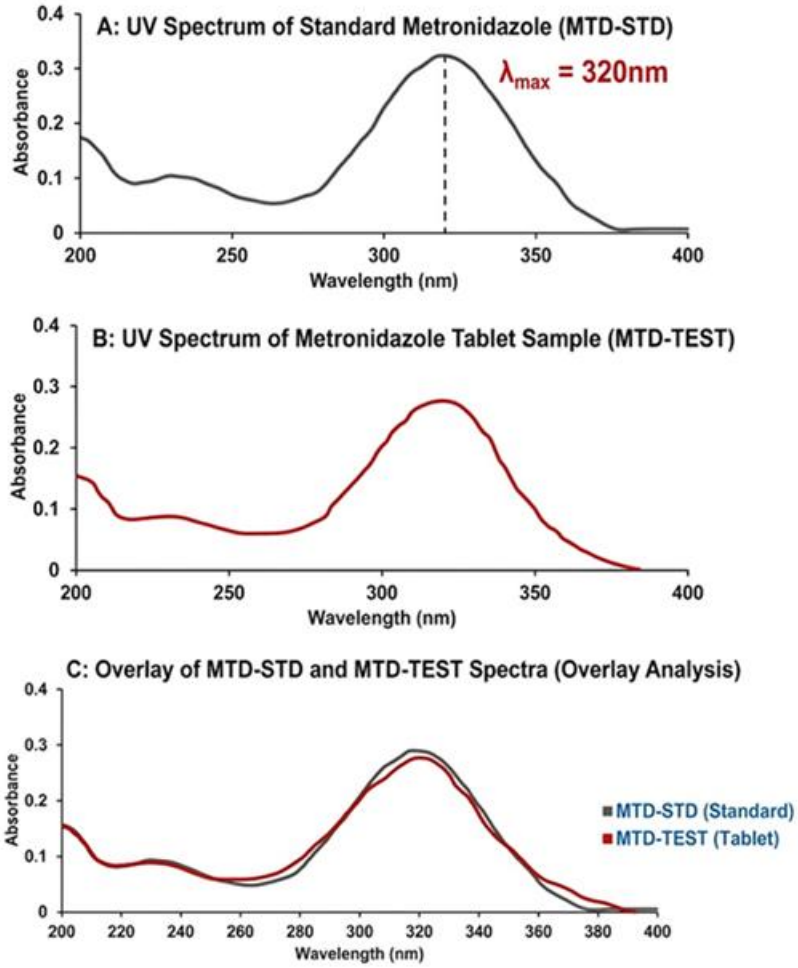

Figure (1): UV-Visible absorption spectra of Metronidazole (20 µg/mL) in 0.1 N NaOH; (A) Standard MTD, (B) Tablet formulation, and (C) Overlaid spectra demonstrating the maximum absorption (λmax) at 320 nm

2.8. Analytical Method Development and Optimization

The selection of an appropriate solvent is crucial for the stability and sensitivity of the spectrophotometric assay. In this study, 0.1 N NaOH was

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utilized as the solvent, providing a stable environment for Metronidazole. The UV scan of MTD in the range of 200–400 nm revealed a distinct maximum absorption peak (λmax) at 320 nm, as shown in Figure (1).

2.9. Spectral Analysis and Specificity

The specificity of the proposed method was confirmed by comparing the absorption spectra of the standard drug (MTD-STD) and the tablet formulation (MTD-TEST).

- Figure 1 (Top/Middle): The individual spectra for the standard and the test sample show a clear, well-defined peak at 320 nm.
- Figure 1 (Bottom): The overlay spectrum demonstrates a perfect alignment between the standard and the test formulation peaks.

This high degree of spectral overlap indicates that the common excipients present in the tablet dosage form (such as binders, fillers, and lubricants) do not interfere with the absorption of Metronidazole at the analytical wavelength of 320 nm. Thus, the method is highly specific for the quantitative estimation of MTD.

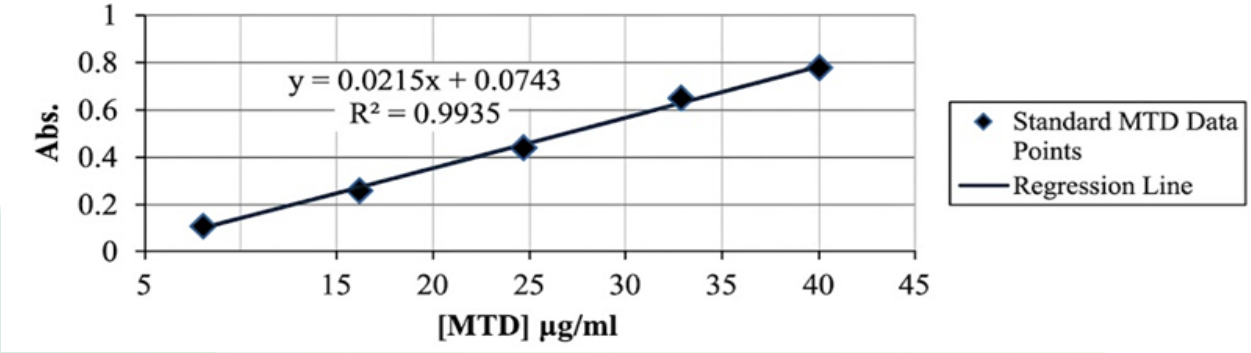

Figure (2): Standard calibration curve of Metronidazole (MTD) in 0.1 N NaOH solvent, showing the linear relationship between concentration (µg/mL) and absorbance at 320 nm.

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Table (1): Evaluation of method precision: Absorbance measurements and standard deviation for Metronidazole (MTD) assay.

Nominal Conc. (µg/mL)	Observed Absorbance (Individual Trials)	Mean Absorbance	Estimated Conc. (µg/mL)	Mean Estimated Conc.	% RSD
10	0.137, 0.138, 0.135	0.137	9.903, 9.949, 9.811	9.888	0.71
15	0.244, 0.245, 0.246	0.245	14.839, 14.885, 14.931	14.885	0.31
20	0.356, 0.358, 0.352	0.355	20.005, 20.098, 19.821	19.975	0.71
25	0.467, 0.461, 0.463	0.464	25.126, 24.849, 24.941	24.972	0.56

Table (2): Statistical validation of accuracy for Metronidazole (MTD) determination, including recovery percentage, relative error, and precision parameters.

Taken Conc. (µg/mL)	Found Conc. (µg/mL)	Recovery (%)	Relative Error (%)	% RSD
10	9.888	98.88	1.12	2.20
15	14.885	99.23	0.77	0.31
20	19.975	99.87	0.13	0.71
25	24.972	99.89	0.11	0.56

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Table (3): Optical characteristics and regression parameters for the spectrophotometric determination of Metronidazole (n=3).

Parameters	Values
Wavelength (λmax, nm)	320
Linearity Range (µg/mL)	8 – 40
Regression Equation	Y=0.0215x−0.0743
Slope (a)	0.0215
Intercept (b)	-0.0743
Correlation Coefficient (r2)	0.9935
Molar Absorptivity (L·mol−1·cm−1)	3681×103
Standard Deviation (SD)	0.0020
Limit of Detection (LOD, µg/mL)	0.3069
Limit of Quantitation (LOQ, µg/mL)	0.9302

3. Results and Discussion

3.1. Spectral Characterization and Qualitative Analysis

The UV spectrophotometric scan, conducted across the wavelength range of 200–400 nm, identified the maximum absorption wavelength (λmax) for the drug sample at 320 nm. A significant correlation was observed between the spectral peaks of the standard material and the commercial tablet formulation at this wavelength ($p < 0.05$), confirming the identity and purity of the active pharmaceutical ingredient. Consequently, 320 nm was established as the primary reference wavelength for all subsequent quantitative estimation procedures.

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3.2. Linearity Study

The results demonstrated that the proposed analytical method exhibits high linearity within the concentration range of 8–40 µg/mL at the tested wavelength. The strong correlation coefficient ($R^2 = 0.9935$) reflects a precise linear relationship between concentration and absorbance, thereby enhancing the reliability of the mathematical model utilized for quantification.

3.3. Method Validation

The developed method was subjected to a rigorous validation protocol covering precision, specificity, linearity, limit of detection (LOD), and limit of quantification (LOQ), in strict accordance with the International Conference on Harmonization (ICH) guidelines for the validation of analytical procedures.

- Accuracy and Recovery: The study revealed high efficiency in recovering the active ingredient from pharmaceutical samples. Utilizing the standard curve equation ($Y = 0.0215x + 0.0743$), the percentage recovery ranged from 99.33% to 99.88%. These excellent results clearly indicate the absence of any chemical interference from the excipients present in the pharmaceutical formulation.
- Relative Error Analysis: The method recorded exceptionally low relative error percentages of 1.1226, 0.7667, 0.1274, and 0.1129 for samples with concentrations of 10, 15, 20, and 25 µg/mL, respectively. This reflects the high precision of the method across varying concentration levels.
- Sensitivity Evaluation: Through statistical regression analysis, the Limit of Detection (LOD) was determined to be 0.3069 µg/mL, while the Limit of Quantification (LOQ) was 0.6302 µg/mL, with a standard deviation (SD) of

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0.0020. These values confirm the high sensitivity of the proposed method and its capability to effectively monitor minute concentrations.

3.4. Precision Study

3.4.1. Inter-day Precision (Intermediate Precision): The inter-day precision of the proposed spectrophotometric method was rigorously evaluated by analyzing the test compounds at two distinct concentration levels over five consecutive days. This assessment aims to determine the reproducibility of the method under varying daily conditions.

- Recovery Efficiency: The analysis revealed a robust recovery range, spanning from 98.26% to 99.87%, which signifies the high reliability of the assay over extended periods.
- Error Analysis: The recorded relative error values were consistently low (less than 2%), demonstrating the exceptional inter-day precision of the developed procedure.
- Statistical Significance: A detailed statistical analysis indicated no significant variations in inter-day precision across the different concentration levels ($p > 0.05$).
- F-Test Validation: The calculated F-values were found to be significantly lower than the F-critical values at a 95% confidence interval ($p < 0.05$). This statistical evidence confirms that the variances between the daily measurements are negligible, further validating the stability and consistency of the proposed method.

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Table (4): Intermediate precision (Inter-day) data for Metronidazole (MTD) assay at two concentration levels (10 and 20 µg/mL) over five consecutive days.

Day	Mean Absorbance (10µg/ml)	Mean Absorbance (20µg/ml)
Day 1	0.138	0.354
Day 2	0.135	0.354
Day 3	0.137	0.355
Day 4	0.135	0.355
Day 5	0.137	0.354

Conc. (µg/ml)	Day	Found Conc. (µg/ml)	% Recovery	% Relative Error	% RSD
10	Day 1	9.949	99.49	0.51	0.030
	Day 2	9.826	98.26	1.74	0.029
	Day 3	9.903	99.03	0.97	0.020
	Day 4	9.826	98.26	1.74	0.016
	Day 5	9.903	99.03	0.97	0.010
20	Day 1	19.928	99.64	0.36	0.048
	Day 2	19.898	99.49	0.51	0.038
	Day 3	19.944	99.72	0.28	0.020
	Day 4	19.975	99.87	0.13	0.035
	Day 5	19.898	99.49	0.51	0.061

a) Intraday validation: By using the suggested process to analyze test chemicals at two distinct concentration levels five times a day, the intraday precision of the proposed method was also ascertained. It was discovered that the recovery percentage ranged from 98.10 to 99.95%. It was evident from the

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low Relative error (<2) figures that the suggested technique had outstanding intraday precision. However, no significant observation was noted on the intraday precision between the concentrations ($p<0.05$). F value were found to be too low than the F critical value ($p<0.05$).

Intraday validation: The results given above clearly show that the suggested approaches produced good outcomes when using MTD in mass. Thus, both the approved method and the suggested procedures were used to analyze the contents of the capsules for MTD. The percentages on the label varied from 99.38 to 99.83% (Table 2). By using statistical analysis, these results were compared to those obtained from the official technique in terms of accuracy (t-test) and precision (F-test). The computed and theoretical values of the t- and F-tests at the 95% confidence level did not differ significantly, indicating comparable accuracy and precision in the analysis of MTD in its capsules. These results clearly show that all of the suggested techniques may be used to analyze the medication in both bulk and capsule form with similar analytical performance.

Table (5): Repeatability (Intra-day precision) data for Metronidazole (MTD) at two concentration levels (10 and 20 µg/mL) based on five independent replicates.

Sample No.	Mean Abs. (10µg/ml)	Mean Abs. (20µg/ml)
S1	0.1350	0.3543
S2	0.1370	0.3547
S3	0.1367	0.3557
S4	0.1390	0.3553
S5	0.1377	0.3543

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Conc. (µg/ml)	Trial	Found Conc. (µg/ml)	% Recovery	Relative Error (%)	% RSD (COV)
10	T1	9.811	98.11	1.89	0.010
	T2	9.903	99.03	0.97	0.010
	T3	9.888	98.88	1.12	0.006
	T4	9.995	99.95	0.05	0.035
	T5	9.934	99.34	0.66	0.015
20	T1	19.928	99.64	0.36	0.029
	T2	19.944	99.72	0.28	0.061
	T3	19.990	99.95	0.05	0.018
	T4	19.975	99.87	0.13	0.059
	T5	19.928	99.64	0.36	0.088

Table (6): Assay validation and pharmaceutical content uniformity of Metronidazole (MTD) in commercial tablet formulations (200 mg and 400 mg)

Tablet Dosage (mg)	Observed Absorbance (Individual Trials)	Mean Absorbance	Found Conc. (µg/mL)	Recovery (%)	Relative Error (%)	SD	% RSD
200	0.351, 0.352, 0.351	0.351	19.79	98.95	01.05	0.049	0.25
400	0.357, 0.352, 0.354	0.3543	19.928	99.64	0.36	0.062	0.31

4. Conclusion

The present study successfully developed and validated a simple, sensitive, and accurate UV-spectrophotometric method for the estimation of Metronidazole (MTD) in both bulk and pharmaceutical dosage forms. The method demonstrated excellent linearity within the concentration range of 8–40 µg/mL at λmax of 320 nm, with a high correlation coefficient (R2=0.9935).

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The validation results, conducted in accordance with ICH guidelines, confirmed that the method is highly precise and repeatable, with recovery percentages reaching up to 99.95% and relative errors consistently below 2%. Statistical comparisons (t-test and F-test) between the proposed method and the official technique showed no significant differences, proving that this approach offers comparable analytical performance for routine quality control.

Due to its cost-effectiveness, speed, and reliability, the proposed spectrophotometric method is highly recommended for the standardized analysis of MTD in pharmaceutical laboratories and industrial applications.

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