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Eco-Analytical validation of miconazole in drug formulations using UV-visible Spectrophotometry for finished drug formulations

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Abstract: UV spectroscopy methods were created to rapidly and accurately estimate miconazole nitrate in a topical formulation. This technique is simple, specific, linear, accurate, and robust. The environmental impact assessment of the suggested approach was executed by applying the GAPI and AGREE tools. Miconazole nitrate dissolves well in water: Isopropyl alcohol, so a standard stock solution was arranged in water: Isopropyl alcohol. The research employed five miconazole nitrate concentrations, varying from 24 to 72 μg/mL, with the drug showing strong absorbance at 210 nm. The method followed Beer's law with a 0.999 correlation coefficient and achieved a 99.4 % recovery rate, indicating high accuracy. Inter-day and intra-day precision both had < 2.0 % relative standard deviation. No interference from foreign particles or excipients was observed. Overall, the validated method is specific, reliable, and reproducible for analyzing miconazole nitrate. This cost-effective method is ideal for routine analysis in the pharmaceutical industry due to its simplicity and precision. The method is extremely environmentally friendly and sustainable, making it suitable for regular use in quality control laboratories with minimal environmental impact. In addition, the results of AGREE and GAPI confirmed the environmental friendliness and environmental sustainability of the developed approach.

Key words: UV spectrophotometry, method development, method validation, and miconazole nitrate

1. Introduction

Miconazole was an imidazole antifungal agent. It was a widely used antifungal drug that effectively treated various fungal infections by inhibiting the biosynthesis of ergosterol, an essential component of fungal cell membranes.[1] This medicine was usually formulated for topical applications with creams, powders, and ointments. Analytical methods to determine miconazole concentrations were critical to ensure the efficacy, stability, and safety of this formulation.[2]

The FDA approved miconazole, an antifungal medication from the imidazole class, in 1974. It is often referred to as 1-[(2RS)-2- [(2,4-dichloro benzyl) oxy]-2-(2,4-dichloro phenyl)ethyl]-1H-imidazole nitrate.[3] It works by inhibiting ergosterol synthesis in fungi, affecting the membrane's barrier function and

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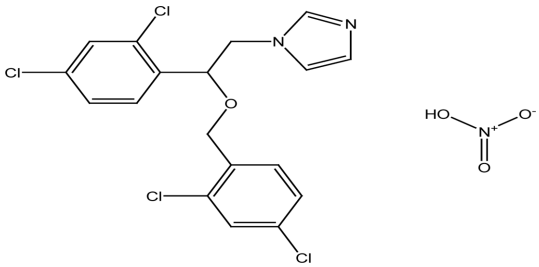

Fig. 1. Structure of miconazole nitrate.

enzymes.[4] This medication is frequently applied for the treatment of the skin infections like athlete's foot, jock itch, and ringworm. It is sometimes administered orally in gel for conditions like intestinal and oropharyngeal candidiasis.[5] Miconazole nitrate, $C_{18}H_{14}Cl_4N_20$, is an antifungal drug in the imidazole class as shown in the *Fig*. 1.

It prevents the production of Cytochrome P-450, 14-α-demethylase, suppressing the conversion of lanosterol into ergosterol necessary for fungus growth.[6] This leads to elevated cellular permeability and leakage of cellular content, with additional antibacterial and anti-dermatophyte properties.[7,8] Miconazole nitrate (MCN) comes in a 2 % strength formulation and is marketed under brands such as Micogel and Miconazole nitrate vaginal cream. UV spectroscopy is a technique that uses the ultraviolet and visible range to analyze chemical compounds based on their electromagnetic radiation absorption.[9,10] This method has various applications such as quantitative estimation of compounds, structural elucidation, and detection of impurities inorganic molecules. To market a drug product, it is essential to determine its safety, quality, and efficacy. Developing and validating an analytical process for the drug substance and its dosage forms is crucial in meeting these requirements, as outlined in the ICH Q2 guidelines. While different analytical techniques exist for estimating Miconazole nitrate, the investigation aims to progress and validate a specific, simple, sensitive, and accurate UV methodology for quantifying miconazole in pharmaceutical dosage forms.[11-17]

Several analytical techniques such as spectrophotometric, chromatographic and electrochemical methods have been developed over time to quantify miconazole. Recent studies have shown significant advances in these methods. High-performance liquid chromatography (HPLC) with ultraviolet (UV) or mass spectrometry (MS) detection was the preferred choice due to its high sensitivity, specificity, and reproducibility.[18] Advances such as ultra-performance liquid chromatography (UPLC) has further reduced analysis time and solvent consumption. Similarly, spectrophotometric techniques, particularly those using derivatization and chemometric methods, offer a cost-effective alternative to routine quality control.[19-22]

Investigations in combined dosage forms highlight the need for simple analytical procedures to efficiently measure multiple components. UV-visible spectrophotometry is a widely used, cost-effective method for analyzing such formulations. Method development focuses on establishing reliable and straightforward analytical procedures, with validation playing a crucial role in pharmaceutical applications. The United States Pharmacopeia (USP) and the ICH provide comprehensive guidelines for the validation of analytical methods in pharmaceutical processes.[23]

Despite significant progress, current analytical methods for the determination of miconazole have limitations. Some commonly identified challenges include:

Many studies focus on specific formulations, such as creams or ointments, without considering differences in other dosage forms or environmental samples. For example, HPLC methods optimized for topical formulations may not be directly applicable to tablet matrices or biological samples due to differences in excipient composition.[24]

Many investigations rely on traditional UV spectrophotometry or minor modifications to existing HPLC methods, which may not represent significant advances over established techniques. Although these traditional methods are reliable, they cannot adequately address emerging challenges such as real-time monitoring or high-throughput analysis.[25]

Despite the high specificity of advanced techniques such as HPLC and GC-MS, they are not always suitable for low-cost, widespread use due to their

high solvent consumption and complex equipment requirements. This limitation is particularly relevant in resource-limited settings.[26]

Many methods claim high specificity, but studies have shown that some excipients or degradation products in the formulation can interfere with miconazole quantification. This highlights the need to further improve analytical selectivity.

The novelty of recent research on miconazole determination is to overcome these limitations by increasing the accuracy, precision, and utility in drug quality control. Optimization and validation efforts are focused on analyzing miconazole in gel formulations while minimizing solvent use to improve environmental sustainability.

In the context of Green Analytical Chemistry (GAC), a major goal is to reduce the environmental impact of analytical methods while maintaining efficiency. Several approaches support sustainability in analytical chemistry:

Metrics and assessment tools such as the Analytical Eco-Scale and HPLC-EAT are widely used to evaluate the environmental performance of analytical methods by considering factors including reagent toxicity, solvent consumption, waste generation, and energy usage; for instance, the Eco-Scale assigns penalty points for hazardous chemicals, excessive waste, and poor waste management, thereby encouraging the adoption of greener alternatives.[27] In addition, multivariate and decision-making approaches such as Principal Component Analysis (PCA) and Self-Organizing Maps (SOM) support the comparison, ranking, and optimization of analytical methods based on environmental impact, assisting in the selection of more sustainable analytical strategies.[28] Furthermore, green solvent selection plays a crucial role in reducing environmental burden, with established guidelines promoting the use of safer and more sustainable solvents such as water, alcohols, and selected esters, while discouraging hydrocarbons and chlorinated solvents, thus significantly minimizing the ecological footprint of analytical procedures.[29]

By integrating these strategies, researchers and industry professionals can develop more environmentally friendly analytical methods, reduce hazardous waste, improve solvent selection, and enable informed decision-making in pharmaceutical analysis.

2. Materials and Methods

2.1. Chemicals and reagents

Every chemical employed for development was of analytical reagent (AR) grade and didn't require any additional purification. The medication substance miconazole was purchased from a commercial source, and Encube Ethical Pvt Ltd was the supplier of the drug items. Isopropyl alcohol of HPLC quality was acquired from Finar Chemicals Ltd.

2.2. Instrumentation

To do the Ultraviolet (UV) analysis, a Shimadzu UV 1800 Spectrophotometer was used. The route length of the quartz cell used was 1.0 cm.

2.3. Preparation of diluent/blank

Combine water and isopropyl alcohol in a 70:30 v/v ratio. And released the gas.

2.4. Preparation of the standard solution

After precisely weighing as well as transferring 24 mg of Miconazole working standard into a 50 mL volumetric flask that had been cleaned and dried, a tiny amount of diluent was added, and the mixture was allowed to sonicate to thoroughly dissolve the components and make up the difference with the diluent.

Additionally, carefully pour 5 mL of the previously created stock solution into a volumetric flask (50 mL) that has been cleaned and dried, then top it over with a diluent (48 μg/ml of Miconazole).

2.5. Preparation of sample solution

1 g of the miconazole sample (cream) was precisely weighed as well as transferred to a volumetric flask (50 ml) that was clean and dry. Volumetric flask. To fully dissolve all the ingredients, add roughly 30 mL of diluent, sonicate it for up to 30 minutes, adjust the volume with the diluent, and thoroughly mix. After a few minutes of cooling at ambient temperature,

strain through a 0.45-micron PTFE filter.

Additionally, carefully pour 6 ml of the previously created stock solution into a volumetric flask (50 mL) that has been cleaned and dried, then top it out with a diluent (48 μg/mL of Miconazole).

2.6. Procedure

UV spectrophotometric methods were used to analyze standard and sample solutions. The drug content of miconazole in cream samples was evaluated using standard measurements at 210 nm

2.7. Method validation

The method validation aimed to show suitability for its intended purpose as per ICH guidelines. It was validated based on ICH guidelines to determine performance characteristics and analytical parameters, ensuring requirements for the intended application. Testing was done with optimized spectroscopic conditions and instruments.

2.8. Specificity

A solution with cream excipients was prepared to analyze possible interfering peaks. For an accurate evaluation, the test solution's spectral pattern must coincide with the Standard solution.

2.9. Linearity

Five concentrations of Miconazole were prepared in the designated concentration range of 24 μg/mL (50 %), 36 μg/mL (75 %), 48 μg/mL (100 %), 60 μg/mL (125 %), 72 μg/mL (150 %), to evaluate the linearity of the suggested approach. Visual examination of a signal plot as a function of analyte concentration is used to assess linearity. The correlation coefficient and %Y-axis should be within the limit, according to the linearity results.

2.10. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of quantification is the lowest concentration of an analyte in a sample that can be measured with accuracy and precision, while the limit of detection is the lowest concentration of an analyte in a sample that can be detected but not always quantified as an exact value. The slope method and the residual standard deviation of the response were used to determine the miconazole nitrate limit of quantitation and limit of detection concentration as per ICH guidelines. This was achieved using calibration curves prepared for linearity studies.

2.11. Accuracy

Recovery tests, which involved adding a known concentration of the working standard to a predetermined concentration of the previously examined cream sample, were used to assess the method's accuracy. By contrasting the region with a previously examined sample, the percentage recovery was determined. Miconazole was made in triplicate in three separate solutions at 50 %, 100 %, and 150 % of its predefined concentration (24, 48, and 72 μg/mL). The percentage average and individual recoveries were computed.

2.12. Method precision

The accuracy of an analytical method is the degree of agreement among a set of measurements taken from many samples of the same homogenous material under specific conditions. Miconazole at a 48 μg/mL concentration was tested in six separate experiments to guarantee the method's repeatability. The percentage RSD (relative standard deviation) and mean area were computed. % RSD ought to be less than 2.0 %.

2.13. Intermediate precision

The assay method's intermediate precision was determined by comparing two independent replication tests conducted on two distinct days. First-day data were extracted from the “repetition” analysis. A second series of tests was conducted using a different device or analyzer. From the data collected each day, the SD, RSD, and mean value difference were computed.

2.14. Solution stability

The absorbance of the reference and test solutions should be measured as soon as possible after preparation and then 24hours later at room temperature. The solution should then be kept at room temperature.

Table 1. Comparable performance for routine analysis

Parameter	Proposed Method	Reported Method 1 (Ref 12)	Reported Method 2 (Ref 13)	Reported Method 3 (Ref 14)	Reported Method 4 (Ref 16)
λmax (nm)	210	232	272	272	205
Linear Range (µg/mL)	24-72	2–10	100–600	80–240	1–12
LOD (µg/mL)	0.45	1.868	1.908	-	0.49
LOQ (µg/mL)	1.20	2.760	5.782	-	1.49
Recovery (%)	99.4	98.36	98.52–101.56	100.0	99.23–100.89
Sample Nature	Pharmaceutical gel	Bulk & Topical Formulation	Topical Formulation	Pharmaceutical cream	Bulk

2.15. Robustness

The robustness of an analytical process is determined by how well it can tolerate small but deliberate adjustments to its parameters and shows how reliable it is under typical operating conditions. Variations in sonication time and wavelength etc. were observed.

2.16. Greenness assessment of the proposed method

A greenness assessment of a suggested technique involves evaluating its environmental impact and overall sustainability. This is increasingly important in various fields, especially analytical chemistry, where minimizing the application of hazardous chemicals, energy consumption, solvent usage, and reducing waste is paramount.[30] By carefully considering these factors and utilizing appropriate greenness assessment tools, you can evaluate the economic effect of your proposed methodology and make informed decisions to minimize its negative effects.[31] green analytical metrics have been utilized to assess the greenness of the suggested method. 1) The GAPI, or Green Analytic Procedure Index 2) Analytical greenness metrics (AGREE)

3. Results

3.1. Comparison with existing methods

To determine the maximum absorption wavelength (λmax), the method development process begins with UV-visible scanning, usually 200–400 nm. For the analyst, this guarantees the best possible sensitivity and specificity. The proposed approach was evaluated against other approaches in the literature in terms of sensitivity, accuracy, and applicability. *Table* 1 illustrates its goal of offering enhanced or comparable performance for routine analysis.

The advantage of the proposed method offers improved sensitivity with lower LOD (0.45 µg/mL) and LOQ (1.20 µg/mL) compared to references 12 and 13. It provides high accuracy (99.4 % recovery) and a suitable linear range (24–72 µg/mL) for routine gel analysis. Additionally, it is effectively applied to pharmaceutical gel formulation, demonstrating better practical applicability than methods limited to bulk or simple topical forms.

3.2. Method validation

Method validation aims to show that the procedure is appropriate for the goals outlined in the ICH recommendations. The aforementioned procedures were verified to suit the needs of their intended use and to determine their performance characteristics (as measured by analytical parameters). Miconazole showed maximum absorbance at 210 nm.

3.3. Specificity

The test solution maxima and spectral patterns matched those of the reference solution, according to a comparison of the two solutions' spectra. The UV scans of the blank, placebo solution, reference solution, test solution as well as overlay spectrum scan are shown in *Figs.* 2 through 6.

3.4. Linearity

Five miconazole concentrations 24, 36, 48, 60, and 72 µg/mL were generated, and *Fig.* 7 illustrates the

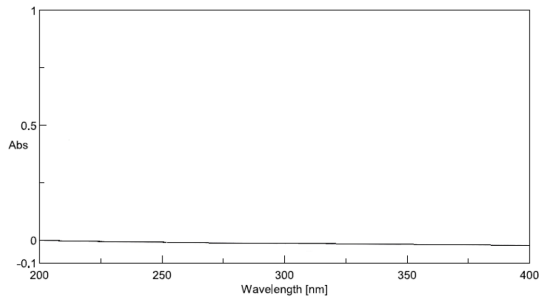

Fig. 2. Scan spectra of blank.

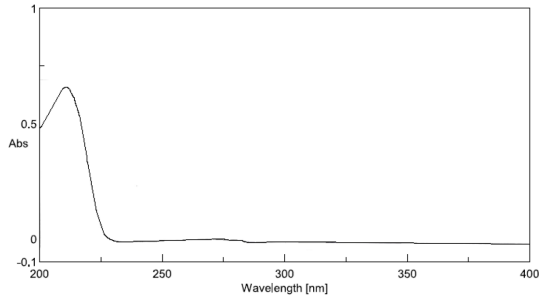

Fig. 3. Scan spectra of Standard.

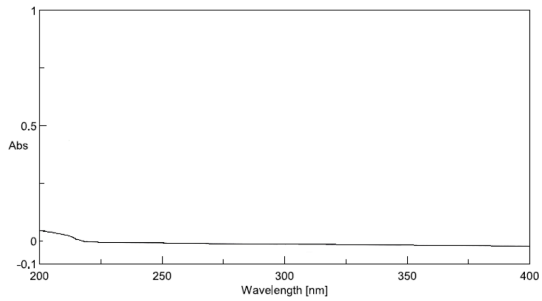

Fig. 4. Scan spectra of Placebo.

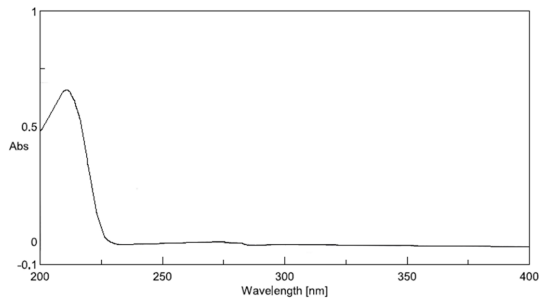

Fig. 5. Scan spectra of the sample.

linearity plot created by plotting the absorbance against concentration. Since the correlation coefficient R as well as the %Y-intercept fell within the acceptable

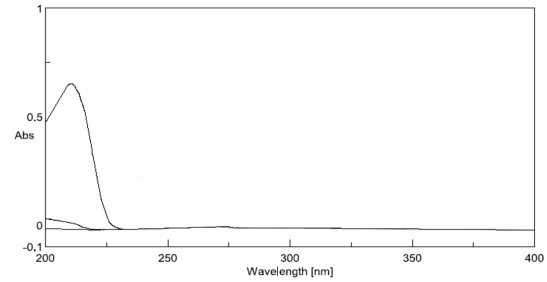

Fig. 6. Overlay spectrum Scan.

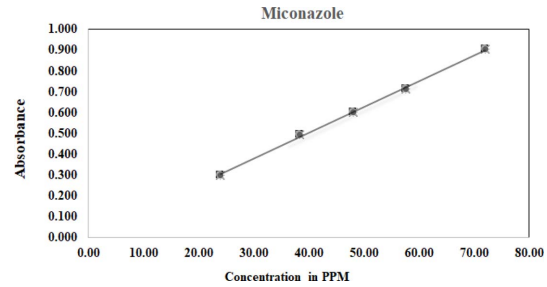

Fig. 7. Linearity plot of miconazole.

Table 2. Observation table for linearity of Miconazole.

Parameter for Linearity	Values	Acceptance criteria
Correlation coefficient R	0.9994	> 0.999
Slope	0.01239	Complies
Y Intercept	0.01	Complies
%Y – axis intercept	1.42	≤ ± 5 %
LOD	0.45	Complies
LOQ	1.20	Complies

range, a linear relationship between 50 and 150% (24–72 μg/mL) for miconazole was found (see *Table* 2). In the 24–72 μg/mL range for miconazole, which is regarded as linear since the %Y-intercept and correlation coefficient should be within the range.

3.5. Limit of detection (LOD) and limit of quantification (LOQ)

Using the calibration curve, the standard deviation of the response (σ) and the slope of the calibration curve (S) were determined. Required calculations were performed using the equations (3.3 × σ)/S for LOD and (10 × σ)/S for LOQ. The limit of detection (LOD) was established as 0.45 μg/mL, while the limit

Table 3. Recovery at different concentration levels

Accuracy level	% Recovery of Miconazole	Mean Recovery
50%	99.4 99.4 98.3	99.0
100%	100.0 100.1 99.9	100.0
150%	99.7 98.9 99.5	99.4
% Overall Recovery		99.4
% RSD		0.51

of quantification (LOQ) was determined as 1.20 μg/mL. These findings are presented in *Table* 1. These results highlight the exceptional sensitivity of the proposed UV technique.

3.6. Accuracy

Since the mean recovery percentage and individual recovery percentage fell between 98 and 102 %, the approach was deemed accurate. *Table* 3 below displays the miconazole recovery percentage.

3.7. Precision

The calculated % RSD of precision (intra-day) and intermediate precision (inter-day) tests are discussed below. For a precise analytical method, the % RSD of assay results of six replicates should not be more than 2. The RSD of the intra-day assay of six replicates was 0.96 % and the percentage assay of miconazole was 99.1 %. for intermediate precision (inter-day) assay and %RSD were 99.5 % and 0.40 % respectively, conducted on 2 different days. A low % RSD value indicates that the method was precise and rugged. The precision of the RSD from six determinations was better within the acceptable range of <2 %, the method's precision was deemed exact according to the precision of 2 %.

Table 4. Solution stability data for miconazole

Time Interval	Standard Area	% Difference	Sample Area	% Difference
0 Hrs.	0.568	-	0.516	-
24 Hrs.	0.521	0.35	0.475	0.40

3.8. Solution stability

At room temperature, by examining the solutions kept, the stability of the sample and standard solutions was investigated. There should be no more than a 2.0 percentage discrepancy between the beginning and ending zones. *Table* 4 presents solution stability data.

3.9. Robustness

All assessed robustness parameters satisfied the system appropriateness criteria, demonstrating the method's resilience. The robustness of the established UV approach is described by the truth that purposefully altering the parameters has no discernible effect on the method's performance. *Table* 5 presents the findings.

3.10. Application to dosage forms

The proposed method was used to analyze miconazole in commercial cream formulations and was statistically compared with a specified spectrophotometric method.[12] The analysis used point and interval hypothesis tests, with Student's T and F values confirming no significant difference at a confidence level of 95 %.[32] Recovery experiments found that the lower (θL) and upper (θU) confidence limits were within ± 2 %, which indicates compliance with regulatory standards. The results of two tablet brands, which were analyzed using both the proposed method and the reference

Table 5. Robustness result for miconazole

Name of Test	Method precision	Change in sonication time		Change in wavelength	
% Assay of Miconazole	99.1	20 minutes 100.4	40 minutes 99.4	218 nm 99.3	222 nm 100.1

Table 6. Results of the statistical analysis

Pharmaceutical Formulation	Proposed Method		Reference Method[12]		t-test	F- test	p-value (t)	θL	θU
	% Recovery	% RSD	% Recovery	% RSD					
Cream and Gel formulation	99.1	0.96	98.36	1.606	0.983	0.363	>0.05	0.988	2.468

method,[12] further confirmed the precision and accuracy, as statistical tests once again revealed no significant differences, which confirmed regulatory compliance. The results of the statistical analysis are presented in *Table* 6.

3.11. Greenness assessment of the proposed method

There were two methods of greenness assessment in the suggested methodology.

3.12. Green analytical procedure index (GAPI)

The Green Analytical Procedure Index (GAPI) is a comprehensive assessment tool used in Green Analytical Chemistry (GAC) to evaluate the environmental impact of analytical procedures. It provides a visual and systematic representation of method greenness through a pentagonal pictogram divided into five major stages of the analytical workflow, including sample preparation, reagents and solvents, instrumentation, energy consumption, and waste generation. Each stage is color-coded from red (high environmental impact) to green (low environmental impact), enabling an intuitive interpretation of the method's environmental profile.[33,34] By assessing each analytical step individually, GAPI offers a transparent and holistic evaluation of the greenness of the proposed method, as illustrated in *Fig.* 8.

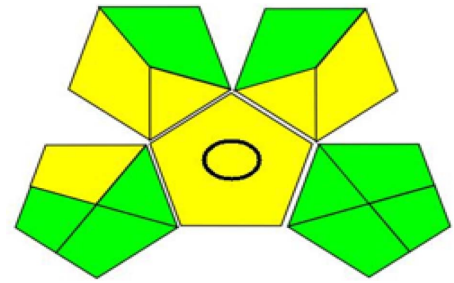

Fig. 8. GAPI Assessment.

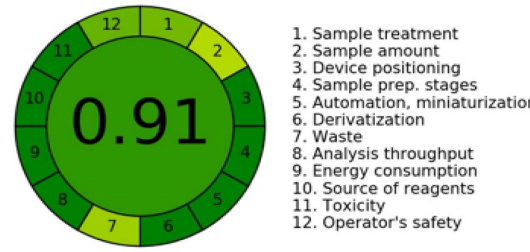

Fig. 9. AGREE Assessment.

3.13. Analytical greenness metrics (AGREE)

AGREE is a quantitative greenness assessment tool based on the 12 principles of Green Analytical Chemistry. The AGREE software integrates these principles into a unified score ranging from 0 to 1, where values closer to 1 indicate a more environmentally friendly analytical method. The evaluation considers key parameters such as sample treatment, solvent and reagent toxicity, waste generation, energy consumption, automation, analysis throughput, and operator safety. In the present study, the proposed method achieved a high AGREE score of 0.91, reflecting excellent compliance with green analytical principles.[35,36] This result confirms that the method is environmentally sustainable, efficient, and suitable for routine pharmaceutical analysis, as shown in *Fig.* 9.

4. Discussion

The developed UV spectrophotometric method for the determination of miconazole in cream formulations was validated in accordance with ICH guidelines and critically compared with the reported reference method using clearly defined analytical criteria, including linearity, accuracy, precision, sensitivity, robustness, statistical equivalence, and environmental impact. The method exhibited excellent linearity over

the concentration range of 24–72 μg/mL, which is appropriate for routine formulation analysis. Accuracy was confirmed through recovery studies (99.1 %), indicating absence of interference from excipients, while precision studies demonstrated a low %RSD value (0.96 %), confirming repeatability and intermediate precision. Statistical comparison using Student's t-test and F-test showed no significant difference between the proposed and reference methods at the 95 % confidence level ($p > 0.05$), establishing their equivalence. The method also demonstrated adequate sensitivity and robustness under minor deliberate variations in analytical conditions. Furthermore, greenness assessment using AGREE and GAPI tools confirmed strong compliance with green analytical chemistry principles. Collectively, these findings demonstrate that the proposed method is reliable, accurate, environmentally sustainable, and suitable for routine quality control analysis of miconazole in pharmaceutical cream formulations.

5. Conclusions

In this study, a simple, accurate, and precise UV spectrophotometric method for quantifying miconazole in pharmaceutical formulations was successfully developed and validated. The method showed excellent linearity in the concentration range of 24–72 μg/mL with a correlation coefficient (R) of 0.9994, which confirmed a strong linearity ($R > 0.999$). The slope (0.01239) and intercept (0.01) met the acceptance criteria, and the percentage Y-intercept (1.42 %) remained well within the allowable limit of $\leq \pm 5$ %. In addition, the detection limit (LOD) and the limit of quantification (LOQ) were 0.45 μg/mL and 1.20 μg/mL, which confirmed the sensitivity of the method. The intraday precision test showed an RSD of 0.96 % with a percentage assay of 99.1 %, while the Interday precision test performed on two different days gave an RSD of 0.40 % with a percentage assay of 99.5 %. The method was further tested for robustness by examining changes in sonication time and wavelength variation, with the percentage of miconazole assays ranging from 99.1% to 100.4 %, which indicates a high reliability of the method. In addition, the environmental impact of the developed method was assessed using the AGREE metrics program, which assesses solvent consumption, waste production, energy consumption, and reagent toxicity. The proposed method achieved a GAC (Green Analytical Chemistry) value of 0.91, which indicates strong environmentally friendly properties. Overall, the developed spectrophotometric UV method provides a precise, accurate, robust and environmentally sustainable approach for the routine analysis of miconazole in pharmaceutical formulations and is therefore ideal for quality control applications in the pharmaceutical industry and regulatory laboratories.

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Conflicts of Interest

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Ethical Approval

The work described has not been previously published and it is not under consideration for

publication elsewhere. This publication is approved by all authors and the responsible authorities where the work has been carried out.

Abbreviation

ICH: International Conference of Harmonization, **UV:** Ultraviolet-visible, **FDA:** Food Drug Administrative, **HPLC:** High-performance liquid chromatography, **PPM:** Parts per million, **GAPI:** Green Analytic Procedure Index, **AGREE:** Analytical greenness metrics

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