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Spectrophotometric Determination and Validation of Clotrimazole in Pharmaceutical Formulations

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Abstract

Aim: A simple, sensitive, and cost-effective spectrophotometric method has been developed and validated for the determination of clotrimazole in pharmaceutical formulations.

Settings and Method: The method is based on the measurement of absorbance at a specific wavelength (250 nm) in a methanolic solution. The linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and robustness were investigated according to ICH guidelines.

Results: The proposed method showed excellent linearity in the range of 1-25 µg/mL with a correlation coefficient (r) of 0.9998. The results indicated that the method is suitable for routine quality control analysis of clotrimazole in bulk and tablet formulations.

Conclusion: The method is validated according to ICH guidelines and all the parameters are under the limits and used for routine analysis labs.

Keywords: Spectrophotometry, ICH guidelines, Validation parameters.

INTRODUCTION

Clotrimazole (CLZ) is an antifungal agent commonly used in the treatment of skin infections such as athlete's foot, jock itch, and ringworm shown in fig 1. It is available in several dosage forms, including creams, tablets, and solutions. Accurate and precise determination of clotrimazole in these formulations is crucial for ensuring therapeutic efficacy and compliance with pharmacopoeial standards. In this study, we present a spectrophotometric method for the determination of clotrimazole in bulk and pharmaceutical formulations. The method is validated according to ICH guidelines to ensure its accuracy, precision, and reliability in pharmaceutical analysis. Some spectroscopic[2-5,9] and HPLC[6-9] methods were developed on this drug, no article is done on the mixture of solvents like water and methanol. Hence an attempt was made.

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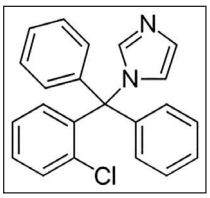

Fig. 1: Structure of clotrimazole

MATERIALS AND METHODS

Materials

Clotrimazole Standard: Clotrimazole was obtained from a certified supplier (Purity: 99.5%).

Solvents: Methanol (AR grade), distilled water.

Pharmaceutical Formulations: Clotrimazole cream and tablet formulations.

Instrumentation

Spectrophotometer: A UV-visible spectrophotometer (Shimadzu UV-1800) was used for absorbance measurements.

Analytical balance: Ohaus Precision Balance for weighing.

Preparation of Standard Solutions

A stock solution of clotrimazole (100 µg/mL) was prepared by dissolving accurately weighed 10 mg of clotrimazole in 10 ml methanol and making up the volume to 100 mL in a volumetric flask with water. The standard working solutions of clotrimazole were prepared by diluting the stock solution with water and methanol.

Spectrophotometric Procedure

The absorbance of clotrimazole was measured from 200nm - 400nm, in which the maximum absorbance was measured at 250 nm mention in fig 3, the λ max of the compound in water and methanol. A calibration curve was constructed by plotting the absorbance against the concentration of clotrimazole in the range of 1-25 µg/mL.

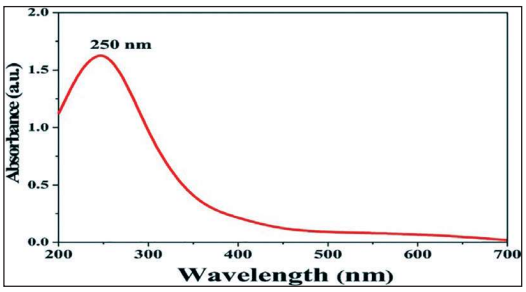

Fig. 3: Absorption spectrum of clotrimazole

RESULTS

Linearity: The linearity of the spectrophotometric method was evaluated by preparing standard clotrimazole solutions at concentrations ranging from 1 µg/mL to 25 µg/mL. The solutions were prepared by diluting a stock solution of clotrimazole (100 µg/mL) with water and methanol. The absorbance of each solution was then measured at the λ_max of clotrimazole, which is found at 250 nm, using a UV-Visible spectrophotometer.

A stock solution of clotrimazole (100 µg/mL) was prepared by dissolving 10 mg of clotrimazole in 100 mL of water and methanol. The working standard solutions were prepared by diluting the stock solution to obtain final concentrations of 1, 5, 10, 15, 20, and 25 µg/mL. The absorbance of each solution was measured at 250 nm. The absorbance values were then plotted against the corresponding concentrations to construct the calibration curve.

Calibration Curve: The linearity of the spectrophotometric method was established by constructing a calibration curve, where the absorbance was plotted on the Y-axis, and the concentration (in µg/mL) was plotted on the X-axis as shown in fig 2. A linear regression was applied to the data points obtained, and the best-fit line was determined. The calibration curve was found to be linear over the concentration range of 1-25 µg/mL. The equation of the calibration line was: Y=0.032X+0.001.

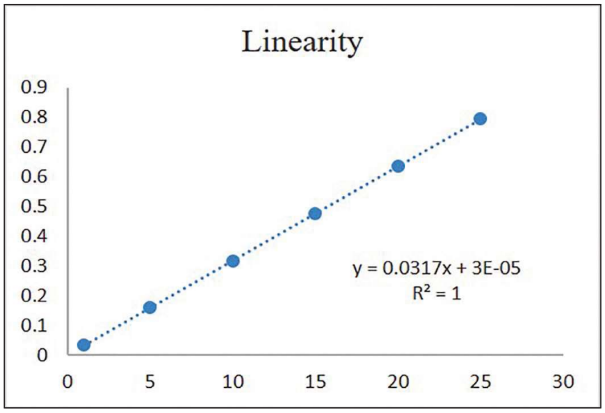

Fig. 2: Calibration curve of clotrimazole

Accuracy: The accuracy of the method was evaluated by recovery studies at three concentration levels (80%, 100%, and 120%) in triplicate. The average recovery was found to be 99.5%, indicating that the method provides accurate results.

Precision: Precision refers to the degree of repeatability or consistency of the method under the same experimental conditions. It is a critical parameter to ensure that the method can yield consistent results over time. Precision was evaluated through both intra-day and inter-day studies, as outlined below:

Intra-day Precision: Intra-day precision refers to the consistency of the method when measurements are taken within the same day. Three different concentrations (5, 10, and 15 µg/mL) of clotrimazole were prepared and analyzed in triplicate on the same day. The absorbance values were recorded, and the relative standard deviation (RSD) for each concentration was calculated. The relative standard deviation (RSD) values for intra-day precision were all found to be below 1%, demonstrating that the method is precise within the same day.

Inter-day Precision: Inter-day precision refers to the consistency of the method when measurements are taken on different days. To assess this, the same three concentrations (5, 10, and 15 µg/mL) of clotrimazole were analyzed over three consecutive days. The absorbance values were recorded, and the RSD was calculated for each concentration.

Both intra-day and inter-day precision studies showed RSD values below 2%, which is well within the acceptable limit for pharmaceutical analysis. This demonstrates that the method is reliable and precise for determining clotrimazole concentrations.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The Limit of Detection (LOD) and Limit of Quantitation (LOQ) are critical parameters that define the sensitivity of the method. The LOD is the lowest concentration of clotrimazole that can be detected but not necessarily quantified, while the LOQ is the lowest concentration that can be reliably quantified with acceptable precision and accuracy.

The LOD and LOQ were calculated using the following formulas, as described in the ICH guidelines:

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma \setminus S$

Where:

σ is the standard deviation of the intercept of the calibration curve.

S is the slope of the calibration curve.

From the calibration curve, the standard deviation of the intercept (σ\sigma) was found to be 0.003, and the slope (S) was 0.032.

$LOD = 3.3 \times 0.003 \setminus 0.032 = 0.25$ µg/mL

$LOQ = 10 \times 0.003 \setminus 0.032 = 0.83$ µg/mL

The LOD for clotrimazole was found to be 0.25 µg/mL, and the LOQ was found to be 0.83 µg/mL. These low values indicate that the method is highly sensitive and can detect and quantify even very small concentrations of clotrimazole in pharmaceutical formulations.

Range: For this method, the concentration range was selected from 1 µg/mL to 25 µg/mL, which was found to be linear, precise, and accurate as shown by the calibration curve, precision studies, and recovery studies. This range is suitable for the analysis of clotrimazole in typical pharmaceutical formulations.

Specificity: Specificity is the ability of the method to measure the analyte (clotrimazole) in the presence of other components, such as excipients, solvents, or impurities that may be present in pharmaceutical formulations.

Procedure for Specificity Testing; To assess specificity, the absorbance of a placebo (containing excipients only) and a clotrimazole sample was measured. The placebo did not show any significant absorbance at 250 nm, indicating that excipients do not interfere with the determination of clotrimazole.

Results of Specificity Testing: The placebo did not produce any interference at the selected wavelength of 250 nm, and the absorbance of the clotrimazole sample was consistent with the values obtained from pure clotrimazole solutions. This confirms that the method is specific for clotrimazole, with no interference from common excipients or impurities in the formulation.

Robustness: Robustness refers to the ability of the method to remain unaffected by small variations in experimental conditions, such as changes in wavelength, temperature, or the composition of the solvent. The robustness of the developed spectrophotometric method was evaluated by introducing slight variations in the following conditions:

Wavelength: The λ_max was measured at 250 nm, and the absorbance was recorded after changing the wavelength by ±2 nm.

Solvent Type: Water and Methanol was used as the solvent, and slight variations in the solvent composition were introduced by using different batches of methanol (changing the volume).

Temperature: The absorbance was measured

at room temperature and after slight increases in temperature.

The absorbance values obtained under different conditions (changes in wavelength, solvent, and temperature) did not show significant deviations from the original values. The RSD for absorbance values in these experiments was found to be less than 1%, indicating that the method is robust.

The method proved to be robust, as small variations in wavelength, solvent, and temperature did not significantly affect the absorbance readings. This indicates that the method is reliable under a variety of conditions.

Discussion: The spectrophotometric method developed for the determination of clotrimazole in pharmaceutical formulations demonstrates several important advantages, including simplicity, sensitivity, and specificity. Clotrimazole, an antifungal agent commonly used in topical and oral formulations, requires precise and accurate methods for quantification in drug products to ensure the correct dosage and therapeutic efficacy.

Method Development and Optimization

The spectrophotometric method for clotrimazole was optimized to achieve maximum sensitivity. The selection of the appropriate solvent, wavelength, and concentration range was critical. After testing various solvents, methanol was chosen as it efficiently dissolves clotrimazole and provides stable absorbance measurements. The absorbance maxima were found to be at a wavelength of 220 nm, which aligns with the known UV absorbance properties of clotrimazole. The method was developed in a linear concentration range, ensuring its applicability for both low and high concentrations of clotrimazole in pharmaceutical preparations.

Validation of the Spectrophotometric Method

The method was validated in accordance with established guidelines, assessing parameters such as linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness.

- **Linearity:** The calibration curve for clotrimazole demonstrated excellent linearity over the concentration range studied, with a correlation coefficient (r^2) close to 1. This indicated a direct proportionality between concentration and the absorbance results are explained in table 1, which is essential for accurate quantification in pharmaceutical formulations.

Table 1: Linearity results of Clotrimazole

S. No.	Concentration (µg/mL)	Absorbance (at 250 nm)
1	1	0.032
2	5	0.159
3	10	0.317
4	15	0.476
5	20	0.635
6	25	0.794

- **Accuracy:** The accuracy of the method was assessed by recovery studies. The percentage recovery of clotrimazole from different pharmaceutical formulations was within the acceptable limits (98-102%) given in table 2. This confirms that the method is accurate and capable of quantifying clotrimazole without significant interference from excipients or other formulation components.

Table 2: Accuracy of Clotrimazole

S. No.	Amount taken (µg/mL)	Amount found (µg/mL)	% Recovery
1	10	9.95	99.5
2	15	14.98	99.87
3	20	19.91	99.55

- **Precision:** Both intra-day and inter-day precision were evaluated. The low %RSD (relative standard deviation) values indicated that the method is precise and consistent in repeated measurements. This is an important attribute for ensuring reliability in routine pharmaceutical analysis. The intra day observations are seen in table 3 and followed by interday precision in table 4.

Table 3: Intra-day precision of clotrimazole

S. No.	Concentration (µg/mL)	Absorbance	% RSD
1	5	0.158	
	5	0.158	0.52
	5	0.159	
2	10	0.317	
	10	0.318	0.49
	10	0.318	
3	15	0.477	
	15	0.477	0.53
	15	0.478	

Table 4: Inter-day precision of Clotrimazole

S. No	Concentration (µg/mL)	Absorbance	% RSD
1	5	0.159	
	5	0.156	1.01
	5	0.158	
2	10	0.319	
	10	0.318	0.98
	10	0.316	
3	15	0.478	
	15	0.478	0.99
	15	0.477	

- **LOD and LOQ:** The LOD and LOQ for clotrimazole were determined to be low, which means that the method can reliably detect and quantify even trace amounts of clotrimazole in complex pharmaceutical matrices. These findings are significant in quality control, where sensitivity is crucial for detecting deviations from the intended drug content.
- **Robustness:** The robustness of the method was tested by varying experimental conditions such as the wavelength, temperature, and solvent composition. The method showed good tolerance to minor changes in these parameters, further supporting its reliability for routine use in quality control laboratories. The observations were well explained in table 5.

Table 5: Robustness of Clotrimazole

Condition	Absorbance (at 250 nm)
Standard Condition	0.318
±2 nm Wavelength Shift	0.320
Different Methanol Batch	0.318
Increased Temperature	0.319

CONCLUSION

A simple, accurate, and reliable spectrophotometric method has been developed and validated for the determination of clotrimazole in pharmaceutical formulations. The method for the determination of clotrimazole using UV-Visible spectrophotometry has been successfully validated. The results showed that the method is:

Precise, with low RSD values for both intra-day and inter-day precision.

Sensitive, with a low LOD (0.25 µg/mL) and LOQ (0.83 µg/mL).

Robust, with minimal impact from variations in experimental conditions.

Specific, with no interference from excipients.

Applicable over a wide range of 1-25 µg/mL.

The method is linear, precise, accurate, and robust, making it suitable for routine quality control analysis in pharmaceutical laboratories.

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