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Research Article

SIMULTANEOUS DETERMINATION OF ITRACONAZOLE AND TERBINAFINE IN PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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ABSTRACT:
A simple, accurate, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the simultaneous estimation of Itraconazole and Terbinafine in tablet dosage forms. Chromatographic separation was achieved using a Syncronis C18 column (150 mm × 4.6 mm, 5 µm) with a mobile phase consisting of 0.01N KH_2PO_4 buffer (pH 4.8) and acetonitrile in the ratio of 35:65 (v/v), at a flow rate of 1 mL/min and a column temperature of 25°C. The detection wavelength was set at 237 nm. The retention times for Itraconazole and Terbinafine were found to be 2.2 min and 3.2 min, respectively. The method showed excellent linearity, accuracy, and precision, with %RSD values of 1.2 and 0.5, and recovery rates of 99.95% and 99.89% for Itraconazole and Terbinafine, respectively. The method is suitable for routine quality control analysis of these drugs in pharmaceutical formulations.
Keywords: Itraconazole, Terbinafine, RP-HPLC, Simultaneous estimation, Tablet dosage forms

INTRODUCTION

Itraconazole is a synthetic triazole antifungal compound with the molecular formula $C_{35}H_{38}Cl_2N_8O_4$. It is a lipophilic molecule that exhibits poor water solubility and is typically administered in a solid dispersion form to enhance its bioavailability. Terbinafine, on the other hand, is an allylamine derivative with the molecular formula $C_{21}H_{25}N$ and exists as a crystalline, white to off-white powder. It is also lipophilic and practically insoluble in water. Both compounds are stable under normal storage conditions and require proper formulation strategies to ensure effective delivery in pharmaceutical preparations.

Itraconazole acts by inhibiting the cytochrome P450-dependent enzyme lanosterol 14-α-demethylase, which is essential for the biosynthesis of ergosterol, a key component of fungal cell membranes. This disruption leads to altered membrane permeability and ultimately, fungal cell death. Terbinafine exhibits its antifungal activity by inhibiting the enzyme squalene epoxidase, also involved in ergosterol synthesis, resulting in the accumulation of toxic squalene within the fungal cell and membrane disruption. Itraconazole is primarily effective against a broad range of systemic and superficial fungal infections, whereas Terbinafine is especially potent against dermatophytes, making their combination effective for treating mixed fungal infections.

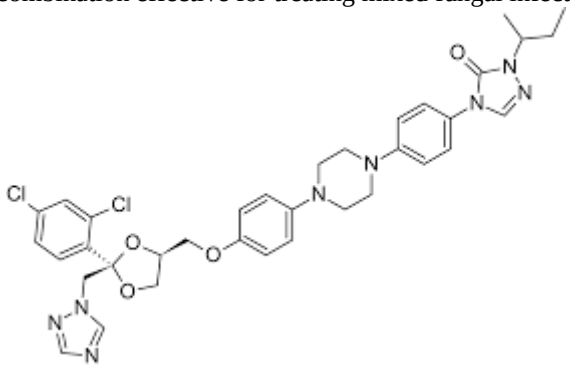

Fig. 1 Chemical structure of Itraconazole

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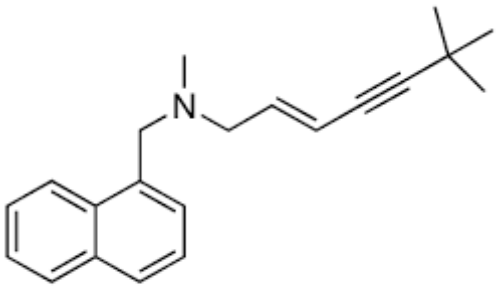

Fig.2 Chemical structure of Terbinafine

The simultaneous estimation of these drugs in combined pharmaceutical dosage forms is essential for quality control and regulatory compliance. Several analytical methods have been reported for individual estimation; however, a simple, robust, and validated method for their simultaneous determination is highly desirable. This study describes the development and validation of an RP-HPLC method for the simultaneous estimation of Itraconazole and Terbinafine in tablet formulations.

Materials and Methods

Chemicals and Reagents

The analysis was carried out using Itraconazole and Terbinafine reference standards, each with a purity greater than 99%, to ensure the accuracy and validity of the method. Tablet dosage forms containing both active pharmaceutical ingredients were used for assay and validation purposes. HPLC-grade acetonitrile served as the organic component of the mobile phase, contributing to effective separation and peak resolution. A 0.01N potassium dihydrogen phosphate (KH_2PO_4) buffer, adjusted to pH 4.8, was used as the aqueous phase to maintain consistent pH and improve analyte stability during the chromatographic run. All other chemicals and reagents used in the study were of analytical grade, ensuring high purity and minimal interference in the analysis.

Preparation of Standard and Sample Solutions

Standard Preparation:

Accurately Weighed and transferred 10mg&25mg of Itraconazole and Terbinafine working Standards into 50ml clean dry volumetric flask, add 3/4th of diluent, sonicated for 30 minutes and make up to the final volume with diluents to prepare stock solutions of 200µg/ml of Itraconazole and 500µg/ml of Terbinafine. From the above stock solutions, 1 ml was pipeted out in to a 10ml Volumetric flask and then make up to the final volume with diluents to get 20µg/ml of Itraconazole and 50µg/ml of Terbinafine .

Sample Preparation:

20 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablets was transferred into a 50 ml volumetric flask, 30ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 100 ml volumetric flask and made upto 100ml with diluent.

Method Validation

The method was validated according to ICH guidelines for the following parameters:

- **Linearity:** Assessed over a suitable concentration range for both drugs.
- **Precision:** Repeatability and intermediate precision were evaluated (%RSD).
- **Accuracy:** Determined by recovery studies at different levels (50%, 100%, 150%).
- **Limit of Detection (LOD) and Limit of Quantitation (LOQ):** Calculated from the regression equation.
- **Specificity:** Assessed by analyzing blank, standard, and sample solutions for interference.
- **Robustness:** Evaluated by small deliberate changes in chromatographic conditions.

Results

Method Development and Optimization

A robust RP-HPLC method was successfully developed for the simultaneous determination of Itraconazole and Terbinafine in tablet dosage forms. Chromatographic separation was achieved using a Syncronis C18 column (150 mm × 4.6 mm, 5 µm) with a mobile phase of 0.01N KH_2PO_4 buffer (pH 4.8) and acetonitrile in a 35:65 ratio. The flow rate was maintained at 1 mL/min, and the column temperature was set at 25°C. The optimized detection wavelength was 237 nm.

The retention times for Itraconazole and Terbinafine were found to be 2.2 min and 3.2 min, respectively, with well-resolved, symmetrical peaks and no interference from excipients or blank solutions. This indicates the selectivity and suitability of the method for routine analysis.

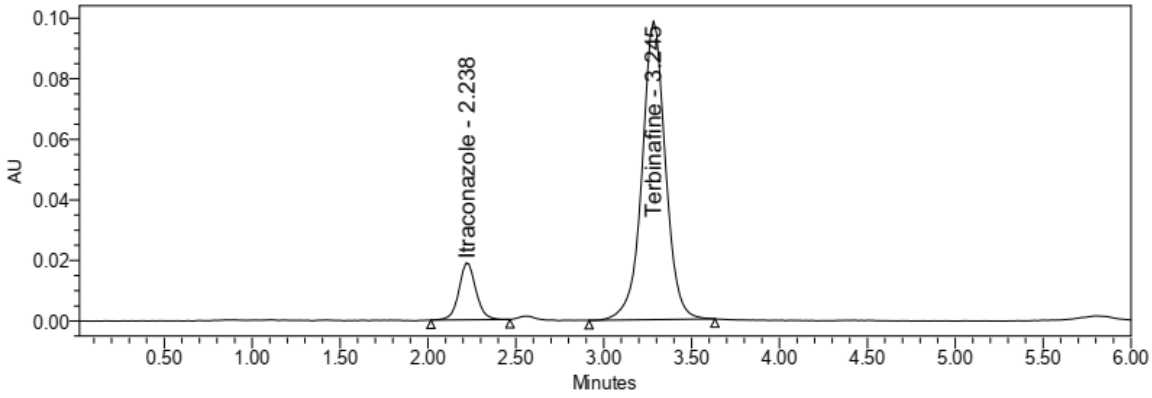

Fig 3. Optimized chromatogram of Itraconazole and Terbinafine

System Suitability and Validation

System suitability parameters were within acceptable limits, confirming the reliability of the chromatographic system. The %RSD of peak areas for Itraconazole and Terbinafine were 1.2 and 0.5, respectively, indicating high precision. The theoretical plate counts and tailing factors also met standard criteria, ensuring consistent performance.

Table: 1 System suitability studies of Itraconazole And Terbinafine method

Property	Itraconazole	Terbinafine
Retention time (t_R)	2.23± 0.2 min	3.24±0.2min
Theoretical plates (N)	2456 ± 542	6314± 240
Tailing factor (T)	1.12 ± 0.11	1.01± 0.12

Linearity

Six Linear concentrations of Itraconazole (5µg/ml to 30µg/ml) and Terbinafine (12.5µg/ml to 75µg/ml) are prepared and Injected. Regression equation of the the Itraconazole And Terbinafine are found to be, y = 5805.3x - 1372 and y = 17370x + 2266 And regression co-efficient was 0.999.

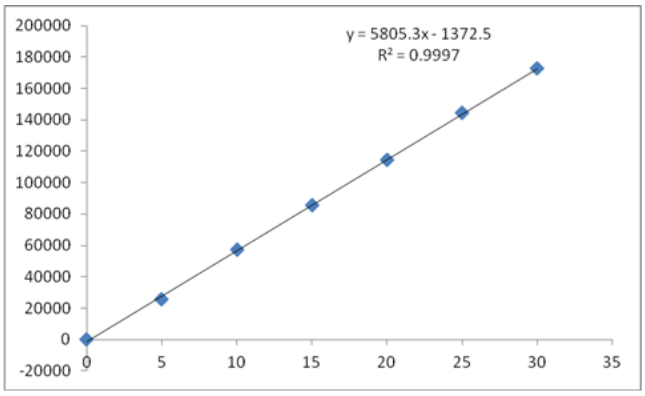

Fig: 4 Calibration curve of Itraconazole

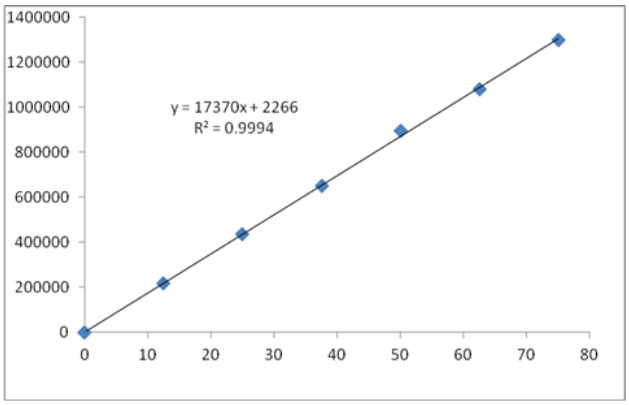

Fig: 5 Calibration curve of Terbinafine

Sensitivity

The limits of detection (LOD) and quantification (LOQ) were determined from the calibration curves. For Itraconazole, the LOD and LOQ were 0.02 µg/mL and 0.06 µg/mL, respectively. For Terbinafine, the LOD and LOQ were 0.06 µg/mL and 0.18 µg/mL, respectively. These low detection limits indicate the method's high sensitivity.

Precision

Intra-day and inter-day precision studies showed %RSD values below 2% for both drugs, confirming the repeatability and reproducibility of the method. The results demonstrate that the method yields consistent results under the same experimental conditions on different days.

Accuracy

Recovery studies were conducted by spiking pre-analyzed samples with known amounts of Itraconazole and Terbinafine at three different levels (50%, 100%, and 150%). The mean recoveries were 101% for Itraconazole and 99.59% for Terbinafine, indicating the accuracy of the method and the absence of interference from excipients.

Table: 2 Accuracy results of Itraconazole and Terbinafine

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	Average
Itraconazole	5	4.98	99.60	101
	10	9.96	99.60	
	15	15.59	103.93	
Terbinafine	25	24.98	99.92	99.59
	50	49.67	99.34	
	75	74.64	99.52	

Robustness

The robustness of the method was evaluated by making deliberate minor changes in chromatographic parameters such as flow rate, mobile phase composition, and detection wavelength. The method remained unaffected by these changes, with no significant variation in results, confirming its robustness.

Specificity

The specificity of the method was established by analyzing blank, standard, and sample solutions. No interfering peaks were observed at the retention times of Itraconazole and Terbinafine, confirming the method's specificity.

Analysis of Marketed Formulations

The developed method was applied to the simultaneous estimation of Itraconazole and Terbinafine in commercially available tablet formulations. The assay results were within the acceptable limits of the labeled claim, demonstrating the applicability of the method for quality control purposes.

Discussion

The developed RP-HPLC method allows for rapid, accurate, and precise simultaneous estimation of Itraconazole and Terbinafine in tablet dosage forms. The method is robust, with excellent linearity, accuracy, and precision, and is suitable for routine quality control analysis. The short retention times and simple mobile phase composition make the method cost-effective and efficient for high-throughput analysis in pharmaceutical laboratories.

Conclusion

A validated RP-HPLC method for the simultaneous determination of Itraconazole and Terbinafine in pharmaceutical dosage forms has been developed. The method is simple, sensitive, and reliable, and can be employed for routine analysis and quality control of these drugs in combined tablet formulations.

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