International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Development and validation of an RP-HPLC method for the quantitative estimation of itraconazole in bulk and capsule dosage forms

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International Journal of Science and Research Archive, 2025, 15(03), 1264-1271

Publication history: Received on 07 May 2025; revised on 17 June 2025; accepted on 20 June 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.15.3.1845

Abstract

This study presents the development and validation of a simple, precise, accurate, and robust Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the quantitative analysis of Itraconazole in bulk and capsule forms. Chromatographic separation was carried out using a C18 column with an optimized mobile phase, flow rate, and detection wavelength to ensure effective resolution and symmetrical peak shapes. The method was validated in accordance with ICH guidelines, assessing parameters such as linearity, precision, accuracy, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ). The developed method demonstrated excellent linearity (R^2 > 0.999), accuracy with recoveries between 98–102%, and precision with RSD below 2%. No interference was observed from excipients or degradation products, confirming the method's specificity. Robustness testing confirmed the system's reliability under minor changes in conditions. Therefore, this RP-HPLC method is suitable for routine quality control of Itraconazole in pharmaceutical formulations

Keywords: Itraconazole; RP- HPLC; Method Development; Method Validation; Bulk Drug; ICH Guidelines; Quality Control; Linearity; Robustness

1. Introduction

Itraconazole is a synthetic antifungal agent belonging to the triazole class, generally specified for the treatment of both systemic and superficial fungal infections. Maintaining the quality of pharmaceutical products containing Itraconazole is pivotal to insure remedial effectiveness and patient safety. Rear Phase High- Performance Liquid Chromatography(RP- HPLC) is a extensively used logical system in the pharmaceutical assiduity, known for its trustability and perfection in both qualitative and quantitative medicine analysis. Chemically, Itraconazole is named 1-(butan-2-yl)- 4-{ 4-(4-(4-{((2R, 4S)- 2-(2,4- dichlorophenyl)- 2-(1H) -1,3-dioxolan-4-yl) methoxy} phenyl) piperazin-1-yl) phenyl}. As a triazole-type antifungal emulsion, it functions primarily by widely inhibiting the fungal cytochrome P-450-dependent enzyme 14α- demethylase. This inhibition disrupts ergosterol conflation, a critical element of fungal cell membranes, thereby plying its antifungal goods.

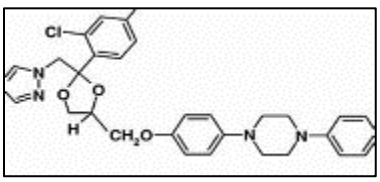

Figure 1 Chemical structure of Itraconazole

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2. Materials and Methods

2.1. Chemical and Reagents:

Itraconazole was obtained as a gift from JNTUH- UCPS Laboratory, Sultanpur, Sangareddy. HPLC grade double distilled water and analytical grade 0.1% Hydrochloric acid, methanol obtained from JNTUH -UCPS Laboratory. HPLC grade water was used to prepare all solutions.

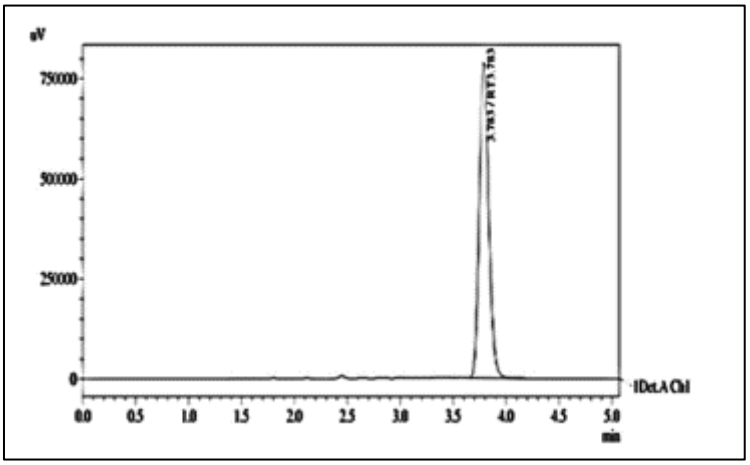

Figure 2 Chromatogram of Standard Itraconazole solution

2.2. HPLC Instrumentation:

A Shimadzu HPLC system equipped with a UV detector and an Enable C18G column (150 mm × 4.6 mm, 5 µm) was used.

2.3. Chromatographic Conditions

- **Mobile Phase**: Methanol: 0.1% Hydrochloric acid (99:1 v/v)
- **Flow Rate**: 1.0 mL/min
- **Detection Wavelength**: 264 nm
- **Injection Volume**: 10 µL
- **Run Time**: 5-7 minutes.

2.4. Preparation of Standard stock solution

10 mg of Itraconazole was accurately weighed and taken in 100 ml clean and dry volumetric flask containing 50 ml of diluent (same as mobile phase) and then sonicated for 15 minutes to dissolve. Later the solution was made up to the mark using the mobile phase. This is considered as working standard solution (100 µg/ml).

2.5. Preparation of stock and working sample solution

Ten capsules were weighed separately and the average weight was determined. The capsule content equivalent to 100 mg of itraconazole was transferred to a 100 ml volumetric flask and dissolved in little portion of mobile phase then volume was made up to the mark with mobile phase. The resulting solution was sonicated for 15 minutes, followed by filtration through 0.2 µl nylon membrane filter to get sample stock solution of 1mg/ml. 1 ml of the above stock solution was pipetted out and made up to 10 ml to get working sample solution equivalent to a concentration of working standard of 100 µg/ml. From this suitable aliquot was prepared and injected. From the calibration curve the concentration was determined.

3. Results and discussion

3.1. HPLC method development and optimization

To optimize chromatographic conditions, various columns, mobile phases, and flow rates were evaluated. Methanol and 0.1% Hydrochloric acid in a 99:1 ratio was selected as the mobile phase, as it provided a stronger response for Itraconazole compared to other tested combinations. Flow rates between 0.9 and 1.1 ml/min were assessed, with 1.1

ml/min identified as the most effective. Under these optimized conditions, the analyte peak was well-defined, free from tailing, and exhibited a retention time (RT) of 3.78 minutes. The final chromatographic parameters are summarized in Table1.

Table 1 Optimized chromatographic parameters

Elution	**Isocratic**
Mobile phase	methanol and 0.1% Hydrochloric acid (99:1)
Column	Dionex C18column
Flow rate	1 ml/min
Detection	264 nm
Injection volume	10µl
Temperature	28 ℃
Retention time	3.78 min
Run time	5-7 min
Concentration	200-600µg/ml

3.2. Validation of the method

After the development and optimization of a method, validation becomes essential to ensure its reliability and accuracy. This process includes assessing the linear range through correlation coefficients, evaluating precision using relative standard deviation (RSD, %), and determining accuracy through percentage recovery and RSD, %. Additionally, sensitivity studies involving the limits of detection (LOD) and quantification (LOQ) are conducted, along with robustness testing to confirm the method's stability under varied conditions.

3.3. System suitability studies: System Suitability Testing

System suitability tests are a critical component of chromatographic method development, used to verify the performance and consistency of the analytical system. Parameters evaluated included retention time (RT), number of theoretical plates (N), tailing factor (T), peak asymmetry (AS), and resolution (RS). The test was conducted using five replicate injections of the standard solution prior to sample analysis.

Acceptance criteria for system suitability in each validation run were as follows:

- Capacity factor (k') > 2.0
- Tailing factor (T) ≤ 2.0
- Theoretical plates (N) > 2000

In all cases, the relative standard deviation (RSD) of the analytical peak area between two consecutive injections was less than 2.0%. The system suitability parameters are summarized in Table 2.

Table 2 System suitability parameters

Parameters	**Values**
Retention time	3.78 min
Theoretical plates	7491.00
Tailing factor	1.231

3.3.1. Linearity

The linearity of the method was evaluated by preparing six series of standard solutions of Itraconazole in the range of 200 – 600 µg/ml in methanol and injecting the solutions into the HPLC system. Excellent correlation between

Itraconazole peak area and concentration was observed with R2 = 0.999 (Figure.3). The regression equation was found to be Y = y = *48935x -1862.5*. Statistical data are presented in table 3 and the calibration curve was shown in figure 3.

Table 3 Linearity results for Itraconazole

S.No	Concentration (µg/ml)	Area AU (n=6)
1	200	1465324
2	300	1467854

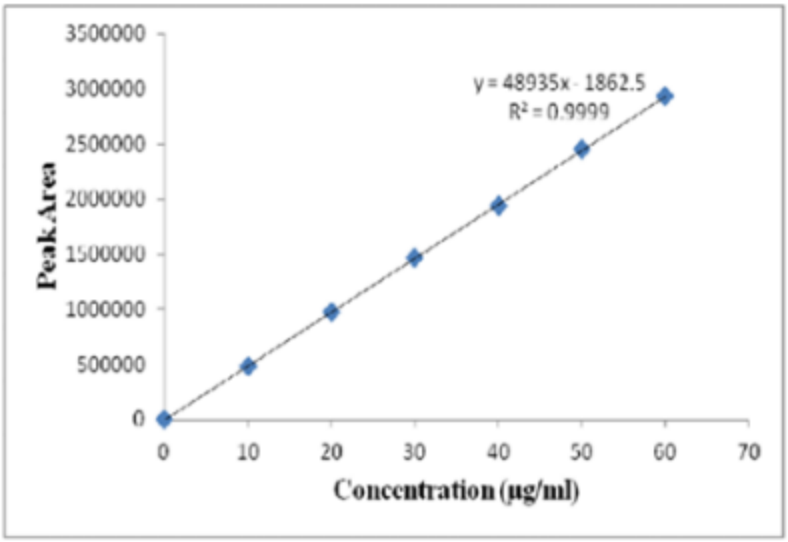

Figure 3 Calibration curve of Itraconazole

3.3.2. Precision:

System precision: (Repeatability) To study precision, five replicate standard solutions of Itraconazole (400µg/ml) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated. Results of system precision studies were shown in table 4.

Table 4 Results of system precision for Itraconazole

S.No	Rt (min)	Peak Area (AU)
1	3.753	1465324
2	3.767	1467854
AVG	3.78	1466589
SD	0.0098	1788.9
%RSD	0.26	0.12

3.3.3. Method precision: (Reproducibility)

The intraday and inter-day precision of the proposed method was determined by analyzing the corresponding responses times on the same day and on different days for concentration of sample solutions of 400µg/ml. The result was reported in terms of relative standard deviation (% RSD). Results of method precision studies were shown in table 5.

Table 5 Results of Method precision for Memantine

S. No	Itraconazole	Standard Area = 1467854 Peak Area % Labelled Claim
1	1465324	99.42
2	1467854	99.88

3.3.4. Accuracy

Accuracy of the method was confirmed by the standard addition method, which was carried out by performing recovery studies at 3 different concentrations 50%, 100% and 150% of these expected, in accordance with ICH guidelines, by replicate analysis (n=3). For a pre analyzed sample solution 100 µg/ml, 50%, 100%, 150% standard drug solution was added and percentage drug content was measured. The closeness of obtained value to the true value indicates that the proposed method is accurate. Recovery studies were shown in table 6.

3.3.5. Limit of Detection and Quantitation:

The LOD and LOQ were calculated based on the S/N ratio of the standard injection. The chromatograms for LOD and LOQ were shown in fig: 4 and 5.

Table 6 Results of recovery studies for Itraconazole

Sample	Area	Sample amount (µg/ml)	Standard added (µg/ml)	Standard recovered* (µg/ml)	%Recovery ± SD*	%RSD
80%	1174038.4	30	24	23.96	99.83 ± 0.03	0.03
100%	1467548	30	30	29.89	99.63 ± 0.043	0.0431
120%	17610576	30	36	35.92	99.77 ± 0.032	0.032

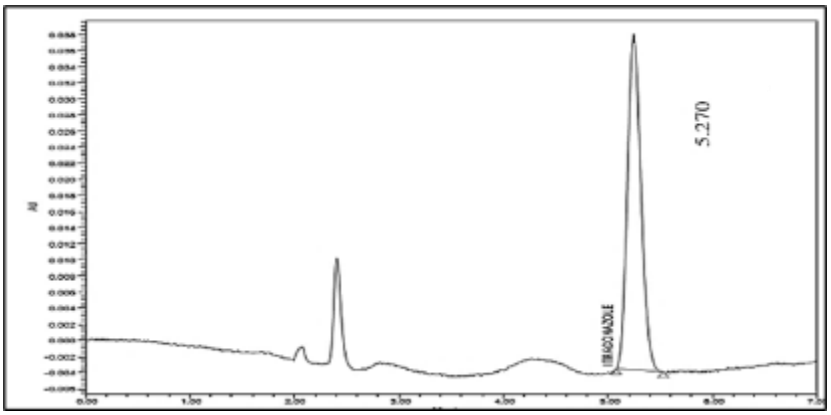

Figure 4 Chromatogram for LOD

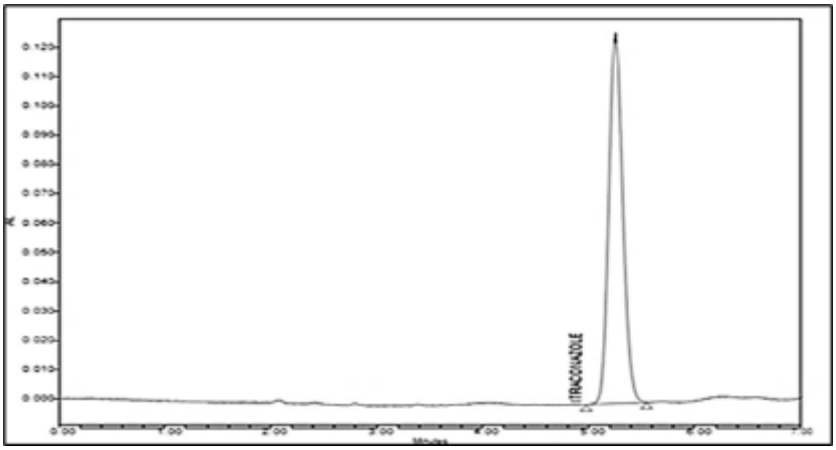

Figure 5 Chromatogram for LOQ

Limit of detection and limit of quantification was calculated based on S/N ratio. The S/N ratio was found to be 645.374091.

- LOD of itraconazole was found to be 1.8594 µg/ml
- LOQ of itraconazole was found to be 6.197 µg/m

3.3.6. Robustness

The robustness test was done to check how small changes in HPLC conditions affect the results. It helps to see if the method still gives accurate results even when there are slight changes.

In this test, we slightly changed the flow rate and the column temperature while using the working standard solution of itraconazole. The results for these changes (flow rate and temperature) are shown in Table 7.

Table 7 Robustness data of Itraconazole

S. No	Condition	Modification	Mean Peak are ± SD*	Mean Rt ± SD*	Mean %RSD (for Peak Area)
1	Flow rate (ml/min)	0.9	1465321±4654	4.213	0.317
		1.1	1398754±4876	3.298	0.347
2	Wavelength (nm)	262	1432876±4321	3.543	0.301
		266	1457642±4562	3.653	0.312

3.4. Assay of pharmaceutical formulation

The proposed validated method was successfully applied to determine Itraconazole in their capsule dosage form and the % Assay results were shown in table 8.

Table 8 Assay study of itraconazole

Sample	Label Claim (mg)	Standard Area*	Sample Area*	Amount found* (mg)	(%) Recovery ± SD*
SPORANOX	100	1467035	1466811	99.98	99.98± 0.03

Table 9 Summary of validated parameters for proposed method

Parameters	Results
Calibration range (µg/ml)	10-60
Detection Wavelength(nm)	264
Mobile phase (methanol: Hydrochloric acid) (V/V)	99:1
Regression equation (Y)	48395x-1862.5
Retention Time(min)	3.78
Slope (b)	48395
Intercept (a)	-1862.5
Correlation coefficient (r^2)	0.999
LOD (µg/ml)	1.8594
LOQ (µg/ml)	6.197

4. Conclusion

A simple, fast, accurate, and reliable RP-HPLC method was successfully developed and validated for the analysis of Itraconazole in both pure form and capsule dosage forms, following ICH guidelines. This method is cost-effective due to its short retention time, which reduces mobile phase usage.

Based on the %RSD values from precision and recovery studies, the method proved to be both precise and accurate. The low limits of detection (LOD) and quantification (LOQ) confirm the method's high sensitivity. Robustness testing showed that small changes in chromatographic conditions do not significantly affect the results, indicating the method is stable.

Compliance with ethical standards

Acknowledgments

The authors are grateful to the Principal of JNTUH University college of pharmaceutical sciences, Sultanpur, Sangareddy-502001 Telangana, India.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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