JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

# METHOD DEVELOPMENT & VALIDATION OF ITRACONAZOLE BY UV-SPECTROSCOPY

**Sandesh S. Chavan*, Prathamesh V. Waske, Dhyanesh S. Chaudhari, Ankita A. Mali, Kiran D. Sable.**
**Rajarambapu College of Pharmacy, Kasegaon (MS) India**

Corresponding Authors
Sandesh Subhash Chavan,
Research Scholars,
Rajarambapu College of Pharmacy, Kasegaon (MS) India

**ABSTRACT**

An artificial triazole antifungal agent is called itraconazole. Itraconazole was prepared in a number of pharmacological forms with different delivery methods. To treat fungal infections in the lungs that have the potential to spread throughout the body, utilize itraconazole capsules. Since it is not yet listed as an official medication in any pharmacopoeia, there aren't many published methods for quality control and stability testing of itraconazole in pharmaceutical formulations. The current study aims to create a spectrophotometric method that is more accurate, straightforward, and cost-effective for analyzing itraconazole in both bulk and capsule dosage forms. Using chloroform as the solvent, UV spectroscopic determination was performed at an absorption maximum of 267 nm. Using the UV spectroscopic method, linearity was determined to be 1-10 µg/ml with a correlation coefficient of 0.999 over the concentration range of itraconazole. The analyses' conclusions were supported by recovery studies and statistical validation. The International Conference on Harmonization guidelines were followed in the study of parameters such as linearity, precision, accuracy, limit of detection, and limit of quantification in order to validate the method.

**KEY WORDS:** Pharmacopoeia, Itraconazole, Quality control, UV spectroscopic method.

## INTRODUCTION

An artificial triazole antifungal agent is called itraconazole. A 1:1:1:1 racemic mixture of four diastereomers—two pairs of enantiomers—each with three chiral centers makes up itraconazole. It could be denoted by any of the following terms: 2-(2, 4-dichlorophenyl) 4-[4-[4-[4-[2-]1-ylmethyl)-1,3-dioxolan-4-yl] methoxy]phenyl] piperazin-1-yl]phenyl]1-Methylpropyl-2--2, 4-triazol-3, -2, 4-dihydro-1 (Fig 1). Its molecular weight is 705.64 and its formula is C35H38Cl2N8O4. [1-4] The powder is white to slightly yellow in color. It dissolves readily in dichloromethane but very slightly in alcohols. Itraconazole is essentially water insoluble and has a strong lipophilic nature. It is only ionized at very low pH levels and is an incredibly weak base (pKa = 3.7). It is a three-chiral, hydrophobic anti-mycotic medication that is used in clinical settings as a stereoisomeric mixture. [5] It is an oral active triazole antifungal agent that shows broad spectrum activity against several fungal species such as Histoplasma capsulatum var. capsulatum, Candida species, Aspergillus species, and dermatophytes. [6,7] Itraconazole works by interacting with 14-α demethylase, a cytochrome P-450 enzyme that is required to change lanosterol into ergosterol. Since ergosterol is a crucial part of the fungal cell membrane, blocking its synthesis increases the permeability of the cell, which allows the contents of the cell to leak out. Moreover, itraconazole may interfere with membrane phospholipids, hinder triglyceride and/or phospholipid biosynthesis, prevent yeast from transforming into mycelial forms, inhibit purine uptake, and inhibit endogenous respiration. Itraconazole is primarily metabolized in the liver by the cytochrome P450 3A4 isoenzyme system (CYP3A4), which produces a number of metabolites, the main one being hydroxyl itraconazole. [8] It was discovered that taking it with food increased its oral bioavailability, with a plasma concentration that was roughly twice that of taking it while fasting. [9] The bioactive metabolite hydroxyl itraconazole is produced by extensive hepatic metabolism, primarily through an oxidative pathway.[10]

Itraconazole was prepared in a number of pharmacological forms with different delivery methods. To treat fungal infections in the lungs that have the potential to spread throughout the body, utilize istraconazole capsules. utilized to treat nail fungal infections. Toenail fungus infections are treated with tablets and capsules. Yeast infections of the mouth, throat, or esophagus (the tube that joins the throat and stomach) are treated with itraconazole oral solution (liquid) [11]. Since it is not yet listed as an official medication in any pharmacopoeia, there aren't many published methods for quality control and stability testing of itraconazole in pharmaceutical formulations. Numerous methods for the analysis of itraconazole in plasma have been reported, including HPLC [12] and LC/MS-MS [13–15], which suffer from either undesired extended run times for chromatography, the need for gradient analysis, or the application of an internal standard Additionally, one spectrophotometric technique [16] has been documented. Itraconazole has been assayed in dosage forms and raw materials using the spectroflourimetry method. To determine the amount of itraconazole in human plasma, utilize the RP-HPLC method. [17–21] This method's chromatographic separation was carried out using a fluorescence detector on an octadecyl silane column.

Nevertheless, the drawback is that it takes a lot of time. The necessity of carrying out prompt and accurate quality-control analyses of pharmaceutical formulations containing itraconazole has been underlined by all of these investigations. We have attempted to create a more accurate, straightforward, and cost-effective spectrophotometric method with improved sensitivity, accuracy, and precision for the analysis of itraconazole in dosage forms and bulk.

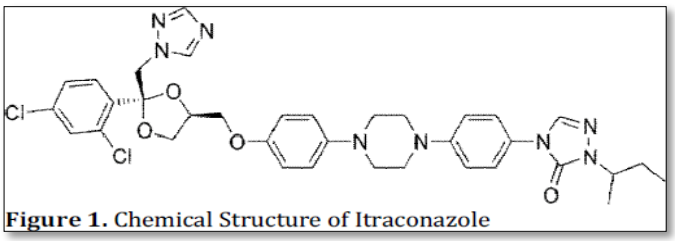



**Figure 1.** Chemical Structure of Itraconazole

### Experimental

**Chemicals and reagents:** Throughout the development and validation of UV spectrophotometric methods, chloroform was employed.

### Instrumentation

Using a double beam UV-visible spectrophotometer (Shimadzu, model 1700) with two matching quartz cells and a 1 cm light

path, the UV spectrophotometric method was used.

**Selection of solvent**

The best solvent for spectrophotometric analysis of itraconazole was found to be chloroform.

**PREPARATION OF STANDARD STOCK SOLUTIONS**

A precisely weighed amount of 20 mg of itraconazole reference standard was put into a 20 ml volumetric flask, dissolved, and then diluted with chloroform until the desired strength was reached in the stock solution, which was 1000µg/ml. One milliliter of the stock solution was diluted to ten milliliters using chloroform to create a 100 µg/ml working standard solution.

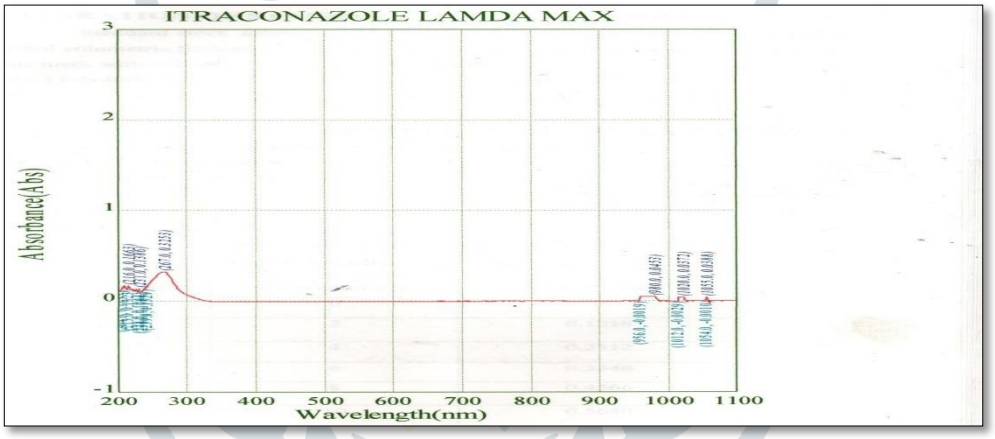
## Preparation of Sample stock solution

Ten capsules were precisely weighed for the drug's dosage form analysis, and the powder was triturated in a mortar to produce a fine powder. After weighing and transferring the 10 mg of capsule powder to a 10 ml volumetric flask, the powder was dissolved in chloroform. The concentration of the capsule solution was eventually diluted to 10µg/ml. These solutions' absorbance was measured at 267 nm. The calibration curve was used to determine how much itraconazole was contained in each capsule. The results are displayed in a table.1.

**Formula: %Purity=Sample absorbance / Standard absorbance X 100**

**Table 1: Assay of Itraconazole Capsule.**

| Brand Name | Lable Claim | Amount prepared | % Purity |
|---|---|---|---|
| Sporanox | 100mg | 10µg/ml | 99.54 |



## METHOD VALIDATION

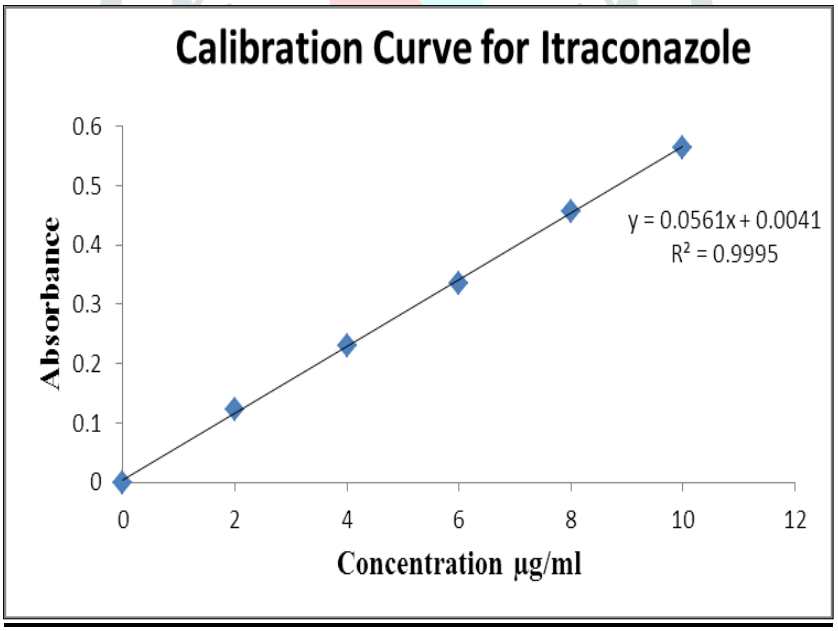
The International Conference on Harmonization (ICH) Q2B guidelines from 1996 were followed in the validation of the method to ascertain its linearity, precision, accuracy, and limit of detection.

## LINEARITY & RANGE

The absorbance versus concentration calibration graphs for the suggested method were found to be linear under experimental conditions within the 0.2–1.0µg/ml range. The statistical analysis of the data collected for the estimation of itraconazole shows that the suggested methods have a high degree of accuracy, as shown by the low coefficient of variation and standard deviation values. Table.2 presents the findings.

**Table 2: Linearity Data of Itraconazole.**

| S. No. | Concentrationµg/ml | Absorbance |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 2 | 0.1316 |
| 3 | 4 | 0.2419 |
| 4 | 6 | 0.3516 |
| 5 | 8 | 0.4615 |
| 6 | 10 | 0.5612 |
| Slope:0.056<br>Regression:0.999 | | |




## ACCURACY

In order to assess the precision of the suggested approach, recovery experiments were conducted by incorporating varying proportions (50%, 100%, and 150%) of a standard bulk Itraconazole sample within the linearity range into a previously examined formulation with a concentration of 10µg. Recovery values as a percentage are computed. Every spike level should have a triplet test prepared, and the assay should be carried out in accordance with the test protocol. The figures are displayed in Table 3.

**Table 3: Accuracy & % Recovery Reading.**

| S. No | Amount Of Sample (µg) | Amount of Standard Added(µg) | Total Amount of Itraconazole | Amount of Itraconazole found | % Recovery | % Mean Recovery | S.D | %RSD |
|---|---|---|---|---|---|---|---|---|
| 50% | 10 | 5 | 15 | 14.95 | 99.6% | 99.7% | 0.0011 | 0.183 |
| 50% | 10 | 5 | 15 | 14.98 | 99.8% | | | |
| 50% | 10 | 5 | 15 | 14.96 | 99.7% | | | |
| 100% | 10 | 10 | 20 | 20 | 100% | 100% | 0.0057 | 0.106 |
| 100% | 10 | 10 | 20 | 20.25 | 101% | | | |
| 100% | 10 | 10 | 20 | 20 | 100% | | | |
| 150% | 10 | 15 | 25 | 24.7 | 99.1% | 99.5% | 0.0040 | 0.403 |
| 150% | 10 | 15 | 25 | 24.9 | 99.6% | | | |
| 150% | 10 | 15 | 25 | 25 | 100% | | | |

**PRECISION**

The degree of agreement between independent test results obtained under ideal circumstances is the definition of a method's precision. Six replicates of the same sample concentration and a standard for both system and method precision were used to determine the precision of the ascertainment.

**SYSTEM PRECISION**

The system precision of the proposed method was ascertained by determination of six replicates of same concentration of standard drug within the Beer's range and finding out the absorbance. We computed the absorbance, standard deviation, and percentage RSD. Table 4 displays the results.

**Table 4: System Precision Readings.**

| S. No | Concentration µg/ml | Absorbance |
|---|---|---|
| 1 | 10 | 0.5440 |
| 2 | 10 | 0.5438 |
| 3 | 10 | 0.5432 |
| 4 | 10 | 0.5428 |
| 5 | 10 | 0.5424 |
| 6 | 10 | 0.5420 |
| Standard Deviation | | 0.00115 |
| %RSD | | 0.2761 |

**METHOD PRECISION**

The approach By measuring the absorbance of six replicates of the same drug sample concentration within Beer's range, the precision of the suggested method was determined. We computed the absorbance, standard deviation, and percentage RSD. The table displays the results.5.

**Table 5: Method Precision Readings.**

| S. No | Concentrationµg/ml | Absorbance |
|---|---|---|
| 1 | 10 | 0.5390 |
| 2 | 10 | 0.5388 |
| 3 | 10 | 0.5385 |
| 4 | 10 | 0.5380 |
| 5 | 10 | 0.5378 |
| 6 | 10 | 0.5374 |
| Standard Deviation | | 0.000619 |
| %RSD | | 0.1149 |

**LIMIT OF DETECTION AND LIMIT OF QUANTITATION**

The lowest concentration of analyte in a sample that can be identified but may not always be quantified as an exact value is known as the detection limit of a particular analytical technique. The lowest concentration of analyte in a sample that can be quantitatively determined with appropriate precision and accuracy is known as the quantification limit of a particular analytical procedure. The relationship 3.3 S and 10 S, respectively, was used to calculate the LOD and LOQ. Here, S stands for slope and sigma represents the standard error of the estimate. The determined LOD and LOQ values for itraconazole were determined to be 0.14 and 0.43 µg/ml, respectively. The outcomes are displayed in Table No. 6.

**Table 6: Limit of Detection and Limit of Quantitation.**

| S. No | Conc. (µg/ml) | Absorbance | Standard Deviation | Slope | Limit of Detection | Limit of Quantification |
|---|---|---|---|---|---|---|
| 1 | ITRACONAZOLE 10 µg/ml | 0.5440 | 0.00115 | 0.051 | 0.2 | 0.5 |
| 2 | | 0.5438 | | | | |
| 3 | | 0.5432 | | | | |
| 4 | | 0.5428 | | | | |
| 5 | | 0.5424 | | | | |

**RUGGEDNESS**

Using the same instrument, two analysts conducted an assay of 10µg/ml of itraconazole under different conditions to assess the robustness of the suggested method. The same ideal circumstances on various days. The results were determined to be repeatable, as there was no discernible variation amongst the analysts. As such, one could characterize the suggested approach as rugged. The table displays the findings.7.

**TABLE 7: RUGGEDNESS**

| S. No | Analysts | Conc. (µg/ml) | Absorbance | Standard Deviation | %RSD |
|---|---|---|---|---|---|
| 1 | Analyst-I | 10 | 0.5390 | 0.000619 | 0.1149 |
| | | 10 | 0.5388 | | |
| | | 10 | 0.5385 | | |
| | | 10 | 0.5380 | | |
| | | 10 | 0.5378 | | |
| | | 10 | 0.5374 | | |
| 2 | Analyst-II | 10 | 0.4882 | 0.000757 | 0.1547 |
| | | 10 | 0.4886 | | |
| | | 10 | 0.4892 | | |
| | | 10 | 0.4897 | | |
| | | 10 | 0.4899 | | |
| | | 10 | 0.4901 | | |

**ROBUSTNESS**

The method's robustness was assessed by making minor adjustments to UV parameters, like varying the wavelength by ± 4. The table displays the findings.8

**TABLE 8: RESULTS OF ROBUSTNESS**

| S. No | Wavelength(nm) | Absorbance |
|---|---|---|
| 1 | 267 | 0.5280 |
| 2 | 271 | 0.5288 |
| 3 | 263 | 0.5272 |

**ANALYSIS OF PHARMACEUTICAL FORMULATIONS**

Without the need for sample extraction or filtration, the calibration curve method of the optimized spectrophotometric approach was used to determine the exact concentration of itraconazole in tablets. Calculating the drug content per tablet involved taking the absorbance value. The table displays the findings.9.

**Table 9: Analysis of Pharmaceutical Formulations.**

| Formulation | Labeled Amount (mg) | Amount Recovered (mg) | % Drug Recovered | Mean | Standard Deviation | %RSD |
|---|---|---|---|---|---|---|
| Sporanox | 100 | 93.26 | 98.315 | 99.838 | 1.9398 | 1.9430 |
| Sporanox | 100 | 102.09 | 102.022 | | | |
| Sporanox | 100 | 96.71 | 99.177 | | | |

## RESULTS AND DISCUSSION

The study's objective is to validate the dosage form of itraconazole using a UV spectrophotometer; it was conducted under ideal circumstances. The validation parameters were verified to yield results that fell within acceptable bounds. Linearity is observed for ittraconazole in the concentration range of 1–10µg/ml. A high degree of method sensitivity was indicated by the observed linearity range's good fit to Beer-Lambert's law and the corresponding regression coefficient (r=0.999). The results were tabulated in Table 2. The percentage of the drug found in formulations and the analysis's findings indicate that the amount of drug was in good agreement with the formulation's label claim. The fact that the percentage RSD is less than indicates that the system and methodology are highly reproducible. The outcomes were totaled. The formulation's analysed solution yielded pure drug recovery values ranging from 98 to 102 percent, indicating the accuracy of the proposed method. A table with the results was tabulated.3. System precision and method precision were used to validate the suggested approach; the data was tabulated in a table and the percent RSD for the system precision of itraconazole was 0.5.4. The precision of the method was tested, and table 6.6 displays the percentage average that was obtained for itraconazole. The outcomes of robustness studies, which involve verifying changes in parameters like wavelength, suggest that the analytical method remains unaltered. The results of the study on ruggedness, which involved two analysts and several systems, are displayed in Table 8.The suggested technique for istraconazole in capsules was sensitive, quick, accurate, precise, and sample-based.

## SUMMARY AND CONCLUSION

The UV-Spectrophotometer methods yielded comparable results for the determination of Itraconazole in bulk drug in capsule form, and the method was found to be fast, affordable, accurate, and precise. It can be used for an accurate and precise analysis of Itraconazole in both pure and capsule form. Studies on interference revealed that other activities and common excipients are typically included in the dosage. The recovery percentage values were nearly 100%, suggesting that the suggested method is accurate and reproducible. It has been effectively used as a quality control tool for the analysis of itraconazole in both bulk and capsule dosage forms.

## REFERENCES

1. De Beule K, Van Gestel J. Pharmacology of Itraconazole, 2001; 61(S1): 27–37.
2. Conway SP, Etherington C, Peckham DG et al. Pharmacokinetics and safety of Itraconazole in patients with cystic fibrosis. Journal of Antimicrobial Chemotherapy, 2004; 53: 841-847.
3. Janssen Pharmaceutical Products (2002). Sporanox® (itraconazole) injection. US Foodand Administration.
4. Kramer M, Marshall S, Denning D et al. Cyclosporine and Itraconazole Interaction inHeart and Lung Transplant Recipients. Annals of Internal Medicine, 1990; 113: 327–329.
5. European Pharmacopoeia. Itraconazole, Strasbourg: France, 4thEd, 2005; 1852-1853.
6. Hay R.J, Dupont B, Graybill J.R. Review Infectious Disease, 1987; 9(Suppl.1): S1-3.
7. Saag M.S, Dismukes W.E. Antimicrobial Agents Chemotherapy, 1988.
8. Mechanism of action available online at URL http://www.drugbank.ca/drugs/DB01167.
9. Zimmermann T, Yeates R.A, Laufen H. European Journal of Clinical Pharmacology,1994; 46: 147.
10. Haria M, Bryson H.M, Goa K.L Drugs, 1996; 51: 585-620.
11. Pharmaceutical applications available online at URLhttp://www.nlm.nih.gov.
12. Shalin K. Parikh, Ankit D. Patel, Dr. J. B. Dave, Dr. C. N. Patel and Dr. D. J. Sen, “Development and validation of UV Spectrophotometric method for estimation of itraconazole bulk drug and pharmaceutical formulation” International Journal of Drug Development and Research, 2011; 3(2): 324-328.
13. Stefanie Redmann, Bruce G. Charles, “A rapid HPLC method with fluorometric detection for determination of plasma itraconazole and hydroxy-itraconazole concentrations in cystic fibrosis children with allergic broncho pulmonary aspergillosis” Biomedical Chromatography, 2006; 20(4): 343–348.

14. D. Vijaya Bharathi, Kishore Kumar Hotha, P.V.Vidya Sagar, Sanagapati Sirish Kumar, Pandu Ranga Reddy, A. Naidu, Ramesh Mullangi, "Development and validation of a highly sensitive and robust LC–MS/MS with electrospray ionization method for simultaneous quantitation of itraconazole and hydroxyitraconazole in human plasma" Science Direct Journal, 2008; 868(1-2): 70-76.

15. Ming Yao, Laishun Chen, Nuggehally R Srinivas, "Quantitation of itraconazole in rat heparinized plasma by liquid chromatography–mass spectrometry" Journal of Chromatography B: Biomedical Sciences and Applications, 2001; 752(1)5: March 2001; 916.

16. Constantinos Kousoulos, Georgia Tsatsou, Constantinos Apostolou, Yannis Dotsikas and Yannis L. Loukas, "Development of a high-through put method for the determination of itraconazole and its hydroxy metabolite in human plasma, employing automated liquid– liquid extraction based on 96-well format plates and LC/MS/MS " Analytical and Bioanalytical Chemistry, 2006; 384(1): 199-207.

17. M. V. Kumudhavalli, "Isocratic RP-HPLC, UV Method Development and Validation Of Itraconazole In Capsule Dosage Form" International Journal of Pharmaceutical Research IJPSR, 2011; 2(12): 3269-3271.

18. Al-Rawithi H, Sameer M, Hussein A, Rajaa Al-Moshen, Ibrahim, Raines, Dale, et al. Determination of Itraconazole and Hydroxy itraconazole in Plasma by High-Performance Liquid Chromatography with Fluorescence Detection, Therapeutic Drug Monitoring, 2001; 23: 445-448.

19. Khoschsorur G, Frueehwirth F, Zelzer S.Isocratic High Performance Liquid Chromatographic Method with UV detection for simultaneous determination of voriconazole and itraconazole and its hydroxyl metabolite in human serum. Antimicrobial agents and Chemotherapy, 2005; 49: 3569-3571.

20. Srivatsan V, Dasgupta A, Kale P, Datla R, Soni D, Patel M, Patel R, and Mavadhiya, C. Simultaneous determination of itraconazole and hydroxyitraconazole in human plasma by high performance liquid chromatography, Journal of Chromatography, 2004; 1031: 307- 313.

21. El-Enany N, El-Sherbilny D, Belal F. Spectrofluorimetric Determination of Itraconazole in Dosage Forms and Spiked Human Plasma. Journal of te Chinese Chemical Society, 2007; 54: 375-382.