WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 15, 959-967. Research Article ISSN 2277–7105

A NOVEL ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ITRACANAZOLE BY UV SPECTROSCOPIC METHOD

Malkapuram Madhavi[*1] and Santhosh Illendula[2]

[*1]Under Graduate Scholar, Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Charlapally, Nalgonda, Telangana.

[2]Associate Professor, Department of Pharmaceutical Analysis, Vijaya College of Pharmacy, Munaganoor, Hayathnagar, Hyderabad, Telangana, India.

Article Received on
10 June 2025,

Revised on 30 June 2025,
Accepted on 20 July 2025,

DOI: 10.20959/wjpr202515-37719

***Corresponding Author**
Malkapuram Madhavi
Under Graduate Scholar, Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Charlapally, Nalgonda, Telangana.

ABSTRACT

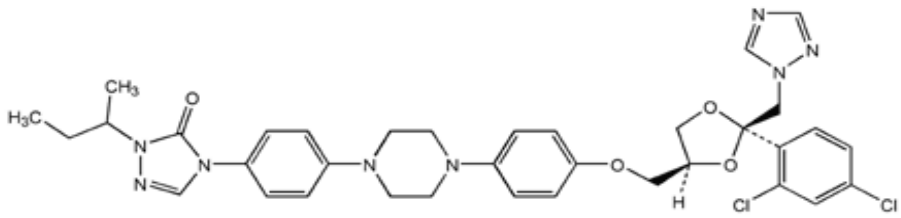
A novel, accurate, precise, and economical UV spectrophotometric method was successfully developed and validated for the estimation of Itraconazole in bulk and pharmaceutical dosage forms. Itraconazole, a synthetic triazole antifungal agent, exhibits poor aqueous solubility, making its analysis challenging using simple techniques. In this study, methanol: water in a ratio of 70:30 was selected as the solvent system based on the drug's solubility profile. The standard solution was scanned in the UV range (200–400 nm), and the maximum absorbance (λmax) was observed at 262 nm. The method was validated in accordance with ICH Q2 (R1) guidelines. It demonstrated excellent linearity in the concentration range of 5–25 µg/mL, with a correlation coefficient (R^2) of 0.999, indicating a strong linear relationship between concentration and absorbance. Accuracy was confirmed through recovery studies, with results ranging from 98% to 102%, proving minimal interference from excipients. Precision studies showed %RSD values below 2%, confirming good repeatability and reproducibility. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated to be 1.25 µg/mL and 3.80 µg/mL, respectively. Robustness testing, performed by making minor variations in wavelength and solvent composition, showed no significant effect on results, confirming the method's reliability. This validated UV spectrophotometric method

is simple, cost-effective, and suitable for routine quality control and academic research applications.

KEYWORDS: Itraconazole, UV spectrophotometry, analytical method development, λmax 262 nm, ICH Q2(R1) guidelines, precision, accuracy, bulk drug estimation, pharmaceutical analysis.

INTRODUCTION

2-butan-2-yl-4-[4-[4-[4-[[(2R, 4S)-2 (2, 4-dichlorophenyl)-2-(1, 2, 4-triazol-1-ylmethyle1, 3-dioxolan-4-ethoxy] phenyl] piper zine -1—yl]""phenyl]-1, 2, 4-triazol-3-one.



itraconazole

MATERIALS AND METHODS

Chemicals and Reagents: Methanol and water.

Instruments: SHIMADZU UV – 1800 UV–VIS spectrophotometer, Electronic Balance **(CITIZEN CTG302-300),** Digital Ultra Sonicator **(SOLTEC),** and P^H Meter **(ELICO TYPE V003),** Distillation unit **(BOROSIL),** Vacuums filtration unit **(BOROSIL).**

Reagents and Solutions

Diluent preparation: Methanol water (70:30).

Preparation of Standard Solutions

Accurately weighed 100 mg of itraconazole was weighed accurately and transferred into 100ml volumetric flask. About 10 ml of diluent was added and sonicated to dissolve. The volume was made up to the mark with the same solvent. The final solution contained about 100µg/ml of itraconazole working standard solution of itraconazole containing 10µg/ml for the method. Finally add those above solutions and prepare the final solution is about 10µg/ml.

Determination of wavelength of maximum absorbance for itraconazole

The absorbance of the final solution scanned in the UV spectrum in the range of 200 to 400nm against solvent mixture as blank.

Optimization of selection of Solvent

It is well known that the solvents do exert a profound effect on the quality and the shape of the peak. The choices of solvents for UV method development are: Methanol, Ethanol Acetonitrile, Dimethyl Sulphide, water solutions etc. First optimize the different solvents. From that solvents methanol and water 70:30 % v/v satisfied all the optimized conditions.

Wavelength Selection

The standard solutions are prepared by transferring the standard drug in a selected solvent or mobile phase and finally diluting with the same solvent or diluent. That prepared solution is scanned in the visible wavelength range of 200-400nm. This has been performed to know the maxima of itraconazole. While scanning the itraconazole solution we observed the maxima at 262 nm. The visible spectrum has been recorded on (SHIMADZU UV-1800 make UV – Visible spectrophotometer model UV-1800.The scanned visible spectrum is attached in the following page. The λ_{max} of the itraconazole was found to be 262 nm in diluents as a solvent system.

METHOD VALIDATION

1. Accuracy

Recovery study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Itraconazole were taken and added to the pre- analysed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in Table-1.

2. Precision

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Itraconazole (API) the percent relative standard deviations were calculated for Itraconazole is presented in the Table-2.

Intermediate Precision

Intra-assay & inter-assay

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Itraconazole revealed that the proposed method is precise. The results were shown in Table-3.

3. Linearity & Range

The calibration curve showed good linearity in the range of 5-25µg/ml, for Itraconazole (API) with correlation coefficient (r^2) was found to be 1, which shows good linearity between above range. The slope was found to be 0.061654 and intercept was found to be 0.0038 which was close to zero intercept.

Standard solutions of itraconazole in the concentration range of 5 µg/ml to 25 µg/ml were obtained by transferring (5, 10, 15, 20 and 25 ml) of itraconazole stock solution (100ppm) to the series of clean & dry 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed.

The absorbance's of the solutions were measured at 260 nm against the solvent system as blank and calibration curve is plotted. The Beer's Lambert- Law is linear in concentration range of 5 to 25 µg/ml at 262 nm for itraconazole. The results were shown in Table-4.

4. Method Robustness

Robustness of the method was determined by carrying out the analysis under different Wavelength i.e., at 260 nm, and 264 nm. The respective absorbance's of 10µg/ml were noted SD < 2%) the developed UV-Spectroscopic method for the analysis of Itraconazole (API). The results were shown in Table-5.

5. LOD & LOQ

The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot.

Robustness of the method was determined by carrying out the analysis under different Wavelength i.e., at 258 nm, and 262 nm. The respective absorbance's of 10µg/ml were noted

SD < 2%) the developed UV-Spectroscopic method for the analysis of Itraconazole (API). The results were shown in Table-5

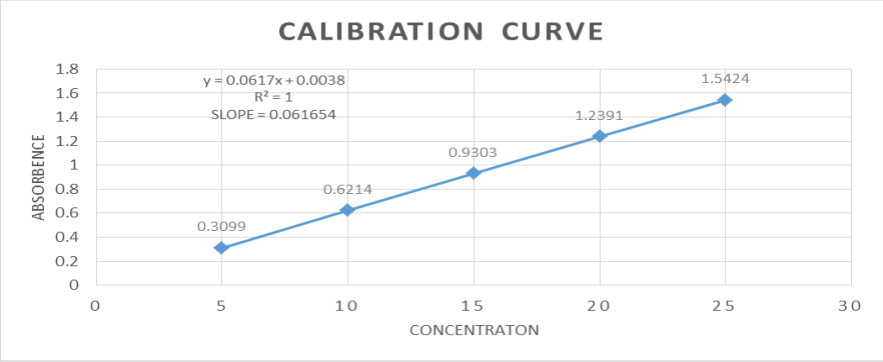
The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.000247561 & 0.00074268414 µg/ml respectively.

RESULTS AND DISCUSSION

The standard solutions of itraconazole with methanol and water 70:30 (10µg/ml) subjected to a scan individually at the series of wavelengths of 200 nm to 400 nm. Absorption maximum of itraconazole was found to be at 262 nm. Therefore, 262 nm was selected as λ_{max} of Itraconazole for the present study. The calibration curve of Itraconazole was found to be linear in the range of 5-25 µ g/ml at 262 nm. Therefore, it was clear that itraconazole can be determined without interference of any irrelevant substance in single component pharmaceutical products. The used technique was initially attempted on bulk drugs in their synthetic sample and concentrations were estimated.

The % recovery was carried out at 3 levels, 80%, 100% and 120% of itraconazole standard concentration. Three samples were prepared for each recovery level. The solutions were then analysed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet dosage form. The newly developed method was validated according to the ICH guidelines and the method validation parameters.

The developed method was subjected to do the various method validation parameters such as specificity, accuracy, precision, linearity and range, limit of detection and limit of quantification, robustness and ruggedness etc.



Calibration Curve for Itraconazole at 262nm.

Table: 01.

Level of Recovery	Sample Conc. (µg/ml)	Absorbance	% Recovery	Mean % Recovery
80%	8	0.4961	99.9	99.4
80%	8	0.4898	98..9	
80%	8	0.4994	99.3	
100%	10	0.6191	99.7	99.6
100%	10	0.6238	98.4	
100%	10	0.6209	99.1	
120%	12	0.7450	99.8	99.3
120%	12	0.7392	99.5	
120%	12	0.7389	99.6	

Accuracy studies

Acceptance criteria: % of recovery should be present in between 98% -102%

Table 02: Repeatability.

S. No.	Conc. (mg/ml)	Wavelength (nm)	Absorbance
1	10	262	0.6193
2	10	262	0.6211
4	10	262	0.6175
5	10	262	0.6129
6	10	262	0.6144
Mean ± S.D.			0.616267
Standard Deviation			0.003174
% RSD			0.57988

Table 03: Intra-Day and Inter-Day Precision.

Conc. taken (µg/mL)	Observed Conc. of Itraconazole (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Absorbance	Statistical Analysis	Con. found (µg/mL)	Statistical Analysis
10	0.6291	Mean = 0.6229 SD = 0.005335 %RSD = 0.8564	0.6154	Mean = 0.6161 SD = 0.003361 %RSD = 0.545427
10	0.6204		0.6132	
10	0.6194		0.6198	

Table 04: Results of Linear Curve.

Concentration (µg/ml)	Absorbance
5	0.3099
10	0.6214
15	0.9303
20	1.2391
25	1.5424

Acceptance criteria: correlation coefficient should not be less than 0.999

Table 06: Robustness.

Concentration(µg/ml)	Wavelength	Absorbance	Statistical Analysis
10	260	0.6104	Mean =0.6152 SD =0.003685 % RSD = 0.599009
10		0.6178	
10		0.6143	
10	264	0.6214	
10		0.6168	
10		0.6135	

Change in wavelength

CONCLUSION

The standard solutions of itraconazole 100 mg and the solvent methanol and water (70:30 % v/v) (10 µg/ml) subjected to a scan individually at the series of wavelengths of 200 nm to 400 nm. Absorption maximum of itraconazole was found to be at 262 nm. Therefore, 262 nm was selected as λ_{max} of itraconazole for the present study. The calibration curve of itraconazole was found to be linear in the range of 5-25 µg/ml at 262 nm. Therefore, it was clear that itraconazole can be determined without interference of any irrelevant substance in single component pharmaceutical products. The used technique was initially attempted on bulk drugs in their synthetic sample and concentrations were estimated.

The % recovery was carried out at 3 levels, 80%, 100% and 120% of itraconazole standard concentration. Three samples were prepared for each recovery level. The solutions were then analysed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet dosage form. The newly developed method was validated according to the ICH guidelines and the method validation parameters.

The developed method was subjected to do the various method validation parameters such as specificity, accuracy, precision, linearity and range, limit of detection and limit of quantification, robustness and ruggedness etc.

SUMMARY

Table 07: Summary of Validation Parameters.

Parameter	Itraconazole	ICH Limits
System Precision	0.579	%RSD was less than 2%
Linearity Range	5-25 µgm/ml	----------
Correlation co-efficient	1	More then 0.999
Accuracy	99.43%	98-102%
Specificity	0.9940	Peak purity angle is less

		than purity threshold
Robustness	0.5990	%RSD was less than 2%
Ruggedness	0.4733	%RSD was less than 2%
LOD	0.00070	Less Than 3.3
LOQ	0.002148	Less Than 10
Intra and inter precision	0.8564 and 0.545427	%RSD was less than 2%
Repeatability	0.579	%RSD was less than 2%

ACKNOWLEDGEMENT

The authors are grateful to the management of Nalanda College of Pharmacy, charlapally, Nalgonda. for providing the facilities to carry out the present research work.

REFERENCE

1. A.H. Beckett and J.B. Stenlake, Practical Pharmaceutical Chemistry, 4th Edn. Part II, CBS Publisher and Distributor, 2002; 270-274.
2. A. Douglas Skoog, F. James Holler and Stanely Crouch, Principles of Instrumental Analysis, 2nd Edn. Saunders Golden Sunburst Series, 1980; 395-397.
2. https://go.drugbank.com/drugs/DB01167.
3. Research Gate https:// www. researchgate.net
4. Chavan SS, Mahaparale PR. UV Spectrophotometric method development and validation of itraconazole in bulk and tablet dosage form. Int J Pharm Sci., 2024; 16(1): 75–79.
5. Soundarya N, Kumar KS, et al. Development and validation of UV spectrophotometric method for estimation of itraconazole in pharmaceutical dosage forms. Int J Pharm Sci Rev Res, 2023; 79(2): 45–49.
6. Santhosh Illendula, Thota sumanjali, D. Sandhya, N.Lavanya, Dr. K N V Rao: Method development and validation of afatinib in bulk and pharmaceutical dosage form by uv spectrophotometric method, Indo American journal of pharmaceutical sciences. 2018; 05(03): 1569-1575.
7. Santhosh Illendula, A. Navyasree, Ch.Ganesh, K. Sneha, Dr. K N V Rao: Method development and validation of axitinib in bulk and pharmaceutical dosage form by uv spectrophotometric method, Indo American journal of pharmaceutical sciences. 2018; 05(03): 1569-1575.
8. Yogeshwar Rao M, Raju K, Reddy MN. Validated spectrophotometric methods for quantitative estimation of itraconazole in formulations. Asian J Pharm Anal. 2023; 13(4): 230–235.

9. Shalin P, Raval M. Development of simple UV spectrophotometric method for estimation of itraconazole in capsules. J Appl Pharm Sci., 2022; 12(10): 160–164.
10. International Conference on Harmonization (ICH). Q2 (R1): Validation of Analytical Procedures: Text and Methodology. ICH Harmonized Tripartite Guideline. Geneva: ICH; 2005.
11. Santhosh Illendula, Maya sudha, Nitesh & KNV Rao; Method development and validation of Ropinirole by using UV spectroscopy method, World Journal of Pharmacy and Pharmaceutical Sciences, WJPPS, Jan 2022; 11(8): 1418-1427.
12. Santhosh Illendula, M Divya, Rajeswar Dutt & KNV Rao; RP HPLC method development & validation for forced degradation studies for the simultaneous estimation of abacavir & lamivudine in pure form & marketed formulation, International Journal of research, May 2019; 08(5): 3342-3364.
13. Indian Pharmacopoeia Commission. Indian Pharmacopoeia 2018. Ghaziabad: IPC; 2018.
14. Ahuja S, Cywinski S. Handbook of Modern Pharmaceutical Analysis. 2nd ed. Academic Press; 2001.
15. Kasture AV, Mahadik KR, Wadodkar SG, More HM. Pharmaceutical Analysis Vol II. 17th ed. Nirali Prakash an; 2013.
16. Mahadik KR, Paradkar AR. Instrumental Techniques and Drug Analysis. 1st ed. Nirali Prakash an; 2012.
17. Indian Pharmacopoeia Commission. Indian Pharmacopoeia 2018. Ghaziabad: IPC; 2018.