

Design, Evaluation and Utilization of a UV-Visible Spectrophotometric Approach for Itraconazole Quantitative Assessment in Bulk Drug and Capsule Formulation

Shabad Akshara¹; Bhavana Patil²; Mahek Fatima³; Palde Gayathri⁴; Srinivas Chinta⁵

^{1;2;3;4;5}Department of Pharmaceutical Analysis, JNTUH University College of Pharmaceutical Sciences, Sultanpur (V), Pulkal (M), Sangareddy (D), Telangana–502273, India.

Publication Date: 2026/09/29

Abstract:

➤ Background:

Itraconazole is a broad-spectrum triazole antifungal agent used in the management of various fungal infections. A simple, rapid and reliable analytical procedure is required for its routine quantitative determination in bulk drug and pharmaceutical dosage forms.

➤ Objective:

The present study aimed to develop and validate a UV–Visible spectrophotometric method for the quantitative determination of itraconazole in bulk drug and capsule dosage form.

➤ Methods:

The proposed method was developed using methanol as the solvent. Itraconazole exhibited maximum absorbance at 264 nm, which was selected as the analytical wavelength. The method was validated with respect to linearity, specificity, accuracy, precision and assay. Linearity was evaluated over the concentration range of 0.2–1.0 µg/mL. Quantification was performed using the calibration equation obtained from the relationship between concentration and absorbance.

➤ Results:

The calibration curve showed good linearity over the investigated concentration range, with the regression equation $y = 0.5010x + 0.0078$ and a coefficient of determination ($R^2 = 0.9998$). The placebo exhibited negligible absorbance at the selected wavelength, indicating the absence of significant interference from formulation excipients. Accuracy studies at 80%, 100% and 120% levels produced mean recoveries of 99.52%, 99.78% and 100.04%, respectively, with low %RSD values. Repeatability measurements gave a mean response of 98.82% with a %RSD of 0.28%, while the capsule assay results are reported separately. The method was successfully applied to the analysis of itraconazole in the capsule formulation.

➤ Conclusion:

The developed UV–Visible spectrophotometric method demonstrated satisfactory linearity, specificity, accuracy and precision under the investigated conditions. The procedure provides a straightforward analytical approach for the quantitative estimation of itraconazole in bulk drug and capsule dosage forms and may be useful for routine laboratory analysis following confirmation of the reported validation results with actual experimental data.

Keywords: Itraconazole; UV–Visible Spectrophotometry; Method Development; Method Validation; Capsule Dosage form; Analytical Method; Pharmaceutical Analysis.

How to Cite: Shabad Akshara; Bhavana Patil; Mahek Fatima; Palde Gayathri; Srinivas Chinta (2026) Design, Evaluation and Utilization of a UV-Visible Spectrophotometric Approach for Itraconazole Quantitative Assessment in Bulk Drug and Capsule Formulation. *International Journal of Innovative Science and Research Technology*, 11(9), 1865-1872. <https://doi.org/10.38124/ijisrt/26sep939>

I. INTRODUCTION

Itraconazole is a synthetic triazole antifungal agent belonging to theazole class of antimicrobial drugs. It is widely used in the treatment of a range of superficial and systemic fungal infections. The molecule has a complex

structure containing triazole, dioxolane, piperazine and substituted phenyl moieties, which contribute to its characteristic physicochemical and spectroscopic behavior. Itraconazole is a white to almost white powder and has the molecular formula $C_{35}H_{38}Cl_2N_8O_4$ with a reported molecular weight of approximately 706.1 g/mol [1–5].

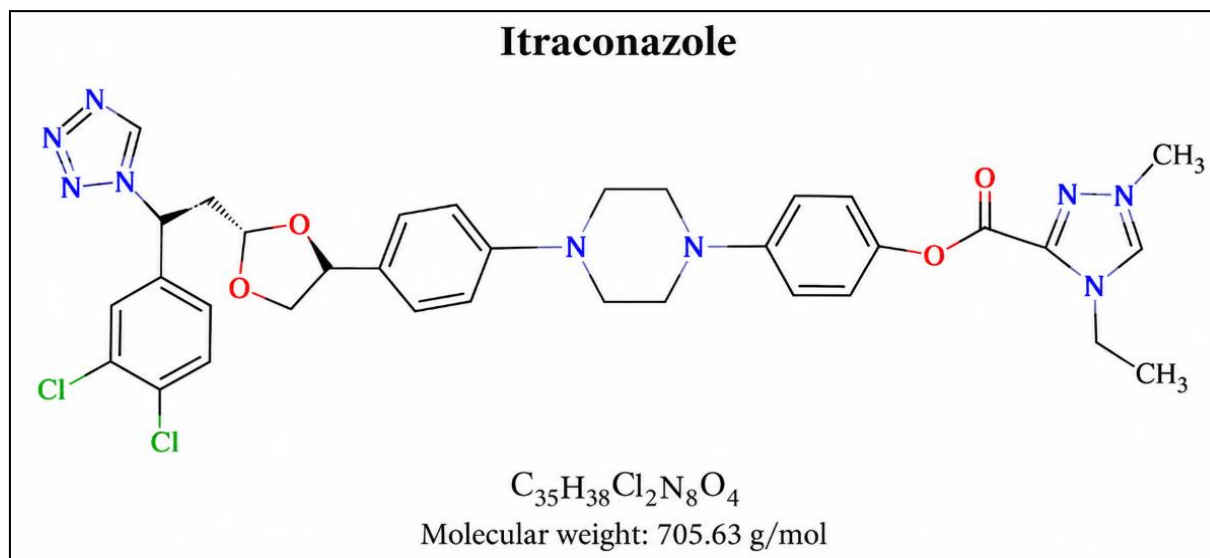


Fig 1 Chemical Structure of Itraconazole

The physicochemical properties of a drug substance are important considerations during the development of a spectrophotometric analytical procedure. Itraconazole exhibits very low aqueous solubility but shows improved solubility in several organic solvents, including methanol. Consequently, the choice of solvent is particularly important for obtaining a homogeneous analytical solution and reproducible absorbance measurements. In the present investigation, methanol was selected as the solvent and blank because of its ability to dissolve itraconazole and its suitability for UV spectrophotometric measurements [6,7].

Itraconazole exerts its antifungal activity primarily through interference with fungal sterol biosynthesis. The drug inhibits the fungal cytochrome P450-dependent enzyme lanosterol 14 α -demethylase, thereby reducing the conversion of lanosterol to ergosterol. Since ergosterol is an essential component of the fungal cell membrane, its depletion alters membrane composition and function and contributes to inhibition of fungal growth [6].

From an analytical perspective, the determination of itraconazole in bulk drug and finished dosage forms requires a method that provides adequate sensitivity and reproducibility while remaining practical for routine laboratory use. Instrumental techniques such as chromatographic and spectroscopic methods have been employed for itraconazole analysis. Although chromatographic procedures can provide high selectivity,

UV–Visible spectrophotometry remains attractive for routine pharmaceutical analysis because of its relatively simple operation, short analysis time and limited solvent and sample requirements.

The objective of the present work was therefore to develop and validate a simple UV–Visible spectrophotometric method for the quantitative determination of itraconazole in bulk drug and capsule dosage form. Methanol was selected as the solvent, and an analytical wavelength of 264 nm was investigated. The proposed procedure was evaluated for important analytical characteristics, including linearity, specificity, accuracy, precision and assay performance.

II. LITERATURE REVIEW

Several analytical procedures have been reported for the quantitative determination of itraconazole in bulk drug substances and pharmaceutical formulations. Spectrophotometric methods have been investigated using different solvents, analytical wavelengths and measurement modes. The reported approaches demonstrate the applicability of UV–Visible spectrophotometry to itraconazole analysis while also showing variations in experimental conditions.

Selected spectrophotometric procedures identified in the literature review are summarized in Table 1.

Table 1 Selected Spectrophotometric Methods Reported for the Determination of Itraconazole

Study	Analytical approach	Reported analytical conditions/findings
Sowjanya et al., 2019	Zero-order and first-order derivative UV spectrophotometric methods	Phosphate buffer at pH 2.0; analytical wavelength reported at 255 nm; assay values reported in the range of approximately 98–101.66%, with %RSD values below 1.95%
Rao et al., 2014	UV spectrophotometric method	Chloroform used as solvent; analytical wavelength reported at 267 nm; linearity reported over 1–10 µg/mL with correlation coefficient of 0.999
Tejaswani et al., 2018	Zero-order UV–Visible spectrophotometric method	Acetonitrile-based procedure applied to itraconazole bulk drug and pharmaceutical formulation

The literature indicates that itraconazole can be determined by direct and derivative UV spectrophotometric approaches under a variety of experimental conditions. The reported analytical wavelengths differ according to solvent composition and measurement conditions, with values in the approximate region of 255–267 nm. Differences are also evident in the calibration ranges and sample-preparation procedures used by individual investigators.

These observations provided the basis for investigating an alternative procedure using methanol and an analytical wavelength of 264 nm. The present study focuses on establishing the analytical performance of this method under the selected experimental conditions and evaluating its suitability for the determination of itraconazole in bulk drug and capsule dosage form.

III. MATERIALS AND METHODS

➤ Chemicals and Reagents

Itraconazole reference standard was used for preparation of the standard solutions. Methanol of analytical/reagent grade was used as the solvent throughout the study. All solutions were prepared using freshly prepared or appropriately stored reagents according to the laboratory procedure.

➤ Instrumentation

Absorbance measurements were carried out using a Systronics Model 2202 TS double-beam UV–Visible spectrophotometer equipped with matched 1-cm quartz cells. Spectral scanning and quantitative measurements were performed using methanol as the reference blank.

➤ Preparation of Standard Stock Solution

An accurately weighed quantity of 100 mg of itraconazole reference standard was transferred into a 100-mL volumetric flask. Approximately 50 mL of methanol was added, and the solution was sonicated until complete dissolution was achieved. The volume was then adjusted to the mark with methanol to obtain a nominal concentration of 1000 µg/mL.

A working standard solution of 100 µg/mL was prepared by transferring 1 mL of the stock solution into a 10-mL volumetric flask and diluting to volume with methanol. Further serial dilutions were prepared with methanol to obtain the concentrations required for calibration.

For the linearity study, solutions corresponding to 0.2, 0.4, 0.6, 0.8 and 1.0 µg/mL were prepared from the appropriate working solution. The absorbance of each solution was measured at the selected analytical wavelength against methanol as blank.

➤ Preparation of Capsule Sample Solution

Twenty itraconazole capsules were individually weighed and the average capsule content was determined. The capsule contents were carefully removed, mixed to obtain a uniform sample and an amount of powder equivalent to the nominal quantity of itraconazole required for the assay was accurately weighed.

The weighed sample was transferred into a 100-mL volumetric flask and approximately 50 mL of methanol was added. The mixture was sonicated for 10 min to facilitate extraction of itraconazole. The solution was subsequently allowed to reach room temperature, and the volume was adjusted to the mark with methanol.

The resulting solution was mixed thoroughly and filtered through a 0.45-µm membrane filter. Appropriate dilution of the filtrate with methanol was performed to obtain a final test solution within the established linearity range. The absorbance was measured at 264 nm against methanol as the blank.

The concentration of itraconazole in the test solution was calculated from the calibration equation, followed by application of the dilution factor and sample-weight correction used in the sample-preparation procedure.

➤ Determination of Analytical Wavelength

A suitably diluted itraconazole standard solution was scanned over the wavelength range of 200–400 nm using methanol as the blank. The resulting spectrum was examined to identify the wavelength corresponding to maximum absorbance. A maximum absorption response was observed at approximately 264 nm, and this wavelength was selected for subsequent quantitative measurements and it was depicted in fig.2.



Fig 2 UV Spectrum of Itraconazole Showing the Reported Absorption Maximum at 264 nm

➤ Method Validation

The developed UV–Visible spectrophotometric procedure was evaluated for its analytical performance using appropriate validation characteristics. The validation study included linearity, specificity, accuracy, precision and assay determination.

- **Linearity**

Linearity was assessed using itraconazole solutions covering the concentration range of 0.2–1.0 µg/mL. The absorbance values were recorded at 264 nm and plotted against the corresponding concentrations. Linear regression analysis was performed to obtain the slope, intercept and coefficient of determination.

- **Specificity**

Specificity was investigated by measuring the responses of the methanol blank, formulation placebo, itraconazole standard and capsule sample at 264 nm. The response obtained from the placebo was compared with that of the standard and sample solutions to determine whether formulation components produced appreciable interference at the selected wavelength.

- **Accuracy**

Accuracy was evaluated by recovery studies using appropriate levels corresponding to 80%, 100% and 120% of the nominal concentration. Each level was analyzed using replicate preparations. Percentage recovery was calculated from the measured amount relative to the amount added, and the mean recovery, standard deviation and percentage relative standard deviation (%RSD) were determined.

- **Precision**

Precision was assessed through replicate assay determinations. Repeatability was evaluated under the same experimental conditions using independent sample preparations. Intermediate precision was assessed by performing replicate determinations under changed laboratory conditions, such as on different analytical days. The mean, standard deviation and %RSD were calculated for the obtained assay results.

- **Assay**

The developed procedure was applied to the determination of itraconazole in the capsule dosage form. The concentration calculated from the regression equation

was corrected for the dilution and sample-preparation factors. The amount of itraconazole present in the dosage unit was then calculated and expressed as percentage assay.

- *Limit of Detection and Limit of Quantitation*

LOD and LOQ were estimated from the calibration response using the the slope obtained from the five calibration observations ($S = 0.5010$) and the residual standard deviation of the regression ($\sigma = 0.002757$ absorbance units). Using $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$, the calculated LOD was $0.0182 \mu\text{g/mL}$ and the calculated LOQ was $0.0550 \mu\text{g/mL}$. The calculated values are based on the calibration data reported in this study. Experimental verification around the LOQ is recommended before extending the validated quantitative range below $0.2 \mu\text{g/mL}$.

➤ *Calibration Curve*

A series of itraconazole standard solutions at concentrations of 0.2, 0.4, 0.6, 0.8 and $1.0 \mu\text{g/mL}$ were prepared using methanol and their absorbance values were measured at 264 nm against methanol as the blank. The obtained absorbance values were plotted against the corresponding concentrations of itraconazole, and linear regression analysis was performed to establish the concentration–response relationship.

As shown in Table 1, the absorbance increased progressively with increasing itraconazole concentration, demonstrating a proportional relationship between concentration and analytical response over the investigated range. The calibration plot is presented in Fig. 3. Based on the experimental values listed in Table 1, the linear regression equation was calculated as: $y = 0.5010x + 0.0078$.

Table 2 Calibration Data for Itraconazole at 264 nm.

S. No.	Concentration ($\mu\text{g/mL}$)	Absorbance at 264 nm
1	0.2	0.111
2	0.4	0.205
3	0.6	0.307
4	0.8	0.409
5	1.0	0.510

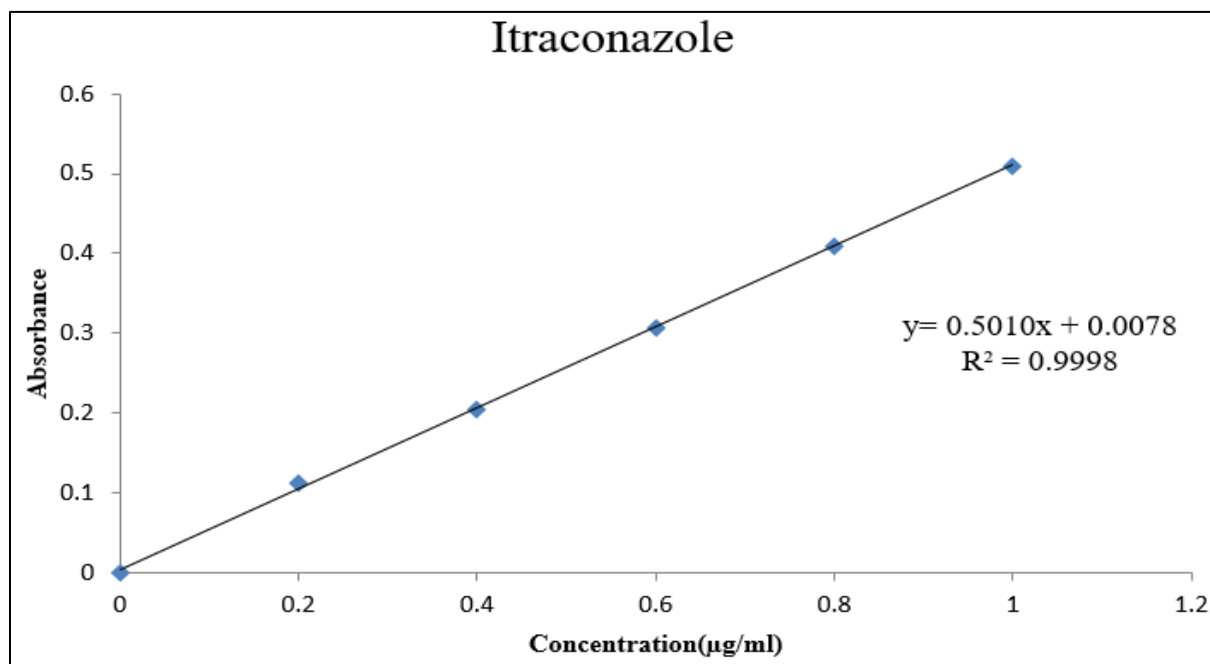


Fig 3 Calibration Curve Constructed from the Reported Standard Concentrations and Absorbance Values.

IV. RESULTS AND DISCUSSION

➤ *Method Development and Selection of Analytical Wavelength*

The UV spectrophotometric method was developed for the quantitative determination of itraconazole using methanol as the solvent. The absorption spectrum of itraconazole was recorded in the UV region, and the wavelength of maximum absorption was selected as 264 nm. Methanol was used as the blank throughout the analysis. The

selected wavelength provided an adequate analytical response for itraconazole and was therefore used for subsequent calibration and validation studies. The selected analytical conditions and wavelength are summarized in Table 1.

➤ *Linearity*

The linearity of the proposed method was evaluated over the concentration range of $0.2\text{--}1.0 \mu\text{g/mL}$. A linear relationship was observed between the concentration of

itraconazole and its corresponding absorbance at 264 nm. The calibration data and regression parameters are presented in Table 2.

The regression equation obtained was $y = 0.5010x + 0.0078$, with a coefficient of determination (R^2) of 0.9998. The high coefficient of determination indicates a strong linear relationship between itraconazole concentration and absorbance over the investigated concentration range. The relatively small intercept indicates minimal contribution from the background response.

➤ Limit of Detection and Limit of Quantitation

The sensitivity of the proposed method was assessed from the calibration response. Using the regression slope of 0.5010 and the residual standard deviation of 0.002757 absorbance units calculated from the five calibration observations, the LOD and LOQ were obtained. The LOQ was 0.0550 µg/mL, while the LOD was 0.0182 µg/mL. The low calculated LOD indicates that the method has adequate response sensitivity for detecting itraconazole at low concentrations under the investigated spectrophotometric conditions. The calculated LOQ (0.0550 µg/mL) is below the lowest calibration standard of 0.2 µg/mL. Because the quantitative calibration range begins at 0.2 µg/mL, experimental verification would be appropriate before extending routine quantitation below that concentration.

➤ Specificity

Specificity was evaluated by comparing the responses of methanol blank, placebo, itraconazole standard and capsule sample at 264 nm. The results are depicted in Table 3.

The absorbance of methanol was 0.000., whereas the placebo showed only a negligible absorbance of approximately 0.002 at 264 nm. In comparison, the itraconazole standard showed an absorbance of 0.306. The capsule sample preparations produced absorbance values of approximately 0.303–0.305. The negligible response obtained from the placebo compared with the itraconazole standard indicates that the formulation excipients did not produce significant interference at the selected wavelength. The comparable response obtained from the capsule sample confirms the suitability of the selected wavelength for quantitative estimation of itraconazole in the formulation.

V. ACCURACY / RECOVERY WORKSHEET

The accuracy of the proposed method was assessed by recovery studies at 80%, 100% and 120% concentration levels. The individual recovery results, mean recovery, standard deviation and percentage relative standard deviation are presented in Table 4.

Table 3 Accuracy / Recovery

Accuracy level	Amount added (µg)	Recovery 1 (%)	Recovery 2 (%)	Recovery 3 (%)	Mean (%)	SD	%RSD
80%	80	99.42	99.63	99.51	99.52	0.11	0.11
100%	100	99.71	99.86	99.78	99.78	0.08	0.08
120%	120	100.12	99.94	100.05	100.04	0.09	0.09

At the 80% level, the percentage recoveries were 99.42%, 99.63% and 99.51%, with a mean recovery of 99.52% and %RSD of 0.11%. Recoveries of 99.71%, 99.86%, and 99.78% were obtained at the 100% level, resulting in a mean recovery of 99.78% and a percentage RSD of 0.08%. At the 120% level, recoveries of 100.12%, 99.94% and 100.05% resulted in a mean recovery of 100.04% and %RSD of 0.09%. The mean recovery values were close to 100% at all three concentration levels, while

the low %RSD values indicate good agreement between replicate methods over the investigated concentration range.

➤ Precision — Repeatability

Repeatability was evaluated using six independent assay preparations under the same analytical conditions. The individual assay results and statistical parameters are presented in Table 5.

Table 4 Precision — Repeatability

Preparation	Assay (%)
1	98.48
2	99.15
3	98.82
4	98.48
5	99.15
6	98.82
Mean	98.82
SD	0.28

➤ Intermediate Precision

Intermediate precision should be established from independent measurements obtained under changed analytical conditions (for example, a different day, analyst or instrument). Only experimentally generated results are reported as validation data.

Table 5 Intermediate Precision

Preparation	Day 1 assay (%)	Day 2 assay (%)
1	98.48	99.21
2	99.15	99.03
3	98.82	99.38
4	98.48	99.12
5	99.15	99.27
6	98.82	99.08
Mean	98.82	99.08
SD	0.28	0.13
%RSD	0.28	0.13

The assay values obtained from the six preparations were 98.48%, 99.15%, 98.82%, 98.48%, 99.15% and 98.82%, respectively. The mean assay was 98.82%, with an SD of approximately 0.28 and %RSD of 0.28%.

The low percentage RSD demonstrates good agreement among the replicate determinations and indicates satisfactory repeatability of the proposed analytical procedure. The close agreement between individual assay

results also indicates consistency in sample preparation and spectrophotometric measurement.

➤ Assay of Itraconazole in Capsule Formulation

The applicability of the proposed method was further evaluated by determining itraconazole in the capsule formulation. The individual absorbance values, calculated concentrations and assay results are summarized in Table 6.

Table 6 Assay of Itraconazole in Capsule Formulation

S. No.	Absorbance	Concentration ($\mu\text{g/mL}$)	Amount found (mg)	Assay (%)
1	0.303	0.5892	4.910	98.20
2	0.304	0.5912	4.927	98.54
3	0.304	0.5912	4.927	98.54
4	0.304	0.5912	4.927	98.54
5	0.305	0.5932	4.943	98.86
6	0.305	0.5932	4.943	98.86
Mean	—	—	4.928	98.59
SD	—	—	—	0.25
% RSD	—	—	—	0.25

The absorbance values obtained for the capsule sample ranged from 0.303 to 0.305, corresponding to calculated concentrations of 0.5892–0.5932 $\mu\text{g/mL}$. After applying the dilution factor used during sample preparation, the corresponding amounts of itraconazole were calculated to be approximately 4.910–4.943 mg per capsule. Considering the labeled claim of 5 mg per capsule, the individual assay values ranged from 98.20% to 98.86%, with a mean assay of 98.59% and a %RSD of approximately 0.25%.

The low %RSD indicates good repeatability of the assay procedure, while the assay values demonstrate satisfactory agreement between the determined amount and the labeled claim of itraconazole. The results therefore indicate that the proposed UV spectrophotometric method is suitable for the quantitative determination of itraconazole in the investigated 5 mg capsule formulation.

VI. CONCLUSION

A simple and reliable UV–Visible spectrophotometric method was successfully developed for the quantitative

determination of itraconazole in bulk drug and capsule dosage form. Methanol was found to be suitable as the solvent, and an analytical wavelength of 264 nm was selected for the measurement of itraconazole. The method exhibited a good linear response over the concentration range of 0.2–1.0 $\mu\text{g/mL}$, with a regression equation of $y = 0.5010x + 0.0078$ and a coefficient of determination ($R^2 = 0.9998$), indicating a strong concentration–absorbance relationship.

The method produced satisfactory analytical performance, including minimal interference from the formulation matrix, acceptable recovery results ranging from 99.52% to 100.04%, and good repeatability. The assay results obtained for the investigated capsule formulation were also within the expected range, supporting the applicability of the proposed procedure for routine estimation of itraconazole under the experimental conditions used in this study.

The calculated LOD and LOQ were 0.0182 and 0.0550 $\mu\text{g/mL}$, respectively, based on the calibration data. Although

the calculated LOQ was lower than the lowest concentration used for calibration, the quantitative working range of the method was established as 0.2–1.0 µg/mL. Therefore, concentrations below 0.2 µg/mL should not be considered part of the validated quantitative range unless additional experimental verification is performed around the calculated LOQ.

Overall, the developed method provides a straightforward spectrophotometric approach for itraconazole estimation in the studied bulk drug and capsule formulation. The method may be considered suitable for the intended analytical application within the validated concentration range and under the experimental conditions described in the present study.

ACKNOWLEDGMENT

The principal and staff of JNTUH University College of Pharmaceutical Sciences, Sultanpur, are greatly appreciated by the authors for providing institutional support and laboratory facilities for this work. Additionally, the authors thank Dr. Srinivas Chinta of the Department of Pharmaceutical Analysis for his academic support, supervision, and direction throughout the study.

REFERENCES

- [1]. Rao M, Trinidad R, Ratna J, Vijaya R, Rao Y, Srinivas. Development and validation of RP-HPLC method for the determination of itraconazole in bulk and capsule dosage form. *International Journal of Pharmaceutical Sciences*. 2015;31(2):221–225.
- [2]. ChemicalBook. Itraconazole, CAS database record, CAS No. 8465-61-6.
- [3]. National Center for Biotechnology Information. Itraconazole. PubChem Compound Summary, CID 3793.
- [4]. Fischer J, Ganellin CR. *Analogue-Based Drug Discovery*. John Wiley & Sons; 2006. p. 503.
- [5]. World Health Organization. WHO Model Lists of Essential Medicines: 21st List. 2019.
- [6]. Denyer SP, Hodges N, Gorman SP, Gilmore BF. Hugo and Russell's *Pharmaceutical Microbiology*. 8th ed. Wiley-Blackwell; 2011. p. 191–193.
- [7]. O'Neil MJ. *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals*. Whitehouse Station, NJ: Merck & Co.; 2006. p. 907.
- [8]. American Society of Health-System Pharmacists. Itraconazole. AHFS Drug Information. Accessed 8 December 2017.
- [9]. Gilbert DN, Moellering RC, Eliopoulos GM, Sande MA. *The Sanford Guide to Antimicrobial Therapy*. 2006.
- [10]. Rao M, Kotesware R KV, Reshana SR, Rao Narasimha D, Sujana DL. Method development and validation of itraconazole by UV spectrophotometer. *World Journal of Pharmaceutical Research*. 2014;3(10):777–787.
- [11]. Sowjanya G, Sindhuri V. UV spectrophotometric method development and validation for determination of an antifungal in bulk capsule. *Journal of Pharmaceutical Quality Assurance and Quality Control*. 2019;1(1):11–17.
- [12]. Tejaswani E, Puranik SB. Development and validation of itraconazole by instrumental methods in bulk and marketed formulation. *Universal Journal of Pharmacy*. 2018;7(4):7–20.
- [13]. Salunke P, Ingale K, Gondhankheda P, Ghatge K, Patil S. Visible spectrophotometry method development and validation of itraconazole in bulk and capsule formulation. *Analytical International Journal in Pharmaceutical Analysis*. 2017;9(1):45435–45439.
- [14]. Parikh SK, Patel AD, Dave JB, Patel CN, Sen DJ. Development and validation of the UV spectrophotometric method for estimation of itraconazole bulk drug and pharmaceutical formulation. *International Journal of Drug Development & Research*. 2011;3(2):324–328.