ISSN Print: 2664-7591
ISSN Online: 2664-7605
Impact Factor: RJIF 8-16
IJPCR 2025; 7(2): 123-128
www.pharmaceuticaljournal.in
Received: 19-06-2025
Accepted: 21-07-2025

M. Swapna
Viswanadha Institute of Pharmaceutical Sciences, Visakhapatnam, Andhra Pradesh, India

Dr. P.V. Madhavi Latha
Viswanadha Institute of Pharmaceutical Sciences, Visakhapatnam, Andhra Pradesh, India

Rama Madhuri
Viswanadha Institute of Pharmaceutical Sciences, Visakhapatnam, Andhra Pradesh, India

Corresponding Author:
M. Swapna
Viswanadha Institute of Pharmaceutical Sciences, Visakhapatnam, Andhra Pradesh, India

UV spectrophotometric quantification of itraconazole using mixed hydrotropic solubulization

M. Swapna, P.V. Madhavi Latha and Rama Madhuri

DOI: https://doi.org/10.33545/26647591.2025.v7.i2b.148

Abstract
A simple, accurate, novel, safe, and precise method was developed for the quantification of Itraconazole (ITZ), a poorly water-soluble drug, in bulk and tablet dosage forms using various hydrotropic reagents. Among the hydrotropic agents tested, a combination of PABA (para-amino benzoic acid) and citric acid in a 1:1 ratio showed the maximum absorbance. Sodium acetate, however, did not exhibit any absorbance above 240 nm, which indicated no interference in the estimation of the drug. ITZ followed Beer's law within the concentration range of 15-75 µg/ml ($r^2 = 0.999$). The standard deviation, coefficient of variation, and standard error for ITZ were found to be satisfactorily low. The developed method was validated according to ICH guidelines, and the values of accuracy, precision, and other statistical analyses were found to be in good accordance with the prescribed values. Therefore, this method can be used for routine monitoring of ITZ in the pharmaceutical industry for the assay of bulk drug and tablets.

Keywords: Itraconazole, statistical validation, PABA, citric acid, solubility enhancement

Introduction
Itraconazole is a broad-spectrum antifungal agent chemically represented as 1-[(2R, 4S)-2-(2,4-dichlorophenyl)-4-(4-fluorophenyl)-1H-1,2,3-triazol-1-yl]-2-(2,4-dichlorophenyl)-1-butanol. It is a triazole derivative used in the treatment of various fungal infections, including systemic and superficial infections. Itraconazole exhibits its antifungal activity by inhibiting the synthesis of ergosterol, a key component of fungal cell membranes. This mechanism of action disrupts the structural integrity of the fungal membrane, leading to cell death. Itraconazole is typically available in oral capsule and suspension forms, but it is known to have limited aqueous solubility, which may affect its bioavailability and therapeutic efficacy. To overcome this limitation, various techniques have been explored to enhance the solubility of itraconazole, one of the most effective of which is hydrotropy. Hydrotropic solubilization involves the use of a mixture of water-soluble compounds to increase the solubility of poorly water-soluble drugs, without the need for organic solvents. Recent studies have shown that hydrotropic agents such as sodium acetate, sodium benzoate, and urea, PABA, Citric acid when used in combination, can significantly improve the aqueous solubility of itraconazole, enabling more efficient drug extraction and analysis. These hydrotropic solutions offer a promising alternative to organic solvents, which are often expensive, toxic, and environmentally harmful. The use of mixed hydrotropic solutions for the solubilization of itraconazole is an emerging area of interest, as it offers a safer and more cost-effective approach for quantitative estimations of the drug, especially in pharmaceutical formulations like tablets and capsules. UV spectroscopic methods, in particular, can be employed to quantitatively estimate itraconazole in such formulations by exploiting the enhanced solubility afforded by hydrotropic solutions. To date, there is a growing body of literature suggesting the potential for mixed hydrotropy to facilitate the accurate and efficient estimation of itraconazole in both its pure and combined dosage forms. This study focuses on the quantitative estimation of itraconazole in pharmaceutical formulations using UV spectrophotometric methods, with the aid of mixed hydrotropic solutions to enhance drug solubility and facilitate the accurate analysis of its concentration. The proposed approach represents a significant advancement in the analysis of itraconazole, especially in formulations where traditional solubilization methods are less effective.

Experimental work

Methanol and HCl: (Preparation of solutions)

Preparation of stock solution: 25mg of Itraconazole was taken in a 25ml volumetric flask, dissolved in 3ml of Methanol and made upto 25ml with0.1N HCl. (1mg/mlor 1000 µg/ml) Preparation of 0.1N HCl solution: 4.25 ml was taken in a 500ml volumetric flask, and then made up to 500ml with distilled water.

Method development determination of λmax of itraconazole

Preparation of solution: 1ml of standard drug solution (1µg/ml) was taken in a 10 ml volumetric flask and make up to 10ml with 0.1N HCl solution.

Linearity data

Table 1: Linearity data of itraconazole

Concentration	Absorbance
15 µg/ml	0.145
30 µg/ml	0.285
45 µg/ml	0.427
60 µg/ml	0.567
75 µg/ml	0.701

Calibration curve of itraconazole using methanol

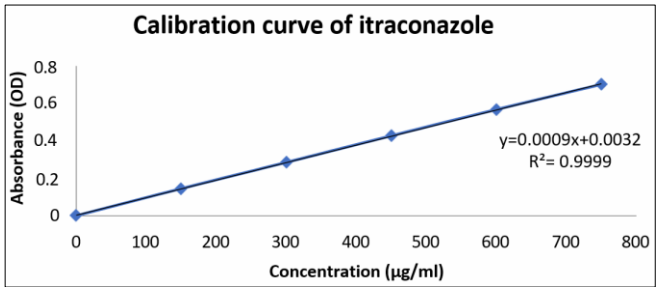

Fig 1: Calibration curve of itraconazole

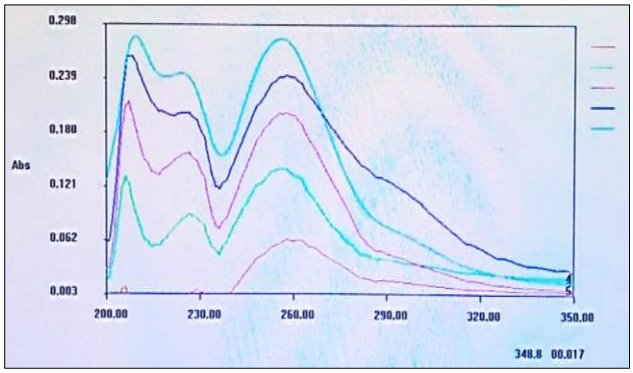

Fig 2: Overlay spectrum of itraconazole

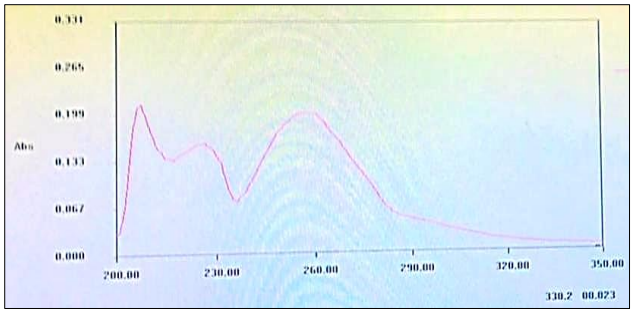

Fig 3: λ_{max} of itraconazole

Single hydrotropes used for itraconazole solubility enhancement

Table 2: Single hydrotropes used for itraconazole solubility enhancement

Reagents	Quantities (1:1)
PABA	25mg
Citric acid	25mg
Urea	25mg
Sodium acetate	25mg

1. Drug + Urea (1:1)

Drug: Itraconazole
Hydrtrope: Urea
Concentration: 90-450µg/ml
R^2 value: 0.998
λ_{max}: 260nm

Linearity data of ITZ+urea

Table 3: Linearity data of Urea+ITZ

Concentration (µg/ml)	Absorbance (OD)
0 µg/ml	0
90 µg/ml	0.165
180 µg/ml	0.323
270 µg/ml	0.492
360 µg/ml	0.662
450 µg/ml	0.822

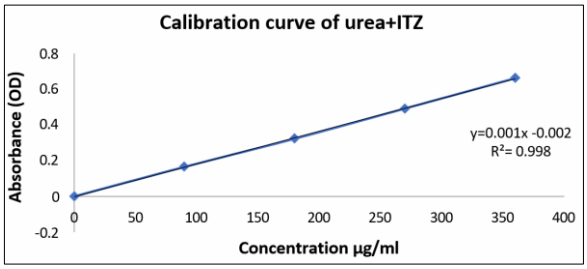

Fig 4: Calibration curve of urea + ITZ

3. Drug + PABA: (1:1)

Hydrotrope: PABA
Concentration: 15-75µg/ml
R^2 value: 0.998
Λ_{max}: 245nm

Linearity data of ITZ+PABA

Concentration (µg/ml)	Absorbance (OD)
0 µg/ml	0
15 µg/ml	0.134
30 µg/ml	0.270
45 µg/ml	0.394
60 µg/ml	0.530
75 µg/ml	0.636

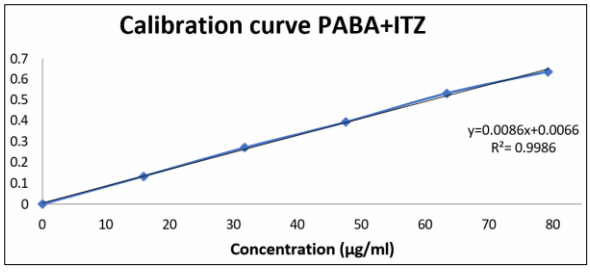

Fig 5: Calibration curve of ITZ +PABA

4. ITZ + Sodium acetate
Drug: Itraconazole
Hydrotrope: Sodium acetate
Concentration: 150-750µg/ml R^2
Value: 0.9993
Λ_{max}: 240nm

Linearity data of ITZ + sodium acetate

Table 5: Linearity data of ITZ + sodium acetate

Concentration (µg/ml)	Absorbance
0 µg/ml	0
150 µg/ml	0.028
300 µg/ml	0.049
450 µg/ml	0.065
600 µg/ml	0.080
750 µg/ml	0.100

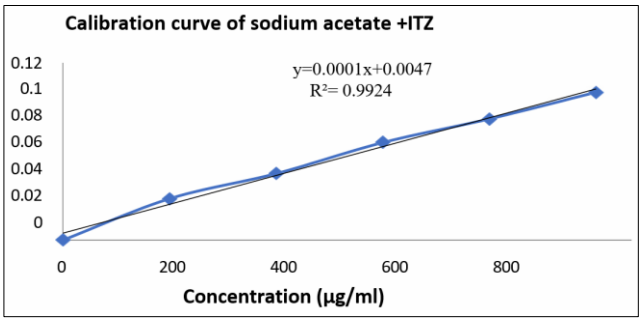

Fig 6: Calibration curve of drug + sodium acetate

UV method development (mixed hydrotropy agents)

1. ITZ + PABA + citric acid: (1:1:1)
Drug: Itraconazole
Hydrotropes: PABA + citric acid
Concentration: 15-75µg/ml
R^2value: 0.999
Λ_{max}: 245nm

Linearity data of ITZ + PABA + citric acid

Table 6: Linearity data of PABA + citric acid

Concentration (µg/ml)	Absorbance (OD)
15 µg/ml	0.174
30 µg/ml	0.350
45 µg/ml	0.519
60 µg/ml	0.690
75 µg/ml	0.869

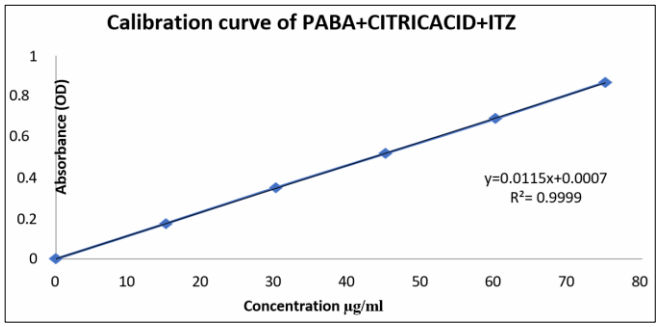

Fig 7: Calibration curve of ITZ + PABA + citric acid

2. ITZ + sodium acetate + PABA
Drug: Itraconazole
Hydrotropes: Sodium acetate, PABA (1:1:1)
Concentration: 70 -350µg/ml
R^2value: 0.995
Λ_{max}: 241nm

Table 7: Linearity data of sodium acetate + PABA

Concentration (µg/ml)	Absorbance (OD)
0 µg/ml	0
70 µg/ml	0.124
140 µg/ml	0.249
210 µg/ml	0.370
280 µg/ml	0.420
350 µg/ml	0.616

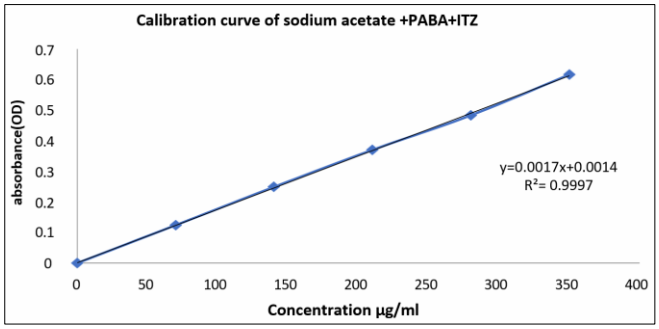

Fig 8: Calibration curve of ITZ + PABA + sodium acetate

3. ITZ + PABA + Urea: (1:1:1)
Drug: Itraconazole
Hydrotropes: PABA, Urea
Concentration: 30-150µg/ml
R^2value: 0.999
Λ_{max}: 241nm

Table 8: Linearity data of Urea + PABA

Concentration (µg/ml)	Absorbance (OD)
0 µg/ml	0
30 µg/ml	0.416
60 µg/ml	0.290
90 µg/ml	0.431
120 µg/ml	0.570
150 µg/ml	0.712

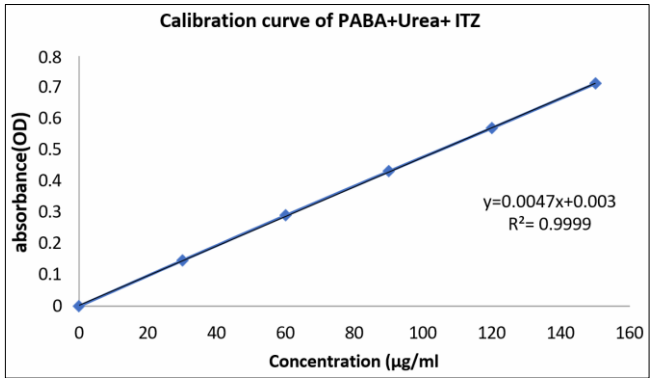

Fig 9: Calibration curve of ITZ + PABA + Urea

4. ITZ + sodium acetate + citric acid (1:1)
Drug: Itraconazole
Hydrotropes: Sodium acetate, citric acid
Concentration: 20-100µg/m
R^2 value: 0.999
Λ_{max}: 242nm

Table 9: Linearity data of sodium acetate + citric acid

Concentration (µg/ml)	Absorbance (OD)
0 µg/ml	0
20 µg/ml	0.108
40 µg/ml	0.210
60 µg/ml	0.312
80 µg/ml	0.416
100 µg/ml	0.531

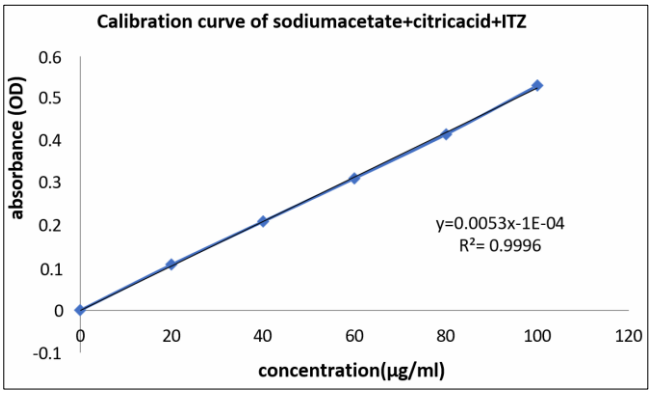

Fig 10: Calibration curve of drug + citric acid + sodium acetate

Linearity
Preparation of linearity solutions

15µg/ml solution: 1.5ml of standard solution was taken in a10mlvolumetric flask; make up to10ml with 0.1N HCl solution.
30µg/ml solution: 3ml of standard solution was taken in a10 ml taken in volumetric flask; make up to 10ml with 0.1N HCl solution.
45µg/ml solution: 4.5ml of standard solution was taken in volumetric flask; make up to 10ml with 0.1N HCl solution.
60µg/ml solution: 6ml of standard solution was taken in volumetric flask; make up to 10ml with 0.1N HCl solution.
75µg/ml solution: 7.5 ml of standard solution was taken, in volumetric flask; make up to 10ml with 0.1N HCl solution.
Criteria: R2 should not be more than 0.999 Precision
Preparation of 45µg/ml solution: 1ml of standard drug solution (1µg/ml) was taken in a 10 ml volumetric flask and make up to 10ml with 0.1N HCl solution.
Intraday precision: 6 determinations of 45 mcg/ml solution were done 3 times in a day and observed for absorbance.
Interday precision: 6 determinations of 45mcg/ml solution were done 3 consecutive days and observed for absorbance

Criteria: % RSD should be less than 2

Robustness: 6 determination sof45mcg/ml solution were done and observed for absorbance at different wave length (nm) with respect to optimized wave length
Criteria: % RSD should be less than LOD
LOQ: Limit of detection: 3.3×σ/S
Quantification: 10×σ/S
Where,
- Σ is standard deviation
- S is slope

Assay: Assay was performed on marketed formulation and % assay was calculated.

$$\%\ \text{Assay} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times \frac{\text{Standard dilution}}{\text{Test dilution}} \times \frac{\text{Average weight}}{\text{Label claim}} \times 100$$

Accuracy: Accuracy was performed a t3different levels i.e.80%, 100%and 120%.

$$\%\ \text{Recovery} = \text{Amount Found} \times 100\ \text{Amount Taken}$$

The % recovery should be between 98% to 102 percent

Results and Discussion

Optimization of tablet + PABA + citric acid
- **Concentration:** 15-75µg/ml
- **Wave length:** 240nm
- **R^2value:** 0.998
- **Hydrotropy reagent:** PABA + citric acid (1:1)

Table 10: Linearity data of PABA + citric acid

Concentration (µg/ml)	Absorbance (OD)
15µg/ml	0.141
30µg/ml	0.291
45µg/ml	0.427
60µg/ml	0.572
75µg/ml	0.701

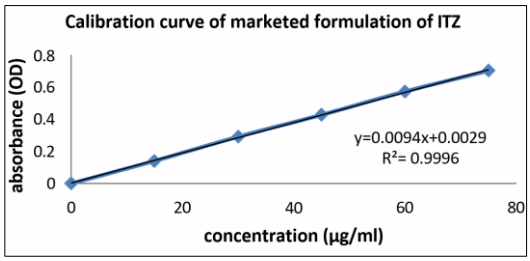

Fig 11: Calibration curve of PABA and citric acid

Optimization of ITZ using PABA and citric acid (1:1:1)
- **Concentration:** 15µg/ml to 75µg/ml
- **Wave length:** 245nm
- **R^2value:** 0.998
- **Hydrotropy reagent:** PABA + citric acid (1:1)

Table 11: Linearity data of PABA and citric acid

Concentration (µg/ml)	Absorbance of PABA + citric acid
15µg/ml	0.174
30µg/ml	0.35
45µg/ml	0.519
60µg/ml	0.69
75µg/ml	0.869

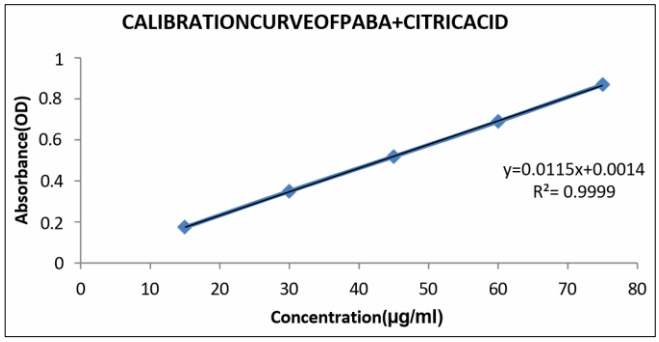

Fig 12: Calibration curve of PABA and citric acid

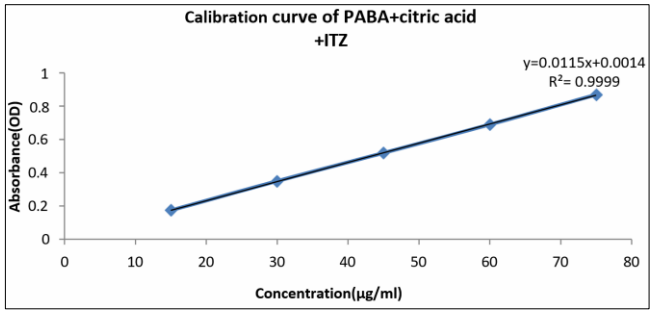

Fig 13: Calibration curve of itraconazole and PABA

Method validation

1. Linearty

Table 12: Linearity data of PABA and citric acid

Concentration (µg/ml)	Absorbance (OD)
15µg/ml	0.174
30µg/ml	0.350
45µg/ml	0.519
60µg/ml	0.690
75µg/ml	0.869

Result

The linearity range was 15-75µg/ml.

The Regression equation (y=mx+ c) obtained was Y=0.011+0.001

Correlation coefficient (R^2) value obtained was 0.999

2. Accuracy

Accuracy was performed at 3 levels i.e. 80%, 100% and 120% Amount Taken = 103.23 X 100

=98.23

=99.82%

Table 13: Accuracy data of itraconazole

Sample	Amount of sample taken(µg/ml)	Spiked level	Amount of pure drug added(µg/ml)	Amount recovered	% Recovery	Mean
Itraconazole	45µg/ml	80%	5	49.709	99.42%	99.61%
		100%	10	5454.8	99.6%	
		120%	15	59.8	99.89999.4%	

Acceptance criteria: Should be between 99% to 101%

Result: The % recovery was found to be 99.82%

3. Precision

Intraday precession

Table 14: Intraday precision data of itraconazole

Concentration	Morning	Afternoon	Evening
45µg/ml	0.519	0.520	0.521
	0.523	0.524	0.525
	0.528	0.529	0.530
	0.533	0.534	0.535
	0.538	0.539	0.540
	0.543	0.545	0.546
	SD	0.000443	
Statistical analysis	%RSD	1.63%	

Interday precesion

Table 15: Interday precision data of itraconazole

Concentration (µg/ml)	Day-1	day-2	day-3
450µg/ml	0.519	0.519	0.521
	0.523	0.524	0.525
	0.528	0.528	0.533
	0.533	0.533	0.538
	0.538	0.539	0.549
	0.549	0.549	0.556
	SD	0.000368	
Statistical analysis	%RSD	1.69%	

Intraday precision: 6 determination of 45µg/ml solution were done 3 times in a day and observed for absorbances.

Interday precision: 6 determinations of 45µg/ml solution were done for 3 consecutive days and observed for absorbances.

Table 16: Precision data of itraconazole

Parameters	PABA + citric acid + itraconazole
Intraday (% RSD)	1.63%
Interday (% RSD)	1.69%

Acceptance criteria: should not be more than 2%

Result

% RSD for intraday precision was found to be 1.63%

% RSD for interday precision was found to be 1.69%

4. Robustness

6 determinations of 45µg/ml solution were done and observed for absorbance at different wavelengths (240nm, 245nm and 260) with respect to optimized wave length (245nm).

Table 17: Robustness data of itraconazole

Concentration (µg/ml)	240nm	245nm	260nm
45µg/ml	0.514	1.798	0.516
	0.521	1.795	0.522
	0.525	1.798	0.527
	0.530	1.795	0.532
	0.532	0.539	0.537
	0.541	0.543	0.542
% RSD	1.59%	1.63%	1.60%

Acceptance criteria: Should not be more than 2%

Result

% RSD for wave length of 240nm was found to be 1.59%
% RSD for wave length of 245nm was found to be 1.63%
% RSD for wave length of 260nm was found to be 1.60%

5. Ruggedness

6 determinations of 45µg/ml solution was done by two different and observed for absorbances.

Table 18: Ruggedness data of itraconazole

Sample	Concentration	Analyst-1	Analyst-2
PABA + citric acid + ITZ	45µg/ml	0.519	0.523
		0.521	0.529
		0.524	0.526
		0.533	0.534
		0.535	0.536
		0.540	0.542
Statistical analysis	SD	0.002638	0.002645
	%RSD	1.64%	1.68%

Acceptance criteria: should not be less than 2%

Result

% RSD for analyst-1 was found to be 1.64%
% RSD for analyst-2 was found to be 1.68%

Table 19: LOD and LOQ data of itraconazole

S. No.	Parameters	Results
1.	Limit of detection (µg/ml)	4.14µg/ml
2.	Limit of quantification (µg/ml)	11.217µg/ml

Summary and conclusion

Table 20: Summary and conclusion

Parameters	ITZ
Wave length (nm)	245 nm
Linearity range (µg/ml)	15-75µg/ml
Regression equation	Y=0.0115x+0.0014
Slope(m)	0.8206
Intercept(c)	
Correlation coefficient	0.999
Precision(%RSD)	1.61%
Interday	1.63%
Intraday	1.69%
Robustness (% RSD)	1.62%
Wave length 240nm	1.59%
Wave length 260nm	1.60%
Wave length 245nmn	1.63%
Ruggeddness (% RSD) Analyst-1	1.65% 1.64%
Analyst-2	1.68%
Limit of detection (µg/ml)	4.14µg/ml
Limit of quantification	11.217µg/ml
Assay (%)	99.01%
Accuracy (% Recovery)	99.6%

Conclusion

The UV spectrophotometric method for the quantification of Itraconazole using mixed hydrotropic solubilization proved to be are liable and efficient analytical technique. The use of mixed hydrotropic agents facilitated the enhancement of Itraconazole solubility, enabling accurate measurement at its characteristic absorbance wavelength. The method exhibited good linearity, precision, and accuracy, making it suitable for routine quality control applications in pharmaceutical formulations. Furthermore, the method is cost-effective, rapid, and does not require the use of organic solvents, aligning with environmentally sustainable practices. Overall, this approach offers a promising alternative for the determination of Itraconazole in various pharmaceutical dosage forms.

References

1. Chatwal G. A textbook of pharmaceutical analysis. 4th ed. p. 2-13.
2. Ravi Shankar S. A textbook of pharmaceutical analysis. 5th ed. p. 2.149-2.184.
3. Ravi Shankar S. A textbook of pharmaceutical analysis. 4th ed. p. 1-1, 1-4, 1-5.
4. Skoog DA, Holler FJ, Crouch SR. Instrumental analysis. p. 13-14.
5. Chatwal GR, Anand SK. Instrumental methods of analysis. 5th ed. Mumbai: Himalaya Publishing House; p. 2.107-2.112.
6. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental methods of analysis. 7th ed. p. 118-154.
7. Khar A. Pharmaceutical drug analysis. 2nd rev. ed. New Delhi: New Age International Publishers; p. 303-304.
8. Maheshwari RK, Jagwani Y. Mixed hydrotropy: Novel science of solubility enhancement. Indian J Pharm Sci. 2011;73(2):179.
9. Kumar VS, Raja C, Jayakumar C. A review on solubility enhancement using hydrotropic phenomena. Int J Pharm Pharm Sci. 2014;6(6):1-7.
10. Namdev B, Venkatachalam S, Jawahar N, Chorsiya A. A brief review on solubility enhancement technique: hydrotropy. Indian J Pharm Educ Res. 2022;56(2):347-355.
11. Padiyar A, Agrawal OP, Rajpoot K, Tekade RK. Hydrotropy, mixed hydrotropy, and mixed solvency as trending concept for solubilization of lipophilic drugs. In: The Future of Pharmaceutical Product Development and Research. London: Academic Press; c2020, p. 145-178.
12. Karnakar N, Ramana H, Amani P, Sri Tharun D, Nagaraju M, Sharma SB. Int J Multidiscip Res Dev. 2020;7:23-24.
13. Ravisankar P, Shaheem Sulthana MD, Babu PS, Afzal Basha SK, Aswini R, Swathi V, *et al*. Comprehensive review of important analytical reagents used in spectrophotometry. Indo Am J Pharm Res. 2017;7(5):8716-8774.
14. Jain R, Jain N, Jain DK, Patel VK, Rajak H, Jain SK. Novel UV spectrophotometer methods for quantitative estimation of metronidazole and furazolidone using mixed hydrotropy solubilization. Arab J Chem. 2017;10(2):151-156.
15. Jain N, Jain R, Thakur N, Gupta BP, Banweer J, Jain S. Novel spectrophotometric quantitative estimation of torsemide in tablets using mixed hydrotropic agent. Der Pharm Lett. 2010;2(3):249-254.
16. Tripathi R, Biradar SV, Mishra B, Paradkar AR. Study of polymorphs of progesterone by novel melt sonocrystallization technique: a technical note. AAPS PharmSciTech. 2010;11:1493-1498.