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DEVELOPMENT AND VALIDATION OF UV- SPECTROPHOTOMETRIC METHOD FOR QUANTITATION OF ITRACONAZOLE FROM ITS CAPSULE DOSAGE FORM

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ABSTRACT

Itraconazole is a synthetic triazole antifungal agent. Itraconazole is formulated into several pharmaceutical forms available into market. Itraconazole capsules are used to treat fungal infection in the lungs that can spread throughout the body. Itraconazole is partially water soluble therefore methods available for assay of Itraconazole in pharmaceutical formulation use organic solvents thus available methods are not cost effective and difficult to routine use. In present work attempt has made to develop a more precise, simple and economical spectrophotometric method with combination of water and methanol as solvent with greater precision, accuracy and suitability for the quantitation of Itraconazole in capsule dosage form. UV spectroscopic determination was carried out at absorption maxima of 254 nm using Methanol: Water (40:60 % v/v) as solvent. In present UV spectroscopic method linearity over the concentration range of Itraconazole was found to be in between 50-150 µg/ml with a correlation coefficient 0.996. Results of the analyses where validated statistically and by recovery studies. The method was validated for the parameters like linearity, range, precision, accuracy, LOD and LOQ as per ICH Q2R1 guidelines.

***Keyword**: Itraconazole, assay, UV spectrophotometric method, ICH Q2R1.*

1. INTRODUCTION

Itraconazole is a synthetic triazole antifungal agent. Itraconazole is a 1:1:1:1 racemic mixture of four diastereomers (tow enantiomeric pairs), each possessing three chiral centers. It may be represented by the following nomenclature 4-4-4-4-2-(2, 4-dichlorophenyl)- 2-(1H-1,2,4-triazole 1-ylmethyl)-1,3- dioxolan-4- yl methoxylphenyl piperazin- 1-ylphenyl-2-(1-methylpropyl)-2, 4-dihydro-1, 2, 4-triazole-3 – one. It has a molecular formula $C_{35}H_{38}C_{12}N_8O_4$ and a molecular weight is 705.64[1-4]. It is a white to slightly yellowish powder. Chemical structure of Itraconazole is shown in **Fig. 1**.

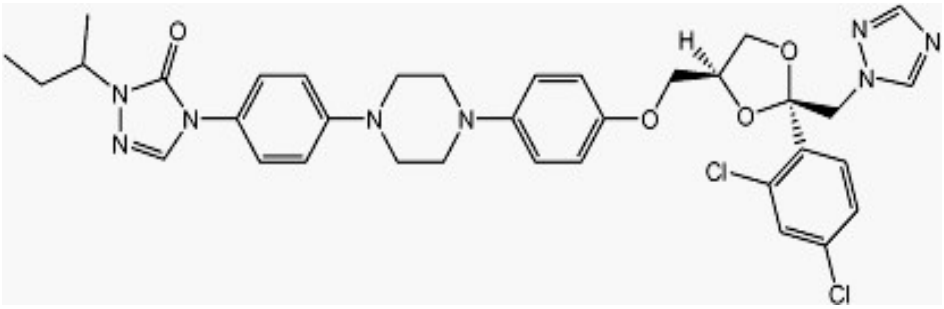

Fig. 1: Chemical structure of Itraconazole

It is very slightly soluble in alcohol, and freely soluble in dichloromethane. Itraconazole is lipophilic in nature and practically poorly soluble in water. It is an extremely weak base (pKa = 3.7) that is ionized only at very low pH. It is a hydrophobic anti mycotic drug with three chiral centers and is used clinically as a stereo isomeric mixture [5]. It is an orally active triazole antifungal agent, which demonstrates broad spectrum activity against a number of fungal species [6, 7, 8, 9, 10].

Itraconazole was formulated in to several pharmaceutical forms through various routes of administration. Itraconazole capsules are used to treat fungal infections in the lungs that can spread throughout the body [11.] For quality control and stability testing of Itraconazole in pharmaceutical formulations, limited methods have been published, because the drug is not yet official in any pharmacopoeia. Several HPLC [12], and LC/MS-MS [13-15] methods have been reported for the analysis of Itraconazole in plasma that suffers from either undesirably long chromatographic run times and requirement for gradient analysis or use of an internal standard one spectrophotometric method [16] have also been reported spectrophotometric method has been used for assay of Itraconazole in raw material and in dosage form. RP-HPLC method is used for determination of Itraconazole in human plasma. [17-21] Chromatographic separation in this method was performed on an octadecylsilane column using fluorescence detector. However, it has the disadvantage of being time consuming. All this study has further emphasized the need to perform rapid and sensitive quality control analysis of pharmaceutical formulations containing Itraconazole.

2. MATERIALS AND METHODS

2.1 Materials

Chemicals and reagents of spectroscopic grade were used. Itraconazole was obtained as gift sample from USV Limited, Govandi, Mumbai, India. Method was developed and validated using double beam UV- visible spectrophotometer (Shimadzu, Model No. 1800) having tow matched quartz cells with 1 cm light path.

2.2 Selection of common solvent:

Methanol and water was selected as a common solvent for developing spectral characteristics of drug. The ration of these solvents was selected after examining the different ratio of the methanol and water.

2.3 Preparation of standard stock solution:

Accurately weighted quantity of 50 mg Itraconazole reference standard was transferred into 100 ml volumetric flask, dissolved and diluted up to the mark with final solvent to obtain a stock solution of 500 µg/ml strength.

2.4 Selection of method wavelength:

The wavelength was selected for estimation of Itraconazole from recording spectrum of reference standard solution in between 200-400 nm. The λ max was found to be 254 nm.

2.5 Preparation of calibration curve

50-150 µg/ml standard solutions were prepared by diluting reference standard stock solution. The absorbance of these solutions was recorded and calibration curve was plotted as concentration Vs absorbance. The regression equation was obtained from calibration curve.

2.6 Preparation of sample stock solution

For analysis of drug in capsule dosage form, 20 capsules were weighed accurately and the powder triturated in mortar to get a fine powder. The capsule powder equivalent to 50 mg was weighed and transferred to 100 ml volumetric flask and dissolved with selected solvent. The capsule solution was diluted to get a final concentration of 100µg/ml. The absorbance of these solutions measured. The amount of Itraconazole per capsule was calculated using the calibration curve.

2.7 Method validation

The method was validated according to International conference of Harmonization (ICH) Q2B, (ICH) Q2R1 guidelines for validation of analytical procedure in order to determine linearity, range, accuracy, precision, LOD, LOQ, ruggedness and robustness.

2.7.1 Linearity and Range

For study of linearity and range the different concentrations of Itraconazole were prepared. The calibration graph of the absorbance versus concentration was plotted to obtained linearity and range of method.

2.7.2 Accuracy

To determine the accuracy of proposed method, recovery studies were carried out at three different levels (50%, 100%, 150%) of assay concentration. The percentage recovery values are calculated. Test was prepared in triplets at each spike level and recovery was done as per assay method. Percent recovery values were calculated.

2.7.3 Precision

The precision is determined by two methods as Intra-day precision and Inter-day precision by preparing three concentrations of the Itraconazole.

Intra-day precision

The Intra-day precision was determined by analyzing Itraconazole at three different time points of the same day. The absorbance, standard deviation, and % RSD were calculated.

Inter-day precision

The Inter-day precision was determined by analyzing Itraconazole at three different time points on different days. The absorbance, standard deviation, and % RSD were calculated.

2.7.4 Limit of detection and limit of quantitation

The LOD and LOQ were calculated by using the relation 3.3 σ/S and 10 σ/S respectively, where σ is the standard error and S is the slope.

2.7.5 Ruggedness

The ruggedness of the proposed method was evaluated by using the same instrument by two different analysts under the same optimized conditions at different days.

2.7.6 Robustness

The robustness of method was determined by introducing small change in UV parameters, such as changing the wavelength +4 and -4.

2.8 Analysis of Capsule formulation

The developed and validated spectrophotometric method was applied for assay of Itraconazole from marketed capsule formulation. Assay was to performed on 20 capsules, taking average weight of capsules equivalent to weight 50 mg was dissolved to 100 ml to

obtain stock of 500 µg/ml. dilutions to were made to obtain 6 sets of 100 µg/ml as assay concentration. Quantitation was done by using regression data.

3. RESULTS AND DISCUSSION

Hand The goal of the study was to develop and validate UV- Spectrophotometric method for quantitation of Itraconazole in capsule dosage form. Method was carried out to optimize the spectrophotometric conditions and validated according to ICH Q2R1 guidelines. Result of the validation parameters were within acceptable limits.

3.1. Selection of common Solvents

Solubility of Itraconazole was studied. It is observed that it is partially soluble in the water and completely soluble in organic solvents like methanol, therefore it is decided to select methanol water as solvent for the experimental work. The selection was made after examining the different ratio of the methanol: water and finally the methanol: water (40:60 % v/v) was selected as solvent for method development.

3.2. Selection of method wavelength

After recording spectrum of reference standard solution 254 nm was selected as working wavelength. The absorption maxima of pure drug Itraconazole was found to be 0.253 at 254 nm in solvent mixture of methanol: water in the ratio 40: 60 % v/v. It is shown in **Fig. 2.**

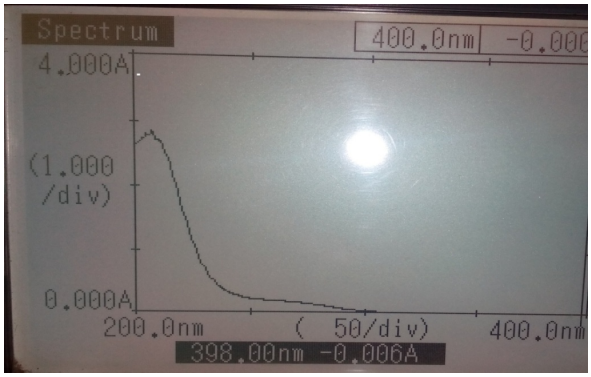

Fig. 2: Selection of method wavelength

3.3. Preparation of calibration curve

Calibration curve of Itraconazole was prepared and found to be linear in the range of 50-150 µg/ml in methanol: water (40: 60 % v/v) with correlation coefficient 0.996. Regression equation was found to be y = mx + c (m = 0.073, c = 0.1714) Concentration Vs absorbance data is given in **Table 1** and graphically presented in **Fig. 3**. Calibration curve statistics is given in **Table 2**.

Table 1: Result for calibration curve of Itraconazole

Concentration µg/ml	Absorbance
50	0.190
60	0.240
80	0.433
100	0.572
120	0.691
140	0.845
150	0.909

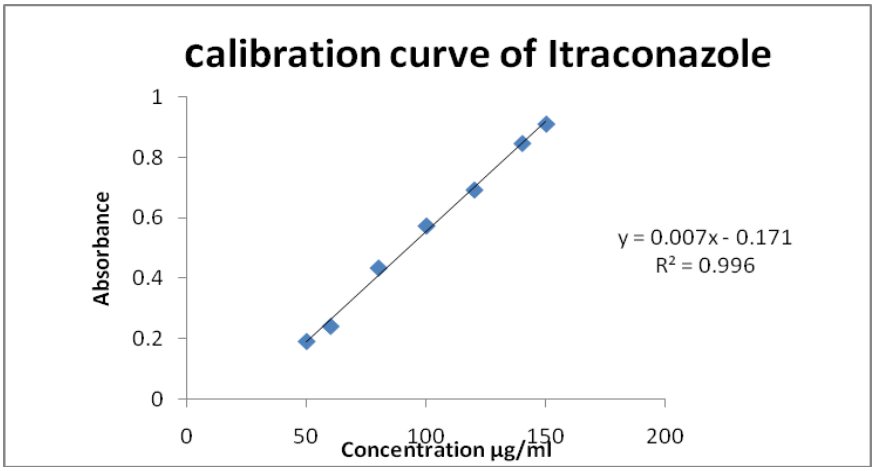

Fig. 3: Calibration curve of Itraconazole

Table 2: Calibration curve statistics

Parameter	Observation
λmax	254 nm
Slope	0.0073
Intercept	0.1714

3.4 Method validation

3.4.1 Linearity and range of Itraconazole

Linearity and range data for Itraconazole is shown in Table 1. From observed values, it was found that method is linear over the range of 50 – 150 µg/ml. The observed linearity range fitted well Beer-Lambert's law and corresponding regression coefficient (r = 0.996) is an indicating of a high degree of method accuracy and reproducibility. The statistical data in Table 2 shows good correlation between the method parameters. An overlain spectrum of Itraconazole is shown in **Fig. 3.**

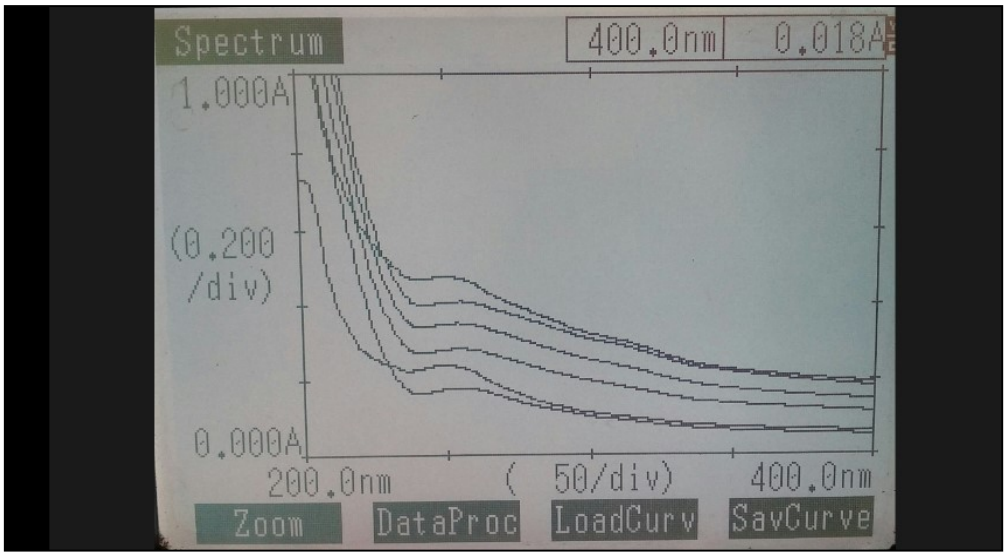

Fig. 3: Overlain Spectra of Itraconazole

3.4.2 Accuracy by recovery studies

Recovery studies were done for accuracy determination at the concentration levels of 50% 100% 150% of assay concentration with triplicate measurement of each to obtain 9 determinations. Percent purity was calculated for each recovery showed the result between 99 -101% of assay values. Thus, method is found to be accurate for quantitation. Results are shown in **Table 3**.

Table 3: Accuracy by recovery studies:

Sr. No.	Theoretical	Absorbance	Practical	% Purity (X)	Mean	S.D.	% RSD
1	50	0.190	49.71	99.42			
2	50	0.189	49.57	99.14	99.45	0.109	0.110
3	50	0.193	49.90	99.80			
4	100	0.540	99.71	99.71			
5	100	0.542	100	100	99.99	0.0813	0.081
6	100	0.544	100.28	100.28			
7	150	0.909	152.42	101.61			
8	150	0.915	153.28	102.18	101.93	0.080	0.082
9	150	0.913	153	102			

3.4.3 Precision

Results for method precision are shown in **Table 4 and 5**.

Table 4: Intra-day precision

Sr. No.	Theoretical	Absorbance	Practical	% Purity (X)	Mean	S.D.	% RSD
1	100	0.592	101.14	101.14	101.02	0.1313	0.122
2	100	0.595	101.58	101.58			
3	100	0.590	101.85	101.85			

Table 5: Inter-day precision

Sr. No.	Theoretical	Absorbance	Practical	% Purity (X)	Mean	S.D.	% RSD
1	100	0.584	100	100	100.42	0.1386	0.130
2	100	0.588	100.55	100.55			
3	100	0.589	100.71	100.71			

Validation of proposed method was verified for intra-day precision and inter-day precision, the % RSD was found to be 0.122 and 0.130 respectively.

3.4.4 Limit of detection and limit of quantitation

LOD and LOQ of method was found to be 3 µg/ml and 30µg/ ml respectively by using standard method

3.4.5 Ruggedness

The ruggedness study of method was performed by operating the developed method by two different analysts. Result of method ruggedness is shown in **Table 6**.

Table 6: Data for method ruggedness

Sr. No.	Analysts	Concentration (µg/ml)	Absorbance	Practical	% Purity	Mean	SD	% RSD
1	Analysts-1	100	0.546	101.42	101.42	101.28	0.2647	0.2587
		100	0.548	101.71	101.71			
		100	0.541	101.71	101.71			
2	Analysts-2	100	0.490	99.42	99.42	99.77	0.2317	0.2545
		100	0.493	99.85	99.85			
		100	0.499	100.04	100.04			

3.4.6 Robustness

The results obtained for robustness studies by deliberately changing parameters such as wavelength by + 4, -4 nm indicated that the developed method is robust for analysis. Results are shown in **Table 7**.

Table 7: Results of robustness

Sr. No.	Wavelength (nm)	Absorbance
1	254	0.5921
2	259	0.5972
3	250	0.5903

3.5 Analysis of Capsule formulation

The developed and validated spectrophotometric method was applied for assay of Itraconazole from marketed capsule formulation. Results recorded for 6 sets of assay concentration are shown in **Table 8**.

Table 8: Analysis of Capsule Formulation

Formulation	Labeled amount (mg)	Amount prepared (µg)	Absorbance	% Purity	Mean	SD	% RSD
Itrajohn	100	100	0.521	97	97.52	0.1841	0.1887
Itrajohn	100	100	0.522	97.14			
Itrajohn	100	100	0.527	97.85			
Itrajohn	100	100	0.525	97.57			
Itrajohn	100	100	0.524	97.42			
Itrajohn	100	100	0.529	98.14			

4. CONCLUSION

The aim of the present research work was to develop and validate UV spectrophotometric method for assay of Itraconazole from capsule dosage form by employing cost effective solvent system and to obtain method parameters within the acceptable limits. Method development has successfully executed the use of water methanol (60:40 % v/v) solvent system without affecting the solubility of Itraconazole. Marketed formulation of Itraconazole has high dose for that developed method is sensitivity enough. The developed

method was fully validated for method parameter such as linearity, range, accuracy, LOD and LOQ, ruggedness, robustness as per ICH Q2R1 guideline. Thus, developed method is economical and easy to handle as compare to available methods and the method is precise, accurate, rapid, rugged and robust as per guidelines. The developed method was successfully applied for the assay of Itraconazole from its capsule dosage form. Thus, it can be used for routine analysis in quality control.

5. ACKNOWLEDGEMENT

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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. Johnlee Pharmaceutical Product. Itrajohn (itraconazole).
4. Kramer M, Marshall S, Denning D et al. Cyclosporine and Itraconazole Interaction in Heart and Lung Transplant Recipient. 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,
10. 1994; 46: 147.
11. Haria M, Bryson H.M, Goa K.L Drugs, 1996; 51: 585-620.
12. Pharmaceutical applications available online at URL http://www.nlm.nih.gov.
13. 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.
14. 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.
15. 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.
16. 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.
17. 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.
18. 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.
19. 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.
20. 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.

21. 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.

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