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RESEARCH ARTICLE

UV-VISIBLE SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF ITRACONAZOLE IN BULK AND CAPSULE FORMULATION

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ABSTRACT

A simple, rapid, accurate and precise UV method was developed and validated for the estimation of Itraconazole in pharmaceutical dosage form. Spectroscopic method was carried out by using acidic ethanol as solvent. Itraconazole detection wavelength was set at 262nm for validation purpose linearity, accuracy, repeatability, precision, LOD, LOQ, and ruggedness parameters were studied. The linearity was found to be in the range of 2-12 µg/ml.

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INTRODUCTION

UV spectroscopy involves the promotion of electrons from the ground state to higher energy state. It is very useful to measure the number of conjugated double bonds and also aromatic conjugation within the various molecules. For visible and ultra-violet spectrum, electronic excitation occurs in the range 200-800 nm and involve the promotion of electrons to the higher energy molecule orbital. A record of the amount of light absorbed by sample as a function of the wavelength of light in nm units is called absorption spectrum which is generally consist of absorption bonds. UV spectroscopy is based on absorption law in which absorption of light by the molecules. These are

1.Lambert's law
2.Beer's law

1.Lambert's law: "When a beam of monochromatic radiation passes through a homogeneous absorbing medium, the rate of decrease of intensity of radiation with thickness of absorbing medium is proportion to the incident radiation." Mathematically, law is expressed as ; $-\frac{dI}{dx} = kI$

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2.Beer's law: "When a beam of monochromatic radiation is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of absorbing solution is proportion to the intensity of incident radiation as well as the concentration of solution."

Mathematically, law is expressed as; $-\frac{dI}{dx} = k'Ic$

Validation: Validation yields a developed process, the product of which should do what the user expects it to do. It is nothing but reproducibility of result validation according to various agencies is spelled as ; As per FDA. "Validation is defined as the collection and evaluation of data from the process design stage throughout production, in which establishes scientific evidence that a process is capable of consistently delivering quality product." As per WHO – The documented act of providing that any procedure, process, equipment, activity or system actually leads to the expected result.

Analytical Validation: Validation of analytical method may be defined as "The process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirement for the intended analytical application". One of the triazole antifungal agent that inhibit cytochrome P-450 dependent enzyme resulting in impairment of ergosterol synthesis. It has been used against histoplasmosis,

blastomycosis, cryptococcal meningitis and aspergillosis. Itraconazole is a synthetic triazole antifungal agent. Itraconazole is a 1:1:1:1 racemic mixture of four diastereomers (two 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- triazol- 1-ylmethyl)- 1,3- dioxolan- 4- yl) methoxy)phenyl) piperazin-1-yl)phenyl)-2-(1-methylpropyl)-2, 4-dihydro-1, 2, 4-triazol- 3-one (Fig 1). It has a molecular formula is C35H38Cl2N8O4 and a molecular weight is 705.64. (Manohar A.potdar *et al*., 2013; Ramesh sawant and SandipHapse, 2011; Supriya S. Mahajan, 2010; Mahajan, 2006) It is a white to slightly yellowish powder. It is very slightly soluble in alcohols, and freely soluble in dichloromethane. Itraconazole is highly lipophilic in nature and practically insoluble 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. (Mehdham *et al*., 2007) It is an orally active triazole antifungal agent, which demonstrates broad spectrum activity against a number of fungal species including dermatophytes, Malassesia further, Candida species, Aspergillus species, and Histoplasma capsulatum var. capsulatum. (KoteswaraRao *et al*., 2014; Trinadha Rao *et al*., 2015) The mechanism of action of itraconazole is it interacts with 14-α demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. Itraconazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis. Itraconazole is metabolized predominately by the cytochrome P450 3A4 isoenzyme system (CYP3A4) in the liver, resulting in the formation of several metabolites, including hydroxyl itraconazole, the major metabolite. (ShalinK.Parikh *et al*., 2011) Its oral bioavailability was found to increase when taken with food, with plasma concentration approximately two times that taken in the fasting state. (PrasunaSundari *et al*., 2013) It is extensively metabolized in the liver, mainly via an oxidative pathway, into the bioactive metabolite hydroxyl itraconazole. (Garg *et al*., 2013) Itraconazole was formulated into 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. Used to treat fungal infections of the fingernails. Tablets and capsules are used to treat fungal infections of the toenails. Itraconazole oral solution (liquid) is used to treat yeast infections of the mouth and throat or of the esophagus (tube that connects the throat to the stomach) (Thangabalan *et al*., 2013) Several HPLC (Kumudhavalli *et al*., 2011), and LC/MS-MS (Savant, 2008; ICH harmonised triparatite guideline, 1994; Mehdham *et al*., 2007) methods have been reported for the analysis of Itraconazole in plasma that suffer from either undesirably

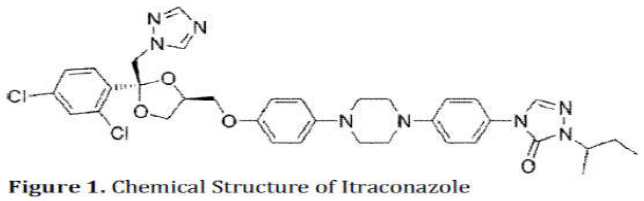

Figure 1. Chemical Structure of Itraconazole

Need and objective of work

1. To develop and validate analytical method of Itraconazole capsule.
2. The development and validation of simple, accurate and precise analytical method of Itraconazole in bulk drug and in pharmaceutical dosage forms by UV-Visible spectrophotometric method.

MATERIALS AND METHODS

Itraconazole bulk drug and capsule (CANDIFORCE 100) purchased from market, Hydrochloric acid and ethanol obtained from college. UV spectrophotometric method was performed on double beam UV-visible spectrophotometer (Shimadzu, model 1600) having two matched quartz cells with 1 cm light path.

Figure 2. Instrument: UV-Visible spectrophotometer

Methodology

Selection of solvent

Acidic ethanol was selected as ideal solvent for spectrophotometric analysis of Itraconazole.

Preparation of standard stock solution (1000µg/ml)

Accurately weighed 100 mg Itraconazole was volumetric flask and dissolved and diluted up to the mark with 0.1N acidic ethanol to give a stock solution having strength 1000µg/ml. 100 µg/ml working standard solution was prepared by diluting 1 ml of stock solution to 10 ml with 0.1N acidic ethanol.

Preparation of (100µg/ml)

To measure the Itraconazole content of capsule (label claim100 mg Itraconazole per capsule, Itaspor capsules), twenty capsules were weighed, the mean weight was determined. A weight of the powder equivalent to100 mg Itraconazole was transferred to a 100 ml volumetric flask containing 50 ml 0.1N acidic ethanol and the mixture was sonicated for 30 min then diluted to 100 ml with0.1N acidic ethanol (1000 µg/ml). The solution was filtered and 1 ml of filtered solution was diluted tenfold to furnish a concentration of 100µg/ml.

Validation of UV spectrophotometric method

Determination of wavelength

The stock solution was diluted with acidic ethanol to obtain solution of concentration 10µg/ml. The absorbance of resulting

solution was scanned in UV spectrometer under the range of 200-400nm. The absorbance maximum was found at wavelength about 262nm.

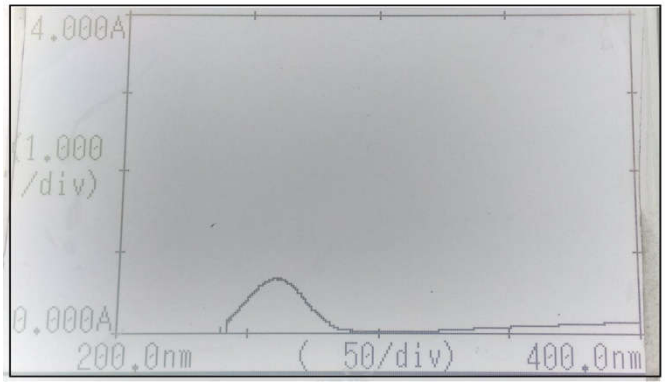

Figure 3. UV Spectra of Itraconazole

Linearity and range

The linearity was determined by analyzing 6 independent levels of calibration curve in the range of 2-12µg/ml. Absorbance of each solution against methanol was recorded at curve of absorbance vs. concentration was plotted and correlation co-efficient and regression line equation for Itraconazole were determined.

Table 1. Linearity

S.No.	Concentration	Absorbance
1	0	0.001
2	2	0.109
3	4	0.196
4	6	0.259
5	8	0.330
6	10	0.444
7	12	0.517

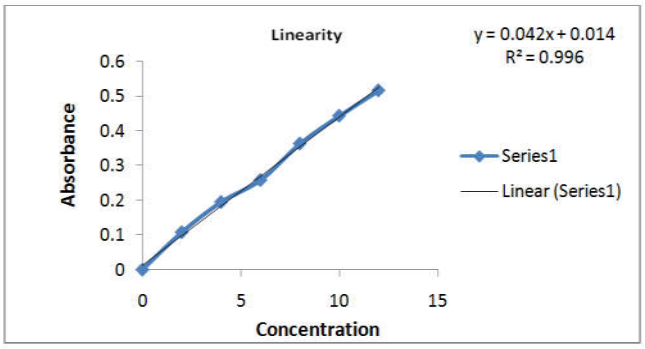

Figure 4. Linearity curve of Itraconazole

Accuracy: Accuracy was determined by performing recovery studies by spiking different concentrations of pure drug in the pre-analyzed powder for infusion samples within the analytical concentration range of the proposed method at three different set at level of 80%, 100% and 120%. The amount of Itraconazole was calculated at each level and % Recoveries were computed.

Precision

Intra-day precision was determined by analyzing Itraconazole (2-6µg/ml) at three different time points of the same day and determined by analyzing Itraconazole (2-6µg/ml) at three different time points on different days and %RSD was calculated.

Table 2. Accuracy 1

80%						
S.No.	Conc.	Amount added	Abs.	Amount found	Amount Recovered	% Recovered
1	6	4.8	0.207	4.60	4.82	100.36
2	6	4.8	0.205	4.55	4.77	99.37
3	6	4.8	0.206	4.57	4.79	99.87
			Mean	4.57	4.79	99.87
			SD	0.02	0.02	0.50
			%RSD	0.52	0.50	0.50

Table 3. Accuracy 2

100%						
S.No.	Conc.	Amount added	Abs.	Amount found	Amount Recovered	% Recovered
1	6	6	0.259	5.83	6.06	100.93
2	6	6	0.258	5.81	6.03	100.53
3	6	6	0.258	5.81	6.03	100.53
			Mean	5.82	6.04	100.66
			SD	0.01	0.01	0.23
			%RSD	0.24	0.23	0.23

Table 4. Accuracy 3

120%						
S.No.	Conc.	Amount added	Abs.	Amount found	Amount Recovered	% Recovered
1	6	7.2	0.31	7.05	7.27	100.97
2	6	7.2	0.309	7.02	7.25	100.64
3	6	7.2	0.314	7.14	7.37	102.29
			Mean	7.07	7.29	101.30
			SD	0.06	0.06	0.87
			%RSD	0.89	0.86	0.86

Table 5. Intraday Precision

S. No.	Conc.	Abs. I	II	III	MEAN	SD	%RSD
1	2	0.111	0.107	0.109	0.11	0.00	1.83
2	4	0.2	0.206	0.206	0.20	0.00	1.70
3	6	0.263	0.257	0.259	0.26	0.00	1.18

Table 6. Intraday Precision

S. No.	Conc.	Abs. I	II	III	MEAN	SD	%RSD
1	2	0.101	0.1	0.102	0.10	0.00	0.99
2	4	0.133	0.13	0.131	0.13	0.00	1.16
3	6	0.163	0.157	0.159	0.16	0.00	1.91

Repeatability

Repeatability was carried out by using a minimum of 6 determinations at one of the test concentration.

Table 6. Repeatability

S.No.	Conc.	Peak Area	Amt Found	%Amt Found
1	6	0.262	5.90	98.41
2	6	0.263	5.93	98.81
3	6	0.262	5.90	98.41
4	6	0.263	5.93	98.81
5	6	0.261	5.88	98.02
6	6	0.265	5.98	99.60
		Mean	5.92	98.68
		SD	0.03	0.54
		%RSD	0.55	0.55

LOD AND LOQ

Detection limit was determined based on the standard deviation of the response & slope of calibration curve.

LOD= 3.3*σ/S and LOQ= 10*σ/S

Where,
σ = the standard deviation of y-intercepts of regression lines
S = the slope of the calibration curve

Table 7. LOD AND LOQ

S.No.	Conc.	Area I	II	III	Mean	SD	%RSD
1	2	0.109	0.105	0.108	0.11	0.00	1.94
2	4	0.198	0.199	0.192	0.20	0.00	1.93
3	6	0.269	0.279	0.271	0.27	0.01	1.94
4	8	0.375	0.388	0.387	0.38	0.01	1.89
5	10	0.444	0.432	0.428	0.43	0.01	1.92
6	12	0.517	0.498	0.509	0.51	0.01	1.88
					Avrg SD	0.01	

LOD =	3.3 * Avrg SD/ Slope	0.785
LOQ =	10 * Avrg SD / Slope	2.38

Ruggedness

The evaluation of Ruggedness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to external factors in method parameters such as instrument, analyst variation.

Table 8. Ruggedness

ANALYST I			ANALYST II		
S. No.	Conc.	Area	S. No.	Conc.	Area
1	6	0.258	1	6	0.256
2	6	0.256	2	6	0.262
3	6	0.265	3	6	0.253
4	6	0.256	4	6	0.256
5	6	0.258	5	6	0.258
6	6	0.262	6	6	0.256
	Mean	0.26		Mean	0.26
	SD	0.00		SD	0.00
	%RSD	1.39		%RSD	1.17

Robustness

Robustness is determined by making small but deliberate variations in method parameters. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters such temperature.

Table 9. Robustness

S. No.	Temperature	Conc.	Absorbance	Mean	SD	% RSD
1	25	6	0.258			
2	25	6	0.256	0.26	0.00	1.82
3	25	6	0.265			
4	30	6	0.256			
5	30	6	0.258	0.26	0.00	1.18
6	30	6	0.262			
7	35	6	0.269			
8	35	6	0.279	0.27	0.01	1.94
9	35	6	0.271			

RESULTS DISCUSSION

The UV method has been developed for the estimation of Itraconazole in bulk & pharmaceutical dosage formulation.

Table 10. Result discussion

S. No.	Validation Parameter	Result
1	Solubility	Acidic ethanol
2	UV detection Maximum wavelength	262 nm
3	Linearity range	2-12 µg/ml
4	Standard regression equation	Y=0.042x+0.014
5	Coefficient of regression (r^2)	0.996
6	% Recovery (n=9)	0.53
7	Precision	
	Interday(n=3)	1.57
	Intraday(n=3)	1.35
8	Repeatability(n=6)	0.55
9	LOD	0.785
	LOQ	2.38
10	Ruggedness (n=6)	1.28

Conclusion

The developed method was simple, accurate, precise and economic in nature and can be used for analysis of marketed formulations.

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