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METHOD DEVELOPMENT AND ANALYTICAL METHOD VALIDATION OF ITRACONAZOLE BY USING UV- VISIBLE SPECTROPHOTOMETRY

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Abstract:

A simple UV-spectrophotometric method was developed for the determination of Itraconazole in pure and its pharmaceutical formulations. Itraconazole exhibited maximum absorption at 261nm in Methanol and obeyed linearity in the concentration range of 2.5-25 ***µg/ml****. The proposed method was statistically validated.All the proposed methods are simple, selective, reproducible, sensitive and accurate with good precision. Some of the methods were proved to be superior to most of the reported methods. All these proposed methods for estimation of selected drugs such as Itraconazole were successfully applied either in bulk or pharmaceutical formulations. The proposed methods can be used as alternative methods to the reported ones for the routine determination of selected drugs under the study in bulk and pharmaceutical dosage forms.*

Keywords: *Itraconazole, UV-Visible Method, Methanol.*

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INTRODUCTION:

The UV-visible spectrophotometric methods which fall in the wavelength region 200-800 nm and fluorimetric methods (may fall in UV & Visible regions) are very simple, cheap & easy to carry out estimations of drugs in bulk form and their formulations. The limitations of many colorimetric or fluorimetric methods of analysis lie in the chemical reaction upon which the procedures are based rather than the instruments available. Many of the reactions involve color or fluorescence of a particular drug are quite selective or can be rendered selective through the introduction of masking agents, control of pH, use of solvent extraction technique, adjustment of oxidation states or by prior removal of interfering ingredients with the aid of chromatographic separate[1-3].

Itraconazole is a potent triazole antifungal agent that is prescribed to patients with fungal infections used for the treatment of mycoses. The drug may be given orally or intravenously.1-3 The IUPAC nomenclature of the drug is as follows: (2*R*,4*S*)-*rel*-1- (butan-2-yl)-4-{4-[4-(4-{[(2*R*,4*S*)-2-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-ioxolan-4-yl]-methoxy}-phenyl)-piperazinyl]phenyl}-4,5-dihydro-1*H*-1,2,4-triazol-5-one.ITZ is used orally in the form of capsules for treatment of dermatophyte infections, occurrence of superficial fungal infections and systemic fungal infections. 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. Spectrofluorimetry method has been used for assay of Itraconazole in raw material and in dosage forms. RP-HPLC [4-6] method is used for determination of Itraconazole in human plasma.4-separation in this method was performed on anoctadecylsilane column using fluorescence detector. However, it has the disadvantage of being time consuming. All these studies have furth emphasized the need to perform rapid and sensitive quality-control analysis of pharmaceutical formulations containing Itraconazole. As these methods are expensive, we have made an attempt to develop a more precise, simple and economical spectrophotometric method with greater precision, accuracy and sensitivity for the analysis of Itraconazole in bulk and dosage forms [7].

MATERIALS AND METHODS:

Itraconazole was obtained as gift sample from Elite chemicals and all reagents were purchased from SD Chemicals Chennai. All materials and reagents used were in analytical grade.

Method Development

A simple UV-Visible Spectrophotometric method was developed for the determination of Itraconazole in pure and its pharmaceutical formulation. Itraconazole exhibiting maximum absorbance at 261nm in Methanol and obeyed linearity in the concentration range of 2.5 to 25 µg/ml. The proposed method was statistically validated.

Instrumentation:

Analytical technologies ltd, T60 UV-Visible Spectrophotometric method was performed with 1-cm quartz cells

Selection of Solvent:

Methanol was selected an ideal solvent for spectrophotometric analysis of Itraconazole.

Scanning and Determination of Maximum Wavelength (λmax)

In order to ascertain the wavelengths of maximum absorption (**λmax**) of the drug, different solutions of the drug (10µg/ml and 20µg/ml) in Methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–380nm against Methanol as blank. The resulting spectrum was presented in Fig 1 and and the absorption curve showed characteristic absorption maximum at 261 nm for Itraconazole.

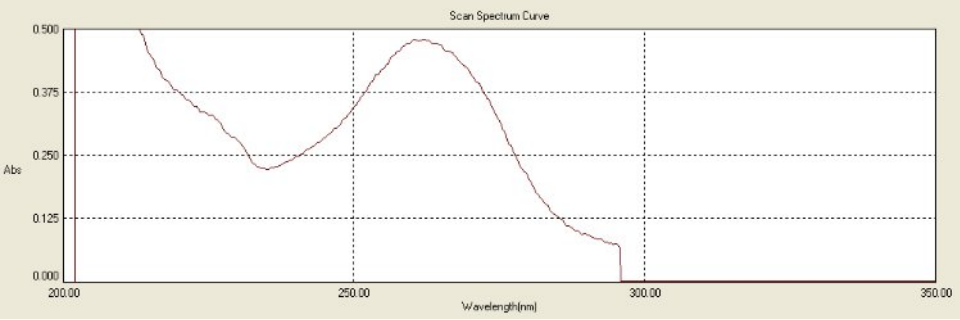

Fig1: Absorption curve for Itraconazole (λmax = 261nm)

Preparation of Stock Solution

Standard stock solution of Itraconazole was prepared by dissolving 10mg of Itraconazole drug in 10ml of Methanol in 10ml of volumetric flask to get a concentration of 1mg/ml (1000µg/ml) solutions.

Preparation of Working Standard Solutions and construction of standard graph

The prepared stock solution was further diluted with Methanol to get working standard solutions of 10µg/ml and 100µg/ml. To construct Beer's law plot for Itraconazole different aliquots of Itraconazole were taken and diluted to 10 ml with Methanol to get the working standard solutions as shown in the table 8.1. The absorbances of each solution were measured at λmax 261 nm against Methanol as blank. The results were shown in table. The standard graph for Itraconazole was plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in Fig 8.1. The drug has obeyed Beer's law in the concentration range of 2.5-20µg/ml.

Estimation of Itraconazole in commercial formulations

For analysis of commercial formulations, 10 capsules containing Itraconazole were taken and powdered. The powder equivalent to 0.047g of Itraconazole was taken in a 10ml volumetric flask, containing 7ml of Methanol and sonicated for 30 minutes. The volume was made up to 10ml with Methanol and filtered to get a solution of concentration 1000µg/ml. This was further diluted with Methanol to get a concentration within the linearity range and the absorbances were measured against the blank at 261nm.

Table 1 : Linearity table of Itraconazole (pure drug) in methanol at 261nm

S.No	Concentration (µg/ml)	Absorbance
1	2.5	0.114
2	5	0.224
3	7.5	0.345
4	10	0.442
5	12.5	0.569
6	15	0.672
7	17.5	0.78
8	20	0.856

Fig 2: Linearity graph of Itraconazole

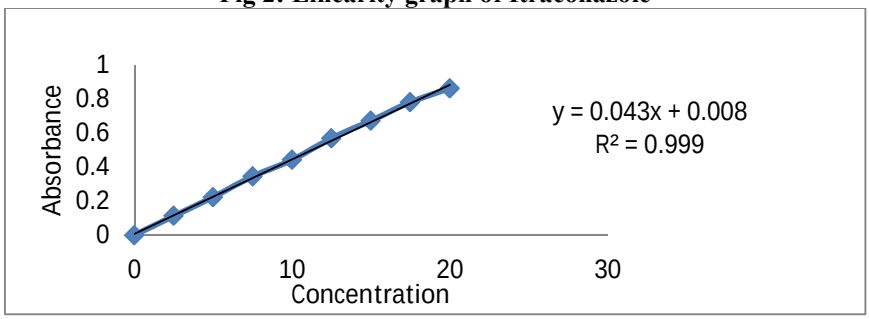

Table 2: Optical characteristics of proposed method.

Parameter	Itraconazole
λmax (nm)	261
Beer's Law limit (µg/ml)	2.5-25
Regression equation (Y)	0.050x-0.053
Slope (a)	0.050
Intercept (b)	0.053
% Range of error 95% confidence limits 99% confidence limits	 0.0021 0.0028
Correlation co-efficient	0.999

Validation

Precision

The precision of the proposed method was ascertained by actual determination of six replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance's by the proposed method. From this absorbance's Mean, Standard deviations, %R.S.D were calculated. The readings were shown in Table 4.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100% and 120%) of bulk samples of Itraconazole within the linearity range were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in Table 5.

Table 3: Amount of Itraconazole in formulation by proposed method.

Sl.No.	Formulation	Drug	Labeled Amount mg	Observed Amount mg Mean±SD	% Recovery
1.	Itromed-100	Itraconazole	47	44.902±0.293	98.546

Table 4: Precision data

S.no	Concentration (µg/ml)	Absorbances (nm)
1	10	0.413
2	10	0.415
3	10	0.411
4	10	0.412
5	10	0.411
6	10	0.413
Mean		0.4125
S.D		0.001751
% R.S.D		0.070776

Table 5: Accuracy data

ACCURACY						
Conc (bulk)	conc(formln)	Abs	%rec	Mean	Sdv	%rsd
8	10	0.726	5.872			
8	10	0.729	5.895	5.88	0.011676	0.416
8	10	0.728	5.887			
10	10	0.836	6.710			
10	10	0.839	6.737	6.725	0.013868	0.377
10	10	0.838	6.729			
12	10	0.912	7.296			
12	10	0.919	7.349	7.329	0.028792	0.369
12	10	0.918	7.345			

SUMMARY:

Pharmaceutical analysis simply means analysis of pharmaceuticals. Today pharmaceutical analysis entails much more than the analysis of active pharmaceutical ingredients or the formulated product. The pharmaceutical industry is under increased scrutiny from the government and the public interested groups to contain costs and at consistently deliver to market safe, efficacious product that fulfill unmet medical needs. The pharmaceutical analyst plays a major rule in assuring identity, safety, efficacy, purity, and quality of a drug product. The need for pharmaceutical analysis is driven largely by regulatory requirements. The commonly used tests of pharmaceutical analysis generally entail compendia testing method development, setting specifications and method validation. Analytical testing is one of the more interesting ways for scientists to take part in quality process by providing actual data on the identity, content and purity of the drug products. New methods are now being development with a great deal of consideration to worldwide harmonization. As a result, new products can be assured to have comparable quality and can be brought to international markets faster.

Pharmaceutical analysis occupies a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities. In industry, the quality assurance and quality control departments play major role in bringing out a safe and effective drug or dosage form. The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in estimation of drugs.

UV-Spectrophotometric method development:

A simple UV-spectrophotometric method was developed for the determination of Itraconazole in pure and its pharmaceutical formulations. Itraconazole exhibited maximum absorption at 261nm in Methanol and obeyed linearity in the concentration range of 2.5-20 **µg/ml**. The proposed method was statistically validated.

All the proposed methods are simple, selective, reproducible, sensitive and accurate with good precision. Some of the methods were proved to be superior to most of the reported methods. All these proposed methods for estimation of selected drugs such as Itraconazole were successfully applied either in bulk or pharmaceutical formulations.

The proposed methods can be used as alternative methods to the reported ones for the routine determination of selected drugs under the study in bulk and pharmaceutical dosage forms.

CONCLUSION:

The proposed method was simple, sensitive and reliable with good precision and accuracy. The proposed method is specific while estimating the commercial formulations without interference of excipients and other additives. Hence, this method can be used for the routine determination of Itraconazole in bulk samples and Pharmaceutical formulations.

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