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EXTRACTIVE SPECTROPHOTOMETRIC METHOD DEVELOPMENT & VALIDATION FOR DETERMINATION OF ISAVUCONAZOLE

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ABSTRACT

A simple, accurate, exact, and rapid extractive spectrophotometric approach was devised for the measurement of Isavuconazole in tablets and biological fluids. Various analytical parameters have been evaluated and the results have been validated by statistical data. It was observed that the drug only reacted with Bromocresol Green. Further the λ_{max} of IVZ was found to be 506.0 nm. The Linearity of IVZ At λ_{max} of 506nm estimated the Correlation Coefficient (r2) as 0.999 with slope as 0.028. Form the results of recovery study it was clearly observed that % recovery at 80%, 100% & 120% as 97.96±1.172, 99.08±0.834 & 99.38±0.469 respectively. The repeatability & reproducibility was observed to be 99.127±0.064 and 99.666±0.039 respectively while the day to day & Analyst to Analyst variation was noted to be 99.530±0.038 and 99.450±0.150 respectively. The assay of tablet formulation in replicate 1 was performed The Conc. found (µg/ml) ranged between196.65 to 198.85. The % Conc. Found was observed to be varied from 98.33 to 99.56. The Mean, SD & % RSD values were observed to be 99.21, 0.501 and 0.505 respectively. The proposed method has been used to identify the Isavuconazole in various medicinal goods with success. The suggested approach is suited to quantify Isavuconazole in pharmaceutical formulations, as shown by the good agreement between extractive spectrophotometric method and high-performance liquid chromatography mass spectrometry (HPLC-MS) for the determination of Isavuconazole in various genuine samples.

Keywords: Isavuconazole, Extractive spectrophotometric, Method development, method validation, Bromocresol Green, Accuracy, Precision

INTRODUCTION

Isavuconazole has been identified in pharmaceutical and biological products using a variety of techniques, including voltammetry, flow injection electrogenerated chemiluminescence, spectrofluorometry, spectrophotometry, high-performance liquid chromatography, and liquid chromatography tandem mass spectrometry. Among them, the spectrophotometric approach provides a number of benefits, including ease of use, speed, and affordability. Spectrophotometry has been used successfully for pharmaceutical analysis, including pharmacodynamic research and quality control of commercialized products. According to the various reactions, spectrophotometric approaches for the determination of isavuconazole could be categorized (Nguyen *et al*., 2018; Du *et al*., 2004).

Ion-pair extraction was used in the well-known spectrophotometric technique. With the use of acid-dye reagents like Sudan III, methyl orange, supracene violet 3B, tropaeolin 000, bromophenol blue, bromothymol blue, bromocresol green, and bromocresol purple, I ion-pair complex formation can be achieved. The ion-pair is extracted into a water-immiscible organic solvent at a particular pH, and the concentration of the resultant ion pair in the organic phase is calculated spectrophotometrically (Malvankar and Shinde, 1991; Jahromi *et al*., 2018).

Isavuconazole is a triazole antifungal with acceptable safety characteristics and a broad spectrum of activity. For the treatment of invasive aspergillosis and mucormycosis, it has FDA and EMA approval. It functions by preventing the production of fungal cell membranes. Patients, particularly those who are immunocompromised, face considerable clinical issues as a result of invasive fungal infections. Isavuconzaole was susceptible to most Candida species, most Aspergillus species, Mucorales, Cryptococcus spp., Fusarium species, dermatophytes, and dimorphic fungi in vitro (Miceli and Kauffman, 2015; Ellsworth *et al*., 2020).

In this study, we examined the extractive spectrophotometric approach for the first time. This method is based on the creation of ionpair complexes between isavuconazole and various colors, followed by extraction into chloroform. Systematically investigated were a few factors that affect complex formation, including pH, shaking time, organic solvent, and dye concentration (Gowda *et al*., 2001; Tabata *et al*., 1996). Thus, present study deals with extractive spectrophotometric method development & validation for determination of isavuconazole.

MATERIALS & METHODS

Chemicals & reagents

Chloroform, Acetone, Sodium hydroxide, hydrochloric acid, Methanol, water and dyes like Methyl orange, Methyl violet, Bromocresol purple, Bromocresol Green, Bromcresol Blue were obtained from S.D Fine Chemicals Mumbai.

Proposed method

In the proposed method following marketed formulation Isavuconazole 200mg was used**.**

Solubility

Solubility of IVZ was determined at 25±1°C. Accurately weighed 10 mg IVZ was added in different 10 ml volumetric flask containing different solvent and placed at mechanical shaker for 8 hrs. After 8 hrs filter both solution were filtered through whatman filter paper No. 41. The filtrates were diluted suitably and analyzed spectrophotometrically against water.

Selection of particular dye

Different dyes used for confirmation of reaction with dye by following procedure:-

Solutions of 100µg/ml of Isavuconazole was prepared in distilled water, in 3 ml of drug solutions add 1 ml dye and extracted with 3ml chloroform and same manner control also prepared shake both the solution and stand aside for 10 min and the colour change compared to control for dye drug reaction.

Linearity range and calibration graph

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 10mg of each drug in 8ml water in 10 ml volumetric flask. The flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark 10ml with distilled water to get a concentration of 1000 µg/ml (Stock-A) for both drug.

Preparation of Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of IVZ and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with distilled water that gave concentration of 100 µg/ml (Stock-B).

Preparation of Working Standard Solution

1) 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with distilled water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for IVZ. Take 2 ml of each aliquots and react with 1ml Aqueous dye solution of Bromocresol Green and Extracted with Chlorofom, shake well and pipette out the lower colored layer and take the abs. using UV vis. Spectroscopy.

Selection of wavelength for linearity

Solutions of 10 µg/ml of IVZ were prepared separately. Both the solutions were scanned react with Bromocresol green in the spectrum mode from 400nm to 800 nm. The maximum absorbance of IVZ was observed at 506.0 nm. IVZ showed linearity in the concentration range of 5-25µg/ml and 10-50µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration.

Analysis of IVZ in synthetic mixture

amount equal to 10 mg of IVZ was taken in 10 ml volumetric flask and sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with water. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with water and extract with dye to get the final concentrations of drug in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentration was obtained from calibration curve method. The procedure was repeated for five times.

Validation

Linearity

To obtain final concentrations of 2, 4, 6, or 10 g/ml, appropriate aliquots of drug working standard solutions can be taken in various 10 ml volumetric sizes and diluted up to the mark with distilled water. After plotting absorbance vs concentration, calibration curves are created, and drug regression equations can be computed (Eticha *et al*., 2018).

Accuracy

By estimating isavuconazole recoveries using the standard adds method at three distinct levels—60, 100, and 140%—the accuracy of the procedure was evaluated. The average recovery percentage was calculated (Chafle and Awale, 2021).

Precision

By measuring the percent relative standard deviation (% RSD), the suggested method's

precision was assessed for intra- and interday precisions. The results of three consecutive days were used to evaluate the intermediate precision, while the results of six times the drug solution prepared in accordance with the protocol were utilized to estimate the intraday precision. Analyzing six samples of the same drug concentrations allowed researchers to gauge the repeatability (intraday) of the approach. To determine the variation, the absorbance of each was measured and reported in terms of relative standard deviation.

RESULTS AND DISCUSSION

Ion pair extractive spectrophotometric methods have attracted a lot of attention for the quantitative measurement of many pharmaceutical preparations because extractive spectrophotometric methods are popular due to their sensitivity in drug assay. These suggested techniques use chloroform as a solvent to extract the Isavuconazole from its tablet forms in order to determine its concentration. Color ion pair complexes that are created are extremely stable. These methods' operating parameters were established by changing one parameter at a time while maintaining the other values constant and evaluating the impact on the color species' absorbance. The numerous variables necessary for these technologies' maximum color development were optimized. The suggested techniques have been statistically and through recovery studies verified.

The result of solubility indicated that Isavuconazole is soluble in water and chloroform. Freely soluble in methanol, and slightly soluble in Hydrochloric acid acetone and sodium hydroxide. Also it was observed that the drug only reacted with Bromocresol Green. Further the λ_{max} of IVZ was found to be 506.0 nm. The Linearity of IVZ At λmax of 506nm estimated the Correlation Coefficient (r2) as 0.999 with slope as 0.028. Form the results of recovery study it was clearly observed that % recovery at 80%, 100% & 120% as 97.96±1.172, 99.08±0.834 & 99.38±0.469 respectively.

For the analysis of precision various parameters like Repeatability, Day to day precision, Analyst-to-Analyst & Reproducibility were taken into account. The repeatability & reproducibility was observed to be 99.127±0.064 and 99.666±0.039 respectively while the day to day & Analyst to Analyst variation was noted to be 99.530±0.038 and 99.450±0.150 respectively. The assay of tablet formulation in replicate 1 was performed The Conc. found (µg/ml) ranged between196.65 to 198.85. The % Conc. Found was observed to be varied from 98.33 to 99.56. The Mean, SD & % RSD values was observed to be 99.21, 0.501 and 0.505 respectively.

Table 1: Solubility of drug in different solvents

Solvent	Solubility of Isavuconazole
Water	Soluble
0.1N HCl	Slightly Soluble
0.1N NaOH	Slightly Soluble
Methanol	Freely Soluble
Chloroform	soluble
Acetone	Slightly soluble

Table 2: List of dyes used for experimentation

S. No.	Name of dye	Reaction
1	Methyl orange	No
2	Methyl violet	No
3	Bromocresol purple	No
4	Bromocresol Green	Yes
5	Bromcresol Blue	No

Table 3: Linearity of IVZ At λmax = 506 nm

Standard Conc. (µg/ml)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean
5	0.145	0.148	0.143	0.146	0.146	0.146
10	0.285	0.284	0.286	0.247	0.246	0.270
15	0.436	0.438	0.437	0.435	0.435	0.436
20	0.567	0.568	0.566	0.568	0.567	0.567
25	0.702	0.703	0.702	0.702	0.703	0.702
Correlation Coefficient (r^2)						0.999
Slope (m)						0.028
Intercept (c)						0.000

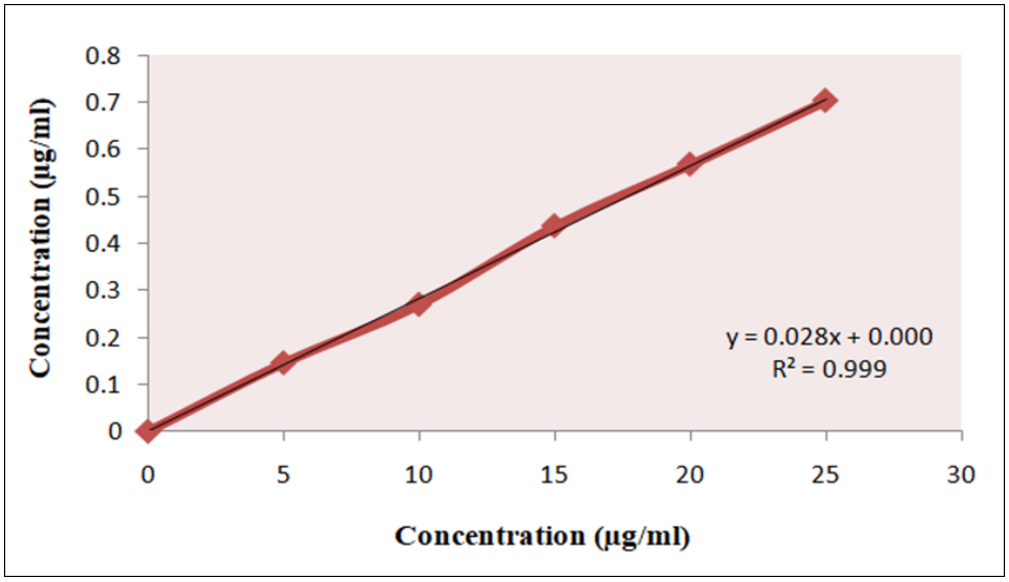

Figure 1: Calibration Curve of IVZ

Table 4: Results of Recovery study

% Level	% Mean±SD*
	Isavuconazole
80%	97.96±1.172
100%	99.08±0.834
120%	99.38±0.469

***** Value of three replicate and five concentrations.

Table 5: Results of precision

Parameter	% Mean±SD*
	Isavuconazole
Repeatability	99.127±0.064
Intermediate precision	
Day to day precision	99.530±0.038
Analyst-to-Analyst	99.450±0.150
Reproducibility	99.666±0.039

***** Value of five replicate and five concentrations

Table 6: Assay of tablet formulation

Conc. present (µg/ml)	Replicate-1	
	Conc. found (µg/ml)	% Conc. found
IVZ	IVZ	IVZ
200	198.85	99.43
200	198.74	99.37
200	196.65	98.33
200	199.12	99.56
200	198.74	99.37
	Mean	**99.21**
	SD	**0.501**
	% RSD	**0.505**

*Average of three replicate and five concentrations

CONCLUSION

In conclusion, the extractive spectrophotometric method developed and validated for the determination of isavuconazole exhibited excellent performance characteristics, including linearity, sensitivity, accuracy, precision, and robustness. The extractive spectrophotometric method developed and validated for the determination of isavuconazole proved to be reliable and accurate. The method demonstrated good sensitivity, precision, and linearity over the specified concentration range. This method can be reliably used for routine analysis of isavuconazole in various samples, such as pharmaceutical formulations or biological matrices.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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