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SIMPLE COST EFFECTIVE METHOD DEVELOPMENT FOR THE ESTIMATION OF ISOCONAZOLE BY USING UV AND HPLC

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ABSTRACT

Isoconazole is a potent antifungal agent used in the treatment of various fungal infections. The accurate estimation of isoconazole for ensuring the quality and efficacy of pharmaceutical formulations containing this drug. In this study, we aimed to develop simple and cost-effective methods for the estimation of isoconazole using UV spectrophotometry and HPLC. The UV spectrophotometric method demonstrated good linearity over the concentration range of 2-10 µg/ml, with a correlation coefficient (r2) of 0.997. Meanwhile, the HPLC method exhibited a correlation coefficient of 255.5, indicating its suitability for isoconazole quantification. Both methods were further evaluated for accuracy through recovery studies at different concentration levels (80%, 100%, and 120%), yielding satisfactory results. Precision studies were conducted to assess the repeatability, intermediate precision, and reproducibility of the UV and HPLC methods. The results confirmed the reliability and consistency of both methods in quantifying isoconazole. Additionally, the assay of tablet formulations using these methods demonstrated accurate quantification of isoconazole content, further validating their suitability for routine quality control analysis. In conclusion, the developed UV and HPLC methods offer reliable options for the estimation of isoconazole in pharmaceutical formulations. These methods are simple, cost-effective, and suitable for routine analysis in pharmaceutical laboratories, ensuring the quality and potency of isoconazole-containing products.

Keywords: Isoconazole, UV spectrophotometry, HPLC, Antifungal agent, Method development, Validation.

INTRODUCTION

Isoconazole is a broad-spectrum antifungal agent commonly used in the treatment of various fungal infections, including dermatophytosis, candidiasis, and pityriasis versicolor. Its accurate quantification is essential for ensuring proper dosage and therapeutic efficacy. Several analytical methods have been developed for the determination of isoconazole in pharmaceutical formulations and biological samples. Among these, UV spectrophotometry and high-performance liquid chromatography (HPLC) are widely employed due to their simplicity, sensitivity, and accuracy (Nagaraja *et al.,* 2019). UV spectrophotometry is a rapid and cost-effective technique commonly used for the quantitative analysis of pharmaceutical compounds. It relies on the measurement of absorbance at specific wavelengths, allowing for the determination of concentration based on Beer-Lambert's law.

UV methods for isoconazole estimation have been reported in the literature, offering simplicity and ease of implementation (Lee *et al.,* 2006). On the other hand, HPLC is a more sophisticated and versatile analytical technique that provides excellent resolution, specificity, and sensitivity. It involves the separation of components in a mixture based on their interaction with a stationary phase and a mobile phase under high pressure. HPLC methods for isoconazole analysis have been developed using various detection techniques, including UV detection, fluorescence detection, and mass spectrometry, offering high accuracy and precision (Tan *et al.,* 2005).

In this study, we aim to develop simple and cost-effective methods for the estimation of isoconazole using UV spectrophotometry and HPLC. These methods will be validated for their specificity, linearity, precision, accuracy, and robustness according to international guidelines (ICH, 2005). The developed methods will be applied to the analysis of commercial formulations containing isoconazole, providing valuable information for quality control and pharmaceutical analysis.

MATERIALS AND METHODS

Method development of drug using UV

Solubility

Solubility of INZ was determined at 25±1°C. Accurately weighed 10 mg INZ 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.

Linearity range and calibration graph

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 10mg of drug in methanol and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark 10ml with mixed hydrotropic agent to get a concentration of 1000µg/ml (Stock-A).

Preparation of Sub Stock Solution (Stock-B)

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

Preparation of Working Standard Solution

0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with methanol. This gave the solutions of 2µg/ml, 4 µg/ml, 6 µg/ml, 8µg/ml and 10 µg/ml respectively for INZ.

Selection of wavelength for linearity

Solution of 10µg/ml of INZ was prepared and was scanned in the spectrum mode from 200nm to 400nm. The maximum absorbance of INZ was observed at 222.0 nm. INZ showed linearity in the concentration range of 2-10µg/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration.

Calibration curve method

Working standard solution from the standard stock solution prepared in concentration 10µg/ml of INZ was scanned in the spectrum mode over the range of 200-400 nm against methanol as blank and the overlain spectra of drug recorded. INZ showed an absorbance peak at 222.0 nm, INZ showed linearity in the concentration range of 2-10 g/ml at their respective maxima. Calibration curve was plotted, absorbance versus concentration drug can be estimated by calibration curve method (Veera *et al.,* 2011).

Method development of drug using HPLC

Mobile Phase Selection

Initially to estimate Isoconazole number of mobile phase in different ratio were tried. Taking into thought the system suitability parameter like RT, Tailing factor, no. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 15mM KH_2PO_4 and Acetonitrile in the ratio of 20:80 adjust the pH 4 with OPA. The mobile phase was filtered through 0.45 µm filter paper to remove particulate matter and then degassed. Flow rate employed for analysis was 1.0 ml/min (Agarwal *et al.,* 2013).

Selection of wavelength

100 mg of Isoconazole was weighed accurately and transferred to a 100 ml volumetric flask, and the volume was adjusted to the mark with the mobile phase. From above solutions of 0.1 ml was transferred to 10 ml volumetric flasks, and make up the volume up to mark. Resulting solution was scanned over UV range (200-400nm), maximum absorbance was found at λ_{max} 222.00 nm (Poola *et al.,* 2002).

Selection of Separation Variable

Standard drug solution of Isoconazole was prepared in different mobile phase and chromatograph was recorded by using different column (5µm) at different chromatographic condition like different flow rate and temperature. Considering the theoretical facts and after several trials separation variables were selected which were constant during whole experiment.

Preparation of Standard Stock Solution

10mg of Isoconazole was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

Preparation of Working Standard Solution

From stock solutions of Isoconazole 1 ml was taken and diluted up to 10 ml from this solution 0.2, 0.4, 0.6, 0.8, 1.0ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with methanol, gives standard drug solution of 2, 4, 6, 8, 10µg/ ml concentration.

Preparation of the calibration curves of the drug

Standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Analysis of cream formulation

Weight equivalent to 10mg of Isoconazole was transferred to 10ml volumetric flask and dissolved in mobile phase. The solution was

shaking vigorously for 20mins and filtered through Whattman filter paper no. 41, then volume was made up to mark with acetonitrile. From the above solution 1ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 100µg/ml. From the above solution 1ml of solution was taken and diluted to 10ml with methanol to get a solution containing 10µg/ml of Isoconazole. The amounts of Isoconazole in tablet formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with tablet formulation (Ayaseelan *et al.,* 2012).

Validation of calibration curve method

Linearity

Linearity of drug was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of INZ preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week.

Analysis of cream formulation by UV

Cream amount equal to 10mg of INZ was taken in 10 ml volumetric flask. Then 4ml methanol was added and the flask was sonicated for about 10 min to solubilize the drug present in cream and the volume was made up to the mark with methanol. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with methanol to get the final concentrations of drug in the working range. The absorbance of final dilutions was observed at selected wavelengths and the concentrations were obtained from calibration curve Method. The procedure was repeated for five times.

Analysis of cream formulation by HPLC

Weight equivalent to 10mg of Isoconazole was transferred to 10ml volumetric flask and dissolved in mobile phase. The solution was shaking vigorously for 20mins and filtered through Whattman filter paper no. 41, then volume was made up to mark with acetonitrile. From the above solution 1ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 100µg/ml. From the above solution 1ml of solution was taken and diluted to 10ml with methanol to get a solution containing 10µg/ml

of Isoconazole. The amounts of Isoconazole in tablet formulation were calculated by extrapolating the value of area from the calibration curve.

RESULTS AND DISCUSSION

In the presented study, two analytical methods, UV spectrophotometry and HPLC, were developed and validated for the estimation of isoconazole. The results of linearity demonstrated that both methods showed a linear response over the concentration range of 2-10 µg/ml. The correlation coefficients (r2) for UV and HPLC methods were found to be 0.997 and 255.5, respectively, indicating good linearity. Additionally, the slope (m) and intercept (c) values obtained for both methods further supported the linearity of the calibration curves. The recovery study results revealed satisfactory accuracy for both UV and HPLC methods at three different concentration levels (80%, 100%, and 120%). The percentage mean recovery values were within the acceptable range, indicating that both methods were capable of accurately quantifying isoconazole in the presence of excipients or matrix components commonly found in tablet formulations.

Precision studies demonstrated good repeatability, intermediate precision (including day-to-day precision and analyst-to-analyst variation), and reproducibility for both UV and HPLC methods. The low values of standard deviation (SD) observed in the precision studies indicate the reliability and robustness of the developed methods for isoconazole estimation.

Furthermore, the assay of tablet formulation results indicated that both UV and HPLC methods were suitable for the determination of isoconazole content in commercial tablet formulations. The percentage assay values obtained were within the acceptable range, confirming the applicability of the developed methods for routine quality control analysis of isoconazole tablets.

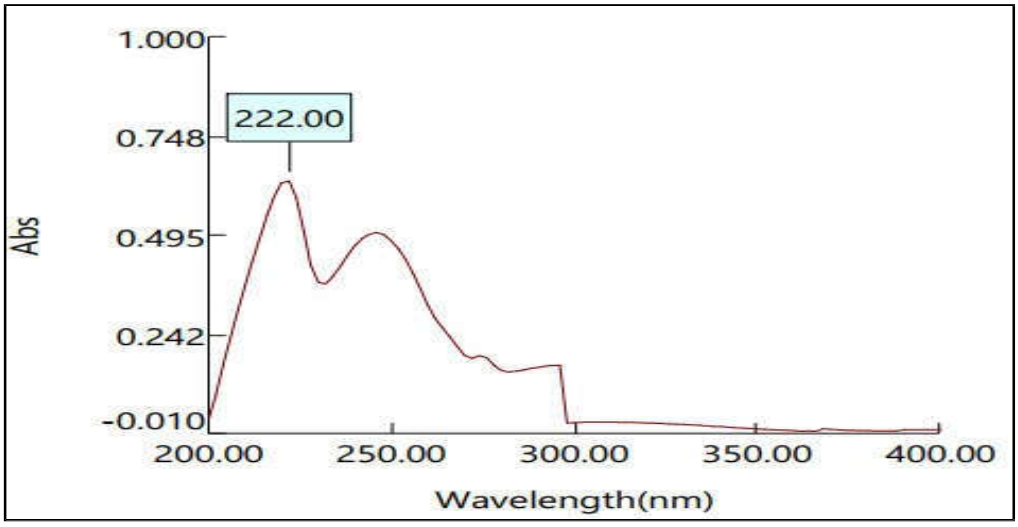

Figure 1: Determination of λ_{max} of Isoconazole

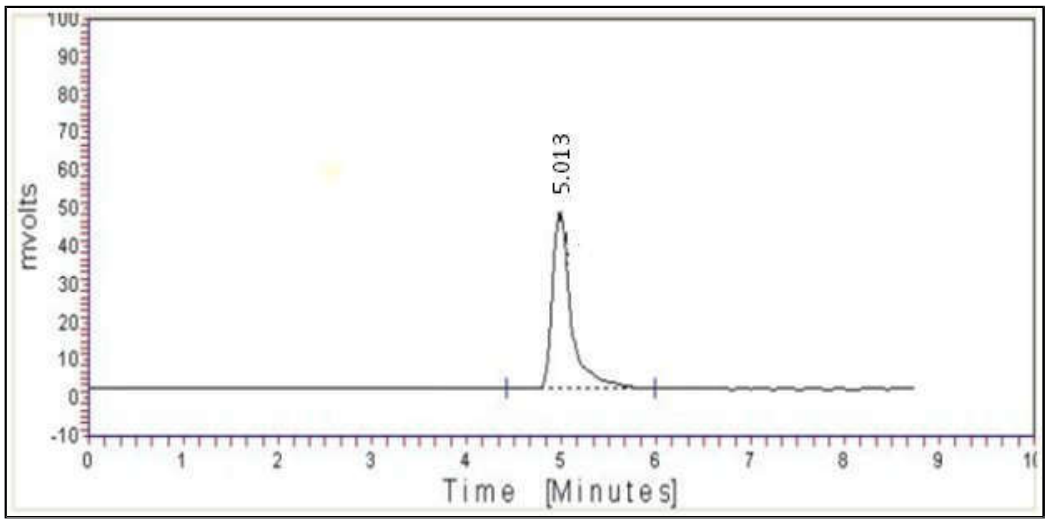

Figure 2: Chromatogram of Isoconazole

Table 1: Results of Linearity

Parameter	UV	HPLC
Concentration (µg/ml)	2-10	2-10
Correlation Coefficient (r^2)*	0.997	255.5
Slope (m)*	0.062	13.56
Intercept (c)*	0.010	0.999

Table 2: Results of Recovery Study

% Level	% Mean±SD*	
	UV	HPLC
80%	98.50±0.851	98.50±1.146
100%	98.06±1.304	96.80±1.114
120%	98.85±0.442	97.11±1.945

Table 3: Results of Precision

Parameter	% MEAN±SD*	
	UV	HPLC
Repeatability	96.58±0.11	99.95±0.165
Intermediate precision		
Day to day precision	97.21±0.13	98.86±0.292
Analyst-to-Analyst	97.32±0.04	97.29±0.499
Reproducibility	97.40± 0.09	98.65±0.144

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

Table 4: Assay of Tablet Formulation

Parameter	% Assay
UV	97.60±1.347
HPLC	98.60±0.854

CONCLUSION

In conclusion, the developed UV spectrophotometric and HPLC methods offer simple, cost-effective, and reliable approaches for the estimation of isoconazole. These methods can be effectively employed for routine analysis in pharmaceutical laboratories to ensure the quality and potency of isoconazole-containing formulations.

DECLARATION OF INTEREST

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

REFERENCES

- Nagaraja, P., Ramesh, M. & Prasad, R. (2019) Development and validation of UV spectrophotometric method for the estimation of isoconazole nitrate in bulk and in pharmaceutical dosage form. *Asian Journal of Pharmaceutical Analysis*, 9, 13–17.
- ICH harmonised tripartite guideline (2005). *Validation of Analytical Procedures: Text and Methodology*. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Vol. Q2, (p. R1).
- Lee, J.K., Choi, Y.H., Choi, S.M. et al (2006) A simple and sensitive high-performance liquid chromatography method for the determination of isoconazole nitrate in human plasma. *Journal of Chromatography. Part B*, 831, 217–221.
- Tan, Q., Wang, Y., Zhuang, L. & Qiu, F. (2005) Determination of isoconazole in rat plasma by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography. Part B*, 817, 217–220.
- Veera, V.S. & Murali, M. (2011) A RP-HPLC Method for the Estimation of ritonavir in Pharmaceutical dosage forms. *Journal of Pharmacy Research*, 4, 3049–3051.
- Agarwal, A., Tiwari, S. & Nagariya, K. (2013) Method development and its validation for quantitative simultaneous determination of latanoprost, timolol and benzalkonium chloride in ophthalmic solution by RP-HPLC. *Journal of Drug Delivery and Therapeutics*, 3.
- Poola, N.R., Bhuiyan, D., Ortiz, S., Savant, I.A., Sidhom, M., Taft, D.R., Kirschenbaum, H. & Kalis, M. (2002) A novel HPLC assay for pentamidine: Comparative effects of creatinine and inulin on GFR estimation and pentamidine renal excretion in the isolated perfused rat

kidney. *Journal of Pharmacy and Pharmaceutical Sciences*, 5, 135–145.

- Jayaseelan, S., Rajasekar, M., Sekar, V. & Perumal, P. (2012) A New Analytical Method Development and Validation for the Simultaneous Estimation of lamivudine and stavudine in Tablet Dosage Form by RP-HPLC Method. *Int. J. Pharm.*Tech. Res, 2, 1539–1542.