RESEARCH ARTICLE

Integration of UV Spectrophotometry Method For Simultaneous Estimation of Fluconazole and Efinaconazole in Nanoparticle Loaded Hydrogel

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ABSTRACT

The goal of this study was to create and validate a UV spectrophotometric method for fluconazole and efinaconazole. Ultraviolet spectroscopy was employed at 255 nm for fluconazole and 268 nm for efinaconazole, using samples prepared in a DMF and phosphate buffer solution with a pH of 4.9. The method demonstrated strong linearity with a correlation coefficient of 0.999. Following ICH guidelines, the validity of the method was assessed for various parameters, including accuracy, precision, limit of detection (LoD), limit of quantification (LoQ), recovery study, and range. The simplicity, time efficiency, and cost-effectiveness of UV spectroscopy were key factors in its selection over HPLC technique, which is known for being expensive and time-consuming with potential susceptibility to various factors affecting determination. Through repeated experiments and meticulous sampling, the method exhibited linearity, accuracy, repeatability, and lack of errors. It was also found to be selective, specific, and cost-effective, affirming its reliability. Moreover, the use of a consistent solvent throughout the experimental work ensured that the method was free from interference by any excipients. Overall, the proposed UV spectrophotometric method was deemed simple, rapid, precise, accurate, and sensitive, making it suitable for routine analysis of fluconazole in both single and combined forms.

Keywords: Fluconazole, Efinaconazole, UV spectroscopy, Nanoparticles, *R. erythropolisalidation*

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INTRODUCTION

The UV spectrophotometric method presented is not only straightforward and swift but also cost-effective, offering a reliable means of determining the fluconazole and efinaconazole in pharmaceuticals. Existing literature indicates that many methods for fluconazole and efinaconazole determination are intricate and lack the desired simplicity. In contrast, our current approach introduces a well-structured UV spectroscopic method with validated parameters, following a straightforward format as seen in previous publications. This UV method is both accurate and uncomplicated, avoiding complex procedures. Additionally, it proves versatile for dissolution studies, having been successfully conducted in the relevant dissolution medium.[1, 2]

Fluconazole, classified as a triazole, is chemically identified as 2-(2,4-Diflurophenyl)-1,3-di(1H-1,2,4-triazole-1-yl)-2-propanol. This compound is extensively employed in treating diverse fungal infections attributed to *Cryptococci, Candida*, and *Coccidia*. Its mechanism involves inhibiting the formation of ergosterol from lanosterol through binding to cytochrome P-450.[1]

Efinaconazole (EFN) is an innovative triazole antifungal compound designed to hinder the fungal cytochrome P450 enzyme (lanosterol-14α demethylase, CYP51). By doing so, it disrupts the biosynthesis of fungal membrane ergosterol, leading to a disruption in membrane integrity and inhibiting fungal growth.[3] Notably, azoles like EFN exhibit a greater affinity for fungal CYP51 compared to mammalian enzymes.

EFN holds the distinction of being the first azole drug sanctioned by the US Food and Drug Administration (FDA) for the topical treatment of onychomycosis in the USA.[4] Its unique characteristic involves a relatively lower binding to

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keratin, allowing for the swift release of bound EFN from keratin. This property facilitates enhanced nail penetration of EFN. Despite these advancements, there remains a need for a topical formulation with increased efficacy and rapid penetration post-application to improve complete cure rates.[5]

The crucial element in drug development lies in the analysis, whether dealing with a drug in bulk or in combination. It is imperative to devise a fitting method to identify any drug, be it in dosage form or bulk form. Method development is essential to facilitate the straightforward determination of the quantity of a specific drug. Validation parameters further confirm that the method established for estimating the mixture of fluconazole and EFN aligns with the necessary validation criteria.[1,6,7]

The objective in developing the UV spectroscopic method was to streamline the process of determining the fluconazole and EFN. Additionally, the goal was to validate the developed method in accordance with ICH guidelines, making it suitable for routine analysis.

MATERIALS AND METHODS

Instrumentation

Spectroscopic analysis was carried out using a Double beam UV-visible spectrophotometer-1800, Shimadzu (Kyoto, Japan) with 10 mm path length matched quartz cells.

Selection of Solvent

The criterion for the selection of solvent is based on solubility. The solubility of fluconazole and efinaconazole was determined by using a variety of solvents.

Preparation of Standard Stock Solution and Working Solution

Accurately weighed 10 mg of pure fluconazole and efinaconazole drug and transferred into a 100 mL volumetric flask and volume was made up to the mark with the DMF and pH 4.9 phosphate buffers. The standard stock solution was obtained having a concentration of 100 µg/mL to get them as a working solution.

Preparation of Calibration Curve

For fluconazole from the working solution, 0.5, 0.75, 1.0, 1.25, 1.5 and 1.75 mL solution was transferred into a series of calibrated 10 mL volumetric flasks, and the volume was made up using DMF and pH 4.9 phosphate buffers. The solutions were scanned in the range of 200 to 400 nm against a blank. Similarly, for fluconazole from the working solution, 0.5, 0.75, 0.6, 0.75, 0.9 and 1.05 solution was transferred into a series of calibrated 10 mL volumetric flasks, and the volume was made up using DMF and pH 4.9 phosphate buffers. The solutions were scanned in the range of 200 to 400 nm against a blank.[8]

Selection of Wavelength

The selection of wavelengths for the estimation fluconazole and efinaconazole suitable diluted stock solution contains 16 µg/ mL of each and the solutions were scanned between 200 to 400 nm by using methanol as blank. From the overlain spectra, by the observation of spectral characteristics of fluconazole and efinaconazole were selected for simultaneous estimation. The wavelengths selected were 255 nm (Fluconazole) and 268 nm (Efinaconazole).

UV Method Validation

Linearity and calibration

- *Simultaneous equation method*

From the working stock solution of fluconazole, pipette out 0.5 to 1.75 mL into a series of six 10 mL volumetric flask and made up to mark with pH 4.9 phosphate buffers to get concentration range of 5 to 17.5 µg/mL. From the working stock solution of efinaconazole, pipette out 0.3 to 1.5 mL into a series of six 10 mL volumetric flask and made up to mark with and pH 4.9 phosphate buffers to get concentration range of 3 to 10.5 µg/mL. The linearity was carried out individually for both the drugs and absorbances of these solutions were measured at 255 and 268 nm.

It involves the calculation of the integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength area is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. In combination drugs λ1 and λ2 denotes the wavelength ranges of the components. The integrated value of absorbance in the wavelength ranges of both the drugs are substituted in the simultaneous equation to get the concentration of the drugs.

$$c_x = \frac{A_2 a_{y1} - A_1 a_{y_2}}{a_{x_2} a_{y1} - a_{x_1} a_{y2}} \quad \text{And} \quad c_y = \frac{A_1 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y1} - a_{x_1} a_{y2}}$$

Validation of Developed Method

Linearity

A calibration curve was plotted between concentration and absorbance. Fluconazole was linear with the concentration range of 5 to 17.5 µg/ mL and efinaconazole showed the linearity in the range of 3 to 10.5 µg/mL at selected wavelengths for both methods.

Recovery studies

In order to ensure the accuracy of the proposed method, recovery studies were carried out. To 50% of the pre-analyzed sample solution, a definite concentration of 2.5, 5 and 7.5 µg/mL standard solution of candesarta1.5, 3 and 4.5 µg/ mL standard solution of simvastatin were added and then its recovery was studied. The absorbance of resulting solutions was measured at their corresponding wavelengths and the percentage recovery was calculated.[9]

Precision

Precision of the method was demonstrated by repeatability studies. Repeatability studies were done by consequently analysing the sample solution for six times. Intraday and inter-

day precision were established by repeating the determination on the same day and on different days, respectively.

Ruggedness

The analysis of formulation confirmed the ruggedness of the method was done by the different analysts. The amount and %RSD were calculated.

Limit of detection and limit of quantification

The linearity studies were carried out for six times. The limit of detection and limit of quantification were calculated by using the average of slope and standard deviation of intercept.[10]

RESULTS AND DISCUSSIONS

Method Validation

The proposed method was validated as per ICH guidelines for its linearity, accuracy, precision, robustness, the limit of detection, the limit of quantitation and ruggedness. The overall results of the validation parameters were compiled in Table 1.

Table 1: Validation parameters for determination of fluconazole and efinaconazole

Parameter	*Values*
Absorption maxima (nm) Fluconazole Efinaconazole	 255 nm 268 nm
Linearity range (µg/mL) Fluconazole Efinaconazole	 5–17.5 3–10.5
Standard regressed equation Fluconazole Efinaconazole	Y= 0.098x + 0.099 Y= 0.068x + 0.099
Corelation coeficient Fluconazole Efinaconazole	 0.999 0.999
Recovery study (%recovery ± SD) Fluconazole Efinaconazole	 103.3 98.92
Precision (%RSD)	Fluconazole Intraday- 0.15 Efinaconazole Intraday – 0.15
LoD (µg/mL) Fluconazole Efinaconazole	 0.003 0.048
LoQ (µg/mL) Fluconazole Efinaconazole	 0.102 0.147

Recovery Studies

The accuracy of the proposed method was estimated by %recovery of the method at the three-level of percentage addition.[11] The %recovery of fluconazole and efinaconazole was found to be in the range of 96.8 to 110.4 for fluconazole and 89.77 to 106% for efinaconazole and was shown in Table 2. The results of the recovery studies undoubtedly demonstrate the accuracy of the proposed method.

Precision

The precision of the proposed method was estimated by the Intraday and Interday Precision at the three-level of percentage addition. The repeatability results indicate the precision over a short interval of time, as well as during interday assessment. The %RSD of Fluconazole and Efinaconazole was found to 0.15 for intraday data were shown in Table 3. The intraday relative standard deviation (RSD) values obtained by the proposed method are within 2% relative standard deviations.

Limit of Detection and Limit of Quantification

The LoD and LoQ study was performed to check the sensitivity of the proposed developed method. The LoD for fluconazole and Efinaconazole were found to be 0.003 and 0.048 µg/mL, and LoQ 0.102 and 0.147 µg/mL,, respectively. From the obtained results, it can be easily interpreted that this UV method is highly sensitive to analyze fluconazole and efinaconazole.

Robustness

The robustness of the developed method shows a non-significant influence of the absorption level through the analysis of the fluconazole and efinaconazole solutions using different glassware and at different temperatures. The result of the robustness study was shown in Table 4.

Ruggedness

As shown in Table 5, it was observed that there were no significant changes in the result by changing an analyst, which confirmed that the developed method is rugged.

Assay of Synthetic Mixture

The prepared physical mixture of fluconazole and efinaconazole with various excipients were analyzed by the developed method. As per ICH guidelines, the assay values for all these formulations were found to be 97.68% for candesartan and 102.29%.

Table 2: Estimation of accuracy by percentage recovery method

Efinac onazole conc	*Conc. of pure fluc onazole*	*Conc. of efinacon azole Test*	*Conc. of flucona zole Test*	*Conc. of efinacona zole obtained*	*Conc. of fluconazole obtained*	*Efinac onazole recover*	*Flucon azole rec overed*	*%Efinac onazole rec overed*	*%Fluconazole recovered*
3	5	1.5	2.5	4.59	7.76	1.59	2.76	106	110.4
3	5	3	5	6.04	9.84	3.04	4.84	101.33	96.8
3	5	4.5	7.5	7.04	12.26	4.04	7.72	89.77	102.93

Table 3: Intraday and interday precision

Concentration µg/mL	*Intraday precision*			
	Fluconazole		*Efinaconazole*	
	Mean ± SD	*% RSD*	*Mean ± SD*	*% RSD*
10 + 6 µg/mL	0.0025	0.1559	0.0025	0.1512

Table 4: Robustness study

Concentration µg/mL	*With different glassware*		*At different temperature*	
	Fluconazole %RSD	*Efinaconazole %RSD*	*Fluconazole %RSD*	*Efinaconazole %RSD*
10 + 6	0.081	0.332	0.031	0.599

Table 5: Ruggedness study

Ruggedness				
Conc. (µg/mL)	*Fluconazole*		*Efinaconazole*	
	Abs.	*%RSD*	*Abs.*	*%RSD*
10 + 6	1.604	0.34	1.654	0.09

CONCLUSION

An effective ultraviolet spectroscopic technique was developed and validated following ICH guidelines to determine the concentration of fluconazole in pharmaceutical dosage forms. The results demonstrated that the proposed method exhibited linearity, accuracy, repeatability, absence of errors, selectivity, specificity, and cost-effectiveness, affirming the reliability of the approach.

Furthermore, the consistent use of the same solvent throughout the experiments revealed that the method remained unaffected by any interference from excipients. Consequently, the proposed method proved successful in the routine analysis of fluconazole and efinaconazole.

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