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Method Development and Validation for Estimation of Luliconazole in Bulk and Formulation by UV-Visible Spectrophotometry

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ABSTRACT

Luliconazole is a broad-spectrum antifungal agent that has shown promising results as an oral agent for the treatment of systemic and invasive aspergillosis. An oral formulation was developed and a simple and rapid UV- visible spectrophotometric method was developed for the determination of Luliconazole. The absorbance maxima were found to be at 296nm as reported in the literature. The method was validated for linearity, precision, accuracy, LOD, and LOQ. The drug was found to be linear within the concentration range of 4-12 µg/ml and the coefficient of correlation was found to be 0.993. The LOD and LOQ were found to be 0.437 and 1.325 respectively. The results obtained for the validation of method indicated that the developed analytical method is cost-effective, accurate, and precise.

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INTRODUCTION

Luliconazole (LCNZ) ((2*E*)-2-[(4*R*)-4-(2,4-chlorophenyl)-1,3-dithiolan-2-ylidene]-2-imidazol-1-ylacetonitrile) also known as NND-502 was first synthesized by Nihon Nohyaku Co Ltd (Osaka, Japan). It is a Biopharmaceutical class II imidazole antifungal agent used to treat broad-spectrum fungal infection caused by *Trichophyton spp., C. albicans, and Aspergillus fumigatus* [1,2]. Luliconazole is the R-enantiomer and has a more potent antifungal agent than Lanoconazole [3]. Luliconazole acts by blocking ergosterol synthesis by inhibiting the enzyme lanosterol demethylase. Azoles inhibit the activity of this enzyme, resulting in lower levels of ergosterol, a component of fungal cell walls, and an accumulation of lanosterol [4,5].

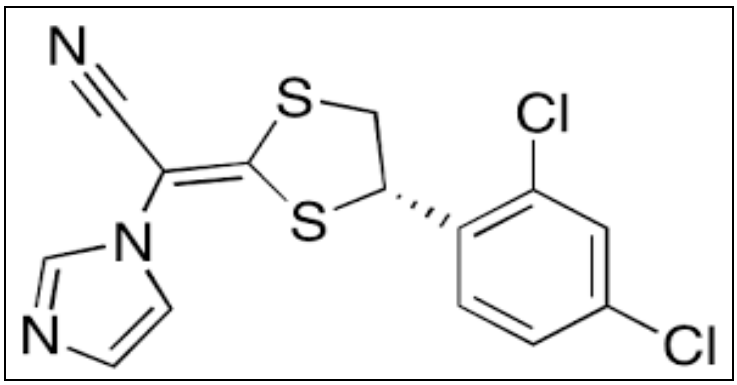

Fig.1 Structure of Luliconazole

Its molecular formula is $C_{14}H_9Cl_2N_3S_2$ and has molecular weight of 354.3 g/mol. Luliconazole has shown promising clinical development results as an oral agent potentially useful for systemic and invasive aspergillosis [1]. A review of the literature for Luliconazole analysis showed existing methods such as an HPTLC approach, an LC-MS/MS method, a few spectroscopic methods, and a few HPLC methods for the quantification of Luliconazole and its related chemicals in formulations and biological samples [6-11]. This study aims to develop and validate a simple, cost-effective UV-visible spectrophotometric method for the estimation of Luliconazole in bulk and tablet formulations.

MATERIALS AND METHOD

Chemicals and reagents

Luliconazole was received as a gift sample from Glenmark Pharmaceuticals Ltd. All chemicals and reagents were of analytical grade and was purchased from Visham Chem.

Instrumentation

Shimadzu Ultraviolet-visible spectrophotometer (UV-1800) was used for the measurement of absorbance. All the analytical weights were measured using Contech electronic balance.

Selection of wavelength for analysis of Luliconazole

Accurately weighed 10 mg of LCNZ and transferred to a 10 ml of volumetric flask and diluted up to the mark with methanol (1000 µg/ml). 1ml of the resulting solution was further transferred to a 10ml volumetric flask and diluted up to the mark with methanol: water (1:1) (100 µg/ml). 1.5 ml of the above solution is transferred to a 10ml volumetric and diluted up to the mark with methanol: water (1:1) to give 15 µg/ml and was scanned using a UV visible spectrophotometer in the range of 200-400nm [12].

Preparation of Standard Stock solution:

Standard stock solution was prepared by weighing 10mg of LCNZ accurately and transferring it to 10ml of volumetric flask and was diluted up to the mark using methanol to give 1000 µg/ml. 1ml of the resulting solution was withdrawn and transferred to 10ml of the volumetric flask and diluted up to the mark with methanol: water (1:1) to give 100 µg/ml as a standard stock solution.

Preparation of working solutions:

Working solutions were prepared by pipetting out 0.4, 0.6, 0.8, 1.0, and 1.2 from the standard stock solution to different 10ml volumetric flask and diluting each volumetric flask with methanol: water to yield a series of concentration ranging 4-12 µg/ml.

Preparation of Calibration curve:

The working solution ranging from 4-12 µg/ml was analyzed for its absorbance using UV-visible spectrophotometry at 296 nm.

Validation of method

The developed method was validated for its parameters such as linearity, accuracy, precision, limit of detection (LOQ) and limit of quantification (LOD) as per ICH guidelines.

1) **Linearity:** The linearity of the sample was analyzed by measuring the absorbance of the series of working solutions ranging from 4-12 µg/ml using UV-visible spectrophotometry at 296 nm.

2) **Accuracy:** The accuracy of the assay was performed at three different levels of recovery 80%, 100%, and 120%. Standard solution (8, 10, 12 µg/ml) of LCNZ was added to a pre-analyzed sample solution of the tablet, each solution was evaluated in triplicate and percent recovery was evaluated.

3) **Precision:** Intra-day precision and inter-day precision (intermediate) was performed. Intra-day precision was determined by analyzing solutions of known concentrations 4, 8, and 12 µg/ml three times a day. Inter-day precision was determined by analyzing the same concentration of the solutions on three different days and % RSD was calculated.

4) **Limit of Detection and Limit of Quantification:** Limits of detection (LOD) and limit of quantitation (LOQ) were used to determine the sensitivity of the proposed technique for LCNZ. Based on the standard deviation of the response and the slope of the created calibration curve, the limit of quantification and limit of detection was determined as per ICH guidelines Q2(R1). The LOD and LOQ are determined by using the following formula:

$$LOD = \frac{3.3*\sigma}{s} \text{ and } LOQ = \frac{10*\sigma}{s}$$

Assay

Twenty tablets of dose 10 mg with tablet weight 120 mg were weighed accurately. The average weight was determined, and tablets were finally powdered using mortar and pestle. Powder equivalent to 10mg was weighed and transferred to a 10ml volumetric flask and diluted up to the mark with methanol to give 1000 µg/ml. From the resulting solution, withdraw 1ml of the solution and transfer it to a 10ml of volumetric flask and dilute it up to the mark with methanol: water (1:1) to give 100 µg/ml. 1 ml of this solution was withdrawn and transferred to a 10ml volumetric flask and diluted it up to the mark with methanol: water (1:1) to give 10 µg/ml and the solution was analyzed for absorbance using UV- visible spectrophotometer at 296nm.

Assay was calculated using the below formula:

$$Assay = \frac{Test\ absorbance}{Standard\ asborbance}\ X\ \frac{Standard\ concentration}{Test\ concentration}\ X\ 100$$

RESULTS AND DISCUSSION

Determination of absorbance maxima:

The absorption maxima of the drug were determined and was found to be 296 nm, identical to the reported value in literature which confirmed the obtained sample.

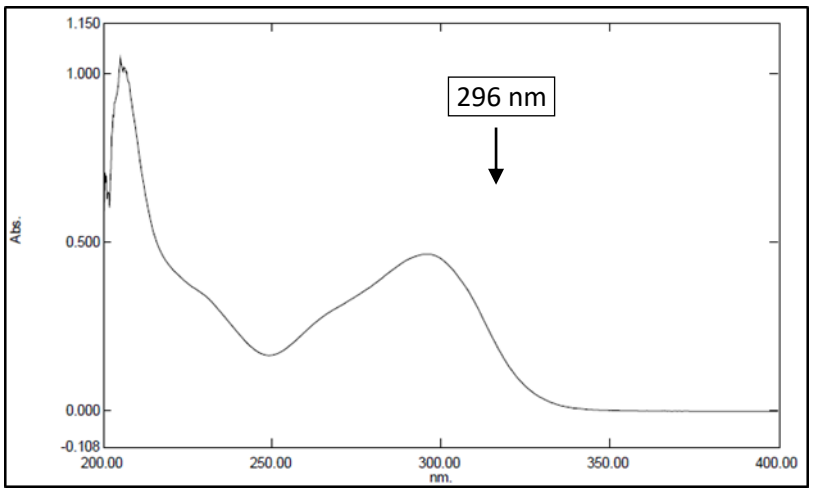

Fig.2. Absorption maxima of Luliconazole in Methanol: water

Calibration curve

The Calibration curve was prepared based on the absorbance of the series of concentrations ranging from 4-12 µg/ml. It is obtained by plotting absorbance Vs Concentration µg/ml data as reported in table 1. The correlation coefficient (r^2) was found to be 0.9993 and the equation of the calibration curve was found to be y=0.0469x + 0.0128 as shown in Fig. 3.

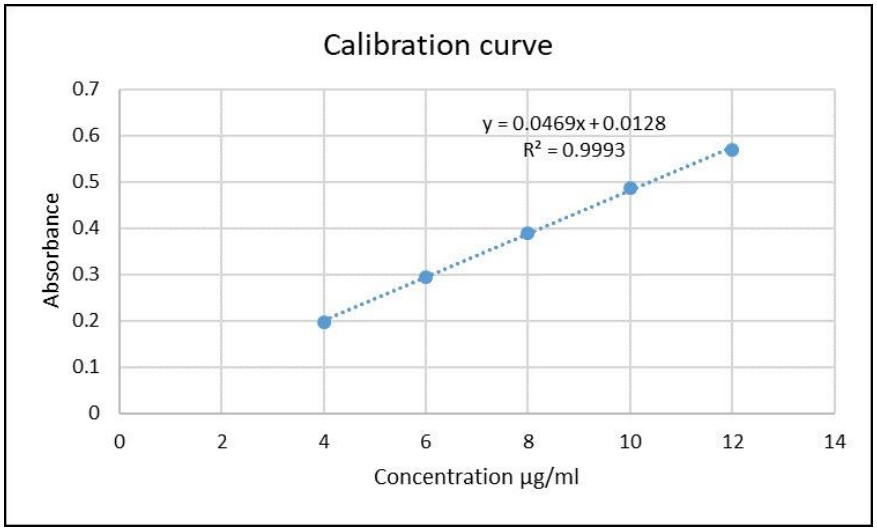

Fig.3. Calibration curve

Table 1. Calibration data

Concentration µg/ml	Absorbance
4	0.197
6	0.295
8	0.39
10	0.486
12	0.57

Validation of the method

1) **Linearity:** The linearity was determined for LCNZ at five different concentrations ranging from 4-12 µg/ml. The drug was found to be linear within the concentration range as shown in Fig.1.

2) Precision: Precision was performed to determine the repeatability and reproducibility of the method. Intra-day and Inter-day precision was performed, and the results are as reported in Table 2 and 3. The %RSD of Intra-day precision and Inter-day precision was less than 2% and hence the method is precise and reproducible.

3) Table 2. Intra-day precision

Concentration (µg/ml)	Intra-day precision					
	Morning (Mean Absorbance)	%RSD	Afternoon (Mean Absorbance)	%RSD	Evening (Mean absorbance)	%RSD
4	0.148	0.6758	0.146	0.393	0.149	0.671
8	0.351	0.591	0.351	0.328	0.350	0.329
12	0.543	0.425	0.549	0.277	0.549	0.210

Table 3. Inter-day precision

Concentration (µg/ml)	Inter-day Precision					
	Day 1 (Mean absorbance)	%RSD	Day 2 (Mean absorbance)	%RSD	Day 3 (Mean absorbance)	%RSD
4	0.148	0.812	0.148	0.778	0.144	0.400
8	0.351	0.197	0.352	0.866	0.351	0.164
12	0.547	0.651	0.543	0.368	0.544	0.742

4) Accuracy*:* The accuracy of the method was determined by spiking the formulation at three different levels (80%, 100%, and 120%) and determining the mean % recovery as reported in Table 4 which was found to be within an acceptable range.

5) Table 4. Mean % recovery of drug

Concentration of drug (µg/ml)	Level of recovery	Mean % recovery
8	80%	99.05
10	100%	99.65
12	120%	100.18

6) Limit of Detection and Limit of Quantification: LOD and LOQ were calculated by using the formula, standard deviation of the y-intercept of the regression line, and the slope of the calibration curve. The LOD and LOQ were found to be 0.437 and 1.325 respectively.

Assay

The assay of Luliconazole tablets was performed having label claim as 10mg and the results were found to be within the usual range (95%-105%) as reported in Table 5.

Table 5. Result of assay

Test absorbance	Standard absorbance	Standard Concentration (µg/ml)	Test Concentration (µg/ml)	Assay
0.478	0.482	10	10	98.35

CONCLUSION

A UV-visible spectrophotometric method was developed for quantification of Luliconazole in tablets and the developed method was validated for linearity, precision (intra-day and inter-day precision), accuracy, LOD, and LOQ parameters. The results obtained indicated that the developed analytical method is cost-effective, accurate, and precise.

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