DOI: 10.12923/cipms-2025-0012 Curr. Issues Pharm. Med. Sci., Vol. 38, No. 2, Pages 81-84

Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: https://czasopisma.umlub.pl/curipms

Reversed phase high-performance liquid chromatography for the evaluation of Luliconazole in bulk and pharmaceutical preparations

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ARTICLE INFO

Received 23 June 2023
Accepted 20 August 2024

Keywords:
cream,
luliconazole,
method development,
rp-hplc,
validation.

ABSTRACT

A new precise economical RP-HPLC analytical approach will serve as the basis for Luliconazole assay determination – both in bulk drug form and topical cream format. The separation took place on a HPLC device. The chromatographic separation was performed on a reverse-phase C-18 column (250 mm × 4.6 mm, 5 µ) using 0.1% OPA: acetonitrile (75:25) as the mobile phase at a flow rate of 1.10 mL/min. The wavelength of 298 nm was employed for detecting the effluents through the system. The physical measurement revealed that Luliconazole exists at 3.112 minutes, and the determined correlation coefficient value for Luliconazole measurement reached 0.996, while Luliconazole measurement in the formulation range from 98% to 102%. The study validated the Luliconazole (LOD) and limit of quantification (LOQ) values of 1.31 µg/mL and 3.98 µg/mL, respectively. Its simplicity, accuracy, rapid execution and ease of implementation revealed a successful application of method for the analysis of Luliconazole in bulk drug and topical cream formulations.

INTRODUCTION

Luliconazole (Figure 1) works against a wide array of infections. It belongs to the dichlorobenzene organic compounds as an antifungal and optically active imidazole medication [1]. Medical research suggests this drug exhibits its strong antifungal properties due to it blocking the fungal cytochrome P450 enzyme thus stopping ergosterol formation while preventing the synthesis of fungal cell walls [2]. The chemical name of Luliconazole is (2E)-2-{(4R)-4-(2, 4-dichlorophenyl)-1, 3-dithiolan-2-ylidene}-2- imidazol-1-yl-acetonitrile. Luliconazole's molecular formula is C14H9CL2N3S2, and its relative molecular mass is 354.267 g/mol. Luliconazole is the first topical broad-spectrum imidazole antifungal agent belonging to the dichlorobenzene class of organic compounds and an optically active R-enantiomer [3]. Different analytical methods such as HPTLC-Densitometry [4], RP-HPLC [5-6], and RP-UFLC [7] were developed for the estimation of Luliconazole in its bulk and dosage forms.

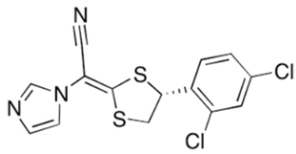

Figure 1. Luliconazole Structure

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MATERIALS AND METHODS

Material

Universal Twin Labs provided Luliconazole as LuleeTM Cream, while Luliconazole Cream 1% w/w (a cream manufactured in the US by Universal Twin labs) was bought from the market. HPLC-grade acetonitrile and HPLC-grade water were obtained from Pravara Rural College of Pharmacy, Pravaranagar (Finar), along with ortho-phosphoric acid (Qualigens). Reagents and chemicals used during RP-HPLC analysis were filtered using 0.45 µm filter paper prior to use.

Equipment and chromatographic parameters

The HPLC analysis of Luliconazole used a reversed phase C-18, (250 × 4.6 mm, 5 µ) and was equipped with an UV detection system and Lab Solution, running Version DB 6.110 software. Chromatographic separation was

successfully carried out at 25°C by employing 0.1% OPA in water made by transferring 1 ml of orthophosphoric acid into 1 000 ml of HPLC grade water and filtering this through 0.45 µm filter paper. An ultrasonic bath was used to degas (sonicated for 5 min for each) the mixture of acetonitrile in the ratio of 75: 25, respectively. The C18 column (250 mm × 4.6 mm, 5 µ) was first equilibrated with the mobile phase, in which the phase was pumped through the column for 30 minutes before injecting the drug solution. A 10 µL injection volume was performed, with a 1.10 mL/min flow rate and detection at 298 nm by UV.

Standard stock solution preparation

In creating the standard stock solution, 100 mg of Luliconazole was weighed accurately and transferred into a 100 mL volumetric flask. Approximately 70 mL of the mobile phase was added and the solution was sonicated for approximately 5 min until completely dissolved. The solution was then brought to the mark with the mobile phase and mixed well. Afterwards, 2.0 mL of this solution was diluted to 100 mL with the mobile phase so as to obtain a final concentration of 20 µg/mL. The sonicated solution was de-gassed before use.

Sample solution preparation

A quantity of Luliconazole cream, equivalent to 100 mg of Luliconazole, was weighed into a 100 mL volumetric flask containing 70 mL of the mobile phase. This was sonicated for 10 min with occasional shaking to ensure complete dissolution. The solution was then diluted up to the mark with the mobile phase and mixed well. Subsequently, 2.0 mL of this solution was diluted to 100 mL with the phase mobile phase to achieve a concentration of 20 µg/mL. Finally, the prepared solution was passed through a 0.22 µm syringe filter before analysis.

Method

Based on the system suitability parameters and the peak shape for Luliconazole, we believed that the applied chromatographic conditions and mobile phase would give satisfactory results. Isocratic mode was used with a flow rate of 1.10 ml/min of acetonitrile, 0.1% OPA (o-phthaldialdehyde) in water 75:25%v/v, being the mobile phase, and 35 to 85 megadaltons was pumped into the column. The wavelength of UV detector used was 298 nm. The injection volume was 10 µL, and the column was maintained at room temperature. The column was equilibrated by the drug solution for 30 minutes before injecting it. Individual portions from the sample solution, the standard solution, as well as a blank (diluent) were separately injected into the equipment. The integral of the chromatograms was calculated through the peak areas.

RESULTS AND DISCUSSION

Method validation

System suitability

The results indicate that system suitability parameters fall within the acceptance criteria, confirming the system's suitability. The injection of the standard solution demonstrated a tailing factor of less than 2.0. Additionally, the theoretical plates for the Luliconazole peak in the initial injection were found to be fewer than 1500 (Table 1).

Table 1. System Suitability assessment of Luliconazole

Parameters	Result
Peak Area	379109
Retention Time	3.112
Tailing factor	1.23
Theoretical plates	4565

Specificity (*Identification, interference & peak purity*)

The last was the injection of the standard solution, sample solution and diluent (blank). The retention time of the peak corresponding to Luliconazole remained the same on both standard and sample solutions. Throughout the Luliconazole retention time, analysis of the data confirmed no blank or placebo interference. In addition, chromatograms of the standard and sample solutions showed Peak Purity Match (Table 2, Figure 2 and Figure 3).

Table 2. Specificity (Recognition and Potential Interference)

Component	Retention time (min)	Area	Theoretical Plates	Asymmetry
Blank	-		-	-
Standard solution	3.105	386068	4408	1.238
Sample solution	3.112	388295	4491	1.214

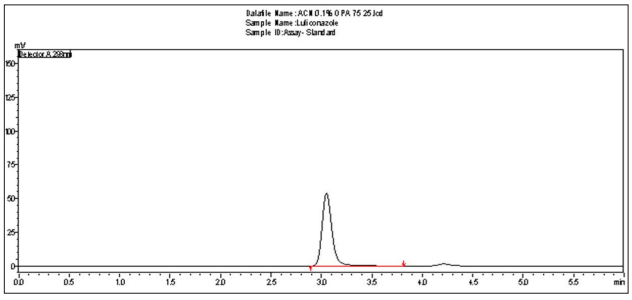

Figure 2. Chromatogram of Standard

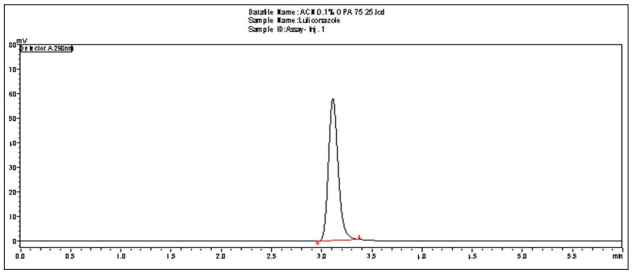

Figure 3. Chromatogram of Sample

Linearity

To discern the linearity, different concentrations of Luliconazole solutions (5 µg/mL~30 µg/mL) were prepared by accurately diluting a stock solution of Luliconazole. The correlation coefficient of 0.9968 indicates a linear response (Figure 4).

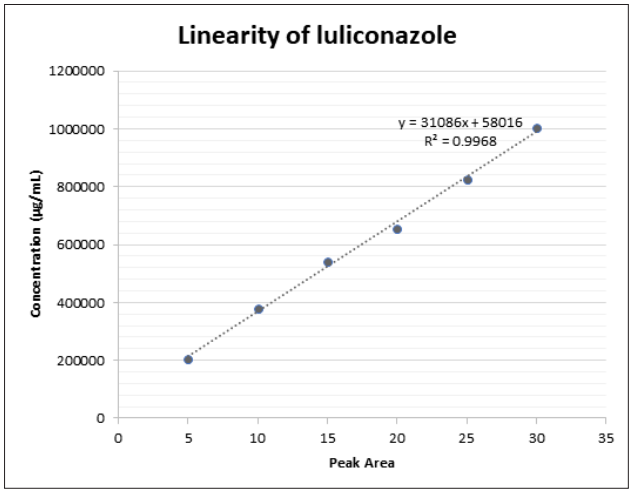

Figure 4. Linearity of luliconazole

% Recovery

Accuracy was assessed for luliconazole at three levels: 50%, 100%, and 150% of the working concentration of the sample solution. Each concentration, including a working concentration of 20 µg/mL Luliconazole, was prepared in triplicate. The data indicates that the mean recovery across 50% to 150% levels falls within the range of 98.0% to 102.0%, while individual recovery values range from 97.0% to 103.0% (Table 3).

Table 3. % Recovery for luliconazole

Levels	Area (m)	Recovered Conc. (µg/mL) (x)	Recovery (%)
50 %	469881	15.2	101.3%
100 %	602751	19.6	98.0%
150 %	783301	25.45	101.8%

Table 4. %RSD in Intraday Precision (Repeatability)

Sr. No.	Conc	Area			RT (min.)			Mean		%RSD	
		Morning	Afternoon	Evening	Morning	Afternoon	Evening	Area	RT (min.)	Area	RT
1	7 µg/ml	260518	260975	259592	3.126	3.122	3.116	260361	3.120	0.27%	0.16%
2	17 µg/ml	599760	598890	596910	3.132	3.129	3.110	598520	3.124	0.24%	0.38%
3	27 µg/ml	923512	920409	919507	3.142	3.128	3.116	921142	3.128	0.22%	0.41%

Table 5. %RSD in Intermediate Precision Luliconazole

Sr. No.	Conc	Area			RT (min.)			Mean		%RSD	
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Area	RT (min.)	Area	RT
1	7 µg/ml	260518	259697	260414	3.126	3.120	3.129	260209	3.124	0.17%	0.12%
2	17 µg/ml	599760	599902	596529	3.132	3.121	3.121	598730	3.124	0.31%	0.20%
3	27 µg/ml	923512	921866	918144	3.142	3.122	3.128	921174	3.130	0.29%	0.32%

Precision

The precision of the method was evaluated for both intraday (repeatability) and interday (intermediate precision) using working solutions of 7, 17, and 27 µg/mL. Each solution was injected under predefined chromatographic conditions, and the results, as presented in Tables 4 and 5, were within the acceptable relative standard deviation (RSD) limit of ≤2%. Additionally, intermediate precision was assessed by analyzing the same sample on two different days by two different analysts, yielding an RSD value of <2% [8].

Robustness

Robustness was evaluated by varying the flow rate and wavelength, followed by recording the chromatograms. The system suitability criteria were met. The percentage difference in peak area under each modified condition was found to be less than 2.0% compared to the control, as shown in Table 6.

Table 6. Robustness for Luliconazole

Changes in Parameter	Values	Retention time of luliconazole	Area	% Difference
Control	As per method	3.068	368124	NA
Change in Flow rate (±0.1 mL/min)	1.0 mL/min	3.258	369088	-0.3
	1.2 mL/min	2.927	366203	0.5
Change in wavelength (±2 nm)	296 nm	3.089	369222	-0.3
	300 nm	3.108	366848	0.3

LOD and LOQ

The limit of quantification for luliconazole measurement was determined to be 3.98 µg/ml. The method revealed capability to measure the drug according to the established values. The Evaluation determined that luliconazole has a Limit of detection (LOD) at 1.31 µg/ml. This method demonstrated the capacity to detect the drug through the acquired numerical data.

CONCLUSION

Both simplicity and high accuracy had been achieved for RP-HPLC analysis of Luliconazole drug in topical cream formulations. The methods exhibited complete resistance against any additives or matrix components. Multiple test parameters, including specificity, along with linearity and % recovery and precision and LOD and LOQ analysis were used for assessment. Analysis of Luliconazole concentrations between 5 µg/ml and 30 µg/ml showed strong linear correlation resulting in an R2 value of 0.9968. Luliconazole was detected during the retention period which lasted for 3.112 minutes. The technical precision expressed through the %RSD of all parameters stayed between 2% and less and, simultaneously, the recovery percentage remained in a permissible range of 97%-103%. The pharmaceutical industry can implement the RP-HPLC Method as it is a convenient approach for drug quality assessments in routine operations.

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