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ORIGINAL ARTICLE

RP-HPLC Method Development and Validation for the Estimation of Luliconazole in Semisolid Dosage Form

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ABSTRACT
Luliconazole (LCZ) is a novel imidazole antibacterial candidate for treating fungal infections on the skin. Its current treatment is limited by extremely poor and sluggish skin absorption, necessitating long-term, repetitive dosing to cure the condition completely. To perform batch release testing and to conduct stability studies of Luliconazole in pharmaceutical semisolid products, a stability-indicating analytical method is required to separate the active pharmaceutical ingredient peak from the peaks of all potential degradation products, process related impurities, potential packaging leachable, excipients, and also separate these compounds from each other. In the current developed method Inertsil ODS -3V was used as stationary phase. Whereas Mixture of 0.1% Perchloric acid buffer and acetonitrile in the ratio 80:20 as Mobile phase A and Mixture of 0.1% Perchloric acid buffer, acetonitrile and methanol in the ratio 20:70:10 as Mobile phase B in a gradient mode used as mobile phase. It is pumped through the chromatographic system at a flow rate of 1.5 ml min^{-1}. The UV detector is operated at 210 nm. The validation study is carried out fulfilling the ICH guidelines Q2 (R1) to prove that the new analytical method, meets the reliability characteristics, and these characteristics show the capacity of an analytical method to keep, throughout the time, the fundamental criteria for validation: selectivity, linearity, precision, accuracy and specificity. The stability indicating method is applied during the working day for the quality control of commercial luliconazole semisolid dosage form to quantify the drug and its degradation products and to check the Tube uniformity test.
Keywords: *Luliconazole, Assay, HPLC, Validation, Accuracy.*

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INTRODUCTION

Fungal pathogens are one of the most common dermatological illnesses worldwide, with over 150 million people suffering from severe fungal infections that significantly affect their lives or are deadly, with a high frequency in developing and underdeveloped countries. Even though fungal pathogens do not cause death, they are the leading source of morbidity and healthcare costs. Opportunistic fungal infections are common, 20–25 percent global frequency, and are linked to everyday activities, poor cleanliness, and inadequate care quality [1-2]. Luliconazole, an optically active R-enantiomer of Lanoconazole, was discovered as a novel imidazole molecule with greater patient compliance, higher effectiveness, and better tolerance due to continuous clinical research for improved topical therapeutics for fungal infection [3-4]. Luliconazole has been clinically evaluated for tinea pedis therapy, cruris, as well as corporis [5]. It has been demonstrated to have antifungal action contrary to dermatophytes and Candida in vitro. Luliconazole was first introduced in Japan in 2005 as a topical antifungal agent. It is now accessible as 1 percent creams and solutions for managing dermatophytosis, candidiasis, and Pityriasis Versicolor [6-8].
To perform batch release testing and to conduct stability studies for cream and ointment pharmaceutical products, a stability-indicating analytical method is required to separate the active pharmaceutical ingredient (API) peak from the peaks of all potential degradation products, process related impurities, potential packaging leachables, excipients, and also separate these compounds from each other. The finished product release specifications should include a content determination test with acceptance

criteria and limits for Luliconazole present in the formulation [9-10]. The drug product stability guideline Q1A (R2) issued by the International Conference on Harmonisation (ICH) suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and, hence, supporting the suitability of the proposed analytical procedures. Analytical method validation ensures that various HPLC analytical techniques shall give reliable and repeatable results; it is a crucial step in developing new dosage forms as it provides information about accuracy, linearity, precision, detection, and quantitation limits. According to the ICH guideline, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." It is now obligatory in the process of drug development to supply the validation data for the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines [11–14].

In the past, numerous techniques for the simultaneous or single measurement of Luliconazole have been developed, which have made use of a variety of equipment, such as the UV spectrophotometer, the high performance liquid chromatography, the HPLC-MS, and the UPLC [15-17]. In contrast, the current approach, which was developed via RP-HPLC for the determination of Luliconazole in dosage forms, was found to be simple, exact, quick, and cost-effective to perform. Although multiple RP-HPLC approaches for measuring LCZ were discovered in various works of literature, they were found to be complex and time-consuming. To promote green chemistry, the present research sought to create a new RP-HPLC technique for the measurement of LCZ in dosage form that was accurate, sensitive, cost-effective, and stability-indicating, while employing the fewest amount of hazardous chemicals possible. According to ICH Q2 guidelines, the validation of the newly designed LCZ, RP-HPLC technique was completed (R1). The accuracy, precision, linearity, specificity, limit of detection (LOD), and limit of quantification (LOQ) of the technique for LCZ, as well as the quantification of LCZ in indicated Semisolid dosage form, were evaluated throughout the development of the method for LCZ [18-20].

MATERIAL AND METHODS

Chemical and reagents

Dr. Reddy's Laboratories provided a gift sample of Luliconazole (99.73 percent pure) (Hyderabad, India). The HPLC-grade solvents used in this study were obtained from Merck Ltd. in Bangalore, India, including acetonitrile, methanol, Perchloric acid and water. All of the chemicals used were of the highest quality for HPLC.

Instruments and chromatography condition

The HPLC was carried out on a Waters 2695 Alliance system equipped with a 2996 photodiode array detector (Waters) (PDA). The standards were resolved on a reverse-phase Inertsil ODS -3V (150 mm× 4.6 mm, 5 μm). Whereas Mixture of 0.1% Perchloric acid buffer and acetonitrile in the ratio 80:20 as Mobile phase A and Mixture of 0.1% Perchloric acid buffer, acetonitrile and methanol in the ratio 20:70:10 as Mobile phase B in a gradient mode used as mobile phase. The selected diluent is mixture of water and acetonitrile in the ratio of 20:80. Before the first injection, the column was saturated for 30 min with the initial mobile phase. The temperature was maintained at 40°C. Injection volume was decided to maintain at 10 μL. The PDA was set by optimizing wavelength to give the best response for two peaks at 210 nm to acquire the chromatogram. A software system called Water Empower 3 was used to collect the chromatographic data for this study. The standard Luliconazole were identified by comparing the retention time and spectra obtained from the sample and standard solutions [20-22].

Selection of lambda max

Lambda max is selected using a UV spectrophotometer, and this decision is critical for the sensitivity of the RP-HPLC technique. By using an optimum wavelength, it is possible to detect an exact absorbance for any medication. In the current investigation, pure CC concentrations of 100 ppm were determined by scanning in the range of 200–400 nm using a high-performance liquid chromatography system (Waters 2695 Alliance system).

Preparation of Luliconazole Standard Stock Solution:

Weigh and transfer accurately about 40 mg of Luliconazole working standard to a 50 mL volumetric flask. Add about 35 mL of diluent, sonicate to dissolve and dilute to volume with diluent and mix well. (Theoretical Concentration: 800 ppm of Luliconazole)

Preparation of Standard Solution:

Pipette out 5.0mL of Luliconazole Standard Stock Solution transfer it into 50 mL volumetric flask and dilute to volume with diluent and mix well. (Theoretical Concentration: 80ppm of Luliconazole)

Preparation of Sample solution:
Weigh and transfer accurately about 2000 mg of sample to a 250mL volumetric flask, add about 150mL of diluent and vortex for 2 minutes. Sonicate for 15 minutes with intermittent shaking. Allow the Sample solution to equilibrate to room temperature. Make up to volume with diluent and mix well. Pipette out 5.0mL of above solution to 25mL volumetric flask and dilute to volume with diluent and mix well. Filter the solution through 0.45µm Teflon filter with discarding first five mL.

Method of analysis
The chromatographic conditions were maintained as previously mentioned, and the baseline stabilisation procedure was carried out for 30 minutes total. Following stabilisation, the repeatability of the Blank and the prepared concentration solution of the standard drug was tested in the respective peak regions of the Blank and the prepared concentration solution of the standard drug. For the purpose of quantification, the solution of the sample was injected. We estimated the response factor based on the standard peak ratio and the sample peak ratio. The same technique was done six times to ensure that the created method was adhering to the established standard of repeatability [23-25].

- **Validation of RP-HPLC method**

Accuracy
Accuracy is defined as the degree of agreement between the measured value and the genuine value. The three separate LCZ standard and sample solutions were obtained from concentrations ranging from 80 to 120 mg in the concentration range. These solutions were injected into the test subjects in order to determine the correctness of our devised procedure. A sample solution was generated for three duplicate concentrations, and the results were quantified in the meanwhile. The percentage of recovery was calculated using the methodology shown below. The recovery rate must be in the range of 98–102 percent in order to be considered satisfactory.

Precision
Precision may be defined as a measurement of real value between different outcomes of the same amount or quantity range. Analyzing six duplicate concentration solutions from 100 ppm on the same day and three separate days allowed researchers to examine intra-day and inter-day fluctuations in the concentrations. Calculation of the percent relative standard deviation (RSD) was accomplished by the use of the following equation: The normal acceptance restriction for percent RSD acceptance is less than 2 percent of the total.

Linearity
For the purpose of evaluating the linearity of LCZ solution, several concentration solutions ranging from 50 to 150 mg/ml (40, 64, 72, 80, 88, 96, and 120 ppm) were injected into the test tube. Each concentration solution was examined six times in the column under the identical conditions by injecting it into the column six times. The linearity calibration curve was developed based on the area peak obtained from the chromatogram and each concentration of LCZ solution used in the experiment. It was possible to calculate the coefficient of correlation (R2) using regression analysis calculations since the slope and intercept values were known. It is recommended that the standard limit of regression analysis be greater than 0.999.

System suitability
The appropriateness of the system was determined in accordance with the United States Pharmacopeia (USP). To determine the metrics such as column efficiency, resolution, peak symmetry factor, percentage coefficient in peak area or height, it was necessary to employ the prepared concentration solution of LCZ for six duplicate injections. A percentage RSD, theoretical plate value, tailing factor, and system accuracy were all calculated using the observed value as a starting point [26-28].

Specificity and selectivity
It was necessary to test the specificity and selectivity of the newly devised approach in order to identify the excipients that were interfering with the estimate of the LCZ. The blank solution, which did not include the LCZ, was produced and injected. The chromatogram produced from the blank was compared to the chromatograms obtained from the standard and sample, and the difference was examined to determine if excipients interfered with the drug quantification.

Robustness and ruggedness
The robustness and ruggedness of the created LCZ RP-HPLC technique were proved by modest modifications in the chromatographic conditions that were used in the development. In order to determine if any impacts were induced by the changes in parameters such as column temperature, flow rate, and the usage of various percentage ratios of mobile phase, the parameters were varied. The difference in chromatographic conditions was taken into consideration in the current investigation.
The following modifications to the Chromatographic conditions will be evaluated:

- Change in column Temperature (±5°C)
- Change in wavelength (±5 nm)
- Change in Flow rate (± 0.1 ml\min) 10% change [29-30].

RESULTS AND DISCUSSION:

Development of RP-HPLC method

The development of an RP-HPLC technique for the measurement of LCZ in dosage form was completed. Mixture of 0.1% Perchloric acid buffer and acetonitrile in the ratio 80:20 as Mobile phase A and Mixture of 0.1% Perchloric acid buffer, acetonitrile and methanol in the ratio 20:70:10 as Mobile phase B in a gradient mode used as mobile phase. A variety of chromatographic conditions, including flow rate, column temperature, and the components ratio in the mobile phase used, were tested in order to generate a crisp and symmetric peak with an appropriate retention period. In order to get a superior peak, the column Inertsil ODS -3V (150 mm× 4.6 mm, 5 µm) was employed. Because of the mobile phase employed in the procedure, the characteristics of the chromatographic settings such as retention duration, theoretical plate number (N), retention factor, and selectivity could be tailored to meet the needs of the researchers. The determination of wavelength was carried out using a Waters 2695 Alliance system equipped with a 2996 photodiode array detector in order to ensure adequate sensitivity of LCZ using the RP-HPLC technique (PDA). In order to discover the wavelength maxima, the standard solution of LCZ was scanned throughout a range of 200–400 nm, with a better peak being identified at 210 nm (Figure 1).

- **Validation of RP-HPLC method**

Accuracy

Placebo of Luliconazole gel was spiked with Luliconazole drug Substance at three different levels: 80%, 100% and 120% of the label claim in triplicate (in total nine determinations) and then proceeded with Sample solution as described under. As demonstrated in Table 2, the information received was deemed to be accurate. According to the findings, the percentage recovery of standard and sample LCZ was 101.2 percent, respectively. It was determined that the acquired result is within the range of typical recovery values (98.0 percent to 102.0 percent).

Precision

In this research, precision was examined in terms of system precision, technique accuracy, and intermediate accuracy. Using six duplicate injections of the same standard from the same vial, the accuracy of the system was measured and quantified in terms of percent relative standard deviation (percent RSD), tailing, plate count, and resolution. The sample was subjected to the above-mentioned method a total of six times. The percent assay for each analyte was represented as a percentage of the standard deviation (percent RSD). Intermediate precision was performed on two different systems, one using a Waters e2695 Alliance system with a 2996 PDA and the other using a 2489 ultraviolet (UV) detector, by different analysts by analyzing six different samples of extract, and the results were expressed as a percent relative standard deviation. The results of this investigation demonstrated a more exact and accurate approach for detecting LCZ in the dose form than previous methods (Table 3).

Linearity and range

The linearity calibration curves were found to have an R2 value of 1.0000. The calibration curve that was drawn in the concentration range of 40–120 ppm was found to be linear in nature (Table 4). The equation and regression coefficient (R2) are presented in Fig. 2. According to the results, the relative standard deviation was in the range of 1.0 to 1. A higher correlation value was discovered between the observation derived from peak value and the concentration of the drug solution than had previously been seen.

A series of Standard preparations of Luliconazole were prepared over a range of 50% to 150% of the working concentration of Luliconazole. (Minimum Five points in the range 80-120% of standard / sample concentration for Assay). Since the working concentration is 80 µg per ml of Luliconazole, the range proposed is about 40 µg per ml to 120 µg per ml of Luliconazole.

System suitability

For the system suitability investigations, a standard LNZ solution with a concentration of 100 ppm was used, and the results were evaluated. The time required for separation under chromatographic conditions was determined to be 9.9 minutes. A variety of frameworks, including the tailing factor, retention duration, theoretical plate number (N), and system accuracy, were determined to be within acceptable limits of 2 percent. The results obtained were within the acceptability requirements specified in the United States Pharmacopeia at the time of testing. Detailed data were displayed in Table 5, which may be seen here.

Specificity and selectivity

When comparing the results of the current study to those of past research studies on the same medication, it was discovered that the retention period with readily accessible mobile phase was superior. The approach was quantified accurately and with high resolution. It was not possible to draw any conclusions from the blank sample. A cost-effective mobile phase consisting of Mixture of 0.1% Perchloric acid buffer and acetonitrile in the ratio 80:20 as Mobile phase A and Mixture of 0.1% Perchloric acid buffer, acetonitrile and methanol in the ratio 20:70:10 as Mobile phase B is utilised in this procedure. Figure 3 shows the retention time for standard and sample LCZ concentrations. The retention time for standard and sample LCZ concentrations was 9.9 minutes. When compared to other approaches that have been developed, the retention time attained was found to be shorter.

Robustness

In order to determine the robustness of the currently developed luliconazole RP-HPLC method, minor variations in chromatographic parameters, such as the rate of flow of mobile phase (1.4 ml/min and 1.6 ml/min), different wavelengths (205 and 215), and temperature of the column (35 degrees Celsius and 45 degrees Celsius), were applied. The collected results did not reveal any statistically significant differences in peak area or retention duration. The percentage recovery of LCZ for the standard solution was almost identical to 99.0 percent, whereas the percentage recovery of LCZ for the sample solution was nearly identical to 99.2 percent. According to the findings, the percent RSD was less than 2.0 percent under various situations, indicating that the current approach is robust and tough. The values of % RSD as shown in Table 6 indicate better robustness of the method [28-30].

Table 1: Details of Gradient program

Time (Minutes)	Mobile Phase A (% v/v)	Mobile Phase B (%v/v)
0	80	20
10	65	35
15	32	68
17	80	20
20	80	20

Table 2: Accuracy studies of developed method

Sample No.	Amount added (mg)	Amount recovered (mg)	% Recovery
Acc. 80% -1	80.40	81.00	100.7
Acc. 80% -2	80.08	80.98	101.1
Acc. 80% -3	80.44	81.12	100.8
Acc. 100% -1	99.86	101.81	102.0
Acc. 100% -2	100.19	101.27	101.1
Acc. 100% -3	99.64	101.33	101.7
Acc. 120% -1	119.51	120.95	101.2
Acc. 120% -2	119.35	121.27	101.6
Acc. 120% -3	119.94	121.13	101.0
Mean			**101.2**
SD			**0.433**
% RSD			**0.43**

Table 3: Method Precision and Intermediate precision Results

Sample	Method Precision % Assay	Intermediate Precision % Assay
1	102.2	103.0
2	101.4	100.4
3	101.7	99.9
4	101.2	100.7
5	101.3	100.8
6	101.5	100.5
Mean	**101.6**	**100.9**
SD	**0.362**	**1.083**
%RSD	**0.36**	**1.07**
Overall Mean	**101.2**	
Overall SD	**0.845**	
Overall %RSD	**0.83**	

Table 4: Linearity of Luliconazole

% Concentration	Concentration (µg per ml)	Response (Area)	Statistical analysis	
50%	40.077	1217552	**Slope**	**30570.24206**
80%	64.124	1949310		
90%	72.139	2196004	**Intercept**	**-8617.79594**
100%	80.155	2440345		
110%	88.170	2686431		
120%	96.186	2932388	**Correlation Coefficient**	**1.0000**
150%	120.232	3665931		

Table 5: System suitability of Luliconazole

Sr.No.	Solution Name	Retention Time	USP Tailing	USP Plate Count
1	Standard	9.946	1.3	75214
2	Sample	4,256	1.3	73113

Table 6: Robustness for Luliconazole

Robustness parameter		% RSD	Remark
		Luliconazole	
Wavelength (nm)	205	1.88	Pass
	210	0.36	Pass
	215	1,27	Pass
Temperature (°C)	35	1,25	Pass
	40	0.36	Pass
	45	1.06	Pass
Flow (mL/min)	1.4	0.84	Pass
	1.5	0.36	Pass
	1.6	0.84	Pass

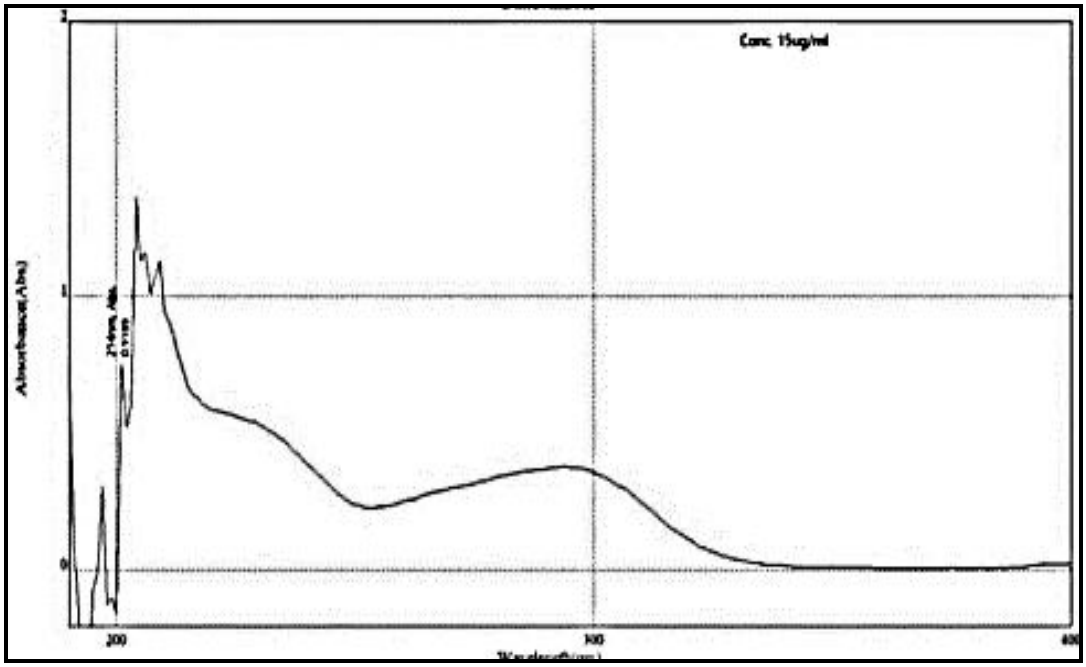

Figure 1: HPLC spectra of LCZ

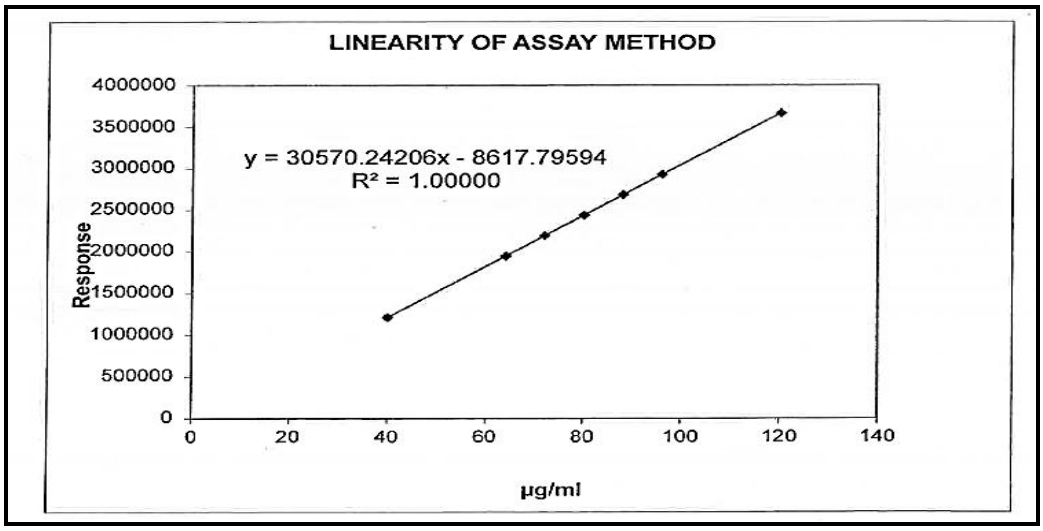

Figure 2: Linearity Graph for Luliconazole

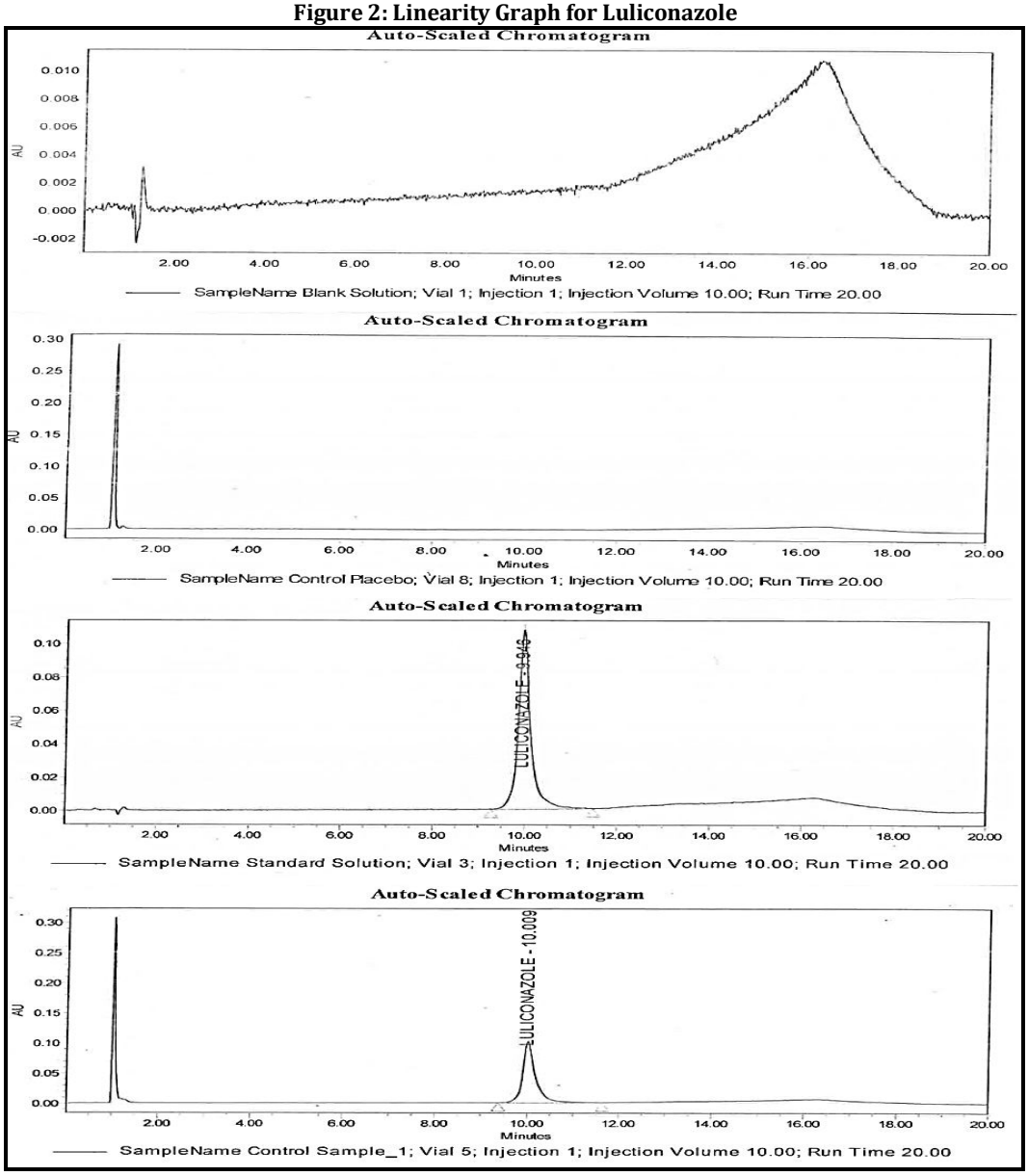

Figure 3: System suitability of Luliconazole

CONCLUSION

The RP-HPLC technique was used to produce an accurate, precise, robust, reliable, and repeatable approach for the quantitative measurement of LCZ in dosage form. The solvent that was utilised as the mobile phase has shown a very high resolution rate while requiring less retention time. The procedure was carried out in accordance with ICH and FDA rules, and the report received fulfilled all of the required standards. The accuracy, precision, and linearity of the procedure were all evaluated in order to ascertain the quality of the drug content. The RP-HPLC technique presented here enables for the measurement of LCZ in a repeatable manner. According to the statistical data, the proposed approach may be effectively used to our regular determination method with success. The specificity report demonstrated that the excipient had no effect on the results. A kinetics investigation using plasma and biological fluids may be included to this research as a further extension. The fact that this innovative approach demonstrated a greater cost-effectiveness ratio when compared to the previously reported studies was a significant finding.

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CONFLICTS OF INTEREST

No conflicts of interest are declared by the authors.

AUTHORS' CONTRIBUTION

Vibhavari M. Chatur & Shashikant N. Dhole were involved in the sample selection, the planning and execution of lab research, the interpretation of data, and the writing of the report. Shashikant N. Dhole's efforts include data analysis and chemical identification. The final document was interpreted and approved by each author.

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