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RESEARCH ARTICLE

Method Development and Validation of Luliconazole Bulk Drug Using Reverse Phase - HPLC Technique

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Abstract

Objective: To develop and validate the RP- HPLC method for determining Luliconazole in bulk and pharmaceutical formulations. **Methods:** Following the International Conference on Harmonization (ICH) guidelines, a sensitive, accurate, and specific reversed-phase HPLC technique was created and validated for the detection of Luliconazole. Using high-performance liquid chromatography, this drug was examined. A better separation of the drug was achieved by using Agilent Eclipse XDB C (4.6mm x 250mm, 5μm) with a mobile phase consisting of a mixture of Acetonitrile and Buffer in HPLC water. The ratio of 30:70 v/v (Formic acid and Acetonitrile) at a flow rate of 1.0 ml/ min and the detection was at the wavelength 295 nm using a Photodiode Arrays Detector. The planned method was able to produce good quality separation of the drug and its degradation products with sharp peaks. **Findings:** Luliconazole had a retention time of 2.38 $\pm$ 0.127 minutes. The technique was linear in the range of 10-100 μg/ml, with a correlation (r^2) of 0.9995 and a run duration of 4 minutes. The method limit of detection (LOD) and limit of quantification (LOQ) were set at 0.1 and 1 g/ml, respectively. The method accuracy and system precision were estimated, and the findings were calculated as percentage RSD values, which were found to be limitations. Luliconazole recovery was 100% confirming the method's efficiency. **Novelty:** The presented approach was innovative and successfully fulfilled all validation requirements, including linearity, robustness, accuracy, reduced retention, and run time. This suggests that the method is appropriate for identifying the stability of luliconazole in bulk and pharmaceutical formulations.

Keywords: RP-HPLC; Transferosome; ICH guidelines; Validation; Luliconazole

1 Introduction

Luliconazole (LUZ) belongs to the imidazole class of drugs renowned for their potent antifungal effects, especially against dermatophytes. The USFDA has approved the commercial formulation of cream since 2013[1]. LUZ is a BCS (Biopharmaceutical Classification System) class II drug, which means it is highly permeable and weakly soluble. The molecular formula for Luliconazole is $C_{14}H_9C_{l2}N_3S_2$. The enzyme lanosterol demethylase, which is crucial for the production of ergosterol, a crucial component of the fungal cell membrane, is thought to be blocked by LUZ, and its disruption can alter the fluidity and integrity of the cell membrane and cell wall. Luliconazole available in the marketed formulation as 1% w/v cream is associated with lower skin permeation and shorter skin retention of the drug[2–4]. To overcome these conditions, targeted drug delivery is selected by using the carrier, which enhances the skin permeation and also prolonged the action of the drug. Here transferosomal patches were selected for drug administration. The goal of the current study was to develop and validate the RP-HPLC (Reverse Phase - High-Performance Liquid Chromatography) method that would meet ICH requirements for the quick, easy, and accurate determination of LUZ[5]. The advancement of analytical techniques helps in the comprehension of critical process parameters and minimizes their effect on accuracy and precision. In the pharmaceutical industry, validation is a critical process that improves the standard of work and helps in the development of medications and other products. The objective of this study was to maintain sensitivity while reducing cost, time, and retention time efficiency. Therefore, it may be a useful technique for routine analysis of luliconazole.

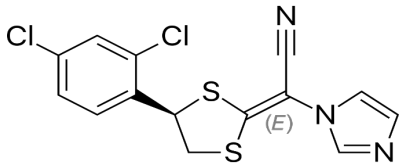

Fig 1. Structure of Luliconazole

2 Methodology

2.1 Chemicals and Reagents

The LUZ is a gratis sample from, Apex Labs Chennai. Acetonitrile HPLC grade was procured from Hi Media. Formic acid and Methanol were purchased from fine chemicals. All other chemicals and reagents used were of analytical grade.

2.2 Instrumentation

An autosampler and PDA detector-equipped water 2489 HPLC system was used for the method development and validation. Agilent Eclipse XDB, size (250mm x 4.6mm, 5m), was used to conduct the analysis at room temperature. With the aid of the Empower 2 program, the data was compiled and evaluated.

2.3 Preparation of solutions

The 1ml formic acid is dissolved in a 1000 ml volumetric flask, the volume is dissolved and diluted to 1000ml with HPLC grade water (Milli- Q water). The pH was adjusted and the buffer was degassed in an ultrasonic water bath and filtered through a 0.45μm filter using vacuum filter using vacuum filtration.

2.3.1 Preparation of mobile phase

The mobile phase was prepared by mixing with 0.1% formic acid and acetonitrile in the ratio of 30: 70 v/v. The mobile phase was degassed in the ultrasonic water bath for 15 min and it can be filtered through a 0.45 μm filter using vacuum filtration.

2.3.2 Preparation of diluent

The mobile phase is used as a diluent.

2.3.3 Preparation of Luliconazole Standard Solution

5 mg of Luliconazole standard was weighed into a 10 ml volumetric flask and diluted to the volume with diluent (mobile phase). Further, 1 ml of the above solution was diluted to 10 ml using a diluent to get the final concentration of 10 μg/ ml.

2.3.4 Preparation of Luliconazole Sample Solution

Transfer 64.5mg of Luliconazole tablet (Each tablet contains 200mg of Luliconazole) transferred into a 100 ml volumetric flask and add 70 ml of diluents, and sonicate to dissolve and make up. Further diluted with the 5ml of the above solution into 50ml volumetric flask and diluted to volume with diluents, then filter through 0.45μ nylon syringe filter[6].

2.3.5 Selection of wavelength for method development

Luliconazole was prepared in stock solution at a concentration of 1000 mg/ml, and then serial dilutions were made using methanol to achieve the concentration of 100 g/ml. By scanning the above-mentioned drug solution between 200 and 400 nm, the wavelength was determined. The scan results revealed that the absorbance peaked at 295 nm. As a result, shows the RP-HPLC detection wavelength.

2.4 Method development

The chromatographic method was developed by performing the trials considering the response as United States Pharmacopoeia (USP) plate count and tailing factor. The variables considered for conducting experiments were column type and mobile phase composition. The wavelength (295 nm), flow rate (1.0 ml/min), and injection volume (10μl) were kept constant throughout the trials.

2.4.1 Construction of Calibration Curve

The standard stock solution was diluted using diluent (Mobile phase) to get the various concentrations of 10, 20, 40, 60, 80, and 100 μg/ ml. The chromatograms were captured under ideal chromatographic circumstances. The chromatograms were used to compute the mean peak areas at various concentration levels. The mean peak areas of the corresponding concentrations were then used to build the linear plot.

2.5 Method Validation

The suggested technique was verified for the ICH-recommended characteristics of specificity, linearity, accuracy, precision, limit of detection, limit of quantitation, robustness, and system applicability[7,8].

2.5.1 Specificity

Spiking a pure particular concentration of a drug with allowable levels of impurities demonstrated specificity, which was explained. A marketed formulation of LUZ from Apex Labs was used to assess specificity in 6 repetitions at a concentration of 100 g/ml, and % RSD (Relative Standard Deviation) was obtained.

2.5.2 System Suitability

System suitability is the process of ensuring system performance before or during the analysis of unknowns. By conducting six replicate analyses of LUZ at a concentration of 100 g/ml, the suitability of the system was evaluated. The peak area and LUZ retention times had to be under 2% to be accepted.

2.5.3 Linearity and Range

The capacity to provide test results that are directly proportionate to analyte concentration is referred to as linearity. To evaluate linearity, three injections of eight different LUZ concentrations (10, 20, and 30) were employed. Peak areas and average concentrations were plotted. Plots of concentrations v_s linear area were made. In order to evaluate the linearity using linear regression analysis, the least square regression approach was used. A correlation coefficient (r^2) value of 0.999 is typically regarded as proof that the data fit the regression line well.

2.5.4 Accuracy

The degree to which the expected and observed values are near to each other is expressed by an analytical procedure's accuracy. By dividing the analyte recovered by the percentage recovery (% R), it is calculated. In order to assess the efficacy of the suggested

procedure, a successful analysis (n=3) for three different concentrations of LUZ solution (50g/ml) was carried out in this example. The experiment data were statistically analyzed using the formula (% Recovery = (Recovered conc/ injected conc) to examine the recovery and validity of the designed technique. The typical recovery should range from 90 to 110% in order to be acceptable.

2.5.5 Precision

Using the devised method and six replicate analyses with a concentration of 100 g/ml of standard LUZ solution, the precision was computed. The precision was expressed as % RSD, and it was discovered to be less than 2%, demonstrating the system's good precision in accordance with USP[9–13].

2.5.6 Robustness

Robustness is an evaluation of the accuracy of an analysis to purposeful changes in technique parameters. It involves measuring changes in analytical circumstances. In the current investigation, robustness was examined by permitting a slight deliberate alteration in injection flow rate, and organic phase buffer concentration was calculated using the RSD of %.

2.5.7 Limit of detection and Limit of quantification

Under certain experimental settings, the LOD is the lowest quantity in a sample that can be detected but not always quantified. The lowest concentration of an analyte that may be identified with reasonable accuracy and precision is known as the LOQ. The formula was used to determine these two parameters.

$$LOD = \frac{3.3\sigma}{S}$$

Where, σ = standard deviation of response and S = slope of the calibration curve.

3 Results and Discussion

The purpose of developing the RP-HPLC method was to separate and measure the significance of any impurities or other excipients[14]. The technique was developed with the purpose of resolving chromatographic peaks for active pharmaceutical ingredients (LUZ). The Agilent Eclipse XDB C (4.6mm x 250mm, 5μm) used a mobile phase of acetonitrile and buffer in HPLC water. The ratio of formic acid and acetonitrile was 30:70 v/v at a flow rate of 1.0 ml/min, with detection at 295 nm using a Photodiode array detector. The approach was linear between 10-200 μg/ml, with a correlation (r^2) of 0.9995 and a run time of 4 minutes. The literature review data reveals that there were no previously published methods available for method development and validation of LUZ transferosomal patches, indicating the novelty of this current work.

3.1 Development of HPLC Method

The chromatogram represents the blank mobile phase and the chromatogram represents LUZ's average retention time of and with no interfering peaks. This is an indication of the specificity of the developed HPLC method (Figure 2).

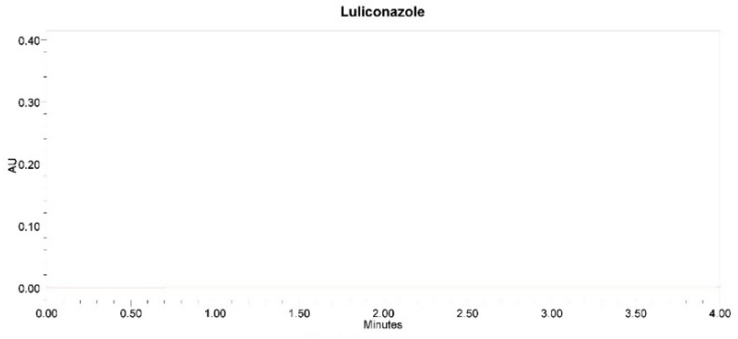

Fig 2. Blank chromatogram

3.1.1 System Suitability

System suitability was assessed by six replicate analyses of LUZ at a concentration of 100μg/ ml. The acceptance criterion was $\pm$2% for the percentage relative standard deviation (% RSD) for the peak area and retention times for LUZ as shown in Table 1.

Table 1. System suitability of Luliconazole sample

S.NO	Name of Drug	RT	Area	% Area	USP plate count	USP tailing
1	LUZ	2.317	2930654	100.0	2358	1.14
2	LUZ	2.313	2977351	100.0	2652	1.69
3	LUZ	2.310	2987251	100.0	2452	1.56
4	LUZ	2.315	2915471	100.0	2164	1.24
5	LUZ	2.316	2933031	100.0	2261	1.48
6	LUZ	2.312	2902356	100.0	2236	1.75

3.1.2 Linearity

From the stock solution 10, 20, 40, 60, 80, and 100 μg/ml solution was made and their chromatograms were recorded chromatograms their respective mean peak areas were calculated and the linearity plot was constructed using the mean peak areas of their respective concentrations. The correlation coefficient (r^2) was found to be **0.9991**. The linearity curve and regression data are shown in Figure 3, and Table 2 respectively.

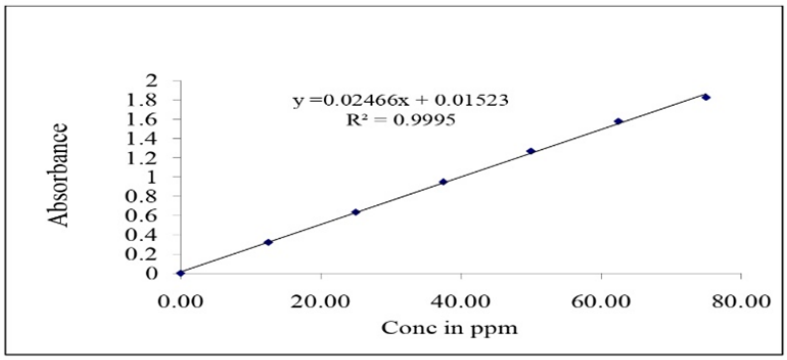

Fig 3. Calibration plot of Luliconazole

Table 2. Regression parameters of Luliconazole

Parameters	Results
Linearity	10-100
Regression equation	Y=0.02466x+0.01523
Slope	0.02466
Intercept	0.01525
Correlation coefficient (r^2)	0.99949

3.1.3 Accuracy

A recovery study was conducted to evaluate the method precision at the three levels of 50%, 100%, and 150%. For each level, a recovery study was conducted three times, and the percentage of recovery and relative standard deviation were computed. LUZ average recovery was found to be 100.1% The accuracy of the chromatogram is shown in Figure 4.

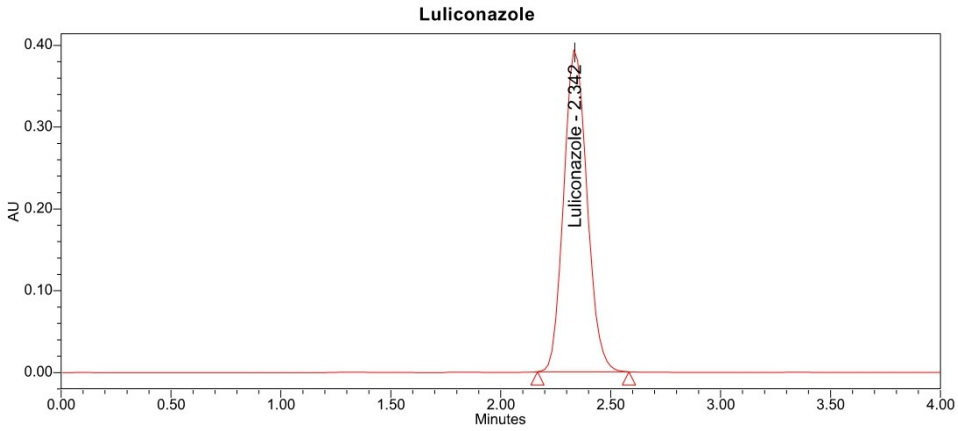

Fig 4. Sample Name LUZ 100%-1; Vial 43; Injection 1; Injection Volume 20.00; Run Time 4.00 Accuracy Data 100%

3.1.4 Precision

Precision was indicated in percentage relative standard deviation (% RSD) which was determined to be less than 2% displaying satisfactory precision of the system. This indicating the current method is precise and reproducible.

3.1.5 Robustness

Robustness was evaluated by permitting the modest change in injection flow rate and organic phase buffer concentration by $\pm$ 7 and % RSD was determined and findings are presented in table. It was found that the concentration of the organic phase was less than 2% when the flow rate was slightly altered.

3.1.6 Limit of Detection and Limit of Quantification

LOD and LOQ were found to be 0.1 and 1 μg/ ml respectively. This expresses the sensitivity of the developed method. The results of LOD and LOQ are shown in Table 3.

Table 3. LOD and LOQ of Luliconazole

S.No	Luliconazole	RT	Area	% Area	USP plate count	USP Tailing
1	LOD	2.346	87289	100.0	2277	1.11
2	LOQ	2.350	295784	100.0	2284	1.56

4 Conclusion

A simple, precise, specific, and exact RP-HPLC method obtained results that met all of the validation criteria for Luliconazole in bulk and therapeutic dosage form. The approach is linear throughout a wide range, cost-effective, and utilizes a mobile phase that can be promptly prepared. The percent RSD of precision was determined to be less than 2%. LUZ retention time was 4 minutes, with a mean recovery of 100%. The LOQ validated the approach's sensitivity. The method was validated in accordance with ICH criteria and it can be used for routine luliconazole analysis.

References

1) Kumar M, Shanthi N, Mahato AK, Soni S, Rajnikanth PS. Preparation of luliconazole nanocrystals loaded hydrogel for improvement of dissolution and antifungal activity. *Heliyon*. 2019;1(5). Available from: https://doi.org/10.1016/j.heliyon.2019.e01688.
2) Rani M, Parekh K, Mehta T, Omri A. Formulation development and characterization of luliconazole loaded—mesoporous silica nanoparticles (MCM—48) as topical hydrogel for the treatment of cutaneous candidiasis. *Journal of Drug Delivery Science and Technology*. 2024;91:105250. Available from: https://doi.org/10.1016/j.jddst.2023.105250.
3) Porto DS, Bajerski L, Malesuik MD, Paim CS. A review of characteristics, properties, application of nanocarriers and analytical methods of Luliconazole. *Critical Reviews in Analytical Chemistry*. 2022;52(8):1930–1937. Available from: https://doi.org/10.1080/10408347.2021.1926219.
4) Shah N, Prajapati R, Gohil D, Aundhia C, Sadhu P, Kardani S. Luliconazole Loaded Niosomal Topical Gel: Factorial Design, in vitro Characterization and Antifungal Study. *Indian Journal of Pharmaceutical, Education & Research*. 2023;57(3s):520–527. Available from: https://doi.org/10.5530/ijper.57.3S.60.

5) Kadam GM, Puyad AL, Kalyankar TM. RP-HPLC method development and validation for the estimation of sacubitril and valsartan in pharmaceutical dosage form. *Research Journal of Pharmacy and Technology*. 2021;14(11):5797–5802. Available from: http://dx.doi.org/10.52711/0974-360X.2021.01008.
6) Patel MM, Patel D, Shah U, Kachhiya HM. A simple, precise, and sensitive RP-HPLC method for quantification of teneligliptin hydrobromide and metformin hydrochloride: development and validation. *Research Journal of Pharmacy and Technology*. 2023;16(2):495–501. Available from: http://dx.doi.org/10.52711/0974-360X.2023.00084.
7) Kumar PS, Manickam MS, Sangeetha G. RP-HPLC method development and validation of Tapentadol hydrochloride in bulk and pharmaceutical formulations. *Journal of Pharmaceutical Research International 2021*;15(11). Available from: https://doi.org/10.5530/ijper.57.3S.60.
8) Dandagi PM, Pandey P, Gadad AP, Mastiholimath VS. Formulation and evaluation of microemulsion based luliconazole gel for topical delivery. *Indian Journal of Pharmaceutical, Education & Research*. 2020;54(2):293–301. Available from: https://doi.org/10.5530/ijper.54.2.34.
9) Urmi KF, Nawaz MS, Islam SM. Analytical quality by design approach to RP HPLC method development and validation for simultaneous estimation of esomeprazole and naproxen in modified-release dosage form. *Future J Pharm Sci*. 2022;8(8). Available from: https://doi.org/10.1186/s43094-021-00396-z.
10) Soni A, Sharma N, S R. Formulation and Evaluation of Luliconazole Suppositories for the Treatment of Candida Infection (vulvovaginal candidiasis). *Journal of Pharmaceutical and Drug Regulatory Affairs*. 2022;p. 31–39. Available from: https://matjournals.co.in/index.php/JPDRA/article/view/1042.
11) Ismail Y, Prasad MV, Shaheedha SM, Habeeb M. A New Stability Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Dolutegravir and Rilpivirine in Bulk and its Dosage Forms: Stability indicating analytical method for simultaneous estimation of Dolutegravir and Rilpivirine by RP-HPLC. *Iranian Journal of PharmaceuticalSciences*. 2019;15(4):53–72. Available from: https://doi.org/10.22037/ijps.v15.40454.
12) Pawar SS, Deshmukh DD, Gorde PL, Gosavi SA. Development and validation of RP - HPLC method for quantitation of luliconazole in bulk and formulation. *International Journal of Health Science*. 2014;6(S2):14944–52. Available from: https://sciencescholar.us/journal/index.php/ijhs/article/view/8962.
13) Patil K, Patil AS, Pawar SP. Method Development and Validation of Luliconazole by UHPLC in Bulk and Topical Dosage Form. *International Journal of Pharmaceutical Sciences*. 2024;2(6):1068–1075. Available from: https://doi.org/10.5281/zenodo.12206014.
14) Carlota RR, Nayak A, Reddy YV, Prathibha KR, Nirma T, Marbaniang D. RP HPLC-Based Method Development, Validation and Stability Indicating Assay of Levosulpiride in Bulk Drug Using Analytical Quality by Design Approach. *Indian Journal of science and technology*. 2023;16(46):4436–4444. Available from: https://doi.org/10.17485/IJST/v16i46.2995.