"ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF LULICONAZOLE IN BULK AND CREAM FORMULATION"

Sagar Vishnu Badak

(M Pharm Chemistry)

Abstract

A new simple, specific, precise & validated Analytical Method has been developed for the determination of Luliconazole in bulk and pharmaceutical formulation. The separation was achieved on a C18 ODS (250×4.6 mm,5µm) or using mobile phase consisting of methanol: water (85:15) at a flow rate of 1.0 ml/min. Detection was carried out at 296nm. The retention time of Luliconazole was found to be 4.2min. the calibration curve found linear between range of 20-60 µg/ml with a regression coefficient of 0. 9998. The percentage recovery of Luliconazole was found to be in the range of 90-110%. The method was validated in accordance with International Conference on Harmonization. LOD & LOQ for Luliconazole were found to be 0.24µg & 0.748µg/ml respectively. Present method is simple, precise and can be used in routine analysis of Luliconazole in bulk and pharmaceutical lotion.

Objective: To develop and validate the RP- HPLC method for determining Luliconazole in bulk and pharmaceutical formulations. Methods: Followingthe 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 ofthe drug and its degradation products with sharp peaks. Findings: Luliconazole had a retention time of 2.38 ± 0.127 minutes. The technique was linear in the range of 10-100 μg/ml, with a correlation (r2) 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

LIST OF ABBREVEATION

ACN	Acetonitrile
AUC	Area under curve

C_{18}	n-octadecyl
Cm	Centimeter
Cef	Cefixime
Conc.	Concentration
DNA	Deoxy ribose nucleic acid
Gm/lit	Grams per litre
HETP	Height Equivalent to Theoretical plate
HPLC	High performance Liquid Chromatography
HPTLC	High performance Thin layer Chromatography
ICH	International Conference of Harmonisation
I.R.	Infra-Red
L.C.	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantitation
MOXI	Moxifloxacin
mg	Miligram
µg	Micro gram
µL	Micro Litre
nm	Nano Meter
RF	Retention Factor
RSD	Relative standard Deviation
RPM	Revolution Per Minute
RP-HPLC	Reverse phase High performance Liquid Chromatography
R_S	Resolution
Rt	Retention Time
TLC	Thin Layer Chromatography
USP	United State of pharmacopeia
UV	Ultraviolet
λmax	Wavelength Maximum

1. INTRODUCTION

Antifungal compose a large & diverse group of drugs used to treat fungal infections. These agents are usually classified as either systemic or topical, although these divisions are somewhat arbitrary since many may be administered in either way. The mechanism of action of Antifungals include inhibition of fungal membrane & Cell wall synthesis, alteration of Fungal membranes, effects o n microtubules and inhibition of nucleic Acid synthesis. The fungal infections caused by invasion of microorganism in epithelial tissue. Over all kind of fungal agents some are not harmful but some are pathogenic in humans. These pathogenic fungi after en try in human body may cause mild to severe infection.

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 $C_{14}H_9Cl_2N_3S_2$ The enzyme lanosterol demethylase, which is crucial for the production ofergosterol, 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 a dvancement of analytical techniques helps in the comprehensionof 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.

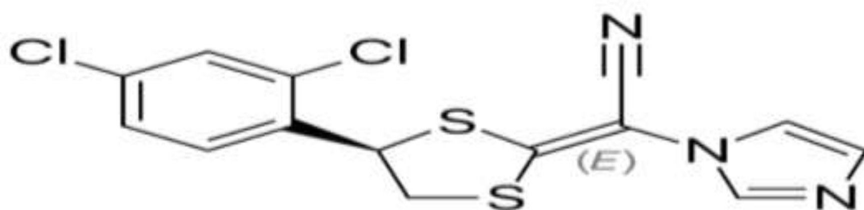

Fig 1. Structure of Luliconazole

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2. Methodology

2.1 Chemicals and ReagentsThe 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 a0.45μm 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 400nm, 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 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)

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2.4.1 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.

2.4.2 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.

2.4.3 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 linearregression analysis, the least square regression approach was used. A correlation coefficient (r2) value of 0.999 is typically regarded as proof that the data fit the regression line well.

2.4.4 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.

2.4.5 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)

2.4.6 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 %.

2.4.7 Limit of detection and Limit of quantificationUnder 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.$LOD = 3.3\sigma S$

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

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 (r2) 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.

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. 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.

The results of present study indicate that the proposed RP - HPLC method is simple, rapid, precise and accurate. The developed RP-HPLC method was found suitable for determination of Luliconazole in marketed formulation without any interference from the excipients. From the above data, it was observed that all the validation parameters meet the predetermined acceptance criteria.

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