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Development And Validation of Stability-Indicating Hptlc and Rp-Hplc Methods for Determination of Luliconazole in Bulk Drug, Tablet, And Semisolid Dosage Forms

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ABSTRACT

The primary objective of this study was to develop and validate stability-indicating HPTLC and RP-HPLC methods for the estimation of luliconazole in bulk, tablet, and semisolid dosage forms. Both methods were designed to meet ICH guidelines, ensuring precision, accuracy, sensitivity, and specificity. In the HPTLC method, separation was achieved using silica gel 60 F254 plates with a methanol:toluene mobile phase, allowing clear resolution of luliconazole from excipients and degradation products. The RP-HPLC method utilized a C18 column with an acetonitrile-water mobile phase (pH 3.5 adjusted with orthophosphoric acid), achieving excellent separation and reproducibility. Force degradation studies subjected luliconazole to hydrolytic (acidic and basic), oxidative, thermal, and photolytic conditions. Clear degradation peaks confirmed the drug's susceptibility to stress while demonstrating the specificity of the methods, as excipients did not interfere with the analysis. Validation parameters—system suitability, robustness, accuracy, precision, and linearity—were within ICH-specified limits. Accelerated stability studies on semisolid dosage forms over six months revealed no significant degradation, indicating the formulation's stability under recommended storage conditions. Overall, the HPTLC method provided a rapid, cost-effective option for routine analysis, while the RP-HPLC method offered higher sensitivity, precision, and robustness. In conclusion, the developed HPTLC and RP-HPLC methods are reliable, efficient, and suitable for regular quality control and stability studies of luliconazole across various dosage forms, ensuring consistent product quality and regulatory compliance throughout the shelf life..

Keywords: *Stability-indicating methods, Luliconazole, HPTLC, RP-HPLC, Force degradation studies, Validation parameters, Quality control*

1. INTRODUCTION

One of the main reasons of skin issues is often environmental fungi. Every year, diseases caused by fungi claim the lives of almost 150 million people globally. These diseases are most common in the third world and the undeveloped countries. Fungal pathogens are the most common cause of sickness and high medical care costs, even though they do not cause death. Opportunistic fungal infections affect 20 to 25 percent of people worldwide and are linked to common causes like unclean surroundings and inadequate medical attention. Luliconazole, also known as the R-enantiomer, is the optically active form of Lanoconazole. During ongoing clinical research for more effective topical treatments for fungal infections, a novel imidazole molecule was found to have improved patient compliance, increased efficacy, and improved tolerability. Laboratory studies have demonstrated that Luliconazole is effective against both dermatophytes and Candida, and it has been shown to cure tinea pedis, as well as cruris and corporis. In 2005, licorice-derived luconazole was introduced as a topical antifungal therapy in Japan.[1] Luliconazole is a member of the imidazole class of drugs, which are highly effective against dermatophytes and have a broad range of antifungal action. Chemically, luliconazole is (2E).(2, 4-dichlorophenyl)-1, 3-dithiolan-2-ylidene -2- [(4R)-4-]1-yl-acetonitrile-2-imidazol. Its formula for structure is [2]:

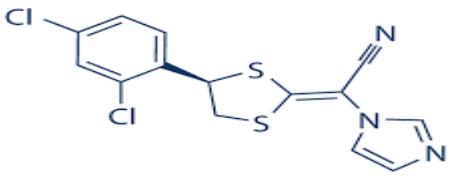


Figure 1. Luliconazol

Antifungals are a broad and varied class of medications used to treat fungal infections. Since many of these drugs can be delivered in either manner, the classifications of systemic and topical are mostly arbitrary. Antifungals work by inhibiting the synthesis of fungal membranes and cell walls, changing fungal membranes, affecting microtubules, and preventing the synthesis of nucleic acids. fungal infections brought on by microorganisms invading epithelial tissue. Generally speaking, some fungi are harmless, but others can cause disease in people. After entering the human body, this harmful fungus can cause infections ranging from mild to severe [3]. A stability-indicating analytical technique that isolates the API peak from the peaks of all potential degradation products, process-related contaminants, potential packaging leachables, and excipients is necessary for pharmaceutical cream and ointment products. Additionally, it must divide these peaks from one another. Furthermore, these chemicals' peaks are isolated from one another. We are able to guarantee the accuracy and dependability of the results from our various HPLC analytical techniques in the product release parameters, approval criteria, and limitations for the amount of luconazole in the formulation validation. This method is a critical phase in the process of creating new dosage forms since it reveals the limitations of accuracy, linearity, precision, detection, and quantitation. The International Conference on Harmonization's (ICH drug) Product Stability Guideline Q1A (R2) suggests stress testing a medication to ascertain its intrinsic stability characteristics. This will enable the evaluation of the suitability of the designated analytical techniques and the identification of degradation byproducts. through the analytical method's process.1[1] However, the sensitivity and precision of TLC were believed to be the primary drawbacks of this analytical technique before high-performance TLC(HPTLC)was developed. Additionally, the employment of semiautomatic and automatic samplers (ATS and Linomat, respectively), the building of an integrated chamber, and the application of the sample as a band rather than a spot all enhanced the procedure. If the measurements were in mass mode, fluorescence mode, or ultraviolet [UV] absorbance mode, the increased sensitivity of HPTLC enabled quantitation limits on the order of nanograms or picograms.In comparison to other methods, it also permits quicker analytical times and lower solvent usage while maintaining respectable selectivity, accuracy, and repeatability. [4]

2. MATERIAL AND METHODS

Chemicals

Analytical Pure working standard Luliconazole was received as gift sample from Manus Aktteva Biopharma LLP, Ahemdabad, India. The pharmaceutical dosage form Lulican® cream, which was labeled to contain 1% was procured from the local pharmacy. Toluene (AR grade), methanol (HPLC grade), Acetonitrile (HPLC Grade) o-phosphoric acid (AR Grade) was obtained from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and Analytical Methods

HPTLC Instrumentation: The current study made use of an HPTLC (Camag) equipment that included a Camag Linomat V sample applicator, a 100 µL Hamilton syringe, a Camag TLC Scanner-III with WinCAT software version 1.4.3, and a Camag twin-trough chamber (10×10 cm) (CAMAG, Muttenz, Switzerland).

HPLC Instrumentation: The HPLC system (JASCO), model PU 2080 plus pump with Rheodyne sample injection port (20 µL), was used to analyze the samples. The HiQsil C8 (4.6 mm × 250 mm × 5µm) column was used for the study, and a PDA detector (MD 2010) was used for detection. Borwin chromatography software (version 1.5) was used for quantification at a wavelength of 298 nm. The optimum mobile phase, used in isocratic mode, included 0.1% OPA: ACN (60: 40, v/v) at a flow rate of 1 mL min-1. Additional tools used in the investigation included a hot air oven (Kumar Laboratory Oven), a vacuum pump (JET-VAC-J1), a photo stability chamber (Newtronic), and a UV-visible spectrophotometer (SHIMADZU UV-1780).

Solvent Selection

The solubility was checked by dissolving the drug in different organic solvents and drug was found to be freely soluble in methanol. Methanol of HPLC grade was used.

Selection of Analytical Wavelength

Following chromatographic development, 200–400 nm bands were scanned. The medication was found to exhibit a significant absorbance at 298 nm. Thus, 298 nm was chosen as the detecting wavelength.[5]

Standard Preparation

In a 10 ml volumetric flask, 10 mg of precisely weighed pure drug was dissolved in methanol, sonicated for 2 minutes, and the volume was increased to 10 ml to obtain 1000 ng/µL stock solution. This was then further diluted with methanol to obtain the final working standard solution, which had a concentration of 100 ng µL-1.

Method Optimization

HPTLC: To improve separation and eliminate luliconazole spots, multiple solvent solutions with varying concentrations of n-hexane, benzene, chloroform, toluene, and methanol were investigated. Ultimately, the ideal mobile phase, which provided a symmetrical peak shape and a satisfactory drug resolution, was determined to be composed of toluene: methanol (9: 1, v/v). At 298 nm, a densitometric measurement was made.

HPLC: Initial tests with HPLC-grade methanol, acetonitrile, and water combined in various ratios as the mobile phase marked the beginning of the analytical method development for the determination of luliconazole. Using a mobile phase that comprised methanol: water and acetonitrile: water in various ratios, such as 50: 50, 40: 60, and 60: 40, the development was started. The ideal mobile phase was ultimately determined to be a solvent mixture consisting of 0.1% orthophosphoric acid: acetonitrile (60: 40, v/v). Excellent clarity and a crisp, well-resolved peak without tailing were provided by the mobile phase that was used. Low noise level and short retention time were attained, along with better baseline stability.

Sample Solution Preparation

HPTLC: After weighing and transferring a quantity equal to 10 mg of luliconazole to a 10 mL volumetric flask with roughly 6 mL of methanol, the mixture was ultrasonically sonicated for 15 minutes, and the volume was adjusted with the methanol. The Whatman paper No. 41 was used to filter the mixture. To obtain a solution with a concentration of 100 ng µL1, one milliliter of this solution was transferred to a 10-milliliter calibrated volumetric flask, and the volume was adjusted with methanol. On a TLC plate, two microliters of the solution were seen. Following chromatographic development, the calibration plot was used to calculate the amount of medication in a sample by measuring the peak areas of the band at 298 nm.

HPLC: After weighing and transferring a quantity equal to 10 mg of luliconazole to a 100 mL volumetric flask with roughly 60 mL of methanol, the mixture was ultrasonically sonicated for 15 minutes, and the volume was adjusted with the methanol. The Whatman paper No. 41 was used to filter the mixture. To obtain a solution with a concentration of 10 µg mL1 for luliconazole, one milliliter of this solution was transferred to a 10-milliliter calibrated volumetric flask, and the volume was adjusted with methanol. The tablet sample solution was injected, the chromatogram was produced, and the peak regions were noted following the establishment of the chromatographic conditions. Six repetitions of the injections were made, and the quantity of each medication contained in each tablet was calculated using the corresponding calibration curve.

Stress Degradation Studies

HPTLC: Stress degradation tests were conducted on bulk drugs under acidic, alkaline, neutral, oxidative, thermolytic, and photolytic conditions in order to demonstrate the stability-indicating nature and selectivity of the suggested technique. The concentration used in the investigation was 1000 ng µL-1.

HPLC: Stress degradation tests were conducted on bulk pharmaceuticals by exposing them to a variety of stress conditions, such as acidic, alkaline, oxidizing agents, photolysis, and dry heat to promote degradation, in order to determine whether the devised approach was stability suggesting.

Acid hydrolysis

HPTLC: One milliliter of Luliconazole stock solution (1000 µg mL-1) was combined with one milliliter of 1 N hydrochloric acid, and the volume was adjusted with solvent to 10 milliliters. At room temperature, the solution was left for two-hour period. To get the final concentration of 100 ng band-1, the acid-stressed sample was neutralized with NaOH. A volume of 5 µL was then added to the plate, and development was carried out under ideal chromatographic conditions.

HPLC: One milliliter of 1 N HCl was added to one milliliter of Luliconazole stock solution (200 µg mL-1) to create the sample. For almost two hours, the solution was left at room temperature. After neutralizing the solution, 10 mL of mobile phase was added, and the mixture was then injected into the system.

Alkaline hydrolysis

HPTLC: Form the stock solution of Luliconazole (1000 µg mL-1), 1 mL solution was mixed with 1 mL of 0.1 N sodium hydroxide and the volume was made with solvent to 10 mL. Solution was placed at room temperature for period of 1 h. 5 µL volume was applied onto plate and development was performed using optimized chromatographic conditions.

HPLC One milliliter of 0.1 N NaOH was added to one milliliter of Luliconazole stock solution (200 µg mL-1) to create the

sample. For approximately one hour, the solution was left at room temperature. After neutralizing the solution, 10 mL of the mobile phase was added, and the mixture was injected into the system.

Oxidative degradation

HPTLC: By combining 1 mL of stock solution with 1 mL of 3% H2O2, oxidative degradation was carried out, and the solvent was adjusted as necessary. Five microliters of the solution were spotted on a TLC plate and developed under ideal chromatographic conditions after being allowed to stand at room temperature for one hour.

Neutral degradation

HPTLC: Neutral hydrolysis study was completed by treating the drug solution with water for 2 h and stressed sample was diluted with solvent to furnish the final concentration of 100 ng µL-1. 5 µL volume was applied onto plate and development was performed using optimized chromatographic conditions.

Photo-degradation

HPTLC: The solid drug powder was exposed UV light up to 200-watt hour square meter-1 to check photolytic degradation. Solution having final concentration 100 ng µL-1 was achieved by dissolving the sample in methanol and 5 µL solution was applied to HPTLC.

Thermal degradation

HPTLC: To conduct the investigation, medication powder was baked for one hour at 80 degrees Celsius. 5 µL of the resultant solution was added to HPTLC after a sample was taken out of the oven at the appropriate intervals and dissolved in methanol to create a solution of 100 ng µL-1.

HPLC: Bulk drug powder was exposed to 80ºC temperature in hot air oven for 1 h. The sample was cooled to room temperature and then 10 mg of powder was weighed and dissolved in methanol to10 mL. Diluted with mobile phase and 20 µg mL-1 concentration injected to system and chromatogram was recorded.

Peroxide induced degradation

HPLC: One milliliter of 3% w/v H2O2 was added to one milliliter of Luliconazole stock solution (200 µg mL-1) to create the sample. For approximately one hour, the solution was left at room temperature. After that, 10 mL of the mobile phase was added to the volume and injected into the system.

Photolysis Sample

HPLC: After being exposed to UV radiation for at least 200 watt hours per square meter, the sample was illuminated with white fluorescent light for at least 1.2 million lux hours. Ten milligrams of powder were weighed and dissolved in ten milliliters of methanol following exposure. To get a chromatogram, a final concentration dilution of 20 µg mL-1 was produced and injected.

Method Validation

In compliance with ICH requirements, the developed method was validated for linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation, and robustness.

Linearity and Range

HPTLC: Using a sample applicator in nitrogen gas, volumes 1, 2, 3, 4, 5, and 6 µL of the standard drug solution (100 ng µL-1) were administered to the TLC plates. Plates were dried after spotting. As previously mentioned, development was completed and separated spots were subjected to densitometric analysis. The calibration curve's regression data showed a good linear connection across the concentration range of 100–600 ng band-1.

HPLC: Linear regression analysis was used to assess the method's linearity, and the drug's linearity was discovered within the concentration range of 5–30 µg mL-1. In order to create calibration standards, the necessary volume of working standard (100 µg mL-1) solution was spiked into several 10 mL volumetric flasks, and the volume was then made up with mobile phase to provide concentrations of 5, 10, 15, 20, 25, and 30 µg mL-1.

Precision

HPTLC

Intra-day precision: It was ascertained by doing three linearity-range analyses of Luliconazole standard solutions at three distinct concentrations (200, 300, and 400 ng band-1) on the same day. Three duplicates of each concentration were observed, and the percentage R.S.D. was calculated.

Inter-day precision: Over the course of a week, standard drug solutions at three distinct concentrations (200, 300, and 400 ng band-1) were examined on three separate days, and the percentage R.S.D. was computed.

HPLC: To determine precision, three duplicates of Luliconazole at three distinct concentrations (10, 15, and 20 µg mL-1) were injected and examined in a single day as well as on three separate days.

Limit of detection (LOD) and Limit of quantitation (LOQ)

HPTLC: LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Specificity

HPTLC: Method specificity was verified by application of Luliconazole formulation on TLC plate which was then developed and scanned at 298 nm.

HPLC: The method's particularity was ascertained by analysing the sample solution and by contrasting the length of retention of sample with that of standard.

Assay of marketed formulation

HPTLC: From the sample stock solution, two microliter volume of prepared sample solution was spotted on the plate, developed and scanned. The amount of drug was calculated from the peak areas recorded.

HPLC: Luliconazole cream sample solution having concentration 10 µg mL-1 was injected and peak areas were recorded. Procedure was repeated for six times.

Accuracy (Recovery studies)

HPTLC: The consistency and accuracy of technique was ensured by recovery studies. Recovery studies were carried out by adding standard drug to pre-analysed sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 200 ng band-1. The drug concentrations were calculated from linearity equation.

HPLC: By adding standard drug solution to pre-analyzed sample solution at three different levels—50%, 100%, and 150%—recovery trials were conducted to verify the method's accuracy. The recovery percentage was calculated using linear formula.

Robustness

HPTLC:The robustness of the method was evaluated, during method development at concentration level of 500 ng band-1 by analyzing the effects of small variation in the composition of mobile phase (± 1 % methanol), change in wavelength (±1 nm). For each parameter, the % R.S.D. of peak area was calculated.

HPLC: The ICH defines method resilience as the ability of a method to remain unchanged through minor, intentional changes in the method's parameters. The parameters that were changed included the mobile phase's inflow rate (± 0.1 mL min-1) and wavelength (± 1 nm), as well as the composition of the mobile phase (± 2% ACN).

System suitability

HPLC: It was assessed by injecting mixed standard solution (10 µg mL-1) of Luliconazole into chromatographic system. The resolution, peak asymmetry, number of theoretical plates was calculated.[6]

Sensitivity

HPLC: The detection and quantitation limits were used to calculate the method's sensitivity. The corresponding regression equation was used to determine them.[7]

Accelerated stability study for HPTLC

For performing accelerated stability studies, the pharmaceutical dosage form Lulican® cream was used which was initially assayed by the developed method and then was exposed to different conditions for accelerated stability, such as 30ºC, 65% RH, 30ºC, 75% RH and 40ºC, 75% RH.

Selected Chromatographic conditions

Mobile phase: Toluene: methanol (9: 1, v/v)

Stationary phase: pre-coated silica gel 60 F254

Wavelength: 298 nm

Preparation of standard stock solution

To create a working standard with a concentration of 1 mg mL-1, 10 mg of the drug was dissolved in 10 mL of methanol to create the standard stock solution, which was then further diluted to reach 100 ng µL-1. On a TLC plate, five microliters of this solution were visible.

Preparation of standard Sample solution

After weighing and transferring a quantity equal to 10 mg of luliconazole to a 10 mL volumetric flask with roughly 6 mL of methanol, the mixture was ultrasonically sonicated for 15 minutes, and the volume was adjusted with the methanol. To obtain the necessary concentration, the solution was diluted with methanol after being filtered using Whatman paper No. 41. On a precoated TLC plate, the sample and standard were applied. Development was carried out using a chosen mobile phase, dried, and scanning was done at 298 nm.[8]

3. RESULTS AND DISCUSSION

Selection of analytical wavelength

Luliconazole's standard stock solution (1000 µg mL-1) was used to create a solution of 10 µg mL-1, which was then scanned at 200–400 nm. At 298 nm, the highest absorption was observed. It was chosen as the analytical wavelength as a result.

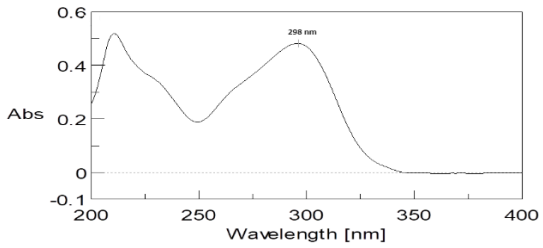


Figure 2. UV absorption spectrum of Luliconazole

Method optimization

HPTLC: The ideal mobile phase, which provided a symmetrical peak shape and a satisfactory drug resolution, was determined to be composed of toluene: methanol (9: 1, v/v). At 298 nm, a densitometric measurement was made. It was determined that the retention factor was 0.39 ± 0.03.(Fig. 3)

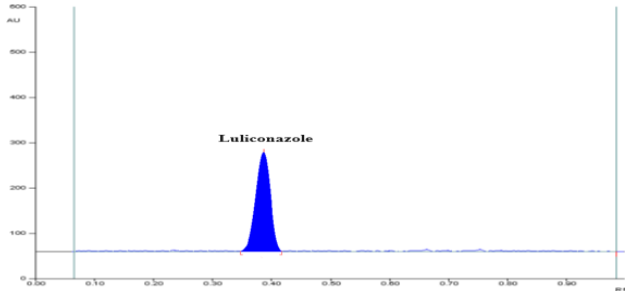


Figure 3. Densitogram of standard solution of Luliconazole

(500 ng band^{-1}, Rf= 0.39±0.03)

HPLC: The most advantageous mobile phase for obtaining a well-resolved drug peak was determined to be 0.1% orthophosphoric acid:acetonitrile (60:40, v/v). Using the suggested method, the retention time was determined to be 4.97 ± 0.02 minutes. Figure 4 shows a representative chromatogram of the Luliconazole standard solution.

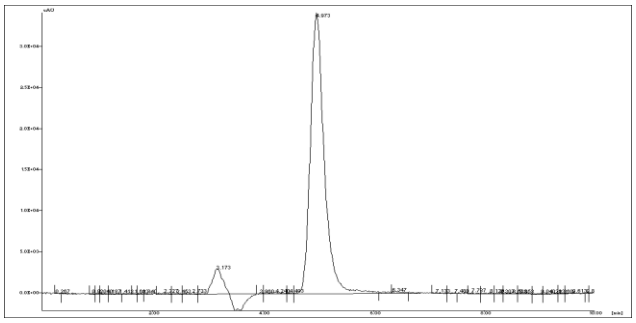


Figure 4. Chromatogram of standard solution (RT= 4.97 min)

Results of stress degradation studies

Acid hydrolysis

HPTLC: In presence of acid, degradation was seen 18.71 % degradation observed for the drug without appearance of peak

for degradation product but peak area was found to be reduced as compared to standard. The densitogram after treatment with acid is shown below. (Fig.5)

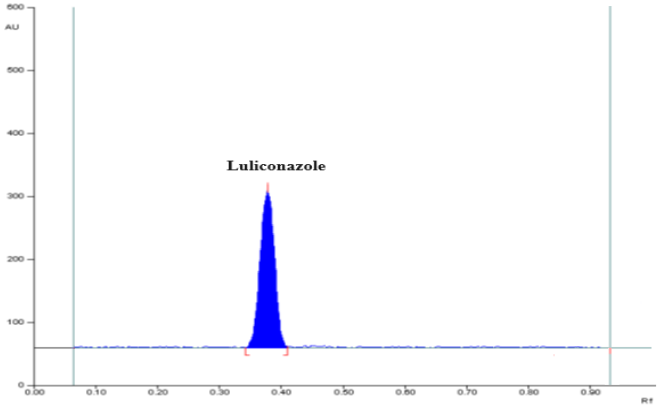


Figure.5 Densitogram of acid treated Luliconazole

HPLC: Marked degradation in the chromatogram was observed. The acid degraded sample showed 17.84 % degradation. No degradation peak was observed but the peak area of drug was considerably reduced as compared to the peak area standard solution. (Fig. 9.3)

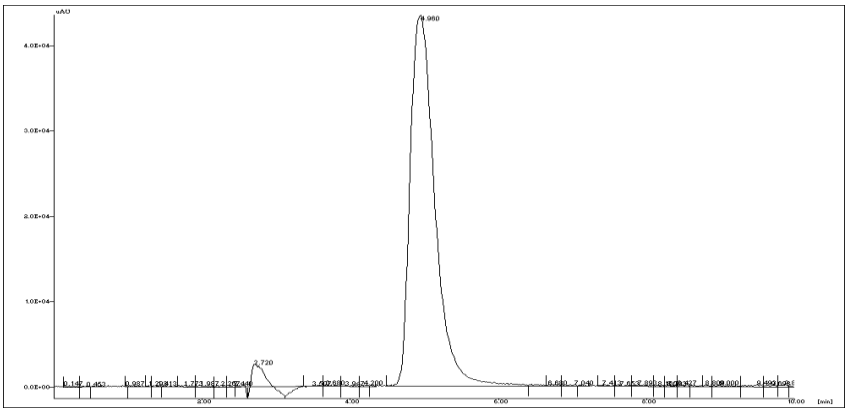


Figure 6. Chromatogram after acid hydrolysis

Alkaline hydrolysis

HPTLC: The drug was found to be susceptible to alkaline condition. About 17.73 % degradation was observed for the drug with reduction in peak area in comparison to standard. The densitogram of alkali treated luliconazole is shown below. (Fig.7)

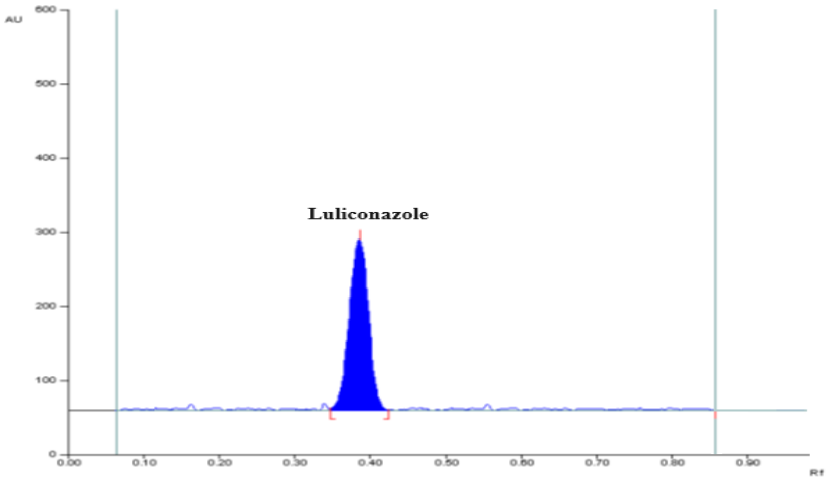


Figure 7. Densitogram of alkali treated Luliconazole

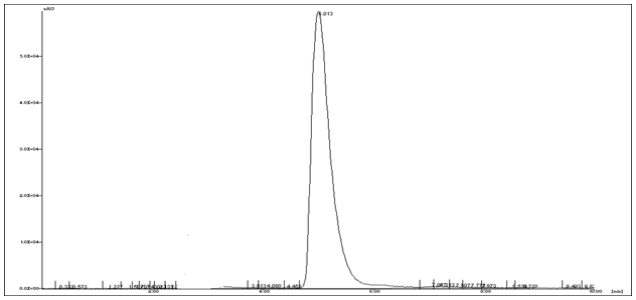


Figure 8. Chromatogram showing alkali degradation

HPLC: About 16.81 % degradation was observed when drug was exposed to base induced degradation. No degradation peak was observed. The representative chromatogram of alkali treated sample is shown in Fig.8.

Neutral degradation

HPTLC: The densitogram obtained after treatment with water showed 12.25 % degradation without appearance any peak for degradation product. (Fig.9.)

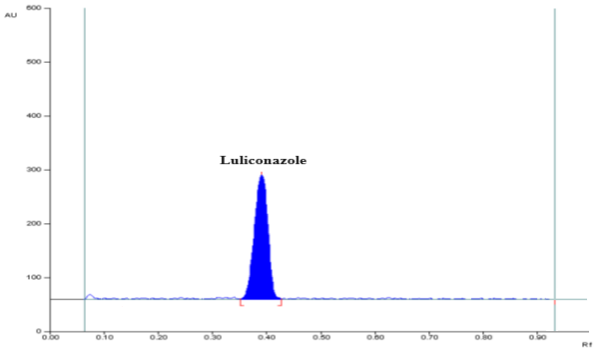


Figure 9. Densitogram obtained after neutral degradation

Oxidative degradation

HPTLC: The obtained densitogram for drug exposed to 3% H2O2 showed 14.61 % of degradation and no additional peak for product of degradation observed. (Fig.10)

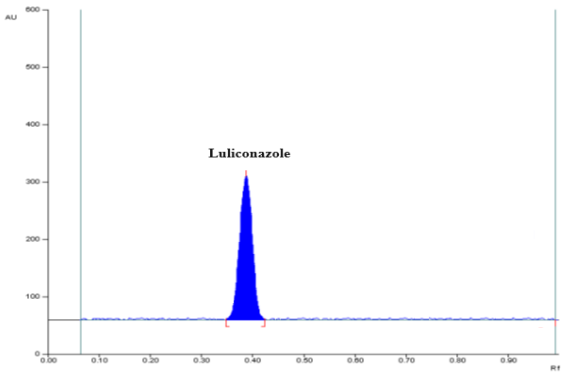


Figure 10. Densitogram for peroxide treated drug

Photo degradation

HPTLC: Drug exposed to UV light showed 14.49 % degradation with decrease in peak area and no extra peak of degradation product was observed. (Fig. 11)

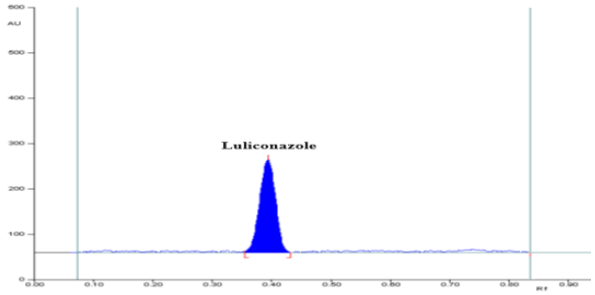
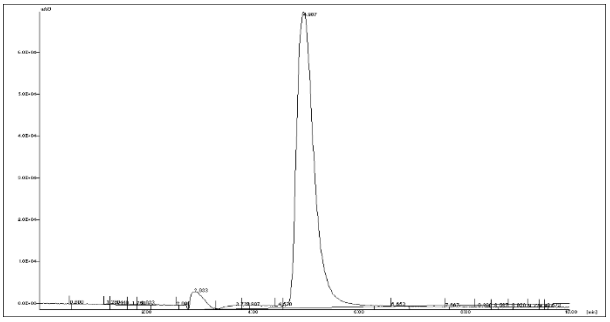


Figure 11. Densitogram obtained after exposure to UV light



HPLC: The chromatogram of the drug exposed to UV light and fluorescence light showed 14.61 % and 17.19 % degradation, respectively without appearance of any peak for degradation product (Fig1.12).

Figure 12. Chromatogram obtained after exposure to UV light

Dry heat degradation

HPTLC: Thermal studies in solid state for drug substance denoted that drug is sensitive to heat. The drug exhibited 13.27 % degradation but no additional peak for degradation product observed in the densitogram. (Fig. 13)

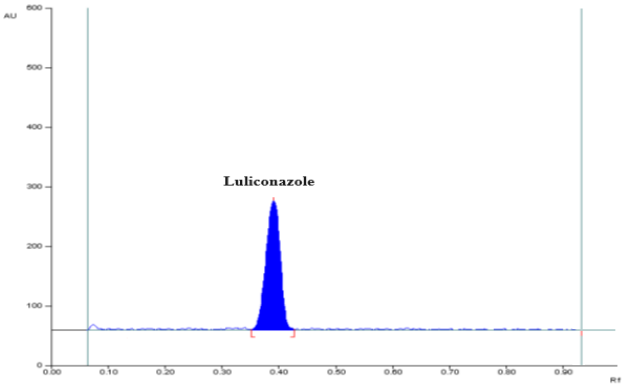


Figure 13. Densitogram obtained after exposure to heat at 80ºC for 1h

The stress degradation results indicated susceptibility of drug to hydrolytic, oxidative, thermal as well as photolytic stress conditions. The findings of degradation studies are represented in Table 2.

Stress conditions/ duration	**% Recovered**	**% Degradation**
Acid / 1 N HCl/ Kept at RT for 2 h	81.29	18.71
Alkali /0.1 N NaOH/ Kept at RT for 1 h	82.27	17.73
Neutral/water/ Kept at RT for 2 h	87.75	12.25
Oxidative /3 % H_2O_2 / Kept at RT for 1 h	85.39	14.61

Dry heat/ 80ºC/ 1 h	86.73	13.27
Photolysis: UV light 200 watt h square meter^{-1}	85.51	14.49

Table 2. Results of stability studies by proposed HPTLC method

HPLC: After exposure to 80ºC for 1 h, drug showed significant degradation with reduction in the peak area of drug indicated 14.28 % degradation. Representative chromatogram obtained for sample subjected to dry heat is shown in Fig.14.

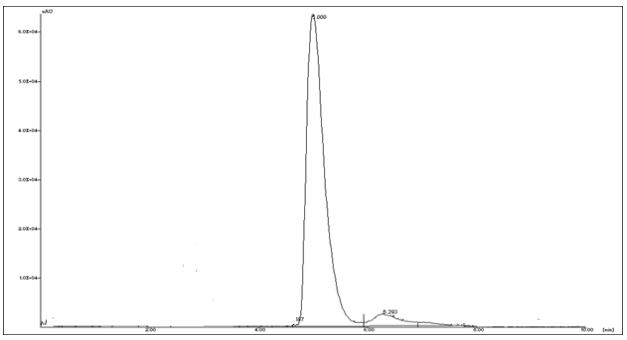


Figure 14.Chromatogram obtained after thermal degradation

The amount of drug recovered and % degradation observed after tested stress conditions are summarized as follows Table 3.

Stress conditions/ duration	**% Recovered**	**% Degradation**
Acid / 1 N HCl/ Kept at RT for 2 h	82.16	17.84
Alkali /0.1 N NaOH/ Kept at RT for 1 h	84.09	16.81
Oxidative /3 % H_2O_2 / Kept at RT for 1 h	84.62	15.38
Dry heat/ 80ºC/ 1 h	85.72	14.28
Photolysis: UV light 200 watt h square meter^{-1}	85.39	14.61

Table 3. Results of stability studies by proposed RP-HPLC method

Peroxide degradation for HPLC

The peroxide treated sample also found susceptible to oxidative degradation and showed 15.38 % degradation is shown in Fig.15

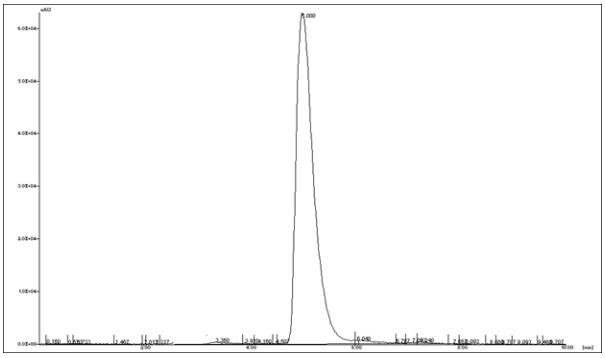


Figure 15. Chromatogram obtained after peroxide degradation

Validation of analytical procedure

Linearity and range

HPTLC: The method was found to be linear and straight-line calibration curves were achieved in the concentration range of 100-600 ng band-1 with high correlation coefficient. The peak area was plotted against the corresponding concentrations to obtain the calibration curve. The results discovered to be linear with regression equation of y = 6.5951x + 555.2 and correlation coefficient (R^2) = 0.995. The densitogram obtained for linearity is shown in Fig. 16. The peak area was plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig. 17.

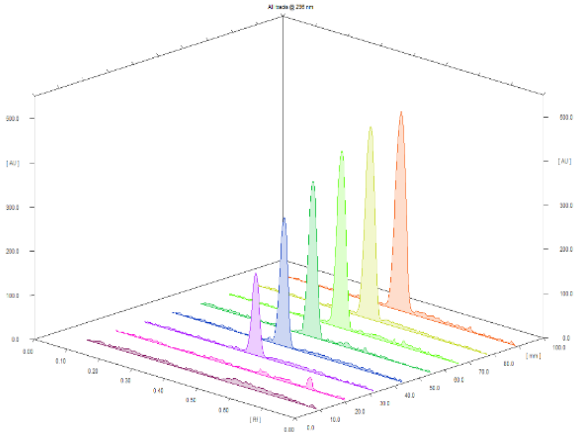


Figure 16. 3D densitogram in concentration range 100-600 ng band^{-1}

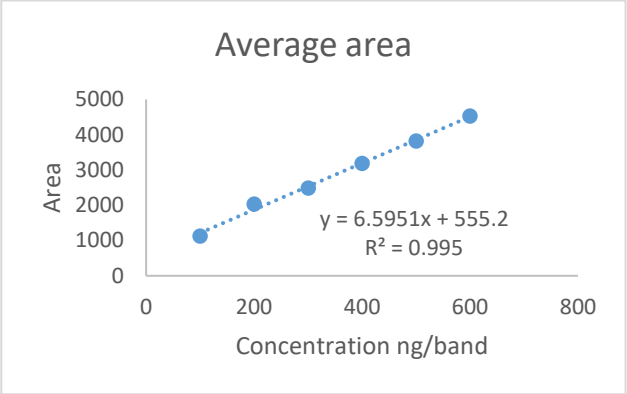


Figure 17. Calibration curve of Luliconazole

Concentration (ng band^{-1})	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Avg. Area
100	2774	2734	2656	2648	2768	2746	2721
200	4516	4597	4395	4533	4655	4586	4547
300	6395	6289	6135	6117	6309	6159	6234
400	7386	7367	7242	7352	7560	7543	7408
500	8567	8645	8286	8325	8601	8589	8502
600	10011	10030	9732	9896	9632	10004	9884

Table 4. Linearity and range of Luliconazole

***n=6**

Parameters	Data
Linearity range	100-600 ng band^{-1}
r^2	0.995

Slope ± S.D.	6.5951
Intercept ± S.D.	555.2

Table 5. Linear regression data for calibration curve

HPLC: The method was found to be linear and straight-line calibration curves were achieved in the concentration range of 5-30 µg mL-1 with high correlation coefficient. The peak area was plotted against the corresponding concentrations to obtain the calibration curve. The results discovered to be linear with regression equation of y = 70457x + 193556 and correlation coefficient (R^2) = 0.996. The calibration curve and overlay spectrum of linearity in the concentration range of 5-30 µg mL-1 is represented in Fig.18.19.

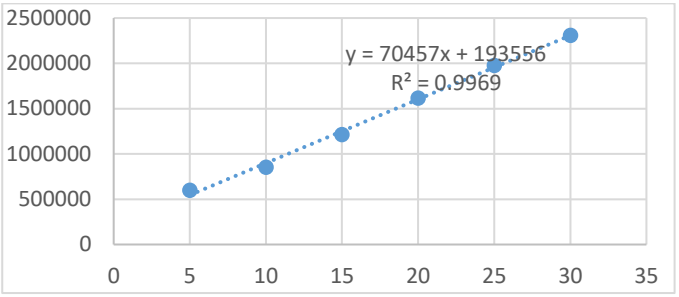


Figure 18 .Calibration curve of Luliconazole

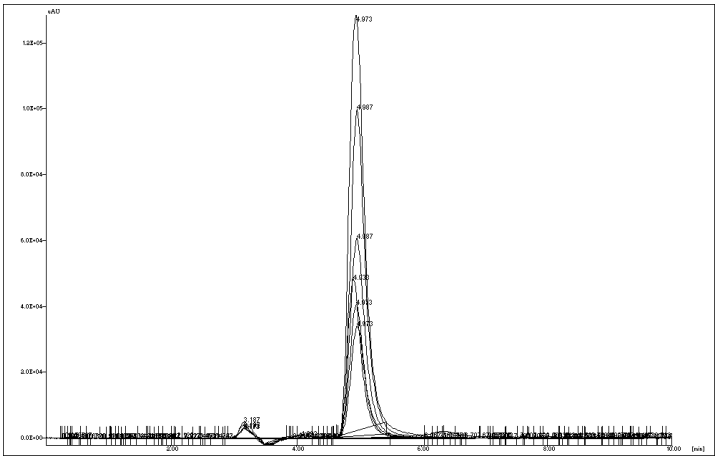


Figure 19 .Linearity spectrum in the concentration range 5-30 µg mL^{-1}

Conc. (µg mL^{-1})	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Mean Area*
5	609707	608718	602309	605678	6076208	608208	607041
10	761211	759318	751310	759932	768965	762421	760526
15	1289041	1303301	1299230	1302018	1302508	1290902	1297833
20	1511830	1512300	1511986	1493220	1514202	1405276	1505276
25	1773058	1778358	1766989	1788976	1777201	1776945	1776905
30	2308797	2307657	2308990	2209567	2304807	2308737	2291426

Table 6. Linearity data of Luliconazole

*Average of six determinations

1. *Parameters*	2. **Data***
3. Linearity range	4. 5-30 µg mL-1
5. r2	6. 0.996
7. Slope	8. 70457
9. Intercept	10. 193556

Table 6. Linearity data of Luliconazole

*Average of six determinations

Precision

HPTLC: Intra and interday deviations in results were examined by recording the values of peak area after application of three different concentrations in linearity range. The % R.S.D. values were in the range 0.65 to 0.82 for intraday (Table 8.4) and 0.69 to 1.20 for interday (Table 8.5) . The lesser % R.S.D. values (< 2) obtained indicated the precision of the developed method

Concentration (ng band^{-1})	**Recovered concentration (ng band^{-1})**	**% Recovery**	**% R.S.D.***
200	199.86	99.92	0.80
300	300.99	100.32	0.65
400	394.80	99.20	0.82

Table 8. Intra-day precision of Luliconazole

***n=3

Concentration (ng band^{-1})	**Recovered concentration (ng band^{-1})**	**% Recovery**	**% R.S.D.***
200	200.06	100.05	1.10
300	300.29	100.09	1.20
400	396.67	99.16	0.69

Table 9. Inter-day precision of Luliconazole

***n=3

HPLC: Intra and interday deviations in results were examined by recording the values of peak area after application of three different concentrations in linearity range. The % R.S.D. values were in the range 0.14 to 0.81 for intraday and 0.15 to 1.04 for interday. The lesser % R.S.D. values (< 2) obtained indicated the precision of the developed method.

11. **Concentration** 12. **applied (µg mL^{-1})**	13. **Average area**	14. **Recovered concentration** 15. **(µg mL^{-1})**	16. **% R.S.D.***
17. 10	**18.** 847811	**19.** 09.90	**20.** 0.14
21. 15	**22.** 1221239	**23.** 15.23	**24.** 0.81
25. 20	**26.** 1510247	**27.** 19.75	**28.** 0.58

Table 10. Intra-day precision

*Average of three determinations

29. Concentration 30. applied (µg mL^{-1})	**31. Average area**	**32. Recovered concentration 33. (µg mL^{-1})**	**34. % R.S.D.***
35. 10	**36.** 842118	**37.** 09.81	**38.** 0.15
39. 15	**40.** 1208230	**41.** 15.18	**42.** 0.23
43. 20	**44.** 1501986	**45.** 19.80	**46.** 1.04

Table 11. Inter-day precision

*Average of three determinations

Limit of detection (LOD) and Limit of quantitation (LOQ)

HPTLC: The detection limit was 20.43 ng band-1 and quantitation limit was 61.91 ng band-1 .

HPLC: Limit of detection (LOD) and limit of quantification (LOQ) was discovered to be 0.72 µg mL-1and 2.20 µg mL-1, respectively.

Specificity

HPTLC: The excipients from formulation did not show any interference with analyte peak and obtained peak purity value was in the limit representing method specificity. The non-interference of degradation product peaks with the drug peak also proved that method is specific.

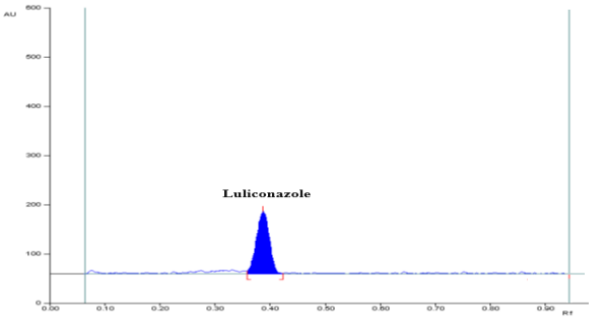


Figure 20. Densitogram of marketed formulation with concentration 200 ng band^{-1} (Rf= 0.39 ± 0.03)

HPLC: Excipients present in formulation did not show any interference with peak of active drug which indicated the specificity of developed method.

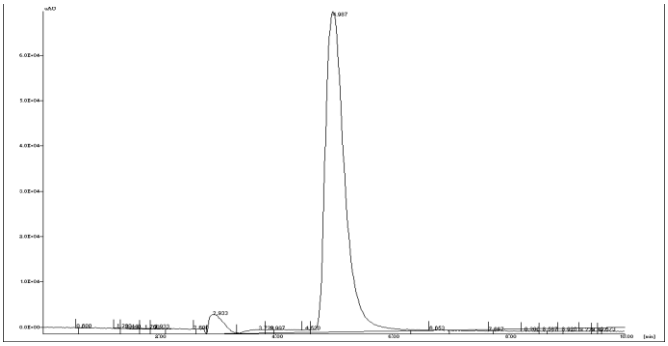


Figure 21. Chromatogram of marketed formulation

Assay of marketed formulation

HPTLC: The % drug amount was found to be 99.56±1.15. (Table 12)

Spotted concentration (ng band^{-1})	Recovered concentration(ng band^{-1})	% Recovery	% R.S.D.*
200	199.05	99.52	1.17

Table 12. Assay of Luliconazole

* Average of six determinations

HPLC: The % drug content was determined from linear equation. The % drug content was found to be 99.07 ± 1.21.

47. Drug	48. Peak area	49. Recovered Concentration(µg mL^{-1})	50. %Drug content	51. % R.S.D.*
52. 53. 54. Luliconazole	55. 852836	56. 09.96	57. 99.62	58. 59. 60. 1.22
	61. 845697	62. 09.85	63. 98.57	
	64. 859764	65. 10.06	66. 100.64	
	67. 835982	68. 09.71	69. 97.13	
	70. 853789	71. 0 9.97	72. 99.76	
	73.	74.	75.	
	76. 846931	77. 0 9.87	78. 98.75	

Table 13. Assay of marketed formulation

***Average of six determinations

Accuracy

HPTLC The % average recovery was 99.76 ±0.75 which indicated accurateness of developed method for determination of drug in semi-solid dosage form. (Table 14.)

Recovery Level	Amount of analyte taken(ng band^{-1})	Amount added(ng band^{-1})	Mean area	Mean Amount recovered(ng band^{-1})	% Mean Recovery±R.S.D.*
80	200	160	2919	358.41	99.55±0.74
100	200	200	3186	398.90	99.72±0.97

120	200	240	3458	440.14	100.02±0.55

Table 14. Results of recovery studies

*Average of three determinations

HPLC: The % mean recovery was found to be 99.14±0.89 which indicated that the method is accurate for estimation of drug in dosage form.

79. **80.** **81.** Level	**82.** Sample conc. taken **83.** (µg mL^{-1})	**84.** Conc. Added **85.** (µg mL^{-1})	**86.** Area	**87.** Conc. Recovered (µg mL^{-1})	**88.** % **89.** Recovery	**90.** % R.S.D. *
91. **92.** 50 %	**93.** **94.** 10	**95.** **96.** 5	**97.** 1200023	**98.** 15.08	**99.** 100.59	**100.** **101.**0.48
			102.1199123	**103.**15.07	**104.**100.50	
			105.1191023	**106.**14.95	**107.**99.71	
108. **109.**100 %	**110.** **111.**10	**112.** **113.**10	**114.**1509820	**115.**19.66	**116.**98.32	**117.** **118.**0.33
			119.1508730	**120.**19.64	**121.**98.24	
			122.1501610	**123.**19.54	**124.**98.71	
125. **126.**150 %	**127.** **128.**10	**129.** **130.**15	**131.**1840058	**132.**24.54	**133.**98.16	**134.** **135.**0.36
			136.1839048	**137.**24.52	**138.**98.10	
			139.1840059	**140.**24.68	**141.**98.75	

Table 15. Recovery studies

*Average of three determinations

Robustness

HPTLC: The deliberate variations made during study demonstrated that peak areas were not affected due to small alterations made in experimental parameters as well as values of R.S.D. were observed within the limit demonstrating robustness of the method. (Table 8.8)

Parameters	**% R.S.D.***
Composition of mobile phase (± 1 % methanol)	0.50
Wavelength (± 1 nm)	0.55

Table 16. Robustness data

*Mean of three determinations

The overall review of validation parameters is given away in table 17.

Sr. No.	**Parameters**	**Results**
1.	Linearity (ng $band^{-1}$)	100-600
2.	Correlation coefficient (r)	0.995
3.	Detection limit (ng $band^{-1}$)	20.43
4.	Quantitation limit (ng $band^{-1}$)	61.91
5.	Accuracy	99.76±0.75

6.	Precision (% R.S.D.)	
	Intra day precision	0.65-0.82
	Inter day precision	0.69-1.20
7.	Robustness	Robust

Table 17. Validation parameters for Luliconazole

HPLC: The method was found to be robust as there were no marked changes in the chromatograms and % RSD was below 2 %.

Drug	**% R.S.D.***		
	Flow Rate(± 0.1 ml/min)	**Wavelength (± 1 nm)**	**Change in mobile Phase composition (± 2% ACN)**
Luliconazole	0.48	1.35	1.76

Table 18. Robustness data

*Mean of three determinations

Accelerated stability testing For HPTLC

The accelerated stability testing of marketed formulation was performed using following conditions viz. 30ºC, 65% RH, 30ºC, 75% RH and 40ºC, 75% RH for period of 6 months. (Table 19 -22)

Sr. No.	**Standard/Sample**	**Rf**	**Amount found (ng band^{-1})**	**% Assay**
1	Standard	0.39	502.70	100.54
2	Sample	0.39	500.73	100.14

Table 19. Initial assay of marketed formulation

The sample exposed to the above conditions in their primary packaging was tested at intervals of 2 months. The results obtained were as follows:

Sr. No.	**Standard/Sample**	**Rf**	**Amount found (ng band^{-1})**	**% Assay**
1	Standard	0.39	498.82	99.76
2	Sample (2M at 30ºC, 65% RH)	0.38	499.23	99.84
3	Sample (2M at 30ºC, 75% RH)	0.39	497.61	99.52
4	Sample (2M at 40ºC, 75% RH)	0.39	498.24	99.64

Table 20. Assay of formulation upon exposure to accelerated conditions for 2 months

RH = Relative humidity

Sr. No.	**Standard/Sample**	**Rf**	**Amount found (ng band^{-1})**	**% Assay**
1	Standard	0.39	501.14	100.22
2	Sample (4M at 30ºC, 65% RH)	0.39	496.51	99.30
3	Sample (4M at 30ºC, 75% RH)	0.38	497.67	99.53

4	Sample (4M at 40ºC, 75% RH)	0.39	495.35	99.07

Table 21. Assay of formulation upon exposure to accelerated conditions for 4 months

Sr. No.	Standard/Sample	Rf	Amount found (ng band^{-1})	% Assay
1	Standard	0.39	500.67	100.13
2	Sample (6M at 30ºC, 65% RH)	0.39	499.52	99.90
3	Sample (6M at 30ºC, 75% RH)	0.39	494.31	98.86
4	Sample (6M at 40ºC, 75% RH)	0.39	494.89	98.97

Table 22. Assay of formulation upon exposure to accelerated conditions for 6 months

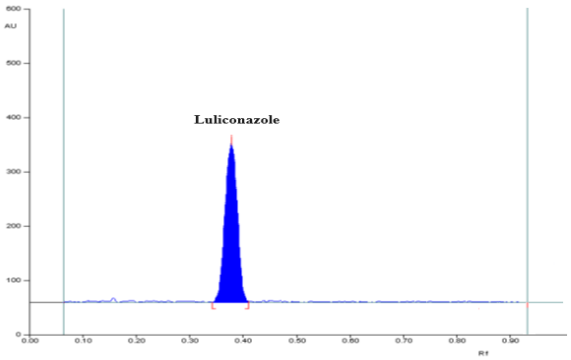


Figure 22 .Densitogram of Luliconazole sample exposed to 40ºC, 75% RH (2 months)

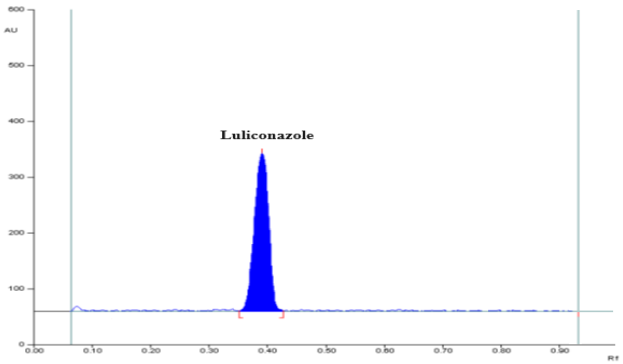


Figure 23. Densitogram of Luliconazole sample exposed to 40ºC, 75% RH (4 months)

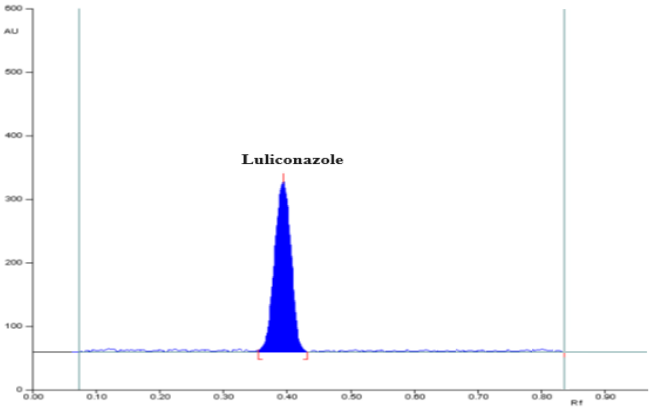


Figure 24. Densitogram of Luliconazole sample exposed to 40ºC, 75% RH(6 months)

The results obtained shown that analyzed marketed formulation retained assay of Luliconazole within limits after performing the analysis at the end of six months.

System suitability HPLC

Reproducibility of developed procedure was checked by carrying out system suitability tests. The parameters such as capacity factor, number of theoretical plates and tailing factor for active substance was in limits suggesting the system suitability. The achieved values for various parameters confirmed appropriateness of the system for the analyzing drug in combination (Table 23).

Table 23. System suitability parameters

Drug	Retention Time(min)	Area (µV. Sec)	Theoretical plates	Resolution	Asymmetry
Luliconazole	4.97	762911	3623.28	3.10	1.32

2 CONCLUSIONS

For the quantification of luliconazole in bulk medication and semi-solid dosage forms, a straightforward, accurate, and precise stability-indicating HPTLC and RP-HPLC method has been created and validated. Both approaches showed the capacity to isolate the active medication from degradation products and excipients under stressful circumstances. The medication was put through a number of stressors to establish its stability-indicating characteristics, including hydrolysis (acid and base), oxidation, heat, and photolysis, in accordance with ICH regulations. The drug's vulnerability to hydrolytic, oxidative, thermal, and photolytic destruction was demonstrated by the data. Crucially, the formulation's excipients had no effect on the analysis, demonstrating the methodologies' specificity. The accelerated stability results showed no degradation peaks, and the tested formulation retained its potency within acceptable limits even after six months of testing, indicating a stable formulation and appropriate packaging. Furthermore, both methods' validation parameters—linearity, accuracy, precision, robustness, and specificity—were substantially within the bounds permitted by ICH recommendations, guaranteeing their dependability for regular quality control. The HPTLC method proved particularly effective for rapid and cost-efficient analysis, while the RP-HPLC method offered high precision and sensitivity, making them complementary for various analytical applications. Both methods can be utilized to monitor the potency and stability of marketed products throughout their shelf life, ensuring compliance with regulatory requirements. These methods not only facilitate quality assurance but also provide critical insights into the stability profile of Luliconazole under different stress conditions. All things considered, the suggested techniques are strong, dependable, and appropriate for the regular examination of luliconazole in semi-solid and bulk drug formulations. The results of this study also highlight how crucial it is to carry out comprehensive stability studies to guarantee the safety and effectiveness of products, highlighting their value for pharmaceutical quality control and legal compliance.

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