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Research Article

Method Development And Validation Of Luliconazole By UHPLC In Bulk And Topical Dosage Form

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ABSTRACT

Analytical chemistry measures a physical or chemical property of a characteristic ingredient of the components of interest to quantitatively analyze the composition of substances and complicated materials in various matrices. The process of verifying that the analytical technique used for a particular test is appropriate for its intended use is known as method validation. In accordance with ICH criteria, metrics such as accuracy, precision, linearity, LOD and LOQ, and repeatability were examined. A brand-new imidazole antifungal called luliconazole is used to treat tinea corporis, tinea cruris, and interdigital tinea pedis. The new, rapid, sensitive, simple, precise and accurate Ultra High Performance Liquid Chromatography (UHPLC) method was development and validation of Luliconazole by UHPLC in bulk and topical dosage form. The column used was waters cortex C18 column (150mm x 4.6mm; 2.7µm) with mobile phase containing the Methanol : Water (90:10% V/V). The retention time was found to be 4.14 min on chromatogram. The detection wavelength was 296 nm and flow rate were 1.0ml/min. The linearity of luliconazole was found to be in the range of 1.0-15.0µg/ml with correlation coefficient (R2) 0.99999. The %RSD for ruggedness was 1.245. The values for LOD and LOQ were 0.073 µg/ml and 0.220 µg/ml. After analysis, it was discovered that the linearity, Range, specificity, Robustness, precision, accuracy, Limit of detection (LOD) And Limit of quantitation (LOQ) complied with the official limits specified in the ICH recommendations.

INTRODUCTION

Luliconazole, also known by the commercial name Luzu, is an imidazole-based antifungal drug. It is recommended as a 1% topical cream to treat ringworm, jock itch, and athlete's foot brought on by dermatophytes like Trichophyton rubrum, Microsporum gypseum and Epidermophyton floccosum. The imidazole class includes it. Though it's unclear exactly how they work to combat dermatophytes[1,2]. The azoles obstruct the ergosterol production pathway's lansterol 14α-demethylase, which prevents lanosterol from

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being converted to ergosterol. An inadequate quantity of ergosterol causes intracellular 14α methyl sterols to accumulate, which inhibits growth and ultimately results in mortality. It exhibits activity against several types of fungi, including Aspergillus, Trichophyton, Tinea, and Epidermatophytes in particular[3,4]. Various analytical techniques, including UV spectrophotometry[5,6], liquid chromatography[7], TLC[8], HPTLC[8,9] , RP-HPLC[10,11,12], RP-UFLC[13] and other techniques[14,15], ingredients and experimental design were released for the purpose of evaluating luliconazole in biological fluids and pharmaceutical formulations. In the current study, we have created a straightforward UHPLC method for quantification of luliconazole in pharmaceutical dosage form and bulk that indicates economic stability and accuracy. The procedure was verified in accordance with ICH recommendations. A new antifungal medication called LCZ was introduced by Ranbaxy Laboratories Ltd. in India. Originally, the molecule was screened from active ingredients associated with the powerful antidermatophytic medication lanoconazole. Luliconazole (LCZ) is a medication that is highly effective against dermatophytes and a member of the imidazole class of drugs. It has a broad range of antifungal activity. Luliconazole is chemically, (2E)-2-[(4R)-4-(2, 4-dichlorophenyl)-1, 3-dithiolan-2-ylidene]-2-imidazol-1-yl-acetonitrile ($C_{14}H_9Cl_2N_3S_2$) with molecular weight 354.28. It is used as an antifungal agent. The double bond next to the dithiolane molecule in the R-enantiomer of luliconazole is arranged in an E configuration, and it has just one chiral center[13]. It has been discovered that luliconazole has a wide range of antifungal action against pathogenic fungi, particularly dermatophytes. 1% creams and solutions are mostly used to treat superficial infections including candidiasis, pytyriasis versicolor, and dermatophytosis. It is recommended for the treatment of dermatophytes like Trichophyton robrum, Microsporum gypseum, and Epidemophyton floccosum that cause ringworms, jock itch, and athlete's foot. An azole antifungal called luliconazole cream is prescribed for the topical management of interdigital tinea pedis, tinea crusis, and tinea corporis. I have created a straightforward, precise, and cost-effective UHPLC method in this study to quantify luliconazole in topical and bulk dose forms. Method validation followed ICH norms[16].

UHPLC is a technology that has the potential to reduce operation costs while also achieving greater resolution, faster analytical times, and increased separation efficiency. Ultra high-pressure liquid chromatography, also known as performance liquid chromatography, involves the use of sub-2 µm particle-packed columns in combination with a system capable of withstanding extremely high pressures (up to 1500 bar). This chromatographic method aims to boost peak capacity and/or sample throughput [17].

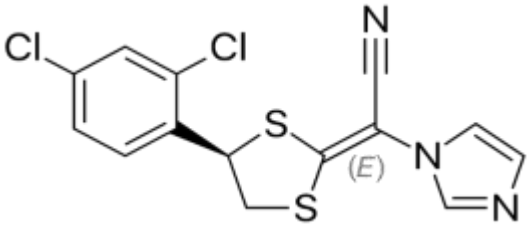

Fig.1: Structure of luliconazole [13]

MATERIAL AND METHODS

Selection of solvent:

Methanol was selected as the solvent for dissolving Luliconazole.

Selection of analytical wavelength:

Methanol as a blank and Luliconazole standard solution (20 PPM) was scanned from 400 nm to 200 nm. Absorption maxima was determined for drug.

UHPLC Method Development and Optimization of chromatographic conditions:

Preparation of standard stock solution for Chromatographic development: Luliconazole Standard stock solution was prepared by transferring 10 mg Luliconazole into a 20 mL clean and dried volumetric flask, added about 15 mL of Methanol to dissolve it completely and made volume up to the mark with methanol. (500 PPM). Further diluted 2 ml of stock solution to 10 mL with Mobile phase. (100 PPM). The standard solutions of Luliconazole was used for UHPLC Method development.

Selection of detection wavelength for UHPLC method development: Analytical wavelength for the examination was selected from the wavelength of maximum absorption from the spectrophotometric analysis and it was 296 nm.

Selection of mobile phase: Mobile phase was prepared by mixing the MEOH: Water in the ratio of 90:10% v/v and the mixture degasified by vacuum filtration using 0.45µ filter and sonication. Optimization can be started only after reasonable chromatogram has been obtained. Reasonable chromatogram means that more or less symmetrical peak on the chromatogram after detection. By slight change in mobile phase composition, the position of peak can be predicted within a range of investigated changes. An optimized chromatogram was the one where peak of Luliconazole was symmetrical and well separated within 8 minute of run time. The Mobile phase was selected on the basis of best separation, theoretical plate and tailing factor, peak shape, peak stability etc. Numbers of trials were taken for selection of mobile phase. Initially different proportions of methanol-water, Methanol-ACN were tried. Finally, a Methanol : water was used in ratio of 90:10v/v.

Optimization of chromatographic conditions: optimized chromatography which is as follows:

Mode: Isocratic
Detector: U.V. Detector
Column Name: Waters cortex C18,
Column Dimension: (150 mm X 4.6 mm i.d.) 2.7 µm.
Column Oven temperature: 40°C
Injection Volume: 20 µl
Wavelength: 296 nm
Mobile phase: Methanol : Water (90:10)
Flow Rate: 1.0 ml/min.
Run time: 8 minutes

VALIDATION OF UHPLC METHOD [6,16]

The developed chromatographic method was validated for system suitability, linearity, range, accuracy, precision, LOD-LOQ and robustness parameters According to Q2A (R1) ICH guidelines. The developed method for estimation of Luliconazole was validated in accordance with ICH guidelines for following parameters.

Linearity And Range: The linearity was determined by analyzing 5 independent levels of calibration curve in the range of 1-15µg/ml. absorbance of each solution against methanol was recorded at 296nm.The calibration curve of absorbance vs conc. Was plotted and correlation co-efficient and regression line equation for luliconazole were determined. Linearity was performed from 10% to 150% of working concentration. Linear regression data as well as calibration curve were shown in table no.1 and 2 under result and discussion section.

Precision: intra-day precision was determined by analyzing luliconazole at 6 different samples of the same day and inter-day precision was determined by analyzing luliconazole at 6 different samples on different days and %RSD was calculated.

The values of % relative standard deviation (% RSD) for both the parameters are shown in table no.3 under result and discussion. Standard deviation or relative standard deviation are commonly used to express the precision of an analytical process.

Accuracy (% Recovery) : By determining the percentage recovery of luliconazole from the topical dosage form, the method's

accuracy was assessed. Recovery studies were carried out by applying the method to topical dosage form containing Luliconazole at 50, 100 and 150% levels. At each level three determinations were carried out and the results are shown in table no.5 under result and discussion. The amount of luliconazole was calculated at each level and % recovery were computed. Accuracy will be conducted in the range from 50 % to 150 % of working concentration. Each accuracy level's solution was created in triplicate. Calculated % Recovery for each sample, Mean % recovery for each level and overall recovery and also calculated % RSD for each level and % RSD for overall recovery.

Robustness: Robustness of the optimized method was studied by changing column wavelength (±3 nm), temperature (±2°C), and flow rate (±10%) during analysis. The sample was injected in triplicate for every condition and % RSD was calculated for each condition is shown in table no. 4 under result and discussion section.

Limit of Detection (LOD) And Limit of Quantitation (LOQ): The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method of linearity. Calibration curves were plotted for each set. These calculations were used to determine LOD and LOQ based on the y-intercept standard deviation and the calibration curve's average slope was used to calculate LOD And LOQ using following formulae.

LOD= 3.3×avg SD/ slope

LOQ=10×avg SD/ slope

Where, SD is the standard deviation of y-intercepts of the calibration curves; S is the mean slope of six calibration curves.

RESULT AND DISCUSSION

UV spectrophotometric Analysis

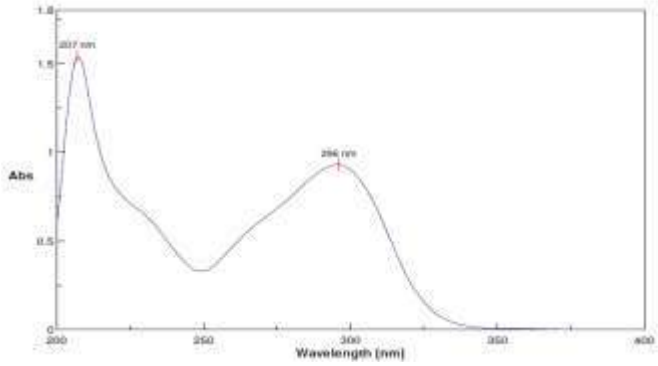

Fig.2: UV Spectrum of Luliconazole

Observation: The standard solution was scanned between 200 nm to 400 nm. Wavelength of maximum absorption was determined for drug. Luliconazole showed maximum absorbance at 296 nm. 296 nm considered as an analytical wavelength for further determination as it reported in research article.

Linearity And Range

Table No.1 Results of UHPLC Linearity Data for Luliconazole:

Level	Conc (µg/mL)	Area	Mean	% RSD
10%	1.00	1022684	1026363	0.470
		1024586		
		1031820		
50%	5.00	4986603	4984377	0.209
		4973025		
		4993503		
100%	10.00	9997342	10008016	0.231
		10034562		
		9992145		
125%	12.50	12473334	12461283	0.371
		12410256		
		12500258		
150%	15.00	14928360	14924836	0.317

		14875896		
		14970253		

Calibration curve for luliconazole

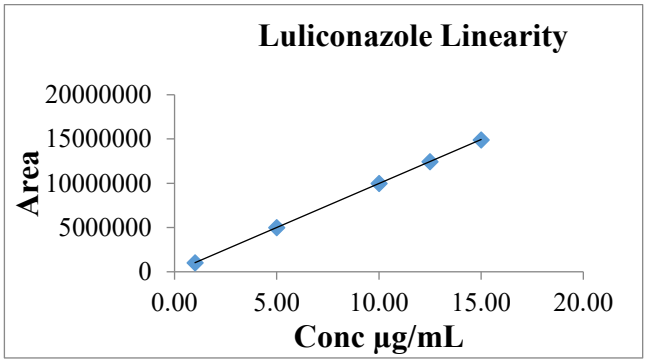

Fig. 3: Calibration Curve of LCZ

Working standard Solution: 10µg/ml concentration. UV range scanned: 200–400 nm using methanol as blank. The value of λ max was found to be 296 nm.

Table 2: Summary UHPLC linearity of Luliconazole.

Sr no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	1.0-15.0 µg/mL	NA
2	Correlation coefficient (R^2)	0.99999	NLT 0.98
3	Intercept	32001.5132	To be report
4	Slope	994134.8835	To be report
5	% RSD for area at each level	NA	NMT 2.0

The respective linear equation for Luliconazole was:

Y = M X + C

Y = 994134.8835 x + 32001.5132

Where, x = concentration of Analyte in µg/mL, y = area of peak, M = Slope, C= Intercept

Precision Studies: Inter-day precision studies were performed using 6 sample. The method is precise as the % RSD values (Table) were found within an acceptable limit.

Table No.3 Result of Intra- day and Inter- Day Precision for Luliconazole

	Sample	Test Sample (mg)	Area	% Assay
Repeatability	Sample 1	1000.8	10063581	100.27
	Sample 2	1001.4	9912501	98.71
	Sample 3	1001.2	9725470	96.87
	Sample 4	1000.3	9855601	98.25
	Sample 5	999.8	10006582	99.81
	Sample 6	1000.3	9812363	97.82
	Mean			**98.62**
	STD DEV			**1.263810**
	% RSD			**1.281**
Intermediate precision (Inter-Day)	Sample 1	1001.4	9924686	98.83
	Sample 2	999.8	10001591	99.76
	Sample 3	1001.6	9785236	97.42
	Sample 4	1000.9	9822360	97.86
	Sample 5	999.8	9718610	96.93
	Sample 6	1000.6	10017593	99.84

	Mean	**98.44**
	STD DEV	**1.225675**
	% RSD	**1.245**
Repeatability Plus Inter-day	**Mean**	**98.531**
	STD DEV	**1.19074**
	% RSD	**1.208**

Robustness of analytical method: One factor was change at one time to estimate the effect. Robustness of method was evaluated for Luliconazole. Irrelevant change in peak area and less variability in retention time were observed. The results of robustness studies are shown in (Table No.4) Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

Table No.4 Result of Robustness study

Change in Parameter	R.T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +3 NM (299 NM)	4.11	9896988	1.23	11540
Wavelength by -3 NM (293 NM)	4.11	9784725	1.21	11637
Flow rate by +10% (1.1mL/min)	3.74	9058659	1.20	10747
Flow rate by -10% (0.9mL/min)	4.56	11100477	1.26	12477
Column oven temp by +2ºC (42 ºC)	4.09	9930145	1.26	11360
Column oven temp by -2ºC (38 ºC)	4.11	9965230	1.23	11413

Accuracy studies for luliconazole: Recovery studies were carried out to verify the developed method's accuracy. A specific concentration of the standard drug (50%, 100%, and 150%) was added to the before examined topical solution, and its recovery was then examined. Validation of recovery studies by statistics, as indicated in (Table No.5)

Table No.5 Result and statistical data of Accuracy of Luliconazole

Level	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
50	5245821	5.23	5.30	98.68	99.30	0.5780
	5185826	5.17	5.20	99.42		
	5199582	5.19	5.20	99.81		
100	10085561	10.06	10.20	98.63	99.25	0.7688
	10041256	10.01	10.00	100.10		
	10024775	10.00	10.10	99.01		
150	14925302	14.88	15.10	98.54	99.67	1.2788
	15404785	15.36	15.20	101.05		
	15152569	15.11	15.20	99.41		

Overall Recovery**:** 99.41 %

% RSD for Overall Recovery: 0.825

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

LOD and LOQ are the lowest quantity of a given compound that can be found and measured using the designed UHPLC method. The signal to noise ratio is that minimum amount which when injected in UHPLC it gives minimum detectable peak area. The value of amount at this point is multiplied by 3 to get LOD and by 10 to get LOQ value. LOD for Luliconazole was found to be 0.073µg/ml & LOQ for Luliconazole was found to be 0.220µg/ml.

CONCLUSION

The UHPLC method was validated as per ICH guidelines and found to be quite simple, accurate, precise, sensitive, economical and reproducible. It can be used for routine analysis for the estimation of Luliconazole in bulk and Topical dosage form. The validation process attests to the suitability of this approach for their formulation-based quantification. It is also used in routine control of the formulations containing this entire compound.

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