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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF LULICONAZOLE IN BULK AND FORMULATION

S. P. Gadhwe*, V. V. Waghmare, S. J. Ithape, P. K. Ramgude, S. S. Telang and P. D. Mali

Dept. of Pharmaceutical Quality Assurance, Sinhgad College of Pharmacy, Vadgaon Bk., Pune, MS, India- 411041.

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***Corresponding Author**
S. P. Gadhwe
Dept. of Pharmaceutical Quality Assurance, Sinhgad College of Pharmacy, Vadgaon Bk., Pune, MS, India- 411041.

ABSTRACT

Luliconazole is a novel imidazole antifungal used in treatment of interdigital tinea pedis, tinea cruris, and tinea corporis. A simple, precise new ultrafast liquid chromatographic method has been developed and validated for the Luliconazole in bulk and pharmaceutical dosage form. The Luliconazole had been analyzed by using RP-HPLC method using Ammonium acetate buffer: ACN at (45:55 v/v) as a mobile phase at 296 nm. The retention time was found to be 5.7 min on chromatogram. The linearity, Precision and Accuracy has been studied and found to be in compliance with official limits mentioned in ICH guidelines.

KEYWORDS: Luliconazole, Anti-fungal, HPLC, Validation, Analysis.

INTRODUCTION

Luliconazole, trade names Luzu among others, is an imidazole antifungal medication. As a 1% topical cream, It is indicated for the treatment of athlete's foot, jock itch, and ringworm caused by dermatophytes such as Trichophyton rubrum, Microsporum gypseum[2] and Epidermophyton floccosum.[1] It belongs to imidazole class. Although the exact mechanism of action against dermatophytes is unknown. The azoles inhibit lansterol 14α-demethylase of the ergosterol synthesis pathway resulting in the inhibition of conversion of lanosterol to ergosterol. Insufficient amount of ergosterol leads to accumulation of intracellular 14α methyl sterols resulting in inhibition of growth, and leads to death.[3] It shows activity against a variety of fungi like Tinea, Aspergillus, Trichophyton and especially on Epidermatophytes.[4] Luliconazole was determined by analytical techniques such as,

HPTLC,[5] TLC,[6] LC-MS.[7] spectrophotometry[7-9] and few HPLC[10-12] methods. In the present study we have developed a simple, accurate and economic stability indicating method for the quantification of Luliconazole in bulk and pharmaceutical dosage form. The method was validated as per ICH guidelines.

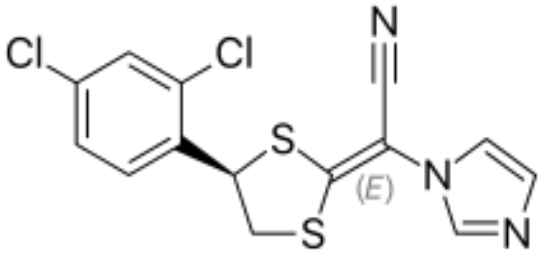

Fig. 1: Structure of luliconazole.

MATERIAL AND METHODS

Selection of analytical wavelength

Detection of wavelength was selected from spectra of Luliconazole obtained by using UV-spectrophotometer. 10µg/ml concentration of the drug samples were prepared in methanol separately and scanned in UV range (200 to 400 nm) using methanol as blank.

RP-HPLC Method Development and Optimization of chromatographic conditions

The standard solutions of Luliconazole (100 µg/ml) was used for RP-HPLC Method development. Different concentrations used for confirmation of drug peak.

A) Selection of detection wavelength

Wavelength of Luliconazole (296 nm) was selected as analytical wavelength from UV absorption spectra.

B) Selection of mobile phase

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 10 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-10mM Ammonium Acetate buffer was used in ratio of 45:55.

Selection of flow rate

Different mobile phase flow rates (0.8, 1, 1.2, 1.5, 1.8, 2 ml/min) were tried. The optimum flow rate for which theoretical plate number was maximum, with best resolution of all peaks and with reasonable run time (15 min) was selected.

Method validation[14]

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

Linearity and Range

Working standard solutions were injected in the range of 5-30µg/ml under the optimized chromatographic conditions and peak areas were calculated at 296 nm. The calibration curve was plotted between areas against concentrations of the drug. Linear regression data as well as calibration curve were shown in table no.27 and 28 under result and discussion section.

Precision (intra-day precision)

Repeatability study was carried out with nine replicates and intermediate (inter-day) precision was carried out with three concentrations of Luliconazole with three replicates. The values of % relative standard deviation (% RSD) for both the parameters are shown in table no. 29 and 30 under result and discussion.

Accuracy studies

The accuracy of the method was determined by calculating percentage recovery of Luliconazole from cream dosage form. Recovery studies were carried out by applying the method to cream dosage form containing Luliconazole at 80, 100 and 120% levels. At each level three determinations were carried out and the results are shown in table no. 32 under result and discussion.

Robustness studies

Robustness of the optimized method was studied by changing column wavelength (±2 nm), temperature (±2°C), and flow rate (±2%) during analysis. The sample was injected in triplicate for every condition and % RSD was calculated for each condition is shown in table no. 31 under result and discussion section.

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

Prepared six sets of concentrations between 5-30µg/ml and the corresponding areas of these sets were measured. Calibration curves were plotted for each set. The standard deviation of the y-intercept and average slope of the calibration curve was used to calculate LOD and LOQ using following formulae.

LOD = 3.3×SD/ Slope LOQ = 10×SD/ Slope

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

RESULTS AND DISCUSSION

The performance of UV, IR, TLC, Melting Point, Solubility, RP-HPLC Method Development and Validation is done. All results of above studies are below in result and discussion.

Physicochemical properties

UV Spectrophotometric studies on luliconazole

A) Selection of Analytical Wavelength:

Wavelength of detection was selected from spectrum of Luliconazole obtained by UV-spectrophotometer. 10µg/ml concentration of drug sample solution was prepared in methanol and scanned in UV range (400 to 200nm) using methanol as blank. When Standard solutions of Luliconazole scanned in UV range, showed absorbance at 290nm, 291nm 293nm, 295nm and 296nm. 296 nm was selected for the further studies.

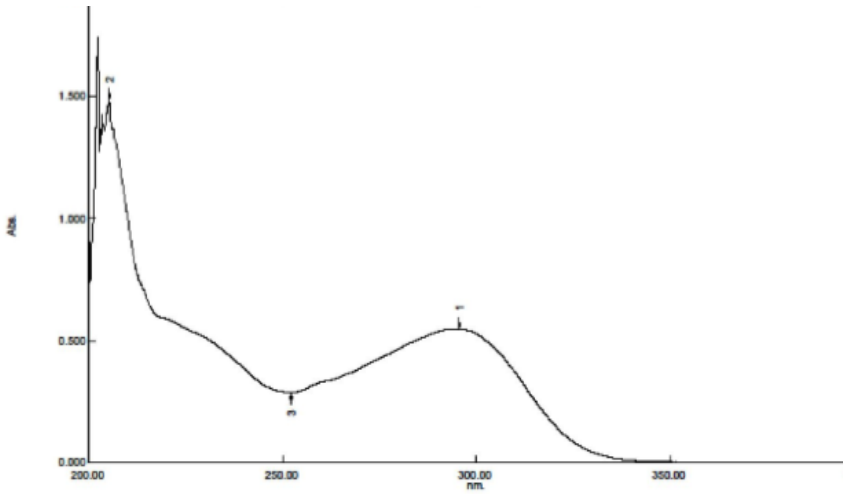

Fig. 2: Selection of analytical wavelength.

This graph shows the wavelength of drug that is 296nm by using UV.

Table 1: Linearity data of luliconazole.

Sr. no.	Conc. (ppm)	Absorbance
1	50	0.27454
2	75	0.41314
3	100	0.53860
4	125	0.65152
5	150	0.78894
6	175	0.90935

Calibration curve for luliconazole

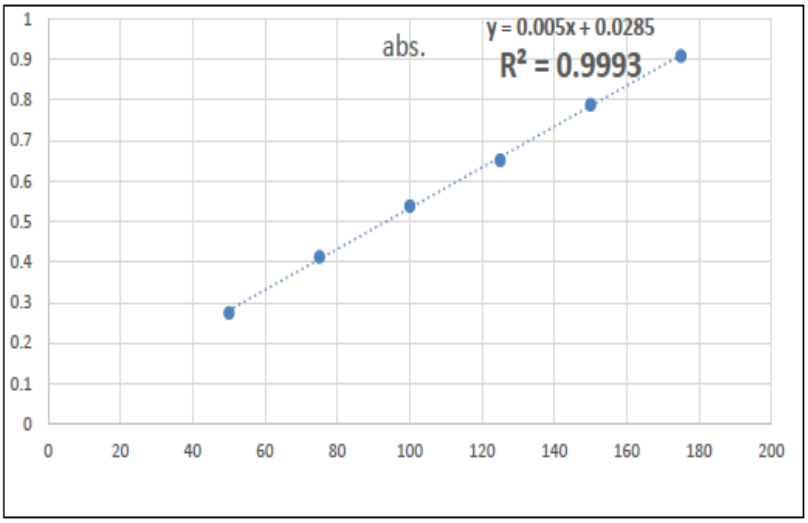

Fig. 3: Calibration Curve of LCZ.

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

Table 2: Linearity regression data for calibration curve.

Parameter	Luliconazole
Linearity and range	50-175 ppm
R^2	0.9993
Equation	Y=0.005x+0.0285

System suitability test parameters

Initially, various chromatographic conditions were tried in order to obtain better separation characteristics by changing mobile phase composition. Mobile phase Ammonium acetate buffer: ACN at (45:55 v/v) and 1ml/min. was selected with UV detection at 296 nm. The retention time of Luliconazole was found to be 5.7 min. Optimized chromatographic conditions are mentioned in table no.03

Table 3: System suitability test parameters.

Parameters	Luliconazole
Column	X Terra C18, (4.6×150mm), 3.5µm particle size
Flow rate	1ml/min
Injection volume	20ul
Retention time	5.7Min
Detection wavelength	296 nm
Mobile phase	Ammonium acetate buffer: CAN (45:55)
Run time	15min.
Diluent	Methanol
Theoretical plates	5457.749
Tailing Factor	0.957

Validation of the analytical method

Validation of developed chromatographic method was done by linearity, range, accuracy, precision, robustness and Limit of detection and Limit of quantification parameters as per ICH guideline Q2 (R1).

1. Linearity and Range

The value of correlation coefficient for Luliconazole was found to be the developed method was linear in the concentration range of 5–30 µg/ml. The linearity of calibration graphs and adherence of the system to Beer's law was validated by determining correlation coefficient and S.D. values which were found to be well within the accepted limits.

Table 4: Linearity data of luliconazole.

Sr. no.	Concentration/ug/ml	Peak area for luliconazole
1	5	386163
2	10	649827
3	15	939754
4	20	1190278
5	25	1449551
6	30	1712350

Calibration curve for luliconazole

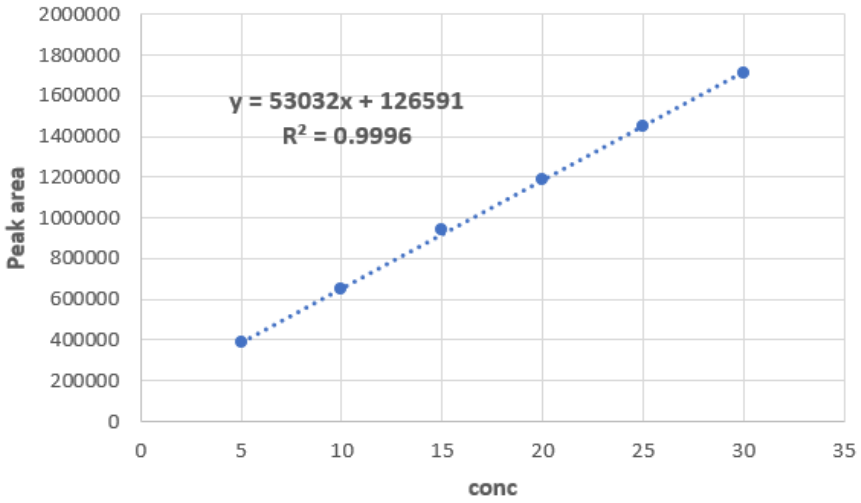

Fig. 4: Calibration Curve of LCZ.

The value of correlation coefficient for Luliconazole indicates the linear relationship between peak areas and concentrations. Therefore, the developed method was linear in the concentration range of 5-30µg/ml.

Linearity regression data for calibration curve

Table 5: Linearity regression data for calibration curve.

Parameter	Luliconazole
Linearity & Range	5-30ug/ml
SD	±496159
R2	0.9996
Y= mx + c	Y=53032x + 126591

Precision studies

Repeatability was studied by nine replicates of the working standard (10 µg/ml) solutions for Luliconazole. Intraday precision studies were performed by repeated injections of standard drug solutions of (10µg/ml). Inter day precision studies were performed using three different concentrations on three different days. The method is precise as the % RSD values (Table) were found within an acceptable limit.

Table 6: Intraday precision.

Precision	Amount ppm	Area	Mean Area ±SD	% RSD
Repeatability Intraday precision (n=9)	10	647023	647256.4± 2178.635	0.33 %
	10	647510		
	10	641776		
	10	647045		

	10	648827		
	10	648718		
	10	648065		
	10	647524		
	10	647023		

Table 7: Intermediate precision.

Precision	Amount ppm	Area	Mean Area ±SD	% RSD
Repeatability Intermediate precision (n=9)	5	396049	391111.667 ±4943.009	1.7
	5	391123		
	5	386163		
	10	657867	652094.333 ±5037.451	0.7
	10	648589		
	10	649827		
	15	963927	955924.667 ±14004.454	1.4
	15	964093		
	15	939754		

Robustness of analytical method

One factor was change at one time to estimate the effect. Robustness of method was evaluated at a concentration level 20µg/ml for Luliconazole. Irrelevant change in peak area and less variability in retention time were observed.

Table 8: Robustness of analytical method.

Factor	Level	Luliconazole (tR in min) Mean tR±SD	%RSD	Peak Area Mean peak area ± SD
A: Change in Wavelength of detection				
294 nm	-2	5.43±0.05	1.06 %	390099±6522.5
296 nm	2	5.5±0.05	1.03 %	398654.7±2893.8
298 nm	+2	5.6±0.1	1.8 %	393270.3±2445.4
B: Change in composition of flow rate				
0.8 ml/min	-2	5.6±0.05	1.01 %	390023.3±7494.2
1ml/min	2	5.73±0.05	1.0 %	398356.7±2113.8
1.2ml/min	+2	5.7±0.1	1.75 %	398831±1406.2
C: Change in temperature of oven				
23	-2	5.8±0.1	1.72 %	394357±557.74
25	2	5.7±0.05	1.0 %	385601.3±2685.05
27	+2	5.83±0.05	0.98 %	399148.3±1061.32

LOD and LOQ

LOD and LOQ are the smallest amount of the particular compound that can be detected and quantified by using the developed HPLC method. The signal to noise ratio is that minimum

amount which when injected in HPLC 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 8.69µg/ml & LOQ for Luliconazole was found to be 26.35µg/ml.

Accuracy studies for luliconazole

The recovery studies were performed on standard solution at 10, 20 and 30 µg/ml concentrations corresponding to 80, 100 and 120 % level. The mean % recovery ±SD values corresponding to 3 levels were found to be 98.06±0.709, 99.71 ± 0.160, 96.58631.

Table 9: Accuracy studies for luliconazole.

Drug	Amount added ppm	Amount recovered ppm	% Recovery	SD	%RSD
Luliconazole	10	9.73	97.3	98.06	0.723
		9.87	98.7		
		9.82	98.2		
	20	19.93	99.65	99.71	0.161
	20	19.92	99.60		
	20	19.98	99.90		
	30	28.76	95.86	96.58	0.653
	30	29.07	96.90		
	30	29.10	97.00		

CONCLUSION

The RP-HPLC method was validated as per ICH guidelines and found to be simple, economical and reproducible. It can be used for routine analysis for the estimation of Luliconazole in bulk and pharmaceutical dosage form.

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