The Pharma Innovation Journal 2019; 8(5): 103-108

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(5): 103-108

www.thepharmajournal.com
Received: 21-03-2019
Accepted: 25-04-2019

Tomal Majumder
Primeasia University, Dhaka, Bangladesh

Md. Razibul Hasan
State University of Bangladesh, Dhaka, Bangladesh

Pritam Roy
State University of Bangladesh, Dhaka, Bangladesh

Ratan Pramanik
State University of Bangladesh, Dhaka, Bangladesh

Md. Nazmul Hasan
State University of Bangladesh, Dhaka, Bangladesh

Correspondence
Tomal Majumder
Primeasia University, Dhaka, Bangladesh

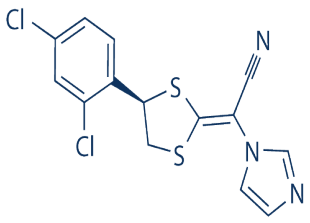
Method development and validation of RP-HPLC method for estimation of luliconazole in marketed formulation (Cream)

Tomal Majumder, Md. Razibul Hasan, Pritam Roy, Ratan Pramanik and Md. Nazmul Hasan

Abstract
A simple, specific, accurate, precise, rapid, robust and selective stability indicating reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for assay and validated for quantification of antifungal drug with its excipients in its topical dosage form. The mobile phase has been used for separation consisting of Water: Acetonitrile (60:40). Column used was C_{18} (4.6 X150 mm, 5µm) with flow rate of 2.0 ml/min. Detection wavelength for Luliconazole was 294 nm. The method has been linear at 60-140% range with r2 0.999. Luliconazole has showed 97-103% recovery. The LOD and LOQ were found to be 0.38µg/ml and 1.06µg/ml respectively. Methanol was used as solvent. The method have been robust under various variation with flow rate, detection wavelength and column oven temperature. Developed method can be used routinely for estimation of drug Luliconazole with its excipients in dosage form and stability sample. The validation of method was carried out as per ICH Guidelines.

Keywords: luliconazole, assay, RP-HPLC, stability, antifungal, validation

Introduction
Luliconazole belongs to imidazole class of drug that possesses a wide spectrum of antifungal activity and is very potent against dermatophytes. Luliconazole is chemically, (2E)-2- [(4R)-4-(2, 4-dichlorophenyl)-1, 3-dithiolan-2-ylidene]-2-imidazol-1-yl-acetonitrile. Its structural formula is:



Luliconazole

The molecular formula is $C_{14}H_9Cl_2N_3S_2$ with a molecular weight of 354.28. Luliconazole is the R enantiomer and contains one chiral center. The double bond adjacent to the dithiolane group is in the E configuration. It is not official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP). Till date no analytical method was reported for quantitative estimation of LCZ except stability indicating method. LCZ is a novel antifungal drug launched in India by Ranbaxy Laboratories Ltd. The compound was originally screened from active compounds related to lanoconazole, a potent antidermatophytic drug. Literature survey reveals that the simple and rapid stability-indicating liquid chromatographic method has been developed and validated for LCZ. The present manuscript describes simple, accurate, precise, reproducible, and economical RP-HPLC method for the estimation of Luliconazole in marketed formulation.

Material, Machine and Methods
Instruments
Shimadzu (Prominence – i LC2030 3D Plus liquid chromatography), Analytical balance (Mettler Toledo, Model: ML204/01).

Chemicals and Reagents

Methanol (Active Fine Chemicals Ltd, Bangladesh), Acetonitrile (Active Fine Chemicals Ltd, Bangladesh), Luliconazole (Viwit Pharmaceuticals Co. Ltd., India).The Lulizol 1% Cream was procured from the local market. Lulizol (Eskayef Pharmaceuticals Limited) 1% Cream contains 1% Luliconazole, an azole antifungal agent, in a white cream for topical application.

Methodology (By HPLC)

Chromatographic system

Column	:	4.6-mm × 150 -cm, L_1 (C_{18})
Wavelength	:	296nm
Flow Rate	:	2.00ml / min
Inject volume	:	20 µl
Temperature	:	40.0 ^{0}C
Run Time	:	20.0 minutes

Preparation of mobile phase

Prepare a filtered and degassed mixture of Water, Acetonitrile (60: 40). Make adjustments if necessary.

Standard preparation

Weigh accurately about 10 mg of working standard of Luliconazole and take into a 100 ml volumetric flask. Add 60 ml of methanol and sonicate to dissolve. Make volume up to the mark with the same solvent and mix well. Filter the solution through Whatman # 1 filter paper and collect the filtrate discarding first few ml. Then take 2 ml of solution into another 50 ml volumetric flask and add methanol upto the mark and mix well. Filter the solution through 0.45 µ membrane filter.

Sample preparation

Weigh accurately about 1.0 g of test sample equivalent to about 10 mg of Luliconazole into a 100 ml volumetric flask. Add about 60 ml of methanol and sonicate in a water bath at 55.0^{0}C to 60.0^{0}C until the sample is completely dispersed, and mix. Cool the solution to below room temperature, mix and dilute with same solvent to volume and mix well. Filter the solution through Whatman # 1 filter paper and collect the filtrate discarding first few ml. Then take 2 ml of solution into another 50 ml volumetric flask and add methanol up to the mark and mix well. Filter the solution through 0.45 µ membrane filter.

Procedure

Equilibrate the system for about 60 minutes with mobile phase flow rate at 2.0 ml/min. Separately inject equal volumes (about 20 µl) of the standard preparation and the sample preparation in to the chromatograph, record the chromatograms, and measure the responses for the major peaks. %RSD of peak area for replicate injections of standard solution should be less than 2.0%.

Calculate the amount of Luliconazole per g of test sample by using the following equation:

$$= \frac{Pu}{Ps} \times \frac{Ws}{100} \times \frac{2}{50} \times \frac{100}{Wu} \times \frac{50}{2} \times \frac{P}{100}$$

= ---------- Luliconazole (mg/g)

Where,

Pu	=	Peak area of test sample solution
Ps	=	Peak area of standard solution
Wu	=	Sample weight taken in g
Ws	=	Standard weight taken in mg
P	=	Potency of standard (as Luliconazole)

Validation parameters

System precision/system suitability

System suitability testing is an integral part of many analytical procedures. System suitability test parameters depend on the type of procedure being validated. System precision is determined by measuring the peak area of standard solution containing 100% working concentration for six times and calculates the % RSD. The % RSD should be less than 2.0%.

Table 1: System precision

	No of measurement	Peak area	Retention time	Theoretical plates (NLT 2000) (NLT 2000)
Standard Concentration (0.2 mg/ml)	1.	119206	14.502	8668
	2.	119646	14.411	8765
	3.	119423	14.286	8782
	4.	119780	14.358	8758
	5.	119473	14.242	8666
	6.	119804	14.329	8611
RSD % (Limit NMT 2.0%)		0.193 %	0.644%	Limit:≥2000

The relative standard deviation of six replicate measurement of standard solution found 0.193 % (limit NMT 2.0%), which indicates that the system is precise to analyze the sample.

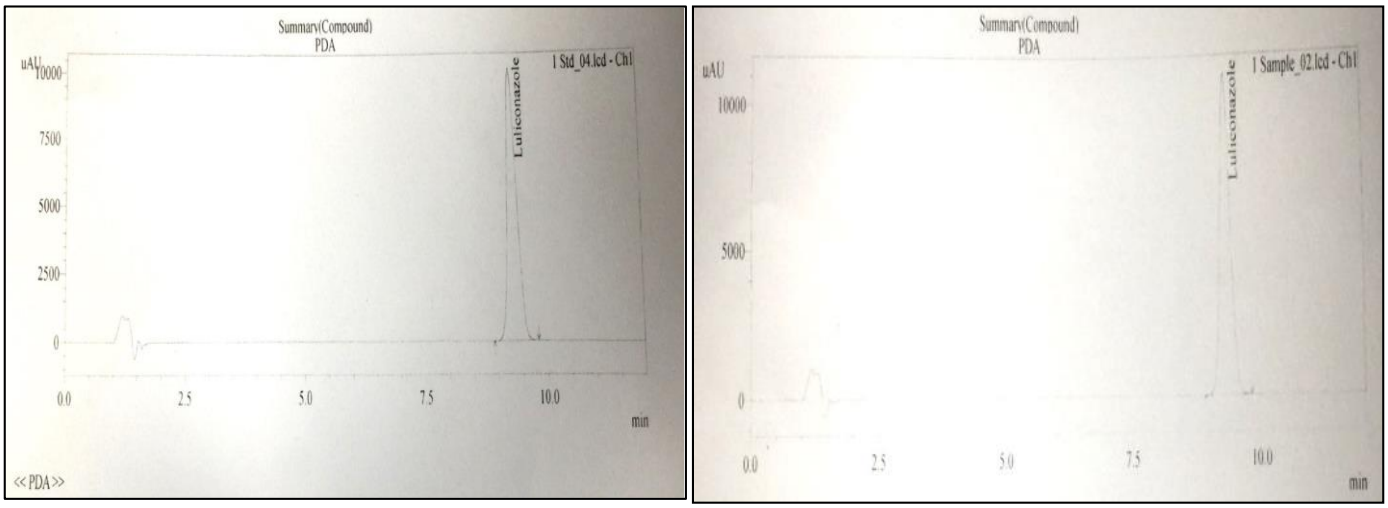


Fig 1: Chromatogram of luliconazole standard & sample

Precision

Repeatability/ method precision

Repeatability was established by analyzing six separate samples at 100% of the working concentration from a same batch Lulizol (Eskayef Pharmaceuticals Limited) 1% Cream Result was calculated against label claim.

Table 2: Data for method precision

S. No.	Sample taken (in g)	Peak area	Results (mg/g)	RSD %
1	1.06	117736	10.07	1.10 (Limit NMT 2.0%)
2	1.08	118095	9.91	
3	1.04	116417	10.15	
4	1.06	116159	9.93	
5	1.05	116453	10.05	
6	1.04	116861	10.18	

The relative standard deviation of assay result of six separate samples from a single batch found 1.10 % (limit NMT 2.0%) which indicates that the method is precise to analyze Luliconazole.

Intermediate precision

Intermediate precision was established by analyzing six separate samples at 100% of the working concentration from a same batch of in different day by different analyst using different machine. Result was calculated against label claim.

Table 3: Data for precision in day 2

S. No	Sample taken (in g)	Peak Area	Results (mg/ g)	RSD %
1.	1.03	116336	9.98	1.34 (Limit NMT 2.0%)
2.	1.02	116555	10.10	
3.	1.03	116536	10.00	
4.	1.05	116396	9.80	
5.	1.01	116521	10.20	
6.	1.03	116388	9.99	

Table 4: Data for intermediate precision

Sample No.	Results (mg/ g)	
	Day-1 (Analyst-01)	Day-2 ((Analyst-02)
1.	10.07	9.98
2.	9.91	10.10
3.	10.15	10.00
4.	9.93	9.80
5.	10.05	10.20
6.	10.18	9.99
Mean	10.05	10.01
Overall mean	10.03	
% RSD	1.10	1.34
RSD % of 12 units	1.22	

The relative standard deviation of assay result of six separate samples from a single batch found 1.10 % in day 1, 1.34 % in day-2 and 1.22 % for 12 results (limit NMT 2.0%) which indicate that the method is precise to analyze the Luliconazole.

Accuracy

Accuracy was established by analyzing nine sample solution of Luliconazole spiked with placebo at 80%, 100% and 120% of the working concentration (Three replicates for each concentration) and the percent recovery was calculated. The percent recovery at each level should be within 97.0% to 103.0%. A linear curve was prepared by plotting amount added Vs amount recovered and correlation co-efficient was calculated.

Standard preparation

10.9 mg working standard of Luliconazole was weighed and taken into 100ml volumetric flask. About 60 ml of methanol was added and Sonicated to dissolve. Volume was made with diluents and mixed well. The solution was filtered through

whatman # 1 filter paper and the filtrate was collected discarding first few ml. 2 ml of this filtered solution was taken into a 50 ml volumetric flask and volume was made up to mark with diluents and mixed well. Again the solution was filtered through 0.45 µ membrane filter.

Placebo stock preparation
1 g of placebo was weighed and taken into 100 ml volumetric flask.60 ml of methanol was added and sonicated to dissolve. Made volume up to the mark with same methanol and mix well. Filtered the solution through Whatman # 1 filter paper and collected the filtrate discarding first few ml.

Sample preparation for 80%
Sample 01
1.6 ml Standard stock preparation + 2 ml Placebo stock preparation →50 ml with methanol. Again the solution was filtered through 0.45 µ membrane filter.

Sample 02
1.6 ml Standard stock preparation +2 ml Placebo stock preparation →50 ml with methanol. Again the solution was filtered through 0.45µ membrane filter.

Sample 03
1.6 ml Standard stock preparation +2 ml Placebo stock preparation →50 ml with methanol. Again the solution was filtered through 0.45 µ membrane filter.

Sample preparation for 100%
Sample 01
2 ml Standard stock preparation + 2 ml Placebo stock preparation →50 ml with methanol. Again the solution was filtered through 0.45 µ membrane filter.

Sample 02
2 ml Standard stock preparation + 2 ml Placebo stock preparation →500 ml with methanol. Again the solution was filtered through 0.45 µ membrane filter.

Sample 03
2 ml Standard stock preparation + 2 ml Placebo stock preparation →50 ml with methanol. Again the solution was filtered through 0.45 µ membrane filter.

Sample preparation for 120%
Sample 01
2.4ml Standard stock preparation + 2 ml Placebo stock preparation →50 ml with methanol. Again the solution was filtered through 0.45 µ membrane filter.

Sample 02
2.4 ml Standard stock preparation +2 ml Placebo stock preparation →50 ml with methanol. Again the solution was filtered through 0.45 µ membrane filter.

Sample 03
2.4ml Standard stock preparation + 2 ml Placebo stock preparation →50 ml with methanol. Again the solution was filtered through 0.45 µ membrane filter.

Table 5: Data for accuracy

Concentration level	Sample No.	Amount added in (mg/ml)	Amount recovered in (mg/ml)	Peak area	% Recovery
80%	Sample-1	0.00349	0.00339	92515	97.1
	Sample-2	0.00349	0.00339	92727	97.1
	Sample-3	0.00349	0.00339	92857	97.1
100%	Sample-1	0.00436	0.00424	116022	97.2
	Sample-2	0.00436	0.00423	115862	97.0
	Sample-3	0.00439	0.00425	116492	97.5
120%	Sample-1	0.00523	0.00511	140090	97.7
	Sample-2	0.00523	0.00512	140259	97.9
	Sample-3	0.00523	0.00511	139839	97.7

The percent recovery was calculated for nine determinations and found within limit. A graphical presentation between amount added VS amount recovered also shows linearity. Thus it has been concluded that the method is accurate to analyze the Luliconazole.

Specificity
The retention time of the sample preparation for assay is concordant with the retention time of standard sample from assay preparation.

Blank effect: To identify the blank effect.

Placebo effect: To identify the placebo effect.

Wt. of placebo: 1 g →100 ml →2 ml→50 ml

	Peak area
Blank	No interference
Placebo	No interference

There is no significant interference of blank and placebo. So the method is specific to analyze Luliconazole.

Linearity
Five different standard solutions were prepared covering a concentration of 60% to 140 % of the working concentration of Luliconazole and all peak area was recorded. A linear curve was prepared by plotting percentage of nominal concentration Vs peak area and correlation co-efficient was calculated. The results obtained correlate with the concentrations resulting in the following calibration curve.

Standard stock solution
10 mg working standard of Luliconazole was weighed and taken into 50 ml volumetric flask. About 60 ml of methanol was added and sonicated to dissolve. Volume was made with methanol and mixed well. The solution was filtered through whatman # 1 filter paper and the filtrate was collected discarding first few ml.

Sample preparation for 60%
1.20 ml Standard stock preparation →50 ml with Methanol .Again the solution was filtered through 0.45 µ membrane filter.

Sample preparation for 80%
1.60 ml Standard stock preparation →50 ml with Methanol. Again the solution was filtered through 0.45 µ membrane filter.

Sample preparation for 100%
2.0 ml Standard stock preparation →50 ml with Methanol. Again the solution was filtered through 0.45 µ membrane filter.

Sample preparation for 120%
2.40 ml Standard stock preparation →50 ml with Methanol. Again the solution was filtered through 0.45 µ membrane filter.

Sample preparation for 140%
2.80 ml Standard stock preparation →50 ml with Methanol. Again the solution was filtered through 0.45 µ membrane filter.

Table 6: Data for linearity

S. No.	Concentration of Luliconazole (mcg/ml)	Percentage of nominal concentration	Peak area
1	2.4	60%	70433
2	3.2	80%	95627
3	4.0	100%	119919
4	4.8	120%	144097
5	5.6	140%	168212

Correlation coefficient : 0.999

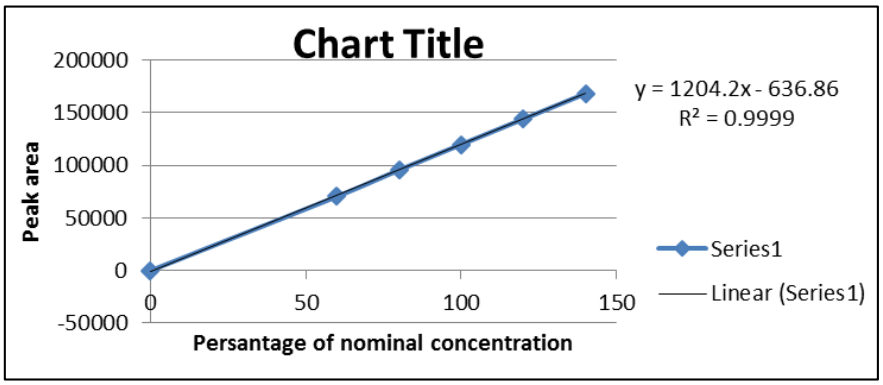


Fig 2: Graphical presentation of linearity

The correlation co-efficient found 0.999. Thus the graph confirms the linearity of the method in the range of 60% to 140%.

Limit of detection and limit of quantification
ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the 3.3 × (SD/Slope) and 10 × (SD/Slope) criteria, respectively; where SD is the standard deviation of y-intercept of regression line and S is the slop of the calibration curve.

Robustness
Robustness of this method was determined by analyzing the same batch of Lulizol (Eskayef Pharmaceuticals Limited) 1% Cream by different flow rate and different wavelength.

Table 7: Data for robustness

S. No.	Changed Flow rate (ml/min)	Peak area	Assay (%)	Changed mobile phase	Peak area	Assay (%)	RSD (%)
1.	Flow rate actual 2.00 ml/min			Wavelength:296 nm			0.49
	Standard	120626	99.4	Standard	120626	99.4	
	Sample	116350		Sample	116350		
2.	Flow rate changed to 1.8 ml/min			Wavelength:298 nm			
	Standard	133443	99.6	Standard	120991	99.6	
	Sample	128890		Sample	116880		
3.	Flow rate changed to 2.20 ml/min			Wavelength:294 nm			
	Standard	109673	100.6	Standard	119133	100.2	
	Sample	105967		Sample	114721		

Table 8: Summary results

S. No.	Validation parameters	Results
1.	System precision/System suitability	RSD 0.193%
2.	Precision	
	Repeatability/ Method Precision	RSD 1.10 %
	Intermediate Precision	RSD 1.22 %
3.	Accuracy	97.1-97.9 %
4.	Linearity	Correlation co-efficient 0.999
5.	LOD(µg/ml)	0.38
6.	LOQ(µg/ml)	1.06
7.	Robustness	RSD 0.49 %
8.	Specificity	No peak Area detected

Conclusion

The results of our 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. Statistical analysis proves that the method is repeatable and selective for the analysis of Luliconazole. It can therefore be conclude that use of the method can save time and money and it can be used in small laboratories with accurate and wide linear range. From the above data it was observed that all validation parameters (like system suitability, precision, accuracy, specificity, linearity, robustness) meet the predetermined acceptance criteria. Thus it has been concluded that the method is validated for the analysis of Luliconazole in Lulizol (Eskayef Pharmaceuticals Limited) 1% Cream.

Acknowledgements

Authors are highly thankful to Department of Pharmacy, State University of Bangladesh and Department of Pharmacy, Primeasia University for providing facilities to complete the work.

References

1. Validation of compendial procedures, USP-42, Chapter-1225.
2. ICH, Q2 (R1) Validation of Analytical Procedure Methodology, International Conference on Harmonization.
3. Tripathi KD, Essential Medical pharmacology, Jaypee Brother Medical Publisher, Six edition pp 684-689.
4. Mohan H. Textbook of Pathophysiology, Jaypee brother's Medical Publishers, Six Edition, 773.
5. Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dales Pharmacology, Churchill Livingstone Elsevier Publication, Six Edition, 692.
6. Hardman G, Limbird L. Goodman and Gilman's-The Pharmacological basis of Therapeutics, Mc Grow Hill Medical Publishing Division, Tenth Edition, 1181-1182.
7. Chatwal GA. Insrtumental Methods of Chemical Analysis, Himalaya Publishing House, First edition, 2.167-2.171.
8. Sharma BK. Instrumental methods of chemical analysis in introduction to analytical chemistry, Nineteenth Edition, Goel publishing House, 2003, 1-4:71
9. Willard HH, Merritt LL, Dean JA, Frank AS. Instrumental method of analysis, New Delhi: CBS publishers and Distributors, Seventh Edition, 1986, 101-105.
10. Skoog DA, Crouch SR, Holler FJ. Analytical Methods. Principles of Instrumental Analysis. California Cengage Learning Inc., sixth Edition, 2006, 6-19.
11. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development, John Wiley and Sons, New York, A Wiley- Inter science Publication, Second Edition, 1997, 21-56,266-288.
12. Sethi PD. HPLC, Quantitative analysis of pharmaceutical formulations, CBS Publishers and Distributors, New Delhi,1996, 3-3.
13. Chung HCC, Lee YC. Analytical method validation and instrument performance verification, Wiley- Inter science Publication, 1999, 51-67.