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Quality by Design based Analytical method Development and Validation for the Estimation of Luliconazole in API and Pharmaceutical Dosage form.

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ABSTRACT

A novel Quality by Design methodology was used to develop and validate a rapid, accurate, precise, simple, efficient and reproducible isocratic Reversed Phase-High Performance Liquid Chromatographic (RP-HPLC) method for the estimation of Luliconazole in bulk and pharmaceutical dosage form. Luliconazole was separated using Kromasil C18 column (250mm×4.6 mm, 5µm particle size), Shimadzu LC2030 HPLC system having UV detector and the mobile phase contained a mixture of 0.01M Ammonium acetate buffer and Acetonitrile (35:65). The flow rate was set to 1.2 ml/min with the responses measured at 294nm. The retention time of Luliconazole was found to be 3.092 min. Central composite design employed for design of experiment and optimization. Desirability value was found to be 0.723 and overall model was found to significant. Linearity was established for Luliconazole in the range of 20-120 µg/ml with correlation coefficient (r2=0.9995). Limit of detection (LOD) and limit of quantitation (LOQ) were evaluated and found to be 1.1700 and 3.5455 respectively. The accuracy values were found to be in the range of 98 –102% and every parameter found with in limit. Validation parameters were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. This method can be used for the estimation and analysis of Luliconazole drug in active pharmaceutical ingredients and pharmaceuticals.

Keywords: 3 level factorial designs, Luliconazole, Development, Validation.

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INTRODUCTION

Luliconazole is chemically 2-[(2E, 4R)-4-(2,4- dichlorophenyl)-1,3- dithiolan2 ylidene]-2 – (1,H imidazole – 1- yl) and having molecular formula is $C_{14}H_{9}C_{l2}N_{3}S_{2}$ with a molecular weight of 354.28 (Luliconazole structure shown in figure 1). LCZ is a wide spectrum of antifungal activity and is very potent against dermatophytes. It is an imidazole antifungal agent and indicated for the topical treatment of interdigital tineapedis, tineacruris, and tineacorporis. From literature survey intended for Luliconazole analysis revealed existing methods which include an LCMS/MS method, a HPTLC method, HPLC methods, and few spectroscopic methods for the quantification of Luliconazole and its Pharmaceutical formulations by biological samples, Assay method, Dissolution method. Since very few HPLC methods were reported, an attempt has been made to novel Quality by Design approach to develop and validate a simple, appropriate and rapid RP-HPLC method for the estimation of Luliconazole in creams.

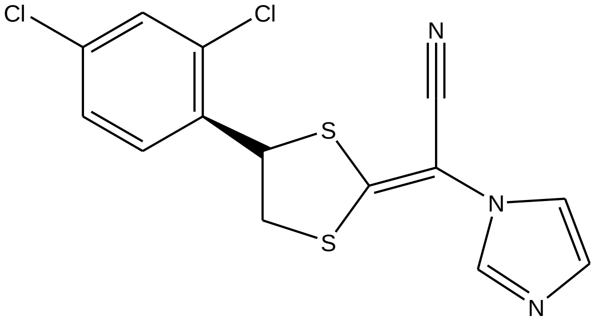

Figure 1: Structure of Luliconazole

MATERIALS AND METHOD:

Chemicals and reagents:

Chemicals: Acetonitrile (HPLC Grade), Orthophosphoric acid (AR Grade), Acetate Buffer (HPLC grade) by Merck Life Science, water (HPLC Grade).

The active pharmaceutical drug and sample used in experiment, shown in table 1.

Table 1: Active Pharmaceutical Drug

Sr.No.	Name	Description
1.	Luliconazole	White powder, antifungal agent. Procured from GHPL, Pune
2.	Lulicoz 1%	Lulicoz 1% drug contain each unit Manufactured by Bioconic Remedies

Instrumentation:

The instrument / equipment used for experimental work is shown in table 2.

Table 2: Instrumentation Details

Sr. No.	Name of Equipment's / Instruments	Model / Specification	Manufacturer
1	HPLC Pump Sample Injection Port UV/Vis Detector Software	Series LC2030 PU2030 Autosampler UV 2075 plus Lab Solutions	Shimadzu (I prominence plus)
2	pH Meter	101	Chemiline
3	Balance	AY-120	Shimadzu
4	Sonicator	UCB-40	Rolex

Design of Experiment

3 level-Factorial designs by Design expert 8 Software.

3 level-is an experimental matrix that has limited application in RSM when the factor number is higher than 2 because the number of experiments required for this design (calculated by expression N = 3k, where N is experiment number and k is factor number) is very large, thereby losing its efficiency in the modeling of quadratic functions. The majority of applications of 3 level-factorial designs are in the area of chromatography.

Selection of Dependent factors

- Retention Time
- Area
- Theoretical Plate
- Asymmetry

Selection of Independent factors

- Mobile Phase
- pH of Mobile phase
- Flow Rate

Columns used

C18 Column

3 level-Factorial design

Factorial design has flexibility to change/add/delete any parameter at any time when our experiment is going on. It provides facility to give standard run at one time at only one mobile phase. Three independent factors have been selected.

Following mobile phases selected

- Water : Methanol

- Water : Acetonitrile
- Buffer : Acetonitrile

3 level-Factorial design facilitate only one mobile phase at a time

- Buffer : Acetonitrile
- pH of Aqueous Phase: 4-6 mmol/L
- Change Flow Rate Range: 0.8-1.2
- Change Mobile phase proportion Range: 60-70% (Consider Organic Phase)

When all above ranges are put in 3 level-Factorial design. It gave 12 run at different pH and Mobile phase proportion.

Followed same procedure for each mobile phase. That is for column C-18 which has three mobile Phase. Total runs of design are 36. After completion of all trails software give one optimize best value for given chromatographic conditions. Optimization means finding an alternative with the most cost effective or highest achievable performance under the given constraints, by maximizing desired factors and minimizing undesired ones. In comparison, maximization means trying to attain the highest or maximum result or outcome without regard to cost or expense.

3 level-Factorial design was utilized for method development in order to evaluate the effects of amount of buffer, buffer pH and flow rate on responses. Total 12 runs were suggested by the software. Factors and responses considered for study were shown in table 3 Ranges considered were based upon previous univariate chromatographic separation studies. For amount of organic range were 60-70 % v/v and for flow rate 0.8 to 1.2 mL min.

Table 3: Run suggested by Software (Design Expert)

Sr. No	Mobile Phase Composition (Organic Phase)	pH of Buffer	Flow Rate
1	65.00	6.00	0.80
2	70.00	5.00	1.20
3	65.00	4.00	1.20
4	65.00	4.00	0.80
5	70.00	6.00	1.00
6	60.00	4.00	1.00
7	60.00	5.00	0.80
8	60.00	5.00	1.20
9	60.00	6.00	1.00
10	70.00	5.00	0.80
11	70.00	4.00	1.00
12	65.00	6.00	1.20

Preparation of mobile phase

65 ml of HPLC grade Acetonitrile and 35 ml of Buffer pH was adjusted to 4.0 with

orthophosphoric acid i.e. in 65: 35 v/v proportions. The solution was filtered through 0.45μ membrane filter and then sonicated in sonicator bath for 10 min.

Preparation of stock solutions of Luliconazole

Stock solution was prepared by dissolving 10 mg Luliconazole in water and then diluted with Diluent in 10 ml of volumetric flask to get concentration of 1000 μg/ml. From the resulting solution 0.2 ml was diluted to 10 ml with Diluent to obtain concentration of 20 μg/ml of Luliconazole and labeled as standard stock Luliconazole.

Selection of detection wavelength

From the standard stock solution further dilutions were done using water and scanned over the range of 200-400 nm and the spectra were overlain. It was observed that drug showed considerable absorbance at 294 nm.

RESULTS AND DISCUSSION

Optimization Result

Screening design for suitable chromatographic condition:

Determination of solvent system based on peak parameters. Methanol: water/ ACN: water and ACN: Ammonium Formate Buffer, these three mobile phases were selected for screening study on C18 columns at pH 4.0 and 6.0. These mobile phases were screened by varying the organic phase composition from 35 to 65 % v/v. Flow rate was varying form 0.8-1.2 ml/min.

Results of various trials, having organic phase composition 65 % v/v are shown in following tables 4.

Table 4: Trials performed on C18 column at mobile phase (65:35 v/v) with aqueous phase pH 4

Sr. no.	Composition	Observation	Remarks
1	Methanol: water	Less peak asymmetry but less theoretical plates	Satisfied
2	ACN: buffer	**Less peak asymmetry with more theoretical plates and good retention time with greater peak height**	**Extremely Satisfactory**
3	ACN: water	Greater peak Asymmetry and lower theoretical plates	Not satisfactory

This methodology is initially based on constructing a desirability function for each individual response. The scale of individual desirability function ranges between i= 0, for completely undesirable response and i =1, for fully desired response. Selection of trial was based on maximum desirability value. Therefore, first trial which was having desirability one (i=1) selected for method optimization. The desirability value presented in table 5.

Table 5: Optimized trials suggested by software based on desirability value

Sr. no.	Amount of Acetonitrile	pH of buffer	Flow rate	Retention time	Tailing factor	Theoretical plates	Desirability
1	65.00	4.0	1.2	3.12	1.07	11483	0.784

Optimized chromatographic conditions:

Mobile phase: Phosphate buffer: Acetonitrile (35: 65 v/v), Analytical column: C_{18} column Kromasil (4.6× 250mm id. particle size 5µm), UV detection: 294 nm, Injection volume: 10 µL, Flow rate: 1.20 mL min^{-1}, Temperature: Ambient, Run time: 5 min

Effect of independent variables on retention time (X):

After applying experimental design, suggested Factorial Model was found to be significant with model F value of 52.03, *p* value less than 0.005 and R^2 value of 0.000. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of % C.V. and adjusted R^2 were 28.87 and 0.000 respectively. The model for response X (Retention time) is as follows:

The equation for Factorial model is as follows Retention Time (X)

$$\mathbf{X = +8.24}$$

Change in pH of buffer showed slightly change in retention time (X), also increase in amount of Acetonitrile showed decrease the retention time. The interpretation is shown in figure 2.

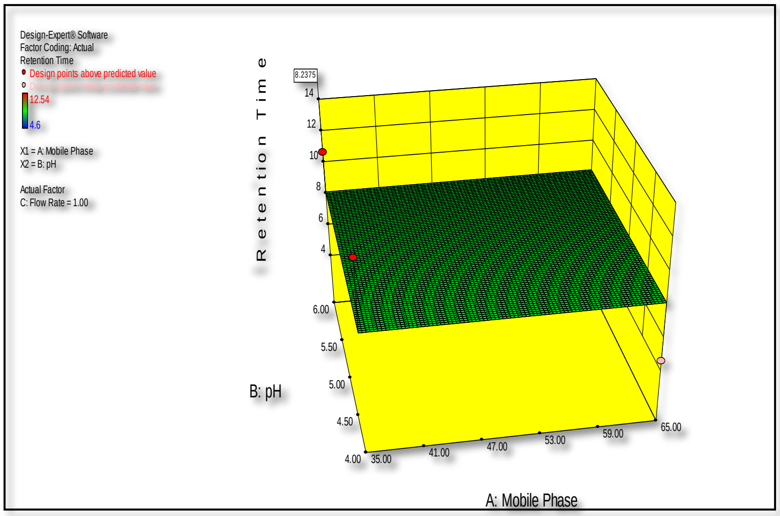

Figure 2: Three-dimensional plot for retention time as a function of pH of buffer and amount of buffer. Constant factor (flow rate- 1.2 mL min^{-1})

Fit summary: Quadratic model was suggested by the software.

ANOVA: ANOVA of developed 3 level factorial models for retention time (Y).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant (Presented in Table 6) In this case All factors are significant model terms.

Table 6: Significance of *p* value on model terms of retention time

Source	Sum of Square	df	Mean Squares
Model	0.000	0	
Residual	86.66	7	12.38
Cor Total	86.66	7	

Effect of independent variables on tailing factor (Y):

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 1.19, *p* value less than 0.005 and R^2 value of 0.0000. There is only a 0.0001% chance that a "Model F-Value" this large could occur due to noise. Values of % C.V. and adjusted R^2 were 24.44 and 0.0000 respectively. The model for response Y (Tailing factor) is as follows

$$Y = +1.37$$

As increases in pH of buffer had antagonistic effect on response while increase in amount of Acetonitrile showed decrease the asymmetric factor. (Figure 3)

Fit summary: Response Surface Linear Model was suggested by the software.

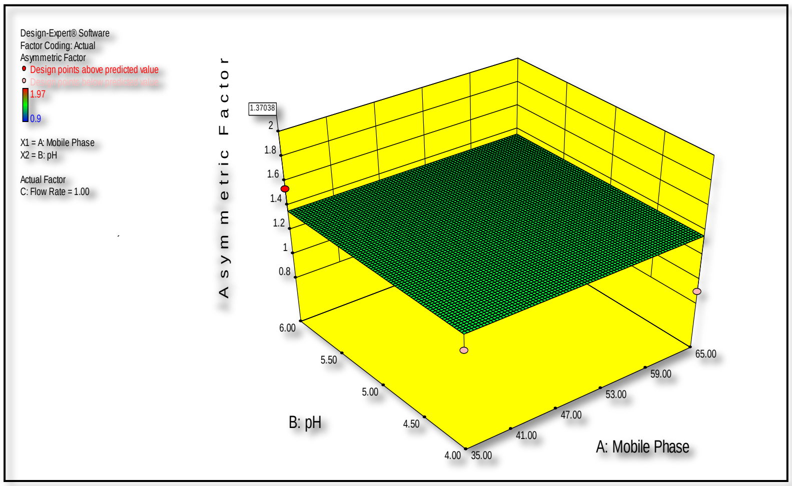

Figure 3:Three-dimensional plot for tailing factor as a function of pH of buffer and % v/v of buffer. Constant factor (flow rate- 1.2 mL min^{-1})

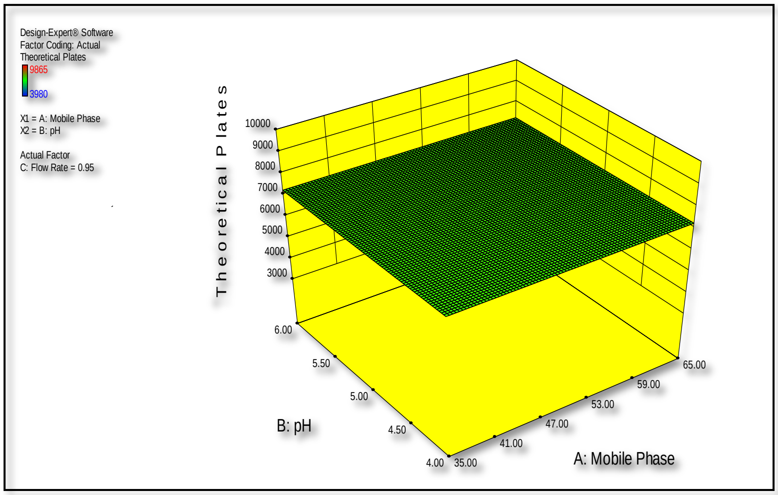

Figure 4: Three-dimensional plot for theoretical plates as a function of pH of buffer and % v/v of buffer. Constant factor (flow rate- 1.2 mL min^{-1})

ANOVA: ANOVA of developed factorial model for tailing factor (Y).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Table 7 shows the Significance of tailing factor.

Table 7: Significance of *p* value on model terms of tailing factor

Source	Sum of Square	df	Mean Squares
Model	0.000	0	0.12
Residual	0.87	7	
Cor Total	0.87	7	

Effect of independent variables on theoretical plates (Z):

After applying experimental design, suggested Response Surface Quaratic Model was found to be significant with model F value of 2.65, *p* value less than 0.005 and R^2 value of 0.000. There is only a 0.0001% chance that a "Model F-Value" this large could occur due to noise. Values of % C.V. and adjusted R^2 were 69.42 and 0.000 respectively. The model for response Z (theoretical plates) is as follows:

$$Z = +7289.13$$

An increases in pH of buffer showed increase in number of theoretical plates (Z), while increase in amount of Acetonitrile showed increases response. Combination of amount of Acetonitrile and pH of buffer showed synergistic effect on it. (Figure 4)

Fit summary: Factorial model was suggested by the software

ANOVA: ANOVA of developed CCD model for theoretical plates (Z).

Values of "Prob > F" (p- value) less than 0.0500 indicate model terms are significant.

In this case model is significant. (Table 8)

Table 8: Significance of *p* value on model terms of theoretical plates

Source	Sum of Square	df	Mean Squares
Model	0.000	0	
Residual	3.074E+007	7	4.392E+006
Cor Total	3.074E+007	7	

Method Validation

The projected RPHPLC method was validated in terms parameters such as specificity, linearity, precision, accuracy, and robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines.

Linearity and Range:

The linearity response was determined by analyzing six independent levels of calibration curve in the range of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml -120 µg/ml for Luliconazole standard. Linearity graph of concentration (as x-value) versus area (as y-value) were plotted and correlation coefficient, y-intercept and slope of the regression were calculated. Linearity Result of Luliconazole shown in table 9, figure 5 and the chromatogram are presented in figure 6 -11. All parameters of linearity data were within limit the Characteristic parameters of Luliconazole for the proposed HPLC method is shown in table 10.

Table 9: Linearity Result of Luliconazole

Linearity Sr. No	Concentration (µg/mL)	Peak Area
1	20	1274862
2	40	2549724
3	60	3774586
4	80	5099448
5	100	6374310
6	120	7649172
Slope		63814.53
Standard Error		22625.31

Table 10: Characteristic parameters of Luliconazole for the proposed HPLC method.

Sr. No	Parameter	Result
1	Calibration range (µg/ml)	20-120
2	Detection wavelength (nm)	294
3	Solvent (ACN: Buffer) v/v	65:35
4	Regression equation (y*)	y = 63815x - 13333
5	Slope (b)	63815
6	Intercept (a)	13333
7	Correlation coefficient(r2)	0.9999
8	Limit of Detection (µg/ml)	1.1700
9	Limit of Quantitation (µg/ml)	3.5455

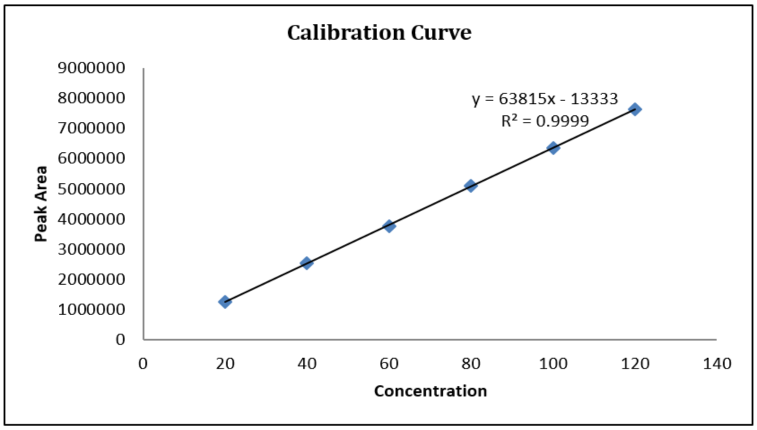

Figure 5: Calibration Curve of Luliconazole

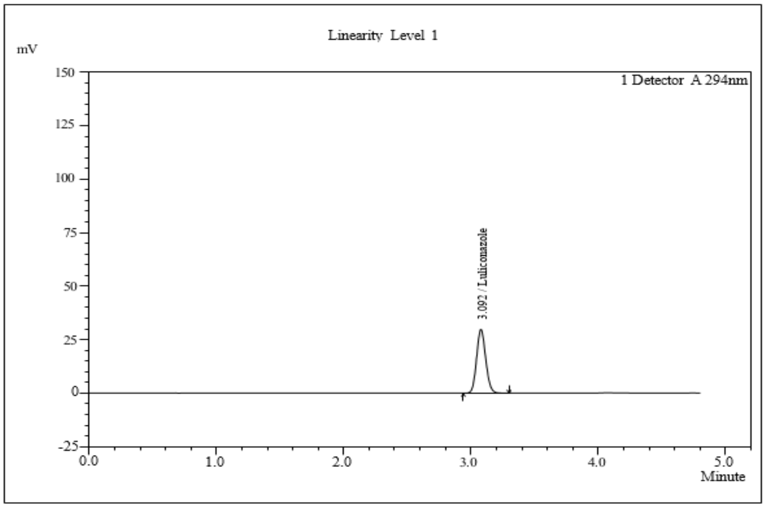

Figure 6: A Typical Chromatogram of Linearity Level 1

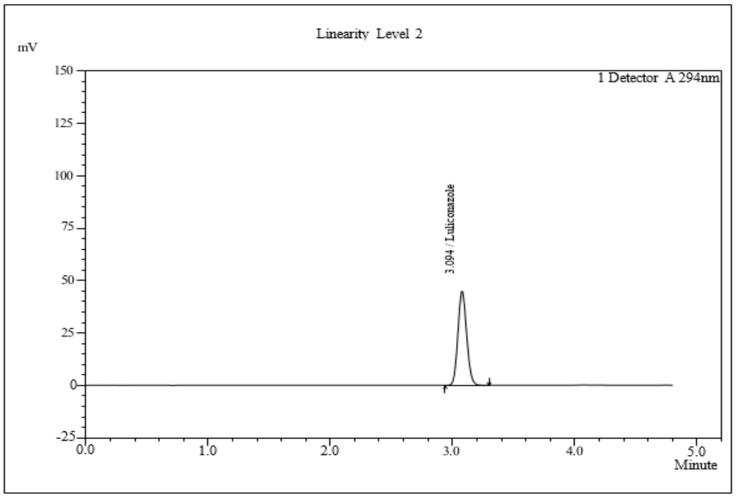

Figure 7: A Typical Chromatogram of Linearity Level 2

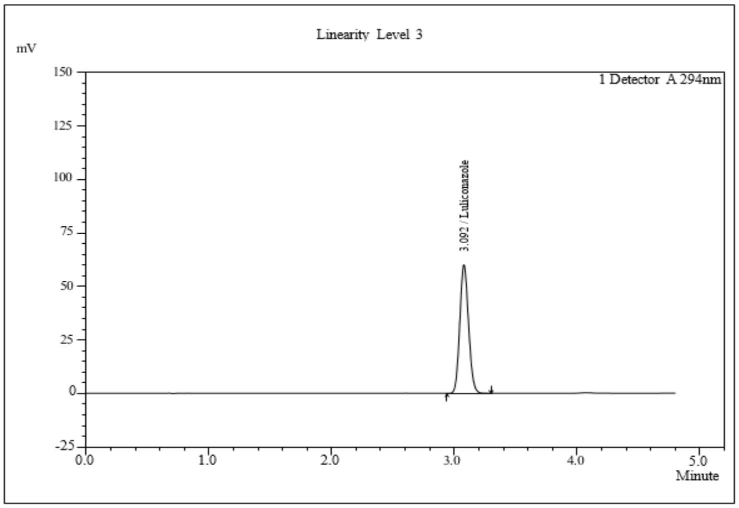

Figure 8: A Typical Chromatogram of Linearity Level 3

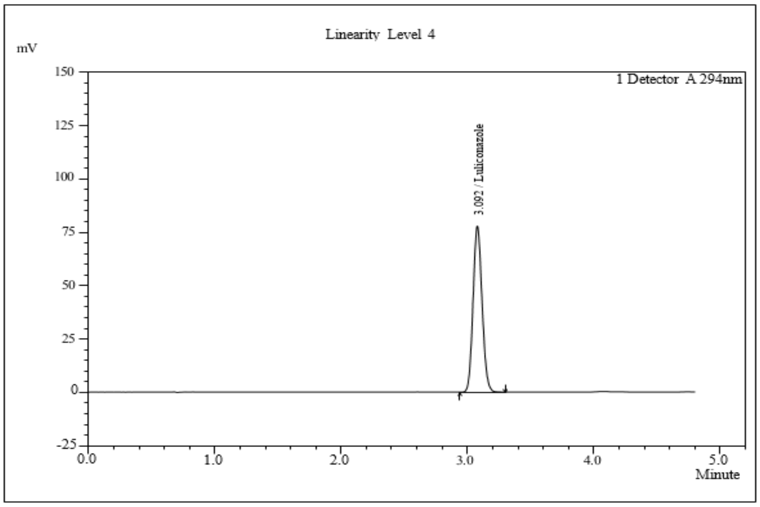

Figure 9: A Typical Chromatogram of Linearity Level 4

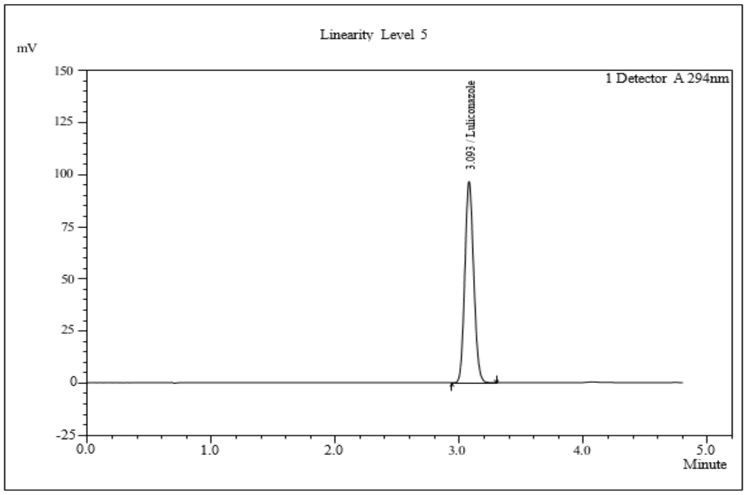

Figure 10:A Typical Chromatogram of Linearity Level 5

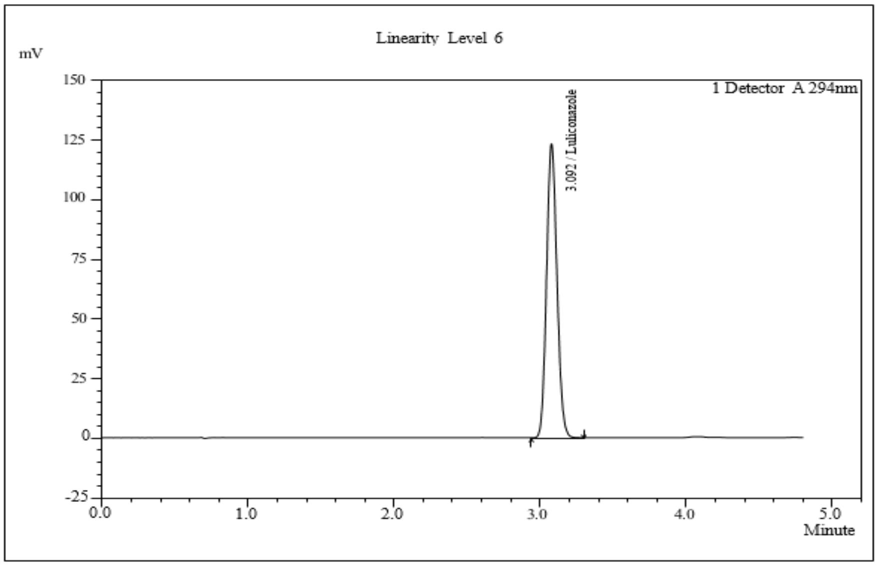

Figure 11: A Typical Chromatogram of Linearity Level 6

System Suitability:

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time, number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 40 µg/ml. The results which are given in Table 11 were within acceptable limits.

Table 11: System suitability studies of Luliconazole by HPLC method

Sr. No.	Properties	Values
1	Retention time	3.092
2	Area	1274862
3	Asymmetry	1.18
4	Theoretical plates	19976

Specificity:

The effect of excipients and other additives usually present in the dosage form of Luliconazole in the determination under optimum conditions was investigated. Luliconazole showed peak at a retention time of 3.092 min. The mobile phase designed for the method resolved the drug very efficiently. The Retention time of Luliconazole was 3.092 ± 0.0098min. The wavelength 294 nm was selected for detection because; it resulted in better detection sensitivity for the drug. The peak for Luliconazole from the tablet formulation was Luliconazole. Specificity data were displayed in specificity table 12a and 12b.

Table 12a: Specificity data

Specificity Sample	Label Claim (mg)	Amount Found	Recovery	Retention Time
Cream	30	29.91	99.7	3.092

Table 12b: Specificity data

Formulation	
Name of Formulation	Luliconazole
Type of Formulation	Cream
Concentration %	1 % w/w (30g)

Precision:

Demonstration of precision was done under two categories. The injection repeatability Interday and Intraday was assessed by using six injections of the standard solution of Luliconazole and the % RSD of the replicate injections was calculated. Method precision was subjected for inter-day and intra-day variations as reported in Table 13 and 14 respectively.

Table 13: Intraday Precision of Luliconazole at 294nm

Intraday Precision						
Sr. No	**Concentration**	**0 hr**	**2hr**	**4hr**	**6hr**	**Average**
1	80	5104687	5105374	5191161	5094476	5123924
2	80	5194674	5084153	5276750	5040219	5148949
3	80	5190566	4289560	5278902	5143220	4975562
4	80	5203201	5026500	5199475	5094540	5130929
5	80	5172104	5110843	5198439	5169350	5162684
6	80	5104784	5140115	5106420	5193222	5136135
Average						5113031
Standard Deviation						62750.9
RSD						1.2273

Table 14: Interday Precision of Luliconazole at 294nm

Interday Precision					
Sr. No	**Concentration (µg/ml)**	**1st Day**	**2nd Day**	**3rd Day**	**Average**
1	80	5130334	5227481	4979046	5112287
2	80	5144895	5303462	5011235	5153197
3	80	5229205	5442258	5044367	5238610
4	80	4873923	5247628	4933169	5018240
5	80	4983057	5326606	4987787	5099150
6	80	5139855	5231135	4823623	5064871
Average					5114392.56
Standard Deviation					69366.48
RSD					1.356

Accuracy:

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of Luliconazole (80 µg/ml) were spiked with 80, 100, and 120 % extra Luliconazole standard and the mixtures were analyzed by the proposed method. Standard deviation of the % recovery and % RSD were calculated and reported in Table 15.

Table 15: Accuracy of Luliconazole at 294nm

Sr.No	Concentration(µg/mL)	Peak area	Found Concentration(µg/mL)	% Recovery
1	64	4105162.4	63.99	99.98
2	80	5131453	79.99	99.99
3	96	1539435.9	96.09	100.09

Robustness:

Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method. A method is robust, if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at four different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of Wavelength, Mobile Phase proportion, Flow rate and pH of buffer had no significant effect on the method and chromatographic response of the 80 µg/ml solution, indicating that the method was robust. The results are shown in Table 16.

Table 16: Robustness of Luliconazole

Robustness					
Sr. No	**Parameter**		**Response**	**Parameter**	**Response**
Methanol : Water(V/V)			**Retention Time (min)**	**Detection Wavelength**(nm)	**Peak Area**
1	64	36	3.035	292	5041321
2	65	35	3.092	294	5099636
3	66	34	3.151	296	5132900
Average			3.093	Average	5091286
Standard Deviation			0.0580	Standard Deviation	37850.36
RSD%			1.875	**RSD%**	0.743
Flow Rate (mL/min)			**Retention Time (min)**	**pH of Buffer** (mmol/L)	**Peak Area**
1	1.1		3.125	4.2	5127708
2	1.2		3.092	4.0	5099809
3	1.3		2.991	4.4	5024188
Average			3.069	Average	5083902
Standard Deviation			0.0698	Standard Deviation	43733.13
RSD%			2.275	**RSD%**	0.8602

CONCLUSION

An RP-HPLC approach (Using Quality by Design) was developed in this work for the quantitative determination of Luliconazole in bulk pharmaceuticals and pharmaceutical formulations using ICH criteria. The suggested RP-HPLC approach using QbD was shown to be simple, selective, sensitive, accurate, precise, and rapid for Luliconazole in bulk and formulations. In a short length of time, the proposed approach demonstrated sensitivity and was repeatable enough for the analysis of Luliconazole. The strategy outperformed most previously published approaches. The movable phases were simple to set up and inexpensive. The sample recoveries in the formulation were consistent with their respective label claims, indicating that excipients in the formulation had no effect on the estimation. As a result, depending on the availability of chemicals and the nature of other substances present in the sample, these approaches can readily be used as an alternative to those previously reported for the routine determination of Luliconazole.

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