Research Article

Development and Validation of a New Stability Indicating Ultra-Fast Liquid Chromatographic (RP-UFLC) Method for the Quantification of Luliconazole

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Abstract

Luliconazole is an antifungal agent. A new stability indicating RP-UFLC method has been developed for the estimation of Luliconazole using Shimadzu UFLC system (PDA detector) with Zorbox C8 column (150 mm × 4.60 mm i.d. 5μm particle size) in bulk and pharmaceutical dosage form and validated as per ICH guidelines. A mixture of tetra butyl ammonium hydrogen sulphate and acetonitrile was used for the chromatographic separation with a flow rate of 1 mL/min. and UV detection at 220 nm. Linearity was observed over the concentration range of 0.1-100 μg/mL with linear regression equation y = 89055x - 1545.2 (R^2 = 0.9998). Forced degradation studies were performed and Luliconazole was found to be more sensitive towards acidic and oxidation conditions. The LOD and LOQ were found to be 0.0311 and 0.0981. The method was validated and found to be precise, accurate and robust.

Keywords: Luliconazole; Forced Degradation Studies; Validation; ICH Guidelines

Introduction

Luliconazole (Figure 1) is an imidazole derivative and chemically named as (2E)-2-[(4R)-4-(2, 4-dichlorophenyl)-1, 3-dithiolan-2-ylidene]-2-imidazol-1-ylacetonitrile $(C_{14}H_9Cl_2N_3S_2)$ with molecular weight 354.28. It is used as an antifungal agent [1]. The R-enantiomer of Luliconazole has only one chiral centre and the double bond which is adjacent to dithiolane moiety is in E configuration [2].

Different analytical methods such as spectrophotometry [3-5], TLC [6], HPTLC [6,7], RP-HPLC [8,9], RP-UFLC [10] and other liquid chromatographic methods involving the estimation of related [11] substances and experimental design [12] were published for the assay of Luliconazole in pharmaceutical formulations and biological fluids. In the present study we have developed a simple, accu-rate and economic stability indicating method for the quantification of Luliconazole in bulk and pharmaceutical dosage form. The method was validated as per ICH guidelines [13].
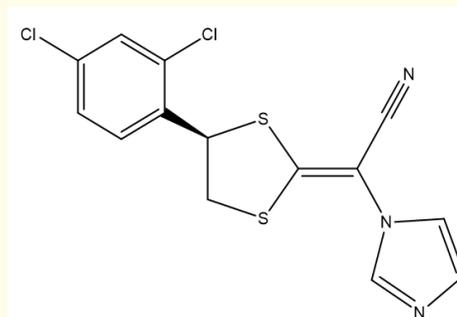

Figure 1: Chemical structure of Luliconazole.

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Chromatographic conditions

Shimadzu Model CBM-20A/20 Alite UFLC system (Shimadzu Co., Kyoto, Japan) equipped with photodiode array detector and Zorbox C8 column (250 mm × 4.60 mm i.d. 5μm particle size) were employed for the chromatographic study. Mobile phase consisting of tetra butyl ammonium hydrogen sulphate and acetonitrile (32:68 v/v) was used with a flow rate of 1.0 mL/min were used as the optimised conditions and the eluted compounds were monitored at 220 nm. Tetra butyl ammonium hydrogen sulphate is an ion pairing agent. The run time was 10 minutes and the sample injection volume was 20μl.

Preparation of 10 mM tetra butyl ammonium hydrogen sulphate solution:

3.395grams of tetra butyl ammonium hydrogen sulphate was accurately weighed and transferred in to a 1000 mL volumetric flask and made up to volume with HPLC grade water and used after filtration through the membrane filter.

Preparation of stock solution of Luliconazole

25 mg of Luliconazole was accurately weighed and transferred in to a 25 mL volumetric flask and dissolved in HPLC grade acetonitrile. The volume was made up to the mark with HPLC grade acetonitrile (1000 μg/mL) and dilutions were made with mobile phase and all the solutions were filtered through 0.45 μm membrane filter prior to injection.

Method validation [13]
Linearity

A series of 0.1-100 μg/mL Luliconazole solutions were prepared on dilution from the stock solution with mobile phase and each of the solution was injected into the UFLC system (n=3) and the chromatogram was recorded. The peak area observed at the retention was noted and further mean peak area was calculated. Calibration curve was drawn by plotting concentration of Luliconazole solutions on the x-axis and the corresponding mean peak area obtained on the y-axis.

LOD and LOQ were calculated from the standard deviation of response and also the slope of the corresponding calibration curve. LOD was calculated as 3.3 times the signal to noise ratio and LOQ is 10 times the signal to noise ratio.

Precision

Precision was evaluated intra-day and inter-day precision studies. Three different concentration solutions (10, 20, 40 μg/mL) of Luliconazole were prepared within the linearity range on the same day (intra-day precision) and on three consecutive days (inter-day precision) and the chromatographic study was performed. The mean peak area (n = 3) and thereby the % RSD was calculated.

Accuracy

Accuracy was measured by spiking the drug formulation solution (50, 100, 150%) with a known concentration of standard drug (n = 3). These studies were carried out and finally the % RSD was calculated from the values obtained followed by the percentage recovery from the linear regression equation.

Robustness

The robustness of the method was proved by incorporating a very small changes in the optimized chromatographic conditions such as mobile phase composition (± 2%; 30:70 v/v and 34:66 v/v), detection wavelength (± 2 nm; 222 and 218 nm) and flow rate (± 0.1 mL; 1.1 and 0.9 mL/min).

Forced degradation studies

Forced degradation studies [14] were performed to determine the stability of Luliconazole towards stress conditions such as acidic hydrolysis, basic hydrolysis, oxidation and thermal degradation. The specificity of the method can be known from the stability studies and therefore Luliconazole was exposed to the following stress conditions and the stability was studied.

Acidic degradation was performed by heating Luliconazole solution with 1 mL of 0.1 N HCl solution at 80^0 for one hour on a water bath. The stressed sample was then cooled, neutralized with 1.0 mL 0.1N sodium hydroxide solution, diluted with mobile phase and then 20 μl of the solution was injected in to the UFLC system.

Alkaline degradation was performed by heating Luliconazole solution with 1.0 mL 0.1N sodium hydroxide solution at 80^0 for one hour on a water bath. The stressed sample was then cooled, neutralized with 1.0 mL of 0.1 N HCl solution, diluted with mobile phase and then 20 μl of the resulting solution was injected in to the UFLC system.

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Thermal degradation was performed by heating the Luliconazole solution at 80ºC for one hour on a water bath and then cooled, diluted with mobile phase and 20 µl of the resulting solution was injected in to the UFLC system.

Oxidative degradation was performed by heating Luliconazole solution with 1.0 mL 30% hydrogen peroxide solution at 80ºC for one hour on a water bath. The stressed sample was then cooled, diluted with mobile phase and then 20 µl of the resulting solution was injected in to the UFLC system.

Assay of Luliconazole in its topical dosage form (Cream)

Luliconazole is available as a topical dosage form i.e. as Cream with brand names LULICON (Glenmark Pharmaceuticals), LULINAZ (Generic) (Labelled claim: 1% w/w) and also as lotion, soap and cream with brand name as LULIFORD (Leeford Healthcare Ltd) (India).

25 mg of Luliconazole cream accurately weighed from two different brands in to two different beakers and heated on a water bath with continuous stirring till it melts and then the contents were quantitatively transferred in to two different 25 ml volumetric flasks and acetonitrile was added. The resulting mixture was

sonicated, filtered and then diluted with the mobile phase. 20 µL of each of these solutions were injected in to the system and the peak area was noted from the resultant chromatogram (n = 3).

Results and Discussion

A new stability indicating RP-UFLC method has been developed for the estimation of Luliconazole in creams. The previously published methods for the assay of Luliconazole were briefly summarised and compared with the present proposed method in table 1.

Method optimisation

Initially a 10 µg/mL of Luliconazole was injected in to the UFLC system with the mobile composition 20: 80 and flow rate 0.8 ml/min and a chromatogram was eluted where the system suitability parameters were not satisfied. During the optimization process the mobile phase composition, flow rate were varied (Table 2) and finally the previous Phenomenex column was replaced with a Zorbox column there by Luliconazole was eluted at 2.211 minutes where the mobile composition was 32: 68 and flow rate was 1.0 ml/min (Trial 5). The chromatograms obtained during the optimisation process (Trials) were shown in figure 2.

Mobile phase (v/v)/ Detection wavelength (nm)	Linearity (µg/mL)	Remarks	Ref
Methanol/296	5-25	Spectrophotometry (AUC)	[3]
0.1N HCl Phosphate buffer (pH 2.0)/294	2-30 10-30	Spectrophotometry	[4]
Methanol/295	3-18	Spectrophotometry (AUC)	[5]
Toluene: Isopropanol: Ammonia (90:10:0.5)/297	1-24 µg/band 0.8-6 µg/band	TLC and HPTLC	[6]
Toluene: Methanol: Ethyl Acetate (6:2.5:0.5)	0.1-0.6 µg/band	HPTLC	[7]
0.1% *ortho* phosphoric acid: Methanol (20:80)/294	0.1-200	RP-HPLC	[8]
Water: Acetonitrile (60:40)/294	2.4-5.6	RP-HPLC	[9]
Phosphate buffer (pH 4): Acetonitrile (60:40)/299	5-100	RP-UFLC (Stability indicating)	[10]
Ammonium acetate buffer: ACN (60:40)/294 Methyl paraben (254 nm) and Related substances (294 nm)	-	RP-HPLC Related substances (Gradient mode)	[11]
Methanol: Water (80:20)/296	2-14	Experimental design	[12]
Tetra butyl ammonium hydrogen sulphate: Acetonitrile (32: 68)/220	0.1-100	RP-UFLC (Stability indicating) (Ion pairing reagent)	Present method

Table 1: Review of literature.

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| Trial	Column	Mobile phase (v/v)	Flow rate (mL/min)	Retention time (min)	Theoretical plates	Tailing factor	Remarks
1	Phenomenex C18 column	20 : 80	0.8	2.175	1091.03	1.451	Theoretical plates < 2000
2	Phenomenex C18 column	25 : 75	0.8	2.548	3207.312	1.576	Peak splitting
3	Phenomenex C18 column	25 : 75	0.6	3.426	3315.824	1.591	Peak splitting
4	Phenomenex C18 column	30 : 70	0.8	2.680	3764.071	1.014	Peak splitting
5	Zorbox C8 column	32 : 68	1.0	2.211	4504.437	1.256	Method optimized

Table 2: Method optimization.
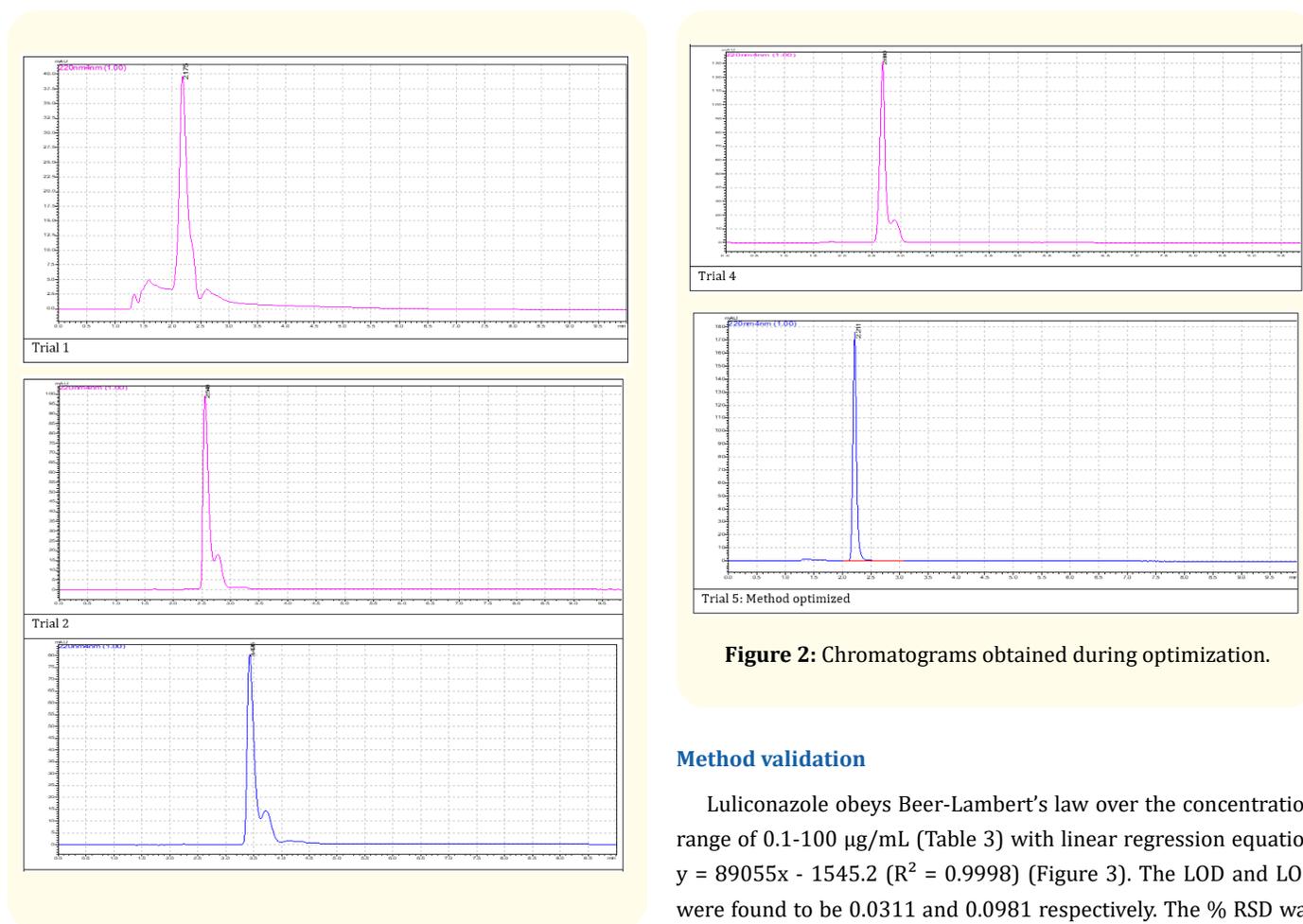
Figure 2: Chromatograms obtained during optimization.

Method validation

Luliconazole obeys Beer-Lambert's law over the concentration range of 0.1-100 µg/mL (Table 3) with linear regression equation y = 89055x - 1545.2 (R^2 = 0.9998) (Figure 3). The LOD and LOQ were found to be 0.0311 and 0.0981 respectively. The % RSD was found to be 0.01-0.24 (Intraday) (Table 4) and 0.23-0.44 (Inter-

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day) (Table 5) in precision studies which is less than 2.0 indicating that the method is precise. The % recovery in accuracy studies was found to be 99.37-99.73% (Table 6) and % RSD was (0.35-0.62) less than 2% indicating that the method is accurate. The % RSD in robustness study was found to be 0.23-1.21 which was less than 2% indicating that the method is robust (Table 7). In all the validation studies the system suitability parameters were within the acceptable criteria.

Conc. (µg/mL)	*Mean peak area	% RSD
0.1	9019	0.28
0.2	17859	0.26
0.5	44234	0.34
1	89596	0.61
2	178957	0.24
5	440856	0.39
10	895496	0.41
20	1786259	0.53
40	3536985	0.81
60	5396547	0.73
80	6989654	0.89
100	8985176	0.92

Table 3: Linearity study of Luliconazole.

*Mean of three replicates.

Conc. (µg/mL)	*Mean peak area	Statistical Analysis *Mean ± SD (% RSD)
10	895496	894626 ± 2178.44 (0.24)
10	896235	
10	892147	
20	1786259	1786117 ± 166.33 (0.01)
20	1786158	
20	1785934	
40	3536985	3537268 ± 544.75 (0.02)
40	3537896	
40	3536923	

Table 4: Intraday precision study of Luliconazole.

*Mean of three replicates.

Conc. (µg/ mL)	*Mean peak area			*Mean ± SD (% RSD)
	Day 1	Day 2	Day 3	
10	895496	896598	896391	896161.67 ± 2061.17 (0.23)
20	1786259	1784692	1785634	1785528.33 ± 5535.14 (0.31)
40	3536985	3537983	3537264	3537410.67 ± 15564.61 (0.44)

Table 5: Inter day precision study of Luliconazole.

*Mean of three replicates.

Spiked Conc. (µg/mL)	Formulation (µg/ mL)	Total Conc. (µg/mL)	*Conc. Obtained (µg/mL) ± SD (%RSD)	% Recovery
10 (50%)	20	30	29.81 ± 0.1033 (0.35)	99.37
	20	30		
	20	30		
20 (100%)	20	40	39.89 ± 0.2473 (0.62)	99.73
	20	40		
	20	40		
30 (150%)	20	50	49.74 ± 0.2039 (0.41)	99.48
	20	50		
	20	50		

Table 6: Accuracy study of Luliconazole.

*Mean of three replicates.

Parameter	Condition	*Mean peak area ± SD (% RSD)
Flow rate (± 0.1 ml/min)	0.9	898962 ± 10877.44 (1.21)
	1.0	
	1.1	
Detection wavelength (± 2 nm)	222	896598 ± 2062.18 (0.23)
	220	
	218	
Mobile phase composition Tetra butyl ammonium hydrogen sulphate: Acetonitrile (± 2 %, v/v)	30: 70	895341 ± 0.0002 (0.34)
	32:68	
	34: 66	

Table 7: Robustness study of Luliconazole (10 **µg**/mL).

*Mean of three replicates.

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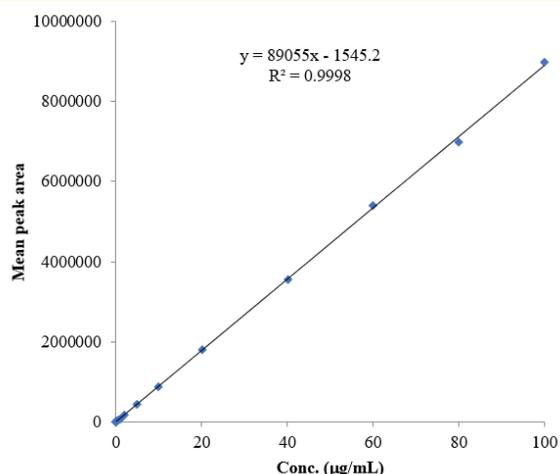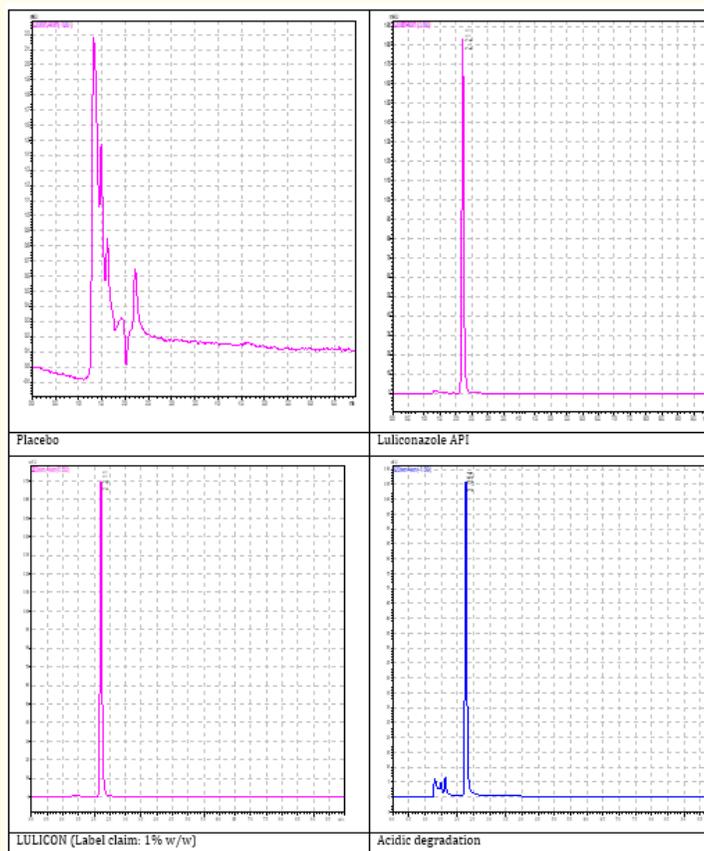
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Figure 3: Calibration curve of Luliconazole.

Forced degradation studies

Luliconazole standard was eluted at 2.236 min with theoretical plates more than 2000 (4776.817) and tailing factor less than 1.5 (1.165) indicating that the system suitability parameters are within the acceptable criteria. During the forced degradation studies Luliconazole was observed to be more sensitive towards acidic degradation and oxidative degradation in comparison to alkaline degradation. In these degradations the more than 15% of Luliconazole has undergone degradation. During alkaline and oxidation degradation reactions extra peaks were observed at 1.616 min and 1.744 min respectively with resolution values 5.518 and 3.550 which is greater than 2. The proposed method is specific as the degradants eluted were not interfering with Luliconazole peak. The % recovery during the degradation studies was with other system suitability parameters were shown in table 8. The corresponding chromatograms observed during the forced degradation studies were shown in figure 4.
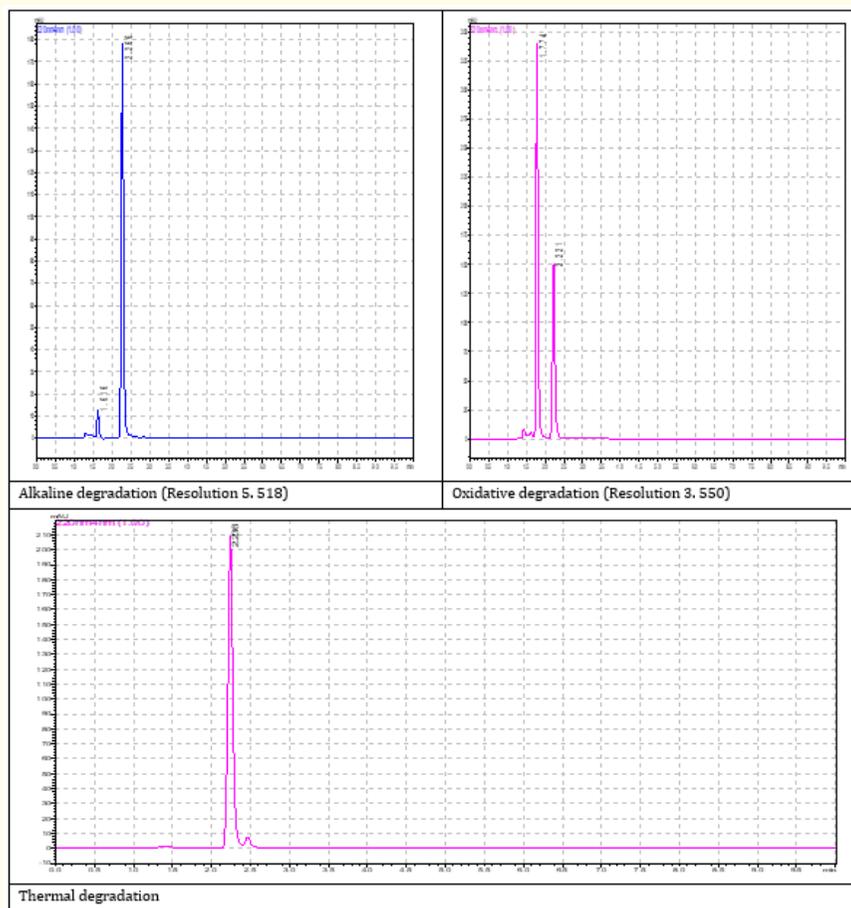
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Figure 4: Representative chromatograms of Luliconazole (10 μg/mL) and its marketed products during degradation studies.

Stress condition	Rt (min)	Mean peak area	% Recovery	% Drug degradation	Theoretical plates (>2000)	Tailing factor (<1.5)
Standard drug	2.236	895496	100	-----	4776.817	1.165
Acidic degradation 0.1N HCl/80⁰/1 hour	2.254	466229	52.06	47.94	5039.192	1.333
Alkaline degradation 0.1N NaOH/80⁰/1 hour	2.266 1.616	754474	84.25	15.75	5632.688	1.227
Oxidative degradation 30% H₂O₂/80⁰/1 hour	2.221 1.744	697398	77.88	22.12	4042.078	1.267
Thermal degradation 80⁰/1 hour	2.211	829463	92.63	7.37	4395.765	1.263

Table 8: Forced degradation studies of Luliconazole.

*Mean of three replicates.

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Assay of Luliconazole in its topical dosage form (Cream)

The assay of Luliconazole was found to be 98.31-99.34 (Table 9) in creams. The individual chromatograms observed for the placebo and the Luliconazole cream formulation were shown in figure 4.

S. No.	Brand name	Label claim (% w/w)	Observed amount (%w/w)	% Recovery*
1	Brand I	1	0.9831	98.31
2	Brand II	1	0.9934	99.34

Table 9: Assay of Luliconazole cream.

Conclusion

The present proposed RP-UFLC method was validated and it is precise, accurate, robust and economical and used for the estimation of pharmaceutical formulations such as creams etc.

Acknowledgement

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