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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR STABILITY INDICATING HPTLC METHOD FOR ASSAY OF LULICONAZOLE IN BULK AND DOSAGE FORM

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ABSTRACT

A quick, precise and accurate method based on HPTLC has been developed for analysis of Luliconazole. The method was developed and validated for the determination of Luliconazole on pre coated silica gel HPTLC plates using Toluene: methanol: GAA Solution (8:2:0.2V/V/V) as a mobile phase with Densitometric detection at 294nm. The method was validated for linearity, precision, accuracy and robustness. Linearity range for LUL was found 100-500ng/band Correlation coefficient was 0.990. The developed method was precise and robust, % RSD was found less than 2%. % recovery was found to be in range of 101.67-103.61%. LOD and LOQ were 15.48ng/b and 46.92ng/b. Stress degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Degradation study was carried out by exposing of working standard solution of LUL with acid (0.1N HCL at 80ºC), base (0.1 N NaOH at 80ºC), hydrogen peroxide (3% H2O2), Distilled water (H2O) for 2 hours while one volumetric flask was exposed to UV light (294 nm) and one volumetric flask was exposed to (800 C) for 24 hours and thermal LUL sample at (80ºC for 1hr) The degradation was found to be resp. (5.17%, 7.20%, 8.18%, 7.82%, 7.58%, 5.72%)

KEYWORDS

Analytical method validation, High-performance thin-layer chromatography, Luliconazole and Stability-indicating assay method.

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INTRODUCTION

The drug Luliconaozole (LCZ) was selected for this study. The (2E)-2-[(4R)-4-(2, 4-dichlorophenyl)-1, 3-dithiolan-2-ylidene]-2-imidazol-1-ylacetonitrile, a completely unique antifungal drug launched in India by Ranbaxy Laboratories Ltd. The products were screened from active compounds associated with the drug luliconazole, a potent antidermatophytic

drug. LCZ possesses a good spectrum of antifungal activity and is extremely potent against dermatophytes [Uchida *et al*, 2004[1]]. Till date no analytical method was reported for quantitative estimation of luliconazole [S. Sonawane *et al*, 2016[2]]. The present study was aimed to establish inherent stability of Luliconazole through stress induced studies under a variety of ICH recommended test conditions and to develop stability indicating HPTLC method Validation of the developed method was administered as per ICH guidelines. The developed method was applied to marketed lotion dosage forms. One of the available chromatographic techniques is HPTLC, which is employed for the identification of constituents, identification and determination of impurities and quantitative determination of active substances. HPTLC a crucial alternative method to HPLC or gas chromatography because the use of recent apparatus like video scanners, densitometers and new chromatographic chambers and simpler elution techniques, high-resolution particle size or chemically modified surface and development of computer programs for method optimization make HPTLC a crucial alternative method to HPLC or gas chromatography. Specifically, HPTLC is one among the perfect TLC technique for the analytical purposes due to its increased accuracy, reproducibility, and skill to document the results, compared with standard TLC. Because of this, HPTLC technologies are also the most appropriate TLC technique for conformity with GMPs. [Mahesh Attimarad *et al*, 2011[3]].

MATERIAL AND METHODS

High performance thin layer chromatography

CAMAG HPTLC SYSTEM

Make: Camag.

Stationary Phase: Silica gel 60 F_{254} plates

Sample Applicator: Camag Linomat V

Gas: Nitrogen

Syringe: Camag 100µl syringe

Development chamber: Camag twin trough glass chamber

UV-Lamp: Camag (D2 and W)

TLC Scanner: Camag TLC scanner III

Software: Win CAT's software

TLC Plates Used

Aluminium plates precoated with silica gel 60 F254 plates (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India).

Sample applicator

Camag Linomat V (Muttenz, Switzerland). Pressure requirement for sample application is 3.5 bar. Dimension: 360mm x 510mm x 410mm (Width x Length x Height).

Syringe

Camag 100µl syringe (Hamilton, Bonaduz, Switzerland).

Development chamber

Camag twin trough glass chamber (10 x 10cm and10 x 20cm)

UV-lamp

It having the wavelength (296nm) [Dimension**:** 477mm x 343mm x 285mm (Length x Width x Height)].

TLC scanner

Camag TLC scanner III densitometer operated in reflectance- absorbance mode. The scanning speed was 5-100mm/s. The source of radiation used was deuterium lamp, halogen tungsten and mercury vapour emitting a continuous UV spectra between 190-800nm (with wavelength accuracy ±1nm). Scanner fitted with grating type of monochromator. General operating temperature range is 18-35^{o}C. [Dimension: 620mm x 620mm x 345mm (Width x Length x Height)].

Standard Drugs

Marketed Formulations

Authentication of Drug

Authentication of Pure Drug Sample of Luliconazole

Authentication of Luliconazole

Test procedure for UV

Accurately weighed quantity (10mg) of Luliconazole was transferred to 10.0ml volumetric flask, added 5ml of methanol and ultrasonicated for 10 minutes, volume was then made up to the mark with methanol (1000µg/ml). From above solution, 1.0ml solution was diluted to 10.0ml with methanol. Further diluted 1.0ml of this solution to 10.0ml with

methanol conc. obtained (10µg/ml). This solution was then scanned in spectrum mode, from 400nm to 200nm, in 1.0cm cell against methanol as blank.

Observation

The wavelength of maximum absorbance was found to be 294nm.

Inference

Luliconazole pure drug sample complies the test.

Melting point test

Reported melting point for Luliconazole is 149 - 154°C.

Observation

Observed melting point for Luliconazole 150 - 152°C.

Inference

Luliconazole passes the test.

Inference

Observed frequencies of pure drug sample of Luliconazole matches with standard values. Hence Luliconazole pure drug sample complies the test.

Method

Development and validation of stability indicating assay method for luliconazole using high performance thin layer chromatography technique

Experimental work

Chromatographic Procedure

Chromatography was performed on 10 × 10cm aluminium TLC plates precoated with 250µm layers of silica gel. Samples were applied in the form of bands, under a continuous flow of nitrogen, by means of a Camag Linomat V sample applicator fitted with 100µL Applicator syringe. A constant application rate of 0.1µL per second was used and the distance between the adjacent bands were also optimized. The plates were then conditioned for 10 min in a presaturated twin-trough glass chamber (10 x 10cm2).

The spotted plate was then dipped in mobile phase (Tolune: Methanol: GAA 8:2:0.2; v/v) and ascending development was performed to a distance of around 80mm from the point of application at ambient temperature. Subsequently after, plates were dried in a current of air with the help of an air dryer and spots was visualized in Camag UV cabinet with dual wavelength UV lamp and densitometric scanning was performed at 296nm with Camag TLC scanner III operated in reflectance-absorbance mode and controlled by WINCATS software.

The slit dimensions (4 × 0.2mm) were also optimized and kept constant throughout the analysis.

Method development

Preparation of standard solutions

A stock solution of LUL was prepared by dissolving accurately about 10mg of LUL with 100mL methanol. Aliquots of this solution were suitability diluted with methanol to get working standard solutions of LUL having concentration of 1000µg /mL.

Selection of Mobile Phase

Aliquot portions of standard stock solutions (0.4µL) were applied on TLC plates in the form of band (band size: 6mm). Different solvents with varying polarity as well as combination of solvent were tried to get well separated bands of the drugs. After trying several permutations and combinations, the solvent system containing Tolune: Methanol: GAA the ratio 8:2:0.2v/v/v was found to be most satisfactory as it gave good resolution.

Selection of wavelength for densitometric evaluation of separated bands

Standard stock solution was applied on TLC plate with the help of CAMAG LINOMAT-V automatic sample applicator, the plate was chromatographed in twin-through glass chamber saturated with mobile phase for 10 minutes. After chromatographic development, the plate was removed and air dried. The separated bands on the TLC plate were scanned over the wavelength range of 200-700nm. The wavelength 294nm was selected for densitometric evaluation of separated bands. The spectrum obtained is depicted in Figure No.11.

Chromatographic conditions

The following chromatographic conditions were optimized by trial and error for effective separation and densitometric evaluation of drugs:

Densitogram of Luliconazole

The Retention factor (Rf) of Luliconazole was 0.62.

Method validation

To prove the reliability and reproducibility, the developed method was validated for following validation parameters.

Analysis of bulk drug

Preparation of Standard Solution

Five sample solutions were prepared and analyzed in following manner

An accurately weighed quantity of 10mg LUL was transferred to 100mL volumetric flasks dissolved and diluted up to the mark with methanol. From this solution, 10.0mL was transferred to 100.0mL volumetric flask and diluted to the mark with methanol (Concentration 10µg/mL). On TLC plate two bands of standard and four bands of sample solution, 1µL each, were applied and the plate was developed and scanned under the optimized chromatographic conditions. After scanning, the peaks obtained for standard and sample were integrated. .The amount of LUL present in applied volume of standard solution was fed to computer. Amount of the drugs present in applied volume of sample solution was obtained by comparison between peak area of standard and sample bands. The total amount of drug estimated in laboratory mixture and percent estimation was calculated by using following formula.

Linearity and range

For establishment of linearity of LUL by proposed method, the calibration curve was obtained at five levels in the concentration range of 100-500ng/spot. For this the different increasing amounts of LUL working standard (0.1µg/mL) was spotted three times on individual plates and analyzed as described. For evaluation of linearity, observed peak area and concentrations were subjected to least square regression analysis to calculate calibration equation and correlation coefficient. The observed linearity confirming adherence of the system to Beer's law. The regression analysis equation was y = 1888.896+16.547X with correlation coefficient (r) was 0.990.

Precision

Precision of the method was verified by repeatability and intermediate precision studies.

Repeatability

In the repeatability studies, six replicates of one concentration of Luliconazole were prepared and spotted on HPTLC plate. From the obtained data, %RSD of Luliconazole were found to be less than 2%. The results of repeatability studies for Luliconazole shown in Table No.9.21.

Intermediate Precision

In the intermediate precision studies, six replicates of one concentration was prepared and spotted on HPTLC plate for 3 consecutive days. From the obtained data, %RSD of Luliconazole were found to be less than 2%. The intermediate precision results of Luliconazole shown in Table No.9.4.

Accuracy

To ascertain the accuracy of proposed method, recovery studies were carried out by standard addition method, as per ICH guidelines.

Preparation of Sample Solutions

An accurately weighed quantity of pre-analysed tablet powder equivalent to about 10mg LUL was transferred individually in nine different 100mL volumetric flasks. To each of the flask following quantities of LUL was added.

Then 5mL methanol was added to each flask and contents of the flask were ultrasonicated for 20 minutes, volume was made up to the mark with methanol. The solution was individually mixed and filtered through Whatman filter paper No. 42. From the filtrate, 1.0mL solution was diluted to 10.0mL with methanol.

On TLC plate two bands of standard and four bands of sample solution, 0.4µL each, were applied and the plate was developed and scanned under the optimized chromatographic conditions. After scanning, the chromatograms obtained for standard and sample were integrated. The result of accuracy study is given in Table No.22.

The accuracy of the method was determined by calculating the recovery of Luliconazole by the standard addition method at three concentration levels (80%, 100% and 120%). The percentage recoveries of Luliconazole were found to be in the range of 101.67-103.61%. The Accuracy results of Luliconazole shown in Table No.9.5. The weight of the lotion taken is 10mg.

Range

Range of Luliconazole was found to be as follows

Luliconazole: 100-500ng/band

LOD and LOQ

Limit of Detection (LOD)

For Luliconazole, LOD was calculated from the formula

LOD = 3.3σ/S

σ = Standard deviation of the response

S = slope of the calibration curve

Limit of detection of Luliconazole = 15.48ng/band

Limit of Quantitation (LOQ)

For Luliconazole, LOQ was calculated from the formula LOQ=10σ/S

σ = Standard deviation of the response S= slope of the calibration curve

Limit of Quantitation of Luliconazole = 46.92ng/band

Robustness

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters such as change in chamber saturation time, change in composition of the mobile phase. This was studied to find out the robustness of the proposed method %RSD was found to be less than 2%. The Robustness result of change in saturation time (±5min) of Luliconazole shown in table.

Analysis of marketed lotion formulation

Brand Name: Lulifin 10ml Lotion

Label Claim: 1%w/v

The % label claim of Luliconazole lotion was found to be 101.994%.

Stress (Forced) Degradation Study

The stress degradation studies for LUL were carried out as per ICH guidelines. Different stress conditions were applied such as acid, base, hydrolytic, oxidative, dry heat (thermal) and light exposure on LUL bulk drug.

The stress studies were carried out by preparing LUL solution of 1mg/mL in respective stressors as described in Table No.25.

Forced degradation study of LUL

10mg LUL was separately transferred to six different 10.0ml volumetric flasks (Flask No. 1, 2, 3, 4, 5 and 6), added 3.0ml of 0.1 N HCl, 0. 1 N NaOH, H_2O to Flask No. 1, 2, 3 respectively. In flask No. 4: 3% H_2O_2 is added and kept at dark for 3 hr and after that heated to remove H_2O_2. Flask No. 1, 2, 3 were then refluxed at 80°C for 1 hr. Flask No. 5 containing LUL was kept at 80°C for 3 hrs to study the effect of heat on drug sample (heat degradation). The forced degradation was performed in the dark to exclude the possible degradative effect of light. Flask No.6 was exposed to ultraviolet radiations at 294nm for 24 hrs in a UV-chamber. All the flasks were removed, the LUL samples were treated and analyzed in similar manner as described under analysis of pure drug.

The typical densitogram for acidic, alkaline, oxide, Neutral, heat and UV exposure, are shown in figure respectively.

FORCE DEGRADATION STUDY OF LICONAZOLE BY HPTLC

Acidic stress degradation

In acidic stress degradation, Luliconazole showed 5.17% degradation was observed on exposure to 0.1N HCl at room temp for 20 min. (Figure No.1).

Alkaline stress degradation

In alkaline stress degradation, Luliconazole showed 7.20% degradation in 0.1N NaOH at room temp for 45 min. (Figure No.2).

Oxidative stress degradation

In oxidative stress degradation, Luliconazole showed 8.18% degradation in 3% H_2O_2 at room temperature for 45 min. (Figure No.3)

Photolytic stress degradation

In photolytic stress degradation, Luliconazole showed 5.72% degradation on exposure to UV light (294 nm) for 24 hrs. (Figure No.4).

Thermal Stress degradation

In thermal stress degradation, Luliconazole showed 7.58% degradation on exposed to 60°C for 45 min. (Figure No.5).

Neutral Stress degradation

In Neutral stress degradation, Luliconazole showed 7.82% degradation in Distilled Water at room temperature for 45 min. (Figure No.6).

SUMMARY AND CONCLUSION

High Performance Thin Layer Chromatography (HPTLC)

Luliconazole

A quick, precise and accurate method based on HPTLC has been developed for analysis of Luliconazole. The method was developed and validated for the determination of Luliconazole on pre coated silica gel HPTLC plates using Toluene: methanol: GAA Solution (8:2:0.2V/V/V) as a mobile phase with Densitometric detection at 294nm. The method was validated for linearity, precision, accuracy and robustness. Linearity range for LUL was found 100-500ng/band Correlation coefficient was 0.990. The developed method was precise and robust, % RSD was found less than 2%. % recovery was found to be in range of 101.67-103.61%. LOD and LOQ were 15.48ng/b and 46.92ng/b.

Stress degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Degradation study was carried out by exposing of working standard solution of LUL with acid (0.1N HCL at 800C), base (0.1 N NaOH at 80^{o}C), hydrogen peroxide (3% H2O2), Distilled water (H2O) for 2 hours while one volumetric flask was exposed to UV light (294nm) and one volumetric flask was exposed to (80^{o}C) for 24 hours and thermal LUL sample at (80^{o}C for 1hr) the degradation was found to be resp. (5.17% 7.20%, 8.18%, 7.82%, 7.58%, 5.72%).

Table No.1: Standard drug

Drug	Manufacturing company
Luliconazole	Concept Pharmaceuticals Ltd

Table No.2: Marketed formulation

Marketed Formulation	Drug	Content	Formulation type	Company
Lulifin	Luliconazole	1%w/v	Lotion	Sun Pharma

Table No.3: List of equipment used

S.No	Equipment/Accessories	Model/Specification	Company
1	Electronic Weighing Balance	AUX-200	Shimadzu
2	HPTLC	CHF47150	Camag
3	Sonicator	UC120WF	Imecoultrasonics

Table No.4: List of Chemicals used

S.No	Materials	Specifications	Source
1	Methanol	HPLC grade	Merck Specialities Pvt. Ltd, Mumbai.
2	Tolune	HPLC grade	Merck Specialities Pvt. Ltd, Mumbai.
3	Glacial acetic acid	HPLC grade	Merck Specialities Pvt. Ltd, Mumbai
4	Ethyl Acetate	HPLC grade	Merck Specialities Pvt. Ltd, Mumbai
5	Conc. HCL	AR grade	Merck Specialities Pvt. Ltd, Mumbai.
6	Hydrogen Peroxide	AR grade	Merck Specialities Pvt. Ltd, Mumbai.
7	NaOH pellets	AR grade	Merck Specialities Pvt. Ltd, Mumbai.
8	Water	HPLC grade	Merck Specialities Pvt. Ltd, Mumbai.

Table No.5: Chromatographic condition

S.No	Stationary phase	Aluminium plates precoated with silica gel 60 F254 Merck
1	Mobile phase	Tolune: Methanol : GAA (8:2:0.2 v/v)
2	Plate size	10cm X 10cm (Thickness: 200µm)
3	Mode of application	Band
4	Band size	6mm (Distance between two bands: 7.7mm)
5	Sample volume	2.1µL
6	Development chamber	Twin-through glass chamber, 10 cm X 10 cm with stainless steel lid.
7	Saturation time	10 minutes
8	Separation technique	Ascending
9	Migration distance	≈ 80mm
10	Temperature	25 ± 50c
11	Scanning mode	Absorbance/Reflectance
12	Slit dimensions	5 X 0.45mm
13	Scanning wavelength	294nm

Table No.6: Preparation of different linearity levels of LUL

S.No	Linearity Level	Volume Applied (µL)	Concentration (ng/spot)
1	I	0.1	100
2	II	0.2	200
3	III	0.3	300
4	IV	0.4	400
5	V	0.5	500

Table No.7: Linearity data of Luliconazole by HPTLC

S.No	Concentration (ng/band) Luliconazole	Rf	Area
1	100	0.61	3399.43
2	200	0.61	5360.12
3	300	0.62	6617.77
4	400	0.63	9069.18
5	500	0.63	9818.35

Table No.8: Statistical data of Luliconazole by HPTLC

S.No	Parameters	Results
1	Linearity range	100-500ng/band
2	Regression equation	y= 1888.896+16.547*X
3	Correlation coefficient	0.990
4	Slope	6.17

Table No.9: Repeatability result of Luliconazole

S.No	Drug	Amount of drug taken	% Mean estimated	S. D.	% R. S. D
1	Luliconazole	10mg	100.155	0.15	0.151
		10mg			
		10mg			

Table No.10: Intermediate precision of Luliconazole (Interday)

S.No	Drug	Amount of drug taken	% Mean estimated	S. D.	% R. S. D
1	Luliconazole	10mg	99.50	1.86	1.870
		10mg			
		10mg			

Table No.11: Intermediate precision of Luliconazole (Intraday)

S.No	Drug	Amount of drug taken	% Mean estimated	S. D.	% R. S. D
1	Luliconazole	10mg	98.66869	1.26	1.28
		10mg			
		10mg			

Table No.12: Preparation of sample solution

Flask No	LUL
1	8mg
2	8mg
3	7mg 8mg
4	10mg
5	10mg
6	10mg
7	12mg
8	12mg
9	12mg

Table No.13: Accuracy results of Luliconazole by HPTLC

S.No	Level of recovery (%)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Recovery	% Recovery Mean	SD	%RSD
1	80	8	8.269749	103.3719	103.321	0.04	0.046
		8	8.262194	103.2774			
		8	8.265216	103.3152			
2	100	10	10.40793	104.0793	103.610	0.83	0.808
		10	10.26438	102.6438			
		10	10.41095	104.1095			
3	120	12	12.29679	102.4732	101.671	0.97	0.960
		12	12.07012	100.5844			
		12	12.23483	101.9569			

Table No.14: LOD and LOQ

S.No	Parameters	LUL (ng/band)
1	Limit of Detection (ng/band)	15.48
2	Limit of Quantification (ng/band)	46.92

Table No.15: Change in Mobile phase composition (±1ml) of Luliconazole

S.No	Chromatographic Changes		
Factor		**Level**	**Rf values**
Mobile phase composition Tolune: Methanol : GAA (6:4:0.1 v/v)			**Luliconazole**
1	6:4:0.1	0	0.68
2	7:3:0.1	+1	0.73
3	5:5:0.1	-1	0.71
Amount of mobile phase (±1ml)			**Luliconazole**
4	9.1	-1	0.41
5	10.1	0	0.68
6	11.1	+1	0.44
Duration of chamber (±1min)			**Luliconazole**
7	5 min	-5 min	0.64
8	10 min	0 min	0.68
9	15 min	+5 min	0.85

Table No.16: % label claim of Luliconazole in lotion by HPTLC

S.No	Weight of drug (mg/ml)	Amount Found (mg/ml)	% label claim	Mean Amount Found (mg/ml)	SD and %RSD
1	10	10.35586	103.5586	101.9943	1.32, 1.300
2	10	10.14437	101.4437		
3	10	10.00069	100.0069		
4	10	10.12298	101.2298		
5	10	10.28194	102.8194		
6	10	10.29076	102.9076		

TableNo.17: Summary of Method validation result by HPTLC

S.No	Parameters	Results
1	Linearity (n=6)	100-500ng/band
2	Correlation coefficient (R^2)	0.990
3	Precision (%RSD)	
4	Intraday Pression (n=9)	1.282
	Intermediate precision (n=9)	1.870
5	Accuracy (%Recovery) (n=9)	101.67-103.61%
6	Limit of Detection (LOD)	15.48ng/band
7	Limit of Quantitation (LOQ)	46.92ng/band
8	Robustness (%RSD)	
	a) Change in saturation time (±5min) (n=3)	
	+5min	0.85
	-5min	0.64
	b) Change in mobile phase composition	
	7:3:0.1	0.73
	5:5:0.1	0.71
	c) Change in mobile phase (±0.1ml) (n=3)	
	9.1	0.41
	11.1	0.44
9	% label claim of Marketed lotion formulation	101.99%

Table No.18: The results of the stress degradation studies of Luliconazole by HPTLC

S.No	Stress Condition	Temp and Time	Percentlabel claim	Rf Value of degraded product
		Luliconazole	Luliconazole	
1	Acid (0.1 N HCl)	Room temp for 30 min	5.17%	0.46
2	Alkali (0.1 N NaOH)	Room temp for 30 min	7.20%	0.44
3	Oxide (3 % H2O2)	Room temp for 30min	8.18%	0.47
4	Neutral(H2O)	Room temp for 30 min	7.82%	0.48
5	Thermal	60ºC for 30 min	7.58%	0.48
6	Photolytic Degradation	24 hr	5.72%	0.46

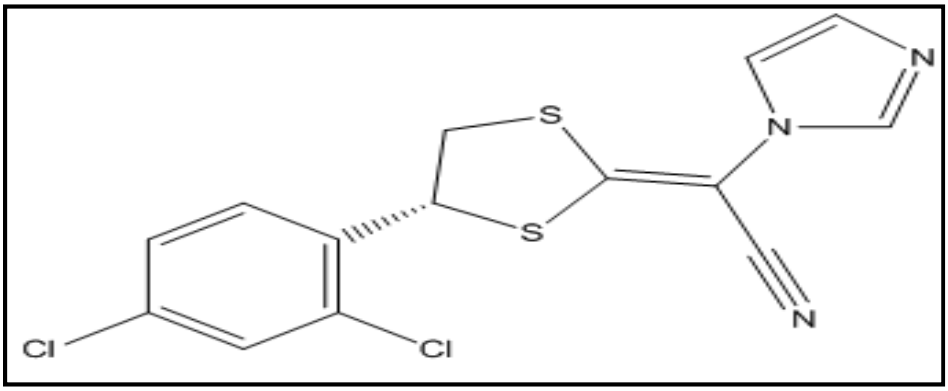

Figure No.1: Structure of luliconazole

Figure No.2: High performance thin layer chromatography

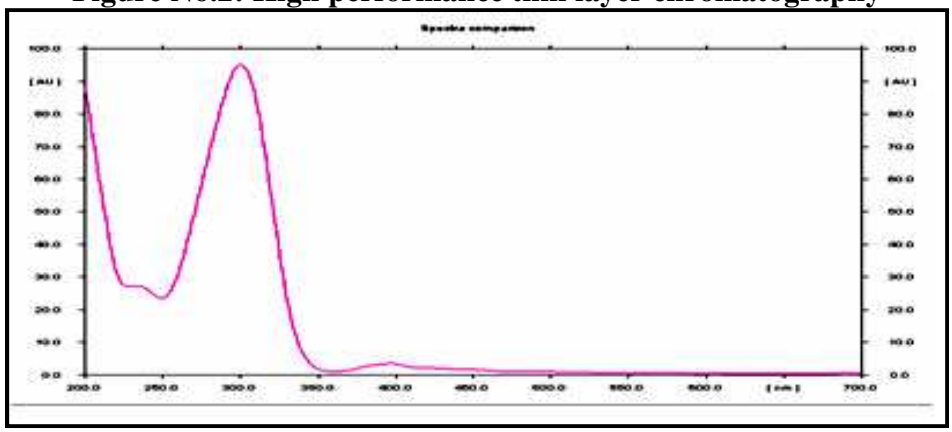

Figure No.3: UV spectra of Luliconazole

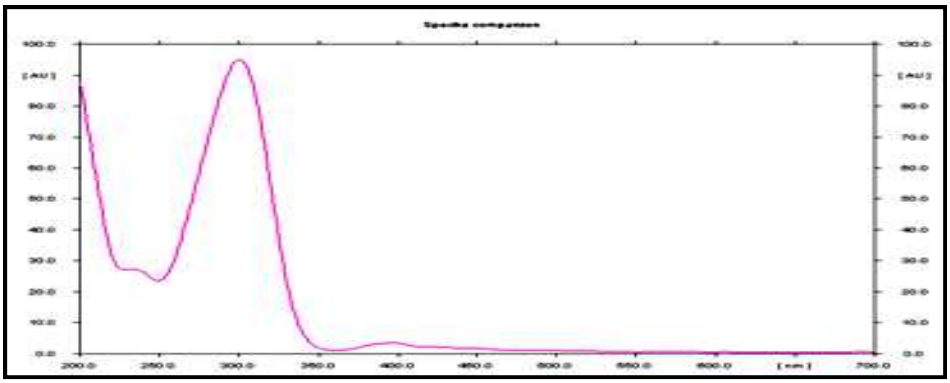

Figure No.4: Luliconazole show the maximum absorbance at 294nm

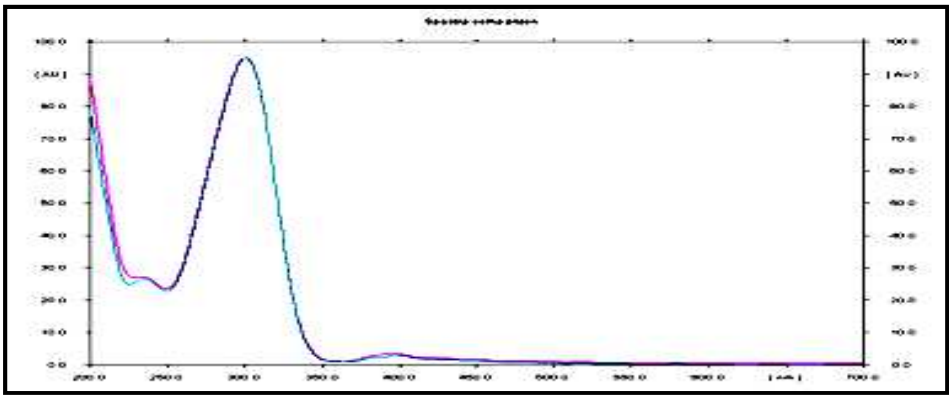

Figure No.5: Overly spectra Std and Lotion

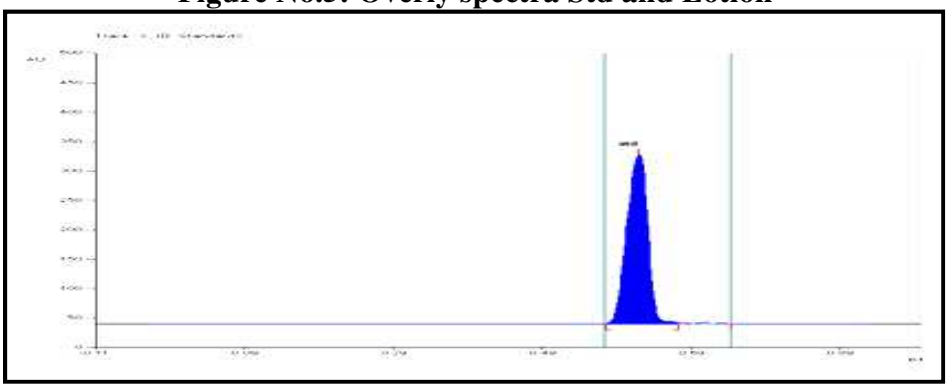

Figure No.6: HPTLC densitogram of LUL

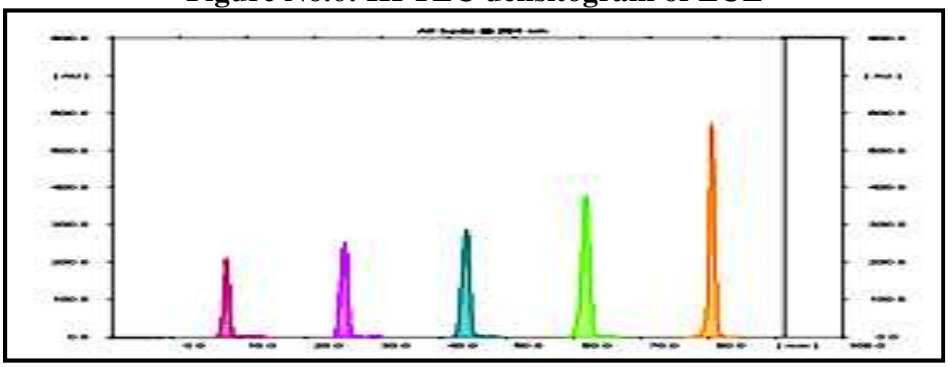

Figure No.7: Densitogram of LUL

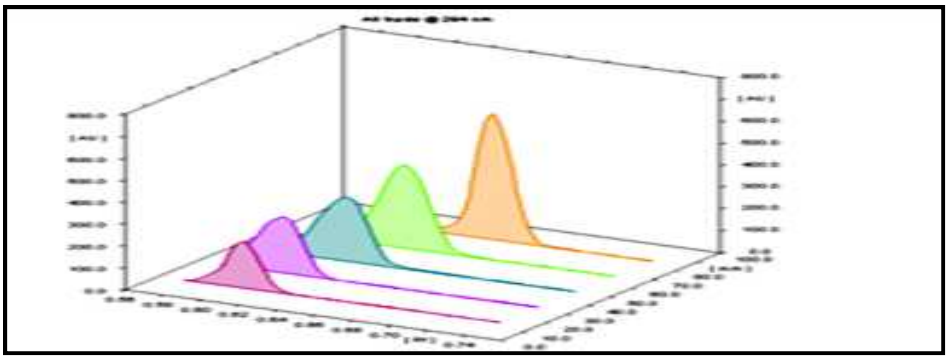

Figure No.8: 3D Densitogram of LUL

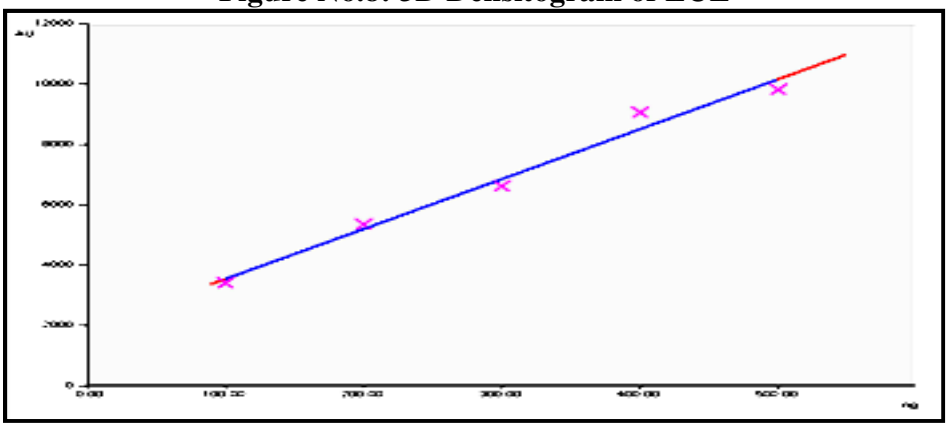

Figure No.9: Calibration plot of LUL

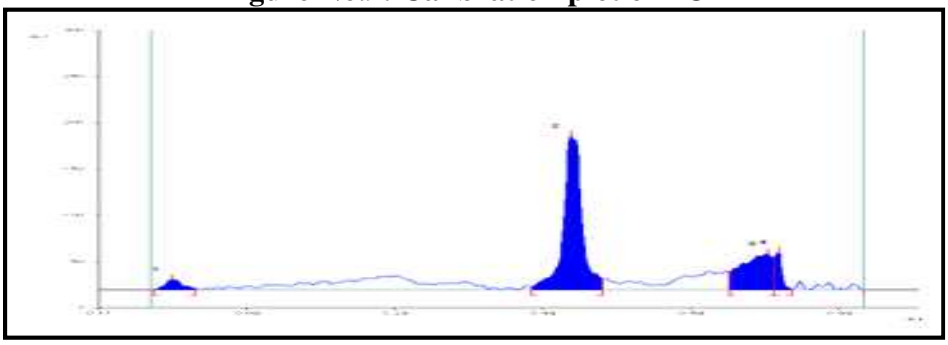

Figure No.10: HPTLC Densitogram of acid degradation of Luliconazole in 0.1N HCl at room temperature after 45 min

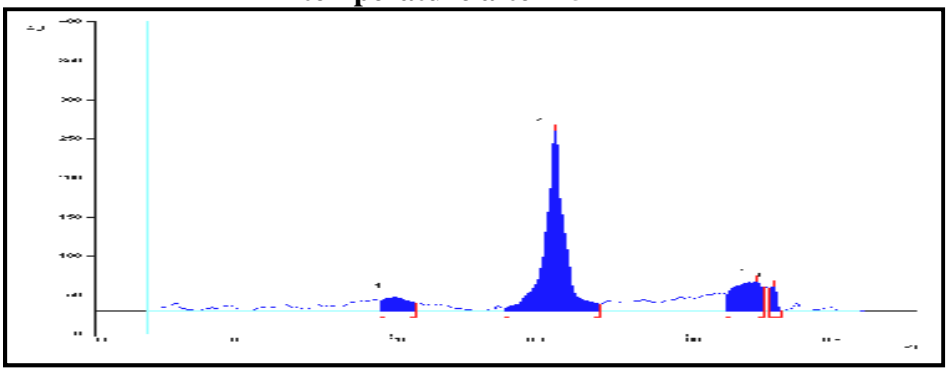

Figure No.11: HPTLC Densitogram of alkaline degradation of Luliconazole in 0.1N NaOH at room temperature after 45min

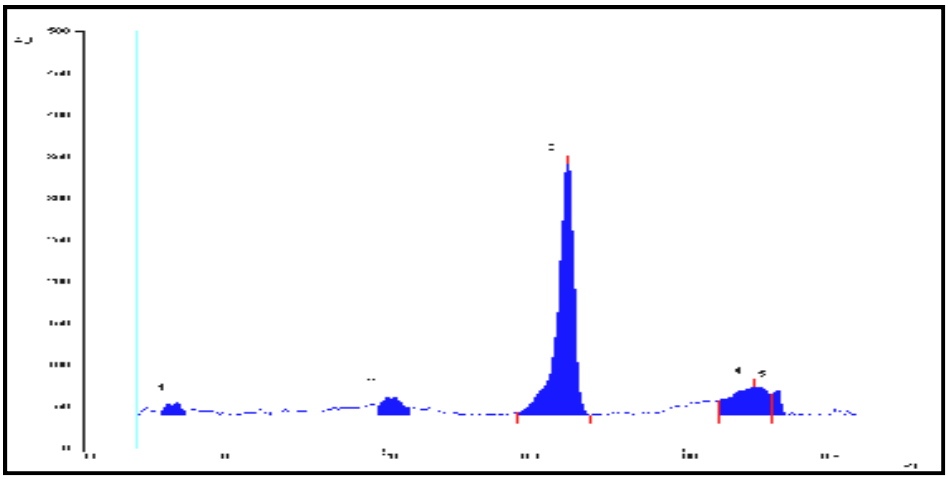

Figure No.12: HPTLC Densitogram of oxidative degradation of Luliconazole in 3% H2O2 at room temperature after 45min

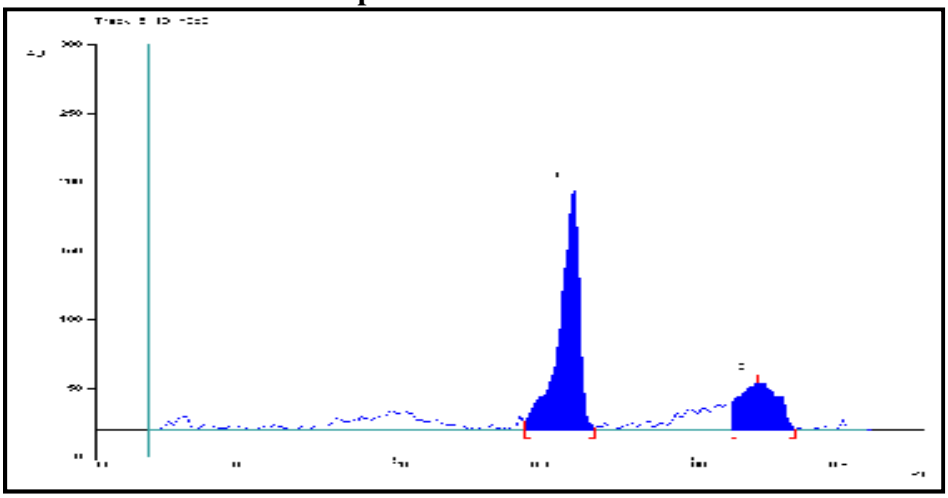

Figure No.13: HPTLC Densitogram of photolytic degradation of Luliconazole on exposure to UV light for 24 hrs

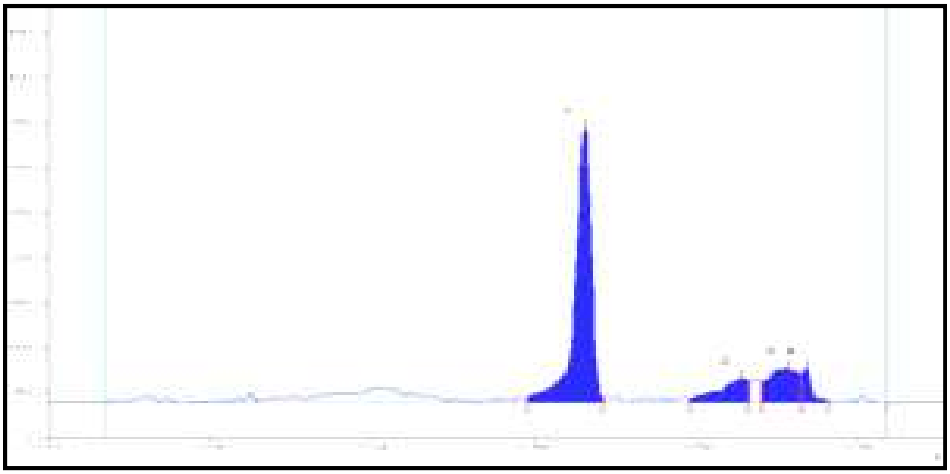

Figure No.14: HPTLC Densitogram of thermal degradation of Luliconazole on exposure to 60°C for 30 min

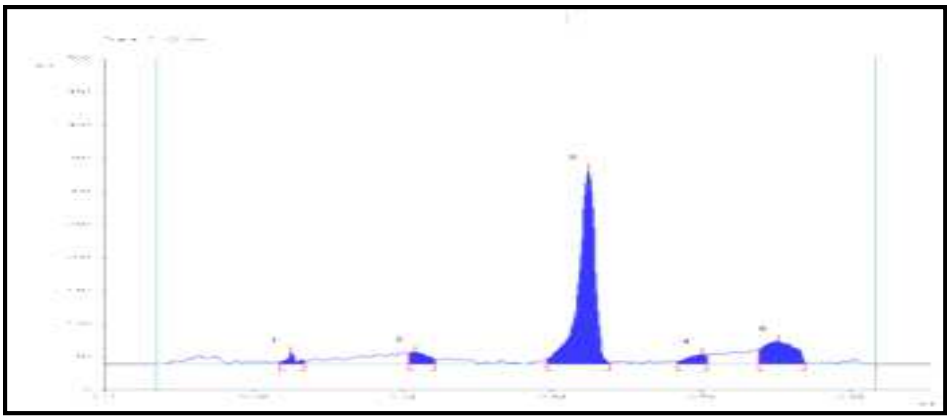

Figure No.15: HPTLC Densitogram of Hydrolytic degradation of Luliconazole in Distilled Water at room temperature after 45 min

CONCLUSION

The proposed HPTLC methods gives well symmetric peaks for Luliconazole. Based on the results obtained it is concluded that these methods are sensitive, accurate, precise and reproducible. The proposed HPTLC methods was also able to selectively quantitate Luliconazole in presence of the degradation product obtained in stability study. ICH guideline were followed throughout method validation and the suggested methods can be applied for routine quality control analysis of pharmaceutical formulation containing Luliconazole.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

BIBLIOGRAPHY

1. Uchida K, Nishiyama Y, Yamaguchi H. *In vitro* antifungal activity of luliconazole NND-502), A novel imidazole antifungal agent, *J. Infect. Chemother,* 10(4), 2004, 216-219.
2. Sonawane S, Gide P. Application of experimental design for the optimization of forced degradation and development of a validated stability-indicating LC method for luliconazole in bulk and cream formulation, *Arab Jo of Chem,* 9(2), 2016, S1428-S1434.
3. Mahesh Attimarad, K. K. Mueen Ahmed, Sree Harsha. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery, *Pharm Methods,* 2(2), 2011, 71-75.
4. Attimarad M, Ahmed M, Bandar E A, Sree H *et al.* High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery, *Pharm Methods,* 2(2), 2011, 71-75.
5. Majumder, Hasan R, Roy P, Pramanik R, Hasan N *et al.* Method development and validation of RP-HPLC method for estimation of luliconazole in marketed formulation (Cream), *The Pharma Innovation Journal,* 8(5), 2019, 103-108T.
6. Sultan M A, El-Alamin M A, Atia M A, Aboul-Enein H Y *et al.* Stability-indicating methods for the determination of luliconazole by TLC and HPTLC -densitometry in bulk powder and cream dosage form, *Journal of Planar Chromatography - Modern TLC,* 2017, 68.
7. Kumar M, Shanthi N, Mahato A K. Qualitative and Quantitative Methods for

Determination of Drug Luliconazole, *International Journal of Research in Advent Technology,* 6(10), 2018, 2321-9637.

8. Malasiya A, Goyal A. Method development and validation of RP HPLC method for assay and related substances of luliconazole in topical dosage form, *International Journal of Pharmaceutical Chemistry and Analysis,* 4(2), 2017, 46-50.
9. Yan H E , Geng P, Wang C, Lian Y, Liu Z, Yang S, Lin Y, Wen C, Ding T *et al.* Pharmacokinetic Study of Luliconazole in Rat by UPLC-MS/MS, *Latin American Journal of Pharmacy,* 34(4), 2015, 810-815.
10. Kumar M, Nithya S, Mahato A K. Qualitative and quantitative methods for determination of drug luliconazole, *International Journal of Research in Advent Technology,* 6(10), 2018, 2321-9637.
11. Cnner's K A. A text book of pharmaceutical analysis, *CBS Publication and Distributors Pvt. Ltd, New Delhi,* 3rd Edition, 2001, 3-6.
12. Denny R C, Barns J D. Vogel's text book of quantitative chemical analysis, *Prentice Hall,* 6th Edition, 2001, 297-305.
13. Ona M. A textbook of validation of analytical method for pharmaceutical analysis, *Mourne Training Publisher Service,* 1st Edition, 2005, 1-2.
14. Bakshi M, Singh M. Development of validated stability indicating assay methods critical review, *J Pharm Biomed Anal,* 28(6), 2002, 1011-1040.
15. https://en.wikipedia.org/wiki/Luliconazole
16. https://www.drugbank.ca/drugs/DB08933
17. Guidelines prepared within the international conference on harmonization of technical requirements for the registration of pharmaceuticals for human use (ICH), *Validation of Analytical Procedure: Text and Methodology Q2 (R1),* 2005, 1-13.
18. International conference of harmonization draft received guidance on impurities in new drug substance, *Federal Register Q3A(R),* 139, 2000, 45085.
19. International conference of harmonization draft received guidance on impurities in new drug substance, *Federal Register Q3B(R),* 139, 2000, 44791.
20. International conference of harmonization impurities, Q3C- guideline for residual solvent, Q3C, *Federal Register,* 62(247), 1997, 67377.
21. International conference of harmonization specification Q6A-test procedure and acceptance criteria for new drug substance and new drug product, *Chemical Substance,* 146, 1999, 67488.
22. ICH, Stability testing of new drug substance and product, *International Conference of Hormonization, Geneva,* 1993.
23. United States Pharmacopoeia: USA 23 NF 18 Inc, *US Pharmacopoeia: USA,* 1995.
24. British Pharmacopoeia, *Her Majesty's Stationary Office: London, UK,* 1998.
25. Indian Pharmacopoeia, *The controller publication, Govt of India, New Delhi,* 2, 2010.
26. Wong A W. Handbook of pharmaceutical analysis by HPLC, *Assay and Stability Testing,* 1st Edition, 2005, 335-339.
27. Chatwal G A. Insrtumental methods of chemical analysis, *Himalaya Publishing House,* 1st Edition, 2019, 2.167-2.171.
28. Sharma BK. Instrumental methods of chemical analysis in introduction to analytical chemistry, *Goel Publishing House,* 71, 9th Edition, 2003, 1-4.
29. Willard H H, Merritt L L, Dean J A, Frank A S. Instrumental method of analysis, *CBS Publishers and Distributors New Delhi,* 7th Edition, 1986, 101-105.
30. Skoog D A, Crouch S R, Holler F J. Analytical methods, Principles of instrumental analysis, *California Cengage Learning Inc,* 6th Edition, 2006, 6-19.
31. Snyder L R, Kirkland J J, Glajch J L. Practical HPLC method development, John Wiley and Sons, *A Wiley- Inter Science Publication, New York,* 2nd Edition, 1997, 21-56, 266-288.

32. Sethi P D. HPLC, Quantitative analysis of pharmaceutical formulations, *CBS Publishers and Distributors, New Delhi,* 1996, 3-3.
33. Chung H C C, Lee Y C. Analytical method validation and instrument performance verification, *Wil- Inter Scie Pub,* 1999, 51-67.
34. Klick S P, Muijselaar T, Eichinger C, Korn T, Gerding A, Debert C, Griend C, Beld G, Somsen G, Jong J. Toward a generic approach for stress testing of drug substances and drug products, *Pharm. Technol,* 29(2), 2005, 48-66.
35. Lundstedt T, Seifert E, Abramo L, Thelin B, Nystrom J, Pettersen J, Bergman R. Experimental design and optimization, *Chemo Intell, Lab. Syst,* 42(1-2), 1998, 3-40.
36. Reynolds D W, Facchine K L, Mullaney J F, Alsante K M, Hatajik T D, Motto M G. Available guidance and best practices for conducting forced degradation studies, *Pharm. Technol,* 2000, 48-56.
37. Desai N J, Maheshwari D G. UV spectrophotometric method for the estimation of luliconazole in marketed formulation (lotion), *Pharma Science Monitor,* 5(2), 2014, 48-54.
38. Li Di Edward H. Kerns. Lipophilicity methods in drug like properties- concepts, structure design and methods from ADME to toxicity optimization, *Academic Press,* 2nd Edition, 2016, 299-306.
39. Khanna D, Bharti S. Luliconazole for the treatment of fungal infections: An evidence-based review, *Core Evid,* 9, 2014, 113-124.
40. Chanapa T, Boonleang J. Simultaneous stability indicating method for determination of cisapride, Methyl paraben, propyl paraben in oral suspension, *Songklankarin Journal of Science and Technology,* 32(4), 2010, 379-385.

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