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Development and validation of RP – HPLC method for quantitation of luliconazole in bulk and formulation

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***Abstract*---**A new simple, specific, precise & validated Analytical Method has been developed for the determination of Luliconazole in bulk and pharmaceutical formulation. The separation was achieved on a C18 ODS (250×4.6 mm,5µm) or using mobile phase consisting of methanol: water (85:15) at a flow rate of 1.0 ml/min. Detection was carried out at 296nm. The retention time of Luliconazole was found to be 4.2min. the calibration curve found linear between range of 20-60 µg/ml with a regression coefficient of 0. 9998. The percentage recovery of Luliconazole was found to be in the range of 90-110%. The method was validated in accordance with International Conference on Harmonization. LOD & LOQ for Luliconazole were found to be 0.24µg & 0.748µg/ml respectively. Present method is simple, precise and can be used in routine analysis of Luliconazole in bulk and pharmaceutical lotion.

***Keywords*---**luliconazole, analytical method validation, HPLC.

Introduction

Antifungal compose a large & diverse group of drugs used to treat fungal infections. These agents are usually classified as either systemic or topical, although these divisions are somewhat arbitrary since many may be administered in either way. The mechanism of action of Antifungals include inhibition of fungal membrane & Cell wall synthesis, alteration of Fungal membranes, effects on microtubules and inhibition of nucleic Acid synthesis. The fungal infections caused by invasion of micro-organism in epithelial tissue. Over all kind of fungal agents some are not harmful but some are pathogenic in humans. These pathogenic fungi after entry in human body may cause mild to severe infection.

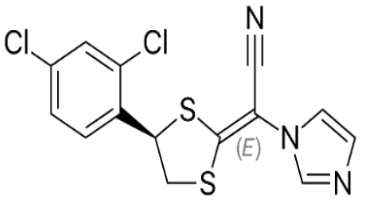

Fig1: Structure of Luliconazole

Materials and Methods

Material

Pure Drug sample of Luliconazole was gifted by industry and used without further purification. Luliconazole formulation (Luliconazole lotion containing 1gm/ml Luliconazole) was procured from the market. All the solvents and chemicals used in the analysis were HPLC grade.

Instrumentation

A double beam UV spectrophotometer (UV- 1800, Shimadzu, Japan) connected to a computer loaded with Lab Solutions Software was used for determination of detection wavelength. Spectra were obtained with instrumental parameters as follows: Wavelength range 200-800nm, HPLC- Shimadzu (Prominence-I) HPLC system with Photodiode array detector, automatic injector, Lab solutions software and C18 (250mmx4.6mm, 5 µm) column. Weighing was done on Endel Digital analytical balance of model EJB-FE300.

Solvent selection

The ideal property of a solvent should be that the solvents completely solubilize the drug. Drug should be economical and stable in selected solvents. Various solvents were studied for the solubility of Luliconazole. The Luliconazole is freely soluble in Methanol, Acetonitrile and poorly soluble in water therefore mobile phase was selected as a solvent of choice for Luliconazole. After the solubility study, Methanol: Water (85: 15) %v/v was selected as common solvent system and used as mobile phase after suitable pre-treatment.

Selection of wavelength

The UV spectrum of Luliconazole in mobile phase has maximum absorption, at 296nm. The absorbance of excipients in lotion did not interfere with Luliconazole

Preparation of Standard Stock Solution

Weigh accurately about 10 mg of working standard of Luliconazole and take into a 100 ml volumetric flask. Add 85 ml of methanol and sonicate to dissolve. Make volume up to the mark with water.

Preparation of sample Solution

Luliconazole lotion (1ml) equivalent to about 10 mg Luliconazole was transferred in a 100 ml volumetric flask containing about 60 ml methanol and sonicated until the sample was completely dispersed and mix. The solution was cooled to room temperature and diluted with same solvent to volume.

Chromatographic condition

Initially to estimate Luliconazole simultaneously number of mobile phases in different ratios were tried as mentioned below, taking into consideration the system suitability parameters like Retention time, tailing factor, no. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Methanol: Water (85: 15). The chromatographic analysis has been performed on C18 ODS (250×4.6 mm,5µm). The total analysis time was selected 10min.

Table No. 1. Summary of chromatographic trials during optimization

Trial No.	Mobile Phase	Flow Rate	Wavelength	Column Temperature	Observation
1	Water: Methanol (50: 50)	1.0ml/min	294 nm	35 ℃	Peak was not Properly Eluted
2	Acetonitrile: Water (70: 30)	2 ml / min	298 nm	35 ℃	Good separation but peak sharpness does not observe.
3	Acetonitrile: water (60: 40)	1 ml / min	296 nm	40 ℃	Tailing Factor is more
4	Acetonitrile: Water (50: 50)	1.2ml/ min	298 nm	40 ℃	Tailing factor is more
5	Methanol: water (85: 15)	1 ml / min	296 nm	40 ℃	Good separation obtained and finalized.

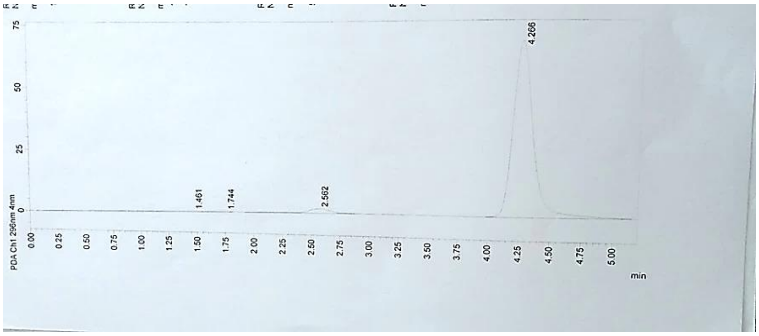

Fig. 2: Representative Chromatogram of Trial 05

Results and Discussion

Method Optimization

Several trials have been taken for accurate and precise method development. After using different solvents, column temperature, flow rates and good peak shape was obtained in HPLC C18 ODS (250×4.6 mm,5µm) column with isocratic mobile phase Methanol: Water (85: 15). The standard solution of Luliconazole in mobile phase was screened over 200 to 400 nm using photodiode array detector. On the basis of peak absorption maxima and peak purity index, 296nm was decided as a detection wavelength which provided the maximum chromatographic compatibility to the method.

Specificity

Retention time of Luliconazole peak in Sample Solution is comparable with standard therefore, the HPLC method for the determination of assay Luliconazole in its lotion dosage form is selective.

Table 2: Specificity

Sr. No	Name	R.T.
1	Luliconazole in standard Solution	4.2
2	Luliconazole in Sample Solution	4.1

Method Validation

Linearity

Linear relationship was observed by plotting drug concentration against peak areas. Luliconazole showed linear response in the concentration range of 2- 20 µg / ml. The corresponding linear regression equation was Y = 15675x – 14241 with square of correlation coefficient (r^2) of 0.999.

Limit of Detection (LOD) & Limit of Quantitation (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision, and variability. The LOD, LOQ were calculated as LOD = 3.3 sigma / S and LOQ = 10 sigma / S where sigma is the standard deviation of the lowest standard concentration and S is the slope of the standard curve. The LOD and LOQ were found to be 0.24µg / ml and 0.748µg / ml, respectively.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Table 3: Repeatability

Conc.	Area		Mean	$(X - \bar{x})$	$(X - \bar{x})^2$
	Morning	Afternoon	(X)		
6 ppm	777620	646993	762269	20225	40905062
6 ppm	725817	757014	741415	629	395641
6 ppm	724321	728493	736407	5637	3177576
6 ppm	717774	734319	726046	15998	2559360
6 ppm	732645	748741	740693	1351	1825201
6 ppm	764893	725984	745438	3394	11519236
			$\bar{x}$ = 742044		$\sum (X - \bar{x})^2$ = 60382076
SD	3475.11				
%RSD	0.468%				

%RSD of Intraday Precision is 0.468%. Therefore, the HPLC method for the determination of assay for Luliconazole in Luliconazole lotion is precise.

Accuracy

To ensure Accuracy of the method, recovery studies were performed by standard addition method at 80%, 100% ,120% level to pre analyzed sample and subsequent solution were reanalyzed. At each level, three dimensions were performed.

Table 4: Table for Accuracy

Accuracy Level	Sample conc.	Std. conc.	Area	Average Area	% Recovery
80%	8 ml	6 ml	1084481	1085480	101 %
	8 ml	6 ml	1083479		
	8 ml	6 ml	1085482		

100%	8 ml	8 ml	1514883	1514884	96 %
	8ml	8 ml	1515879		
	8ml	8 ml	1513881		
120%	8ml	10 ml	1749451	1750453	102%
	8ml	10 ml	1751449		
	8ml	10 ml	1750452		

The HPLC method for the determination of assay for Luliconazole in Luliconazole lotion is found to be accurate.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions as per ICH guidelines. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC Method Developed and system suitability parameters were found to be within acceptable limits. Results are shown in table indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions.

Table 5: flow Rate

FLOW RATE					
LF 0.6 ml / min.		MF 0.8 ml /min.		HF 1.2 ml / min.	
Conc.	Area	Conc.	Area	Conc.	Area
6 ppm	1256527	6 ppm	791606	6 ppm	640555
6 ppm	1219726	6 ppm	823857	6 ppm	652136
Mean	1238126		807731	646345	
% R.S.D.	2.0%		2.0%	1.26%	

Table 6: Table for Wavelength

Conc.	Area	
	Y1 = 298	Y1 = 284
6 ppm	774238	685598
6 ppm	743818	657988
Mean	759028	671793
%RSD	1.9%	1.4%

Conclusion

The results of present 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. From the above data, it was observed that all the validation parameters meet the predetermined acceptance criteria. Thus it has been concluded that the method is validated for the analysis of Luliconazole in 1% lotion.

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