Advances in Bioresearch
Adv. Biores., Vol 15 (1) January 2024: 155-161

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.1.155161

Advances in Bioresearch

ORIGINAL ARTICLE

Stability Indicating RP-HPLC Assay Method for Luliconazole in Pure and Formulations

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ABSTRACT
A simple, rapid, accurate and precise RP-HPLC method was developed for the determination of luliconazole in pure and dosage forms. Separation of the luliconazole was achieved on a reverse phase Symmetry C-18 Column (4.6 x 250 mm, 5µm) using the mobile phase of Formic acid, Methanol, and acetonitrile in the ratio of 50:30:20 (%v/v) with a flow rate of 1.0 mL/min and detection at 292nm. The method showed a linear response in the range of 10-50µg/mL and retention time was 5.272 min. The method was statistically validated for linearity, accuracy, precision and selectivity as per ICH guidelines. The drug was subjected to stress conditions of hydrolysis (acid and base), oxidation, photolysis and thermal degradation to show the stability-indicating power of the developed RP-HPLC method. The present method can be successfully used for routine quality control analysis of luliconazole and stability studies.
Keywords: *Luliconazole, RP-HPLC, Validation.*

Received 24.09.2023 Revised 14.10.2023 Accepted 21.12.2023

How to cite this article:
M. Latha, P. Sumalatha, M. Durga Bhavani, G. Ravi Kumar, M. Subba Rao. Stability Indicating RP-HPLC Assay Method for Luliconazole in Pure and Formulations. Adv. Biores., Vol 15 (1) January 2024: 155-161

INTRODUCTION

Luliconazole [1-3] is chemically (−)(E)-[4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-1-imidazolylacetonitrile (**Fig. 1**) is used to treat skin infections such as athlete's foot, jock itch, and ringworm. Luliconazole is an azole antifungal that works by preventing the growth of the fungus.

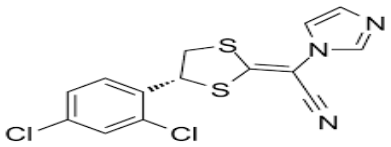

Figure 1. Structure of luliconazole

Only three HPLC methods [4-6] were reported and published for the analysis of luliconazole in bulk and pharmaceutical dosage form using several analytical techniques. Extensive literature survey revealed that there is no rapid stability-indicating HPLC method for the determination of luliconazole in pure and pharmaceutical dosage forms and this made the author in the present research work to develop a suitable, single and rapid stability-indicating HPLC method for the determination of luliconazole in

formulations. The present research paper describes the development and validation of a stability-indicating liquid chromatographic analytical method for assay of luliconazole in pure and in formulations.

MATERIAL AND METHODS

Instrumentation

The present analysis was performed on HPLC system (Waters Alliance 2695 separations module) equipped with 600e controller pump, 776 auto sampler. The HPLC system was equipped with "PEAK" software. A Techcomp-2301 model UV/Visible spectrophotometer with HITACHI software was used for wavelength scanning. on Dell computer. Waters Symmetry C-18 Column (4.6 x 250 mm, 5µm) purchased from Waters Corporation (Bedford, MA, USA) was used in the present assay. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. Dona analytical balance was used for weighing the reagents in the present assay.

Chemicals and Reagents

All the chemicals were analytical grade. Formic acid, Methanol and acetonitrile used were HPLC grade and were purchased from Merck Specialties Private Limited, Mumbai, India. luliconazole (API-99% pure) was provided by Ranbaxy laboratory, Hyderabad and Commercial formulation [Luzihit-1.0% cream; Nihit Pharmaceuticals Private Limited] of luliconazole are purchased from local market. Milli-Q water was used throughout the experiment

Preparation of mobile phase

The mobile phase in the present assay is prepared by dissolving Formic acid, Methanol, and acetonitrile in the ratio of 50:30:20 (%v/v). This mobile phase is filtered and degassed prior to the assay.

Preparation of diluent

Mobile phase is used as diluent in the present assay.

Preparation of standard stock solution

10 mg of the standard drug luliconazole (API) was weighed accurately and was dissolved in 10 mL mobile phase to obtain concentration of 1000µg/mL into a 10mL volumetric flask with the mobile phase. Then it was filtered through membrane filter paper. This standard stock solution is used to prepare necessary concentrations to construct calibration curve by proper dilution. All the above volumetric flasks of working standard solutions were wrapped with aluminium foil and stored in the dark.

Preparation of sample solution

Weigh accurately about 1.0g of test sample [Luzihit-1.0% cream; Nihit Pharmaceuticals Private Limited] equivalent to about 1.0g of luliconazole was placed into a 100ml volumetric flask. Add about 50ml of methanol and sonicate in a water bath at 55^{o}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. Formulation solution having 1000µg/mL concentration of luliconazole was obtained. The drug peak area was referred to the regression equation to get the sample concentration and % nominal label claimed.

RESULTS AND DISCUSSION

Method development (method optimization studies)

In developing the new RP-HPLC method a systematic study of the effect of various factors [i.e, the influence of column, aqueous and organic phase for mobile phase, mobile phase proportion, wavelength, diluent, concentration of analyte and other chromatographic parameters] was carried out by varying one parameter at a time and keeping all other conditions constant.

From these studies it was revealed that Waters Symmetry C-18 Column (4.6 x 250 mm, 5µm) having 5µm particle size was used as stationary phase for luliconazole among the other columns because of its advantages of high degree of retention, high resolution capacity, better reproducibility, ability to produce lower back pressure and low degree of tailing. A good symmetrical peak for luliconazole was obtained, when water was replaced by Formic acid, Methanol, and acetonitrile as organic phase in mobile phase. Preliminary trials on mobile phase proportion were carried to provide good resolution for luliconazole using different compositions of mobile phase. From these trails the proportion of Formic acid, Methanol, and acetonitrile in the ratio of 50:30:20 (%v/v) was finalized as it gave good symmetrical peak for luliconazole.

The appropriate wavelength for determination of luliconazole was scanned by UV-visible spectrophotometer and was observed that the maximum absorbance (λ_{max}) was obtained at 292nm. At this wavelength luliconazole offered high response with good linearity. The best separation with adequate resolution and symmetric peak of luliconazole was obtained with the injection volume of 20µL at a flow rate of 1.0 ml/min for the mobile phase respectively.

On this finalized chromatographic conditions, obtained chromatogram of luliconazole exhibited good peak symmetry with higher theoretical plates. The representative chromatogram of luliconazole is shown in **Fig.4**.

METHOD VALIDATION

After fixing the optimization studies the developed method was validated as per ICH guidelines which include system suitability, specificity, linearity, accuracy test, precision, robustness, ruggedness, sensitivity, limit of detection and quantification.

System suitability

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions of luliconazole. The values obtained demonstrated the suitability of the system for the analysis of luliconazole in dosage forms and the results of these studies were summarized in **Table.1**.

Table 1: System suitability condition:

Sample	Retention time	Area	Tailing factor	Theoretical plates
Luliconazole	5.272min	892887.4	1.27	4054

Specificity

The specificity of the proposed method for luliconazole was studied and calculated basing on the resolution factor of the peak and was found to be free of interference from the excipients used in pharmaceutical formulation and it indicated the specificity of the system.

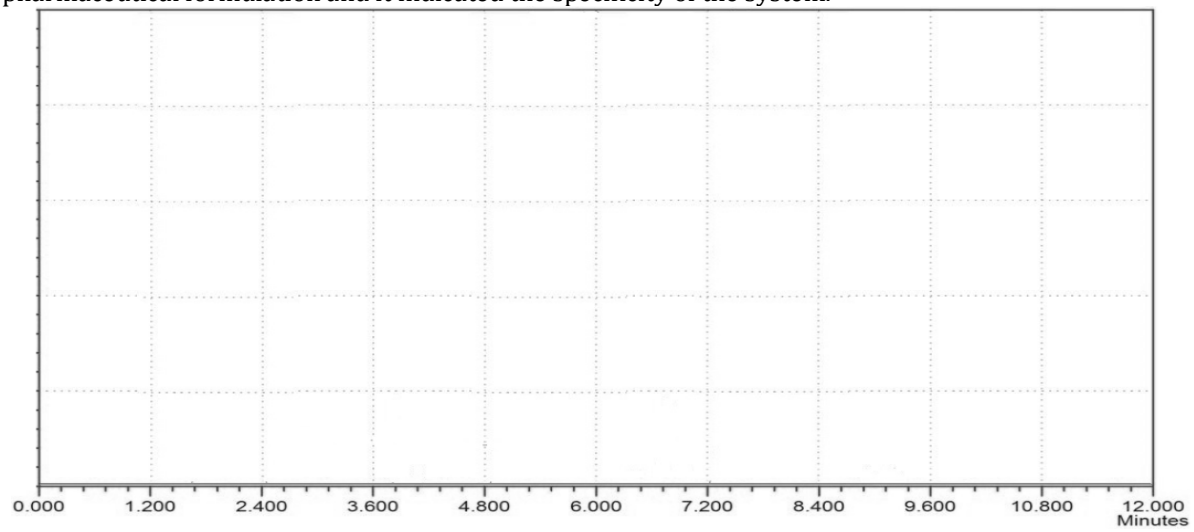

Figure 2. Blank chromatogram

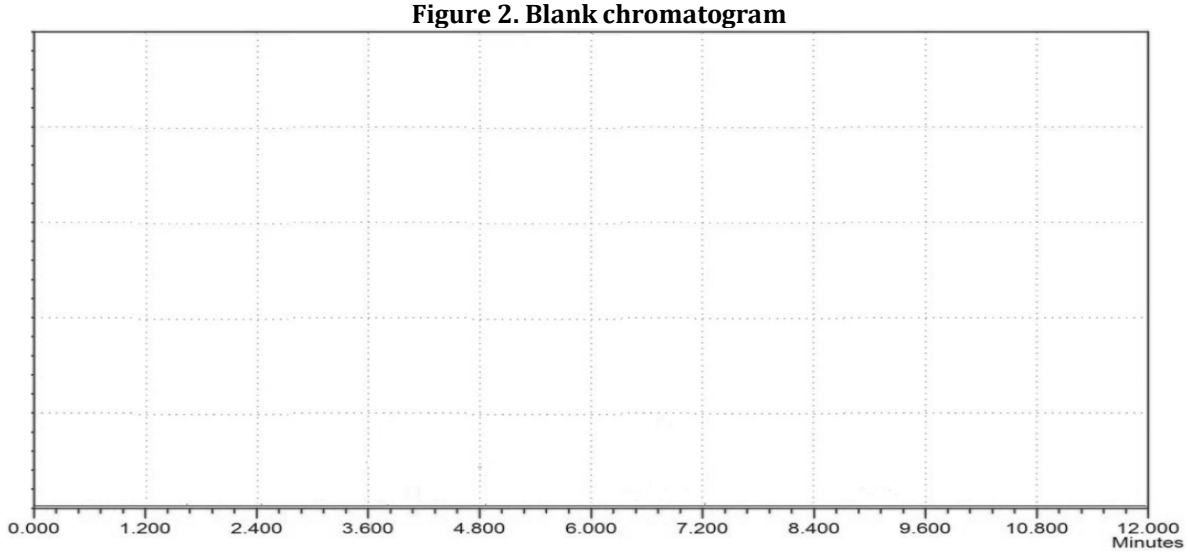

Figure 3. placebo chromatogram

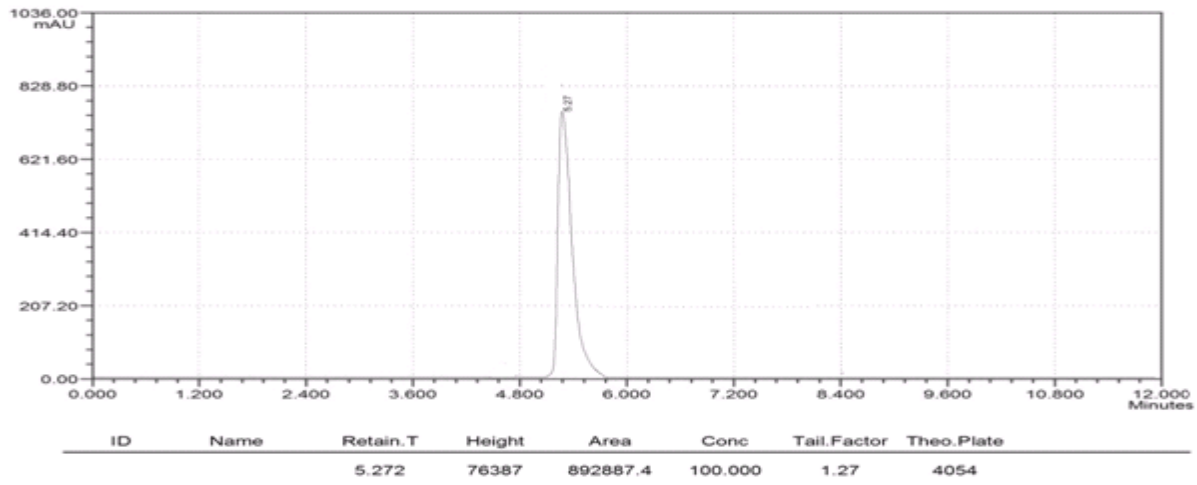

Figure 4. System suitable chromatogram

Forced degradation studies

In the present study above said drug was subjected to various stress degradation studies as per the ICH recommended guidelines. As luliconazole is soluble in methanol all solutions of luliconazole for use in forced degradation studies were prepared in methanol. This is done by subjecting luliconazole standard reference powder to acidic (0.1N HCl), basic (0.1N NaOH), oxidizing (3% H_2O_2), and photo stability stress conditions.

The chromatograms of obtained under acidic stress, basic stress and photo stability stress conditions revealed that luliconazole was found to be more stable did not showed any degradation and is eluted from the column respectively. The oxidative stress studies revealed that luliconazole was not fully degraded and its degradation products were eluted separately at different retention times respectively.

From the respective chromatographs**,** it was observed that the degradation products did not interfere in the detection analysis of luliconazole establishing the high stability of the developed method.

Table 2. showing stress degradation results

Condition	No. of deg. Products	Degra. Time	Rt	% Degraded	% Recovery	Tf	Tp
Acid	03	48 Hrs	5.26	13.78	86.21	1.95	4848
Base	04	48 Hrs	5.27	10.59	89.40	1.35	4940
Photolytic	03	48 Hrs	5.27	6.17	93.82	2.12	4445
UV light	02	48 Hrs	5.26	10.2	89.71	1.50	4669
Thermal	03	48 Hrs	5.26	9.90	90.09	1.72	4970
Peroxide	04	48 Hrs	5.26	11.46	88.53	3.39	4845
Aqueous	02	48 Hrs	5.26	7.39	92.60	1.33	4705

Linearity

For linearity studies concentration levels corresponding to 50, 75, 100,125 and 150% of test solution [10µg/ml - 50µg/ml] of luliconazole were prepared separately and 20 µL of each concentration was injected into the prescribed HPLC system and the response was read at 292nm and the corresponding chromatograms were recorded.

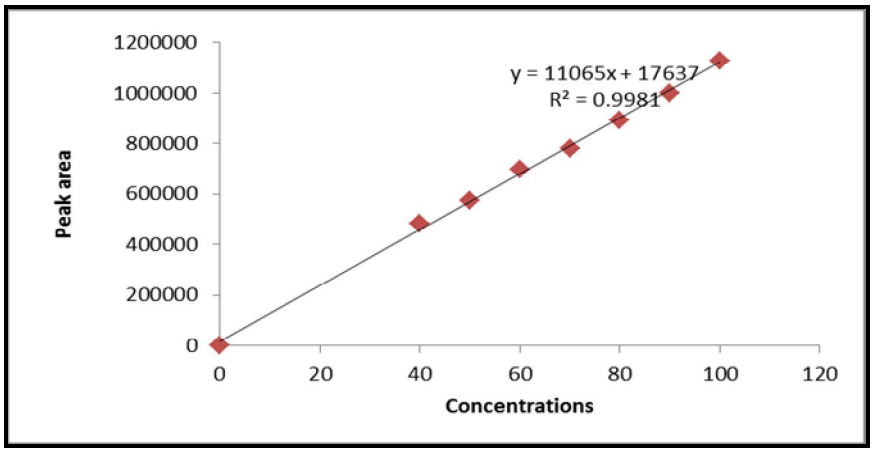

Figure 5. Calibration curve of Luliconazole

Table 3. Results of Linearity

Level	Concentration In µg/mL	peak area
Level – 1	10	486584
Level – 2	20	575994
Level – 3	30	698640
Level – 4	40	781303
Level – 5	50	892887
Level – 6	60	1000592
Range: 40 to 100µg/mL		Slope :11065 Intercept :17636 Correlation coefficient: 0.9981

From these chromatograms a calibration curve was constructed by plotting the peak areas of the drug versus concentration of luliconazole (**Fig. 5.)**. The linear regression equation for the calibration curve of luliconazole was found to be Y= 11065x+17636with a coefficient of regression r^2=0.9981 respectively. The calibrated results of luliconazole were tabulated in **Table 3.** respectively.

Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by calculating the signal to noise (S/N) ratio. The LOD and LOQ values of luliconazole were found to be 9.8 µg/ml and 21.0 µg/ml respectively.

Table 4. Limit of detection and limit of quantification

Parameter	Measured Value
Limit of Quantification	9.8µg/mL
Limit of Detection	21.0µg/mL

Precision

Precision of the proposed method was determined by repeatability (intra-day precision). It was expressed as % relative standard deviation (%RSD).The percent relative standard deviation (% RSD) was calculated and it was found to be 0.50, which are within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in **Table 5.**

Table 5. Results of Precision studies

Concentration in	Intraday precision	Interday precision
50µg/mL	843626	848965
	841753	844489
	845255	833314
	842773	848567
	848269	835286
	840057	837786
%RSD	**0.50**	**0.78**

Accuracy

The accuracy of this present proposed method was assessed by determination of recovery for three concentrations in triplicate (corresponding to 50, 100, 150 % level of test solution concentration) of luliconazole covering the within the linearity range of the proposed method. The percent recovery was calculated and results are compiled in **Table 6.** and these results indicated a high degree of accuracy of the proposed method for determination of luliconazole.

Table 6. Results of recovery

Spike Level	Target Conc.(µg/mL)	Spiked conc.(µg/mL)	Final Conc.(µg/mL)	Conc. Obtained	% Assay
50%	40	20	60	59.79	99.64
	40	20	60	59.51	99.18
	40	20	60	60.86	101.43
100%	40	40	80	79.42	99.28
	40	40	80	79.73	99.66
	40	40	80	80.80	101.01
150%	40	60	100	99.89	99.89
	40	60	100	99.26	99.26
	40	60	100	100.08	100.08

Ruggedness and Robustness

The ruggedness of the present RP-HPLC method was determined by carrying out the experiment by different analysts using different columns of similar types. The percentage of assay (%RSD) of different preparations assay values with two different analysts and columns were within the limits respectively **Table 7.**

Table 7. Robustness results

S.No.	Parameter	Condition	Mean area	% difference
1	Unaltered	NA	892887	NA
2	Mobile phase	Formic acid: MeOH:ACN 45:30:25 (v/v) 45:10:45 (v/v)	909158 902808	1.82 1.11
3	Mobile phase pH	4.0 5.0	901869 904040	1.00 1.24
4	Wavelength	290 294	891205 906156	-0.18 1.48

Robustness of the method was determined by small deliberate changes in flow rate, and temperature. The robustness limit for flow rate variation and temperature variation were well within the limit, revealing that the proposed method is robust under given set of defined experimental conditions **Table 8.**

Table 8. Ruggedness results

Concentration	Luliconazole Peak Area
50µg/mL	816921
	816196
	815916
	805677
	823061
	810820
%RSD	**0.72**

Assay of Luliconazole in formulation

The proposed RP-HPLC method has been validated for the assay of luliconazole in formulations as per guidelines of ICH. Luzihit-1.0% cream; Nihit Pharmaceuticals Private Limited were procured from local pharmacy. Weigh accurately about 1.0g of sample [Luzihit-1.0% cream] equivalent to about 1.0g of

luliconazole was placed into a 100ml volumetric flask. Add about 50ml of methanol and sonicate in a water bath at 55°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. Formulation solution having 1000µg/mL concentration of luliconazole was obtained. The drug content in the formulation was quantified using the regression equation and the chromatogram and the results are reported in **Table 9.** respectively.

Table 9. Results of Assay

S.No.	Brand name	Available form	Label claim	Concentration	Amount found	% Assay
1.	LUZIHIT	Cream 1%w/w	1.0g	80 µg/mL	79.12 µg/mL	98.9

Conclusions

In conclusion, a simple, accurate and stability indicating RP-HPLC method has been developed and validated for the analysis of luliconazole in formulations. Based on peak purity results, obtained from the analysis of force degradation studies, it can be concluded that the absence of co eluting peak along with the main peak of luliconazole indicated that the developed method is specific for the estimation of luliconazole in formulated products. Statistical analysis proved that the method is suitable for the analysis of luliconazole pure and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of luliconazole and can be conveniently used for the routine assay of luliconazole by the pharmaceutical manufacturing units.

ACKNOWLEDGEMENTS

The authors are thankful to Pharma Train, Hyderabad, India for providing gift sample of Luliconazole for the present research and the Department of chemistry, Principal, University Science College, Acharya Nagarjuna University, Guntur for providing all facilities to carry out the present work.

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