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ORIGINAL ARTICLE

RP-HPLC Method Development and Validation for the Estimation of Luliconazole and Curcuminin Control Release Formulations

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ABSTRACT
This work has been done with a motto to develop a simple, accurate, precise, reproducible and economic reverse phase HPLC method for Luliconazole (LCZ) and Curcumin (CCM) in bulk drug as well as in formulations. In the current developed method C8H column (4.6 X 250 mm) was used as stationary phase and 0.1 % Orthophosphoric acid &Acetonitrile in a gradient mode as mobile phase. It is pumped through the chromatographic system at a flow rate of 1.00 ml min^{-1}. The UV detector is operated at 254 nm. The validation study is carried out fulfilling the ICH guidelines Q2 (R1) to prove that the new analytical method, meets the reliability characteristics, and these characteristics show the capacity of an analytical method to keep, throughout the time, the fundamental criteria for validation: selectivity, linearity, precision, accuracy and specificity. The stability indicating method is applied during the working day for the quality control of commercial Luliconazole and Curcumin tablets to quantify the drug and its degradation products and to check the content uniformity test.
Keywords:*Luliconazole and Curcumin, Assay, HPLC, Validation, Accuracy*

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INTRODUCTION

Luliconazole is a novel topical antifungal imidazole with broad-spectrum and potent antifungal activity used in the treatment of superficial mycoses. Superficial mycoses are not fatal, but they constitute a serious problem for patient's quality of life given the considerable discomfort and/or cosmetic deformity they cause. These diseases are found worldwide and affect 20 to 25% of the world's population [1]. Dermatophytosis is the most common infection among the superficial mycoses [2-8]. Curcumin 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6- heptadiene-2,5-dione is a yellow-colored phenolic pigment obtained from the powdered rhizome of Curcuma longa Linn. (Family: *Zingiberaceae*), from ancient it was been used for relieving pain and inflammation since ancient times in traditional medicine. Extensive researches have also revealed the potent anti-inflammatory effects of curcumin. It blocks the synthesis of certain prostaglandins, reduces pro-inflammatory cytokine synthesis, and inhibits pro-inflammatory arachidonic acid as well as neutrophil aggregation when inflammatory conditions occur. However, the oxygen radical scavenging activity of Curcumin has also been observed in its anti-inflammatory effects. Curcumin is unstable at basic pH and undergoes alkaline hydrolysis in alkali/higher pH solution. Decomposition of Curcumin in Hydrolytic decomposition is reported in in-vitro physiological conditions (isotonic phosphate buffer, pH 7.2). It undergoes photodegradation while exposing to light in solution as well as in solid form [9-13].

Analytical method validation ensures that various HPLC analytical techniques shall give reliable and repeatable results; it is a crucial step in developing new dosage forms as it provides information about accuracy, linearity, precision, detection, and quantitation limits. According to the ICH guideline, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended

purpose." It is now obligatory in the process of drug development to supply the validation data for the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines [14–18].

Literature survey revealed that there is a very limited number of analytical methods available for estimation of Luliconazole, such as validated stability-indicating LC method for Luliconazole in bulk and cream formulation, the analytical methods developed for Luliconazole includes LC-MS/MS method for the determination of the Luliconazole in human toenails, RP HPLC Method for Assay and related Substances of Luliconazole in Topical Dosage form UV Spectrophotometric Method For The Estimation of Luliconazole In Marketed Formulation (Lotion) [19-21]. A variety of analysis techniques for the quantification of total and isolated curcuminoids in different matrices have been reported, especially spectrophotometric methods for the determination of total curcuminoids. High-pressure liquid chromatography with UV detection (HPLC–UV) is the most common method for the determination of curcuminoids and curcumin in turmeric samples, biological samples, or dosage forms. Due to the very labile characteristics of curcuminoids, C18 columns are preferred for HPLC analysis. These methods reported by HPLC–UV for luliconazole and curcuminoids, especially those in older literature, have several disadvantages, including unsatisfactory separation times, poor resolution, complicated solvent mixtures with gradient elution, and long analysis times [22-25].

However, reviews on different analytical methods of curcuminoids and luliconazole are extremely limited. There is no HPLC analytical method for the simultaneous determination of curcuminoids and luliconazole. Therefore, this study aimed to develop and validate an efficient HPLC method for the simultaneous determination of LCZ and CCM. Moreover, this new method could also be used for the routine analysis of LCZ and CCM in pharmaceutical dosage forms, provided it is completely validated and rapid. The newly developed method was different both in terms of methodology and aim in comparison to previously reported methods in the literature. The method was validated according to guidelines and applied for the assay of LCZ and CCM from their combination Semisolid dosage form.

MATERIAL AND METHODS

Chemicals

HPLC-grade solvents such as Methanol, acetonitrile, and orthophosphoric acid were obtained from Merck Ltd. Bangalore India. Water obtained from the Milli-Q water system. Curcumin was purchased from Natural Remedies Ltd. Bangalore India and Luliconazole was procured as a gift sample Glenmark Pharmaceutical Pvt. Ltd., Mumbai, Maharashtra.

Preparation of the standard solution Luliconazole:

20.0 mg Luliconazole was weighed and transferred into a 100 mL volumetric flask. 20 mL of diluent (100% Methanol) was added and sonicated in an ultrasonic water bath for 15 minutes. The solution was cooled and volume was made with diluent.

Preparation of the standard solution Curcumin:

20.0 mg Curcumin was weighed and transferred into a 100 mL volumetric flask. 20 mL of diluent was added and sonicated in an ultrasonic water bath for 15 minutes. The solution was cooled and volume was made with diluent.

Preparation of the Mixture of the standard solution of Luliconazole (LCZ) and Curcumin (CCM):

5 mL resulting solution of Luliconazole (LCZ) and 10 mL of Curcumin (CCM) were added into 25 mL volumetric flask. Volume was made with diluent. 2 ml resulting solution was diluted up to 10 mL with diluent. The resulting solution was used as the mixed standard solution.

Preparation of test solution

About 1000 mg Luliconazole and Curcumin Cream was taken into a 100 mL volumetric flask. 70 mL of diluent was added and then sonicated in the ultrasonic water bath for 20 minutes. Allow to come to room temperature before adding diluent and thoroughly mixing. Allow for the settling of any remaining solids. A 0.45 µ Prefilter + PTFE Syringe filter should be used to remove the supernatant solution. The first 2-3 mL of filtrate should be discarded, and the remaining filtrate should be discarded. Obtained 4 mL of clear filtrate, dilute to volume, then combine in a 100 mL volumetric flask with diluent.

Chromatographic conditions for HPLC

HPLC was performed using a Waters 2695 Alliance system with a 2996 photodiode array detector (PDA) and 2489 UV/Visible detector (UV). The standards as Luliconazole and Curcumin were resolved on a reverse-phase columnInertsil ODS-C8 Column, 5µ (4.6 X 250 mm, (Mumbai, India). The mobile phase was prepared from 0.1 % Orthophosphoric acid (solvent-A) and Acetonitrile (100%) (solvent-B). The gradient program used is given in **Table 1**. The mobile phase flow rate was kept at 1.0 ml/min. The selected diluent is Methanol. Before the first injection, the column was saturated for 30 min with the initial mobile

phase. The temperature was maintained at 30°C. Injection volume was decided to maintain at 10 µL. The PDA was set by optimizing wavelength to give the best response for two peaks at 254 nm to acquire the chromatogram. The standard Luliconazole and Curcuminwere identified by comparing the retention time and spectra obtained from the sample and standard solutions. The present work was performed in an air-conditioned room maintained at 10°C [17-19].

Preparation of Calibration Graph

The linearity of peak area response for Luliconazole and Curcuminwas determined from 50 % to 150 % level of working concentration for Luliconazole and Curcumin. The stock solutions of luliconazole and Curcuminwere diluted in seven different known concentrations. Graphs of concentration (as x-value) versus area (as y-value) were plotted.

Validation of HPLC method

The proposed HPLC method was validated in terms of specificity, precision, accuracy, the limit of detection (LOD), the limit of quantification (LOQ), standard solution stability, sample solution stability, and robustness as per the International Conference on Harmonization (ICH Q2 (R1)) guidelines[20-23].

Specificity

The specificity of the method was studied by assessment of peak purity of Luliconazole and Curcuminusing the Waters empower software and diode array detector and represented in terms of purity angle, purity threshold, and purity flag.

Precision

Precision was studied in terms of system precision, method precision, and intermediate precision.

System precision

System precision was carried out by six replicate injections from the same vial of standard and was expressed in terms of percent relative standard deviation (% RSD) tailing, plate count, and resolution.

Method precision

The sample was analyzed six times by mentioned procedure. The % assay for each analyte was expressed in terms of % RSD.

Intermediate precision

Intermediate precision was performed on different systems, one the Waters e2695 Alliance system with a 2996 PDA and the other a 2489 ultraviolet (UV) detector by different analysts by analyzing six different samples of extract and was expressed in terms of % RSD.

Recovery studies

The accuracy of the method was determined from recovery studies by adding a known amount of each standard at the 80%, 100%, and 120% levels to the pre-analyzed sample followed by replicate quantitative analyses by the proposed method.

Robustness

The robustness of the method was determined by a slight deviation in the method parameters. The parameters selected were deviation in column chemistry, wavelength, column temperature, flow rate, and mobile phase gradient. The retention time of Luliconazole and Curcumin was determined and % RSD using system suitability parameters was observed.

Polyherbal tablet formulation was analyzed to determine the contents of Luliconazole and Curcuminas per the method described under chromatographic conditions by HPLC. All analysis was repeated three times and results were expressed in mean ± SD.

RESULTS AND DISCUSSION

The composition of the mobile phase in the HPLC method was optimized by testing different solvent compositions of varying polarity, column chemistry, column temperature, and pH of mobile phase, and the best results were obtained by using the present method, which produces highly symmetrical peaks showing good resolution between each standard and other peaks [Figure 1]. The scanning wavelength selected was 254 nm to provide comparable results and at this wavelength, all analytes showed an optimum response. Luliconazole and Curcumin were satisfactorily resolved with retention times about 17 and 21 minutes respectively.

The calibration graph was in 50 % to 150 % level of working Luliconazole and Curcumin, with acceptable correlation coefficients 0.9990 (40-140 µg/ml) [Table 2].The graph for each standard is given in Figure 2.

The values of system precision, method precision, and intermediate precision are given against sample application and scanning of peak area and are expressed in terms of % RSD. For system precision %RSD values were found to be 0.21 and 0.56 % for Luliconazole and Curcumin respectively. Method precision was done and %RSD value was found to be 0.30% and 0.34% for Luliconazole and Curcumin respectively. For intermediate precision %RSD values between the two analysts were found to be 0.89% and 0.43%for

Luliconazole and Curcumin respectively [Table 3].For the values of system precision, method precision, and intermediate precision, the %RSD values showed that the proposed method provides an acceptable level of system precision, method precision, and intermediate precision.

The peak purity of each analyte was assessed by comparing their respective spectra at peak start, peak apex, and peak end positions of the spot from standard and extracts. The purity angle and purity threshold values are given in table [Table 4]

The given method was optimized by doing robustness. The peak area for each analyte was calculated for each parameter and % RSD was found to be less than 2%. The values of % RSD as shown in Table 5 indicate better robustness of the method.

The recovery study was carried out by spiking a known amount of standards into placebo solution at 80%, 100%, and 120% of working concentration. The overall recovery percent were found to be for Luliconazole and Curcumin between 98.0% to 102.0%. [Table 6]

Table 1: Details of Gradient program

Time (minute)	Flow (mL/minute)	% solvent A	% solvent B
0	1.0	50	50
20	1.0	40	60
21	1.0	10	90
30	1.0	10	90
31	1.0	50	50
35	1.0	50	50

Table 2: Linearity of Luliconazole and Curcumin

% Level	Conc. of Curcumin (ppm)	Average Peak area of Curcumin	Conc. of Luliconazole (ppm)	Average Peak area of Luliconazole
50	10.08	587594	32.40	1536126
65	13.44	777203	43.20	2075742
80	16.80	975697	54.00	2583291
100	20.16	1150894	64.80	3014198
110	23.52	1373196	75.60	3577181
130	26.88	1589750	86.40	4093473
150	33.60	1948623	108.00	5856339
r^2	**0.9993**		**0.9790**	
Slope of Regression line	58511		46519	

Table 3: Method precision and intermediate precision for Luliconazole and Curcumin

Name of Analyte	Sr. No.	Assay (% w/w, Analysis-1) MP	Assay (% w/w, Analysis-2) IP
Curcumin	1	100.5	98.8
	2	100.9	100.5
	3	101.0	99.9
	4	100.4	102.7
	5	100.7	100.9
	6	100.2	100.8
	Average	**100.6**	**100.6**
	% RSD	**0.30**	**1.28**
	Overall % RSD	**0.89**	
Luliconazole	1	100.6	101.2
	2	101.1	101.9
	3	101.1	101.1
	4	100.5	101.9
	5	101.4	101.6
	6	101.0	101.4
	Average	**101.0**	**101.5**
	% RSD	**0.34**	**0.34**
	Overall % RSD	**0.43**	

Table 4: Specificity of Luliconazole and Curcumin

Sample Name	Retention Time (Min)	Purity Angle	Purity Threshold	Peak Purity
Blank (diluent)				
Luliconazole and Curcumin	ND	NA	NA	NA
Standard solution				
Curcumin	16.66	0.116	0.280	Pass
Luliconazole	20.86	0.321	0.453	Pass
Worst Case Placebo				
Luliconazole and Curcumin	ND	NA	NA	NA

Table 5: Robustness for Luliconazole and Curcumin

Robustness parameter		% RSD		Remark
		Luliconazole	Curcumin	
Wavelength (nm)	249	0.36	0.34	Pass
	254	0.26	0.30	Pass
	259	0.58	0.32	Pass
Temperature (°C)	25	0.33	0.31	Pass
	30	0.60	0.39	Pass
	35	0.26	0.30	Pass
Flow (mL/min)	0.9	0.76	0.32	Pass
	1.0	0.49	0.30	Pass
	1.1	0.34	0.31	Pass

Table 6: Recovery for Luliconazole and Curcumin

Analyte	Recovery level	% Recovery	Average % Recovery
Curcumin	80% - 1	98.68	98.72
	80% - 2	98.86	
	80% - 3	98.62	
	100% - 1	101.65	100.35
	100% - 2	99.55	
	100% - 3	99.84	
	120% - 1	99.60	100.30
	120% - 2	99.43	
	120% - 3	101.86	
Luliconazole	80% - 1	98.79	98.98
	80% - 2	98.83	
	80% - 3	99.30	
	100% - 1	102.67	101.04
	100% - 2	99.75	
	100% - 3	100.71	
	120% - 1	99.25	99.59
	120% - 2	98.63	
	120% - 3	100.90	

Figure 1: Chromatograms for Blank, Standard and Sample

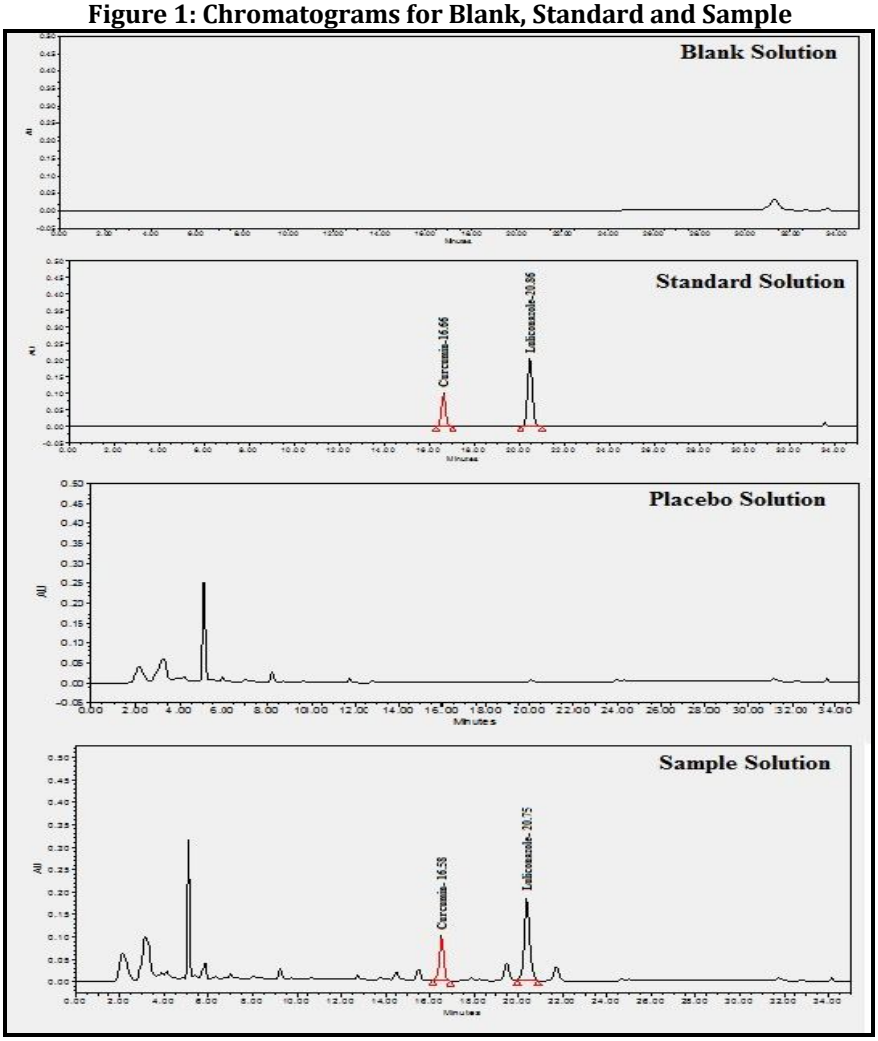
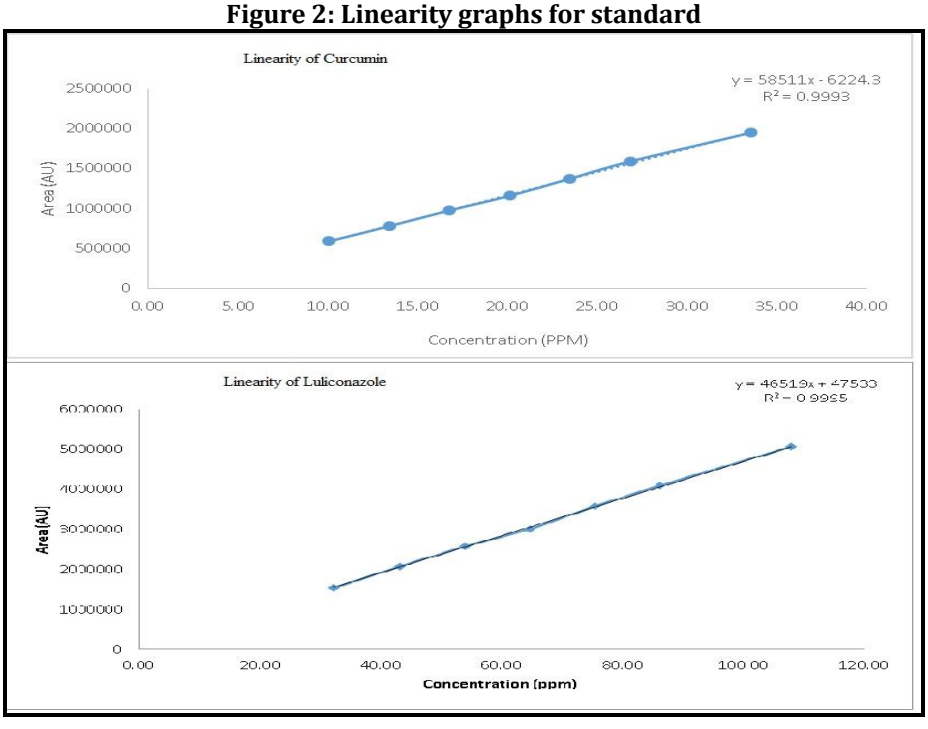


Figure 2: Linearity graphs for standard



CONCLUSION

The present investigation resulted in the development of an RP-HPLC-UV-DAD analysis method for Luliconazole and Curcumin that was validated in terms of linearity, precision, accuracy, specificity, system suitability, and robustness. The presented method in addition to its novelty for determination of two ingredients i.e. Luliconazole and Curcumin at single wavelength is sufficiently rapid, simple, and sensitive as well as precise and accurate that complies with ICH guidelines. The assay of the two active ingredients was not interfered by the excipients in the Tablet. Therefore, the proposed analytical method is recommended for the routine analysis of Luliconazole and Curcuminas such, or in various dosage forms. In addition, the method can be applied in many developing countries or field stations where advanced analytical equipment are not available.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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