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ORIGINAL ARTICLE **OPEN ACCESS**

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

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ABSTRACT
The goal of this work was to come up with a simple, accurate, precise, repeatable, and cost-effective reverse phase HPLC method for Luliconazole (LCZ) and Curcumin (CCM) in bulk drug and formulations. As the stationary phase, a 4.6 x 250 mm C8H column was used, and 0.1% Orthophosphoric acid and acetonitrile were used in a gradient mode as the mobile phase. At a flow rate of 1.00 ml min1, it is pumped through the chromatographic system. At 254 nm, the UV detector is in use. The validation study is done according to the ICH guidelines to show that the new analytical method meets the reliability characteristics. These characteristics show that an analytical method can keep the basic criteria for validation: selectivity, linearity, precision, accuracy, and sensitivity, over time. During the work day, the method is used to test the quality of commercial Luliconazole and Curcumin tablets by measuring how much of the drug and its breakdown products there are, as well as checking the content uniformity test.
Keywords: *Luliconazole, Curcumin, Assay, HPLC, Validation and Accuracy.*

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INTRODUCTION

A brand-new antifungal cream made with imidazole called luconazole has just been made. It works against many different types of fungi, which means it can be used to treat superficial mycoses. Even though most superficial mycoses don't kill people, the pain they cause and the way they change a person's appearance can have a big effect on their quality of life. Between 25% and 35% of the world's population has one or more of these diseases [1]. The vast majority of superficial fungal infections are caused by dermatophytes [2–8]. Curcumin is a yellow phenolic pigment that comes from the ground rhizome of a plant called Curcuma longa Linn. Scientists call it 1, 7-bis-(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-2, 5-dione, which is the name of its chemical name. (Genus: Zinziber) Since ancient times, this herb (Zinziberaceae) has been used to reduce pain and inflammation. Several studies have also shown that curcumin is a powerful anti-inflammatory. It stops the production of prostaglandins, cytokines, arachidonic acid, and prostaglandin precursors that cause inflammation. This keeps neutrophils from gathering during an inflammatory response and stops prostaglandin precursors from being made. Curcumin's ability to get rid of oxygen radicals has been linked to its ability to reduce inflammation, but this doesn't mean that the opposite is also true. When curcumin is mixed into a solution with a higher pH, it goes through a process called alkaline hydrolysis, which breaks it down and makes it unstable. Curcumin breaks down when it is in contact with water. There are records of the physiological in-vitro conditions that led to this finding (isotonic phosphate buffer, pH 7.2). Photo degradation is the name for what happens when something is in a liquid or solid state and is exposed to light [9–13].

The validation of analytical techniques makes sure that the results from the many different HPLC analytical methods are reliable and consistent. During this process, the limits of accuracy, linearity, precision, detection, and quantitation are shown. This is a key step in the process of making new dosage forms, so it is called a "disclosure step." According to the ICH guideline, validation is needed to show that an analytical process works well for the purpose it was made for. As part of the process of making a new medicine, the validation data for the new drug must be sent to the right people. Both ICH and USP have made rules about how to check an analysis method [14–18].

Based on a review of the relevant literature, there aren't that many different ways to test luconazole. An LC method that has been approved for use can be used to test how stable both the bulk and cream forms

of luconazole are. An LC-MS/MS method has also been used to figure out how much Luliconazole is in a person's toenails. This adds to the growing number of analytical methods that have been set up for this chemical. RP The development and use of a high-performance liquid chromatography (HPLC) method for the analysis of topical doses of liconazole and related substances UV Evaluation of a spectrophotometric method for figuring out how much Luliconazole is in a lotion that can be bought [19–21] Particularly, spectrophotometric methods have been used a lot to measure total curcuminoids in a wide range of matrices. There have also been reports of other ways to measure isolated curcuminoids. People have talked a lot about using spectrophotometric methods to measure the total amount of curcuminoids. High-pressure liquid chromatography with ultraviolet (HPLC-UV) detection is the best way to find curcuminoids and curcumin in turmeric, biological samples, and pharmaceutical dosage forms, respectively. On the other hand, since curcuminoids are very volatile, it is strongly recommended to use HPLC analysis with C18 columns. Some of the problems with these HPLC-UV methods for Luliconazole and curcuminoids, especially those from older literature, are long separation times, low resolution, difficult solvent mixtures with gradient elution, and long analysis times [22–25]. There are a number of ways to find these problems. Curcuminoids and Luliconazole, on the other hand, have not been tested in a way that has been fully evaluated. HPLC can't analyses both curcuminoids and luconazole at the same time, for example. The goal of this study was to come up with and test a good HPLC method for measuring both LCZ and CCM at the same time. This was the point of this research. This cutting-edge method could be used to analyses LCZ and CCM in pharmaceutical dosage forms on a regular basis, as long as it is first thoroughly tested and then has a high throughput. The unique strategy was very different from the ways that had been described in the past, both in terms of how it worked and what it tried to accomplish. After the method was tested to make sure it works well, it was used to figure out how much LCZ and CCM were in the combination Semisolid dosage form.

MATERIAL AND METHODS

Chemicals

We bought methanol, acetonitrile, and Orthophosphoric acid from Merck Ltd. These solvents are all HPLC-safe. Milli-Q was used to acquire water. Natural Remedies Ltd. in Bangalore, India, gave us curcumin, while Glenmark Pharmaceutical Pvt. Ltd. in Mumbai, Maharashtra, gave us luconazole. Both companies are from India.

Preparation of the standard solution Luliconazole:

A 100-ml volumetric flask containing 20.0 mg luconazole was weighed. After adding 20 mL diluent, the mixture was sonicated for 15 minutes. After making the solution, a diluent was added to make it more concentrated.

Preparation of the standard solution Curcumin:

20.0 milligramme of curcumin were measured out and transferred to a volumetric flask with a 100 milliliter capacity. The mixture was then sonicated in an ultrasonic water bath for 15 minutes after being added 20 mL of diluent. The prepared solution was allowed to cool before a diluent that would increase its concentration was added to it.

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

5 ml of a solution containing Luliconazole (LCZ) and 10 ml of a solution containing curcumin (CCM) were added to a volumetric flask with a capacity of 25 ml. Volume increased as a result of the usage of a diluent. The final volume of the solution, which was 2 mL at first, was increased to 10 mL by adding diluent. As a result, a combination was created that served as a standard for other blended solutions.

Preparation of test solution

Five milliliters of a Luliconazole (LCZ) solution and ten milliliters of a curcumin solution were respectively added to a volumetric flask that had a capacity of twenty-five milliliters (CCM). The inclusion of a diluent resulted in an increase in the volume of the mixture as a whole. Following the addition of the diluent, the volume of the solution was brought up to its ultimate value of 10 mL; this represented an increase from the solution's original volume of 2 mL. This directly led to the development of a combination that, in the future, would come to serve as the standard by which all other kinds of blended treatments would be measured.

Chromatographic conditions for HPLC

HPLC analysis was performed on a Waters 2695 Alliance system equipped with a 2996 PDA and 2489 UV/Visible detector (UV). To accomplish a separation between the reference compounds Luliconazole and curcumin, a reverse-phase column was used (Inertsil ODS-C8 Column, 4.6 x 250 mm, 5). (India's Mumbai). Acetonitrile (solvent-B) was diluted to 100% and Orthophosphoric acid (solvent-A) was diluted to 0.1% to generate the mobile phase. (Solvent-B). An inventory of the relevant software is provided in **Table 1.** The mobile phase flow rate was maintained constant throughout the experiment at 1 ml/min.

The medium used to lower the original substance's concentration will be methanol. The first mobile phase was initially infused into the column for thirty minutes prior to the injection. Throughout the day, the temperature remained constant at 30 degrees Celsius. After some consideration, a permanent injection volume of ten litres (L) was chosen. A chromatogram was produced after modifying the PDA's wavelength to offer the greatest response to the double peak at 254 nm. We were able to identify the presence of Luliconazole and curcumin in the standard solution by comparing the retention durations and spectra of the sample solution with those of the standard. The temperature in the room where this procedure was performed was a chilly 10 degrees Celsius [17-19].

Preparation of Calibration Graph

The peak area response for both Luliconazole and curcumin was evaluated from 50% to 150% of the working concentration, and it was discovered that it was linear over this concentration range. That's what happened. By weakening the initial stock solutions of licorice root extract (curcumin) and Nystatin (a fungicide), seven distinct strengths were produced. It was necessary to compare the two variables of concentration (represented by the x-value) and area in order to create graphs (represented by the y-value).

VALIDATION OF HPLC METHOD

The proposed HPLC method was subjected to a battery of tests in accordance with the guidelines established by the International Conference on Harmonization (ICH) [20-23] to validate its specificity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), stability of the standard solution, stability of the sample solution, and robustness.

Specificity

In order to evaluate the sensitivity of the approach, peak purity measurements were performed on Luliconazole and curcumin using the empower software from Waters and a diode array detector. The results were portrayed through three different types of visualizations: a purity angle, a purity threshold, and a purity flag.

Precision

Precision was looked at in terms of how well a system works, how well a method works, and how well a method works.

System precision

In order to evaluate the precision of the measurement, six separate injections from a single vial containing the same standard were administered. The relative standard deviation (RSD), the number of plates, and the level of detail were all reported as results. Tailing was expressed as a percentage of RSD.

Method precision

The sample was analyzed six times using the specified procedure. Each analytes assay findings were expressed as a percentage of the relative standard deviation.

Intermediate precision

On two distinct platforms, work of a level of accuracy that is somewhere in the middle was carried out. Both the PDA, model number 2996, and the UV detector, model number 2489, were included in the Waters e2695 Alliance system. On each system, six different extract samples were analyzed, and the results were given as a percent relative standard deviation (RSD).

Recovery studies

The precision of the procedure was evaluated with the use of research on data recovery. A sample that had already undergone analysis was supplemented with quantities of each standard at the levels of 80%, 100%, and 120% respectively. The sample underwent a second examination, this time utilizing the newly developed technique.

Robustness

Simply adjusting some of the settings allowed us to demonstrate how robust the method was. It was decided that the separation factors would consist of shifts in column chemistry, wavelength, temperature, flow rate, and mobile phase gradient. We determined how long Luliconazole and Curcumin persist in the body by using system suitability variables, and we analyzed the percentage of RSD.

HPLC chromatography was utilised in order to provide an accurate reading of the quantities of Luliconazole and curcumin that were contained within a Polyherbal tablet formulation. The computations were carried out on three separate occasions, and the findings are presented below in the form of means and standard deviations.

RESULTS AND DISCUSSION

Numerous solvent compositions with various polarities, column chemistries, column temperatures, and mobile phase pH were examined in order to determine the best mobile phase composition for the HPLC procedure. The symmetrical peaks created by the current procedure and the clear separation it allowed

between each standard peak and the others show that it delivered the most promising outcomes. These two characteristics demonstrate the efficacy of the method **(Figure 1).** In order to standardize the results, we scanned at 254 nm, the wavelength at which all of the analytes had their greatest response. After being removed, Luliconazole and curcumin remained in the body for about 17 minutes each. The correlation coefficients for Luliconazole and curcumin were determined to be good at 0.9990 (40-140 g/ml), as shown by the calibration graph in **[Table 2]**. **Figure 2** is available online and contains graphs for each norm.

Table 1: Details of Gradient programme

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**		**0.9995**	
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

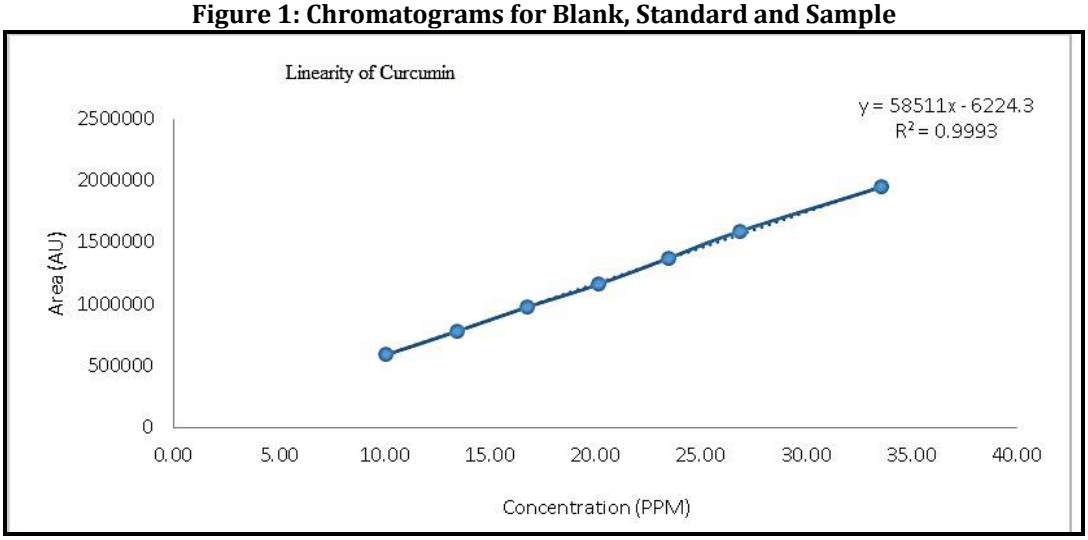
Table 5: Robustness for Luliconazole and Curcumin

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



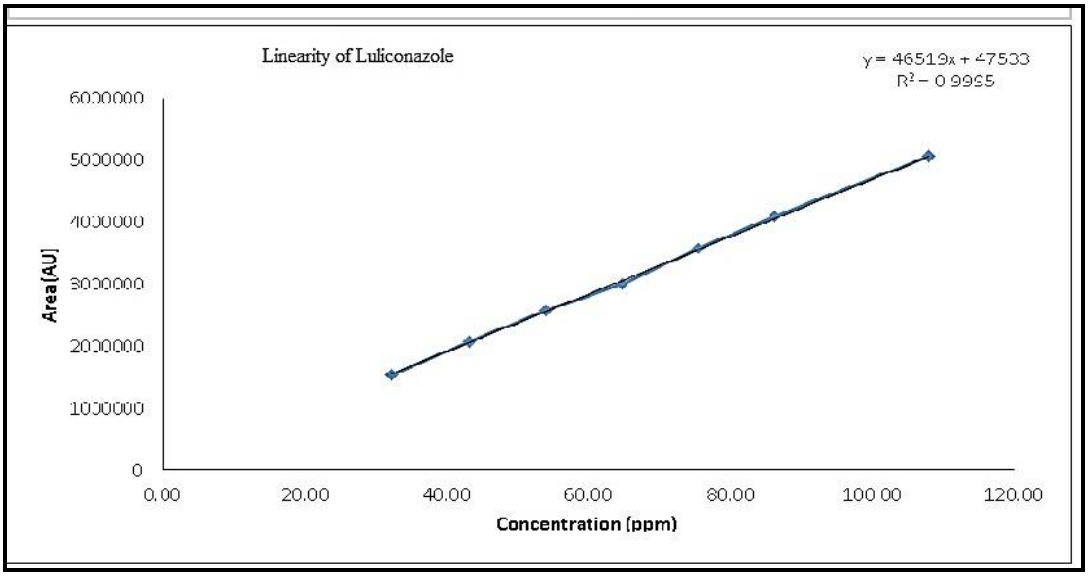


Figure 2: Linearity graphs for standard

For sample application and peak area scanning, the system accuracy, method precision, and intermediate precision data are all reported as a percentage relative standard deviation (RSD). Curcumin's RSD value was 0.56%, whereas the relative standard deviation (RSD) values for luconazole's and its system precision were 0.21 and 0.56%, respectively. The fact that the percent RSD for Luliconazole and curcumin were 0.30 and 0.34, respectively, when we looked at them shows how accurate our technique is. Luliconazole had a value of 0.89 percent, while curcumin had a value of 0.43 percent when the RSD values from the two experts were compared. These two numbers both fall inside the moderate precision level (Table 3). The methodology that was proposed delivers a level of system, method, and intermediate precision that is sufficient, as shown by the results of the % RSD. By analyzing the spectra of the standard spots at the start, peak, and end of the peak and contrasting them with the spectra of the extracts, the peak purity of each analyte was ascertained. For your convenience, Table 4 includes a purity angle image and purity threshold values.

The proposed approach was strengthened by the use of robustness. After computing the peak area of each analyte for each parameter, a relative standard deviation (RSD) of less than 2% was discovered. In terms of the percentage of RSD readings it generates, Table 5 shows how the approach has gotten more stable. The placebo solution was supplemented with 80%, 100%, and 120% of the working concentration of the active ingredient in order to conduct the recovery trial. The results showed that there was a 98.0% to 102.0% success rate when luconazole and curcumin were combined. [Table 6]

CONCLUSION

An RP-HPLC-UV-DAD technique was created and effectively validated within the parameters of this study for the analysis of Luliconazole and curcumin in terms of linearity, precision, accuracy, specificity, system applicability, and robustness. In addition to being novel, the technique that has been shown to be capable of identifying the quantities of two distinct compounds (Luliconazole and curcumin) at a single wavelength is also simple, sensitive, and accurate enough to meet the requirements set forth by the ICH. The usage of the other components of the tablet had no effect whatsoever on the precision of the dosage calculations for the two active chemicals. As a result, it is advised to apply the proposed analytical approach for the routine analysis of Luliconazole and curcumin in a range of dosage forms as well as alone. Even though many third-world nations and field stations lack access to highly advanced analytical equipment, they can nevertheless benefit from the technique.

AUTHORS' CONTRIBUTION

XYZ contribution included collecting samples, designing and performing laboratory work, analyzing the results, and preparing the paper. ABC contribution included data interpretation and identification of the compounds. All the authors have read the final manuscript and approved the submission.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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