ORIGINAL ARTICLE

Quantitative Assessment of Luliconazole and Gallic Acid Simultaneously in Formulated Emulgel by UV Spectrophotometric Methods

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Abstract

Introduction: Three new UV spectrophotometric approaches specifically, simultaneous equation (SME), absorbance ratio (ASR), and first derivative (zero crossing) spectroscopic techniques were developed and validated for simultaneous estimation of luliconazole (LCZ) and gallic acid (GLA) in emulgel formulation which were simple, sensitive, precise, and accurate. **Materials and Methods:** In SME method, absorbance was measured at 299 and 259 nm for both the drugs. LCZ and GLA were estimated at 299 and 266 nm in the ASR method. First derivative (zero crossing) method depended on the change of UV spectra in to first derivative spectra followed by measurement of first derivative signal at 249 and 259 nm for LCZ and GLA, respectively, using 4 nm as wavelength interval (Δλ) and 4 as scaling factor. Developed methods were validated according to ICH guidelines including parameters, namely, specificity, linearity, range, precision and accuracy, limit of detection, and limit of quantification. **Results and Discussion:** All three techniques showed direct relation of absorbance in the concentration range of 1-30 µg/ml for both the drugs. Good repeatability, low intra and inter-day variability, indicate that precise agreement within the value. Recovery studies for both drugs ranged from 97 to 102 percent, recommending that the methods are effective. Results of method validation parameters such as linearity and range, precision, and accuracy adhere to ICH guideline acceptable limit. **Conclusion:** All the developed methods were found to be quick, profoundly accurate and financially effective; and henceforth can be valuable for simultaneous estimation of LCZ and GLA in emulgel formulation for routine quality control analysis.

Key words: Absorbance ratio, antifungal, first derivative (zero crossing) spectroscopic methods, formulated emulgel, gallic acid, simultaneous equation

INTRODUCTION

Antifungal medications treat fungal infections by destroying or preventing hazardous fungi from growing in the body. Antibiotic resistance can occur, when bacteria and fungi gain the ability to resist medications targeted to kill them.[1,2] When fungus no longer respond to antifungal treatments, this is known as antifungal resistance. Antifungal resistance is becoming more common so responsibility in preventing fungal infections and minimizing antifungal resistance is of everyone. Natural products, whether as pure phytocompounds or as standardized plant extracts, provide virtually limitless possibilities for novel medication development due to their chemical variety. Novel techniques to utilize phytocompounds will be the future prospect for new drug development and improved antifungal therapy.[3,4]

Luliconazole (LCZ) is chemically described as {(2*E*)-2-[(4*R*)-4-(2, 4-dichlorophenyl) -1, 3-dithiolan-2-ylidene]-2-imidazol-1-ylacetonitrile works by inhibiting the enzyme lanosterol demethylase.[5] LCZ acts by inhibiting lanosterol

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demethylase which is needed for the synthesis of ergosterol that is a major component of the fungus cell membranes, which leads to alteration in the fungal cell membranes. The strong clinical antifungal activity of LCZ is possibly attributable to a combination of strong *in vitro* antifungal activity and favorable pharmacokinetic properties in the skin.[6]

Gallic Acid (GLA) is 3, 4, 5-trihydroxybenzoic acid a naturally occurring low molecular weight triphenolic compound having strong antioxidant activity. It showed various activities such as antioxidant, anti-inflammatory, neuroprotective, antitumor, anticancer, and antipyretic.[7,8] GLA has been suggested by several publications to have antifungal properties against *Candida albicans* planktonic cultures and biofilms. Moreover, it is reported that GLA also suppressed the growth of various clinical isolates of *Candida* spp., and it was postulated that the fungicidal effect was related to the reduction of ergosterol production, a key component of the fungal membrane.[9-11]

Based on literature survey it is hypothesis that the combination of LCZ and GLA in formulated emulgel can be effective in controlled management of inflammation and skin infections caused by *Staphylococcus aureus* and *C. albicans*. Chemical structures of both the drugs are shown in Figure 1. Literature survey reveals various analytical methods for the estimation of LCZ using UV spectrophotometry,[12,13] high performance liquid chromatography (HPLC),[14-17] and high performance thin layer chromatography (HPTLC).[18] Various method for the estimation of GLA using UV spectrophotometry,[19,20] HPLC,[21-23] and HPTLC.[24] However, the development of simultaneous estimation of LCZ and GLA in combined dosage form has not yet been reported by any method. Hence, this manuscript is the first to describe the development and validation of some simpler, sensitive, precise, accurate, and cost effective UV spectroscopic methods for the simultaneous determination of LCZ and GLA in combined emulgel formulation.

MATERIALS AND METHODS

Chemicals and reagents

LCZ reference standard used throughout the experiment was received as gift sample from Tirupati Medicare Ltd., Sirmour, Himachal Pradesh, India and GLA was obtained from Simson Pharma Ltd., Mumbai, Maharashtra, India. AR grade methanol was used as solvent and procured from Loba Chemie Pvt. Ltd., Mumbai, India.

Instruments

Shimadzu double beam UV visible spectrophotometer (UV-1800, UV Probe, Shimadzu Corporation, Kyoto, Japan) with matched quartz cell of 1 cm path length was used throughout the experiment. Highly sensitive electronic balance Adventurer Pro AVG264C, Ohaus Corporation, Pine Brook, NJ, USA was used for weighing purpose.

Preparation of standard solution

Stock solution of LCZ and GLA was prepared individually by weighing accurately 10 mg of standard drugs and transferred to a 10 ml volumetric flask separately. Standard drugs were diluted to 10 ml with methanol to get the concentration of the drugs 1000 µg/ml. Further dilutions were made to get required concentration with phosphate buffer saline (PBS).

Procedure

Simultaneous equation (SME) and absorbance ratio (ASR) method

Standard stock solutions containing 1000 µg/ml of LCZ and GLA were suitably diluted separately with PBS to obtain the drug solutions containing 10 µg/ml. Both the solutions were scanned in the UV region (200–400 nm) and spectra were recorded. Based on the spectral pattern, SME and ASR methods[25] were chosen for the estimation of both the drugs. From the overlain spectra [Figure 2], 299 nm (λ_{max} of LCZ)

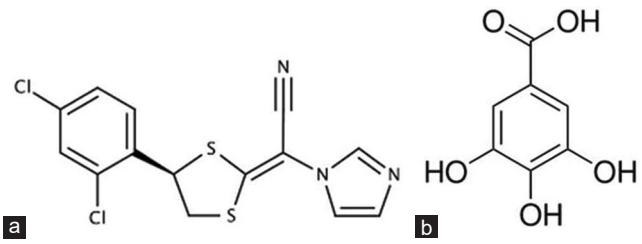

Figure 1: Chemical structures of (a) Luliconazole and (b) Gallic acid

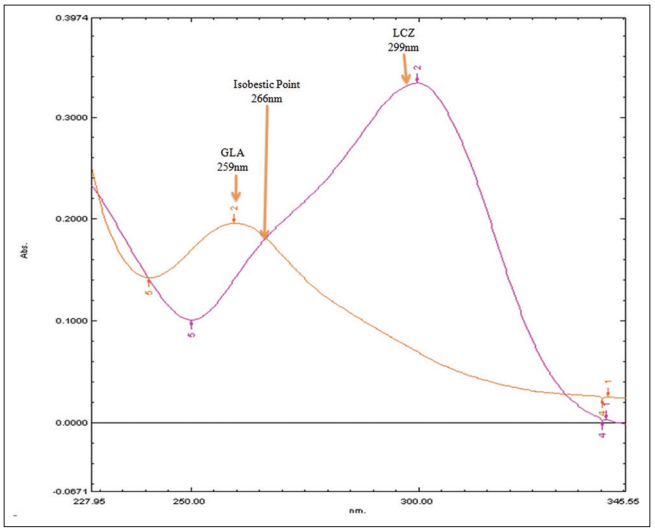

Figure 2: Overlain UV spectra of Luliconazole and Gallic acid standard solution (10 µg/ml)

and 259 nm (λ_{max} of GLA) were selected for SME method. In case of ASR method, 266 nm (isobestic point) and 299 nm (λ_{max} of LCZ) were selected, which showed excellent linearity and therefore used for simultaneous determination [Figure 2].

Varying concentrations ranging from 1 to 30 µg/ml of GLA and LCZ were prepared by diluting respective stock solutions. All the solutions were scanned in the UV region and absorbance's were noted at 259 and 299 nm for SME; 266 and 299 nm for ASR method [Figure 3]. Absorptivity values were calculated for GLA and LCZ at their relevant wavelengths by applying following formula:

Absorptivity = absorbance/concentration (g/100 ml)

Absorptivity value of individual solution at their respective wavelength was calculated and average absorptivity value [Table 1] at specific wavelength of particular drug was used for calculating concentration of drugs.

Zero crossing derivative (ZCD) method

The normal UV spectra of LCZ and GLA were transformed into first and second derivative spectra. Based on the spectral pattern and zero crossing points, first ZCD method was chosen for the study. First derivative spectra showed typical zero-crossing points at 249 nm for LCZ and 259nm for GLA applying 4 nm as wavelength interval (Δλ) and 4 as scaling factor. After assessing overlain spectra, 259 nm and 249 nm were selected for further studies [Figure 4].

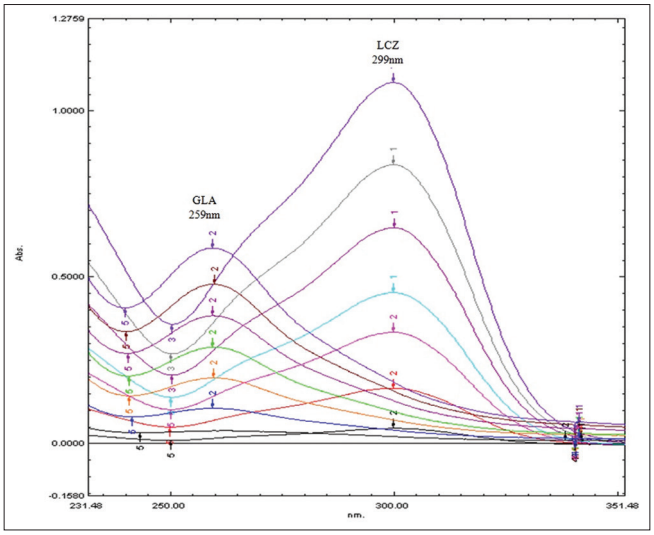

Figure 3: Overlain UV spectra of Luliconazole and Gallic acid (1–30 µg/ml) for simultaneous equation and absorbance ratio methods

Analysis of sample solution

1 g of emulgel equivalent to, 1 mg of LCZ, and 1 mg of GLA were weighed accurately and transferred to 25 ml volumetric flask containing 15 ml methanol. The volumetric flask was heated in water bath at 60°C for 5 min and then the solution was centrifuged for 15 min at 800 rpm and volume was made up with methanol. The supernated solution of 5ml was diluted to 10 ml in volumetric flask with phosphate buffer saline to obtained the concentration of 20 µg/ml of LCZ and GLA of formulated emulgel solution. Using the developed SME, absorption correction and ZCD methods the concentrations of LCZ and GLA present in formulated gel were calculated.

SME method

After scanning the sample solution (formulation) between 200 and 400 nm, responses were noted at 299 and 259 nm. The unknown concentration of drugs present in the sample solution was estimated by solving following formula

$$Cx = \frac{A_2 ay_1 - A_1 ay_2}{ax_2\, ay_1 - ax_1\, ay_2}$$

$$Cy = \frac{A_2 ax_2 - A_2 ax_1}{ax_2\, ay_1 - ax_1\, ay_2}$$

Where Cx and Cy are the concentrations of LCZ and GLA, ax_1 and ax_2 are absorptivities of LCZ at 299 nm and 259 nm, respectively. ay_1 and ay_2 are absorptivities of GLA at 299 nm and 259 nm, respectively. A_1 and A_2 are the absorbance's of sample solution at 299 nm and 259 nm.

ASR method

The unknown concentration of drugs in the sample solution was estimated by ASR method applying following formula:

Table 1: Average absorptivity values for SME and ASR method

SME				ASR			
Avg. absorptivity*				Avg. absorptivity*			
LCZ		GLA		LCZ		GLA	
259 nm	299 nm	259 nm	299 nm	266 nm	299 nm	266 nm	299 nm
140	347	221	88	183	347	208.7	88

*(*n*=6) Average of six determinations

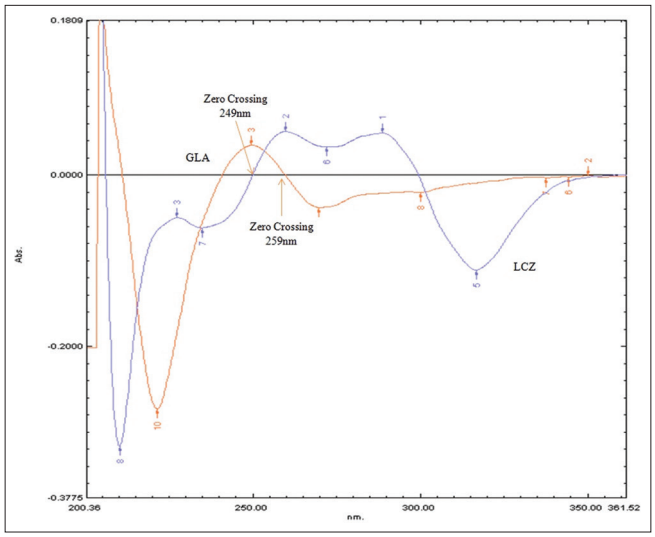

Figure 4: Overlain 1st derivative (zero crossing) UV spectra of Luliconazole and Gallic acid standard solution (10 µg/ml)

$$Cx = \frac{Qm - Qy}{Qx - Qy} \times \frac{A_1}{a_{x1}} \quad Cy = \frac{Qm - Qx}{Qy - Qx} \times \frac{A_1}{a_{y1}}$$

Where, ax_1 and ax_2 are absorptivities of LCZ at 299 and 266 nm, respectively. ay_1 and ay_2 are absorptivities of at 299 and 269 nm. $QM = A_2/A_1$, $Qx = ax_2/ax_1$, $Qy = ay_2/ay_1$.

A_1 and A_2 are the absorbance of sample solution at 299 and 266 nm. Cx and Cy are the concentrations of LCZ and GLA, respectively, in sample solution.

ZCD method

Sample solution was scanned in the UV region (200–400 nm) and spectrum was recorded and transformed into their 1st derivative spectra and amplitude was measured at 259 and 249nm. The unknown concentration of drugs present in the sample solution was estimated using regression equation.

Validation of spectroscopic methods[26]

The developed methods were validated in accordance with "International Conference on Harmonization" guidelines (ICH, 2005).

Specificity

Interaction between emulgel excipients used in the formulation and drug substance were check for specificity parameter. All the emulgel excipients were mixed in proportion and diluted using methanol and filtered using Whatman filter paper no 41. All the solutions (Placebo, standard, and formulation) were scanned in the UV region and compared to assess the interference among excipients and drugs.

Linearity and range

Linearity and range of all the three methods were checked by analyzing all the standard solutions separately containing LCZ and GLA (1,5,10,15,20,25 and 30 µg/ml) using PBS as solvent and absorbance's were noted at 299 and 259 nm for SME method; 299 and 266 nm for ASR method; 259 and 249 nm for 1st ZCD method.

Precision

Precision of the methods was evaluated by performing repeatability, intra-day and inter-day studies of standard solutions (LCZ and GLA: 15 and 15 µg/ml) 6 times, three different concentration within linearity range (LCZ and GLA: 5, 15, 25 and 5, 15, 25 µg/ml) 3 times on same day and 3 times on different day, respectively. The absorbance's were measure of both the drugs solution at 299 and 259 nm in SME method; 299 and 266 nm for ASR method; 259 and 249 nm for ZCD method, respectively, and % RSD was calculated.

Accuracy

To ensure the suitability and reliability of the projected methods, recovery studies were performed by standard addition method. To an equivalent quantity of pre-analyzed sample solution (LCZ and GLA: 1:1 %w/w), a known concentration of standard LCZ and GLA was added at 50, 100, and 150% level and the resulting solutions were reanalyzed by projected methods and % recoveries were calculated by applying following formula:

%Recovery = (Amount of drug found after addition of standard drug - Amount of drug found before addition of standard drug)/(Amount of standard drug added) × 100.

Limit of detection (LOD) and limit of quantification (LOQ)

Sensitivity of the proposed methods was determined in terms of LOD and LOQ. The LOD and LOQ of LCZ and GLA were calculated applying following equation as per ICH guidelines.

$$LOD = 3.3 \times \frac{\sigma}{S} \quad LOQ = 10 \times \frac{\sigma}{S}$$

Where σ = The standard deviation of the response,

S = The slope of the calibration curve.

RESULTS AND DISCUSSION

Three UV spectroscopic methods, namely, SME, ASR, and ZCD spectroscopic methods were developed and validated for simultaneous estimation of LCZ and GLA in emulgel formulation. In SME method, absorbance was measured at 299 and 259 nm and in ASR method 299 and 266 nm was used for the detection and quantification of LCZ and GLA. ZCD method was based on the transformation of UV-spectra in to first derivative spectra and followed by measurement of first derivative signal at 259 and 249 nm for LCZ and GLA, respectively, using 4 nm as wavelength interval (Δλ) and 4 as scaling factor. Comparative overlain spectra of placebo, drug solutions, and emulgel solution showed that there was no hindrance between them [Figure 5]. Linear relation was established for LCZ and GLA in the concentration range of 1–30 µg/ml for all the methods. Overlain spectra of LCZ and GLA are shown in Figures 3 and 6. Calibration graphs were plotted using absorbance of standard drug solution versus concentration for SME and ASR method. 1st derivative signal of standard drug solution versus concentration was used to plot calibration curve for ZCD method. Regression analysis was performed by applying least square method for calculating values of slope, intercept, and correlation coefficient for LCZ and GLA at their relative wavelengths are mention in Table 2.

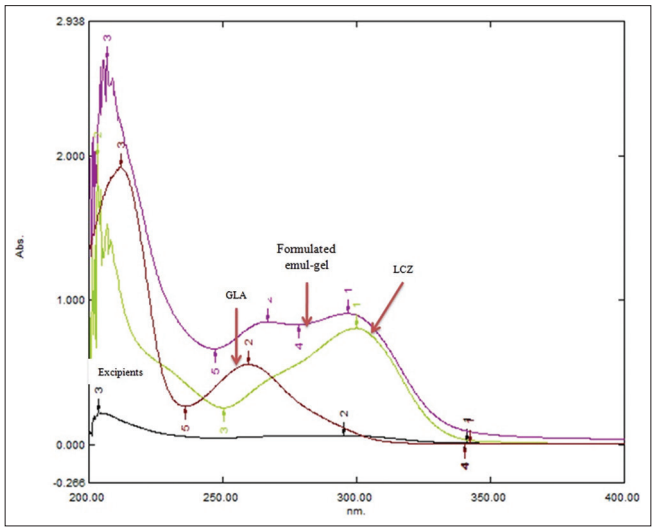

Figure 5: Overlain UV spectra of excipient solution of emulgel, standard solution of Luliconazole, Gallic acid and emulgel formulation (20 µg/ml)

Outcome of precision studies was evaluated in terms of % RSD, follows ICH guideline acceptable limits (<2), which shows good repeatability, low intra and inter-day variability, indicating an excellent precision of the developed methods [Table 2]. The outcome of recovery studies ranged from 97% to 102% for both the drug suggests suitability of the proposed methods [Table 3]. Percentage recovery indicates that there was no interference from emulgel excipients. Moreover, low LOD and LOQ values prove the sensitivity of the proposed methods [Table 2]. The projected methods were successfully applied for the quantitative determination of LCZ and GLA in emulgel formulation. Sample solutions were analyzed 6 times and experimental values were found to be within 96 and 100% [Table 4] for both the drugs and hence the developed methods can be used for the simultaneous determination of both the drugs in combined emulgel formulation. For statistical significance evaluation, the significance level was established at $P < 0.05$, and all three procedures were assessed using one-way ANOVA followed by Bonferroni all tests. The assay findings showed that there was no significant difference between all of the developed procedures as shown in Table 5.

Table 2: Summary of linear regression and method validation data for the proposed methods

Parameters	SME				ASR				ZCD	
	LCZ		GLA		LCZ		GLA		LCZ	GLA
Wavelengths (nm)	259	299	259	299	266	299	266	299	259	249
Linearity range (µg/ml)	1–30									
Correlation coefficient	0.9991	0.9991	0.9995	0.9992	0.9998	0.9991	0.9994	0.9992	0.9993	0.9995
Slope	0.0145	0.033	0.0188	0.0059	0.0182	0.039	0.0172	0.0076	0.0026	0.0164
Intercept	0.0054	0.0013	0.0109	0.0125	0.0011	0.0018	0.0137	0.0129	0.0012	0.0096
LOD (µg/ml)	0.03	0.006	0.02	0.06	0.02	0.008	0.02	0.08	0.005	0.02
LOQ (µg/ml)	0.11	0.02	0.05	0.17	0.05	0.03	0.06	0.19	0.02	0.05
Specificity	No interferences									
Precision (% RSD)*										
Repeatability of measurement (n=6)*	0.51	0.94	0.92	0.55	0.72	0.94	0.45	0.80	1.01	1.09
Intra-day**	0.67	0.72	0.72	0.65	0.83	0.74	0.78	0.46	0.86	0.65
Inter-day **	0.88	0.94	0.46	0.93	0.61	1.06	0.67	0.69	0.72	0.86

*n=6; **n=3 number of determinations, % RSD: Percentage relative standard deviation, ZCD: Zero crossing derivative

Table 3: Recovery data of the proposed methods

Drugs	Level (%)	Recovery (%)*			RSD (%)		
		SME	ASR	ZCD	SME	ASR	ZCD
LCZ	50	98.83±0.69	99.04±0.29	99.76±0.36	0.70	0.29	0.36
	100	99.40±0.65	99.69±0.31	99.03±0.72	0.66	0.31	0.72
	150	99.22±0.45	99.81±0.39	99.47±0.58	0.45	0.39	0.58
GLA	50	99.22±0.76	98.93±0.63	100.2±0.62	0.76	0.63	0.62
	100	99.68±0.78	99.26±0.70	100.36±0.26	0.78	0.70	0.26
	150	99.33±0.53	99.53±0.62	99.47±0.96	0.54	0.62	0.96

*Mean±SD (*n*=3), SD: Standard deviation, % RSD: Percentage relative standard deviation, LCZ: Luliconazole, GLA: Gallic acid, SME: Simultaneous equation, ASR: Absorbance ratio, ZCD: Zero crossing derivative

Table 4: Results of formulation analysis using different methods

Method	Drug	Labeled amount (w/w %)	Found amount (w/w %)*	% Drug found*	%RSD*
SME	LCZ	1	1.001±0.016	100.013±1.552	1.552
	GLA	1	0.985±0.015	98.501±1.539	1.563
ASR	LCZ	1	0.996±0.017	99.600±1.706	1.713
	GLA	1	0.966±0.015	96.633±1.474	1.526
ZCD	LCZ	1	1.007±0.019	100.667±1.90	1.896
	GLA	1	0.999±0.017	99.933±1.704	1.705

*Mean±SD, *n*=6, % RSD: Percentage relative standard deviation, LCZ: Luliconazole, GLA: Gallic acid, SME: Simultaneous equation, ASR: Absorbance ratio, ZCD: Zero crossing derivative

Table 5: Results of statistical comparison using one-way ANOVA and Bonferroni multiple comparison tests for SME, ASR, and ZCD spectroscopic methods

Drugs	Simultaneous Equation Method	Absorbance Ratio Method	First Derivative Method
LCZ	100.00±1.552	99.600±1.706	100.667±1.90
GLA	98.50±1.539	96.633±1.474	99.933±1.704

All values are expressed in Mean±SD (*n*=6), LCZ: Luliconazole, GLA: Gallic acid, SME: Simultaneous equation, ASR: Absorbance ratio, ZCD: Zero crossing derivative

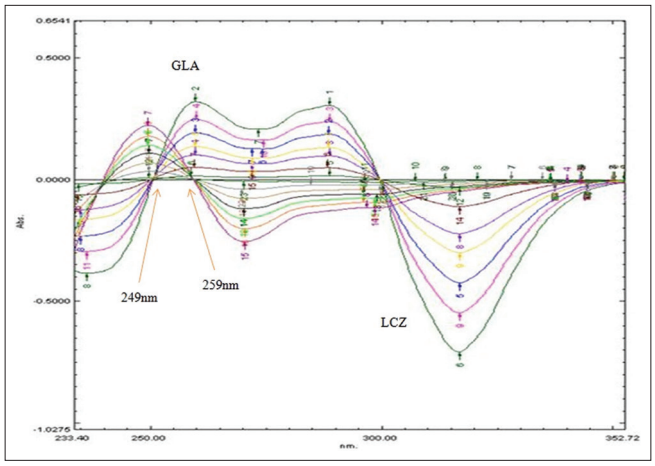

Figure 6: Overlain 1st derivative (zero crossing) UV spectra of Luliconazole and Gallic acid (1–30 µg/ml) for zero crossing derivative method

CONCULSION

UV spectroscopy analysis method, an cost effectiveness, less time consuming method has developed for quantify LCZ and GLA phytoconstituent present in formulated emulgel. Furthermore, the obtained data of validation parameter were within the acceptable range of linearity, precision, and reproducibility for the simultaneous estimation of LCZ and GLA. This proposed method can be applied for simultaneous estimation of cited drug in formulation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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