

Simultaneous Estimation of Luliconazole and Methyl Paraben in Pharmaceutical Formulation by UV Spectroscopy using Absorption Ratio Method

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Abstract

For the simultaneous quantification of luliconazole (LUZU) and methyl paraben (MP) in formulation, a straightforward, precise UV spectrophotometric approach was developed and validated. The method was developed using absorbance ratio method. For luliconazole and methyl paraben, the absorbance was measured at 297 nm and 274 nm, respectively. The method was discovered to be linear, sensitive, exact and accurate. ICH guidelines were followed in developing the procedure.

Keywords: *Q- absorbance ratio method, methyl paraben, luliconazole, and UV spectrophotometric method.*

Introduction:

Spectrophotometry is the most widely employed techniques for quantitative analysis of drug molecules. It involves measuring the amount of ultraviolet radiation absorbed by a substance in solution.

Any molecule has either n, or combination of these electrons. These bonding (0) and non-bonding (5) electrons absorb the characteristics radiation and undergoes transition from ground state to excited state.

When a molecule absorbs ultraviolet radiation of frequency, the electron in that molecule undergoes transition from a lower to higher energy level, the energy difference is given by

$$\Delta E = h\nu$$

The amount of energy required depends on the difference in energy between ground state E_0 and excited states E_1 of electrons.

$$E_1 - E_0 = h\nu$$

The two fundamental laws governing the friction of incident radiation absorbed by matter are

- Beer's law
- Lambert's law

Q-Absorbance ratio method

The absorbance ratio method is a modification of the simultaneous equations procedure. In the quantitative assay of two components in admixture by the absorbance ratio method, absorbance's are measured at two wavelengths, one being the λ -max of one of the components (λ_2) and other being a wavelength of equal absorptivity of two components (λ_1), i.e. an isoabsorptive point.

For a substance that obeys beer's law at all wavelength, the ratio of absorbance at any two wavelengths is a constant which is independent of concentration or path length

For example, two different dilution of the same substance give the same absorbance ratio

This ratio is Q-value i.e $Q=A_1/A_2$

In the USP, this ratio is referred to as Q value

$$C_x = (Q_m - Q_x / Q_x - Q_y) \times A_1 / a_{x1}$$

$$C_y = (Q_m - Q_y / Q_y - Q_x) \times A_1 / a_{y1}$$

Where, A_1 and A_2 are absorbance of mixture at λ_1 and λ_2

A_{x1} and a_{x2} are absorptivities of X drug at λ_1 and λ_2

A_{y1} and a_{y2} are absorptivities of sample at λ_1 and λ_2

$$Q_m = A_2 / A_1$$

$$Q_x = a_{x2} / a_{x1} \text{ and } Q_y = a_{y2} / a_{y1}$$

An imidazole antifungal is licconazole (LUZU). It is advised for the treatment of dermatophytes includes *Trichophyton rubrum*, *Microsporum gypseum*, and *Epidermophyton floccosum* that cause ringworm, jock itch, and athlete's foot. Luliconazole is effective against *Tinea Candida*, *Trichophyton*, *Aspergillum*, and *Epidermophyton*. By blocking the action of the enzyme lanosterol demethylase, azole prevents the production of ergosterol, which is a component of fungal cell membranes, and causes a deposition of lanosterol in its place.

A common anti-fungal agent included in many cosmetic and personal care products is methylparaben (MP). To stop the growth of mould and other hazardous microorganisms, it is added to cosmetics. Additionally, it serves as a food preservative.

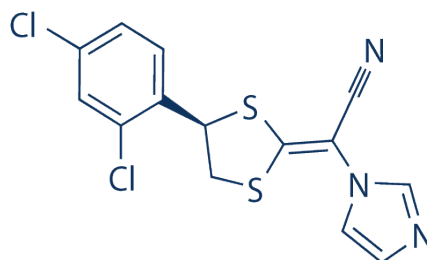


Figure 1. Structure of Luliconazole

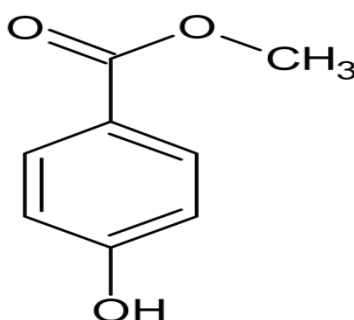


Figure 2. Structure of Methyl Paraben

Literature review reveals that there are few analytical techniques are available for estimation of LUZU and MP alone as well as in combine dosage form such as UV, RP-HPLC and Spectrophotometric. Keeping this objective in mind an attempt has been made to develop and validate a method for simultaneous estimation of luliconazole and methyl paraben in pharmaceutical formulation by UV spectroscopy using absorption ratio method which was not developed for the combination earlier. However, no references have been found for simultaneous estimation of luliconazole and methyl paraben in pharmaceutical formulation by UV spectroscopy using absorption ratio method. Both these methods are simple, accurate and precise for estimation of luliconazole and methyl paraben in cream and do not require any complicated sample treatment like heating or organic solvent extraction and costly instrument like HPLC.

Materials and Methods

Instruments:

Instrument used was an UV-Visible Spectrophotometer, make: Shimadzu, Model UV-1800 with a pair of 1cm matched quartz cells and 1cm path length. All weighing was done on analytical balance (Contech Instruments ltd). A sonicator (PCI Analytics 6.5 li200H) was used in the study. Calibrated glass wares were used throughout the work.

Chemicals and Reagents:

LUZU: Hetero labs Limited, Hyderabad

M.P: Hetero labs Limited, Hyderabad

Formulation of LUZU and MP: Sun Pharma industries Limited

Selection of Solvent: Methanol was selected as solvent for studying spectral character

Preparation of Standard Stock Solution**Preparation of Standard Stock Solution of Luliconazole:**

The stock solutions of LUZU were prepared by dissolving 10mg of drug in 10 ml volumetric flask and add 10 ml of methanol and volume was made up to the mark with solvent (1000 μ g/ml). From the above solution 1ml was transfer to 10ml volumetric flask and volume was made to the mark with the solvent (100 μ g/ml).From the above solution 1ml was transfer to 10ml volumetric flask and volume was made to the mark with the solvent (10 μ g/ml).

Preparation of Working Standard Solution of Luliconazole

The stock standard solutions (10 μ g/ml) of LUZU were further diluted to obtain final concentration 2, 4, 6, 8, 10 μ g/ml respectively.

Preparation of Standard Stock Solution of Methyl Paraben

The stock solutions of MP were prepared by dissolving 10mg of drug in 10 ml volumetric flask and add 10 ml of methanol and volume was made up to the mark with solvent (1000 μ g/ml). From the above solution 1ml was transfer to 10ml volumetric flask and volume was made to the mark with the solvent (100 μ g/ml).From the above solution 1ml was transfer to 10ml volumetric flask and volume was made to the mark with the solvent (10 μ g/ml).

Preparation of Working Standard Solution of Methyl Paraben

The stock standard solutions (10 μ g/ml) of MP were further diluted to obtain final concentration 2, 4, 6, 8, 10 μ g/ml respectively.

Selection of Analytical Wavelength

To determine wavelength for measurements, standard spectra of LUZU and MP were scanned between 200-400nm against methanol.

The absorbance maxima were obtained at 297nm and 256nm for LUZU and MP respectively and Iso-absorptive point were obtained at 274nm.

Preparation of Calibration Curve

Calibration Curve for Luliconazole

Calibration curve for LUZU consist of different concentrations of standard LUZU solution ranging from 2-10 μ g/ml. The solutions were prepared by pipetting out 0.2, 0.4, 0.6, 0.8, 1.0ml volumetric flask and the volume was adjusted to mark with methanol.

The absorbance of solutions was measured at 297nm and 274nm against methanol as a blank

Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity.

Calibration Curve for Methyl Paraben

Calibration curve for MP consist of different concentrations of standard MP solution ranging from 2-10 μ g/ml. The solutions were prepared by pipetting out 0.2, 0.4, 0.6, 0.8, 1.0ml volumetric flask and the volume was adjusted to mark with methanol.

The absorbance of solutions was measured at 297nm and 274nm against methanol as a blank

Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity.

Preparation of Sample Solution

10gm of cream (LULIFORT, containing 1mg of luliconazole and 0.01% of methyl paraben each) were weighed and transferred into 100ml beaker of which contain methanol and this mixture was heated on water bath till the cream base gets melted. And finally volume was made up to the mark with the solvent.

1ml of sample stock solution (1000 μ g/ml) was transferred into the 10ml volumetric flask and the volume was made up to the mark with respective solvent i.e., methanol to get the desired concentrations of 100 μ g/ml.

Validation

Linearity and Range

The linearity of analytical method is the ability to elicit test results that are directly proportional to the concentration of analyte in the sample within the range. The linearity response was determined by analyzing 5 independent levels of concentration curve in the range of 2-10 μ g/ml for LUZU and MP respectively. The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient and regression line equations for LUZU and MP calculated.

Precision

Repeatability

Repeatability of an analytical method was performed by analyzing the six replicates of single concentration of 6µg/ml of mixed standard solution. The absorbance of samples were measured at 274nm and the % Relative Standard deviation (RSD) was calculated

Intra- Day Precision

The intraday precision of the analytical method was determined for both the drugs at different concentration levels (4.8µg/ml, 6µg/ml, and 7.2µg/ml) by analyzing the three replicates of each sample three times on the same day at different time intervals at 296nm and 274nm. The %RSD was calculated.

Inter- Day Precision

The inter day of the method was performed by analyzing three replicates of three different concentration samples (4.8µg/ml, 6µg/ml, and 7.2µg/ml) for three consecutive days at 297nm and 274nm. The %RSD was calculated.

Limit of Detection (LOD)

The LOD is estimated from the set of 5 calibration curves used to determined method linearity.

The LOD may be calculated as,

$$\text{LOD} = 3.3\sigma / S$$

Where σ = the standard deviation of y intercept of the calibration curve

S = the slope of the calibration curve

Limit of Quantification (LOQ)

The LOQ is estimated from the set of 5 calibration curves used to determined method linearity.

The LOQ may be calculated as,

$$\text{LOQ} = 10\sigma / S$$

Where σ = the standard deviation of y intercept of the calibration curve

S = the slope of the calibration curve

Accuracy

Accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

For Drug Substance

Accuracy for drug substance was determined on samples of drug solutions at varying concentration levels in the range of 80-120% by analyzing the three replicates of each sample as a batch in a single assay. The %RSD was calculated

For Drug Product (Recovery Studies)

Accuracy for drug product was determined on samples to which known amount of (4µg/ml) standard drugs are added to different concentration levels in the range of 80-120%. The % recovery was calculated.

Q –Value Method

In Q - value method, Q – values of LULICONAZOLE and METHYL PARABEN were calculated by taking ratio of absorbance values at 297nm and 274nm. The Q- values are substituted in the standard equations used in the Q- value method for calculation of individual concentrations of LULICONAZOLE and METHYL

$$C_x = \frac{Q_m - Q_x}{Q_x - Q_y} \times \frac{A_1}{AX_1}$$

$$C_y = \frac{Q_m - Q_x}{Q_x - Q_y} \times \frac{A_1}{AX_1}$$

Where,

$$Q_m = A_2/A_1, Q_x = AX_2/AX_1 \text{ and } Q_y = AY_2/AY_1$$

C_x and C_y are concentrations of LULICONAZOLE and METHYL PARABEN

A_2 and A_1 are sample absorbance at 297nm and 274nm (Isobestic point) respectively

AX_2 and AY_1 are absorbance of luliconazole at 297nm and 274nm

AY_2 and AY_1 are absorbance of methyl paraben e at 297nm and 274nm

Q_x and Q_y are Q-values of LULICONAZOLE and METHYL PARABEN respectively.

Values obtained: $AX_2 = 6.06$, $AX_1 = 3.93$, $AY_1 = 4.24$, $AY_2 = 0.774$.

$Q_x = 1.54$, $Q_y = 0.18$

Results and Discussion

A reliable Q-absorption ratio method was developed for simultaneous estimation of Luliconazole and Methyl paraben in pharmaceutical formulation by UV spectroscopy using absorption ratio method. Beer's law is obeyed in the concentration range of 2-10 μ g/ml for both the compounds with r^2 0.999. The limit of detection was found to be 0.17 μ g/ml and 0.154 μ g/ml for luliconazole and 0.118 μ g/ml and 0.18 μ g/ml for methyl paraben. The limit of quantification was found to be 0.5 μ g/ml and 0.46 μ g/ml for luliconazole and 0.35 μ g/ml and 0.54 μ g/ml for methyl paraben. luliconazole and methyl paraben has showed 95-99.5% recovery. Isobestic point was found at 274nm The proposed method was also evaluated by the assay of synthetic mixture containing luliconazole and methyl paraben. The % assay was found to be 100.6% for luliconazole and 100.08% for methyl paraben.

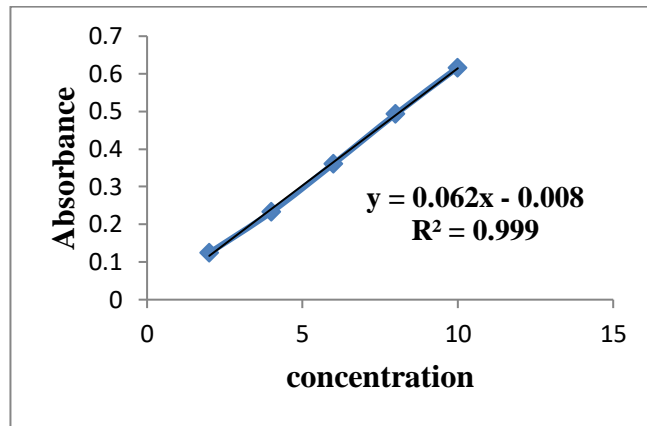


Figure 3. Calibration Curve of Luliconazole at 297nm

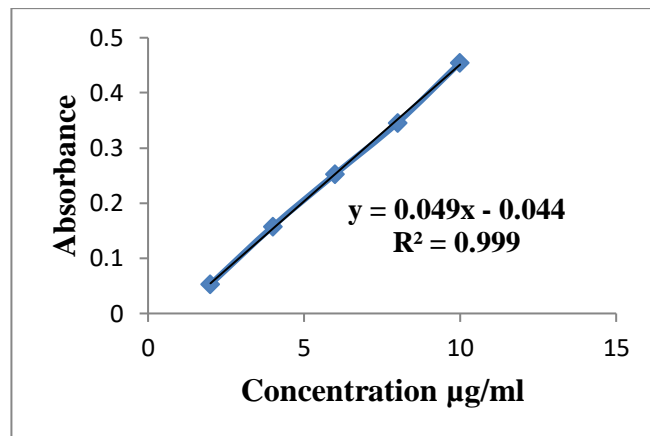


Figure 4. Calibration Curve of Luliconazole at 274nm

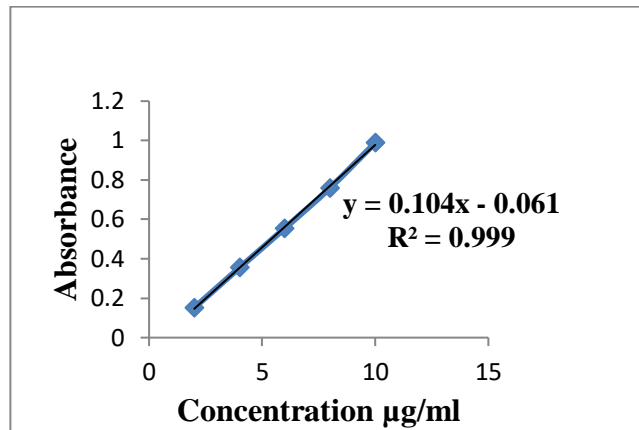


Figure 5. Calibration Curve of Methyl Paraben at 297nm

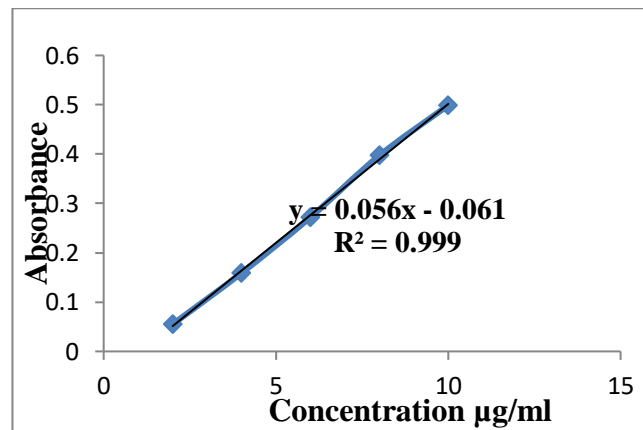


Figure 6. Calibration Curve of Methyl Paraben at 274nm

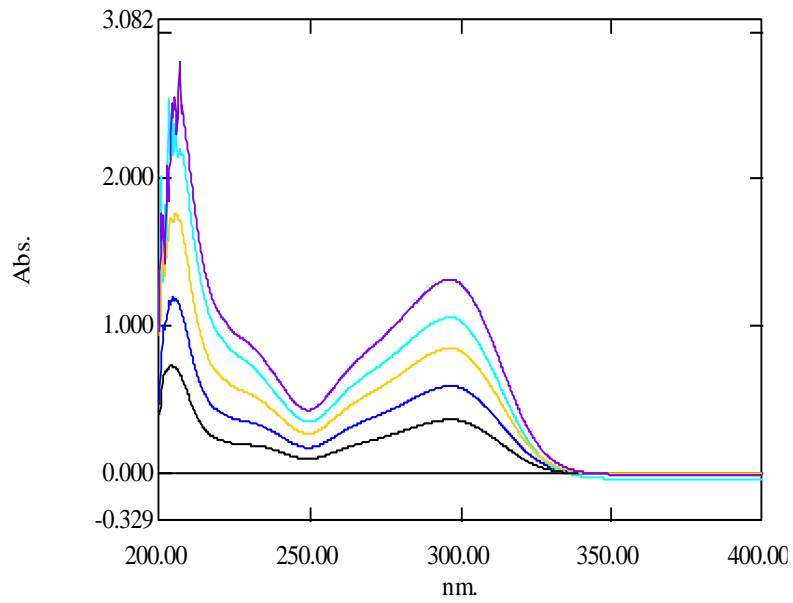


Figure 7. Overlay Absorption Spectrum of Luliconazole

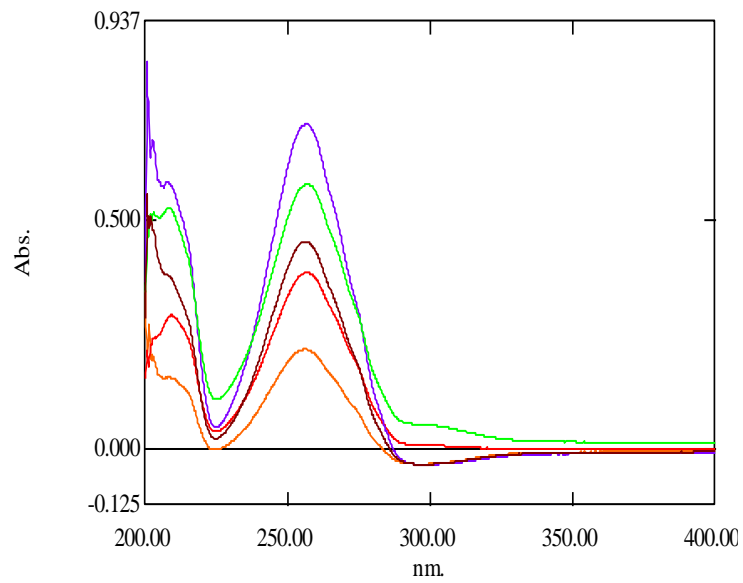


Figure 8. Overlay Absorption Spectrum of Methyl Paraben

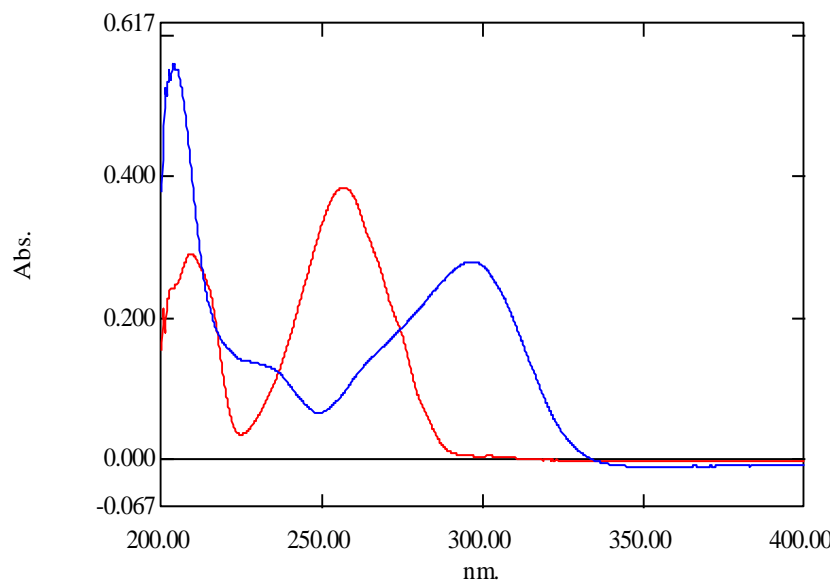


Figure 9. Overlay Spectrum of Luliconazole and Methyl Paraben

Table 1. Recovery Study (Accuracy) for Drug Substance

Formulation	Level	Concentration (µg/mL)	Mean ± Standard Deviation	%RSD
LULIFORT	80	4.8	0.219±0.0004	0.18
	100	6	0.233±0.00094	0.38
	120	7.2	0.253±0.0017	0.66

Table 2. Recovery Study (Accuracy) for Drug Product Luliconazole

Drug	Level	Amount Of Sample Added (µg/mL)	Concentration Of Standard Solution (µg/mL)	Concentration Found (µg/mL)	%Recovery
LUZU	80	4.8	6	9.96	99.6
	100	6	6	11.94	99.5
	120	7.2	6	12.18	92.3

Table 3. Recovery Study (Accuracy) for Drug Product Methyl Paraben

Drug	Level	Amount Of Sample Added ($\mu\text{g/mL}$)	Concentration Of Standard Solution ($\mu\text{g/mL}$)	Concentration Found ($\mu\text{g/mL}$)	%Recovery
MP	80	4.8	6	9.79	97.9
	100	6	6	95.5	95.5
	120	7.2	6	12.5	95.2

Table 4. Optical Regression Characteristics and Validation Parameters

Parameters	Luliconazole		Methyl Paraben	
	Wavelength for Quantification	297	274	297
Concentration Range ($\mu\text{g/ML}$)	2-10	2-10	2-10	2-10
Regression Equation (Y)	$Y=0.062x+0.008$	$Y=0.049x+0.044$	$Y=0.104x-0.061$	$Y=0.056x-0.061$
Slope (M)	0.062	0.049	0.104	0.056
Intercept (C)	0.008	0.044	0.061	0.061
Correlation Coefficient (R^2)	0.999	0.999	0.999	0.999
LOD ($\mu\text{g/mL}$)	0.17	0.154	0.118	0.18
LOQ ($\mu\text{g/mL}$)	0.5	0.46	0.35	0.54

Conclusion

The proposed spectroscopic method was found to be simple, sensitive, accurate and precise for determination of LUZU and MP in combined dosage form. The limit of detection was found to be 0.17 μ g/ml and 0.154 μ g/ml for luliconazole and 0.118 μ g/ml and 0.18 μ g/ml for methyl paraben. The limit of quantification was found to be 0.5 μ g/ml and 0.46 μ g/ml for luliconazole and 0.35 μ g/ml and 0.54 μ g/ml for methyl paraben. Luliconazole and methyl paraben has showed 95-99.5% recovery. Luliconazole and methyl paraben has showed 95-99.5% recovery. The method utilizes easily available and cheap solvent for analysis of LUZU and MP. Hence, the method is economic for estimation of LUZU and MP in combined dosage form. The common excipients and additives are usually present in the combined dosage form do not interfere in the analysis of LUZU and MP in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in mixture or combined pharmaceutical formulation.

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