“Robust and Rapid” UV Spectroscopic Method for Estimation of Luliconazole and Clobetasol Propionate Drug Combination

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Authors’ contributions

This work was carried out in collaboration between both authors. Author BS designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author HJ managed the analyses of the study, guided the entire research work. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i45A32748
Editor(s):
(1) Dr. Sawadogo Wamtinga Richard, Ministry of Higher Education, Scientific Research and Innovation, Burkina Faso.
(2) Dr. Farzaneh Mohamadpour, University of Sistan and Baluchestan, Iran.
Reviewers:
(1) Nohad Alomari, Knowledge University, Iraq.
(2) Jyothi Penta, HITC College of Pharmacy, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74511

Received 15 July 2021
Accepted 25 September 2021
Published 01 October 2021

ABSTRACT

Objective: The new combination for Luliconazole and Clobetasol Propionate was approved for the treatment of variety of skin disease. The main objective of this research is development and validation of novel, simple, fast and responsive derivative spectroscopic process for simultaneous estimation of newly approved combination Luliconazole (LLZ) and Clobetasol Propionate (CLP).

Methodology: Here in this first derivative spectroscopic method, the absorbance of LLZ and CLP was taken at 312nm (ZCP of CLP) and 249nm (ZCP of LLZ) respectively. Establishment of linearity was in a concentration varies from 10-50 µg/ml for Luliconazole and 5-25µg/ml for Clobetasol Propionate.

Results: From the method developed above the R² value observed for LLZ and CLP is 0.9988 and 0.9961. Statistical validation of accuracy and reproducibility was done for planned procedure with the help of recovery studies. The mean % recovery of Luliconazole and Clobetasol Propionate was

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found to be 99.45 % and 99.43% respectively. For LLZ the Limit of detection is 0.0054 µg/ml and limit of quantification is 0.0164 µg/ml and for CLP the Limit of detection is 0.0009µg/ml and limit of quantification 0.0027µg/ml.

Conclusion: From research work the method development was done and shows fast, precise, exact and easily accessible laboratory procedure for routine evaluation of combination drugs.

**Keywords:** Clobetasol propionate; glucocorticosteroids; luliconazole; UV spectroscopic method.

### 1. INTRODUCTION

Luliconazole is highly potent anti-inflammatory drug frequently prescribed in the treatment of rheumatic and inflammatory condition. Luliconazole is chemically2E)-2-[(4R)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-2-imidazol-1-ylacetonitrile, (Fig. 1) an imidazole antifungal agent, in which imidazole moiety is involved into the ketene dithioacetate structure. [1] It works against fungal infection like tinea pedis, tinea curies, and tinea corporis by slowing the growth of fungi. Luliconazole showed more prominent power opposes to Trichophytonrubrum, Trichophytonmentagrophytes, Trichophytononsurans than the available standard drugs like Terbinafine, clotrimazole. Luliconazole is a white powder, poorly water-soluble drug having molecular weight 354.267 g/mol [2].

![Fig. 1. Chemical structure of Luliconazole](image)

Clobetasol propionate is whitish to cream in color and having crystalline nature that is water insoluble and is derivative of prednisolone having high affinity towards glucocorticoid receptor than mineralocorticoid receptors. Chemically, clobetasol propionate is 21-chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate (Fig.2) and is a synthetic corticosteroid having activity on cytoplasmic glucocorticoid receptor which mediate gene expression. Clobetasol Propionate exert its effect by releasing anti-inflammatory phospholipase A2 Protein.by this way it regulates arachidonic acid which is inflammatory precursor [3-4].

![Fig. 2. Chemical structure of Clobetasol Propionate](image)

The evaluation of the text concerning quantitatively evaluation for Luliconazole and Clobetasol Propionate exhibits a few simultaneous analytical estimations of Clobetasol 17-Propionate with different drugs had been mentioned in the reported methods. [5-13]. To date, no research had been mentioned to estimate the mixed dosage of Clobetasol Propionate and Luliconazole along with the UV however in our previously published method the costly HPLC instrument were used [14]. The aim of this study is development and validation of fast, steady, precise and economic derivative spectrophotometry process for evaluating the Luliconazole and Clobetasol Propionate combination.

### 2. MATERIALS AND METHODS

#### 2.1 Reagent and Chemical

Solvent used is AR grade Methanol. Standard and Pure drugs sample of Luliconazole (LLZ) and Clobetasol Propionate (CLP) was obtained as gift sample from Kantam pharma, Chhatral, and Farbe Pharma, Ankleshwar, Gujrat, India.
2.2 Instruments

For the recording of derivative spectra of standard and test samples of LLZ and CLP, “Shimadzu UV-Vis-2450 and UV/Vis-1900 double beam UV-vis spectrophotometer” was used having fixed slit width, i.e. 2nm and quartz cell of 1 cm. Sartorius CD2250 balance helps in weighing of samples used in the process and for sonication, Sonicator (D120/2H, TRANS-O-SONIC) was used. Calibration of all instruments and glassware were done and all volumetric glassware used are belongs to class ‘A’.

2.3 First Derivative Method Specification

The mode used is Spectrum with fast scan speed ranging the wavelength from 200-400nm and derivative order is first with scaling factor 1.

2.4 Test Solution Preparation Procedure

Solution of synthetic mixture was prepared as per literature [15]

- Luliconazole -200mg
- Clobetasol Propionate-100mg
- Cetosteryl alcohol-50mg
- Liquid paraffin -50 mg
- Propylene glycol- Q. S

The drug powder was taken equivalent to 10mg of synthetic mixture in a volumetric flask capacity 100 ml and dissolved in the synthetic mixture in methanol (25 ml) with the help of sonicator for a time limit of 15 min. The volume was made up with methanol up to 100 ml and diluted up to 100 ml and shaken and the solid particle of the residues was filter out before the dilution [15].

2.5 Preparation of Stock Solution

LLZ and CLP standard stock solution of 100 μg/ml were prepared. Weight around 10 mg of each drug and transfer to a volumetric flask of 100 ml, dissolved the drugs methanol (25 ml) and volume make up with methanol up to 100 ml in a calibrated volumetric flask and diluted with the various concentrations like CLP 10-50 μg/ml and for LLZ 5-25 μg/ml. [15].

2.6 Determination of Absorption Maxima (λ max)

For the determination of absorption maxima scanning of LLZ (10 μg/mL) and CLP (5 μg/mL) standard solutions were done separately ranging between 200-400 nm. The absorbance maxima of LLZ observed at 297 nm and for CLP at 254nm as depicted in Fig.3 with the blue and red graph hump.

![Overlaid zero order spectra of LLZ and CLP](image)
2.6.1 Derivative spectroscopy

By observing both drugs overlain spectrum from Fig.4, first order spectrum was chosen for estimation of both drugs, which was converted from the previous Fig 3. Selection of wavelength for quantitation were 312 and 249 nm for LLZ (zero cross for CLP) and CLP (zero cross for LLZ) respectively.

For LLZ and CLP the calibration curve was plotted and the concentration vary from 10 to 50 μg/ml at 312nm for LLZ and 5-25 μg/ml at 249 nm for CLP shown in Fig.4. Each drug concentration that is present in the mixture is evaluated opposite to calibration curve in quantitation mode.

2.6.2 Validation

The validation of developed method was done as per ICH Guide line in terms of linearity, precision, accuracy, robustness, ruggedness, limit of detection, and limit of quantitation assay [16].

2.6.3 Linearity

From LLZ and CLP 100 μg/ml standard solution, appropriate dilutions were prepared using methanol as solvent for getting the working standard solutions of LLZ and CLP of 10-50 μg/ml and 5-25 μg/ml respectively at wavelength 312nm for LLZ and 249nm for CLP by using derivatized spectra.

2.6.4 Precision

For the developed method the precision done was in terms of intra and inter day studies. Sample preparation was done for same batch in nine findings with 3 concentrations, i.e. 10, 20 and 30 μg/ml for LLZ and for CLP 5, 10, 15 μg/ml, three replicates each on same day and for consecutive 3 days, and method precision was evaluated from % RSD result.

2.6.5 Accuracy

External standard addition method was used for determination of accuracy; 50 mg of mixture was weighted accurately from the synthetic mixture. Four volumetric flasks were taken each of 100 ml and addition of synthetic mixture equivalent to 20 mg of LLZ into it. First flask (1) used as placebo and rest flasks number (2, 3 and 4) spike with 80, 100 and 120 % of Solid API. Repetition of same method was done for CLP as mentioned in below described all the tables. In 100 ml volumetric flask the content was taken and dissolves it with methanol 25 ml with the help of sonicator for 15 min and volume makeup up to 100 ml with propylene glycol. Filter the solution with Whatman filter paper no 42. Data obtained from nine evaluations over 3 concentration level cover the complete range and %recovery also evaluated.

2.7 Limit of Detection and Quantitation

The LOD and LOQ of the developed procedure were assessed analysing 10 replicates of standard solutions containing concentrations 10μg/ml for LLZ and 5μg/ml for CLP.[16]

2.8 Robustness & Ruggedness

Robustness and ruggedness of the process was evaluated by specifying the method to a bit but deliberate make changes in conditions of method, specifically like change in wavelength,
change in equipments. The data of robustness and ruggedness evaluation is shown in Table 5.

3. RESULTS AND DISCUSSION

The analysis of the LLZ and CLP was done accurately and conveniently by this first order derivative spectroscopic method. The detection wavelengths selected for quantitation were 312 nm for LLZ (zero crossing point for CLP) and 249 nm for CLP (zero crossing point for LLZ). Both the drugs obey the Beer’s law with the concentration range 10-50μg/ml for LLZ and, 5-25 μg/ml for CLP with R² value of 0.9988 for LLZ and 0.9961 for CLP (Fig. 5, Table 1).

The concentration and absorbance are given in the below Table1. which is depicted with the %RSD value. The concentration ration of both the drug were kept fix as 2:1 (LLZ: CLP).

The data obtained within one day is often called intraday (within one day) precision. The Percentage RSD was found in the range of 0.131– 0.881 for intra-day precision (Table 2).

To analyze the long long term accuracy the inter-day precision data was calculated with the %RSD of 0.131-0.920 for, which conclude the method as a precise and robust. Moreover, the low %RSD value signifies the results very well as précised experiments (Table 3).

![Calibration Curve of mixture at 312 nm](image)

![Calibration Curve of mixture at 249 nm](image)

Fig. 5. Calibration curve (Curve a for LLZ and b for CLP) for mixture at 312 nm & 249 nm

| Concentration (µg/ml) in 2:1 | At 312 nm (n=6) | %RSD | At 249 nm (n=6) | %RSD |
|----------------------------|----------------|------|----------------|------|
| 10, 5                      | -0.014±0.00004 | 0.291| 0.006±0.00005  | 0.865|
| 20, 10                     | -0.031±0.00008 | 0.262| 0.010±0.00007  | 0.759|
| 30,15                      | -0.045±0.00004 | 0.924| 0.016±0.00001  | 0.883|
| 40, 20                     | -0.060±0.00038 | 0.647| 0.021±0.00004  | 0.194|
| 50, 25                     | -0.074±0.00007 | 0.101| 0.027±0.00004  | 0.303|

*All the data were taken n=3

| Concentration (µg/ml) | Abs. At 312 nm (Mean) | %RSD | Abs. At 249 nm (Mean) | %RSD |
|-----------------------|-----------------------|------|-----------------------|------|
| LLZ and CLP           |                       |      |                       |      |
| (µg/ml) in 2:1        |                       |      |                       |      |
| 10, 5                 | -0.014                | 0.586| 0.006                 | 0.865|
| 20, 10                | -0.031                | 0.131| 0.010                 | 0.408|
| 30, 15                | -0.045                | 0.881| 0.016                 | 0.256|

*All the data were taken n=3
Table 3. Interday precision data for estimation of LLZ and CLP*  

| Conc. (µg/ml) | Abs. at 312 nm (Mean) | %RSD | Abs. at 249 nm (Mean) | %RSD |
|--------------|-----------------------|------|-----------------------|------|
| LLZ: CLP (2:1) | | | | |
| 10:5 | -0.014 | 0.599 | 0.0059 | 0.920 |
| 20:10 | -0.031 | 0.131 | 0.0090 | 0.413 |
| 30:15 | -0.044 | 0.920 | 0.0160 | 0.256 |

*All the data were taken n=3

Table 4. Recovery data of LLZ and CLP*  

| Spiked level (µg/ml) | % Recovery ± SD |
|----------------------|----------------|
| LLZ | CLP | LLZ | CLP |
| 0% | 98.80±0.0902 | 0.0912 | 100.10±0.0862 | 0.0862 |
| 80% | 99.66±0.0828 | 0.0831 | 99.50±0.1730 | 0.1736 |
| 100% | 99.65±0.1006 | 0.1009 | 99.10±0.0699 | 0.0706 |
| 120% | 99.70±0.0121 | 0.0121 | 99.04±0.0126 | 0.0127 |

*All the data were taken n=3

Table 5. LOD & LOQ Data for estimation of LLZ & CLP  

| Drugs | LOD (µg/ml) | LOQ (µg/ml) |
|-------|------------|-------------|
| Luliconazole | 0.0054 | 0.0164 |
| Clobetasol Propionate | 0.0009 | 0.0027 |

Table 6. Ruggedness and Robustness data of LLZ and CLP *  

| Parameters | At 249 nm LLZ+CLP (Mean) %RSD | At 312 nm LLZ+CLP (Mean) %RSD |
|------------|-------------------------------|-------------------------------|
| Different instrument | Instrument. 1 0.015 0.562 -0.044 0.855 |
| | Instrument. 2 0.016 0.617 -0.045 0.223 |

| ROBUSTNESS | RUGGEDNESS |
|------------|------------|
| Change wavelength 247nm & 310nm | 247nm & 310nm 0.15 0.259 -0.044 0.897 |
| 251nm & 314nm | 251nm & 314nm 0.16 0.768 -0.044 0.855 |
| Change Ratio 10:5 | 10:5 0.006 0.091 -0.013 0.643 |
| 5:10 | 5:10 0.005 0.846 -0.013 0.708 |
| 20:10 | 20:10 0.010 0.903 -0.030 0.136 |
| 10:20 | 10:20 0.010 0.994 -0.030 0.172 |

*All the data were taken n=3

Accuracy of the result gives the descriptor value of the closeness to the actual value. The precision and accuracy is important parameter to define the experiments characters. In the previous section the method was found precise and in this section the analysis of accuracy were done using the recovery data as prime descriptor to define accuracy as depicted in the below Table 4. where the concentration of the LLZ and CLP were taken 20 and 10 µg/ml.

The result expressed in the Table 4, with the high recovery of the data, suggest the accuracy of the method with the assigned drug combination ratio. It also depicts the method versatility as per the combination is concern. The limits of detection (LOD) and quantification (LOQ) are defined as the lowest concentration of the analyte that can be reliably detected and quantified, respectively. Usually, the LOD and LOQ refer to the limits associated with 95% probability of obtaining a correct result. The data given in the below Table 5, shows the lower limit of the experiment as the sensitive.
Table 7. Summary of validation parameters

| Sr.no. | Parameter                        | Luliconazole          | Clobetasol Propionate |
|--------|----------------------------------|-----------------------|-----------------------|
| 1      | Wave length Max (λ max)          | 312 nm                | 249.9 nm              |
| 2      | Linearity (µg/ml) (n=6)          | 10 to 50 µg/ml        | 5 to 25 µg/ml         |
| 3      | Regression equation              | Y= -0.0015x-0.0001    | Y=0.0005x+0.0001       |
| 4      | Correlation coefficient (r²)     | 0.9988                | 0.9961                |
| 5      | Accuracy(%Recovery) (n=3)        | 99.45                 | 99.43                 |
| 6      | **Precision**                    |                       |                       |
|        | Intra-day (%RSD) (n=3)           | 0.131-0.881           | 0.256-0.865           |
|        | Inter-day (%RSD) (n=3)           | 0.131-0.920           | 0.256-0.920           |
| 7      | LOD (µg/ml) (n=10)               | 0.0054                | 0.0009                |
| 8      | LOQ (µg/ml) (n=10)               | 0.0164                | 0.0027                |
| 9      | **Robustness**                   |                       |                       |
|        | Different Instrument (%RSD) (n=3)| 0.223-0.855           | 0.562-0.617           |
| 10     | **Ruggedness**                   |                       |                       |
|        | Change in Wavelength (%RSD) (n=3)| 0.855-0.897           | 0.259-0.768           |
|        | Change in Ratio(%RSD) (n=3)      | 0.136-0.708           | 0.091-0.994           |
| 11     | % Purity                         | 99.98                 | 98.75                 |

The terms robustness and ruggedness define the ability of an analytical method to remain unaffected by small variations in the method parameters (mobile phase composition, column age, column temperature, etc.) and influential environmental factors (room temperature, air humidity, etc.). The data in the below Table 6 confirms the Ruggedness and Robustness of the method.

The method quantification analysis of the drug in the define parameters were concluded with the 98-99 % of assay value (Table 7). The results of the optimized methods have been summarized with the results in the below Table 7, with the drug Luliconazole and Clobetasol propionate individual as well as their defined ratios. The regression equation Y=mx+c was used to calculate the slop as m and intercept as c as in the Luliconazole the slop was -0.0015 and in Clobetasol slop was found as 0.0005, however the intercept was -0.001 for Luliconazole and 0.0001 for Clobetasol.

### 4. CONCLUSION

The developed UV spectroscopic method for the drug combination of Luliconazole and Clobetasol Propionate was found appropriate with the correlation value of 0.99; moreover, the accuracy data with recovery studies also confirms reliability of the method. The developed method founds to be rapid, precise accurate with 99% % recovery of drug combination. The lower value of Limit of detection and (LOD) and limit of quantification (LOQ) strongly recommends as the sensitive method with the ease and low cost because of using UV spectroscopy, instead of HPLC method. The broadness of the experiment could also be utilized in the laboratory for the various concentration combinations. In the future the method may get the deserve place in the analysis of the drug combination with the concern dosage form.

### DISCLAIMER

The marketed products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

### CONSENT

It is not applicable.

### ETHICAL APPROVAL

It is not applicable.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74511

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