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Original Article

COMBINATION EFFECT OF LULICONAZOLE AND CLOBETASOL FOR TREATMENT OF SKIN AILMENTS

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ABSTRACT

Objective: The new combination for Luliconazole and Clobetasol Propionate was approved for the treatment of a 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).

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

Results: From the method developed above the R^2 value observed for LLZ and CLP is 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 found to be 99.45 % and 99.43%, respectively. For LLZ the Limit of detection is 0.9988 µg/ml and limit of quantification is 0.0009µg/ml and for CLP the Limit of detection is0.0164µg/ml and limit of quantification 0.0027µg/ml.

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

Keywords: Antifungal agent, Clobetasol propionate, Glucocorticosteroids, Luliconazole, Derivative spectroscopic method

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INTRODUCTION

Luliconazole

It is a 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, an imidazole antifungal agent, in which imidazole moiety is involved into the ketene dithioacetate structure [1]. It works against fungal infection like tineapedis, tinea curies, and teniacorporis by slowing the growth of fungi. Luliconazole showed more prominent power opposes to *Trichophytonrubrum, Trichophytonmentagrophytes, Trichophytontonsurans* than the available standard drugs like Terbinafine, clotrimazole. Luliconazole is a white powder, poorly water-soluble drug having molecular weight 354.267 g/mol.

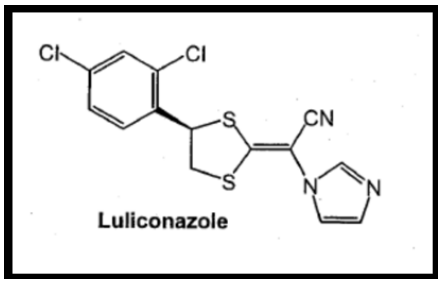

Fig. 1: Chemical structure of luliconazole [2]

Clobetasol propionate

Appearance 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 and is a synthetic corticosteroid having activity on cytoplasmic glucocorticoid receptor which mediate gene expression. Clobetasol Propionate exerts its effect by releasing anti-inflammatory Phospholipase A2 Protein; in this way it regulates Arachidonic acid which is inflammatory precursor [3, 4].

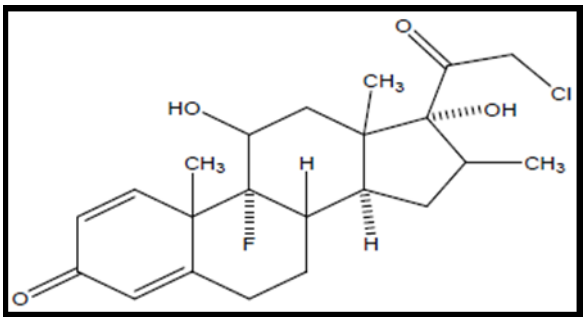

Fig. 2: Chemical structure of clobetasol propionate

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 with inside the literature [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.

MATERIALS AND METHODS [15, 16]

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 Kantampharma, Chhatral, and FarbePharma, Ankleshwar.

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. 2 nm 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'.

First derivative method specification

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

Test solution preparation procedure

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

Luliconazole-200 mg

Clobetasol Propionate-100 mg

Cetosteryl alcohol-50 mg

Liquid paraffin-50 mg

Propylene glycol-Q. S

Take powder equivalent to 10 mg of the synthetic mixture in a volumetric flask capacity 100 ml. Dissolve the synthetic mixture in methanol (25 ml) with the help of Sonicator, by sonicating for a time limit of 15 min. Volume make up with methanol up to 100 ml and Dilute up to 100 ml and shaken vigorously with the filtration and dilution.

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 drug's methanol (25 ml) and volume make up with methanol up to 100 ml in a calibrated volumetric flask. Different dilutions were prepared from this stock solution.

Determination of absorption maxima (λmax)

For the determination of absorption maxima scanning of LLZ (10 µg/ml) and CLP (5 µg/ml) standard solutions were done separat1ely ranging between 200-400 nm. The absorbance maxima of LLZ were observed at 297 nm and for CLP at 254 nm as depicted in fig. 3 with the blue and red graph hump.

Derivative spectroscopy

By observing both drug's overlain spectrum from fig. 4, first drug derivative spectrum was chosen for estimation of both drugs. Selection of wavelengths for quantization were 312 and 249 nm for LLZ (zero cross for CLP) and CLP (zero cross for LLZ), respectively.

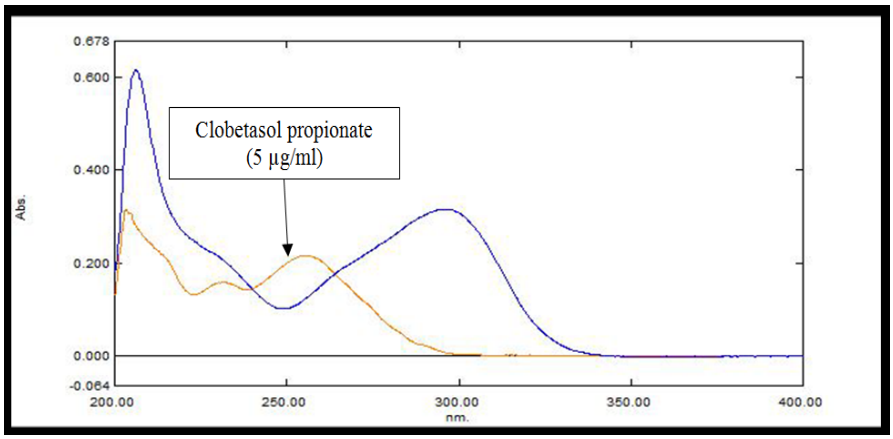

Fig. 3: Overlain zero-order spectra of llz and clp

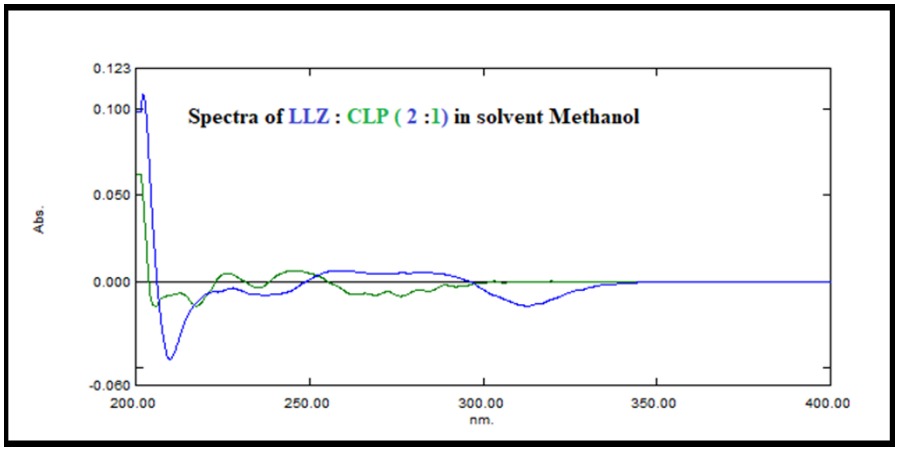

Fig. 4: Overlain 2nd order spectra of LLZ and CLP

For LLZ and CLP the calibration curve were plotted and the concentration vary from 10-50 µg/ml at 312 nm 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.

Validation

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

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 312 nm for LLZ and 249 nm for CLP by using derivatized spectra. Five replicate analyses were carried out.

Precision

For the developed method, the precision done was in terms of intra and inter-day studies. Sample preparation was done for the 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 the same day and for consecutive 3 d. Method precision was evaluated from % RSD result.

Accuracy

External standard addition method was used for determination of accuracy; 50 mg of the 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 Table. In 100 ml volumetric flask the content was taken and dissolves it with methanol 25 ml with the help of a 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 levels cover the complete range and %recovery was also evaluated.

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.

Robustness and ruggedness

Robustness and Ruggedness of the process were evaluated by specifying the method to a bit, but deliberate make changes in conditions of the method, specifically like Change in Wavelength, Change in equipments. The data of Robustness and Ruggedness evaluation is shown in table no 5.

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 Beer 's law with the concentration range 10-50µg/ml for LLZ and, 5-25 µg/ml for CLP with R^2 value of 0.9988 for LLZ and 0.9961 for CLP (fig. 5, table 1).

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

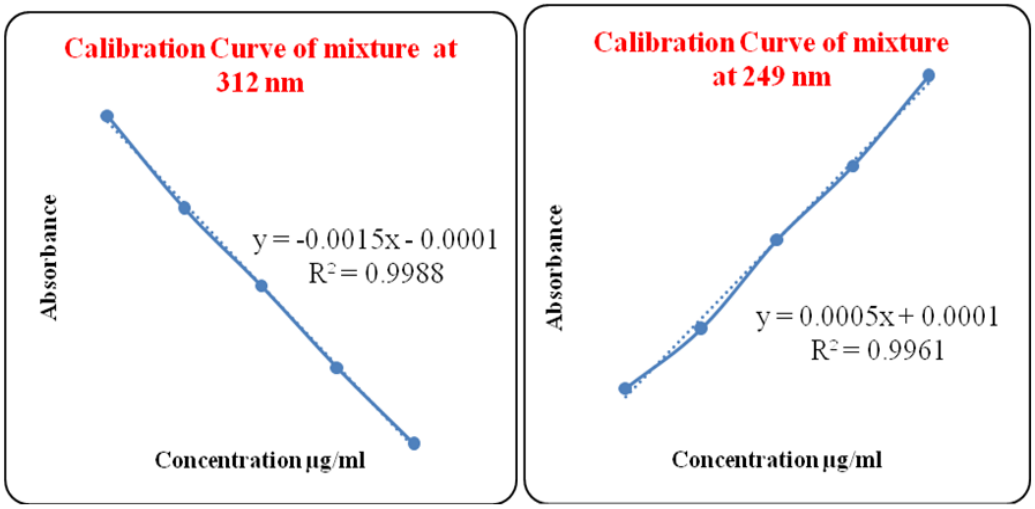

Fig. 5: Calibration curve for mixture at 312 nm and 249 nm

Table 1: Calibration data for mixture at 312 and 249 nm

Con. LLZ and CLP	At 312 nm (n=6)		At 249 nm (n=6)	
(µg/ml) in 2:1	Abs.±SD mean	%RSD	Abs.±SD mean	%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±0.00040	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, 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).

Table 2: Intraday precision data for estimation of LLZ and CLP*

Conc. (µg/ml) LLZ and CLP (µg/ml) in 2:1	Abs. At 312 nm (mean)±% RSDTZN	%RSD	Abs. At 249 nm (mean)	%RSD
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, To analyze the long-term accuracy the inter-day precision data was calculated with the %RSD of 0.131-0.920 for, which conclude the method as precise and robust. Moreover, the low % RSD value signifies the results very well as précised experiments (table 3)

Table 3: Interdayprecision data for estimation of LLZ and CLP*

Conc. (µg/ml) LLZ: CLP	Abs. at 312 nm (Mean)	%RSD	Abs. at 249 nm (mean)	%RSD
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

Accuracy of the result gives the descriptor value of the closeness to the actual value. The precision and accuracy is an important parameter to define the experiment's 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 a 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.

Table 4: Recovery data of LLZ and CLP*

Spiked level (µg/ml)			% Recovery±SD			
	LLZ	CLP	LLZ	%RSD	CLP	%RSD
0%	-	-	98.80±0.0902	0.0912	100.10±0.0862	0.0862
80%	16	08	99.66±0.0828	0.0831	99.50±0.1730	0.1736
100%	20	10	99.65±0.1006	0.1009	99.10±0.0699	0.0706
120%	24	12	99.70±0.0121	0.0121	99.04±0.0126	0.0127

*All the data were taken n=3

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 5: LOD and LOQ Data for estimation of LLZ and CLP

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

The terms robustness and ruggedness defines 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 confirms the Ruggedness and Robustness of the method.

Table 6: Ruggedness and robustness data of LLZ and CLP*

Robustness Parameters		At 249 nm LLZ+CLP (Mean)	%RSD	At 312 nm LLZ+CLP (Mean)	%RSD
Different instrument	Inst. 1	0.015	0562	-0.044	0.855
	Inst. 2	0.016	0.617	-0.045	0.223
RUGGEDNESS					
Change wavelength	247 nm and310 nm	0.015	0.259	-0.044	0.897
	251 nm and314 nm	0.016	0.768	-0.044	0.855
Change Ratio	10:5	0.006	0.091	-0.013	0.643
	5:10	0.005	0.846	-0.013	0.708
	20:10	0.010	0.903	-0.030	0.136
	10:20	0.010	0.994	-0.030	0.172

*All the data were taken n=3, the method quantification analysis of the drug in the define parameters were concluded with the 98-99 % of assay value (table 7).

Table 7: Assay data for estimation of LLZ and CLP

Drugs	% Assay±SD
Luliconazole	99.98±0.011
Clobetasol Propionate	98.75±0.009

The results of the optimized methods have been summarized with the results in the below table 8, with the drug Luliconazole and Clobetasol propionate individual as well as their defined ratios.

Table 8: Summary of validation parametrs

S. No.	Parameter	Luliconazole	Clobetasol propionate
1	Wavelength Max (λ max)	312 nm	249. 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 (r^2)	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	Assay	99.98	98.75

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 the reliability of the method. The developed method was 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 recommended as the sensitive method with 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.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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