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Original Research Article

Development and validation of RP-HPLC method for simultaneous estimation of luliconazole and beclomethasone dipropionate in topical dosage form

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ABSTRACT

Background: It was found that there was no official method for simultaneous determination of luliconazole and beclomethasone dipropionate.
Aim & Objective: A new, straightforward, reliable, and highly precise HPLC technique for regular quality control analysis of the combination of Luliconazole and Beclomethasone dipropionate in a topical dosage form.
Material & Methods: For the purpose of separation, 0.1% Glacial acetic acid: Acetonitrile (30:70) was utilised as the mobile phase. Column used was C18 (4.6 X150mm, 5μm) with flow rate of 1.0ml/min. Methanol was used as solvent.
Results: Detection wavelength was 290nm and 242nm, measured at an Rt of 6.6 and 10.2 min. 13 minutes running time, Linearity and range were observed at concentrations from 5μg/ml to 150μg/ml and 0.4μg/ml to 4.8μg/ml of luliconazole and beclomethasone dipropionate respectively. The method developed was linear with a correlation coefficient of 0.999.
Conclusion: Validation of the method was performed according to ICH guidelines for assay, linearity and range, precision, limit of detection, limit of quantitation, and forced degradation test.

1. Introduction

Luliconazole belongs to the imidazole class of medicines renowned for their potent antifungal effects, especially against dermatophytes.[1] It has a wide range of antifungal activities. Its chemical formula is $C_{14}H_9C_{l2}N_3S_2$, and it weighs 354.28 in molecular mass. Chemically, it is known as (2E)-2-[(4R)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-2-imidazol-1-yl-acetonitrile.[2] In its molecular structure, the E-configured double bond is adjacent to the dithiolane group. Luliconazole is a crystalline, off-white to pale yellow powder and is soluble in solvents like methanol, acetonitrile, dimethylformamide, and dimethyl sulfoxide.[3] While, the precise mechanism through which luliconazole acts against dermatophytes remains uncertain, it is believed to impede the production of ergosterol by inhibiting the enzyme lanosterol demethylase Azoles, including luliconazole, inhibit this enzyme's activity, leading to reduced ergosterol levels, a vital constituent of fungal cell membranes. This disruption results in an accumulation of lanosterol.

Beclomethasone dipropionate, a potent anti-inflammatory medication, is a synthetic corticosteroid.[4] It goes by the chemical names 1,4-diene-17,21-diyldipropionate and 9a-chloro-11b-hydroxy-16b-methyl-

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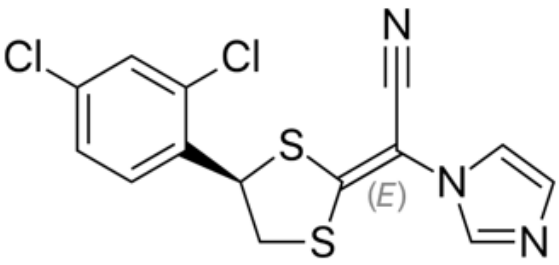

Figure 1: Structure of Luliconazole

3,20-dioxopregnane. Its molecular weight is 521.042, with a chemical formula of $C_{28}H_{37}ClO_7$. This substance appears nearly white crystalline powder, with a pKa of 13.6 and a melting range of 117–120°C.[5,6] In terms of its mechanism of action, Beclomethasone dipropionate interacts with DNA at the cellular nucleus level, influencing gene transcription by either stimulating or inhibiting it. This drug functions as a factor that regulates gene expression, which means that it may change how certain genes are expressed in response to particular hormone signals.[7,8]

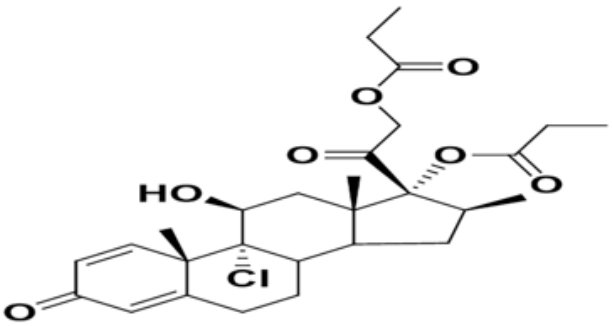

Figure 2: Structure of Beclomethasone dipropionate

Luliconazole and beclomethasone dipropionate is a well-known marketed as Luliford-B cream.

2. Materials and Methods

2.1. Chemicals and reagents

The Luliconazole working standard, with a stated potency of 98.27% on a dried basis, was obtained from the Central Drug Testing Laboratory (CDTL) in Mumbai. Similarly, the Beclomethasone dipropionate working standard, with a claimed potency of 98.13%, was also sourced from the Central Drug Testing Laboratory in Mumbai. For the Luliford-B cream, a marketed formulation by Leeford Healthcare Ltd., the composition includes Luliconazole at a concentration of 1% w/w and Beclomethasone dipropionate at a concentration of 0.025% w/w. Acetonitrile (ACN) and Methanol of high-performance liquid chromatography (HPLC) grade was procured from Merck Life Science. Glacial Acetic Acid was sourced from Finar Chemicals located in Gujarat. Ultra-purified water of HPLC grade was obtained from Milli-Q®. Additionally, a high-flow nylon membrane filter with a pore size of 0.45μm from Axiva Sichem Pvt. Ltd.

2.2. Instrumentation

UV-visible spectrometer from Lab India. All UV spectrophotometric measurements were performed using Win Lab software. Chromatography was performed using a Thermo Scientific Dionex Ultimate 3000 with Chromeleon 7.2.6 software and LC instrument control. The Sartorius Analytical Balance was used for all weighing. The column utilized had a particle size of 4.6mm, 25cm and 5μm and was inert enough to last.

2.3. Choice of diluent (solvent)

Based on the solubility and chemical nature of luliconazole and beclomethasone dipropionate, the HPLC grade methanol was selected as a diluent for preparing standard and sample solutions.

2.4. Choice of wavelength

A standard solution of 10μg/mL of Luliconazole and Beclomethasone dipropionate was scanned under UV within the 400-200nm range against diluent as a blank. Luliconazole showed maximum absorbance at 290nm and Beclomethasone dipropionate showed maximum absorbance at 242nm.

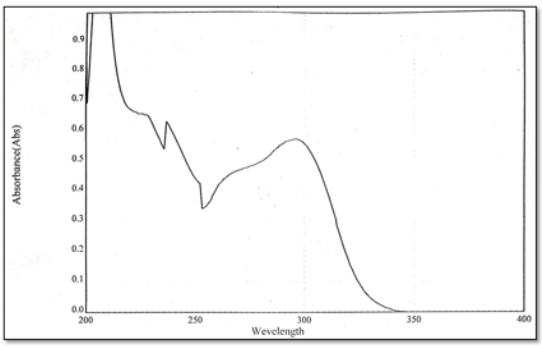

Figure 3: Wavelength for Luliconazole and Beclomethasone dipropionate

3. Preparation of Standard Solution

Transferred approximately 10mg of Luliconazole working standard in a 10ml volumetric flask, sonicated it to dissolve

it with an adequate amount of diluent, and then adjusted the volume (1000ppm standard stock solution). Weighed out around 10 mg of Beclomethasone dipropionate working standard, dissolved it with enough diluent by sonication, and adjusted the volume in a 100ml flask (100ppm standard stock solution). Then take 2 ml of 1000 ppm of stock solution of luliconazole and 5ml of 100 ppm of stock solution of beclomethasone dipropionate and make up to 20 ml with diluent (100ppm of luliconazole and 2.5ppm of beclomethasone dipropionate).

3.1. Analysis of marketed formulation

Transferred equivalent 1g of cream (equivalent 1%w/w luliconazole and 0.025%w/w beclomethasone dipropionate) to 100 ml flask, sonicated for 15 minutes with an adequate amount of diluent, and then filtering through a 0.45μ nylon filter to create volume.

3.2. Preparation of mobile phase (MP)

1% glacial acetic acid (GAA : Acetonitrile (ACN) (30: 70)

3.3. Optimization chromatographic conditions

Column: Hemochrom C18(4.6mm × 250mm × 5μm)
MP: 0.1% Glacial acetic acid: ACN (30:70v/v)
Flow rate: 1ml/min
Wavelength: 242nm, 290nm
Injection: 10μl
Run time: 13 mins
Column temperature: 35ºC

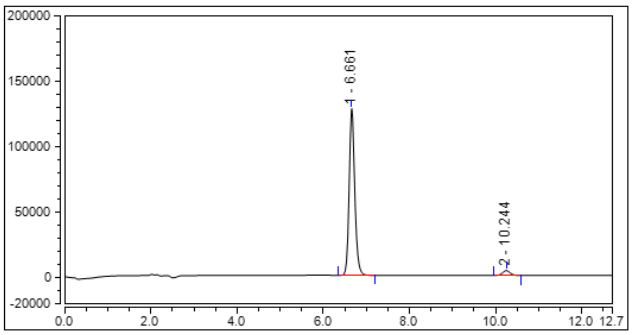

Figure 4: Chromatogram of standard solution of luliconazole and beclomethasone dipropionate

4. Result and Discussion

5. Method validation

The method that was created underwent validation in compliance with the standards established by aspects including system suitability, linearity, precision (both repeatability and intermediate precision), accuracy, assay, robustness, limit of detection (LOD), limit of quantification (LOQ), and a stability indicating study. These evaluations were carried out following specific procedures.[8,9]

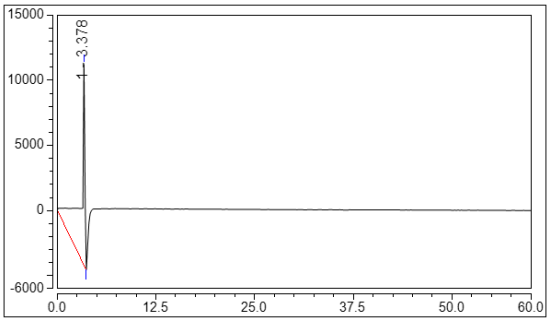

Figure 5: Chromatogram of blank

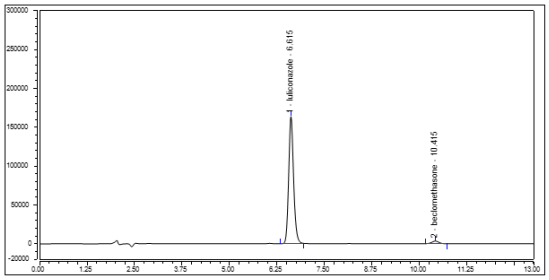

Figure 6: Analysis of formulation

5.1. System suitability

The table labelled as Table 1 presents the parameters for the system suitability test regarding luliconazole and beclomethasone dipropionate in the developed method.[10] The percent relative standard deviation (% RSD) for retention time, tailing factor, theoretical plate count, peak area, and for both luliconazole and beclomethasone dipropionate was determined to be within the specified limit of 2%. This outcome suggests that the system is appropriate and functions suitably.[11]

Additionally, the number of theoretical plates and tailing factor for both compounds met the acceptance criteria of being ≥ 2000 and ≤ 2.0, respectively. This demonstrates efficient column performance and an optimal composition of the mobile phase.[12]

5.2. Linearity

The linearity of the analytical method was assessed using linear regression analysis, employing the least squares method.[13] This evaluation involved the preparation of standard solutions for luliconazole and beclomethasone dipropionate at various concentration levels. After measuring the peak area of the final solutions, the

Table 1: Result from system suitability study

Parameters	Luliconazole		parameters	Beclome- thasone dipropionate	
	Mean (n=5)	%RSD		Mean (n=5)	% RSD
Retention time (t_R)	6.5955	0.56	Retention time (t_R)	10.4	0.35
Peak area (A)	24440. 66407	0.33	Peak area (A)	701.58	0.41
Tailing factor (T)	1.145	1.20	Tailing factor (T)	1.02	1.24
No. of theoretical plates (N)	13340	0.44	No. of theoretical plates (N)	1.240	0.55

peak area was plotted against the amount present of each drug to create a calibration curve. (Figures 7 and 8). In the range, the reaction was discovered to be linear 5-150μg/ml for luliconazole and 0.4-4.8μg/ml for beclomethasone dipropionate.

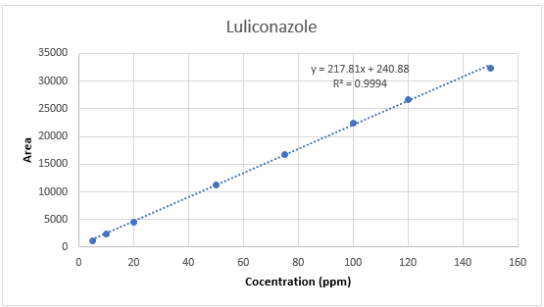

Figure 7: Linearity graph of luliconazole

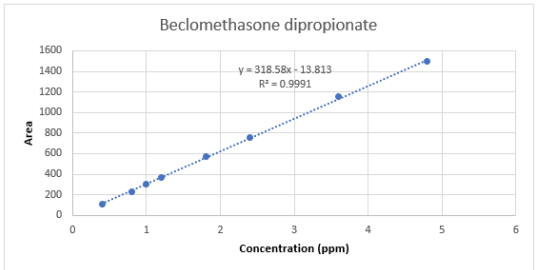

Figure 8: Linearity graph of beclomethasone dipropionate

5.3. Limit of detection and limit of quantification

LOD and LOQ was determined in accordance with the ICH guidelines.[14] These values were calculated using the slope and standard deviation derived from the calibration curve. (Table 1)

Table 2: Result of regression analysis of luliconazole and beclomethasone dipropionate

Parameter	Luliconazole	Beclomethasone dipropionate
Linearity range (μg/ml)	5-150	0.4-4.8
Regression equation (y=mx+c)	217.81x + 240.88	318.58x - 13.813
Slope (m)	217.81	318.58
Intercept (c)	240.88	13.813
Correlation coefficient (R^2)	0.9994	0.9991
LOD (μg/ml)	0.526312	0.002

Result of regression analysis ofluliconazole and beclomethasone dipropionate

5.4. Accuracy

For the accuracy assessment, we conducted experiments using various concentrations of luliconazole and beclomethasone dipropionate. This involved adding a known quantity of luliconazole and beclomethasone dipropionate standards to a fixed amount of the previously analyzed luliconazole and beclomethasone dipropionate sample. The accuracy data was represented as a percentage, reflecting the luliconazole and beclomethasone dipropionate content in the actual samples. We determined the recovered amounts of luliconazole and beclomethasone dipropionate, calculated the % recovery, and computed the %RSD for each concentration level. The summary of these results is provided in Table 2, offering a concise overview of the accuracy assessments conducted.

5.5. Precision

5.5.1. Repeatability

In compliance with the provided guidelines, a total of five standard solutions, all possessing equivalent concentrations, were meticulously formulated and subsequently introduced into the HPLC instrument. The result was given in Table 3.

5.6. Precision (Day to Day variability)

To assess the precision of measurements, intra-day precision involved conducting three replicates of luliconazole and beclomethasone dipropionate peak area measurements on the same day under consistent conditions. Conversely, three separate test runs on different days were used to establish the inter-day precision. The data was then analyzed by calculating the percent relative standard deviation (%RSD), which provides a measure of the variability or consistency in the results. The results were subsequently reported in Table 4.

Table 3: Accuracy data for both drugs

Accuracy studies as per standard addition method for Luliconazole					Accuracy Studies as per standard addition Method for Beclomethasone dipropionate			
Level	**Average**	**SD**	**% RSD**	**Mean Recovery (%)**	**Average**	**Standard deviation**	**% RSD**	**Mean Recovery (%)**
100%	98.5	0.238	0.241		95.6	0.723	0.756	
110%	99.1	0.400	0.404	99.08	99.4	0.379	0.381	**98.50**
120%	99.9	0.173	0.173		98.8	0.557	0.564	
130%	99.2	0.441	0.445		99.8	0.265	0.265	

Table 4: Precision (Repeatability)

System Precision		
	Luliconazole	**Beclomethasone**
Standard	**Area**	**Area**
1	23275.5061	699.699
2	23285.1	694.8
3	23046.8487	696.4257
4	23187.7206	706.8269
5	22866.9885	702.4764
Mean	23132	700
SD	176.4965909	4.800767437
RSD	0.763	0.686
Limit: NMT 2.0 %		

Table 5: Intermediate precision

	Day-1		**Day-2**	
Sr. No	**Luliconazole**	**Beclomethasone Dipropionate**	**Luliconazole**	**Beclomethasone Dipropionate**
1	98.46	96.22	98.08	96.6
2	98.44	97.63	98.6	96.65
3	98.45	96.87	98.54	96.64
Average	98.45	96.91	98.41	96.63
Standard Deviation	0.01	0.71	0.28	0.03
% RSD	0.010	0.728	0.289	0.027
Limit	NMT 2.0%		NMT 2.0%	

5.7. Assay

The method we developed was utilized to determine the levels of luliconazole and beclomethasone dipropionate in a topical dosage form. The quantities of these drugs present in the cream were analyzed based on the peak area obtained. The drug content was computed by averaging the results of six separate determinations. The assay results are presented in Table 5, demonstrating the measured drug concentrations in the formulation.

6. Results

6.1. Robustness

Robustness of an analytical method pertains to its capacity to maintain stability and consistency when deliberately subjected to minor variations in method parameters. This assessment offers valuable insights into the method's reliability when used in typical conditions. The evaluation of robustness involves analysing the analytical solution while

Table 6: Result of analysis of formulation

Assay Sr No.	% Assay Luliconazole	% Assay Beclomethasone
1	97.2172	96.5932
2	98.1561	98.41
3	98.879	96.0517
4	97.4098	99.1325
5	97.2667	98.4213
6	98.4129	95.3345
Average	97.89028333	97.32386667
SD	0.631678836	1.400301254
% RSD	0.645292683	1.438805611

Table 7: Robustness data for both drugs

Luliconazole Parameter	Change in parameter	% Evaluation	Mean	SD	% RSD	Limit
	28:72	97.67				
Mobile Phase Ratio	30:70	97.22	97.29	0.28	0.29	NMT 2.0%
	32:68	96.99				
	0.8	97.21				
Flow	1	98.88	98.30	0.77	0.79	NMT 2.0%
	1.2	98.81				
	33	97.44				
Temperature (° C)	35	98.16	97.64	0.37	0.38	NMT 2.0%
	37	97.32				
	288	98.29				
wavelength	290	97.27	97.82	0.42	0.43	NMT 2.0%
	292	97.92				
Beclomethasone dipropionate						
Parameter	**Change in parameter**	**% Evaluation**	**Mean**	**SD**	**% RSD**	**Limit**
Mobile Phase Ratio	28:72	95.87	96.13	0.33	0.34	NMT 2.0%
	30:70	96.59				
	32:68	95.94				
Flow	0.8	95.27	96.09	0.69	0.72	NMT 2.0%
	1	96.05				
	1.2	96.95				
Temperature (° C)	33	95.37	96.27	1.52	1.58	NMT 2.0%
	35	98.41				
	37	95.02				
Wavelength	240	98.29	97.30	1.49	1.53	NMT 2.0%

intentionally adjusting certain physical parameters like flow rate, temperature, and wavelength.[15] In this process, one parameter is deliberately altered while the others are held constant, and the resulting chromatogram profile is carefully observed and recorded. This systematic approach aids in quantifying the method's ability to endure slight alterations in these parameters, reinforcing its dependable performance as shown in Table 6.

7. Conclusion

The RP-HPLC method proposed in this study was effectively validated according to the criteria outlined by the International Conference on Harmonisation (ICH). The validation encompassed key parameters such as specificity, linearity, system suitability, precision, assay, accuracy, LOD, LOQ, and robustness. The method was characterized by its simplicity, speed, cost-effectiveness, and precision. All validation parameters fell within the acceptable range specified by the guidelines. Notably, no existing chromatographic combination method was identified in the literature for the analysis of luliconazole and beclomethasone dipropionate topical dosage form. Consequently, our efforts were directed towards creating a straightforward, swift, accurate, and precise method of chromatography with reverse phases for the estimation

of luliconazole and beclomethasone dipropionate topical formulation. This developed method has been effectively employed in the quality control analysis of luliconazole and beclomethasone dipropionate topical dosage form.

8. Source of Funding

None.

9. Conflict of Interest

None.

10. Acknowledgment

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