Development and Validation of a Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Luliconazole and Clobetasol Propionate in Topical Cream Formulation

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***Abstract*—A stability-indicating Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Luliconazole and Clobetasol Propionate in a topical cream formulation. The chromatographic separation was achieved using a C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of acetonitrile and water (70:30, v/v), adjusted to pH 3 with orthophosphoric acid. The method was optimized for a flow rate of 1.0 mL/min and detection at 254 nm. The method was fully validated according to ICH Q2(R1) guidelines, including specificity, linearity, accuracy, precision, robustness, and sensitivity (LOD and LOQ). Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions demonstrated the stability-indicating nature of the method. The linearity was established over the concentration ranges of 50–150% for both drugs with correlation coefficients (R^2) greater than 0.999. The recovery was found to be within 98–102%, confirming the accuracy of the method. The method exhibited good precision with %RSD values less than 2.0. The validated RP-HPLC method is robust, reliable, and suitable for routine quality control analysis and stability testing of Luliconazole and Clobetasol Propionate in topical cream formulations.**

Keyword— Lariocidin A, antimicrobial resistance, novel antibiotics, bacteriocins, drug discovery.

I. INTRODUCTION

In the treatment of mixed fungal infections with accompanying inflammation, topical formulations that include antifungal and anti-inflammatory drugs have become increasingly important. Clobetasol Propionate is a strong corticosteroid that is used to lessen inflammation and itching, while luliconazole, a new imidazole antifungal, has broad-spectrum efficacy against dermatophytes. When used together, they improve patient compliance and therapeutic efficacy in the treatment of a range of dermatological disorders.

Developing a reliable analytical technique that can precisely quantify both active pharmaceutical ingredients (APIs) in a combination dose form is crucial for quality control and regulatory compliance. The precision, sensitivity, and repeatability of reversed-phase high-performance liquid chromatography (RP-HPLC) make it the method of choice for many.

There is little research on a validated, stability-indicating RP-HPLC method for the simultaneous evaluation of clobetasol propionate and liconazole in topical cream formulations, despite the fact that there are numerous analytical techniques for individual estimate. In order to guarantee the formulation's stability throughout time, a stability-indicating approach efficiently separates the APIs from degradation products in addition to quantifying them.

In compliance with ICH recommendations, the objective of this work is to develop and validate a precise, accurate, and stability-indicating RP-HPLC method for the simultaneous measurement of clobetasol propionate and liconazole in a topical cream.

RESEARCH DESIGN:

This study follows an experimental research design aimed at developing and validating a stability-indicating RP-HPLC method for the simultaneous estimation of Luliconazole and Clobetasol Propionate in topical cream formulations. The key steps include:

1. METHOD DEVELOPMENT:

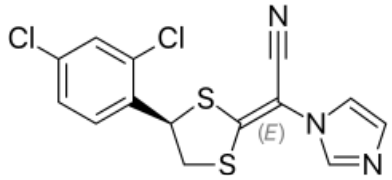
- Selection of appropriate chromatographic conditions (column type, mobile phase composition, flow rate, detection wavelength).
- Optimization of separation parameters to achieve clear resolution between Luliconazole, Clobetasol Propionate, and any possible degradation products.

2. Forced Degradation Studies:
   - Subjecting the topical cream formulation to various stress conditions (acidic, basic, oxidative, thermal, photolytic) to induce degradation.
   - Analyzing the degraded samples to confirm the method's ability to separate drugs from their degradation products, establishing the method as stability-indicating.
3. Method Validation:
   - Validation of the developed method according to ICH Q2(R1) guidelines, including assessment of specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ).
4. Application:
   - Application of the validated method for routine quality control of marketed topical cream formulations containing both active ingredients.

DRUG PROFILE

1. Luliconazole

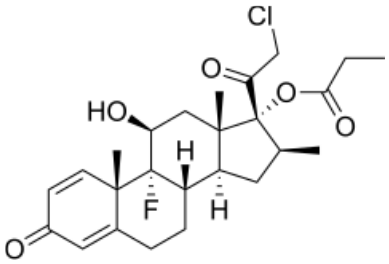
- Chemical Name: (2E)-1-(2,4-dichlorophenyl)-N-(1H-imidazol-1-ylmethyl)-N-methyl-2-(2,4-dichlorophenyl)ethanamine
- Molecular Formula: C14H9Cl2N3S
- Chemical Structure:



- Molecular Weight: 354.21 g/mol
- Pharmacological Class: Imidazole antifungal
- Mechanism of Action: Inhibits fungal lanosterol 14α-demethylase, disrupting ergosterol synthesis, leading to fungal cell membrane damage and death.
- Indications: Treatment of dermatophytic infections such as tinea pedis, tinea cruris, and tinea corporis.
- Dosage Form: Topical cream, typically 1% w/w concentration.
- Solubility: Practically insoluble in water, soluble in organic solvents like methanol and ethanol.

2. Clobetasol Propionate

- Chemical Name: (11β,16β)-21-chloro-9-fluoro-11,17-dihydroxy-16-methylpregna-1,4-diene-3,20-dione 17-propionate
- Molecular Formula: C25H32ClFO5
- Chemical Structure:



- Molecular Weight: 466.97 g/mol
- Pharmacological Class: Super potent corticosteroid
- Mechanism of Action: Binds to glucocorticoid receptors to modulate inflammatory response, suppressing immune cell activity and reducing inflammation, itching, and redness.
- Indications: Used topically for inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses.
- Dosage Form: Topical cream, ointment, or lotion, commonly at 0.05% w/w concentration.
- Solubility: Slightly soluble in water, soluble in alcohol and chloroform.

II. MATERIALS AND INSTRUMENTS

Category	Materials / Instruments	Details / Specifications
Active Drugs	Luliconazole	Pure standard, pharmaceutical grade
	Clobetasol Propionate	Pure standard, pharmaceutical grade
Formulation	Topical cream formulation	Marketed sample containing Luliconazole and Clobetasol Propionate

Category	Materials / Instruments	Details / Specifications
Chemicals & Reagents	HPLC-grade Methanol	Mobile phase component
	HPLC-grade Acetonitrile	Mobile phase component
	Water (Milli-Q or distilled)	Mobile phase and sample preparation
	Ortho-phosphoric acid	pH adjustment of mobile phase
	Hydrogen peroxide	For oxidative degradation studies
	Hydrochloric acid	For acidic degradation studies
	Sodium hydroxide	For alkaline degradation studies
Instruments	RP-HPLC System	Equipped with UV detector, e.g., Shimadzu or Agilent
	Analytical balance	Precision ±0.1 mg
	Sonicator	For sample dissolution
	pH Meter	For mobile phase pH measurement
	Hot air oven	For thermal degradation studies
	UV Cabinet	For photolytic degradation studies
	Micropipettes and volumetric glassware	For accurate sample and reagent measurement

Materials and Instruments

2.1 Materials and Reagents

- Luliconazole: Reference standard (purity ≥ 99%), obtained from a certified pharmaceutical manufacturer or supplier.
- Clobetasol Propionate: Reference standard (purity ≥ 99%), procured from a validated source.
- Topical Cream Formulation: Commercial or laboratory-prepared formulation containing both Luliconazole and Clobetasol Propionate.
- Acetonitrile: HPLC-grade, purchased from Merck or equivalent.
- Potassium dihydrogen phosphate (KH_2PO_4): Analytical reagent (AR) grade, used to prepare phosphate buffer.
- Orthophosphoric Acid (H_3PO_4): For pH adjustment of phosphate buffer.
- Milli-Q Water: Used for buffer preparation and dilution, filtered through 0.22 µm membrane.
- Methanol: HPLC-grade, used for sample dissolution and dilution.
- Filter paper and syringe filters: 0.45 µm and 0.22 µm membrane filters (PVDF or nylon) for sample and mobile phase filtration.

2.2 Instruments and Equipment

- HPLC System: Shimadzu LC-20AD or equivalent, equipped with:
  - UV/Visible Detector or Photodiode Array Detector (PDA)
  - Auto-injector and gradient pump
  - Column oven and software for data acquisition (e.g., LabSolutions, Empower)
- Column:
  - Reversed-phase C18 column (e.g., Inertsil ODS-3, 250 mm × 4.6 mm, 5 µm particle size)
- Ultrasonic Bath: For sonication of samples (e.g., Bandelin or equivalent)
- Analytical Balance: With readability of 0.1 mg (e.g., Mettler Toledo or Sartorius)
- pH Meter: Calibrated digital pH meter for buffer preparation (e.g., Eutech Instruments)
- Volumetric Flasks, Pipettes, and Glassware: Class A, calibrated
- Water Purification System: Milli-Q or equivalent system for preparing HPLC-grade water
- Temperature-controlled Oven: For thermal degradation studies
- UV Cabinet or Light Source: For photolytic degradation studies as per ICH Q1B

Challenges and Limitations

Despite the successful development and validation of the proposed RP-HPLC method, several challenges and limitations were encountered during the study:

1. Matrix Complexity of Topical Formulations

Topical creams contain various excipients such as emulsifiers, stabilizers, and thickening agents, which can interfere with chromatographic separation. Extensive sample preparation and optimization of extraction techniques were required to ensure that these excipients did not affect the accuracy and specificity of the method.

2. Solubility and Extraction Efficiency

Achieving complete extraction of both Luliconazole and Clobetasol Propionate from the cream base was challenging due to differences in polarity and solubility. Multiple trials were conducted to optimize the solvent system for effective drug recovery without degrading the analytes.

3. Forced Degradation Conditions

Standardized stress conditions, especially oxidative and alkaline degradation, led to rapid degradation of Clobetasol Propionate, making it difficult to quantify residual drug in some conditions. This required careful control of degradation duration and conditions to avoid total degradation while still demonstrating stability-indicating capability.

4. Close Retention Times of Degradation Products

Some degradation products had retention times close to that of the parent drugs, especially under acidic and oxidative stress. Additional method optimization was necessary to improve resolution and ensure proper identification and quantification of analytes.

5. Limited Stability Data

While the method was successfully validated, long-term stability studies of the formulation were not conducted within this research timeline. The application of this method for extended stability testing over months or years still needs to be explored.

6. Instrument and Column Variability

Minor differences in HPLC systems or column batches (even of the same specifications) could potentially affect retention time and resolution. Method robustness was confirmed to some extent, but full inter-laboratory reproducibility was not evaluated.

Future Directions

- Evaluation of method transferability across different laboratories and HPLC systems.
- Application of the method in real-time and accelerated stability studies.
- Extension of the method to other dosage forms (e.g., lotion, gel).
- Incorporation of mass spectrometry (LC-MS) for confirmation of degradation product structures.

III. CONCLUSION

For the simultaneous measurement of clobetasol propionate and luliconazole in a topical cream formulation, a straightforward, precise, accurate, and stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) approach was successfully designed and validated. The technique proved to be resilient to minor changes in chromatographic conditions and showed outstanding linearity, precision, and accuracy.

Crucially, forced degradation experiments demonstrated the method's stability-indicating character by confirming its capacity to separate the active medicinal components from their corresponding degradation products. Regular quality control, batch release testing, and long-term stability studies of topical formulations incorporating these two medications can all benefit from the suggested technique. When used in dermatological pharmaceutical preparations, it can improve regulatory compliance and product quality assurance.

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