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# A comprehensive review on analytical, stability-indicating and *In-Vitro* evaluation strategies for Luliconazole and Salicylic Acid in topical formulations

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**Abstract**
Topical antifungal combinations containing Luliconazole and Salicylic acid have emerged as efficient options for the management of chronic dermatophytic infections involving thickened or hyperkeratinized lesions. Analytical method development and validation ensure formulation quality, stability, and therapeutic reliability. This review compiles official and non-official analytical approaches, forced degradation studies, stability-indicating methods, and *in-vitro* evaluation strategies applicable to Luliconazole and Salicylic acid, either alone or in combination. Data from pharmacopoeias, peer-reviewed publications, patents, and regulatory reports (2010–2025) were critically examined. Emphasis is placed on ICH Q1A(R2) and Q2(R2) guidelines, which provide the basis for method validation and stress testing. Analytical tools such as RP-HPLC, UPLC, LC–MS/MS, UV-Visible spectroscopy, capillary electrophoresis, and green analytical methodologies are reviewed with discussion on method optimization, validation parameters, and Quality-by-Design (QbD) applications. This comprehensive review provides analytical scientists and formulation developers with consolidated knowledge to establish robust, sensitive, and regulatory-compliant procedures for Luliconazole–Salicylic acid topical formulations.

**Keywords:** Luliconazole, Salicylic Acid, rp-hplc, stability-indicating method, ICH guidelines, analytical validation, forced degradation

## Introduction

Pharmaceutical quality assurance relies on precise and validated analytical techniques to ensure the purity, potency, and safety of formulations throughout their life cycle. In topical preparations, active pharmaceutical ingredients (APIs) may undergo degradation due to environmental and formulation-related factors such as pH, oxidation, or exposure to light. Hence, development of stability-indicating analytical methods (SIAMs) is vital for routine quality control and regulatory submission.

Luliconazole is an imidazole derivative with potent broad-spectrum antifungal activity, whereas Salicylic acid serves as a keratolytic and exfoliating agent that facilitates deeper drug penetration through the stratum corneum. Their combination is clinically valuable in treating fungal infections accompanied by hyperkeratosis, such as tinea cruris and tinea corporis.

The analytical estimation of these compounds is challenging due to distinct physicochemical properties: Luliconazole is lipophilic and unstable under alkaline conditions, whereas Salicylic acid is weakly acidic and highly susceptible to photodegradation. A validated analytical approach must therefore achieve selective separation of degradation products without interference. This review provides a consolidated account of official, non-official, patented, and innovative analytical methodologies, with particular attention to RP-HPLC, forced degradation, and QbD-based optimization strategies.

## Drug Profile

### 1. Luliconazole

- **Chemical Name:** 2-[(2E,4R)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-2-(1H-imidazol-1-yl) acetonitrile
- **Molecular Formula:** $C_{14}H_9Cl_2N_3S_2$ Molecular Weight: 354.27 g/mol
- **λmax:** ≈ 296 nm (in methanol)
- **Solubility:** Soluble in methanol, DMSO, DMF; practically insoluble in water
- **Mechanism:** Inhibits lanosterol 14-demethylase, disrupting ergosterol synthesis and fungal cell membrane integrity.

### 2. Salicylic Acid

- **Chemical Name:** 2-Hydroxybenzoic acid
- **Molecular Formula:** $C_7H_6O_3$ Molecular Weight: 138.12 g/mol
- **λmax:** ≈ 270 nm
- **Solubility:** Slightly soluble in water; freely soluble in alcohol and acetone
- **Mechanism:** Breaks down keratin by disrupting cell-to-cell adhesion and shows mild anti-inflammatory effects.

## Rationale for Combination

Hyperkeratotic fungal infections hinder topical antifungal absorption. Incorporation of Salicylic acid with Luliconazole aids in desquamation and enhances drug diffusion. The combination provides dual benefits: (i) fungicidal activity from Luliconazole, and (ii) keratolytic exfoliation from Salicylic acid, enabling effective drug penetration.

Analytically, simultaneous quantification of these two drugs in a single dosage form is complex due to overlapping UV spectra and solubility differences. A validated, stability-indicating RP-HPLC method must therefore employ a carefully optimized mobile phase, pH, and wavelength for selectivity.

## Analytical and Stability-Indicating Methods

### 1. Official Pharmacopoeial Methods

**Luliconazole:** Listed in the *Indian Pharmacopoeia (IP 2022)* [1]. The official method employs RP-HPLC using a C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of 7 % sodium perchlorate monohydrate: methanol (20: 80 v/v),

flow rate 0.6 mL/min, detection at 295 nm. System suitability parameters include theoretical plates > 5000 and tailing factor < 2.

**Salicylic acid:** Monographed in IP 2022, BP 2025, and USP 2025. The HPLC assay uses methanol-water or phosphate buffer systems with UV detection between 270–280 nm. It is also assayed by acid-base titrimetry and derivative spectrophotometry for quality control.

These official protocols set baseline conditions but may not suffice for combination analysis where overlapping peaks and matrix effects demand customized separation.

### 2. Reported Non-Official and Peer-Reviewed Methods

Numerous authors have proposed validated chromatographic and spectroscopic methods (2015–2025) for the assay of Luliconazole and Salicylic acid either individually or together.

| Sr No | Drug/Matrix | Method | Mobile Phase | λmax (nm) | Key Findings | Reference |
|---|---|---|---|---|---|---|
| 1 | Luliconazole (cream) | RP-HPLC | Methanol: Water (50:50) | 296 | Linear 5–50 µg/mL; RSD < 2 % | Panthi *et al*., 2022 [4] |
| 2 | Luliconazole (formulation) | UV | Methanol: Water (70:30) | 297 | Rapid & simple method | Suryawanshi *et al*., 2021 [5] |
| 3 | Salicylic acid (API) | RP-HPLC | Methanol: Water (50:50) | 270 | Validated stability-indicating | Tandel *et al*., 2018 [6] |
| 4 | Luliconazole + Salicylic acid (Tablet) | UV | Methanol | 230 | Simultaneous estimation | Shaikh *et al*., 2023 [7] |
| 5 | Luliconazole + Salicylic acid (Ointment) | RP-HPLC | Phosphate buffer pH 3: Acetonitrile (55:45) | 295 | Forced degradation resolved 5 peaks | GTU Project Report, 2024 [12] |

Reported methods demonstrate excellent linearity ($R^2 > 0.999$), precision (%RSD < 2), and accuracy within 98–102 %, confirming their reliability for routine use. RP-HPLC remains the most preferred technique because of its simplicity and reproducibility.

### 3. Patents and Research Projects

Several patents highlight innovative approaches:

- **EP 2745692 A1:** Antifungal formulation containing Luliconazole.
- **WO 2017203456 A1:** Stable topical compositions of Luliconazole with corticosteroids.
- **US 10703744 B2:** Process for preparation of Luliconazole.
- **US 3183169 A / US 3359307 A:** Synthesis and purification of Salicylic acid.

Research projects (2023–2025) from Indian universities report stability-indicating RP-HPLC methods using methanol-phosphate buffer mobile phases validated per ICH Q2(R2). Parameters optimized include mobile phase pH (3.0–4.0), column temperature (25–30 °C), and flow rate (1 mL/min).

### 4. Analytical Method Validation and ICH Guidelines

The validation of an analytical method as per **ICH Q2(R2)** ensures accuracy, precision, specificity, linearity, range, LOD, LOQ, and robustness. Key concepts are:

- **Linearity:** Calibration range spanning 50–150 % of nominal concentration must yield $R^2 \geq 0.999$.
- **Accuracy:** Mean recovery between 98–102 %.
- **Precision:** Intra-day and inter-day %RSD ≤ 2.
- **LOD/LOQ:** Calculated by σ/S (standard deviation of response/slope of calibration).
- **Robustness:** Minor changes in flow rate or mobile phase ratio should not affect results.

**ICH Q1A(R2)** guidelines govern stability testing of drug substances and products under stress conditions—acidic, alkaline, oxidative, thermal, and photolytic. These conditions aid in identifying potential degradants and establishing the stability-indicating nature of the method.

### 5. Forced Degradation and Stability Indication

Forced degradation is performed to prove specificity of a method. Typical stress conditions for Luliconazole and Salicylic acid:

- **Acidic hydrolysis:** 0.1 N HCl, 60 °C, 30 min → Luliconazole shows 2 % degradation; Salicylic acid hydrolyzes to phenol derivatives.
- **Alkaline hydrolysis:** 0.1 N NaOH, 60 °C, 1 h → Rapid degradation of Luliconazole.
- **Oxidative stress:** 3 % $H_2O_2$, 25 °C, 1 h → Both show moderate oxidation.
- **Photolytic stress:** UV light (254 nm, 24 h) → Salicylic acid degrades significantly.
- **Thermal stress:** Dry heat (60 °C, 24 h) → minor degradation (< 5 %).

Peak purity analysis using diode-array detector (DAD) confirms absence of co-eluting impurities. This demonstrates the method's stability-indicating capability.

### 6. Alternative Analytical Techniques

- **UPLC:** Enables faster analysis with higher resolution due to sub-2 µm particles; suitable for degradation studies.
- **LC–MS/MS:** Ideal for trace level bioanalytical estimation in skin permeation studies.
- **UV–Derivative Spectroscopy:** Useful for simultaneous estimation without chromatographic separation.
- **FT-IR and DSC:** Used for drug–excipient compatibility studies prior to method development.
- **Capillary Electrophoresis:** A green analytical approach that reduces solvent usage and analysis time.

These techniques complement HPLC for different analytical objectives, from rapid screening to trace quantification.

## *In-Vitro* Evaluation and Validation Parameters

### 1. Dissolution and Diffusion Studies

*In-vitro* release testing assesses drug availability from topical formulations. Standard conditions include USP Type II apparatus, 900 mL of phosphate buffer (pH 5.8 or 7.4), 75 rpm, 37 ± 0.5 °C, with sample withdrawal at 5-, 10-, 20-, 30-, 45-, and 60-minute intervals.

Detection wavelengths used are 296 nm for Luliconazole and 270 nm for Salicylic acid. Data are processed for % drug release and kinetic modeling (zero-order, first-order, Higuchi, and Korsmeyer-Peppas).

**2. Validation as per ICH Q2(R2)**

| Parameter | Acceptance Criteria | Typical Results |
|---|---|---|
| System Suitability | %RSD ≤ 2, Resolution > 1.5 | Complied |
| Linearity | $R^2 \geq 0.999$ | 0.9994 |
| Accuracy | 98–102 % | 99.5 % |
| Precision | RSD ≤ 2 % | 1.2 % |
| LOD/LOQ | Low values | 0.012 µg/mL, 0.039 µg/mL |
| Robustness | No significant variation | Pass |

**Quality by Design (QbD) in Analytical Development**

The QbD approach, endorsed by ICH Q8(R2) ** and Q9**, applies statistical tools to understand the relationship between method variables and critical quality attributes. Steps include:

1. **Defining Analytical Target Profile (ATP):** e.g., accurate quantification of both actives within 10 min with RSD < 2 %.
2. **Risk Assessment:** Identifying critical parameters such as pH, mobile phase composition, flow rate, and column temperature using Ishikawa diagram or FMEA.
3. **Design of Experiments (DoE):** Using Box–Behnken or Central Composite Design to optimize chromatographic factors.
4. **Method Operable Design Region (MODR):** Establishing ranges ensuring method robustness.
5. **Control Strategy:** Routine monitoring of method parameters to maintain consistency.

Adopting QbD shortens development time, enhances understanding, and ensures reproducibility across laboratories.

**Discussion**

The analytical evaluation of the Luliconazole–Salicylic acid combination is complex due to differences in polarity, pKa, and degradation pathways. Forced degradation confirms specificity and helps in identifying potential degradants.

Among available techniques, RP-HPLC remains the most versatile and economical. UPLC and LC–MS/MS offer enhanced sensitivity and resolution but are cost-intensive.

Application of QbD principles and green analytical chemistry concepts significantly improves sustainability. Using eco-friendly solvents like ethanol or water-methanol mixtures instead of acetonitrile reduces environmental impact.

Further, the integration of Process Analytical Technology (PAT) and automation can provide real-time quality assurance. Adoption of these advanced tools ensures analytical robustness, regulatory compliance, and consistency in product quality.

**Conclusion**

The development of analytical and stability-indicating methods for Luliconazole and Salicylic acid is essential for ensuring consistent product performance and compliance with regulatory standards. RP-HPLC remains the method of choice for simultaneous estimation and stability studies, validated under ICH Q1A(R2) and Q2(R2) guidelines.

Emerging technologies such as UPLC, LC–MS/MS, and green analytical methods offer future potential for rapid and sustainable analysis. Incorporation of QbD and PAT into analytical method development ensures better process understanding and reproducibility.

Further research should focus on *in-vitro–in-vivo* correlation, microfluidic analytical platforms, and green solvent innovations to strengthen analytical control over topical antifungal formulations.

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