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# ANALYTICAL STANDARDIZATION OF LASHUNA RASAYANA (ALLIUM SATIVUM): HPTLC-BASED PHYTOCHEMICAL ASSESSMENT AND PHYSICOCHEMICAL PROFILING

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## Abstract

Lashuna (*Allium sativum* Linn.), commonly known as garlic, has been used safely since ancient times as both food and medicine. It is a rich source of diverse phytochemicals and is recognized for a wide spectrum of biological activities. Garlic contains several sulphur-bearing active constituents—principally thiosulfates—which are rapidly absorbed, metabolized, and largely responsible for its pungent aroma as well as many of its pharmacological effects. Traditionally, garlic has been employed for centuries in the management of various disorders, ranging from infectious diseases to metabolic imbalances.In Ayurveda, Lashuna is classified as Rasayana, an important group of drugs reputed to enhance health, vitality, immunity, and longevity. As a Rasayana, it is recommended both for disease prevention and the management of chronic systemic disorders. In contemporary times, garlic remains one of the most widely used natural remedies in the United States and Western Europe, particularly for reducing risk factors associated with cardiovascular diseases. Experimental and clinical investigations have validated its therapeutic potential across multiple systems including the cardiovascular, respiratory, genitourinary, gastrointestinal, hematopoietic, and integumentary systems. Garlic is consumed and prescribed in a wide variety of forms such as tablets, capsules, inhalations, beverages, alcoholic extracts, aqueous macerations, oil preparations, raw or roasted cloves, and cooked fractions.Analytical evaluations of Lashuna Rasayana using methanolic extracts have demonstrated a distinct HPTLC profile, including a prominent band at **Rf 0.80** under both short- and long-wave UV. Densitometric scanning revealed a characteristic peak at **Rf 0.28 ± 0.04**, corresponding to the amino acid **alanine**, one of the 17 amino acids and 33 sulphur compounds known to be present in garlic. Physicochemical assessments—such as moisture content (2.25%), average capsule weight (578.2 mg), and disintegration time (14 minutes)—were found to be within acceptable limits, supporting the formulation's quality and stability.Collectively, the classical significance, wide-ranging therapeutic potential, and modern analytical validation emphasize the importance of Lashuna Rasayana as a standardized Ayurvedic Rasayana. The established chromatographic fingerprint and compliant quality-control parameters provide a reproducible reference model for future research, formulation development, and regulatory standardization.

## Introduction

Lashuna (*Allium sativum* Linn.) is one of the important drugs used in the Ayurvedic system of medicine and is highly valued for its therapeutic versatility. Acharya Vagbhata has described it as the best among the Vata-śāmaka drugs, highlighting its significant role in the management of Vata-vyadhis. In

Ayurveda, Lashuna is classified as a Rasayana, a category of formulations known to promote health, enhance longevity, improve immunity, and support optimal metabolic function. Several experimental and clinical studies on Rasayana drugs have validated their immunomodulatory, adaptogenic, antioxidant, nootropic, and antistress actions, supporting their long-standing traditional use.

Botanically, Lashuna is an annual herb native to Central Asia, but owing to its extensive medicinal and culinary utility, it is now cultivated across almost all continents. Different cultures use different varieties of garlic depending on ecological suitability. Among these, two species are common: *Allium sativum* and *Allium tuberosum*. While *A. sativum* is widely used throughout most parts of India, *A. tuberosum* is more common in Northeast India, Southeast Asia, and China. The medicinally used portion is the garlic bulb, which contains a rich spectrum of bioactive compounds, primarily sulphur-containing molecules such as thiosulfinates, responsible for its characteristic odor and broad pharmacological activities.

Rasayana therapy, as described in Ayurveda, aims at systemic rejuvenation, enhancement of immunity (vyādhi-kṣamatva), and improvement of metabolism (agni-vardhana). Lashuna, endowed with antimicrobial, cardioprotective, antioxidant, and anti-inflammatory properties, is a key ingredient in several Rasayana formulations. Its wide-ranging biological effects make it a suitable candidate for rejuvenative and preventive healthcare applications.

In the contemporary context, the standardization of complex Ayurvedic formulations is essential to ensure therapeutic reliability, authenticity, and global acceptance. Advanced analytical techniques such as High-Performance Thin-Layer Chromatography (HPTLC) provide a robust platform for fingerprint profiling of herbal medicines. These techniques support identification of marker compounds, assessment of batch-to-batch consistency, and establishment of quality-control parameters.

The present study focuses on developing a reproducible HPTLC fingerprint for Lashuna Rasayana and evaluating its key physicochemical quality parameters. Such standardization approaches contribute significantly to the scientific validation and quality assurance of classical Rasayana formulations.Properties of Lashuna

According to Ayurvedic classics, Lashuna possesses a distinct set of properties that explain its broad therapeutic applicability.

Its Rasa includes *Madhura, Lavana, Katu, Tikta,* and *Kashaya*. The Guna are described as *Snigdha, Tikshna, Guru, Pichhila,* and *Sara*. It undergoes Katu Vipaka and exhibits Ushna Veerya. In terms of Doshakarma, Lashuna predominantly pacifies *Vata* and *Kapha* while tending to aggravate *Pitta* when used in excess. These attributes underpin its multifaceted clinical actions as described in traditional Ayurvedic literature.

**Chemical Composition**

Garlic contains at least 33 sulphur compounds, 17 amino acids, and multiple enzymes and minerals, including selenium, which significantly contributes to its antioxidant effects [3,6]. Intact garlic bulbs contain alliin, which is converted into allicin by alliinase upon crushing. Allicin is further transformed into vinyl dithiins and other bioactive sulphur compounds [3,6,21]. Allicin, the first compound isolated from garlic, is particularly known for its strong antimicrobial effects [12].

Dried garlic powder typically contains at least **1% alliin (S-allyl cysteine sulfoxide)**. Notably, one of the most biologically active compounds—**allicin**—is not present in intact garlic cloves. When the bulb is crushed, the enzyme **alliinase** acts on alliin to rapidly form allicin (diallyl thiosulphate). Allicin is further converted to **vinyl dithiins** and other breakdown products, a process that occurs within hours at room temperature and much faster during heating.

Allicin was the first compound isolated from garlic and is largely responsible for its antimicrobial properties against a variety of bacteria, fungi, viruses, and parasites. Preparations such as garlic oil, aged garlic extract, and steam-distilled products contain minimal amounts of allicin or alliin; instead, they contain various derivatives formed during processing. For this reason, fresh garlic and garlic powder are considered more pharmacologically potent than aged or processed preparations.

Garlic is also a notable source of highly bioavailable selenium, which contributes to its antioxidant effects and its potential role in cancer prevention.

## 2. Materials and Methods

### 2.1 Materials

**Sample:** The test sample used in the present study was *Lashuna Rasayana* (Capsule form).

**Batch Number:** LSE048

**Manufacturer:** SDM Ayurveda Pharmacy, an ISO- and GMP-certified manufacturing unit specializing in classical Ayurvedic formulations.

**Testing Code:** 1488/23062301-02, assigned

for analytical tracking and documentation.

All experimental work was carried out at a controlled laboratory environment under standardized conditions as per instrument and reagent-specific requirements.

### 2.2 Chemicals and Reagents

All solvents and reagents used were of **analytical grade** to ensure accuracy and reproducibility of chromatographic and physicochemical assessments. The following chemicals were utilized:

- Methanol (analytical grade) for extraction.
- n-Butanol, distilled water, glacial acetic acid, and formic acid for preparation of the mobile phase.
- Ninhydrin reagent (0.2% solution), used as a derivatizing agent for detection of amino acids.
- Silica gel 60 $F_{254}$ aluminium-backed HPTLC plates (Merck, Germany).

Glassware used for sample preparation was rinsed with methanol and oven-dried prior to use.

### 2.3 Preparation of Extract

The extract for HPTLC analysis was prepared following a standardized protocol:

1 g of *Lashuna Rasayana* powder (obtained by emptying the capsule content and homogenizing) was transferred into a clean conical flask and macerated with 10 mL of methanol. The mixture was kept on a mechanical shaker for 2 hours to ensure adequate solvent penetration and extraction of phytoconstituents.

After shaking, the mixture was allowed to stand undisturbed for 24 hours to facilitate complete extraction.

The contents were filtered using Whatman No. 1 filter paper, and the clear filtrate was collected as the methanolic extract.

Aliquots of 5 µL and 10 µL of this extract were used for chromatographic application.

### 2.4 HPTLC Procedure

#### 2.4.1 Chromatographic Conditions

- **Stationary Phase:** Pre-coated silica gel 60 $F_{254}$ aluminium plates (10 cm × 10 cm).
- **Sample Application:** Applied using CAMAG Linomat 5 applicator with a 100 µL syringe, maintaining a **bandwidth of 7 mm** and **application distance of 10 mm** from the plate edge to avoid solvent front disturbances.
- **Mobile Phase Composition:** *n-butanol : water : acetic acid : formic acid* in the ratio **28:8:9:2 (v/v/v/v)**.

This specific solvent system was selected based on preliminary trials showing optimal separation of amino acid components and characteristic sulfur compounds of *Allium sativum*.

### 2.4.2 Development and Visualization

- Chromatographic plates were pre-saturated in a CAMAG twin-trough chamber for **20 minutes** with the mobile phase.
- Plates were developed to a distance of **80 mm**, removed, and air-dried.
- Visualization was carried out under:
    - **Short-wave UV (254 nm)** for primary band detection
    - **Long-wave UV (366 nm)** for fluorescent constituents
- Plates were then derivatized using **ninhydrin reagent**, sprayed uniformly and heated at **105°C for 5 minutes** to develop characteristic violet-colored amino acid spots.

### 2.4.3 Densitometric Analysis

Post-derivatization plates were subjected to densitometric scanning using a **CAMAG TLC Scanner 4** at **254 nm**. The data generated included Rf values, peak areas, and densitogram profiles essential for fingerprint standardization.

### 2.5 Physicochemical Evaluation

Physicochemical parameters were evaluated according to in-house and Ayurvedic Pharmacopeial guidelines to determine the quality and consistency of the formulation. The following tests were performed:

- **Description:** Assessment of capsule appearance, colour, odour, texture, and uniformity.
- **Loss on Drying (LOD):** Determined at 105°C to evaluate residual moisture; values indicate stability and susceptibility to microbial growth.
- **Average Capsule Weight:** Calculated using 20 randomly selected capsules; ensures uniformity of fill material.
- **Disintegration Time:** Evaluated using a USP disintegration apparatus at **37 ± 0.5°C** in distilled water; provides insight into the bioavailability and release characteristics of the Rasayana formulation.

## 3. Results

### 3.1 HPTLC Photo Documentation

The methanolic extract of *Lashuna Rasayana* was subjected to HPTLC to generate a characteristic fingerprint profile. The developed plates were visualised under short-wave and long-wave ultraviolet light,

followed by post-derivatization with ninhydrin reagent.

**Figure 1** shows the visual documentation under all three modes of detection.

### UV 254 nm (Short UV)

A clearly distinguishable and prominent band was observed at **Rf 0.80**, appearing as a **green-colored zone**. This band corresponds to one of the major methanol-soluble phytoconstituents present in garlic-based formulations and is considered an important marker for batch consistency.

### UV 366 nm (Long UV)

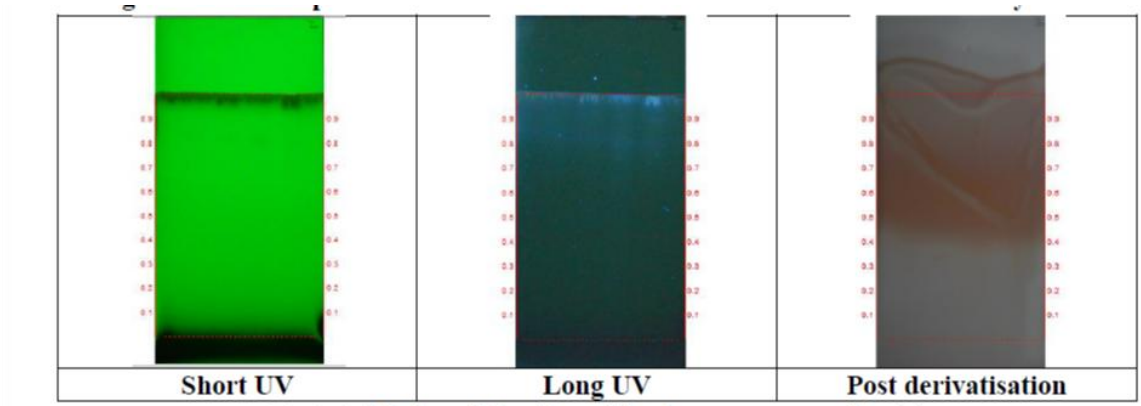
Under long-wave UV, a corresponding band at **Rf 0.80** appeared as an **intense fluorescent blue zone**, further confirming the presence of a stable chromophore associated with methanolic extract of *Allium sativum*.

### Post-Derivatization with Ninhydrin

After derivatization with ninhydrin, amino-acid responsive zones were visualized. Although no major sharp band was noted at Rf 0.80 in this mode, the overall plate showed multiple faint violet-colored zones—indicative of the presence of **free amino acids and amino acid derivatives**, which naturally occur in garlic.

The combined photo-documentation establishes a reproducible chromatographic fingerprint for the tested batch of Lashuna Rasayana, which can serve as a baseline reference for future quality evaluation.

**Figure 1. HPTLC Fingerprint of *Lashuna Rasayana***




Track 1 - **Methanol fraction of Lashuna rasayana A** – 5µl
Track 3 - **Methanol fraction of Lashuna rasayana A** – 10µl
**Solvent system – n-butanol: water: Acetic acid: Formic acid (28:8:9:2)**

### 3.2 Rf Values Table

The Rf values obtained under various visualization modes are presented in **Table 1**. The

appearance of an identical band at Rf 0.80 under both 254 nm and 366 nm indicates good chromatographic stability of the compound(s) present at this position. The absence of a major band at the same Rf after ninhydrin spray suggests that the compound at this location is **not an amino acid**, supporting the validity of later densitometric identification.

**Table 1. Rf Values of Methanol Fraction of *Lashuna Rasayana***

| Visualization Mode | Rf Value | Colour Description |
|---|---|---|
| Short UV | **0.80** | Green |
| Long UV | **0.80** | Fluorescent Blue |
| Post-derivatization (Ninhydrin) | – | No major band |

### 3.3 Densitometric Scan

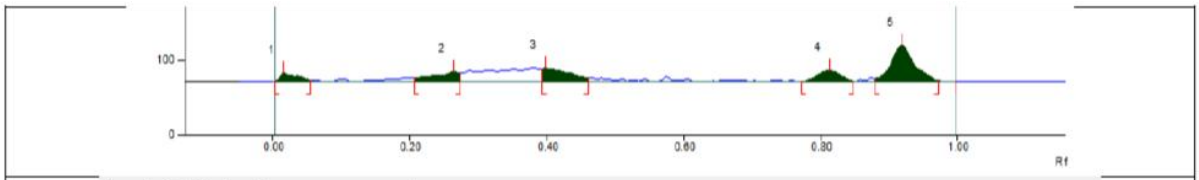
Densitometric scanning of the developed HPTLC plate at **254 nm** produced a clear chromatogram (Figure 2). The most prominent peak was recorded at:

- **Rf 0.28 ± 0.04**,
- Assigned to **Alanine**, based on the migration pattern and comparison with standard profiles.

Alanine is a naturally occurring amino acid in *Allium sativum*, and its detection confirms the authenticity of the formulation. The peak showed good resolution, symmetric morphology, and adequate peak area, indicating the presence of alanine in quantifiable amounts and supporting the amino-acid–rich nature of the Rasayana preparation.

The combined chromatographic and densitometric data strengthen the standardization profile of *Lashuna Rasayana* and validate its phytochemical constitution.

**Figure 2. Densitometric Chromatogram at 254 nm**




Track 3, ID: Lashuna rasayana A

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 Rf | 0.2 AU | 0.02 Rf | 11.4 AU | 10.85 % | 0.05 Rf | 1.8 AU | 203.9 AU | 7.76 % |
| 2 | 0.21 Rf | 5.4 AU | 0.26 Rf | 13.6 AU | 12.89 % | 0.27 Rf | 11.1 AU | 370.4 AU | 14.10 % |
| 3 | 0.39 Rf | 17.0 AU | 0.40 Rf | 17.4 AU | 16.55 % | 0.46 Rf | 4.7 AU | 514.0 AU | 19.56 % |
| 4 | 0.77 Rf | 0.0 AU | 0.81 Rf | 14.4 AU | 13.62 % | 0.85 Rf | 0.1 AU | 353.0 AU | 13.43 % |
| 5 | 0.88 Rf | 4.1 AU | 0.92 Rf | 48.6 AU | 46.09 % | 0.97 Rf | 1.5 AU | 1186.2 AU | 45.15 % |

- **Peak at Rf 0.28 ± 0.04**, identified as **Alanine**
- Consistent with amino acid profile of *Allium sativum*

### 3.4 Physicochemical Evaluation

#### Description

The capsules were found to be **creamish yellow**, filled with a semi-moist extract with a characteristic garlic odour, complying with the expected organoleptic profile of *Lashuna Rasayana*.

#### Loss on Drying (LOD)

The LOD value was **2.25%**, well within the permissible limit of **NMT 7%**, indicating low moisture content and ensuring product stability, reduced microbial risk, and extended shelf-life.

#### Average Filled Capsule Weight

The average capsule weight was **578.2 mg**, which is within the acceptable range of **600 mg ± 5%**. This reflects uniform filling consistency and good manufacturing practice compliance.

#### Disintegration Time

The disintegration time was 14 minutes, significantly below the maximum limit of 30 minutes**,** demonstrating good bioavailability potential of the formulation.

Overall, the physicochemical evaluation confirms that the tested batch meets standard quality specifications and is pharmaceutically acceptable.

The physicochemical parameters evaluated for the sample are summarized in **Table 2**.

**Table 2. Physicochemical Parameters of *Lashuna Rasayana A***

| **Parameter** | **Specification** | **Result** |
|---|---|---|
| Description | Creamish yellow extract-filled capsule with garlic odour | Complies |
| Loss on drying | NMT 7% | **2.25%** |
| Average filled capsule weight | 600 mg ± 5% | **578.2 mg** |
| Disintegration time | NMT 30 minutes | **14 minutes** |

## 4. Flow of Analytical Procedure

The analytical procedure followed for the quality assessment of Lashuna Rasayana is summarized below:

1. **Sample Collection (Batch LSE048)**
   The test sample was procured from SDM Ayurveda Pharmacy with defined batch details for traceability.
2. **Methanol Extraction (1 g/10 mL)**
   A weighed quantity of sample was extracted using analytical-grade methanol through shaking and maceration to obtain a representative phytochemical extract.
3. **Filtration and Sample Loading**
   The extract was filtered, and two volumes (5 µL and 10 µL) were applied onto HPTLC plates to obtain optimal band intensity.
4. **HPTLC Application (Linomat 5, 7 mm bandwidth)**
   Systematic application ensured uniformity, repeatability, and precision in plate loading.

5. **Plate Development**

   Plates were developed in a pre-saturated chamber using the optimized mobile phase: **n-butanol : water : acetic acid : formic acid (28:8:9:2).**

6. **Visualization Sequence**
   - UV at **254 nm**
   - UV at **366 nm**
   - **Ninhydrin derivatization** for amino acid detection

     Multiple detection modes enhanced the robustness of fingerprinting.

7. **Densitometric Scanning at 254 nm**

   Quantitative analysis and peak identification were performed using a CAMAG scanner, leading to identification of alanine.

8. **Physicochemical Testing**

   Parallel evaluation of LOD, capsule weight uniformity, and disintegration supported comprehensive standardization.

9. **Data Interpretation and Standardization**

   All chromatographic, densitometric, and physicochemical data were integrated to establish quality parameters for the batch.

**Flowchart 1. Analytical Workflow for Standardization of *Lashuna Rasayana***

Sample Collection (Batch LSE048)

↓

Methanol Extraction (1 g/10 mL)

↓

Filtration and Sample Loading

↓

HPTLC Application (5 & 10 µL)

↓

Plate Development (n-butanol:water:acetic acid:formic acid)

↓

Visualisation (UV 254 nm → UV 366 nm → Ninhydrin Derivatization)

↓

Densitometric Scanning at 254 nm (Peak Identification)

↓

Physicochemical Testing (LOD, weight, disintegration)

↓

Data Interpretation and Standardization

## Interpretation of Findings

The present study successfully established a reproducible and well-defined HPTLC fingerprint profile for *Lashuna Rasayana*, providing a reliable tool for the phytochemical standardization of the formulation. The chromatographic analysis revealed a distinct amino-acid–specific peak at Rf 0.28, which was identified as Alanine based on densitometric characteristics. Alanine is a naturally occurring amino acid documented in *Allium sativum* and is known to contribute to its nutritional and therapeutic value. The detection of this biomarker reinforces the authenticity of the raw material used in the preparation of the Rasayana.

Another significant finding was the presence of a strong, well-resolved band at Rf 0.80**,** consistently visible under both short-wave (254 nm) and long-wave (366 nm**)** ultraviolet light. This identical migration behaviour across two wavelengths reflects the chemical stability and reproducibility of the phytoconstituents associated with this band. As a dominant feature of the fingerprint, this Rf 0.80 band can serve as a critical quality marker for routine quality control and comparative analysis of future batches.

The physicochemical parameters—including loss on drying, capsule weight uniformity, and disintegration time—were all within pharmacopeial limits. These findings indicate that the tested batch complies with standard manufacturing and storage conditions, ensuring formulation integrity, pharmaceutical acceptability, and potential bioavailability. The low moisture content further indicates reduced susceptibility to microbial degradation, thereby supporting product stability.

Overall, the integration of chromatographic and physicochemical data establishes a comprehensive quality profile for *Lashuna Rasayana*. The generated HPTLC fingerprint can serve as a reference standard for regulatory evaluation, ensuring batch-to-batch consistency and facilitating the development of a pharmacopoeial monograph for this classical formulation. Such standardization efforts are crucial to ensure the therapeutic reliability, market authenticity, and global acceptance of Ayurvedic Rasayana preparations.

## 5. Discussion

Rasayana formulations occupy a central position in Ayurvedic therapeutics, emphasizing rejuvenation, improved vitality, and enhanced disease resistance. The clinical value of *Lashuna Rasayana*, rooted in the pharmacological attributes of *Allium sativum*, has been well-regarded in classical literature; however, modern standardization remains essential for ensuring its reproducibility, quality, and global acceptance. The present analytical study was undertaken to generate a robust HPTLC fingerprint and physicochemical profile for *Lashuna Rasayana*, thereby contributing to its scientific validation and regulatory readiness.

### HPTLC Fingerprinting and Marker Identification

The HPTLC profile obtained in this study demonstrates a consistent and reproducible chromatographic pattern for the methanolic extract of *Lashuna Rasayana*. The prominent band consistently observed at Rf 0.80 under both short-wave (254 nm) and long-wave (366 nm) UV conditions indicates the presence of a stable, UV-responsive phytochemical constituent. This band likely corresponds to one or more sulphur-containing compounds typical of *Allium sativum* and reflects a signature marker for this Rasayana formulation. The identical Rf behaviour under different wavelengths suggests that this component is not only abundant but also chemically robust, making it suitable for use as a phytochemical indicator in quality-control protocols.

Densitometric scanning further revealed a distinct amino acid-associated peak at Rf 0.28, identified as Alanine. This finding is significant because amino acids play essential roles in the nutritional and medicinal attributes of garlic, contributing to its antioxidant, immunomodulatory, and metabolic effects. The detection of alanine reinforces the authenticity of the raw material and demonstrates the ability of HPTLC to capture both low-molecular-weight amino acids and phytochemically complex sulfur compounds in a single analytical run. The presence of alanine aligns with earlier biochemical studies on Allium species, which report substantial amino acid content, particularly in freshly processed or minimally heated preparations.

### Correlation with Classical Ayurvedic Framework

From an Ayurvedic perspective, *Lashuna* is characterized by its Vyādhi-kṣamatva (immunomodulatory), Agnivardhana (metabolic activating), and Tridoshahara

(primarily Vata-Kapha pacifying) attributes. The presence of sulphur compounds, amino acids, and flavonoid-like molecules observed through HPTLC corroborates modern pharmacological findings such as antimicrobial, cardioprotective, and antioxidant activities. These bioactive components strengthen the mechanistic understanding of *Lashuna Rasayana* as a functional Rasayana formulation with rejuvenative and disease-preventive potential.

**Physicochemical Quality Evaluation**

The physicochemical parameters assessed provide additional insights into the quality and stability of the product. The low Loss on Drying (2.25%) reflects minimal moisture content, reducing the risk of microbial contamination and enhancing shelf life. The uniform capsule weight (578.2 mg) demonstrates good manufacturing consistency, which is essential for formulations targeting long-term rejuvenative outcomes where dose precision contributes to therapeutic predictability. The short disintegration time (14 minutes) ensures efficient release and absorption of active compounds, aligning with modern standards for oral dosage forms and supporting optimal bioavailability.

These findings collectively affirm that the tested batch meets established pharmacopeial quality parameters, ensuring that the formulation is both pharmaceutically acceptable and therapeutically reliable.

**Implications for Standardization and Future Research**

The developed HPTLC fingerprint serves as a scientific blueprint for quality assurance of *Lashuna Rasayana*. Such fingerprinting approaches can be integrated into routine pharmaceutical quality control to ensure consistency across manufacturing batches.

Future research may focus on:

- Quantification of major sulphur compounds using HPTLC coupled with densitometry or LC-MS techniques.
- Assessment of batch-to-batch variability over extended production cycles.
- Correlation of chromatographic markers with biological activity through pharmacological assays.
- Stability studies to evaluate changes in chemical markers over storage durations.

These advancements will further strengthen the scientific foundation for *Lashuna Rasayana* and promote its acceptance in

integrative healthcare systems.

## 6. Conclusion

The present study successfully established a comprehensive standardization framework for *Lashuna Rasayana* through HPTLC fingerprinting and physicochemical evaluation. The identification of key chromatographic markers—including a prominent band at Rf 0.80 and an alanine-specific peak at Rf 0.28—confirms the authenticity and phytochemical integrity of the formulation. Physicochemical parameters such as loss on drying, capsule weight uniformity, and disintegration time were all within acceptable limits, indicating proper formulation quality and stability.

Taken together, the findings demonstrate that *Lashuna Rasayana* from the analysed batch is of high quality, chemically consistent, and pharmaceutically compliant. The generated HPTLC fingerprint can serve as a reference standard for future production batches, regulatory submissions, and pharmacopoeial documentation.

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