

Standardization and Estimation of Lupeol in *Uraria picta* by HPLC and HPTLC Techniques

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Received: 12th Mar, 2026 | Revised: 24th Mar, 2026 | Accepted: 14th Apr, 2026 | Available Online: 30th Apr, 2026

ABSTRACT

The present study focuses on the standardization and quantitative estimation of lupeol in *Uraria picta* using High-Performance Liquid Chromatography (HPLC) and High-Performance Thin Layer Chromatography (HPTLC) techniques. Lupeol, a bioactive triterpenoid, is widely known for its pharmacological properties and is considered an important marker compound for quality control of herbal drugs. In the HPLC method, separation was achieved using a C18 column with a mobile phase of methanol:acetonitrile (90:10 v/v) at a flow rate of 1 mL/min and detection at 210 nm. Lupeol showed a well-defined peak at a retention time of 4.112 ± 0.2 minutes. The hydroalcoholic extract of *Uraria picta* exhibited a corresponding peak, confirming the presence of lupeol, with a quantified assay value of 0.119%. For HPTLC analysis, optimized chromatographic conditions using n-hexane:ethyl acetate (80:20 v/v) as the mobile phase resulted in a distinct spot of lupeol at an R_f value of 0.43. The % lupeol content in the extract was found to be 0.276%. The results obtained from both techniques demonstrated good specificity and reliability. The developed methods are simple, precise, and suitable for routine quality control analysis of *Uraria picta*. The study highlights the importance of using complementary analytical techniques for effective standardization of herbal drugs.

Keywords: *Uraria picta*, Lupeol, HPLC, HPTLC, Standardization, Phytochemical analysis, Triterpenoid, Herbal drug, Chromatography, Quality control.

How to cite this article: Malakar RK, Somkuwar S, Singhai AK. Standardization and Estimation of Lupeol in *Uraria picta* by HPLC and HPTLC Techniques. *Int J Drug Deliv Technol.* 2026;16(39s): 832-837. DOI: 10.25258/ijddt.16.39s.114

Source of support: Nil.

Conflict of interest: None

Introduction

Medicinal plants have been widely utilized in traditional systems of medicine due to their rich content of bioactive phytoconstituents. Standardization of herbal drugs is essential to ensure their quality, safety, and efficacy, as variations in phytochemical composition may occur due to geographical, environmental, and processing factors. Among various phytoconstituents, triterpenoids such as lupeol have gained considerable attention due to their diverse pharmacological activities including anti-inflammatory, antioxidant, anticancer, hepatoprotective, and antimicrobial properties (Dar et al., 2023; Hlatshwayo et al., 2023).

Uraria picta (family: Fabaceae), commonly known as "Prishnaparni," is an important medicinal plant used in

Ayurvedic formulations such as Dashamoola. It has been traditionally employed for the treatment of inflammatory disorders, fever, wounds, and respiratory ailments. Phytochemical investigations of *Uraria picta* have revealed the presence of flavonoids, glycosides, steroids, and triterpenoids, among which lupeol is considered a significant bioactive marker compound responsible for many of its therapeutic effects (Faujdar et al., 2023; Bhusare et al., 2021).

Standardization and quantitative estimation of lupeol in *Uraria picta* are crucial for ensuring consistency and reproducibility of herbal formulations. Advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC) and High-Performance Thin Layer Chromatography (HPTLC) are widely used for

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the qualitative and quantitative analysis of phytoconstituents. HPLC offers high sensitivity, accuracy, and reproducibility, making it suitable for precise quantification, while HPTLC provides a simple, rapid, and cost-effective method for fingerprint profiling and simultaneous analysis of multiple samples (Mangal et al., 2026; Shah et al., 2025).

The combination of HPLC and HPTLC techniques provides a comprehensive approach for the standardization of herbal drugs by ensuring both qualitative identification and quantitative estimation of marker compounds. Therefore, the present study aims to develop and validate HPLC and HPTLC methods for the standardization and estimation of lupeol in *Uraria picta*. This will help in establishing reliable quality control parameters and contribute to the development of standardized herbal formulations.

Material and Methods

Material

The plant material, *Uraria picta*, was collected and authenticated, and the hydroalcoholic extract was prepared using suitable extraction methods. Standard lupeol was procured from a certified supplier for analytical comparison. HPLC-grade methanol and acetonitrile were used for chromatographic analysis, while analytical-grade n-hexane and ethyl acetate were used for HPTLC studies. Precoated silica gel 60 F₂₅₄ HPTLC plates were employed as the stationary phase. All solvents and reagents used were of analytical or HPLC grade. Distilled water was used throughout the study. The analysis was carried out using standard laboratory instruments including an HPLC system equipped with a UV detector and an HPTLC system with a densitometric scanner.

Methods

Extraction by Soxhlet extraction method

50 gm dried powdered aerial parts of *Uraria picta* has been extracted with hydroalcoholic solvent (ethanol: water, 70: 30 v/v) using soxhlet extraction method for 48 hrs, filtered and dried using vacuum evaporator at 40°C.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of extract recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it

into percentage. The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{100} \times \text{Weight of powder drug Taken}$$

Phytochemical screening

The qualitative phytochemical analysis helps in detecting the presence or absence of these metabolites, while quantitative estimation provides insights into their concentration. Such screening not only establishes the scientific basis for the traditional use of plants but also guides further isolation, purification, and characterization of active principles. Therefore, phytochemical screening serves as a foundation for pharmacological investigations, standardization of herbal formulations, and discovery of new lead compounds for drug development. The chemical tests were performed for testing different chemical groups present in extracts (Kokate, 1994).

Identification of marker compound (Lupeol) in *Uraria picta* extract by HPLC

Reagents and chemicals

Lupeol was kindly provided by Phyto Drug Pvt Ltd, Pilukhedi. Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade water from Merck Ltd, New Delhi, India. The HPLC system (Waters) consisted of a pump, a U.V. Visible detector, a Thermo C₁₈ (250 X 4.6 mm, 5µm) column, a N2000 Chromatography Data System.

Table 1: Selection of Separation Variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5 µm
Bonded Phase	Octadecylsilane (C ₁₈)
Mobile Phase	
Methanol	90
Acetonitrile	10
Flow rate	1ml/min
Temperature	Room temp.
Sample Size	20 µl
Detection wavelength	210 nm
Retention time	4.112± 0.2 min

Preparation of standard solution

10mg of Lupeol was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm. From stock solutions of Lupeol 1 ml was

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taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25µg/ml concentration.

Analysis of extract

10 mg of extract were taken in 10 ml volumetric flask and dilute upto the mark with Methanol; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. The resulting solution was again filtered using 0.45µ membrane filter and then sonicated for 10 min (Dahiya et al., 2025).

Quantitative estimation of Lupeol by HPTLC

Instrumentation

Sample application was performed using a CAMAG Linomat V automatic sample applicator (MuttENZ, Switzerland) on aluminum-backed HPTLC plates precoated with silica gel 60 F₂₅₄ (10.0 × 10.0 cm; E. Merck KGaA, Germany). The chromatographic separation was carried out in a CAMAG twin-trough development chamber pre-saturated with the mobile phase consisting of n-Hexane: ethyl acetate (80:20, v/v). The solvent front was allowed to migrate up to a distance of 70.0 mm using 10.0 mL of the mobile phase. After development, the plates were dried in an oven at 60°C for 5 min.

Sample bands were applied with the first track positioned at 15.0 mm from the plate edge, maintaining a distance of 11.6 mm between adjacent tracks. Densitometric scanning was performed using a CAMAG TLC Scanner III controlled by WinCATS software (version 1.4.3.6336). Scanning was carried out in absorption mode using a deuterium–tungsten (D₂ & W) lamp at a wavelength of 366 nm. The scanning parameters included slit dimensions of 5.00 × 0.30 mm (micro), scanning speed of 20 mm/s, data resolution of 100 µm/step, scan start position at 5.0 mm, and scan end position at 75.0 mm. The optical system was optimized for light, with second-order optical filtering, automatic detector mode, remission measurement type, and a photomultiplier tube (PMT) voltage set at 443 V.

Pure Standards

Reference standards of Lupeol (99.0% purity) were procured from Himedia Pvt. Ltd., India.

Standard Solution Preparation

Standard stock solutions were prepared by accurately weighing 10 mg each of Lupeol transferring them into

separate 10 mL volumetric flasks. The volume was adjusted with methanol to obtain a concentration of 1 mg/mL. The solutions were stored in tightly closed containers under refrigerated conditions. From this stock solution, 1 mL was further diluted to 10 mL with methanol to obtain stock solution A. Calibration curves were prepared in accordance with the International Conference on Harmonization (ICH) guidelines.

Sample Preparation

A stock solution of the sample was prepared by dissolving 10 mg of the dried extract in methanol and making up the volume to 10 mL in a volumetric flask. The solution was stored under refrigerated conditions until analysis.

Calibration Curve

Calibration curves were constructed in accordance with ICH guidelines. Standard Lupeol solutions in the concentration range of 1–5 µL were applied in triplicate as bands of 6.0 mm width on aluminum-backed HPTLC plates precoated with silica gel 60 F₂₅₄ (10.0 × 10.0 cm). The bands were applied using a CAMAG Linomat V applicator, maintaining a distance of 11.7 mm between adjacent tracks, 12 mm from the plate edge, and 8 mm from the bottom of the plate. The application rate was kept constant at 15µL/s (Gurupriya et al., 2018).

Chromatographic development was carried out in a CAMAG twin-trough chamber pre-saturated for 20 min with the mobile phase consisting of n-Hexane: ethyl acetate (80:20v/v). The plates were developed to a distance of 70 mm, followed by air-drying. Densitometric scanning was performed using a CAMAG TLC Scanner III at a wavelength of 254 nm in absorption mode. Lupeol was well resolved with an R_f value of 0.66. Peak areas were recorded, and calibration curves were generated by plotting peak area against applied concentration. Linear regression analysis was employed to establish the calibration equation and assess linearity.

Sample Assay

Sample and standard solutions were applied to the TLC plates and developed under identical chromatographic conditions. After development and drying, the analytes were well resolved from other constituents. Densitometric scanning was performed at 366nm, and peak areas corresponding to Lupeol were recorded for quantitative estimation.

Results and Discussion

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The present study successfully developed and optimized analytical methods for the standardization and estimation of lupeol in *Uraria picta* using both HPLC and HPTLC techniques. These methods provided reliable, accurate, and reproducible results for the identification and quantification of the marker compound.

In the HPLC method, the use of a C18 column with a mobile phase consisting of methanol and acetonitrile (90:10) resulted in efficient separation of lupeol with a sharp and well-defined peak at a retention time of 4.112 ± 0.2 minutes. The chromatogram of the hydroalcoholic extract also showed a peak at the same retention time, confirming the presence of lupeol in the extract. The quantitative estimation revealed a % assay of 0.119%, indicating that lupeol is present in measurable amounts in *Uraria picta*.

The HPTLC method further supported the findings of the HPLC analysis. The optimized mobile phase system of n-hexane: ethyl acetate (80:20 v/v) provided good resolution and separation of lupeol with an R_f value of 0.43. The extract showed a corresponding spot at the same R_f value, confirming the identity of lupeol. The % lupeol content determined by HPTLC was found to be 0.276%, which is comparatively higher than that obtained by HPLC. This variation may be attributed to differences in sensitivity, sample preparation, and detection techniques between the two methods.

The developed methods demonstrated good specificity, as no interfering peaks were observed at the retention time or R_f value of lupeol. The consistency of results obtained from both techniques confirms their suitability for routine quality control and standardization of *Uraria picta*. The higher sensitivity of HPTLC for fingerprint analysis and the precision of HPLC for quantification complement each other, providing a comprehensive analytical approach.

The study establishes validated HPLC and HPTLC methods for the estimation of lupeol, which can be effectively applied for the standardization of herbal formulations containing *Uraria picta*. These methods ensure the quality, safety, and efficacy of herbal products by confirming the presence and quantity of bioactive marker compounds.

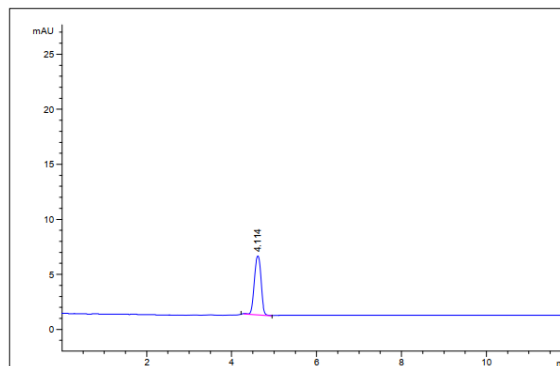


Figure 1: Chromatogram of standard Lupeol

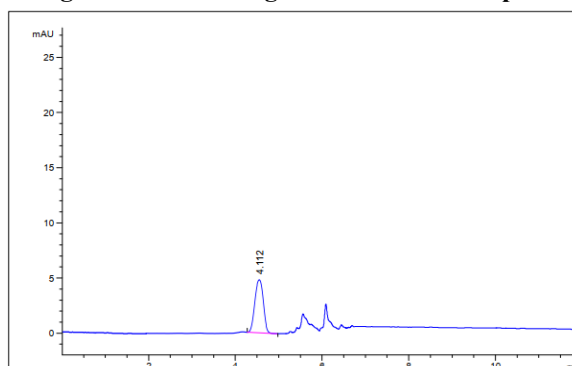


Figure 2: Chromatogram of Hydroalcoholic extract of *Uraria picta*

Table 2: Quantitative estimation of Lupeol in *Uraria picta* extract

S. No.	Standard/ Extracts	RT	Area	% Assay
1.	Lupeol	4.112		
2.	Hydroalcoholic Extract	4.112	325.698	0.119

Table 3: Optimized chromatographic conditions for Lupeol and extract

Parameter	Conditions
Mobile phase	n-Hexane: ethyl acetate (80:20v/v)
Stationary phase	Precoated silica gel 60 F ₂₅₄ HPTLC plates (100 mm × 100 mm, layer thickness 0.2 mm)
Plate size	10.0 × 10.0 cm
Chamber saturation time	20 min
Distance run	70 mm
Temperature	27 ± 2 °C
Application mode	Band
Band length	6.0 mm
Distance between tracks	11.6 mm

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Detection wavelength	366 nm
Scanning speed	20 mm/s
Data resolution	100 $\mu\text{m}/\text{step}$
Light source	Deuterium–Tungsten (D ₂ & W) lamp
Measurement mode	Absorption
Retardation factor (Rf) of Lupeol	0.43
Diluent	Methanol

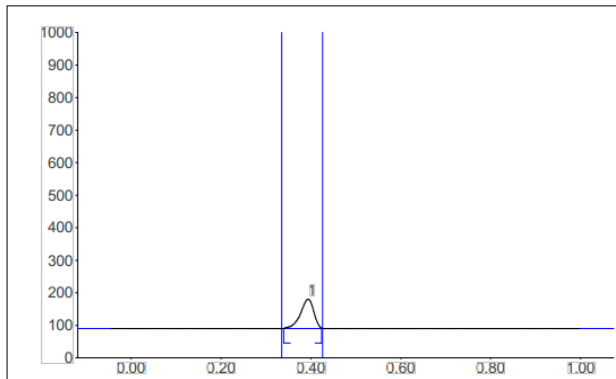


Figure 3: Chromatogram of standard Lupeol

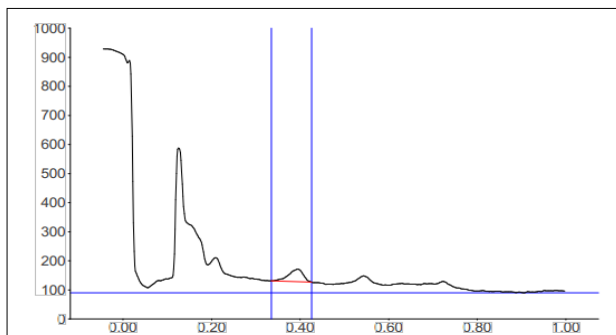


Figure 4: Chromatogram of *Uraria picta* extract

Table 4: Results of % Lupeol content in *Uraria picta* extract

Extract	Area	Rf	% Lupeol content
<i>Uraria picta</i> extract	1126.85	0.43	0.276

Conclusion

The present study successfully developed and validated simple, reliable, and reproducible HPLC and HPTLC methods for the standardization and quantitative estimation of lupeol in *Uraria picta*. Both techniques effectively confirmed the presence of lupeol, with well-defined retention time and Rf values, ensuring

specificity of the methods. The HPLC method provided accurate quantification, while HPTLC offered efficient fingerprint profiling and comparative analysis. The results demonstrated that *Uraria picta* contains measurable amounts of lupeol, supporting its therapeutic potential. The developed methods are suitable for routine quality control and standardization of herbal formulations containing *Uraria picta*, ensuring consistency, safety, and efficacy of the plant-based products.

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