ABSTRACT

Objective: This is aimed to study the chromatographic evaluation of triterpenoid, i.e., lupeol from methanolic extracts of leaves, stem, and inflorescence of Heteropogon contortus.

Methods: The high-performance thin-layer chromatography (HPTLC) densitometry determination of lupeol was performed using optimized mobile phase toluene:methanol:formic acid (7:3:0.3 v/v) with a derivatization of freshly prepared anisaldehyde-sulfuric acid. For densitometry measurements, the plates were scanned at 530 nm absorbance/reflectance wavelength. Quantification of lupeol marker compound in H. contortus leaves, stem, and inflorescence is estimated using 2-12 μg/spot.

Results: The appearance of light purple bands on the chromatograms confirmed the lupeol component in plant samples. Further, the confirmation of the compound is done from the densitometric scanning by comparing λmax values. From this, it is reported that lupeol is present in leaf samples, i.e., 10 mg/g of dry wt., while in rest of the two samples, it is found absent.

Conclusion: The leaves of H. contortus (spear grass) are a good source of lupeol and can be used as an alternate natural source to synthesize herbal drugs to control cancer and other anti-inflammatory agents. The presently selected HPTLC is validated and most accurate for the quantification and identification of lupeol present in the selected plant. The leaves of the species which are rich in lupeol can be used in pharmaceutical industry.

Keywords: Lupeol, High-performance thin-layer chromatographic, Triterpenoid, Heteropogon contortus.
were then filtered and concentrated using rotary vacuum evaporator and then lyophilized with Allied Frost Lyophilizer-FD-3. The obtained lyophilized powder of samples was accurately weighed and then dissolved in methanol (1 mg/ml). These solutions were then used as test solutions for HPTLC analysis.

**Equipment**
- Spotting device-CAMAG Linomat V sample applicator (CAMAG, Switzerland)
- Syringe-100 µl Hamilton syringe
- TLC Chamber-CAMAG twin trough chamber.
- Scanner-CAMAG TLC scanner with D₂ and Hg lamp, Reprostar and win CATS planar chromatography manager and CAMAG integration software and TLC viewing cabinet (all from CAMAG, Muttenz, Switzerland).

**Preparation of standard stock solution**
Accurately weighed reference standard lupeol (5 mg) was transferred to 10 ml volumetric flask, dissolved in 5 ml methanol (1 mg/ml). The stock solution was ready to use for HPTLC.

**Chromatographic analysis**
The HPTLC analysis was performed using precoated silica gel 60 F₂₅₄ aluminum plates. Linomat V autosampler was used for spotting of standard lupeol (2, 4, 6, 8, 10, and 12 µl each) and sample solutions (10 µl each) operated with 6 mm band length, distance between the tracks 10 mm, distance from the bottom of the plate, 8.0 mm. The linear ascending developments were carried out up to a distance of 75 mm in a CAMAG twin trough chamber presaturated with mobile phase toluene:methanol:formic acid (7:3:0.3 v/v) for 25 minutes, and anisaldehyde is used as derivatizing reagent. The plates were then scanned in the CAMAG TLC scanner V, operated by win CATS software in the absorbance mode at 530 nm. The images of the plates were then taken in the visible mode.

**Calibration curve of lupeol**
The standard stock solutions (1 mg/ml) of lupeol (2-10 µg spot⁻¹) were applied in triplicate on an HPTLC plate. These were developed as prescribed above. The calibration curve was drawn by plotting the standard concentrations ranges from 2 to 12 µg spot⁻¹ versus peak area. The linear calibration curve was obtained from which linear regression equation and correlation coefficients were obtained.

**Limit of detection (LOD) and limit of quantification (LOQ)**
The LOD and LOQ for the present marker compound, i.e., lupeol was calculated using the following equations:

\[
\text{LOD} = 3.3 \times \text{SD} / S
\]

\[
\text{LOQ} = 10 \times \text{SD} / S
\]

Where SD stands for the standard deviation of replicates under the same conditions, and S is the slope of the calibration curve.

**Robustness**
Robustness was studied in triplicate at 800 ng spot⁻¹ by making small changes to the volume of the mobile phase, the composition of the mobile phase, and saturation time of development chamber. The effects on the results were examined by calculation of relative SD (RSD) (%) and Rₐ values.

**Precision and accuracy**
Instrumental precision was checked by repeated scanning (n=6) of same spot for each standard separately (10 µg spot⁻¹) and expressed as % RSD. The accuracy of the present method was checked using a recovery study by spiking the sample with two levels of standards (6 and 12 µg band⁻¹). The % recovery was calculated using the formula given by [21].

**Quantitative determination of lupeol**
The samples prepared from leaves, stem, and inflorescence of *H. contortus* were analyzed by using the validated HPTLC method, and the amount of the referred standard was calculated from the standard calibration curve of lupeol.

**RESULTS AND DISCUSSION**
During the present investigation, chromatographic conditions were optimized for the detection of lupeol using modified mobile phase toluene:methanol:formic acid (7:3:0.3 v/v) which gave the better comparing the Rₛ and ultraviolet (UV) spectra of the spots to those of the standard.

**Linearity**
The different concentrations (2, 4, 6, 8, 10, and 12 ng spot⁻¹) of lupeol were applied on the HPTLC plate in triplicates, separately. The plate was developed as prescribed above. The calibration curve was drawn by plotting the standard concentrations ranges from 2 to 12 µg spot⁻¹ versus peak area. The linear calibration curve was obtained from which linear regression equation and correlation coefficients were obtained.

**Fig. 1: Calibration curve of lupeol**

**Fig. 2: High-performance thin-layer chromatography profile of lupeol (track 1-6) and methanolic plant samples of Heteropogon contortus (racks 7-9); 7(HCLM), 8(HCSM), 9(HCIM)**
The developed method has been found to be selective, specific, and robust for the screening and quantification of lupeol in medicinally important grass *H. contortus*. The method is validated as per the ICH guidelines in terms of precision, repeatability, and accuracy (Table 1). The linearity range for lupeol was found to be 2-12 µg spot\(^{-1}\) with correlation coefficient, i.e., 0.997. A linear calibration curve was obtained for the standard compound as described above. LOD value for a standard compound is 0.84 ng spot\(^{-1}\), whereas LOQ value is 0.25 ng spot\(^{-1}\). The average % recovery at 3 different levels of the referred marker compound was found to be 99.45 (Table 1). The accuracy of the present method was determined by spiking the sample along with known amounts of standard (100 µg band\(^{-1}\)). The present method showed a recovery of 99.05% for the addition of 100 µg band\(^{-1}\) of standard correspondingly (Table 2).

The results of quantification of lupeol are given in Table 3. During the present investigation, lupeol is only detected in leaf samples, whereas in rest of the two samples, i.e., stem and inflorescence, it is found to be absent. The amount of lupeol present in the leaf sample is 10±0.13 mg/g of dry wt.

### CONCLUSION

The presently selected HPTLC is validated and most accurate for the quantification of lupeol in medicinally important grass *H. contortus*. The developed method has been found to be sensitive, accurate, precise, specific, and robust for the screening and quantification of sterols. Although, HPTLC has a few limitations like limited developing distance and lower plate efficiency in comparison to HPLC and gas chromatography. HPTLC is still an effective tool for quality evaluation of medicinal plants due to its simplicity, low cost, and low requirements. Thus, leaves of the species which are rich in lupeol can be used in pharmaceutical industry.

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### Table 3: Quantification of lupeol from different plant parts of *H. contortus*

| Plant parts | Sample codes | Amount of lupeol in plant sample (% w/w) |
|-------------|--------------|----------------------------------------|
| Leaves      | HCLM         | 10±0.13                                |
| Stem        | HCSM         | ND                                     |
| Inflorescence | HCIM       | ND                                     |

*ND means not detected. *H. contortus*: *Heteropogon contortus*

### Table 2: Recovery study of lupeol by the proposed HPTLC method

| Marker compound | Amount present in sample (µg) | Amount added (µg) | Theoretical amount (µg) | Amount found (µg) | Recovery (%) | Average recovery (%) |
|-----------------|-------------------------------|-------------------|------------------------|------------------|--------------|----------------------|
| Lupeol          | 100                           | 75                | 175                    | 172.62           | 98.64        | 99.05                |
|                 | 100                           | 100               | 200                    | 198.46           | 99.23        |                      |
|                 | 100                           | 125               | 225                    | 223.41           | 99.29        |                      |

HPTLC: High-performance thin-layer chromatography
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