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HPTLC densitometric evaluation by simultaneous estimation of galangin in *Alpinia galanga* and *Alpinia officinarum*

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ABSTRACT

The aim of the work is to develop a simple, rapid, selective and cost effective HPTLC method for the determination of galangin in Alpinia galanga & Alpinia officinarum. The HPTLC densitometric technique was therefore, selected for the quantitative and qualitative determination of galangin in Alpinia galanga and Alpinia officinarum respectively. There are different analytical methods were used to isolate constituents from Alpinia galanga and Alpinia officinarum. Literature survey reveals that no HPTLC method so far is reported for the determination of galangin in Alpinia galangal & Alpinia officinarum. The present study describes HPTLC method for the qualitative and quantitative estimation of galangin. Both the methods were found to be simple, precise, specific, reproducible, sensitive and accurate and can be used for the quantitation of galangin and routine quality control of raw materials and formulations containing galangin.

Key words: *Alpinia galangal, Alpinia officinarum*, HPLC, HPTLC, galangin and 3-O-methyl galangin.

INTRODUCTION

Alpinia galanga Linn. is a perennial aromatic rhizomatous herb and an important crop plant of family Zingiberaceae, which is cultivated in India, China, Thailand, Malaysia and Indonesia. [1] Galanga (also called galangal, galingale, or galangal) is a species of the ginger family [Zingiberaceae]. There are two different species of galanga, smaller galanga [*Alpinia officinarum* Hance] and greater galanga (*Alpinia galanga* Willd.). *Alpinia galanga* is known to possess analgesic activity, [2] immunostimulant activity, [3] aphrodisiac activity, [4] antifungal, anti-inflammatory and antiretroviral agents [5] and anticancer. [6]

Alpinia officinarum is a perennial herb, belonging to family Zingiberaceae, originated in China and mainly cultivated in Southeast Asia. Phytochemicals have been found to be associated with the herb which includes quercetin, kaemferol, isorhamnetin, kaemferide, galangin, alpinol, and galangal. The plant has been reported to possess potent anti-inflammatory, antibacterial, antifungal, antiviral, diuretic, and anticancer properties. [7]

Galangin is an important constituent from *Alpinia galangal* & *Alpinia officinarum.* There are different analytical methods were used to isolate constituents from *Alpinia galanga* & *Alpinia officinarum*. Reversed phase high performance liquid chromatography-diode array detection (RP-HPLC-DAD) method used for quantification of *Alpinia officinarum* preparations by using galangin as a marker. [8]

High-performance liquid chromatographic (HPLC) method was developed for the assessment of two major bioactive flavonoids: galangin and 3-O-methyl galangin in *Alpinia officinarum* Hance. [9]

Ultra-performance liquid chromatography (UPLC) coupled to electrospray ionization (ESI+) tandem mass spectrometry (MS) was developed to identify and characterize the diarylheptanoids in the supercritical fluid extract (SFE) of *Alpinia officinarum.* [10]

Diarylheptanoids were isolated from the ethanol extract from the rhizomes of *Alpinia officinarum* Hance. The structural identification of these compounds was mainly achieved by spectroscopic methods. Optical rotations were measured on a JASCO P-1020 spectropolarimeter. UV spectra were obtained on a Shimadzu UV- 260 spectrophotometer. IR spectra were recorded on an Avatar 360-ESP spectrophotometer. 1H and 13C NMR spectra were recorded on a DRX-400 spectrometer. EI-MS on an Agilent 5973 N mass spectrometer and HR-ESI-MS were carried out on a Bruker APEX 7.0 TESLA FT-MS apparatus. Normal-phase column chromatography followed by semi-preparative reversed-phase HPLC has been used to isolate diarylheptanoids from the rhizomes of *Alpinia officinarum*, The levels of these diarylheptanoids in root material were determined quantitively by HPLC with UV detection and the assay methods so developed were simple, rapid and accurate. [11]

A novel diarylheptanoid bearing flavonol moiety, named officinin A, along with two known compounds galangin and kaempferide were isolated from the rhizomes of *Alpinia officinarum* Hance. The structure elucidation was accomplished by HR- ESI–MS, 1D and 2D NMR methods. [12]

A novel dimeric diarylheptanoid, named alpinin A was isolated from the rhizomes of *Alpinia officinarum* Hance and its structure was elucidated on the basis of spectral analysis, including HR-IT-TOF-MS, 1D and 2D NMR. [13]

Simultaneous determination of alpha-pinene, beta-pinene, eucalyptol and alpha-terpineolin in essential oil from Alpinia officinarum is carried out by GC [14]

MATERIALS AND METHODS

2.1 Analytical studies

[A] *Alpinia galanga* and *Alpinia officinarum*

2.1.1 Development of HPTLC protocol for the active constituents from *Alpinia galanga* and *Alpinia officinarum*

2.1.1.1 Apparatus

The spotting device used was Linomat IV automatic sample spotter (Camag, Muttenz, Switzerland); the syringe was 100 µL (Hamilton Bonaduz, Switzerland); The TLC chamber was a glass twin trough chamber (20 × 10 × 4 cm) (Camag, Switzerland); the densitometer was a TLC Scanner 3 linked to WINCATS software (Camag, Switzerland); the HPTLC plates of 20 × 10 cm, 0.2 mm thickness, precoated with silica gel 60F254 (E. Merck Kga A, Cat. no. 1.05548, Darmstadt, Germany) were used.

2.1.1.2 Preparation of standard solution of galangin

Stock solution of galangin was prepared by dissolving 10 mg of accurately weighed galangin in 10 mL methanol. From this stock solution, standard solutions of 100 ppm/mL were prepared in 10 mL volumetric flasks and adjusting the volume with methanol.

2.1.1.3 Preparation of sample solution

[A] *Alpinia galanga*

The dried extracts of *Alpinia galanga* (10 mg) were transferred to 10 mL volumetric flask and the volume was made upto 10 mL with methanol to furnish the final concentration 100 µg/mL.

[B] *Alpinia officinarum*

The dried extracts of *Alpinia officinarum* (10 mg) were transferred to 10 mL volumetric flask and the volume was made upto 10 mL with methanol to furnish the final concentration 100 µg/mL.

2.1.1.4 Calibration curve for galangin

The standard solution of galangin was applied in triplicate on a HPTLC plate (20×10 mm) in 2,4,8,12,16 and 20 µL quantity. The plate was developed in a solvent system hexane-ethylacetate-acetic acid (7.5:2:0.5 v/v) at 25±2ºC temperature and 40% relative humidity up to a distance of 8 cm. After development, the plate was dried in air and scanned at 254 nm wavelength. The peak area was recorded. Calibration curve was prepared by plotting peak area vs. concentration.

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2.1.1.5 Validation of the method

The proposed method was validated as per the recommendations laid down by International Conference on Harmonization (ICH) guidelines (ICH Q2A, 1994; ICH Q2B, 1996).

2.1.1.5.1 Precision

Instrumental precision was checked by repeated scanning (n = 6) of the same spot of galangin (240 ng/spot) and was expressed as coefficient of variance (% CV) of the peak areas. Variability of the method was studied by analyzing aliquots of standard solution of galangin (240, 320, 400 ng/spot) on the same day (intraday precision) and on different days (interday precision) and the results were expressed as % CV.

2.1.1.5.2 Repeatability

The repeatability of the method was affirmed by analyzing 240 ng/spot of standard solution of galangin after application on the HPTLC plate (n = 6) and analyzing them as described in the preparation of calibration plot, which was expressed as % CV.

2.1.1.5.3 Robustness

Mobile phases having different composition like hexane-ethyl acetate-acetic acid (7.5:2:0.5 v/v), (7.0:2.5:0.5 v/v), (8.0:1.5:0.5 v/v) etc., were tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of ± 5%. The plates were prewashed by methanol and activated at 60ºC for 5, 10 and 15 min respectively. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 30, 60 and 90 min. Robustness of the method was studied at three different concentration levels: 240, 320, 400 ng/spot for galangin.

2.1.1.5.4 Specificity

The specificity of the method was ascertained by analysis of standard and samples. The identities of the bands of galangin in the chromatogram obtained from the samples were confirmed by comparison of Rf values and spectra of the band with standard. The peak purity was assessed by comparing the standard and sample spectra acquired at the peak start [S], peak apex [M] and peak end [E] of the bands.

2.1.1.5.5 Accuracy

Accuracy of the method was tested by performing recovery studies at three levels (80%, 100% and 120%). The percent recovery as well as average percent recovery was calculated.

2.1.1.5.6 Limit of detection and Limit of quantification

LOD represents the lowest concentrations of galangin that can be detected, whereas the LOQ represents the lowest concentrations of CPT that can be determined with acceptable precision and accuracy. LOD and LOQ were determined on the basis of signal-to-noise (S/N) ratio. The known concentrations of standard solution of CPT were diluted and applied along with methanol as blank until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.

RESULTS AND DISCUSSION

3.1 HPTLC studies of galangin for *Alpinia galanga* and *Alpinia officinarum*

For the analysis of raw herbal materials and herbal preparations, HPTLC is superior to other instrumental analytical techniques because it is simple, economical and requires minimum sample clean up. The time required for sample analysis in HPTLC is much less as compare to HPLC, liquid chromatography and electro-spray mass spectrometry. In HPLC, one sample is injected at a time and after every injection there is a washing period. On the other hand, in HPTLC more than one sample is applied on a plate and quantified in a single run. The HPTLC densitometric technique was therefore, selected for the quantitative and qualitative determination of galangin in *Alpinia galanga* and *Alpinia officinarum* respectively. Out of number of solvent systems tried, the one containing hexane-ethylacetate-acetic acid (7.5:2:0.5 v/v) gave the best resolution of galangin with the retention factor (Rf) of 0.46. HPTLC profile of galangin in *Alpinia galanga* and *Alpinia officinarum* at wavelength of 254 nm are illustrated in Figure 3.1a.

Fig. 3.1a HPTLC cromatogram of galangin in *Alpinia galanga* and *Alpinia officinarum*

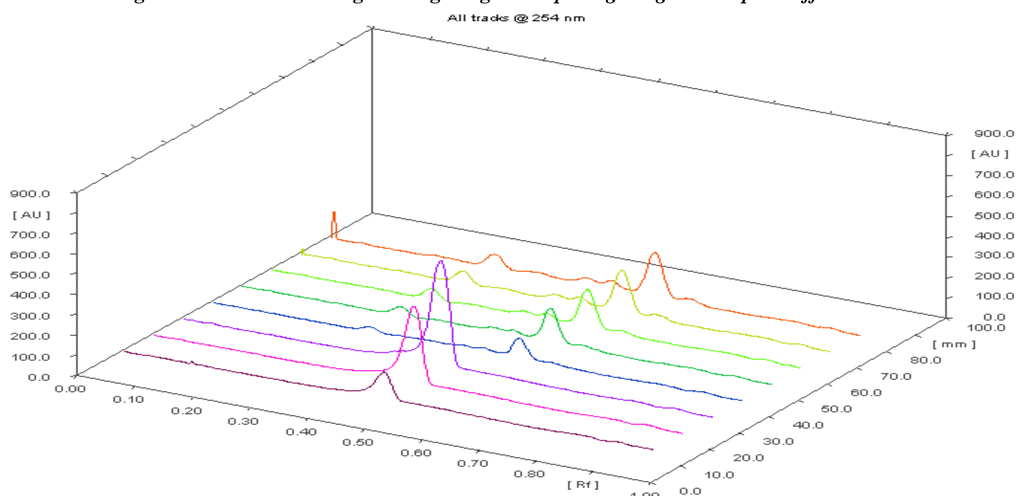
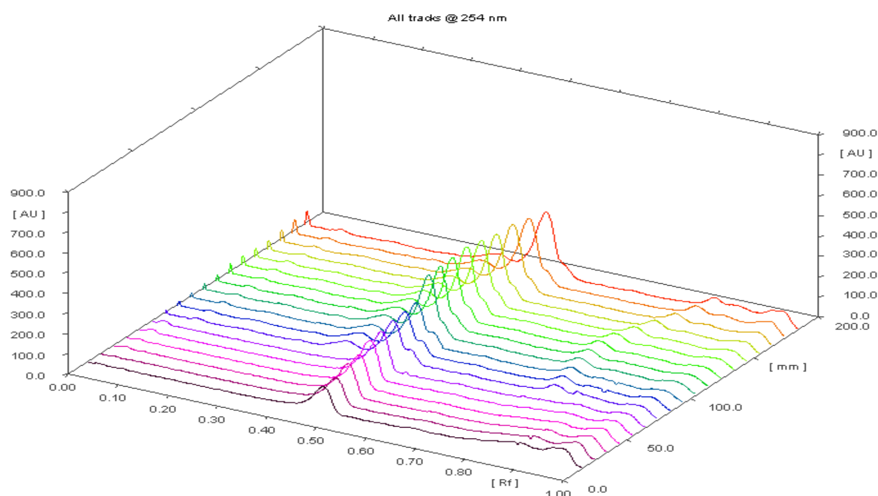


Fig. 3.1b HPTLC cromatogram of galangin in *Alpinia officinarum*



3.2 Linearity of galangin

Linear regression revealed good relationship between the concentration of standard solutions and the peak response within the concentration range of 200 to 2000 ng/spot with a correlation coefficient (r^2) of 0.999 (y=7.765x + 3739). Fig 3.2a shows linearity and densitogram of galangin.

Fig 3.2a: Linearity of galangin

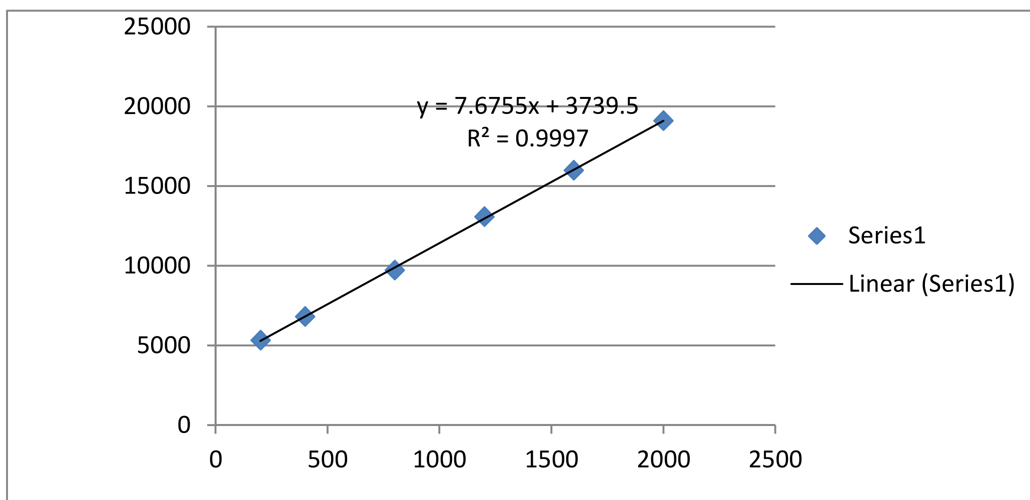
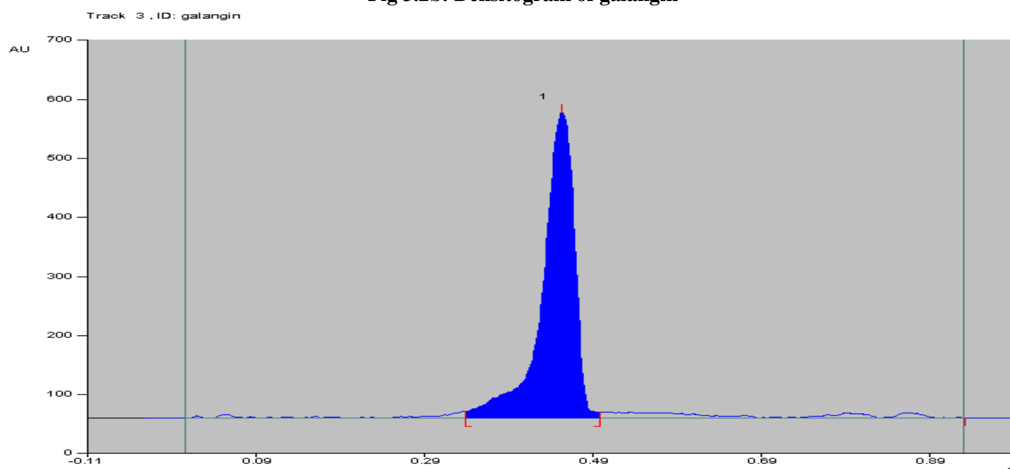


Fig 3.2b: Densitogram of galangin



3.3. Limit of detection (LOD) and Limit of quantification (LOQ) for galangin
Limit of detection (LOD) and limit of quantification were calculated to determine sensitivity as 3.3 σ/s and 10 σ/s, respectively. Where σ is the standard deviation of the response [y- intercept] and S is the slope of linearity plot. The LOD and LOQ were obtained with the signal-to-noise ratio of 3.3 and 10. The LOD and LOQ were found to be 45.14 and 136.80 ng/spot for galangin. This indicated that the new method exhibited a good sensitivity for the quantification of galangin.

3**.4. Precision**
The precision and the repeatability at three different concentration levels reflect the robustness of the method. The intraday and interday precision results are presented in Table 3.4.

Table 3.4 Intraday and interday precision of galangin

Standard drug	Nominal concentration [a]	concentration obtained [a]		Precision obtained [a]	
		Intra day	Inter day	Intra day	Inter day
Galangin	400	403.87	399.78	0.99	0.92
	800	797.39	797.22	0.20	0.57
	1200	1200.52	1198.87	0.37	0.15

3.5. Robustness
The standard deviation of peak areas was calculated for each condition and percentage Relative Standard Deviations (% RSD) was found to be less than 2%. These low values of % RSD was indicative of the robustness of the method.

Table 3.5 Robustness of galangin

Parameters	Galangin	
	Concentration found	% RSD
Mobile phase (Ethyal Acetate) composition (± 0.1 mL)	1.73	0.02
Amount of mobile phase (± 5 %)	15.53	0.16
Time from band application to chromatography (+ 10 min)	27.40	0.28
Time from chromatography to scanning (+ 15 min)	5.86	0.06

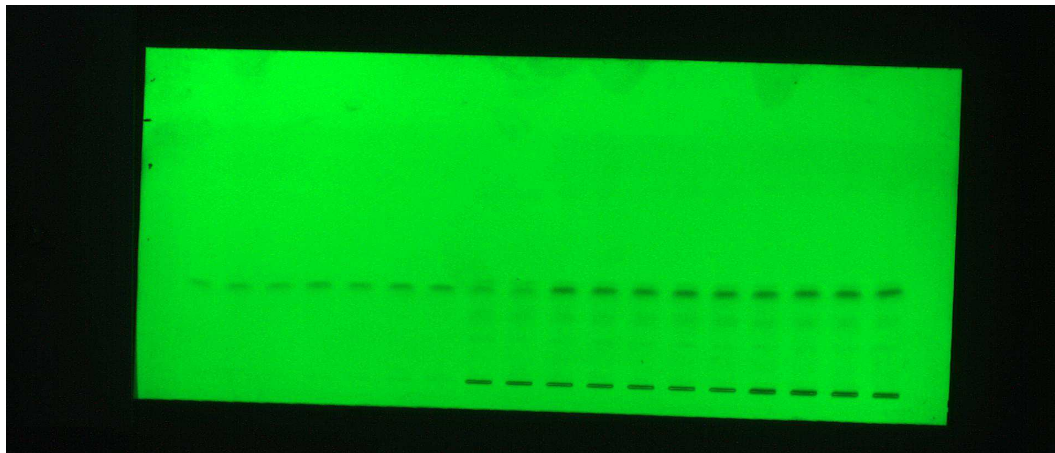
3.6. Specificity
The peak purity for galangin was assessed by comparing visible spectra acquired at the peak start (S), peak apex (M) and peak end (E) of the peaks obtained from scanning of bands. The results obtained were *r (*S, M) = 0.999 and *r (*M, E) = 0.998 respectively. Peak purity data showed that peak obtained for galangin was pure.

3.7. Accuracy
The accuracy of the method was evaluated by the recovery study. Both the percent recovery and average percent recovery was calculated. After the addition of standard galangin to same amount of the sample solution at three different concentration levels, the percentage recovery of galangin was found to be 100.04%, 100.39% and 100.25% with an average of 100.22%. The results are presented in Table 3.7.

Table 3.7 Accuracy of galangin

Amount Taken	Amount added [a]	Amount found [a]		% Recovery ± % R.S.D.	
GEL	galangin	galangin	SD	GEL	RSD
300	240	540.24	1.012046054	100.04	0.187333069
300	300	602.35	1.585084698	100.39	0.263152175
300	360	661.63	3.113434044	100.25	0.470571215

Fig. 3.7 Accuracy of galangin



3.8 Method validation parameters

Table 3.8: Method validation parameters for the quantitation of galangin by proposed HPTLC method

Parameters	Galangin
Linearity range	45.14 and 136.80 ng/spot
Correlation coefficient	0.999
Limit of detection	45.14
Limit of quantitation	136.80
Specificity	**Specific**
Robustness	**Robust**

CONCLUSION

The present study describes HPTLC method for the qualitative and quantitative estimation of galangin. The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation.

Both the methods were found to be simple, precise, specific, reproducible, sensitive and accurate and can be used for the quantitation of galangin and routine quality control of raw materials and formulations containing galangin.

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