HPTLC-UV fingerprints of *Gelsemium elegans* and koumine contents determined by densitometry compared to UPLC-MS/MS

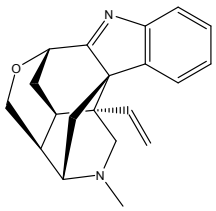
From left: Zhao Qin Yeap, Dr. Mun Fei Yam, Chiew Hoong Ng, Dr. Chu Shan Tan

Dr. Yam won the Xie Peishan Award for the Young Researcher at HPTLC 2018 Bangkok for his excellent lecture held. He is senior lecturer and researcher at the School of Pharmaceutical Sciences, University of Science, Penang, Malaysia, where he works with his team on hypertension bioassays and chemical fingerprinting. After exploring the robustness of HPTLC in chemical fingerprinting in previous projects, he is now testing the method against UPLC-MS/MS to find out if HPTLC is already good enough for the fingerprinting of herbs. The need for a cheap, robust, accurate and easy to implement technique for solving quality control issues in traditional medicine via chemical fingerprinting is exactly why HPTLC is the preferred method.

Introduction

Gelsemium elegans, also known as "heartbreak grass", is a flowering plant genus of the *Gelsemiaceae* family, found in China and Southeast Asia. There are drawbacks in consuming this plant for long periods of time. Since there are different amounts of alkaloids present in different parts of the plant, it is important to chemically distinguish these parts to both assess the pharmaceutical properties of the herb and to avoid over-consumption. The content of the alkaloid koumine present in different plant parts (stem, root and leaf) was determined by HPTLC. UPLC-MS/MS was used for comparison.

HPTLC is a straightforward versatile technique applied in pharmaceutical research for both qualitative and quantitative assessment of chemical constituents. HPTLC is the only chromatographic technique that presents the outcome as an image, *e.g.*, the simple presentation of separated components by UV light. The visible outcome and simplicity of the HPTLC technique allow inexperienced analysts to easily run the chromatographic procedure. Analysis time is relatively short, and numerous samples can easily be analyzed side by side on the plate, making HPTLC the method of choice for simple and rapid evaluation.



Structural formula of koumine

Chromatogram layer

HPTLC plates silica gel 60 F_{254} (Merck), 20×10 cm

Standard solutions

Methanolic solutions of koumine (125, 250, 500, 1000 and 2000 µg/mL)

Sample preparation

100 mg of dried, powdered *Gelsemium elegans* were mixed with 1 mL of ethanol – water 7:3 and sonicated for 10 min. The mixture was centrifuged for 5 min and the supernatant was used for analysis. The samples were spiked by adding a known amount of koumine (250 µg/mL for stem or leaf, and 500 µg/mL for root) at the ratio of 1:1 (100 µL:100 µL).

Sample application

Bandwise with Automatic TLC Sampler (ATS 4), 15 tracks, band length 8 mm, distance from the side 15 mm, distance from lower edge 8 mm, application volume 2 µL for sample and standard solutions

Chromatography

In the Automatic Developing Chamber (ADC 2) with chamber saturation (with filter paper) for 20 min and conditioning of the plate at 33% relative humidity for 10 min (using a saturated solution of magnesium chloride), development with chloroform – methanol – water 30:10:1, migration distance 70 mm from lower plate edge, drying for 5 min

Documentation

With TLC Visualizer under UV 254 nm and UV 366 nm

Densitometry

TLC Scanner 4 with *visionCATS* software, spectra recording from 200 to 400 nm, absorption measurement at 220 nm, slit dimension 5 mm x 0.20 mm, scanning speed 20 mm/s, polynomial regression, evaluation by peak area

UPLC-MS/MS

Separation on a ACQUITY UPLC BEH C18 column (100 mm x 2.1 mm, particle size 1.7 µm) with a gradient based on acetonitrile and 1% formic acid in water using a Waters ACQUITY UPLC I-Class

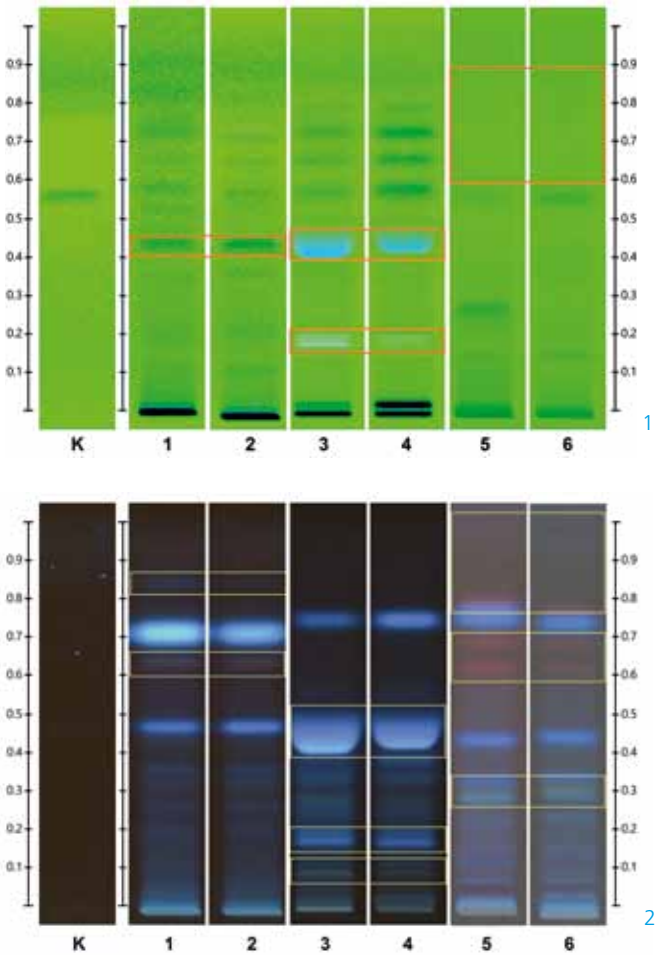
Results and discussion

Samples spiked with koumine showed the same hR_F value as the koumine standard zone, well separated from matrix components. At UV 254 nm, the chromatograms of samples exhibited a zone similar in hR_F (55 to 58) and color to the koumine standard zone, which proper assignment was underlined by the recorded HPTLC-UV spectra of the respective sample zones compared to the koumine standard zone. The optimum wavelength at 220 nm for densitometric evaluation was confirmed by the UV spectrum. Polynomial calibrations in the working range of 125–2000 µg/mL led to determination coefficients $R^2 > 0.9995$.

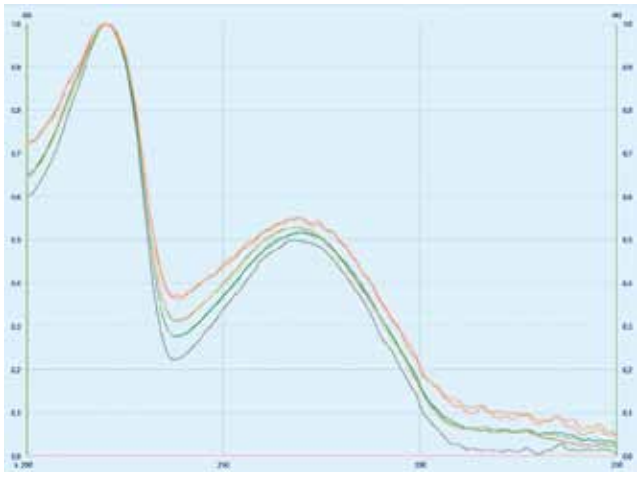
On the one hand, the stem fingerprints showed a dark zone (hR_F 40) at UV 254 nm as well as light pink (hR_F 62) and light blue fluorescent zones (hR_F 82) at UV 366 nm. These bands can only be seen in the fingerprints of the stem, but not in those of the root and leaf. Hence, these zones can be used as markers to differentiate the stem extract from others.

On the other hand, the root fingerprints showed blue zones (hR_F 18 and 40) at UV 254 nm, whereas a yellow (hR_F 10), two blue (hR_F 15 to 20) and a broad blue fluorescent zone(s) (hR_F 40 to 50) were detected at UV 366 nm, which featured a characteristic fingerprint for the differentiation of the root extract from others.

Besides the fact that the leaf fingerprint at UV 254 nm did not show any zones at hR_F 60 to 90, which are observed for the stem and root extracts (at least four zones), the absence of these bands can be used as a discrimination of the leaf extract. At UV 366 nm, two red band patches were exclusively observed from hR_F 58 to 70 and hR_F 78 to 100 for leaf extract as well as a faint yellow zone at hR_F 32 (not in stem and root fingerprints). These markers additionally can differentiate the leaf extract from others.



Chromatograms at UV 254 and 366 nm of Gelsemium elegans extracts (tracks 1/3/5: stem/root/leaf from Fu Jian province versus 2/4/6: stem/root/leaf from Guang Xi province) compared to koumine (K)



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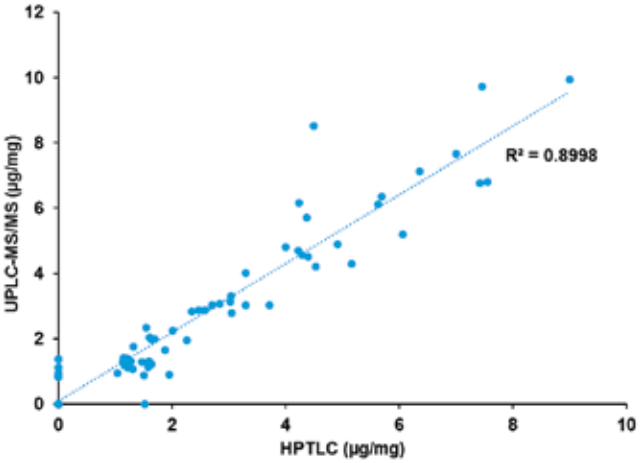
HPTLC-UV spectra of koumine standard compared to the 6 sample zones

The quantity of koumine present in the plant was determined by HPTLC-UV and UPLC-MS/MS. The validation of both methods showed good results in terms of precision (2.7% for HPTLC and 2.4% for UPLC-MS/MS), reproducibility (2.6% for HPTLC and 3.1% for UPLC-MS/MS) and recovery (101.3% for HPTLC and 104.4% for UPLC-MS/MS). The koumine results correlated with a determination coefficient R^2 of 0.8998, proving that the results from both methods can be cross checked. The actual amounts differed due to the orthogonality of the methods, *e. g.*, different solvent systems, stationary phases and detection principles.



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Correlation of the koumine contents determined by UPLC-MS/MS versus HPTLC-UV

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CAMAG Derivatizer

The Derivatizer is used for automated reagent transfer in the derivatization of thin-layer chromatograms. Due to its unique "micro droplet" spraying technology, the Derivatizer ensures homogeneity and reproducibility in applying derivatization reagents. Most of the common reagents are suitable.

To meet the diverging physicochemical properties of different reagents, *e. g.* acidity and viscosity, four different color-coded spray nozzles are available with six spraying modes to be selected by the user.

In addition to the significantly increased homogeneous reagent distribution, the Derivatizer offers other advantages compared to manual spraying:

- Environmentally friendly and safe handling through a closed system
- Intuitive handling and easy cleaning
- Low reagent consumption (2–4 mL) through efficient operation
- Reproducible and user-independent results