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Marker compounds in Java tea characterized by HPTLC

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The subject of this article brings together two companies who have a common interest in sharing the importance of knowledge about excellent phytochemical reference materials, medicinal plants and their constituents, and the use of HPTLC as a standardized analytical method. EXTRASYNTHESE is an independent French company with a catalogue of hundreds of reference materials which can be used for regulatory and quality testing, analyzed predominantly with HPTLC. The activities of CAMAG's laboratory in the analysis of phytochemicals are well documented in its dedication to the world-wide recognition and acceptance of HPTLC as the standard method for plant analysis. In this article the focus is on specific markers in method development for the analysis of complex plant extracts.

Introduction

Orthosiphon is an Indonesian medicinal plant which is widely used as an herbal tea commonly known as Java tea. *Orthosiphon* contains high levels of phenolic compounds such as sinensetin and rosmarinic acid. Sinensetin is therefore selected as a reference substance in the monograph of the European Pharmacopoeia, but it can be found in many other herbs as well.

Through investigation of HPTLC fingerprints of flavonoids from *Orthosiphon* species, besides sinensetin, other 5, 6-dimethoxy flavones, and 5-hydroxy 6-methoxy flavones could be identified, which could be used as specific markers for the identification.

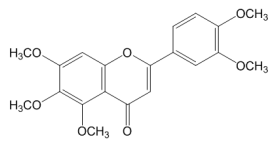
EXPERIMENTAL

Chromatography layer

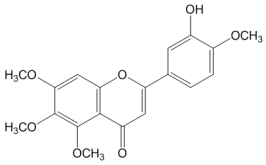
HPTLC plates silica gel 60 F_{254} (Merck), 20×10 cm

Standard solutions

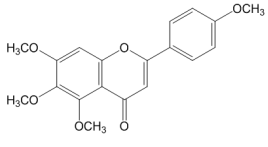
Methanolic solutions (1 mg/mL) of sinensetin and eight other reference substances (Extrasynthese); the selection of substances was made on the basis of Extrasynthese's research on flavonoid components and publications by Tezura *et al.* (2000) [1], Sumaryono *et al.* (1991) [2] and Akowuah *et al.* (2004) [3].



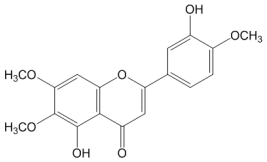
Sinensetin
CAS# 2306-27-6



Eupatorin-5-methylether
CAS# 21764-09-0



Scutellarein tetramethylether
CAS# 1168-42-9



Eupatorin
CAS# 855-96-9

Sample preparation

0.5 g of each powdered drug was mixed with 5 mL of methanol and sonicated for 10 min. After centrifugation the supernatant was used as test solution.

Sample application

Bandwise with Automatic TLC Sampler (ATS 4), 15 tracks, band length 8 mm, track distance 11.4 mm, distance from left edge 20 mm, distance from lower edge 8 mm.

Chromatography

In the ADC 2 with chamber saturation (with filter paper) for 20 min and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride, development with toluene, ethyl acetate, methanol (55:40:5) to the migration distance of 70 mm (from the lower edge), drying for 5 min.

Documentation

With the TLC Visualizer under UV 254 nm and UV 366 nm

Densitometry

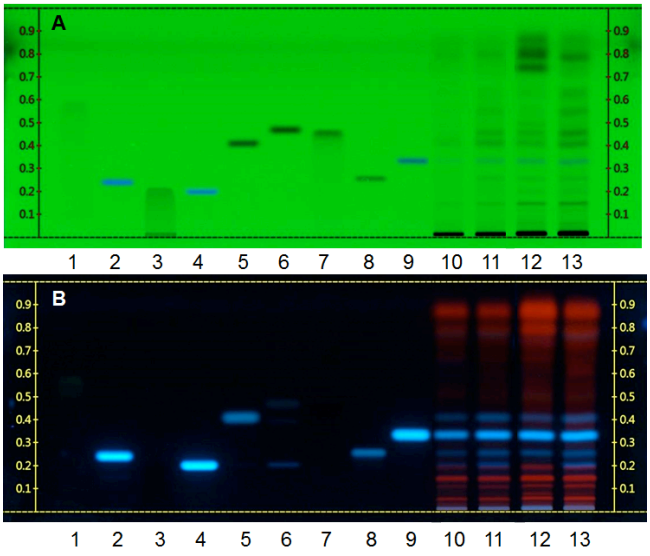
TLC Scanner 4 and visionCATS, absorption measurement at 254 nm, slit dimension 5 mm x 0.30 mm, scan speed 20 mm/s, spectra recording from 190 to 600 nm

Mass spectrometry

Elution of zones with TLC-MS Interface (oval head 4 x 2 mm) into a single mass spectrometer (expression CMS, Advion, Ithaca, NY). Data processing and evaluation of mass spectra was performed with *Advion Mass Express 2.0* and *Advion Data Express 2.0.50.9* (Advion)

RESULTS AND DISCUSSION

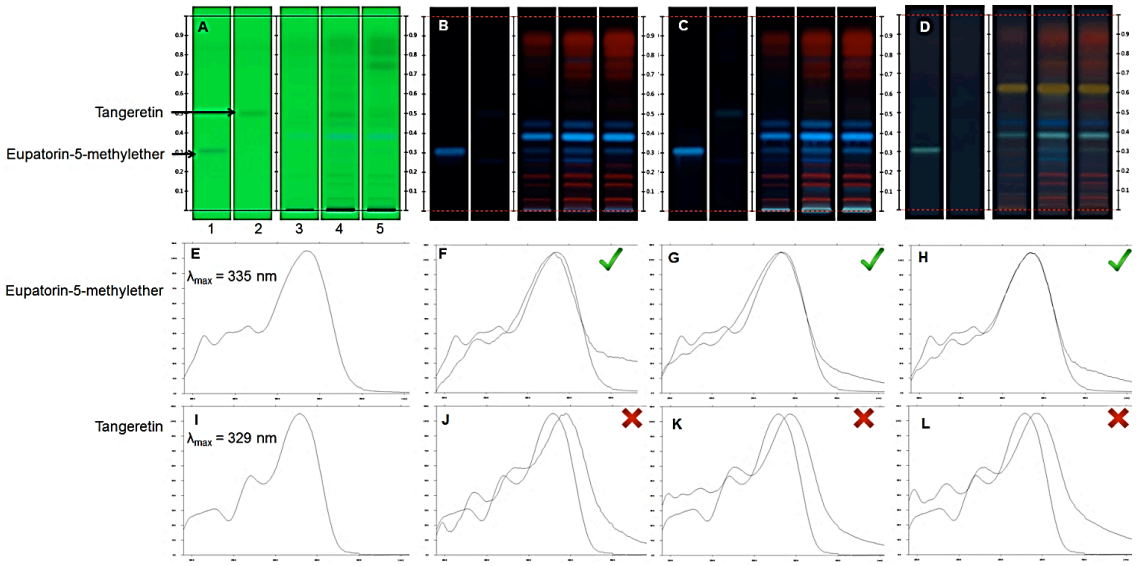
The mobile phase of the European Pharmacopoeia Monograph No. 1229 was used for the separation of 9 available standards (rhamnazin, apigenin-4,5,7-trimethylether, 6-methoxyluteolin, luteolin tetramethylether, scutellarein tetramethylether, tangeretin, eupatorin, eupatorin-5-methylether, sinensetin). Only rhamnazin and 6-methoxyluteolin have shown a tailing.



Revelation under UV 254 nm and UV 366 nm; track 1: rhamnazin; 2: apigenin-4,5,7-trimethylether; 3: 6-methoxyluteolin; 4: luteolin tetramethylether; 5: scutellarein tetramethylether; 6: tangeretin; 7: eupatorin; 8:eupatorin-5-methylether; 9: sinensetin; 10-13: Orthosiphon aristatus

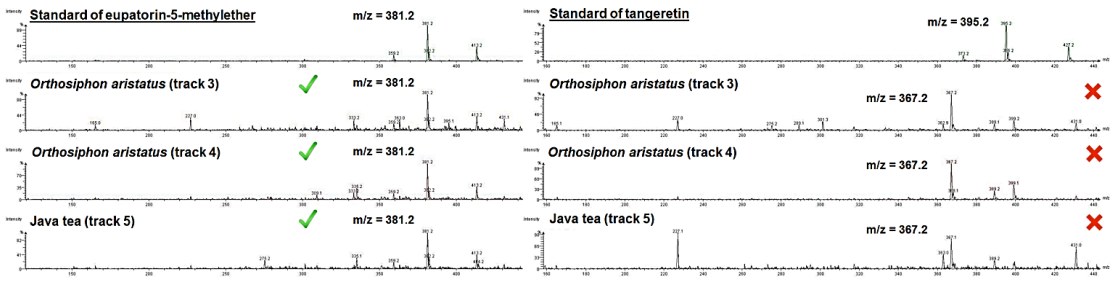
In order to confirm the presence of the selected substances in the samples, different detection modes were tested and the UV spectra recorded. For example, following development with toluene, ethyl acetate, methanol (55:40:5), eupatorin-5-methylether and tangeretin were detected under UV 254 nm and 366 nm prior derivatization, and under UV 366 nm after a two-step derivatization (natural product, then anisaldehyde-sulfuric acid).

Prior and after derivatization (A-D), the chromatograms of test solutions exhibited a zone similar in hR_F and color to the zone due to eupatorin-5-methylether. This presence was also confirmed by comparing the UV spectra of standard and those of zones at the same position in the samples (E-H). Concerning the zone corresponding to tangeretin, the chromatograms of test solutions do not seem to have a zone at similar hR_F R_F (A). The image under UV 366 nm after derivatization A (D) shows tangeretin as a faint blue fluorescence which is not seen in test solutions at this position. The UV spectra of relevant positions in the test solutions confirm that tangeretin cannot be identified in the three samples of Java tea (I-L).



Eupatorin-5-methylether (track 1) and tangeretin (track 2) in 3 samples of Java tea (track 3 and 4: Orthosiphon aristatus (Blume) Miq; track 5: Java Tea documented under UV 254 nm (A), under UV 366 nm (B), under UV 366 nm after derivatization with natural product reagent (C), under UV 366 nm after derivatization with anisaldehyde in addition (D), UV spectra of eupatorin-5-methylether (E), and comparison with the corresponding zones in the three samples (F-H), as well as tangeritin (I), and comparison to the corresponding zones in the three samples (J-L)

HPTLC-MS allows a further confirmation of determined substances. The presence of eupatorin-5-methylether and the absence of tangeretin in the 3 samples could be confirmed by mass spectrometry.



HPTLC-MS spectra of eupatorin-5-methylether and tangeretin and comparison with the corresponding zones in the samples

CONCLUSION

Together with the already established sinensetin, scutellarein tetramethylether, eupatorin, and eupatorin-5-methylether have been determined as specific markers suitable for the identification of *Orthosiphon aristatus* (Blume) Miq. by HPTLC. The presence of these four markers in the flavonoid profile is specific for Java tea.

References

Further information is available from the authors.

[1] Y. Tezuka *et al.*, Chem. Pharm. Bull. 48 (2000) 1711-1719

[2] G.A. Akowuah *et al.*, Food Chem. 87 (2004), 559-566

[3] W. Sumaryono *et al.*, Planta Medica 57 (1991) 176–180

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