CAMAG Laboratory: Method Development in Practice

Adulteration of St. John's Wort Products

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Introduction

Hypericum perforatum L., known as St. John's Wort, is the principal active ingredient of Herbal Medicinal Products commonly used for treating depression. In 2012 products made with powdered St. John's Wort plant material and/or extracts were in the top ten biggest selling botanical dietary supplement (DS) in the USA's mass market channels. Due to their popularity there is potential for the profit motive being the driving force in the adulteration of St. John's Wort products.

Any analytical method used for quality control under cGMP should not only be specific enough to confidently pass or fail samples during identification, but also be able to identify presence of a different species or any other adulterants. Below an easy HPTLC method to detect such adulteration is presented [1].

Sample preparation

St. John's Wort extract (0.5 g) or powdered St. John's Wort drug (1.0 g) were suspended in 10 mL methanol and sonicated for 10 min at 60 °C. The suspensions were centrifuged for 5 min and the supernatants used for analysis.

Standard solutions

Stock solutions were prepared individually in methanol at a concentration of 0.5 mg/mL.

Chromatography layer

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

(B) HPTLC plates silica gel 60 RP-18 W (Merck), 20 × 10 cm

Sample application

Bandwise with Automatic TLC Sampler (ATS 4), 15 tracks, band length 8.0 mm, distance from left edge 20.0 mm, distance from lower edge 8.0 mm, application volume 2.0 µL

Chromatography

In the Automatic Developing Chamber (ADC 2) with chamber saturation (with filter paper) 20 min and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride, development with ethyl acetate – dichloromethane – formic acid – acetic acid – water 100:25:10:10:11 (A) and methanol – 5% aqueous sodium sulfate 3:4 (B) to the migration distance of 70 mm (from the lower edge), drying for 5 min

Postchromatographic derivatization

The plate (A) was heated at 100 °C for 3 min and while still hot immersed into Natural Products reagent (NP, 1.0 g of 2-aminoethyl diphenylborinate dissolved in 200 mL ethyl acetate), dried and immersed into polyethylene glycol reagent (PEG, 10 g of polyethylene glycol 400 dissolved in 200 mL dichloromethane); for both, immersion speed 3 cm/s, immersion time 0 s

Documentation

With the TLC Visualizer under white light illumination and at UV 366 nm prior to and after derivatization

Densitometry

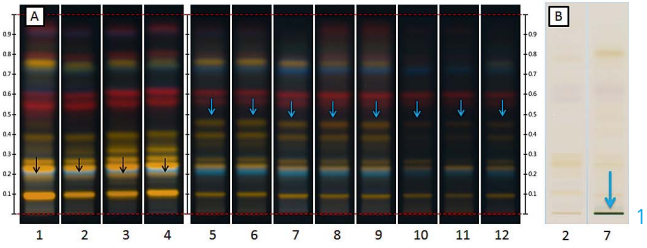
TLC Scanner 4 and visionCATS, absorption measurement at 433 nm (Tartrazine), 524 nm (Amaranth), 480 nm (Sunset yellow) and 632 nm (Brilliant blue), spectra recording between 400 and 800 nm

Mass spectrometry

Elution of zones with TLC-MS Interface 2 (oval elution head, 4.0 x 2.0 mm) at a flow rate of 0.5 mL/min with methanol (with 0.1% ammonium hydroxide) into an ESI-MS (ACQUITY QDa, Waters, USA) and detected in negative ionization mode.

Results and discussion

During a routine investigation of St. John's Wort ingredients and products, the HPTLC Association method for identification of St. John's Wort was used [2]. Some HPTLC fingerprints featured an additional yellow fluorescent zone with variable intensity between the R_F 0.4 and 0.5 at UV 366 nm after derivatization. This zone was absent in the reference samples of extracts and raw materials. At the same time a yellow fluorescent zone, which was present in the reference materials, was absent in those samples. Furthermore the overall intensity of several zones in the test samples was lower than that of corresponding zones of materials with confirmed identity. A closer inspection of the chromatogram under white light illumination prior to derivatization revealed a bluish zone at the application position in the test samples but not in the reference materials.



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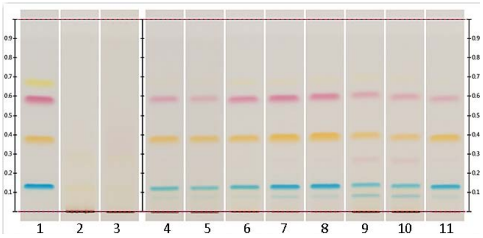
St. John's Wort chromatograms at UV 366 nm after derivatization with NP/PEG reagent (A) and under white light illumination prior to derivatization (B); tracks 1 and 2 dry extracts, tracks 3 and 4 herb reference materials, tracks 5–12 extract with an additional yellow fluorescent zone of variable intensity (blue arrow)

This blue zone suggested the presence of an adulterant, possibly a dye in the samples in question. The hypothesis was tested by employing a method for the analysis of water-soluble food colorants [3] using a wettable reverse phase (RP-18 W). Investigated under white light illumination, the extracts showed zones corresponding in position and color to those of Tartrazine, Amaranth, Sunset yellow and Brilliant blue, in contrast to reference materials.



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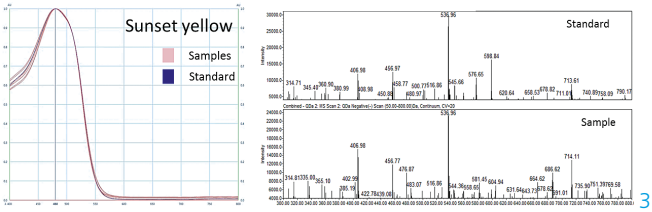
St. John's Wort chromatograms under white light illumination; track 1: Tartrazine, Amaranth, Sunset yellow, Brilliant blue (with decreasing R_F values), tracks 2 and 3: extracts, tracks 4–11: adulterated extracts

With the aim of confirming the identity of the dyes detected in some St. John's Wort samples, UV and MS spectra of the separated zones were recorded and compared to those obtained from standards with matching R_F values. Good correlation was observed. Quantitative evaluation of the amount of individual dyes and their ratio in various samples suggested the presence of a fixed dye mixture.



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Left: comparison of the UV spectra of Sunset yellow with the corresponding zone in an adulterated sample; right: mass spectra of amaranth and the corresponding zone in an adulterated sample (m/z 537 [M-3Na+2H]⁻)

The current HPTLC Association method is suitable for identifying St. John's Wort ingredients and products. Other types of St. John's Wort that show differences in the flavonoid fingerprint can be discriminated. In this study 8 out of 37 samples of St. John's Wort were found adulterated with dyes and contained material with a different fingerprint.

Further information is available from the author.

[1] D. Frommenwiler *et al.*, J. AOAC 99 (2016) 1204–1212

[2] HPTLC identification method for St. John's wort herb (*Hypericum perforatum*), HPTLC Association, www.hptlc-association.org

[3] M. Werther, CBS 88 (2002) 7

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