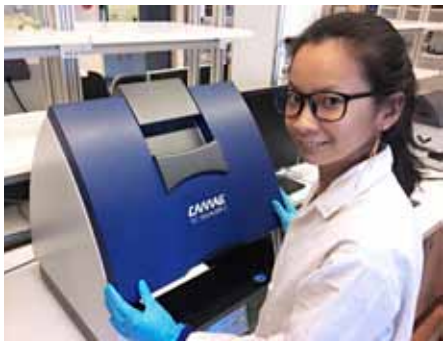
Quality control of cosmetic products by HPTLC



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CAMAG has started a collaborative project with DSM (Switzerland), Waters (USA), Sederma (France), and Extrasynthese (France) on the analysis of cosmetic products by HPTLC. Two different analytical tasks are described here, detection of UV filter substances with different lipophilicity and identification of plant extracts used as ingredients in cosmeceuticals.

Introduction

Cosmetics represent a huge market which is in constant evolution, always looking for innovation. Synthetic or natural, hydrophobic or hydrophilic actives are commonly incorporated into cosmetic matrices to create and/or improve products with properties desirable for the consumer. Cosmetic products are often complex mixtures, difficult to analyze because of possible interferences by their different components. Therefore, the incorporation of active ingredients into the various formulations requires the development of suitable analytic methods for quality control. In the past years regulations (*e. g.* EC 1223/2009 in Europe) have continuously tightened and portend to become even more restrictive [1]. The safety of a cosmetic product is obviously based on the safety of its ingredients in the finished product, and also on their safety at different stages of the manufacturing process. Hence toxicity testing has been focused on ingredients, particularly on those that are intended to react with their biological matrices [2]. The first example (A, [3]) is a general method for detection and identification of UV filter substances in sun cream by HPTLC and HPTLC-MS.

Example B describes a method for the detection of the major phenolic markers specific to Edelweiss species (*Leontopodium spp.*) in order to qualify different sources and grades of raw materials as well as glycerol-based cosmetic ingredients [4]. Since the Alpine Edelweiss is protected, the cosmetic industry uses cultivated plant material of which mainly antioxidant compounds are extracted.

For cosmetics both methods are fast and easy for characterization of UV filters and plant material, even those from different origins and qualities.

Chromatogram layer

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

Sample preparation

(A): 100 mg sun cream were mixed with 5 mL of THF. The mixture was homogenized for 30 s by vortexing and extracted in an ultrasonic bath for 10 min at room temperature. 2 mL water and 3 mL methanol were added and the mixture vortexed again for 30 s. After centrifugation for 10 min at 25 °C the supernatant was collected for application.

(B): 500 mg powdered Edelweiss sample (or 125 mg dry extract) was suspended in 5 mL methanol, sonicated for 10 min, centrifuged for 5 min and the supernatant applied. Glycerol sample (1 g) was extracted with 4 mL water, stirred vigorously and centrifuged for 15 min. The supernatant was loaded on an Oasis HLB SPE cartridge (Waters; conditioned with 5 mL ethanol and equilibrated with 5 mL water), washed with 10 mL water and eluted with 4 mL ethanol. This extract was filled up to 5 mL with ethanol and used for application.

Standards

(A) Octocrylene, avobenzone, octisalate, and ensulizole in tetrahydrofuran (2 mg/mL)

(B) Methanolic solutions of chlorogenic acid, apigenin, luteolin, luteolin-4-O-glucoside, and luteoline-7-O-glucoside are prepared at 1 mg/mL in methanol. Leontopodic acids A and B, cynarine, and 3,5-dicaffeoylquinic acid (each 0.75 mg/mL)

Sample application

Bandwise with Automatic TLC Sampler (ATS 4), 15 tracks, band length 8 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume 2 µL

Chromatography

In the 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), migration distance 70 mm (from the lower edge), drying for 5 min

(A) Development with heptane – ethyl acetate 8:2, then second development without saturation and conditioning with isopropanol to a migration distance of 28 mm

(B) Development with butyl acetate – formic acid – water 28:10:0.3

Note: During method development a better separation of Leontopodic acids A and B was obtained when a small amount of water was added as modifier. Larger amounts are not miscible with butyl acetate.

Postchromatographic derivatization

(B) The plate was heated at 100 °C for 3 min and while still hot immersed into Natural Products reagent (1.0 g of 2-aminoethyl diphenylborinate dissolved in 200 mL ethyl acetate; (immersion speed 3 cm/s, immersion time 0 s).

Documentation

With TLC Visualizer (A) under UV 254 nm and (B) under UV 366 nm after derivatization

Densitometry

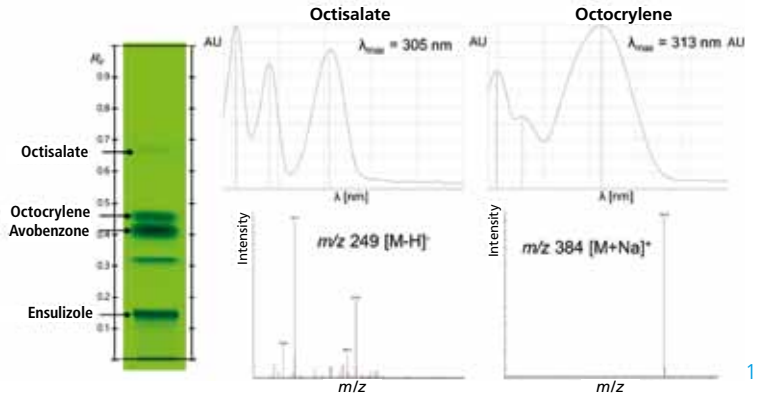
TLC Scanner 4 with *visionCATS*, absorption measurement at 254 nm, slit dimension 5.00 × 0.30 mm, scanning speed 20 mm/s, spectra recording from 190 to 450 nm

Mass spectrometry

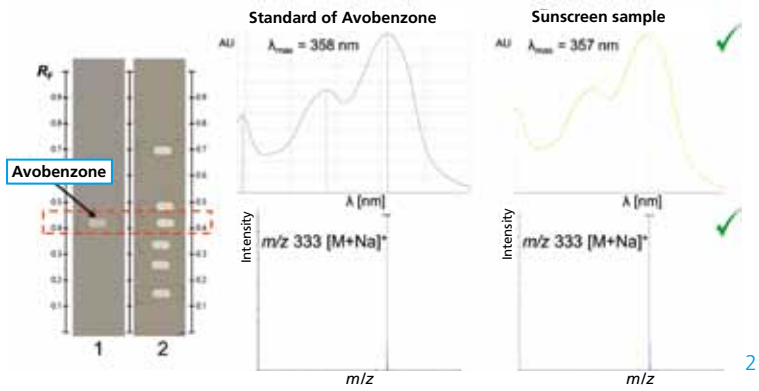
Elution of zones with TLC-MS Interface 2 (oval elution head) at a flow rate of 0.5 mL/min acetonitrile – water 95:5 (with 0.1% formic acid) into a single quadrupole mass spectrometer (ACQUITY QDa, Waters, USA) and detected in the positive and negative ionization mode. Data processing and evaluation of mass spectra with Empower (Waters).

Results and discussion

(A) A sunscreen sample as well as the standards were analyzed and identified by MS.

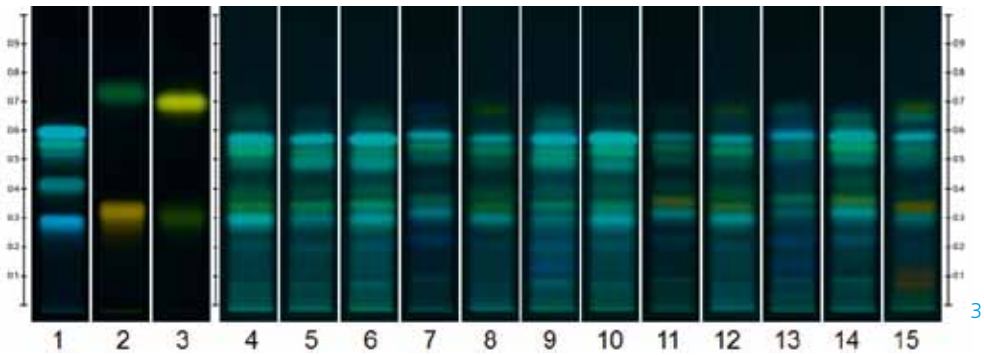


HPTLC chromatogram under UV 254 nm; UV spectra (190–450 nm) and mass spectra (m/z 50–500) of the standards



HPTLC chromatograms under white light, UV spectra (190–450 nm) and mass spectra (m/z 50–500) of avobenzone (track 1) and target zone (track 2)

(B) A set of reference substances and powdered samples of Edelweiss were analyzed in order to check the specificity of these markers for the different Edelweiss species.



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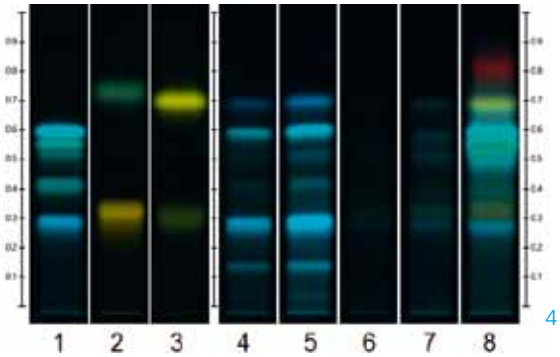
HPTLC chromatograms under UV 366 nm after derivatization. Track 1: chlorogenic acid, cynarine, leontopodic acid B, leontopodic acid A, 3,5-dicaffeoylquinic acid, track 2: luteolin-7-O-glucoside, apigenin, track 3: luteoline-4-O-glucoside, luteolin (each with increasing R_F), tracks 4–15: powdered samples of different Edelweiss species

Two authentic glycerol based samples (Majestem® Sederma and another manufacturer) and a powdered dry extract were analyzed. The profile of the purchased glycerol sample (tracks 6–7) has shown a very low content of Edelweiss extract (less intense zones).



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HPTLC chromatograms under UV 366 nm after derivatization. Track 1: chlorogenic acid, cynarin, leontopodic acid B, leontopodic acid A, 3,5-dicaffeoylquinic acid, track 2: luteolin-7-O-glucoside, apigenin, track 3: luteoline-4-O-glucoside, luteolin (each with increasing R_F), tracks 4–5: glycerol sample (Majestem®, 2 and 5 µL), tracks 6–7: purchased glycerol sample (2 and 5 µL), track 8: powdered dry extract (Extrasynthese)

Both methods proved suitable for analysis of the ingredients of interest. The recommended mobile phase for UV filter substances ensures a sufficient separation of the active substances from other ingredients. Method B demonstrates an excellent resolution of complex cosmeceutical plant extracts with simple sample preparation.

[1] Council Regulation No. 358/2014, Official Journal of the European Union, 2014.

[2] W. Schwack and C. Stiefel, CBS 111 (2013) 7 and 9

[3] CAMAG Application Note A-103.1: Detection of UV filters in cosmetic products (sunscreen) by HPTLC and confirmation by HPTLC-MS

[4] CAMAG Application Note A-104.1: HPTLC fingerprint of Edelweiss plants and extracts used as ingredients in cosmeceuticals

Further information is available on request from the author.

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