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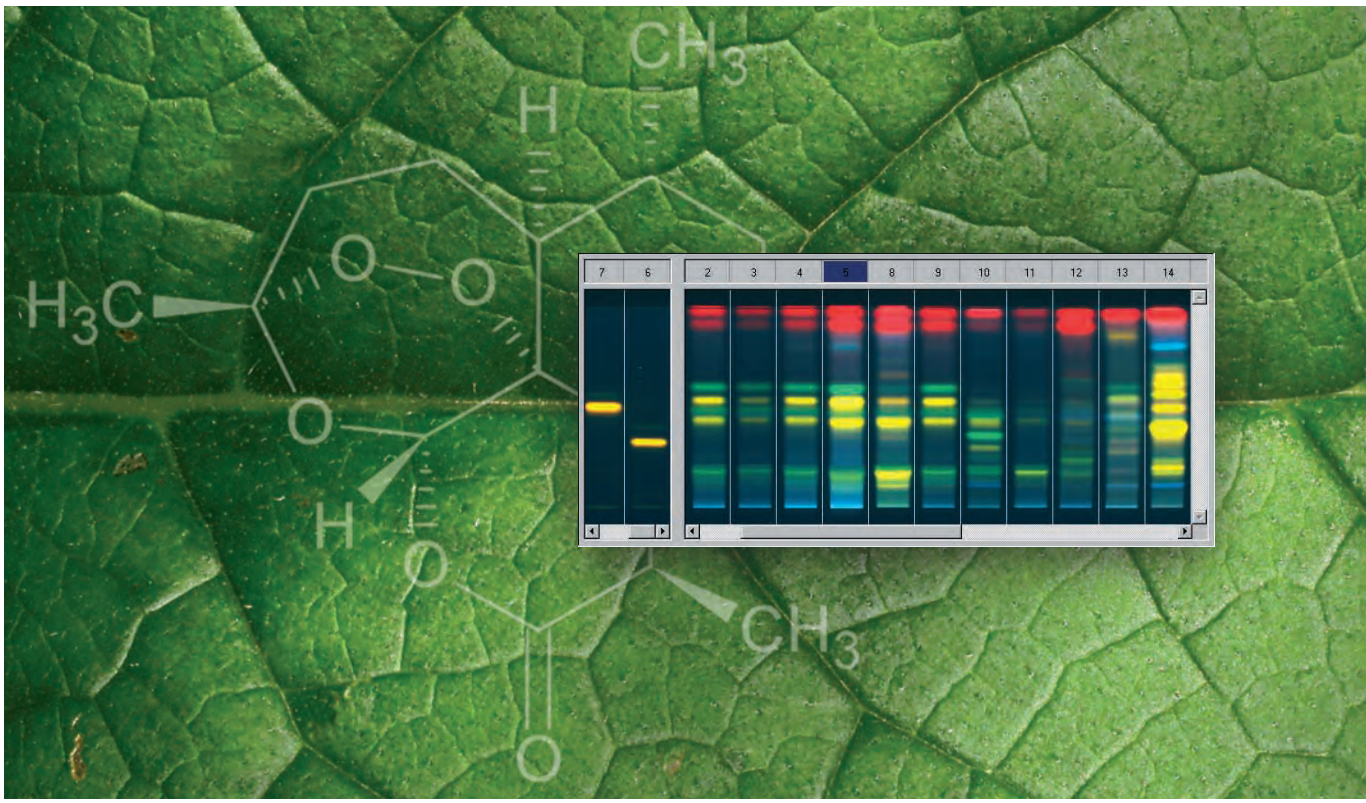

Analysis of herbals using Planar Chromatography – one step ahead due to its image feature

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Determination of acrylamide in drinking water

▲ *Alexander Alpmann, doctorial candidate in the research group supervised by Dr. G. Morlock*

The research group of Professor Dr. Wolfgang Schwack*, Institute of Food Chemistry, University of Hohenheim, Stuttgart, is working in the field of planar chromatography as well as in other research fields (see CBS 93). The flexibility of the technique is impressive time after time, particularly for solving difficult problems in a simple way.

Introduction

Polyacrylamide is used e.g. in the paper, cosmetic, textile and construction industries as well as a flocculating agent in the treatment of drinking water. Due to its high solubility in water, the monomer acrylamide (AA) can be found in ground and drinking water. The AA concentration maximal allowed is stated at 0.1 µg/L in the EU directive 98/83/EC due to its cancerogenity.

The employment of HPLC-MS/MS according to DIN 38413-6, however, is non-profitable for smaler laboratories due to the high instrumental costs involved. Besides this, the recording of the protonated AA molecule (72 Da) can be interferred by matrix fragments due to the very low molecular mass. Additionally AA can not be detected in traces (ng/L) in the UV range. Hence, a cost-effective and selective alternative for routine analysis is based on the derivatization of AA with a fluorophor. This is performed prechromatographically at the starting zone of the HPTLC plate.

Sample preparation

Water samples (500 mL) are spiked with 250 µL N,N-dimethylacrylamide (1 ng/µL in methanol) as internal standard (IS), extracted by solid phase extraction with spherical activated carbon (Bakerbond Carbon) and 5 times eluted with 2 mL methanol – acetonitril 1:1 each. The combined eluate is reduced to ca. 1 mL in a rotary evaporator and subsequently under a gentle stream of nitrogen.

Standard and derivatization solution

Ultrapure water (500 mL each) is spiked with 50 to 200 µL AA solution (1 ng/µL in methanol) and 250 µL IS solution and treated as described above. For the blank, just ultrapure water is used.

The derivatization reagent dansulfinic acid which is not commercially available can be readily synthesized according to Scully et al. [1] and was used as 3.2 µg/µL methanolic solution.

Layer

HPTLC plate silica gel 60 (Merck) 10 x 10 cm

Sample application

As 6 x 3 mm area using the Automatic TLC Sampler 4 equipped with the heated spray nozzle (40 °C), application volume 100 µL for samples and standards, 8 tracks, track distance 10 mm, first and lower application position 12 and 8 mm, respectively, application speed 350 nL/s, overspraying of the starting zones with 20 µL derivatization solution

Derivatization

On the TLC Plate Heater at 120 °C for 1 h.

Chromatograpy

In the twin trough chamber with ethyl acetate after focussing with methanol (migration distance 70 mm (lower plate edge), migration time 15 min). After 2 min drying the plate is dipped in a polypropylene glycol solution (25 % in *n*-hexane) for fluorescence enhancement using the Chromatogram Immersion Device III (dipping time 1 s, dipping speed 5 cm/s).

Documentation

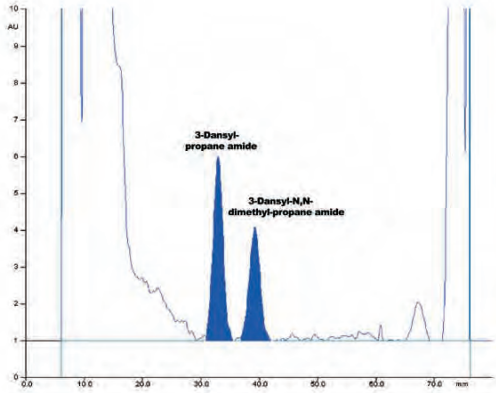
With DigiStore 2 System at UV 366/>400 nm

Densitometric evaluation

TLC Scanner 3 in fluorescence mode at UV 366/ >400 nm, linear calibration via peak area

Results and discussion

AA can selectively be detected without interfering matrix after derivatization with dansulfinic acid to 3-dansylpropane amide (DPA). The performance of sample preparation is ascertained by correction with IS (derivatized to 3-dansyl-N,N-dimethyl-propane amide).



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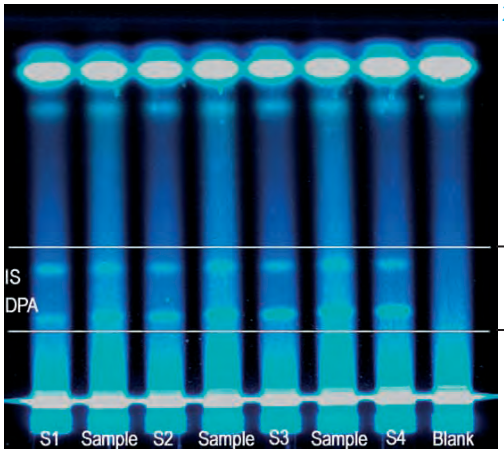
▲ *Fluorescence scan of an ultrapure water sample spiked with AA (0.2 µg/L)*

The mean within-run precision (*RSD*, *n* = 3 at 3 different concentration levels each) were established to be 4.8 % and the mean recovery over 3 concentration levels (0.1, 0.2 and 0.3 µg/L) was 96 % (corrected by the IS).



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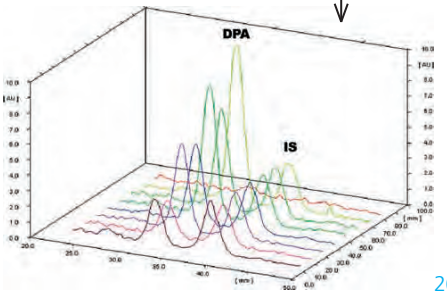
▲ *Plate image of ultra-trace analysis of AA (as DPA) in drinking water*



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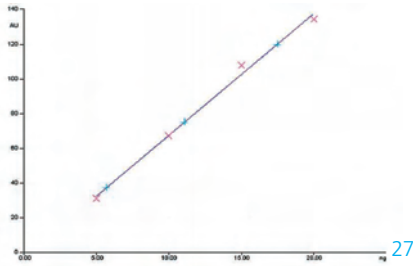
Fluorescence scan in the region marked of standards (0.1–0.4 µg/L, S1-S4), spiked blank samples (0.1–0.3 µg/L) and blank sample ▲

The regression in the working range of 0.1 to 0.4 µg/L was linear showing a relative standard deviation of ± 5.2 % (r = 0.9957).



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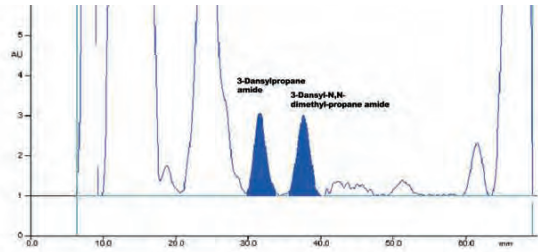
▲ *Linear calibration of DPA (5–20 ng/zone or 0.1–0.4 µg/L)*

LOQ was calculated to be 0.08 µg/L AA in drinking water and thus enables a reliable control of the limit value at 0.1 µg/L according to 98/83/EC.



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▲ *Densitogram of a sample spiked below the LOQ at 0.05 µg/L*

The method comparison with HPLC-MS/MS showed comparable results for the ultra-trace analysis of AA in ground water and proves the efficiency of the new method at the ultra-trace level:

Method comparison	HPLC-MS/MS AA [µg/L]	HPTLC-FLD AA [µg/L]
Ground water sample 1	<0.05	<0.05
Ground water sample 2	0.07	0.09
Ground water sample 3	0.18	0.24
Ground water sample 4	0.59	0.60

When relevant, additionally mass spectra can be recorded by online extraction (ca. 1 min/zone). Due to derivatization the protonated molecule of a higher mass (*m/z* 307) is highly advantageous because it can be detected with less interference compared to AA at *m/z* 72 and with a simple MS system (instead of the MS/MS).

[1] F. Scully et al. Environ. Sci. Technol. 18, 787, 1984

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