Hypernations in planar chromatography

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Scope

Initially advocated by T. Hirschfeld, in 2007 the term hypernation was coined by I.D. Wilson and T.A. Brinkman to place all of the required spectrometers into a single system so that all of the spectroscopic information is obtained in a single run. Hypernation represents a logical, rapid and efficient strategy for obtaining **the maximum possible information out of a single separation**. The major problems associated with column-based hypernations are capital cost and strategies for dealing with the large amounts of data that such systems produce. The complexity of the instumentation increases, which makes them difficult to operate in a routine way. A single eluent that is optimal for all detectors is difficult to obtain. Differences in sensitivity between spectroscopic techniques and spectrometers are challenging as well.

Results and Discussion

All these problems are much less challenging in HPTLC-based hypernations because of the open system that is (1) highly adaptive to different sensitivities, (2) cost-effective by modular instrumentation, (3) generating less data due to targeted access to points-of-care on the plate, and (4) directly accessible for the respective optimal solvent because the eluent is evaporated after chromatography and not impacting the different detectors. The latter is extremely relevant for effect-directed detection with bioassays.

Existing hypernations are

- ✓ HPTLC/UV/Vis/FLD
- ✓ HPTLC/UV/Vis/FLD/MS
- ✓ HPTLC/UV/Vis/FLD/EDA(bioactivity)/HRMS
- ✓ HPTLC/UV/FTIR
- ✓ HPTLC/UV/Vis/FLD/FTIR ATR
- ✓ TLC/Vis/SERS

For example in natural products search (Fig.1)

1. **30 sponges** (reduced sample preparation → just lyophilized, dissolved and centrifuged) were screened matrix-robust **at one go** for natural bioactive secondary products (UV/Vis/FLD/EDA images),
2. **high producers** of bioactive products were **identified**,
3. information about the **range of bioactive products** produced by the sponge was given, and
4. the **sum formula** was obtained from bioactive zones of interest.

All this information was obtained **by a single separation for 30 sponges in parallel** using HPTLC/UV/Vis/FLD/EDA(bioactivity)/HRMS [1,2].

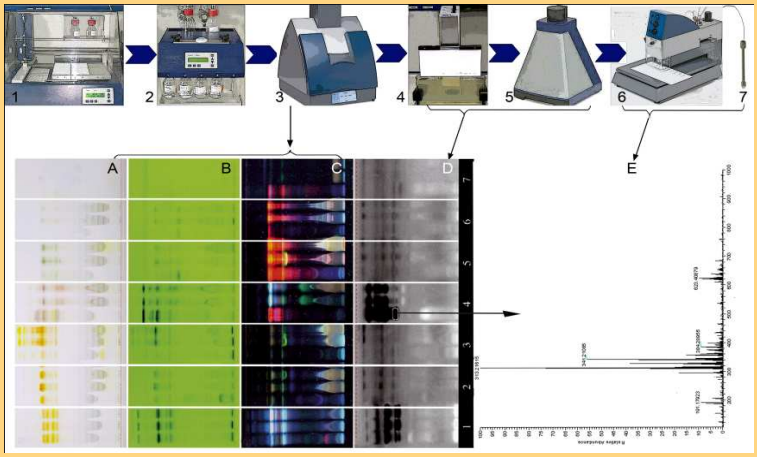

Fig. 1: Natural products search: HPTLC/UV/Vis/FLD/bioactivity/HRMS answered 4 question (left side) for 30 samples with a single separation; automated devices used (top), sponge samples analyzed (bottom),

Another example is given in the field of phospholipids' analysis (Fig. 2):

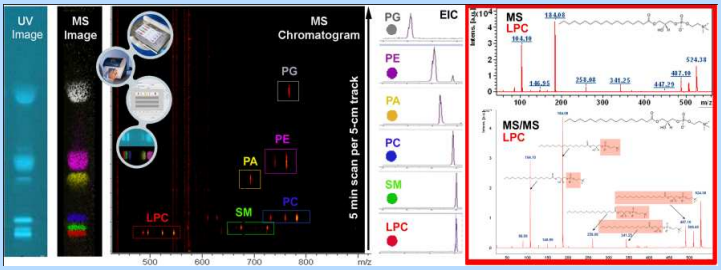

Fig. 2: HPTLC/FLD/MALDI-TOF MS for phospholipid analysis: after the MS scan (in the MS chromatogram *m/z* versus migration distance were depicted), MS/MS can be performed highly targeted from bands of interest as shown for lysophosphatidylcholin (LPC)

1. HPTLC-MALDI-TOF MS imaging allowed to generate images alike the image after derivatization with primuline, but additionally **overlapping lipids can be distinguished** in the ion density image.
2. The MS scan along the chromatographic track (5 min/track) provided density **plots of migration distance versus *m/z* ions**.
3. On particular bands, **MS/MS analysis provided further specificity** in assigning lipids with regard to their respective sn1/sn2 substituents and their head groups; all obtained by a single separation. [3,4]

A last example is given in the field of food chemistry (Fig. 3):

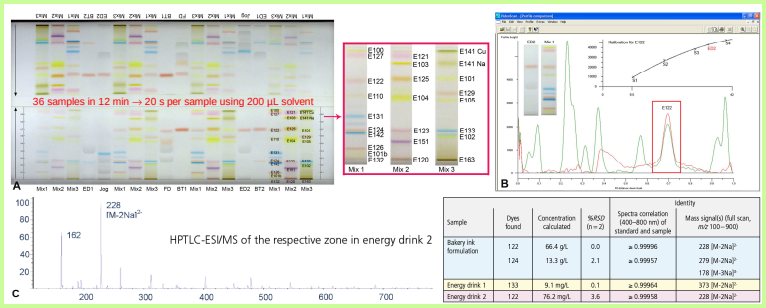

Fig. 3: Screening (A) and digital quantification (B) of food samples for water-soluble dyes → spectral confirmation in case of need for positive findings (C)

1. Many samples (diluted, degassed and/or centrifuged) were **screened matrix-robust for 25 water-soluble dyes** at one go**,**
2. **positive findings were quantified** digitally through the image, and
3. **in case of need**, positive findings were **confirmed** by Vis spectra library search or mass spectra.

All this information was reached **by a single separation for many food samples in parallel** using HPTLC/UV/Vis/FLD/MS [5,6].

Conclusion

⇒ Depending on the task at hand, hypernations can readily be selected as required. Information is obtained for many samples in parallel.

⇒ Although rarely found, the decisive advantages of HPTLC-based hypernations valuably assist researcher. To make analysts familiar with this technique **HPTLC knowledge should be trained and taught**.

Literature: [1] A. Klöppel, W. Gasse, F. Brümmer, G. Morlock, J. Planar Chromatogr. 21 (2008) 431-436. [2] A. Klöppel, F. Brümmer, A. Kolm, G. Morlock, CAMAG Bibliogr. Service CBS 102 (2009) 4-7. [3] M. Schuerenberg *et al.*, IMSC 2009, Bremen, Poster PMM 386. [4] Bruker Daltonics Application Note MT-101. [5] G. Morlock, C. Oellig, J. AOAC Int., 2009, 745-756. [6] G. Morlock, C. Oellig, CAMAG Bibliogr. Service CBS 103 (2009) 5-9.