Why HPTLC in the age of ultra-rapid HPLC separations?

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Scope

Parallel chromatography (the development of 46 runs from both plate sides in one run of 15 min) and the stacked system (15 min-intervals of the step-stacked system) easily achieve 1000 runs in a 8-hour shift. The resulting runs are complete within a 20 second time-frame with about 300 µL solvent consumption for each run. This method compares favorably with rapid separations performed on the state-of-the-art ultra-rapid HPLC separations where, for example, a 2 min gradient would perform 720 runs in 24 hours. It even has some advantages over state-of-the-art instrumental methods — obviously not in terms of separation efficiency, but in terms of ingenuity [1, 2].

The role of "retro" TLC

It is surprising that TLC is so widely undervalued and regarded as antiquated. Most analysts do not use the method's full potential. Most TLC applications are aimed at the screening stage in food or pharmaceutical analysis, but the method is capable of much more. There is generally a **lack of confidence** in the method because of the **lack of experience in advanced** versions of this technique? This is also reflected by the general lack of research activity in planar chromatography. The method works quantitatively if instrumentation is used and the analysis is performed properly, but the technique will only become more popular if there is a greater level of advanced training: much greater than existing "retro" TLC.

Key performance data of HPTLC

HPTLC and HPLC are both liquid chromatographic methods serving similar fields of application. HPTLC is usually regarded as less precise than HPLC. But how could it be otherwise, if in general, scant attention is paid to its performance?

Using HPTLC the **repeatabilities** of the analysis of sucralose in various matrices was ≤ 4.4 % (n = 3), of active ingredients in energy drinks ≤ 1.5 %, for ITX at trace levels in milk products, yoghurt and fat ≤ 6.4 %, of HAA ≤ 4.4 % and of acrylamide in water at the ultra-trace level inclusive derivatization 4.6 %. All these repeatabilities were surely appropriate in food analysis.

For validated methods **correlation coefficients** of calibration plots are usually better than 0.998 and **limits of detection** (LODs) are also appropriate. In milk-based confection the LOD of 1 mg/kg fully meets the required limits of sucralose according to the European legislation (10 - 3000 mg/kg depending on the food). For acrylamide in drinking water the LOD of 0.025 µg/L is suited for monitoring the EU limit value of 0.1 µg/L or the WHO or EPA limit value of 0.5 µg/L. Also for polycyclic aromatic hydrocarbons (PAH) or pesticides in water appropriate LODs are obtained to monitor the respective limit values of 0.1 µg/L.

Selectivity can be guaranteed by automatic purity control of the UV/VIS spectra or by the recording of mass spectra. Why not using HPTLC if sufficient selectivity is given?

Impact of HPTLC

As the matrix compounds of milk, cakes, sweets, chocolate and drinks are strongly retarded at the origin of the adsorbent, **rapid sample treatment** is allowed. A heavy matrix fixed at the starting region (Fig. 1) does not matter in single use HPTLC, but it does if fixed on an HPLC column.

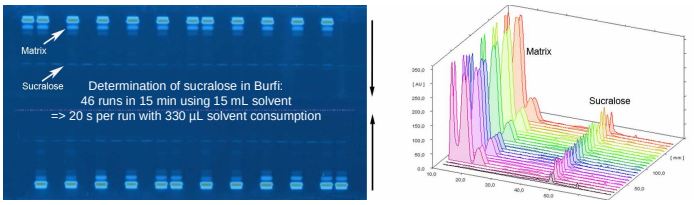

Fig. 1: Parallel chromatography of 46 runs under identical environmental conditions

Therefore, HPLC methods for sucralose analysis usually employ solid-phase extraction (SPE) for sample preparation, but this is not necessary for HPTLC.

HPTLC is **cost-effective**. A simple derivatization step allows the use of simple detectors for the detection of analytes without a chromophore. **Selective derivatizations** (Fig. 2) solve analytical tasks which could otherwise only be managed by HPLC/MS(-MS) or the use of special detectors.

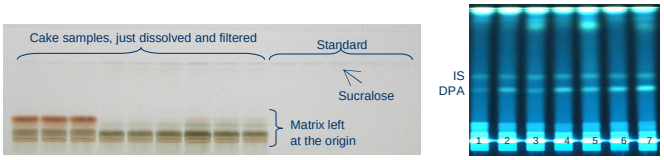

Fig. 2: Documentation of the analysis of *Left* sucralose in different cakes in the visible range after derivatization with aniline/diphenylamine o-phosphoric acid reagent and *Right* acrylamide (as DPA) in drinking water samples (0.1 - 0.2 µg/L) after derivatization with dansulfinic acid

The old adage, a picture is worth a thousand words, applies to the power of **digital image comparisons** in HPTLC that can now also be used for rapid quantification (Fig. 3).

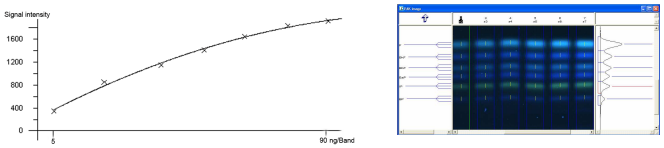

Fig. 3: *Left* Calibration of BaP by *Right* digital quantification of the plate image under UV 366/>400 nm

HPTLC enables **effect-directed detection** and a highly-targeted, **lower-cost HPTLC-MS** where the separation solvent can be chosen independently from MS. It enables **concentration during application** by up to a factor of 10,000 and tolerates minimal sample preparation. HPTLC gives **information on what is left** at the origin of the adsorbent. This would never be noticed in HPLC. It is the most flexible chromatographic method and working station and the question arises why not taking use of these advantages.

Conclusion

Konfuzius once said: Reaching the water source one must swim against the mainstream.

Planar chromatography is impressive for solving challenging questions in a simple way. A complex matrix can be left at the starting zone on the plate, allowing reduced sample preparation, but generally not on the head of a column. Selective derivatizations solve analytical tasks, which could otherwise only be managed by HPLC/MS or special detectors. The role of TLC as a qualitative tool must be reconsidered. Using appropriate instrumentation the method is quantitative. This technique can be validated to fulfill the requirements for a reliable analysis at the required detectability limits in many application fields complying with regulated environments such as cGMP or cGLP.

Thanks to CAMAG and Merck for material support granted.

[1] G. Morlock, W. Schwack, J Planar Chromatogr 20 (2007) 399-406
[2] G. Morlock, W. Schwack, LCGC Europe July (2008)