Advance your Knowledge in Planar Chromatography

It's Not What it Used to be

In 2013, planar chromatography will celebrate its 75th anniversary [1]. It seems to be an old technique with minor contribution to analytical tasks of today and, based on its versatility in the performance level, controversy is an ongoing issue for planar chromatography. It depends on the level of advanced knowledge and experience of the analyst, whether it is considered as a unique chromatographic technique for challenging analytical problems or as a dispensable inferior version of liquid column chromatography.

Introduction

Planar chromatography never hit the critical mass necessary to promote the method itself, or obtained satisfactory teaching and consequently respective recognition and funding. The statement "Most people approach TLC with feeling rather than with knowledge, with the inevitable consequence of hazardous results and disorientation about TLC's real potential" [2] is still up-to-date. Despite those obstacles, high-performance thin-layer chromatography (HPTLC) significantly developed further in the last decades. Unrivalled features through the planar, image-giving format attracted analysts' interest for true sample comparisons. Standardization of the method made chromatograms comparable which paved the way for the establishment of image databases. The combined hyphenation to bioassays and high resolution mass spectrometry allowed the rapid, direct link to the compounds indicating the effect (effect-directed analysis). Such an effective strategy could revolutionize natural product search. It can even be concluded that planar chromatography still has brilliant potential left. Not only the high flexibility with regard to hyphenations, but also the new discipline office chromatography will impact further the progress of planar chromatography through combining innovative print & media technologies with chromatography [3, 4].

Automation

For all HPTLC steps automation is available and often the crucial precondition for a successful solution of the analytical task. All instrumentation can be operated in a strictly regulated environment by a common software platform, which makes routine use comfortable. Nowadays the labor time is reduced to the plate transfer time between the automated working stations, which takes extra time of just some minutes for up to 40 samples in parallel on one plate if compared to HPLC. Today migration distances (hR_F values) show precisions of ± 0.5 to 1 mm using intelligent instrumentation compared to commonly used trough chamber developments. Using standardized HPTLC methods based on automated developing chambers with control of the plate activity, the chromatograms are comparable worldwide over the years.

Questions on similarities or differences between samples, pass or fail specification, finding important samples out of a multitude etc. are ideally solved by the reliable HPTLC image. All at once, retention data (hR_F), color intensities (signal intensities) and color nuances (absorption wavelengths) can readily be compared as image or subjected to multivariate data analysis. High sample throughput and cost-efficiency combined with reliability (verification of the HPTLC results by comparison to established methods) made HPTLC apparently superior for quality control analysis on the example of the hydroxymethylfurfural (HMF) content in honey, which is

GDCh course 335/12: High-Performance Thin-Layer Chromatography Mass Spectrometry (HPTLC-MS)
November 28, 2012 in Stuttgart, Germany

Focus of the course
Recognize the power of HPTLC
- Overview on planar chromatographic hyphenations (hyphenated HPTLC)
- HPTLC-MS and differentiation between desorption-based and elution-based coupling approaches
- HPTLC-UV/Vis/FLD-ESI-MS with experiments
- HPTLC-UV/Vis/FLD-bioassay-ESI-MS with experiments
- HPTLC-UV/Vis/FLD-ATR -FTIR with experiments
- TLC-HPLC-DAD-MS with experiments
- HPTLC-UV/Vis/FLD-MALDI-TOF-MS with experiments
- HPTLC-UV/Vis/FLD-DART-MS with experiments
- Discussion of the different hyphenations

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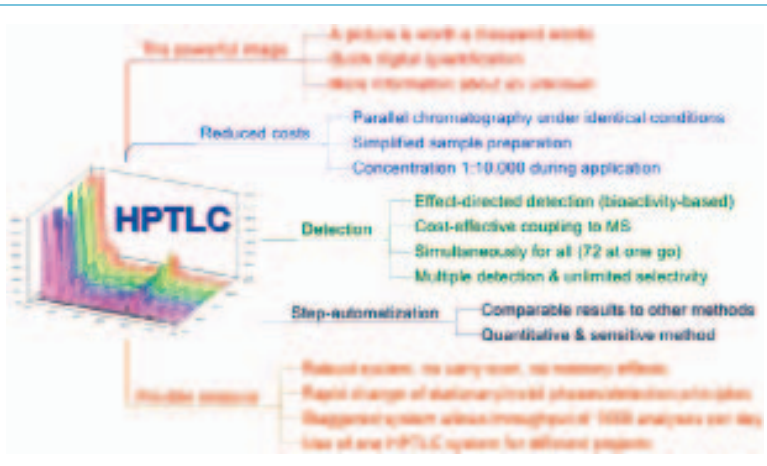

Fig. 1: Unrivalled features of planar chromatography owed to the planar format

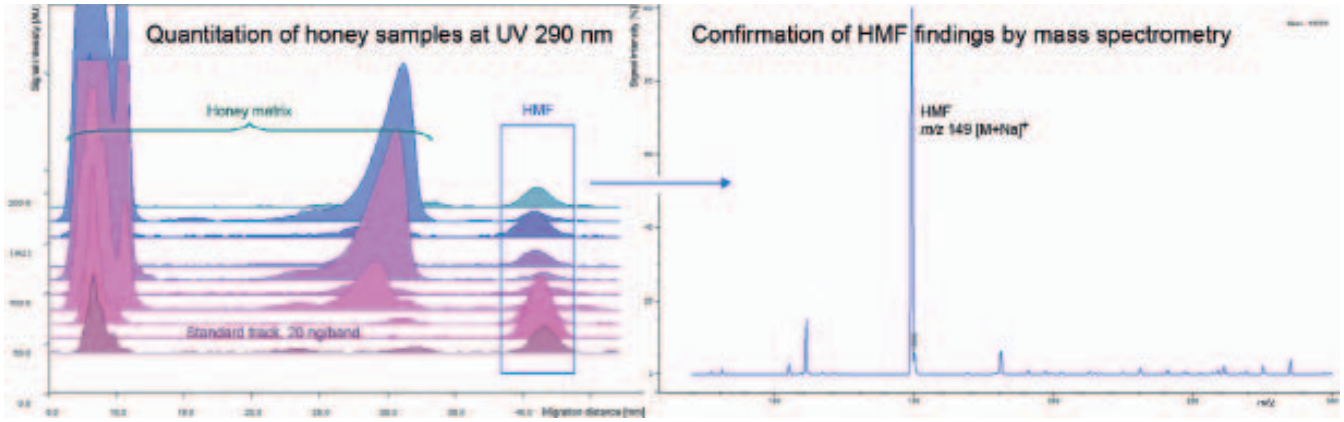

Fig. 2: Fast and reliable HPTLC analysis of hydroxymethylfurfural (HMF) in honey samples

an indicator of its freshness [5] (fig. 2). Many other method comparisons underline the reliability [6, 7, 8].

Application of HPTLC

In recent surveys [9, 10] fast HPTLC separations were discussed with a similar rapidness compared to fast HPLC. It was demonstrated by parallel analyses of up to 46 runs within 15 min, which means 20 seconds per run, consuming 200 µl mobile phase per run [5] (fig. 3). For example, in case of a food scandal, HPTLC can easily cope with an increased sample throughput of 1.000 samples per 8-h day, when synchronizing the automated HPTLC steps in a 20-min interval.

The rudimental, limited teaching only of TLC leads to a significant lack of advanced HPTLC knowledge and of its practical knowledge on many beneficial features (fig. 1). For example the full capability of detection is generally not taken advantage of. Options like application of high volumes via area application, e.g., for matrix-rich samples, zone focusing via automated multiple development (AMD 2 system), use of reduced thicknesses, or employment of more sensitive derivatization reagents, would offer sensitivity gains up to a factor of, e.g., 1000 in case of need, but are rarely applied.

Ambient mass spectrometry is a relatively new field of high interest and rapid growth, being attractive for hyphenations with planar chromatography. Direct sample access at ambient conditions and the feasibility to obtain mass spectra free of contamination from zones of interest on a HPTLC plate within one minute or even seconds highly contributes to the progress of planar chromatography.

Linking planar chromatography with mass spectrometry (MS) was a merit of the last decade and is still developed further [11]. The invention of ion sources working under ambient conditions and atmospheric pressure enormously eased the introduction of a planar object.

Upcoming meetings would be an ideal opportunity to recognize whether HPTLC would be a suitable solution to one´s own analytical problems or challenges. Some important dates are:

Analysts can get advanced knowledge on HPTLC and its hyphenations at the new GDCh course, 28th November 2012 in Stuttgart, Germany (see text box).

More than 310 participants from over 40 nations attended the recent HPTLC 2011 symposium in Basle. The next, HPTLC 2014, will take place in Lyon, 2nd - 4th July (www.hptlc.com).

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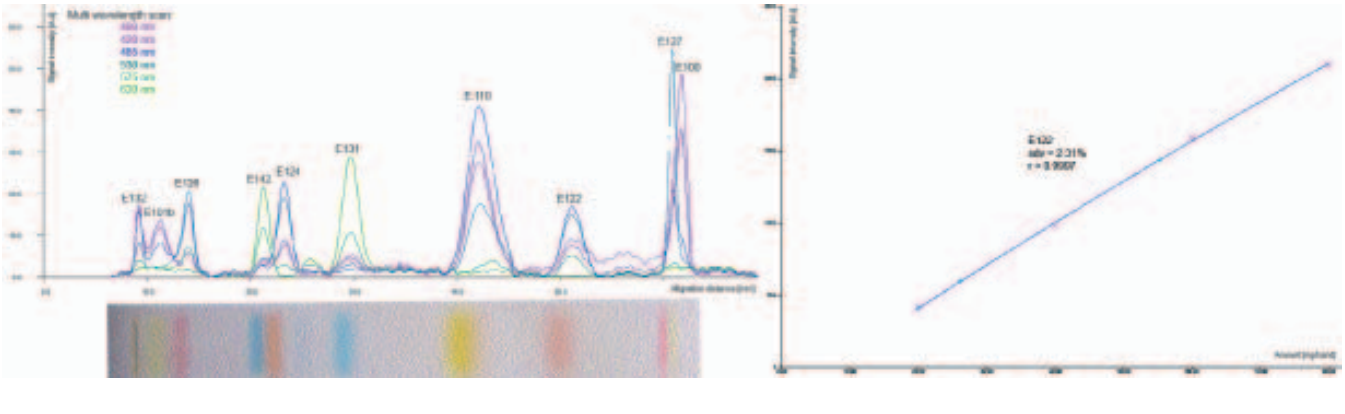

Fig. 3: Multi wavelength scan of a food additive mixture (absorption curve overlay of 6 wavelengths); exemplarily shown is the calibration function of E122 (x) found in a fruit drink sample (+)