Comparison of different ionization techniques (ESI, DART, APGD) for coupling of mass spectrometry with planar chromatography (HPTLC/MS)

Part 1 **Gertrud E. Morlock** **University of Hohenheim, Stuttgart, Germany**

Scope

Rapid and cost-effective analyses are two major trends in analytical chemistry which are easily achieved by planar chromatography. High-performance thin-layer chromatography (HPTLC), the most important planar chromatographic method, operates in an offline mode, however, is fully automated in its single steps (controlled by a common software platform). **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 high sample throughput. The resulting runs are complete within a 20 seconds time-frame with about 300 µL solvent consumption for each. Thus **1000 chromatographic runs can be performed in an eight-hour shift** [1, 2]. But, what are the advantages of coupling HPTLC with MS? How can it be performed?

Results and discussion

In contrast to online column techniques the stepwise automated planar chromatographic method allows the evaluation of the runs first. Then, after quantification, MS can be employed **highly targeted**. The recording of mass spectra just for interesting zones or positive findings **reduces costs substantially** for several applications, but there are other advantages too, for example, when using electrospray ionization (ESI), the **mobile phase** can be chosen **independently from MS** because it evaporates after the chromatography. Compared to column techniques, planar chromatography enables more **comprehensive information** on unknown samples because also substances strongly retarded by the adsorbent are applicable for MS. These substances are mostly out of focus in column techniques because they will not reach the detector during the run. Today w**ithin a minute or even within seconds** mass spectra can be obtained from zones on the plate. The coupling technique applied depends on the user's preferences. Besides MALDI and DESI, the latest desorption-based techniques for coupling HPTLC with MS by ambient ion sources, such as *Direct Analysis in Real Time* (DART) and *Atmospheric Pressure Glow Discharge* (APGD), are now compared to an *extraction-based interface* followed by *Electrospray-Ionization* (ESI) (Fig. 1).

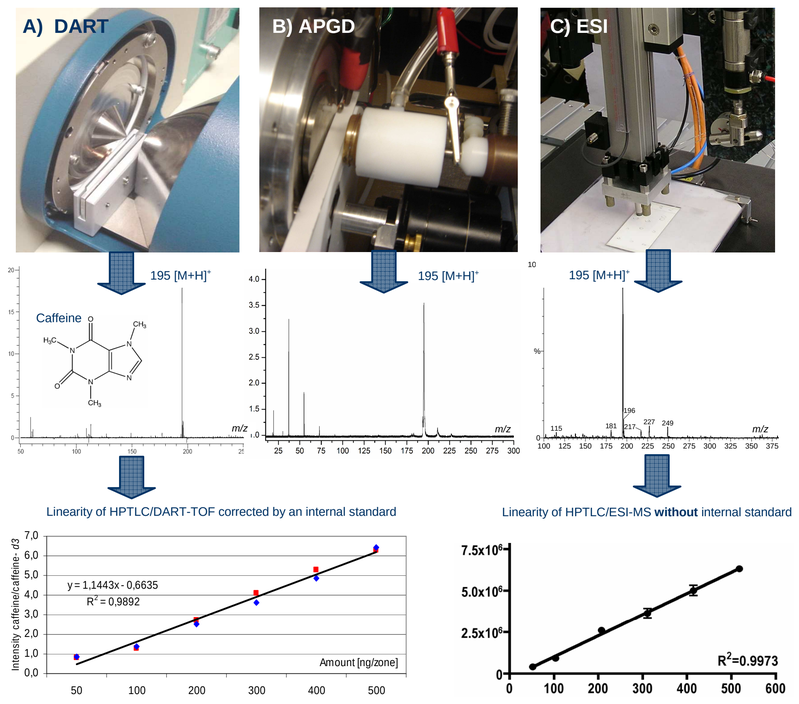

Fig. 1: Comparison of different ion sources, i. e. (A) DART, (B) APGD and (C) ESI, for HPTLC/MS coupling and their mass spectra (full scan) of caffeine *@ m/z* 195 $[M+H]^+$. The analytical response (calibration ranged between 50 – 500 ng/zone) of HPTLC/DART-TOF had to be corrected by the internal standard caffeine-*d3 @ m/z* 198 $[M+H]^+$, whereas HPTLC/ESI-MS showed a linear correlation without the use of an internal standard; experimental details see [3, 4].

University of Hohenheim, Institute of Food Chemistry, Garbenstr. 28, D-70599 Stuttgart, www.ilc.uni-hohenheim.de

Comparison of different ionization techniques (ESI, DART, APGD) for coupling of mass spectrometry with planar chromatography (HPTLC/MS)

Part 2 **Gertrud E. Morlock** **University of Hohenheim, Stuttgart, Germany**

The three techniques clearly showed the protonated molecule of caffeine $[M+H]^+$. For **HPTLC/DART-TOF** the plate was cut along the track or substance assignment window. The zones of interest were placed into the excited helium gas stream and within seconds mass spectra were obtained [3]. **HPTLC/APGD-TOF** coupling worked similar, with the exception that the after glow of an helium plasma was used [5]. Desorption systems generally require an automated plate positioning or/and internal standards for correction of the precision and linearity. Whereas the extraction-based interface used for **HPTLC/ESI-MS** worked well without any internal standard [3] which was proven for the analysis of caffeine in pharmaceutical and food samples [4]. The results obtained by HPTLC/ESI-MS were **highly reliable** and statistically comparable to the results of the validated HPTLC/UV method (Table 1).

Table 1: Comparative caffeine quantification in an energy drink and pharmaceutical sample by HPTLC/ESI-MS versus HPTLC/UV.

Sample	Caffeine in pharmaceuticals (mg/tablet) Mean ± SD (*%RSD*)	Caffeine in energy drinks (mg/100 mL) Mean ± SD (*%RSD*)
HPTLC/ESI-MS (n = 6)	102.09 ± 5.76 (5.6)	32.91 ± 1.60 (4.9)
HPTLC/UV (n = 5)	101.98 ± 2.30 (2.3)	33.71 ± 0.96 (2.8)
Labeled	100	32

The interface can easily be connected to existing HPLC/MS systems and within a minute the respective mass signal of an HPTLC zone was obtained. The system was modified for extraction from glass plates [6], and a positioning error of up to 5 % was found when working with the manual instead of the automated interface [7].
Detectability of the three coupling techniques was shown to be down to the ng/zone- or even pg/zone-range. Desorption techniques generally desorbed just an aliquot at the surface of the zone. However, the extraction-based interface was shown to elute the complete zone including its depth profile [8] and LODs in the lower pg/zone-range were obtained comparable to HPLC/MS.

The **spatial resolution** was shown to be better than 2 mm for HPTLC/DART-TOF and HPTLC/APGD-TOF. For HPTLC/ESI-MS it was limited to 2 or 4 mm depending on the plunger geometry.

Conclusions

Recent HPTLC/MS approaches, working under ambient conditions, extract or desorb zones of interest directly from an HPTLC plate and allow sensitive mass spectrometric signals within a minute or even within seconds. They enable cost-reduced and highly targeted recording of mass spectra.

Desorption techniques (DART, APGD)

☺ Dry desorption process ↔ DESI
☺ No extra plate preparation ↔ SALDI, SELDI, MALDI, SIMS
☺ Simple spectra ↔ MALDI, SIMS
☹ Works quantitatively only with internal standard or automated plate positioning
→ ☺ Potential to scan the whole track

Extraction techniques (ESI)

☺ Interface universally adjustable to existing HPLC/MS systems
☺ Interface with manual or automated zone positioning
☺ Detects down to the lower pg/zone-range comparable to HPLC/MS
☺ Works highly quantitative without the use of an internal standard

Thanks to Landesstiftung Baden-Württemberg (Project No. P-LS-E2/25), Dr. Luftmann, University of Münster, Germany, Dr. Leiss, Merck, Germany, and Mr. Rolli, CAMAG, Switzerland, for financial and material support.

[1] G. Morlock, M. Vega (2007) J Planar Chromatogr 20, 411; [2] G. Morlock, W. Schwack (2008) LCGC Eur June, in print; [3] G. Morlock, Y. Ueda (2007) J Chromatogr A 1143, 243; [4] H. Luftmann, M. Aranda, G. Morlock (2007) Rapid Commun. Mass Spectrom. 21, 3772; [5] G. Morlock, F. Andrade, G. Hieftje, in preparation; [6] A. Alpmann, G. Morlock (2006) Anal Bioanal Chem 86, 1543; [7] M. Aranda, G. Morlock (2007) Rapid Commun Mass Spectrom 21, 1297; [8] U. Jautz, G. Morlock (2006) J Chromatogr A, 1128, 244.

University of Hohenheim, Institute of Food Chemistry, Garbenstr. 28, D-70599 Stuttgart, www.ilc.uni-hohenheim.de