Hyphenating HPTLC and Direct Bioautography to Direct Analysis in Real Time Mass Spectrometry

MS Surface Scanning and the Influence of Bioassay Matrices

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The desorption-based ionization technique DART-MS was modified and utilized for quantitative scanning and substance characterization also subsequent to direct bioautography.

Direct Analysis in Real Time mass spectrometry (DART-MS) is a novel desorption-based ionization technique that is classified under ambient ionization techniques. It uses heated metastable helium or nitrogen for desorption and ionization of target compounds from various sampling surfaces. The first commercial DART ion source, the DART-100 (Ionsense, Saugus, MA) had a linear gas flow towards the MS system and was continuously improved to realize different hyphenations and applications. In 2006, the recording of DART-MS spectra from HPTLC zones started with a manual sample introduction into the linear gas stream [1] and was further developed to quantitative surface scanning of HPTLC chromatograms in 2012 [2]. Therefore, the interface geometry as well as the source cap and transfer tube design of the new DART-SVPA-3DS model was optimized to improve the signal intensity by a factor of 34 [3]. A homogeneous scan track was provided by this interface design and reliable quantification performance without internal standard was achieved. For repeated scanning along butyl paraben bands, a mean precision of 2.7% was obtained. Surface scanning was enabled with a spatial resolution at full width at half maximum of 0.8 mm and a scan track width of 3 mm [4]. Comparing extracted ion chromatograms (EIC) of mass-selective DART-MS scanning with densitograms at 254 nm revealed a better resolution for four adjacent parabens. Quantification was independent on the separation performance and on potential coeluting substances with similar absorption spectra and hR_F values (Fig. 1).

Additionally to scanning along HPTLC tracks, the scanning along a substance windows at specified hR_F values is possible (Fig. 2). The benefit of parallel separation of many samples was enhanced by such a rapid scanning along one substance window for mass selective quantification [3]. Hence, the fast DART-MS scanning was used in combination with multivariate data analysis for the profiling and classification of French propolis [5].

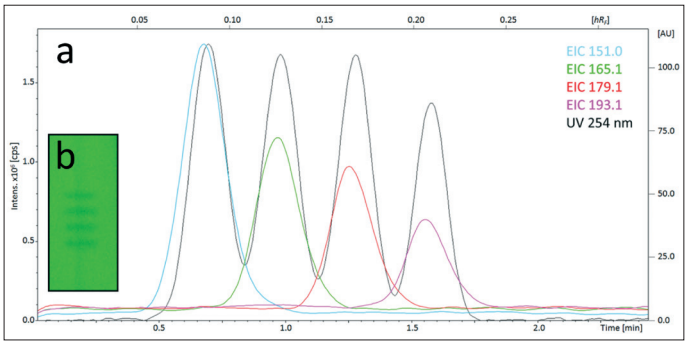


Fig. 1: Comparison of DART-MS scanning versus densitometry: (a) the resolution and peak shape was shown for individual EIC signals and densitogram peaks at UV 254 nm for four adjacent parabens (b, each 120 ng/band). Decreasing EIC signal intensities correlated with increasing molecular weight due to decreasing desorption and ionization rate. Reprinted with permission from [4]. Copyright 2015 John Wiley & Sons.

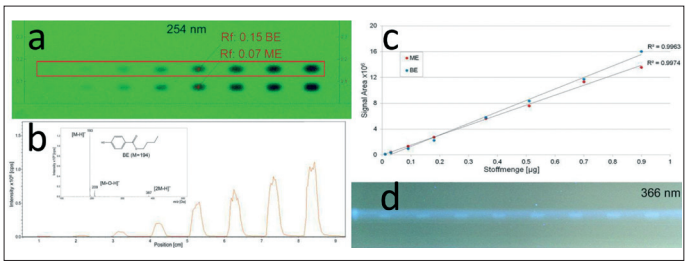


Fig. 2: Performance of HPTLC-DART-MS: (a) scanning along the substance window of methyl and butyl paraben (ME and BE 10-900 ng/band each, documented at UV 254 nm) to obtain (b) mass spectra, respective EICs and (c) calibration plots for ME (R^2= 0.9974) and BE (R^2=0.9963); (d) visualization at UV 366 nm of the fresh scan track along the substance window. Reprinted with permission from [3]. Copyright 2015 John Wiley & Sons.

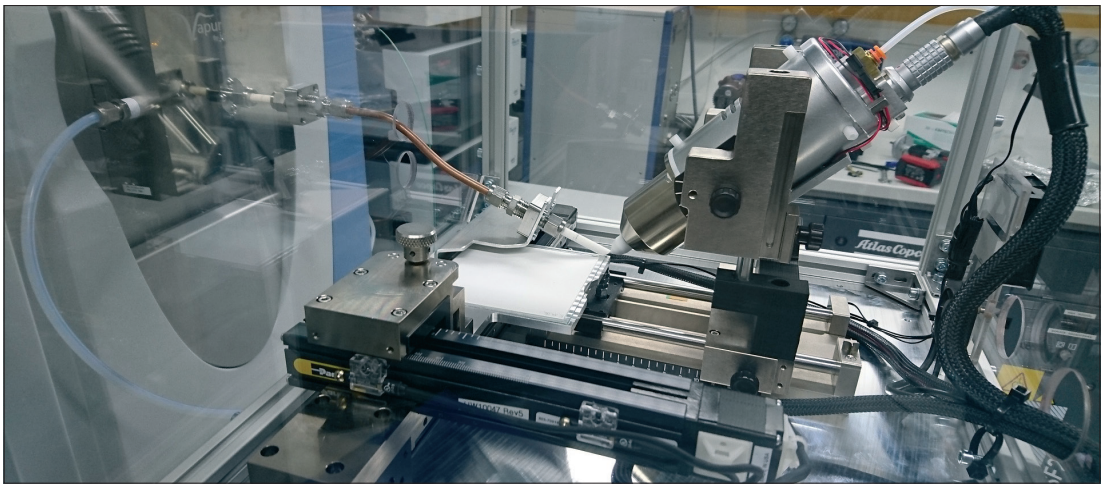


Fig. 4: The desorption-based ionization technique DART-MS

Combining direct bioautography (DB) data with scanning DART-MS results was helpful for tracking and identification of antibacterial compounds in the essential oil of *Tanacetum vulgare* L. [6]. After chromatography, one plate part was subjected to the *Aliivibrio fischeri* bioassay, whereas mass spectra and EIC profiles were recorded from the second identical plate part (Fig. 3). The same instrumental setup was used as part of the effect-directed discovery of bioactive compounds in *Solidago virgaurea* L. [7].

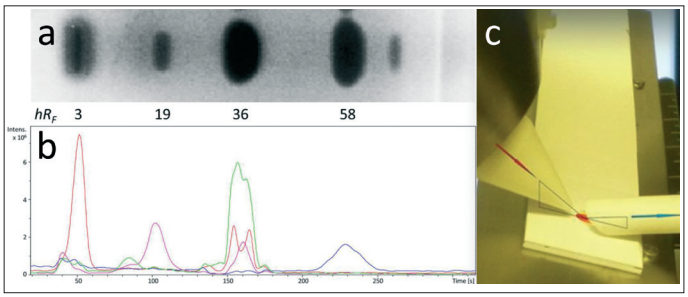
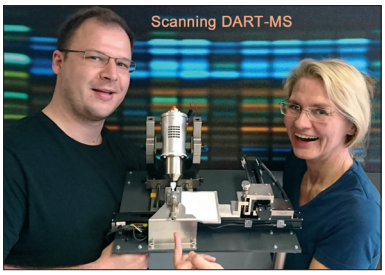


Fig. 3: Essential oil of *Tanacetum vulgare* L. analyzed by (a) *Aliivibrio fischeri* bioassay and (b) DART-MS scanning showing the overlaid EICs of the target compounds, (c) recorded with the modified interface. Reprinted with permission from [3,6]. Copyright 2015 Elsevier and 2015 John Wiley & Sons.

The direct hyphenation of DB with DART-MS was shown for the three bioassays of *Aliivibrio fischeri, Bacillus subtilis* and the planar yeast estrogen screen (pYES). The discriminating properties of the DART desorption and ionization enabled the detection of target compounds in the presence of the bioassay matrices [8]. The mean DART-MS signal intensity was decreasing by 72% for the pYES matrix and by 90% for the *Aliivibrio fischeri* matrix. This influence was notable; nevertheless, the overall DART-MS performance and detectability of the parabens were sufficient for reliable quantitation in the nanogram range. Quantification was investigated for two hand creme samples, with and without applied bioassays on normal and reversed phase layers. The mean deviation was 4.6% and represented a streamlined technique for detection of bioactive compounds prior to mass spectrometric characterization or quantification.

[1] Morlock, G.E., Schwack, W, Anal. Bioanal. Chem. 385 (2006) 586-595
[2] Chernetsova, E.S., Revelsky, A.I., Morlock, G.E., Rapid Commun. Mass Spectrom. 25 (2011) 2275-2282
[3] Häbe, T.T., Morlock, G.E., Rapid Commun. Mass Spectrom. 29 (2015) 474-484
[4] Häbe, T.T., Morlock, G.E., Rapid Commun. Mass Spectrom. 30 (2016) 321-332
[5] Chasset, T., Häbe, T.T., Ristivojevic, P., Morlock, G.E., J. Chromatogr. A 1465 (2016) 197-204
[6] Móricz, A.M., Häbe, T.T., Böszörményi, A., Ott, P.G., Morlock, G.E., J. Chromatogr. A 1422 (2015) 310-317
[7] Moricz, A.M., Ott, P.G., Häbe, T.T., Darcsi, A., Boszormenyi, A., Alberti, A., Kruzselyi, D., Csontos, P., Beni, S., Morlock, G.E., Anal. Chem. 88 (2016) 8202-8209
[8] Häbe, T.T., Jamshidi-Aidji, M., Macho, J., Morlock, G.E., in submission (2017)



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