Research Article

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Some new features of Direct Analysis in Real Time mass spectrometry utilizing the *desorption at an angle* option

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The present study is a first step towards the unexplored capabilities of Direct Analysis in Real Time (DART) mass spectrometry (MS) arising from the possibility of the *desorption at an angle*: scanning analysis of surfaces, including the coupling of thin-layer chromatography (TLC) with DART-MS, and a more sensitive analysis due to the preliminary concentration of analytes dissolved in large volumes of liquids on glass surfaces. In order to select the most favorable conditions for DART-MS analysis, proper positioning of samples is important. Therefore, a simple and cheap technique for the visualization of the impact region of the DART gas stream onto a substrate was developed. A filter paper or TLC plate, previously loaded with the analyte, was immersed in a derivatization solution. On this substrate, owing to the impact of the hot DART gas, reaction of the analyte to a colored product occurred. An improved capability of detection of DART-MS for the analysis of liquids was demonstrated by applying large volumes of model solutions of coumaphos into small glass vessels and drying these solutions prior to DART-MS analysis under ambient conditions. This allowed the introduction of, by up to more than two orders of magnitude, increased quantities of analyte compared with the conventional DART-MS analysis of liquids. Through this improved detectability, the capabilities of DART-MS in trace analysis could be strengthened. Copyright © 2011 John Wiley & Sons, Ltd.

Direct Analysis in Real Time Mass Spectrometry (DART-MS), reported for the first time by Cody *et al.* in 2005,[1] is a widely used method, with its main advantage being the minimization or even absence of the need for sample preparation. Several reviews have described the developments in DART-MS, as well as its benefits and limitations.[2–5] In the majority of early publications samples were manually introduced into the DART ionization region, and, because of the severe response dependence on the positioning of the sample in the ionization region, only analyte identification or chemical profiling of samples could be performed.[6–10] More recently the possibility of DART-MS quantitation was also developed,[11–13] usually employing special sampling devices. In addition, the coupling of DART-MS with high-performance thin-layer chromatography (HPTLC) was first demonstrated in 2006.[14–18] However, due to the fixed, horizontally aligned supply of the gas flow from the DART ionization source to the MS inlet, the introduction of HPTLC/TLC plates as cut strips was inconvenient for quantitation. The repeatability was very low due to the manual positioning, but it was shown to be reliable if an internal standard was used for correction of the positioning.[15]

The sensitivity of DART-MS was, however, 1–2 orders of magnitude lower than that of electrospray ionization (ESI)-MS.[2,11] Several attempts to decrease the detection limits of DART-MS for liquids were made: increasing the surface of a sampling probe by coiling several turns of wire around the needle, providing a 4-fold benefit in sensitivity,[19] micro solid-phase extraction (SPE; microextraction by packed sorbent, MEPS) combined with DART-MS,[20,21] and stir-bar sorptive extraction prior to DART-MS.[22] In our opinion, the coiled wire approach with an increased sampling surface, and thus an increased liquid volume, is very attractive, because it does not require additional steps which might complicate the analysis. However, the 4-fold sensitivity increase would not compensate for the 1–2 orders of magnitude difference from the sensititivity of ESI-MS. The limitation of the microSPE and stir-bar sorptive extraction based approaches is the greater cost- and time-consuming sample preparation.

In 2009, a new version of the DART ion source was introduced by the manufacturer (IonSense, Saugus, MA, USA), which allowed the angle of the DART gas stream to be adjusted and the use of a motorized rail. No experimental research papers have yet been published on the advantages provided by this source. The angled source should, however, significantly extend the general capabilities of DART-MS due to the introduction of and access to wide surfaces (pointwise, linearly or even in three different dimensions). The present work has focused on these new capabilities of DART-MS arising from the *desorption at an angle* option. First, a method for visualization of the impact region of the DART gas stream

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was developed, which is essential for the proper positioning of the adjustable DART ion source and of the sample substrate. Hereinafter under 'visualization' we mean the development of a visible impact region on the target due to the color change at the respective zone. Secondly, the overall DART-MS detectability of liquids was increased by the application of large volumes of analytes on glass surfaces and their subsequent analysis after drying.

EXPERIMENTAL

Reagents and chemicals

Coumaphos (*O*,*O*-diethyl-*O*-3-chloro-4-methyl-2-oxo-2*H*-chromen-7-yl phosphorothioate, Sigma-Aldrich, Steinheim, Germany) was >97% pure. 5-Hydroxymethylfurfural (Fluka, Seelze, Germany) was of a purity of >97%. The white sugar was bought in a local shop (Stuttgart, Germany). Methanol was HPLC-grade (Sigma-Aldrich). Ultra-pure water (18 MΩ * cm) was produced by a Synergy system from Millipore (Schwalbach, Germany). Other solvents (technical grade) were purchased from BASF (Ludwigshafen, Germany) and distilled prior to use.

For derivatization, 2-naphthol, *p*-aminobenzoic acid, diphenylamine (all Merck, Darmstadt, Germany), aniline (Fluka, Buchs, Switzerland) and phosphoric acid (Sigma-Aldrich) were used.

The aniline diphenylamine *o*-phosphoric acid reagent was prepared according to Morlock and Sabir:[25] 1.5 g aniline and 1.5 g diphenylamine were dissolved in 150 mL acetone and 15 mL *o*-phosphoric acid were added dropwise. The reagent stored in the refrigerator was stable for at least 1 month.

For the 2-naphthol sulfuric acid reagent,[23] 2 g 2-naphthol were dissolved in 180 mL ethanol and 12 mL sulfuric acid (50%) were added dropwise to avoid an accelerated exothermal reaction. The reagent stored in the refrigerator was stable for at least 1 year.

For the *p*-aminobenzoic acid reagent,[23] 1 g 4-aminobenzoic acid was dissolved in 36 mL pure acetic acid, and 40 mL water, 2 mL phosphoric acid (85%) and 120 mL acetone were then added. This reagent stored in the refrigerator was stable for at least 1 month.

Sample preparation

Visualization of the impact region of the DART gas stream on filter paper and TLC plates

Samples for visualization experiments were prepared by treating filter paper (Whatman, Maidstone, UK) or TLC plates silica gel 60 (20 × 10 cm, layer thickness ca. 250 µm, Merck) with a 25 mg mL^{-1} aqueous solution of white sugar (thereinafter: sugar). The 2 × 5 cm^2 paper strips were automatically immersed into the sugar solution using a chromatogram immersion device (CAMAG, Muttenz, Switzerland) and then dried with a hair-dryer. The TLC plate substrates were prepared applying the sugar solution areawise using the automatic TLC sampler 4 (ATS4, CAMAG). The volume applied was 100 µL on an application area of 2 × 20 cm^2.

After treatment with the sugar solution, the TLC plate or paper strip was automatically immersed into the aniline diphenylamine *o*-phosphoric acid reagent, the 2-naphthol sulfuric acid, or the *p*-aminobenzoic acid reagent, as described,[23] and dried with a hair-dryer. The substrates prepared with the aniline diphenylamine *o*-phosphoric acid reagent and 2-naphthol sulfuric acid were used within 0.5 to 3 h for the visualization experiments, because prolonged storage resulted in darkening of the substrate due to the slow reaction of the reagent with the sugar at room temperature.

Model samples for large-volume application

For the large-volume application, small glass vessels were prepared by cutting the bottoms from standard 2 mL glass vials (Agilent, Waldbronn, Germany). The inner height of the walls of each vessel was ca. 3 mm; the i.d. was ca. 10 mm. The model solutions of coumaphos in methanol were prepared at levels of 0.4, 2.0, 4.8, 16.0 and 40.0 µg mL^{-1}. These solutions and the volumes 1, 5, 12, 40 or 100 µL were pipetted into separate clean glass boats. For concentration, these vessels were kept at ambient conditions for 0.5 to 5 min depending on the volume.

Instrumental conditions

The optimized version of the DART ion source (DART-SVP A) was equipped with a motorized rail (IonSense), allowing the samples (e.g., TLC strips) to be introduced horizontally into the ionization region with a controlled speed in the range of 0.2–10 mm s^{-1}. The ion source was coupled to a G1956B MSD single quadrupole mass spectrometer (Agilent) via a Vapur vacuum interface (IonSense). The evacuation was performed using a diaphragm vacuum pump MZ 2 (Vacuubrand, Wertheim, Germany). The DART ion source was operated using the typical, recommended conditions:[1] positive ionization mode, needle voltage of 4000 V, voltages at electrodes 1 and 2 of 100 and 250 V, respectively. For operation of the DART source, helium gas (99.999%) was employed, whereas nitrogen gas was used in the standby mode. Two external flowmeters (Analyt-MTC, Müllheim, Germany) were installed to control the vacuum flow adjusted to 12.0 L min^{-1}, and the helium gas flow adjusted to 3.2–3.4 L min^{-1}. For DART operation, the DART Control software (IonSense) was used. The movement of the motorized rail was manually controlled using the 'move-to-the-left' and 'move-to-the-right' buttons in the software program and selecting the speed of movement in the range of 0.2–10 mm s^{-1}. For data acquisition and processing, LC/MSD Chemstation B.02.01-SR1(260) software (Agilent) was used.

For the visualization experiments, the DART ion source was operated at 150, 200, 250 and 300 °C. After the visualization reaction, the images of the substrates were acquired using the DigiStore 2 documentation system, a special system for digital documentation of TLC or HPTLC plates. The images were captured under UV light at 366 nm and at white light illumination (both in reflectance mode). That system consisted of a Reprostar 3 illuminator with a Baumer optronic DXA252 digital camera controlled by the winCATS software, version 1.4.5 (all CAMAG). For optimal positioning of HPTLC plates, the following coordinates of the angled DART ion source were selected: horizontal coordinate 2.6 cm; vertical coordinate 5.1 cm; angle 30°.

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For the experiments with coumaphos model solutions, the ion source was operated at 300 °C. The DART-MS analysis of the residue in the small glass vessels was performed keeping the DART source at 30° to the entrance of the Vapur interface. The small glass vessels were positioned with respect to the DART gas stream using the following optimal coordinates: horizontal coordinate 2.5 cm, vertical coordinate 5.3 cm. Single ion monitoring (SIM) measurements of coumaphos were carried out using its characteristic $[M+NH_4]^+$ ion at *m/z* 380.

RESULTS AND DISCUSSION

Visualization of the impact region of the DART gas stream

The optimized DART ion source (DART SVP-A), which allows *desorption at an angle*, is equipped with measuring rulers for horizontal, vertical and angled positioning (Fig. 1). However, it is difficult to suggest common positioning coordinates, suitable for all cases, because the optimal coordinates will depend on the type of mass spectrometer coupled with the DART source and the configuration of the Vapur interface, e.g., the ceramic tube length and its i.d. The dimensions of a substrate also have to be taken into account. For example, the thicknesses of a filter paper and a TLC plate are different; therefore, the focus point of the DART gas stream will vary. On the other hand, in each individual case the proper positioning of the DART source depending on the substrate is a prerequisite for an optimal detectability.

Hence for guiding the gas focus positioning, it is necessary to develop an approach for visualization of the impact region of the DART gas stream on the target for the determination of optimal coordinates of the DART ion source and the sample prior to performing any analysis. In this study the visualization was based on a chemical reaction upon heating. The respective impact region became colored and was thus visualized. The first attempts were performed with an unmodified, plain piece of filter paper placed beneath the DART gas stream. It was expected that, at an internal DART source temperature of 300 °C, pure heating would yield a brownish color based on charring. However, such an approach was not at all suitable: keeping the filter paper at a 1 cm distance from the DART ion source outlet for 20 s did not result in any color change. This could be explained by the fast cooling of the gas outside the DART source when entering atmospheric conditions. As has been reported previously,[24] the actual temperature at the sample surface can differ from the internal DART ion source temperature by 70–140 °C. Subsequent attempts at visualization employed a microchemical reaction. As a cost-effective substrate, the derivatization of sugar was selected.[25] A piece of filter paper or a strip of TLC plate was loaded with the aqueous sugar solution, dried and then immersed into the aniline diphenylamine *o*-phosphoric acid reagent. For application of the sugar solution onto the TLC plate, an automated application device was used which allowed us to calculate the amount applied on the carrier as 62.5 µg/cm^2. As has been reported in HPTLC studies,[25] the chemical derivatization reaction lasted 5–10 min at 110 °C and resulted in the fast color change of the respective zones. This reaction was used for the visualization process. The chemicals for the reaction were applied in two steps as the mixture of both in a common solution was not stable: sedimentation of sucrose occurred immediately after mixing the two solutions due to the low solubility of sucrose in acetone. However, preparation of the reagent with water instead of acetone as the solvent would help in this respect. In the case of filter paper, the suggested procedure is very simple and can easily be performed without any special equipment, with the exception of the device for automatic dipping which is known to be important for homogenous reagent application onto a carrier. As the most frequently used temperatures of the DART ion source are between 150 and 300 °C,[2] this temperature range was of primary interest in the visualization experiments.

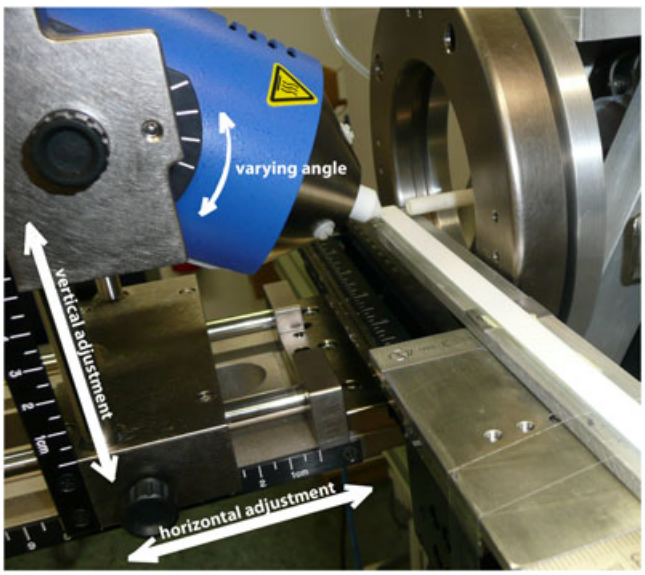

Figure 1. The new version of the DART ion source (called DART *SVP-A)*, which allows *desorption at an angle*, suitable for horizontal, vertical and angled positioning.

At temperatures in the range of 200–300 °C, the treated filter paper and TLC strip quickly turned brownish at the impact region, clearly indicating the focus of the DART gas stream. The photographic images of the paper and TLC strips were collected and documented using the DigiStore 2 documentation system at 366 nm, as well as under white light illumination, both in the reflectance mode (Fig. 2).

Directing the DART gas stream (T_{DART} = 300 °C) for only 3 s onto the treated filter paper resulted in the visualization of the impact region as an ellipsoidal spot, highly suited to the proper determination of the positioning coordinates. The impact region was bright and clearly visible. At 250 and 200 °C the color change was weaker at the same exposure time, but still satisfactory for positioning. At 150 °C, no color change of the substrate, treated with the aniline diphenylamine *o*-phosphoric acid reagent, was observed.

To establish the TLC-HPTLC/DART-MS hyphenation, linear scanning of the plate by DART-MS could be very attractive. Therefore, the exact linear positioning of the strip would be crucial when using the motorized rail. Due to the peculiarities of the linear rail construction, the plate strip had to be placed non-symmetrically in the horizontal direction on the carrier. In our first attempts at visualization of the impact region, a 2 × 20 cm^2 strip of a plate was fixed by

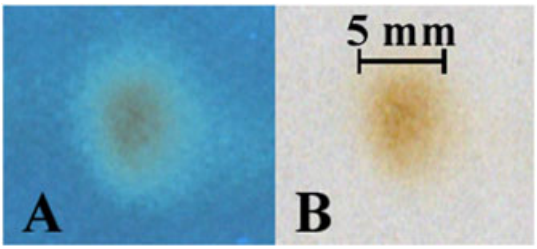

Figure 2. Visualization of the DART gas stream on a filter paper due to the chemical derivatization reaction of sugar with the aniline diphenylamine *o*-phosphoric acid reagent upon heating. DART was operated at 300 °C. The distance from the DART outlet to the filter paper surface was 1 cm. The exposure time of the paper to gas stream was ca. 3 s. The images were acquired using the DigiStore 2 documentation system (A) at 366 nm and (B) under white light illumination (corresponds to the image visible by eye), both in the reflectance mode.

Figure 3. Visualization of the DART gas stream on the TLC plate strip, treated with the aniline diphenylamine *o*-phosphoric acid reagent. DART was operated at 300 °C, the speed of the linear rail movement was 5 mm s^{-1}, the pauses for the DART gas stream visualization were ca. 2 s measured by the laboratory second meter. The image was acquired using the DigiStore 2 Documentation System under white light illumination in the reflectance mode.

Scotch tape onto a homemade 10 × 5 cm-wide stainless steel carrier table with ruler marks, which was mounted on the small (4.5 × 4.1 cm) motorized, linear rail table. The residual 10-cm TLC plate strip part was hanging over one carrier side. During the linear scan, this overhang caused vertical overbalance. Thus, the vertical coordinates of the impact region differed by 1 to 2 mm on the same plate, depending on its horizontal coordinate. A typical linear scan at a rate of 5 mm s^{-1} was employed for simulation of the scanning of a TLC-HPTLC plate in the so-called substance window (all compounds with the same hR_F-value, Fig. 3). Every 8–12 mm the linear movement was stopped for 2 s and the gas beam was kept onto the substrate, thus generating equidistant impact regions. The descending character of the visualized impact region on the plate strip, which can be seen in Fig. 3, reflects the pronounced dependence of the beam focus on the proper vertical alignment of the analyzed surface. This makes it clear that an ideally flat carrier table connected to the motorized linear rail must be used for analysis from such surfaces. Although such a table is currently unavailable on the market, it would have high potential for quantitative studies.

As we also desired to establish a method for color change at 150 °C, the possibility of visualization of the DART impact region using another sugar derivatization reagent, namely 2-naphthol sulfuric acid,[25] was investigated. At 150 °C this reagent rapidly gave the clear violet color of the respective substrates, which could also be visualized by measuring the fluorescence at 366 nm (Fig. 4). The experimental setup for

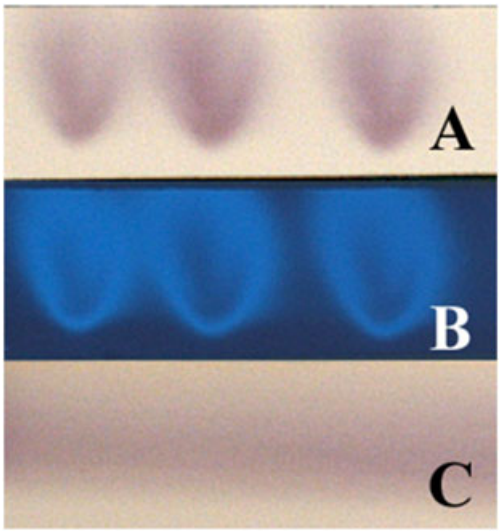

Figure 4. Visualization of the DART gas stream at 150 °C on the TLC plate strip (ca. 3 × 1 cm sections are shown), treated with the 2-naphthol sulfuric acid reagent. The speed of the linear rail movement was 2 mm s^{-1} (A, B) or 1 mm s^{-1} (C); the pauses for the DART gas stream visualization (A, B) were ca. 10 s measured by the laboratory second meter. The images were acquired using the DigiStore 2 Documentation System in the reflectance mode under white light illumination (A, C) or at 366 nm (B).

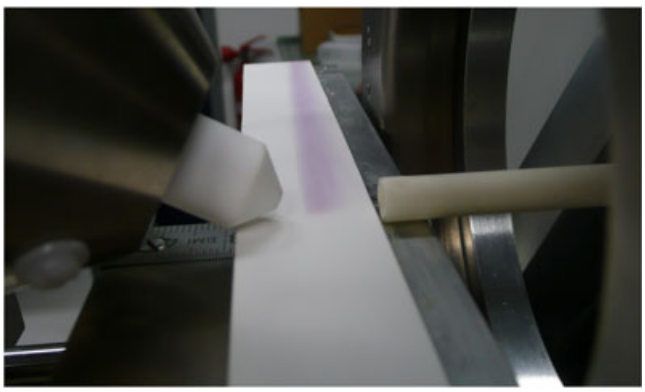

Figure 5. Experimental setup with the DART ion source with the *desorption at an angle* option (DART SVP-A). The visualization was performed in real-time using the substrate treated with the 2-naphthol sulfuric acid reagent.

the visualization study is illustrated in Fig. 5. At higher temperatures the color change of the substrate treated with 2-naphthol sulfuric acid was too intense; the TLC plate loaded with 62.5 µg/cm^2 sugar and treated with this reagent immediately colored when subjected to the DART gas stream at temperatures between 200 and 300 °C. However, by treating the TLC plate with lower quantities of sugar, it would also be possible to use this reagent at higher temperatures. For a more efficient protocol, it was found that, for dipping the respective plate piece, a mixture (1:1) of the 2-naphthol sulfuric acid reagent with the sugar solution can be used directly, thus simplifying the substrate preparation procedure. However, due to reduced sucrose adsorption onto the TLC plate by this procedure, the intensity of the color was weaker than

in the two-step procedure. The (1:1) mixture of the 2-naphthol sulfuric acid reagent with the sugar solution was found to be stable for at least 2 weeks after mixing if stored refrigerated.

The third reagent tested, *p*-aminobenzoic acid, did not show any color change or fluorescence at 366 nm when used at 150 °C. This was explained by the slower reaction rate than with the 2-naphthol sulfuric acid reagent. The slower reaction rate is also evident for derivatization of the components of the HPTLC zones, for which this reagent usually needs a longer heating time than the other two reagents until the color changes. At 300 °C the *p*-aminobenzoic acid reagent showed fluorescence at 366 nm. However, the two other reagents were found to be generally better suited for the visualization of the DART impact region due to the intense color visible in white light.

Following from this study, by using different combinations of quantity of sugar on a plate and varying the reagent type and quantity, one can choose the most appropriate conditions for the visualization of the DART impact region in any specific analysis. Of course, other derivatization reactions are also possible.

After these studies, in which the settings for the ion beam alignment onto the substrate were established, mass spectra of 5-hydroxymethylfurfural (HMF) from the HPTLC plate were recorded with the optimized coordinates selected (Fig. 6). The *desorption at an angle* and the use of a linear rail allow the prompt reliable recording of spectra from surfaces. The gain in reliability will be described in detail in a forthcoming paper.

Large-volume application in DART analysis

Using the common DART ionization source with a horizontal gas flow, the liquid samples are usually supplied to the ionization region using a glass capillary (e.g., the Dip-It tips, IonSense). The limitations of such a sampling method are that the sample volume captured by the tip:

- is very low. In our experience, it depended on the viscosity of the solvent and was usually less than 1 μL.
- varies with the filling height (volume) of a liquid sample inside the vial. Even when using an autosampler, it was difficult to supply the same amount of liquid for each measurement.

With the optimized, angled DART SVP-A, new ways of sampling for liquids are possible. For example, it is possible to use small glass vessels for sampling to overcome the disadvantages of handling liquids in DART-MS (listed above). By using liquids in small glass vessels, it would be possible to control the sample volume by pipetting, as well as to apply higher volumes in order to increase the analyte quantity in the ionization region. The latter advantage would be especially important for the determination of trace amounts of analytes, when detection by the routine DART-MS procedure where the liquid is introduced on glass sticks is not satisfactory. For example, the amount of analyte retained on the tip of the glass stick can be below the amount detected by DART-MS.

Residues of coumaphos in small glass vessels were used as model samples to evaluate the large-volume capabilities of DART-MS with the *desorption at an angle* option. Methanolic solutions of coumaphos were pipetted and then dried in the homemade, cut glass vessels which were used as support surfaces for different volumes of model solutions (Fig. 7). As coumaphos is a non-volatile compound with a melting point at 91 °C, we expected that its quantity on a glass surface would not decrease upon solvent drying. Due to the high vapor pressure of methanol (13.02 kPa at 20 °C) it was possible to dry solutions of 1–100 μL by leaving them on a glass surface in ambient conditions over 0.5–5 min and without

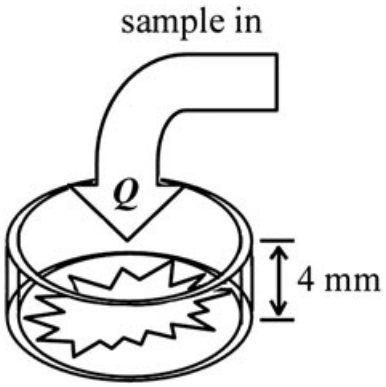

Figure 7. Schematic view of a small glass vessel used as a carrier for large volumes of liquid samples.

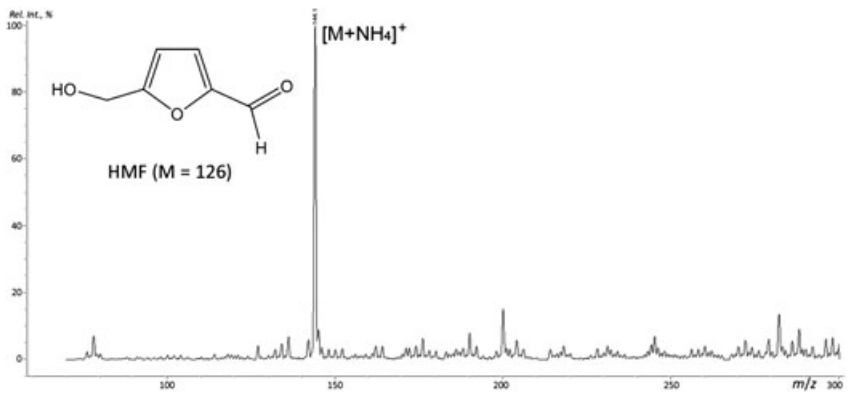

Figure 6. DART mass spectrum for a zone on the HPTLC plate containing 1 μg 5-hydroxymethylfurfural, recorded at an angle of 30° to the surface of the plate.

evaporation in a nitrogen flow. Such nitrogen-assisted evaporation was undesirable, because, according to preliminary experiments, it resulted in the distribution of the analyte on the periphery of the nitrogen flow focus. Consequently, the homogeneity of substance distribution and the repeatability of analyte response were decreased.

The experimental setup included two series of 20 samples each. For each series, the coumaphos residue was calculated to be 2, 24, 80 and 200 ng. Each quantity was represented by five equally prepared samples (4 × 5 samples in total). The first series was prepared by application of different volumes of the same analyte concentration, while the second was prepared by pipetting the same volumes of different analyte concentrations (Fig. 8). This setup allowed the confirmation of the complete recovery of coumaphos from such samples with a large initial volume. It was thus possible to compare the responses of the same analyte quantities applied either in fixed small (5 μL) or varying large (1–100 μL) volumes of liquid solutions.

DART mass spectra of methanolic coumaphos solutions were initially obtained by manual introduction on a glass stick (Fig. 9(A)). The DART ion source temperature of 300 °C was found to be optimal for recording coumaphos mass spectra. The spectra of model solutions contained two abundant signals at *m/z* 363 and 380, which corresponded to the $[M+H]^+$ and $[M+18]^+$ ions of coumaphos, respectively. Depending on the concentration of coumaphos in these solutions, the relative intensity of the $[M+18]^+$ ion was ca. 2–3 times higher than that of $[M+H]^+$. Hence, for further quantitative experiments in the SIM mode, the *m/z* 380 ion was monitored (Fig. 9(B)). This ion is assumed to correspond to $[M+NH_4]^+$ of coumaphos as the formation of abundant $[M+NH_4]^+$ ions has been widely reported in DART-MS with or without using ammonia vapors as a dopant.[2] In our experiments, no special supply of ammonia to the ionization region was arranged. The peak heights for both series were comparable for identical quantities of coumaphos on the glass surfaces, although obtained in two different ways (Fig. 10). It was also possible to use the peak areas for measurements, but this drastically increased the analysis times for higher quantities due to the peak tailing until the desorption of the whole analyte quantity was complete (up to 5 min per sample or even more). Therefore, we do not recommend the use of peak area measurements for high-throughput analysis. We found that the recovery of coumaphos from the samples with the same quantities applied on the surface was comparable when applying either small or large volumes, and that no loss of analyte from the glass vessels occurred upon drying. The precision (%RSD, n = 5) was in the range of 26–56%. These relatively high values could be caused by the non-optimal shape of the flat-bottomed glass carrier causing the analyte distribution on that bottom to be wide and inhomogeneous. Conically bottomed vessels or carriers could provide focusing of the residue upon drying, and these requirements are already partially met in the DAP-it™ glass inserts which were suggested in 2011 for the DART-MS analysis of liquids.[26] However, the liquid volume applied to such inserts is restricted to 10 μL, and to date no studies have been published on the use of such inserts for the analysis of liquids.

The limit of quantification (LOQ, signal-to-noise (S/N) ratio of 10) of coumaphos was evaluated from the 0.4 μg mL^{-1} coumaphos solution applied as 5 μL (mean S/N ratio of 20) and was calculated to be ca. 1.0 ng analyte on the glass surface. However, the LOQ evaluated from the same coumaphos quantity applied as 1 μL of 2 μg mL^{-1} (mean S/N ratio of 50) solution was ca. 0.4 ng analyte on the glass surface. This

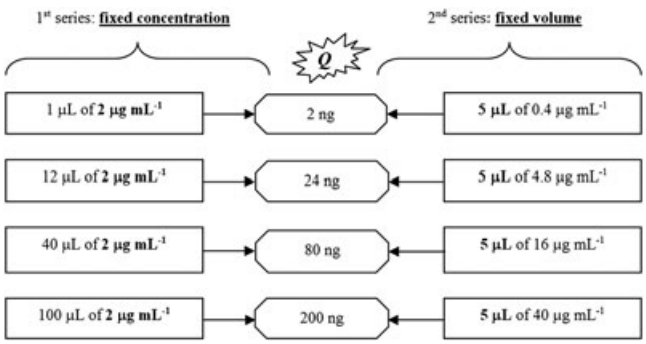

Figure 8. Preparation of two series of model samples containing equal quantities (*Q*) of coumaphos dried in small glass vessels: (*left*) a fixed analyte concentration was applied in different volumes and (*right*) a fixed volume of different analyte concentrations was applied.

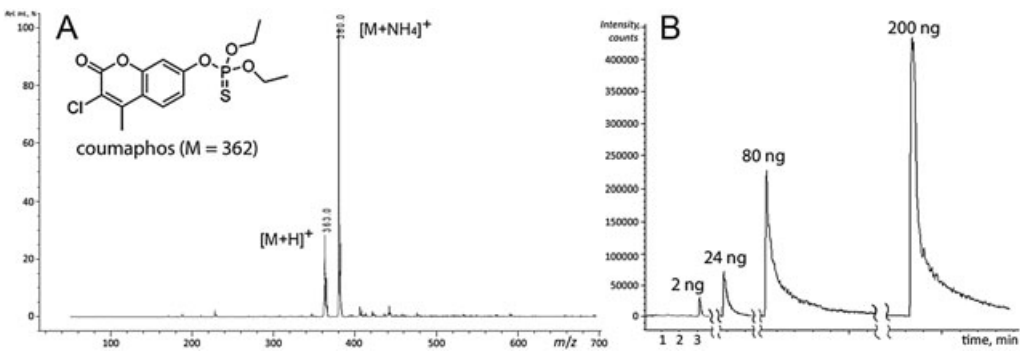

Figure 9. Mass spectrum of a methanolic coumaphos solution (40 μg mL^{-1}) applied on a glass stick (sample volume <1 μL) (a) and DART chronogram recorded in the SIM mode for coumaphos residues analyzed from the small glass vessels, when the large volumes of solutions were applied (b).

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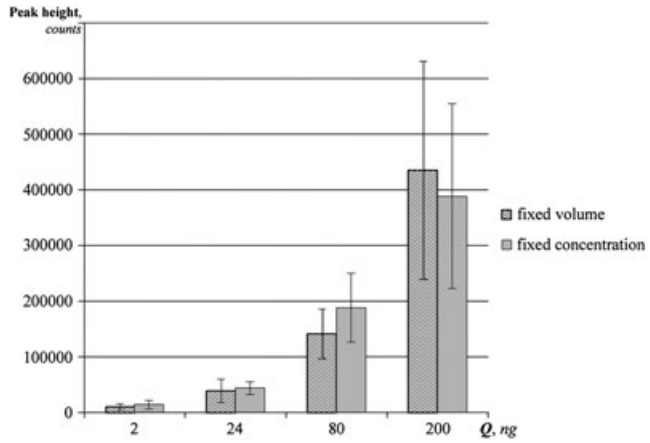

Figure 10. The dependence of signal intensities (peak heights, SIM mode at *m/z* 380) on the quantity of analyte on a glass surface for both series of model samples (n = 5).

could be explained by the better focusing of the analyte on the flat surface of the vessel bottom due to its lower volume. Hence, one might expect the best detectability for analytes in large volumes to be provided by the use of conically bottomed glass vessels due to the improved focusing of the residue and, therefore, more homogeneous and complete ionization of the whole analyte quantity.

CONCLUSIONS

The present study is a first step towards the completely new and unexplored capabilities of DART-MS arising from the possibility of the *desorption at an angle* scanning analysis of surfaces, including the coupling of planar chromatography with DART-MS, and a more sensitive analysis due to the concentration of analytes from large volumes of liquids on glass surfaces.

For the analysis of different surfaces and proper positioning of samples, a simple approach for the visualization of the DART stream on a surface was suggested based on the chemical reaction on a filter paper or TLC plate upon heating. This approach, when employed using filter paper, does not reqire any high-cost equipment or reagents. It allows the proper coordinates of the DART ion source and a sample to be selected for most favorable conditions for ionization from a sample surface. Such visualization experiments should be performed *before* the analysis of real samples. Especially for scanning a whole sample track by DART-MS coupled with planar chromatography, such a visualization approach is useful due to the possibility to optimize the coordinates of the DART ion source. Until now, no completely satisfactory solution has been suggested for the coupling of planar chromatography with DART-MS. The existing setup could be convenient for the scanning of a TLC or HPTLC strip vertically along its track, which means 'one scan – one separation (one sample)' (only one separated sample can be analysed by such single scanning). Upon sample application, a band length in TLC and HPTLC typically ranges between 4 and 8 mm. Therefore, the chromatographed bands could be more robustly scanned in this (horizontal) direction rather than perpendicular to them (vertical) where the band width will be only 1 or 2 mm. However, for the development of an efficient quantitative HPTLC/DART-MS coupling, the important option, which is not yet possible, would be horizontal scanning of a plate along the socalled hR_F substance window. In this way analyte signals could rapidly be obtained from all samples on the plate. However, this is not yet possible because of the absence of a technical solution, which would embody the motorized rail equipped with a flat carrier for TLC or HPTLC plates with a suitable fixation providing its excellent alignment in all dimensions. In addition, a slight reduction in the DART outlet cone diameter could improve the spatial resolution and this will be investigated in further studies.

The other important possibility, explored in the current study, was the up to more than two orders of magnitude improved detectability in the DART-MS analysis of liquids by analyzing analyte residues from the large volumes applied on glass surfaces. Application volumes up to 100 μL or more can be used. Compared with the conventional DART-MS analysis of liquids, this means a step towards a HPLC/MS-like detectability for DART-MS analysis. The proposed approach for the DART-MS analysis of large volumes of liquids and employing the *desorption at an angle* option can be useful for the determination of trace concentrations of analytes in liquids. However, depending on the complexity of the sample compositions, the influence of any matrix effects has to be considered, because the matrix components will be concentrated on a glass surface together with the analytes. Limitations are given by the volatility of compounds or low vapor pressure of the solvent.

It is obvious that the coupling of planar chromatography with DART-MS is restricted by the thickness of the layer, the diffusion of analytes into its depth, and the geometry of the zone. Single zones on the TLC or HPTLC plate could be almost completely eluted in about 100 μL of solvent by the TLC-MS Interface (CAMAG). Such 100 μL eluates can also be considered in the approach suggested for large volumes and concentrated in small glass vessels. The benefit might be a gain in sensitivity over the direct DART-MS analysis of zones on the TLC or HPTLC plate. Such an off-line coupling would provide the ionization of the whole analyte quantity and could be beneficial in specific cases, although collecting eluates and concentrating them on glass surfaces seems to be not as user-friendly as direct analysis.

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