Implementation of an enclosed ionization interface for the analysis of liquid sample streams with direct analysis in real time mass spectrometry

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Rationale: The development of an interface to analyze liquid sample streams with direct analysis in real time mass spectrometry (DART-MS) is of great interest for coupling various analytical techniques, using non-volatile salts, with MS. Therefore, we devised an enclosed ionization interface and a sample introduction system for the versatile analysis of liquid samples with DART-MS.

Methods: The sample introduction system consists of a nebulizer, a spray chamber and a transfer line, while the confined ionization interface is created by implementing a cross-shaped housing between ion source outlet and mass spectrometer inlet. Methodical studies of the effects of various setup parameters on signal intensity and peak shape were conducted, while its diverse applicability was demonstrated by coupling with high-performance liquid chromatography (HPLC) for the analysis of alcohols, organic acids and furanic compounds.

Results: The confinement of the ionization interface results in a robust setup design with a well-defined ionization region for focusing of the sprayed sample mist. Thereby, an increase in analyte signal intensity by three orders of magnitude and improved signal stability and reproducibility were obtained in comparison with a similar open ionization interface configuration. Additionally, the successful quantification of alcohols could be demonstrated as well as the compatibility of the setup with HPLC gradient elution.

Conclusions: A versatile setup design for the analysis of liquid sample streams with DART-MS was devised for monitoring reactions or hyphenating analytics with MS. The design minimizes interferences from the laboratory surroundings as well as allows for safe handling of hazardous and toxic chemicals, which renders it suitable for a broad range of applications.

1 | INTRODUCTION

Mass spectrometry (MS) is a powerful technique capable of providing valuable information for species identification and structure elucidation. Therefore, it is of high interest to combine MS with various analytical separation tools, since it additionally offers the possibility of increased selectivity and often also improved sensitivity in comparison with typically applied detectors such as ultraviolet...
Among these ambient ionization techniques, direct analysis in real time (DART), developed in 2005 by Cody et al., turned out to be applicable to a broad variety of separation techniques, as analytes can be introduced within matrices of all physical states (solid, liquid or gaseous). The simplest combination of DART-MS with chromatography is the analysis of thin-layer chromatography (TLC) plates. Thereby, the gas stream of the DART source is used to desorb the analytes from the separated spots on the TLC plate and ionize the sample for compound identification. Furthermore, a hyphenated technique between gas chromatography (GC) and DART-MS has been realized, by positioning the outlet of a GC column directly in front of the MS orifice within the ionization region. Pioneering work on online coupling of high-performance liquid chromatography (HPLC) with DART-MS has been performed by Klampfl and co-workers. They developed an interface which introduces a liquid jet of HPLC effluent into the ionization region via a capillary and they applied it for the sensitive quantification of parabens. Additionally, the insusceptibility of DART towards ion suppression by non-volatile phosphate buffers was demonstrated as well as the superiority of this ion source in handling complex matrices. Chang et al. adapted this setup to perform chiral normal-phase liquid chromatography by adjusting the flow rate of the effluent with a splitting Tee junction. The latest innovation to couple liquid chromatography (LC) with DART-MS presents a thermal-assisted gasification injector as the interface between chromatography and MS. The high tolerance of DART towards non-volatile salts was also explored by coupling DART-MS with capillary electrophoresis (CE) and surface plasmon resonance (SPR). For this purpose, an interface based on a spray tip was developed with the tip positioned between the ion source and the inlet to the mass spectrometer to generate a fine ionizable mist of effluent in the ionization region.

In this work, we introduce an enclosed interface for analyzing liquid sample streams with DART-MS, based on the introduction of a cross-shaped housing between the DART ion source and the mass spectrometer inlet. Confined DART interfaces have already been applied for the detection of volatiles from fruits and human breath as well as for the detection of gaseous species evolving during thermal desorption and pyrolysis DART-MS. In these cases, a Tee junction was implemented between the ion source and the mass spectrometer inlet to introduce the gaseous species to the ionization region and thereby prevent random gas diffusion for increased ionization efficiency. Moreover, the confinement of the interface resulted in an increased sample-to-sample reproducibility in comparison with classical open-air DART analyses. Additionally, a closed interface for maximum reproducibility has been recently developed for the analysis of solid-phase microextraction (SPME) fibers with DART-MS. It is noteworthy that the numerous advantages of interface enclosure have not only been explored for DART-MS, but also for applications with other ambient ionization techniques such as protein analysis with desorption electrospray ionization (DESI). In contrast to the previously described enclosed DART interfaces, the setup presented here introduces a nebulized stream of liquid into the confined ionization region and has already been successfully applied for the coupling of an electrochemical cell to DART-MS for real-time product analysis during electrochemical reactions. This work provides deeper insights into the effects of various setup properties such as the enclosed interface, the spray chamber and the introduction system parameters on the analyte signal intensity, reproducibility and susceptibility to interferences from the surroundings. Furthermore, the universal applicability of the setup for the analysis of liquid sample streams is emphasized by demonstrating the coupling with HPLC during isocratic and gradient elution. Additionally, the performance of the system as a quantitative detector is showcased for a series of six simple alcohols.

2 | EXPERIMENTAL

2.1 | Chemicals and reagents

Methanol, ethanol, 2-propanol and acetic acid were purchased from Merck. 1-Butanol, propionic acid, 5-hydroxymethylfurfural, 5-methylfurfural and acetonitrile (HPLC gradient grade) were purchased from Sigma Aldrich. 1-Propanol, acrylic acid and sodium dihydrogen phosphate dihydrate were purchased from Alfa Aesar. 2-Butanol and phosphoric acid (85%) were purchased from Honeywell Research Chemicals. Formic acid and 2,5-dihydroxydimethylfuran were purchased from VWR Chemicals and 5-methylfurfuryl alcohol was purchased from Acros Organics. All standard solutions were prepared using ultrapure (18 MΩ cm) water from a Milli-Q IQ 7000 system (Merck).

2.2 | Instrumentation

All experiments were performed on a JMS-T100LP AccuTOF LC-plus 4G mass spectrometer (JEOL, Tokyo, Japan) with a time-of-flight mass analyzer with resolving power of ±10,000 (measured at nominal m/z 609 according to full width at half maximum (FWHM) definition). A DART-SVP ion source (IonSense, Saugus, MA, USA) was mounted on
the mass spectrometer and operated with helium gas (Air Liquide, Grade 5.0) for all measurements. The sample introduction system includes a concentric PTFE nebulizer (PFA-ST, Perkin Elmer) and a baffled, cyclonic glass spray chamber with an inner volume of 40 mL (for NexION 300/350, Perkin Elmer). For investigations on the effect of the inner volume of the spray chamber on the analysis, the former spray chamber was replaced by a cyclonic, baffled glass spray chamber with an inner volume of 20 mL (for NexION PC3, Elemental Scientific). As nebulizing gas, either oxygen (Air Liquide, Grade 4.8), synthetic air or argon (Air Liquide, Grade 4.8) was used. A stainless-steel transfer line and a modified stainless-steel union cross for 6 mm tubing (Swagelok) completed the setup. For the direct analysis of liquid samples, a Reglo ICC peristaltic pump (Ismatec) was coupled to the nebulizer in order to create a stream of the liquid sample, which is necessary for the application of the here presented setup. The coupling of the setup with HPLC was performed with an Infinity II 1260 instrument (Agilent Technologies, Waldbronn, Germany). Headspace GC/MS was performed on a Clarus 580 gas chromatograph coupled to a Clarus SQ 8 T mass spectrometer and equipped with a TurboMatrix 40 headspace autosampler (Perkin Elmer).

### 2.3 Analytical methods

All alcohols were analyzed in the positive mode of the DART-MS setup using an orifice 1 voltage of $+50 \text{ V}$, the spray chamber with 40 mL inner volume and a flow rate of the liquid sample of 0.8 mL min$^{-1}$. Optimal values for the flow rate of oxygen as nebulizing gas, the helium gas flow rate and the ion source temperature were found to be 0.4 L min$^{-1}$, 3.0 L min$^{-1}$ and 250°C, respectively, and were used for all experiments, if not stated otherwise. A mixture of six alcohols (methanol, ethanol, 1-propanol, 2-propanol, 1-butanol and 2-butanol) was separated on a Hi Plex H column (7.7 × 300 mm, particle size 8 μm; Agilent Technologies) using ultrapure water at a flow rate of 0.8 mL min$^{-1}$ as the eluent while the column temperature was kept constant at 70°C and 50 μL was defined as the injection volume.

The amount of analyte that could be recovered from the liquid drain of the spray chamber was quantified by a method adapted from a procedure previously applied for atomic absorption spectroscopy. A 50 mL volumetric flask was used to collect all liquid from the drain of the spray chamber, while 5 mL of an aqueous solution containing 1000 mg L$^{-1}$ methanol, ethanol and 2-propanol were subjected to the system using the parameters described above. Afterwards, pure water was passed through the system in the same way until the 50 mL flask was filled and the decay in the alcohol signal was monitored by DART-MS. To determine the amount of alcohol recovered, 1 mL of the diluted alcohol solution was quantitatively analyzed by headspace GC/MS (for instrumental details, see section 2.2). The crimped headspace vial was thermostated for 15 min at 65°C before the vapor was injected into an Elite-624Sil MS column (30 m × 0.32 mm × 1.8 μm; Perkin Elmer) for separation. In the gas chromatograph, a temperature program was performed starting with a 5 min hold at 35°C, followed by a ramp at 2°C min$^{-1}$ to 45°C, finalized by a ramp at 40°C min$^{-1}$ to 90°C. The compound detection was performed with electron ionization mass spectrometry (70 eV) using a quadrupole mass analyzer.

For the analysis of organic acids, the DART ion source was operated in the negative mode at 300°C with a helium flow rate of 2.5 L min$^{-1}$ and an orifice 1 voltage of $–35 \text{ V}$ was set at the mass spectrometer. Oxygen was chosen as the nebulizing gas and operated at a flow rate of 0.9 L min$^{-1}$ while the liquid sample flow rate was set to 0.5 mL min$^{-1}$. The separation of the organic acid mixture (formic acid, acetic acid, propionic acid, acrylic acid) was performed on an Acclaim Organic Acid (OA) column (4 × 250 mm, particle size 5 μm; ThermoFisher Scientific). A freshly prepared 10 mM phosphoric acid buffer (pH 2.6) was used as the eluent at a flow rate of 0.5 mL min$^{-1}$. The column was kept thermostated at 25°C and an injection volume of 50 μL was chosen. In order to investigate the effect of the sample introduction system on the mass chromatograms, the diode-array UV detector of the HPLC system was implemented between the outlet of the HPLC column and the inlet to the nebulizer and set to a wavelength of 210 nm.

The four furanic compounds (5-hydroxymethylfurfural, 2,5-dihydroxymethylfuran, 5-methylfurfural and 5-methylfurfuryl alcohol) were separated by gradient elution on an InfinityLab Poroshell 120 EC-C18 column (3 × 150 mm, particle size 2.7 μm; Agilent Technologies). Ultrapure water and acetonitrile were used as eluents and the amount of acetonitrile was increased from initially 3% to finally 25% during a 15 min run. The eluent flow rate was kept constant at 0.5 mL min$^{-1}$; the temperature of the column was maintained at 25°C and the injection volume was 10 μL. The UV detector was implemented before the introduction system into the mass spectrometer and set to wavelengths of 210 nm and 285 nm to monitor the elution of the compounds. The DART ion source was operated in the positive mode at 300°C with a helium flow rate of 3.0 L min$^{-1}$. An orifice 1 voltage of $+40 \text{ V}$ was chosen and the spray chamber with 40 mL inner volume was used with oxygen at a flow rate of 0.9 L min$^{-1}$ as the nebulizing gas.

### 3 RESULTS AND DISCUSSION

#### 3.1 Setup design for direct introduction of a liquid stream into the DART-MS ionization region

The setup for analyzing streams of liquid with DART-MS is composed of two parts (Scheme 1). The first part is the sample introduction system which consists of a nebulizer, a spray chamber and a transfer line. Its purpose is to spray the stream of analyte-containing liquid and transfer the fine part of the generated mist towards the ionization region. The second part is the cross-shaped housing, which is introduced between the DART ion source and the mass spectrometer inlet to obtain an enclosed ionization interface.
The nebulizer and the spray chamber are commercially available items, originally designed for the inlet system of inductively coupled plasma mass spectrometry (ICP-MS) and adapted to our purpose. The nebulizer is used to create an aerosol of the liquid sample inside the spray chamber. The latter ensures that a constant stream of homogeneous fine mist droplets is supplied through the transfer line to the ionization region and thereby reduces baseline noise and spikes. Both parts can operate at liquid flow rates between 0.1 and 2.0 mL min\(^{-1}\) and are stable against all common solvents, which renders them highly suitable for coupling with, e.g., HPLC. A stainless-steel transfer line connects the upper outlet of the spray chamber with the implemented housing that encloses the ionization region. Since applications such as electrochemistry, HPLC or CE require the handling of non-volatile mobile phases, the transfer line can be converted into a salt trap by heating it with a resistance wire to elevated temperatures.\(^{28}\) Thereby, the majority of the salt precipitates on the transfer line inner walls, so that the orifice and the ion source are protected, while the volatile compounds continue on to the ionization region.

A modified stainless-steel union cross functions as the housing to generate an enclosed ionization environment for the nebulized sample. It is connected to the outlet of the transfer line from one side and to an exhaust line at the opposing thread, while the two remaining threads were removed from the union cross. The so-generated housing is held tightly in place by pressing it between the conical ceramic DART ion source outlet and the conical mass spectrometer inlet orifice, resulting in a fixed distance of 11 mm between ion source and orifice (Scheme 1). Thereby, a well-defined ionization region is generated in the center of the housing, since the sprayed liquid stream is directed perpendicular to the helium stream within the crosspiece. Moreover, the implementation of the housing renders the whole design rather robust and reproducible, as it confines the distance between the ion source, the mass spectrometer inlet and the position of the mist inlet into the housing, based on the housing geometry. Additionally, the waste outlet of the housing is directly connected to an exhaust, which enables the use of hazardous chemicals without the risk of exposure to the user. For ideal operation of the setup and high reproducibility, the whole system needs to be equilibrated at least 1 h in advance in order to allow for all the temperatures to stabilize, as the housing temperature is indirectly controlled by the temperature set at the DART ion source. The setup presented here also enables a facile way of sample recovery from the liquid waste drain of the spray chamber, which allows for applications such as preparative HPLC, where sample retrieval for further processing or analysis is required. It was found that methanol (MeOH), ethanol (EtOH) and 2-propanol (2-PrOH) could be retrieved by 95%, 91% and 88%, respectively, from the spray chamber waste with high reproducibility (relative standard deviations (RSDs) <3.3% from three consecutive measurements). Thus, it can be concluded that the majority of the analyte can be retrieved from the spray chamber waste, while the actual percentage of sample regained is dependent on the compound and will most likely be influenced by the nebulizer and spray chamber model and the applied analytical conditions as well (e.g. nebulizing gas flow rate and liquid sample flow rate).

3.2 Effects of the enclosed interface

In order to study the effects of the enclosed ionization region on the detection sensitivity and the interferences from the surrounding laboratory atmosphere, comparative experiments on an open ionization interface configuration were performed. Therefore, the stainless-steel union cross was removed, while the positions of the outlet of the transfer line and the DART ion source were kept unchanged. Exemplarily, we investigated the effect of the housing on the EtOH and 2-PrOH signal intensity, which was defined as the increase in MS signal observed upon switching from a sample containing only the background matrix (in this case water) to a sample containing the analyte within the background matrix (in this case aqueous alcohol solutions). In the positive ionization mode of the DART-MS setup, EtOH was detected with highest sensitivity in its protonated form at \(m/z\) 47 as \([M + H]^+\) (where \(M\) is the analyte molecule), while 2-PrOH was detected at \(m/z\) 43, which corresponds to the most abundant \([M + H – H_2O]^+\) carbocation, since longer chain alcohols tend to lose water upon protonation.\(^{28}\) It was observed that the MS response obtained by introducing a 1000 mg L\(^{-1}\) EtOH or 2-PrOH sample into the open configuration system was comparable to the signal increase obtained from 1 mg L\(^{-1}\) of analyte in the confined ionization interface configuration (Figure S1, supporting information). To deduce if this effect is caused by the enclosure of the ionization region or by the oxygen-based ionization environment in the enclosed system, experiments with air instead of oxygen as the nebulizing gas were performed for direct comparison and similar results were obtained. This demonstrates that it was truly the enclosed ionization environment that led to an increase in signal intensity by approx. three orders of magnitude, for both analytes, in
comparison with the open configuration. The reason for this strongly enhanced detection sensitivity is the improved analyte ionization efficiency in the enclosed configuration, since the sample mist is being focused in the ionization region by the housing. In addition, a smoothening of the baseline noise and a more stable analyte signal were observed when the housing was implemented (Figure S1, supporting information). Considering the sample-to-sample reproducibility, RSDs from three repetitive injections of 1 mg L\(^{-1}\) EtOH and 2-PrOH in the enclosed ionization interface configuration were found to be below 4%. In case of the open configuration on the other hand, RSDs of approx. 20% were determined for three successive injections of 1000 mg L\(^{-1}\) EtOH and 2-PrOH. This beneficial effect of the housing on the reproducibility of the analysis is a result of the defined geometry and confinement of the ionization region as well as of the protection from disturbances from the surroundings. To further evaluate the influence of the housing regarding interferences from the surroundings, EtOH and 2-PrOH were chosen as typical volatile compounds from a lab environment to study their interference on the MS analysis in the open and in the enclosed configuration. Therefore, the two solvents were brought in close vicinity to the ionization region, within a well-controlled distance of approx. 13 cm, while pure water was continuously supplied to provide a stable background. The absolute interference of the two alcohols was quantified by evaluating the increase in the respective mass signals caused by the presence of the alcohols in comparison with the background when no alcohol was close. In the open configuration, the proximity of 1 mL EtOH resulted in a 5.8-fold higher interference at \(m/z\) 47 in comparison with the enclosed ionization region. For 1 mL of 2-PrOH, the open system experienced a 3.4-fold higher interference than the enclosed system at \(m/z\) 43. This shows that the housing provides a certain amount of protection for the ionization region and is able to reduce interferences from the laboratory surroundings significantly. Furthermore, the benefits of the confined ionization region regarding the detection of an actual analyte in the presence of an external disturbance were studied. Therefore, EtOH was brought close to the ionization region and its influence on the detection of 2-PrOH in the open and in the enclosed configuration was monitored (Figure S2, supporting information). Here, the baseline noise caused by the presence of EtOH disturbed the analysis of 1000 mg L\(^{-1}\) 2-PrOH in the open system severely, while the interference on the 1 mg L\(^{-1}\) 2-PrOH signal in the closed system was minimal. Thus, the confinement of the ionization region with a housing yields improved baseline stability and minimizes the effects of external disturbances.

### 3.3 Effects of the variable setup parameters

The sample introduction system as well as the DART-MS setup itself offer various parameters such as ionization mode, type and flow rate of the nebulizing gas, helium gas flow rate, ion source temperature and mass spectrometer tuning parameters to optimize the detection sensitivity and signal intensity for the compounds of interest. In the following, we studied the effect of the nebulizing gas type on the signal intensity of a series of four alcohols, namely MeOH, EtOH, 2-PrOH and 2-butanol (2-BuOH). Therefore, we introduced an aqueous analyte mixture containing 1 mg L\(^{-1}\) of each alcohol into the system and analyzed the increase in the respective mass signals (MeOH and EtOH detected as [M + H]\(^+\) at \(m/z\) 33 and 47, 2-PrOH and 2-BuOH detected as [M + H – H\(_2\)O]\(^+\) at \(m/z\) 43 and 57) using either argon or oxygen as the nebulizing gas.

Figure 1 reveals that all compounds were detected with lower intensity when argon was used as nebulizing gas in comparison with oxygen. This can be related to the ionization mechanism, where the oxygen pathway, which plays a prominent role in the formation of the protonating hydronium species, is favored when oxygen is used as the nebulizing gas.\(^{30}\) The same effect could be observed in the analysis of organic acids in the negative mode (Figure S3, supporting information), which strengthens the hypothesis of a relation to the ionization mechanism, since also the negative mode relies on an oxygen pathway for the deprotonation of analyte molecules. Using oxygen as the nebulizing gas instead of argon had a stronger impact on the signal than on the noise level, resulting in improved signal-to-noise (S/N) ratios for all studied alcohols when oxygen was used. Figure 1 also shows that the choice of nebulizing gas has different quantitative effects on the signal intensity of the four investigated compounds. While the EtOH signal is decreased by a factor of 19 using argon as nebulizing gas in comparison with oxygen, the signal for 2-BuOH is lowered only by a factor of 1.2. Thus, a general statement on the quantitative improvement in analyte signal intensity by using oxygen as the nebulizing gas instead of argon cannot be made. Since the highest signal intensities for all four analytes were obtained using oxygen, it was chosen as nebulizing gas for the following parameter optimization experiments in order to determine maximum sensitivity conditions.

In the following, the dependency of the signal intensity of the four alcohols on the flow rates of the nebulizing gas (oxygen) and the DART gas (helium) as well as on the DART ion source temperature was investigated. When varying the oxygen nebulizing gas flow rate...
between 0.2 and 0.6 L min\(^{-1}\), opposing trends for the different analyte compounds could be observed (Figure 2A). While the MeOH and EtOH signals exhibited an approx. 5-fold decrease in intensity when the oxygen flow was increased from 0.2 L min\(^{-1}\) to 0.6 L min\(^{-1}\), 2-BuOH showed a corresponding increase in the MS response by a factor of 1.5. For 2-PrOH, a maximum is observed at 0.4 L min\(^{-1}\), while higher and lower nebulizing gas flow rates resulted in up to 1.5-fold decreases in the signal. These opposing trends do not allow for a simultaneous parameter optimization for all four compounds, so that a trade-off must be made in order to analyze all compounds concurrently with reasonable sensitivity. In this case, 0.4 L min\(^{-1}\) was determined as the optimum nebulizing gas flow rate for the simultaneous analysis of these alcohols.

Similar optimization procedures were performed to find the ideal parameters for the helium flow rate and the DART ion source temperature (Figures 2B and 2C). These experiments also showed opposing trends in the signal intensities of the different alcohols. Therefore, trade-offs between the optimum conditions of the different alcohols were made and compromise values of 3.0 L min\(^{-1}\) helium flow and 250°C ion source temperature were determined in order to analyze all compounds simultaneously. Furthermore, the appropriate ionization mode and mass spectrometer settings must be chosen as well to achieve optimal setup performance and ionization efficiency of the target compounds. These experiments demonstrate that a parameter optimization procedure must precede each compound analysis, since optimum condition values cannot be generalized for all possible analytes of interest. For applications such as LC/MS, where all compounds elute gradually, improved signal intensities can likely be achieved by programming ion source temperature gradients and flow rate ramps for the DART and nebulizing gas to match the optimal conditions found for each compound.

### 3.4 Application of the setup for the quantification of alcohols

To demonstrate the performance of the above-mentioned design as a quantitative detector and to show its broad applicability, we coupled our setup with HPLC and analyzed a standard mixture containing six C1–C4 alcohols, such as MeOH, EtOH, 1-propanol (1-PrOH), 2-PrOH, 1-butanol (1-BuOH) and 2-BuOH. Figure 3 shows the extracted ion chromatograms (EICs) obtained for the six alcohols using the selected parameters detailed in the previous section.

As expected from reversed-phase HPLC characteristics, the elution order of the alcohols was determined by the carbon chain length with MeOH eluting first and the C4 alcohols last. Furthermore, secondary alcohols eluted before primary ones, which can be seen in the EICs displaying the PrOH and BuOH peaks. Here, the two isomers appear at the same m/z value and distinction between primary and secondary alcohols is only possible by HPLC separation, since the comparably soft ionization mechanism of DART does not yield any compound-specific fragments, in this case. The separation was highly reproducible with retention time shifts below 0.01 min, determined
from repetitive injections. Additionally, it is noteworthy that the separation was performed with water as the only eluent. This was necessary to ensure sufficient protonation of the alcohols for detection, as organic solvents usually show high proton affinity and thereby suppress ionization of the analyte, as observed for various other LC/MS ion sources as well.\textsuperscript{31,32} In contrast to e.g. ESI,\textsuperscript{32} this setup had no difficulties operating with a pure aqueous mobile phase.

To determine the limits of detection (LODs) of the alcohols and the linear ranges of detection, calibration experiments were performed by injecting standard mixtures containing the six alcohols in the concentration range from 0.1 to 75 mg L\textsuperscript{-1}. A S/N ratio of 3 was used to estimate the LODs, while the baseline noise was calculated using the peak-to-peak definition. All results, presented in Table 1, are averaged from at least three injections.

For all alcohols, linear ranges of at least one decade were found, which proves the capability of the setup to operate quantitatively. The analysis of ions that are formed upon ionization of the analytes with lower efficiency than the ions investigated here additionally offers the possibility to extend the upper limits of the linear ranges. Furthermore, within the determined linear ranges, the obtained calibration curves correlated excellently with the linear fits, represented by the regression coefficients close to 1. At a concentration level of 5 mg L\textsuperscript{-1}, RSDs of the three measurements were equal to or less than 5.1% for all compounds, which demonstrates again the high reproducibility of the setup. As noticed from section 3.3, the signal intensity is dependent on the chosen setup parameters and, in this case, a trade-off in sensitivity for all alcohols was made in order to analyze all compounds simultaneously. Therefore, the LODs determined here are representative for the above-mentioned parameters and can be improved if the conditions are optimized for each alcohol separately. The higher LOD for MeOH than EtOH, which is unexpected after contemplating the signal intensities shown in Figure 1, is the result of the higher level of baseline noise at m/z 33 than m/z 47 (see Figure 3).

### 3.5 Effects of the sample introduction system

As already observable during the analysis of alcohols, the peaks in the EICs exhibit noticeable tailing. To further investigate if the observed tailing is due to the separation on the column or to the sample introduction system, an HPLC/DART-MS analysis was performed with a UV detector inserted between the outlet of the HPLC column and the inlet to the nebulizer, to enable a comparison of the UV and extracted ion chromatograms. As UV-active compounds, four organic acids, namely formic acid (FA), acetic acid (AA), propionic acid (PA) and acrylic acid (AcrA), were chosen to be analyzed using 10 mM phosphoric acid buffer (pH 2.6) as the eluent. The acidic conditions ensure that the organic acids are present in their protonated forms on the HPLC column to avoid peak splitting due to pH equilibria around the pKa values of the acids. Furthermore, we exploit the ability of the setup to handle liquid streams containing non-volatile species by using here a 10 mM phosphoric acid buffer. Higher concentrations

| LOD [μg L\textsuperscript{-1}] | Linear range [mg L\textsuperscript{-1}] | Equation | R\textsuperscript{2} |
|--------------------------------|--------------------------------------|----------|----------------|
| MeOH 159                      | 0.5–10                               | y = 94,993x + 4339 | 0.9996 |
| EtOH 61                       | 0.5–20                               | y = 99,940x + 28,292 | 0.9982 |
| 1-PrOH 50                     | 0.5–10                               | y = 587,072x + 115,864 | 0.9976 |
| 2-PrOH 31                     | 0.1–5                                | y = 1,004,930x + 96,243 | 0.9991 |
| 1-BuOH 48                     | 0.5–20                               | y = 524,335x - 241,421 | 0.9966 |
| 2-BuOH 82                     | 0.5–20                               | y = 340,624x - 134,509 | 0.9979 |
are also possible, as already demonstrated in our recent work on electrochemical real-time mass spectrometry, where we detected electrochemically generated CO₂ reduction products in the presence of 0.1 M KHCO₃ electrolyte.²⁴ The ionization of the organic acids was performed in the negative mode of the DART-MS setup, as organic acids possess highly acidic protons, so that proton abstraction is the most favored ionization mechanism here.

The obtained UV chromatogram of the organic acids and the corresponding EICs are presented in Figure 4. As expected, the primary ion species generated from the organic acids is \(\text{[M - H]}\)⁻.³³ In the UV chromatogram, the PA peak is shown as a shoulder/tail to the AcrA peak, due to insufficient separation under the given conditions and the weak UV absorption of the PA in comparison to AcrA. In the ion chromatograms, on the other hand, the AcrA and PA signals can easily be differentiated based on their different molecular masses. A comparison of the UV chromatogram and the EICs showed that all compounds display Gaussian peak shapes in the UV detector, while considerable peak tailing is observed in the MS signals. Hence, we conclude that the sample introduction system is responsible for the observed tailing in the ion chromatograms and it is not caused by the analyte separation on the column. The tailing in the introduction system leads to a decrease in chromatographic resolution³⁴ (calculated from the peak width at half height) from e.g. 3.3 between the FA and AA peaks in the UV chromatogram to 0.8 in the MS signals. However, for purely analytical purposes, where peak identification and quantification is required, the decrease in resolution is not problematic as long as all components of the mixture can be detected at unique \(m/z\) values, as in the case of Figure 4, so that no signal overlap occurs.

For ICP-MS analyses, it is known that the spray chamber size, shape and material have a considerable influence on the analyte detection.³⁵ Since the sample introduction system used here is adapted from ICP-MS, we decided to investigate the effect of the inner volume of the spray chamber on the peak tailing and signal intensity.

Figure 5 depicts the EICs obtained using two different quartz cyclonic spray chambers of 20 and 40 mL inner volume after the injection of 50 μL of 10 mg L⁻¹ FA. A 2.4-fold decrease in signal

![Figure 5](image)
intensity was observed when the 20 mL spray chamber was used in comparison with the 40 mL spray chamber. Nevertheless, the peak broadening, which was defined as the time from the beginning of the peak until the baseline value was reached again, was reduced by a factor of 2.8, which is favorable for obtaining ideal baseline-separated peak resolution. For AA, AcrA and PA, similar trends were found, although the decrease in peak intensity and broadening was less pronounced (Tables S1 and S2, supporting information). In order to evaluate the peak tailing independently of the peak intensity, tailing factors were calculated by dividing the back peak width by the front peak width measured at 10% of the peak height. For the different compounds, tailing factors between 3.5 (for FA in the 20 mL spray chamber) and 8.3 (for PA in the 40 mL spray chamber) were found, which provide a quantitative measure for the severeness of the tailing observed for the peaks in the EICs (a symmetric, Gaussian peak has a tailing factor of 1). Comparing the tailing factors for the different compounds in the 20 mL and 40 mL spray chambers, decreases in the tailing factors for FA, AA and PA by factors of 1.4, 1.2 and 1.3, respectively, confirm the trend of lesser tailing with the smaller spray chamber inner volume, while a 1.1-fold improvement in the peak asymmetry was observed for the AcrA peak (Table S3, supporting information). Therefore, it can be concluded that an increase in the inner volume of the spray chamber is favorable for high signal intensities, but has predominantly negative effects on the peak shape. Consequently, a trade-off between peak broadening and signal intensity must be made for real-life applications depending on the requirements of the envisaged analysis. Further work should be devoted to improving the spray chamber washout behavior without compromising peak intensities, since the spray chambers used here have been designed for ICP-MS applications and thus have not been optimized for our purpose of organic compound analysis. Therefore, the characteristics of the spray chamber (geometry and material) should be studied and optimized, as well as the effects of the nebulizer and the nebulizing gas flow rate, to decrease the retention time of the analytes in the spray chamber, without loss in signal intensity.

3.6 Application of the setup for gradient elution

Finally, the suitability of the setup for operating under gradient elution was demonstrated, which is crucial for many LC/MS applications. As exemplary analytes, 5-hydroxymethylfurfural (HMF) and three of its hydrogenation products were chosen, since these kinds of reactions are of high interest for the valorization of biomass-based platform chemicals. Figure 6 presents the chromatogram obtained for the separation of 10 mg L\(^{-1}\) HMF, 2,5-dihydroxymethylfuran (DHMF), 5-methylfurfural (5-MF) and 5-methylfurfuryl alcohol (MFA) using a binary water/acetonitrile gradient.

Similar to the previously analyzed C\(_3\) and C\(_4\) alcohols, the furanic compounds containing a hydroxy group were detected in the positive mode as \([M + H - H_2O]^+\) with HMF giving a signal at m/z 109, DHMF at m/z 111 and MFA at m/z 95. 5-MF, on the other hand, was protonated to yield a \([M + H]^+\) species at m/z 111, which coincides with the m/z value for DHMF detection. The linear gradient elution from initially 3% to finally 25% of acetonitrile resulted in a gradual change in the baselines in the EICs, clearly visible in Figures 6C and 6D. Nevertheless, this change in baseline did not prevent the detection of all investigated compounds with a good S/N ratio at a concentration level of 10 mg L\(^{-1}\). The two UV chromatograms at 210 nm and 285 nm, respectively and C–E, simultaneously recorded EICs demonstrate again the Gaussian peak shapes obtained for all compounds after the separation on the HPLC column. In the EICs, peak tailing was primarily observed for the two late-eluting compounds MFA and 5-MF, while the HMF and DHMF peaks were less affected. Thus, the effect of the introduction system on the peak shape was once more validated as well as the dependency of the peak broadening on the analytical conditions and compound of interest. In contrast to the detection of the alcohols, the presence of an organic compound in the eluent did not prevent the protonation of the analytes in this case, due to the higher proton affinity of the...
furanics. The injection of a blank, following the analysis of the furanic compounds, demonstrated additionally that no analyte carry-over is being caused by the sample introduction system (Figure S4, supporting information).

4 | CONCLUSIONS

This work describes the implementation of an enclosed ionization interface for the analysis of liquid sample streams with DART-MS. It was demonstrated that the enclosure of the ionization region by a housing results in improved analyte signal intensities and minimized interference from the surroundings. Furthermore, it renders the whole design robust and reproducible and enables the safe use of toxic and hazardous chemicals. Additionally, the sample introduction system offers various parameters to allow for optimization of the system for a broad variety of compounds. Therefore, this design can be used and adapted for the coupling of various analytics or reaction processes for which MS detection is of interest. By the analysis of a simple series of alcohols, it was demonstrated that the setup is also suitable for compound quantification, while its compatibility with gradient elution was shown for an analysis of four furanic compounds. Further development of this configuration should deal with the optimization of the sample introduction system, particularly of the spray chamber, to minimize peak tailing and optimize detection sensitivity. Since only a fraction of the nebulized liquid stream is directed to the mass spectrometer, while the majority can be recollected from the spray chamber waste outlet, the setup can also be used for preparative HPLC purposes or further processing of the sample.

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