Laser Ablation Electrospray Ionization Hydrogen/Deuterium Exchange Ambient Mass Spectrometry Imaging

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Supporting Information

ABSTRACT: Identification and confirmation of known as well as unknown (bio)chemical entities in ambient mass spectrometry (MS) and MS imaging (MSI) mostly involve accurate mass determination, often in combination with MS/MS or MS n work flows. To further improve structural assignment, additional molecular information is required. Here we present an ambient hydrogen/deuterium exchange (HDX) laser ablation electrospray ionization (LAESI) MS method in which, apart from the accurate mass and MS/MS data, the number of exchangeable protons in (un)known molecules is obtained. While eventually presenting ambient HDX-LAESI-MSI, samples were not preincubated with deuterated solvents, but instead HDX occurred following fusion of ablated sample material with microdroplets generated by ESI of deuterated solvents. Therefore, the degree of HDX was studied following ablation of nondeuterated sample solutions of melamine and monosaccharides. From these experiments, it was concluded that the set-up used could provide meaningful HDX data in support of molecular structure elucidation by significantly reducing the number of structure options from a measured elemental composition. This reduction was demonstrated with an unknown accurate m/z value obtained in the analysis of an orange slice, reducing the possible number of molecular structures having the same elemental composition by 87% due to the number of H/D exchanges observed. Next, deuterated and nondeuterated MS/MS experiments showed the number of exchangeable protons in the substructures from deuterated neutral losses in the product ion spectra, confirming the compound to be arginine. Finally, the potential of ambient HDX-LAESI-MSI was demonstrated by the imaging of (secondary) plant metabolites in a Phalaenopsis petal.

KEYWORDS: laser ablation electrospray ionization, hydrogen deuterium exchange, ambient mass spectrometry, structure elucidation, mass spectrometry imaging

INTRODUCTION

Mass spectrometry (MS) studies commonly aim to find the identity and/or quantity of molecules in a sample. In mass spectrometry imaging (MSI) the localization of molecules is studied, mainly in plant or animal tissue such as lipid profiles and the accumulations of drugs and their metabolites.1–4 Tissue samples often require laborious pretreatment steps and vacuum conditions in order to be compatible with matrix-assisted laser desorption ionization (MALDI) MSI analysis.5 However, sample pretreatment can largely affect the outcome of MSI due to analyte losses and/or delocalization of molecules.6,7 Additionally, samples can be disrupted or damaged by vacuum conditions.6,9 In contrast, ambient MS allows the acquisition of mass spectra from samples in their native environment, without any sample pretreatment.10–13 Structural identification in ambient MS(I) is largely dependent on accurate mass measurements, often combined with MS/MS or MS n approaches.14 The accurate mass is acquired with high-resolution instruments, such as time-of-flight and Orbitrap MS, and provides the elemental composition of (bio)molecules.15 This elemental composition usually still yields numerous candidates of chemical substances.
in database searches. MS/MS approaches can then further elucidate the (bio)molecular structure, but with hundreds of molecular structure options this requires the availability of numerous standard substances and large spectral libraries thereof. Alternatively, orthogonal methods like ion mobility (IMS) or online reactions can be used during ambient MS(I) data acquisition to obtain additional information for final structure elucidation, but these are not or only partly useful for elucidation of unknown entities and are typically applied for improved selectivity and signal-to-noise ratio in targeted analysis. The development of methods to obtain additional molecular structural information is thus of utmost importance for reducing the number of structure options and thereby increasing the certainty in, as well as the speed of, identification in ambient MS(I).

A well-known approach that may assist in the identification of unknown entities is hydrogen/deuterium exchange (HDX) MS. HDX-MS is a method to obtain the number of exchangeable hydrogens in a molecule, present in, e.g., –NH and –OH moieties. The m/z value of any (un)known molecule or fragment thereof will increase accordingly with each included deuteron, providing valuable information on the number of exchangeable protons. This information can then be used to reduce the number of structure options for identical elemental compositions, limiting the need for hundreds of MS/MS experiments on numerous standards for comparison. Common HDX procedures rely on prolonged exchange reactions by dissolving the samples or standards in deuterated solvents, the addition of a deuterated gas to trapped gas-phase ions for exchange reactions inside the vacuum of the mass spectrometer or, classically, using a deuterated reaction gas in chemical ionization (CI). However, gas-phase HDX of trapped ions is hard to combine with the wide m/z range of non-targeted MSI common in, e.g., spatially resolved metabolomics studies. CI, or desorption atmospheric pressure chemical ionization (DAPCI), is only compatible with volatiles and very low molecular weight molecules, limiting its use for surface and tissue analysis. Dissolving/diluting tissue samples with deuterated solvents causes delocalization of analytes and hampers spatial resolution in surface analysis. Hence, a combination of HDX with ambient MSI would be a valuable addition for structure identification in surface analysis, including tissue. Apart from an initial DART set-up for ambient gas-phase HDX, ambient MS has not been explored for HDX yet.

Laser ablation electrospray ionization (LAESI) is an ambient MS ionization technique that can also be used for imaging. LAESI-MS applicability was demonstrated in the analysis of tissue, food contaminants, synthetic materials, and single cells. Furthermore, LAESI-MS was proven viable for performing and/or monitoring online time-resolved reactions. In LAESI-MS analysis, ablated sample material is continuously extracted by charged microdroplets that are produced by an orthogonally placed electrospray emitter, prior to MS analysis. In an HDX-LAESI-MS approach the ESI solvent can easily be replaced with a deuterated one, resulting in a simple ambient MS HDX method that would enable imaging without the need to expose the sample surface to deuterated solvents and with a higher spatial resolution than in DART. As shown in previous ESI studies, the problem of deuterium—hydrogen back-exchange would be minimal as a result of the continuous ESI spray directed towards the MS inlet. Obviously, various deuterium donors might be exploited, although in the present work D2O was mostly used. HDX reactions of exchangeable hydrogens are expected to be rapid due to the liquid microdroplet environment and to be characterized by up to complete conversion for at least small molecules.

In this research, we report a novel ambient MS approach, viz. HDX-LAESI, for (bio)molecular structure elucidation and confirmation. First, the degree of HDX conversion was studied for nondeuterated standard solutions of small molecules and critically compared with literature. Next, the applicability of HDX-LAESI-IMS-MS in (bio)molecular structure identification was shown for biomolecules like arginine and oligosaccharides, detected directly from an orange slice. Arginine was investigated in full detail by HDX-LAESI-MS/MS experiments. The deuterated neutral losses provided additional evidence of molecular (sub)structures. Finally, the feasibility and added-value of ambient HDX-LAESI-MSI in spatially resolved metabolomics was demonstrated by the imaging of arginine in an orange slice and secondary plant metabolites within and outside the purple pigment regions of a Phalaenopsis petal.

## EXPERIMENTAL SECTION

### Materials

Ultrapure water (H2O) 18.2 MΩ cm at 25 °C was freshly produced with a Millipore (Molsheim, France) Integral 3 system. Deuterium oxide (D2O) 99.9% atom D, methanol-d4 (MeOD) 99.8%/atom D, melanine, and l-arginine were purchased from Sigma-Aldrich ( Zwijndrecht, The Netherlands). A new ampule of D2O was used for each analysis. An orange was obtained from a local supermarket. An orchid (Phalaenopsis) was purchased from a local plant store.

### Ambient HDX-LAESI-MS

A Protea Biosciences (Morgan-town, WV) LAESI DP-1000 system was coupled to a Waters (Manchester, U.K.) Synapt G2-S traveling wave ion mobility (TWIM) time of flight mass spectrometer (TOFMS) and used for all experiments. Masslynx v.4.1 SCN 883 (Waters) was used to control the experimental settings of the Synapt G2-S, which was operated in positive ion TOFMS resolution mode (mass resolution approximately 18000 FWHM, mass accuracy 5 ppm) with a scan time of 1 s, source temperature 150 °C, sample cone 40 V, and source offset 80 V. In the case of oligosaccharide analysis, TWIM was applied using the following experimental conditions: the IMS wave velocity was set at 1200 m/s and wave height was 40 V. Driftscope v2.7 (Waters) was used to individually select drift time regions corresponding to oligosaccharides (Figure S1). Selected drift time data—containing all m/z information residing within the selection—were exported back into Masslynx and background-subtracted. The LAESI DP-1000 system was equipped with a 2940 nm mid-IR laser and controlled by LAESI desktop software v.2.0.1.3 (Protea Biosciences). Sample solutions were put in a 96-well plate. At each well 35 laser pulses were applied at a repetition rate of 5 Hz to produce a plume of ablated sample material, which was continuously extracted with charged microdrops generated from an orthogonally placed electrospray emitter prior to MS analysis. The electrospray solvent was either H2O or D2O at a flow rate of 3 μL/min. Electrospray voltage was set at ~4 kV in order to have a stable signal. Background-subtracted mass spectra were generated using the “combine spectrum” function in Masslynx. 5 scans were combined and 20 scans of electrospray background were subtracted.

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### instrumentation

- **LAESI**
  - Integral 3 system
  - Deuterium oxide (D2O) 99.9% atom D
  - Methanol-d4 (MeOD) 99.8%/atom D
  - Melanine
  - l-arginine

- **TWIM**
  - Masslynx v.4.1 SCN 883
  - Scan time: 1 s
  - Source temperature: 150 °C
  - Sample cone: 40 V
  - Source offset: 80 V

- **Laser Parameters**
  - Wave length: 2940 nm
  - Mid-IR Laser
  - Control via LAESI desktop software v.2.0.1.3

- **Sample Preparation**
  - Orange slice
  - Orchid (Phalaenopsis)

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### methodology

- **HDX-LAESI-MS**
  - Combined approach for (bio)molecular structure elucidation
  - Suitable for nondeuterated standard solutions of small molecules
  - Critically compared with literature

- **Results**
  - HDX reactions of exchangeable hydrogens rapid in liquid microdroplet environment
  - Characterized by up to complete conversion for small molecules
  - Arginine investigated in detail
  - Oligosaccharides detected from orange slice

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### Conclusion

The novel ambient HDX-LAESI-MS approach presents a valuable tool for (bio)molecular structure elucidation, providing additional evidence of molecular (sub)structures. It demonstrates feasibility and added-value over ambient HDX-LAESI-MSI in spatially resolved metabolomics, with applications in understanding (bio)molecular structure within and outside purple pigment regions of a Phalaenopsis petal.
Figure 1. LAESI-MS and HDX-LAESI-MS of melamine and monosaccharides. (A, B) LAESI-MS (ESI solvent was H2O) and HDX-LAESI-MS (ESI solvent was D2O) of an aqueous melamine solution, respectively. (C) Chemical structure of protonated melamine. (D) LAESI-MS and HDX-LAESI-MS of hexoses in 20× diluted (with H2O) orange juice, respectively. (C) Structure of the most abundant (cationized) monosaccharide (D-fructose) in oranges.

Ambient HDX-LAESI-MSI of an Orange Slice and Phalaenopsis Petal. An orange was sliced with a kitchen knife and placed, on a glass slide, onto the temperature-controlled sample stage maintained at 4 °C. (HDX-)LAESI-MS data were acquired from a 14 × 17 pattern (238 sample locations) in a sampling area defined from an optical image. The Phalaenopsis experiment was imaged in negative ion LAESI mode with D2O/MeOD 1:9 as ESI solvent. Data were acquired from a 14 × 9 pattern (126 sample locations) in a sampling area, containing both white and purple patches, defined from an optical image. For both the orange and the petal MSI experiments 20 laser pulses were applied at each location with a frequency of 10 Hz and 3 s dwell time per analyses location. The laser spot size was approximately 200 μm in diameter, and the in-between spot interval was 1 mm. These experimental settings resulted in total analysis times of 43 and 20 min, respectively. Proteaplot v2.0.8.5 (Protea Biosciences) was used to create maximum intensity ion maps.

RESULTS AND DISCUSSION

Ambient HDX-LAESI-MS of Small Molecules in Solution. The feasibility of ambient HDX-LAESI-MS was first investigated in nondeuterated standard solutions with the model compounds melamine, comprising multiple −NH groups, and monosaccharides, containing multiple −OH groups in order to observe the effects of HDX on simple molecules containing these widely abundant functional groups that can rapidly exchange protons for deuterons. The selection of these specific two molecules allowed us to study the effect of HDX on −OH (shown with monosaccharides) and −NH (shown with melamine) separately. As −OH and −NH moieties are the major source of rapid exchangeable protons in biomolecules, these compounds are considered a good model system for a much wider variety of compounds in life sciences. Melamine has an elemental composition of C3N6H6 and holds six exchangeable −NH1 protons (chemical structure is included in Figure 1A) that should experimentally result in a maximum m/z increase of 7 for the [M-d6 + D]+ ion. Parts A and B of Figure 1 present LAESI-MS background-subtracted mass spectra of a 100 μM aqueous melamine solution with H2O (Fig. 1A) and D2O (Fig. 1B) as ESI solvents. When H2O was used as the ESI solvent an [M + H]+ ion was detected at m/z 127.08. When D2O was used as the ESI solvent an m/z distribution from 128.08 to 134.12 was observed. These values correspond to protonated melamine [M-d6 + H]+ and a stepwise increase of the deuterium content up to the completely hydrogen/deuterium (H/D) exchanged value at m/z 134.12 for [M-d6 + D]+. These results correspond nicely with previously reported liquid- and gas-phase melamine HDX studies. Following elemental composition assessment based on accurate mass measurement, a SciFinder elemental composition search was performed to obtain structural isomer options. C3N6H6 initially resulted in 25 different constitutional isomers, however, when only structures comprising six proton exchange sites were included, the number of structure options was substantially reduced to only five, even without any additional MS/MS experiment. The number of proton exchange sites were simply included in the SciFinder search using its "refine by property value" function and selecting "H Donors".

The most common C6 monosaccharides have an elemental composition of C6H12O6 and are detected as sodium adducts in positive mode ESI. Thus, they contain five exchangeable −OH protons (as an example, the structure of a cationized monosaccharide, D-fructose, is included in Figure 1C). As a consequence, HDX is expected to result in a maximum m/z shift of +5 Da. Parts C and D of Figure 1 depict LAESI-MS (with H2O as ESI solvent) and HDX-LAESI-MS (with D2O as ESI solvent) background-subtracted mass spectra of C6 monosaccharides in 20× diluted orange juice. With LAESI-MS a single m/z value is detected at 203.05, corresponding to cationized monosaccharides [M + Na]+. HDX-LAESI-MS, however, shows a clear increasing isotope pattern ranging from m/z 203.05 for [M-d6 + Na]+ up to the fully H/D exchanged at m/z 208.08 for [M-d6 + Na]+. These results are in line with the number of exchanges for the previously observed [M-d4 + D]+ monosaccharide ion in an atmospheric pressure chemical ionization study when the sample was dissolved and incubated in D2O at forehand. A SciFinder elemental composition search was performed (on C6H12O6) and 450 results were obtained including all stereoisomers. 95 of the obtained isomers could be eliminated following the selection of structures having five proton exchange sites. Both melamine and monosaccharides demonstrate that ambient HDX-LAESI-MS is a simple and expeditious technique for a significant simplification of the structure search for molecules having identical elemental composition and comprising N−H or O−H bonds. Most importantly, despite the relatively short HDX-LAESI process (versus common prolonged sample incubation in deuterated solvents) the exchange still proceeds up to
completion, yielding great promise for HDX-LAESI-MSI applications in which sample pre-incubation is either impossible or undesired.

Ambient HDX-LAESI-MS of Metabolites in an Orange Slice. An abundant class of biomolecules in fruits are oligosaccharides. Oligosaccharides contain a substantial number of exchangeable protons and are therefore an excellent class of biomolecules to evaluate ambient HDX-LAESI-MSI for real biological samples. Oligosaccharides are detected with (HDX-)LAESI-MS in positive ion mode as sodium adducts, so theoretical m/z values of 365.1054 for $[\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{Na}]^+$, 527.1583 for $[\text{C}_{18}\text{H}_{32}\text{O}_{16} + \text{Na}]^+$, 689.2111 for $[\text{C}_{24}\text{H}_{42}\text{O}_{21} + \text{Na}]^+$, etc. are expected to be observed in LAESI-MS upon HDX for $5 + 3$ enzymes of oligosaccharides. With HDX-LAESI-MS these m/z values can be shifted up to a maximum of $5 + 3 \times (n - 1)$, in which $n$ is the number of monomers from $n = 1$ onwards, resulting in maximum m/z value shifts upon HDX of $+8$, $+11$, $+14$, etc. for di-, tri-, and tetrasaccharides, respectively. Figure 2 presents mass spectra of oligosaccharides obtained with LAESI-TWIM-MS and HDX-LAESI-TWIM-MS from the surface of an orange slice. Figure 2A shows an m/z value of 689.21 for $[\text{C}_{24}\text{H}_{42}\text{O}_{21} + \text{Na}]^+$, and Figure 2B depicts HDX-LAESI-TWIM-MS analysis. (A, inset) C shows the ESI solvent background and D shows the orange mass spectrum including background. Obviously, the mass-resolving power is insufficient in D to baseline separate the cationized oligosaccharide from the background interferences.

LAESI approach. This ion at m/z 175.1189 relates to an endogenous compound and provides a nice example that HDX can strongly facilitates its structure elucidation. Based on the exact mass only one elemental composition (comprising: $\text{C}_{6}\text{H}_{15}\text{N}_{4}\text{O}_{2}$) was obtained within 10 ppm (3.4 ppm) mass accuracy: $\text{C}_{6}\text{H}_{15}\text{N}_{4}\text{O}_{2}\text{H}_x$ so obviously a protonated substance rather than a cationized species. A SciFinder elemental composition search, excluding substances comprising isotopes and substances without any references, resulted in 103 chemical structures. HDX-LAESI-MS showed up to eight exchanges to occur (Figure 3B), including the deuteration. Due to the seven detected exchangeable protons for $[\text{M}-d_7 + H]^+$ the search was refined to only include compounds comprising seven proton donors. As a result, from the initial 103 options only 13 structures fulfill this requirement (Table S1), including several stereoisomers. Upon excluding individual stereoisomers, which cannot be resolved by MS alone, 10 out of the remaining 11 substances have only 1–5 references according to SciFinder with various origins such as, e.g., synthesis, patents, and review articles. One candidate substance, however, is arginine, which is obviously well documented with approximately 100000 references. This thus induces the hypothesis that the ion in the orange slice belongs to arginine. Instead of verifying MS/MS experiments on all the 103 options prior to HDX, the experimental verification could—due to the large reduction of background interferences, using HDX can shift the m/z value of a molecule to obtain enhanced mass accuracy.

In principle, the sensitivity of the HDX-LAESI-MS method should be lowered due to additional signals of incomplete H/D exchange (e.g., signals numbered 6–13 in Figure 2B). In this particular example, however, the sensitivity has increased as a result of the m/z shift away from background interferences: the original signal was barely distinguishable from the background signal. On the contrary, it could also happen that due to H/D exchange the signal would (partly) overlap with previously not-interfering background or matrix signals. So, any increase or decrease in sensitivity following HDX-LAESI-MS would be strongly analyte, matrix, and background specific.

To demonstrate the added value of HDX for unknown structure identification in biological samples, one out of the many detected ions from the orange slice (m/z value 175.1189, Figure 3A) was used as an example with the current HDX-
options following HDX—be achieved by comparison of the orange sample with only an arginine standard solution. The obtained (HDX-)LAESI-MS/MS fragmentation spectra of m/z 175.1 (H2O) and m/z 183.2 (D2O) are presented in Figure S4. The arginine reference solution and the orange sample show similar fragmentation spectra, and both nicely correspond with literature.52 Furthermore, the proposed elemental compositions for the observed neutral losses from m/z 175.1 and 183.2 are confirmed by the corresponding number of H and D atoms, thus providing additional evidence that the detected m/z value 175.1198 is indeed the amino acid arginine (Figure S4). These results show that the developed HDX approach is also applicable to endogenous amino acids present in a real sample matrix.

As an initial imaging feasibility experiment, (HDX-)LAESI-MSI was performed on the same orange slice. The laser ablation x−y coordinates were used to create 2D ion maps of arginine and the fully H/D exchanged arginine LAESI-MSI signals, and both were superimposed on an optical image of the orange (Figure 3C,D). The area inside the red box was imaged and for both ions, m/z 175.12 for [M-d0 − H]+ and m/z 183.17 for [M-d7 + D]+, images were only obtained in the pulp area of the orange slice and not on the peel, in accordance with expectations (apart from a few minor slicing artifacts due to orange juice wetting the knife’s edge).

**Ambient HDX-LAESI-MSI of (Secondary) Metabolites in a Phalaenopsis Petal.** In order to show the potential of ambient untargeted HDX-LAESI-MSI in plant metabolomics, a Phalaenopsis petal sample was imaged for secondary plant metabolites. Luteolin-type flavonoids are expected to show colocalization with anthocyanins (purple pigments), whereas apigenin-based flavonoids are reported to be absent in these purple regions.53 Figure 4 presents the 2D ion maps and (HDX-)LAESI-MSI spectra of an apigenin-based flavonoid. Figure 4A presents an optical image of the petal including the laser x-y locations marked as red dots, whereas Figure 4B,C shows the apigenin-derived flavonoid LAESI-MSI, m/z 563.1 for [M-H]−, and HDX-LAESI-MSI, m/z 571.2 for [M-d8−H]−, 2D ion maps superimposed on this optical image, respectively. Indeed, the apigenin-type flavonoid shows an inverse-distribution with the purple pigments in the petal, demonstrating that similar ion distributions are obtained with both LAESI-MSI and HDX-LAESI-MSI experiments. The obtained (HDX-)LAESI-MSI mass spectra are presented in Figure 4D,E. The number of H/D exchanges (Figure 4E) are clearly observed to be 8, with the small signal at m/z 572.2 to be a 13C isotope in a similar ratio as the (LAESI-MSI) isotope signal observed in Figure 4D. Figure 4D also shows the proposed molecular structure, comprising eight exchangeable protons for [M − H]− (please note that the positions of the glycoside bonds are putative), and nicely corresponds with literature.53 As the data was recorded in an untargeted approach, many signals were obtained and a larger m/z range of the analysis is provided in Figure S5. One of the observed m/z values, 609.1480, relates to a luteolin-type flavonoid and was processed similarly as the apigenin-type flavonoid (Figure 5).
As expected, instead of an inverse-localization the resulting 2D ion maps (Figures 5A–C) now showed co-localization with the purple patches of the petal. Additionally, also these (HDX-)LAESI-MSI mass spectra (Figure 5D,E) show the proposed molecular structure (Fig 5D) and an up to fully H/D exchanged isotope distribution (Figure 5E). Both Figures 4 and 5 thus present 2D ion maps corresponding with the co-localization and distribution of the compounds within (Figure 5E) or outside (Figure 4) the purple patches for both LAESI-MSI as well as HDX-LAESI-MSI, demonstrating the feasibility of the HDX-MSI approach.

The larger m/z range presented in Figure S5 shows that untargeted HDX-LAESI-MSI is suitable especially for the more abundant analytes in a sample. Analytes with a low signal-to-noise ratio could, however, be concealed by matrix or background signals. It is therefore recommended to compare both LAESI-MSI(1) with HDX-LAESI-MSI(1) for each sample to extract the highest amount of information. Additionally, in case the number of H/D exchanges would be unclear, either due to interfering signals, incomplete exchange or limited ion abundance; as might be observed in Figure 5E, a minimum number of exchanges (e.g., 10 in Fig 5E) could still be used to reduce the number of structural options in a database search.

Finally, targeted (HDX-)LAESI-MS/MS experiments for the identification of the apigenin-type flavonoid were performed on the white areas of the petal, and the obtained spectra are shown in Figure S6. The proposed molecular structure (Fig. 4D) showed apigenin with an O-glycoside as well as a C-glycoside modification. The MS/MS spectra indeed show the m/z values of apigenin itself (the aglycon) at 269.0454 for [M-d0 − H]+ (Figure S6A) and the expected 2 H/D exchanges to obtain m/z 271.0581 for [M-d10 − H]+ (Figure S6B). Also, the presence of the O-glycoside was confirmed by the neutral loss of 162.0531 (C10H16O5) showing m/z 401.0877 (Figure S6A) and, upon HDX-MSI, 165.0715 (C5H13D2O3) showing m/z 406.1202 (Figure S6B). Proposed (fragment) structures for m/z 563.1408, 401.0877, and 269.0454 as well as 571.1917, 406.1202, and 271.0581 are provided in Figure S7. C-Glycosides (in contrast to O-glycosides) are known to show several glycoside ring cleavages in MS/MS experiments.54-56 These cleavages were also observed by both the deuterated as well as the nondeuterated precursors and are presented (along with the O-glycoside and the apigenin fragments) in Table S2. The apigenin-type flavonoid, comprising apigenin with O-glycoside and C-glycoside modifications, analyzed direct from plant tissue, could be observed and additionally confirmed by the mass difference between neutral losses and H/D exchanged neutral losses. These experiments clearly show the feasibility and benefit of HDX-LAESI-MSI for structure elucidation in spatially resolved plant metabolomics.

**CONCLUSIONS**

Here, we developed a novel ambient HDX-LAESI-MSI approach to acquire additional molecular structural information for the identification and confirmation of (bio)molecules. Like all ambient MS approaches, this method provides a simple and rapid analysis of samples under ambient conditions but now with the benefit of trivially obtainable additional chemical structural information. Ambient HDX-LAESI-MS in non-deuterated solutions of the model compounds melamine and monosaccharides revealed that information on the maximum number of H/D exchanges significantly reduced the number of structures in elemental composition database searches. Mass spectrometric investigation of an orange slice revealed the presence of, among others, oligosaccharides, and the amino acid arginine. The latter was investigated in detail, and it was proven that combining accurate mass, MS/MS, and literature data with HDX led to the unambiguous structural elucidation of that compound. Additionally, a significant increase in mass accuracy was observed for an oligosaccharide as a result of the HDX reaction m/z shift away from an unresolved background interference. The feasibility of the method in support of structure elucidation in ambient MSI of (secondary) plant metabolites was clearly demonstrated in an orange slice and a *Phalaenopsis* petal. As a future hardware simplification, the use of a dual or θ ESI sprayer—one with H2O and the other with D2O—can be considered in the LAESI set-up.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jasms.9b00082.

Figures and tables showing additional MS data (PDF)

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**Notes**

The authors declare no competing financial interest.
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