Infrared Laser Ablation Atmospheric Pressure Photoionization Mass Spectrometry

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Ⓢ *Supporting Information*

ABSTRACT: In this paper we introduce laser ablation atmospheric pressure photoionization (LAAPPI), a novel atmospheric pressure ion source for mass spectrometry. In LAAPPI the analytes are ablated from water-rich solid samples or from aqueous solutions with an infrared (IR) laser running at 2.94 μm wavelength. Approximately 12 mm above the sample surface, the ablation plume is intercepted with an orthogonal hot solvent (e.g., toluene or anisole) jet, which is generated by a heated nebulizer microchip and directed toward the mass spectrometer inlet. The ablated analytes are desolvated and ionized in the gas-phase by atmospheric pressure photoionization using a 10 eV vacuum ultraviolet krypton discharge lamp. The effect of operational parameters and spray solvent on the performance of LAAPPI is studied. LAAPPI offers ~300 μm lateral resolution comparable to, e.g., matrix-assisted laser desorption ionization. In addition to polar compounds, LAAPPI efficiently ionizes neutral and nonpolar compounds. The bioanalytical application of the method is demonstrated by the direct LAAPPI analysis of rat brain tissue sections and sour orange (*Citrus aurantium*) leaves.
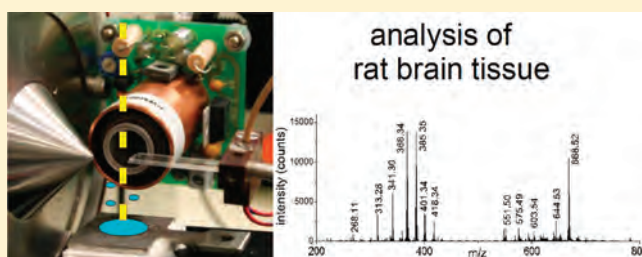
Desorption ionization techniques are a fast growing field of mass spectrometry (MS) for the rapid analysis of solid samples and surfaces. Desorption ionization includes several techniques, such as secondary ion mass spectrometry, fast atom bombardment, matrix-assisted laser desorption ionization (MALDI),[1−3] laser desorption electron impact,[4] and the more recently introduced desorption electrospray ionization (DESI),[5] to name a few. MALDI, which uses an ultraviolet laser for analyte desorption and ionization, has an established role in the analysis of large biomolecules.[6,7] DESI, introduced in 2004, has proved its potential in the analysis of compounds directly from diverse untreated surfaces.[8] In DESI, the charged solvent droplets, generated by electrospray, pick up the analytes from the sample surface and ionize them in a process similar to conventional electrospray. Whereas MALDI and DESI are best suited for ionic and polar compounds, their ionization efficiency for neutral and less polar compounds may be poor. In vacuum, nonpolar and neutral analytes can be analyzed by secondary ion mass spectrometry or laser desorption electron impact. Techniques that rely on different ionization mechanisms, e.g., chemical ionization (desorption atmospheric pressure chemical ionization, DAPCI),[9] metastables in plasma (direct analysis in real time, DART),[10] and photoionization (desorption atmospheric pressure photoionization, DAPPI),[11] can enable ambient ion production from neutral and nonpolar compounds. Among these methods, DAPPI has been demonstrated to provide high ionization efficiency for nonpolar as well as polar compounds. DAPPI employs a heated solvent spray for analyte desorption

and 10 eV ultraviolet light for photoionization. In DAPPI, polar compounds can be ionized via proton transfer and less polar compounds via charge exchange with ions derived from the spray solvent.[12] However, as analytes are desorbed thermally from the substrate, DAPPI is not suitable for large molecules and thermolabile compounds, such as peptides and proteins.

Mass spectrometric imaging (MSI) is a promising application of desorption ionization techniques for the mapping of molecular distributions on surfaces.[6,7] Molecular images showing the chemical identity of multiple compounds cannot be obtained by other analysis techniques. To date, mainly MALDI and DESI have been applied in the MSI of biological surfaces. Although MALDI is effective in peptide and protein analysis with high to moderate lateral resolution (5−300 μm), the local analysis of small molecules suffers from matrix interferences. DESI has been applied to the imaging of phospholipids, drugs, and drug metabolites in tissue with resolutions ranging from hundreds of micrometers down to 40 μm.[13] As it requires no matrix, DESI is better suited for the analysis of small molecules than MALDI. To analyze nonpolar and neutral compounds from biological matrixes by DESI, however, selective, reactive spray additives are needed.[14,15] DAPPI has been demonstrated for the direct imaging of nonpolar compounds, i.e., cholesterol from rat brain[16] and nonpolar analytes from *Salvia* leaf.[16] Unfortunately the lateral

resolution of DAPPI is relatively low, ~1 mm, because the desorption of analytes relies on thermal energy that is delivered by heated gas flow. Thus, the MSI of nonpolar compounds with high sensitivity and high lateral resolution has remained a challenge.

In this contribution, we combine infrared (IR) laser ablation with photoionization to introduce a novel ambient ionization technique for the analysis of compounds with a wide range of polarities. Mid-IR laser ablation has been previously combined with electrospray in laser ablation electrospray ionization (LAESI)[17] and chemical ionization in IR-LAMICI.[18] The mid-IR laser is operated at 2.94 μm wavelength to transfer energy to the water molecules in the sample. This leads to partial vaporization and ejection of solid sample material or liquid droplets from the surface layer.[19] As artificial matrix is not used, the background interferences in the low mass range are minimized. In LAESI, the ablated sample is picked up by an electrospray plume and the analytes are ionized similar to conventional electrospray. In IR-LAMICI the analytes are ionized by a metastable plume. Recently, 30 μm lateral resolution has been achieved with LAESI, when an optical fiber tip was used to focus the laser beam.[20] IR-LAMICI has been applied to the analysis of tablets and algal tissue[18] and the applications of LAESI include two and three-dimensional tissue imaging[17,21−23] and the analysis of small cell populations and even single cells.[20,24] We show that laser ablation atmospheric pressure photoionization (LAAPPI) provides finely localized ablation and high ionization efficiency for both polar and nonpolar compounds, readily produces radical cations ($M^{+\bullet}$) that have not been observed in LAESI or IR-LAMICI, and can be successfully applied for the analysis of tissue samples.

■ EXPERIMENTAL SECTION

Chemicals and Biological Tissues. Toluene (HPLC grade, 99.9%), anisole (≥99.0%), methanol (LC−MS grade), dehydroepiandrosterone (DHEA, ≥99%), verapamil hydrochloride (verapamil, 99%), estrone (99%), cholecalciferol (≥98%), glyceryl trioctanoate (tricaprylin, ≥99%), α-tocopherol, and bradykinin fragment 1-8 acetate hydrate (bradykinin 1-8, peptide content 86%) were from Sigma-Aldrich (St. Louis, MO). Water (HPLC grade) was from Ricca Chemical Company (Arlington, VA) and cholesterol (96%) from Alfa Aesar (Ward Hill, MA). Nitrogen nebulizing gas was obtained via the mass spectrometer nebulizer gas line from refrigerated industrial grade liquid nitrogen (99.8%, GTS-Welco, Inc., Allentown, PA) in a tank, regulated with a needle valve (Swagelok, Solon, OH) and measured with a panel-mounted single flow tube (Aalborg, Orangeburg, NY).

Stock solutions of the analytes were prepared at 10 mM concentration in methanol except the 5 mg/mL solution of bradykinin fragment 1-8 acetate hydrate (corresponding to 4.8 mM of the peptide) was prepared in water. The stock solutions were diluted in a water/methanol (1:1, v/v) mixture to prepare the working solutions, except that bradykinin 1-8 was diluted in water. The 5−50 μL aliquots of the liquid samples were applied onto microscope glass slides for the LAAPPI analysis.

The rat brain samples were prepared and sectioned as described elsewhere.[23] Rats were treated according to the Guide for the Care and Use of Laboratory Animals (NIH). To minimize tissue dehydration during the MS analysis, the rat brain section was kept cold, approximately at −4 °C determined by an infrared thermometer (845, Testo Inc., Sparta, NJ) on a Peltier cooling stage. The cooling stage was built in-house with a ceramic thermoelectric module (Ferrotec Corp., Bedford, NH).

Sour orange (*Citrus aurantium*) saplings (20 in. tall), obtained from the USDA laboratory in Weslaco, TX, were maintained in natural light and watered twice a week with ~2 L of tap water prior to the analysis. The *Citrus aurantium* leaves were collected just before analysis, rinsed with deionized water to remove possible dust particles, dried with lint free tissue, and attached onto a microscope glass slide with adhesive tape.

Mass Spectrometer. A JEOL AccuTOF JMS-T100LC mass spectrometer (JEOL Ltd., Peabody, MA) was used for mass analysis. The inlet cone (orifice) temperature was set to 150 °C and voltage to 20 V. The ion optics was optimized for the MH^+ ions of DHEA and verapamil using microchip APPI[25] for the ionization. The data acquisition time was set to 1 s. To efficiently transfer the ions with different m/z values to the time-of-flight (TOF) analyzer, the peaks voltage setting of the ion guide was optimized. The reported analyte and sample spectra were recorded using an ion guide peaks voltage of 1200 V, and the reactant ion spectra were recorded at 500 V. The m/z values in rat brain analysis were internally calibrated using cholesterol.

LAAPPI Ion Source. A LAAPPI ion source was constructed according to the schematic in Figure 1. All mechanical parts such as stands and microscope glass slide holder were from Thorlabs (Newton, MA). A sample stage, either the Peltier stage or a microscope glass slide, was positioned in front of the mass spectrometer ~12 mm below the inlet orifice. An infrared laser beam was guided with two gold-coated mirrors (PF10-03-M01, Thorlabs) to pass vertically in front of the mass spectrometer inlet and focused with a 50 mm focal length planoconvex CaF_2 lens (Infrared Optical Products, Huntington, NY) to a small spot at the sample surface. The beam was produced by an optical parametric oscillator by converting the output of a Nd:YAG laser (Vibrant IR, Opotek, Carlsbad, CA) in the form of 5 ns pulses at 2.94 μm and 10 Hz. This way the laser energy was conveyed to the water molecules of the sample causing its ablation.[19] The spot size was measured by exposing thermal paper (multigrade IV, Ilford Imaging Ltd., U.K.) to the focused beam. The laser beam produced approximately 0.08 mm^2 circular marks (~300 μm in diameter) on the paper. The laser pulse energy at the target was 0.3 to 2.5 mJ/pulse, which, for the given focusing conditions, corresponded to a fluence of 0.4 to 3.5 J/cm^2.
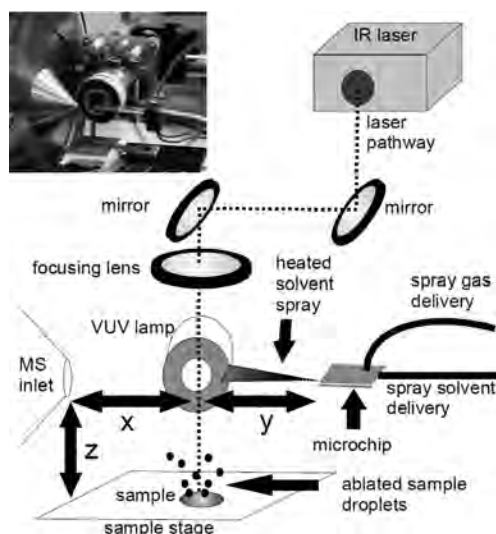

Figure 1. A schematic representation of the LAAPPI ion source (not to scale) with a photo of the ionization region shown in the inset.

Approximately 12 mm above the sample, the ablation plume was intercepted by a hot narrow solvent jet directed toward the mass spectrometer inlet. A heated nebulizer microchip (Aalto University, Espoo, Finland) was used to generate the jet. The microchips and their fabrication[26] and the microchip holder[27] have been previously described in detail. Toluene (ionization energy (IE) 8.8 eV),[28] anisole (IE 8.2 eV),[28] toluene/anisole (9:1, v/v), and toluene/methanol (1:9, v/v, methanol IE 10.8 eV)[28] were used as spray solvents. They were introduced to the microchip using a syringe pump (Physio 22 by Harvard Apparatus, Holliston, MA) and vaporized inside the microchip with the aid of nitrogen gas flow (70−300 mL/min) and high temperature (~240 °C, HY 3005 power supply by RSR Electronics). The sample plume and the solvent spray mixture were ionized with 10.0 and 10.6 eV photons produced by a radio frequency (rf) krypton discharge vacuum ultraviolet (VUV) photoionization lamp (PKR 100 from Heraeus Noblelight, Cambridge, U.K.), which was positioned orthogonally to the ablation plume. The VUV lamp was powered by a C210 13 MHz rf source (Heraeus Noblelight), which gives a typical rf lamp power input of 0.5 W. The rf power source was operated using a HY 3005 dc power supply (RSR Electronics) at 15.0 V and 0.06−0.09 A.

■ RESULTS

LAAPPI Setup. The geometry of the LAAPPI ion source had a significant effect on the overall sensitivity and stability of the ion signal. The geometry was optimized by analyzing a 100 μM mixture of verapamil, DHEA, and cholecalciferol (see Figure S1 in the Supporting Information for analyte structures) with toluene/anisole (9:1) as the spray solvent. The best sensitivity was achieved when the VUV lamp was positioned orthogonally with respect to the laser beam, and the distance of the VUV lamp from the ablated plume and the solvent spray, i.e., from microchip to mass spectrometer inlet axis ($x + y$ in Figure 1), was kept as short as possible (~5 mm) to minimize the spreading of the divergent light beam and the absorption of the VUV light by the ambient air. The optimum distance of the microchip nozzle tip from the laser beam (y in Figure 1) was approximately 1−2 mm, and the signal intensity was significantly reduced at longer distances (by ~80% when y was 4 mm). A short y distance ensures that the temperature of the solvent spray is high enough to vaporize the ablated sample droplets because the temperature of the solvent spray decreases rapidly when the spray exits the microchip.[29] The optimum distance of the laser beam, and thus the ablated sample plume, from the mass spectrometer inlet orifice (x in Figure 1) was ~10 mm, allowing the efficient vaporization of the ablated sample droplets and the subsequent gas-phase photoionization reactions to occur. The distance of the sample plate from the solvent spray axis (z in Figure 1) did not have a significant effect on the analyte signal between 7 and 21 mm, and ~12 mm was used in the experiments. Optimum direction of the solvent spray was directly on-axis to the mass spectrometer inlet, whereas spraying by only 2 mm off-axis reduced the analyte signal intensities by ~70%. The optimum solvent spray temperature was 200−250 °C (when y was ~2 mm and the N$_2$ gas flow rate was 300 mL/min). The optimum flow rate of the spray solvent depended on the solvent composition. Typically the signal intensity grew when the flow rate was raised, but the background ion signal also increased. To obtain a high signal-to-noise ratio for the analytes, the solvent flow rate
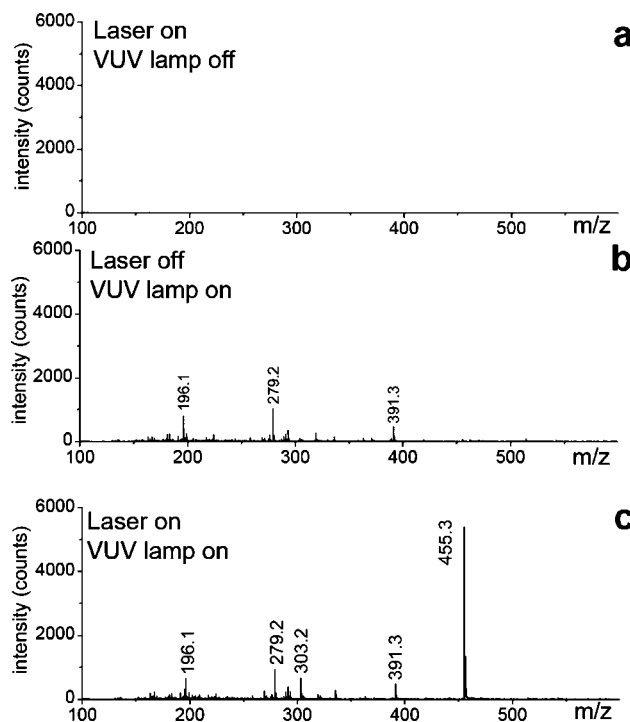

Figure 2. LAAPPI mass spectra of 100 μM verapamil (M_r = 454.6) obtained with toluene (1 μL/min) as the spray solvent with (a) the IR laser on and the VUV lamp off, (b) the IR laser off and the VUV lamp on, and (c) both the IR laser and the VUV lamp on.

was set in the range between 0.5 and 10 μL/min, depending on the used spray solvent.

Ablation and Ionization. The contribution of the IR laser and the VUV lamp to the performance of the LAAPPI source was studied by using verapamil as the sample and toluene as the spray solvent. When the IR laser was turned on and the VUV lamp was turned off, no signal was detected indicating that the IR laser was not ionizing the analyte (Figure 2a). When the IR laser was turned off and the VUV lamp was turned on, the spectrum showed abundant ions originating only from toluene and the ambient environment (Figure 2b), indicating that the sample (verapamil) is not desorbed by the hot spray solvent jet alone. When the IR laser and the VUV lamp were both turned on, intense signal of the protonated verapamil was detected (Figure 2c) indicating that the sample is ablated by the IR laser and the ablated analytes are photoionized by the VUV lamp.

Effect of Spray Solvent and Sample Solvent on Ionization. The spray solvent (S) is known to have a major role in atmospheric pressure photoionization (APPI)[30−32] and DAPPI.[12] As LAAPPI employs a similar VUV lamp for ionization to these techniques, also in LAAPPI the UV light ionizes the abundant gaseous low IE (<10 eV) solvent (S) species present in the ion source (Scheme 1, reaction 1), and the analytes (M) are ionized

Scheme 1. Photoionization Reactions (S = Solvent, M = Analyte)

$$S + h\nu \rightarrow S^{+\bullet} + e^- \quad (1)$$

$$M + S^{+\bullet} \rightarrow M^{+\bullet} + S \quad (2)$$

$$M + S^{+\bullet} \rightarrow MH^+ + [S\text{-}H]^\bullet \quad (3)$$
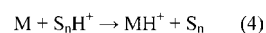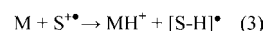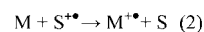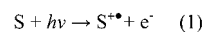
$$M + S_nH^+ \rightarrow MH^+ + S_n \quad (4)$$

Table 1. Analyte Species Observed with LAAPPI Using Different Spray Solvents

	observed ion species (m/z, relative intensity (%) in parentheses[a])			
spray solvent	verapamil	DHEA	cholecalciferol	estrone
toluene	455 (100) MH$^+$, 303 (42)	289 (100) MH$^+$, 271 (62) [MH − H$_2$O]$^+$	384 (62) M$^{+\bullet}$, 385 (100) MH$^+$	270 (100) M$^{+\bullet}$, 271 (26) MH$^+$
toluene/methanol (1:9,v/v)	455 (100) MH$^+$	289 (100) MH$^+$, 271 (58) [MH − H$_2$O]$^+$, 253 (18)	385 (100) MH$^+$	271 (100) MH$^+$
anisole	455 (27) MH$^+$, 303 (100)	289 (100) MH$^+$	384 (100) M$^{+\bullet}$, 385 (19) MH$^+$	270 (100) M$^{+\bullet}$
anisole/toluene (1:9, v/v)	455 (33) MH$^+$, 303 (100)	289 (100) MH$^+$	384 (100) M$^{+\bullet}$, 385 (32) MH$^+$	270 (100) M$^{+\bullet}$

[a]Values for MH$^+$ ions have been corrected by taking into account the natural abundance of the ^{13}C isotope peak for M$^{+\bullet}$.

via gas-phase reactions with the solvent ions. In our preliminary studies, toluene, anisole, toluene/anisole (9:1, v/v) and toluene/methanol (1:9, v/v) were used as the spray solvents and verapamil, DHEA, cholecalciferol, and estrone as the analytes. Similarly to APPI[33] and DAPPI,[11] the ionization in LAAPPI is thought to occur via charge exchange (Scheme 1, reaction 2) or proton transfer reactions (Scheme 1, reactions 3 and 4) depending on the proton affinity (PA) and the IE of the spray solvent and the analyte (Table 1). Toluene, anisole and toluene/anisole (9:1, v/v) behaved quite similarly producing protonated molecules (MH$^+$) of verapamil and DHEA that have relatively high PAs (980[34] and 825 kJ/mol,[35] respectively), and MH$^+$ and/or radical cation (M$^{+\bullet}$) of cholecalciferol and estrone having low IEs (7.55[36] and 8.7 eV,[37] respectively). However, compared to proton transfer, the charge exchange reaction was more favored with anisole than with toluene, as the PA of anisole is higher than that of toluene. When the toluene/methanol (1:9) mixture was used as the spray solvent, the analytes produced only MH$^+$ ions. This is because the radical cation of toluene formed in photoionization (Scheme 1, reaction 1) transfers a proton to a methanol cluster, as described earlier for APPI.[33] Thus, the radical cation of toluene is neutralized, and charge exchange (Scheme 1, reaction 2) is not possible. Ionization then occurs by proton transfer from the protonated methanol (cluster) to the analyte (Scheme 1, reaction 4).

Water molecules are always transferred to the gas-phase together with the analytes because the mid-IR laser ablation process is based on the ejection of a water-rich sample. Sometimes it may be necessary to add organic solvent to the sample to increase the solubility of less polar compounds. Since the organic modifier may have an effect on the ionization reactions, we studied the effect of sample solvents, water, and water/methanol (1:1, v/v) on the reactant ion composition (Figure 3). Toluene was used as the spray solvent. When the IR laser was turned on for a short period and water/methanol (1:1, v/v) was used as a solvent, the time-resolved intensities of the reactant ions (Figure 3a−e) and the reactant ion spectra (Figure S2 in the Supporting Information) show that the intensity of protonated water and the radical cation of toluene decreased, whereas the intensities of protonated toluene and a protonated molecule (and dimer) of methanol increased. This is because the protonated water molecule and the radical cation of toluene are neutralized by proton transfer to the ablated methanol (Scheme 1, reaction 4) or more probably to methanol clusters.[33] This shows that both the spray solvent and the sample solvent have an effect on the reactant ion composition in LAAPPI. When pure water was used as a sample and the laser was fired, the combined intensity of all measured ions (Figure 3a) and the intensities of methanol and toluene reactant ions decreased (Figure 3b,d). However, there
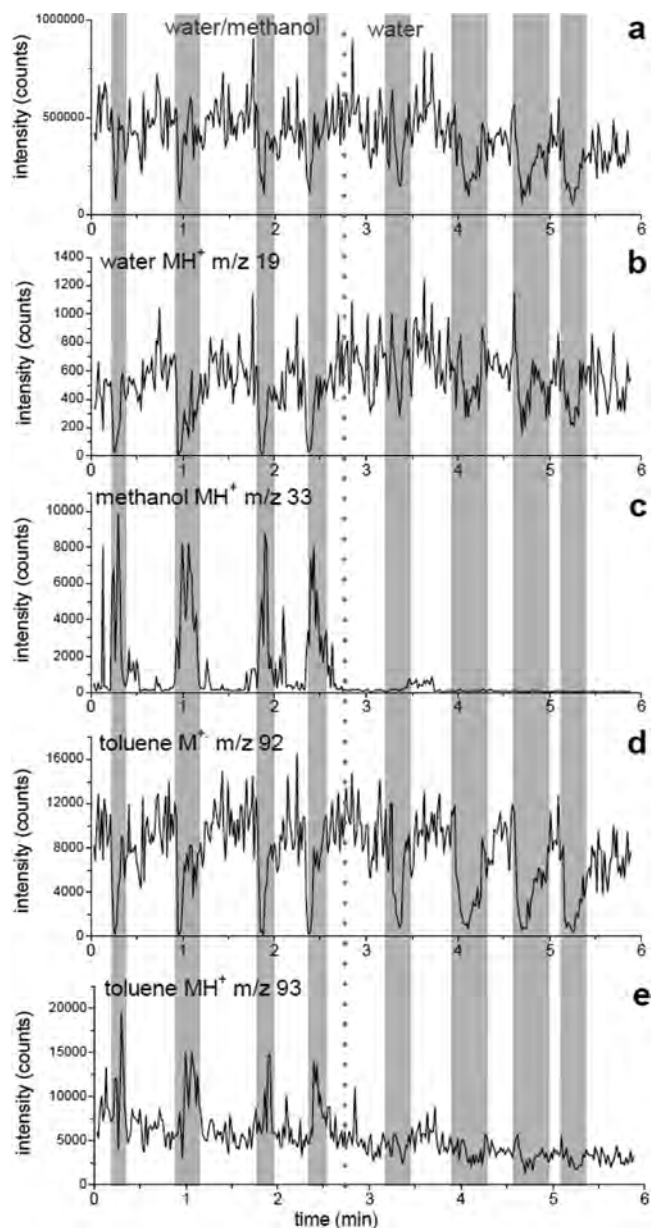

Figure 3. Time resolved intensities of (a) all measured ions at m/z 10−1500 and (b) water MH$^+$, (c) methanol MH$^+$, (d) toluene M$^{+\bullet}$, and (e) toluene MH$^+$ ions for sample solvents water/methanol (1:1) (0−2.7 min) and water (3−6 min) measured by LAAPPI. Toluene was the spray solvent with a flow rate of 0.5 μL/min. The gray bars show when the laser was turned on.

were no significant changes in the relative abundances of the solvent reactant ions (Figures S2c and S2d in the Supporting Information).

Table 2. Limits of Detection (LOD), Signal Area Linearities, and Relative Signal Area Repeatabilities (RSD) at 100 μM for DHEA and Verapamil Using Toluene/MeOH (1:9) at a Flow Rate of 8 μL/min As the Spray Solvent

		LOD		linearity			
analyte	monitored ion	measured concentration	estimated absolute amount	studied range	R^2	n	RSD (%, $n = 4$) at 100 μM
DHEA	MH$^+$ at m/z 289.1	5.0 μM	3.3 pmol	5 μM–1 mM	0.99	6	25
verapamil	MH$^+$ at m/z 455.3	250 nM	170 fmol	250 nM–1 mM	0.99	9	27

This indicated that the flow of ablated microdroplets may, to some extent, hinder the spray solvent flow to the mass spectrometer by displacing it.

Quantitative Performance of LAAPPI. The quantitative performance of LAAPPI was studied by determining the limits of detection (LOD), linearities, and repeatabilities for DHEA and verapamil (Table 2) by monitoring the time-resolved signal heights of the MH$^+$ ions. For these experiments, 10 μL of liquid sample was applied on the microscope glass slide and subjected to LAAPPI analysis until all liquid was ablated. Toluene/methanol (1:9) was used as the spray solvent. The concentrations of DHEA and verapamil at the LOD (S/N $\geq$ 3) were 5 μM and 0.25 μM, respectively. Absolute LODs in LAAPPI can be estimated by the amount of sample needed to produce a spectrum (1 s and 10 laser shots each) with analyte S/N $\geq$ 3. As each analyzed sample produced approximately 15 spectra, about 0.67 μL of the sample was consumed per spectrum. Thus, the absolute LODs were estimated to be 3.3 pmol and 170 fmol for DHEA and verapamil, respectively. The LOD for verapamil was similar to what had been reported for LAESI,[17] and the estimated absolute LOD was similar to what had been estimated for acetaminophen in IR-LAMICI.[18] Linearities of the LAAPPI signal were studied from the LODs to 1 mM concentration. The correlation coefficients (R^2) of the calibration curves plotted on log–log-scales were 0.99 indicating good linearity of the method. The relative standard deviations (RSD) for four DHEA and verapamil samples at 100 μM were 25% and 27%, respectively, indicating semi-quantitative performance as it is often observed for direct ambient analysis methods, when not using an internal standard.

Biological Applications. The feasibility of LAAPPI in the analysis of compounds of biological interest was tested with standard samples of α-tocopherol, cholesterol, triglyceride tricaprylin, and bradykinin 1-8 (Figure 4, see Figure S1 in the Supporting Information for the analyte structures). Toluene was used as the spray solvent. The smaller molecules, α-tocopherol, cholesterol, and tricaprylin, showed molecular weight specific ions (MH$^+$, M$^{+\bullet}$, or [M $-$ H]$^+$) and specific fragment ions. α-Tocopherol, having delocalized π-electrons, produced abundant M$^{+\bullet}$ and MH$^+$ ions and a fragment at m/z 165.1, whereas tricaprylin produced only MH$^+$ and an abundant fragment ion at m/z 327.3 formed by the loss of one fatty acyl chain. Cholesterol produced [M $-$ H]$^+$ ions formed by hydride abstraction or rapid hydrogen loss from the protonated molecule, and abundant fragment ions at m/z 368.4 and m/z 369.4 indicating the loss of a hydroxyl group. Bradykinin 1-8 produced only sequence specific fragment ions at lower m/z values. These spectra (Figure 4) show that in addition to polar compounds, LAAPPI is capable of efficiently ionizing relatively nonpolar compounds such as cholesterol and tricaprylin, which are difficult to ionize with electrospray-based techniques. However, larger and more labile compounds dissociated in LAAPPI under the chosen experimental conditions, most probably due to the relatively high
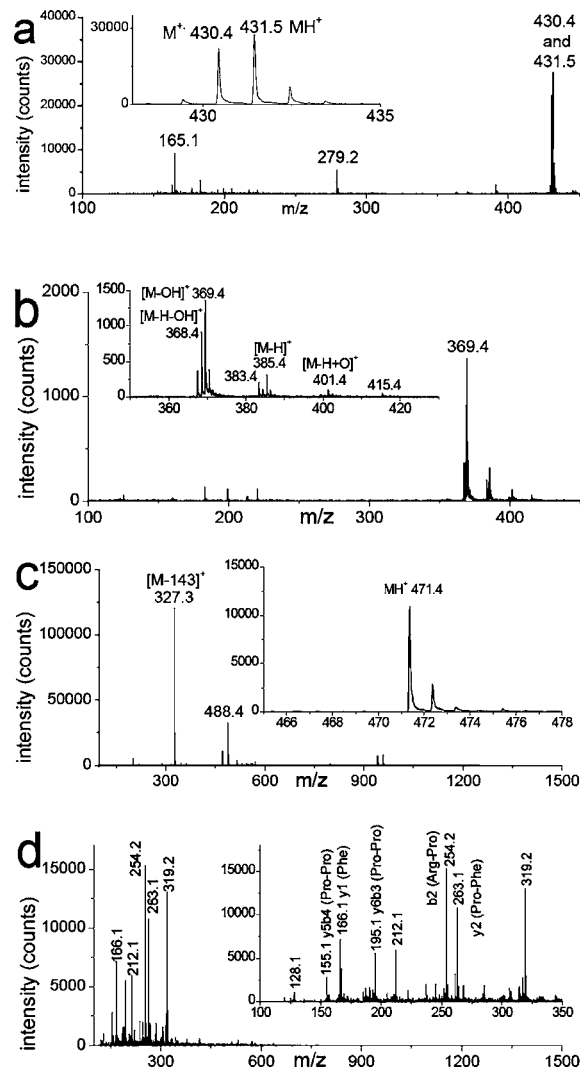
Figure 4. LAAPPI mass spectra of (a) 100 μM α-tocopherol, (b) 100 μM cholesterol (background subtracted), (c) 100 μM tricaprylin (background subtracted), and (d) 480 μM bradykinin 1-8 (background subtracted).

temperature in the system, which was caused by the hot solvent jet (~240 °C).

The feasibility of LAAPPI in the direct analysis of biological samples was demonstrated by studying *Citrus aurantium* leaf and rat brain section samples. Random, discrete spots on these sample surfaces were subjected to LAAPPI analysis. The majority of the leaf tissue of *Citrus aurantium* showed ions mainly below m/z 300 (Figure 5a). The ions are most likely due to volatile terpenes[38,39] and other leaf metabolites. For example, the ion at m/z 196.15 could be the M$^{+\bullet}$ of linalyl acetate and an ion at m/z 153.13 the [M $-$ H]$^+$ of linalool (Table S1 in the Supporting Information), which would indicate a common chemotype (chemical phenotype) of
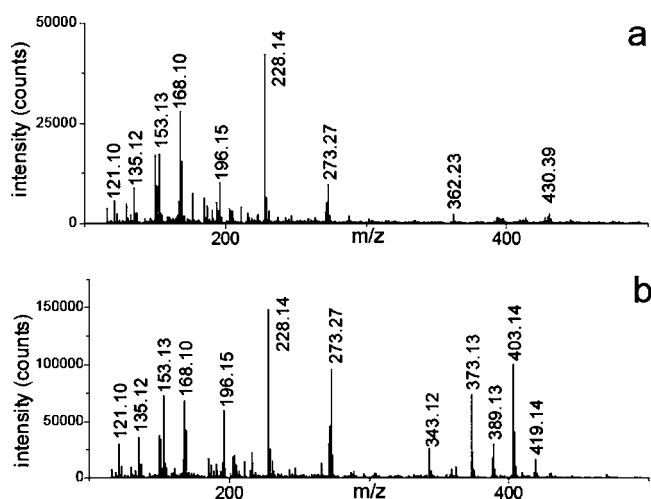
Figure 5. LAAPPI mass spectra obtained in the analysis of a *Citrus aurantium* leaf from (a) most of the leaf tissue and (b) certain studied spots with additional peaks. The solvent background has been subtracted from both spectra. The spray solvent was toluene with a flow rate of 0.5 μL/min. See Table S1 in the Supporting Information for the putative peak assignments.

Citrus aurantium.[38] Some of the studied spots showed additional abundant ions at *m/z* 343.12, 373.13, 389.13, 403.14, and 419.14 (Figure 5b), where the latter four ions correspond to protonated O-methylated flavones, such as tangeritin, 5-demethyl nobiletin, nobiletin, and flavonol natsudaidain, respectively. These ions were previously found in *Citrus aurantium* leafs.[40] Because the O-methylated flavones have nonpolar structures, they are expected to accumulate in the oil glands of the leaf,[41] which correlates with the lack of the corresponding ion signals in the spectra from most of the studied leaf tissue (Figure 5a). The presented mass spectra (Figure 5) show that LAAPPI offers a rapid analysis method for nonpolar compounds in plant tissue.

Figure 6 presents a typical LAAPPI spectrum measured directly from a rat brain section. The spectra showed abundant ions at *m/z* 385.35 and at *m/z* 386.35 corresponding to [M − H]$^+$ and M$^{+\bullet}$ for cholesterol. The M$^{+\bullet}$ ion was not detected from the cholesterol standard in water/methanol (1:1) solution (Figure 4b) because, as discussed above, the proton transfer from the toluene radical cation to methanol may suppress the charge exchange reaction. The rat brain sample matrix does not contain methanol; therefore, the radical cation of toluene remains in the system making the charge exchange reaction with cholesterol possible. The ions at *m/z* 368.34 and *m/z* 369.35 are also seen in the cholesterol standard spectrum
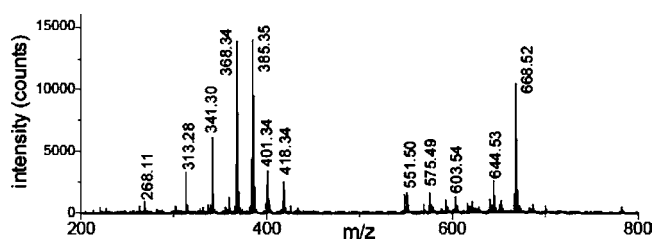
(Figure 4b) and are most likely formed by the loss of a hydroxyl group. In addition to the possible cholesterol species, the brain spectra (Figure 6) showed ions at *m/z* 551.50, 575.49, 577.52, 579.53, and 603.53, the masses corresponding to triglyceride fragments (Table S2 in the Supporting Information) that are formed by the loss of one fatty acyl chain, as observed for the tricaprylin standard (Figure 4c). The comparison between the rat brain spectrum measured by LAAPPI to that measured earlier with LAESI[23] shows that nonpolar compounds such as cholesterol and triglycerides are ionized more efficiently by LAAPPI. On the other hand, LAESI is able to efficiently ionize phospholipids, which were not detected as molecular weight specific ions in LAAPPI (Figure 6). On the basis of these initial experiments, LAAPPI and LAESI[23] ionize different biomolecules from the rat brain tissue; therefore, the two techniques can be viewed as complementary tools in bioanalysis.

■ CONCLUSIONS

In this contribution, we presented a novel laser ablation photoionization technique, LAAPPI, for the ambient analysis of nonpolar and neutral compounds, such as cholesterol and triglycerides. LAAPPI showed molecular weight specific ions and structure specific fragments for low molecular weight (<500 amu) compounds. Particularly, when a suitable spray solvent, e.g., toluene or anisole, was used, analytes with low IE could be ionized via charge exchange resulting in the formation of radical cations (M$^{+\bullet}$). This enabled the detection of nonpolar compounds, which cannot be efficiently ionized by proton transfer due to their low proton affinities. High molecular weight, thermolabile compounds fragmented due to the high temperature of the ion source. LAAPPI was found to be linear over three decades with R^2 0.99 and signal RSD ≤ 27%, showing adequate quantitative performance. The sensitivity of LAAPPI was comparable to other ambient ionization methods, e.g., LAESI. We demonstrated that LAAPPI could produce spectra from approximately 0.08 mm^2 spots of biological samples, such as plant leaf and rat brain tissue sections. The analysis of rat brain tissue showed that the ionized constituents in LAAPPI differed from LAESI, making the two techniques complementary tools for bioanalysis. The presented data also suggested that LAAPPI could be applied in MSI of biological samples and, similarly to LAESI, could also be utilized in single cell analysis.

■ ASSOCIATED CONTENT

ⓢ Supporting Information
Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Figure 6. LAAPPI mass spectrum obtained from a 200 μm thick rat brain section with toluene as the spray solvent at a flow rate of 0.5 μL/min.

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Infrared laser ablation atmospheric pressure photoionization mass spectrometry

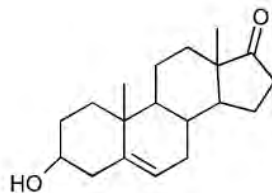*A. Vaikkinen,[a, b] B. Shrestha,[b] T.J. Kauppila,[a] A. Vertes[b]* and R. Kostiainen[a]**

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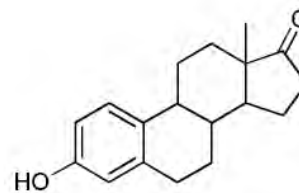
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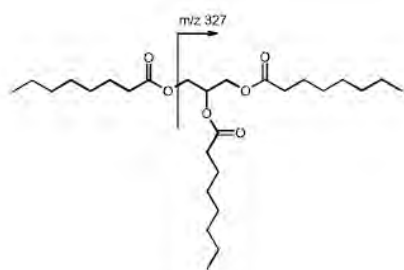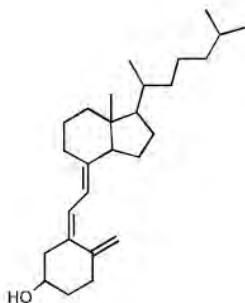Washington University, Washington, District of Columbia 20052, USA
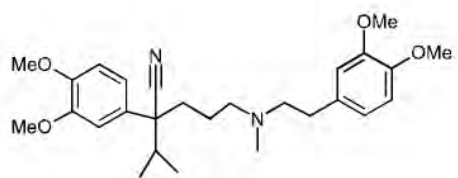
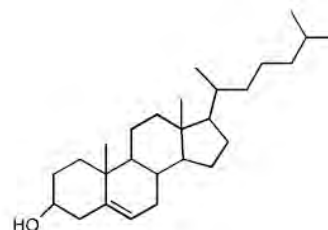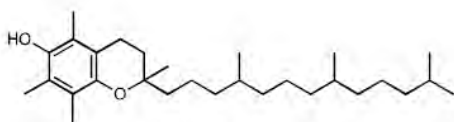**Figure S1:** Structures of the analytes studied by laser ablation atmospheric pressure photoionization (LAAPPI) mass spectrometry (MS).
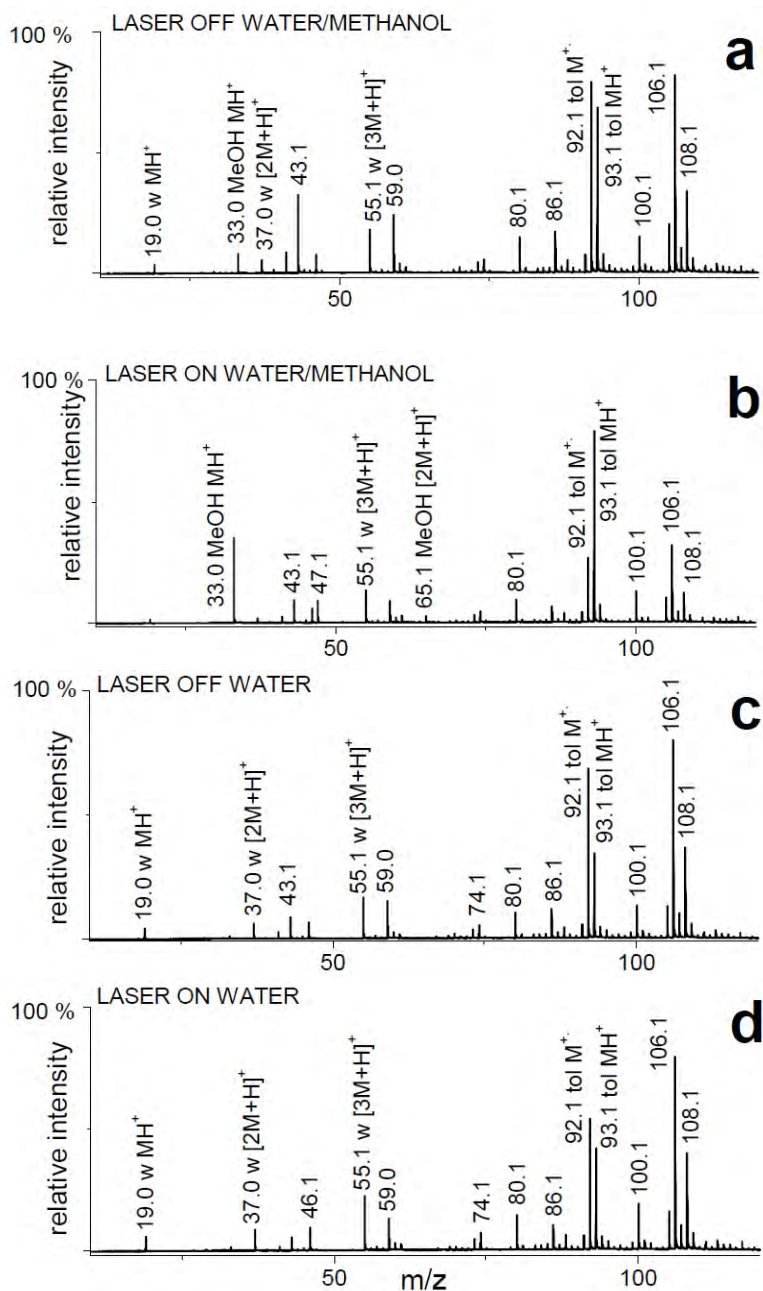
Figure S2. Solvent reactant ion spectra of water/MeOH with a) the laser off (0.0-0.2 min) and b) laser on (0.2-0.3 min), and water with c) the laser off (2.7-3.1 min) and d) the laser on (3.2-3.4 min). Total and selected ion chromatograms for solvent ions are presented in Figure 3. w = water, MeOH = methanol, tol = toluene. Toluene was the spray solvent with a flow rate of 0.5 μL/min.

Table S1: Ions observed in LAAPPI analysis of *Citrus aurantium* leaf and putative assignments of the ions based on literature on leaf phytocompounds. Observed in Fig. 5a refers to ions observed from the majority of the leaf (spectrum presented in Figure 5a), and Observed in Fig 5b refers to ions observed from only some of the studied spots (spectrum presented in Figure 5b). x means that the ion was observed.

ions observed by LAAPPI-MS			Citrus aurantium leaf oil components reported in literature		
observed *m/z*	Observed in 5a	Observed in 5b	compound (ion corresponding to observed *m/z* in parenthesis)	Calculated *m/z*	Δ*m/z*
121.1023	x	x			
135.1210	x	x			
137.1360	x	x	monoterpenes[1, 2]: limonene, myrcene, ocimene, phellandrene, pinene, sabinene, terpinene, terpinolene (MH+)	137.1325	0.0035
150.0949	x	x	thymol[1] (M+·)	150.1039	-0.0090
153.1288	x	x	geraniol,[2] nerol,[2] terpinen-4-ol,[2] terpinene,[1] terpineol,[2] linalool[1, 2] ([M-H]+)	153.1274	0.0014
168.1028	x	x	synephrine[3] (MH+)	168.1019	0.0009
196.1469	x	x	linalyl acetate,[1, 2] neryl acetate,[1, 2] geranyl acetate[1, 2] (M+·)	196.1458	0.0011
228.1392	x	x			
273.2654	x	x			
343.1246		x			
373.1347		x	tangeritin[4] (MH+)	373.1282	0.0065
389.1286		x	5-demethyl nobiletin[4] (MH+)	389.1231	0.0055
403.1351		x	nobiletin[4] (MH+)	403.1387	-0.0036
419.1372		x	natsudaidain[4] (MH+)	419.1336	0.0036

Table S2: Selected ions observed in rat brain LAAPPI spectra and their putative assignments. The given values for observed *m/z* have been subjected to internal calibration using cholesterol [M-H]$^+$ ion *m/z* as the lock mass, and the Δ*m/z* marked with * is the deviation of the internally and externally calibrated values. Trigyceride fragment refers to loss of fatty acid similar to what was observed for tricaprylin (Fig. 4c) and FAs to the fatty acids of the fragment.

observed *m/z*	putative assignment (ion in parenthesis)	calculated *m/z*	Δ*m/z*
368.3448	cholesterol [M-H-OH]$^+$	368.3448	0
369.3522	cholesterol [M-OH]$^+$	369.3516	0.0006
383.3359	cholesterol [M-3]$^+$, oxocholesterol [M-H$_2$O]$^+$	383.3308	0.0051
385.3465	cholesterol [M-H]$^+$	385.3465	0.0107*
386.3538	cholesterol M$^{+\cdot}$	386.3543	-0.0005
401.3372	cholesterol [M-H+O]$^+$ or ketocholesterol MH$^+$	401.3414	-0.0042
418.3398	cholesterol [M+O$_2$]$^+$ or dihydroxycholesterol M$^{+\cdot}$	418.3441	-0.0043
419.3530	cholesterol [MH+O$_2$]$^+$ or dihydroxycholesterol MH$^+$	419.3520	0.0010
551.5011	trigyceride fragment (FAs 16:0, 16:0)	551.5033	-0.0022
575.4947	trigyceride fragment (FAs 16:0, 18:02 or 16:01, 18:01)	575.5034	-0.0087
577.5184	trigyceride fragment (FAs 16:0, 18:01)	577.5190	-0.0006
579.5314	trigyceride fragment (FAs 16:0, 18:0)	579.5346	-0.0032
603.5269	trigyceride fragment (FAs 18:0, 18:02 or 18:01, 18:01)	603.5347	-0.0078

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