Surface-modified Wooden-tip Electrospray Ionization Mass Spectrometry for Enhanced Detection of Analytes in Complex Samples

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Abstract

Replacement of capillary with solid substrates for electrospray ionization mass spectrometry (ESI-MS) has created many new possibilities on sample loading and ionization, and exploring the relationship between surface activity of solid substrates and analytical properties (i.e., sensitivity and selectivity) is important to further investigation and development of solid-substrate ESI-MS. In this study, we investigated the analytical properties of solid-substrate ESI-MS systematically and comprehensively using wooden tips modified with hydrophobic ($-C_{18}$), basic ($-NH_2$) and acidic ($-SO_3H$) functional groups via both extractive sampling and direct loading methods. Analytes with different chemical properties were applied to investigate the effects on surface activity of solid substrate. Our results showed that analytes not strongly retained on solid-substrate surface and thus were readily sprayed out for detection in direct loading method, since the surfaces having relatively weaker interactions with target analytes. While for extractive sampling method, analytes strongly retained on solid-substrate surface could be selectively enriched and detected, and subsequent washing step could effectively remove unbound components for reducing interference. In addition, we demonstrated that selective enrichment of target analytes in complex mixtures and on-surface sample cleanup could be effectively performed if appropriate modified WT were applied. Overall, the insights on the effects of surface-analyte interactions on the analytical features obtained in this study could aid the development of surface-modified strategies for enhancing the analytical capability of solid-substrate ESI-MS

Introduction

Electrospray ionization mass spectrometry (ESI-MS) is one of the most important analytical techniques for analysis of biological and chemical molecules. However, conventional ESI, which makes use of a tiny capillary for sample introduction, could be easily susceptible to clogging and interference from salts and impurities, leading to its limited capability in direct analysis of complex samples, e.g, biological fluids, natural products, environmental samples, etc. ESI-MS analysis of these samples usually requires various procedures, such as extraction, enrichment and chromatographic separation that could be time-consuming and laborious. Due to these limitations, development of ionization techniques for rapid and direct analysis of complex samples has attracted considerable research interests.

Replacement of capillary with solid substrates has been approved to facilitate ESI-MS analysis by improving the sampling and ionization processes.[1] In solid-substrate ESI, samples are applied on the open surface of solid substrates, which could be metal tips,[2-4] aluminum foils,[5] polymer tips,[6,7] thin layer chromatography (TLC) plates[8,9] and cellulose materials such as wooden tips[10] and paper.[11] Upon application of a high voltage to the solid substrate, spray ionization could be induced and a mass spectrum could be acquired. The open surface sampling in solid-substrate ESI avoids the problem of clogging easily encountered in conventional capillary-based ESI.[2-6,10,11] In addition, on-surface separation of sample is allowed for materials having separating properties, such as paper[12], wooden tips[7,10,13] and TLC plates.[8] Sequential ionization resulting in separated detection of samples was observed for various materials including metal needles and wooden tips.[10,13,14] These characteristics enable rapid and direct analysis of raw complex mixtures including liquid, powder and

viscous samples, e.g., biological fluids, foods and herbal materials.

Compared to conventional ESI in which the ion signals are mainly determined by the ionization conditions, e.g., solvent composition, solvent flow rate, ion source temperature and desolvation gas flow, the mass spectral features obtained by solid-substrate ESI could be influenced by much more factors, such as efficiency of on-surface extraction, on-surface separation, diffusion of solvent on the surface….etc, which are to large extent governed by interactions between analytes and solid-substrate surface.[6,13,15,16] Surface modification of solid substrates has been an effective strategy for improving the detection selectivity and sensitivity of solid-substrate ESI. For example, Deng et al., demonstrated the use of wooden tips coated with different materials for enhanced detection of compounds such as perfluorinated compounds (PFCs);[17,18] paper spray using modified papers was developed for analysis of therapeutic drugs in dry blood spots, pesticides in milk, illicit drugs in raw urine, and proteins in protein gel, etc;[15,19-27] stainless-steel sheets coated with biocompatible C_{18} polyacrylonitrile were used for extraction/cleanup and ionization of samples in complex mixtures.[28-30]

Understanding the chemical properties of solid-substrate surface for analytical features (i.e., sensitivity and selectivity) is of great benefits to the further development of solid-substrate ESI. In this study, we performed surface modification of wooden tips (WT) with hydrophobic ($-C_{18}$), basic ($-NH_2$) and acidic ($-SO_3H$) groups to investigate the analytical properties of solid-substrate ESI in details. WT can produce more durable ion signals compared to metal tips and papers,[10,13] and its surface is rich with hydroxyl groups for modification of functional groups through silanization reactions.[17,18] The functional groups allow molecular interactions

with analytes through hydrophobic (-C_{18}) or electrostatic (-NH_2 and –SO_3H) interactions. Analytes with different chemical properties were applied to investigate the effects on surface activity of solid substrate via both extractive sampling and direct loading WT-ESI-MS methods.

Experimental Section

Reagents and materials

Dimethyl formamide (DMF), octyl β-D-glucopyranoside (OG), sodium sinapine, sodium hydroxide (NaOH), sodium pyrosulfite ($Na_2S_2O_5$), lysozyme, hexane, trimethoxyoctadecylsilane, sodium chloride (NaCl), 3-aminopropyltriethoxysilane and trimethoxy(7-octen-1-yl) silane were purchased from Sigma (St. Louis, MO). Cocaine and cocaine-d_3 were purchased from Alfasan (Woerden, Holland). Methanol was purchased from Tedia (Fairfield, OH). Formic acid was purchased from Fluka (Buchs, Germany). Gramicidin D was purchased from International Laboratory U.S.A. (South San Francisco, CA). Tetrapeptide Gly-Gly-His-Ala (GGHA) was purchased from Bachem Bioscience Inc (King of Prussia, PA). Wooden tips (BestBuy brand toothpick) were purchased from a supermarket in Hong Kong (ParknShop).

Sampling with wooden tips

Two sampling methods, i.e., extractive sampling (Figure S-1a) and direct loading (Figure S-1b), were investigated using various surface-modified wooden tips (the details for fabrication and characterization of surface-modified wooden tips are shown in Supporting Information and Figure S-2) and unmodified wooden tips. In extractive sampling for liquid samples,

wooden tips were soaked in liquid samples for 5 minutes with vortexing to extract analytes (Figure S-1a). In extractive sampling for raw potato, wooden tips were punctured into the sample for 10 seconds to adhere a certain amount of semi-solid sample material (Figure S-1a). After sample extraction, the loaded wooden tip was washed by dipping the tip into milliQ water for 2 cycles of 10 seconds. The extraction time for sampling analytes from liquid samples semi-solid samples were selected at 5 minutes and 10 seconds, respectively. (the details see Figure S-3 and S-4 in the Supporting Information). The length of wooden tips for sampling was optimized at 1.0 cm (Figure S-5). For ESI-MS analysis, 5.0 μL of elution solvent, i.e., methanol with 0.1 % formic acid for positive ion mode and pure methanol for negative ion mode, was added onto the wooden tip connected with a high voltage. In direct loading method, 5.0 μL of liquid sample was directly loaded onto the wooden tip connected with a high voltage for analysis (Figure S-1b). All the experimental time was recorded using an automatic watch (Longines, Switzerland).

Mass spectrometry

Wooden-tip electrospray ionization mass spectrometry (WT-ESI-MS) analysis was performed as described previously.[10] Briefly, wooden tips were mounted on the nanoESI source of a quadrupole time-of-flight (Q-ToF) mass spectrometer (QToF2, Waters, Milford, MA) or a triple quadrupole mass spectrometer (Quattro Ultima, Waters, Milford, MA) (Figure S1c) unless specified otherwhere. The distance between the tip end of wooden tip and the MS inlet was ~ 0.8 cm. The ionization voltage and cone voltage were set at 3.5 kV and 30 V, respectively.

For conventional ESI-MS analysis, sample solutions were infused into the mass spectrometer

with a syringe pump (KD Scientific Inc., Holliston, MA, USA) at a flow rate of 5 μL/min. Nitrogen desolvation gas and cone gas were set at 250 L/h and 75 L/h, respectively.

For direct ionization mass spectrometric analysis of potato, the sample was cut into V-shape and placed in the front of the MS inlet with a clip connected with high voltage.[31]

Results and discussion

Analytical properties of ESI-MS with surface-modified wooden tips

To investigate the analytical properties of ESI with different surface-modified wooden tips, analytes with different chemical properties were mixed together and subjected to analysis. First, methanolic solution of 1 μg/ml of sinapine, an alkaloid with a positively charged quaternary amine group, was mixed with methanolic solution of 30 μg/ml of a relatively hydrophobic tetrapeptide, Gly-Gly-His-Ala (M.W. 310 Da) (Figure 1a). The peak intensity ratio of the two components, *m/z* 310 for sinapine ($[M]^+$) and *m/z* 341 for Gly-Gly-His-Ala ($[M+H]^+$), was found to be 1:1 when this mixture was analyzed with conventional ESI (Figure 1b). Interestingly, when this mixture was analyzed by ESI with wooden tips of different surfaces, mass spectral results significantly different from conventional ESI were obtained. When the mixture was directly loaded onto unmodified-WT and SO_3H-WT (direct loading method), the peak intensity ratio between sinapine and Gly-Gly-His-Ala ($R_{sinapine/Gly\text{-}Gly\text{-}His\text{-}Ala}$) became remarkably lower than conventional ESI-MS (Figure 1c). These observations were believed to be because that the electronegative –OH and $–SO_3H$ groups on the unmodified-WT and SO_3H-WT surfaces, respectively, interacted considerably strongly with the positive

charged on the sinapine, increasing the retention of this analyte on the tip surfaces and reducing its tendency to spray out for detection. The stronger interaction between the more electronegative -SO_3H group and the sinapine analyte led to the lower $R_{sinapine/Gly\text{-}Gly\text{-}His\text{-}Ala}$ obtained from SO_3H-WT, as compared with that of the unmodified-WT (Figure 1c). When the mixture was directly loaded onto the C_{18}-WT, the $R_{sinapine/Gly\text{-}Gly\text{-}His\text{-}Ala}$ slightly increased, a result that is consistent with the fact that the hydrophobic -C_{18} group tended to retain the relatively hydrophobic tetrapeptide (Figure 1c). For the NH_2-WT, the $R_{sinapine/Gly\text{-}Gly\text{-}His\text{-}Ala}$ was almost 1:1 as similar to conventional ESI, revealing that there was no significant interaction between the analyte and the NH_2 group on the tip surface (Figure 1c).

Interestingly, the mass spectral results obtained by the extractive sampling method were significantly different from those obtained by the direct loading method. When the unmodified-WT and SO_3H-WT were applied in extractive sampling for the same mixture, the $R_{sinapine/Gly\text{-}Gly\text{-}His\text{-}Ala}$ became much higher than that obtained by conventional ESI, which was the opposite of that observed by the direct loading method (Figure 1d). For the C_{18}-WT, contrary to the direct loading method, the $R_{sinapine/Gly\text{-}Gly\text{-}His\text{-}Ala}$ was much lower than that obtained by conventional ESI (Figure 1d). These results showed that the interactions between analytes and tip surface, i.e., electrostatic interaction between electronegative groups on the tip surface and positively charged sinapine and hydrophobic interaction between -C_{18} group on tip surface and Gly-Gly-His-Ala, would lead to selective enrichment of analytes with particular chemical properties. After washing away the loosely bound components and addition of elution solvent, analytes strongly interacting with the solid-substrate surfaces

could be selectively detected. For the NH_2-WT, similar to that of the direct loading method, the peak intensity ratio between two components was almost 1:1 as for conventional ESI, indicating the limited interaction between the analytes and the tip surface (Figure 1d).

A mixture of an aliphatic amino acid, i.e., alanine, and an acidic amino acid, i.e., aspartic acid, was further applied to investigate the analytical behavior of solid-substrate ESI (Figure 2a). Similarly, the mixture was prepared such that the intensity ratio of the two amino acids was 1:1 when analyzed with conventional ESI (Figure 2b). When the mixture was analyzed with the direct loading method, the intensity ratios of the two components for unmodified-WT, C_{18}-WT and SO_3H-WT were not largely different from those for conventional ESI (Figure 2c), indicating the insignificant interactions between the analytes and the solid-substrate surfaces. Interestingly, the intensity ratio between alanine and aspartic acid ($R_{Ala/Asp}$) became remarkably higher for NH_2-WT (Figure 2c), revealing that the basic surface of this wooden tip tended to retain the acidic aspartic acid, lowering its tendency to spray out for detection. When the extractive sampling method was applied, the results obtained for unmodified-WT, C_{18}-WT and SO_3H-WT were similar to those for the direct loading method, consistent with the limited interactions between solid-substrate surfaces and analytes (Figure 2d). For NH_2-WT, however, the $R_{Ala/Asp}$ became significantly lower than those of other wooden tips (Figure 2d), indicating that the basic NH_2 group of the solid-substrate surface significantly enriched the acidic aspartic acid in the mixture and led to selective detection of this component after washing and addition of the elution solvent.

In addition to the chemical property of solid-substrate surface, the effect of elution solvent on the analytical features was also investigated. With the use of the extractive sampling method, a mixture of alanine and aspartic acid was analyzed with NH_2-WT using different proportions of ACN and water as the elution solvent. As shown in Figure S-6, the intensity ratio between alanine and aspartic acid ($R_{Ala/Asp}$) was ~ 10 when pure ACN was used as elution solvent, indicating that this relatively nonpolar solvent system was less effective in eluting the relatively polar aspartic acid from the polar NH_2 surface. However, as the proportional of water, which is relatively polar, increased, the $R_{Ala/Asp}$ decreased accordingly and eventually reached a significantly low level, i.e., ~0.05, when 100% of water was applied as the elution solvent (Figure S-6). These data revealed that the high polarity of water allowed more effective elution of the relatively polar aspartic acid retained on the NH_2-WT surface. The change in spectral features with the composition of the elution solvent was not due to variation in ionization efficiency, as the $R_{Ala/Asp}$ were similar when the mixture was analyzed by conventional ESI using these five solvents with ACN compositions from 100% to 0% (Figure S-7).

Apart from simple mixtures, the analytical properties of the surface-modified wooden tips were also investigated with a raw potato sample, which is a more complex sample containing numerous ingredients with a broad range of chemical properties, e.g., organic acids, fatty acids, lipids, sugars, glycoalkaloids and free amino acids. The hard and slim properties of wooden tips permit direct puncturing into solid objects for sampling. The mass spectral results obtained with different wooden tips as well as direct ionization mass spectrometry (DI-MS),[31] which allowed direct extraction and ionization of compounds from the potato, were compared to investigate the effect of the tip surface activity. When the raw potato was analyzed with

DI-MS in positive ion mode, various components including arginine, sucrose, phosphatidylcholines (PC) and glycoalkaloids (solanine and chaconine) were detected (Figure 3a) and their identities were confirmed by tandem mass spectrometry (Figure S-8). When the potato was sampled with the unmodified-, C_{18}-, SO_3H- and NH_2-WT through puncturing and the adhered semi-solids were directly analyzed in positive mode by adding extraction solvent without washing, the mass spectra obtained for all wooden tips were similar to each other and similar to that obtained by DI-MS (Figure S-9). Interestingly, the results were significantly different if a washing step was involved between sampling and addition of the elution solvent. For the hydrophobic C_{18}-WT, the hydrophobic PCs, which were only detected as minor components with other wooden tips and DI-MS, could be predominately detected if the washing step was involved (Figure 3b). On the other hand, the relatively basic chaconine was the only detected component for the acidic SO_3H-WT, while the acidic ascorbic acid, which was not detected in other wooden tips and DI-MS, was predominately detected for the basic NH_2-WT after washing (Figure 3b). For DI-MS in negative ion mode, two organic acids, malic acid and citric acid, were predominately detected and some fatty acids, e.g., palmitic acid and stearic acid, and sucrose were detected as minor components (Figure 3c). The identities of these compounds were confirmed by tandem mass spectrometry (Figure S-10). Similarly, analysis with unmodified and various surface-modified wooden tips without washing gave similar mass spectral results as DI-MS (Figure S-11). After washing with water, however, the two relatively hydrophobic fatty acids, palmitic acid and stearic acid, became the major components in the mass spectrum for the hydrophobic C_{18}-WT (Figure 3d), while the mass spectra obtained with other wooden tips were not significantly different from that of DI-MS.

The results shown above indicated that the interactions between analytes and solid-substrate surface were important factors affecting the analytical properties of solid-substrate ESI and the influences of these interactions were different for different sampling methods. In the direct loading method, the analyte-substrate surface interactions tended to retain analytes on the substrate and prevent them to spray out for detection. Therefore, selective and enhanced detection of target analytes in mixtures could be achieved with surfaces having relative weak interaction with the target analytes and relatively strong interactions with other components, such that the interested components could be effectively sprayed out for detection and interference from other components could be reduced. In the extractive sampling method, conversely, tip surface with chemical groups having strong interactions with target analytes tended to selectively enrich and enhance the detection of interested analytes, indicating the different roles of surface-analyte interactions in selective detection. The chemical property of elution solvent could also be a critical factor affecting the detection sensitivity and selectivity. Incorporating a washing step between sampling and addition of elution solvent was demonstrated to allow effective removal of loosely bound components and selective and enhanced detection of more strongly retained components.

Selective enrichment of target analytes in complex mixtures

Based on the insights obtained above, we applied surface-modified wooden tips to directly analyze cocaine, a commonly abused drug, in raw human oral fluids. A raw oral fluid sample (0.5 mL) spiked with 0.1 μg/mL of cocaine was extracted using unmodified-WT, C_{18}-WT, NH_2-WT, and SO_3H-WT, and then analysis by Q/TOF-MS after a washing step for removing the interfering matrix. When sample was analyzed by unmodified-WT, C_{18}-WT and NH_2-WT,

no significant signal for the target analyte could be obtained (Figure 4a-c), most probably due to the incapability of these wooden tips in extraction of the interested compound. However, when SO_3H-WT was applied for extractive sampling, predominate signal of cocaine was observed (Figure 4d). Cocaine is basic in nature (pK_a=8.61), therefore the wooden tip with acidic surface (e.g., SO_3H-WT) was adopted for extractive sampling, revealing that the acidic nature of the -SO_3H group enabled effective extraction of the basic analyte. When samples containing different concentrations of cocaine (1 – 200 µg/L) and a fix amount of the corresponding deuterium-labelled internal standard, i.e., cocaine-d3 (10 µg/L), were extracted with SO_3H-WT and then analyzed with a triple quadrupole mass spectrometer operating under multiple-reactions monitoring (MRM) mode, the signal intensity of cocaine (*m/z* 304→182) showed a linear relationship with the sample concentration and the ion signal intensity of cocaine-d_3 (*m/z* 307→185) were similar in various no obviously fluctuated in different samples (Figures 4e & 4f). The signal duration was continuing about 0.7 minutes (Figures 4e & 4f) and the first 0.5 min was recorded for establishing standard curve and quantitative detection due to the signal is the most stability at this duration (Figure S-12a). As showed in Figure 4g, a desirable linearity, R^2 = 0.9978, was obtained for three orders-of-magnitude of concentration range (1 – 200 µg/L) The limit-of-detection (LOD) (S/N = 3) and limit-of-quantitation (S/N = 10) were determined to be 0.01 ppb and 0.1 ppb, respectively. Moreover, the matrix effect for detection of cocaine in oral liquid samples also was investigated in this study. As shown in the Figure S-12b, the mass spectrum of raw oral liquid with spiked 100 µg/L of cocaine obtained by infusion ESI-MS showed that an abundance of matrices signals were detected and the signal of cocaine was seriously suppressed. In the extractive sampling of cocaine (100 µg/L) in different concentrations of matrices with SO_3H-WT, the quantitative results (Figure S-12c) showed that the cocaine signals were increased

with gradual dilution of matrices, and was reached the best signal in the pure water, revealed that the matrices could affect the extraction of analytes, probably that the properties of some matrices in oral fluid are similar to analytes. In addition, the reproducibility and recovery of cocaine when SO_3H-WT was used to concentrate it in the different concentration of cocaine (6, 30 and 150 ng/mL) also were investigated (Table S-1), showed that acceptable RSD and recovery were found to be at 5.3-16.5 % and 102.5 – 120.4 %, respectively. These resultes showed that desirable analytical performance could be achieved by selecting an appropriate surface material for extractive sampling.

Surface-modified wooden tips for on-surface sample pretreatment

The applicability of surface-modified wooden tips for on-surface cleanup of protein and peptide samples with high concentration of salts and detergents, commonly contaminants interfering ESI-MS analysis, was explored. Wooden tips modified with -C_{18} group, a commonly used hydrophobic material having stronger retention for proteins and peptides than salts and detergents, was applied here for sample pretreatment and the extractive sampling approach was applied. When a sample containing 40 μM lysozyme and 100 mM of NaCl was analysed with conventional ESI and unmodified-WT, the mass spectra showed only predominant ion signals for the salt clusters and no signal for the protein component (Figure 5a). When the sample was applied onto the C_{18}-WT, washed with water, and eluted with spraying solvent, the mass peaks of the protein components were clearly observed with much less interference from the salt clusters (Figure 5a). Compared to the C18-solid-phase extraction (SPE) (the experimental details are shown in the Supporting Information, the protein signal obtained by C_{18}-WT is slightly lower than C18-SPE (Figure S-13), probably

the C18-SPE (100 mg) has larger surface area than C_{18}-WT. Similar results were obtained for a sample containing a membrane peptide, gramicidin D and 2.5% (w/w) octyl β-D glucopyranoside detergent (OG). Only strong ion signals of the OG detergent and no signal for the interested peptide were observed for conventional ESI and unmodified-WT (Figure 5b). After sampling with C_{18}-WT and the cleanup process, the interested peptide could be predominated detected and the interfering signals from the detergent were much reduced (Figure 5b). When the other surface-modified wooden tips, i.e., NH_2-WT and SO_3H-WT, were applied, however, no significant ion signal for the interested protein or peptide was obtained, which is believed to be because that the proteins and peptides could not be effectively retained on these wooden tips and were lost during the washing step (Figures 5a & 5b). These data showed that surface modification with suitable functional groups could allow effective on-surface sample cleanup and enhanced detection of analytes in interfering matrix.

Conclusions

In this study, the analytical properties of solid-substrate ESI were investigated in details with the use of surface-modified wooden tips, having hydrophobic, acidic and basic properties, respectively. Direct loading and extractive sampling, with these surface-modified wooden tips were applied for investigation of the effects of surface properties on the mass spectral features The results showed that surface modification allowed selective and enhanced detection of analytes with both the direct loading and extractive sampling methods, yet the roles of surface-analyte interactions were different for the two sampling methods. Particularly for the extractive sampling methods, the detection selectivity could be further influenced by the

chemical property of the elution solvent and incorporation of a washing step after sampling. In addition, we demonstrated that selective and enhanced detection of target analytes in complex mixtures and effective on-surface sample cleanup could be readily achieved if appropriate surface modification materials were adopted. Overall, this study allowed understanding on the effects of surface-analyte interactions on the analytical features of solid-substrate ESI. Development of surface-modification strategies for further advanced applications will be carried out later on.

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Figure captions

Figure 1. ESI-MS analysis of a mixture of sinapine and tetrapeptide Gly-Gly-His-Ala in positive ion mode. (a) Chemical structures of sinapine and Gly-Gly-His-Ala. (b) A mass spectrum obtained with conventional ESI-MS. (c) & (d) Mass spectra obtained with various wooden tips using (c) direct loading and (d) extractive sampling methods respectively.

Figure 2. ESI-MS analysis of a mixture of alanine and aspartic acid in positive ion mode. (a) Chemical structures of alanine and aspartic acid. (b) A mass spectrum obtained with conventional ESI-MS. (c) & (d) Mass spectra obtained with various wooden tips using (c) direct loading and (d) extractive sampling methods respectively.

Figure 3. (a) & (b). Mass spectra obtained from DI-MS analysis of a potato sample in (a) positive and (b) negative ion mode. (c) & (d). Mass spectra obtained from ESI-MS analysis of potato by extractive sampling with different wooden tips involving the washing step in (c) positive and (d) negative ion modes.

Figure 4. Analysis of cocaine in raw oral fluid. (a)-(d) Extractive sampling with (a) unmodified-WT, (b) C_{18}-WT, (c) NH_2-WT and (d) SO_3H-WT. (e) & (f). SRM chromatograms of d_3-cocaine internal standard (10 ppb) and cocaine at different concentrations (1-200 ppb), respectively, obtained by extractive sampling with SO_3H-WT. (g) A calibration curve obtained for the data shown in (e) & (f).

Figure 5. ESI-MS analysis of solutions containing (a) 40 μM lysozyme with 100 mM NaCl and (b) 5 μM gramincidin D with 2.5 % (w/w) detergent with conventional ESI and extractive sampling with different wooden tips.

Figures

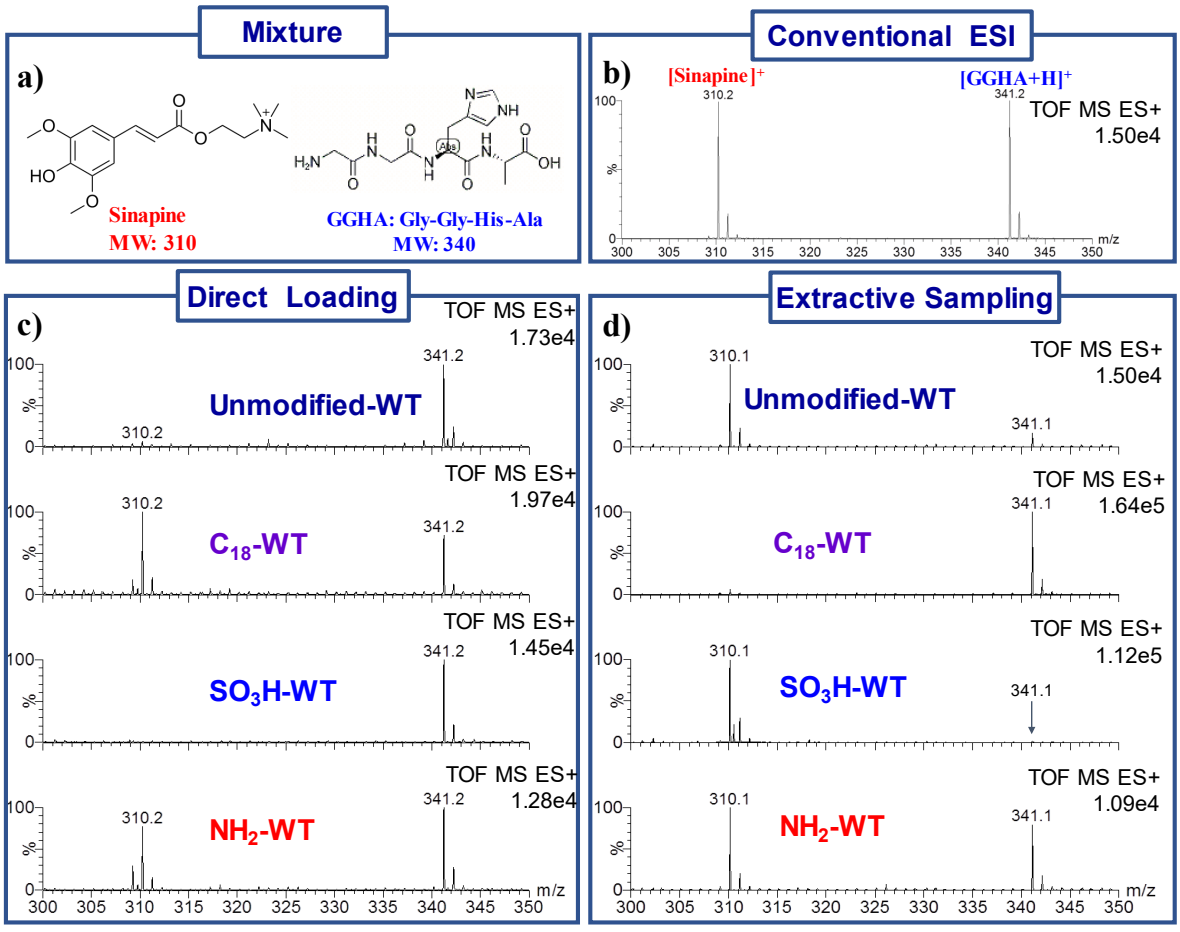


Figure 1

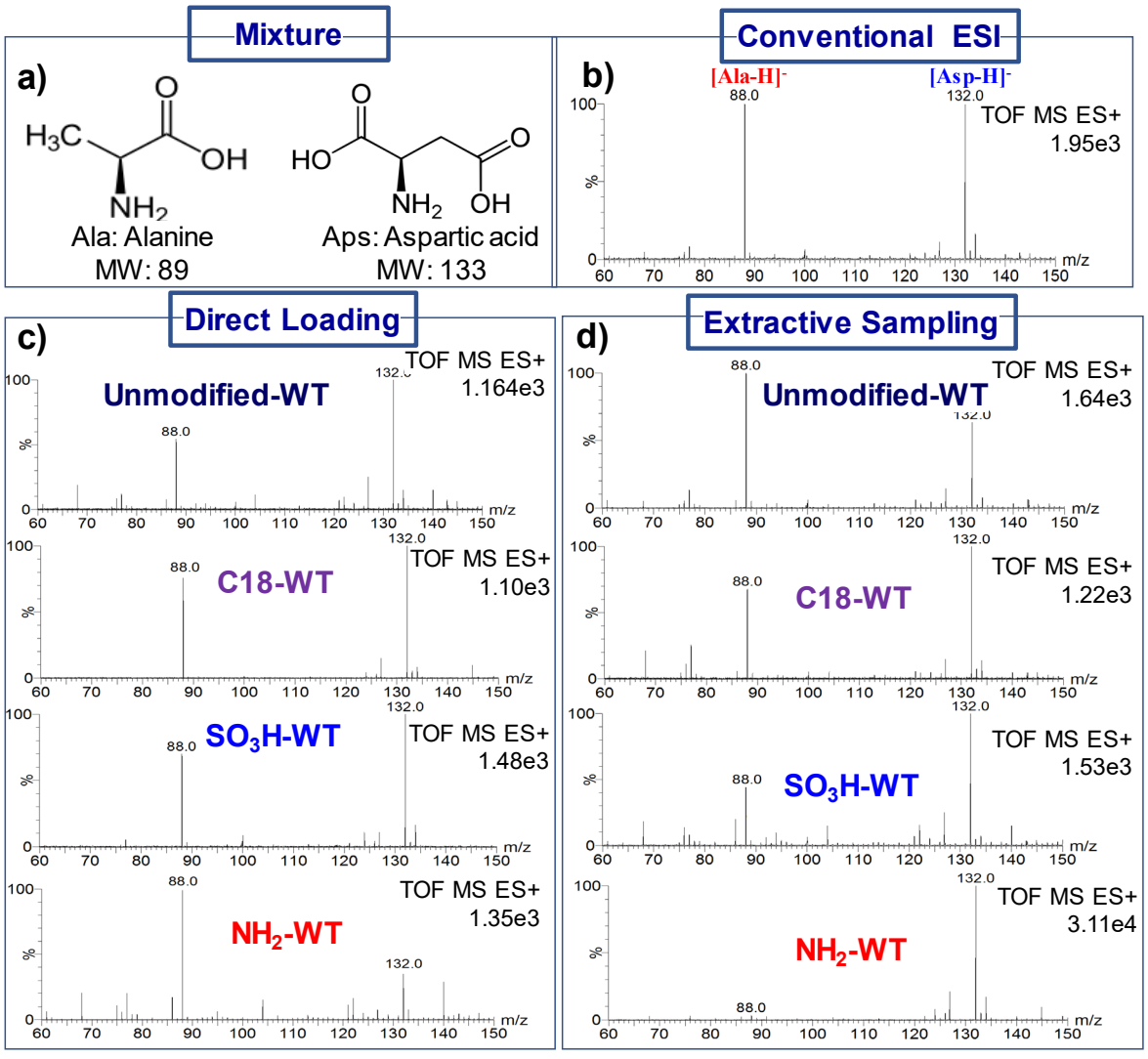

Mixture
a)
Ala: Alanine
MW: 89
Aps: Aspartic acid
MW: 133
Conventional ESI
b)
[Ala-H]-
[Asp-H]-
88.0
132.0
TOF MS ES+
1.95e3
Direct Loading
c)
Unmodified-WT
TOF MS ES+
1.164e3
C18-WT
TOF MS ES+
1.10e3
SO3H-WT
TOF MS ES+
1.48e3
NH2-WT
TOF MS ES+
1.35e3
Extractive Sampling
d)
Unmodified-WT
TOF MS ES+
1.64e3
C18-WT
TOF MS ES+
1.22e3
SO3H-WT
TOF MS ES+
1.53e3
NH2-WT
TOF MS ES+
3.11e4
m/z

Figure 2

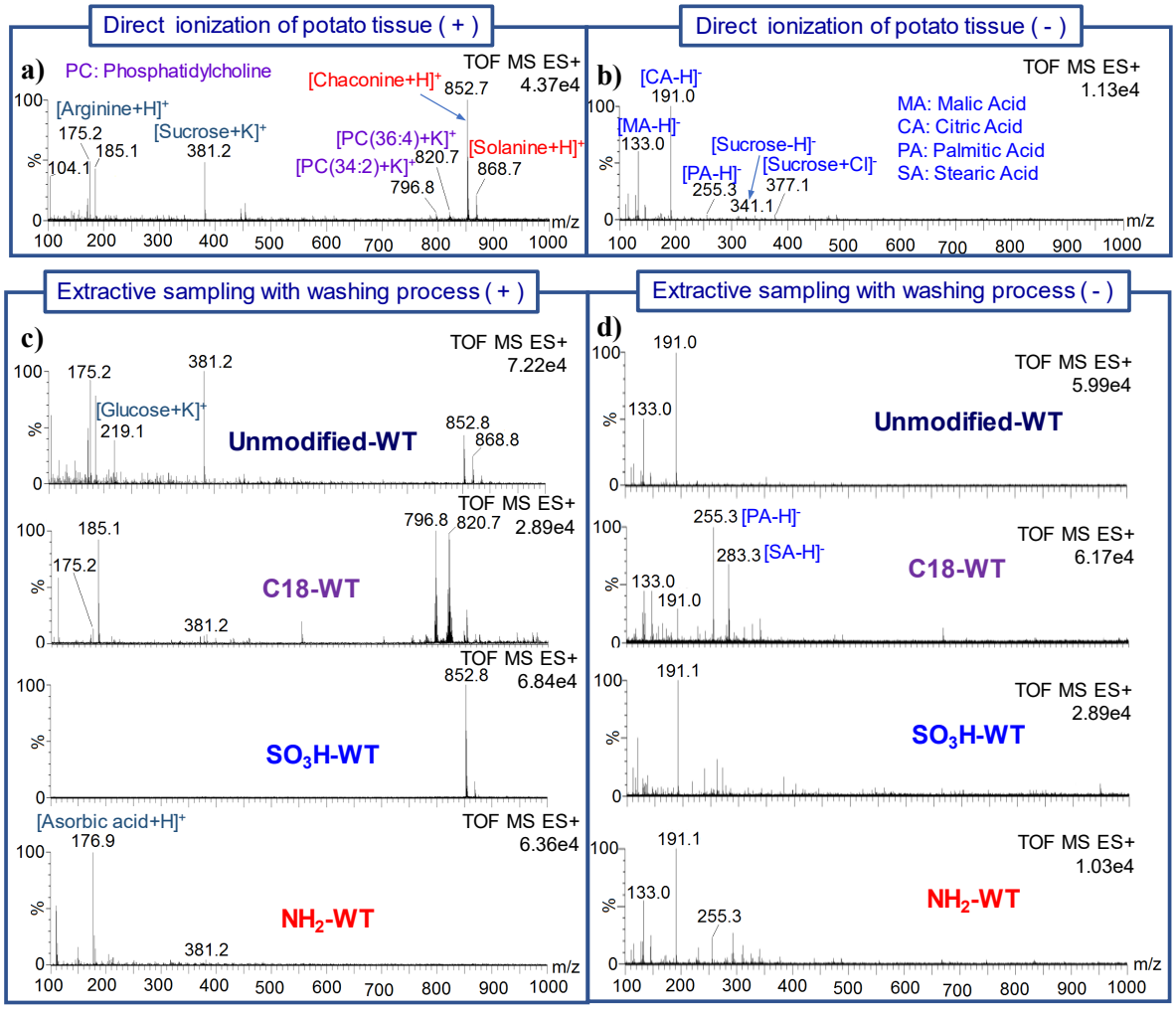

Direct ionization of potato tissue (+)
a)
PC: Phosphatidylcholine
[Arginine+H]+
175.2
185.1
104.1
[Sucrose+K]+
381.2
[Chaconine+H]+
852.7
[PC(36:4)+K]+
820.7
[PC(34:2)+K]+
796.8
[Solanine+H]+
868.7
TOF MS ES+
4.37e4
m/z
Direct ionization of potato tissue (-)
b)
[CA-H]-
191.0
[MA-H]-
133.0
[PA-H]-
255.3
[Sucrose-H]-
341.1
[Sucrose+Cl]-
377.1
MA: Malic Acid
CA: Citric Acid
PA: Palmitic Acid
SA: Stearic Acid
TOF MS ES+
1.13e4
m/z
Extractive sampling with washing process (+)
c)
175.2
381.2
[Glucose+K]+
219.1
Unmodified-WT
852.8
868.8
TOF MS ES+
7.22e4
185.1
175.2
C18-WT
796.8
820.7
381.2
TOF MS ES+
2.89e4
SO3H-WT
852.8
TOF MS ES+
6.84e4
[Asorbic acid+H]+
176.9
NH2-WT
381.2
TOF MS ES+
6.36e4
m/z
Extractive sampling with washing process (-)
d)
191.0
Unmodified-WT
133.0
TOF MS ES+
5.99e4
255.3 [PA-H]-
283.3 [SA-H]-
C18-WT
133.0
191.0
TOF MS ES+
6.17e4
191.1
SO3H-WT
TOF MS ES+
2.89e4
191.1
NH2-WT
133.0
255.3
TOF MS ES+
1.03e4
m/z

Figure 3

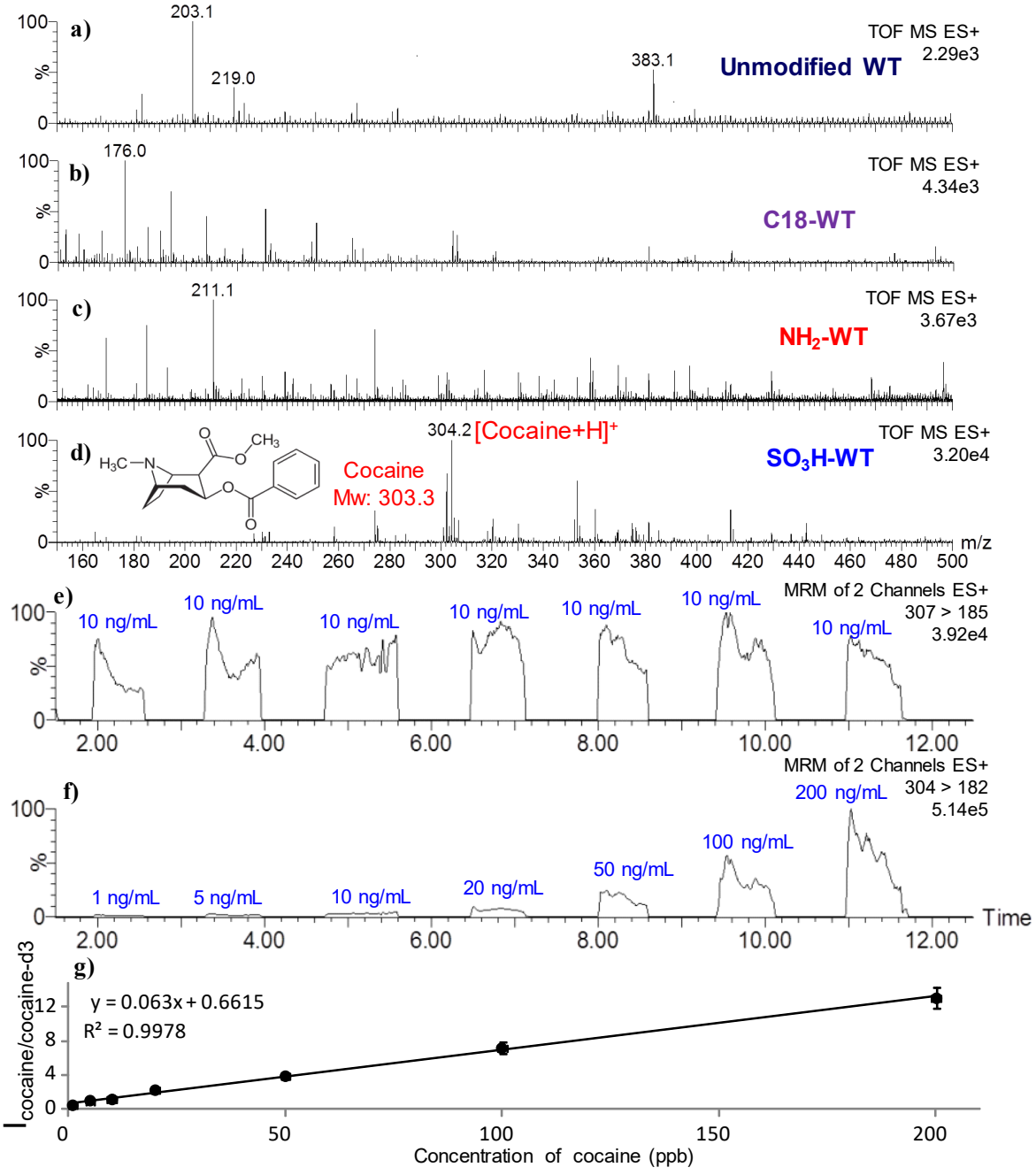

a)
203.1
219.0
383.1
Unmodified WT
TOF MS ES+
2.29e3
b)
176.0
C18-WT
TOF MS ES+
4.34e3
c)
211.1
NH2-WT
TOF MS ES+
3.67e3
d)
304.2 [Cocaine+H]+
Cocaine
Mw: 303.3
SO3H-WT
TOF MS ES+
3.20e4
m/z
e)
10 ng/mL
MRM of 2 Channels ES+
307 > 185
3.92e4
f)
1 ng/mL
5 ng/mL
10 ng/mL
20 ng/mL
50 ng/mL
100 ng/mL
200 ng/mL
MRM of 2 Channels ES+
304 > 182
5.14e5
Time
g)
y = 0.063x + 0.6615
R² = 0.9978
Icocaine/cocaine-d3
Concentration of cocaine (ppb)

Figure 4

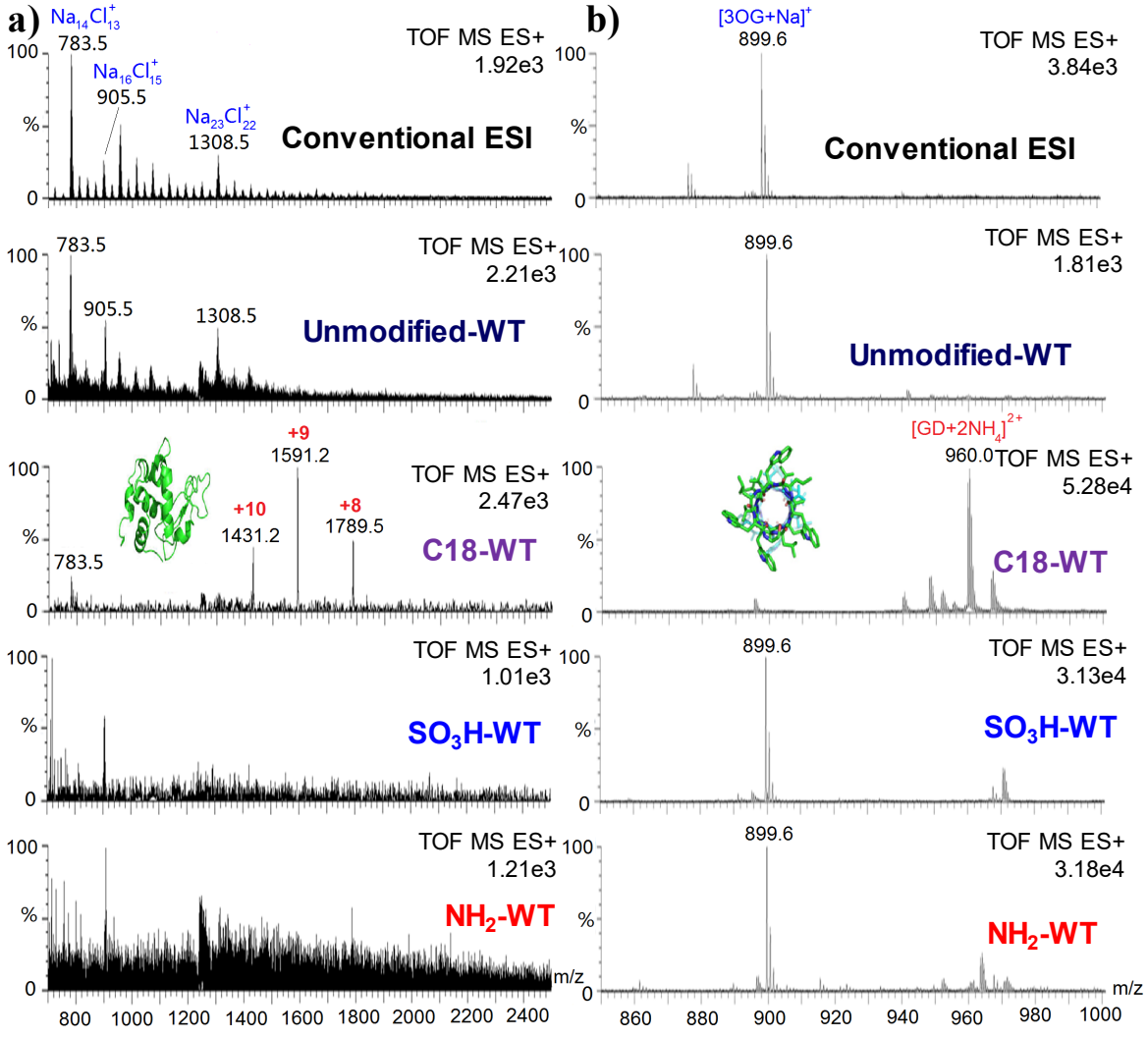
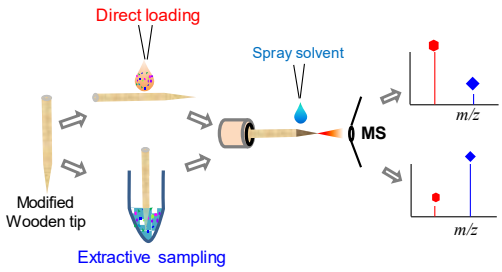

a)
$Na_{14}Cl_{13}^+$
783.5
$Na_{16}Cl_{15}^+$
905.5
$Na_{23}Cl_{22}^+$
1308.5
TOF MS ES+
1.92e3
Conventional ESI
783.5
905.5
1308.5
TOF MS ES+
2.21e3
Unmodified-WT
+9
1591.2
+10
1431.2
+8
1789.5
783.5
TOF MS ES+
2.47e3
C18-WT
TOF MS ES+
1.01e3
SO_3H-WT
TOF MS ES+
1.21e3
NH_2-WT
100
%
0
m/z
800 1000 1200 1400 1600 1800 2000 2200 2400
b)
$[3OG+Na]^+$
899.6
TOF MS ES+
3.84e3
Conventional ESI
899.6
TOF MS ES+
1.81e3
Unmodified-WT
$[GD+2NH_4]^{2+}$
960.0
TOF MS ES+
5.28e4
C18-WT
899.6
TOF MS ES+
3.13e4
SO_3H-WT
899.6
TOF MS ES+
3.18e4
NH_2-WT
100
%
0
m/z
860 880 900 920 940 960 980 1000

Figure 5

TOC Only


Direct loading
Spray solvent
MS
m/z
m/z
Modified
Wooden tip
Extractive sampling