

# Solvent-Assisted Electrospray Ionization for Direct Analysis of Various Compounds (Complex) from Low/Nonpolar Solvents and Eluents

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## S Supporting Information

**ABSTRACT:** Electrospray ionization (ESI) is a powerful ionization technique with a wide range of applications. However, the analytes in low/nonpolar solvents cannot be analyzed directly in electrospray ionization–mass spectrometry (ESI-MS), because low/nonpolar solvents are incompatible with ESI, because of their low conductivity. To circumvent this problem, we introduce an electrospray-based ionization method termed solvent-assisted electrospray ionization (SAESI). With the help of electrospray solvents at the tip of the spray needle, compounds in “non-electrospray ionization-friendly” solvents can be ionized directly using solvent-assisted electrospray ionization–mass spectrometry (SAESI-MS). The key features that the assistant solvent can be chosen flexibly and makes little interference to samples lead to better ionization performance in detection of organic reaction intermediates and real-time analysis of polymers and chiral drugs separated by gel permeation chromatography (GPC) and normal phase liquid chromatography (NPLC). Furthermore, it can achieve online hydrogen/deuterium (H/D) exchange reaction and even mitigate the signal suppression caused by strong acid modifiers in liquid chromatography. In addition, all parts of this device are commercially available and it only requires two parameters to be optimized, which makes SAESI easy to handle.



Molecular weights provide key information for the identification of organic compounds. As a tool of weighing molecules, mass spectrometry requires a proper ionization method as the first step. Electrospray ionization (ESI),<sup>1,2</sup> being a soft ionization technique, makes it possible for direct identification of organic reaction intermediates, which is of great importance for studying the reaction mechanisms.<sup>3–10</sup> Besides, it is ideally suited to be coupled with liquid separation modes for rapid online detection of routinely separated complex mixtures, because of its continuous-flow sampling mode.<sup>11,12</sup> Unfortunately, the choice of solvent is limited in ESI. Nonpolar or low polarity solvents such as dichloromethane or hexane, which are usually the optimal conditions for organic reactions and liquid separation such as normal phase liquid chromatography (NPLC) and gel permeation chromatography (GPC), is incompatible with ESI, because of their low conductivity. Thus, analytes in low/nonpolar solvents cannot be analyzed directly in electrospray ionization–mass spectrometry (ESI-MS). For successful analysis of low/nonpolar solvent systems, adding the polar solvents or ionic liquids directly or through a T-junction in the post column is a general method,<sup>13–16</sup> but it changes the conditions of prior experiments that may disturb the intermediate ions of reactions or compromise the separation efficiency. Recently, some new electrospray-based ionization methods, such as extractive electrospray ionization (EESI),<sup>17–19</sup> continuous flow–extractive desorption electrospray ionization (CF-EDESI),<sup>20,21</sup> and paper spray (PS),<sup>22,23</sup> were developed and reported for the

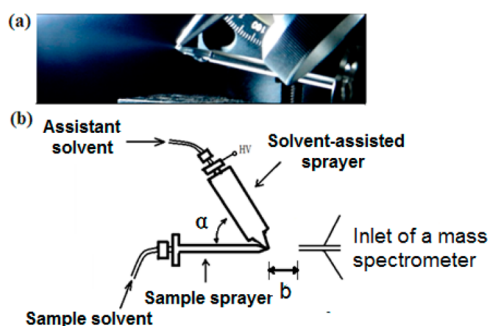
direct analysis of analytes from “non-ESI-friendly” solvents. In these ionization methods, sample introduction is independent of ionization optimization, so nonpolar solvent systems can be ionized in their original state. However, these methods still need complicated instruments or multiparameter adjustment.

Herein, we introduce an alternative electrospray-based ionization method termed solvent-assisted electrospray ionization (SAESI) that permits the ionization of analytes in low/nonpolar solvent directly. The instrumental image and schematic representation of SAESI apparatus are shown in Figure 1. SAESI contains two separated sprayers: a sample sprayer and a solvent-assisted sprayer with a high-voltage power supply. The dual sprayers are in contact at the tip and positioned at an angle with respect to each other. The zero distance between the tips of two sprayers enables the charged solvent and the sample solution to meet immediately at the tips of spray needles to form a stable spray plume. A polar solvent, assisting in ionization, is just mixed with the sample in the Taylor cone in a short time period, which makes little interference with their original state. The angle ( $\alpha$ ) between the two sprayers and the distance ( $b$ ) between the tip of sprayers and the inlet to the mass spectrometer are easily adjustable, to some extent (see Figure S1 in the Supporting Information). All parts of this device are commercially available,

Received: July 18, 2014

Accepted: August 19, 2014

Published: August 19, 2014



**Figure 1.** (a) Photographic image of SAESI apparatus. The stable sprayer plume is visible under strong illumination when using methanol as the assisting solvent and dichloromethane as the sample solvent. (b) Schematic representation of the SAESI. The angle ( $\alpha$ ) between the two sprayers is  $30^\circ$ – $75^\circ$  ( $45^\circ$  is the optimized angle) and the distance ( $b$ ) between the tip of sprayers and the inlet to the mass is 5–10 mm (6 mm is the optimized distance).

and it only requires two parameters to be optimized, which makes SAESI convenient and easy to handle. In previous literature, several techniques were reported to mix the analytes with reagents in the Taylor cone for altering the protein charge state distributions, online mass tagging of phosphopeptides, studying interfacial complexes, and so on.<sup>24–30</sup> All of these dual-channel devices were developed in electrospray microchips or in borosilicate theta glass capillaries, which are hardly achieved in common laboratory settings. In the present report, to the best of our knowledge, this is the first time commercial electrospray needles have been used as dual-channel sprayers for direct analysis of various low/nonpolar solvent systems.

## EXPERIMENTAL SECTION

**Chemicals and Materials.** Compound **1**,  $\text{Ph}_3\text{PAuNTf}_2$ , polystyrene (Mw 1300), benzoin, naringenin,  $\text{D}_2\text{O}$ , benzaldehyde, methyl acrylate, and DABCO were purchased from Sigma–Aldrich (Germany). Compounds **S**<sub>2</sub> and **S**<sub>3</sub> were synthesized and verified by nuclear magnetic resonance (NMR).<sup>31</sup> Dichloromethane, methanol, water, acetonitrile, tetrahydrofuran, hexane, isopropanol (all chromatography grades) were obtained from Merck (Darmstadt, Germany).

**Mass Spectrometric Experiments.** The SAESI-MS, EESI-MS, ESI-MS, and subsequent tandem mass spectroscopy (MS/MS) experiments were performed on a Finnigan TSQ Quantum Access triple-quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Nitrogen was used as the sheath gas and auxiliary gas, and argon was used as the collision gas. The basic ESI, EESI, SAESI conditions were as follows: vacuum,  $2.8 \times 10^{-6}$  Torr; spray voltage,  $\pm 3000$ – $3800$  V; capillary temperature,  $270^\circ\text{C}$ ; sheath gas pressure, 0–30 arbitrary units; and auxiliary gas pressure, 0–30 arbitrary units. The collision energy depended on the dissociation capability of the precursor ions. Data acquisition and analysis were carried out with the Xcalibur software package (Version 2.0, Thermo Fisher Scientific). The injection speed was 3–12  $\mu\text{L}/\text{min}$ . When using SAESI, the angle ( $\alpha$ ) between the two sprayers is  $30^\circ$ – $75^\circ$  and the distance ( $b$ ) between the tip of sprayers and the inlet to the mass is 5–10 mm.

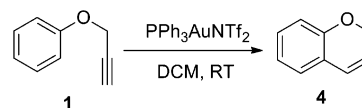
The GPC and NPLC separations in GPC-SAESI-MS and NPLC-SAESI-MS experiments were carried out in an Accela ultrahigh-performance liquid chromatography system (Thermo Fisher Scientific, Waltham, MA) with an analytical pump, an autosampler, an optional PDA detector, and a solvent platform.

The GPC separation was using a Agilent PL gel Organic GPC column (PL gel  $5\ \mu\text{m}$  MIXED-D,  $300 \times 7.5\ \text{mm}$ ). The eluent was tetrahydrofuran, and the flow rate was 1 mL/min. Postcolumn effluent splitting was achieved using a stainless steel T-junction and a fixed length of 0.13 mm-i.d. PEEK tubing that supplied a flow of  $\sim 50\ \mu\text{L}/\text{min}$  to the SAESI source. The NPLC separation was performed using a  $150\ \text{mm} \times 2\ \text{mm}$ ,  $5\ \mu\text{m}$  ChiralPak AD-H column with mobile-phase solvents of 8:2 hexane/IPA at a flow rate of 0.7 mL/min under isocratic conditions. Data acquisition and analysis were carried out with the Xcalibur software package (Version 2.0, Thermo Fisher Scientific).

## RESULTS AND DISCUSSION

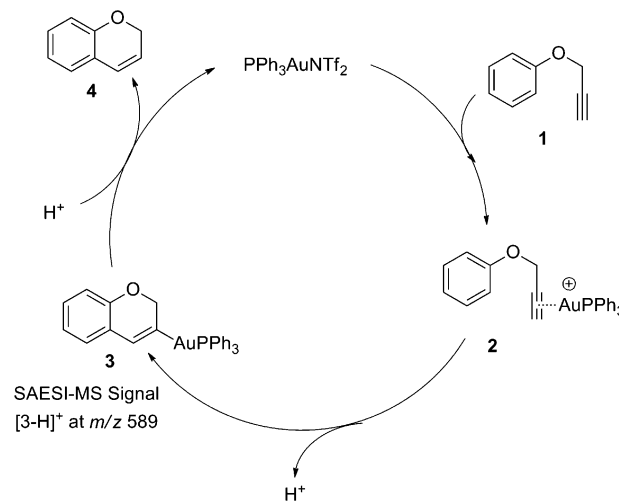
To demonstrate the advantages of SAESI in analysis of low/nonpolar systems, gold-catalyzed cycloisomerization reactions in  $\text{CH}_2\text{Cl}_2$  were analyzed. The cycloisomerization of aryl propargyl ethers into 2H-chromenes catalyzed by  $\text{Ph}_3\text{PAuNTf}_2$  (Scheme 1) is an efficient method to build the benzopyran

### Scheme 1. Cycloisomerization of Phenyl Propargyl Ether Catalyzed by $\text{Ph}_3\text{PAuNTf}_2$ in Dichloromethane at Room Temperature



moiety, which is characterized in many natural products.<sup>32</sup> A plausible mechanism of this reaction is shown in Scheme 2.<sup>33</sup>

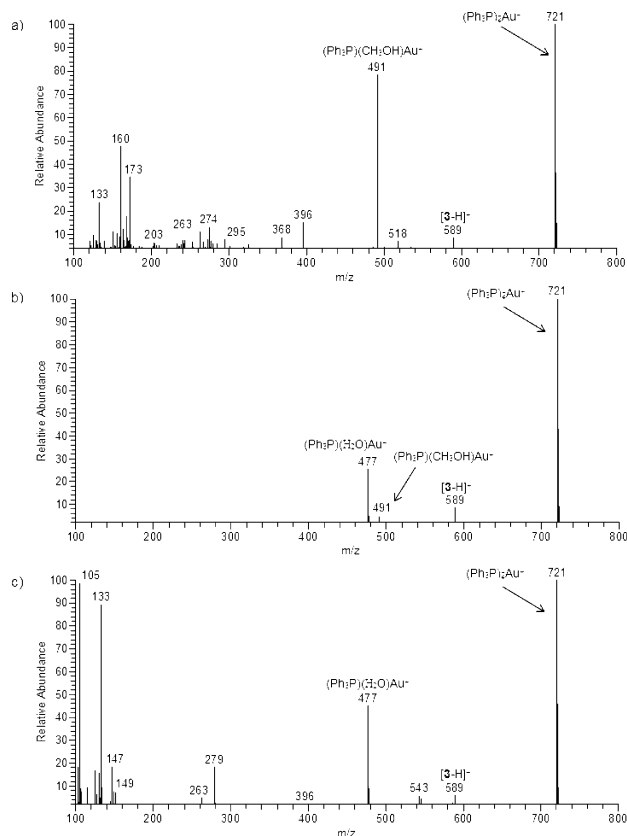
### Scheme 2. Proposed Mechanism of Cycloisomerization of Phenyl Propargyl Ether



Coordination of the gold catalyst to triple bond of **1** occurs to form the intermediate **2**. Then, **2** is cyclized through nucleophilic attack of the carbon on the aromatic ring. Subsequent protodeauration of intermediate **3** releases the gold catalyst back into the catalytic cycle. To the best of our knowledge, until now, the intermediates have not been identified by direct observation and characterization.

First, we studied this reaction using the classic method: ESI-MS. Because of the coordination to gold catalysts, polar solvents such as methanol and acetonitrile are not appropriate for this reaction.<sup>34</sup> Thus, dichloromethane, which is served as

the proper solvent for the reaction, cannot be replaced by methanol and acetonitrile. While dichloromethane is less amenable to ESI, the reaction solution should be diluted by methanol before being introduced into the ESI ion source. One millimole (1 mmol) phenyl propargyl ether and 0.01 mmol  $\text{Ph}_3\text{PAuNTf}_2$  were mixed in  $\text{CH}_2\text{Cl}_2$  for 5 min. Electrospray ionization of the reaction solution diluted with methanol in the positive mode led to the  $[3-\text{H}]^+$  cation at  $m/z$  589,  $(\text{Ph}_3\text{P})_2\text{Au}^+$  ( $m/z$  721), and  $(\text{Ph}_3\text{P})(\text{CH}_3\text{OH})\text{Au}^+$  ( $m/z$  491) (Figure 2a), which were further characterized by the collision-



**Figure 2.** (a) ESI-MS spectrum, (b) SAESI-MS spectrum, and (c) EESI-MS spectrum of the reaction solution of compound **1** and  $\text{PPh}_3\text{AuNTf}_2$  in  $\text{CH}_2\text{Cl}_2$ . The solution of the catalyst was prepared by dissolving 7.4 mg of  $\text{Ph}_3\text{PAuNTf}_2$  (0.01 mmol) in 0.5 mL of dichloromethane. The solution was mixed with 0.5 mL of 132.0 mg of **1** (1 mmol) in dichloromethane for 5 min. The reaction solution then was diluted by methanol for ESI-MS experiments or by dichloromethane for the EESI-MS and SAESI experiments. In the EESI-MS experiment, methanol was used as an ionizing solvent spray. The angle between the two sprayers was  $\sim 45^\circ$ , and the distance between the sprayers was  $\sim 2$  mm. The sprayers-to-inlet distance was  $\sim 6$  mm.

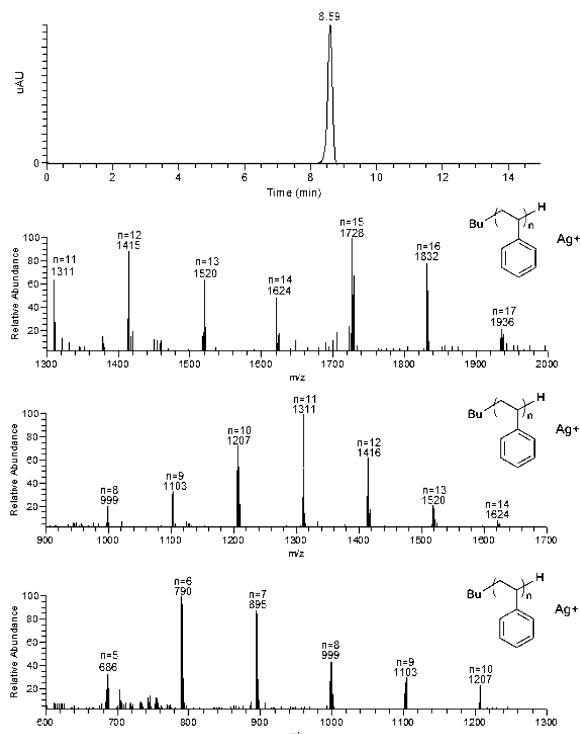
induced dissociation experiments (see Figure S2 in the Supporting Information). As the ion of  $m/z$  589 disappeared after 1 h of reaction (Figure S3 in the Supporting Information), it is reasoned that it is a reaction intermediate rather than a side product. The cyclized  $\sigma$ -gold intermediate **3** is neutral and easily loses a hydrogen anion to form a more stable ion  $[3-\text{H}]^+$  in the ESI condition. Other ions are solvent cluster ions ( $m/z$  133  $[\text{3CH}_3\text{OH}+2\text{H}_2\text{O}+\text{H}]^+$  (Figure 2a)<sup>35</sup> or related with the complex formed by the Au cation reacting with the residual impurities. It may complicate the reaction intermediate identification, especially when the reaction mechanism is unexplored.

In the SAESI-MS experiment, dilution with methanol was unnecessary to the reaction solution. For efficient ionization, methanol was introduced into solvent-assisted sprayer to mix with reaction solution in the Taylor cone. The angle ( $\alpha$ ) between two sprayers was adjusted between  $30^\circ$  and  $75^\circ$  (see Figure S4 in the Supporting Information). Upon sensitivity and stability, we chose  $45^\circ$  as an optimal angle. The distance  $b$  between the tip of sprayers and the inlet to the mass ( $5\text{--}10$  mm) was also optimized, and 6 mm is the best distance. Similar to that in ESI-MS, peaks corresponding to gold-catalysis adducts  $[\text{Au}(\text{PPh}_3)_2]^+$  cation at  $m/z$  721,  $[\text{Au}(\text{PPh}_3)(\text{H}_2\text{O})]^+$  cation at  $m/z$  477, and  $[\text{Au}(\text{PPh}_3)(\text{CH}_3\text{OH})]^+$  cation at  $m/z$  491, and an intermediate at  $m/z$  589, are observed clearly in the spectrum of SAESI-MS (Figure 2b). Strikingly, the spectrum shows no chemical noises or disturbed ions which were found in the spectrum of ESI-MS, simplifying the analysis of the spectrum. When three other polar solvents (acetonitrile, water, and acetone) were used as assistant solvents, a similar clean spectrum was obtained (see Figure S5 in the Supporting Information). All of these polar solvents can be used as assistant solvents to help the ionization of low-polar reaction solution likely by increasing the conductivity of the solution in the Taylor cone. Meanwhile, short-time scale mixture with polar solvent would not bring about unexpected ions, providing wide selection range of assistant solvent. Although it is not known clearly why the spectrum of SAESI-MS is so clean with little disturbed ions, avoiding dilution with methanol is presumably the most critical point to decrease the appearance of unknown ions (see Figure S6 in the Supporting Information).<sup>35–37</sup> This gold-catalyzed reaction was also detected by EESI-MS when using methanol as an electrospray solvent (Figure 2c). However, other than the ion related to the intermediate, there is chemical noise, especially in the low mass area, which is suspected to be caused by impurity compounds in background or some complex ion–molecule reactions. In addition, precise adjustment of the distance between two sprayers makes it difficult to operate. By comparison, SAESI can avoid unexpected ion–molecule reactions and has fewer parameters to be adjusted. Upon these advantages, two other Au-catalyzed reactions in  $\text{CH}_2\text{Cl}_2$  were also detected successfully by SAESI-MS, validating the intermediate  $[3-\text{H}]^+$  (see Figures S7 and S8 in the Supporting Information). Furthermore, since the assistant solvent makes little interference to the reaction solution in SAESI, some less-polar reaction participants, such as the product in the Morita–Baylis–Hillman (MBH) reaction that is hardly ionized by ESI-MS, can also be detected by SAESI-MS by adding traces of acid and salt in the assistant solvent (see Figure S9 in the Supporting Information).<sup>38–40</sup> The easy handling and clear spectrum character make SAESI-MS a potential candidate to be used for the online real-time monitoring of key intermediates and less-polar species of chemical reactions encountered in the low/nonpolar solvent.

Beyond detecting the intermediates of reactions, SAESI-MS is also applicable to be coupled with separation methods such as GPC and NPC for real-time analysis of low/nonpolar eluents separated online. One example is the detection of polystyrene (Mw 1300) by GPC-UV/SAESI-MS. Being nonpolar and nonfunctional, polystyrene is hardly ionized by ESI-MS. The use of silver(I) tetrafluoroborate as a doping salt to facilitate efficient ionization of polystyrene has been reported.<sup>41</sup> However, it was difficult to combine this method with GPC, because adding Ag salt in the mobile phase is destructive to the GPC column. Herein, using GPC-UV/SAESI-MS,  $\text{CH}_3\text{OH}$



containing 0.2 mg/mL  $\text{AgBF}_4$  dopant served as an assistant solvent just mixing with separated polystyrene from GPC eluent (THF) only in the Taylor cone to realize effective ionization with little disturbance to GPC separation. Figure 3

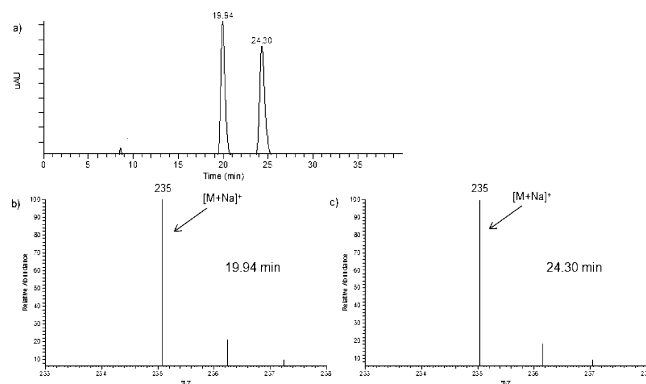


**Figure 3.** GPC-UV/SAESI-MS of PS ( $M_w$  1300) (0.5 mg/mL): (a) UV chromatogram, (b) SAESI-MS spectrum taken at 8.34 min, (c) SAESI-MS spectrum taken at 8.59 min, and (d) SAESI-MS spectrum taken at 8.75 min. The PDA detector is connected with SAESI-MS in series. The effluent was split after the PDA detector such that 50  $\mu\text{L}/\text{min}$  of GPC effluent was directed to the SAESI-MS ion source. Methanol containing 0.2 mg/mL  $\text{AgBF}_4$  dopant was injected into solvent-assisted sprayer by syringe pump at 50  $\mu\text{L}/\text{min}$ . The angle between the two sprayers was  $\sim 45^\circ$ .

illustrates the UV chromatogram and three representative mass spectra at different points in eluent time of polystyrene from online GPC-UV/SAESI-MS analysis. Ions are shown as  $[\text{M} + \text{Ag}]^+$  and few fragmentations are observed. As expected, lower and lower molecular weights of polystyrene ( $M_w$  1300) are detected as eluent time increases, which is in accordance with the GPC separation principle. The coupling of GPC and SAESI-MS is time-saving for characterization of the polymer and has a potential for determination of chemical composition and calculation of molecular weight averages of more-complicated polymers with a broad molecular distribution which are hardly characterized by MS or GPC independently.<sup>42,43</sup>

Another example is the analysis of chiral drugs by NPLC-UV/SAESI-MS. For more-efficient chiral separation, normal phase LC more frequently uses nonpolar solvents such as hexane as a mobile phase.<sup>44</sup> The incompatibility of the mobile phase with ESI-MS restricts the combination of NPLC with ESI. For coupling NPLC to MS, SAESI can be used as a good interface. To verify the feasibility of this method, racemic benzoin and naringenin dissolved in solvents (the hexane:*i*-PrOH ratio is 8:2, which is the same as the mobile phase in NPLC) were detected by SAESI-MS. When  $\text{CH}_3\text{OH}$  was used

as an assistant solvent,  $[\text{M} + \text{Na}]^+$  of benzoin in positive ionization mode and  $[\text{M} - \text{H}]^-$  of naringenin in negative ionization mode are obtained (see Figure S10 in the Supporting Information). When using water as the assistant solvent, the ion signal intensity increases by a factor of  $\sim 10$  (see Figure S11 in the Supporting Information). Although water is immiscible with hexane, the signal is relatively stable. Then, benzoin was used as a model to achieve the coupling of NPLC and MS. Here, we used UV analysis and MS as a double detector, because UV methods are more accurate for quantitative analysis and MS methods are more readily for qualitative analysis. Figure 4 shows the NPLC-UV/SAESI-MS data for racemic

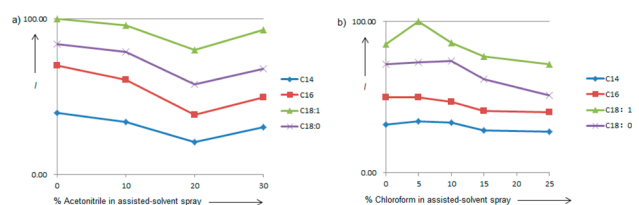


**Figure 4.** NPLC/UV/SAESI-MS of benzoin: (a) UV chromatogram, (b) SAESI-MS taken at 19.94 min, and (c) SAESI-MS taken at 24.30 min. The PDA detector is connected with SAESI-MS in series. The effluent was split after the PDA detector such that 50  $\mu\text{L}/\text{min}$  of NPLC effluent was directed to the SAESI-MS ion source. Methanol was injected into a solvent-assisted sprayer by a syringe pump at a rate of 50  $\mu\text{L}/\text{min}$ . The angle between the two sprayers was  $\sim 45^\circ$ .

benzoin with UV output (Figure 4a) and two representative mass spectra (Figures 4b and 4c) at different eluent times. When racemic benzoin was separated and flowed out of NPLC, it can be characterized directly by MS online. In addition, using  $\text{D}_2\text{O}$  as an assistant solvent, SAESI-MS can determine the presence and number of exchangeable hydrogen(s) through the H/D exchange reaction<sup>45</sup> in the Taylor cone (see Figure S12 in the Supporting Information). NPLC-UV/SAESI-MS provides a powerful tool for enantioselective qualitative and quantitative analysis of chiral drugs. Meanwhile, combining with online H/D exchange, it also has the potential to facilitate structural elucidation of drugs.

Although SAESI is reminiscent of EESI, there are significant differences between them. Since there is zero distance between the two sprayers, the time and position of mixing of solutions in SAESI are different from that in EESI. To seek information on the ionization mechanism preliminarily, experiments that used by Zenobi and co-workers to study the mechanism of EESI<sup>46</sup> were carried out here. Four fatty acids (1  $\mu\text{g}/\text{mL}$ ) were dissolved in methanol and the assistant solvent contained acetonitrile or chloroform ranging from 0 to 30%, respectively, for investigation using SAESI with negative ionization mode. In EESI, the ion abundance of fatty acids decreased with increased ACN in ESI spray, because fatty acids have poor solubility in ACN.<sup>46</sup> With increased percentage of chloroform in ESI spray, the opposite result was obtained, because fatty acids have good solubility in chloroform.<sup>46</sup> By contrast, in SAESI, the percentage of ACN or chloroform in the assistant solvent does not have much effect on the signal of fatty acids (Figure

S), indicating that extraction is not the main process in SAESI. Considering the conductivity of acetonitrile and chloroform,



**Figure 5.** Effect of (a) ACN concentration and (b) chloroform concentration in the assistant solvent on the signals of fatty acids in SAESI-MS. Fatty acids (1  $\mu\text{g/mL}$ ) were dissolved in methanol.

both extraction efficiency and conductivity of assistant solvent are supposed to have an effect on ionization efficiency. The SAESI is also believed to have some similarities with fused-droplet ESI (FD-ESI).<sup>47</sup> FD-ESI is also a two-step electrospray ionization method, relying on the interaction between charged particles generated by ESI and aerosol particles carrying the sample.<sup>48,49</sup> In FD-ESI, the sample solution is first nebulized to form aerosols before being fused with the charged droplets, which is more similar to EESI.<sup>47</sup> By contrast, the ionization process in SAESI occurs in the Taylor cone before nebulization of the sample solution, which indicates that the ionization process is different from that of FD-ESI.

## CONCLUSIONS

In summary, SAESI-MS provides a desirable method for direct analysis of various mixtures in low/nonpolar solvents, including the detection of intermediates of organic reactions, as well as online analysis of polymers in GPC eluents and chiral drugs separated by NPLC. The solvent assisting in ionization mixed with the sample in the tip in a short time period, minimizing the interference with original reactions from the assisting solvent and being a satisfying feature for online-reaction monitoring. Meanwhile, it is an outstanding interface between separated techniques and MS as SAESI offers good tolerance to nonpolar eluents. The selection of assistant solvents can be flexible to improve ionization efficiency, decrease ion suppression effect (see Figure S13 in the Supporting Information), and even perform online H/D exchange, indicating that it has a great potential to couple with more-separated techniques that are incompatible with ESI. Also, it is easily implemented, since few parameters need to be adjusted. Further experiments to obtain a better understanding of the ionization process are underway.

## 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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### Author Contributions

The manuscript was written through contributions of all authors.

### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

We thank the National Science Foundation of China (Grant Nos. 21275155 and 2117225).

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