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Extractive Electrospray Ionization Mass Spectrometry toward in Situ Analysis without Sample Pretreatment

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A homemade novel nanoextractive electrospray ionization (nanoEESI) source has been characterized for in situ mass spectrometric analysis of ambient samples without sample pretreatment. The primary ions generated using a nanospray emitter interact with the neutral sample plume created by manually nebulizing liquid samples, allowing production of the analyte ions in the spatial cross section of the nanoEESI source. The performance of nanoEESI is experimentally investigated by coupling the nanoEESI source to a commercial LTQ mass spectrometer for rapid analysis of various ambient samples using positive/negative ion detection modes. Compounds of interest in actual samples such as aerosol drug preparations, beverages, milk suspensions, farmland water, and groundwater were unambiguously detected using tandem nanoEESI ion trap mass spectrometry. The limit of detection was low picogram per milliliter levels for the compounds tested. Acceptable relative standard deviation (RSD) values (5−10%) were obtained for direct measurement of analytes in complex matrixes, providing linear dynamic signal responses using manual sample introduction. A single sample analysis was completed within 1.2 s. Requiring no sheath gas for either primary ion production or neutral sample introduction, the nanoEESI has advantages including readiness for miniaturization and integration, simple maintenance, easy operation, and low cost. The experimental data demonstrate that the nanoEESI is a promising tool for high-throughput, sensitive, quantitative, in situ analysis of ambient complex samples, showing potential applications for in situ analysis in multiple disciplines including but not limited to pharmaceutical analysis, food quality control, pesticides residue detection, and homeland security.

Fast, reliable, sensitive detection of trace amounts of compounds such as explosives, environmental toxins, and chemical and biological warfare agents are of increasing importance. Compared with lab analysis of field samples, in situ analysis allows early warning of food safety or spot harmful release, saving time and resources, promoting public safety and homeland security, and avoiding sample contamination during the sample transportation. Nowadays, in situ analysis is an accelerated trend in analytical chemistry, especially for pharmaceutical products screening, food analysis, environment monitoring, and homeland security.

To date, several techniques including laser induced fluorescence,[1,2] laser-induced phosphorescence,[3] surface-enhanced Raman spectroscopy,[4] electrochemical sensor,[5] and portable photometer[6,7] are available for in situ analysis. Mass spectrometry is a promising tool for in situ analysis, due to its high throughput, high sensitivity, and high specificity. Several miniature mass analyzers[8] such as the ion trap,[9−11] quadrupole,[12] and time-of-flight[13] have been developed for in situ analysis. Alternatively, reducing or removing the sample preparation step before the analysis of samples with complex matrixes is required for fast, in situ analysis.

Ambient ionization methods are of great interest for in situ analysis because the analytes can be easily ionized and introduced to the mass spectrometer under ambient conditions with reducing or removing the sample pretreatment process. Direct ambient ionization was pioneered by desorption electrospray ionization (DESI),[14−18] which allows the determination of compounds directly at ambient conditions, removing solvent extraction or

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(1) Hottle, J. R.; Huisman, A. J.; DiGangi, J. P.; Kammrath, A.; Galloway, M. M.; Coens, K. L.; Keutsch, F. N. *Environ. Sci. Technol.* **2009**, *43*, 790–795.
(2) Thornton, J. A.; Wooldridge, P. J.; Cohen, R. C. *Anal. Chem.* **2000**, *72*, 528–539.
(3) Huisman, A. J.; Hottle, J. R.; Coens, K. L.; DiGangi, J. P.; Galloway, M. M.; Kammrath, A.; Keutsch, F. N. *Anal. Chem.* **2008**, *80*, 5884–5891.
(4) Brosseau, C. L.; Gambardella, A.; Casadio, F.; Grzywacz, C. M.; Van Duyne, J. R. P. *Anal. Chem.* **2009**, *81*, 3056–3062.
(5) Zhang, R. H.; Hu, S. M.; Zhang, X. T.; Wang, Y. *Anal. Chem.* **2008**, *80*, 8807–8813.
(6) Yang, R.; Chen, H.; Zhang, H.; Yu, A.; Jin, Q. *Chem. Res. Chin. Univ.* **2002**, *23*, 1501–1503.
(7) Chen, H.; Xu, S.; Yu, A.; Jin, Q. *Chem. Res. Chin. Univ.* **2002**, *23*, 1873–1876.
(8) Badman, E. R.; Cooks, R. G. *J. Mass Spectrom.* **2000**, *35*, 659–671.
(9) Li, M.; Fei, Q.; Chen, H.; Jin, W.; Jiang, J.; Jin, Q.; Zhou, J.; Xu, X. *Chin. J. Sci. Instrum.* **2007**, *28*, 1147–1152.
(10) Badman, E. R.; Cooks, R. G. *Anal. Chem.* **2000**, *72*, 3291–3297.
(11) Tabert, A. M.; Griep-Raming, J.; Guymon, A. J.; Cooks, R. G. *Anal. Chem.* **2003**, *75*, 5656–5664.
(12) Holkeboer, D. H.; Karandy, T. L.; Currier, F. C. *J. Vac. Sci. Technol., A* **1998**, *16*, 1157–1162.
(13) Hughes, P. G.; Votava, O.; West, M. B. A.; Zhang, F.; Kable, S. H. *Anal. Chem.* **2005**, *77*, 4448–4452.
(14) Takats, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. *Science* **2004**, *306*, 471–473.
(15) Chen, H.; Talaty, N.; Takats, Z.; Cooks, R. G. *Anal. Chem.* **2005**, *77*, 6915–6927.
(16) Chen, H.; Li, M.; Zhou, J. G.; Fei, Q.; Jiang, J.; Jin, Q. H.; Zhang, T. M.; Zhang, X. *Chem. J. Chin. Univ. Chin.* **2006**, *27*, 1439–1442.
(17) Wiseman, J. M.; Ifa, D. R.; Venter, A.; Cooks, R. G. *Nat. Protoc.* **2008**, *3*, 517–524.
(18) Zhen, X.; Bo, Q.; Lin, G. X.; Lai, C. F.; Hai, L. *Prog. Chem. Chin.* **2008**, *20*, 594–601.

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other sample preparation steps such as chromatography, solid phase microextraction prior to the analysis. DESI is one of the ambient ionization methods with the advantage that the ionization process occurs under ambient conditions in which the samples are easily accessible during the analysis process. Surface desorption atmospheric pressure chemical ionization (DAPCI) was initially developed to probe the mechanism of DESI using toluene for the generation of primary ions.[19] Recently, a new design of the DAPCI source with a corona discharge source has been built up, and even the ambient air can be directly used as a chemical reagent for the generation of primary ions.[20] Extractive electrospray ionization (EESI)[21−28] has been initially developed to analyze liquid and gas samples, as a useful tool for real time, in situ analysis with high sensitivity and specificity. In the EESI method, the compounds of interest are extracted from the solution or gas sample and then ionized by the primary ions produced by electrospray. Alternatively, many techniques including direct analysis in real time (DART),[29−31] low-temperature plasma (LTP),[32,33] electrospray assisted laser desorption/ionization (ELDI),[34−36] and easy ambient sonic ionization (EASI)[37−39] have been reported for rapid analysis of ambient, complex samples with minimal or no sample pretreatment.

In this work, a concept of nanoextractive electrospray ionization (nanoEESI) mass spectrometry has been illustrated with merits for rapid, in situ analysis with high sensitivity, specificity, and throughput. Herein, a nanospray emitter is used to generate the primary ions, so the sheath gas is avoided and then the number of parameters needed to be optimized during the analysis process is reduced. A simple, disposable, manual sample injector is used to introduce the samples, which gives the additional advantage that contamination and memory effects are significantly decreased. This new source could be implemented to a portable mass spectrometer for in situ analysis because it works without sheath gas or discharge gas and the instrument is simplified, and so is the operation process.

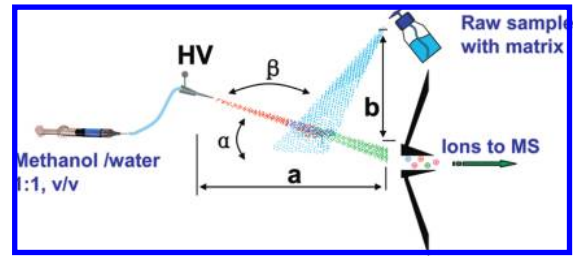


Figure 1. Schematic diagram of the nanoEESI source. The schematic is not proportionally scaled.

EXPERIMENTAL SECTION

Methanol (A.R. grade) used was bought from Chinese Chemical Reagent Co. Ltd. (Shanghai, China), and the water used was deionized. The samples such as sulbutamol aerosol, terbutaline aerosol, milk, Red Bull beverage, paraquat, and β-cypermethrin were bought in local super markets; cyclotrimethylenetrinitramine (RDX) was bought from Accu Standard, Inc. (New Haven, CT), and 2,4,6-trinitrotoluene (TNT) was purchased from Chem. Service, Inc. (West Chester, PA). All the chemicals were directly used without further treatment.

All the experiments were carried out using a Thermo Finnigan LTQ-XL mass spectrometer (San Jose, CA) coupled with a homemade nanoEESI source. The principle and structure of nanoEESI is shown in Figure 1. The novel nanoEESI is composed of a nanospray emitter used for generating the primary ions and a disposable manual sample injector for introducing the neutral sample. The nanospray emitter was composed using a commercial silica capillary nanoemitter with the inner diameter of 5 ± 1 μm (Pico Tip). The nanospray emitter was placed 4 cm (a) far from the inlet of the mass spectrometer. The aerosol generated using the manual sample injector was introduced to the nanoEESI source through a Teflon tube (i.d., 1 cm), which was appropriately fixed to the nanoEESI source, allowing reproducible sample introduction. The distance (b) between the manual sample injector and the inlet of the mass spectrometer was optimized and chosen as 8 cm because a short distance results in bigger droplets intersected with primary ions, while a longer distance leads to a wider sample plume distribution. A sensitivity loss is expected in both cases. The angle (α) formed between the nanospray emitter and the inlet of the mass spectrometer was 30°, and the angle (β) formed between the nanospray emitter and the sample injector was 135°. The contamination of the inlet capillary of the mass spectrometer was reduced by spraying the samples toward the opposite direction of the inlet of the mass spectrometer. A high voltage of ±5 kV was supplied to the nanospray emitter for either the positive ion detection mode or the negative ion detection mode. A methanol and water mixture solution (1:1, v/v) was delivered to the nanoelectrospray emitter by a syringe pump at a flow rate of 0.05−0.10 μL/min to generate the primary ions. The samples were loaded into a disposable manual sample injector,

(19) Takats, Z.; Cotte-Rodriguez, I.; Talaty, N.; Chen, H.; Cooks, R. G. *Chem. Commun.* **2005**, 1950–1952.
(20) Chen, H. W.; Zheng, J.; Zhang, X.; Luo, M. B.; Wang, Z. C.; Qiao, X. L. *J. Mass Spectrom.* **2007**, *42*, 1045–1056.
(21) Chen, H.; Zenobi, R. *Nat. Protoc.* **2008**, *3*, 1467–1476.
(22) Chen, H. W.; Venter, A.; Cooks, R. G. *Chem. Commun.* **2006**, 2042–2044.
(23) Chen, H. W.; Wortmann, A.; Zhang, W. H.; Zenobi, R. *Angew. Chem., Int. Ed.* **2007**, *46*, 580–583.
(24) Chen, H.; Yang, S.; Wortmann, A.; Zenobi, R. *Angew. Chem., Int. Ed.* **2007**, *119*, 735–738.
(25) Chen, H. W.; Sun, Y. P.; Wortmann, A.; Gu, H. W.; Zenobi, R. *Anal. Chem.* **2007**, *79*, 1447–1455.
(26) Zhou, Z.; Jin, M.; Ding, J.; Zhou, Y.; Zheng, J.; Chen, H. *Metabolomics* **2007**, *3*, 101–104.
(27) Chen, H.; Hu, B.; Hu, Y.; Huan, Y. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 719–722.
(28) Chen, H.; Hu, B.; Hu, Y.; Huan, Y.; Zhou, Z.; Qiao, X. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 719–722.
(29) Cody, R. B.; Laramée, J. A.; Durst, H. D. *Anal. Chem.* **2005**, *77*, 2297–2302.
(30) Cody, R. B. *Anal. Chem.* **2009**, *81*, 1101–1107.
(31) Kpegba, K.; Spadaro, T.; Cody, R. B.; Nesnas, N.; Olson, J. A. *Anal. Chem.* **2007**, *79*, 5479–5483.
(32) Harper, J. D.; Charipar, N. A.; Mulligan, C. C.; Cooks, R. G.; Ouyang, Z. *Anal. Chem.* **2008**, *80*, 9097–9104.
(33) Zhang, Y.; Ma, X. X.; Zhang, S. C.; Yang, C. D.; Ouyang, Z.; Zhang, X. R. *Analyst* **2009**, *134*, 176–181.
(34) Cheng, C. Y.; Yuan, C. H.; Cheng, S. C.; Huang, M. Z.; Chang, H. C.; Cheng, T. L.; Yeh, C. S.; Shiea, J. *Anal. Chem.* **2008**, *80*, 7699–7705.
(35) Peng, I. X.; Loo, R. R. O.; Shiea, J.; Loo, J. A. *Anal. Chem.* **2008**, *80*, 6995–7003.
(36) Shiea, J.; Yuan, C. H.; Huang, M. Z.; Cheng, S. C.; Ma, Y. L.; Tseng, W. L.; Chang, H. C.; Hung, W. C. *Anal. Chem.* **2008**, *80*, 4845–4852.
(37) Haddad, R.; Catharino, R. R.; Marques, L. A.; Eberlin, M. N. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 3662–3666.
(38) Haddad, R.; Milagre, H. M. S.; Catharino, R. R.; Eberlin, M. N. *Anal. Chem.* **2008**, *80*, 2744–2750.
(39) Haddad, R.; Sparrapan, R.; Kotiaho, T.; Eberlin, M. N. *Anal. Chem.* **2008**, *80*, 898–903.

through which 0.1 mL sample for each time was nebulized toward the charged plume by squeezing the trigger. The sample aerosol lasts about 1.5 s in the nanoEESI source. The analytes in the samples with complex matrixes were ionized directly when the neutral sample plume was intersecting the primary ion beam generated by the nanospray emitter; the analyte ions were then introduced to the mass spectrometer by the forces created by the electric field and the vacuum of the LTQ instrument. The temperature of the inlet capillary was maintained at 180 °C during the analysis process. The ion injection time was set to 100 ms for the LTQ-MS instrument. To perform collision-induced dissociation (CID), ions of interest were isolated, using an m/z window width of 1 unit. Helium was used as the collision gas, and the CID energy was 10−30% with a duration time of 30 ms for tandem mass spectrometry. The other parameters used in the experiment were the default values of the mass spectrometer. All the mass spectra were obtained using the Xcalibur software of the LTQ instrument.

RESULTS AND DISCUSSION

Characterization of nanoEESI. To characterize the new ionization source, the analysis of L-arginine with a concentration of 1.0×10^{-9} g/mL in wastewater was performed using nanoEESI in the positive ion detection mode. As a result, the mass spectrum with good signal-to-noise (S/N = 300) was rapidly recorded (Figure 2a) without cleaning up the matrix. Among the many signals contributed by the matrixes of the wastewater, the peak at m/z 175 corresponded to the protonated arginine molecules. The product ion spectrum of the mass selected ions of m/z 175 (inset of Figure 2a) generated ionic fragments of m/z 157, 158, 130, 116, and 60 by the loss of H_2O, NH_3, [NH_3, CO],[40] $HN{=}C(NH_2)_2$ and $CH_2{=}CH{-}CH_2{-}CH(NH_2){-}COOH$, respectively. The signal was detected instantly once the arginine wastewater solution was nebulized by the manual injector. The signal reached 90% of the maximal height within 200 ms (2 scans) and decreased rapidly within 2 scans. Interestingly, no peak tailing was observed in the single ion chromatogram of the protonated arginine (Figure 2b), which meant that contamination and memory effects were avoided using this method. The sampling time was about 100 ms, and the peak width observed in the selected ion chromatogram was only 1.2 s, which suggested 50 samples could be analyzed in 1 min. This proves rapid, high-throughput analysis for the samples with complex matrixes can be obtained using the nanoEESI source as no sample pretreatment is required. The limit of detection (LOD) for the analysis of arginine was determined to be 11 pg/mL (S/N ≥ 3). Reproducible measurements were performed using an arginine solution at 100 pg/mL, providing repeatable signal abundances in the mass spectra. The signal ion current chromatograms were also reproducible (partially shown in Figure 2b), providing a relative standard deviation (RSD) of 6.9% for 10 measurements.

Quantitative analysis of trace amounts of arginine in the wastewater solution was also demonstrated using the nanoEESI LTQ mass spectrometry. For each measurement, a reproducible ion current chromatogram and mass spectra (data not shown) were recorded by the commercial LTQ mass spectrometer. The plot of the relative mass spectrometric abundance of the protonated arginine molecules vs the concentrations shows a good linear relationship with a linear correlation coefficient of 0.998. As an example by using the reported method, a linear dynamic range from 1 to >100 ppb (Figure 2c) was illustrated for quantitative analysis of arginine in matrixes without sample pretreatment. These data show the potential of nanoEESI for rapid, in situ, quantitative detection of analytes in complex matrixes with high sensitivity, specificity, and throughput.

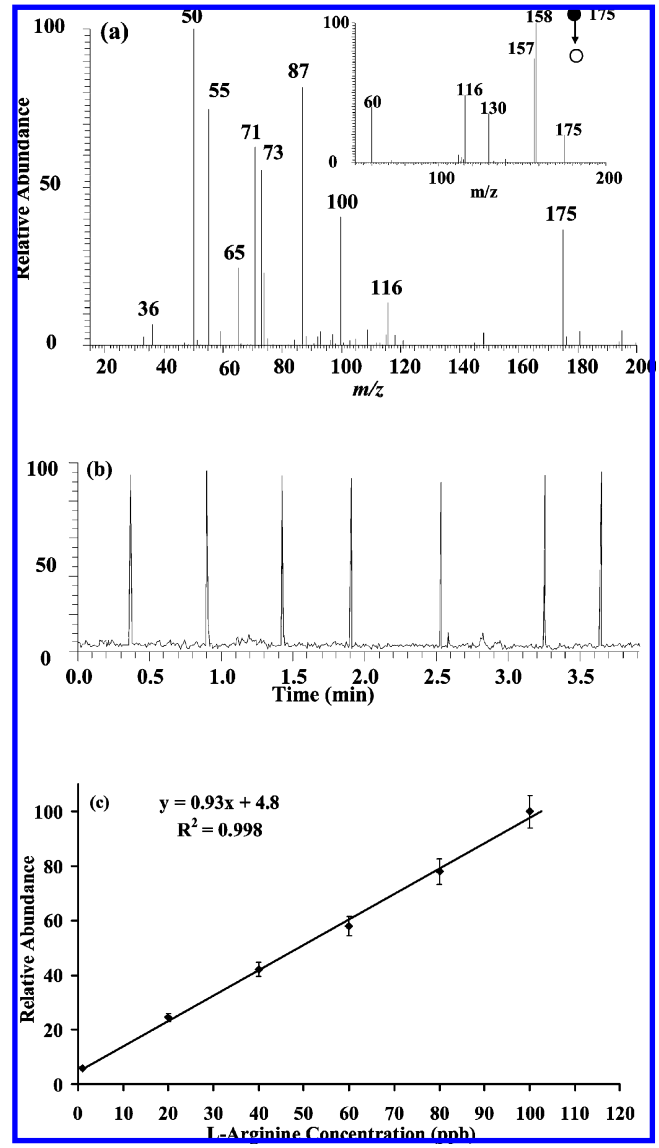


Figure 2. Analysis of waste water samples spiked with L-arginine using nanoEESI in the positive ion mode: (a) a full scan nanoEESI mass spectrum (inset shows MS^2 spectrum of m/z 175), (b) single ion chromatogram of m/z 175, and (c) plot of the relative mass spectrometric abundance of protonated arginine (m/z 175) vs concentration, showing the linear dynamic range between 1 and 100 ppb; the error bars designate the 5% of the mean values.

The characterization of nanoEESI in the negative ion detection mode has been demonstrated by direct analysis of a dilute L-aspartic acid (1.0×10^{-9} g/mL) wastewater solution without sample pretreatment. Among many background signals, L-aspartic acid was detected as deprotonated molecules (m/z 132) with a high signal-to-noise ratio (S/N > 10^3) (Figure 3). In the CID mass spectrum (inset of Figure 3), the ions of m/z 132 produced major fragments of m/z 115, 114, and 88 by the

(40) Shek, P. Y. I.; Zhao, J.; Ke, Y.; Siu, K. W. M.; Hopkinson, A. C. *J. Phys. Chem. A* **2006**, *110*, 8282–8296.

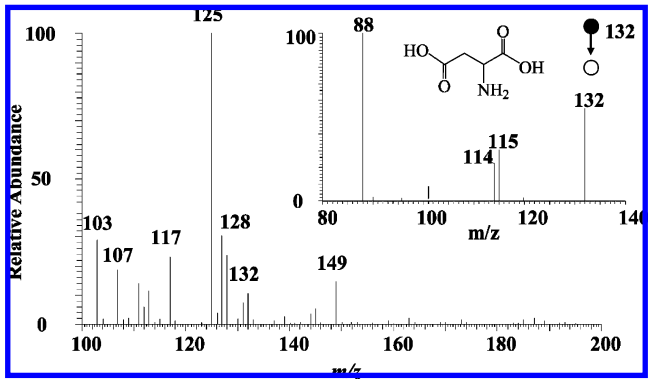


Figure 3. Mass spectrum of wastewater samples spiked with L-aspartic acid (1.0×10^{-9} g/mL) using nanoEESI in the negative ion detection mode (inset shows the MS^2 spectrum of m/z 132).

loss of NH_3, H_2O, and CO_2, respectively. The relative abundance of the peak at m/z 88 was much higher than those observed at m/z 115 and m/z 114, suggesting that the CO_2 cleavage was favored under the experimental conditions. These data confirmed that the aspartic acid was successfully detected using the nanoEESI-MS. Our data showed a low LOD (10 ppt, S/N = 3, RSD = 8%, $n = 8$) for detection of aspartic acid in the negative ion detection mode.

APPLICATIONS

Pharmaceutical Analysis. Pharmaceutical analysis in complex matrixes is one of the most challenging topics in modern analytical chemistry.[41,42] High-throughput pharmaceutical analysis was demonstrated using DESI-MS for solid tablets.[15] Besides tablets, many pharmaceutical preparations are in liquid phase or heterogeneous liquid mixtures. Aerosol drugs are typical examples widely used for lung/airway diseases. As a demonstration, fast detection of the active components in the raw sulbutamol aerosol, terbutaline aerosol were performed using nanoEESI without any sample preparation. Figure 4a shows the spectrum recorded using the sample of the sulbultamol aerosol drug by nanoEESI in the positive ion detection mode. The signal at m/z 240 was corresponding to the protonated sulbutamol. In the CID experiments, the precursor ions of m/z 240 generated product ions of m/z 222 and 166 by the loss of H_2O and $CH_2{=}C(CH_3)_2$ (Figure 4b), successively. In the MS^3 spectrum of m/z 222 (inset of Figure 4b), the precursor ions yielded the major fragments of m/z 204, 166, and 148 by the loss of H_2O, $CH_2{=}C(CH_3)_2$, and [H_2O, $CH_2{=}C(CH_3)_2$], respectively. These data confirmed the successful detection of the active components in the aerosol drug samples. The limit of detection for the analysis of sulbutamol was determined to be 10 pg/mL (S/N = 3), providing good sensitivity for trace pharmaceutical sample analysis.

Active components in drug preparations are usually sensitive to light, heat and acidity of the environment. The quality of drug might be degraded significantly during the transportation, storage, and improper usage. Expired drugs may lose some of their potencies and become toxic; therefore, it is very dangerous for patients to have the expired drugs. It is urgent to develop sensitive, in situ analysis techniques to fast screen impurities in drug preparations on the market. The capability of the nanoEESI for the drug quality control was demonstrated by detecting the expired sulbutamol aerosol drug. To prepare the expired drug, the sulbultamol aerosol drug was heated at 93 °C in a water-bath for 3 h. The expired sulbutamol aerosol was then directly analyzed using nanoEESI in the positive ion detection mode, resulting in mass spectra differing from those recorded using authentic sulbutamol aerosol drugs. As shown in Figure 4c, a new peak at m/z 86 showed up while the relative intensity of the signal at m/z 268 increased in the mass spectra obtained using the heated sample. The new peak at m/z 86 was interpreted as the major pyrolysis product of sulbutamol (i.e., $(CH_3)_3CNHCH_2^+$), which generated major fragments of m/z 69 and m/z 30 (Figure 4d), by the loss of NH_3 and $CH_2{=}C(CH_3)_2$, respectively. The peak at m/z 268 could correspond to the product of the condensation reaction through which sulbutamol and ethanol (i.e., the solvent of sulbutamol aerosol) molecules lost intermolecular water. The structure of the ion (m/z 268) was confirmed by CID experiments, in which the precursor ions (m/z 268) yielded fragments at m/z 250, 222, 212, and 155 by the loss of H_2O, CH_3CH_2OH, $CH_2{=}C(CH_3)_2$, and [$CH_2{=}NC(CH_3)_3$, H_2O], respectively. Note that a major fragment of m/z 222 was detected in the CID spectrum of the protonated subultamol (Figure 4b). In the multiple-stage CID experiments, the identical fragmentation patterns (Figure 4e) were observed using precursor ions (m/z 222) generated either from the protonated sulbutamol molecule (m/z 240) or from the protonated resultant of the condensation reaction (m/z 268). These data further confirm that the ion of m/z 268 is the product of the condensation reaction between sulbutamol and ethanol.

Sulbutamol may induce the disorder of metabolism to some extent for certain patients with asthma, so another pharmaceutical, terbutaline aerosol, is used in such cases as a substitute for sulbutamol. Herein, nanoEESI has been also used for the analysis of terbutaline aerosol. The terbutaline aerosol was sprayed to the nanoEESI directly in the positive ion detection mode without any sample pretreatment, and the resulting mass spectrum is shown in Figure 5a. The signal at m/z 226 corresponds to the protonated terbutaline, and the structure was confirmed by the CID experiment (as shown in Figure 5b), in which the precursor ion m/z 226 generated the ions of m/z 208, 170, and 152, by the loss of H_2O, $CH_2{=}C(CH_3)_2$, and [H_2O, $CH_2{=}C(CH_3)_2$], respectively. The limit of detection for the analysis of terbutaline was determined to be as low as 9 pg/mL (S/N = 3).

Food Analysis. Food safety is of worldwide concern. Recently, the finding that large number of dairy products adulterated with melamine that were on the market has drawn intensive attention from an international audience,[43] who require protection from inferior foods. Therefore, development of a novel technique for rapid, sensitive, in situ detection of trace amounts of regulated compounds such as melamine in food is of paramount importance. Gas chromatography/mass spectrometry (GC/MS) has been recommended by the U.S. Food and Drug Administration (FDA) for the detection of melamine in pet food.[44] Alternatively, several other methods such as liquid chromatography/mass spectrometry (LC/MS),[45,46] capillary electrophoresis,[47] laser enhanced Raman

(41) Kataoka, H. *Curr. Pharm. Anal.* **2005**, *1*, 65–84.
(42) Lim, C. K.; Lord, G. *Biol. Pharm. Bull.* **2002**, *25*, 547–557.
(43) http://en.wikipedia.org/wiki/2008_baby_milk_scandal.
(44) http://www.fda.gov/cvm/GCMSMelamine.htm.

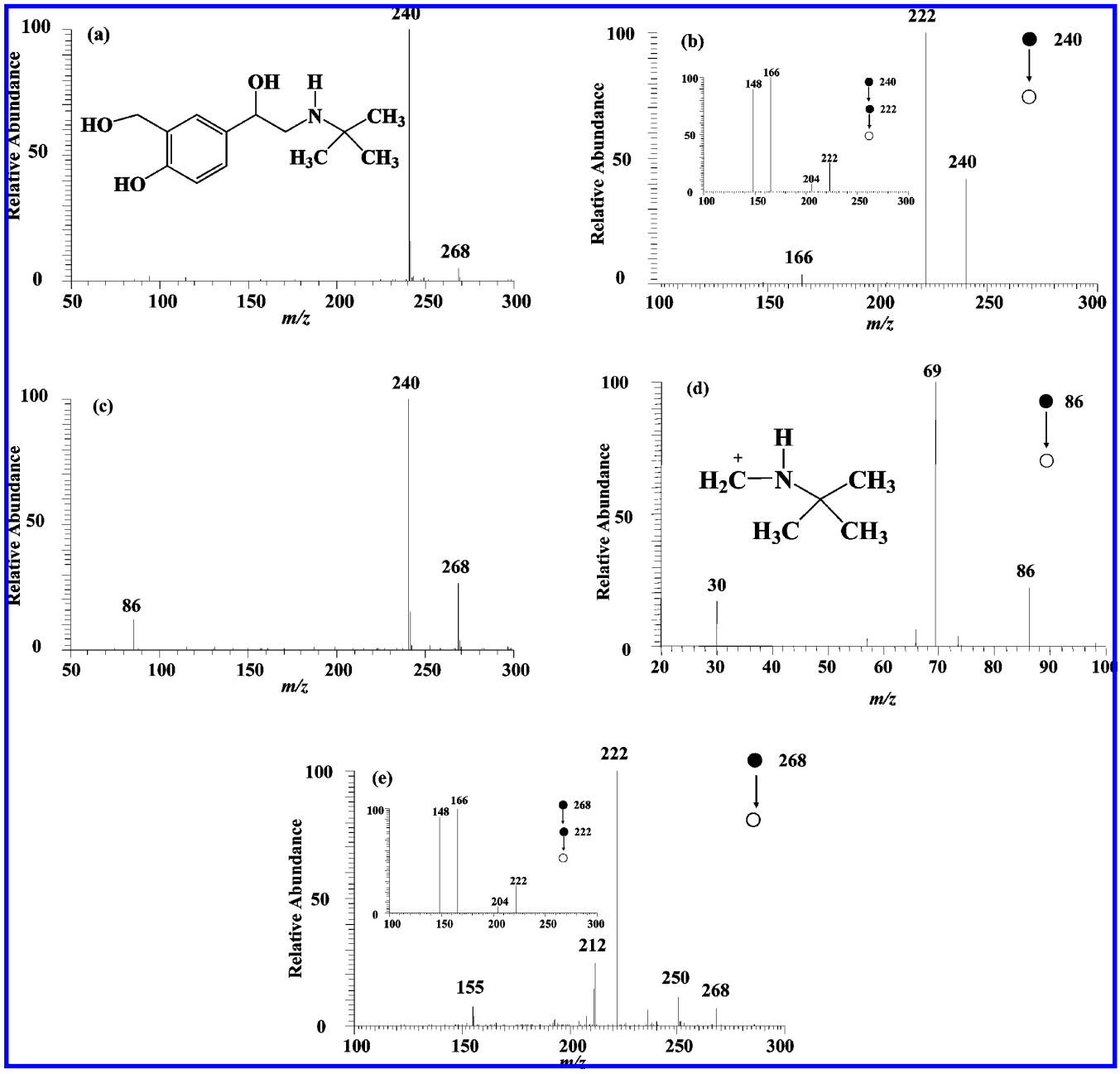


Figure 4. Analysis of sulbultamol aerosol drug preparations using nanoEESI in the positive ion detection mode: (a) full scan mass spectrum of sulbultamol aerosol, (b) MS^2 spectrum of protonated sulbultamol (inset shows the MS^3 spectrum of *m*/*z* 222), (c) mass spectrum of heat-induced expired sulbutamol, (d) MS^2 spectrum of the pyrolysis product (*m*/*z* 86), and (e) MS^2 spectrum of *m*/*z* 268 (inset shows the MS^3 spectrum of *m*/*z* 222).

spectrometry,[48] and enzyme-linked immunosorbent assay (ELISA)[49] have been used for the detection of melamine in food with various matrixes. Because of the complicated matrixes, extensive sample pretreatment, such as extraction, preconcentration, and derivatization, which are time-consuming processes, is usually required. Ambient mass spectrometry techniques including surface desorption atmospheric chemical ionization (DAPCI),[50,51] extractive electrospray ionization,[52] and low-temperature plasma probe[53] are available for the detection of trace amounts of melamine with minimal or no sample pretreatment. Compared with the ambient ionization methods mentioned above, the nanoEESI allows the experiment to be performed without sheath gas or discharge gas, which makes it attractive for in situ analysis

(45) Filigenzi, M. S.; Tor, E. R.; Poppenga, R. H.; Aston, L. A.; Puschner, B. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 4027–4032.
(46) Heller, D. N.; Nochetto, C. B. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 3624–3632.
(47) Yan, N.; Lei, Z.; Zhu, Z.; Chen, X. *J. Agric. Food Chem.* **2009**, *57*, 807–811.
(48) Lin, M.; He, L.; Awika, J.; Yang, L.; Ledoux, D. R.; Li, H.; Mustapha, A. *Sens. Instrum. Food Qual. Saf.* **2008**, *2*, 66–71.
(49) Garber, E. A. E. *J. Food Prot.* **2008**, *71*, 590–594.
(50) Yang, S.; Ding, J.; Zheng, J.; Hu, B.; Li, J.; Chen, H.; Zhou, Z.; Qiao, X. *Anal. Chem.* **2009**, *81*, 2426–2436.
(51) Yang, S.; Chen, H.; Yang, Y.; Hu, B.; Zhang, X.; Zhou, Y.; Zhang, L.; Gu, H. *Chin. J. Anal Chem.* **2009**, *37*, 315–318.
(52) Zhu, L.; Gamez, G.; Chen, H.; Chingin, K.; Zenobi, R. *Chem. Commun.* **2009**, 559–561.
(53) Huang, G.; Ouyang, Z.; Cooks, R. G. *Chem. Commun.* **2009**, 556–558.

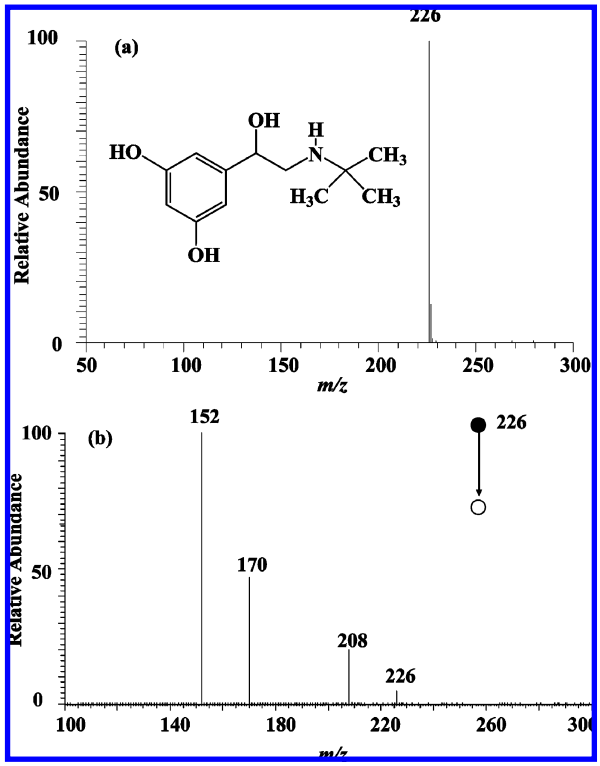


Figure 5. Analysis of terbutaline aerosol drug preparations using nanoEESI in the positive ion detection mode: (a) mass spectrum of terbutaline aerosol and (b) MS^2 spectrum of protonated terbutaline.

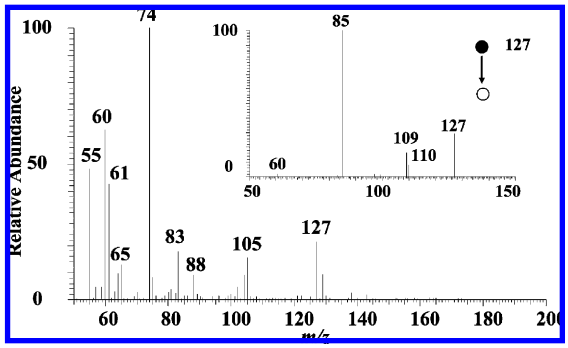


Figure 6. Mass spectrum of liquid milk spiked with melamine (0.2 mg/mL). The inset shows the MS^2 spectrum of m/z 127.

for the food samples with complex matrixes. Figure 6 shows a mass spectrum recorded using a raw liquid cow milk sample spiked with melamine with a concentration of 0.2 mg/L. The signal at m/z 127 corresponds to the protonated melamine. Upon CID, the precursor ions (m/z 127) generated ions of m/z 110, 109, 85, and 60 as the major fragments (inset of Figure 6), by the loss of NH_3, $[NH_3 + H^{\bullet}]$,[50] NH_2CN, and C_2N_3H, respectively, which is in agreement with previous results.[50,51]

Beverages including energy drinks and sport drinks are becoming more and more popular among students, athletes, and active persons because of their attribution of energy-giving properties. Usually these drinks are composed of several compounds such as vitamins, caffeine, and taurine in various complex matrixes, which makes it difficult to determine all the relevant compounds directly and simultaneously. Sample pretreatment such as degassing should be performed prior to the analysis by

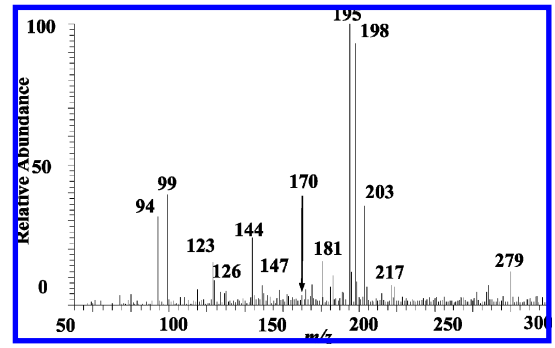


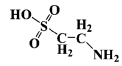
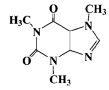
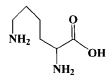
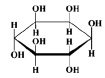
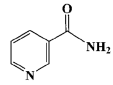
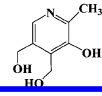
Figure 7. Mass spectrum for the analysis of Red Bull using nanoEESI in the positive ion detection mode.

the conventional methods including high-performance liquid chromatography (HPLC)[54] and planar chromatography/mass spectrometry.[55] So fast, in situ quality monitoring is of importance for the manufacturers, sellers, and costumers. The direct analysis of Red Bull beverage containing low levels of functional components such as taurine, caffeine, lysine, inositol, nicotinamide, and vitamin B6 was demonstrated using nanoEESI without any sample pretreatment. The mass spectrum recorded for the analysis of red bull beverage is shown in Figure 7. The ions at m/z 126, 195, 147, 198, 123, and 170 are corresponding to the protonated molecules of taurine, caffeine, lysine, inositol, nicotinamide, and vitamin B6, respectively. The structures of these components were confirmed by CID, and the fragment ions are listed in Table 1.

Pesticide Residues Detection. Nowadays, the public has more concerns about the toxic effects of pesticides being widely used for control of insects and weeds, which leads to serious contamination of a large volume of the biosphere. Several methods, including mass spectrometry[56] reversed-phase liquid chromatography/gas chromatography (RPLC/GC),[57] are available for the detection of pesticides in the environment. However, these methods suffer from low sensitivity and are time-consuming due to the multiple-step sample pretreatment required. Rapid, sensitive, on spot detection is difficult using any of these methods mentioned above. NanoEESI mass spectrometry can address these problems for pesticide residue detection. Paraquat and β-cypermethrin were selected as representatives of the heterocyclic pesticides and pyrethroid pesticides in this study. The mass spectrum collected from the farmland water spiked with paraquat (200 ng/mL) and β-cypermethrin (45 ng/mL) using the nanoEESI source showed signals at m/z 186, 93 and m/z 416, which correspond to the paraquat radical ions and protonated β-cypermethrin, respectively. The peaks at m/z 93 and 185 could correspond to the doubly charged paraquat ions and deprotonated paraquat ions. The relative abundances of the doubly charged paraquat ions and deprotonated paraquat are much lower than the results recorded by electrospray mass spectrometry.[58] This is probably because the high voltage was directly applied to the ESI source, so that the doubly charged ions were generated easily and the high

(54) Sawab, Y.; Tagami, T.; Yamasaki, K. *J. Health Sci.* **2008**, *54*, 661–664.
(55) Aranda, M.; Morlock, G. *J. Chromatogr., A* **2006**, *1131*, 253–260.
(56) Nanita, S. C.; Pentz, A. M.; Bramble, F. Q. *Anal. Chem.* **2009**, *81*, 3134–3142.
(57) Perez, M.; Alario, J.; Vazquez, A.; Villén, J. *Anal. Chem.* **2000**, *72*, 846–852.
(58) Marr, J. C.; King, J. B. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 479–483.

Table 1. Functional Compounds Found in Red Bull Using NanoEESI-MS

Compounds	Molecular structure	Molecular weight	Ions observed	Product ions (MS/MS, *m/z*)	Neutral species lost in MS/MS experiment
Taurine		125	$[M+H]^+$	108, 109	H_2O, NH_3
Caffeine		194	$[M+H]^+$	138, 110,	$CH_3N{=}C{=}O$, [$CH_3N{=}C{=}O$, CO]
Lysine		146	$[M+H]^+$	129, 130, 119, 84	H_2O, NH_3 , CO, [NH_3, HCOOH]
Inositol		180	$[M+NH_4]^+$	163, 180, 181	[H_2O, NH_3], H_2O, NH_3
Nicotinamide		122	$[M+H]^+$	106, 95, 81, 80, 67	NH_3, CO, [CO, •HCH•], O=C=NH, [CO, 2•HCH•]
Vitamin B6		169	$[M+H]^+$	152	H_2O

voltage cleaved the C−H bond to produce the deprotonated paraquat. While ions are produced using nanoEESI, the neutral analytes are prevented from touching the high voltage directly. As a result, the spectra recorded by nanoEESI are more relevant to the species in the solution. To confirm the structures of those ions, the CID experiments were performed, and the tandem mass spectra for the ions *m/z* 186 and *m/z* 416 are shown in parts b and c of Figure 8, respectively. The product ion spectrum of *m/z* 186 yields the main fragments at *m/z* 171 by the loss of $CH_3^{\bullet}$. The other signals observed in the MS^2 spectrum are *m/z* 157, 145, and 131, probably generated by the loss of $HN{=}CH_2$, $CH_2{=}N{-}CH^{\bullet}$, and [$HN{=}CH_2$, C_2H_2], respectively. As shown in Figure 8c, the tandem mass spectrum of *m/z* 416 shows the fragments at *m/z* 388, by the loss of CO, and the product ions of *m/z* 191 and *m/z* 226 are generated by the cleavage of the C−O bond in the ester group. The limit of detection (LOD) for the paraquat was determined as 10 ng/mL (S/N = 3), which is much lower than the effluent standard (0.1 μg/mL) of paraquat for industry.[59] The limit of detection (LOD) for β-cypermethrin was determined as 6 ng/mL (S/N = 3), which is also lower than the recommend effluent standard (0.01 μg/mL) of β-cypermethrin.

Explosives Analysis. In situ analysis of explosives is of paramount importance in public safety and environment applications.[60] Several techniques[60] including X-rays, enzyme-linked immunosorbent assays, and voltammetry methods have been reviewed for the analysis of explosives. Mass spectrometry[19,32,61,62] is a preferable tool for the determination of explosives because of its high sensitivity and specificity. Recently, ambient mass spectrometry, such as desorption electrospray ionization,[19,62] low-temperature plasma probe,[32,33] direct analysis in real time (DART),[29] and extractive electrospray ionization,[28] shows its capability for the direct analysis of explosives samples with various complex matrixes. However, these ambient ionization methods need sheath gas as a pneumatic gas or discharge gas for the generation of the primary ions. A gasless nanospray emitter rather than a conventional ESI probe is used in the nanoEESI, which avoids the use of the sheath gas or discharge gas, allowing the simplification of the instrument and the operation. The ability of the nanoEESI technique for the explosive analysis has been demonstrated by recording the mass spectra and tandem mass spectra of TNT/RDX in a negative/positive ion detection mode using groundwater samples spiked with trace amounts of explosives (1 ppt). As shown in Figure 9a, the peak at *m/z* 223 corresponds to the protonated RDX molecules, which generate the main fragment ions of *m/z* 207, 177, 205, 163, and 191 by the loss of O, NO_2, H_2O, $-N-NO_2$, and O_2, respectively, in the MS/MS spectrum (inset of Figure 9a). The peak at *m/z* 227 in the mass spectrum collected using 2,4,6-trinitrotoluene samples (1 ppt) without sample pretreatment corresponds to the radical anion of TNT, which was confirmed by the CID mass spectrum (inset of Figure 9b). The most abundant signal observed in the CID spectrum is *m/z* 183, with a loss of 44, which has been reported previously.[19,28] The structure of *m/z* 183 is still unclear, and it might be formed by the loss of $NOCH_2$[28] from the precursor ions. The product ion spectrum of the radical anion also yields the ions at *m/z* 212, 210, 197, and 167 by the loss of $CH_3^{\bullet}$, $HO^{\bullet}$, $NO^{\bullet}$, and $2NO^{\bullet}$, respectively. These data confirmed that trace amounts of explosives were detected by nanoEESI-MS without any sample pretreatment.

(59) *Effluent Standards of Pollutants for Heterocyclic Pesticides Industry*, GB21523-2008.
(60) Yinon, J. *Trends Anal. Chem.* **2002**, *21*, 292–301.
(61) Martin, A. N.; Farquar, G. R.; Gard, E. E.; Frank, M.; Fergenson, D. P. *Anal. Chem.* **2007**, *79*, 1918–1925.
(62) Justes, D. R.; Talaty, N.; Cotte-Rodriguez, I.; Cooks, R. G. *Chem. Commun.* **2007**, 2142–2144.

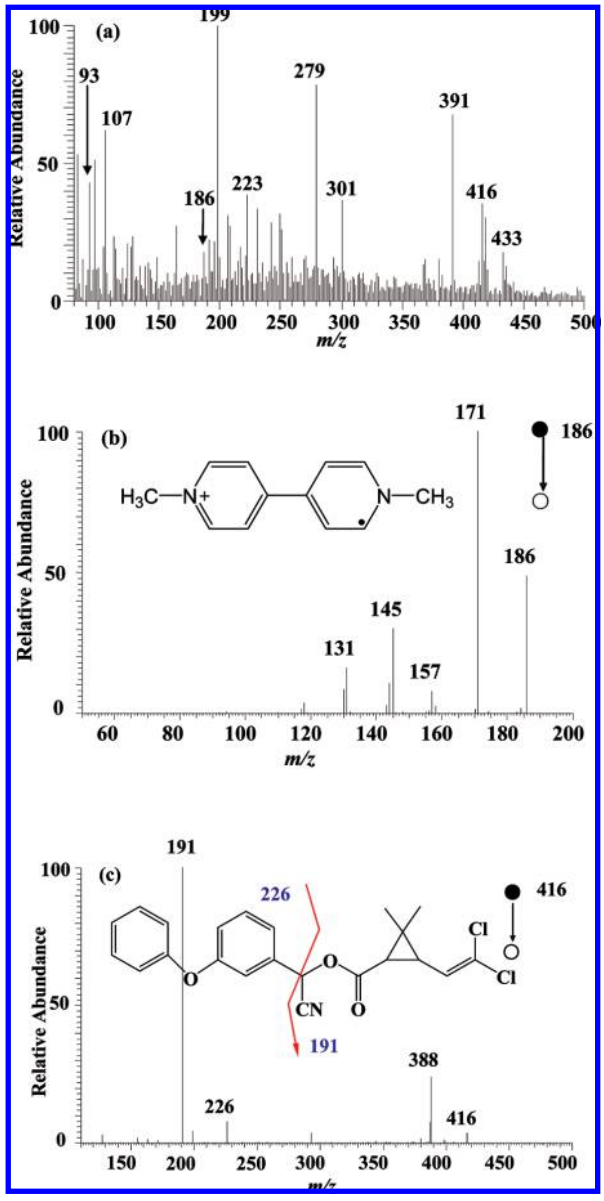


Figure 8. Analysis of farmland water spiked with paraquat (200 ng/mL) and β-cypermethrin (45 ng/mL) using nanoEESI in the positive ion detection mode: (a) full scan mass spectrum of the farmland water sample, (b) MS^2 spectrum of *m*/*z* 186, and (c) MS^2 spectrum of *m*/*z* 416.

CONCLUSIONS

A gasless nanoESI rather than the conventional ESI probe has been used to produce the primary ions for extractive electrospray ionization of analytes in the neutral sample plumes, which is manually introduced using a sample injector. Consequently, a novel nanoEESI source has been developed for rapid analysis of various ambient samples without any sample pretreatment. The nanoEESI source was characterized by rapidly detecting trace amounts of amino acids in water with complex matrixes in the positive/negative ion detection mode. High sensitivity, specificity, and throughput detection has been demonstrated for a wide range of applications including drug quality analysis, food safety regulation, pesticide residues detection, and explosives screening. Alternatively, nanoEESI allows the experiments to be performed without any sheath gas or discharge gas, so the instrument and the operation as well are significantly simplified. This makes nanoEESI suitable for coupling to portable mass spectrometers, resulting in platforms based on nanoextractive electrospray ionization mass spectrometry, and toward in situ analysis without sample pretreatment.

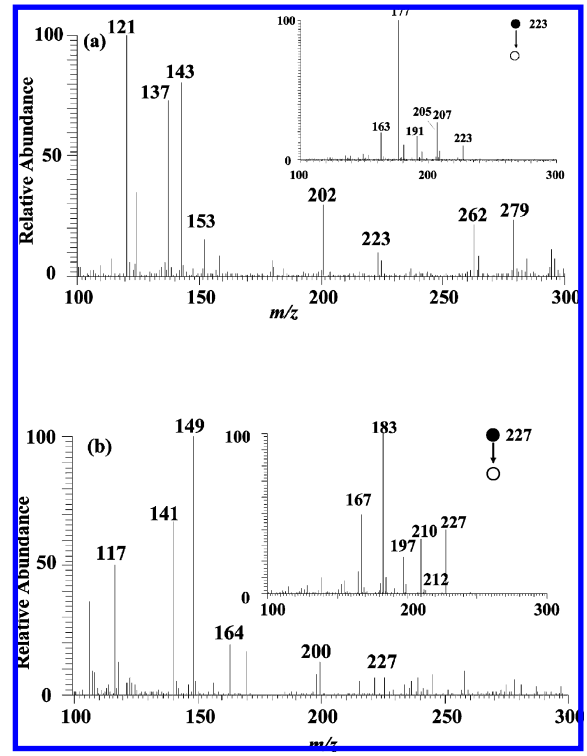


Figure 9. Explosives detection in groundwater using nanoEESI-MS: (a) mass spectrum of RDX in the positive ion detection mode (the inset shows the MS^2 of *m*/*z* 223) and (b) mass spectrum of TNT in the negative ion detection mode (the inset shows the MS^2 of *m*/*z* 227).

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