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Rapid Differentiation of Tea Products by Surface Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry

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Protonated water molecules generated by an ambient corona discharge were directed to impact tea leaves for desorption/ionization at atmospheric pressure. Thus, a novel method based on surface desorption chemical ionization mass spectrometry (DAPCI-MS) has been developed for rapid analysis of tea products without any sample pretreatment. Under the optimized experimental conditions, DAPCI MS spectra of various tea samples are recorded rapidly, and the resulting mass spectra are chemical fingerprints that characterize the tea samples. On the basis of the mass spectral fingerprints, 40 tea samples including green tea, oolong tea, and jasmine tea were successfully differentiated by principal component analysis (PCA) of the mass spectral raw data. The PCA results were also validated with cluster analysis and supervised PCA analysis. The alteration of signal intensity caused by rough surfaces of tea leaves did not cause failure in the separation of the tea products. The experimental findings show that DAPCI-MS creates ions of both volatile and nonvolatile compounds in tea products at atmospheric pressure, providing a practical and convenient tool for high-throughput differentiation of tea products.

KEYWORDS: Mass spectrometry; desorption atmospheric pressure chemical ionization; tea; differentiation; principal component analysis; cluster analysis

INTRODUCTION

With an annual production of 1.8×10^6 t of dry leaves and a daily worldwide consumption of (18–20) $\times$ 10^6 cups (6 oz), tea is the most worldwide beverage (*1*), besides water. Tea is made from the leaves of the *Camellia sinensis* plant. Most teas can be classified into three main types: (1) black tea, (2) oolong tea, and (3) green tea, based on the depth of oxidation and the procedure to process the leaves. Although the quality of tea depends on many factors (e.g., leaf variety, growing environment, manufacturing procedures, post handle conditions, infusion preparation, etc.), the quality is usually measured in a traditional way by using a sensory method (*2–4*), which requires highly trained specialists to grade the tea quality based on the tea aroma (flavor), the taste of the brew, and the appearance of tea leaves. Sensory methods are simple and convenient to use; however, these methods lack precision and accuracy, even when executed by specialists with plenty of experience. Variation of tea quality results in significant difference in function, for example, pharmaceutical activity (*1, 5, 6*), taste, and, thus, the price. Therefore, authentication of tea quality grades, from raw materials to finished products, is of primary importance for both consumers and industries so that tea adulteration and unfair competition can be avoided.

Numerous efforts have been made to evaluate tea quality on the basis of qualitative and quantitative analysis of chemical constituents by instrumental techniques such as gas chromatography (*7–10*), high performance liquid chromatography (*11–16*), gas chromatography–mass spectrometry (*8, 17, 18*), atmospheric pressure chemical ionization mass spectrometry (APCI-MS) (*19–21*), optical spectroscopy (*22–24*), and nuclear magnetic resonance (NMR) spectroscopy (*25* ,*26*). Among these techniques, mass spectrometry, providing high specificity and sensitivity, is widely used for tea analysis (*7, 8, 14, 19, 24, 27–29*). However, to date, a multiple-step procedure for sample pretreatment is required when mass spectrometry is employed for tea analysis. Typically, dried tea leaves need to be ground and followed with extraction, centrifugation, and drying processes. Before gas chromatography separation, analyte derivatization must be completed with various incubation temperatures and times. For electrospray ionization mass spectrometry (ESI-MS) and APCI-

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MS, analyte derivatization is not required, but sample extraction and separation is still necessary (*14, 24, 27, 28*). Usually, the tedious sample pretreatment makes high-throughput tea analysis and online inspection impossible.

Recently, ambient mass spectrometry, represented typically by desorption electrospray ionization (DESI) (*30–32*), is of increasing interest for direct detection of trace amounts of components present in complex matrices with minimal sample pretreatment. Examples have been demonstrated for the fast detection of various ambient samples, including active components in pharmaceutical preparations (*31*), explosives on surfaces (*30*), alkaloids in plants (*30, 33*), Sudan dyes in foods (*32*), metabolites in urine (*34*), and for direct imaging of animal tissues (*35*). In a typical DESI experiment, a high pressure (c.a. 200 PSI) sheath gas is used, and a methanol/water solution (1:1, v/v) is electrosprayed directly onto samples to maintain a stable signal. The direct analysis in real time (DART) technique (*36, 37*) is operated without spraying methanol but with inert gases such as helium and was recently found to be less sensitive than DESI (*37*), especially for compounds absorbed chemically by solid surfaces. For liquid sample such as raw urine, milk, and wastewater, it can be directly monitored continuously by extractive electrospray ionization (EESI) (*38–41*) mass spectrometry without contamination by toxic chemicals. These results indicate that tea infusion could be analyzed directly by EESI-MS; however, it requires an extra step to make the tea brew.

Surface desorption atmospheric pressure chemical ionization (DAPCI) has been demonstrated for direct surface analysis with enhanced sensitivity (*37, 42*) with chemical reagents such as toluene. Recently, DAPCI was also implemented in an LTQ-MS instrument using ambient air as a reactive reagent for sensitive detection of various analytes (*43, 44*) in clothes, pharmaceutical preparations, or on human skin. In this study, a surface desorption chemical ionization is performed directly on tea leaves using protonated water as primary ions, which are generated by a corona discharge of ambient air with high moisture content. The DAPCI-MS and MS/MS spectra of tea products are recorded in an LTQ-MS instrument, and the mass spectral fingerprints are used successively for pattern recognition analysis, providing a way for rapid differentiation of tea products with neither sample pretreatment nor toxic chemical contamination.

MATERIALS AND METHODS

All tea samples were randomly selected from Chinese green tea, oolong tea, and jasmine tea. All of these tea samples were made from the leaves of the *Camellia sinensis* plant. Chinese green tea is the least processed tea, for which the raw plant leaves are steamed, roasted, or pan-fried almost immediately, leaving little chance for the leaves to ferment. As a result, green tea tends to be lighter in color and has a delicate 'green' character. Oolong tea is the product of a very labor-intensive process. Once picked, the plant leaves are gently rolled to allow the tea oils to react with oxygen in the air for oxidation. This process turns the leaves darker and produces the fragrant and fruitful aromas characteristic of oolong tea. The leaves are rolled into their final shapes and heated to dry as final products. Jasmine tea is processed in a procedure similar to making green tea, but with the addition of jasmine flowers (0.2% weight). All of the tea samples were prepared by their manufactures in China. Fourty samples (as shown in **Table 1**) were bought from a local tea outlet (Nanchang, China) and were directly used without further treatment. Each measurement was performed with 500 mg tea of leaves, which were held by a piece of clean filter paper. Chemicals such as caffeine (A.R. grade), methylsalicylate (A.R. grade), and indole (A.R. grade) were bought from Chinese Chemical Reagent Co. Ltd. (Shanghai, China). All chemicals were directly used without any pretreatment except for dissolution and dilution with deionized water when it was necessary.

Table 1. Tea samples investigated by DAPCI-MS[a]

tea samples	oolong tea					green tea					jasmine tea				
manufacturer A	1	2	3	4	5	1	2	3	4	5					
manufacturer B	1	2	3	4	5	1	2	3	4	5					
manufacturer C	1	2	3	4	5	1	2	3	4	5					
manufacturer D						1	2	3	4	5	1	2	3	4	5

[a] Note: the processes utilized by different manufacturers to make oolong tea, and green tea as well, were different. The individual samples from the same manufacturer were marked with different numbers (e.g. oolong tea AO1–AO5), and they were made using the same procedure but with different series numbers for each tea product.

Experiments were carried out using a commercial linear ion trap mass spectrometer (LTQ-XL, Finnigan, San Jose, CA, USA) installed with a homemade surface desorption chemical ionization source (schematically shown in **Figure 1**). A cylindrical electrode (stainless steel, 5 cm in length and 0.2 mm in diameter), with a cone on one end (ca. 20 μm in diameter at the tip) and an insulator on the other end, was inserted into a fused-silica capillary (0.5 mm i.d.). The capillary and the sharp needle were coaxially fixed to a union tee (Swagelok, OH, USA) using a silica ferrule. The length of the capillary was about half of the tee tube. The capillary and the sharp needle were carefully arranged such that about 5mm of the sharp needle was exposed to air and that the capillary was open inside the tee tube. The cylindrical electrode was securely fixed, at the insulated end, to the other end of the tee tube. A gas line was mounted to the tee tube at the unoccupied end. Gases, carrying water vapor as a reactive reagent, could come out through the tee tube along the sharp needle. When a high voltage is applied to the sharp needle, a corona discharge occurs to generate primary ions of the reactive reagent in the ambient air. The source assembly was coupled to the LTQ-MS in such a way that the electrode tip was placed coaxially in front of the ion inlet of the LTQ instrument. The distances between the discharge tip and the MS inlet was 5–10 mm, depending on the size of samples. The distance between the discharge tip and tea sample was about 1–3 mm. The sensitive area for sampling was estimated to be about 10 mm^2. The primary ions were accelerated by the electric field created by the high voltage on the needle. These ions then impacted the surface of the sample for desorption/ionization so that ions of analytes form the sample were created at ambient pressure. The analyte ions were then introduced into the LTQ mass analyzer for mass analysis through the ion guide system of the LTQ instrument. The LTQ-MS instrument was set to work in positive ion detection mode, and the corona discharge voltage was +3–4 kV with a discharge current of 1–2 mA. The temperature of the heating capillary of the LTQ-MS was maintained at 200 °C. The voltages for the heating capillary, tube lenses, conversion dynode, detectors, etc. were left at default values. Further optimization was not performed.

All of the mass spectra were recorded as peak profile with an average time of 1 min and were background subtracted. Collision induced dissociation (CID) was performed to the precursor ions of interest, isolated with 1 *m*/*z* unit, with 20–35% collision energy. MS/MS spectra could be collected with a recording time of more than 1 min if necessary. Compounds of interest were identified using MS and CID data-matching of the unknown compounds against authentic standards.

Pattern recognition was performed based on principal component analysis (PCA) and cluster analysis (CA) of the mass spectral raw data (formatted into .txt files), using commercially available software MVSP (version 3.1, Kovach Computing Services, Wales, UK). As a standard feature of the LTQ-MS instrument, the mass spectra were exported as text files and then saved in software such as Excel. To ensure the accuracy of the data conversion, each *m*/*z* unit (e.g., *m*/*z* 80–81) was sampled by 11 points. All text data were arranged using the *m*/*z* values as independent variables and the signal intensities as dependent variables. The data matrix was loaded automatically from the Excel file to the MVSP software. This is also a standard feature of the MVSP software. Once the data were loaded successfully into the MVSP software, they were used directly either for PCA or CA processing without any further treatment. To minimize the deviation caused by

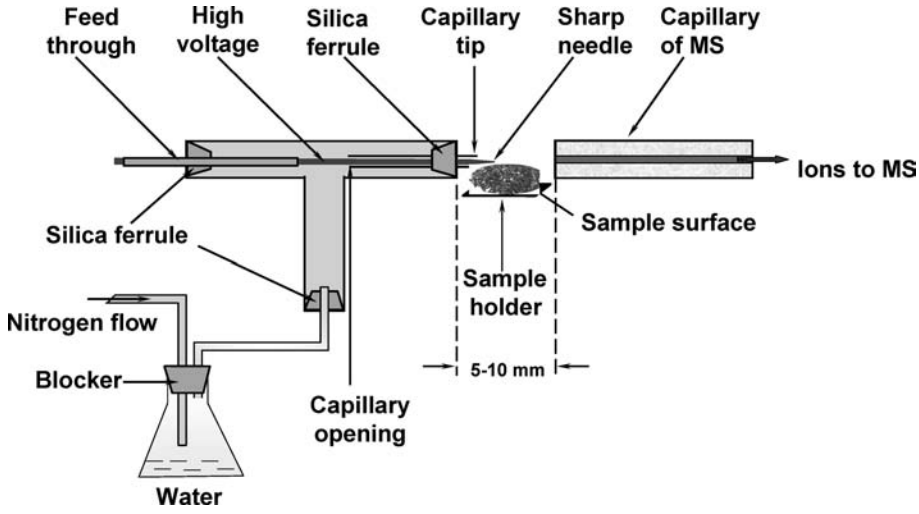

Figure 1. Schematic diagram of the DAPCI source for tea analysis.

individual measurements of the same sample, normalization of the mass spectra data was selected during the PCA or CA process. Note that the "centre data" function of the MVSP software was also enabled for the PCA process. The principal components for output were automatically determined by the software based on Kaiser's rule, and the accuracy for PCA was set to be high (1×10^{-8}). Typically, the first two principal components represent about 85% of the total variance. For supervised PCA, mass spectral profile data of 10 peaks of interest (i.e., the differential peaks found in PCA loading plots obtained with the full mass spectral data sets) were selected manually and were then imported to MVSP for PCA as described above. CA was done, using the same mass spectral raw data that were used for PCA, to output the percent similarity of different tea samples based on an unweighted pair group method with arithmatic mean (UPCMA) algorithm, which is a commercial feature of the MVSP software. UPGMA employs a sequential clustering algorithm, in which local topological relationships are identified in order of similarity. The input for UPCMA is the data collection of mass spectra, and the output is a rooted tree in sequence of similarity.

RESULTS AND DISCUSSION

Optimization of the DAPCI source. DAPCI is a variant of chemical ionization, for which a chemical reagent is necessary to generate the primary ions as ionization reagent (*37, 42, 43*). Theoretically, a wide variety of compounds can be used as reactive reagents for corona discharge to generate the primary ions. To avoid any potential contamination by toxic chemicals, only water vapor was used in this work as the reagent to produce primary ions such as H_3O^+. As reported previously (*43, 44*), DAPCI source can work properly without a gas flow if the moisture of the ambient air is high enough (ca. 60% relative humidity (R.H.)). In this study, because the air moisture was about 40% R.H., saturated water vapor (25 °C) was introduced to the DAPCI source by a nitrogen gas flow (shown in **Figure 1**). Experimentally, it was found that the signal intensity increased along with an increase in pressure of the nitrogen gas, and a stable signal was achieved when the gas pressure reached 15 PSI. Further increase of the gas pressure results in no prominent effect on the signal intensity. Thus, the experiments were done with a carrier gas pressure of 20 PSI.

Optimization of the DAPCI configuration was performed prior to the experiments, which was described in detail elsewhere (*43*). Briefly, a high, level, and stable signal was obtained under the following conditions: the voltage for corona discharge was 3.5–4.5 kV; the distance between the corona discharge tip and the sample surface was 2–3 mm; and the distance between the tip and the LTQ-MS inlet was about 5–10 mm. If the corona discharge voltage is lower than 3.5 kV, a less stable, low-intensity signal is produced; probably because the primary ions are not sufficiently produced or accelerated. However, if the corona discharge voltage is higher than 5 kV, the signal intensity decreases slightly again. Most likely, an increased corona discharge voltage accelerates the ions more than necessary. The ions with extra energy can not be properly introduced to the mass analyzer, because the ion introduction system (i.e., the ion optics system) of the LTQ-MS instrument was tuned for ions without extra energy, and the more energetic ions would be neutralized on the surface of the electrodes. Thus, a signal drop was observed. The distance between the corona discharge tip and the sample surface was carefully optimized and determined to be 2–3 mm. The signal could be eliminated if the tip reached the tea surface, especially when the corona discharge voltage was low ($\leq$1 kV). The extinguishing of signal was caused by the quenching of the corona discharge. The distance from the discharge tip to the sample surface can be 2–5 mm if the voltage for the corona discharge is about 3.5–4.5 kV, which enables a robust discharge at the ambient conditions. The distance between the tip and the MS inlet has little effect on the signal intensity, so it can be 5–10 mm or even longer, depending on the sample size. Note that the tip was coaxially arranged to the MS inlet in all the cases; the off-center position should be avoided to ensure a good signal.

Representative Mass Spectra. The tea products generated their mass spectral fingerprints using DAPCI-MS without any sample pretreatment. Similar mass spectra were recorded from all types of tea samples, providing numerous peaks in the mass range *m/z* 30–600. The compounds detected in the low mass range ($m/z \leq 80$) are relatively small molecules, that is, typical volatile compounds. These compounds present no essential difference in the mass spectra, probably because all of the tea samples have very similar volatile molecular profiles. In fact, all of the oolong tea samples, and all the green tea products as well, can not be individually distinguished with a sensory method without sample pretreatment (e.g., making a tea brew to taste). The mass spectral fingerprints recorded from each tea product are quite different in the mass range from *m/z* 80 to 600 in terms of peak density and signal intensity. Some representative mass spectral fingerprints are shown in **Figure 2**. Fewer peaks were detected in the mass range over *m/z* 300, possibly because the large molecules can not be efficiently desorbed from the leaves, because of the low vapor pressure of

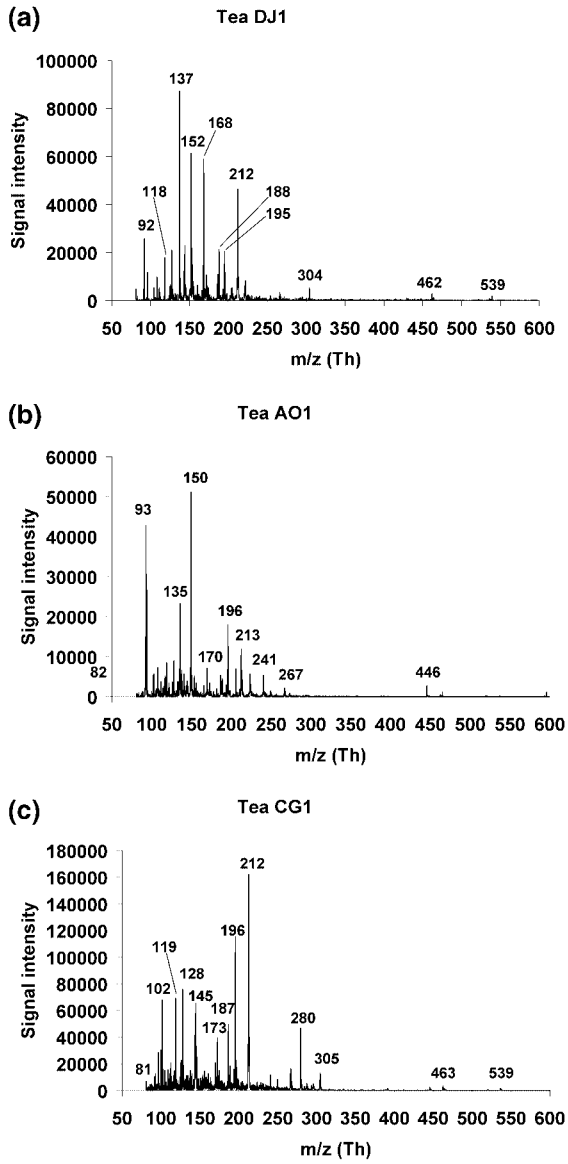

Figure 2. Typical DAPCI mass spectra obtained from different tea samples: (**a**) Jasmine tea, (**b**) oolong tea, and (**c**) green tea.

the large molecules and the roughness of the twisted surfaces, which might further prevent efficient desorption of high molecular weight species from the tea samples. However, the compounds, detected in the mass range from *m/z* 80 to 300, are characteristic mass spectral fingerprints of the tea products.

As reported previously (*37*, *42–44*), nonvolatile and semivolatile compounds can be detected as protonated molecules using DAPCI mass spectrometry. Because volatile and nonvolatile compounds are present on the surfaces of tea products, they can be simultaneously detected by DAPCI-MS. For example, caffeine (MW = 194) and indole (MW = 117), typical semivolatile compounds, were detected from tea samples (**Figure 2a**) as protonated molecules at *m/z* 195 and 118, respectively. In the CID experiments, ions of *m/z* 195 generated two major fragments of *m/z* 138 and 110 by the loss of CH_3NCO and CO, successively. This fragmentation pattern is in agreement with previous observations (*27*, *28*) and with the data obtained using a reference compound; thus, the assignment of the peak at *m/z* 195 to protonated caffeine is confirmed. Using a reference compound, the assignment of the peak at *m/z* 118 was also confirmed by the CID mass spectrum, in which a major fragment of *m/z* 91 was generated by the loss of HCN. Methyl salicylate (MW = 152), a typical volatile compound, was detected as protonated molecules (*m/z* 153) from all of the jasmine tea products (shown as a small peak in **Figure 2a**). In the CID mass spectrum, the precursor ions (*m/z* 153) gives major fragments of *m/z* 121, 93, and 135 by the loss of methanol, CH_3OCOH, and water, respectively. These data are consistent with the CID spectrum obtained with APCI-MS using authentic methyl salicylate. Note that numerous compounds were successfully detected from the tea products; according to the literature, most of these compounds were identical to those already reported in previous studies (*5*, *7*, *8*, *10*, *11*, *14*, *21*). Theoretically, all of the peaks detected could be identified by multiple-stage tandem mass spectrometry, plus the use of reference compounds for improved confidence if necessary. However, it is beyond the scope of this work to interpret all of the detected peaks.

Differentiation of Oolong Tea Products. Oolong tea is a traditional Chinese type of tea somewhere in between green and black in oxidation, ranging from 10 to 70% oxidation (*1*). As a result, oolong tea has a unique taste and a nuanced flavor profile. Usually, tea connoisseurs classify the oolong tea by its aroma (often fragrant or flowery), taste, and aftertaste (often melony). Depending on the manufacturing process, the quality of oolong tea can be varied in a wide range. As the first demonstration, 3 types of oolong tea samples (i.e., AO1, BO2, CO3), made from the same material by different manufacturers (i.e., by manufacture A, B, and C, respectively), were chosen for experiments. All of the samples were black-green and were shaped as long curly leaves. The major difference lies in the manufacturing process. Oolong tea BO2 and CO3 were samples fermented for a long time (2 days) and dried at 70 and 60 °C, respectively. Oolong tea AO1 was a sample fermented for a short time (1 day) and dried at 60 °C. Apparently, the quality difference between these oolong teas is not distinguishable, from the appearance and flavor of the tea product, to an ordinary consumer.

In contrast, DAPCI-MS provides mass spectral fingerprints of the oolong tea products, and the difference between the samples tested can be easily visualized by PCA. A score plot of PCA of the three oolong tea samples is shown in **Figure 3**. Clearly, all three samples are classified into three clusters, which are separated in PC 1 and PC 2. A single sign designates one mass spectrum averaged with multiple scans. As shown in **Figure 3**, the distribution of these signs in each cluster is restricted in a narrow scope. This indicates that the precision of the mass spectrometric measurement was good enough that the sample differentiation could not be failed by different measurements. The distance between clusters of the sample BO2 and those of the sample CO3 is much shorter than that between other samples (**Figure 3a**). The distribution of the clusters indicates that (1) the separation of the samples, in the PC 1 and PC 2 plane, is mainly based on the fermentation time, and (2) the roasting temperature contributed less to differentiation of the quality of the three oolong tea samples. However, this does not mean that the roasting temperature is not an important role for controlling the quality of the sample. For instance, in the three-dimensional (3-D) plot of the PCA results (**Figure 3b**), all the three oolong tea samples are well separated in the space along PC 3. From **Figure 3b**, it is clear that the 3-D PCA score plot is better than the 2-D plot for visualizing the difference between all three tea samples, because the difference between the BO2 and the CO3 samples is well-separated in the 3-D space. Furthermore, PCA provides differential peaks distinguishing these oolong tea samples in the PCA loading plots

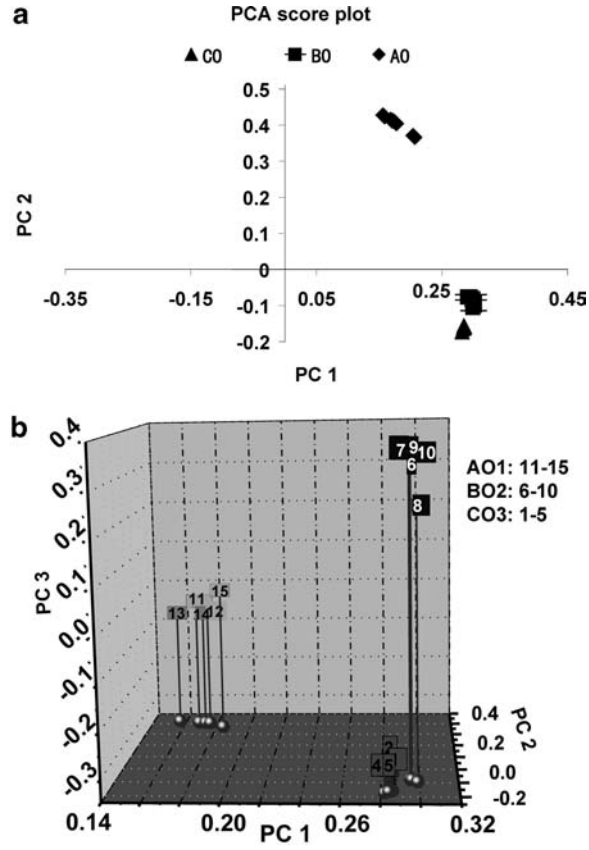

Figure 3. Separation of three oolong tea samples by PCA. (**a**) 2-D PCA score plot; (**b**) 3-D PCA score plot. The percentages of variance explained by PC1, PC2, and PC3 are 59.7, 26.2, and 13.0%, respectively.

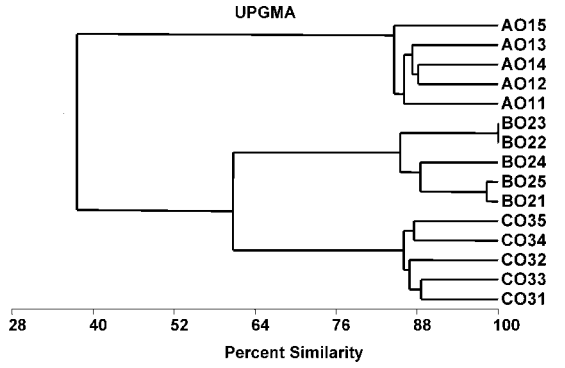

Figure 4. Cluster analysis results of DAPCI-MS raw data recorded from three oolong tea products.

(**Figure S1** in the Supporting Information). Potentially, these peaks can be used as molecular markers to evaluate oolong tea products. It is conceivable that such molecular markers could be monitored individually for online monitoring of tea quality in the industry, using a simple and cheap mass spectrometric sensor (*45*) tuned to one or several diagnostic signals.

Cluster analysis (CA) is also widely used to classify samples. In this study, CA was used to cross-check the results obtained in PCA. **Figure 4** shows the CA results of the three oolong tea samples tested by DAPCI-MS. Basically, the samples are all oolong tea products, so that they have a similarity of about 36%. The BO2 and CO3 samples are about 60% similar, thus they are of the most similar among the three samples. This is in agreement with the PCA results (**Figure 3**). In the CA results, the similarity varies from 100 to 88% for each sample. Theoretically, for a homogeneous tea sample, the similarity should be 100% for each sample because there is only one sample tested with multiple measurements. In fact, the oolong tea, and green tea as well, has no chemically homogeneous surfaces because the tea products are twisted tea leaves. Consequently, the deviation of the similarities for a single sample might be resulted from the chemically heterogeneous tea surfaces, with additional contribution from the DAPCI-LTQ-MS measurements. To evaluate the deviation contributed by the LTQ-MS instrument, multiple tries for analysis of the same sample were made in three days. As a result, the fingerprints of the same sample obtained before and after the three days were very similar. For example, the relative standard deviation of the peaks at *m/z* 93 and *m/z* 150 from the sample AO1 were calculated to be 3.8% ($n = 10$) and 3.2% ($n = 15$), respectively. Thus, it is believed that the LTQ-MS instrument made a minor contribution to the deviation of the similarity. The homogeneity of tea samples can be improved if the tea leaves are carefully ground to be uniform powders (e.g., black tea powders); and the quantitative repeatability, especially for the low molecular weight species, can be enhanced using a quadrupole mass filter or time-of-flight mass analyzer rather than an ion trap. However, as demonstrated here with either PCA or CA, the deviation of the homogeneity of the tea products and/or the DAPCI-LTQ-MS measurements can not change the separation patterns of the samples. Thus, it is not necessary to precisely perform quantitative measurements of the abundance of specific ions to differentiate tea products on the basis of pattern recognition of the mass fingerprints. On the other hand, accurate quantification of specific ions will be necessary for tea product differentiation, if the mass fingerprints over a wide range are not available when a simple mass sensor rather than a mass spectrometer is used.

Differentiation of Multiple Tea Samples. Some flower tea products such as jasmine tea are becoming more popular. Another set of 40 samples, including 3 types of oolong tea samples (15 individual sample), 4 types of green tea samples (20 individual sample), and 1 type of jasmine tea samples (5 individual sample), was used to validate the feasibility of DAPCI-MS for rapid differentiation of tea products using statistical approaches. Note that each type of tea sample consisted of five individual sample of the same type of tea products (shown in **Table 1**). **Figure 5a** shows the 2-D PCA score plot of the 40 samples tested. Apparently, all of the samples are classified into eight clusters in the PC 1 and PC 2 plane. These clusters are separated from each other, which corresponds to the eight types of tea products. For a given cluster, the distance between each individual sample is significantly shorter than those between different clusters (i.e., types of tea). The difference between every single sample (e.g., AO1, AO2, etc.) of a certain type of product (e.g., AO, BO, etc.) does not hinder the differentiation of the samples based on their mass spectral fingerprints. The difference between different types of tea products (e.g., AO, BO, AG, DJ, etc.) is clearly visualized in the PCA score plot. Although jasmine tea was made from green tea and jasmine flowers, the unique jasmine fragrant makes the jasmine tea easily distinguishable from any other tea products without jasmine flowers by a sensory method. This is also reflected by the PCA results obtained using the DAPCI mass spectral fingerprints. More interestingly, all four types of green tea, which are not distinguishable by a sensory method, are differentiated successfully in the PCA plot. This finding shows that DAPCI-MS combined with PCA is a useful tool for the differentiation of multiple tea products based on their mass spectral fingerprints. The success of differentiation of all of these tea products is also shown in a 3-D PCA score plot (**Figure**

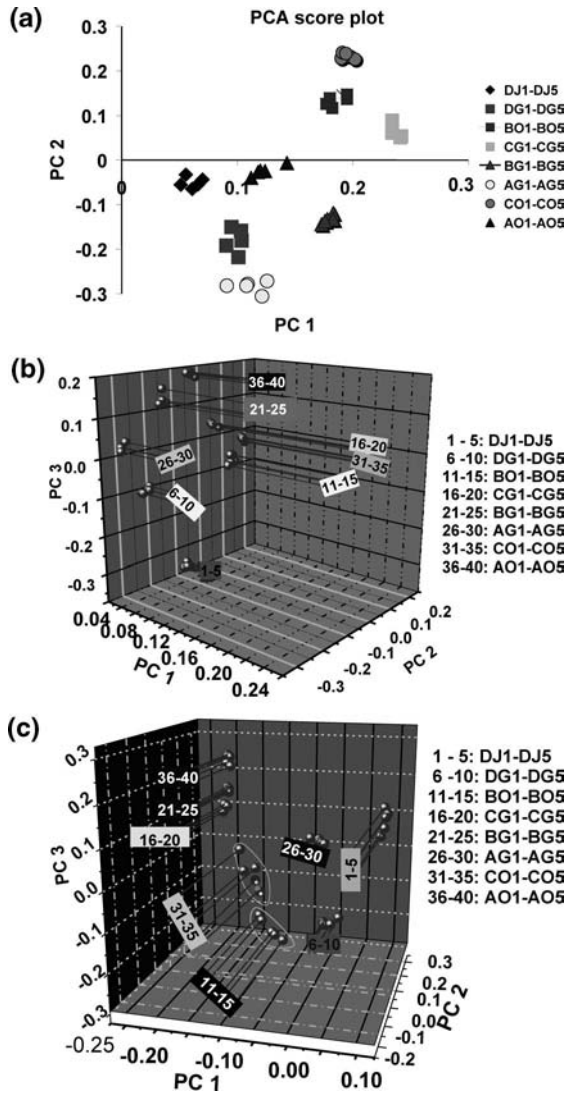

Figure 5. Score plots of PCA results for 40 tea products. (**a**) separation of oolong tea, green tea, and jasmine tea in a 2-D plane; (**b**) separation of oolong tea, green tea, and jasmine tea in a 3-D space. The percentages of variance explained by PC1, PC2, and PC3 are 71.8, 15.4, and 8.6%, respectively. (**c**) Separation of oolong tea, green tea, and jasmine tea by supervised PCA in a 3-D space. The percentages of variance explained by PC1, PC2, and PC3 are 59.7, 20.3, and 13.0%, respectively. Each sign notifies a DAPCI-MS spectrum recorded using a single sample, but averaged with five measurements. Each measurement was of an average time of 1 min.

5b), where the similarities and differences between all 40 tea products are clearly visualized in the 3-D space.

The PCA loading plots of the 40 samples are shown in **Figure S2** of the Supporting Information. The major differential peaks (i.e., peaks of significant abundances in the PCA loading plots, which correspond to ions that contribute most to differentiation of the samples) shown in the PCA loading plots are potentially useful as molecular markers for quality evaluation of tea products. For this reason, typical differential peaks at *m/z* 100, 128, 137, 138, 145, 150, 152, 195, 196 and 212 were selected as molecular markers, because they were outstanding in the PCA loading plots, for supervised PCA to separate all the 40 tea products. As expected, all of the samples are discriminated in the PCA score plot (**Figure 5c**). This indicates that tea products could be differentiated using some characteristic ions (e.g., ions of biomarkers) rather than a full mass spectrum. This is of interest for the tea industry, because the high cost of current commercial mass spectrometers prevents wide applications of this technique in the industry. Potentially, a simple mass spectrometer-based sensor can be designed, with significantly reduced size and cost, to selectively monitor specific ions of interest. Therefore, the high-cost mass spectrometers can be replaced by cheap, miniature mass sensors with low power consumption. Such an instrument would be particularly useful in tea industry, especially when the simple mass spectrometer is featured with atmospheric pressure desorption sampling/ionization techniques.

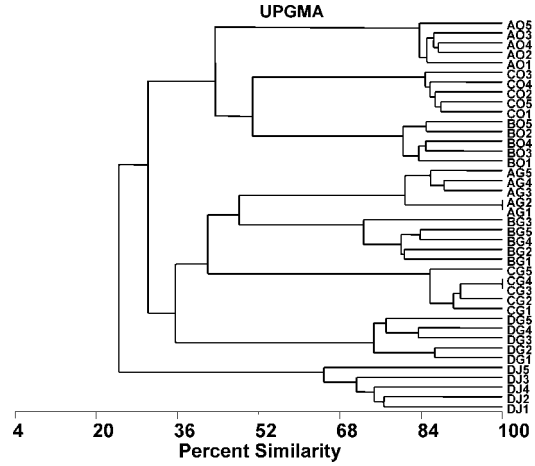

Figure 6. Results of cluster analysis using DAPCI-MS raw data recorded from 40 tea samples.

Cluster analysis (shown in **Figure 6**) was also performed using the same data set as those used in PCA (**Figure 5**). All 40 tea samples have a similarity about 24%. This reflects the fact that the samples tested are intrinsically similar to each other because they are all tea samples. The 40 tea samples are clustered into three groups such as jasmine tea, green tea, and oolong tea. The jasmine tea is far separated from all the other products. This indicates that, to a mass spectrometer, jasmine tea is far dissimilar to the oolong tea or green tea products. The 4 types of green tea samples (i.e., AG, BG, CG, and DG) are clustered in one group, with similarities of 36% and 28% to jasmine tea and oolong tea, respectively. In the green tea group, the AG sample group is more similar to the BG sample group, with a similarity of 50%. Similar to the CA results obtained with three samples (**Figure 4**), the similarities of each sample within a given group are about 84%, which is probably contributed jointly by the deviation of the measurement and the difference between the individual samples. Compared with the PCA score plots in **Figure 5**, the CA provides similar classification of the 40 samples, indicating that the chemical fingerprints of tea products, recorded using DAPCI-MS without sample pretreatment, can be used directly for quality assessment based on pattern recognition using either PCA or CA.

Analysis Speed. Generally, mass analysis, even tandem mass analysis, can be done within seconds. To date, the bottleneck of throughput for mass spectrometric analysis remains in the sampling introduction and ionization process prior to mass analysis. Because no sample pretreatment is required, direct ambient sampling/ionization techniques (*31–39*) substantially increase the throughput of mass spectrometry, providing real-world capabilities for high-throughput analysis of complex samples. As demonstrated here, no sample pretreatment is required by DAPCI-MS to profile the chemical fingerprints of tea products. A mass spectral fingerprint of tea product can be recorded within 10–20 ms if the sample is loaded prior to the

sampling process. Otherwise, the analysis speed is most likely to be controlled by sample reloading process. The analysis speed can be significantly enhanced if a transport belt is used (*31*). So far, 10 samples can be profiled within 15 s using the transferring belt to load samples. Promisingly, the throughput can be further improved for industry process monitoring.

ABBREVIATIONS USED

APCI, atmospheric pressure chemical ionization; CA, cluster analysis; DAPCI, surface desorption atmospheric pressure chemical ionization; DESI, desorption electrospray ionization; ESI, electrospray ionization; EESI, extractive electrospray ionization; PCA, principal component analysis; MS, mass spectrometry; NMR, nuclear magnetic resonance.

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Supporting Information Available: PCA loading plots for three oolong tea samples and all 40 tea samples are shown in **Figures S1 and S2**, respectively.

LITERATURE CITED

(1) Zhu, Y. X.; Huang, H.; Tu, Y. Y. A review of recent studies in China on the possible beneficial health effects of tea. *Int. J. Food Sci. Tech.* **2006**, *41* (4), 333–340.

(2) Glabasnia, A.; Hofmann, T. Sensory-directed identification of taste-active ellagitannins in American (*Quercus alba* L.) and European oak wood (*Quercus robur* L.) and quantitative analysis in bourbon whiskey and oak-matured red wines. *J. Agric. Food Chem.* **2006**, *54* (9), 3380–3390.

(3) Liang, Y. R.; Zhang, L. Y.; Lu, J. L. A study on chemical estimation of pu-erh tea quality. *J. Sci. Food Agric.* **2005**, *85* (3), 381–390.

(4) Kolanowski, W.; Jaworska, D.; Weissbrodt, J. Importance of instrumental and sensory analysis in the assessment of oxidative deterioration of omega-3 long-chain polyunsaturated fatty acid-rich foods. *J. Sci. Food Agric.* **2007**, *87* (2), 181–191.

(5) Miketova, P.; Schram, K. H.; Whitney, J. L.; Kerns, E. H.; Valcic, S.; Timmermann, B. N.; Volk, K. J. Mass spectrometry of selected components of biological interest in green yea extracts. *J. Agric. Food Chem.* **1998**, *61* (4), 461–467.

(6) Amatatongchai, M.; Hofmann, O.; Nacapricha, D.; Chailapakul, O.; Demello, A. J. A microfluidic system for evaluation of antioxidant capacity based on a peroxyoxalate chemiluminescence assay. *Anal. Bioanal. Chem.* **2007**, *387* (1), 277–285.

(7) Pongsuwan, W.; Fukusaki, E.; Bamba, T.; Yonetani, T.; Yamahara, T.; Kobayashi, A. Prediction of Japanese green tea ranking by gas chromatography/mass spectrometry-based hydrophilic metabolite fingerprinting. *J. Agric. Food Chem.* **2007**, *55* (2), 231–236.

(8) Mizukami, Y.; Kohata, K.; Yamaguchi, Y.; Hayashi, N.; Sawai, Y.; Chuda, Y.; Ono, H.; Yada, H.; Yoshida, M. Analysis of acrylamide in green tea by gas chromatography–mass spectrometry. *J. Agric. Food Chem.* **2006**, *54* (19), 7370–7377.

(9) Cordella, C.; Moussa, I.; Martel, A. C.; Sbirrazzuoli, N.; Lizzani-Cuvelier, L. Recent developments in food characterization and adulteration detection: Technique-oriented perspectives. *J. Agric. Food Chem.* **2002**, *50* (7), 1751–1764.

(10) Bilia, A. R.; Flamini, G.; Taglioli, V.; Morelli, I.; Vincieri, F. F. GC-MS analysis of essential oil of some commercial Fennel teas. *Food Chem.* **2002**, *76* (3), 307–310.

(11) Repolles, C.; Herrero-Martinez, J. M.; Rafols, C. Analysis of prominent flavonoid aglycones by high-performance liquid chromatography using a monolithic type column. *J. Chromatogr. A* **2006**, *1131* (1–2), 51–57.

(12) Neilson, A. P.; Green, R. J.; Wood, K. V.; Ferruzzi, M. G. High-throughput analysis of catechins and theaflavins by high performance liquid chromatography with diode array detection. *J. Chromatogr. A* **2006**, *1132* (1–2), 132–140.

(13) Chen, H. X.; Zhang, M.; Xie, B. J. Quantification of uronic acids in tea polysaccharide conjugates and their antioxidant properties. *J. Agric. Food Chem.* **2004**, *52* (11), 3333–3336.

(14) Del Rio, D.; Stewart, A. J.; Mullen, W.; Burns, J.; Lean, M. E. J.; Brighenti, F.; Crozier, A. HPLC-MSn analysis of phenolic compounds and purine alkaloids in green and black tea. *J. Agric. Food Chem.* **2004**, *52* (10), 2807–2815.

(15) Yao, L. H.; Jiang, Y. M.; Datta, N.; Singanusong, R.; Liu, X.; Duan, J.; Raymont, K.; Lisle, A.; Xu, Y. HPLC analyses of flavanols and phenolic acids in the fresh young shoots of tea (*Camellia sinensis*) grown in Australia. *Food Chem.* **2004**, *84* (2), 253–263.

(16) Wang, H. F.; Provan, G. J.; Helliwell, K. HPLC determination of catechins in tea leaves and tea extracts using relative response factors. *Food Chem.* **2003**, *81* (2), 307–312.

(17) Peng, C. F.; Kuang, H.; Li, X. Q.; Xu, C. L. Evaluation and interlaboratory validation of a GC-MS method for analysis of pesticide residues in teas. *Chem. Pap.* **2007**, *61* (1), 1–5.

(18) Jacques, R. A.; Freitas, L. D.; Peres, V. F.; Dariva, C.; de Oliveira, J. V.; Caramao, E. B. Chemical composition of mate tea leaves (*Ilex paraguariensis*): A study of extraction methods. *J. Sep. Sci.* **2006**, *29* (18), 2780–2784.

(19) Wright, J.; Wulfert, F.; Hort, J.; Taylor, A. J. Effect of preparation conditions on release of selected volatiles in tea headspace. *J. Agric. Food Chem.* **2007**, *55* (4), 1445–1453.

(20) Desai, M. J.; Armstrong-K, D. W. Analysis of derivatized and underivatized theanine enantiomers by high-performance liquid chromatography/atmospheric pressure ionization-mass spectrometry. *Rapid Commun. Mass Spectrom.* **2004**, *18* (3), 251–256.

(21) Zeeb, D. J.; Nelson, B. C.; Albert, K.; Dalluge, J. J. Separation and identification of twelve catechins in tea using liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry. *Anal. Chem.* **2000**, *72* (20), 5020–5026.

(22) Baranska, M.; Schulz, H.; Joubert, E.; Manley, M. In situ flavonoid analysis by FT-Raman spectroscopy: Identification, distribution, and quantification of aspalathin in green rooibos (*Aspalathus linearis*). *Anal. Chem.* **2006**, *78* (22), 7716–7721.

(23) Seetohul, L. N.; Islam, M.; O'Hare, W. T.; Ali, Z. Discrimination of teas based on total luminescence spectroscopy and pattern recognition. *J. Sci. Food Agric.* **2006**, *86* (13), 2092–2098.

(24) Pelillo, M.; Bonoli, M.; Biguzzi, B.; Bendini, A.; Toschi, T. G.; Lercker, G. An investigation in the use of HPLC with UV and MS-electrospray detection for the quantification of tea catechins. *Food Chem.* **2004**, *87* (3), 465–470.

(25) Van Dorsten, F. A.; Daykin, C. A.; Mulder, T. P. J.; Van Duynhoven, J. P. M. Metabonomics approach to determine metabolic differences between green tea and black tea consumption. *J. Agric. Food Chem.* **2006**, *54* (18), 6929–6938.

(26) Le Gall, G.; Colquhoun, I. J.; Defernez, M. Metabolite profiling using H-1 NMR spectroscopy for quality assessment of green tea, *Camellia sinensis* (L.). *J. Agric. Food Chem.* **2004**, *52* (4), 692–700.

(27) Venzie, J. L.; Castro, J.; Krishna, M. V. B.; Nelson, D. M.; Marcus, R. K. Electron-impact and glow-discharge ionization LC-MS analysis of green tea tincture. *Anal. Bioanal. Chem.* **2007**, *387* (1), 321–333.

(28) Ford, M. J.; Deibel, M. A.; Tomkins, B. A.; Van Berkel, G. J. Quantitative thin-layer chromatography/mass spectrometry analysis of caffeine using a surface sampling probe electrospray ionization tandem mass spectrometry system. *Anal. Chem.* **2005**, *77* (14), 4385–4389.

(29) Menet, M. C.; Sang, S. M.; Yang, C. S.; Ho, C. T.; Rosen, R. T. Analysis of theaflavins and thearubigins from black tea extract by MALDI-TOF mass spectrometry. *J. Agric. Food Chem.* **2004**, *52* (9), 2455–2461.

(30) Takats, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* **2004**, *306* (5695), 471–473.

(31) Chen, H. W.; Talaty, N. N.; Takats, Z.; Cooks, R. G. Desorption electrospray ionization mass spectrometry for high-throughput analysis of pharmaceutical samples in the ambient environment. *Anal. Chem.* **2005**, *77* (21), 6915–6927.

(32) Chen, H. W.; Zhang, X.; Luo, M. B. Desorption electrospray ionization mass spectrometry for fast detection of Sudan dyes in foods without sample pretreatment. *Chin. J. Anal. Chem.* **2006**, *34* (4), 464–468.

(33) Talaty, N.; Takats, Z.; Cooks, R. G. Rapid in situ detection of alkaloids in plant tissue under ambient conditions using desorption electrospray ionization. *Analyst* **2005**, *130* (12), 1624–1633.

(34) Chen, H. W.; Pan, Z. Z.; Talaty, N.; Raftery, D.; Cooks, R. G. Combining desorption electrospray ionization mass spectrometry and nuclear magnetic resonance for differential metabolomics without sample preparation. *Rapid Commun. Mass Spectrom.* **2006**, *20* (10), 1577–1584.

(35) Wiseman, J. M.; Ifa, D. R.; Song, Q. Y.; Cooks, R. G. Tissue imaging at atmospheric pressure using desorption electrospray ionization (DESI) mass spectrometry. *Angew. Chem. Int. Ed.* **2006**, *45* (43), 7188–7192.

(36) Cody, R. B.; Laramee, J. A.; Durst, H. D. Versatile new ion source for the analysis of materials in open air under ambient conditions. *Anal. Chem.* **2005**, *77* (8), 2297–2302.

(37) Williams, J. P.; Patel, V. J.; Holland, R.; Scrivens, J. H. The use of recently described ionisation techniques for the rapid analysis of some common drugs and samples of biological origin. *Rapid Commun. Mass Spectrom.* **2006**, *20* (9), 1447–1456.

(38) Chen., H. W.; Wortmann, A.; Zhang, W. H.; Zenobi, R. Rapid in Vivo Fingerprinting of Non-volatile Compounds in Breath by Extractive Electrospray Ionization Quadrupole Time-of-flight Mass Spectrometry. *Angew. Chem., Int. Ed.* **2007**, *46* (4), 580–583.

(39) Zhou, Z.; Jin, M.; Ding, J.; Zhou, Y.; Zheng, J.; Chen, H. Rapid detection of atrazine and its metabolite in raw urine by extractive electrospray ionization mass spectrometry, *Metabolomics* **2007**, DOI: 10.1007/s11306–006–0050–2.

(40) Chen, H. W.; Venter, A.; Cooks, R. G. Extractive electrospray ionization for direct analysis of undiluted urine, milk and other complex mixtures without sample preparation. *Chem. Commun.* **2006**, (19), 2042–2044.

(41) Gu, H.; Chen, H.; Pan, Z.; Jackson, A. U.; Talaty, N.; Xi, B.; Kissinger, C.; Duda, C.; Mann, D.; Raftery, D.; Cooks, R. G. Monitoring Diet Effects from Biofluids and Their Implications for Metabolomics Studies. *Anal. Chem.* **2007**, *79* (1), 89–97.

(42) Takats, Z.; Cotte-Rodriguez, I.; Talaty, N.; Chen, H. W.; Cooks, R. G. Direct, trace level detection of explosives on ambient surfaces by desorption electrospray ionization mass spectrometry. *Chem. Commun.* **2005**, (15), 1950–1952.

(43) Chen, H. W.; Lai, J. F.; Zhou, Y. F.; Huan, Y. F.; Li, J. Q.; Zhang, X.; Wang, Z. C.; Luo, M. B. Surface Desorption atmospheric pressure chemical ionization mass spectrometry: instrumentation and characterization. *Chin. J. Anal. Chem.* **2007**, *35* (8), 1036–1041.

(44) Chen, H. W.; Zheng, J.; Zhang, X.; Wang, Z. C.; Luo, M. B.; Qiao, X. L. Surface Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry for Direct Ambient Sample Analysis without Toxic Chemical Contamination, *J. Mass Spectrom.* **2007**,DOI 10.1002/jms.1235.

(45) Zhang, C.; Chen, H.; Guymon, A. J.; Wu, G.; Cooks, R. G.; Ouyang, Z. Instrumentation and methods for ion and reaction monitoring using a non-scanning rectilinear ion trap. *Int. J. Mass Spectrom.* **2006**, *255–256* (1), 1–10.

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