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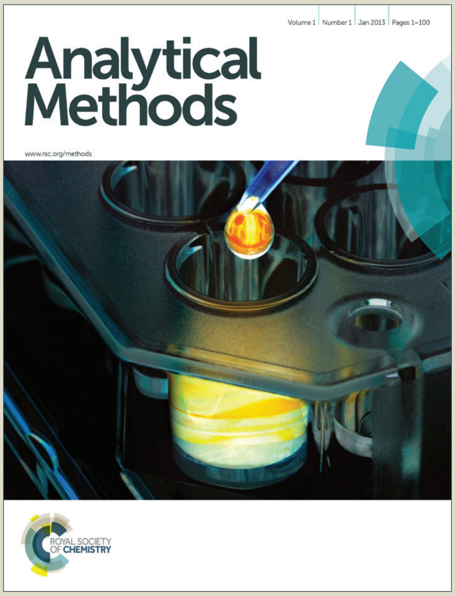

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Wooden-tip Electrospray Ionization Mass Spectrometry for trace analysis of toxic and hazardous compounds in Food Samples

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Wooden-tip electrospray ionization (Wooden-tip ESI) is applied to the rapid, in situ, direct qualitative and quantitative trace analysis of toxic and hazardous compounds in food samples. To evaluate the potential of wooden-tip ESI mass spectrometry (MS) in food analysis, pesticide, toxicant, date-rape drug, and illicit additive were detected in various food sample. Wooden-tip ESI-MS experiments were performed using wooden tip sampling and ionization, with examination by MS and tandem mass spectrometry (MS/MS). The results proved that wooden-tip ESI allows the detection and confirmation of traces of toxic and hazardous compounds in food. In addition, selected analystes in beverage was obtained at absolute levels as low as 10 pg ~level. Quantitation of analystes in liquid and powder sample were also evaluated. Single sample analysis was completed within 2 min. The data obtained shown that the wooden-tip ESI-MS is a promising tool for rapid analysis for food samples.

Introduction

Food safety is of the worldwide concern. Development of novel technique for rapid, reliable, sensitive detection of trace amounts of analytes in food samples is of increasing importance, particularly those involving analysis of complex samples.[1-3] Many analytical techniques applied in the food analysis such as liquid chromatography-mass spectrometry (LC-MS),[3] gas chromatography-mass spectrometry (GC-MS)[4] have been well routinely established. Mass spectrometry is one of most powerful analytical tools for food analysis, due to its high throughput, high sensitivity, and high specificity. Compared with lab analysis of field samples, direct sample analysis enables rapid identifications of suspicious samples, and allows early warning, saving time and resources. However, sample preparation and chromatography separations, which are time-consuming and complicated procedures, are usually required before MS detection. Developing protocols of rapid response are highly considerable in food analysis.

In the last ten years, MS studies have focused on the development of ambient ionization MS since the desorption electrospray ionization (DESI)[5] and direct analysis in real time (DART)[6] have been introduced. Many ambient ionization techniques have been developed,[7-11] and many of those are successfully applied in food analysis.[12-14] Development of ESI techniques with solid substrates have been introduced since ESI with copper wire was developed in the late 1990s.[15] To date, many materials such as metal needle,[16] paper,[17] toothpick,[18] metal foil,[19] and other materials,[20-22] have been developed as ESI emitters for sampling and ionization of various samples. The ESI using wooden tips (common toothpicks) are readily available with very low prices and can be directly mounted on a nanoESI device without any hardware modification,[18] showed that the wooden-tip could be use for many applications due to its convenient sampling and rapid analysis, e.g., pharmaceutical analysis,[23] metabolites analysis,[24, 25] forensic analysis,[26] and plant identification.[27]

In this work, the wooden-tip ESI-MS was used for rapid analysis of food samples. The porous surface, slim tip and hardness of wood tips are conveniently sampling in complicated conditions, and are generated subsequently electrospray ionization for MS detection when high voltage were applied and organic solvent was added. Food samples can be directly sampled by wooden tips with multiple applications. Furthermore, the sampling technique with porous wooden tip is a versatile method that could be the sampling liquids, vicious, and powder samples, which is the highly desirable feature for food analysis. In addition, the disposed wooden tip gives the additional advantages that cost are significantly decreased in analysis of a large sum of samples.

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Experimental

Materials

Methanol, ethanol, and formic acid used were bought from Chinese Chemical Reagent Co. Ltd (Shanghai, China). Sudan I (Solvent Yellow 14). beta-cypermethrin was purchased from Jiangsu Huangma Agrochemicals Co., Ltd (Lianyungang, China). Diazepam was purchased from AccuStandard, Inc. (New Haven, CT, USA). The tissue of *Gelsmium elegans*, a gift from Prof. X. Zhang at Luohe Traditional Chinese Medical Hospital (Luohe, China), was used for this research purpose only. The hot spot,[28, 29] which is prepared by hot soup and food material that is one of the most popular foods in south and southwest China,[27, 28] was brought from the Staff Canteen of Jiangxi Provincial Maternal and Child Health Hospital (Nanchang, China). The wooden tips (toothpick, the original body length is 5.0 cm, the body size are ~ 2.0 mm, the original tip size are 0.2 ~ 0.5mm, were manufactured by SunChua wood and bamboo company, Zhejiang, China), Coco cola drink (produced by Zhongliang Coca-Cola Beverage (Jiangxi) Co., Ltd., Nanchang, China), apple juice drink (produced by Jiangxi Huiyuan Juice Co., Ltd, Fuzhou, China), and red pepper powder (powder size 0.2 mm, produced by Zhihongda Agricultural products Co., Ltd, Zhaoqing, China) were purchased from the local Hongkelong Supermarket (Nanchang, China). The length of wooden tip was cut as 2.0 cm in this study. Diazepam Tablets, production of Shandong Xinyi Pharmaceutical Co., Ltd. (Dezhou, China), were supplied by the Jiangxi Provincial Maternal and Child Health Hospital (Nanchang, China). Water used in this study was Milli-Q water.

Wooden-tip Sampling

Wooden tip were cut a sharp tip (tip size: 0.1 mm) and were washed with methanol (1.0 ml) before use as previously described.[19, 25, 26] For sampling of liquid sample (i.e., soup, beverage) and viscous sample (i.e., residual drink), 1.0 cm of dry wooden tip was directly immersed in the sample about 10 seconds, then about 10 µL sample was sampled by the porous wooden tips (which was calculated by original volume minus the volume of after sampling). Before sampling of powder sample (i.e., pepper powder), the whole wooden tip was immersed in ethanol (1.0 ml) about 10 seconds, then powder samples (~ 0.2 mg) were wiped by the wet wooden tip-end.

Wooden-tip ESI-MS

All the MS experiments were performed using an AB SCIEX Qtrap 3200 mass spectrometer (Framingham, MA, USA) coupled with a nanospray device (Framingham, MA, USA) as previous described.[18] The wooden tip was placed in the front of MS inlet, the distance between MS inlet and wooden-tip-end was typically 0.8 cm. When a high voltage of 3.5 kV was supplied to the wooden tips and 5.0 µL solvent (methanol with 0.1% formic acid) was added on tip-end for generation of spray ionization, the analytes were sprayed out from the wooden-tip-end and then were introduced to the mass spectrometer for detection. The signal duration was about 2 minutes. The temperature of the MS inlet was maintained at 80°C. MS/MS was performed on the interest ions with appropriate collision energy (15-35 eV). MS/MS spectra could be recorded with a recording time. To perform the quantitative detection, selected-reaction monitoring (SRM) mode was used. To measure the LODs, in SRM mode, 10 µl liquid blank sample or 0.2 mg blank powder was loaded on wooden tip to detect the noise response, and then the 10 µl liquid samples or 0.2 mg powder samples spiked with low concentration of analyst was loaded on wooden tip to detect the signal response, the LOD was determined when the ration of signal-to-noise was measured at 3.The other parameters used in the experiment were the default values of the mass spectrometer.

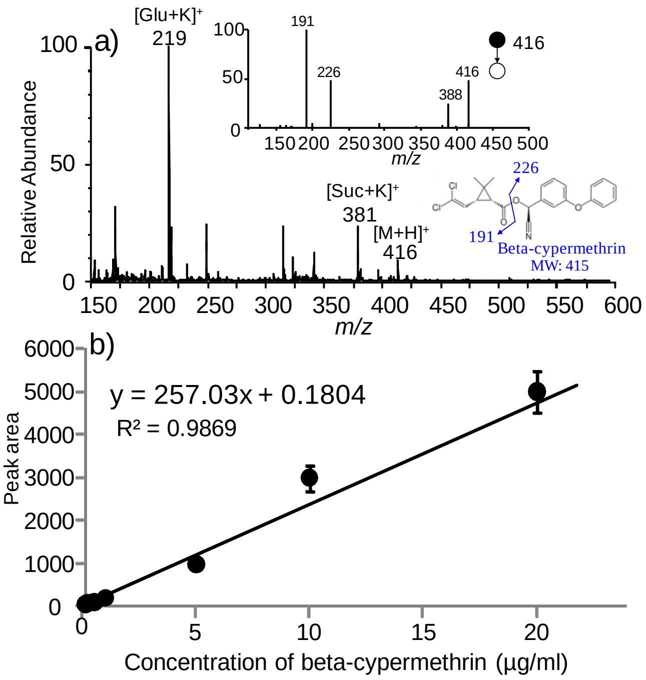

Figure 1 Detection of beta-cypermethrin in apple juice beverage with spiked 1µg/ml. a) full mass spectrum in the positive ion detection mode, inset shows the MS/MS mass spectrum of *m/z* 416, b) the plot of the peak area of ion pair beta-cypermethrin (*m/z* 416>191) vs the concentration(0.1 ~ 20.0 µg/ml).

Results and discussion

The first demonstration is the sampling and detection of pesticides in beverage (Figure 1). Beta-cypermethrin was selected as the representative pesticide in this study. Mass spectrum collected from the apple juice drink spiked with d beta-cypermethrin (0.5 µg/ml) using the wooden-tip ESI-MS, showed signals at *m/z* 219, 381 and *m/z* 416, which were corresponding to the potassium-glucose adduct $[M+K]^+$, potassium-sucrose adduct $[M+K]^+$ and protonated beta-cypermethrin $[M+H]^+$, respectively. Upon the MS/MS experiments (in inset of Fig.1b), the MS/MS mass spectra for the ion at *m/z* 416 shows the fragment ion at *m/z* 388, by the loss of CO, and the fragment ions of *m/z* 191 and *m/z* 226 are yielded by the cleavage of C-O bond in the ester group of cypermethrin (in inset of Fig.1b), which was agreement with previous studies.[30] Based on the characteristic signal of beta-cypermethrin (416 > 191), the linearity of the response was obtained at R^2=0.98 over the dynamic range 0.1-20 µg/ml of beta-cypermethrin in beverage (Fig. 1b); and the relative standard deviation (RSD) of 9.5 % was obtained for 6 measurements (10 µg/ml). The limit of detection (LOD) (S/N=3) for the beta-cypermethrin was determined as 30.0 ng/mL (absolute amount: 30.0 pg), which is much lower than the food standard (GB 2763-2012) of beta-cypermethrin in food.[31] Compared this results with the of previous studies on beverage analysis[30] which required a special container to deposit the liquid sample, these data demonstrated that trace amounts of pesticide in beverage could be simply sampled and be rapidly detected by wooden-tip ESI-MS.

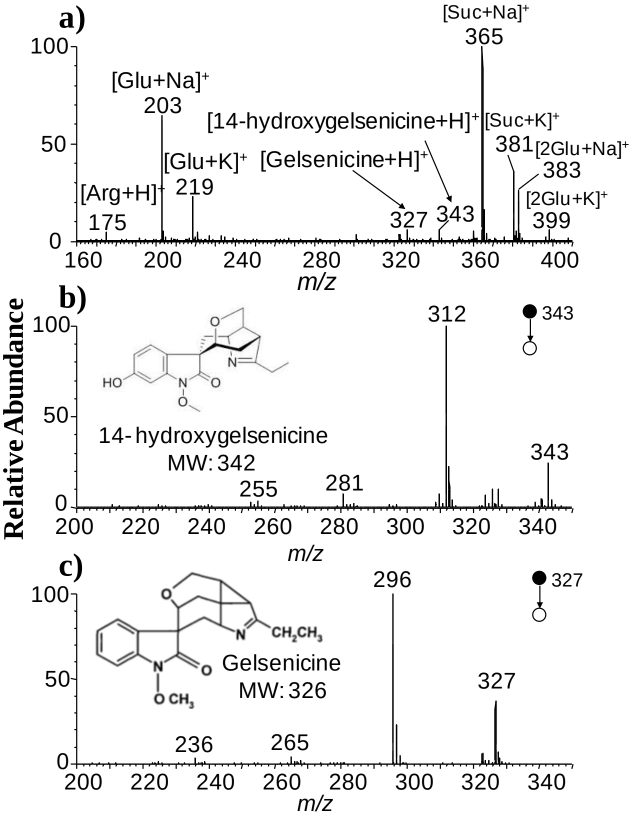

Figure 2 Analysis of hot soup samples spiked with Gelsemium elegant using wooden-tip ESI-MS in the positive ion mode. a) full mass spectrum, MS/MS mass spectra of ions at b) *m/z* 343 and c) *m/z* 327

The wooden tip sampling is useful for direct sampling of daily foods. For example, it is reported that many cases of food poisoning were caused by herbal *Gelsemium elegant* in South China, the identification of the toxicants in gastric content or residual food, however, are normally performed by GC-MS,[32-35] which are time consuming processes due to sample collection and sample pre-treatment. The wooden tip allows direct sample the hot soup (~ 95 °C), which makes it attractive for rapid analysis of the food samples with complex matrices. Figure 2a displayed a mass spectrum of raw hot soup (0.5 L) spiked with 2.0 mg tissue of *Gelsemium elegant*. Many nutritional ingredients such as amino acid, sugar and salt, were observed. The peaks at *m/z* 327 and *m/z* 343 are corresponding to the protonated molecules of gelsenicine and 14-hydorxygelsencine, Upon MS/MS experiments, these ions were confirmed by their fragment ions as shown in Figure 2b and Figure 2c, and were agreement with previous studies.[36] The analytical procedures, including wooden-tip sampling of soup, ESI-MS analysis, and MS/MS identification of compound, were completed in within minutes. To our knowledge, there is no report on direct sampling of hot soup by other ambient ionization techniques.

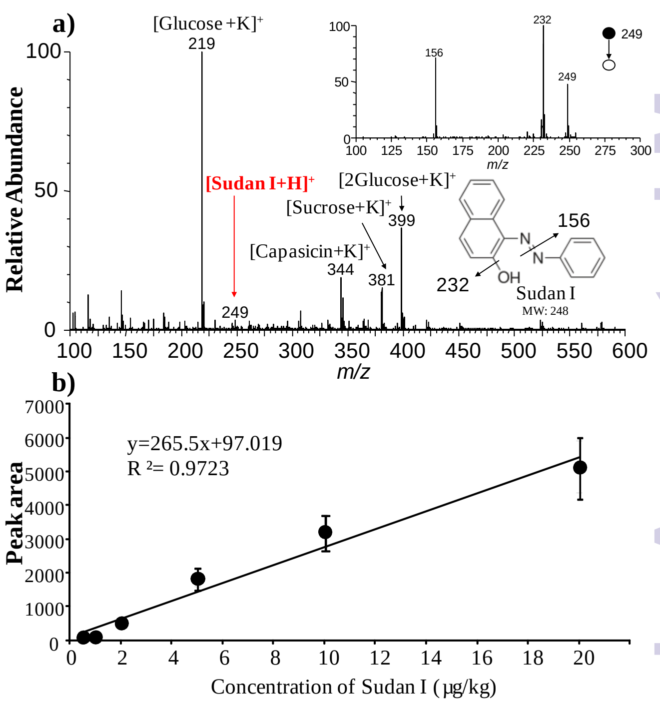

Figure 3 Analysis of red pepper powder with spiked 5 µg/kg Sudan I. a) full mass spectrum in the positive ion detection mode, inset shows the MS/MS mass spectrum of *m/z* 249, b) the plot of the peak area of ion pair Sudan I (*m/z* 249>156) vs concentration, showing the linear dynamic range between 0.5 ~ 20 µg/kg.

This method is also useful to detect a trace amount of analytes in powder sample. For example, red pepper high in capsaicin and vitamin C are becoming more and more popular among diet. However, Sudan I is illegally used as colorant in food and in many countries owing to it is carcinogenic potential for humans.[37] The red pepper samples are usually powders that makes sample pretreatment such as extracting, should be performed prior to the analysis.[38] So, fast quality monitoring is of importance for public concerns. The direct sampling and detection of Sudan I in powder pepper (5.0 µg/kg) using wet wooden tip was demonstrated. The main functional components in pepper including glucose, sucrose, and capsaicine were recorded in the mass spectrum as shown in Figure 3a. The peak at *m/z* 249 is corresponding to the protonated molecules of Sudan I. Upon the MS/MS experiment, the protonated Sudan I yield the ions at *m/z* 232 and *m/z* 156 by the loss of HO and aniline, respectively (inset of Fig. 3a). The Quantitative detection of Sudan I in red pepper powder was evaluated without addition of an internal standard. 0.2 mg powder per sample spiked with different concentration of Sudan I were prepared in Eppendorf tube and were sampled by wet wooden tip-end. Based on the characteristic signal of Sudan I (249 > 156), the linearity of the response (judged by the correlation coefficient) was R^2=0.97 over the dynamic range 0.5-20 µg/kg of Sudan I spiked (Fig. 3b). The LOD (S/N=3) for the detection of Sudan I was determined to be 0.1 µg/kg (absolute amount: 0.02 pg) by SRM. Reproducible measurements were evaluated (1.0 µg/kg), providing a relative standard deviation (RSD) of 23.2 % for 6 measurements. Compared the detection of Sudan dye in powder sample with previous work on the detection of Sudan dye in liquid sample by desorption atmospheric pressure chemical ionization,[39] the quantitative information could be provided by wooden-tip ESI-MS, and the sensitivity could also be comparable the detection of Sudan in pepper powder by paper spray ionization mass spectrometry.[40]

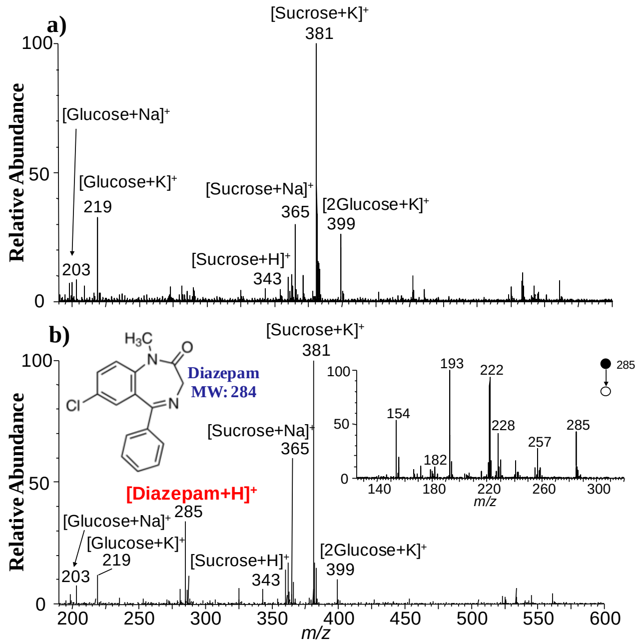

Figure 4 a) full mass spectrum of soft drink; b) mass spectrum of detection of diazepam in soft drink, the inset shows the MS/MS spectrum of *m/z* 285.

Finally, experiments were performed to quickly detect date-rape-drug in a soft drink, which is of paramount importance to human safety and evidence collection, but challenges analytical science in terms of sensitivity and feasibility. Drug-facilitated sexual assaults have reported widespread media coverage, and sedatives are the most popular drugs used for this purpose.[41] The diazepam is one of sedatives and is commonly used to anxiety, panic attacks, insomnia etc, which can impair short-term memory and increased feelings of sleepiness[42] and thus was used for sexual crime.[43] To simulate a crime scene, a can of Coca-Cola™ drink (350 mL) spiked with the powders of a piece of Diazepam Tablets™ (2.5 mg) was prepared. Then, the can of drink were poured out to simulate drank. However, the residual drink (~20.0 µL), which was quickly changed to viscous state (10.0 µL) at the air after 4.0 hours, can also be sampling by wooden tips, and then be ionization by wooden tip ESI-MS. As shown in Figure 4a, the peaks of sugars were dominated in the sample of Coca-Cola™. However, the interest peak at *m/z* 285 was significantly raised in the drink with spiked diazepam, which is well matched by protonated molecule of diazepam (MW: 284). The MS/MS experiment was conducted to further confirm the diazepam. As shown in the inset of Figure. 4b, the fragment ions at *m/z* 257, 228, 222, 193, 182 and 154 was produced by the loss of CO, CH_3NCO, COHCl, toluene, COHCl+benzonitrile and benzonitrile+CO, respectively. Those fragments are identical to those obtained using authentic compounds and is good agreement with previous studies.[44] LOD (S/N=3) and RSD (n=6) for the detection of diazepam in drink were found to be 30 ng/mL (absolute amount: 30.0 pg) and 8.7 %.

Conclusions

In this study, wooden-tip ESI-MS was implemented with litter sample pretretment for rapid analysis of food samples. A trace amount (10 pg level as total amount) of analytes present in the complex food samples, including liquid beverage, hot soup, powder food, and viscous drink samples, were successfully detected as protonated molecular ions, followed by MS/MS for identification. Compared the wooden-tip EIS with other ambient ionization techniques and conventional method for analysis of raw food samples, wooden-tip ESI-MS has the advantages of easy fabrication, convenient use, efficient sampling, and high throughput with minor sample pretreatment. In addition, the disposed wooden tip could be minimized in cost, and expendable to allow it suitable for analysis of a large amount of samples. Further improvement and applications of this method will be performed to establish it as a new general approach for food safety.

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