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Direct analysis of herbal powders by pipette-tip electrospray ionization mass spectrometry

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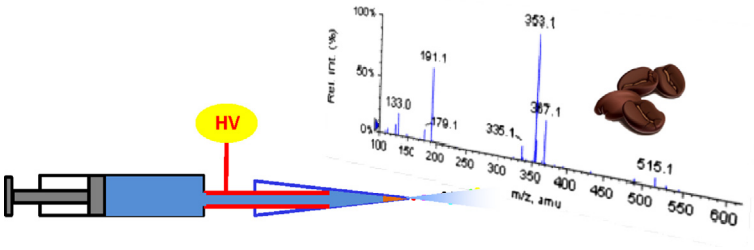
HIGHLIGHTS

- Combination of pipette tips with syringe and syringe pump for direct analysis of herbal powders by mass spectrometry.
- Online extraction and rapid ESI-MS analysis of various herbal powders with stable, durable and reproducible signals.
- Rapid differentiation of closely related herbal species.
- Rapid quantitation of caffeine contents in tea samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Conventional electrospray ionization mass spectrometry (ESI-MS) is widely used for analysis of solution samples. The development of solid-substrate ESI-MS allows direct ionization analysis of bulky solid samples. In this study, we developed pipette-tip ESI-MS, a technique that combines pipette tips with syringe and syringe pump, for direct analysis of herbal powders, another common form of samples. We demonstrated that various herbal powder samples, including herbal medicines and food samples, could be readily online extracted and analyzed using this technique. Various powder samples, such as *Rhizoma coptidis*, lotus plumule, great burdock achene, black pepper, *Panax ginseng*, roasted coffee beans, *Fructus Schisandrae Chinensis* and *Fructus Schisandrae Sphenantherae*, were analyzed using pipette-tip ESI-MS and quality mass spectra with stable and durable signals could be obtained. Both positive and negative ion modes were attempted and various compounds including amino acids, oligosaccharides, glycosides, alkaloids, organic acids, ginosensides, flavonoids and lignans could be detected. Principal component analysis (PCA) based on the acquired mass spectra allowed rapid differentiation of closely related herbal species.

1. Introduction

Plants are important natural resources for foods and herbal medicines. Analysis of chemical compositions and bioactive components of plants is an essential part of analytical chemistry and natural product research. Such analysis typically involves various experimental steps including extraction, separation and characterization, which can be time-consuming and labor-intensive. Mass spectrometry (MS) is a powerful tool for analysis of various samples. In recent years, great efforts have been made to allow direct analysis of samples, including plant samples, by mass spectrometry. Direct analysis of plant tissues by MS can now be achieved by using techniques such as secondary ion mass spectrometry (SIMS), matrix-assisted laser

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desorption/ionization (MALDI), desorption electrospray ionization (DESI) and electrospray-assisted laser desorption/ionization (ELDI, or laser ablation electrospray ionization LAESI) [1–5]. These techniques allow *in situ* profiling of phytochemicals on surfaces of plant tissues, and the latter two are ambient ionization techniques that can be performed under ambient pressure and require little or no sample preparation [6–10]. Recently, direct ionization techniques were developed for analysis of plant and animal tissues [11–14]. These techniques involved connection of a high voltage to the tissue sample, and with application of solvents if necessary, spray ionization could be induced from the tip of the tissue sample and a mass spectrum regarding chemical components of the sample could be obtained.

Herbal powders are commonly present as food, supplementary products, medicines, *etc.* For example, many household products such as tea, coffee and pepper are usually sold and used in powder forms, and many herbal medicines are prepared in powder forms for convenient uses. Development of a simple and rapid method for analysis of these herbal powders is thus highly desirable for their quality control and food safety. However, there have been few studies about direct analysis of herbal powders by mass spectrometry [15–17]. In our group, attempts have been made to use solid-substrate electrospray ionization mass spectrometry (solid-substrate ESI-MS) [12,15], *e.g.*, wooden-tip ESI-MS, for direct analysis of herbal powders. Although ion signals with desired intensity could be readily obtained, the reproducibility and duration of signals needed to be further improved, particularly for comparison of different species and for quantitation purpose. In this study, by combining common and disposable pipette tips with syringe pump and ESI-MS (termed pipette-tip ESI-MS herein), we demonstrated that herbal powders could be readily analyzed and stable and durable signals could be obtained. The method is simple, rapid, only requires a small amount of powder samples, and allows quantitative measurements of chemical components in the samples.

2. Experimental

2.1. Materials

Fructus Schisandrae Chinensis (FSC) and *Fructus Schisandrae Sphenantherae* (FSS) samples were purchased from licensed pharmacy stories in Hong Kong and mainland China and further confirmed by Ms. Dawn Tung Au, an expert in authentication of herbal medicines. Other herbal medicine samples were purchased from licensed pharmacy stores in Hong Kong. Tea samples (green tea, black tea, Pu Erh tea, Iron Buddha tea and jasmine tea) and other herbal food samples were purchased from local supermarkets. Water was distilled water prepared using a Milli-Q system (Millipore Laboratory, USA). All other solvents were of HPLC grade and purchased from Tedia (Fairfeild, OH, USA). Caffeine was purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Preparation of samples and standard solutions

The herbal samples used for the analysis in this study were either directly obtained in powder forms or homogenized to powders. The particle sizes of the analyzed samples were typically smaller than 40 mesh. For qualitative analysis, approximate 1 mg of sample powder was filled into a pipette tip for direct analysis.

For direct quantitation of caffeine in the five tea samples using pipette-tip ESI-MS/MS, standard addition method was applied. Caffeine standard solutions used in the standard addition method were prepared at a concentration of 4.00% (w/v) first and then diluted to concentrations of 1.00% and 2.00% with the same solvent (methanol/water, 80/20, v/v). The accurately measured 1 mg of tea powder sample was filled into a pipette tip and spiked with 1 μL of standard caffeine solutions (0, 1.00%, 2.00% and 4.00%). These moist powder samples with spiked caffeine were dried at 60 °C for 5 min and cooled to room temperature for MS analysis.

For quantitation of caffeine in tea samples with ultra performance liquid chromatography ESI-MS/MS (UPLC–ESI-MS/MS), caffeine standard solutions (2, 4, 6, 12, 24, 48 ng mL^{-1}) for construction of calibration curve (peak area *versus* caffeine concentration) were prepared by diluting a 4800 ng mL^{-1} caffeine stock solution with methanol/water (50/50, v/v). Each tea sample solution was prepared as follows: 100 mg of accurately weighed tea powder was extracted with 5.00 mL 50/50 (v/v) methanol/water and sonicated for 30 min at room temperature, and then filtered through filter paper. This procedure was repeated twice, and then the filtrate was combined and centrifuged at 4000 rpm for 10 min using a C-28A centrifuge (BOECO, Germany). The supernatant was diluted to exactly 20.00 mL and 150 μL of this solution was further diluted to 1.00 mL, both with methanol/water (50/50, v/v). The final solution was filtered through a 0.20 μm hydrophilic PTFE syringe filter (Millex-LG). All solutions were stored at −20 °C prior to MS analysis.

2.3. Instrumentation and setup

The designed pipette-tip ESI ion source was assembled and installed as shown in Fig. 1. The blunt point needle (i.d. 410 μm and o.d. 720 μm) of a glass syringe (250 μL, Hamilton) was inserted into the pipette tip (0.1–10 μL, Sorenson) to form a ~2 μL space which could be filled with a variety of sample powders. A very small degreasing cotton swab was normally placed in front of samples to retain the powders. Methanol/water/formic acid (50/50/0.1, v/v/v) was used as the extraction and spraying solvent if not specified elsewhere. A syringe pump (Harvard Pump Apparatus 11 Plus) was employed to supply solvents with adjustable flow rates. A high voltage (typically 5.5 kV) was applied to the stainless steel syringe needle and conducted to the pipette tip end through the solvent to induce electrospray ionization (see Fig. S-1 in Supporting Information). Mass spectrometric measurements were performed in positive ion mode unless specified.

For qualitative analysis, the pipette-tip ESI ion source was coupled with a quadrupole time-of-flight (Q-TOF) mass spectrometer (Applied Biosystems, QStar Pulsar). The mass spectrometer was operated with a curtain gas flow of 30 A.U., and the spray voltage (IS), first declustering potential (DP1), focusing potential (FP) and second declustering potential (DP2) were set to optimum values in both positive and negative ion modes. For differentiation of herbal samples, spectra from the first 60 scans (1 min) were accumulated for principal component analysis (PCA) using SPSS software (version 18.0, SPSS Inc., USA).

For direct quantitation of caffeine contents in the tea samples, the pipette-tip ESI ion source was coupled with a triple quadrupole mass spectrometer (Waters, Quattro Ultima) operated in multiple reaction monitoring (MRM) mode. The capillary voltage, cone voltage and source temperature were set at 4.0 kV, 30 V and 150 °C respectively. The selected reaction m/z 195 → m/z 138 was monitored with a collision energy of 19 eV and a dwell time of 0.5 s for quantitation, while another selected reaction m/z 195 → m/z 110 was monitored with a collision energy of 21 eV and the same dwell time for further confirmation. The inter-channel delay time and inter-scan delay time were set at 0.02 s and 0.1 s respectively. For quantitation, data was acquired only for the first 2 min for each sample. The obtained monomodal chromatographic peak was then smoothed and integrated to obtain the peak area for quantitation. Data acquisition and processing were performed with MassLynx™ V4.0 software (Waters, USA).

Conventional quantitation of caffeine contents in the tea samples was performed on a triple quadrupole mass spectrometer,