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Full Length Article

Sampling and profiling caffeine and its metabolites from an eyelid using a watercolor pen based on electrospray ionization/mass spectrometry

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ABSTRACT

A novel and non-invasive sampling method was developed for use in electrospray ionization/mass spectrometry (ESI/MS). The sampling tool, a watercolor pen (brush) that had been rinsed with ethanol, was used to collect analytes from the eyelids of volunteers. Following this, the brush was quickly moved to the front of the mass inlet, where the ethanol as well as the analytes evaporated very fast and escaped from the brush surface. When the analytes make contact with the ESI plume, which arises from the ESI needle tip, they are ionized and then detected by a mass spectrometer. The findings show that this new technique is applicable for the analysis of caffeine and its metabolites in samples obtained from eyelids after the volunteers consumed coffee. The methodology is simple and economical, non-invasive, and is suitable for use in monitoring the levels of caffeine and its metabolites over an extended period of time.

1. Introduction

Ambient pressure ionization mass spectrometry (API-MS) is currently in widespread use because of its simplicity and straightforward nature [1–7]. Based on the electrospray ionization (ESI) process, API-MS is ideal for use in various fields of chemistry, including biology, clinical assessment, the pharmaceutical industry, medicine and forensic science. Hence, a wide variety of methods have been developed for use in this area, including desorption electrospray ionization, electrospray-assisted laser desorption ionization, paper spray-mass spectrometry and swab touch spray mass spectrometry [8–13]. Furthermore, developing new ionization sources for in-vivo sampling and direct electrospray biocompatible solid-phase microextraction to API-MS have also been reported [14–17]. In order to further innovate the performance of API-MS, various sampling methods, including the use of a toothpick [24], a medical swab [13], an agarose hydrogel and a metal probe have also been developed [18–24]. We report herein on a novel sampling method for use in API-MS. The method involves the use of a watercolor pen (brush) to collect samples from the eyelids of four volunteers after they consumed coffee. Following this, the brush was moved to the front of the mass inlet. Since the pen brush was rinsed with ethanol prior to use, caffeine and its metabolites, as well as ethanol evaporated and escaped from the brush surface. When these substances make contact with the ESI plume that arises from the ESI needle tip, the analytes are ionized and then detected by a mass spectrometer. This is different from DESI (desorption electrospray ionization) or EASI (easy ambient sonic spray ionization), in which the analytes are only being "washed away" by the ESI spray [25–27]. Our method developed herein is simple and straightforward. Details of the procedures for collecting a sample from an eyelid, the optimized location for the pen brush and the concentration of caffeine and its metabolites on eyelids are also reported.

2. Experimental section

2.1. Reagents

The standard sample of caffeine (1,3,7-trimethylxanthine) was purchased from Hayashi Pure Chemical (Osaka, Japan). Methanol was purchased from Merck (Darmstadt, Germany); ethanol was obtained from J. T. Baker (Pennsylvania, USA). Coffee beans (Arabica, Brazil) were purchased from a local coffee shop. Samples for analysis were collected from the eyelids of four volunteers.

2.2. Apparatus

A Finnigan LCQ Deca XP Plus mass spectrometer was used in the study. The mass signal was recorded under a selected ion monitoring (SIM) mode (m/z = 195 and 181 for caffeine and its metabolites,

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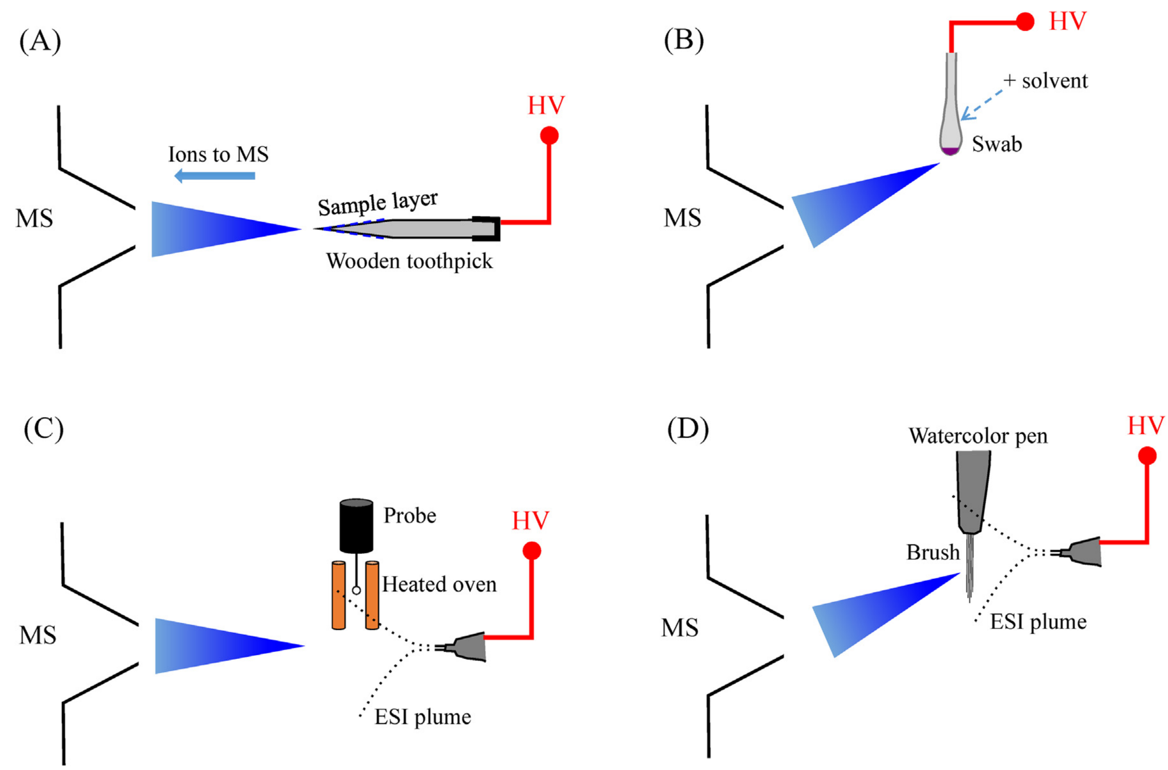

Fig. 1. Four schematic diagrams showing the sampling and ionization methods used in API-MS, including ours. (A) wooden tip spray/mass spectrometry; (B) touch spray-mass spectrometry using a swab; (C) sample collection was achieved by using a metal probe based on the thermal desorption electrospray ionization mass spectrometry; (D) The use of a watercolor pen, as proposed in this study, for sampling based on ESI.

respectively). An Xcalibur data system was used for data collection, and the collected data were converted to ASCII text files. A syringe pump (KDS100) was used to supply auxiliary liquid. Gas chromatography/mass spectrometry (GC/MS) (Agilent6890/HP5972) was used to determine the concentration of caffeine in a cup of coffee. Samples were weighed on an analytical balance (Cubis MSA 125P -100-DA, Sartorius, Germany). Watercolor pens (synthetic brush hair; model, #000) were obtained from a local art shop. The average weight of the watercolor pens were ~2 g. The weight of the actual brush was ~5 mg; one brush bundle contains ~300 hairs that are capable of holding approximately 6 mg of ethanol. The length and the circumference of the brush were 5 and 1 mm, respectively.

2.3. Administration of caffeine

Caffeine was administered to the volunteers under the certificate of Research Ethics Committee (*REC*) approval of National Taiwan Normal University (REC Number: 201702HM004). The eyelid was selected as the source for collecting analytes since it contains numerous blood vessels and collecting metabolites that accumulate in the area can be easily done. This method is non-invasive and the analytes are not easily affected by diet. To obtain valid samples from eyelids, the volunteers consumed three cups of coffee (155 mL; caffeine, 225 mg), one cup, early in the morning at 10:00, the second cup in the afternoon at 14:00 and the third cup in the evening at 17:00. The volunteers were not restricted in any way and were allowed to function in a normal manner during the day with free access to food and water. All samples for analysis were collected over a period of ~10 h at 10 min intervals.

3. Results and discussion

In order to compare traditional methods with our method developed in this study, Fig. 1 shows four schematic diagrams of different sampling and ionization methods, including the one developed here. As shown in frame (A), in the wooden tip spray/mass spectrometry, which was first reported in 2011, a wooden toothpick was used for the loading and ionization of samples [24]. Sample collection can be done by simply dipping the toothpick into the sample solution, even slurry samples and powdered samples can be used. Furthermore, the porous nature and hydrophilic property of wood permits the sample solution to adhere effectively to the sampling device, resulting in the production of durable ion signals. However, a toothpick is quite hard, which makes it difficult to scrap analytes from a surface. A high voltage needs to be applied to the toothpick, which requires alignment skills. Frame (B) shows a schematic diagram of touch spray-mass spectrometry, in which a swab is used for sampling (either a medical or a cotton swab). In contrast to a toothpick, a swab is very useful for collecting analytes from soft tissues, such as the throat, oral fluid or any living object [13]. However, it is also necessary to apply a high voltage, also requiring alignment skills for this method. On the other hand, sample collection can also be done by using a metal probe, as shown in frame (C). By modifying a welding torch, thermal desorption electrospray ionization mass spectrometry (TD-ESI) is suited for the rapid characterization of thermally stable chemical compounds in solid or liquid states. In this case, a metallic sampling probe (60 mm long, 2.5 mm in diameter) was used [21]. The sampling probe was dipped into the sample solution and then removed quickly (drained gastric lavage fluid or an organic solvent), and then inserted promptly into the TD-ESI source, so that alignment skills are not required. However, the probe is composed of metal tubing, which is not suitable for collecting a sample from soft body tissues. Furthermore, the desorption temperature of the TD-ESI source was usually set at 280 °C. As a result, it is not applicable for use in conjunction with thermal decomposition products. In contrast to the three methods described above, we propose a novel method, in which a watercolor pen is used for sample collection. As can