

Full Length Article

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



Yea-Wenn Liou, Kai-Yin Chang, Cheng-Huang Lin*

Department of Chemistry, National Taiwan Normal University, 88 Sec. 4, Tngchow Road, Taipei, 11677, Taiwan

ARTICLE INFO

Article history:

Received 9 May 2017

Received in revised form 17 July 2017

Accepted 19 August 2017

Available online 26 August 2017

Keywords:

Watercolor pen

Non-invasive sampling

ESI/mass spectrometry

Caffeine

Metabolite

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.

© 2017 Elsevier B.V. All rights reserved.

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,

* Corresponding author.

E-mail address: chenglin@ntnu.edu.tw (C.-H. Lin).

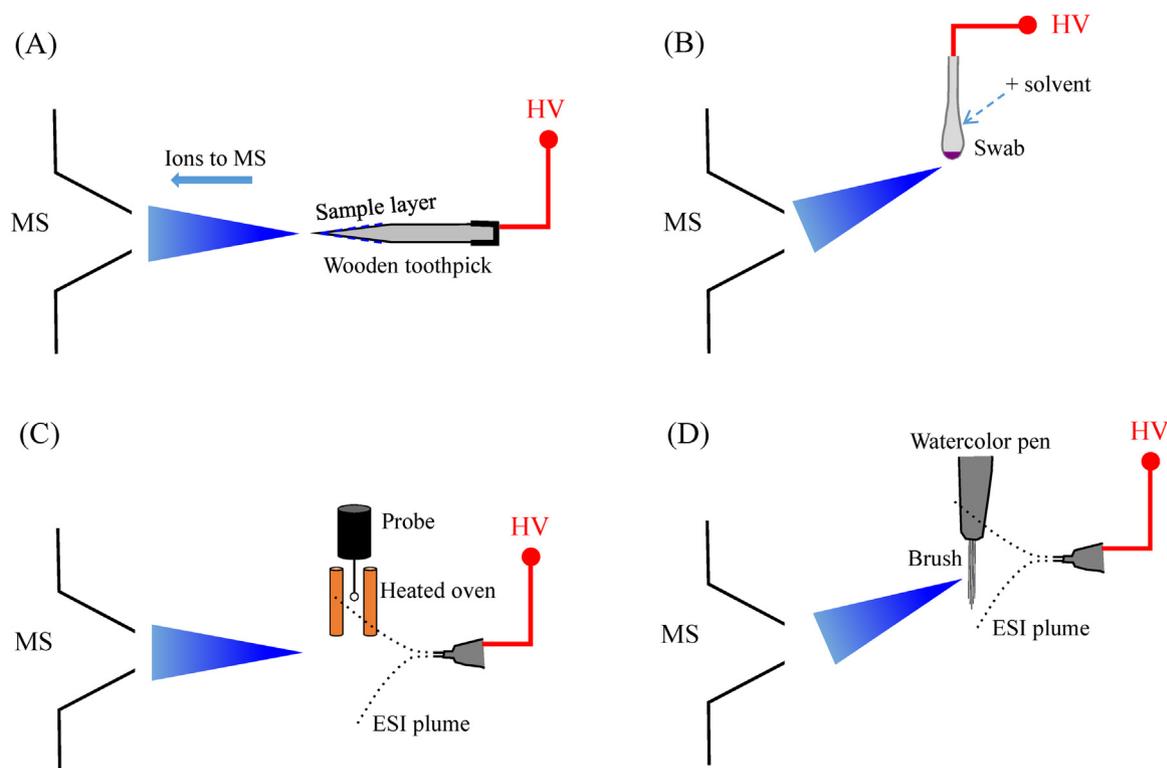


Fig. 1. Four schematic diagrams showing the sampling and ionization methods used in API-MS, including ours. (A) wooden tip spray/mass spectrometry; (B) touch spray-mass spectrometry using a swab; (C) sample collection was achieved by using a metal probe based on the thermal desorption electrospray ionization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

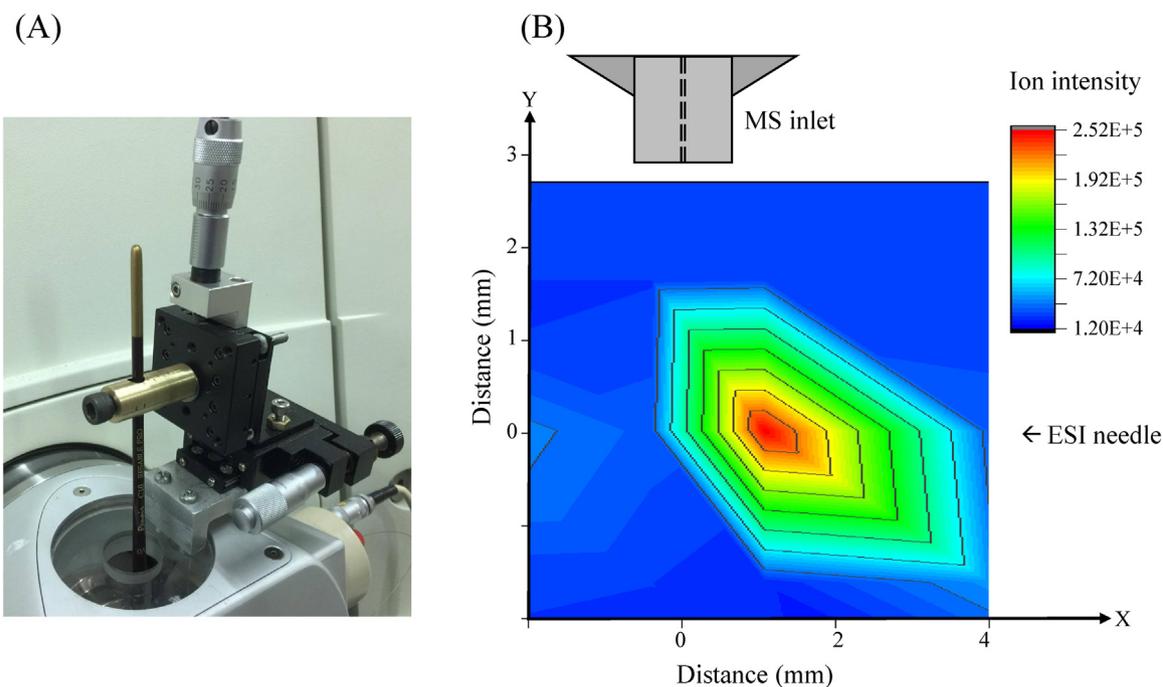


Fig. 2. Photo (A), a picture of the sampling brush-spray ionization/mass spectrometry set up used in this study. The sampling brush was located between the mass inlet and the ESI needle (not shown in the picture). (B), relationship between ion intensities and the location of the brush. In this case, caffeine was used as the test sample (concentration levels, 10 $\mu\text{g}/\text{mL}$) and the ESI voltage was +4.5 kV. The position, indicated in red, denotes the optimized location. (For interpretation of the references to colour in this figure legend and text, the reader is referred to the web version of this article.)

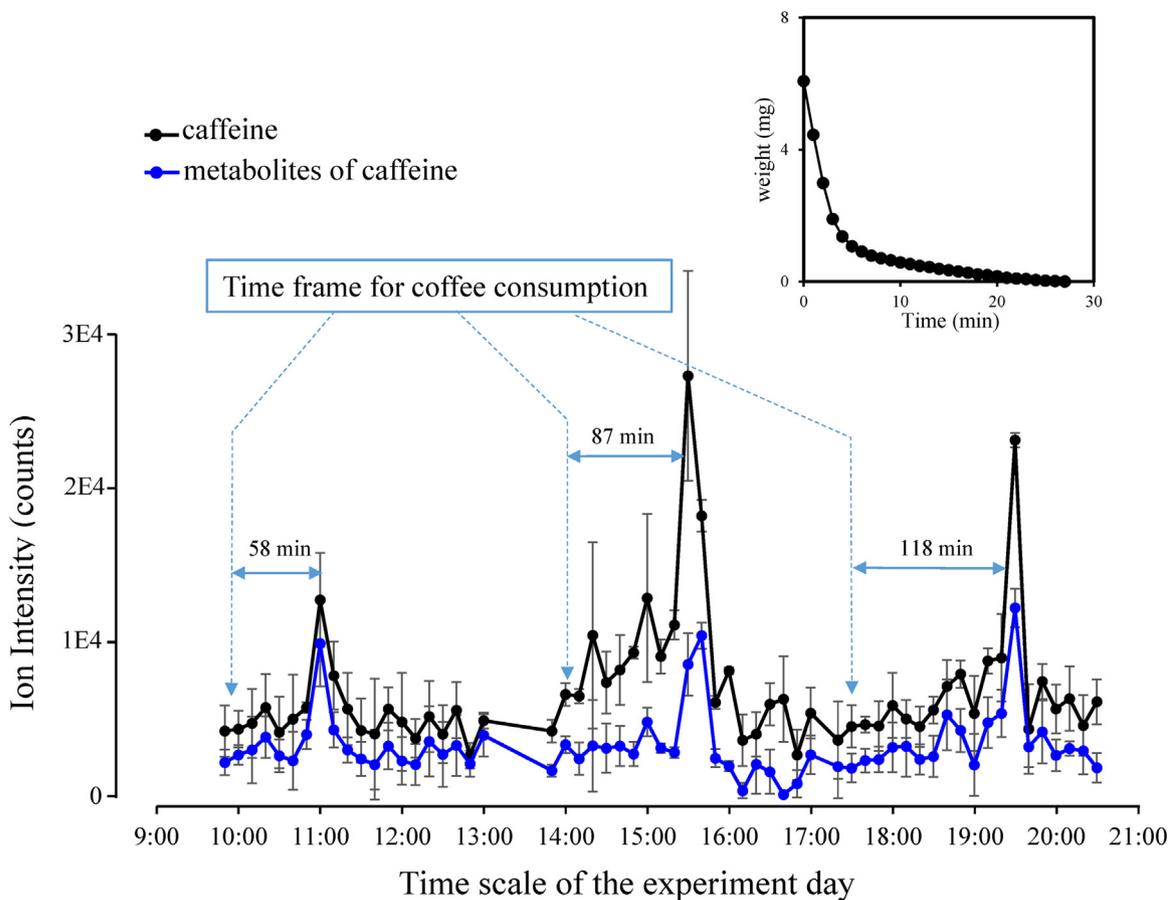


Fig. 3. Monitoring results obtained for volunteer (I). The levels of caffeine and metabolites are described as a black line (upper) and a blue line (below), respectively. The inset shows the relationship between time (min, X-axis) and the weight of ethanol absorbed by the brush (mg, Y-axis), respectively. (For interpretation of the references to colour in this figure legend and text, the reader is referred to the web version of this article.)

Table 1
Average observation time obtained by three measurements (in minute \pm standard deviations) and ratios for caffeine and its metabolites based on various subjects. Data were obtained from various subjects by the use of a sampling brush based on electrospray ionization/mass spectrometry.

Subjects	F or M (age/weight ^a)	morning	afternoon	evening	ratio of caffeine to metabolites
I	F (26/45)	58 \pm 4	88 \pm 5	118 \pm 9	2.0 \pm 0.7
II	F (25/52)	50 \pm 0	85 \pm 5	110 \pm 0	1.5 \pm 0.4
III ^b	F (44/50)	30 \pm 0	65 \pm 6	100 \pm 10	1.7 \pm 0.4
IV	M (24/56)	53 \pm 5	73 \pm 5	110 \pm 0	1.6 \pm 0.5

^a Weight, in kg.

^b Heavy coffee drinker.

be seen in frame (D), a watercolor pen (brush) was rinsed with ethanol, and the analytes were collected by sweeping them onto the ethanol-saturated brush. The collected analytes, along with the ethanol, rapidly evaporate and escape from the brush surface. An ESI plume is then used to ionize these molecules. In the meantime, the analytes are also “washed away”, but we did not prove it in this study. The ESI needle tip approaches from the other direction to produce electro-sprayed/charged droplets from a regular ESI stainless needle. Once the materials (mostly neutral), evaporate from the surface of the brush, they meet the electrospray plume and ionization occurs under ambient conditions. We were very surprised to find that a watercolor pen can provide both functions, i.e. sampling and volatilization of the samples for ionization. When this was discovered, we realized that many applications would be possible. Since the position of the pen brush is very important in acquiring sufficient ions for detection, ionization efficiencies were investigated when the brush was placed in various positions. Photo (A), in Fig. 2 shows the actual position of the watercolor pen and the holding stage. The glass window of the observation port was replaced with an acrylic plate. To determine the optimal position, a special 3D-stage was constructed. The sampling brush was located between the mass inlet and the ESI needle. The XY-stage was set on the acrylic plate and a hole (1 in. in diameter) was prepared to allow the sampling brush to pass through and move. Meanwhile, the brush was held in place with a screw, this permitting the brush to be adjusted up and down in the Z-direction. Fig. 2(B) shows the relationship between ion intensities and the location of the brush. In this case, caffeine was used as the test sample (concentration levels, 10 μ g/mL) at an ESI voltage of +4.5 kV. It is clear that the position, indicated by a red color, i.e. from the view of the mass inlet in the vertical and horizontal distances by \sim 3 mm and 1.5 mm, respectively, is the optimized spot. Hence, this position was used in subsequent experiments. Fig. 3 shows the results obtained from volunteer (I). Data are presented as the average during four days obtained from the same volunteer. This is the reason for why the observation time of caffeine and its metabolites is not 10 min interval. It is well known that three types of metabolites are produced from caffeine (194.19 g/mol), namely, paraxanthine, theobromine and theophylline, respectively [28,29]. Although paraxanthine represents the major metabolite of caffeine in humans, it was not possible to verify this in this study, without chromatographic separation. Using the SIM mode, it was possible to quantify caffeine, 194.19 g/mol and metabolites, 180.17 g/mol, the levels of which were recorded as a black line (upper) and a blue line (below), respectively (Fig. 3). In this case, the volunteer consumed the first cup of coffee, after which analytes were collected from the eyelid over a period of \sim 10 h at 10 min intervals. The inset in Fig. 3 shows the relationship between time (min, X-axis) and the weight of ethanol absorbed by the brush (mg, Y-axis), respectively (conditions: temperature, 26 °C; relative humidity, 41%). We found that ethanol evaporates very rapidly from the brush surface, so that we routinely completed the MS experiments within 3 min after sampling to acquire better ion signals. Before using the brush, it was rinsed with ethanol and was then used to wipe the eyelid back and forth 10 times (distance, \sim 2 cm). Following this, the brush was

transferred to the front of ESI plume. In order to speed up the process, 4 watercolor pens were used in this case. To beginning with, the first one was used to wipe the eyelid and then moved to the front of the MS inlet for measurement; it only took a few minutes. After this, the first one was cleaned by washing with ethanol, followed by a \sim 35 min ultrasound treatment. Following this, the second, third and fourth watercolor pen was used for the next measurement, respectively. As it can be seen in Fig. 3, the maximum value (caffeine, black line) appears 58 min later (observation times: 50, 60, 60 and 60 during four days, respectively). The above process was repeated until noon and the volunteer then consumed a second cup of coffee. The caffeine levels reached a maximum value 88 min later. After dinner the volunteer consumed the last cup of coffee and this time the maximum value was reached after 118 min. This method represents a simple and rapid method for pharmacokinetics studies of a variety of metabolites, which could be very useful for new drug development. Meanwhile, the population of metabolites during the day can be determined by changing the SIM mode from $m/z = 195$ (caffeine) to $m/z = 181$ (metabolites), as shown by the blue line. It is clear that the population of metabolites parallels that for caffeine. The level of caffeine is higher than that for metabolites, with the ratio ranging from 1.5–2.0. The results obtained from the four volunteers are summarized in Table 1. We have no knowledge of the factors that cause the delay in the levels of caffeine in samples obtained from eyelids during the day, but it is clear that caffeine is metabolized faster in the morning than in the afternoon. The metabolic rate is usually slowest at night. It is interesting to note and volunteer (III) is a heavy coffee drinker but also exercises each day. This explains why her metabolic rate was faster than the others. Irrespective of this fact, the focus of this study was on the use of a watercolor pen for sample collection, which opens a new field, and the results indicate that it represents a potentially useful new methodology that has generally applicability in the field of mass spectrometric analysis.

4. Conclusion

The development of novel method for sampling for use in ESI-MS analyses is described. Using a watercolor pen, analytes can be collected either by sweeping the surface of the eyelid of a subject or by simply pipetting a solution of an analyte onto the brush or simply dipping the brush into a solution of an analyte. The methodology is simple and economical, non-invasive, and is suitable for use in monitoring the levels a variety of metabolites produced by a subject over an extended period of time. We provide initial proof of the feasibility and potential for the method, but extensive work needs to be done in order to prove the suitability of the method for use in pharmacokinetics studies. Further applications are currently being explored.

Acknowledgment

This work was supported by grants from the Ministry of Science and Technology (Taiwan) under Contract 103-2113-M-003-001-MY3.

References

- [1] R.G. Cooks, Z. Ouyang, Z. Takats, J.M. Wiseman, Ambient mass spectrometry, *Science* 311 (2006) 1566–1570.
- [2] D.N. Correa, J.M. Santos, L.S. Eberlin, M.N. Eberlin, S.F. Teunissen, Forensic chemistry and ambient mass spectrometry: a perfect couple destined for a happy marriage, *Anal. Chem.* 88 (2016) 2515–2526.
- [3] D.T. Snyder, C.J. Pulliam, Z. Ouyang, R.G. Cooks, Miniature and fieldable mass spectrometers: recent advances, *Anal. Chem.* 88 (2016) 2–29.
- [4] R. Haddad, R. Sparrapan, T. Kotiaho, M.N. Eberlin, Easy ambient sonic-spray ionization-membrane interface mass spectrometry for direct analysis of solution constituents, *Anal. Chem.* 80 (2008) 898–903.
- [5] E.T. Jansson, M.T. Dulay, R.N. Zare, Monitoring enzymatic reactions in real time using venturi easy ambient sonic-spray ionization mass spectrometry, *Anal. Chem.* 88 (2016) 6195–6198.
- [6] X.C. Lin, X.N. Wang, L. Liu, Q. Wen, R.Q. Yu, J.H. Jiang, Surface enhanced laser desorption ionization of phospholipids on gold nanoparticles for mass spectrometric immunoassay, *Anal. Chem.* 88 (2016) 9881–9884.
- [7] R.B. Cody, J.A. Laramée, H.D. Durst, Versatile new ion source for the analysis of materials in open air under ambient conditions, *Anal. Chem.* 77 (2005) 2297–2302.
- [8] Z. Takats, J.M. Wiseman, B. Gologan, R.G. Cooks, Mass spectrometry sampling under ambient conditions with desorption electrospray ionization, *Science* 306 (2004) 471–473.
- [9] C.Y. Cheng, C.H. Yuan, S.C. Cheng, M.Z. Huang, H.C. Chang, T.L. Cheng, C.S. Yeh, J. Shiea, Electrospray-assisted laser desorption/ionization mass spectrometry for continuously monitoring the states of ongoing chemical reactions in organic or aqueous solution under ambient conditions, *Anal. Chem.* 80 (2008) 7699–7705.
- [10] S.Y. Lin, M.Z. Huang, H.C. Chang, J. Shiea, Using electrospray-assisted laser desorption/ionization mass spectrometry to characterize organic compounds separated on thin-layer chromatography plates, *Anal. Chem.* 79 (2007) 8789–8795.
- [11] J. Liu, H. Wang, N.E. Manicke, J.M. Lin, R.G. Cooks, Z. Ouyang, Development characterization, and application of paper spray ionization, *Anal. Chem.* 82 (2010) 2463–2471.
- [12] K.S. Kerian, A.K. Jarmusch, R.G. Cooks, Touch spray mass spectrometry for in situ analysis of complex samples, *Analyst* 139 (2014) 2714–2720.
- [13] A.K. Jarmusch, V. Pirro, K.S. Kerian, R.G. Cooks, Detection of strep throat causing bacterium directly from medical swabs by touch spray- mass spectrometry, *Analyst* 139 (2014) 4785–4789.
- [14] G.A. Gómez-Ríos, C. Liu, M. Tascon, N. Reyes-Garcés, D.W. Arnold, T.R. Covey, J. Pawliszyn, Open port probe sampling interface for the direct coupling of biocompatible solid-phase microextraction to atmospheric pressure ionization mass spectrometry, *Anal. Chem.* 89 (2017) 3805–3809.
- [15] P. Nemes, A. Vertes, Ambient mass spectrometry for in vivo local analysis and in situ molecular tissue imaging, *Trends Anal. Chem.* 34 (2012) 22–34.
- [16] H. Chen, S. Yang, A. Wortmann, R. Zenobi, Neutral desorption sampling of living objects for rapid analysis by extractive electrospray ionization mass spectrometry, *Angew. Chem. Int. Ed.* 46 (2007) 7591–7594.
- [17] C.R. Ferreira, K.E. Yannell, A.K. Jarmusch, V. Pirro, Z. Ouyang, R.G. Cooks, Ambient ionization mass spectrometry for point-of-care diagnostics and other clinical measurements, *Clin. Chem.* 62 (2016) 1–12.
- [18] V. Pirro, A.K. Jarmusch, M. Vincenti, R.G. Cooks, Direct drug analysis from oral fluid using medical swab touch spray mass spectrometry, *Anal. Chim. Acta* 861 (2015) 47–54.
- [19] E.P. Dutkiewicz, H.Y. Chiu, P.L. Urban, Micropatch-arrayed pads for non-invasive spatial and temporal profiling of topical drugs on skin surface, *J. Mass Spectrom.* 50 (2015) 1321–1325.
- [20] E.P. Dutkiewicz, H.Y. Chiu, P.L. Urban, Probing skin for metabolites and topical drugs with hydrogel micropatches, *Anal. Chem.* 89 (2017) 2664–2670.
- [21] C.W. Lee, H. Su, Y.D. Cai, M.T. Wu, D.C. Wu, J. Shiea, Rapid identification of psychoactive drugs in drained gastric lavage fluid and whole blood specimens of drug overdose patients using ambient mass spectrometry, *J. Mass Spectrom.* 6 (2017) S0056.
- [22] E.P. Dutkiewicz, J.D. Lin, T.W. Tseng, Y.S. Wang, P.L. Urban, Hydrogel micropatches for sampling and profiling skin metabolites, *Anal. Chem.* 86 (2014) 2337–2344.
- [23] Z. Yu, L.C. Chen, H. Suzuki, O. Ariyad, R. Erra-Balsells, H. Nonami, K. Hiraoka, Direct profiling of phytochemicals in tulip tissues and in vivo monitoring of the change of carbohydrate content in tulip bulbs by probe electrospray ionization mass spectrometry, *J. Am. Soc. Mass Spectrom.* 20 (2009) 2304–2311.
- [24] B. Hu, P.K. So, H. Chen, Z.P. Yao, Electrospray ionization using wooden tips, *Anal. Chem.* 83 (2011) 8201–8207.
- [25] N. Talaty, C.C. Mulligan, D.R. Justes, A.U. Jackson, R.J. Noll, R.G. Cooks, Fabric analysis by ambient mass spectrometry for explosives and drugs, *Analyst* 133 (2008) 1532–1540.
- [26] D.R. Justes, N. Talaty, I. Cotte-Rodriguez, R.G. Cooks, Detection of explosives on skin using ambient ionization mass spectrometry, *Chem. Commun.* 214 (2007) 2142–2144.
- [27] M.F. Riccio, S.A. Saraiva, L.A. Marques, R. Alberici, R. Haddad, J.C. Moller, M.N. Eberlin, R.R. Catharino, Easy mass spectrometry for metabolomics and quality control of vegetable and animal fats, *Eur. J. Lipid Sci. Technol.* 112 (2010) 434–438.
- [28] J.A. Carrillo, J. Benitez, Clinically significant pharmacokinetic interactions between dietary caffeine and medications, *Clin. Pharmacokinet.* 39 (2000) 127–153.
- [29] M. Huang, J.Y. Gao, Z.G. Zhai, Q.L. Liang, Y.M. Wang, Y.Q. Bai, G.A. Luo, An HPLC-ESI-MS method for simultaneous determination of fourteen metabolites of promethazine and caffeine and its application to pharmacokinetic study of the combination therapy against motion sickness, *J. Pharm. Biomed.* 62 (2012) 119–128.