https://doi.org/10.1016/j.aca.2019.05.044

Direct Coupling of Solid Phase Microextraction with Electrospray Ionization Mass Spectrometry: A Case Study for Detection of Ketamine in Urine

Bin Hu,[a,b,c] Bo Zheng,[a,b] Daniel Rickert,[d] German Augusto Gómez-Ríos,[d,e] Barbara Bojko,[d,f] Janusz Pawliszyn,[d]* Zhong-Ping Yao[a,b]*

[a]State Key Laboratory of Chemical Biology and Drug Discovery, Food Safety and Technology Research Centre and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong Special Administrative Region, China

[b]State Key Laboratory of Chinese Medicine and Molecular Pharmacology (Incubation) and Shenzhen Key Laboratory of Food Biological Safety Control, Shenzhen Research Institute of The Hong Kong Polytechnic University, Shenzhen 518057, China

[c]Institute of Mass Spectrometer and Atmospheric Environment, and Guangdong Provincial Engineering Research Center for On-line Source Apportionment System of Air Pollution, Jinan University, Guangzhou 510632, China

[d]Department of Chemistry, University of Waterloo, Waterloo, ON N2L 3G1, Canada

[e]Current address: Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823, United States

[f]Current address: Department of Pharmacodynamics and Molecular Pharmacology, Faculty of Pharmacy, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, 85-067 Bydgoszcz, Poland

*Corresponding authors:
J. Pawliszyn:
Department of Chemistry, University of Waterloo, Waterloo, ON N2L 3G1, Canada
E-mail: janusz@uwaterloo.ca; Tel.: +1 519 8884641; Fax: +1 519 7460435.

Z. P. Yao:
Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong
E-mail: zhongping.yao@polyu.edu.hk; Tel.:+852 34008792; Fax: +852 23649932.

Abstract

Electrospray ionization mass spectrometry (ESI-MS) is a commonly used technique for analysis of various samples. Solid phase microextraction (SPME) is a simple and efficient technique that combines both sampling and sample preparation into one consolidated step, preconcentrating extracted analytes for ultra-sensitive analysis. Historically, SPME has been coupled with chromatography-based techniques for sample separation prior to analysis, however more recently, the chromatographic step has been omitted, with the SPME device directly coupled with the mass spectrometer. In this study, direct coupling of SPME with ESI-MS was developed, and extensively validated to quantitate ketamine from human urine, employing a practical experimental workflow and no extensive hardware modification to the equipment. Among the different fibers evaluated, SPME device coated with C_{18}/benzenesulfonic acid particles was selected for the analysis due to its good selectivity and signal response. Different approaches, including desorption spray, dripping, desorption ESI and nano-ESI were attempted for elution and ionization of the analytes extracted using the SPME fibers. The results showed that the desorption spray and nano-ESI methods offered better signal response and signal duration than the others that were evaluated. The analytical performance of the SPME-nano-ESI-MS setup was excellent, including limit of detection (LOD) of 0.027 ng/ml, limit of quantitation (LOQ) of 0.1 ng/ml, linear range of 0.1-500.0 ng/ml (R^2=0.9995) and recoveries of 90.8-109.4% with RSD 3.4-10.6% for three validation points at 4.0,40 and 400.0 ng/ml, far better than the performance of conventional methods. The results herein presented, demonstrated that the direct coupling of SPME fibers with ESI-MS-based systems allowed for the simple and ultra-sensitive determination of analytes from raw samples such as human urine.

1. Introduction

Mass spectrometry (MS) is a powerful tool for analytical and research purposes, and electrospray ionization (ESI) is one of the most commonly used ionization techniques in MS [1]. Conventional ESI uses a capillary for sample delivery and ionization. Development of nano-ESI- facilitates consumption of smaller volume of samples and tolerance of more complex matrices [2]. In recent years, use of solid substrates for sample delivery and ambient ionization has attracted a lot of attention [3-14]. Solid-substrate ESI-MS allows convenient sample loading and direct analysis of various forms of samples [8, 9, 12, 15-24], including viscous liquids [25], powders [26, 27], and bulky solids [28], as well as quantitative analysis [29]. Moreover, *in vivo* and real-time detection [5, 30, 31] using solid-substrate ESI-MS has been reported to provide transient and dynamic information about the metabolic processes, with minimal or negligible sample preparation. Unlike other ambient ionization techniques that typically require additional settings such as solvent spray, plasma or a laser, the assembly and operation of solid-substrate ESI-MS devices is practical and straightforward. Therefore, due to the minimal modification required to commercial mass spectrometers systems, these techniques have encouraged experts in the booming fields of proteomics, metabolomics, and molecular diagnostics to consider its applications [32].

Although solid-substrate ESI-MS allows direct analysis of samples in their original state, given that the entire raw sample is directly loaded on the substrate, severe suppression/enhancement of the signals of the target compounds can occur. Therefore, solid substrates that allow selective extraction/enrichment and ionization are ideal for improving analysis of complex matrices [7, 33, 34]. Recently, surface-modified blades [33] and wooden tips [7, 35, 36] were developed for selective and sensitive detection of analytes from mixtures using solid-substrate ESI-MS. In these studies, blades and wooden tips were modified with specific functional groups on the

surfaces and were used as solid phase microextraction (SPME) probes for sampling. SPME is a commonly used technique for sampling/sample pretreatment [37], and SPME fibers with various coating materials and various configurations have been developed and commercially available [17, 38-40]. SPME is typically coupled to MS with gas chromatography (GC-MS) [41] or liquid chromatography (LC-MS) [37, 41, 42] for detection of the extracted and enriched analytes. Detection of analytes extracted on SPME fibers directly by mass spectrometry has been also explored [43, 44], and ionization techniques such as matrix-assisted laser desorption ionization (MALDI) [45, 46], direct analysis in real-time (DART) [47-49], desorption electrospray ionization (DESI) [50], and nano-ESI [51] have been attempted. Likewise, SPME fibers have also been coupled with direct electrospray probe (DEP) that was made of copper coil [52], thermal desorption technique [48], direct infusion chamber [53], open port probe (OPP) [54], microfluidic open interface (MOI) [55] and the capillary gap sampler [56] . Unsurprisingly, the development of simple and efficient techniques for coupling of SPME fibers and ESI-MS is still an on-going field of research [17].

Conventionally, detection and quantitation of drugs-of-abuse in body fluids requires intensive sample pretreatment workflows and time-consuming chromatographic separation prior to MS analysis [57-59]. Due to the high analytical demand, development of rapid and reliable methods for analysis of drugs-of-abuse is highly desirable. Recently, we developed a wooden-tip ESI-MS (WT-ESI-MS) method [20] for direct analysis of drugs-of-abuse in urine and oral fluids [29, 60]. Our results demonstrated that WT-ESI-MS was an efficient method for rapid detection and quantitation of ketamine and its metabolite norketamine in urine and oral fluid. However, the performance of WT-ESI-MS for analysis of the drug in body fluids was not as effective as those offered-by conventional methods, mainly due to the interferences and signal suppression during the ionization process. Therefore, by replacing the wooden tip with a SPME fiber that

is capable of selectively extracting and enriching analytes, ionization and quantitation of ketamine and its metabolite are expected to be significantly improved.

In this study, we explored the direct coupling of SPME fibers with multiple ESI-MS formats (termed SPME-ESI-MS), ultimately applying this approach for the analysis of ketamine in urine, in order to validate the method and to detect ketamine excreted in urine following the intake of ketamine [61]. The SPME fibers were directly mounted onto a standard nano-ESI ion source without any hardware modification and desorption and elution solvent was introduced by a spray emitter for SPME-ESI-MS analysis, or the desorption step was preformed directly inside the nano-ESI emitter, a method similar to some of the previous studies [22-24]. Employing a C_{18}/benzenesulfonic acid (mixed-mode) coated SPME fiber, SPME-ESI-MS demonstrated better analytical performance than that of conventional methods. Furthermore, it was elucidated in this study that desorbing the fibers directly into the nano-ESI emitter prior to the ionization event resulted in the lowest LOQs and the best figures of merit out of the four formats that were evaluated. Succinctly, this study demonstrates that SPME fibers could be conveniently coupled with ESI-MS for rapid and sensitive detection of ketamine in urine samples.

2. Experimental

2.2 Reagents and materials

Ketamine and internal standard ketamine-d_4 were purchased from Alfasan (Woerden, Holland). Methanol and acetonitrile were the products of Tedia (Fairfield, OH, USA). Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). For the SPME-ESI-MS experiments, formic acid (FA) and ammonium acetate (AA) were purchased from Sigma-Aldrich (Saint Louis, MO, USA), and LC-MS grade methanol (MeOH), acetonitrile (ACN),

isopropanol (IPA) and water were purchased from Fisher Scientific (Bartlesville, OK, USA). Individual working stock standard solutions were prepared in MeOH at a concentration of 100 μg/mL and stored at -80°C. SPME fibers tested in this study were kindly provided by Supelco (Bellefonte, PA, USA), with fused silica fibers coated with PDMS (polydimethylsiloxane, 100 μm), PDMS/DVB (polydimethylsiloxane/ divinylbenzene, 60 μm), and PA (polyacrylate, 85 μm) as well as Bio-SPME fibers (200 μm nitinol wire) coated with C_{18} (octadecyl, 50 μm) and mixed-mode (C_{18}/benzenesulfonic acid, coating thickness: 40 μm, coating length: 10 mm) [37, 40, 62] as the coating, respectively. The specifications of the nano-ESI emitters were as follows: coated Glasstip (1.0/0.78, o.d./i.d., mm; 4 μm tip) were obtained from New Objective Inc. (Woburn, MA, USA). Urine samples were collected from healthy volunteers. A urine sample spiked with 1.0 μg/mL ketamine was used to optimize the analytical procedures. Pure water used in this study was produced by the Milli-Q water-purification system (Milford, MA, USA).

2.2 Mass spectrometry

The qualitative analysis of ketamine was carried on a Q-TOF2 mass spectrometer (Waters, Milford, MA, USA), while the quantitative data were acquired with multiple reaction monitoring (MRM) mode on a triple quadrupole mass spectrometer (MS) (Waters Quattro Ultima, Milford, MA, USA). A 3.5 kV high voltage was applied onto the SPME fibers to initiate the ESI event; Cone voltage was 30 V; Source temperature was set to 80 °C. For the MRM experiments, ion pairs *m/z* 238 > 125 and *m/z* 242 > 129, for ketamine and d_4-ketamine (internal standard), respectively, were chosen and the peak heights were used for the quantitation, similar to those in the previous study [29]. The collision energy was 25 eV, and the dwell time was set at 0.2 second. For the SPME-nano-ESI experiments, the quantitative data was acquired in the MRM mode on a Thermo Scientific TSQ Vantage triple quadrupole MS (San Jose, CA, USA). A 1.3 kV voltage was applied to initiate ionization, and was

maintained for 10s. The same ion pairs stated above were used, and the MS dwell time was fixed at 0.1s. Data processing on the TSQ Vantage was done using Thermo Scientific Trace Finder v4.1 (San Jose, CA, USA).

2.3 SPME-ESI-MS

The whole analytical procedure of SPME-ESI-MS analysis can be divided in 4 basic steps, including cleaning/conditioning (Figure 1a), sampling/extraction (Figure 1b), washing (Figure 1c), and elution/ionization (Figure 2). Briefly speaking, the SPME fibers were immersed into 1 mL solvent (water/methanol, 1/1, v/v) for 5 min with vortex to remove background compounds and condition the solid phase coating. Then, the SPME fiber (with the whole 10 mm coating part) was immersed into 300 μL of urine sample (1.0 ml of Eppendorf tube) and vortex for 10 minutes (speed: ~1500 rpm). Subsequently, the fiber was rinsed by pure water for 50 seconds before the SPME fiber was mounted onto the nano-ESI ion source for detection (Figure 2).

As shown in Figure 2, the setup of SPME-ESI-MS was simple and convenient, without any hardware modification. SPME fibers were mounted onto a commercial nano-ESI ion source (Waters, Milford, MA, USA) by an inner screw and with the aid of a conductive elastomer, similar to the setup of nano-ESI needles for nano-ESI analysis. SPME fiber's direction and distance from the MS inlet could be readily adjusted by using the settings of the nano-ESI ion source. Four different approaches: desorption spray, dripping, desorption electrospray ionization (DESI), and direct insertion of the SPME fiber into the nano-ESI emitter filled with desorption solvent were attempted for desorption and ionization of the extracted analytes in this study. As shown in Figure 2a, in the desorption spray approach, a high voltage was applied directly onto the SPME fiber, and desorption solvent (water/methanol/formic acid, 1/1/0.001,

v/v/v) was sprayed onto the SPME fiber. As shown in Figure 2, the vertical distance (d1) and horizontal distance (d2) between the SPME fiber and MS inlet was 6 mm, respectively; the spray emitter was placed on the side of the SPME fiber with an optimized distance (d3) and angle of 3 mm and 45°, respectively. The flow rate of desorption solvent was 5 μL/min and the nitrogen gas pressure rate was 10 psi. In the dripping approach (see Figure 2~~1~~b), desorption solvent was directly dripped onto the SPME fiber by a pipette. In the DESI approach (see Figure 2~~1~~c), all the settings were identical with those of the desorption spray approach, except that the high voltage was applied onto the spray emitter instead of the SPME fiber. In the SPME-ESI-MS, the mass spectra were obtained by averaging the data acquired in 2 minutes in desorption spray and DESI mode, while the duration in dropping mode is ~10 seconds. In the nano-ESI approach (Figure 2d), the fiber was placed directly inside the nano-ESI emitter that was pre-filled with 4 μL of 95:5 MeOH: H_2O (0.1 % formic acid, 12 mM ammonium acetate). The nano-ESI emitter was placed 3 mm back from the center of the MS inlet, and the desorption was performed for 3 minutes. Following the desorption, a 1.3 kV high voltage was applied to the nano-ESI emitter, initiating a spray event that lasted for 10s.

3. Results and Discussion

3.1 Optimization of SPME-ESI-MS

Figure 3a is a typical ESI-MS spectrum of ketamine obtained by SPME-ESI-MS analysis of 1 μg/ml ketamine aqueous solution with the dripping method. Ketamine was observed as the protonated molecule at *m/z* 238 in the spectrum. In this study, a urine sample spiked with 1 μg/ml ketamine was used to optimize the conditions and establish the analytical protocol. Different types of SPME fibers and various experimental settings were attempted and their influences to the ketamine signals were investigated and evaluated.

As shown in Figure 3b, GC-based fibers (PDMS, PDMS/DVB and PA) provided low signal intensities for ketamine in urine, which was believed to be mainly caused by the nonpolar properties of the coating materials that might not effectively extract ketamine from the urine samples. It was found that electrospray plume was difficult to generate from these tips even if a higher voltage (e.g., 4.5 kV) was applied. LC-based SPME fibers (C18 and mixed-mode) used an electrically conductive material as a support (i.e., nickel-titanium metal alloy), facilitating generation of electrospray and thus good detection of ketamine signals from the fibers. Higher ketamine signals were obtained using the mixed-mode fiber in comparison to the C18 one, due to higher extraction efficiency of this coating. Indeed, the mixed-mode fiber contained C18 and benzenesulfonic acid on the fiber surface and could have both hydrophobic and ion-dipole interactions with ketamine that contains phenyl and amide groups (inset of Figure 3a). Consequently, further experiments were performed using the mix-mode fiber in this study.

Four approaches, i.e., desorption spray, dripping, DESI, and nano-ESI, as described in the Experimental Section, were attempted for desorption and ionization of the analytes extracted on the SPME fibers. For the dripping approach that could be conveniently used, the solvent was added manually using a pipette and typically only a limited volume (typically 1.0 μL) of solvent could be held by the fiber for analyte elution and ionization, leading to poorer signal intensities and duration. For the DESI approach, charged droplets were generated and sprayed onto the surface of the SPME fiber for the elution and ionization of the retained analytes; the results revealed that the desorption and ionization efficiency was quite low with this approach [63]. In the desorption spray approach, the solvent was sprayed onto the SPME fiber surface in a continuous and uniform manner, which provided sufficient solvent volume and duration for analyte elution, and the high voltage was directly applied onto the fiber to more efficiently

induce spray ionization, leading to good signal response and signal duration. Finally, in the nano-ESI approach, the fiber was desorbed into an small volume of solvent (~4 μL), resulting in extremely high sensitivity and low background noise, as listed in Table S1. Coupled with the spray stability, nano-ESI was deemed to be the best approach and was thus employed for subsequent quantitative analysis.

Extraction time, washing time as well as elution and ionization solvent were also optimized for SPME-ESI-MS analysis of ketamine in urine using the mixed-mode fiber. As shown in Figures 4 a and b, the extraction was found to reach equilibrium at 10 minutes, and the optimal washing time was determined as 50 seconds with water as the washing solvent for removing polar interference compounds (Figure S1). Various solvents were attempted to elute and ionize the ketamine analyte retained on the SPME fiber, and water/methanol/formic acid (1/1/0.001, v/v/v) was found to be the most efficient one as indicated by the ketamine signals detected using the desorption spray mode. In the nano-ESI experiments, prior experiments and publications have deemed that 95:5 MeOH: H_2O + 0.1% FA & 12 mM AA was the optimal spray solvent for quantitative analysis of small molecule pharmaceuticals [51]. For this reason, this solvent composition was chosen for the respective analysis.

3.2 Performance for analysis of ketamine in urine using SPME-ESI-MS

Figure 5a is a spectrum obtained by analyzing a urine sample spiked with 100 ng/ml ketamine with SPME-ESI-MS using the optimized conditions as shown above. Protonated molecule of ketamine (*m/z* 238), confirmed by MS/MS spectrum (Figure 5b),[10] was predominately observed in the spectrum with very few interference signals such as the peak at *m/z* 114 ($[Creatinine+H]^+$). In our experiments, the target analyte was selectively enriched by the SPME fiber, and loosely attached matrix components were removed during the washing process.

Furthermore, the use of nanoESI allowed reduced ion signal suppression. The above results indicated that the SPME-ESI-MS protocol could efficiently enrich ketamine in urine, significantly reduce matrix effect, and enhance the signal of ketamine.

The limit of detection (LOD) and limit of quantitation (LOQ) of the SPME-nano-ESI format for analysis of ketamine in urine were measured. The signal/noise ratio (S/N) was obtained by comparing the intensity of the "spiked" sample to that of the "blank" one (Figure S3), i.e., $(I_{analyte}/I_{IS})_{spiked}/(I_{analyte}/I_{IS})_{blank}$, where $I_{analyte}$ and I_{IS} were the MRM chromatogram intensities (peak areas) of analyte and internal standard (i.e., d_4-ketamine), respectively, and the subscripts "blank" and "spiked" represented the samples spiked with the internal standard only and spiked with both analyte and internal standard, respectively. As shown in Table 1, the LOD (S/N$\geqslant$3) and LOQ (S/N$\geqslant$10) of the present method for detection of ketamine in urine were ~0.027 ng/mL and 0.1 ng/ml, respectively. Since the lowest calibration point (0.1 ng/mL) generated a S/N of ~10.8, the LOD was approximated. No tailing was observed for the technique since no signals were detected when the high voltage was not being applied. As shown in Figure S2, the signals was observed only between 0.1 and 0.3 min with the application of the high voltage. These results determined that the LOD and LOQ of SPME-nano-ESI-MS for detection of ketamine in urine could be at least 2000 folds better than those obtained with WT-ESI-MS (LOD of 20 ng/ml and LOQ of 50 ng/ml) [29] and far better than those obtained with most conventional methods (LOD of 0.5-25 ng/ml and LOQ of 1.5-50 ng/ml for using GC-MS [64, 65], and LOD of 0.03-5 ng/ml and LOQ of 0.1-3.17 ng/ml for using LC-MS [66-68]).

The calibration curve for quantitation was constructed with ten sets of experimental data. Each set of data was obtained by detecting triplicate samples containing different levels of the analyte (0.1, 0.5, 1.0, 2.5, 5.0, 25.0, 50.0, 100.0, 250.0 and 500.0 ng/ml) and a fixed amount of

internal standard (20.0 ng/ml). As shown in Figure 6, excellent linearity (R^2=0.9995) was obtained over the concentration range from 0.1 to 500.0 ng/ml. The implementation of the internal standard effectively compensated the deviations during the extraction/desorption/ionization processes and enabled such an excellent linearity. Such low concentration could not be achieved for quantitation with other SPME-ESI-MS, WT-ESI-MS or conventional methods [64-68].

The precision and accuracy of the SPME-ESI-MS approach were evaluated by quantitation of ketamine in urine samples spiked with ketamine of low, middle and high concentrations. Each sample was analyzed three times and the data were averaged for comparison. Recoveries of SPME process was determined by spiking samples of different concentrations (i.e., 4, 40, and 400 ng/ml), i.e., $(I_{analyte}/I_{IS})_{spiked}/(I_{analyte}/I_{IS})_{calculation}$, where $I_{analyte}$ and I_{IS} were the MRM chromatogram intensities of ketamine and d_4-ketamine, respectively; and the subscripts "spiked" and "calculation" represented the experimental value of the spiked samples (i.e., 4, 40, and 400 ng/ml) and back-calculation using the line of best fit from the calibration curve in Figure 5. For 4.0, 40 and 400.0 ng/ml spiked samples, the recoveries were in the range of 90.8-109.4 % with RSD 3.4-10.6 % (n=3) (Table 2). The figures of merit for the accuracy and precision using the nano-ESI technique revealed that the accuracy and precision obtained were acceptable for bioanalytical sample analysis. This technique is applicable for analysis of different urine samples containing different levels of matrix, since the selective extraction of the SPME fibre allows effective removal of matrix and the use of an internal standard can correct the deviations from the samples and the analysis.

The entire extraction and desorption procedure for SPME-nano-ESI-MS took approximately 13 minutes to analyze one fiber, and this could be greatly reduced using high-throughput

extraction protocols or a more comprehensive automated nano-ESI interface [69]. This analysis time is much shorter than the analysis time of several hours per sample required with the conventional methods. Another notable feature of SPME fibers is their re-usability. Our experimental results showed that a SPME fiber could be reused over 200 times with no obvious degradation of performance. This allows much reduced costs and good reproducibility of results since the same fiber can be used for the experiments.

4. Conclusions

Direct coupling of SPME with ESI-MS has been developed in this study. The setup of SPME-ESI-MS was optimized, and SPME fibers placed directly inside the nano-ESI emitter was employed to provide stable and sensitive analytical signal of attained analytes. Such direct coupling avoids chromatographic separation, makes use of the rapid and selective extraction capability of SPME, and integrates with nanoESI-MS that has good ionization efficiency and tolerance to ion suppression. We demonstrated that SPME-nano-ESI-MS could provide analytical performance much better than most conventional methods, with minimal compromise in terms of total analysis time. These results indicated that SPME-ESI-MS could be a simple and efficient method for rapid, sensitive and selective detection of analytes in mixture samples. Given the popularity of both SPME and ESI-MS, as well as the easy setup of the coupling technique, SPME-ESI-MS is expected to have great potentials for sample analysis in various areas.

Acknowledgments

The authors acknowledge the financial supports from Beat Drugs Fund (BDF140029), National Natural Science Foundation of China (Grants No. 8187130081 and 21804053) and Hong Kong Research Grants Council (CRF Grant No. C5031-14E). The supports from the University

Research Facility in Chemical and Environmental Analysis (UCEA) and the University Research Facility in Life Sciences (ULS) of Hong Kong Polytechnic University are also acknowledged.

References

[1] J. Fenn, M. Mann, C. Meng, S. Wong, C. Whitehouse, Electrospray Ionization for Mass-Spectrometry of Large Biomolecules, Science, 246 (1989) 64-71.

[2] M. Wilm, M. Mann, Analytical properties of the nanoelectrospray ion source, Anal Chem, 68 (1996) 1-8.

[3] C.M. Hong, C.T. Lee, Y.M. Lee, C.P. Kuo, C.H. Yuan, J. Shiea, Generating electrospray from solutions predeposited on a copper wire, Rapid Commun Mass Spectrom, 13 (1999) 21-25.

[4] K. Hiraoka, K. Nishidate, K. Mori, D. Asakawa, S. Suzuki, Development of probe electrospray using a solid needle, Rapid Commun Mass Spectrom, 21 (2007) 3139-3144.

[5] B. Hu, P.-K. So, Z.-P. Yao, Analytical Properties of Solid-substrate Electrospray Ionization Mass Spectrometry, J Am Soc Mass Spectrom, 24 (2013) 57-65.

[6] M.-Z. Huang, C.-C. Zhou, D.-L. Liu, S.-S. Jhang, S.-C. Cheng, J. Shiea, Rapid Characterization of Chemical Compounds in Liquid and Solid States Using Thermal Desorption Electrospray Ionization Mass Spectrometry, Anal Chem, 85 (2013) 8956-8963.

[7] J. Deng, Y. Yang, L. Fang, L. Lin, H. Zhou, T. Luan, Coupling Solid-Phase Microextraction with Ambient Mass Spectrometry Using Surface Coated Wooden-Tip Probe for Rapid Analysis of Ultra Trace Perfluorinated Compounds in Complex Samples, Anal Chem, 86 (2014) 11159-11166.

[8] B. Hu, P.-K. So, Z.-P. Yao, Electrospray ionization with aluminum foil: A versatile mass spectrometric technique, Anal Chim Acta, 817 (2014) 1-8.

[9] M.K. Mandal, K. Yoshimura, S. Saha, Z. Yu, S. Takeda, K. Hiraoka, Biomolecular Analysis and Biological Tissue Diagnostics by Electrospray Ionization with a Metal Wire Inserted Gel-Loading Tip, Anal Chem, 86 (2014) 987-992.

[10] 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.

[11] B. Hu, G.Z. Xin, P.K. So, Z.P. Yao, Thin layer chromatography coupled with electrospray ionization mass spectrometry for direct analysis of raw samples, J Chromatogr A, 1415 (2015) 155-160.

[12] H. Wang, J. Liu, R.G. Cooks, Z. Ouyang, Paper Spray for Direct Analysis of Complex Mixtures Using Mass Spectrometry, Angew Chem Int Edit, 49 (2010) 877-880.

[13] R.D. Espy, S.F. Teunissen, N.E. Manicke, Y. Ren, Z. Ouyang, A. van Asten, R.G. Cooks, Paper Spray and Extraction Spray Mass Spectrometry for the Direct and Simultaneous Quantification of Eight Drugs of Abuse in Whole Blood, Anal Chem, 86 (2014) 7712-7718.

[14] D.T. Snyder, C.J. Pulliam, Z. Ouyang, R.G. Cooks, Miniature and Fieldable Mass Spectrometers: Recent Advances, Anal Chem, 88 (2016) 2-29.

[15] R.T. Kelly, J.S. Page, I. Marginean, K. Tang, R.D. Smith, Dilution-Free Analysis from Picoliter Droplets by Nano-Electrospray Ionization Mass Spectrometry, Angew Chem Int Edit, 48 (2009) 6832-6835.

[16] Y. Ren, M.N. McLuckey, J. Liu, Z. Ouyang, Direct Mass Spectrometry Analysis of Biofluid Samples Using SlugFlow Microextraction Nano-Electrospray Ionization, Angew Chem Int Edit, 53 (2014) 14124-14127.

[17] N. Reyes-Garces, E. Gionfriddo, G.A. Gomez-Rios, M.N. Alam, E. Boyaci, B. Bojko, V. Singh, J. Grandy, J. Pawliszyn, Advances in Solid Phase Microextraction and Perspective on Future Directions, Anal Chem, 90 (2018) 302-360.

[18] G.A. Gomez-Rios, M. Tascon, J. Pawliszyn, Coated blade spray: shifting the paradigm of direct sample introduction to MS, Bioanalysis, 10 (2018) 257-271.

[19] L. Fang, J.W. Deng, Y.Y. Yang, X.W. Wang, B.W. Chen, H.T. Liu, H.Y. Zhou, G.F. Ouyang, T.G. Luan, Coupling solid-phase microextraction with ambient mass spectrometry: Strategies and applications, Trac-Trends Anal Chem, 85 (2016) 61-72.

[20] B. Hu, P.-K. So, H. Chen, Z.-P. Yao, Electrospray Ionization Using Wooden Tips, Anal Chem, 83 (2011) 8201-8207.

[21] B. Hu, Y.-H. Lai, P.-K. So, H. Chen, Z.-P. Yao, Direct ionization of biological tissue for mass spectrometric analysis, Analyst, 137 (2012) 3613-3619.

[22] S. Aksyonov, P. Williams, Electrospray ionization using disposable plastic pipette tips, Rapid Commun Mass Spectrom, 15 (2001) 1890-1891.

[23] M.K. Mandal, K. Yoshimura, S. Saha, S. Ninomiya, M.O. Rahman, Z. Yu, L.C. Chen, Y. Shida, S. Takeda, H. Nonami, Solid probe assisted nanoelectrospray ionization mass spectrometry for biological tissue diagnostics, Analyst, 137 (2012) 4658-4661.

[24] M.K. Mandal, K. Yoshimura, S. Saha, Z. Yu, S. Takeda, K. Hiraoka, Biomolecular analysis and biological tissue diagnostics by electrospray ionization with a metal wire inserted gel-loading tip, Anal Chem, 86 (2013) 987-992.

[25] B. Hu, Z.P. Yao, Detection of native proteins using solid-substrate electrospray ionization mass spectrometry with nonpolar solvents, Anal Chim Acta, 1004 (2018) 51-57.

[26] G.Z. Xin, B. Hu, Z.Q. Shi, Y.C. Lam, T.T.X. Dong, P. Li, Z.P. Yao, K.W.K. Tsim, Rapid identification of plant materials by wooden-tip electrospray ionization mass spectrometry and a strategy to differentiate the bulbs of Fritillaria, Anal Chim Acta, 820 (2014) 84-91.

[27] Y.Y. Yang, J.W. Deng, Z.P. Yao, Field-induced wooden-tip electrospray ionization mass spectrometry for high-throughput analysis of herbal medicines, Anal Chim Acta, 887 (2015) 127-137.

[28] H.Y. Wong, M.Y. Wong, B. Hu, P.K. So, C.O. Chan, D.K. Mok, Z.P. Yao, Rapid differentiation of Ganoderma species by direct ionization mass spectrometry, Anal Chim Acta, 999 (2018) 99-106.

[29] P.-K. So, T.-T. Ng, H. Wang, B. Hu, Z.-P. Yao, Rapid detection and quantitation of ketamine and norketamine in urine and oral fluid by wooden-tip electrospray ionization mass spectrometry, Analyst, 138 (2013) 2239-2243.

[30] J.W. Deng, W.Y. Li, Q.X. Yang, Y.H. Liu, L. Fang, Y.H. Guo, P.R. Guo, L. Lin, Y.Y. Yang, T.G. Luan, Biocompatible Surface-Coated Probe for in Vivo, in Situ, and Microscale Lipidomics of Small Biological Organisms and Cells Using Mass Spectrometry, Anal Chem, 90 (2018) 6936-6944.

[31] P.K. So, B. Hu, Z.P. Yao, Mass spectrometry: towards in vivo analysis of biological systems, Mol Biosyst, 9 (2013) 915-929.

[32] G.A. Harris, A.S. Galhena, F.M. Fernandez, Ambient Sampling/Ionization Mass Spectrometry: Applications and Current Trends, Anal Chem, 83 (2011) 4508-4538.

[33] G.A. Gómez-Ríos, J. Pawliszyn, Development of coated blade spray ionization mass spectrometry for the quantitation of target analytes present in complex matrices, Angew Chem Int Edit, 53 (2014) 14503-14507.

[34] S. Ahmad, M. Tucker, N. Spooner, D. Murnane, U. Gerhard, Direct Ionization of Solid-Phase Microextraction Fibers for Quantitative Drug Bioanalysis: From Peripheral Circulation to Mass Spectrometry Detection, Anal Chem, 87 (2015) 754-759.

[35] B. Hu, P.K. So, Y.Y. Yang, J.W. Deng, Y.C. Choi, T.G. Luan, Z.P. Yao, Surface-Modified Wooden-Tip Electrospray Ionization Mass Spectrometry for Enhanced Detection of Analytes in Complex Samples, Anal Chem, 90 (2018) 1759-1766.

[36] Y.-N. Yao, B. Hu, Analyte-Substrate Interactions at Functionalized Tip-Electrospray Ionization Mass Spectrometry: Molecular Mechanisms and Applications, J Mass Spectrom, (2018) doi:10.1002/jms.4300.

[37] E. Boyaci, K. Gorynski, A. Rodriguez-Lafuente, B. Bojko, J. Pawliszyn, Introduction of solid-phase microextraction as a high-throughput sample preparation tool in laboratory analysis of prohibited substances, Anal Chim Acta, 809 (2014) 69-81.

[38] D. Vuckovic, X. Zhang, E. Cudjoe, J. Pawliszyn, Solid-phase microextraction in bioanalysis: New devices and directions, J Chromatogr A, 1217 (2010) 4041-4060.

[39] B. Bojko, E. Cudjoe, G.A. Gomez-Rios, K. Gorynski, R. Jiang, N. Reyes-Garces, S. Risticevic, E.A.S. Silva, O. Togunde, D. Vuckovic, J. Pawliszyn, SPME - Quo vadis?, Anal Chim Acta, 750 (2012) 132-151.

[40] E.A.S. Silva, S. Risticevic, J. Pawliszyn, Recent trends in SPME concerning sorbent materials, configurations and in vivo applications, Trac-Trends Anal Chem, 43 (2013) 24-36.

[41] E.A. Souza-Silva, J. Pawliszyn, Direct Immersion Solid-Phase Microextraction with Matrix-Compatible Fiber Coating for Multiresidue Pesticide Analysis of Grapes by Gas Chromatography-Time-of-Flight Mass Spectrometry (DI-SPME-GC-ToFMS), J. Agric. Food Chem., 63 (2015) 4464-4477.

[42] N. Reyes-Garces, B. Bojko, J. Pawliszyn, High throughput quantification of prohibited substances in plasma using thin film solid phase microextraction, J Chromatogr A, 1374 (2014) 40-49.

[43] M. Walles, Y. Gu, C. Dartiguenave, F.M. Musteata, K. Waldron, D. Lubda, J. Pawliszyn, Approaches for coupling solid-phase microextraction to nanospray, J Chromatogr A, 1067 (2005) 197-205.

[44] J. Deng, Y. Yang, X. Wang, T. Luan, Strategies for coupling solid-phase microextraction with mass spectrometry, Trac-Trends Anal Chem, 55 (2014) 55-67.

[45] H. Tong, N. Sze, B. Thomson, S. Nacson, J. Pawliszyn, Solid phase microextraction with matrix assisted laser desorption/ionization introduction to mass spectrometry and ion mobility spectrometry, Analyst, 127 (2002) 1207-1210.

[46] Y. Wang, B.B. Schneider, T.R. Covey, J. Pawliszyn, High-performance SPME/AP MALDI system for high-throughput sampling and determination of peptides, Anal Chem, 77 (2005) 8095-8101.

[47] F.S. Mirnaghi, J. Pawliszyn, Reusable Solid-Phase Microextraction Coating for Direct Immersion Whole-Blood Analysis and Extracted Blood Spot Sampling Coupled with Liquid Chromatography-Tandem Mass Spectrometry and Direct Analysis in Real-Time Tandem Mass Spectrometry, Anal Chem, 84 (2012) 8301-8309.

[48] A. Rodriguez-Lafuente, F.S. Mirnaghi, J. Pawliszyn, Determination of cocaine and methadone in urine samples by thin-film solid-phase microextraction and direct analysis in real time (DART) coupled with tandem mass spectrometry, Anal Bioanal Chem, 405 (2013) 9723-9727.

[49] G.A. Gomez-Rios, J. Pawliszyn, Solid phase microextraction (SPME)-transmission mode (TM) pushes down detection limits in direct analysis in real time (DART), Chem. Commun., 50 (2014) 12937-12940.

[50] P.A. D'Agostino, C.L. Chenier, J.R. Hancock, C.R.J. Lepage, Desorption electrospray ionisation mass spectrometric analysis of chemical warfare agents from solid-phase microextraction fibers, Rapid Commun Mass Spectrom, 21 (2007) 543-549.

[51] G.A. Gomez-Rios, N. Reyes-Garces, B. Bojko, J. Pawliszyn, Biocompatible Solid-Phase Microextraction Nanoelectrospray Ionization: An Unexploited Tool in Bioanalysis, Anal Chem, 88 (2016) 1259-1265.

[52] C.P. Kuo, J. Shiea, Application of direct electrospray probe to analyze biological compounds and to couple to solid-phase microextraction to detect trace surfactants in aqueous solution, Anal Chem, 71 (1999) 4413-4417.

[53] J. Rios-Gomez, B. Fresco-Cala, M.T. Garcia-Valverde, R. Lucena, S. Cardenas, Carbon Nanohorn Suprastructures on a Paper Support as a Sorptive Phase, Molecules, 23 (2018).

[54] G.A. Gomez-Rios, C. Liu, M. Tascon, N. Reyes-Garces, 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.

[55] M. Tascon, M.N. Alam, G.A. Gomez-Rios, J. Pawliszyn, Development of a Microfluidic Open Interface with Flow Isolated Desorption Volume for the Direct Coupling of SPME Devices to Mass Spectrometry, Anal Chem, 90 (2018) 2631-2638.

[56] S. Ghiasikhou, M.F. da Silva, Y. Zhu, R. Zenobi, The capillary gap sampler, a new microfluidic platform for direct coupling of automated solid-phase microextraction with ESI-MS, Anal Bioanal Chem, 409 (2017) 6873-6883.

[57] S. Strano-Rossi, E. Castrignanò, L. Anzillotti, G. Serpelloni, R. Mollica, F. Tagliaro, J.P. Pascali, D. di Stefano, R. Sgalla, M. Chiarotti, Evaluation of four oral fluid devices (DDS®, Drugtest 5000®, Drugwipe 5+® and RapidSTAT®) for on-site monitoring drugged driving in comparison with UHPLC–MS/MS analysis, Forensic Sci Int, 221 (2012) 70-76.

[58] N.S. Nosseir, G. Michels, P. Binder, M.H.J. Wiesen, C. Mueller, Simultaneous detection of ketamine, lorazepam, midazolam and sufentanil in human serum with liquid chromatography-tandem mass spectrometry for monitoring of analgosedation in critically ill patients, J. Chromatogr. B, 973 (2014) 133-141.

[59] M. Thevis, T. Kuuranne, H. Geyer, W. Schaenzer, Annual banned-substance review: analytical approaches in human sports drug testing, Drug Test. Anal., 7 (2015) 1-20.

[60] T.-T. Ng, P.-K. So, B. Hu, Z.-P. Yao, Rapid detection and quantitation of drugs-of-abuse by wooden-tip electrospray ionization mass spectrometry, J Food Drug Anal, (2019) 10.1016/j.jfda.2018.1009.1002.

[61] P. Adamowicz, M. Kala, Urinary excretion rates of ketamine and norketamine following therapeutic ketamine administration: Method and detection window considerations, J Anal Toxicol, 29 (2005) 376-382.

[62] D. Vuckovic, J. Pawliszyn, Systematic Evaluation of Solid-Phase Microextraction Coatings for Untargeted Metabolomic Profiling of Biological Fluids by Liquid Chromatography-Mass Spectrometry, Anal Chem, 83 (2011) 1944-1954.

[63] J.H. Kennedy, C. Aurand, R. Shirey, B.C. Laughlin, J.M. Wiseman, Coupling Desorption Electrospray Ionization with Solid-Phase Microextraction for Screening and Quantitative Analysis of Drugs in Urine, Anal Chem, 82 (2010) 7502-7508.

[64] M.-K. Huang, C. Liu, J.-H. Li, S.-D. Huang, Quantitative detection of ketamine, norketamine, and dehydronorketamine in urine using chemical derivatization followed by gas chromatography–mass spectrometry, J. Chromatogr. B, 820 (2005) 165-173.

[65] E.-m. Kim, J.-s. Lee, S.-k. Choi, M.-a. Lim, H.-s. Chung, Analysis of ketamine and norketamine in urine by automatic solid-phase extraction (SPE) and positive ion chemical ionization-gas chromatography-mass spectrometry (PCI-GC-MS), Forensic Sci Int, 174 (2008) 197-202.

[66] J.Y.K. Cheng, V.K.K. Mok, Rapid determination of ketamine in urine by liquid chromatography-tandem mass spectrometry for a high throughput laboratory, Forensic Sci Int, 142 (2004) 9-15.

[67] C.-Y. Chen, M.-R. Lee, F.-C. Cheng, G.-J. Wu, Determination of ketamine and metabolites in urine by liquid chromatography-mass spectrometry, Talanta, 72 (2007) 1217-1222.

[68] M.C. Parkin, S.C. Turfus, N.W. Smith, J.M. Halket, R.A. Braithwaite, S.P. Elliott, M.D. Osselton, D.A. Cowan, A.T. Kicman, Detection of ketamine and its metabolites in urine by ultra high pressure liquid chromatography-tandem mass spectrometry, J. Chromatogr. B, 876 (2008) 137-142.

[69] M. Tascon, G.A. Gomez-Rios, N. Reyes-Garces, J. Poole, E. Boyaci, J. Pawliszyn, High-Throughput Screening and Quantitation of Target Compounds in Biofluids by Coated Blade Spray-Mass Spectrometry, Anal Chem, 89 (2017) 8421-8428.

Table 1. Response of ketamine at low concentrations in urine as detected by SPME-ESI-MS

Spiked ketamine (ng/mL)	Signal-to-noise
0.1	10.8
0.5	52.4
1.0	87.7
2.5	216.7
5.0	420.6

Table 2. Precision and accuracy for detection of ketamine in urine by SPME-ESI-MS

Spiked ketamine (ng/mL)	Accuracy (n=3), %	Precision (n=3), %
4.0	90.8	8.1
40.0	109.4	3.4
400.0	104.8	10.6

Figure captions

Figure 1. Procedures for SPME sampling: (a) cleaning/conditioning, (b) sampling/extraction, (c) washing.

Figure 2. Schematic diagram of SPME-ESI-MS with different settings for applying elution and ionization solvent: (a) desorption spray (with a top view photo), (b) dripping, (c) DESI, (d) nanoESI.

Figure 3. (a) Mass spectra obtained by analysis of 1 μg/ml ketamine aqueous solution with SPME-ESI-MS; (b) Effects of different SPME fibers for detection of ketamine.

Figure 4. Effects of various factors on SPME-ESI-MS signal of ketamine: (a) extraction time, (b) washing time. Each data point was the average of three individual measurements, where the error bar represented the standard deviation.

Figure 5. (a) Mass spectra obtained by analysis of a urine sample spiked with 100 ng/ml ketamine using SPME-ESI-MS under optimized conditions, (b) MS/MS spectrum of ion at *m/z* 238.

Figure 6. Quantitative analysis of ketamine in human urine. Blue markers represent calibration points, and orange markers represent validation points. The following extraction parameters were used: 10 min extraction time, 1500 rpm orbital agitation, and room temperature. Each data point was the average of three individual measurements (n=3), where the error bar depicts the standard deviation.

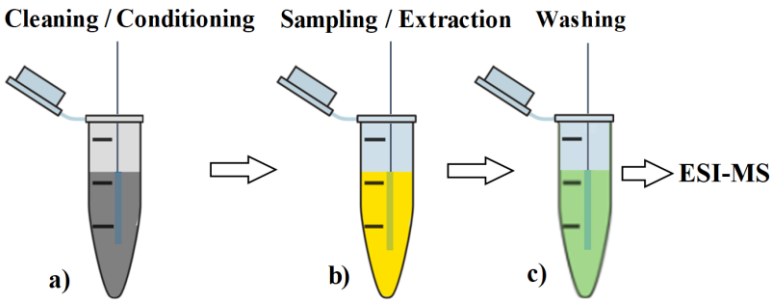
Cleaning / Conditioning
Sampling / Extraction
Washing
ESI-MS
a)
b)
c)

Figure 1

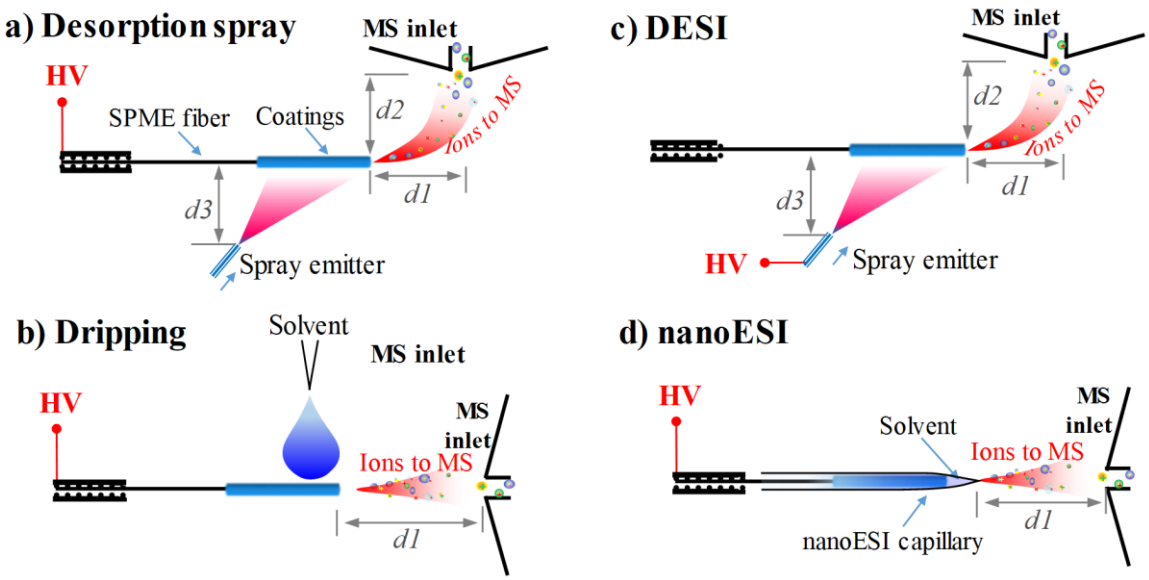
a) Desorption spray
MS inlet
HV
SPME fiber
Coatings
d2
Ions to MS
d3
d1
Spray emitter
c) DESI
MS inlet
d2
Ions to MS
d3
d1
HV
Spray emitter
b) Dripping
Solvent
MS inlet
HV
MS inlet
Ions to MS
d1
d) nanoESI
HV
Solvent
MS inlet
Ions to MS
d1
nanoESI capillary

Figure 2

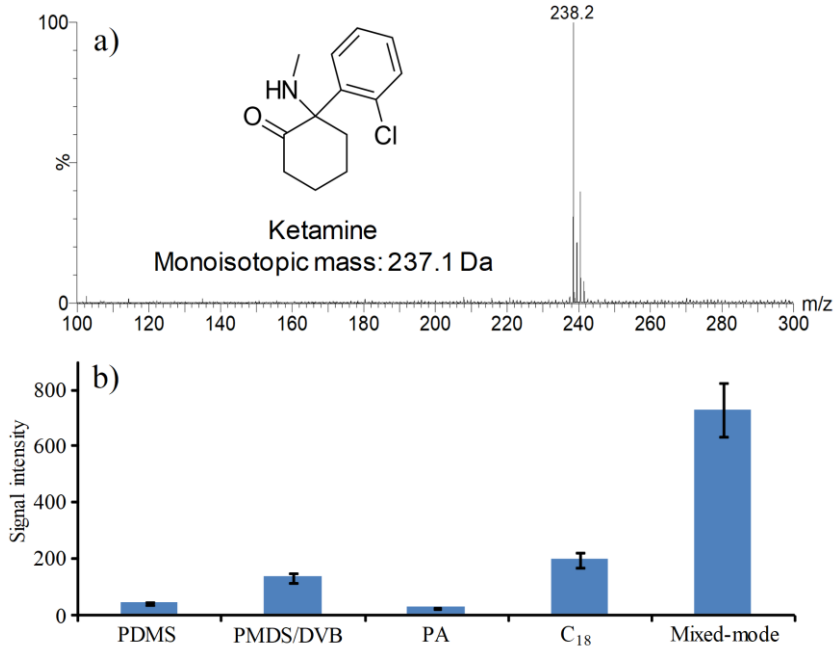
a)
100
%
0
238.2
HN
O
Cl
Ketamine
Monoisotopic mass: 237.1 Da
100
120
140
160
180
200
220
240
260
280
300
m/z
b)
Signal intensity
800
600
400
200
0
PDMS
PMDS/DVB
PA
C18
Mixed-mode

Figure 3

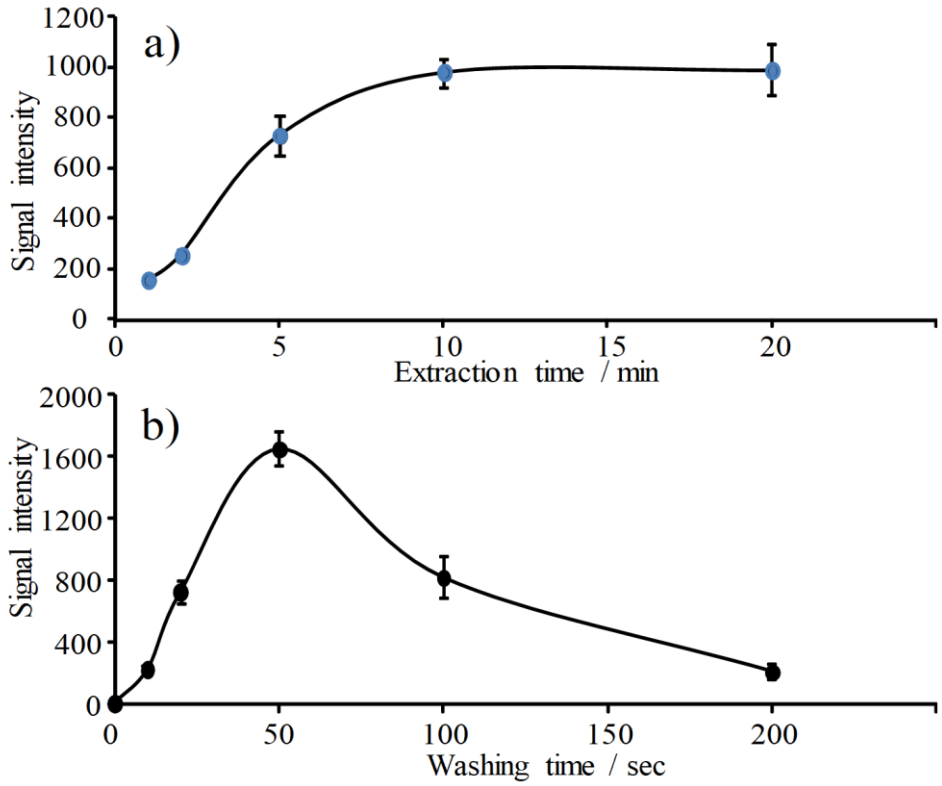
a)
Signal intensity
1200
1000
800
600
400
200
0
0
5
10
15
20
Extraction time / min
b)
Signal intensity
2000
1600
1200
800
400
0
0
50
100
150
200
Washing time / sec

Figure 4

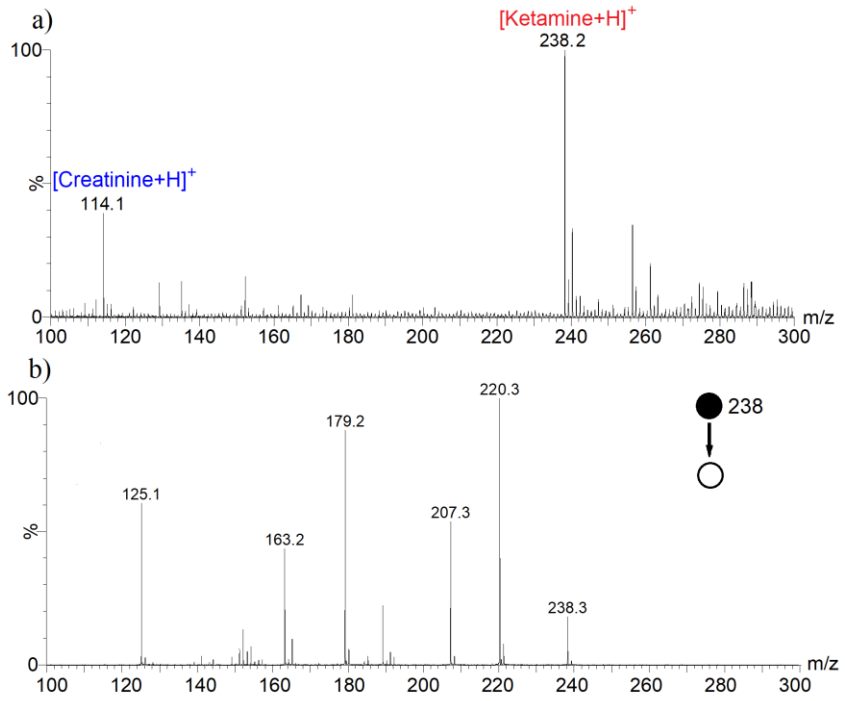
a)
[Ketamine+H]+
238.2
100
%
[Creatinine+H]+
114.1
0
m/z
100 120 140 160 180 200 220 240 260 280 300
b)
100
220.3
238
179.2
125.1
207.3
%
163.2
238.3
0
m/z
100 120 140 160 180 200 220 240 260 280 300

Figure 5

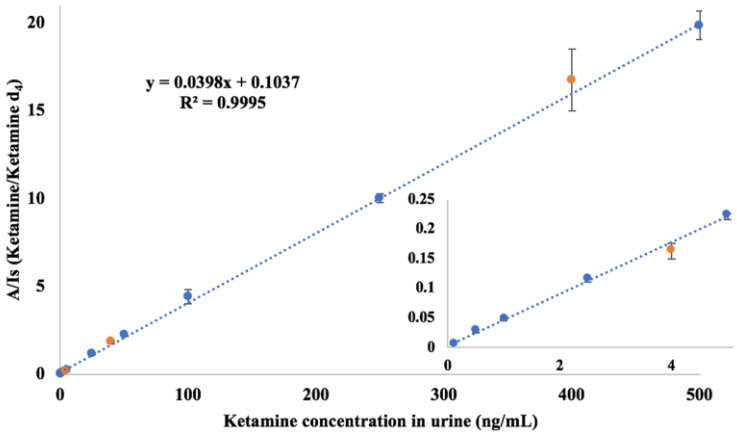
y = 0.0398x + 0.1037
R² = 0.9995
A/Is (Ketamine/Ketamine d4)
Ketamine concentration in urine (ng/mL)
0
5
10
15
20
0
100
200
300
400
500
0.25
0.2
0.15
0.1
0.05
0
0
2
4

Figure 6

Supplementary Material

Direct Coupling of Solid Phase Microextraction with Electrospray Ionization Mass Spectrometry: A Case Study for Detection of Ketamine in Urine

Bin Hu,[a,b,c] Bo Zheng,[a,b] Daniel Rickert,[d] German Augusto Gómez-Ríos,[d,e] Barbara Bojko,[d,f] Janusz Pawliszyn,[d]* Zhong-Ping Yao[a,b]*

[a]State Key Laboratory of Chemical Biology and Drug Discovery, Food Safety and Technology Research Centre and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong Special Administrative Region, China

[b]State Key Laboratory of Chinese Medicine and Molecular Pharmacology (Incubation) and Shenzhen Key Laboratory of Food Biological Safety Control, Shenzhen Research Institute of The Hong Kong Polytechnic University, Shenzhen 518057, China

[c]Institute of Mass Spectrometer and Atmospheric Environment, and Guangdong Provincial Engineering Research Center for On-line Source Apportionment System of Air Pollution, Jinan University, Guangzhou 510632, China

[d]Department of Chemistry, University of Waterloo, Waterloo, ON N2L 3G1, Canada

[e]Current address: Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823, United States

[f]Current address: Department of Pharmacodynamics and Molecular Pharmacology, Faculty of Pharmacy, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, 85-067 Bydgoszcz, Poland

*Corresponding authors:
J. Pawliszyn:
Department of Chemistry, University of Waterloo, Waterloo, ON N2L 3G1, Canada
E-mail: janusz@uwaterloo.ca; Tel.: +1 519 8884641; Fax: +1 519 7460435.

Z. P. Yao:
Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong
E-mail: zhongping.yao@polyu.edu.hk; Tel.:+852 34008792; Fax: +852 23649932.

Table S1. Comparison of LOD and LOQ obtained by different SPME-ESI-MS modes for detection of ketamine in raw urine samples.

SPME-ESI-MS mode	**LOD (ng/mL) (S/N ≥ 3)**	**LOQ (ng/mL) (S/N ≥ 10)**
Desorption spray	0.1	1.0
DESI	0.5	2.0
Dripping	0.5	2.0
nanoESI	0.027	0.1

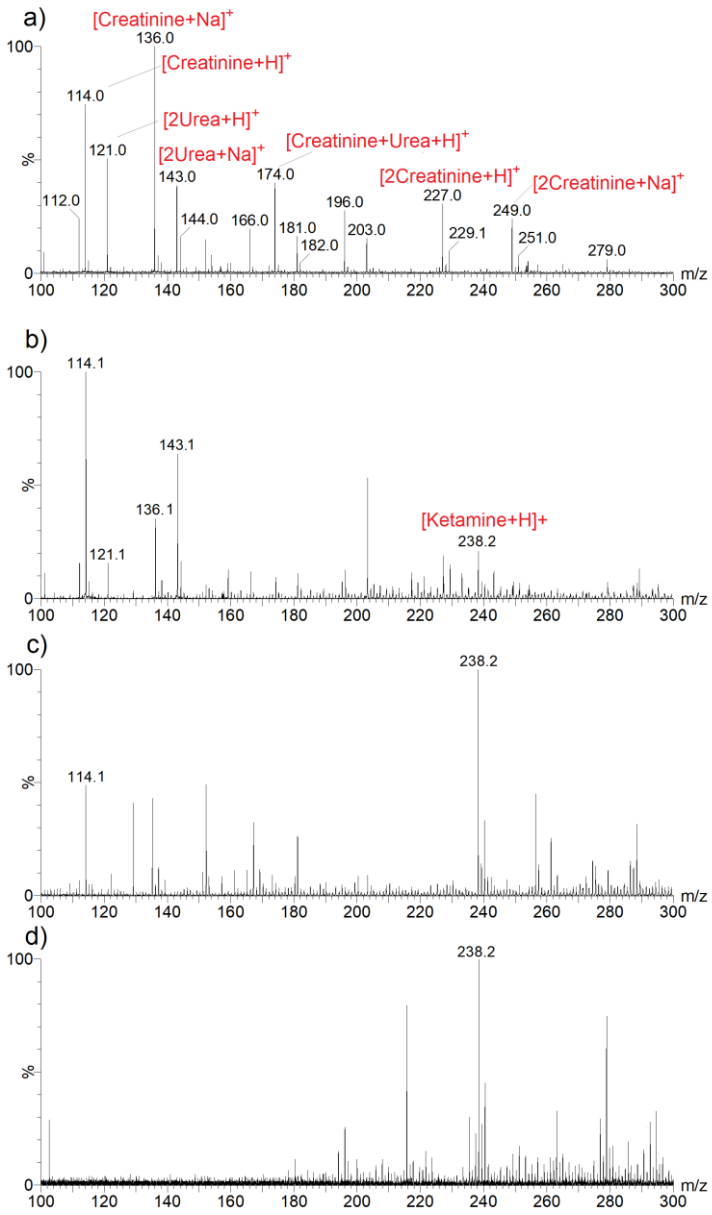

Figure S1. Mass spectra obtained by analysis of 100 ng/ml ketamine aqueous solution with SPME-ESI-MS under different conditions (a) extraction time at 1 min and washing 0 sec; (b) extraction time at 1 min and washing 50 sec; (c) extraction time at 5 min and washing 50 sec; (b) extraction time at 10 min and washing 200 sec.

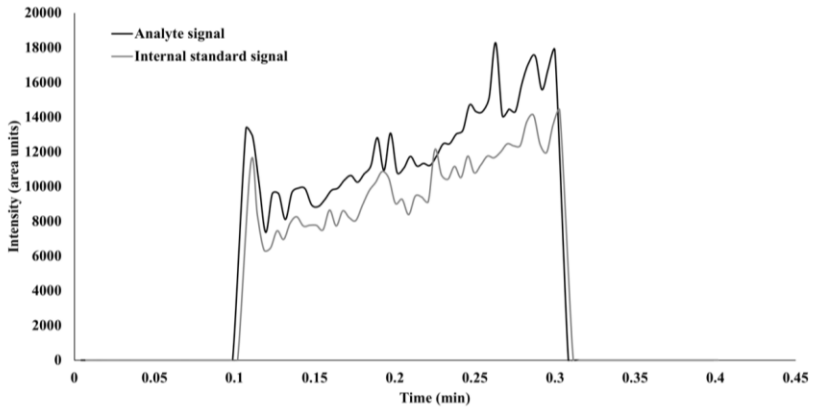

Figure S2. Representative overlaid extracted ion chromatograms depicting the analyte and internal standard signals that were used to create the A/I ratios.

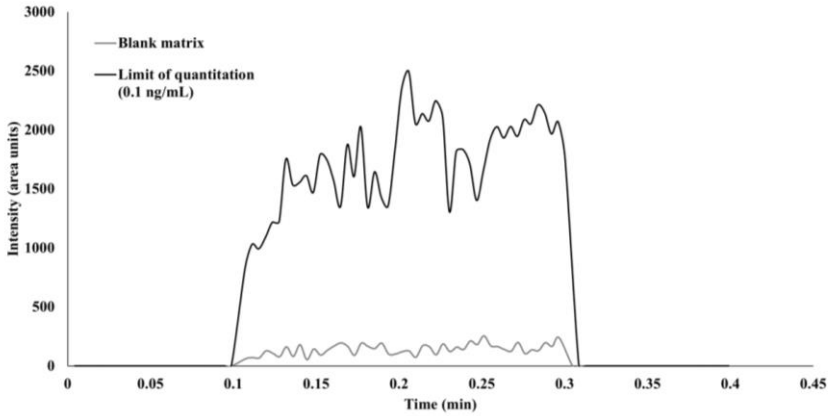

Figure S3. Representative overlaid extracted ion chromatograms depicting the signal to noise ratio at the limit of quantitation.