Mass Spectrometry

DOI: 10.1002/anie.201407057

Development of Coated Blade Spray Ionization Mass Spectrometry for the Quantitation of Target Analytes Present in Complex Matrices**

*German Augusto Gómez-Ríos and Janusz Pawliszyn**

Abstract: *Coated blade spray (CBS) is a technology based on solid-phase microextraction (SPME) that has been designed for the quick extraction/cleanup of analytes from complex matrices and direct desorption/ionization under ambient mass spectrometry conditions. The entire analytical process can be completed in less than 3 min and enables limits of quantitation in the low picogram-per-milliliter region to be reached.*

The rapid development of ambient ionization techniques during the beginning of the 21st century[1–4] has enabled the introduction of new applications of solid-phase microextraction (SPME). As recently reviewed,[5] different geometries of SPME have been coupled to direct analysis in real time (DART), desorption electrospray ionization (DESI), surface-enhanced laser desorption–ionization (SELDI), and matrix-assisted laser desorption–ionization (MALDI) in a broad range of applications.[6–13] We report herein the development of a novel SPME configuration that allows its use, without further modifications, as an ambient ionization method for mass spectrometry.

Indeed, coated blade spray (CBS) was conceived as an ideal compromise between sample preparation and direct coupling to mass spectrometry. In essence, the device comprises a stainless-steel sheet ($\varnothing \leq 200$ μm) cut as a "gladius sword" and coated with the biocompatible polymer C_{18}-polyacrylonitrile (C_{18}-PAN). As a sample-preparation method, the SPME coating ($\varnothing \leq 80$ μm) simultaneously isolates and enriches the analytes present in the matrix without removing the matrix itself.[14–22] Furthermore, since the coating is matrix-compatible and tuned for the extraction of analytes of interest in their free form, the technique enables the cleaning up of undesirable artefacts that might cause ion suppression or enhancement, which are typically observed if the matrix is placed directly in front of a mass spectrometer.[15–17] In this ambient ionization technique, the coated blade acts as a solid-substrate ESI source;[2,3] ions of the extracted/preconcentrated analytes are generated by applying a high electric field to a blade prewetted with a small volume (≤ 20 μL) of the desorption solution (see Video S1 in the Supporting Information). As summarized in Figure 1, the analytical process consists of three simple steps: First, a preconditioned blade is inserted in a vial containing the sample matrix (300–1500 μL), and quick extraction/enrichment is performed by agitating the sample at high speed (vortex agitation at 3200 rpm, $t \leq 1$ min); next, the blade is rapidly rinsed in a vial containing water ($t \leq 10$ s) in an aim to remove matrix components adhered to the coating surface; finally, the blade is installed on the blade holder, in which a spring-loading system enables the straightforward connection of a high voltage (HV) to the blade and the ready and fast replacement of the blade between experiments (see Figure S1 in the Supporting Information). Similarly to other methodologies based on solid subtrate-electrospray ionization (ESI),

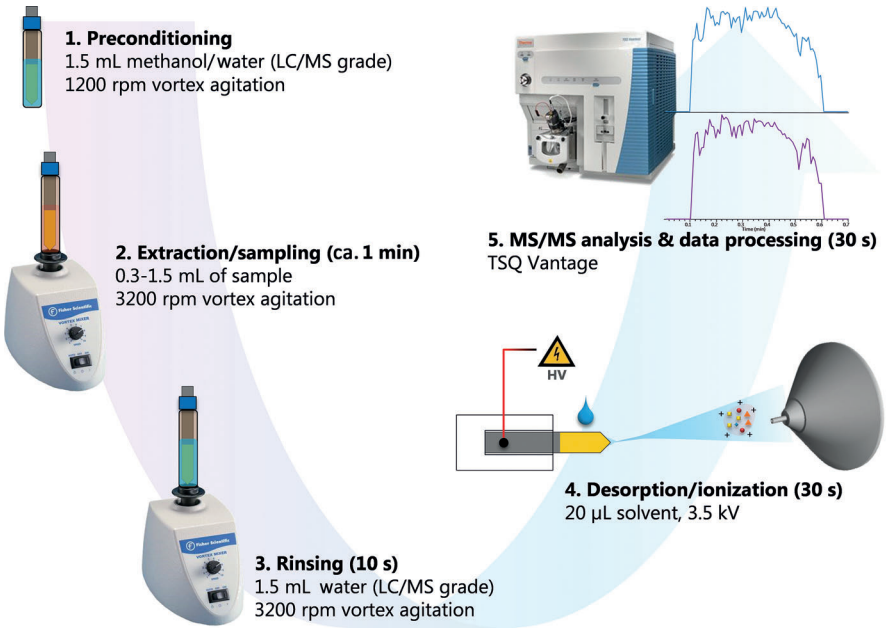

Figure 1. Experimental setup for blade-spray extraction and desorption/ionization.

[*] M. Sc. G. A. Gómez-Ríos, Prof. J. Pawliszyn
Department of Chemistry, University of Waterloo
200 University Avenue, Waterloo, N2L 3G1 (Canada)
E-mail: janusz@uwaterloo.ca
Homepage: http://spme.uwaterloo.ca

[**] This research was financially supported by the National Sciences and Engineering Research Council of Canada (NSERC) and Supelco. We also want to express our sincere gratitude to the Science Shop of the University of Waterloo for their technical assistance, as well as to M. Sc. Nathaly Reyes-Garcés and Dr. Barbara Bojko for scientific discussions.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201407057.

the duration and intensity of the analyte signal is affected by the amount extracted, the volume and chemical features of the desorption solvent, the wetting time (time preceding the application of a high voltage), and the spraying voltage.[23–28]

Given that coated blade spray merges sample preparation and ambient ionization, the selection of adequate desorption/ionization conditions depends on the following: 1) the chemistry of the analyte and its affinity for the desorption solvent; 2) the kinetics of analyte partitioning between the coating layer and the solvent drop (elution efficiency of the analytes); and 3) the spray efficiency of the solvent at the tip of the blade.[4,26] The optimization of such parameters by experimental design resulted in the use of 17.5 μL of methanol, a wetting time of 37.5 s, and a voltage of 3.5 kV as the most favorable conditions for the analysis of diazepam (DZP). However, these values are valid only for the compound examined in the present study, and during the development of a new blade-spray analytical method, all these parameters must be addressed thoroughly by the analyst.

In view of the fact that CBS and paper spray (PS) have similarities in terms of the design of the devices (differences and resemblances between PS and CBS are summarized in Table S1 of the Supporting Information),[4] an analogous ionization mechanism is plausible.[25] There are three key similarities between the techniques: First, the spraying tip consists of a macroscopically sharp point with a tip optimally angled at less than 90°. Second, the desorption solution transports the analytes towards the tip once a high potential is applied between the device and the mass-spectrometer inlet. Third, the electric field created between the blade and the MS inlet induces a charge that accumulates at the vertex of the device, thus causing ionization, as previously described for ESI.[24,25] However, provided that the CBS is made of a conductive material, such as stainless steel, rather than a porous nonconductive substrate, such as paper, it could be assumed that a steady electric field is produced at the CBS tip. As a result of the stable electric-field gradient between the metal tip and the MS inlet, more reproducible and efficient ion formation during the spray process is expected. Consequently, the high sensitivity of the CBS method is presumed to result not only from the enrichment factor provided by SPME, but also from the enhancement in ionization. As in PS,[26] corona discharge can occur in CBS when electrical potentials above 4.5 kV are applied to the metal blade (particularly when the solvent has been depleted). Although we have not yet thoroughly explored the mechanisms governing CBS ionization, the protocol for the analysis of complex mixtures is simple and rapid. A better understanding of the fundamentals involved in coated blade spray mass spectrometry should enable improvements to be made in the performance of the technique and widen its scope of application.[2,3,26]

Since their conception, ambient ionization methods have been developed to circumvent several steps in the analytical process.[1] Indeed, sample-preparation approaches coupled to MS have been erroneously viewed by practitioners of ambient mass spectrometry as unnecessary, byzantine, and tedious.[1,4] Conversely to what is normally believed,[1] SPME extraction/enrichment can be performed in a short period of time, and the limit of detection (LOD) of the method is generally constrained by the instrumental capabilities rather than by the intrinsic features of the coating.[14,15,19,20] For example, a time period of 15 s is enough for the extraction of a quantifiable amount of an analyte at low ppb levels by a traditional LC/MS method (see Figure S2; ppb = parts per billion). The direct coupling of SPME to MS can easily surpass these detection limits, since the desorption/dilution step inherent to most SPME–LC methods is removed from the analytical procedure. Furthermore, since extractions are carried out at preequilibrium,[19] the amount of analyte collected is controlled by the convection conditions (i.e. boundary layer), the extraction time, and the surface area of the extracting phase. Hence, in an identical sampling setting, CBS can exceed the sensitivity of other SPME geometries owing to its high surface area.[20] By merely increasing the interaction time between the coating and the sample matrix from 15 s to 1 min, lower LODs can be achieved. Limits of quantitation (LOQs) as low as 1 part per trillion (1 ppt) were reached upon extraction for 1 min from 1.5 mL of phosphate-buffered saline (PBS) spiked with cocaine (calibration functions were constructed on the basis of the signal ratio of the analyte and its isotopologue (A/I_s) in three independent CBS experiments; Figure 2). Furthermore, the linear dynamic range of the method, evaluated up to 1 ppm, showed astounding linearity. Beyond any doubt, high concentration levels are not a limitation for SPME.[19] Indeed, in cases in which the affinity of the coating for the analytes is high and analytes are present at concentrations superior to 50 ppb, shorter extraction times (≤ 30 s) could be used.

The remarkable features of blade-spray technology, in comparison to other ambient mass spectrometry devices, include its reusability and intra-/interdevice reproducibility.[11,21] Extractions performed with three independent blades ($n = 12$) from 1.5 mL of a PBS solution containing 10 ppb of DZP showed intra-/interblade relative standard deviations (RSDs) lower than 1.8 % (see Table S2). Thus, the production of coatings with reproducible characteristics resulted in excellent reproducibility and repeatability. Besides, since the extraction phase normalized the sample matrix placed in front of mass spectrometer, the extraction of only small molecules (i.e. analytes of interest) in amounts corresponding to their free concentration in the sample ensured no measurable changes in matrix effects and therefore the reproducible response of the instrument. Furthermore, carry-over was negligible when a cleaning step was implemented once the extraction/desorption–ionization cycle was completed. A mixture of methanol (MeOH, 50 %), isopropanol (IPA, 25 %), and acetonitrile (ACN, 25 %) was found in preliminary experiments to be an ideal solution to get rid of most residual analytes from preceding extractions.[22] The cleaning step should be optimized according to the chemical nature of the coating and its affinity for the analyte of interest. In cases in which large variability in the sample concentration exists between different samples (e.g. low ppt to high ppb or even ppm levels), blades should be constrained to a single use. Otherwise, a small amount of analyte (a few picograms) could potentially linger on the blade after the desorption/cleaning

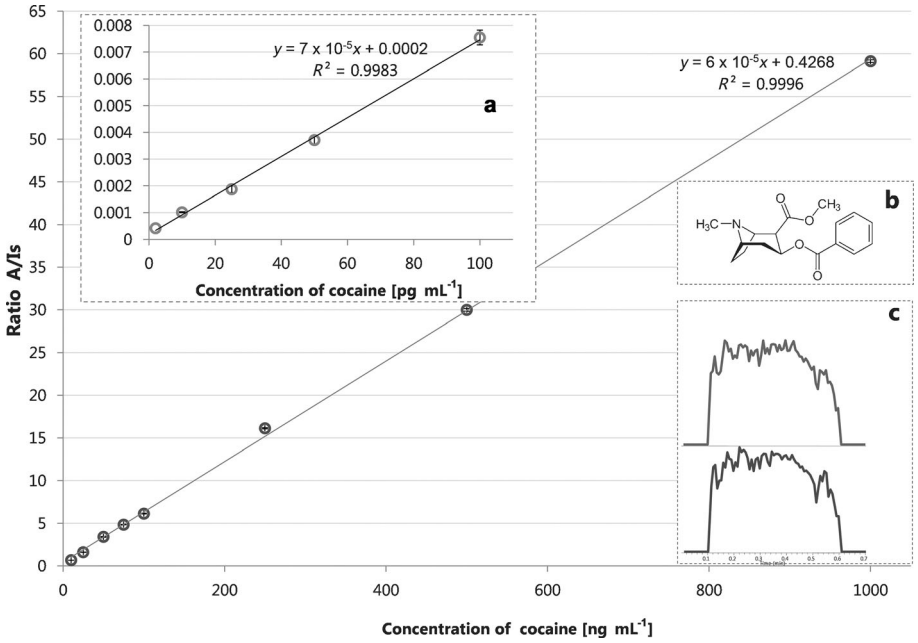

Figure 2. Quantitative analysis of PBS spiked with cocaine (2 pg ml^{-1} to 1 μg mL^{-1}) and its isotopologue [D_3]cocaine (14.5 ng mL^{-1}). Bars represent the standard deviation of analyses for three replicates with independent blades. a) Plot showing the low-concentration range. b) Molecular structure of cocaine. c) Ion chronogram of cocaine (top) and [D_3]cocaine (bottom) for an acquisition time of 30 s.

cycle and lead to possible false positive results. When working with compounds with a high affinity for the coating[18–20] that are present in the sample at concentrations higher than 50 ppb, shorter extraction times ($\leq$ 30 s) are recommended to diminish the amount of analyte enriched; this precaution would guarantee complete removal of the nonsprayed analytes during the cleaning step. Furthermore, by the use of thin coatings, efficient equilibration of the analytes is possible (faster extractions), together with more effective desorption/ionization. For this reason, blades coated with a consistent and reproducible thinner layer of sorbent are under development in our laboratory.[14]

In contrast to PS, another exceptional characteristic of CBS is the mechanical strength provided by the use of a stiff substrate in the creation of the blades. As a result, deformation/damage of the device does not occur, regardless of the sample dimensions. Thus, CBS could be used to perform extractions from small volumes (e.g. a few microliters, such as an extracted blood spot)[11] up to large volumes (e.g. hundreds of liters, as in the on-site monitoring of a watercourse/lake).[14] Additionally, in analogy with thin-film microextraction (TFME),[21] CBS can be implanted into tissue for the in vivo monitoring of endogenous and exogenous substances.[14,20,21] Indeed, the geometry of the device for in vivo applications might not be limited to a flat surface. For example, a cylindrical coated device (i.e. a metal pin with $\varnothing \leq$ 150 μm) could provide mildly intrusive access to tissue,[14] which is inaccessible by other modern methods (e.g. DESI), with minimal damage.[15–17] Succinctly, contrary to most ambient ionization techniques, SPME-based spray approaches can be used for in situ, in vivo, ex vivo, and on-site applications independently of the sample characteristics (e.g. volume, structure, complexity, and viscosity) with truly minimal sample preparation.

An exclusive characteristic of blade spray is its capability for reproducible and independent desorption/ionization from each side of the blade (see Figure S3). Hence, analysis in duplicate of each sample from a single extraction is feasible when the blade is coated with the same extraction phase on both sides. Indeed, multiple attempts towards the development of a comprehensive coating for SPME have been made.[14–18] The universal approach for complete coverage, solid-phase extraction (SPE), consists of combining two or more extraction phases with different affinities for the analytes, for example, coatings that undergo hydrophobic and hydrophilic interactions. However, when using this procedure, a compromise often has to be made with regard to the chemical nature of the two coatings, and generally an intermediate phase is obtained. The device presented herein can be coated with a different polymeric phase on each side, thus covering two different ranges of polarities and consequently providing a genuinely comprehensive analysis in a single experiment.[14,17,20] Because of these features, CBS appears to be a practical tool for metabolomics studies; however, further research regarding its reproducibility and quantitation capabilities without the use of an internal standard (I_s) is still required prior to its application for untargeted analysis in complex matrices. As we recently reported,[29] once the SPME geometrical characteristics that affect the desorption/ionization of the analytes are controlled, reproducible quantitation without an internal standard is feasible. Therefore, our ongoing research is primarily focused on optimizing the geometry of CBS and the coating characteristics to provide run-to-run and batch-to-batch reproducible results even when an internal standard is not used for signal correction. Considering that CBS, as a SPME device, reduces matrix effects and provides matrix normalization (i.e. analytes are extracted/preconcentrated in a well-defined noninterfering matrix, which is the coated sorbent), precise geometrical conditions (e.g. a thin homogenous layer of particles)[29] will facilitate its application as a quantitative tool in metabolomics research.[14,17]

Given the simplicity and speed of analysis with blade spray, the technique can be said to be an ideal device for the screening of pharmaceutical drugs or illicit compounds in biological samples. Blade spray was used for the quantification of cocaine and diazepam in urine and plasma. Exceptional linearity was observed in both matrices (Figure 3). Similar to the results obtained in PBS, LOQs of 0.5 and 2 pg ml^{-1} were determined for cocaine in plasma and urine,

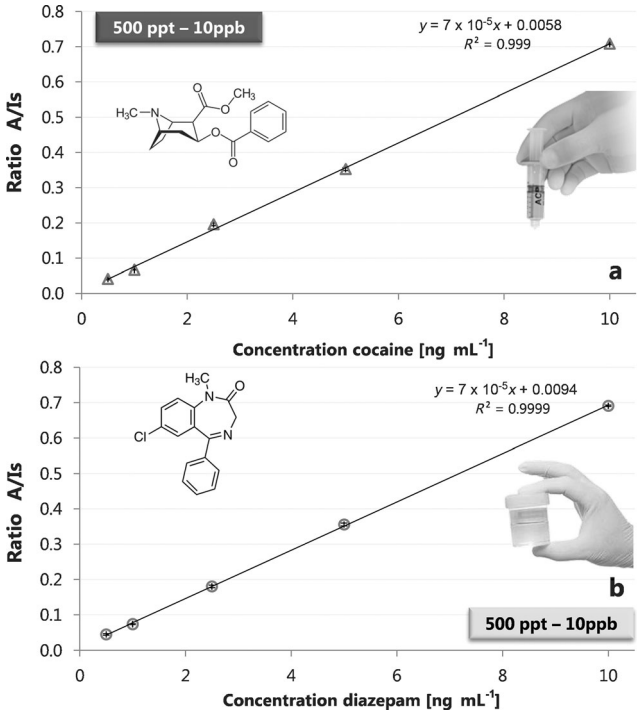

Figure 3. a) Quantitative analysis of plasma spiked with cocaine (500 pg ml^{-1} to 10 ng mL^{-1}) and its isotopologue [D_3]cocaine (14.5 ng mL^{-1}). b) Quantitative analysis of urine spiked with diazepam (500 pg ml^{-1} to 10 ng mL^{-1}) and its isotopologue [D_5]diazepam (16 ng mL^{-1}).

respectively. As expected, in a comparison made with nanopure water spiked with target analytes (see Figure S4), it was found that the rinsing step was critical in the diminishment of ionization suppression from salts (e.g. from urine/PBS) and the attachment of biomolecules to the coating surface (e.g. from plasma). In summary, by using a microextraction device to extract/transfer the analytes from the sample matrix to the MS system, matrix effects for analytes with low binding are significantly minimized, and detection limits are similar independent of the matrix (e.g. cocaine, 5% protein binding).[22] Indubitably, sample cleanup provided by CBS is convenient not only for quantitation purposes, but also to extend the operative time of the mass spectrometer by minimizing instrument maintenance and providing steady instrumental sensitivity. In contrast to the results for cocaine, the LOD and LOQ for DZP in plasma were 15 and 50 ppt, respectively. Certainly, the quantification limit is higher than that in urine and PBS (LOQ: 5 ppt). However, 98% of the DZP in the sample is bound to plasma proteins, and, as an SPME device, CBS equilibrates only with the free portion of the analyte in the sample.[14] The total analysis time (extraction from a complex matrix without pretreatment, rinsing, desorption/ionization, peak integration, and quantitation of total concentration) is less than 3 min per sample when the blades are operated manually.

There is currently much effort directed towards the development of powerful LC–MS/MS or GC–MS/MS methods that enable the analysis of controlled substances in complex matrices. Because of the complexity of the samples, such procedures entail cumbersome and extensive sample-preparation steps. Consequently, approaches that enable fast, quantitative, and direct analysis are in high demand.

As a proof of concept, the blade spray technique was used to screen 21 compounds controlled by the World Anti-Doping Agency (WADA) and the United Nations Office on Drugs and Crime (UNODC).[22,30] The selected reaction monitoring (SRM) mode was used to uniquely identify each substance.[30] On the basis of the results obtained for cocaine and DZP in PBS (Figure 2), LOQs were tentatively estimated for all compounds (see Table S3). Although the desorption/ionization conditions were not optimized for each analyte, all substances were detected at 20 ppb, and hypothetical LODs lower than 5 ppt were found for 14 compounds (e.g. clenbuterol, 6-acetylcodeine, and toremifene; see Figure S6). As blade spray derives its selectivity and sensitivity from the chemical and physical properties of the coating used, current research is directed towards the development of coatings with higher affinity for the target compounds to provide lower limits of detection.[14,20] The ability to use CBS to simultaneously screen multiple substances of interest in a single analysis without sacrificing sensitivity or increasing the analysis time is an outstanding characteristic of this technique. Thus, the application of CBS for the concomitant monitoring of numerous pesticides in food commodities, personal-care products in wastewater, or doping substances used in high-performance competitions is foreseen in the near future.[5]

Other major future applications of CBS are in situ applications within the medical field. The ability to deliver a rapid prognostic metric of a clinical condition is certainly important in the critical-care setting (emergency and surgery units).[14,28] Thus, molecular diagnostic and prognostic instruments, which are able to provide doctors with fast and reliable results, are highly desired in such facilities for the personalized diagnosis and treatment of patients. Although CBS reusability is undoubtedly beneficial in fields in which high throughput is needed and hundreds of samples need be processed on a daily basis,[21] blade spray is also envisioned as a splendid single-use device for trace analysis. For this reason, our group is currently working on the development of a cost-effective disposable device. By coupling disposable blade spray (dCBS) to portable/readily deployable mass spectrometers,[14,31] fast and reliable "real-time" measurements will be provided on site.[15–17] Thus, owing to its speed, sensitivity, selectivity, simplicity and linear dynamic range, dCBS–MS/MS will be a useful tool not only for point-of-care therapeutic drug monitoring, but also in diverse forensic, food, medical, and environmental applications.[2,3,5,14] Indeed, the many advantages of using CBS and other SPME–MS configurations[5,11,12,29] will certainly encourage analytical scientists around the world to choose these swifter sample-preparation approaches prior to direct MS analysis over traditional, more time-consuming methods.

Experimental Section

All experiments were carried out with a TSQ Vantage mass spectrometer (Thermo Scientific, San Jose, CA, USA). To guarantee the accurate positioning of the blades in front of the mass

spectrometer during all experiments, an in-house ionization source was built at the University of Waterloo (see Figure S5). The 3D moving stage (Newport Corporation, Irvine, CA) not only adjusts the position with a precision of 0.02 mm in each dimension (25 mm moving path), but also tunes the spraying tip at different angles in the Z dimension ($\pm 0.01°$ per moving mark). To ensure optimum ion transmission, the position of the blade tip should not be offset by more than 5 mm in all directions from the center of the ion-transfer capillary. Coated blades were prepared in house by spraying a C_{18}-polyacrylonitrile (C_{18}-PAN) solution according to a protocol developed in our laboratory.

Received: July 9, 2014
Revised: October 21, 2014
Published online: November 10, 2014

Keywords: ambient ionization · coated blade spray · complex matrices · mass spectrometry · trace analysis

[1] R. G. Cooks, Z. Ouyang, Z. Takats, J. M. Wiseman, *Science* **2006**, *311*, 1566 – 1570.
[2] A. R. Venter, K. A. Douglass, J. T. Shelley, G. Hasman, E. Honarvar, *Anal. Chem.* **2014**, *86*, 233 – 249.
[3] M. E. Monge, G. A. Harris, P. Dwivedi, F. M. Fernández, *Chem. Rev.* **2013**, *113*, 2269 – 2308.
[4] A. K. Badu-Tawiah, L. S. Eberlin, Z. Ouyang, R. G. Cooks, *Annu. Rev. Phys. Chem.* **2013**, *64*, 481 – 505.
[5] J. Deng, Y. Yang, X. Wang, T. Luan, *TrAC Trends Anal. Chem.* **2014**, *55*, 55 – 67.
[6] H. Tong, N. Sze, B. Thomson, S. Nacson, J. Pawliszyn, *Analyst* **2002**, *127*, 1207 – 1210.
[7] Y. Wang, M. Walles, B. Thomson, S. Nacson, J. Pawliszyn, *Rapid Commun. Mass Spectrom.* **2004**, *18*, 157 – 162.
[8] Y. Wang, B. B. Schneider, T. R. Covey, J. Pawliszyn, *Anal. Chem.* **2005**, *77*, 8095 – 8101.
[9] M. Walles, Y. Gu, C. Dartiguenave, F. M. Musteata, K. Waldron, D. Lubda, J. Pawliszyn, *J. Chromatogr. A* **2005**, *1067*, 197 – 205.
[10] J. H. Kennedy, C. Aurand, R. Shirey, B. C. Laughlin, J. M. Wiseman, *Anal. Chem.* **2010**, *82*, 7502 – 7508.
[11] F. S. Mirnaghi, J. Pawliszyn, *Anal. Chem.* **2012**, *84*, 8301 – 8309.
[12] A. Rodriguez-Lafuente, F. S. Mirnaghi, J. Pawliszyn, *Anal. Bioanal. Chem.* **2013**, *405*, 9723 – 9727.
[13] N. Strittmatter, R.-A. Düring, Z. Takáts, *Analyst* **2012**, *137*, 4037 – 4044.
[14] B. Bojko, E. Cudjoe, G. A. Gómez-Ríos, K. Gorynski, R. Jiang, N. Reyes-Garcés, S. Risticevic, E. A. S. Silva, O. Togunde, D. Vuckovic, J. Pawliszyn, *Anal. Chim. Acta* **2012**, *750*, 132 – 151.
[15] E. Cudjoe, B. Bojko, I. Delannoy, V. Saldivia, J. Pawliszyn, *Angew. Chem. Int. Ed.* **2013**, *52*, 12124 – 12126; *Angew. Chem.* **2013**, *125*, 12346 – 12348.
[16] D. Vuckovic, I. De Lannoy, B. Gien, R. E. Shirey, L. M. Sidisky, S. Dutta, J. Pawliszyn, *Angew. Chem. Int. Ed.* **2011**, *50*, 5344 – 5348; *Angew. Chem.* **2011**, *123*, 5456 – 5460.
[17] D. Vuckovic, S. Risticevic, J. Pawliszyn, *Angew. Chem. Int. Ed.* **2011**, *50*, 5618 – 5628; *Angew. Chem.* **2011**, *123*, 5734 – 5745.
[18] D. Vuckovic, E. Cudjoe, F. M. Musteata, J. Pawliszyn, *Nat. Protoc.* **2010**, *5*, 140 – 161.
[19] S. Risticevic, H. Lord, T. Górecki, C. L. Arthur, J. Pawliszyn, *Nat. Protoc.* **2010**, *5*, 122 – 139.
[20] E. A. S. Silva, S. Risticevic, J. Pawliszyn, *TrAC Trends Anal. Chem.* **2013**, *43*, 24 – 36.
[21] D. Vuckovic, *TrAC Trends Anal. Chem.* **2013**, *45*, 136 – 153.
[22] E. Boyaci, K. Gorynski, A. Rodriguez-Lafuente, B. Bojko, J. Pawliszyn, *Anal. Chim. Acta* **2014**, *809*, 69 – 81.
[23] H. Wang, J. Liu, R. G. Cooks, Z. Ouyang, *Angew. Chem. Int. Ed.* **2010**, *49*, 877 – 880; *Angew. Chem.* **2010**, *122*, 889 – 892.
[24] J. Liu, H. Wang, N. E. Manicke, J.-M. Lin, R. G. Cooks, Z. Ouyang, *Anal. Chem.* **2010**, *82*, 2463 – 2471.
[25] R. D. Espy, N. E. Manicke, Z. Ouyang, R. G. Cooks, *Analyst* **2012**, *137*, 2344 – 2349.
[26] R. D. Espy, A. R. Muliadi, Z. Ouyang, R. G. Cooks, *Int. J. Mass Spectrom.* **2012**, *325 – 327*, 167 – 171.
[27] Q. Yang, H. Wang, J. D. Maas, W. J. Chappell, N. E. Manicke, R. G. Cooks, Z. Ouyang, *Int. J. Mass Spectrom.* **2012**, *312*, 201 – 207.
[28] L. Shen, J. Zhang, Q. Yang, N. E. Manicke, Z. Ouyang, *Clin. Chim. Acta* **2013**, *420*, 28 – 33.
[29] G. A. Gómez-Ríos, J. Pawliszyn, *Chem. Commun.* **2014**, *50*, 12397 – 12940.
[30] A. Thomas, H. Geyer, W. Schänzer, C. Crone, M. Kellmann, T. Moehring, M. Thevis, *Anal. Bioanal. Chem.* **2012**, *403*, 1279 – 1289.
[31] L. Li, T. Chen, Y. Ren, P. I. Hendricks, R. G. Cooks, Z. Ouyang, *Anal. Chem.* **2014**, *86*, 2909 – 2916.

