An Interface for Reproducible, Multi-shot Direct Analysis of Solid-phase Microextraction Samples

G. Asher Newsome*, Gwénaëlle Kavich, Alba Alvarez-Martin

Smithsonian Institution Museum Conservation Institute, 4210 Silver Hill Rd., Suitland MD

*corresponding:

G. Asher Newsome

Smithsonian Institution Museum Conservation Institute

Suitland, MD 20746-2863

T. 301-238-1223

Email. newsomeg@si.edu

Fax: 301-238-3709

Abstract

An enclosed interface that joins a direct analysis in real time (DART) probe, solid phase microextraction (SPME) fiber, and the inlet of a high-resolution mass spectrometer is described. Unlike other systems to couple SPME sampling to ambient mass spectrometry, the interface is able to perform discrete analyses on different areas of a single SPME fiber device for up to three technical replicate measurements of one sampling event. Inlet flow speed and desorption temperature are optimized, and reproducibility is demonstrated between replicate analyses on the same derivatized SPME fiber and with sequential fiber sampling events, yielding analyte measurement center of variance (CV) from 3-6%. Conditioning is also performed with the enclosed DART. The interface is a straightforward addition to commercially available technologies, and machine diagrams for custom components operated with SPME/DART/MS equipment are included.

Introduction

Solid phase microextraction (SPME)[1,2] is a popular, commercialized sampling technique developed for rapid analysis in the laboratory and at a remote site. SPME methodology is based on a two-step process in which an adsorbtive or absorptive polymer coating on a solid substrate is exposed to a gaseous or liquid sample. Molecules with higher affinity for the coating than the sample matrix are selectively extracted over a sampling time of seconds to days[3] depending on the analyte, then desorbed and transferred to a mass spectrometer. SPME is commonly coupled to gas chromatography-mass spectrometry (GC-MS), where analytes are desorbed in a heated GC inlet for column separation, or liquid chromatography-mass spectrometry, where the coating contacts the mobile phase.[4]

Direct analysis in real time (DART)[5] uses a heated, continuous flow of post-plasma gas to desorb and ionize analytes from a surface. By using the selectivity of high resolution mass analysis or selected reaction monitoring to discern analytes, DART is operated without chromatographic separation. However, the sample surface must fit in the 1-2 cm gap between the MS inlet and DART probe in transmission mode, or fit close to the MS inlet in reflection mode.[6] The small volume of the SPME coating makes it well-suited to interface with ambient MS methods.[7] SPME sampling has been coupled to DART, using the high-temperature gas to desorb analytes from a coated fiber,[8] mesh,[9] or consumable tip[10] in front of an MS inlet. The methodology combines the ability of DART-MS to analyze samples rapidly with the delivery of analytes via SPME from a remote origin to the small ionization region. Unlike desorption with a GC inlet, the accessibility of the atmospheric-pressure DART source also allows ion signal to be changed by environmental conditions or air currents in the laboratory,[11] and small differences in positioning within the gas stream expose the sample surface to significant but difficult-to-reproduce temperature variation.[12]

Enclosure of the sample ionization space affords a measure of control over conditions, and rapid analysis further reduces inter-sample signal variation. A new interface is described here to enclose and mount a commercial, re-usable SPME fiber and DART probe for maximum reproducibility. In addition to precisely controlling the thermal environment for analyte desorption, the interface includes an electronically-controlled shutter[12] to expose the SPME coating to the heated DART flow after the substrate has been placed in position, creating a thermal chamber[13,14] that is discontinuous and powered by the ambient ionization source itself. Although various coatings and substrates are commercially available, a conventional SPME fiber

device able to be used with a portable holder or autosampler[15] is chosen for this study for simplicity. Because the diameter of the orifice in the DART probe cap is less than one-third the length of the commercial SPME coating, three discrete sample areas on a single fiber are able to be analyzed in seconds for technical replicate measurements of a single analyte sampling event. The DART flow is also used to condition the SPME coating more quickly than a GC inlet. SPME coatings are derivatized to enhance the extraction of substances with poor chromatographic behavior, high reactivity and/or low volatility, or thermal instability,[16] and the methodology is demonstrated here with the small, polar analyte acetic acid.

Materials and Methods

Saturated solutions of 1-pyrenyldiazomethane (PDAM) were prepared by adding 0.65 mg of crystalized PDAM (Molecular Probes, Fisher Scientific, Waltham, MA) to 1.30 mL of hexane in a 1.5 mL glass vial sealed with a PTFE septum. The solution was vortexed thoroughly until few solids remained suspended. The solution was stored at 4 °C, used at room temperature with solids settled to the bottom of the vial, and discarded after four days. 1 mL volumes of acetic acid (J.T. Baker, Fisher Scientific, Waltham, MA) at various concentrations in water were prepared in 20 mL glass vials (5182-0838, Agilent, Santa Clara, CA) with a PTFE/silicone septa with an 20 mm aluminum crimp cap (9301-0719 and 9301-0718, Agilent, Santa Clara, CA), and used at room temperature.

All experiments were performed with 50/30 µm DVB/CAR/PDMS fibers (57328-U, Sigma-Aldrich, St. Louis, MO) and a manual fiber holder (57330-U, Sigma-Aldrich, St. Louis, MO). The fiber holder was modified to alternate between use with a standard GC heated inlet (Agilent 6890, Santa Clara, CA), a CombiPAL autosampler (PAL Systems, Switzerland), and a custom-designed interface joining a DART probe cap and MS inlet. For CombiPAL operations, the central outer-threaded holder shaft was removed from the larger, inner-threaded needle guide. With the fiber holder assembly mounted in the autosampler (Supporting Information Figure S1), position relative to reagent and sample vials was calibrated to 0.1 mm. Two additional slots were machined into the fiber holder shaft using a 1/16” end mill, creating three positions each 3.12 mm apart (hereafter termed positions A, B, and C) for the set screw.

The CombiPAL autosampler and PDAM-derivatized SPME fibers were used to sample acetic acid in the headspace for all mass spectrometry experiments. . The fiber was derivatized by dipping into the PDAM solution for 10 s to avoid MS signal saturation and minimize conditioning time. The derivatized fiber was then exposed to the headspace 2.5 cm above a 1 mL, 1 M acetic acid solution in a sealed 20 mL vial for 10 s. The fiber holder assembly was manually removed from the autosampler for MS analysis.

Analysis was performed with an LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, Waltham, MA). A Vapur flange (IonSense, Saugus, MA) differentially pumped by a MZ2NT diaphragm pump (Vacuubrand, Essex, CT) was mounted over the MS inlet capillary. The ceramic transfer tube in the flange was replaced with a 64 mm o.d., 40 mm long stainless steel tube. The total flow rate from the MS vacuum and supplemental pump was adjusted with a needle valve and measured at the transfer tube with a flow calibrator (Defender 510, Mesa Labs, Butler, NJ). A platform connected to the base of the Vapur flange was used to mount a stainless steel SPME-DART-MS interface. The Vapur transfer tube was inserted 8.7 mm into a fitted port in the rear of the interface.

The interface similarly holds the other components of the analysis system in set positions (Supporting Information Figure S2). An IonSense DART 100 source with a 2.5 mm i.d. flat

ceramic cap, operated in positive mode by an SVP controller, and with housing and shielding removed was custom-mounted from above with multidimensional translation stages. The DART cap was inserted 8.7 mm into a fitted port in the front of the interface. Post-plasma gas flows 8.0 mm through a 3.4 mm diameter through-hole from the DART cap to the Vapur transfer tube. When stabilized at the temperature setpoint, the DART outputs a continuous gas flow at 2.5 L/min. Further mounting and schematic details are given in Supporting Information.

To prevent exposure of the SPME fiber to the continuous DART flow during setup, a 0.25 mm thick stainless steel shutter is inserted through a 1.6 mm slot in the side of the interface in between the DART cap and the fiber. The shutter vane is attached to a spring-loaded solenoid pull actuator mounted to the side of the interface. The DART flow escapes from the interface enclosure through either side of the slot when the shutter is closed. An Arduino was used to trigger shutter opening[12] for 5 s, during which time 32 mass spectra were acquired at 30k resolving power. Shutter open and close motions each took 70 ms. To ensure that the shutter remained vertical in the slot, a guide track for the actuator was attached to the back of the interface. SPME plunger position was adjusted while the shutter was closed. MS data was monitored with the Xcalibur Qual Browser.

After MS analysis of analytes from one or more SPME fiber positions, the fiber was conditioned in a 250 °C gas flow. The fiber assembly was either moved to a heated GC inlet to condition the entire fiber or exposed for time periods in the SPME-DART-MS interface to condition positions A, B, and C individually. The fiber was cooled to near room temperature before being derivatized again.

Machine diagrams for the SPME-DART-MS interface, needle guide, actuator mount, and shutter vane, as well as a design for a multi-slot fiber holder shaft for use with a commercial assembly, are available at https://doi.org/10.25573/data.11373972.v1. Principal machining was performed according to design by eMachineShop. The 1.6 mm slot in the interface and the additional slots in the SPME fiber holder shaft were machined in-house on a benchtop end mill.

Results and Discussion

To maximize the thermal desorption efficiency of derivatized acetic acid from a SPME fiber without damaging the fiber coating, the environment around the fiber should be at the manufacturer-prescribed upper limit of 250 °C. The DART probe has flow temperature settings up to 500 °C, but the feedback measurement references the gas temperature within the probe which is significantly higher than rapidly-cooling gas exiting the probe cap. To accurately replicate experimental conditions and ensure the fiber is not damaged, a SPME fiber holder assembly was inserted into the top mount, and a k-type thermocouple with a 0.38 mm junction was inserted into the interface through a vent hole and positioned in the flow pathway in place of a SPME fiber (Supporting Information Figure S3). Using a constant supplemental pump flow setting, the temperature at the fiber position was measured at various helium plasma temperature settings with the shutter closed and open (Figure 1). The maximum temperature was recorded within 3-4 seconds of shutter opening, and a 250 °C measurement was recorded at a 450 °C helium setting which was used for all subsequent experiments. The steel SPME-DART-MS interface itself is significantly warmed by its contact with the hot ceramic cap of the DART, but the temperature at the fiber position remains below 145 °C at any setting with the shutter closed.

The speed setting on the supplemental pump has the potential to affect SPME-DART-MS sampling as well. The combined suction of the MS vacuum and supplemental pump at different

speed settings was recorded (Supporting Information Table S1), although the precise measurement was observed to be dependent on the gap between the transfer tube and MS inlet capillary. The maximum temperature measured by thermocouple was largely insensitive to supplemental pump speed over the range tested (Supporting Information Figure S4). MS signal for both the acetic acid analyte and the derivatizing reagent significantly decreased almost exponentially with increasing pump speed (Figure 2). As a result the minimum supplemental pump speed necessary to achieve manufacturer-recommended ion trap pump performance was used for subsequent experiments. The supplemental pump needle valve was dialed to 0.2 for a total suction of 2.48 L/min, giving near parity to sample ions transported into the MS capillary and lost to the supplemental pump. DART-MS of a sample-loaded SPME fiber produced protonated molecule signal from derivatized acetic acid at *m/z* 274.0987 ([PDAM + acetic acid + H]$^+$) and from the derivatizing agent at *m/z* 215.0853 ([PDAM + H]$^+$), as well as oxygenated adducts of each (Supporting Information Figure S5). The greater residence time of desorbed analyte material in the hot source region from low pumping speeds produced greater relative abundance of the oxygenated analyte ion (Supporting Information Figure S6). The oxygenated analyte ion signal was less stable than the protonated molecule and was not considered in data analyses.

The performance of the optimized SPME-DART-MS interface was demonstrated with successive derivatization/headpsace sampling/DART-MS analysis/fiber conditioning experiments using the same SPME fiber for an entire sample set. The fiber holder slots evenly space apart the areas for DART desorption, leaving 0.60 mm gaps separating each desorption area and at the top and bottom of the 10.0 mm fiber. The shaft collar is precisely positioned on the fiber holder so that when the assembly is threaded in the mount fully to the collar, the end of the fiber in position C extends no more that 0.60 mm past the orifice of the DART cap. The desorption areas on the fiber are analyzed in order A, B, C, first exposing the part of the SPME coating closest to the plunger and subsequently drawing the fiber upward, to ensure that rising heat does not contribute to analyte desorption from fiber areas that have not yet been exposed. Averaging analyte signal between the three desorption areas on a single fiber could produce a CV as low as three percent if the whole fiber had been conditioned in a 250 °C GC inlet for up to 60 minutes. Reproducibility between repeated headspace sampling experiments using the same fiber and GC conditioning time was comparatively poor, even as CVs between the three positions on a single fiber remained small.

The key to high reproducibility between repeated headspace sampling experiments, in addition to the three positions on each fiber, was precisely equivalent conditioning. The completeness of SPME fiber conditioning could be measured by analyzing the fiber with DART-MS after the conditioning step without re-derivatization. If the conditioning was incomplete, a notable abundance of residual [PDAM + acetic acid + H]$^+$ signal was detectable. The DART itself was used to condition[17] the discrete sampling positions on the fiber with extended exposure to the post-plasma flow. Analyte MS signal was recorded during DART conditioning and monitored with the ICIS peak detection algorithm (Figure 3). Each sampling position was exposed to the DART flow for 2.5 min after the ICIS algorithm ceased regular detection of residual analyte ion peaks. The exposure time required for equivalent conditioning was not precisely equal for each sample position or headspace sampling replicate. Higher headspace concentrations had higher residual analyte signal after the equal-time analysis phase, requiring longer conditioning exposure. Higher headspace concentrations were also more likely to produce disparity between the conditioning exposure time required at positions A, B, and C. Rising heat

within the SPME-DART-MS interface from prolonged conditioning at position A reduced residual analyte signal at positions B and C and the subsequent discrete conditioning times. For the headspace concentrations tested, the total conditioning time for all three sampling positions varied between eight and twenty minutes. Conditioning with the DART was far more efficient than the GC inlet because the heated gas flow rate of the former was greater by three orders of magnitude.

Using equivalent DART conditioning, reproducibility across replicate experiments was measured. Analyte signal for three consecutive fiber analyses was nearly equal at each of the three sampling positions, and average signal for all nine replicates had a CV of 5% (Table 1). Signal from the derivatizing agent in the same replicates was two orders of magnitude greater, magnifying any disparities. Although the individual sampling positions had CVs of 2-3%, the overall CV for positions A, B, and C was 11%. Such a high degree of reproducibility for analyte signal is normally only possible with liquid immersion sampling[9] and not headspace sampling without the interface. The reproducibility experimentally demonstrated the performance limits of either the polymer coating or the derivatizing agent on the SPME fiber. Mean values for three replicate fibers were within one standard deviation, but the addition of a fourth replicate fell outside the standard deviation of the first measurement to show a gradual but nonetheless linear decrease in performance (Supporting Information Figure S7).

Uptake curves are often used to show the adsorption of analytes onto a SPME fiber as a function of sampling time[18,19] and are characterized by a sharp increase, a transition point, and a more gradual rise. To conform to the rapidity of DART, an uptake curve was constructed using 10 s analyte sampling experiments of headspace over serially-diluted solutions. The actual headspace concentration is not calculated, but the concentrations used to produce the headspace are used to plot Supporting Information Figure S8. Analyte signal as recorded with the SPME-DART-MS was linear over a significant range, below which signal dropped sharply, and above which signal levels plateaued when the derivatized fiber surface was at capacity.

Conclusions

The SPME-DART-MS interface replicates the thermal properties of a GC-MS inlet while conferring the benefits of atmospheric pressure analysis and high resolution mass spectrometry without chromatography. The integration of a mechanical shutter into the interface separates in time the positioning of the fiber and analyte desorption. Three technical replicate analyses can be performed on different areas of the same SPME fiber with no functional increase in experiment time, which would be particularly advantageous when the initial SPME sampling requires more time than the analysis. The single-digit percent CVs of the replicates (both for analyses on the same fiber and for multiple fibers) and the reproducible performance along a SPME uptake curve make the methodology suitable for more quantitative analysis than the qualitative work presented here. Other practical considerations also recommend the SPME-DART-MS interface methodology. Direct analysis without chromatography allows the conditioning desorption of residual analyte to be monitored in real time to precisely tune for maximum fiber-to-fiber reproducibility. By not using a septum like a GC-MS inlet, the interface eliminates the possibility of stripping the SPME coating by pulling the fiber through while extended from the needle mount. The relative brevity of DART exposure for analysis and conditioning may increase the service lifetime of a SPME fiber compared to use with GC-MS. To prevent long-term buildup of siloxanes and desorbed materials without using a replaceable surface liner, it would be

advantageous to coat interface surfaces with a commercial, high-temperature non-stick coating like SilcoNert.

Considering the rapidity of analysis, other changes to the experimental design could be implemented for more technical replicates per fiber with little additional time. A DART with a smaller diameter orifice cap[20], or other high temperature, small diameter post-plasma probe like pin-to-capillary flowing atmospheric pressure afterglow[21], could analyze 4-8 discrete sample areas on a 10 mm SPME fiber. The SPME-DART-MS interface can also accommodate a significantly longer fiber, which would create more replicate areas and enable the study of SPME adsorption kinetics[22] over a longer time scale.

Associated Content

Supporting Information is available free of charge on the ACS Publications website at DOI: xxx.

Acknowledgements

The authors thank Christopher Maines and the National Gallery of Art, Washington, DC, for donation of the CombiPAL, and Tim Flug of CTC Analytics for CombiPAL maintenance recommendations. AAM would like to acknowledge the Smithsonian Postdoctoral Fellowship in the Conservation of Museum Collections and the Smithsonian's Museum Conservation Institute Federal Funds for financial support.

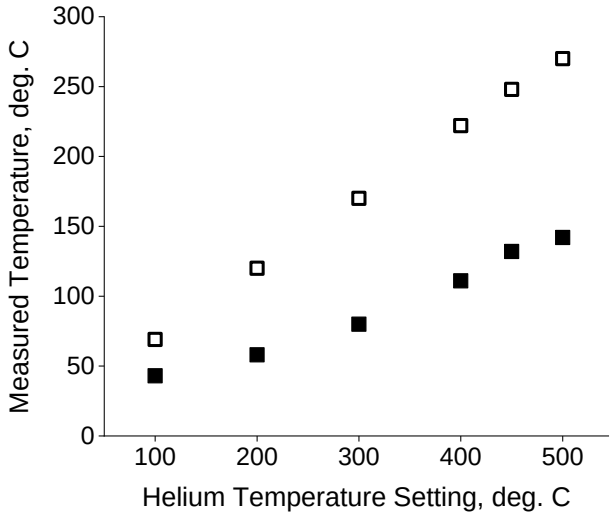

Figure 1. Maximum temperature at fiber exposure position in interface with shutter open (open squares) and closed (closed squares) observed at different DART helium temperature settings, with 2.48 L/min suction from MS and supplemental pump.

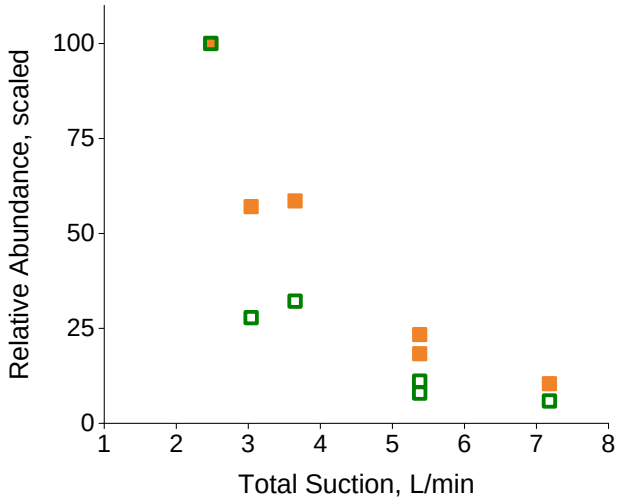

Figure 2. Scaled relative signal from protonated derivatizing agent $[PDAM + H]^+$ (orange squares) and derivatized analyte $[PDAM + acetic\ acid + H]^+$ (open green squares) observed at differing amounts of total suction.

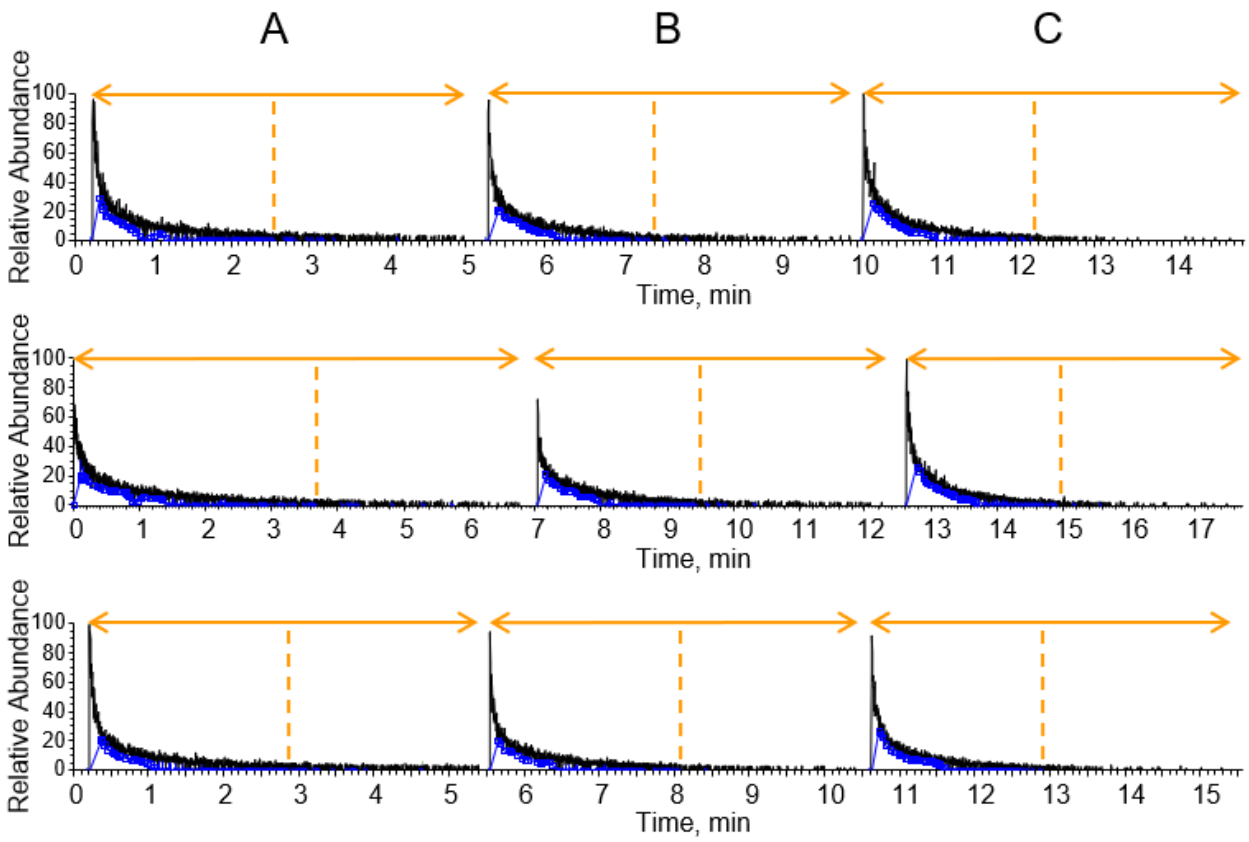

Figure 3. Chronogram showing the decrease in [PDAM + acetic acid + H]$^+$ signal (black trace) at positions A, B, and C as a function of DART cleaning time for three successive SPME fibers. The timespans during which the shutter was open are denoted by solid arrows. The time points at which the ICIS algorithm ceased regular peak detection (blue trace) are denoted by dashed lines.

Table 1. Analyte signal at positions A, B, and C from three consecutive SPME-DART experiment replicates.

	[PDAM + acetic acid + H]$^+$			[PDAM + H]$^+$		
position	A	B	C	A	B	C
average signal area	2.2E+08 ± 1E+07	2.3E+08 ± 1E+07	2.35E+08 ± 9E+06	3.36E+10 ± 7E+08	2.98E+10 ± 9E+08	2.63E+10 ± 7E+08
CV per position	0.050	0.058	0.040	0.020	0.029	0.026
overall CV	0.053			0.108		

References

(1) Arthur, C. L.; Pawliszyn, J. *Anal Chem* **1990**, *62*, 2145-2148.
(2) Gomez-Rios, G. A.; Mirabelli, M. F. *Trac-Trend Anal Chem* **2019**, *112*, 201-211.
(3) Lattuati-Derieux, A.; Thao, S.; Langlois, J.; Regert, M. *J Chromatogr A* **2008**, *1187*, 239-249.
(4) Chen, J.; Pawliszyn, J. B. *Anal Chem* **1995**, *67*, 2530-2533.
(5) Cody, R. B.; Laramee, J. A.; Durst, H. D. *Anal Chem* **2005**, *77*, 2297-2302.
(6) Gross, J. H. *Anal Bioanal Chem* **2014**, *406*, 63-80.
(7) Deng, J.; Yang, Y.; Wang, X.; Luan, T. *Trac-Trend Anal Chem* **2014**, *55*, 55-67.
(8) Cajka, T.; Riddellova, K.; Tomaniova, M.; Hajslova, J. *J Chromatogr A* **2010**, *1217*, 4195-4203.
(9) Gomez-Rios, G. A.; Pawliszyn, J. *Chem Commun* **2014**, *50*, 12937-12940.
(10) Li, F.; Musselman, B. In *Analysis of Drugs of Abuse*; Springer, 2018, pp 97-106.
(11) Newsome, G. A.; Ackerman, L. K.; Johnson, K. J. *J Am Soc Mass Spectr* **2016**, *27*, 135-143.
(12) Newsome, G. A.; Kayama, I.; Brogdon-Grantham, S. A. *Anal Methods-UK* **2018**, *10*, 1038-1045.
(13) Mirabelli, M. F.; Wolf, J. C.; Zenobi, R. *Anal Chem* **2016**, *88*, 7252-7258.
(14) Reyes-Garces, N.; Gionfriddo, E.; Gomez-Rios, G. A.; Alam, M. N.; Boyaci, E.; Bojko, B.; Singh, V.; Grandy, J.; Pawliszyn, J. *Anal Chem* **2018**, *90*, 302-360.
(15) Zini, C. A.; Lord, H.; Christensen, E.; de Assis, T. F.; Caramao, E. B.; Pawliszyn, J. *J Chromatogr Sci* **2002**, *40*, 140-146.
(16) Stashenko, E. E.; Martinez, J. R. *Trac-Trend Anal Chem* **2004**, *23*, 553-561.
(17) Mirnaghi, F. S.; Pawliszyn, J. *Anal Chem* **2012**, *84*, 8301-8309.
(18) Semenov, S. N.; Koziel, J. A.; Pawliszyn, J. *J Chromatogr A* **2000**, *873*, 39-51.
(19) Hook, G. L.; Kimm, G. L.; Hall, T.; Smith, P. A. *Trac-Trend Anal Chem* **2002**, *21*, 534-543.
(20) Habe, T. T.; Morlock, G. E. *Rapid Commun Mass Sp* **2016**, *30*, 321-332.
(21) Shelley, J. T.; Wiley, J. S.; Hieftje, G. M. *Anal Chem* **2011**, *83*, 5741-5748.
(22) Wang, J. X.; Tuduri, L.; Millet, M.; Briand, O.; Montury, M. *J Chromatogr A* **2009**, *1216*, 3031-3037.

For Table of Contents Only