Coupling Desorption Electrospray Ionization with Solid-Phase Microextraction for Screening and Quantitative Analysis of Drugs in Urine

ARTICLE *in* ANALYTICAL CHEMISTRY · SEPTEMBER 2010

Impact Factor: 5.64 · DOI: 10.1021/ac101295g · Source: PubMed

5 AUTHORS, INCLUDING:

Joseph H Kennedy
Prosolia
36 PUBLICATIONS **560** CITATIONS
SEE PROFILE

Justin Wiseman
Prosolia, Inc.
45 PUBLICATIONS **4,346** CITATIONS
SEE PROFILE

Anal. Chem. **2010,** *82,* 7502–7508

Coupling Desorption Electrospray Ionization with Solid-Phase Microextraction for Screening and Quantitative Analysis of Drugs in Urine

Joseph H. Kennedy,[†] Craig Aurand,[‡] Robert Shirey,[‡] Brian C. Laughlin,[†] and Justin M. Wiseman*[,†]

Prosolia, Inc., Indianapolis, Indiana, and Supelco/Sigma Aldrich, Bellefonte, Pennsylvania

Direct analysis of silica C_{18}-coated solid-phase microextraction (SPME) fibers using desorption electrospray ionization mass spectrometry (DESI-MS) for the purpose of analyzing drugs from raw urine is presented. The method combines a simple, inexpensive, and solvent-less sample preparation technique with the specificity and speed of DESI-MS and MS/MS. Extraction of seven drugs from raw urine is performed using specially designed SPME fibers coated uniformly with silica-C_{18} stationary phase. Each SPME device is inserted into unprocessed urine under gentle agitation and, then, removed, rinsed, and analyzed directly by DESI-MS (MS/MS). Rapid screening over a wide mass range is afforded by coupling the method with a time of flight (TOF) mass spectrometer while quantitative analysis is performed using selected reaction monitoring (SRM) using a triple quadrupole mass spectrometer. The performance of the SPME DESI-MS/MS method was evaluated by preparing calibration standards and quality control (QC) samples of the seven drug compounds from urine over a range from 20 to 1000 ng/mL, with the exception of meprobamate which was prepared from 200 to 10000 ng/mL. The calibration curves constructed for each analyte had an $R^2 > 0.99$. The range of precision (%CV) and accuracy values (% bias) for low QC samples was 1−11% and 3−38%, respectively. Precision and accuracy values for high QC samples range from 0.9 to 8% and −31 to −8%. Results from urine specimens of actual exposure to drugs screened using the SPME DESI-MS/MS method showed good agreement with the conventional immunoassays and GC/MS analysis. Liquid desorption of the SPME fiber followed by LC/MS/MS also showed good agreement with the SPME DESI-MS/MS method.

The nonmedical use of prescription drugs and abuse of illegal substances presents a serious health concern. The U.S. Department of Health and Human Services estimates that in 2008, 20.1 million Americans aged 12 or older were current users of illicit drugs, a category including both nonmedical use of prescription drugs and illegal substances such as cocaine, marijuana, LSD, etc.[1] Consequently, screening for drugs of abuse in biological samples, such as urine, has become increasingly important not only for illicit substances but also because of the nonmedical use of prescription medications. Urine is the preferred matrix for measuring drugs and metabolites primarily because it is physically noninvasive to collect and high specimen volumes are afforded with urine sampling. Typically, urine is first screened by an enzyme immunoassay (EIA) or enzyme linked immunosorbent assay (ELISA) for presumptive positive specimens followed by gas chromatography mass spectrometry (GC/MS) or liquid chromatography mass spectrometry (LC/MS) to determine the presence of the predefined analytes above threshold concentrations.

The advent of the ambient mass spectrometry methods,[2] such as desorption electrospray ionization (DESI)[3] and direct analysis in real time (DART),[4] presents an opportunity to explore the potential for streamlining sample analysis by minimizing upfront sample preparation when employing these methods. DESI combines the attributes of electrospray ionization (ESI) and the throughput of desorption/ionization (DI) methods. DESI is an atmospheric pressure DI method that uses high velocity charged droplets to effect desorption and ionization of the condensed-phase analyte present on a surface. The DESI DI process was described early on based on empirical observations[3,5] and later supported by phase-Doppler anemometry experiments[6] and simulations.[7,8] The applications of the technique are extensive and have been reviewed previously.[2,9,10]

* To whom correspondence should be addressed. Address: Prosolia, 351 West 10th Street, Suite 316 Indianapolis, Indiana 46202. Tel: 317-278-6110. E-mail: Wiseman@prosolia.com.

[†] Prosolia, Inc.

[‡] Supelco/Sigma Aldrich.

(1) Substance Abuse and Mental Health Services Administration; Results from the 2008 National Survey on Drug Use and Health: National Findings, (2009); Office of Applied Studies, N. S. H.-. HHS Publication No. SMA 09−4434.

(2) Cooks, R. G.; Ouyang, Z.; Takats, Z.; Wiseman, J. M. *Science* **2006**, *311*, 1566–1570.

(3) Takats, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. *Science* **2004**, *306*, 471–473.

(4) Cody, R. B.; Laramee, J. A.; Durst, H. D. *Anal. Chem.* **2005**, *77*, 2297–2302.

(5) Myung, S.; Wiseman, J. M.; Valentine, S. J.; Takats, Z.; Cooks, R. G.; Clemmer, D. E. *J. Phys. Chem. B* **2006**, *110*, 5045–5051.

(6) Venter, A.; Sojka, P. E.; Cooks, R. G. *Anal. Chem.* **2006**, *78*, 8549–8555.

(7) Costa, A. B.; Cooks, R. G. *Chem. Commun. (Cambridge, U.K.)* **2007**, 3915–3917.

(8) Costa, A. B.; Cooks, R. G. *Chem. Phys. Lett.* **2008**, *464*, 1–8.

(9) Van Berkel, G. J.; Pasilis, S. P.; Ovchinnikova, O. *J. Mass Spectrom.* **2008**, *43*, 1161–1180.

(10) Weston, D. J. *Analyst* **2010**, *135*, 661–668.

10.1021/ac101295g
Published on Web 08/09/2010

Quantitative measurements are rapidly obtained by DESI-MS^n using ion trap mass analyzers[11,12] or triple quadrupole instruments.[13] Often times, however, the quantitation of analytes in biological matrices is improved by employing an extraction step where the analyte is removed from interfering matrix components. Simple protocols, such as liquid−liquid extraction or protein precipitation, have facilitated drug quantitation in plasma[13] while more sophisticated solid-phase extraction and affinity-based ambient mass spectrometry methods have also been developed.[14] Should sample purification be necessary in the development of analytical methods based on DESI or any other ambient desorption/ionization method, it is highly desirable that the sample preparation procedure has the following features: (1) sampling and sample preparation are combined into one step; (2) minimal sample transfer steps to eliminate analyte losses through nonspecific adsorption; (3) the extraction method is not labor intensive and can be automated; (4) the extraction method is rapid and facilitates direct coupling to the ambient DI method; (5) the analytes are stable in the extraction phase. A recent development that addresses some of these considerations is the advent of paper spray ionization, wherein the sample is collected on common filter paper media and eluted from the paper in the presence of an electric field induced by a high voltage causing an electrospray to form at the "tip" of the paper.[15,16] Examples were shown of analyzing imatinib directly from 0.4 μL of dried blood on filter paper and dyes separated by paper chromatography.

Solid-phase microextraction (SPME)[17] is a simple sample preparation technique that minimizes the use of solvents and couples the sampling and sample preparation into one step. Since its first introduction in 1990, numerous developments in theoretical treatment, applications, and chemistry and design of the SPME probes have been demonstrated.[18−23] Conventionally, SPME uses a thin polymer film coating on a fine fused silica capillary fiber or metal wire to adsorb compounds of interest from either the condensed or gas phases. Typical SPME techniques consist of extraction of analytes by immersion into a sample or from the headspace above a sample, followed by thermal desportion in the injection port of a GC or GC/MS. Due to solvent incompatibility of the coatings, conventional SPME phases require thermal desorption to release the analytes. Conventional SPME phases can swell when placed into organic solvents resulting in decreased mechanical stability and erratic extraction efficiencies. The versatility of the technique has led to the development of many different configurations,[24−26] support chemistries,[27] and extraction phases.[28,29] More recently, a new line of SPME fibers has been developed for the extraction of analytes from biological fluids followed by solvent desorption. The newly developed SPME fibers consist of C_{18} functionalized silica particles bonded to the fiber core using a biocompatible polymeric binder.[30] The resulting SPME fiber is compatible with organic solvents and is amenable to direct insertion into biological fluids. The biocompatible fibers reduce surface adhesion of proteins and other macromolecules while allowing the extraction of small molecule analytes. This configuration enables direct extraction of the biological sample without any special pretreatment.

The approach of this study involves the use of SPME fibers functionalized with silica-C_{18} chromatography stationary phase for analyte enrichment from urine specimens combined with DESI-MS (MS/MS) for rapid analysis. The coupling of SPME and DESI has been previously demonstrated using conventional SPME fibers coated with polydimethylsiloxane (PDMS) for the analysis of chemical warfare agents extracted from the gas phase.[31] In the present study, drugs are extracted from unprocessed urine using the biocompatible SPME fibers under pre-equilibrium conditions and analyzed directly by the DESI-MS technique. With this approach, the extraction of multiple individual samples is conducted simultaneously with total extraction times of 5−16 min for multiple analytes, requiring low volumes of reagents and employing the high specificity and sensitivity of MS (and MS/MS) detection. In this report, the SPME-DESI-MS and MS/MS method is applied to the analysis of unprocessed urine for cocaine, benzoylecgonine, cocaethylene, meprobamate, norfentanyl, methadone, and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). Cocaine and its metabolites, benzoylecgonine and cocaethylene, norfentanyl, the primarily metabolite of fentanyl, and methadone and its metabolite, EDDP, were selected as representative therapeutic and drugs of abuse. The objective of this research was to explore the feasibility for rapid extraction of drugs and direct desorption analysis using DESI-MS/MS as combined technologies for a comprehensive drug testing method in urine.

EXPERIMENTAL DETAILS

Chemicals and Reagents. Acetonitrile and HPLC grade water were obtained from Burdick and Jackson (Muskegon, MI), and concentrated formic acid was obtained from J.T. Baker-Mallinckrodt Baker Inc. (Phillipsburg, NJ). Meprobamate (MB), meprobamate-d_7 (d_7-MB), norfentanyl (NF), norfentanyl-d_5 (d_5-NF),

(11) Ifa, D. R.; Manicke, N. E.; Rusine, A. L.; Cooks, R. G. *Rapid. Commun. Mass. Spectrom.* **2008**, *22*, 503–510.
(12) Takats, Z.; Wiseman, J. M.; Cooks, R. G. *J. Mass Spectrom.* **2005**, *40*, 1261–1275.
(13) Kennedy, J. H.; Wiseman, J. M. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 309–314.
(14) Chipuk, J. E.; Gelb, M. H.; Brodbelt, J. S. *Anal. Chem.* , *82*, 16–18.
(15) Wang, H.; Liu, J.; Cooks, R. G.; Ouyang, Z. *Angew. Chem., Int. Ed. Engl.* **2010**, *49*, 877–880.
(16) Liu, J.; Wang, H.; Manicke, N. E.; Lin, J. M.; Cooks, R. G.; Ouyang, Z. *Anal. Chem.* **2010**, *82*, 2463–2471.
(17) Pawliszyn, J. *Solid Phase Microextraction: Theory and Practice*; Wiley-VCH, Inc.: Weinheim, Germany, 1997.
(18) Pawliszyn, J.; Pedersen-Bjergaard, S. *J. Chromatogr. Sci.* **2006**, *44*, 291–307.
(19) Pawliszyn, J. *Anal. Chem.* **2003**, *75*, 2543–2558.
(20) Pawliszyn, J. *Adv. Exp. Med. Biol.* **2001**, *488*, 73–87.
(21) Pawliszyn, J. *J. Chromatogr. Sci.* **2000**, *38*, 270–278.
(22) Pawliszyn, J.; Smith, R. *Applications of Solid Phase Microextraction*; The Royal Society of Chemistry: London, 1999.
(23) Lord, H.; Pawliszyn, J. *J. Chromatogr., A* **2000**, *885*, 153–193.
(24) Kataoka, H.; Narimatsu, S.; Lord, H. L.; Pawliszyn, J. *Anal. Chem.* **1999**, *71*, 4237–4244.
(25) Zhang, X.; Oakes, K. D.; Cui, S.; Bragg, L.; Servos, M. R.; Pawliszyn, J. *Environ. Sci. Technol.* **2010**, *44*, 3417−3422.
(26) Hutchinson, J. P.; Setkova, L.; Pawliszyn, J. *J. Chromatogr., A* **2007**, *1149*, 127–137.
(27) Kaykhaii, M.; Dicinoski, G. W.; Smedley, R.; Pawliszyn, J.; Haddad, P. R. *J. Chromatogr., A.* **2010**, *1217*, 3452−3456.
(28) Jiang, R.; Zhu, F.; Luan, T.; Tong, Y.; Liu, H.; Ouyang, G.; Pawliszyn, J. *J. Chromatogr., A* **2009**, *1216*, 4641–4647.
(29) Yuan, H.; Mullett, W. M.; Pawliszyn, J. *Analyst* **2001**, *126*, 1456–1461.
(30) Vuckovic, D.; Shirey, R.; Chen, Y.; Sidisky, L.; Aurand, C.; Stenerson, K.; Pawliszyn, J. *Anal. Chim. Acta* **2009**, *638*, 175–185.
(31) D'Agostino, P. A.; Chenier, C. L.; Hancock, J. R.; Jackson Lepage, C. R. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 543–549.

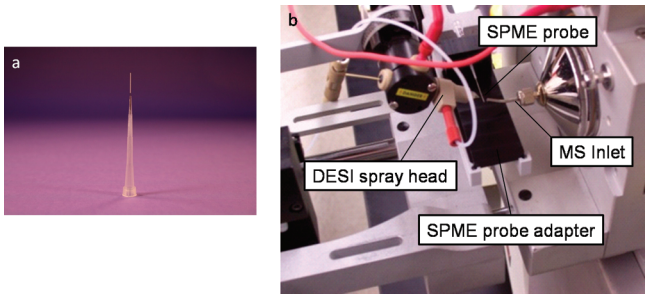

Figure 1. (a) Photograph of the coupling of SPME and DESI to a Thermo Scientific TSQ Quantum Discovery MAX triple quadrupole mass spectrometer. The SPME attachment is removable and can be placed into the 76 × 26 mm sample tray on the DESI ion source. (b) Photograph of the SPME fiber assembly.

cocaine (COC), cocaine-d_3 (d_3-COC), benzoylecgonine (BE), benzoylecgonine-d_3 (d_3-BE), cocaethylene (CE), cocaethylene-d_3 (d_3-CE), methadone (MD), methadone-d_3 (d_3-MD), EDDP, and EDDP-d_3 were purchased as 1 mg/mL individual solutions from Cerilliant (Round Rock, Texas). Drug-free urine samples were obtained from a healthy volunteer. Previously analyzed urine case samples were provided by a local forensic toxicology laboratory.

Supplies. Solid-phase microextraction (SPME) fibers were supplied by Supelco/Sigma Aldrich (Bellefonte, PA). SPME fibers were coated with silica based C_{18} stationary phase with film thickness of 45 μm on 0.008 in. diameter stainless steel wire. The SPME fibers were mounted in 20 μL Eppendorf pipet tips with approximately 1 cm of fiber exposed as illustrated in Figure 1a.

Standard and Sample Preparation. Stock labeled internal standard solutions at a concentration of 10 000 ng/mL were prepared in methanol and added to each sample at a final concentration of 200 ng/mL. Spiked urine samples were prepared containing NF, COC, CE, BE, MD, and EDDP over a concentration range of 20−1000 ng/mL. MB was spiked in urine over a concentration range of 200−10 000 ng/mL.

Analyte Extraction. The SPME fibers were conditioned in 100% methanol prior to immersion into the spiked urine samples. Care was taken to ensure the SPME coating remained solvated prior to sample extraction. Each SPME fiber was suspended into 1 mL of the urine sample. The urine samples were then mixed under gentle agitation using a Vortexer for 15 min. The SPME fibers were then removed from the samples and quickly rinsed with HPLC grade water prior to analysis by DESI-MS.

DESI-MS and MS/MS Analysis. All experiments were performed using an Omni Spray 1-D DESI ion source (Prosolia, Indianapolis, IN) coupled to either a TSQ Quantum Discovery MAX triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA) or an LCT Premier time-of-flight mass spectrometer (Waters, Milford, MA) operated in the positive ion mode. The DESI source operating parameters were as follows: spray voltage, 3.5 kV; solvent flow rate, 10 μL/min; nebulizing gas (nitrogen, 99.9995%) pressure, 125 psi; tip-to-SPME fiber distance was approximately 1 mm. The DESI spray solvent was 80:20:0.1 (v/v/v) acetonitrile/water/formic acid.

In a traditional DESI experiment, the spray is directed toward a planar surface at an angle of approximately 45 degrees to the surface. An extended ion transfer capillary with a slight downward bend is used to collect secondary droplets and ions scattered from the interaction of the spray plume with the surface being analyzed. In the present work, the extended ion transfer tube was positioned with the bend pointing upward and the DESI spray plume was directed toward the SPME fiber representing a three-dimensional surface, placed directly between the MS inlet and the DESI sprayer (Figure 1b). This configuration, which is analogous to the so-called transmission-mode DESI approach,[32] proved to be more convenient for inserting the SPME fiber into the spray plume and also resulted in increased S/N (data not shown) versus fixing the fiber to a planar surface for analysis by the conventional approach. Following extraction, each fiber assembly was secured in a prototype device for positioning the SPME fiber in the DESI spray and analyzed using a 1-D automated DESI ion source (Prosolia, Inc.) coupled to a Waters LCT Premier time-of-flight mass spectrometer in full scan mode or a Thermo TSQ Quantum Discovery Max triple quadrupole mass spectrometer in selected reaction monitoring (SRM) mode. The SRM settings for each compound are summarized in Table 1. In each case, a 7 mm length of the SPME fiber was analyzed using a surface scan rate of 100 μm/sec.

Table 1. Selected Reaction Monitoring (SRM) Parameters

compound	parent ion (m/z)	product ion (m/z)	CE[a] (eV)	tube lens (V)
meprobamate	219	158	10	57
meprobamate-d_7	226	165	10	57
norfentanyl	233	84	19	99
norfentanyl-d_5	238	84	19	99
EDDP	278	234	33	71
EDDP-d_3	281	234	33	71
benzoylecgonine	290	168	22	103
benzoylecgonine-d_3	293	171	22	103
cocaine	304	182	30	92
cocaine-d_3	307	185	30	92
methadone	310	265	12	58
methadone-d_3	313	268	12	58
coca-ethylene	318	196	21	121
coca-ethylene-d_3	321	199	21	121

[a] Collision energy.

RESULTS AND DISCUSSION

SPME extraction followed by direct analysis using DESI-MS was first applied to a mixture of drug compounds spiked into urine. COC, MB, NF, BE, MD, EDDP, and CE were prepared at 500 ng/mL in urine, along with their deuterated analogs which were prepared at 200 ng/mL, and extracted using the biocompatible silica-C_{18} SPME fiber for 15 min. Figure 2 shows the DESI mass spectrum recorded on the LCT Premier time of flight (TOF) MS after direct insertion of the SPME fiber into the spray plume of the DESI source. COC, NF, MD, EDDP, and CE are readily seen in the DESI mass spectrum, along with their deuterated analogs (Figure 2a), while MB and BE are seen at significantly lower abundance (Figure 2b,c). The absolute abundance for each of the compounds varies greatly due to differences in extraction efficiency, desorption efficiency, and ionization efficiency; these

(32) Chipuk, J. E.; Brodbelt, J. S. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 1612–1620.

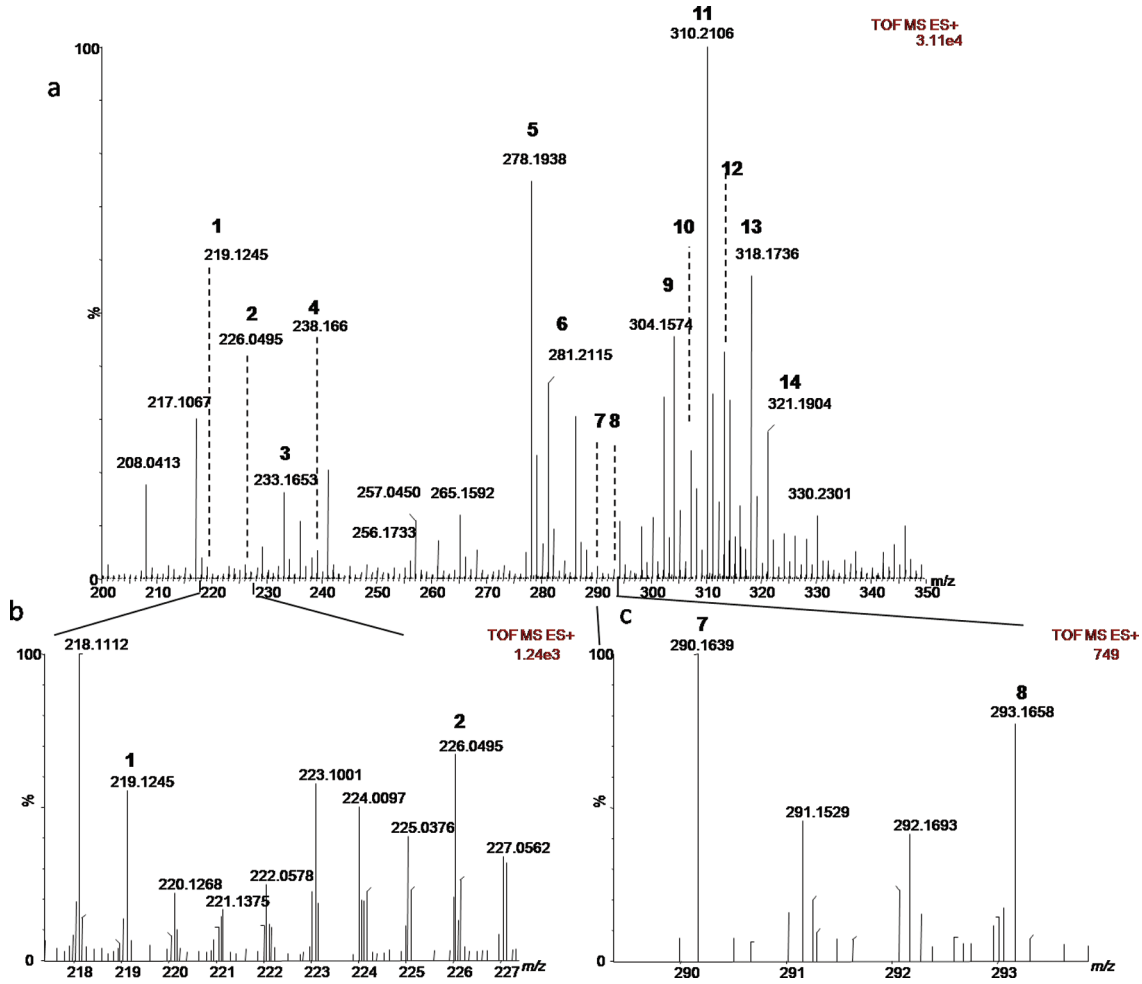

Figure 2. Positive ion DESI mass spectrum of a drug mixture extracted from unprocessed urine using C_{18} SPME fiber for 15 min showing the $[M + H]^+$ ions of (1) meprobamate; (2) meprobamate-d_7; (3) norfentanyl; (4) norfentanyl-d_5; (5) EDDP; (6) EDDP-d_3; (7) benzoylecgonine; (8) benzoylecgonine-d_3; (9) cocaine; (10) cocaine-d_3; (11) methadone; (12) methadone-d_3; (13) cocaethylene; (14) coca-ethylene-d_3. Data was recorded using a Waters LCT Premier.

effects are normalized with the addition of the stable isotope labeled internal standard. In this experiment, pre-equilibrium extraction conditions were chosen to minimize sampling time. In pre-equilibrium conditions, the amount of analyte extracted from the matrix is controlled by diffusion of the analyte from the bulk liquid of high concentration into the static stationary phase of low concentration, thus allowing quantitative measurements to be obtained using isotopically labeled internal standards.[33]

The performance of the SPME DESI-MS/MS method was evaluated by preparing calibration standards and quality control (QC) samples of the seven drug compounds in urine over a range from 20 to 1000 ng/mL, with the exception of MB which was prepared over a range from 200 to 10000 ng/mL. Precision and accuracy were evaluated for all of the compounds using SRM with positive ion detection in order to assess the performance of each method. Figure 3 shows the selected ion chronograms (SIC) for the analysis of COC (m/z 304 → 182) and d_3-COC (m/z 307 → 185) after three consecutive scans of the SPME fiber. As the fiber is introduced into the DESI spray, there is a sharp rise in ion current and the signal is sustained for the length of the scan (i.e., 7 mm) and then decays quickly when the fiber is removed. By scanning the charged-droplet beam across the fiber, ion chronograms are constructed and the peak areas are recorded. Multiple fibers are scanned sequentially, and the integrated peak areas are used for quantitative determination and the creation of the standard curve. As shown in Figure 3, after consecutive scans of the fiber surface, the ion currents for COC and d_3-COC do not decrease as expected after each successive scan. This result indicates that desorption of the analytes from the SPME fiber is not exhaustive under the chosen experimental conditions. The fact that the signal intensities remain relatively constant after successive analyses suggests that a "wicking" effect may be occurring whereby as the outermost molecules in the boundary layer are desorbed the molecules embedded within the stationary phase migrate to the boundary layer and are desorbed in the next scan.

Method performance parameters are summarized in Table 2. The limit of detection (LOD) was calculated as the mean of the blank response plus three standard deviations, and the lower limit of quantitation (LLOQ) was calculated as mean of the blank response plus ten standard deviations. All SPME fibers were analyzed in triplicate, and the average peak area ratio (PAR) of the analyte to the labeled internal standard was recorded. The linearity of the

(33) Ai, J. *Anal. Chem.* **1997**, *69*, 1230–1236.

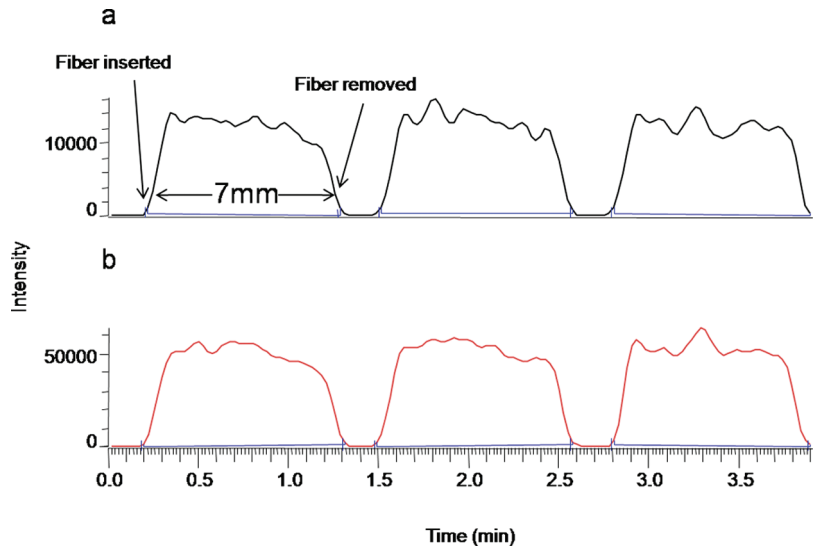

Figure 3. Selected ion chronograms (SICs) of cocaine (*m*/*z* 304 → 182) and cocaine-d_3 (*m*/*z* 307 → 185) in urine recorded using the automated DESI source at 100 μm/s surface velocity, coupled to the Thermo Scientific TSQ Quantum Discovery MAX triple quadrupole mass spectrometer operated in positive ion mode using SRM.

Table 2. SPME DESI-MS/MS Method Performance[a]

compound	concentration range (ng/mL)	R^2	LOD/LLOQ (ng/mL)	low QC% bias	low QC% CV	high QC% bias	high QC% CV
cocaine	20−1000	0.9997	25	6.2	1.3	−11.2	1.9
benzoylecgonine	20−1000	0.9960	50	38.6	11.2	−31	7.6
coca-ethylene	20−1000	0.9973	30	28	5.4	−22	1.7
methadone	20−1000	0.9998	10	5.5	1.9	−10.9	1.5
EDDP	20−1000	0.9999	10	3	1.2	−7.9	2.5
norfentanyl	20−1000	0.9991	30	19.8	3.1	−18	0.96
meprobamate	200−10000	0.9986	160	38	7.6	22	5.7

[a] The low and high QC concentrations were 50 and 500 ng/mL, respectively, for all analytes except meprobamate, which were 500 and 5000 ng/mL, respectively.

method was excellent with $R^2 > 0.99$ in all cases. Method precision (%CV) was based on the percent relative standard deviation of the mean of three determinations of each QC sample.

The method accuracy (% bias) for the low QC samples had a range of 3 to 38% and the high QC samples exhibited a range of −7.9 to −31%. With the exception of BE and CE, the accuracy of the method fell within 100 ± 20%. The high variability and poor accuracy for benzoylecgonine suggests that the extraction procedure may not be optimal for this analyte. Method precision ranged from 1% to 11% for low QC and 0.96% to 7.6% for high QC samples. To further characterize the method for drug analysis, urine samples containing drugs from actual use were obtained from a local forensic toxicology laboratory and analyzed using conventional GC/MS, liquid desorption of the SPME fibers followed by HPLC-MS/MS, and the SPME DESI-MS/MS method described herein. The samples were labeled randomly such that no patient identifying information was transmitted. Urine samples were screened for COC (by analysis of BE), MD, and EDDP using an EIA approach commonly employed in the industry for such tests. MB and NF were screened using ELISA. MB, NF, MD, and EDDP were confirmed by UPLC/MS/MS at the forensic lab. The presence of COC in urine was confirmed by analysis of BE by GC/MS. Positive SPME DESI-MS/MS results were reported based on the detection of the analytes at a level greater than the LLOQ. The concentration range and number of positive results from these analytes as calculated in the urine samples by DESI-MS/MS and the forensic toxicology lab are summarized in Table 3. These results show 100% agreement on all 19 samples screened between the SPME DESI-MS/MS and the respective screening and confirmation method for each analyte. Further, Figure 4a illustrates the relationship of BE results obtained from SPME-DESI-MS/MS and GC/MS performed at the forensics toxicology lab. Notably, the GC/MS results were calculated versus a single

Table 3. Drug Screening Results from SPME DESI-MS/MS and Forensic Toxicology Lab

analyte	range detected by SPME DESI-MS/MS (ng/mL)	number of positive results by SPME DESI-MS/MS	number of positive results reported by the toxicology laboratory[a]
meprobamate	0	0	0
norfentanyl	0	0	0
EDDP	(13−57 824)	4	4
methadone	(18−17 840)	4	4
benzoylecgonine	(74−24 178)	19	19
cocaine	(163−460)	3	NT[b]
coca-ethylene	(50−158)	2	NT

[a] Benzoylecgonine, methadone, and EDDP were screened using EIA while meprobamate and norfentanyl were screened using ELISA. Meprobamate, norfentanyl, methadone, and EDDP were confirmed by UPLC-MS/MS. Cocaine results were confirmed via testing for benzoylecgonine using GC/MS. [b] NT: Not tested.

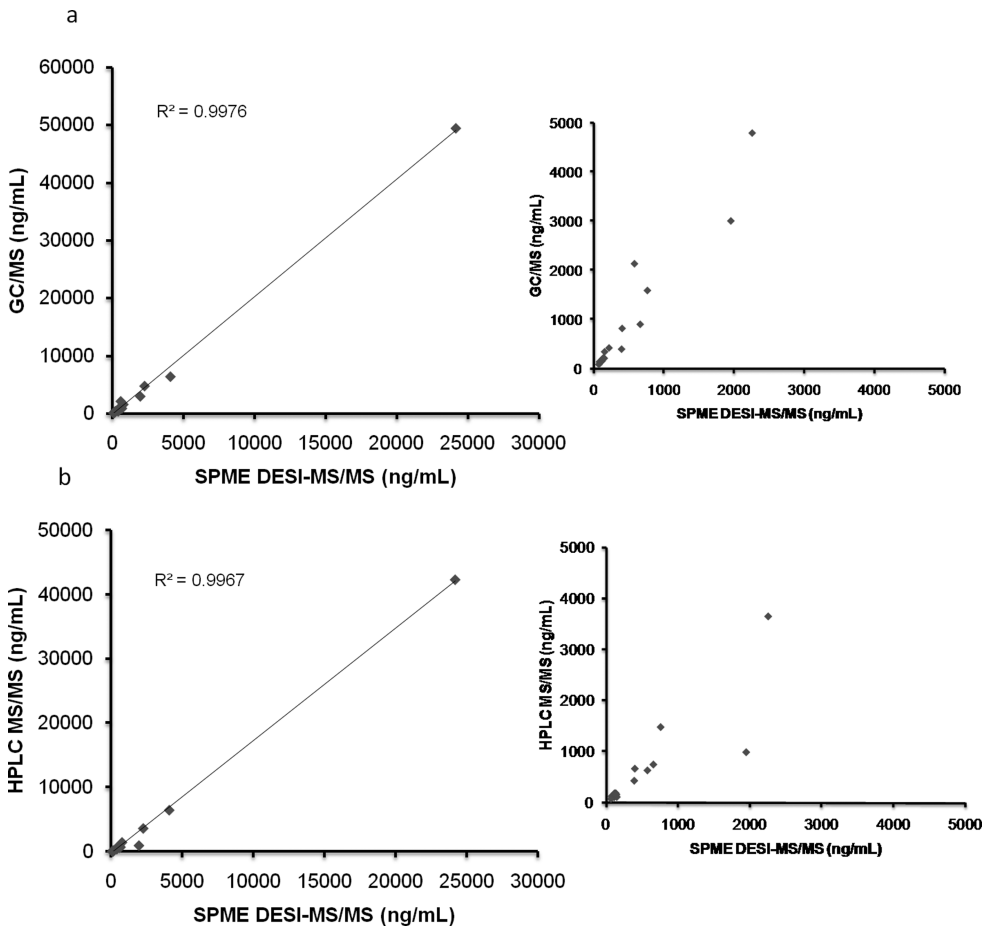

Figure 4. Correlation plots of SPME DESI-MS/MS results for benzoylecgonine with (a) GC/MS and (b) liquid desorption of SPME followed by LC-MS/MS. The concentration range from 0 to 5000 ng/mL is shown as insets.

point calibration where SPME DESI-MS/MS results were calculated from a five point calibration curve. The concentration of BE in several samples was outside the calibration range, results which were beyond our control and not anticipated given the uncertainty associated with analyzing authentic patient samples. Although the calculated concentrations from the two techniques differed, both techniques confirmed the same trend in results with an $R^2 = 0.9976$ showing good agreement between the two approaches. Liquid desorption of the SPME fibers after extraction from urine and analysis by LC/MS/MS was also performed (see Supporting Information for the experimental description of the liquid desorption SPME LC/MS/MS method). In this case, both DESI-MS/MS and LC-MS/MS results were calculated from full calibration curves and better agreement in the absolute concentration values was obtained. Figure 4b shows the correlation for BE in the 19 urine samples between the SPME DESI-MS/MS and the SPME LC/MS/MS methods having an $R^2 = 0.9967$.

The SPME-DESI-MS/MS results are influenced by the extraction, desorption, and ionization efficiency of the analytes. Even though the desorption of the analytes by DESI is suggested not to be exhaustive, for this application, the sensitivity is shown to be sufficient. The absolute sensitivity of DESI technique is at times inferior to the state of the art GC/MS or LC/MS/MS. However, in many cases such as in the present example, the sensitivity is adequate for the application and allows the scientist to obtain information rapidly with fewer resources. Furthermore, the fact that the analytes are not completely removed during the analysis presents the opportunity for re-examination of the fibers for investigational purposes if warranted. Alignment of the SPME fiber within the spray plume was determined to be a critical aspect for consistent analyte measurements. In some cases, SPME fibers that had a slight bend or did not protrude coaxially from the pipet tip presented a challenge in keeping the fiber accurately positioned in the DESI source. The ability to maintain the accurate position of the SPME probe in the spray plume can cause a loss in sensitivity and contributes to the error in the measurements presented here. It is also important to note that the DESI-MS/MS data presented on the forensic case samples were recorded from unprocessed urine samples and none of the samples was enzymatically hydrolyzed to convert glucoronide-conjugated metabolites that may be present in the urine back to the free drug.

CONCLUSION

The present study has demonstrated the feasibility of the use of C_{18} coated SPME fibers in combination with DESI-MS/MS for rapid extraction of a multitude of illicit drugs and pain medications in untreated urine. This study demonstrates the potential for determining the concentrations of drug molecules in urine using SPME DESI-MS/MS. The data herein represents an important step toward the development of more routine screening and quantitative DESI-MS/MS procedures for the analysis of biological samples. SPME combines sampling and sample preparation into a single step and is shown to be a suitable format for direct analysis using DESI-MS. Features of

the method include simplicity, rapid extraction and analysis, selectivity, and the ability to reanalyze the fibers for further investigations.

The compelling analytical features and versatility of the method should not restrict its use to the examination of urine for drugs of abuse. In fact, it is foreseeable that this method may be more suitable in situations such as in on-site water sampling and testing for pharmaceuticals, pesticides, herbicides, etc. or in pharmaceutical process monitoring. Indeed, the combination of SPME and DESI is an enabling approach to the implementation of field portable mass spectrometers for such purposes, since it combines rapid sampling and sample preparation with the capability of direct analysis.

ACKNOWLEDGMENT

We gratefully acknowledge the assistance and insights from Joshua Gunn and Brenda Sweeney of AIT laboratories in Indianapolis, Indiana, and of Michael Balogh, Hilary Major, and Steve Smith of Waters Corp. for the loan of the Waters LCT Premier.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review May 18, 2010. Accepted July 26, 2010.

AC101295G