Analysis on the Go: Quantitation of Drugs of Abuse in Dried Urine with Digital Microfluidics and Miniature Mass Spectrometry

Andrea E. Kirby,†,∥ Nelson M. Lafrenière,†,∥ Brendon Seale,‡ Paul I. Hendricks,§ R. Graham Cooks,§ and Aaron R. Wheeler,*,†,‡

†Department of Chemistry, University of Toronto, 80 St George Street, Toronto, Ontario M5S 3H6, Canada
‡Institute of Biomaterials and Biomedical Engineering, 164 College Street, Toronto, Ontario M5S 3G9, Canada
§Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907, United States

ABSTRACT: We report the development of a method coupling microfluidics and a miniature mass spectrometer, applied to quantitation of drugs of abuse in urine. A custom digital microfluidic system was designed to deliver droplets of solvent to dried urine samples and then transport extracted analytes to an array of nanoelectrospray emitters for analysis. Tandem mass spectrometry (MS/MS) detection was performed using a fully autonomous 25 kg instrument. Using the new method, cocaine, benzoylecgonine, and codeine can be quantified from four samples in less than 15 min from (dried) sample to analysis. The figures of merit for the new method suggest that it is suitable for on-site screening; for example, the limit of quantitation (LOQ) for cocaine is 40 ng/mL, which is compatible with the performance criteria for laboratory analyses established by the United Nations Office on Drugs and Crime. More importantly, the LOQ of the new method is superior to the 300 ng/mL cutoff values used by the only other portable analysis systems we are aware of (relying on immunoassays). This work serves as a proof-of-concept for integration of microfluidics with miniature mass spectrometry. The system is attractive for the quantitation of drugs of abuse from urine and, more generally, may be useful for a wide range of applications that would benefit from portable, quantitative, on-site analysis.

Drug abuse is an epidemic that impacts the social and economic well-being of people all around the world.1,2 While urine, blood, hair, sweat, and saliva have been used to probe for the presence of illicit and abused drugs,3 urine is the most preferred sample because of its ease of collection and high concentrations of drugs and metabolites.4,5 A recent trend is the archiving of urine samples as dried spots on paper,6 with advantages of long-term storage and analysis (similar to what has been reported for dried blood spots7). Urine samples are routinely analyzed for stake-holders in a wide range of settings including the workplace, the military, athletics, and the criminal justice and health care systems.8,9 This has driven the development of portable immunoassays for drugs of abuse that are compact, inexpensive, and compatible with a wide range of operating environments.10,11 However, for most applications, the imperfect nature of immunoassays, which have non-negligible rates of false negatives and positives (resulting in the use of high cutoff levels),5,12 requires that initial test results be confirmed by a second analysis in the laboratory. This two-tiered system of analysis (first in the field, second in the laboratory) results in significant costs associated with maintaining the chain of custody and ensuring proper storage and transportation.4,13

Laboratory analysis for drugs of abuse (the second tier) is typically implemented by gas chromatography (GC) or high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS).8,9 These methods are extremely precise and reproducible, representing the analytical “gold standard” for confirmatory analysis.8,13,14 However, these methods are slow (often requiring extensive sample processing prior to analysis), must be performed by well-trained technicians, and require use and maintenance of expensive laboratory equipment. Various strategies have been used in an attempt to reduce analysis times, including “dilute-and-shoot” protocols in which the sample is simply diluted and then analyzed by HPLC—MS/MS.15,16 While this circumvents sample processing, these protocols are not ideal for quantifying low concentrations and the methods require chromatographic separation which is often the rate-limiting step in the analysis (30–60 min per sample). Perhaps most importantly, samples must be transported to and from the lab, adding days to weeks to a process that might otherwise be completed in minutes.

A potential solution to the time-consuming two-tiered analysis system is to bring gold-standard analyses into the

Received: April 9, 2014
Accepted: May 19, 2014
Published: June 6, 2014

© 2014 American Chemical Society

dx.doi.org/10.1021/ac5012969 | Anal. Chem. 2014, 86, 6121−6129

6121
field, effectively eliminating the need for prescreening with error-prone immunoassays. The recent emergence of miniature (or “mini”) mass spectrometers makes this prospect a possibility. Several kinds of mini MS systems have been developed, using quadrupoles,17 ion traps,18−20 time-of-flight (TOF) analyzers,21,22 and magnetic sectors.23 While mini MS systems benefit from small size and low power consumption, the trade-off is a loss of specificity,24 which can be problematic for real-world samples. Specificity limits can be overcome by implementing MS/MS and selected reaction monitoring (SRM), permitting targeted analysis of specific mass transitions upon fragmentation. The mini mass spectrometer systems developed at Purdue University20,25 are particularly attractive for the analysis of complex mixtures because they combine MS/MS analysis with ambient ionization techniques. The latest generation of these instruments was recently implemented in “backpack” format,26 as a fully autonomous lightweight instrument (12 kg, including attendant pumps, power supply, detector, mass analyzer, and ion source) that can be carried or worn comfortably by one person.

For on-site analysis of drugs of abuse, mini mass spectrometers must be combined with miniaturized systems for sample collection and processing and/or chromatographic separations. One option for portable sampling is microfluidics, which typically relies on the transport of liquid samples in enclosed microchannels. A range of microchannel-based methods for analyzing drugs of abuse have been reported,27−31 but only a few of these methods are compatible with MS,29−31 and those that are require on-chip chromatography or other separations prior to detection. More importantly, these methods have only been coupled to laboratory scale MS instruments to date. A second option is paper spray ionization,32 in which samples embedded in paper are eluted in solvent and analyzed directly by mass spectrometry with no separations. Paper spray has been used to analyze drugs of abuse,33 but it is likely limited to simple processes comprising one or a few steps. A third option is digital microfluidics (DMF), a technique in which droplets of reagents and samples are manipulated electrodynamically on an array of electrodes.34 DMF is useful for combining complex, multistep sample preparation with direct analysis by mass spectrometry,35−39 but (as is the case for microchannels), all systems reported previously have relied on bench-scale mass spectrometers in a laboratory setting. We propose that a portable system relying on DMF for sample preparation and mini MS for analysis would be a powerful new tool for single-tier quantification of drugs of abuse.

Here we report a digital microfluidic platform used for the extraction and quantification of drugs of abuse in urine by tandem mass spectrometry. This work is novel in several respects, being (a) the first microfluidic method integrated with a mini MS, (b) the first microfluidic method capable of analyzing dried urine, and (c) the first quantitative analysis of drugs of abuse in urine using a mini MS. We propose that this innovation (a) is particularly attractive for a wide range of applications for portable analysis; there are many ongoing efforts to couple microfluidics to miniature mass spectrometers for portable analysis,40 but to our knowledge, this report constitutes the first description of such a system in the scientific literature.

EXPERIMENTAL SECTION

Reagents and Materials. Cocaine, benzoylecgonine (BZE), and codeine were purchased as 1 mg/mL stock solutions in methanol (MeOH) from Sigma-Aldrich (Oakville, ON, Canada), and solid mepivacaine was obtained from the same source. Cocaine (and its deuterated internal standard, cocaine-d$_3$), was also obtained from Cerilliant Corporation (Round Rock, TX) as a 1 mg/mL stock solution in MeOH. Urine (pooled male donor) was obtained from BioChemed Services (Winchester, VA). HPLC grade methanol (MeOH) and deionized water (dH$_2$O) with a resistivity of 18 MΩ cm at 25 °C were used in all experiments. All drugs were diluted to the appropriate concentration in MeOH or urine prior to experiments. Glass substrates coated with 200 μm chromium were from Telic Company (Valencia, CA) and indium-doped tin oxide (ITO) coated glass substrates were from Delta Technologies, Ltd. (Loveland, CO). Parylene-C dimer was from Specialty Coating Systems (Indianapolis, IN) and Teflon-AF 1600 was from Dupont (Wilmington, DE).

DMF Device Fabrication, Assembly, and Operation. Devices were fabricated at the University of Toronto Nanofabrication Centre (TNFC). Device bottom plates bearing
patterned chromium electrodes and contact pads covered with Parylene-C and Teflon-AF were formed using photolithography and wet etching as described previously. As shown in Figure 1, bottom plates had four analysis zones spaced 24.44 mm apart, each comprising one loading reservoir (7 mm \times 7 mm), two thin actuation electrodes (2 mm \times 5 mm), two large extraction electrodes (7 mm \times 7 mm), one plus-shaped electrode (7 mm \times 5 mm), and two large actuation electrodes (7 \times 7 mm), with inter-electrode gaps of 40 \mu m.

DMF device top plates were formed from ITO-coated glass substrates coated with 50 nm Teflon-AF and patterned using a lift-off technique, as described previously. Briefly, top plates were formed such that when complete, they were globally coated with Teflon-AF with four 2 mm diameter circular regions of exposed ITO (known as “anchors”) spaced 24.44 mm apart. When used for analysis, a 20 \mu L aliquot of urine spiked with an appropriate concentration of drug was spotted onto each of the four anchors and dried on a hot plate at 100 °C for \sim 10 \text{ min}.

DMF devices were assembled with a urine-spotted ITO-glass top plate and a patterned chromium-glass bottom plate separated by a spacer formed from four pieces of double-sided tape (spacer thickness \sim 360 \mu m) such that each dried urine zone on the top plate aligned with a central thin electrode on an analysis zone (Figure 1a), and the top plate was aligned with the outer edge of the reservoir electrodes on the device bottom plate to facilitate solvent loading. Droplets were actuated on assembled devices by applying driving potentials \sim 135 \text{ VRMS} between the top-plate electrode (ground) and successive electrodes on the bottom substrate, managed by the open-source DropBot control system (described in detail elsewhere).

**DMF-Driven Urine Extraction.** In a typical experiment, one 22 \mu L aliquot of extraction solvent (neat MeOH for conductivity measurements, MeOH containing 50 ng/mL mepivacaine as the internal standard for lab-scale MS analysis, or MeOH containing 100 ng/mL cocaine-d_{3} as the internal standard for mini MS analysis) was loaded into each of the four reservoirs. Each aliquot was then driven to its respective dried urine zone and incubated for 5 min, including 15 cycles of actuating the droplet back-and-forth and incubating. Extract droplets were then split from the hydrophilic dried urine zones and driven to the destination electrode for analysis.

**Conductivity Measurements.** For each conductivity measurement, \sim 80 \mu L of pooled methanolic extract generated by DMF from four samples of neat urine or urine spiked with 50 ng/mL cocaine (representing a total urine volume of 80 \mu L) was collected off the device and diluted in 13 mL of diH_{2}O. For comparison, 80 \mu L aliquots of diH_{2}O, pure methanol, pure urine, and urine containing 50 ng/mL cocaine were also diluted in 13 mL aliquots of diH_{2}O. Diluted solutions were evaluated using an H270G conductivity meter (Hach Company, Loveland, CO). Four replicates were prepared and evaluated for each condition.

**Lab-Scale DMF-MS/MS Experiments.** DMF devices were interfaced to a Thermo LTQ linear ion trap mass spectrometer (Thermo Scientific, Waltham, MA) using pulled glass capillary nanoESI emitters sandwiched between the top and bottom substrates of the device as described previously. Briefly, a nanoESI emitter (5 cm long, 360 \mu m o.d., 50 \mu m i.d., 30 \mu m pulled tip i.d., New Objective Inc., Woburn, MA) was manually inserted between the two plates of the DMF device. When the extract droplet was driven to the rear of the emitter, it was filled by capillary action in \sim 1 \text{ s}. The emitter was positioned \sim 3 \text{ mm} from the grounded inlet of the mass spectrometer, and spray voltage was applied to the ITO-glass top plate of the DMF device, making contact with the solution to be sprayed. Once the first sample was analyzed, a second emitter was inserted into the second extract droplet, the device moved laterally to align the next emitter with the MS inlet, and the process repeated until all four samples were analyzed. All analytes were analyzed in positive ion mode at a capillary temperature of 250 °C. Parameters including spray potential (+1.6–2.0 kV), capillary voltage, tube lens voltage, and collision energy were tuned for each analyte to provide optimum signal. Spectra were obtained by averaging 10 acquisitions (at a rate of 6 acquisitions/s).

**DMF-Mini-MS/MS Experiments.** Mini MS experiments were performed with a Mini 12 mass spectrometer, described in detail elsewhere. Briefly, the Mini 12 is a 25 kg, 19.6 in. \times 22.1 in. \times 16.5 in. mass spectrometer with a self-contained

<table>
<thead>
<tr>
<th>Table 1. MS/MS Conditions for the Analytes and Internal Standards Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>analyte</td>
</tr>
<tr>
<td>cocaine</td>
</tr>
<tr>
<td>benzoylecgonine (BZE)</td>
</tr>
<tr>
<td>codeine</td>
</tr>
<tr>
<td>mepivacaine</td>
</tr>
<tr>
<td>cocaine-d_{3}</td>
</tr>
</tbody>
</table>

*aUsed as an internal standard. *Used only for Mini MS/MS.
Vacuum system and an average power consumption of \(\sim 65\, \text{W}\).

The 4 mm \(\times\) 5 mm rectilinear ion trap (RIT) was operated with an rf driving frequency of 1 MHz, and ions were trapped for MS/MS analysis with a notched stored waveform inverse Fourier transform (SWIFT) using activation frequencies of 102.25 kHz (for \(m/z\) 304) and 100.85 kHz (for \(m/z\) 307). For DMF-Mini-MS/MS experiments, samples were extracted and introduced into nanoESI capillaries as above and sprayed into the instrument using the discontinuous atmospheric pressure interface (DAPI) described in detail elsewhere,\(^4\) with a pulse time of 15 ms.

**MS/MS Data Collection and Processing.** The mass transitions for each analyte and internal standard are listed in Table 1. For lab-scale MS/MS analyses, precursor and product ion peak heights were recorded for the analyte and the internal standard in series using Thermo Finnigan’s Xcalibur software (version 2.0). Four replicates were evaluated for each condition, and calibration curves were generated by plotting the analyte: internal standard product ion peak height ratios as a function of concentration. Linear regressions were generated using IGOR Pro (version 5.0.4.8, WaveMetrics, Inc., Lake Oswego, OR). Limits of quantification (LOQ) were calculated as the concentration of analyte corresponding to the average response from blank measurements (extracts from dried urine containing no analyte) plus 10 times the standard deviation of the average blank response. For mini MS/MS analysis, cocaine and cocaine-\(d_3\) precursor ions were isolated and fragmented simultaneously, allowing for coincident collection of product ion scans. Product ion peak areas were extracted from raw data using PTC Mathcad (PTC, Needham, MA). The calibration curve was generated and the LOQ was calculated as above using peak areas rather than peak heights.

**Dip-DMF-MS/MS Analysis.** Device top plates were manually immersed in a beaker of urine spiked with 1000 ng/mL cocaine. When the top plate was removed from the urine, small droplets of urine (\(\sim 3\, \mu\text{L}\)) adhered to the hydrophilic zones and were dried for \(\sim 5\) min on a hot plate at 100 °C before analysis. Lab-scale DMF-MS/MS extraction and analysis was performed as above using 50 ng/mL mepivacaine as internal standard.

## RESULTS AND DISCUSSION

**Sample Processing.** A digital microfluidic system was developed to extract analytes from dried urine. As far as we are aware, this is the first microfluidic device (of any format) to be used for this application; we speculate that the “dried-sample” format might be difficult to integrate with enclosed micro-channels because of the risk of channel clogging. The device is shown in Figure 1 and has four extraction modules distributed in a format similar to that reported previously for other applications.\(^3\) In a typical analysis, four 20 \(\mu\text{L}\) urine samples were loaded onto the device and then processed (extracted, desalted, and mixed with internal standards) in parallel and analyzed sequentially by nanoESI MS/MS via embedded pulled-glass capillary emitters.

In initial experiments, samples were loaded and dried directly onto Teflon-coated device bottom plates (as was done previously with blood samples), but this strategy was abandoned after observing that sections of the dried urine would break off during extraction, resulting in suspended dried urine particles in the extract droplet which interfered with...
A new strategy was developed to circumvent the problem of droplet movement and analysis. To do this, a fluorocarbon liftoff technique developed originally for on-chip cell culture to form hydrophilic sample "anchors" on the device top plate. Several circular anchor sizes were evaluated; for the work reported here, 2 mm diameter anchors were used as they offered the best compromise between keeping the dried sample adhered to the device surface while facilitating facile splitting of extract droplets from the sample. We propose that the strategy of using hydrophilic anchors for dried samples may be useful for other applications in the future.

Figure 2 illustrates the steps involved in extraction from a dried urine zone, and a video depicting this process can be found in the Supporting Information. A 22 μL aliquot of extraction solvent was loaded into the reservoir (Figure 2A), driven to the dried urine zone (Figure 2B), and extracted for 5 min by cycling the droplet between the adjacent extraction electrodes (Figure 2C–E). After extraction, the droplet was split from the dried urine zone and driven to the final electrode for analysis, where it filled the nanoESI emitter by capillary action (Figure 2F). As shown, a large fraction of the solvent droplet (~90% estimated by area) was delivered to the emitter.

Figure 4. Lab-scale tandem mass spectrometry analysis of drugs extracted from urine in DMF devices. Representative product ion spectra illustrate (A) the cocaine transitions 304 → 182 (primary product ion) as well as 304 → 286 (primary product ion) as well as 300 → 282 (primary product ion), and (C) the codeine transitions 300 → 215 (primary product ion), as well as 300 → 282 (primary product ion). Calibration curves are linear over 2 orders of magnitude for (D) cocaine, LOQ = 51 ng/mL, R² = 0.9924, (E) BZE, LOQ = 21 ng/mL, R² = 0.9989, and (F) codeine, LOQ = 39 ng/mL, R² = 0.9805. The error bars represent ±1SD, n = 4. Insets illustrate the lower end of the calibration curves; the blue lines represent the LOQs.
while the remainder stayed adhered to the hydrophilic anchor (a phenomenon known as passive dispensing). After the nanoESI emitter was filled, electrospray was generated by applying spray voltage to the top-plate electrode. The internal standard was included in the extraction solvent droplet (mepivacaine or cocaine-de) to compensate for samples losses and evaporation.

The salt content of urine can be troublesome for ESI MS analysis, as high salt concentrations cause ion suppression. For this reason, standard urine analysis protocols include an extraction step (liquid—liquid or solid phase extraction) prior to analysis. In the method presented here, we developed a procedure for extracting dried urine zones with MeOH. The mechanism is depicted in Figure 3A; drugs are extracted into MeOH droplets, while some of the salt is left behind in the dried urine zone (exploiting the low solubility of salts in MeOH). This procedure is fast and automated and eliminates steps that are traditionally used in liquid—liquid extraction procedures such as centrifugation to remove particulates and separation of the extract from the liquid urine sample.

To evaluate the efficacy of the DMF extraction method for separating analytes of interest from the salty urine matrix, we compared the conductivities of samples before and after extraction. As shown in Figure 3B, the conductivities of unextracted urine and urine extracts are 82.0 ± 6.7 and 25.5 ± 4.0 μS/cm, respectively, representing nearly 70% reduction in conductivity. As expected, this trend was observed for samples with and without analyte. The blank sample conductivity was 5.0 ± 1.4 μS/cm, which suggests that the salt content in the extracts is not zero; in the future, if additional desalting is necessary, this procedure might be performed multiple times in series (i.e., extract from urine, move and dry to form an intermediate dried spot, extract again). Alternatively, a liquid extraction might be replaced with a DMF solid-phase extraction.

**Quantitative Analysis.** As a first step toward a portable analysis system, methods were developed to quantify three analytes (cocaine, BZE, and codeine) in dried urine on DMF devices using a lab-scale tandem mass spectrometer (m/z transitions and other parameters listed in Table 1). Four urine samples can be analyzed using this method (including extraction and quantification) in ~15 min. Representative spectra for cocaine, BZE, and codeine extracted from dried urine on-device are shown in Figure 4A–C; high intensity product ion peaks are observed as well as peaks representing the precursor ion minus the loss of water. Calibration curves (Figure 4D–F) were generated by fitting lines of regression to the intensity ratios of drug product ions relative to those of the internal standard as a function of drug concentration in the urine samples. As shown, the curves are linear over multiple orders of magnitude, with $R^2$ values of 0.9924, 0.9982, and 0.9805, for cocaine, BZE, and codeine, respectively. The precision at each concentration is illustrated in error bars corresponding to the standard errors of those measurements, with % RSD values ranging from 6.4—57.0%, 6.0—30.4%, and 4.7—27.9% for cocaine, BZE, and codeine, respectively. The LOQs, defined as the concentration corresponding to the response of the blank plus 10 times the standard deviation of the blank response were 51 ng/mL, 21 ng/mL, and 39 ng/mL for cocaine, BZE, and codeine, respectively. These LOQ values are approximately 10× higher than those reported for conventional laboratory methods (e.g., Otero-Fernandez et al. report LOQs of 0.87, 3.1, and 5.0 ng/mL for cocaine, BZE, and codeine, respectively), but they are well below the standard cutoff values for quantifying drugs of abuse in urine for confirmation analysis. When viewed in context of the speed of the new system (15 min from dried sample to answer), this performance is attractive, even in laboratory settings. However, our primary goal in this work was to develop a portable method, as described below.

---

**Figure 5.** DMF-Mini-MS/MS analysis of dried urine spots. (A) Picture of setup, with numbers indicating (1) Mini-12 miniature mass spectrometer, (2) Digital microfluidic “DropBot” automation system, (3) MS interface, and (4) DMF device. (B) Mass spectrum illustrating the transitions of cocaine (10 ng/mL) and internal standard, cocaine-de (50 ng/mL). (C) Calibration curve for cocaine, LOQ = 40 ng/mL, $R^2 = 0.9990$. The error bars represent ±1SD, $n = 4$. Inset illustrates the lower end of the calibration curve; the blue line represents the LOQ.
After optimizing the method using a lab-scale mass spectrometer, the DMF system was combined with the fully autonomous 25 kg Mini 12 mass spectrometer. The experimental setup is shown in Figure 5A and is similar to that described above for lab-scale MS analysis. A DMF device was positioned in front of the mass spectrometer; once a sample was extracted and filled a nanoESI emitter, the extract was introduced to the Mini 12 via the discontinuous atmospheric pressure interface (DAPI). The Mini 12 MS system is controlled using house-built software, and ions are isolated with a notched stored waveform inverse Fourier transform (SWIFT). Since ion isolation is performed manually by tuning the frequency notch for each ion under a unique set of conditions, it is desirable to limit the time used to complete ion isolation and activation per analysis. Using an analyte/internal standard pair with very close precursor ion m/z ratios (m/z 304 for cocaine and m/z 307 for cocaine-d₃) permits the simultaneous isolation of both precursor ions for MS/MS analysis. In this case, the isolation window was set to m/z 302–309, allowing both m/z 304 and m/z 307 to be isolated for sequential fragmentation (Table 1). As shown in Figure 5B, the product ion scan for a representative Mini 12 MS/MS analysis contains peaks for both the cocaine and cocaine-d₃ product ions at m/z 182 and m/z 185, respectively, as well as unfragmented precursor ions at m/z 304 and m/z 307.

A calibration curve for cocaine extracted from dried urine on DMF devices with analysis by the Mini 12 MS was generated by fitting a line of regression to the intensity ratio of the cocaine product ion peak area relative to that of cocaine-d₃ as a function of cocaine concentration for seven different urine concentrations (Figure 5C). The calibration curve is linear over the 3 orders of magnitude, has an R² value of 0.9990, and an LOQ of 40 ng/mL. The precision at each concentration is illustrated in error bars corresponding to the standard errors of those measurements, with % RSD values ranging from 12.3 to 55.7%. Interestingly the LOQ of this new portable DMF-Mini-MS/MS method is lower than the minimum required performance limit (MRPL) outlined by the United Nations Office on Drugs and Crime for laboratory quantification of cocaine in urine: 50 ng/mL. More importantly, the LOQ of the new method is far lower than the 300 ng/mL cutoff levels reported for the portable immunoassays that are routinely used for on-site analysis of drugs in urine. An advantage of immunoassays (e.g., the Alere Triage TOX Drug Screen) is their low cost; they are often formed from paper substrates and are less expensive than the glass DMF devices used here. However, we note that methods were recently reported for forming low-cost DMF devices from paper, an innovation that may eventually enable single-use DMF tests with consumables costs comparable to those of immunoassays.

The results shown in Figure 5 validate the use of DMF extraction coupled to the Mini 12 MS for quantitation of cocaine in urine. Future work will focus on developing a fully field-deployable system for on-site quantitative analysis of drugs of abuse in urine and other biological samples, taking advantage of the backpack mass spectrometer format to exploit the ease of portability. Toward this goal, we developed a rapid sampling system that we call “Dip-DMF,” illustrated in Figure 6A–C. Dip-DMF exploits the difference in surface energies between the ITO anchors and the bulk Teflon-AF surface of the devices used here to quickly and easily deposit urine samples onto the device top-plate. As shown, arrays of sample droplets can be generated in seconds using Dip-DMF, which (after drying) can be analyzed using the methods described herein (Figure 6D).

**CONCLUSION**

In summary, we have developed a digital microfluidic platform coupled to a miniature mass spectrometer for the quantification of drugs of abuse in urine. The figures of merit for the new technique are compatible with the performance criteria for laboratory analyses established by the United Nations Office on Drugs and Crime. The proof-of-concept results presented here suggest the possibility of a new paradigm for drug screening in which a single-tier test performed in the field might replace the two-tier system (one in the field and a second in the laboratory) used today. More generally, we propose that the combination of microfluidics and miniature mass spectrometry represents a powerful new tool for portable, on-site “laboratory quality” analysis for a wide range of applications.

**ASSOCIATED CONTENT**

Supporting Information

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

**AUTHOR INFORMATION**

Corresponding Author

*E-mail: aaron.wheeler@utoronto.ca. Phone: (416) 946 3864. Fax: (416) 946 3865.

dx.doi.org/10.1021/ac5012969 Anal. Chem. 2014, 86, 6121−6129
Author Contributions
A.E.K. and N.M.L. contributed equally to this work.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Christopher Pulliam (Purdue Univ.) and Prof. Jacob T. Shelley (Kent State Univ.) for assistance with collecting Mini MSD data, Prof. Zheng Ouyang (Purdue Univ.) for help with Mini 12 instrumentation, Elena Gritzan (Univ. of Toronto) for initial dried urine on DMF experiments, Dr. Kihwan Choi (Univ. of Toronto) for help with conductivity measurements, Dr. Dean Chamberlain (Univ. of Toronto) for helpful discussions regarding Dip-DMF, and Prof. James H. Watterson (Laurentian Univ.) for helpful discussions regarding internal standard selection. We thank the Natural Sciences and Engineering Research Council of Canada (NSERC) and the U.S. National Science Foundation (Grant CHE1307264) for financial support. A.E.K. thanks NSERC and N.M.L. thanks the Ontario Graduate Scholarship (OGS) program for graduate fellowships, and A.R.W. thanks the Canada Research Chair (CRC) program for a CRC.

REFERENCES