PORTABLE DIGITAL MICROFLUIDIC/MASS SPECTROMETRY METHOD FOR QUANTIFICATION OF DRUGS OF ABUSE IN URINE

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ABSTRACT

We present a new digital microfluidic (DMF) method for the multiplexed quantification of drugs of abuse in urine, coupled to a portable mass spectrometer (MS). The protocol is characterized by the quick, simple and automated quantification of drugs in dried urine. Advantages over traditional macroscale methods include shorter extraction times, portability for on-site analysis, and reduced workflow complexity. This automated 5 minute extraction protocol represents the first combination of DMF with portable MS and may represent the first combination of microfluidics in any format with portable MS.

KEYWORDS: Digital Microfluidics, Mass Spectrometry, Urine, Drugs, Quantification

INTRODUCTION

The last decade has seen an increase in drug screening in the workplace, the military, athletics, and the criminal justice and health care systems [1]. Urine is a well-established test substance characterized by its ease of collection, and high concentrations of drugs and metabolites [2]. Key limitations to the traditional analysis of urine include a reliance on repeated immunoassays for each suspected drug of abuse, and the complexity of subsequent confirmatory analysis by an orthogonal method such as high performance liquid chromatography (HPLC) coupled with tandem MS (MS/MS). Moreover, traditional methods are characterized by manual, labour-intensive reagent-handling procedures.

DMF is a droplet based fluid handling technique [3] that has been used previously to analyze constituents in dried blood spot samples [4]. The technique is characterized by the manipulation of small amounts of fluids (i.e., dispensed from reservoirs, split, merged and mixed) on an open surface by applying a series of electrical potentials to an array of electrodes [5, 6]. Droplets are actuated by electromechanical forces generated on free charges in the droplet meniscus (in the case of conductive liquids) or on dipoles inside of the droplet (in case of dielectric liquids). The reconfigurable nature of DMF enables unique opportunities, such as interfaces with portable analytical systems.

Here, we introduce a digital microfluidic method for the multiplexed extraction and quantification of drugs of abuse in urine, coupled to a portable MS. We propose this technique will have similar performance to traditional methods of analysis, but with the important and unique capacity to provide reliable, multiplexed quantification in the field, without the need for multiple screening assays and HPLC.

EXPERIMENTAL

The new methods described here rely on a multiplexed device design (Fig 1:A) featuring four independent extraction zones (Fig 1:B) for the parallel extraction of drugs from dried urine. As shown in Fig 1:C, urine samples are immobilized and allowed to dry on microfabricated hydrophilic sites [7] in the device top plate. DMF was used to automate all of the steps required for the extraction of drugs from urine, including delivery of extraction solvent, delivery of internal standards, incubation, and delivery to spray capillaries, which are directly interfaced to a mass spectrometer using a custom nanoelectrospray ionization (nESI) capillary interface [4].

Fig 1: Device schematic and method. (A)Top view schematic of DMF device. (B) Top view schematic of individual extraction zone. (C) Side-view schematic of DMF device, not to scale.
Several different drugs of abuse were extracted from urine and analyzed using this DMF-MS/MS method. Briefly, human urine was spiked with different concentrations of drug and spotted onto the hydrophilic regions of the top plate, and left to dry overnight at room temperature. With the urine dried and the DMF device assembled, ~22 L of extraction solvent was actuated to the dried urine, actively cycled back and forth 15 times, and left to extract for a total of five minutes. The extract was then actuated to the pulled glass capillary emitter sandwiched between the top and bottom plates of the device for analysis by MS/MS. Each drug was extracted and analyzed separately. The extraction solvent was methanol spiked with an appropriate concentration of internal standard (mepivicaine). Experiments were performed using an LTQ linear ion trap mass spectrometer and a Mini-12 portable MS (Fig 2).

For experiments performed with the LTQ, spray potentials were varied in the range of +1.5-2.0 kV, and MS/MS collision energies ranged from 25-40 eV. The Mini-12 portable MS, developed at Purdue university, is built on a small form-factor chassis that measures 28 x 20 x 19 cm and weighs 17 kg. It has a self-contained vacuum system comprising a KNF diaphragm roughing pump (5 L/min) and a Pfeiffer HiPace 10 turbo molecular pump (10 L/s). The mini-12 uses a 5 x 4 mm rectilinear ion trap (RIT) as the mass analyzer and has a mass range of m/z 80 to 800 with full width half mass (FWHM) of 1-2 amu. The discontinuous atmospheric pressure interface (DAPI) of the mini-12 enables the introduction of externally generated ions into the mass analyzer at atmospheric pressures with minimal pumping requirements [8].

RESULTS AND DISCUSSION
The newly developed DMF-MS/MS method was applied to a variety of different drugs of abuse. Figure 3 shows a collection of tandem mass spectra that were generated from urine samples extracted on DMF.

Fig 2: Picture of a portable mini-12 mass spectrometer coupled to DMF via a discontinuous atmospheric pressure interface (DAPI). Each DMF device can accommodate four samples which are analyzed in series.

Fig 3: Tandem mass spectra of fragment ions (with mass transitions in parentheses) from selected drugs extracted from urine. (A) Morphine, (B) Cocaine, (C) Oxycodone, (D) Codeine.
Calibration curves were implemented on device with appropriate concentrations of internal standards, and were constructed over physiologically relevant concentrations in order to evaluate linear dynamic range and limits of detection. Results for morphine are presented in Figure 4. Optimization and evaluation of the DMF-MS/MS method was conducted on the LTQ. Figure 5 shows a spectrum for cocaine collected with the mini-12 mass spectrometer, marking the successful combination of digital microfluidics and portable mass spectrometry.

CONCLUSION
We present a new automated method for quantitative drug analysis in urine using a combination of digital microfluidics and tandem mass spectrometry. The use of a portable, miniature mass spectrometer promises to be useful for a wide range of applications for analysis in the field.

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Fig 4: Calibration curve of morphine extracted from urine on device. N=4. R² = 0.9994. LOD = 6.46 ng/mL. LLOQ = 11.28 ng/mL.

Fig 5: Mass spectrum of cocaine collected with the mini-12 mass spectrometer.