Comprehensive Surveillance of Drugs of Abuse

A Method for Rapid and Accurate Urine Drug Testing in an Opioid Crisis

Chronic pain have fuelled an opioid crisis in North America. Due to the high risk of dependence and intoxication, there exists an urgent need for a rapid and low-cost analytical method to reliably screen for opioids and other drugs of abuse (DoA). This information is critical for the therapeutic monitoring of high-risk patients at methadone clinics and pain management centres. Here, we present a high-throughput method (<3 min/sample) for comprehensive surveillance of narcotic drugs and their metabolites above their recommended screening cut-off concentrations in urine when using multi-segment injection-capillary electrophoresis-mass spectrometry (MSI-CE-MS).

Background
The opioid crisis is an emerging public health emergency as reflected by the alarming increase in opioid-related deaths and addictions across North America. This is largely attributed to their widespread use as analgesics for the treatment of chronic pain [1]. This problem is further compounded by the concurrent use of by other medications prone to abuse and poisoning, which are increasingly used without a prescription (e.g., benzodiazepenes). The adulteration of illicit drugs has also contributed to a rise in accidental drug overdose fatalities due to access to highly potent yet inexpensive synthetic opioids on the illegal drug market, such as fentanyl [2]. In this context, methadone maintenance programs offer a harm reduction strategy to treat opioid addiction as methadone acts as a long-acting opioid agonist that inhibits pain while attenuating opioid withdrawal symptoms without generating euphoric-like sensations. However, optimum dose regimes are critical for successful treatment outcomes given large between-subject variability in methadone metabolism. For example, fast metabolizers who rapidly form the major inactive metabolite of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrlidine (EDDP), may not receive adequate treatment efficacy thereby increasing their chances for relapse [3]. As a result, frequent treatment monitoring of high-risk patients at methadone clinics or pain management centres is needed to confirm drug therapy adherence while simultaneously revealing potential use of other
narcotic drugs.

New methods that can additionally screen for other classes of drugs of abuse (DoA) and their metabolites in urine is also important to prevent adverse methadone-drug interactions in real-world patients due to recent drug exposures, habitual diet and/or lifestyle changes [4].

**Drug Surveillance by MSI-CE-MS**

Traditional methods used for workplace testing and therapeutic drug monitoring rely on an immunoassay screen for detection of major drug classes in urine followed by a confirmatory test using liquid chromatography-tandem mass spectrometry (LC-MS/MS) [5]. Major disadvantages of this two-tiered approach for urine drug testing include the poor specificity and sensitivity of immunoassays that can only provide presumptive results, whereas LC-MS/MS suffers from low sample throughput for a limited number of defined drug panels. Recently, we introduced multi-segment injection-capillary electrophoresis-mass spectrometry (MSI-CE-MS) as a single-step strategy that allows for rapid and non-targeted drug surveillance. This method is achieved via multiplexed electrophoretic separations together with full-scan data acquisition using high resolution mass spectrometry [6]. Additional improvements in sample throughput and resolution are achieved by programming serial hydrodynamic injections of samples between short segments of electro-kinetic separations of BGE spacers, which enables the identification and quantification of virtually an unlimited panel of drugs of abuse and their isomers in human urine [7]. Figure 1 highlights the analysis of thirteen independent urine samples performed within the same run (<30 min) by MSI-CE-MS, where a synthetic opioid (methadone) and its faster migrating yet inactive metabolite (EDDP) are resolved based on differences in their intrinsic electrophoretic mobilities in free solution [6,7]. In this case, cationic drugs migrate with steady-state mobilities when using an isocratic buffer system under strongly acidic conditions; similarly, stable ionization conditions are maintained throughout the separation when using a coaxial sheath liquid interface, unlike conventional gradient elution programs in LC-
MS/MS. Importantly, each run in MSI-CE-MS includes two quality control (QC) samples at the start (injection sample#1) and end (sample#13) of the injection sequence that comprise a standard drug panel mixture prepared in synthetic urine at two defined screening cut-off concentrations (e.g., 1.5× and 5×) as internal references. In addition, a blank sample (sample#6) is included together with a randomized set of ten patient urine samples to confirm the lack of sample carry-over between injections. All urine samples were enzyme hydrolyzed and subsequently diluted five-fold in deionized water after the addition of deuterated internal standards (d-IS), which are selected to avoid cross-interferences with other drugs within the panel [6]. Extracted ion electropherograms for the intact protonated molecular ions ([M+H]$^+$) corresponding to methadone (m/z 310.216) and EDDP (m/z 278.190) together with traces for their matching d-IS are also shown in Figure 1. In this case, one patient (A41, sample#11) among the ten urine samples analyzed was confirmed to be adhering to the methadone maintenance therapy. Confident assignment of this screen-positive urine drug test is achieved after satisfying four criteria in MSI-CE-MS, including co-migration of methadone with its matching d-IS, which helps reject potential isobaric interferences from the urine matrix when relying solely on accurate mass. Additional criteria include the reliable detection of methadone with low mass error (<10 ppm) with ion responses that exceed minimum screening cut-off concentrations (>50 ng/mL) after correction for urine dilution together with detection of one or more urinary metabolites in the same sample, such as EDDP. The detection of EDDP not only confirms patient drug adherence that overcomes sample tampering (e.g., methadone adulteration), but also provides an assessment of the metabolic status of individual patients if the dosage and time of administration of methadone are known. For instance, a high urinary EDDP-to-methadone ratio infers a strong metabolizer if methadone was recently administered, and such patients may require a higher dose regime to ensure therapeutic efficacy. Expanded screening for other classes of narcotics (e.g., oxycodone), illicit drugs (e.g., cocaine), as well as prescribed or self-medicated drugs prone to abuse (e.g., valium) can also be performed by MSI-CE-MS as required for patient safety and optimal treatment of opioid addiction.

**Future Work and Perspective**

MSI-CE-MS offers a cost-effective approach for the rapid screening of narcotic drugs and their metabolites in urine, which is useful for reliable therapeutic monitoring of patients physically dependent on prescription opioids and illicit opioids, such as heroin. In addition, medicinal cannabis offers an alternative analgesic for treatment of chronic neuropathic pain [8], which has shown promising efficacy for treatment of opioid use disorder as compared to methadone maintenance therapy. Current work is focused on rigorous validation of MSI-CE-MS for routine screening of large panels of drugs of abuse at incremental costs in cases
where timely yet accurate information on drug metabolism is critical for patient outcomes, including psychiatric medicine, emergency care and pain management.

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