Detection of 21 Opioids by LC-MS/MS from dried urine spots

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Introduction: Dried specimens offer a convenient and economical approach for collection, storage and transport of clinical specimens. Although, dried blood spots have been used as the specimen of choice in clinical laboratories (Biochemical Genetics) for years, dried urine spots (DUS) have not been established for routine use in clinical laboratories. Traditionally, drugs of abuse in clinical laboratories are detected by immunoassay (IA) and confirmed by GC-MS, which is time consuming and expensive. LC-MS/MS can offer a one-step detection and confirmation method for detection and measurement of drugs.

Objectives: To develop a LC-MS/MS method to detect multiple drugs in DUS in one-step.
Methods: 21 opioids (morphine, hydromorphone, oxymorphone, codeine, hydrocodone, oxycodone, heroin, 6- mono-acetyl morphine (6-MAM), fentanyl, norfentanyl, naloxone, tramadol, and meperidine, and 8 others added on) were analyzed in dried urine spot extraction. Briefly, 15 µL of de-identified urine samples containing the above drugs was spotted onto Whatman 903 Protein Saver Cards (GE Healthcare Biosciences). Drugs were extracted using a mixture of methanol, acetonitrile and water (4:4:1). Extracts, evaporated at RT, reconstituted in 5% acetonitrile before injecting into the LC-MS/MS. Urine samples were spiked by standards ranging from 100-2000 ng/mL. HPLC: Agilent 1290 (Palo Alto, CA) with a Restek Ultra Biphenyl column (100mm x 2.1 mm x 5µm). Mobile phase A was 1 mM ammonium formate and 0.1% formic acid in water; Mobile phase B was 0.1% formic acid in methanol. Separation was achieved using a gradient elution program starting at 99% A for 1 minute, decreasing to 95% A for one minute, and then decreasing to 50% A until 13.6 minutes, holding at 50% A until 15.6 minutes, and returning to 99% an over 0.1 minutes. Flow rate was 0.5 mL/min and the injection volume was 10 µL. Mass spectrometric detection was performed using an Agilent 6460 triple quadrupole mass spectrometer. Source parameters were optimized as follows: Electrospray voltage +2500V, Sheath gas temperature 380 °C, sheath gas flow 11 L/min, Nebulizer gas was 30 psi, Source gas temperature was 300 °C and the gas flow was 9 L/min. MRM transitions for all analytes were selected and optimized using the Agilent Optimizer software using 1000 ng/mL of each analyte in methanol. Data analysis was performed using Agilent MassHunter Quantitative analysis software

Results: Our preliminary recovery results from DUS by LC/MSMS were: Morphine, 97%; Oxymorphone, 97%, hydromorphone, 100%; naloxone, 100%; codeine, 100%; 6-MAM, 99%; hydrocodone, 99%; oxycodone, 100%; heroin, 98%; fentanyl, 97%; norfentanyl, 97%; tramadol, 100%; and meperidine, 97%. These results matched those generated from identical liquid urine same by traditional immunoassay followed by GC/MS. 8 other opioids are currently being run in tandem with the aforementioned 13, results will follow.

Conclusions: We modified a LC-MS/MS method to detect 13 opioids from DUS, simultaneously. DUS offers a convenient and economical approach for specimen collection, transportation and storage. This one-step LC-MS/MS detection confirmation method eliminates the need for the traditional multi-step approach of immunoassay and GC-MS and can greatly reduce the operational cost. More drugs of abuse are being tested.