Testing and Quantification of a Representative Panel of Illicit Drugs in Urine and Serum Using LC-Time-of-Flight Mass Spectrometry

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1 Introduction

The panel of drug compounds used in this work are examples of commonly studied molecules in this compound class. The full list of compounds is displayed in **Table 1**. Immunoassays have traditionally been used, for specimen testing but are well known for potential false negatives. Additionally, immunoassays are not always sensitive enough to detect low levels of drugs in challenging matrices, and can often not identify specific drugs within drug classes, due to their lack of specificity. GC/MS, which is also used extensively for compounds in these classes, offers its own challenges Many compounds are polar and often thermally labile, requiring derivatization prior to enhance volatility. Thermally labile compounds may be misidentified due to common EI fragments with other compounds. Unlike GC/MS, LC/MS typically does not require derivatization of samples, and is ideally suited for the rapid analysis of these compounds.

In 2011, guidelines were altered to allow the use of LC/MS instruments for urine quantitative confirmatory analysis. Among the LC techniques, LC/MS/MS is often used to quantitate compounds in a variety of matrices, due to its sensitivity and selectivity. However, triple quadrupole techniques can have a high cost and lack the ability to easily identify new or unknown compounds. We present an alternative workflow to test and quantify such compounds in a research setting, utilizing a rapid LC method with time-of-flight mass spectrometry (TOF).

Table 1: List of analytes.

Compound
Amphetamine (AMP)
Methamphetamine (MAMP)
3,4-Methylenedioxyamphetamine (MDA)
3,4-Methylenedioxymethamphetamine (MDMA)
3,4 Methylenedioxy-N-ethylamphetamine (MDEA)
Phencyclidine (PCP)
Codeine (COD)
Morphine (MOR)
6-Acetylmorphine (6-AM)
Benzoylecgonine (BZE)
Tetrahydrocannabinol carboxylic acid (THC-COOH)

2 Experimental

All standards were purchased from Cerilliant (Round Rock, TX).

Urine Sample Preparation: Urine was diluted 1:1 with water and directly injected, no sample extraction was required.
Urine Calibration Curves: Urine blanks were spiked with the 11 drugs in **Table 1**, 300 ng/ml of deuterated internal standard (IS), and then diluted 1:1 with water. The deuterated MDMA IS was used as an IS for both MDA and MDMA. Each calibration level was injected five times.

FX-15 UHPLC-AxION 2 TOF Conditions for Urine Analysis

Mobile Phase A: Water with 10 mM ammonium formate
Mobile Phase B: 95:5 ACN:water with 0.05 % formic acid
Gradient: 97.5% A for 2.5 min, linear gradient to 25% A in 4.5 min, linear gradient to 5% A in 3 min, and a 1.5 min hold at 5% A
Flow: 0.25 mL/min, Injection volume: 10 µL, full loop
Column: PerkinElmer Brownlee™ SPP C18 (2.1x50 mm, 2.7 µm), SPP C18 guard column (2.1 mm x 5 mm, 2.7 µm)
Column Temperature: 30 °C
Diverter Valve: LC flow diverted to waste for first 1.8 min
Ionization Source: PerkinElmer Ultraspray™ 2 (Dual ESI)
Ionization Mode: Positive
Lock Mass Ions: 118.0863 and 322.0481

Serum Sample Preparation: 0.3 mL of serum was protein precipitated with 0.6 mL of acetonitrile. The sample was vortexed for 10 s and centrifuged at 10000 rpm for 15 min. 0.40 mL of supernatant was removed and dried with a rotary vacuum dryer for 1 hour at room temperature. The dried sample was reconstituted in 0.8 mL of water.
Serum Calibration Curve: A 0.3mL serum aliquot was spiked with different levels of the 11 compounds in **Table 1** and 500 ng/mL deuterated IS. The deuterated MDMA IS was used as an IS for both MDA and MDMA. Each calibration level was injected five times.

FX-15 UHPLC-AxION 2 TOF Conditions for Serum Analysis:

Mobile Phase A and B: Same as urine analysis
Gradient: 96% A for 1 min, linear gradient to 0% A in 4.5 min and 2 min hold at 0% A
Flow: 0.25 mL/min, Injection volume: 20 µL, partial loop
Column and Temperature: Same as urine analysis
Diverter Valve: LC flow diverted to waste for first minute
Ionization Source and Mode: Same as urine analysis
Lock Mass Ions: Same as urine analysis

3 Results

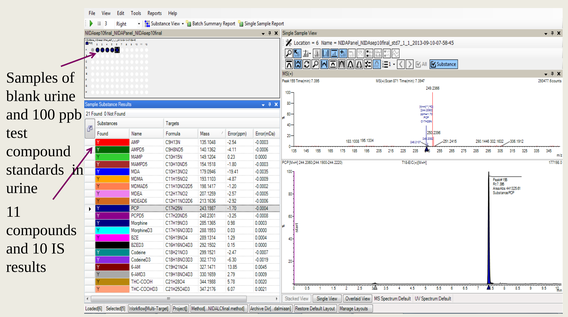


Figure 1: Fast visual analysis of the presence or absence of the compounds in urine using AxION Solo software.

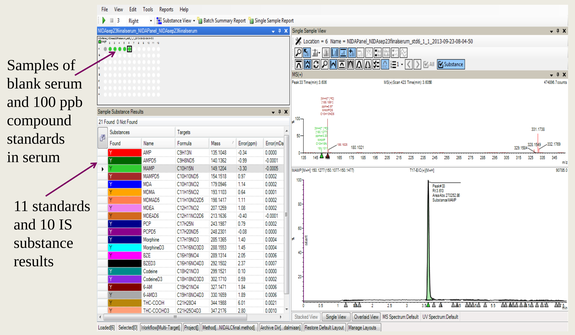


Figure 2: Fast testing of compounds in serum using AxION Solo software.

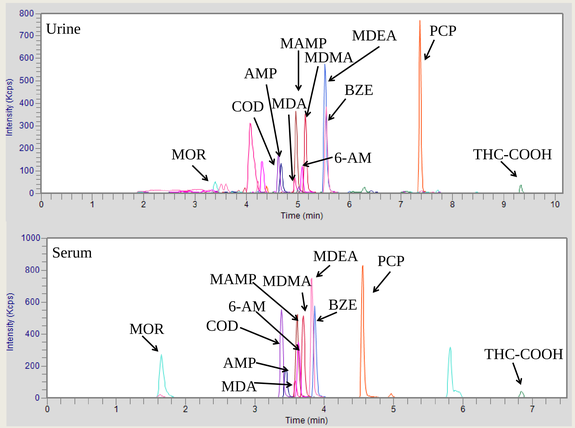


Figure 3: EICs of 300 ng/mL standards in urine (top) and serum (bottom).

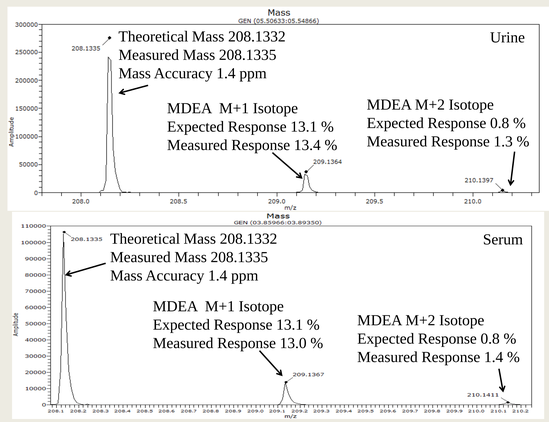


Figure 4: Mass accuracy and isotopic distributions observed with the AxION 2 TOF for MDEA in urine (top) and serum (bottom).

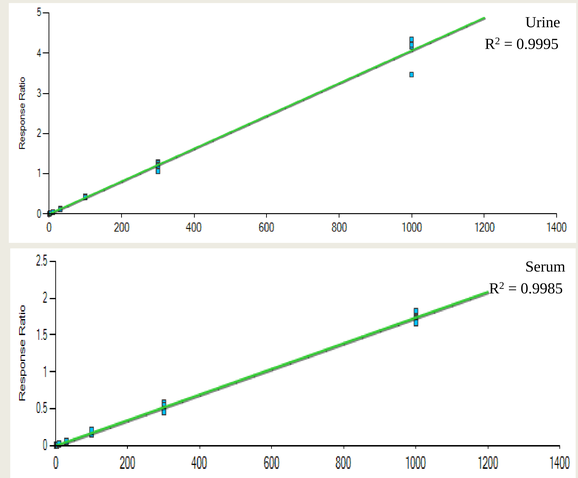


Figure 5: MDEA linear calibration curve in urine (top) and serum (bottom). In urine and serum the concentration of the MDEA standards used in the curve ranged from 1-1000 ng/mL. The R^2 value was 0.9995 and 0.9985 for urine and serum, respectively. These curves represent good linearity over 3 orders of magnitude.

Table 2: List of the limits of quantitation (LOQs) for each analyte in urine and serum.

Compound	LOQ in Urine	LOQ in Serum
AMP	10 ng/mL	10 ng/mL
MAMP	3 ng/mL	3 ng/mL
MDA	10 ng/mL	10 ng/mL
MDMA	3 ng/mL	1 ng/mL
MDEA	1 ng/mL	1 ng/mL
PCP	1 ng/mL	1 ng/mL
BZE	3 ng/mL	10 ng/mL
6-AM	10 ng/mL	1 ng/mL
MOR	10 ng/mL	1 ng/mL
COD	3 ng/mL	1 ng/mL
THC-COOH	10 ng/mL	10 ng/mL

4 Conclusion

The detection limits of the compound panel analyzed by the TOF were approximately 1.5-2000 times lower than those required by regulatory guidelines for these types of compound, and quite adequate for research purposes. In addition to the adequate quantitative dynamic range of the AxION® 2 TOF, which rivals capabilities of the triple quadrupole instruments, TOF collects ‘all the ions, all the time’, and provides full spectrum information which allows for screening of non-target compounds. TOF-MS can be used to discover ‘unknowns’ post acquisition with no extra method development or cost, and with no reanalysis of the sample.

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