Title: Urine Drug Surveillance in Philadelphia, PA, with Emphasis on Xylazine and its Metabolites

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ABSTRACT:

Introduction:

Over the past decade, the heroin supply in the United States has diminished as the fentanyl supply has greatly increased along with the presence of various adulterating agents, including most notably xylazine. Xylazine is a veterinary sedative not intended for human use; however, currently more than 90% of recreational opioid samples (primarily fentanyl) collected and tested from Philadelphia, PA, also contain xylazine. The Center for Forensic Science Research and Education recently partnered with the Philadelphia Department of Public Health to pilot expanded analysis of urine samples collected in the Police Assisted Diversion (PAD) program. This program engages individuals who encounter law enforcement for non-violent low-level offenses to provide referrals to behavioral health services, social services, public benefits, and medical services. PAD clients who received an assessment for behavioral health services provided urine samples, which were analyzed during this study.

Objectives:

This study involved three main objectives: 1) qualitative analysis of urine samples by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) to determine drug prevalence, 2) comparison of the test results to BTNX™ Rapid Response Xylazine Test Strips (XTS) to determine effectiveness, and 3) characterization of xylazine and its known metabolites, 4-hydroxy xylazine and 2,6-xylidine, to determine the appropriate urine biomarker.

Methods:

From May through October 2022, 412 urine samples were collected and sequestered for testing. Samples were prepared for LC-QTOF-MS analysis using two extraction procedures – a basic liquid-liquid extraction and an acidic liquid-liquid extraction. Instrumental analyses were completed using two LC-QTOF-MS platforms: a SCIEX TripleTOF™ 5600+ and a SCIEX X500R. Datafiles were processed against an internal library database of more than 1,000 analytes. Standard reference materials for xylazine and its metabolites were acquired for addition to that database.

Testing with the BTNX Rapid Response™ XTS (manufacturer listed cutoff: 1,000 ng/mL) was conducted following the manufacturer's guidelines. A positive control (2,000 ng/mL) and negative control (drug-free urine) were prepared alongside the authentic urine samples. Results were tabulated and compared to prior qualitative LC-QTOF-MS results for further data analysis.

Results:

Based on the LC-QTOF-MS results, approximately 90% of all urine samples screened positive for fentanyl, with fluorofentanyl (65%) being the primary fentanyl analog detected. Methamphetamine, cocaine, and benzoylecgonine were present in approximately 70% of samples. Approximately 25%

screened positive for carboxy-THC. Novel psychoactive substances (NPS) were not frequently detected, but 8-aminoclonazolam (4%) was observed. The LC-QTOF-MS results showed 88% of the urine samples screened positive for xylazine, and of those positive samples, 53% also contained 4-hydroxy xylazine. Only 13% of the positive samples also contained 2,6-xylidine, while the remaining 46% of positive samples did not contain either metabolite. All samples contained parent xylazine.

Comparison of XTS urinalysis results with LC-QTOF-MS results showed acceptable performance. The sensitivity was calculated to be 87%, the specificity was 80%, and the accuracy was 86%. Of note, the qualitative LC-QTOF-MS results were categorized as positive/negative and were not evaluated quantitatively against the cutoff; therefore, a higher number of false negatives (n=48) may be reported due to increased sensitivity of the LC-QTOF-MS assay vs. the XTS. Additionally, the sample population was highly saturated with xylazine positivity leading to a higher proportion of true positives (n=314) compared to true negatives (n=40).

Discussion/Conclusion:

Our urine surveillance showed that Philadelphia, PA, is experiencing high positivity for xylazine, with fentanyl being the primary drug in urine samples from this population. Methamphetamine and cocaine were also commonly detected. Metabolite characterization showed that 4-hydroxy xylazine was the primary metabolite; however, xylazine remains the appropriate biomarker for toxicology testing. Overall, the performance of the XTS was adequate for detecting xylazine in the urine samples analyzed when compared to comprehensive LC-QTOF-MS drug screening.