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

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Overview

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 98% of dope (recreational opioid powder, primarily fentanyl) collected and tested from Philadelphia, PA, by the CFSRE, 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

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

Xylazine Metabolism

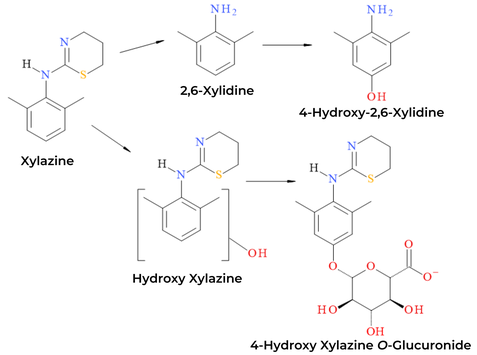

Figure 1: Phase I and II metabolism of xylazine to most common metabolites.

Xylazine is metabolized (Figure 1) via *N*-dealkylation, oxidation, and/or hydroxylation to phase I metabolites, followed by glucuronidation, acetylation, or sulfation to phase II metabolites. 2,6-Xylidine is also a metabolite of lidocaine and thus, is not selective for xylazine.

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 1,100 drug targets. Standard reference materials for xylazine and its available metabolites were acquired for addition to our 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.

LC-QTOF-MS Qualitative Results

Approximately 90% of all urine samples screened positive for fentanyl, with fluorofentanyl (65%) being the primary fentanyl analogue 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 (Figure 2).

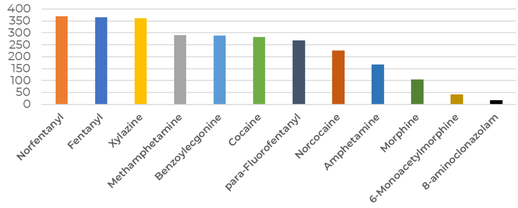

Figure 2: Prevalence of most common drugs detected.

The LC-QTOF-MS results (Figure 3) showed that **88%** of the urine samples screened positive for **xylazine**. Of those positive samples, 11% contained both metabolites with parent xylazine present. Only 41% contained hydroxy xylazine and 2% contained 2,6-xylidine. The remaining 46% of positive samples did not contain either metabolite. **No metabolites were present without xylazine.**

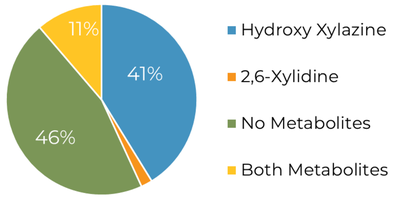

Figure 3: Prevalence of xylazine metabolites with parent in QTOF qualitative results.

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Xylazine Test Strip Qualitative Results

Table 1: Xylazine test strip positive cutoff evaluation.
(Lot: DOAA2306042, Exp.: 2025-06-05)

Xylazine Conc.	1	2	3
10,000 ng/mL	+	+	+
5,000 ng/mL	+	+	+
2,000 ng/mL	+	+	+
1,000 ng/mL	+	+	+
500 ng/mL	-	-	-
100 ng/mL	-	-	-
0 ng/mL	-	-	-

Table 2: Evaluation of xylazine test strips.
(Lot: DOA2211187, Exp.: 2024-11-24, Lot: XYL2303001-S, Exp.: 2025-03-06)

Characteristic	Count
False Negative (FN)	48
False Positive (FP)	10
True Negative (TN)	40
True Positive (TP)	314
Total	**412**
ROC	**Percent (%)**
Sensitivity	87%
Specificity	80%
Accuracy	86%

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 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 the comprehensive LC-QTOF-MS drug screening.