Development of LC-MS/MS Xylazine Assay for Investigation of Xylazine Prevalence in New Haven, CT Region

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BACKGROUND

Xylazine is a veterinary tranquilizer that has been implicated in an increasing number of overdose deaths nationwide due to its use as an adulterant in recreational drugs. It is an α2-agonist not responsive to naloxone that was involved in 10% of all drug overdose deaths in Connecticut in 2020. Currently, there is no available FDA-approved immunoassay for xylazine detection. We aim to develop an LC-MS/MS assay that detects xylazine and determine the xylazine prevalence in patients who tested positive for cocaine (COC), opiates (OPI) and/or fentanyl (FEN) by urine drug screen in the New Haven, CT region. We also examine the value of measuring the metabolite 2,6-dimethylaniline.

METHODS

- We performed a retrospective review of 131 randomly chosen urine specimens that screened positive by immunoassay for COC, OPI or FEN at Yale New Haven Hospital between September 2023 and February 2024
- Internal standard: Norfentanyl-d5
- Mobile phase A: 0.1 % formic acid in water
- Mobile phase B: 0.1 % formic acid in acetonitrile
- Strong Needle = 0.1% Formic Acid in 80/20 Acetonitrile/CLRW
- Weak Needle = 65/35 Methanol/CLRW
- Linearity: 1 – 1000 ng/mL
- IRB approval received

Gradient				
Column: Acquity UPLC HSS T31.8 µm, 2.1 x 100 mm				
Column temperature: 50ºC				
Step	Time (min)	Flow (mL/min)	% A	% B
1	Initial	0.4	90	10
2	1.00	0.4	90	10
3	2.00	0.4	45	55
4	3.00	0.4	45	55
5	3.10	0.4	90	10
6	4.00	0.4	90	10

Transitions		
Compound Name	Parent > Quantifier	Parent > Qualifier
Xylazine	221.19 > 89.9	221.19 > 164.02
Norfentanyl-d5	238.12 > 55.74	N/A

Table 1: LC-MS/MS Method Gradient and Transitions

RESULTS

- Xylazine was detected in a total of **37 out of 131** patient samples (**28%)**
- Patient characteristics of the xylazine positive samples are similar to that of the overall population that screen positive for cocaine, opiates and/or fentanyl

Patient Characteristics		
	Xylazine Positive Samples (n = 37)	All Samples (n = 131)
Male	21 (58%)	77 (60%)
Female	15 (42%)	52 (40%)
Age		
Median	44	43
Minimum	16	16
Maximum	61	76
Patient Status		
Inpatient	22 (60%)	64 (49%)
ED	11 (30%)	52 (40%)
Outpatient	4 (11%)	15 (11%)

Table 2: Patient characteristics of xylazine positive samples compared to all samples in cohort

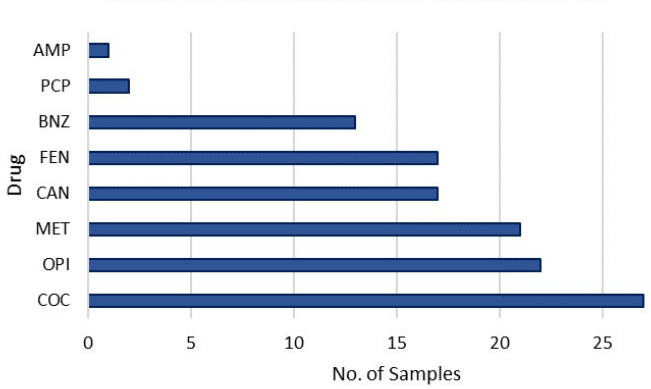

Figure 1:Number of samples with each drug present in cohort of xylazine positive samples. AMP: Amphetamine, BNZ: Benzodiazepine, CAN: Cannabinoid, COC: Cocaine, FEN: Fentanyl, MET: Methadone, OPI: Opiate, PCP: Phencyclidine

- Measurement of 2,6-dimethylaniline does not add value
- Xylazine shows 13 – 24% prevalence in samples positive for just cocaine, opiates or fentanyl and 40 – 50% prevalence in samples with > 1 co-occurring substance

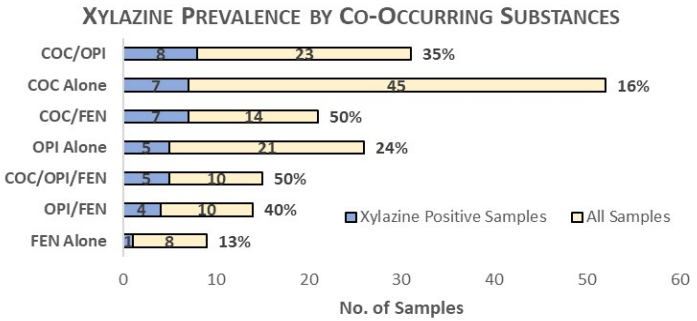

Figure 2: Total number of samples that screened positive for COC, OPI, FEN or any combination of those and the number that tested positive for xylazine in each category

CONCLUSIONS

- Our data indicates there is a xylazine prevalence of approximately 28% among patients who screen positive for cocaine, opiates or fentanyl in the New Haven, CT region. This is consistent with previous literature.
- There is a greater prevalence of xylazine in samples with multiple co-occurring illicit substances

CHALLENGES

- Xylazine-d6 is not available in the US.
- Presence of high concentrations of commonly co-ingested drugs causes ion suppression
- Ion suppression may not be at same level as target drug, making quantitation difficult
- The following other internal standards were assessed in regards to level of ion suppression compared to xylazine: Methadone-d9, EDDP-D3, Ketamine-D4, PCP-D5, Lidocaine-D10
- Proposed solutions: find IS that suppresses at same level OR separate out other substances chromatographically