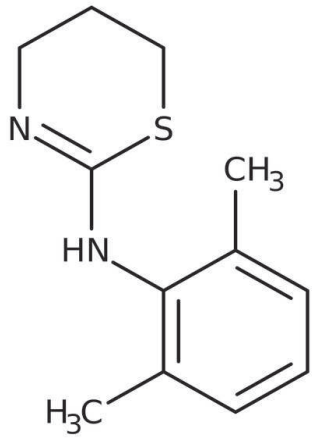
Analysis of Xylazine, Opioids, and Other Common Adulterants in Blood and Urine by SPE and LC-MS/MS



UCT Part Numbers

CSDAU206
Clean Screen® DAU
200 mg, 6 mL

SCS27-DA1021
SelectraCore® DA Column
100 x 2.1 mm, 2.7 µm

SCS27-DAGDC21
SelectraCore®
DA Guard Column
5 x 2.1 mm, 2.7 µm

SLGRDHLDR-HPOPT
Selectra®
Direct Connect
Guard Holder

SPHPHO6001-10
Select pH Buffer Pouch
100 mM Phosphate Buffer
pH 6.0

Introduction:

Xylazine is a veterinary sedative that has been emerging as a popular adulterant in the illicit drug market. It is most commonly seen with powders and tablets containing fentanyl.[1,2] Drug powders or tablets can easily be "cut" with other substances. Some of these substances have pharmacological activity of their own, adding to the effects intended by the main drug. Substances added to drug samples that are pharmacologically active are considered to be adulterants. It is becoming increasingly popular for fentanyl samples to be adulterated with xylazine. This drug combination is commonly called "Tranq".[1]

Although xylazine is not an opioid, its use with fentanyl is having a significant impact on the opioid epidemic for a number of reasons. It can induce a state of unconsciousness, worsen addiction and potentially increase the risk of fatal overdose. Unfortunately, its effects are not counteracted by naloxone.[1,2] Of all of the fentanyl-positive samples tested by the DEA, 23% of powder samples and 7% of tablet samples also contained xylazine. The trends of xylazine usage parallel to fentanyl, indicating that it is likely to persist.[3] This application note details a robust and effective method for the simultaneous analysis of fentanyl, fentanyl analogs, xylazine, and other common adulterants by SPE and LC-MS/MS.

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Sample Pretreatment:

Urine: In a test tube add 1 mL urine sample + 500 µL MeOH (optional) + 2.5 mL of 100 mM phosphate buffer pH 6.0 + ISTDs

Note: Include a hydrolysis procedure to recover conjugated analytes

Blood: In a test tube add 0.5 mL whole blood sample + 3 mL of 100 mM phosphate buffer pH 6.0 + ISTDs

SPE Procedure:

1. Condition Column

a) 1 x 3 mL MeOH
b) 1 x 3 mL DI H_2O
c) 1 x 3 mL 100 mM phosphate buffer pH 6

2. Load Sample

a) Load at 1 to 2 mL/minute

3. Wash Column

a) 1 x 3 mL 100 mM HCl in DI H_2O
b) 1 x 3 mL MeOH

4. Dry Column

a) Dry for at least 10 minutes under full pressure or vacuum

5. Elute

a) 1 x 3 mL MeOH:NH_4OH (98:2) or DCM:IPA:NH_4OH (78:20:2)

Note: Make elution solvent fresh daily

6. Evaporate

a) Evaporate eluate at 40°C, starting at 5 psi and increasing the pressure slowly over 30 minutes

7. Reconstitute

a) 1 mL MeOH:H_2O (5:95) or other appropriate solvent and volume

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LC-MS/MS Parameters	
LC-MS/MS	Shimadzu Nexera LC-30AD with MS-8050
UHPLC Column	SelectraCore® DA Column 100 x 2.1 mm, 2.7 µm (PN: **SCS27-DA1021**)
Guard Column	SelectraCore® DA Guard Column 5 x 2.1 mm, 2.7 µm (PN: **SCS27-DAGDC21**)
Column Temperature	40°C
Flow Rate	0.4 mL/min
Injection Volume	5 µL
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	Methanol

Gradient Program		
Time (min)	**Mobile Phase A (%)**	**Mobile Phase B (%)**
0	95	5
3-7	55	45
8-9	0	100
9-12	95	5

MRM Table						
Analyte	**RT (min)**	**Parent Ion (m/z)**	**Product Ion 1 (m/z)**	**CE (V)**	**Product Ion 2 (m/z)**	**CE (V)**
Morphine	2.17	285.6	165.1	41	152.1	55
Procaine	2.88	236.6	120.0	25	100.2	16
6-MAM	3.12	327.9	165.2	40	211.1	28
Lidocaine	3.13	234.7	86.2	15	58.1	34
Norfentanyl	3.75	233.0	84.1	19	55.1	35
Xylazine	3.94	220.9	89.9	23	164.0	27
4-ANPP	5.02	280.8	188.1	18	105.0	32
para-Fluorofentanyl	5.71	355.0	188.2	25	105.1	40
meta-Fluorofentanyl	5.90	355.0	188.2	24	105.1	39
Fentanyl	6.02	337.3	188.2	20	105.1	37
ortho-Fluorofentanyl	6.31	355.0	188.2	25	105.1	39
Quetiapine	6.75	384.0	253.1	24	221.1	39
Acepromazine	8.35	326.8	86.1	21	58.1	40

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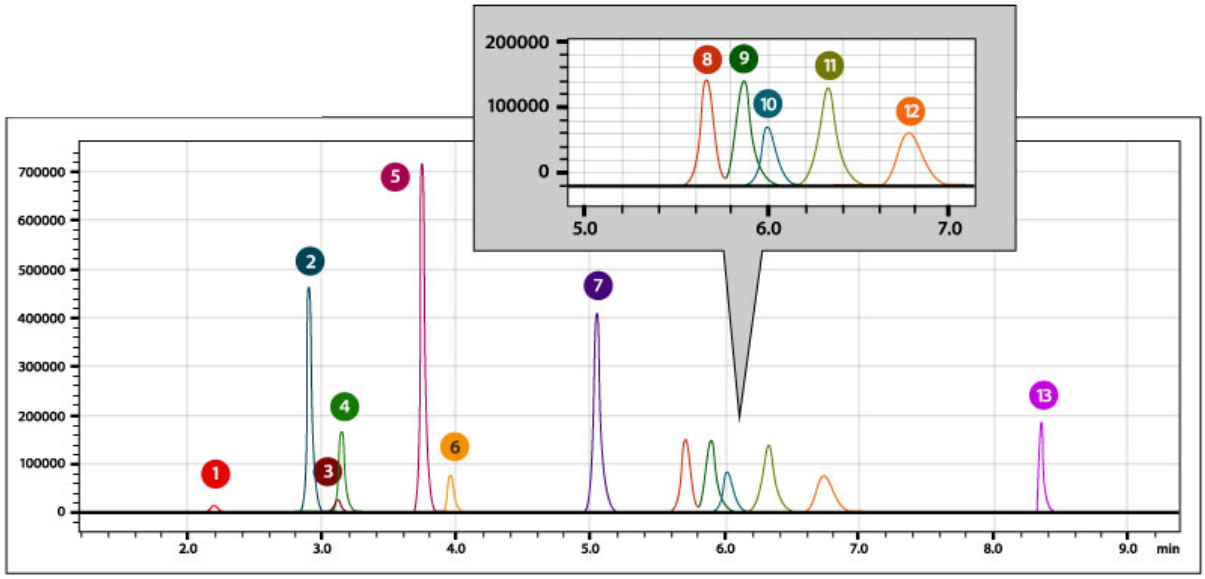
Chromatogram:



1 Morphine	5 Norfentanyl	9 meta-Fluorofentanyl	13 Acepromazine
2 Procaine	6 Xylazine	10 Fentanyl	
3 6-MAM	7 4-ANPP	11 ortho-Fluorofentanyl	
4 Lidocaine	8 para-Fluorofentanyl	12 Quetiapine	

Figure 1: Chromatogram of a solvent standard mix prepared at 25 ng/mL.

Calibration Curve Examples:

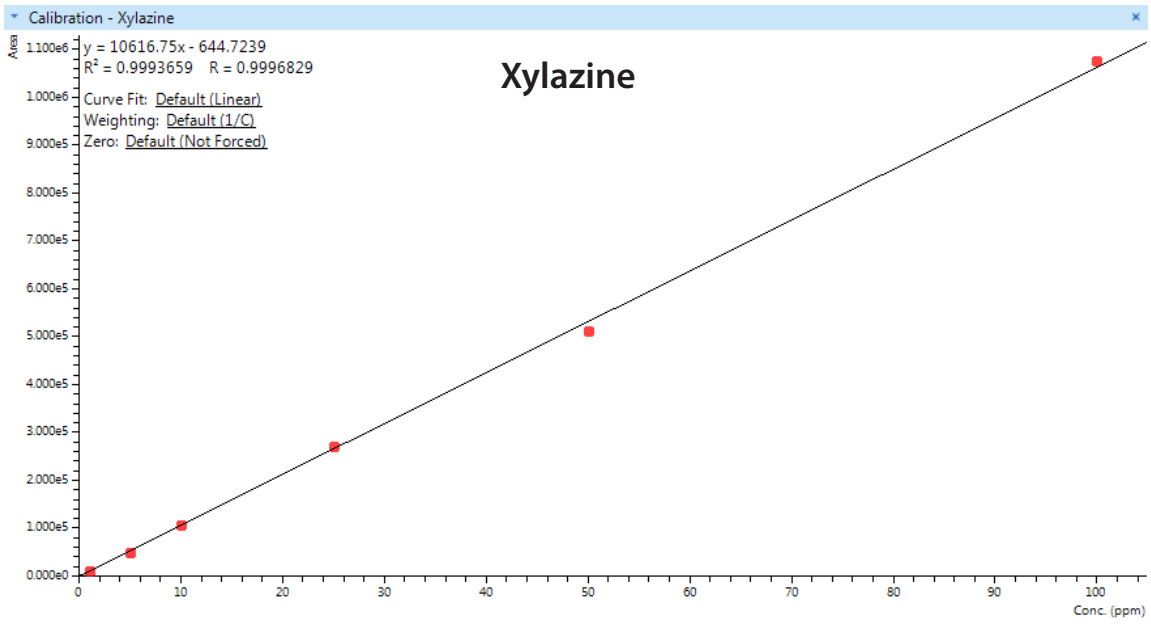


Figure 2a: Example of a 6-point solvent calibration curve for Xylazine with linear equation and R^2 value (1, 5, 10, 25, 50 and 100 ng/mL)

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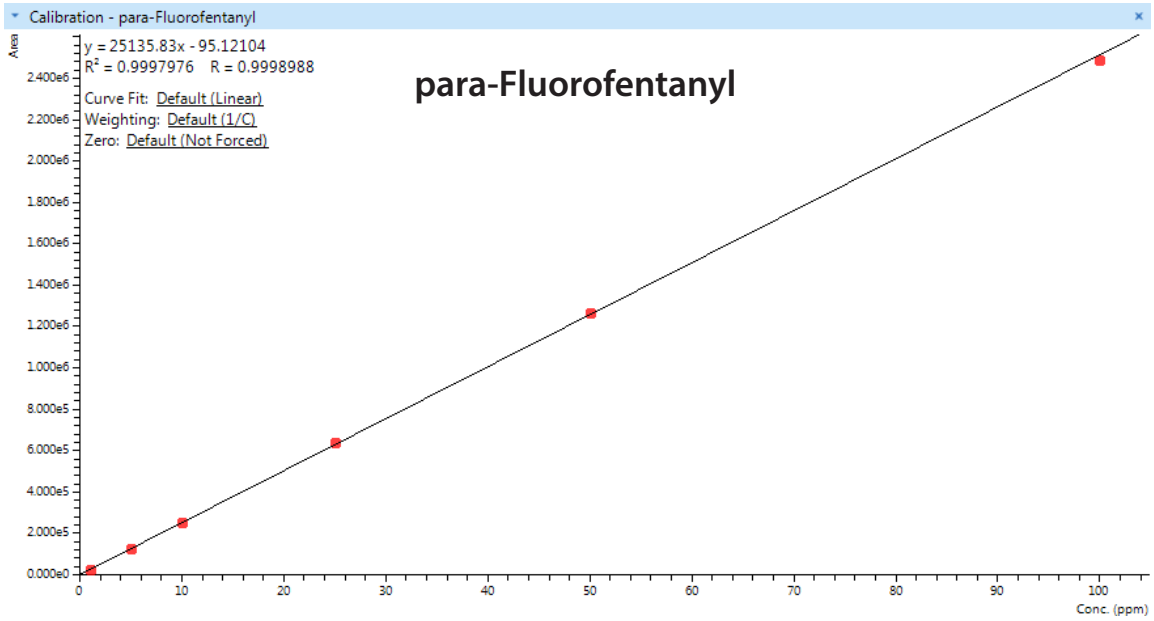

Figure 2b: Example of a 6-point solvent calibration curve for para-Fluorofentanyl with linear equation and R^2 value (1, 5, 10, 25, 50 and 100 ng/mL)

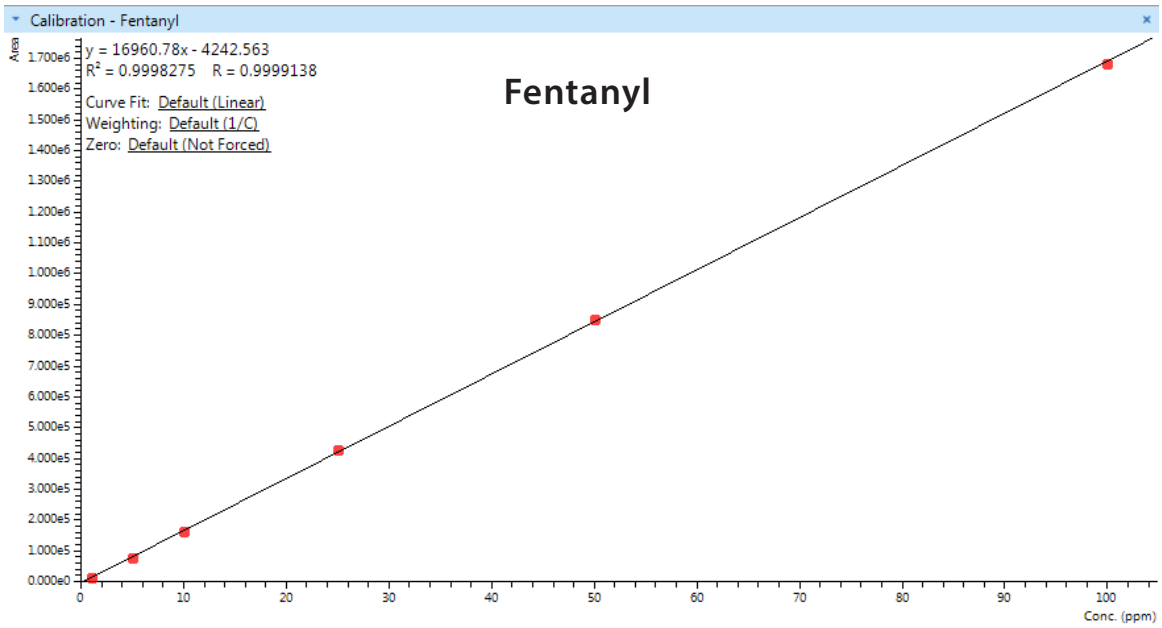

Figure 2c: Example of a 6-point solvent calibration curve for Fentanyl with linear equation and R^2 value (1, 5, 10, 25, 50 and 100 ng/mL)

Results:

Urine									
n=3	5 ng/mL			25 ng/mL			80 ng/mL		
Analyte	Recovery	Matrix Effects	RSD	Recovery	Matrix Effects	RSD	Recovery	Matrix Effects	RSD
Morphine	119%	-15%	8%	90%	-24%	13%	100%	-25%	2%
Procaine	107%	1%	9%	96%	0%	8%	88%	-4%	4%
6-MAM	96%	-20%	17%	96%	-22%	2%	92%	12%	6%
Lidocaine	116%	12%	10%	96%	17%	5%	93%	-9%	5%
Norfentanyl	94%	-23%	19%	90%	-6%	2%	91%	-25%	10%
Xylazine	91%	-17%	2%	109%	-13%	5%	96%	-25%	13%
4-ANPP	89%	-24%	18%	105%	-17%	5%	91%	-5%	17%
Para-Fluorofentanyl	117%	-12%	16%	102%	-17%	7%	91%	-21%	20%
Fentanyl	114%	-5%	14%	104%	-12%	6%	91%	-21%	18%
Quetiapine	98%	-18%	4%	104%	11%	4%	95%	-25%	13%
Acepromazine	114%	-28%	2%	114%	-42%	4%	102%	-44%	12%

**Recoveries were calculated by comparing pre vs. post spiked samples. Matrix effects were calculated by comparing post spiked samples to solvent calibrators.*

Blood									
n=3	5 ng/mL			25 ng/mL			80 ng/mL		
Analyte	Recovery	Matrix Effects	RSD	Recovery	Matrix Effects	RSD	Recovery	Matrix Effects	RSD
Morphine	113%	-5%	20%	94%	14%	1%	93%	5%	2%
Procaine	103%	-8%	19%	96%	-16%	2%	104%	-6%	5%
6-MAM	110%	-6%	1%	86%	-18%	8%	86%	6%	2%
Lidocaine	113%	-10%	20%	94%	-4%	2%	93%	-5%	3%
Norfentanyl	101%	-8%	18%	85%	-7%	6%	94%	-22%	8%
Xylazine	101%	-9%	18%	83%	-6%	12%	97%	-21%	11%
4-ANPP	92%	-25%	16%	84%	7%	7%	92%	-25%	6%
Para-Fluorofentanyl	92%	-20%	19%	87%	-16%	8%	91%	-24%	7%
Fentanyl	102%	-23%	20%	84%	-20%	9%	89%	-22%	7%
Quetiapine	109%	-7%	18%	86%	-9%	6%	93%	-11%	5%
Acepromazine	94%	-40%	5%	71%	-48%	12%	91%	-51%	15%

**Recoveries were calculated by comparing pre vs. post spiked samples. Matrix effects were calculated by comparing post spiked samples to solvent calibrators.*

Conclusion/Discussion:

A full SPE and LC-MS/MS method was developed and optimized to achieve the highest recoveries of the analytes with the lowest matrix effects. Like most drugs of abuse, xylazine is slightly basic and ionizable at a pH less than 7, making this veterinary drug easy to integrate into an existing fentanyl or opioid panel that is potentially already in use at drug testing laboratories. It also makes this panel of drugs an excellent candidate for UCT's flagship mixed-mode Clean Screen® DAU, which combines cation exchange and reverse phase functionalities.

This method yields high recoveries ranging from 80 to 119% at low, medium, and high concentrations in both blood and urine. Aside from acepromazine, which was not the focus of this panel, relative standard deviations (RSDs) were ≤20% and matrix effects were within ±25%. The LC-MS/MS method features the separation of the three fluorofentanyl isomers, para-, meta-, and ortho-fluorofentanyl, to ensure that misidentifications will not be made. This application note provides a procedure that can be readily implemented by drug testing laboratories to simultaneously monitor fentanyl, fentanyl analogs, xylazine, and other common adulterants.

References:

[1] Congressional Research Service (2023, February 2) Xylazine: Considerations for Federal Control. CRS Insight. https://www.everycrsreport.com/

[2] Holt, Andrew C, et. al. (2023, January 6) Widespread Distribution of Xylazine Detected Throughout the United States in Healthcare Patient Samples. Journal of Addiction Medicine. https://journals.lww.com/journaladdictionmedicine.

[3] DEA Joint Intelligence Report. (2022 October) The Growing Threat of Xylazine and its Mixture with Illicit Drugs. US DOJ DEA. https://www.dea.gov/.

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