The Development and Validation of a Quantitative Method for the Analysis of Fentanyl Containing "Dope" Samples

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DISCLOSURE & ACKNOWLEDGEMENTS

- I am a paid employee of FRFF / CFSRE, a 501(c)(3) non-profit research and educational facility.
- I have no conflicts of interest in this presentation
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- The opinions, findings, conclusions, and/or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of NIJ, the CDC or other federal, state, local, or private agencies.

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- Husband and dad
- Senior Scientist at CFSRE, outside of Philadelphia, PA
- PhD – Chemistry; FIU Miami, FL
- Background in analytical chemistry
- Research interests in method development, data analysis and process improvement
- NPS Discovery
  - HRMS and HRMS-MS development
  - Drug Checking/Surveillance

OUTLINE

- Method development
  - Background on project
  - Procedures
- Method Validation
  - Design
  - Results
  - Summary
  - Limitations
- Results of Sample Analysis
- Conclusions


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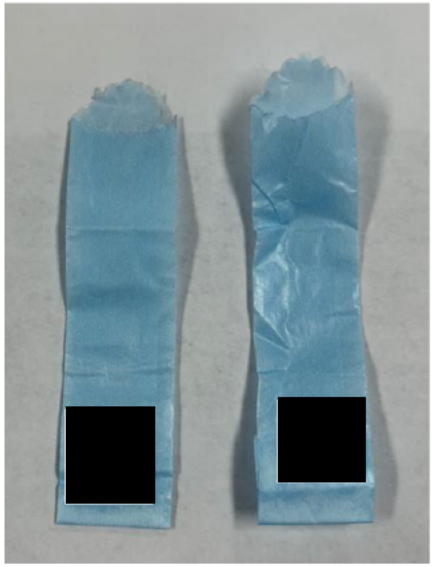
DISCOVERY
Method Development

PROJECT BACKGROUND

- CFSRE performs drug analysis for PDPH for surveillance of existing and emerging public health threats in the drug supply.
- Previously an estimation of relative abundance was performed using relative response ratios
  - Useful, but not ideal
  - Previously no attempt was made to correct for sampling variability
- Without at least one common quantitative measurement, comparing potency is not possible
- Quant panel was designed from our experience with the analysis of Philly's drug samples (ongoing since 2020).



METHOD DEVELOPMENT

- Work with Drug checking screening workflow:
  - Screen by GC-MS
  - Confirm by LC-QToF-MS
- Purpose: to quantify fentanyl in a variety of drug types
- Method specifications:
  - Target compounds:
    - Methamphetamine, Lidocaine, Levamisole, Xylazine, Cocaine, 4-ANPP, para-Fluorofentanyl, & Fentanyl
    - w/ Internal standard
  - Target quantitative range 100% - 1%, lower if possible
  - Minimal sample preparation
  - Must use current GC-MS hardware and chromatographic parameters in use for screen

SAMPLE PREPARATION PROCEDURES

- Weigh approximately 3 mg of sample to a test tube
- Suspend sample in 2mL methanol, vortex thoroughly
- Dilute as needed with methanol and transfer a final volume of 500 µL
- Add 200 µL internal standard (20 µg/mL N-propyl-amphetamine)
- Extract with 0.75 mL of 0.1 N NaOH and 0.5 mL $CHCl_3$.
- Basic compounds extracted to organic phase (bottom layer)

INTEGRATION WITH CURRENT INSTRUMENTAL METHODS

- The following parameters were used, but were not optimized for this method:
- Instrument: Agilent 6890 GC and Agilent 5975 MS
- Column: 12m Agilent DB-1, 0.2mm diameter, 0.33µm film
- MS parameters: Full scan, 40-550 amu; 0.8 min solvent delay
- GC parameters: 50°C - 340°C, at 30°C/min, held for 2.33 min
- Inlet parameters: 1 µL, splitless

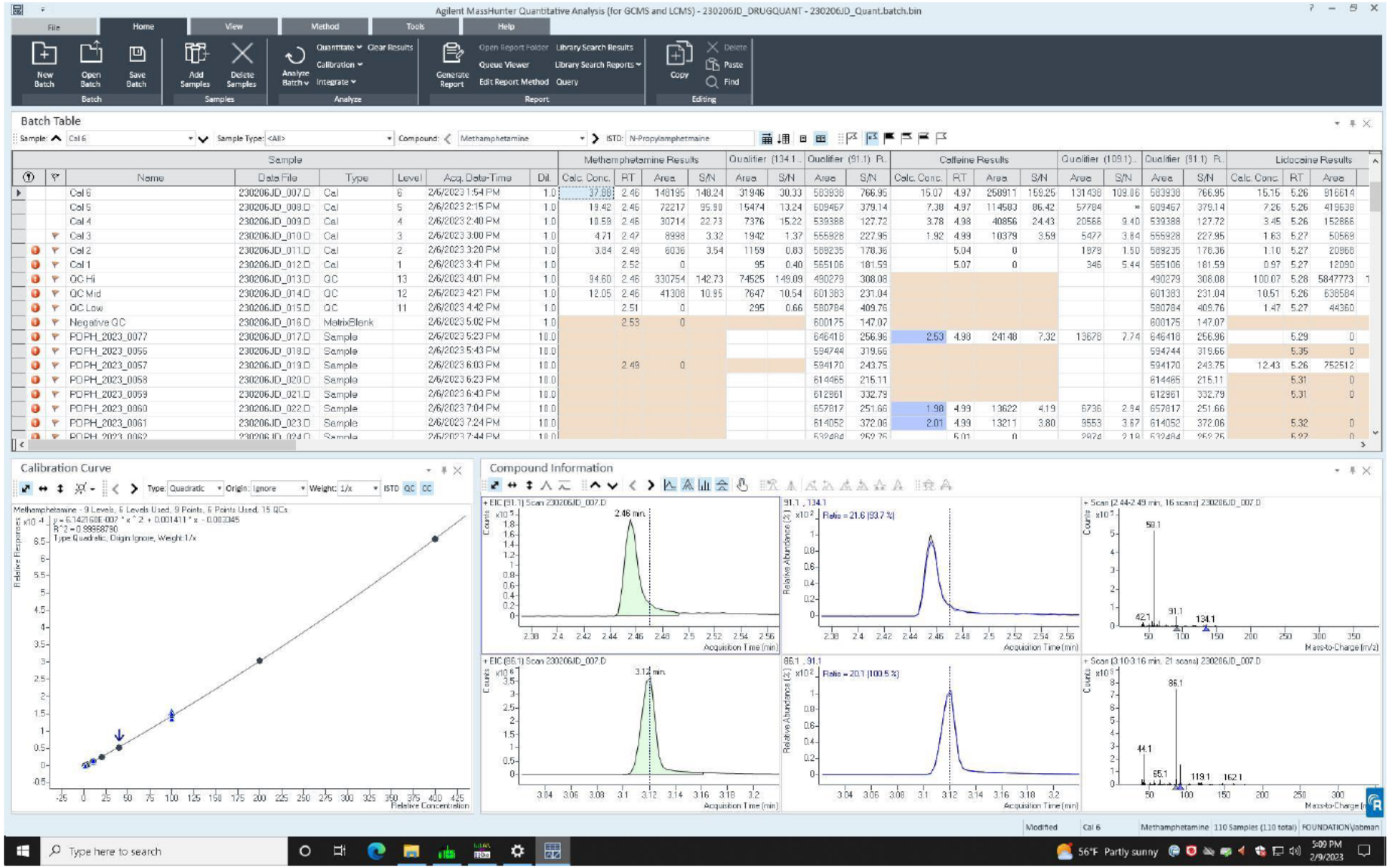
Method Validation

METHOD VALIDATION

- Linearity – assessed different calibration models over 5 days
- Accuracy – checked with CRM standards at concentration of 100µg/mL and 10µg/mL
- Precision – intra-day and inter-day precision were calculated
- Dilution – dilutions up to 10x of original preparation were evaluated
- Matrix Effect : $\left(\frac{Fortified\ extracted\ blank\ sample}{Neat\ sample}\right) - 1$
- Recovery : $\frac{Extracted\ sample}{Neat\ sample}$
- Process Efficiency : $\frac{Extracted\ sample}{Fortified\ extracted\ blank\ sample}$
- Post-extraction stability was checked but failed for cocaine.

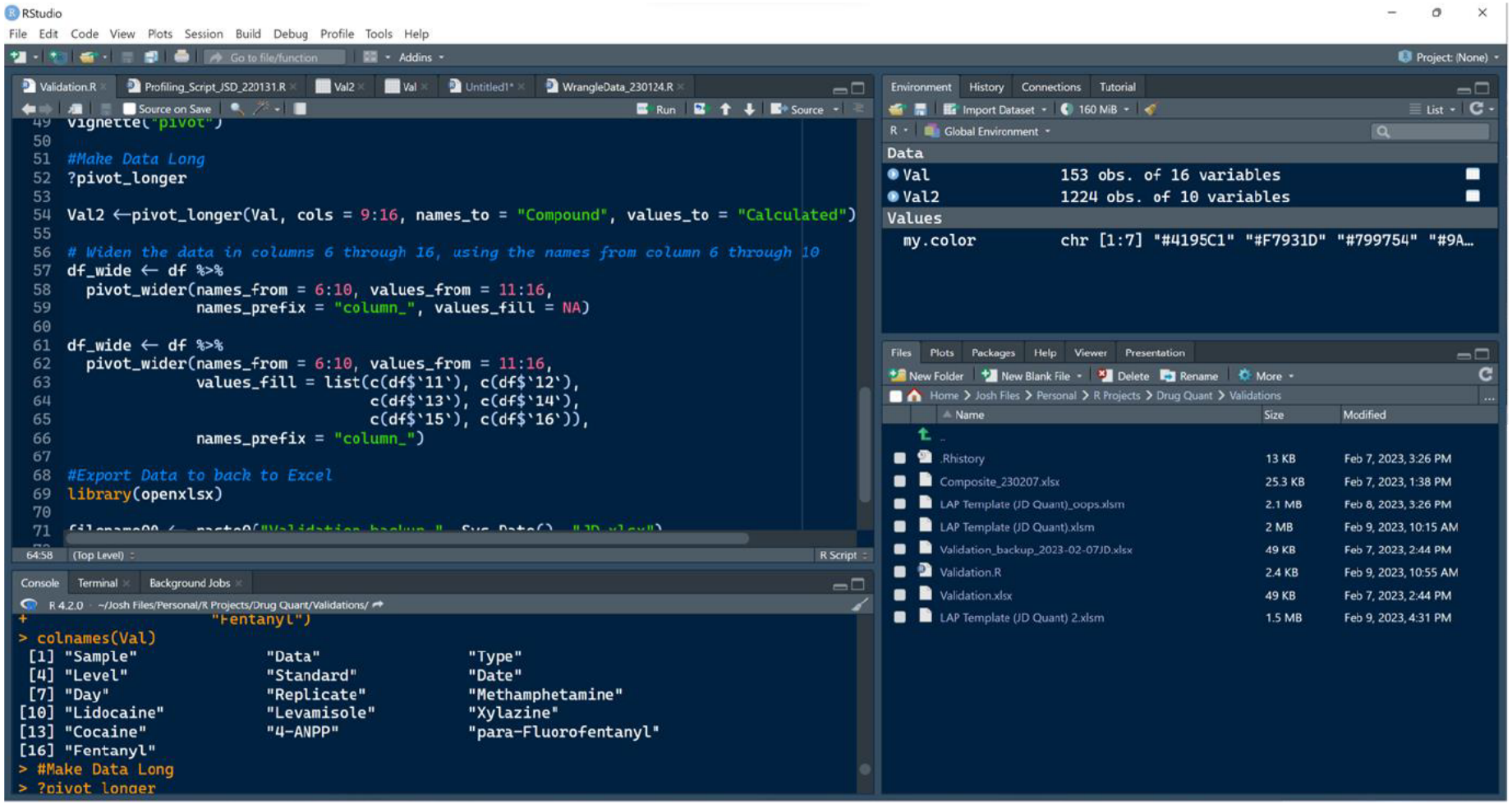
WORKFLOW

- Begins in Agilent MassHunter 10 Quantitative Analysis



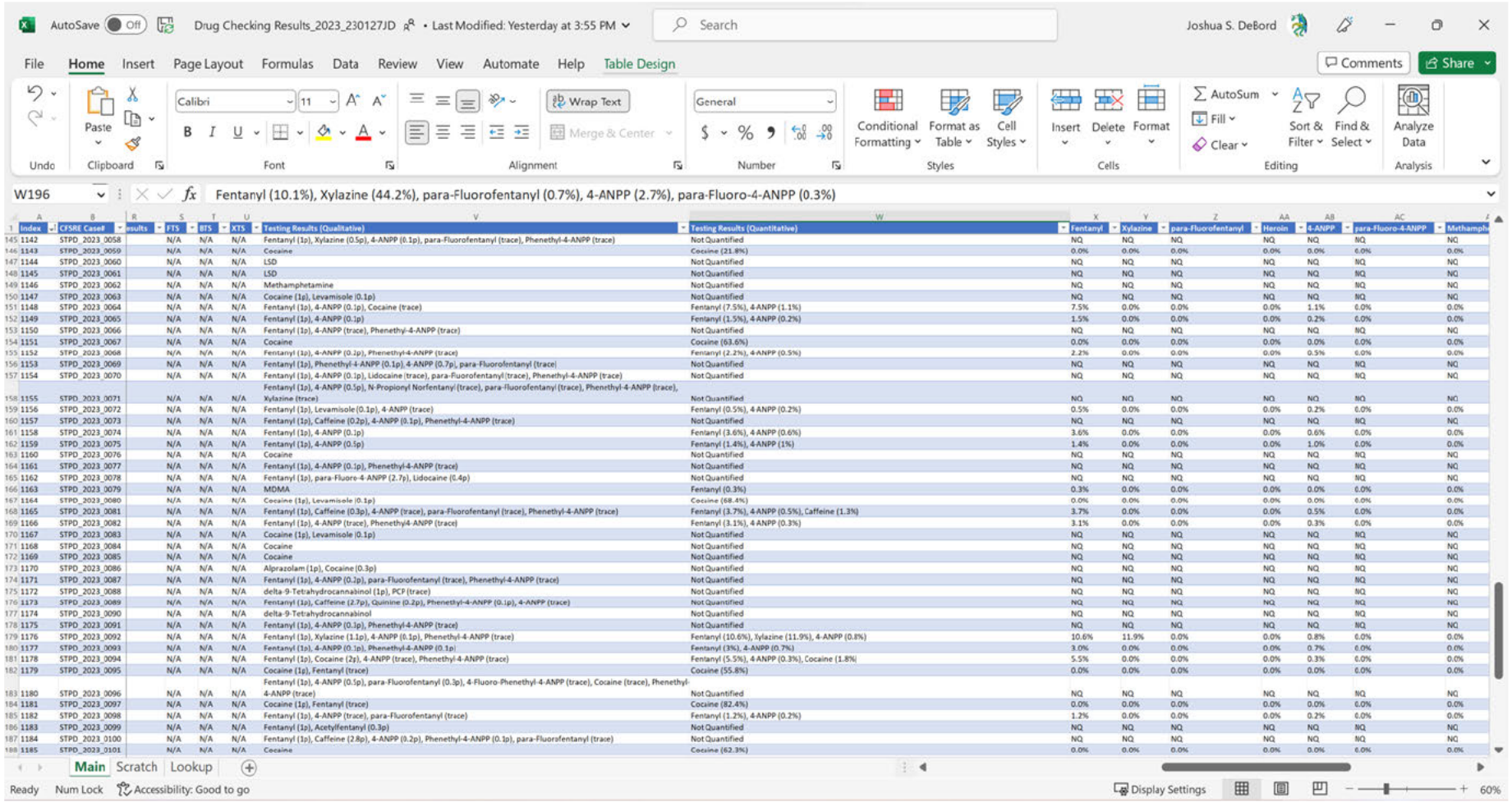
WORKFLOW

- Begins in Agilent MassHunter 10 Quantitative Analysis
- Exported to .csv, cleaned, manipulated and joined by R



WORKFLOW

- Begins in Agilent MassHunter 10 Quantitative Analysis
- Exported to .csv, cleaned, manipulated and joined by R
- Exported to .xlsx, data tabulated for review and sharing in Excel



LIMITATIONS

- Coelution of Levamisole and Xylazine which share some common ions
  - Negatively affected sensitivity for both compounds
  - Negatively affected accuracy at lower concentrations for both compounds
  - Levamisole, if present, could be approximated by a response ratio to something that is quantified instead
- This also negatively affected data review time
  - Response for levamisole when xylazine is at moderate or greater concentrations and like likewise for xylazine when levamisole is present
  - Easily reviewed and flagged by ion ratio filters but still required a manual review.
- Our balance capabilities could be [will be] improved

RESULTS OF METHOD VALIDATION

Validated parameters for compound identification.

Compound	RT (min)	Quant m/z	Qual 1 ion	Ratio 1 ±20%	Qual 2 ion	Ratio 2 ±30%*
Methamphetamine	2.45	91.1	65.1	52.3%	134.1	22.0%
N-Propylamphetamine	3.11	86.1	65.1	7.5%	91.1	28.8%
Lidocaine	5.27	86.1	58.1	8.8%	120.1	4.0%*
Levamisole	5.49	101	73.1	101.7%	121.0	81.6%
Xylazine	5.55	177	130.1	97.3%	145.1	103.3%
Cocaine	6.3	303.2	94.1	137.0%	105.1	110.0%
4-Anilino-N-Phenethylpiperidine	7.01	146.1	118.1	16.0%	189.2	87.0%
Para-fluorofentanyl	7.62	263.2	164.1	38.0%	207.1	25.0%
Fentanyl	7.71	245.2	146.2	48.0%	189.2	31.0%

RESULTS OF METHOD VALIDATION

Validatated quantitative parameters

Compound	Calibration Range (µg/mL)	Model	Weighting	LOQ (µg/mL)	Admin LOD (µg/mL)	R^2	Average of y-intercept	Calculated LOD (µg/mL
Methamphetamine	4 - 400	Quadratic	1/x	4.0	4.0	0.999	-0.56	1.72
Lidocaine	4 - 150	Quadratic	1/x	4.0	4.0	0.999	-1.35	1.77
Levamisole	4 - 150	Quadratic	1/x	4.0	1.5	0.999	-0.98	1.22
Xylazine	15 - 400	Quadratic	1/x	15.0	4.0	0.999	-0.46	2.63
Cocaine	8 - 400	Quadratic	1/x	8.0	8.0	0.999	0.16	4.6
4-Anilino-N-Phenethylpiperidine	4 - 150	Quadratic	1/x	4.0	4.0	0.999	-0.91	2.28
Para-fluorofentanyl	4 - 150	Quadratic	1/x	4.0	4.0	0.999	-1.39	2.77
Fentanyl	4 - 150	Quadratic	1/x	4.0	4.0	0.999	-1.10	2.1

RESULTS OF METHOD VALIDATION

Validaiton Summary

Compound	Accuracy (%)		Precision (%)		Process Efficiency (%)		Matrix Effect (%)		Recovery (%)	
	High	Low	High	Low	High	Low	High	Low	High	Low
Methamphetamine	3.3	7.6	7.9	7.3	96	108	3	4	98	112
Lidocaine	1.5	9.2	4.5	4.5	96	100	2	-3	98	97
Levamisole	1.1	16.4	6.7	16.3	92	95	10	18	101	112
Xylazine	5.0	3.9	5.5	6.5	94	98	1	-1	96	98
Cocaine	3.0	11.4	10.2	13.8	42	41	4	0	43	41
4-Anilino-N-Phenethylpiperidine	0.5	7.4	5.4	7.2	96	98	1	-2	97	96
Para-fluorofentanyl	0.7	9.3	8.0	8.8	95	97	4	1	98	98
Fentanyl	2.0	4.5	7.5	8.8	94	97	4	1	98	98

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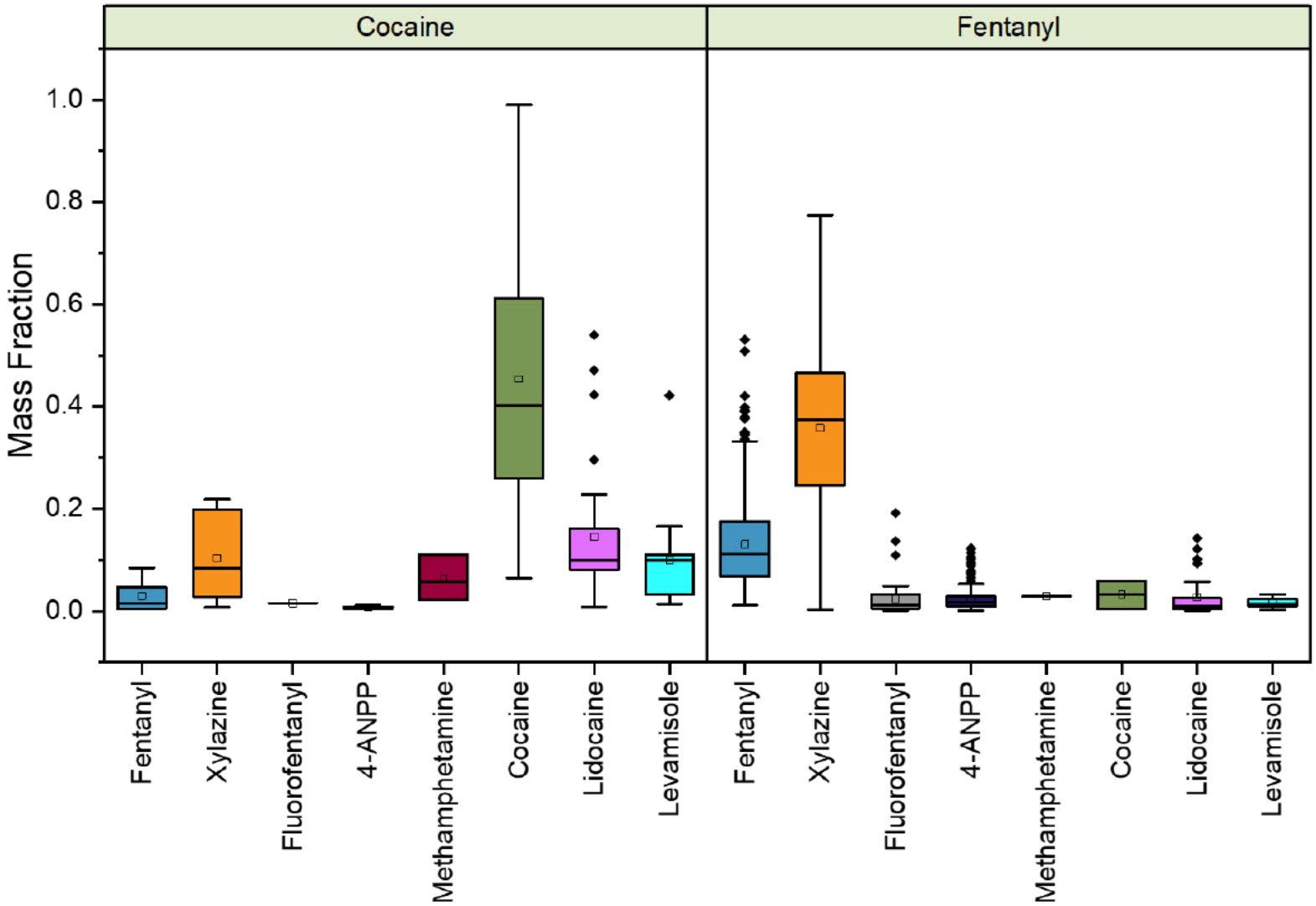
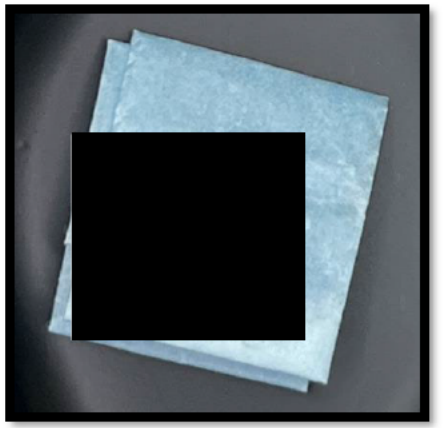
Sample Results

POTENCY EVALUATION POSSIBLE IN "REAL-TIME"

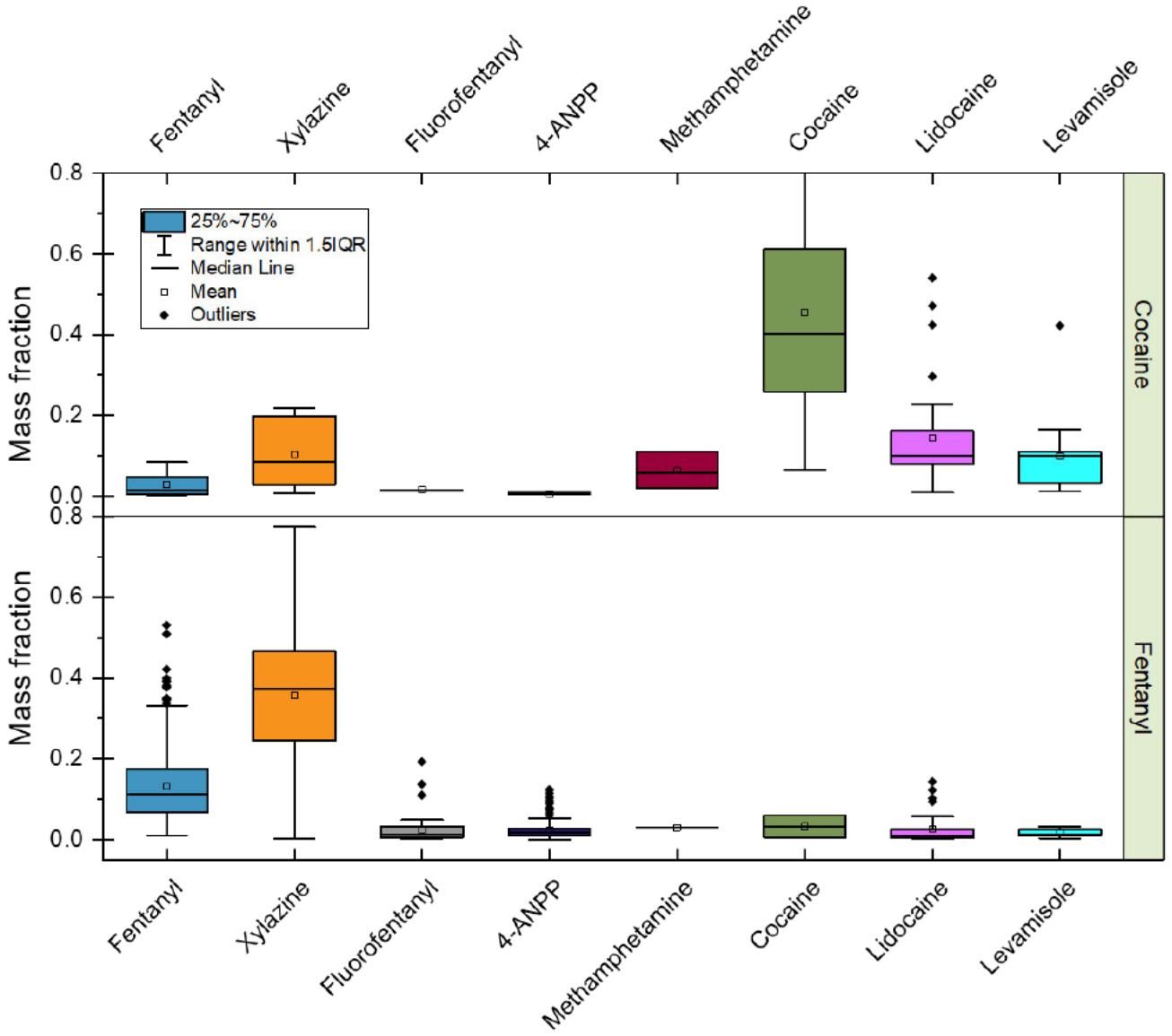
- Without a quantitative anchor for ground truth, potency comparisons are lacking.
- Quantitative measurement with this method is reasonably easy allowing for fast turnaround.
- Suitable for a variety of drug types
- Using the quantitative results of fentanyl and fluorofentanyl
  - Approximate relative abundance of adulterants and impurities
  - Approximate relative abundance of concurrently observed NPS, such as nitazene analogs
  - Provide a public health assessment on relative opioid potencies and compare between samples.
  - Begin to explain why particular samples lead to adverse drug events.

Compound distributions for cocaine (n=66) and fentanyl (n=241) samples





Compound distributions for cocaine (n=66) and fentanyl (n=241) samples



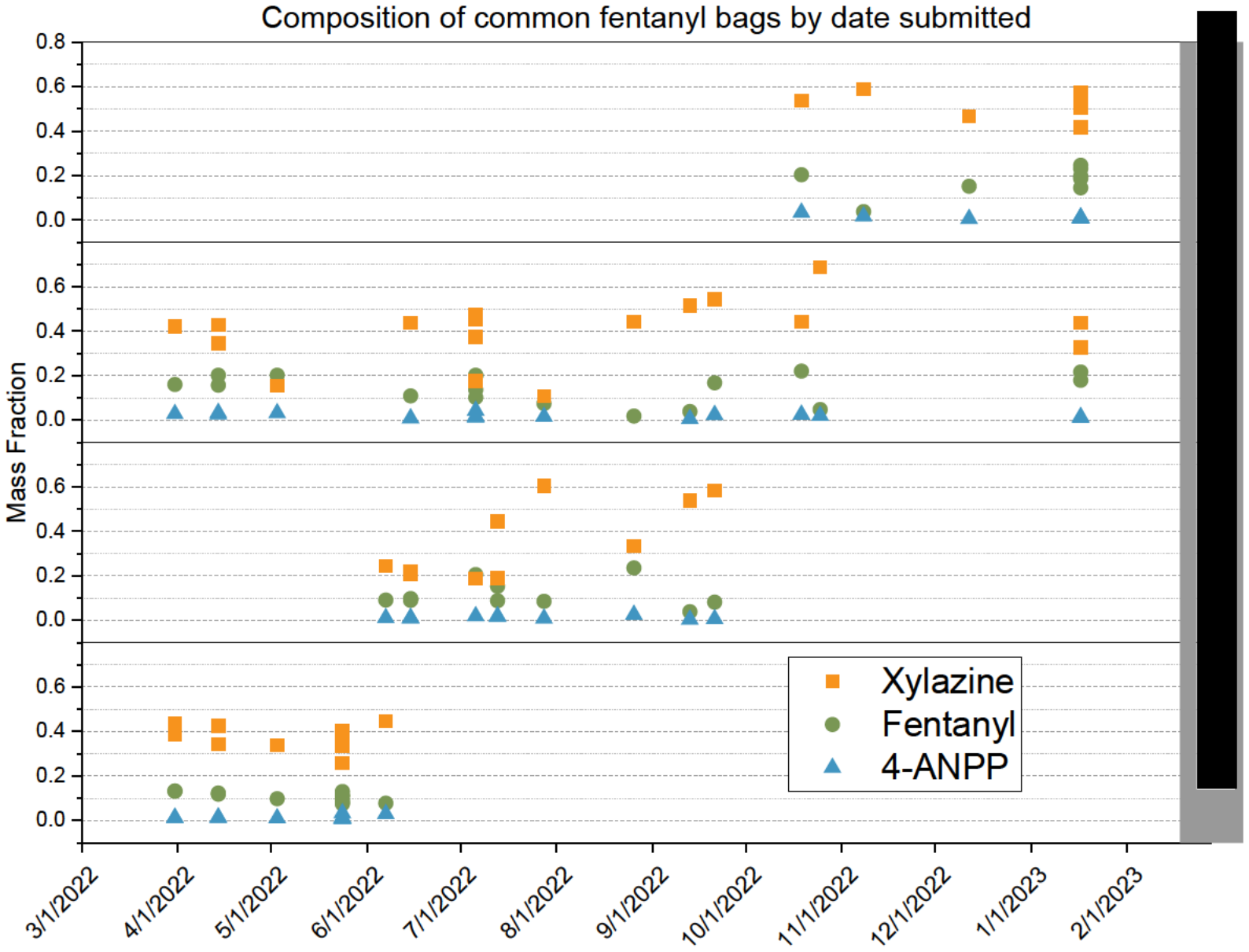
Compound	N	Mean	Standard Deviation
Fentanyl	7	2.93%	2.95%
Xylazine	6	10.37%	8.78%
Fluorofentanyl	1	1.53%	--
4-ANPP	4	0.69%	0.31%
Methamphetamine	3	6.31%	4.51%
Cocaine	66	45.44%	25.45%
Lidocaine	31	14.51%	12.60%
Levamisole	15	9.93%	10.09%

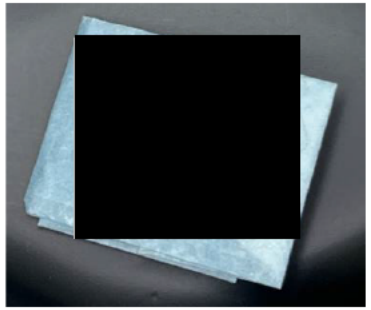
Compound	N	Mean	Standard Deviation
Fentanyl	241	13.06%	8.99%
Xylazine	229	35.76%	16.71%
Fluorofentanyl	47	2.38%	3.61%
4-ANPP	229	2.31%	2.16%
Methamphetamine	1	2.87%	--
Cocaine	2	3.21%	3.92%
Lidocaine	29	2.70%	3.85%
Levamisole	5	1.64%	1.15%

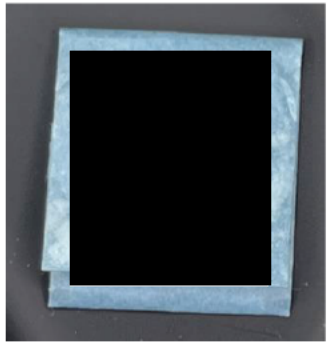
Descriptive statistics on quantitative values (mass percentage) of samples with predominant cocaine (top) and of samples with predominant fentanyl (bottom).

Compound	N	Mean	Standard Deviation	Lower 95% CI of Mean	Upper 95% CI of Mean	Minimum	1st Quartile (Q1)	Median	3rd Quartile (Q3)	Maximum
Fentanyl	7	2.93%	2.95%	0.20%	5.66%	0.38%	0.41%	1.47%	4.70%	8.43%
Xylazine	6	10.37%	8.78%	1.15%	19.58%	0.77%	2.82%	8.42%	19.88%	21.88%
Fluorofentanyl	1	1.53%	--	--	--	1.53%	1.53%	1.53%	1.53%	1.53%
4-ANPP	4	0.69%	0.31%	0.20%	1.19%	0.43%	0.51%	0.60%	0.87%	1.15%
Methamphetamine	3	6.31%	4.51%	-4.88%	17.51%	2.13%	2.13%	5.73%	11.09%	11.09%
Cocaine	66	45.44%	25.45%	39.18%	51.70%	6.52%	25.93%	40.21%	61.14%	99.00%
Lidocaine	31	14.51%	12.60%	9.89%	19.14%	0.89%	8.09%	10.03%	16.18%	54.01%
Levamisole	15	9.93%	10.09%	4.34%	15.52%	1.34%	3.27%	9.98%	11.06%	42.18%

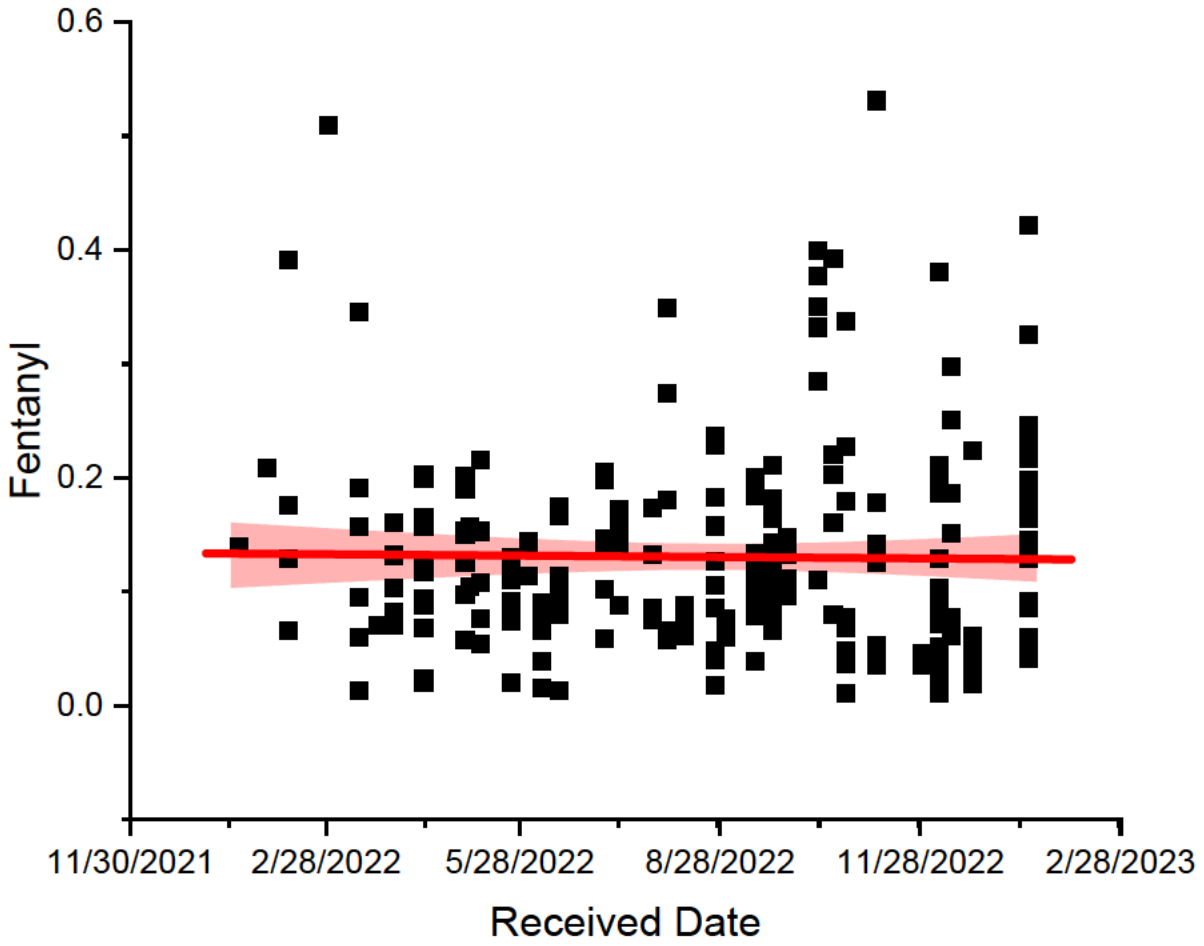
Compound	N	Mean	Standard Deviation	Lower 95% CI of Mean	Upper 95% CI of Mean	Minimum	1st Quartile (Q1)	Median	3rd Quartile (Q3)	Maximum
Fentanyl	241	13.06%	8.99%	11.92%	14.20%	1.09%	6.77%	11.21%	17.51%	53.08%
Xylazine	229	35.76%	16.71%	33.59%	37.94%	0.24%	24.53%	37.48%	46.52%	77.44%
Fluorofentanyl	47	2.38%	3.61%	1.32%	3.44%	0.16%	0.51%	1.17%	3.28%	19.21%
4-ANPP	229	2.31%	2.16%	2.03%	2.59%	0.10%	0.98%	1.72%	2.84%	12.33%
Methamphetamine	1	2.87%	--	--	--	2.87%	2.87%	2.87%	2.87%	2.87%
Cocaine	2	3.21%	3.92%	-32.02%	38.43%	0.43%	0.43%	3.21%	5.98%	5.98%
Lidocaine	29	2.70%	3.85%	1.23%	4.16%	0.18%	0.43%	1.03%	2.54%	14.30%
Levamisole	5	1.64%	1.15%	0.22%	3.07%	0.31%	1.00%	1.33%	2.35%	3.22%

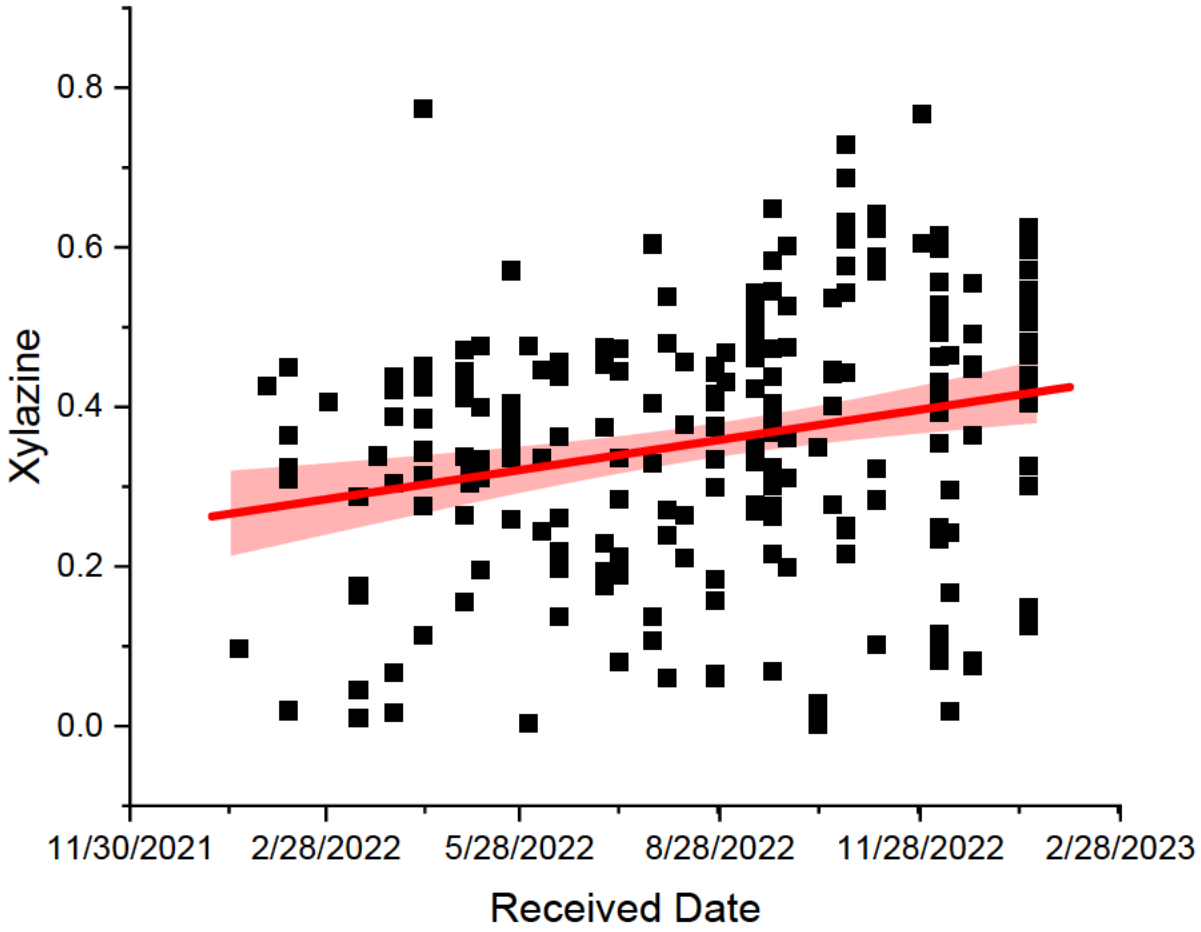

Composition of common fentanyl bags by date submitted
Mass Fraction
0.8
0.6
0.4
0.2
0.0
Xylazine
Fentanyl
4-ANPP
3/1/2022
4/1/2022
5/1/2022
6/1/2022
7/1/2022
8/1/2022
9/1/2022
10/1/2022
11/1/2022
12/1/2022
1/1/2023
2/1/2023

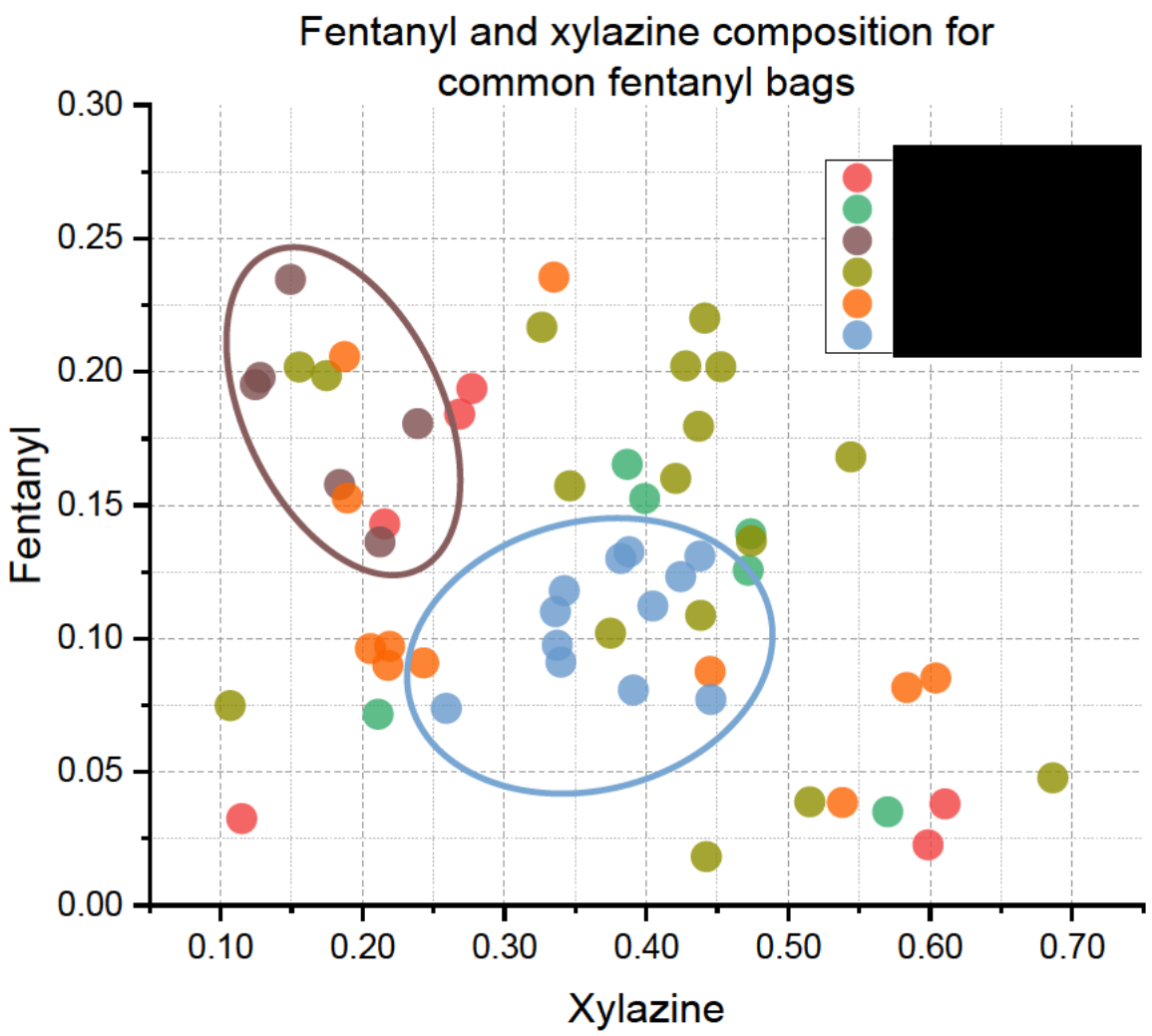


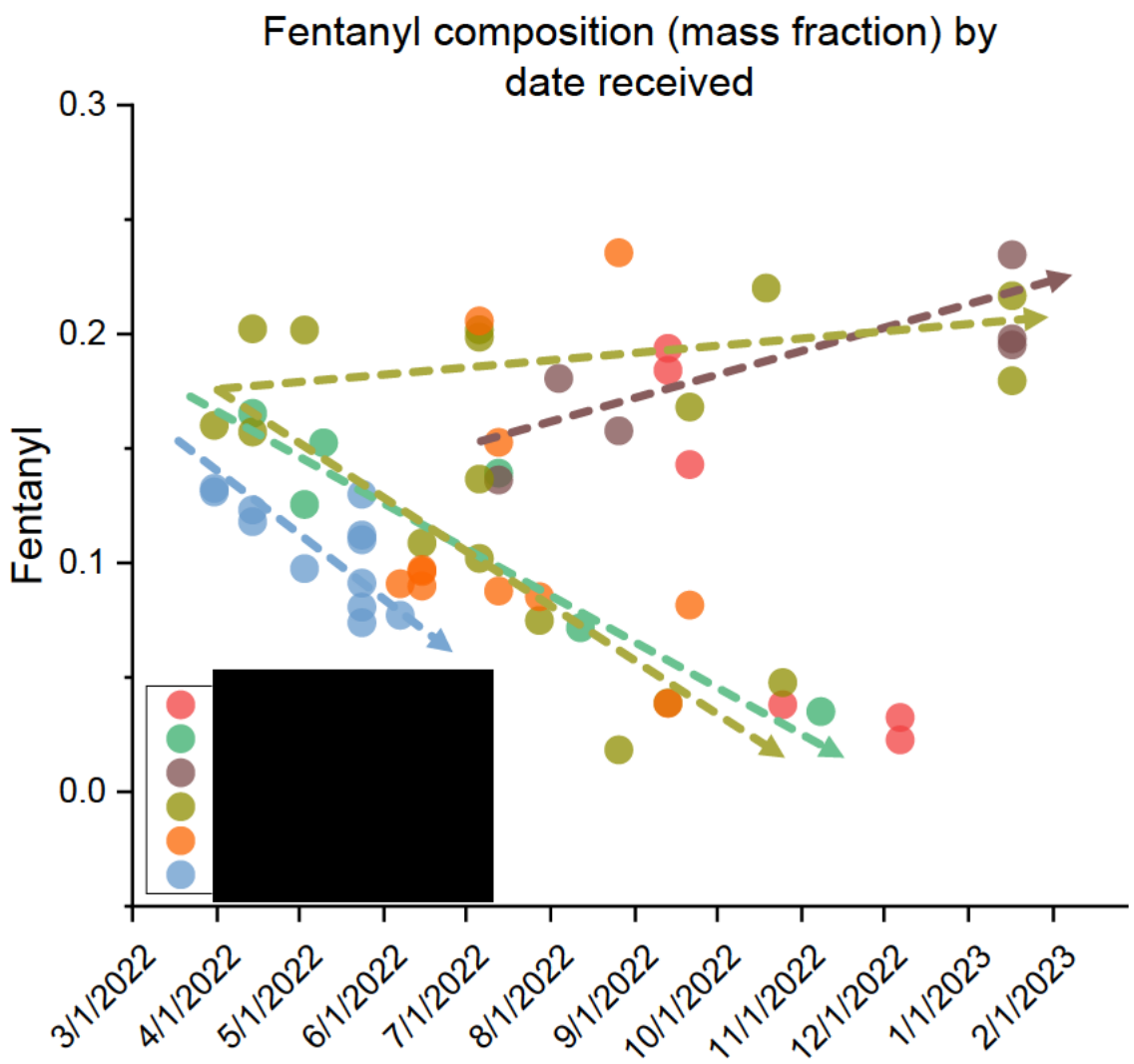


General yearly observations in Philly fentanyl samples for 2022






Fentanyl and xylazine composition for common fentanyl bags
Fentanyl
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
Xylazine
[redacted]


Fentanyl composition (mass fraction) by date received
Fentanyl
0.3
0.2
0.1
0.0
3/1/2022
4/1/2022
5/1/2022
6/1/2022
7/1/2022
8/1/2022
9/1/2022
10/1/2022
11/1/2022
12/1/2022
1/1/2023
2/1/2023
[redacted]

CONCLUSIONS

- A simple extraction and analytical method using common instrumentation to quantify fentanyl and related compounds in common drug types.
- Ability to determine/compare potency in street drugs in near real-time is vital to harm reduction, public health advancement, and informing policy
- We have begun to inform public health partners on their relative potencies of opioid samples within two weeks of collection.
- In Philadelphia, among the samples we've tested:
  - Some batches of fentanyl have shown a tendency to decrease in fentanyl concentration overtime, but further studies are needed
  - We have observed xylazine to have increased in concentration over the past year approximately 10% to a new average mass % of 40. Xylazine is still ubiquitous and is detected in ~95% of powder fentanyl samples from PDPH.
  - Fentanyl average concentration has not changed and is still an average of ~13%

Thank you!

Questions?

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