

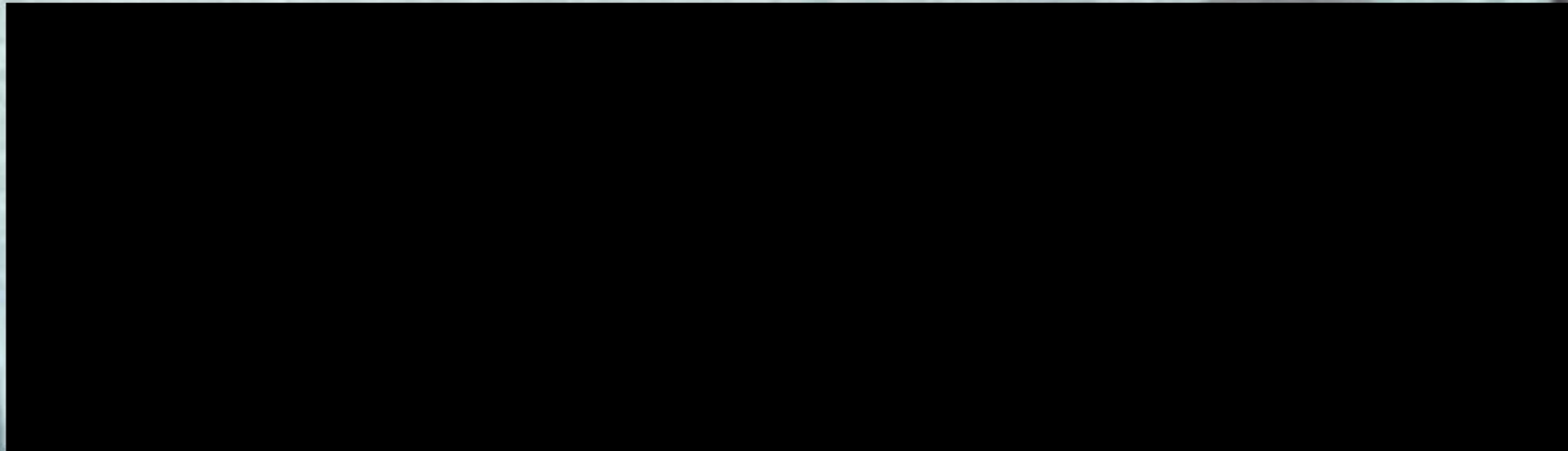
# The Development and Validation of a Quantitative Method for the Analysis of Fentanyl Containing “Dope” Samples

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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
- CFSRE / NPS Discovery gratefully acknowledges the support of the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice
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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.

## JOSHUA DEBORD

- 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



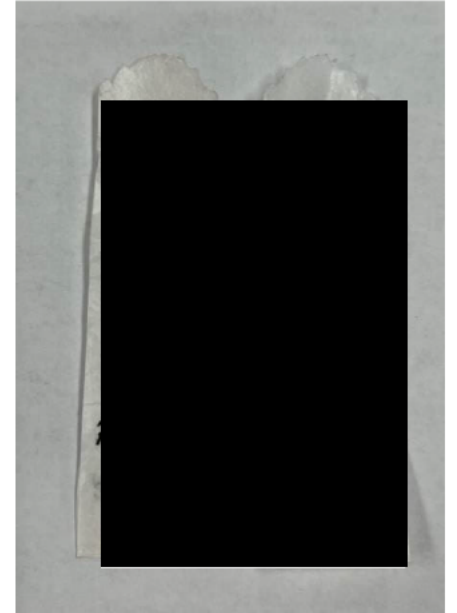


cfsre | NPS DISCOVERY | Method Development



## 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  $\mu$ L
- Add 200  $\mu$ L internal standard (20  $\mu$ g/mL N-propyl-amphetamine)
- Extract with 0.75 mL of 0.1 N NaOH and 0.5 mL  $\text{CHCl}_3$ .
- 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, 0.33 $\mu$ m 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  $\mu$ L, 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 :  $\left( \frac{\textit{Fortified extracted blank sample}}{\textit{Neat sample}} \right) - 1$
- Recovery :  $\frac{\textit{Extracted sample}}{\textit{Neat sample}}$
- Process Efficiency :  $\frac{\textit{Extracted sample}}{\textit{Fortified extracted blank sample}}$
- Post-extraction stability was checked but failed for cocaine.

# WORKFLOW

- Begins in Agilent MassHunter 10 Quantitative Analysis

The screenshot displays the Agilent MassHunter 10 Quantitative Analysis software interface. The main window shows a 'Batch Table' with columns for Sample Name, Data File, Type, Level, Acq. Date-Time, Dil., and various results for Methamphetamine, Cocaine, and Lidocaine. Below the table, there are three panels: 'Calibration Curve' showing a linear plot of Relative Response vs. Relative Concentration; 'Compound Information' showing EIC (Extracted Ion Chromatogram) and mass spectra for Methamphetamine at 2.46 min, 3.12 min, and 3.12 min; and a mass spectrum for Cocaine at 2.44-2.49 min. The interface includes a menu bar (File, Home, View, Method, Tools, Help) and a toolbar with icons for file operations, analysis, and reporting.

Sample	Name	Data File	Type	Level	Acq. Date-Time	Dil.	Calc. Conc.	RT	Area	S/N	Qualifier (134.1)	Qualifier (91.1) R.	Cocaine Results	Qualifier (109.1)	Qualifier (91.1) R.	Lidocaine Results										
Cal 6	230206JD_007.D	Cal	6	2/6/2023 1:54 PM	1.0	37.68	2.46	148195	140.24	31946	30.33	503938	766.95	15.07	4.97	250911	158.25	131438	109.06	503938	766.95	15.15	5.26	916614		
Cal 5	230206JD_008.D	Cal	5	2/6/2023 2:15 PM	1.0	19.42	2.46	72217	95.90	15474	13.24	609467	379.14	7.38	4.97	114583	86.42	57784	9.40	609467	379.14	7.26	5.26	419638		
Cal 4	230206JD_009.D	Cal	4	2/6/2023 2:40 PM	1.0	10.59	2.46	30714	22.73	7376	15.22	539388	127.72	3.78	4.98	40856	24.43	20566	9.40	539388	127.72	3.45	5.26	152866		
Cal 3	230206JD_010.D	Cal	3	2/6/2023 3:00 PM	1.0	4.71	2.47	8998	3.32	1942	1.37	555928	227.95	1.92	4.99	10379	3.59	5477	3.84	555928	227.95	1.63	5.27	50589		
Cal 2	230206JD_011.D	Cal	2	2/6/2023 3:20 PM	1.0	3.84	2.48	6036	3.54	1159	0.83	589235	178.36	5.04	0		1879	1.50	589235	178.36	1.10	5.27	20968			
Cal 1	230206JD_012.D	Cal	1	2/6/2023 3:41 PM	1.0	2.52	0	0	0	95	0.40	555106	181.59	5.07	0		346	5.44	555106	181.59	0.97	5.27	12090			
QC Hi	230206JD_013.D	QC	13	2/6/2023 4:01 PM	1.0	94.60	2.46	330754	142.73	74525	149.09	490279	308.08							490279	308.08	100.07	5.28	5847773		
QC Mid	230206JD_014.D	QC	12	2/6/2023 4:21 PM	1.0	12.05	2.46	41308	10.95	7647	10.54	601383	231.04							601383	231.04	10.51	5.26	636584		
QC Low	230206JD_015.D	QC	11	2/6/2023 4:42 PM	1.0	2.51	0	0	0	295	0.66	580784	409.76							580784	409.76	1.47	5.27	44360		
Negative QC	230206JD_016.D	MatrixBlank		2/6/2023 5:02 PM	1.0	2.53	0	0	0	0	0	600175	147.07							600175	147.07					
POPH_2023_0077	230206JD_017.D	Sample		2/6/2023 5:23 PM	10.0							646418	256.96	2.53	4.93	24140	7.32	13678	7.74	646418	256.96			5.29	0	
POPH_2023_0056	230206JD_018.D	Sample		2/6/2023 5:43 PM	10.0							594744	319.66							594744	319.66			5.35	0	
POPH_2023_0057	230206JD_019.D	Sample		2/6/2023 6:03 PM	10.0		2.48	0				594170	243.75							594170	243.75			12.43	5.26	752512
POPH_2023_0058	230206JD_020.D	Sample		2/6/2023 6:23 PM	10.0							614465	216.11							614465	216.11			5.31	0	
POPH_2023_0059	230206JD_021.D	Sample		2/6/2023 6:43 PM	10.0							612961	332.79							612961	332.79			5.31	0	
POPH_2023_0060	230206JD_022.D	Sample		2/6/2023 7:04 PM	10.0							657617	251.66	1.98	4.93	13622	4.19	6736	2.94	657617	251.66			5.32	0	
POPH_2023_0061	230206JD_023.D	Sample		2/6/2023 7:24 PM	10.0							614052	372.06	2.01	4.93	13211	3.80	9553	3.67	614052	372.06			5.32	0	
POPH_2023_0062	230206JD_024.D	Sample		2/6/2023 7:44 PM	10.0							539484	959.76							539484	959.76			6.92	0	

# WORKFLOW

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

The screenshot shows the RStudio interface with the following components:

- Source Editor:** Contains R code for data manipulation:
 

```

vignette("pivot")
#Make Data Long
?pivot_longer
Val2 <- pivot_longer(Val, cols = 9:16, names_to = "Compound", values_to = "Calculated")
# Widen the data in columns 6 through 16, using the names from column 6 through 10
df_wide <- df %>%
  pivot_wider(names_from = 6:10, values_from = 11:16,
             names_prefix = "column_", values_fill = NA)
df_wide <- df %>%
  pivot_wider(names_from = 6:10, values_from = 11:16,
             values_fill = list(c(df$`11`, c(df$`12`),
                                c(df$`13`, c(df$`14`),
                                c(df$`15`, c(df$`16`))),
             names_prefix = "column_")
#Export Data to back to Excel
library(openxlsx)
file_name00 <- paste0("Validation_backup_", Sys.Date(), ".xlsx")
      
```
- Environment:** Shows loaded objects:
 

Object	Size
Val	153 obs. of 16 variables
Val2	1224 obs. of 10 variables
- Files Panel:** Shows a directory listing for 'Validations':
 

Name	Size	Modified
.Rhistory	13 KB	Feb 7, 2023, 3:26 PM
Composite_230207.xlsx	25.3 KB	Feb 7, 2023, 1:38 PM
LAP Template (JD Quant)_oops.xlsm	2.1 MB	Feb 8, 2023, 3:26 PM
LAP Template (JD Quant).xlsm	2 MB	Feb 9, 2023, 10:15 AM
Validation_backup_2023-02-07JD.xlsx	49 KB	Feb 7, 2023, 2:44 PM
Validation.R	2.4 KB	Feb 9, 2023, 10:55 AM
Validation.xlsx	49 KB	Feb 7, 2023, 2:44 PM
LAP Template (JD Quant) 2.xlsm	1.5 MB	Feb 9, 2023, 4:31 PM
- Console:** Shows the output of the `colnames(Val)` command:
 

```

> colnames(Val)
[1] "Sample"      "Data"      "Type"
[4] "Level"      "Standard"  "Date"
[7] "Day"        "Replicate" "Methamphetamine"
[10] "Lidocaine"  "Levamisole" "Xylazine"
[13] "Cocaine"    "4-ANPP"    "para-Fluorofentanyl"
[16] "Fentanyl"
      
```

# 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

The screenshot shows an Excel spreadsheet with the following data structure:

Index	CFSRE Case#	Results	FIS	BTS	XIS	Testing Results (Qualitative)	Testing Results (Quantitative)	Fentanyl	Xylazine	para-Fluorofentanyl	Heroin	4-ANPP	para-Fluoro-4-ANPP	Mthamph
145	1142	STPD_2023_0058	N/A	N/A	N/A	Fentanyl (1p), Xylazine (0.5p), 4-ANPP (0.1p), para-Fluorofentanyl (trace), Phenethyl-4-ANPP (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
146	1143	STPD_2023_0059	N/A	N/A	N/A	Cocaine	Cocaine (21.8%)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
147	1144	STPD_2023_0060	N/A	N/A	N/A	LSD	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
148	1145	STPD_2023_0061	N/A	N/A	N/A	LSD	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
149	1146	STPD_2023_0062	N/A	N/A	N/A	Methamphetamine	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
150	1147	STPD_2023_0063	N/A	N/A	N/A	Cocaine (1p), Levamisole (0.1p)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
151	1148	STPD_2023_0064	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (0.1p), Cocaine (trace)	Fentanyl (7.5%), 4-ANPP (1.1%)	7.5%	0.0%	0.0%	0.0%	1.1%	0.0%	0.0%
152	1149	STPD_2023_0065	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (0.1p)	Fentanyl (1.5%), 4-ANPP (0.2%)	1.5%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%
153	1150	STPD_2023_0066	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (trace), Phenethyl-4-ANPP (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
154	1151	STPD_2023_0067	N/A	N/A	N/A	Cocaine	Cocaine (63.6%)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
155	1152	STPD_2023_0068	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (0.2p), Phenethyl-4-ANPP (trace)	Fentanyl (2.2%), 4-ANPP (0.5%)	2.2%	0.0%	0.0%	0.0%	0.5%	0.0%	0.0%
156	1153	STPD_2023_0069	N/A	N/A	N/A	Fentanyl (1p), Phenethyl-4-ANPP (0.1p), 4-ANPP (0.7p), para-Fluorofentanyl (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
157	1154	STPD_2023_0070	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (0.1p), Lidocaine (trace), para-Fluorofentanyl (trace), Phenethyl-4-ANPP (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
158	1155	STPD_2023_0071	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (0.1p), N-Propionyl Norfentanyl (trace), para-Fluorofentanyl (trace), Phenethyl-4-ANPP (trace), Xylazine (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
159	1156	STPD_2023_0072	N/A	N/A	N/A	Fentanyl (1p), Levamisole (0.1p), 4-ANPP (trace)	Fentanyl (0.5%), 4-ANPP (0.2%)	0.5%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%
160	1157	STPD_2023_0073	N/A	N/A	N/A	Fentanyl (1p), Caffeine (0.2p), 4-ANPP (0.1p), Phenethyl-4-ANPP (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
161	1158	STPD_2023_0074	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (0.1p)	Fentanyl (3.6%), 4-ANPP (0.6%)	3.6%	0.0%	0.0%	0.0%	0.6%	0.0%	0.0%
162	1159	STPD_2023_0075	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (0.1p)	Fentanyl (1.4%), 4-ANPP (1%)	1.4%	0.0%	0.0%	0.0%	1.0%	0.0%	0.0%
163	1160	STPD_2023_0076	N/A	N/A	N/A	Cocaine	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
164	1161	STPD_2023_0077	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (0.1p), Phenethyl-4-ANPP (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
165	1162	STPD_2023_0078	N/A	N/A	N/A	Fentanyl (1p), para-Fluoro-4-ANPP (2.7p), Lidocaine (0.4p)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
166	1163	STPD_2023_0079	N/A	N/A	N/A	MDMA	Fentanyl (0.3%)	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
167	1164	STPD_2023_0080	N/A	N/A	N/A	Cocaine (1p), Levamisole (0.1p)	Cocaine (68.4%)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
168	1165	STPD_2023_0081	N/A	N/A	N/A	Fentanyl (1p), Caffeine (0.3p), 4-ANPP (trace), para-Fluorofentanyl (trace), Phenethyl-4-ANPP (trace)	Fentanyl (3.7%), 4-ANPP (0.5%), Caffeine (1.3%)	3.7%	0.0%	0.0%	0.0%	0.5%	0.0%	0.0%
169	1166	STPD_2023_0082	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (trace), Phenethyl-4-ANPP (trace)	Fentanyl (3.1%), 4-ANPP (0.3%)	3.1%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%
170	1167	STPD_2023_0083	N/A	N/A	N/A	Cocaine (1p), Levamisole (0.1p)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
171	1168	STPD_2023_0084	N/A	N/A	N/A	Cocaine	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
172	1169	STPD_2023_0085	N/A	N/A	N/A	Cocaine	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
173	1170	STPD_2023_0086	N/A	N/A	N/A	Alprazolam (1p), Cocaine (0.3p)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
174	1171	STPD_2023_0087	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (0.2p), para-Fluorofentanyl (trace), Phenethyl-4-ANPP (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
175	1172	STPD_2023_0088	N/A	N/A	N/A	delta-9-Tetrahydrocannabinol (1p), PCP (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
176	1173	STPD_2023_0089	N/A	N/A	N/A	Fentanyl (1p), Caffeine (2.7p), Quinine (0.2p), Phenethyl-4-ANPP (0.1p), 4-ANPP (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
177	1174	STPD_2023_0090	N/A	N/A	N/A	delta-9-Tetrahydrocannabinol	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
178	1175	STPD_2023_0091	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (0.1p), Phenethyl-4-ANPP (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
179	1176	STPD_2023_0092	N/A	N/A	N/A	Fentanyl (1p), Xylazine (1.1p), 4-ANPP (0.1p), Phenethyl-4-ANPP (trace)	Fentanyl (10.6%), Xylazine (11.9%), 4-ANPP (0.2%)	10.6%	11.9%	0.0%	0.0%	0.2%	0.0%	0.0%
180	1177	STPD_2023_0093	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (0.1p), Phenethyl-4-ANPP (0.1p)	Fentanyl (3%), 4-ANPP (0.7%)	3.0%	0.0%	0.0%	0.0%	0.7%	0.0%	0.0%
181	1178	STPD_2023_0094	N/A	N/A	N/A	Fentanyl (1p), Cocaine (2p), 4-ANPP (trace), Phenethyl-4-ANPP (trace)	Fentanyl (5.5%), 4-ANPP (0.3%), Cocaine (1.8%)	5.5%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%
182	1179	STPD_2023_0095	N/A	N/A	N/A	Cocaine (1p), Fentanyl (trace)	Cocaine (55.8%)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
183	1180	STPD_2023_0096	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (0.1p), para-Fluorofentanyl (0.3p), 4-Fluoro-Phenethyl-4-ANPP (trace), Cocaine (trace), Phenethyl-4-ANPP (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
184	1181	STPD_2023_0097	N/A	N/A	N/A	Cocaine (1p), Fentanyl (trace)	Cocaine (82.4%)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
185	1182	STPD_2023_0098	N/A	N/A	N/A	Fentanyl (1p), 4-ANPP (trace), para-Fluorofentanyl (trace)	Fentanyl (1.2%), 4-ANPP (0.2%)	1.2%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%
186	1183	STPD_2023_0099	N/A	N/A	N/A	Fentanyl (1p), Acetyl-fentanyl (0.3p)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
187	1184	STPD_2023_0100	N/A	N/A	N/A	Fentanyl (1p), Caffeine (2.8p), 4-ANPP (0.2p), Phenethyl-4-ANPP (0.1p), para-Fluorofentanyl (trace)	Not Quantified	NQ	NQ	NQ	NQ	NQ	NQ	NQ
188	1185	STPD_2023_0101	N/A	N/A	N/A	Cocaine	Cocaine (62.3%)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

## 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 $\pm$ 20%	Qual 2 ion	Ratio 2 $\pm$ 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

Validated quantitative parameters

Compound	Calibration Range (µg/mL)	Model	Weighting	LOQ (µg/mL)	Admin LOD (µg/mL)	R <sup>2</sup>	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



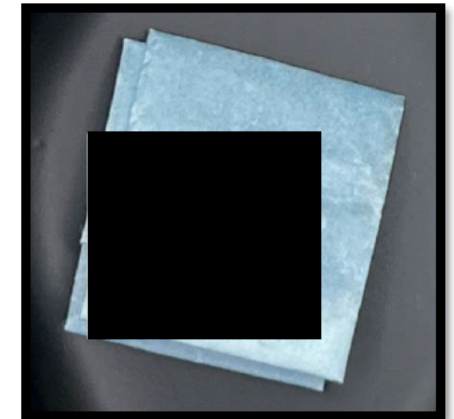
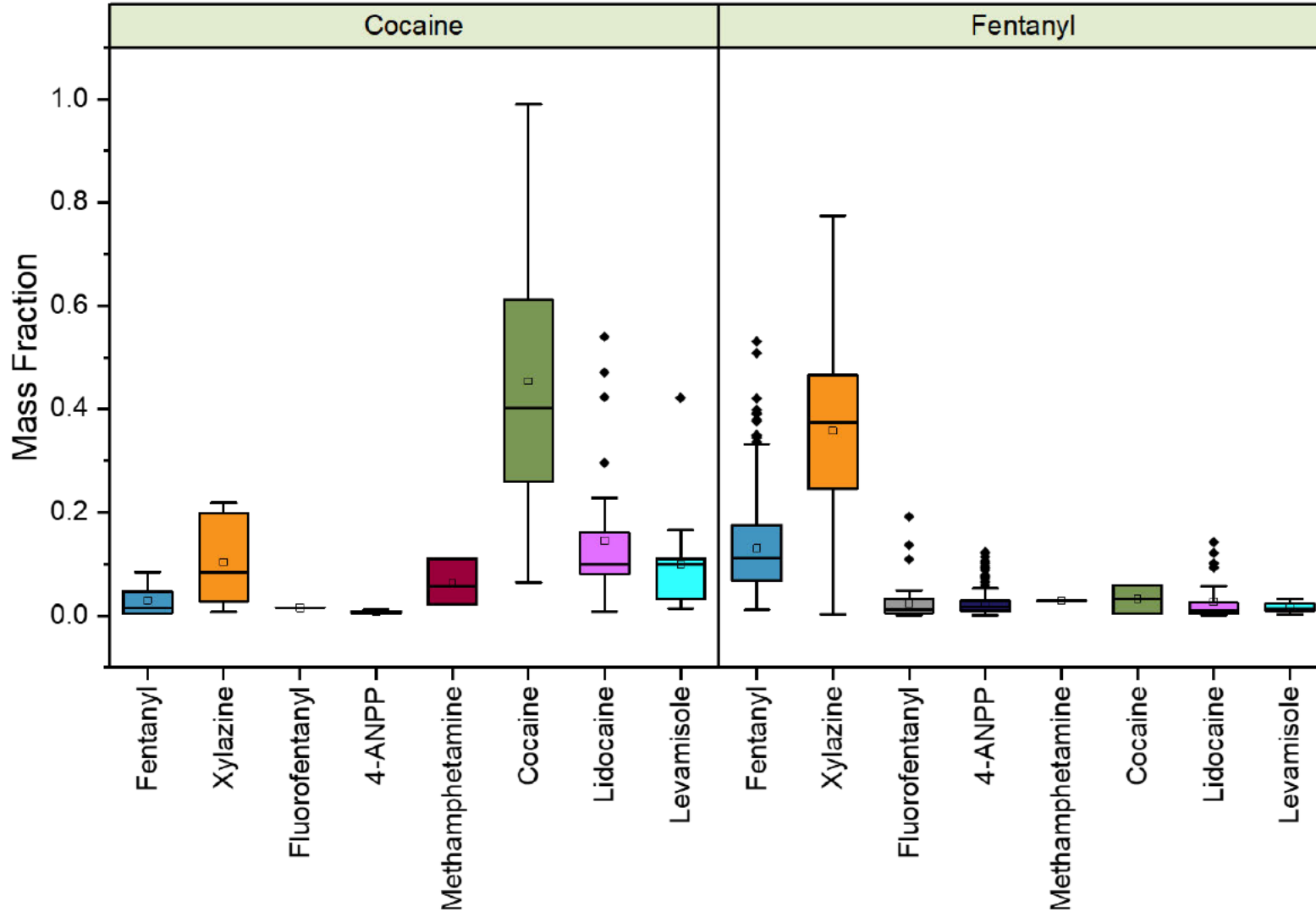
## Sample Results



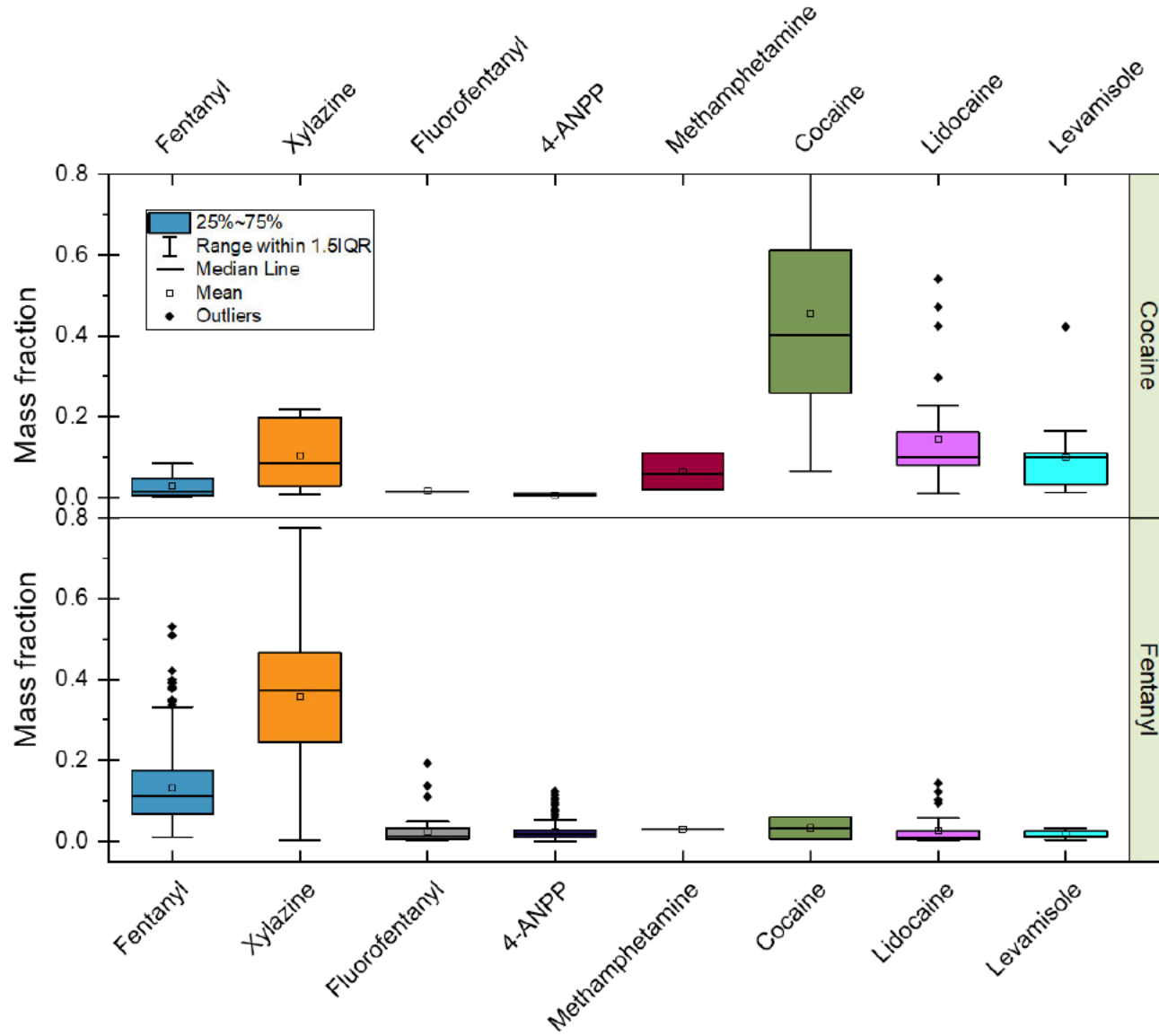
## POTENCY EVALUATION POSSIBLE IN “REAL-TIME”

- 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%

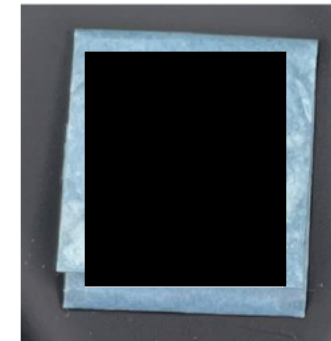
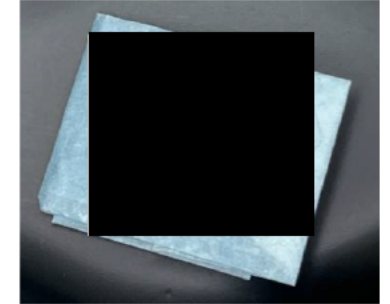
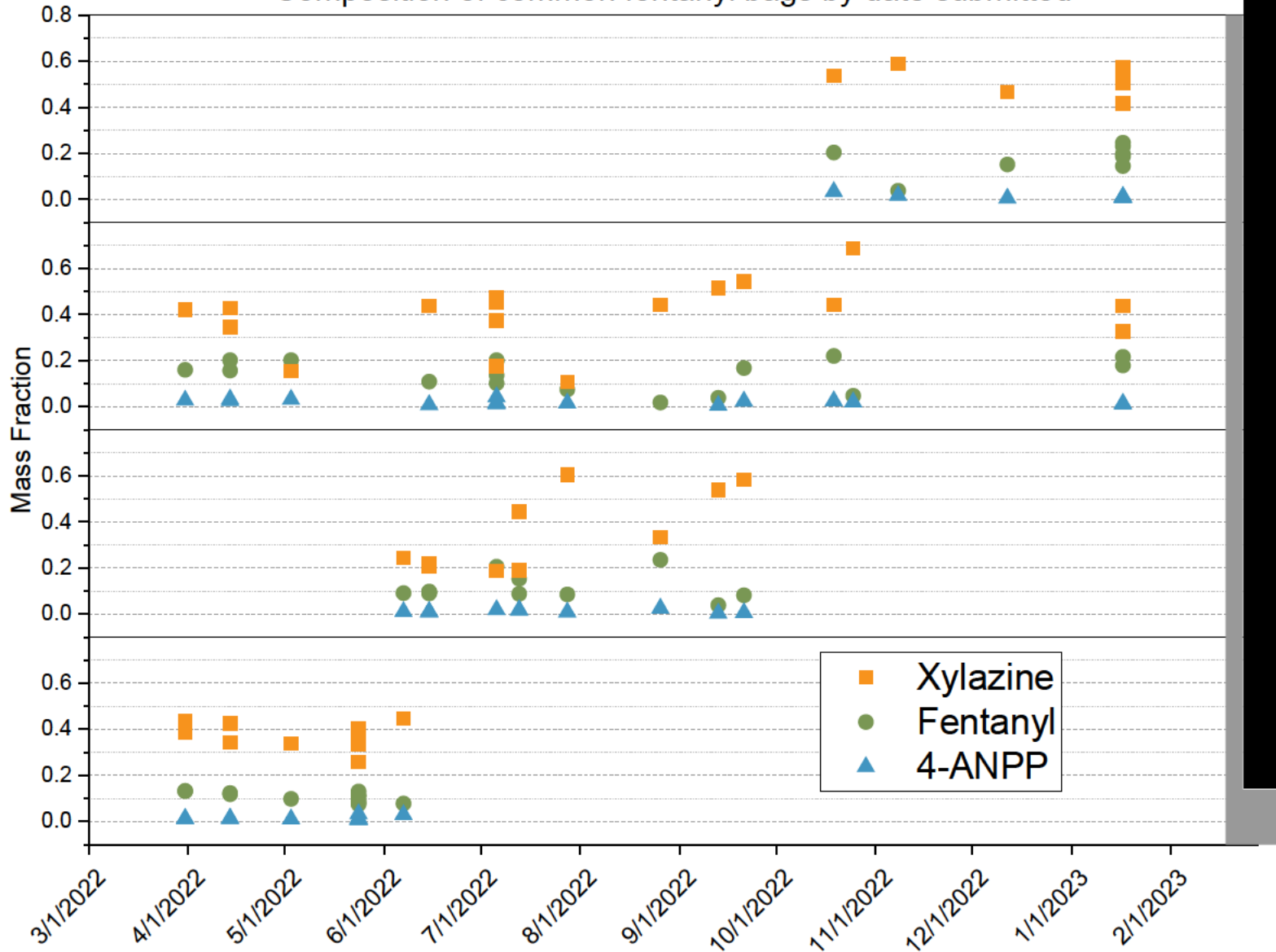
Compound	N	Mean	Standard Deviation
Fentanyl	241	13.06%	8.99%
Xylazine	229	35.76%	16.71%
Fluorofentanyl	47	2.38%	3.61%
4-ANPP	229	2.31%	2.16%
Methamphetamine	1	2.87%	--
Cocaine	2	3.21%	3.92%
Lidocaine	29	2.70%	3.85%
Levamisole	5	1.64%	1.15%

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%

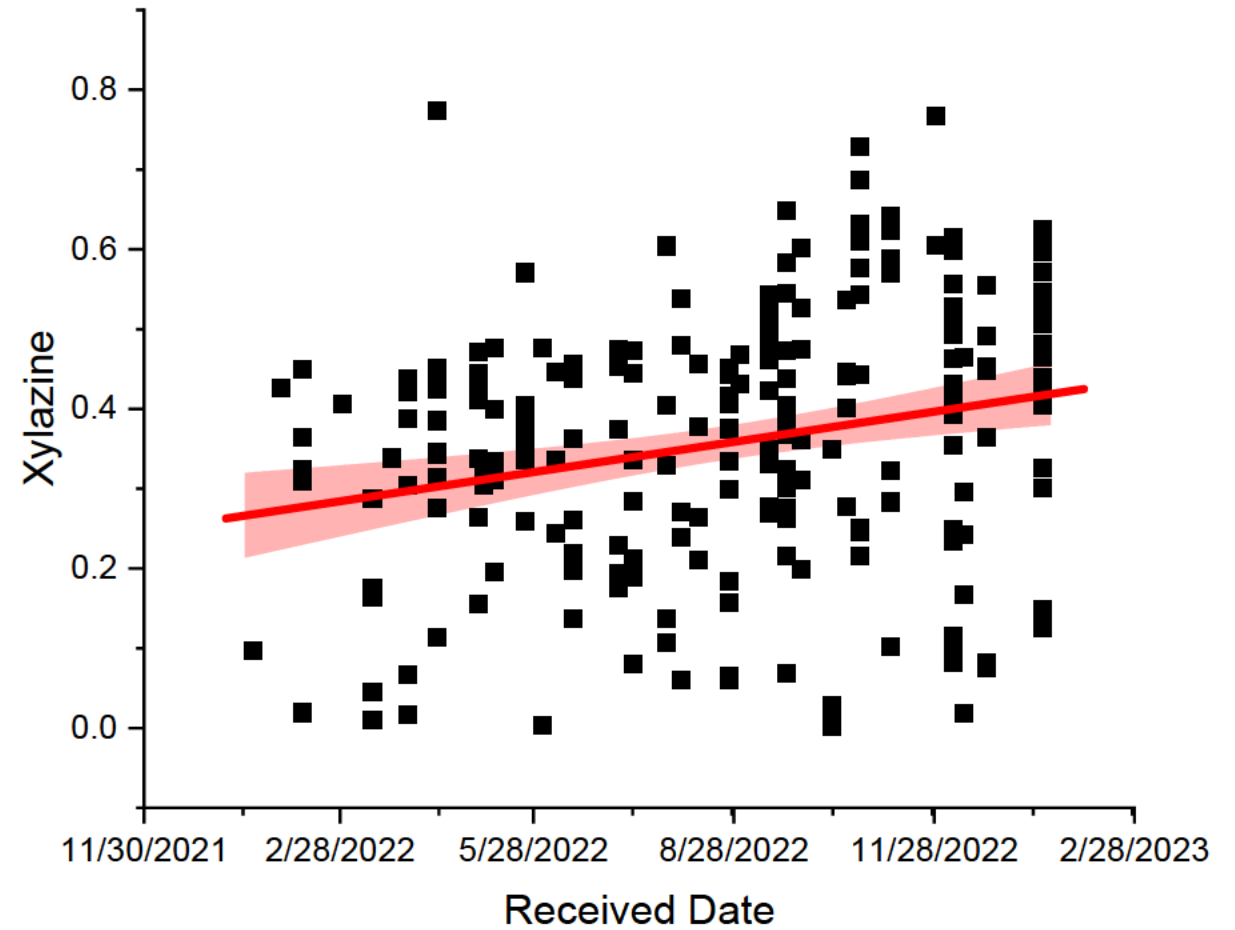
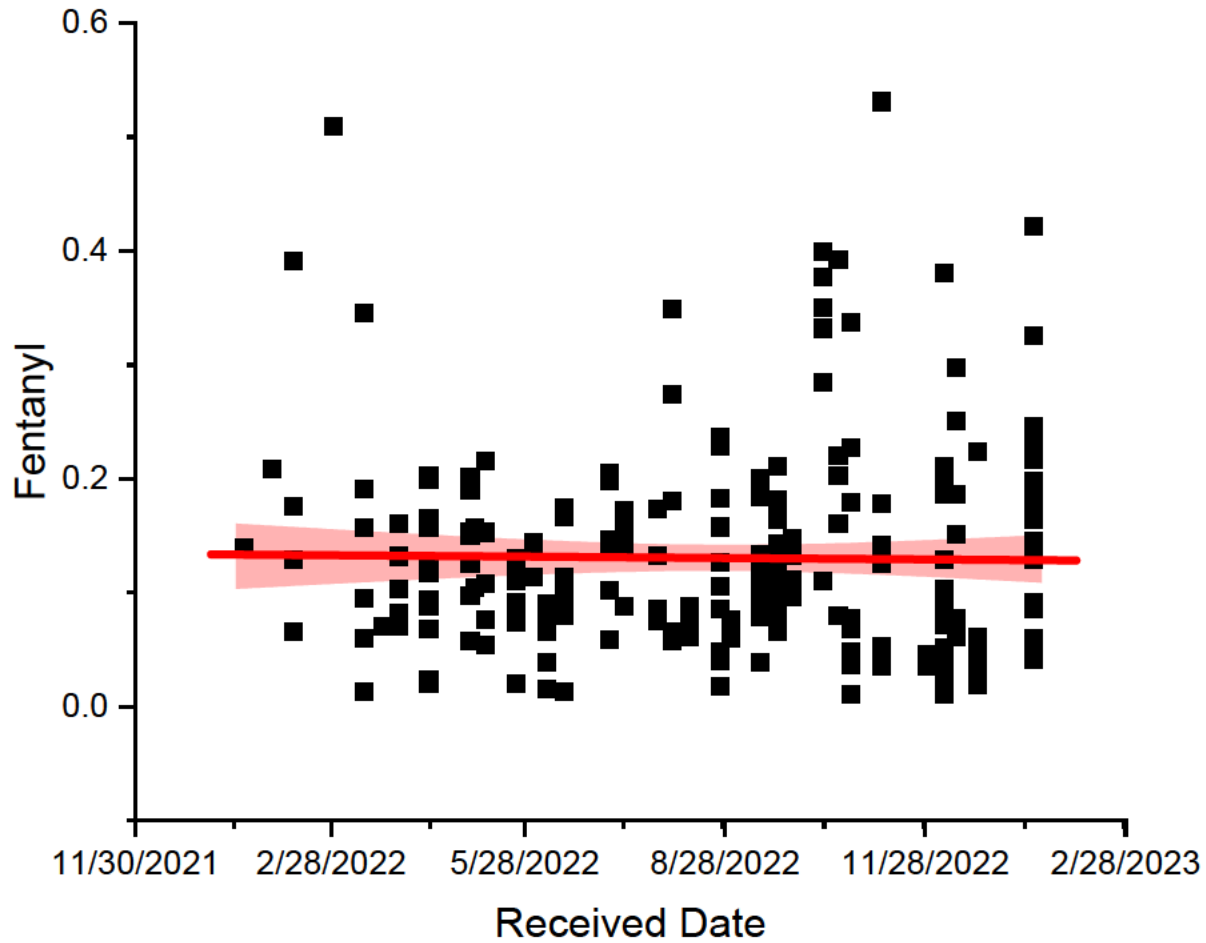
Compound	N	Mean	Standard Deviation	Lower 95% CI of Mean	Upper 95% CI of Mean	Minimum	1st Quartile (Q1)	Median	3rd Quartile (Q3)	Maximum
Fentanyl	241	13.06%	8.99%	11.92%	14.20%	1.09%	6.77%	11.21%	17.51%	53.08%
Xylazine	229	35.76%	16.71%	33.59%	37.94%	0.24%	24.53%	37.48%	46.52%	77.44%
Fluorofentanyl	47	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

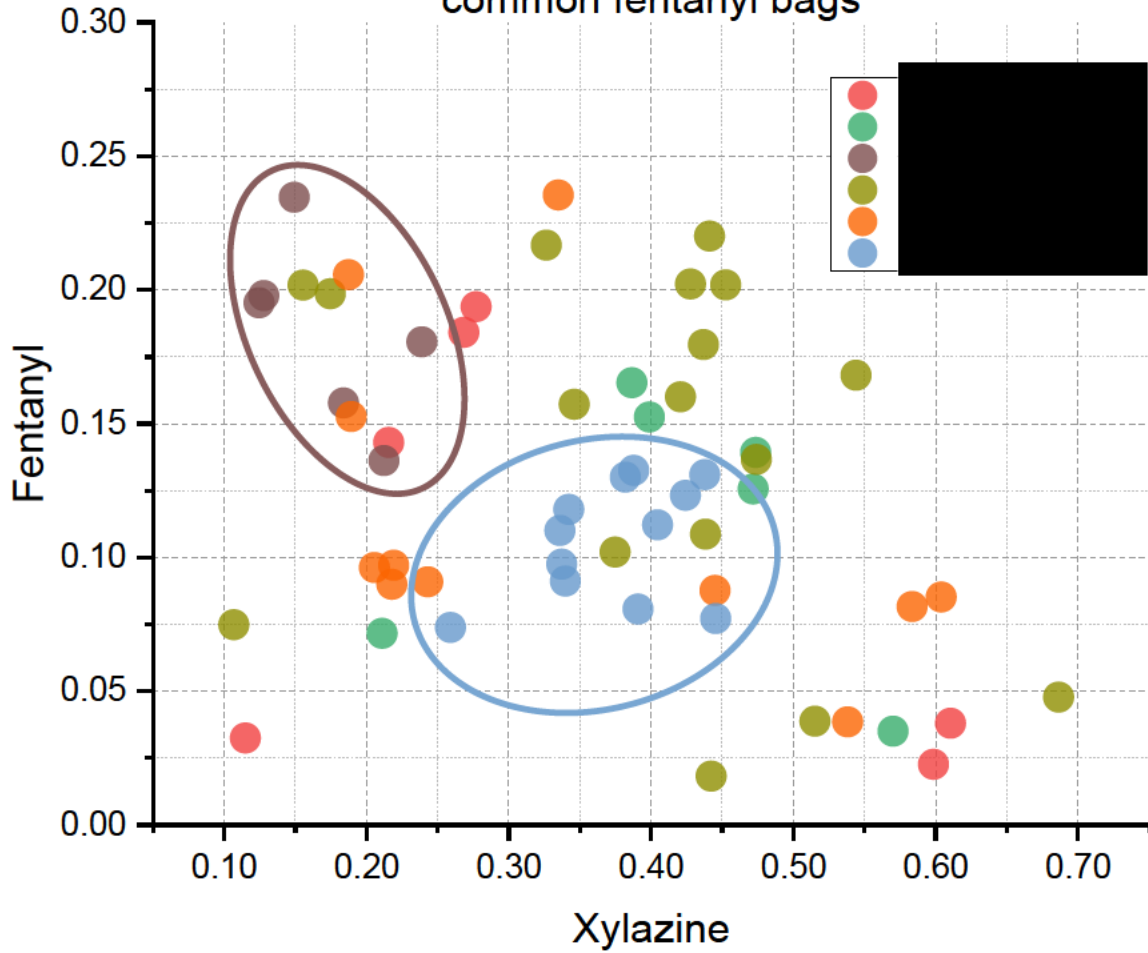




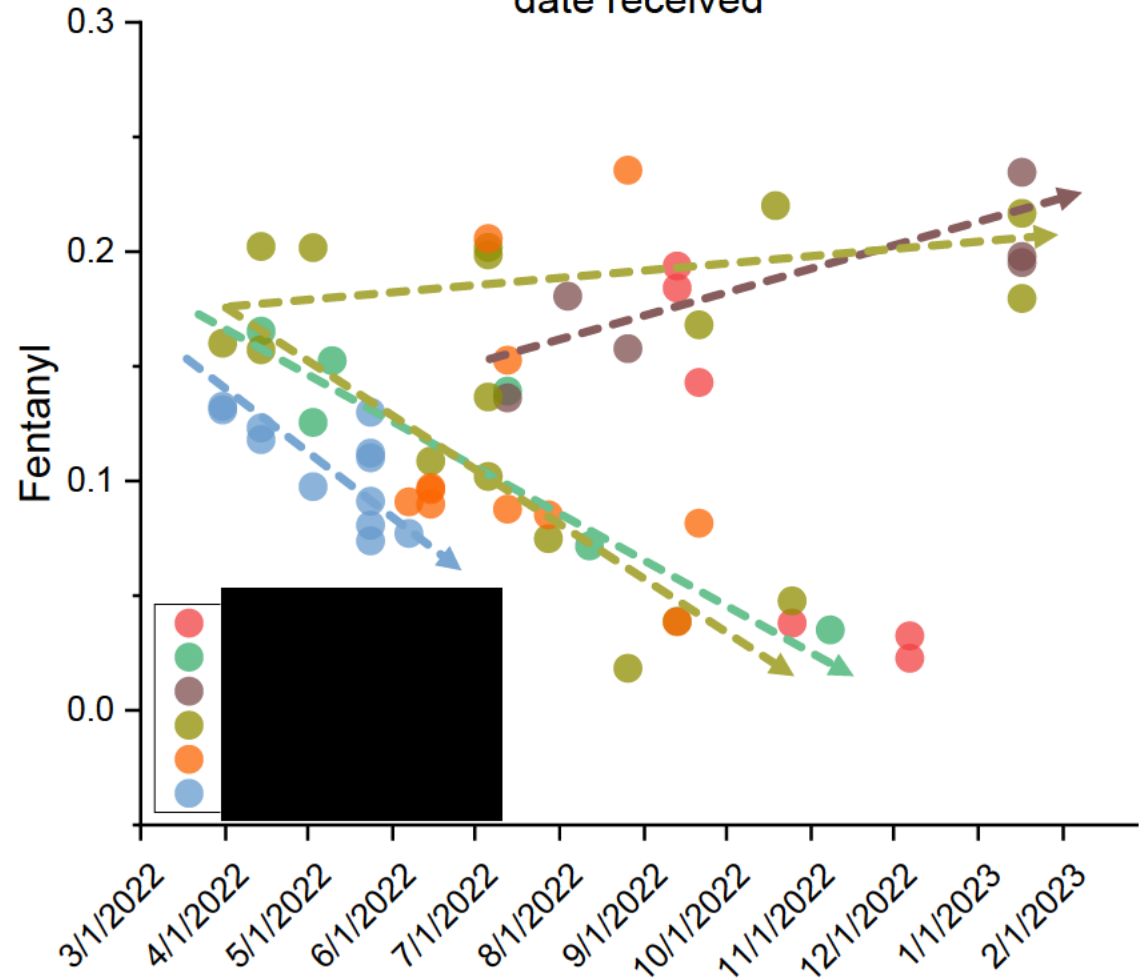
# General yearly observations in Philly fentanyl samples for 2022



Fentanyl and xylazine composition for common fentanyl bags



Fentanyl composition (mass fraction) by date received



## 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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