

# Comparative Analysis of Opioids by UPLC-MSMS and GC-TOF

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# Overview

- PHILIS
- Why Opioids
- Instrumentation
- Extraction Methods
- Results
- Discussion
- Future Development

## PHILIS

- Mobile
- Support EPA
- Onsite
- Real-time
- Cost effective



## Mission

Provide analytical testing support for consequence management during an incident of national significance or natural disaster

## Current Capabilities

- Chemical warfare agents
- Industrial toxins
- Volatile organics (8260D)
- Semivolatile organics (8270D)
- DRO
- PCB
- TO-17
- Pesticides
- Herbicides

## Why Opioids?

- Toxicity
- Increase in opioid incidence
- Weaponization
- Proper PPE
- Proper decontamination measures
- Proactive

# Instrumentation

## UPLC-MSMS

- Vanquish Binary Pump
- Vanquish Autosampler
- Vanquish DAD Detector
- Vanquish Column Compartment
- ThermoFisher Altis-TQD



## GC-TOF

- Agilent 7890B GC system
- Agilent 7693 Autosampler
- LECO Pegasus-BT TOF mass spec.



# UPLC-MSMS Parameters

Mobile Phases

A: 0.01%FA + 2.5mM NH<sub>4</sub>OFA in Water

B: 100% MeOH

5uL injection

**Method Update: 1uL injection**

Column Compartment			
Column	Waters Acquity HSS T3 column, 2.1 mm x 150 mm, 1.8 $\mu$ m particle size		
Column temp (C)	50		
Air	Forced		
Fan	5		
Preheater Left ( C )	50		
UPLC Gradient			
Line No.	Time (min)	Flow Rate (mL/min)	%B
1	0	Run	
2	0	0.25	10
3	0.5	0.25	10
4	6	0.25	60
5	8	0.25	98
6	12	0.25	98
7**	12	0.25	10
8	New Row		
9	14	STOP RUN	
TSQ Altis Parameters			
Global Parameters		Scan Parameters	
Ion Source Properties		SRM Properties	
Method duration (min)	14	Polarity	Positive
Ion source type	H-ESI	Use cycle time	Yes
Spray voltage	Static	Cycle Time (sec)	0.8
Positive ion (V)	500	Use calibrated RF lens	No
Negative ion (V)	2500	Q1 resolution (FWHM)	0.7
Sheath gas (arb)	50	Q3 resolution (FWHM)	0.7
Aux gas (arb)	10	CID gas (mTorr)	1.5
Sweep gas (arb)	1	Source fragmentation	0
Ion transfer tube (C)	325	Chromatographic peak width (s)	6
Vaporizer (C)	350	Use chromatographic filter	Yes
		Use Retention time reference	No

# SRM Table

SRM Table

Compound	Start Time (min)	Time (min)	End Time (min)	Precursor (m/z)	Product (m/z)	Collision Energy (V)	Min Time (ms)	Dwell Time (ms)	RF Lens (V)	Use	Quan	Ion
Acetylentanyl	5.5		9.5	323.175	105.125	36.13	30.61		71		False	
Acetylentanyl	5.5		9.5	323.175	188.137	22.73	30.61		71		True	
Acetylentanyl	5.5		9.5	323.175	202.137	22.48	30.61		71		False	
Fentanyl	5.5		9.5	337.25	105.196	37.48	30.61		72		False	
Fentanyl	5.5		9.5	337.25	188.208	22.94	30.61		72		True	
Fentanyl-D5	5.5		9.5	342.27	105.125	36.17	30.61		70		False	
Fentanyl-D5	5.5		9.5	342.27	188.208	22.82	30.61		70		True	
Heroin	5.5		9.5	370.088	211.125	31.12	30.61		79		False	
Heroin	5.5		9.5	370.088	268.054	28.88	30.61		79		True	
Heroin	5.5		9.5	370.088	328.155	26.4	30.61		79		False	
Remifentani I	5.5		9.5	377.138	285.125	19.28	30.61		62		False	
Remifentani I	5.5		9.5	377.138	317.137	15.95	30.61		62		True	
Remifentani I	5.5		9.5	377.138	345.125	12.54	30.61		62		False	
Heroin-d9	5.5		9.5	379.175	212.125	31.58	30.61		89		False	
Heroin-d9	5.5		9.5	379.175	272.226	29.47	30.61		89		True	
Heroin-d9	5.5		9.5	379.175	335.208	27.49	30.61		89		False	
Sulfentanil	5.5		9.5	387.175	111.125	36.97	30.61		72		False	
Sulfentanil	5.5		9.5	387.175	238.125	18.9	30.61		72		True	
Sulfentanil	5.5		9.5	387.175	355.208	19.2	30.61		72		False	
Carfentanil	5.5		9.5	395.3	246.155	21.47	30.61		71		False	
Carfentanil	5.5		9.5	395.3	335.238	18.44	30.61		71		True	
Carfentanil	5.5		9.5	395.3	363.208	13.26	30.61		71		False	

# GC-TOF Parameters

Column: Restek Rxi-5Sil MS  
30m, 0.25mm ID, 0.25um df

4mm single gooseneck liner

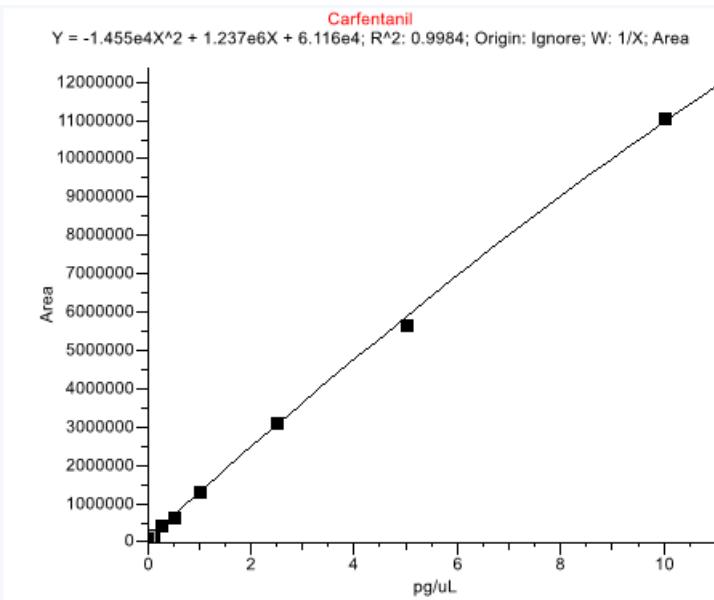
1uL injection

Inlet flow(s):	Rate (mL/min <sup>2</sup> )	Target Flow (mL/min)	Duration (min)
1	Initial	1.2	Entire Run

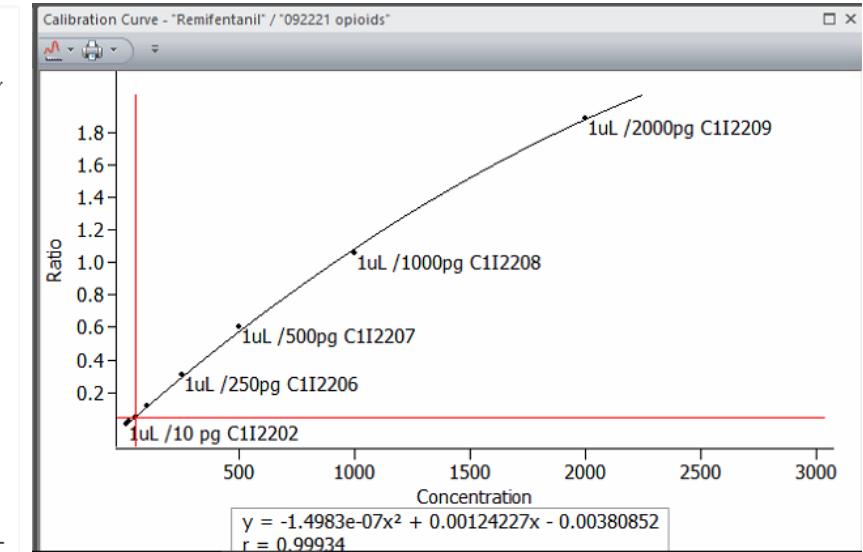
Inlet Parameters	
Injection Type:	Pulsed Spitless
Select the column mode:	Constant Flow
Septum Purge Flow (mL/min):	3
Septum Purge Mode	Standard
Inlet Pulse Pressure ( psi )	80
Inlet Pulse Duration ( minutes )	1.5
Inlet Purge Time (sec)	90
Inlet Purge Flow (mL/min)	20
Splitless Inlet Total Flow (mL/min)	21.2
Inlet Gas Saver	Yes
Inlet Gas Saver Flow ( mL/min )	15
Inlet Gas Saver Time ( minutes )	7

GC Temp Program			
Line No.	Rate (C/min)	Target Temp (C)	Duration (min)
1	Initial	110	1.5
2	40	250	0
3	30	300	0
4	25	330	4.2

# Calibration Ranges



UPLC-MSMS



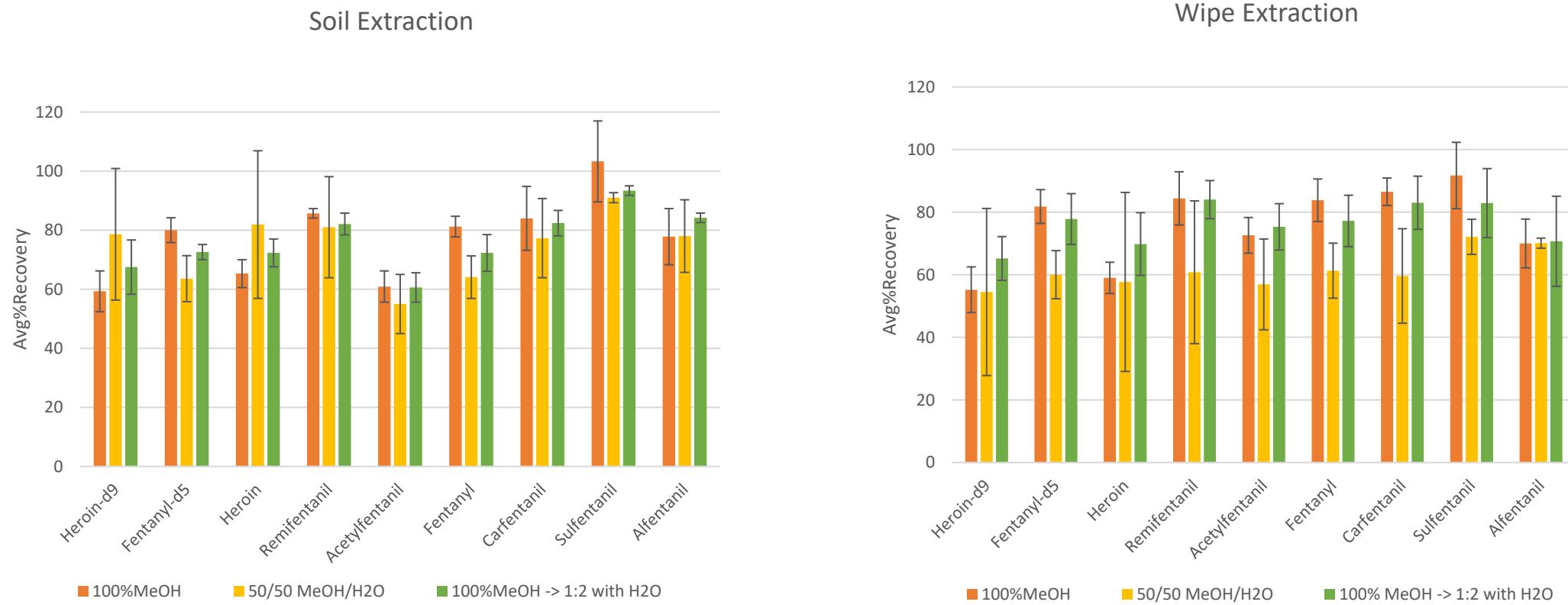
GC-TOF

## Linear Dynamic Range

	UPLC-MSMS	GC-TOF	Units
Low	0.025	10	pg/uL
High	10	2000	pg/uL

# Solvent Extraction Recoveries

## UPLC-MSMS

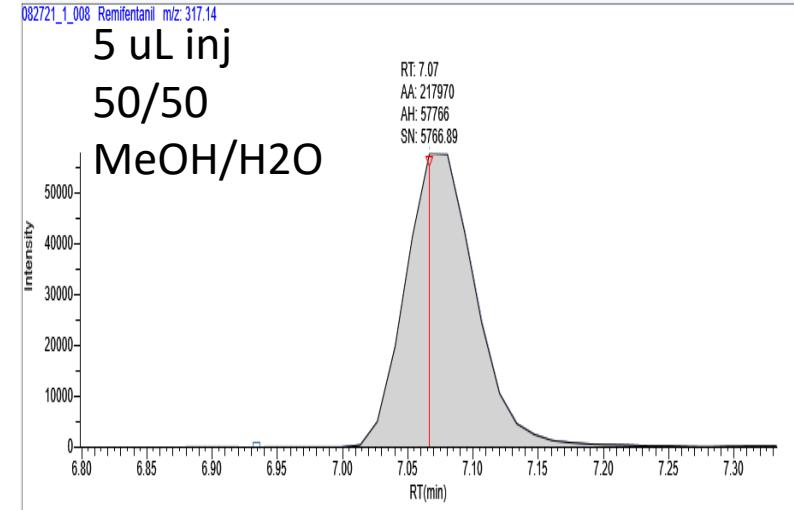
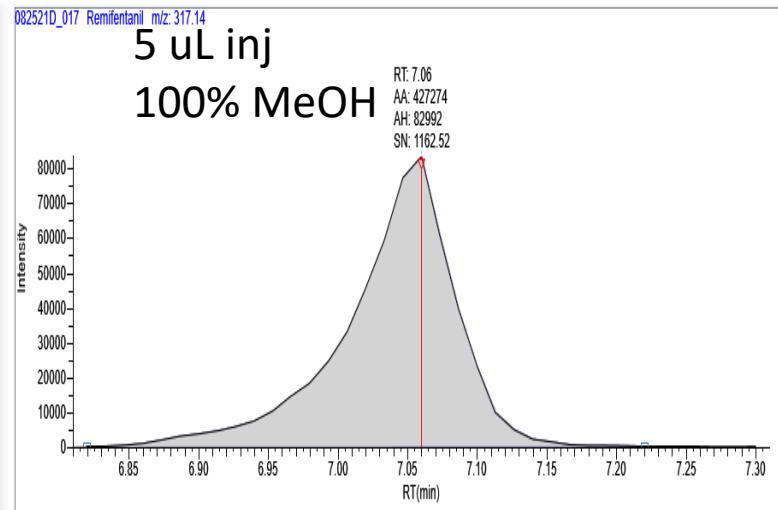


# Solvent Extraction

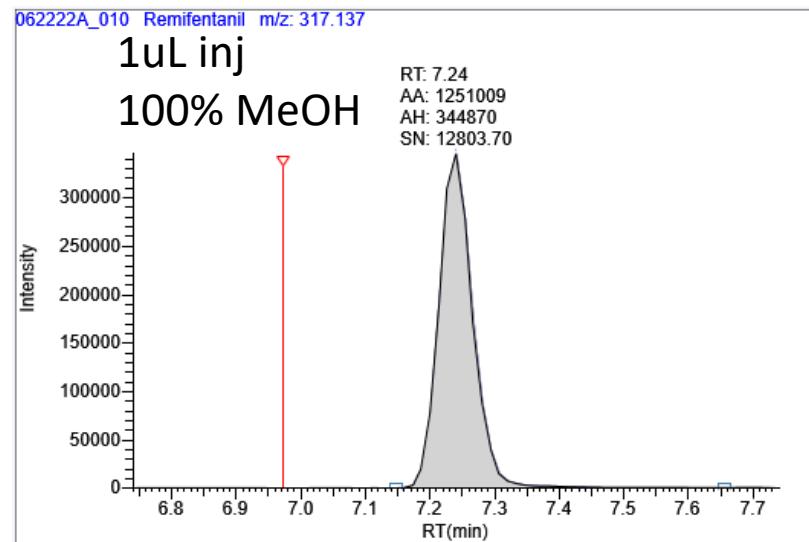
## Chromatography

### UPLC-MSMS

During the initial development of the study



Method Update: Current Parameter



## Sample Preparation

### Matrices of Interest

- Soil
- Water
- Wipe

## Preparation of Soil

### UPLC-MSMS

- 5 g of soil
- 15 mL of MeOH
- Vortexed and shaken
- Syringe driven filter
  - Method Update: Filtration removed and now centrifuged
- Direct injection
- Sample prep time ~30 minutes
- Analytical run time 14 min
- Injection to injection 14.5 mins

### GC-TOF

- 30 g of soil
- BUCHI extractor, MeOH ~40 mL
- Concentrate to 1 mL
- Sample prep time ~270 min
- Analytical run time 12 min
- Injection to injection ~15min



# Soil Recoveries

## Raw Data

### • UPLC-MSMS

File	082721_1_006	082721_1_007	082721_1_008	082721_1_009	Average	% Recovery	Std dev	% RSD
Analyte	pg/uL							
Heroin-d9	3.80	3.40	3.19	3.11	3.38	67.51	0.31	9.18
Fentanyl-d5	0.38	0.36	0.36	0.35	0.36	72.55	0.01	2.57
Heroin	3.82	3.68	3.54	3.43	3.62	72.32	0.17	4.70
Remifentanil	0.42	0.42	0.40	0.39	0.41	82.10	0.02	3.66
Acetyl fentanyl	0.32	0.31	0.30	0.28	0.30	60.55	0.02	5.04
Fentanyl	0.39	0.36	0.34	0.35	0.36	72.30	0.02	6.17
Carfentanil	0.44	0.40	0.42	0.40	0.41	82.40	0.02	4.32
Sufentanil	0.47	0.46	0.46	0.47	0.47	93.40	0.01	1.58
Alfentanil	0.42	0.43	0.42	0.41	0.42	84.15	0.01	1.57

Expected recoveries: 0.5 (5.0 heroin) pg/uL on instrument

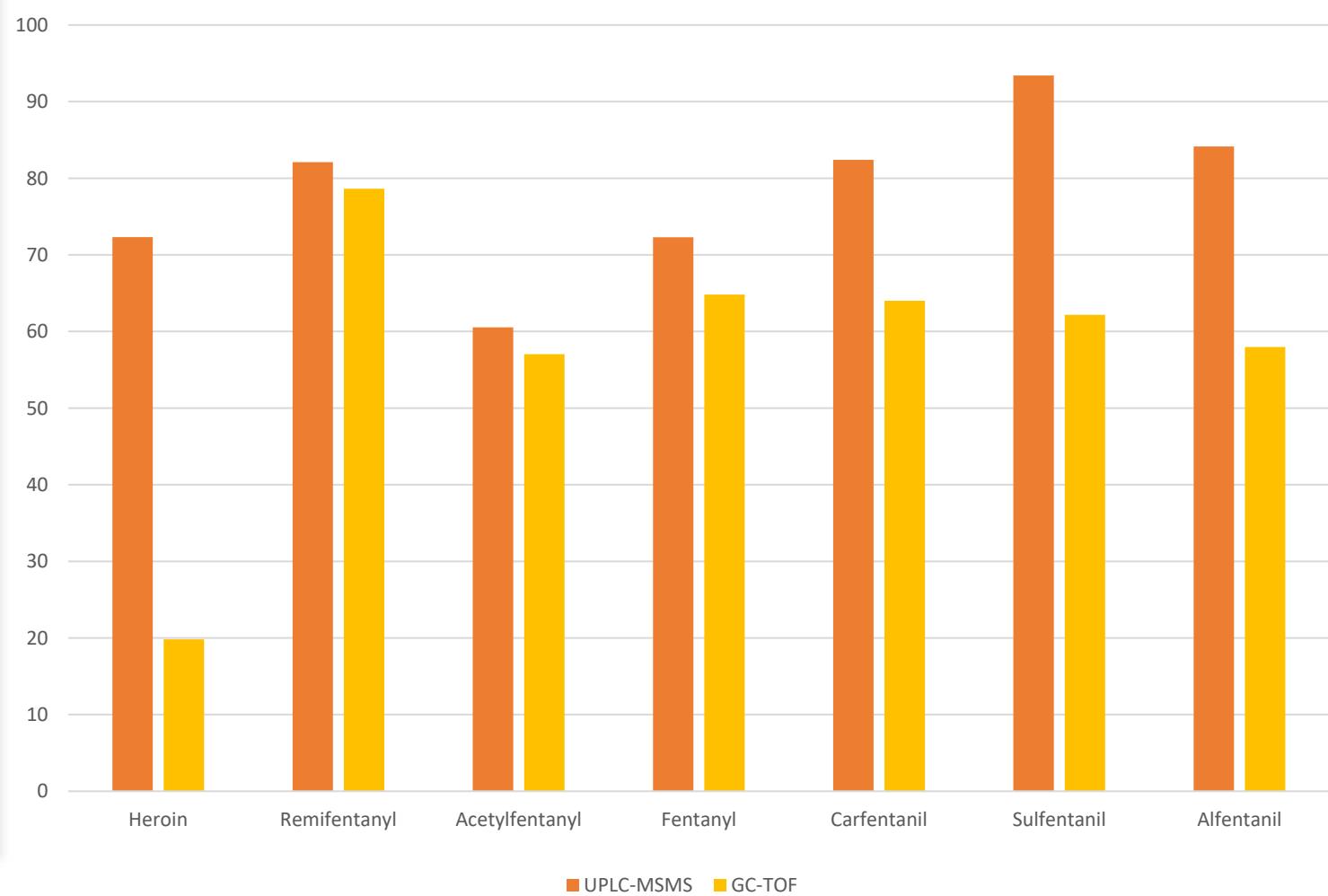
### • GC-TOF

File	C1I2801-MRL5	C1I2801-MRL6	C1I2801-MRL7	C1I2801-MRL8	Average	Recovery	Std dev	% RSD
Analyte	pg/uL							
Carfentanil-d5	32.58	38.50	26.04	24.44	30.39	60.78	6.45	21.23
Acetyl fentanyl	31.35	35.53	24.67	22.53	28.52	57.04	6.00	21.02
Alfentanil	31.37	36.61	25.07	22.88	28.98	57.97	6.23	21.49
Carfentanil	34.71	40.39	27.30	25.61	32.00	64.01	6.85	21.40
fentanyl	33.46	44.10	26.16	25.90	32.41	64.81	8.55	26.38
Heroin	105.52	106.26	94.12	90.50	99.10	19.82	7.98	8.06
Remifentanil	43.35	49.16	32.38	32.38	39.32	78.64	8.35	21.25
Sufentanil	33.54	41.18	26.04	23.57	31.08	62.17	7.96	25.59

Expected recoveries: 50.0 (500.0 heroin) pg/uL on instrument

# Soil Recoveries

Average% Recoveries  
Soil



# Preparation of Water

## UPLC-MSMS

- 2 mL of water
- Syringe driven filter
  - Method Update: Filtration removed, now centrifuged
- Direct injection
- Sample prep time ~10 mins
- Analytical run time 14 min
- Injection to injection 14.5 mins

## GC-TOF

- 50 mL of water
- 5 mL of MeCl<sub>2</sub>
- 5-minute shake
- 1 mL aliquot taken
- Sample prep time ~25 min
- Analytical run time 12 min
- Injection to injection ~15min

# Water Recoveries

## Raw Data

### • UPLC-MSMS

File	082521D_011	082521D_012	082521D_013	082521D_014	Average	% Recovery	Std dev	% RSD
Analyte	pg/uL							
Heroin-d9	8.06	8.46	8.43	8.16	8.27	83.16	0.20	2.38
Fentanyl-d5	0.71	0.82	0.64	0.82				
Heroin	7.47	8.21	8.26	7.51	7.86	79.03	0.43	5.50
Remifentanil	0.67	0.66	0.69	0.64	0.66	66.81	0.02	3.06
Acetyl fentanyl	0.75	0.88	0.71	0.83	0.79	79.82	0.08	9.72
Fentanyl	0.73	0.84	0.77	0.83	0.79	79.62	0.05	6.65
Carfentanil	0.59	0.65	0.75	0.65	0.66	66.36	0.07	10.23
Sufentanil	0.62	0.68	0.78	0.68	0.69	69.27	0.06	9.43
Alfentanil	0.65	0.51	0.65	0.61	0.61	60.80	0.07	10.97

Expected recoveries: 0.95 (9.5 heroin) pg/uL on instrument

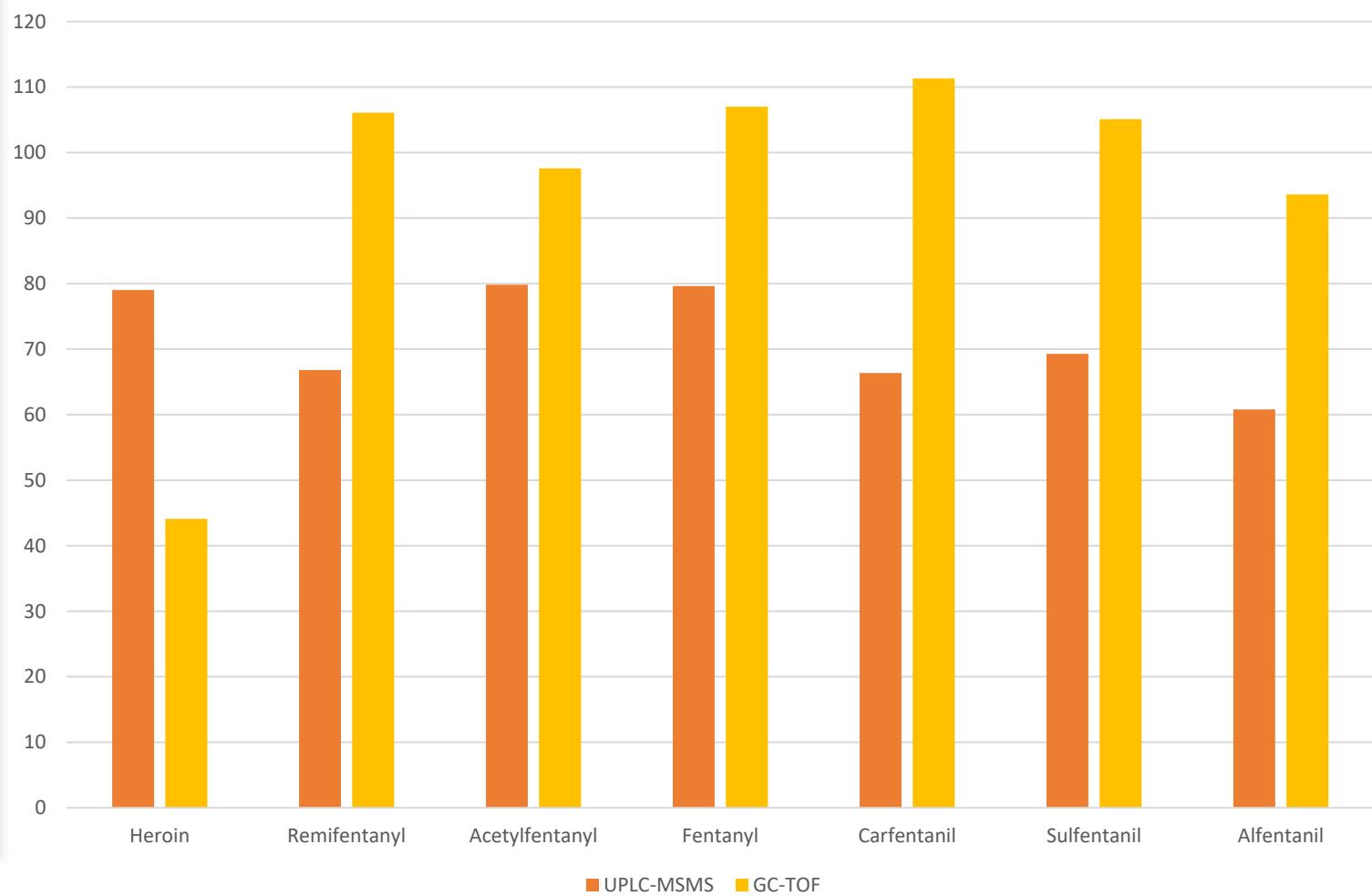
### • GC-TOF

File	C1J0101-MRL1	C1J0101-MRL2	C1J0101-MRL3	C1J0101-MRL4	Average	% Recovery	Std dev	% RSD
Analyte	pg/uL							
Carfentanil-d5	21.12	23.37	22.11	21.17	21.94	109.71	1.06	4.81
						0.00		
Acetyl fentanyl	19.11	21.21	19.34	18.41	19.52	97.59	1.20	6.13
Alfentanil	18.78	20.48	18.44	17.19	18.72	93.61	1.36	7.25
Carfentanil	21.91	23.25	22.17	21.67	22.25	111.25	0.70	3.13
fentanyl	20.88	22.60	21.36	20.73	21.39	106.96	0.85	3.97
Heroin	86.03	90.19	89.60	87.27	88.27	44.14	1.96	2.22
Remifentanil	20.51	22.78	21.41	20.14	21.21	106.05	1.17	5.54
Sufentanil	21.01	22.28	20.67	20.05	21.00	105.01	0.94	4.47

Expected recoveries: 20.0 (200.0 heroin) pg/uL on instrument

# Water Recoveries

Average% Reoveries  
Water



# Preparation of Wipes

## UPLC-MSMS

- 1 wipe
- 15 mL of MeOH, vortexed
- 15 minute shake
- Syringe driven filter
  - Method Update: Filtration removed, now centrifuged
- Direct injection
- Sample prep time ~25 minutes
- Analytical run time 14 mins
- Injection to injection 14.5 mins

## GC-TOF

- 1 wipe
- BUCHI extractor, MeOH ~40 mL
- Concentrate to 1 mL
- Sample prep time ~240 min
- Analytical run time 12 min
- Injection to injection ~15min

# Wipe Recoveries

## Raw Data

### • UPLC-MSMS

File	082721_1_011	082721_1_012	082721_1_013	082721_1_014	Average	% Recovery	Std dev	% RSD
Analyte	pg/uL							
Heroin-d9	3.07	3.49	3.06	3.42	3.26	65.15	0.23	7.01
Fentanyl-d5	0.35	0.42	0.37	0.42	0.39	77.75	0.03	8.12
Heroin	3.26	3.84	3.13	3.74	3.49	69.83	0.35	10.01
Remifentanil	0.41	0.43	0.39	0.45	0.42	84.00	0.03	6.13
Acetylfentanyl	0.35	0.40	0.35	0.40	0.38	75.30	0.03	7.41
Fentanyl	0.35	0.42	0.37	0.40	0.39	77.20	0.03	8.21
Carfentanil	0.38	0.45	0.39	0.44	0.41	82.95	0.04	8.50
Sulfentanil	0.39	0.44	0.37	0.47	0.41	82.90	0.05	10.97
Alfentanil	0.34	0.41	0.29	0.37	0.35	70.65	0.05	14.39

Expected recoveries: 0.5 (5.0 heroin) pg/uL on instrument

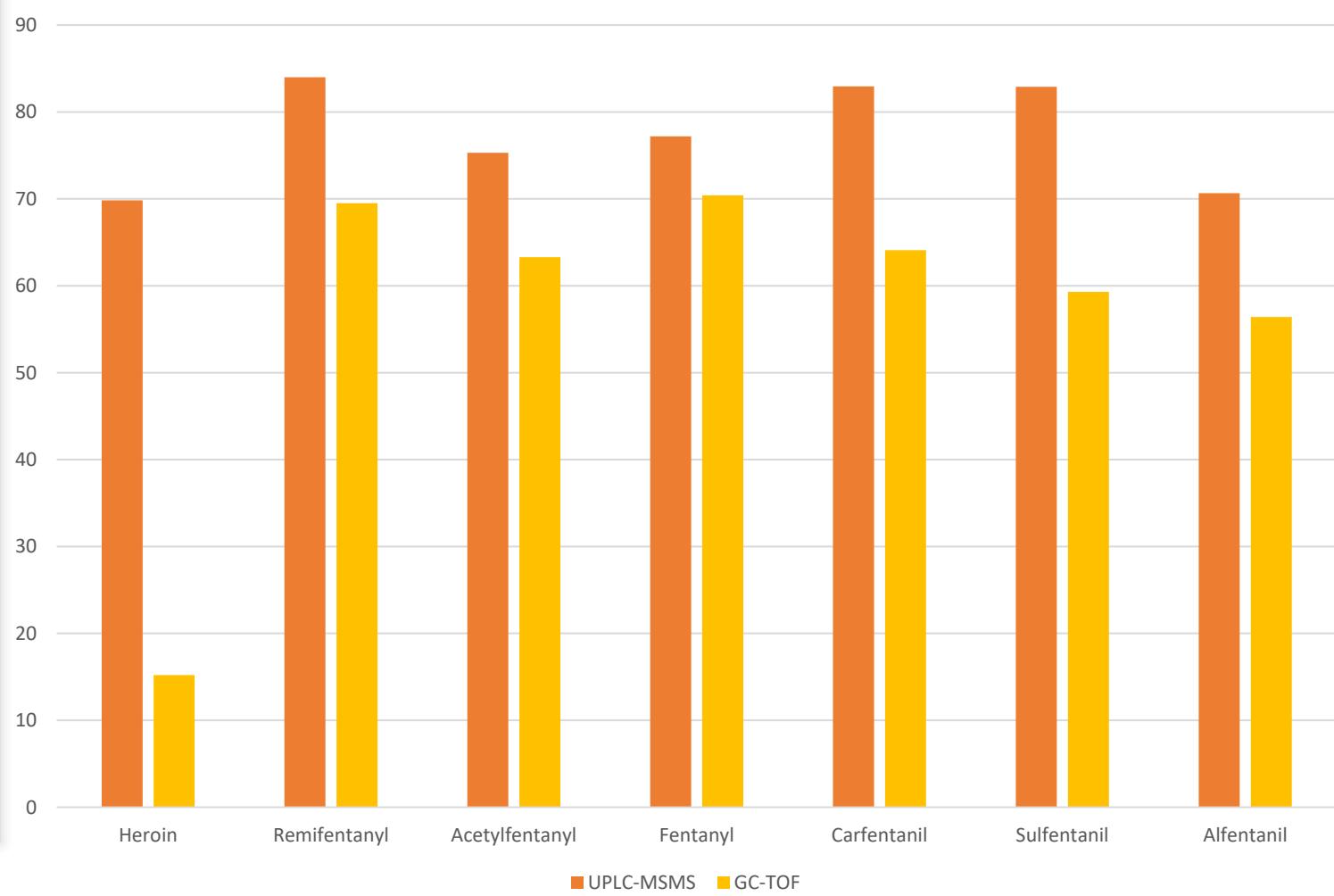
### • GC-TOF

File	C1I2801-MRL5	C1I2801-MRL6	C1I2801-MRL7	C1I2801-MRL8	Average	% Recovery	Std dev	% RSD
Analyte	pg/uL							
Carfentanil-d5	30.35	37.52	26.79	33.09	31.94	63.88	4.53	14.18
Acetylfentanyl	30.83	38.11	25.29	32.42	31.66	63.33	5.27	16.66
Alfentanil	26.76	34.93	22.68	28.41	28.20	56.39	5.10	18.07
Carfentanil	30.83	37.95	26.89	32.58	32.06	64.13	4.59	14.32
fentanyl	32.9	41.62	30.76	35.6	35.22	70.44	4.70	13.36
Heroin	78.23	78.74	71.49	74.79	75.81	15.16	3.37	4.45
Remifentanil	35.29	40.35	27.92	35.4	34.74	69.48	5.12	14.75
Sufentanil	28.55	35.52	24.56	30.05	29.67	59.34	4.54	15.29

Expected recoveries: 50.0 (500.0 heroin) pg/uL on instrument

# Wipe Recoveries

Average% Reoveries  
Wipe



# Comparison Discussion

## Instrumentation

Analyte	Reporting Limits					
	Soil ug/kg		Water ug/L		Wipe ug/wipe	
	UPLCMSMS	TOF	UPLCMSMS	TOF	UPLCMSMS	TOF
Heroin	3.75	3.33	0.25	10	0.03	0.1
Remifentanil	0.375	0.33	0.025	1	0.0015	0.01
Acetyl fentanyl	0.375	0.33	0.025	1	0.0015	0.01
Fentanyl	0.375	0.33	0.025	1	0.0015	0.01
Carfentanil	0.375	0.33	0.025	1	0.0015	0.01
Sulfentanil	0.375	0.33	0.025	1	0.0015	0.01
Alfentanil	0.375	0.33	0.025	1	0.0015	0.01

Linear Dynamic Range			
	UPLC-MSMS	GC-TOF	Units
Low	0.025	10	pg/uL
High	10	2000	pg/uL

## Comparison Discussion

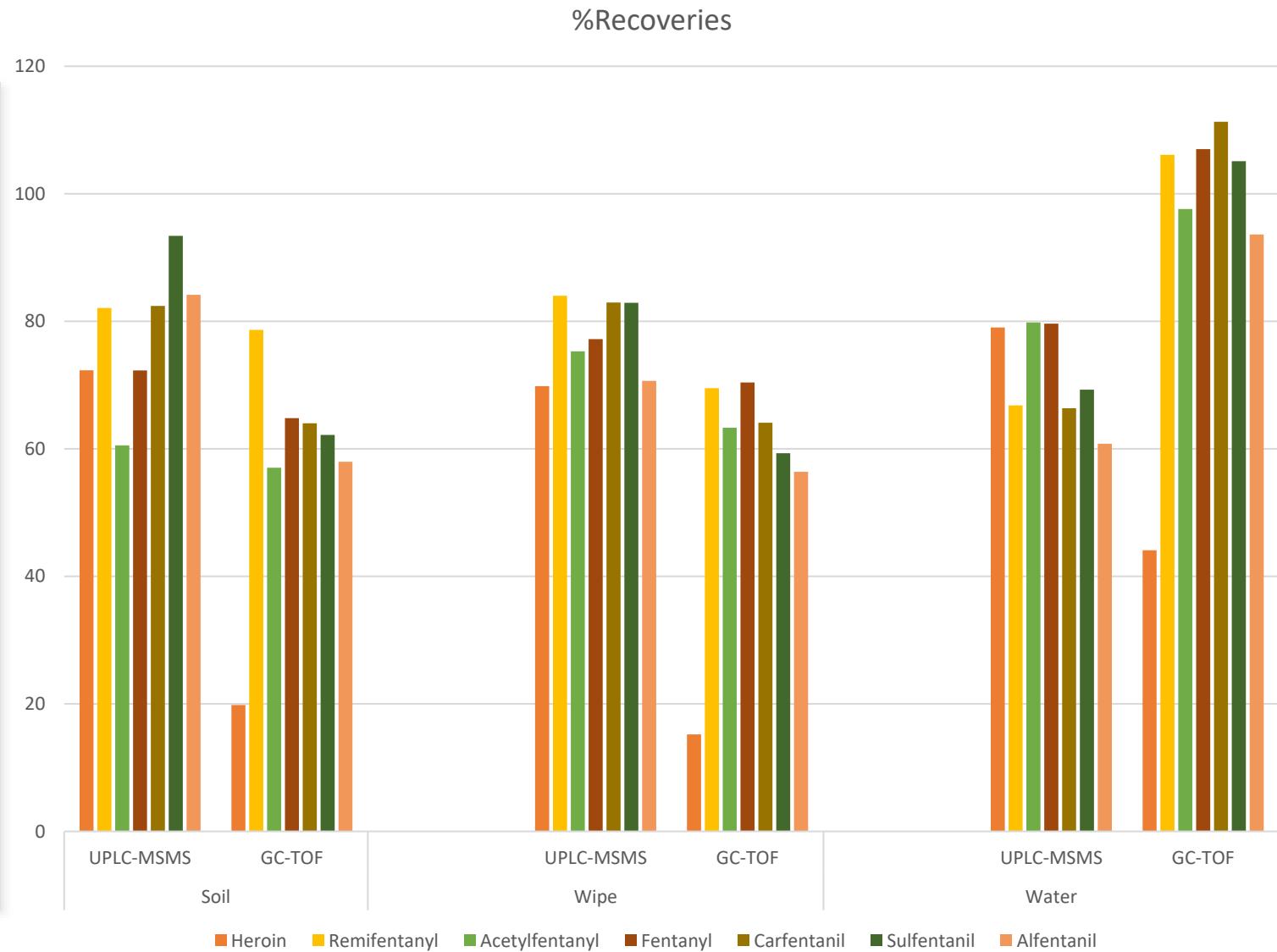
### Sample prep time

	<b>Sample prep time (min)</b>	
	UPLC-MSMS	GC-TOF
<b>Soil</b>	30	270
<b>Water</b>	10	25
<b>Wipe</b>	25	240

	UPLC-MSMS	GC-TOF
<b>Analytical run time (min)</b>	14	12
<b>Injection to injection (min)</b>	14.5	15

## Comparison Discussion

%Recoveries

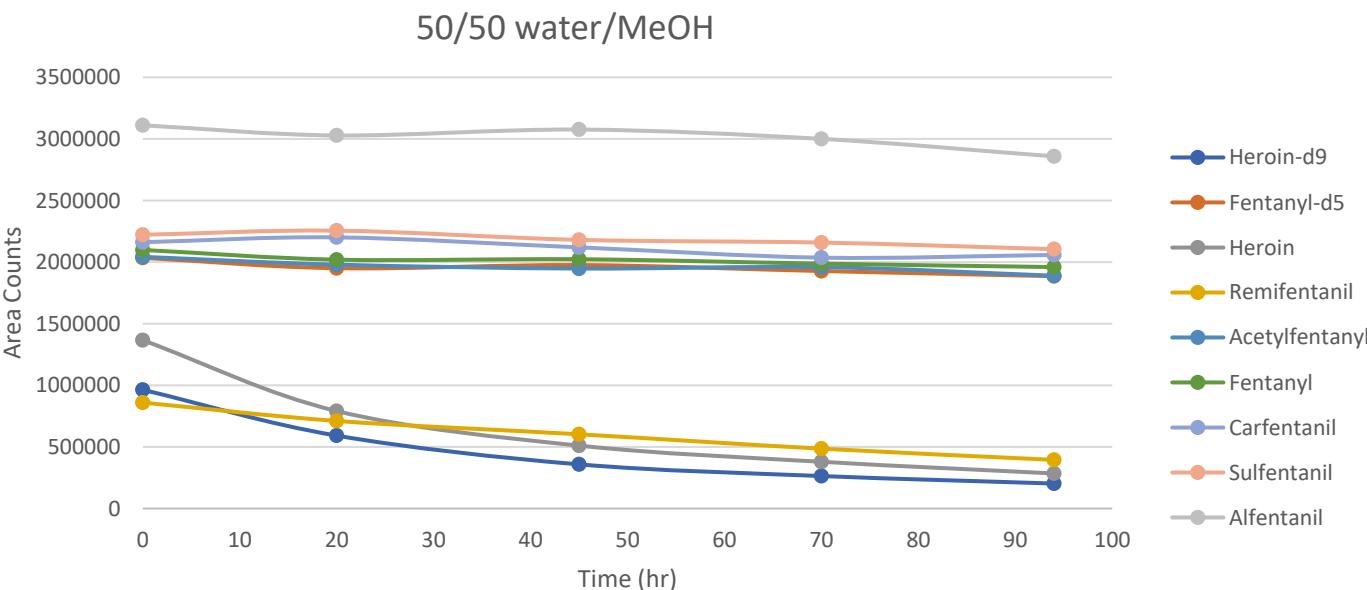
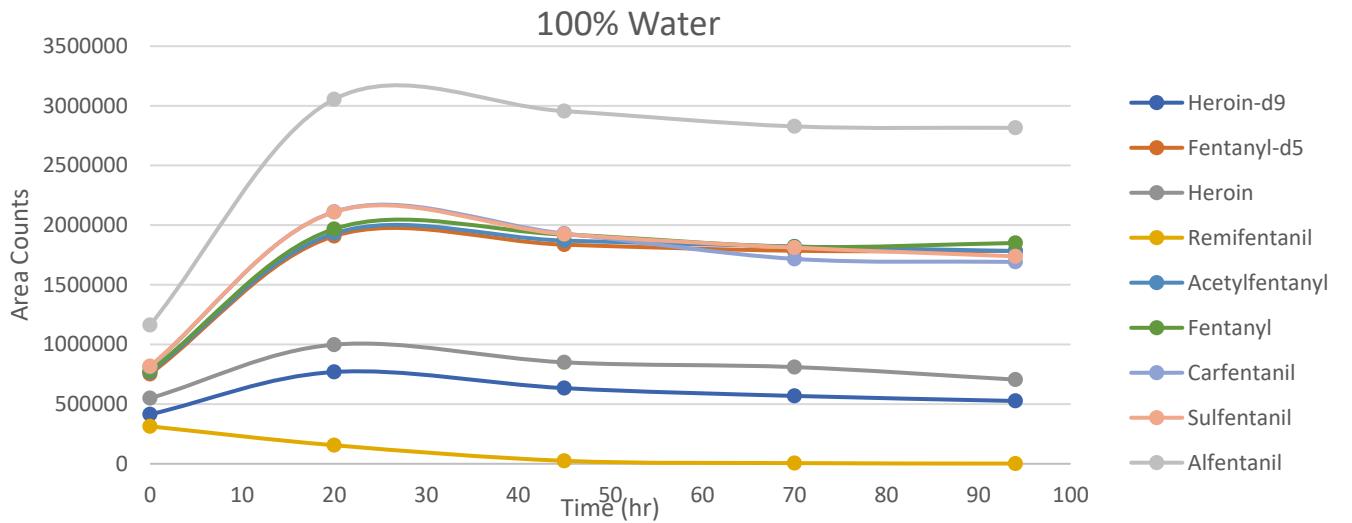


## Comparison Discussion

- Mobile Lab
- Rapid analysis
- UPLC-MSMS vs GC-TOF
- Cost effective

# Future developments

## Stability Study of Opioids



## References

- Carfentanil, PubChem, Section 12.1.1 Toxicity. [Carfentanil | C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>3</sub> - PubChem \(nih.gov\)](#)
- Doyle R., Andrada D., Sanchez-Woehler A., Espinosa D. Quantitative analysis of 40 Fentanyl, its Precursors, Analogues and Metabolites in Urine, Oral fluids and Blood using LC-MS/MS for forensic use TP189. Poster resource. [Quantitative Analysis of 40 Fentanyl, its Precursors, Analogues, and Metabolites in Urine, Oral Fluids, and Blood Using LC-MS/MS for Forensic Use \(thermofisher.com\)](#)
- Travis J., Garcia J., PHILIS contract SOP L-A-310 Rev.2, Standard Operating Procedure for Opioids on Soil, Water and Wipes, by ALTIS UPLC/MS/MS. Internal document, 09/08/2021
- Morekas G., CSS-inc Quality Assurance Policy. Internal document, 04/18/2017
- Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, EPA publication SW-846, Third Edition, Final updates I (1993), II (1995), IIA (1994), IIB (1995), III (1997), IIIA (1999), IIIB (2005), IV (2008), and V (2015).

# Questions

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Feel free to contact me via e-mail

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