

Quantification of U-47700 and Metabolites in Plasma by LC-MS/MS

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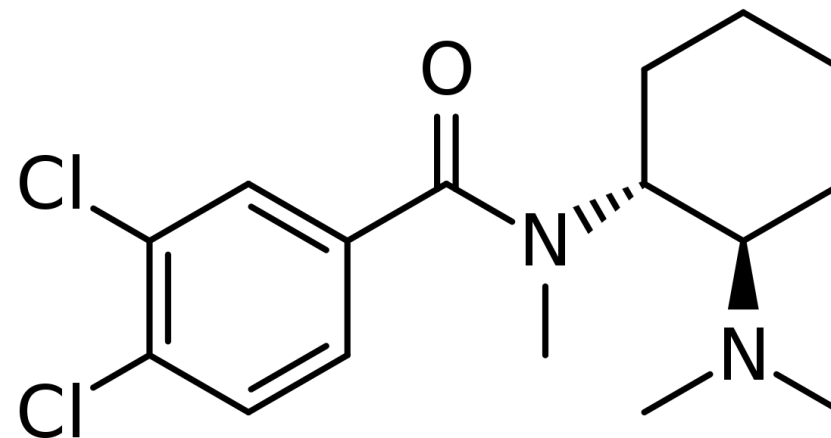
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Disclaimer

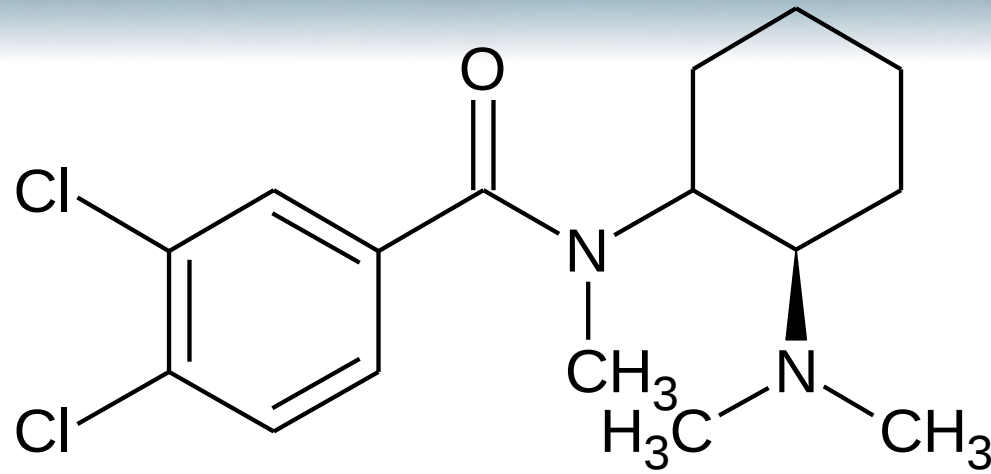
- The authors have no conflicts of interest to disclose.

U-47700

- Novel Synthetic Opioid
- 1970s – Upjohn Company
- Selective agonist on the μ -opioid receptor
- Analgesic and therapeutic properties
- 7.5x more potent than morphine
- Schedule I
- 2 metabolites

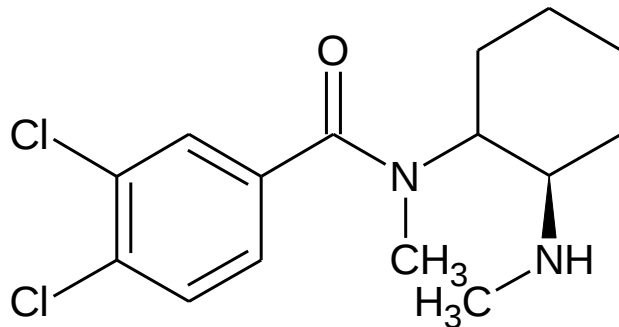


Metabolism



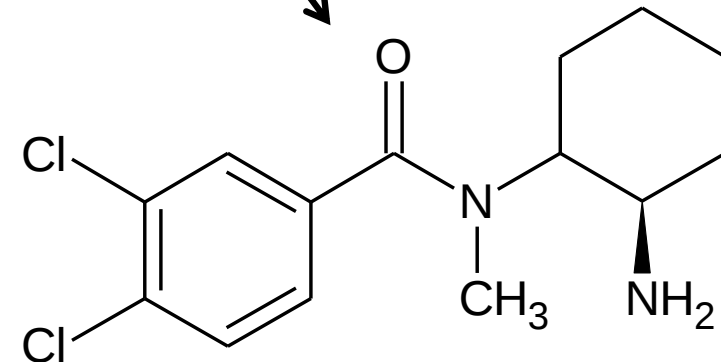
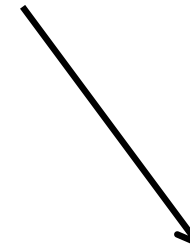
U-47700

N-demethylation



N-desmethyl-U-47700 (dm-U4)

N-demethylation



N,N-didesmethyl-U-47700 (ddm-U4)

Prevalence

- Emerged onto the radar in 2016
 - 46 confirmed deaths in US, November 2016 (DEA)
 - 88 forensic laboratory cases (DEA)
- Commonly mixed with fentanyl & heroin – “Gray Death”
- Famous Case: Death of Prince



Previous Literature

Study	Instrument	Matrix	LOD	LOQ	Conc. Range	Acute or Fatal
Elliot (<i>DTA</i> 2016)	LC-MS	Blood, urine	50ng/mL	312.5ng/mL	1,460ng/mL	Fatal*
Mohr (<i>JAT</i> 2016)	LC-MS/MS	Blood	0.5ng/mL	1ng/mL	17-490 ng/mL	Fatal*
Shoff (<i>JAT</i> 2017)	LC – Ion trap MS	Blood, urine	0.5ng/mL	-	-	Fatal
Seither (<i>JAT</i> 2017)	LC-MS/MS	Blood	0.1ng/mL	-	-	Acute
Fleming (<i>JAT</i> 2016)	LC-MS/MS LC-QToF	Urine	1ng/mL	1ng/mL	<LOQ, 140, 224ng/mL	Acute
Jones (<i>Clin Tox</i> 2016)	LC-MS	Serum, urine	Not listed	Not listed	228, 394ng/mL	Acute*

Objectives

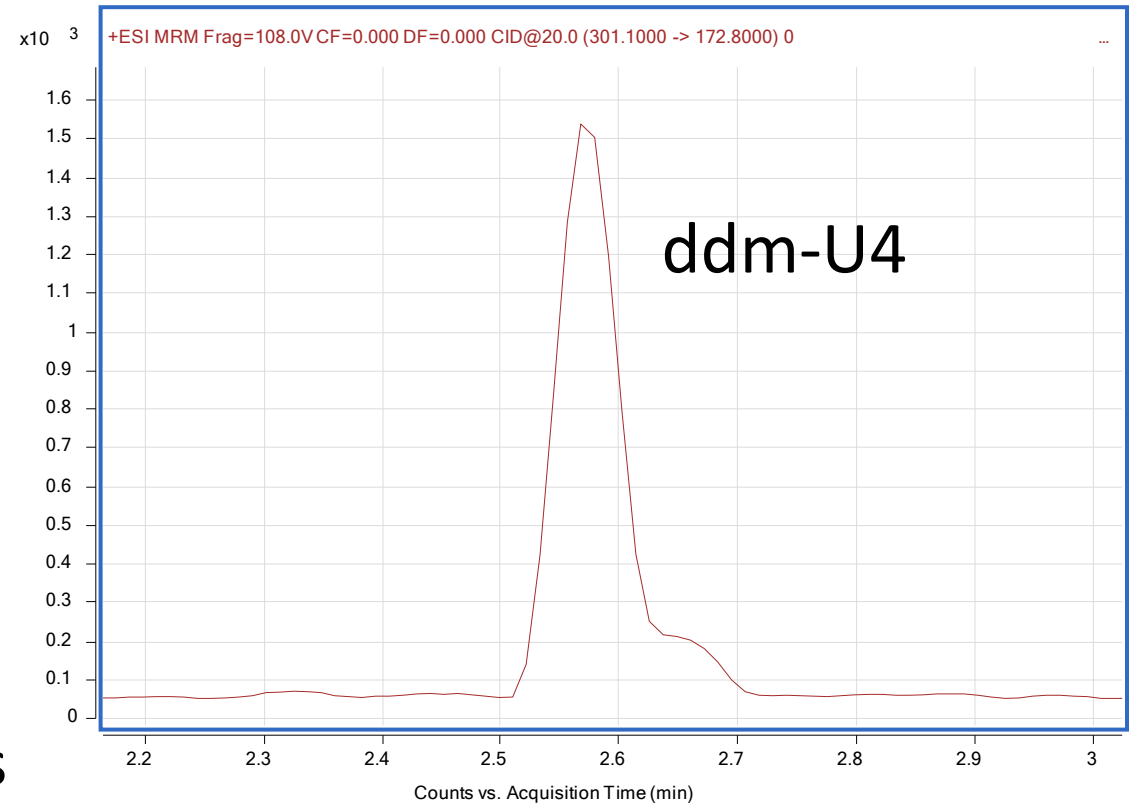
- To successfully optimize an extraction method for U-47700 and its metabolites from plasma.
- To successfully separate U-47700 and its metabolites using liquid chromatography.
- To validate a method for U-47700 and its metabolites to be quantified using tandem mass spectrometry using a small sample volume (<100μL).

Optimized Instrument Conditions

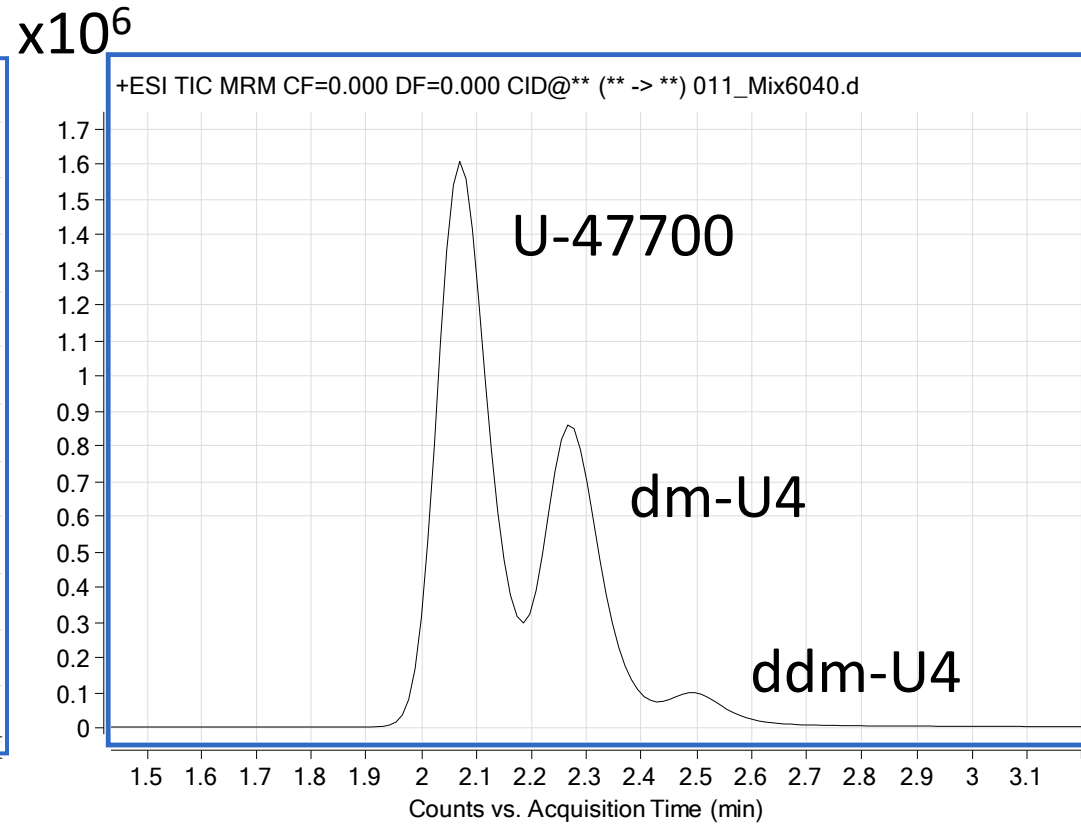
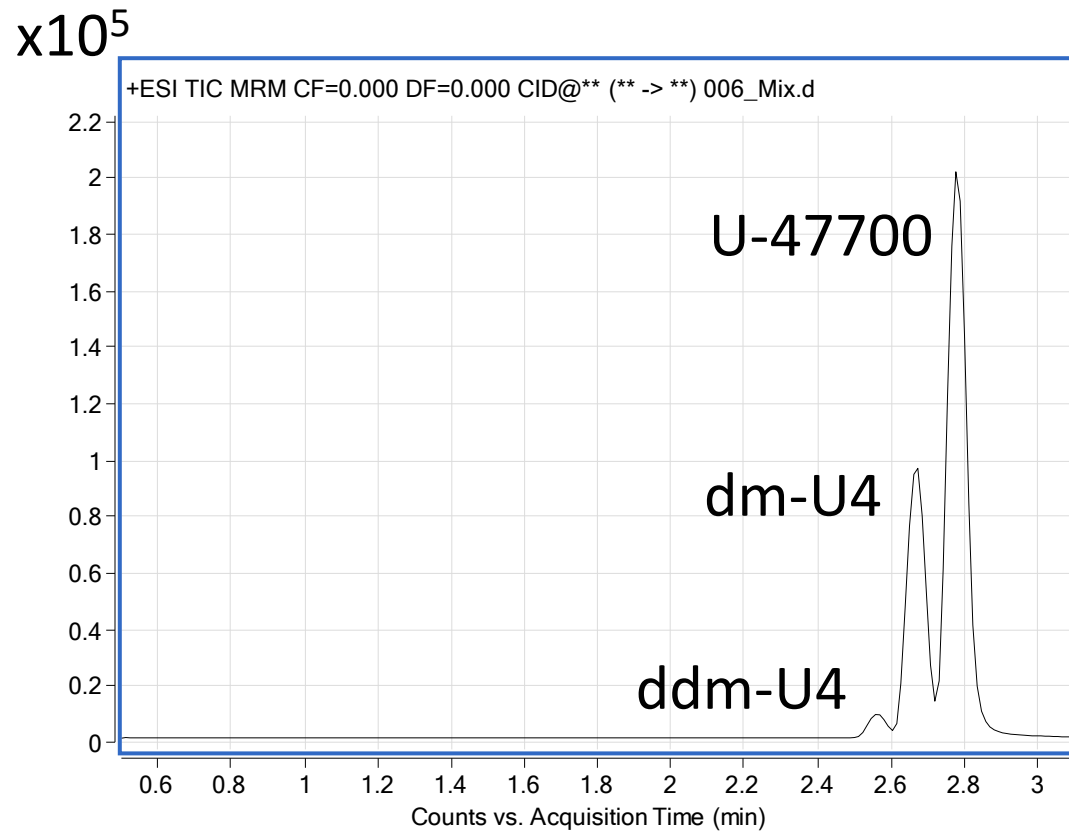
- Agilent 1290 Infinity Liquid Chromatograph
- Agilent 6470 Triple Quadrupole Mass spectrometer
- Agilent ZORBAX Eclipse Plus C18 column (1.8 μm , 2.1 x 50 mm) with matching guard.
- Isocratic elution
 - Mobile phase A:B at 60:40
 - A: 5mM ammonium formate with 0.05% formic acid in water
 - B: 0.1% formic acid in methanol
 - 0.4 mL/min flow rate
- Multiple Reaction Monitoring (MRM) mode

Method Development

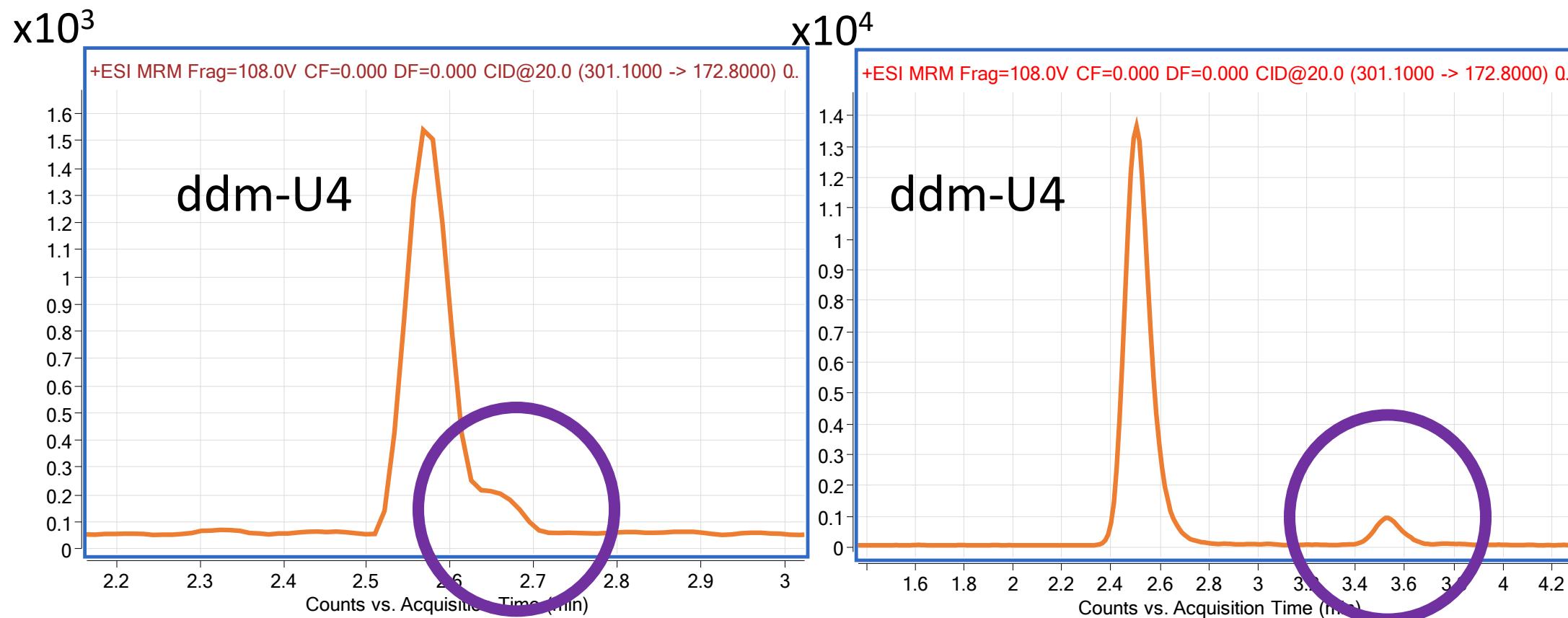
- Chromatography
 - Column:
 - Poroshell C18 EC column
 - ✓ Zorbax C18+ Eclipse
 - Mobile Phase:
 - Gradient to isocratic
 - Acetonitrile to methanol
- Extraction Method:
 - Reconstitution and Injection Volumes



TIC (ACN v MeOH)

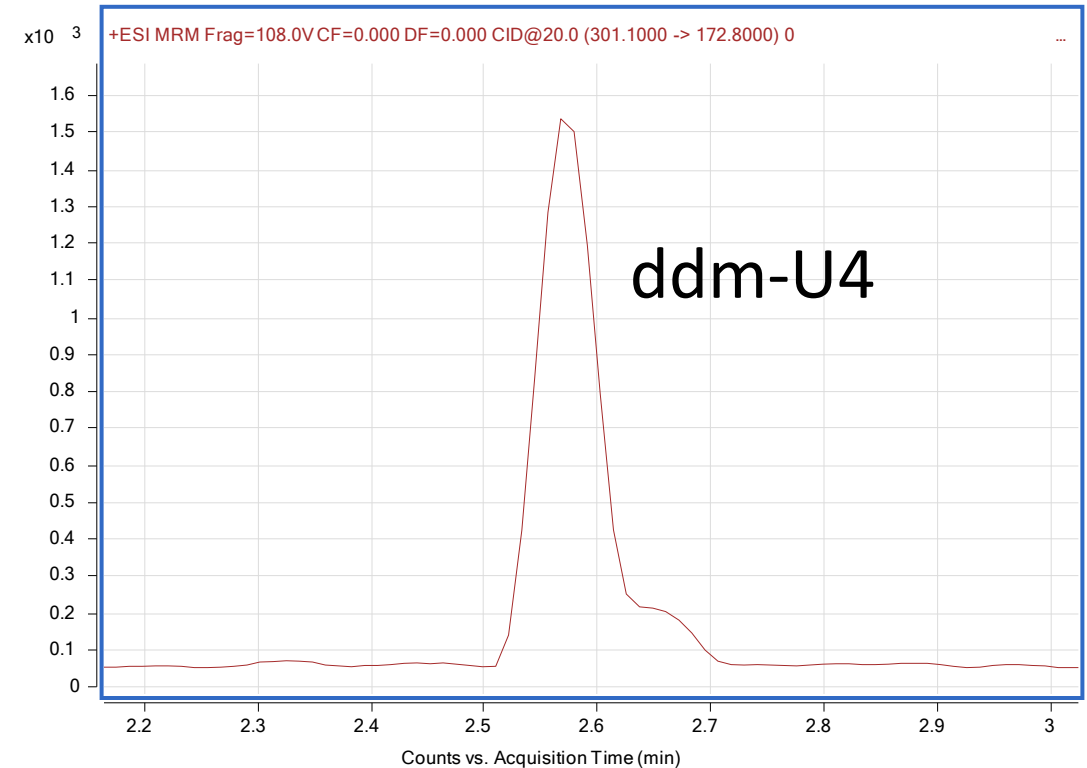


N,N-Didesmethyl (ACN vs MeOH)

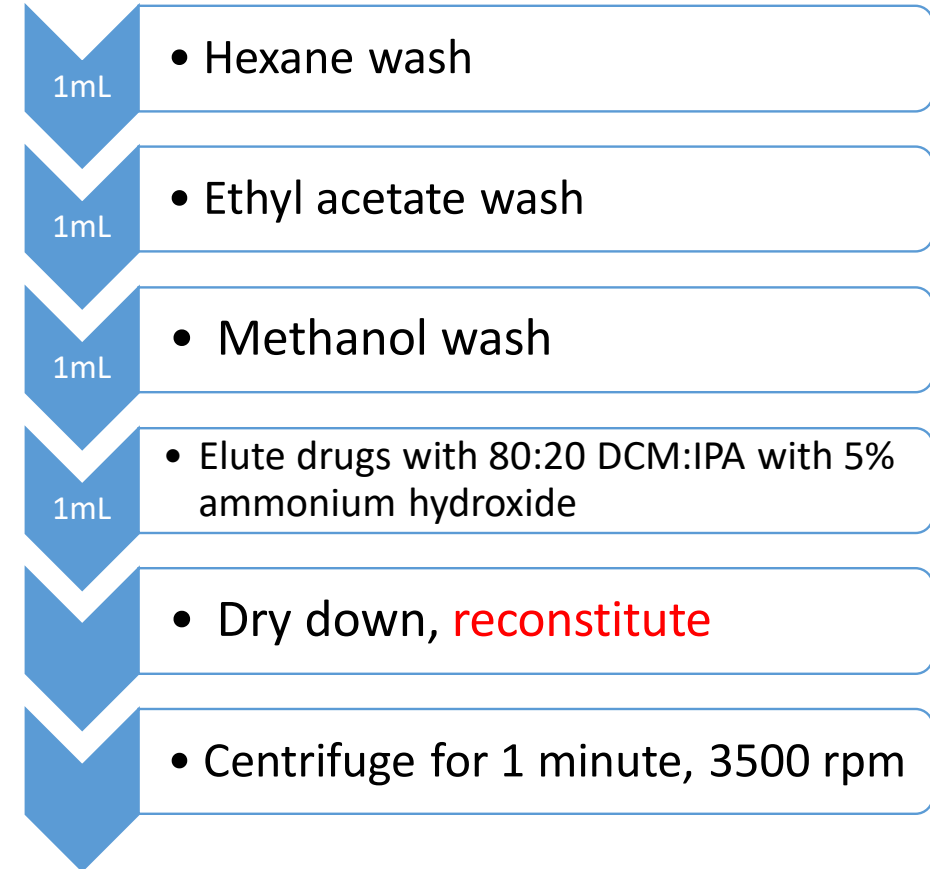
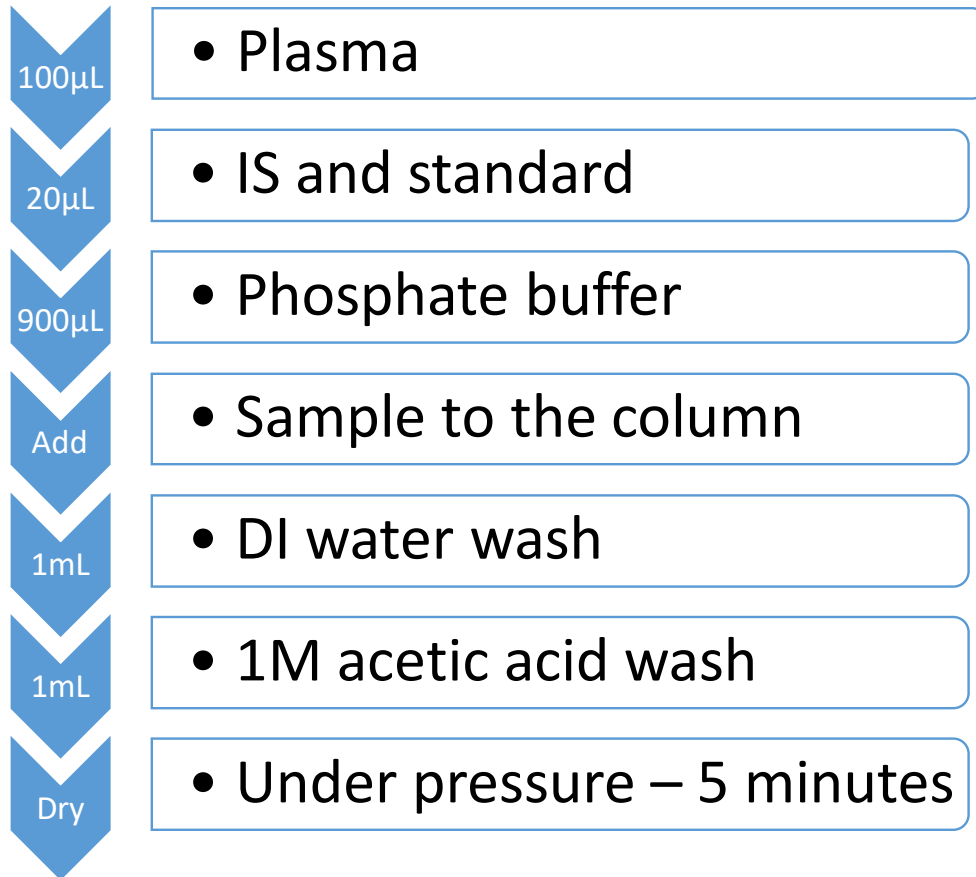


Method Development

- Chromatography
 - Column:
 - Poroshell C18 EC column
 - ✓ Zorbax C18+ Eclipse
 - Mobile Phase:
 - ✓ 80:20 gradient to 60:40 isocratic
 - ✓ Acetonitrile to methanol
- Extraction Method:
 - Reconstitution and Injection Volumes
 - Reconstitution volume: 50µL
 - Injection volume: 10µL



Extraction Method



Method Validation

- SWGTOX Quantitative Validation

Cross-Validation

- Blood QCs analyzed against plasma curves

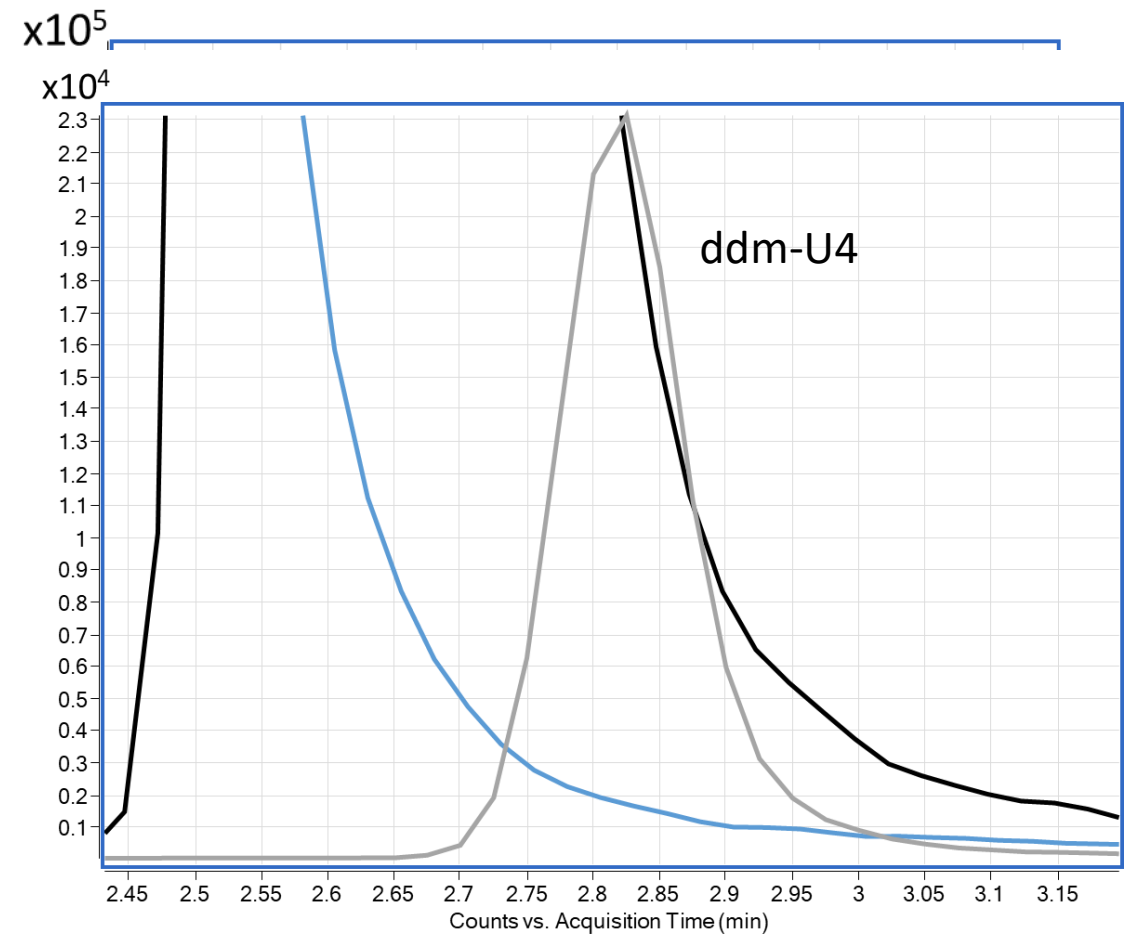
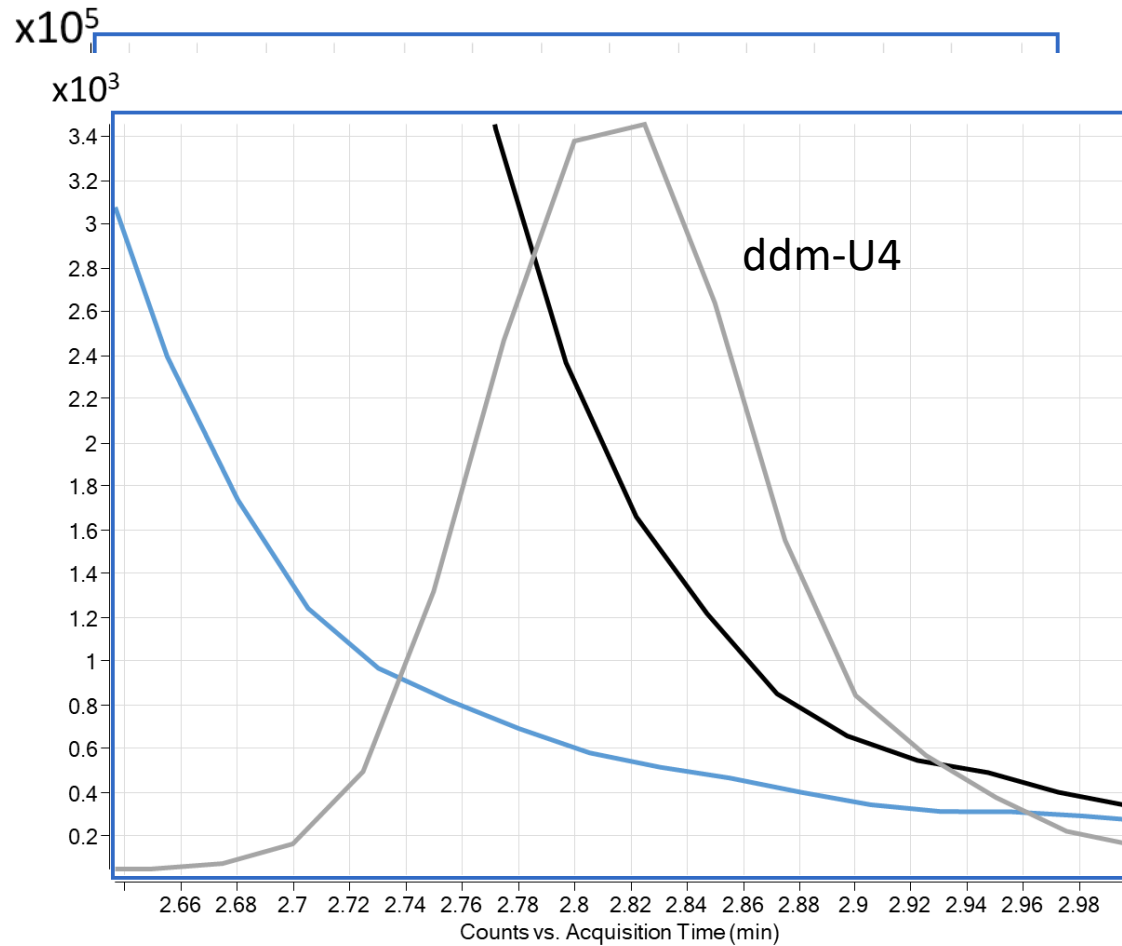
Analyte (n=3)	Bias (%)		Precision (%CV)		Extraction Efficiency (%)	Matrix Effect (%)
	<i>LQC</i>	<i>HQC</i>	<i>LQC</i>	<i>HQC</i>	<i>MQC</i>	<i>MQC</i>
U-47700	-7.3	-0.7	2.4	1.4	74%	135%
N-desmethyl-U-47700	-8.4	5.4	4.7	1.1	77%	123%
N,N-didesmethyl-U-47700	8.8	13.3	2.7	3.6	74%	115%

Authentic Postmortem Blood* Samples

	U-47700	N-desmethyl-U-47700	N,N-didesmethyl-U-47700
Sample	ng/mL	ng/mL	ng/mL
Sample 1	1367	1177	658
Sample 4	145	465	402
Sample 7	144	258	195
Sample 9	208	109	8.1
Sample 11	543	33.0	1.6
Sample 13	38.5	34.0	0.3
Sample 17	1.1	ND	ND
Sample 18	4.2	15.8	17.0
Sample 20	431	1400	361
Sample 22	68.3	56.7	16.4
Sample 23	114	8.0	0.7
Sample 25	46.6	28.0	12.1
Sample 27	50.7	14.1	12.5
Sample 28	207	225	85.5
Sample 30	126	253	129

Authentic Samples

- Parent-Metabolite Drug Chromatograms



Conclusions

- Study proven to be a valid method to detect and quantify U47700 and its metabolites
 - LOD and LOQ are comparable to previous studies
- Beneficial for forensic laboratories facing the opioid epidemic
- Inclusion of metabolites may widen detection window
- Future study to investigate pharmacokinetics of U-47700 in a rat model

Acknowledgements

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Questions?

