Quantification of Novel Synthetic Opioids in Blood Using LC-MS/MS

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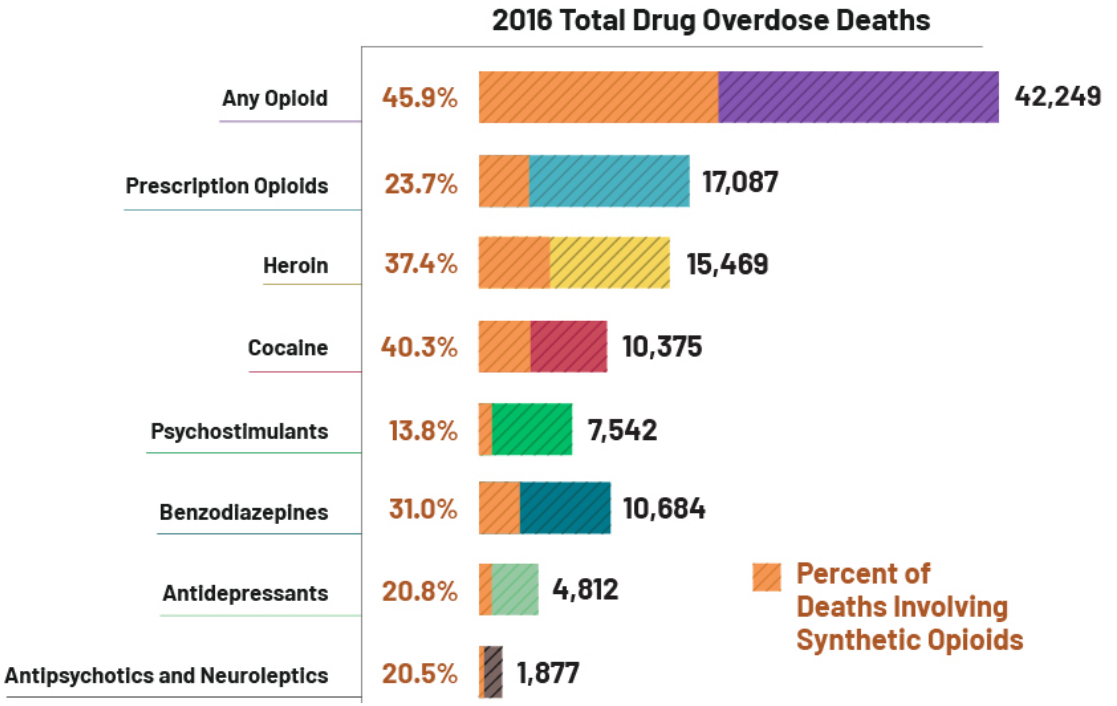
Disclaimer

- Authors have no conflicts to disclose.

Introduction

- HHS declared the opioid epidemic a public health emergency
- According to CDC, 66.4% of fatal overdoses in 2016 involved opioids
  - From 2015-2016, the number of fatal overdoses involving synthetic opioids doubled



NIDA - Fentanyl and Other Synthetic Opioids Drug Overdose Deaths

Targeted Analytes

Analyte	Receptor Agonist	Potency Relevant to Morphine
U-47700	μ, κ-opioid	7.5x
AH-7921	μ, κ-opioid	Equipotent
U-49900	Unknown	Unknown
U-50488	κ-opioid	Unknown
MT-45	μ, κ, δ-opioid	Equipotent
W-18	Reported to lack opioid receptor activity	Speculated to be 10,000x
W-15	Reported to lack opioid receptor activity	Unknown

Objective

Quantification method

- Develop and optimize
- Validate (SWGTOX guidelines)
- U-47700, AH-7921, U-49900, U-50488, MT-45, W-18, and W-15
- Blood
- SPE
- LC-MS/MS

SPE Development and Optimization

Acidic/neutral elution solvents

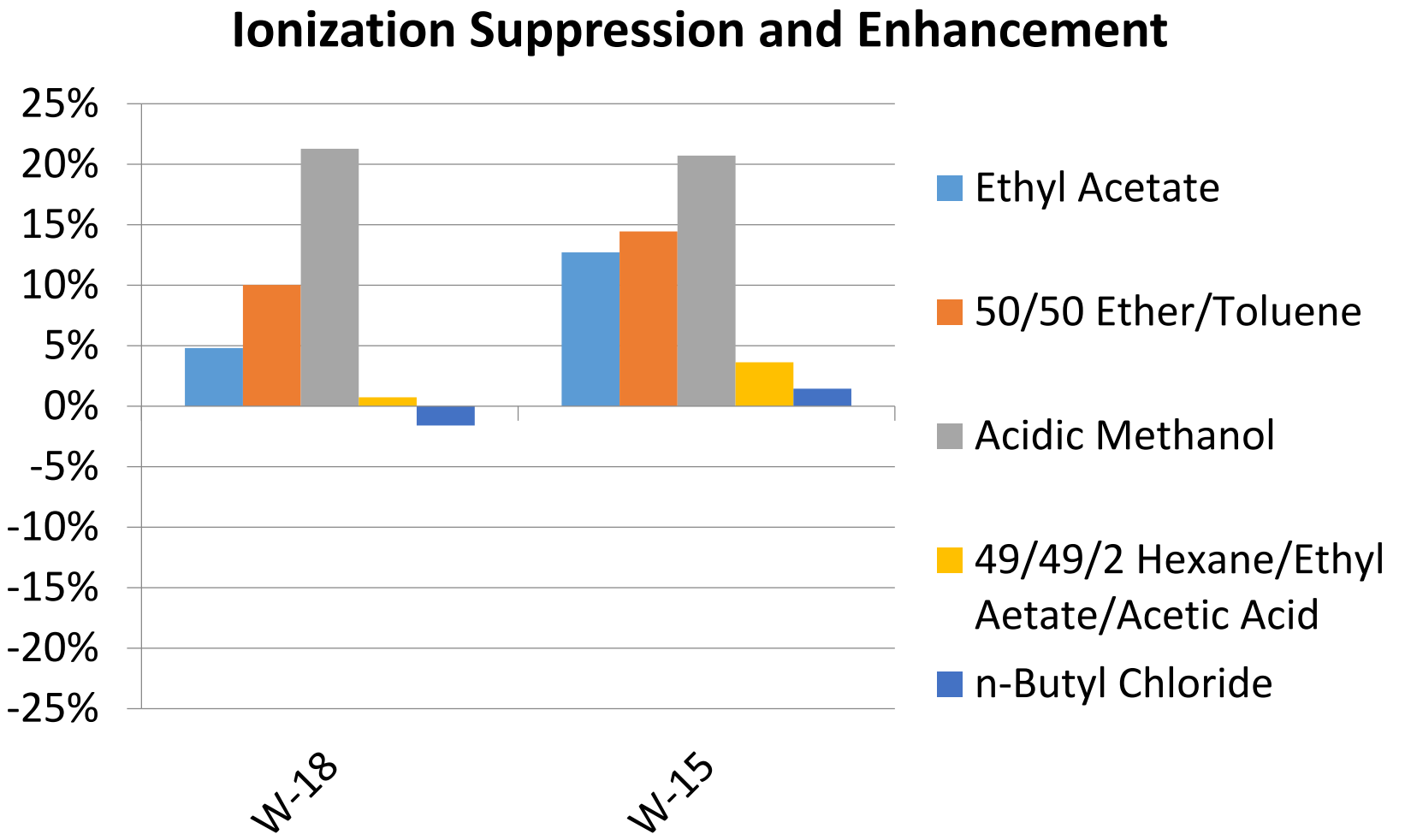
- Ethyl acetate
- 50/50 ether/toluene
- Acidic methanol
- 49/49/2 hexane/ethyl acetate/acetic acid
- n-butyl chloride

Basic elution solvents

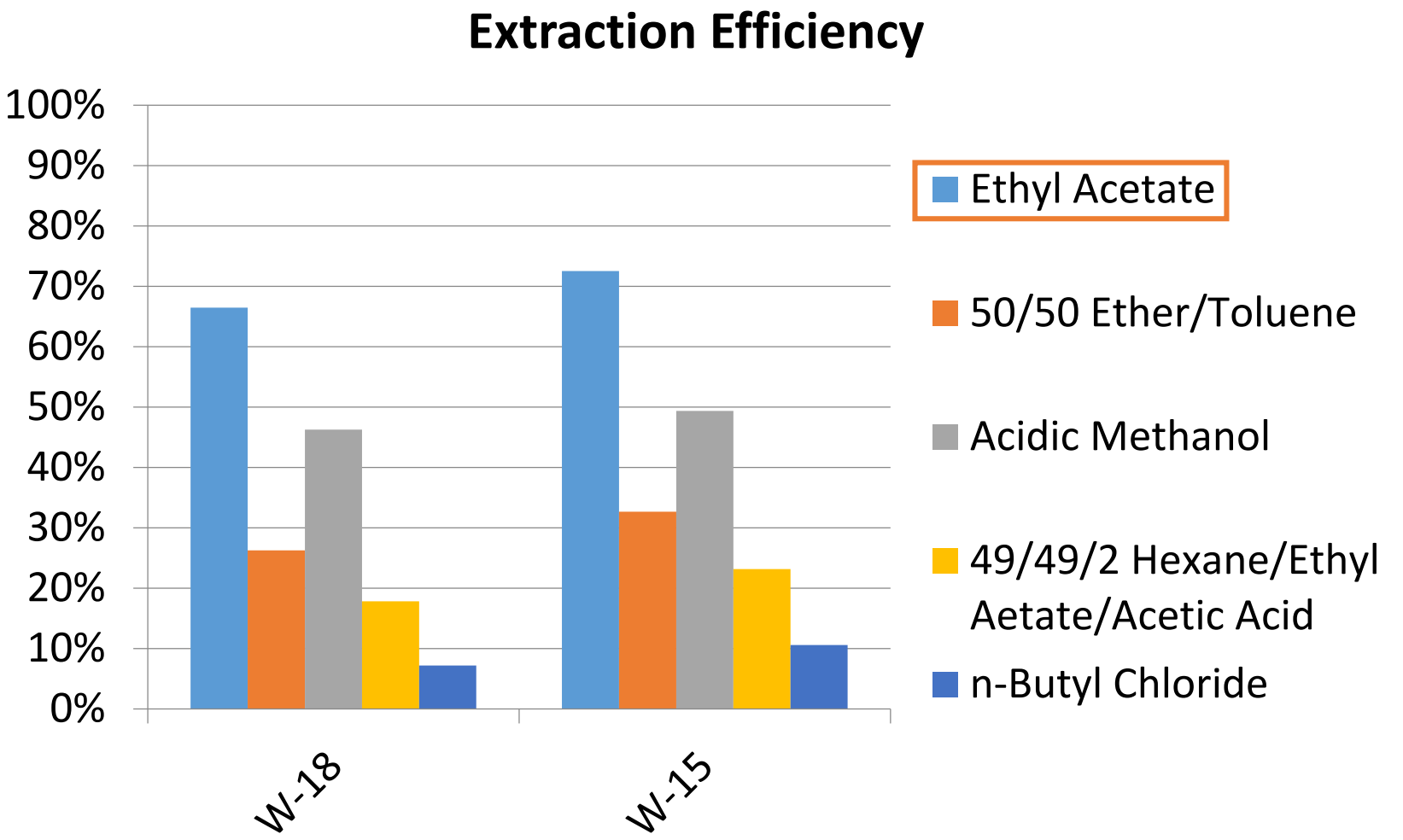
- 2% ammonium hydroxide in ethyl acetate
- 5% ammonium hydroxide in 80/20 DCM/IPA

Matrix effects and extraction recoveries were calculated for each analysis

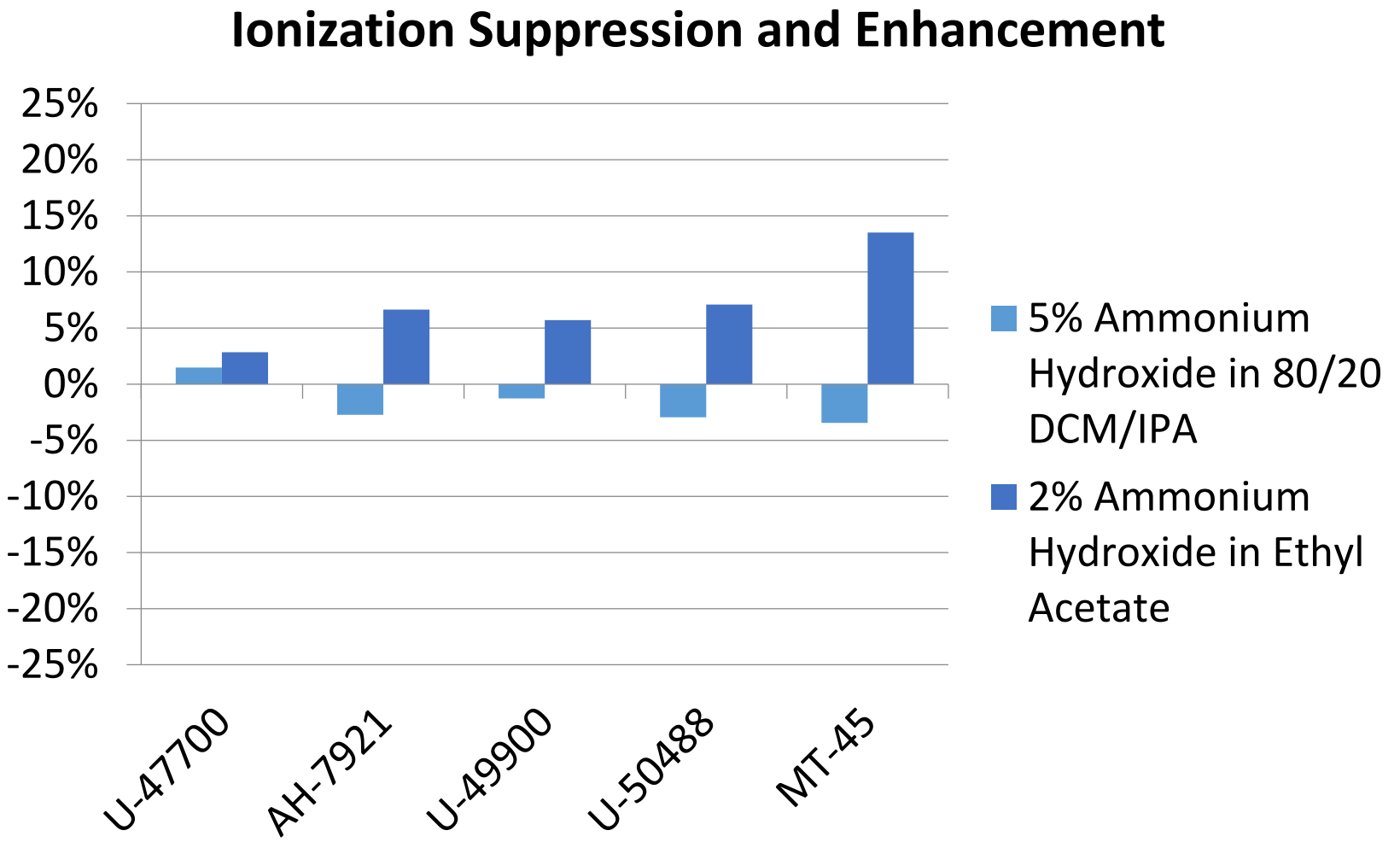
Acidic/Neutral Elution Solvents



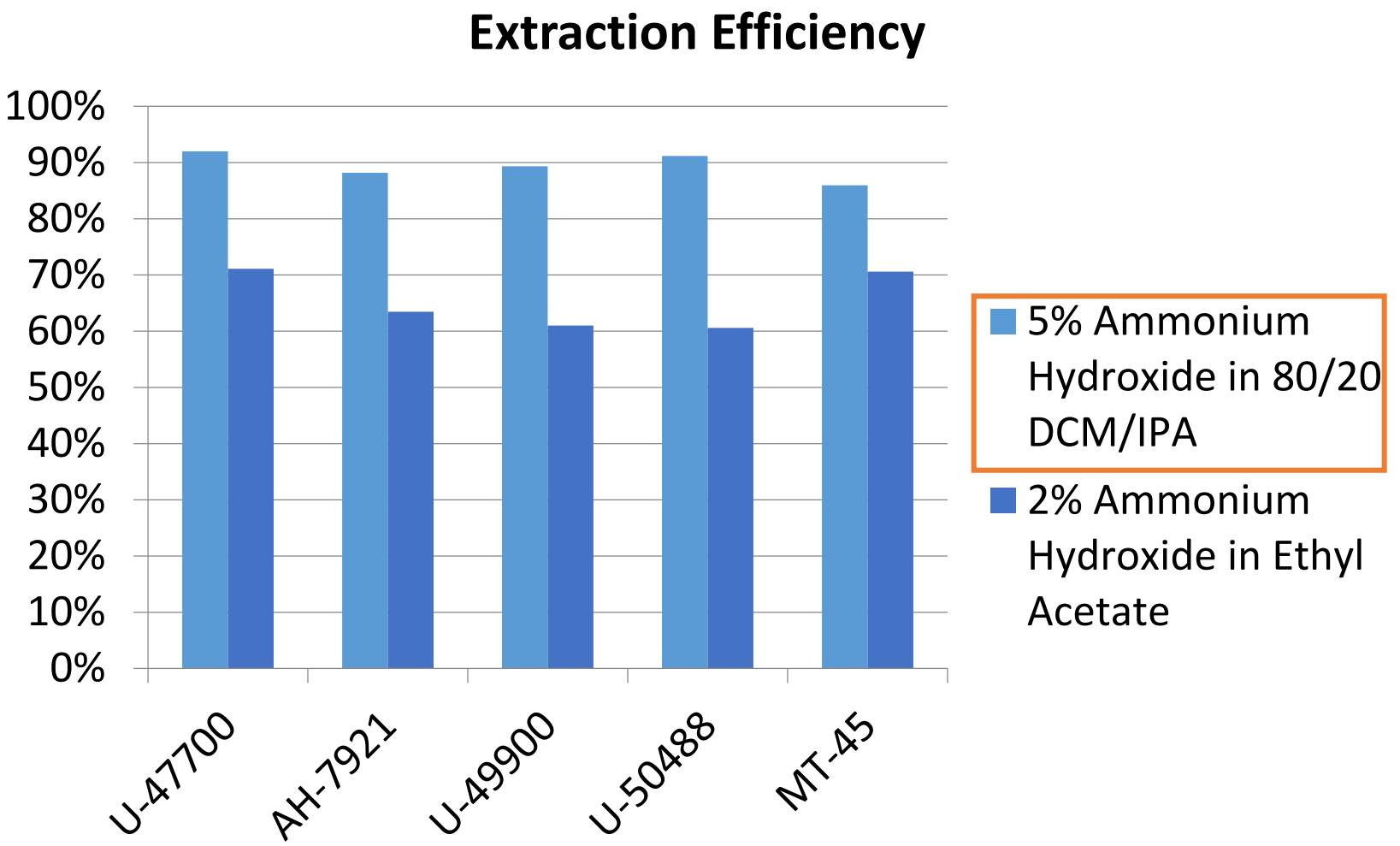
Acidic/Neutral Elution Solvents



Basic Elution Solvents



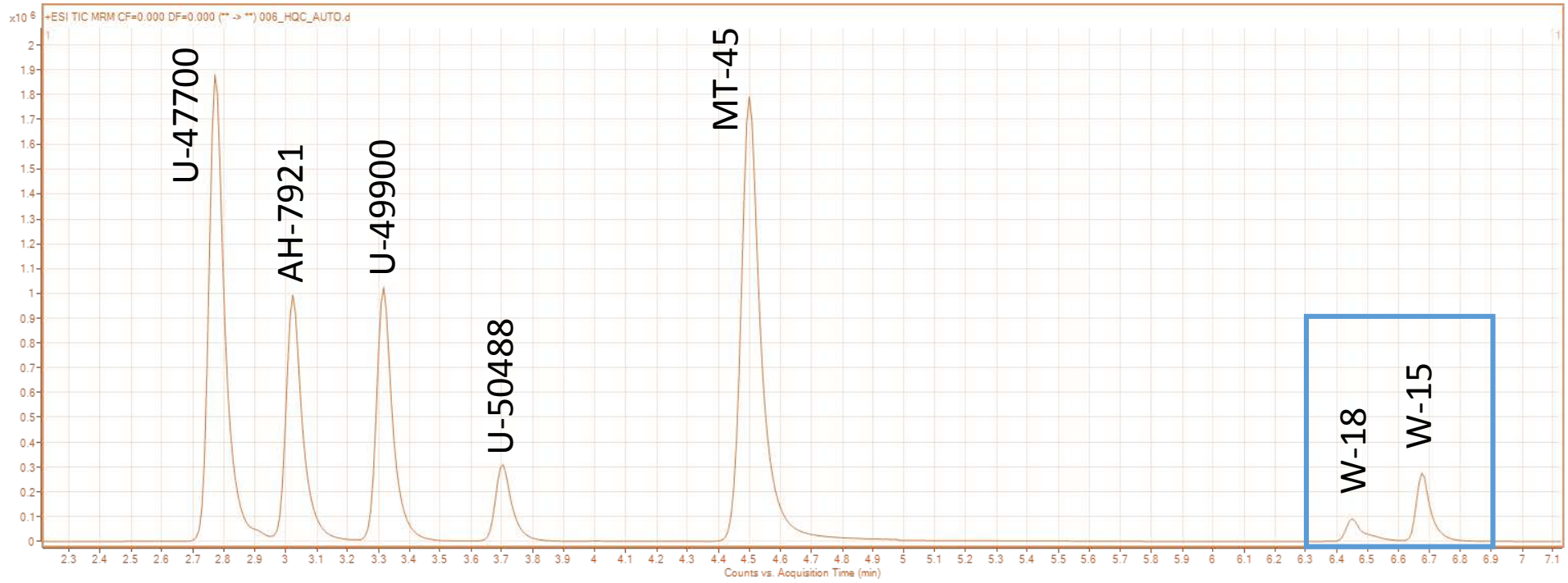
Basic Elution Solvents



LC-MS/MS Development and Optimization

Addition of ammonium formate to the aqueous mobile phase

- Improved peak shape and response for W-18 and W-15



Optimized SPE Method

0.5 mL blood + 2.5 mL phosphate buffer

↓

Add sample to column (Cerex® Clin II SPE column)

↓

2 mL washes (DI water, 1 M acetic acid)

↓

Dry for 5 mins under positive pressure

↓

2 mL wash (hexane)

↓

2 mL acidic/neutral drug elution (ethyl acetate)

↓

2 mL washes (methanol, DCM)

↓

2 mL basic drug elution (5% ammonium hydroxide in 80/20 DCM/IPA)

↓

Evaporate

↓

Reconstitute in 80:20 mobile phase

↓

Centrifuge

Optimized LC-MS/MS Method

Instrumentation

- Agilent 1290 Infinity II Liquid Chromatograph
- Agilent 6470 Triple Quadrupole Mass Spectrometer

Separation

- Mobile phase A: 0.05% formic acid + 5 mM ammonium formate in water
- Mobile phase B: 0.1% formic acid in acetonitrile
- Gradient elution
- Agilent InfinityLab Poroshell 120 EC-C18 column

Data acquisition

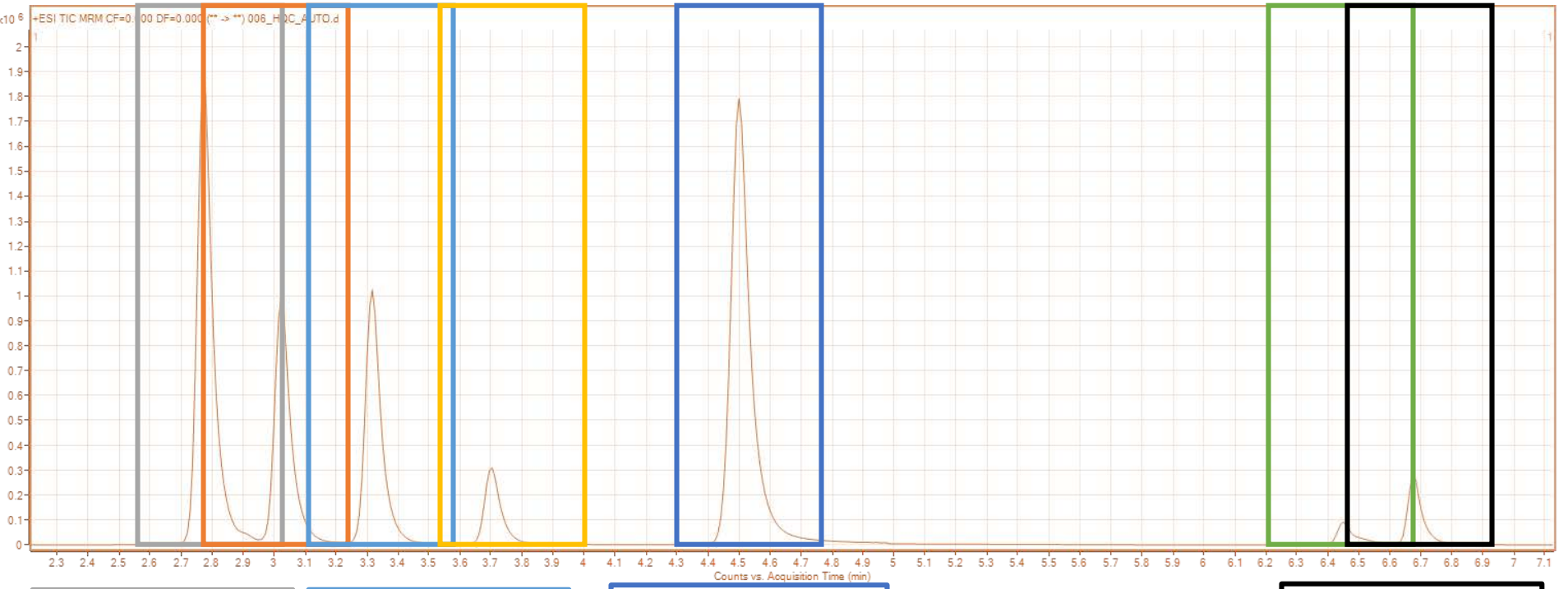
- Positive electrospray ionization
- Dynamic multiple reaction monitoring (dMRM)
- Agilent MassHunter Workstation version B.07 software

dMRM

AH-7921
329.2 → 144.9
329.2 → 46.2

U-50488
369.1 → 158.9
369.1 → 112.1

W-18*
422.1 → 111.0
422.1 → 75.1



U-47700*
329.2 → 172.9
329.2 → 144.9

U-49900
357.1 → 172.9
357.1 → 144.9

MT-45*
349.5 → 181.0
349.5 → 77.1

W-15
377.1 → 111.0
377.1 → 75.1

Validation Parameters

Calibration model

- 7 non-zero calibrators (6 for W-18)
- $R^2 > 0.99$
- Linear range
  - 0.25–100 ng/mL
  - 1–100 ng/mL (W-18)

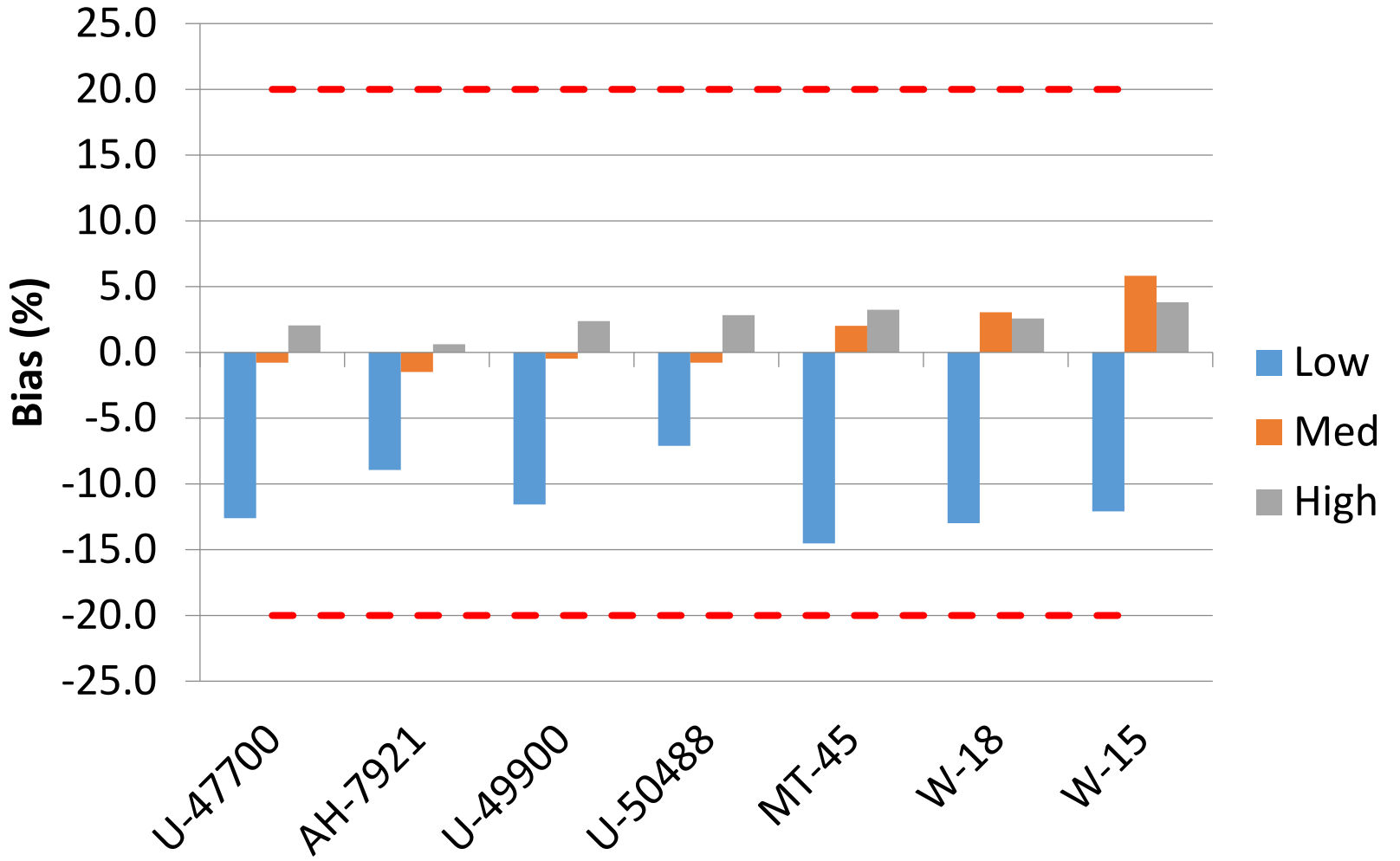
LOD

- 0.125 ng/mL
- 0.25 ng/mL (W-18)

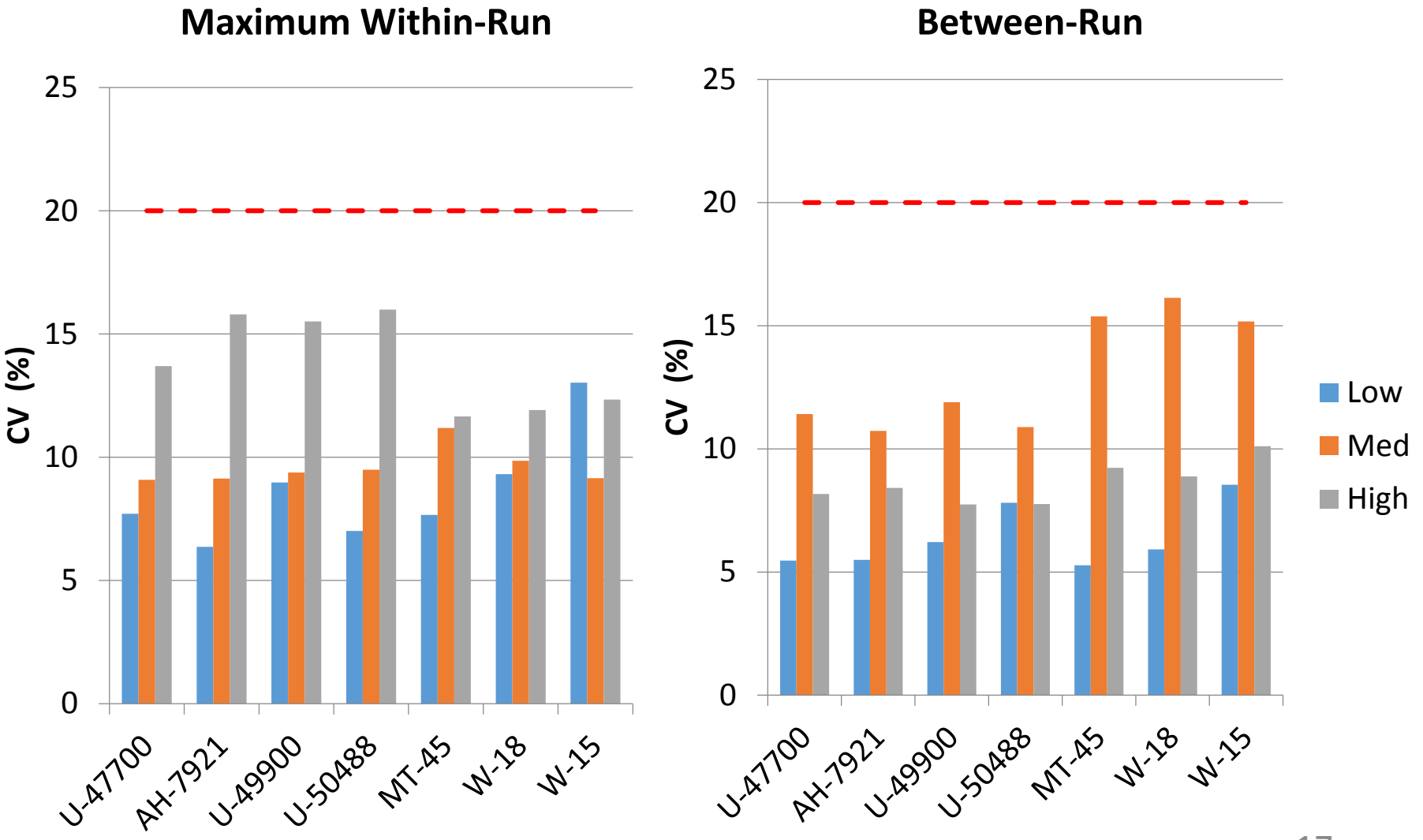
LOQ

- 0.25 ng/mL
- 1 ng/mL (W-18)

Bias


25.0
20.0
15.0
10.0
5.0
0.0
-5.0
-10.0
-15.0
-20.0
-25.0
Bias (%)
U-47700
AH-7921
U-49900
U-50488
MT-45
W-18
W-15
Low
Med
High

Precision


Maximum Within-Run
Between-Run
CV (%)
25
20
15
10
5
0
U-47700
AH-7921
U-49900
U-50488
MT-45
W-18
W-15
Low
Med
High

Validation Parameters

Interference studies

- Common drugs of abuse (32)
- 3 sources of blank matrix
- Negative samples (ISTD only)

Matrix effects

- 6 matrix sources
- ±21%
  - 28.4% (U-50488)
- ≤13% CV

Extraction efficiency

- 62.6-96.6%

Validation Parameters

Dilution integrity

- 10-fold
- ±18%
  - -23.8% (W-18)
- < 3% CV

Carryover

- None detected following high calibrator

Stability

- All analytes were found to be stable (±17%) under each condition
  - 24h room temperature
  - 72h at 4°C
  - 72h in the autosampler

Authentic Samples

Postmortem blood samples (n = 30)

- Cardiac
- Peripheral
- Subclavian
- Femoral
- Iliac

Positive for U-47700

- 15 samples
- Range: 3.2-1448 ng/mL
- Mean: 214 ng/mL

Sample	U-47700 Concentration (ng/mL)	Other Opioids
1	1448	Furanyl Fentanyl
4	135	-
7	133	-
With Other Opioids: 266 ng/mL (3.2-1448 ng/mL) Without Other Opioids: 155 ng/mL (43.1-354 ng/mL)		
18	4.2	Oxycodone, Oxymorphone, 4-ANPP (Despropionyl Fentanyl), Furanyl Fentanyl
20	354	-
22	60.6	Furanyl Fentanyl, 4-ANPP (Despropionyl Fentanyl)
23	101	Fentanyl
25	43.1	-
27	53.7	4-ANPP (Despropionyl Fentanyl), Furanyl Fentanyl
28	118	-
30	109	-

Conclusion

Development and optimization

- Improved matrix effects and extraction efficiency
- Improved chromatography

Validation

- Performed in accordance with SWGTOX guidelines
- Parameters that exceeded requirements
  - U-50488 matrix effects
  - W-18 dilution integrity bias

Conclusion

First method reported

- Simultaneous quantification of these synthetic opioids in blood
- Quantification of W-18 and W-15 in blood

Method applications

- Driving under the influence cases
- Fatal drug intoxication cases

Questions?

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