Comprehensive Drug Screening Using Supported Liquid Extraction (SLE) and Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS)

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Disclaimer

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OVERVIEW

- Challenges of New Psychoactive Substances (NPS)
- High Resolution Mass Spectrometry (HRMS)-based Drug Screening
- Standards for Analytical Testing
- Comprehensive Drug Screening Method
  - Validation
    - Supported liquid extraction (SLE) method using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS)
  - Applicability
    - Authentic specimen analysis

Challenges of NPS

- Transitory nature
  - Maintaining relevant drug screening protocols
- Analytical Challenges
  - Structural
    - Isobars, isomers, and stereoisomers
  - Pharmacological
    - Potency
    - Low concentrations
  - Reference Material
    - Availability
    - Development

https://www.unodc.org/documents/scientific/NPS-Leaflet_WEB_2020.pdf

HRMS-based Drug Screening

- **Non-targeted screening (increased scope of testing)**
- Increased specificity
- **Retrospective data analysis**
- Elucidate chemical formula and structural information
- HRMS
  - LC-QTOF-MS (Agilent 6530)
    - Merge TOF high mass resolution and LC/MS/MS mass filtering and fragmentation

Standards for Analytical Testing

- **Recommendations for DUID Investigations**
  - Tier I
    - Commonly encountered drugs
    - Essential for routine testing
  - Tier II
    - Regionally specific drugs
    - Require advanced instrumentation
  - "Tier III"
    - New group created
    - Includes all other compounds not identified as Tier I or II
- **Standards for Analytical Scope and Sensitivity of Testing**
  - ANSI/ASB Std. 119
    - Postmortem
  - ANSI/ASB Std. 120
    - DUID
  - ANSI/ASB Std. 121
    - DFC (in draft)

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Article

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Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities—2021 Update

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ANSI/ASB Standard 120, First Edition 2021

Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations

Method Validation

Method Validation Overview

- Optimization and validation of supported liquid extraction protocol in whole blood using LC-QTOF-MS (Agilent 6530)
  - Drug list
    - 209 total drugs
    - 10 internal standards
    - Concentrations range from < 1ng/mL to 50,000 ng/mL
    - All Tier I drugs targeted at recommended screening cutoffs
    - Tier II/III drugs targeted at forensically significant concentrations
- Completed method validation with guidance from the ANSI/ASB Std. 036
  - Carryover
  - Interferences
  - Limit of detection
  - Matrix effects
  - Processed sample stability
  - Extraction efficiency (recovery)
  - Reproducibility

Optimized Drug List

Drug Class	N
Amphetamines	8
Analgesics/NSAIDs	4
Anticonvulsants	9
Antidepressants/SSRIs	18
Antihistamines	8
Antihypertensives	2
Barbiturates	18
Cannabinoids	3
Cathinones	12
Designer Amphetamines	13
Designer Benzodiazepines	15

Ten Deuterated Internal Standards

Drug Class	N
Dissociatives	3
Fentanyl-Analogs	17
Hallucinogens/Psychedelics	6
Hypnotics/Sedatives	5
Miscellaneous	4
Muscle Relaxants	7
Novel Synthetic Opioids	11
Opioids	19
Stimulants	6
Synthetic Cannabinoids	11
Total	209

Supported Liquid Extraction (SLE)

- SLE
  - Novelty in forensic toxicology
  - Modified liquid/liquid extraction (LLE)
    - Advantages of general drug screen
    - No disadvantages of LLE
  - Diatomaceous earth
    - Inert material retains elements of sample and matrix
  - Selective analyte elution
    - Sample pretreatment
      - Non-ionized analyte form
    - Immiscible organic solvent
    - Parallels LLE

Optimized SLE Protocol

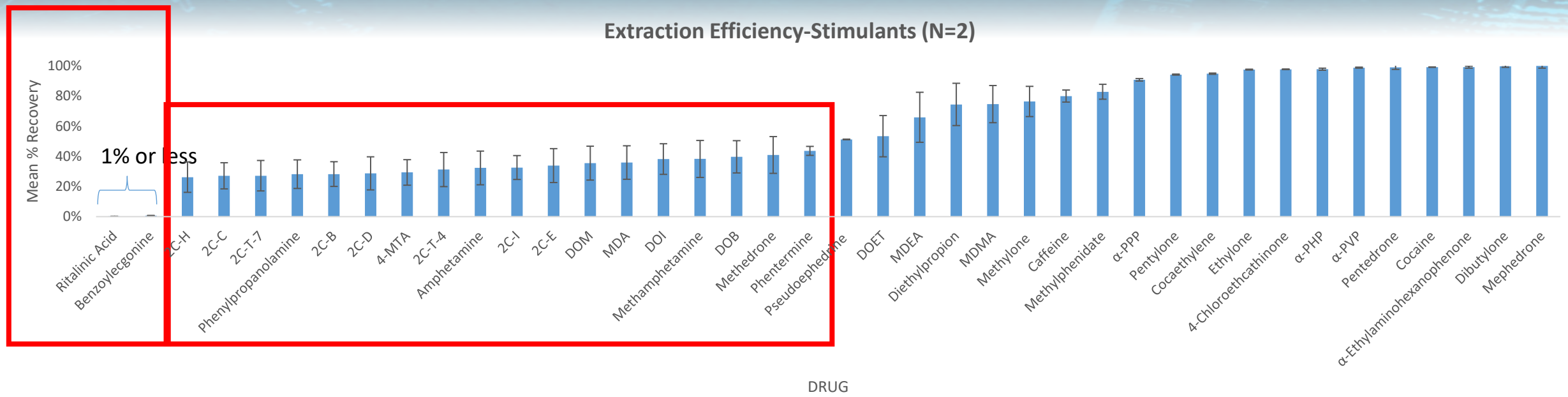
- **Spike 600 µL of blood with selected drugs**
- **Add 300 µL of 0.1M acetic acid**
- Centrifuge samples 4000 rpm for 10 mins.
- Load supernatant on 1mL SLE column (Biotage SLE+)
- Wait 5 mins.
- Add 3 mL of **70:23:7 Hexane:ETAC:IPA**
- Apply vacuum for 30 secs.
- Add 3 mL of **70:23:7 Hexane:ETAC:IPA**
- Apply vacuum for 5 mins.
- Add 30 µL of acidic methanol
- Evaporate under nitrogen at 40°C
- **Reconstitute in 20 µL 60:40 (MPA:MPB)**
  - A: 5mM ammonium formate; 0.01% FA in DIW
  - B: 0.01% FA in acetonitrile
- Centrifuge extracts 4000 rpm for 10 mins.
- Transfer to autosampler vials
- **Inject in positive and negative electrospray ionization**

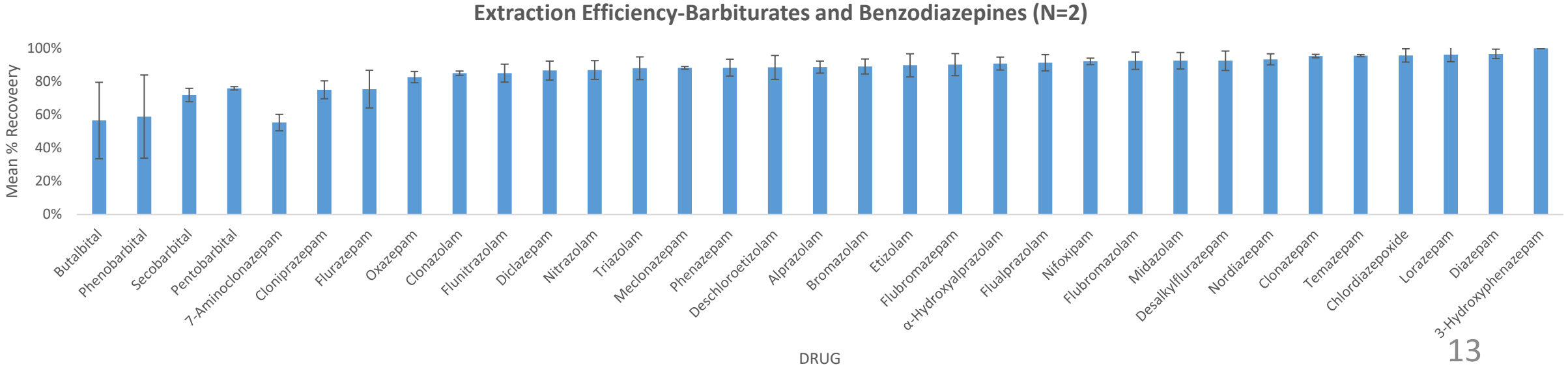
Extraction Efficiency (Recovery)

- Drugs with low recoveries
  - Analgesics/NSAIDs
  - Gabapentin
  - Cetirizine
  - Psilocin
  - GHB
  - BE
  - Ritalinic acid
  - Amphoteric compounds
- Low extraction efficiencies tolerated for drugs encountered at high concentrations
- Extraction methodology optimized for most challenging **essential** drugs
  - THC-COOH
  - Zwitterions (i.e. BE)

Drug Category	Extraction Efficiency (%) [N=2] at the Decision Point
Amphetamines/SMAs	28-75%
Anticonvulsants	21-92%
Antidepressants	58-100%
Antihistamines	72-97%
Antihypertensives	68-98%
Antipsychotics	67-97%
Barbiturates	57-76%
Benzodiazepines	55-97%
Cannabinoids	14-82%
Cathinones	41-101%
Designer Amphetamines	26-53%
Designer Benzodiazepines	75-100%
Dissociatives	90-100%
Fentalogs	71-80%
Hallucinogen/Psychedelics	29-92%
Hypnotics/Sedatives	82-92%
Miscellaneous	69-96%
Muscle Relaxants	63-96%
Novel Synthetic Opioids	82-94%
Opioids	16-100%
Stimulants	80-99%
Synthetic Cannabinoids	53-100%

Extraction Efficiency (Recovery)





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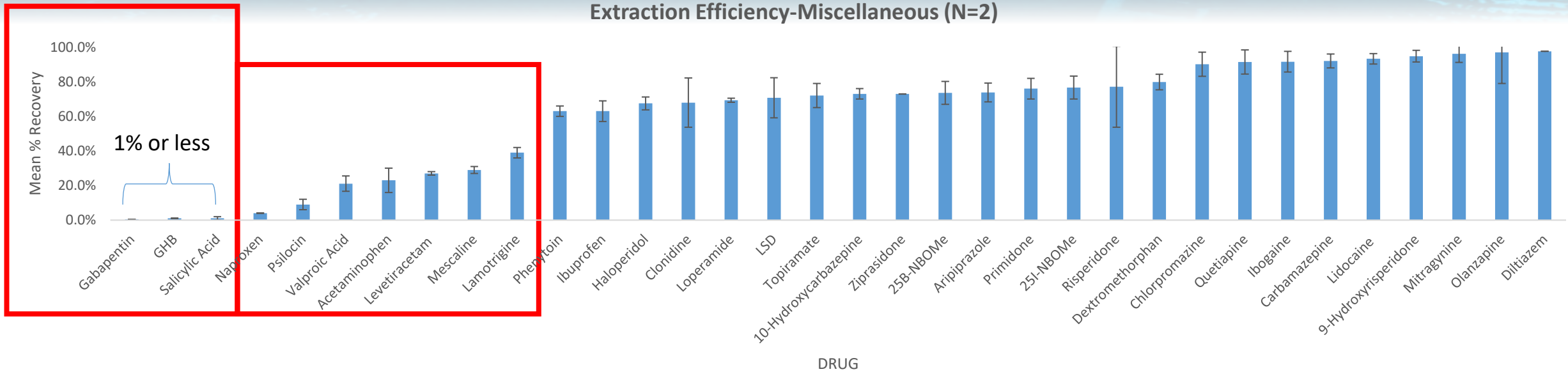

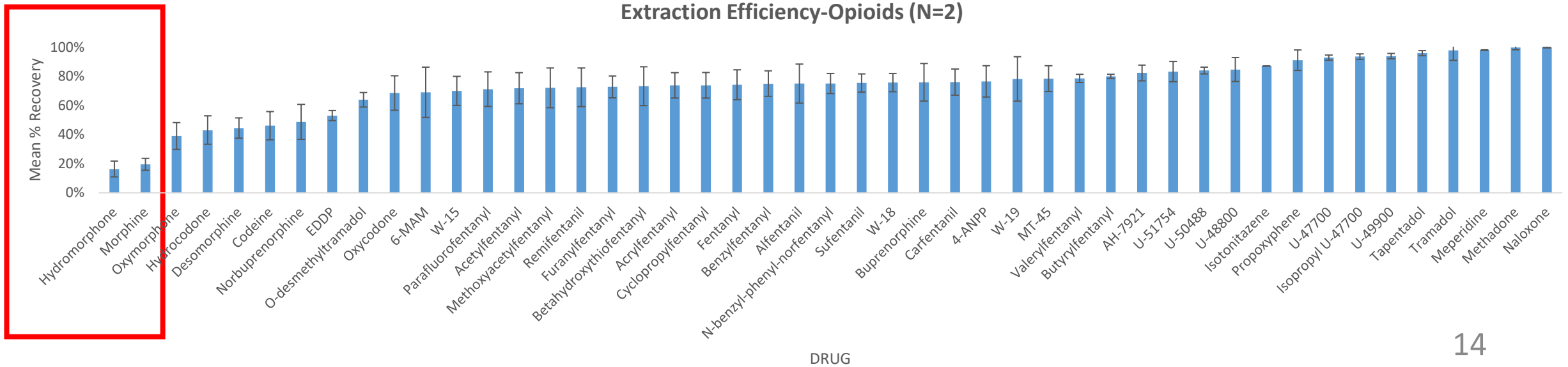

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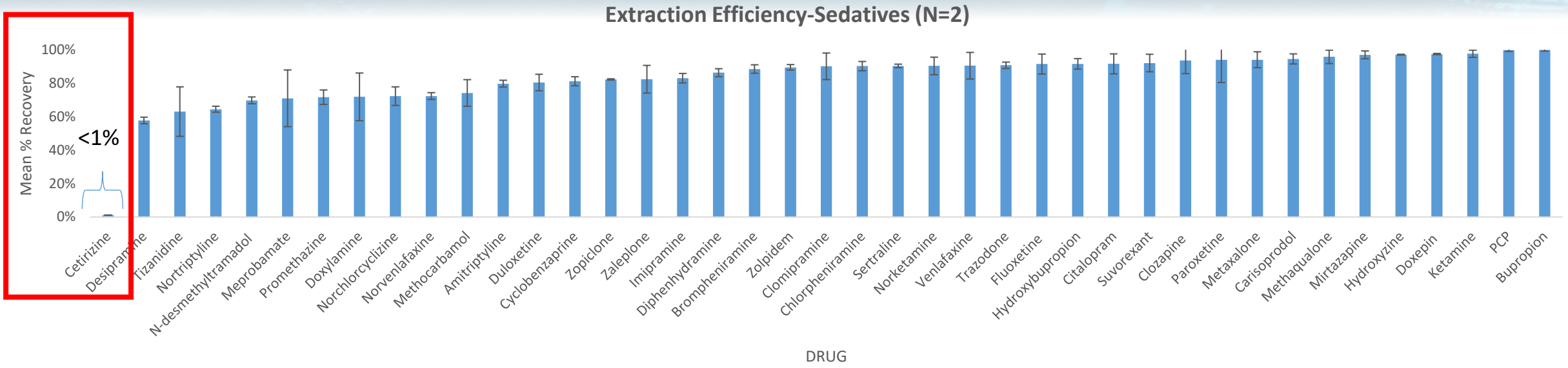
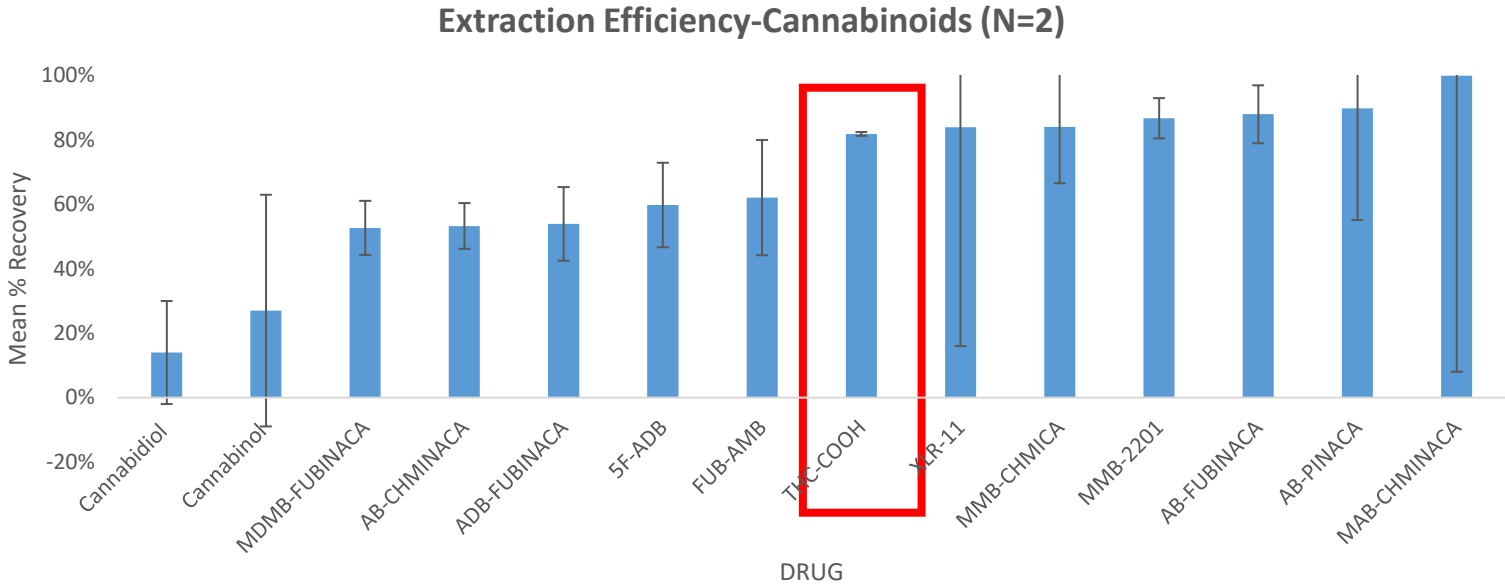




Limit of Detection (LOD)-Tier I Drugs

DRUG	CutOff (ng/mL)	LOD (ng/mL)	Mass Accuracy	S/N Ratio	Cutoff/ LOD
Amphetamine	20	2	-0.4	13	10
Methamphetamine	20	2	-1.6	294	10
MDMA	25	2.5	-1.2	268	10
MDA	25	12.5	-1.1	60	2
Lorazepam	10	1	2.3	> 1000	10
Alprazolam	10	1	0.3	86	10
Clonazepam	10	1	1.0	130	10
7-Aminoclonazepam	10	2.5	0.3	343	4
α-Hydroxyalprazolam	10	5	-2.9	19	2
Oxazepam	50	5	-0.7	105	10
Temazepam	50	5	1.3	149	10
Nordiazepam	50	5	0.3	300	10
Diazepam	50	5	-0.1	526	10
THC-COOH	10	10	0.6	22	1
Fentanyl	1	1	-1.1	115	1
Zolpidem	10	1	2.5	24	10
Carisoprodol	500	5	-2.0	225	100
Meprobamate	100	10	2.4	106	10
6-MAM	5	0.5	-2.3	127	10
Norbuprenorphine	1	1	1.4	109	1
Buprenorphine	1	1	0.0	93	1
Oxymorphone	10	1	-0.4	12	10
Codeine	10	1	-2.3	133	10
Oxycodone	10	1	1.4	45	10
Hydrocodone	10	1	-0.2	69	10
Hydromorphone	10	5	-1.6	31	2
Methadone	50	5	-1.6	567	10
Morphine	10	10	-0.5	552	1
Tramadol	100	25	-3.1	3902	4
O-desmethyltramadol	100	50	-8.8	855	2
Cocaine	50	5	-1.9	900	10
Cocaethylene	50	5	0.7	971	10
Benzoylecoginine	50	50	14	8	1

- Serial dilution (N=1)
- LODs 1 to 100-fold the cutoff concentration
  - Tier I at recommended concentrations (ANSI/ASB 120)
  - Tier II/III no defined concentrations
    - Data not shown
- Acceptance Criteria
  - Mass Accuracy ± 10ppm
  - RT within ± 2%
  - S/N Ratio > 3
  - Fragment ions
    - Ratios within ± 20%
    - Coelution score > 60

Carryover

- Challenging for > 200 analytes
- Wide range of cutoff concentrations
  - < 1 ng/mL to 50,000 ng/mL
- Partitioned drugs by concentration
  - 6 drug mixes made
- Assessed drug mixes at multiple levels to evaluate relevant carryover for each analyte
  - 1 to 400 times the cutoff concentrations

Matrix Effects-Tier I Drugs

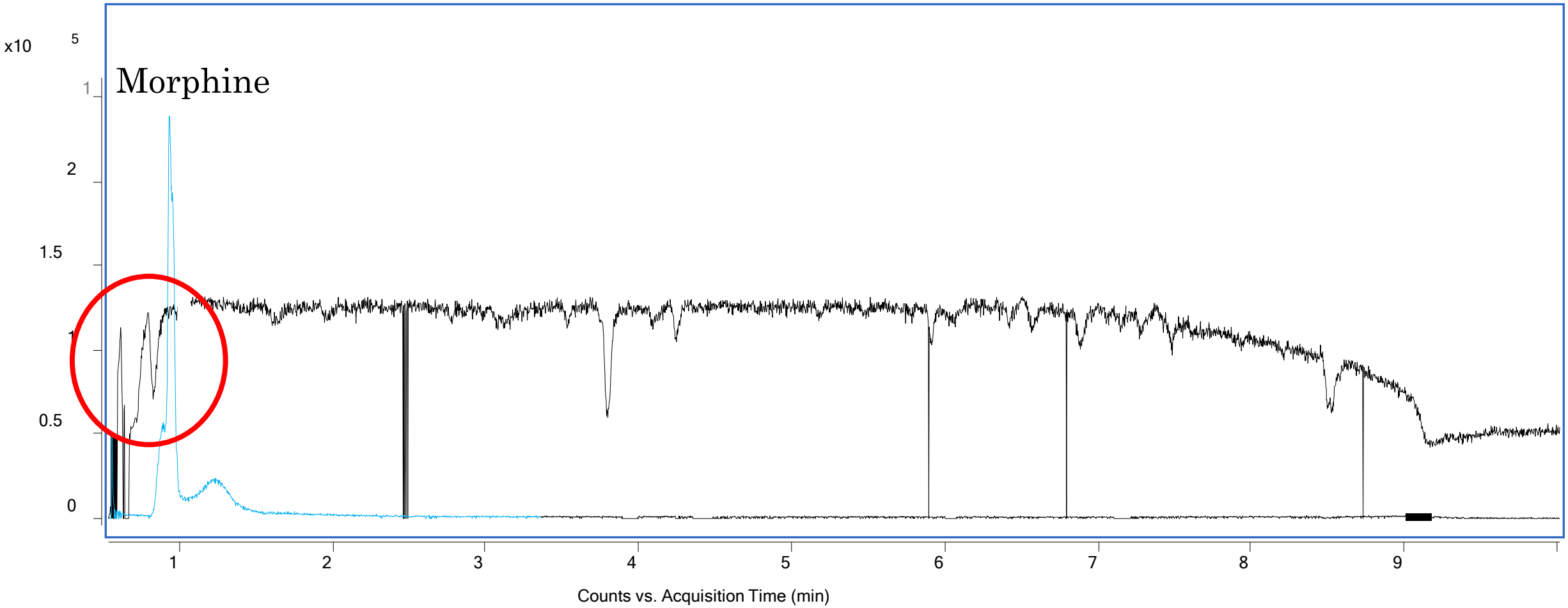
- Single analytes exhibited
  - ion suppression and enhancement
  - Matrix effects influenced by analytes in solution
  - Separated analytes by RT due to analyte mediated suppression/enhancement
    - Analyzed 10 distinct blood matrices in duplicate

- Tier I Drugs (screen) in **BOLD**
  - Performed post-extraction addition and post-column infusion
- Internal standards
  - Performed post column infusion

- ANSI/ASB Std. 036
  - %Matrix effects ± 25% or %CV ± 20%

Drug	Conc. (ng/mL)	Avg. ME (N=10)	% CV
6-MAM	5	-19%	
7-Aminoclonazepam	10	22%	
α-Hydroxyalprazolam	10	11%	
Alprazolam	10	7%	
Amphetamine	20	11%	
Benzoylecgonine	50	11%	
Buprenorphine	1	10%	
Carisoprodol	500	-1%	
Clonazepam	10	-17%	
Cocaethylene	50	6%	
Cocaine	50	8%	
Codeine	10	11%	
Diazepam	50	-10%	
Fentanyl	1	-1%	
Hydrocodone	10	23%	
Hydromorphone	10	14%	
Lorazepam	10	**49%**	**32%**
MDA	25	17%	
MDMA	25	6%	
Meprobamate	100	13%	
Methadone	50	-2%	
Methamphetamine	20	8%	
Morphine	10	-4%	
Norbuprenorphine	1	**-32%**	**-32%**
Nordiazepam	50	**-27%**	**-71%**
O-desmethyltramadol	100	-1%	
Oxazepam	50	9%	
Oxycodone	10	44%	12%
Oxymorphone	10	25%	32%
Temazepam	50	**30%**	**36%**
THC-COOH	10	-97%	-2%
Tramadol	100	6%	
Zolpidem	10	17%	

Post Column Infusion



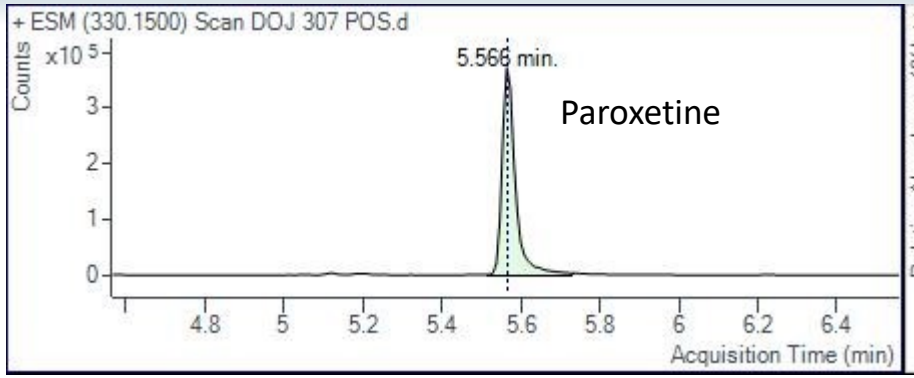
Additional Parameters

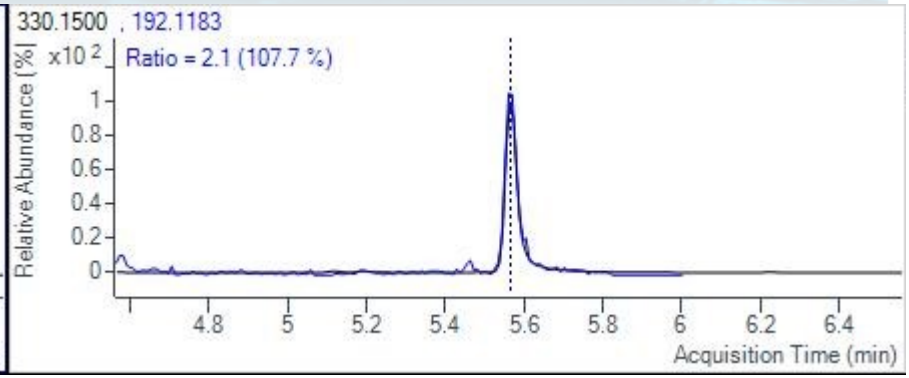
- **Matrix interferences:**
  - Observed for salicylic acid using bovine blood
  - Suspected contaminant from feed
  - No interference in human blood
  - No matrix interferences for remaining 208 drugs (inclusive of ISTDs)
- **Processed sample stability (0-72h)**
  - All drugs detectable for 24 hours
  - Variable (drug/class-dependent) stability thereafter
    - Loss of signal for select amphetamines, cannabinoids, synthetic cannabinoids, and psilocin
- **Reproducibility** at the cutoff (TIER I) or targeted concentration (TIER II/III) using independently sourced matrices (n=10) for all 209 drugs
  - Evaluated average S/N ratio
    - All drugs acceptable at > 3
    - All other acceptance criteria were met

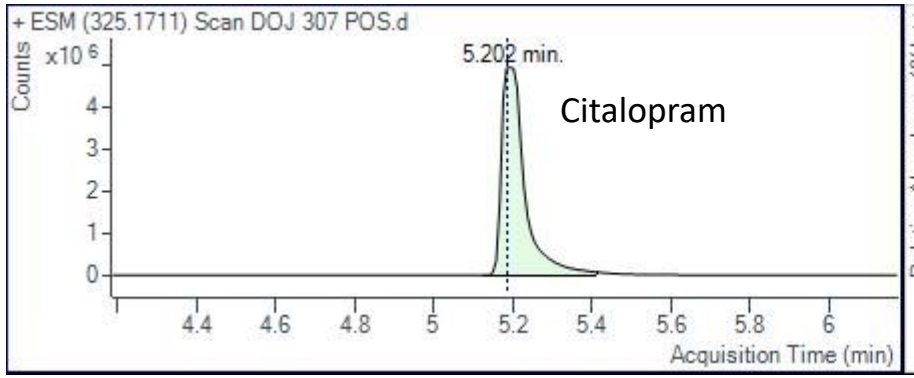
Applicability and Authentic Specimen Analysis

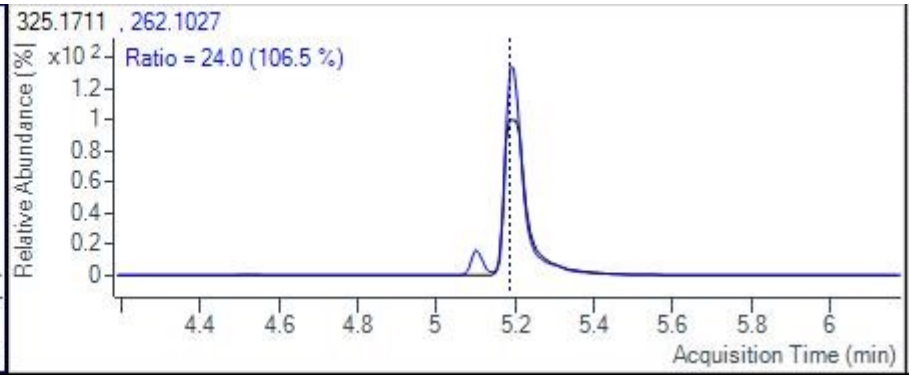
Specimen F

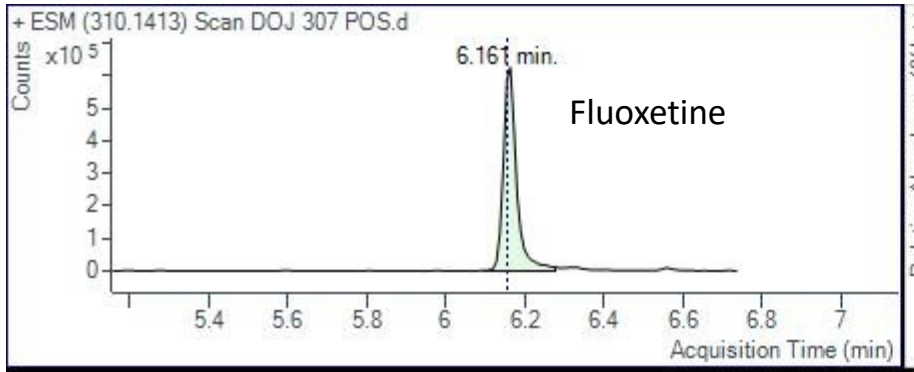
- **Immunoassay Results**
  - NEGATIVE
- **LC-QTOF-MS Results**
  - Paroxetine
  - Citalopram
  - Fluoxetine
  - Doxylamine
  - Trazodone
  - DXM

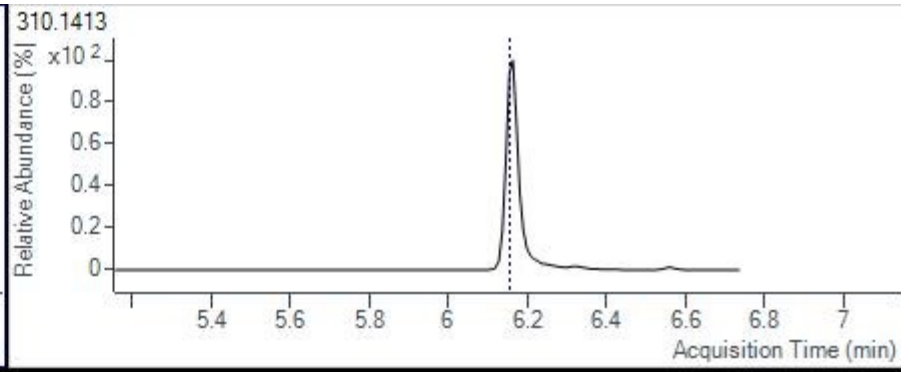












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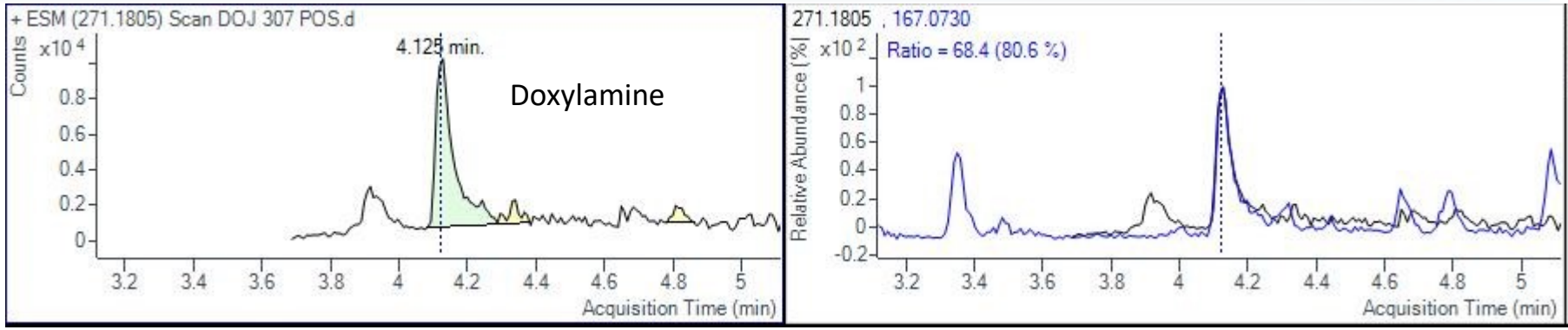
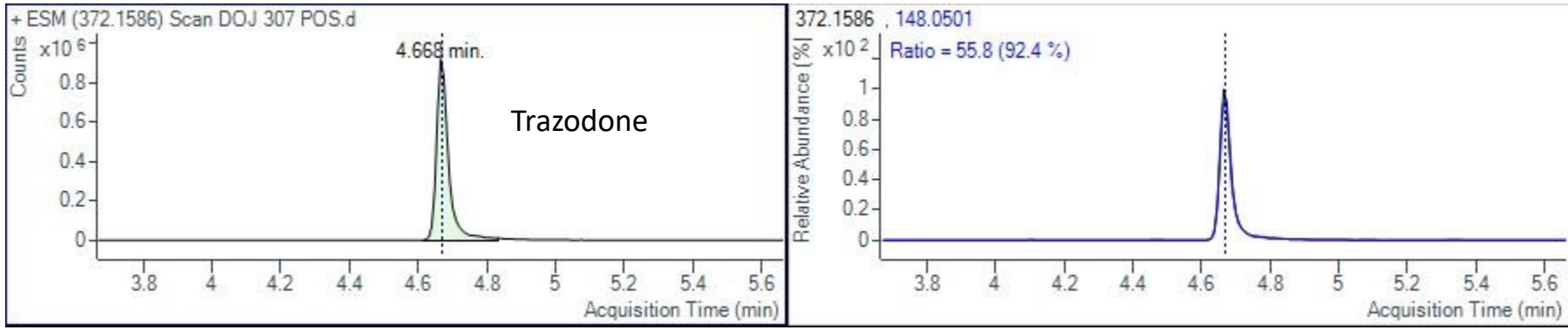
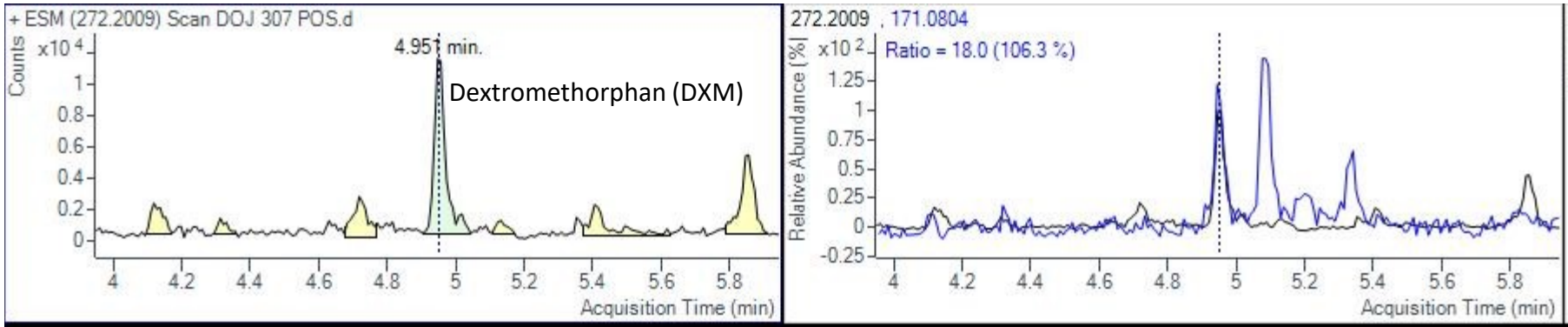
  - Doxylamine
  - Trazodone
  - DXM







Conclusion

- Traditional drug screening techniques
  - Cannot keep pace with NPS
- Unpredictability of the illicit drug market and drug legislation
  - Shifts the patterns of use
  - New substances emerge as replacements
  - Circumvent prosecution with legal highs
- HRMS-based screening
    - Broadens analytical capabilities and scope of drug testing
    - Improves delivery of forensic toxicology services

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Questions

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