INSTITUTE FOR FORENSIC
RESEARCH TRAINING AND INNOVATION

Sam Houston State University

LC/MS/MS Approaches for Identifying emerging NPS…

Considerations for Targeted LC-MS Assays

Current Trends in Forensic Toxicology Virtual/Online Symposium
Hosted by RTI and ForensicED
Sponsored Agilent Technologies
May 23, 2018

Overview

- Comparison of targeted LC-QTOF-MS and LC-MS-MS approaches
- Specific challenges in forensic toxicology
- Analytical considerations for quantitative assays
- Method development and validation considerations
- Advantages of the HRMS approach

Generally Held Beliefs

- **LC-MS-MS**
  - More sensitive
  - Preferred technique for targeted quantification
  - Abundance of published methods
  - Widely accepted
  - Well understood
- **LC-QTOF-MS**
  - Preferred technique for qualitative identification (e.g. metabolites)
  - Less widely utilized for targeted quantification
  - More complex data acquisition
  - Fewer published methods for quantification
  - Steeper learning curve

Realities….

- How does LC-QTOF-MS performance measure up?
- Think beyond assay sensitivity…
- Under what situations might LC-QTOF-MS be advantageous?
- Potentially outperform LC-MS-MS?

General Challenges

- Proliferation of NPSs
  - Massive burden for method development
  - *Quantitative* analysis much-needed (to establish interpretive knowledge base)
- Many compounds with similar structures
  - Isomers
  - Constantly evolving
- Method development and validation time consuming
  - Isotopically labelled internal standards and metabolites may not be available

Question…

Can LC-QTOF-MS outperform LC-MS-MS for quantitative analysis?

Answer…

Sometimes……

Examples

- Provide examples related to assay performance for methods developed and validated in our laboratory
- Instances where LC-QTOF-MS outperformed LC-MS-MS during validation experiments
- Made us re-think our approach and commonly held beliefs
- Common themes/factors in quantitative assay performance
- Can we make better choices up-front? Before development?

Side-by-Side Comparisons

- LC-QTOF-MS
  - Agilent 6530 Accurate-Mass Q-TOF MS
  - 1290 Infinity Binary LC System
- LC-MS-MS
  - Agilent 6470 QQQ
  - 1290 Infinity Binary LC System

#1
Cathinones in Blood and Urine

ELSEVIER

Journal of Chromatography B

Volume 1035, 1 November 2016, Pages 91-103

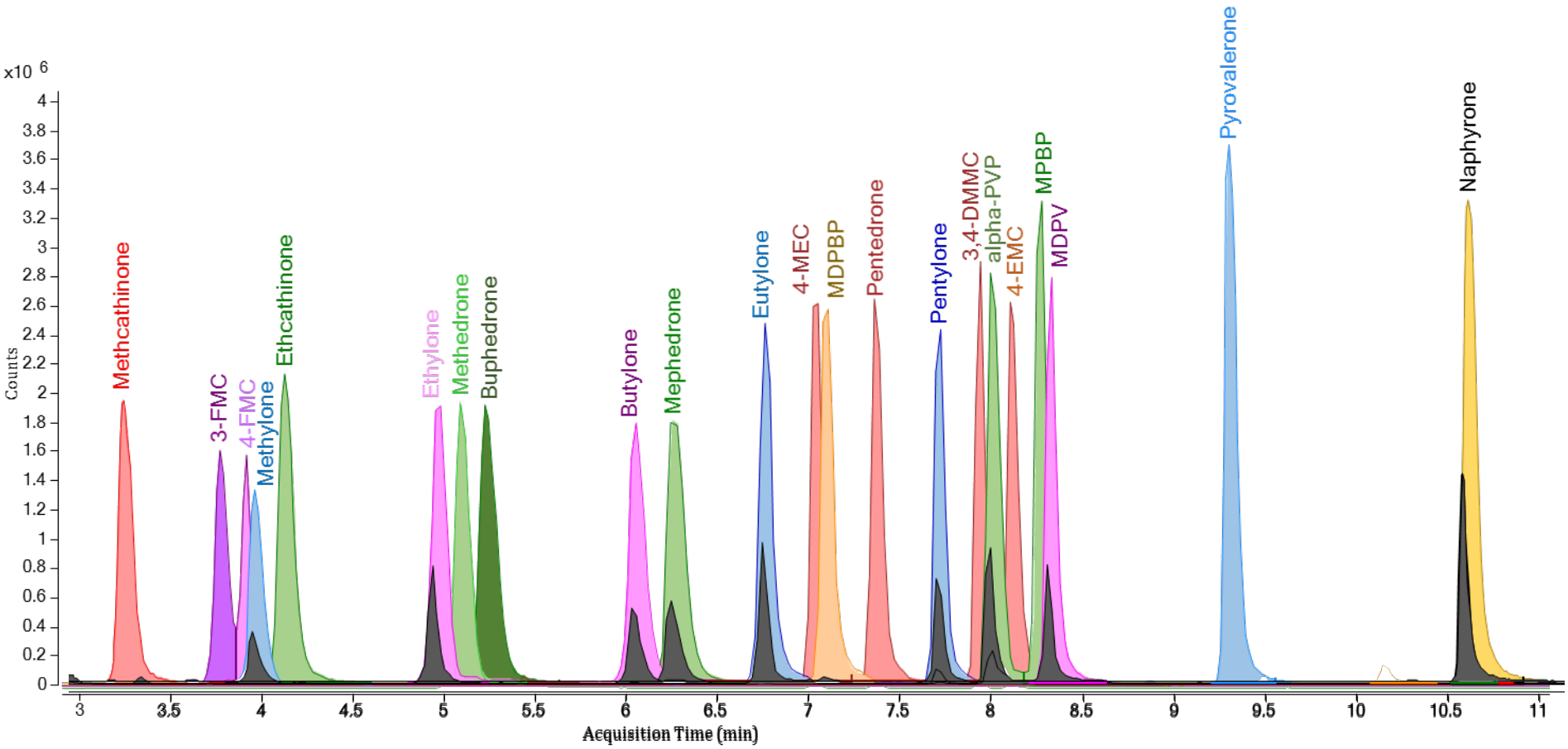
Identification and quantification of synthetic cathinones in blood and urine using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry

Lindsay Glicksberg, Kelsie Bryand, Sarah Kerrigan PhD

Cathinones in Blood & Urine

- Targeted assay for 22 cathinones in blood & urine
- Nine deuterated IS available at time of assay
- Isolation using SPE
- Quantitative analysis by LC-QTOF-MS
- Poroshell 120 EC-C18 (2.1x100 mm, 2.7 µm)
- MP FA (0.1%) in DIW/ACN
- Validation in accordance with SWGTOX recommendations

Chromatographic Separation



For Forensic Use

Validation Summary

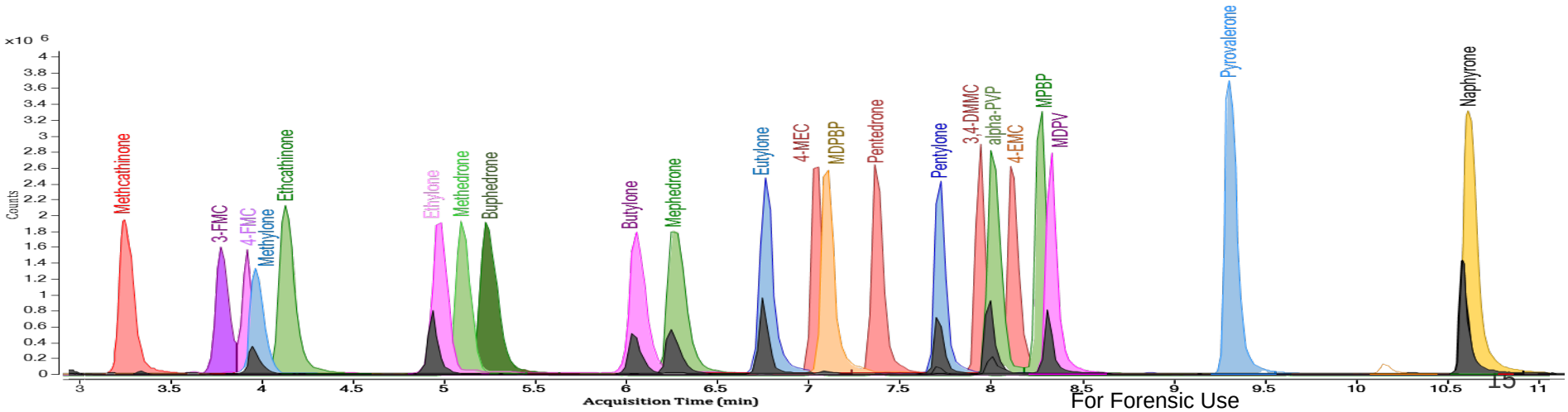
Parameter	Blood	Urine
Calibration Model	Quadratic, 1/x	
Limit of Detection	1 – 5 ng/mL	0.25 – 5 ng/mL
Limit of Quantitation	1 – 5 ng/mL	0.25 – 5 ng/mL
Inter-assay Precision (n=15)	3 – 12%	2 – 12%
Intra-assay Precision (n=3)	0 – 17%	0 – 11%
Bias (n=15)	-7 – 11%	-3 – 12%
Accuracy (n=15)	93 – 111%	97 – 112%
Analytical Recovery (n=4)	81 – 91%	84 – 104%
Matrix Effects (n=10)	-15 – 3%	-21 – -1%
Dilution Integrity	2- and 4- fold	
Interference Studies (>50 drugs)	No qualitative interferences observed in either matrix	

Interference Studies

- Matrix interferences (n=10)
- Internal standard
- Other drugs (>50)
  - 25 amphetamine-type
- Qualitative interferences
  - NEG and POS QCs
- **Quantitative interferences**
  - Using POS QCs (10 & 100 ng/mL) with 10- and 100-fold excess of other drug (1,000 ng/mL)

Quantitative Interferences Cathinone Assay

Drug [M+H]	IS	RT (min)	Bias	Bias (10:1)	Bias (100:1)	Interferent
4-MEC [192]	Mephedrone-D3	7.20	-8.1	-55%	-75%	Ketamine (7.25 m)
MDPBP [262]	Eutylone-D5	7.28	4.3	-41%	-77%	Ketamine (7.25 m)
MPBP [232]	Naphyrone-D5	8.44	-1.0	-40%	-25%	Cocaine (8.5 m)
3,4-DMMC [192]	Methylone-D3	8.13	-0.7	-51%	-71%	2C-C (8.2 m)
Ethcathinone [178]	Butylone-D3 (6.31 min)	4.30	0.0	+49%	+89%	2C-H (6.27 m)



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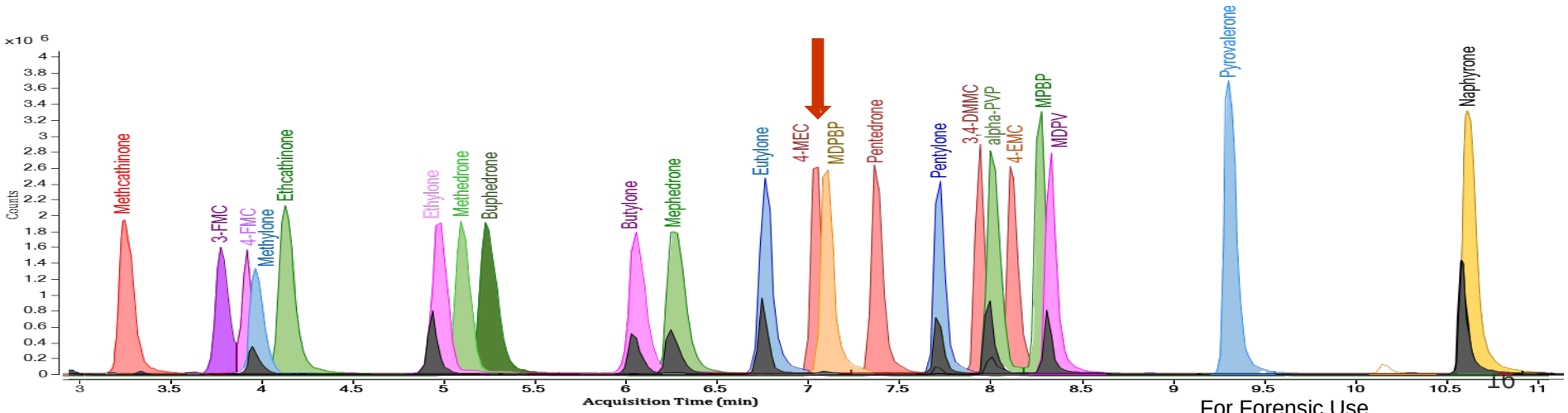

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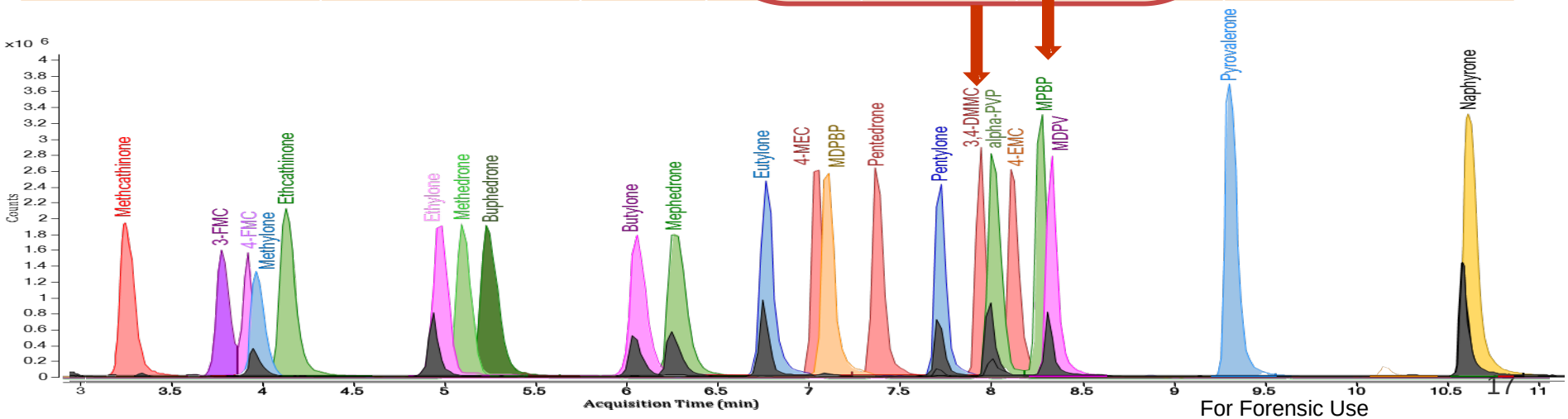

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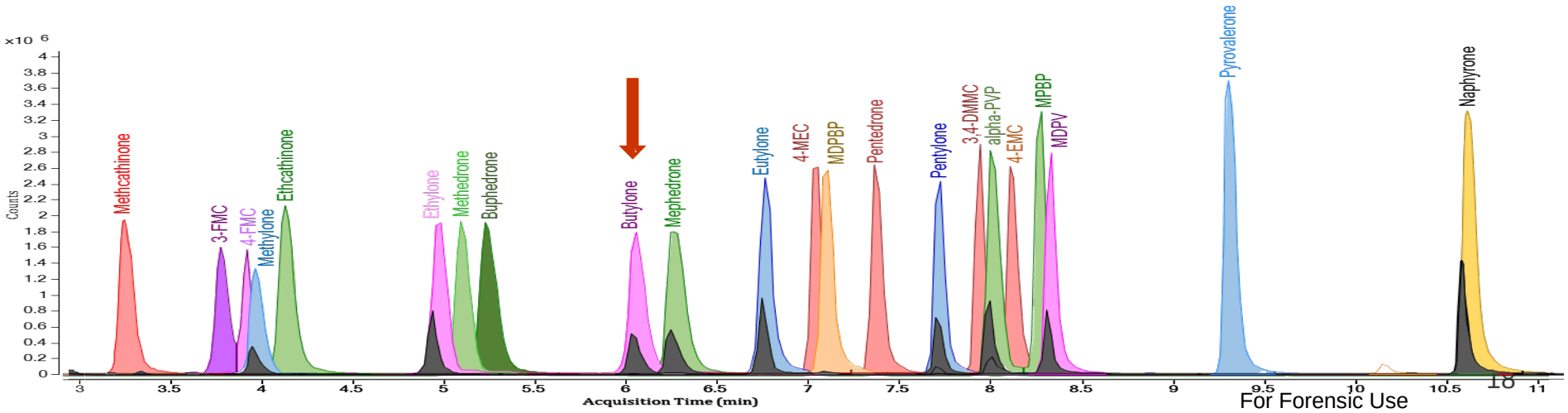

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- Interferences independent of precursor ion
- Significant bias caused by coeluting/closely eluting drug (*negative bias*)
- Magnitude of bias increased with increasing [interference]
- **Decreased ionization efficiency (competition from interferent)**
- *Positive bias* due to coelution of interferent with the IS
- **Highlights the importance of isotopically labelled internal standards**

Problem….

- No qualitative interferences present
- Quantitative interference present due to coeluting drug
  - 11 minute run (not fast LC)
  - Impossible to eliminate possibility of *any* coeluting substance
- No indication of interference from RT, ion ratios
  - Interference therefore invisible to the analyst/reviewer
- **More attention to quantitative interferences needed in future assay development**
- LC-QTOF-MS and LC-MS-MS equally susceptible?

#2
Suvorexant (Belsomra®) in Blood & Urine

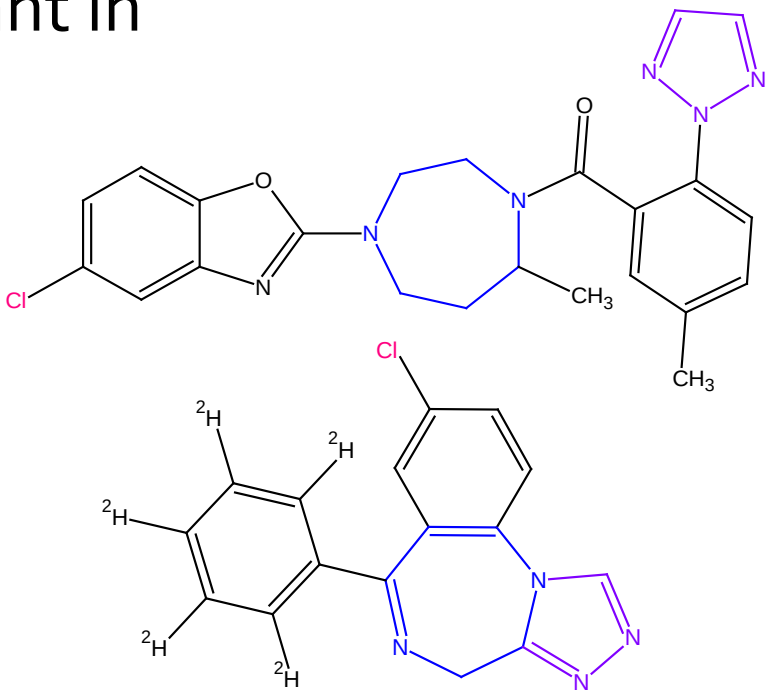
Identification of Suvorexant in Urine Using Liquid Chromatography–Quadrupole/Time-of-Flight Mass Spectrometry (LC–Q/TOF–MS) FREE

Sydney Sullinger, Kelsie Bryand, Sarah Kerrigan ✉

Journal of Analytical Toxicology, Volume 41, Issue 3, 1 April 2017, Pages 224–229,
https://doi.org/10.1093/jat/bkw132
Published: 30 December 2016

Suvorexant in Blood & Urine

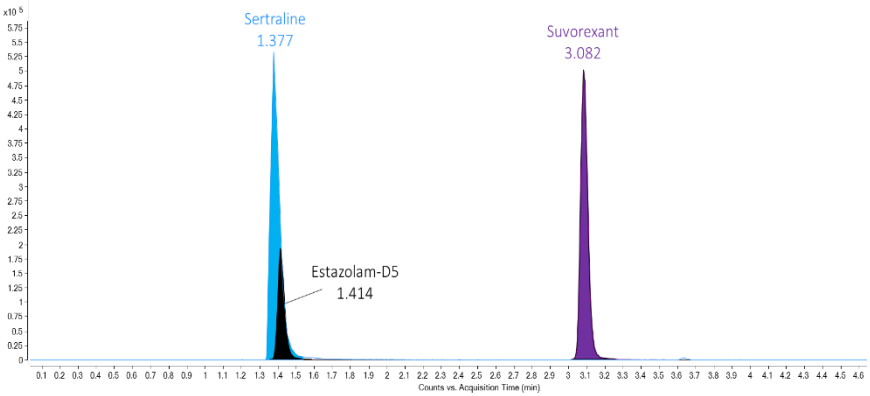
- Targeted assay for suvorexant in blood & urine
- NO deuterated IS available
  - Estazolam-D5 selected

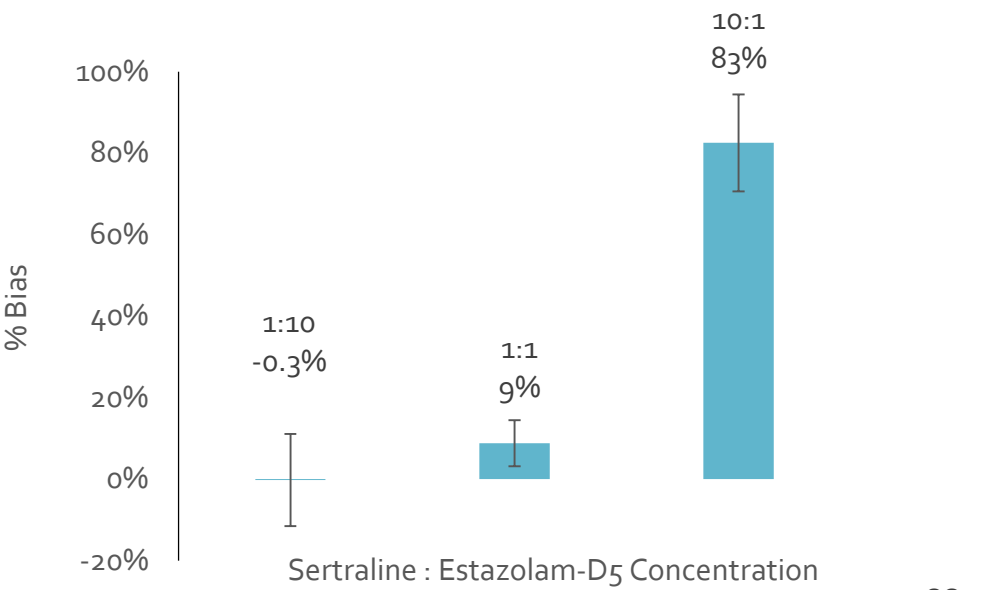


- Isolation using LLE
- LC-QTOF-MS
- Validation in accordance with SWGTOX recommendations

Quantitative Interference (Blood)

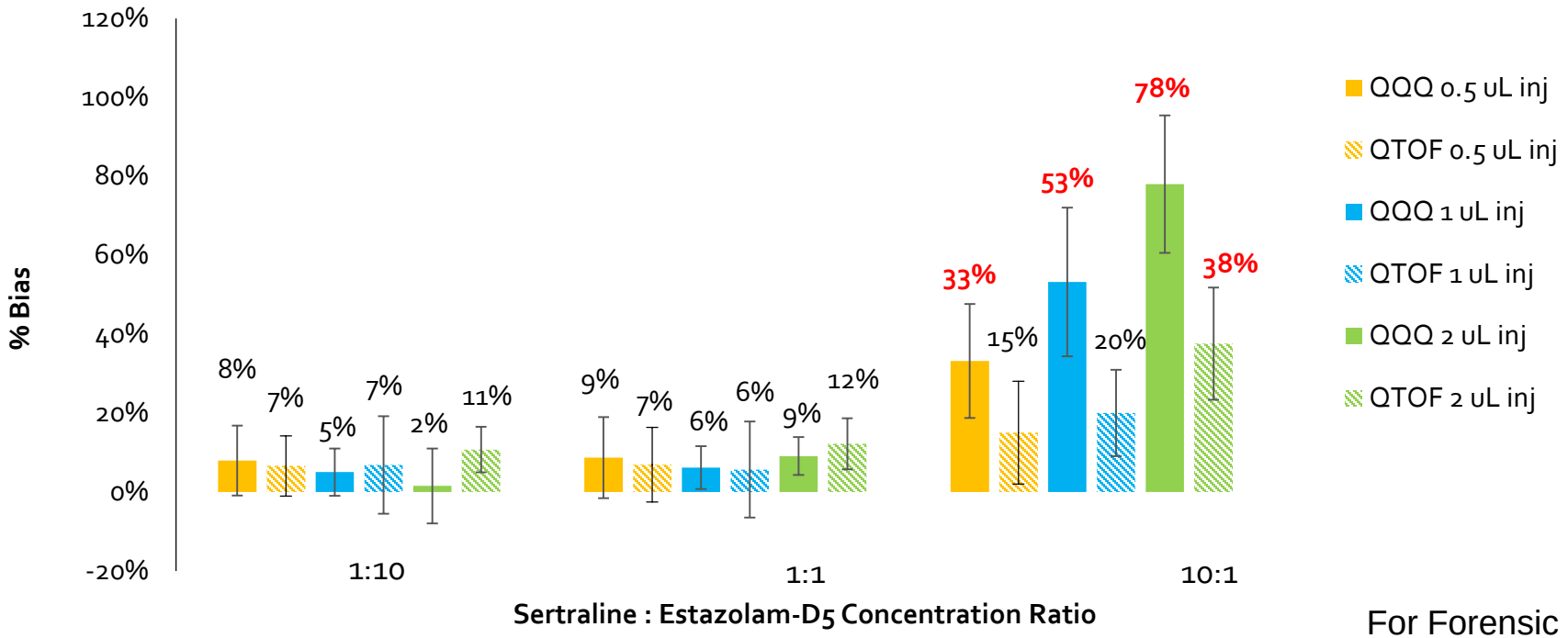
- No qualitative interference
- Sertraline similar RT to IS (estazolam-D5) – 100 ng/mL
- Sertraline:IS 1:10, 1:1, and 10:1 (n=3)
- Significant bias at 10:1 sertraline:estazolam-D5 concentrations





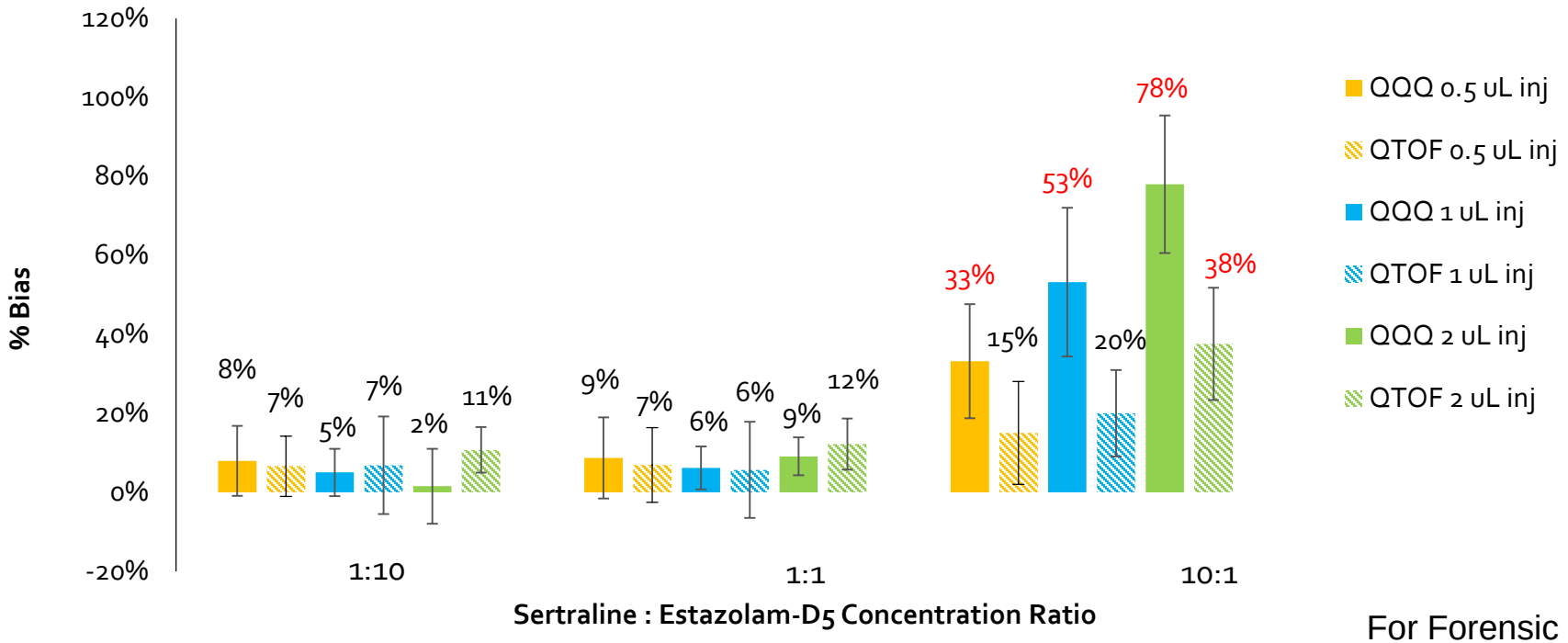
Minimize Quantitative Interferences?

- QTOF & MSMS comparison?
- Minimize the effect?



QTOF vs. MS/MS

- QTOF slightly less susceptible
- Reduced injection volume helps



QTOF vs. MS/MS

- Ion suppression > 25% observed using LC-MS/MS but not LC-Q/TOF-MS during method validation

Average Matrix Effect (n=10)	Suvorexant		Estazolam-D5	
	QTOF	***QQQ***	*QTOF*	*QQQ*
Low QC (20 ng/mL)	16%	**-35%**	19%	12%
High QC (100 ng/mL)	15%	**-26%**	11%	6%

Desomorphine in Urine

- LC-MS-MS (LOQ 0.5 ng/mL)
  - No quantitative interferences (66 drugs, incl. 24 opioids)

Interferences	Desomorphine	Mean Conc.	Accuracy	Bias
500 ng/mL	0 ng/mL	0	-	-
	5 ng/mL	5.3 ± 0.04	95%	5%
	50 ng/mL	51.0 ± 0.22	98%	2%
	500 ng/mL	516.3 ± 8.9	97%	2%

- LC-QTOF-MS (LOQ 0.5 ng/mL)
  - No quantitative interferences (66 drugs, incl. 24 opioids)

Interferences	Desomorphine	Mean Conc.	Accuracy	Bias
500 ng/mL	0 ng/mL	0	-	-
	5 ng/mL	4.6 ± 0.06	108%	7%
	50 ng/mL	46.3 ± 0.86	108%	-8%
	500 ng/mL	467.1 ± 22	107%	-7%

Summary

- LC-MS may be susceptible to quantitative interferences when isotopically labelled IS not available (NPSs)
  - Vigilance needed
  - Considerations for experimental design
    - Large number of drugs
    - Excess concentration (concentration-dependent bias)
  - Mitigation possible
- LC-QTOF-MS out-performed LC-MS-MS in some validation criteria

Summary

- Improved detection limits for LC-MS-MS? Does not always hold true
- LC-QTOF-MS targeted quantitations can offer comparable sensitivity
- QTOF-MS was less susceptible to ME in some assays using side-by-side comparisons
- QTOF-MS slightly less susceptible to quantitative drug interferences
  - Avoid fast LC methods for quantitative analysis wherever possible
  - Use isotopically labelled IS wherever possible
  - LC-MS more susceptible to drug interferences than GC-MS due to inhibited ionization in ESI (competition)
  - Robust interference studies are needed
  - Some correction for these phenomena can be made during method development

Conclusions

- Quantitative interferences can easily go undetected
- SWGTOX validation requires only *qualitative* interference studies, not *quantitative*
- Ion ratios, retention times are unaffected
- **No way to identify an unknown quantitative interference in actual casework**
- Must be carefully evaluated during method development/validation
- Interference (in source) *i.e.* pre-mass filtering, therefore possible using both LC-MS/MS and LC-QTOF-MS

Acknowledgements

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