

Innovation Applied

Quantitative Screening of 46 Illicit Drugs in Urine using
Exactive Ultrahigh Resolution and Accurate Mass system

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Forensic Toxicology Use Only

Presentation Overview

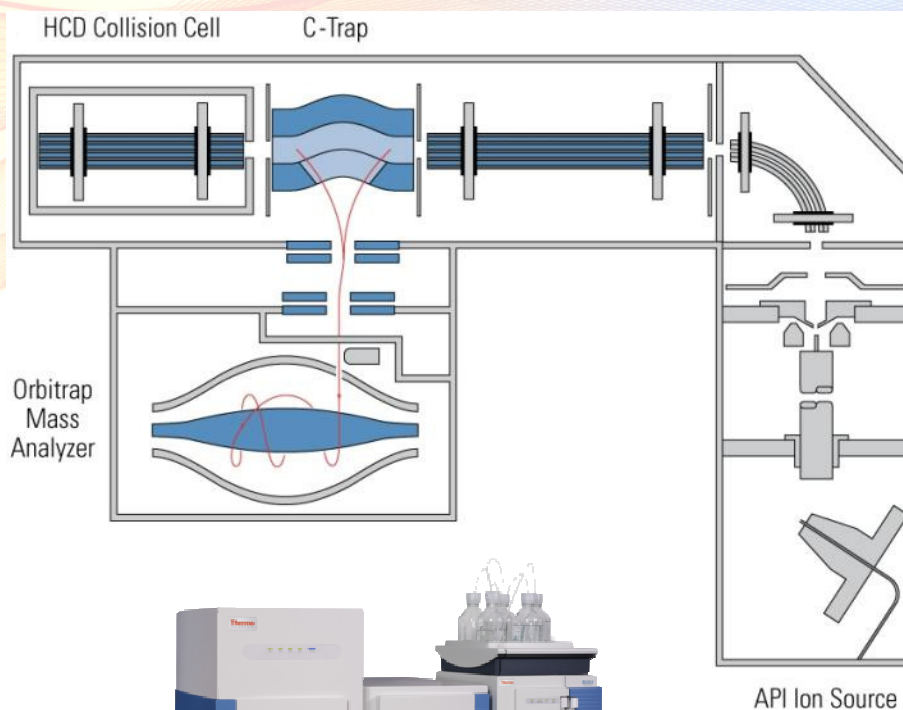
- Anabolic androgenic steroids landscape
- Triple quadrupole MS/MS characteristics and performance
- AAS behavior in ESI-MS
- Sample preparation methods
- Orbitrap high resolution mass spectrometry
- Quantitative analysis of AAS in OF using Q Exactive
- Testosterone in serum using TurboFlow and Q Exactive
- Summary

Presentation overview

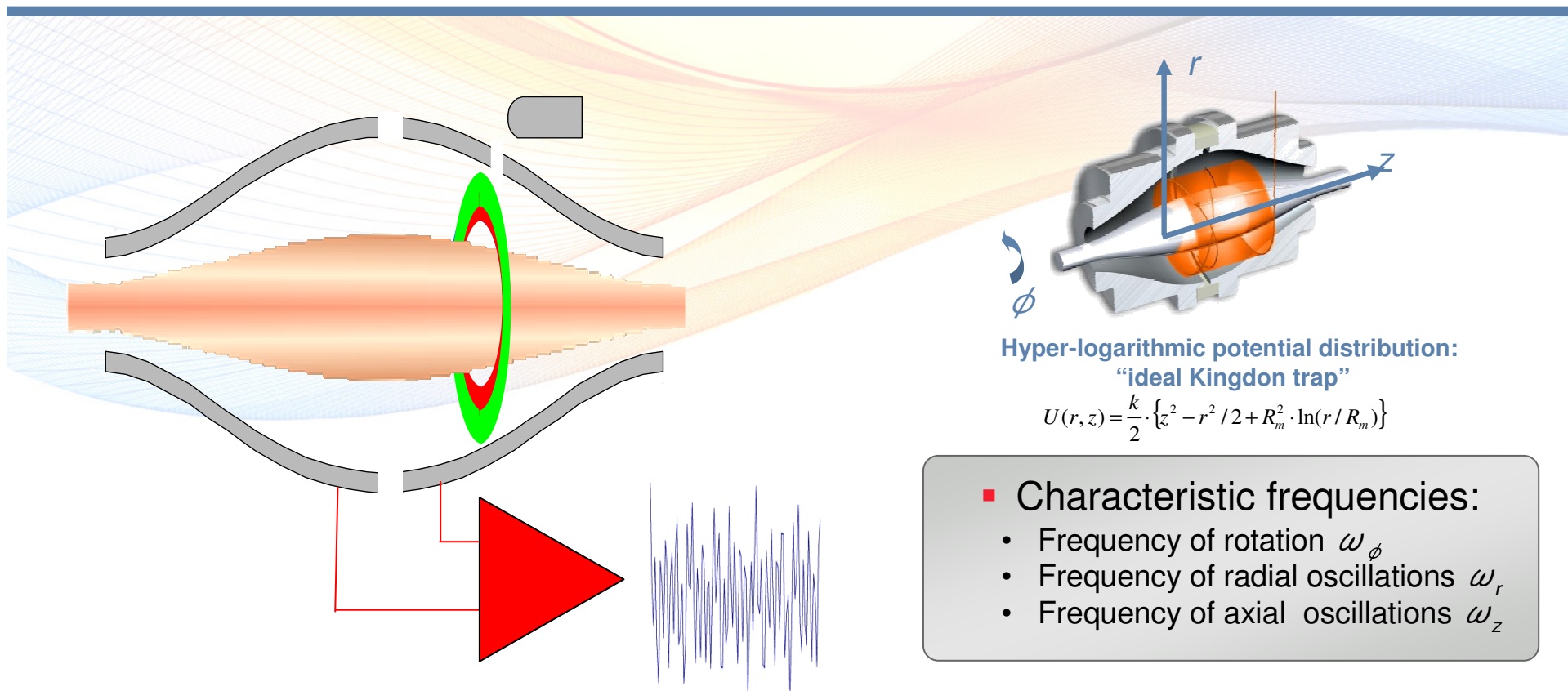
- Exactive system introduction
- Principle of operation
- Method overview and salient features
- Urine analysis results
- Conclusions
- Appendix

Exactive Benchtop LC-MS

- Resolution
 - 100,000 at 1 scan per second
 - 10,000 at 10 scans per second
- Mass accuracy
 - Sub ppm
- Sensitivity
 - 500 fg Buspirone with S/N >10:1
- Dynamic range
 - >10,000 within a spectrum
- Scan speed
 - Up to 10 scans per second
- Mass range
 - m/z 50 - 4000
- Polarity switching
 - One positive and one negative scan < 1 second (25K Resolution)



Orbitrap – Principle of Operation



Hyper-logarithmic potential distribution:
“ideal Kingdon trap”

$$U(r, z) = \frac{k}{2} \cdot \{z^2 - r^2 / 2 + R_m^2 \cdot \ln(r / R_m)\}$$

■ **Characteristic frequencies:**

- Frequency of rotation ω_ϕ
- Frequency of radial oscillations ω_r
- Frequency of axial oscillations ω_z

$$\omega_z = \sqrt{\frac{k}{m/q}}$$

$$\omega_\phi = \frac{\omega_z}{\sqrt{2}} \sqrt{\left(\frac{R_m}{R}\right)^2 - 1} \quad \omega_r = \omega_z \sqrt{\left(\frac{R_m}{R}\right)^2 - 2}$$

Makarov A. *Anal. Chem.* 2000, 72, 1156-

1162.

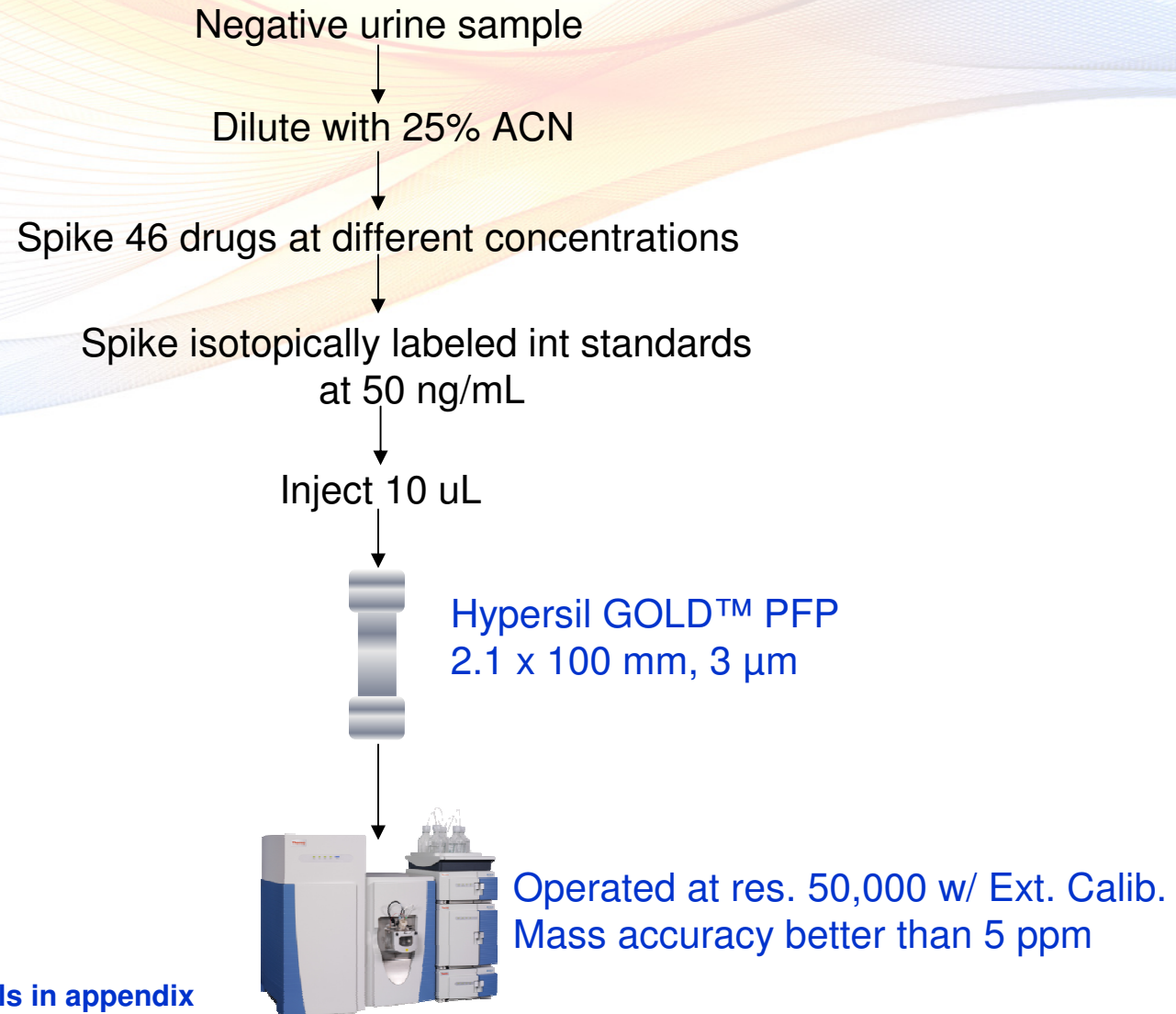
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Method overview and salient features

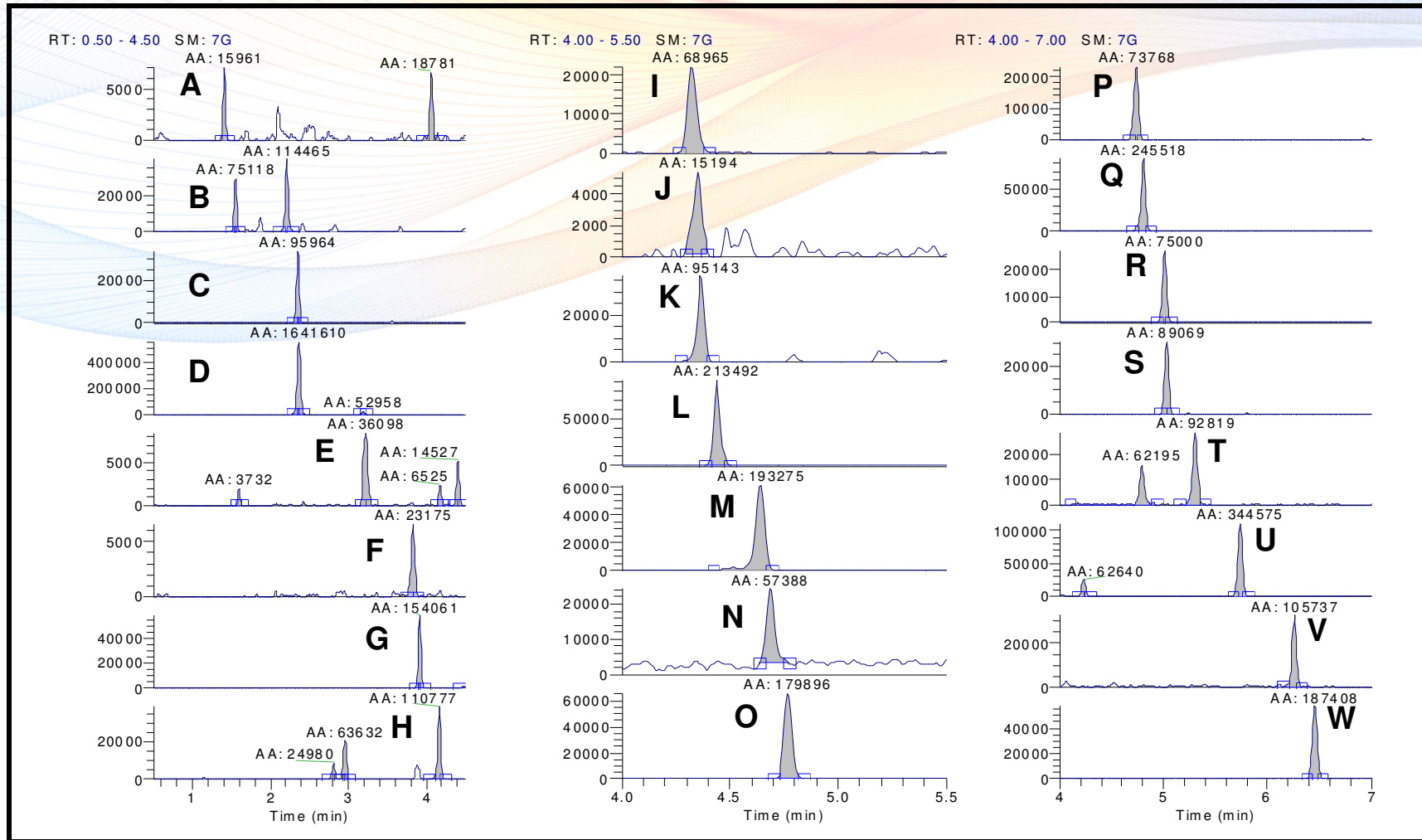
- Negative standard urine diluted with 25% ACN spiked with 46 drugs at different concentrations.
- Isotopically labeled internal standards at constant 50 ng/mL.
- No sample preparation. A 10 uL aliquot directly injected onto reverse phase column.
- Exactive operated at a resolution of 50,000 FWHH
- External calibration mass accuracy better than 5 ppm.
- Analysis time <10 minutes

Note: LC/MS method details in appendix

Method overview and salient features



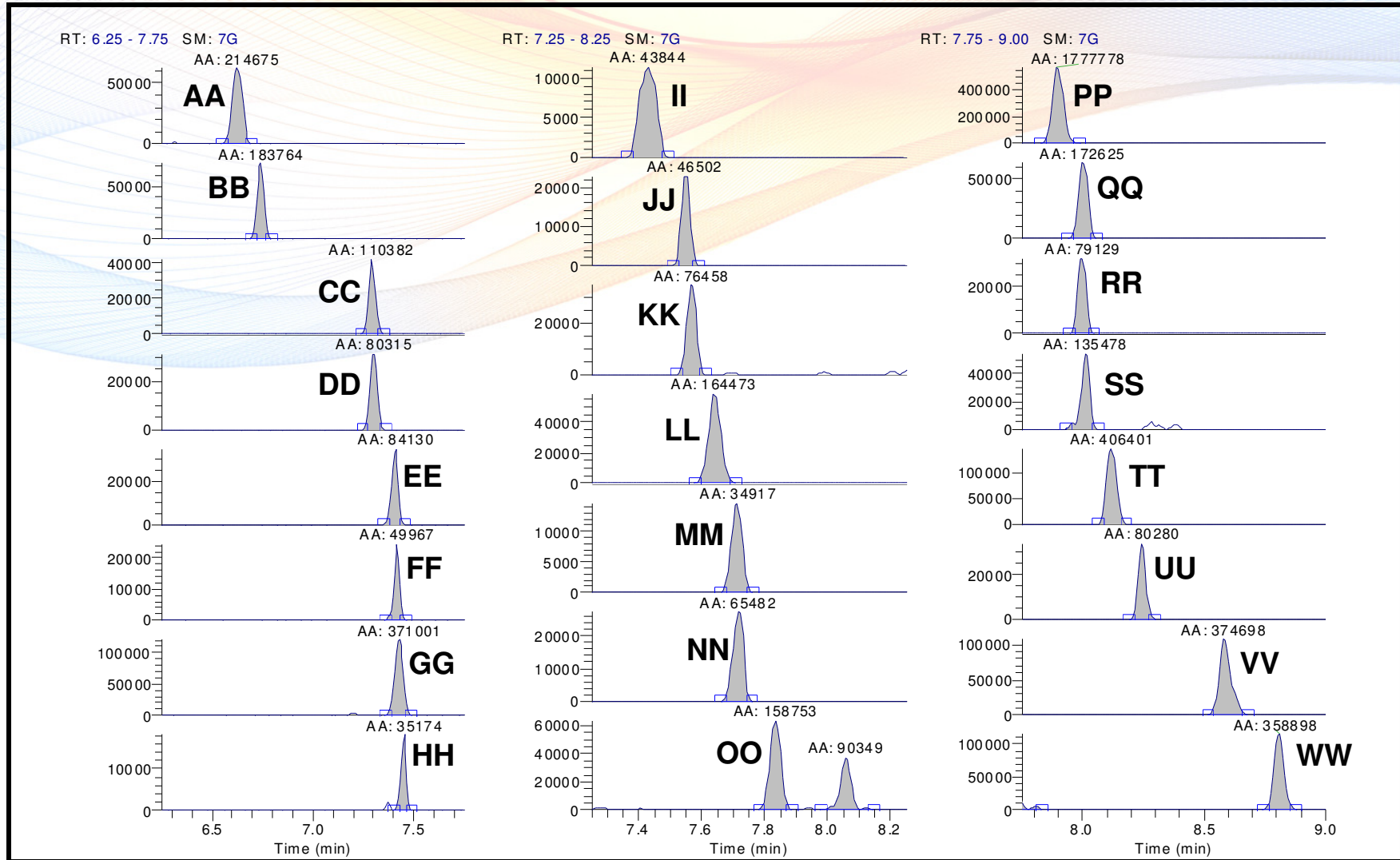
Results - XIC@ ± 5 ppm at LOQs in urine



Note: Drug names and LOQs on slide 9 & 10

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Results - XIC @ ± 5ppm at LOQs in urine.



Note: Drug names and LOQs on slide 9 & 10

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List of drugs and mass errors @ LOQ

	Drug of Abuse	RT (min)	Exact m/z	Measured m/z	Error (ppm)	LOQ (ng/mL)
A	Nicotine	1.40	163.12298	163.12288	-0.6	0.5
B	Cotinine	1.55	177.10224	177.10204	-1.2	0.5
C	Morphine	2.35	286.14377	286.14362	-0.5	1.25
D	Hydromorphone	3.19	286.14377	286.14374	-0.1	1.25
E	Ephedrine	3.23	166.12264	166.12256	-0.5	1.25
F	Amphetamine	3.82	136.11208	136.11203	-0.4	2.5
G	Codeine	3.91	300.15942	300.15891	-1.7	1.25
H	Noroxycodone	4.17	302.13868	302.13828	-1.3	1.25
I	Methamphetamine	4.32	150.12773	150.12761	-0.8	1.25
J	MDA	4.36	180.10191	180.10181	-0.6	0.5
K	Oxycodone	4.36	316.15433	316.15384	-1.5	0.5
L	6-Acetylmorphine	4.44	328.15433	328.15384	-1.5	1.25
M	Hydrocodone	4.64	300.15942	300.15891	-1.7	1.25
N	MDMA	4.69	194.11756	194.11742	-0.7	0.5
O	Norketamine	4.77	224.08367	224.08351	-0.7	1.25
P	7-Amino-clonazepam	4.75	286.07417	286.07367	-1.7	0.5
Q	Benzoyllecgonine	4.81	290.13868	290.13806	-2.1	1.25
R	Ketamine	5.01	238.09932	238.09877	-2.3	0.5
S	Norfentanyl	5.03	233.16484	233.16470	-0.6	0.5
T	MDEA	5.31	208.13321	208.13304	-0.8	0.5
U	7-Amino-flunitrazepam	5.73	284.11937	284.11929	-0.3	0.5
V	Normeperidine	6.26	234.14886	234.14865	-0.9	0.5
W	Meperidine	6.45	248.16451	248.16428	-0.9	0.5
AA	Cocaine	6.62	304.15433	304.15405	-0.9	0.5

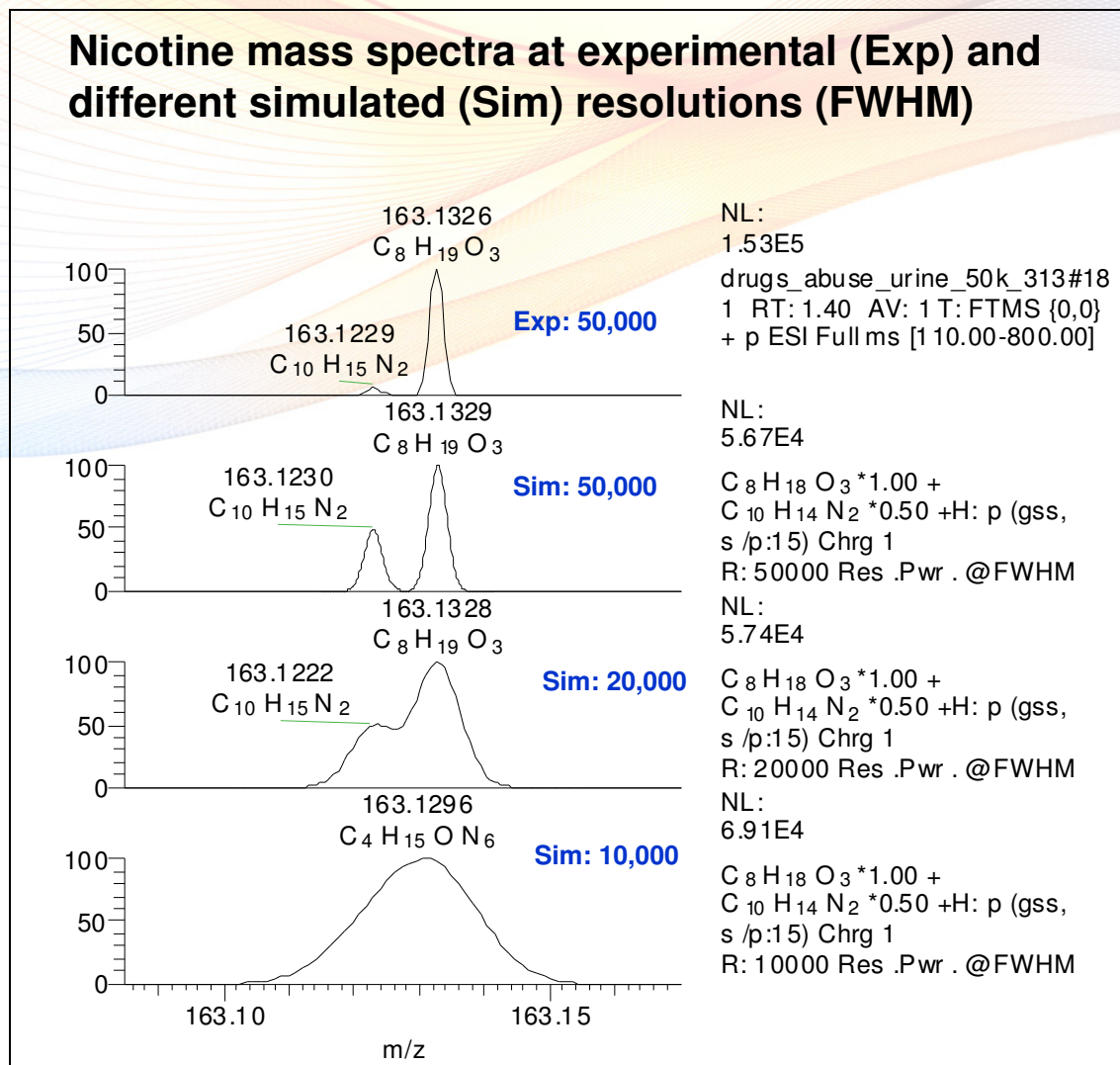
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List of drugs monitored and mass errors @ LOQ

	Drug of Abuse	RT (min)	Exact m/z	Measured m/z	Error (ppm)	LOQ (ng/mL)
BB	Norbuprenorphine	6.74	414.26389	414.26257	-3.2	2.5
CC	alpha-Hydroxymidazolam	7.29	342.08039	342.07971	-2.0	1.25
DD	Oxazepam	7.30	287.05818	287.05768	-1.7	5
EE	alpha-Hydroxytriazolam	7.41	359.04609	359.04562	-1.3	2.5
FF	alpha-Hydroxyalprazolam	7.42	325.08507	325.08447	-1.8	2.5
GG	Cocaethylene	7.43	318.16998	318.16962	-1.1	0.5
HH	Lorazepam	7.45	321.01921	321.01797	-3.9	2.5
II	PCP	7.44	244.20598	244.20518	-3.3	0.5
JJ	Nitrazepam	7.54	282.08732	282.08658	-2.6	2.5
KK	2-Hydroxyethylflurazepam	7.57	333.08006	333.07889	-3.5	1.25
LL	Midazolam	7.64	326.08548	326.08493	-1.7	0.5
MM	Nordiazepam	7.71	271.06327	271.06265	-2.3	0.5
NN	Clonazepam	7.72	316.04835	316.04791	-1.4	2.5
OO	Temazepam	7.83	301.07383	301.07349	-1.1	2.5
PP	Fentanyl	7.90	337.22744	337.22635	-3.2	2.5
QQ	Alprazolam	8.00	309.09015	309.08957	-1.9	1.25
RR	Triazolam	7.99	343.05118	343.05103	-0.4	0.5
SS	Flunitrazepam	8.01	314.09355	314.09293	-2.0	1.25
TT	Buprenorphine	8.12	468.31084	468.31006	-1.7	1.25
UU	Diazepam	8.24	285.07892	285.07819	-2.6	0.5
VV	EDDP	8.58	278.19033	278.18991	-1.5	0.5
WW	Methadone	8.81	310.21654	310.21628	-0.8	0.5

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Need for 50,000 resolution



Note: Experimental Nicotine at RT 1.40 min; exact mass = 163.1230

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Conclusions

- The method is capable of easy confirmatory and quantitative analysis of 46 illicit drugs at LOQs of 0.5 – 5 ng/mL in urine.
- Ultrahigh mass resolution of 50,000 FWHM provided the necessary selectivity to identify 46 drugs in urine in 9 minutes with minimal sample preparation
- Analyte confirmation is achieved by utilizing better than 5 ppm mass accuracy with external mass calibration.
- Good quantitative accuracy achieved by isotopically-labeled standard.
- Nicotine example demonstrates the need for $\geq 50,000$ resolution in addition to exact masses.

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- Appendix

LC/MS Method

HPLC system

- Accela UHPLC™ Pump, PAL autosampler (CTC Analytics)

• LC Column

- 2.1 x 100 mm, 3 µm Hypersil GOLD™ PFP
- Injection Volume = 10 µL

• Mobile Phase

- (A) 5 mM NH₄OOC + 0.1% HCOOH, (B) ACN + 0.1% HCOOH

• Flow Rate

- 0.35 mL/min

• **MS system**

- Exactive™ (Thermo Scientific)
- Heated ESI; Polarity: Positive; Scan Range: m/z 110 – 800
- Resolution: 50,000 FWHM;
- External mass calibration