Advantages of Ultra-High-Resolution Q Exactive Mass Spectrometer in Analysis of Unlimited Number of Compounds in Urine Quantitative Screening Application for Forensics

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Introduction

Implementation of ultra-high-resolution mass spectrometers for quantitative forensic toxicology allows for unlimited number of analytes, short acquisition times and simple sample preparation. At the same time, ultra-high-resolution mass spectrometry provides high confidence in reported hits. Quantitative screening in forensic toxicology applications is important because it allows reporting of only those compounds with concentrations above specified threshold, reinjection of samples following those with concentration above carry-over limit, and appropriate sample dilution, if required, for confirmatory quantitative analysis.

Instrumentation

Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system

Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap MS

Methods

Sample Preparation

Enzymatic hydrolysis followed by liquid-liquid extraction.

A 1 mL aliquot of urine (spiked calibrator, QC or donor sample) was spiked with internal standard (Tolbutamide), and incubated with 10,000 U/mL beta-glucuronidase enzyme in pH 5.5 buffer for 60 minutes at 60 °C. The resulting mixture was basified with sodium carbonate and extracted with ethylacetate:hexane (1:1). The organic supernatant was evaporated to dryness under nitrogen at 37 °C. The residue was reconstituted in 100 µL of 20% methanol and 10 µL of the sample was analyzed by LC-MS.

LC Method

The column used was a Thermo Scientific™Hypersil™ GOLD PFP 100 x 2.1 mm, 5 µm. Mobile phase was 10 mM ammonium acetate in water (A) and methanol (B). Both solvents were Fisher Scientific™ Optima™ grade. The LC gradient was as follows:

Time (min)	%A	%B	Flow rate (mL/min)
0	95	5	0.75
0.5	95	5	0.75
3.6	60	40	0.75
6.1	5	95	0.75
6.15	0	100	0.85
7.1	0	100	0.85
7.15	95	5	0.85
9.0	95	5	0.85

Mass Spectrometry Method

The Q Exactive benchtop orbitrap mass spectrometer was equipped with a HESI source and operated in positive ionization mode. The MS method consisted of 2 scan events: Full scan from 130–472 *m/z*+ (R = 70K) and all ion fragmentation (AIF) scan from 50–472 *m/z*+ (R = 70K). The AIF spectra were collected with stepped collision energy of 70 ± 50%

Method Validation

The method was validated for 37 representative compounds from different drug classes (Table 1). Calibration standards (0.05–1000 ng/mL) and QC samples (2, 10, 50 ng/mL) were prepared in pooled negative urine.

Matrix effects were evaluated by spiking urine from 15 different donors at concentrations of 10 ng/mL (opioids) , 20 ng/mL (benzodiazepines) or 100 ng/mL (amphetamines) and then processing the samples as described in sample preparation above. Percent recovery was calculated against samples at the same concentrations prepared in water instead of urine.

Data Analysis

Data was acquired and processed with Thermo Scientific™ TraceFinder™ software version 3.1. Full-scan data (chromatograms reconstructed with *m/z* accuracy of 5 ppm) were used for analyte detection and quantification. AIF spectra were used for compound confirmation. Calibration ranges and LOQ's were evaluated based on concentration accuracy and data precision. Back-calculated concentration had to be within 30% for the LOQ.

Results

- Examples of data collected with the method are presented in Figure 1.
- Representative chromatograms for the lowest calibration standards of selected analytes are presented in Figure 2.
- Linearity ranges, method precision and % recovery in spiked urine samples from 15 different donors are presented in Table 1.
- Examples of calibration curves are shown in Figure 3.

FIGURE 1. Representative chromatograms and spectra of tramadol and diazepam in pooled urine spiked at 100 ng/mL. Shown are the full-scan chromatogram of parent compound for quantitation, chromatogram of fragments from AIF spectra reconstructed with *m/z* accuracy of 5 ppm, and AIF spectra for the scan collected at the apex of the peak. Chromatograms are reconstructed in stick mode to demonstrate sufficient number of scans across the peak.

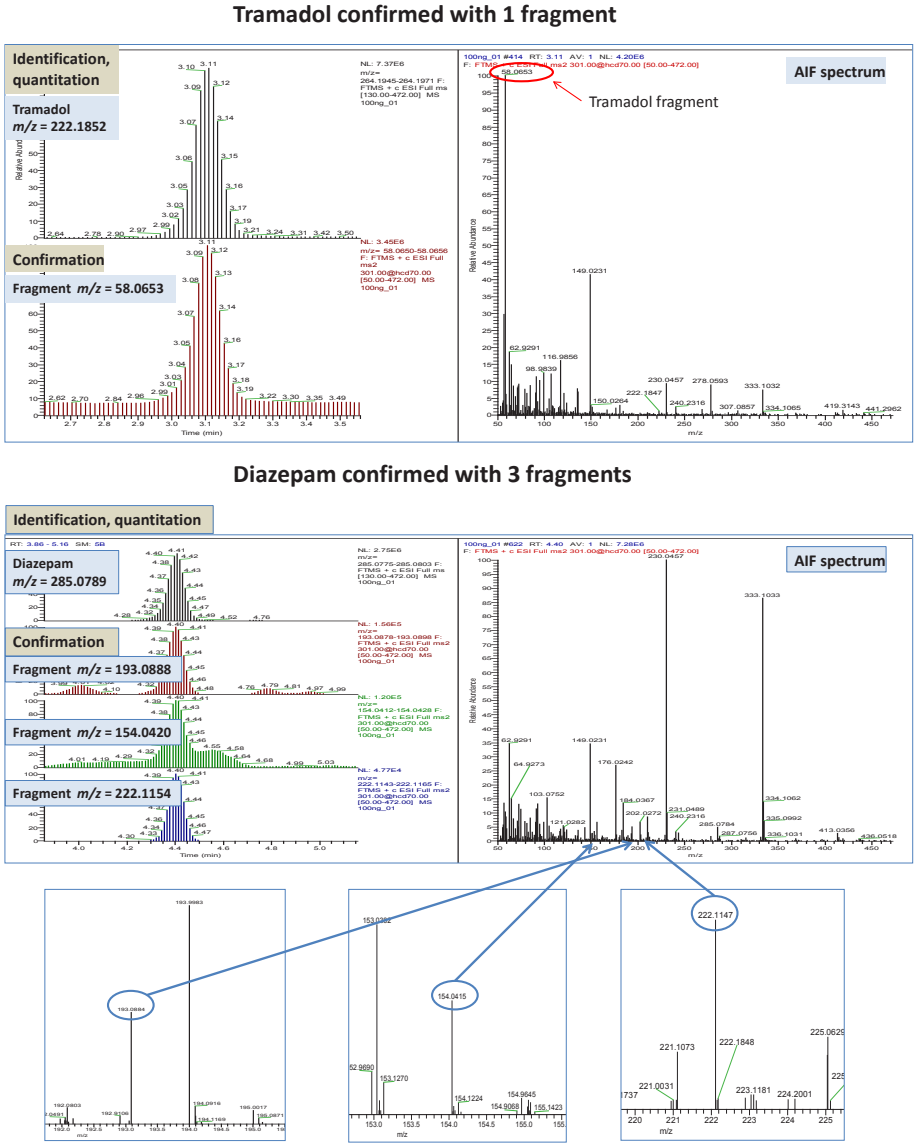


FIGURE 2. Chromatograms of the lowest calibration standards for selected analytes as displayed in TraceFinder software.

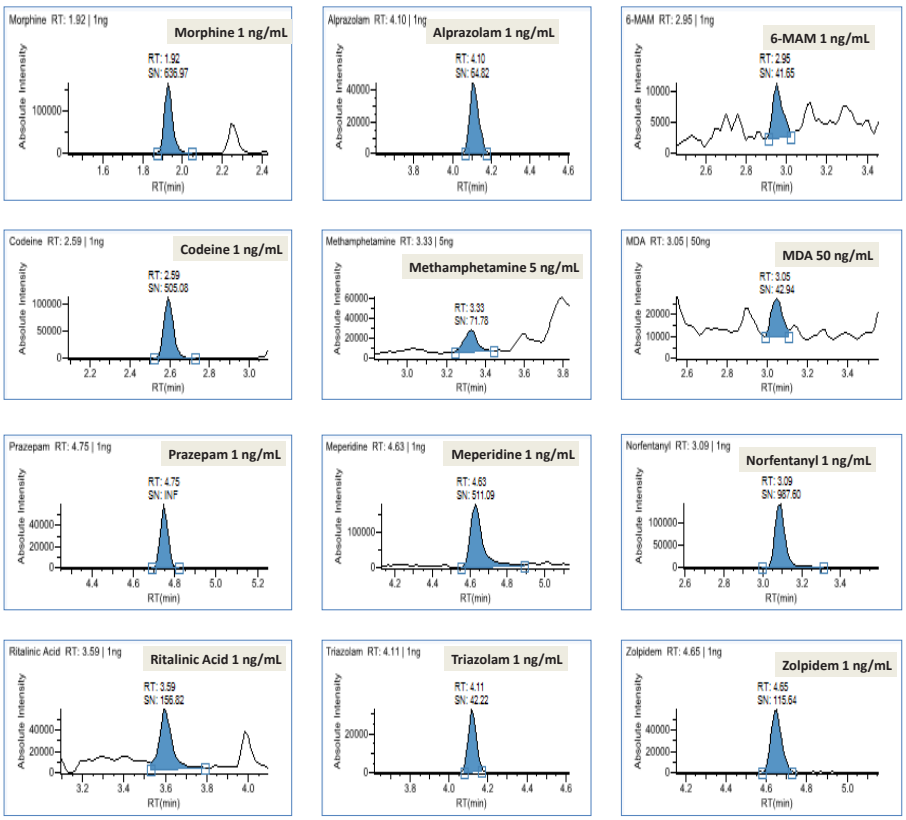
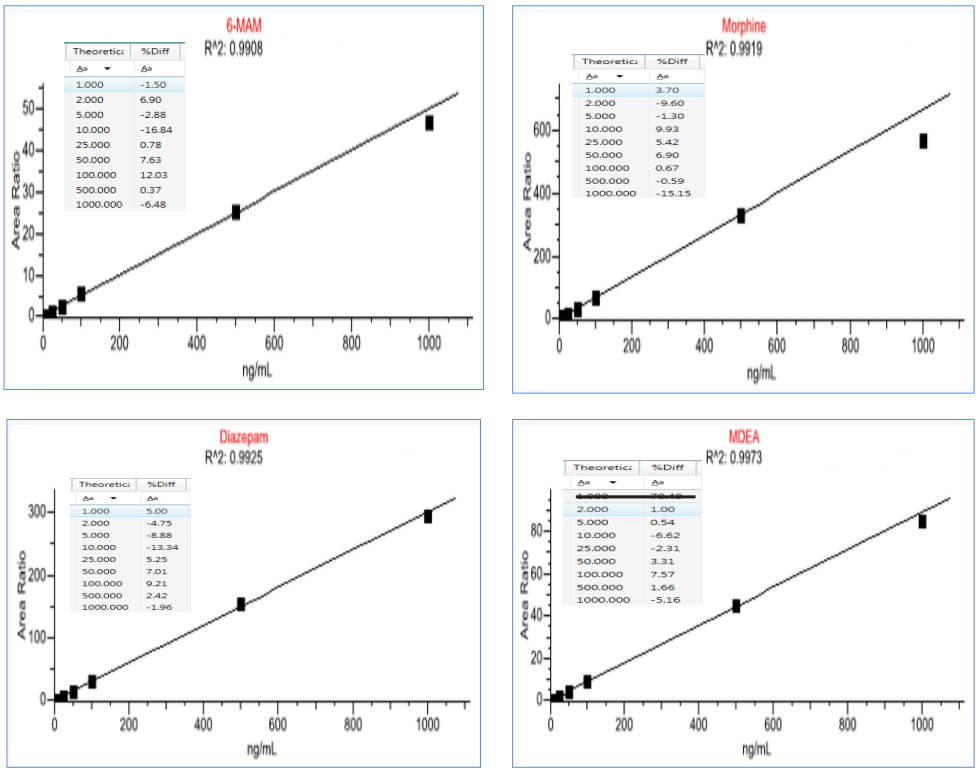


Table1. Linearity ranges, method precision and % recovery in spiked urine samples from 15 different samples.

Compound	Calibration range (ng/ml)	Precision 2 ng/mL (%RSD)	Precision 10 ng/mL (%RSD)	Spike Recovery	Compound	Calibration range (ng/ml)	Precision 2 ng/mL (%RSD)	Precision 10 ng/mL (%RSD)	Spike Recovery
6-MAM	1–1000	10	9.6	97.6–127	Midazolam	1–1000	20.8	9.9	104–126
7-aminoclonazepam	1–1000	8.1	9.1	87.0–118	Morphine	1–1000	16.2	10.6	99.2–126
Alprazolam	1–1000	10.5	8.9	96.5–110	Nordiazepam	1–1000	16.8	8.7	92.4–110
Clonazepam	1–1000	13	6.8	91.4–118	Norfentanyl	1–1000	13.1	10.1	96.8–119
Clorazepate	1–1000	22	8.7	92.4–107	Norhydrocodone	1–1000	14	9.6	97.5–118
Codeine	1–1000	13.6	7.7	96.4–113	Normeperidine	1–1000	8.5	8.9	105–123
Diazepam	1–1000	11.8	8	98.9–116	Noroxycodone	1–1000	15.7	7	95.6–128
Dihydrocodeine	1–1000	13.6	9.7	97.7–112	Desmethyltramadol	1–1000	12.4	8.7	95.6–121
Flurazepam	1–1000	9.5	12.9	92.7–111	Oxazepam	1–1000	10.5	9.2	90.2–124
Hydrocodone	1–1000	8.4	8.1	94.0–117	Oxycodone	1–1000	19.3	12.2	99.0–126
Hydromorphone	1–1000	13.6	7.7	96.8–121	Oxymorphone	1–1000	12.8	8.4	93.2–117
Lorazepam	2–1000	18.2	12.7	86.5–122	Prazepam	1–1000	8.5	6.7	96.1–114
MDA	50–1000	NA	11.1*	88.7–112	Ritalinic Acid	1–1000	7.1	10	98.0–122
MDEA	2–1000	21.5	13.8	106–128	Tapentadol	1–1000	11.9	11.1	98.5–116
MDMA	25–1000	NA	16.9*	106–136	Temazepam	1–1000	10.5	9.8	91.5–114
Meperidine	1–1000	13.7	13.2	103–125	Tramadol	1–1000	12.1	11.9	107–129
Methadone	5–1000	NA	15.6	80.4–128	Triazolam	1–1000	8.5	8.9	96.6–110
Methamphetamine	5–1000	NA	18.1	104–138	Zolpidem	1–1000	14	12.2	99.4–116
Methylphenidate	1–1000	8.5	13.3	110–123					

* Precision obtained for QC sample at concentration of 50 ng/mL

FIGURE 3. Example of calibration curves and calibration standard accuracy tables for selected compounds. Note: tolbutamide was used as internal standard for all analytes.



Conclusion

- A method was developed for quantitative screening which can be used for analysis of a virtually unlimited number of compounds in forensic toxicology.
- Method linearity, precision and accuracy meet requirements for quantitative screening in forensic toxicology laboratories.
- Method is robust: limited matrix effects were observed.
- Method can be multiplexed on dual channel LC system resulting in an analytical time of less than 5 minutes.

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