Demonstration of Screening of Over 300 Compounds in Urine Using Triple Quadrupole Mass Spectrometer and Software for Rapid Data Analysis

Kristine Van Natta, Marta Kozak
Thermo Fisher Scientific, San Jose, California, USA

Poster Note 64641

Overview

Purpose: To evaluate a screening method for 300 plus compounds on a fast-scanning triple quadrupole mass spectrometer. Compounds tested were selected from different classes of chemicals: opiates, benzodiazepines, amphetamines, stimulants, therapeutic drugs, designer drugs, antibiotics, environmental toxins and others.

Methods: Hydrolyzed and diluted urine samples were analyzed using a 13-minute chromatography method followed by SRM detection. Two or three SRM transitions were collected for each analyte.

Results: Over 90% of the screened compounds were identified at concentration of 100 ng/mL or less.

Introduction

Forensic toxicologists need tools for rapid and confident screening for large numbers of compounds. Triple quadrupole mass spectrometers are becoming the instruments of choice in many forensic toxicological laboratories, supplanting traditional immunoassays because they offer better specificity within compound classes, can test for a wider range of compounds, and more compounds can easily be added to the test menu. In order to generate results as fast as immunoassays, mass spectrometry methods must contain a larger number of analytes in one analytical run. Additionally, software is needed for easy and rapid data analysis of the large panels.

Methods

Sample Preparation

• Urine samples spiked with 300+ compounds, in groups of 10, were fortified with Tolbutamide-d9 internal standard, hydrolyzed with ß-glucuronidase enzyme and diluted with water. The final dilution factor was 30. A 10-µL aliquot of sample was analyzed.

LC method

- Thermo Scientific™ Transcend ™ II TLX system operated in LX mode.
- Chromatographic column: Thermo Scientific™ Accucore™ Phenyl Hexyl, 100 x 2.1 mm, 2.6 µm
- A: 2mM ammonium formate, 0.1% formic acid in water
- B: 2mM ammonium formate, 0.1% formic acid in acetonitrile/methanol (50/50, v/v)
- LC gradient: 13.5 min

Step	Start	Sec	Flow	Grad	%A	%B
1	0.00	60	0.50	Step	99.0	1.0
2	1.00	540	0.50	Ramp	1.0	99.0
3	10.00	90	0.80	Step	1.0	99.0
4	11.50	120	0.80	Step	99.0	1.0

Thermo SCIENTIFIC

Methods (cont.)

Mass Spectrometry

- Thermo Scientific™ TSQ Endura™ mass spectrometer
- HESI source
- Data acquisition experiment: SRM transitions in time windows with polarity switching. Two or three SRM transitions were collected for each analyte. Figure 1 presents screen capture of SRM table in data acquisition method.

Figure 1. SRM transitions table in data acquisition method (ending rows). 842 SRM transitions were collected with a cycle time of 0.3 sec.

SRM Table

	Compound	Retention Time (min)	RT Window (min)	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
818	aminodarone	8.52	.5	Positive	646.055	58.086	59	245
819	aminodarone	8.52	.5	Positive	646.055	100.084	35	245
820	aminodarone	8.52	.5	Positive	646.055	276.059	40	245
821	THC-OH	8.83	.5	Positive	331.175	295.053	20	101
822	THC-OH	8.83	.5	Positive	331.175	313.213	15	101
823	THC-COOH-d9-POS	8.93	.5	Positive	354.25	308.262	22	94
824	THC-COOH-d9-POS	8.93	.5	Positive	354.25	335.263	17	94
825	THC-COOH-d9-POS	8.93	.5	Positive	354.25	336.153	17	94
826	THC-COOH-d9-NEG	8.93	.5	Negative	352.275	308.294	22	143
827	Tolbutamide-d9-POS_8.9	8.95	.5	Positive	280.2	121.051	37	65
828	Tolbutamide-d9-POS_8.9	8.95	.5	Positive	280.2	149.033	18	65
829	Tolbutamide-d9-POS_8.9	8.95	.5	Positive	280.2	150.039	18	65
830	THC-COOH_NEG	8.95	.5	Negative	343.2	297.231	27	125
831	THC-COOH_NEG	8.95	.5	Negative	343.2	299.254	21	125
832	THC-COOH_POS	8.95	.5	Positive	345.2	193.125	28	127
833	THC-COOH_POS	8.95	.5	Positive	345.2	299.205	21	127
834	THC-COOH_POS	8.95	.5	Positive	345.2	327.163	17	127
835	AM2201	8.96	.5	Positive	360.17	127.033	44	169
836	AM2201	8.96	.5	Positive	360.17	155.025	26	169
837	JWH-081	9.57	.5	Positive	372.175	157.024	40	170
838	JWH-081	9.57	.5	Positive	372.175	184.934	28	170
839	JWH-122	9.67	.5	Positive	356.2	141	42	178
840	JWH-122	9.67	.5	Positive	356.2	169	28	178
841	JWH-019	9.72	.5	Positive	356.2	127	43	178
842	JWH-019	9.72	.5	Positive	356.2	155	26	178

Data Processing

- Data were acquired with Thermo Scientific™ TraceFinder™ software, version 3.2 and analyzed with Thermo Scientific ToxFinder™ software, version 1.0.
- Thermo Scientific™ ToxFinder™ software identified compounds based on retention time and ion ratio. Figure 2 presents a screen capture of the ToxFinder data processing method.

Figure 2. ToxFinder data processing method. Compounds are identified based on retention time and ion ratio. Ion ratio accuracy criteria are specified in the method table.

Method evaluation

Limits of Detection

Pooled donor urine was spiked with compounds in groups of 10 to concentrations of 5, 10, 50, 100 and 500 ng/mL, processed as described in Sample Preparation section, and analyzed.

Results

More then 90% of analyzed compounds were detected at a concentration of 100 ng/mL. Figure 3 presents a ToxFinder data review page for Nordiazepam which was identified based on retention time and two ion ratios. Figure 4 presents percent of compounds detected at each analyzed concentration. Table 1 presents limits of detection for selected compounds.

Figure 3. Data review page in ToxFinder software. Nordiazepam in donor urine sample was identified based on retention time and ion ratio.

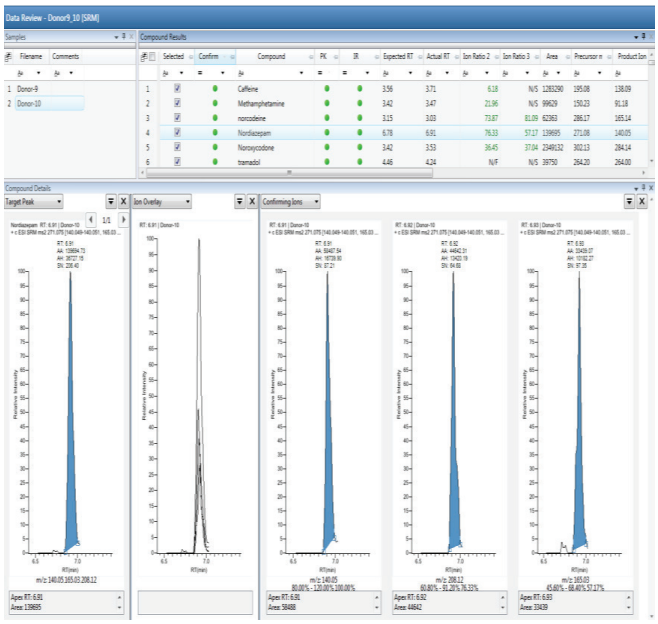

Figure 4. Percent of compounds detected at specified concentration.

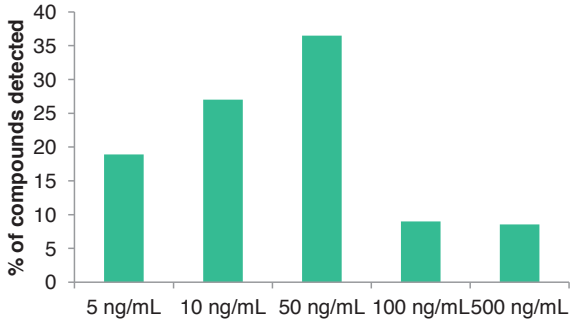

Table 1. Limits of detection for selected compounds.

Analyte	LOQ (ng/mL)	Analyte	LOQ (ng/mL)
Caffeine	10	Trazodone	50
Methamphetamine	10	Methyprylon	100
MDMA	5	Acebutolol	50
Gabapentin	50	Benzociane	500
Ritalinic Acid	50	Enalapril	50
Theophylline	100	Brompheniramine	5
LSD	10	Cimetidine	10
Benzoylecgonine	5	Betaxolol	100
Bromazepam	50	Erythromycin	100
Clobazam	10	Fluphenazine	500
Diazepam	10	Isoniazid	100
Flunitrazepam	50	Mepivocaine	5
Lorazepam	50	Quetiapine	50
Desalkylflurazepam	50	Procainamide	5
Meprobamate	100	Tetracaine	10
Zaleplon	50	Albuterol	5
Dextromethorphan	5	N-desmethylselegiline	5
Fentanyl	5	Sotalol	5
Hydrocodone	10	Papaverine	10
Methadone	5	Timolol	10
Naloxone	10	Clozapine N-Oxide	50
Meperidine	5	Ibogaine	100
Normeperidine	10	Sulpride	10
Naltrexone	50	Cisapride	50
Amitriptyline	50	Buspirone	50
Citalopram	10	Mirtazapine	5
Paroxetine	50	Glafenine	10
Sertraline	50	Terbutaline	5

Conclusion

- We successfully demonstrated a screening method for 300+ compounds in urine samples.
- Limits of detection for more then 90% of analyzed compounds were 100 ng/mL or lower.
- Analyte retention time and ion ratio used for confirmation ensured high selectivity of the method.

For forensic use only.

www.thermoscientific.com

Thermo Fisher Scientific, San Jose, CA USA is ISO 13485 Certified.

ISO 13485

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Canada +1 800 530 8447
China 800 810 5118 (free call domestic) 400 650 5118
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 10 3292 200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 45 453 9100
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00
Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Spain +34 914 845 965
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
UK +44 1442 233555
USA +1 800 532 4752

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

PN64641-EN 1015S