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Simultaneous analysis for forensic drugs in human blood and urine using ultra-high speed LC-MS/MS

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Introduction

In Forensic Toxicology, LC/MS/MS has become a preferred method for the routine quantitative and qualitative analysis of drugs of abuse. LC/MS/MS allows for the simultaneous analysis of multiple compounds in a single run, thus enabling a fast and high throughput analysis. In this study, we report a developed analytical system using ultra-high speed triple quadrupole mass spectrometry with a new extraction method for pretreatment in forensic analysis. The system has a sample preparation utilizing modified QuEChERS extraction combined with a short chromatography column that results in a rapid run time making it suitable for routine use.

Methods and Materials

Sample Preparation

Whole blood sample preparation was carried out by the modified QuEChERS extraction method [(1)] using Q-sep™ QuEChERS Sample Prep Packets purchased from RESTEK (Bellefonte, PA).

1) Add 0.5 mL of blood and 1 mL of distilled water into the 15 mL centrifugal tube and agitate the mixture using a vortex mixer.
2) Add two 4 mm stainless steel beads, 1.5 mL of acetonitrile and 100 µL of acetonitrile solution containing 1 ng/µL of Diazepam-d5. Then agitate using the vortex mixer.
3) Add 0.5 g of the filler of the Q-sep™ QuEChERS Extraction Salts Packet.
4) Vigorously shake the tube by hand several times, agitate well using the vortex mixer for approximately 20 seconds. Then centrifuge the tube for 10 minutes at 3000 rpm.
5) Move the supernatant to a different 15 mL centrifugal tube and add 100 µL of 0.1 % TFA acetonitrile solution. Then, dry using a nitrogen-gas-spray concentration and drying unit or a similar unit.
6) Reconstitute with 200 µL of methanol using the vortex mixer. Then move it to a microtube, and centrifuge for 5 minutes at 10,000 rpm.
7) Transfer 150 µL of the supernatant to a 1.5 mL vial for HPLC provided with a small-volume insert.

[ref.] (1) Usui K et al, Legal Medicine 14 (2012), 286-296

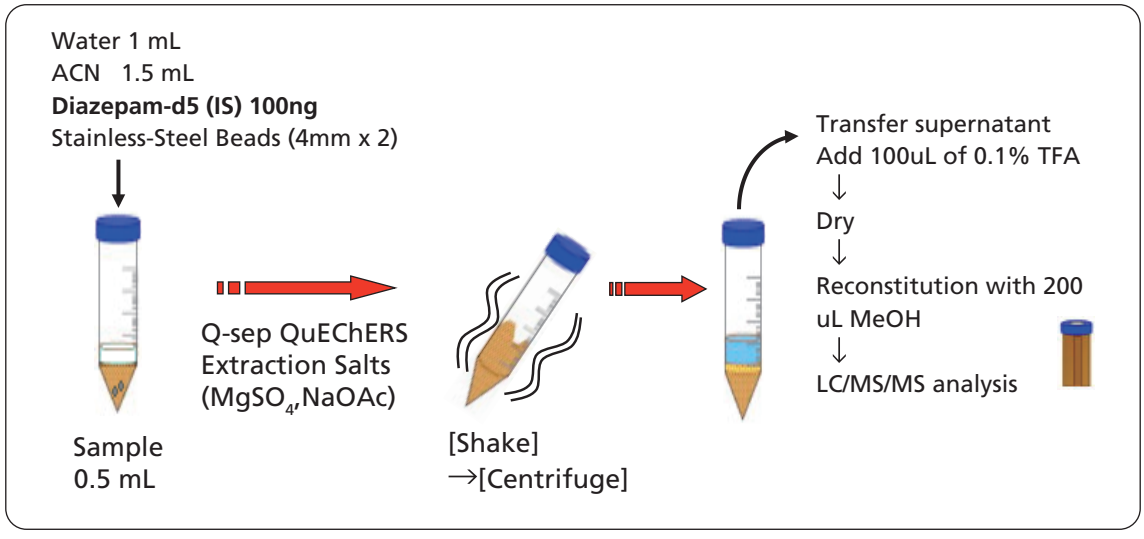

Figure 1 Scheme of the modified QuEChERS procedure

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LC-MS/MS Analysis

Treated samples were analyzed using a Nexera UHPLC system coupled to a LCMS-8050 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan) with LC/MS/MS Rapid Tox. Screening Database. The Database contains product ion scan spectra for 106 forensic and toxicology-related compounds of Abused drugs, Psychotropic drugs and Hypnotic drugs etc (Table 1) and provides Synchronized Survey Scan® parameters (product ion spectral data acquisition parameters based on the MRM intensity as threshold) optimized for screening analysis.

Samples were separated on a YMC Triart C18 column. A flow rate of 0.3 mL/min was used together with a gradient elution.

Analytical Conditions

HPLC (Nexera UHPLC system)

Column	: YMC Triart C18 (100x2mm, 1.9µm)
Mobile Phase A	: 10 mM Ammonium formate - water
Mobile Phase B	: Methanol
Gradient Program	: 5%B (0 min) - 95%B (10 min - 13min) - 5%B (13.1 min - 20 min)
Flow Rate	: 0.3 mL / min
Column Temperature	: 40 °C
Injection Volume	: 5 uL

Mass (LCMS-8050 triple quadrupole mass spectrometry)

Ionization	: heated ESI
Polarity	: Positive & Negative
Probe Voltage	: +4.5 kV (ESI-Positive mode); -3.5 kV (ESI-Negative mode)
Nebulizing Gas Flow	: 3 L / min
Drying Gas Pressure	: 10 L / min
Heating gas flow	: 10 L / min
DL Temperature	: 250 °C
BH Temperature	: 400 °C
MRM parameter	:

Analytes	Ret. Time	Q1 m/z	Q3 m/z	Collision Energy
Diazepam-d5	9.338	290.15	154.05	-27
		290.15	198.20	-34
Alprazolam	8.646	309.10	281.10	-24
		309.10	205.10	-41
Atropine	5.378	290.15	124.15	-23
		290.15	93.20	-30
Estazolam	8.408	295.05	267.15	-24
		295.05	205.25	-37
Ethyl loflazepate	9.350	361.15	259.10	-30
		361.15	287.15	-19
Etizolam	8.786	343.05	314.10	-24
		343.05	138.15	-36
Haloperidol	8.253	376.15	165.15	-24
		376.15	123.10	-39

Analytes	Ret. Time	Q1 m/z	Q3 m/z	Collision Energy
Risperidone	7.993	411.20	191.05	-28
		411.20	69.05	-55
Triazolam	8.573	343.05	315.00	-27
		343.05	308.20	-25
Amobarbital (neg)	8.093	225.15	42.00	25
		225.15	182.00	14
Barbital (neg)	5.243	183.10	42.10	21
		183.10	140.10	15
Phenobarbital (neg)	6.762	231.10	42.20	19
		231.10	85.10	14
Thiamylal (neg)	8.883	253.00	58.10	23
		253.00	101.00	16

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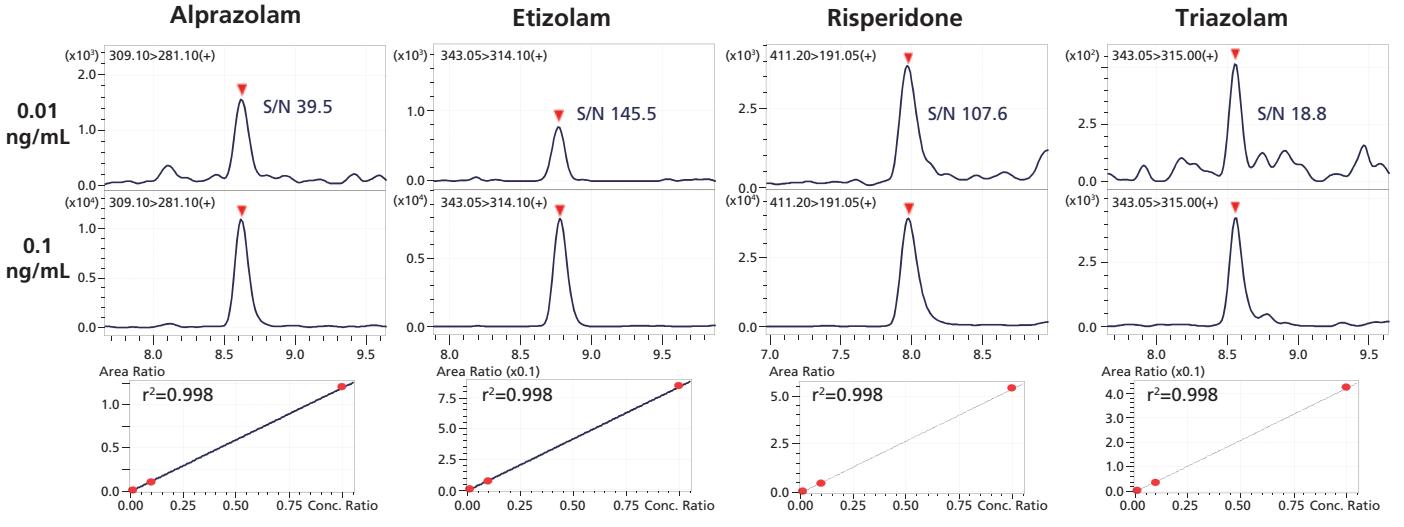
Type	Event#	+/−	Compound Name m/z	Time (4.236 min – 10.415 min)
MRM	1	+	Atropine 290.15>124.15, 290.15>93.20	
MRM	2	+	Risperidone 411.20>191.05, 411.20>69.05	
MRM	3	+	Haloperidol 376.15>165.15, 376.15>123.10	
MRM	4	+	Estazolam 295.05>267.15, 295.05>205.25	
MRM	5	+	Triazolam 343.05>315.00, 343.05>308.20	
MRM	6	+	Alprazolam 309.10>281.10, 309.10>205.10	
MRM	7	+	Etizolam 343.05>314.10, 343.05>138.15	
MRM	8	+	D_Diazepam-d5 290.15>154.05, 290.15>198.20	
MRM	9	+	Ethyl loflazepate 361.15>259.10, 361.15>287.15	
MRM	10	−	Barbital (neg) 183.10>42.10, 183.10>140.10	
MRM	11	−	Phenobarbital (neg) 231.10>42.20, 231.10>85.10	
MRM	12	−	Amobarbital (neg) 225.15>42.00, 225.15>182.00	
MRM	13	−	Thiamylal (neg) 253.00>58.10, 253.00>101.00	

positive

negative

Figure 2 LCMS-8050 triple quadrupole mass spectrometer

Results and Discussion



Conc.	Area	Accuracy	%RSD
0.01	9,004	112.1	6.57
	8,288	105.1	
	9,519	119.3	
0.1	75,236	89.6	6.04
	75,983	89.6	
	74,023	80.6	
1	829,519	99.9	2.53
	831,098	99.6	
	849,597	104.2	

Conc.	Area	Accuracy	%RSD
0.01	4,865	114.4	8.71
	5,109	119.9	
	4,321	105.7	
0.1	48,038	84.0	1.82
	49,152	85.1	
	54,497	87.0	
1	604,640	103.7	2.22
	581,207	99.2	
	579,390	101.2	

Conc.	Area	Accuracy	%RSD
0.01	29,832	108.4	5.14
	32,436	116.7	
	30,461	110.8	
0.1	335,202	91.3	4.74
	309,273	83.7	
	343,172	85.6	
1	3,826,373	102.8	1.66
	3,718,854	99.4	
	3,705,165	101.4	

Conc.	Area	Accuracy	%RSD
0.01	3,047	107.0	5.63
	3,064	109.2	
	3,356	118.5	
0.1	27,991	94.8	7.83
	25,542	85.7	
	26,317	81.5	
1	288,776	99.0	1.96
	297,332	101.5	
	294,788	102.9	

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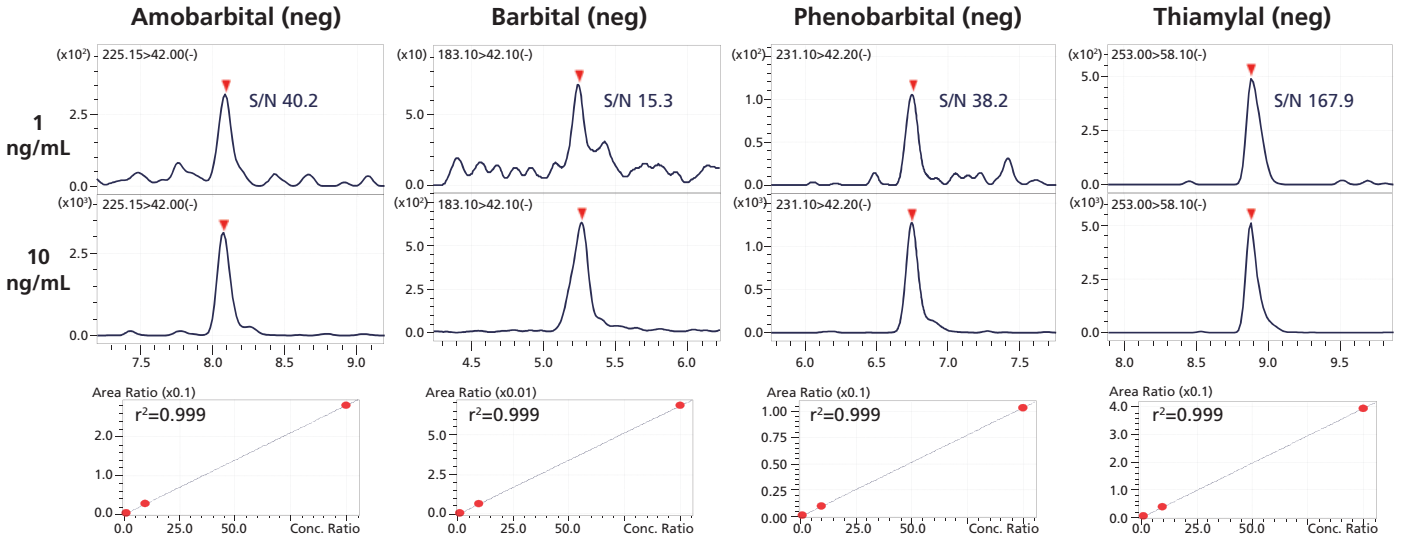

Conc.	Area	Accuracy	%RSD
1	1,837	100.2	4.53
	1,862	99.1	
	2,041	105.8	
10	21,685	99.6	5.30
	22,169	102.4	
	20,654	92.5	
100	227,698	101.3	1.62
	223,480	98.3	
	225,079	100.9	

Conc.	Area	Accuracy	%RSD
1	521	108.7	7.10
	464	96.6	
	509	103.4	
10	5,078	95.6	2.38
	5,033	95.4	
	5,424	99.4	
100	55,420	101.4	1.42
	55,658	100.8	
	53,484	98.7	

Conc.	Area	Accuracy	%RSD
1	725	106	9.82
	693	100.2	
	617	91	
10	7,909	98.8	5.82
	8,564	107.5	
	7,939	96.7	
100	81,987	99.2	0.85
	83,274	99.7	
	82,656	100.8	

Conc.	Area	Accuracy	%RSD
1	2,520	107	8.99
	2,192	95.3	
	2,288	97.5	
10	30,808	101.4	1.68
	29,623	98.3	
	31,379	100.6	
100	318,233	100.7	0.71
	317,214	99.3	
	313,399	100	

Figure 3 Results of 8 drugs spiked in human whole blood using LCMS-8050

In this experiment, two different matrices consisting of human whole blood and urine were prepared and 18 drugs were spiked into extract solution. Calibration curves constructed in the range from 0.01 to 1 ng/mL for 12 drugs (Alprazolam, Aripiprazole, Atropine, Brotizolam, Estazolam, Ethyl loflazepate, Etizolam, Flunitrazepam, Haloperidol, Nimetazepam, Risperidone and Triazolam) and from 1 to 100 ng/mL for 6 drugs (Bromovalerylurea, Amobarbital, Barbital, Loxoprofen, Phenobarbital and Thiamylal). All calibration curves displayed linearity with an R2 > 0.997 and excellent reproducibility was observed for all compounds (CV < 12%) at low concentration level.

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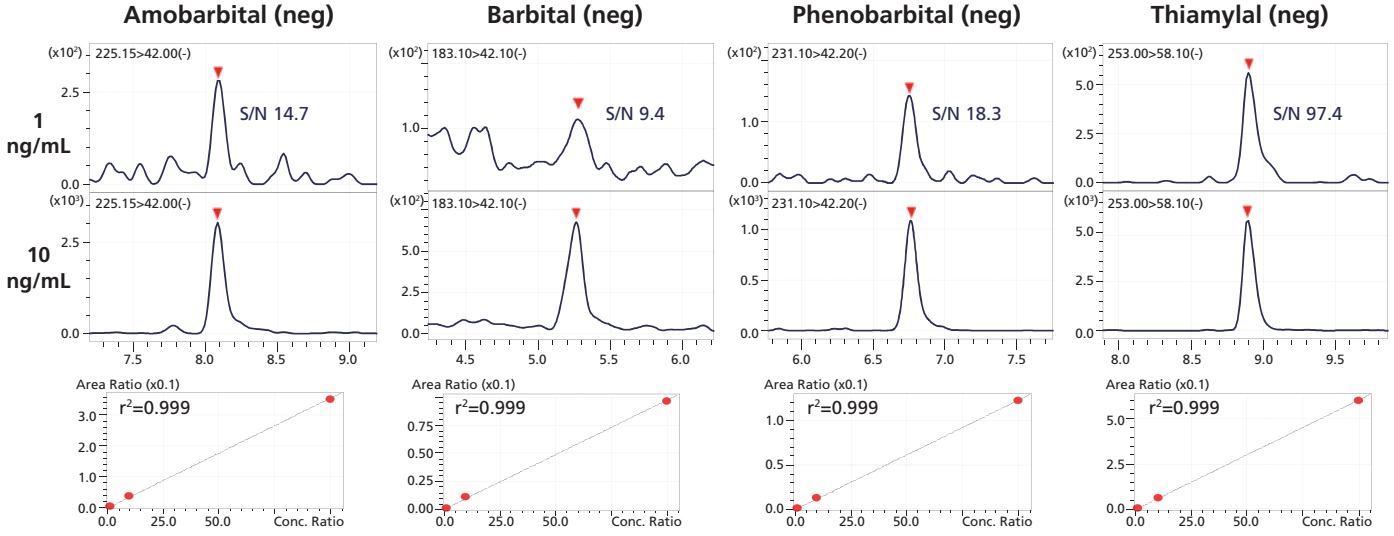

Conc.	Area	Accuracy	%RSD
1	1,468	102.2	12.73
	1,233	86.6	
	1,245	87.6	
10	17,241	104.4	5.10
	20,546	114.7	
	18,689	106.9	
100	211,917	96.8	3.34
	251,963	103	
	234,789	97.9	

Conc.	Area	Accuracy	%RSD
1	651	93.6	2.77
	695	96.1	
	654	89	
10	4,989	105.2	2.07
	5,613	109.6	
	5,443	108.6	
100	55,392	92.6	5.98
	69,481	104	
	66,327	101.3	

Conc.	Area	Accuracy	%RSD
1	612	103.6	8.16
	545	89.4	
	609	99.3	
10	5,656	97.9	4.24
	6,632	106.1	
	6,384	104.4	
100	71,965	95.2	4.95
	88,685	105	
	82,091	99.1	

Conc.	Area	Accuracy	%RSD
1	3,142	95.1	4.54
	3,470	100.5	
	3,153	91.4	
10	27,257	94.9	8.15
	34,377	110.8	
	32,933	108.5	
100	365,563	98.5	4.15
	431,826	104.1	
	390,719	96.1	

Figure 4 Results of 4 drugs spiked in human urine using LCMS-8050

Conclusions

- The validated sample preparation protocol can get adequate recoveries in quantitative works for all compounds ranging from acidic to basic.
- The combination of the modified QuEChERS extraction method and high-speed triple quadrupole LC/MS/MS with a simple quantitative method enable to acquire reliable data easily.

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