

# Rapid screening method covers common 161 forensic drugs by ultra-high speed LC/MS/MS with synchronized survey scanning

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### Introduction

LC/MS/MS has become an essential tool for the routine analysis in the field of forensic toxicology. It allows you to conduct the simultaneous analysis of multiple compounds in a single run which makes it possible to carry out a fast and high throughput analysis. In this study, we report a newly developed rapid screening method covers common 161 forensic drugs in Japan using ultra-high speed triple

quadrupole mass spectrometry. This method also incorporates with information of calibration curve for all 161 using two internal standards. The use of synchronized survey scanning (MRM triggered product ion scanning) output both quantitative and qualitative results in a single run.

### Methods and Materials

Sample preparation was carried out by the simplified QuEChERS extraction method. After the above mentioned sample preparation, extracts were measured using a Nexera UHPLC system and

LCMS-8040 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan) operated with Shimadzu proprietary "Synchronized Survey Scanning" data acquisition mode.

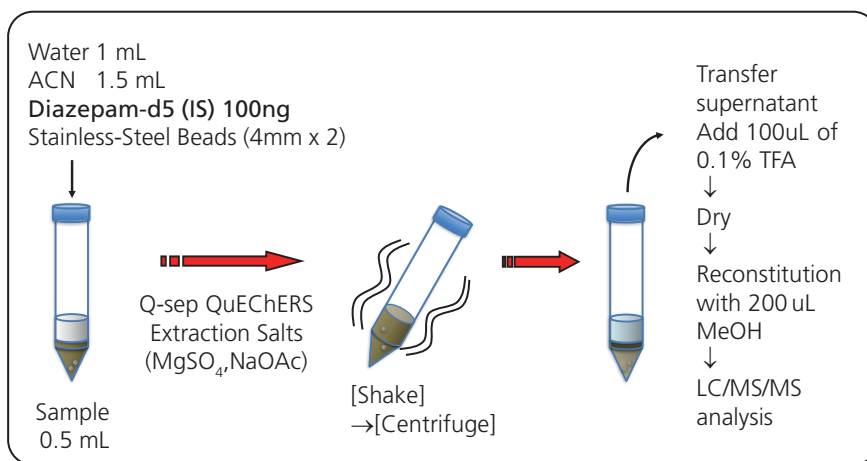


Figure 1 Scheme of the modified QuEChERS procedure

Extracts were separated on a Phenomenex kinetex XB-C18 (100x2mm, 2.6µm) at a column temperature of 40 °C for 15 min. A flow rate of 0.3 mL/min was used with a binary gradient system. 10mM ammonium formate

with 0.1% Formic acid in water and methanol were used for mobile phases. Semi-quantification was carried out by internal standard using diazepam-d5 and Phenobarbital-d5.

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## Analytical Conditions

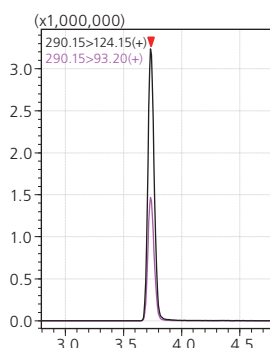
HPLC (Nexera UHPLC system)	
Column	: Phenomenex Kinetex (2.1 mmI.D. x 100 mmL., 2.6µm)
Guard Column	: Phenomenex SecurityGuard Ultra C18 2.1mmID
Mobile Phase A	: 10mmol/L Ammonium formate + 0.1% Formic acid - Water
Mobile Phase B	: 10mmol/L Ammonium formate + 0.1% Formic acid - Methanol
Gradient Program	: 5%B (0 min) – 95%B (7.5-10 min) –5% (10.01-15 min)
Flow Rate	: 0.3 mL / min
Column Temperature	: 40 °C
Injection Volume	: 5 µL

Mass (LCMS-8040 triple quadrupole mass spectrometry)	
Ionization	: ESI
Polarity	: Positive & Negative
Nebulizing Gas Flow	: 3 L / min
Drying Gas Pressure	: 10 L / min
DL Temperature	: 250 °C
BH Temperature	: 400 °C

Type	+/-	Compound Name	m/z	Time (0.000 min - 9.724 min)
MRM	+	Caffeine	195.05>138.05, 195.05>42.10	
- Product Ion Scan	+		> 20.00:205.00	
MRM	+	Atropine	290.15>124.15, 290.15>93.20	positive
- Product Ion Scan	+		> 20.00:300.00	
MRM	-	Barbital (neg)	183.10>42.10, 183.10>140.10	negative
- Product Ion Scan	-		> 20.00:193.00	
MRM	+	Lidocaine	235.00>86.10, 235.00>58.10	
- Product Ion Scan	+		> 20.00:245.00	
MRM	+	Mepivacaine	247.10>98.10, 247.10>70.10	
- Product Ion Scan	+		> 20.00:257.00	

[MRM]



ID#	Name	Ret. Tim	Area	Conc.	Unit
23	Atropine	3.736	12108310	0.155	ng/ul

[Product Ion Scan]

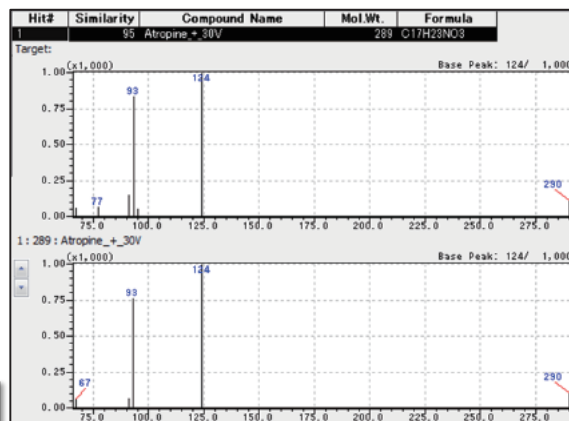


Figure 2 MRM Chromatograms and library searching/product matching of Atropine using “Synchronized Survey Scanning” data acquisition mode

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## Result

Table 1 Data Summary of 161 drugs for forensic

ID	Name		Area	CV	Conc. (ng/uL)	Recovery (%)
(IS)	Diazepam-d5		6,346,323	0.8%	0.2	79%
(IS)	Phenobarbital-d5 (neg)		431,365	1.1%	10.0	96%
1	7-Aminoclonazepam	B	19,452,499	0.8%	0.9	67%
2	7-Aminoflunitrazepam	B	18,056,878	1.3%	0.9	75%
3	7-Aminonimetazepam	B	3,859,824	0.3%	0.9	62%
4	7-Aminonitrazepam	B	45,031,657	0.8%	0.9	71%
5	8-Hydroxyetizolam (M-III)	B	6,394,039	1.1%	1.0	65%
6	9-Hydroxyrisperidone	A	15,360,791	0.7%	0.1	80%
7	Acetaminophen	B	3,780,130	0.4%	0.9	76%
8	Acetylpheneturide	B	33,379,387	0.9%	1.8	2%
9	Aconitine	B	4,105,344	3.3%	0.9	72%
10	Allylisopropylacetylurea	B	3,487,595	5.1%	1.0	59%
11	alpha-Hydroxyalprazolam	B	1,784,842	0.2%	1.4	91%
12	Alpha-Hydroxybrotizolam	B	215,820	7.0%	1.1	55%
13	alpha-Hydroxymidazolam	B	9,228,593	0.9%	1.1	71%
14	Alpha-Hydroxytriazolam	B	236,632	7.8%	1.2	114%
15	Alprazolam	A	6,312,246	1.7%	0.1	83%
16	Amitriptyline	B	23,047,854	1.4%	0.9	77%
17	Amobarbital (neg)	C	326,561	2.4%	11.4	59%
18	Amoxapine	B	10,879,786	2.3%	1.0	57%
19	Amphetamine	B	36,409,412	0.6%	0.8	54%
20	Aripiprazole	A	3,705,924	2.5%	0.1	86%
21	Atropine	A	4,233,550	2.4%	0.1	67%
22	Barbital (neg)	C	96,291	5.6%	8.4	64%
23	Benzoyl ecgonine	B	33,258,665	1.6%	1.0	66%
24	Biperiden	B	92,376,036	0.7%	1.0	71%
25	Blonanserin	A	29,731,727	1.4%	0.1	74%
26	Bromazepam	B	1,817,080	2.9%	1.1	77%
27	Bromocriptine	A	285,682	3.9%	0.1	86%
28	Bromovalerylurea	C	2,248,327	7.4%	12.6	73%
29	Bromperidol	A	8,860,525	0.9%	0.1	76%
30	Brotizolam	A	1,060,293	3.0%	0.1	73%
31	Bupivacaine	B	89,798,275	0.4%	0.9	77%
32	Caffeine	C	35,018,865	4.8%	10.9	47%
33	Carbamazepine	B	86,131,310	0.3%	0.9	84%
34	Carpipramine	B	93,409,751	1.2%	1.0	75%
35	Chlordiazepoxide	B	26,885,377	0.5%	1.0	71%
36	Chlorpheniramine	B	88,928,160	0.5%	0.9	75%
37	Chlorpromazine	B	30,638,922	1.9%	0.7	28%
38	Clobazam	B	16,276,060	4.7%	1.1	69%

ID	Name		Area	CV	Conc. (ng/uL)	Recovery (%)
39	Clocapramine	B	87,885,543	0.6%	1.0	73%
40	Clomipramine	B	71,391,110	1.7%	1.1	68%
41	Clonazepam	B	4,456,595	2.0%	1.0	68%
42	Clotiazepam	B	39,697,763	0.9%	1.0	69%
43	Clozapolam	B	7,056,201	1.0%	1.0	20%
44	Clozapine	B	88,320,002	1.6%	1.0	56%
45	Cocaine	B	30,818,570	2.4%	0.9	74%
46	Codeine	B	1,809,561	1.1%	0.9	78%
47	Colchicine	A	1,081,576	3.7%	0.1	104%
48	Desipramine	B	87,657,880	1.3%	1.0	70%
49	Desmethylclotiazepam	B	12,807,827	2.9%	1.0	76%
50	Desmethyldiazepam	B	1,991,930	2.6%	0.9	65%
51	Dextromethorphan	B	31,609,360	1.3%	1.0	74%
52	Diazepam	B	9,489,963	0.5%	0.9	88%
53	Diclofenac	B	3,019,210	1.9%	1.1	57%
54	Dihydrocodeine	B	5,116,815	1.0%	0.9	65%
55	Diltiazem	B	31,651,195	3.2%	0.9	74%
56	Diphenhydramine	B	77,475,542	2.5%	1.0	68%
57	Diprophyline	B	5,467,498	1.6%	1.0	73%
58	Diquat	C	4,133,180	1.7%	4.6	30%
59	Donepezil	B	86,996,851	1.2%	1.0	76%
60	Dosulepin	B	16,633,544	0.6%	1.1	68%
61	Duloxetine	B	54,224,544	2.0%	1.0	68%
62	Ecgonine methyl ester	B	6,247,156	3.1%	0.7	24%
63	Ephedrine	B	11,379,097	1.7%	0.9	59%
64	Escitalopram	B	84,329,488	0.4%	1.0	73%
65	Estazolam	A	3,001,004	0.6%	0.1	78%
66	Ethenamide	B	15,846,851	2.8%	0.9	72%
67	Ethyl loflazepate	A	3,790,211	0.9%	0.1	147%
68	Etizolam	A	1,785,029	1.6%	0.1	95%
69	Fenitrothion (MEP)	B	56,710	2.4%	0.7	45%
70	Fludiazepam	B	19,005,501	2.1%	0.9	67%
71	Flunitrazepam	A	1,928,431	3.1%	0.1	70%
72	Flurazepam	B	26,399,077	1.2%	0.9	79%
73	Fluvoxamine	B	41,243,571	1.3%	0.9	63%
74	Gabapentin	C	17,535,785	0.6%	8.8	38%
75	Glibenclamide	B	37,050,136	2.6%	1.0	89%
76	Gliclazide	B	35,614,336	2.3%	1.0	69%
77	Glimepiride	B	29,462,383	1.4%	1.1	110%
78	Haloperidol	A	9,224,082	0.8%	0.1	76%

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ID	Name		Area	CV	Conc. (ng/uL)	Recovery (%)
79	Haloxazolam	B	2,552,322	1.3%	1.1	16%
80	Hydroxyzine	B	92,434,639	1.2%	0.9	75%
81	Ibuprofen	C	17,877	8.1%	26.7	24%
82	Imipramine	B	91,607,654	0.0%	1.0	67%
83	Ketamine	B	32,915,241	0.8%	0.9	56%
84	Lamotrigine	C	26,043,679	1.1%	10.1	69%
85	Levetiracetam	C	178,318,455	0.7%	9.2	63%
86	Levomepromazine	B	28,966,443	2.0%	0.6	35%
87	Lidocaine	A	15,587,912	0.5%	0.1	78%
88	Lorazepam	B	3,029,344	1.9%	1.2	91%
89	Lormetazepam	B	13,232,638	1.2%	1.0	116%
90	Loxoprofen (neg)	C	12,260	4.0%	5.9	21%
91	Malathion	B	10,503,659	3.6%	0.9	60%
92	Maprotiline	B	42,940,407	2.2%	0.9	72%
93	MDA	B	5,017,066	1.4%	0.9	61%
94	MDMA	B	41,678,893	1.5%	0.9	70%
95	Medazepam	B	25,509,966	1.0%	1.0	73%
96	Mefenamic acid	C	83,829,657	0.6%	11.9	49%
97	Memantine	B	29,642,093	2.6%	1.1	61%
98	Mepivacaine	B	73,360,863	1.7%	0.9	75%
99	Mequitazine	A	1,298,976	3.7%	0.1	66%
100	Metformin	C	60,820,590	1.0%	7.9	17%
101	Methamphetamine	B	46,165,784	1.7%	0.8	63%
102	Methomyl	B	11,562,809	0.8%	1.0	61%
103	Methylephedrine	B	16,736,508	1.1%	0.9	51%
104	Methylphenidate	B	82,689,585	1.9%	1.0	58%
105	Mexazolam	A	3,697,305	1.0%	0.1	71%
106	Mexiletine	B	19,716,158	0.6%	1.0	58%
107	Mianserin	B	27,837,925	1.1%	1.0	62%
108	Midazolam	B	18,888,384	2.9%	1.0	69%
109	Milnacipran	B	56,833,849	0.9%	0.8	77%
110	Mirtazapine	B	45,436,986	0.1%	1.0	71%
111	Morphine	C	12,755,018	1.1%	8.8	56%
112	Mosapramine	B	34,681,834	1.2%	0.9	78%
113	Nemonapride	B	94,056,627	0.3%	0.9	73%
114	Nicotine	B	14,630,277	1.5%	0.9	43%
115	Nimetazepam	A	2,645,033	1.8%	0.1	73%
116	Nitrazepam	B	3,743,601	1.4%	2.0	70%
117	Nortriptyline	B	24,212,882	1.7%	1.1	69%
118	Olanzapine	B	1,013,283	2.4%	0.0	6%
119	Oxazepam	B	3,759,101	1.5%	1.2	98%
120	Paliperidone	B	88,522,356	1.1%	0.9	81%
121	Paroxetine	B	12,978,555	4.0%	0.9	64%

ID	Name		Area	CV	Conc. (ng/uL)	Recovery (%)
122	Pemoline	B	29,399,939	1.5%	0.9	79%
123	Pentazocine	B	50,112,012	0.3%	0.9	76%
124	Pentobarbital (neg)	C	259,013	9.6%	9.0	67%
125	Perospirone	B	23,962,968	2.0%	0.9	74%
126	Perphenazine	A	2,762,174	0.9%	0.1	51%
127	Phenobarbital (neg)	C	120,532	9.5%	11.3	64%
128	Pimozide	A	4,946,965	0.7%	0.1	68%
129	Pioglitazone	B	101,995,993	0.3%	1.0	76%
130	Primidone	C	24,541,578	2.2%	10.8	72%
131	Promethazine	B	2,950,749	0.7%	0.0	40%
132	Propicyazine	B	54,091,137	1.5%	0.7	44%
133	Propofol (neg)	D	13,839	9.7%	411.0	5%
134	Quazepam	B	5,472,384	1.9%	0.9	57%
135	Quetiapine	B	67,509,364	0.7%	1.1	72%
136	Risperidone	A	19,540,418	1.6%	0.1	79%
137	Ropivacaine	B	95,255,477	0.3%	1.0	76%
138	Salicylic_acid (neg)	C	3,745,146	0.8%	7.7	59%
139	Sertraline	B	34,443,151	1.0%	1.0	66%
140	Sildenafil	B	10,071,805	1.8%	1.1	81%
141	Spiperone	A	10,274,630	1.8%	0.1	76%
142	Sulpiride	B	40,428,373	0.6%	0.9	70%
143	Tadalafil	B	5,707,353	3.0%	1.0	88%
144	Tandospirone	B	69,674,885	1.0%	0.9	80%
145	Temazepam	B	29,174,105	2.2%	1.0	110%
146	THC	A	75,575	7.1%	0.1	34%
147	THC-COOH	A	130,124	7.5%	0.1	60%
148	Thiamylal (neg)	C	556,729	2.4%	8.0	72%
149	Timiperone	A	12,938,642	1.6%	0.1	81%
150	Tofisopam	B	23,628,199	0.6%	0.9	88%
151	Topiramate	C	1,796,628	2.4%	10.0	72%
152	Trazodone	B	31,660,079	0.4%	0.9	71%
153	Triazolam	A	1,445,813	2.0%	0.1	86%
154	Trihexyphenidyl	B	96,637,783	0.7%	1.0	64%
155	Valdenafil	B	26,100,227	1.5%	0.9	100%
156	Valproic Acid (neg)	D	1,017,379	1.4%	398.0	22%
157	Warfarin	B	22,708,164	0.8%	0.9	90%
158	Zolpidem	B	42,558,363	2.5%	0.9	72%
159	Zopiclone	B	3,561,411	3.3%	0.1	63%
160	Zopiclone N-oxide	B	120,279	2.9%	0.0	27%
161	Zotepine	B	85,889,002	0.4%	1.0	66%

A:0.1 ng/uL, B:1 ng/uL, C:10 ng/uL, D:500 ng/uL (N=3)

## Rapid screening method covers common 161 forensic drugs by ultra-high speed LC/MS/MS with synchronized survey scanning

We evaluated this method couple to modified QuEChERS using standard drugs spiked into human whole blood (Table 1). The peak area was calculated for each compound and the percentage recovery was confirmed. Percentage recovery was 68.3 %. Calibration curves constructed in the range from 0.001 to 1 ng/uL for 26

Some different matrices (blood, muscle, liver, etc.) were prepared and 24 drugs were spiked into extract solution (Table 2). There is little matrix effect compare some matrices to STD using modified QueChERS extraction.

drugs (Group A), from 0.01 to 10 ng/uL for 114 drugs (Group B), from 0.1 to 100 ng/uL for 19 drugs (Group C) and from 10 to 10,000 ng/uL for 2 drugs (Group D) . All calibration curves displayed linearity with an  $R^2 > 0.995$  and excellent reproducibility was observed for most compounds ( $CV < 10\%$ ) at low concentration level.

Moreover we confirmed that all compounds were identified from the MS/MS spectra by matching against the highest scoring MS/MS spectra library in terms of degree of similarity.

Table 2 Data Summary of 24 drugs in some matrices

	Diazepam-d5		Phenobarbital-d5		Alprazolam		Amobarbital		Aripiprazole		Atropine		Barbital	
	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.
STD	7,646,723	0.08	55,108	4.0	476,667	0.003	31,587	2.29	249,832	0.002	562,848	0.004	10,082	2.00
Whole Blood	6,370,025	0.08	31,016	4.0	438,044	0.003	19,289	2.37	259,356	0.003	431,082	0.004	7,283	2.43
Muscle	7,240,401	0.08	48,419	4.0	454,751	0.003	24,711	1.99	270,451	0.003	474,380	0.003	9,375	1.91
Liver	6,487,690	0.08	44,904	4.0	447,094	0.003	28,862	2.64	266,590	0.003	499,792	0.004	9,649	2.34
Heart Muscle	7,230,086	0.08	44,068	4.0	438,791	0.003	19,239	1.65	283,732	0.003	536,799	0.004	11,252	2.62

	Bromovalerylurea		Brotizolam		Colchicine		Caffeine		Diquat		Estazolam		Ethyl loflazepate	
	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.
STD	243,307	0.33	93,214	0.003	75,825	0.003	5,207,845	0.55	12,438,039	4.68	197,725	0.003	281,784	0.003
Whole Blood	232,721	0.38	85,165	0.004	67,276	0.003	15,626,330	1.98	2,375,643	1.06	184,777	0.003	220,217	0.003
Muscle	261,680	0.38	101,188	0.004	69,562	0.003	4,191,943	0.47	2,362,832	0.93	198,174	0.003	301,005	0.003
Liver	252,611	0.41	96,975	0.004	74,458	0.004	4,542,206	0.56	3,464,484	1.53	196,355	0.003	5,561	0.000
Heart Muscle	315,893	0.46	88,961	0.003	73,791	0.003	4,547,260	0.51	2,765,936	1.09	227,409	0.003	318,874	0.003

	Etizolam		Flunitrazepam		Haloperidol		Lidocaine		Morphine		Nimetazepam		Pentobarbital	
	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.
STD	130,546	0.003	117,431	0.002	893,593	0.004	2,635,289	0.004	3,762,167	0.88	214,988	0.003	24,708	1.81
Whole Blood	107,060	0.003	104,105	0.002	767,357	0.004	1,913,088	0.003	1,816,263	0.51	149,626	0.002	19,804	2.51
Muscle	135,056	0.003	120,207	0.003	874,515	0.004	1,915,631	0.002	1,661,476	0.41	200,654	0.003	24,082	2.00
Liver	130,302	0.003	97,176	0.002	877,570	0.004	2,253,460	0.004	1,798,734	0.50	165,956	0.002	25,413	2.33
Heart Muscle	123,522	0.003	136,984	0.003	866,232	0.004	2,022,358	0.003	1,981,075	0.49	203,639	0.003	19,266	1.78

	Phenobarbital		Risperidone		Salicylic aci		Thiamylal		Triazolam	
	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.	Area	Conc.
STD	5,932	1.19	1,592,942	0.003	762,058	5.44	56,512	2.07	206,916	0.004
Whole Blood	2,422	0.78	1,265,373	0.003	277,390	3.44	32,842	2.14	173,303	0.004
Muscle	6,439	1.39	1,475,856	0.003	485,341	3.85	29,493	1.24	232,892	0.005
Liver	5,254	1.22	1,478,555	0.003	535,338	4.79	46,147	2.11	218,486	0.005
Heart Muscle	4,653	1.11	1,457,905	0.003	489,892	4.43	42,548	2.08	208,833	0.004

(Conc. unit : ng/uL)

## Rapid screening method covers common 161 forensic drugs by ultra-high speed LC/MS/MS with synchronized survey scanning

### Conclusions

We developed rapid screening method for 161 drugs for forensic purposes with synchronized survey scanning capable of both qualitative and quantitative results in a single run. The combination of the developed method with ultra-high speed LC/MS/MS and modified QuEChERS enable to strengthen the efficiency of drug screening.