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Strategies for Multiple-Target Screening using LC-MS/MS with Merged Spectrum Database for Forensic Toxicology

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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 developed Multiple-Target Screening (MTS) method for forensic toxicology to reduce false positive and negative using MS/MS spectral library database. MTS method consists of multiple reaction monitoring (MRM) and product ion scans at three collision energies to confirm the compound identification based on mass spectral library searching. The mass spectral library was created using certified reference materials from over 1,200 compounds for forensic toxicology.

Methods and Materials

Biological sample preparation was carried out by the modified QuEChERS extraction method. Treated samples were measured using a Nexera UHPLC system and LC-MS/MS (Shimadzu Corporation, Japan). Samples 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 together with a binary gradient system. 10mM acetic formate with 0.1% Formic acid in water and methanol were used for mobile phases.

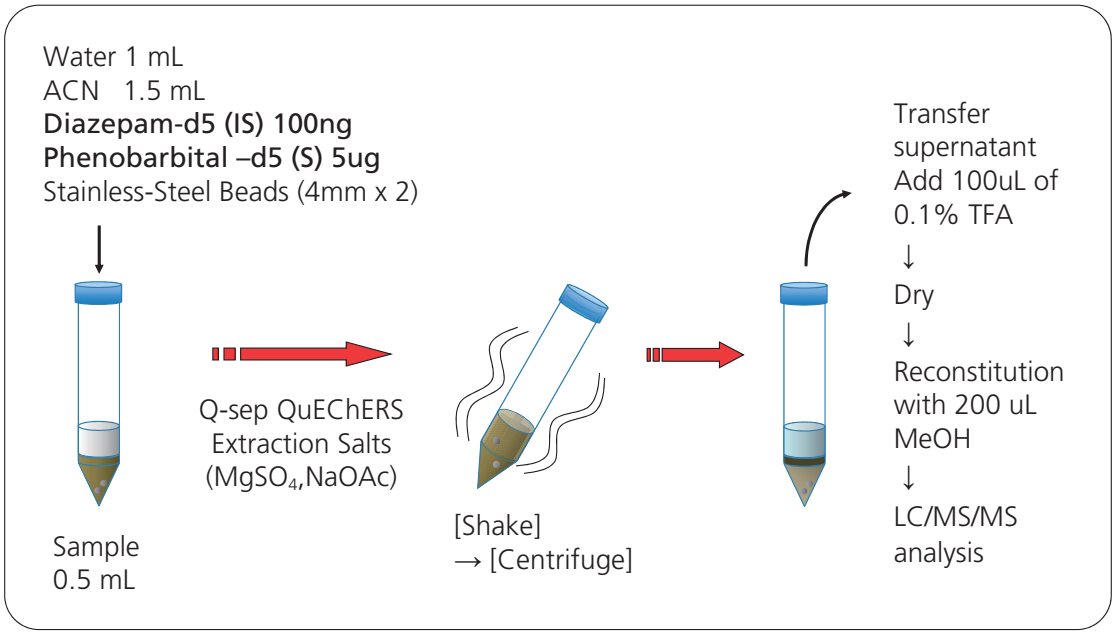

Figure 1 Scheme of the modified QuEChERS procedure

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Analytical Conditions

HPLC (Nexera UHPLC system)	
Column	: Phenomenex Kinetex (2.1 mmI.D. x 100 mmL., 2.6um)
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
LCMS (triple quadrupole mass spectrometry)	
Ionization	: ESI
Polarity	: Positive & Negative
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
Interface Temperature	: 300 °C

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The MTS parameters were set to a single MRM per compound with threshold triggered MS/MS at 3 collision energies (15, 30, 45V) enabling confirmation of fragmentation widely. Library searching was performed on all CE spectral data in addition to a merged-CE spectrum.

Type	Event#	+/−	Compound Name m/z	Time (1.623 min – 8.982 min)
MRM	21	+	Bromovalerylurea 223.00>17	
|– Product Ion Scan	22	+	> CE:-15.0, 20.00:1000.00	
|(Product Ion Scan)	23	+	> CE:-30.0, 20.00:1000.00	
|(Product Ion Scan)	24	+	> CE:-45.0, 20.00:1000.00	
MRM	25	+	Chlorpheniramine 275.00>23	
|– Product Ion Scan	26	+	> CE:-15.0, 20.00:1000.00	
|(Product Ion Scan)	27	+	> CE:-30.0, 20.00:1000.00	
|(Product Ion Scan)	28	+	> CE:-45.0, 20.00:1000.00	
MRM	29	+	7-aminonimetazepam 266.10	
|– Product Ion Scan	30	+	> CE:-15.0, 20.00:1000.00	

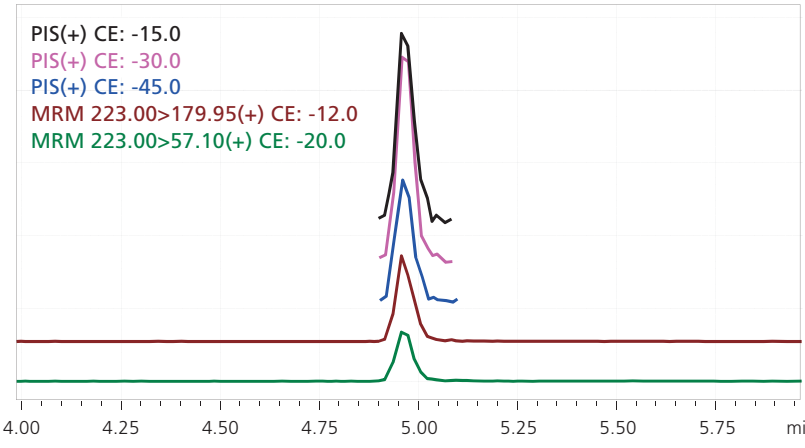
MRM Acq. Time: 3.986 - 5.986 min Compound Name: Bromovalerylurea

Ch	Precursor m/z	Product m/z	Pause Time	Dwell Time	Q1 Pre Bias	CE	Q3 Pre Bias	Use for Survey
Ch1	223.00	179.95	1.0	1.0	-11.0	-12.0	-20.0	☑
Ch2	223.00	57.10	1.0	1.0	-11.0	-20.0	-23.0	☑
Ch3								
Ch4								
Ch5								

Result

The MTS parameters were set to a single MRM per compound with threshold triggered MS/MS at 3 collision energies (15, 30, 45V) enabling confirmation of fragmentation widely. Library searching was performed on all CE spectral data in addition to a merged-CE spectrum.

One primary MRM is used for quantitation together with scanning data for compound identification and confirmation. Merged spectrum generates a similarity index score as opposed to a reference ion tolerance.



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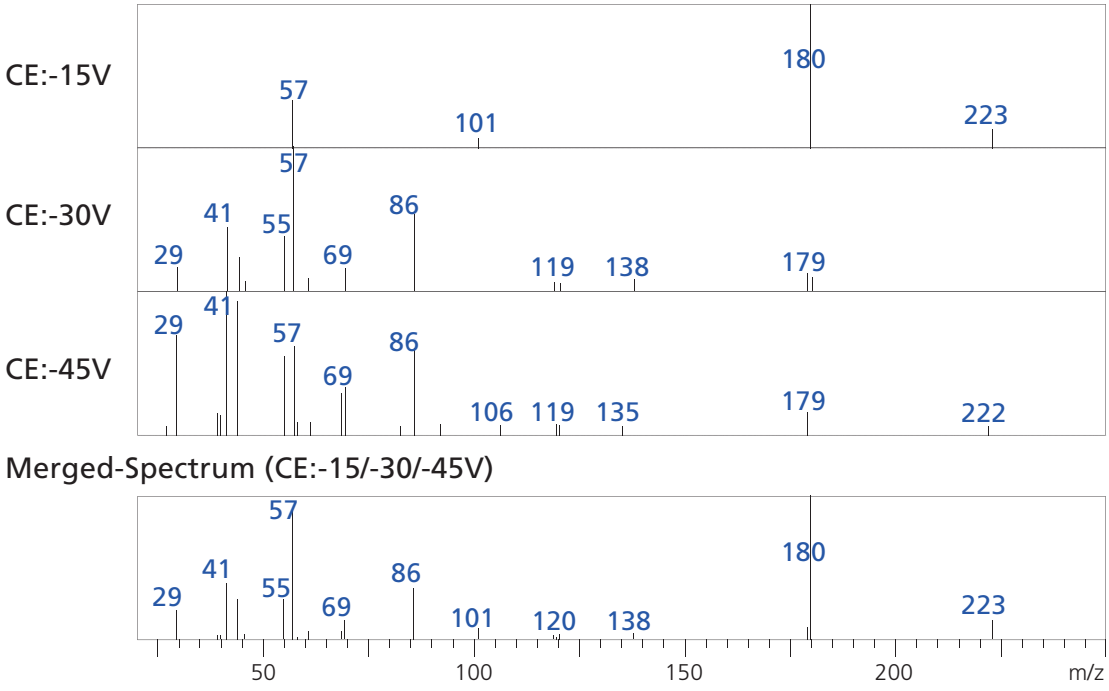

We evaluated the MTS method couple to modified QuEChERS using 115 standard drugs spiked into human whole blood or urine. Most compounds can be identified as the first hit in a spectral based library matching with merged-CE spectrum.

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Table 2 Similarity Index of 115 drugs in whole blood or urine

compound	whole blood		urine	
	1ng/mL	10ng/mL	1ng/mL	10ng/mL
7-Aminoclonazepam	91	91	92	94
7-Aminoflunitrazepam	87	85	81	86
7-Aminonimetazepam	74	79	65	68
7-Aminonitrazepam	95	95	95	95
8-hydroxyetizolam (M-III)	94	94	94	94
Acetaminophen	98	97	98	98
Acetylpheneturide	94	94	94	96
Allylisopropylacetylurea	92	94	96	89
Alprazolam	98	98	99	99
Amitriptyline	79	77	75	77
Amoxapine	92	88	91	93
Aripiprazole	81	63	80	82
Atropine	93	90	92	94
Biperiden	98	98	99	98
Blonanserin	92	94	94	92
Bromazepam	84	91	84	86
Bromovalerylurea	84	87	79	86
Bromperidol	96	97	98	96
Brotizolam	64	66	84	78
Bupivacaine	98	98	98	98
Caffeine	74	74	74	75
Carbamazepine	94	88	89	92
Carpipramine	95	96	96	96
Chlordiazepoxide	87	87	90	90
chlorpheniramine	95	95	95	95
Chlorpromazine	98	99	99	99
Clobazam	72	71	79	77
Clocapramine	97	97	95	98
Clomipramine	99	97	99	97
Clonazepam	94	91	87	76
Clotiazepam	85	78	83	83
Clozapine	94	95	94	95
Desipramine	96	91	87	93
Desmethylclotiazepam	78	90	99	95
Desmethyldiazepam	86	93	91	70
Dextromethorphan	69	74	68	69
Diazepam	83	81	77	84
diclofenac	96	95	96	96
Diltiazem	87	91	91	92
Diphenhydramine	89	87	87	88
Diprophyline	93	92	92	92
Diquat	93	93	93	93
Donepezil	97	97	97	97
Dosulepin	80	87	72	72
Duloxetine	100	100	100	100
Escitalopram	95	97	95	90
Estazolam	74	77	88	68
Ethenzamide	93	93	95	93
Ethyl loflazepate	88	88	91	88
Etizolam	93	91	76	75
Fludiazepam	95	96	93	91
Flunitrazepam	100	85	96	88
Flurazepam	97	97	95	96
Fluvoxamine	93	94	95	95
Gabapentin	92	90	96	90
Glibenclamide	96	96	94	96
Gliclazide	93	92	96	94
Glimepiride	91	91	90	91

compound	whole blood		urine	
	1ng/mL	10ng/mL	1ng/mL	10ng/mL
Haloperidol	96	98	92	91
Hydroxyzine	96	95	96	96
Imipramine	96	95	93	97
Lamotrigine	91	95	92	91
Levetiracetam	92	93	92	93
Levomepromazine	96	97	98	99
Lidocaine	100	99	99	99
malathion	97	96	97	97
malathion	97	98	97	96
Maprotiline	85	85	82	92
Mefenamic acid	87	83	86	86
Mefenamic acid_neg	99	99	100	100
Memantine	96	92	91	93
Mepivacaine	98	98	98	98
Metformin	93	90	90	90
methomyl	91	92	93	93
methomyl	83	91	92	89
Mexazolam	79	68	88	88
Mianserin	81	79	88	83
Midazolam	80	78	83	76
Milnacipran	87	89	89	86
Mirtazapine	89	91	90	90
Mosapramine	87	88	85	87
Nemonapride	95	95	96	94
Nicotine	88	86	87	87
Nimetazepam	89	79	90	92
Nortriptyline	93	92	93	89
Olanzapine	85	84	85	83
Paroxetine	86	80	92	92
Pemoline	93	95	99	94
Pentazocine	85	87	84	81
Perospirone	98	98	98	99
Perphenazine	85	88	98	94
Pimozide	87	72	81	83
Pioglitazone	94	94	95	94
Primidone	95	95	94	95
Promethazine	91	96	94	99
Propericiazine	92	92	91	94
Quazepam	97	93	96	95
Quetiapine	96	92	95	95
Risperidone	99	99	97	97
Ropivacaine	97	98	98	98
Salicylic_acid (neg)	98	100	100	98
Sertraline	97	96	96	95
Spiperone	96	97	82	63
Sulpiride	97	97	98	97
Tandospirone	87	87	91	87
Temazepam	70	78	75	69
Timiperone	96	97	99	99
Tofisopam	100	99	100	94
Trazodone	92	93	93	91
Triazolam	66	66	66	72
Trihexyphenidyl	98	99	99	99
Warfarin	92	87	89	91
Zolpidem	81	81	81	78
Zopiclone	93	89	92	87
Zotepine	100	100	100	100

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Conclusions

A MRM triggered product ion spectra method with merged-CE spectrum matching to identify compounds in biological samples is effective for forensic toxicology.

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