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Development of a generic approach to drugs of abuse screening using fast polarity switching MRM triggered product ion scanning on the fly

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Introduction

In recent years the need for forensic, toxicological and clinical analyses have increased, and as a consequence of sample complexity, analysis has become increasingly challenging due to a growing trend in the use of illicit drugs and non-medicinal prescription drugs. Screening applications requires rapid and unambiguous results that can be achieved using a generic analysis method designed for a large number of target compounds. To meet this need, a universal high speed MRM triggered product ion scanning method with fast polarity switching was applied to simultaneously screen, quantitate and confirm by reference to an MS/MS data base containing the majority of drugs of abuse available in Japan.

Materials and Methods

Table 1 List of compounds for Forensic method.

[Abused Drugs]
- Amphetamine
- Benzoyl ecgonine
- Cocaine
- Codeine
- Dihydrocodeine
- Ecgonine methyl ester
- Ephedrine
- Ketamine
- MDA
- MDMA
- Methamphetamine
- Methylephedrine
- Methylphenidate
- Morphine
- Sildenafil
- THC
- THC-COOH

[Psychotropic Drugs]
- Amitriptyline
- Amoxapine
- Aripiprazole
- Chlorpromazine
- Clomipramine
- Dosulepin
- Fluvoxamine
- Haloperidol
- Imipramine
- Levomepromazine
- Maprotiline
- Mianserin
- Mirtazapine
- Nortriptyline
- Olanzapine
- Paroxetine
- Promethazine
- Quetiapine
- Risperidone
- Sertraline
- Sulpiride
- Trazodone
- Zotepine

[Hypnotic Drugs]
- 7-Aminoflunitrazepam
- 7-Aminonimetazepam
- 7-Aminonitrazepam
- 8-Hydroxyetizolam
- Allylisopropylacetylurea
- alpha-Hydroxybrotizolam
- alpha-Hydroxytriazolam
- Alprazolam
- Amobarbital (neg)
- Barbital (neg)
- Bromazepam
- Bromovalerylurea
- Brotizolam
- Diazepam
- desmethyldiazepam
- Estazolam
- Ethyl loflazepate
- Etizolam
- Flunitrazepam
- Flurazepam
- Hydroxyzine
- Lorazepam
- Lormetazepam
- Midazolam
- Nimetazepam
- Nitrazepam
- Oxazepam
- Pentobarbital (neg)
- Phenobarbital (neg)
- Quazepam
- Temazepam
- Thiamylal (neg)
- Triazolam
- Zolpidem
- Zopiclone

[Medical Drugs]
- Acetaminophen
- Acetylpheneturide
- Atropine
- Biperiden
- Bupivacaine
- Carbamazepin
- Chlorpheniramine
- Clonazepam
- Dextromethorphan
- Diclofenac
- Diltiazem
- Diphenhydramine
- Diprophyline
- Ethenzamide
- Glibenclamide
- Glimepiride
- Ibuprofen (neg)
- Lidocaine
- Loxoprofen (neg)
- Mepivacaine
- Mexiletine
- Pancuronium
- Pentazocine
- Salicylic_acid (neg)
- Trihexyphenidyl
- Vecuronium
- Warfarin

[Pesticides]
- Diquat
- Fenitrothion (MEP)
- Glufosinate
- Malathion
- Methomyl
- Paraquat

[Natural Toxines]
- Aconitine
- Colchicine
- Tetrodotoxin

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Samples were analyzed using a Nexera UHPLC system coupled to a LCMS-8030 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan) with LC/MS/MS Method Package for Forensic Toxicology. Database contains product ion scan spectra for 286 forensic and toxicology-related compounds such as 87 Abused drugs, 105 Psychotropic drugs, 70 Hypnotic drugs and others. This library provides Synchronized Survey Scan parameters (product ion spectral data acquisition parameters based on the MRM intensity as threshold) optimized for screening analysis.
The simple quantitative method included the most frequently analyzed 111 components of Abused drugs, Psychotropic drugs and Hypnotic drugs for method validation (Table 1).
Samples were separated using a Shim-pack FC-ODS using a gradient elution with ammonium formate and methanol.

Analytical Conditions

HPLC (Nexera UHPLC system)

Column	: Shim-pack FC-ODS (2.0 mmI.D. x 150 mmL., 3 um)
Mobile Phase A	: 10 mM ammonium formate
Mobile Phase B	: Methanol
Gradient Program	: 5%B (0 min) - 95%B (15-20 min) - 5%B (20.01 - 30 min)
Flow Rate	: 0.3 mL / min
Column Temperature	: 40°C
Injection Volume	: 5 uL

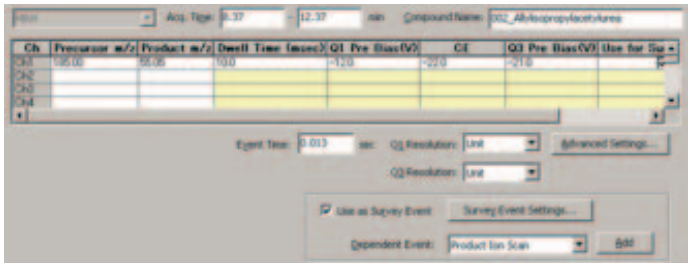
Mass (LCMS-8030 triple quadrupole mass spectrometry)

Ionization	: ESI
Polarity	: Positive & Negative
Probe Voltage	: +4.5 kV (ESI-Positive mode); -3.5 kV (ESI-Negative mode)
Nebulizing Gas Flow	: 1.5 L / min
Drying Gas Pressure	: 10 L / min
DL Temperature	: 250°C
BH Temperature	: 400°C

Analysis of several drugs was performed using fast polarity switching and high speed data acquisition LC/MS/MS. This was achieved using Synchronized Survey Scan® which refers to the execution of MS/MS scanning triggered by survey scan signals (in this case, MRM). Therefore, during the elution of a peak in MRM analysis, a full-product ion mass spectrum can also be obtained.

Type	Event#	+/−	Compound Name m/z	Time (0.000 min – 15.050 min)
MRM	23	+	017_Lidocaine 235.00>86.10	
|- Product Ion Scan	24	+	017_Lidocaine 100.00 > 50.00:245.0	Positive
MRM	25	+	012_Aconitine 646.00>104.95	
|- Product Ion Scan	26	+	012_Aconitine 100.00 > 50.00:656.0	
MRM	41	−	007_Pentobarbital (neg) 225.15>42.	
|- Product Ion Scan	42	−	007_Pentobarbital (neg) 100.00 > 5	Negative
MRM	43	−	003_Amobarbital (neg) 225.15>42.0	
|- Product Ion Scan	44	−	003_Amobarbital (neg) 100.00 > 50.	
MRM	27	+	024_Vecuronium 557.50>100.10	
|- Product Ion Scan	28	+	024_Vecuronium 100.00 > 50.00:56	

MRM parameter



Product Ion Scan parameter

Fig. 1 User Interface of MRM-Product Ion Scan setting at LabSolutions software.

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Results

MS/MS Library Matching

MRM chromatograms of four compounds (each 1000 ng/mL) spiked into urine and analyzed by Nexera coupled to LCMS-8030 following sample preparation (Fig. 2). These product ion scans were searched against the MS/MS library and the four previously identified peaks were assigned a high hit score. The assay generates both MRM and Product Ion Scan data (MS/MS) due to the speed of data acquisition from the LC/MS/MS system. This results in quantitative data and library searching / product matching data to help with product ion confirmation. Fast polarity switching helps to provide information rich product ion spectra resulting in better detection and identification for each compound.

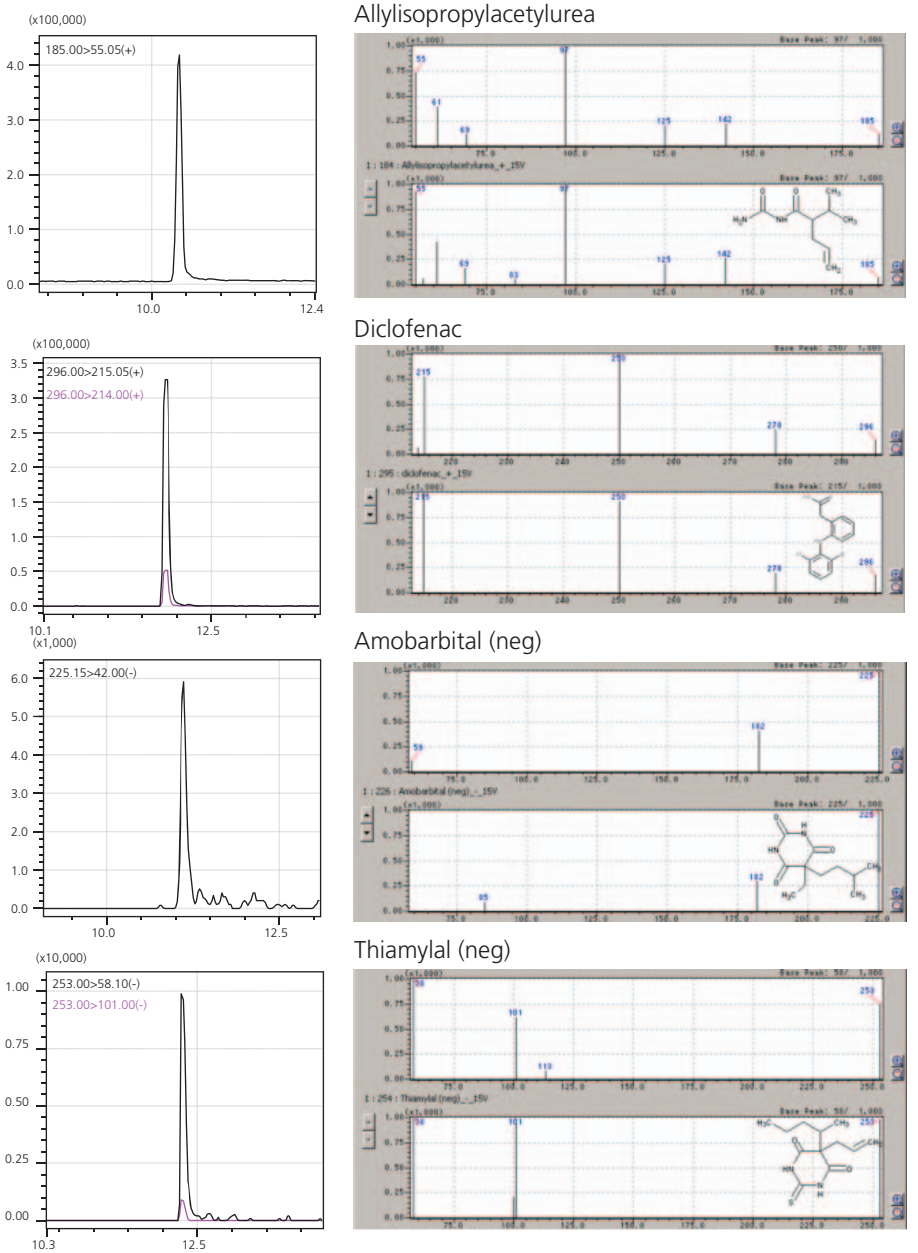

Fig. 2 MRM - Product Ion Scan screening data about 4 compounds.

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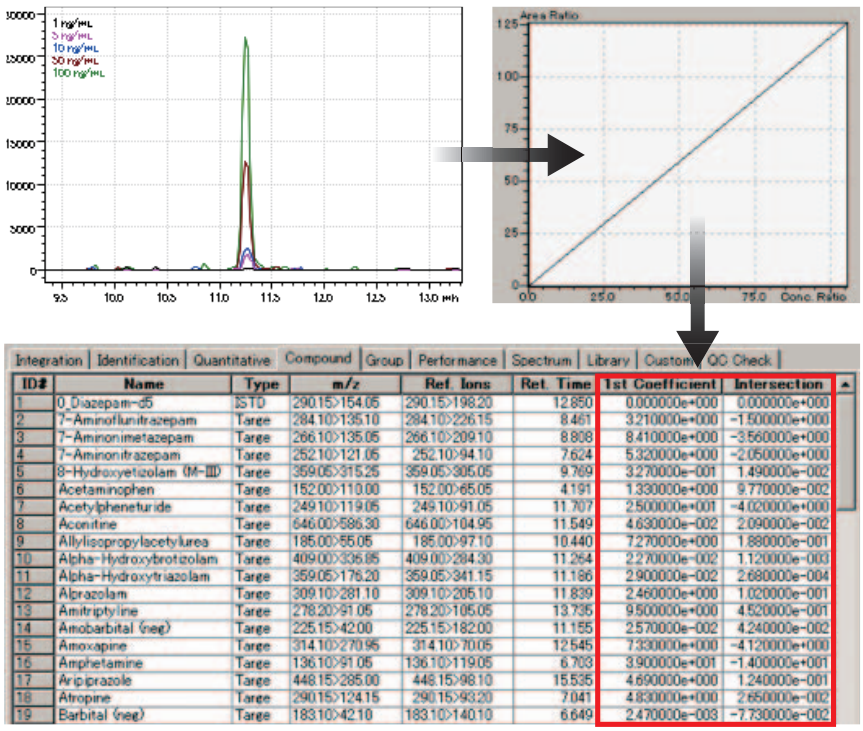
Simple Quantitative Method for Forensic analyses

Based on the chromatogram obtained by injection of a fixed volume of individual reference standard solutions, the ratio of peak area of the reference standard was calculated and compared to that of the internal standard (Diazepam-d5). The resulting calibration curve was prepared by plotting the ratios of the amount of the reference standard to that of the internal standard. 1st coefficient and intersection were calculated from the calibration curve and were registered to the LCMS method (Fig. 3).



ID#	Name	Type	m/z	Ref. Ions	Ret. Time	1st Coefficient	Intersection
1	0_Diazepam-d5	ISTD	290.15>154.05	290.15>198.20	12.850	0.000000e+000	0.000000e+000
2	7-Aminoflunitrazepam	Targe	284.10>135.10	284.10>226.15	8.461	3.210000e+000	-1.500000e+000
3	7-Aminonimetazepam	Targe	266.10>135.05	266.10>209.10	8.808	8.410000e+000	-3.560000e+000
4	7-Aminonitrazepam	Targe	252.10>121.05	252.10>94.10	7.624	5.320000e+000	-2.050000e+000
5	8-Hydroxyetizolam (M-III)	Targe	359.05>315.25	359.05>305.05	9.769	3.270000e-001	1.490000e-002
6	Acetaminophen	Targe	152.00>110.00	152.00>65.05	4.191	1.330000e+000	9.770000e-002
7	Acetylpheneturide	Targe	249.10>119.05	249.10>91.05	11.707	2.500000e+001	-4.020000e+000
8	Aconitine	Targe	646.00>586.30	646.00>104.95	11.549	4.630000e-002	2.090000e-002
9	Allylisopropylacetylurea	Targe	185.00>55.05	185.00>97.10	10.440	7.270000e+000	1.880000e-001
10	Alpha-Hydroxybrotizolam	Targe	409.00>335.85	409.00>284.30	11.264	2.270000e-002	1.120000e-003
11	Alpha-Hydroxytriazolam	Targe	359.05>176.20	359.05>341.15	11.186	2.900000e-002	2.680000e-004
12	Alprazolam	Targe	309.10>281.10	309.10>205.10	11.839	2.460000e+000	1.020000e-001
13	Amitriptyline	Targe	278.20>91.05	278.20>105.05	13.735	9.500000e+000	4.520000e-001
14	Amobarbital (neg)	Targe	225.15>42.00	225.15>182.00	11.155	2.570000e-002	4.240000e-002
15	Amoxapine	Targe	314.10>270.95	314.10>70.05	12.545	7.330000e+000	-4.120000e+000
16	Amphetamine	Targe	136.10>91.05	136.10>119.05	6.703	3.900000e+001	-1.400000e+001
17	Aripiprazole	Targe	448.15>285.00	448.15>98.10	15.535	4.690000e+000	1.240000e-001
18	Atropine	Targe	290.15>124.15	290.15>93.20	7.041	4.830000e+000	2.650000e-002
19	Barbital (neg)	Targe	183.10>42.10	183.10>140.10	6.649	2.470000e-003	-7.730000e-002

Fig. 3 Registration of 1st coefficient and intersection by calibration curve.

The method was validated using 12 of 111 compounds, between 0,05 ng/mL and 5 ng/mL, spiked into whole blood and treated with solid phase extraction (Table 2). The results from this method indicated a high quantitative performance and could prove useful as rapid confirmation and simple quantitative analysis.

Table 2 The calculated results of 12 compounds in whole blood using LC-MS/MS (n=2 average).

		0.05 ng/uL		0.5 ng/uL		5 ng/uL	
Compounds	R.T	Area	Conc.	Area	Conc.	Area	Conc.
Diazepam-d5 (IS)	12.987	396,803	[0.500]	342,441	[0.500]	77,460	[0.500]
Alprazolam	11.857	114,210	0.038	918,575	0.525	2,497,911	6.72
Aripiprazole	15.592	59,975	0.025	700,323	0.205	7,120,340	3.07
Atropine	7.225	327,992	0.084	3,105,470	0.935	17,445,635	10.92
Brotizolam	11.987	42,175	0.044	325,945	0.502	1,043,787	7.49
Colchicine	9.794	21,970	0.015	159,050	0.270	696,217	3.72
Estazolam	11.464	128,563	0.048	1,078,497	0.639	5,580,490	5.49
Ethyl loflazepate	13.068	85,673	0.028	489,250	0.272	1,012,405	2.68
Etizolam	12.092	73,746	0.032	575,984	0.421	2,519,896	5.67
Flunitrazepam	11.229	77,218	0.060	545,933	0.649	1,816,819	7.12
Haloperidol	12.011	616,938	0.048	5,378,666	0.610	28,654,837	7.10
Risperidone	11.778	783,134	0.038	6,811,884	0.510	30,675,213	6.72
Triazolam	11.728	34,424	0.042	283,935	0.550	746,810	6.78

Conclusion

- A high speed LC/MS/MS data acquisition system was applied to drug screening in forensic, toxicological and clinical analysis.
- To achieve a highly specific and sensitive detection method in screening and quantitation, an MRM triggered product ion scanning method using a polarity switching speed of 15msec and a scan speed of 15,000u/sec was applied to 111 components including illicit drugs, psychotropics, hypnotics, pesticides and other substances.
- As the MRM acquisition time was very fast, this enabled product ion spectra to be generated in both positive and negative ionization mode which could be matched against a user library of compounds as an automated aid to screening and compound identification.

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