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High-sensitivity and simultaneous analysis of Psychoactive drugs using LC-MS/MS with full-automated pretreatment system

ASMS 2017 WP358

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Introduction

LC-MS/MS has become a preferred method for the routine analysis for forensic toxicology. LC-MS/MS allows for the simultaneous analysis of multiple compounds in a single run, thus enabling a fast and high throughput analysis. In recent years that it seems the number of incident and accident is increasing caused by dosed with psychotropic drugs and the number of drug testing with LC-MS/MS is also increasing to investigate the cause of death. However, manual sample preparation often involves several complicated manual steps which can introduce error into the results. In this study, we investigated the processing capability to analyze serum, whole blood and urine spiked sixty psychotropic drugs by LC-MS/MS with automated sample preparation unit.

Group 1. Eight Barbiturate drug and Bromovalerylurea

Allobarbital Amobarbital Barbital Pentobarbital Phenobarbital
Secobarbital Thiamylal Thiopental Bromovalerylurea

Group 2. twelve Tri-/Tetra-cyclic antidepressant

Amitriptyline Amoxapine Clomipramine Desipramine Dosulepin Imipramine
Lofepramine Maprotiline Mianserin Nortriptyline Promethazine Setiptiline

Group 3. Thirty-nine Benzodiazepines and their metabolites

Alprazolam Bromazepam Brotizolam Chlordiazepoxide Clorazepic acid Clotiazepam Cloxazolam Diazepam Estazolam Ethyl loflazepate Etizolam Fludiazepam Flunitrazepam Flurazepam Flutazolam Flutoprazepam Haloxazolam Lorazepam Lormetazepam Medazepam Mexazolam Midazolam Nimetazepam Nitrazepam Oxazolam Prazepam
Quazepam Rilmazafone Tofisopam Triazolam Zolpidem

7-Aminoflunitrazepam 7-Aminonimetazepam 7-Aminonitrazepam
α-Hydroxyetizolam (M-VI) α-Hydroxyalprazolam α-Hydroxybrotizolam
α-Hydroxytriazolam Zolpidem M-1

Figure 1 Target drugs

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Methods and Materials

The analysis of 60 psychoactive drugs (eight Barbiturate drug, thirty-nine Benzodiazepines and their metabolites, twelve Tri-/Tetra- cyclic antidepressant and bromovalerylurea) were performed using a fully automatic LCMS preparation unit (CLAM-2000, Shimadzu) online with HPLC-LCMS (NexeraX2-LCMS-8060, Shimadzu).

Samples were trapped on Imtakt Unison UK-C18 (10x2mm, 3.0µm), then separated by Imtakt Unison UK-C18 (75x2mm, 3.0µm) with a binary gradient system. Water with ammonium formate and methanol were used for mobile phases.

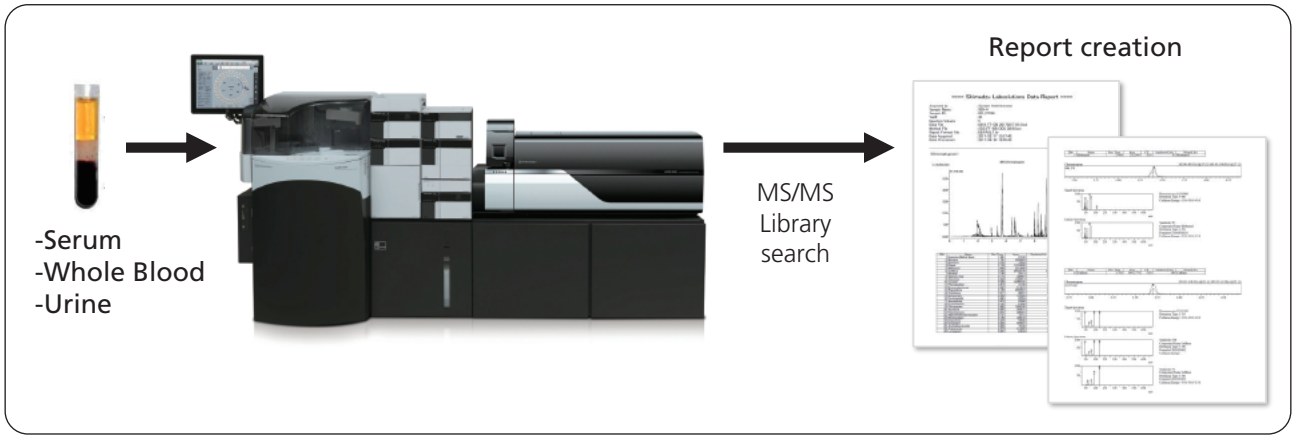

Figure 2 CLAM-2000 and LCMS-8060 system

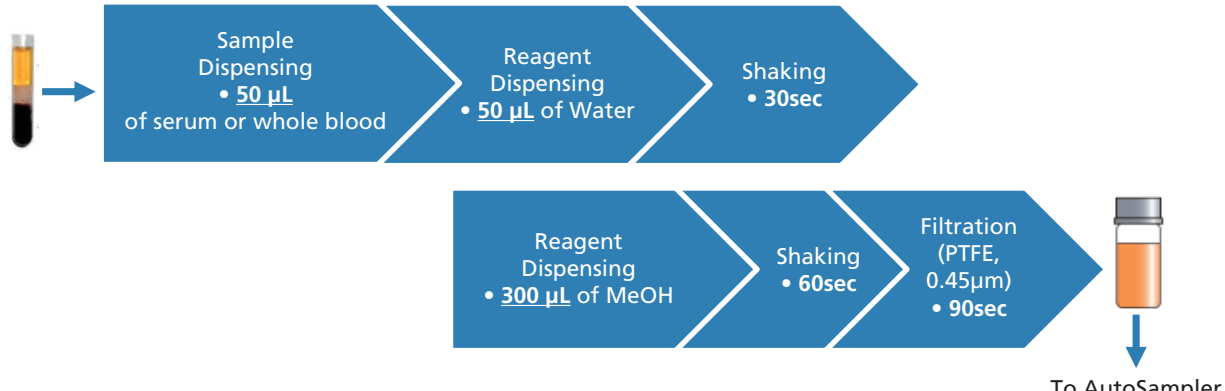

Figure 3 Analytical flow of serum and whole blood

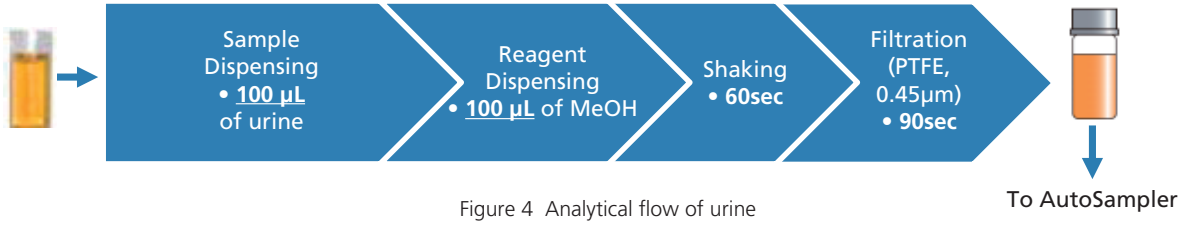

Figure 4 Analytical flow of urine

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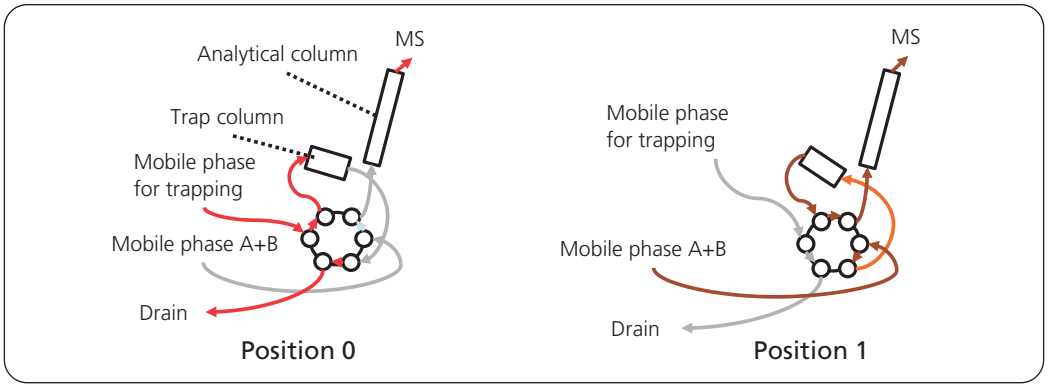

Figure 5 Flow diagram of trapping system

LC/MS/MS conditions (Nexera system and LCMS-8060)	
Ionization	: ESI, Positive/Negative MRM mode
Trap column	: Unison UK-C18 (10×2 mm, 3 µm, Imtakt)
Analytical column	: Unison UK-C18 (75×2 mm, 3 µm, Imtakt)
Mobile phase for traping	: 5% MeOH / 0.1% Formic acid
Mobile phase A	: 10mM Ammonium formate, 5% Methanol
B	: 10mM Ammonium formate, 95% Methanol
Time program	: B conc. 0 % - (1 min) - 5 % - (7 min) - 95 % (3 min)

Result

Recovery rate

Usually LC-MS/MS analysis of biological samples require some manual preparation steps such as protein precipitation, solid phase extraction or liquid/liquid extraction before the injection. With the aim to reduce the operator involvement, to increase the throughput and the data quality, we completely eliminated the manual sample preparation procedure by the use of a novel automatic preparation unit including precipitation, filtration, incubation, shaking and pipetting. Serum and whole blood spiked with sixty psychoactive drugs were pretreated with organic solvent and filtration by the unit. On the other hands, urine spiked with their drugs were only filtration. The treated samples were trapped for cleaning and concentration, then separated by Unison UK-C18 in HPLC Unit.

The recovery of whole blood spiked with sixty psychoactive drugs were more than 70% and the recovery of serum and urine spiked with them were more than 80% except Lofepramin. We completed analysis of their psychoactive drugs in several biological matrices using the automated sample preparation system coupled to LC-MS/MS

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Concentration in sample (μg/mL)	Serum			Whole blood			Urine		
	0.1	1	10	0.1	1	10	0.1	1	10
Allobarbital (neg)	81%	84%	80%	67%	77%	76%	90%	103%	89%
Amobarbital (neg)	81%	86%	80%	78%	79%	78%	88%	103%	92%
Barbital (neg)	84%	82%	91%	73%	84%	79%	87%	102%	89%
Pentobarbital (neg)	77%	78%	80%	72%	80%	76%	95%	109%	92%
Phenobarbital (neg)	77%	83%	92%	70%	74%	81%	92%	101%	86%
Secobarbital (neg)	69%	92%	80%	67%	73%	77%	78%	107%	88%
Thiamylal (neg)	76%	83%	84%	72%	79%	77%	76%	97%	84%
Thiopental (neg)	75%	84%	81%	68%	75%	77%	81%	110%	95%
Bromovalerylurea	71%	84%	84%	75%	79%	79%	91%	104%	92%

Concentration in sample (μg/mL)	Serum			Whole blood			Urine		
	0.01	0.1	1	0.01	0.1	1	0.01	0.1	1
Amitriptyline	74%	69%	80%	64%	66%	70%	87%	84%	102%
Amoxapine	72%	73%	80%	66%	66%	73%	90%	86%	101%
Clomipramine	67%	70%	74%	63%	65%	83%	82%	78%	99%
Desipramine	74%	72%	77%	57%	60%	89%	90%	83%	103%
Dosulepin	70%	68%	80%	63%	64%	77%	88%	86%	100%
Imipramine	76%	70%	81%	64%	67%	87%	86%	84%	100%
Lofepramine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Maprotiline	68%	68%	76%	56%	58%	67%	91%	83%	101%
Mianserin	79%	84%	76%	78%	77%	81%	90%	85%	111%
Nortriptyline	71%	73%	80%	55%	64%	70%	91%	83%	100%
Promethazine	71%	69%	80%	89%	104%	116%	89%	84%	103%
Setiptiline	83%	75%	83%	68%	70%	83%	87%	87%	106%

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Concentration in sample (μg/mL)	Serum			Whole blood			Urine		
	0.01	0.1	1	0.01	0.1	1	0.01	0.1	1
Alprazolam	69%	70%	82%	78%	75%	86%	98%	93%	106%
Bromazepam	73%	74%	78%	82%	76%	77%	103%	96%	112%
Brotizolam	72%	72%	86%	79%	73%	85%	98%	93%	108%
Chlordiazepoxide	76%	73%	81%	77%	73%	82%	99%	93%	111%
Clorazepic acid	84%	70%	80%	78%	77%	90%	97%	91%	126%
Clotiazepam	73%	73%	74%	81%	76%	85%	96%	93%	116%
Cloxazolam	90%	79%	82%	81%	80%	89%	N.A	77%	83%
Diazepam	82%	77%	77%	80%	74%	83%	96%	94%	109%
Estazolam	72%	73%	79%	80%	74%	82%	99%	95%	110%
Ethyl loflazepate	82%	72%	81%	76%	73%	80%	90%	92%	112%
Etizolam	77%	73%	80%	81%	75%	95%	105%	95%	103%
Fludiazepam	79%	76%	79%	74%	74%	87%	95%	93%	115%
Flunitrazepam	79%	74%	80%	76%	73%	82%	100%	95%	111%
Flurazepam	75%	73%	93%	73%	73%	97%	92%	93%	99%
Flutazolam	75%	71%	79%	70%	70%	81%	92%	97%	111%
Flutoprazepam	82%	79%	73%	79%	77%	92%	96%	89%	106%
Haloxazolam	56%	64%	87%	55%	68%	75%	86%	89%	122%
Lorazepam	76%	73%	76%	86%	70%	76%	115%	85%	103%
Lormetazepam	75%	73%	78%	75%	73%	79%	95%	89%	106%
Medazepam	80%	70%	79%	77%	76%	91%	96%	90%	123%
Mexazolam	81%	77%	73%	83%	75%	103%	79%	93%	123%
Midazolam	74%	70%	80%	79%	72%	84%	94%	91%	104%
Nimetazepam	75%	73%	81%	76%	75%	85%	105%	98%	115%
Nitrazepam	80%	73%	77%	75%	74%	77%	97%	93%	107%
Oxazolam	N.A.	73%	89%	N.A.	73%	88%	77%	70%	81%
Prazepam	78%	82%	73%	81%	78%	86%	94%	90%	107%
Quazepam	73%	78%	70%	81%	77%	81%	86%	88%	217%
Rilmazafone	78%	76%	82%	71%	73%	70%	98%	97%	126%
Tofisopam	73%	71%	82%	78%	75%	85%	101%	93%	109%
Triazolam	72%	70%	83%	80%	76%	85%	93%	93%	110%
Zolpidem	71%	72%	87%	76%	74%	95%	99%	97%	102%
α-Hydroxyalprazolam	84%	70%	76%	81%	72%	77%	96%	95%	110%
α-Hydroxybrotizolam	70%	74%	79%	74%	70%	76%	94%	89%	107%
α-Hydroxyetizolam (M-VI)	79%	70%	79%	71%	70%	80%	86%	90%	104%
7-Aminoflunitrazepam	75%	72%	78%	79%	75%	79%	94%	91%	108%
7-Aminonimetazepam	75%	74%	78%	81%	75%	80%	93%	90%	104%
7-Aminonitrazepam	74%	71%	78%	77%	73%	88%	94%	90%	103%
α-Hydroxytriazolam	74%	72%	78%	79%	75%	79%	109%	92%	106%
Zolpidem M-1	74%	71%	86%	76%	74%	95%	99%	98%	103%

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Conclusions

These results shows the capability of the system for large sample set analyses with improved accuracy and precision by eliminating human error associated with manual sample handling.

* Disclaimer: LCMS-8060 and CLAM-2000 are not registered as a Class I device, and it is available for Research Use Only (RUO). Not for use in diagnostic procedures.

First Edition: June, 2017

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