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Screening and Semi-Quantitation by LC/MS/MS in Whole Blood Using Rapid Tox Screening

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User Benefits

- A simple extraction procedure using a commercially available Micro Volume QuEChERS kit.
- LabSolutions Insight Library Screening makes it possible to confirm the result of spectrum library search.
- A quick way to screen for 231 forensic compounds and confirm the semi-quantitative results without standard samples.

■ Introduction

Forensic toxicology is an important tool in the field of forensic medicine and death investigation. Since investigations are often time sensitive, a rapid analysis system to provide toxicologists and medical examiners with timely information would be beneficial. Recent developments in the robustness, sensitivity, and affordability of LC/MS/MS systems have made their presence in forensic laboratories a necessity. A quick and robust screening system with the ability to semi-quantitate whole blood samples called LC/MS/MS Rapid Toxicology Screening System has been developed. This system contains the method parameters for the identification and semi-quantitation of 231 forensically relevant compounds. The increased sensitivity of these systems allows for analysis of only 100 microliters of whole blood using a Micro Volume QuEChERS kit. This article details the sample pretreatment and results from analysis of an actual autopsy whole blood sample using the LC/MS/MS Rapid Toxicology Screening System Ver.3.01.

Fig. 1 LC/MS/MS Rapid Toxicology Screening system

■ Sample Pretreatment

The sample processing flow is shown in Figure 2 and the details of the procedure are described below. To the Micro Volume QuEChERS kit containing the extraction salts, 300 µL of acetonitrile and 200 µL of distilled water were added and mixed thoroughly. A 5 µL volume of an internal standard (ISTD) solution containing 4 ng/µL Diazepam-d5 and 200 ng/µL Phenobarbital-d5 was added. 100 µL of whole blood was added, immediately mixed, and centrifuged (15,000 x g, 10 min, room temperature). 100 µL of the supernatant was transferred to a new 1.5 mL plastic tube. To this was added 20 µL of a 0.1% TFA-acetonitrile solution and mixed. The solution was evaporated to dryness under a stream of nitrogen gas. After drying, the sample was reconstituted in 500 µL of methanol. After centrifugation (15,000 x g, 10 min, room temperature), the sample was transferred to an autosampler vial for measurement.

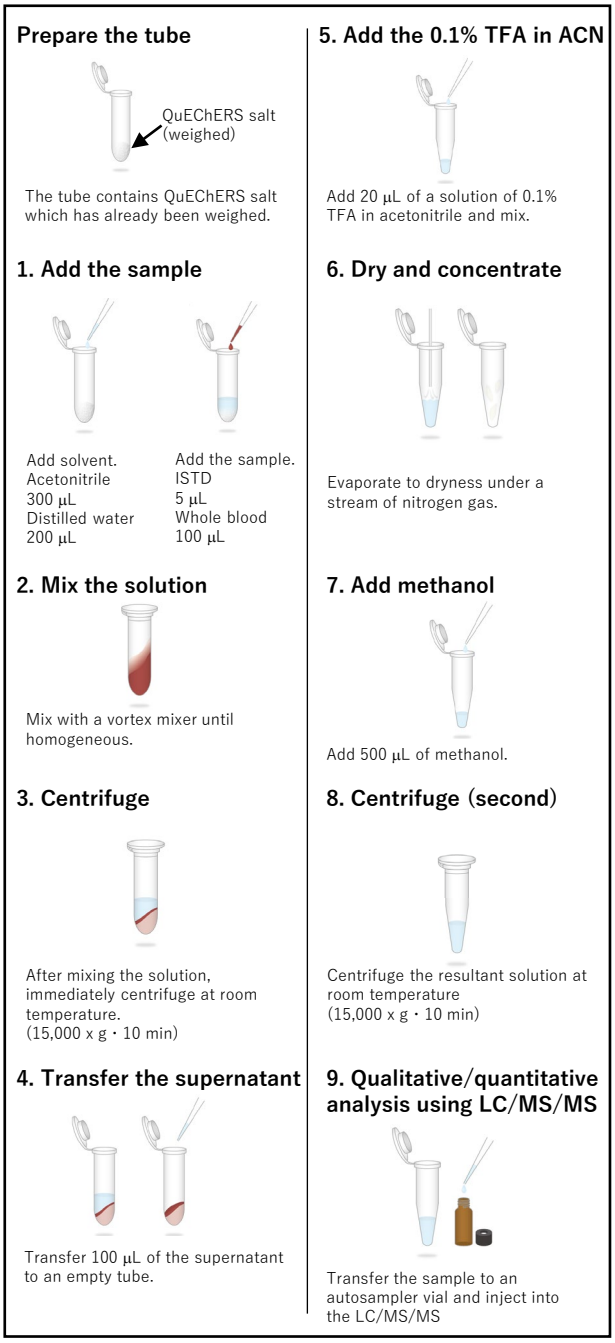

Fig. 2 Sample pretreatment flow using Micro Volume QuEChERS kit

■ Measurement Conditions and Samples

The extracted samples were analyzed using the Nexera™ X2 system and LCMS-8045. The analysis column used was a Shim-pack Velox™ SP-C18 (2.1 mm I.D. x 100 mm L., 2.7 µm). A synchronized survey scan (SSS) was used for qualitative analysis of the samples and a multiple reaction monitoring (MRM) method for quantitative analysis. Table 1 shows the common UHPLC conditions used for both methods, and Table 2 shows the different MS/MS conditions for each method.

Table 1 Analytical condition of UHPLC

UHPLC (Nexera™ X2 system)	
Column	: Shim-pack Velox SP-C18 (2.1 mm I.D. x 100 mm L., 2.7 µm, Shimadzu) P/N: 227-32003-03
Mobile phase	: A) 10 mM ammonium formate and 0.1% formic acid in water B) 10 mM ammonium formate and 0.1% formic acid in methanol
Mode	: Gradient elution (15 min)
Flow rate	: 0.3 mL/min
Injection volume	: 5 µL

Table 2 Analytical condition of MS/MS

SSS method	
Ionization	: ESI (Positive/Negative switching)
Mode	: MRM and Product Ion Scan (231 Compounds)
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 10.0 L/min
Heating gas flow	: 10.0 L/min
DL temp.	: 250 ℃
Block heater temp.	: 400 ℃
Interface temp.	: 300 ℃

MRM method	
Ionization	: ESI (Positive/Negative switching)
Mode	: MRM (229 Compounds)
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 10.0 L/min
Heating gas flow	: 10.0 L/min
DL temp.	: 250 ℃
Block heater temp.	: 400 ℃
Interface temp.	: 300 ℃

■ Autopsy Blood Sample

A whole blood sample from a case autopsied at the Department of Forensic Medicine, Faculty of Medicine, Fukuoka University was used as the test sample. Prior to analysis using semi-quantitative method, quantitation using a fully validated LC/MS/MS method with 9-point calibration curves from reference-spiked calibrators for each detected compound was performed.[1)] The detectable compounds and their quantitative values are shown in Table 3.

Table 3 Compounds detected in the autopsy blood sample and the quantitative results calculated by previous research

Compound name	Quantitative results (ng/µL)
7-Aminoflunitrazepam	0.0059
Biperiden	0.002
Haloperidol	0.0033

■ Result of Identification by SSS method

The LC/MS/MS Rapid Toxicology Screening System includes two method files: SSS and MRM. Both methods contain calibration curve information for each compound, and can calculate semi-quantitative results without a standard sample. The SSS method performs MRM analysis for each compound and runs product ion scans for compounds that exceed a set intensity threshold. The results can be easily viewed with LabSolutions Insight Library Screening. From the data of autopsy blood samples acquired by SSS method, three compounds (7-Aminoflunitrazepam, Biperiden, Haloperidol) were detected from the sample.

The library search results for each compound are shown in Table 4. Figure 3 shows an example of library search results processed by the LabSolutions Insight Library Screening software.

Table 4 The results of library screening

Compound name	Similarity (Maximum: 100)
7-Aminoflunitrazepam	61
Biperiden	84
Haloperidol	95

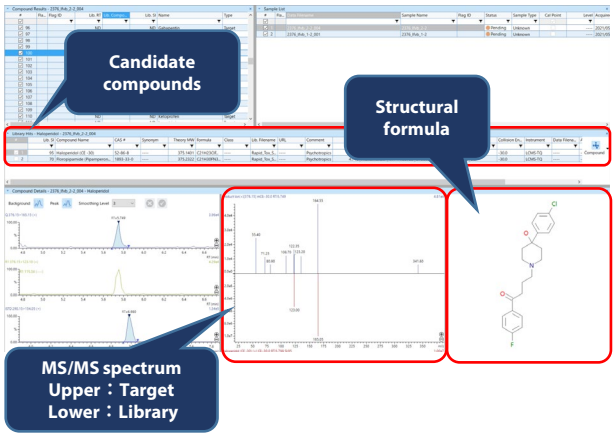

Fig. 3 Example of LabSolutions Insight Library Screening

■ Semi-quantitation result by MRM method

The MRM method provides better quantitative accuracy especially at lower concentrations compared to the SSS. Three compounds identified by the SSS method were analyzed by the MRM method, and the chromatograms are shown in Figure 4. The errors between the semi-quantitative results and the quantitative results in Table 3 are shown in Table 5.

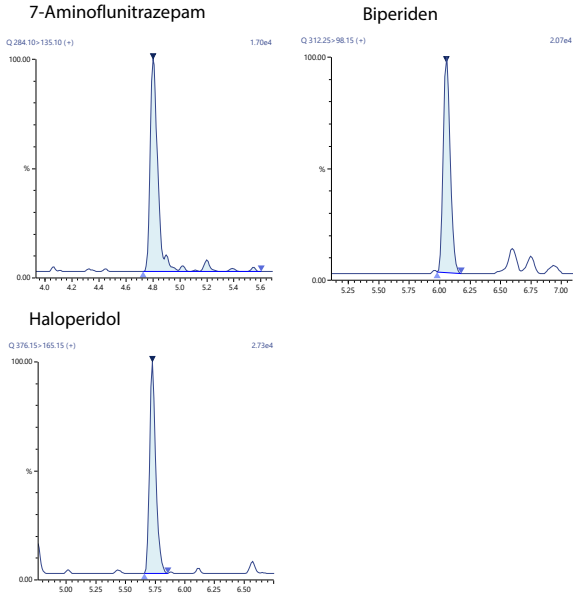

Fig. 4 Chromatograms of target compounds detected from the autopsy blood sample

Table 5 The semi-quantitative results by the MRM method

Compound name	Semi-quantitative results(ng/µL)	Error from quantitative results(%)
7-Aminoflunitrazepam	0.0051	14
Biperiden	0.0007	65
Haloperidol	0.0034	3

■ Conclusion

The LC/MS/MS Rapid Toxicology Screening system was effective in detecting the compounds of forensic interest from an actual autopsy blood sample taken from a case autopsied at the Fukuoka University Department of Forensic Medicine. The semi-quantitative results determined by stored calibration curves in the method package were very close to the results determined in the previous research[1] for two compounds (7-Aminoflunitrazepam and Haloperidol). The result for Biperiden was below the Lower Limit of Quantitation (LLOQ) for the Rapid Toxicology Screening method, which could explain the inaccuracy for this compound. These findings demonstrated the utility and convenience of the Rapid Tox Screening system when applied to an actual autopsy blood sample, both with regard to sensitive screening of compounds at very low concentrations, and the accuracy of the semi-quantitation from the registered calibration curves.

■ List of compounds registered in the method

Table 6 shows the list of compounds registered in the LC/MS/MS Rapid Toxicology Screening System.

Table 6 Registered compounds in the Rapid Toxicology Screening system

7-Aminoclonazepam	7-Aminoflunitrazepam	7-Aminonimetazepam
7-Aminonitrazepam	8-Hydroxyetizolam	Acetaminophen
Aconitine	Alfa-methyltryptamine	Allylisopropylacetylurea
alpha-Hydroxyalprazolam	alpha-Hydroxymidazolam	alpha-Hydroxytriazolam
Alprazolam	Amitriptyline	Amlodipine
Amobarbital	Amoxapine	Amphetamine
Ampicillin	Aripiprazole	Atenolol
Atomoxetine	Atorvastatin	Atropine
Azelnidipine	Azilsartan	Barbital
Benzoyl ecgonine	Biperiden	Blonanserin
Brexpiprazole	Bromazepam	Bromocriptine
Bromovalerylurea	Bromperidol	Brotizolam
Bupivacaine	Caffeine	Candesartan
Carbamazepine	Carbazochrome	Carpipramine
Carvedilol	Chlordiazepoxide	Chlorpheniramine
Chlorpromazine	Chlorpromazine-M	Cibenzoline
Clobazam	Clocapramine	Clomipramine
Clonazepam	Clotiazepam	Cloxazolam
Clozapine	Cocaine	Codeine
Colchicine	DDVP	Delorazepam
DEP	Desipramine	Desmethylclotiazepam
Desmethyldiazepam	Dextromethorphan	Diazepam
Dibucaine	Diclofenac	Dihydrocodeine
Diltiazem	Diphenhydramine	Diprophyline
Diquat	Domperidone	Donepezil
Dosulepin	Droperidol	Duloxetine
Ecgonine methyl ester	Ephedrine	Escitalopram
Estazolam	Ethenzamide	Ethyl loflazepate
Etizolam	Famotidine	Fludiazepam
Flufenamic acid	Flunitrazepam	Fluphenazine
Flurazepam	Fluvoxamine	Furosemide
Gabapentin	Glibenclamide	Gliclazide
Glimepiride	Haloperidol	Haloxazolam
Hydroxymethylbrotizolam	Hydroxyzine	Ibuprofen
Imidapril	Imipramine	Irbesartan
Isopropylantipyrine	Ketamine	Ketoprofen
Lamotrigine	Levetiracetam	Levomepromazine
Lidocaine	Lorazepam	Lormetazepam
Losartan	Loxoprofen	Malathion
Maprotiline	MDA	MDMA
Medazepam	Mefenamic acid	Memantine
Mepivacaine	Mequitazine	Metformin
Methamphetamine	Methomyl	Methylephedrine
Methylphenidate	Mexazolam	Mexiletine
Mianserin	Midazolam	Milnacipran
Mirtazapine	Morphine	Mosapramine
Naftopidil	N-Desmethyl clobazam	N-Desmethyl zopiclone
N-Desmethylmirtazapine	Nemonapride	Nicardipine
Nicotine	Nicotine-M	Nifedipine
Nimetazepam	Nitrazepam	Norephedrine
Nortriptyline	Noscapine	Olanzapine
Olmesartan	Oxazepam	Oxypertine
Paliperidone	Papaverine	Paroxetine
Pemoline	Pentazocine	Pentobarbital
Perospirone	Perphenazine	Phenobarbital
Phenytoin	Pimozide	Pioglitazone
Pipamperone	Piroxicam	Pitavastatin
Pranlukast	Primidone	Procaine
Prochlorperazine	Promethazine	Propericiazine
Propofol	Propranolol	Quazepam
Quetiapine	Risperidone	Ropivacaine
Rosuvastatin	Salicylamide	Salicylic acid
Secobarbital	Sertraline	Setiptiline
Sildenafil	Silodosin	Sitagliptin
Solifenacin	Spiperone	Spironolactone
Sulfamethoxazole	Sulpiride	Sultopride
Suvorexant	Tadalafil	Tandospirone
Telmisartan	Temazepam	Tetracaine
THC	THC-COOH	Thiamylal
Timiperone	Tofisopam	Topiramate
Tramadol	Trandolapril	Trazodone
Triazolam	Trihexyphenidyl	Trimethoprim
Trimipramine	Urapidil	Valproic acid
Valsartan	Vardenafil	Venlafaxine
Verapamil	Warfarin	Zaleplon
Zolpidem	Zolpidem _M-1	Zonisamide
Zopiclone	Zopiclone-N-oxide	Zotepine

■ Reference

1) Waters B et.al., "Tissue distribution of suvorexant in three forensic autopsy cases", J. Anal. Toxicol. 42 (2018) 276-283.

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01-00255-EN First Edition: Dec. 2021

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