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

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1. 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. A quick and robust screening LC/MS/MS system with the ability to semi-quantitate whole blood samples 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 study details the sample pretreatment and results from analysis of an actual autopsy whole blood sample.

2. Sample Pretreatment

The sample processing flow shown in Figure 1 was performed in this study. An internal standard (ISTD) solution containing 4 ng/μL Diazepam-d5 and 200 ng/μL Phenobarbital-d5 was used for quantitative analysis.

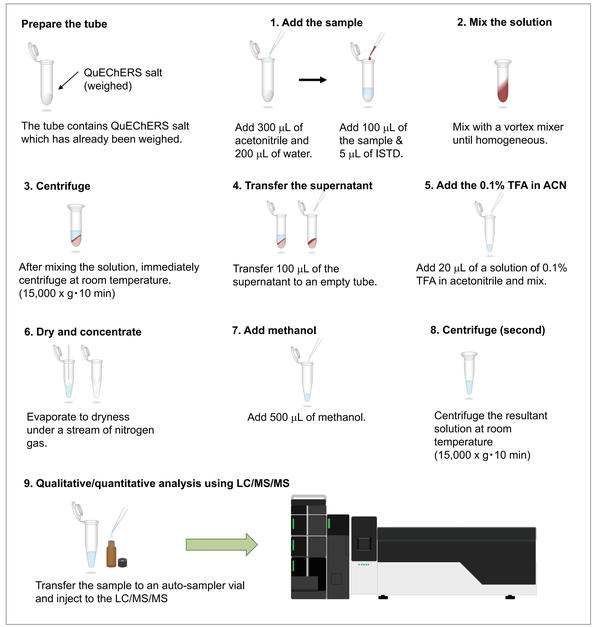

Fig.1 Sample pretreatment flow using Micro Volume QuEChERS kit

3. Method and Sample

3-1. Measurement Conditions

Target compounds in the autopsy blood sample were extracted by the Micro Volume QuEChERS kit containing the extraction salts. 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) that automatically performs MS/MS when a precursor exceeds the threshold was used for qualitative analysis of the samples and a multiple reaction monitoring (MRM) method for quantitative analysis. The methods contain target information (MRM transition, retention time, calibration curve information) for 231 compounds and can screen all compounds simultaneously in a single run.

Table 1 Analytical condition of UHPLC

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 °C
Block heater temp.	:400 °C
Interface temp.	:300 °C
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 °C
Block heater temp.	:400 °C
Interface temp.	:300 °C

3-2. 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 this 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/mL)
7-Aminoflunitrazepam	0.0059
Biperiden	0.002
Haloperidol	0.0033

4. Results

The developed 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.

4-1. Result of Identification by SSS method

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 2 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

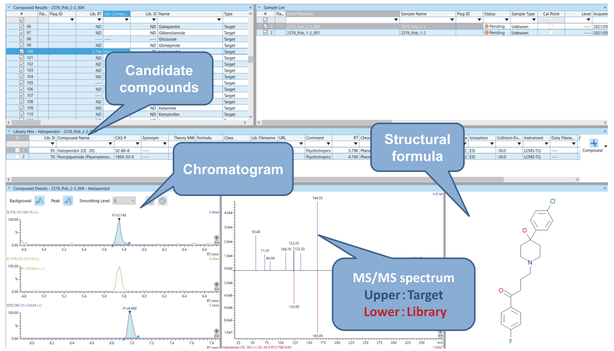

Fig. 2 Example of LabSolutions Insight Library Screening

4-2. 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 3. The errors between the semi-quantitative results and the quantitative results in Table 3 are shown in Table 5. Some other peaks were also detected, but they were not targets since the MS/MS patterns obtained by SSS method were found to be inconsistent with the library data.

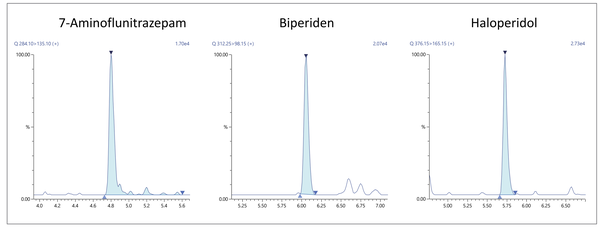

Fig. 3 Chromatograms of target compounds detected from the autopsy blood sample

Table 5 The semi-quantitative results by the MRM method

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

5. Conclusions

The 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 regards to sensitive screening of compounds at very low concentrations, and the accuracy of the semi-quantitation from the registered calibration curves.

6. 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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