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Quadrupole Time-of-Flight Liquid Chromatograph Mass Spectrometer LCMS-9030

Application News

Qualitative Analysis of Drugs in Blood Using LCMS-9030 and MS/MS Spectral Libraries

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User Benefits

- Qualitative screening of drugs using LCMS-9030 can be performed in 15 minutes.
- LabSolutions Insight Discovery and High Resolution Accurate Mass Library for Forensic Toxicology Ver. 2 simplify the analysis of measurement data .

■ Introduction

Many forensic toxicological analyses are related to overdoses of prescription or over-the-counter drugs, and the majority of drugs consumed are psychiatric drugs such as sleeping drugs, antidepressants, and anti-anxiety drugs. Hikiji et al.[1)] reported 30 drugs that are at high risk of causing death due to overdose. In addition, Asano et al.[2)] reported the top 20 psychotropic drugs left in the places where bodies were discovered, and according to that, sleeping drugs, anti-anxiety drugs, antidepressants, and antipsychotic drugs were particularly common. Many of the drugs listed in both reports are the same and are considered important targets for forensic toxicological analysis. For this reason, forensic toxicological analysis requires a workflow that can reliably identify the important related components without any omissions.

This application introduces an example of drug qualitative screening using the LCMS™-9030 quadrupole time-of-flight (QTOF) mass spectrometer, LabSolutions Insight Discovery, and "High Resolution Accurate Mass Library for Forensic Toxicology Ver. 2" (Fig. 1).

Fig. 1 High Resolution Accurate Mass Library for Forensic Toxicology Ver. 2

■ Sample Preparation and Analytical Conditions

The blood test samples were prepared by spiking a total of 25 drugs into bovine whole blood at a concentration of 0.02 µg/mL. Namely, in addition to the common 18 drugs reported in both Hikichi et al. and Asano et al., the following drugs were included: Paliperidone, which is an active metabolite of Risperidone and has recently become more commonly used as a substitute for Risperidone; sleep medications such as Lemborexant, Suvorexant, and Ramelteon, all of which have seen an increase in usage in recent years; and Acetaminophen, Chlorpheniramine, and Diphenhydramine, which are components of cold medicines and whose overdose has become a concern among young people. Table 1 lists these 25 drugs. The concentration of the spiked drugs was determined based on Brotizolam, which had the lowest toxic concentration among the 21 (out of the 25) drugs for which toxic levels are documented in reference[3)].

Table 1 25 Drug Compounds Spiked in Bovine Whole Blood

#	Compounds	#	Compounds	#	Compounds
1	Acetaminophen	10	Flunitrazepam	19	Quetiapine
2	Alprazolam	11	Haloperidol	20	Ramelteon
3	Bromazepam	12	Lemborexant	21	Risperidone
4	Brotizolam	13	Levomepromazine	22	Sulpiride
5	Chlorpheniramine	14	Lorazepam	23	Suvorexant
6	Chlorpromazine	15	Nitrazepam	24	Zolpidem
7	Diazepam	16	Olanzapine	25	Zopiclone
8	Diphenhydramine	17	Paliperidone		
9	Etizolam	18	Paroxetine		

Sample Preparation

The Micro Volume QuEChERS kit (P/N: S225-37870-91) was used for sample preparation. The workflow is shown in Fig. 2: 200 µL of water, 300 µL of acetonitrile, and 100 µL of drug-spiked bovine whole blood were added to the Micro Volume QuEChERS kit and thoroughly stirred then centrifuged. The supernatant was collected and then dried. The residue was redissolved in 40 µL of methanol to make the sample for analysis.

Fig. 2 Workflow of Sample Preparation

LC/MS/MS Analytical Conditions

Qualitative analysis by LC/MS/MS was performed using Nexera™ and LCMS-9030. Analytical conditions are shown in Table 2.

Table 2 LC/MS/MS Analytical Conditions

System:	Nexera XS
Column:	Shim-pack Velox™ SP-C18*1 (100 mm × 2.1 mm I.D., 2.7 µm)
Temperature:	40 °C
Injection Volume:	2 µL
Mobile Phases:	10 mM ammonium formate + 0.1 % formic acid in Water 10 mM ammonium formate + 0.1 % formic acid in MeOH
Flowrate:	0.3 mL/min
Mode:	Gradient elution (15 min)
System:	LCMS-9030 (ESI Positive)
Nebulizing Gas:	3 L/min
Drying Gas:	10 L/min
Heating Gas:	10 L/min
DL Temp.:	250 °C
Heat Block Temp.:	400 °C
Interface Temp.:	300 °C
Mode:	DDA*2, 3
MS Scan Range:	*m/z* 100-600
MS/MS Scan Range:	*m/z* 50-600

*1 P/N: 227-32003-03
*2 Ions detected in blank analysis were set to the excluded ion list.
*3 Mass correction was performed by external standard.

■ Data Analysis

The acquired data was subjected to a library search using LabSolutions Insight Discovery, which can perform analyses on multiple acquired data in batch format, reducing the time and effort required for data analysis. The libraries used were the ODS method libraries contained in "High Resolution Accurate Mass Library for Forensic Toxicology Ver. 2" (S225-45370-91).

■ Qualitative Screening Using LCMS-9030

The library similarity and mass errors of 25 drugs added to bovine whole blood are shown in Table 3, the measured spectra of some drugs in Fig. 3, and the MS chromatogram of the acquired data in Fig. 4. Although there were many matrix-derived peaks in the sample and some drugs had retention times that were very close to each other, all the spiked drugs were identified, and their library similarity was 82-99. The difference between the measured and theoretical precursor ion *m/z* values for each drug was within 1 ppm.

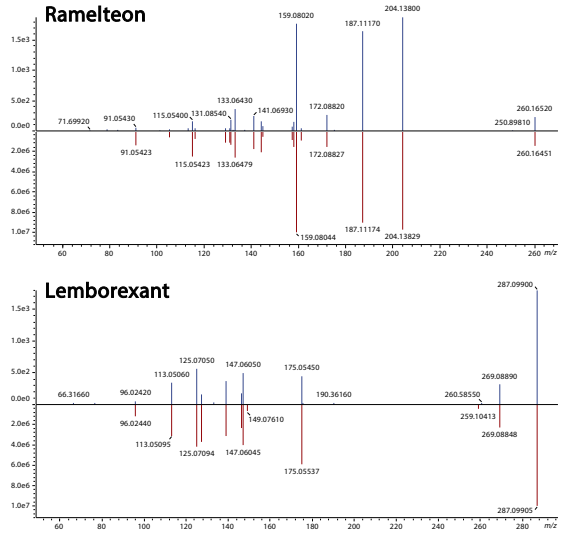

Fig. 3 (Upper Panel) Measured MS/MS Spectrum of Standard-Spiked Bovine Whole Blood (Standards were Spiked at 0.02 µg/mL) and (Lower Panel) MS/MS Spectrum of Each Drug in the Library

Table 3 Library Similarity and Mass Error of 25 Drug Compounds Spiked in Bovine Whole Blood

#	Compounds	Library Similarity	Mass Error (ppm)
1	Acetaminophen	98	-0.79
2	Alprazolam	96	-0.39
3	Bromazepam	94	-0.60
4	Brotizolam	95	-0.20
5	Chlorpheniramine	96	-0.29
6	Chlorpromazine	82	-0.38
7	Diazepam	97	0.00
8	Diphenhydramine	98	-0.51
9	Etizolam	99	-0.44
10	Flunitrazepam	98	-0.13
11	Haloperidol	95	-0.24
12	Lemborexant	91	0.51
13	Levomepromazine	99	-0.21
14	Lorazepam	96	-0.28
15	Nitrazepam	95	-0.07
16	Olanzapine	94	-0.19
17	Paliperidone	98	-0.05
18	Paroxetine	96	0.15
19	Quetiapine	94	-0.08
20	Ramelteon	97	0.04
21	Risperidone	92	-0.05
22	Sulpiride	99	-0.26
23	Suvorexant	99	0.11
24	Zolpidem	96	0.10
25	Zopiclone	94	-0.05

■ Conclusion

Qualitative screening with LCMS-9030 was performed on bovine whole blood spiked with 25 drugs. All the spiked drugs were identified with a library similarity of more than 80 and high mass accuracy from a sample containing many matrix-derived components. These results demonstrate the usefulness of the LCMS-9030 and the "High Resolution Accurate Mass Library for Forensic Toxicology Ver. 2" in the qualitative screening of drugs.

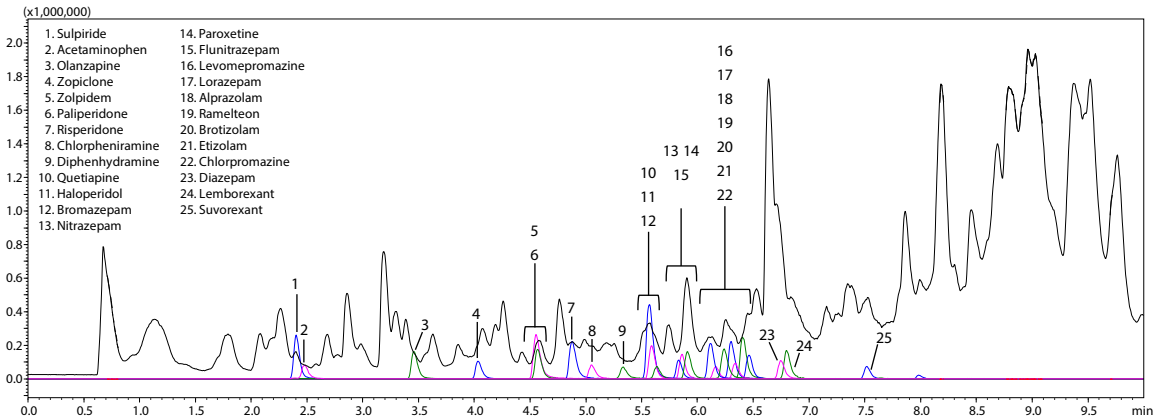

Fig. 4 TIC (black) and XIC (Blue, Pink, Green) for Each Drug in Standard-Spiked Bovine Whole Blood (Standards were Spiked at 0.02 µg/mL). XICs are shown as magnification ×10 for Lorazepam and Suvorexant and magnification ×5 for the other drugs.

<References>

1) W. Hikiji, et al. Identification of Psychotropic Drugs Attributed to Fatal Overdose : A Case-control Study by Data from the Tokyo Medical Examiner's Office and Prescriptions. Psychiatria et neurologia Japonica. 2016, vol. 118, No. 1, p. 3-13.
2) M. Asano, et al. Statistical Observations of Psychotropic Drugs in Postmortem Inspections (Transfer from Japanese), Japan Medical Journal. 2018, No. 4925, p. 49-53. (in Japanese)
3) M. Schulz, et al. Therapeutic and toxic blood concentrations of more than 1100 drugs and other xenobiotics. Critical Care. 2020, vol. 24, p. 195-198.

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