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Application News

DPiMS™ QT Probe Electrospray Ionization Kit
LCMS™-9030 Quadrupole Time-of-Flight Liquid Chromatograph Mass Spectrometer

Qualitative/Quantitative Analysis of Drugs in Whole Blood by DPiMS QT and Nexera™ installed LCMS-9030

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User Benefits

- Qualitative screening using the DPiMS QT kit can be conducted with a sample preparation time of about 5 min and measurement time of approximately 0.5 min.
- Simple switching to the Nexera ultra-high performance liquid chromatograph is possible.
- Once data are acquired, it is possible to extend the target compounds later without storing samples.

■ Introduction

In analysis of drugs and poisons in forensic medicine and scientific criminal investigations, it is necessary to identify drugs and report the quantitative results quickly. For this, development of an efficient workflow that enables exhaustive qualitative/quantitative analysis of all relevant components is required.

This Application News introduces a workflow consisting of qualitative analysis using a combination of an LCMS-9030 quadrupole time-of-flight mass spectrometer and DPiMS QT which is a probe kit of electrospray ionization unit (Fig. 1, left) followed by quantitative analysis by a combination of an LCMS-9030 and a Nexera X3 ultra-high performance liquid chromatograph (Fig. 1, right). Use of the DPiMS QT and Nexera with one LCMS-9030 enables quick completion of the entire process from high-speed qualitative screening of drugs to quantitation. By reducing the number of samples necessary in the quantitative analysis, this technique also makes it possible to achieve high efficiency in the measurement and reduce the consumable items used in the quantitative analysis.

Fig. 1 DPiMS™ QT and LCMS™-9030 (Left), and Nexera™ and LCMS™-9030 (Right)

■ Sample Preparation and Analysis Conditions

In this experiment, spiked human whole blood samples were prepared by spiking human whole blood with 7 drugs. Table 1 shows the information for the 7 drug compounds. Calibration curves for use in the quantitative analysis by LC/MS were prepared using calibration point samples of spiked human whole blood samples with concentrations of 1, 5, 10, and 100 ng/mL. Quantitation was carried out with the LC/MS after qualitative screening with the DPiMS QT kit using the 50 ng/mL spiked sample as an unknown sample.

Table 1 7 Drug Compounds Spiked in Human Whole Blood

#	Compounds	Formula	MW
1	7-Aminonimetazepam	C16H15N3O	265.1215
2	Aconitine	C34H47NO11	645.3149
3	Brotizolam	C15H10BrClN4S	391.9498
4	Clotiazepam	C16H15ClN2OS	318.0594
5	Donepezil	C24H29NO3	379.2148
6	Fluvoxamine	C15H21F3N2O2	318.1555
7	Lidocaine	C14H22N2O	234.1732

Qualitative Screening by DPiMS QT

Screening results for blood, urine, and other biological samples can be obtained in total time of about 6 min with the DPiMS QT. After simple sample preparation (Fig. 2), the measurement time by the DPiMS QT is approximately 0.5 min. In the DPiMS QT, the sample adhering to the probe surface is ionized by applying a voltage to the probe tip while the probe is moved vertically, and the sample is then introduced directly into the mass spectrometer. Table 2 shows the analysis conditions.

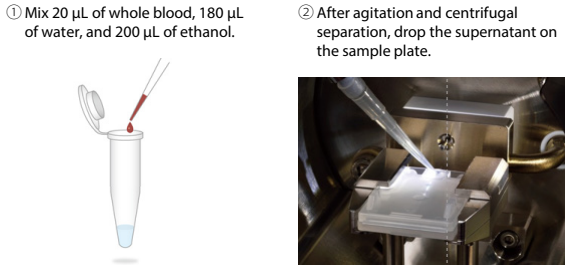


Fig. 2 DPiMS™ QT Sample Preparation

Table 2 Analysis Conditions of DPiMS QT and LCMS-9030

System	: DPiMS QT+LCMS-9030
Polarity	: Positive
DL temp	: 250 ˚C
Heat block temp	: 50 ˚C
Interface Voltage	: 3.5 kV
TOF-MS	: *m/z* 100-800
Measurement Time	: 0.5 min

Quantitative Analysis by Nexera

In the quantitative analysis with the Nexera HPLC, a Micro Volume QuEChERS kit (P/N: S225-37870-91) was used for a sample preparation. A Diazepam-d5 (2 µg/mL) solution was prepared with methanol and used as the ISTD. Fig. 3 shows the flow of sample preparation. In this experiment, 200 µL of water, 300 µL of acetonitrile, 100 µL of the biological sample, and 2 µL of the ISTD were added to the Micro Volume QuEChERS kit. After sufficient stirring, the sample was centrifuged and the supernatant was used as the analysis sample. Table 3 shows the analysis conditions.

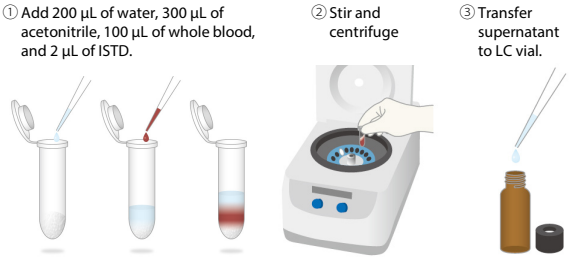


Fig. 3 Flow of Sample Preparation Using Micro Volume QuEChERS Kit

Table 3 Analysis Conditions of Nexera and LCMS-9030

System	: Nexera X3
Column	: Shim-pack Velox™ SP-C18 (100 mm × 2.1 mm I.D., 2.7 µm)
Temperature	: 40 °C
Injection volume	: 1 µL
Mobile phases	: 10 mM ammonium formate + 0.1 % formic acid in Water 10 mM ammonium formate + 0.1 % formic acid in MeOH
Flow rate	: 0.3 ml/min
Time program (%B)	: 5 % (0 min) → 95 % (7.5-10 min) → 5 % (10.01-15 min)
System	: LCMS-9030 (ESI Positive)
Nebulizing gas	: 3 L/min
Drying gas	: 10 L/min
Heating gas	: 10 L/min
DL temp	: 300 °C
Heat block temp	: 250 °C
Interface temp	: 400 °C
TOF-MS	: *m/z* 20 - 800

■ Qualitative Screening by DPiMS QT

Fig. 4 shows the TIC of the spiked human whole blood sample (50 ng/mL) obtained by DPiMS QT. Because the DPiMS QT method includes the time for sampling on the plate and the time when the probe approaches the inlet and introduces ions, the target compounds are detected intermittently.

LabSolutions™ Insight Explore was used in this analysis. The detection results for each compound can be verified by using the screening function by the molecular formula or the *m/z* of the target compounds. Here, the composition of the measured mass spectrum was estimated. Fig. 5 shows the measured mass spectrum (top) and theoretical mass spectrum (bottom) for the spiked human whole blood sample (50 ng/mL). The mass error (ppm) was calculated from the theoretical *m/z* and measured *m/z* of each drug. The mass error was -1.0 ppm to 1.9 ppm, showing stable mass accuracy in the matrix. Use of the DPiMS QT enabled simple screening of drugs in the human whole blood with a sample preparation time of about 5 min and measurement time of 0.5 min, and narrowed the range of samples and compounds requiring quantitative analysis. Following this screening, the quantitative analysis was conducted by switching to the Nexera.

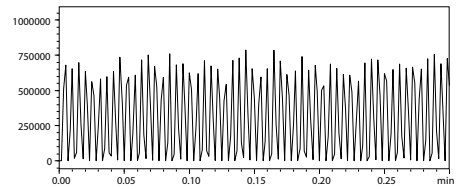
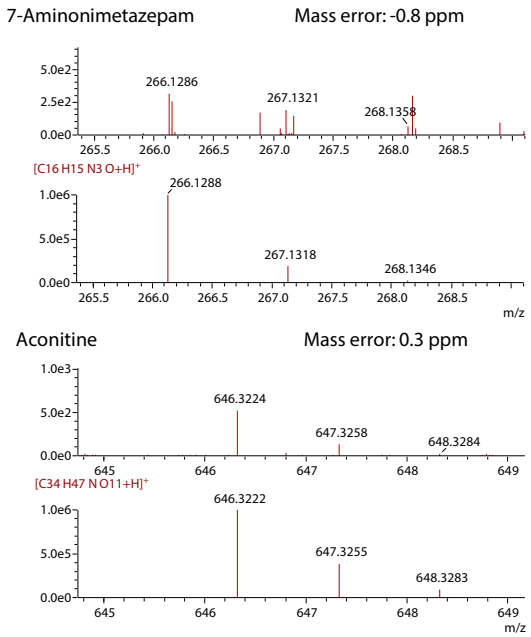


Fig. 4 TIC of Spiked Human Whole Blood Sample (50 ng/mL) Obtained by DPiMS™ QT



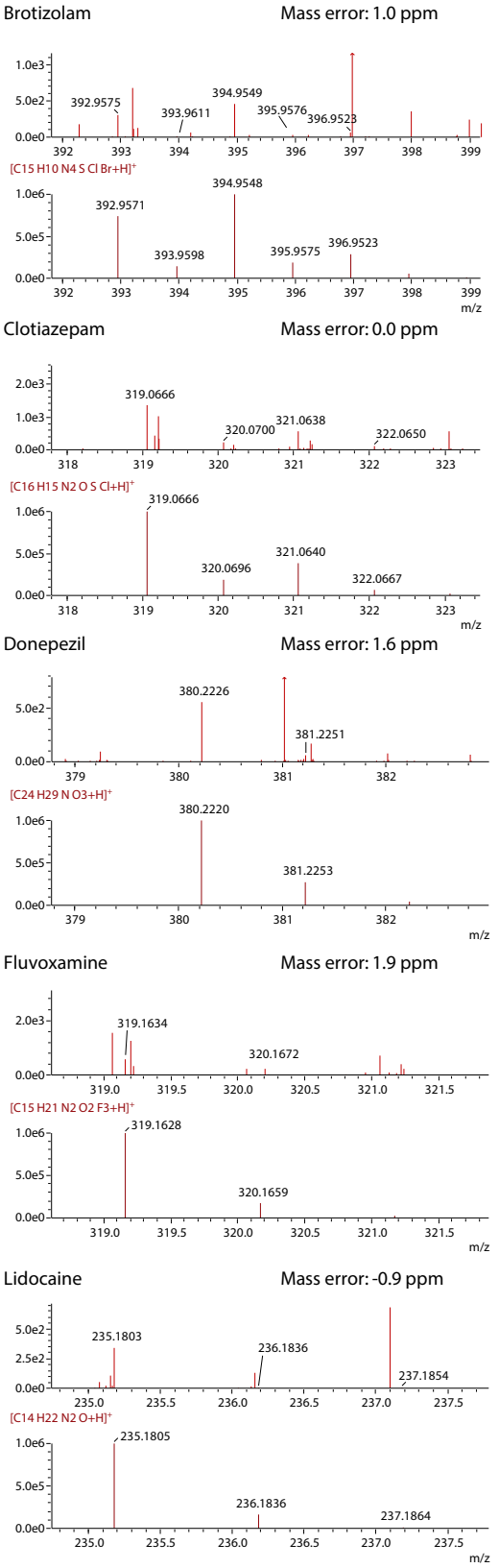
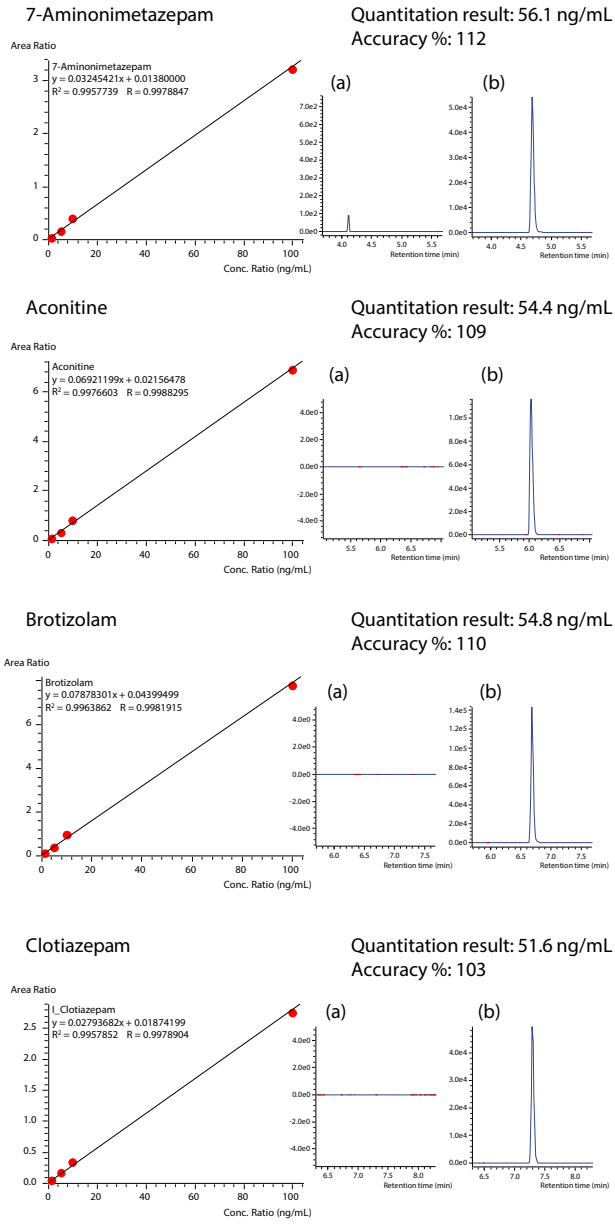


Fig. 5 (Top) Measured Mass Spectra of Spiked Human Whole Blood Sample (50 ng/mL) and (Bottom) Theoretical Mass Spectra of Drugs

■ Quantitative Analysis by Nexera

Calibration curves were prepared for each drug using human whole blood spiked with 1, 5, 10, and 100 ng/mL of the 7 drug compounds as the calibration point samples, and a quantitative analysis of a spiked human whole blood sample (50 ng/mL) was carried out using these calibration curves. Fig. 6 shows the quantitation results and the mass chromatograms of the blank and the spiked sample (50 ng/mL). Good linearity was obtained in the set concentration range. The accuracy of the quantitation results of the spiked sample (50 ng/mL) was within 100 ±15 %.



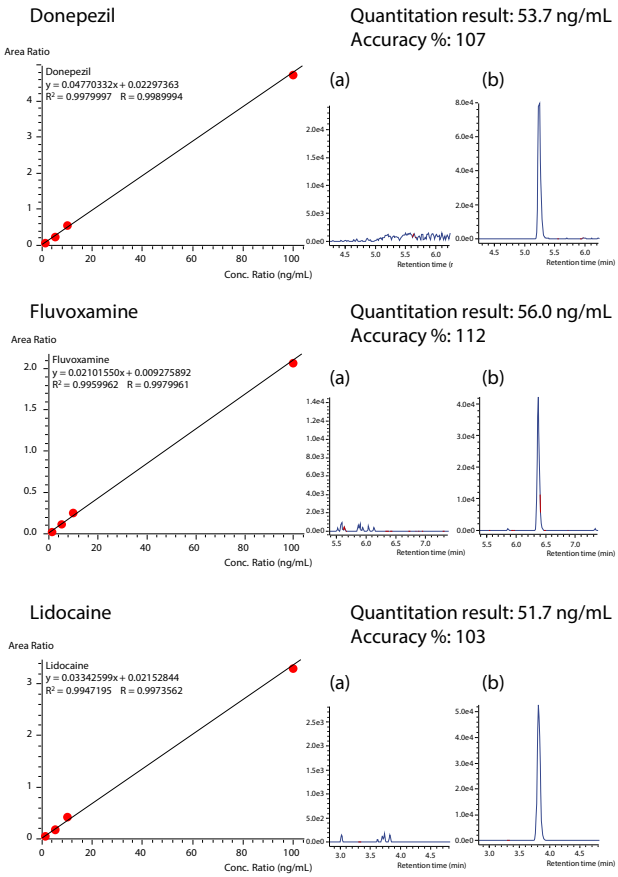


Fig. 6 Calibration Curves of Drugs Using Spiked Human Whole Blood Samples (1 to 100 ng/mL), and Quantitation Results and Mass Chromatograms of (a) Blank and (b) Spiked Human Whole Blood Sample (50 ng/mL)

■ Conclusion

High-speed qualitative screening using a LCMS-9030 and DPiMS QT and a quantitative analysis by switching to a Nexera HPLC were conducted using human whole blood samples spiked with 7 drug compounds. In the DPiMS QT measurement, all of the drugs were detected with a sample preparation time of approximately 5 min and measurement time of 0.5 min. In the quantitative analysis using the Nexera, good linearity was obtained for all compounds in the concentration range of 1 to 100 ng/mL, and satisfactory accuracy of within 100 ±15 % was obtained in the quantitation results of a spiked whole blood sample (50 ng/mL). It is possible to narrow down the candidates that require quantitation by high-speed qualitative screening using DPiMS QT, and then proceed to the quantitative analysis by simple switching to the Nexera.

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