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Technical Report

Effectiveness of Metabolomics Research Using Gas Chromatograph / Quadrupole Mass Spectrometer with High-Sensitivity and High-Speed Scanning

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Abstract:

Gas Chromatography/Mass Spectrometry (GC/MS) has been recognized as a core technology for metabolomics research for the comprehensive analysis of low-molecular weight compounds in living organisms, and is widely used for biomarker discovery and quality assessment. To fulfill the demand for a high-throughput, high-sensitivity analytical system for GC/MS metabolomics research, we developed a high-speed quadrupole mass spectrometer (Q/MS), and conducted studies to determine the effectiveness of this instrument. In an experiment using a standard sample consisting of 10 amino acids, acquisition was possible over a wide quantitation range of 3.5–4.5 orders of magnitude. In analysis of the plasma from a mouse with acute inflammation, identification and quantitation of 168 compounds were possible, and using common inflammation markers, we were able to characterize the convergence process of the acute inflammatory response, which previously was not possible from the perspective of the metabolome. With the advent of this GC-Q/MS, further development in the study of metabolomics can be expected.

Keywords: metabolomics, gas chromatography, quadrupole mass spectrometer, scan speed

1. Introduction

Due to the high stability of the gas chromatograph/mass spectrometer (GC/MS), and the high repeatability of the quantitative data that is generated, GC/MS is recognized as a core analytical technique in metabolomics research. In metabolomics studies using GC/MS, comprehensive analysis of hydrophilic low-molecular weight compounds is primarily conducted for quality evaluation and biomarker discovery.

GC/MS metabolomics studies have been frequently conducted using a gas chromatograph connected to a time-of-flight (TOF) mass spectrometer (GCTOF/MS). This provides the major advantage of high speed data acquisition obtained with a time-of flight instrument, enabling (1) high-throughput analysis, in this case, Fast GC, and (2) acquisition of abundant data consisting of more than 30 points for each peak, permitting accurate data processing using such features as peak-top discrimination and deconvolution to ensure acquisition of highly repeatable qualitative and quantitative data. For this reason, the GC-TOF/MS, with its narrow gas chromatography 1 – 3 second peak width, has become an indispensable instrument for metabolomics research. However, due to the high cost of time-of-flight instruments and their narrow quantitation range (dynamic range), there has been adequate incentive to develop a more practical analytical technique.

Here, along with the newly released Shimadzu GCMS-QP2010 Ultra quadrupole mass spectrometer capable of high-speed scan measurement, we investigated the usefulness of this instrument (below, GC-Q/MS). In this report, we verified the quantitation range of the instrument using a standard sample containing 10 types of amino acids, and also report the results of a metabolomics analysis of the blood plasma of a mouse in the state of acute inflammation.

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2. Experiment

Sample Preparation of Standard Solution of 10 Amino Acids

Ten types of amino acids were used, including asparagine, glycine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, and valine. The solutions were prepared so that the respective concentrations were 10 nM, 50 nM, 100 nM, 500 nM, 1 µM, 5 µM, 10 µM, 50 µM, 100 µM, 500 µM, 1 mM, 5 mM, 10 mM, and 50 mM. Then, three 150 µL aliquots of each of these solutions were dispensed into separate vials, and then lyophilized. In addition, 10 µL ribitol (0.2 mg/mL) was added to each sample as an internal standard. Then, 100 µL of methoxyamine (20 mg/mL, pyridine) was added to the post-lyophilized dried samples, which were then incubated for 90 minutes at 30 °C while centrifuging at 1,200 rpm. In addition 50 µL of MSTFA was added, and incubated for 30 minutes at 37 °C while centrifuging at 1,200 rpm.

Sample Preparation of Acute Inflammation-Affected Mouse Plasma

The mice were obtained from CLEA Japan. To induce inflammation, Lipopolysaccharide (LPS) derived from *Escherichia coli* O111:B4 (L2630; Sigma-Aldrich Co, St. Louis, Missouri, USA) was administered intraperitoneally into 20 mice at a dosage of 1 mg/kg body weight. One hour after administration of LPS, all of the blood was collected from 10 of the 20 mice, and 24 hours after LPS administration, all of the blood was collected from the remaining 10 mice. As a negative control, 8 mice were administered 200 µL phosphate buffered saline (PBS) intraperitoneally, and 1 hour later, all the blood was collected from the 8 mice. The collected blood was immediately treated with heparin (sodium heparin, Mochida Pharmaceutical, Japan, 5,000 units/5 mL), and after centrifuging at 3,000 × *g* at 4 °C for 10 minutes, the supernatant plasma was transferred to 1.5 mL Eppendorf tubes, then frozen in liquid nitrogen and stored at –80 °C until experimentation was conducted.

Then, in preparation for GC/MS analysis, 50 µL of plasma was transferred to a 1.5 mL Eppendorf tube, and 250 µL of a mixed solution ($MeOH/H_2O/CHCl_3$, 2.5/1/1) was added. In addition, 10 µL of 0.5 mg/mL 2- isopropylmalic acid was prepared as an internal standard, and added to the tube. Incubation was then conducted for 30 minutes at 37 °C while centrifuging at 1,200 rpm, which was followed by separation centrifuging for 3 minutes at 4 °C while centrifuging at 16,000 rpm. After transferring 225 µL of the supernatant to a newly prepared 1.5 mL Eppendorf tube, 200 µL Milli-Q water was added, and vortexing was conducted. After centrifuging at 16,000 rpm at 4 °C for 3 minutes, 125 µL of each supernatant was transferred to a newly prepared 1.5 mL Eppendorf tube. To remove any remaining methanol, centrifuge concentration was conducted for 1 hour and the sample was freeze-dried overnight.

Derivatization was conducted by adding 80 µL Methoxyamine solution (20 mg/mL, pyridine), and after thoroughly vortexing, sonication was conducted for 20 minutes. Then, incubation was conducted for 90 minutes at 37 °C while centrifuging at 1,200 rpm. Next, 40 µL MSTFA was added, and incubation was conducted for 30 minutes at 37 °C while centrifuging at 1,200 rpm. Finally, after centrifuging at 16,000 rpm at 4 °C for 3 minutes, 80 µL of supernatant was transferred to a vial for analysis, and analysis was conducted by GC/MS.

Analytical Conditions

The GC/MS analytical conditions are shown in Table 1.

Table 1 GC/MS Analytical Conditions

Instruments	
GC-MS	:GCMS-QP2010 Ultra
Autoinjector	:AOC-20i + s
Column	:CP-SIL 8 CB low bleed MS (30 m × 0.25 mm I.D. df = 0.25 µm, Agilent)
Analytical Conditions	
GC	
Injection Temp.	:230 °C
Column Temp.	:80 °C (2 min) – (15 °C/min) – 330 °C (6 min)
Injection Mode	:Split
Carrier Gas	:He (Constant Linear Velocity)
Linear Velocity	:39 cm/sec
Purge Flow	:5 mL/min
Injection Volume	:1 µL
MS	
Ion Source Temp.	:200 °C
Interface Temp.	:250 °C
Acquisition Mode	:Scan
Scan Range	:*m/z* 85 – 500
Event Time	:0.05 sec
Scan Speed	:10000 u/sec (ASSP™)

3. Results

We identified the compounds from the obtained analytical data based on the retention times and a mass spectrum database of standard compounds. In addition, we prepared a data matrix containing the compound name, retention time, quantitation ion, and peak height for each sample peak detected (standardized based on the peak height of the internal standard substance). The results of GC-Q/MS analysis of the amino acid sample mixture demonstrated the acquisition of all 10 amino acids within a wide quantitation range of 3.5–4.5 orders of magnitude. (Fig. 1)

All 168 compounds were identified from the mouse plasma analysis data. The results obtained from subjecting the acquired data matrix to principle component analysis are shown in Fig. 2. Also, Table 2 lists the 35 metabolites detected with significant differences among all of the identified compounds.

Principal component analysis indicated that the PBS processed group (control), the group treated 1 hour after LPS administration, and the group treated 24 hours after LPS could be distinguished from one another from their metabolic profile. Regarding the convergence process of the acute inflammatory response, this can be seen as an important process in the recovery from tissue inflammation, but presently, that molecular mechanism remains largely unknown. In particular, while a variety of changes have been observed in the process of convergence, such as the decrease of neutrophils and the increased influx of macrophages into various tissues such as the lungs, up to now, from a clinical standpoint, the convergence mechanism with respect to inflammation markers in blood has not been elucidated. These results suggest that the convergence process in acute late stage inflammation in mice, which previously could not be measured with frequently used inflammation markers, can be measured using blood plasma. Further, as shown in Table 2, abundant metabolite information can be acquired using this instrument. In particular, in plasma metabolome analysis using GC/MS, except for glucose, the intensity of all the metabolite peaks is low, making it difficult to conduct peak identification and quantitation. Therefore, there is a need for a high-sensitivity analytical system. The results presented here suggest that the newly released GC-Q/MS can provide high-resolution phenotypic analysis of metabolites.

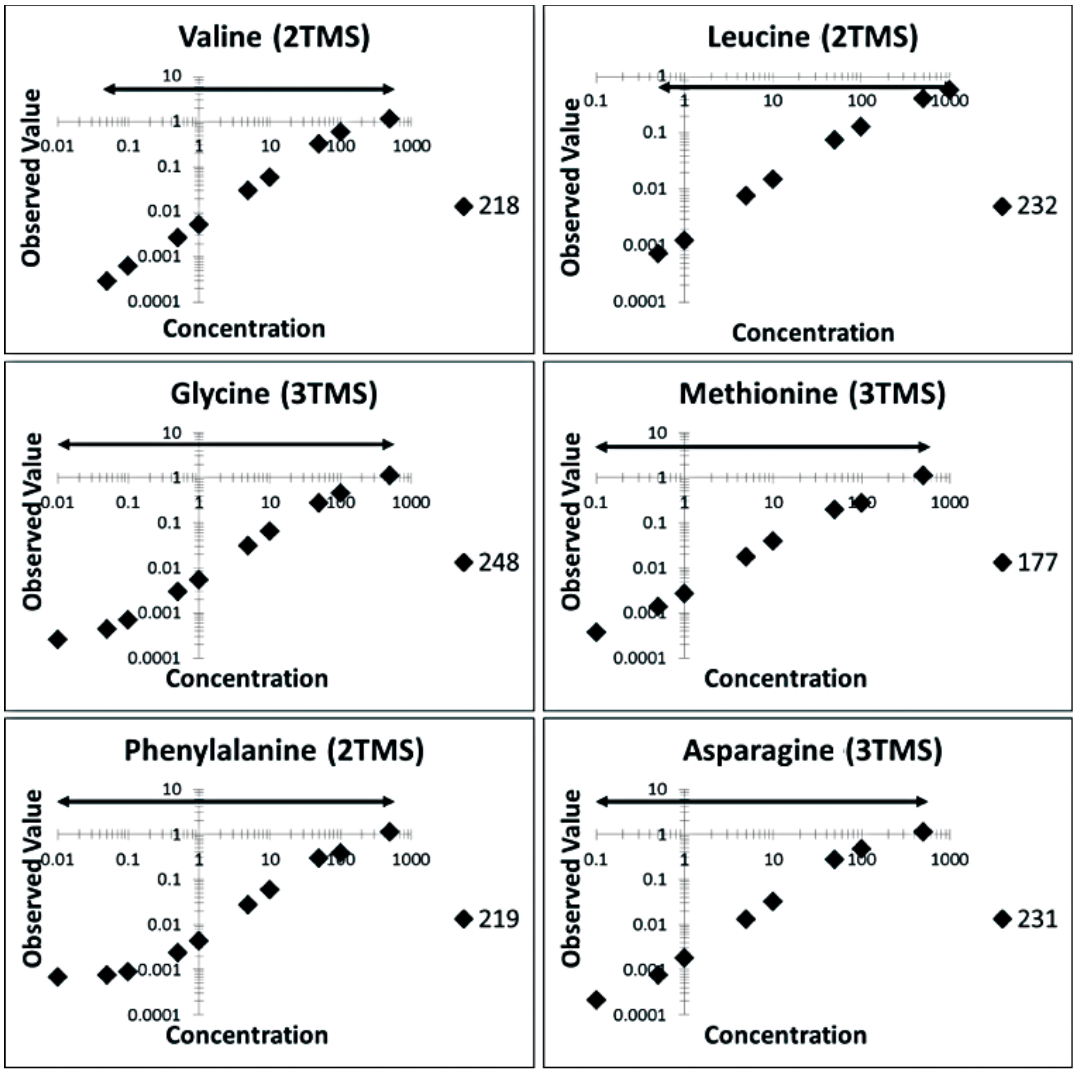

Fig. 1 Example of Quantitation Range of Analyzed Amino Acid Mixture

The Y-axis of the quantitation range in the amino acid mixture analysis data represents the relative peak intensity of the ribitol internal standard substance, and the X-axis represents the concentration submitted to analysis. The legend shows the *m/z* value used for quantitation of the compound.

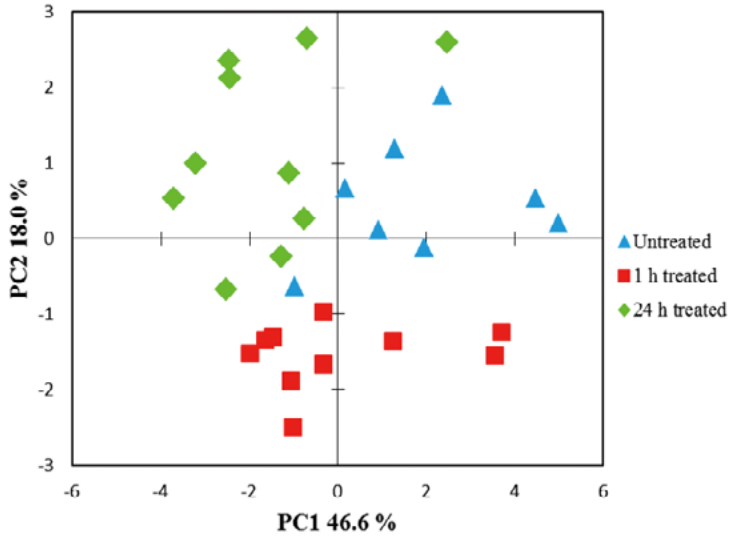

Fig. 2 Principal Component Analysis of Plasma in Mouse with Acute Inflammation

Table 2 Compounds Detected with Significant Difference Among 3 Groups

	Mean ± SD			*P* value		
Compound Name	A (n = 8)	B (n = 10)	C (n = 10)	AB	BC	CA
2-Aminoadipic acid	6.8 ± 3.1 × E-03	1.0 ± 0.2 × E-02	2.3 ± 1.2 × E-02	0.0593[w]	0.0181[w]	0.0028[w]
2-Hydroxybutyric acid	2.4 ± 0.5 × E-03	1.6 ± 0.3 × E-03	3.5 ± 0.8 × E-03	0.0027	0.0023	0.7742
3-Hydroxybutyric acid*	7.0 ± 2.3 × E-01	3.4 ± 0.8 × E-01	5.2 ± 1.0 × E-01	0.0156	0.0104[w]	0.0027[w]
Acetylsalicylic acid	2.9 ± 1.2 × E-03	2.3 ± 0.9 × E-03	4.0 ± 0.8 × E-03	0.5794	<0.0001	<0.0001
Aconitic acid	1.2 ± 0.1 × E-03	9.1 ± 1.9 × E-04	1.3 ± 0.3 × E-03	0.4878	<0.0001	<0.0001
Alanine (2TMS)*	1.8 ± 0.3 × E+00	1.2 ± 0.1 × E+00	2.1 ± 0.5 × E+00	0.0002	<0.0001[w]	0.007
Arabinose	5.2 ± 1.2 × E-03	3.5 ± 0.4 × E-03	5.4 ± 1.3 × E-03	0.0729	0.0001	<0.0001[w]
Arabitol	5.5 ± 2.1 × E-02	9.1 ± 2.3 × E-02	2.5 ± 0.9 × E-01	0.0026[w]	0.0004	0.0711[w]
Asparagne (3TMS)	2.6 ± 0.8 × E-02	1.4 ± 0.3 × E-02	2.2 ± 0.4 × E-02	0.0064	0.0052	0.6297
Behenic acid	6.6 ± 1.4 × E-04	4.9 ± 2.4 × E-04	1.1 ± 0.3 × E-03	0.0012	<0.0001	0.4514
Citrulline	4.1 ± 1.0 × E-03	3.4 ± 0.5 × E-03	4.8 ± 0.7 × E-03	0.0006	<0.0001	0.5445
Cystathionine	1.2 ± 0.2 × E-01	7.5 ± 1.7 × E-02	1.1 ± 0.1 × E-01	0.0018	0.0004	0.1968
DethioBiotin	1.1 ± 0.3 × E-03	1.7 ± 0.9 × E-03	3.6 ± 2.0 × E-03	0.0248	<0.0001	0.0083
Glycine (3TMS)	1.1 ± 0.2 × E+00	8.3 ± 1.1 × E-01	1.1 ± 0.1 × E+00	0.2472	0.0004	0.0392
Heptadecanoic acid*	1.3 ± 0.1 × E-02	1.3 ± 0.1 × E-02	1.9 ± 0.1 × E-02	0.0004	<0.0001	0.0003[w]
Homoserine	1.3 ± 0.1 × E-03	1.2 ± 0.3 × E-03	2.0 ± 0.2 × E-03	0.0013	0.0184	<0.0001
Icosanoic acid	4.5 ± 1.0 × E-03	5.5 ± 1.2 × E-03	9.8 ± 2.5 × E-03	0.0129	<0.0001	0.0384
Inositol	3.8 ± 0.8 × E-01	2.9 ± 0.4 × E-01	3.7 ± 0.6 × E-01	0.0028	0.004	0.5411
Isoleucine (2TMS)	3.1 ± 0.7 × E-01	2.0 ± 0.5 × E-01	3.3 ± 0.3 × E-01	0.0003	0.0001[w]	0.1287
Leucine (2TMS)*	9.3 ± 2.2 × E-02	5.6 ± 1.5 × E-02	9.8 ± 1.4 × E-02	0.005	0.0002[w]	<0.0001[w]
Methionine (3TMS)	1.9 ± 0.3 × E-02	1.0 ± 0.5 × E-02	2.3 ± 0.7 × E-02	0.0043	0.0043[w]	0.0794[w]
N-Acetyl-Valine	2.5 ± 0.5 × E-03	1.8 ± 0.5 × E-03	3.4 ± 0.6 × E-03	0.0177	<0.0001	0.0713
Phenylalanine (2TMS)	1.3 ± 0.1 × E-01	9.9 ± 1.8 × E-02	1.9 ± 0.3 × E-01	0.0046[w]	0.001[w]	0.7157
Prolinamide	1.8 ± 1.2 × E-03	5.6 ± 2.5 × E-03	8.5 ± 2.4 × E-03	0.0305[w]	0.0006	0.1121
Proline (2TMS)	4.2 ± 1.2 × E-02	2.9 ± 0.7 × E-02	5.4 ± 1.2 × E-02	0.0597	<0.0001	0.0027
Rhamnose	3.4 ± 1.2 × E-03	5.5 ± 1.4 × E-03	1.4 ± 0.4 × E-02	0.1749[w]	0.002	0.0006
Sarcosine*	3.2 ± 0.7 × E-03	2.1 ± 0.6 × E-03	4.6 ± 2.1 × E-03	0.003[w]	<0.0001	<0.0001
Serine (3TMS)*	2.4 ± 0.7 × E-01	1.7 ± 0.5 × E-01	2.9 ± 0.4 × E-01	0.0036	0.0002[w]	<0.0001[w]
Threonic acid	1.4 ± 0.3 × E-02	1.1 ± 0.1 × E-02	1.6 ± 0.3 × E-02	<0.0001	<0.0001	0.5716
Threonine (3TMS)	1.4 ± 0.4 × E-01	1.1 ± 0.3 × E-01	2.1 ± 0.4 × E-01	0.0085[w]	0.0003	0.3221
Thymine*	7.7 ± 3.0 × E-04	9.6 ± 2.5 × E-04	1.7 ± 0.5 × E-03	0.0011	<0.0001	0.0101
Tryptophan (3TMS)	6.8 ± 1.9 × E-02	9.6 ± 1.6 × E-02	2.2 ± 0.4 × E-01	0.1005	<0.0001	0.0005
Tyrosine (3TMS)	5.1 ± 0.8 × E-01	2.5 ± 0.5 × E-01	5.3 ± 1.0 × E-01	0.0846	0.0001	0.0854
Valine (2TMS)	6.5 ± 1.1 × E-01	4.4 ± 1.0 × E-01	7.9 ± 1.0 × E-01	0.0001	0.0002	0.2049
Xylitol	6.9 ± 1.8 × E-03	8.6 ± 2.0 × E-03	2.2 ± 0.7 × E-02	0.0855	0.0003[w]	0.0001[w]

Compounds detected with a significant difference in at least one pair among the 3 groups are listed. For cases in which a significant difference was detected among all of the three groups, an asterisk (*) is appended to the compound name. The Welch's t-test results are indicated using a W superscripted to the p-value. Correction of the p-value in multiple comparisons was conducted using the Bonferronimethod ($p < 0.017$).

4. Conclusion

Use of the newly released high-speed scanning quadrupole mass spectrometer GCMS-QP2010 Ultra (Shimadzu) permits high-throughput analysis and data point acquisition equivalent to that of a time-of-flight mass spectrometer. Further, the results of this study using a mixture of amino acids and mouse plasma demonstrate the high sensitivity and high dynamic range of this instrument, and suggest that the time-course changes in the state of acute inflammation can be measured using the metabolome. Here, by demonstrating the high sensitivity and high throughput metabolomics analysis that is possible with this extremely versatile quadrupole mass spectrometer, metabolomics studies can be expected to become more widespread using the GC-Q/MS.

References

1) Tsugawa H, Bamba T, Shinohara M, Nishiumi S, Yoshida M, Fukusaki E. Practical Non-targeted Gas Chromatography/Mass Spectrometry-based Metabolomics Platform for Metabolic Phenotype Analysis. *J Biosci Bioeng.* 2011 Sep;112 (3) :292-8.

Shimadzu GC/MS and the Quantitation of Biomolecules

Because biomolecules such as amino acids, organic acids, and fatty acids do not possess many volatile polar functional groups, they are difficult to measure directly by gas chromatography/mass spectrometry (GC/MS). It is therefore necessary to first convert these substances to volatile compounds by derivatizing these polar groups. Due to the considerable time and effort required for these tasks, this analysis tends to be avoided.

However, due to the advantages of GC/MS over other types of chromatography, such as the high resolution as compared with liquid chromatography, increased attention to GC/MS has led to its applicability for measurement of biological substances. In particular, high resolution is easily achieved with GC/MS by using a capillary column, and even if there is overlapping of contaminant and analyte peaks, appropriate selection of the fragment ion that is generated using the electron ionization (EI) method permits quantitation of the target substance without any adverse effects from the contaminant. In addition, since ion suppression, which is a problem with LC/MS/MS, is less likely to occur, this method is applicable for quantitation of samples containing large amounts of contaminants.

The Shimadzu GCMS-QP2010 Series offers excellent features and performance for bioanalysis.

1. The GCMS-QP2010 Series has the sensitivity required to permit measurement of biomolecules at very low concentrations.
2. Biological samples contain large amounts of contaminants. Measurement of such samples by GC/MS is a problem due to contamination of the ion source. The GCMS-QP2010 Series is quite resistant to contamination, but even when the ion source becomes contaminated, it is easily cleaned.
3. Batch analysis of biological samples typically involves a compli cated parameter setting procedure, however, the GCMS-QP2010 Series GC/MS metabolite database includes method files that incorporate the optimal analytical conditions and quantitation parameters.

GCMS-QP2010 Ultra

Gas Chromatograph Mass Spectrometer

GCMS-QP2010 Ultra Features

1. High sensitivity
2. Easy maintenance
3. Compound identification using retention indices

GC/MS Metabolite Database

(Amino Acids, Fatty Acids, Organic Acids)

The GC/MS Metabolite Database is a library designed for the GCMSsolution Workstation, the software package used with the GCMS-QP2010 Series gas chromatograph mass spectrometer. The mass spectrum library with retention indices significantly reduces the number of candidate compounds, thereby improving the reliability of results.

This database contains 4 types of method files including information such as analytical conditions, mass spectra, and retention indices, 4 types of libraries including compound information along with CAS numbers, mass spectra, and retention indices, as well as a handbook (published library information).

The methods and libraries contain spectra for a wide range of metabolite-related substances, including amino acids, fatty acids, and organic acids. There are 261 spectra that were obtained using the electron ionization method, and 50 spectra that were obtained using the chemical ionization method.

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