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Application News

Gas Chromatograph Mass Spectrometer GCMS-TQ™8050 NX
High Performance Liquid Chromatograph Mass Spectrometer LCMS-8060NX

GC/MS and LC/MS Analysis of Metabolites in Hepatitis Model Mouse Serum

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User Benefits

- Multi-omics Analysis Package can be used to analyze data obtained by GC/MS and LC/MS measurements.
- Multi-omics Analysis Package enables multivariate analysis, including principal component analysis.
- Acquired data can be easily visualized using a metabolic map template..

■ Introduction

Metabolomics is technology used to comprehensively measure all metabolites in living organisms. Metabolomics is used in various fields. In the medical field, it is used to search for biomarkers and determine pathogenesis. A variety of analytical instruments are used to measure metabolites. In particular, chromatographs and mass spectrometers are often used in combination because they can measure various metabolites with high sensitivity. Liquid chromatography/mass spectrometry (LC/MS) and gas chromatography/mass spectrometry (GC/MS) differ in detectable compounds, and both methods can be used to obtain complementary data.

This article describes using GC/MS and LC/MS data analysis methods with Multi-omics Analysis Package[1]. As samples, serum was measured from mice treated with a choline-deficient (CD) and a methionine- and choline-deficient (MCD) diet, known as nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH) models, respectively. By using Multi-omics Analysis Package, GC/MS and LC/MS data were integrated and visualized on a single metabolic map.

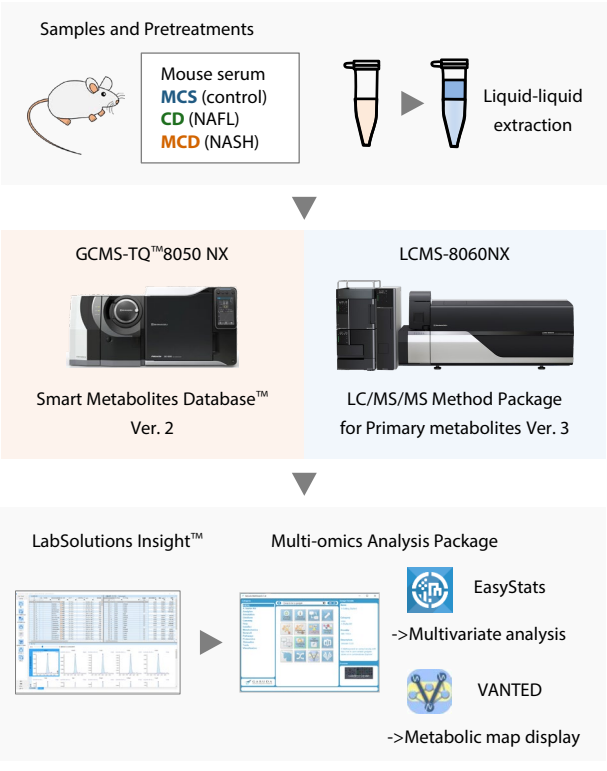

Fig. 1 Overall Workflow

■ Samples and Pretreatments

Serum samples were obtained from mice treated with a methionine- and choline-deficient (MCD, n = 6) diet, a choline-deficient (CD, n = 6) diet , and a methionine- and choline-supplemented (MCS, n = 5) diet. Mice treated with MCD diet are known as representative NASH models[2]. Serum samples were pretreated as shown in Fig. 2. 2-Isopropylmalic acid dissolved in ultrapure water was used as the internal standard. Derivatization for GC/MS was performed according to the Pretreatment Procedure Handbook for Metabolites Analysis[3].

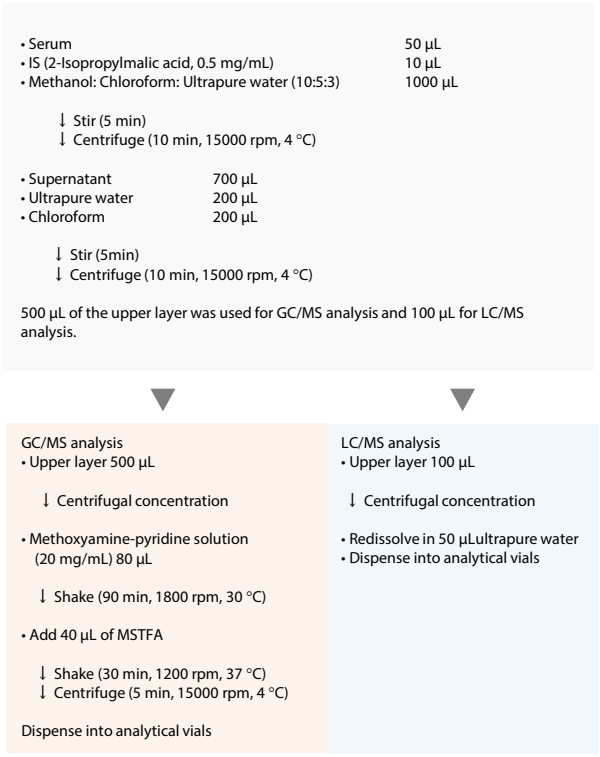

Fig. 2 Pretreatment Process Flows

■ Analytical Conditions

"Smart Metabolites Database Ver. 2" was used for GC/MS analysis. "LC/MS/MS Method Package for Primary Metabolites Ver. 3" was used for LC/MS analysis, with MRM event added for the 2-isopropylmalic acid added as an internal standard. The analysis conditions for GC/MS and LC/MS are shown in Table 1.

Table 1 Analytical Conditions

GC-MS:	**GCMS-TQ8050 NX**
Autoinjector:	**AOC20i Plus / 20s Plus**
GC	
Column:	BPX-5 (30 m, 0.25 mm I.D., 0.25 µm) P/N: 054101
Injection Temp.:	250 °C
Column Oven:	60 °C (2 min)→ 15 °C/min → 330 °C (3 min)
Injection Mode:	Split
Split Ratio:	30
Carrier Gas:	He
Carrier Gas Control:	Linear Velocity (39.0 cm/sec)
Injection Volume:	2 µL
MS	
Mode:	MRM
Ion Source Temp.:	200 °C
Interface Temp.:	280 °C
HPLC:	**Nexera™ X3**
Column:	Shim-pack™ GIST PFPP (2.1 mm I.D. x 150 mm, 3 µm) P/N: 227-30858-07
Column Oven:	40 °C
Solvent A:	0.1% Formic acid in water
Solvent B:	0.1% Formic acid in acetonitrile
Mode:	Gradient elution
Flowrate:	0.25 mL/min
Injection Volume:	1 µL
MS:	**LCMS-8060NX**
Ionization:	ESI positive/ negative (IonFocus™)
Mode:	MRM
Nebulizing Gas:	3.0 L/min
Drying Gas:	10.0 L/min
Heating Gas:	10.0 L/min
DL Temp.:	250 °C
Heat Block Temp.:	400 °C
Interface Temp.:	270 °C

■ Data Analysis

Data obtained by GC/MS and LC/MS were processed using LabSolutions Insight. LabSolutions Insight enables efficient waveform processing for GC/MS and LC/MS in a similar manner. GC/MS and LC/MS data were each corrected with internal standards and the resulting area ratios were used for subsequent analyses.

The corrected area values were further analyzed using the "Multi-omics Analysis Package." This software can analyze a large amount of data obtained by mass spectrometry using various methods, such as multivariate analysis, graphing volcano plots, and displaying metabolic maps. In addition, it includes visualization templates for various GC/MS and LC/MS method packages, allowing smooth visualization of the obtained data.

In this analysis, a metabolic map that integrates GC/MS and LC/MS was used, but metabolic maps were also provided for "Smart Metabolites Database Ver. 2" and "LC/MS/MS Method Package for Primary Metabolites Ver. 3" so that a metabolic map can be selected based on objectives (Fig. 3).

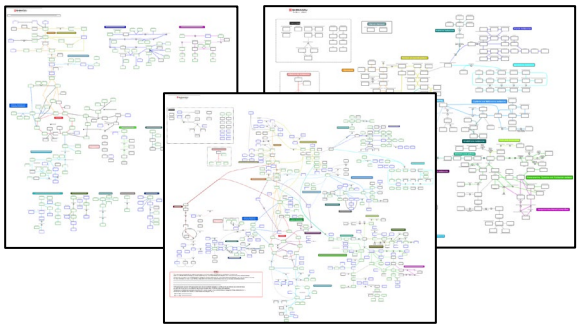

Fig. 3 Metabolic Map Templates
(From left to right: Metabolic maps for Smart Metabolites Database, integrated GC/MS and LC/MS analysis, and LC/MS/MS Method Package for Primary Metabolites)

■ Metabolites Detected

As shown in Fig. 4, a total of 122 compounds were detected using GC/MS and LC/MS. GC/MS detected 93 components, mainly amino acids, organic acids, and sugars. LC/MS detected 58 components, mainly amino acids, organic acids, and nucleobases. Twenty-nine compounds, including amino acids and organic acids, were detected by both methods. For metabolites detected by both GC/MS and LC/MS, the LC/MS data, which offered higher sensitivity, were used in subsequent data analysis.

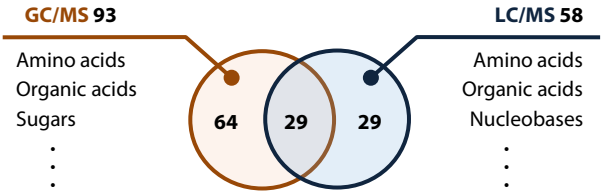

Fig. 4 Metabolites Detected by GC/MS and LC/MS

■ Principal Component Analysis Results

Principal component analysis was performed using GC/MS and LC/MS data (Fig. 5). Three groups were separated on the score plot. The MCD group was separated on the first principal component axis, whereas the CD group was separated on the second principal component axis.

Loading plots showed that small amino acids, such as glycine, alanine, serine and 2-aminobutyric acid, were more abundant in the MCD group. On the other hand, glucose and related metabolites were found to be lower in the MCD group. Sulfur-related metabolites, such as methionine and methionine sulfoxide, were also found to be more abundant in the CD group.

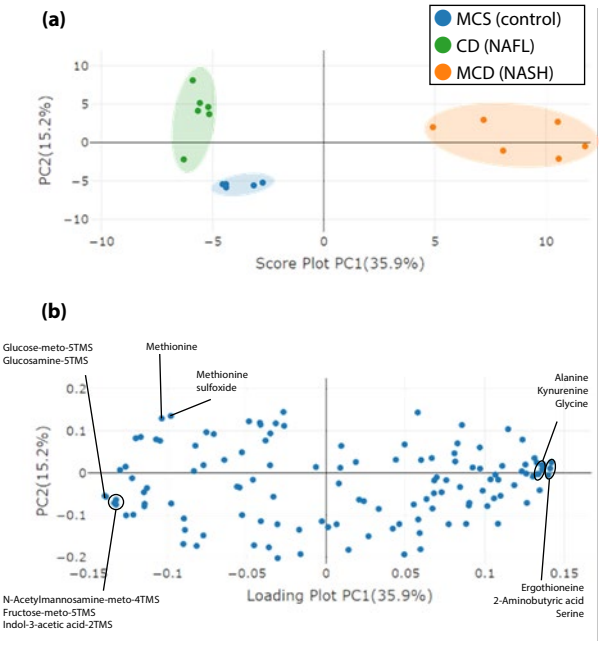

Fig. 5 Principal Component Analysis Using EasyStats
(a) Score plot, (b) Loading plot

■ Metabolic Map Display

The obtained data were visualized on a metabolic map using VANTED (Fig. 6). The names of metabolites identified by GC/MS, LC/MS, and both methods are indicated in blue, black, and green, respectively. This metabolic map includes key metabolic pathways, including glycolysis, amino acid metabolism, the TCA cycle, and the urea cycle.

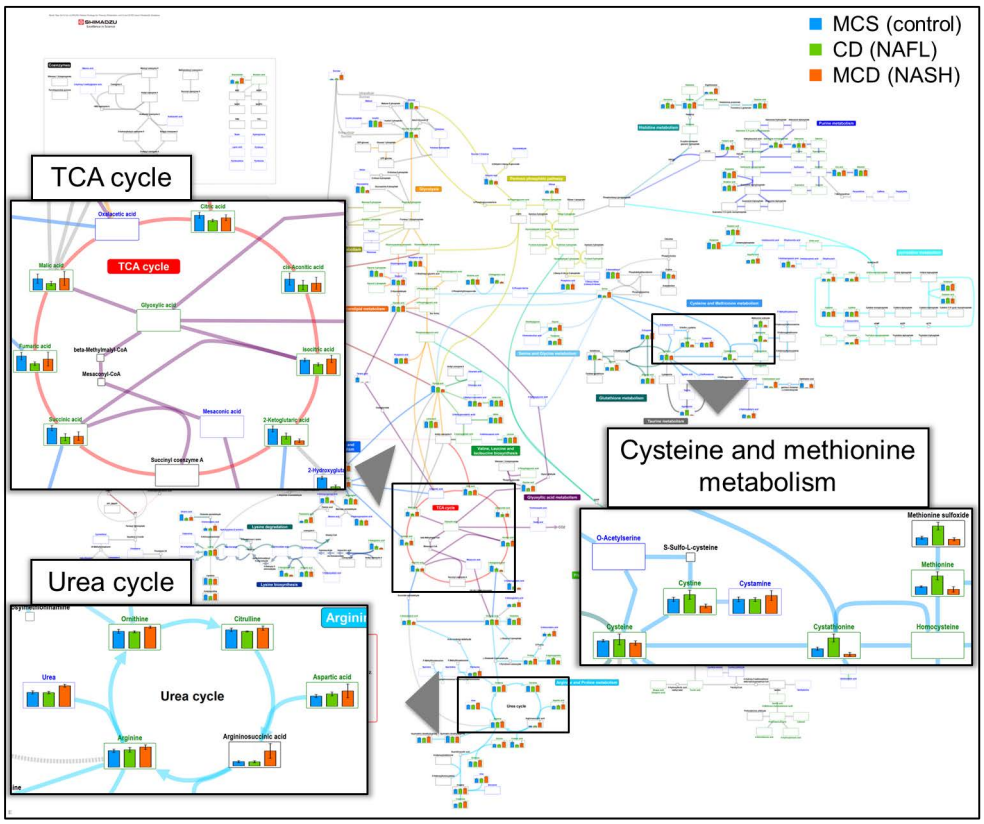

Fig. 6 Metabolic Map Display using VANTED (Overall)

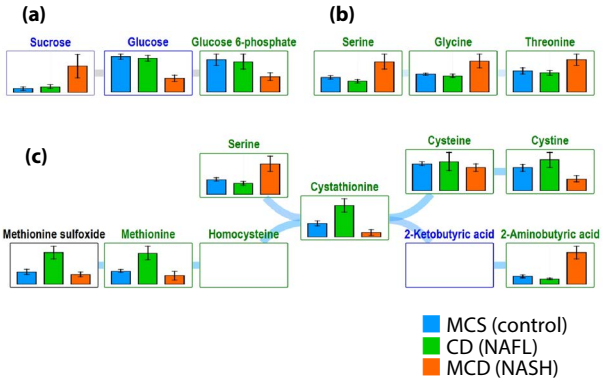

Fig. 7 Metabolic Map Display using VANTED (Excerpt)
(a) Sugar-related metabolites, (b) amino acids, and (c) sulfur-related metabolites

Some of the metabolites that changed among the three groups are shown in Fig. 7, which shows how metabolic maps can be edited to suit given objectives.

As in the previous study[4], glucose and other sugars decreased in the MCD group. Amino acids such as serine, glycine, and threonine increased in the MCD group. Sulfur-related metabolites also increased in the CD group.

■ Conclusion

Analysis using GC/MS and LC/MS detected 122 compounds in mouse serum. Area value data from both methods were integrated and visualized on a single metabolic map. The metabolic map display showed that sugars, amino acids, and sulfur-related metabolites were altered in the CD and MCD groups.

Using a metabolic map template makes it easy to visualize the obtained data. The Multi-omics Analysis Package provides strong support for interpreting metabolomic data results.

■ Acknowledgment

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