SHIMADZU
Excellence in Science

Application News

High Performance Liquid Chromatograph Mass Spectrometer LCMS-2050

Simultaneous Analysis of Hydrophilic Metabolites in Feces Using a Single Quadrupole Mass Spectrometer

Takanari Hattori and Yasuko Shibayama

User Benefits

- The single quadrupole LC-MS system allows for simple, simultaneous analysis of hydrophilic metabolites in feces.
- The Multi-omics Analysis Package offers a simple tool for data analysis including multivariate analysis, and it allows users to find characteristic compounds with ease.

■ Introduction

Human intestines are home to around 1,000 different species of approximately 100 trillion intestinal bacteria. It is becoming increasingly clear that these intestinal bacteria help maintain and improve the health of their host, and research into intestinal microbiota has been attracting increasing interest. In the medical domain, the relationship between intestinal microbiota and diseases, such as colorectal cancer, and the effects of intestinal microbiota on drug efficacy and the immune system are being investigated. In the food domain, the beneficial effects of lactic acid bacteria found in functionally enhanced foods are also being investigated. Since short-chain fatty acids and other metabolites produced by intestinal bacteria affect the host, analysis of these metabolites is important for the research of intestinal bacteria.

This Application News describes simultaneous analysis of hydrophilic metabolites in feces using a single quadrupole LC-MS. Application News No. 01-00601-EN describes a method for the analysis of short-chain fatty acids and organic acids, and combining that method with the method described here offers an approach for a comprehensive analysis of metabolites in feces.

■ Samples

Table 1 provides details about the monkey feces analyzed in this article, and Fig. 1 shows a schematic map of where the feces were collected. Feces were collected from locations A, B, and C from five monkeys. The feces were frozen at the collection site and stored at -80 °C until analysis. For further details on the collection of the samples, please see the research by Tsuchida et al.[1)]

Table 1 Details of Samples

Sample Name	Collection Point	Monkey	Time to Freezing
S1	Location A	Unknown	Between half a day and 1 day
S2	Location B	4-year-old male	Immediately after defecation
S3	Location B	2-year-old male	Immediately after defecation
S4	Location C	Adult female	Immediately after defecation
S5	Location C	Adult female	Immediately after defecation

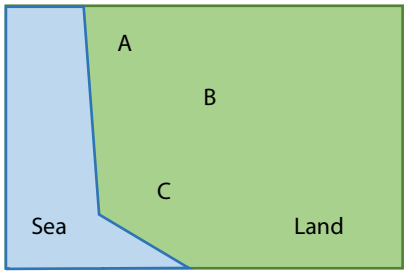

Fig. 1 Schematic Map of Collection Points

■ Sample Pretreatment

The sample pretreatment procedure is shown in Fig. 2. 700 µL of phosphate-buffered saline (PBS) was added to 100 mg of feces. The mixture was mixed and cooled on ice. After centrifugation, the supernatant was filtrated by ultrafiltration. The filtrate was then diluted 10-fold with ultrapure water containing internal standard (2-morpholinoethanesulfonic acid, MES). The final concentration of MES was 10 µmol/L.

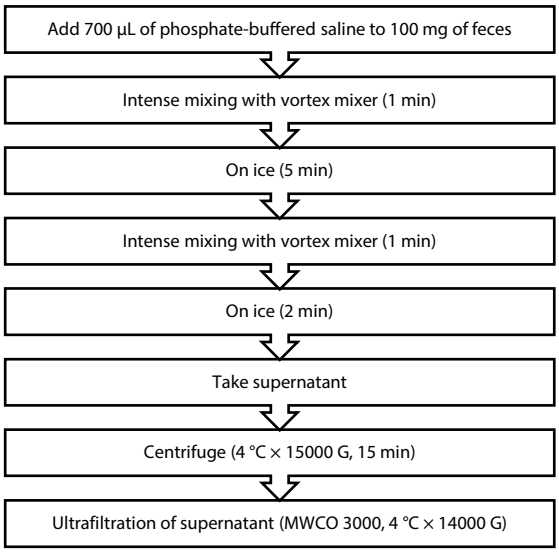

Fig. 2 Sample Pretreatment of Feces

■ Instruments and Analytical Conditions

As shown in Fig. 3, analysis was performed on the system, which combined the Nexera™ series and the LCMS-2050. The LCMS-2050 single quadrupole mass spectrometer is compact, but it affords excellent ease of use and performance. The LCMS-2050 is equipped with a heated DUIS™ dual ion source, which combines the benefits of electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI), and it covers a mass range of *m/z* 2 to 2000. These features make the system ideal for the simultaneous analysis of metabolites with a wide range of physical properties.

Fig. 3 Nexera™, LCMS-2050 System

Table 2 shows the analytical conditions of HPLC and MS. The analytical method was constructed for the single quadrupole LC-MS from the LC/MS/MS Method Package for the Primary Metabolites Ver. 3. In the analytical method, targeted compounds were 143 hydrophilic metabolites, including amino acids, organic acids, nucleosides, and nucleotides.

Table 2 Analytical Conditions

HPLC Conditions (Nexera XR)	
Column:	Shim-pack™ GIST PFPP*
	(2.1 mm I.D. × 150 mm L., 3.0 µm)
Mobile Phases:	A) 0.1 % Formic acid in water
	B) 0.1 % Formic acid in acetonitrile
Mode:	Gradient elution
Flow Rate:	0.25 mL/min (17–19 min, 0.5 mL/min)
Injection Volume:	3 µL
MS Conditions (LCMS-2050)	
Ionization:	ESI/APCI (DUIS), Positive and Negative mode
Mode:	SIM (143 events)
Nebulizing Gas Flow:	2.5 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
Desolvation Temp.:	500 °C
DL Temp.:	250 °C

* P/N: 227-30858-07

■ Results of Analysis

As a result of simultaneous analysis of hydrophilic metabolites, 66 compounds were detected. The main metabolites were amino acids, organic acids, and nucleoside metabolites. The number of metabolites detected in each sample is shown in Table 3.

Table 3 Number of Detected Compounds

S1	S2	S3	S4	S5
44	52	52	55	48

The Multi-omics Analysis Package was used to perform principal component analysis, based on the peak area ratio of each compound to the internal standard. The results of this analysis are shown in Fig. 4. From the score plot (Fig. 4 top), it was found that the samples were grouped by their location (monkey troop). The loading plot (Fig. 4 bottom) showed that 4-aminobutyric acid (GABA), carnitine, and choline were characteristic of S1; purine-related compounds, such as xanthine and hypoxanthine, were characteristic of S2 and S3; and organic acids, such as citric acid and caffeic acid, were characteristic of S4 and S5. The differences in metabolites were estimated from the differences in foods eaten before fecal collection.

■ Conclusion

This Application News described a simultaneous analysis of hydrophilic metabolites in feces using a single quadrupole LC-MS system. Although widely targeted metabolomics is typically performed using a triple quadrupole LC-MS system, this study demonstrated a single quadrupole LC-MS was useful for widely targeted metabolomics of feces. The single quadrupole LC-MS is inexpensive and simpler to operate than triple quadrupole LC-MS. So, it is suitable for users with less experience in mass spectrometry. The spread of simultaneous analysis of hydrophilic metabolites using a single quadrupole LC-MS system is expected to further advance research on intestinal bacteria.

We would like to express our sincere gratitude to Professor Kazunari Ushida at the College of Bioscience and Biotechnology, Chubu University, for providing the monkey feces samples and for his guidance in the preparation of this Application News.

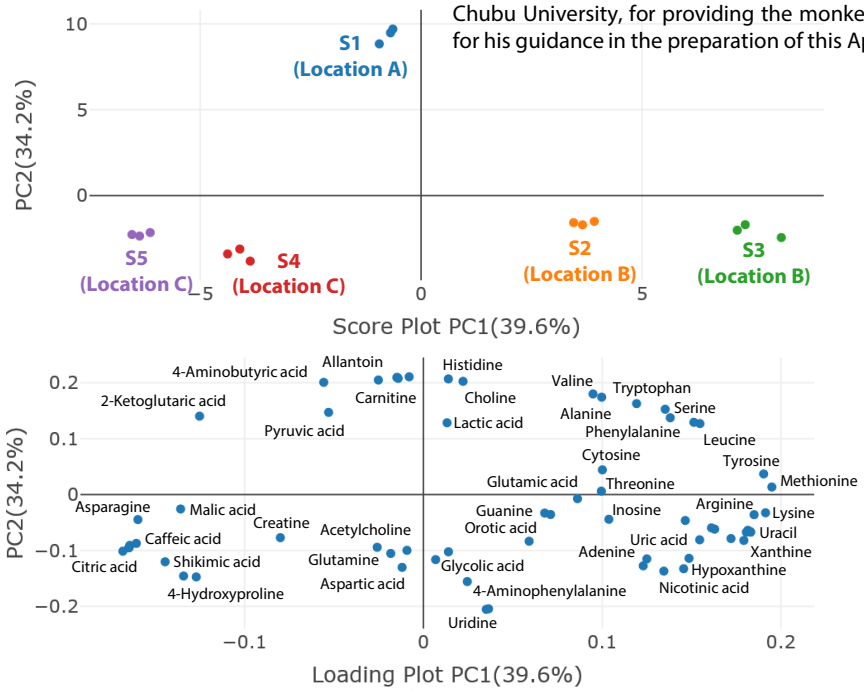

Fig. 4 Results of Principal Component Analysis

< References >

1) S. Tsuchida, T. Hattori, A. Sawada, K. Ogata, J. Watanabe, K. Ushida: Fecal metabolite analysis of Japanese macaques in Yakushima by LC-MS/MS and LC-QTOF-MS, J Vet Med Sci., 83 (6) 1012–1015 (2021).

< Related Application News Articles >

1. Improvement of Productivity in Research on Intestinal Microbiota by Shim-pack™ Fast-OA High-Speed Organic Acid Analysis Column Application News No. eL555
2. Analysis of Short-Chain Fatty Acids and Organic Acids in Feces Using a Single Quadrupole Mass Spectrometer Application News No. 01-00601-EN

01-00600-EN First Edition: Nov. 2023

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/