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High Performance Liquid Chromatograph Mass Spectrometer LCMS-2050

Application News

Analysis of Short-Chain Fatty Acids and Organic Acids in Feces Using a Single Quadrupole Mass Spectrometer

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User Benefits

- The single quadrupole LC-MS system allows for sensitive analysis of short-chain fatty acids and organic acids 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 the analysis of short-chain fatty acids and organic acids in feces using a single quadrupole LC-MS. Application News No. 01-00600-EN describes a method for the simultaneous analysis of hydrophilic metabolites including amino acids, organic acids, and nucleoside metabolites, and combining that method with the method described here offers 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

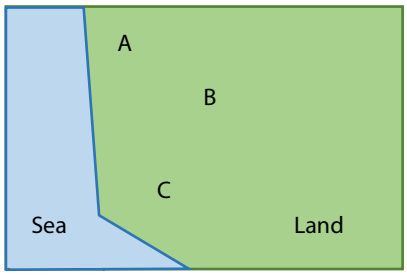

Fig. 1 Schematic Map of Collection Points

■ Sample Pretreatment

After extracting compounds from feces by the procedure shown in Fig. 2, short-chain fatty acids and organic acids were derivatized to improve the retention of analytes in C18 column and the sensitivity of MS detection. 3-nitrophenylhydrazine (derivatizing reagent), pyridine (catalyst), and 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (condensing agent) were used for the derivatization. These reagents were added to the extracts containing 2-ethylbutyric acid (internal standard) and reacted for 30 min at room temperature.

The reacted extract was then diluted five-fold with an aqueous methanol solution containing formic acid.

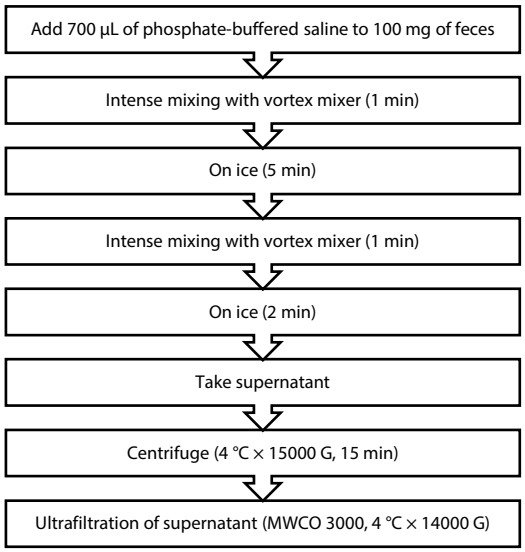

Fig. 2 Sample Pretreatment of Feces (Extraction)

■ 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 wide mass range of *m/z* 2 to 2000. These features also make the system ideal for the simultaneous analysis of metabolites other than short-chain fatty acids and organic acids.

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 system from the LC/MS/MS Method Package for Short Chain Fatty Acids. In the analytical method, targeted compounds were 6 short-chain fatty acids with a carbon number between two and five, such as acetic acid, propionic acid, and butyric acid, and 16 organic acids associated with central metabolic pathways, such as lactic acid, pyruvic acid, and succinic acid. These compounds are considered key compounds for research into intestinal bacteria.

Table 2 Analytical Conditions

HPLC Conditions (Nexera XR)	
Column:	Mastro 2 C18 (2.0 mm I.D. × 150 mm L., 3.0 µm)
Mobile Phases:	A) 0.1 % Formic acid in water B) Acetonitrile
Mode:	Gradient elution
Flow Rate:	0.35 mL/min
Injection Volume:	3 µL
MS Conditions (LCMS-2050)	
Ionization:	ESI/APCI (DUIS), Positive and Negative mode
Mode:	SIM (23 events)
Nebulizing Gas Flow:	2.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	5.0 L/min
Desolvation Temp.:	500 °C
DL Temp.:	200 °C

■ Results of Analysis

The mixed extract of S1-S5 was derivatized and the solution was analyzed six times to confirm the reproducibility of the method.

As shown in Table 3, 20 short-chain fatty acids and organic acids were detected and results with good reproducibility were obtained.

Table 3 Reproducibility

Compound	%RSD, n=6	
	Retention time	Peak area
Lactic acid	0.084	0.8
beta-Hydroxybutyric acid	0.139	1.4
Acetic acid	0.074	0.6
Propionic acid	0.101	0.3
Isobutyric acid	0.053	0.9
Butyric acid	0.058	1.0
2-Hydroxyglutaric acid	0.029	4.8
Succinic acid	0.036	2.4
Isovaleric acid	0.024	0.8
Fumaric acid	0.030	2.8
Valeric acid	0.021	1.4
Maleic acid	0.012	2.4
2-Ethylbutyric acid	0.013	1.4
Glyoxylic acid	N.D.	N.D.
Pyruvic acid	0.006	1.9
2-Oxobutyric acid	N.D.	N.D.
alpha-Ketoglutaric acid	0.015	4.5
Glycolic acid	0.122	1.5
Malic acid	0.046	0.9
Malonic acid	0.020	2.8
Isocitric acid	N.D.	N.D.
Citric acid	0.012	1.9
Oxaloacetic acid	0.008	3.7

As a result of the five monkey feces (S1-S5), 21 short-chain fatty acids and organic acids were detected. Table 4 shows the detected compounds. The compounds marked with "+ (LC-MS)" were not detected when the same samples were analyzed by LC (Application News No. eL555). The LC-MS was able to detect compounds that were not detected due to the low sensitivity of LC. Fig. 4 shows mass chromatograms of the S3 sample. Acetic acid, propionic acid, and butyric acid are considered key compounds for research into intestinal bacteria and were all detected with good sensitivity. This LC-MS method could analyze isomers, such as isobutyric acid/butyric acid and isovaleric acid/valeric acid, due to sufficient separation by LC.

Table 4 Detected Compounds

Compound	S1	S2	S3	S4	S5
Lactic acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
beta-Hydroxybutyric acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
Acetic acid	+	+	+	+	+
Propionic acid	+ (LC-MS)	+	+	+	+
Isobutyric acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
Butyric acid	+ (LC-MS)	+	+	+	+
2-Hydroxyglutaric acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
Succinic acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
Isovaleric acid	+ (LC-MS)	+	+	+ (LC-MS)	+ (LC-MS)
Fumaric acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
Valeric acid	+ (LC-MS)	+	+	+	+
Maleic acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
Glyoxylic acid	N.D.	N.D.	N.D.	N.D.	N.D.
Pyruvic acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
2-Oxobutyric acid	+ (LC-MS)	N.D.	N.D.	N.D.	N.D.
alpha-Ketoglutaric acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
Glycolic acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
Malic acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
Malonic acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
Isocitric acid	N.D.	N.D.	N.D.	N.D.	+ (LC-MS)
Citric acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)
Oxaloacetic acid	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)	+ (LC-MS)

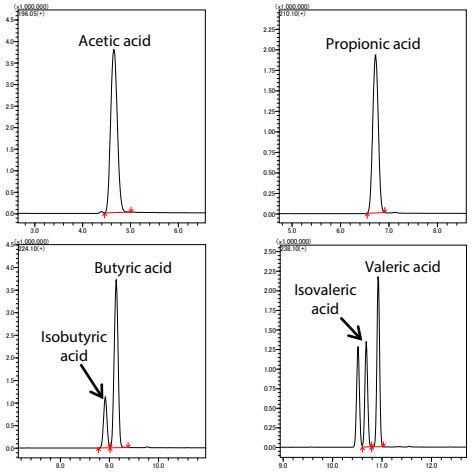

Fig. 4 Mass Chromatograms (S3)

The peak area ratio of each compound to the internal standard was used to perform principal components analysis and hierarchical clustering analysis with the Multi-omics Analysis Package. The results of these analyses are shown in Figs. 5 and 6. S1 was significantly different from other monkey feces, with higher amounts of pyruvic acid, lactic acid, 2-oxobutyric acid, and α-ketoglutaric acid and lower amounts of acetic acid, propionic acid, and butyric acid. S2 and S3, which were collected in the same location (the same monkey troop), showed similar trends, perhaps due to the same foods. S4 and S5 were collected in the same location, but the trend was different on the second principal component (PC2) axis. S5 contained a large amount of organic acids such as citric acid and isocitric acid. The differences in metabolites were estimated from the differences in foods eaten before fecal collection or the differences in intestinal microbiota.

■ Conclusion

This Application News described an analysis of short-chain fatty acids and organic acids in feces using a single quadrupole LC-MS system. Although these compounds in feces can be analyzed by LC, LC-MS enables sensitive analysis. Using an LC-MS, low concentrations of short-chain fatty acids and organic acids in feces were detected. The single quadrupole LC-MS is inexpensive and simple to operate. So, it is suitable for users with less experience in mass spectrometry. The spread of analysis of short-chain fatty acids and organic acids in feces 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.

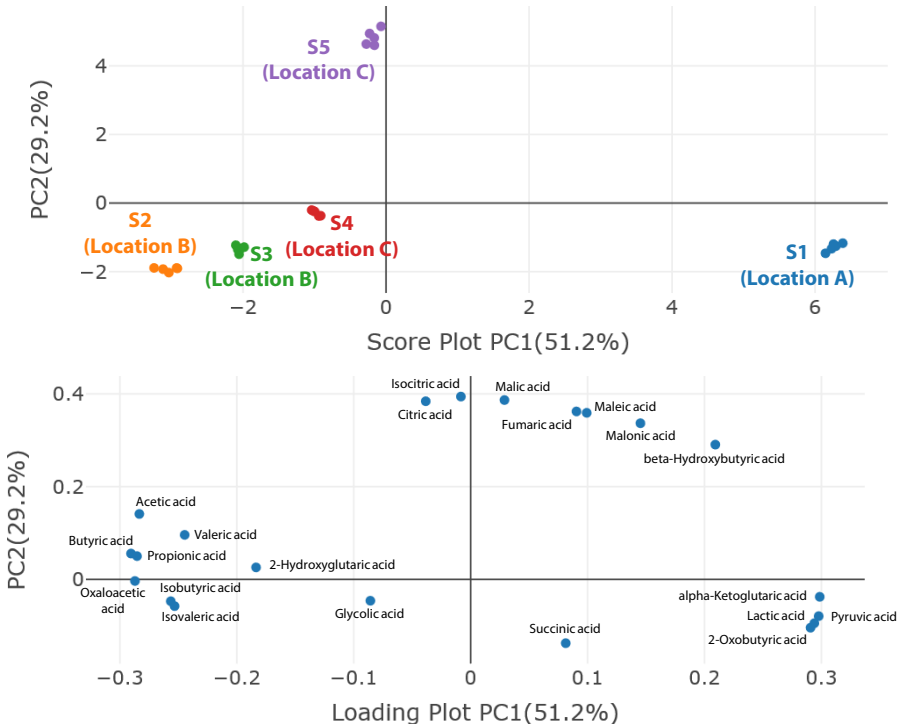

Fig. 5 Results of Principal Component Analysis

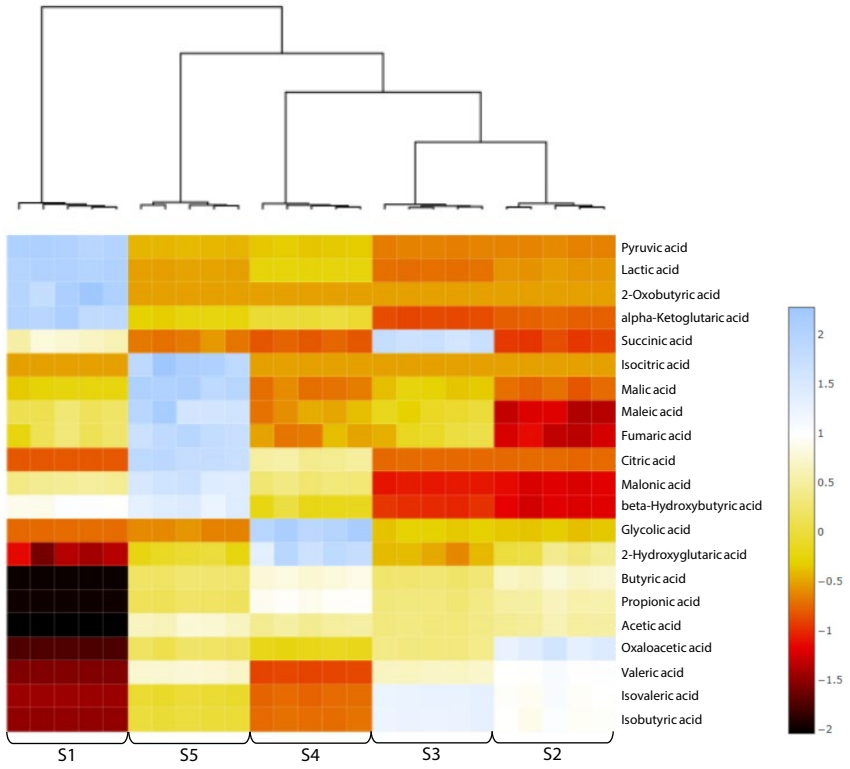

Fig. 6 Results of Hierarchical Clustering Analysis

< 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).

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