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Application News

No. L516

High Performance Liquid Chromatography

Applying an Organic Acid Analysis System to Intestinal Flora Research

In recent years, it is becoming clear that intestinal flora in the gut contribute to the maintenance and preservation of the health of their hosts. Therefore, when examining the effects of intestinal flora on the host, the metabolites produced by intestinal flora can be considered to be related to these benefits. [*1]

While LC-MS may be used for analysis due to the large number of substances present in these metabolites, formic acid and acetic acid cannot be detected since LC-MS employs these organic acids in the mobile phase.

In contrast, the post-column pH buffered electric conductivity detection method, which combines the ion exclusion mode and post-column reaction detection method performed using HPLC, employs neither formic acid nor acetic acid in the mobile phase, and this enables both highly sensitive and selective detection of organic acids including formic acid and acetic acid.

This article introduces an example of extracting metabolites produced by intestinal flora from mouse feces and measuring the short-chain fatty acids contained in the metabolites using an organic acid analysis system.

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Analysis of Standard Sample Solution

Table 1 lists the analysis conditions. Fig. 1 shows the result of injecting 10 µL of standard solution (20 mmol/L each).

Table 1 Analysis Conditions

Column	:	Shim-pack SCR-102H (300 mmL. × 8.0 mm I.D.) × 2
Guard column	:	Guard column SCR-102H (50 mmL. × 6.0 mm I.D.)
Mobile phase	:	5 mmol/L p-toluenesulfonic acid
Flow rate of mobile phase	:	0.8 mL/min
pH buffering solution	:	5 mmol/L p-toluenesulfonic acid 20 mmol/L Bis- Tris 0. 1 mmol/L EDTA
Flow rate of pH buffering solution	:	0.8 mL/min
Column temp.	:	45 °C
Detection	:	Electro conductivity detector (CDD-10Avp)
Injection vol.	:	10 µL

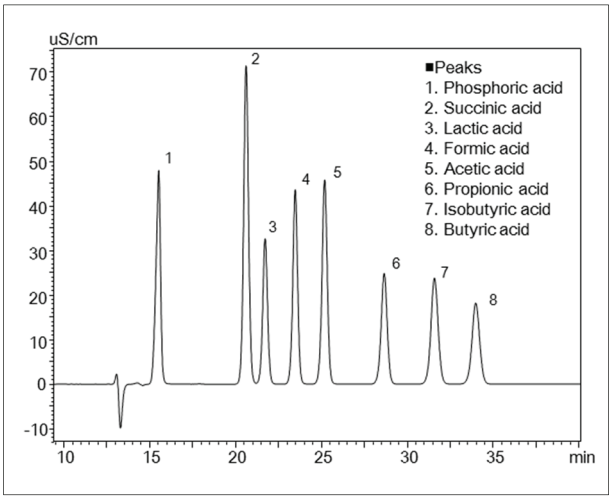

Fig. 1 Chromatogram of Standard Solution

Calibration Curve

Fig. 2 shows the calibration curve of propionic acid created through analysis with the conditions in Table 1. The calibration curve was created over the ranges of 0.1 to 2.0 mmol/L and 1.0 to 20 mmol/L. Favorable linearity was obtained with a coefficient of determination (R^2) of 0.999 or higher for each component.

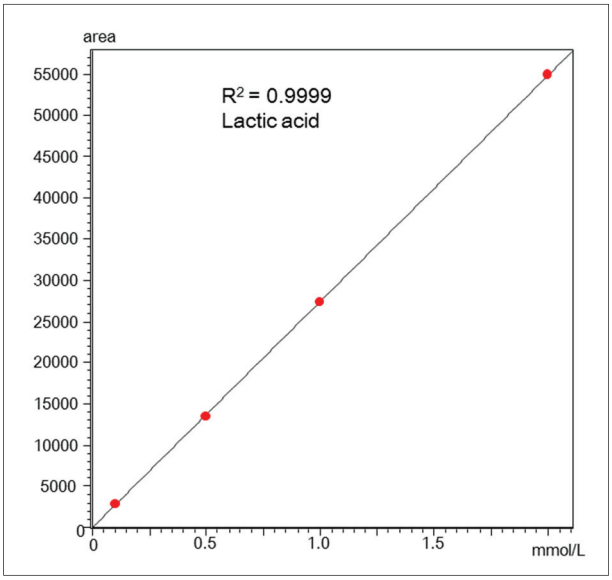

Fig. 2 Linearity of Calibration Curve

Pretreatment

Fig. 3 shows a flowchart of the pretreatment process used to extract metabolites from the mouse feces. *1

Feces were collected from a C57BL/6J mouse bred under standard conditions. 50 mg of fresh feces were added to 450 µL of phosphate buffered saline and agitated. The supernatant from the agitated mixture was then separated by centrifugation and ultrafiltrated to obtain the measurement sample. Note

Analysis of Mouse Fecal Extract

Fig. 4 shows the result of injecting 10 µL of mouse fecal extract. Seven components were detected that include acetic acid and formic acid, which are difficult to detect using LC-MS.

Table 2 lists the quantitative result for each component.

Table 2 Quantitative Results

No.	Compounds	:	Quantitative Value (mmol/L)
1	Phosphoric acid	:	7.88
2	Succinic acid	:	0.07
3	Lactic acid	:	0.26
4	Formic acid	:	0.13
5	Acetic acid	:	2.69
6	Propionic acid	:	0.59
8	Butyric acid	:	0.61

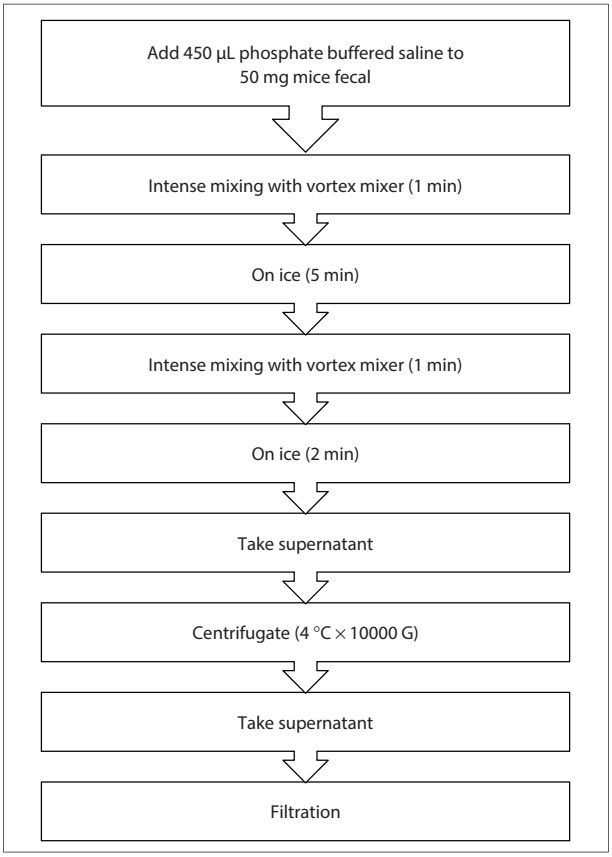

Fig. 3 Pretreatment Flowchart

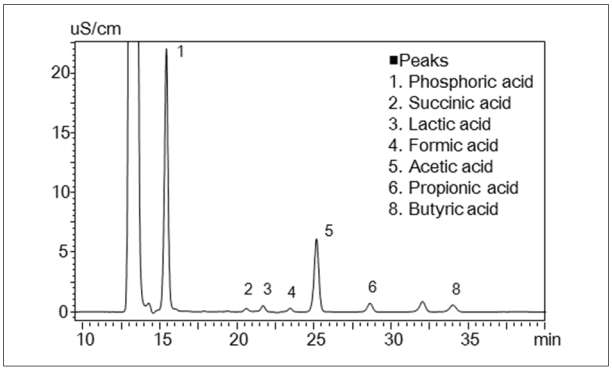

Fig. 4 Chromatogram of Mouse Fecal Extract

References

*1 M. Matsumoto, R. Kibe, T. Ooga, Y. Aiba, S. Kurinara, E. Sawaki, Y. Koga, Y. Benno: Scientific Reports 2, 223 (2012).

*2 Seiko Hoshi, "Methods for Measuring Organic Acids in the Contents of the Large Intestine" in *Nutritional Physiology and Enteric Bacteria of the Digestive Tract*, 11-25 (2011).

Note: If feces cannot be agitated uniformly, pulverize them with a mortar and pestle for homogenization and then perform agitation with a vortex mixer.

* The sample used in this measurement was provided by Mr. Matsumoto from Kyodo Milk Industry Co., Ltd.

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