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Application News
No. L547

High-Performance Liquid Chromatography

Monitoring Organic Acids during Fermentation with Shim-pack™ Fast-OA High-Speed Organic Acid Analytical Column

Organic acids are attracting attention not only as taste and flavor components in food, but also as raw materials for pharmaceuticals and chemical products, and have been analyzed in various fields. Organic acid analysis by HPLC has multiple separation modes such as ion exclusion, ion exchange, and reverse phase, and there are options for detection such as UV method and conductivity detection method. Ion-exclusion columns and post-column pH-buffered electrical conductivity detection are often used because they selectively detect organic acids while avoiding the matrix effects from the sample. However, the long analysis times are a problem. There is demand for shorter analysis times, especially in bio-production research and intestinal microbiota analysis, which involve analyzing only a limited number of target components. Furthermore, in the monitoring of fermentation conditions, it is necessary to quickly determine the amount of organic acids produced as metabolites by microorganisms in order to control the cultivation in accordance with their growth conditions. Here we report a case of monitoring organic acid content during fermentation using an ion exclusion column, Shim-pack Fast-OA.

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■ Analysis of Standard Sample

Using an acidic mobile phase in the ion-exclusion mode, Shim-pack Fast-OA columns separate solutions based on the pKa value of each sample component. The Shim-pack SCR-102H column also separated organic acids based on the same principle, but the Shim-pack Fast-OA column elutes organic acids more faster due to an optimized design, which includes an functional group bonding rate.

The analytical conditions are shown in Table 1, and the chromatograms of the five components of the standard organic acid mixture are shown in Fig. 1. It was confirmed that acetic acid could be eluted within 10 minutes.

Fig. 2 shows the elution times of the five standard organic acid mixtures and the pKa of each compound. As for the five components in this study, we found that the ion exclusion effect of the Shim-pack Fast-OA was effective and that the separation was achieved according to the difference in pKa.

Table 1 Analytical Conditions

Column:	Shim-pack Fast-OA 2 column in series (100 mm L × 7.8 mm I.D.)
Guard column:	Shim-pack Fast-OA (G) (10 mm L × 4.0 mm I.D.)
Mobile phase:	5 mmol/L p-toluenesulfonic acid
Flowrate:	0.8 mL/min
pH buffering solution:	5 mmol/L p-toluenesulfonic acid 20 mmol/L Bis-Tris 0. 1 mmol/L EDTA
Flowrate:	0.8 mL/min
Column temperature:	30 °C
Detection:	Conductivity detector (CDD-10Avp)
Injection volume:	10 µL

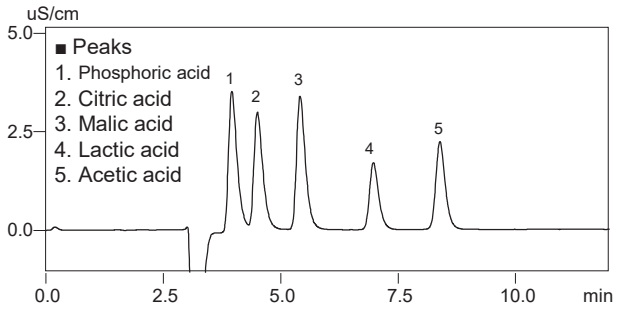

Fig. 1 Chromatogram of Standard Sample

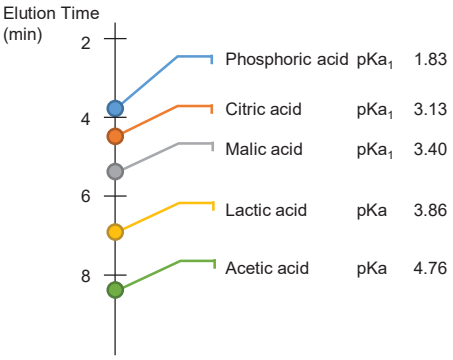

Fig. 2 Elution Time and pKa Values for Target Compounds (pKa values are from the Biology Data Book and Handbook of Chemistry)

■ Linearity and Reproducibility

Each organic acid component was evaluated in terms of contribution to linearity over the concentration range including 10, 50, 100, 500, and 1000 mg/L and in terms of area reproducibility for repeated analysis at 10 mg/L, the lowest concentration in the calibration curve. The results are shown in Table 2. Good results were obtained, with contribution rate (r^2) values higher than 0.9999, and RSD values lower than 1.5 %.

Table 2 Linearity and Area Reproducibility

	Linearity (r^2)	Area (%RSD)
Phosphoric acid	0.99994	0.981
Citric acid	0.99998	0.975
Malic acid	0.99999	0.777
Lactic acid	0.99997	1.322
Acetic acid	0.99999	1.190

■ Sample Pretreatment

Organic acids were extracted from commercially available yogurt drinks by adding 5 mmol/L p-toluenesulfonic acid aqueous solution (mobile phase) and chloroform for protein removal and delipidation. The sample was filtered and analyzed by HPLC. The recovery rate was evaluated using yogurt to which the standard sample was added to make a standard sample concentration of 50 mg/L. The pretreatment protocol is indicated in Fig. 3. The resulting chromatogram is shown in Fig. 4, with corresponding recovery rates shown in Table 3. Selectively detecting organic acids by the pH-buffering method, excellent 96 to 118 % recovery rates were achieved.

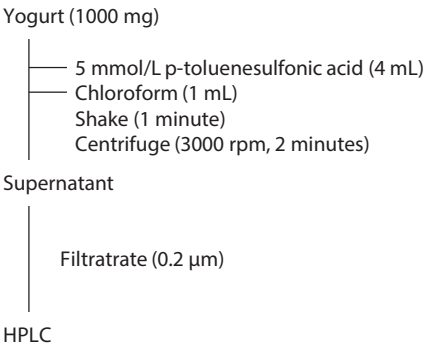

Fig. 3 Pretreatment Protocol

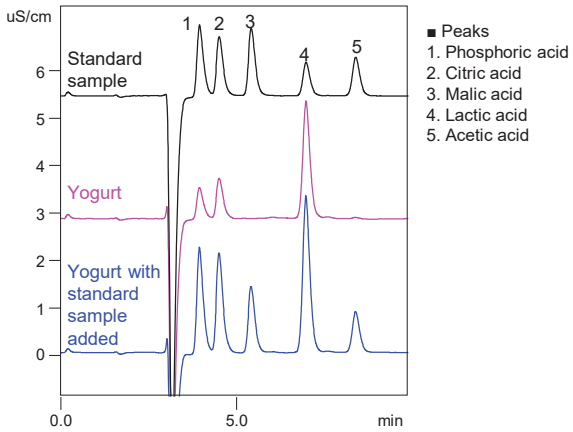

Fig. 4 Chromatogram of Pretreated Yogurt

Table 3 Recovery Rate of Each Component in Spiked Yogurt

	Recovery (%)
Phosphoric acid	102.0
Citric acid	102.2
Malic acid	96.3
Lactic acid	117.1
Acetic acid	100.9

■ Example of Monitoring Fermentation

Figure 5 shows the results of an evaluation of changes over time of phosphoric acid, citric acid, and lactic acid, which are the main acidic substances contained in home-fermented yogurt.

In general, microorganisms are said to metabolize the lactose to produce lactic acid, which gives yogurt its sour flavor as the fermentation progresses. In actuality, we confirmed that lactic acid increased from about 3.5 hours after the start of cultivation.

The metabolic and fermentation status of microorganisms can be checked by monitoring the organic acid contents present in environments where microorganisms are active (fermented foods or culture media).

With the method described above, the analysis time is 12 minutes, and it takes only about 20 minutes from sampling to confirmation of the results. This makes it possible to contribute to detailed fermentation monitoring and control, which was previously difficult to achieve.

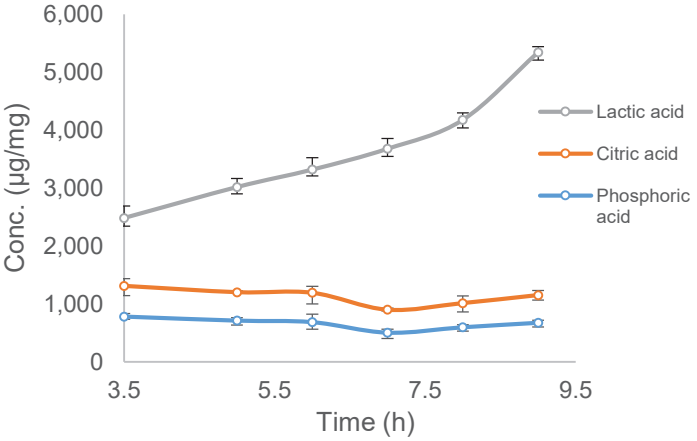

Fig. 5 Change in Acid Content in Yogurt Over Time (N = 3)

■ Summary

Using a Shim-pack Fast-OA column to analyze yogurt, we were able to confirm the primary organic acids in about 12 minutes. These results from rapid analysis of organic acids suggest that the method provides an effective means of ensuring an adequate number of repeated analyses for multianalyte processing and quickly controlling the fermentation process based on the fermentation status.

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