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Application News

No.L442

High Performance Liquid Chromatography

Effect of Column Temperature on Organic Acid Separation

Shimadzu's organic acid analysis system utilizes ion exclusion chromatography to first separate the target components, then introduce a pH buffer solution by the post-column method to adjust the pH to near neutral, and detect the organic acids using a conductivity detector to permit quantitation of the various organic acids detected.
To increase the peak capacity for separations with large numbers of components, this system is often equipped with two columns connected in series. Although the temperatures of these columns are usually set to the same value, setting their temperatures to different values permits adjustment of the separation conditions to improve the resolution of closely eluting organic acids. Here we report the results of analysis of a mixture of organic acids using a dual-column, dual-temperature method.

Adjusting Separation by Changing Column Temperature - Part 1

When a sample contains numerous organic acids, it may be difficult to separate all of the components using the standard analytical conditions designed for the organic acid analysis system (Table 1).
In this situation, it may be possible to adjust the separation of the organic acids from each other by changing the column temperature and mobile phase concentration, but in some occasions complete separation may not be achievable even if the temperatures of the two columns are changed together due to differences in the retention behaviors of the many organic acids.
Fig. 1 shows chromatograms of a standard mixture of organic acids run at column temperatures of 25 °C and 48 °C. With a column temperature of 25 °C, the peaks of succinic acid and lactic acid, and of fumaric acid and acetic acid, respectively, were not completely separated. However, while the peaks of succinic acid and lactic acid, and fumaric acid and acetic acid were separated using a column temperature of 48 °C, the peaks of phosphoric acid and α-ketoglutaric acid, and fumaric acid and formic acid were not separated. The separation status of the various peaks is summarized in Table 2.

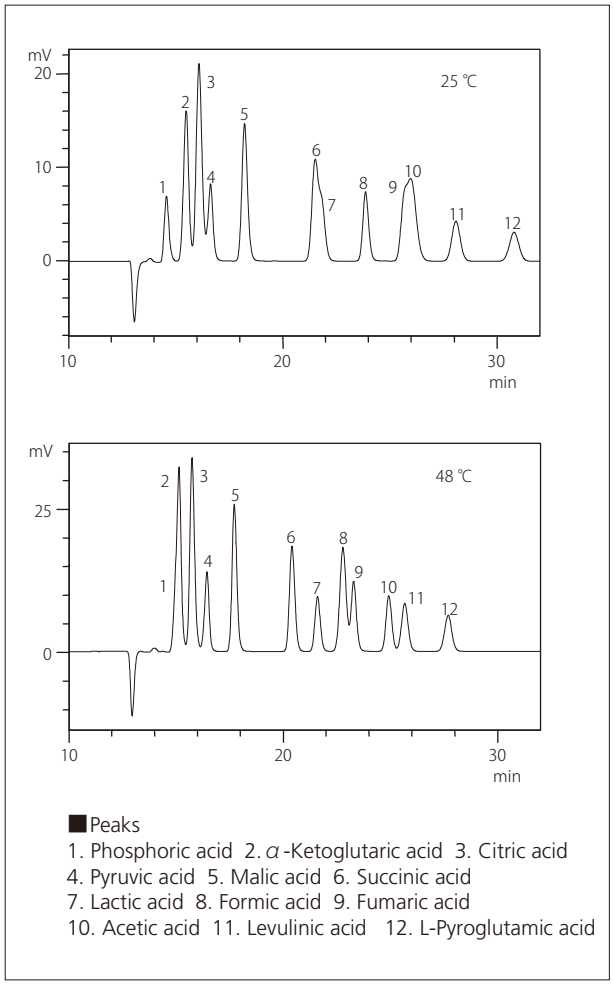

Fig. 1 Chromatograms of Organic Acid Mixture at Column Temperatures of 25 °C and 48 °C

Table 1 Standard Analytical Conditions

Column	: Shim-pack SCR-102H, 2 columns in series (300 mm L. × 8.0 mm I.D., 7 µm)
Mobile Phase	: 5 mmol/L p-TSA
pH Buffer Solution	: 5 mmol/L p-TSA, 20 mmol/L Bis-Tris, 0.1 mmol/L EDTA-4H
Flowrate of Moble Phase	: 0.8 mL/min
Flowrate of pH Buffer Solution	: 0.5 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 10 µL
Detection	: CDD-10A$_{VP}$

Table 2 Separation of Main Peaks

	Succinic acid and Lactic acid	Fumaric acid and Acetic acid	Phosphoric acid and α-ketogultaric acid	Formic acid and Fumaric acid
25 °C	**Not separated**	**Not separated**	Separated	Separated
48 °C	Separated	Separated	**Not separated**	**Not separated**

Adjusting Separation by Changing Column Temperature - Part 2

In order to achieve the desired separation of the different acids at their ideal temperatures, analysis was conducted using two column ovens (CTO-20AC) as shown in Fig. 2. The first oven, with Column 1, was set to a temperature of 25 °C, and the second oven, with Column 2, set to 48 °C. The results are shown in Fig. 3. Compared to the incomplete separation obtained when both column temperatures were set to either 25 °C or 48 °C, much better separation of the components was demonstrated.

Similarly, we conducted analysis with the first column oven set to a temperature of 48 °C and the second to 25 °C. In this case, we also confirmed that separation was better than when both columns were set to the same temperature.

This demonstrates that complex separation behavior can be controlled by setting the two column temperatures to different values. Further, we showed that components which are difficult to separate using the standard organic acid analysis conditions can be analyzed more effectively with a dual-column and dual-temperature method.

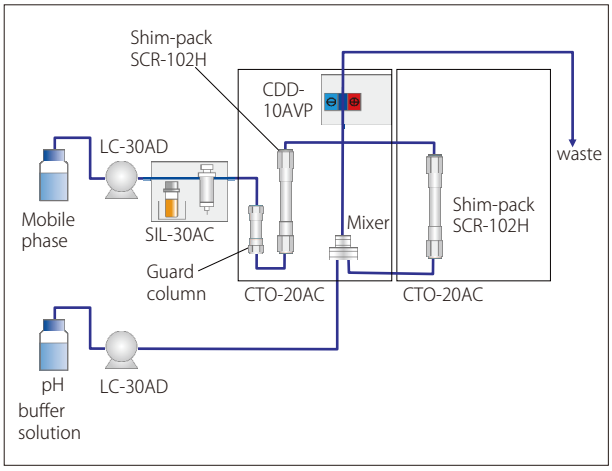

Fig. 2 Flow Diagram

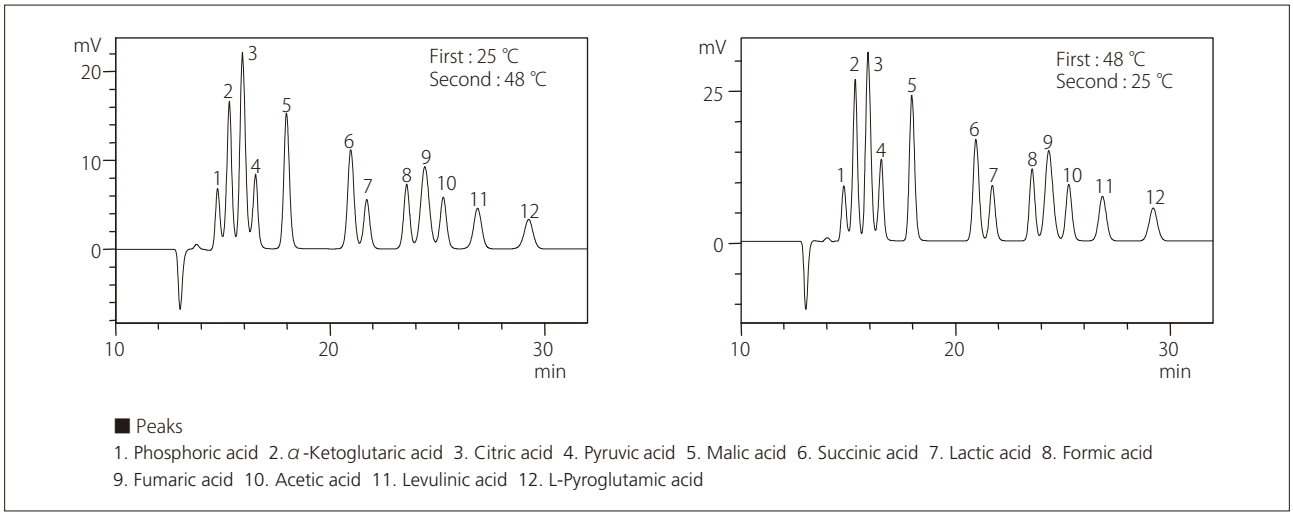

Fig. 3 Chromatograms of Organic Acid Mixture Analyzed Using Two Columns Set to Different Temperatures

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