

Application News

No. L515

High Performance Liquid Chromatography

Reducing Organic Acid Analysis Times Using SIL-30AC Autosampler

Organic acid counter-ions found in food and drugs are considered important components as they decide the flavor of food and properties of drugs. Food in particular contains a large number of substances analyzable with LC-MS, though LC-MS is unable to detect formic acid and acetic acid in foods due to its use of formic acid and acetic acid in the mobile phase.

Combining ion exclusion mode HPLC with post-column reaction detection (post-column pH-buffered electrical conductivity detection) is an approach that does not use formic acid or acetic acid in the mobile phase, and therefore allows the selective and highly sensitive detection of formic acid-containing and acetic acid-containing organic acids. However, this approach uses 2 columns to achieve high resolution separation, which increases time to detection. Another problem with this technique is the large number of constituents targeted by food analysis, and the time required to analyze standard reference solutions for calibration curve creation.

This article describes using the overlapping function of SIL-30AC to shorten the time required for continuous analysis of organic acids.

■ Overlap Injection

Fig. 1 compares a normal analysis cycle and an analysis cycle with overlap injection. Because organic acid analysis systems required around 10 minutes for target component elution, analysis times can be reduced by injecting the next sample just before the final target component is eluted in the previous analysis cycle, while column stability is maintained.

■ Standard Mixture Analysis

Table 1 shows the analytical conditions used. Fig. 2 shows the results of a repeat injection of 10 μ L standard mixture solution (20 mmol/L per component). The normal analysis shown at the top of Fig. 2 required 40 minutes per analysis and 80 minutes to analyze the 2 injections, while the bottom of Fig. 2 shows using the SIL-30AC pretreatment function successfully reduced the analysis time by 25 % by requiring just 30 minutes for a single analysis and using an overlap time of 10 minutes.

Table 1 Analytical Conditions

Column	: Shim-pack SCR-102H (300 mm L. \times 8.0 mm I.D.) \times 2
Guard Column	: Guard column SCR-102H (50 mm L. \times 6.0 mm I.D.)
Mobile Phase	: 5 mmol/L p-Toluenesulfonic acid
Flowrate of Mobile Phase	: 0.8 mL/min
pH Buffering Solution	: 5 mmol/L p-Toluenesulfonic acid, 20 mmol/L Bis-Tris and 0.1 mmol/L EDTA
Flowrate of pH Buffering Solution	: 0.8 mL/min
Column Temp.	: 45 $^{\circ}$ C
Detection	: Electro conductivity detector (CDD-10Avp)
Injection Vol.	: 10 μ L

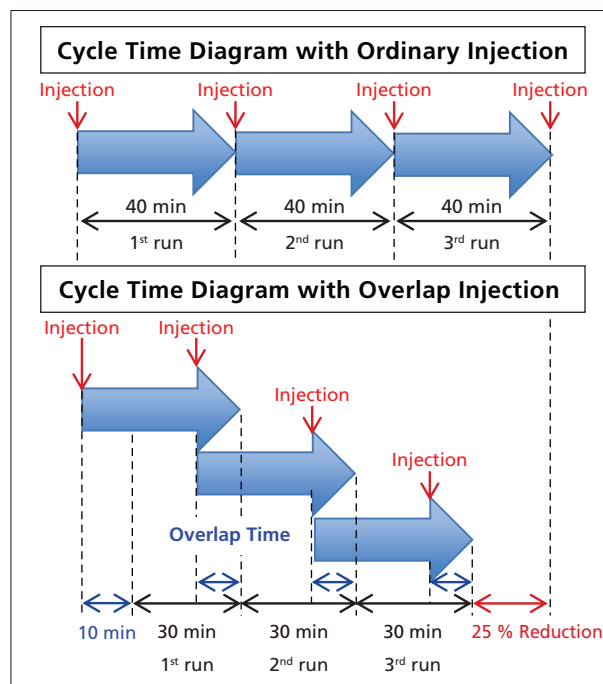


Fig. 1 Model of Overlap Process

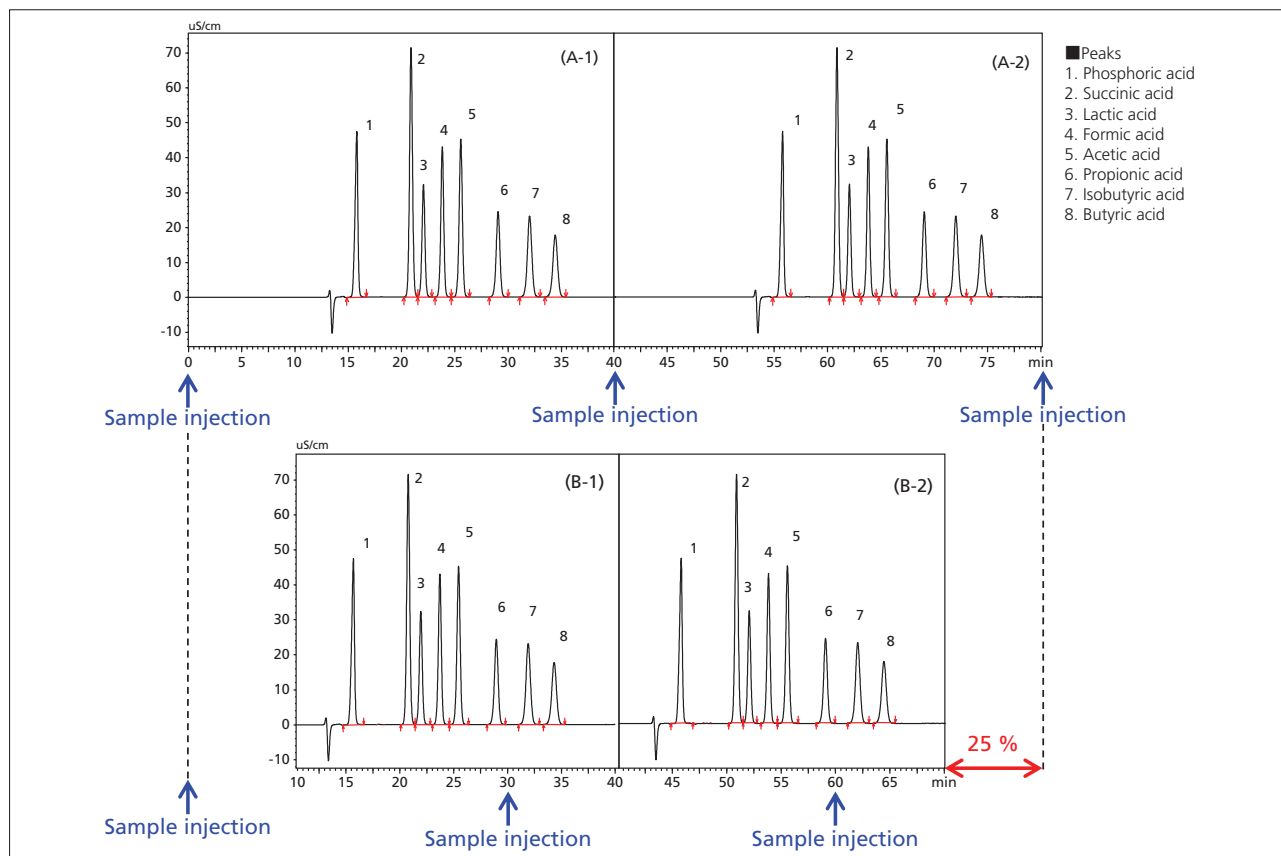


Fig. 2 Standard Mixture Chromatograms (20 mmol/L)
(A-1): Conventional Analysis (1st), (A-2): Conventional Analysis (2nd)
(B-1): Analysis by Overlap Injection (1st), (B-2): Analysis by Overlap Injection (2nd)

■ **Calibration Curve Linearity**

Fig. 3 shows the calibration curve obtained using the conditions in Table 1. The calibration curve was plotted in the range of 2.5 to 20 mmol/L. Good linearity was obtained as shown by a coefficient of determination (R^2) of 0.9999 or higher for all components.

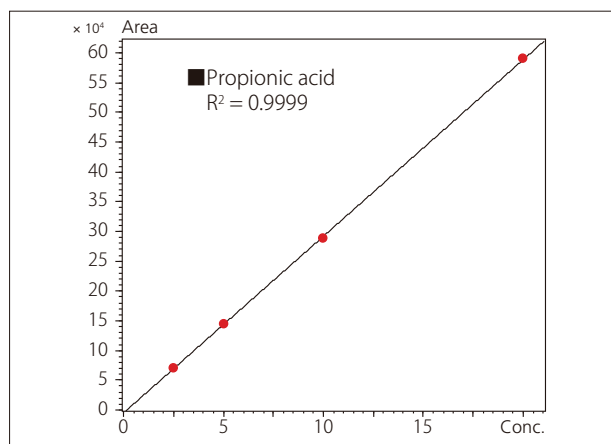


Fig. 3 Calibration Curve

■ **Repeatability**

Table 2 shows %RSD (n = 6) values for peak area, which is an indicator of repeatability. Repeatability was good as shown by a peak area %RSD of 2 % or lower for all compounds.

Table 2 Peak Area Repeatability

Compounds	%RSD Peak Area
Phosphoric acid	1.70
Succinic acid	1.72
Lactic acid	1.83
Formic acid	1.36
Acetic acid	1.71
Propionic acid	1.77
Isobutyric acid	1.84
Butyric acid	1.45