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Application News

No. L565

High Performance Liquid Chromatography

Shortening of Cycle Time in Organic Acid Analysis by Overlapped Injection

Organic acids are not only known as acid taste components in foods, but are also used as food additives with antibacterial and antioxidative effects. Pharmaceutical products contain organic acids as counterions of the active ingredient, and analysis of organic acids is carried out to measure the solubility and stability of the active ingredient. Regardless of the field, instruments that enable organic acid analysis with high repeatability in a shorter time are demanded, as large numbers of samples are handled for quality control of products.

Use of the ion exclusion mode in HPLC in combination with the post-column pH-buffered electroconductivity detection method enables detection of organic acids with high sensitivity and selectivity. On the other hand, when attempting to achieve high resolution with this technique, detection requires considerable time because two columns are used.

In continuous analysis, the overlapped injection of SIL-40 series autosamplers for Nexera™ ultra high performance liquid chromatographs makes it possible to inject the following sample while the preceding sample is being analyzed. This advance injection technique realizes a reduction of the total analysis time in continuous analyses equivalent to the time until elution of the preceding target component. This article introduces an example of shortening of the analysis time in a continuous analysis of organic acids using the overlapped injection of the SIL-40C.

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Overlapped Injection

Fig. 1 shows a schematic diagram of the typical analysis cycle and the analysis cycle in overlapped injection. Since elution of the target component requires approximately 10 min in this analysis, the analysis time can be shortened by injecting the following sample overlapping that time.

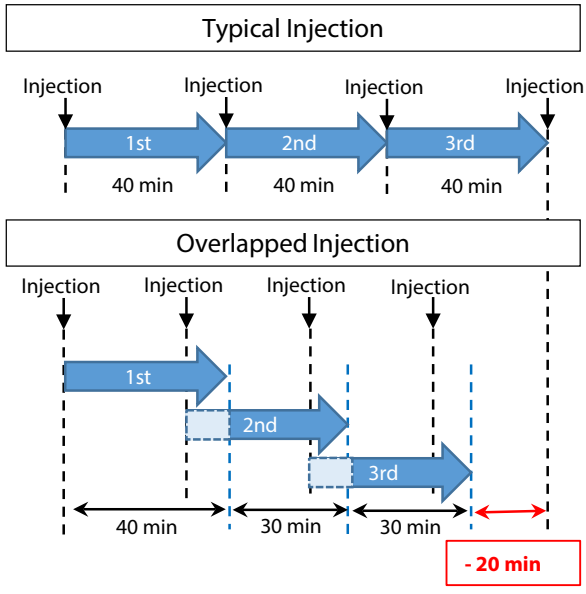

Fig. 1 Schematic Diagram of Overlapped Injection

Fig. 2 Nexera™ Organic Acid Analysis System

Mobile Phase Reagent Set for Organic Acid Analysis

A mobile phase reagent set for organic acid analysis was used in this analysis. As shown in Fig. 3, the set consists of a preadjusted mobile phase and a pH buffering reagent, and can be used immediately as soon as opened. The set not only saves the time and cost of solution preparation *1, but also reduces the risk of pH control errors, enabling stable analysis at all times.

Fig. 3 Mobile Phase Reagent Set for Organic Acid Analysis

*1 Cost saving of approximately ¥70,000/y (estimated by Shimadzu Corporation).

Example of Standard Sample Solution

Table 1 shows the analysis conditions, and Fig. 4 shows the results of 3 repeated injections of the standard solution (2.0 mmol/L of each organic acid). In the typical analysis shown in the upper part of Fig. 4, each analysis required 40 min, resulting in a total time of 120 min for the 3 repeated analyses. However, as shown in the lower part of Fig. 4, the analysis time was successfully shortened by 20 min by using the overlapped injection of the SIL-40C. Because the time axis of the chromatograms when using the overlapped injection shows the time from after injection, it is not necessary to change the retention time setting of each component on the control software.

Table 1 Analysis Conditions

Column	:	Shim-pack™ SCR-102H (300 mm × 8.0 mm I.D., 7 µm) × 2
Guard column	:	Guard column SCR-102H (50 mm × 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*-toluene sulfonic acid, 20 mmol/L Bis-Tris and 0.1 mmol/L EDTA
Flow rate of pH buffering solution	:	0.8 mL/min
Column temp.	:	45 °C
Injection vol.	:	10 µL
Detection	:	Conductivity detector (CDD-10A$_{VP}$)

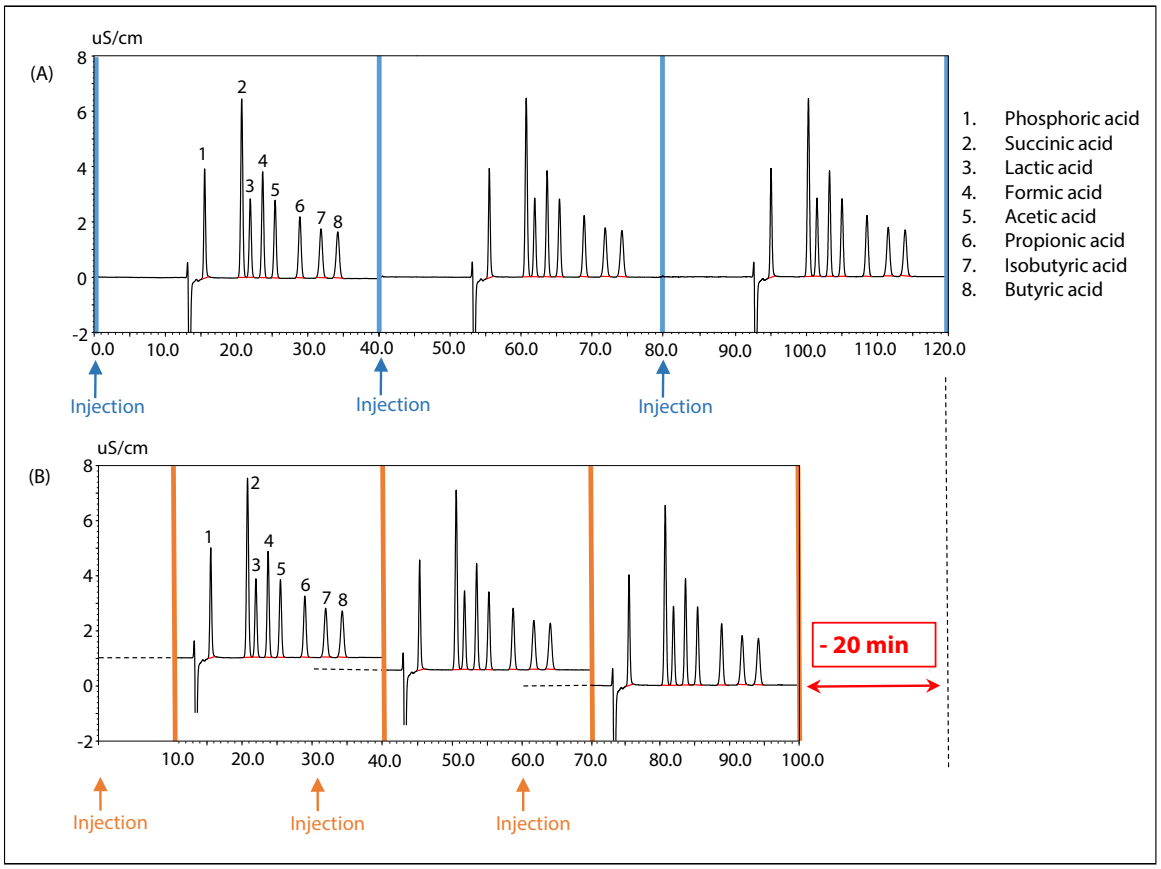

Fig. 4 Chromatograms of Standard Solution (2.0 mmol/L each)
(A): Typical Injection (1st - 3rd Injections), (B): Overlapped Injection (1st - 3rd Injections)

Linearity of Calibration Curve and Repeatability of Peaks

Fig. 5 shows the calibration curve of the analysis using the overlapped injection under the conditions in Table 1.

The calibration curves were prepared in the range of 2.0 to 20.0 mmol/L. Good linearity with a regression coefficient r^2 = 0.999 or higher was obtained for all components.

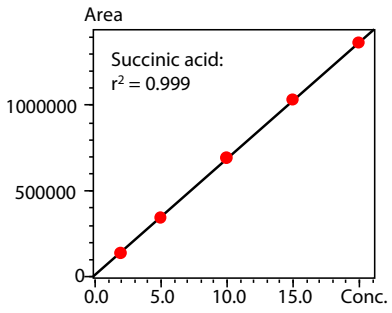

Fig. 5 Linearity of Calibration Curve

Table 2 shows a comparison of the %RSD (n = 3) of the peak area for typical injection and overlapped injection. Satisfactory repeatability of 0.5% or less was obtained under both conditions.

Table 2 Repeatability of Peak Areas of Standard Solution Containing 2.0 mmol/L of Each Compound (n = 3)

	%RSD of peak area	
Compound	Typical	Overlapped
Phosphoric acid	0.26	0.20
Succinic acid	0.10	0.10
Lactic acid	0.16	0.47
Formic acid	0.16	0.17
Acetic acid	0.20	0.15
Propionic acid	0.22	0.13
Isobutyric acid	0.49	0.48
Butyric acid	0.33	0.18

Conclusion

A continuous analysis of a standard solution was conducted using the overlapped injection of an SIL-40C autosampler installed in a Nexera organic acid analysis system. In a continuous analysis of 3 samples, the analysis time could be reduced by 20 min. In a continuous analysis of large numbers of samples, for example, an analysis of 20 samples, it is possible to shorten the analysis time by 3 h or more. At the same time, it is also possible to increase the number of samples that can be analyzed with one mobile phase reagent set for organic acid analysis.

First Edition: Mar. 2021

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