

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No. L213

Applications of HPLC Organic Acid Analysis System

- Comparison with UV Detection -

For the analysis of organic acids by HPLC, separation by ion exclusion chromatography and detection at around 210nm (UV) has conventionally been applied as a convenient and simple method of analysis. The drawback of this system, however, is that wavelength setting is deprived of selectivity for the detection of organic acids; it is apt to be interfered with from foreign substances mixed with the target analytes.

The HPLC organic acid analysis system that has been developed to overcome the above-mentioned

shortcoming detects with the electric conductivity detector taking advantage of the character of organic acids, i.e., being charged with negative electricity, contributing to the improvement of selectivity. In this article, in order to show the characteristics, a culture medium for microorganisms which was supposed to be full of lots of foreign substances and yoghurt were subjected to analysis by the UV detection method and by this HPLC organic acid analysis system. Shown below are the chromatograms for comparison.

■ Analysis of Culture Medium for Microorganisms

20 μ L each of two kinds of filtered culture medium for microorganisms was injected into the analyzer and was subjected to analysis. Figure 1(A) and Figure 3 (B) show chromatograms by the HPLC organic acid analysis system, and Figure 2 (A) and 4 (B) show chromatograms by the UV detection method. Although many peaks were presented by the UV detection method, few of the many peaks of the chromatograms obtained by the UV detection method were consistent

in retention time with the results of determination using the standard samples. On the other hand, interference from foreign substances was greatly reduced by the HPLC organic acid analysis system, making possible quantitative and qualitative analyses of organic acids.

The samples were by courtesy of Hokkaido Can Co., Ltd.

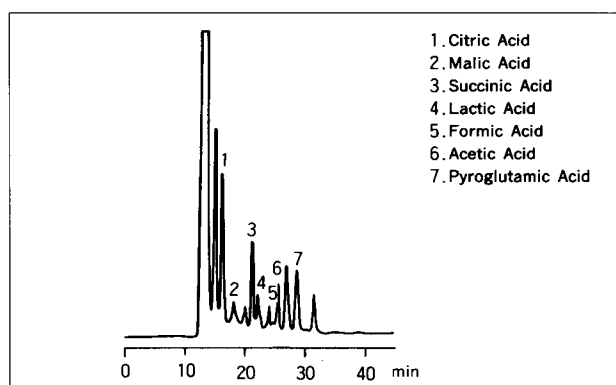


Fig.1 Chromatogram of Culture Solution "A"
(Organic Acid Analysis System)

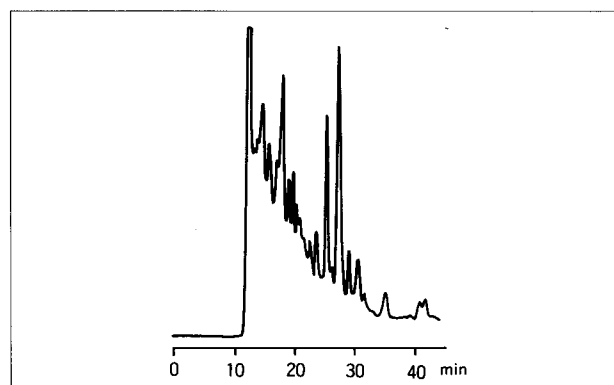


Fig.2 Chromatogram of Culture Solution "A"
(UV Detection)

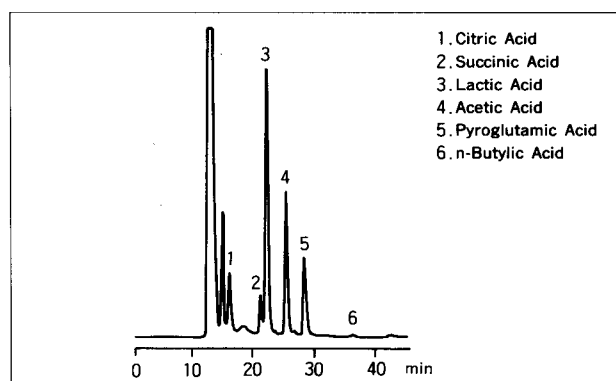


Fig.3 Chromatogram of Culture Solution "B"
(Organic Acid Analysis System)

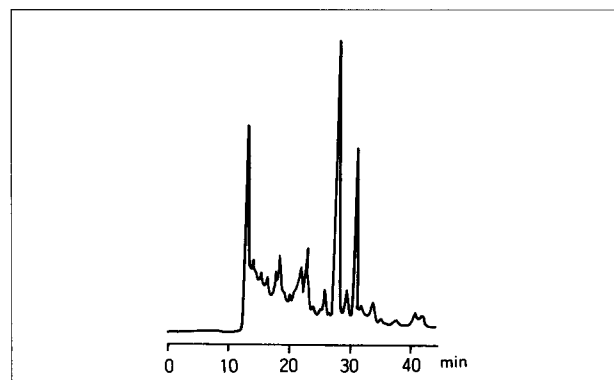


Fig.4 Chromatogram of Culture Solution "B"
(UV Detection)

■ Analysis of Yoghurt

After pretreatment of yoghurt as shown in Table 1, 50 μ L of the sample was subjected to analysis. Different from the culture medium for microorganisms, foreign substances contained in yoghurt are limited, and high concentration components like citric acid and lactic acid can be quantitated by the UV detector. But, by the UV detection, as shown in the zoomed chromatogram of Figure 6, peaks of low concentration components (in this data, succinic acid data (peak 2) and formic acid (peak 4) are not separated from peaks of foreign substances, making quantitation impossible.

By the organic acid analysis system, selectivity is greatly improved as shown in Figure 5, making possible quantitation of these components.

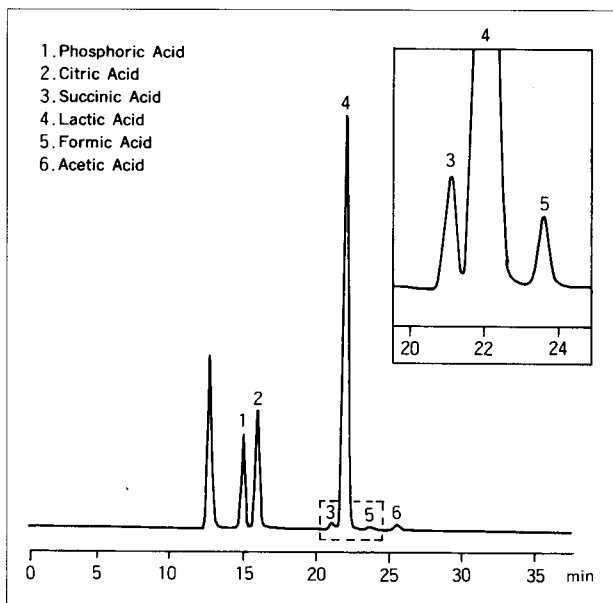


Fig.5 Chromatogram of Yoghurt
(by the Organic Acid Analysis System)

Yogurt, 5g
← Dilute to 20mL with H ₂ O
Shake, 5min.
Centrifuge, 12,000r.p.m., 5min.
Supernatant
Inject, 50 μ L.

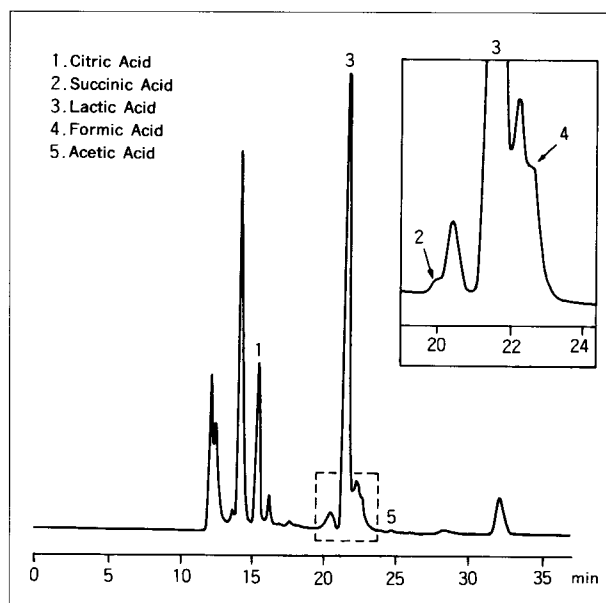


Fig.6 Chromatogram of Yoghurt
(by the UV Detection)

Table 2 Analytical Conditions-Organic Acid Analysis System

for Separation

Column : Shim-pack SCR-102H
(8.0mm I.D. \times 300mm L.) \times 2
Mobile Phase : 5mM *p*-toluenesulfonic acid
Flow Rate : 0.8mL/min.
Temperature : 45°C (Fig.1,3), 40°C (Fig.5)

for Detection

Reagent : 5mM *p*-toluenesulfonic acid,
20mM Bis-Tris and
100 μ M EDTA-2Na
Flow Rate : 0.8mL/min.
Temperature : 48°C (Fig.1,3), 43°C (Fig.5)
Detector : CDD-6A

Table 3 Analytical Conditions-UV Detection

for Separation

Column : Shim-pack SCR-102H
(8.0mm I.D. \times 300mm L.) \times 2
Mobile Phase : 5mM HClO₄
Flow Rate : 0.8mL/min.
Temperature : 60°C

for Detection

Detector : SPD-6A(210nm)

 **SHIMADZU**

SHIMADZU CORPORATION, International Marketing Division

3, Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan Phone: 81(3)3219-5641 Fax: 81(3)3219-5710
Cable Add: SHIMADZU TOKYO