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Application News

No.L490

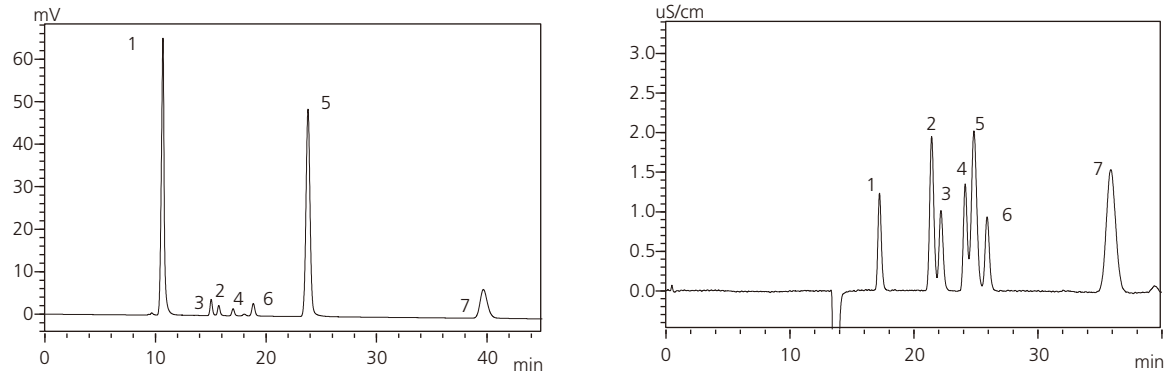
High Performance Liquid Chromatography

Analysis of Organic Acids in Culture Medium Using Post-Column pH Buffering Organic Acid Analysis System

The shift from emphasis on fossil fuels in recent years has been emphasized with increasingly active research into the bioproduction and manufacture of new energy sources and chemical products. In the field of bioproduction, microbial cells serve as factories. In the process by which sugars are metabolized to produce the desired compounds in the culture medium, organic acids such as pyruvic acid, lactic acid, acetic acid, formic acid and succinic acid are also produced as metabolites. Accurate identification and quantification of these organic acids makes it possible to accurately grasp the metabolic state of the cell, for example elucidation of the flow of carbon introduced into the system. To improve production of the compounds of interest by the microorganism's cells, results obtained from analysis of the culture medium to identify and quantify the metabolites make it possible to conduct an in-depth search for modification targets among the microorganism's genes. This, therefore, elevates the importance of analysis of organic acids in the culture medium to further the study of bioproduction.
Organic acid analysis is often conducted using an ultraviolet absorbance detector. However, when analyzing media, which typically comprise complex matrices, the risk of contaminant components overlapping target components increases. Here, we introduce an example of analysis of organic acids in a culture medium using post-column pH buffered electrical conductivity detection in combination with a dual column oven, a system that provides both improved separation and high-selectivity detection.

Post-Column pH Buffered Electrical Conductivity Detection with Dual Column Oven System

Ion-exclusion chromatography using an acidic aqueous solution is used for the separation of the organic acids, but when using an electrical conductivity detector to monitor changes in ion quantities, high-sensitivity detection is difficult due to increased background noise originating from the mobile phase and suppression of the dissociation of the organic acids. With the system mentioned here, after separation of the organic acids by the column, the mobile phase pH is adjusted to near-neutral by mixing with buffer, thus improving detection sensitivity due to dissociation of the organic acids.
Previously, an example of improved organic acid separation was described in Application News No. L442, in which two columns were used to achieve better separation than the incomplete separation obtained with a single column. This method offers better separation of organic acids for use in bioproduction, thereby permitting more accurate qualitative and quantitative analysis.
In organic acid analysis by UV detection, peak intensities differ significantly among substances even when present at the same concentration. This can be attributed to the differences in absorption coefficient, which is dependent on the number of double bonds in the compound, and can complicate the determination of the appropriate dilution factor of the sample. On the other hand, in organic acid analysis using electrical conductivity detection, the peak intensities will be uniform. Fig. 1 shows a comparison of the chromatograms of a standard solution obtained using UV detection and electrical conductivity detection. The analytical conditions used for UV detection are shown in Table 1, and those for electrical conductivity detection are shown in Table 2.



■ Peaks
1. Pyruvic acid, 2. Succinic acid, 3. Lactic acid, 4. Formic acid, 5. Fumaric acid, 6. Acetic acid, 7. Pimelic acid (I.S.)

Fig. 1 Comparison of Chromatograms of Organic Acid Standard Solution
Left: UV Detector Right: Conductivity Detector

Comparison of UV Detection and Electrical Conductivity Detection for Biomass Analysis

Fig. 2 shows the results of analysis of a culture medium using the analytical conditions listed in Table 1. Due to the presence of Polypeptone™ and yeast extract in the analyzed culture medium, substances other than the organic acids of interest were also detected when using UV detection. Furthermore, not only is it difficult to achieve separation of the contaminant and target substances, partial deterioration of baseline stability is also evident. Fig. 3 shows the results of analysis of the same culture medium using the analytical conditions listed in Table 2, which in this case includes use of post-column pH buffered electrical conductivity detection.

Using this analytical method, the organic acid components are clearly detected with almost no adverse influence due to impurities.

Table 1 Analytical Conditions with UV Detector

Column	: Ion Exclusion Column
Mobile Phase	: 0.75 mmol/L Sulfuric Acid
Flowrate of Moble Phase	: 0.8 mL/min
Column Temp.	: 40 ˚C
Injection Vol.	: 10 µL
Detection	: UV 205 nm

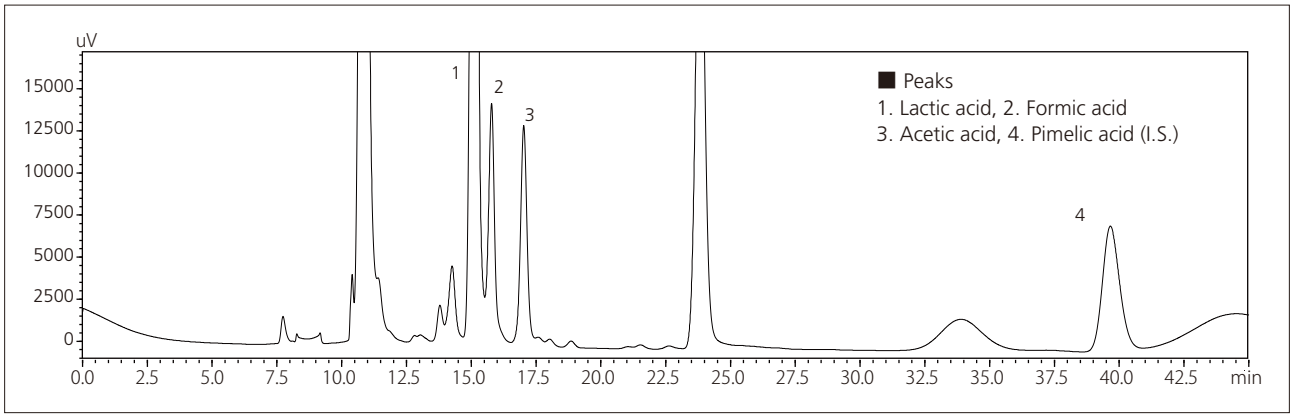


Fig. 2 Chromatogram of Culture Medium Using UV Detector

Table 2 Analytical Conditions with Conductivity Detector

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*-Toluenesulfonic acid
pH Buffering Solution	: 5 mmol/L *p*-Toluenesulfonic acid, 20 mmol/L Bis-Tris, 0.1 mmol/L EDTA-4H
Flowrate of Moble Phase	: 0.8 mL/min
Flowrate of pH Buffering Solution	: 0.8 mL/min
Column Temp.	: 48 ˚C, 25 ˚C
Injection Vol.	: 10 µL
Detection	: Conductivity Detector

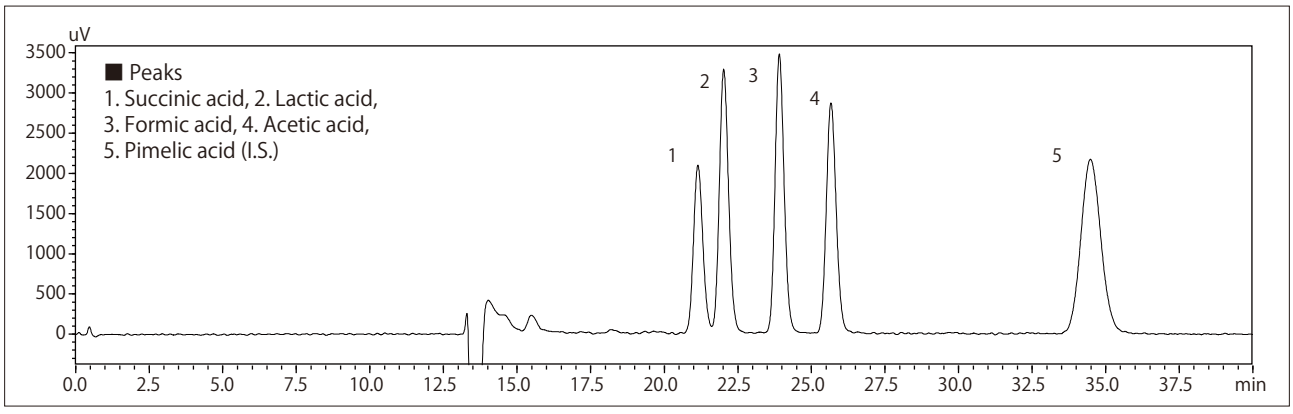


Fig. 3 Chromatogram of Culture Medium Using Conductivity Detector

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