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Application News

High Performance Liquid Chromatography

Monitoring the Fermentation Progress in Bioethanol Production Using the Prominence-i Integrated HPLC with Differential Refractive Index Detection

No. HPLC-011

Bioethanol is made of biomass like corn or sugar canes. Since biomass is an organic compound, it releases carbon dioxide when it is burned. However, carbon dioxide is absorbed by the photosynthesis of raw materials. Therefore, it can be interpreted that the carbon dioxide amount in the atmosphere is maintained as a whole. This thinking called "Carbon Neutral" has attracted attention from the standpoint of environmental protection. From this viewpoint, bioethanol is considered a renewable and clean energy source.

The United States established the Renewable Fuels Standard (RFS) program by the Energy Policy Act of 2005 and enacted the Energy Independence and Security Act in 2007. These policies set annual usage amounts of bioethanol and accelerated its production.

HPLC is now a commonly used technique for monitoring the progress of fermentation in the bioethanol production laboratory. In the bioethanol production plant, the HPLC system is typically used to profile the carbohydrate, alcohol, and organic acid contents of the fermentation broth. These compounds display almost no ultraviolet absorption, and are therefore typically detected using a differential refractive index detector.

This application shows an example of the analysis of fermentation broth by using a refractive index detector (RID-20A) and a LC-2030C LT integrated high-performance liquid chromatograph.

This system can determine the quantity of these compounds in one analysis. This information can be used to evaluate the progress of the fermentation and what intervention may be necessary at a future time to maximize the production of ethanol and minimize the production of further oxidation products.

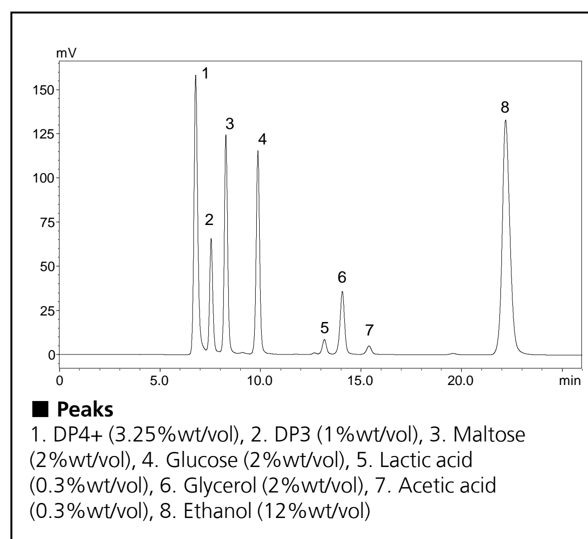
■ Calibration of the HPLC system by use of a standard test solution

The previous monitoring system uses the Phenomenex® Rezex ROA-Organic Acid column and dilute Sulfuric acid as a mobile phase. A 5μL aliquot of test standard was injected on the HPLC operating at a flowrate of 0.6mL/min. Table 1 shows the analytical conditions. Fig. 1 shows the chromatogram of a standard solution of the components of interest (DP4+, DP3, Maltose, Glucose, Lactic acid, Glycerol, Acetic acid, Ethanol). The effect of the amylases can be checked from the early eluting sugar peaks. The endpoint of the fermentation progress can be determined by the later eluting peaks.

Baseline resolution is achieved for all peaks within 24 minutes. This allows the user to obtain, directly, the weight percent for the analytes of the broth samples.

Table 1: Analytical Conditions

System	: LC-2030C LT
Column	: Rezex ROA – Organic Acid H+ (7.8 x 300 mm)
Mobile Phase	: 0.005N Sulfuric acid aq.
Flowrate	: 0.6mL/min
Detector	: RID-20A



■ **Peaks**
1. DP4+ (3.25%wt/vol), 2. DP3 (1%wt/vol), 3. Maltose (2%wt/vol), 4. Glucose (2%wt/vol), 5. Lactic acid (0.3%wt/vol), 6. Glycerol (2%wt/vol), 7. Acetic acid (0.3%wt/vol), 8. Ethanol (12%wt/vol)

Figure 1: Chromatogram of standard solution

■ Analysis of samples at various fermentation times

Figure 2 gives examples that monitor the progress of several fermentation times of corn. These samples were provided by East Kansas Agri-Energy (Garnett, KS). Figure 2 (a) is a comparison of samples that have two different fermentation times. Figure 2 (b) shows time-dependence concentration of each component. The area of the sugar peaks is reduced with fermentation time. In contrast, the area of ethanol increases over time. These variations indicate ethanol fermentation is obviously occurring in real samples.

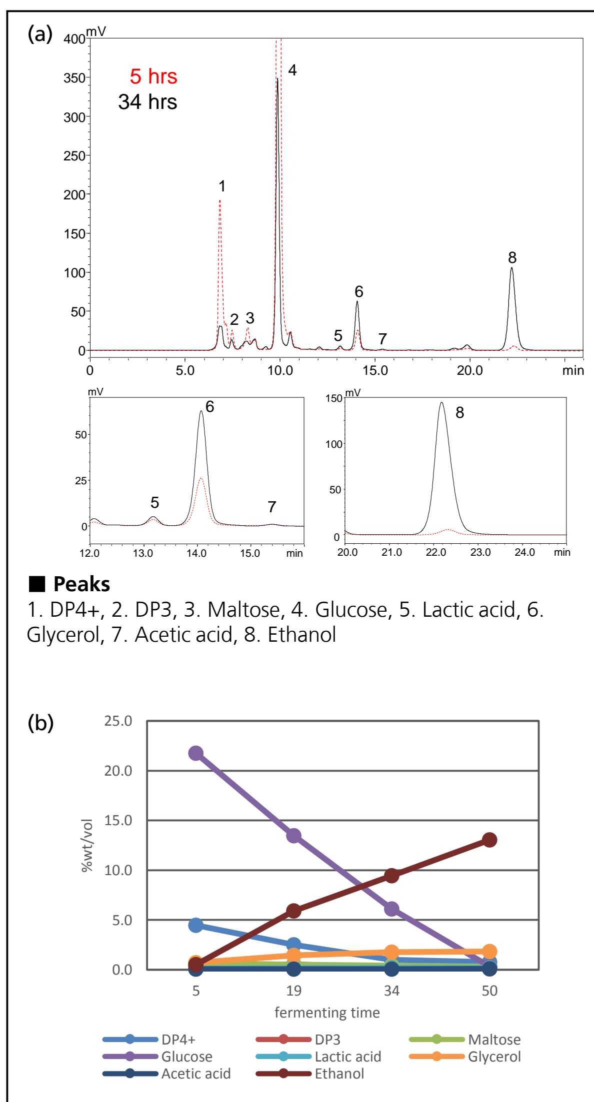


Figure 2: Chromatogram of an actual fermentation sample
Fermenting time : (a) 5 hours and 34 hours
(b) Time-Dependent Process of each Components

■ Reduction of analysis time and cost

Figure 3 shows the chromatogram of the standard solution analyzed using a short column. This analysis increased throughput and decreased the usage of mobile phase compared with the analysis data that used the 300 x 7.8 mm column. This analysis permits faster real-time responses reduces production costs.

Figure 4 shows the chromatogram of an actual fermentation sample after 34 hours have passed from starting the fermenting step. The shorter column decreases analysis time and usage of mobile phase.

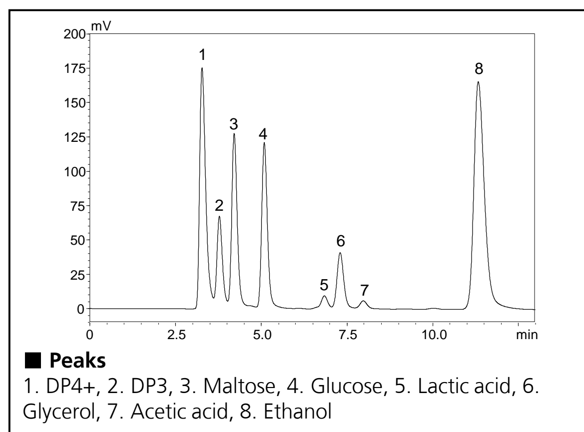


Figure 3: Chromatogram of the standard solution with the shorter column

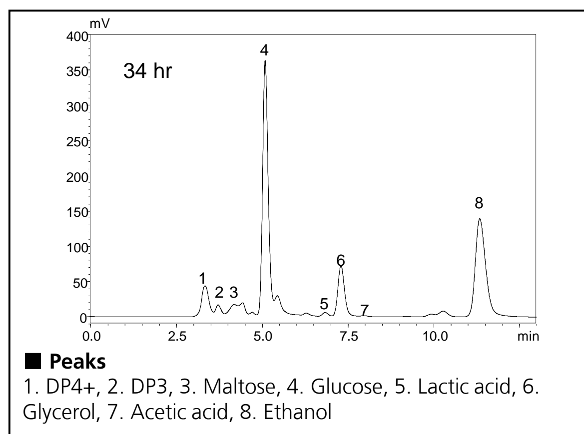


Figure 4: Chromatogram of an actual fermentation with the shorter column

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