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Application News

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High Performance Liquid Chromatography

Analysis of Oligosaccharides in Japanese Sake Using an Evaporative Light Scattering Detector

An evaporative light scattering detector (ELSD) is an HPLC detector often called a "universal" detector because it can detect almost all non-volatile sample components, including those that do not absorb light. Although a differential refractive index detector (RID) can also be used for analysis of compounds with no chromophore, the ELSD removes any potential interference from the solvent peak that is eluted at the column void volume because detection occurs after volatilization and evaporation of the mobile phase. In addition, an ELSD detector has an advantage over an RID with its capability for analysis using gradient elution conditions.

Here we introduce an example of analysis of oligosaccharides in Japanese sake using the Nexera-i integrated high-performance liquid chromatograph, which includes a built-in UV detector. The ELSD-LT Ⅱ evaporative light scattering detector was connected directly to the Nexera-i through an A/D acquisition board.

Analysis of a Standard Mixture of Isomaltooligosaccharides

A differential refractive index detector is typically used in the analysis of sugars, but it is limited because gradient elution cannot be used, therefore resulting in longer run times. Fig. 1 shows the chromatogram obtained from the analysis of a standard mixture of isomaltooligosaccharides using isocratic conditions, and Table 1 shows the analytical conditions that were used.

Table 1 Analytical Conditions: Isocratic Elution

Column	: Asahipak NH_2P-50 4E (250 mm L. × 4.6 mm I.D.)
Mobile Phase	: A : 10 mM Ammonium Acetate Buffer
	B : Acetonitrile
	Isocratic
	B 70 %
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Detection	: ELSD-LT Ⅱ
	Temperature : 40 °C
	Gain : 7
	Nebulizer Gas : N_2
	Gas Pressure : 350 kPa

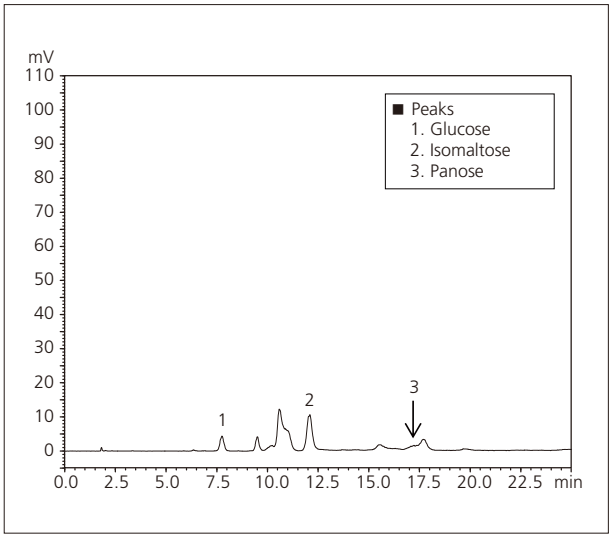

Fig. 1 Standard Solution of Isomaltooligosaccharides: Isocratic Elution

Under isocratic conditions, components that have long retention times tend to have broader peak shapes and diminished sensitivity because of the less intense response. Fig. 2 shows an example where a gradient was used for the same sample. The analytical conditions are described in Table 2. Using gradient elution with the ELSD permits separation of many components with high sensitivity because the narrower peaks produce a much higher signal.

Table 2 Analytical Conditions: Gradient Elution

Column	: Asahipak NH_2P-50 4E (250 mm L. × 4.6 mm I.D.)
Mobile Phase	: A : 10 mM Ammonium Acetate Buffer
	B : Acetonitrile
	Linear Gradient
	B 70 % → 40 %, 25 min
Flowrate	: 1.0 mL/min
Column Temp.	: 40 °C
Detection	: ELSD-LT Ⅱ
	Temperature : 40 °C
	Gain : 7
	Nebulizer Gas : N_2
	Gas Pressure : 350 kPa

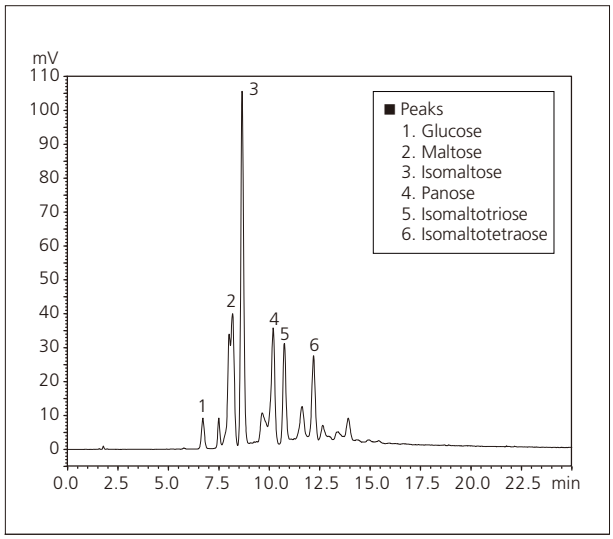

Fig. 2 Standard Solution of Isomaltooligosaccharides: Gradient Elution

Quantitative Analysis of Oligosaccharides Included in Sake

We used the ELSD to conduct quantitative analysis of glucose, isomaltose and panose that are present in sake. Pretreatment of the sake sample was performed according to the steps shown in Fig. 3. The calibration curves that were generated based on standard samples analyzed using the analytical conditions shown in Table 2 are shown in Fig. 4. Because the ELSD is not a spectroscopic detector, it does not obey Beer's law with a linear correlation between absorbance and concentration. Instead, a log-log plot of peak area and analyte quantity produces a linear response. Within the concentration range including 100, 200, 400, 1000, and 2000 mg/L (for glucose, 200, 400, 800, 2000, 4000 mg/L), excellent linearity was obtained with R^2 greater than 0.999. Fig. 5 shows the chromatogram of sake obtained using the conditions shown in Table 2. The quantitation results calculated using the calibration curves generated using the standard samples are shown in Table 3.

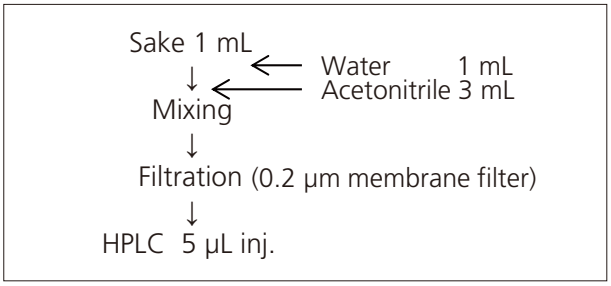

Fig. 3 Pretreatment of Sake

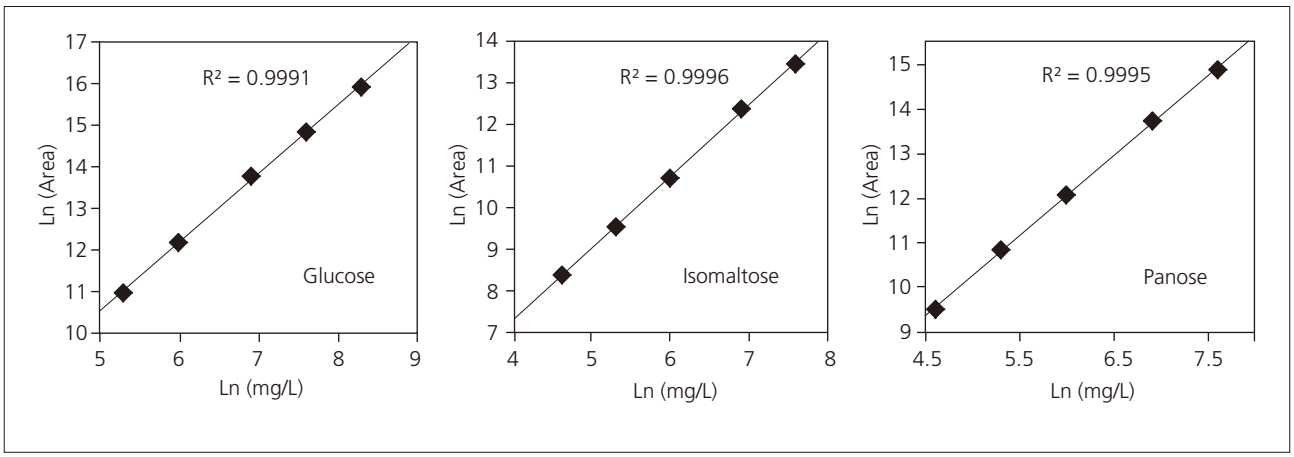

Fig. 4 Calibration Curves (Each injection 5 µL)

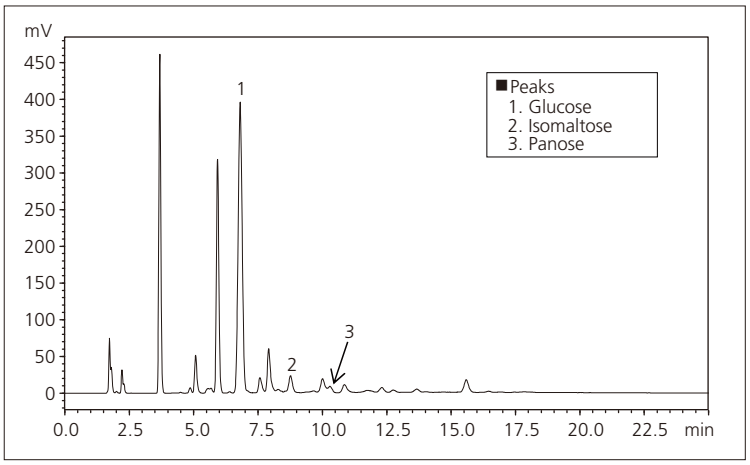

Fig. 5 Chromatogram of Sake

Table 3 Quantitation Results and Repeatability (n = 3) of Oligosaccharides in Japanese Sake

	Area	%RSD	Conc.
Glucose	432×10^4	0.6	13110 mg/L
Isomaltose	228×10^3	2.1	5130 mg/L
Panose	909×10^2	5.9	1410 mg/L

Note: The listed concentrations are the values obtained after converting the results obtained from the 5 µL measurement samples to the undiluted source solution.

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