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i-Series LC-2050C 3D, High Performance Liquid Chromatograph

Application News

Simultaneous Analysis of Monosaccharides and Oligosaccharides in Beer Using ELSD-LT III

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User Benefits

- Simultaneous analysis of saccharides by gradient elution can be performed using ELSD-LT III evaporative light scattering detector.
- Quantitative determination of Malto-oligosaccharides and isomalto-oligosaccharides in beer can be done owing to satisfactory separation of those saccharides each other.
- Dynamic range expansion function enables quantitative determination at optimum sensitivity without manual consideration of sensitivity settings even if there are large differences in concentration of target compounds in beer.

■ Introduction

Saccharide in beer is formed mainly through the saccharification of starch contained in malt and other raw materials, and one of the factors that determine the flavor of beer.

Evaporative Light Scattering Detector (ELSD) is suitable for the detection of no-UV/VIS absorbing compounds such as saccharides. ELSD is also applicable to gradient elution, allowing simultaneous analysis of multiple compounds.

In this Application News, we introduce a simultaneous analysis of monosaccharides and oligosaccharides contained in beer. Integrated high-performance liquid chromatograph i-Series LC-2050C with an ELSD-LT III was employed for this study. Hydrophilic interaction chromatography (HILIC), which is often used in saccharide analysis, enabled gradient elution using water and acetonitrile.

■ Analysis of Mixed Standard Solution

Three mixed standard solutions of fructose/sucrose, maltooligosaccharides (M1-M10), and isomaltooligosaccharides (I2-I5) were analyzed individually to confirm the separation of each compound. For the analysis of maltooligosaccharides, a mixed solution of ten compounds kit from Senshu Kagaku (P/N: BC-GM, concentration not disclosed) was used. For the analysis of fructose/sucrose and isomaltooligosaccharides, mixed solutions individually prepared with a mixture of water and acetonitrile (3:7) to make concentration of 50 mg/L for each were used.

Fig. 1 shows the overlaid chromatograms of respective mixed standard solutions, and Table 1 shows the analytical conditions. All the target compounds were analyzed within 50 minutes. Fig. 2 also shows an enlarged chromatograms of the retention time from 34 to 43 minutes (within the dotted box) in Fig. 1. Maltooligosaccharides and isomaltooligosaccharides were successfully separated.

Table 1 Analytical Conditions

System	:	i-Series LC-2050C
Column	:	Shodex HILICpak VG-50 4E (250 mm×4.6 mm I.D., 5 µm)
Mobile phase	:	A) Water B) Acetonitrile
Flow rate	:	1.0 mL/min
Time program	:	88%B (0-12 min)→83.5%B (25 min) →50%B (50 min)→88%B (50.1-60 min)
Mixer	:	40 µL
Column temp.	:	45 ℃
Injection volume	:	20 µL
Vial	:	SHIMADZU LabTotalTM for LC 1.5 mL, Glass*1
Detection (ELSD)	:	ELSD-LT III
Gain	:	Wide
Filter	:	4 sec.
Drift tube temp.	:	40 ℃
Nebulizer gas	:	N_2
Gas pressure	:	350 kPa

*1：227-34001-01

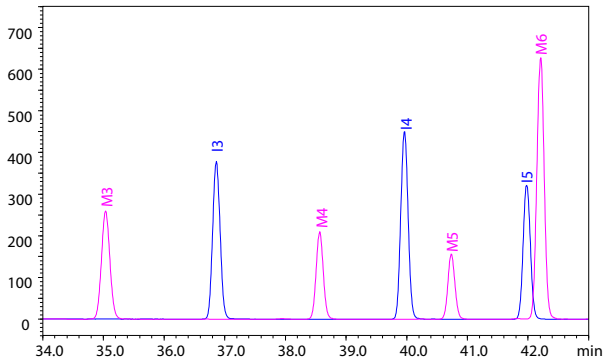

Fig.2 Separation of maltooligosaccharides and isomaltooligosaccharides (Enlarged figure of dotted box in Fig. 1)

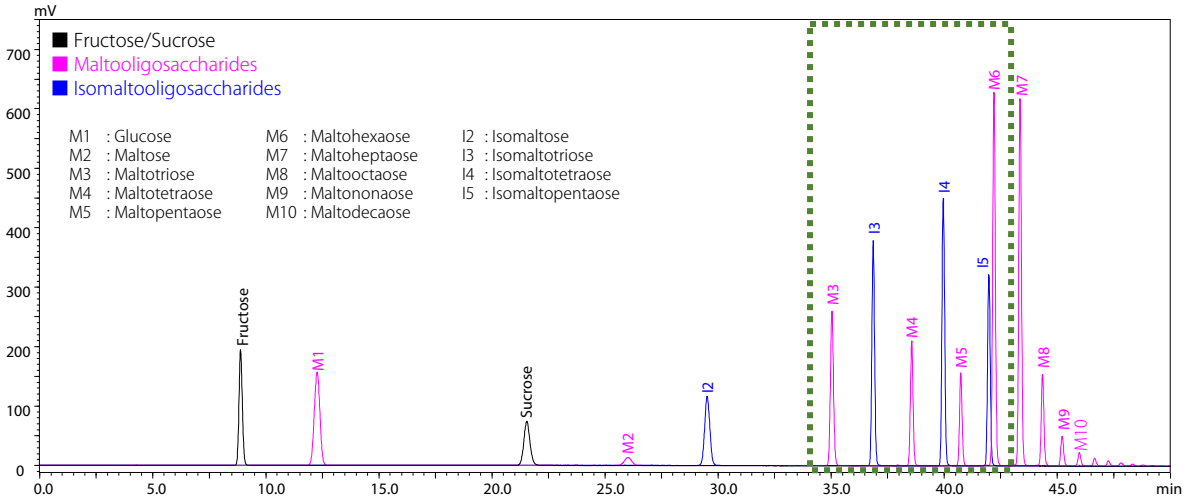

Fig.1 Chromatograms of respective mixed standard solutions (overlayed)

■ Calibration Curve

Standard solutions of 5, 10, 50, 100, and 200 mg/L were prepared for all the compounds of fructose/sucrose, maltooligosaccharides (M1 to M7), and isomaltooligosaccharides (I2 to I5). Calibration curves for all compounds prepared from the results of these analyses are shown in Fig. 3-5. Note that ELSD response is exponential to logarithm of the concentration, so the calibration curve is generally plotted using both logarithmic axes. the coefficients of determination were greater than 0.997 for all the target compounds.

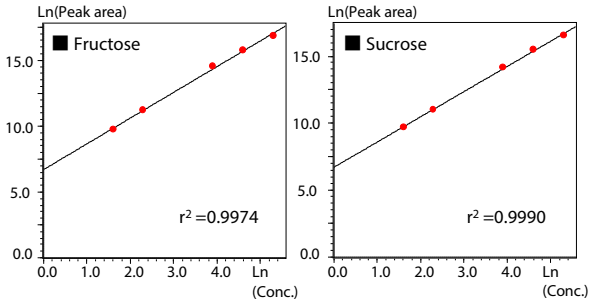

Fig. 3 Calibration curves for fructose and sucrose

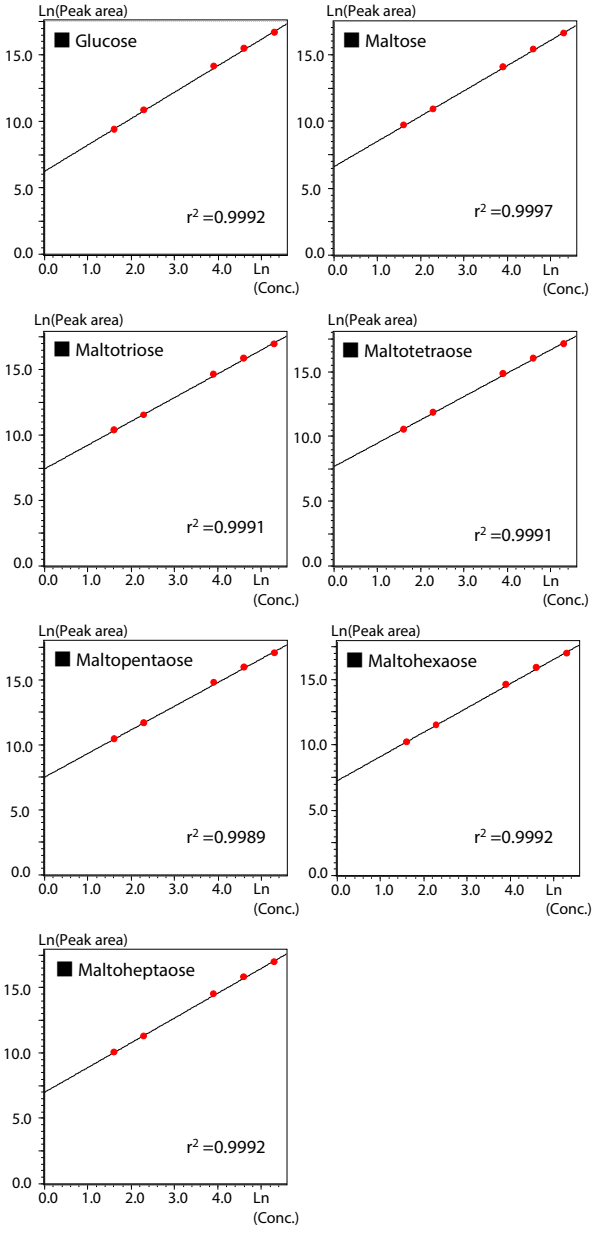

Fig. 4 Calibration curves for maltooligosaccharides (M1～M7)

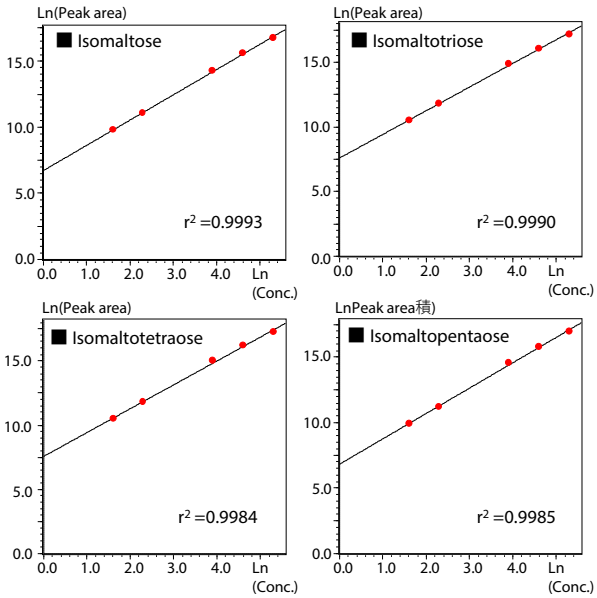

Fig. 5 Calibration curves for isomaltooligosaccharides

■ Repeatability

Repeatability evaluation was conducted by five times consecutive analyses of three mixed standard solutions of each 50 mg/L used for creating calibration curves. Table 2 and 3 show the of retention time and peak area respectively in relative standard deviation (%RSD). Satisfactory repeatabilities within 0.2% and 4% for retention time and peak area were obtained respectively for all the target compounds.

Table 2 Repeatabilities of retention time (n=5)

Compound	Average of retention time (min)	%RSD
Fructose	8.9	0.07
Sucrose	21.6	0.07
Glucose	12.4	0.11
Maltose	26.2	0.13
Maltotriose	35.1	0.05
Maltotetraose	38.6	0.04
Maltopentaose	40.8	0.04
Maltohexaose	42.3	0.04
Maltoheptaose	43.4	0.04
Isomaltose	29.5	0.13
Isomaltotriose	36.9	0.04
Isomaltotetraose	40.0	0.02
Isomaltopentaose	42.0	0.02

Table 3 Repeatabilities of peak area (n=5)

Compound	Average of peak area	%RSD
Fructose	2,013,719	0.63
Sucrose	1,392,641	1.38
Glucose	1,558,588	3.22
Maltose	1,430,044	3.12
Maltotriose	2,550,256	2.04
Maltotetraose	3,258,277	3.82
Maltopentaose	3,029,923	3.78
Maltohexaose	2,812,045	2.56
Maltoheptaose	2,666,494	3.28
Isomaltose	1,827,958	2.81
Isomaltotriose	3,356,850	2.57
Isomaltotetraose	3,794,042	1.74
Isomaltopentaose	2,744,466	2.67

■ Analysis of beer

● Sample analysis

Ten types of commercial beer (beer A-G, sugar-free beer H, low-malt beer I, and alcohol-free beer J) were prepared as samples. The ten samples were decarboxylated, diluted 20-fold with a water/acetonitrile = 1/1 mixture, filtered through a 0.2 µm membrane filter, and subjected to HPLC.

Fig. 6-15 show the chromatograms of respective beer samples. Peak identifications are indicated for compounds detected at concentrations above the lowest point of the calibration curve (5 mg/L). The ELSD-LT III's dynamic range extension function, which automatically optimizes sensitivity parameters, allowed simultaneous analyses of beer samples with large differences in concentration of contained target compounds.

Comparing the chromatograms of beer A, B, and C, the types and concentrations of contained saccharides were similar. On the other hand, dark colored beer D and craft beer E, F, and G tended to have higher concentrations of certain compounds compared to those in beer A-C. In sugar-free beer H, all the target compounds were present in trace amounts and were virtually undetectable. In addition, low-malt beer I had a chromatogram similar to that of beer A-C, whereas alcohol-free beer J contained more monosaccharides than those of beer A-C. Thus, a characterization of various types of beer was able to be conducted by comparing resulting chromatograms of monosaccharides and oligosaccharides.

M1 : Glucose
M2 : Maltose
M3 : Maltotriose
M4 : Maltotetraose
M5 : Maltopentaose
M6 : Maltohexaose
M7 : Maltoheptaose
I2 : Isomaltose
I3 : Isomaltotriose
I4 : Isomaltotetraose
I5 : Isomaltopentaose

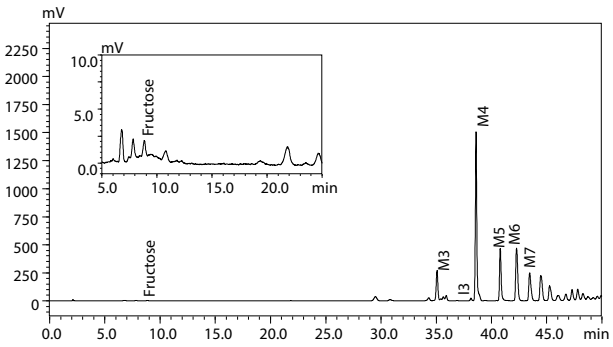

Fig. 6 Chromatogram of beer A

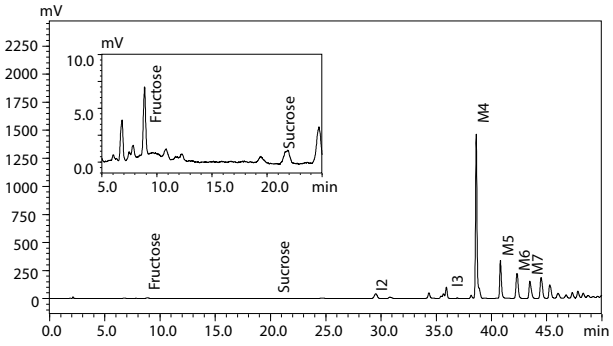

Fig. 7 Chromatogram of beer B

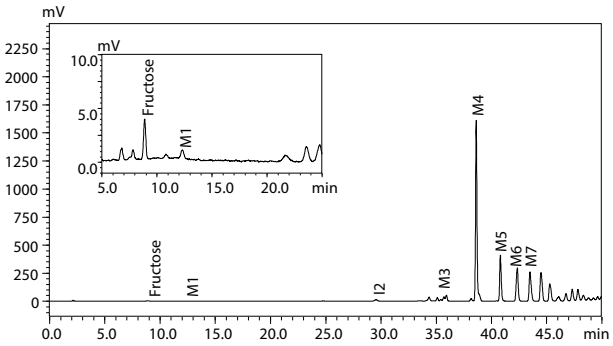

Fig. 8 Chromatogram of beer C

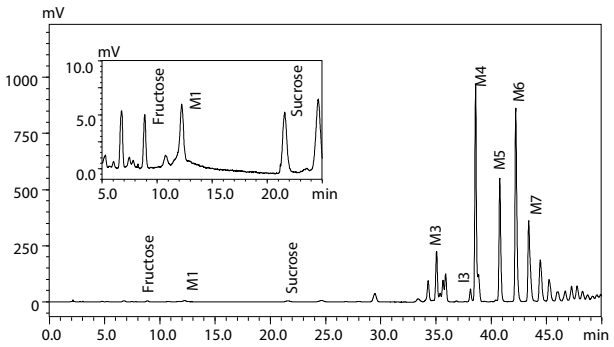

Fig. 9 Chromatogram of beer D

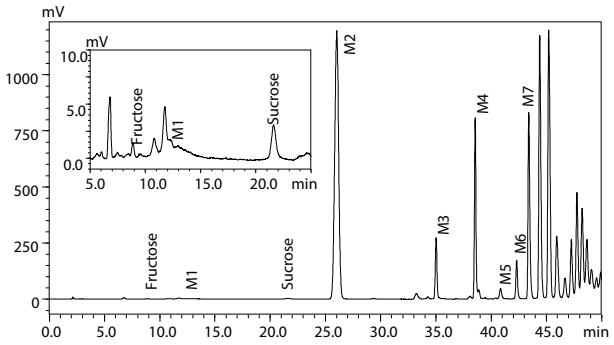

Fig. 10 Chromatogram of beer E

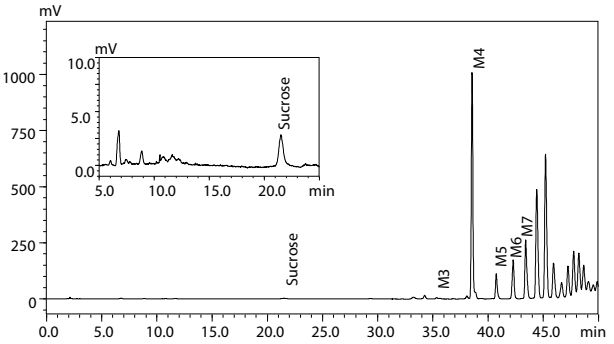

Fig. 11 Chromatogram of beer F

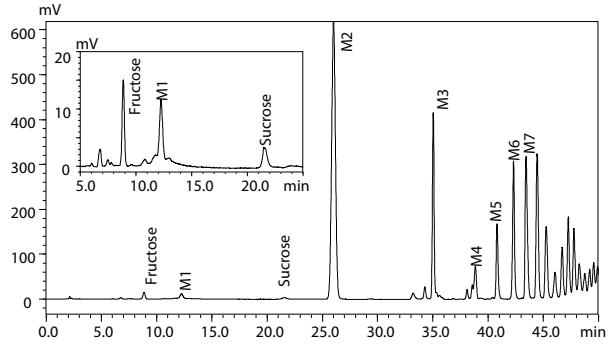

Fig. 12 Chromatogram of beer G

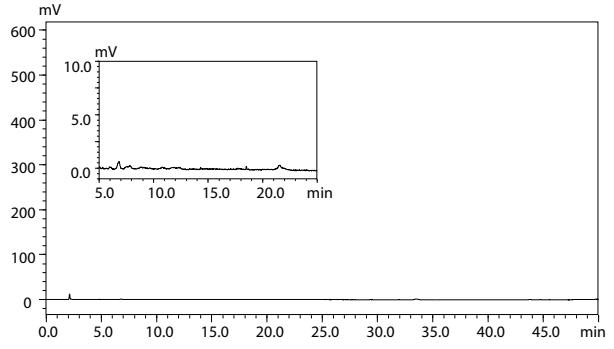

Fig. 13 Chromatogram of sugar-free beer H

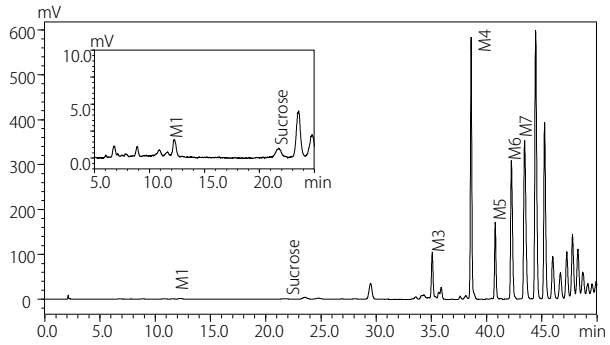

Fig. 14 Chromatogram of low-malt beer I

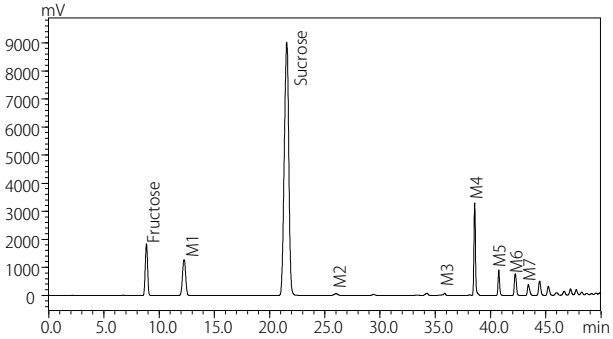

Fig. 15 Chromatogram of alcohol-free beer A

● Repeatability

Five consecutive analyses were performed on Beer A to determine monosaccharides and oligosaccharides and to evaluate repeatability. Table 4 shows the results of quantitative determination of each compound after pretreatment. Table 5 and 6 show the repeatabilities of retention time and peak area in terms of relative standard deviation (%RSD) respectively. Satisfactory repeatabilities within 0.2% and 4% for retention time and peak area were obtained respectively for all the target compounds.

Table 4 Concentrations of respective compounds in beer A

Compound	Average of concentration (mg/L)
Sucrose	10.3
Maltotriose	62.7
Maltotetraose	135.2
Maltopentaose	78.1
Maltohexaose	92.7
Maltoheptaose	76.3
Isomaltotriose	5.6

Table 5 Repeatabilities of retention time of respective compounds in beer A (n=5)

Compound	Average of retention time (min)	%RSD
Sucrose	21.6	0.11
Maltotriose	35.0	0.02
Maltotetraose	38.6	0.01
Maltopentaose	40.8	0.02
Maltohexaose	42.2	0.02
Maltoheptaose	43.4	0.02
Isomaltotriose	36.8	0.02

Table 6 Repeatabilities of peak area of respective compounds in beer A (n=5)

Compound	Average of peak area	%RSD
Sucrose	67,674	3.52
Maltotriose	3,115,892	1.26
Maltotetraose	15,084,463	1.56
Maltopentaose	5,169,858	0.50
Maltohexaose	6,369,978	0.94
Maltoheptaose	4,047,583	1.82
Isomaltotriose	46,088	1.17

● Spike-and-recovery rate

Fructose, sucrose, and maltooligosaccharides (M1-M7) standards were added to the Beer A sample to make 50 mg/L each, prepared and analyzed six times to evaluate spike-and-recovery rates. For the compounds that were not contained virtually in Beer A, the calculations were performed to consider that original concentrations in Beer A were 0 mg/L. As shown in Table 7, satisfactory results of recoveries ranging from 90 to 110% were obtained for all target compounds.

Table 7 Spike-and-recovery rates of respective compounds in beer A

Compound	Average of recovery(%)
Fructose	92.6
Sucrose	91.2
Glucose	108.6
Maltose	97.9
Maltotriose	103.5
Maltotetraose	93.6
Maltopentaose	97.0
Maltohexaose	93.4
Maltoheptaose	102.5

■ Conclusion

Simultaneous analysis of monosaccharides and oligosaccharides in beer was performed. The use of ELSD-LT III for detection enabled gradient elution of water and acetonitrile, resulting in satisfactory separation of fructose, sucrose, maltooligosaccharides, and isomaltooligosaccharides. In addition, satisfactory results were obtained for linearity, repeatabilities of both retention time and peak area, and spike-and-recovery rate.

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