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Application News

No. L571

High Performance Liquid Chromatography

# Analysis of 10 kinds of Maltooligosaccharides in a Soft Drink by ELSD-LT III

Saccharides are mainly analyzed by ligand exchange chromatography or hydrophilic interaction chromatography. HILIC can be applied to oligosaccharides having larger retention as well as monosaccharides and disaccharides. The combination of HILIC and gradient elution provides simultaneous analysis of saccharides in relatively short time. Saccharides show very narrow UV absorption wavelength range, from 190 nm to 195 nm. Therefore, a refractive detector (RID) is commonly used for this analysis. However, gradient elution cannot be used with RID because the baseline drifting derived from the change of mobile phase composition during gradient elution is practically unacceptable. So, RID is not suitable for a simultaneous separation of compounds that show widely different retention behaviors due to expected long analysis time without gradient elution. Evaporative light scattering detector (ELSD) is one of universal detector that detects the scattering light from the target compounds after nebulizing and evaporating the mobile phase. ELSD provides reduced analysis time and simultaneous separation of compounds that show widely different retention due to applicability to gradient elution.

The individual amounts of saccharides contained in foods are often grately different. Simultaneous analysis of such components requires different optimized sensitivity settings for individual compounds, and it is normally tedious procedures. “Wide function”, a new feature of ELSD-LT III used in this article, automatically optimizes a parameter that is related to sensitivity and a single method file can be used for data acquisition regardless of sample concentration, from low to high.

Here, 10 kinds of maltoorigosaccharides (G1～G10) in a soft drink were analyzed simultaneously by ELSD-LT III.

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## Simultaneous Analysis of 10 kinds of Maltoorigosuccharides

Table 1 shows the analytical conditions for 10 different maltoorigosaccharides. Fig. 1 shows the obtained chromatogram. Here, mixture of 10 maltoorigosaccharides, G1 ～ G10(Unknown concentration, Manufactured by Senshu Scientific co., ltd., model No. BC-GM) was used. 10 oligosaccharides of G1～G10 were able to be eluded within 20 minutes by gradient elution.

**Table 1 Analytical conditions**

| | |
|---|---|
| System | : Nexera™ XR |
| Column | : Shodex Asahipak NH2P-50 4E (250 mm x 4.6 mm I.D., 5 µm) |
| Mobile Phase | : A) Water<br>B) Acetonitrile |
| Time Program | : B. Conc. 70% (0 min) →40% (25 min) →70% (25.01 min) →70% (30 min) |
| Flow Rate | : 1 mL/min |
| Column Temp. | : 40 ℃ |
| Injection Vol. | : 10 µL |
| Vial | : LabTotal Vial for LC 1.5 mL, Glass*1 |
| Detection | : ELSD-LT III |
| Gain | : Wide |
| Filter | : 4 sec |
| Drift Tube Temp. | : 40 ℃ |
| Nebulizer Gas | : $N_2$ |
| Gas Pressure | : 350 kPa |

*1 P/N: 227-34001-01

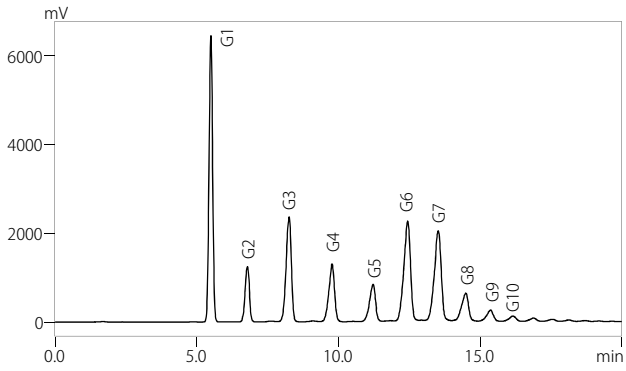


**Fig. 1 Chromatogram of Mixture of 10 maltoorigosacchrides (G1～G10)**

## Linearity and Repeatability

The standard solution of the mixture of oligosaccharides, G1～G7, were analyzed to create the respective calibration curve. Fig. 2. shows the obtained chromatogram (0.05 g/L each). Fig. 3 shows the calibration curves. The response of ELSD was plotted on double logarithmic axes because the logarithm of ELSD response is in proportion to the logarithm of concentration. The calibration curve of G1 was created using 5 different concentrations of 0.05, 0.10, 1.00, 1.50, 2.00 g/L. The calibration curves of other 6 compounds were created using 5 different concentration of 0.01, 0.05, 0.10, 0.25, 0.50. Table 2 shows the concentration ranges and linearities of respective calibration curves. Table 3 shows the repeatability. The repeatability was confirmed using repeated analyses at 0.05 g/L(n=6). From Table 2, the repeatability of retention time and area both showed good results.

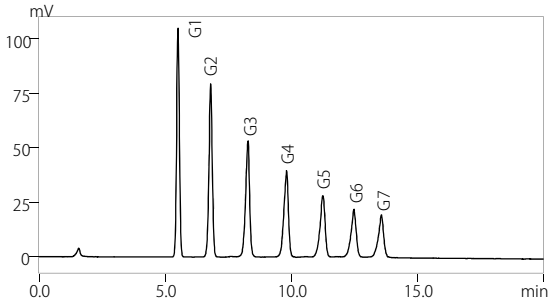


**Fig. 2 Chromatogram of Standard Solutions of 7 Maltoorigosaccharides Mixture (G1～G7) (0.05 g/L each)**

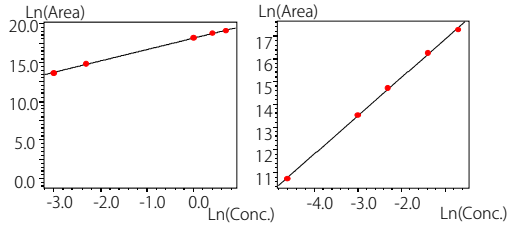


**Fig. 3 Calibration curves (Left: G1, Right: G2)**

**Table 3 Concentration Range and Linearity of the Calibration Curves**

| Compounds | Calibration Concentration range (g/L) | Linearity ($r^2$) |
|---|---|---|
| G1 | 0.05～2.00 | 0.9982 |
| G2 | 0.01～0.50 | 0.9991 |
| G3 | | 0.9997 |
| G4 | | 0.9996 |
| G5 | | 0.9995 |
| G6 | | 0.9993 |
| G7 | | 0.9995 |

**Table 4 Repeatability of 0.05 g/L(n=6)**

| Compounds | Retention Time (%RSD) | Area (%RSD) |
|---|---|---|
| G1 | 0.08 | 2.05 |
| G2 | 0.09 | 1.24 |
| G3 | 0.10 | 1.46 |
| G4 | 0.05 | 1.71 |
| G5 | 0.07 | 2.20 |
| G6 | 0.10 | 1.79 |
| G7 | 0.08 | 1.24 |

## Simultaneous Analysis of Oligosaccharides in a Soft Drink

This Analysis was carried out under the same analytical conditions shown in Table 1. A soft drink was filtered with a 0.2 µm membrane filter and diluted 20 times with water to analyze. Fig. 4 shows the chromatogram of the soft drink.

Table 4 shows the determination result of maltooligosaccharides in the soft drink. The concentration of G1 is much bigger than those of other maltooligosaccharides. Therefore, the calibration curve of G1 was created using different concentration range.

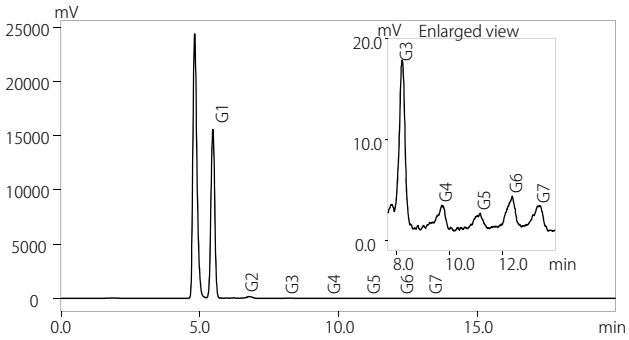


**Fig. 4 Chromatograms of a Soft Drink**

**Table 5 Determination result of Maltoorigosaccharides in the Soft Drink**

| Compounds | Concentration*1 (g/L) |
|---|---|
| G1 | 1.59 |
| G2 | 0.10 |
| G3 | 0.03 |
| G4 | 0.02 |
| G5 | 0.01 |
| G6 | 0.02 |
| G7 | 0.02 |

*1 Determination result of Sample Diluted 20 Times

## Wide function

A Wide function(Fig. 5) is newly equipped in ELSD-LT III. Using this function, the parameter related to sensitivity is automatically optimized. Therefore, a single method file are able to be used for data acquisition regardless of sample concentration even largely different concentration of target compounds are co-existing in a sample solution.


## Conclusion

Analysis of 10 maltooligosaccharides were carried out to confirm the separation performance of this method. Then the determination of maltooligosaccharides (G1～G7) in a soft drink was also carried out. 10 oligosaccharides in largely different concentrations were able to be determined without sensitivity adjustment using Wide function of ELSD-LT III.

First Edition: Sep. 2020

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