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Application News

No. L572

High Performance Liquid Chromatography

High Speed Analysis of Monosaccharides and Disaccharides in Soft Drinks by ELSD-LT III

Saccharides show very narrow UV absorption wavelength range, from 190 nm to 195 nm because hydroxyl group is the only major functional group that show UV absorption in the structure. 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.

Monosaccharides and disaccharides are mainly analyzed by anion-exchange chromatography or ligand exchange chromatography. However it takes a long time to analyze. So, in this article, monosaccharides and disaccharides were separated in short time by hydrophilic interaction chromatography (HILIC) and were detected by ELSD .

"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, high speed analysis of monosaccharides and disaccharide in soft drinks were carried out by ELSD-LT III.

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Analysis of 5 Standard Compounds

Table 1 shows the analytical conditions for 5 standard compounds of different saccharides (Fructose, Glucose, Sucrose, Maltose, Lactose). Fig. 1 shows the obtained chromatogram (1000 mg/L each). It is recommended to wash the column well before use. 5 saccharides eluded within 5 minutes by gradient elution.

Table 1 Analytical Conditions

System	:	Nexera™ XR
Column	:	Shinwa Chemical Industries Ltd. ULTRON AF-HILIC-CD (HT) (100 mm × 3 mm I.D., 2 µm) ULTRON AF-HILIC-CD (HT) Guard Cartridge (5 mm × 2 mm I.D., 2.0 µm)
Mobile Phase	:	A) 10 mmol/L Ammonium Acetate B) Acetonitrile
Time Program	:	B. Conc. 85% (0-0.5 min) → 82% (3-5 min) → 85% (5.01-8 min)
Flow Rate	:	0.8 mL/min
Column Temp.	:	45 °C
Injection Vol.	:	2 µL
Vial	:	LabTotal Vial for LC 1.5 mL, Glass *1
Detection	:	ELSD-LT III
Gain	:	Wide
Filter	:	4 sec
Drift Tube Temp.	:	40 °C
Nebulizer Gas	:	N_2
Gas Pressure	:	350 kPa

*1: P/N: 227-34001-01

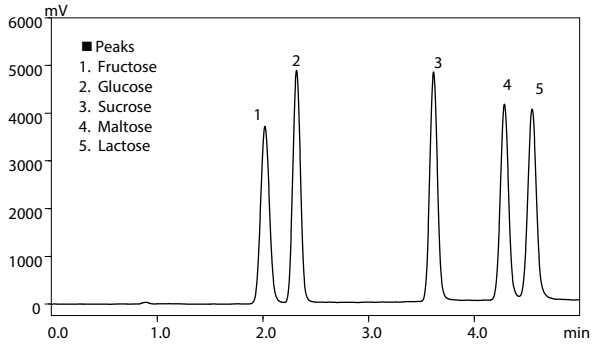

Fig. 1 Chromatogram of 5 Standard Sacchrides (1000 mg/L each)

Repeatability

Table 2 shows the repeatability confirmed by repeated analyses at 250 mg/L (n=6).

Table 2 Repeatability of 250 mg/L(n=6)

Compounds	Retention Time (%RSD)	Area (%RSD)
Fructose	0.05	2.33
Glucose	0.03	1.66
Sucrose	0.04	3.18
Maltose	0.03	2.28
Lactose	0.02	3.56

Linearity

Fig. 2 shows the calibration curves. Table 3 shows the concentration ranges of 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 curves of Fructose and Glucose were created using 6 different concentrations.

Table 3 Concentration Ranges of the Calibration Curves

Compounds	Calibration Concentration range (mg/L)
Fructose	500, 1000, 2000, 2500, 3000, 4000
Glucose	
Sucrose	50, 100, 250, 750, 1000, 1500
Maltose	
Lactose	

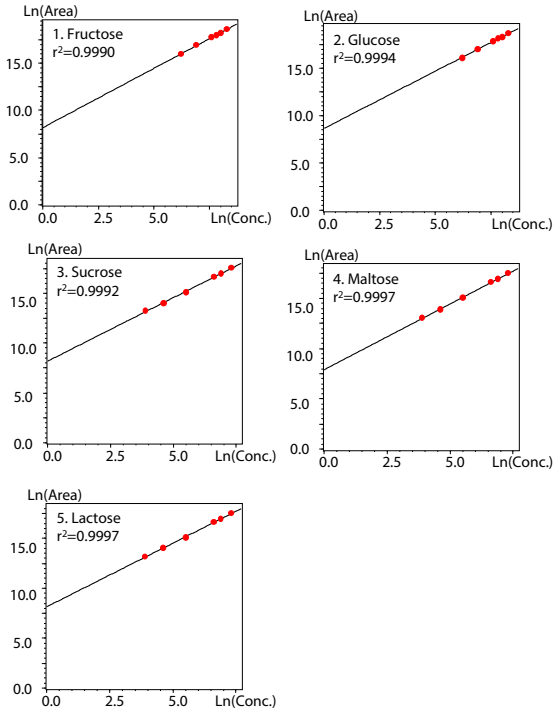

Fig. 2 Calibration Curves

High Speed Analysis of 5 Saccharides in Soft Drinks

This Analysis was carried out under the same analytical conditions shown in Table 1.

Fig. 3 shows the chromatogram of the soft drink A. Table 4 shows the determination result of saccharides in the soft drink A. The sample was filtered with a 0.2 µm membrane filter and diluted 20 times with water/acetonitrile (50:50) to analyze.

Fig. 4 shows the chromatogram of the soft drink B. Table 5 shows the determination result of saccharides in the soft drink B. After filteration with a 0.2 µm membrane filter and 20 times dilution with water/acetonitrile (50:50), obtained supernatant was analyzed.

5 saccharides were analyzed without sensitivity adjustment by reason of Wide function of ELSD-LT III.

The high speed analysis of 5 saccharides in soft drinks was able to be carried out.

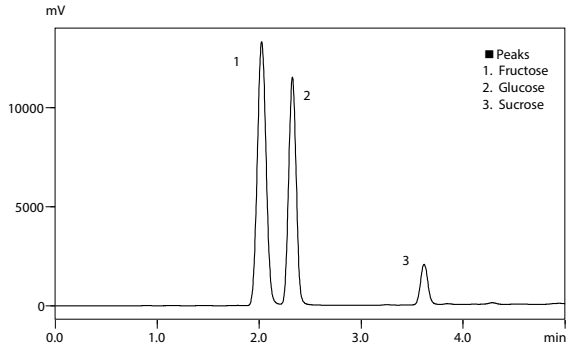

Fig. 3 Chromatogram of Soft Drink A

Table 4 Determination Result of Saccharides in Soft Drink A

Compounds	Retention Time (%RSD)	Area (%RSD)	Concentration*1 (mg/L)
Fructose	0.08	1.24	2920
Glucose	0.06	0.81	2113
Sucrose	0.08	1.06	510

*1: Average concentration in 20-times diluted sample (n= 6)

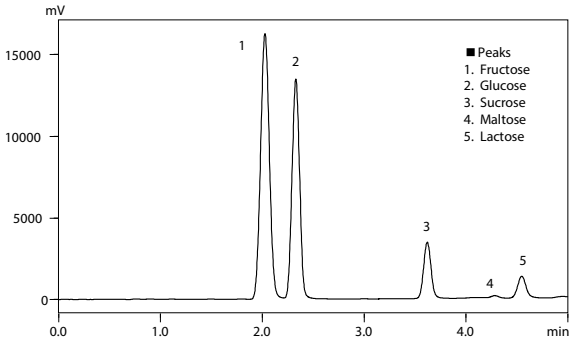

Fig. 4 Chromatogram of Soft Drink B

Table 5 Determination Result of Saccharides in Soft Drink B

Compounds	Retention Time (%RSD)	Area (%RSD)	Concentration *1 (mg/L)
Fructose	0.03	1.63	3448
Glucose	0.04	2.07	2432
Sucrose	0.05	3.21	786
Maltose	0.01	7.60	70
Lactose	0.04	1.29	424

*1: Average concentration in 20-times diluted sample (n= 6)

Conclusion

High speed analysis of monosaccharides and disaccharides were carried out to confirm the separation performance of this method. Then the determination of monosaccharides and disaccharides in soft drinks was also carried out. 5 saccharides were able to be determined simultaneously without sensitivity adjustment using Wide function of ELSD-LT III.

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