SHIMADZU APPLICATION NEWS

LA190-E016A

● HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No.L294A

Application of Evaporative Light Scattering Detector (Part 2) Analysis of Oligosaccharides in Beer

The Evaporative Light Scattering Detector (ELSD-LT) is universal detector for HPLC. It makes a light scattering measurement using a photomultiplier of target analytes that have been dried of mobile phase through evaporation. It detects most non-volatile compounds in sensitivity depending on their mass. The ELSD-LT is particularly suited for such applications as sugars, fats and surfactans that have low absorbance and are difficult to detect using UV detector.
This Application News introduces an example of analyzing oligosaccharides contained in beer, as an example of using the ELSD-LT for sugar analysis.

■ Comparison of ELSD-LT and RID in Sugar Analysis

In general, Refractive Index Detectors (RID) is used for analyzing sugars. However, RID has following disadvantages:
- detection sensitivity is insufficient;
- the baseline is easily affected by changes of the room temperature or flow rate;
- the number of substances that can be separated at one time is limited because gradient elution cannot be conducted.

Although it requires a volatile mobile phase, the ELSD has advantage in sensitivity and baseline stability, and is also capable of gradient elution.

Table 1 shows typical separation methods for sugar analysis, which is properly used by the analysis purpose. The nomal phase methods, which is suited to separating both monosaccharides and oligosaccharides, is widely used in the food industry. Table 1 also indicates the mobile phase normally used with each methods and the applicability of ELSD. The nomal phase method is suited to ELSD because it mainly uses volatile acetonitrile for the mobile phase.

Fig. 1 shows the comparison of sensitivity between the ELSD-LT and RID in sugars analyzing with the nomal phase method. A standard sample solution (5µL) containing 0.1g/L of fructose, glucose, sucrose and maltose was injected. Table 2 shows the analytical conditions. The ELSD-LT has better baseline stability and higher sensitivity for sugars.

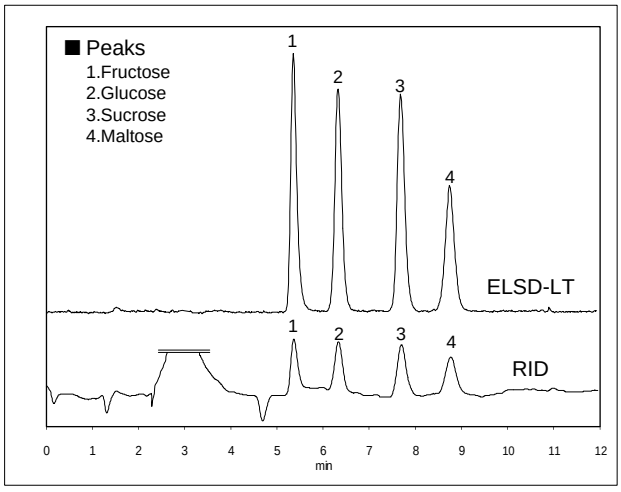

Fig.1 Chromatogram for Standard Mixture of 4 Sugars

Table 1 Separation Methods for Sugars

◎ Nomal phase	: Acetonitrile-water
△ Size exclusion	: Water, buffer solution
○ Ligand exchange	: Water
× Anion exchange	: Sodium hydroxide
× Borate complex anion exchange	: Borate buffer solution

ELSD applicability

◎: Optimal ○: Applicable △: Possible with water ×: Not applicable

Table 2 Analytical Conditions

Column	: NH2P- 50 (250mmL. × 4.6mmI.D.)
Mobile Phase	: Acetonitrile/ Water =7 / 3 (v /v %)
Flow Rate	: 1.0 mL/min
Column Temp.	: 30 ˚C
Detection	: ELSD-LT
	Temperature : 35 ˚C
	GAIN : 7
	Nebulizer Gas : N_2
	Gas Pressure : 350kPa
	: RID-10A

■ Analysis of Oligosaccharides in Beer

Oligosaccharides can be efficiently separated by combining the ELSD-LT with gradient elution.

Fig. 2 shows an example of analyzing oligosaccharides in beer using the isocratic and gradient elution methods. Table 3 shows the analytical conditions (condition (1) for isocratic elution and (2) for gradient elution). The beer was filtered through a membrane filter and 10μL was injected. Oligosaccharides exist in branched (1→6 glycoside bonds) or linear (1→4 glycoside bonds) structures, and they are eluted together. The chromatogram indicates elution times for monosaccharide, disaccharide, trisaccharide, and heptasaccharide linear oligosaccharides. As shown in the figure, the gradient elution method efficiently separates up to 20 forms. Fig. 3 through 6 show analysis examples of commercially marketed beers under the same gradient elution conditions.

Table 3 Analytical Conditions

Column	: NH2P- 50 (250mmL. × 4.6mmI.D.)
Mobile Phase	: (1)Acetonitrile/ Water=6 / 4 (v /v %)(Fig.2)
	(2)A:Acetonitrile
	B:Water
	Linear gradient B 30%→60%(Fig.3~Fig.6)
Flow Rate	: 1.0 mL/min
Column Temp.	: 40˚C
Detection	: ELSD-LT
	Temperature : 35˚C
	GAIN : 7
	Nebulizer Gas : N_2
	Gas Pressure : 350kPa

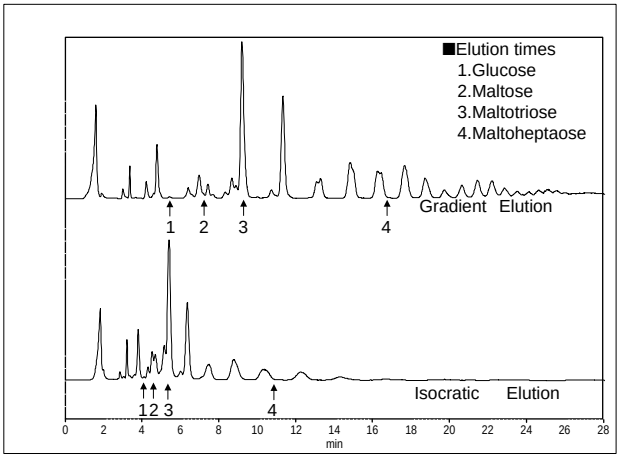

Fig.2 Chromatogram of Oligosaccharides in Beer

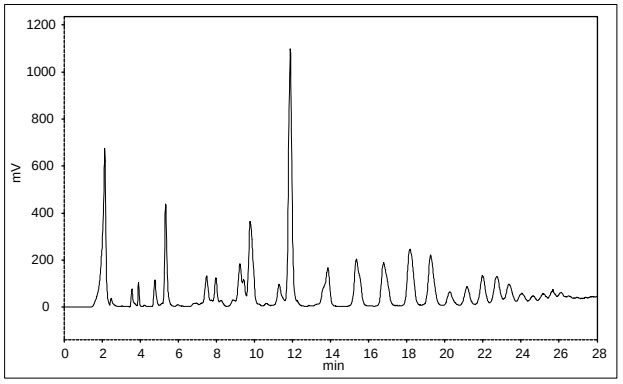

Fig.3 Chromatogram of Beer Sample A

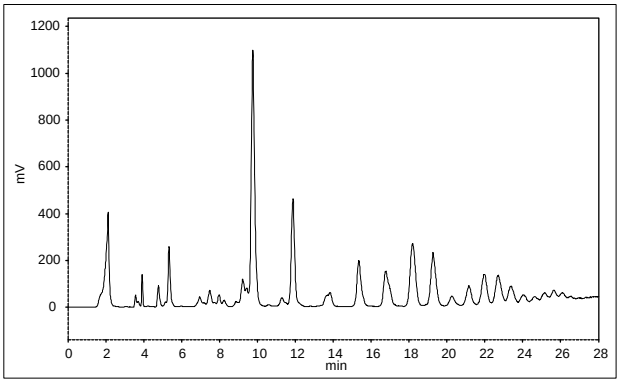

Fig.4 Chromatogram of Beer Sample B

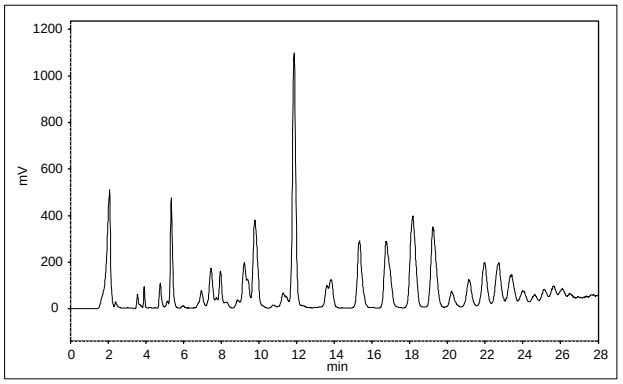

Fig.5 Chromatogram of Beer Sample C

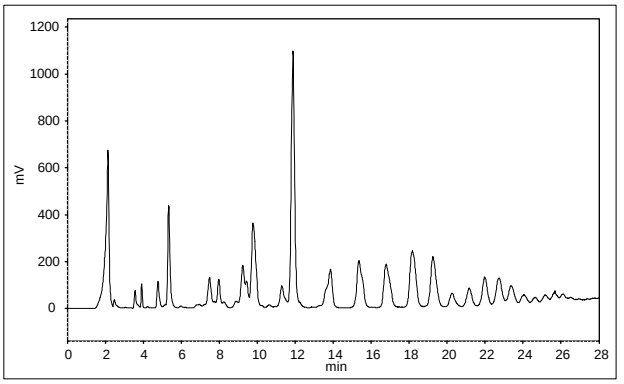

Fig.6 Chromatogram of Beer Sample D

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