

## Application News

No. L593

High Performance Liquid Chromatography Nexera™ lite, ELSD-LT III

### Simultaneous Determination of 15 Saccharides Without Interference by Salt in Seasonings

N. Iwata

#### User Benefits

- ◆ The gradient elution method enables highly sensitive analysis of 15 saccharides from monosaccharides to oligosaccharides.
- ◆ It is possible to separate glucose and galactose, which are known to coelute.
- ◆ Quantitation of sugars that exist in small amounts is possible by desalination, even when analyzing foods with large salt contents.

#### ■ Introduction

The relationship of sugars to factors that cause diabetes, obesity, allergies, and dementia has become clear. Although limiting sugar intake is effective for prevention and treatment of these conditions, seasonings tend to be overlooked when considering sugar limits, even though some seasonings actually contain large amounts of sugar.

In addition to sugar, some seasonings also contain a large amount of salt. In quantitative analysis of samples with high salt contents, salt frequently interferes with the analysis of sugars when using detectors with limited selectivity, such as a differential refractive index detector (RID) or an evaporative light scattering detector (ELSD). In this article, quantitation accuracy was improved by desalination of the real samples in the sample preparation process.

This article introduces analyses in which 15 saccharide compounds were separated by hydrophilic interaction liquid chromatography (HILIC) and detected with an ELSD-LT III detector. In addition, analyses of the saccharides in real samples of six types of commercially-available seasonings are also introduced.

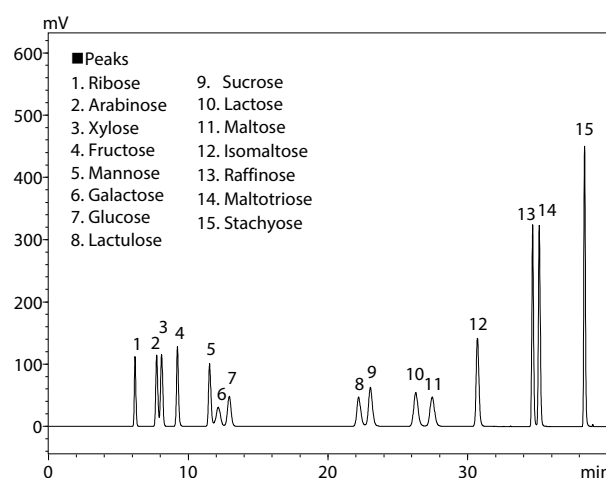


Fig. 1 Chromatogram of the Mixed Standard Solution of 15 Saccharides (100 mg/L Each)

#### ■ Analysis of Mixed Standard Solution of 15 Saccharides

The target compounds were 15 saccharides ranging from monosaccharides to tetrasaccharides (Table 1). Fig. 1 shows a chromatogram of the mixed standard solution of these 15 saccharides (100 mg/L each, prepared with 70% acetonitrile solution). Table 2 shows the analytical conditions. All 15 saccharides were able to be eluted in approximately 40 minutes by the gradient elution method. Although galactose and glucose are stereoisomers with the same molecular formula and are known to coelute in LC analysis, these two compounds were able to be separated under the analytical conditions in this article.

Table 1 Target Compounds

| Compound | Saccharide  |
|----------|-------------|
| 1        | Ribose      |
| 2        | Arabinose   |
| 3        | Xylose      |
| 4        | Fructose    |
| 5        | Mannose     |
| 6        | Galactose   |
| 7        | Glucose     |
| 8        | Lactulose   |
| 9        | Sucrose     |
| 10       | Lactose     |
| 11       | Maltose     |
| 12       | Isomaltose  |
| 13       | Raffinose   |
| 14       | Maltotriose |
| 15       | Stachyose   |

Table 2 Analytical Conditions

|                  |   |
|------------------|---|
| System           | : Nexera lite   |
| Column           | : Shodex HILICpak VG-50 4E<br>(250 mm × 4.6 mm I.D., 5 μm)                      |
| Flow rate        | : 1.0 mL/min  |
| Mobile phase     | : A) Water<br>B) Acetonitrile   |
| Time Program     | : 88%B (0-12 min) → 83.5%B (25 min)<br>→ 60%B (40-45 min) → 88%B (45.10-55 min) |
| Column temp.     | : 45 °C   |
| Injection volume | : 10 μL   |
| Vial             | : SHIMADZU LabTotal™ for LC 1.5 mL, Glass <sup>*1</sup>                         |
| Detection (ELSD) | : ELSD-LT III   |
| Gain             | : Wide  |
| Filter           | : 4 sec   |
| Drift Tube Temp. | : 40 °C   |
| Nebulizer Gas    | : N <sub>2</sub>  |
| Gas Pressure     | : 350 kPa   |

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

■ Repeatability

Table 3 shows the relative standard deviation (%RSD) of the retention times and peak areas based on six repeated analyses of the standard solution containing 100 mg/L each of the saccharides. The %RSD of retention times and peak areas were 0.2% or less and 6.3% or less, respectively.

Table 3 %RSD based on Six Repeated Analyses

|    | Compound    | Retention time | Peak area |
|----|-------------|----------------|-----------|
| 1  | Ribose      | 0.09           | 6.22      |
| 2  | Arabinose   | 0.06           | 5.23      |
| 3  | Xylose      | 0.04           | 5.44      |
| 4  | Fructose    | 0.04           | 6.12      |
| 5  | Mannose     | 0.00           | 4.66      |
| 6  | Galactose   | 0.15           | 5.71      |
| 7  | Glucose     | 0.09           | 6.04      |
| 8  | Lactulose   | 0.07           | 4.70      |
| 9  | Sucrose     | 0.04           | 3.68      |
| 10 | Lactose     | 0.05           | 3.80      |
| 11 | Maltose     | 0.05           | 3.18      |
| 12 | Isomaltose  | 0.04           | 2.59      |
| 13 | Raffinose   | 0.02           | 1.22      |
| 14 | Maltotriose | 0.02           | 1.15      |
| 15 | Stachyose   | 0.02           | 2.99      |

■ Calibration Curves

The linearities of the calibration curves of the 15 saccharides were good. The contribution ratio  $r^2 = 0.998$  or greater. The results were plotted as a log-log graph because the intensity of ELSD is an exponential response to concentration. Fig.2 shows the calibration curves, and Table 4 summarizes the concentration ranges of the calibration curves and the contribution ratio.

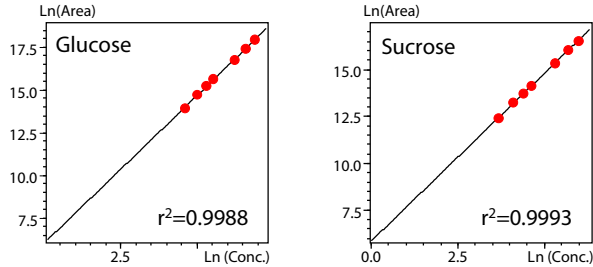


Fig. 2 Calibration Curves

Table 4 Concentration Ranges of Calibration Curves and Contribution Ratio ( $r^2$ )

|    | Compound    | Conc. range (mg/L) | $r^2$  |
|----|-------------|--------------------|--------|
| 1  | Ribose      | 10-80              | 0.9982 |
| 2  | Arabinose   | 10-80              | 0.9991 |
| 3  | Xylose      | 10-80              | 0.9988 |
| 4  | Fructose    | 100-1000           | 0.9980 |
| 5  | Mannose     | 10-100             | 0.9991 |
| 6  | Galactose   | 40-400             | 0.9992 |
| 7  | Glucose     | 100-1000           | 0.9988 |
| 8  | Lactulose   | 10-80              | 0.9986 |
| 9  | Sucrose     | 40-400             | 0.9993 |
| 10 | Lactose     | 10-80              | 0.9998 |
| 11 | Maltose     | 10-400             | 0.9991 |
| 12 | Isomaltose  | 10-400             | 0.9990 |
| 13 | Raffinose   | 10-100             | 0.9993 |
| 14 | Maltotriose | 10-80              | 0.9997 |
| 15 | Stachyose   | 10-80              | 0.9999 |

■ Analysis of BBQ Sauce

The sample used here was commercially-available BBQ sauce. Fig. 3 shows the sample preparation protocol. The BBQ sauce was extracted with a 70% acetonitrile solution to make 20 mL using a volumetric flask. Extracts were then prepared by centrifugation and filtration.

Fig. 4 shows the chromatogram. Fructose, glucose, and maltose were detected from the BBQ sauce.

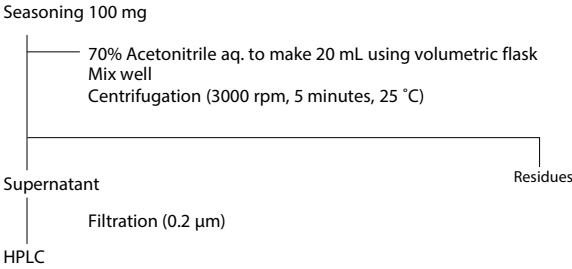


Fig. 3 Sample Preparation Protocol (BBQ Sauce)

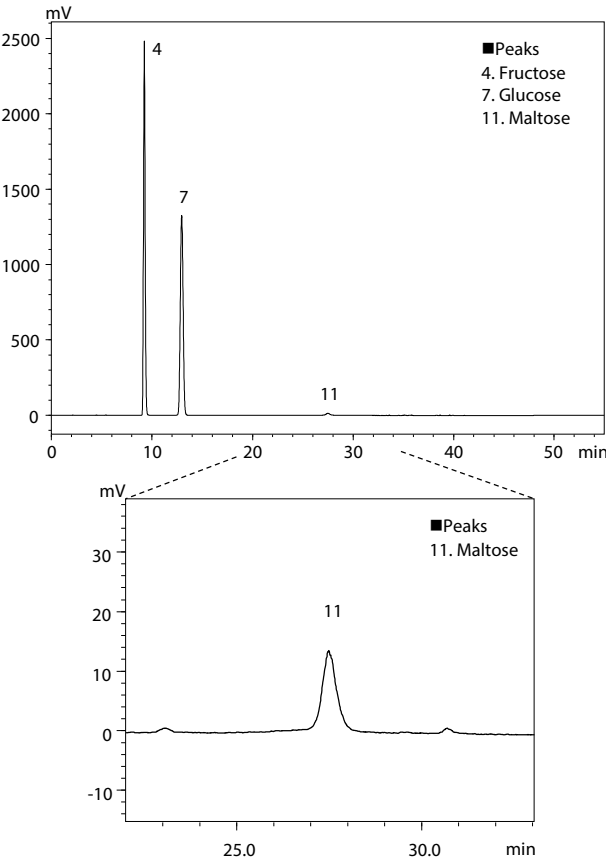


Fig. 4 Chromatogram of BBQ Sauce

## ■ Analysis of Steak Sauce and Ketchup

The samples used here were commercially-available steak sauce and ketchup. Fig. 5 shows the sample preparation protocol. The steak sauce and the ketchup were extracted with 70% acetonitrile solution to make the respective volumes using volumetric flasks shown in Fig. 5. Desalination was carried out with a solid phase extraction (SPE) cartridge in the cation exchange mode because these samples contained salt, which would interfere with quantitation of saccharides that existed in small amounts. The SPE cartridge used here was a Maxi-Clean™ SPE 0.5 mL IC-H (H<sup>+</sup> type) manufactured by S\*Pure Pte. Ltd. As the operation method, the supernatant obtained in the previous process was loaded in the SPE cartridge, which had been conditioned with ultrapure water. The initial 2 mL of the eluate was discarded, and the following eluate was filtered with a 0.2 µm membrane filter.

Fig. 6 and Fig. 7 show the chromatograms of the two samples. The peak appeared at approximately 30 minutes is salt. Desalination with the IC-H made it possible to quantitate the small amounts of maltose and isomaltose, which had been hidden by the salt.

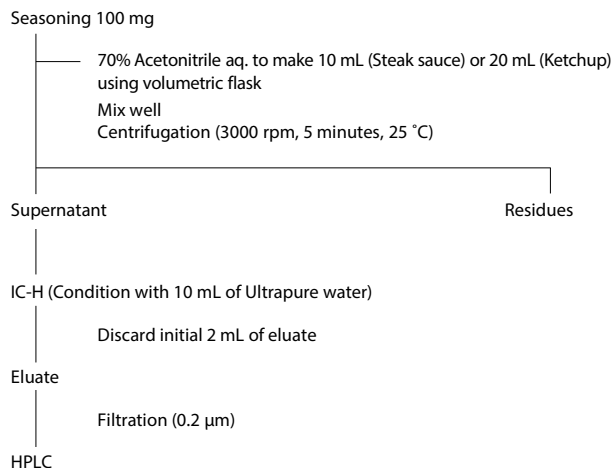


Fig. 5 Sample Preparation Protocol (Steak Sauce and Ketchup)

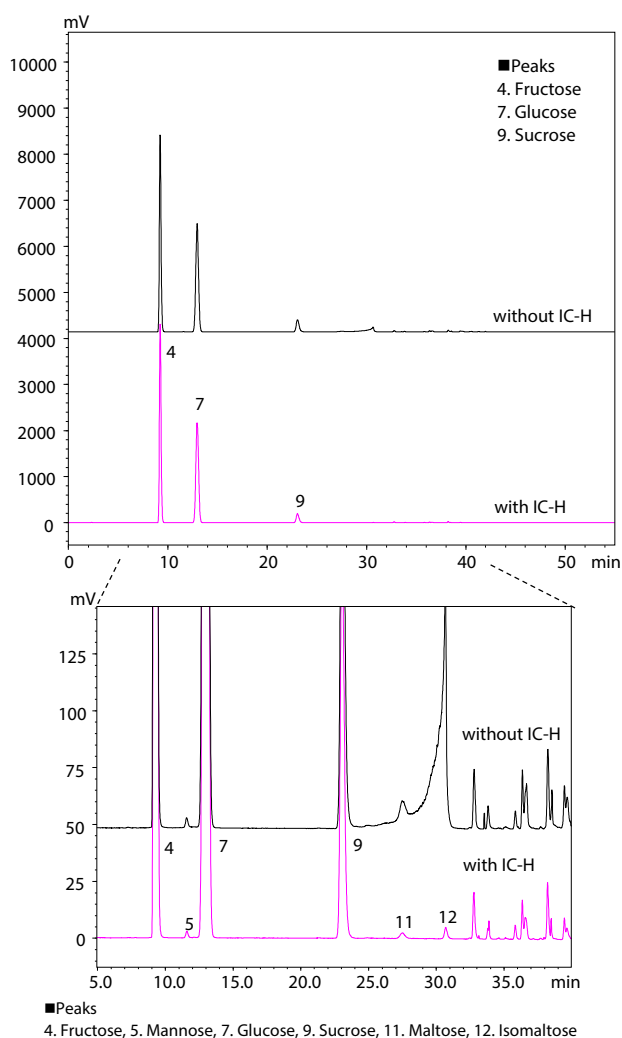


Fig. 6 Chromatograms of Steak Sauce

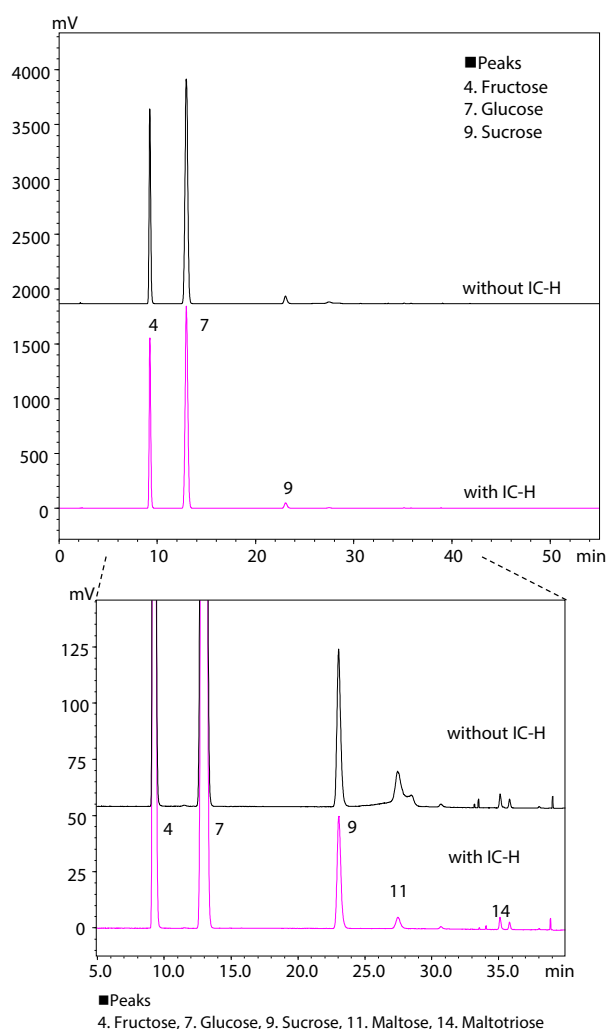


Fig. 7 Chromatograms of Ketchup

## ■ Analysis of Soy Sauce and Black Vinegar

The samples used here were commercially-available soy sauce and black vinegar. Fig. 8 shows the sample preparation protocol. The soy sauce and black vinegar were extracted with 70% acetonitrile solution to make 5 mL using volumetric flasks, respectively. Desalination was carried out with the IC-H because these samples, like the steak sauce, have high concentrations of salt.

Fig. 9 and Fig. 10 show the chromatograms of the two samples. As a result of desalination with the IC-H, it was possible to confirm that these samples contained no saccharides which were hidden by the high concentration of salt. Furthermore, although galactose and glucose were coexisted in the soy sauce, separation and determination of these two compounds were possible.

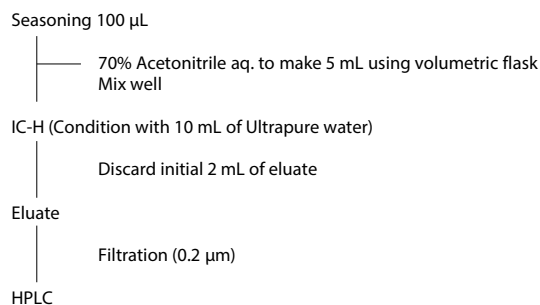


Fig. 8 Sample Preparation Protocol (Soy Sauce and Black Vinegar)

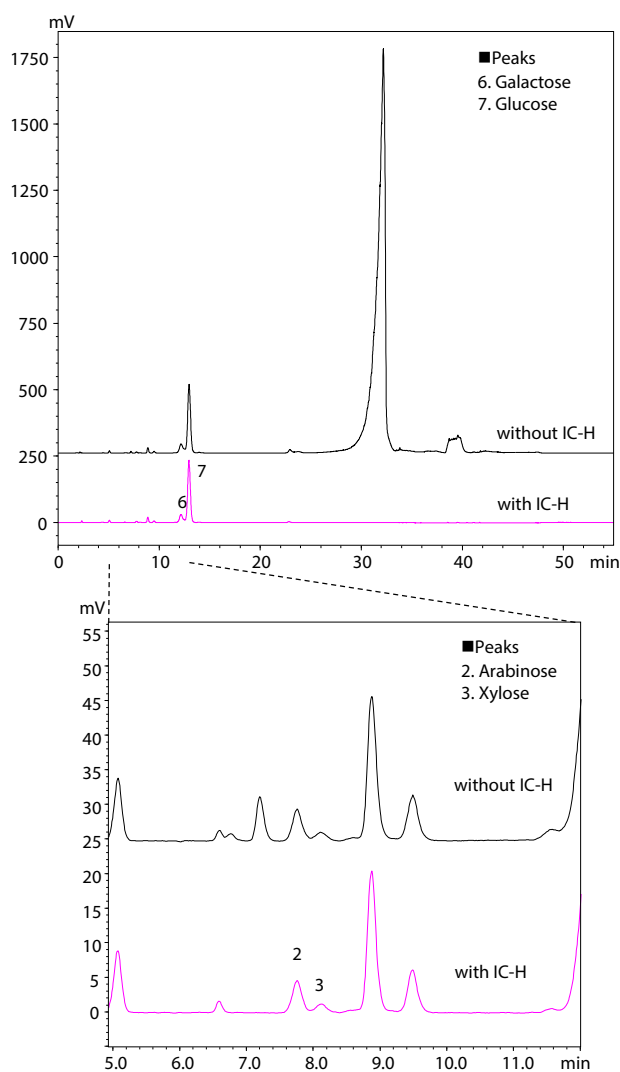


Fig. 9 Chromatograms of Soy Sauce

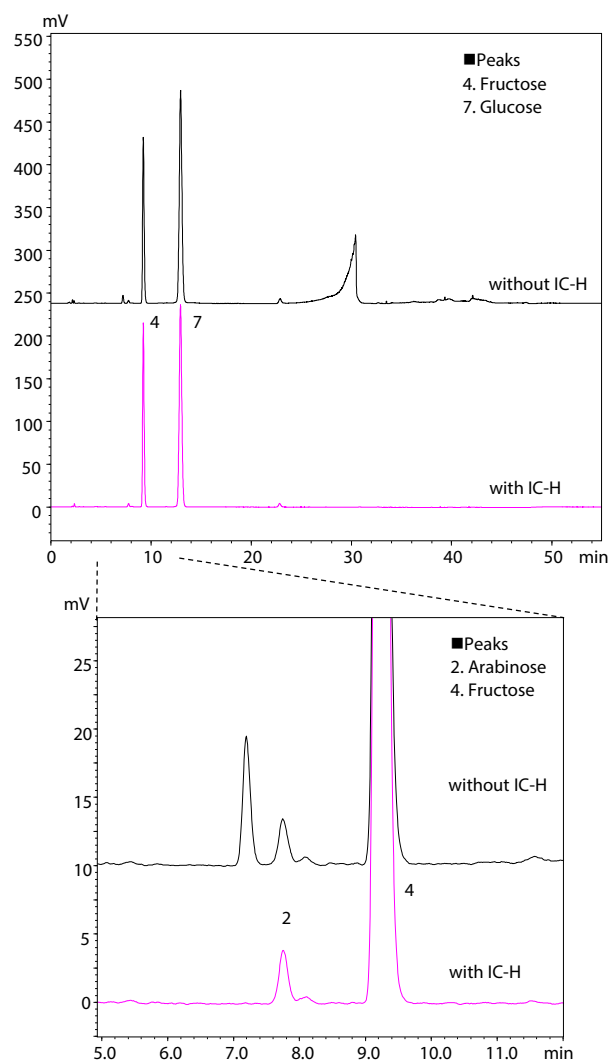


Fig. 10 Chromatograms of Black Vinegar

## ■ Recovery Rates

Five ketchup samples were spiked with standards of fructose, glucose and sucrose to make concentration of 2000 mg/100 g each, and then sample preparation was performed. Table 5 shows the average recovery rates obtained from the results of five samples.

Table 5 Recovery Rates (N=5)

| N                 | Recovery rates (%) |                |                |
|-------------------|--------------------|----------------|----------------|
|                   | Fructose           | Glucose        | Sucrose        |
| 1                 | 82.0               | 80.0           | 93.3           |
| 2                 | 92.5               | 91.7           | 99.2           |
| 3                 | 83.2               | 81.5           | 88.1           |
| 4                 | 88.2               | 80.8           | 94.1           |
| 5                 | 82.8               | 80.3           | 95.3           |
| Average<br>(%RSD) | 85.8<br>(0.91)     | 82.9<br>(0.64) | 94.0<br>(2.05) |

## ■ Analysis of Balsamic Vinegar

The sample used here was commercially-available balsamic vinegar. Fig. 11 shows the sample preparation protocol. The balsamic vinegar was extracted with 70% acetonitrile solution to make 20 mL using a volumetric flask. Extracts were then prepared by filtration.

Fig. 12 shows the chromatogram. Fructose and glucose were detected in the balsamic vinegar.

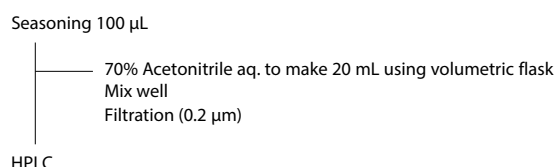


Fig. 11 Sample Preparation Protocol (Balsamic Vinegar)

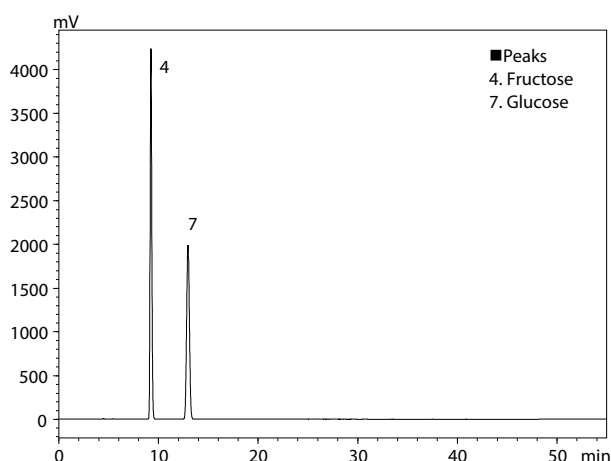


Fig. 12 Chromatogram of Balsamic Vinegar

## ■ Concentration of Saccharides in Seasonings

Table 6 shows the concentrations of the saccharides in the six seasonings. In this table, concentration means the concentration after sample preparation.

Table 6 Concentration of Saccharides in Six Seasonings

| Compounds      | Concentration (mg/L) |             |         |           |               |                  |
|----------------|----------------------|-------------|---------|-----------|---------------|------------------|
|                | BBQ sauce            | Steak sauce | Ketchup | Soy sauce | Black vinegar | Balsamic vinegar |
|                | Dilution ratio       |             |         |           |               |                  |
|                | 200                  | 100         | 200     | 50        | 50            | 200              |
| 2 Arabinose    | N.D.                 | N.D.        | N.D.    | 21.2      | 18.5          | N.D.             |
| 3 Xylose       | N.D.                 | N.D.        | N.D.    | 12.0      | N.D.          | N.D.             |
| 4 Fructose     | 541.6                | 772.7       | 398.5   | N.D.      | 116.1         | 757.0            |
| 5 Mannose      | N.D.                 | 17.1        | N.D.    | N.D.      | N.D.          | N.D.             |
| 6 Galactose    | N.D.                 | N.D.        | N.D.    | 95.8      | N.D.          | N.D.             |
| 7 Glucose      | 596.6                | 803.9       | 710.1   | 214.9     | 210.2         | 757.0            |
| 9 Sucrose      | N.D.                 | 182.8       | 84.6    | N.D.      | N.D.          | N.D.             |
| 11 Maltose     | 56.6                 | 22.1        | 31.7    | N.D.      | N.D.          | N.D.             |
| 12 Isomaltose  | N.D.                 | 17.2        | N.D.    | N.D.      | N.D.          | N.D.             |
| 14 Maltotriose | N.D.                 | N.D.        | 12.5    | N.D.      | N.D.          | N.D.             |

## ■ Conclusion

A total of 15 saccharides including monosaccharides, disaccharides and oligosaccharides were separated by hydrophilic interaction liquid chromatography (HILIC) and detected by the ELSD-LT III detector.

Steak sauce, ketchup, soy sauce and black vinegar contained salt. Therefore, the salt in those samples was removed by using a H<sup>+</sup> type SPE cartridge in the sample preparation process. As a result, quantitation of the small amounts of saccharides that had been hidden by the salt was possible. This experiment also confirmed that no saccharides were hidden by the high concentration of salt.

Therefore, it is considered that foods containing salt other than seasonings are able to be applied by homogenization because this pretreatment and analytical methods were able to be applied to paste-like seasonings.

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