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Application News

Analysis of Saccharides Using Integrated HPLC and Size Exclusion-Ligand Exchange (Ca Type) Column

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User Benefits

- Ca-type size exclusion-ligand exchange column retain sugar alcohols more strongly than Na-type column and can separate sugar alcohols and monosaccharides more selectively.
- Just water can be employed as mobile phase, requiring no mobile phase preparation.
- The integrated HPLC system allows for the installation of a refractive index detector without changing the footprint.

■ Introduction

Saccharides are the main component of sweetness in food and an important source of energy. However, excessive saccharide intake has been reported to increase the risk of lifestyle-related diseases such as obesity and dental caries[1)]. Monitoring the amount of saccharide in food plays an important role in quality control and nutritional management.

Since saccharides are hydrophilic compounds, reversed phase chromatography, which provides mutual separation of analytes based on hydrophobic interaction, is not suitable for saccharide analysis. In this study, ligand exchange chromatography is applied to saccharides separation. The fundamental principle of this separation mode is size exclusion chromatography, in which analytes are separated based on differences in molecular size in solution. Furthermore, saccharides are retained through complex formation between metal counterions and the hydroxyl groups of saccharides and eluted through ligand exchange with water molecules in the mobile phase. Saccharides can be reliably separated by utilizing the difference in complex formation between metal cations on the stationary phase surface and respective saccharides.

Here, Saccharide analysis using size exclusion-ligand exchange (Ca-type) column with integrated HPLC is introduced.

■ Analyses of standard solutions

Shim-pack™ SCR-101C column was used for analysis. The retention of saccharides varies depending on the metal counterions. When the packing material with calcium as the counterion is employed, the retention of saccharides tends to be stronger than when sodium is employed as the counterion. Consequently, more selective retention of sugar alcohols and monosaccharides can be accomplished.

Saccharide anomers may be separated to form two peaks at low temperature in ligand exchange chromatography. So, around 80 ˚C column temperature is generally required.

There is no need to prepare the mobile phase and simple analysis can be done since just water is used as the mobile phase. Detection was performed using a differential refractive index detector.

Table 1 shows the analytical conditions, and Fig. 1 shows the appearance of integrated HPLC used for analysis. Fig. 2 shows the chromatogram of a mixed standard solution of four saccharides (sucrose, glucose, fructose, and sorbitol).

Table 1 Analytical conditions

System	: LC-2070
Column	: Shim-pack SCR-101C *1 (300 mm × 7.9 mm I.D., 10 µm)
Guard column	: Shim-pack SCR-101C (G) *2 (50 mm × 4 mm I.D., 10 µm)
Mobile phase	: Water
Column temperature	: 80 ˚C
Flow rate	: 1.0 mL/min
Injection volume	: 10 µL
Vial	: TORAST Vial *3
Detection	: Refractive index detector (RID-20A) Polarity +, Cell temp. 40 ˚C, Response 1.5 sec

*1 P/N：S228-17889-91, *2 P/N：S228-17891-91, *3 P/N：GLCTV-901

Fig. 1 Appearance of integrated HPLC
(1)LC-2070 (2)RID-20A (3)Reservoir tray

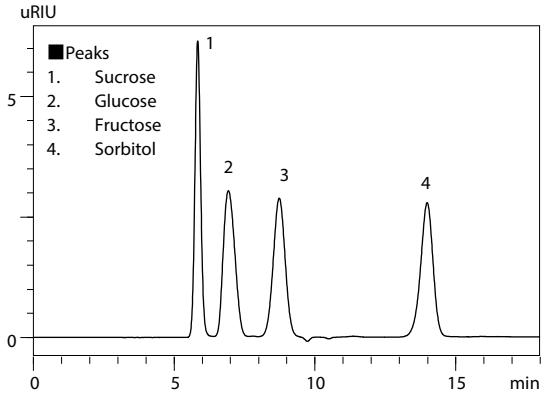

Fig. 2 Chromatogram of four saccharides (each 1000 mg/L)

■ Calibration curves

Table 2 shows coefficients of determination of respective calibration curves of saccharides created from the analyses of mixed standard solutions. Calibration curves were created in the concentration range of 20 to 2000 mg/L. All the calibration curves showed good linearity with coefficients of determination of r2 = 0.9999 or higher.

Table 2 Coefficients of determination of calibration curves

Compound	Linearity(r^2)
Sucrose	0.9999
Glucose	0.9999
Fructose	0.9999
Sorbitol	0.9999

■ Method repeatability

Table 3 shows the relative standard deviation of retention times and peak areas obtained by six times consecutive analyses of mixed standard solution of 50 mg/L for all saccharides. Relative standard deviations of retention times were less than 0.2%, and those of area values were less than 1.2%.

Table 3 Method repeatability for six times consecutive analyses (Each 50 mg/L)

Compound	Retention time(%RSD)	Peak area(%RSD)
Sucrose	0.00	0.77
Glucose	0.12	1.2
Fructose	0.09	0.79
Sorbitol	0.04	0.94

■ Analyses of real samples

Figs. 3 and 4 show the chromatograms of soft drink and energy drink. Soft drink and energy drink were diluted 50 times with water. The diluted samples were thoroughly mixed and analyzed after being filtered through 0.2 µm membrane filter[*4]. Table 4 shows saccharides contents of respective samples.

*4 Pall Acrodisc 13 mm with 0.2 um PVDF P/N：4455T

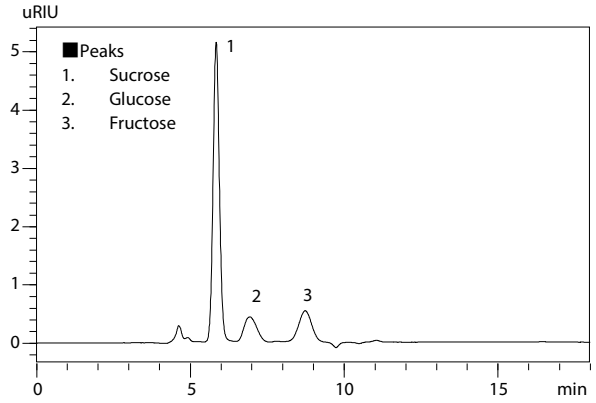

Fig. 3 Chromatogram of soft drink

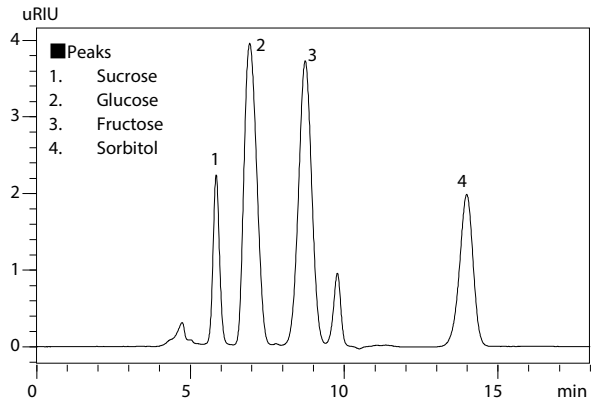

Fig. 4 Chromatogram of energy drink

Table 4 Saccharide contents in each sample

Sample	Unit	Sucrose	Glucose	Fructose	Sorbitol
Soft drink	mg/L	4.2×10^4	6.9×10^4	9.2×10^4	N.D
Energy drink	mg/L	1.8×10^4	6.5×10^4	6.5×10^4	3.6×10^4

N.D.=Not Detected

500 mg/L of standard sucrose, glucose, fructose, and sorbitol were spiked to respective samples and evaluated spike and recovery rates. Table 5 shows the results of the spike and recovery tests. Satisfactory results of recovery rates from 86 to 99% for all compounds were obtained.

Table 5 Spike and recovery rates from each sample

Sample	Sucrose	Glucose	Fructose	Sorbitol
Soft drink	90	97	96	98
Energy drink	94	94	86	91

■ Conclusion

Simultaneous analyses of saccharides in soft drink and energy drink were conducted. Saccharides were detected using integrated HPLC connected to differential refractive index detector. Separation was performed using a Shim-pack SCR-101C column in size exclusion -ligand exchange mode. Not only monosaccharides such as glucose and fructose, but also sorbitol, a type of sugar alcohol, was able to be separated. Satisfactory results were obtained for the linearity of the calibration curves for respective compounds, the reproducibility of retention times for standard solutions, the spike and recovery rates.

<References>

1) World Health Organization (2015)
Guideline：Sugars intake for adults and children
〈http://apps.who.int/iris/bitstream/10665/149782/1/9789241549028_eng.pdf〉

<Related Applications>

1. Analysis of saccharides using integrated HPLC and size exclusion-ligand exchange (Na type) column 01-00931-EN

01-00932-EN First Edition: Oct. 2025

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