SHIMADZU
Excellence in Science

Application News

LCMS-2050 High Performance Liquid Chromatograph Mass Spectrometer

Simultaneous Analysis of Saccharides in Non-sugar Beverages Using Single Quadrupole Mass Spectrometer

Natsuki Iwata and Miho Kawashima

User Benefits

- The LCMS-2050 can detect saccharides with higher selectivity and sensitivity than differential refractive index detectors or evaporative light scattering detectors.
- The analysis is unaffected by the matrix, allowing simultaneous determination of trace amounts of saccharides.

■ Introduction

In 2015, the World Health Organization (WHO) issued guidelines that daily sugar intake should be kept below 25 g.[1)] As a standard value stipulated in the Food Labeling Standards of the Consumer Affairs Agency of Japan, a beverage can be labeled as "non-sugar" or "sugar free" if the saccharide content is less than 0.5 g per 100 mL.[2)]

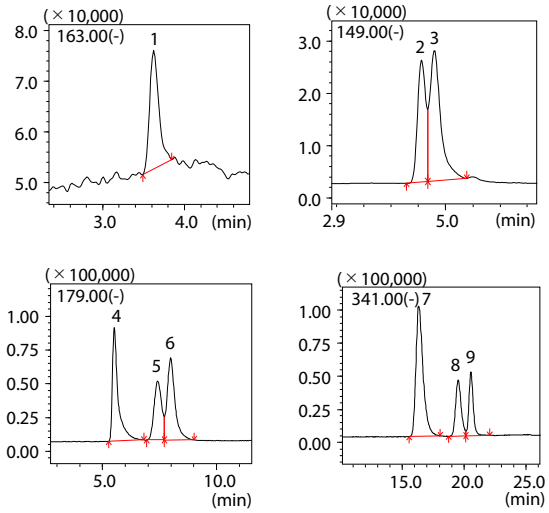
Sugars are usually detected using a refractive index detector or an evaporative light scattering detector since these compounds have little or no UV absorption. However, if the sugar concentration is low, as in non-sugar beverages, a mass spectrometer with high selectivity and sensitivity is more effective.

This article describes analyses of saccharides in non-sugar beverages separated by hydrophilic interaction liquid chromatography (HILIC) and detected with a single quadrupole mass spectrometer LCMS-2050.

■ Analysis of Mixed Standard Solution

The target compounds were nine saccharides that included both monosaccharides to disaccharides.

Fig. 1 shows chromatograms of the mixed standard solution of these nine saccharides (0.5 mg/L each, prepared with 75 % acetonitrile solution). Tables 1 and 2 show the analytical conditions. All nine saccharides were eluted in approximately 22 minutes by the gradient elution method.



■Peaks 1. Rhamnose, 2. Arabinose, 3. Xylose, 4. Fructose, 5. Galactose, 6. Glucose, 7. Sucrose, 8. Lactose, 9. Maltose

Fig. 1 Chromatograms of Mixed Standard Solution (0.5 mg/L each)

Table 1 HPLC Analytical Conditions

System	:	Nexera™ XR
Column	:	Shodex HILICpak VG-50 2D (150 mm × 2.0 mm I.D., 5 µm)
Flowrate	:	0.2 mL/min
Mobile Phase	:	A) 2.5 mmol/L Ammonium bicarbonate aq. B) 25 mmol/L Ammonium bicarbonate aq. / Acetonitrile=10:90
Time Program	:	99 %B (0-11 min)→77 %B (23-27 min) →99 %B (27.1-38 min)
Mixer	:	180 µL
Column Temp.	:	45 °C
Injection Volume	:	1 µL
Vial	:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*1

*1 P/N: 227-34001-01

Table 2 MS Analytical Conditions

Ionization	: ESI/APCI (DUIS™), Negative mode
Mode	: SIM (*m/z* 149, 163, 179, 341)
Nebulizing Gas Flow	: 3.0 L/min
Drying Gas Flow	: 5.0 L/min
Heating Gas Flow	: 7.0 L/min
Desolvation Temp.	: 400 °C
DL Temp.	: 150 °C
Interface Voltage	: -2.0 kV

■ Repeatability

Table 3 shows the repeatability (%RSD) of the retention time and the peak area for each 0.5 mg/L mixed standard solution in six repeated analyses.

Table 3 Repeatability (%RSD) in Six Repeated Analyses

Compound	Retention time	Peak area
Rhamnose	0.44	4.71
Arabinose	0.43	3.35
Xylose	0.49	3.42
Fructose	0.52	2.46
Galactose	0.53	2.60
Glucose	0.61	1.90
Sucrose	0.37	3.40
Lactose	0.16	2.09
Maltose	0.14	2.21

■ Calibration Curve

The calibration curves for the nine target compounds were highly linear, with coefficients of determination (r^2) of 0.998 or greater. Fig. 2 shows the calibration curves of rhamnose and arabinose. Table 4 shows the concentration ranges of the calibration curves and coefficients of determination for all the target compounds.

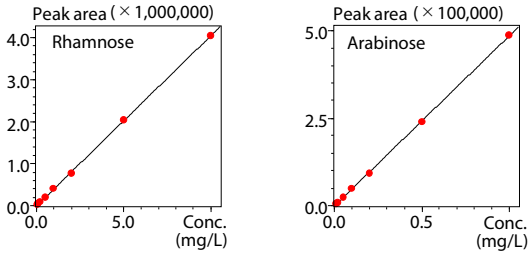


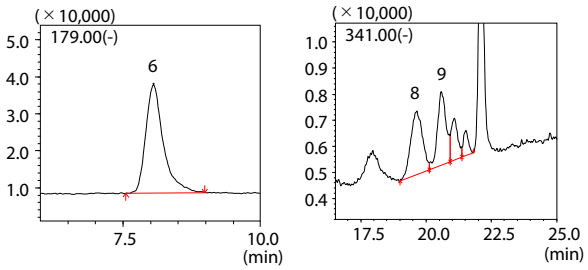
Fig. 2 Calibration Curves

Table 4 Concentration Ranges of Calibration Curves and Coefficients of Determination (r^2)

Compound	*m/z*	Conc. Range (mg/L)	r^2
Rhamnose	163	0.05-10	0.999
Arabinose	149	0.01-1	0.999
Xylose	149	0.01-1	0.999
Fructose	179	0.005-1	0.998
Galactose	179	0.01-1	0.998
Glucose	179	0.01-1	0.998
Sucrose	341	0.005-1	0.999
Lactose	341	0.01-1	0.999
Maltose	341	0.01-1	0.999

■ Analysis of Non-sugar Beverage

Four commercially available non-sugar beverages containing carbon dioxide were degassed for 5 minutes and filtered through 0.2 µm membrane filters. Filtrates were then diluted 1000-fold with 75 % acetonitrile solution before analysis by HPLC. Chromatograms of non-sugar beverage A are shown in Fig. 3. Concentration and content are shown in Table 5. The saccharide content of each beverage was less than 0.5 g/100 mL.



■Peaks 6. Glucose, 8. Lactose, 9. Maltose

Fig. 3 Chromatograms of Non-sugar Beverage A

Table 5 Concentration and Content

Compound	Concentration*2 (mg/L)			
	A	B	C	D
Fructose	n.d.	n.d.	n.d.	0.021
Glucose	0.228	0.225	0.100	0.017
Lactose	0.028	0.033	n.d.	n.d.
Maltose	0.031	0.028	n.d.	n.d.
Total	0.287	0.286	0.100	0.038
	Content (g/100 mL)			
	A	B	C	D
Total	0.029	0.029	0.010	0.004

*2 n.d.: not detected

■ Recovery Rate

After preparation, four samples were spiked with standards of the nine saccharides to make the concentration of 0.5 mg/L each, and then analyses were performed. Table 6 shows the recovery rate of each compound. It was confirmed that accurate determination was possible even in the presence of a matrix because good results (within 90 to 110 %) were obtained for all compounds.

Table 6 Recovery Rate (%)

Compound	Recovery rate (%)			
	A	B	C	D
Rhamnose	107.4	105.8	102.0	99.1
Arabinose	101.3	99.7	102.6	112.1
Xylose	96.8	99.0	103.2	105.5
Fructose	100.0	99.2	100.7	107.8
Galactose	96.0	100.4	103.2	109.6
Glucose	90.7	89.9	94.3	105.0
Sucrose	104.8	103.4	103.6	104.4
Lactose	105.6	100.5	102.7	105.0
Maltose	104.3	105.3	103.4	108.1

■ Conclusion

Determination of trace amounts of saccharides was possible using a single quadrupole mass spectrometer. The LCMS-2050 is expected to contribute to research and development in food engineering including the study for non-sugar beverages.

References
1) Guideline: Sugars Intake for Adults and Children. Geneva: World Health Organization; 2015.
2) Cabinet Office Ordinance No. 10. Food labelling standards. Japan: Consumer affairs agency; 2015.

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/

01-00330-EN First Edition: Jun. 2022