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Using a Single Quadrupole Mass Spectrometer for Simultaneous Analysis of Sugars in Sugar Free Drinks

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1. Overview

A newly developed single quadrupole mass spectrometer, LCMS-2050, contributed to a highly sensitive analysis method for sugars with excellent separation.

2. Introduction

The World Health Organization (WHO) recommends adults reduce their daily intake of free sugars to less than 25 g. A beverage can be indicated as sugar free if it contains less than 0.5 g of sugar per 100 mL according to Japan Consumer Affairs Agency's Food Labeling Standard.

Due to the low ultraviolet absorption, refractive index (RI) detectors and evaporative light scattering detectors (ELSD) are often used in sugar analysis. However, when the concentration of the analyte is low, such as in sugar free drinks, a mass spectrometer with higher selectivity and sensitivity is needed.

A method utilizing hydrophilic interaction chromatography (HILIC) and a newly developed single quadrupole mass spectrometer, LCMS-2050, is developed to analyze sugars in sugar free beverages.

Figure 1. LCMS-2050 external view

3. Methods

Mixed standard solutions of nine monosaccharides and disaccharides (rhamnose, arabinose, xylose, fructose, galactose, glucose, sucrose, lactose, and maltose) were prepared in 75% aqueous acetonitrile (ACN) in concentrations of 0.005-10 mg/L for each analyte. The solutions were then injected (1 µL) into a LCMS-2050 system. A HILIC column (150 mm x 2.0 mm I.D., 5 µm) was used for separation. The chromatographic separation of the nine analytes was achieved by a gradient elution in 22 minutes. In the single quadrupole mass spectrometer, ESI/APCI (DUIS™) ionization was set in negative mode and selected ion monitoring (SIM) was used to monitor *m/z* 149, 163, 179, 341.

Table 1. LC-MS 2050 Analytical Conditions

Column:	Shodex HILICpak VG -50 2D (150 mm x 2.0 mm I.D., 5 µm)	Nebulizing Gas:	3.0 L/min
Mobile phase:	A: 2.5 mmol/L Ammonium bicarbonate in water B: 25 mmol/L Ammonium bicarbonate H_2O/ACN = 10: 90	Ionization:	ESI /APCI (DUIS™) negative
Time program:	99% B (0 -11 min) → 77% B (23 -27 min) → 99% B (27.1 -38 min)	Mode:	SIM (*m/z* 149, 163, 179, 341)
Flow Rate:	0.2 mL/min	Desolvation Temp.:	400 °C
Oven Temp.:	45°C	DL Temp.:	150 °C
Inj. volume:	1.0 µL	Interface voltage:	-2.0 kV

4. Results

4-1. Analysis of the mixed standard solution

The target ingredients are 9 monosaccharides and disaccharides. Figure 2 shows the chromatogram of the nine-compound mixed standard solution (0.5 mg/L each, prepared in 75% aqueous acetonitrile), and Tables 1 shows the analytical conditions. By the gradient elution method, 9 compounds could be eluted in 22 minutes.

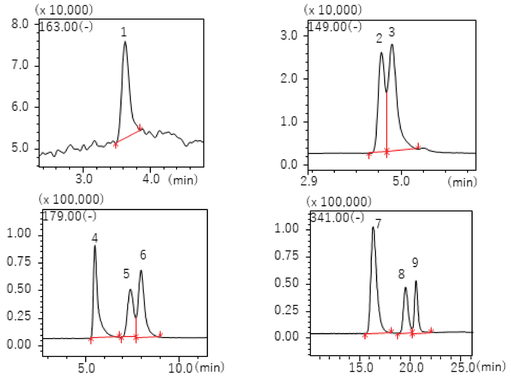

Figure 2. Chromatograms of the mixed standard solutions. Peaks 1. Rhamnose, 2 Arabinose, 3. Xylose, 4. Fructose, 5. Galactose, 6. Glucose, 7. Sucrose, 8. Lactose, 9. Maltose

4-2. Reproducibility

Reproducibility (% RSD) of retention times and peak areas of the nine monosaccharides and disaccharides in the mixed standard solution was evaluated with six replicates analyses. Reproducibility of retention times was less than 1% for all nine analytes, and reproducibility of peak areas was less than 5% for all nine analytes.

Table 2. Reproducibility of 6-time repeat analysis (% RSD)

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

4-3. Calibration curve

A calibration curve was created for the 9 compounds of interest, and good linearity was obtained with $r^2 \geq 0.998$ for all compounds. Figure 3 shows the calibration curves for rhamnose and arabinose, and Table 3 shows the calibration ranges and contribution ratios for all compounds.

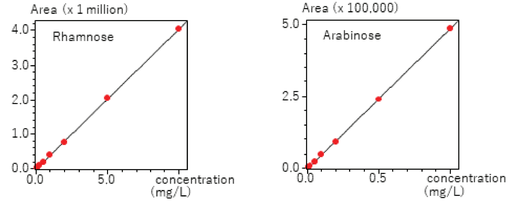

Figure 3. Calibration curves of rhamnose and arabinose

Table 3. Calibration curve concentration range and contribution rate (r^2)

Compound	*m/z*	Cal. 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

4-4. Analysis of sugar free beverages

Sugar free beverages containing 4 types of carbon dioxide were degassed for 5 minutes and filtered through a 0.2 µm membrane filter. The sample was then diluted 1000 times with 75% aqueous acetonitrile. Figure 4 shows the chromatograms of sugar free beverage A and Table 4 shows the quantitative results. All beverages contained less than 0.5 g of sugar per 100 ml.

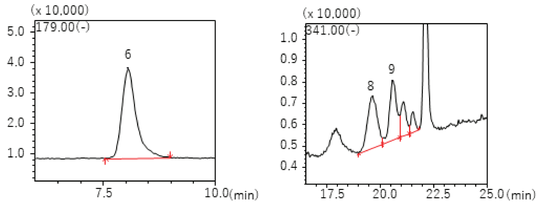

Figure 4. Chromatograms of mixed standard solutions. Peaks 6. Glucose, 8. Lactose, 9. Maltose

Table 4. Quantitative results

Compound	Concentration (mg/L)			
	A	B	C	D
Fructose	n.d. *	n.d.	n.d.	n.d.
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

* n.d.: not detected

4-5. Additive recovery test

After the pretreatment, the standard solution of saccharides was added to the sample solution at 0.5 mg/L. Table 5 shows the recovery rate of each component. Good results were obtained for all components within approximately 90 ~ 110%, and it was confirmed that accurate quantification was possible even under the matrix.

Table 5. Additive recovery rate (%)

Compound	Recovery rates (%)			
	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

5. Conclusions

A single quadrupole mass spectrometer, LCMS -2050, was used to separate and quantify trace amounts of sugars. LCMS -2050 is expected to contribute to research and development in the food sector, including the sugar free beverage market.