LAAN-A-LM-E045

SHIMADZU

Application News

Liquid Chromatography Mass Spectrometry No.C74

Quantitative Analysis of Carbohydrates by LC/MS

A variety of separation and detection methods are available for the HPLC analysis of saccharides. Typical separation techniques for carbohydrate analysis include normal phase distribution and ligand exchange. Differential refractive index detection and post-column fluorescence derivatization are known to be effective for detection.
In carbohydrate analysis using LC/MS, derivatization is often used to improve detection sensitivity. However, to circumvent this cumbersome derivatization operation for ESI, solvent is added after column using APCI to allow ionization of low-polarity compounds. This solvent addition promotes ionization, thereby allowing stable ion detection. Here we introduce examples of quantitative analysis of carbohydrates in foods using post-column solvent addition.

■ Analysis of Carbohydrates Using LCMS-2020

Fig. 1 shows mass chromatograms of carbohydrates. Methanol – chloroform (4:1) was used as the solvent that was added after the column.
This solvent encourages the formation of the chloride ion adduct ($[M+Cl]^-$) preferentially, allowing stable detection of the ion.
Since post-column solvent addition means that substances eluting from the column (including mobile phase) are mixed with the solvent, a connector is used between the column and interface (in this case, the APCI), and a separate pump is used for delivery of the solvent.

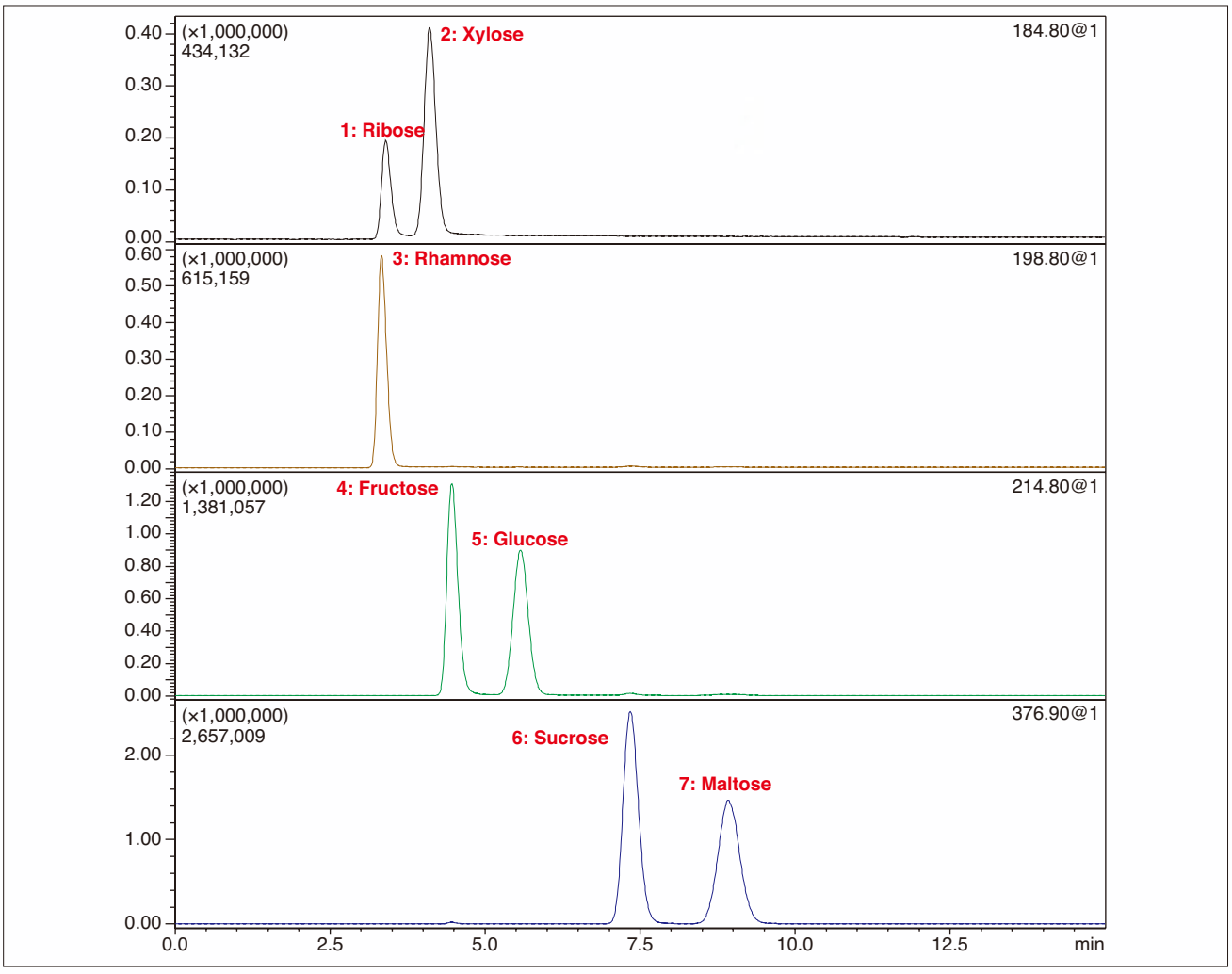

Fig. 1 LC/MS (SIM) Analysis of Carbohydrates Using Post-Column Solvent Addition (100 mg/L each)
(1: Ribose, 2: Xylose, 3: Rhamnose, 4: Fructose, 5: Glucose, 6: Sucrose, 7: Maltose)

■ Calibration Curves

Table 1 shows the quantitation results for each substance. The RSD (%) values shown in the table correspond to the RSD (%) for the smallest concentrations used in generating the calibration curves.

Table 1 Calibration Curves

	MW	*m/z*	Range (mg/L)	Correlation Coefficient (R)	RSD (%)
Ribose	150.1	184.8 [M+Cl]-	0.5-100	0.9999	3.40
Xylose	150.1	184.8 [M+Cl]-	0.1-100	0.9996	5.13
Rhamnose	164.2	198.8 [M+Cl]-	0.5-100	0.9999	5.04
Fructose	180.2	214.8 [M+Cl]-	0.1-100	0.9999	2.81
Glucose	180.2	214.8 [M+Cl]-	0.5-100	0.9999	0.51
Sucrose	342.3	376.9 [M+Cl]-	1-100	0.9996	10.22
Maltose	342.3	376.9 [M+Cl]-	5-100	0.9996	4.92

■ Analysis of Foods Using the LCMS-2020

Fig. 2 and 3 show the results of food analysis. The samples were each diluted 10-fold using distilled water, and then ultrafiltrated (Microcon YM-3, MILLIPORE). The recovered sample was further diluted 1000-fold in distilled water, and 1 µL of each sample was injected. The carbohydrate concentrations in the foods used for analysis were 360 mg/mL (glucose) and 41.7 mg/mL (maltose) in the mirin (sweet sake), and 8.6 mg/mL (fructose), 12.1 mg/mL (glucose), and 32.4 mg/mL (sucrose) in the soft drink.

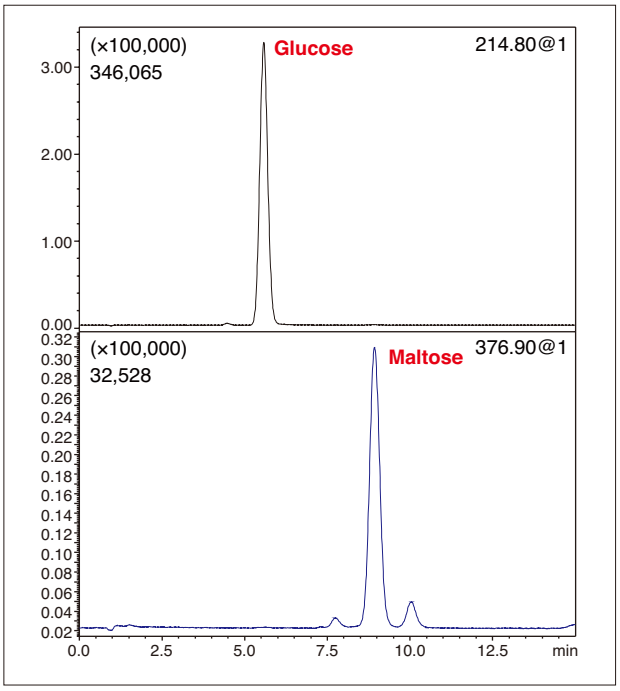

Fig. 2 Sweet Sake (1 µL injected)

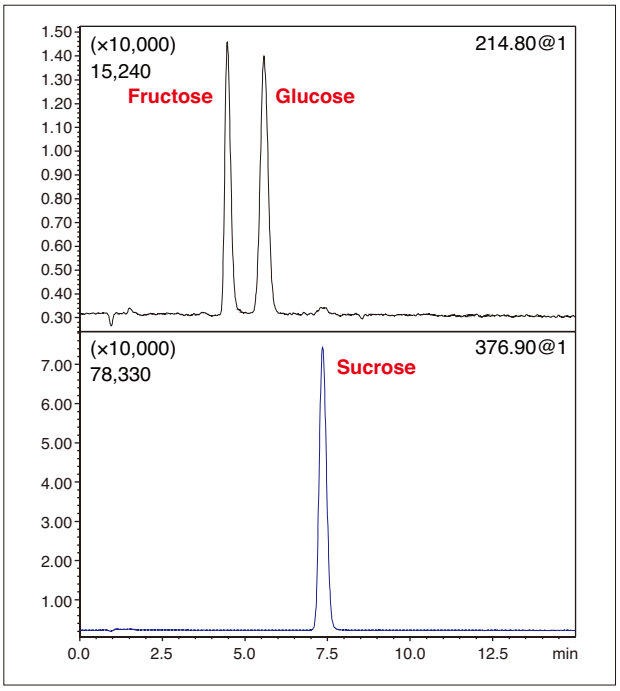

Fig. 3 Soft Drink (1 µL injected)

Table 2 Analytical Conditions

Column	: Shodex Asahipak NH2P-50 (150 mmL. × 2.0 mmI.D., 5 µm)	MS	: LCMS-2020
Mobile Phase	: acetonitrile/water (3:1)	Probe Voltage	: -3.5 kV (APCI-Negative mode)
Flow Rate	: 0.2 mL/min	Probe Temperature	: 400 °C
Post Column Solvent	: methanol-chloroform (4:1)	Nebulizing Gas Flow	: 4.0 L/min
Flow Rate	: 0.075 mL/min	Drying Gas Flow	: 5.0 L/min
Column Temperature	: 30 °C	DL Temperature	: 250 °C
Injection Volume	: 1 µL	Block Heater Temperature	: 200 °C
		DL, Q-Array Voltages	: default values
		Event Time	: 0.5 sec
		SIM	: *m/z* 184.8 (Ribose, Xylose) *m/z* 198.8 (Rhamnose) *m/z* 214.8 (Fructose, Glucose) *m/z* 376.9 (Sucrose, Maltose)

SHIMADZU

SHIMADZU CORPORATION. International Marketing Division

3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan Phone: 81(3)3219-5641 Fax. 81(3)3219-5710

Cable Add.:SHIMADZU TOKYO