SHIMADZU

Application News

Liquid Chromatography Mass Spectrometry No.C61A

LC-MS Analysis of Carbohydrates Using Post Column Addition of Solvent for Improved Ionization Efficiency

Typically, in the HPLC analysis of saccharides, two different modes of chromatography can be used: aqueous normal phase, or ligand exchange mode. Due to the difficulty in completely separating all of the saccharides, these are used in a complementary manner.
Since salts that are not easily volatilized cannot be used in LC-MS analysis, the suitability of the HPLC conditions for LC-MS analysis depends on the choice of the mobile phase. Because acetonitrile - water (or ammonium acetate in water) is generally used as the mobile phase in the aqueous normal phase mode, and only water is used as the mobile phase in the ligand exchange mode, these can be considered suitable mobile phases for LC-MS analysis. While these conditions are very suitable for the HPLC separation of mixed carbohydrates, there can be difficulty in observing an ion which up to then has been easily confirmed, or there may be a loss of sensitivity due to the separation column or the HPLC conditions.

Fig.1 shows, on the left side, an example of analysis of a saccharide (at 200 μg/mL) with a mass of 162 using the ligand exchange column (ionization method: negative ion APCI). The ion at the *m/z* of the deprotonated molecule is barely noticeable. The chloride adducts $[M+Cl]^-$ show up at *m/z* 197, and *m/z* 199 for the isotopic chloride adduct. The formate adduct will be seen at *m/z* 207, while the acetate adduct can be seen at *m/z* 221. Since no acid additive is used in the mobile phase, it is assumed that some leftover acid remains in the LC system, including the column. Residual chlorine ions similarly may be left in LC system, or it is likely that they originate from impurities in the analytical sample. Therefore, in analysis where an ion donor related to the ionization is not proactively added, sensitivity may be inconsistent due to the occurrence of various molecular ions, or the intensity ratios may be affected by slight changes in the environment, resulting in poor repeatability.
Fig.1 shows, on the right side, an example of analysis in which a chloride ion donor was added proactively at the post-column stage, with the intent of promoting ionization. Since the chloride ion adduct is given priority, sensitivity and stability are improved. Chloroform (or methylene chloride) is used to provide a source of chloride ions by the APCI source, and to ensure that the solvent would mix very quickly with the mobile phase. In this case, methanol - chloroform (4:1) are added in the post-column stage.
Fig.2 shows the mass chromatograms of typical saccharides. LC-MS has the advantage of being able to easily distinguish between saccharides with nearby retention times, and to provide good detection levels, and whether they are reducing or non-reducing sugars.

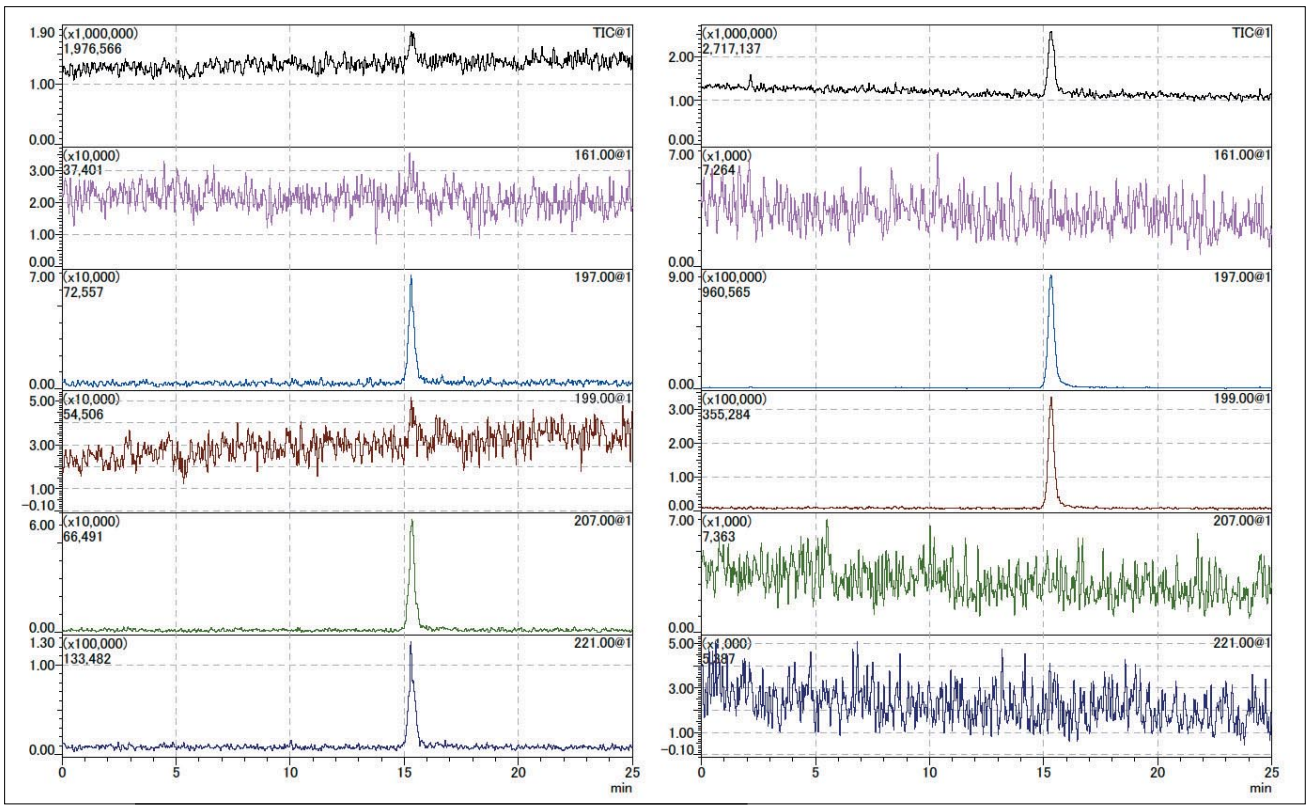

Fig.1 Effect of Adding Solvent Post Column (Left: not added, Right: methanol-chloroform (4:1 v/v) added post column)

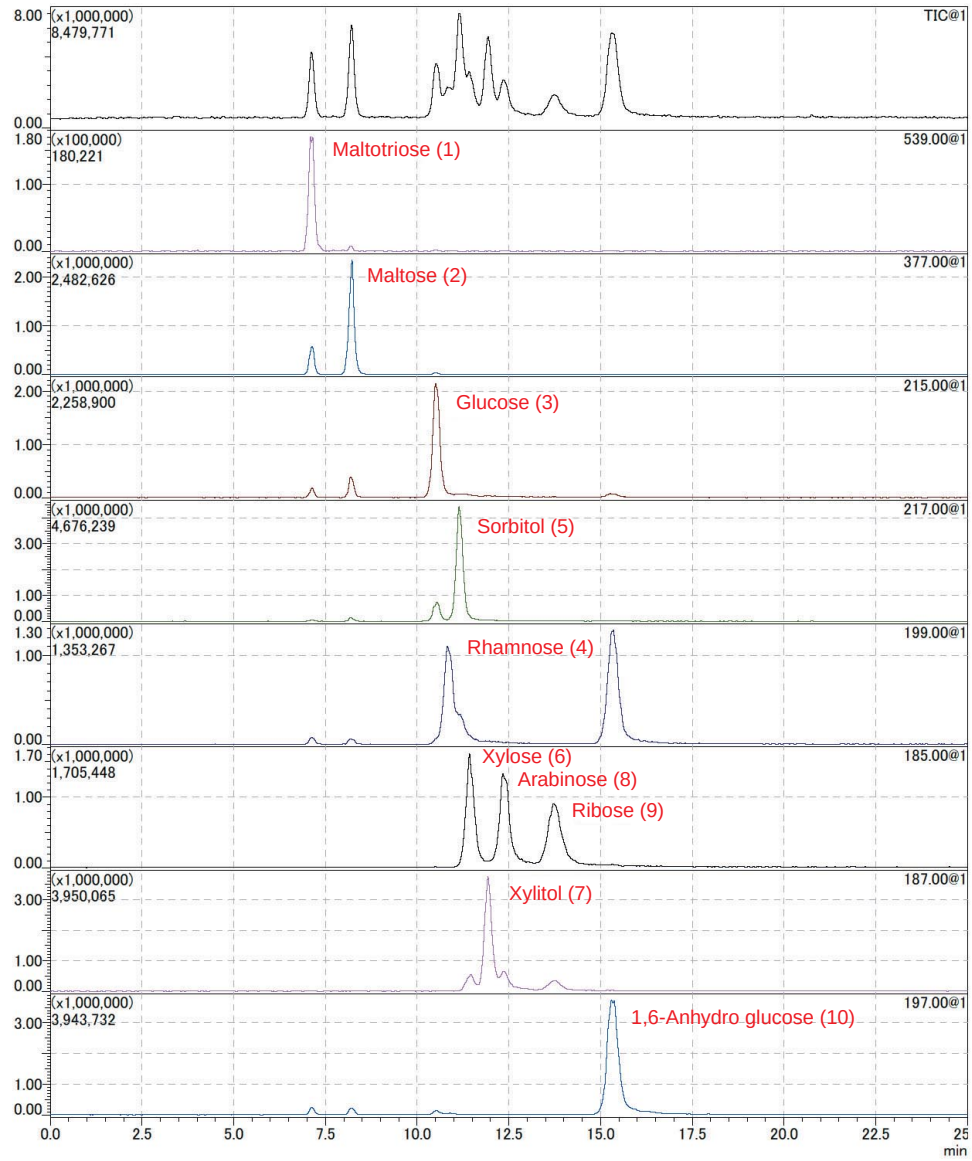

Fig.2 2 LC-MS Analysis of Typical Saccharides Using Post-Column Addition of Solvent (500 ng/mL each)
(1: Maltotriose, 2: Maltose, 3: Glucose, 4: Rhamnose, 5: Sorbitol, 6: Xylose, 7: Xylitol, 8: Arabinose, 9: Ribose, 10: 1,6-Anhydro glucose)

Table 1 Analytical Conditions for LC-MS

Column	: Shim-pack SPR-Na (G) + Shim-pack SPR-Na (7.8 mm I.D. × 50 + 250 mmL.)		
Mobile Phase	: Water		
Flow Rate	: 0.6 mL/min		
Post Column Solvent	: Methanol-Chloroform (4:1)		
Flow Rate	: 0.2 mL/min		
Injection Volume	: 3 µL	Column Temperature	: 80˚C
Probe Voltage	: -3.5 kV (APCI-Negative mode)		
Probe Temperature	: 400˚C		
CDL Temperature	: 250˚C	Block Heater Temperature	: 200˚C
Nebulizing Gas Flow	: 2.5 L/min	Drying Gas Flow	: 0.01 MPa
CDL Voltage	: Scan mode		
Q-array DC Voltage	: Scan mode	Q-array RF Voltage	: Scan mode
Scan Range	: *m/z* 150-600 (0.5 sec/scan)		

NOTES:
✻This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.

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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

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