SHIMADZU

Application News

Liquid Chromatography Mass Spectrometry No.C70

Analysis of Water-Soluble Vitamins with Multi-Mode ODS Column Using LCMS-2020

Dietary guidelines and nutritional supplements are a significant concern to health conscious consumers. Upper and lower limits of daily intake of such functional foods have been specified for 12 vitamins and 2 minerals.

Water-soluble vitamins are one class of nutrients whose measurement is important to the food and nutritional supplement industries. Due to their high polarity, their retention is extremely weak when using reversed-phase chromatography. Historic use of ion-pair reagents when conducting LCMS analysis of such weakly retained analytes has resulted in reduced sensitivity.

Here we present an example of analysis of 8 water-soluble vitamins using a cation exchange-anion exchange multi-mode ODS column, (Scherzo SM-C18, Imtakt Corporation) in conjunction with the LCMS2020 mass spectrometric detector. The gradient consisted of formic acid / ammonium formate buffer and acetonitrile mobile phases, components typical for high sensitivity reverse-phase LCMS analysis.

Fig. 1 shows the chromatograms of the 8 water-soluble vitamins, Fig. 2 shows the mass spectra, and Fig. 3 shows the respective calibration curves. The linearity in all cases was excellent, with coefficient of repeatability values all greater than $R^2 = 0.99$.

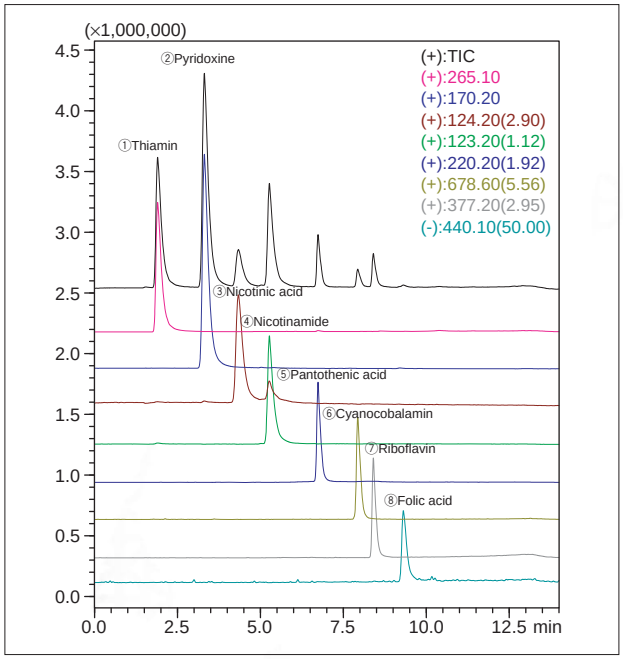

Fig. 1 Chromatograms of 8 Water-Soluble Vitamins

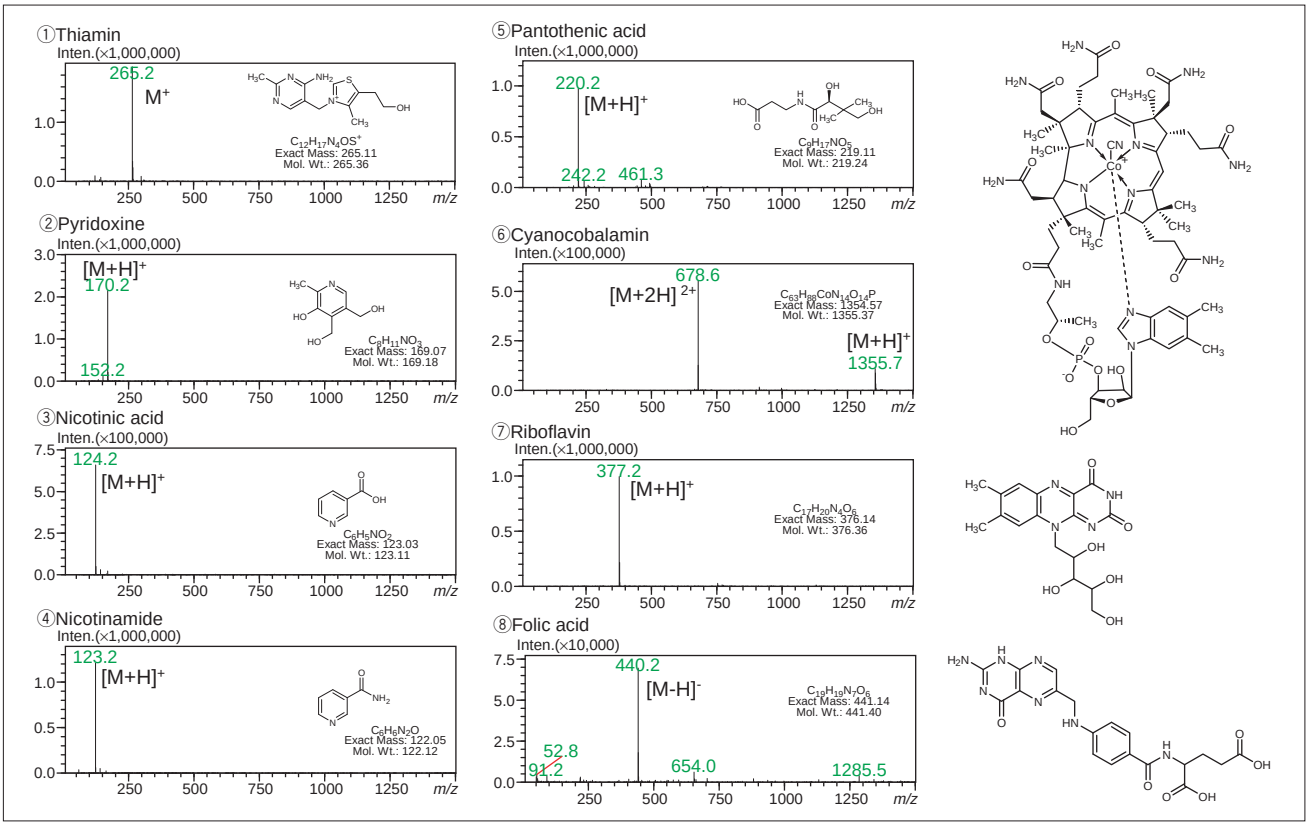

Fig. 2 Mass Spectra of Water-Soluble Vitamins

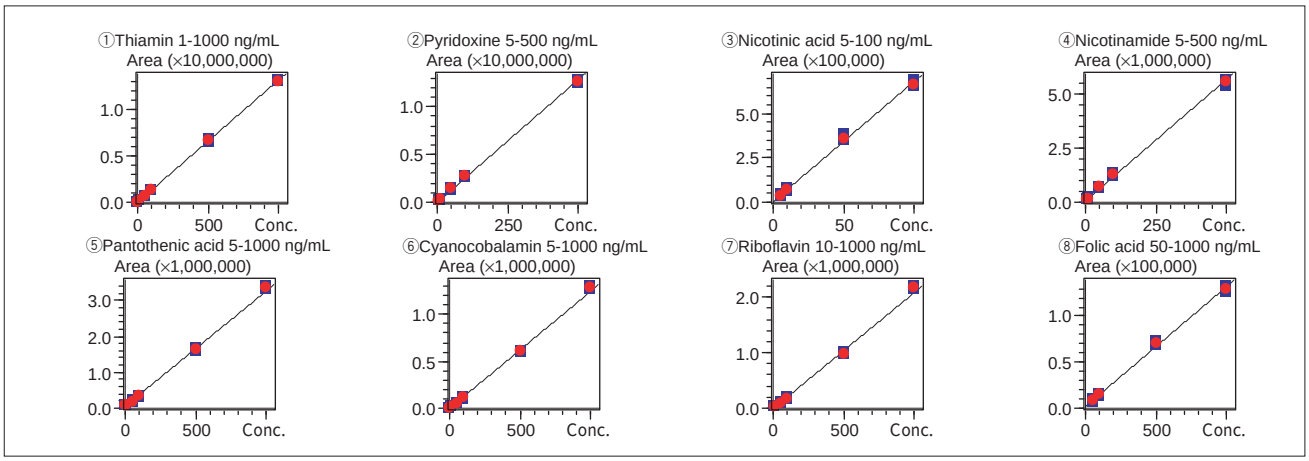

Fig. 3 Calibration Curves of Water-Soluble Vitamins

■ Quantitative Analysis of Water-Soluble Vitamins from Cereal

One gram of a commercial functional food product was prepared according the procedure shown in Fig. 4, and quantitation of the water-soluble vitamins was conducted.

Fig. 5 shows the SIM chromatograms obtained from analysis of the cereal extract solutions. The content of ⑥ cyanocobalamin (vitamin B_{12}) in the sample was below the method detection limit. Actual sample analysis requires confirmation of extraction efficiency, daily quality control etc., but this analysis confirmed that quantitation was possible without almost any interference from impurities and demonstrated the LCMS-2020 to be a suitably selective and sensitive detector.

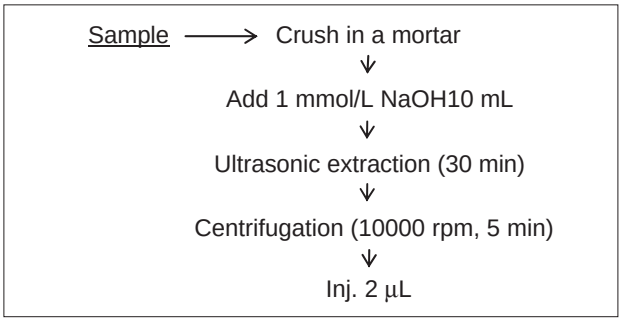

Fig. 4 Sample Preparation

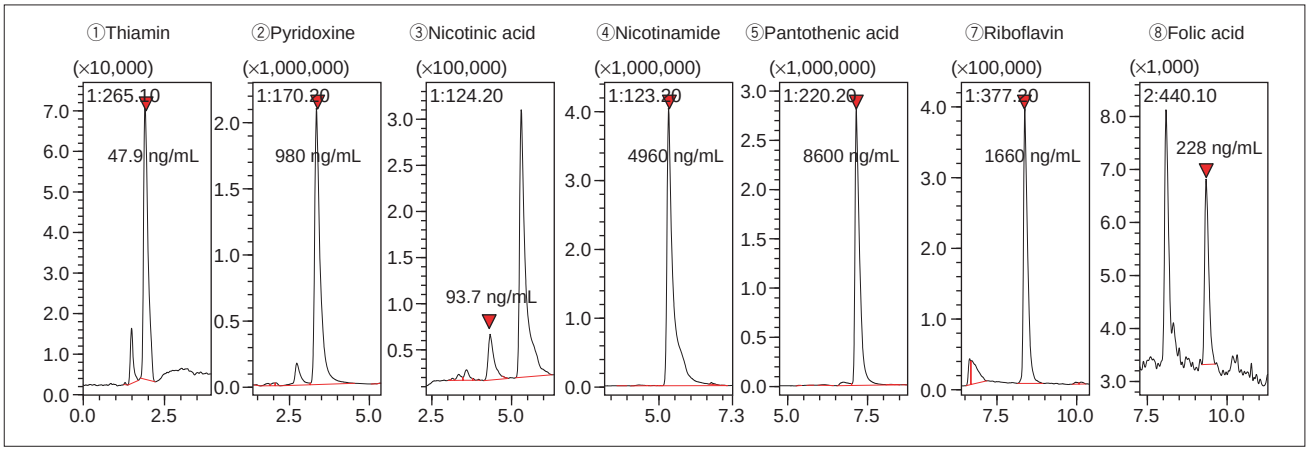

Fig. 5 SIM Chromatograms of Extract from Cereal

Table 1 Analytical Conditions

Column	: Imtakt Scherzo SM-C18 (150 mmL. × 2.0 mmI.D., 3 µm)	Probe Voltage	: +4.5 kV (ESI-Positive mode), -3.5 kV (ESI-Negative mode)
Mobile Phase A	: 5 mmol/L ammonium formate + 0.1 % formic acid-water	Nebulizing Gas Flow	: 1.5 L/min
Mobile Phase B	: acetonitrile	Drying Gas Flow	: 10 L/min
Gradient Program	: 0 %B (0 min) - 55 %B (10 min) - 0 %B (10.01 - 20 min)	DL Temperature	: 250 ˚C
Flow Rate	: 0.2 mL/min	Block Heater Temp.	: 450 ˚C
Injection Volume	: 2 µL	DL Voltage/Q-array Voltage	: using default values
Column Temp.	: 40 ˚C		

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