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Application News

LCMS™-8060NX High Performance Liquid Chromatograph Mass Spectrometer

Analysis of Water-Soluble Vitamins in Foods by LCMS-8060NX Triple Quadrupole Mass Spectrometer

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User Benefits

- Simultaneous quantitative analysis of water-soluble vitamins with largely different contents is possible.
- Because the IonFocus™ unit introduces ions into the MS with high efficiency, both excellent system robustness and high sensitivity analysis are achieved when moving the ion spray away from the ion inlet port to mitigate contamination.
- The analysis results show a high recovery rate even in analysis of complex food matrices such as modified milk powder (powdered infant formula) and milk raw material.

■ Introduction

Vitamins are classified as water-soluble vitamins or fat-soluble vitamins depending on their nature.

Although the general analysis techniques for water-soluble vitamins include microbiological methods (bioassay), fluorescence methods, and the HPLC method, with the exception of HPLC, only one vitamin can be measured by those analysis methods. Thus, the time and labor required with those methods is a drawback. The HPLC method enables simultaneous measurement of multiple vitamins, but depending on the vitamin, the content in foods may be trace amount, and in some cases, this is an obstacle to analysis in complex matrices.

With the LC/MS/MS method, simultaneous quantitative analysis of vitamins with higher efficiency is possible because vitamins can be analysed with selectivity and high sensitivity even in complex matrices.

However, contamination of the MS is a problem when measuring complex matrices like those of foods. As one solution to this problem, the effects of contaminants can be minimized by separating the ionization unit from the MS ion introduction unit. The IonFocus Unit of the LCMS-8060NX efficiently introduces only ions into the MS and enables high sensitivity analysis while minimizing contamination of the MS unit because the ionization unit is separated from the MS ion introduction unit.

In this article, we report the development of an analysis method for four water-soluble vitamins and an example of simultaneous analyses of the vitamins in food products using the LCMS-8060NX.

■ Measurement Samples

The sample solutions[*1] used here were prepared by acid hydrolysis of four modified milk powders and milk raw material.

■ Analysis Conditions

Table 1 shows the LSMS analysis conditions, and Table 2 shows the MS/MS parameters.

Although the MS/MS parameters for each compound were selected by the automatic MRM optimization function, the collision energies of nicotinamide and pantothenic acid were set to different values from the selected optimum values to adjust the conditions to a suitable calibration curve range for the concentrations of the sample solutions used in the quantitative analysis.

In the LCMS-8060NX IonFocus unit used in the mass spectrometer, focus electrodes efficiently introduce only ions into the MS while expelling unnecessary neutral particles. For this analysis, the probe position was set to 4.5 mm, and high sensitivity was realized by the effect of the IonFocus unit while minimizing contamination of the MS when moving the ion spray away from the ion inlet port.

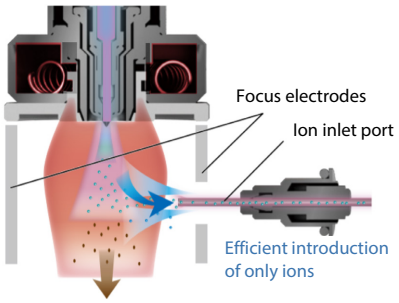

Fig. 1 Concept of IonFocus Unit

Table 1 LCMS Analysis Conditions

[HPLC conditions] (Nexera™ X3)	
Column	: L-column3 C18 Metal-free (150 mmL. × 2.0 mm I.D., 3.0 µm)
Mobile phases	: A) 10 mmol/L Ammonium bicarbonate in H_2O B) Acetonitrile
Gradient Program	: B 2% (0-3 min) – B 60% (4-5 min) – B 98 % (5.01-9 min) – B 2 % (9.01-13 min)
Flow rate	: 0.35 mL/min
Column Temp.	: 40 ˚C
Injection volume	: 1 µL
[MS conditions] (LCMS-8060NX)	
Ionization	: ESI (Positive mode)
Probe Voltage	: -0.5 kV
Mode	: MRM
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 25.0 L/min
Heating gas flow	: 15.0 L/min
DL/ Heat Block Temp.	: 250 ˚C/300 ˚C
Interface Temp.	: 400 ˚C
Probe position	: 4.5 mm

Table 2 MS/MS Parameters

Compound	Quantitative MRM transition (*m/z*)	Qualitative MRM transition (*m/z*)
Biotin	245.10>227.00	245.10>96.95
Nicotinic acid	124.00>80.00	124.00>78.05
Nicotinamide	123.00>79.90	123.00>77.90
Pantothenic acid	220.10>89.90	220.10>202.15

■ Analysis Results of Standard Samples

Linearity was confirmed by three repeatability analyses of the standard samples prepared for the calibration curves. Fig. 2 shows the chromatograms at the lowest concentrations of the calibration points and the calibration curves prepared by the external standard method. Table 3 summarizes the range of the calibration curves.

The calibration points were decided based on accuracy of within 80 to 120% for all calibration points and confirmation of peak area repeatability of 20% or less. Satisfactory linearity was obtained, as the correlation coefficient R = 0.998 or higher for all components.

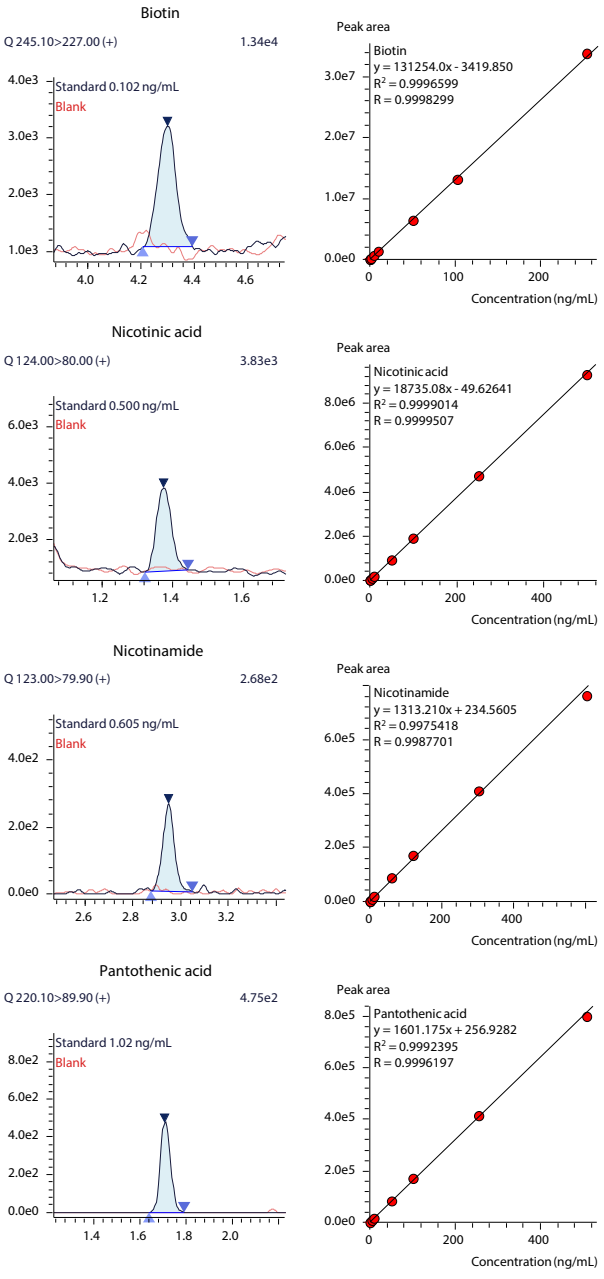

Fig. 2 Chromatograms and Calibration Curves

Table 3 Range of Calibration Curves of Water-Soluble Vitamins (ng/mL)

Compound	Calibration curve (ng/mL)			Correlation coefficient (R)
Biotin	0.102	-	254	0.999
Nicotinic acid	0.500	-	500	0.999
Nicotinamide	0.605	-	605	0.998
Pantothenic acid	1.02	-	510	0.999

■ Analysis Results of Milk Raw Material

Three repeated analyses of each sample solution were carried out, and a quantitative analysis of the water-soluble vitamins was conducted. Fig. 3 shows the chromatograms when the milk raw material samples were analyzed. Table 4 shows the results of a comparison of these quantitative values and the values obtained by the bioassay method.

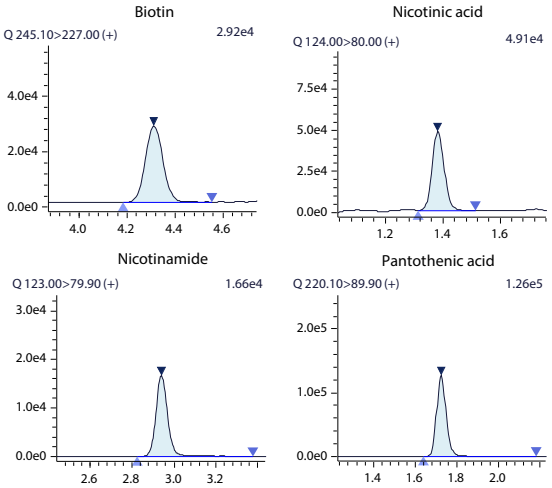

Fig. 3 Chromatograms of Water-Soluble Vitamins in Milk Raw Material

Table 4 Comparison of LC/MS/MS Method and Bioassay Results

Compound	LC/MS/MS	Bioassay
Biotin	56.24	60.8
Niacin*	242.45	274.7
Pantothenic acid	1.14	1.33

* The values for niacin are the totals of nicotinic acid and nicotinamide.

■ Results of Spike-and-Recovery Tests of Modified Milk Powder and Milk Raw Material

Each of the sample solutions was spiked with water-soluble vitamins, and spike-and-recovery tests were conducted. Table 5 shows the test results.

Table 5 Recovery Rates of Sample Solutions

Compound	Modified milk powder 01	Modified milk powder 02	Modified milk powder 03	Modified milk powder 04	Milk raw material
Biotin	79.4%	86.0%	52.9%	84.4%	76.1%
Nicotinic acid	90.6%	112.0%	91.2%	89.7%	102.8%
Nicotinamide	89.1%	98.0%	115.6%	92.4%	88.8%
Pantothenic acid	80.8%	103.6%	103.2%	87.5%	98.7%

■ Conclusion

A simultaneous analysis method for water-soluble vitamins using LC/MS/MS was developed. Accurate quantitative analysis of water-soluble vitamins in complex matrices, exemplified here by modified milk powder and milk raw material, was possible by using this analysis method.

*1 We wish to express our deep appreciation to MORINAGA MILK INDUSTRY CO., LTD. for providing the sample solutions used in this paper.

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01-00033-EN First Edition: Jul. 2021