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Liquid Chromatograph Mass Spectrometer LCMS-8060NX

Application News

Development of a Simultaneous Analysis Method for Allergens in Food Using a Triple Quadrupole Mass Spectrometer

Yuki Ito and Tetsuo Iida

User Benefits

- Enables simultaneous analysis of seven specific ingredients (wheat, buckwheat, eggs, milk, peanuts, crustaceans (shrimp and crab)) and soy allergen, which is considered equivalent to specific ingredients.
- Using this analytical method, it is possible to perform simultaneous analysis of food allergens in processed foods.

■ Introduction

Food allergies are known to be caused by excessive immune responses to specific proteins (allergens) in food and have become a pressing issue for public health and the food industry. To prevent health hazards caused by food allergies, strict regulations regarding food labeling have been enacted in many countries. In Japan, considering the extent and frequency of past health damages, labeling is mandatory for eight specified ingredients (wheat, buckwheat, eggs, milk, peanuts, shrimp, crab, and walnuts) in packaged processed foods, while labeling is recommended for 20 other ingredients that are considered equivalent to specific ingredients.

Currently, ELISA (Enzyme-linked immunosorbent assay) and PCR (Polymerase chain reaction) are widely used as detection methods, allowing for the detection of allergenic food ingredients through relatively simple operations. However, ELISA is known to carry the risk of false positives due to cross-reactivity with similar substances. Furthermore, simultaneous analysis using ELISA is limited, and when testing a wide range of food ingredients, it is necessary to conduct measurements in multiple steps using different measurement kits. Moreover, since PCR detects DNA rather than proteins, it is difficult to distinguish between milk and beef, and it is also challenging to detect egg whites, which do not contain DNA.

Against this background, measurement methods for food allergens using liquid chromatography mass spectrometry are gaining attention due to their high selectivity and sensitivity, as well as the potential for simultaneous analysis of multiple allergens.

This Application News introduces a method for the simultaneous analysis of seven specific ingredients (wheat, buckwheat, eggs, milk, peanuts, crustaceans (shrimp and crab)) and soy allergen, which is considered equivalent to specific ingredients, in processed foods using the Nexera™ X3 ultra-fast liquid chromatograph and the LCMS-8060NX triple quadrupole mass spectrometer (Fig. 1).

Fig. 1 Nexera™ X3 and LCMS-8060NX

■ Sample Preparation and Analysis Conditions

Standard samples of the seven specific ingredients (wheat, buckwheat, eggs, milk, peanuts, crustaceans (shrimp and crab)) and the soy allergen, which is considered equivalent to specific ingredients, were obtained from the Saika Technological Institute Foundation's "Food-Derived Allergen Extract."

Processed foods used in this study included commercially available pre-packed curry, baby food, and udon noodles, with allergen information listed on the packaging shown in Table 1.

After extracting proteins from each sample, they underwent reduction and alkylation, trypsin digestion, and purification using solid-phase columns, followed by LC/MS/MS analysis (Fig. 2). The HPLC conditions and MS conditions are shown in Table 2.

Table 1 Food Allergen Information

Processed food	Allergens listed on the package
Pre-packaged curry	No mention of specific ingredients or soybeans
Baby food	No mention of specific ingredients or soybeans
Udon noodles	wheat

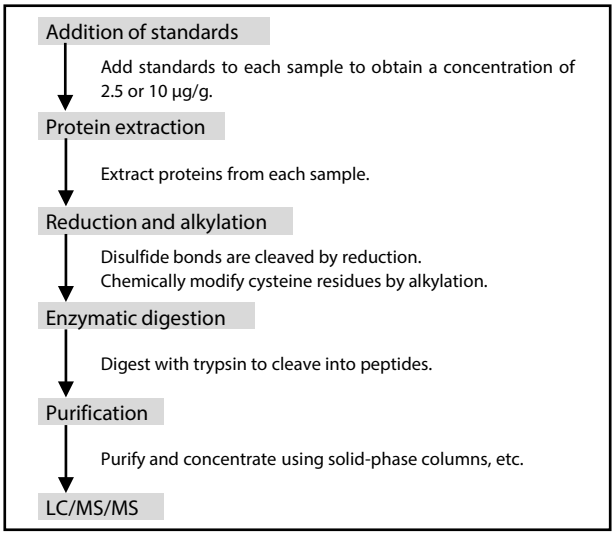

Fig. 2 Workflow for Sample Preparation

Table 2 Analysis Conditions

UHPLC (Nexera X3 system)	
Column	: Shim-pack™ GIST-HP C18-AQ [Metal free] (2.1 mmI.D. × 100 mmL., 1.9 µm) P/N: 227-30936-02
Mobile Phase A	: 0.1 % Formic acid in water
Mobile Phase B	: 0.1 % Formic acid in acetonitrile
Gradient Program	: B Conc. 2 % (0 min) → 15 % (6 min) → 40 % (10.5 min) → 95 % (10.65 min) → 95 % (12 min) → 2 % (13.5 min) → 2 % (20 min)
Flow Rate	: 0.5 mL/min (0.35 mL/min for 16 min – 18 min)
Column Temp.	: 40 °C
Injection Volume	: 3 µL

MS (LCMS-8060NX)	
Ionization	: IonFocus™ ESI (Positive)
Mode	: MRM
Nebulizing Gas Flow	: 2.0 L/min
Drying Gas Flow	: 3.0 L/min
Heating Gas Flow	: 17.5 L/min
DL Temp.	: 150 °C
Block Heater Temp.	: 300 °C
Interface Temp.	: 250 °C

■ Simultaneous Analysis of Seven Specified Ingredients and One Equivalent Ingredient

To simultaneously analyze the allergenic peptides derived from the eight ingredients mentioned above, we developed a method with 48 MRM transitions targeting 17 peptides. When analyzing the allergen mixed standard for the eight ingredients, all peptides eluted within 10.5 minutes, as shown in Fig. 3 (A), demonstrating good peak shapes and separation patterns. Fig. 3 (B) shows the linearity of the crustacean allergenic peptide (AGGLTLER) as an example. Table 3 shows a list of MRM transitions of allergenic peptides analyzed.

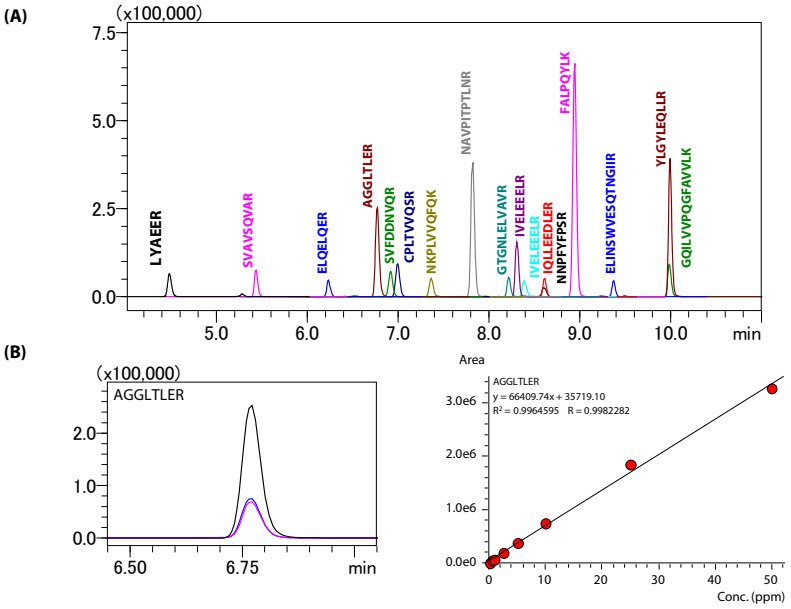

Fig. 3 (A) Mass Chromatogram of a Peptide Mixture Derived from Eight Allergenic Foods
(B) Enlarged Mass Chromatogram of a Crustacean-Derived Peptide and its Calibration Curve (0.1 – 50 ppm)

Table 3 MRM Transitions of Target Allergenic Peptides

Food	Peptides	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (V)
Wheat	SVAVSQVAR	458.75	730.40	-16.0
			560.30	-18.0
	ELQELQER	522.75	674.35	-18.0
			802.40	-23.0
			432.20	-17.0
Buckwheat	SVFDDNVQR	540.00	746.20	-19.0
			631.10	-21.0
	GQILVVPQGFAVVLK	784.50	958.60	-23.0
			1057.80	-24.0
Eggs	ELINSWVESQTNGIIR	930.30	1116.50	-35.0
			888.40	-34.0
	LYAEER	391.10	667.10	-15.0
			504.10	-13.0
Milk	YLGYLEQLLR	634.35	991.55	-23.0
			771.45	-22.0
			658.40	-24.0
			277.15	-20.0
			334.20	-24.0
	NAVPITPTLNR	598.35	911.55	-17.0
			600.35	-25.0
			701.40	-24.0
	FALPQYLK	490.30	648.35	-17.0
			761.45	-18.0
			832.50	-15.0
Peanuts	NNPFYFPSR	571.25	669.35	-25.0
			506.25	-21.0
			229.10	-14.0
	GTGNLELVAVR	564.80	686.40	-19.0
			557.40	-23.0
			444.30	-23.0
Crustaceans (shrimp, crab)	IQLLEEDLER	629.35	1016.55	-23.0
			903.45	-20.0
			790.35	-23.0
	IVELEEELR	565.30	917.45	-18.0
			788.40	-21.0
			675.35	-20.0
	AGGLTLER	408.75	518.30	-17.0
			745.40	-17.0
			688.40	-17.0
Soy	NKPLVVQFQK	600.85	958.55	-24.0
		400.90	453.30	-13.0
			649.35	-14.0
	CPLTVVQSR	530.30	689.40	-23.0
			802.50	-23.0
			588.35	-23.0
	GIGTIISSPYR	582.30	722.40	-18.0
			993.55	-21.0
			835.45	-21.0

■ Analysis of Allergens in Processed Foods

We analyzed food samples with and without the addition of eight allergen standards to confirm whether the developed method could be applied to food samples. No peaks derived from allergenic peptides were detected in the pre-packaged curry and baby food analyzed this time. A peak derived from wheat was detected in the udon noodles, but no peaks derived from other allergenic peptides were detected. These results were consistent with the information listed on the package of the processed food. As an example, mass chromatograms of peptides derived from buckwheat and wheat are shown in Figs. 4 (A) and (B).

■ Conclusion

We introduced a method for the simultaneous analysis of seven specific ingredients (wheat, buckwheat, eggs, milk, peanuts, crustaceans (shrimp and crab)) and soy, which is considered equivalent to specific ingredients, using a triple quadrupole mass spectrometer.

In order to simultaneously analyze eight allergenic peptides, we developed a method with 48 MRM transitions targeting 17 types of peptides. We analyzed allergens present in three types of commercially available processed foods and were able to detect them accurately as stated on the packaging. Therefore, the analytical method presented in this article has been shown to be useful for simultaneously analyzing food allergens in food products.

<Acknowledgments>
We would like to thank the Saika Technological Institute Foundation for their cooperation in preparing this Application News.

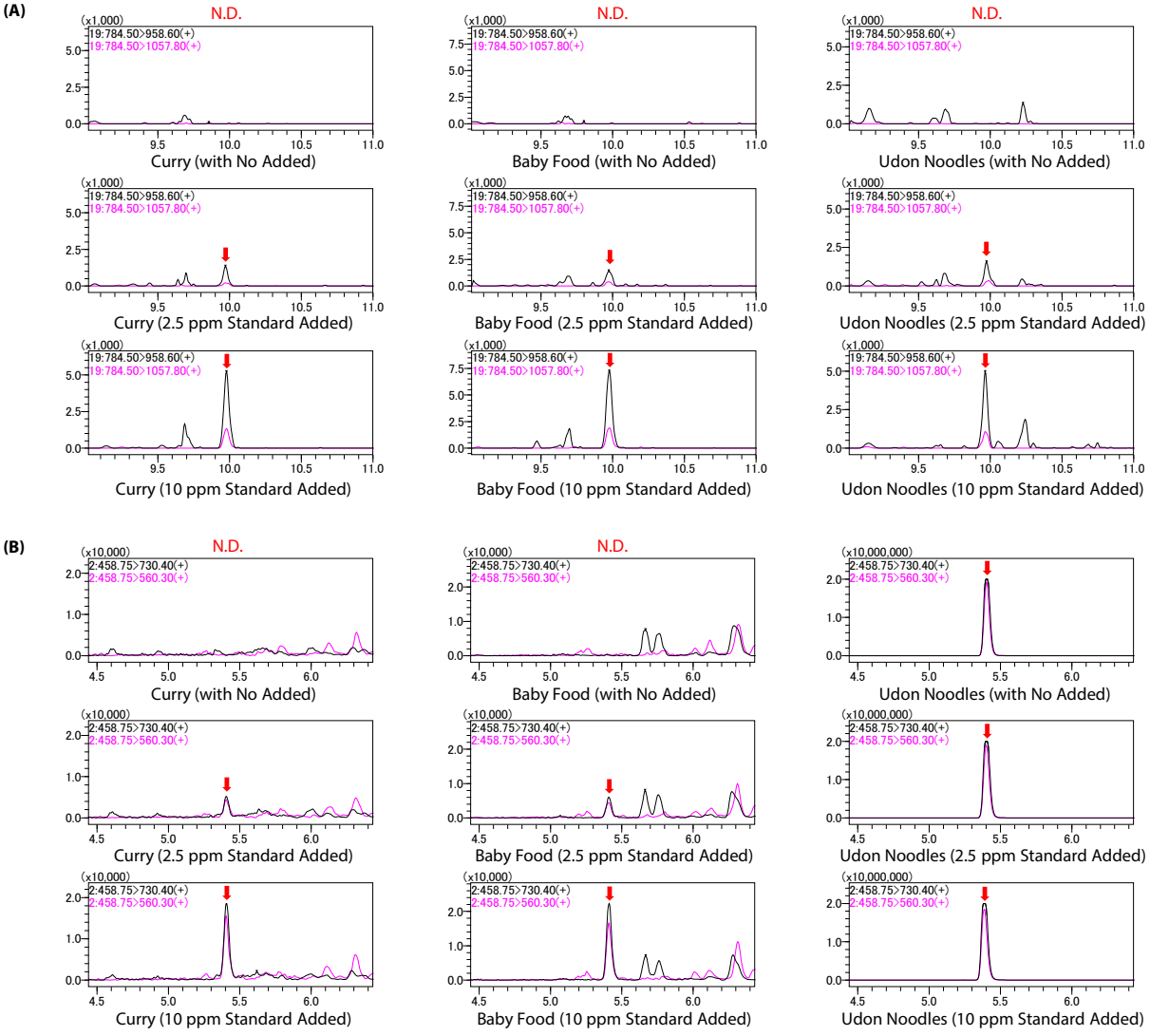

Fig. 4 (A) Mass Chromatogram of Buckwheat-Derived Peptides
(B) Mass Chromatogram of Wheat-Derived Peptides

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01-00665-EN First Edition: Mar. 2025

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