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

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1. Introduction

Food allergies result from immune responses to specific food proteins and are a major public health concern. To mitigate health risks, many countries enforce strict food labeling regulations. In Japan, mandatory labeling is required for eight ingredients—wheat, buckwheat, eggs, milk, peanuts, shrimp, crab, and walnuts—in processed foods, with recommendations for 20 other ingredients. Current detection methods include ELISA and PCR, which are simple but have limitations. ELISA risks false positives and cannot analyze multiple ingredients simultaneously, while PCR detects DNA but struggles to differentiate between substances like milk and beef or detect egg whites lacking DNA. Liquid chromatography mass spectrometry offers high selectivity and sensitivity for allergen detection, enabling simultaneous analysis of multiple ingredients. This application introduces a method for detecting seven specific ingredients and soy allergen in processed foods using the Nexera™ X3 liquid chromatograph and LCMS-8060NX mass spectrometer triple quadrupole mass spectrometer (Fig. 1).

Fig. 1 Nexera™ X3 and LCMS-8060NX

2. Sample Preparation and Analysis Conditions

Standard samples of the seven specific ingredients (wheat, buckwheat, eggs, milk, peanuts, crustaceans such as 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-packaged curry, baby food, and udon noodles, with allergen information listed on the packaging. 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 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

3. Simultaneous Analysis of Seven Specified Ingredients and One Equivalent Ingredient

To simultaneously analyze allergenic peptides from the eight ingredients mentioned above, we developed a method with 48 MRM transitions targeting 17 peptides. When analyzing the allergen mixed standard for these eight ingredients, all peptides eluted within 10.5 minutes, as shown in Fig. 3, demonstrating good peak shapes and separation patterns. Fig. 4 shows the linearity of the crustacean allergenic peptide (AGGLTLER) as an example.

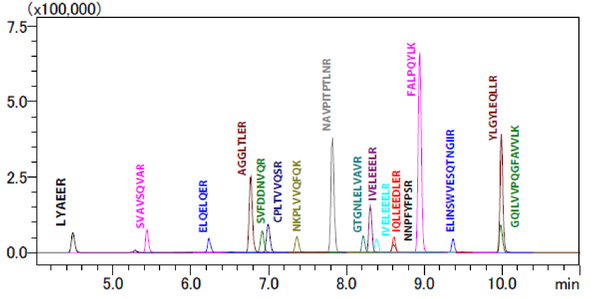

Fig. 3 Mass Chromatogram of Peptide Mixture - Eight Allergenic Foods

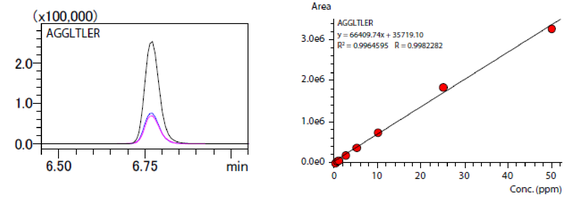

Fig. 4 Enlarged Mass Chromatogram of a Crustacean-Derived Peptide and its Calibration Curve (0.1 – 50 ppm)

4. Analysis of Allergens in Processed Foods

We analyzed food samples with and without the addition of eight allergen standards to verify whether the developed method could be applied to food samples. No peaks from allergenic peptides were detected in the pre-packaged curry and baby food analyzed this time. A peak from wheat was detected in the udon noodles, but no peaks from other allergenic peptides were observed. These results matched 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. 5 and 6.

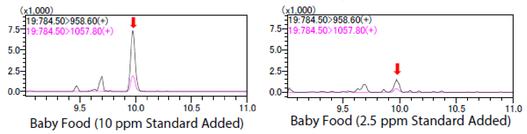

Fig. 5 Mass Chromatogram of Buckwheat-Derived Peptides

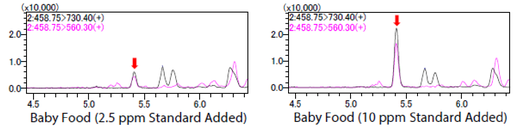

Fig. 6 Mass Chromatogram of Wheat-Derived Peptides

5. 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 these ingredients, using a triple quadrupole mass spectrometer. To analyze eight allergenic peptides simultaneously, we developed a method with 48 MRM transitions targeting 17 peptide types. We tested allergens in three types of commercially processed foods and successfully detected them as indicated on the packaging. Therefore, the analytical method described in this article has proven useful for the simultaneous detection of food allergens in food products.

<Acknowledgments>

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