SHIMADZU
Excellence in Science

Application News

No.C141

Liquid Chromatography Mass Spectrometry

High Sensitivity Analysis of Peanut Allergen in Cumin and Spice Mix [LCMS-8060]

Food allergens are a major public health concern. Among them, peanut allergy is one of the common food allergies. To avoid unexpected contact with food allergens, food labels are strictly used to indicate the presence of specific allergens. With the increasing awareness of food allergies, the presence of undeclared peanut in cumin lead to huge recalls in recent years. Although ELISA is the most commonly used technique to detect allergens, its false-positive rate is a major concern due to its cross-reactivity. We developed a method with high specificity and sensitivity to overcome this issue by using a high sensitivity triple quadrupole mass spectrometer to detect peanut allergen Ara h1 (Fig.1) in commercially available spices and seasonings.

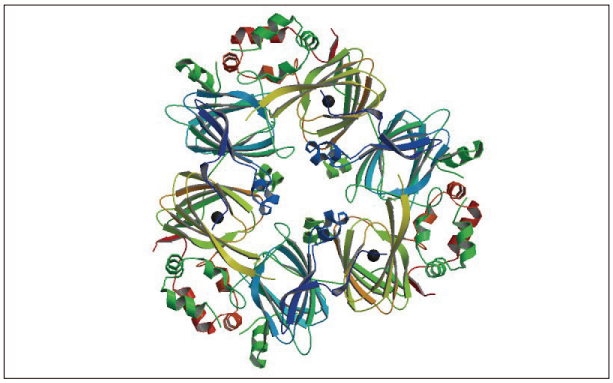

Fig. 1 Structure of Ara h1 [3S7I] (68kDa) Vicilin Like Protein

Sample Preparation

Commercially available defatted peanut flour was purchased and used for the initial development work. The test samples were ground and protein content was enriched by liquid-liquid extraction. Extracted proteins were denatured, reduced and alkylated before subjecting to tryptic digestion to obtain peptides that were quantitated as proxies of original protein abundance.
Cinnamon, cumin, chilli pepper, ginger, garlic, mustard seed, nutmeg, oregano, rosemary, sage, turmeric and thyme were selected as test food samples for evaluating cross-reactivity and sensitivity of the developed method. Food samples were pretreated as above with or without 2 ppm peanut powder.

Selection of MRM Transitions Using Skyline

Ara h1 is known as is known as the sensitizing allergen in 95 % of peanut allergy. Tryptic digest of protein extracted from peanuts were analyzed by monitoring theoretically calculated transitions of peptides based on amino acid sequences of two clones P17 and P41B of Ara h1.

MRM transitions for each clone was determined by using Skyline (MacCoss Lab Software). The transition list, which contained more than ten peptides for each clone, was reviewed by removing several peptides that could be susceptible by post translational modification and Maillard reaction during food processing.
Finally, nine peptides including three common peptides to both clones were selected based on sensitivity. Three transitions were set for each peptide.

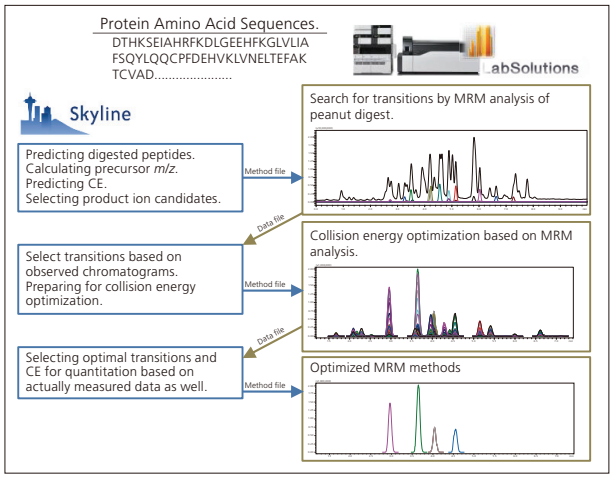

Fig. 2 Workflow of MRM Transition Optimization Using Skyline

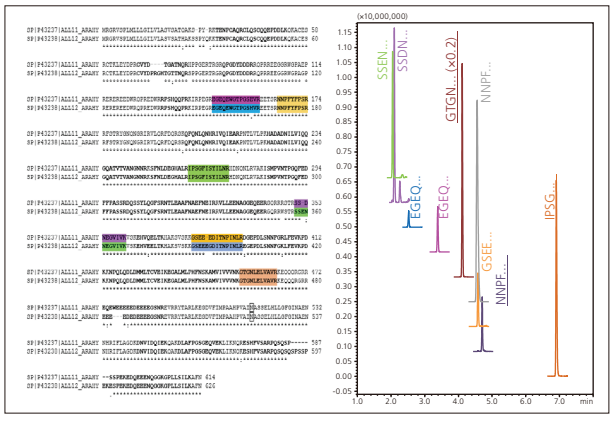

Fig. 3 AA Sequences of P17/P41B and Nine MRM Chromatograms

Table 1 Analytical Conditions

System	: Nexera X2	System	: LCMS-8060
Column	: Shim-pack XR-ODS Ⅱ (50 mm L. × 2 mm I.D., 1.6 µm)	Ionization	: Heated ESI
		Probe Voltage	: +1 kV (positive ionization)
Column Temperature	: 40 °C	Temperature	: Interface: 250 °C
Mobile Phases	: A: Water + 0.1 % formic acid		Desolvation Line: 150 °C
	B : Acetonitrile		Heater Block: 200 °C
Flowrate	: 500 µL/min	Gas Flow	: Nebulizing Gas: 3 L/min
Gradient	: 2 %B (0.00 min) > 25 %B (7.00 min) > 95 %B (7.10-8.00 min) > 2 %B (8.10-10.00 min)		Heating Gas: 20 L/min
			Drying Gas: 5 L/min
Injection Volume	: 10 µL		

Table 2 MS/MS Acquisition Parameters

MRM Transitions	Name	Polarity	Quan	Qual1	Qual2
	EGEQEWGTPGSEVR	+	780.85 > 802.40	780.85 > 644.35	780.85 > 316.10
	NNPFYFPSR	+	571.25 > 669.35	571.25 > 506.25	571.25 > 229.10
	IPSGFISYILNR	+	690.40 > 765.45	690.40 > 211.15	690.40 > 502.25
	SSDNEGVIVK	+	524.25 > 515.35	524.25 > 359.25	524.25 > 175.05
	GSEEEDITNPINLR	+	793.90 > 726.45	793.90 > 612.40	793.90 > 402.25
	GTGNLELVAVR	+	564.80 > 686.40	564.80 > 557.40	564.80 > 444.30
	EGEQEWGTPGSHVR	+	784.85 > 652.35	784.85 > 555.30	784.85 > 316.10
	SSENNEGVIVK	+	588.30 > 515.35	588.30 > 359.25	588.30 > 246.20
	GSEEEGDITNPINLR	+	822.40 > 726.45	822.40 > 612.40	822.40 > 402.25

Dwell Time	: 41 to 130 msec depending upon the number of concomitant transitions to ensure to have at least 15 points per peak (max total loop time 400 msec).
Pause Time	: 3 msec
CID Pressure	: 300 kPa
Quadrupole Resolution	: Q1: Unit Q3: Unit

Interface Optimization

Ionization parameters optimization was performed using companion software ISSS (Interface Setting Support Software, Shimadzu Corp.). As a result, sensitivity was improved more than twofold compared to default values.

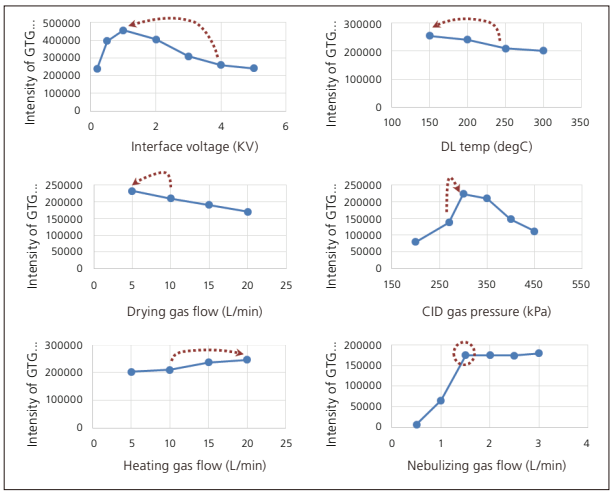

Fig. 4 Interface Optimization Results

Effect of Surfactant During Digestion

A higher intensity of peptides by addition of a surfactant during tryptic digestion was expected due to improved digestion efficiency. However, the intensity of peptides were relatively worse by adding surfactant. Thus, no surfactant was used for tryptic digestion.

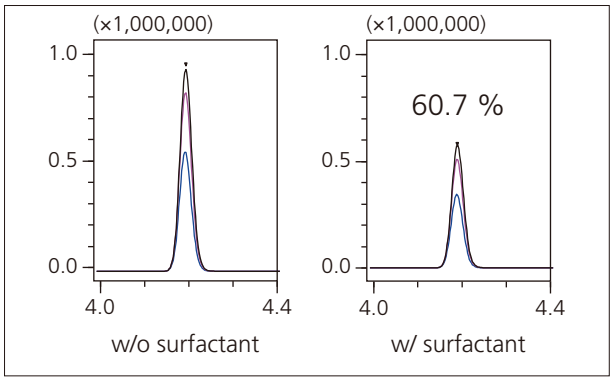

Fig. 5 Difference of the Chromatograms of Peptide GTG... by Addition of Surfactant

Peanut Allergen in Other Nuts

Walnuts, cashew nuts, and almonds were analyzed to test specificity. These nuts were spiked with 2 ppm (2 mg/kg) of peanut before sample preparation. The spiked peanut peptides were successfully detected and any obvious peak was detected in blank samples.

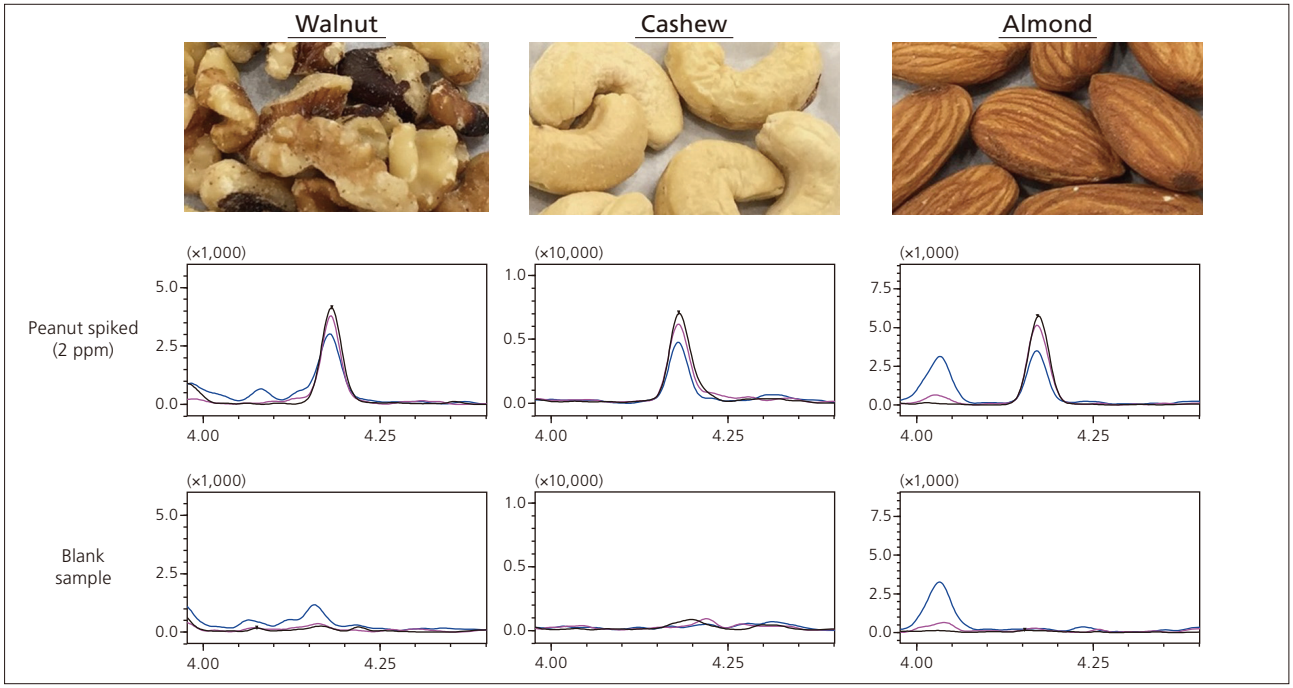

Fig. 6 Chromatograms of Peptide GTG… in Other Kind of Nuts With or Without Spiking with Peanuts

Detection of ARA h1 in Spice Mixes and Seasonings

Several spice mixes and seasonings were analyzed using sample preparation and analytical conditions described here. Peaks of tryptic peptides of Ara h1 from samples without spiking of peanut peptides were detected.

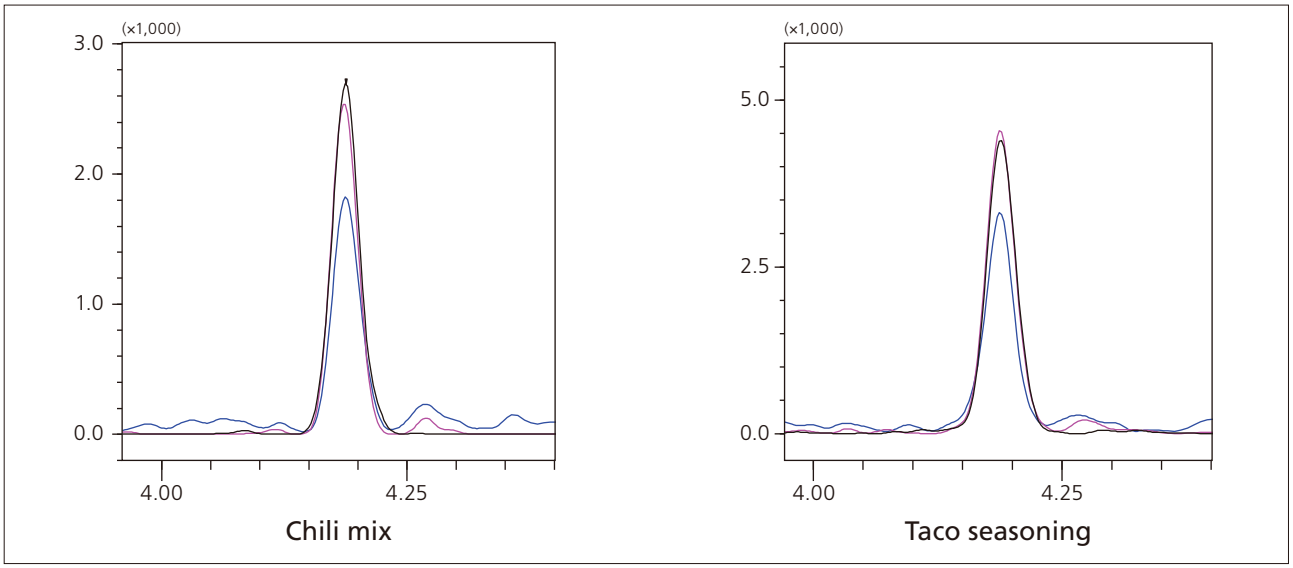

Fig. 7 Detected Peaks of Peptide GTG… in Chili Mix and Seasoning

Peanut Allergen in Spices

Contaminated spice samples were prepared and analyzed to confirm that the low amount of peanuts added into the various spices can be detected. Peptides of Ara h1 were successfully observed from the spice samples spiked with 2 ppm of peanuts. It was also confirmed that there are no obvious false-positive peaks from the blank samples.

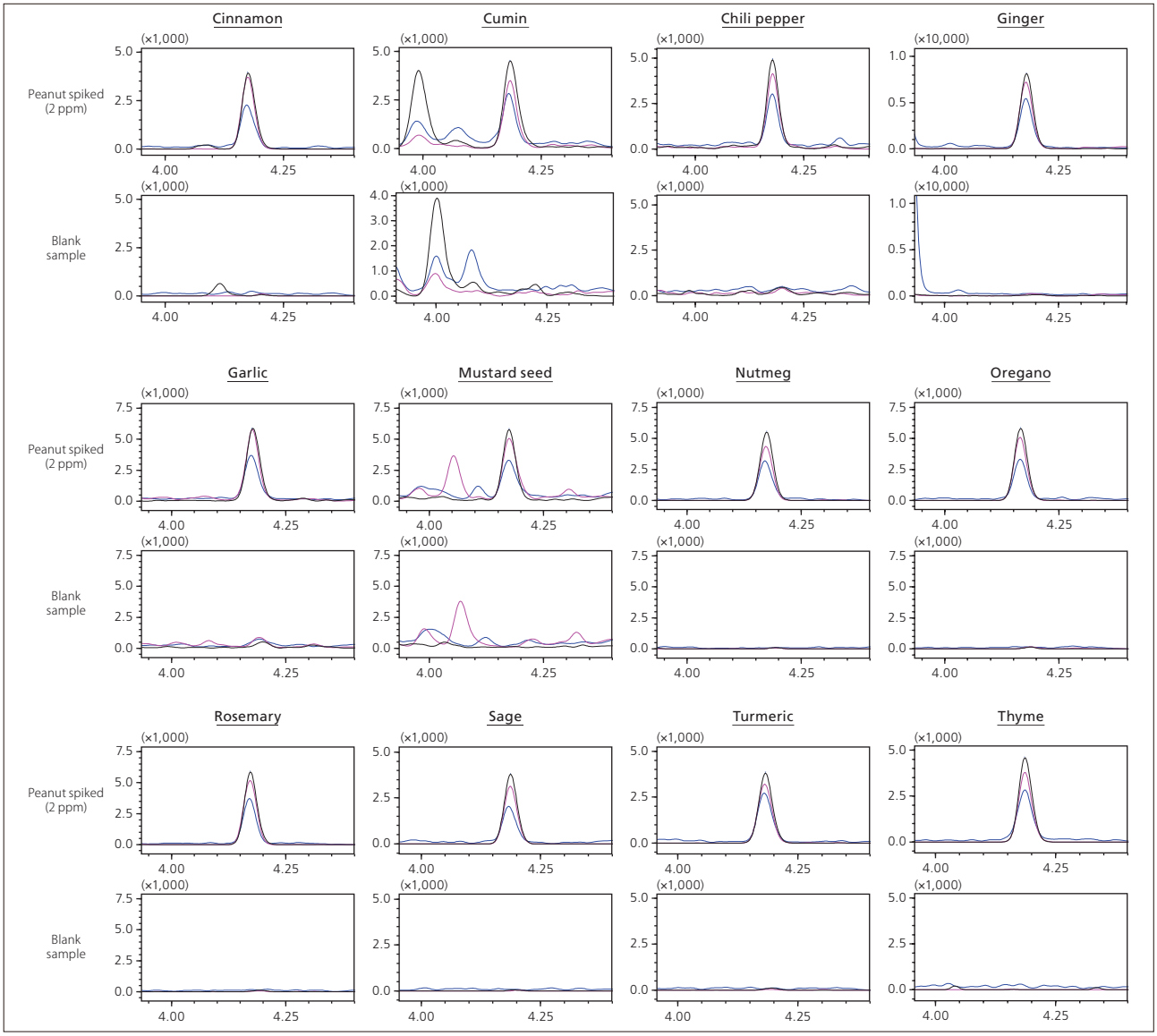

Fig. 8 Chromatograms of Peptide GTG... in Spices With or Without Spiking with Peanuts

Conclusion

A method for the analysis of Ara h1 in spices and seasonings was successfully developed.
The combination of the developed method and a high sensitivity triple quadrupole mass spectrometer enabled the detection of 2 ppm or lower of peanut allergen Ara h1 in spices and seasonings.

First Edition: Dec. 2016

Shimadzu Corporation
www.shimadzu.com/an/