Simultaneous analysis of multiple food allergen and its detection from processed food

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1. Introduction

Food allergy becomes a public health concern and its prevalence is currently estimated up to 10%. The Food Allergen Labeling and Consumer Protection Act (FALCPA) requires that food manufacturers label food products intentionally containing eight major allergenic food ingredients such as milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybeans are identified as the major food allergens. While ELISA is frequently used technique to detect allergenic proteins, liquid chromatograph tandem mass spectrometry (LC-MS/MS) becomes an alternative technique in terms of high selectivity, sensitivity, and capability to analyze multiple allergens simultaneously. It is also expected that LC-MS/MS technique can overcome existing issue of ELISA such as false detection of allergens in processed foods.

2. Materials and methods

Sample preparation

Commercially available allergenic food materials were purchased at local grocery store and used for development of analytical methods. The samples were ground into fine powders with a dry-ice by GM-200 (Retsch). 0.5 g of each ground samples was transferred into 15 mL tube. 5 mL of hexane was added for removal of oils and fats from samples, and after centrifugation, supernatant was discarded. Proteins were extracted by using the extraction buffer containing 50 mM Tris-HCl (pH8.0) 2M Urea and protease inhibitors. Aliquot of extract containing 200 µg of proteins were denatured, alkylated, and digested into peptides by traditional in-solution protein digestion technique. Digested peptides were desalted by SPE, lyophilized, and stored until analysis.

LC/MS analytical conditions

LC/MS analysis was conducted by using Shimadzu Nexera X2^{TM} UHPLC coupled to triple quadrupole mass spectrometer LCMS-8050. 0.1 % formic acid in water (A) and acetonitrile (B) were used for mobile phase at a flow rate of 0.5 mL/min. Shim-pack XR-ODS III (2.0 mmID x 75 mmL., 1.6 µm) was used as analytical column. The high pressure gradient elution was set as follows: 2%B (0.0 min), 15%B (4.0 min), 40%B (7.0 min), 95%B (7.1-8.0 min), 2%B (9.1-10.0 min). Peptides were detected by MRM acquisition. Other parameters for mass spectrometer were set as follows: positive mode electrospray ionization, nebulizing gas flow of 3 L/min, heating gas flow of 20 L/min, drying gas flow of 5 L/min, interface temperature of 250 °C, DL temperature of 150 °C, heat block temperature of 200 °C.

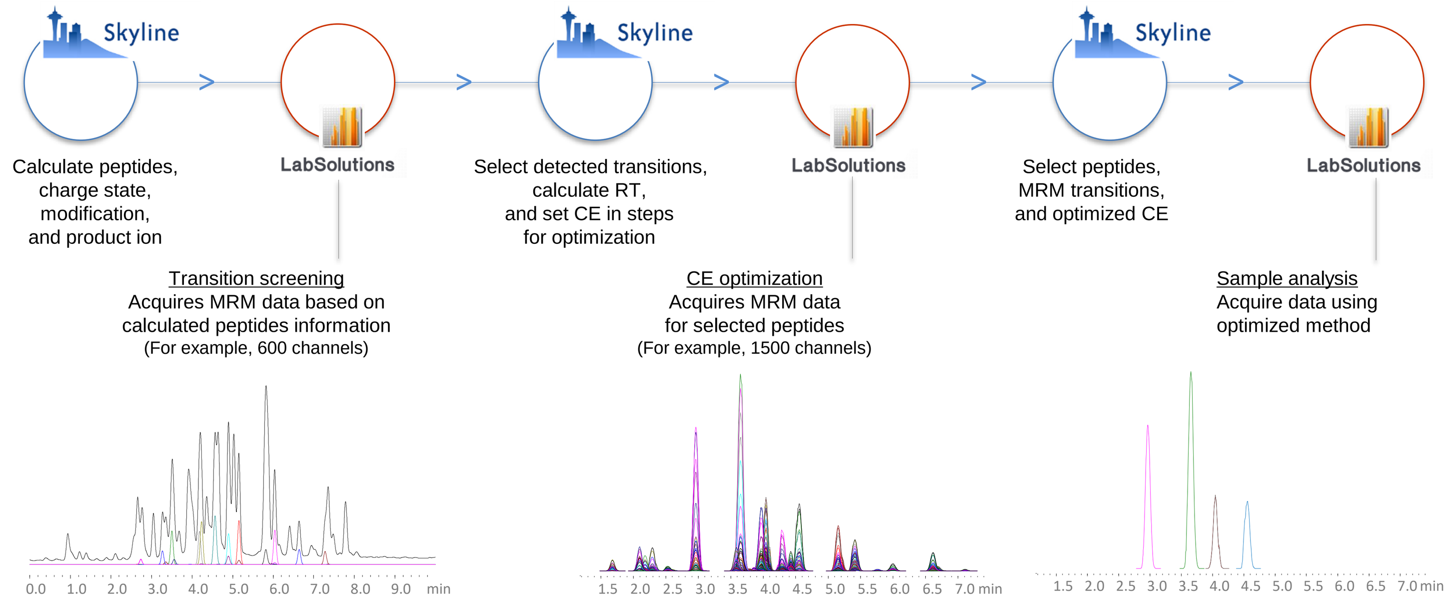

Figure 1 Work flow of MRM transition optimization using Skyline.

3. Result

Detection of allergenic proteins by LC-MS/MS

We developed the LC-MS/MS method to detect 13 food materials consisting eight foods which FDA requests to label. The target food materials were common wheat, milk, egg, cod, shrimp, lobster, soybean, peanut, brazil nut, cashew nut, walnut, hazel nut and almonds. Low molecular glutenin subunit (Tri a 26) and high molecular glutenin subunit (Tri a 36) were selected to monitor common wheat. Among candidates of peptides to monitor, several peptides commonly were removed from candidate list due to its homology to other grains. For example, AQQLAAQLPAMCR, ELQESSLEACR and LPWSTGLQMR were found from both barley and rye. In addition to informatics approach, we analyzed eight grains including common wheat and durum wheat as an experimental confirmation. Calibration curve was prepared by plotting a peak ratio of light and heavy-labeled peptides against a concentration of food ingredients. 245 transitions were finally selected to monitor unlabeled peptides derived from 13 food materials.

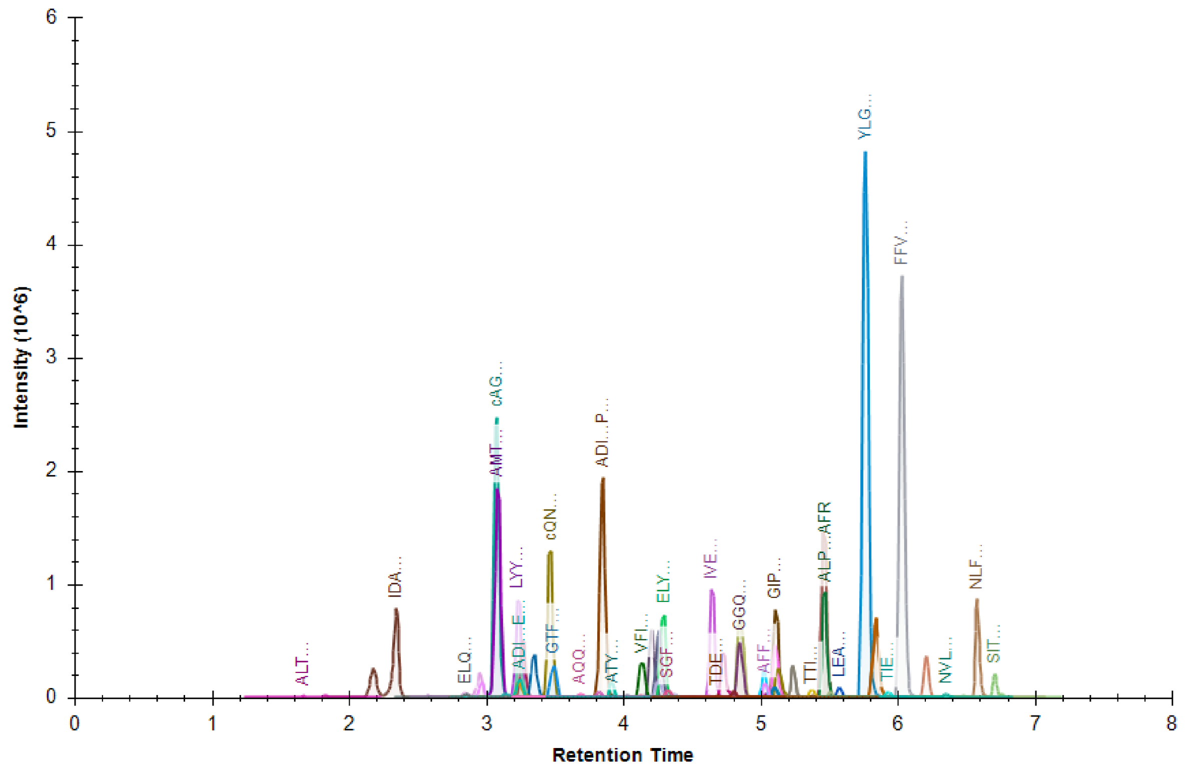

Figure 2 Chromatogram of a mixture of milk, eggs, cod, shrimp, lobster, almonds, brazil nuts, cashew nuts, hazelnuts, walnuts, peanuts, wheat, and soybeans.

Quantitation of wheat proteins

Quantitation of the food ingredient is more challenging because there are several factors to be determined such as extraction efficiency, proteolysis efficiency, and matrix effect etc. It is also difficult to calculate the amount of a food ingredient based on quantitation result of several proteins. We have used reference materials to mitigate these issues and improve the traceability. The linear range was set 3.9 – 250 mg/kg and its R2 value was >0.995.

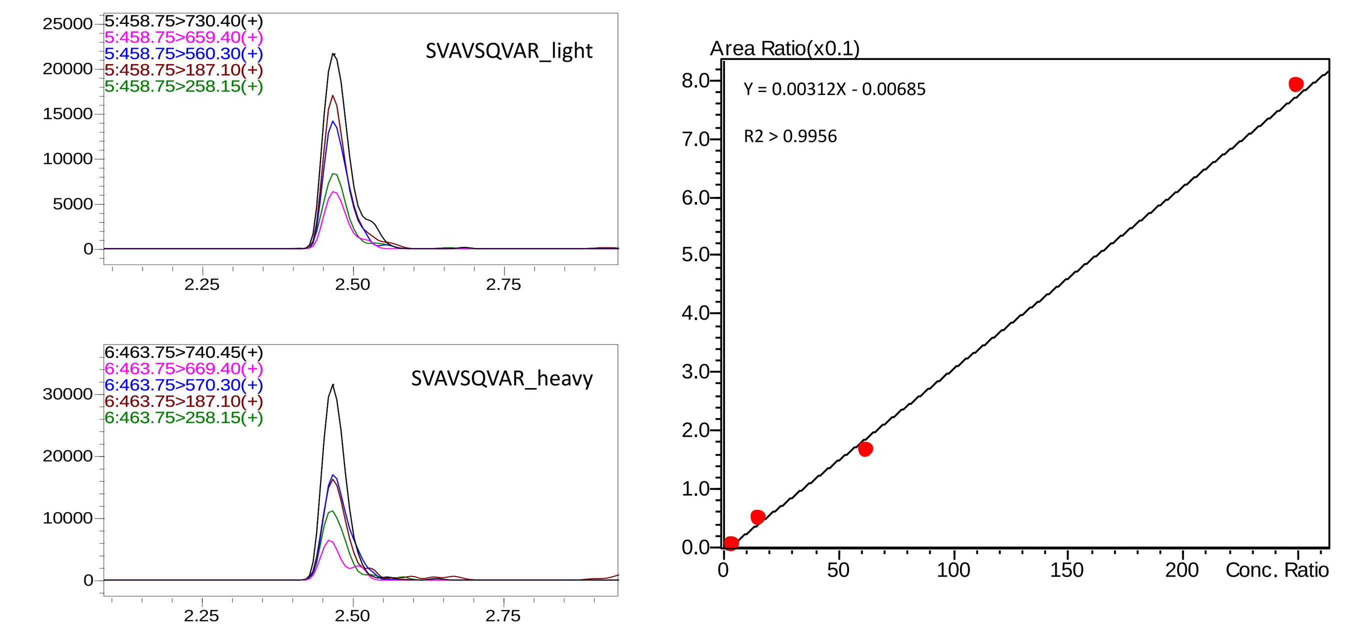

Figure 3 Quantitation of wheat based on SRM1567b wheat flour.

Detection of signature peptides from thermally processed food.

To evaluate the performance of this method, we analyzed peptides prepared from raw food materials and thermally processed foods. All of targeted peptides were successfully detected. These results confirm that this developed method could be used for monitoring of the 13 allergenic food ingredients from both raw material and thermally processed food.

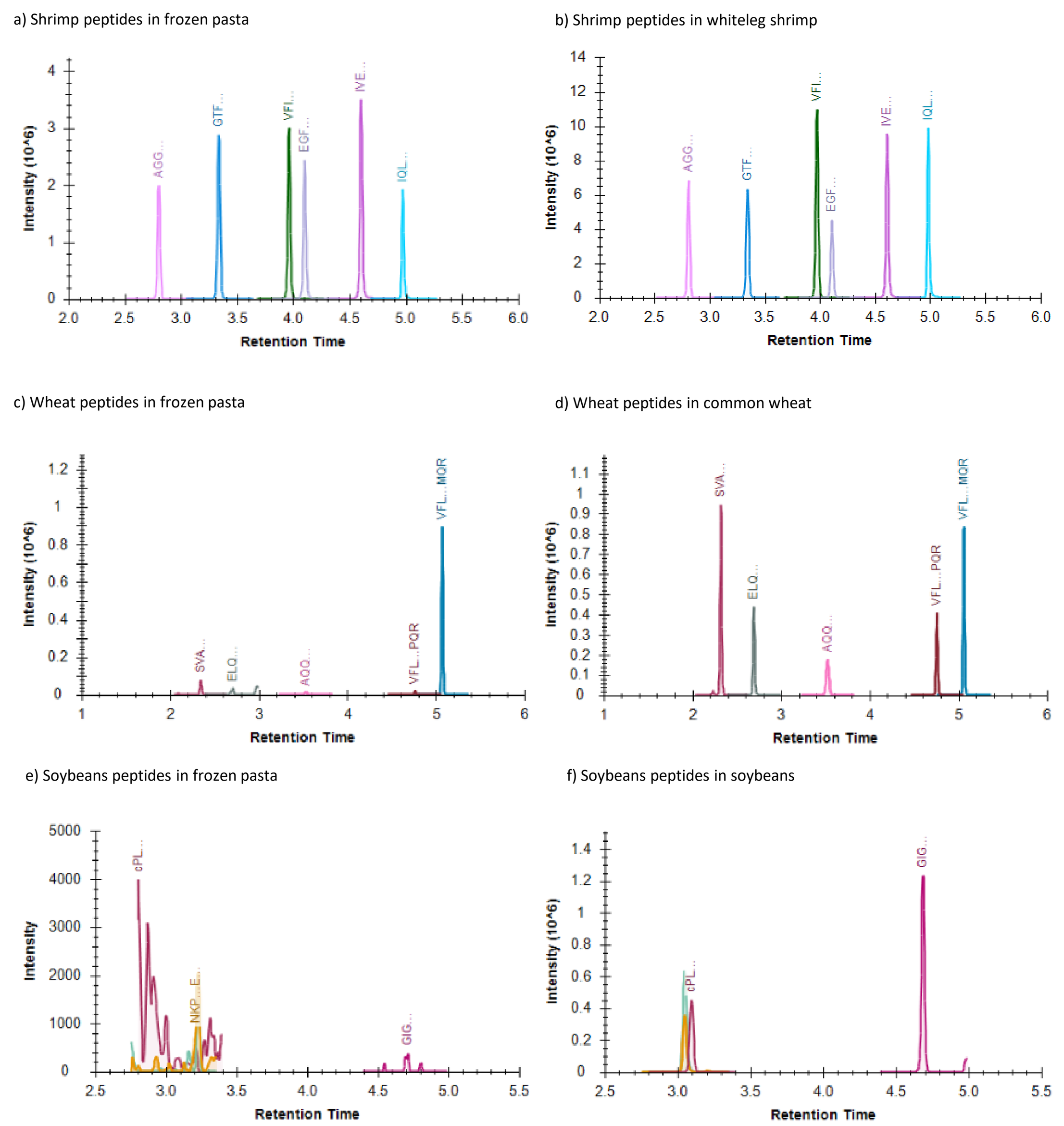

Figure 4. Comparison of chromatograms of each ingredients in pasta and row materials.

Table 2 Peptide search result of tryptic peptides among the genus *Triticum.*

	Triticum aestivum	Triticum aestivum subsp. tibeticum	Triticum aestivum subsp. yunnanense	Triticum compactum	Triticum dicoccoides	Triticum dicoccon	Triticum macha	Triticum monococcum	Triticum monococcum subsp. aegilopoides	Triticum monococcum subsp. monococcum	Triticum polonicum	Triticum spelta	Triticum timopheevii	Triticum timopheevii subsp. araraticum	Triticum turgidum	Triticum turgidum subsp. durum	Triticum urartu
ACQQVMDQQLR	X											X					
AQQLAAQLPAMCR	X				X			X		X	X	X	X	X	X	X	X
AQQPATQLPTVCR	X		X	X								X					
ELQELQER	X											X					
ELQESSLEACR	X	X	X	X	X	X			X	X		X	X	X	X	X	X
GGSFYPGETTPPQQLQQR	X											X					
IFWGIPALLK	X											X					
ILPTMCSVNVPLYR	X						X										
LEGGDALSASQ	X									X		X					
LPWSTGLQMR	X	X	X	X	X	X			X	X		X	X	X	X	X	X
MEGGDALSASQ	X		X	X	X	X			X	X		X	X		X	X	X
QGSYYPGQASPQQPGQGQQPGK	X	X	X	X								X					
QQPGQGQHPEQGK	X																
QQQIPVIHPSVLQQLNPCK	X				X		X	X	X				X		X	X	X
QVVDQQLAGR	X	X	X	X	X	X			X			X	X	X	X	X	
QYEQTVVPPK	X	X	X	X	X							X			X	X	
SQMLQQSICHVMQQQCCQQLR	X	X			X		X	X	X						X		X
SVAVSQVAR	X	X	X	X								X					
TTTSVPFGVGTGVGAY	X				X		X										
VFLQQQCIPVAMQR	X	X			X			X	X				X		X	X	X
VFLQQQCSPVAMPQR	X				X				X	X							X

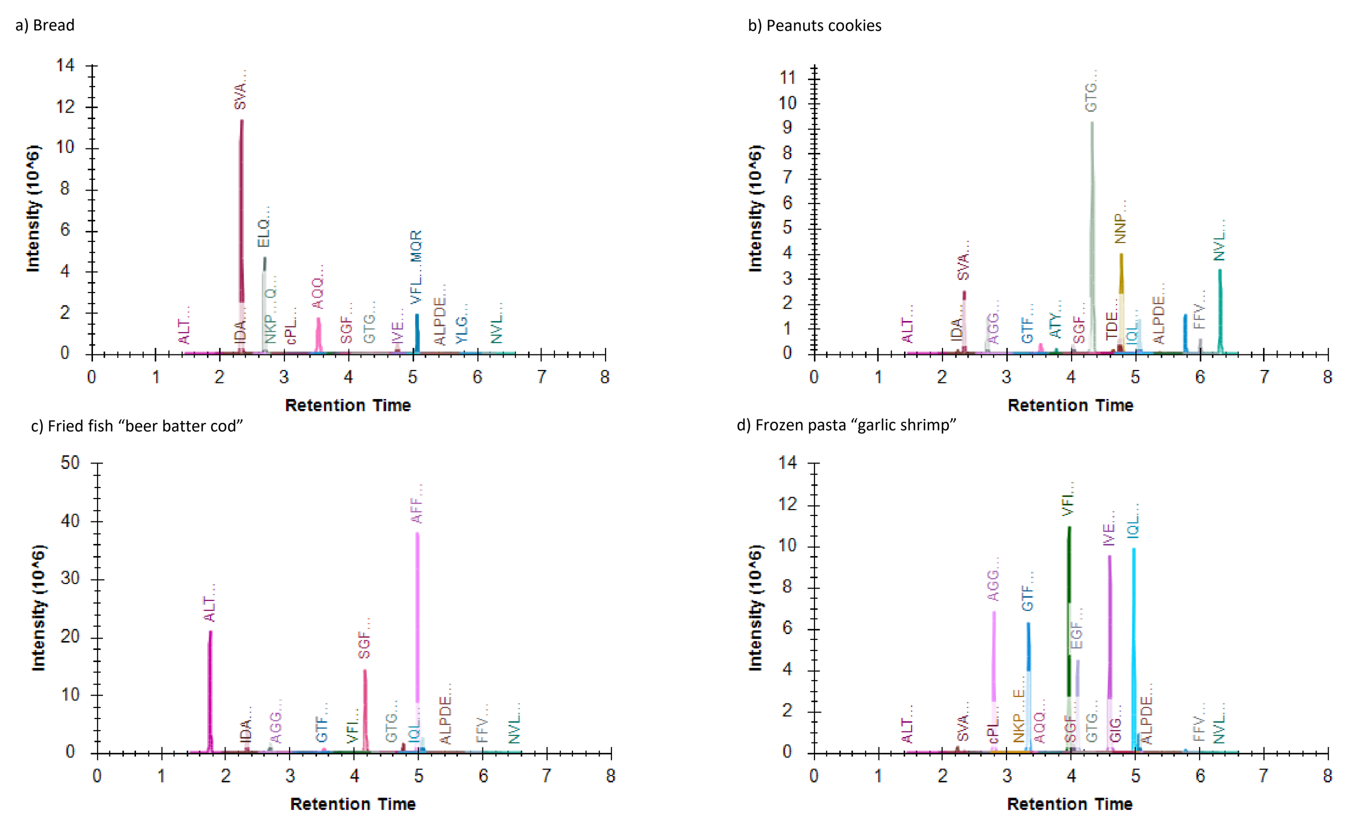

Figure 5. Chromatograms of four thermally processed foods: (a) bread, (b) cookies, (c) fried fish, and (d) frozen pasta.

Table 3 Summary of food labeling and analytical results of thermally processed foods (bread, peanuts cookie, fried fish, and frozen pasta)

Food	Allergens	Bread		Peanuts cookie[a]		Fried fish "Beer butter cod"		Frozen pasta "garlic shrimp"	
		Label	Detect	Label	Detect	Label	Detect	Label	Detect
Wheat	High molecular weight glutenin (Tri a 26)	x	x	-	x	x	x	x	x
	Low molecular weight glutenin (Tri a 36)		x		x		x		x
Milk	Caseins (Bos d 8)			-	x	x	x	x	x
	Beta-lactoglobulin (Bos d 5)				x		x		x
Eggs	Ovalbumin (Gal d 2)	x	x	-	x				
	Ovotransferrin (Gal d 3)		x		x				
Peanuts	Cupin, vicillin-type, 7S globulin (Ara h 1)			-	x				
Soybeans	Trypsin inhibitor (Gly m TI)			-				x	
Atrantic cod	Beta-parvalbumin (Gad m 1)			-		x	x		
Whiteleg shrimp	Tropomyosin (Lit v 1)			-				x[b]	x
	Myosin, light chain 2 (Lit v 3)								x
	Sarcoplasmic CBP (Lit v 4)								x

[a] No allergen information was provided.
[b] It was declared as shrimp.

4. Conclusion

- ✓ The method including 245 MRMs for monitoring of 13 food materials was developed.
- ✓ Detection of allergenic food materials from thermally processed foods were evaluated.]

LCMS-8050 triple quadrupole mass spectrometer

The product and application are Research Use Only. Not for use in human clinical diagnostics or in vitro diagnostic procedures.