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Simultaneous analysis of major allergens in food matrices by high sensitive mass spectrometer

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1. Introduction

Food allergy is an abnormal overreaction of immune system to a particular protein in food. It is becoming a major concern for public health and food industries. Typical food allergens are proteins and peptides. The signs and symptoms may range widely from itching, red skin, swelling, anaphylaxis etc. There is no cure for food allergy at present, so people with allergy must avoid food triggers. To avoid unexpected contact with food allergens, food labels are strictly used to indicate presence of specific allergens. The Food Allergen Labeling and Consumer Protection Act (FALCPA) identified eight foods or food groups as major allergens which include milk, eggs, fish (e.g., bass, flounder, cod), crustacean shellfish (e.g., crab, lobster, shrimp), tree nuts (e.g., almonds, walnuts, pecans), peanuts, wheat and soybeans and FALCPA mandates that the labels of foods containing eight major food allergens declare the presence of allergens. ELISA (enzyme-linked immunosorbent assay) and PCR (Polymerase chain reaction) are most commonly used technique to detect allergenic foods due to relatively simple handling. Even so, cross-reactivity of ELISA can raise a the risk of false positive results. Additionally, ELISA requires separated analysis for each target. Since PCR assay is based on detection of DNAs rather than allergenic proteins, milk cannot be distinguished from beef and will be difficult to detect food contains egg white. Therefore, it is important to determine allergens in food by using more reliable detection method. Recently, liquid chromatography mass spectrometry becomes an alternative technique to detect allergenic proteins with high selectivity, sensitivity, and capability to analyze multiple allergens simultaneously. We developed a method to detect 31 peptides derived from eight allergens. We analyzed commercially available samples such as bread and gluten free bread etc to evaluate this method. We did not detected any peptides derived from gluten in gluten free bread and gluten free cracker. And we could detect peptides of 20 ppm wheat fortified to gluten free bread. We could detect other allergens shown on the label from commercial available food matrices.

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 in fine powders by GM-200 (Retsch). 0.5 - 1 g of each ground samples was transferred into 50 mL tube. Hexane was used for removal of oils and fats from samples. Proteins were extracted by using the extraction buffer containing 50 mM Tris-HCl (pH8.0) 2M Urea and protease inhibitors. Aliquot of extract containing 100 – 250 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 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 min), 40%B (7 min), 95%B (7.10-8.00 min), 2%B (9.10-10.00 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.

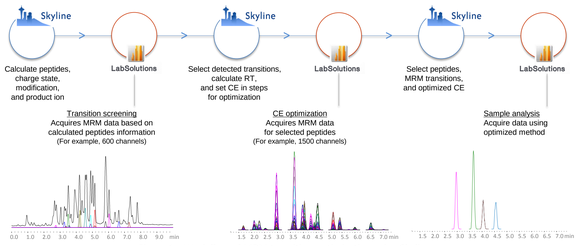

Figure 1 Work flow of MRM transition optimization using Skyline.

3. Result

Detection of allergenic proteins by LC-MS/MS

To establish analytical method, we selected MRM transitions of signature peptides by using Skyline (Figure 1) based on their peak intensity, peak shape, and similarity to other peptides of target proteins. As a result of method development, we finally selected 150 MRM transitions for monitoring 33 peptides derived from 13 proteins as allergenic proteins of eight foods or food groups. As Figure 2 shows, all of peptides were eluted within 6.5 min with good separation. Figure 2 also shows the linearity of peptides.

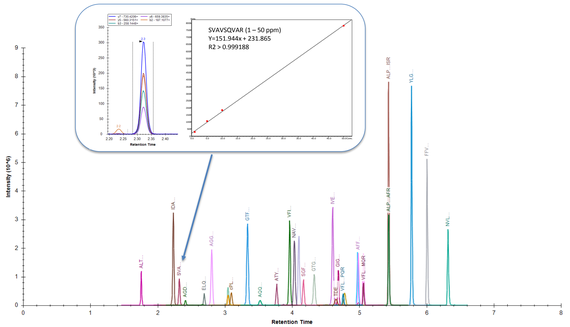

Figure 2 Chromatogram of peptides mixture derived from eight food allergens, and magnified view of five MRM transitions for wheat peptides and its calibration curve.

Table 1 The target food matrices, protein name, peptides, and UniProt ID found in same food.

Food *(Binomial name)*		Protein name (IUIS name)	Peptides	Uniprot ID
Milk *(Bos taurus)*		Caseins (Bos d 8)	FFVAPFPEVFGK	P02662, B5B3R8
			YLGYLEQLLR	P02662, B5B3R8
			NAVPITPTLNR	P02662, B5B3R8
			FALPQYLK	P02663
		Beta-lactoglobulin (Bos d 5)	IDALNENK	P02754, G5ES57, B5B0D4
Egg *(Gallus gallus)*		Ovalbumin (Gal d 2)	NVLQPSSVDSQTAMVLVNAVFK	P01012
		Ovotransferrin (Gal d 3)	ATYLDCIK	P02789, Q4ADJ7, Q4ADJ6, E1BQC2, Q4ADG4, A0A1D5P4L7
			TDERPASYFAVAVAR	P02789, Q4ADJ7, Q4ADJ6, E1BQC2, Q4ADG4, A0A1D5P4L7, A0A1L1RSU6
Fish	Atrantic cod *(Gadus morhua)*	Beta-parvalbumin (Gad m 1)	ALTDAETK	P02622, A5I873, Q90YL0
			AFFVDQDK	Q90YL0, A5I873
			SGFIEEDELK	Q90YL0, A5I873
Crustacean shellfish	Whiteleg shrimp *(Litopenaeus vannamei)*	Tropomyosin (Lit v 1)	IQLLEEDLER	B4YAH6
			IVELEEELR	B4YAH6
		Myosin, light chain 2 (Lit v 3)	EGFQLMDR	B7SNI3
			GTFDEIGR	B7SNI3
		Sarcoplasmic calcium-binding protein (Lit v 4)	VFIANQFK	C7A639
			AGGLTLER	C7A639
Tree nuts	Almonds *(Prunus dulcis)*	Amandin, 11S globulin legumin-like protein (Pru du 6)	ALPDEVLQNAFR	E3SH28, Q43607
			ALPDEVLQNAFR	E3SH29
Peanuts *(Arachis hypogaea)*		Cupin Vicillin-type, 7S globulin (Ara h 1)	NNPFYFPSR	P43237, P43238, E5G076, B3XXL2, N1NG13, Q6PSU3
			GTGNLELVAVR	P43237, P43238, B3XXL2, Q6PSU6, Q6PSU3, N1NG13, Q6PSU5, E5G076, Q6PSU4
Wheat *(Triticum aestivum)*		High molecular weight glutenin (Tri a 26)	ELQELQER	P10388, P08489 and 22 others in wheat
			SVAVSQVAR	P10387, P08488, and 21 others in wheat
			ACQPAITQLPTVCR	P10387, P08488, and 21 others in wheat
		Low molecular weight glutenin GluB3-23 (Tri a 36)	VFLQQQCSPVAMPQR	P10385 and 71 others in wheat
			VFLQQQCSPVAMPQR	P10386, P04729, P04730 and 114 others in wheat
Soybeans (Glycine max)		Trypsin inhibitor (Gly m TI)	CPLTVVQSR	P01070, P01071, P25272 and 13 others
			NKPLVVQFQK	P01070, P01071, P25272 and 8 others
			NKPLVVEFQK	P25273

Allergens in cooked food matrices

Chromatograms of commercially available food matrices were shown below. A mixture of eight allergenic food and seven cooked food were analyzed. As summarized in Table 3, even we missed soybeans from several food, these data shows that we could detect expected allergens from actual samples overall.

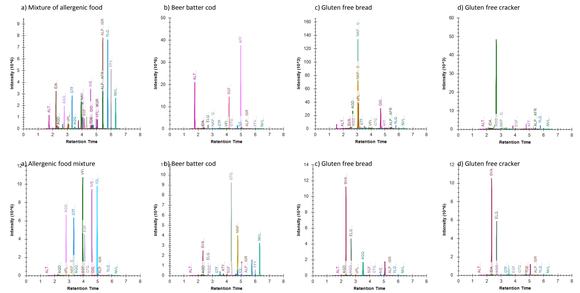

Figure 3 Chromatograms of seven cooked food matrices and mixture of allergenic food as positive control.

Table 2 The results of seven cooked food samples.

Food	Allergens	Gluten free bread		Gluten free cracker		Bread		Cracker		Peanuts cookies		Frozen fish "fried cod"		Frozen pasta "garlic shrimp"	
		Label	Detect	Label	Detect	Label	Detect	Label	Detect	Label	Detect	Label	Detect	Label	Detect
Milk	Caseins (Bos d 8)			x	x			(x)		-	x	x	x	x	x
	Beta-lactoglobulin (Bos d 5)				x						x		x		x
Egg	Ovalbumin (Gal d 2)			x	x	x	x	(x)		-	x				
	Ovotransferrin (Gal d 3)				x		x				x				
Atrantic cod	Beta-parvalbumin (Gad m 1)									-		x	x		
Whiteleg shrimp	Tropomyosin (Lit v 1)														x
	Myosin, light chain 2 (Lit v 3)									-				x'	x
	Sarcoplasmic CBP (Lit v 4)														x
Almonds	Amandin (Pru du 6)							(x)		-					
Peanuts	Cupin, vicillin-type, 7S globulin (Ara h 1)			x				(x)		-	x				
Wheat	High molecular weight glutenin (Tri a 26)					x	x	x	x	-	x	x	x	x	x
	Low molecular weight glutenin (Tri a 36)						x	x	x	-	x		x	x	x
Soybeans	Trypsin inhibitor (Gly m TI)	x	x	x				x		-			x		

'Labeled as "Crustacean shellfish (Shrimp)"

"Gluten-free" food samples

As a part of evaluation of the method, we analyzed bread containing gluten and gluten-free bread. In US, as one of the criteria for using the claim "gluten-free", FDA set a gluten limit of less than 20 ppm in foods that carry this label. Then, we also analyzed gluten free-bread spiked with wheat extract at 10 ppm. As shown in Figure 4, those level of glutens was detected successfully.

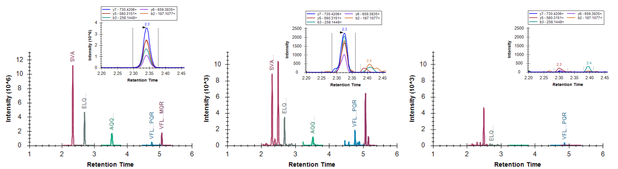

Figure 3 Chromatograms of seven cooked food matrices and mixture of allergenic food as positive control.

Similarity to other food ingredients

In method development, we performed peptides search for amino acid sequences of theoretically calculated peptides by using UniProt database. Since gluten is a major protein in grains, those peptides sequences are commonly preserved in other edible grains as well (Table 3). To avoid miss identification of food ingredients, we selected the sequences not found in Barley or Rye as significant peptides. On the other hand, these peptides are also found in some sort of goat grass.

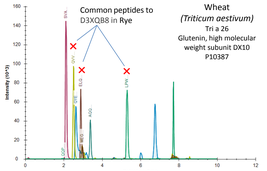

Table 1 Peptides search result of predicted peptides

Analyzed wheat peptides (P10387)	Positions	Barley	Rye
ACQPATQLPTVCR	624-636		
ELQESSLEACR	33-43	x	x
LPWSTGLQMR	54-63	x	x
MEGGDALSASQ	637-647		x
QGSYYPGQASPQQPGQGQQQPGK	135-156		x
QQPGQGQQHPEQGK	469-481		x
QVVDQQLAGR	44-53		x
QYEQTVVPPK	86-95		
SVAVSQVAR	75-85		

x: found, blank: not found

Figure 3 Work flow of MRM transition optimization using Skyline.

4. Conclusion

- Major food allergens were successfully detected by LC-MS/MS method.
- The method contains 150 MRMs for 31 peptides of 13 allergenic proteins identified in 8 foods.
- The presence of allergenic ingredients in cooked meal could be detected.

LCMS-8050 triple quadrupole mass spectrometer