

Labeled Peptide-Free UHPLC–MS/MS Method Used for Simultaneous Determination of Shrimp and Soybean in Sauce Products

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ABSTRACT: Unintentional missing of shrimp and soybean allergen information on precautionary food allergen labeling often occurs in sauce products. To avoid food allergies, sensitive and time-saving analytical methods are urgently needed. However, the currently reported methods usually employed labeled peptides for isotope internal standard quantitation, and the matrix effect caused by protein extraction or digestion can not be corrected. In this study, a labeled peptide-free standard addition method was developed for simultaneous determination of shrimp and soybean in sauce products using ultrahigh-performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS). Through the rational selection of stable peptides, satisfying mean recoveries and relative standard deviations of the chosen peptides are achieved. The limit of quantifications of each peptide ranged from 0.25 to 5 μg tropomyosin/g sauce and from 1 to 10 μg Gly m 6/g sauce, respectively. Using the labeled peptide-free UHPLC–MS/MS method, not only ideal experimental results were obtained surpassing those obtained with labeled peptides, but also the reagents were economized and shortening of the sample preparation time was achieved.

KEYWORDS: shrimp, soybean, allergen, UHPLC–MS/MS, labeled peptide-free standard addition

INTRODUCTION

Food allergy is considered as a global health issue, and is associated with some adverse immunological reactions. About 6% of children and 3–4% of adults in western countries are affected by food allergy, and the prevalence appears to be increasing.¹ There are eight allergens of foods that cause 90% of worldwide food allergy incidences.² These foods include peanut, tree nuts, shellfish, fish, wheat, soybean, milk, and egg.

Sauce is a flavorful food and prepared from soybean, shrimp, tomatoes, potatoes, mushrooms, chillies, and so forth.³ Sauce is the most commonly used condiment for daily cooking in houses or restaurants.⁴ Soybean and shrimp are increasingly used as important protein sources and flavoring agents in sauce production.⁵ The allergy problem caused by soybean and shrimp affects the quality of life and may even threaten lives. To prevent severe allergic reactions, the patients should avoid allergen-suspicious food. Food allergen labeling is also mandated by several countries, including U.S.,⁶ European Union,⁷ Canada,⁸ and Australia.⁹ Unfortunately, incomplete and inaccurate food labeling often occurs in processed food due to the allergen contamination from the same production lines.¹⁰ Besides, no regulatory threshold is set for allergenic compounds in food samples.¹¹ Thus, highly sensitive and robust analytical methods to quantify soybean and shrimp in sauce are required for improving labeling and reducing the risk of casual consumption of allergens by food-allergic patients.¹²

The reported analytical methods for allergen detection are polymerase chain reaction,¹³ colorimetric,¹⁴ surface plasmon resonance,¹⁵ electrochemical,¹⁶ enzyme-linked immunosorbent assay,^{17,18} etc. These methods either lack of sensitivity or specificity, and the ability to simultaneously analyze low

amounts of allergens in complex food matrices is another challenge for the existing methods. Ultrahigh-performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) is considered as a good tool and frequently used to detect different kinds of allergens in many foodstuffs.^{19–21} The matrix effect and analyte loss during the sample preparation for UHPLC–MS/MS are usually corrected by employing the “short or long isotope-labeled peptides quantitative strategy”. In addition to the high cost of labeled internal peptide standards, this strategy can not compensate the matrix effect and loss caused by incomplete protein extraction or digestion.¹² To solve these problems, a “standard addition strategy” without the use of isotope-labeled peptides was proposed and validated by Planque et al.²² Then, the just mentioned authors improved the method by combining the standard addition strategy with a labeled internal standard for the quantification of milk, soy, peanut, and egg allergens in eight food products, and satisfying recoveries meeting the AOAC recovery regulation of 60–120% and lower relative standard deviations (RSDs) were achieved.²³ It is disappointing that this quantification strategy using a single calibration curve is still not allowed for the quantification of all target allergens. Additionally, the existing reported methods showed

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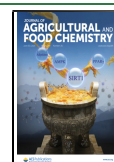


Table 1. MS/MS Parameters Used for Detection of Shrimp and Soy Allergens

allergen	protein name	peptide sequence	retention time (min)	charge state (z)	precursor (m/z)	transition (fragments)	Q1 pre bias (V)	CE	Q3 pre bias (V)
shrimp	tropomyosin	IVELEELR	4.904	2+	565.30	675.30 (y5+)	−28.0	−27.0	−30.0
						917.20 ^a (y7+)	−34.0	−21.0	−38.0
		LAEASQAADER	2.520	2+	688.80	520.25 (y4+)	−34.0	−33.0	−36.0
						706.15 (y6+)	−40.0	−23.0	−34.0
						777.25 ^a (y7+)	−38.0	−26.0	−38.0
		SITDELDQTFSELGY	7.988	2+	903.25	326.20 ^a (y3+)	−20.0	−22.0	−23.0
						655.30 (y6+)	−20.0	−19.0	−32.0
		ALSNAEGEVAALNR	4.283	2+	708.00	544.30 (y5+)	−20.0	−26.0	−28.0
						829.35 ^a (y8+)	−20.0	−27.0	−32.0
		soybean	Gly m 6	LNALKPDNR	2.337	2+	520.85	289.20 ^a (y2+)	−26.0
501.25 (y4+)	−26.0							−23.0	−26.0
VFDGELQEGR	3.897			2+	573.30	602.10 ^a (y5+)	−32.0	−24.0	−40.0
						903.60 (y8+)	−40.0	−23.0	−20.0
NNNPFSLVPPQESQR	6.746			3+	625.50	744.30 (y6+)	−24.0	−28.0	−28.0
						841.35 ^a (y7+)	−24.0	−21.0	−24.0
						940.40 (y8+)	−22.0	−24.0	−36.0
FYLAGNQEQEFLK	5.535			2+	794.10	1093.45 ^a (y9+)	−22.0	−27.0	−32.0
						1164.45 (y10+)	−22.0	−27.0	−34.0
SQSDNFEYVSFK	5.018			2+	725.95	381.20 ^a (y3+)	−22.0	−26.0	−27.0
		643.30 (y5+)	−22.0			−29.0	−34.0		
		1235.45 (y10+)	−22.0			−24.0	−36.0		

^aUsed for quantification.

that large amounts of reagents are consumed in the sample preparation procedure. Especially in the digestion of protein, a level of mg trypsin per sample was added.^{22–24}

By reason of the foregoing, this study aims at developing a cost-effective and environment-friendly method for the detection of shrimp and soybean in sauce samples by utilizing a standard addition strategy without the usage of labeled peptides. Therefore, reasonable peptide selection and detailed working condition optimization were performed, and the effect of heating processing was investigated to reduce the analysis time and consumption of reagents, and as a result, an improvement in both accuracy and precision of the method was achieved. To the best of our knowledge, this is the first report on simultaneous determination of shrimp and soybean in complex sauce samples based on UHPLC–MS/MS.

MATERIALS AND METHODS

Chemicals and Reagents. Natural shrimp tropomyosin (purity > 95%) and natural Gly m 6 (purity > 95%) were purchased from INDOOR biotechnologies (Charlottesville, USA). Dithiotreitol (DTT), iodoacetamide (IAA), and trypsin from porcine were from Sigma-Aldrich (St. Louis, MO, USA). The Oasis HLB solid-phase extraction column (3 cm³, 60 mg) was purchased from Waters (Milford, Massachusetts, USA). Urea (purity 99%), ammonium bicarbonate (purity 99%), and formic acid (FA, purity 99%) were obtained from J&K Chemicals (Beijing, China). LC–MS grade acetonitrile (ACN) was from Merck (Darmstadt, Germany). Ultrapure water was prepared using a Milli-Q ultrapure system (Millipore, Bedford, MA). 1 M tris(hydroxymethyl)aminomethane-hydrochloric acid (Tris-HCl) buffer (pH 8.0) was purchased from Solarbio LIFE SCIENCES (Beijing, China).

Preparation of Noncontaminated Sauce. Chilli sauce was prepared by mixing chopped chilli (60%) and chopped garlic (40%) purchased from local supermarkets and finely ground. The mixture was then cooked with fire for 10 min.

Preparation of Incurred Sauce. Tropomyosin is the major allergen among crustaceans and identified as a heat-stable IgE-binding allergen in shrimps.^{5,25} Gly m 6 is also an important IgE-binding

soybean allergen. Therefore, tropomyosin and Gly m 6 are selected as target standards in the preparation of incurred sauce.²⁶ Two main proteins of shrimp (tropomyosin) and soybean (Gly m 6) were added to a mixture of chilli and garlic to obtain a theoretical calculated protein concentration of 500 µg/g for tropomyosin and Gly m 6. Different levels of contaminated sauce were prepared by serial dilutions of contaminated sauce with noncontaminated sauce to reach the following levels: 1, 10, 20, 50, and 100 µg/g for tropomyosin and Gly m 6. The sauce samples were homogenized using a Waring HGB2WTS3 blender (Torrington, USA).

Extraction and Enzymatic Digestion of Samples. The protocol described in Planque et al. was used with some modifications.²² 200 mg of chilli sauce was weighed into a 2.0 mL polypropylene tube, and 1.0 mL of 200 mM Tris-HCl buffered (containing 6 M urea) at pH 8 was added. The extraction was performed by shaking samples for 30 min at 200 rpm using an air oscillator (Donglian Electronic Technology Development Co., Ltd, Harbin, China). Afterward, the sample was ultrasonically extracted for 20 min and centrifuged at 8600g for 10 min. 500 µL of the supernatant was transferred to another 2.0 mL polypropylene tube and diluted with 500 µL of 200 mM ammonium bicarbonate. Protein was reduced by adding DTT at a final concentration of 5 mM for 60 min at 37 °C, and subsequently alkylated by adding a solution of IAA at a final concentration of 10 mM for 30 min in the dark at room temperature. The protein concentrations in samples were determined using the Bradford Protein Assay Kit (BIO-RAD, Shanghai, China). Working details of the Bradford Protein Assay Kit were as follows: 5 µL of the sample solution was pipetted into microplate wells, and 250 µL of the Bradford dye reagent was added. After incubation at room temperature for 5 min, the absorbance of samples at 595 nm was measured using a Thermo Scientific Multiskan FC microplate reader (USA). The sample concentration was determined by using the standard curve created by plotting the 595 nm values (y-axis) versus bovine serum albumin concentration in µg/mL (x-axis). Then, the samples were digested with trypsin (1 mg/mL in 1 mM HCl) at a ratio of 1 µg trypsin to 20 µg protein and incubated at 37 °C for 8 h. The enzymatic reaction was quenched by the addition of 1 µL of 99% FA.

Purification of Samples. The obtained peptides were purified on a water HLB solid phase extraction column. Before use, the column

was conditioned with 1 mL of 80% ACN and 1 mL of ultrapure water. Samples were loaded on the column, which was rinsed twice with 2 mL of 0.1% FA solution. Peptides were eluted with 1 mL of 80% ACN (containing 0.1% FA). After evaporation under a nitrogen flow in a water bath set at 40 °C, the peptides were dissolved in 200 μ L of 5% ACN/water (5/95, v/v). The resulting solution was centrifuged at 8600g for 10 min at 4 °C. 100 μ L of the upper layer was transferred to a glass vial and then analyzed by UHPLC–MS/MS.

UHPLC–MS/MS Parameters for Peptide Analysis. Peptide analysis was performed by using an UHPLC system (Nexera X2, Shimadzu, Japan) coupled with a Shimadzu LC–MS 8060 triple quadrupole mass spectrometer (Kyoto, Japan). Peptides were separated on a Waters ACQUITY Peptide CSH C₁₈ column (2.1 mm $\times$ 100 mm; 1.7 mm; MA, USA). The mobile phase was consisted of water (A) and ACN (B), where A and B both contain 0.1% FA. The gradient elution program was used. The percentage of B was increased linearly to 30% over 6.5 min. Afterward, the solvent B was increased linearly to 95% at 8.0 min. The column was washed with 95% B for 2.0 min, and finally returned to the initial composition over 0.1 min. The column was equilibrated for 2 min. The column temperature and sampler temperature were set at 40 and 10 °C, respectively. The injection volume and flow rate were 10 μ L and 0.3 mL min^{−1}, respectively.

MS/MS analysis was conducted in positive electrospray ionization mode with multiple reaction monitoring (MRM). Interface temperature, desolvation line temperature, and heat block temperature were set as 300, 250, and 400 °C, respectively. The nebulizing gas (nitrogen) flow rate was 3 L min^{−1}. Drying gas and heating gas flow rates were both 10 L min^{−1}. The MRM parameters for peptides are listed in Table 1.

RESULTS AND DISCUSSION

Selection of Marker Peptides. The selection of marker peptides was similar to those reported previously.^{24,27} First, the FASTA files of each protein were searched using the Uniprot database, and then the files were imported in Skyline. Compared to theoretical peptides generated by using Skyline to the experimental results of digested peptides detected by UHPLC–MS/MS in incurred sauce, targeted peptides must be unique to background proteome matrices so as to avoid false positive.²⁸ The lengths of peptides should contain at least eight amino residues and not contain methionine and/or cysteine residues. As shown in Table 4, four kinds of tropomyosin peptides and five kinds of Gly m 6 peptides were selected as allergen indicators of shrimp and soybean for analyzing the specificity and sensitivity of detection, respectively. However, the peptide selection should be further verified in the method validation part, in terms of accuracy and precision.

Optimization of Extraction, Digestion, and Cleanup Conditions. In order to get the maximal signal intensity and reproducible experiment results, the conditions of protein extraction and enzymatic digestion should be optimized. Seven extracting solutions were compared: 50 mM Tris-HCl buffered at pH 8.0, 50 mM Tris-HCl buffered at pH 8.0 containing 2 M urea, 200 mM Tris-HCl buffered at pH 8.0, 200 mM Tris-HCl buffered at pH 8.0 containing 2 M urea, 200 mM Tris-HCl buffered at pH 8.0 containing 6 M urea, 50 mM NH₄HCO₃, and 50 mM NH₄HCO₃ containing 2 M urea. As shown in Figure S1, the peak area was higher for six out of nine peptides when using 200 mM Tris-HCl buffered at pH 8.0 containing 6 M urea. As a chaotropic, high-molarity buffer, urea was often used in the extracting process of thermally processed food.²¹ Prior to the reduction of disulfide bonds by DTT, the three-dimensional structures of proteins were disrupted by urea.

Higher concentrations of urea break hydrophobic interaction and hydrogen bonding more completely than lower concentrations, hence 200 mM Tris-HCl buffer at pH 8.0 containing 6 M urea shows a higher peak area. Then, three previously reported extraction modes were optimized, including shaking,²⁷ ultrasonic extraction,²⁹ and mix mode.²⁴ Figure S2 presents the results obtained in different extraction modes. The mix mode group of each peptide is obviously better than the other two groups. A possible explanation for this might be that higher extraction efficiency was achieved by using mix mode. The dosage of enzyme is important for protein enzyme catalysis, various protein to enzyme ratios (m/m) were investigated after protein extraction. From Figure S3, it can be seen that the peak area of the peptide increases with dosage of enzyme, six out of nine peptides reached the highest when the protein to enzyme ratio was 20. Then, the peak area showed a decrease at a ratio of 10. The results may indicate that excess enzymes will lead to mutual enzyme cleavage or nonspecific enzyme digestion.³⁰ The digestion process is very time-consuming, and thus the digestion time has been optimized in this study. Figure S4 shows that peak areas of each peptide increase with the increase of the digestion time, until it reaches an equilibrium. For the peptides IVELEEELR, LAEASQAADER, and ALSNAEGEVAALNR, the optimum digestion time was 16, 20, and 16 h, respectively. However, the peak area of these peptides at optimum time is not much higher than that at 8 h. Considering shortening the whole analysis time, 8 h was selected for further research. Interestingly, there was some decline in the peak area when the digestion time was longer than the optimum time. It may be explained that degradation of peptides occurred in the reaction process. In this study, the whole digestion time of 8 h was obviously less than the reported at least 12 h approach.^{20,27,28}

Quantification Strategies for Allergens. Though MS/MS has a high sensitivity, the matrix effect still has a great impact on allergen detection and might cause ionization suppression or a retention time shift.¹⁹ Labeled peptides are usually used as internal standards to compensate the matrix effect during allergen extraction, digestion, and purification in MS analysis of allergens.^{24,27} However, some researchers found the use of labeled peptides can not correct for losses occurring during protein extraction and digestion.²² To solve this problem, labeled proteins are viewed as the “gold standard” to correct the matrix effect in the entire process, because they have the same physical or chemical properties with the native proteins. Disappointingly, labeled proteins are limited by their prohibitive cost.²³ Currently, a quantitative method in which an allergen standard was added for foodstuff (standard addition method), which was proposed by Planque et al.,²² was considered as the best way to quantify allergens. Peptides were quantified by establishing calibration curves in the matrices spiked at five different concentrations of tropomyosin and Gly m 6 standards. The concentration of each protein was calculated by dividing the intercept by the slope. However, recovery and precision of the reported standard addition method hardly meet the requirements of AOAC.²² In order to develop a standard addition method with ideal experimental results, detailed optimization of working conditions and rational selection of stable peptides were performed in this study.

Effects of Heating Processing. The chemical structures of proteins may be modified by the heating process in the

Table 2. Comparison of Calibration Curve Slopes

protein name	peptide sequence	calibration curve slopes		processing effect ^a (%)
		incurred	spiked	
tropomyosin	IVELEELR	2791	5523	49.5
	LAEASQAADER	215	739	70.9
	SITDELDTFSELSGY	108	346	68.8
	ALSNAEGEVAALNR	512	1306	60.8
Gly m 6	LNALKPDNR	10703	53213	79.9
	VFDGELQEGR	3207	4079	21.4
	NNNPFSLVPPQESQR	1659	3522	52.9
	FYLAGNQEQEFLK	4469	8935	50.0
	SQSDNFEYVSFK	7640	14286	46.5

^aProcessing effect = (1 – slope of the incurred samples/slope of the spiked samples) × 100%.

chilli sauce preparation. To investigate the effects of thermal processing, the protocol was similar to that reported by Pilolli et al.²⁹ Two sets of matrix-matched calibration curves were constructed by analyzing two kinds of chilli samples: (i) chilli sauce was incurred with the protein standard before heating at 90 °C for 50 min and (ii) blank sauce was spiked with protein standard after heating. Matrix-matched calibration curves consisted of five points (10, 20, 50, 100, and 500 µg/g). Then, the slopes of the incurred and spiked matrix-matched calibration curve for each peptide were compared. As shown in Table 2, heating has a major influence on the slopes of chilli sauce samples. The processing effect ranged from 21.4 to 79.9%, which indicated that the detection sensitivity of the developed method for each peptide dropped to different extents. According to some reports,^{21,29} allergic proteins may react with matrix components, resulting in chemical modification of single peptide sequence or/and a reduction of protein solubility/extractability. This evidence supports the necessity of preparing an incurred chilli sauce sample when developing and validating a method.³¹

Method Validation. To validate the method, sensitivity, specificity, accuracy, and precision were evaluated by analyzing incurred chilli sauce samples. The limit of quantification (LOQ) was defined as the minimum contaminated concentration of protein in the prepared chilli samples that can be detected at signal-to-noise ratios of 10. The specificity of this method was validated by analyzing noncontaminated sauce samples. As is exhibited in Figures 1 and 2, no interference was observed at the retention time of each target peptide, showing the good specificity of the developed method.³² The LOQs were as follows: 0.25 µg tropomyosin/g sauce for IVELEELR and SITDELDTFSELSGY, 0.5 µg tropomyosin/g sauce for LAEASQAADER, 5 µg tropomyosin/g sauce for ALSNAEGEVAALNR, 10 µg Gly m 6/g sauce for LNALKPDNR, 1 µg Gly m 6/g sauce for VFDGELQEGR, NNNPFSLVPPQESQR, and FYLAGNQEQEFLK, and 5 µg Gly m 6/g sauce for SQSDNFEYVSFK. The obtained results demonstrated the high sensitivity of this method. Method accuracy and precision were expressed as recoveries and RSD, respectively. A standard addition method was used to measure recoveries at three contaminated protein levels. Three technical replicates of recovery experiments were performed to calculate RSDs of intra- and interday. As is shown in Table 3, mean recoveries for peptides SITDELDTFSELSGY, ALSNAEGEVAALNR, NNNPFSLVPPQESQR, and FYLAGNQEQEFLK were all out of the recoveries of 60–120% set by SMPR 2016.002 “Standard Method Performance Requirements for Detection

and Quantitation of Selected Food Allergens”, especially for SITDELDTFSELSGY, recoveries can be reached as high as 195.3–233.3% along with RSDs ranging from 21.2 to 104.0%. Recovery of NNNPFSLVPPQESQR was 94.6–331.3%. On the other hand, recoveries of ALSNAEGEVAALNR and FYLAGNQEQEFLK were only 25.0–59.3% with RSDs below 111.2%. Both the recoveries and RSDs of the above peptides can not meet the requirements of SMPR 2016.002. It is worth noting that recoveries of SQSDNFEYVSFK were in the range of 81.4–115.0%, but the poor precision indicates this peptide is not suitable for allergic protein quantification. The reason why recoveries of these peptides showed a high variability may be the incomplete release by proteolysis and/or poor stability in sauce samples.³¹ Thus, considering the accuracy and precision of each peptide, the rest of the four peptides (IVELEELR, LAEASQAADER, LNALKPDNR, and VFDGELQEGR) were chosen as quantitative peptides. Though the results of some peptides were not satisfied, they can be applied to assist in qualitative analysis. The selected peptides, which are consistent with previous reports,^{27,28} are the most suitable for the development of an LC–MS/MS method for the detection of tropomyosin and Gly m 6 in the sauce samples.

Detection of Shrimp and Soybean in Sauce Samples.

There are many types of sauces, made from different ingredients containing a high carbohydrate content, a fat content, a high protein content, or a mix of them. The purpose of this study is to develop a routine method to detect all kinds of sauce samples. Several processed sauce commodities purchased on from supermarkets in Beijing were detected using the established method. The results are listed in Table 4. The table illustrates that shrimp and soybean are usually used as raw materials or additives in sauce samples. The majority of labels on samples are consistent with the presence of allergens, which indicates the applicability of the developed UHPLC–MS/MS method. In a mushroom sauce sample, soybean was not listed on the label but detected. Unintended contamination with soybean may occur in the production facility by cross-contact.²⁷ This issue may pose a great threat on public health, and it also reminds us of the importance to establish reliable analytical methods for allergen detection.

An environment-friendly and cost-effective UHPLC–MS/MS method was developed to simultaneously analyze shrimp and soybean in sauce samples without using labeled peptides. Peptide selection of each allergen proteins was based on the results generated from Skyline software and the experimental results from the trypsin-digested proteins in sauce samples. The most important part is choosing accurate and precise

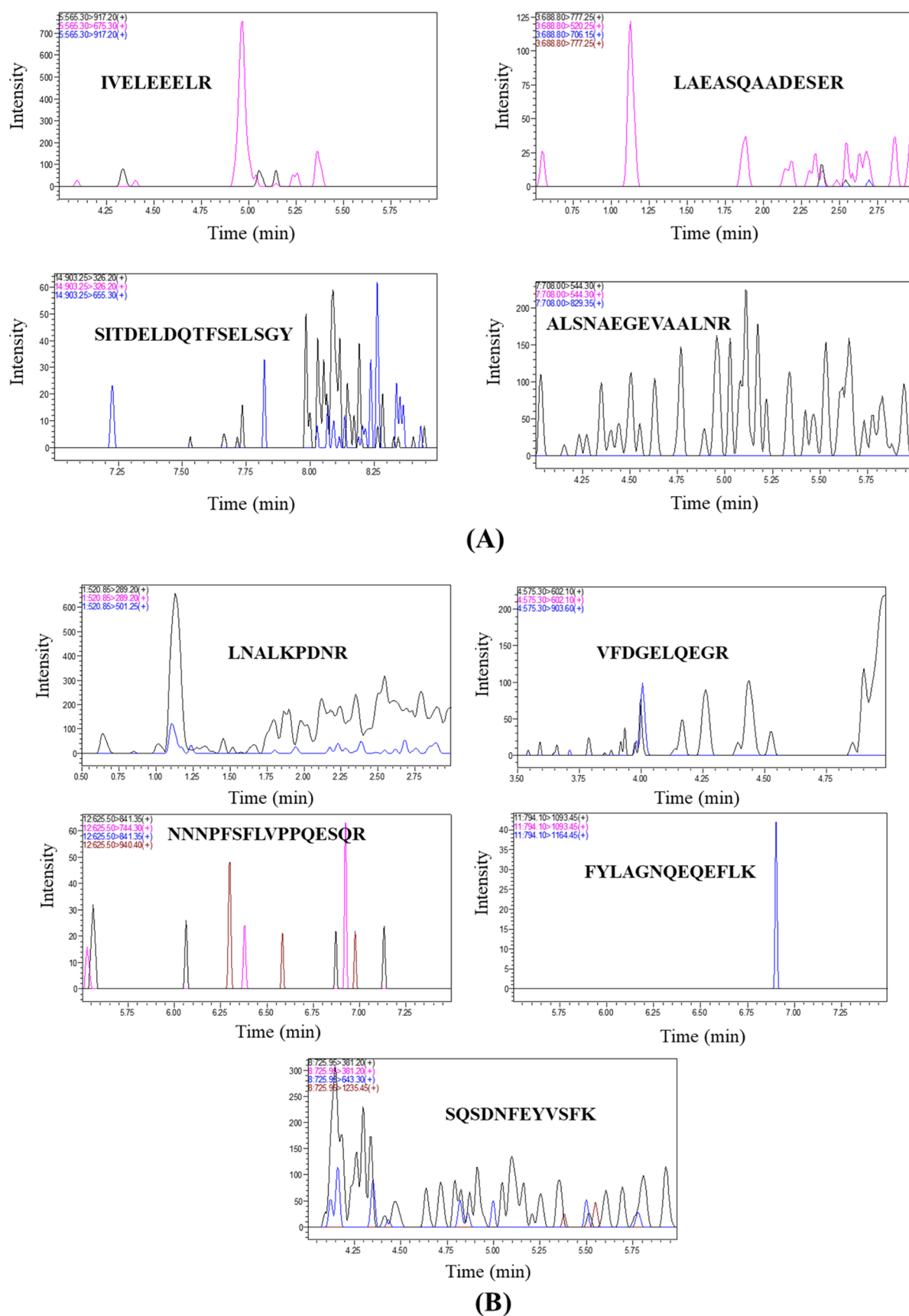


Figure 1. Chromatograms of each peptide for (A) tropomyosin and (B) Gly m 6 in noncontaminated sauce.

peptides for quantity analysis of allergen proteins. For every sauce sample, only 1 mL extract solvent, a 1:20 enzyme to

protein ratio, and a digestion time of 8 h were used, resulting in a cost-saving and time-saving sample preparation. Even if

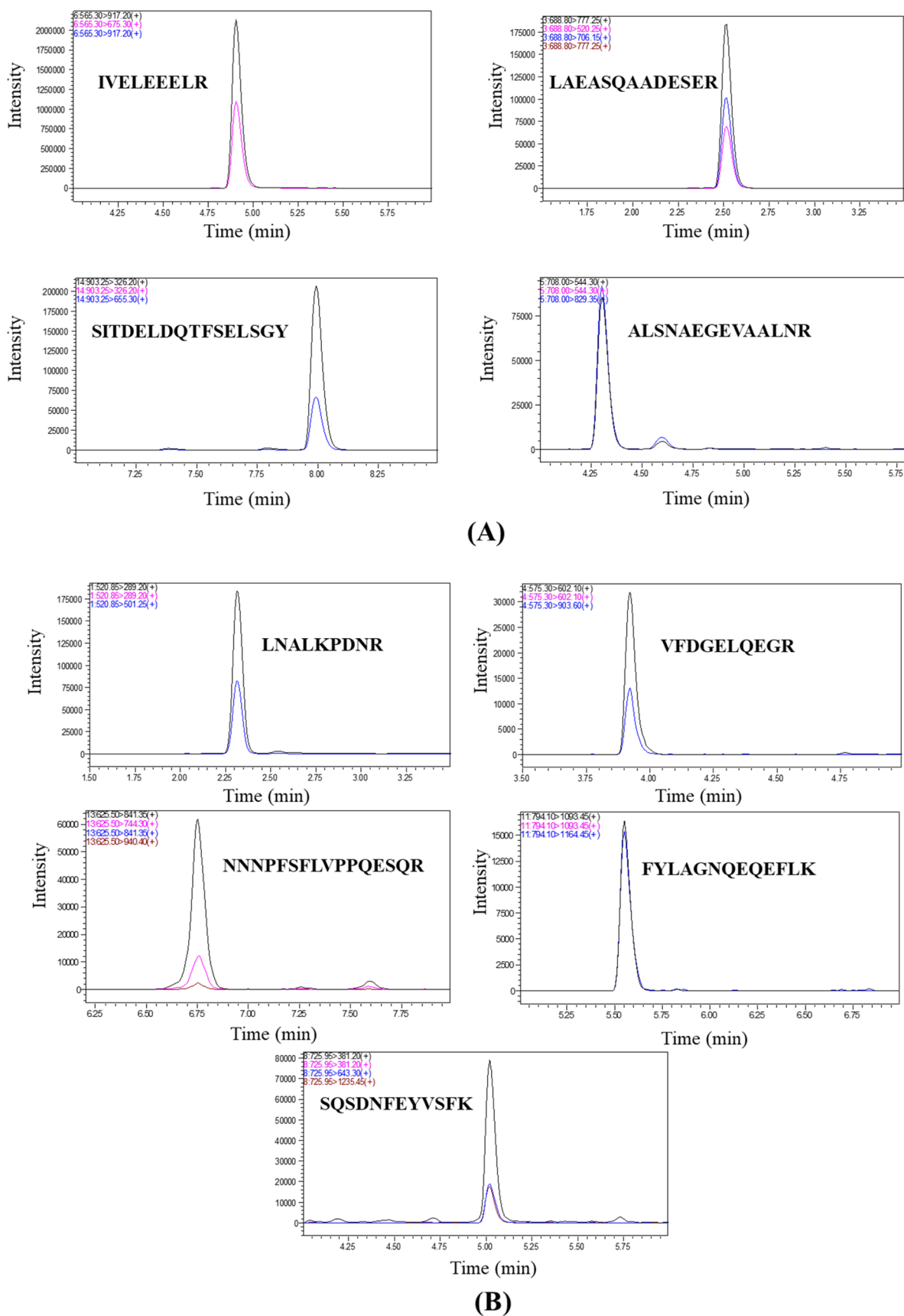


Figure 2. Chromatograms of each peptide for (A) tropomyosin and (B) Gly m 6 in incurred sauce with a targeted protein concentration of 50 $\mu\text{g/g}$.

Table 3. Mean Recoveries ($n = 3$) and RSD ($n = 3$) for Each Peptide of Protein

allergen	protein name	peptide sequence	LOQ ($\mu\text{g/g}$)	contaminated levels ($\mu\text{g/g}$)	mean recoveries (%)	intraday RSD (%)	interday RSD (%)
shrimp	tropomyosin	IVELEELR	0.25	20	96.4	15.2	31.0
				100	102.9	4.4	19.2
				500	117.1	3.8	20.3
		LAEASQAADER	0.5	20	101.9	12.0	21.5
				100	81.9	0.4	15.9
				500	117.8	1.5	13.6
		SITDELDQTFSELGY	0.25	20	227.6	104.0	96.1
				100	195.3	45.2	21.6
				500	233.3	21.5	49.8
		ALSNAEGEVAALNR	5.0	20	57.6	50.0	36.4
				100	25.0	31.6	111.2
				500	47.4	3.5	110.1
soybean	Gly m 6	LNALKPDNR	10.0	20	63	14.0	11.2
				100	108.3	4.9	6.3
				500	93.6	7.4	10.1
		VFDGELQEGR	1.0	20	100.9	11.1	19.9
				100	118.9	2.5	17.0
				500	76.0	1.0	5.3
		NNNPFSFLVPPQESQR	1.0	20	153.6	67.3	34.0
				100	94.6	0.2	1.6
				500	331.3	0.8	7.5
		FYLAGNQEQEFLK	1.0	20	51.3	42.1	93.3
				100	49.7	9.0	71.8
				500	59.3	12.1	35.0
		SQSDNFEYVSFK	5.0	20	81.4	94.5	24.6
				100	85.4	81.9	11.1
				500	115.0	13.8	48.7

Table 4. Presence of Allergens Detected by UHPLC–MS/MS on Different Sauce Commodities^c

Sample type	classification	food label		UHPLC–MS/MS method ($\mu\text{g/g}$)	
		shrimp	soybean	tropomyosin ^a	Gly m 6 ^b
broad bean paste	protein	-	✓	-	267.8
peanut paste	protein	-	-	-	-
shrimp sauce	protein	✓	-	463.2	-
mushroom sauce	carbohydrate	-	-	-	124.3*
chilli sauce	carbohydrate	-	✓	-	6.6
spaghetti sauce	carbohydrate	-	-	-	-
thai sweet chilli sauce	carbohydrate	-	-	-	-
salad sauce	carbohydrate	-	-	-	-
black pepper sauce	carbohydrate	-	-	-	-
hazelnut cocoa sauce	carbohydrate	-	-	-	-
barbecue sauce	fat	-	-	-	-
thousand island dressing	fat	-	-	-	-
spare rib sauce	fat	-	✓	-	7.4

^aTropomyosin was quantified by using the peptide LAEASQAADER. ^bGly m 6 was quantified by using the peptide LNALKPDNR. ^c- allergens were not listed on the label or not detected; ✓ allergens were listed on the label; and * allergens were not listed on the label but detected.

the heating process has a great impact on allergen peptide detection and no labeled peptides were used, good linearities of calibration curves and low LOQs were still obtained. The applicability of this method was also demonstrated by analyzing 13 kinds of commercially available processed sauce products.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.1c02008>.

Extracting solution optimization of each peptide for (A) tropomyosin and (B) Gly m 6 in contaminated sauce (100 μg protein/g); extraction mode optimization of each peptide for (A) tropomyosin and (B) Gly m 6 in contaminated sauce (100 μg protein/g); dosage of enzyme optimization of each peptide for (A) tropomyosin and (B) Gly m 6 in contaminated sauce (100 μg protein/g); and digestion time optimization of each peptide for (A) tropomyosin and (B) Gly m 6 in contaminated sauce (100 μg protein/g) (PDF)

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