

Analytical Methods

Simultaneous determination of multi-allergens in surimi products by LC-MS/MS with a stable isotope-labeled peptide

Xue Huang^{a,1}, Zitong Zhu^{b,1}, Heng Feng^a, Qiumei Zhang^a, Hong Zhang^{a,*}^a School of Food Science and Biological Engineering, Zhejiang Gongshang University, Hangzhou, Zhejiang 310018, China^b Comprehensive Technology Service Center of Quzhou Entry Exit Inspection and Quarantine Bureau, Quzhou, Zhejiang 324003, China

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ABSTRACT

A novel LC-MS/MS method for simultaneous quantification of the allergens of soy, milk and egg in surimi products was established based on three signature peptides, namely EAFGVNMQIVR (soy glycinin G2), YLGY-LEQLLR (milk α -S1-casein), GGLEPINFQTAADQAR (egg ovalbumin) and a stable isotope-labeled peptide EAFGVNMQI* (I^* , $^{13}\text{C}_6$, ^{15}N) VR. After protein extraction and tryptic digestion, four selected marker peptides were measured by HPLC-MS/MS. The determination coefficient R^2 was higher than 0.9914 at the range of 0.5–200 ng/mL and both the intra and interday precision RSD were less than 6.7% for three peptides. Limit of quantitation was shown as 0.054 $\mu\text{g/g}$ for soy, 0.024 $\mu\text{g/g}$ for milk and 0.032 $\mu\text{g/g}$ for egg. Current validated method was successfully applied to analyze surimi products, which can not only provide accurate quantification information of allergens for sensitive consumers, but also it may be used for label management for surimi market.

1. Introduction

Surimi products are kinds of gelatinous foods, which are made of raw surimi or frozen surimi as raw materials, salt and other excipients through a series of process of crushing, chopping, mixing, molding and gelation. There are various types, such as fish ball, fish sausage, fish cake, simulated crab meat and other seafood products (Park & Park, 2005). Due to its high nutritional value and delicious taste, it is deeply loved by consumers and thus improve its economic value (Alakhrash, Anyanwu, & Tahergorabi, 2016).

To improve the gel properties of surimi products, it is likely to add some non-muscle proteins as elastic enhancer, which mainly are soy isolate protein, whey protein and egg ovalbumin (Rawdkuen & Benjakul, 2008; Jafarpour, Hajiduon, & Aie, 2012; Tran & Benjakul, 2018). While all these proteins are just common types that cause food allergies for sensitive individuals. It is said that about 8% of children and 4% of adults in the world suffer from food allergy, which has been one of the major threats to human health (Tang & Mullins, 2017). For sensitive individuals, even a small dose of allergens could cause unpleasant reactions ranging from slight to severe (Ho, Wong, & Chang, 2012). The most effective way to prevent food allergy is to avoid eat foods that contain allergic proteins. In order to protect those sensitive consumers, EU, USA, Australia, New Zealand and other developed

countries have adopted compulsory labeling technology for food allergens (Popping & Diaz-Amigo, 2017).

To date, there are two main methods being used to detect allergens in food: antibody-based enzyme-linked immunosorbent assay (ELISA) (Liu, Da, Yang, Wang, & Wang, 2012; Ma et al., 2010) and DNA-based polymerase chain reaction (PCR) (Hiemori et al., 2000; Xiang, Haas, Zeece, Markwell, & Sarath, 2004). However, ELISA has obvious shortcomings in terms of reproducibility, especially in detecting processed foods (Poms, Agazzi, Bau, Brohee, & Capelletti, 2005). ELISA kits are restricted to detect single allergen at a time. Though DNA-based methods, like PCR, are specific and sensitive, it can't detect proteins directly. What's worse, a negative result does not mean the absence of proteins, which may cause a false-negative result (Holzhauser & Röder, 2015). Thus, both of the methods are limited to apply to detect the food allergens (Hengel & Arjon, 2007).

Some researchers have tried to combine proteomics with liquid chromatography-tandem mass spectrometry technologies to analyse allergic proteins precisely. Na Lin (Lin, Chen, Zhang, Li, & Fu, 2018) used an isotope-labeled internal standard method to detect the major royal jelly protein 1 (MRJP 1) for the first time. Planque M (Planque et al., 2017) detected ten allergens in complex matrix, namely chocolate, ice cream, cookie and sauce by UPLC-MS/MS, as an alternative to the ELISA method. Gu, Shuqing (Gu et al., 2017) developed a SPE-LC-

* Corresponding author.

E-mail address: hongzh1316@zjgsu.edu.cn (H. Zhang).¹ These two authors contributed equally to this study.

MS/MS method to screen multiple allergens in chocolates. Liquid chromatography-mass spectrometer technique has advantage of analyzing proteins in complex food matrices owing to its high selectivity and sensitivity (Ippoushi, Sasanuma, Oike, Kabori, & Maeda-Yamamoto, 2016; Zhang et al., 2014). The main steps of LC-MS/MS method for protein analysis are selecting appropriate signature peptide after trypsin digestion, optimizing instruments parameters and validating method in terms of linearity, sensitivity and accuracy.

Based on the strategy mentioned above, the signature peptides of soy, milk and egg, as well as a stable internal standard peptide were to be selected and used in surimi products. As we know, there are no reports on the method of detecting multiple allergic peptides simultaneously by LC-MS/MS in surimi products. The paper is to develop a novel LC-MS/MS method to simultaneously detect proteins of soy, milk and egg in surimi products. Specificity, linearity and accuracy of the method would be validated. Then the new method was applied for surimi products on sale.

2. Materials and methods

2.1. Materials

Dithiothreitol (DTT), ammonium bicarbonate (NH_4HCO_3), iodoacetamide (IAA) (analytical grade) were purchased from Sigma-Aldrich. Formic acid (HPLC grade) was obtained from Tedia (Fairfield, USA). Trypsin (sequencing grade) was purchased from Sigma-Aldrich. Acetonitrile (HPLC-MS grade) was obtained from Merck (Darmstadt, Germany). Milli-Q Water was prepared for LC-MS/MS analyses (Millipore, MA, USA). BCA protein quantitation kit and Tris-HCl were from Bio-Rad.

The signature peptides including EAFGVNMQIVR (molar mass 1262.49, amino acid residues 228–238 of soy glycinin G2), YLGYLEQLLR (molar mass 1267.50, amino acid residues 106–115 of milk powder α -S1-casein) and GGLEPINFQTAADQAR (molar mass 1687.84, amino acid residues 128–143 of egg ovalbumin) and a stable isotope-labeled peptide EAFGVNMQI* (1^* , $^{13}\text{C}_6$, ^{15}N) VR (molar mass 1270.49) were chemically synthesized by China Peptide Co., Ltd (Suzhou, China) with purity higher than 95%.

Soy protein isolate powder was purchased from Bio-Rad. Egg white powder was obtained from Shanghong Co., Ltd (Henan province, China). Milk powder was from Yili Industrial Group Co., Ltd (Inner Mongolia, China). Surimi products like fish ball, fish sausage, fish tofu, simulated crab meat from different bands were obtained from Yonghui supermarket in Hangzhou, China. All samples were kept at -20°C until next step.

2.2. Preparation for surimi blank sample matrices

Bighead (*Aristichthys nobilis*) was obtained from local supermarkets. After removing viscera, skin and bones, the cleaned fish muscles were

minced, subpacked and then stored at -20°C .

2.3. Preparation of the standard protein working solution

Stock solution (1 mg/mL) of marker peptide EAFGVNMQIVR was dissolved in 17% ACN/83% H_2O (V/V) and isotope-labeled internal standard (IS) peptide EAFGVNMQI* (1^* , $^{13}\text{C}_6$, ^{15}N) VR was prepared in 33% ACN/67% H_2O (V/V). Signature peptide YLGYLEQLLR (1 mg/mL) was dissolved in 25% ACN/75% H_2O (V/V). Signature peptide GGLEPINFQTAADQAR (1 mg/mL) was dissolved in 33% ACN/67% H_2O (V/V). All dissolution conditions were based on the instructions of China Peptide Co., Ltd to make the peptide dissolve completely. Stock solutions were stored at -20°C .

The linearity was set as follows: 0.5, 1, 5, 10, 20, 50, 100 and 200 ng/mL for three signature peptides. All standard curves were diluted with blank surimi enzymatic hydrolysate, adding 25 ng/mL IS peptide. Calibration curve was adopted by internal standard method.

2.4. Sample preparation

Briefly, the blank surimi sample in 2 g was extracted with 5 mL 0.2 M Tris-HCl (pH 7.5) extraction buffer and shaken at 60°C , 200 rpm for 2 h. After centrifugation at 8000 rpm/min for 15 min at 4°C , the supernatant was evaluated by BCA protein quantitation kit. The protein supernatant of 500 μL was reduced by 10 μL 50 mM DTT solution for 30 min at 56°C . Then adding 30 μL 50 mM IAA solution for 30 min, the alkylation was left in the dark at room temperature. Trypsin (1:50, w/w, enzyme to substrate ratio) was added and incubated for 16 h in 37°C . To end enzymatic hydrolysis, 5 μL formic acid was added. The digested mixture was diluted by the initial mobile phase to make the concentration of IS was 25 ng/mL and centrifuged at 10,000 g for 15 min at 4°C . The supernatant was filtered by a 0.22 μm filter membrane before LC-MS/MS analysis.

2.5. LC-MS/MS parameters

Data were acquired by using a LC-30AD (Shimadzu, Japan) and QTRAP 4500 MS/MS system (AB Sciex, USA). LC separation was achieved by using Agilent Eclipse XDB-C18 column (100 mm $\times$ 2.1 mm, 1.8 μm) at 40°C , 20 μL injection volume and 0.3 mL/min flow rate. Gradient mobile phase (solvent A: 0.15% v/v formic acid in water and solvent B: ACN) was as follows: 0–13 min, linear gradient 97–78% A; 13–16 min, linear gradient 78–10% A; 16–16.1 min, linear gradient 10–97% A; 16.1–21 min, 97% A continued for 5 min before the next injection.

Multiple reaction monitoring (MRM) data were optimized with positive ESI mode. Ionspray voltage of Q1 (the first quadrupole) and Q3 (the third quadrupole) was 5.5 KV, and the ion temperature was at 550°C . An auxiliary gas, spray gas and curtain gas were set at 55, 30, 55 psi, respectively. Specific parameters of the method were shown in

Table 1
Parameters of MRM for Targeted Peptides.

Protein	Sequence	Precursor ion	Declustering Potential (IV)	Product ions	Collision energy(eV)	Ions type
GlycininG2 (Soy)	EAFGVNMQIVR	632.4 ²⁺	95	760.4*	30	Y ₆
				916.4	33	Y ₈
				859.4	30	Y ₇
α -S1-casein (milk)	YLGYLEQLLR	634.4 ²⁺	96	991.4*	27	Y ₈
				771.5	27	Y ₆
				658.2	29	Y ₅
Ovalbumin (egg)	GGLEPINFQTAADQAR	844.6 ²⁺	80	666.3*	36	Y ₁₂ ²⁺
				1331.9	40	Y ₁₂
				1121.5	45	Y ₁₀
Isotope-labeled IS	EAFGVNMQI*VR	636.1 ²⁺	105	767.4*	34	Y ₆
				923.3	33	Y ₈

*Ion for quantification.

Table 1.

2.6. Method validation

To verify the proposed LC-MS/MS approach for quantitation of soy, milk and egg allergens in surimi products, a set of parameters were taken into account. LOD and LOQ were calculated as 3- and 10-times of the signal-to-noise (S/N) in the tryptic hydrolysates, respectively. Eight points in the range of 0.5–200 ng/mL spiked with a certain concentration of IS peptide (25 ng/mL) were investigated. Intra-day and inter-day precision were assessed as the relative standard deviation (RSD) by calculating recoveries obtained from three spiked levels at 1, 5, 50 ng/mL, respectively. The intra-day and inter-day precisions were calculated by analyzing spiked samples through the whole procedure at different times on the same day ($n = 3$) and on the consecutive days ($n = 4$), respectively. Recovery experiments were performed with blank surimi tryptic hydrolysate spiked with different concentrations of targeted peptides and fixed IS peptide. The ratio of measured and added concentrations was calculated as recovery.

2.7. Calculation formula of allergic proteins in surimi

$$X = \frac{\delta c V \frac{M1}{M2}}{m}$$

where X ($\mu\text{g/g}$) is the content of allergic proteins in surimi samples; m (g) is the weight of surimi samples, δ is the dilution factor, c (ng/mL) is the calculated concentration of signature peptides in surimi samples, V (mL) is trypsin enzymatic volume, $M1/M2$ is the ratio of molar mass between allergic protein and the corresponding signature peptide. All the experimental data were calculated as the mean values $\pm$ SD. Analyst version 1.6.3 software (AB Sciex, USA) was used for data processing.

3. Results and discussion

3.1. Selection of targeted peptides

The proper marker peptides are crucial factor for protein detection in LC-MS/MS method. There are some common principles for the selection of target peptide markers (Kamiie et al., 2008). Firstly, length between 8 and 20 amino acids is suitable, excluding cysteine and methionine. Secondly, the selected peptides should not contain more than one amino acid lysine (K) or arginine (R) to make sure the cleavage efficiency and reproducibility. Thirdly, good stability and high signal intensity should meet the requirements. In addition, the matrix effect including exogenous and endogenous interferences in complex food-stuffs should be taken into consideration.

Based on the principles and previous studies mentioned above (Angelis, Pilolli, & Monaci, 2016; Boo, Parker, & Jackson, 2017), two tryptic peptides of each allergen proteins were chosen at the beginning (shown in Fig. 1). The six candidate peptides were detected by LC-MS/MS analysis. For soy, the type of product ions for peptides EAFGVNMQIVR and FYLAGNQEQEFLK were y_6 , y_7 , y_8 and a_2 , b_3 , y_{11} , respectively. In MS analysis of peptides fragments, it gives priority to y -ions than b -ions. Taken the economic cost into account, the peptide EAFGVNMQIVR was determined and synthesized as the signature peptide of Glycinin G2 (molar mass 54391). For milk, the peptide YLGYLEQLLR was chosen, for reason that it had higher signal intensity than the peptide HQGLPQEVNENLLR. Similarly, the peptide YLGYLEQLLR had a series of y -ions, namely y_8 , y_6 and y_5 , which can be used as quantitative and qualitative ions. Therefore, the peptide YLGYLEQLLR as the signature peptide of α -S1-casein (molar mass 24529) was synthesized. For egg, the peptide GGLEPINFQTAADQAR of ovalbumin (molar mass 42881) was selected simply because it had higher response

than peptide LTEWTSSNVMEER. Moreover, in the MS analysis, doubly charged ions were preferred than single ones. The double charged ion y_{12}^{2+} was the highest intensity ion in peptide GGLEPINFQTAADQAR, which was used as quantitative ion. The specificity of the three candidate peptides was proved by using BLAST search in UniProt (www.uniprot.org). Lastly, it was proved that the three peptides were absent in the blank surimi samples by LC-MS/MS analysis.

3.2. Matrix effect

In LC-MS/MS method, the accuracy is largely influenced by ion efficiency, especially in complex matrices (Bantscheff, Schirle, Sweetman, Rick, & Kuster, 2007; Brun, Masselon, Garin, & Dupuis, 2009). Compared with the pure solvent, the presence of matrix can affect the ionization of compounds in some way, either enhancement or inhibition. Therefore, the matrix effect was taken into consideration in our work. The matrix effect (ME) was calculated as the ratio of the slope of matrix calibration curve dissolved in blank surimi enzymatic hydrolysate and the slope of standard curve dissolved in initial mobile phase. The results showed that the ME for peptides EAFGVNMQIVR, YLGYLEQLLR and GGLEPINFQTAADQAR were 94%, 45.97% and 91.74%, respectively (Fig. S1). The matrix effect had little effect on detecting peptides of soy and egg. However, the signal intensity of milk peptide was obviously inhibited by the blank matrix. Thus, to compensate the matrix effect, the matrix calibration curves were used in our method.

3.3. Selection of isotope-labeled internal standard peptide

A stable isotope-labeled peptide EAFGVNMQI* (I^* , $^{13}\text{C}_6$, ^{15}N) VR was used as an internal standard (IS) to minimize the variability of ionization efficiency. The difference between IS and the signature peptide EAFGVNMQIVR was the amino acid I, which was labeled with ^{13}C and ^{15}N . IS peptide can monitor the mass spectrometric environment so that it can guarantee the other three peptides keep in the same physicochemical condition. Some studies had been used internal method to quantify major royal jelly protein 1 (Lin et al., 2018) and allergenic plant traces (Huschek, Bönick, Löwenstein, Sievers, & Rawel, 2016). According to the recommended MRM transition selection rules (Yocum & Chinnaiyan, 2009), y -ions are prior to b -ions for that all transitions can contain isotope labeled sites. There is only one amino acid I in the isotope labeled peptide. And the amino acid I was included in y_6 , y_8 and y_7 fragment ions, which are used as the quantitative and qualitative ions for allergic protein Glycinin G2. Therefore, the isotope-labeled amino acid I was selected and synthesized.

3.4. Selection of MRM transitions

MRM transitions were chosen complied with the rules proposed by Yocum and Chinnaiyan (Yocum & Chinnaiyan, 2009). In short, when ESI was used, it gives priority to doubly charged precursor and y -ions. Meanwhile, a larger m/z , which can facilitate selectivity and exclude low mass ion overlap, was chosen.

As expected in Fig. 2, peptide EAFGVNMQIVR showed higher intensity of y -ions than b -ions in Glycinin G2 tryptic products. The intensity of singly charged ions y_6^{1+} (760.4), y_8^{1+} (916.4) and y_7^{1+} (859.4) were higher than other ions, so they were used for the further analysis. Since the intensity of y_6^{1+} (760.4) was markedly the highest ions in the marker peptide, it was chosen as the quantification ion. The other two ions were used as the qualitative ions. Similarly, y_6^{1+} (767.4), y_8^{1+} (923.3) of isotope-labeled peptide EAFGVNMQI* (I^* , $^{13}\text{C}_6$, ^{15}N) VR were used as the quantification ions. For milk, though the singly charged ion a_2^{1+} (249.1) was highest intensity in the peptide YLGYLEQLLR, it has less than four amino acids, which will be easily destroyed by high energy. The singly charged ion y_8^{1+} (991.4) was the highest y -series ion, which was chosen as quantitative ion. Meanwhile,

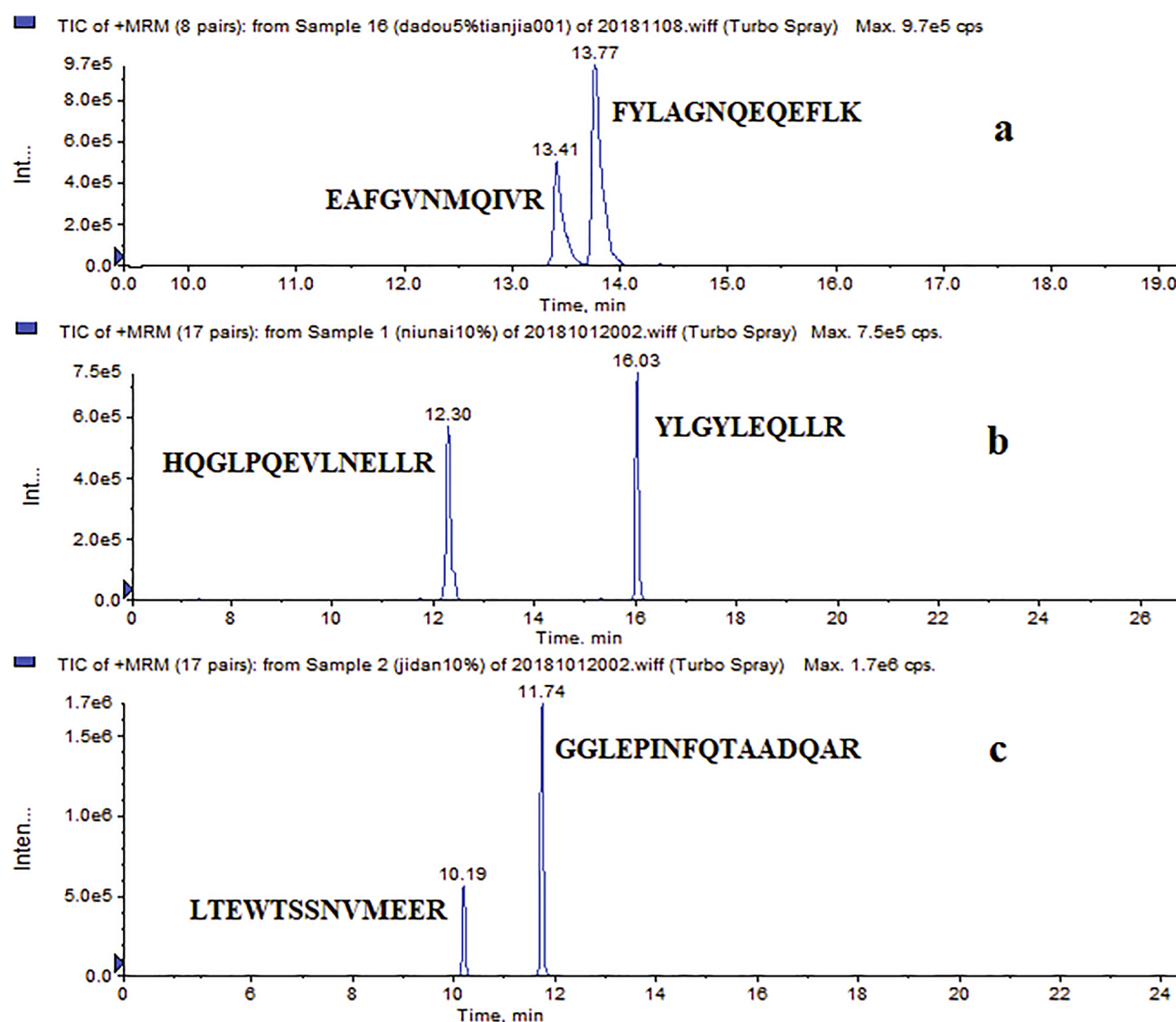


Fig. 1. Selection of target peptides in three proteins. (a) TIC spectrum of EAFGVNMQIVR and FYLAGNQEQLK in soy Glycinin G2; (b) TIC spectrum of YLGYLEQLLR and HQGLPQEVLNENLLR in milk α -S1-casein; (c) TIC spectrum of LTEWTSSNVMEER and GGLEPINFQTAADQAR in egg ovalbumin.

y_6^{1+} (771.5) and y_5^{1+} (658.2) ions were used as qualitative ions. For peptide GGLEPINFQTAADQAR, the doubly charged ion y_{12}^{2+} (666.4) was determined as quantitative ion rather than the higher intensity of the singly charged ion y_{12}^{1+} (1331.2) according to the aforementioned rules. Then, the singly charged ions y_{12}^{1+} (1331.2) and y_{10}^{1+} (1121.2) were used as qualitative ions (See Fig. 3).

The three peptides and its corresponding isotope-labeled peptide were successfully detected in the enzymatic hydrolysates (Fig. S2). Subsequently, the collision energy (CE) and the declustering potential (DP) of each peptide were optimized. The optimal MRM parameters were shown in Table 1.

3.5. Method validation

The method parameters including the linearity, sensitivity, accuracy and precision were validated. Good linearity was calculated by plotting the ratio of analyte/IS peptide peak area versus the ratio of analyte/IS concentration in the range of 0.5–200 ng/mL. The determination coefficients (R^2) was as good as 0.9914. Sensitivity was calculated by LOD ($S/N = 3$) and LOQ ($S/N = 10$). The result showed that the Glycinin G2, α -S1-casein and ovalbumin could be quantified in surimi products were at 0.054 μ g/g, 0.024 μ g/g and 0.032 μ g/g, respectively.

Precision was evaluated in three levels of signature peptides at 1 ng/mL, 5 ng/mL and 50 ng/mL with a stable IS peptide at 25 ng/mL

(results showed in Table 2). The recoveries were 97–108.0% in peptide EAFGVNMQIVR, 73.2–83.0% in peptide YLGYLEQLLR, and 80.8–98.7% in peptide GGLEPINFQTAADQAR. RSD values in intra- and inter-day were less than 6.6% and 6.7%, respectively. All data showed that the established method in our work was reliable and valid so that it can be used to quantify the allergic proteins of soy, milk and egg in surimi products.

3.6. Sample analysis

To verify the established method, soy, milk and egg were determined in 30 surimi samples from the local market, family workshops and supermarket (Hangzhou, China). Samples were pretreated and analyzed in line with the aforementioned method. The selected three signature peptides and a stable isotope-labeled peptide were successfully detected. Total allergic proteins of these samples were shown in Table 3. In this study, it is worth noting that in sample 7, the Glycinin G2 was detected as 0.22 μ g/g. In sample 15, 16 and 21, the α -S1-casein were detected as 0.27 μ g/g, 0.27 μ g/g and 0.28 μ g/g, respectively. In sample 11, 12, 19 and 23, the content of ovalbumin were 0.05 μ g/g, 0.15 μ g/g, 0.49 μ g/g and 0.19 μ g/g, respectively. However, there were no corresponding labels for the aforementioned samples. The sample preparation, centrifugation had little effect on the detection of the signature peptides. The trypsin was sufficient, so the digestion yields

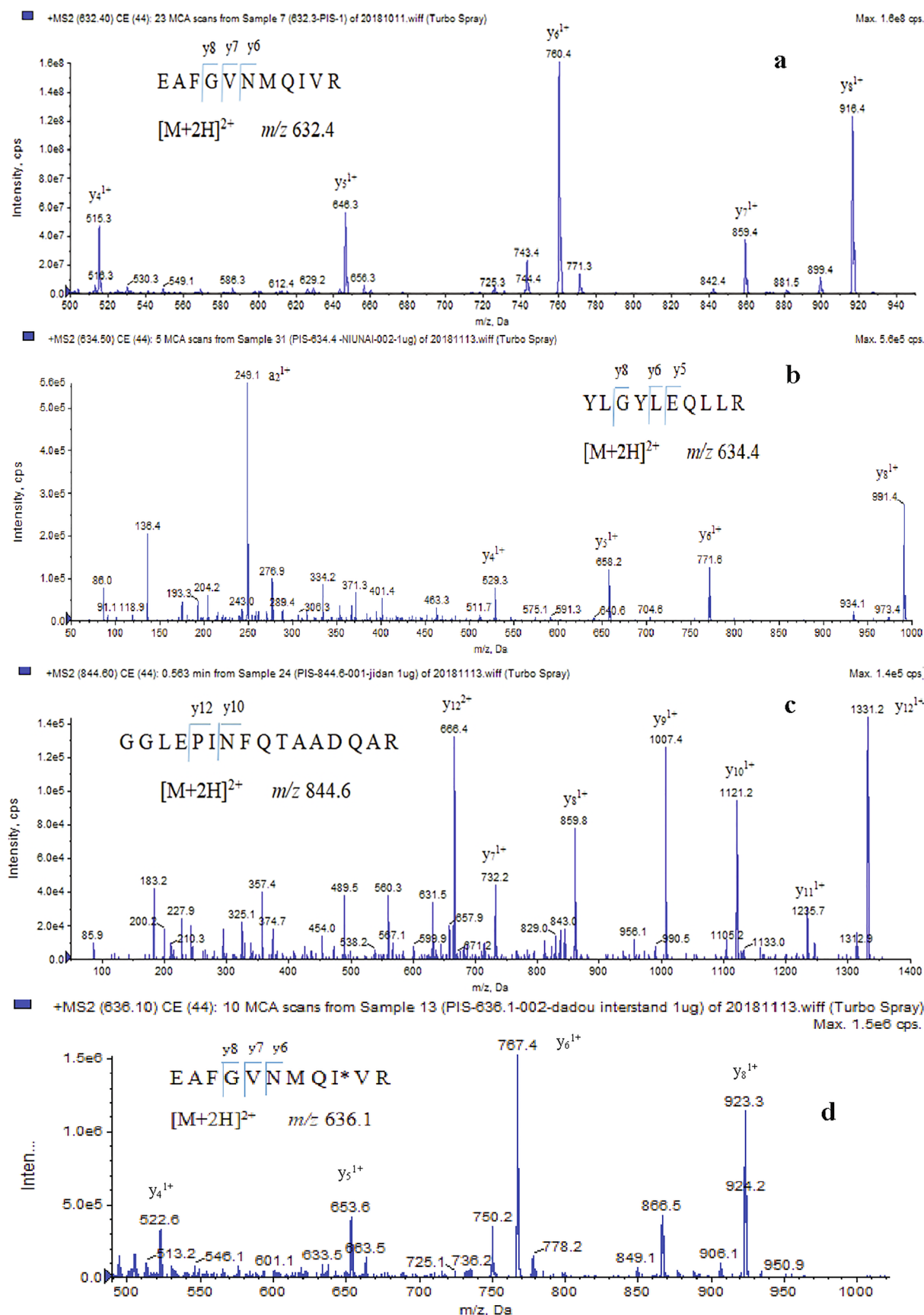


Fig. 2. Fragment ions of signature peptides from tryptic products. (a) MS/MS spectrum of peptide of EAFGVNMQIVR; (b) MS/MS spectrum of peptide of YLGYLEQLLR; (c) MS/MS spectrum of peptide of GGLEPINFQTAADQAR; (d) MS/MS spectrum of peptide of EAFGVNMQI*VR.

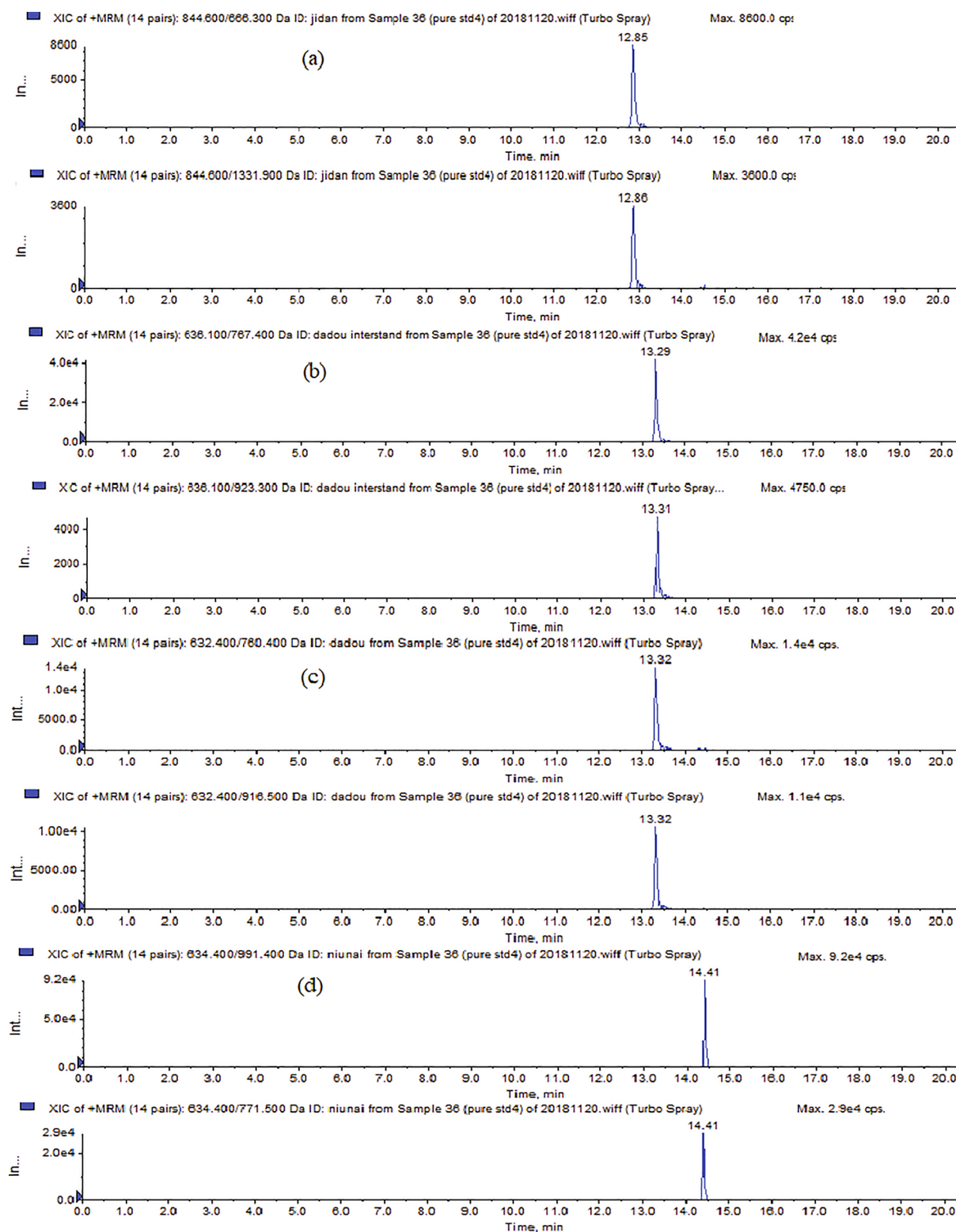


Fig. 3. MRM quantitative ion-pairs of tryptic products. (a) Mass transitions of the signature peptide GGLEPINFQTAADQAR, m/z 844.6/666.3 and 844.6/1331.9. (b) Mass transitions of the signature peptide EAFGVNMQIVR, m/z 632.4/760.4 and 632.4/916.5. (c) Mass transitions of the internal standard peptide EAFGVNMQI*VR, m/z 636.4/767.4 and 632.4/923.5. (d) Mass transitions of the signature peptide YLGYLEQLLR, m/z 634.4/991.4 and 634.4/771.5.

were the same and the peptides were complete. Therefore, the abnormal results implied that the manufacturers may add more than one other proteins in processing of surimi products just like the meat processing, to form the gel texture and cut costs. And the different brands of surimi products have different proportions of protein additives. On the other hand, the hidden allergens may enter into the products via

sharing the same production line, which was hard to find out and posed a potential danger to sensitive consumers.

To date, manufacturers just provide a list of ingredients or vague information that “may contain”. The ubiquitous allergen labels may confuse consumers. What’s worse, a large proportion of allergic consumers tend to ignore precautionary labeling even if they have a history

Table 2
Results of linearity, LOD, LOQ, precision and accuracy for target peptides.

Peptide	Linearity	LOD/LOQ ($\mu\text{g/g}$)	Spiked conc (ng/mL)	Recovery, %	Intra-day RSD, %	Inter-day RSD, %
EAFGVNMQIVR	$y = 2.8478x - 0.3047$ $R^2 = 0.9987$	0.016/0.054	1	100.7	5.0	5.4
			5	97	6.0	6.7
			50	108	3.1	5.1
YLGYLEQLLR	$y = 1.8229x - 0.3445$ $R^2 = 0.9914$	0.007/0.024	1	83	6.6	2.7
			5	76.3	3.0	5.9
			50	73.2	2.9	1.2
GGLEPINFQTAADQAR	$y = 0.7832x - 0.0659$ $R^2 = 0.999$	0.009/0.032	1	98.7	6.1	2.6
			5	81.2	3.7	3.9
			50	80.8	4.3	3.6

of anaphylaxis (Reese et al., 2015). Therefore, the accurate and quantitative detection methods for food allergy is essential and of great importance. The established LC-MS/MS method with a stable isotope-labeled peptide has been successfully detected the trace amount of allergic proteins in soy, milk and egg.

4. Conclusions

A novel, sensitive and multiple-allergen LC-MS/MS method was established for determining soy glycinin G2, milk α -S1-casein and egg ovalbumin in surimi products. The determination coefficient R^2 was higher than 0.9914 at the range of 0.5–200 ng/mL and both the intra and interday precision RSD were less than 6.7% for three peptides. The limit of quantitation was 0.054 $\mu\text{g/g}$ for soy, 0.024 $\mu\text{g/g}$ for milk and 0.032 $\mu\text{g/g}$ for egg.

To our knowledge, this paper is the first to simultaneously detect tree potentially allergenic proteins in surimi products with a isotope-

labeled peptide. Owing to its low limits of quantification, the method could be used as a tool for multi-allergen detection or production line monitoring of surimi products to reduce the crossing contamination. Precise and quantitative data provided by LC-MS/MS method can help sensitive people take targeted preventive and therapeutic measures. It can be used for label management for surimi products as well. In the future, more work should be done about allergens in complex food matrix using the established method.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 3
Determination of allergens in samples.

Sample Name	Declared allergen containing	Detected content(μg allergic protein/g) ^a		
		Soy	Milk	Egg
1	soy, milk, egg	1.93 $\pm$ 0.05	1.78 $\pm$ 0.02	0.96 $\pm$ 0.03
2	—b, —b, —b	N	N	N
3	soy, milk, egg	9.91 $\pm$ 0.16	5.55 $\pm$ 0.18	2.75 $\pm$ 0.07
4	—b, —b, —b	N	N	N
5	soy, milk, egg	25.65 $\pm$ 0.32	11.19 $\pm$ 0.06	8.49 $\pm$ 0.17
6	soy, —b, egg	5.12 $\pm$ 0.16	N	0.84 $\pm$ 0.01
7	—b, —b, egg	0.22 $\pm$ 0.01	N	0.15 $\pm$ 0.007
8	—b, —b, —b	N	N	N
9	soy, —b, egg	14.63 $\pm$ 0.22	N	1.68 $\pm$ 0.08
10	—b, —b, —b	N	N	N
11	soy, —b, —b	3.18 $\pm$ 0.04	N	0.05 $\pm$ 0.003
12	soy, —b, —b	8.25 $\pm$ 0.10	N	0.15 $\pm$ 0.01
13	—b, —b, —b	N	N	N
14	soy, —b, —b	3.53 $\pm$ 0.02	N	N
15	soy, —b, egg	0.56 $\pm$ 0.05	0.27 $\pm$ 0.005	0.24 $\pm$ 0.005
16	soy, —b, egg	1.73 $\pm$ 0.06	0.27 $\pm$ 0.001	0.76 $\pm$ 0.06
17	—b, —b, —b	N	N	N
18	—b, —b, egg	N	N	0.2 $\pm$ 0.003
19	soy, —b, —b	3.25 $\pm$ 0.07	N	0.49 $\pm$ 0.05
20	soy, —b, —b	1.15 $\pm$ 0.07	N	N
21	soy, —b, —b	0.79 $\pm$ 0.02	0.28 $\pm$ 0.001	N
22	—b, —b, —b	N	N	N
23	soy, —b, —b	0.49 $\pm$ 0.04	N	0.19 $\pm$ 0.001
24	soy, —b, —b	2.17 $\pm$ 0.08	N	N
25	soy, —b, —b	3.01 $\pm$ 0.33	N	N
26	—b, —b, —b	N	N	N
27	soy, milk, egg	4.35 $\pm$ 0.11	3.08 $\pm$ 0.04	1.37 $\pm$ 0.02
28	soy, —b, —b	5.74 $\pm$ 1.57	N	N
29	soy, —b, —b	13.44 $\pm$ 3.02	N	N
30	—b, —b, —b	N	N	N

^aData were shown as means $\pm$ SD, n = 3.

^bmeans not declare in the label.

N means not be detected.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2020.126580>.

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Simultaneous determination of multi-allergens in surimi products by
LC-MS/MS with a stable isotope-labeled peptide

Xue Huang^{a,1}, Zitong Zhu^{b,1}, Heng Feng^a, Qiumei Zhang^a, Hong Zhang^{a,*}

*^a School of Food Science and Biological Engineering, Zhejiang Gongshang University,
Hangzhou, Zhejiang 310018, China*

*^b Comprehensive technology service center of Quzhou entry exit inspection and
Quarantine Bureau, Quzhou, Zhejiang 324003, China*

***Corresponding Author**

Prof. Hong Zhang

Phone: +86 13858001588.

E-mail address: hongzh1316@zjgsu.edu.cn

¹These two authors contributed equally to this study.

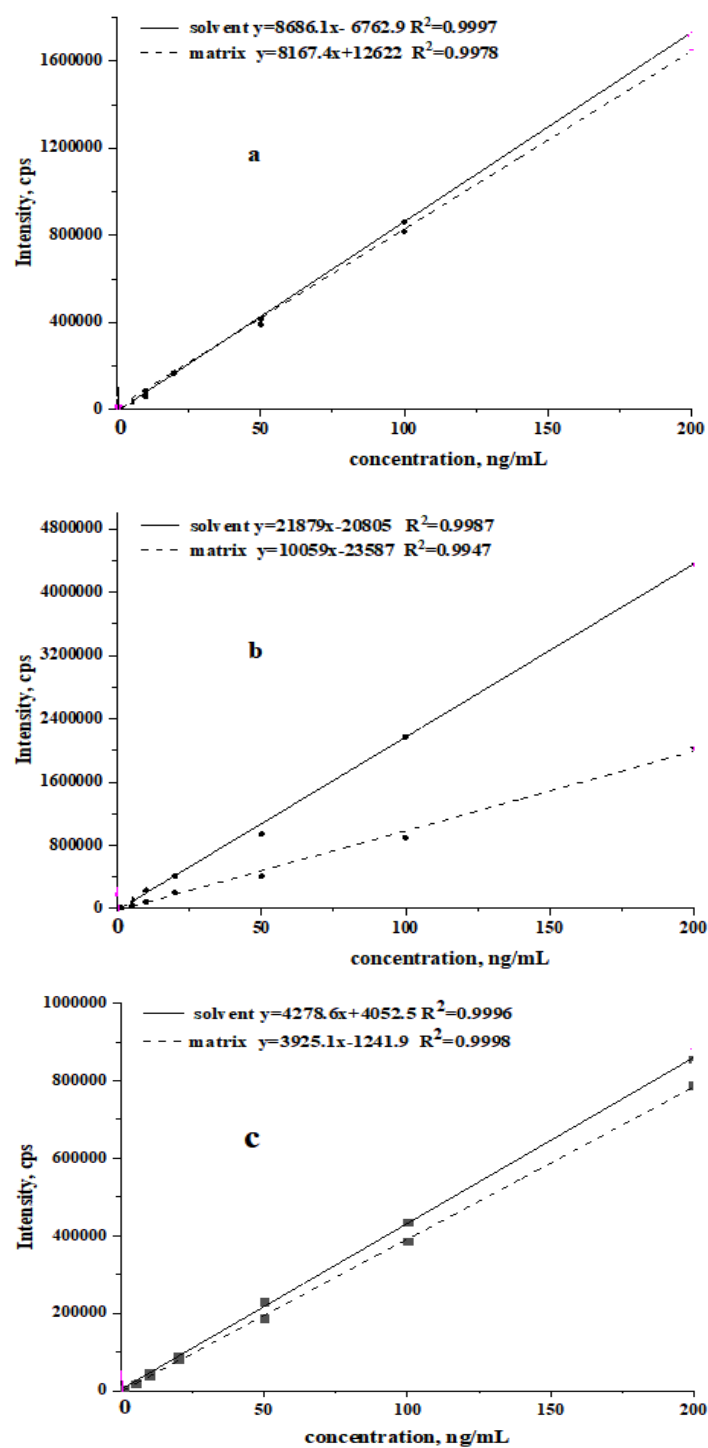


Figure S1. Matrix effect of three target peptides. (a) ME for soy peptide EAFGVNMQIVR; (b)

ME for soy peptide YLGYLEQLLR; (c) ME for soy peptide GGLEPINFQTAADQAR.

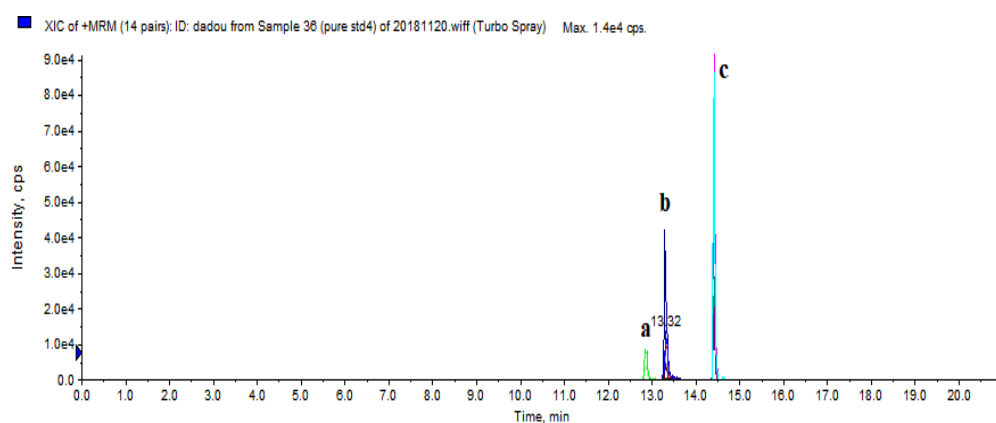


Figure S2. The chromatogram of four peptides from tryptic sample. (a) the chromatogram of peptide GGLEPINFQTAADQAR; (2) the chromatogram of peptide EAFGVNMQIVR and isotope-labeled peptide EAFGVNMQI*VR; (c) the chromatogram of peptide YLGYLEQLLR.