PO-CON1665E

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# High sensitive analysis of peanut allergen in cumin and spice mix

ASMS 2016 ThP118

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## Introduction

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 one of 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 (Figure 1) in commercially available spices and seasonings.

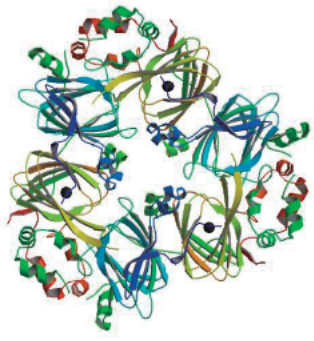

Figure 1 the structure of Ara h1 [3S7I] (68kDa) Vicilin like protein

Figure 2 LCMS-8060 triple quadrupole mass spectrometer

## Materials and methods

Commercially available defatted peanut flour was purchased and used for the initial development work. The test samples were ground and digested into peptides by trypsin digestion followed by reduction, alkylation, liquid-liquid extraction, and denaturation. UHPLC/MS/MS using Shimadzu Nexera X2 coupled to a LCMS-8060 system was used to detect peptides of the allergen. In-silico digestion using Skyline software was used for selection of target peptides and transitions. Several spices and spice mixes were used to test cross-reactivity of developed method. We used peanut powder for the method optimization and spiking test. This peanut powder contains 90% less fat and it is commercially available. All of samples including peanut powder, other nuts, and spices were purchased from a local grocery store.

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| Analytical conditions | |
|---|---|
| Instrument | : Nexera X2 with LCMS-8060 system |
| Mobile phase A | : 0.1 % formic acid in water |
| Mobile phase B | : Acetonitrile |
| Flow rate | : 0.5 mL/min |
| Gradient program | : 2%B (0.00 min) > 25%B (7.00 min) > 95%B (7.10-8.00 min) > 2%B (2.10-10.00 min) |
| Injection vol. | : 10 µL |
| Column temp. | : 40 °C |
| Nebulizing gas flow | : 3 L/min |
| Heating gas flow | : 20 L/min |
| Drying gas flow | : 5 L/min |
| Interface temp. | : 250 °C |
| DL temp. | : 150 °C |
| Heat Block temp. | : 200 °C |

# Result

Ara h1 is known as a common allergen of peanuts. We analyzed tryptic digest of protein extracted from peanuts by monitoring theoretically calculated transitions of peptides based on amino acid sequences of two clones P17 and P41B of Ara h1. 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. Since food samples contain various concentrations of matrix including lipids and salts, liquid-liquid extraction was performed before tryptic digestion.

## Selection of MRM transitions for Ara h1

MRM transitions for each clone was determined by using Skyline. Nine peptides including three common peptides to both clones were selected based on sensitivity. Three transitions were set for each peptide.

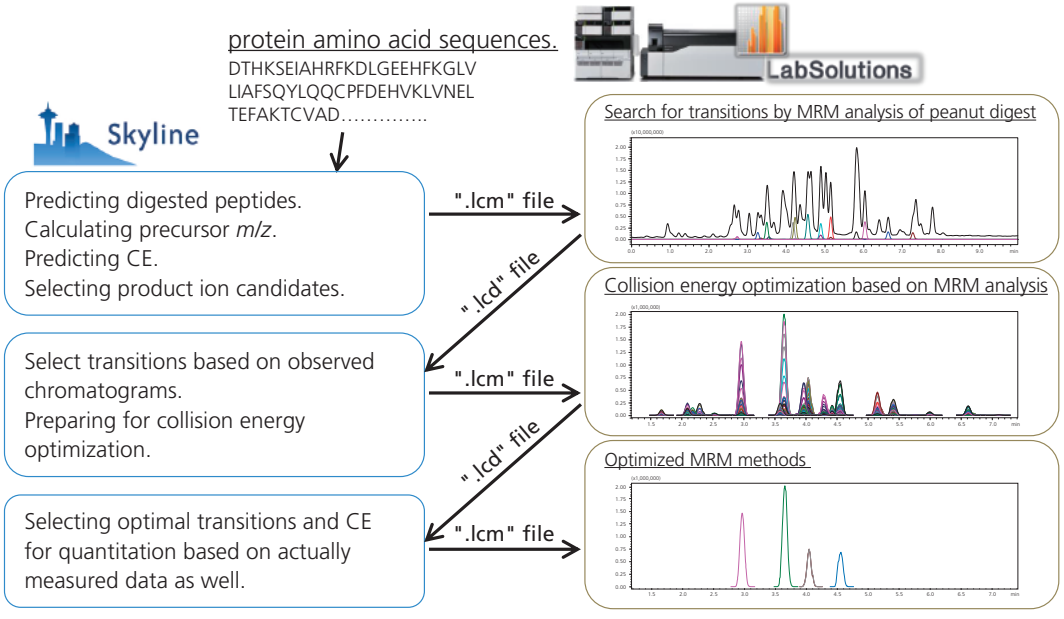


Figure 3 Work flow of MRM transition optimization using Skyline.

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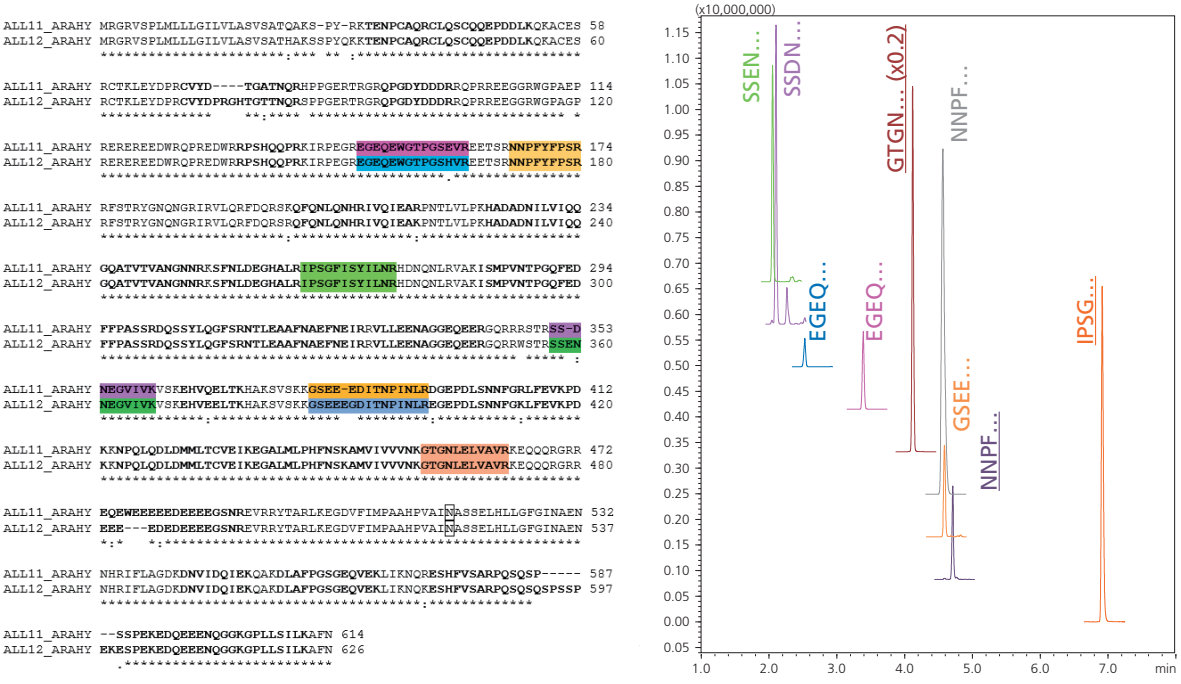


Figure 4 AA sequences of P17/P41B and nine MRM chromatograms.

## 2 ppm peanuts spiked in other nuts

We analyzed walnuts, cashew nuts, and almonds to test specificity. The peptides spiked peanuts were detected successfully and any obvious peak was not detected in blank samples.

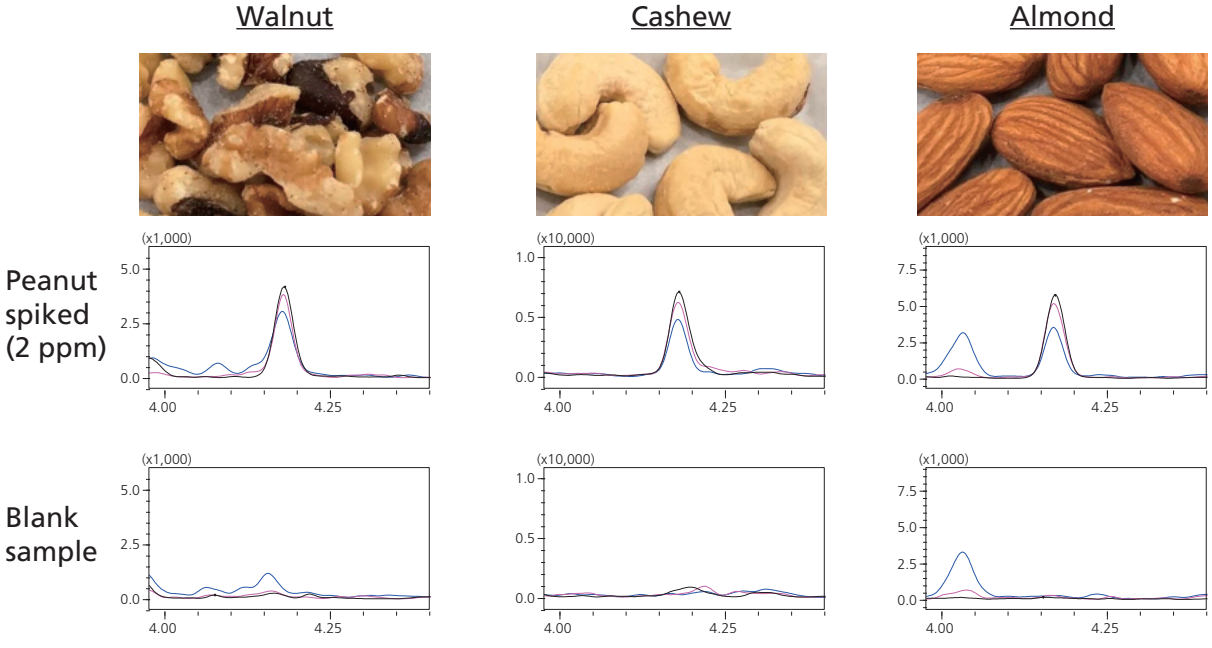


Figure 5 Chromatograms of peptide GTG… in other kind of nuts with or without spiking of peanuts.

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## 2 ppm peanuts spiked in spices

We prepared contaminated spice samples 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.

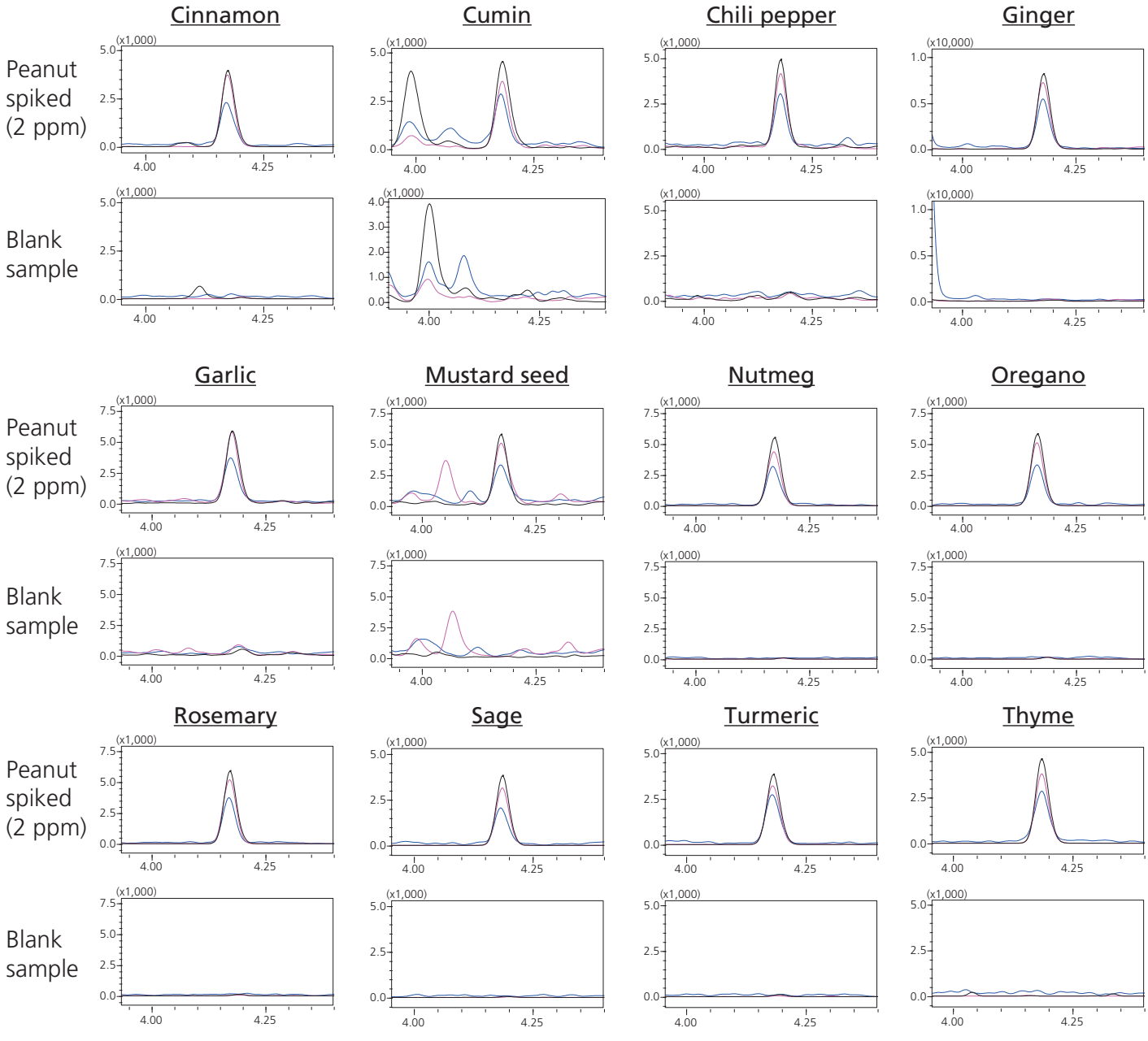


Figure 6 Chromatograms of peptide GTG… in spices with or without spiking of peanuts.

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## Detection of Ara h1 in spice mixes and seasonings

We analyzed several spice mixes and seasonings and detected peaks of tryptic peptides of Ara h1 from samples without spiking of peanut peptides.

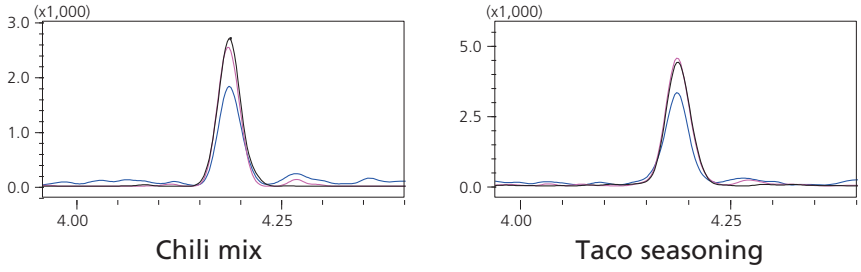


Figure 7 The peaks of peptide GTG… in Chili mix and seasoning.

## Interface optimization to achieve better sensitivity

As a result of interface optimization, sensitivity was improved more than twofold.

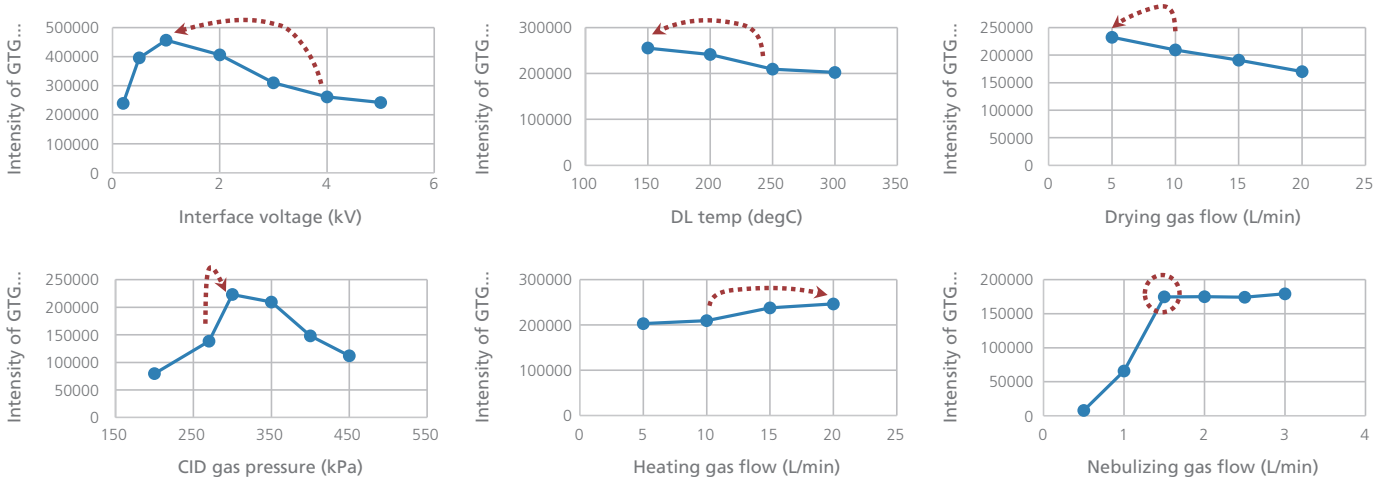


Figure 8 Interface optimization result.

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## Effect of surfactant during digestion

We expected that intensity of peptides would become higher by addition of a surfactant during tryptic digestion due to improved digestion efficiency. However, the intensity of peptides were relatively worse by adding surfactant. So we did not use any surfactant for tryptic digestion.

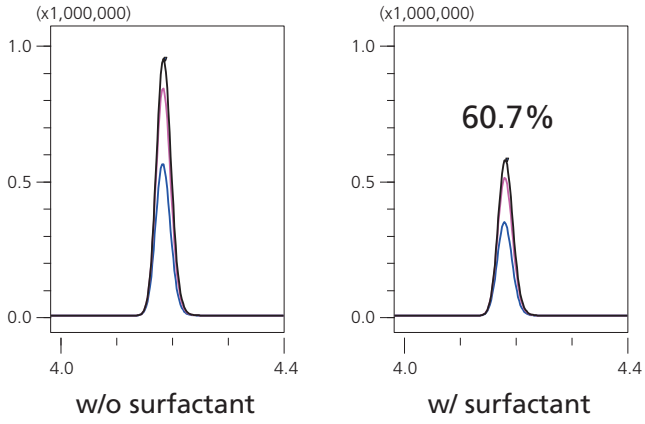


Figure 9 Difference of the chromatograms of peptide GTG… by addition of surfactant.

# 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.

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

First Edition: June, 2016

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