LC-MS/MS Analysis of Emerging Food Contaminants

Detection of Peanut and Almond Allergens in Spices

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Introduction

Recent findings (in February 2015) of allergens in spices caused the recall of many food products in North America and Europe. The US Food and Drug Administration (FDA) advised people who are highly allergic to peanuts to consider avoiding products that contain ground cumin or cumin powder, because some shipments of these products have tested positive for undeclared peanut protein. The Food Standards Agency (FSA) has issued a further allergy alert following confirmation that a batch of paprika was the most likely source of undeclared almond protein in three food products which had been recalled. According to the European Rapid Alert System for Food and Feed (RASFF) portal, additional food products containing Cayenne pepper and Pilli-Pilli powder were found to contain undeclared traces of peanuts. Another recall of cumin containing product was triggered by the Canadian Food Inspection Agency (CFIA).[1-4]

This was the latest in a string of spices being recalled for possible nut protein findings. It remains unclear whether the contamination is accidental or deliberate.

It is important that consumers know food is safe and authentic. Potential weaknesses in the food supply chain need to be identified and counter measures need to be taken to strengthen consumer protection. Accurate and reliable analytical methods are needed to monitor the food supply chain and to allow correct labeling of food products.

Traditionally enzyme-linked immunosorbent assay (ELISA) based methods are used for food allergen testing. However, it is well known that ELISA can generate variable results, including false negative and false positive results that can occur due to the technique's limited sensitivity and selectivity. In addition, each allergen requires a separate test kit for the identification of an individual allergen. A multi-allergen screening method would be invaluable to increase the throughput and efficiency in allergen testing.

Here we present a method to detect the presence of peanut and almond in spices. Samples were extracted and then the allergenic proteins were reduced, alkylated and digested using trypsin. The extract containing peptides from the digested

proteins were filtered and analyzed by LC-MS/MS using a reverse phase chromatography and positive polarity electrospray ionization (ESI). The SCIEX QTRAP® 4500 system used for this study was operated in Multiple Reaction Monitoring (MRM) mode to achieve high selectivity of detection. In MRM mode characteristic transitions of peptides breaking into compound-specific fragment ions are monitored. At least 12 transitions (3 transitions for 4 peptides) were monitored per allergen to minimize potential false positive results caused by matrix interferences. The QTRAP® 4500 system also allows the acquisition of full scan MS/MS spectra which can be searched against mass spectral libraries to further increase the confidence in identification.

Experimental

Samples

Samples of cumin and paprika were obtained from local supermarkets. Store-bought roasted and raw peanuts and almonds were used for spiked experiments.

Sample preparation

The sample preparation method was based on previous work of Lock et al. The complete protocol is available in the iMethod™ Application for Allergens in Baked Goods (version 1.0).[5-6]

The analytical workflow is shown in Figure 1.

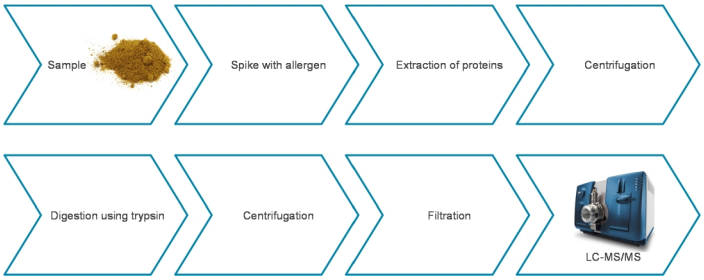

Figure 1. Sample preparation workflow

LC Separation

A Shimadzu UFLC$_{XR}$ system was used for analysis. Separation was achieved using a Phenomenex Kinetex 2.6u XB-C18 100A (30 x 1.0 mm) column with a mobile phase consisting of water and acetonitrile containing 0.1% formic acid and a 15 min gradient from 98/2 to 2/98 (A/B%). The LC column was held at 30°C. The flow rate was set to 300 µL/min and the injection volume to 30 µL.

MS/MS Detection

A SCIEX QTRAP® 4500 system with Turbo V™ source with ESI probe was used in positive polarity. The ion source temperature was set to 500°C.

MRM transitions were obtained from *in-silico* and protein ID experiments. Specificity and cross reactivity was evaluated by injecting extracts of roasted and raw almonds and peanuts as well as spiked extracts of spices. The final list of MRM transitions used in this study is shown in Table 1.

Table 1. Retention times (RT) and MRM transitions used for the detection of almond and peanut

Allergen (Peptide)	RT (min)	Q1	Q3
Almond (Peptide 1)	9.7	830.4	738.4
Almond (Peptide 1)	9.7	830.4	1035.5
Almond (Peptide 1)	9.7	830.4	922.5
Almond (Peptide 2)	8.3	571.8	369.2
Almond (Peptide 2)	8.3	571.8	858.5
Almond (Peptide 2)	8.3	571.8	743.4
Almond (Peptide 3)	7.7	698.3	732.4
Almond (Peptide 3)	7.7	698.3	879.5
Almond (Peptide 3)	7.7	698.3	936.5
Almond (Peptide 4)	10.1	780.8	1154.7
Almond (Peptide 4)	10.1	780.8	848.5
Almond (Peptide 4)	10.1	780.8	1186.7
Peanut (Peptide 1)	8.2	688.8	300.2
Peanut (Peptide 1)	8.2	688.8	930.6
Peanut (Peptide 1)	8.2	688.8	1077.5
Peanut (Peptide 1)	8.2	688.8	833.4
Peanut (Peptide 2)	8.4	564.4	686.6
Peanut (Peptide 2)	8.4	564.4	557.5
Peanut (Peptide 3)	8.5	793.9	827.5
Peanut (Peptide 3)	8.5	793.9	612.4
Peanut (Peptide 3)	8.5	793.9	726.4
Peanut (Peptide 4)	8.9	571.3	913.5
Peanut (Peptide 4)	8.9	571.3	669.3
Peanut (Peptide 4)	8.9	571.3	506.3

Results and Discussion

Qualitative Allergen Screening using MRM

Example chromatograms of spiked extracts are presented in Figure 2.

Figure 2a shows the results for 10 mg of roasted and raw almond spiked into 1 g of paprika, and Figure 2b shows the results for 10 mg of roasted and raw peanut spiked into 1 g of cumin.

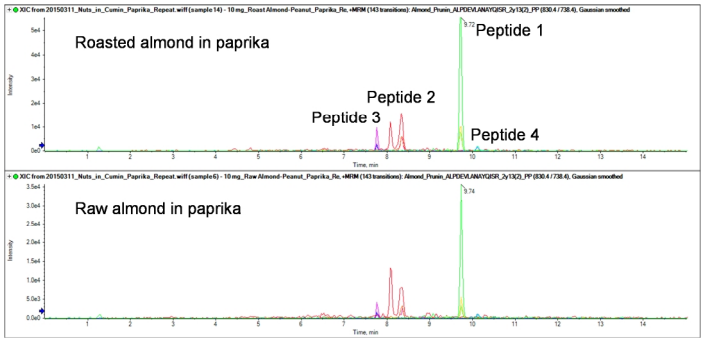

Figure 2a. Detection of almond in extracts of paprika (spiked at 10 mg/g)

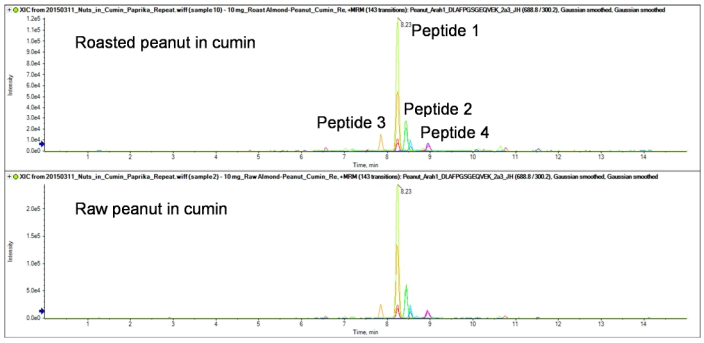

Figure 2b. Detection of peanut in extracts of cumin (spiked at 10 mg/g)

Identification of target compounds or peptides is typically based on MRM ratio calculation when utilizing LC-MS/MS.

There is the possibility of protein modification during food product, transportation, storage, and processing. The monitoring of 12 MRM transitions corresponding to 4 different peptide fragments per allergen provides high confidence in identification since different characteristic peptides of the allergen are monitored simultaneously. This procedure greatly reduces the possibility of false negative results.

MultiQuant™ software automatically calculates MRM ratios and MRM ratio tolerances. MRM transitions outside the tolerance will be flagged to identify outliers quickly. The MRM tolerances are also displayed in the Peak Review (see Figures 3a and 3b).

The MRM ratio measured from raw and roasted almonds and peanuts spiked into spices was typically well below 30%.

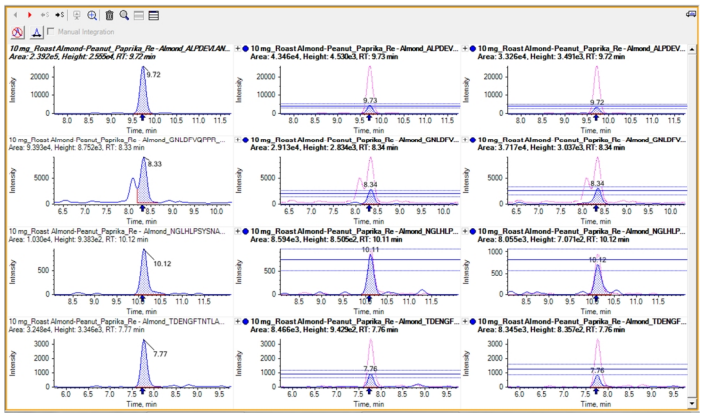

Figure 3a. Identification of almond in a paprika extract based on multiple MRM ratios

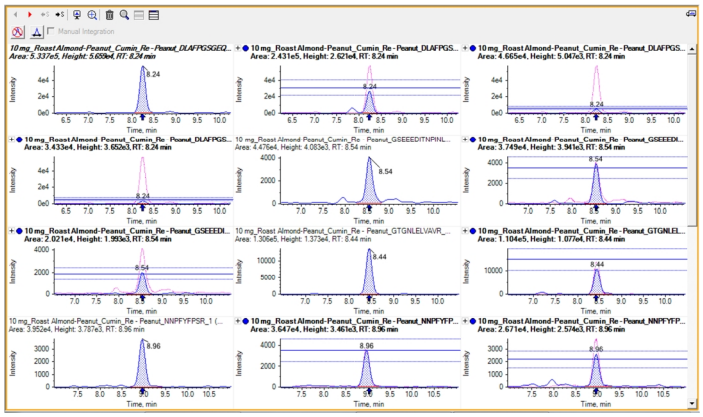

Figure 3b. Identification of peanut in a cumin extract based on multiple MRM ratios

Quantitation of Allergens in Spices

LC-MS/MS is a well know technique for the accurate and reproducible quantitation.

In this study initial quantitative results were obtained by spiking almond and peanut in spices (1, 10, and 100 mg/g) and analyzing samples following the complete sample preparation and LC-MS/MS workflow.

Example calibration lines are presented in Figure 4.

Figure 4a shows the results for roasted almond spiked into paprika and Figure 2b shows the results for roasted peanut spiked into cumin. Good accuracy and coefficients of correlation >0.999 were achieved for all transitions.

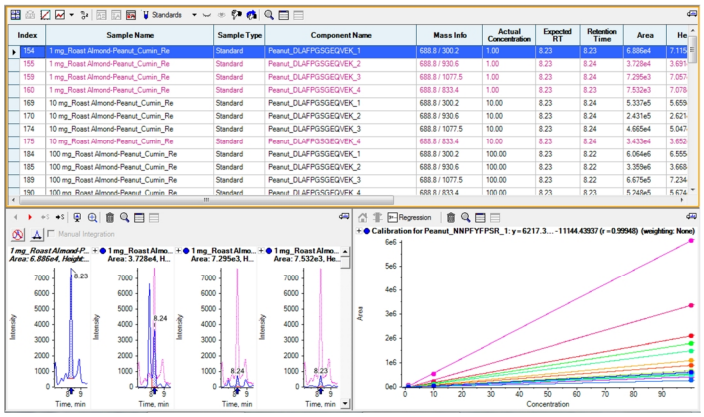

Figure 4a. Quantitative results of analyzing almond spiked into paprika powder

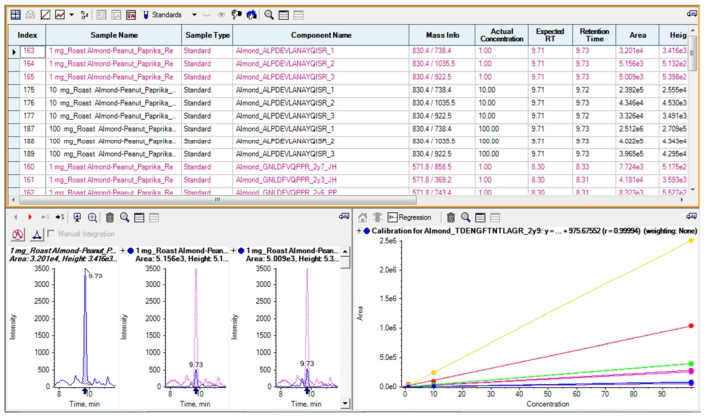

Figure 4b. Quantitative results of analyzing peanut spiked into cumin powder

Identification using MS/MS Scanning

The SCIEX QTRAP® 4500 system allows collecting MRM and MS/MS full scan data simultaneously using information dependent acquisition (IDA).

An example chromatogram with acquired MS/MS spectra for two peptides of peanut is presented in Figure 5. The spectra can be searched against mass spectral libraries which increases the confidence in identification when analyzing complex food samples.

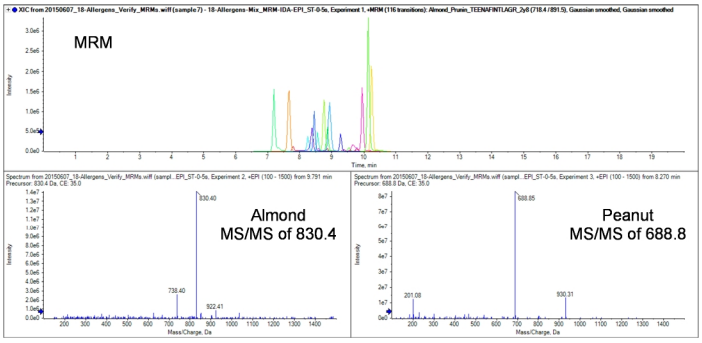

Figure 5. High confidence in identification using MS/MS full scan

Multiplexing of Allergens by LC-MS/MS

LC-MS/MS has the additional advantage of performing multi-allergen screening, unlike ELISA where different allergens are detected by separate kits.

In our laboratory LC-MS/MS was successfully applied to simultaneously screen for multiple food allergens, including egg, milk, gluten, peanut, tree nuts, soy, sesame, and mustard. An example of detecting a total of 18 allergens with a single analysis is presented in Figure 6.

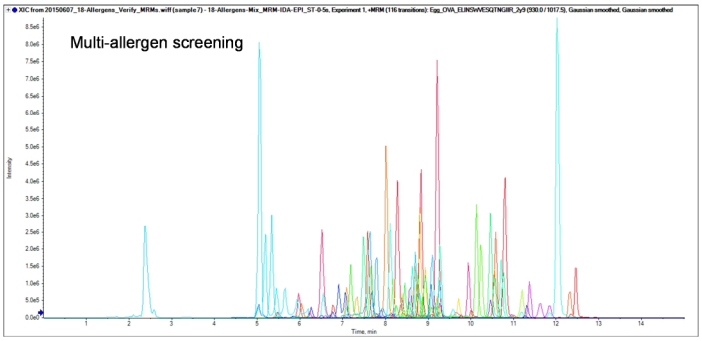

Figure 6. Multi-allergen screening by LC-MS/MS, detection of a total of 18 allergens in a single analysis

Summary

An LC-MS/MS method for the detection of almond and peanut in spices was presented.

Samples were extracted and then the allergenic proteins were reduced, alkylated and digested using trypsin. The digested extract was filtered and analyzed by LC-MS/MS using a SCIEX QTRAP® 4500 system operated in MRM mode.

Good linearity for quantitation was achieved when analyzing almond and peanut spiked into paprika and cumin at different concentrations.

Allergen identification was achieved through the monitoring of 12 characteristic MRM transitions per allergen. MRM ratios were calculated automatically using MultiQuant™ software and MRM ratios were typically well below 30%. The QTRAP® 4500 system also allows the acquisition of full scan MS/MS spectra which further increase the confidence in identification.

LC-MS/MS has the additional advantage of performing multi-allergen screening, unlike ELISA where different allergens are detected by separate kits.

References

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