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Identification and Quantitative Analysis of Egg Allergen Peptides Using Data Independent Ion Mobility Mass Spectrometry

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Abstract

In this application note, a discovery proteomic workflow has been applied to determine marker peptides that can be used for the quantitative analysis of allergenic proteins within food.

Benefits

- HDMS^E provides both qualitative and quantitative information in a single experiment.
- Utilizing ion mobility as part of the workflow provides enhanced specificity and therefore confidence of identifications returned.
- Method provides potential means for multi-allergen detection.

Introduction

Food allergies arise from an abnormal immunological response to certain foods. Proteins are the main candidates for triggering allergic reactions. Egg-based proteins are one of the most frequent causes of adverse reactions in food. Since many processed foods contain egg as a raw ingredient, the ability to assess changes in protein structure and detection through the manufacturing cycle is important.

Food allergen analysis using LC-MS/MS is a current hot topic for many food scientists and there are two approaches that can be used to generate a quantitative method. The first approach is to perform in silico digestion of proteins, based on fasta sequences available in databases (e.g. UniProt), providing a list of potential peptides and MRM transitions. This methodology requires further investigation to determine which MRMs are the most specific and sensitive at detectable response levels for post-food processing, sample treatment, and during the ionization process. The alternative is to perform a discovery omics experiment using a high resolution instrument, such as a QTof mass spectrometer and use the data observed from this experiment to generate a targeted method.

In this study, the second approach has been applied and focuses on identifying and quantifying known allergenic proteins from raw and cooked egg samples. Proteins extracted from raw and cooked egg samples were digested using trypsin and label-free protein expression data were acquired with Waters SYNAPT G2-S*i* using an ion mobility data independent approach (whereby the collision energy was switched between low and elevated energy states during alternate scans).

Utilizing ion mobility as part of the workflow provides enhanced specificity and therefore confidence of identifications returned, even in the presence of complex matrices, such as processed food samples. Precursor and product ions were associated by means of retention and drift time alignment. Although egg proteins were the focus of this work, other allergenic proteins that are also extracted using the sample preparation could be investigated, providing a potential means for multi-allergen detection.

The acquired data were processed using Progenesis QI for Proteomics and searched against a Gallus Gallus (Uniprot) database. The results generated allowed for relative quantification to be established. The results of this study showed that a significant proportion of proteins identified were expressed when comparing cooked and raw egg sample sets, which included known allergenic proteins (e.g. apovitellenin I). Peptides identified in both sample sets allowed for MRM transitions to be generated and a quantifiable value assigned.

Experimental

Sample preparation

Proteins were extracted from egg-based samples using phosphate buffer saline (PBS) and a BCA assay used to determine initial protein concentrations and afterward normalized proteins concentration to 1 mg/ml. Samples were reduced and alkylated before overnight digestion using trypsin. Prior to LC-MS analysis, samples were filtered using a 0.22 µm filter to remove any particulates and diluted appropriately using 0.1% formic acid (Figure 1).

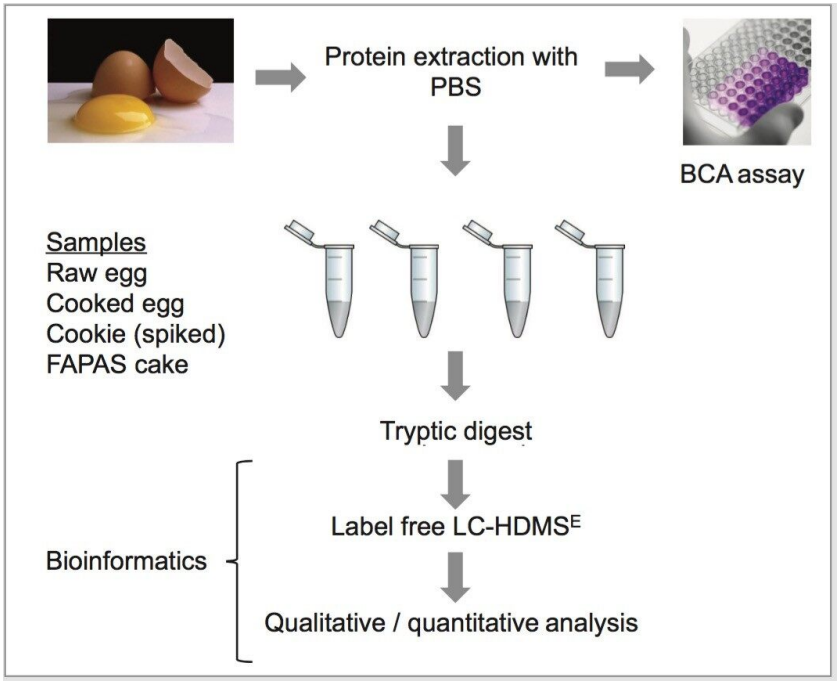

Figure 1. Experimental design study for egg allergen proteins.

LC-MS conditions

Label-free LC-MS was used for qualitative and quantitative peptide analyses. Experiments were conducted using a 90 min gradient from 5 to 40% acetonitrile (0.1% formic acid) at 300 nL/min using an ACQUITY UPLC M-Class System configured with an ACQUITY UPLC Peptide BEH C_{18} nanoACQUITY Column 10K psi, 130Å, 1.7 µm, 75 µm X 150 mm, part no. 186003543. Data were acquired in data independent analysis (DIA) utilizing a SYNAPT G2-S*i* Mass Spectrometer enabled with ion mobility functionality.

Bioinformatics

The LC-MS peptide data were processed and searched with Progenesis QI for Proteomics Software. A species specific Gallus Gallus (Uniprot) database was used. Fixed and variable modifications included carbamidomethyl C and met-oxidation respectively in addition to a protein false discovery rate of 4%.

ACQUITY UPLC M-Class System with the SYNAPT G2-Si.

Results and Discussion

Protein identification and quantification for raw and cooked egg

Proteins extracted from raw and cooked egg samples were analyzed to identify, quantify, and investigate the variance between potential allergenic markers. A total of 95 and 84 proteins were identified for raw and cooked respectively. A subset of those proteins identified are highlighted in Table 1 with their respective amounts found in both the raw and cooked egg extracts.

Allergenic protein	Raw (ng/µL)	Cooked (ng/µL)
P01005: Ovomucoid (OVM)	344.0	333.0
P01012: Ovalbumin (OVA)	44.3	43.8
P02659: Apovitellenin (APO)	12.7	5.2
P02789: Ovotransferrin (OVT)	29.7	5.8
P00698: Lysozyme (LYS)	6.5	1.0

Table 1. Typical allergenic proteins identified and quantified from raw and cooked egg extracts.

The SYNAPT G2-S*i* utilized data independent analysis with ion mobility ($HDMS^E$) enabled. The advantage of $HDMS^E$ mode is that it maximizes the number of identified proteins through increased peak capacity and overall specificity. Example low energy (relating to precursor ions) and high energy (relating to fragment ions) are presented in Figure 2.

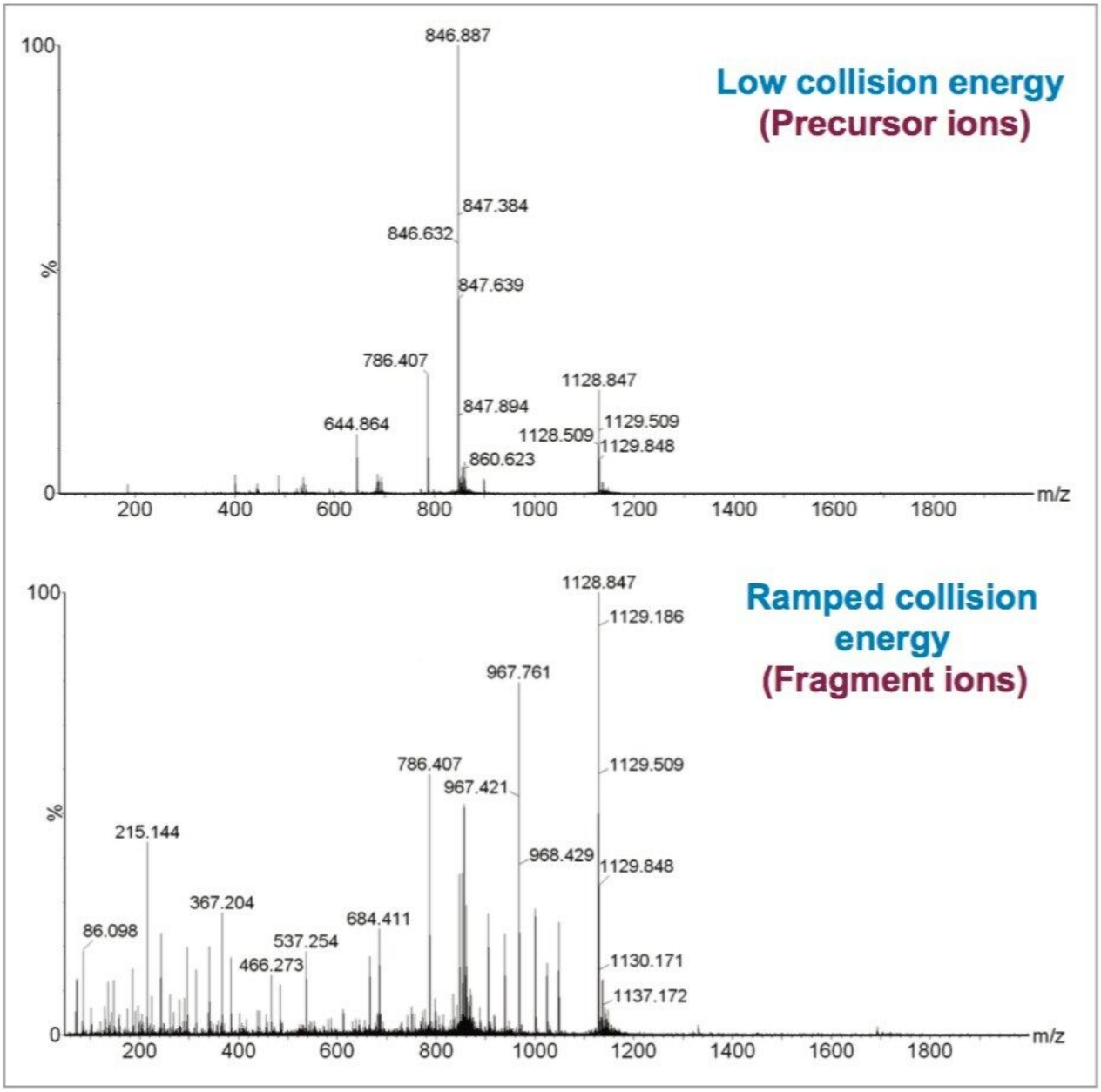

Figure 2. OVM diagnostic marker identifiable at 1 ppm (m/z 846.624, ELAAVSVDCSEYPKPDCTAEDRPLCGSDNK).

Ion mobility was enabled to provide enhanced specificity for the experiment. This results in cleaner spectra (important when analyzing complex food matrices), and provides the ability to separate similar species, as shown in Figure 3, where example spectra are shown with ion mobility deactivated (off) and activated (on).

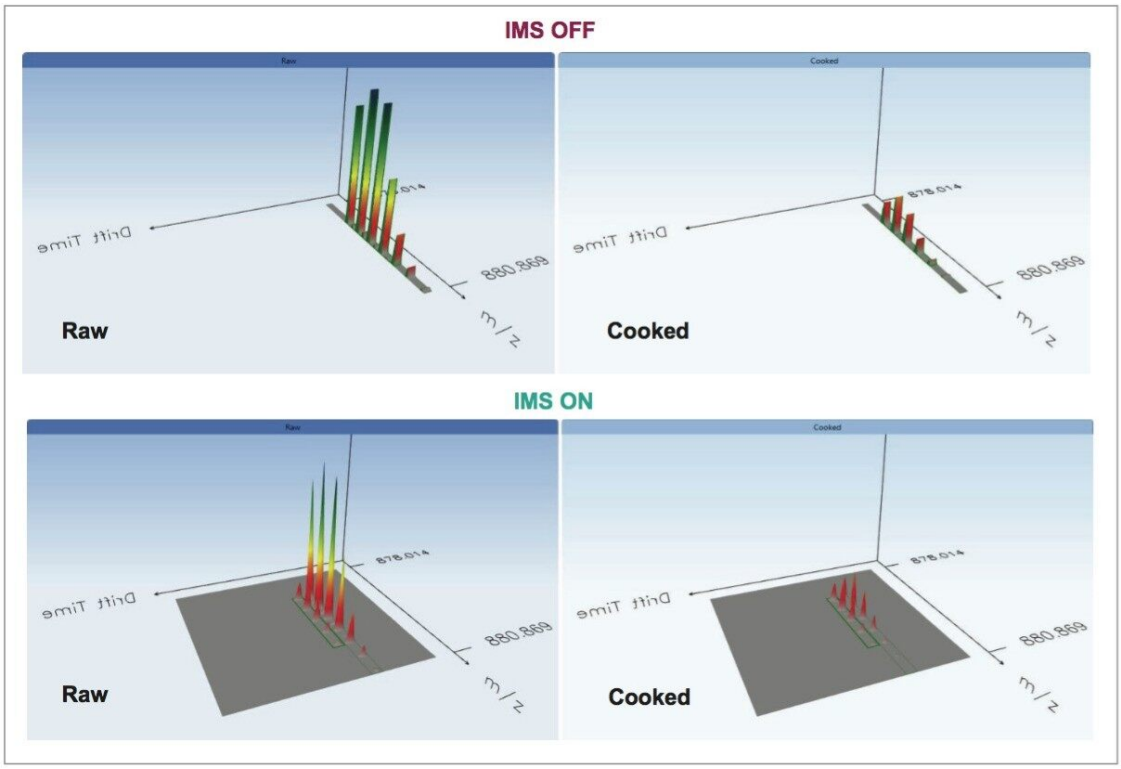

Figure 3. Effect of IMS separation demonstrated for the overlapping OVT peptide (m/z 878.7726, AIANNEADAISLDGG). Upper traces show overlapping species for both raw and cooked egg samples. The implementation of IMS (lower trace) allows separation of precursors with the same m/z, resulting in the identification of two distinct species.

Using the SYNAPT G2-S*i* in HDMS^E mode made it possible to obtain high peptide sequence coverage in the presence of cake matrix (Figure 4).

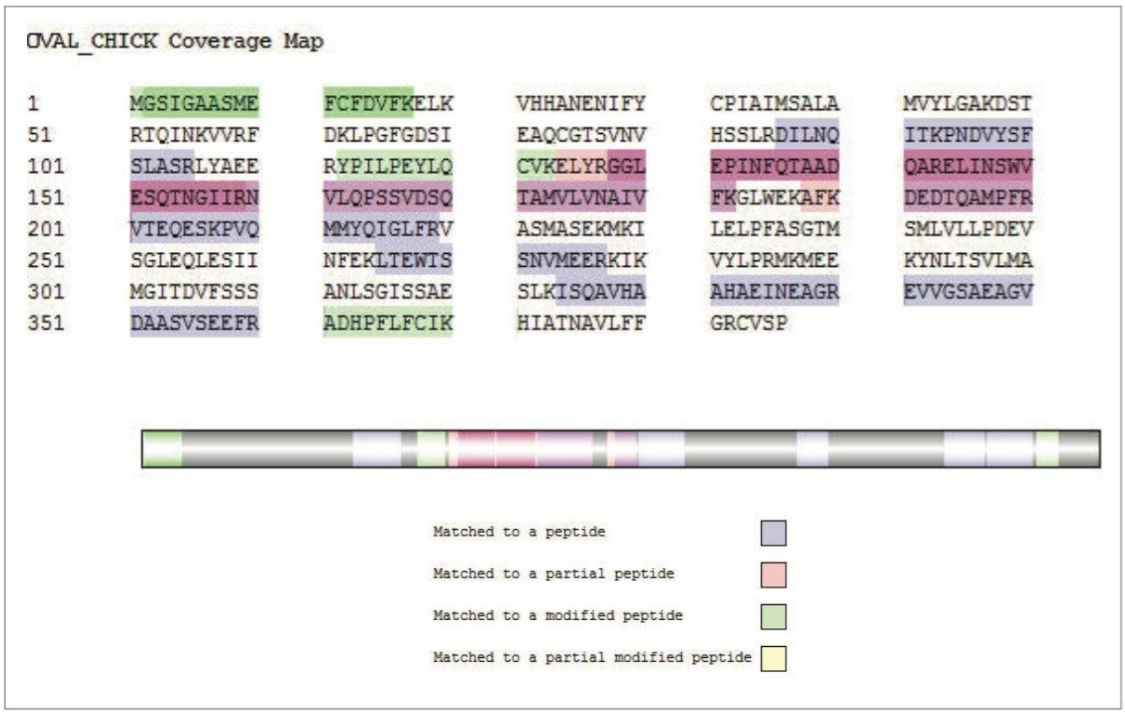

Figure 4. P01012, ovalbumin (OVA), and gallus gallus chicken peptides identified in the presence of cake matrix (51.8% sequence coverage).

Utilizing ion mobility to reduce background matrix effects

To assess the capabilities and advantages of implementing ion mobility into the analytical workflow, a dilution series of cooked egg ranging from 500 to 1 ppm was spiked into cookie matrix, which was maintained at the same concentration throughout (Figure 5).

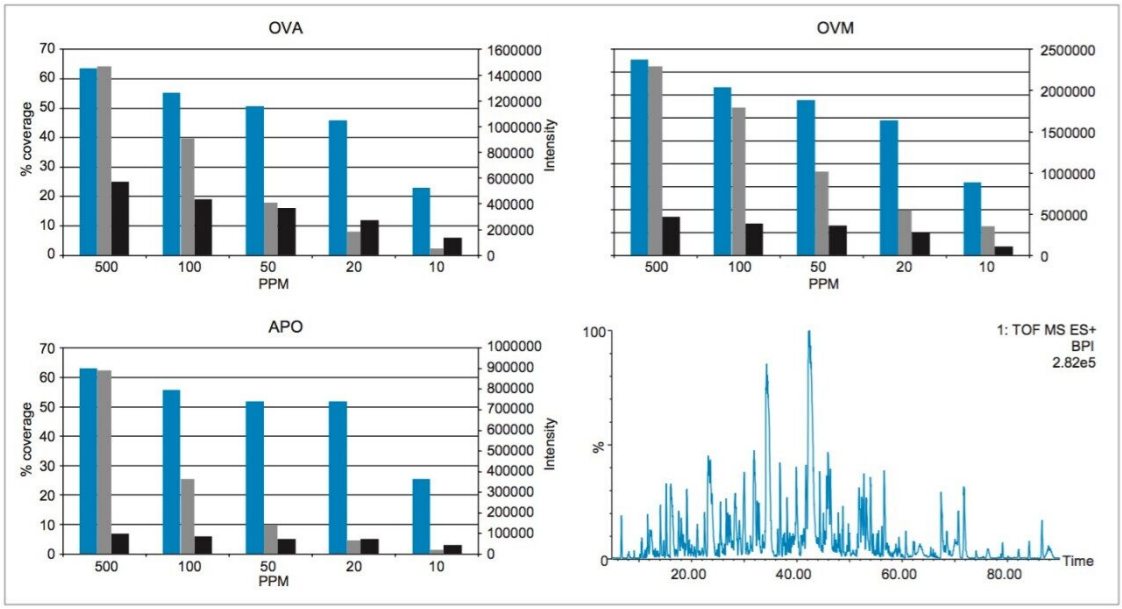

Figure 5. Identified allergenic proteins for serially diluted cooked egg in the presence of cookie matrix. Number of peptides (black), sum of intensity for the top three most abundant peptides (grey), and sequence coverage (blue). An example chromatogram is provided for 50 ppm of cooked egg in the presence of the cookie matrix.

FAPAS test sample – cake mix

Test material was supplied for FAPAS Proficiency Test material T2770 in the form of a cake mix obtained from a retail source which was free from egg and milk but contained gluten. Royal icing sugar was used to introduce egg white protein.

The major allergen identified was ovalbumin (OVA), and the sequence coverage (percentage of peptides identified that make up the protein sequence) in the presence of cake matrix was high at 51.8%.

Initial results from the FAPAS proficiency study were conducted using ELISA, which quantified egg between 39.6 to 62.1 ppm (mean = 47.7 ppm). The LC-MS label-free experiments corresponded with the ELISA findings, quantifying at 58 ppm. The nature of HDMS^E also allows for multi-allergens to be detected and quantified as part of this experiment.

Conclusion

A discovery proteomic workflow has been applied to determine marker peptides that can be used for the quantitative analysis of allergenic proteins within food. A label-free proteomic approach has been applied for the analysis of egg-based allergens, by implementing HDMS^E to provide both qualitative and quantitative information in a single experiment.

Ion mobility as part of the workflow is shown to provide enhanced specificity and therefore confidence of identifications returned, even in the presence of complex matrices, such as processed food samples.

Although only egg proteins were the focus of this work, other proteins relating to other allergens were observed, providing a potential means for multi-allergen detection.

References

1. Commission Directive No 2007/68/EC of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC (Official Journal, L310, 28/11/2007, 001–0014).
2. Li GZ, Vissers JP, Silva JC, Golick D, Gorenstein MV, Geromanos SJ. Database searching and accounting of multiplexed precursor and product ion spectra from the data independent analysis of simple and complex peptide mixtures. ***Proteomics***. Mar;9(6):1696–719, 2009.

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720005891, February 2017