Detection of Egg and Milk Allergens in Baked Foodstuffs

Detection of Egg and Milk Allergens in Baked Foodstuffs

A novel approach to identify multiple allergens in a food sample using a single procedure with reliable direct analysis by LC-MS/MS

An Overview of the verified iMethod™ Application for Egg and Milk Allergens Version 1.0 for Cliquid® Software

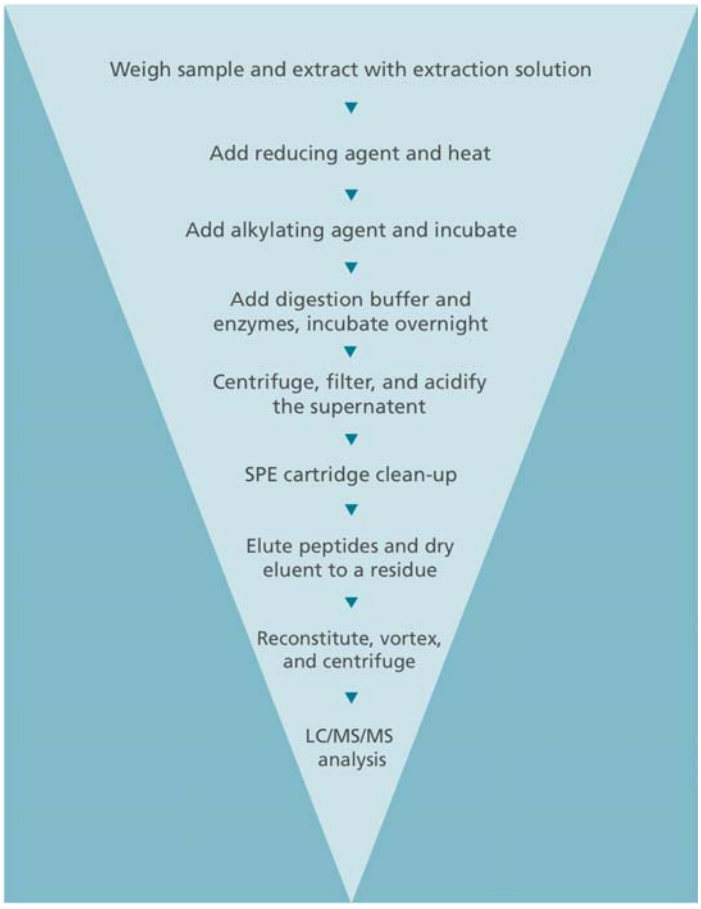
Introduction

Food allergies are on the rise across the world. Interestingly, the number of children with food allergies has reportedly increased by 18% from 1997 to 2007, according to the U.S. Centers for Disease Control and Prevention. Allergic reactions to consuming specific food proteins can range from mild rashes and hives to severe anaphylaxis or loss of consciousness. Given that there is no cure for food allergies, avoidance of the allergen is the only way to prevent a reaction, making accurate food labeling an essential resource for allergen sufferers.

Since allergens are almost exclusively proteins, protein-based methods are most typically used to identify allergens in food products. The most common approaches, ELISA and PCR, exhibit a host of limitations such as matrix interferences, allergen cross-reactivity, and susceptibility to false positive and false negative results. Furthermore, regulations will likely become more stringent for food labeling in the future, and a reliable, robust, and accurate quantitative approach to allergen detection in foods will be a necessity.

In our new Allergen iMethod™ Application, we present a novel analytical analysis method that allows the accurate and reliable identification of multiple allergens in a baked food product using a single sample preparation procedure with direct analysis of the allergen peptides by LC-MS/MS. This procedure produces highly reliable and accurate semi-quantitative results and is the only of its kind to allow for the determination of multiple allergens in a single analysis.

Figure 1. Sample preparation overview for analysis of milk and egg allergens in food samples.



Method Overview

This application identifies 2 different egg peptides and 5 different milk peptides. The basic outline of the sample preparation procedure for this analysis is shown in Figure 1. Briefly, allergenic proteins are extracted from the sample, then proteins are reduced and alkylated prior to undergoing digestion into small peptide fragments. The digested sample is cleaned using solid-phases extraction to remove unwanted matrix components, and the final eluted peptides are analyzed by LC-MS/MS.

This method was optimized using the following specific supplies and equipment beyond basic lab equipment and reagents: (1) Phenomenex Strata-X 33 um plyermeric reverse phases SPE

cartridges, (2) Phenomenex Synergi Hydro-RP 80A analytical HPLC column 4 um, 150 x 2.1 mm), (3) Shimadzu Prominence LC system, and (4) API 4000 Q TRAP® MS/MS system.

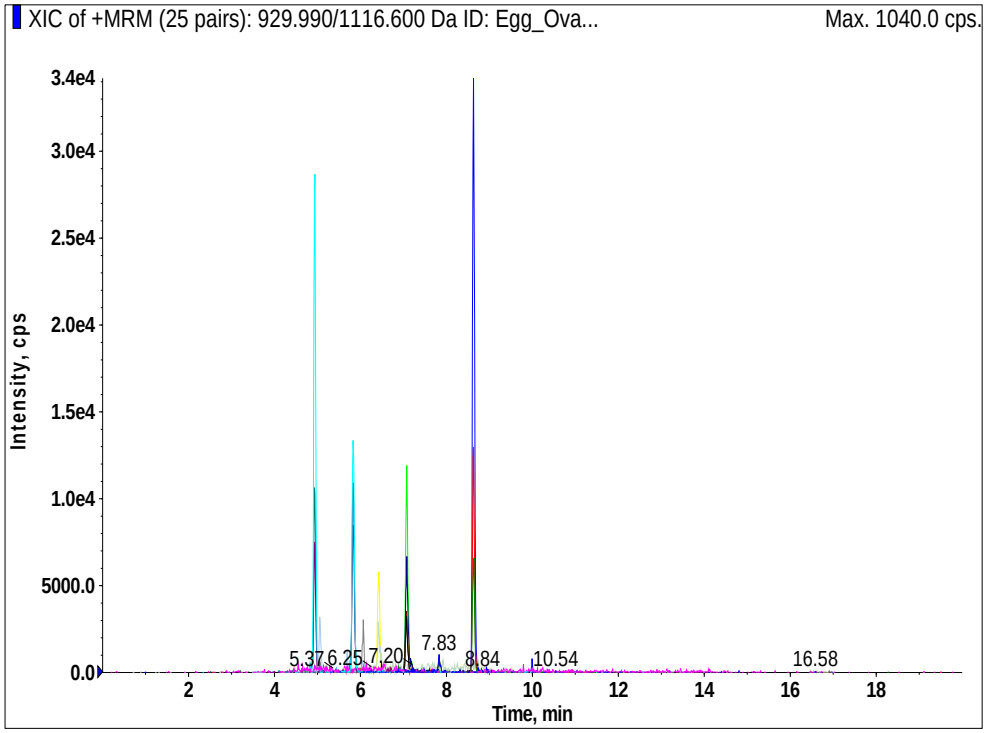
Additional standards, reagent, and supply requirements for this procedure can be supplied by contacting support@absciex.com.

Method data and results

The method performance was verified in multiple labs using both fortified control samples as well as reference test materials provided by FAPAS (T2779A, T2779B, and T2785). A sample chromatogram of the mixed allergen peptide MRMs and the accuracy and precision results obtained in the method verification are shown in Figure 2 and Table 1. Results show that both egg and milk allergens can be accurately identified in this procedure, with accuracies ranging from 80 to 110% for both egg and milk peptides, and method limits of detection ranging from 0.1 to 20 ug/g for the various peptides.

These results show that this method is accurate, reliable, and robust for the identification of milk and egg allergenic peptides in baked food products.

Figure 2. Sample chromatogram of MRMs of egg and milk peptides detected at 50 ug/g in a rye bread sample.



Additional technical details or questions surrounding this method can be provided by contacting customer support at support@absicex.com.

Ordering information

Visit our website for ordering information www.absciex.com.

Table 1. Outline of results obtained for this iMethod Application.

	Approximate LOD (ug/g)	% Accuracy, 20 ug/g	%CV	% Accuracy, 50 ug/g	% CV	Calibration range (ug/g)	Correlation Coefficient
Egg Protein 1 Peptide A	6.5	**86**	29	**102**	22	10 to 100	0.9836
Egg Protein 1 Peptide B	7.9	**108**	8	**99**	5	10 to 100	0.9958
Milk Protein 1 Peptide A	11.8	**100**	11	**100**	13	0 to 50	0.9840
Milk Protein 1 Peptide B	2.8	**87**	17	**102**	23	0 to 50	0.9755
Milk Protein 1 Peptide C	16.7	**89**	22	**102**	25	0 to 50	0.9721
Milk Protein 1 Peptide D	0.1	**95**	34	**101**	31	10 to 100	0.9630
Milk Protein 2 Peptide A	4.9	**84**	15	**103**	15	10 to 100	0.9703

Publication number: 5720112-01.

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
www.absciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
www.absciex.com/offices