INTRODUCTION

Food allergy is a life-long, systemic adverse reaction to food components typically a protein or peptides. The prevalence and severity of food allergies seem to be rising and have been estimated at approximately 10% worldwide, though the prevalence varies by region. At present, there is no cure for food allergies and all treatments are by avoidance. At present, there is no cure for food allergies and future treatments may involve oral desensitization, a strategy that is approved in Europe, but not yet approved by the FDA. However, oral desensitization is expensive and only for a few specific allergenic proteins and requires hospitalization. Therefore, a method that is able to accurately and reliably identify the presence of multiple allergens would be valuable for food screening.

Here, we present a LC-MS/MS method that is designed for the simultaneous detection and screening of 13 food allergens. These allergens were chosen based on the list highlighted by The Codex Alimentarius. The Codex Alimentarius is the food standards commission for the United Nations' Food and Agriculture Organization and the World Health Organization. Many plant allergens are common in nuts, cereal grains, and spices. The list was further refined by removing sequences that were too similar to sequences from other allergens. This method was designed to detect at least 10 ppm of 13 proteins. In this study, samples were extracted from the allergen proteins and then alkylated the free cysteine residues to prevent reformation of the bridges and to improve the stability of the proteins. After centrifuging, 500 µL of the supernatant was removed. A reducing reagent was then added to the supernatant to reduce the disulfide bonds. The samples were then run on a LC-MS/MS system and the results were analyzed using ProteinPilot. The LC-MS/MS method where each MRM is monitored only across its expected retention time, decreasing the number of concurrent transitions at any one time and resulting in a cycle time that is 10 times longer than that needed to scan all 13 transitions at any one time. The results were validated using dilution series for two allergens in bread and cookie matrices. In general, there was good agreement between the LC-MS/MS and ELISA methods for the bread and cookie samples that had these allergens labeled as one of the ingredients. Other allergens were identified in these analyses, including egg and milk. From this study, we have developed a LC-MS/MS method that is capable of multiplexing 13 allergens across a single LC-MS/MS run, thereby significantly increasing the potential to screen and differentiate more allergens than ever before in any technique which shows that LC-MS/MS is an effective method to identify and quantify food allergens in food matrices. The LC-MS/MS method that was developed in this study is being used under license. © 2015 AB SCIEX.

MATERIALS AND METHODS

Sample Preparation

Incurred bread and cookie samples were prepared by spiking 10, 50, 100 and 500 ppm each of the 13 allergens into 500 g of bread and 500 g of cookie matrices before baking. The sample matrices were divided and between four and six samples were run in each matrix for quality assurance. Homogenization, digestion with trypsin and filtration (Figure 4). The calibration curves of Protein 1 peptide 1 of hazelnut and peanut in cookie matrix. The calibration curves of Protein 1 peptide 1 of hazelnut and peanut in bread matrix. The calibration curves of Protein 1 peptide 1 of hazelnut and peanut in cookie matrix. The calibration curves of Protein 1 peptide 1 of hazelnut and peanut in bread matrix. The calibration curves of Protein 1 peptide 1 of hazelnut and peanut in cookie matrix. The calibration curves of Protein 1 peptide 1 of hazelnut and peanut in bread matrix.

CONCLUSIONS

From this study, we have developed a LC-MS/MS method is capable of multiplexing 13 allergens across a variety of different allergenic proteins including egg, milk, peanut, soy, and tree nuts. The methods are capable of detecting 1 ppm or less of allergens in bread and cookie matrices. This method is being used under license. © 2015 AB SCIEX.

REFERENCES


TRADEMARKS/LICENSING

For Research Use Only. Not for use in diagnostic procedures. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX is being used under license © 2015 AB SCIEX.