A cross-platform (PRM/SRM) targeted method for quantitation of peanut residues

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Introduction

- Correct labeling of **allergens in food** is a crucial part of preventing avoidable allergic reactions.
- The detection and quantitation of food allergens in food products is essential to enable allergen control and therefore adequate labeling.
- Targeted mass spectrometry has a nascent but growing role in confirmation of allergen detection using traditional methodology (primarily ELISA).
- In order to become widely-implemented, MS detection methods for allergens should be suitable for employment on a variety of instrument platforms.
- Here we describe a method using 3 peptide targets, initially developed for PRM use, but implemented using both high-resolution (PRM) and triple-quadrupole (SRM) workflows. We describe how different peptide targets have differing performance, and to what degree high-resolution methods may be transferrable to low-resolution platforms.

Goals

- Controlled **comparison of PRM and MRM** workflows using an existing targeted method for peanut detection.
- Identification of **peptide, transition and food matrix** dependent differences between PRM and MRM detection.

Methods

- Samples (raw and roasted peanut) were prepared as previously described (Marsh et al, 2020).
- Detection of peanut in raw and roasted peanut was performed using PRM (Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ MS coupled to Thermo Scientific™ UltiMate™ 3000 RSL liquid chromatography (UPLC) system.
- For SRM, a Thermo Scientific™ TSQ Altis™ triple-quadrupole MS coupled to a Thermo Scientific™ Vanquish™ Duo liquid chromatography (UHPLC) system was used.
- Both workflows employed a Thermo Scientific™ Hypersil Gold™ C18 1.9 µm, 100 x 1 mm reverse phase column.
- We previously identified suitable targets based on their robustness to roasting, and recovery from these matrices (see table below).
- Stable isotope labeled (SIL) peptides were used to derive molar peptide quantity. Data analysis was performed using Skyline.

Peptide	Protein of origin	Transitions measured
NLPQQCGLR	Ara h 2	y7+, y5+, y7++
QIVQNLR	Ara h 3	y5+, y4+, y3+
SPDIYNPQAGSLK	Ara h 3	y9+, y7+, b3+

Peanut peptide detection linearity – MRM and PRM

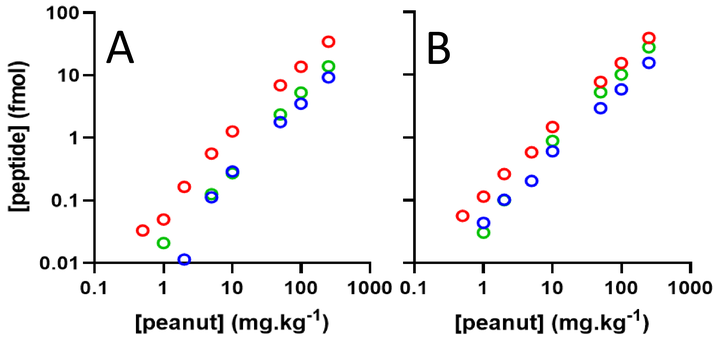

***Figure 1**. Detection of three peptides in peanut concentrations of 0.2 to 250 mg.kg^{-1} peanut extract using MRM (A) and PRM (B). Data show the sum of three transitions/peptide (light/heavy ratio).*

Red – NLPQQCGLR
Blue – QIVQNLR
Green - SPDIYNPQAGSLK

- Detection of our three target peanut peptides in extracts of raw peanut was broadly equivalent when using MRM or PRM.
- PRM allowed for maintenance of linearity at low concentrations of analyte, ultimately resulting in lowered limit of detection for the PRM assay.
- Detection of lower concentrations of peanut using MRM resulted in under-reporting of target peptide.
- Below, we further examine the loss of transitions at lower analyte concentrations

Loss of transition detection at lower analyte concentrations

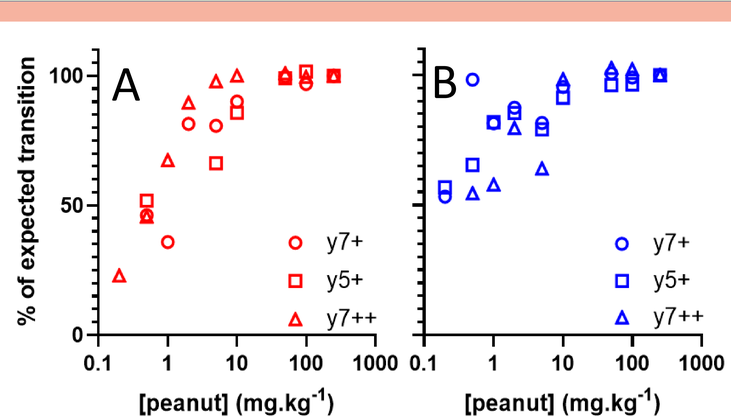

***Figure 2.** % of expected transition recovery of the peptide NLPQQCGLR (of that recovered at 250 mg.kg^{-1} peanut) at differing analyte concentration for MRM experiments (A) and PRM experiments (B)*

- Lower determined amounts of peanut at low analyte concentrations occurs through loss of individual transitions in both MRM and PRM, although MRM losses are greater.
- The magnitude of this effect is dependent on both parent and transition ion, and to some degree on thermal processing and presence of a food matrix (data not shown).

Discussion

- Here, we demonstrate analyte concentration dependent loss of transition intensity that is considerably more prevalent in MRM than in PRM. We suggest that this phenomenon may be due to interference from non-target ions in peanut.
- There are few examples of targeted method transfer between high-resolution and low-resolution platforms, and thus little understanding of how the capabilities of each platform affect allergen detection in food.
- Ronein et al (2015) showed similar performance characteristics comparing MRM and PRM performance for the quantitation of apolipoprotein A-I in human blood.
- The required utility of targeted measurements in many types of food matrices is a peculiarity of food analysis.
- Given our inability to predict the presence of potentially interfering ions from the food matrix, the higher discriminatory performance of high-resolution methods such as PRM may be an advantage.

Conclusions and Future Work

- Loss of transition ion intensity at low analyte concentration is potentially problematic for allergen detection in foods where such low-level quantitation is required.
- As more collaborative method trials emerge, we expect performance comparison of high- and low-resolution platforms to produce a clearer picture of the benefits of both workflows.

Acknowledgements

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Mass spectrometry was conducted on a Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer placed at UNL, and on a Thermo Scientific™ TSQ Altis™ triple-quadrupole MS at Thermo Scientific, San Jose, CA.

References

- Marsh J et al (2020). Food Chem. 313, 126019
- Ronsein GE et al (2015). J. Proteomics. 113, 388–399