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Validation study of a targeted LC-MS/MS method for the detection of milk and egg allergens in cake flour

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Summary

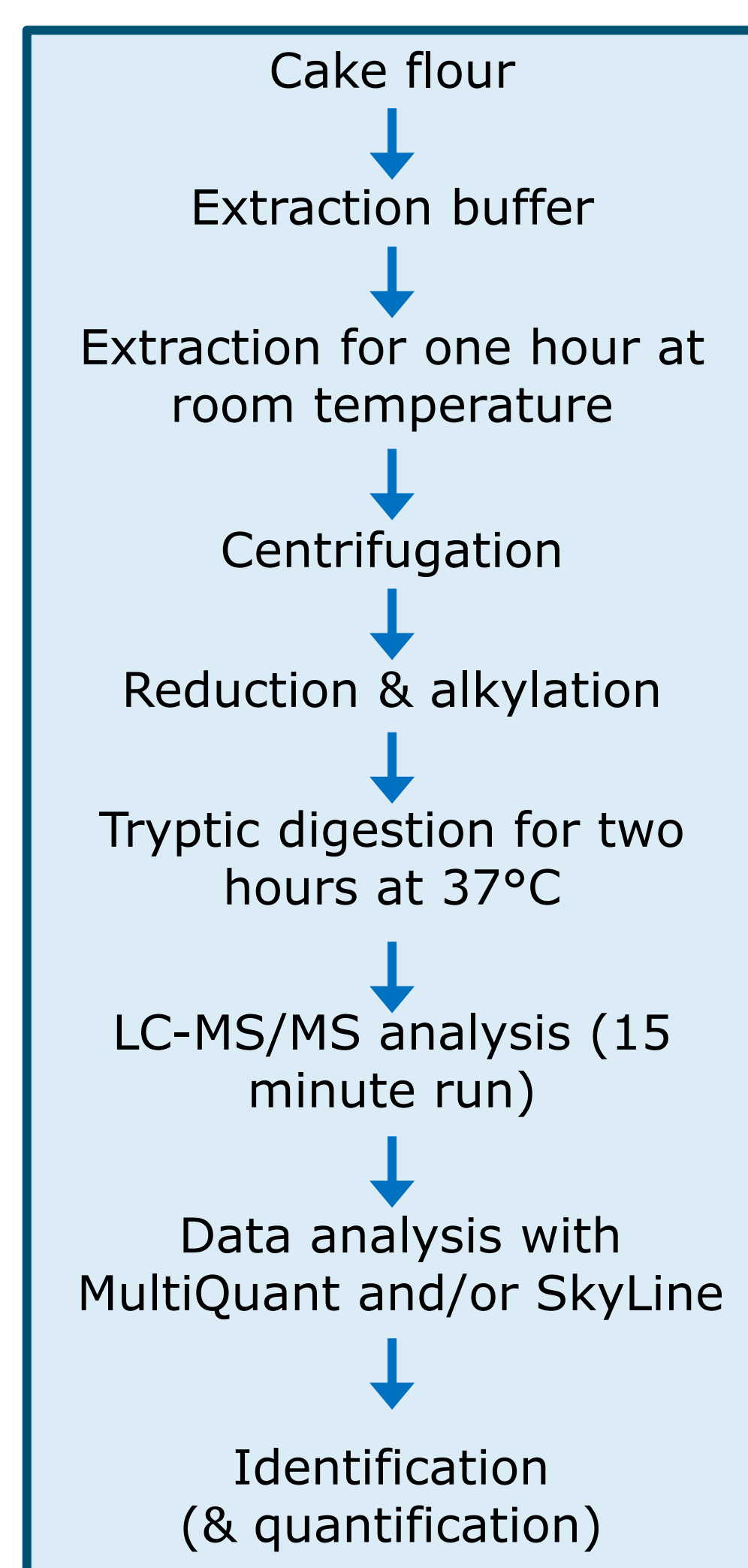
- Validation study of a targeted **LC-MS/MS method** to determine the **presence of milk and egg allergens** in **cake flour**
- Seven** different **cake flours** were spiked with milk and egg proteins at levels of **0, 5, 10 and 50 ppm** and analysed in triplicate
- Detection** of milk and egg proteins in six out of seven samples **at all spiked levels**
- Good reproducibility** at 10 and 50 ppm (CV <20%). At 5 ppm a higher variation is observed (CV 50-60%).

Introduction

Food allergies are generally estimated to affect 1-10% of the world population. Since 2003, legislation in the European Union prescribes labelling food products for the presence of 14 food allergen groups. It is expected that in the coming years also threshold levels will be prescribed. Consequently reliable, accurate and sensitive methods are needed to screen food products for the presence of allergens and assure of their absence when not included on the labelling.

Allergens are commonly detected by immunoassays. However, when positive samples lead to prosecution, additional information is needed to stand in court. Liquid chromatography tandem mass spectrometry (LC-MS/MS) can be used to confirm the presence of the (allergenic) protein in the suspected samples: it gives sequence information of the protein (the identity) and at the same time can be used to quantify the amount of the protein present.

Method¹



Experimental setup

- Seven** different blank **cake flours** from different retailers, including one gluten free flour, were spiked with **milk and egg proteins** at levels of 0, 5, 10 and 50 ppm.
- The experiments and measurements were carried out in **triplicate** on three different days by three different people.
- Targeted LC-MS/MS analysis** was carried out using a Waters Acquity system with a Phenomenex Core Shell column coupled to a QTRAP6500 mass spectrometer
- Data analysis** was performed using MultiQuant and SkyLine² software packages

General observations

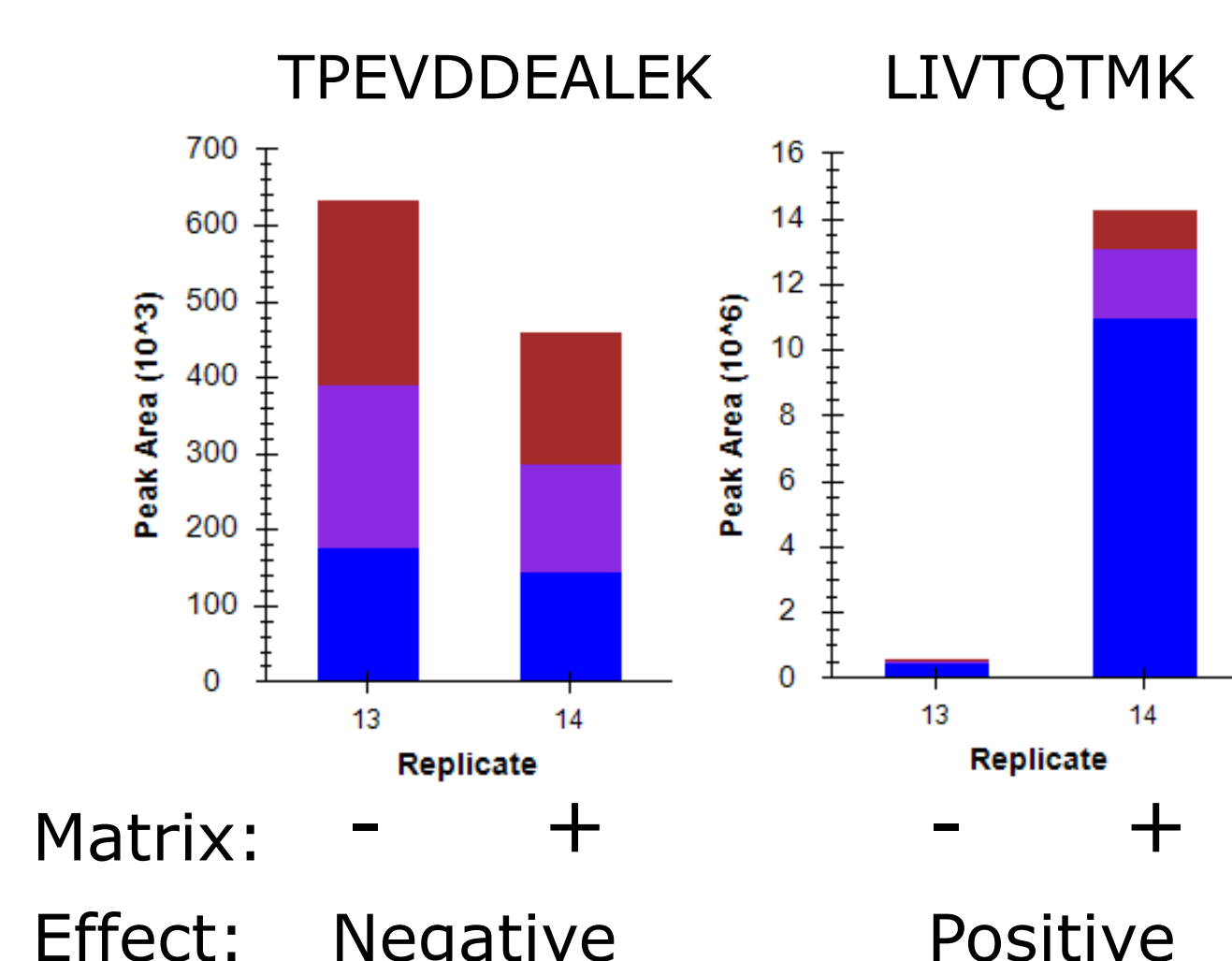
- Different (optimal) digestion time for ovalbumin and other four proteins: longer incubation favours detection of ovalbumin, but results in lower response or absence of peptides from other proteins
- Significant differences in response are observed for peptides from standards with and without matrix (see below)



Selected proteins and peptides for milk and egg allergens

Milk	Egg
<i>α-S1-casein</i>	<i>Lysozyme</i>
FFVAPFPEVFGK	FESNFNTQATNR
HQGLPQEVNLNLLR	HGLDNYR
<i>α-S2-casein</i>	<i>Ovalbumin</i>
NAVPIPTLNR	ELINSWVESQTNGIIR
FALPQYLK	LYAEER
	HIATNAVLFFGR
<i>β-lactoglobulin</i>	LTEWTSSNVMEER
TPEVDDEALEK	
LIVTQTMK	
VYVEELKPTPEGDLEILLQK	

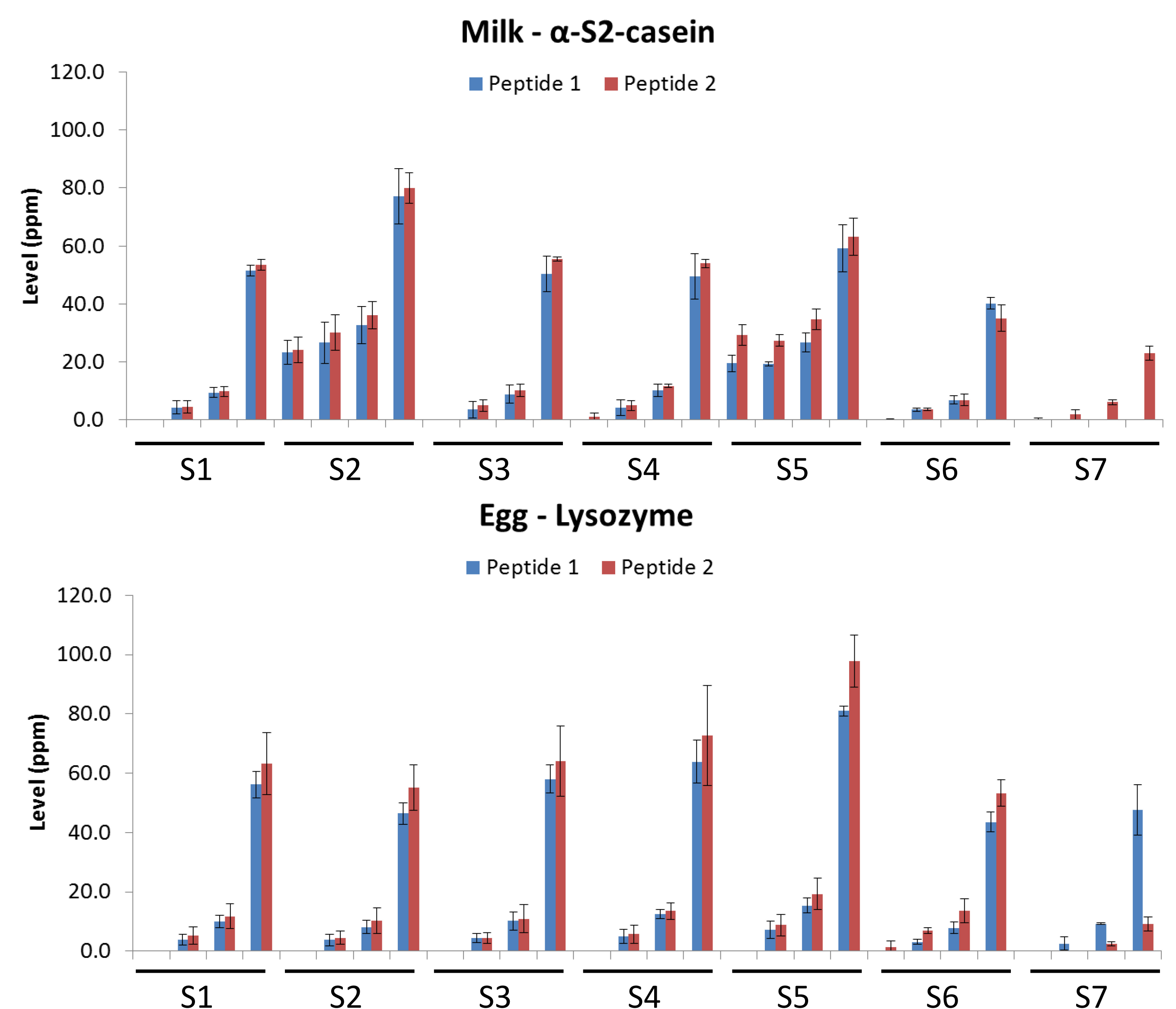
Example: Effect detection β-lactoglobulin peptides with and without matrix



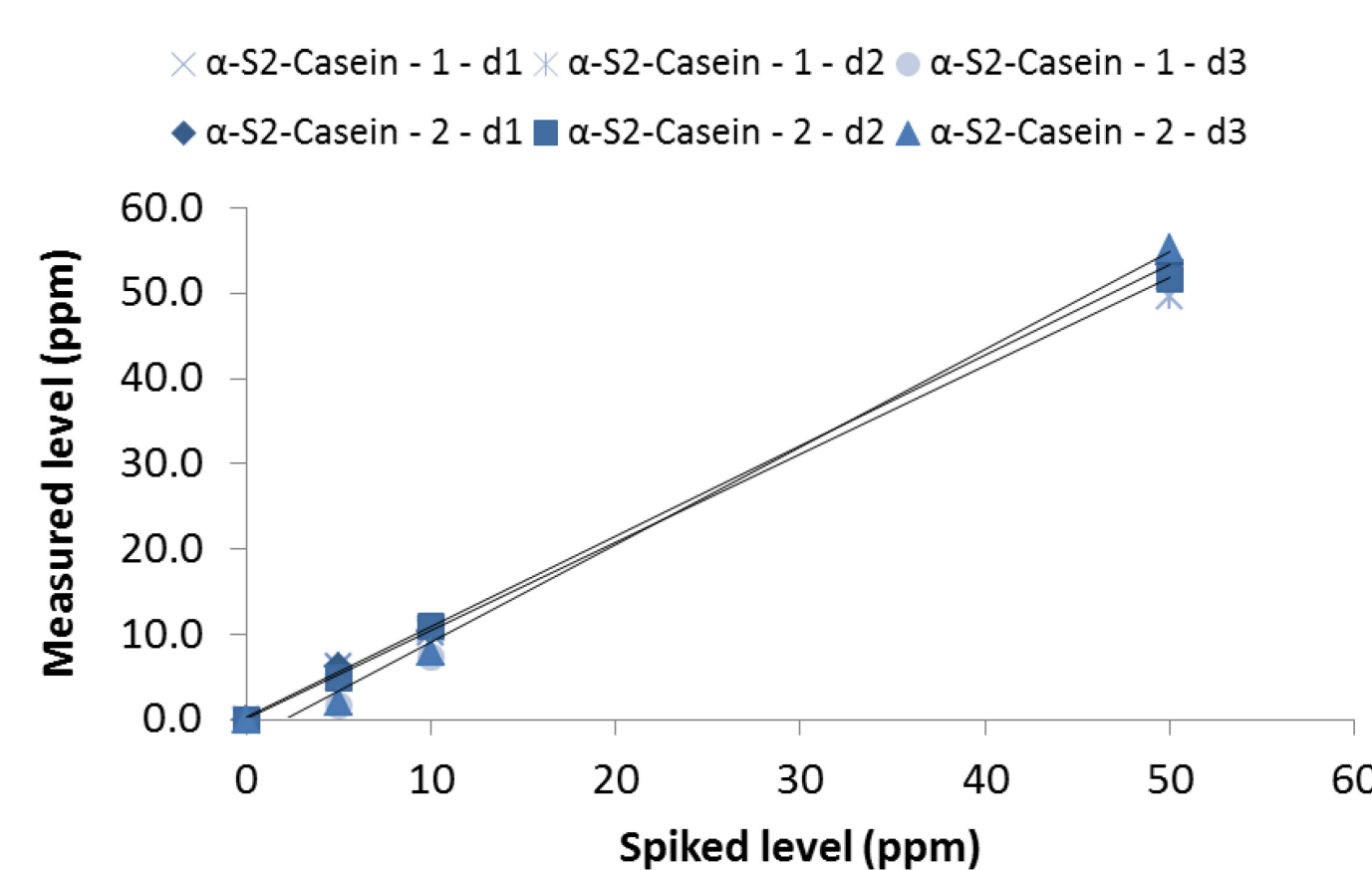
Detection of milk and egg in cake flour

- The variation in response *between* sample types is 30-67.5%
- Flours S2 and S5 seem to contain milk proteins that were not listed as an ingredient
- Flour S7 is a gluten free flour and this matrix has a great effect on the peak shapes and detected peptides levels

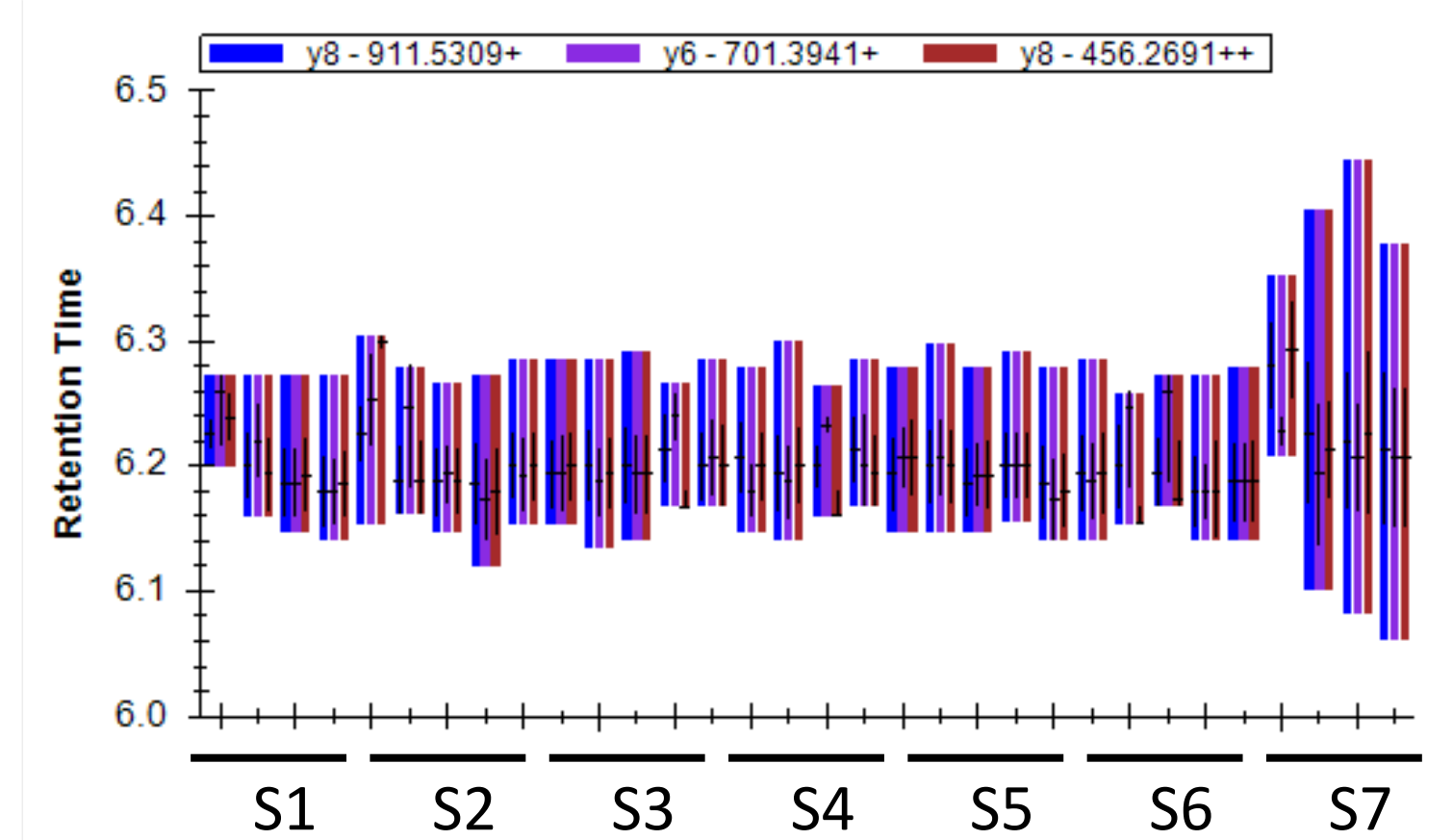
Detected levels of α-S2 casein (milk) and lysozyme (egg) in seven different cake flours spiked at 0, 5, 10 and 50 ppm



Linearity and reproducibility of detection of α-S2-casein at 0, 5, 10 and 50 ppm in cake flour S1



Stability of retention time of α-S2-casein peptide 1 (NAVPIPTLNR) in seven cake flours



Conclusions

- This validation study shows the possibility to confirm the presence of milk and egg allergens in cake flours using mass spectrometry
- Good reproducibility *within* one sample type at 10 and 50 ppm (CV < 20%)
- Variability at 5 ppm level *within* one sample type is 50-60%
- The variation in response *between* sample types is 30-67.5%
- Standard addition needed for quantification³
- Isotopically labelled proteins as internal standard would be favoured³
- Method can be used (after further optimization) for routine control to check and quantify the presence of allergens

References

- Method was adopted from iMethod developed by AB Sciex UK
- MacLean *et al.*, *Bioinformatics* **2010**, 26 (7), 966-968
- As concluded before by studies of AB Sciex UK and FDA



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