Absolute quantification of milk traces in food matrices using liquid chromatography combined with mass spectrometry

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Milk allergy is one of the most common food allergies in young children. As there is no cure for milk allergy, the only prevention is avoidance of milk consumption even in trace amounts. To protect allergenic consumers the availability of accurate and correct methods for the detection of food allergens is crucial for the food industry. For many allergens immunoaffinity assays are available and methods of choice for their detection. They are relatively fast and easy to use in routine analysis. However, the effectiveness of immunoassays strongly depends on the quality of the antigen used as target molecule and the quality of the antibodies directed against. Furthermore, food processing can modify these target proteins and affects their binding affinity to the related antibody leading to false negative or underestimated results.

Mass spectrometry, especially the targeted detection of selected molecules, known as selective or multi reaction monitoring, combines high selectivity with accurate quantification. To establish a confirmatory method for the quantification of milk traces in food, a method based on liquid chromatography combined with triple quadrupole mass spectrometry was used. Precise absolute quantification of this approach was achieved by the use of stable isotope-labelled peptide internal standards to compensate for variation in recovery and the influence of matrix effects. To this end absolute quantification multiple ion monitoring acquisition was used in combination with stable isotope dilution using synthetic peptides as external and internal quantitative standards. Two tryptic peptides represented in both, beta Lactoglobulin A and beta Lactoglobulin B, were selected as quantitative markers and synthesized containing N-terminal isotopically-coded amino acids (^{13}C)Arg or (^{13}C)Lys.

A simple sample preparation including reduction and alkylation of cysteine residues was established without any immunoaffinity enrichment step for infant formula matrix. Stable isotope labelled standard peptides were added to the extracted samples immediately following tryptic digestion. Instrumental parameters were empirically optimised in order to generate the most abundant precursor ions and y-ion fragments for maximum specificity and sensitivity. For the calibration curve the corresponding non-labelled synthetic peptides were used. Linear responses for beta Lactoglobulin (BLG) were obtained with fmol-level limits of quantification even in protein rich matrices like infant formula. Including FAPAS samples, obtained results were compared with ELISA directed against BLG. Values for LOD of ~0.6 BLG mg/kg and LOQ of ~5 mg BLG/kg infant formula indicate that the described LC-MS/MS approach can serve as confirmatory method for the determination of BLG.