



RIKILT

WAGENINGEN UR

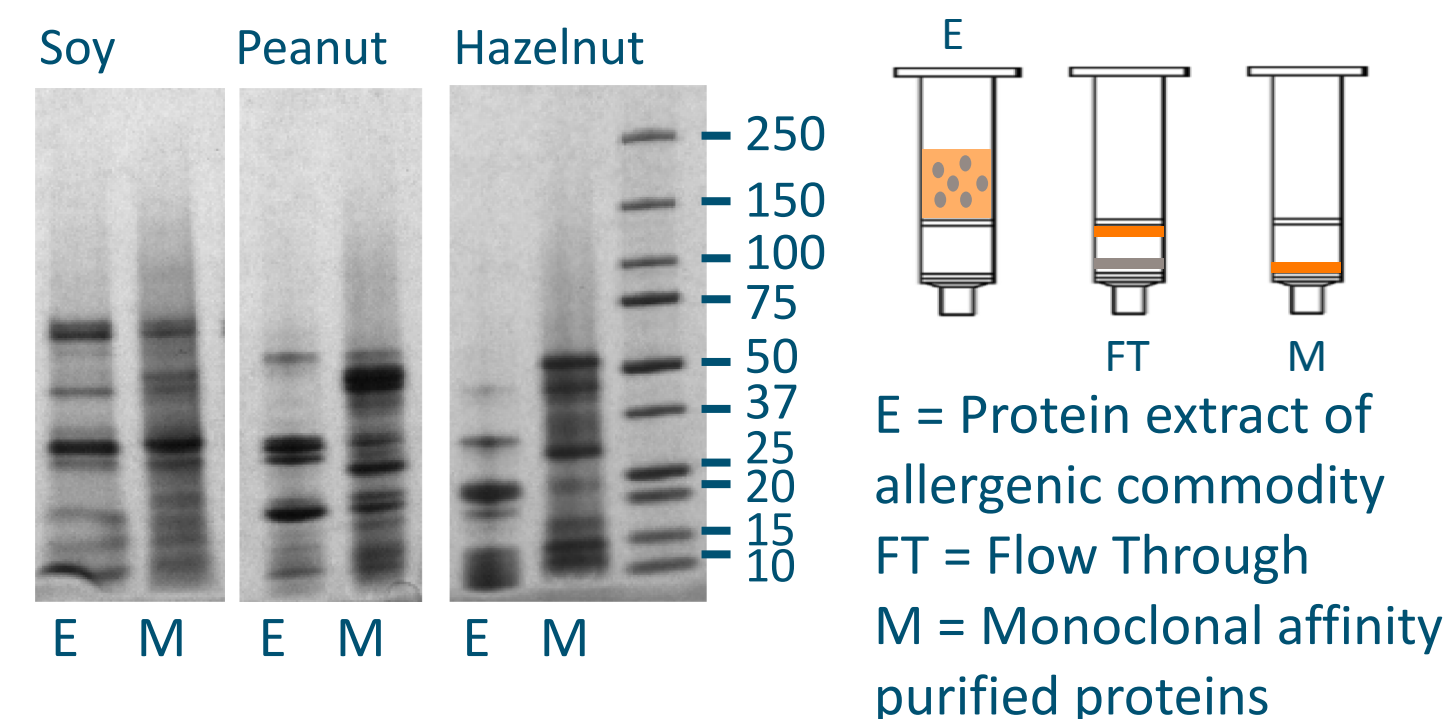
Protein analysis in food by mass spectrometry

An overview

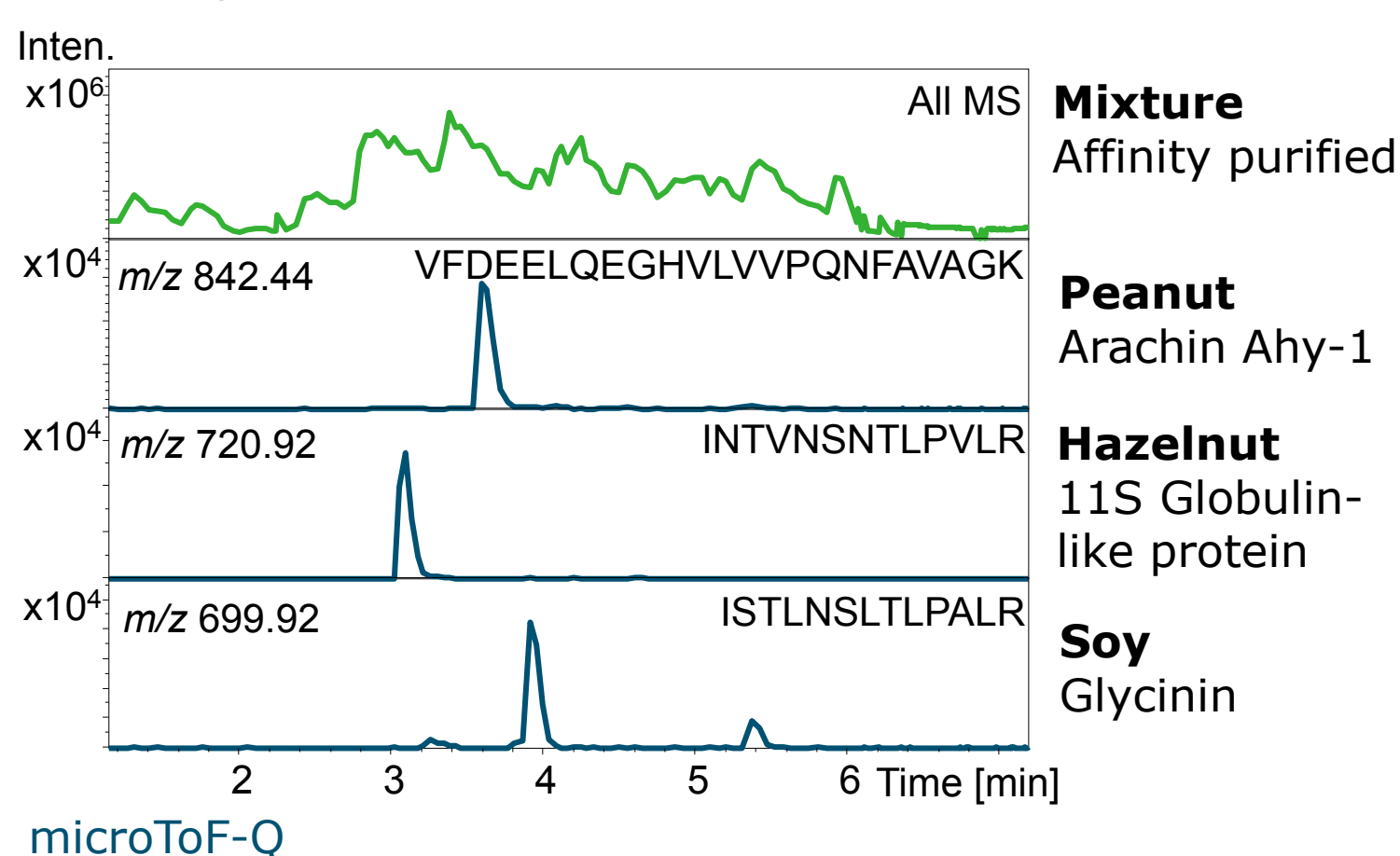
Merel A. Nessen, Dick Hooijerink, Monique G.E.G. Bremer, Vicky M. Manti, Marleen M. Voorhuijzen, Jeroen P. van Dijk, Klaas L. Wubs, Marco H. Blokland, Saskia S. Sterk

Immunoaffinity purification

Enrichment Monoclonal Antibodies (MAB) columns



LC-MS/MS of Combined MAB column



The presence of allergens in food can cause severe problems for allergic people. Immunobased screening methods are available, but confirmation of the detected allergens is still needed. A multiplex immunobased LC-MS/MS method directed against peanut, hazelnut and soy is under development.

In a pilot study the enrichment of proteins by immunoaffinity purification and identification by LC-MS/MS is shown.

Aim

- Development of multiplex (targeted) MS method using immunoaffinity purification

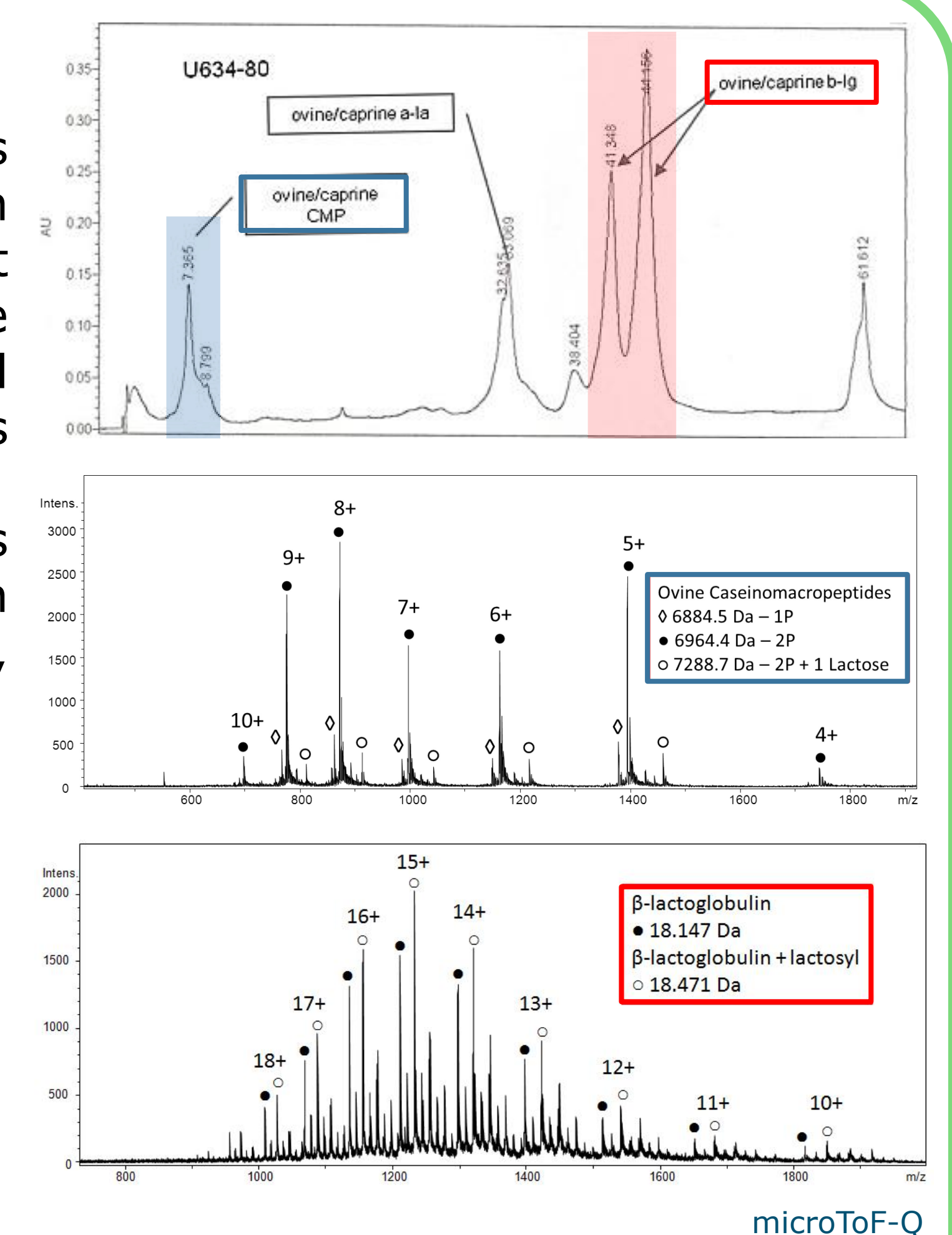
Allergens

Milk serum proteins

Whey powders and concentrates are by-products with a high nutritional value and great potential. They have shown to have functional properties and antimicrobial activity, which is related to the protein composition. For industrial applications it is important to have information on the individual protein composition, glycation and phenotypes.

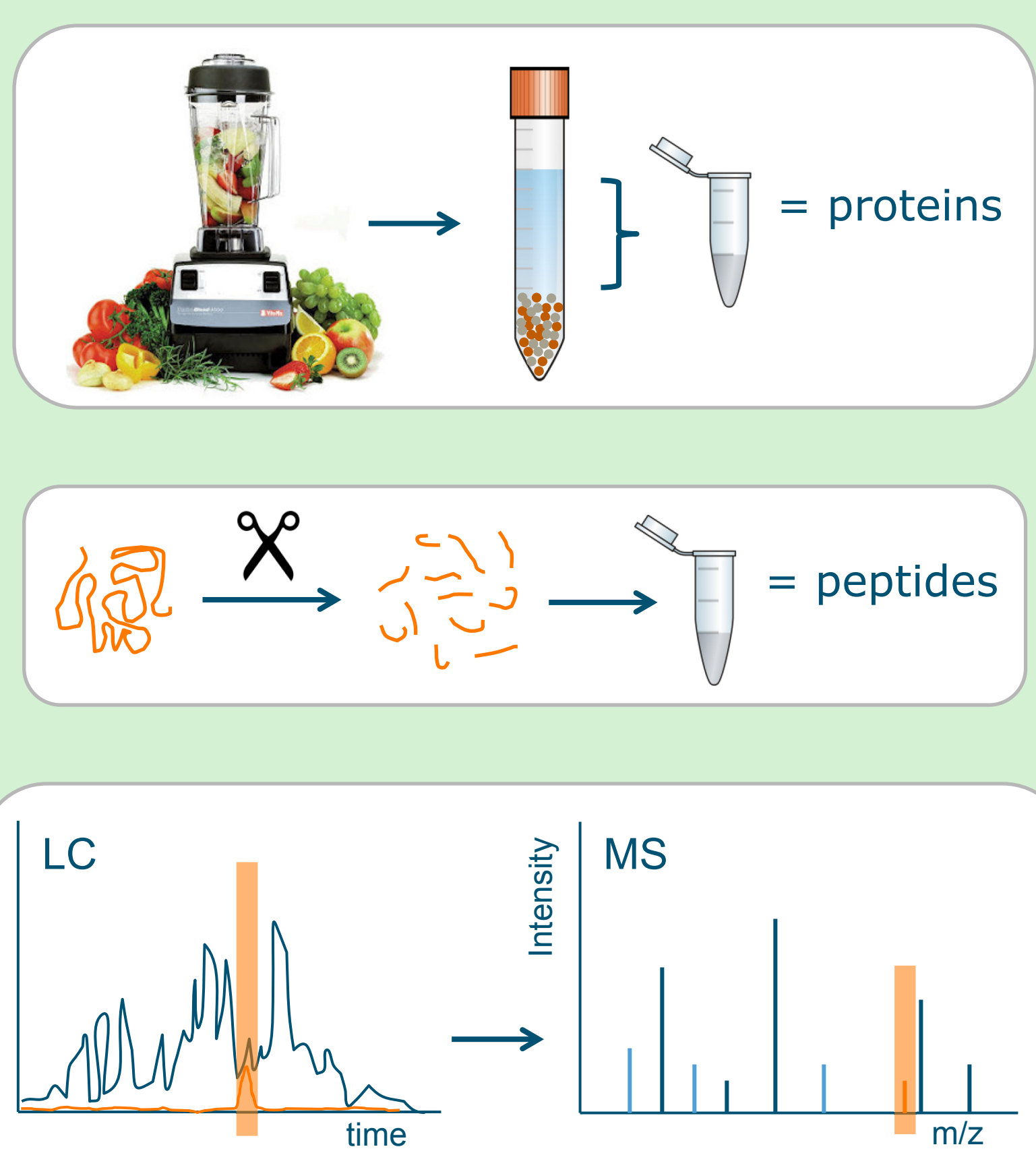
Aim

- Identification of the caseinomacropetides, α -lactalbumin, β -lactoglobulin in caprine and ovine milk
- Identification of the degree of lactosylation and phosphorylation

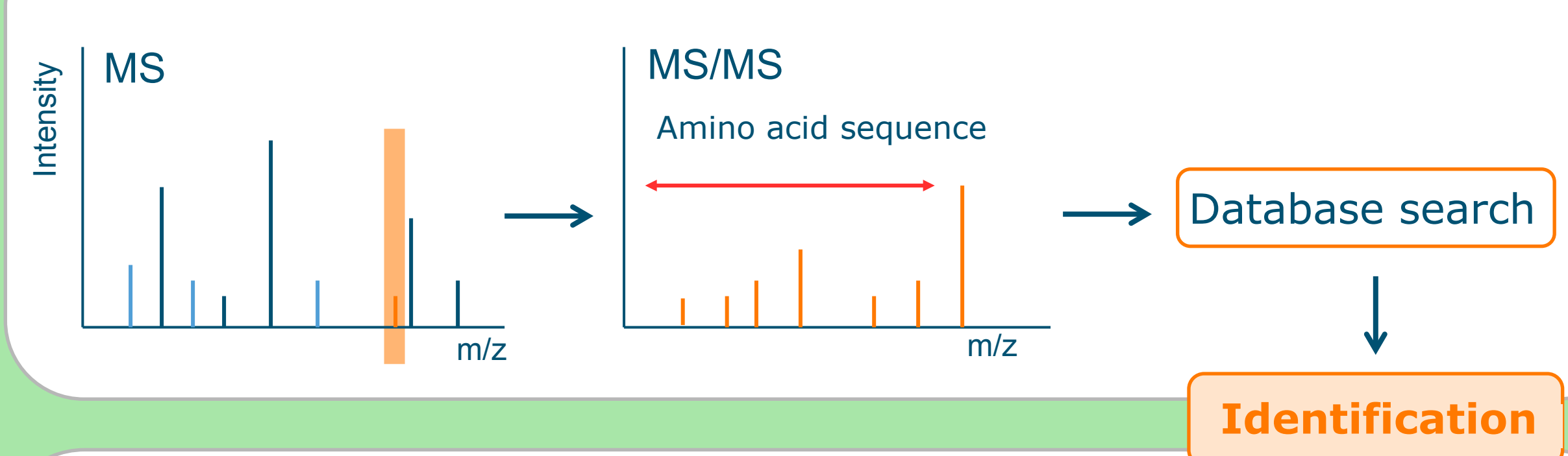


General approach MS detection of proteins

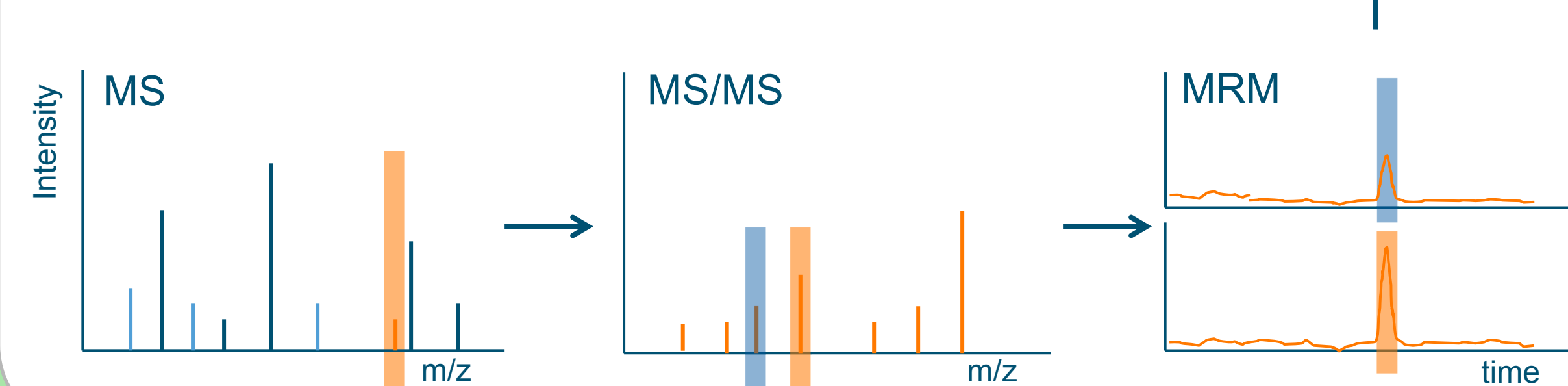
Food/feed
protein extraction
fractionation
(enzymatic) digestion
fractionation
(C18) clean-up
LC-MS/MS detection
identification and/or quantification



Untargeted MS/MS analysis



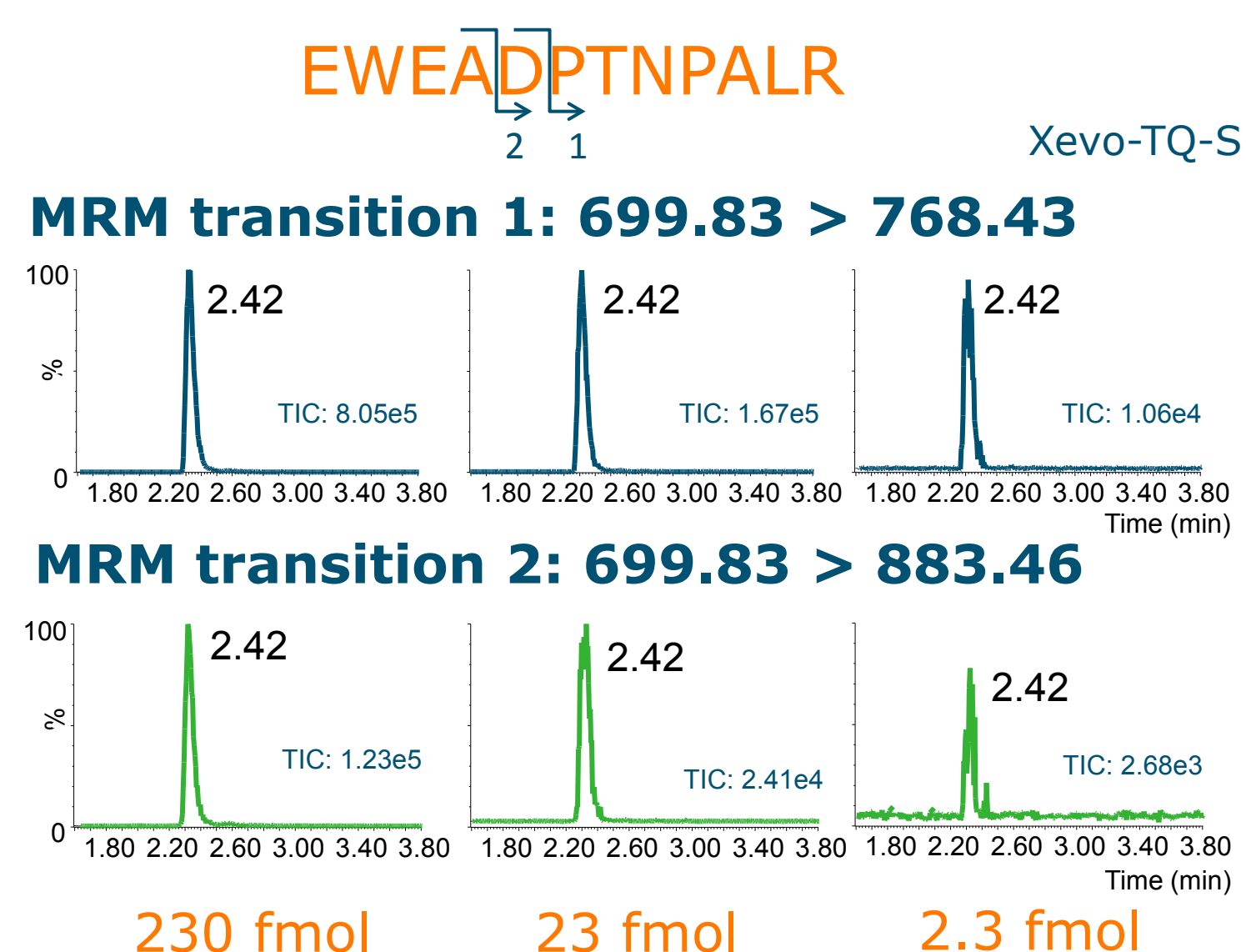
Targeted MS/MS analysis



MS analysis Cry proteins

- Cry1Ab (Mon810): 818 amino acids
- Selection of 4 unique tryptic peptides for Cry1Ab (& Cry1Ac)

Example



- No Cry1Ab from maize yet
- Improve sample preparation by inclusion of strong anion exchange

(Unauthorized) GMOs

Cry proteins derived from *Bacillus thuringiensis* are one of the most commonly used group of toxins in insect-resistant transgenic crops. Presence of GM crops in bulk or in food products can be detected by (1) screening using ELISA kits directed against proteins, or (2) DNA techniques, confirming presence of specific (known) GM crops. However, food processing might influence the detection. In addition unknown GM events will be missed.

Aim

- Development of (targeted) MS based method to detect Cry protein in GM maize

Growth hormone

A recombinant version of the growth hormone somatotropin (rbST) is administrated to cow cattle to increase milk production in US, Central and South Americas. However, it's usage is forbidden in EU, Canada, Australia, New-Zealand and Japan and resistance to its usage is growing in US.

A Biosensor Immunoassay has been developed to screen for (r)bST in serum and milk.^{1,2} Still a confirmation method is needed.

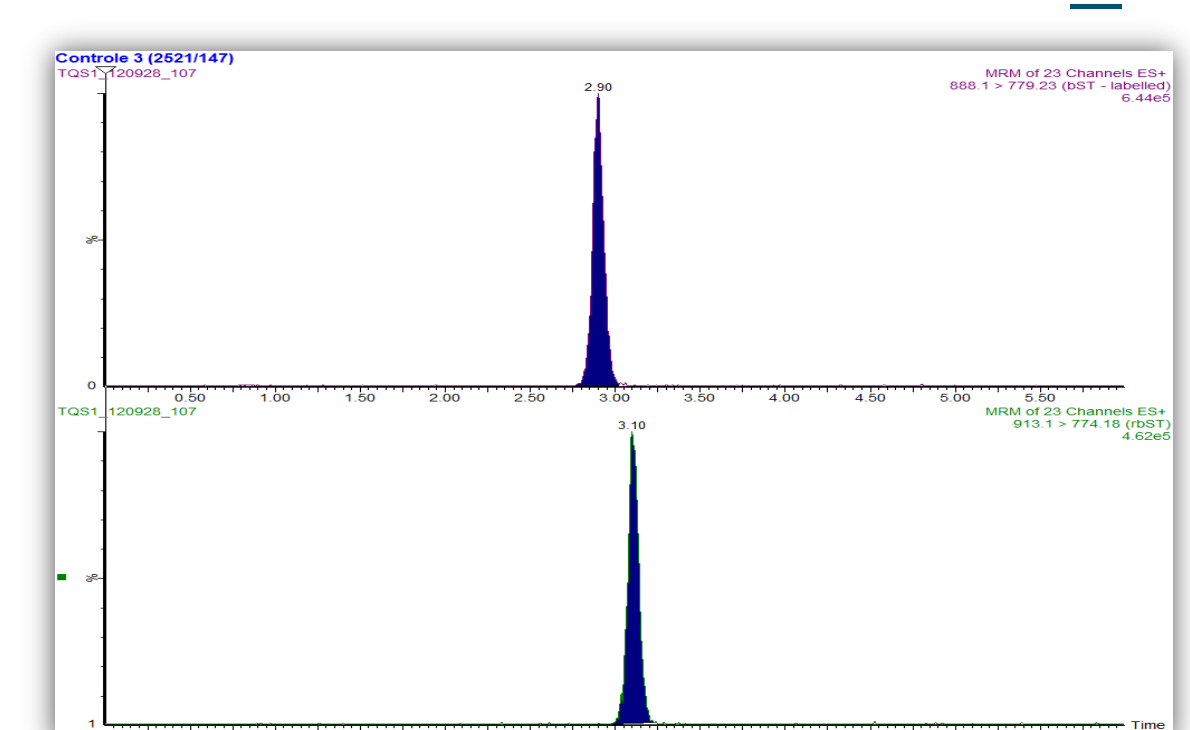
Aim

- Development of confirmation method for rbST based on LC-MS/MS

Approach

- Difference in N-terminal amino acid between bST and rbST leads to retention time shift and mass difference in LC-MS/MS

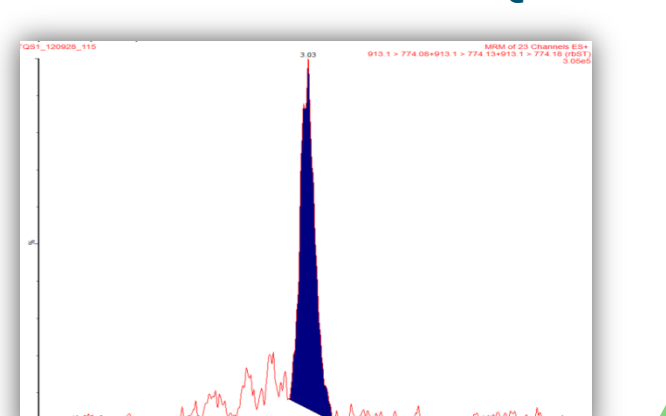
bST: **A**FPAMSLSGLFANAVLR



rbST: **M**FPAMSLSGLFANAVLR

Example

- Confirmation of presence of rbST to 50 ng/mL in serum



RIKILT Wageningen UR
P.O. Box 230, 6700 AE Wageningen, The Netherlands
Contact: merel.nessen@wur.nl
T + 31 (0)317 48 02 81
www.rikilt.wur.nl

This research was funded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation

References

- ¹ Smits, NGE, et al., *Analyst*, accepted
- ² Ludwig, SKJ, et al., *Food Control* **2012**, 26, 68-72