Tackling matrix effects of feed extracts for the detection of ruminant by-products by targeted MS

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Introduction

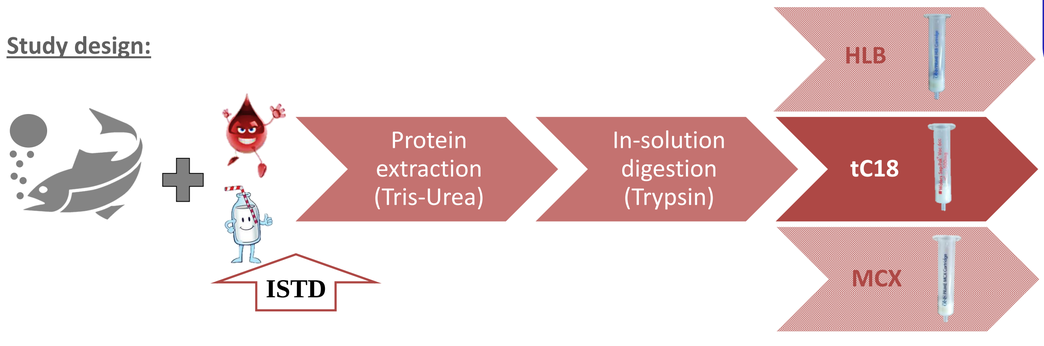
Context: Animal by-products are an interesting source of feed materials. However, since the BSE ("mad cow disease") crisis, their use has been strictly regulated. Official controls are based on a combination of light microscopy and PCR but sometimes these methods are unable to distinguish unauthorised and authorised materials. **UHPLC-MS/MS** method was therefore developed to address the analytical gaps [1]. This method has the advantage to be species and tissue specific.

Objective: The current sample preparation provides a fast, simple and powerful method suitable for routine. But an evaluation of the method on a large number of samples in order to assess its applicability has highlighted that some feeds have major effects on peptide signal (**ion suppression**).
The subject of this study was to evaluate other types of **SPE cartridge** in order to optimise the **purification step regarding the matrix effects**.

Materials & study design

Materials:
- ✓ Feed matrices: 2 aquafeeds = AQF01 = AQF02
- ✓ Adulterant (level): Ruminant blood meal (1 %) & bovine milk powder (0.1 %)
- ✓ Internal Standard (ISTD): AAVTAFWGK* (*Lysine $^{13}C_6$ $^{15}N_2$)

Study design:



Method

Biomarkers:
- ✓ 4 peptides for ruminant haemoglobin detection [1]
- ✓ 6 peptides for milk protein (casein or beta-lactoglobulin) detection [1]

Sample preparation protocol:
- ✓ Extraction: 200mM TRIS-HCl pH 9.2, 2M Urea
- ✓ Reduction (200 mM DTT) and alkylation (400 mM IAA)
- ✓ Digestion: trypsin
- ✓ Purification: Sep-Pak tC18, Oasis PRiME HLB or Oasis PRIME MCX cartridges (Waters)

UHPLC-MS/MS:
- ✓ Waters Acquity system (C18 BEH Column; 2.1 x 100 mm)
- ✓ Waters Xevo TQ-S micro

Targeted approach MRM mode

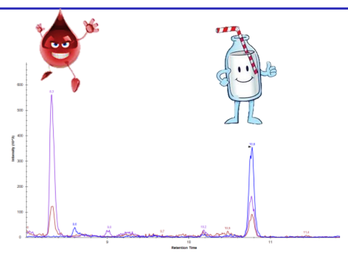


Figure 1: Proteins extracted from adulterated samples were digested. Extracts were then purified by using one type of SPE cartridge and analysed by LC-MS/MS for the detection of bovine haemoglobin, bovine casein and bovine beta-lactoglobulin peptides.

Results

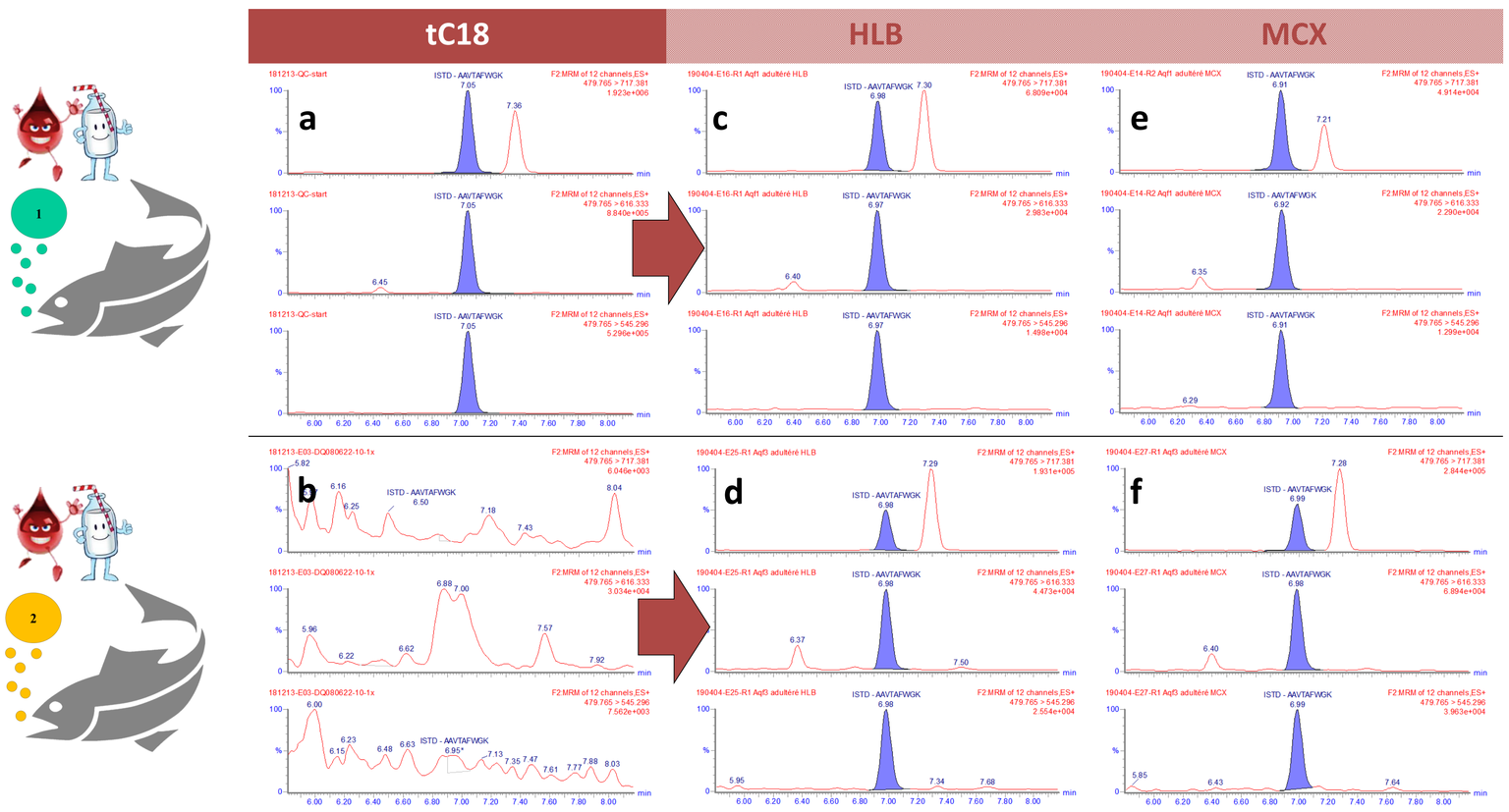


Figure 2: (a & b) give the chromatograms of the three MRM transitions of the internal standard (AAVTAFWGK*) in adulterated AQF01 & AQF02 after tC18 purification. Ion suppression is observed in AQF02 for all transitions and the same result is observed for all peptide biomarkers (10/10). With HLB (c & d) or MCX (e & f) SPE, peaks are observed for all transitions of AAVTAFWGK* in both AQFs.

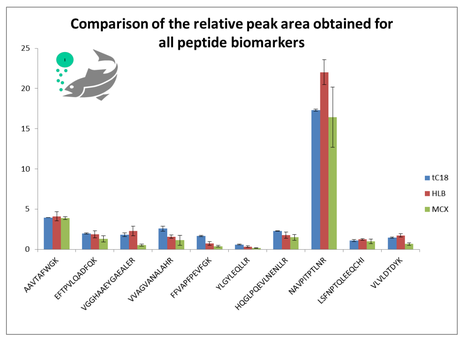


Figure 3: The comparison of the relative peak area obtained in AQF01 for the different type of SPE shows that tC18 and HLB give better results than MCX.

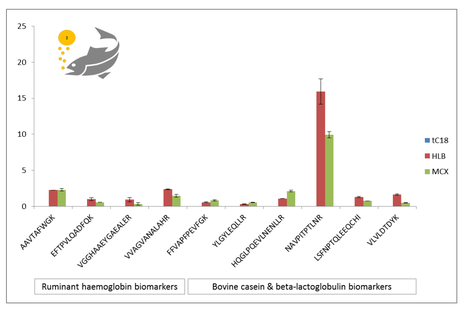


Figure 4: In AQF02, no signal is observed for all peptide biomarkers by using tC18. The comparison of relative peak area for the other cartridges reveals that HLB gives better results in most case, and for haemoglobin peptides in particular.

Conclusion and perspectives

Matrix effect is a major concern in liquid chromatography mass spectrometry (LC-MS/MS). Ion suppression was observed in AQF02 by using the validated method with tC18 SPE cartridge. In order to try to eliminate or minimise this effect, other SPE cartridges were tested. This preliminary test shows that, in case of signal suppression, Oasis PRiME HLB cartridge could be a good alternative to purify the samples. Other samples have to be tested in order to confirm this observation.

References

[1] Lecrenier, M. C. et al. (2018). A mass spectrometry method for sensitive, specific and simultaneous detection of bovine blood meal, blood products and milk products in compound feed, Food Chem. 245 (Suppl. C) (2018) 981–988.