Targeted proteomics method comparison: SRM, PRM and SWATH-MS to quantify proteins in bovine muscle tissues

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Background: Targeted proteomics has become an approach of choice to validate and precisely/absolutely quantify protein biomarkers. Besides selected reaction monitoring (SRM), alternative methods have emerged over the last few years, among which parallel reaction monitoring (PRM) and sequential windowed acquisition of all theoretical spectral (SWATH-MS). These approaches perform differently in terms of instrumentation, multiplexing, and performances such as sensitivity, selectivity and accuracy (Borràs and Sabidó, 2017). A careful consideration of these points is required to select the best-suited targeted proteomics method.

In this study, we have evaluated and benchmarked three targeted methods to precisely quantify beef tenderness biomarkers in muscle tissues. Meat tenderness and lipid content (marbling) guide customer choice. Controlling these quality criterions by relying on protein biomarkers presents a high agronomical and economical interest (Picard *et al.*, 2015; Ceciliani *et al*., 2017).

Methods: Twenty peptides, corresponding to ten previously identified protein biomarkers of beef adiposity and tenderness, were quantified in two muscles, *Longissimus thoracis* and *Rectus abdominis*, using a SRM assay on a triple quadrupole mass spectrometer (MS) (TSQ Vantage, Thermo Fisher Scientific), a PRM assay on a quadrupole-Orbitrap MS (Q Exactive Plus, Thermo Fisher Scientific) and a SWATH-MS method on a quadrupole-time-of-flight MS (TripleTOF 6600, Sciex). Analyses were performed on a cohort of 64 samples of bovine muscles, covering a wide dynamic range of adiposity and tenderness. Limits of quantification for each peptide were determined by establishing isotope-dilution calibration curves.

Results and Conclusions: We showed that (i) PRM performs better than SRM and SWATH-MS regarding sensitivity, (ii) SRM and SWATH-MS exhibit similar – and enhanced while comparing to PRM – dynamic range performance, and (iii) PRM and SRM selectivity is better than SWATH-MS one for the low-concentrated peptides. We also highlighted the advantage of PRM and DIA-SWATH over SRM for post-acquisition data refinement.

Keywords: Quantitative Proteomics, Selected Reaction Monitoring, Parallel Reaction Monitoring, Data-Independent Analysis.