QUANTITATIVE PROTEOMICS for clinical diagnostics

Maria Colombo & Véronique Mainfroid detail how accurate peptide quantification supports the transfer of MS-based quantitative proteomics to the clinic

Despite the identification and validation of numerous potential biomarkers for medical diagnostics, only a few of them actually progressed towards clinical use.

ELISA remains the gold standard to quantify proteins, however it is often limited because of the lack of availability of antibodies with high specificity and sensitivity.

The development of more direct detection methods aims at solving this intrinsic issue linked with immunoassays. Indeed, over the past decade, mass spectrometry (MS)-based quantitative proteomics has gained considerable interest[1], as it is the most promising technology for allowing amino acid-based biomarker quantification. As such, multiple reaction monitoring (MRM) is considered today as a major technology for accurate quantitative proteomics[2].

Absolute biomarker quantification

The development of strategies based on stable isotope-labelled standards makes it possible to use MRM for absolute biomarker quantification: the quantity of the protein biomarker is determined by comparing the MS signal generated by a tryptic peptide of the biomarker with the signal from a peptide equivalent, heavy-isotope labelled and spiked into the sample. Moreover, it is well adapted to multiplexing and to high-throughput assays. MRM uses the strategy based on stable isotope-labelled standards for absolute quantification.

MS-based quantitative proteomics

Because quantitative proteomics relies on the ability to detect small changes in protein abundance, all steps of the MRM process must be under tight control, including instrumentation, sample preparation and biomarker analysis and together, their imprecision cannot exceed the biomarker variability in test samples[3,4]. Accordingly, major technological improvements were done at each step of the MRM process.

High-resolution instruments are continuously developed, providing the reliability and precision required for the detection of low abundance biomarkers, further pushing the limits of detection and quantification of protein biomarkers in complex samples. Fig. 1. shows the MS signals obtained for two 15-mers peptides selected from proteins sharing more than 95% identity, peptides differing from 1 amino-acid used for protein variant analysis.

Sample preparation also deserves particular attention, because biomarkers may be extremely diluted, while the sample matrix may significantly interfere with biomarker detection. Depletion, fractionation and improved liquid chromatography (LC) technologies are therefore continuously evolving.

Isotope-labelled peptides

Stable isotope-labelled peptides keep a central position. The quantification of protein biomarkers using MRM is indeed only achievable if the peptides spiked into the sample perfectly match the endogenous biomarker equivalent. While very complex peptides can be

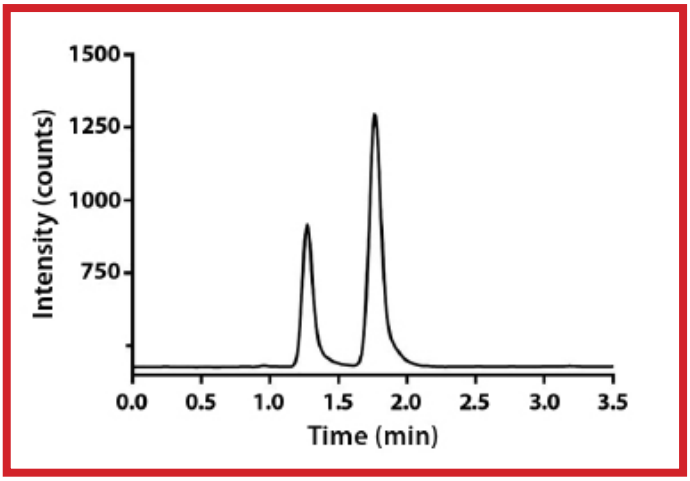

Fig. 1. UPLC MS-MS signal of 15-mer peptides which sequences present one amino-acid variant. Peptides selected from protein showing more than 95% homology

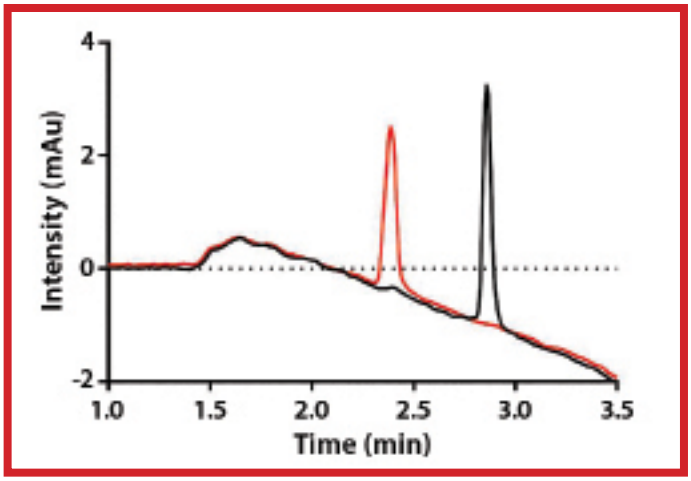

Fig. 2. UPLC signal of tagged-peptides (black line) and after tryptic digestion (red line)

synthesised by expert chemists, the limitation remains on how accurately they can be quantified, as they are intended to be used as internal calibrators. Amino acid analysis (AAA), which is the traditional peptide quantification method, offers limited reproducibility and precision. As such, the seminal publication from M. Louwagie et al.[5], which describes a rapid, sensitive and reliable quantification method for peptides and proteins, offers an extremely important and promising achievement in the peptide field.

This method, AAA-MS, avoids using derivatisation and chromatographic separation of amino acids, and is based on a rapid microwave-assisted acidic hydrolysis followed by high-resolution MS analysis of amino acids (ESI-based). This technology proved to be 100-fold more sensitive than the classical AAA.

Tagged peptides

Alternative quantification can be made using tagged peptides. In this case the absolute quantification is based on the inherent spectral properties of the proprietary tag. The Quant-Tag is coupled to the C-term of the peptide via an Arginine (R) or Lysine (K) residue and can be released by trypsin digestion.

The precise molecular mass of the tag can be used in assessing the cleavage efficacy, and hence in setting the optimal trypsinisation conditions of a sample using UPLC-MS-MS methods. To do so, the tagged peptide must be spiked into the sample prior to trypsin digestion.

Fig. 2. shows the UPLC signals obtained with a tagged peptide before and after cleavage. The Quant-Tag could be used as a reporter of the trypsin digestion efficacy: a peak corresponding to the tag mass is indicative of trypsin digestion.

Accurate peptide quantification

With these technologies, fully calibrated peptide standards can now be offered, highly improving the global analytical performance of MRM. Fig. 3. shows the similarity of signal obtained with the two different quantifications methods using tag spectral properties of mass spectroscopy amino-acid analysis.

It therefore appears that we do have everything in hand to achieve the goal of transposing MRM into clinics, offering hope that MS-based protein biomarker detection and quantification will become effective in routine assays in a very near future.

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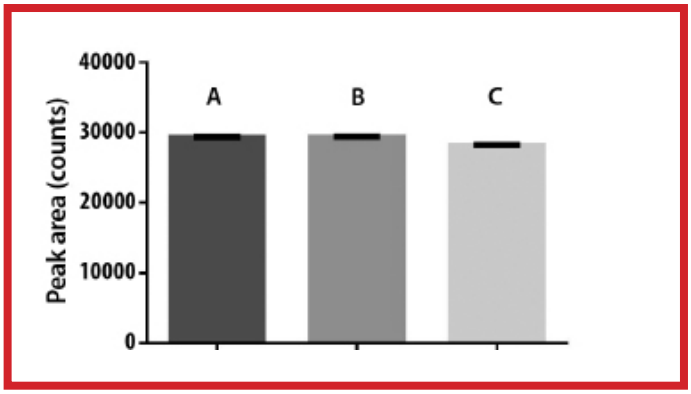

Fig. 3. UPLC-MS-MS signals comparison from peptide quantity obtained after tryptic digestion of tagged-peptide (spectral quantification) (A); non-tagged control peptide (AAA-MS quantification) that has followed the tryptic digestion process (B) and non-tagged control peptide (C)

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