Analysis of proteins from biological matrices using affinity based sample clean-up and LC-MS/MS

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Targeted protein determination using LC-MS/MS after a typical bottom-up pretreatment results in high specificity and the possibility to differentiate between different (iso)forms in one single step. This offers a more reliable alternative to traditional immuno-metric assays.
Determination of low abundance proteins (i.e biomarkers) in biological matrices by LC-MS/MS requires efficient sample clean-up. This can be achieved with affinity extractions based on conventional antibodies as well as synthetic antibodies. Immunoaffinity sample clean-up using monoclonal antibodies *targeting the protein* of interest has been demonstrated to enable biomarker determination in the low picomolar range[1,2] (protein extractions). In recent years affinity based sample clean-up techniques *targeting a proteotypic peptide* both using conventional antibodies (peptide extractions/epitope fishing) and synthetic antibodies (molecularly imprinted polymers) have been introduced[3,4].
The aim of the present paper is to give an overview of affinity based sample clean-up techniques targeting proteins and/or their proteotypic peptides and their use in determination of low abundance proteins in biological matrices using LC-MS/MS. The differences and similarities between the techniques will be highlighted through examples.
In conclusion, each approach has its own distinct advantages and limitations which are important to be aware of: The main advantage of protein affinity enrichment being the possibility to get isoform information of the whole protein using a single antibody, while the main advantage of peptide enrichment is a better clean-up and a more appropriate use of stable isotope labelled peptide standards for quantitation.

References:

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