Combined ESI and ICP-MS approach for the quantification of bio-molecules using natural element tags

Daniel Pröfrock and Andreas Prange

GKSS Research Centre, Institute for Coastal Research, Marine Bioanalytical Chemistry, Max Planck Str. 1, 21502 Geesthacht, Germany
daniel.proefrock@gkss.de

The quantification of proteins or peptides as well as their post translational modified counterparts represents an ongoing challenge within the field of bio- analysis. The utilization of ICP-MS for the determination of covalently bound (hetero)elements, which are naturally present in nearly all proteins, or which have been introduced via chemical reactions, represents an emerging strategy within the field of absolute protein or peptide quantification.
Reversed phase LC is the method of choice for the separation of peptides. Unfortunately even at low flow rates, as used in nano or capillary-LC, changes in the elemental response with changing gradient composition due to carbon related effects on the ionization behavior of an element can be observed, which complicates accurate quantification.
Capillary LC hyphenated to ICP-MS has been used for the element specific detection of the separated peptides. The developed instrumental setup utilizes a matched reversed gradient sheath flow, which is mixed post column with the flow of the RP column. Due to the mixing of both gradients a stable elemental response over the whole chromatographic separation has been achieved, which is an essential pre-requisite for the application of ICP-MS for quantification of phosphorylated peptides via their hetero(atom) content, especially when no matched calibration standards are available or in general when utilizing mono isotopic element tags for quantification.
In addition capillary LC-ESI-QTRAP-MS has been used to identify and to elucidate the tag stoichiometry of the separated peptides.
In comparison to other techniques the developed instrumental setup helps to maintain a constant elemental response during the whole chromatographic separation and therefore eliminates gradient related effects. For the separation of a model peptide retention time and peak area RSDs of 0.05% and 7.6% respectively have been obtained (n=6). Detection limits for phosphorus of 6.24 µg L^{-1} which corresponds to 6.24 pg P have been realized. Simple inorganic phosphorus standards have been used for the quantification of either model peptides or tryptic protein digests. The expected as well as the quantified values were in good agreement resulting in calculate recoveries of 93 % (tryptic digests) or 102 % (model peptides) indicating the potential of the proposed setup for quantitative peptide analysis.
This approach helps to overcome the problems related with the application of mono-isotopic element-tags and ICP-MS for bio-molecule quantification.