

Absolute and Relative Protein Quantification with the Use of Isotopically Labeled p-Hydroxymercuribenzoic Acid and Complementary MALDI- and ICP-MS Detection

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Chemical labelling with subsequent mass spectrometric detection represents a common approach for protein quantification. Whereas most methods make use of stable isotope labels from naturally elements like ^2D , ^{13}C , ^{15}N , or ^{18}O , artificially introduced metals have gained interest as alternative markers. This work presents the application of p-hydroxymercuribenzoic acid (pHMB) as labelling reagent for cysteine-containing proteins. As proof of concept, insulin was chosen as model protein and two different workflows were employed to its absolute and relative quantification with the use of complementary MALDI-MS and ICP-MS. Based on the synthesis of isotopically labelled ^{199}Hg -pHMB, and thus, on the label-specific isotope dilution concept, a differential labelling procedure can be either applied to the comparative study of two different samples (relative quantification) or to the absolute quantification of insulin. In both cases, final detection by MALDI-MS followed by isotope pattern deconvolution was applied to extract the quantitative data from the mass spectra. Good agreement with the expected values was obtained for the relative insulin quantification, and the recovery for insulin applying the absolute quantification workflow was between 90 and 110 % with a RSD better than 5 % in the fmol and pmol range.