

HETEROATOM-TAGGED PROTEOMICS VIA ICP-MS.

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Many known proteins contain metals in their structures. Those elements can be present as the prosthetic group of conjugated proteins (like metalloenzymes) whose function depends on the presence of the metal ion on their structure or just forming stable macromolecular complexes for their storage and/or transportation through living systems. The characterization of such structures is of extraordinary importance to assess the biological role of trace elements in living systems from bioinorganic and clinical points of view. For this purpose, specific heteroatom tagged investigations (using trace element speciation) can provide valuable information that is clearly complementary to classical molecule-selective detection.

Using Fe as elemental target, this presentation will illustrate some examples on the development of quantitative strategies to determine clinical biomarkers related to chronic alcohol abuse, hemochromatosis and long-term monitoring of diabetes mellitus. Thus, the first part of the talk will deal with the development and validation of an alternative method for the determination via HPLC-ICP-MS of carbohydrate deficient transferrin sialoforms (CDTs) by means of Fe stable isotopes. In the second part, using the combination of Fe isotope dilution and isotope pattern deconvolution methods, the accurate determination of serum iron, unsaturated iron binding capacity and transferrin saturation will be addressed simultaneously.

The last part will be devoted to the determination of glycated Hb (GHb), which is a post-translational non-enzymatic modification of haemoglobin (Hb). The amount of GHb depends on the average concentration of blood glucose over the previous 120 days, the normal life of the red blood cell. Therefore, the measurement of GHb is used in diabetic patients for monitoring mid to long-term glycemic control and for evaluating possible risks in their development of diabetes. Thus, the separation of GHb from the non-glycated form is conducted by using a cation exchange column (Mono S 5/50 GL) and a gradient of ammonium acetate (0-250 mM, pH 5.7). The detection of GHb was carried out simultaneously by VIS at 415 nm (specific of heme group) and by ICP-MS for specific monitoring of iron. The use of post-column addition of a solution of isotopically labelled iron (^{57}Fe , 95%) in 25 mM sodium citrate/citric acid (pH =4) will allow the quantification of GHb and total Hb, separately, in hemolysates.