Metallo protein quantitation by LC-ICP-MS

In LC-MS/MS in recent years there has been a shift from small molecules to peptide and protein quantitation. Also for (LC-)ICP-MS such a shift is set in motion. Accurate determination of concentrations of metalloproteins and metal ions bonded to proteins -as direct indication for the effectiveness of pharmaceutical interventions- is rapidly replacing analyses of proxy indicators for disease progression or stabilization. Direct analyses for instance instead of total element determination from which the measured protein concentrations, obtained by other analytical technique, are subtracted. The chromatographic analyses, requirements and results obtained on two metalloprotein systems, iron bound to transferrin and copper bound to ceruloplasmin, will be presented as examples.

Iron bound to transferrin, of interest amongst others to assess the effectiveness of iron replacement in iron deficient patients, requires separation of iron bound to transferrin from the iron supplemental medication and other iron bonding proteins such as albumin. Often not only the amount of iron bound to transferrin is requested, but also the total amount of iron that can be bound to transferrin – the total iron binding capacity. Unless high concentrations are present and total iron binding is not requested, free iron is not a concern for this assay.

Wilson disease is a metabolic disorder in which copper is insufficiently bound to ceruloplasmin leading to severe chronic effects early in live. The key parameter in both diagnosis and tracking of disease progression is the amount of non-ceruloplasmin bound copper. The “golden standard” to determine this is by measuring the total amount of copper and measuring the concentration of ceruloplasmin (with a fixed number of copper atoms per molecule of ceruloplasmin) to determine the amount of non-ceruloplasmin bound bopper. Multiple approaches, based on chromatography or ultra-filtration that were implemented will be presented which are used to determine one or more of the relevant copper concentrations. The pros and cons of the methods will be shared as well.