Analysis of Intact Proteins by HILIC-MS

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Recent developments in separation science and mass spectrometry are now allowing to extend LC-MS methods to the analysis of large proteins (typically up to 50 kDa and in some over 150 kDa) in their intact form, avoiding digestion steps. Such analysis enables to monitor the distribution of proteins in functional proteoforms such as phosphorylation, acetylation, and glycosylation.

Glycosylation is particularly important as post-translational modification as a large portion of the mammalian protein is glycosylated and about one-third of the approved biopharmaceuticals are glycoproteins. The attachment of glycans to a protein is known to affect the tertiary structure and physiochemical properties of the protein, such as protein stability, solubility, and folding. For biopharmaceuticals and biotechnological products, changes in the glycosylation pattern can, therefore, result in a change of the activity or stability of the product.

Currently, the typical workflow for intact-protein analysis involves reversed-phase LC (RPLC)-MS. However, RPLC offers limited to no selectivity towards glycosylation making the MS analysis of proteins having a high degree of diversity in their glycosylation complicated and in some case hindering the detection of low abundance proteoforms due to ion suppression and dynamic range of the measurement.

Here we discuss strategies to enable the analysis of proteins and protein glycoforms using HILIC-MS. By selecting the appropriate injection conditions, gradient slope, eluent additives and ion-source conditions, highly efficient protein separations can be achieved while maintaining good MS performance. We also describe a capillary HILIC-MS method to allow detection of low abundant proteins.

Examples of characterization of model proteins as well as biotechnological products and protein samples from clinical samples will be discussed.

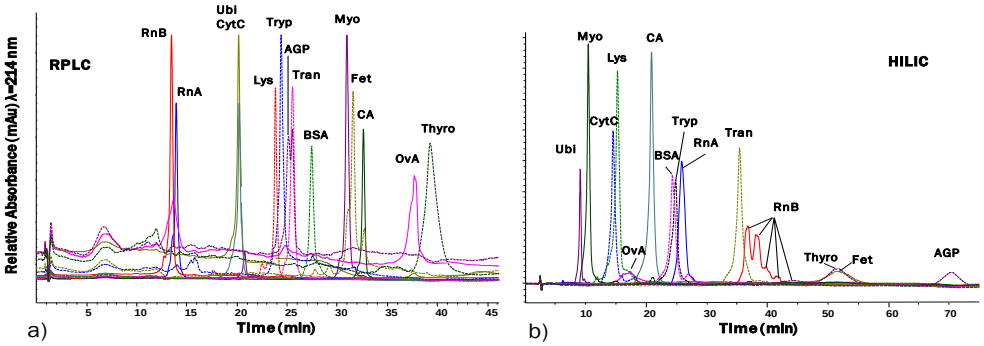

Figure 1. Comparison of the different selectivity of RPLC and HILIC for proteins: overlay of the chromatograms from the injection of protein standards on a RPLC (a) and HILIC columns (b) using the same mobile phases but opposite gradient slopes.