Abstract (English)

A large part of biopharmaceuticals currently on the marked consist of recombinantly expressed proteins, thoroughly purified for obtained a highly pure and stable product. The aim of the purification is to remove, among others, host-derived biological material, such as DNA and proteins. Host cell proteins (HCPs) are of special analytical interest, as they are able to induce undesired immune responses and lower the stability of the target protein. The allowed HCP amounts vary depending on the type of protein being expressed, however it is required to thoroughly monitor the HCP content. No guidelines exist for determination of HCP amount, but antibody-based strategies such as enzyme linked immunosorbent assay (ELISA) is currently the gold standard.

Advanced liquid chromatography systems combined with mass spectrometry (LC-MS) has, in recent years, shown great potential as a complementary method for HCP characterization. Besides not requiring expensive and time-consuming antibodies, LC-MS allows for discrimination of individual HCPs, making it possible to study the intrinsic HCP composition and dynamics. The biggest hurdle in LC-MS-based HCP studies is the vast dynamic range, arising from the presence of low-abundant proteins in a matrix consisting of a single, high-abundant protein. The most effective method to overcome this hurdle is to use advanced chromatographic strategies, such as multidimensional chromatography and systems with a high loadability.

The goal of the presented Ph.D. project was to develop a general and robust method for identification and quantification of HCPs, using LC-MS. For evaluation of the work, we used a biopharmaceutical protein provided by Statens Serum Institut (SSI, DK). The HCP content of this product has not previously been studied by MS, but has been thoroughly benchmarked by ELISA, making the sample optimal for validation. We evaluated different high performance liquid chromatography (HPLC) columns and several pre-fractionation strategies. Furthermore we developed a method for rapid and robust identification and quantification of HCPs, using a combination of data dependent acquisition (DDA), data independent acquisition (DIA) and label-free quantification. The obtained results allowed us to determine the purity of the protein, which was in accordance with previously obtained ELISA data. We developed a protocol for stabilization and purification of crude trypsin, yielding a heat-stable and specific product, useful for digestion of large amounts of sample in proteomics studies, applicable for HCP- and PTM-characterization, with low costs.