Mass Spectrometric Evaluation of Host Cell Protein Patterns in Biopharmaceutical Products

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Developing and producing recombinant biopharmaceuticals in mammalian cells requires unambiguous monitoring of **HCPs impurities**. Immunoassays are still the method of choice for release testing, for which it is recommended to demonstrate the suitability of antisera in a QM-regulated environment to meet regulatory demands. However, peptide analysis by MS has been proven to be a valuable tool by providing complementary data for HCP characterization to identify and quantify HCP. In particular, only little is published about identities of HCP present in marketed biopharmaceutical products.

Here, we evaluated identities of HCP in marketed recombinant IgG products and quantified individual HCP, which are suspected to be critical for the product stability and quality.
For shotgun MS samples were reduced, carbamidomethylated and proteolytically cleaved using trypsin. **LC-ESI-MS** measurements were applied using an UltiMate 3000 RSLCnano coupled to a QExactive HF-X Orbitrap MS. Proteins were identified and relatively quantified using MS Amanda and Proteome Discoverer software.

First, we analyzed HCP in Drug Product (DP) of different market approved mAb products. Detection of HCP with low abundance is still critical and the risk of false positive detection exists. Additional validation of detected HCP peptides in DS by using heavy isotope synthetic peptides is crucial.

Stable Isotope-labeled standard peptides were synthesized and spiked into the sample with a known concentration. The PRM approach allows for validation of the presence of individual HCP with high confidence and determination of the absolute protein amount. The absolute amount of the specific HCP was determined using the labeled peptides.