Quantitative LC-MS/MS for Host Cell Protein Analysis in Biopharmaceutical Development and Production

Introduction

Production of biopharmaceutical drugs depends on cultivation or fermentation of appropriate host organisms. The use of genetically modified host organisms inherently leads to the presence of process-related impurities like DNA, lipids or proteins. Host Cell Proteins (HCPs) can negatively affect function and stability of the product or have adverse effects on the patient. Therefore, to ensure safety and efficacy of the final drug product, it is highly important to remove these impurities.

Host cell proteins (HCP)

Being required by the host cell for proliferation, gene transcription, protein expression etc., any endogenous protein can be part of the highly heterogenous class of host cell proteins. Enzymes like proteases can degrade the therapeutic product, potentially impacting efficacy, stability, potency and shelf life. Other proteins can cause unwanted side effects or trigger an immune response in patients.

Thus, even in trace amounts, host cell proteins can substantially impact the quality of the therapeutic product, making their removal critical for market approval.

Regulatory demands

Regulatory agencies demand identification, quantification and risk analysis of all residual host cell derived contaminations during the drug approval process. Even for approved drugs, HCPs need to be routinely monitored and quantified in every production batch of a drug to confirm quality and safety of the product.

In the past years, expectations of regulatory agencies have increased, demanding for more elaborate analyses for detecting and controlling process-related impurities.
Guidance on HCP control is provided by the International Council for Harmonization (ICH), the U.S. Food and Drug Administration, the United States Pharmacopeia (USP) and the European Pharmacopeia.

However, though these regulatory standards provide information on appropriate analysis methods, they do not provide information on acceptable HCP levels as the associated risk depends on multiple factors (dosage, route of administration, frequency of use, etc.) and needs to be established individually for each new drug substance.

Best practices for HCP analysis in biopharmaceutical production

Currently, the best way to address regulatory demands would be a combination of ELISA and LC-MS/MS.

For many years, enzyme-linked immunosorbent assays (ELISA) have been the gold standard for HCP monitoring in biopharmaceuticals because of their high sensitivity, specificity and their high throughput and automation capability. However, ELISA comes with limitations at it strongly depends on the performance of the antibodies. Some proteins may not be detected due to poor immunoreactivity or weak antibody-antigen binding. Total HCP levels can be quickly determined during the production process and in the purified drug substance but it is not possible to determine abundance of individual proteins. Thus, even if the total levels of HCP decrease, the abundance of critical HCPs may remain high. Also, development, re-production and validation of a new ELISA is a time-consuming process.

By addressing these issues, mass spectrometry (MS) has emerged in the past years as orthogonal method. Able to identify thousands of individual proteins with a single measurement, MS provides a more in-depth analysis of HCPs with high sensitivity. This allows unbiased protein detection at every step of the production process. Supported by reference standards for selected proteins, MS facilitates quantification of critical HCPs while keeping track of the overall presence of HCPs.

Taken together, implementing a robust HCP assay as early as possible is highly important to avoid delays in later development stages.

Controlling HCP abundance during production

Working with biological systems is associated with a high degree of variability and manufacturers can influence the abundance and expression profile of HCPs already before starting the purification process by adjusting cell culture density, temperature, duration of expression etc.

After lysis of the cells, HCPs are released with the biopharmaceutical drug product and need to be removed during the purification process. Manufacturers have numerous options to influence removal of HCPs e.g. buffer selection, pH, temperature. Thus, exact identification and quantification of HCPs already at early stages of the drug development process is key to allowing focussed optimization of the production process and to ensure consistent quality of the product.

After finalizing the production process, routine quality control procedures need to be established to maintain the validation of the product. This requires highly sensitive and specific analytical methods that are able to detect changes in the abundance of specific HCPs even at very low concentrations.

HCP analysis by quantitative LC-MS/MS

Mass spectrometry enables different approaches to HCP analysis. Untargeted MS measurements using either DIA (data independent acquisition) or DDA (data dependent acquisition) allow highly sensitive identification of all proteins present in a sample (given the sequence information of the organism) and are

perfectly suited for analyses at early development stages or in absence of a suitable ELISA.

Targeted MS measurement like multiple reaction monitoring (MRM) enables highly sensitive and specific detection of pre-selected proteins and allows for quantitative monitoring of critical HCPs at all stages of the production process. However, to avoid overlooking unexpected HCPs, it is advisable to additionally perform data acquisition in either DDA or DIA mode.

HCPs can be quantified relative to the main protein or in absolute amounts.
Relative quantification is easy to implement and does not depend on qualified reference materials. However, as the amount of the product protein is very high compared to and the low amounts of HCPs, the concentration of HCPs in the sample could be outside of the linear range of the measurement method. Thus, relative quantification may be suitable for HCP measurements at early development stages but may be insufficient for quality control of the final product.
Alternatively, HCPs can be quantified relative to a labelled or unlabelled exogenous protein that is spiked in the sample at a known concentration prior to enzymatic digestion.
Absolute protein quantification facilitates highly specific measurement of HCP levels even at low amounts. However, this method relies on external reference standards. These standards can be stable isotope labelled (SIL) versions of known HCPs that are spiked in the sample at known concentrations. Though SIL proteins are the gold standard of reference proteins, production is time consuming and costly. Synthesized SIL peptides are an alternative option. These peptides are usually added at known concentrations after enzymatic digest. To ensure reliable quantification, it is recommended to use at least three peptides for each target HCP. Depending on the number of target HCP, this may require a lot of pipetting work preparing complex peptide mixes.
QconCAT reference standards combine the advantages of complete proteins with individual peptides. By concatenating selected signature peptides into a synthetic protein, QconCATs can be co-digested with the analyte sample, releasing SIL peptides in a strict 1:1 stoichiometry for absolute quantification of pre-selected critical HCPs. Being able to comprise of up to 50 peptides, this enables the quantification of dozens of proteins with a single reference standard.

Whatever protein standard one decides for, each standard must be validated by characterisation of purity, concentration, detection limits etc. Guidelines for method setup can be found in the recently published USP General Chapter <1132.1>.

HCP Analyses by PolyQuant

Despite all the advantages, MS has to offer in HCP analysis, it is not easy to implement and requires substantial investments for setting up a MS facility and training the personnel.

PolyQuant's established workflows combine tools from proteomics, bioinformatics and our proprietary QconCAT technology for standard analyses or development of customized assays for HCP monitoring during drug development and production processes.

Our QconCAT reference standards facilitate simultaneous absolute quantification of

dozens of HCPs in a single experiment and can be custom-made for any production organism. For common production organisms we offer ready-made standards for quantifying common and high-risk HCPs.

Our HCP analysis services usually start with unbiased protein detection using high resolution LC-MS/MS. After risk analysis of the detected proteins, custom assays for targeted measurement and quantification of common and risk-related HCPs are developed for routine assays. Thus, HCPs can be controlled already at a very early time point in product development.

Simultaneously, our technique allows monitoring of other quality parameters of protein products, like sequence integrity and post-translational or chemical modifications. During the course of product development, our protocol can be easily customized to also allow for absolute quantification of uncommon HCPs and observed protein modifications. All developed assays can be validated according to GMP guidelines.

Example workflow for HCP quantification employing QconCAT reference standards