Application note

Quality control of recombinant proteins and medical products using LC-MS/MS – Quantification of Host Cell Proteins

Introduction

Therapeutically relevant biomolecules are mostly produced recombinantly in an appropriate expression system.

Before approving a biologic or proteinaceous drug compound, regulatory agencies demand that all residual host cell proteins (HCP) are identified and quantified.

This is of high importance as HCPs could be even at low levels potentially harmful or allergenic, causing unwanted side effects or reduced stability of the product.

The most commonly used method in pharmaceutical industry currently is ELISA. However, this method often relies on polyclonal antibodies directed against the most common HCPs or against several proteins at once. The detection and quantification of known HCPs is highly dependent on the quality of the antibodies and gives no detailed information, which proteins have been detected.

In contrast to ELISA, our LC-MS/MS technology supports both exact identification of formerly unknown custom-specific HCPs and absolute quantification of common HCPs. The technology is suitable for every production organism, as well as cell-free production and can be applied to complex matrices. With our standardized assay, HCPs can be controlled already at a very early time point in product development. Our custom-made isotope labelled standards enable us to set up GMP conform routine measurements for any proteinaceous contaminant.

Simultaneously, our technique allows to monitor other quality parameters of protein products, like sequence integrity of 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.

Workflow

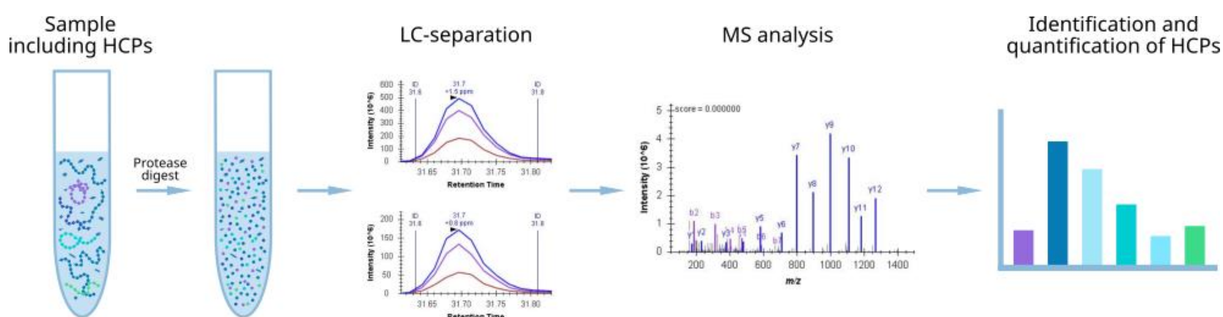

Fig. 1 Illustration of the workflow for HCP analysis using LC-MS/MS. A sample containing HCP contaminations is digested with an appropriate protease. After LC separation the peptides are detected using high resolution mass spectrometry.

In this application note we demonstrate on three examples how LC-MS/MS can be used in quality control of purified proteins.

Example 1:

Early detection of impurities of a recombinant protein

Production of a highly expressing protein was induced in bacteria (*E.coli*). The cells were lysed under denaturing conditions and centrifuged to remove the cell debris.

5 µg crude extract and 5 µg protein standard were reduced, alkylated and denatured prior to digestion with LysC and Trypsin. The measurement was performed on a highly sensitive QExactive Plus instrument (ThermoFisher) in untargeted mode.

The detected peptides covered 100% of the protein sequence.
275 HCPs were identified, the purity of the protein was determined as 79.23%.

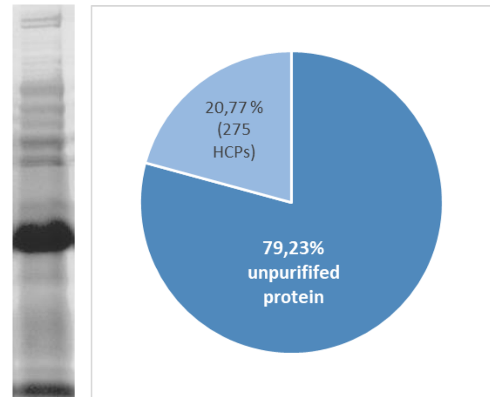

Fig. 2 A) 5 µl of cell extract were resolved via 12.5% SDS-PAGE and stained with Coomassie Brilliant Blue. B) Graphical illustration of the relative amount of protein vs detected HCPs.

Example 2:

Detection of impurities of a His-tagged recombinant protein purified via IMAC affinity chromatography

The target protein was expressed in bacteria (*E.coli*) and purified using IMAC followed by dialysis to an appropriate storage buffer.

For quality control, 5 µg purified recombinant protein and 5 µg protein standard were treated as described above.

The detected peptides covered 99.3% of the protein sequence.
79 HCPs were identified, the purity of the purified protein was determined as 96.66%.

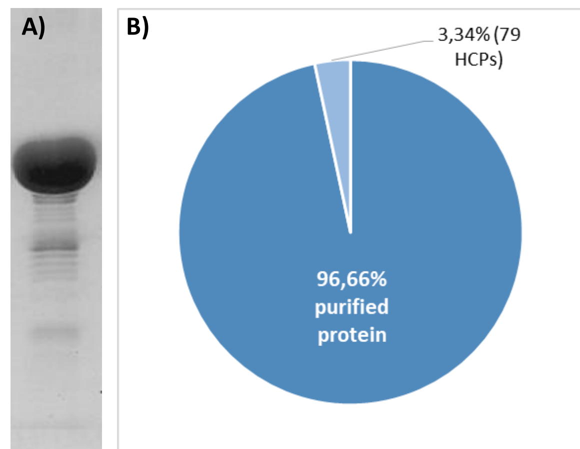

Fig. 3 A) 5 µl of IMAC purified protein solution were resolved via 12.5% SDS-PAGE and stained with Coomassie Brilliant Blue. B) Graphical illustration of the relative amount of protein vs detected HCPs.

Example 3:

HCP identification and quantification for production process optimization and routine quality control

For routine analysis we perform a method development project for detection and quantification of HCPs of a biologic in a complex matrix:

- Identification of contaminating proteins of the production organism
- Verification of the identified contaminating proteins
- Selection of suitable peptides and production of a QconCAT reference standard
- Targeted measurement using the QconCAT standard to confirm selected proteins
- Routine measurement of samples/ transfer to GMP

Results from a typical project:

In an initial analysis, three different batches of a highly purified protein were analysed with two independent LC-MS/MS measurements each, resulting in the identification of 7 proteins that were selected for production of a tailored QconCAT reference standard.

Using this QconCAT standard as reference for targeted measurement, five of the seven HCPs could be validated and used for monitoring production process performance (Fig. 4). The final method was transferred to a GMP certified laboratory.

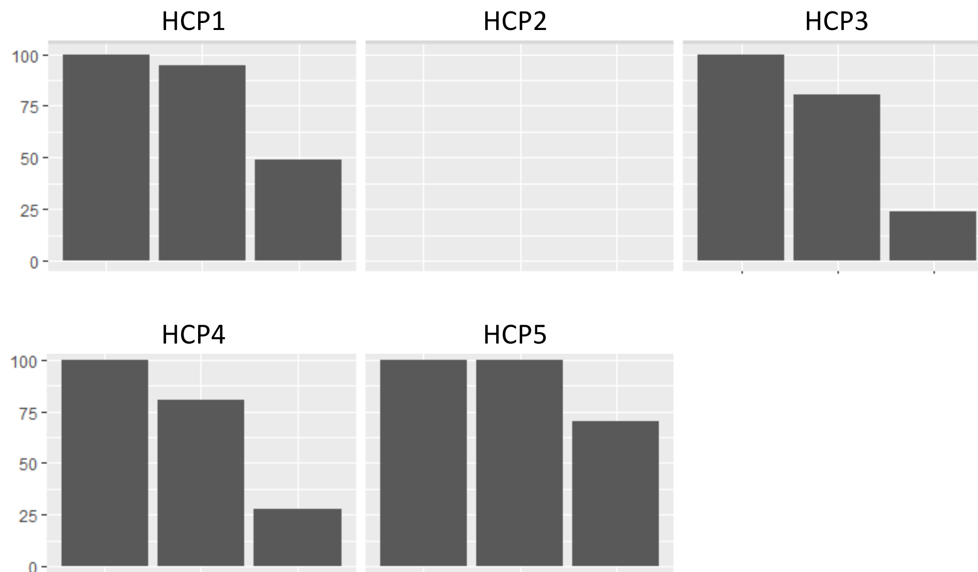

Fig. 4 Quantification of HCPs. Samples of three independent purifications were analysed to monitor effects of adaptations in the production procedure. All HCPs were quantified using targeted LC-MS/MS, normalized over the internal standard and subsequently normalized to the highest measured expression of a HCP. HCP2 was identified in the untargeted assays and verified but was not present in the tested batches in the targeted mode.

Summary

- Antibody-free detection and quantification of host cell proteins
- Protein identification with high accuracy and sensitivity
- Fast determination of relative amounts
- Determination of absolute amounts of protein impurities using QconCAT reference standards with a wide dynamic range
- Surveillance of process performance at any production stage from early R&D up to routine testing at e.g. a GMP laboratory
- Fast production of custom-made QconCATs for individual purification projects for analysis of target proteins and HCPs in a single assay
- HCP detection in difficult matrices

References

1) **Multiplexed absolute quantification for proteomics using concatenated signature peptides encoded by QconCAT genes**. *Pratt JM, Simpson DM, Doherty MK, Rivers J, Gaskell SJ, Beynon RJ.* Nat Protoc. 2006;1(2):1029-43.
2) **Enhanced Detection of Low-Abundance Host Cell Protein Impurities in High-Purity Monoclonal Antibodies Down to 1 ppm Using Ion Mobility Mass Spectrometry Coupled with Multidimensional Liquid Chromatography.** *Doneanu CE, Anderson M, Williams BJ, Lauber MA, Chakraborty A, Chen W.* Anal Chem. 2015 Oct 20;87(20):10283-91.

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