PREOMICS ANAQUANT

Application Note

Unique LC-MS workflow for accurate quantification and detection of individual host cell proteins

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Introduction

A critical challenge in biotherapeutics manufacturing is the control of process-related impurities, such as host cell proteins (HCPs). HCPs co-purified with the drug substance (DS) can affect the safety and efficacy of the drug and shorten its shelf life.

For decades, the method of choice for detection has been immunoassays, such as enzyme-linked immunosorbent assays (ELISA). However, even if ELISA is widely used; it only allows a global quantification of HCPs. Biotherapeutics require a more profound understanding of HCPs by supplementing ELISA with an orthogonal technique (LC-MS)[1].

MS techniques can be used effectively to identify and quantify specific differences in HCP profiles.

There is an unmet need to provide high throughput and robust LC-MS analysis of HCPs for the design and optimization of downstream purification processes.

Here, we present an HCP workflow as a universal LC-MS method for identifying and quantifying individual HCPs that can be easily implemented into process control for biotherapeutics production.

Keywords

Host cell proteins, HCPs, protein identification, accurate quantification, single protein quantification, biotherapeutics, biologics, LC-MS, DDA, iST, sample preparation, biologics purification, HCP-PROFILER

Key takeaways

Complete and easy-to-use standardized HCP workflow

From sample preparation, to the detailed reporting of qualitative and quantitative values of individual process-related impurities.

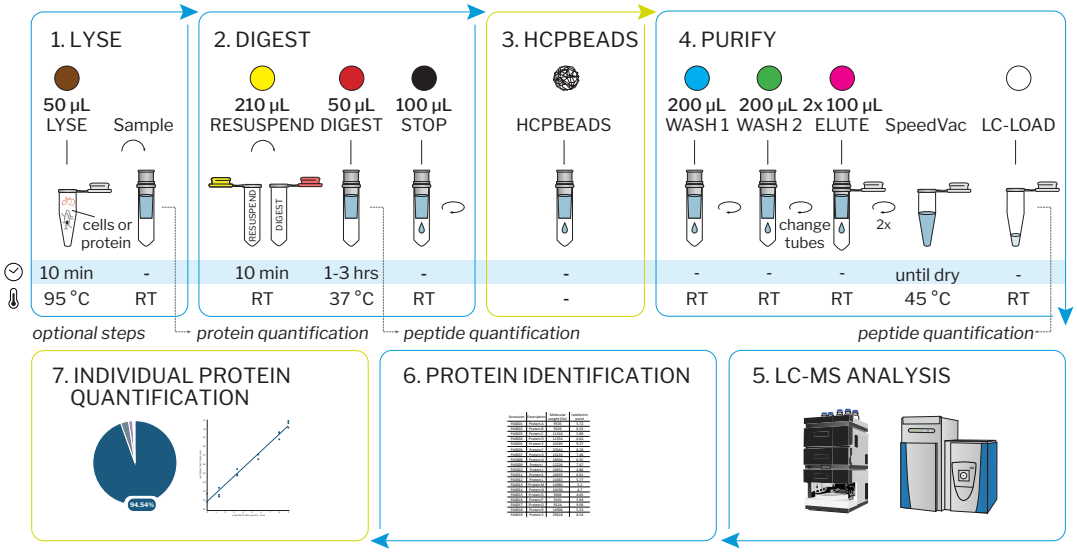

Figure 1 | Fully standardized HCP workflow for monitoring process-related impurities.
From sample preparation to a detailed report. The report graphically illustrates the qualitative and quantitative analysis of a DS sample.

Materials and methods

Three batches of DS were selected for this experiment and were processed using the iST-BCT kit (PreOmics GmbH, DE). Before the peptide cleaning step of iST-BCT, the HCPBEADS[2] (Anaquant, FR) was added.

Large-scale DS batch analysis with data-dependent analysis (DDA) was performed on a Q-Exactive HF instrument (Thermo-Fisher Scientific, San Jose, CA), coupled to an RSLC Ultimate 3000 nanosystem liquid chromatography (Thermo-Fisher Scientific, San Jose, CA) system. A PepMap™ RSLC C18 analytical column, 2 µm, 0.075 mm ID x 500 mm (Thermo-Fisher Scientific, San Jose, CA), was used for peptide separation. Solvent A was water containing 0.1% formic acid, and solvent B was acetonitrile containing 0.1% formic acid. Peptides were eluted with a gradient from 3% to 40% of solvent B over 60 minutes at a 300 nL/min flow rate.

MS/MS spectra were assigned to a peptide sequence using a database search strategy with the X!Tandem search engine. A decoy strategy was used to ensure a false discovery rate (FDR) of 1%. TOP3[3] peptide signals extracted from Proline were processed using the HCP-PROFILER application (Anaquant, Lyon, FR) to create a detailed analysis report.

Results and discussion

Identifying and quantifying individual HCPs is one of the most challenging tasks when developing biotherapeutics. Together with the Anaquant team, PreOmics created a novel HCP workflow that is easily implemented into process control for biotherapeutics production. Figure 1 illustrates a step-by-step HCP workflow, from sample preparation to a data report, to analyze three DS batches collected from biotherapeutic production.

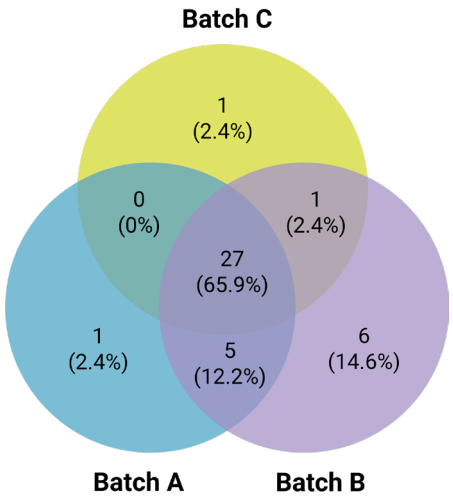

Figure 2 | Number of identified HCPs in three different DS batches. The 27 HCPs were identified in all three DS batches; in addition, specific HCP per batch were identified; in Batch A and C, one unique HCP; in Batch B, a total of six unique HCPs.

In total, 35 HCPs were present in the production of DS batches. Out of these, 27 HCPs were shared within the different batches, and 8 HCPs were specific per batch (1 for Batch A, 6 for Batch B, and 1 for Batch C), as is represented in Figure 2. This variation could be a result of biological or technical variants[4] between batches.

One of the key benefits of the proposed HCP workflow is the quantitative evaluation of every HCP in the batch. After identifying 35 HCPs present in all batches, the HCP-PROFILER was used to evaluate individual HCPs quantitatively. Figure 3 shows the three calibration curves from the internal standard (HCPBEADS) implemented after the digestion set (Figure 1) during DS sample preparation. HCPBEADS is a mixture of 54 peptides in samples corresponding to 18 "proteins" with three peptides per protein and three per calibration point. The calibration curve slope is composed of six points, and the determination coefficient is used as sample preparation and analysis quality control (QC) to ensure comparison consistency between samples/batches. All DS batches passed the QC validation parameters based on selected parameters, and the correlation between the three calibration curve slopes demonstrates the reproducibility of the sample preparation.

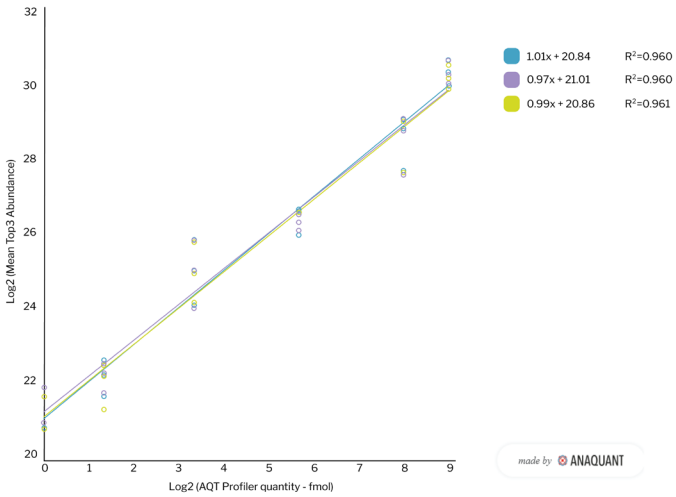

Figure 3 | HCPBEADS calibration curve in three different DS batches. The calibration curve for Batch A is in blue, for Batch B in purple and Batch C in green.

Each detected impurity (HCP) in the three DS batches is quantified due to having passed QC checks. Figure 4 quantitatively illustrates the drug proportion of the biotherapeutic of interest and the individual HCPs in DS batch A. The histogram plot (Figure 4) demonstrates a quantitative zoom of 5,69% HCPs co-purified with DS; it shows the quantity of each HCP present in batch A. The most abundant HCP is an impurity A with an amount of 0.92 ng. An exact quantity of process-related impurity enables quick evaluation of its effect on the immunogenicity and efficacy of the therapeutic protein. Thus, completing the purification processes.

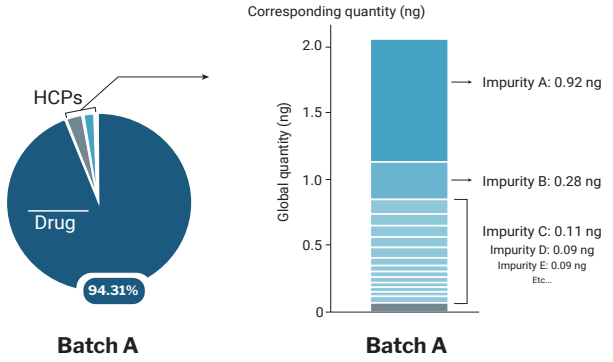

Figure 4 | Example of the purity of batch A with the individual HCP identification and quantification. The pie graph represents the drug proportion in DS of batch A. The histogram graph shows the HCP total quantity in ng divided in each protein and their corresponding quantity (ng).

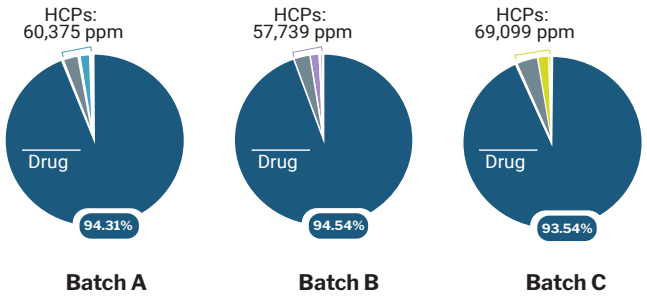

Figure 5 | Drug substance purity comparison between three different batches. The data were normalized using the HCP-PROFILER solution for precise batch-to-batch comparison. The global quantity of HCPs is reported in ppm per batch.

All three DS batches are compared in Figure 5. For batch-to-batch comparison, the data were normalized by the HCP-PROFILER solution.

The total amounts of HCPs per batch were 60,375 ppm Batch A, 57,739 ppm Batch B, and 69,099 ppm Batch C.

Conclusions

Host cell impurities and contaminants must be closely monitored to ensure drug purity, manufacturing process consistency, and patient safety. Even residual amounts of HCPs can affect product quality, efficacy, and patient safety.

This work has demonstrated that the iST Sample Preparation Kit and HCP-PROFILER enable a complete and easy-to-use HCP workflow, from sample preparation, to detailed reporting. Altogether, it accurately identified HCPs present in the selected DS batches. Furthermore, the quantity of individual HCP and total amount of HCPs detected in the DS batch were reported.

The presented HCP workflow :

- Provides a fully standardized protocol to detect impurities from the manufacturing of biotherapeutics
- Is able to identify and quantify individual process-related impurities
- Provides reproducible, accurate, and reliable quantitative data for particular impurity.

Products

Product	Quantity	Manufacturer	Product Code
iST-BCT kit 8x	8 reactions	PreOmics GmbH	P.0. 00103
iST-BCT kit 96x	96 reactions	PreOmics GmbH	P.0. 00121

Ordering information

PreOmics GmbH

http://www.preomics.com/quote
order@preomics.com

ANAQUANT

https://www.anaquant.com/contact/
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Acknowledgment

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For information about HCP-PROFILER and HCPBEADS contact ANAQUANT.

*HCP-PROFILER and BEADS can be implemented in your lab based on a collaboration. Additionally, the ANAQUANT team offers services to analyze process-related impurities.

References

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