How to future-proof the analysis of host cell proteins in biopharma using microflow LC-MS/MS

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Host cell proteins (HCPs) are a common class of residual process-related impurities in biologic drug products. They can affect the quality, efficacy and safety of the product as well as induce or enhance immunogenicity. As such, they are an obligatory critical quality attribute (CQA). CQAs are physical, chemical, biological, or microbiological properties or characteristics that must be defined, measured, and monitored to ensure that their presence in the final drug product is within acceptable prespecified limits. The successful clearance of HCPs is also therefore used as a benchmark to demonstrate the robustness of the biomanufacturing process. As HCPs vary widely from biologic product to product, and as the products themselves are highly diverse, these HCP CQA limits are set by regulatory authorities on a case-by-case basis.

Increasing demand for better ways to detect and remove HCPs

The demand for improved HCP detection and clearance methods is growing, as biologic drugs move to dominate the global market for medicines [1]. Moreover, rapidly emerging new modalities such as gene therapies present even greater complexity when it comes to HCP impurities, as their biomanufacturing involves more diverse sources of HCP contamination.

The preferred, regulatory compliant method for HCP analysis remains ligand-binding assays (LBAs), namely enzyme-linked immunosorbent assays (ELISAs), due to their ease, speed and sensitivity. However, there are several limitations with ELISAs, which are challenging biopharma companies, especially those developing and manufacturing next generation biotherapeutics like gene therapies.

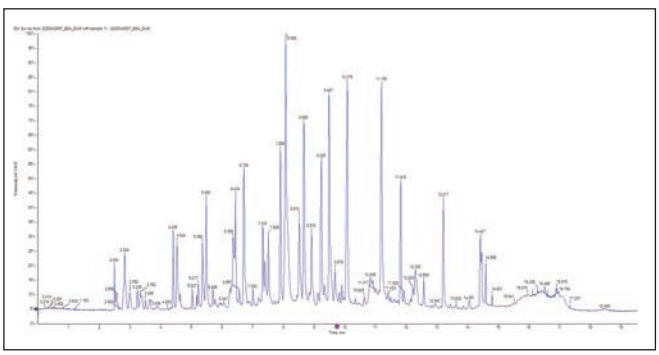

Figure 1. Chromatogram of HCP analysis using novel method based on LC-MS/MS with data-independent SWATH Acquisition [1]

One of these key limitations is the poor coverage provided by the detection antibodies, which have been raised against human proteins that are not very immunogenic. Another issue is the lack of commercially available ELISAs that are sufficiently specific to detect the various types of HCPs that need to be monitored and removed from next-gen therapies. To address this, process-specific ELISAs can be developed but this can take too long. The usual 1.5- to 2-year development time required could hinder the progress of a novel therapeutic reaching the market and clinic, and for one-time treatments such as gene therapies, being first to market could make or break the success of that product.

An orthogonal method for detecting HCP clearance

In response to these pain points expressed by biopharma manufacturers regarding ligand binding assays (LBAs), other approaches have been explored to HCP analysis. One method in particular provides unique advantages and addresses the limitations of ELISAs. Liquid chromatography (LC) using a microflow gradient coupled with tandem mass spectrometry (MS/MS) is increasingly being used for HCP detection and is becoming an expected orthogonal standard in the successful development of complex biologics. In close collaboration with these clients and MS instrument vendors - Sciex, several new HCP analyses based on LC-MS/MS with data-independent acquisition (DIA) have been developed *(see Figure 1)* [1]. As this is a generic approach, LC-MS/MS with DIA is able to detect any protein. The application of DIA using techniques like SWATH Acquisition ensures that the assay has a wide dynamic range and is thus able to detect proteins at even very low abundance within the complex matrix of a high-volume biological sample.

With these HCP assays, a profile of all the proteins present in a sample can be obtained, including the absolute quantification, molecular weight, hydrophobicity, and isoelectric point (pI) of each protein *(see Figure 2)*. This approach is very precise, providing highly reproducible and robust quantification and identification of each protein over time. It is tolerant to complex samples in different buffers and so, enables the comparison of process steps and manufacturing batches. The generic nature of the LC-MS/MS approach makes it easily applicable to analyse a broad range of biologics, including many new modalities. Moreover, compared with LBAs, the additional information provided by LC-MS/MS assays - such as the identification of the specific HCPs, more easily facilitates the modification of the biomanufacturing process to eliminate the HCP impurities. It has been found, time and time again, that microflow LC-MS/MS with SWATH Acquisition is able to provide the coverage, specificity and sensitivity needed by biologics developers for the precise detection of HCPs and monitoring of HCP clearance in the development of their next-gen biotherapeutics, requiring only approximately 2 weeks to optimise the protocol to fit the biomanufacturing processes [2].

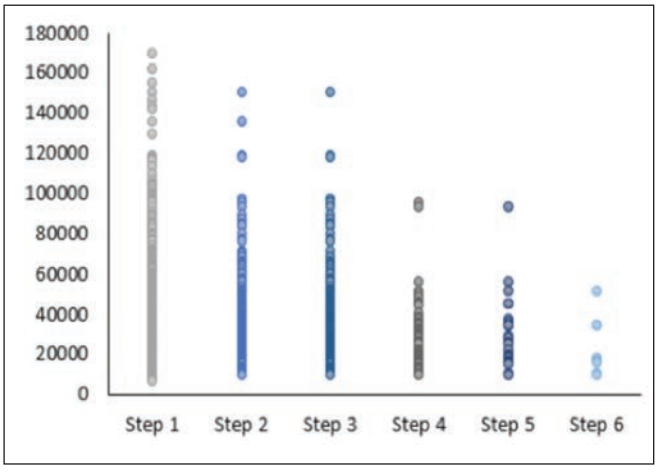

Figure 2. An example of the molecular weight (Daltons) of all identified HCPs in test samples of a drug substance taken throughout a six-step purification process, analysed using a microflow LC-MS/MS assay with SWATH Acquisition [1].

The regulatory landscape of the future

The increasing prevalence of new expression systems in biomanufacturing, new modalities in biotherapeutics, and new analytical technologies such as LC-MS/MS, is likely to lead regulators towards the requirement for more HCP data from biopharma manufacturers. In anticipation of this move, and given the long development cycles for biotherapeutics, many biologics manufacturers are managing their risk and already asking laboratories to perform HCP profiling using LC-MS assays with advance DIA techniques such as Scanning SWATH Acquisition.

This enables them to preserve a comprehensive, 4-dimensional digital record of their HCP data for potential future requirement by regulatory bodies. It has been found that these LC-MS assays are also of great utility when it comes to informing the selection of commercially available ELISAs as well as supporting such decisions with validation data.

The LC-MS assays provide information regarding the coverage, specificity and sensitivity of the ELISAs. This additional information can support as well as complement the data obtained using the LBAs, particularly as LBAs remain a regulatory compliant method for HCP analysis while LC-MS is not (yet). In the meantime, it seems prudent to include LC-MS analysis for HCP detection, identification and monitoring, as part of a risk management strategy for the development of any new biotherapeutic, as this could translate into substantial cost and time savings, as well as minimise potential setbacks further along the drug development process.

Author information

Dr Thomas Kofoed, PhD, is co-founder and CEO of Alphalyse. He has more than 15 years of management experience from the biotech industry and has set up business relationships with hundreds of biopharmaceutical companies. As CEO of Alphalyse his main emphasis is putting together a diverse team of highly skilled employees creating an innovative environment with focus on quality and customer needs.

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