Beyond ELISA

Is Mass Spectrometry the Future of Impurity Analysis?

Thomas Kofoed with Margarita Sabater, Bryant McLaughlin, and Søren Skov Jensen

Impurity characterization of biopharmaceuticals is changing. Health authorities increasingly require orthogonal data to support enzyme-linked immunosorbent assays (ELISAs), pushing for thorough, reliable documentation of downstream purification to reduce process-related impurities such as host-cell proteins (HCPs). With the upcoming US Pharmacopeia (USP) General Chapter <1132.1> on HCP measurement by liquid chromatography–mass spectrometry (LC-MS), LC-MS is set to become an essential method for identifying and quantifying protein impurities, offering the levels of detail and coverage that ELISA lacks.

Thomas Kofoed of Alphalyse, a contract research organization (CRO) specializing in LC-MS–based HCP analysis, interviewed three chemistry, manufacturing, and control (CMC) specialists about current and future applications of LC-MS for impurity analysis in the biopharmaceutical industry. The interviewees were Margarita Sabater of Genmab, Bryant McLaughlin of BCM Solutions, and Søren Skov Jensen of Genmab at the time of the interview.

A Critical Quality Attribute

Kofoed: What are your HCP-analysis needs as CMC managers?

Sabater: HCPs are critical quality attributes (CQAs) of biologic products because they affect drug quality and safety. We must optimize manufacturing processes to remove or decrease HCPs to acceptable levels. I need an analytical method that can

measure HCP levels at multiple process steps to assess the effectiveness of HCP clearance.

Skov Jensen: I would like to know which HCPs are present in my product, especially if some are problematic, as early as possible. I need to know how my ELISA reagent performs, whether the HCP reference standard is representative of my process, and how my process performs over time and after process changes.

McLaughlin: In some well-known cases, including the initial phase 3 trials of lebrikizumab, HCPs went undetected by platform ELISAs and caused significant setbacks during clinical trials. I need to know if a high-risk HCP is present in my product and if it could diminish drug efficacy or cause instability.

ELISA Limitations

Kofoed: ELISAs are the industry standard for measuring HCPs. Have you encountered problems using them?

Sabater: For decades, ELISA was the only technology sensitive enough to measure HCPs. ELISA can provide good control of HCPs, but HCP-antisera coverage must be assessed against process-specific samples, HCP immunogens, and reference standards. That is particularly important after process changes because samples might not have the same HCP profile. Furthermore, HCP-coverage analyses, such as two-dimensional difference gel electrophoresis (2D-DIGE), are not always representative of actual ELISA conditions.

Skov Jensen: When using ELISA to detect residual proteins, we do not know whether our product contains problematic HCPs or whether the assay can even detect the relevant HCPs. Traditional HCP-coverage analysis provides only a percentage number, with no warning of potential product stability or quality issues down the line.

McLaughlin: The benefit of HCP–ELISA assays is that most good manufacturing practice (GMP) quality-control (QC) laboratories are equipped to run such tests. But in my experience, ELISAs often fail to achieve adequate coverage percentage, accuracy, and sensitivity. Even a good ELISA detects only 80–90% of potential HCPs.

LC-MS Benefits

Kofoed: What are the main benefits of LC-MS as an orthogonal analysis?

Sabater: By combining MS-based analysis with ELISA, we can identify individual HCPs, assess their impact on product quality, and target them in purification. The ELISA–MS coverage analysis informs us of which individual HCPs the ELISA antibodies recognize.

Skov Jensen: LC-MS analysis provides detailed knowledge of specific HCPs in a product, the process performance and consistency in HCP

clearance, the effects of process changes on product purity and stability, and which HCPs that the ELISA reagent covers. Adding LC-MS makes an HCP-control strategy much more robust.

McLaughlin: LC-MS analysis can confirm whether any HCPs present are high risk and whether HCP profiles are the same between batches. If we use two different HCP-ELISA kits with the same drug substance and get disparate results, LC-MS can be used to compare them for the best coverage — or even be used instead of ELISA if a suitable kit is not available.

Submission Experience

Kofoed: Requirements from the regulatory authorities largely drive the criteria for HCP characterization. Have you included LC-MS results in US Food and Drug Administration (FDA) submissions, and were they approved?

McLaughlin: Yes, I have included LC-MS HCP results in my regulatory submissions to support comparability assessments, impurity description, and justification of the HCP specifications. I have even provided ELISA-MS HCP coverage data for the coverage characterization, forgoing 2D gels. In each case, there were no concerns or follow-up requests from the FDA.

Sabater: We included ELISA-MS coverage results in our latest biologics application license (BLA) submission of the Epkinly bispecific antibody (bsAb) to demonstrate the appropriateness of commercial HCP-ELISA kits to measure process-specific HCPs; it was approved.

USP Guidance

Kofoed: How do you think the new USP General Chapter <1132.1> will affect the use of LC-MS for HCP analysis?

Sabater: We can expect regulatory authorities to be more critical of HCP-ELISA data in submissions. I hope that the new USP chapter will empower developers to move away from submitting release methods based only on ELISA percentages and instead toward submitting a list of specific HCPs identified with LC-MS throughout process development, characterization, and validation.

From left to right, top to bottom: Margarita Sabater, Bryant McLaughlin, Søren Skov Jensen, and Thomas Kofoed

Showing process consistency that is supported by characterization and comparability studies could remove the need to report ELISA quantity levels altogether.

McLaughlin: I anticipate that regulatory authorities will consider LC-MS more valuable as an orthogonal method than other HCP technologies and ask sponsors to supplement ELISA data with LC-MS analyses.

Skov Jensen: I hope it changes how we think of MS use in control strategies and that the industry adopts such methods for both development and QC purposes. Regulatory authorities are likely to request more information from MS-based methods.

Outsourcing LC-MS

Kofoed: Why did you choose to outsource LC-MS–based HCP analysis rather than doing it in house?

Skov Jensen: Capacity is one of several reasons. But most importantly, analyzing HCPs by LC-MS is not a trivial task, even for an experienced LC-MS laboratory. Instead of trying to build in-house knowledge, I reached out to the Alphalyse team, whose specific knowledge of HCP analysis by LC-MS helped to create a more elaborate HCP-control strategy, increasing our understanding of the process and ELISA performance from downstream process to final product.

McLaughlin: My company had in-house MS capabilities, but we felt that MS-based HCP analysis was a unique specialty. In terms of efficiency and data quality, it made more sense to work with an experienced CRO rather than to attempt the experiments ourselves. In addition, Alphalyse performs analyses aligned with the recommendations of the new USP General Chapter <1132.1>, and we expect that regulatory authorities will ask sponsors to present data incorporating those recommendations. The collaboration has been a convenient way to integrate USP guidelines into our programs.

A Strategic Approach

Kofoed: In your opinion, what is the best strategy for impurity analysis?

Skov Jensen: Do it as early as possible. I have seen several projects where HCPs nearly caused delays. A relatively targeted, well-defined standard impurity-characterization package could save developers from delays later in project development. An iterative approach also provides more information and increased understanding of process performance regarding the clearance of problematic HCPs through reproducible LC-MS characterization of different projects.

Sabater: LC-MS technology enables data-driven process optimization. It allows us to identify specific HCPs that could induce immunogenicity in patients or affect product quality, so that they can be individually targeted, reduced, and controlled during process-development phases.

McLaughlin: Combining MS-based analysis with HCP–ELISAs is a must in biologics CMC development. I recommend using LC-MS to identify and quantify HCPs in drug substances and at multiple steps in the purification process as well as for ELISA characterization, and to initiate such activities early in development.

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