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TECH INSIGHTS

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# Getting the Most from Host Cell Protein Analysis

Host cell proteins (HCPs), which cellular expression systems release during their normal lifecycle, become problematic when they co-purify with product during downstream processing. Many HCPs are benign, but some are immunogenic, others may interact unfavorably with the drug substance and others, like proteases and lipases, can reduce effective product dosage through direct action on the drug or by de-stabilizing formulation buffer components . Since HCP determinations address concerns for both product quality and safety, they constitute a significant component of a biopharmaceutical drug developer's overall risk-management strategy.

Conventional HCP analysis methods were developed at a different time, and for feedstock streams that differed significantly in concentration and composition from what are commonly encountered today. This article describes a novel HCP analysis approach, Antibody Affinity Extraction™ (AAE™), which was commercialized by Cygnus Technologies to address shortcomings in existing HCP analysis methodology.

## Why analyze HCPs?

Regulations governing HCPs are found in guidances by the International Conference of Harmonization (ICH Q11), the U.S. Food and Drug Administration (21CFR610.13), and in 42 U.S.C. 262. Regulations require developers to report HCP levels, preferably within previously agreed upon accepted ranges. In the United States, the Food and Drug Administration requires that HCPs exist at levels below 100 ppm in formulated product, and below 10 ng per dose. That goal has become more challenging as upstream product titers continue rising, thereby shifting the burden of clearing, characterizing, and quantifying HCPs to downstream processing and associated analytics.

Multi-analyte enzyme-linked immunosorbent assay (ELISA) is the standard method for HCP analysis. ELISA is a sensitive and selective assay that may also be configured for high throughput, but it has to be based on an anti-HCP antibody, which is reactive or covers the majority of HCPs present in a given process. ELISA kits do not recognize all potential HCPs, for example weakly- or non-immunoreactive species. A stoichiometric excess of HCP relative to antibody may result in dilution-dependent non-linearity with HCP ELISA products. Even if antibodies for an HCP are present, conditions for antibody-HCP binding may not be right, or the HCP may exist as multiple epitopes, only some of which may be immunoreactive.

## Challenges and solutions

Researchers from Genentech identified the challenges of applying standard protein analysis protocols to HCPs as (1) low HCP levels relative to that of product proteins, (2) assay suitability for quantifying a large number of low-abundance HCPs, and (3) the dependence of HCP levels on process conditions. Since processes change between development and manufacturing stages, it may be necessary to validate methods several times during a product's lifecycle.

The Genentech group noted that the broad concentration dynamic range of HCPs, which persists into downstream processing, "means that no one technology is able to detect and monitor all those HCPs present". Regulatory agencies around the world recommend the use of orthogonal methods for HCP analysis to support process development and validation.

Cygnus Technologies developed AAE to address weaknesses in HCP characterization as they related to risk mitigation. The AAE workflow involves the following steps:

- Immobilizing the anti-HCP antibody on a chromatography support, typically Sepharose®.
- Extracting the HCP sample and eluting until no additional binding occurs.
- Pooling, buffer-exchanging, and concentrating the eluted HCP sample back to its original sample volume.
- Recovering HCPs and analyzing by 2D PAGE silver stain
- Using spot-matching to compare identities and concentrations of HCPs so identified to those in the original sample. Spot matching is also possible using differential gel electrophoresis, in which both samples are labeled with fluorophores and run on the same gel.

Unlike 2D PAGE fractionation followed by western blot (2D WB), where sensitivity is limited by loading capacity and destruction of native HCP epitopes by harsh chemical treatment, AAE sensitivity is 100 times higher due to its ability to extract HCPs in its native conformation and concentrate large sample volumes. In a typical study comparing AAE to 2D WB, AAE showed coverage of 92% of the individual CPs compared to 55% for 2D WB. AAE specificity is also much higher than for 2D WB. Non-specific binding to the AAE column is less than 0.1% compared with 2D WB, where up to half of all spots will react. Importantly, AAE is suitable for both upstream and downstream samples, 2D WB is not.

### Effective Enrichment of HCPs and Depletion of DS

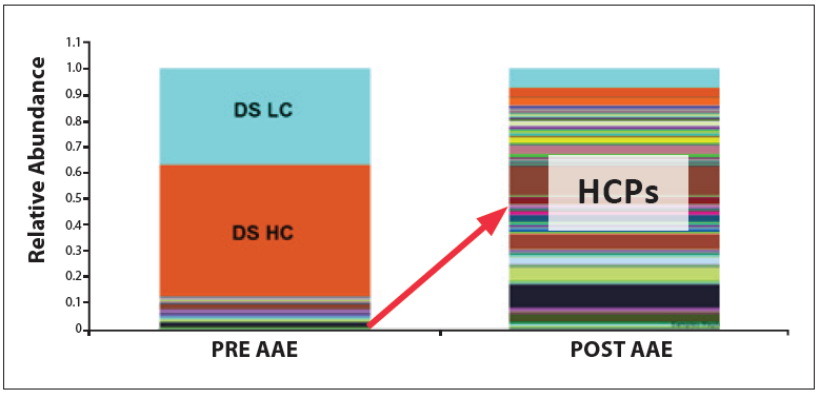


*Figure 1. The relative abundance of DS and HCPs according to their extracted ion chromatograms were normalized to one and graphed in a stacked bar chart. The sample analyzed before AAE (Pre- AAE, left) shows the drug substance heavy chain (DS HC) in orange and the drug substance light chain (DS LC) in light blue. After AAE (Post-AAE, right) the relative abundance of HCPs has increased relative to DS.*

And while current regulatory guidelines still reference and allow for use of the less sensitive 2D western blot to qualify HCP ELISA for antibody coverage to HCPs present upstream in the purification process, this method lacks the sensitivity and specificity to detect the most important and clinically relevant HCPs, which are those that co-purify with the final drug substance.

When coupled with AAE, mass spectrometry (MS) can identify more than 1000 individual HCPs, while allowing investigators to monitor changes in HCP concentrations during various purification steps. MS also complements 2D electrophoresis by providing a second level of identification and quantitation for known HCPs. ELISA provides an overall HCP concentration value. MS does the same but also identifies and quantifies individual HCPs. From here one can calculate HCP molecular weight and isoelectric points, which can assist in designing purification trains that minimize problematic HCP ending up in the drug product.

## AAE Enriches Known Problematic CHO HCPs

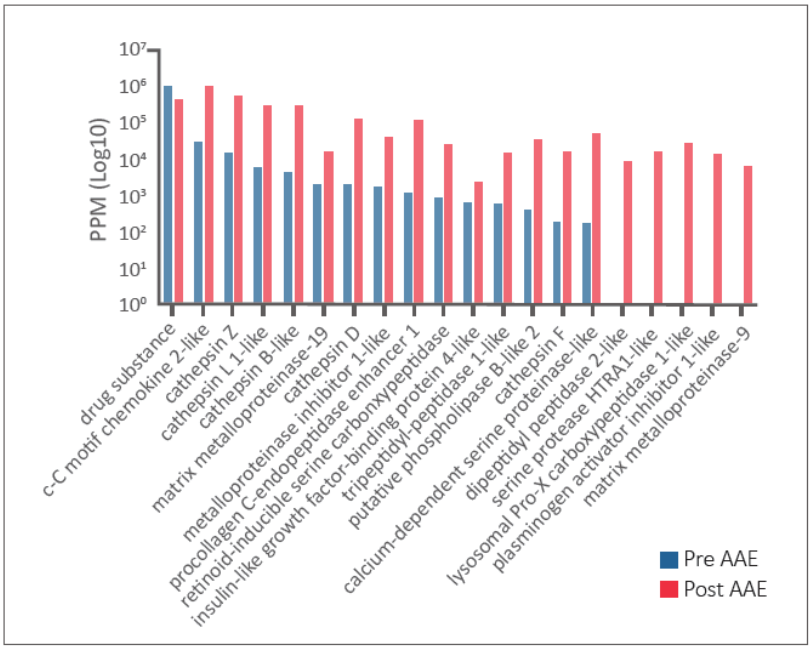


*Figure 2.* **Drug substance (DS)** *was analyzed before and after AAE by MS. PPM quantification was calculated relative to the most abundant protein present in each sample. For the Pre-AAE sample, known problematic CHO HCPs were quantified relative to the DS. For the Post-AAE sample, CHO HCPs were quantified relative to the most abundant protein, C-C motif chemokine 2.*

In a recent white paper Cygnus scientists reported how AAE empowers MS identification of Chinese hamster ovary (CHO) HCPs in the presence of drug substance. Without AAE enrichment, MS lacked the sensitivity to detect five of twenty known, problematic CHO HPCs, most likely due to their low abundance and interference from the drug substance. Spectroscopists can overcome such problems by adjusting the MS ion exclusion window, but instead of merely applying a mass filter AAE overcomes this effect by enriching samples in HCPs by up to 240-fold, thus preserving the original sample characteristics.

## On risk management

FDA's risk-based manufacturing initiative simultaneously empowers and challenges drug-makers. The freedom to incorporate risk and risk-mitigation strategies, however, comes at the cost of demonstrating a higher degree of process understanding than ever before. The potential to achieve this knowledge rests squarely on the robustness and suitability of analytics.

A multi-company research group summarized what this means for HCPs, writing that "the complexity and diversity of residual HCP composition in drug substances and limited knowledge...on the effect of individual HCPs detected in biologics create significant challenges. The main difficulty is in effectively determining at what level — and more important, for which specific HCPs — they could be considered safe or low risk."

In a follow-up paper this group noted that "no practical approach has been made available for HCP risk management during bioprocess development." The authors identified dilution non-linearity and "products with unusual stability profiles or degradation pathways—an acknowledgement of increasing product complexity—as barriers to implementing a robust risk-mitigation strategy. They write that, in addition to adopting purification steps that minimize HCPs, developers must "adopt relevant and robust analytical assays for HCP measurement and characterization."

And, a University College London group advises that these analytics be applied “as soon as is feasible in the product lifecycle.”

ELISA-based HCP detection and quantification remains the gold standard for monitoring downstream purification process consistency, HCP removal, and final drug substance purity. MS improved the ability to identify and quantify HCPs significantly, but too often falls short with very low-abundance species. Antibody affinity extraction complements MS characterization of HCPs by concentrating HCPs and providing near-optimal conditions for MS to work its wonders. Cygnus Technologies, the commercial developer of AAE, is eager to share its experience and expertise in solving HCP characterization projects.

## About the Author

### Angelo DePalma

Angelo DePalma earned his Ph.D. in organic chemistry from Stony Brook University and was previously senior scientist at Schering-Plough. He has written extensively on biotechnology, biomanufacturing, medical devices, pharmaceutical commerce, laboratory instrumentation, and advanced materials.