AAE-MS As an Orthogonal Method in HCP Analytics

Jared Isaac, Alla Zilberman, Kerry Wooding, Jacob Stubbs, and Eric Bishop

Back to Contents

Host-cell proteins (HCPs) copurify with biological drug substance (DS) and can pose potential risks for patients. Although many HCPs are benign, some are immunogenic, others may interact with a DS and diminish its efficacy, and others can interfere with DS stability. Thus, the quantity and nature of residual HCPs in a DS generally are considered critical quality attributes (CQAs) that constitute a significant part of a biopharmaceutical drug developer's risk-management strategy (**1**, **2**).

Mass spectrometry (MS) can be a powerful complementary method for demonstrating the suitability of an enzyme-linked immunosorbent assay (ELISA) for HCP identification and quantitation. The following study provides an example of how Cygnus Technologies' antibody affinity extraction (AAE) method followed by MS can be applied to evaluate an HCP ELISA's suitability. We also show how AAE-MS methods can be used to identify and quantify HCPs using in-process and DS samples.

Methods

Sample Preparation: We cultured CHO-K1, CHO-S, and DG44 Chinese hamster ovary (CHO) cell lines, then pooled clarified harvests from those processes to create a single sample. A Coomassie staining assay determined that the sample was at pH 8.1 and had a total protein concentration of 1.31 mg/mL. Before AAE analysis, we ran the sample through a 0.2-µm filter.

Figure 1: Virtual two-dimensional gel of 3G polyclonal antibody coverage (Cygnus Technologies, 3G-0016-1-AF, F550-1 CHO HCP 3G enzyme-linked immunosorbent assay) against Chinese hamster ovary (CHO) host-cell proteins (HCPs)

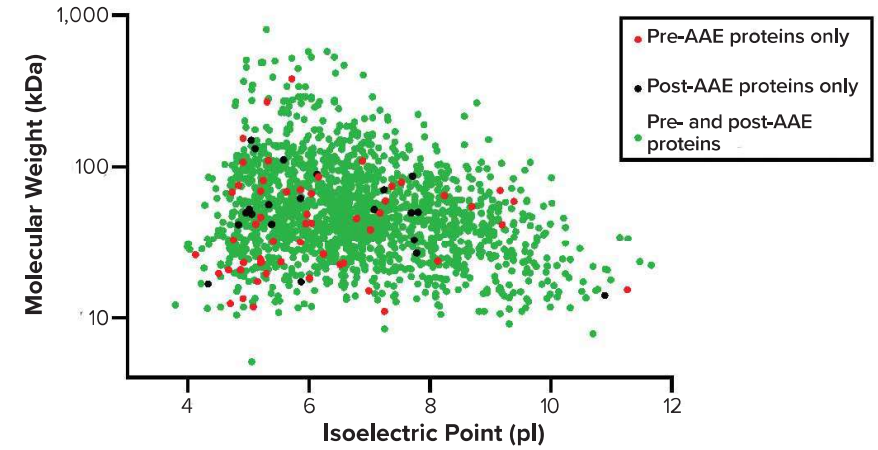


Table 1: Anti-CHO HCP 3G polyclonal antibody coverage (Cygnus Technologies, 3G-0016-1-AF, F550-1)

	Number of Protein Identifications				Antibody Coverage (%)	
Sample Type	Total	Unique to Each Fraction	Total Unique	Matching	Lower Boundary	Upper Boundary
Pre-AAE (all HCPs)	1,673	55	1,694	1,618	97%	98%
Post-AAE (immunoreactive HCPs)	1,639	21				

AAE Fractionation: Anti-CHO HCP 3G polyclonal antibodies (Cygnus Technologies, 3G-0016-1-AF) were immobilized covalently on an AAE chromatography support. The packed column was conditioned to prevent significant antibody leaching while minimizing nonspecific binding. Using a Cytiva ÄKTA pure 25-L fast-protein liquid chromatography (FPLC) system, we loaded an HCP-containing sample onto the column to bind HCPs and collect elution fractions.

Liquid Chromatography–Mass Spectrometry (LC-MS): Protein samples from before and after AAE treatment were precipitated, dissolved, reduced, alkylated, digested with trypsin, desalted, and concentrated. Data were acquired using a Vanquish ultrahigh-performance liquid chromatography (UHPLC) system and an Orbitrap Eclipse Tribrid mass spectrometer, both from Thermo Fisher Scientific. HCPs were identified by two peptides per protein from triplicate runs, and data were screened against the Cygnus Technologies CHO HCP database using Proteome Discoverer software (Thermo Fisher Scientific).

Polyclonal ELISA Antibody Coverage Calculation: We generated a virtual two-dimensional (2D) gel graph from MS data using GraphPad Prism 9 software (Figure 1). Red dots represent proteins that were found in the pre-AAE sample only; black dots indicate those that appeared only in the post-AAE sample; green dots signify proteins that were detected in both samples.

Polyclonal ELISA antibody coverage is represented by a range between the lower and upper coverage-boundary calculations:

- **Lower Boundary:** post-AAE proteins ÷ unique proteins
- **Upper Boundary:** post-AAE proteins ÷ pre-AAE proteins
- **Unique Proteins:** (pre-AAE proteins + post-AAE proteins) – matching proteins.

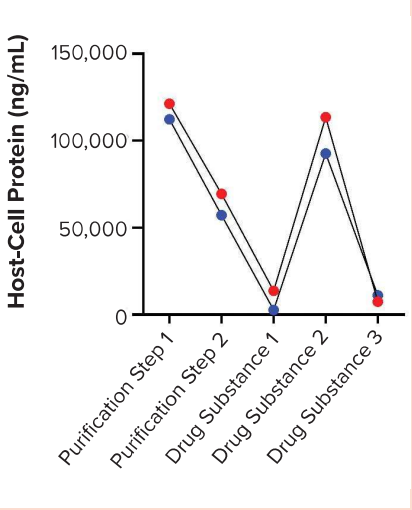
Results and Discussion

HCP Antibody Coverage Analysis: Using an AAE column, we used AAE-MS to analyze the CHO HCP sample. LC-MS detected 1,673 such impurities in the sample. In the AAE elution fraction, 1,639 were detected, representing 97–98% coverage (Table 1). Impurities identified in virtual 2D gels of the pre- and post-AAE samples fell into the same ranges of molecular weight and isoelectric point (pI) as those identified by LC-MS, and the detected proteins covered the majority of the CHO proteome (Figure 1, Table 1).

LC-MS and ELISA As Confirmatory Orthogonal Methods: We applied the Cygnus Technologies LC-MS/MS method and a CHO HCP 3G ELISA to quantify the number of CHO HCPs present in a client's purification process (Figure 2). Samples accounted for purification steps 1 and 2 and different lots of DS. LC-MS and ELISA together confirmed a breakdown of the purification process for the second DS lot, highlighting the need for further investigation by the client.

AAE-MS Identification of HCPs in a DS: MS is a powerful complementary method to ELISA for identification and

Figure 2: Enzyme-linked immunosorbent assay (ELISA, rendered below in blue) and liquid chromatography with tandem mass spectrometry (LC-MS, red) can serve as confirmatory orthogonal methods.



Back to Contents

quantification of HCPs; however, drug products often mask HCPs by a factor of 10^4–10^6. The AAE method can be highly effective at enriching HCPs and depleting DS.

Figure 3 shows the relative abundance of DS and HCPs according to extracted ion chromatograms that were normalized to one for rendering in a stacked bar graph. Peptides associated with the DS heavy chain (HC) are shown in orange, those affiliated with its light chain (LC) are rendered in blue, and HCPs are presented in assorted colors. Analysis of pre-AAE samples demonstrates that most peptides therein belong to the DS. However, following AAE enrichment (right side of the figure), the relative abundance of HCPs increased significantly as that of the DS HC and LC decreased.

MS identification enables accurate prediction of HCP MW and pI. Such data can be graphed into a virtual 2D gel to visualize HCP content in DS samples and downstream purification groups to develop appropriate strategies (Figure 4, Table 2). By focusing on a "potentially problematic HCP" in a quadrant of the virtual 2D gel, chromatography groups can apply column chemistries to eliminate HCPs of interest.

Figure 3: Antibody affinity extraction (AAE) technology enriches host-cell proteins (HCPs) and depletes drug substance (DS); LC = light chain, HC = heavy chain.

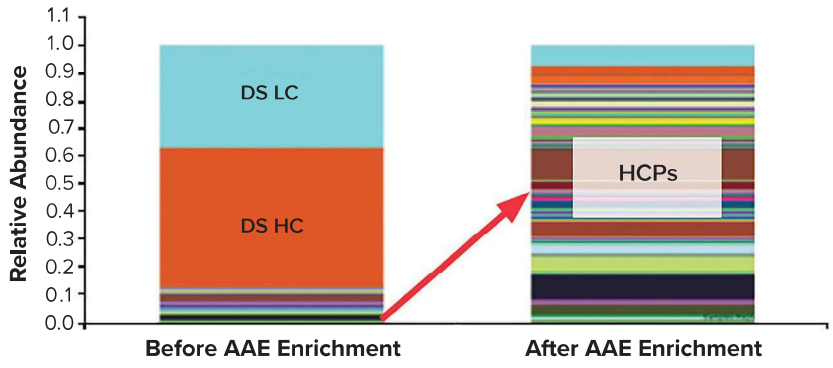


Figure 4: Virtual two-dimensional gel of Chinese hamster ovary (CHO) host-cell proteins (HCPs) in a drug-substance (DS) sample

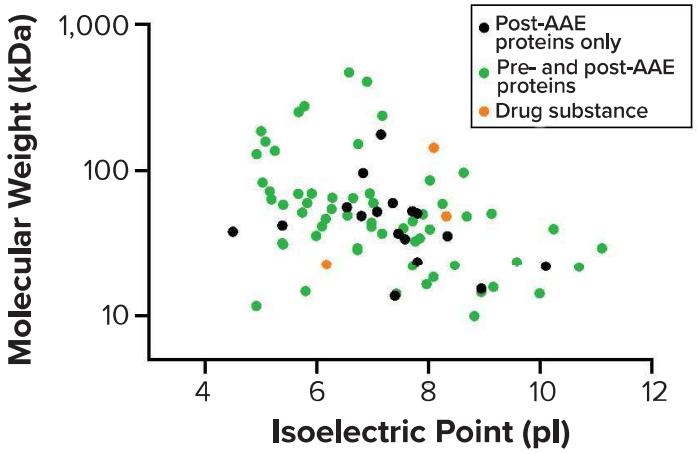


Table 2: Number of host-cell proteins (HCPs) identified in four independent drug substances (DSs) before and after antibody affinity extraction (AAE) enrichment; all experiments applied CHO HCP antibody 3G-0016-1-AF.

DS	HCPs		Coverage
	Pre-AAE	Post-AAE	
1	13	28	100%
2	70	174	100%
3	62	79	100%
4	15	45	100%

The Value of MS in HCP Analytics

MS plays an important role in HCP analytics from submission of an investigational new drug (IND) application through postmarketing activities, when the criteria for evaluating process changes, performing risk assessments, and characterizing reagents all evolve. Although complete characterization of downstream HCPs is not part of current regulatory guidelines, such information is recognized widely as a "value add" by proactive manufacturers and regulators because it helps to ensure drug-product safety and efficacy.

References

1 Lalonde M-E, Durochet Y. Therapeutic Glycoprotein Production in Mammalian Cells. *J. Biotechnol.* 251, 2017: 128–140; https://dx.doi.org/10.1016/j.jbiotec.2017.04.028.

Back to Contents

2 Levy NE, et al. Identification and Characterization of Host Cell Protein Product-Associated Impurities in Monoclonal Antibody Processing. *Biotechnol. Bioeng.* 111(5) 2014: 904–912; https://doi.org/10.1002/bit.25158.

3 Vanderlaan M, et al. Experience with Host Cell Protein Impurities in Biopharmaceuticals. *Biotechnol. Prog.* 34(4) 2018: 828–837; https://doi.org/10.1002/btpr.2640.

4 Valente KN, Lenhoff AM, Lee KH. Expression of Difficult-to-Remove Host-Cell Protein Impurities During Extended Chinese Hamster Ovary Cell Culture and Their Impact on Continuous Bioprocessing. *Biotechnol. Bioeng.* 112(6) 2015: 1232–1242; https://doi.org/10.1002/bit.25515.

About the Authors

Corresponding author ***Jared Isaac, PhD, MBA,*** *is a senior scientist,* ***Alla Zilberman*** *is vice president of technical marketing and business development,* ***Kerry Wooding*** *is a scientist,* ***Jacob Stubbs*** *is an associate scientist, and* ***Eric Bishop*** *is vice president of research and development at Cygnus Technologies, part of Maravai LifeSciences; 4332 Southport Supply Road SE, Southport, NC 28461; jared@cygnustechnologies.com; https://www.cygnustechnologies.com.*

Back to Contents