CYGNUS TECHNOLOGIES

part of Maravai LifeSciences

Antibody Affinity Extraction™ and Mass Spectrometry Services

Empower your host cell protein identification with advanced orthogonal methods

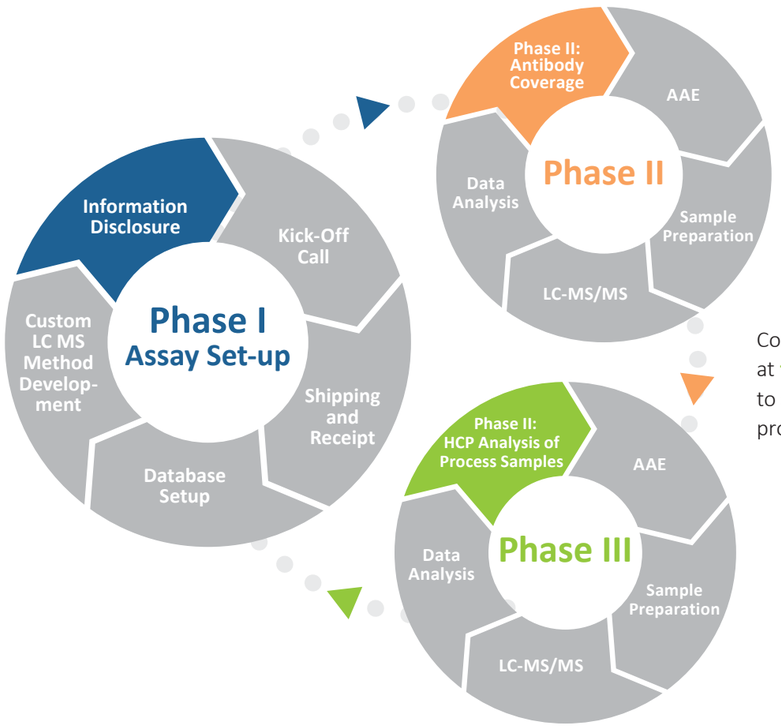
Cygnus AAE-MS HCP Analysis Services

Host cell proteins (HCPs) constitute a major group of impurities for biological drugs produced using cell culture technology. Even at nanogram per milligram concentrations of HCP to drug substance (DS), HCPs can elicit undesired immune response, interfere with drug safety and efficacy, or impact DS stability. A broadly-reactive HCP ELISA should be used during purification to ensure HCP removal and to demonstrate process consistency and final DS purity. Even a well-qualified generic or process-specific ELISA, however, does not provide information about the identities of the HCPs present in a DS.

Building on more than 20 years' experience in HCP analysis, Cygnus advanced orthogonal **Antibody Affinity Extraction™ (AAE™)** and **AAE-MS services** are designed to detect and identify HCP impurities to help optimize your downstream purification processes and identify any potential HCP impurities in your DS. In addition, AAE-MS represents a more objective and direct method to qualify the ELISA as fit for purpose of detecting those HCP that co-purify with your drug substance.

Advance your HCP analysis and de-risk your downstream purification process development with Cygnus AAE-MS Services:

- ✓ HCP antibody coverage analysis by AAE-MS
- ✓ Process monitoring by identifying and quantifying HCPs in process samples
- ✓ Identification and quantification of hitch-hiker HCPs in the final DS
- ✓ Actionable insights into downstream process development and purification improvements with predictive theoretical values for isoelectric point and molecular weight of HCPs detected in process samples and final DS



Contact the experts at Cygnus Technologies at **techsupport@cygnustechnologies.com** to discuss your current and future host cell protein analysis projects!

Why use AAE-MS approach?

Identification and quantification of HCPs by mass spectrometry (MS) is a powerful complementary method to ELISA, however drug products often mask HCPs by a factor of 10^4-10^6. To improve MS sensitivity, Cygnus Technologies employs our novel Antibody Affinity Extraction (AAE) method as a sample preparation step to enrich HCPs and eliminate most of the DS in a sample.

Effective Enrichment of HCPs and Depletion of DS

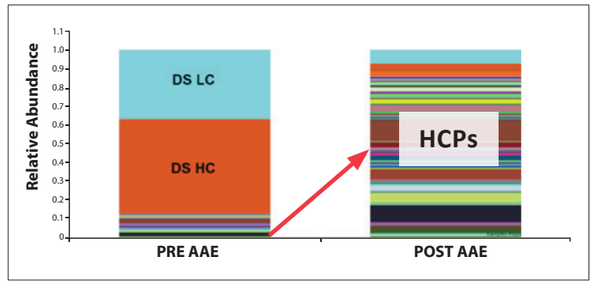


Figure 1. The relative abundance of DS and HCPs according to their extracted ion chromatograms were normalized to one and graphed in a stacked bar chart. The sample analyzed before AAE (Pre- AAE, left) shows the drug substance heavy chain (DS HC) in orange and the drug substance light chain (DS LC) in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

MS Provides PPM, IEP, and MW Data

Integration of orthogonal methods for comprehensive HCP analysis provides data throughout DS purification to inform process development teams of how to modify purification process to ensure DS purity. Analysis of the HCP profile differences from lot to lot demonstrates process consistency within a given process. Comparing HCP content qualitatively and quantitively across different purification methods provides guidance on what HCPs are being affected by various purification methods. It also demonstrates comprehensive quality control data for regulatory agencies.

AAE Enriches Known Problematic CHO HCPs

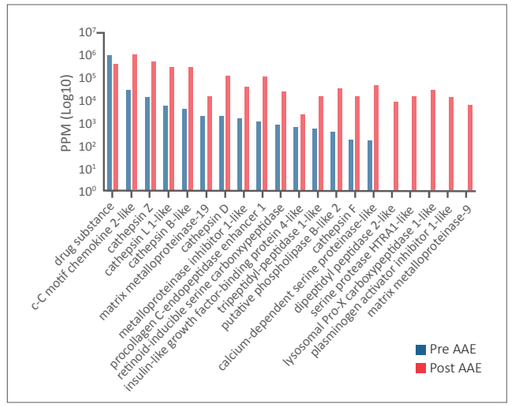


Figure 2. Drug substance (DS) *was analyzed before and after AAE by MS. PPM quantification was calculated relative to the most abundant protein present in each sample. For the Pre-AAE sample, known problematic CHO HCPs were quantified relative to the DS. For the Post-AAE sample, CHO HCPs were quantified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

Protein	PPM	mg/mL	IEP	MW
drug substance	1000000	2.9700	8.49	49607
c-C motif chemokine 2-like	29827	0.0886	9.32	15858
cathepsin Z	14646	0.0435	7.52	34028
cathepsin L 1-like	5712	0.0170	5.94	37298
cathepsin B-like	4116	0.0122	5.73	37504
matrix metalloproteinase-19	2179	0.0065	7.71	58942
cathepsin D	2130	0.0063	6.54	44111
metalloproteinase inhibitor 1-like	1665	0.0049	8.84	22401
procollagen C-endopeptidase enhancer 1	1111	0.0033	8.16	50446
retinoid-inducible serine carbonxypeptidase	829	0.0025	5.31	51255
insulin-like growth factor-binding protein 4-like	659	0.0020	6.77	27726
tripeptidyl-peptidase 1-like	572	0.0017	5.94	61450
phospholipase B-like 2	388	0.0012	5.90	65541
cathepsin F	176	0.0005	6.94	51641
calcium-dependent serine proteinase-like	166	0.0005	4.73	77412

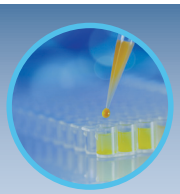
The PPM, mg/mL, isoelectric point and molecular weight of CHO HCPs are shown relative to the DS. Isoelectric point and MW are predictive theoretical values.

Integration of Advanced Orthogonal Methods for HCP Characterization

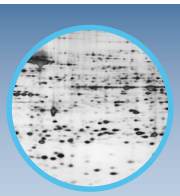
Step 1: HCP antibody and ELISA development using proper upstream source of antigen

Step 2: HCP antibody coverage analysis using AAE followed by 2D-PAGE or MS

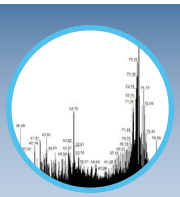
Step 3: ELISA qualification by spike recovery, precision, and sensitivity studies



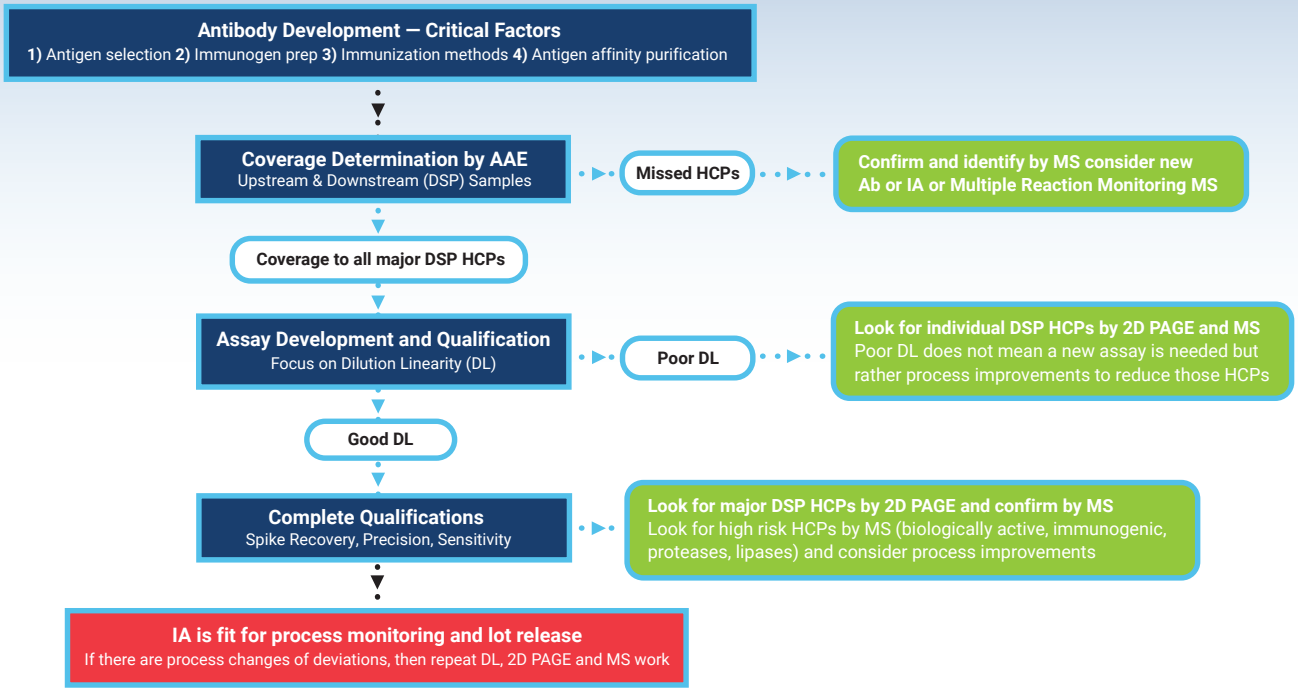
Immunoassay (IA)



Antibody Affinity Extraction (AAE)



Mass Spectrometry (MS)



Step 4: Mass Spectrometry

- ✓ AAE enables removal of DS interference and enrichment of HCPs. While HCPs can be identified and quantified by discovery and targeted MS, mass spectrometry will fail to detect lower abundance HCPs without their enrichment.
- ✓ In the cases where there is a lack of dilution linearity by ELISA, or individual HCPs, these HCPs should be identified and quantified by mass spectrometry.
- ✓ If HCPs are identified by MS and are not being accurately quantified by the total HCP ELISA, then consider development of a mono-specific ELISA or a targeted MS method, or optimize the purification process to better remove HCPs.
- ✓ If there are no detectable individual HCPs in a DS by 2D PAGE, the ELISA shows low total HCP that is confirmed by a "reference" ELISA, and if downstream and DS samples show dilution linearity, then MS serves as a confirmatory orthogonal method.

Contact us at **techsupport@cygnustechnologies.com** to discuss your current and future HCP analysis projects!

Trust Your Assays. Trust Your Results.

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