FRONTAGE
YOUR DRUG DEVELOPMENT PARTNER

HOST-CELL PROTEIN TESTING:

Perspective of Current and Future Techniques

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TABLE OF CONTENTS

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Introduction

Biologics are produced using living cells or organisms. The living cells can be derived from prokaryotic, eukaryotic, and mammalian cells that are genetically engineered to encode the protein of therapeutic use. During the protein expression process, thousands of endogenous proteins, or host-cell proteins (HCPs), are produced and needed to maintain cellular function and regulation, as well as the protein of interest. In cells, the HCPs have a wide range of functions such as growth, proliferation, survival, gene transcription, and protein synthesis, but they are unwanted impurities in the biologics product. Due to their highly diverse physicochemical characteristics, these HCPs can co-purify with the drug substance, and—despite purification steps meant to remove them from the therapeutic protein of interest—low levels can remain in the final drug product, contaminating it.

HCPs CAN CO-PURIFY WITH DRUG SUBSTANCES, RESULING IN LOW LEVELS OF CONTAMINATION

Good Manufacturing Practices (GMP) require that manufacturers demonstrate that they have cleared drug substances of residual HCPs to the lowest level possible prior to releasing their drug products. The methodologies for meeting this challenge are changing rapidly as new instrumentation and techniques are becoming available to laboratories. The following paper outlines the latest developments, focusing on emerging best practices.

Residual HCPs Are Problematic

HCPs, which are process impurities in biologics, pose a high safety risk for patients; it is well known that "the presence of HCP contaminants can result in allergic reactions and other immunopathological effects."[1] HCPs can, for instance, trigger the following pathways to protein degradation:

- Carboxypeptidase derived from a CHO cell line can catalyze the removal of C-terminal lysine and arginine of IgG
- Asparagine endopeptidase can mediate the deamidation of asparagine residue, a common mechanism generating acidic charge variants
- Cathepsins can cause cleavage of IgG, generating C-terminal heavy chain fragments
- Beta-galactosidase is involved in removal of the terminal galactose linked to N-acetylglucosamine in N-linked glycan, and thereby affecting the complement dependent cytotoxicity (CDC) of IgG

HCPs can also negatively impact the stability of the product by causing degradation of the excipients in the formulation, so the presence of HCPs in drug products is often a critical quality attribute. For example, it was known that phospholipase A2 can degrade polysorbate 20 and 80, which are commonly used excipients in the formulation of antibodies, resulting in loss of long-term stability.

Regulators Key in on HCPs

Regulators both in the US and the EU require manufacturers to remove or minimize HCPs in their final biologic products and that they must identify and quantify those that remain. The US Food and Drug Administration (FDA) expects, "contaminants introduced by the recovery and purification process should be below detectible levels using a highly sensitive analytical method."[2] In the EMA, "... residual HCPs have to be tested on a routine basis. As such, it is currently required that HCPs be routinely monitored at the purified bulk level, using suitable analytical assays. Results from batch to batch should be consistent and meet specification limits."[3] "In general, levels up to 10ng residual host cell DNA per dose, depending on the host cell type and the route of administration, and 100ppm for residual host cell protein are acceptable."[4]

Regulators do not, however, specify what tests to use in identifying and quantifying residual HCPs. Indeed, many of the latest methods were not invented when the latest guidance was issued.

[1] ICH Guidance for Industry S6, "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, July 1997.

[2] FDA, "Points to Consider in the Manufacture and Testing of Monoclonal Products for Human Use," 1997.

[3] CPMP/BWP/382/97, The European Agency for the Evaluation of Medicinal Products, Human Medicines Evaluation Unit, June 10, 1997.

[4] Wang, Weihong, Ph.D., Hauri, Phillipp, "Process contaminents in biopharmaceuticals: Host cell DNA and protein," accessed at https://www.eurofins.com/biopharma-services/media/pharma-newsletters/pharma-services-newsletter-03-october-2012/contaminants-in-biopharmaceuticals/

HCPs are Abundant, Heterogeneous and Hard to Detect

The first challenge posed by HCPs is simply that there are thousands of different types of them; HCP composition and abundance is unique to the host cell and the manufacturing process used in the production of the biologic.[5] The second difficulty is that HCPs can be present at extremely low levels (residual amounts of about 1-100 ppm), making them difficult to detect. Analytical methods must have the sensitivity and resolution to be able to separate and detect individual HCPs.

In addition, different HCPs have different impacts on the drug and in the body—not all of which are well understood. It is currently difficult to link specific types of HCPs and concentrations to immunogenicity in humans.[6] Manufacturers should, therefore, as a precaution, assume the worst-case scenario and strive to clear HCPs to the greatest degree possible, based on sensitive and orthogonal assays. Assays should enable manufacturers to identify and monitor the amount of individual HCPs in the purification process.

HCPs pose a particular challenge for biosimilar manufacturers, as they are required to compare process impurities between the proposed product and the reference listed drug. However, the manufacturer of the proposed biosimilar product is likely to have a different manufacturing process (i.e., different cell line, raw materials, equipment, processes, process controls, and acceptance criteria) from that of the reference product and no direct knowledge of the manufacturing process for the reference product. The biosimilar manufacturer has no way of knowing what cell culture conditions, media, or purification process the innovator company used to produce the reference listed drug. Thus, a biosimilar will naturally generate a different profile of residual HCP from the reference listed drug, and the biosimilar sponsor must use its own null cell culture to develop a customized immunoassay for HCP testing, which may not be appropriate for measuring HCPs levels in the reference listed drug.

Several Testing Options Exist

In the early stages of drug development, most sponsors use generic kits based on enzyme-linked immunosorbent assay (ELISA) to detect HCPs. A variety of ELISA kits are commercially available to detect HCPs in a number of different cell lines such as E.coli, CHO, BHK, HEK, HeLa, and A549. However, the particular cell substrate, culture, and purification process used to generate the commercial kit is often quite different from that of the drug under development, so these kits may not be able to detect the full range of protein impurities that are retained in the production process.

In later stages of development, when investigational products are prepared for clinical trials, more sensitive immunoassays are needed. Sponsors often contract with specialty labs for the development of a custom-prepared immunoassay as soon as they have settled on the manufacturing and purification steps. This has traditionally been a very expensive, protracted process. Creating a customized, process-specific immunoassay requires the use of a null culture (which is the production cell minus the product-coding gene) to generate HCPs. These are processed using the same purification chromatographic columns and steps as in the production process, without the recombinant protein of interest. In other words, the same cell line and the medium culture, minus the product protein, is used to generate the null cell culture substrate. (The commonly used ELISA is customarily based on polyclonal antibodies raised to the HCPs used to synthesize the product.)

This process of developing customized polyclonal antibodies using the null cell substrate without the product-encoding gene can take as much as nine months. It involves extracting HCPs, fractioning them into high and low molecular weight antigens, and immunizing animals, as well as purifying and conjugating antibodies.

Table 1 lists the common methods available for analyzing and characterizing HCPs. Each method clearly has advantages and disadvantages, although the industry is moving toward the use of mass spectrometry (MS) to both identify and quantify host cell proteins, as is discussed below.

[5]Wang, F., Richardson, D., and Shameem, M., "Host-Cell Protein Measurement and Control" BioPharm International 28 (6) 2015
[6] Wang, F., Richardson, D., and Shameem, M., "Host-Cell Protein Measurement and Control" BioPharm International 28 (6) 2015

Table 1: Analytical and Characterization Methods for HCP Testing

METHOD	USE	ADVANTAGES	DISADVANTAGES	SENSITIVITY AND QUANTIFICATION
2-D Gel Electrophoresis	Separate HCP impurities, suitable for comparability and similarity, but not suitable for routine use	Detect individual spot of HCP impurities by molecular weight and pI	Require special skills, difficult to reproduce protein spots	Semi-quantitative of spot intensity at approximately 10 ng detection limit
ELISA	Suitable for routine use, including process validation and lot release	Highly sensitive to HCP impurities based on binding to polyclonal Ab (pAb)	Require process-specific custom pAb at clinical stage; doesn't separate individual HCP impurities	Quantitative of total amount of protein at approximately 10 ng detection limit
2D-Liquid Chromatography MS/MS	Reversed phase separation of proteolytically cleaved HCPs; suitable for routine use as well as comparability and similarity	Identify and quantify individual HCP impurities by MS/MS	Require expensive instrument and data processing software	Quantitation of individual HCP impurities at approximately 0.5 – 100 ppm detection range
Capillary Electrophoresis MS/MS	Electrophoretic separation of proteolytically cleaved HCPs; suitable for routine use characterization	Quantify individual HCP impurities by MS/MS	Require expensive instrument and data processing software	Quantify individual HCP protein at approximately 0.5 – 100 ppm detection range

Source: USP <1132> Residual Host Cell Protein Measurement in Biopharmaceuticals.

Best Practices Are Emerglng with New Testing Methods

Fortunately, advances in MS have allowed new methods to be developed for testing for HCPs. Using MS, the protein impurities, along with the drug substance, are digested enzymatically to generate peptides. The peptides are then separated on two-dimensional liquid chromatography (2DLC) or capillary electrophoresis (CE). The peptides are injected into the MS instrument for analysis using a data-independent acquisition (DIA) method (e.g., the MSE method). The peptides can then be identified by the signature of the mass values from the MS/MS. (Signature mass values are named in a database/library.)

MS technology offers the advantage of being able to identify individual host cells proteins and to quantify them, regardless of the cell line, cultures, or purification steps used. It measures them all. And, the final results are a quantification of individual HCPs, rather than a lump sum of their total quantity. (In contrast, immunoassays can only generate an overall number of HCPs.)

One particularly valuable DIA method is Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra (SWATH-MS). The SWATH-MS technique offers several benefits:

- It uses the signature peptides derived from different HCPs to identify a wide range of proteins, and it can quantify them as well.
- It is highly sensitive, so can detect HCP impurities at sub-ppm levels.
- It doesn't depend on the null cell culture, nor does it depend on the precursor ions.

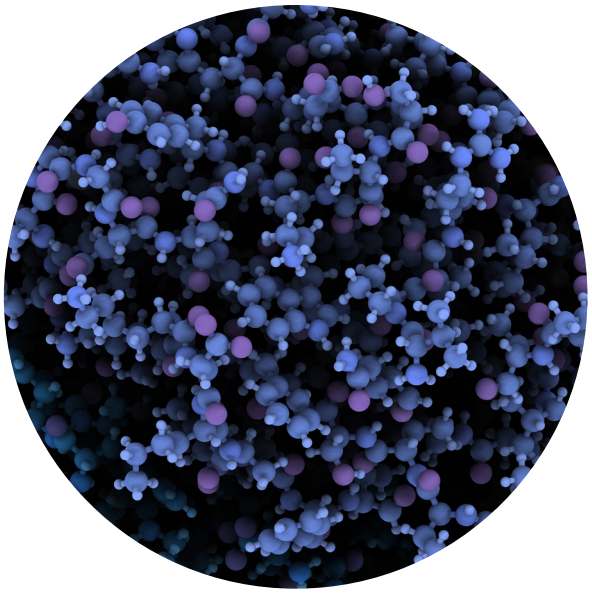
- Developing the method takes mere weeks, and it can be used even after changes in cell culture conditions, media, and purification chromatographic columns.
- Because it directly identifies a range of HCPs and their relative levels, it makes it possible to monitor ho well specific protein impurities are cleared in downstream purification steps.

MS Technology Is the Way of the Future

Laboratory methodologies have progressed substantially since the FDA released its guidance on HCPs nearly two decades ago.

Using MS/MS to quantify individual HCP impurities that co-purify with drug substances provides a sound orthogonal method that overcomes the shortcomings of ELISA. It is highly sensitive and can both quantify and qualify HCPs. It is also less tedious and far less time-consuming and expensive than developing a customized ELISA. Given its advantages, it is today's best practice approach, and several sponsors have already benefited from it. We believe that eventually MS/MS will be the common method of choice for sponsors.

We foresee that in time, the cost will come down still more as MS equipment becomes more specialized for individual applications. (Currently labs must buy all-purpose machines.) At the same time, the industry needs to conduct bridging studies comparing ELISA and MS/MS methods and to develop a library of HCPs for different cell lines.



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