ProtaGene
Analytical Excellence

APPLICATION NOTE

Mass Spectrometry-based Host Cell Protein Analysis in Biologics Development

Highlights

Advantages of LC-MS based HCP profiling for development of complex biologics includes:

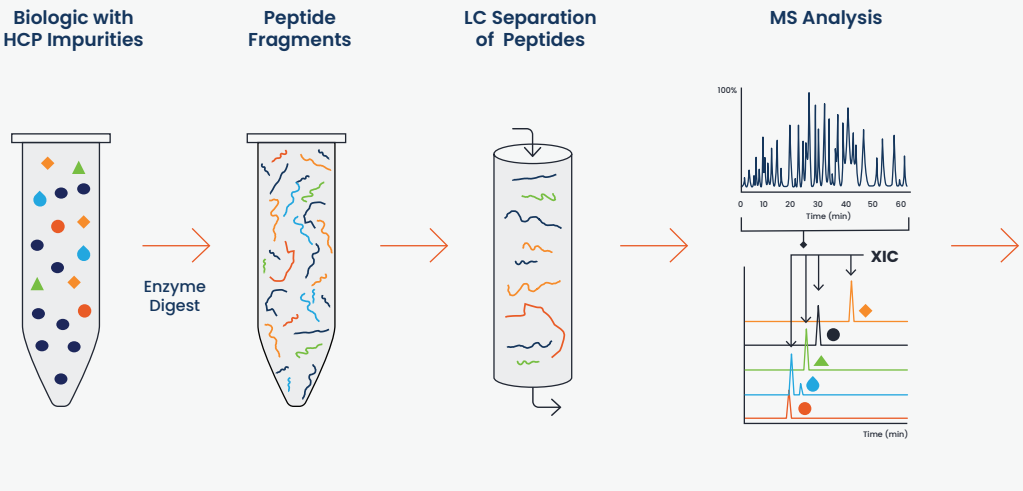
- Sensitive Detection of Peptides
- Excellent Accuracy and Precision
- Fast Estimate of Concentration Based on Selected Peptides
- Rapid Assessment of Relative Amounts
- Effective Comparison Among Samples
- No Custom Immunoreagents Required

Introduction

Host cell proteins (HCPs) are impurities present in all therapeutics derived from biological sources, and as a critical quality attribute (CQA), they must be characterized in detail and controlled through the manufacturing process and in the final drug product.

The individual protein contaminants that make up the HCP profile may vary significantly among individual biologic products, including cell and gene therapies and expression systems, even for very closely related molecules. As such, the HCP profile must be analyzed to identify even low levels of potentially concerning species in a biologic drug.

LC-MS Approach to HCP Profiling



HCP Identification & Quantitation for Decision Making

HCP	CP Level	OK
Carbonic Anhydrase	72	✓
Protein Disulfide Isomerase	67	●
Protein A	34	▼
Serum Albumin	29	✓
Action, Aytoplasmic	26	✓
Polyubiquitin C	18	✓
Lipoprotein Lipase	12	✓
Cathepsin D	9	✓

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Europe
Inselwiesenstraße 10
74076 Heilbronn Germany

North America
4 Burlington Woods Drive
Burlington, MA 01803 USA

ProtaGene.com
info@protagene.com

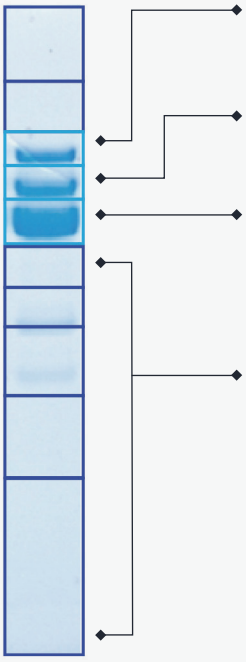
To ensure consistency of product manufacture and shelf-life stability, and to minimize potential adverse clinical reactions, significant attention must be paid to identifying HCPs that remain in a biologic product following purification.

Once identified, HCPs are often monitored and the levels controlled by process development decisions, to consistently yield a safe and efficacious product.

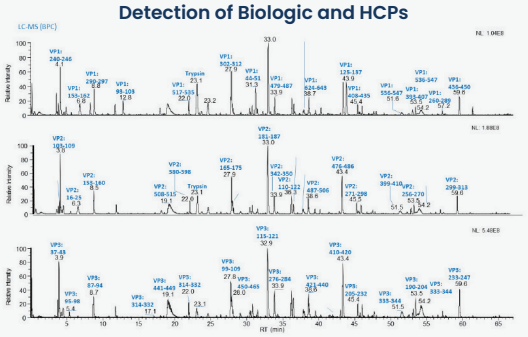
While ELISA methods have historically been the main approach to detecting total HCP content, LC-MS-based HCP detection methods have increasingly become an expected orthogonal standard in successful biologic development.

LC-MS Characterization

Process Gel Bands



Detection of Biologic and HCPs



Identified HCPs and Levels

PROTEIN #	ACCESSION #	DESCRIPTION	SAMPLE 1	SAMPLE 1	SAMPLE 1
1	P0DMV9	Heat shock 70 kDa protein 1B	1719	157	< 10
2	P12268	Inosine-5'-monophosphate dehydrogenase 2	1664	10	30
3	P50990	T-complex protein 1 subunit theta	735	< 10	ND
4	P78371	T-complex protein 1 subunit beta	527	< 10	< 10
5	P49368	T-complex protein 1 subunit gamma	373	< 10	< 10
6	Q13347	Eukaryotic translation initiation factor 3 subunit I	366	< 10	< 10
7	P38159	RNA-binding motif protein, X chromosome	348	ND	29
8	P38646	Stress-70 protein, mitochondrial	343	< 10	ND
9	Q99832	T-complex protein 1 subunit eta	321	< 10	< 10
10	P17987	T-complex protein 1 subunit alpha	309	< 10	< 10
11	P48643	T-complex protein 1 subunit epsilon	196	< 10	< 10
12	Q92688	Acidic leucine-rich nuclear phosphoprotein 32 family member B	162	76	88
13	P12532	Creatine kinase U-type, mitochondrial	152	129	140
14	O00487	26S proteasome non-ATPase regulatory subunit 14	150	< 10	< 10
15	P28331	NADH-ubiquinone oxidoreductase 75 kDA subunit, mitochondrial	139	ND	ND
16	P68104	Elongation factor 1-alpha 1	122	17	24
17	Q9Y265	RuvB-like 1	116	12	< 10
18	P60709	Actin, cytoplasmic 1	110	< 10	< 10
19	P0CG48	Polyubiquitin-C	270	101	121

HCP Profiling by LC-MS

As a modern approach with advanced instrumentation, HCP profiling by LC-MS provides comprehensive identification and relative quantitation of these impurities down to low single-digit ppm. LC-MS analysis of HCPs has quickly developed into a set of approaches that can be performed to profile the diverse set of species and target quantitation of specific components. HCP characterization can be conducted at an early stage to assess the profile quickly and then may be applied with greater rigor at a later stage to support BLA/ regulatory filings.

The most basic method framework typically involves subjecting the sample to enzymatic digestion, followed by LC separation of the resulting peptide fragments with high-resolution MS detection. The MS data is then analyzed to enable identification and quantitation of HCP impurities. A comparison of HCP profiles from multiple lots can be used to assess process consistency and differences in profiles from samples generated using different process parameters to provide information that guides ddecision-making in process development.

To completely identify the HCPs in any given test article, a combination of two MS data acquisition settings (methods) may be required. Data-dependent MS acquisition targets known peptides for identification from a pre-existing library are used because it is fast, robust, and sensitive, which is well-matched for monitoring impurities found at low levels. Data independent acquisition (DIA) is used to identify untargeted HCPs, and the results of DIA can be verified and provide quantitation using parallel reaction monitoring (PRM).

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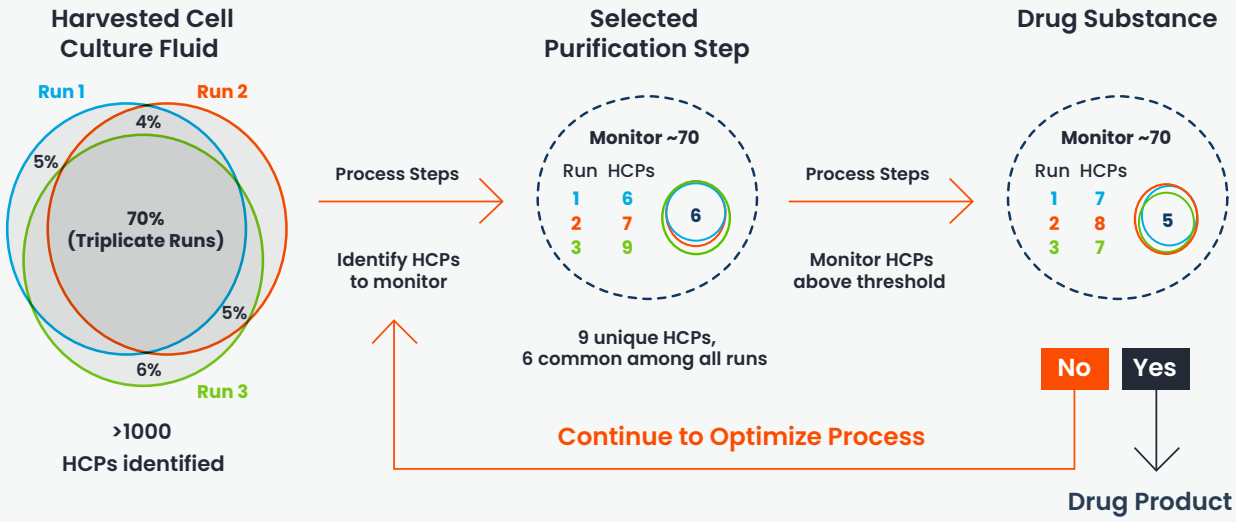
Partner with ProtaGene for Successful LC-MS HCP Profiling

To obtain accurate information using LC-MS profiling of HCPs to guide decision making depends on a few key aspects.

We take a rigorous approach to ensure high-quality results for all projects through:

- Initial Development and Optimization of the Method for Each Unique Product/System
- Thoughtful Attention to Individual Sample Handling and Preparation
- Generation of a Complete Peptide Library for the Host Cell Source

HCP Profiling and Comparability by Process Step



HCP profiling is applied to a wide range of biologic products, from antibodies to enzymes to gene/cell therapies, which are manufactured using diverse cell types. There are substantial differences in the possible HCP impurities derived from different host cell sources, and we have developed complete peptide libraries for common expression systems, including CHO, HEK, and *E. coli*. We have also worked with our clients on unique host cell systems to develop customized, corresponding libraries.

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Europe
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