Mass Spectrometric Evaluation of Host Cell Protein Patterns in Biopharmaceutical Products

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Introduction

Developing and producing recombinant biopharmaceuticals in mammalian cells requires unambiguous monitoring of HCPs impurities. Immunoassays are still the method of choice for release testing, for which it is recommended to demonstrate the suitability of antisera in a QM-regulated environment to meet regulatory demands. However, peptide analysis by MS has been proven to be a valuable tool by providing complementary data for HCP characterization to identify and quantify HCP. In particular, only little is published about identities of HCP present in marketed biopharmaceutical products.

Mass Spectrometry in HCP Analysis

Here, we evaluated identities of HCP in marketed recombinant IgG products and quantified individual HCP, which are suspected to be critical for the product stability and quality.

First, we analyzed HCP in Drug Product (DP) of different market approved mAb products. Detection of HCP with low abundance is still critical and the risk of false positive detection exists. Therefore, we conclude additional validation of detected HCP peptides in DS for example by using heavy isotope synthetic peptides is crucial.

Stable Isotope-labeled standard peptides (SIS) were synthesized and spiked into the sample with a known concentration. The PRM approach (parallel reaction monitoring) allows for validation of the presence of individual HCP with high confidence and determination of the absolute protein amount. The absolute amount of the specific HCP was determined using the SIS peptides.

Principle of MS-based HCP identification and label-free quantification

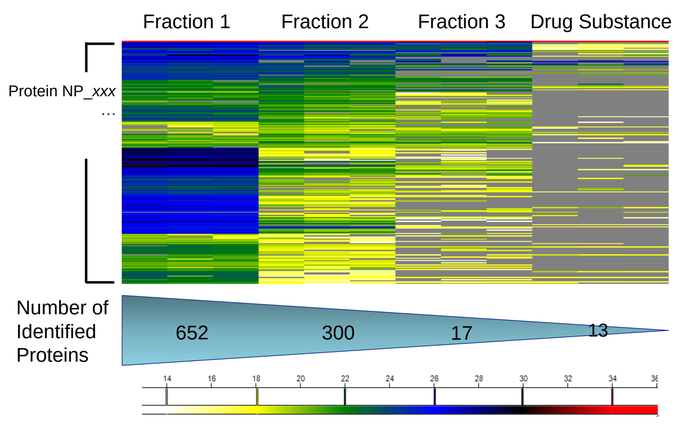
Label-free quantification is a method in mass spectrometry that aims to determine the relative amount of proteins in two or more biological samples.

It allows the unbiased discovery of HCP impurities and its quantification.

PPS is using this approach for evaluation of downstream processing steps to elucidate effectiveness.

Process Sample Harvest / Protein A Eluate / DSP → **Digestion** → High quality **LC-MS** (Vanquish, HF-X) → MS/MS Spectra → Database Searches → **Identification** of Proteins (HCPs)

LC-MS Data → Feature Alignment → **Detection** & **Quantification** of low abundant Proteins

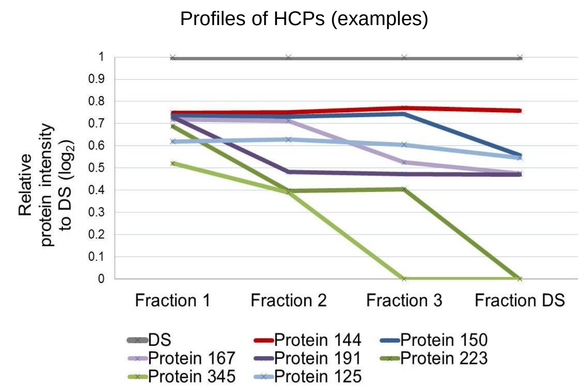


Monitoring of HCPs in Downstream Process

Label-free quantification was used for monitoring of HCPs in fractions obtained from downstream process steps.

Generated Heat Map lists the identified HCPs and shows their abundance in different DSP fractions.

Exemplary profiles demonstrate the variable behavior of the HCPs across the purification steps.



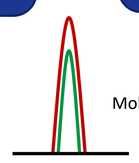
=> ***Mass Spectrometric Evaluation of Upstream and Downstream Process Influences on Host Cell Protein Patterns in Biopharmaceutical Products***

Falkenberg et al. 2019, Biotechnology Progress

HCP Quantification using internal standard (PRM)

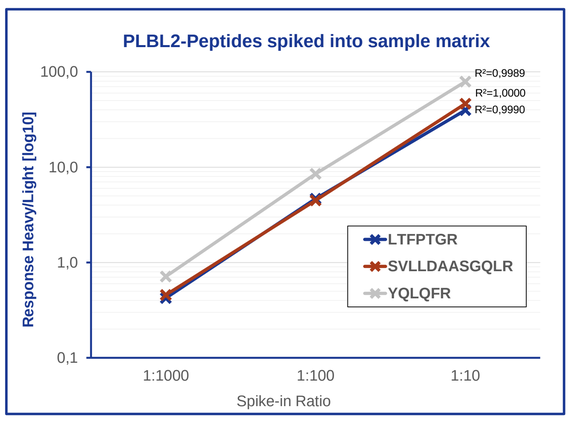
Process Sample Harvest/Protein A Eluate/DSP → **Digestion** → High quality **PRM-LC-MS** (Vanquish, HF-X) → **Absolute Quantification**

Add **synthetic peptides** ($^{15}N/^{13}C$ labeled)



$$\text{Mol HCP} = \frac{\text{AUC HCP}}{\text{AUC IS}} \times (\text{Mol IS})$$

Host Cell Proteins of special interest (examples)
Peroxiredoxin-1
Phospholipase-B-like 2 (PLBL2)
Cathepsin L1
Phosphoglycerate kinase 1
Glyceraldehyde-3-phosphate dehydrogenase
Elongation factor 1-alpha 1
Serine protease HTRA1
≥ 2 peptides per protein



The use of internal standard (IS) combined with mass spectrometric LC-PRM (parallel reaction monitoring) is a reliable method for absolute quantification.
The robust nature of this approach offers the technical potential for validation and usage as GMP release testing for biopharmaceuticals to address the purity.

An LC–SRM experiment measures the abundance of a specific fragment ion (extracted ion chromatograms, XIC) from both, the native HCP peptide and the IS peptide as a function of reverse-phase chromatographic retention time (peak area). The absolute quantification is determined by comparing the abundance of the known IS peptide with the native HCP peptide.

Here, for seven HCPs of special interest synthetic peptides were generated and the absolute amount of each HCP was determined using SRM.

For each peptide the best fragments for quantification down to sub-ppm-level were determined.

LOQ and LOD were defined for each HCP.

The linearity of the absolute quantification was determined by spike-in heavy peptides into a sample matrix with ratios from 1:1000 to 1:10.

The response of the heavy/light-ratio was linear in the investigated range of three magnitudes and can be determined below 0.3 fmol/µg in relation to the Drug Substance.

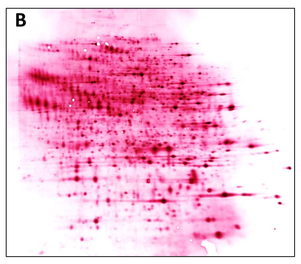
Characterization of HCP antiserum with 2D-PAGE supported by MS

2D-PAGE is addressing the characterization of denatured (linear) epitopes of HCP antisera.

PPS superior 2D-PAGE techniques combined with Western Blotting is a most powerful technique to demonstrate antisera coverage.

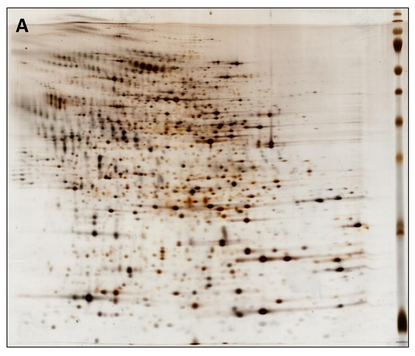
Spot-ratios (% coverage): Blotting efficiency

89 %

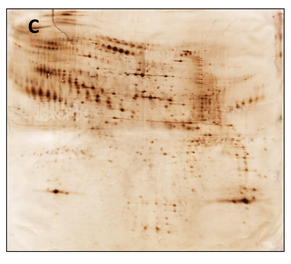


2D-Western Blot Colloidal Gold stained
1762 detected spots

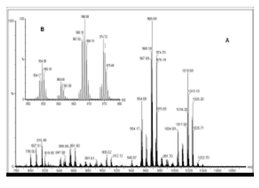
2D-SDS-gel Silver stained
1980 detected spots



Spot-ratios (% coverage):
Antiserum coverage 81 %



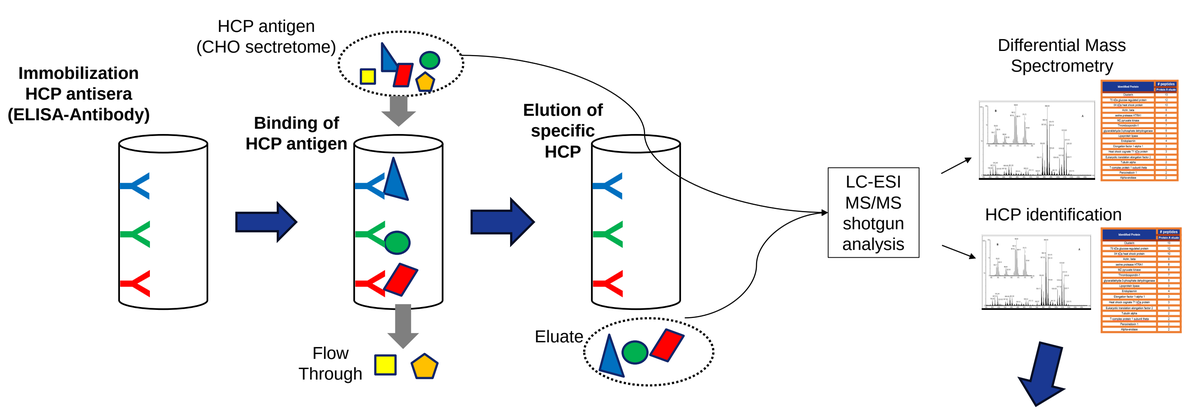
2D-Western Blot Immunodetected
1597 detected spots



Mass spectrometry spot identification

Detection gaps are identified using mass spectrometry

Characterization of HCP antiserum with Affinity Chromatography



Affinity chromatography combined with mass spectrometric based HCP identification is used to specify the antigen coverage.

Affinity chromatography is addressing the characterization of non-denatured (native) epitopes of HCP antisera.

Thus, this method can be used as a complementary approach to 2D-PAGE for linear epitope characterization.