High Coverage Process Specific HCP Identification and Quantification
Using Mass Spectrometry

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BACKGROUND

Regulatory trend towards increasingly deeper HCP characterization
• Regulatory framework: 42 USC 262, ICH Q8B, ICH Q9
• Current practice of using immunoassays has well-recognized gaps; little is known about individual HCPs
• Post-market commitments for development of an HCP assay with improved coverage was required for
3 of 8 BLAs approved in 2014 due to insufficient characterization of HCPs

Mass spectrometry is playing a greater role in characterization of HCPs
• "Immunoassay and (increasingly) mass spectrometry are highly complementary and the most
powerful methods for monitoring residual HCP levels in samples and confirming their absence in
final DS." - USP 1132

HCP IDENTIFICATION

LC-MS/MS Identification & Relative Quantification of HCP

<table>
<thead>
<tr>
<th>Process Quality Controls (PQC)</th>
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<tbody>
<tr>
<td>Highly characterized, defined nature of HCP</td>
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<tr>
<td>Process Enzyme: Trypsin, V8, Glu-C</td>
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Identification of HCP

- Mass spectrometry
- Characterization of HCP sequences
- Characterization of HCP peptide sequences

Comparison of HCP content in Biosimilars vs Innovators

Data shown from two experiments comparing Biosimilars vs Innovators using either
different manufacturing replicates (A) or lots (B).

Demonstration of HCP Clearance During DS Purification

- Use of mass spectrometry shows a decreasing number and concentration of HCPs across the purification process. Protein (A) and spectra (B) data shown.

EXTENSIVE FRACTIONATION PROVIDES INCREASED HCP COVERAGE

- Use of two orthogonal fractionation methods typically provides 30-50% more
HCP peptide identifications and 5-20% more protein identifications
- Venn diagrams showing degree of non-overlap from use of two different
fractionation methods on different DS in various host systems (protein level)

HCP QUANTIFICATION (ABSOLUTE)

- Targeted Multiplexed LC-MRM/MS Assay Development
  • Prioritize list of HCPs
  • Select 55 signature peptides per HCP
  • Use synthetic isotope-labeled peptides to develop LC-MRM/MS
    assay conditions
  • Final optimized assay monitors two fragments (transitions) per peptide

Absolute Quantification of HCP using LC-MRM/MS

- Calibration curves (BIA or pooled DS)
- Spike light peptides: (Low, Mid, High)
- Spike light peptides: (≥7 non-specific sequences)

Quantitative Assessment of HCP Clearance

- Data shows successful clearance of two individual HCPs across the purification process
- Two peptides from the same protein yield similar results

SUMMARY

Caprion’s Mass Spectrometry Platform has been used for the following
types of HCP client studies:
• Characterization of in-process samples and DS
• Demonstration of HCP clearance
• Characterization of HCP from DS expressed in various host expression systems
• Comparability studies of Biosimilars vs Innovators

Mass Spectrometry Platform Features and Applications for HCP

- Extensive Fractionation
- LC/MS/MS Analysis
- Assay qualification
- Absolute quantitation of individual HCPs
- Relative quantitation of identified HCPs
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- Relative quantitation of identified HCPs

Applications for Process Development and Manufacturing

- Monitoring of purification process
- Demonstration of HCP Clearance
- Compare culture media, process improvements
- Evaluation of batch reproducibility and scale-up

HCP QUANTIFICATION

- Study samples (Individual DS)
- QC samples (pooled DS)
- Digestion
- Detailed/ MRM analysis

Endogenous levels, Precision and Accuracy
- Quantitative measurement using validated assay

Calibration curves (BIA or pooled DS)
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