Roundtable Session – Table 6 – MS for HCP

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Abstract:

Host Cell Proteins (HCPs) are a critical concern in the biopharmaceutical industry, as their presence can compromise product safety, efficacy, and regulatory compliance. Accurate, comprehensive, and sensitive detection and characterization of HCPs are therefore essential for downstream processing, product release, and ongoing process validation. Traditional immunoassays, while valuable, often lack the breadth and specificity needed to fully understand the complexity of HCP profiles. In contrast, mass spectrometry (MS) has emerged as a transformative analytical platform, enabling in-depth exploration of HCP landscapes with high resolution and throughput.

This round table aims to facilitate an engaging discussion on the latest advancements in MS-based HCP analysis, including innovative sample preparation workflows, advances in instrumentation, and quantification methods. We will examine the integration of MS with proteomics approaches, bioinformatics tools, and database development, which collectively enhance the detection, identification, and quantification of HCPs across various bioprocess stages. Attendees will share insights into overcoming analytical challenges such as dynamic range, matrix effects, and data interpretation.

The session will also emphasize the strategic importance of continuous improvement and innovation in analytical technologies, as well as the critical role they play in advancing bioprocess understanding and optimizing manufacturing workflows. Additionally, the potential for MS to provide detailed molecular insights into host cell protein profiles will be discussed as a pivotal factor in developing and manufacturing biotherapeutics.

Discussion Questions:

1. **How are advances in mass spectrometry instrumentation changing your HCP analyses?**
    a. **NOTES:**
        i. Dynamic Range & Sensitivity: HCP analysis faces challenges due to the large dynamic range and need for highly sensitive assays.
        ii. Sample Preparation & Software: Complex sample prep and advanced software are required to process and interpret data.

iii. Instrumentation Advances: Implementation of high-speed mass spectrometry (e.g., Astral MS) has improved capabilities, allowing for narrower m/z windows and more comprehensive data acquisition.
iv. Impact: New MS platforms (e.g., Astral MS) enable higher resolution, throughput, and sensitivity, allowing for deeper exploration of HCP profiles.

b. What are the major bottlenecks still facing HCP analyses?

i. NOTES:

1. Bottlenecks: Despite progress, challenges remain with dynamic range, matrix effects, and data interpretation. Sample preparation and data processing are still bottlenecks, especially for large datasets and complex samples

2. How do you balance the tradeoffs between high throughput and sensitivity of analyses?

a. NOTES:

i. Tradeoffs: Labs must balance the need for high throughput (processing many samples) with the sensitivity required to detect low-abundance HCPs.
ii. Workflow Innovations: Standard flow and nano-spray techniques are compared to optimize both throughput and sensitivity. Automation and software improvements are helping, but manual validation is still time-consuming

3. What quantitation methods are you currently using in your lab? Relative to biotherapeutic, protein spikes, absolute quantitation?

a. NOTES:

i. Current Practices: Labs use a mix of quantitation strategies:
    1. Surrogate peptide libraries
    2. Protein spikes
    3. Absolute quantitation with reference standards (e.g., BSA)
    4. Relative quantitation to biotherapeutics
ii. Challenges: Digestion efficiency varies by protein and cell line. Some organizations manufacture their own reference proteins for reliability

4. At what stage in drug development are you implementing HCP analyses?

a. NOTES:

i. Implementation: HCP analysis is performed at multiple stages:
    1. Early purification (to inform process development)
    2. Regulatory filings (to meet compliance thresholds)
    3. Investigation of specific issues (e.g., immunogenicity, degradation)

b. What benefits do you see in earlier applications?

i. NOTES:

1. Early-stage analysis helps tailor purification strategies and optimize manufacturing workflows. Routine testing often uses ELISA, but MS is increasingly requested for deeper investigation

5. What roles do bioinformatics, AI, and advanced computational methods play in MS analyses of host cell proteins?

a. NOTES:

i. Role: Integration of MS with proteomics, bioinformatics, and database development enhances detection, identification, and quantification of HCPs.

ii. Current State: AI is promising for database mining and annotation, but not yet fully reliable for advanced analysis. Automation is improving efficiency, but manual review remains essential for high-confidence identification

Additional Topics Discussed:

6. Database Management & Collaboration

a. NOTES:

i. Internal Databases: Labs use tools like Skyline and custom databases to track HCPs, often tailored to specific cell lines or products.

ii. Collaboration: Sharing databases and software solutions is encouraged to improve coverage and risk assessment. There is interest in developing comprehensive, community-driven resources

7. Sample Prep & Digestion

a. NOTES:

i. Methods: Shotgun digestion, native and denaturing approaches, and spiking of internal standards are common. Consistency and avoiding detector saturation are ongoing challenges

8. Biological Relevance & Risk Assessment

a. NOTES:

i. Interpretation: Analytical chemists rely on biologists to determine which HCPs are relevant for further investigation. Databases of known problematic HCPs (immunogenic, enzymatic, etc.) are being developed and shared

9. **Continuous Improvement & Innovation**
    a. **NOTES:**
        i. Strategic Importance: The session emphasized the need for ongoing innovation in analytical technologies to advance bioprocess understanding and optimize manufacturing workflows.
        ii. Molecular Insights: MS provides detailed molecular insights into HCP profiles, which is pivotal for developing and manufacturing biotherapeutics