Table 3: Deep Dive into HCPs and Polysorbate/Product Degradation

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Scope:

Host cell proteins (HCPs) present a potential safety risk in biopharmaceutical products, not only as impurities, but also as active enzymes that can modify products and degrade formulation excipients such as polysorbates. Consequently, the identification and quantification of HCPs and the detection and characterization of excipient degradation products are critical quality issues in the manufacture of biotherapeutics. In this roundtable, we intend to deep dive into characterization and analytical challenges in detecting trace level of HCP and polysorbate/product degradation.

Questions for Discussion:

1. What are the main analytical challenges in detecting impurities in biopharmaceutical formulations?
    a. Sample prep? Analyte detection? Data analysis?
    b. What special challenges do HCPs present?
    c. What special challenges do PS80 degradation products present?
2. How does MS enable us to meet these challenges?
    a. What are current best practices?
    b. Are there any emerging MS-based approaches?
3. Can bioprocess control and/or purification strategies mitigate risks related to the presence of HCPs and polysorbate/product degradation?
    a. What should be monitored to control PS degradation?
    b. Should specific HCPs be monitored?
    c. Should we move away from PS and consider other excipients (e.g. poloxamer)?
4. What are the expectations of regulatory agencies?
    a. Should MS be used in combination with other techniques?

Discussion Notes:

- Who is doing HCP analysis by MS?
    - 5 persons answer yes
- What is the sample prep for HCP analysis?

    - DIA
    - Should we precipitate mAb? If so, we might precipitate some HCPs.
    - Someone else: no, you just reduce your sensitivity if you don't crash.
    - If HCPs are found in the pellet of crashed mAb, then they are found in the supernatant
- What is the method of choice for HCP analysis?
    - It depends on sample prep
- What samples are we dealing with? DS? PAP? AEX? CEX?
    - All type of samples
- What are the criteria to accept an HCP?
    - 2 unique peptides + MSMS data – basic criteria for publication and making sure there is no interferences
    - Do not trust MS only data
    - What about replicates?
        - Between 1-2 replicates
        - Randomized digestion is known but not common
        - Use of ref standard to account for variation in MS
- Anybody has tried different instrument to analyze the same set of samples for HCP analysis?
    - Different instrument will lead to different sensitivity
- What do we consider better for an instrument? More HCPs?
    - Spiking is a good idea to help to establish the lowest LOQ
    - Exploris helps reducing the number of ions entering the MS
- Uses of LC-MRM to do quantification?
    - Some are doing relative quantitation using a known concentration spiked sample
    - Finding the right peptides/proteins to do quantification is complicated
- Use of ion mobility for analysis?
    - Improve quality of peptide matching and align MSMS and MS data

- If you know that you have an HCP but cannot find 2 unique peptides, what to do?
    - Some cases are limited by the number of unique peptides
- Specific HPC to specifically monitor? Any methods have been developed?
    - Scoring in terms of criticality and abundance
    - Intern knowledge (database) to know which HCP impacts negatively the process
    - Top5 HCPs are analyzed by MRM
    - Relative and absolute quantification goes hand in hand to quantify HPCs in process