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

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Scope:
Evolving evidence has indicated that degradation of product/excipient (i.e. polysorbate) components can occur during biologics storage. The challenge increases with higher cell densities and titer fermentation in bioprocess, and higher drug concentrations in formulation. Enzymatic activity from residual host cell enzymes such as lipases, hydrolases and esterases plays a major role for polysorbate degradation. Their high activity makes them active at very low concentration constitutes a major analytical challenge in the biopharmaceutical industry. Through recent advances in analytical technologies, especially LC-MS, our capability to identify and quantify trace levels of process related HCP has strengthened our capacity to observe and better understand product/excipient degradation. In this roundtable, we intend to deep dive into characterization and measurement of trace level of HCP and polysorbate/product degradation

Discussion Notes:

- PS 80 is used as stabilizer, contains fatty acid component.
- It is necessary to understand how PS80 degredates and what the degradation products do to the mAB.
- Degradation products may change the pH.
- The biggest challenge is to detect the lipases below the 1 ppm level, peptides from the therapeutic protein are too dominant and mask the enzymes.
- To put samples on accelerated stability is the fastest way to detect if you have an active lipase in you product; identify lipases with MS.
- Can you distinguish if degradation is chemically or enzymatically?
    - Polysorbate degradation is mainly due to enzymatical degradation under typical formulation condition.
    - Lipase activity assays can be a way.
    - Chemical induced oxidation can be detected by MS. Looking for oxidized vinyl ester fragment ions (M+14 Da and/or M+16 Da).
    - Enzymatic degradation can be reduced by inhibitors.
- There is a big difference between PS20 and PS80 in terms of degradation.

Commercial polysorbates often contain different kinds of esters in different levels. PS80 with 99% pure in oleic acid content is available on the market. There is also a large difference in the composition of PS80 from different vendors due to various sources of fatty acids used in manufacturing PS80.

- How to avoid PS poluting the mass dector?

    - Answer: Digest the product, dilute out the PS from the sample, so it is not a problem for the MS. High temperature precipitation step in sample preparation is also possible.
- PS80 coelutes with tryptic peptides; free fatty acids, as degradation products, also elute in the middle of the RP-LC chromatogram.
- Who has experience with a single quad method for degradation products?
    - Answer: We use the QDa from Waters.
- What do you monitor when you want to control PS degradtion, what is considered in PS, what are you looking for?
    - Answer: Fatty acids, stearic acid, oleic acid, lauric acid are typically monitored.
- Which lipases tend to be problematic in CHO system?
    - Answer: Serine hydrolases, esterases, lipoproatein lipase, LPLA2.

A reference is provided: Anal Chem, 93 (23) (2021), pp 8161–8169

3. Identification and quantitation of high-risk HCPs for polysorbate/product degradation.

Source of HCPs: Mammal/Insect/Yeast/Bacterial

Types of HCPs that might contribute to polysorbate degradation

Strategies to remove or deactivate HCPs.

Detection and measurement of residual HCP in DS/DP.

Assays to identify and quantify ppb levels of high-risk HCPs by LC-MS and their respective LOD/LOQ

- Detection of HCPs: Nanoflow LC is a difficult technique to detect HCPs. Any other option?

Proteomics or 2D LC online or offline with subsequent library search is an option to identify HCPs.

- Do you know good resources on information about HCP analysis?
    - Answer: There are lots of resources that cover the topic, you need to take information from different sources. Most vendors have a software designed for HCP analysis.
- Does anyone have experience with E. Coli HCPs?
    - Answer: Usually the concentration of HCPs in E. Coli is very low.

4. Process control strategies to mitigate polysorbate/product degradation during product development

Upstream

Downstream

Formulation

Analytical toolbox

5. HCP/lipases risk assessment strategy

How do you present high-risk HCP information to regulatory agencies?
What are the expectations from the regulators?

Ref:

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