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, which presents a concern from a product quality perspective. The challenge increases with higher cell densities and titer fermentation in bioprocess, and higher drug concentrations in formulation. 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 trace level of HCP and polysorbate/product degradation.

Questions for Discussion:

1. How often have you seen significant polysorbate/product degradation in your products under development? How to differentiate it is chemical or enzymatical degradation? What is the root cause investigation strategy?
2. Which LC-MS workflow (DDA/PRM/MRM/DIA) do you use for ID and quantify high-risk HCPs for polysorbate/product degradation? What is the LOD/LOQ? How to identify and quantify ppb level of high- risk HCPs by LC-MS? Besides LPLA2, LPL and liver carboxylesterase B-1- like protein for polysorbate degradation, which other enzymes have been identified for polysorbate degradation? Is PLBL2 responsible for polysorbate degradation?
3. What has done to characterize the raw material polysorbate (i.e. LC, LC-MS, peroxide analysis)? Are there any control limits for purity or peroxides going into the formulation? Have you used LC-MS to characterize polysorbate degradation products?
4. What is your HCP/lipases risk assessment strategy? How do you present high-risk HCPs information to regulatory agencies? What are the expectations from the regulators?
5. What are the process control strategies for polysorbate/product degradation during product development? Any experiences on cell line engineering/clone selection/upstream/downstream/formulation to remove or deactivate high-risk HCPs? What is your analytical toolbox besides LC-MS for high-risk HCPs? Do you have generic and high-throughput enzyme activity assays to assess and screen those high-risk HCPs?

Discussion Notes:

Re Q1

- Polysorbate degradation has been widely observed in industry for mAb programs under development.
- Polysorbate degradation is mostly due to enzymatical degradation since chemical degradation has extremely low rate at typical formulation conditions at close to neutral pH.
- PS-20 promotes the formation of visible particle since it releases saturated fatty acid, which has poor solubility. In contract, PS-80 promotes the formation of subvisible particle.

Re Q2

Methods

- HCP ID and semi-quan: Some companies report sub-ppm range with the use a limited digestion sample preparation method (Lihua Huang, Anal Chem 2017). A leading company achieved ppb level of lipases (LOD/LOQ of ~ 20 – 50 ppb). High dynamic range of the MS method is the key factor for the success (limit digestion, increase accumulation time, tighten the mass range to filter out interfering signals). Another company claims to double the identifications using the limited digestion method.
- A variety of instruments have been used: Fusion, Fusion Lumas, QE HF-X, G2-XS, etc. No much experience about TIMS-TOF Pro: may have some edges over QE HF-X, good for complex sample, raw file is large,
- For HCP quantitation (MRM): TripleTOF from Sciex, QQQ from Waters, ~ 1 ppm
- SISCAPA assay for AP-MS has the potential to be an ultrasensitive assay
- Activity-based protein profiling method has the potential to address challenges on both abundance and activity of trace levels of active lipases

Lipases

~ 5 lipases have been studied to have impact on PS degradation.

- PLBL2 is not considered to be the cause for polysorbate degradation at formulation conditions since no PS degradation is observed even at high concentration (a few hundred ppm) from multiple companies
- In contrast, LPLA2 is a more active enzyme, and is highly effective even at low concentration of 100 ppb in spiked-in experiment. LPLA2 preferentially degrades mono-ester.
- PLBL2 is immunogenic since it triggers neutralizing antibody. However, it has not caused issues in clinically trails, as it has no impact on safety and PK/PD. PLBL2 level is relatively high in IgG4 mAbs.
- There are a few less-effective lipases inhibitors which are not applicable in formulation. However, there are a few effective esterase inhibitors.

Re Q3

- Generally, raw material is not characterized before going into the formulation, and there is no general limit for the PS raw material used for formulation.

- LC-MS is widely used as the characterization method (in-source fragmentation and monitor fatty acid), and a HPLC CAD method is used as the release method to quantitate PS degradation
- One company is in the process of patenting a LC-MS method with in-source fragmentation, and the resulted free fatty acid serves as the reporter ion of quantitation on a QDa instrument.

Re Q4

- Most companies avoid testing marketed products, only work on new programs under development. It is a problem if a new process introduces a new HCP. Companies often don't want to present lipase related info to agencies, but agencies may ask, so it good to have data ready.
- One company shared that action limit (DS/DP release) by the polysorbate content assay is part of the control strategy from mfg or release points of view. The company has seen more requests of providing PS content data to show its levels remain constant during shelf life. There are more and more scrutiny from agencies with regard to PS control strategy.
- Control strategy should be phase appropriated, less critical for early phase programs, easier to justify; for a late phase program, if PS degradation happens, some investigation is required to understand root cause to prepare the potential questions from regulatory agencies. One company provided an example by responding to agencies that "we detected that (lipase), was low"..
- Another opinion on PS shelf life estimation: Shelf life is determined by the product stability using stability data. It is not directly related to the DP itself, so we can put it as a characterization assay, and mainly rely on particle size measurement to determine shelf life.

Re Q5

Process control strategy and analytical tools to monitor, remove, or deactivate high risk HSPs

- One company shared using cell line engineering to knockout a few lipases (4 out of the 5 knowns), while maintain similar productivity and PQ, patent under application, will be shared with public soon
- One company shared different clones seems to have no impact on HCP/lipase profiles,
- Some impact of formulation temperature on lipase activity.
- An enzymatic lipase activity will be ideal to guide process development
- HCP specific ELISA or MRM assay for downstream besides proteomics
- Knockout of CHO secreted antigens? (Such as abeta which binds toCHO antibodies)

Can we use LC-MS to support DOE study and cases of large volumes of samples? Seems to be hard, mostly of the HCP monitoring is supported by ELISA assay. A company is trying to make use of a high-throughput enzymatic assays.