Table 5: Analysis of Polysorbate and Its Degradation Products

SESSION 1:
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SCRIBE: Hilde van Hattum, *Janssen Infectious Diseases and Vaccines*

SESSION 2:
FACILITATOR: Anders Lund, *Sanofi*
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SCOPE:
Polysorbates are widely used excipients in biopharmaceuticals due to their positive effects on protein stability and their ability to minimize protein surface adsorption at liquid-air, liquid-solid and liquid-liquid interfaces while at the same time displaying low toxicity.

However, polysorbates are not uniform compounds, and commercially available polysorbates must rather be regarded as a heterogeneous mixture of molecules with variable chain length, size and diverse esterified fatty acids.
Their complex chemical nature enables multiple degradation pathways such as (auto-) oxidation, chemically or enzymatically driven hydrolysis induced by heat, light, headspace, HCP impurities etc… which might in turn contribute to the destabilization of the protein over time. Therefore, it is crucial to develop scientifically sound control strategies for polysorbate content and its degradation products.

This roundtable aims to discuss analytical and regulatory challenges with respect to supplier lot variability, degradation and comparability.

QUESTIONS FOR DISCUSSION:
Analytics
1. Is there a preferred method portfolio for the analysis of content and degradants of polysorbates for quality control and extended characterization?
2. HCP impurities and their enzymatic potential...preferred substrates? Kinetics?

Guidances
1. What are the agency expectations with respects to control of polysorbate degradation?
2. Is it sufficient to show control of degradation?
3. To which extent is it necessary to identify the root cause and include in submissions?

Alternatives
1. Are there alternative detergents to consider? Polaxamer analysis?

DISCUSSION NOTES:
Session 1:
Analytics:
1) Is there a preferred method portfolio for the analysis of content and degradants of polysorbate for quality control and extended characterization?

There is no preferred method as all methods have their pro's and con's

The fluorescence micelle assay (FMA) is currently the standard. FMA does, however, not detect monoester, so fluorescence is underestimating PS degradation. Micelles are not formed by fatty acids but they can be captured. So, FMA does not display true stability indicating properties
'Mixed mode single peak method' is validated for certain products, using both CAD and ELSD detection. However, protein interference is major problem as it sticks to column causing high background. The ELSD cannot be used for stability indication. Mass spectrometry will give more info than fluorescence, however MS not always available.
FDA, in some cases, has requested additional FMA data to compare to LC methods.
To forward the LC methods the proteins should be removed by for example precipitation or SPE columns. Variety in these columns pose a challenge however. Additionally, more effort should be directed to the lowering of the LLOQ to enhance stability indication.

2) Is there a concern about the raw materials? What incoming goods testing is performed?

Interestingly it seems that the PS ChP (Chinese standard PS) 98.0 % quality PS 80 is inherently less stable than the crude mixtures because it is more prone to oxidation.
There is a need for discussion with the raw material vendors (Croda, Avantor, a Chinese supplier) to have more control over the quality of PS-80/PS-20. For example, concerning the new line of 'superrefined' PS, containing less ketones. However, the pharma industry is only using a little amount of the amount of PS used in general. The pharma industry should agree on what should be quality should be delivered by the suppliers. Difficult to align as impurities have different impact on the different DS/DP. However, if there are common concerned it should be expressed by the industry. Patrick Gariedel and Klaus Wuchner proposed the need for a pharma consortium to start an aligned discussion with the raw material vendors gaining more openness on for example the synthesis methods and the quality. Roche, Lonza, Janssen, Sanofi and AbbVie are positive about a consortium. Friederike Junge will discuss with CASSS organizing committee if their platform could be used as a starting point for this consortium

Incoming goods test with compendial method with internal standards, however the resolution of these one-peak-methods is too low to detect impurities. Testing is not performed on the amount of free fatty acids in the raw material. This could be interesting as free fatty acids can cause nucleation to sub visible particles. However, practically, it is not feasible to check for all impurities that are continuously found. For example, Novartis recently published a paper on ketone impurities, this could also be tested, but others will find other impurities, many assays to be performed for incoming goods, not feasible.

3) HCP (host cell protein) impurities and their enzymatic potential…. Preferred substrates? Kinetics?

Different enzymes cleave different species and it is very difficult to check that. If the degradation products are checked for interaction with the API, then the impact of these degradants is covered. However, there are differences in batches and suppliers of PS, which will have different outcomes. Retains of PS could be kept; however this is very labor intensive.
It is imported to check the effect of free fatty acids to the DS and DP, however, it is not necessary to perform these test in a standard QC package.
Preferred substrates, cocktails of substrates used. Differences of substrates for enzymes, not all PS 80 products are targeted the same, meaning the degradation profile is very complex. Kinetics depends on the enzyme. Lack phases, sometimes it takes very long before products are formed.

How are the fatty acids exactly formed? Root causes of enzymatic degradation is very hard to establish. Experience of agencies asking for this? Show scientific evidence that it is not harming your product. This is very hard. Hundreds of particles, but not everything is analyzed, some things stick to filters, proteins etc. It is very hard to control.
Spiking free fatty acid to product to see if particle formation takes place? This is not easily done, the prerequisites for particle formation are different. It will not be representative.
One could use inhibitors against enzymatic degradation, however with the uncertainty which inhibitors to use these tests are difficult and not representable.

Guidance:

1) What are the agency expectation with respect to control of polysorbate degradation?

Do we have enough knowledge to draw conclusions about the impact of degradants? It depends on the molecule, if it is prone to modification.
What should we know? Interaction with the protein. This should not be a regular QC check but a characterization assay. This work should be done to justify that you do not monitor during life time. If you control the CQA of the DS/DP to make sure nothing happens due to PS degradation.
Add the method as an IPC on the finished product method. Quantify (one peak method), it is not reported. But if the authorities ask, then it can be given.
Why is PS degradation more interesting now in comparison to 5 years ago? Not a clear answer, interaction with free fatty acids causing nucleation and hence subvisible particles.

2) Is it sufficient to show control of degradation?

No questions have been asked to any of the participants concerning the control strategy. Most important is the monitoring of PS itself by whatever assay available.

3) To which extent is it necessary to identify the root cause and include in submissions?

Not been performed.

Session 2:
Methods used among companies:

- mixed-mode charged aerosol detector
- mixed-mode evaporative light scattering detector
- mixed mode Columns: Oasis MAX
- reversed phase –CAD
- Reversed Phase Mass spec
- 2D: MM-RP
- Free Fatty Acids (FFA) Analysis
- Fluorescence micelle assay (FMA)

- Issues to remove protein with high protein concentrated formulations; approach to remove protein: protein precipitation, washing with buffers (e.g. PBS)

Which Polysorbate (PS) is used among companies?
Standard Croda PS, PS ChP (Chinese standard PS); super refined PS was tested by some companies

Is a single PS lot used (PS lot independent standard) throughout the analyses of DS batches produced with different PS lots?

- A single lot (PS standard, PS lot independent) is used by some companies as standard for calibration purposes in mixed mode method
- A high inter-manufacturer variability in PS species is observed. Less lot to lot variability regarding PS species is observed when PS is used from one manufacturer
- For characterization purposes (PS species characterization) the same PS lot should be used that was used in production

Which PS degradation pathway is observed among companies?

- Oxidation:

Oxidation can be controlled by the right handling of PS during production
Oxidation of PS80 in PS80-Histidin Formulation induced by a metal-ion (Fe)

- Hydrolysis:

FFA particles have been observed
Target specificity of enzymes (e.g. lipases): some lipase are specific for poly-esters, some are specific for mono-esters

Alternatives for PS:
Poloxamer, sugars