ASMS 2014 Workshop

Title: Characterization of Biologics by Mass Spectrometry

Names Presiding: Li Tao, Alain Balland, Jason Hogan

Organized by: Biotherapeutics Interest Group

Number of Attendees: ~ 150

Summary:

The Protein Therapeutics Interest Group (PTIG) workshop, entitled “Mass Spectrometry-based Characterization of Biotherapeutics”, was held from 5:45 PM to 7:00 PM on Wednesday, Jun. 18, 2014. As many had mentioned in 2013 that they were unable to get into the small room reserved for the workshop that year, a large room was made available this time. The meeting was well attended and the discussion was animated, illustrating a high interest for debate on the impact of MS technology in the development and commercialization of biotherapeutics. About 150 people showed up at the workshop. By the time it was ended at 7:10 pm, there were about 130 people actively discussing on related topics.

The PTIG workshop was organized around 4 areas of interest for the biopharmaceutical industry: role of MS in biopharmaceutical development, characterization of charge isoforms (both topics presented by Li Tao), color in purified drug substance (presented by Alain Balland) and sequence variants (presented by Jason Hogan). In the last segment of the forum the debate moved to the use of mass spectrometry for release of biopharmaceuticals in the regulated environment. These topics generated a lively discussion well aligned with the goals of the workshop of promoting open exchanges between attendees.

1. **Biologics market and role of mass spectrometry** (discussion led by Li Tao, Bristol-Myers-Squibb)

The role of mass spectrometry in protein therapeutics development was discussed. The discussion focused on the increasing role of mass spectrometry in biosimilar development in anticipation of off-patent status for many of the blockbuster biologics in the next five years. The discussion was focused on the critical role of mass spectrometry in the characterization of molecular variants which will be the center piece of biosimilar strategies. Ideas were also exchanged, particularly in regards to the characterization of protein therapeutics. Ideas were also shared about the extent of extended characterization for biologics comparability studies.

2. **Charge isoform characterization** (discussion led by Li Tao, Bristol-Myers-Squibb)

The charge carrying groups of protein therapeutics and their characterization was discussed. The discussion focused on pros and cons of different techniques as compared to mass spectrometry in terms of sensitivity, accuracy, and robustness. It was agreed that imaged capillary focusing (iCIEF) and ion-exchange chromatography (IEX) provide a different analytical perspective in detecting overall charge profile shift caused by a modification, such as deamidation, that can happen on multiple residues, where mass spectrometry based peptide mapping has the capability to zero in on individual residues. Discussion also touched on the use of traditional gel electrophoresis and practices such as cutting bands from the gel and performing in-gel digestion and peptide mapping analysis. A few of the attendees believe the gel based techniques should be abolished, while other believe the gel based electrophoresis techniques still provide unique value and should still be in the toolbox for biologics characterization. Characterization on antibody-drug conjugates (ADC) by MS and cation-exchange chromatography (CEX) were discussed briefly. Techniques by other chromatographic methods for ADC analysis such as Micellar Electrokinetic Chromatography (MEKC) and its potential advantages were also briefly mentioned.

3. **Color in purified drug substance** (discussion led by Alain Balland, Amgen)

The presence of unwanted coloration in a drug substance is a sporadic phenomenon becoming more prevalent as effective concentrations of the final product increase, notably in the case of subcutaneous injections of therapeutic antibodies. Understanding and control of such events is not always obvious. A recently published case of pink color in a drug substance due to association with a culture medium component (vitamin B12) was presented to illustrate these concepts (see attached slides). In the discussion a comment was made about not having experienced any color issue, illustrating the random nature of these unwanted events. The strategy to avoid color in purified drug substance was discussed. When observed in bioprocesses the best option is to understand the root cause and use this knowledge to control the phenomenon before it appears. Color in drug substance is unlikely to be eliminated by purification strategies as colored contamination is often tightly associated to the product or the result of modification of the product itself. Mass spectrometry should play a prominent role in the characterization of those modifications (amino acid oxidation, metal-induced free radical reactions and cross-linking).

4. **Sequence variants** (discussion led by Jason Hogan, Bristol-Myers-Squibb)

The o observation of amino acid sequence variants in biologic products has become common given the increased dynamic range of current mass spectrometry instrumentation. Sequence variants can be caused by DNA mutations, translational errors, or transciptional errors resulting in an incorrect amino acid replacing the correctly encoded amino acid. The presence of amino acid sequence variants is unavoidable and these heterogeneous molecules are unlikely to be removed by purification strategies. Amino acid variants are usually observed at low levels, most of them below 1%. Comparison of the observed amino acid switch against the DNA sequence may offer clues to the cause. Systematic analysis of the purified drug substance can offer insights on the manufacturing process as some amino acid mis-incorporations result from starvation of specific amino acids in the culture media. Discussions of amino acid variants were limited, but they focused on the levels of sequence variants considered safe for biologics product lot release. Safe levels of sequence variants are difficult to determine, since it is a daunting task to assess the safety impact of a specific sequence viariant. However, it is certain that mass spectrometry will continue to play a key role in amino acid variant analysis due to its superior sensitivity and comprehensive coverage than liquid chromatography based techniques.

5. **MS for release of biologics** (discussion led by Alain Balland, Amgen)

The use of mass spectrometry for the release of biologics generated a good debate. In general it is understood that with the high level of information provided by mass spectrometry it would be logical to see this technology implemented in Quality Control for release. Progress has been made in this direction and it is anticipated that this method will be implemented in 2015 in regulatory filings submitted to the FDA. When this happens the regulators will decide if this approach aligns well with the QbD principles they want to encourage.

Some participants are doubtful that introducing MS in a regulated environment is such a great idea (too complex, validation issues, QC analysts training).

Comments were made that companies have in the past been reluctant at bringing advanced techniques into QC for cost reasons. MS in QC, especially using Orbitrap technology, is unlikely to be cost effective (at least in the beginning before the technology cost goes down).

Can the technology, if pushed by one company, become a common standard that others will have to adopt to release their drugs?

After a few rounds of heated debate, no consensus was reached regarding whether or not mass spectrometry should be used in biologics release and stability studies. The disagreement centered on the comprehensiveness of the data that mass spectrometry can generate versus its cost and robustness during release testing. In light of the great interest of the audience and impact to the mass spectrometry society, it makes sense to continue discussing this topic in next year’s workshop.

Other comments made:

- Use of peak area vs spectral height.
- Error generated by non-homogeneous isotopic distribution, deamidated/non-deamidated mixture. Error can be corrected by software treatment.
- Minimum characterization required for biosimilarity. Comment was made about a recent publication describing what is needed. Concept of “fingerprinting” advocated by FDA, most likely to be fulfilled by sophisticated and high resolving MS techniques. USP (still referencing TLC in its monograph) welcomes these advances.

Slides presented: