Table 2: Multi-Attribute Method 2020 – Challenges in Using MAM to Replace Conventional QC Methods

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Scope:

Peptide map analysis with high resolution mass spectrometry is increasingly common in product characterization and the identification of critical quality attributes (CQAs) of biotherapeutic proteins. A liquid chromatography-mass spectrometry (LC-MS) based peptide map method, named multi-attribute method (MAM), has been proposed for QC release testing of protein therapeutics.

MAM consists of a targeted quantitation function and a non-targeted feature known as new peak detection (NPD). The targeted function provides relative quantitation of multiple PQAs in a single analysis, while NPD is a data processing approach performing differential analysis of LC-MS chromatograms, which can be used to detect unexpected peaks in clinical batches compared to reference standard. MAM has been discussed with FDA's Emerging Technology Team, which works with drug developers to facilitate the adoption and implementation of novel technologies. It is suggested that MAM could be used as a replacement for at least several conventional QC methods, including hydrophilic interaction liquid chromatography for glycan profiling, ion exchange chromatography for charge variant analysis, and reduced capillary electrophoresis-sodium dodecyl sulfate for clipped variant analysis. There has been broad interest in the implementation of MAM for QC testing of therapeutic proteins in the biopharmaceutical industry.

Questions for Discussion:

1. What are the important aspects of risk assessment for using MAM to replace conventional QC method?
2. Challenges in method validation: precision, LOD/LOQ, system suitability
3. How to successfully use New Peak Detection to detect changes in products? Impact of parameter settings, false positive detections, etc.
4. Comparing MAM with conventional method helps to understand advantages and disadvantages of MAM as control system method. How to compare MAM and a conventional method, such as IEC, when their results do not co-relate?
5. How should system suitability be assessed?
6. Any concerns for sample preparation variability?
7. Any new and unique MAM applications?

Discussion Notes:

One participant had successfully been granted an FDA approval on the use of MAM in cGMP environment. Most of the questions were asked to this participant regarding their experience.

Question: What was the general timeline for the FDA approval in cGMP setting?

Parallel testing, comparison of MAM with conventional methods and initial discussion with FDA started in 2016. Many discussions and communications over a few years. There was an on-site visit in 2018, and in 2019, they received a positive response from FDA.

Question: Is the sample preparation robust enough in the QC environment? Manual vs. Automation?

Manual preparation is used in two of the labs that completed validation and the one who received the FDA approval in the cGMP environment, but automation would be ideal. Collaboration with vendors would hopefully bring better solutions for sample prep in the future.

Question: Would it be easier to work with the FDA ETT and go through the approval process once one molecule is approved?

Though the various aspects of the validation such as CQAs may be different, it should be easier.

Question: Which conventional methods could we replace by MAM? Experience so far?

Charge variant analysis (CEX) for deamidation, glycation and other modifications, HILIC glycan analysis, rCE-SDS for clips. The HILIC glycan analysis is an easy method to replace as they co-relate very well. ELISA binding assay for identity was also replaced by MAM by providing sequence coverage.

Questions: Some methods correlate well with MAM, but some do not. What should be done when they do not? In particular, the charge variant assay is difficult to correlate.

It is important to go deeper into data. There may be some exotic modifications that are not identified. It is sometimes difficult to compare at peptide level vs. protein level.

Question: How should one set the release criteria?

Once you have PQAs, the criticality should be assessed. This will lead to specifications to be set. Once you have the specs for conventional methods, they can be translated to MAM as well. A study needs to be done to translate or bridge.

Question: What is the real advantage of MAM in purity check? It is difficult to weed out the false positives or method induced variabilities.

First, during the validation, the robustness should be assessed. One participant published a paper (review) in Trends in Biotechnology addressing the issues by describing the sources of variability. He recommended to read this review. Methods should be in place to investigate such peaks.

Another participant still finds new peaks hard to address. Some of them are easy to identify, but some are not, and they require time consuming investigations. Perhaps the new peak detection technology is not mature enough?

What should be the criteria or threshold? 10X difference? 5X difference?

During the validation process, you need to know the limitation of your method and depending on the specifications you set… 0.5% or 1 etc, then your method needs to be more sensitive than that. Your specifications will guide the number.

Question: For new peak detection, high mass resolution a good thing?

The answer may be molecule specific. There is also some tradeoff between sensitivity and resolution.

Question: Any new MAM related developments at FDA for new guidelines?

There will be a few presentations and publications in the pipeline.

Question: Is anyone using UV for validation?

No one.

Question: Is anyone using more than one enzyme for identifying clips or other modifications in a QC environment?

No one currently, but if necessary, that could be included. Having said that, it would be more difficult to implement than using just one enzyme.

Question: Should "non-reduced MAM" be used in the QC environment?

It has been used in process development in many labs. It may be possible.

Question: Is digestion necessary for monitoring PQAs? Is subunit analysis good enough for attribute analytics?

Oxidation and glycation were monitored in the past, but oxidation is difficult as the peak can be influenced by the sodiated species.