Table 5: Future of CE with MAM Used in QC for Lot Release/Stability?

Session 1:	Session 2:
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Scope:
Recently, mass spectrometry-based methods intended to simultaneously monitor product quality attributes have been introduced to more deliberately assess the quality all along biopharmaceuticals manufacturing processes. As such, these multi-attribute methods (MAM) could offer potential to replace several traditional chromatographic and electrophoretic assays, including CE-based methods, currently used for characterization and release of biopharmaceuticals. This roundtable aims to discuss future of CE applications (CE-SDS, cIEF, etc) with potential of MAM to be used in QC for lot release and stability monitoring.

Questions for Discussion:
1. What are the requirements and challenges for implementation of multi-attribute methods to support QC release/stability?
2. How the role of CE-based methods may change as result of potential implementation of multi-attribute methods in QC for release/stability monitoring?

Discussion Notes:

Session 1:

1. What kind of CE is affected by MAM? What is MAM?

- New trend towards MAM example Amgen 2015 mAbs paper on MAM.
- At the CASSS MS meeting there is much discussion on MAM.
- One MS method can get streamline testing get more info from one run.
- Could view CE methods in competition with MS.

2. Challenges for MAM:

- MS however not thought of as QC user friendly.
- Time and cost of MS is expensive.
- Belief that training and qualifying QC analyst on software is not there yet.
- MS used as characterization focus.
- Instrument software, reporting, troubleshooting.
- If something goes wrong want straight forward solutions that are resolved and fixed quickly.
- Do you use a Porsche to drive into a lake? Is this MAM in QC world perspective?

3. What is purpose of MAM? Is MAM purposeful? MAM comes to QC for quality attribute but

is there a reason for this if have other for example CE methods.

- There was a recent ASMS Thermo talk on high throughput formulation workflow where many samples were able to be analyzed by MAM directly in 96 well plates.

4. MAM robustness questions on repeatability of values. Established methods have established repeatability expectations.

- MAM needs development on these expectations. Molecule as whole attribute vs MS attribute. Post translational modification directly checked.

5. MAM Mamconsortium.org

- One leader is Richard Rodgers was at Amgen and now Just pharmaceuticals.

- Concept is use direct infusion high accuracy mass spec to monitor protein clips, degradants, process impurities, glycans,id method, deamidation and glycation in one instrument. MAM presentation is public knowledge on website.

- Doug Richardson is another MAM leader (Merck) supporting MAM.

- Regulatory is part of consortium / CDER emerging technology team.

6. Question on validation group support as MAM is thought of as characterization assay. When will MAM be adopted as routine QC assay?.

- Rebuttal: mass spec is a mature technology and supporting software are already used in other industries.

- Question is how to bridge the current methodologies to MAM. One option is to start early phase with both technologies and anticipate overtime there will a buildup of data and confidence.

- CZE 1988 start publishing papers on possible use in pharma but didn't take off until 2000 timeframe. Early adopters' vs mainstream adopters. Challenge then was what benefit of CE over current HPLC technologies was. HPLC and CE live together now. Expectation that in x number of years MAM will coexist with HPLC and CE as MAM technology evolves. Thinking back, early 1990s expectation for Biologics was monitoring using gel methods.

- As establishment of new attribute based methodologies grows the expectations to adapt and adopt new technologies becomes the new norm. IEF and SDS are now not the performed norm for testing strategy and replaced by CE and HPLC options.

- Expectation that MAM will go from new technology to norm in the next 20 years. In processing testing by MAM method maybe beneficial at first.

7. Challenges: How do you process data quickly in MAM?

- Analyze one method and good data processing software. To do this need connection between good software programming and science. Want automatic data processing with end result.

- Worry is MAM will have a data analysis time constraint. What are the requirements to bring method to QC? Validation should be applied and ICH guidelines towards Quantitation or identity methods could also be applied.

8. How to incorporate invalid assay approach? Example what if fails for one attribute but passes for another.

- Will need to define in methodology. What do you validate? All attributes or some attributes. Could you be challenged on attributes not selected?

- Difficult to correlate for example charge profiling phenotype to MAM method. MAM is very specific. Which detected parameters are important to define and track on molecules? Evaluate differences of product to standard and degree of change.

9. What does MS data tell us in addition to HPLC data that needs trending?

- Is it feasible for routine testing analyst to handle MAM data processing load?

- Performing high resolution MS analysis yields a large quantity of data that needs proper software and understanding for roper processing. Question on sensitivity to low quantity attributes in MS detection in MAM approach.

- Does MAM require 2DLC and MS/MS? How does press a button technologies compare to analyst training requirements for mass spec? Can you push a button high resolution mass spec? Job safety....need knowledge set to understand software and have proper data interpretation.

10. Clinical aspects of MS analysis are already routine in regulated environment.

- Not so far-fetched to envision MS in routine environment. Chances are high for adoption of mass spec in our routine testing biopharma industry. New tools needed and time to get there. MAM still maybe future looking.

11. Regulatory has advantage of different perspective and are privileged to see how new technologies are implemented at different companies through filings.

12. Is MAM economically positive? Only big innovators and afford to buy high resolution mass specs in high quantity for QC testing. Could this be viewed as strategy to stop biosimilar? Have requirement to show comparability by MAM method and innovator raises analytical procedures requirement bar higher

<u>Session 2:</u>

10 participants present at Session 2:

Most of participants shared an assessment that Multi-Attribute Method (MAM) is intended to provide a single assay that could potentially replace multiple traditional assays for either release and/or characterizations purposes. The peptide mapping is expected to be a primary type of MAM, some participants left possibility that some other assays could be also considered as MAM methods.

An example of MAM from used for release and stability monitoring of early program by single participant has been discussed. It is important to note that in this case MAM is supplemented with in parallel use of traditional assays (IEX, CE-SDS, N-glycan assay) due to regulatory expectations. In general, consensus seems to be that MAM is to be developed during early stage (even before final clone selection) so that necessary knowledge is built up before it can be ultimately used for drug substance/drug product monitoring.

Some of the challenges in implementation of MAM attribute are direct correlation between traditional assays that monitor change on the entire molecule, such as CE-SDS, icIEF/IEC vs site-specific results

obtained by MAM. Also correlation could be molecule dependent since traditional assays and MAM may not always monitor same product qualities.

In conclusions, participants seem to agree that in foreseeable future it would be difficult to fully replace CE based methods with MAM for reasons listed above. Even if MAM gets implemented in QC, CE is likely to be used as characterization assay,

Some other points expressed by participants are as follows:

- a holistic approach that traditional assays provide is hard to drop completely, especially as "unknown" attribute could be detected on stability, for example, thus traditional assays and MAM may complement each other for its faster identification.
- how to link measured information on a single detected peptide for protein fragmentation, for example, to total purity obtained by CE-SDS and how to report it?
- how to set-up specifications for MAM method, including threshold for new peak detection?