**Table 11: Peaks, Lumps and Humps - Strategies for Identifying and Characterizing That Anomaly in Your Forced Deg Sample**

**Facilitator:** Alexander Kozintsev, *Genentech, a Member of the Roche Group*

**Scribe:** Romesh Rao, *Seattle Genetics, Inc.*

**Scope:**

The roundtable will primarily be a discussion regarding contemporary techniques and workflows for identifying new anomalies in degraded samples. This discussion will be supplemented with considerations for which approaches are common and/or appropriate depending on the phase of development. Time-permitting, the roundtable discussion may expand to strategies for the controlling of anomalies identified as pCQA's, such as the implementation of new methods and/or controls.

**Questions for Discussion:**

1. When you see a new peak, lump, or hump in a non-MS analysis of your forced degradation sample, what kinds of workflows and techniques do you use to identify it?
2. Does your approach to identifying anomalies and the extent of the characterization work that you perform change depending on development phase? Additionally, who is involved in determining the approach?
3. When you have identified a forced degradation anomaly as a pCQA but it does not appear in other sample panels, how do you control for it?

**Discussion Notes:**

**1.** When you see a new peak, lump, or hump in a non-MS analysis of your forced degradation sample, what kinds of workflows and techniques do you use to identify it?
   - **a.** Approaches to identifying new peaks in a non-MS analysis vary from company to company and are driven by the technologies available. While fractionation and purification to make the material amenable to MS analysis remains the most popular option, 2D LC-MS approaches are becoming increasingly utilized. As 2D systems become more user friendly the implementation of such systems to directly analyze new peaks in non-MS compatible separations will increase. Currently the usage of this technology appears to be limited to larger companies due to the rather niche space it occupies.
   - **b.** The traditional LCMS workflows-Top Down, Middle Down, and Bottom Up-are heavily utilized. The consensus was that a Top Down approach was the most appropriate as it reduces the scope of subsequent, higher resolution techniques, like peptide mapping.
   - **c.** Peptide mapping is generally performed once a solid understanding of what the peak might manifest as at the peptide level is gained.

**2.** Does your approach to identifying anomalies and the extent of the characterization work that you perform change depending on development phase? Additionally, who is involved in determining the approach?
   - a. Development phase clearly limits the scope of characterization work that is performed to identify anomalies. In all cases an attempt is made to identify the new peak with resources dedicated based on the following:
     - i. Level of impurity/anomaly
     - ii. Relevance
     - iii. Development stage (i.e. early or late)
     - iv. The factors above are used to determine how much effort is put into the characterization work. This decision is generally not made by the MS analyst,

but rather a cross-functional team to ensure there is a comprehensive understanding of the risk associated with the identified anomaly.

v. As expected, there was no consensus on what constitutes an anomaly with respect to levels of the impurity or anomaly. However, there was agreement that context is everything. For example, the original non-MS assay in which the anomaly is found heavily drives the decision on how much further characterization is needed and the extent to which the anomaly should be explained.

**3.** When you have identified a forced degradation anomaly as a pCQA but it does not appear in other sample panels, how do you control for it?

a. Retains of the sample with the force degradation anomaly are typically run with test samples as a positive control to ensure the anomaly is or isn't present

i. If the anomaly can be generated and/or driven to higher levels through force degradation this can also be a strategy to create a positive control sample.

b. Once the anomaly has been characterized it may be monitored with more sensitive orthogonal MS methods to ensure either the absence or presence of the anomaly as opposed to monitoring by the original assay in which it was detected.