

## **Table 7: Timing and Frequency of Forced Degradation and Variant Characterization Studies**

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**Key Words:** Forced, Degradation, Characterization

### **Table Scope:**

Forced degradation (FD) is an integral part of biotherapeutics development from early-stage candidate selection to post-approval. FD provides an opportunity to gain an in-depth understanding of the biochemical and biophysical properties of the molecule, including the major degradation pathways that are not observed from stability studies performed in real time. Typically, FD involves subjecting the biotherapeutic to elevated temperatures, increased photo-exposure, higher/lower pHs, and oxidative conditions to simulate the environmental forces that a product undergoes during manufacturing and storage. Mass spectrometry and the analytical release methods provide a full understanding of potential degradation pathways after FD studies. Even though FD studies are performed at relatively harsh conditions within a short period of time, the information gathered supports real time stability and period of use.

FD is performed initially during the molecular design phase to support sequence liability identification, manufacturability assessments, and formulation development. During process and product development, FD at different temperatures from -80C to 40C supports the formulation nomination. FD reveals structure/function relationships when a loss in potency is observed, supporting critical quality attribute assessments. FD also contributes to establishment of stability-indicating analytical methods and optimization of the manufacturing process in terms of parameters and hold times. FD is also necessary in late-stage comparability studies to compare degradation pathways following a manufacturing process and/or site change.

### **Discussion Notes:**

#### **1. Forced Degradation:**

**Question 1:** What scientific approaches and product characterization experiments are utilized?

- Peptide mapping, intact mass, sometimes subunit mass.
- Peptide mapping is usually performed at pH 7-8, rarely low pH. Consensus that most groups default to pH ~8 and use low pH by special circumstance only.
- Company A: every team involved in characterization goes through a set strategy on how the molecule is stressed and then there is something called the "CQA council" where a meeting occurs for each molecule to discuss what is considered a CQA for that molecule. This happens at all stages and the bar gets higher the later phase you go.
- Important to have two lines of evidence to confirm peptide mapping to make sure something is not an artifact of digestion.

#### **2. Elucidation of Forced Degradation and Monitoring:**

**Question 2:** What are the timings of the forced degradation studies being performed? (Early vs late-stage development) Are the FD conditions the same for all?

- **Early stage:** In-silico hot spot prediction is performed after sequence lock and before any FD is ever performed. The widest range of stresses are performed at early stage. First FD study is performed in discovery on several candidates (6-8) that were generated through transient transfection. The top 2-3 candidates that make it out of the FD study move on to cell-line development.
- **Formulation changes:** If there is a formulation change some attendees would do a forced degradation study and others would only conduct a stability study.
- **Late stage:** Should already know your CQAs and most attendees do not often find it necessary to do FD studies at time of site change or process change. Usually just perform regular comparability and your QC methods should already be stability indicating. Often necessary to do a FD study on your final product/process prior to filing BLA.

**Question 3:** What mass spectrometry assays are used in FD studies?

- Generally, peptide mapping, subunit, and intact mass.

**Question 4:** What orthogonal methods are used to support forced degradation studies?

- SEC-UV, CE-SDS, icIEF, IEX, HIC, potency, FcRN

**Question 5:** Discuss challenges in aggregating forced degradation data during process development.

- MS results are generally not databased and difficult to visualize using tools like Spotfire. There is a strong desire by nearly all attendees to be able to database results, but it's a long road.
- Desire to database all of the results that are generated so that informed decisions can be made on future programs.

**Question 6:** How are CQAs determined?

- CQA council at one company
- Defined scoring matrices to help score the criticality of an attribute at most other companies.

### 3. Variant Characterization

**Question 7:** What do we consider a variant?

- Wide range of what is considered a variant, including all of the following:
  - DNA level misincorporation, sequence variant.
  - Charge variants
  - Glycoforms
  - Clipping/fragmentation

**Question 8:** What types of MS variant characterization are performed?

- Sequence variant analysis by peptide mapping
- Sequence variant, glycoform variant, at intact level
- Charge variant characterization coupled to MS (IEX-MS and iciEF-MS)

**Question 9:** When is variant characterization performed?

- Very common during clone selection
- Deep characterization at process lock, but faster variant characterization methods are leveraged during process development (IEX, HIC, SEC, etc.) and only sent to MS in certain circumstances.
- Routine to test during site change, process change, etc.
- After process lock, uncommon unless something new pops up.