Table 10: Forced Degradation

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Scope:
Forced degradation studies are used to characterize prospective drug substance/drug product under extreme conditions and can be used to guide candidate molecule selection, analytical method development, formulation development, and comparability studies. It is required to demonstrate specificity of stability indicating methods and provides an insight into degradation pathways and degradation products of the drug substance. At present, regulatory guidance is very general and does not fully define the design and implementation of forced degradation studies. In this session, we will discuss the scope of forced degradation studies and the appropriate application of mass spectrometry to characterize product quality changes and degradation pathways that become evident under stress conditions.

Questions for Discussion:
1. General scope of Forced Degradation Study
   a. How and why does FDA require the force degradation study?
   b. How can we utilize the forced degradation study on the analytical method development?
2. Selection of Analytical Methods
   a. What instrumentation is most appropriate for the analysis of degraded species?
   b. What follow-on experiments are necessary to evaluate pharmacokinetics of degraded products?
   c. What is advantage and disadvantage of application of mass spectrometry?
3. What are the specific challenges and concerns for small molecule and biologic drugs? We would like to talk about the difference according to the bullet points below
   a. The FDA guideline
   b. The stress conditions
   c. Analytical methods
4. Selection of Stress Condition
   a. What stress stability conditions are most relevant/essential for determining drug degradation pathways?
   b. Are there conditions that should be considered which are currently outside regulatory requirements/guidelines?
   c. How many data points should we choose for each condition?
   d. How to determine the optimized conditions?
Discussion Notes

Why does the FDA require degradation studies?

- Understand the chemical behavior of the molecule in extreme conditions
- Ensure patient safety and product integrity under stress

When in development are forced degradation studies appropriate?

- Value in starting early but also incurs additional costs
  - Avoid significant delay resulting from late discovery of issues
  - Must be balanced against the throughput and effort required

**Example:** Would thermal stability testing be useful during clone selection?

**Pro**

- Rapid analysis
- Low sample requirements (0.5 ug)
- Orthogonal method to disqualify clones

**Con**

- Throughput concerns
  - Individually fast to run but time consuming the aggregate
  - Increased data analysis requirements

Guidelines for forced degradation studies

- Conditions are not strictly defined by the FDA
  - Relevant conditions (and levels) depend on the program and must be set by innovator
    - Thought must be paid to the reasonable storage and use conditions

- Data point requirements vary based on development stage
  - Fewer time points in early programs
  - More granularity in late stage
  - Minimum of two timepoints with strong preference to 3+
    - Linear regression was not seen as an important requirement

- Analytical platforms must be sensitive to low (but relevant) levels of product variation
  - For filing, some level of degradation is desirable.
    - Increase intensity until detectable level of change
o Avoid complete degradation of product
  ▪ 20% was agreed as a reasonable upper limit on degradation/modification target

Stresses to consider
• Heat, pH, photo, agitation, and oxidative stress are commonly tested
• Thermal stress is the most common condition and requires the longest longitudinal studies
  o Common to have 6+ month studies
    ▪ Timelines can be compressed by application of additional thermal stress

Analytical requirements and techniques
• MS based methods
  o Peptide maps are used to evaluate degradation species of proteins
    ▪ High sensitivity for detection of changes
    ▪ Observed PTMS are then correlated to orthogonal potency and binding data
  o Tiered approaches
    ▪ MAM methods to locate hotspots
      • Directed methods (MS and non-MS) to follow up on observed species
• Orthogonal methods
  o HIC and iceEF as alternatives to peptide maps based on prior identifications
  o HPLC affinity methods can be used evaluate total oxidation levels

Limitations of MS for evaluation of product degradation
• Mass spectrometry is not currently a release assay
  o Too many variables to readily move to QC
    ▪ Acquisition options are highly variable based on context and requirements
    ▪ Complex instruments and software
    ▪ Difficult to maintain stable instrument sensitivity/quantification
      • Ambient temperature, sample prep effects, LC variance, etc.
Requires expert knowledge for optimal results

- Unable to evaluate insoluble aggregates
  - These are critical to the evaluation of product stability

Some programs/materials require additional considerations

Case Study: Antibodies (and ADCs)
- Particular focus required for CDR
  - Potency and binding disruption should be correlated to MS detected features
    - Information can provide feedback for formulations

Case Study: Virus capsid
- Initial degradation conditions based on protein workflows
- Not amenable to MS analysis
  - 10 megadalton size
  - High heterogeneity

Case Study: Small molecules
- Assumed to be more stable than proteins
  - No representative at the table had experience with an example