Table 8: Specification Setting for CE Methods

Session 1:

Facilitator: Bernd Moritz, *F. Hoffmann-La Roche Ltd*

Scribe: Steffen Kiessig**,** *F. Hoffmann-La Roche Ltd*

Session 2:

Facilitator: Bazarra Damdinsuren, *CDER, FDA*

Scribe: Nomalie Jaya, *Seattle Genetics, Inc*

Scope:

For ensuring therapeutic efficacy and patient safety, resolution and specification of critical quality attributes (CQAs) is important. CE based methods are well established in the QC environment of most biopharmaceutical companies. They are mainly used for charge (CZE, IEF), size (CE-SDS) and specificity (ACE) heterogeneity testing for release and stability testing under GMP, but also for process characterization / process validation studies (PC/PV) and for formulation development. CE-SDS can differentiate between low molecular weight (LMW), main and high molecular weight (HMW) forms whereas IEF and CZE can differentiate between acidic, main and basic forms. ACE can separate specific compounds. CE-SDS can be performed either under reduced or under non-reduced conditions. For a comprehensive control system, the stability indicating potential and the goal of the control strategy (e.g. consistency versus stability) should be considered.

Questions for Discussion:

1. What are the company's application areas for CE based methods?
2. How do companies handle the setting of specifications for CE based methods? What is the underlying CQA assessment? May IEC be preferred over IEF and CZE due to easier peak characterization tools?
3. How do companies handle the setting of specifications for orthogonal methods like SEC and CE-SDS or reduced and non-reduced CE-SDS? When is ACE included? What are the thoughts about setting acceptance criteria only for the method that is more suitable (e.g. HMWs only for SEC and LMWs only for CE-SDS) or to omit one of these methods (e.g. reduced CE-SDS)?
4. How do companies handle the setting of CE specifications in dependence on the development stage of the product?
5. What health authority feedback has been received?

Discussion Notes:

Session 1:

As broad consensus, it was concluded that specifications are set phase dependent. In early stage acceptance criteria are often "generic" or "platform. With growing product experience, specifications are re-considered and adapted, towards late stage rather tightened, based on product-specific data.

In this context it was agreed that "report value" is considered acceptable, e.g. for charge heterogeneity species, in order to gain product experiences, enabling more thorough acceptance criterion definitions based on.

Special focus must be on the patient. Hence, considerations of potency, immunogenicity, safety and PK data of minor forms is strongly recommended.

Molecule-specific requirements

- Some molecules may have limited assays available, especially in early stage development.

  Example: CE-SDS non reduced might be used for drug antibody ratio determination when not much other assays are available.

- Special requirements of new or non-standard molecular formats must be considered as required. Example: Acceptance criterion for special LMW peak in CE-SDS non reduced, in addition to sum of LMW forms, if this one species requires special attention.

CE-SDS: non-reduced, reduced, or both?

Commonly agreed was that both assays are standard for monoclonals. Non-reduced does provide information about all covalently linked species and might result in the more concise picture for low molecular species by CE-SDS. During stability testing non-reduced CE-SDS is often more stability indicating than reduced CE-SDS, i.e. a higher degradation rate is often observed with non-reduced CE-SDS. That can be attributed to the higher mass of non-reduced fragments that result in higher relative peak intensities. In addition, in contrast to reduced CE-SDS, non-reduced CE-SDS separations resolve partly reduced compounds that have lost single light and heavy chains. After reduction this compounds merge with the light and the heavy chain peaks and therefore cannot be separated by reduced CE-SDS. However, in some cases also reduced CE-SDS may be useful. It was recommended to compare both assays at least in early stage in order to confirm suitability of method of choice. The use of CE-SDS non-reduced as leading assay with CE-SDS reduced as additional characterization assay seems suitable. However, the decision is case by case and strongly driven by product requirements regarding control strategy. It was agreed that picking one of the two is acceptable when superior stability indicating of one CE-SDS assay has been shown or is known for the class of molecule. This position is defendable approach for filings with good data basis, CQA information, also considering the clinical safety profile. It was also discussed that in special cases both assays might be needed in order to address all CQAs.

How to select the proper CQAs?

CQAs are essential part of specification setting, driving method selection, tested attribute, and definition of acceptance criteria. In early phase, due to limitations in timing, e.g. only 12 to 16 months between discovery handover and IND filing, as well as product experience, CQA definitions have also limited basis. Accordingly, CQA (re-)assessment is considered stage dependent, i.e. frequent re-evaluation and refining with increasing level of molecule knowledge is recommended. Highest detail level is expected in late stage.

How to define acceptance criteria appropriately?

Limits are strongly assay and CQA dependent. Using size species assessment of an IgG as an example, CE-SDS non-reduced is used for purity analysis with acceptance criterion. Impurities can be controlled indirectly by the main compound. Limit definition is phase and CQA dependent.

Especially in early phase, the use of platform specifications and limits, based on internal experience or literature data is acceptable. Mostly, early phase limits leave room for uncertainties during development and are tightened and adapted as more product specific knowledge becomes available. In all phases the following impact factors are considered for limit definition:

- toxicological and clinical experience, also from similar products
- potency
- safety
- PK
- immunogenicity

Over all, the supply of safe and potent products for patients is in focus.

Grouping peaks, e.g. Sum of LMW by CE-SDS or Acidic Region by cIEF, is accepted as CQA assessment and toxicological / clinical experience allows.

Numerical limits might be difficult to be defined in early stage. Reporting like REPORT VALUE for charge based methods accompanied with COMPARABLE TO REFERENCE STANDARD e.g. for cIEF may be acceptable, enabling the generation of product knowledge.

Sizing methods

Size species are characterized usually by a combination of SE-HPLC for HMW species and CE-SDS for LMW species. LMW species are more difficult to get by SE-HPLC due to limitations in resolution in this mass range. CE-SDS delivers information on HMW only for the covalently bound sub-fraction, which might be an important additional information but does not provide the full picture for HMW species. Due to different method performance CE-SDS was deemed to be more important for low molecular weight forms (LMW) than SEC and the other way round for high molecular weight forms (HMW). This implies that the HMW part of CE-SDS and the LMW part of SEC are often not relevant. Therefore, the primary use of CE-SDS for LMW species and that of SEC for the HMW species was deemed reasonable. Generally, the use of orthogonal methods should be considered in early phases as needed in order to ensure that CQA-driven selected species are addressed appropriately.

CZE over (i)cIEF or IE-HPLC

Both methods have pro's and con's, selection should be made based on product-specific data, e.g. method robustness, resolution of critical species, etc.

CZE is advantageous regarding resolution and robustness since no ampholytes are used, hence no impact of batch-to-batch inconsistency is expected.

CZE and IEF have some limitations in peak fractionation and potentially following characterization due to low sample volumes applied.

icIEF is mostly method of choice for ADCs, e.g. for DAR determination, due to its ease of (QC) application and suitable resolution of species of interest.

IE-HPLC is common for monoclonals due to easy scale up and peak fractionation capabilities for characterization purpose as well as the easy transfer potential (LC hardware).

It was agreed that charge heterogeneity assay selection is always a product specific decision and preference might change from project to project.

<u>Session 2:</u>

- The group primarily focused the discussion on size variants as monitored by CE-SDS and charge variants
- What are the approaches used for early vs late phase specifications?
    - Clinical Specifications are set wider due to limited manufacturing history, limited batch size, lack of structure function knowledge to understand impact of specification limit on safety/efficacy. Many sponsors only have 1 GMP batch at initiation of Phase 1. Difficult to calculate statistical limits with only a few batches
    - Some companies use stress material to determine attribute changes and the impact of attribute on activity. This can inform an early specification

    - Authorities are consistently asking for quantitative specs even on IND enabling material. Qualitative (profile comparable to reference) is not adequate unless there is a clear definition on what profile comparable to references means.
    - Many sponsors have report results for charge-based methods, but all have received feedback to implement quantitative specifications. Encouraged to apply risk-based specifications
    - For late phase, quantitative specifications are expected based on a scientific and reasonable approach. Likely have greater manufacturing history with more data sets, better understanding of structure/function relationship to potential clinical signals and may have sufficient data to derive statistical limits
    - Early specifications can change with increased manufacturing experience and information on assay variability. Specs can be always be widened with the right justification
    - Setting specs is a complicated discussion, with potential variability in process and analytical methods during program lifetime should attempt to come up with a good story based on statistical approach etc.
- How to bridge between new and old methods?
    - Use forced degraded material as well as ensure that sufficient retains are available from various manufacturing campaigns.
- Defininf appropriate specifications
    - icIEF used as an id method should have a pI criteria associated with identity along with other criteria such as peak profile pattern, no new/missing peaks etc. If charge based id assay is in control strategy may need a second orthogonal method to show appropriate controls.
    - If using the R and NR-CE methods for monitoring impurities (fragments) and purity important to ask "what can the method see? What are we loosing? Are we placing the specification on the appropriate attribute?
    - If acidics have been categorized as Non-CQAs. How would you place a specification on that attribute?
    - Many sponsors implement one sided specifications for charge variants but the health authorities are requesting a spec based on range for charge variants to allow for greater process consistency.
- Appropriate selection of methods for attribute monitoring
    - Health authorities never ask for 1 method over another. It is up to the sponsor to demonstrate that appropriate methods are used to monitor product quality
    - The method should be fit for purpose
    - Methods may need to be updated over the lifecycle of the program because of inability to select for critical attributes.
    - If a sponsor still has an outdated method on the control strategy (i.e. SDS-PAGE) they may be asked for justification since CE-SDS is widely implemented in place for SDS-PAGE for improved resolution and sensitivity.
    - Understand difference between assay acceptance criteria vs. sample acceptance criteria