Table 2: CE in Forced Degradation

Session 1:	Session 2:
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Scope:

Forced degradation of therapeutic products result in physical and chemical modifications. Capillary electrophoresis (CE) methods have become an increasingly essential part of the analytical control strategy for monitoring modifications resulting from degradation. Automated instrumentation, powerful separation efficiency, low sample requirements, and fast analysis times have made CE a great analytical tool for drug characterization. Various separation modes such as cIEF, CE-SDS, CZE, and CE-MS all have their own unique capabilities and challenges. The goal of this roundtable discussion is to connect new and experienced CE users to share commonly used strategies to understand the pathways of forced degradation using CE-based methods.

Questions for Discussion:

1. Which CE methodologies are generally used to monitor degradation pathways (e.g. CE-SDS, iCIEF, cIEF, CZE)?
2. What instrument platforms regularly accompany these methods? 3. What type of physical and chemical degradation products are monitored using CE based methods?
3. Are there certain common modifications that are not detected by CE methods? If so why and how do you get around it?
4. If fragmentation is monitored via CE, how do you distinguish between method induced fragmentations (e.g. denaturation at high temp, use of reductants etc.) vs actual forced degraded condition?
5. What is the most sensitive/commonly used method to detect degradation product in your control strategy?
6. What orthogonal methods do you use for identification of the degradation product where physical and chemical changes are detected by CE based methods (e.g. CZE-MS or others)?
7. What are the conditions generally used for forced degradation of DS for analytical method development vs. extended characterization which uses much more parameters like pH, photo stability, later in the development stage?

Discussion Notes:

Session 1:

The majority of the participants of this roundtable session were from industry. Most of them worked already on forced degradation and/or had projects in the pipeline where forced degradation studies will be applied. The molecules which are worked with are mainly mAbs, bispecific mAbs or fusion proteins.

The favorite instrumentation are PA800+ (SCIEX) and iCE3 (Protein Simple). One participant mentioned the quality of data for PA800+ is unbeatable in comparison with other systems.

The peak integration is still done manually (independent form the software) with a lot of experience and knowledge about historic data. One note from a participant has to be mentioned here: "*A drop in A% is not the end of the world as long as you can explain it."*

The main CE methods to monitor degradation pathways used are CE-SDS and cIEF. cIEF is used as trigger for some investigation if something is seen by cIEF. cIEF is also regarded of valuable use for biosimilar/originator comparison.

As orthogonal methods to cIEF/CE-SDS CEX (with pH gradient), SEC, CD and Mass spectrometry were mentioned. Also two dimensional methods are preferred. CE-MS was mentioned with the restriction that this technique is not used as long the "conventional" methods are working.

A discussion about the clinical relevance of these studies arose ("*are generated in-vitro data representative for in-vivo?* "). The participants came to the conclusion that CE is a good beginning with MS at the backend. Charge based methods are indicative for changes and very sensitive. Although these methods cannot tell the difference, but that something is going on. This is seen as a start for the investigation of the degradation pathway. If there are information about the molecule one can go for this special site.

The CE application which has more advantage than any HPLC application is seen in CE-SDS for the detection of fragmentation. Non-reducing SDS is applied mainly in early phase, reducing CE-SDS in later phase. The non-reducing CE-SDS is favored by the participants as non-reducing CE-SDS generally gives better resolution and might also be used for fragmentation detection. Reducing CE-SDS is mostly used if non-reducing CE-SDS can`t tell something reduced can or for special cases (monitor e.g. additional amino acids). The most focused or biggest concern lies on fragmentation of the molecule. The limit of non-reducing CE-SDS lies in partial reduction leading to HL or HHL peaks. This stability indicating factor can sometimes not captured. Short note: The LIF detector is only be used if sensitivity is too low.

A question arises about the use of CE methods as quantitative methods and how specifications are set. The only solution was seen in the experience from known data. In case of HMWs (CE-SDS), release assay are applied for fragments, not for aggregates.

In case of reducing CE-SDS assays are applied on fragments (early stage: purity, late stage/commercial: specs on fragments).

The baseline stability was also mentioned as a big issue. The protein formulation or even the aperture (2 instead of 8 nm) was identified as possible problems. In that case the Maurice instrument was mentioned having a low baseline noise. On the other hand peaks regarded are not so good resolved (e.g. shoulder) compared to other vendors.

Finally the question was discussed if there are difference strategies in conducting a forced degradation study between release methods or high throughput methods (e.g. clone selection)? Participants identified different factors like decision makers, phase, and number of candidates.

Concluding, one participant summed the round table discussion up in two easy questions:

It's about monitoring the CQAs: Is the method capable doing that? What is science telling us?

<u>Session 2:</u>

Eight Scientists participated in the discussion from pharmaceutical companies.

How to degrade material? What methodologies? How to monitor the modifications and what modification properties are you monitoring? What orthogonal methods do you use to characterize peaks?

- Thermal stress - used as first step to create stability samples to prove if method is stability indicating
- CE-SDS-R and NR and iCIEF are major methods for characterizing purity/fragments and charge variants in early phase method development. Instead of icIEF, some companies have IEX was used as a charge variants method
- Need to use chromatography methods later (if iCIEF was used) to characterize the peaks

What orthogonal methods do you use to characterize peaks?

- iCIEF as screening tool for formulation development/analytical development

- Broad pH screening in formulation buffer
- Protocols used for generated forced degraded material
    - Temperature first, then pH, photo-oxidative stress
    - 40 degrees C for ADC's and regular mAbs
- 14 days, sampling at time intervals
- Product dependent
- Stressed samples not for formal stability study, only for method development
- One batch of thermally degraded material for all method development
- Using agitation can cause increase in HMW
- Don't need the material to be fully degraded for method development, just need to prove stability indicating
- Develop methods and FDDS (forced degraded drug substance) simultaneously as formulation is being developed

What methods do you use to characterize FDDS?

    - CE-SDS
- Aggregates and LMWs
- Decrease in main percent area
- Integration strategy?
- IgG peak is purity for NR (NGIgG is impurity in NR method)
- LC+HC is purity for R (NGHC is impurity in reduced method)
- At what point do you characterize peak ID?
    - Late stage

What methodology do you use for charge-based separation?

- iCE or IEX
- Mostly iCE, but back and forth push back for HPLC methods
- Peak ID using CEX, but high throughput of iCE is desirable
- Usually just iCE or IEX, but not both methods
- Characterization group will characterize peaks, don't need characterization for each method
- IEX does not give as good resolution in most cases as iCE, especially for ADC's
- cIEF is emerging technology for CE-MS methods
    - CE infinity to collect fractions for MS. However, it was not implemented as a routine technique. It is not QC friendly either.

How to distinguish between method induced fragments vs. actual forced degraded conditions?

- Orthogonal methods to confirm during method development
- CE using laser induced fluorescence
    - Higher sensitivity
    - more sample manipulation which causes more method induced impurities