Table 09: The Good, The Bad and The Ugly for Charge Heterogeneity: Which Electrophoretic Method to Pick and When?

Session 1:

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Session 2:

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Scope:

Evaluation of charge heterogeneity is a basic expectation for development of a new molecular candidate. This technique may be expected for introduction into routine use for testing at time of release, and on stability. Moreover, it may play a key role in establishment of reference materials, cell banks, as well as be used to establish comparability during changes in manufacture throughout development. For this reason, this technique reflects a variety of "must haves" with respect to its operation. Specifically, the method should be quickly to be implemented early in development, able to be transferred to commercial release facilities, and sufficiently precise.

Questions for Discussion:

1. Does your company have a preferred specific methodology (e.g., CEX-HPLC, iCIEF?)?
2. What advantages do you find certain platforms have in unique situations throughout development?
3. How much refinement is done for a method throughout development?
4. What are your expectations for its routine performance?

Discussion Notes:

Session 1:

What is your preferred methodology for charge heterogeneity?

- IEC is preferred, but screened side-by-side with iCE for robustness
  - find iCE faster in development
  - originally had push-back when iCE first brought in because it was not understood, but now we're finding we have more problems with IEC during the product life cycle
- iCE cIEF was the platform method originally, but now have a platform IEC method as well
  - ultimately choose between them based on which provides better separation—what resolves the CQAs
  - late-stage development favors IEC because it permits fractionation
  - want to be able to take method developed in early phase through the product life cycle
- ultimately you need to choose your method based on your CQAs

How about CZE?

- was able to separate by CZE, fractionate by IEC, and re-inject for peak characterization
- as sample degrades, lose peak resolution in sample more quickly than in other assays
- use CZE for sample identification—CZE has good specificity

Linking charge heterogeneity to CQAs

- fractionation still doesn't provide native molecule that could be passed to bioassay

- should we focus on characterization of individual peaks vs. regions (acidic/basic)?
- methods are improving towards being able to do direct analysis, but still not there yet
    - intact mass will not give the amino acid position of a PTM
- isomerization is invisible to charge heterogeneity

What are the pros and cons of each method?

	iCE	IEC	CZE

Pros	• Method development speed—a big advantage
	  ○ just screen ampholytes, urea, focusing time
	• ease of use
	  ○ Maurice even easier to use than iCE
	• high throughput/fast run time
	• less variable than IEC
		• seamless integration with Empower
		• fraction collection
		• multi-use instrument (SEC/IEC/RFLC) saves bench space and money at small sites
		• don't need solubilizer additives (more streamline materials)
		• can use mixed mode separation (pH, buffer, and salt)
			• simplicity
			  ○ not every molecule is a mAb
			  ○ can just use a neutral capillary and citrate buffer and get decent results
			• can couple to mass spec

Cons	• no direct connection to Empower
	  ○ internal quality system drive to validated system/data integrity
	  ○ want driver to capture metadata as well as all acquired data
	• molecules don't play nice at their pIs
	  ○ urea suffers from degradation, but no other additive is better
	• bad ampholyte lots
	  ○ build lot variability into qualification and robustness
		• adds more complexity to development
		• potential lack of robustness
		  ○ columns are susceptible to metal poisoning
		  ○ vendors changing column manufacture without warning
		• certain types of molecules just don't run by IEC
			• too many possibilities of buffer, pH, additives, coating, capillary length, voltage

<u>Session 2:</u>

Company Preferences: First to try?

• Co. #1: IEX (usually CEX) and iCIEF (iCE3) run in parallel early in development. Historically IEX was preferred but iCE3 now generally favored due to faster, easier development.

• Co. #2: historically started with iCIEF, now IEC for ADC molecules. CZE as a last resort. Have not transferred iEC method to QC yet.

- Co. #3 – utilize CEX characterization scientists. LC is preferred - allows for Peak IDs early in development.
- Phase I peak ID if possible. Easier peak ID than iCIEF.
- Co. #4: Platform approach is iCE. Easy transfer to QC. Limited Development. (mAbs) Phase I peak ID if possible. Easier peak ID than iCIEF.

Biogen: Platform approach is iCE. Easy transfer to QC. Limited Development. (mAbs)

General difficulty in generating comparable iCE data from site to site for multiple companies. Remember to consider CZE as an alternative to IEX and iCIEF.

Platform methods:

Platform methods are desirable for generally increased efficiency, but most molecules require further development.

- Method development generally easier on the iCE3.

Method Performance History (IEX and CIEF):

- Gather control chart data early and often using Reference material
- Increase variability by using multiple instruments, column lots, reagent lots, analysts, etc.
- Utilize control charts to grade method performance and adjust method conditions/language as needed to tighten variability
- Based on control chart data, consider a control sample if needed
- LC may be easier to qualify 2 different columns relative to CIEF dependence on vendor reagents

Trouble Spots:

- IEX sensitive to system contamination/corrosion in some cases. May cause difficulty in busy labs where instruments are shared between multiple techniques. High Salt systems may become corroded and interfere with IEX.
- IEX column lot ot lot variability: May cause significant changes in profile. In some cases may need to hoard a certain column lot to ensure supply.
- Baseline noise can be excessive in iCIEF, especially with certain ampholytes. Fluorescence detection may limit this in some cases (Maurice – see Tom Niedringhaus method development talk)
- ICIEF (iCE3) data export – must export data to Empower for integration which requires additional SPV. However new Waters driver will allow for Empower control of Maurice.
- Data integration – many different softwares used. However, all agree that total manual integration is very difficult to achieve. Processing methods can be set to get 90% of the way there, but manual tweaking of integrations is often needed. This is particularly difficult with stability samples that introduce new peaks in the chromatogram.

System Suitability:

What is appropriate system suitability? How to set initial system suitability requirements?

In early development, visual comparisons can be good enough.

With additional data construct control chart and set criteria based on historical values.

Be sure to incorporate as much variability as possible to avoid criteria that are too tight.

Include detailed integration instructions and appropriate figures to aid in integration.