Table 6: New CE-based Methods in the QC Lab - What Are the Challenges and Learnings?

Scope:
CE has been a continuously evolving analytical methodology since the 1980s. The development of new CE separation procedures, the coupling of these systems to more sensitive and versatile detection systems, and the advances in miniaturization technology have allowed the application of CE to the resolution of new and complex analytical problems, overcoming the traditional disadvantages associated with this method (Ramos-Payán et al. 2018). For use of CE-based methods in a QC-environment, however, these techniques need to provide QC-able data (e.g. reliable, robust,&[hellip]). What is considered a new CE-based method? What are main advantages and disadvantages with respect to implementation in a QC-environment? This will be discussed in current roundtable session.

Questions for Discussion:
1. What kind of new CE techniques are used in QC-laboratories?
2. What samples are measured with these techniques?
3. Which detectors do the different companies couple to CE instruments? e.g. Fluorescent-based detectors or MS? And what are the challenges to implement new CE techniques in a QC-environment?
4. What are the benefits of using new CE in a QC-environment?

Discussion Notes:
Which CE instruments are used in QC?
SCIEX
- PA800
- PA800 plus

Caliper
- LabChip

Protein simple
- Maurice
- iCE3

Prince Technologies
- PrinCE

ThermoFisher
- 3500 (multi capillary system)

Conventional instruments are beneficial because they could be used for different applications. The iCE3 for example, is only limited to cIEF. The question if conventional system would then be better for implementation in QC was left open.

Multi capillary systems in QC:
Vendors wondered if it would be good to build the same multi capillary system in which you can do development and QC in one instrument using only a single capillary for use in QC and multi capillary function in development. From the user side it might be the other way around. Development is not high throughput, so use single capillary in development and then bridge to a multi capillary system in QC to handle the great number of samples.

Should we allow a trade-off between speed and resolution?
The Caliper LabChip is faster than a traditional instrument, however, it does have a lower resolution. This raised the question if we would allow a trade-off between speed and resolution. The general opinion is to not sacrifice resolution for speed, a loss in resolution might only be accepted if a non-CQA peak is affected.

The importance of resolution in QC GMP batch release was questioned, since all samples are purified samples. When a method is stability indicating, resolution is very important.

What CE techniques are used?
The most frequently used technique is CE-SDS. Also, cIEF is used.

Even though other techniques such as CZE could offer a lot, they are not often used. Frequent users of CZE are Roche and Solvias. The main reason for this is the lack of possibilities for characterization, since fraction collection and coupling to an MS are difficult. If coupling to MS would be easier, it would probably be more frequently used.

In addition, small migration time shifts which are normal in CZE, but not in LC, seem to scare people off. The design of the method could help, an internal standard could be added for more precision.

Could CZE be applicable in QC?
CZE is not brand new in QC and it has potential. When CZE is compared to cIEF, CZE is more robust and can obtain higher resolution. For chiral separations, good methods are available. However, the buffers are complicated. An issue for CZE is that not one platform method is in place. Another named example is that the pI cannot be determined with CZE. This could be solved by internal markers, however, this is not used.

Kit applications
CE kits are frequently used. The use of kits brings risks; the composition is only known by the vendor and could change without knowing it. A kit could have fluctuations, electrolytes could be of different quality. Another risk is the possibility that the kit goes off the market and is no longer available.

A frequently heard argument for using kits is that some buffers are difficult to prepare and therefore hard to implement in a QC environment. An easy to make buffer would be doable.

An issue with kits is the limited flexibility. For example, for the Caliper kit protocols almost no development is possible. Only the dye and gel ratio could be adjusted. CE users would like to have more flexibility with kits. Preferably they would like to know the composition of the kit, however, vendors will not provide this. Vendors suggested development kits that allow for screening for gel type and gel ratios (e.g. for larger proteins > 200 kDa), allow for different detergents such as SHS instead of SDS for difficult to denature proteins (presentation of Jeff Beckman from BMS) and different reducing agents. They also suggested a 'cookbook' with recipes to mix/dilute their gels for specific applications.

Chip based systems
All companies on the table tried to establish microchip CE in QC. CRO even drove it to GMP for one of its projects with only limited success.

Everyone seemed to agree that microchip CE is not yes suited for QC or GMP environment, due to difficulties in reproducibility of dye/protein interaction as well as chip instabilities and high failure rates.

Microchip CE at the moment is only suited for development purposes and areas where a lot of samples have to be analyzed, such as process development support. For routine analysis, the use of traditional instruments is preferred.

Ramos-Payán et al. 2018, Recent trends in capillary electrophoresis for complex samples analysis: A review. Electrophoresis.39(1):111-125. doi: 10.1002/elps.201700269.