**Table 1: Alternative Separation Strategies – What Works, and What Is a Work-in-progress**

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**Scope:**

With the wide and increasing scope of molecules in the biopharmaceutical pipeline, alternate separation strategies are becoming a beneficial and sometimes necessary characterization and analysis tool in place of, or in addition to, existing platforms such as routine RPLC-MS or standalone CE methods. From early research to late stage development, multiple considerations are taken into account when considering implementation such as cost, value added, necessity, regulatory compliance, and future proofing among others. We will discuss these technologies as they exist today and drive a conversation around where they can and should exist tomorrow, and how to get there.

**Questions for Discussion:**

1. What are the biggest challenges to adopting these technologies?
2. What drives the consideration and implementation of these alternative strategies?
3. Which parameters define what "works" vs what is a "work in progress"?
4. What unique advantages do these technologies offer that would make it a 'requirement'?
5. What of these platforms do you currently have in use and what has that experience been (and what could be improved?

**Discussion Notes:**

4 people attended, three vendors and a CRO

What are the alternative separation strategies? Alternative to LC. Discussed CE, ion mobility.

CE is good at separating background matrix, but coupled with MS is not very reproducible. Robustness is an issue.

People would like to see a robust icIEF-MS solution for identification of charge isoforms. There is no good tool for this at the moment.

Alternative techniques are needed as orthogonal tools for characterization. Sometimes LC can not be used, thus – unique applications. For example, CE-MS was the only technique used by one company (Biogen?) for adenovirus proteins monitoring.

What would motivate people to use alternative strategies? Something CE can uniquely solve. CE provides more flexibility in terms of buffers (vs. LC). CE may be a good tool for the new upcoming modalities. Different molecular properties can/will utilize alternative separations. At the same time, LC utilizes different type of columns and separation mechanisms; CE – not much choice, low capacity. Implementation in QC (GMP) environment is also a limiting factor. If not implementable in QC – not appealing, especially for CRO/CMO.

Ion mobility is commercialized. But applications are narrow. Good for certain molecules/applications, not universal. Robustness and QC implementation are not there. Not routine or a platform tool.

CZE-MS is still relatively new compared to LC-MS. With introduction of new modalities and maturation of the field we may see more applications. Native MS and pH gradient with MS may be areas for implementation of alternative separations. cIEF mobile phase is not MS friendly. People are working on approaches helping to deal with salty matrixes.

Many questions remain: how do we define success? What works vs. work in progress? Are the alternative separations useful for coupling with MS, and if yes, are the applications limited? Can only solve a specific problem, no universal use? What is that LC can not solve? What are the unique needs and applications? Would oligonucleotides use more CE-MS than LC-MS?

CE-MS vs LC-MS: CE can be 10x more sensitive because it's nanoflow. But concentration needs to be sufficient, otherwise need to focus.

Other aspects: customer trust. The techniques (ion mobility) has not been widely demonstrated. More than one application and wider implementation needs to be demonstrated. Unique applications where all else fails.

CE is very fast; MS can be limiting. Right instrumentation needs to be coupled with CE.

Vendors see some growth of implementation of alternative separations in biotech and biopharma recently.