Table 3: When Is It Time to Change the HPLC Stationary Phase in an HPLC/MS Lab?

Facilitator: Sree Survajj, *Amgen Inc.*

Scribe: Terry Cyr, *Health Canada*

Scope:

Stationary phase is a critical component of the LC/MS workflows such as peptide mapping, subunit analysis and top-down. C18 columns are the most broadly used HPLC columns for these applications as they perform very well for reproducibly separating many compounds in a predictable and robust manner. However, there are applications where these columns are not suitable impacting the outcome and quality of the data. In this session, we will discuss when it is appropriate to try alternate stationary phases and which may be best applied to which compound classes.

Questions for Discussion:

1. Reasons for changing: lack of separation, not sufficiently robust, insufficient coverage, peptide recovery
2. Alternate phase: non-C18 polymer, HILIC, forward phase, graphitized carbon, size exclusion, ion exchange, solid versus porous core
3. Alternate solvents & modifiers, solvents – alcohols, pH, ionic strength, ion pairing strength
4. HPLC or UPLC: robustness, sensitivity

Discussion Notes:

The intent of this table was originally to explore the decision of when to change the HPLC stationary phase. Specifically when alternatives to the almost universal C18 reversed phase should be entertained.

Our group was very small and the answer to this was easily arrived at:

- When the critical components in the mixture are not being separated and standard modifications do not adequately improve the situation then change stationary phase. Standard modifications being: column temperature, exact C18 being used (HSS, BEH CSH, BioBasic…), choice of mass spec friendly ion pair (FA, TFA, DFA), and mobile phase solvent (acetonitrile, methanol, propanol…)

- The alternate stationary phase obviously depends on the problem to be solved: size = organic size exclusion (GPC), charge = ion exchange, very polar compounds = hydrophilic interaction chromatography and porous graphitic carbon.

When there is an issue with stationary phase it often coincidental with trouble shooting issues:

- Unstable baseline is firstly necessary to the group quickly moved to the subject of troubleshooting (there are many pages online dedicated to this topic)

There was also a clear set of ongoing problems related to:

- Carry over is still a problem and is influenced by the type of analyte and the design of the HPLC

- Baseline stability as related to noise can be mitigated by not using 100% pure solvents in A versus B gradients (95% H2O and 5% acetonitrile in A and the reverse for B may help). Depending on the HPLC this is not always easily changed.

- On-column oxidation may be explored by using direct infusion or alternate ionization systems such as MALDI.