High-performance (or pressure) liquid chromatography, more commonly known as HPLC, can be a useful tool to assist in the improvement or simplicity of mass spectral detection and analysis. However, it is often the case where the optimal conditions for the chromatography are less than ideal for the mass spec, and vice versa. Developing the initial chromatographic method with the ideal mass spec parameters in mind proves immensely helpful for the facilitation of the best overall method development.

**Rules of thumb**

- The absolute solvent flow to the mass spec ion-source is typically preferred—and often required—to be less than 0.6 mL/min. Most modern ion-source interfaces are pneumatically assisted, and can handle upwards of 0.8-1.0 mL/min; however, keeping with the typical range of 0.2-0.6 mL/min, the HPLC column internal diameter is generally preferred to be 2.0-3.0mm, where the desired flow rates for the mass spec will correspond to ideal linear velocities through the HPLC column.

- All mobile phase modifiers must be volatile, as they will need to be removed either through desolvation or evaporation. The ease of removal of the total eluent composition typically correlates to the facilitation of the ions of interest being generated and the subsequent detection sensitivity.

- Chromatographic separation of the injected components—particularly those away from the void, commonly referred to as the ion-suppression zone—generally improves the mass spec sensitivity and ease of structural elucidation in LC/MS/MS.

**Common challenges**

- HPLC system parameters, particularly the total system volume, suggest that 4.6mm ID
columns with greater than 1.0 mL/min flow rates are the most universally usable conditions for HPLC analysis. On most HPLC systems, reducing the length and ID of the system connective tubing and other areas of system volume can be easily performed to make amenable 0.2-0.6 mL/min flow rates on 2.0-3.0mm ID columns. Otherwise, splitting the flow rate post-column (part to waste, and part to the mass spec) can be a solution if greater than 1.0 mL/min flow rates are necessary for the chromatography.

• Non-volatile buffers (such as phosphate buffer) and ion-pair reagents (such as alkylsulfonates) often lead to the ideal chromatography, particularly for ionizable compounds that otherwise will retain poorly by reversed phase and/or yield poor peak shapes. Volatile organic acid buffers such as formic or acetic acid can often be used as a mass-spec friendly substitute, as can TFA (volatile acidic ion-pair reagent) and TEA (volatile basic ion-pair reagent). Developing the initial HPLC method with these ideas is preferable, as the chromatographic selectivity can potentially change with the use of different buffers.

• Polar analytes are a common challenge for LC/MS, as they retain poorly under reversed phase conditions that are optimum for mass spec detection (volatile buffers and high organic percentages that are easy to remove). Basic analytes can be better retained on modern pH stable silica-based reversed phase columns under basic conditions (neutralizing the otherwise charged compounds), yielding better chromatography and elution under conditions more ideal for the mass spec. Hydrophilic Interaction Liquid Chromatography (HILIC) is an old idea that has resurfaced recently, and is gaining popularity for mass spec analysis of polar analytes that are difficult to retain by reversed phase. The retention mechanism is based on polar interactions and partitioning between a polar stationary phase and an acetonitrile-rich mobile phase that is ideal for mass spec detection.
Technical Tip: Principle HPLC Conditions for LC/MS

Related resources:

- Reversed Phase Chromatography
- Normal Phase Chromatography
- Hydrophilic Interaction Chromatography
- Ion Exchange Chromatography
- Ion Exclusion Chromatography
- Chiral HPLC
- HPLC and Pharmaceutical Industry
- HPLC for the Food and Beverage Industry

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