Good HPLC Practices

Technical Tip #1:

HPLC Column Care: Based upon decades of HPLC Technical Service experience, the number one mistake, made by experienced and novice chromatographers alike, is the improper changing of mobile phase solvents, such as in the two scenarios below. Incorrect washing procedures often lead to precipitated buffer in the column that can cause changes in retention and high back pressure.

Don't Do:

Scenario 1: An analyst stores his column in high percent organic solvent. He places the column on the HPLC and begins to equilibrate the column with a buffered mobile phase.

Do:

Wash the column with > 50 percent D.I. H_2O before beginning the equilibration with buffer.

Don't Do:

Scenario 2: An analyst finishes his method development project that used a buffered mobile phase. He begins pumping a high percent organic through the column to store it in that solvent.

Do:

Wash the column with > 50 percent D.I. H_2O before beginning the high percent organic wash.

Technical Tip #2:

To obtain the highest efficiency and greatest sensitivity, use HPLC flow rates that provide the optimum linear velocity (OLV) for the particular column employed. This is more important when using larger particle size packings (e.g., 5µm and larger), and less of an issue when using smaller particle packings (e.g., 3µm and smaller), or core-shell particles. The OLV is related to the optimum flow rate (OFR), and is dependent on the internal diameter (i.d.) of the HPLC column:

Column i.d. (mm)	Typical OFR (mL/min)
2.1	0.21
3	0.43
4	0.76
4.6	1.0
10	4.7

III. Technical Tip #3:

Running HPLC chromatograms at elevated temperatures can provide several advantages:

- Lowers mobile phase solvent viscosity and column/system backpressure
- Enhances peak efficiency and sensitivity
- Helps to prevent perturbations in baseline due to room temperature fluctuations
- Provides somewhat faster elution times
- Helps to provide more reproducible chromatographic results

However, when performing chiral separations, a lower column temperature is often advantageous in obtaining greater resolution between enantiomers.

IV. Technical Tip #4:

The standard operating pH range for most silica-based HPLC columns is pH 2 to 8. However, some C18 phases (e.g., UCT Selectra® C18) contain high carbon loading (19% C) that enables the analyst to run methods at an elevated pH (pH 10 or even pH 12) for an extended period of time, if necessary. The higher carbon load is thought to partially shield the silica backbone from alkaline hydrolysis.

V. Technical Tip #5:

Particularly in performing LC-MS method development work, try using Hydrophilic Interaction Liquid Chromatography (HILIC) instead of Reversed Phase Mode (RP) chromatography. The HILIC Mode uses higher concentrations of organic in the mobile phase that allows more efficient desolvation in the mass spectrum instrument. This typically results in increased sensitivity. There are a variety of columns available that can perform HILIC separations, such as the Selectra® PFPP and Selectra® Mixed-Mode I phases.

V1. Technical Tip #6:

When a mass spectrometer is used as the LC detector (LC-MS), the mobile phase must be volatile in order for the LC-MS interface to adequately vaporize it. If you utilize a buffer that is non- volatile, such as phosphate or citrate, a large quantity of it could pass into ESI or APCI, initiating a large amount of salt formation in the MS. This build up has been known to quench ionization in electrospray LC-MS, leading to lower sensitivity of analytes.

Buffers	pH Range	LC-MS Compatible
Phosphate (pK_1)	1.1 – 3.1	X
Phosphate (pK_2)	6.2 – 8.2	X
Phosphate (pK_3)	11.3 – 13.3	X
Acetate*	3.8 – 5.8	YES
Citrate (pK_1)	2.1 – 4.1	X
Citrate (pK_2)	3.7 – 5.7	X
Citrate (pK_3)	4.4 – 6.4	X
Trifluoroacetic acid (0.1%)	2.0	YES
Phosphoric acid (0.1%)	2.0	X
Formic acid (0.1%)	2.7	YES
Ammonium formate	2.7 – 4.7	YES
Ammonium bicarbonate	6.6 – 8.6	YES
Borate	8.3 -10.3	YES
*suitable for LC-MS as ammonium acetate		