HPLC Tips & Tricks: Mobile Phase Preparation Part 2 - Buffers

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When analysing samples containing ionizable compounds, the buffer can be one of the most important variables controlling the retention in an HPLC separation. The pH of the mobile phase determines the presence of ionizable compounds (analytes and matrix) to be in either an ionized or non-ionized state. The ionized species in reverse phase (RP) chromatography always elute from the column earlier than the non-ionized species. Changing the pH can also increase the selectivity for effective separation of closely eluting or overlapping peaks. Run-to- run variability in pH results in a separation inconsistency. Buffers prevent pH variations. Therefore, the proper buffer choice, in terms of buffering species, ionic strength, and pH, is the most critical step in HPLC method development when ionizable substances are analysed.

TIPS FOR CHOOSING AN LC BUFFER

Buffer Selection

The choice of the appropriate buffer for an application is governed by the buffer characteristics such as pK_a, pH range, and UV cut-off. As a rule, buffers should be used for a pH within +/- 1 unit of their pK_a value. Within this range, buffers resist any deliberate attempts of change in pH. The buffer's capacity is at its maximum when its pH is equal to its pK_a. The UV cut-off value also needs to be considered, as the detection wavelength should not interfere with the buffer absorbance (significant absorbance: trifluoroacetic acid <220 nm; formic acid, acetic acid <240 nm). For the best results with an ionizable analyte of interest, use a buffer with a pH at least 2 units away from the analyte's pKa. If the pH of the mobile phase is too close to the analyte's pK_a, split peaks or shoulders might be observed due to the presence of both species in the sample. For several ionizable analytes of interest, it is preferable to choose a pH value wherein all the analytes exist in the same form, either ionized or non-ionized.

Measuring Buffer pH

pH of the buffer is the pH of the aqueous portion before the organic mobile phase part is added. The addition of an organic solvent can shift the pH either up or down (pH shift should be consistent for the same buffer). It is not so important to know the exact pH value of the buffer in an organic medium, but it is important to have a consistent pH value (because pK_a of your analytes is also determined in aqueous phase, and we do not know the individual pKa shifts either).

Chemical Purity

The quality/purity of mobile phase additives (buffers, salts, acids, and bases) along with organic solvents utilized in an HPLC experiment must be adapted to the detector sensitivity and elution protocol.

Chemical Compatibility

Buffer composition, along with mobile phase pH, must be chosen in agreement with column housing material and nature of the stationary as well as different parts of LC instrument (pumps, tubing's, etc.) phase to prevent corrosion or degradation of either.

MS Compatibility

Introducing inorganic buffer salts into a mass spectrometer soon fouls the system. Examples of suitable volatile buffers are ammonium acetate, ammonium formate, and ammonium citrate. pH modifiers like formic acid and acetic acid should be used to control pH and help ionization for LC-MS.

Buffer Solubility

Ideally, the buffer should be completely water-soluble (RP methods) and should not precipitate during the analysis when mixed with a chosen organic solvent. Buffer concentration must therefore be carefully chosen to avoid precipitation at higher concentrations in the organic solvent. If neglected, this can create operational problems with the pumps and instigate HPLC column blockage or backpressure rise.

Buffer Ionic Strength

In case of ionic interactions between analytes and stationary phase, the ionic strength of the buffer must be chosen in a way that compounds are eluted. The required ionic strength of the buffer depends on the stationary phase. Besides elution strength, the viscosity of the buffer plays an important role in terms of its suitability for use in HPLC analyses.

Buffer Concentration

Ideally, the lowest concentration that gives reproducible results should be chosen. Higher concentrations lead to a faster elution of polar molecules. Generally, the buffer concentration should not be lower than 5 mM. Below this concentration, the solution may not perform as a buffer (depending on analyte concentration and buffering capability). Raising the buffer concentration can increase viscosity and the risk of buffer precipitation, which in turn can increase column back pressure.

Commonly, the concentration should be kept in the 5 to 100 mM range. A concentration higher than 100 mM of mineral salt buffers wears out the pump's movable parts faster, therefore a back-seal wash is recommended to be installed.

It can be observed that buffers play a crucial role in a majority of HPLC separations. Method development often requires careful selection of buffers and adequate care in their preparation. So, the general rules to be kept in mind are— buffer solutions must be homogeneous, clear, and free from any particles. If stored, please keep in mind that buffers have a limited lifetime, so consider preparing them daily.

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5.33001	**Acetic acid 100%** for LC-MS LiChropur™		Expand
5.33002	**Formic acid 98% - 100%** for LC-MS LiChropur™		Expand
5.33003	**Ammonia solution 25%** for LC-MS LiChropur™		Expand
5.33004	**Ammonium acetate** for LC-MS LiChropur™		Expand
80457	**Trifluoroacetic acid** eluent additive for LC-MS, LiChropur™, ≥99.0% (GC)		Expand
00922	**Difluoroacetic acid** for LC-MS LiChropur™, ≥97.5% (GC)		Expand
5.33005	**Ammonium hydrogen carbonate** for LC-MS LiChropur™		Expand
1.00030	**Acetonitrile** gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur		Expand
1.06007	**Methanol** gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur		Expand
1.00029	**Acetonitrile** hypergrade for LC-MS LiChrosolv®		Expand
1.06035	**Methanol** hypergrade for LC-MS LiChrosolv®		Expand
1.15333	**Water** for chromatography (LC-MS Grade) LiChrosolv®		Expand
1.59002	**Acetonitrile with 0.1% (v/v) Formic acid** hypergrade for LC-MS LiChrosolv®		Expand
1.59014	**Acetonitrile with 0.1% (v:v) trifluoroacetic acid** hypergrade for LC-MS LiChrosolv®		Expand
1.59013	**Water with 0.1% (v/v) Formic acid** hypergrade for LC-MS LiChrosolv®		Expand