

## HPLC buffer precipitation

One of the recurring problems that most questions we receive is about high pressures in an HPLC column.

After asking some questions and detecting some obvious symptoms such as a “clogged” column, guard column or injector, we often end up concluding that the problem lies in buffer precipitation.

There are some pieces of advice that we would like to share to avoid these «annoyances»:

- Take into account the solubility of the buffer in the organic eluent. For example, orthophosphoric acid is insoluble in concentrations of organic solvent above 80% to 90%. In this case, limit your gradient to 70% organic solvent.
- The use of buffer in reverse phase is due to the need to keep the analytes in their molecular form, because if the mechanism used is the hydrophobic interaction, we optimize the separation with neutral molecules. Therefore, if we are working with a given sample concentration, we do not need more than a few nanomoles of buffer to prevent them from being in ionic form. Adjust the buffer concentration to the minimum necessary — sometimes buffer concentrations 10 to 100 times less are more than enough.
- Use another buffer: as long as the pH of the solution is 1 to 2 units below the pKa of the most acidic component (the one with the lowest pKa), we guarantee that our analytes are in their neutral form. Both formic acid and acetic acid are excellent alternatives to orthophosphoric acid, being much more soluble in organic solvents.