Basic HPLC/UHPLC Record Keeping

Since liquid chromatography involves mixing chemicals, record keeping is required. This means the details of each run need to be recorded in a laboratory notebook (paper or electronic). Here is a list of the details that need to be saved.

date of run:
instrument name:
column:
mobile phase A:
mobile phase B:
method: (method name, flow rate, gradient)
File name of run: (so the data can be found on the HPLC/UHPLC computer)

Each mobile phase solution label should include: contents, date, who prepared it.

Because most HPLC/UHPLCs are used by more than one operator, identity of the mobile phases is an important variable to be documented.

Switching mobile phases requires priming/purging to avoid air bubbles and pressure problems. Find out how to do this for each HPLC/UHPLC instrument you use. Take notes and put them in your notebook.

At the end of a run, make sure some of the sample is not still on the column. There are basically three ways to do this - three ways to get the column back to the same starting point for each run. Operators may have to use more than one of these strategies for optimal results.

1) at the end of the gradient add a slow gradient back to the starting point
2) add a “flush” run with solvent that clears the sample followed by an equilibrating step that brings the mobile phases back to the starting point. The success of flushing with different solvent combinations depends heavily on the samples present.
3) inserting blank runs between runs - this documents carry over. If carry over is present, one solution is to run more blanks. It is a good idea to start a series of runs with one or two blanks to make sure of the previous operator’s samples are not present.

Sample preparation for HPLC/UHPLC: When using a reversed phase column, the more aqueous phase is by convention mobile phase A. If possible the sample should be in mobile phase A - the main phase at the beginning of the run.

Note: “UPLC” belongs to Waters Corporation as they were the first to market columns and pumps designed for the high pressures needed for success.

References:

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