Getting Started With a New RPLC Column

Care and Use Information
Before you install and use your new reversed phase column, we recommend that you first review the column’s care and use information (data sheet), which is shipped in the box with the column. If you don’t have a copy of your column datasheet, you can obtain a copy from the pertinent column product page on the Agilent Technologies website. Be sure to check for any pH range usage information, any minimum % organic solvent stipulations, and other product use recommendations.

Solvent Compatibility
It is important to ensure that the solvent(s) currently in your HPLC system is (are) compatible and miscible with the shipping solvent in the new column. Columns are usually shipped in the solvent used for QC testing the column, and which is typically either a mixture of acetonitrile/water or methanol/water. Install fresh HPLC grade water on one of the pump channels and prime that channel per instrument recommendations. Install fresh acetonitrile or methanol (as per QC test conditions or your method conditions) and prime the pump channel per instrument recommendations. Ensure that the system is set to pump 100% acetonitrile or methanol before proceeding.

Flushing With Strong Solvent
Connect only the inlet end of the new column to the LC system, and connect spare waste or connecting tubing to the column outlet and allow the column flush effluent to flow to waste. Flush the column with at least 10 column volumes of 100% methanol or 100% acetonitrile—whichever is more convenient. (For a 4.6 mm i.d. column, the column volume in mL is 0.1 x column length in centimeters, so, for a 4.6 x 150 mm column, 10 column volumes is 10 x 1.5 mL or 15 mL.) This initial flush with strong solvent will remove any traces of strongly retained components and solvents from column manufacture and packing, and it will also provide for a thorough solvation of
the bonded phase packing. Next, connect the column outlet end to the detector, and pump 100% organic solvent at the same flow rate until a flat baseline (e.g., at 20 - 50 mAUFS) has been established for about 5-10 minutes.

**Establish Reference Point for Column Performance**

Before you equilibrate your column with the desired mobile phase, it’s also practical and useful to carry out some initial reference runs for the column or for your method. A well-behaved analyte, such as toluene (made up at 1-3 mg/mL in 60:40 ACN/water or 70:30 methanol/water, with 5 microliter injection), can be analyzed using the same mobile phase at 1 mL/min. (for a 4.6 mm i.d. column) to provide reference chromatograms for the new column. In the future, if the column or instrument performance is in question, a similar sample can be reanalyzed and chromatograms and performance reports compared with those obtained initially. Peak and chromatographic performance (retention time and peak area repeatability, plate count, peak efficiency, peak width) can be used to determine whether the column (or guard column/column) has a problem. Similar baseline and comparison runs made using your favorite or a particular method and respective analytes can also help you determine whether your column/system method performance has begun to decline by comparison of historical results.

**Column Equilibration and Transition to Desired Mobile Phase**

At this point, the column should be equilibrated with your isocratic mobile phase or the starting mobile phase for your gradient analysis, unless you are using a “true” buffered mobile phase (e.g., sodium acetate/acetic acid, potassium phosphate monobasic/phosphoric acid, etc.) for your isocratic or gradient method. If a buffered mobile phase will be used, first equilibrate the column (15-20 column volumes) with your starting organic modifier/aqueous component ratio, but minus the buffer. For example, if the starting gradient composition will be 10:90 acetonitrile/25 mM, pH 7.0 phosphate buffer, you should flush the column first with about 10-15 column volumes of 100% acetonitrile, followed by equilibration with 15-20 column volumes of 10:90 acetonitrile/water, then with 15-20 column volumes of the starting mobile phase (10:90 ACN/25 mM phosphate (pH 7.0)).

If your mobile phase consists of an organic solvent plus a mobile phase additive—such as acetonitrile/water or methanol/water with TFA (trifluoroacetic acid), acetic acid, formic acid, or phosphoric acid (pH 2.9 or lower) or other additives without an inorganic or organic conjugate acid or base salt, you can usually skip the step with the organic modifier/water equilibration.
Establish Method Performance With New Column

Now, for an isocratic separation, consider making several sample solvent blank injections followed by 2-3 standard runs to assess column equilibration. For a gradient separation, it is a good practice to run two or more blank gradients (with or without sample solvent injection), followed by two or more standard or audit sample runs to assess baseline and retention time consistency. These runs should get the column and system ready for high quality data generation.

These practices, if followed consistently, will help you to achieve superior performance from your column more quickly and predictably. In addition, a number of these steps can be automated using the Agilent ChemStation sequencing features, and can provide you with more time for other hands-on activities.