MICROSOLV TECHNOLOGY CORPORATION
HPLC Columns and Chromatography Accessories

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Suggestions for HPLC method development with silica hydride columns – Tips & Suggestions

Generic tips for developing HPLC methods using Cogent TYPE-C™ columns

It is easy to use a Cogent™ HPLC column that is made with TYPE-C Silica™ in ANP, RP or ONP.

The value of these columns is found in the wide range of solvents that can be used with them and the unique and strong retention they can have for polar compounds while at the same time retaining non polar compounds. Selection of the column (ie. Diamond Hydride, Phenyl Hydride, Amide, Diol, Bidentate C18, Bidentate C8, UDC-Cholesterol, UDA *(wcx)* or Silica-C) will be determined by your compounds of interest. Mixtures that will be highly polar and do not contain non polar compounds might be better suited with the Diamond Hydride, Phenyl Hydride, Amide or Silica-C where compounds with both polar and non polar might be better separated on the Bidentate C18, Bidentate C8, UDC-Cholesterol, Phenyl Hydride or Amide.

Step 1: Mobile Phase Selection. After you have properly installed the column, conditioned it according to our suggestions, it is a good idea to start with a typical gradient run. We suggest starting with an acidified mobile phase of water and acetonitrile. Acidify the mobile phase with up to 0.5% of an acid such as formic or acetic acid. If you are not using LCMS, TFA is another good candidate as long as you do not exceed 0.01% concentration.

Step 2: Equilibrate (Reversed Phase). Run about 6 column volumes of the mobile phase in Step 1 at 95% water.

Step 3: Reversed Phase Gradient. Set up your instrument to run a shallow gradient from 95% water to 40% water for 20 minutes. This long and shallow gradient will be very beneficial for determining the optimal gradient or isocratic method to run your mixture with later. For sharper peaks and less retention, run a shorter (Steeper) gradient from the same starting points to end points.

Step 4: Equilibrate (ANP). Equilibrate the column by running 100% acetonitrile then equilibrate the column at 90/10 acetonitrile / DI water for approximately 5 minutes before starting the gradient in step 5.

Step 5: ANP Gradient. Set up your instrument to run a shallow gradient using the same mobile phase in Step 3 to run from 90% acetonitrile to 40% acetonitrile for 20 minutes. This long and shallow gradient will be very beneficial for determining the optimal gradient or isocratic method to run your mixture with later. For sharper peaks and less retention, run a shorter (steeper) gradient from the same starting points to end points.

Step 6: Compare the Reversed Phase and ANP Data. Evaluate both gradient runs for retention time, peak shape and **elution** order as retention on the column is compound specific where some compounds will not retain in Step 3 (Reverse Phase) and some do not retain in Step 5 (**Aqueous Normal Phase**). One column could produce an isocratic run which retains both polar and non polar compounds.

Note: *The Cogent Bidentate C8™ and Bidentate C18™ columns have a unique quality in that they sometimes can retain polar compounds not retained on other*

columns while run at 100% water. You could insert an isocratic run at 100% water after Step 3 and before Step 4.

COGENT HPLC Columns™

Attachment:

COGENT TYPE-C Quick Start Guide Download File