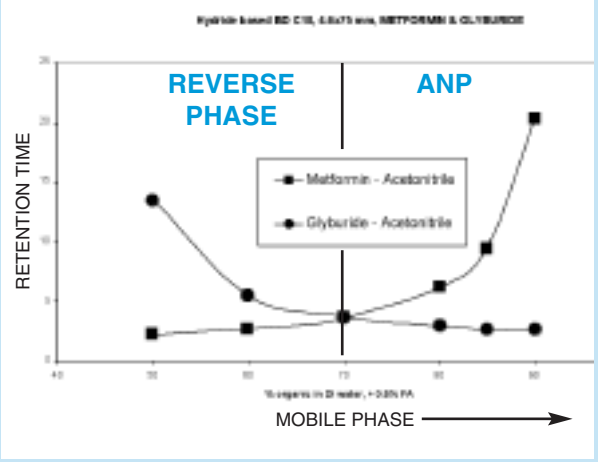
Introduction

Cogent TYPE-C™ Silica Based Bonded Stationary Phases

Cogent Type C™ Silica based, bonded stationary phases (based on silica hydride surfaces as opposed to silanols that are present on all other commercial stationary phases) have the ability to retain polar solutes at high concentrations of the organic component while maintaining an aqueous component in the mobile phase. The Cogent Type-C™ phases (Bidentate C18, Bidentate C8, UDC-Cholesterol and Silica-C) all possess this capability. The exact composition of the mobile phase where aqueous normal phase (ANP) retention begins depends on the solute, as well as the stationary phase selected.

Because of the attached organic group that is part of the stationary phase, most Type-C columns can also retain nonpolar compounds based on the typical reversed phase mechanism. The diagram below illustrates how this dual retention capability can work for both polar (metformin) and nonpolar (glyburide) compounds:



Quick Start Instructions

It is easy to use a Cogent HPLC column that is made with TYPE-C Silica.

The power of these columns is found in the wide range of solvents they can be used with and the unique and strong retention they can have for polar and non polar compounds. Selecting which TYPE-C column to use (C18, C8, Cholesterol 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 by the Silica-C where samples with both polar and non polar compounds might be better suited to the Bidentate C8, C18 or UDC Cholesterol.

Step 1. After you have properly installed and conditioned the column it is a good idea to start with a typical reverse phase gradient run. We suggest starting with an acidified mobile phase of Water as component A and Acetonitrile as component B. Acidify both components with up to 0.5% of an acid such as Formic, Methyl Phosphonic or Acetic Acid. If you are not using LCMS, TFA (up to 0.1%) is another good candidate.

Step 2. Run about 6 column volumes of the mobile phase in Step 1 at 95% Water.

Step 3. Set up your instrument to run a shallow gradient from 95% Water (A) to 40% Water (A) over 20 minutes on a 75mm long column. For longer or shorter columns,modify the gradient time proportionally.
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 the column by running 100% Acetonitrile for approximately 2 minutes for the 75mm long column.

Step 5. Set up your instrument to run a shallow gradient using the same mobile phase to run from 90% Acetonitrile (B) to 40% Acetonitrile(B) over 20 minutes for a 75mm long column. For longer or shorter columns modify the gradient time proportionally.
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. Evaluate both gradient runs for retention time, peak shape and elution order. Since analyte retention on these columns is compound and method specific some compounds may not retain in Step 3 (Reverse Phase) and some may not retain in Step 5 (Aqueous Normal Phase). However, one column could produce an isocratic run which retains both polar and non polar compounds.

Note: The Cogent Bidentate C8, C18 and UDC-Cholesterol columns have a unique quality in that they may retain polar compounds not retained on other columns while run at 100% Water without loss of retention with continued use. You could insert an isocratic run at 100% acidified Water after Step 3 and before Step 4.

If these strategies are not working, please consult our customer service support at **technical.service@mtc-usa.com**.

Optimizing Suggestions:

If you do not find satisfactory results or once you have established some retention and selectivity, you may need to optimize the method for your specific objectives. We have listed some suggestions below.

If you are not satisfied with the results, please contact our technical support team at technical.service@mtc-usa.com

1. For some organic acids that do not retain at low pH in an ANP mobile phase, you can try a high pH ANP mobile phase for retention. A rule of thumb is that for acids you would want to be from 1-2 pH units above the pKa. Some examples of additives that may work for you are Ammonium Acetate, Ammonia, Sodium Acetate, Ammonium Formate or Sodium Formate.

2. The choice of solvents that you make for ANP will have different resolving powers for Acids and Bases. Acetone, Acetonitrile, THF, Ethanol, Acetonitrile/Methanol, Methanol and then Water will have a descending strength of retention in ANP.

3. All Cogent TYPE-C columns all have the ability to perform in ANP. Depending on your sample mixture and matrix, different columns will produce different peak shapes, retention power and selectivity. Ranging from Silica-C™, Bidentate C8, UDC-Cholesterol and Bidentate C18, the amount of reverse phase will increase in this order.

4. Equilibration between gradient runs. Although the Cogent TYPE-C Columns all equilibrate quickly, the mobile phase solvents you are using will determine how much equilibration time you may need between runs. The hydride surfaces of these columns have a preference for Methanol and therefore, it may take longer to equilibrate if methanol is used in a gradient end and not in the beginning. To equilibrate, use the starting point of your gradient as the solvent composition for equilibration.

Use Aqueous Normal Phase To Achieve Normal Phase Separation with Reverse Phase Solvents

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Cogent™ TYPE-C Silica™ BASED HPLC COLUMNS

Quick Method Development Strategy for Cogent TYPE-C™

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METHOD DEVELOPMENT