Figure 4 shown on page 11 illustrates the dual retention capability of TYPE-C silica-hydride phases. In this example the non-polar molecule glyburide is eluted with reversed-phase retention only, since retention decreases with increasing percentage acetonitrile. For the polar molecule metformin, retention increases with increasing amount of acetonitrile — typical normal-phase behaviour but with an aqueous containing mobile phase. For the example of glyburide and metformin, co-elution of the two compounds would occur at 70% acetonitrile, with a reversal of elution order above this value.

The effect of temperature in ANP is opposite to reversed-phase for many solutes.

The less hydrophobic modified phases such as Cogent Diamond Hydride™, Cogent Phenyl Hydride™, Cogent Bidentate C8™ show the best performance for separation by ANP. Bonded phases which are more hydrophobic show weaker ANP characteristics. Greater ANP separation is generally achieved using acetonitrile rather than methanol.

Typical Application Areas

ANP is particularly useful for the analysis of polar compounds and in most cases offers a preferable alternative to polar embedded or HILIC (Hydrophilic Interaction Liquid Chromatography) phases. The technique is widely used and referenced in metabolomic profiling, Natural Products and many others.

Advantages of TYPE-C Silica Phases with Aqueous Normal-Phase

- Retains polar and hydrophilic compounds not retained by reversed-phase
- Precision run to run is unsurpassed by leading column brands
- Enhanced LC-MS sensitivity
- Better for prep chromatography due to the high volatility of mobile phases and higher yields

Method Development Strategy for Selection of Reversed-Phase or Aqueous Normal-Phase

STEP 1.	After installation and conditioning of the column, it is a good idea to start with a 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% formic or acetic acid. If you are not using LC-MS, TFA (up to 0.1%) is another option.
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 to 40% water 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 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 point to end point.*
STEP 4.	Equilibrate the column by running 100% acetonitrile for approximately 2 minutes for the 75mm long column. Adjust run time according to your column length.
STEP 5.	Set up your instrument to run a shallow inverse gradient using the same mobile phase composition as in Step 1 to run from 90% acetonitrile to 40% acetonitrile over 20 minutes for a 75mm length column. For longer or shorter columns, modify the gradient time proportionally. *This long and shallow gradient will be 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 point to end point.*
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 (reversed-phase) and some may not retain in Step 5 (ANP). However, one column could produce an isocratic run which retains both polar and non-polar compounds.