Advantages of TYPE-C™ Silica for Reversed-Phase (RP) HPLC

- **Can be used with 100% aqueous mobile phases**
  Many Type B bonded phases have limitations on the percentage of water they can tolerate in order to avoid 'phase collapse' or 'pore de-wetting'. The presence of direct silicon-carbon (Si-C) bonds in TYPE-C silica phases, with minimal silanol presence, overcomes this issue and all TYPE-C silica-hydride phases can be used with 100% water.
- **Improved pH stability**
  Lack of end capping and the strong Si-C (replaces typical siloxane bonds) bonds in the bonded TYPE-C silica phases make them immune to ligand cleavage under acidic conditions.
- **More retentive for hydrophobic compounds**
  A higher concentration of organic solvent (acetonitrile or methanol) is used to achieve retention data comparable to other non TYPE-C silica-hydride columns, which is a benefit for LC-MS.
- **Resistant to most additives such as PIC reagents**
  Some generic or USP methods specify the inclusion of a potentially damaging reagent in the mobile phase, such as a PIC reagent, which tends to shorten column lifetime. However, because of their chemical resistance, methods can be transferred to Cogent TYPE-C silica without worry of damage to the column and increase instrument "up-time".
- **No bleed of bonded phases or endcapping**
  The strong Si-C bonds minimize ligand cleavage, a benefit for LC-MS. In addition, there is no endcapping. Improves LCMS signal to noise.
- **No "on-column" degradation of analytes due to acidity**
  The lack of silanols reduces the surface acidity and hence reduces the risk of analyte degradation. Great for natural products or bio-active compounds.
- **Fast equilibration-Fast Methods-More Data**
  Extremely fast equilibration between gradient runs enables methods to be "green" and developed, with considerable cost savings in solvent usage. Typically, Type B silica HPLC columns requires more than 15-20 column volumes to equilibrate. Cogent TYPE-C silica columns only require 1-4 column volumes to equilibrate. This feature makes them excellent for LC-MS. Time savings are extremely high especially during method development or high throughput screening.

Method Development Strategy for Reversed-Phase HPLC

STEP 1.	After installation 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.	If sufficient retention of polar components is not achieved, or it is suspected that further 'unknowns' may be present, consider ANP (see step 5, page 12).