
What are the main differences between HILIC and Aqueous Normal Phase?

There are many important differences between HILIC columns and Cogent TYPE-C Silica™ columns but for the sake of brevity, we will only address the main differences.

The Cogent TYPE-C™ silica columns (silica hydride) perform similarly to HILIC columns as far as polar compound retention in a “Normal Phase” elution order is concerned. With both types of columns using higher than 70% organic composition of the mobile phase and with an inverse gradient for retention of polar compounds and that is where the similarities end.

It is well known that HILIC columns perform separations based on variation of normal phase chromatography in a water rich environment. Simply put, polar compounds partition into and out of the **hydration shell** created by adsorbed water on the ordinary silica surface of the stationary phase. As the acetonitrile concentration is greater than the water layer the charged polar analytes are retained by the combination of ionic interaction with the silanols under the water layer and the partitioning effect. The combination of these two mechanisms is mainly responsible for retention of polar compounds in HILIC mode. Also, often “salting” the mobile phase with acids and bases is required to get many polar compounds to retain by using relatively high concentrations of buffers. As rule of thumb, the higher the salt, the higher the better the retention.

Cogent columns perform **Aqueous Normal Phase (ANP)** separations based on a variation of normal phase chromatography in an **extremely low water** environment based mainly on adsorption chromatography and without partitioning. Polar compounds and some non polar compounds are retained by an ionic interaction with hydroxyl ions in the adsorbed mobile phase layer on the surface of the silica hydride. As the water concentration increases in the mobile phase increased the charged polar analytes are eluted from the column. Since silica hydride is slightly hydrophobic and will readily adsorb or desorb the mobile phase as it changes, there is no hydration shell on the silica surface to manage making equilibration extremely fast. Since there is little to no variation of the stationary phase or the chromatographic environment from run to run the **precision** of RT is easily accomplished.

The HILIC stationary phase which is made of ordinary chromatographic silica and is extremely hydrophilic and factors like temperature, pressure, mobile phase additives, number of residual silanols on the silica surface, broken bonds with end capping and stationary phases makes

managing the hydration shell challenging and at the very least, time consuming. It is often recommended by the HILIC column manufacturers to perform 60 column volumes to get the columns ready for use and then 20-25 column volumes of equilibration between runs to achieve **precision**. Some manufacturers will recommend to allow the columns to “rest” for 10-15 minutes after equilibration to gain performance. Mostly HILIC columns are performing outside of their natural state. Cogent column stationary phase is made of silica hydride and is relatively hydrophobic and does not produce a water layer. Also silica hydride surface has been shown to adsorb the mobile phase and concentrate the aqueous portion near the silica surface. This layer is very thin and easily desorbed when the mobile phase concentration changes. It is only necessary to perform 3-5 column volumes between gradient runs to achieve extraordinary **precision**. There is no need to allow these columns to rest and you can make another injection very quickly. Silica hydride columns operate extremely well with any HPLC solvent and can be used in ANP, normal phase or reversed phase with minimal hysteresis and no damage to columns.

It has been said that HILIC is a great idea and product if you have patience, extra time and maybe an extra instrument. With Cogent TYPE-C columns, you can expect...

What you can expect from Cogent Columns with polar compound methods

Fast Equilibration between gradient runs (Typically 3-5 columns volumes)

Extraordinary **precision** of RT compared to HILIC

Low salt needed for excellence compared to HILIC

Long column lifetime compared to most silica based columns

Uses same MP concentration as many Open Access LC Systems inversely

Retain Polar and Non Polar in same run

Perform 3 Modes of Chromatography compared to one on HILIC columns

Low Hysteresis when changing chromatographic modes

See also: [ANP Retention Mechanism](#)

See also: [Comparison of the efficiency in ANP vs. HILIC](#). See also: [Wikipedia definition of ANP](#)

