COGENT

HPLC Columns™

SINCE 2003

Works great with Polar & Non-polar compounds.

Preferred in Metabolomics & Bioanalytical labs worldwide.

Achieve robust methods with very fast equilibration.

CPS analitica for Chemistry

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MICROSOLV TECHNOLOGY CORPORATION

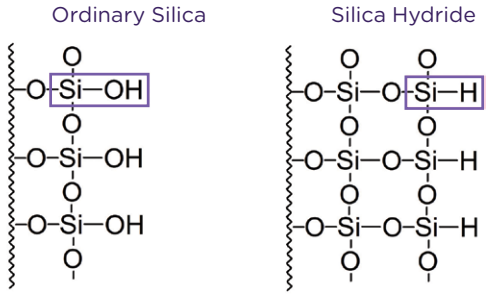
What is Cogent TYPE-C™ Silica?

VALUE PROPOSITION:

Improve your method development, run time and column lifetime by using Cogent TYPE-C™ silica-hydride technology. Get the competitive edge in your industry and make an impact on your company's bottom line by lowering the cost of analysis and possibly providing 'greener' applications by taking advantage of the time and solvent savings these columns can provide. Cogent™ columns bring modern technology to your lab for less money, while making challenging separations more robust and reliable. Using these columns is simple. The on-boarding process and lifetime support makes bringing them to the lab a smooth (even enjoyable) and scientifically valid process.

The introduction of Cogent silica-hydride technology offers a considerable advance in HPLC column technology. TYPE-C silica consists of high purity, low metal content silica particles that have been manufactured so that their surface layer is populated with silicon hydride (Si-H) instead of silanols (Si-OH). These phases are formed from a high purity Type B silica backbone, by replacing >95% of the surface silanols with Si-H (see Figure 1). It can be seen that the internal structure of silica-hydride and 'ordinary' silica is essentially the same, in that the siloxane bonds leading to rigidity and strength are the same. The difference is that the surface silanols are replaced with Si-H, which create a stable hydrophobic surface. The lack of silanols on the surface also means that endcapping is not required.

Figure 1.

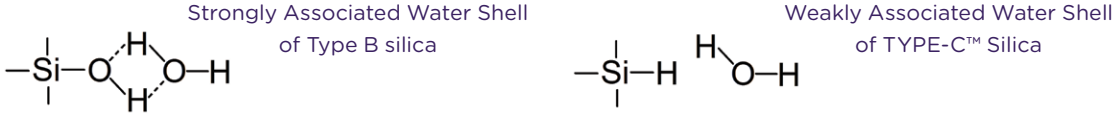


Same basic structure with different surface chemistries

TYPE-C silica-hydride has all the physical advantages of Type B silica, such as spherical shape, low metal content, high purity, high mechanical strength, narrow pore size distribution and ease of chemical modification. However, TYPE-C silica-hydride products also have many advantages over the Type B silica columns.

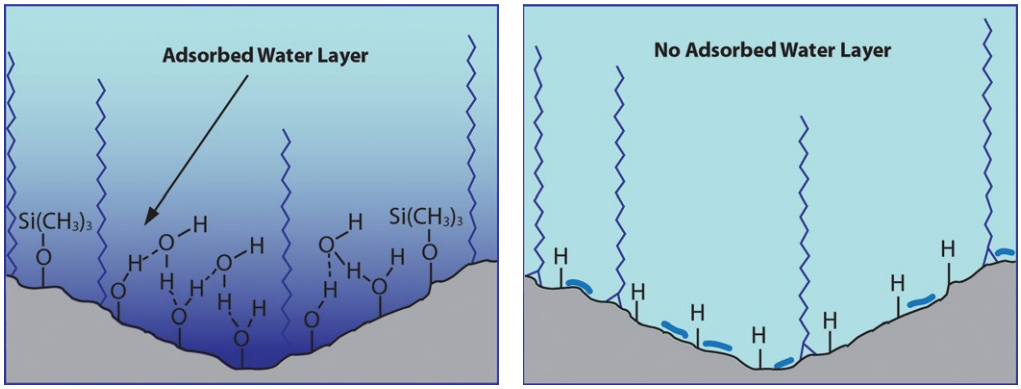
Due to the unique hydride surface, Cogent TYPE-C silica can bond with any chemical moiety which possesses either a terminal double or triple bond. Due to the resulting strong chemical bond between silicon and carbon, these bonded phases show increased stability and improved resistance to conditions that may cause hydrolysis in Type B silica columns.

Figure 2.



Additionally, the surface silanols that are present in all Type A and Type B silicas, even after bonding and extensive endcapping, form a strong association with water, resulting in a 'hydration shell' surrounding the silica (see Figure 2). This adsorbed water layer does not desorb unless it is baked at 600°C and kept under non-aqueous conditions. However, the silica-hydride particles of TYPE-C silica have different adsorption characteristics with only a weak attraction for water. These effects can more easily be visualised from Figure 3 on page 7.

Figure 3. Ordinary HPLC Columns Cogent TYPE-C Silica HPLC Columns



TYPE-C silica phases can be used in three different modes of HPLC: Classic reversed-phase (RP), organic normal-phase (ONP) with non-polar solvents (such as hexane) and normal-phase elution with aqueous solvents. This last technique is referred to as Aqueous Normal-Phase (ANP), which is a powerful technique for the separation of polar compounds. Due to this ability to be used in three different modes of HPLC, the selectivity power of any one phase is vastly increased and one column can be used to separate polar and non-polar compounds at the same time or in different runs.

Features and Benefits of TYPE-C Silica

TYPE-C silica columns offer you the chromatographer many features and benefits:

Feature	Chromatographer's Benefits
Silicon-Carbon bonds instead of Siloxane	More stable and durable
Si-H replaces Si-OH	Rapid equilibration between gradients
Weakly associated hydration shell	Water friendly columns, easy to use
Temperature stability increased	Use temperature as a selectivity tool without damage to the column
Free of salts	Contaminant free surface
Use 100% water on C18	Without loss of retention with time
Lack of pH hysteresis	Quickly change mobile phases and pH buffers
Perform ANP and RP at the same time	Separate polar and non-polar compounds in the same run. Unknown-unknowns are more likely to be identified using dual mode
Retain polar compounds at extremely high organic content	Increases sensitivity of LC-MS using ESI
Use non-polar solvents	Retain and separate compounds which are insoluble in water
Low affinity for water	Run NP separations without problems of moisture in solvents
Use high % organic content in mobile phases	Inject sample diluent (high organic) directly on to column – saves sample prep time
Bonded phase that performs ANP, RP and ONP	Get the performance of HILIC columns on a stable, robust phase
High efficiency and stability	Good peak shapes and long-lasting columns, leading to reduced column costs

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

VALUE PROPOSITION:

Improve your method development, run time and column lifetime by using Cogent TYPE-C™ silica-hydride technology in the Reversed-Phase mode. Take a competitive edge in your industry and make an impact on your company's bottom line by lowering the cost of analysis. Produce 'greener' applications and take advantage of the time and solvent savings these columns can provide. Cogent™ HPLC columns can make your difficult methods more robust and reliable. Using these columns is simple and the lifetime support from MicroSolv makes bringing them to the lab a smooth (even enjoyable) and valid process.

Reversed-phase (RP) HPLC is the most commonly used HPLC technique and in many cases is the first choice for method development of small molecules.

In RP chromatography analytes partition between a non-polar (hydrophobic) stationary phase and a polar mobile phase (the opposite or 'reverse' of normal-phase). In general terms, analytes elute according to their hydrophobicity, with the more polar compounds eluting first and the less polar compounds eluting last. Mobile phases generally consist of a binary mixture of water and polar organic solvent, such as acetonitrile or methanol. Retention times increase as the percentage of the most polar solvent (water) increases. Typical bonded phases for RP include alkyl hydrocarbons, with C18 being the most common.

Due to its unique silica surface, Cogent TYPE-C silica can be bonded via a hydrosilation reaction with many chemical moieties which possess either a double or triple bond. The resulting direct chemical bonds between silicon and carbon make these phases much more stable than other columns and resistant to conditions that can cause hydrolysis such as very low pH. Phases show excellent lot to lot consistency, precision from run to run and little or no silanol activity. This results in greatly improved column lifetime which in turn relates to lower costs and more throughput in your lab.

All TYPE-C silica stationary phases display some degree of RP behaviour. Even the unmodified Cogent Silica-C™ can retain non-polar compounds due to the hydride surface being slightly hydrophobic. As the hydrophobicity of the stationary phases is increased by having greater surface coverage of bonded organic ligands, retention of non-polar compounds increases, just as with other (Type B) reversed-phase materials. The main TYPE-C™ silica columns recommended for RP separations are Cogent Bidentate C18™, Cogent Bidentate C8™ and Cogent UDC-Cholesterol™, but Cogent Phenyl Hydride™ and Cogent Diol™ may also be used.

Cogent TYPE-C phases are ideal for generic or USP methods, as separations can easily be transferred on to these columns.

Application areas:

Reversed-phase HPLC and LC-MS are widely utilized in the majority of industry sectors, including food and beverage, pharmaceutical, clinical, environmental, forensic and others. Cogent TYPE-C silica columns can provide benefits in all these fields.

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).

Cogent TYPE-C™ Silica for Organic Normal-Phase (ONP) HPLC

VALUE PROPOSITION:

Improve your method development, run time and column lifetime by using Cogent TYPE-C silica-hydride technology. Get the competitive edge in your industry and make an impact on your company's bottom line by lowering the cost of analysis and possibly providing 'greener' applications by taking advantage of the time and solvent savings these columns can provide. Cogent™ columns bring modern technology to your lab for less money, while making challenging separations more robust and reliable. Using these columns is simple. The on-boarding process and lifetime support makes bringing them to the lab a smooth (even enjoyable) and scientifically valid process.

Background: In ordinary normal-phase chromatography, sample mixtures are separated into their components by adsorption/desorption of the analytes on to a polar stationary phase, using a non-polar or moderately polar mobile phase. The rate at which individual solutes migrate through these columns is mainly a function of their polarity. For normal-phase on ordinary silica a 100% organic solvent is typically used. In this system, the least polar analytes elute first, whereas the most polar analytes have strong interactions with silanol groups and elute last.

Normal-phase separations have typically been performed on ordinary Type B unbonded silica or bonded phases, such as cyano or amino. Unbonded silica supports are hygroscopic in nature and retain water quite strongly. Water is adsorbed by organic solvents to varying extents, depending on atmospheric conditions and type of solvent used. Therefore, Type B silica can adsorb water from the mobile phase due to the presence of the free silanols, to create a 'hydration shell'. As the water content increases, the analyte retention times can change, resulting in longer equilibration times and lack of reproducibility. In such analyses, measures need to be taken to tightly control the water content of the mobile phases.

This problem is overcome by the use of Cogent TYPE-C silica phases, as the lack of silanols and the silicon-hydride groups (Si-H) on the silica surface virtually eliminates the adsorption of water avoiding the resultant 'hydration shell' which is very difficult to manage. This makes them an excellent choice for Organic Normal-Phase, enabling greater speed and a wider range of solvents to be used. The weaker water adsorption also accounts for the little or no hysteresis observed when changing from ONP to ANP or RP.

Application Areas:

Normal-phase HPLC may be used for the analysis of polar analytes such as amines, acids, metal complexes, isomers and water labile compounds and fats.

Advantages of TYPE-C Silica for Organic Normal-Phase HPLC

- **No significant hydration shell**
  The lack of silanols minimizes the adsorption of water, making chromatography more reproducible
- **Suitable for preparative HPLC**
  Solvents easy to evaporate. Greater stability and reproducibility
- **Fast column equilibration**
  Reduces solvent consumption therefore "greener"

Method Development Strategy for Organic Normal-Phase HPLC

STEP 1.	Run a gradient of hexane with 5% to 95% ethyl acetate.
STEP 2.	Modify the gradient to improve component resolution or develop an isocratic separation.

Cogent TYPE-C™ Silica for Aqueous Normal-Phase (ANP) for Polar Compounds

VALUE PROPOSITION:

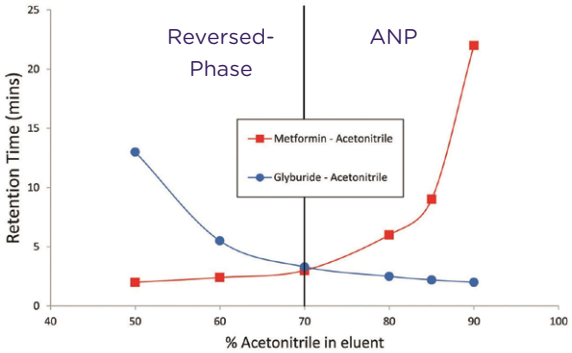
An important advantage for you with Cogent™ HPLC columns is Aqueous Normal-Phase (ANP) chromatography. This valuable technique presents a key opportunity for chromatographers to improve method development time, application run time and column lifetime when separating polar compounds or non polar bio-active compounds. This technique offers improvements in run to run precision and solutions to the problem of separating closely related compounds. Aqueous Normal-Phase must be developed on Cogent TYPE-C™ Silica columns to gain significant improvements over HILIC or IEX type methods. Take a competitive edge in your industry and company by helping lower your cost of analysis, with reduced run time and reduced solvent usage. Using these columns is simple. The on-boarding process and lifetime support makes bringing them to the lab a smooth (even enjoyable) and scientifically valid process.

ANP is a technique involving the mobile phase region between Reversed-Phase (RP) and Organic-Normal-Phase (ONP). TYPE-C silica-hydride phases have the ability to retain compounds in both the reversed-phase and normal-phase modes since the mobile phases contain high concentrations of organic solvent (acetonitrile or acetone) with a lower quantity of water. Therefore the mobile phase for ANP is both 'aqueous' and 'normal' (being less polar than the stationary phase). Thus polar solutes (such as acids and amines) are most strongly retained in ANP, with retention decreasing as the amount of water in the mobile phase increases. ANP therefore shows elution order patterns similar to that of NP (most polar last) but with mobile phase conditions similar to RP (see table below).

	Reversed-Phase (RP)	Organic Normal-Phase (ONP)	Aqueous Normal-Phase (ANP)
Analytes	Most polar analytes elute first, least polar last	Most polar analytes elute last, least polar first	Most polar analytes elute last, least polar first
Mobile Phase	Polar organic and aqueous mobile phase e.g. water/MeCN, water/MeOH	Non polar organic or moderately polar organic e.g. hexane	Polar organic and water e.g. water/MeCN, water/acetone
Columns	Non polar bonded phases e.g. C18, C8, Phenyl, UDC-Cholesterol, Amide	Unbonded silica or polar bonded columns e.g. Silica-C, Diol, C18	TYPE-C silica columns. e.g. Silica-C, Diamond Hydride, Diol, Amide, Phenyl, UDA, UDC-Cholesterol

Typically, the amount of the non-polar component (acetonitrile) in the mobile phase must be 60% or greater with the exact point of increased retention depending on the solute and the organic component of the mobile phase. A true ANP phase will be able to function in both the reversed-phase and normal-phase modes with only the amount of water in the mobile phase varying.

Figure 4.



RP and ANP retention capability

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

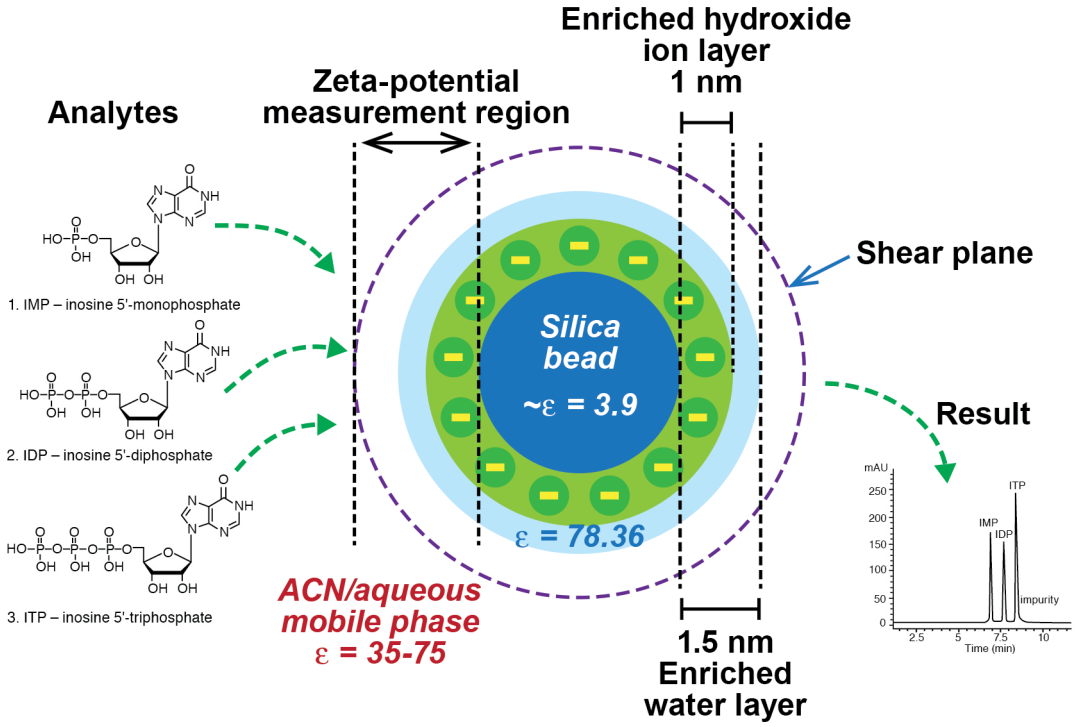
STEP 1.	After installation and conditioning of the column, it is a good idea to start with a gradient run. We suggest starting with an acidified mobile phase of water as component A and acetonitrile as component B. Acidify both components with up to 0.5% formic or acetic acid. If you are not using LC-MS, 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.

ANP Mechanism[1]

The precise mechanism of ANP retention is an active area of investigation as of the publication of this catalog. However, a recent study involving zeta potential measurements to help characterize the surface has demonstrated that the water layer on a silica-hydride surface is, on average, only 0.5 of a monolayer, in contrast to 7–8 monolayers for ordinary Type B silica. This low amount of water on the surface precludes a partitioning process.

In addition, it has been determined that the TYPE-C silica surface possesses a negative charge. Instead of this charge being the result of surface silanols, as is the case for the ordinary unbonded silica used for HILIC methods, it has been ascribed to the presence of excess hydroxide ions adsorbed on the surface, derived from the aqueous component of the mobile phase (see Figure 5). Hydroxide ions from the surrounding liquid accumulate on the slightly hydrophobic silica-hydride surface. The mechanism of ANP is therefore thought to be a combination of ion attraction for positively charged species or ion displacement for negatively charged compounds. For polar neutral compounds a displacement/adsorption effect for retention is most likely.

Figure 5.



ANP vs HILIC

Cogent TYPE-C silica columns perform similarly to HILIC (Hydrophilic Interaction Liquid Chromatography) columns in that they both show increased retention times for polar compounds (when using > 70% organic composition of the mobile phase) compared to reversed-phase HPLC. Both column types perform separations that are based on variations of normal-phase, but they each have different retention mechanisms and various other different properties.

HILIC stationary phases are typically more polar than TYPE-C silica bonded phases, which are relatively non-polar.

On HILIC columns, retention of polar compounds is achieved by partitioning in and out of the adsorbed water layer surrounding the stationary phase surface. As the acetonitrile concentration increases, the water layer decreases and the charged polar analytes are retained by a combination of cation-exchange with the silanols under the water layer and the partitioning effect.

continued on next page

1 C. Kulsing, Y. Yang, C. Munera, C. Tse, M.T. Matyska, J.J. Pesek, R.I. Boysen, M.T.W. Hearn, Analytical Chimica Acta 817 (2014) 48-60
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C. Kulsing, Y. Nolvachai, P.J. Marriott, R.I. Boysen, M.T. Matyska, J.J. Pesek, M.T.W. Hearn, J. Phys. Chem. B, 119 (2015) 3063-3069

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With Cogent TYPE-C™ silica columns, charged polar compounds elute in a similar order as on HILIC columns. Since there are virtually no silanols on these columns, the polar compounds are retained more by the adsorptive character of the silica-hydride surface, rather than by a partition mechanism. In addition, the non-polar ligand of the TYPE-C silica phases will also retain non-polar compounds.

As a result of the weak association of water with the TYPE-C silica-hydride, there is a lack of a hydration shell at high organic content. This allows the column to equilibrate and change more rapidly than HILIC columns. This is a significant advantage for rapid gradients.

Another significant advantage of ANP over HILIC is reproducibility. Historically HILIC as a technique has suffered from a reputation for poor gradient method reproducibility. One of the main causes of this can be attributed to the variability in the thickness of the hydration shell surrounding the silica surface of Type B silicas. Conversely TYPE-C silica-hydride phases used in ANP do not suffer from this because the enriched hydroxide ion water layer is much more stable, resulting in improved method reproducibility.

The Cogent TYPE-C silica columns are also more versatile, as they can be used in RP, ANP and ONP modes, without hysteresis or damage to the columns. HILIC columns can only retain polar compounds and are not suitable for RP analyses.

Key Advantages of ANP over HILIC:

- Polar and non-polar compounds can be separated in the same isocratic run
- Precision run to run, day to day, batch to batch
- Equilibration time is much faster
- TYPE-C silica-hydride columns can perform ANP, RP and ONP, whereas HILIC columns generally can only perform HILIC separations
- TYPE-C silica-hydride columns offer low bleed for LC-MS

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