

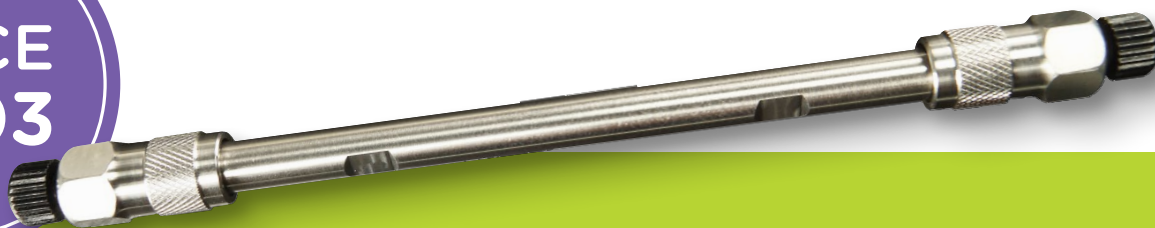


# COGENT

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## HPLC Columns™

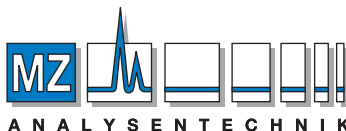
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**MICROSOLV** TECHNOLOGY CORPORATION



# MICROSOLV TECHNOLOGY CORPORATION

Leaders in Separation & Purification Technologies

MicroSolv has been designing and producing solutions for separation and purification technologies since our inception in 1992. Partnering with innovators at academic institutions and with inventors within the industry, we have introduced many ground breaking products that lead to better methods and better results for laboratories.

At our core is the Cogent™ brand of HPLC columns. These unique columns have helped to change the way polar compounds have been analyzed since their introduction in 2003. Based on silicon-hydride surface features, they are the only columns capable of producing reliable results in Aqueous Normal Phase (ANP). This mode of chromatography opened up an entire range of analysis and purification in bioanalytical labs as well as chemical purification.

Since 2003, we have been hard at work creating new successful phases for more and more difficult, closely related compounds from complex mixtures. This new catalog with our new logo and branding is our presentation of many new phases, column hardware and applications.

I hope you enjoy reading this catalog and that you find technologies that help you advance your laboratory and become more technology competitive.

Bill Ciccone



President  
MicroSolv Technology Corporation

**“Working with Cogent Bidentate C18 was easy and fast, the precision of the column from run to run was remarkable. I placed this into our method with great results. The column lifespan is incredible!”**

Allen Looney, Director of Quality  
Generic Pharma Company

**“MicroSolv continues to push the boundaries of contemporary LC with their suite of Cogent TYPE-C silica™ columns. The Diamond Hydride™ series columns in particular are an excellent choice for the analysis of polar compounds.”**

Dr Reinhard Boysen, MRACI CChem, Research Fellow  
School of Chemistry, MONASH UNIVERSITY

**“I evaluated Cogent Bidentate C18 and the UDC-Cholesterol columns against conventional HPLC columns for the separation of structurally-similar compounds. I found that the C18 was the most retentive and the UDC-Cholesterol has some very interesting shape recognition characteristics. These columns perform very well and offer different selectivities compared to other stationary phases.”**

Dr. Mark Powell, Director  
Mark Powell Scientific Ltd.

**“I have used the Cogent range of chemistries, particularly the Cogent Diamond Hydride, for this group’s bioanalytical LC-MS and found the selectivities attainable to be distinctive and extremely useful and they continue to be a valuable part of our toolbox for production of rugged quantitative methodologies, especially multi-analyte.”**

Robert MacNeill, Head of Method Development  
Pharmaceutical CRO

**“As the inventor of silica-hydride HPLC stationary phases, it has been my great pleasure to work with the Cogent columns in many research papers from many disciplines and applications. I have very much enjoyed working with scientists and helping them attain results they could not get without these columns. I look forward to continuing this program and working with more challenges.”**

Professor Joseph Pesek  
Department of Chemistry, San Jose State University

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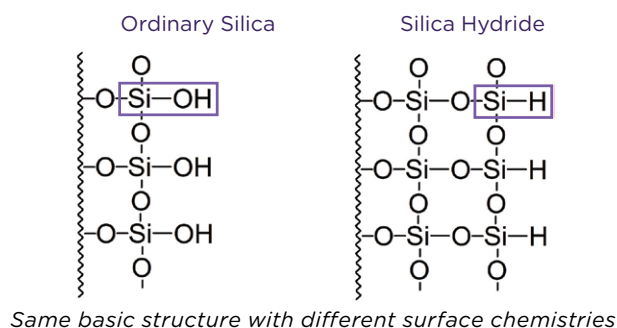
# What is Cogent TYPE-C™ Silica?

## 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.

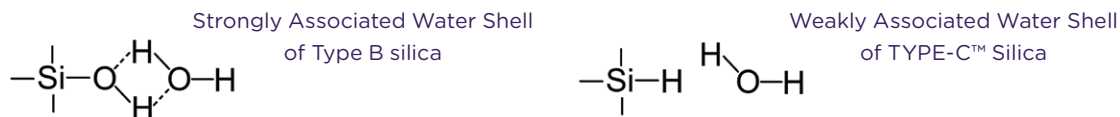
Figure 1.



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.

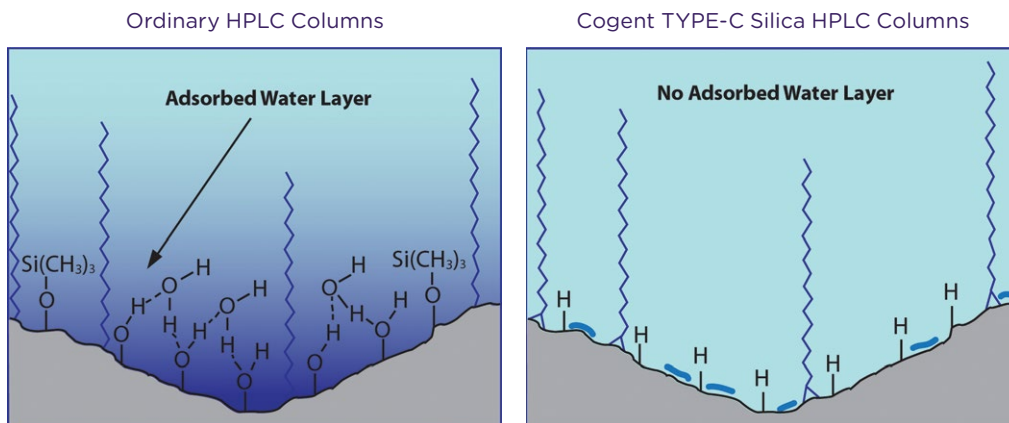
Due to the unique hydride surface, Cogent TYPE-C silica can bond with any chemical moiety which possesses either a terminal double or triple bond. Due to the resulting strong chemical bond between silicon and carbon, these bonded phases show increased stability and improved resistance to conditions that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



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-hydrate 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-hydrate 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-hydrate 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)
<b>Analytes</b>	Most polar analytes elute first, least polar last	Most polar analytes elute last, least polar first	Most polar analytes elute last, least polar first
<b>Mobile Phase</b>	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
<b>Columns</b>	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.

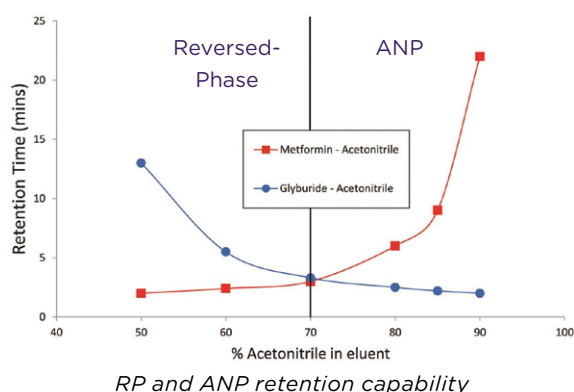


Figure 4 shown on page 11 illustrates the dual retention capability of TYPE-C silica-hydride phases. In this example the non-polar molecule glyburide is eluted with reversed-phase retention only, since retention decreases with increasing percentage acetonitrile. For the polar molecule metformin, retention 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.*

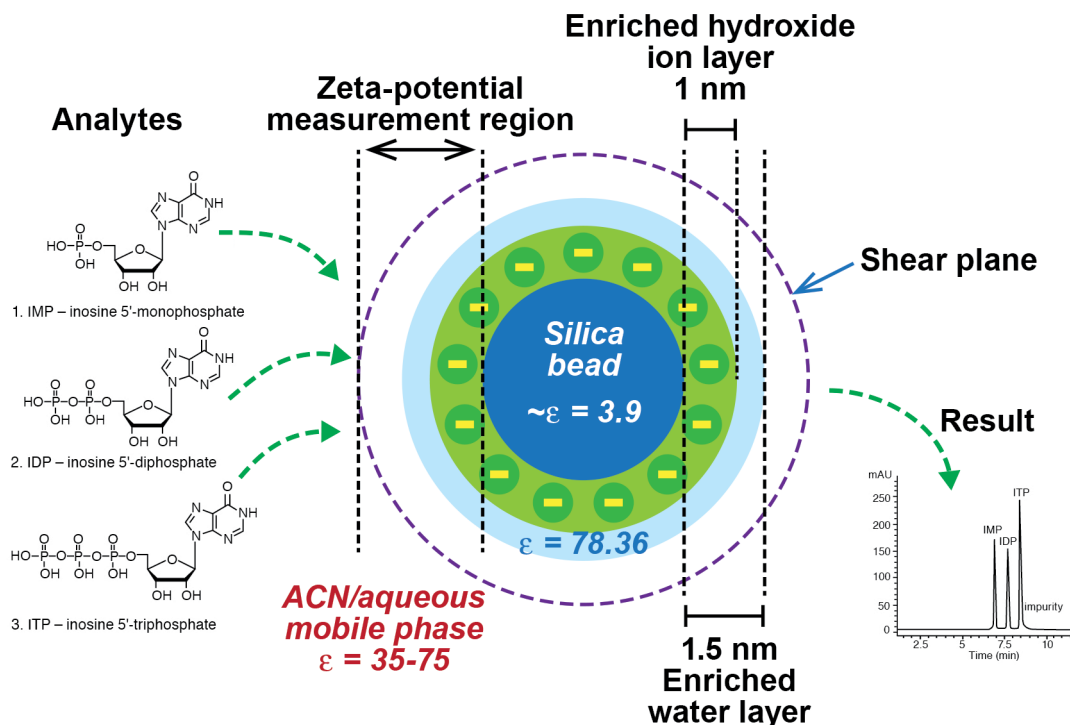
**STEP 6.** Evaluate both gradient runs for retention time, peak shape and elution order. Since analyte retention on these columns is compound and method specific, some compounds may not retain in Step 3 (reversed-phase) and some may not retain in Step 5 (ANP). However, one column could produce an isocratic run which retains both polar and non-polar compounds.

# ANP Mechanism<sup>1</sup>

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  
 J.J. Pesek, M.T. Matyska, N. Salehi, *Current Chromatography* 2 (2015) 41-47  
 C. Kulsing, Y. Yang, M.T. Matyska, J.J. Pesek, R.I. Boysen, M.T.W. Hearn, *Analytica Chimica Acta* 859 (2015) 79-86  
 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

continued from previous page

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-hydrate 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-hydrate, 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-hydrate 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-hydrate columns can perform ANP, RP and ONP, whereas HILIC columns generally can only perform HILIC separations
- TYPE-C silica-hydrate columns offer low bleed for LC-MS



**COGENT**  
HPLC Columns™

**Cogent LCMS Columns**

Works great with Polar & Non-polar compounds.	Preferred in Metabolomics & Bioanalytical labs worldwide.	Achieve robust methods with very fast equilibration.
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**Made with Silica Hydrate**

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[www.mtc-usa.com](http://www.mtc-usa.com)

# Cogent Bidentate C18™

## Highly Retentive

Cogent Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m <sup>2</sup> /g)	Carbon Load (%)	Endcapped	Optimum pH Range	Recommended Max. Temp. (°C)	USP Code
<b>Bidentate C18</b>	4	100	390	18-19	No	2.0 – 10.0	80	L1
<b>Bidentate C18 2.6™</b>	2.2	120	340	20	No	2.0 – 10.0	80	L1

For further details on 2.6 columns, please see page 25. For ordering information, see page 30.

Using proprietary bonding technology, the straight chain C18 hydrocarbon is bonded directly to the TYPE-C silica-hydride surface at two separate points of attachment with direct silicon-carbon bonds. This Si-C bond is virtually indestructible and confers great stability and longevity to these columns. This unique bonding technology and subsequent lack of silanols eliminates the need for endcapping thus making the columns even more durable.

Columns are suitable for use with mobile phases containing from 100% water to 100% organic solvent, although their greatest merits and most common usage are in reversed-phase separations. This is a very hydrophobic phase, showing increased retention compared to Type B silica bonded C18 phases. The use of 100% aqueous buffers can give excellent selectivity for highly polar organic acids and bases without the need for ANP.

Cogent Bidentate C18 can be considered as a laboratory workhorse column since it is so stable and the column lifetime is considerably greater than other ordinary C18 columns. It is ideal for generic or USP methods which specify the use of a C18 (or USP L1) column.

These methods can be transferred to Cogent Bidentate C18 without requiring revalidation of a GMP method. Note that a higher concentration of organic solvent may be required if it is desired to achieve retention data comparable to a non TYPE-C C18 column.

Figure 6.

### Bidentate C18

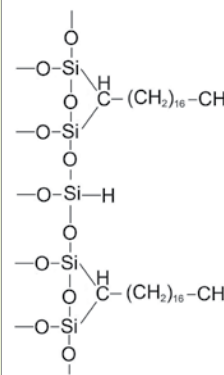
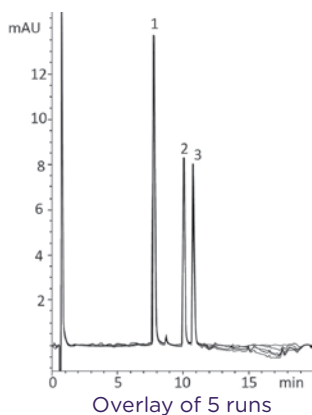


Figure 7.



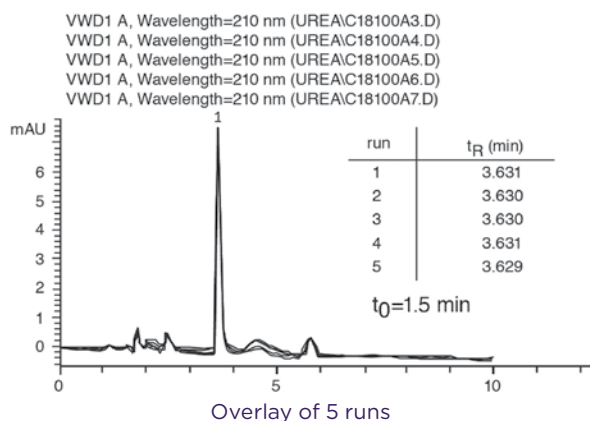
### Method Conditions

Column: Cogent Bidentate C18, 2.2µm, 120Å  
 Catalog No.: 40218-05P-2  
 Dimensions: 2.1 x 50mm  
 Mobile Phase: A: DI H<sub>2</sub>O / 0.1% formic acid (v/v)  
 B: Acetonitrile / 0.1% formic acid (v/v)  
 Gradient:

time (min.)	%B
0	10
1	10
15	30
16	30
17	10

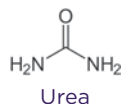
Post Time: 3 min  
 Injection vol.: 0.2µL  
 Flow rate: 0.3mL/min  
 Detection: UV 254nm (Perkin-Elmer instrument)  
 Peaks: 1. Eriocitrin  
 2. Naringin  
 3. Diosmin

Figure 8.



### Method Conditions

Column: Cogent Bidentate C18, 4µm, 100Å  
 Catalog No.: 40018-15P  
 Dimensions: 4.6 x 150mm  
 Mobile Phase: A: DI water (ISOCRATIC Run: 100% A)  
 Injection vol.: 10µL  
 Flow rate: 0.5mL/min  
 Detection: UV 210nm  
 Peak/Sample: Urea (1mg/mL in DI water)



# Cogent Bidentate C8™

## Less Retentive RP

Cogent Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m <sup>2</sup> /g)	Carbon Load (%)	Endcapped	Optimum pH Range	Recommended Max. Temp. (°C)	USP Code
<b>Bidentate C8</b>	4	100	390	13-14	No	2.0 – 8.0	80	L7
<b>Bidentate C8 2.6™</b>	2.2	120	340	11-12	No	2.0 – 8.0	80	L7

For further details on 2.6 columns, please see page 25. For ordering information, see page 30.

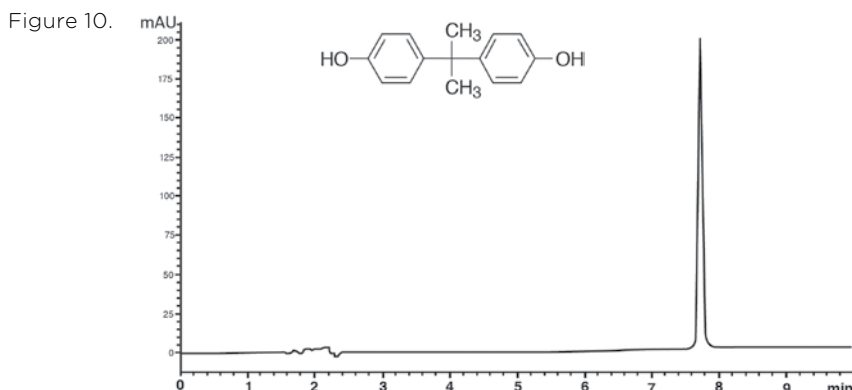
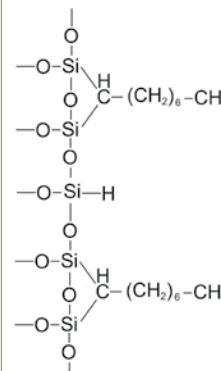
As with the Cogent Bidentate C18 phase, the Cogent Bidentate C8 phase is bonded directly to the TYPE-C silica-hydride surface with two separate points of attachment, using proprietary bonding technology. The phase produces columns that are very stable, efficient and can be operated in three different modes – reversed-phase, normal-phase and ANP, although reversed-phase is the predominant separation technique with this phase.

Cogent Bidentate C8 is less hydrophobic than the Bidentate C18 phase and is an excellent choice for complex mixtures of slightly hydrophilic and hydrophobic compounds. Polar compounds may be retained at low pH for bases and neutral pH for acids. Fast equilibration between gradient runs enables a faster turn-around of samples.

Bisphenol A is a synthetic compound used in a variety of consumer products. It is believed to be an endocrine disruptor, so requires sensitive assays for biological and environmental monitoring. Cogent Bidentate C8 shows good retention of Bisphenol A, with only one column of mobile phase required for equilibration after the gradient. Figure 10 shows the reproducibility of this assay in an overlay of 5 runs.

Figure 9.

### Bidentate C8

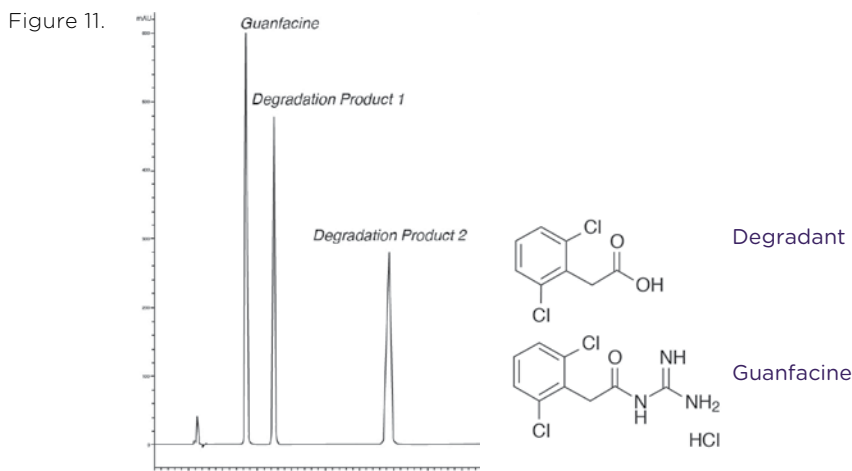


### Method Conditions

Column: Cogent Bidentate C8, 4µm, 100Å  
 Catalog No.: 40008-75P  
 Dimensions: 4.6 x 75mm  
 Mobile Phase: A: DI H<sub>2</sub>O / 0.1% formic acid (v/v)  
 B: Acetonitrile / 0.1% formic acid (v/v)  
 Gradient: 

time (min.)	%B
0	30
2	30
6	90
8	90
9	30

  
 Injection vol.: 5µL  
 Flow rate: 0.5mL/min  
 Detection: UV 275nm  
 Peaks: Bisphenol A



### Method Conditions

Column: Cogent Bidentate C8, 4µm, 100Å  
 Catalog No.: 40008-15P  
 Dimensions: 4.6 x 150mm  
 Mobile Phase: 30% Acetonitrile, 70% DI Water w/Conc Phosphoric Acid (1mL/L), 1 g/L SDS  
 Temperature: 25°C  
 Injection vol.: 20µL  
 Flow rate: 1.5mL/min  
 Detection: UV 220nm  
 Peaks: 1. Guanfacine  
 2. Primary Degradant 1  
 3. Primary Degradant 2



**NEW** Cogent Amide™  
For Sugars and Amines

Cogent Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m <sup>2</sup> /g)	Carbon Load (%)	Endcapped	Optimum pH Range	Recommended Max. Temp. (°C)	USP Code
Amide	4	100	390	2-3	No	2.5 - 7.5	80	L68

For ordering information, see page 30.

Using our proprietary bonding technology, Cogent Amide has an amide functional group bonded to the silica-hydride surface direct silicon-carbon bonds. Since the ligand is attached to the surface with direct silicon carbon bonds the bonded phase does not hydrolyze in acidic conditions. Also, it does not make a strong association with acetone.

This column is very stable and efficient and is recommended for reversed-phase or aqueous-normal-phase separations of biomolecules, including carbohydrates, peptides, polysaccharides or tryptic digests.

Figure 12.

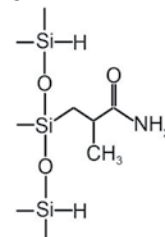
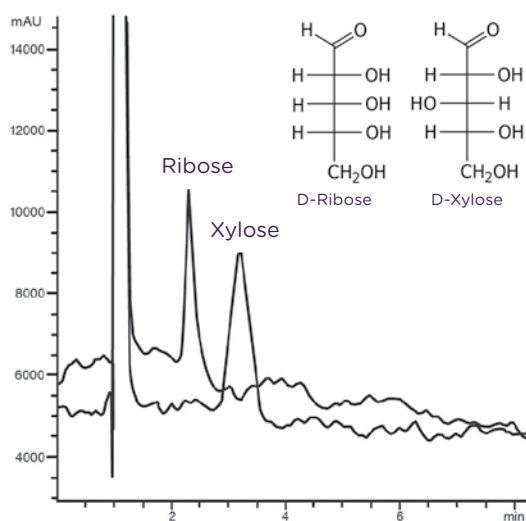


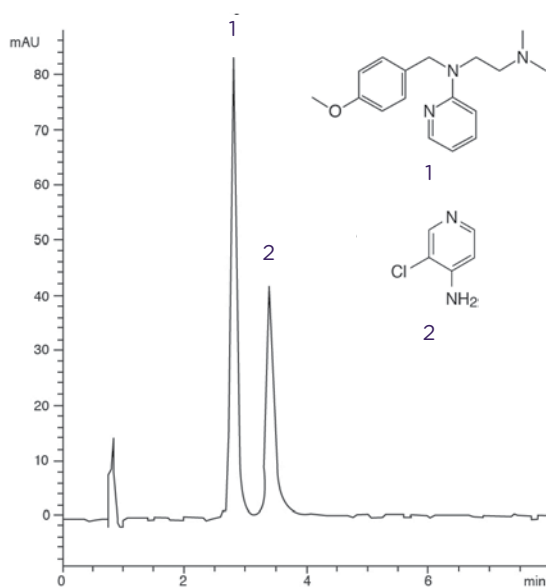
Figure 13.



**Method Conditions**

Column: Cogent Amide™, 4µm, 100Å  
 Catalog No.: 40036-05P  
 Dimensions: 4.6 x 50mm  
 Mobile Phase: 92% Acetonitrile/ 8% DI water/ 0.1% trimethylamine (TEA) (v/v)  
 Injection vol.: 2µL  
 Flow rate: 0.7mL/min  
 Detection: Refractive Index  
 Sample: 2mg/mL ribose and xylose reference standards in diluent of 50% acetonitrile/ 50% DI water/ 0.1% TEA (v/v).  
 Peaks: 1. D-Ribose  
 2. D-Xylose

Figure 14.



**Method Conditions**

Column: Cogent Amide™, 4µm, 100Å  
 Catalog No.: 40036-05P  
 Dimensions: 4.6 x 50mm  
 Mobile Phase: A: 90% DI H<sub>2</sub>O/ 10% acetonitrile/ 0.1% formic acid (v/v)  
 B: Acetonitrile/ 0.1% formic acid (v/v)  
 Gradient: 

time (min.)	%B
0	90
1	90
7	50
8	90

  
 Injection vol.: 2µL  
 Flow rate: 1.0mL/min  
 Detection: 244nm  
 Sample: 100mg/L pyrilamine and 4-amino-3-chloropyridine reference standards in diluent of 50/50 solvent A/solvent B. Peak identities confirmed with individual standards.  
 Peaks: 1. Pyrilamine  
 2. 4-Amino-3-chloropyridine

# Cogent Phenyl Hydride™

## For Aromatics

Cogent Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m <sup>2</sup> /g)	Carbon Load (%)	Endcapped	Optimum pH Range	Recommended Max. Temp. (°C)	USP Code
Phenyl Hydride	4	100	390	10-12	No	2.0 - 8.0	80	L11
Phenyl Hydride 2.6™	2.2	120	340	9-10	No	2.0 - 8.0	80	L11

For further details on 2.6 columns, please see page 25. For ordering information, see page 31.

Ordinary phenyl columns include a Si-O-Si-C4-Phenyl ligand which makes them susceptible to hydrolysis and short column lifetime.

Cogent Phenyl Hydride columns have a silica-hydride surface similar to other TYPE-C silica phases and have a 4 carbon chain with a terminal phenyl group. Attached directly to the silica-hydride surface via a single, direct silicon-carbon bond these columns offer extraordinary robustness and reliability.

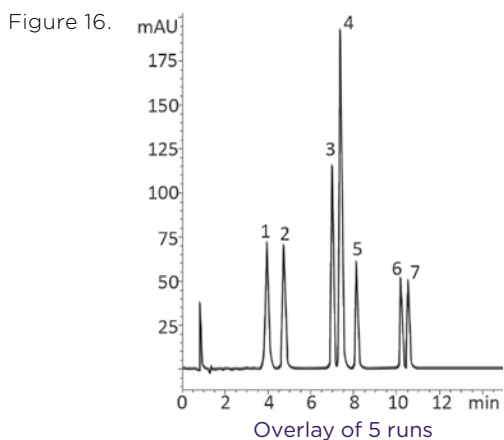
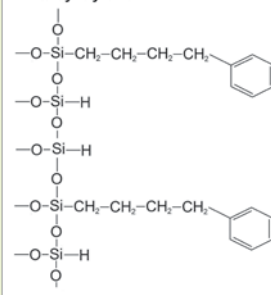
Cogent Phenyl Hydride also has the advantage of not having end-capping that could bleed into the mass spectrometer, making these columns ideal for LC-MS.

This column is a popular choice of laboratories working with small peptides, sulfonyl, azide and other aromatic compounds that are closely related. An excellent choice for analytes with rigid aromatic rings, as it offers π-π interactions as a separation mechanism.

Two applications of Cogent Phenyl Hydride in the reversed-phase mode are shown below, for cough syrup ingredients and cefprozil isomers.

Figure 15.

### Phenyl Hydride

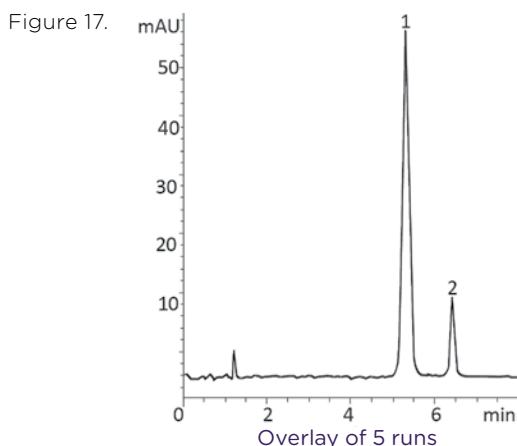


### Method Conditions

Column: Cogent Phenyl Hydride, 4µm, 1 00Å  
 Catalog No.: 69020-7.5P  
 Dimensions: 4.6 x 75mm  
 Mobile Phase: A: DI water/ 0.1% TFA (v/v)  
 B: Acetonitrile/ 0.1%TFA (v/v)

Gradient: time (min.)	%B
0	5
2	5
11	50
12	5

Post Time: 3 min  
 Injection vol.: 2µL  
 Flow rate: 1.0mL/min  
 Detection: UV 210nm (0-6 min), 230nm (6-15 min)  
 Peaks: 1. Acetaminophen  
 2. Pseudoephedrine  
 3. Guaifenesin  
 4. Benzoic Acid  
 5. Methyl Paraben  
 6. Dextromethorphan  
 7. Propyl Paraben



### Method Conditions

Column: Cogent Phenyl Hydride, 4µm, 1 00Å  
 Catalog No.: 69020-7.5P  
 Dimensions: 4.6 x 75mm  
 Mobile Phase:  
 A: DI water/ 0.1% TFA (v/v)  
 B: Acetonitrile/ 0.1%TFA (v/v)

Gradient: time (min.)	%B
0	5
6	20
7	5

Post Time: 1 min  
 Injection vol.: 10µL  
 Flow rate: 1.0mL/min  
 Detection: UV 280nm  
 Peaks: 1. Cefprozil (Z-isomer)  
 2. Cefprozil (E-isomer)

# Cogent UDC-Cholesterol™

## Liquid Crystal

Cogent Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m <sup>2</sup> /g)	Carbon Load (%)	Endcapped	Optimum pH Range	Recommended Max. Temp. (°C)	USP Code
UDC-Cholesterol	4	100	390	13-14	No	2.0 – 8.0	80	L101
UDC-Cholesterol 2.6™	2.2	120	340	13-14	No	2.0 – 8.0	80	L101

For further details on 2.6 columns, please see page 25. For ordering information, see page 32.

The Cogent UDC-Cholesterol phase is bonded directly to the TYPE-C silica hydride surface with direct silicon-carbon bonds. These Si-C bonds make these columns hydrolytically stable. They produce unique selectivity for many compounds, especially at lower temperatures and can separate some compounds based on molecular shape. For this phase mobile phase composition and temperature are powerful tools for controlling and optimizing separations in Aqueous-Normal-Phase (ANP) and Reversed-Phase (RP) modes, since the cholesterol ligand can act as a liquid crystal and can change geometry.

In buffered aqueous/methanol these columns exhibit typical RP properties, but changing to buffered aqueous/acetonitrile additionally enables ANP and ONP selectivity mechanisms.

The Cogent UDC-Cholesterol phase is particularly useful for the separation of steroid hormones. Temperature can be used to affect the retention of these steroids, which generally decreases with increasing temperature. The separation selectivity may also change.

Figure 18.

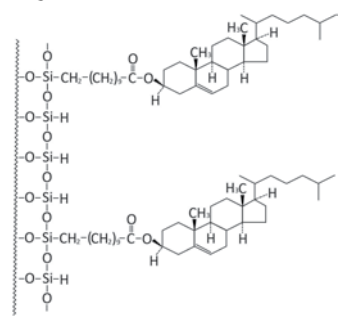


Figure 19.

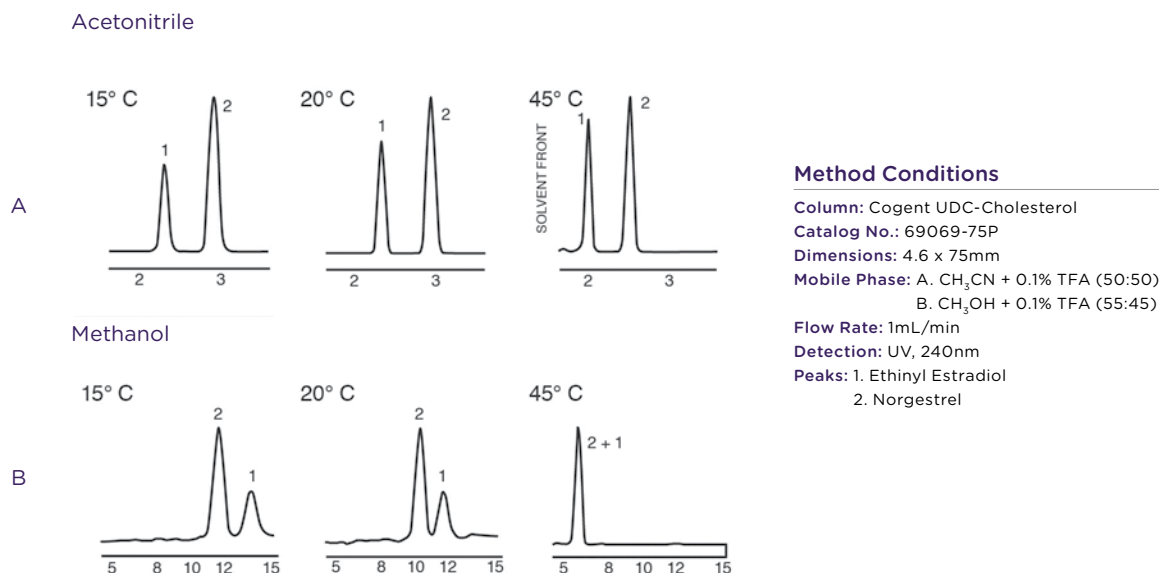


Figure 19 shows that when used with acetonitrile as organic modifier, Cogent UDC-Cholesterol produced similar results for the separation of the steroids ethinyl estradiol and norgestrel at the three different temperatures shown, with negligible effects on selectivity and retention. However, using methanol as modifier, relative retention times differ considerably at the three different temperatures and elution order is reversed. This indicates that when used with methanol, the fundamental selectivity mechanism is based on shape recognition.

# Cogent Diamond Hydride™

## For Polar & Bio-active Compounds

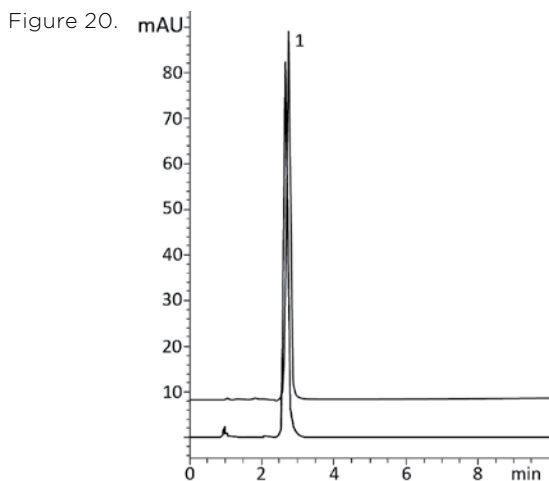
Cogent Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m <sup>2</sup> /g)	Carbon Load (%)	Endcapped	Optimum pH Range	Recommended Max. Temp. (°C)	USP Code
Diamond Hydride	4	100	390	2	No	2.5 - 7.5	80	N/A
Diamond Hydride 2.6™	2.2	120	340	2	No	2.5 - 7.5	80	N/A

For further details on 2.6 columns, please see page 25. For ordering information, see page 31.

Cogent Diamond Hydride columns have a silica-hydride surface similar to other TYPE-C silica phases, but uniquely have a very small amount of carbon (< 2%) impregnated on the surface that adjusts the surface to an important level affecting the adsorption and desorption of solvent, creating excellent peak shapes and phenomenal precision run to run for many types of compounds.

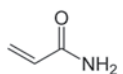
This column is a popular choice of scientists working in metabolomics and bioanalysis with LC-MS for compounds such as un-derivatized amino acids, organic acids, carbohydrates and very polar small isobaric molecules, as well as polar and non-polar peptides. Precise methods are easily developed even with complex sample matrices including biological fluids such as plasma, urine, saliva and other bio matrices. The use of very low salt required to elute compounds makes this column also a favoured choice for prep and process methods.

Cogent Diamond Hydride is designed mainly for Aqueous Normal-Phase (ANP) separations. This phase has high hydrophilic retention and shows excellent peak shape over a wide range of polar compounds that is unsurpassed by any column on the market.



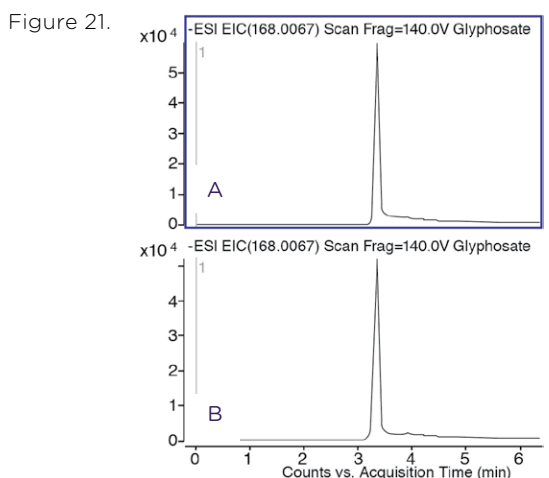
### Method Conditions

Column: Cogent Diamond Hydride, 4µm, 100Å  
 Catalog No.: 70000-7.5P  
 Dimensions: 4.6 x 75mm  
 Mobile Phase: Acetonitrile/ 0.1% formic acid  
 Injection vol.: 1µL  
 Flow rate: 1.0mL/min  
 Detection: 205nm  
 Sample.: 100mg/L acrylamide in mobile phase diluent  
 Peak: 1. Acrylamide



Acrylamide

Figure 20 shows an overlay of two different lots.

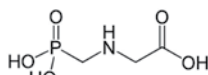


### Method Conditions

Column: Cogent Diamond Hydride,  
 4µm, 100Å  
 Catalog No.: 70000-15P-2  
 Dimensions: 2.1 x 150mm  
 Mobile Phase: A: DI water + 5mm  
 ammonium acetate  
 B: 90% acetonitrile/10%  
 DI water/10mm

Gradient:	time (min.)	%B
	0	80
	1	80
	1.1	5
	5	5
	6	80

Post Time: 5 min  
 Injection vol.: 10µL  
 Flow rate: 0.5mL/min  
 Detection: ESI - neg - Agilent 6210 MSD TOF mass spectrometer  
 Sample Prep: Glyphosate: 168.0067m/z (M - H)  
 Figure A: injection #1, RT = 3.365 min  
 Figure B: injection #5, RT = 3.366 min  
 Sample stock solution was purchased from Sigma (1000mcg/mL). Sample for analysis was made by diluting the stock 1:100 in 30:70 solution A and B.



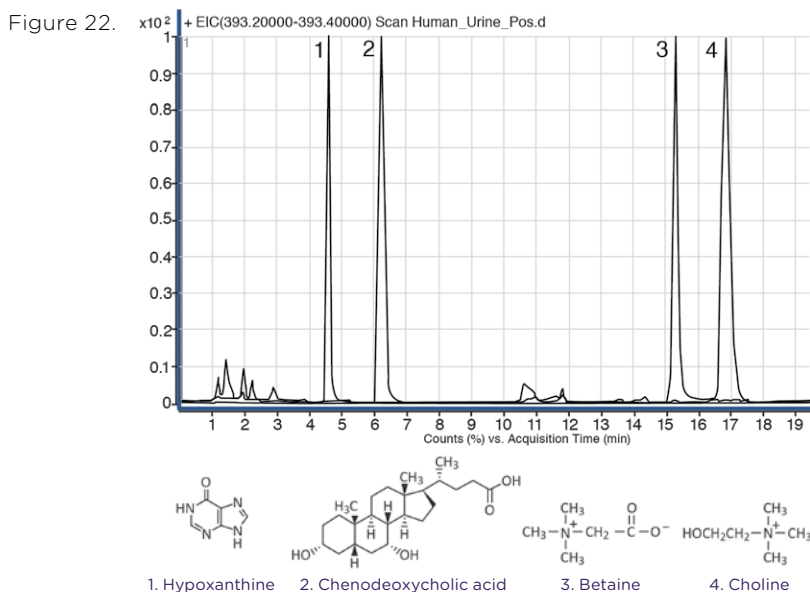
Glyphosate

# Metabolomics Kit – Diamond Hydride and Bidentate C18

## For Polar and Nonpolar Compounds

Metabolomics is a powerful tool for profiling differences in the composition of structurally diverse molecules in complex biological mixtures, including amino acids, sugars and phosphosugars, biogenic amines and lipids. LC-MS is typically used to assist identification and quantification of many of these components.

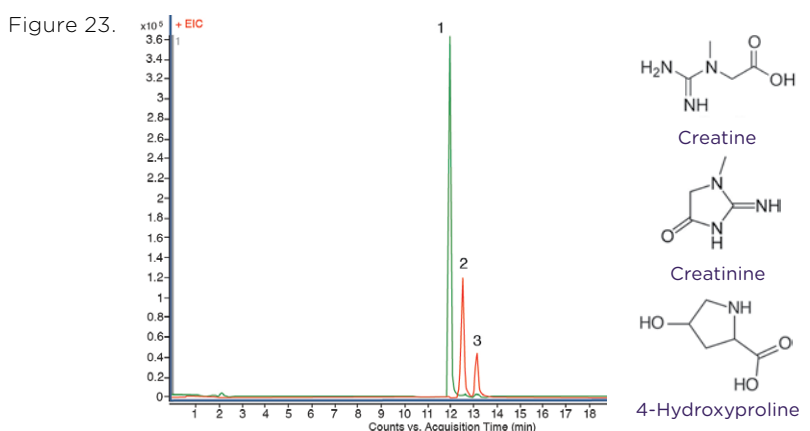
The following two examples illustrate the separation of metabolites in urine using Aqueous Normal-Phase (ANP) HPLC with MS detection.



### Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å  
 Catalog No.: 70000-15P-2  
 Dimensions: 2.1 x 150mm  
 Mobile Phase: A: DI water + 0.1% formic acid  
                   B: Acetonitrile + 0.1% formic acid  
 Flow rate: 0.4mL/min  
 Detection: ESI – pos – Agilent 6210 MSD TOF mass spectrometer  
 Sample: Human Urine – after simple extraction  
 Peaks: 1. Hypoxanthine; 137.04580m/z (M+H)<sup>+</sup>, RT = 4.98 min  
 2. Chenodeoxycholic acid; 393.29990m/z (M+H)<sup>+</sup>, RT = 6.23 min  
 3. Betaine; 118.08680m/z (M+H)<sup>+</sup>, RT = 15.27 min  
 4. Choline; 104.10754m/z (M+H)<sup>+</sup>, RT = 16.82 min

Figure 22: EIC – extracted ion chromatogram of selected compounds (1,2,3,4)



### Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å  
 Catalog No.: 70000-7.5P  
 Dimensions: 4.6mm x 75mm  
 Mobile Phase: A: DI water + 0.1% formic acid  
                   B: Acetonitrile + 0.1% formic acid  
 Flow rate: 0.4mL/min  
 Detection: ESI – pos – Agilent 6210 MSD TOF mass spectrometer  
 Sample: Synthetic urine  
 Compounds: 1. Creatinine - Cm M+H 114.0662, RT = 11.98 min  
 2. Creatine - Cr M+H 132.0768, RT = 12.52 min  
 3. 4-Hydroxyproline M+H 132.0655, RT = 13.16 min

The Cogent Metabolomics Kit offers a combination of two TYPE-C silica-hydride columns. If you are scouting for unknown compounds such as very polar and very hydrophobic compounds, these kits contain the tools you need. A Cogent Diamond Hydride™ for polar compounds and a Cogent Bidentate C18™ column for hydrophobic compounds provide the ability to identify almost any compound when used in tandem. The kits contain one each of these two columns, in the same dimensions.

### Ordering Cogent Metabolomics Kits

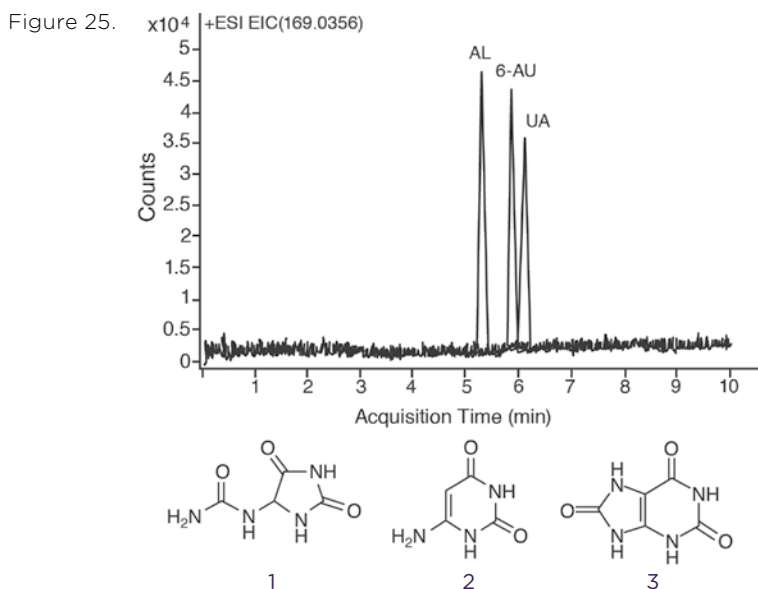
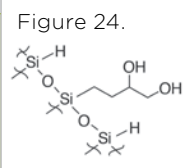
Column Dimensions	Catalog Numbers 4µm Phases	Catalog Numbers 2.2µm Phases
1.0 x 100mm	43000-10P-1	43200-10P-1
2.1 x 50mm	43000-05P-2	43200-05P-2
2.1 x 100mm	43000-10P-2	43200-10P-2
2.1 x 150mm	43000-15P-2	43200-15P-2
4.6 x 100mm	43000-10P	-
4.6 x 150mm	43000-15P	-

**NEW** Cogent Diol™  
Unique Separation & SFC

Cogent Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m <sup>2</sup> /g)	Carbon Load (%)	Endcapped	Optimum pH Range	Recommended Max. Temp. (°C)	USP Code
Diol	4	100	390	9-10	No	2.0 – 8.0	80	N/A
Diol 2.6™	2.2	120	340	9-10	No	2.0 – 8.0	80	N/A

For further details on 2.6 columns, please see page 25. For ordering information, see page 31.

The Cogent Diol HPLC stationary phase structure has a single, direct silicon-carbon point attachment to the silica-hydride surface. The phase is suitable for the reversed-phase or ANP analysis of polar compounds and is LC-MS compatible. Columns show extremely fast equilibration between gradient runs and have been successfully used in studies of pathways in human pathology and many other studies. Cogent Diol is also suitable for use in SFC.

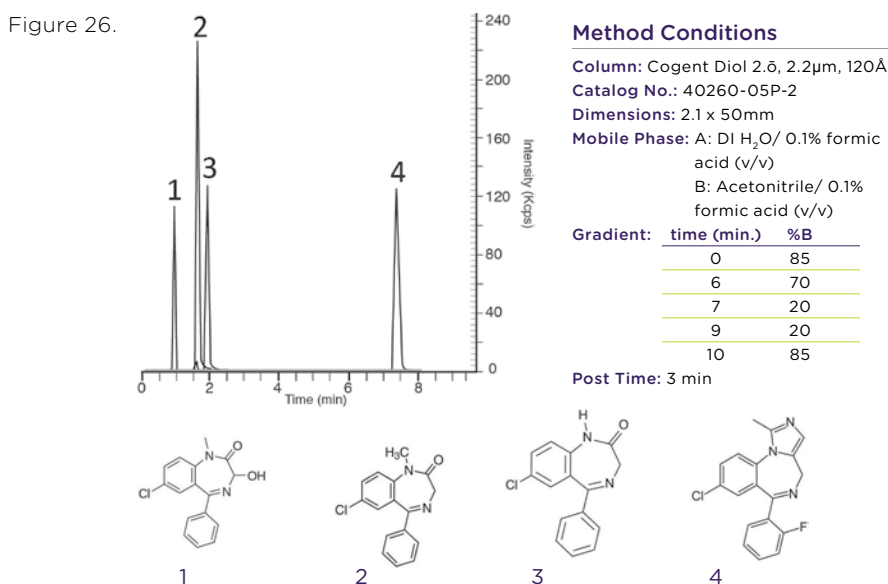


#### Method Conditions

Column: Cogent Diol, 4µm, 100Å  
 Catalog No.: 40060-15P-3  
 Dimensions: 3.0 x 150mm  
 Mobile Phase: A: DI H<sub>2</sub>O / 0.1% formic acid (v/v)  
 B: Acetonitrile / 0.1% formic acid (v/v)

Gradient: time (min.)	%B
0	95
6	30
7	30
8	95

Injection vol.: 1µL  
 Flow rate: 0.4mL/min  
 Sample: Standards of the uric acid and its main metabolites were prepared in DI water at concentrations of 20 micro g/mL each. The sample for injection (a mixture of the three compounds) was diluted by a factor of 3.  
 Peaks: 1. Allantoin 159.0513m/z [M+H]<sup>+</sup>  
 2. 6-aminouracil 128.0455m/z [M+H]<sup>+</sup>  
 3. Uric acid 169.0356m/z [M+H]<sup>+</sup>



#### Method Conditions

Column: Cogent Diol 2.6, 2.2µm, 120Å  
 Catalog No.: 40260-05P-2  
 Dimensions: 2.1 x 50mm  
 Mobile Phase: A: DI H<sub>2</sub>O / 0.1% formic acid (v/v)  
 B: Acetonitrile / 0.1% formic acid (v/v)

Gradient: time (min.)	%B
0	85
6	70
7	20
9	20
10	85

Post Time: 3 min

Injection vol.: 1µL  
 Flow rate: 0.4mL/min  
 Detection: ESI - Pos - Perkin Elmer AxION 2 TOF mass spectrometer  
 Peaks: 1. Temazepam 301.0739m/z [M+H]<sup>+</sup>  
 2. Diazepam 285.0790 [M+H]<sup>+</sup>  
 3. Nordiazepam 271.0633 [M+H]<sup>+</sup>  
 4. Midazolam 326.0855 [M+H]<sup>+</sup>

Figure 26 shows the separation of four benzodiazepines in urine by ANP.

# Cogent UDA™

## Undecanoic Acid (wcx)

Cogent Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m <sup>2</sup> /g)	Carbon Load (%)	Endcapped	Optimum pH Range	Recommended Max. Temp. (°C)	USP Code
UDA	4	100	390	14-15	No	2.0 - 8.0	80	L85
UDA 2.6™	2.2	120	340	14-15	No	2.0 - 8.0	80	L85

For further details on 2.6 columns, please see page 25. For ordering information, see page 32.

Cogent UDA is a unique selectivity phase in which the silica-hydride surface is bonded, via a double attachment, to an eleven carbon chain terminating in a carboxylic acid (undecanoic acid). This gives the phase weak cation-exchange properties in addition to aqueous normal-phase.

The terminal carboxylic acid group contributes some additional selectivity to the phase which can be useful for closely related compounds. When the mobile phase pH is greater than 5.9, the acid group becomes 90% negatively charged and at pH 6.9 it is 99% charged, and so it may act as a cation-exchanger. Below pH 4.9 the group does not have significant ion-exchange properties. Cogent UDA is a good choice for scientists working with closely related compounds and with LC-MS. Precise methods are easily developed, even with complex sample matrices, with very little desalting required.

Figure 27.

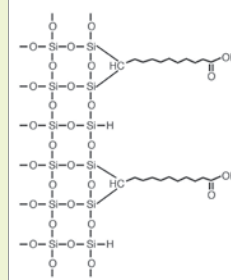
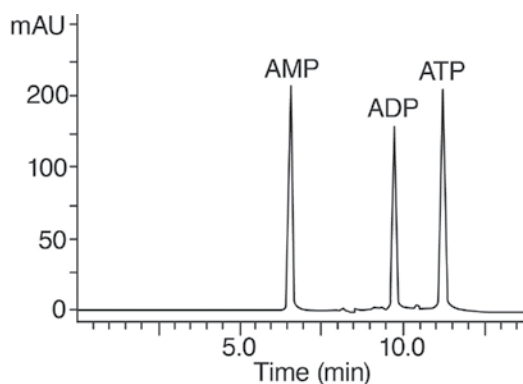


Figure 28.



### Method Conditions

Column: Cogent UDA, 4µm, 100Å  
 Catalog No.: 40031-05P-2  
 Dimensions: 2.1 x 50mm  
 Mobile Phase: A: DI H<sub>2</sub>O / 16.0mm ammonium acetate  
 B: 90% acetonitrile/10% DI H<sub>2</sub>O / 16.0mm ammonium acetate

Gradient: time (min.)	%B
0	95
0.5	95
10	70
15	30
20	30
20.1	95

Temperature: 25°C  
 Injection vol.: 1µL  
 Flow rate: 0.4mL/min  
 Detection: UV 254nm  
 Peaks:  
 1. AMP - adenosine 5'-monophosphate  
 2. ADP - adenosine 5'-diphosphate  
 3. ATP - adenosine 5'-triphosphate

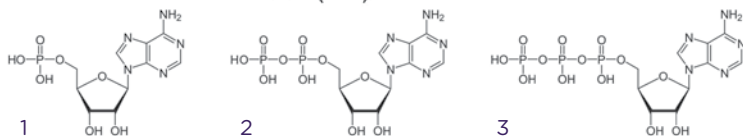
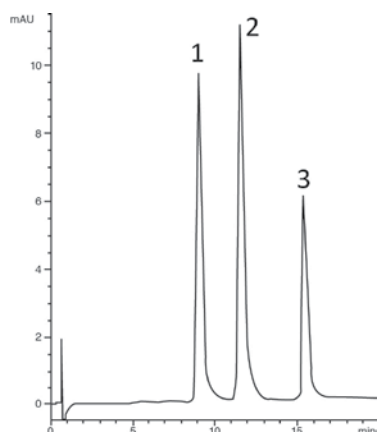


Figure 28 shows the separation of adenine nucleotides. The analytes are eluted in the order of increasing polarity.

Figure 29.



### Method Conditions

Column: Cogent UDA 2.6, 2µm, 120Å  
 Catalog No.: 40231-05P-2  
 Dimensions: 2.1 x 50mm  
 Mobile Phase: A: 0.1% formic acid in water  
 B: CH<sub>3</sub>CN - 10mm ammonium acetate (95/5)

Gradient: time (min.)	%B
0	100
1	100
17	40
19	40
20	100

Injection vol.: 0.5µL  
 Flow rate: 0.3mL/min  
 Detection: UV, 254nm  
 Peaks: 1. 4-Amino-3-chloro-pyridine  
 2. Metformin  
 3. Cetylpyridinium chloride

Figure 29 shows the analysis of three amine containing compounds using an increasing pH gradient with 0.1% formic acid. Separation occurs via a combination of ANP and weak cation-exchange mechanisms.

# Cogent Silica-C™

## Unmodified & For Normal Phase

Cogent Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m <sup>2</sup> /g)	Carbon Load (%)	Endcapped	Optimum pH Range	Recommended Max. Temp. (°C)	USP Code
Silica-C	4	100	390	0	No	2.0 - 7.0	60	L3
Silica-C 2.6™	2.2	120	340	0	No	2.0 - 7.0	60	L3

For further details on 2.6 columns, please see page 25. For ordering information, see page 32.

Cogent Silica-C is an un-bonded phase with a silica-hydride surface. It is manufactured to be completely free of carbon and will adsorb and desorb mobile phase solvents very differently from ordinary silica, resulting in significant benefits to the chromatographer.

As these columns have virtually no silanols remaining (less than 2%), they do not have a strong association with water and will not have the expected hydration shell of other silica based columns (see Figure 3 on page 7). This enables them to be used for normal-phase HPLC without having to attempt to control the moisture contents of the solvents, which is a great feature leading to much more reproducibility even when you transfer the method. In addition, Cogent Silica-C columns last longer and equilibrate much faster. Gradient analyses can be performed more easily with Cogent Silica-C columns than with ordinary silica columns.

Cogent Silica-C exhibits good retention of very polar compounds. The phase is an excellent choice for normal-phase and preparative chromatography, because it produces unique selectivity and is extremely stable. In addition to normal-phase, unbonded Cogent Silica-C columns can be used in the ANP mode for the analysis of polar compounds. These columns are therefore more versatile and more convenient than 'ordinary' silica columns.

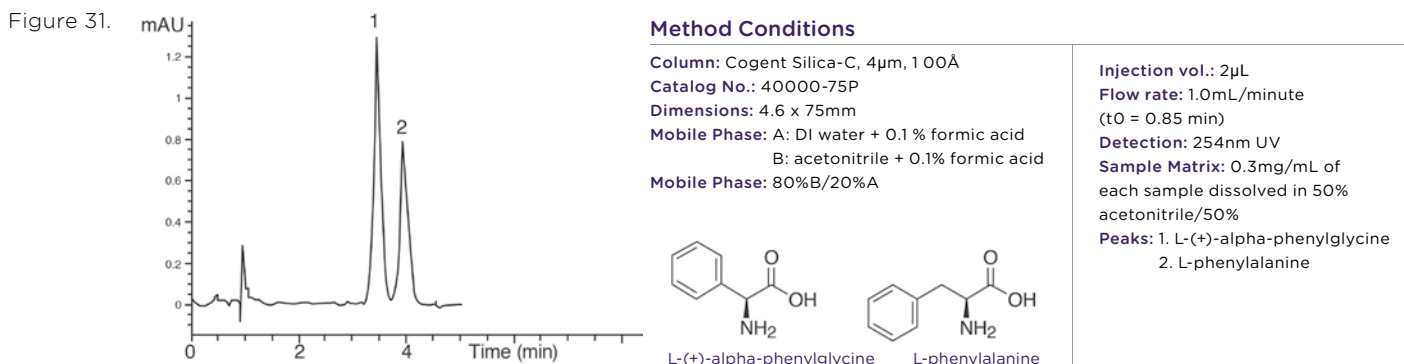
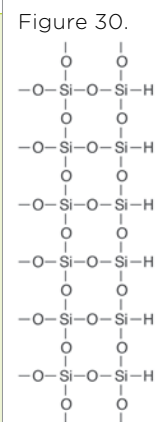
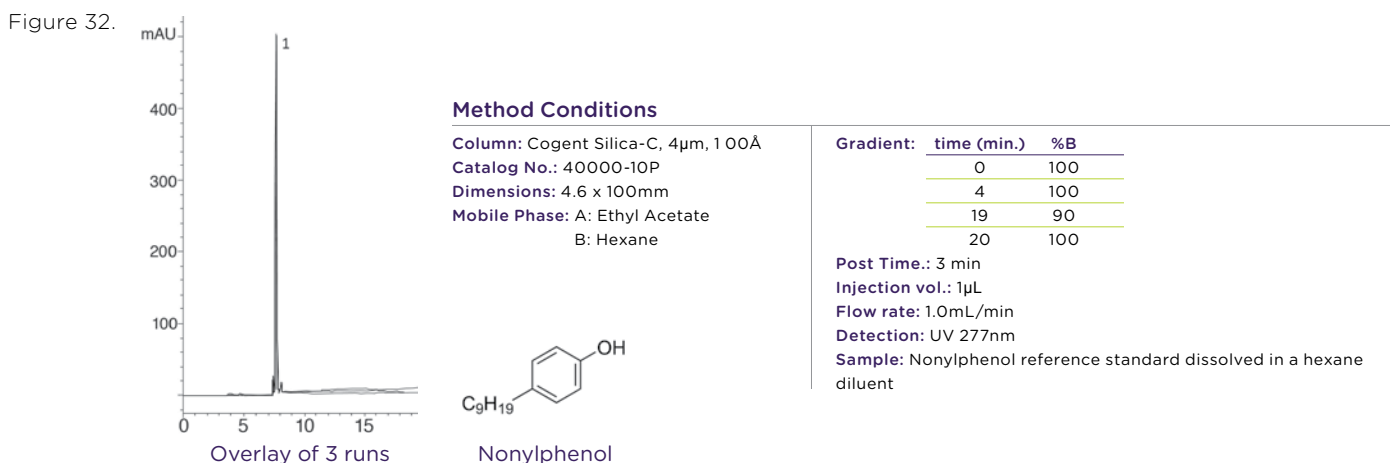


Figure 31 illustrates applicability of Cogent Silica-C in the separation of two underivatized aromatic amino acids by ANP, whereas Figure 32 shows the analysis of nonylphenol and minor isomers by normal-phase HPLC.





# Cogent 2.0™ Columns

## High Efficiency-Low Pressure Near UHPLC

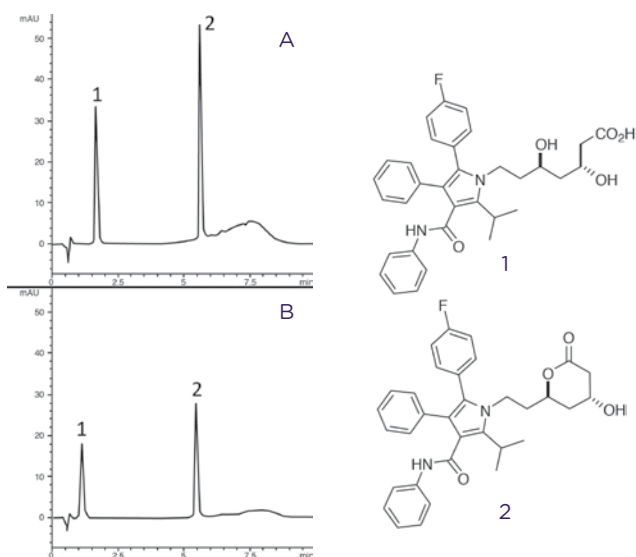
The Cogent 2.0 line of silica-hydrate columns have an average particle size of 2.2µm and show increased efficiencies compared to the 4µm phases. These columns are considered to be 'near-UHPLC' phases. Whereas the maximum recommended pressure for use with Cogent 4µm columns is 4,500psi, the maximum recommended pressure for 2.2µm columns (2.1mm and 3.0mm i.d.) is 9,000psi.

With UHPLC columns, as particle size decreases, peak efficiency increases significantly. However, the main drawback of using sub 2µm columns is the increase in pressure encountered, generally necessitating specialised equipment that can withstand the high pressure. In addition, extra-column broadening becomes more significant, requiring low i.d. tubing and minimized lengths between modules.

A near-UHPLC phase therefore represents a good value between the advantages of both smaller and larger particle size columns. TYPE-C silica 2.0 columns are fully compatible with UHPLC instrumentation.

**NOTE:** Guard columns packed with Cogent 2.0 materials are not generally provided due to pressure restrictions of the hardware used. Instead, the use of a precolumn inline filter is recommended.

Figure 33.



### Method Conditions

Column: Fig. A: Cogent Bidentate C18 2.0, 2.2µm, 120Å  
Fig. B: Cogent Bidentate C18, 4µm, 100Å

Catalog No.: Fig. A: 40218-05P-2  
Fig. B: 40018-05P-2

Dimensions: 2.1 x 50mm

Mobile Phase: A: DI H<sub>2</sub>O / 10mm ammonium acetate  
B: 90% acetonitrile / 10% DI water / 10mm ammonium acetate

Gradient:	time (min.)	%B
	0	40
	1	100
	6	100
	7	40

Flow rate: 0.3mL/min

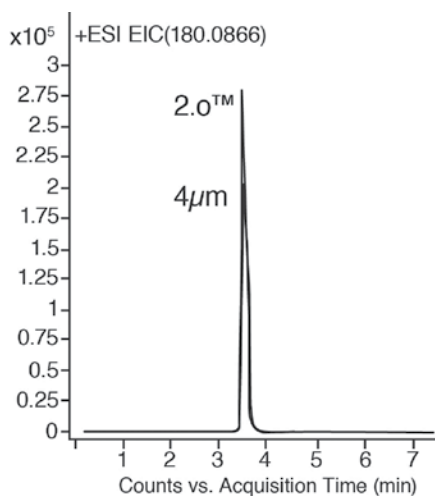
Detection: UV 248nm

Peaks: 1. Atorvastatin  
2. Atorvastatin lactone

Peak:	Efficiency (N/m)	
	4µm	2.2µm
1	88420	143780
2	206920	481460

Figure 33 illustrates the increased efficiency and sensitivity obtained by converting a reversed-phase method for atorvastatin from a 4µm Cogent Bidentate C18 column to a 2.2µm phase. The retention times of both analytes are very comparable.

Figure 34.



### Method Conditions

Columns: Cogent Diamond Hydride, 4µm, 100Å  
Cogent Diamond Hydride 2.0, 2.2µm, 120Å

Catalog No: 70000-05P-2  
70200-05P-2

Dimensions: 2.1 x 50mm (both)

Mobile Phase: A: DI H<sub>2</sub>O + 0.1% formic acid  
B: Acetonitrile + 0.1% formic acid

Gradient:	time (min.)	%B
	0	80
	5	30
	7	30
	8	80

Post Time.: 4 min

Flow rate: 0.3mL/min

Figure 34 shows a comparison of the efficiency and sensitivity of an ANP gradient method for glucosamine obtained with 4µm and 2.2µm Cogent Diamond Hydride columns.

For part number details of Cogent 2.0 columns, please see pages 30-32

# Cogent TYPE-C™ Guard Columns

## Column Protection

Guard columns/cartridges can be important for extending the lifetime of your analytical column. Without using a guard cartridge, particulates may collect in an HPLC column's inlet frit or in the first millimeter of the packed bed. This will result in degradation of the column's performance, manifesting itself as peak tailing, split peaks and/or increased back pressure. Instrument downtime may occur while the costly column is replaced or restored using a suitable clean-up flushing process. In addition, some compounds may irreversibly adsorb to the stationary phase, resulting in permanent damage to the column and causing shifting retention times and loss of peak resolution.

The use of a guard cartridge placed between the column and the injector valve enables damaging contaminants to be trapped on the disposable cartridge, thereby prolonging the lifetime of the analytical column. Ideally the guard cartridge should be packed with the same stationary phase material as the analytical column to be protected.

Figure 35. The 1.0mm i.d. and 2.0mm i.d. guard cartridges are for use with 1.0mm and 2.1mm i.d. analytical columns respectively, whereas the 4.0mm i.d. guard cartridges are for protection of 3.0mm and 4.6mm i.d. analytical columns. The hand-tightened holder allows for easy installation.



## Code Key for Ordering Guard Columns

The following guard cartridge products are available for 4µm Cogent columns. To obtain the correct description and catalog number for your required Cogent product, replace 'phase type' with the required phase description (e.g. Diamond Hydride) and "XXXXX" with the following codes:

- Amide: **40036**
- Bidentate C8: **40008**
- Bidentate C18: **40018**
- Diamond Hydride: **70000**
- Diol: **40060**
- Phenyl Hydride: **69020**
- Silica-C: **40000**
- UDA: **40031**
- UDC-Cholesterol: **69069**

Catalog Numbers	MicroSolv Guard Product Description
XXXXX-HG1	Cogent Replacement Guard Columns Kit, 'phase type' 4µm. Includes 5 each Hichrom 2.0mm x 10mm Guard Columns in individual cases.
XXXXX-HG2	Cogent Replacement Guard Columns Kit, 'phase type' 4µm. Includes 5 each Hichrom 4.0mm x 10mm Guard Columns in individual cases.
XXXXX-HG3	Cogent Guard Column Kit, 'phase type' 4µm. Includes one Universal Holder and 5 each Hichrom 4.0mm x 10mm Guard Columns.
XXXXX-HG4	Cogent Guard Column Kit, 'phase type' 4µm. Includes one Universal Holder and 5 each Hichrom 2.0mm x 10mm Guard Columns.
XXXXX-HG5	Cogent Guard Column, 'phase type' 4µm. Holder required. 2.0mm x 10mm. 1 each
XXXXX-HG6	Cogent Guard Column, 'phase type' 4µm. Holder required. 4.0mm x 10mm. 1 each
XXXXX-HG7	Cogent Replacement Guard Columns Kit, 'phase type' 4µm. Includes 5 each Hichrom 1.0mm x 10mm Guard Columns in individual cases.
XXXXX-HG8	Cogent Guard Column Kit, 'phase type' 4µm. Includes one Universal Holder and 5 each Hichrom 1.0mm x 10mm Guard Columns.
XXXXX-HG9	Cogent Guard Column, 'phase type' 4µm. Holder required. 1.0mm x 10mm. 1 each
82000-GH	Guard cartridge holder 1 each for 10mm length, 1.0, 2.0 or 4.0mm i.d. cartridges
47450-06	Column coupler, for use with 1.0mm to 21.2mm i.d. Hichrom hardware columns (pictured on page 36)

## Cogent TYPE-C™ Columns used in LC-MS

LC-MS is frequently the method of choice for the analysis of both small molecules and larger biomolecules due to the sensitivity, selectivity and robustness of the technique. Increasingly lower concentrations of analytes are required to be measured from complex sample mixtures or matrices. LC-MS shows improved detection limits and may require reduced sample preparation compared to UV or other HPLC detection systems. In many cases the use of LC-MS eliminates or reduces the need for time consuming derivatization of compounds lacking UV chromophores. LC-MS is widely used in pharmaceutical, clinical, forensic, environmental, food & beverage and many other application areas.

Any build-up of matrix (e.g. plasma) on a column is less of a concern with Cogent columns due to the low level of surface silanols and the lack of water shell. Short washing protocols can be incorporated into a method to remove contaminants and keep the column clean and in service.

Although acetone is not useful for LC-UV due to its high UV cut-off, it may be used instead of acetonitrile in LC-MS analyses with TYPE-C silica-hydride HPLC columns. It is more environmentally friendly and easier to recover and reuse. Acetone and acetonitrile can be used interchangeably for the LC-MS analysis of amino acids, although similar results are not obtained for all compound types.

Cogent TYPE-C silica phases are particularly useful for the LC-MS analysis of polar and non-polar compounds. They have several features making them ideal for LC-MS analyses.

- **Higher percentage composition of organic solvent (e.g. acetonitrile).** The high percentage of organic solvent makes the mobile phase more volatile and generally increases ionization sensitivity.
- **Use LC-MS friendly buffers.** The aqueous component of the mobile phase used with TYPE-C silica-hydride phases usually contains from 0.1 to 1.0% formic and or 1.0 to 2.0% acetic acid, which is compatible with LC-MS. For negative ionization mode it is recommended to use 10 to 16mM ammonium formate or ammonium acetate. Higher concentration of this buffer will result in contamination of the source. When these buffers are used as an additive in solvent B (organic solvent), add 1 to 3% of DI water to assure miscibility.
- **Fast equilibration.** Due to no hydration shell. This leads to faster runs and less solvent consumed, resulting in savings in cost and time. For gradient runs, the cycle time is reduced.
- **Low column bleed.** The strong Silicon-Carbon bonds and Si-H surface result in very low levels of bleed due to ligand cleavage. Additionally, the lack of end-capping means that there is no bleed from any end-capping agent.
- **Increased column lifetime.** The stable robust Silicon-Carbon bonds of the TYPE-C silica-hydride bonded phases enable columns to have much longer lifetimes.

Figure 36.



## Cogent™ Mini Columns – Fast LC and LC-MS

Cogent TYPE-C™ Mini Columns (20, 30 and 50mm length) for fast LC and LC-MS are designed to analyze large numbers of samples in as short a time as possible, without major sacrifice to column resolution. Quality Control environments and high throughput labs, in particular, benefit from the use of such columns. These columns are packed to analytical specifications and individually tested for packing dynamics and reproducibility.

The use of shorter columns enables the system to be run at high flow rates for fast LC or used for ‘ballistic type’ gradients for LC-MS. The columns equilibrate extremely fast compared to standard columns and exhibit very long lifetimes.

By reducing the column length from 250mm to 50mm, a saving of 80% in analysis time may typically be observed. Simultaneously, a beneficial reduction in solvent usage is achieved.

Figure 37.



Contact us for further advice and technical support on how to convert methods between different column lengths, i.d.s and particle sizes by emailing [technical@mtc-usa.com](mailto:technical@mtc-usa.com) or visit our online Chrom User Community from our website.

Phase	4.6 x 20mm	4.6 x 30mm	4.6 x 50mm	2.1 x 20mm	2.1 x 30mm	2.1 x 50mm
Cogent UDC-Cholesterol™	69069-02P	69069-03P	69069-05P	69069-02P-2	69069-03P-2	69069-05P-2
Cogent UDC-Cholesterol 2.0™	-	-	-	69269-02P-2	69269-03P-2	69269-05P-2
Cogent UDA™	40031-02P	40031-03P	40031-05P	40031-02P-2	40031-03P-2	40031-05P-2
Cogent UDA 2.0™	-	-	-	40231-02P-2	40231-03P-2	40231-05P-2
Cogent TYPE-C Silica™	40000-02P	40000-03P	40000-05P	40000-02P-2	40000-03P-2	40000-05P-2
Cogent TYPE-C Silica 2.0™	-	-	-	40200-02P-2	40200-03P-2	40200-05P-2
Cogent Phenyl Hydride™	69020-02P	69020-03P	69020-05P	69020-02P-2	69020-03P-2	69020-05P-2
Cogent Phenyl Hydride 2.0™	-	-	-	69220-02P-2	69220-03P-2	69220-05P-2
Cogent Diol™	40060-02P	40060-03P	40060-05P	40060-02P-2	40060-03P-2	40060-05P-2
Cogent Diol 2.0™	-	-	-	40260-02P-2	40260-03P-2	40260-05P-2
Cogent Diamond Hydride™	70000-02P	70000-03P	70000-05P	70000-02P-2	70000-03P-2	70000-05P-2
Cogent Diamond Hydride 2.0™	-	-	-	70200-02P-2	70200-03P-2	70200-05P-2
Cogent Bidentate C8™	40008-02P	40008-03P	40008-05P	40008-02P-2	40008-03P-2	40008-05P-2
Cogent Bidentate C8 2.0™	-	-	-	40208-02P-2	40208-03P-2	40208-05P-2
Cogent Bidentate C18™	40018-02P	40018-03P	40018-05P	40018-02P-2	40018-03P-2	40018-05P-2
Cogent Bidentate C18 2.0™	-	-	-	40218-02P-2	40218-03P-2	40218-05P-2
Cogent Amide™	40036-02P	40036-03P	40036-05P	40036-02P-2	40036-03P-2	40036-05P-2

See pages 30-32 for analytical and preparative columns.

## **NEW** Cogent TYPE-C™ Columns Preparative and Process Chromatography

At the semi-preparative or preparative scale, many laboratories use larger particles (e.g. 10–20µm). In contrast, Cogent columns can use the same 4µm spherical particles as in the corresponding analytical columns. Using this 4µm material, columns up to 30mm i.d. can be packed.

A higher silica surface area generally enables greater sample loadability. However, at the semi-preparative/preparative level, analyte solubility may become the limiting factor on loading capacity. This can be more of a significance in Reversed-Phase (RP) compared to Aqueous-Normal Phase (ANP) or Organic Normal-Phase (ONP).

Since peaks tend to broaden significantly at high loading, the maximum loading capacity will be lower for closely eluting peaks. If there is good selectivity between the target compounds and others in the mixture, it is possible to greatly overload the column without fear of contamination and hence prepare much more material per run. Cogent TYPE-C silica columns provide unique selectivity and solvent compatibility options for many separations, which can offer benefits in this area.

In a study of a complex natural product sample, the same on-column loading was achieved using a Cogent 4µm, 21.2 x 250mm column as with a Type B silica 30 x 250mm column with 15µm particles. Flow rates less than 10mL/min were used with the Cogent column, meaning that the method could be used on a standard analytical HPLC system.

There are several key advantages and overall cost savings to using TYPE-C silica-hydrate columns for preparative work.

- **No need to purchase a high cost preparative LC system.** Simply use your analytical HPLC system at high flow rates with a 4µm, 21.2 x 250mm Cogent TYPE-C silica column.
- **Use less solvent in LC method.** The higher the column i.d. the more volume per minute needed to achieve the same linear flow. This is significant when comparing a 21.2mm i.d. Cogent column with a 30mm i.d. column with 15µm particles.
- **HIGHER YIELD, Less solvent evaporation time.** Cogent columns can be used in the ANP mode, which uses a high percentage of organic solvent and a low percentage of water. This leads to faster solvent evaporation from collected fractions. In contrast RP methods using a high water content mobile phase have slower solvent evaporation rates.
- **HIGHER YIELD, Less salt to elute.** Cogent columns used in ANP have produced as much as a 10 fold increase in yield due to low salt concentrations needed to elute compounds thus reducing “dry down” attempts to collect salt free compounds.
- **Can use columns in 3 modes.** The capability of being used in RP, ONP and ANP increases the chances of success in preparative chromatography, by increasing the dynamic range of solubility of analytes without hysteresis or damage to columns.

Preparative columns packed with 10µm–20µm phases are available on request.

Figure 38.



Please see page 30–32 for ordering details.

# Cogent HPLC Column Catalog Numbers - Ordering information



## Cogent Amide™

Technical information see page 17

4µm Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	40036-02P-1	40036-03P-1	40036-05P-1	40036-75P-1	40036-10P-1	40036-15P-1	40036-25P-1
2.1	40036-02P-2	40036-03P-2	40036-05P-2	40036-75P-2	40036-10P-2	40036-15P-2	40036-25P-2
3.0	40036-02P-3	40036-03P-3	40036-05P-3	40036-75P-3	40036-10P-3	40036-15P-3	40036-25P-3
4.6	40036-02P	40036-03P	40036-05P	40036-75P	40036-10P	40036-15P	40036-25P
7.75	-	-	40036-MP50	40036-MP75	40036-MP100	40036-MP150	40036-MP250
10.0	-	-	40036-SP50	40036-SP75	40036-SP100	40036-SP150	40036-SP250
21.2	-	-	40036-P21-50	40036-P21-75	40036-P21-100	40036-P21-150	40036-P21-250
30.0	-	-	40036-P30-50	40036-P30-75	40036-P30-100	40036-P30-150	40036-P30-250

<sup>1</sup> For guard cartridges, please see page 26. For PEEK Columns, please see page 33.

## Cogent Bidentate C8™

Technical information see page 16

4µm Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	40008-02P-1	40008-03P-1	40008-05P-1	40008-75P-1	40008-10P-1	40008-15P-1	40008-25P-1
2.1	40008-02P-2	40008-03P-2	40008-05P-2	40008-75P-2	40008-10P-2	40008-15P-2	40008-25P-2
3.0	40008-02P-3	40008-03P-3	40008-05P-3	40008-75P-3	40008-10P-3	40008-15P-3	40008-25P-3
4.6	40008-02P	40008-03P	40008-05P	40008-75P	40008-10P	40008-15P	40008-25P
7.75	-	-	40008-MP50	40008-MP75	40008-MP100	40008-MP150	40008-MP250
10.0	-	-	40008-SP50	40008-SP75	40008-SP100	40008-SP150	40008-SP250
21.2	-	-	40008-P21-50	40008-P21-75	40008-P21-100	40008-P21-150	40008-P21-250
30.0	-	-	40008-P30-50	40008-P30-75	40008-P30-100	40008-P30-150	40008-P30-250

<sup>1</sup> For guard cartridges, please see page 26. For PEEK Columns, please see page 33.

## 2.0™ Phase<sup>1</sup>

4µm Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	40208-02P-1	40208-03P-1	40208-05P-1	40208-75P-1	40208-10P-1	40208-15P-1	-
2.1	40208-02P-2	40208-03P-2	40208-05P-2	40208-75P-2	40208-10P-2	40208-15P-2	-
3.0	40208-02P-3	40208-03P-3	40208-05P-3	40208-75P-3	40208-10P-3	40208-15P-3	-

## Cogent Bidentate C18™

Technical information see page 15

4µm Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	40018-02P-1	40018-03P-1	40018-05P-1	40018-75P-1	40018-10P-1	40018-15P-1	40018-25P-1
2.1	40018-02P-2	40018-03P-2	40018-05P-2	40018-75P-2	40018-10P-2	40018-15P-2	40018-25P-2
3.0	40018-02P-3	40018-03P-3	40018-05P-3	40018-75P-3	40018-10P-3	40018-15P-3	40018-25P-3
4.6	40018-02P	40018-03P	40018-05P	40018-75P	40018-10P	40018-15P	40018-25P
7.75	-	-	40018-MP50	40018-MP75	40018-MP100	40018-MP150	40018-MP250
10.0	-	-	40018-SP50	40018-SP75	40018-SP100	40018-SP150	40018-SP250
21.2	-	-	40018-P21-50	40018-P21-75	40018-P21-100	40018-P21-150	40018-P21-250
30.0	-	-	40018-P30-50	40018-P30-75	40018-P30-100	40018-P30-150	40018-P30-250

<sup>1</sup> For guard cartridges, please see page 26. For PEEK Columns, please see page 33.

## 2.0™ Phase<sup>1</sup>

4µm Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	40218-02P-1	40218-03P-1	40218-05P-1	40218-75P-1	40218-10P-1	40218-15P-1	-
2.1	40218-02P-2	40218-03P-2	40218-05P-2	40218-75P-2	40218-10P-2	40218-15P-2	-
3.0	40218-02P-3	40218-03P-3	40218-05P-3	40218-75P-3	40218-10P-3	40218-15P-3	-

## Cogent Diamond Hydride™

Technical information see page 20

4µm Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	70000-02P-1	70000-03P-1	70000-05P-1	70000-75P-1	70000-10P-1	70000-15P-1	70000-25P-1
2.1	70000-02P-2	70000-03P-2	70000-05P-2	70000-75P-2	70000-10P-2	70000-15P-2	70000-25P-2
3.0	70000-02P-3	70000-03P-3	70000-05P-3	70000-75P-3	70000-10P-3	70000-15P-3	70000-25P-3
4.6	70000-02P	70000-03P	70000-05P	70000-75P	70000-10P	70000-15P	70000-25P
7.75	-	-	70000-MP50	70000-MP75	70000-MP100	70000-MP150	70000-MP250
10.0	-	-	70000-SP50	70000-SP75	70000-SP100	70000-SP150	70000-SP250
21.2	-	-	70000-P21-50	70000-P21-75	70000-P21-100	70000-P21-150	70000-P21-250
30.0	-	-	70000-P30-50	70000-P30-75	70000-P30-100	70000-P30-150	70000-P30-250

<sup>1</sup> For guard cartridges, please see page 26. For PEEK Columns, please see page 33.

2.0™ Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	70200-02P-1	70200-03P-1	70200-05P-1	70200-75P-1	70200-10P-1	70200-15P-1	-
2.1	70200-02P-2	70200-03P-2	70200-05P-2	70200-75P-2	70200-10P-2	70200-15P-2	-
3.0	70200-02P-3	70200-03P-3	70200-05P-3	70200-75P-3	70200-10P-3	70200-15P-3	-

**NEW**

## Cogent Diol™

Technical information see page 22

4µm Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	40060-02P-1	40060-03P-1	40060-05P-1	40060-75P-1	40060-10P-1	40060-15P-1	40060-25P-1
2.1	40060-02P-2	40060-03P-2	40060-05P-2	40060-75P-2	40060-10P-2	40060-15P-2	40060-25P-2
3.0	40060-02P-3	40060-03P-3	40060-05P-3	40060-75P-3	40060-10P-3	40060-15P-3	40060-25P-3
4.6	40060-02P	40060-03P	40060-05P	40060-75P	40060-10P	40060-15P	40060-25P
7.75	-	-	40060-MP50	40060-MP75	40060-MP100	40060-MP150	40060-MP250
10.0	-	-	40060-SP50	40060-SP75	40060-SP100	40060-SP150	40060-SP250
21.2	-	-	40060-P21-50	40060-P21-75	40060-P21-100	40060-P21-150	40060-P21-250
30.0	-	-	40060-P30-50	40060-P30-75	40060-P30-100	40060-P30-150	40060-P30-250

<sup>1</sup> For guard cartridges, please see page 26. For PEEK Columns, please see page 33.

2.0™ Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	40260-02P-1	40260-03P-1	40260-05P-1	40260-75P-1	40260-10P-1	40260-15P-1	-
2.1	40260-02P-2	40260-03P-2	40260-05P-2	40260-75P-2	40260-10P-2	40260-15P-2	-
3.0	40260-02P-3	40260-03P-3	40260-05P-3	40260-75P-3	40260-10P-3	40260-15P-3	-

## Cogent Phenyl Hydride™

Technical information see page 18

4µm Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	69020-02P-1	69020-03P-1	69020-05P-1	69020-75P-1	69020-10P-1	69020-15P-1	69020-25P-1
2.1	69020-02P-2	69020-03P-2	69020-05P-2	69020-75P-2	69020-10P-2	69020-15P-2	69020-25P-2
3.0	69020-02P-3	69020-03P-3	69020-05P-3	69020-75P-3	69020-10P-3	69020-15P-3	69020-25P-3
4.6	69020-02P	69020-03P	69020-05P	69020-75P	69020-10P	69020-15P	69020-25P
7.75	-	-	69020-MP50	69020-MP75	69020-MP100	69020-MP150	69020-MP250
10.0	-	-	69020-SP50	69020-SP75	69020-SP100	69020-SP150	69020-SP250
21.2	-	-	69020-P21-50	69020-P21-75	69020-P21-100	69020-P21-150	69020-P21-250
30.0	-	-	69020-P30-50	69020-P30-75	69020-P30-100	69020-P30-150	69020-P30-250

<sup>1</sup> For guard cartridges, please see page 26. For PEEK Columns, please see page 33.

2.0™ Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	69220-02P-1	69220-03P-1	69220-05P-1	69220-75P-1	69220-10P-1	69220-15P-1	-
2.1	69220-02P-2	69220-03P-2	69220-05P-2	69220-75P-2	69220-10P-2	69220-15P-2	-
3.0	69220-02P-3	69220-03P-3	69220-05P-3	69220-75P-3	69220-10P-3	69220-15P-3	-

## Cogent Silica-C™

Technical information see page 24

4µm Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	40000-02P-1	40000-03P-1	40000-05P-1	40000-75P-1	40000-10P-1	40000-15P-1	40000-25P-1
2.1	40000-02P-2	40000-03P-2	40000-05P-2	40000-75P-2	40000-10P-2	40000-15P-2	40000-25P-2
3.0	40000-02P-3	40000-03P-3	40000-05P-3	40000-75P-3	40000-10P-3	40000-15P-3	40000-25P-3
4.6	40000-02P	40000-03P	40000-05P	40000-7.5P	40000-10P	40000-15P	40000-25P
7.75	-	-	40000-MP50	40000-MP75	40000-MP100	40000-MP150	40000-MP250
10.0	-	-	40000-SP50	40000-SP75	40000-SP100	40000-SP150	40000-SP250
21.2	-	-	40000-P21-50	40000-P21-75	40000-P21-100	40000-P21-150	40000-P21-250
30.0	-	-	40000-P30-50	40000-P30-75	40000-P30-100	40000-P30-150	40000-P30-250

<sup>1</sup> For guard cartridges, please see page 26. For PEEK Columns, please see page 33.

2.0™ Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	40200-02P-1	40200-03P-1	40200-05P-1	40200-75P-1	40200-10P-1	40200-15P-1	-
2.1	40200-02P-2	40200-03P-2	40200-05P-2	40200-75P-2	40200-10P-2	40200-15P-2	-
3.0	40200-02P-3	40200-03P-3	40200-05P-3	40200-75P-3	40200-10P-3	40200-15P-3	-

## Cogent UDA™ (wcx)

Technical information see page 23

4µm Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	40031-02P-1	40031-03P-1	40031-05P-1	40031-75P-1	40031-10P-1	40031-15P-1	40031-25P-1
2.1	40031-02P-2	40031-03P-2	40031-05P-2	40031-75P-2	40031-10P-2	40031-15P-2	40031-25P-2
3.0	40031-02P-3	40031-03P-3	40031-05P-3	40031-75P-3	40031-10P-3	40031-15P-3	40031-25P-3
4.6	40031-02P	40031-03P	40031-05P	40031-7.5P	40031-10P	40031-15P	40031-25P
7.75	-	-	40031-MP50	40031-MP75	40031-MP100	40031-MP150	40031-MP250
10.0	-	-	40031-SP50	40031-SP75	40031-SP100	40031-SP150	40031-SP250
21.2	-	-	40031-P21-50	40031-P21-75	40031-P21-100	40031-P21-150	40031-P21-250
30.0	-	-	40031-P30-50	40031-P30-75	40031-P30-100	40031-P30-150	40031-P30-250

<sup>1</sup> For guard cartridges, please see page 26. For PEEK Columns, please see page 33.

2.0™ Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	40231-02P-1	40231-03P-1	40231-05P-1	40231-75P-1	40231-10P-1	40231-15P-1	-
2.1	40231-02P-2	40231-03P-2	40231-05P-2	40231-75P-2	40231-10P-2	40231-15P-2	-
3.0	40231-02P-3	40231-03P-3	40231-05P-3	40231-75P-3	40231-10P-3	40231-15P-3	-

## Cogent UDC-Cholesterol™

Technical information see page 19

4µm Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	69069-02P-1	69069-03P-1	69069-05P-1	69069-75P-1	69069-10P-1	69069-15P-1	69069-25P-1
2.1	69069-02P-2	69069-03P-2	69069-05P-2	69069-7.5P-2	69069-10P-2	69069-15P-2	69069-25P-2
3.0	69069-02P-3	69069-03P-3	69069-05P-3	69069-75P-3	69069-10P-3	69069-15P-3	69069-25P-3
4.6	69069-02P	69069-03P	69069-05P	69069-7.5P	69069-10P	69069-15P	69069-25P
7.75	-	-	69069-MP50	69069-MP75	69069-MP100	69069-MP150	69069-MP250
10.0	-	-	69069-SP50	69069-SP75	69069-SP100	69069-SP150	69069-SP250
21.2	-	-	69069-P21-50	69069-P21-75	69069-P21-100	69069-P21-150	69069-P21-250
30.0	-	-	69069-P30-50	69069-P30-75	69069-P30-100	69069-P30-150	69069-P30-250

<sup>1</sup> For guard cartridges, please see page 26. For PEEK Columns, please see page 33.

2.0™ Phase <sup>1</sup>	Length (mm)						
i.d. (mm)	20	30	50	75	100	150	250
1.0	69269-02P-1	69269-03P-1	69269-05P-1	69269-75P-1	69269-10P-1	-	-
2.1	69269-02P-2	69269-03P-2	69269-05P-2	69269-75P-2	69269-10P-2	69269-15P-2	-
3.0	69269-02P-3	69269-03P-3	69269-05P-3	69269-75P-3	69269-10P-3	69269-15P-3	-

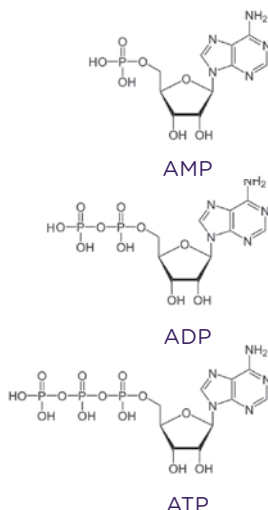
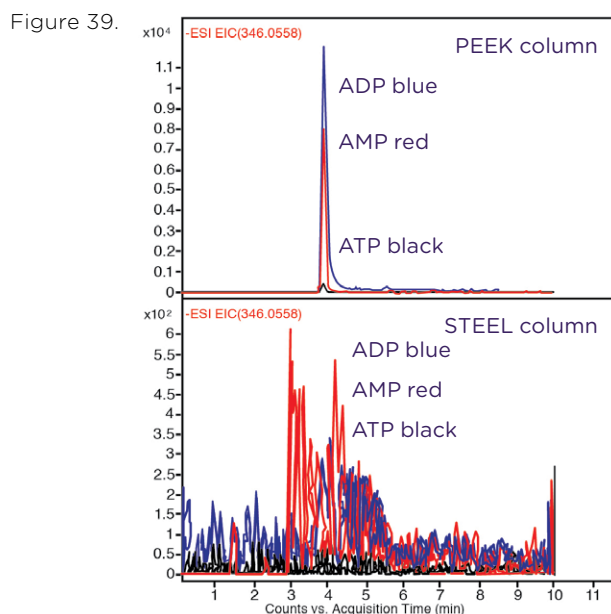


# NEW Cogent™ PEEK Columns

## Metal Free – No Stainless Steel

Cogent™ PEEK HPLC columns are ideal for analyzing compounds that may react with or be degraded by stainless steel. They are more inert to many biological compounds as well as compounds containing chelating groups or those that are ionized in solution. For these compounds the peak shapes may be sharper and the peak area more reproducible from run to run, resulting in improved quantitation.

These PEEK columns also have inert PEEK frits, which minimize adsorption and degradation for susceptible compounds. Column efficiencies and back pressures are similar for the stainless steel and PEEK columns. Maximum pressure rating is 4,500psi.



### Method Conditions

**Column:** Cogent Diamond Hydride™, 4µm, 100Å, PEEK  
**Catalog No.:** 70000-10K-2

**Dimensions.:** 2.1 x 100mm

**Mobile Phase:** A: DI water/10mm ammonium acetate  
 B: 97% Acetonitrile/3% DI water/  
 10mm ammonium acetate (v/v)

Gradient:	time (min.)	%B
	0	90
	6	20
	8	20
	9	90

**Injection vol.:** 1µL

**Flow rate:** 0.4mL/min

**Detection:** ESI - NEG - Agilent 6210 MSD TOF mass

**Peaks:** 1. AMP (adenosine monophosphate), 346.0558m/z [M-H]<sup>-</sup>  
 2. ADP (adenosine diphosphate)  
 3. ATP (Adenosine triphosphate), 505.9885m/z [M-H]<sup>-</sup>

### Catalog Numbers

To obtain the correct catalog number for your required PEEK column, please replace "XXXXX" with the 5 digit codes listed below.

#### For 4µm particle size columns:

- Amide: **40036**
- Bidentate C8: **40008**
- Bidentate C18: **40018**
- Diamond Hydride: **70000**
- Diol: **40060**
- Phenyl Hydride: **69020**
- Silica-C: **40000**
- UDA: **40031**
- UDC-Cholesterol: **69069**

#### For 2.2µm particle size columns:

- Amide: **40236**
- Bidentate C8: **40208**
- Bidentate C18: **40218**
- Diamond Hydride: **70200**
- Diol: **40260**
- Phenyl Hydride: **69220**
- Silica-C: **40200**
- UDA: **40231**
- UDC-Cholesterol: **69269**

Figure 40.



Cogent All PEEK HPLC Columns

i.d. (mm)	Length (mm)						
	20	30	50	75	100	150	250 <sup>1</sup>
2.1	XXXXX-02K-2	XXXXX-03K-2	XXXXX-05K-2	XXXXX-75K-2	XXXXX-10K-2	XXXXX-15K-2	XXXXX-25K-2
4.6	XXXXX-02K	XXXXX-03K	XXXXX-05K	XXXXX-75K	XXXXX-10K	XXXXX-15K	XXXXX-25K

<sup>1</sup> 250mm is not available in 2.2µm

# Column Accessories

## MicroSolv AQ™ Advanced Quality Syringe Filters

All AQ Filters are color coded for pore size and membrane making it easy for analysts and technicians to identify the proper filter for each method.

MicroSolv AQ brand of syringe filters are made of all virgin polypropylene and are extremely rugged and safe. Due to the advanced design of the housing and filter support system, they resist bursting even when applying extra pressure during filtering of very viscous or heavily particulated solutions.

### Nylon Membrane Filters: Aqueous Compatible

Nylon is the most popular membrane used in syringe filters for analytical testing. It is extremely durable, resists tearing as well as having the lowest extractables of all filtration membranes leaving your HPLC baseline clean and quiet. The MicroSolv nylon goes further than most of our competitors as a superior membrane because our membrane **does not carry any charge on it**. A charged membrane will extract ionized compounds from solution thereby changing the content uniformity of the analytes tested and producing false and non reproducible results.

#### Typical Applications:

1. Acidic solutions (not >1N)
2. Alcohols
3. Aqueous Based HPLC and Dissolution Testing
4. Basic solutions
5. Esters
6. Ionized compounds in solution
7. Not compatible with Aldehydes or Ketones
8. Nylon is the most “universal” syringe filter there are many other uses.

Figure 41.



Figure 42.



### PTFE Membrane Filters: Use with Organic Solvents

PTFE is a popular membrane used in syringe filters for analytical testing. It is extremely durable, resists tearing, and tolerates elevated temperatures as well as being chemically resistant to solvents. The MicroSolv PTFE goes further than most of our competitors as a superior membrane because our membrane has an excellent flow rating making it easier to filter through it. Also, these PTFE filters **do not have any surfactants** that can extract out contaminants as “hydrophilic” PTFE filters will. Any extractable can cause a noisy baseline in HPLC or can cause side reactions or can be a problem for some biological samples.

Figure 43.



Figure 44.



#### Typical Applications:

1. Acidic solutions
2. Alcohols
3. Non Polar Based Solvents
4. Basic or acidic solutions
5. Esters, Aldehydes, Ketones, Halogenated
6. Not compatible with Some Proteins
7. PTFE is the most chemically inert to solvents

#### Catalog Numbers

Nylon	4mm	13mm	25mm	PTFE	4mm	13mm	25mm
0.2um	58022-N04	58022-N13	58022-N25	0.2um	58022-P04	58022-P13	58022-P25
0.45um	58045-N04	58045-N13	58045-N25	0.45um	58045-P04	58045-P13	58045-P25

## Male Nuts - Finger Tightened and Stainless Steel

MicroSolv supplies a wide range of high quality male nut connectors for high and low pressure HPLC. A selection of the different types of connectors is outlined below.

**The Endure™ Carbon-PEEK** high pressure fittings offer extra durability. They are more robust to higher temperatures, solvent exposure and over-tightening than standard PEEK fittings. They are available in a variety of dimensions, all with built-in ferrules.

**CombiHead™** Flat and Hex fittings are designed for finger-tightening when access is limited. A flat area is provided to wrench the last ¼ turn, assuring that the high pressure fitting is sealed and therefore less likely to leak.

**Stainless Steel Nuts** Stainless steel male nuts are available in 3 lengths to meet different requirements. All the fittings are manufactured from SS316L.

Figure 45.



The Endure™ Fittings

Figure 46.



CombiHead™ Flat & Hex Fittings

Catalog Numbers	Description
47301-01	Fittings, HPLC, male nuts, Endure brand carbon PEEK. 1 piece fingertight 10mm knurled head 21mm total length 13mm thread length. 10-32 10/pack.
47301-03	Fittings, HPLC, male nuts, Endure brand carbon PEEK. 1 piece fingertight 8mm knurled head 21mm total length 12mm thread length. 10-32 10/pack.
47301-04	Fittings, HPLC, male nuts, Endure brand carbon PEEK. 1 piece fingertight 6mm narrow hex head 20.5mm total length 13mm thread length. 10-32 10/pack.
47301-05	Fittings, HPLC, male nuts, Endure brand carbon PEEK. 1 piece fingertight large knurled head 21mm total length 12mm thread length. 10-32 10/pack.
47301-10	Fittings, HPLC, male nuts, Endure brand carbon PEEK. 1 piece fingertight 15mm winged / knurled head 21mm total length 13mm thread length. 10-32 10/pack.
47320-26	Male Nuts, HPLC, Peek, 1 piece, 8mm narrow hex head, total length 28mm, 14mm thread length, 10-32, natural color. 10/pack.
47320-11N	Male Nuts, HPLC, Combihead, Peek, 1 piece, Part knurled & part flat 12mm head. Finger tightening and/or wrench. Total length 22mm, 8mm thread length, 10-32, natural color. 10/pack.
49590-01	Male Nuts, HPLC, 316L stainless steel. 10-32 hex head for 1/16" tubing and use with short Rheodyne threads. 10/pack.
49590-02	Male Nuts, HPLC, 316L stainless steel. 10-32 hex head for 1/16" tubing and for use with medium Rheodyne threads. 10/pack.
49590-03	Male Nut, HPLC, 316L stainless steel. 10-32 hex head for 1/16" tubing and for use with extra-long Rheodyne threads. 10/pack.

Please inquire about our full line of male nuts and ferrules for HPLC.

### Column End Plugs

Column end plugs are used to prevent HPLC columns from drying out when not in use. Made of hard nylon, these end stoppers are available in packs of 10 and in different colours.

Figure 47.



Column End Plugs

### Column Couplers

Column couplers are used to connect two HPLC columns in series.

Figure 48.



Column Couplers

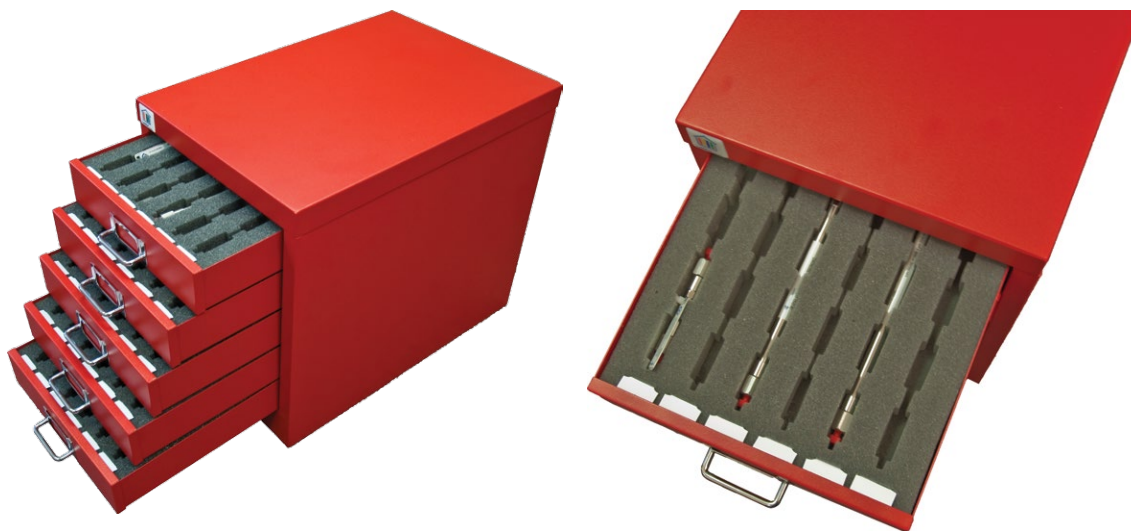
Catalog Numbers	Description
49027-01B	End plugs for HPLC columns. Nylon 10-32 Thread. Blue. 10/Pack.
49027-06K	End plugs for HPLC columns. Nylon 10-32 Thread. Black. 10/Pack.
49027-04W	End plugs for HPLC columns. Nylon 10-32 Thread. White. 10/Pack.
47450-06	Column coupler, for use with 1.0mm to 21.2mm i.d. Hichrom hardware columns.

### Column Storage Storage Cabinet

The Column Storage Cabinet storage system is simple and compact and prevents HPLC columns from getting lost or damaged. It also keeps columns organized for quick retrieval. The column storage cabinet is a steel benchtop unit that can hold up to 30 columns of up to 30cm in length. The five drawer units contain foam cut-outs to accommodate 5, 12.5, 15 or 30cm length columns. The units are stackable.

Catalog Numbers	Description
50100-30	Storage cabinet for HPLC columns. Holds up to 30 columns that are up to 30cm long and up to 10cm OD. Red color with 5 drawers with foam cut outs to hold columns, 13" x 16 x 18" (WxHxD). Supplied with rubber feet. 26lbs. 1 each.

Figure 49.



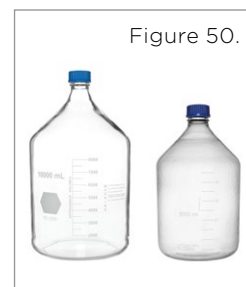
Store columns not in use

## Mobile Phase Reservoir and Storage Bottles

### Safety-Coated:

These borosilicate glass bottles are designed for safety. They are coated in an epoxy plastic on the outside that will not break if dropped, even when full of solvent. Although 1L and 2L are the most common sizes, 5L and 10L bottles are also available. These bottles have 45mm standard screw top necks. Compatible with many mobile phase filtration devices, you can safely filter directly into these bottles and cap them for storage or transportation.

These solvent bottles are made from Type-33 borosilicate glass and have white enamel graduations and write-on patches. Due to their superior chemical stability, these bottles are useful for storage and delivery of reagents and both aqueous and/or organic solvents. Bottles with volumes of 100mL to 10L are supplied.



Catalog Numbers	Description
58802-01	HPLC mobile phase reservoir bottle. Graduated borosilicate glass. 1 liter capacity. 45mm Screw top plastic coated. A solid screw cap & pouring ring included. 1 Each.
58819-05	HPLC mobile phase reservoir bottle. Graduated borosilicate glass. 2L capacity. 45mm screw top plastic coated. A solid screw cap & pouring ring included. 1 Each.
58802-05	HPLC mobile phase reservoir bottle. Graduated borosilicate glass. 5L capacity. 45mm screw top plastic coated for safety. A solid screw cap & pouring ring included. 1 Each.
58802-10	HPLC mobile phase reservoir bottle. Graduated borosilicate glass. 10L capacity. 45mm screw top plastic coated for safety. A solid screw cap & pouring ring included. 1 Each.

### Non-Coated Mobile Phase Delivery Bottles:

MicroSolv Technology provides a low cost option for solvent bottles made from Type-33 borosilicate glass that are excellent for the use of HPLC and GC Solvents. Due to their superior chemical stability, these bottles are useful for storage and delivery of your reagents and solvents that are both aqueous and/or organic in nature. These bottles are also useful as medial bottles.

*Supplied with white enamel markings, a solid blue cap and a drip free sealing ring. Other color caps are available.*



Catalog Numbers	Description
58807-050	HPLC mobile phase reservoir and storage bottle, 50mL. 1 Each.
58807-050-CS	HPLC mobile phase reservoir and storage bottles, 50mL. 10/case.
58807-100	HPLC mobile phase reservoir and storage bottle, 100mL. . 1 Each.
58807-100-CS	HPLC mobile phase reservoir and storage bottles, 100mL. 10/case.
58807-1L	HPLC mobile phase reservoir and storage bottle, 1 Liter. 1 Each.
58807-1L-CS	HPLC mobile phase reservoir and storage bottles, 1 Liter. 10/case.
58807-250	HPLC mobile phase reservoir and storage bottle, 250mL. 1 Each.
58807-250-CS	HPLC mobile phase reservoir and storage bottles, 250mL. 10/case.
58807-2L	HPLC mobile phase reservoir and storage bottle, 2 Liter. 1 Each.
58807-2L-CS	HPLC mobile phase reservoir and storage bottles, 2 Liter. 5/case.
58807-500	HPLC mobile phase reservoir and storage bottle, 500mL. 1 Each.
58807-500-CS	HPLC mobile phase reservoir and storage bottles, 500mL. 10/case.

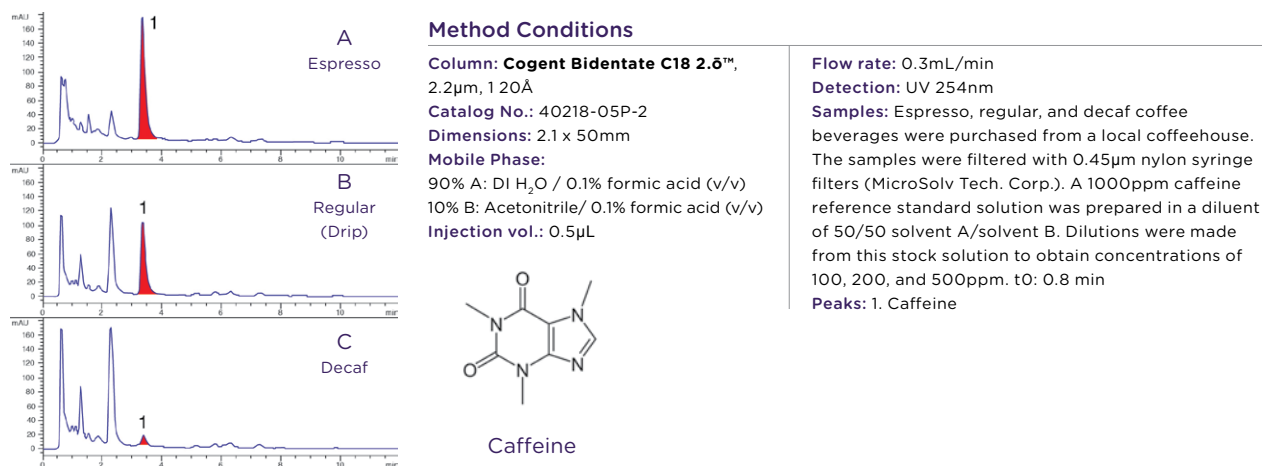
# Applications of Cogent TYPE-C™ Columns

## Food and Beverage Applications

For many other Food and Beverage applications go to [www.mtc-usa.com](http://www.mtc-usa.com) and click on Knowledge Base.

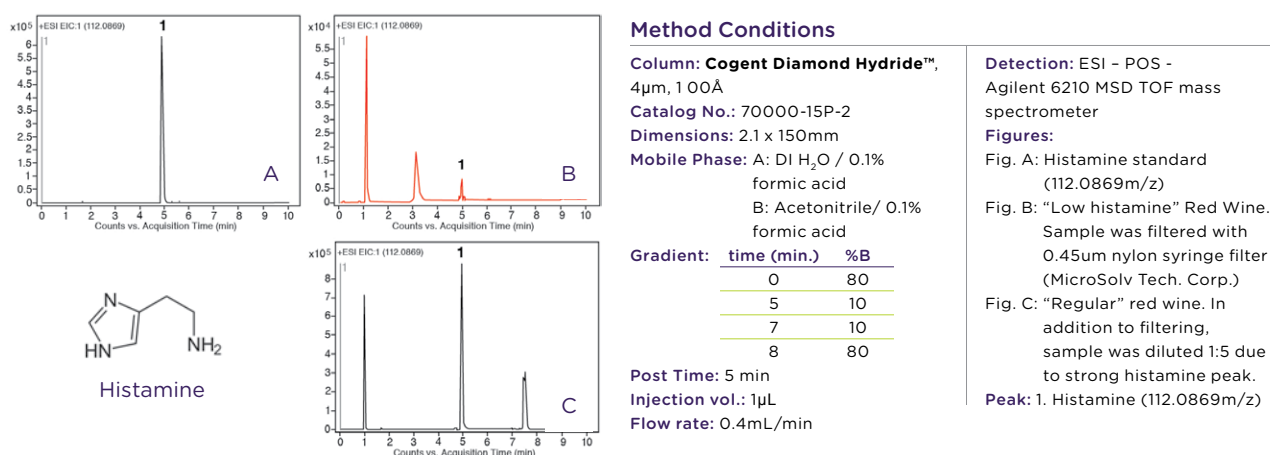
### Caffeine Content in Coffee Beverages by RP

Figure 52.



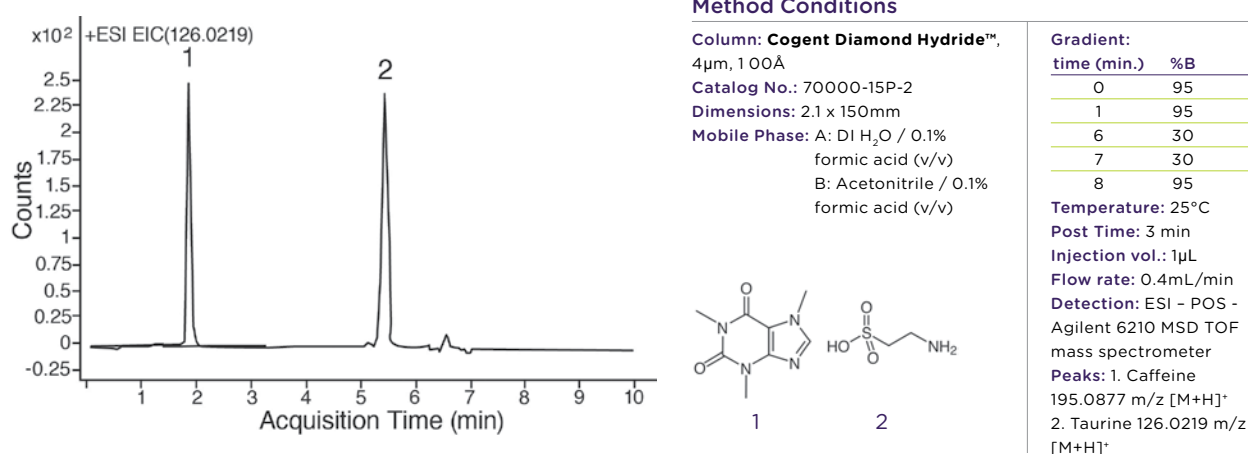
### Histamine Content of Red Wine by ANP

Figure 53.



### Red Bull Energy Drink by ANP

Figure 54.



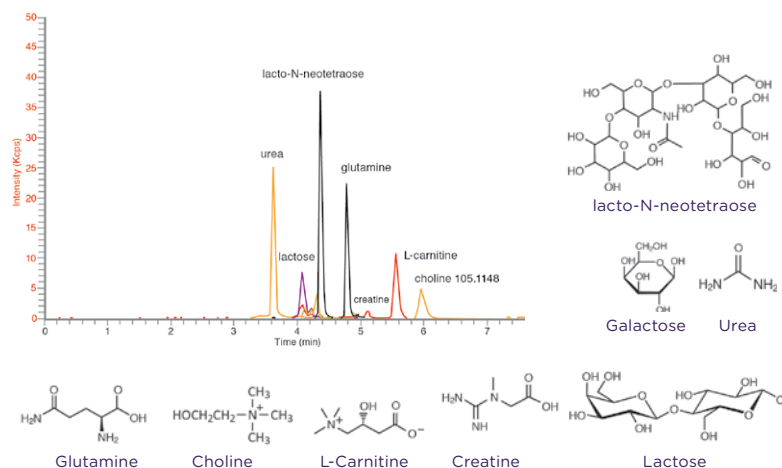
# Applications of Cogent TYPE-C™ Columns

## Food and Beverage Applications continued

For many other Food and Beverage applications go to [www.mtc-usa.com](http://www.mtc-usa.com) and click on Knowledge Base.

### Milk Extract using ANP

Figure 55.



#### Method Conditions

Column: **Cogent Diamond Hydride™**, 4µm, 100Å  
Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150mm

Mobile Phase: A: DI H<sub>2</sub>O / 0.1% formic acid

B: Acetonitrile/ 0.1% formic acid

Gradient:	time (min.)	%B
	0	90
	1	90
	7	20
	11	20
	12	90

Post Time: 3 min

Injection vol.: 1µL

Flow rate:

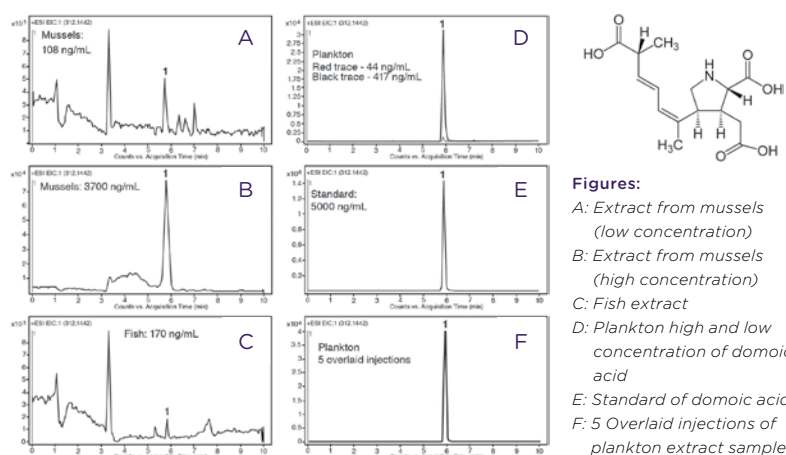
0.4mL/min

Detection: ESI - Pos - Perkin Elmer, Flexar SQ 300 mass spectrometer

Sample: Milk extract reconstituted in 65µL of 80% acetonitrile

### Domoic Acid in Seafood Samples by ANP

Figure 56.



#### Method Conditions

Column: **Cogent Diamond Hydride™**, 4µm, 100Å  
Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150mm

Mobile Phase:

A: 50% MeOH/ 50% DI H<sub>2</sub>O/ 0.1% formic acid

B: Acetonitrile/ 0.1% formic acid

Gradient:	time (min.)	%B
	0	95
	7	30
	8	30
	10	95

Post Time: 5 min

Injection vol.: 1µL

Flow rate:

0.4mL/min

Detection: ESI - POS - Agilent

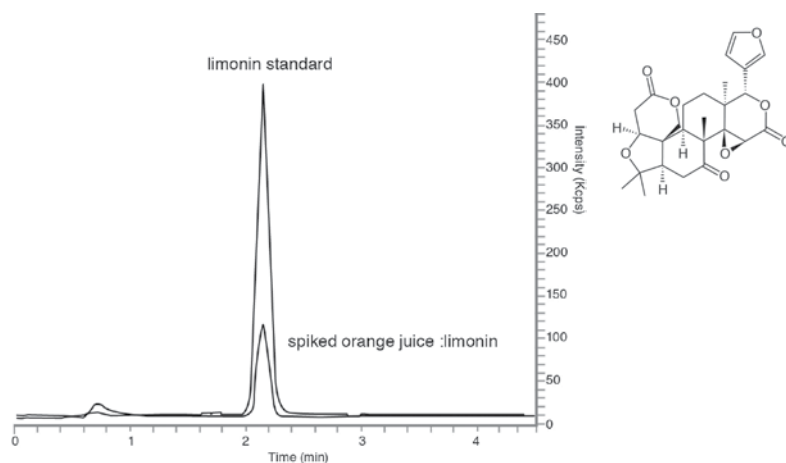
6210 MSD TOF MS

Samples: Samples from Dr. R.Kudela, Univ. of Calif., Santa Cruz, Dept. of Marine Sciences. Samples were injected as received

Peak: Domoic acid 312.1442m/z (M + H)<sup>+</sup>

### Limonin in Orange Juice by RP

Figure 57.



#### Method Conditions

Column: **Cogent Bidentate C18™**, 4µm, 100Å  
Catalog No.: 40018-15P-2

Dimensions: 2.1 x 150mm

Mobile Phase: 50% A: DI H<sub>2</sub>O / 0.1% formic acid

50% B: Acetonitrile/ 0.1% formic acid

Temperature: 25°C

Injection vol.: 1µL

Flow rate: 0.5mL/min

Detection: ESI - Pos - Perkin Elmer, Flexar SQ 300 mass spectrometer

Samples: Standard: 5ppm of limonin in 20% DI water +0.1% formic acid/40% acetonitrile/ 40% methanol. Spiked orange juice preparation: Orange juice was spiked with 2.5ppm limonin, filtered, and injected.

Peaks: 1. Limonin standard, 471.2m/z [M+H]<sup>+</sup>

2. Spiked orange juice: limonin, 471.2m/z [M+H]<sup>+</sup>

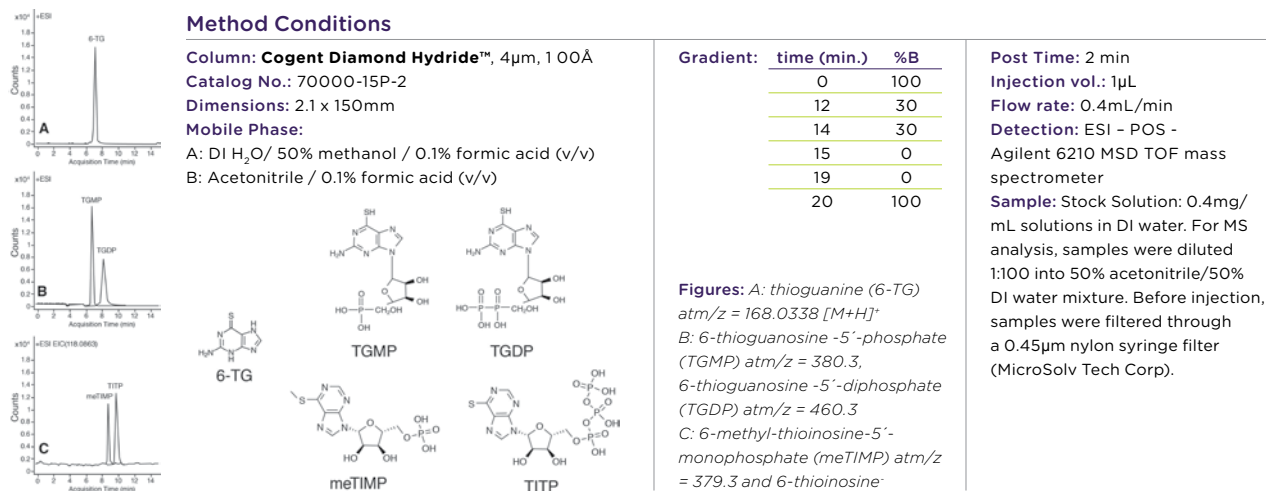
# Applications of Cogent TYPE-C™ Columns

## Clinical Applications

For many other Clinical applications go to [www.mtc-usa.com](http://www.mtc-usa.com) and click on Knowledge Base.

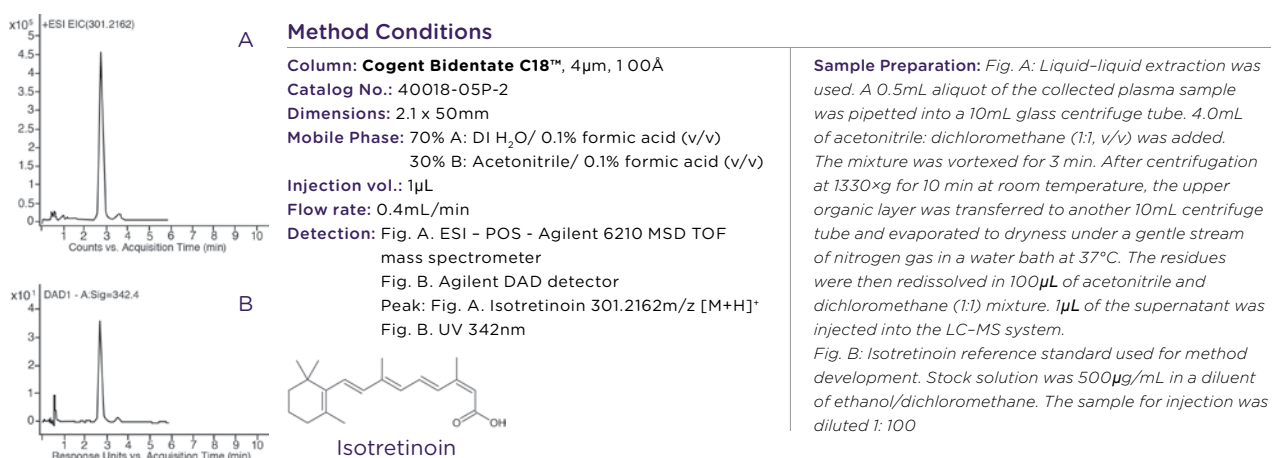
### Analysis of Thiopurines by ANP LC-MS

Figure 58.



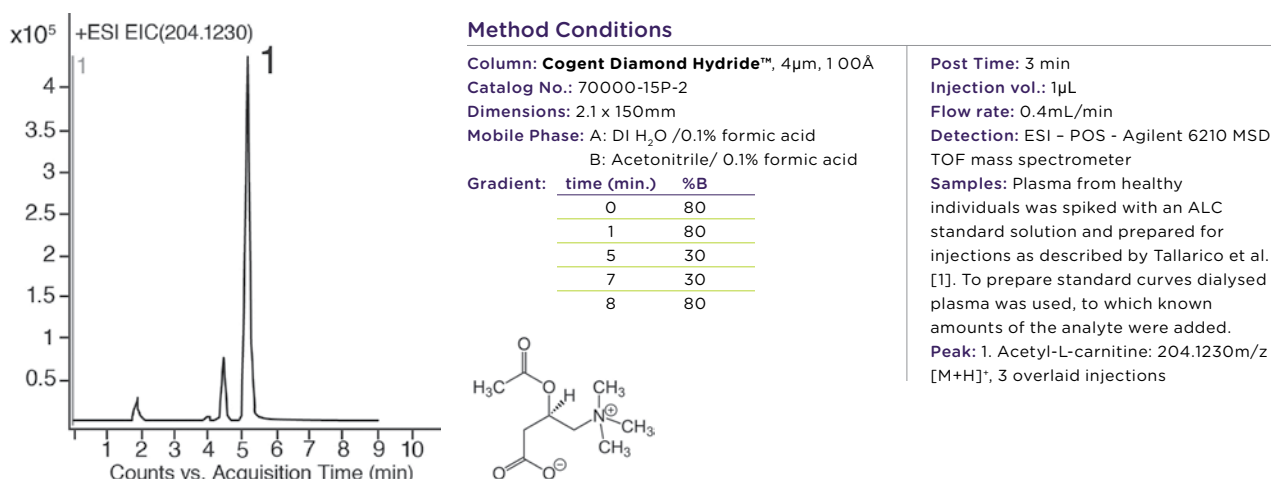
### Isotretinoin in Human Plasma by ANP LC-MS

Figure 59.



### Acetyl-L-Carnitine in Plasma by ANP LC-MS

Figure 60.





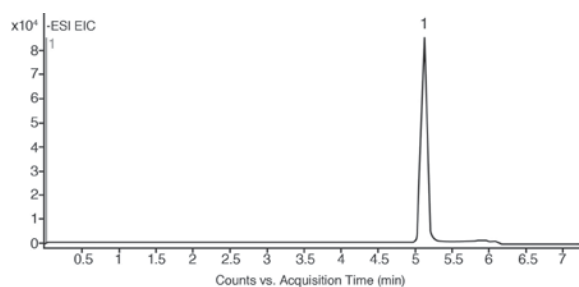
# Applications of Cogent TYPE-C™ Columns

## Clinical Applications continued

For many other Clinical applications go to [www.mtc-usa.com](http://www.mtc-usa.com) and click on Knowledge Base.

### 3-Hydroxy-3-Methylglutaric Acid (HMG) in Urine by ANP LC-MS

Figure 61.

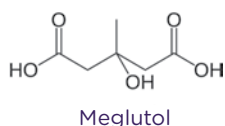


#### Method Conditions

Column: **Cogent Diamond Hydride™**, 4µm, 100Å  
 Catalog No.: 70000-15P-2  
 Dimensions: 2.1 x 150mm  
 Mobile Phase: A: DI H<sub>2</sub>O / 10mm ammonium formate  
 B: 95% acetonitrile/5% DI water/10mm ammonium formate (v/v)

Gradient:	time (min.)	%B
	0	95
	1	95
	5	30
	7	30
	8	95

Post Time: 3 min  
 Injection vol.: 1µL  
 Flow rate: 0.4mL/min  
 Detection: ESI - NEG - Agilent 6210 MSD TOF mass spectrometer  
 Peak: 1. 3-hydroxy-3-methylglutaric acid 161.0455m/z [M-H]<sup>-</sup> in urine sample

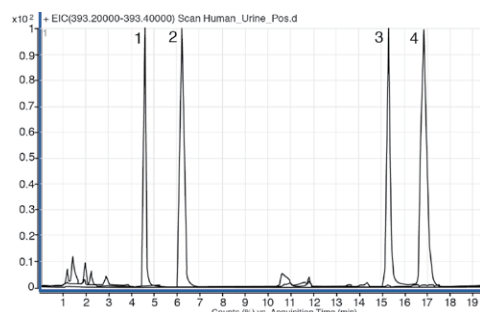


#### Discussion

A selective, specific, and sensitive method based on LC-MS analysis has been developed for the determination of 3-hydroxy-3-methylglutaric acid (a.k.a. meglutol) in urine samples. The method can be also used in the analysis of plasma samples after precipitation of plasma proteins with acetonitrile. The retention was achieved using a Cogent Diamond Hydride™ column. This method can be used for screening of large numbers of urine or plasma samples, due to simple sample preparation and rapid equilibration of the Cogent columns when gradient analysis is used.

### Metabolites in Human Urine by ANP LC-MS

Figure 62.



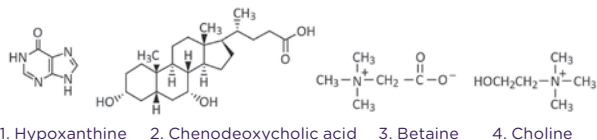
#### Method Conditions

Column: **Cogent Diamond Hydride™**, 4µm, 100Å  
 Catalog No.: 70000-15P-2  
 Dimensions: 2.1 x 150mm  
 Mobile Phase:  
 A: DI water + 0.1% formic acid  
 B: Acetonitrile + 0.1% formic acid

Gradient:	time (min.)	%B
	0.0	95
	0.2	95
	30.0	50
	35.0	50
	35.1	95
	40.0	95

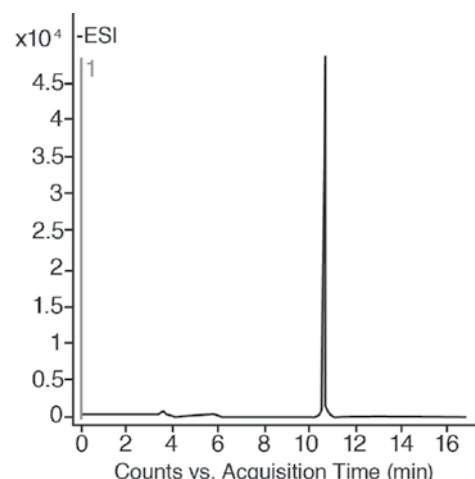
Flow rate: 0.4mL/min  
 Detection: ESI - pos - Agilent 6210 MSD TOF mass spectrometer

Sample: Human Urine - after simple extraction  
 Peaks: 1. Hypoxanthine; 137.04580m/z (M+H)<sup>+</sup>, RT = 4.98 min  
 2. Chenodeoxycholic acid; 393.29990m/z (M+H)<sup>+</sup>, RT = 6.23 min  
 3. Betaine; 118.08680m/z M<sup>+</sup>, RT = 15.27 min  
 4. Choline; 104.10754m/z (M+H)<sup>+</sup>, RT = 16.82 min  
**Figure:** EIC - extracted ion chromatogram of selected compounds (1,2,3,4)



### Galactose-1-Phosphate by ANP LC-MS

Figure 63.

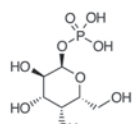


#### Method Conditions

Column: **Cogent Diamond Hydride™**, 4µm, 100Å  
 Catalog No.: 70000-15P-2  
 Dimensions: 2.1 x 150mm  
 Mobile Phase:  
 A: DI H<sub>2</sub>O / 0.1% formic acid (v/v)  
 B: 90% acetonitrile/10% DI water/16.5mm ammonium acetate (v/v)

Gradient:	time (min.)	%B
	0	95
	1	95
	3	85
	6	85
	7	75
	9	75
	10	50
	12	50
	13	30
	15	30
	15.01	95

Post Time: 5 min  
 Injection vol.: 1µL  
 Flow rate: 0.4mL/min  
 Detection: ESI - NEG - Agilent 6210 MSD TOF mass spectrometer  
**Sample: Stock Standard:** 1mg/mL galactose-1-phosphate in DI water, stored at -20°C  
**Peaks:** Galactose-1-phosphate, 259.0224m/z (M-H)<sup>-</sup>



#### Discussion

This method is useful as a quantitative screening or routine clinical test to detect infants suspected of having a defect of galactose metabolism. It can also be used to monitor blood levels of galactose-1-phosphate in children with galactosemia who are on a lactose-free diet.

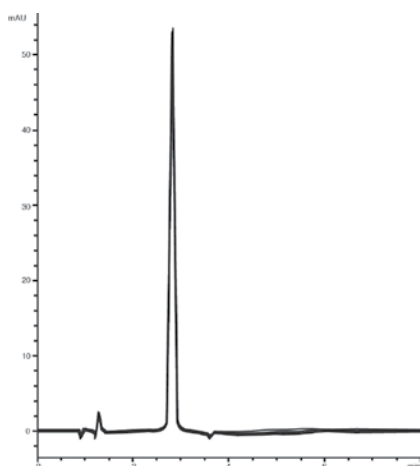
# Applications of Cogent TYPE-C™ Columns

## Pharmaceutical Applications

For many other Pharmaceutical applications go to [www.mtc-usa.com](http://www.mtc-usa.com) and click on Knowledge Base.

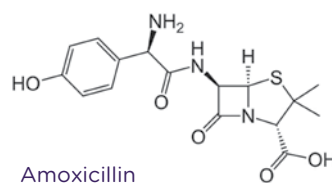
### Amoxicillin Assay – Orthogonal Method on USP Assay

Figure 64.



#### Method Conditions

Column: **Cogent Diamond Hydride™**, 4μm, 1 00Å  
 Catalog No.: 70000-7.5P  
 Dimensions: 4.6 x 75mm  
 Mobile Phase: A: DI water + 10mm ammonium acetate  
 B: 90% Acetonitrile/ 10% DI water/  
 10mm ammonium acetate

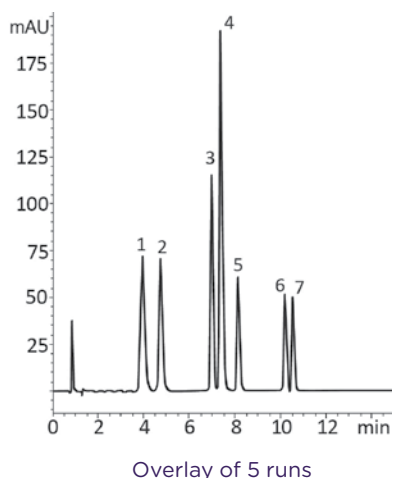


Gradient: time (min.)	%B
0	100
4	90
5	100

Injection vol.: 2μL  
 Flow rate: 1.0mL/min  
 Detection: UV 230nm  
 Sample: Stock Solution: 1mg/mL amoxicillin trihydrate USP RS in 50/50 A/B diluent.  
 Working Solution: A 100μL aliquot of the stock was diluted to 0.1mg/mL using 900μL 50/50 A/B diluent

### Cough Syrup Ingredients

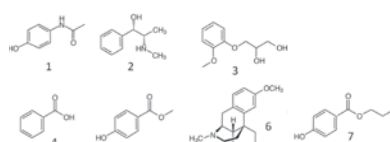
Figure 65.



#### Method Conditions

Column: **Cogent Phenyl Hydride™**, 4μm, 1 00Å  
 Catalog No.: 69020-7.5P  
 Dimensions: 4.6 x 75mm  
 Mobile Phase: A: DI H<sub>2</sub>O / 0.1% TFA (v/v)  
 B: Acetonitrile/ 0.1%TFA (v/v)

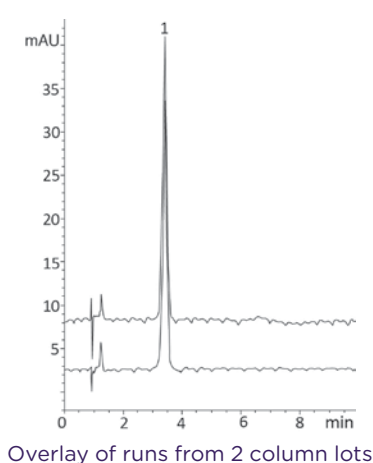
Gradient: time (min.)	%B
0	5
2	5
11	50
12	5



Post Time: 3 min  
 Injection vol.: 2μL  
 Flow rate: 1.0mL/min  
 Detection: UV 210nm (0-6 min), 230nm (6-15 min)  
 Sample: Stock Solution: 1mg/mL solutions of each analyte were made using a 50/50 solvent A/ solvent B diluent (v/v).  
 Working Solution: 0.1mg/mL dilutions were made of the stock solutions and used for peak identity confirmations. A 0.1mg/mL mixture of all the analytes was also made from the stock solutions.  
 Peaks: 1. Acetaminophen  
 2. Pseudoephedrine  
 3. Guaifenesin  
 4. Benzoic Acid  
 5. Methyl Paraben  
 6. Dextromethorphan  
 7. Propyl Paraben

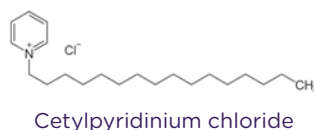
### Cetylpyridinium Chloride by ANP

Figure 66.



#### Method Conditions

Column: **Cogent Diamond Hydride™**, 4μm, 1 00Å  
 Catalog No.: 70000-7.5P  
 Dimensions: 4.6 x 75mm  
 Mobile Phase: 4% DI H<sub>2</sub>O / 96% Acetonitrile/ 0.1% TFA (v/v)  
 Injection vol.: 2μL  
 Flow rate: 1.0mL/min  
 Detection: UV 215nm  
 Sample: 1mg cetylpyridinium chloride USP reference standard was dissolved in 1mL of 50/50/0.1 DI water/acetonitrile/formic acid. This stock solution was diluted 1:10 for HPLC injections using the same diluent.  
 Peak: 1. Cetylpyridinium chloride



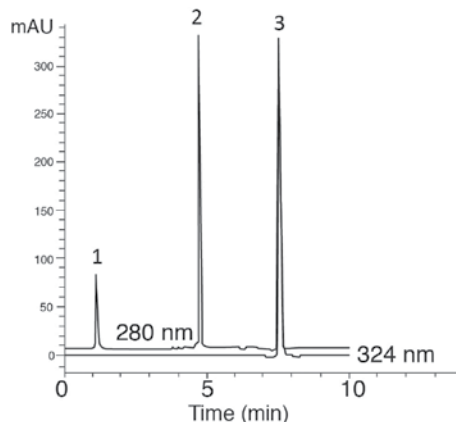
# Applications of Cogent TYPE-C™ Columns

## Pharmaceutical Applications continued

For many other Pharmaceutical applications go to [www.mtc-usa.com](http://www.mtc-usa.com) and click on Knowledge Base.

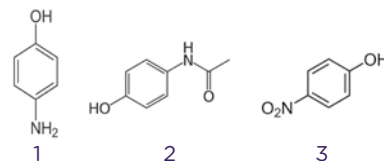
### Acetaminophen and Major Impurities by RP

Figure 67.



#### Method Conditions

Column: **Cogent Bidentate C18™**, 4µm, 100Å  
 Catalog No.: 40018-75P  
 Dimensions: 4.6 x 75mm  
 Mobile Phase: A: DI H<sub>2</sub>O/ 0.1% formic acid  
 B: Acetonitrile/ 0.1% formic

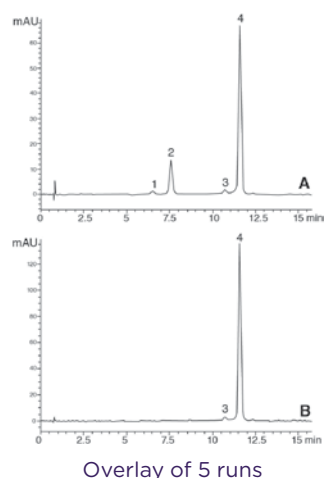


Gradient:	time (min.)	%B
	0	0
	1	0
	4	30
	6	30
	6.01	10
	10	10

Post Time: 3 min  
 Injection vol.: 5µL  
 Flow rate: 1.0mL/min  
 Detection: UV 280 (4-aminophenol, acetaminophen) and 324nm (4-nitrophenol)  
 Peaks: 1. 4-aminophenol 1.072 min  
 2. acetaminophen 4.668 min  
 3. 4-nitrophenol 7.588 min

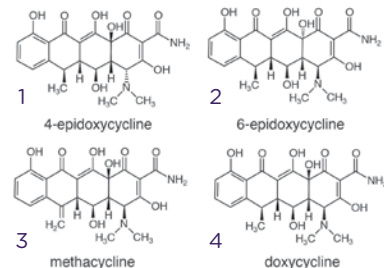
### Doxycycline and Epimers using Shape Selectivity

Figure 68.



#### Method Conditions

Column: **Cogent UDC Cholesterol™**, 4µm, 100Å  
 Catalog No.: 69069-7.5P  
 Dimensions: 4.6 x 75mm  
 Mobile Phase: A: DI H<sub>2</sub>O/ 0.1% TFA  
 B: Acetonitrile/ 0.1% TFA

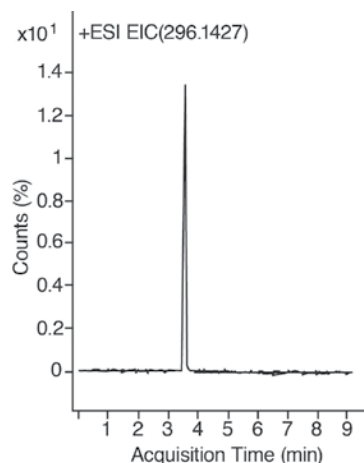


Gradient:	time (min.)	%B
	0	5
	12	30
	13	5

Temperature: 25°C  
 Post Time: 3 min  
 Injection vol.: 20µL  
 Flow rate: 1.5mL/min  
 Figures: A: Doxycycline forced degradation extract  
 B: Doxycycline non-degraded extract  
 Peaks: 1: 4-epidoxycycline  
 2: 6-epidoxycycline  
 3: methacycline  
 4: doxycycline

### Sumatriptan Tablets by LC-MS

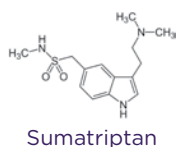
Figure 69.



#### Method Conditions

Column: **Cogent Diamond Hydride 2.6™**, 2.2µm, 120Å  
 Catalog No.: 70200-05P-2  
 Dimensions: 2.1 x 50mm  
 Mobile Phase: A: DI H<sub>2</sub>O / 0.1% (v/v) formic acid  
 B: Acetonitrile/ 0.1% (v/v) formic acid  
 Gradient:

time (min.)	%B
0	90
4	30
6	30
7	90



Post Time: 3 min  
 Injection vol.: 1µL  
 Flow rate: 0.4mL/min  
 Detection: ESI - POS - Agilent 6210 MSD TOF mass spectrometer  
 Sample: Six 25mg strength tablets of Imitrex® were crushed and a portion containing 40mg of the API was weighed out. The powder was suspended in an A:B (1:1) solvent mixture, vortexed, and filtered through a disposable 0.45µm filter (MicroSolv Tech Corp.). Sample for injection was diluted to final concentration of 0.0005µg/mL.  
 Peak: Sumatriptan 296.1427m/z [M+NH<sub>3</sub>]<sup>+</sup>

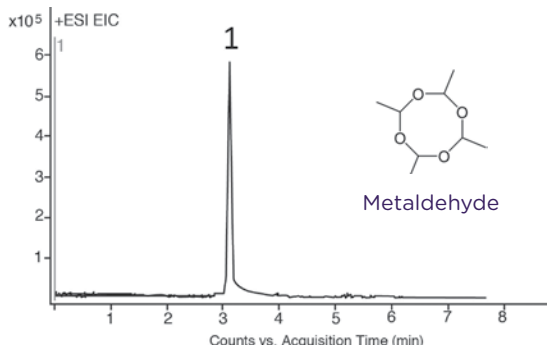
# Applications of Cogent TYPE-C™ Columns

## Environmental Applications

For many other Environmental applications go to [www.mtc-usa.com](http://www.mtc-usa.com) and click on Knowledge Base.

### Metaldehyde in Slug Pellets by RP LC-MS

Figure 70.



#### Method Conditions

**Column:** Cogent Bidentate C18 2.0™, 2.2µm, 120Å

**Catalog No.:** 40218-05P-2

**Dimensions:** 2.1 x 50mm

**Mobile Phase:**

A: DI H<sub>2</sub>O / 0.1% formic acid

B: Acetonitrile/ 0.1% formic acid

**Gradient:**

time (min.)	%B
0	10
3	100
6	100
7	10

**Post Time:** 3 min

**Injection vol.:** 1µL

**Flow rate:** 0.4mL/min

**Detection:** ESI - POS - Agilent 6210 MSD TOF mass spectrometer

**Sample:** Slug pellets were ground (3.25% metaldehyde) and 800mg was transferred to a 25mL volumetric flask. A portion of 50/50 solvent A/solvent B diluent was added and the flask was sonicated 10min. Then it was diluted to mark, mixed thoroughly, and filtered with a 0.45µm nylon syringe filter (MicroSolv Tech. Corp.).

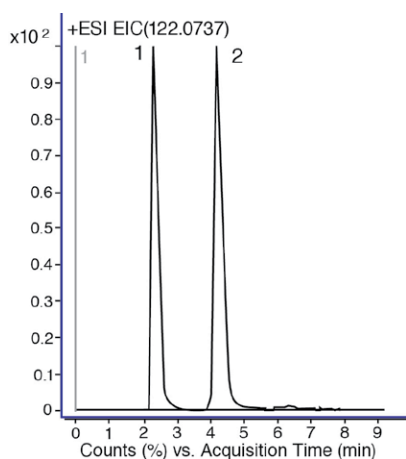
**Peak:** 1. Metaldehyde (177.1121m/z)

#### Discussion

As it has no double bonds, metaldehyde is a non-UV absorbing compound. Therefore other detection methods need to be investigated besides conventional UV-HPLC. LC-MS was found to be well-suited to its analysis by searching for the EIC corresponding to the [M+H]<sup>+</sup> ion. Good retention and peak shape were observed for this analyte using the Cogent Bidentate C18 2.0™ column.

### Chlormequat and Mepiquat Plant Growth Regulators

Figure 71.



#### Method Conditions

**Column:** Cogent Diamond Hydride™, 4µm, 100Å

**Catalog No.:** 70000-15P-2

**Dimensions:** 2.1 x 150mm

**Mobile Phase:** A: DI water + 20mm

ammonium acetate, pH

adjusted to pH 3.3 with

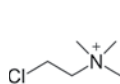
formic acid

B: Acetonitrile

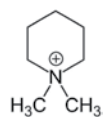
**Mobile Phase:** 70% A

**Post Time:** 5 min

**Flow rate:** 0.5mL/min



Chlormequat



2. Mepiquat

**Detection:** ESI - pos. - Agilent 6210 MSD TOF mass spectrometer. CQ and MQ are already charged in solution and under ESI conditions the mass spectra show abundant molecular ion (M)<sup>+</sup>.

**Sample Peaks:**

1. Chlormequat (CQ) 122.0737m/z (M)<sup>+</sup>

2. Mepiquat (MQ) 114.1277m/z (M)<sup>+</sup>

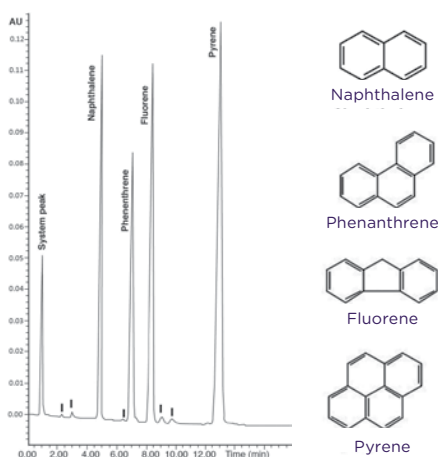
Samples for injection were diluted 1:1000 in the mobile phase.

#### Discussion

This note shows a new, sensitive and selective LC-MS method with low ionic strength mobile phase for the analysis of CQ and MQ residues. The method can be used in analysis of many samples including food. The selectivity and sensitivity of the method can be increased by using LC-MS-MS instruments and adequate product ions (CQ 122m/z to 58m/z and 63m/z, MQ 114m/z to 98m/z and 58m/z).

### Polycyclic Aromatic Hydrocarbons

Figure 72.



#### Method Conditions

**Column:** Cogent Bidentate C18™, 4µm, 100Å

**Catalog No.:** 40018-75P

**Dimensions:** 4.6 x 75mm

**Mobile Phase:** Acetonitrile/DI Water 70:30

**Injection vol.:** 1µL

**Flow rate:** 0.5mL/min

**Detection:** UV at 254nm

**Samples:** 1. Naphthalene

2. Phenanthrene

3. Fluorene

4. Pyrene

1. Impurities or decomposition products 1mg of each sample was dissolved in 1mL of the mobile phase.

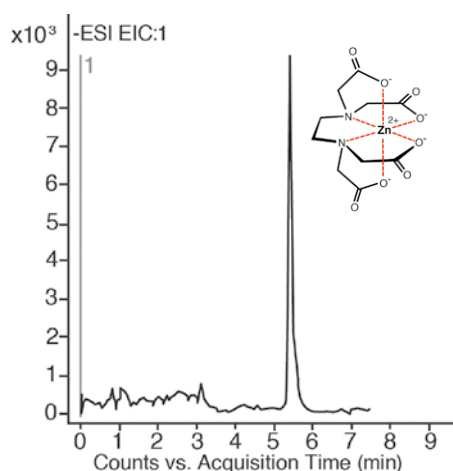
# Applications of Cogent TYPE-C™ Columns

## Environmental Applications continued

For many other Environmental applications go to [www.mtc-usa.com](http://www.mtc-usa.com) and click on Knowledge Base.

### Zinc-EDTA Complex by ANP LC-MS

Figure 73.



#### Method Conditions

**Column:** Cogent Diamond Hydride™, 4µm, 100Å  
**Catalog No.:** 70000-15P-2  
**Dimensions:** 2.1 x 150mm  
**Mobile Phase:**  
 A: DI H<sub>2</sub>O/ 0.1% (v/v) formic acid  
 B: Acetonitrile/ 0.1% (v/v) formic acid  
**Gradient:**

time (min.)	%B
0	90
5	20
8	20
9	90

**Post Time:** 2 min  
**Injection vol.:** 1µL  
**Flow rate:** 0.4mL/min  
**Detection:** ESI - NEG - Agilent 6210  
 MSD TOF mass spectrometer

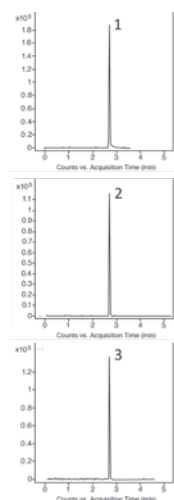
**Sample:** A soil sample was spiked with Zn-EDTA complex at a level of 2000µM. After extraction with DI water (shaking for 24 hours), the sample was filtered using a 0.45µm syringe filter (MicroSolv Tech. Corp.) and diluted with acetonitrile 1:10 before injection.  
**Peak:** Zn-EDTA complex 354.7m/z t<sub>0</sub>: 0.9 min

#### Discussion

Using conventional analytical methods, retention of metal-EDTA complexes is accomplished using ion pair reversed phase chromatography. However, the ion pair agents used in the mobile phase are not compatible with mass spectrometry. In this LC-MS method using the Diamond Hydride™ column, only formic acid is needed in the mobile phase in order to obtain retention of a Zinc-EDTA complex. The figure shows an EIC of the analyte spiked in a soil extract matrix.

### Glufosinate Herbicide and Metabolites by ANP LC-MS

Figure 74.



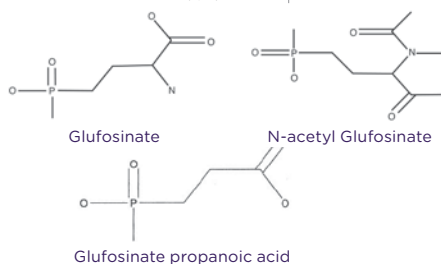
#### Method Conditions

**Column:** Cogent Diamond Hydride 2.6™, 2.2µm, 120Å  
**Catalog No.:** 70200-05P-2  
**Dimensions:** 2.1 x 50 mm  
**Mobile Phase:**  
 A: DI H<sub>2</sub>O/ 10mM ammonium acetate  
 B: 95% Acetonitrile / 5% DI water / 10mM ammonium acetate (v/v)

**Gradient:**

time (min.)	%B
0	90
1	90
1.2	5
5	5
6	90

**Post Time:** 3 min  
**Injection vol.:** 1 microL  
**Flow rate:** 0.4 mL/min  
**Detection:** ESI - NEG - Agilent 6210 MSD TOF mass spectrometer  
**Samples:** Glufosinate (1720.64 ppm), N-acetylglufosinate (639.2 ppm), and glufosinate propanoic acid (1302.5 ppm) stock solutions were diluted 1:100 with 4:1 DI water: methanol.  
**Peak:** 1. Glufosinate m/z 180.0431 [M-H]<sup>-</sup>  
 2. N-acetyl Glufosinate m/z 222.00 [M-H]<sup>-</sup>  
 3. Glufosinate Propanoic Acid m/z 151.00 [M-H]<sup>-</sup>

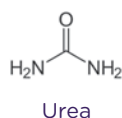
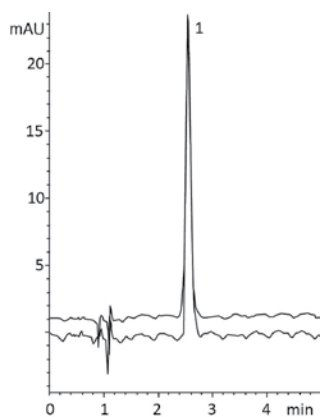


#### Discussion

Analysis of these compounds can be problematic with other methods and poor peak shape may occur. In contrast, the peaks obtained with the Diamond Hydride 2.0™ column are very sharp and symmetrical. The column is a near-UHPLC phase and consequently efficiency is very good. The method can be applied to food products containing these types of compounds.

### Urea by ANP LC-MS

Figure 75.



#### Method Conditions

**Column:** Cogent Diamond Hydride™, 4µm, 100Å  
**Catalog No.:** 70000-7.5P  
**Dimensions:** 4.6 x 75mm  
**Mobile Phase:** 5% DI H<sub>2</sub>O / 95% Acetonitrile/ 0.1% (v/v) trifluoroacetic acid (TFA)  
**Injection vol.:** 1µL  
**Flow rate:** 1.0mL/min  
**Detection:** UV 205nm  
**Sample:** 1mg/mL urea reference standard in diluent of 50% acetonitrile/ 50% DI water/ 0.1% TFA.  
**Peak:** 1. Urea

#### Discussion

Urea is very difficult to retain by conventional HPLC methods. It is highly polar and therefore shows little or no reversed phase retention. On the other hand, it can be readily retained past the solvent front when using the Diamond Hydride™ column and a simple isocratic mobile phase. Furthermore, the peak shape for the compound is symmetrical and does not exhibit tailing or fronting. Data from two column lots is shown in the overlay, illustrating the lot-to-lot precision of the stationary phase.

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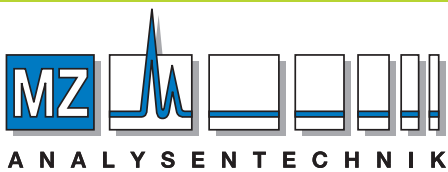
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