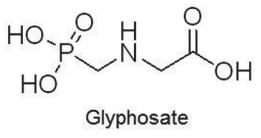
Cogent™ HPLC Columns

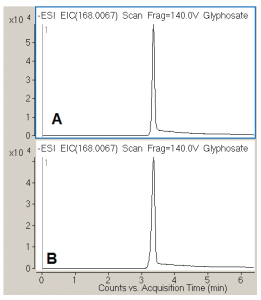
# ENVIRONMENTAL & FOOD APPLICATIONS

# APPLICATION NOTES

## Glyphosate: Herbicide

### ANP Retention of an Extremely Polar Compound




**Method Conditions**

**Column:** Cogent Diamond Hydride™ 4µm, 100Å.
**Catalog No.:** 70000-15P-2
**Dimensions:** 2.1 x150 mm
**Solvents:** A: DI water + 5 mM ammonium acetate
B: 90% acetonitrile/10% DI water/10 mM ammonium acetate
**Gradient:** 0.0 min – 80.0% B 5.0 min 5%B
1 .0 min 80%B 6.0 min to 80%B
1.1 min to 5%B
**Post Tme:** 5 min
**Flow Rate:** 0.5 mL/min.
**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 (1000 mcg/mL). Sample for analysis was made by diluting the stock 1:100 in 30:70 solution A and B.
**Detection:** ESI – neg - Agilent 6210 MSD TOF mass spectrometer.

**Discussion**

A reliable method for the determination of glyphosate is presented. Analysis was performed using a Cogent Diamond Hydride™ HPLC column which provides very reproducible retention and fast equilibration even when a gradient analysis is used. The use of LC-MS detection allows avoiding time consuming derivatization of this compound which is lacking a chromophore for UV detection. The method shown, with ANP retention avoids derivatization, which is required when an ordinary C18 column is used.

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**www.MTC-USA.com or**
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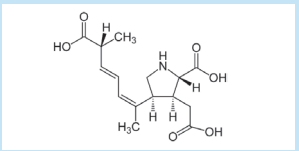
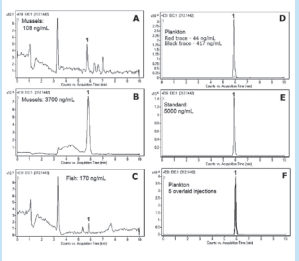
## Domoic Acid in Seafood Samples

### Domoic Acid (DA) from Seafood without Derivatization




A – Extract from mussels (low concentration)
B – Extract from mussels (high concentration)
C – Fish extract
D – Plankton high and low concentration of domoic acid
E – Standard of domoic acid
F – 5 Overlaid injections of plankton extract sample

**Method Conditions**

**Column:** Cogent Diamond Hydride™, 4µm, 100A
**Catalog No.:** 70000-15P-2
**Dimensions:** 2.1 x 150 mm
**Solvents:** A: 50% MeOH/ 50% DI $H_2O$/ 0.1% formic acid
B: Acetonitrile/ 0.1% formic acid
**Gradient:**

| time (min.) | %B | time (min.) | %B |
|---|---|---|---|
| 0 | 95 | 8 | 30 |
| 7 | 30 | 10 | 95 |

**Post Time:** 5 min
**Injection Vol.:** 1 microL
**Flow Rate:** 0.4 mL/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 312.1442 m/z $(M + H)^+$
**$t_0$:** 0.9 min

**Discussion**

Several seafood and plankton samples were tested for domoic acid (DA) which, as a highly polar compound, is poorly retained on most reversed phase columns. This developed method based on HPLC and MS detection does not require derivatization of DA. The concentration of this potent toxin varied quite substantially between the samples (from 40 ng/mL in plankton to 3700 ng/mL in mussels). Despite different sample matrices, it was possible to detect and analyze domoic acid in a wide range of concentrations.

The results were very repeatable (see 5 overlaid injections in Figure F). The detection limit depends on the instrument used. The method can be used by regulatory agencies responsible for monitoring the occurrence of toxins such as the analysis of domoic acid in seafood samples.

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**www.MTC-USA.com or**
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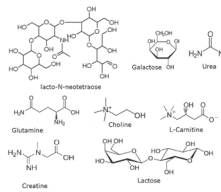
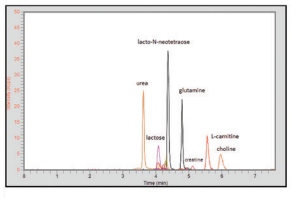
## Milk Extract

### Analysis of Composition using LC-MS




**Method Conditions**

**Column:** Cogent Diamond Hydride™, 4µm, 100A
**Catalog No.:** 70000-15P-2
**Dimensions:** 2.1 x 150 mm
**Mobile Phase:** A: DI $H_2O$ / 0.1% formic acid (v/v)
B: Acetonitrile / 0.1% formic acid (v/v)
**Gradient:**

| time (min.) | %B |
|---|---|
| 0 | 90 |
| 1 | 90 |
| 7 | 20 |
| 11 | 20 |
| 12 | 90 |

**Post Time:** 3 min
**Injection Vol.:** 1 µL
**Flow Rate:** 0.4 mL/min
**Detection:** ESI – Pos – Perkin Elmer, Flexar SQ 300 mass spectrometer
**Sample:** Milk extract reconstituted in 65 micoL of 80% acetonitrile/ 20% DI water.
**$t_0$:** 0.9 min

**Discussion**

Milk components are polar compounds that are not well retained or resolved by traditional reversed-phase chromatography. In addition, many of the compounds do not contain strong chromophores, resulting in low specificity and sensitivity in UV detection. The analysis using the Cogent Diamond Hydride™ column and MS detection provides separation and detection of all the compounds of interest. This column is an excellent choice for analysis of polar compounds in biological matrices.

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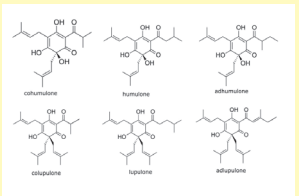
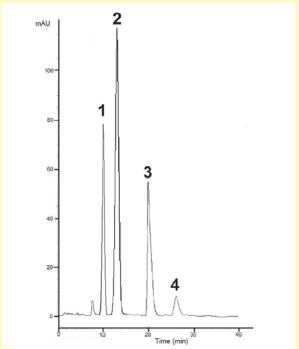
## Analysis of Alpha & Beta Acids in Hops

### Robust, Precise Method for Beer Samples




**Method Conditions**

**Column:** Cogent Bidentate C18™, 4µm, 100A
**Catalog No.:** 40018-75P
**Dimensions:** 4.6 x 75 mm
**Mobile Phase:** 80% A: DI $H_2O$/ 0.1% formic acid
20% B: Methanol
**Injection Vol.:** 5 microL
**Flow Rate:** 0.5 mL/min
**Detection:** UV 326 nm
**Sample:** International Calibration Extract 3 (ICE-3), containing 44.65% alpha acids (cohumulone - 13.88%, humulone/adhumulone - 30.76%) and 24.26% beta acids (colupulone - 13.44%, lupulone/adlupulone 10.84%), was obtained from the American Society of Brewing Chemists. The sample was prepared by dissolving 0.1482g of ICE-3 standard in 50.00 mL of the mobile phase. The solution was then filtered with a 0.45 microm nylon syringe filter (MicroSolv Tech. Corp). Samples for calibration curves were prepared and diluted 1:20. Final concentrations were 0.005–0.05 mg/mL.
**Peaks:** 1. cohumulone 2. humulone/adhumulone
3. colupulone 4. lupulone/adlupulone
**$t_0$:** 1.5 min

**Discussion**

The effectiveness of the presented method is evident from the fact that data obtained were in a good agreement with the literature values. This method also has the added bonus of precision (%RSD = 0.1 and below), which is typical for Cogent Type-C Silica™ columns.

The constructed calibration curves for alpha and beta acids in beer showed good linearity ($R^2$ = 0.9999).

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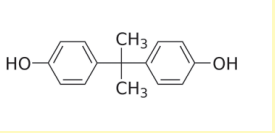
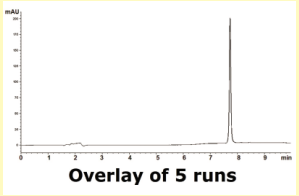
# APPLICATION NOTES

## Fast & Precise Bisphenol A (BPA) Method

### Toxic Substance Found in Consumer Products






#### Method Conditions

**Column:** Cogent Bidentate C8™, 4µm, 100Å
**Catalog No.:** 40008-75P
**Dimensions:** 4.6 x 75 mm
**Mobile phase:** A: DI $H_2O$/ 0.1% formic acid
B: Acetonitrile/ 0.1% formic acid
**Gradient:**

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

**Flow rate:** 0.5 mL/min
**Peaks:** Bisphenol A
**Injection Volume:** 5 µL
**Detection:** UV 275 nm
**$t_0$:** 0.9 min

#### Discussion

Bisphenol A (BPA) is a challenging compound for analysis by HPLC. Biological monitoring of this environmental and health toxicant is a necessary process for surveillance as well as risk assessment. As can be seen from the accompanying chromatograms, a Cogent Bidentate C8™ column was an excellent choice for the analysis of BPA. The peak shape is symmetrical with high efficiency.

The repeatability of the analysis is also remarkable as can be seen in the figure, which shows five overlaid injections of the compound. In addition, the method equilibrates rapidly with only 1 minute post time after the gradient.

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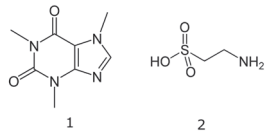
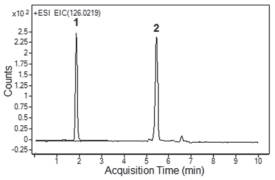
## Red Bull® Energy Drink

### LC-MS Separation of Taurine & Caffeine




#### Method Conditions

**Column:** Cogent Diamond Hydride™, 4µm, 100A
**Catalog No.:** 70000-15P-2
**Dimensions:** 2.1 x 150 mm
**Solvents:** A: DI $H_2O$ / 0.1% formic acid (v/v)
B: Acetonitrile/ 0.1% formic acid (v/v)
**Gradient:**

| time (min.) | %B | time (min.) | %B |
|---|---|---|---|
| 0 | 95 | 7 | 30 |
| 1 | 95 | 8 | 95 |
| 6 | 30 | | |

**Temperature:** 25 °C
**Post Time:** 3 min
**Injection Vol.:** 1 microL
**Flow Rate:** 0.4 mL/min
**Detection:** ESI – POS - Agilent 6210 MSD TOF mass spectrometer.
**Peaks:** 1. Caffeine 195.0877 m/z $[M+H]^+$
2. Taurine 126.0219 m/z $[M+H]^+$
**$t_0$:** 0.9 min

#### Discussion

A method combining solid phase extraction with high performance liquid chromatography–electrospray ionization tandem mass spectrometry was developed for the highly sensitive and accurate screening of caffeine and taurine in energy drinks. The matrix pretreatment by SPE sample extraction was essential in the analysis of taurine. The matrix did not interfere with the analysis of caffeine (data not shown). This method has been successfully applied to screening of caffeine and taurine in commercial Red Bull™ samples, and it is valuable to ensure the safety of food and beverages.

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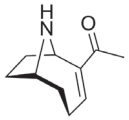
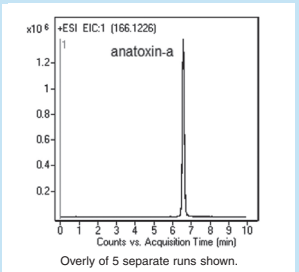
## Anatoxin-a (ANTX-A)

### Potent Neurotoxin





Overly of 5 separate runs shown.

#### Method Conditions

**Column:** Cogent Diamond Hydride™, 4µm, 100A
**Catalog No.:** 70000-15P-2
**Dimensions:** 2.1 x 150 mm
**Solvents:** A: 50% MeOH/ 50% DI $H_2O$/ 0.1% formic acid
B: Acetonitrile/ 0.1% formic acid
**Gradient:**

| time (min.) | %B | time (min.) | %B |
|---|---|---|---|
| 0 | 70 | 6 | 30 |
| 5 | 30 | 7 | 70 |

**Post Time:** 5 min
**Injection Vol.:** 1 microL
**Flow Rate:** 0.4 mL/min
**Temperature:** 25 °C
**Detection:** ESI – POS - Agilent 6210 MSD TOF MS
**Sample:** **Stock standard solution:** (0.5 mg/mL) of Anatoxin-a fumarate was prepared in DI water and stored at -20 °C.
**Working solution:** Sample for injection was diluted 1:100 using 50%A/50%B solvent mixture. It was stored in the dark at 4 °C.
**Peak:** Anatoxin-a, 166.1226 m/z (M + H)$^+$
**$t_0$:** 0.9 min

#### Discussion

Anatoxin-a is a low molecular weight, secondary amine and can be difficult to retain with reversed-phase columns. This application note illustrates a very simple method for retention of Anatoxin-a which does not require derivatization. The retention achieved is good, and the excellent repeatability is demonstrated by the overlay of five injections shown in the figure. If desired, a lower retention time of Anatoxin-a can be obtained by changing the starting concentration of solvent B in the gradient to 60%, designing a steeper gradient, or using a shorter column (e.g. 2.1 x 50 mm).

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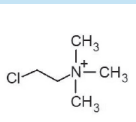
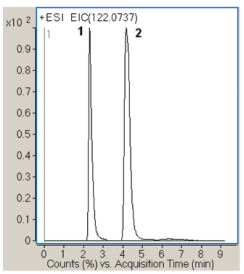
## Chlormequat & Mepiquat

### Plant Growth Regulators


1. Chlormequat


2. Mepiquat




#### Method Conditions

**Column:** Cogent Diamond Hydride™ 4µm, 100Å.
**Catalog No.:** 70000-15P-2
**Dimensions:** 2.1x150mm
**Solvents:** A: DI water + 20 mM ammonium acetate, pH adjusted to pH 3.3 with formic acid
B: Acetonitrile
**Mobile Phase:** 70%A
**Post Tme:** 5 min
**Flow Rate:** 0.5 mL/min.
**Sample Peaks:** 1. Chlormequat (CQ) 122.0737 m/z (M)+
2. Mepiquat (MQ) 114.1277 m/z (M)+
Sample stock solutions were purchased from Sigma. Samples for injection were diluted 1:1000 in the mobile phase.
**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)+.

#### Discussion

Analysis of CQ and MQ can be challenging since they are extremely hydrophilic and are only weakly retained in reversed-phase HPLC which makes their separation impossible. In addition these compounds are retained too strongly in normal phase (non polar solvents) chromatography Due to this situation the preferred method of analysis for these compounds was ion-pair chromatography using high ionic strength (> 150 mM) ion-pair reagents. Buffers used are at very high concentration which makes coupling with MS very difficult if not impossible (source contamination). 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 instrument and adequate product ions (CQ 122 m/z to 58 m/z and 63 m/z, MQ 114m/z to 98 m/z and 58 m/z). This method has high repeatability (precision) and reliability of the results.

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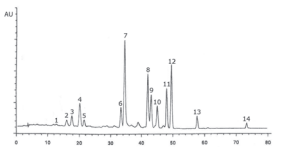
# APPLICATION NOTES

## Separation of Carotenoids

### Use of Shape Selectivity to Resolve Similar Compounds



**Peaks:**
1. Astaxanthin
2. Capsanthin
3. Lutein
4. Zeaxanthin
5. Canthaxanthin
6. β-Cryptoxanthin
7. Echinenone
8. 15-cis β-Carotene
9. 13-cis β-Carotene
10. α-Carotene
11. trans β-Carotene
12. 9-cis β-Carotene
13. δ-Carotene
14. Lycopene

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#### Method Conditions

**Column:** Cogent C30™, 5µm, 200Å
**Catalog No.:** 71030-25P
**Dimensions:** 4.6 x 250 mm
**Mobile Phase:** A: 81/15/4 Methanol / MTBE / DI $H_2O$ (v/v)
B: 6/90/4 Methanol / MTBE / DI $H_2O$ (v/v)

**Gradient:**

| time (min.) | %B |
|---|---|
| 0 | 0 |
| 90 | 100 |

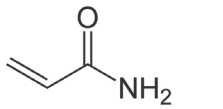
**Temperature:** 20 °C
**Flow Rate:** 1.0 mL/min
**Detection:** UV 450 nm
**Sample:** Reference standards of each analyte in a mixture.

#### Discussion

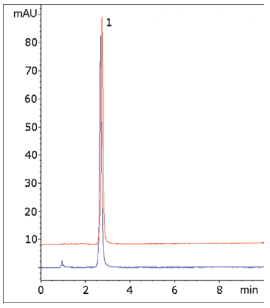
Compounds of the carotenoid family are very lipophilic and often have subtle differences in structure. Resolution of these compounds can be difficult with a typical C8 or C18 column because of the similarities. The Cogent C30™ stationary phase on the other hand can further differentiate by analyte shape in addition to reversed phase interactions. At lower temperatures, the long alkyl chains become more rigid and steric effects become significant, leading to greater selectivity.

## Acrylamide

### Easy & Precise Retention



Acrylamide



Overlay of runs from 2 column lots

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#### Method Conditions

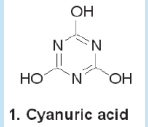
**Column:** Cogent Diamond Hydride™, 4µm, 100A
**Catalog No.:** 70000-7.5P
**Dimensions:** 4.6 x 75 mm
**Mobile Phase:** Acetonitrile/ 0.1% formic acid
**Flow Rate:** 1.0 mL/min
**injection Vol.:** 1 µL
**Sample:** 100 mg/L acrylamide in mobile phase diluent.
**Peak:** 1. Acrylamide
**Detection:** 205 nm
**$t_0$:** 1.0 min

#### Discussion

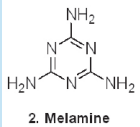
Acrylamide is difficult to retain with conventional reversed phase methods due to its polar nature. With the Diamond Hydride™ column however, retention is readily achievable using a simple isocratic mobile phase. The overlay in the figure illustrates lot-to-lot reproducibility of the stationary phase material. Injections using columns of two different lots of material are shown in the figure.

## Cyanuric Acid and Melamine

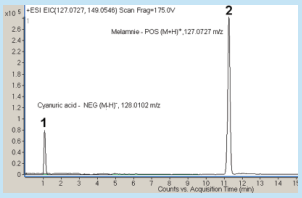
### Simple ANP Method



1. Cyanuric acid



2. Melamine



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#### Method Conditions

**Column:** Cogent Diamond Hydride™ 4µm, 100Å.
**Catalog No.:** 70000-15P-2
**Dimensions:** 2.1 x150 mm
**Solvents:** A: DI water + 0.1% acetic acid
B: Acetonitrile + 0.1% acetic acid

**Gradient:**

| Time | %B |
|---|---|
| 0.0 | 100 |
| 15.00 | 50 |

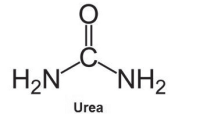
**Post Tme:** 5 min
**Flow Rate:** 0.4 mL/min. t0 = 0.8 min
**Sample Prep:** 1. Cyanuric acid 1.5 microg/mL: NEG (M-H)- 128.0102 m/z analyzed in Neg mode, RT = 1.278 min
2. Melamine 3 microg/mL: POS (M+H)+ 127.0727 m/z analyzed in Pos mode, RT = 11.260 min
**Detection:** ESI – NEG for cyanuric acid and ESI – POS for melamine: - Agilent 6210 MSD TOF mass spectrometer.
Note: When an instrument with a dual ion source is used both samples can be analyzed simultaneously.

#### Discussion

The benefits of using the method in this application note include: 1. Short equilibration time between gradient runs 2. Excellent repeteability of the results. 3. Use of LC-MS detection eliminates the need for derivatization (as is needed with a GC-MS method), 4. Higher flow rates without compromising the efficiency (higher sample throughput possible) 5. Columns used in this method have very long life and are good for over 2000 injections of real life samples. When analyzing melamine alone, a simple isocratic method can be used. When using the Cogent Diamond Hydrdide™ column melamine is retained at 80% B mobile phase. The resulting peak shape is very symmetrical.

## Analysis of Urea

### Fast, Reproducible HPLC Method



Urea

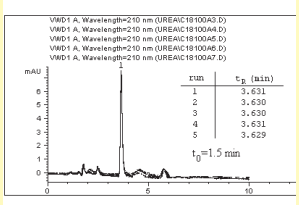


Figure above show 5 consecutive runs using 100% water mobile phase without any loss of precision.

**For more information**
**www.MTC-USA.com or**
**technical@hichrom.co.uk**

#### Method Conditions

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

#### Discussion

A Cogent Bidentate C18™ column was successfully used to retain Urea when a 100% DI water mobile phase was used. Since this hydrophobic column was made on TYPE-C Silica™ DI water can be used as the complete mobile phase without fear of phase "dewetting" and subsequent loss of retention time. The presented method has the advantage of being very simple, without time consuming derivatization or reaction steps when compared to other methods using ordinary C18 columns. Urea can be determined in complex mixtures using this very fast, reproducible (see insert on the chromatogram) method which could also be useful in the analysis of biological samples for diagnostic purposes.

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MICRO SOLV™

Cogent™ HPLC Columns

## Cogent™ TYPE-C™ silica LC phases

Cogent™ TYPE-C™ silica LC phases have the ability to retain polar solutes at high concentrations of organic solvent by aqueous normal-phase (ANP) and non-polar compounds under reversed-phase (RP) conditions. These revolutionary columns use patented bonding technology to create a surface populated by silicon-hydride functional groups instead of silanols. The lack of surface silanols leads to fast equilibration times, excellent peak shape and extended column lifetimes for a wide range of analytes. These application notes demonstrate the unique abilities of Cogent TYPE-C silica LC columns for a range of clinical analysis applications. Further application notes are available at www.MTC-USA.com or from Hichrom Limited at technical@hichrom.co.uk


Cogent TYPE-C columns can be operated in 3 modes of chromatography: reversed-phase (RP), normal-phase (NP) and aqueous normal-phase. The surface silanols that are present in all Type A and B silicas, even after bonding and extensive endcapping, form a strong association with water resulting in a 'hydration shell' surrounding the silica. However, the silica hydride particles of TYPE-C silica are only slightly hydrophobic and therefore have a weak attraction for water allowing them to be used in aqueous normal-phase (ANP) mode, which unlike HILIC, does not require a 'water-rich' environment in order to operate.

## Aqueous Normal Phase (ANP) and Reversed-Phase (RP) Separations

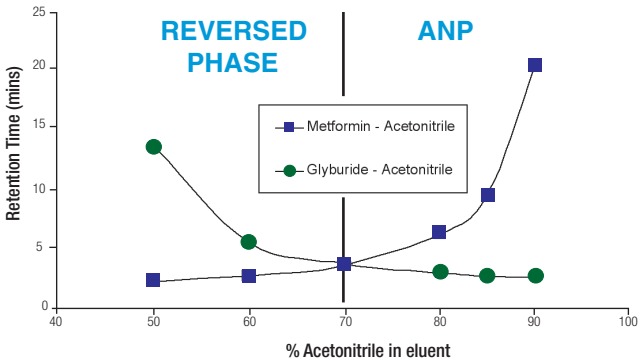


Figure 1. Dual RP and ANP retention capability

Cogent TYPE-C silica based phases (Bidentate C18, Bidentate C8, UDC-Cholesterol, Diamond Hydride, Phenyl Hydride, UDA, Diol and Silica-C) have the ability to operate in ANP mode which enables the retention of polar solutes at high concentrations of the organic component whilst maintaining an aqueous component in the eluent. The exact point in the composition of the eluent where ANP retention begins depends on the solute as well as the stationary phase. In addition, TYPE-C columns can also retain non-polar compounds based on a typical reversed-phase mechanism. Figure 1 illustrates the dual retention capability for both polar (metformin) and non-polar (glyburide) compounds. In this case, with an eluent composition of less than 70% acetonitrile, glyburide and metformin are both retained by a reversed-phase mechanism, with the metformin eluting first. With increasing percentages of acetonitrile, the retention of metformin increases significantly due to ANP mechanisms and now elutes after glyburide.

**For further technical advice and additional application notes on Cogent TYPE-C Silica LC columns, contact MicroSolv Technologies, USA, www.MTC-USA.com or global distributor Hichrom Limited, UK www.hichrom.co.uk, technical@hichrom.co.uk**