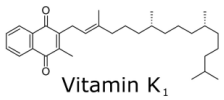
Cogent™ HPLC Columns

CLINICAL APPLICATIONS

APPLICATION NOTES

Vitamin K Isomers by Reversed Phase

Phytonadione separation by shape selectivity



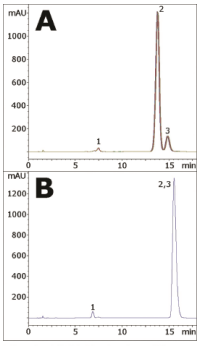


Fig. A: UDC Cholesterol™ column, 5-run overlay
Fig. B: ordinary C8 column

Method Conditions

Column:	Cogent UDC Cholesterol™ 4µm, 100A
Catalog No.:	69069-15P
Dimensions:	4.6 x 150 mm
Solvents:	A: 50% DI H_2O/ 50% MeOH/ 0.1% formic acid B: 97% Acetonitrile/ 3% DI H_2O /0.1% formic acid

Gradient:

time (min.)	%B	time (min.)	%B
0	80	16	80
15	92		

Post Time:	2 min
Flow rate:	1.5 mL/min
Detection:	UV 254 nm
Temperature:	12 °C
Sample:	Stock Solution: 10 µL/mL Phytonadione in acetonitrile diluent. (The solution was vortexed for 10 minutes.) Working Solution: Stock solution was diluted 1:10 with acetonitrile.
Peak:	1. Impurity 2. Phytonadione (E isomer) 3. Phytonadione (Z isomer)
t_0:	1.0 min

Discussion

Using the USP methodology, assay of Vitamin K, (phytonadione) is done in organic normal phase (ONP) with a bare silica column. This can be inconvenient for many laboratories which may not have a dedicated ONP instrument and therefore must spend both time and solvents to convert their reverse phase system to ONP for the analysis. In reverse phase, adequate separation between the E and Z isomers of phytonadione may not be obtained using conventional alkyl chain-based stationary phases (as Figure B illustrates). However, a resolution of 1.5 was obtained between isomers using the UDC Cholesterol™ column (shown in Figure A), which meets the USP requirement for resolution. The UDC Cholesterol™ is able to separate the two isomers on the basis of shape selectivity.

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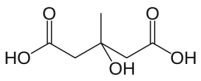
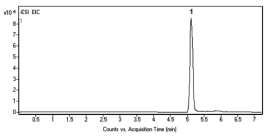
3-Hydroxy-3-Methlglutaric acid (HMG) in Urine

Important biomarker for diabetes and HMG disorder



Meglutol



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 / 10 mM ammonium formate B: 95% acetonitrile/5% DI water/10 mM ammonium formate (v/v)

Gradient:

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

Post Time:	3 min
Injection Vol.:	1 microL
Flow Rate:	0.4 mL/min
Detection:	ESI – NEG - Agilent 6210 MSD TOF mass spectrometer.
Peak:	1. 3-hydroxy-3-methylglutaric acid 161.0455 *m/z* $[M-H]^-$ in urine sample.
t_0:	0.9 min

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.

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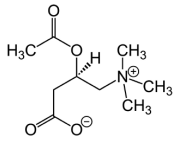
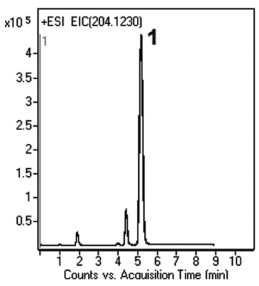
Acetyl-L-Carnitine (ALC) in Plasma

Excellent LC-MS method in spiked plasma sample





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 B: Acetonitrile/ 0.1% formic acid

Gradient:

time (min.)	%B	time (min.)	%B
0	80	7	30
1	80	8	80
5	30		

Post Time:	3 min
Injection Vol.:	1 microL
Flow Rate:	0.4 mL/min
Detection:	ESI – POS - Agilent 6210 MSD TOF mass spectrometer
Sample:	Plasma from healthy individuals was spiked with an ALC standard solution and prepared for injections as described by Tallarico et al. [1]. To prepare standard curves dialysed plasma was used, to which known amounts of the analyte were added.
Peak:	1. Acetyl-L-carnitine: 204.1230 m/z $[M+H]^+$, 3 overlaid injections.
t_0:	0.9 min

Discussion

The method presented in this application note was designed to be suitable for the routine analysis of plasma samples obtained from animal and human pharmacokinetics studies in which ALC is administered. The calibration curve prepared in plasma samples showed good linearity (R^2 = 0.999). The precision of the method was demonstrated by low %RSD (0.2 and below). The advantages over other published LC-MS methods are the short equilibration time between runs for gradient runs and excellent repeatability. Also, the method uses high organic content in the mobile phase, which is more suitable for MS.

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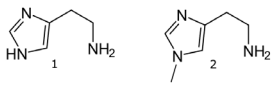
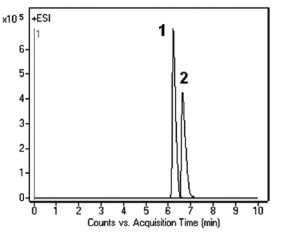
Histamine and Methylhistamine

No Derivatization Required



Histamine Methylhistamine



Method Conditions

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

Gradient:

time (min.)	%B
0	70
2	65
6	10
8	10
9	70

Post Time:	2 min
injection Vol.:	1 microL
Flow Rate:	0.4 mL/min
Detection:	ESI – POS - Agilent 6210 MSD TOF mass spectrometer
Peaks:	1. Histamine 112.0869 m/z $[M+H]^+$ 2. Methylhistamine 126.1026 m/z $[M+H]^+$
t_0:	0.9 min

Discussion

Various assay methods for histamine (HA) and/or its metabolite (MHA) in biological samples have been developed. However, most of them require postcolumn (for detection purposes) or precolumn (to achieve retention) derivatization. The method presented here provides separation of these two compounds yet doesn't require derivatization. The method used in this application note was able to solve the inherently difficult problem of analysis of two biogenic amines with close physicochemical properties.

A successful validation of the assay was indicated by the high linearity of calibration curves and the low inter- and intraday variation coefficients.

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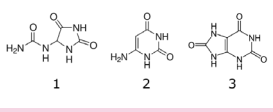
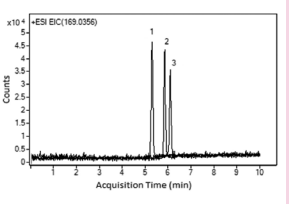
APPLICATION NOTES

Uric acid and Metabolites

LC-MS Method for Allantoin, 6-Aminouracil, and Uric Acid (UA)





Peaks:
1. Allantoin 159.0513 m/z $[M+H]^+$
2. 6-aminouracil 128.0455 m/z $[M+H]^+$
3. Uric acid 169.0356 m/z $[M+H]^+$

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Method Conditions

Column: Cogent Diol™, 4µm, 100A
Catalog No.: 40060-15P-3
Dimensions: 3.0 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	time (min.)	%B
0	95	7	30
6	30	8	95

Flow Rate: 0.4 mL/min
Injection Vol.: 1 µL
Sample: Standards of uric acid and its main metabolites were prepared in DI water at concentrations of 20 microg/mL each. The sample for injection (a mixture of the three compounds) was diluted by a factor of 3.
Detection: ESI – Pos - Agilent 6210 MSD TOF mass spectrometer.
t_0: 0.9 min

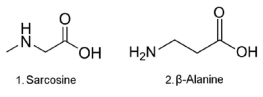
Discussion

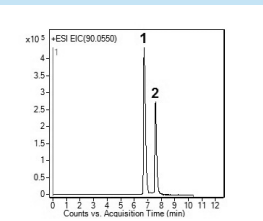
The presented method for analysis of UA and its highly polar metabolites is simple and doesn't require mobile phase additives or pre-column derivatization. The peaks are symmetrical and the MS signal is not diminished, as is the case when ammonium formate or acetate is used as a mobile phase additive. The analyzed metabolites are signature end products for UA degradation in the presence of oxidants and can therefore be used as biomarkers for different disease states.

Cogent™ columns can have very fast equilibration between gradient runs and can be successfully used in studies of pathways in human pathology.

Sarcosine

Separation of potential urine biomarker from isobaric β-alanine





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Method Conditions

Column: Cogent Diamond Hydride™ 4µm, 100Å.
Catalog No.: 70000-15P-2
Dimensions: 2.1 x 150 mm
Solvents: A: 50% isopropyl alcohol/ 50% DI water/ 0.1% acetic acid
B: 97% acetonitrile/3% DI water/ 0.1% acetic acid

Gradient:

time (min.)	%B	time (min.)	%B
0	75	5	65
3	75	10	20
4	65	12	75

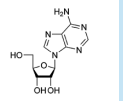
Post Tme: 5 min
Injection Vol.: 1 microL
Flow Rate: 0.6 mL/min
Temperature: 50 °C
Sample: 10 mg/L ea. of sarcosine and beta-alanine in 50:50 A:B.
Detection: ESI – POS - Agilent 6210 MSD TOF mass spectrometer

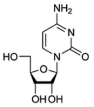
Discussion

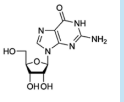
This developed LC-MS method can separate sarcosine from beta-alanine in serum and urine samples without using labor-intensive sample derivatization. Since sarcosine is considered a potential biomarker for prostate cancer risk and aggressiveness, it is essential to resolve and accurately quantify this compound in the presence of isobaric (same m/z) beta-alanine. This objective is achieved using a Cogent Diamond Hydride™ column and a simple gradient method presented in this application note. The developed method is sensitive, specific, quantitative, and reproducible (%RSD = 0.1). It can be used in large scale studies with numerous samples (high throughput of the method due to simple sample preparation).

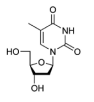
Separation of Nucleobases by HPLC

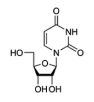
Easy, Efficient and Precise

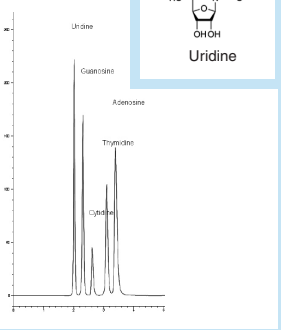

Adenosine


Cytidine


Guanosine


Thymidine


Uridine



Method Conditions

Column: Cogent Diamond Hydride™ 4µm, 100Å.
Catalog No.: 70000-7.5P
Dimensions: 4.6 x 75 mm
Mobile phase: DI Water + 0.1% Acetic Acid
Temperature: 30° C
Flow rate: 1.0 mL/min.
Injection Volume: 5ul
Detection: UV: 254nm

Discussion

This is an easy to use isocratic method for the separation of nucleosides as shown. The major advantage of this method is that even under 100% aqueous conditions, with good selectivity, there is no loss of retention with repeated runs as experienced with C18 columns. Highly efficient, these columns can be used in reverse phase as shown or in aqueous normal phase using high organic (ACN) composition with water.

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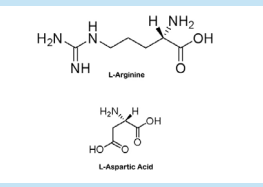
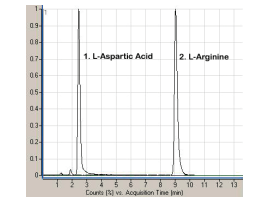
Easy Amino Acid Method

Reduced Sample Prep and Handling





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L – aspartic acid (Asp) and L - arginine (Arg) in synthetic urine

Method Conditions

Column: Cogent Diamond Hydride™ 4µm, 100Å.
Catalog No.: 70000-15P-2
Dimensions: 2.1 x150 mm
Solvents: A: DI water + 0.1% formic acid
B: Acetonitrile + 0.1% formic acid + 0.005% TFA
Mobile Phase: Isocratic: 90% B / 10% A
Flow rate: 400µL/min.
Sample: 1mg/mL of each:
1. L – aspartic acid and
2. L - arginine dissolved in 50% acetonitrile/ DI water/0.1 % TFA.
Sample for injection: Stock solution diluted 1:100 with the mobile phase.
Peaks (Compounds)**:** 1. L–aspartic acid, 134 m/z, RT = 2.45 min
2. L–arginine, 175 m/z, RT = 9.05 min
Detection: ESI – pos - Agilent 6210 MSD TOF mass spectrometer.

Discussion

Two amino acids are adequately retained and can be easily quantified in this method with very little sample prep. There is no need for any derivatizing with this method because of the combination of the mass spectrometric detection and the Aqueous Normal Phase (ANP) method. Also, this method produces reliable and reproducible results. The column lifetime with this method is exceptionally good..

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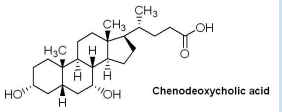
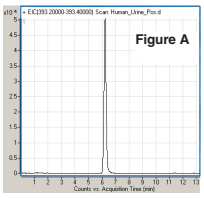
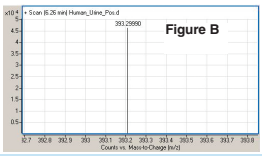
APPLICATION NOTES

Bile Acids from Urine







Determination of Chenodeoxycholic acid (CDCA) (bile acid) in human urine using ANP (inverse gradient)

Method Conditions

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

Time	%B	Time	%B
0.0	95	10.0	50
0.2	95	11.0	50
7.0	85	11.1	95
8.0	85	14.0	95

t_0 = 1.44 min

Flow rate: 0.4 mL/min.
Sample: Human urine – after simple extraction
Analyte: Chenodeoxycholic acid 393.29994 m/z $(M+H)^+$, RT = 6.26 min
Detection: ESI – pos- Agilent 6210 MSD TOF mass spectrometer.
Figure B: EIC – extracted ion chromatogram of selected compound and corresponding spectrum

Discussion

The presence of an important bile acid (chenodeoxycholic acid-CDCA) in human urine was detected using a simple mobile phase, a Cogent Diamond Hydride HPLC column and an Agilent TOF MS instrument. The column is an excellent choice for LC-MS analysis due to its very low carbon content (~2%) background spectrum that is extremely low. In addition the special surface of the column helps to provide a fast equilibration while using a gradient.

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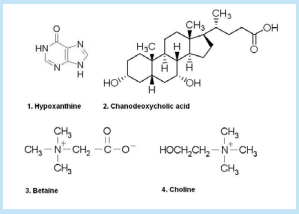
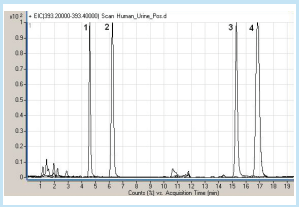
Metabolites in Human Urine





Simple LCMS friendly method that is reproducible using (ANP) Aqueous Normal Phase LCMS.

Method Conditions

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

Time	%B	Time	%B
0.0	95	35.0	50
0.2	95	35.1	95
30.0	50	40.0	95

Flow rate: 0.4 mL/min.
Peaks: 1. Hypoxanthine;
137.04580 m/z $(M+H)^+$, RT = 4.98 min
2. Chenodeoxycholic acid;
393.29990 m/z $(M+H)^+$, RT = 6.23 min
3. Betaine;
118.08680 m/z $(M+H)^+$, RT = 15.27 min
4. Choline;
104.10754 m/z $(M+H)^+$, RT = 16.82 min

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

Sample: Human Urine – after simple extraction
Detection: ESI – pos - Agilent 6210 MSD TOF mass spectrometer.

Discussion

This method can be used for routine assays of urinary purines (hypoxanthine), bile acids (chenodeoxycholic acid) and nutrients (betaine, choline) in biological fluids. The method is very sensitive (due to the high content of organic component (acetonitrile) in the MP and the use of "MS friendly" (formic acid), reproducible (% RSD for gradient analysis is below 0.5%) and accurate (MW to 3-4 decimal points).

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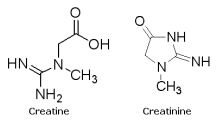
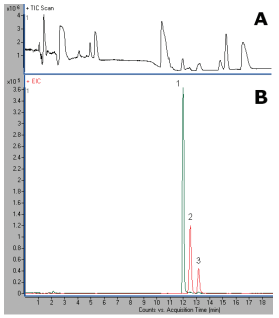
Metabolites in Urine





A- TIC – Total ion chromatogram of synthetic urine sample
B- EIC – Extracted ion chromatogram of selected compounds (1,2,3)

Isobaric compounds (creatine and 4-hydroxyproline) separated from other components of synthetic urine.

Method Conditions

Column: Cogent Diamond Hydride™ 4µm, 100Å.
Catalog No.: 70000-7.5P
Dimensions: 4.6mm x 75mm
Solvents: A: DI water + 0.1% formic acid
B: Acetonitrile + 0.1% formic acid
Mobile phase: Gradient

Time	%B	Time	%B
0.0	95	35.0	50
0.2	95	35.1	95
30.0	50	40.0	95

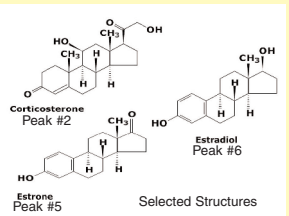
Flow rate: 0.4 mL/min.
Sample: Synthetic urine
Compounds: 1. Creatinine - Cm
M+H 114.0662, RT = 11.98 min
2. Creatine - Cr
M+H 132.0768, RT = 12.52 min
3. 4-Hydroxyproline
M+H 132.0655, RT = 13.16 min
Detection: ESI – pos - Agilent 6210 MSD TOF mass spectrometer.

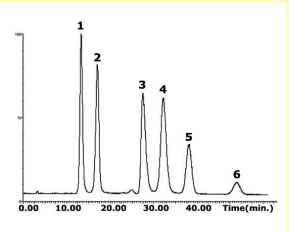
Discussion

Creatine and Creatinine are typically very difficult to separate from each other. A simple gradient with an MS friendly mobile phase was used to resolve these two compounds. In addition 4-hydroxyproline which has a very similar mass weight as creatine was also easily separated. A powerful combination of an Agilent MSD-TOF instrument (4 digit mass accuracy) and a Cogent Diamond Hydride™ LCMS column takes away guessing from the analysis of metabolites in a complex mixture as shown.

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

Using LC-MS to Measure Steroids in Clincal Studies





Method Conditions

Column: Cogent UDC Cholesterol™, 4µm, 100Å. Reverse Phase Mode.
Catalog No.: 69069-7.5P
Dimensions: 4.6 x 75 mm
Mobile phase: 50:50 MeOH/DI water + 0.5% formic acid
Flow rate: 0.5 mL/minute
Injection Volume: 5 µL
Peaks:

Solute	Parent ion m/z	Other peaks
1. Andrenosterone		
	301	283 [MH^+- H_2O]
	333	[MH^+ + O_2]
2. Corticosterone	347	329 [MH^+ - H_2O]
	311	[MH^+ - 2H_2O]
	379	[[MH^+ + O_2]
3. 4-androstene-3,17-dione	287	319 [[MH^+ + O_2]
4. 11-alpha-acetoxyprogesterone	373	313 [MH^+ - H_3CHOOH]
5. Estrone	271	253 [MH^+- H_2O]
6. Estradiol	273	254 [MH^+- H_2O]

Concentraion: 1 mg of each in methanol + DI water
Detection: APCI, Single Ion monitoring.

Discussion

Serum corticosterone concentrations are of clinical significance in adrenal dysfunction. Its measurement is sometimes used to diagnose apparent mineralcorticoid excess syndrome. It can also be used as a bio marker of malignancy in adrenal tumors.

Using the Cogent UDC Cholesterol column allows the simultaneous measurement of the two main estrogen fractions, estrone and estradiol in breast tumor tissue. Highly sensitive assays for estrone and estradiol for measuring low levels of estrogen in postmenopausal women, and monitoring estrogen levels in women receiving hormone replacement therapy can also be developed using the method presented in this note.

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

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

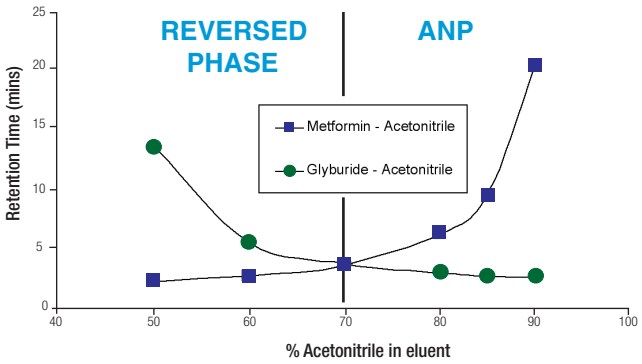


Figure 1. Dual RP and ANP retention capability

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