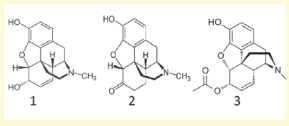
Cogent™ HPLC Columns

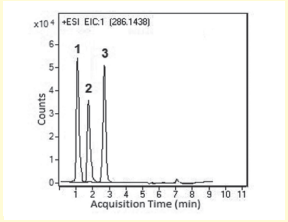
PHARMACEUTICAL APPLICATIONS

APPLICATION NOTES

Morphine, Hydromorphone and 6-MAM

LC-MS Analysis in Plasma Samples





Method Conditions

Column: Cogent Bidentate C18 2.ō™, 2.2µm, 120A
Catalog No.: 40218-05P-2
Dimensions: 2.1 x 50 mm
Mobile Phase: A: DI H_2O / 0.1% formic acid (v/v)
B: 50% Acetonitrile / 50% Methanol / 0.1% formic acid (v/v)
Gradient:

time (min.)	%B
0	5
4	50
5	90
6	90
7	5

Post Time: 3 min
Flow Rate: 0.4 mL/min
Injection Vol.: 1 µL
Peaks: 1. Morphine 286.1438 m/z $[M+H]^+$
2. Hydromorphone 286.1438 m/z $[M+H]^+$
3. 6-Monoacetylmorphine (6-MAM) 328.1543 m/z $[M+H]^+$
Detection: ESI – POS - Agilent 6210 MSD TOF mass spectrometer.

Discussion

The Cogent Bidentate C18 2.ō™ column was successfully used in the analysis of an important class of drugs in plasma samples. The presented procedure after validation can be used as a routine analysis of plasma samples (or whole blood samples – after changing the extraction procedure) for the presence of morphine, hydromorphone, or 6-MAM (indicator of heroin use).

APP A-310
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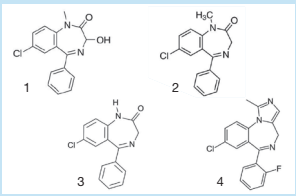
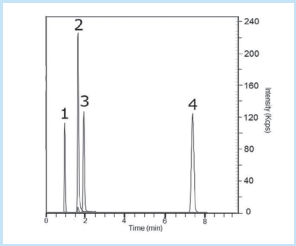
1,4-Benzodiazepines in Urine

LC-MS with SPE





Peaks:
1. temazepam 301.0739 m/z $[M+H]^+$
2. diazepam 285.0790 $[M+H]^+$
3. nordiazepam 271.0633 $[M+H]^+$
4. midazolam 326.0855 $[M+H]^+$

APP A-305
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technical@hichrom.co.uk

Method Conditions

Column: Cogent Diamond Hydride 2.ō™, 2.2µm, 120A
Catalog No.: 70200-05P-2
Dimensions: 2.1 x 50 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	85	9	20
6	70	10	85
7	20		

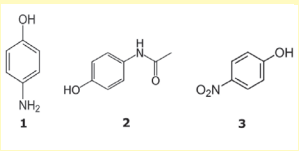
Post Time: 3 min
Flow Rate: 0.4 mL/min
Injection Vol.: 1 µL
Sample: Extraction method: Spiked urine sample was loaded into SPE cartridge I (Clean Screen Xcel™ purchased from UCT Bristol, PA, USA) and eluted with 0.78 mL of acetonitrile, 200 microL of 2-propanol, 20 microL of ammonia. After the elution, the sample was dried under N_2 gas and dissolved in 100 microL of 50% methanol/50% DI water/0.1% formic acid. Before injection, the 10ppm spiked sample was filtered through a 0.45 µm nylon syringe filter (MicroSolv Tech Corp).
Detection: ESI – Pos – Perkin Elmer AxION 2 TOF mass spectrometer.
t_0: 0.9 min

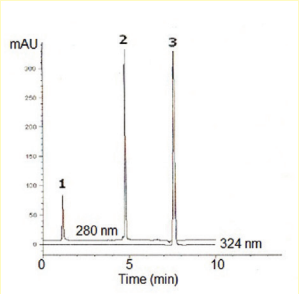
Discussion

The Cogent Diamond Hydride 2.ō™ column was successfully used in analysis of 1,4-benzodiazepines in urine samples after SPE extraction. Four available compounds were well retained and separated. The procedure could be used for determination of this class of compounds in urine samples and other body fluids.

Acetaminophen Impurities Method

Robust and Easy APAP Method





Method Conditions

Column: Cogent Bidentate C18™, 4µm, 100A
Catalog No.: 40018-75P
Dimensions: 4.6 x 75 mm
Solvents: A: DI H_2O/ 0.1% formic acid
B: Acetonitrile/ 0.1% formic acid
Gradient:

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

Post Time: 3 min
Injection Vol.: 5 microL
Flow Rate: 1.0 mL/min
Detection: UV 280 (4-aminophenol, acetaminophen) and 324 nm (4-nitrophenol)
Peaks: 1. 4-aminophenol 1.072 min
2. acetaminophen 4.668 min
3. 4-nitrophenol 7.588 min
t_0: 0.9 min

Discussion

Acetominophen and two of its major impurities were analyzed using the Cogent Bidentate C18™ column and a simple mobile phase. The peak shapes were very high. The repeatability of the results was extremely good (%RSD = 0.01).

APP A-249
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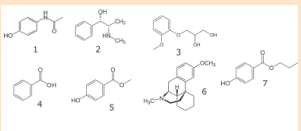
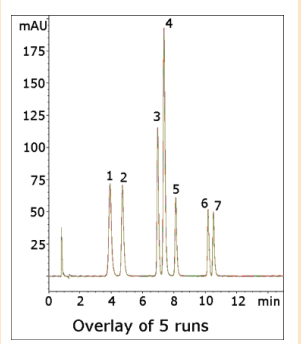
Cough Syrup Ingredients

Separation of Antitussives, Analgesics, Decongestants and Preservatives





Peaks
1. Acetaminophen
2. Pseudoephedrine
3. Guaifenesin
4. Benzoic Acid
5. Methyl Paraben
6. Dextromethorphan
7. Propyl paraben

APP A-174
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www.MTC-USA.com or
technical@hichrom.co.uk

Method Conditions

Column: Cogent Phenyl Hydride™ 4µm, 100Å
Catalog No.: 69020-7.5P
Dimensions: 4.6 x 75 mm
Mobile Phase: A: DI H_2O / 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
Flow Rate: 1.0 mL/min
Injection Vol.: 2 µL
Sample: **Stock Solution:** 1 mg/mL solutions of each analyte were made using a 50/50 solvent A/solvent B diluent (v/v).
Working Solution: 0.1 mg/mL dilutions were made of the stock solutions and used for peak identity confirmations. A 0.1 mg/mL mixture of all the analytes was also made from the stock solutions.
Detection: UV 210 nm (0-6 min), 230 nm (6-15 min)
t_0: 0.9 min

Discussion

Cold and cough formulations may contain a number of components such as antitussives (dextromethorphan), decongestants (pseudoephedrine, guaifenesin), analgesics (acetaminophen), and preservatives (methyl paraben, propyl paraben, benzoic acid). The method illustrates not only excellent separation between a variety of these compounds, but also that symmetric peak shapes can be obtained in each case. Dextromethorphan in particular is often problematic in terms of tailing due to the tertiary amine. The method is also very reproducible, as the five run overlay demonstrates.

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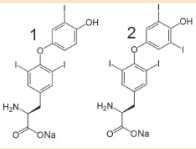
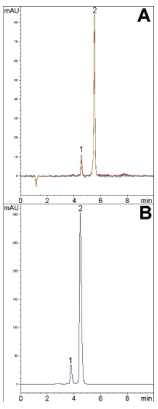
APPLICATION NOTES

Assay Method for Levothyroxine

Superior Resolution, Reproducibility & Peak Shape





APP A-128
For more information
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technical@hichrom.co.uk

Method Conditions

Column: **Fig. A:** MTC Phenyl Hydride™, 4µm, 100A
Fig. B: Type B silica-based Cyano, 5µm, 100A
Catalog No.: **Fig. A:** 69020-7.5P **Fig. B:** N/A
Dimensions: **Fig. A:** 4.6 x 75 mm **Fig. B:** 4.6 x 250 mm
Mobile Phase: **Fig. A:** A: DI water/ 0.1% formic acid
B: 97% Acetonitrile/ 3% DI water/ 0.1% formic acid
Fig. B: 60% DI water/ 40% acetonitrile/ 0.05% phosphoric acid

Gradient: **Fig. A**

time (min.)	%B
0	20
6	50
7	20

Temperature: **Fig. A:** 35 °C **Fig. B:** ambient
Flow rate: **Fig. A:** 1.0 mL/min **Fig. B:** 1.5 mL/min
Injection volume: **Fig. A:** 2 µL **Fig. B:**100 µL
Sample: Mix of levothyroxine and liothyronine standards

Stock Solutions: 0.4 mg levothyroxine or liothyronine dissolved with 1 mL 10 mM NaOH in 50:50 DI water: methanol. Working Solution (**Fig. A**): Aliquots of stock solutions were mixed and diluted with 50:50 A:B to obtain concentrations of 40 mg/L and 4 mg/L for levothyroxine and liothyronine respectively. Working Solution (**Fig. B**): Aliquots of stock solutions were mixed and diluted with the mobile phase to obtain concentrations of 10 mg/L and 0.2 mg/L for levothyroxine and liothyronine respectively.

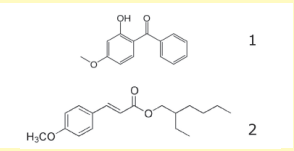
Peaks: 1. liothyronine sodium
2. levothyroxine sodium

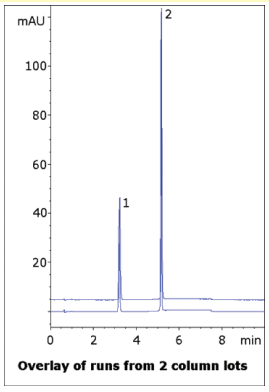
Discussion

The USP assay method for levothyroxine requires that a resolution of not less than 5.0 must be demonstrated between levothyroxine and related compound liothyronine. A chromatogram obtained from following the USP method using a Type-B silica based L10 column is shown in Figure B. The average resolution between the two compounds over five runs is 2.8, which does not satisfy the system suitability for resolution for this assay. Figure A shows the five-run overlay obtained from a method developed with the Cogent Phenyl Hydride™ column. The average resolution in this case was 5.3. In addition, the peak shapes and reproducibility were far superior for the Phenyl Hydride™ method.

Oxybenzone and Octinoxate

Separation of Two APIs in Chap Stick® Extract





APP A-216
For more information
www.MTC-USA.com or
technical@hichrom.co.uk

Method Conditions

Column: Cogent Bidentate C18™, 4µm, 100A
Catalog No.: 40018-75P
Dimensions: 4.6 x 75 mm
Solvents: A: DI H_2O/ 0.1% formic acid (v/v)
B: Acetonitrile/ 0.1% formic acid (v/v)

Gradient:

time (min.)	%B
0	60
1	60
4	100
6	100
7	60

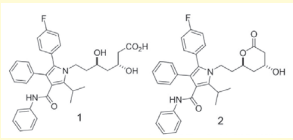
Post Time: 3 min.
Temperature: 40°C
Injection Vol.: 2 microL
Flow Rate: 1.0 mL/min
Detection: UV 288 nm
Peaks: 1. Oxybenzone
2. Octinoxate
t_0: 0.9 min

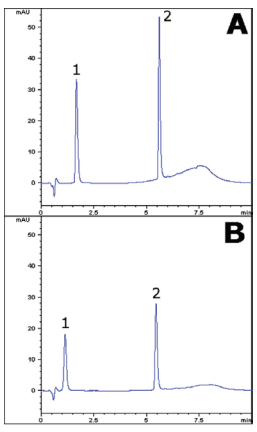
Discussion

This method shows how two common ingredients found in sunscreens and lip balms can be separated using the Bidentate C18™ column. The two compounds are very hydrophobic, so a mobile phase gradient with significant organic content was used in order to avoid excessive retention. Likewise, a highly organic diluent should be used to adequately extract the compounds from the lip balm material. The figure shows an overlay of two runs from different column lots, demonstrating the lot-to-lot reproducibility of the Bidentate C18™ stationary phase.

Atorvastatin Method Transfer

Use of Near-UHPLC 2.2µm Column





APP A-308
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technical@hichrom.co.uk

Method Conditions

Column: Fig. A: Cogent Bidentate C18 2.ō™, 2.2um, 120A
Fig. B: Cogent Bidentate C18™, 4um, 100A
Catalog No.: Fig. A: 40218-05P-2; Fig. B: 40018-05P-2
Dimensions: 2.1 x 50 mm
Mobile Phase: A: DI H_2O / 10 mM ammonium acetate
B: 90% acetonitrile / 10% DI water / 10 mM ammonium acetate

Gradient:

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

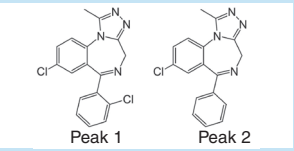
Flow Rate: 0.3 mL/min
Detection: UV 248 nm
Sample: 40 mg strength Lipitor® tablet was ground and added to a 50 mL volumetric flask with a portion of solvent B diluent. The solution was sonicated 10 min and diluted to mark with solvent B. It was then filtered through a 0.45 µm nylon membrane (*MicroSolv Technology Corp. Eatontown, NJ, USA*). The filtrate was diluted 4x in a diluent of 50/50 solvent B/ 1N HCl. It was heated in a dry bath for 10 min at 85°C.
Peaks: 1. Atorvastatin
2. Atorvastatin lactone

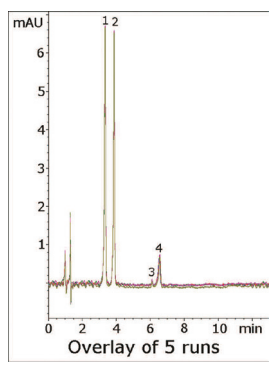
Discussion

This application note demonstrates how data obtained on a 4um Bidentate C18™ column (Fig. B) can be adapted for a 2.2um phase (Fig. A). The retention times of both analytes are very comparable. The method produces excellent separation of the API and its main acid degradant. It is also LC-MS compatible and so could be used in clinical applications involving plasma samples.

Alprazolam (Xanax®)

Robust Separation of API from USP Internal Standard





APP A-183
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technical@hichrom.co.uk

Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100A
Catalog No.: 70000-7.5P
Dimensions: 4.6 x 75 mm
Solvent: A: DI H_2O/ 0.1% formic acid (v/v)
B: Acetonitrile/ 0.1% formic acid (v/v)

Gradient:

time (min.)	%B
0	95
1	95
6	50
7	95

Post Time: 3 min
injection Vol.: 1 µL
Flow Rate: 1.0 mL/min
Peaks: 1. Triazolam (internal standard)
2. Alprazolam (API)
3, 4. Impurities
Detection: 254 nm
t_0: 0.9 min

Discussion

The USP assay method for Alprazolam uses a bare silica column and a complex mobile phase consisting of acetonitrile, chloroform, butyl alcohol, and acetic acid. In this method a simple LC-MS compatible mobile phase is used and produces excellent peak shapes for both the API and its USP internal standard.

Furthermore, a resolution of 4.3 was obtained between the two peaks, which meets the USP system suitability of $R_s \geq 2.0$. Two impurity peaks are also observed, which further illustrates the resolution capabilities of the column.

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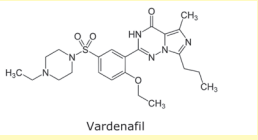
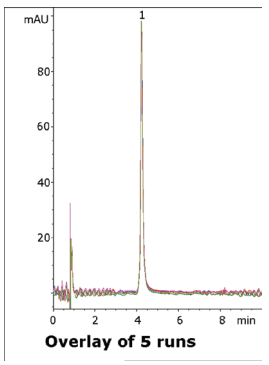
APPLICATION NOTES

Vardenafil (Levitra®)

LC-MS Compatible Assay Method



Vardenafil



Overlay of 5 runs

APP A-266
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www.MTC-USA.com or
technical@hichrom.co.uk

Method Conditions

Column: Cogent UDA™, 4µm, 100A
Catalog No.: 40031-7.5P
Dimensions: 4.6 x 75 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	90	6	40
1	90	7	90

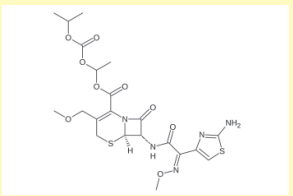
Post Time: 3 min
Injection Vol.: 2 microL
Flow rate: 1.0 mL/min
Detection: UV 210 nm
Sample: 20mg strength Levitra® tablet was ground and added to a 25mL volumetric flask. A portion of 50/50 solvent A/solvent B diluent was added and the flask was sonicated 10 min. Then it was diluted to mark and mixed. A portion was filtered through a 0.45 µm nylon syringe filter (MicroSolv Tech Corp).
Peak: 1. Vardenafil
t_0: 0.7 min

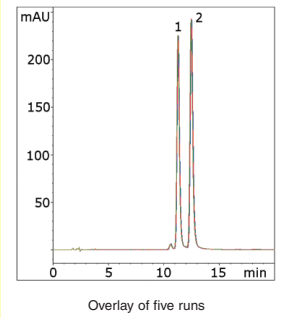
Discussion

Vardenafil in a tablet formulation can be readily assayed with this LC-MS compatible gradient method. The peak tailing factor was close to unity. The compound has several amine groups which can produce tailing with ordinary HPLC columns that have a number of surface silanols. The MS-compatible mobile phase means that the method can be adapted to more complex samples such as plasma. Five runs are shown in the figure to illustrate the repeatability of the data.

Cefpodoxime Proxetil

USP Assay Method





Overlay of five runs

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technical@hichrom.co.uk

Method Conditions

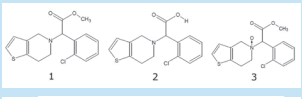
Column: Cogent Bidentate C18™, 4µm, 100Å
Catalog No.: 40018-25P
Dimensions: 4.6 x 250 mm
Mobile Phase: 60% 20 mM ammonium acetate/ 40% acetonitrile
Temperature: 30 °C
Flow rate: 1.0 mL/min
Injection Vol.: 20 µL
Detection: UV 235 nm
Sample: Stock Solution: A 200 mg strength cefpodoxime proxetil tablet was ground and added to a 100 mL volumetric flask. The flask was diluted to mark with the mobile phase and sonicated. A portion was then filtered with a 0.45 micron nylon syringe filter (MicroSolv Tech Corp).
Working Solution: 100 µL of the stock solution was diluted with 900 µL of the mobile phase.
Peaks: 1. Cefpodoxime Proxetil, S-epimer
2. Cefpodoxime Proxetil, R-epimer
t_0: 1.9 min

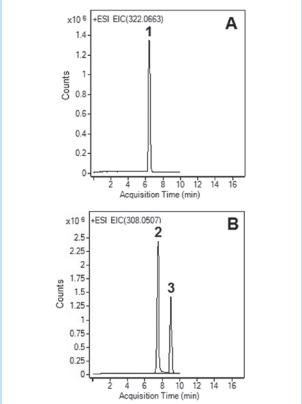
Discussion

The USP assay method for cefpodoxime proxetil specifies a resolution of not less than 2.5 must be obtained between the two epimers of the prodrug. Following the method using a Cogent Bidentate C18™ column, the average resolution was calculated to be 2.8. In addition, the R epimer tailing factor must be not more than 1.5. Again, this data meets this requirement with a tailing factor of 1.2. Finally, the data shows good repeatability with a retention time %RSD from five runs of 0.2%.

Forced Degradation of Clopidogrel

Separation of API and Degradants





APP A-258
For more information
www.MTC-USA.com or
technical@hichrom.co.uk

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	60
2	95	8	95

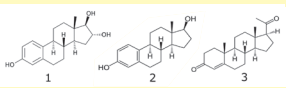
Temperature: 25 °C
Post Time: 3 min
Injection Vol.: 1 microL
Flow Rate: 0.4 mL/min
Detection: ESI – POS - Agilent 6210 MSD TOF mass spectrometer.
Peaks: 1. API: Clopidogrel, m/z 322.0663 $[M+H]^+$
2. Degradant: Clopidogrel Acid, m/z 308.0507 $[M+H]^+$
3. Degradant: Clopidogrel N-oxide, m/z 338.02 $[M+H]^+$
t_0: 0.9 min

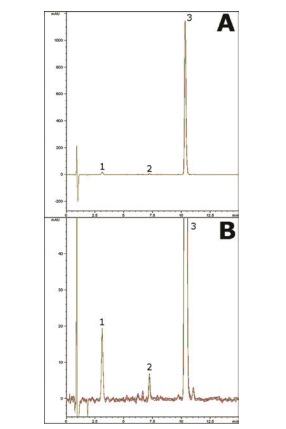
Discussion

A sensitive, selective, and rapid ANP HPLC–MS method was developed for the simultaneous quantification of clopidogrel (Plavix®) and its degradants. Separation of the drug and the degradation products under stress conditions was successfully achieved on a Cogent Diamond Hydride™ column. The method was transferred from UV-HPLC method. The developed method can be used for the determination of clopidogrel in commercial tablets for quality control with an application to a content uniformity test. The method is also stability-indicating as it is suitable for the determination of clopidogrel in the presence of its degradation products under all stress conditions using HCl, NaOH, light and hydrogen peroxide.

Hormone Replacement Capsule

Separation of Estriol, Estradiol and Progesterone





APP A-175
For more information
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Method Conditions

Column: Cogent UDC Cholesterol™ 4µm, 100A
Catalog No.: 69069-7.5P
Dimensions: 4.6 x 75 mm
Solvents: A: DI H_2O/ 0.1% formic acid (v/v)
B: Acetonitrile/ 0.1% formic acid (v/v)

Gradient:

time (min.)	%B
0	33
2	33
11	65
12	33

Post Time: 3 min
Flow rate: 1.0 mL/min
Detection: UV 210 nm
Injection Vol.: 10 µL
Peaks: 1. Estriol
2. Estradiol
3. Progesterone

Discussion

This gradient method features a separation of the three components of a hormone replacement formulation. Excellent separation is obtained between the three compounds using the Cogent UDC-Cholesterol™ column.

Figure A shows a five run overlay of the formulation extract injections, demonstrating the excellent run-to-run repeatability of the method.

Figure B shows a zoomed-in view so that the estriol and estradiol peaks, which are present in much lower concentration than progesterone, can be seen clearly. Figure B also shows separation of an impurity from the progesterone peak.

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

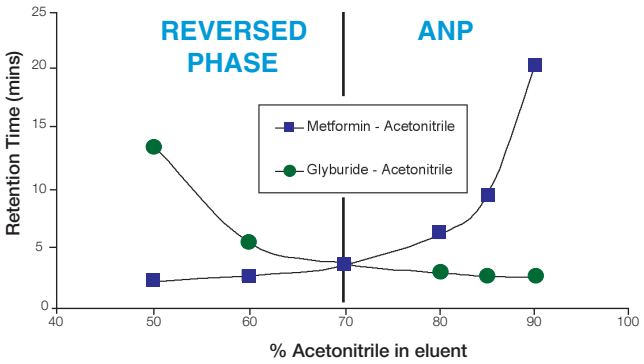

Figure 1. Dual RP and ANP retention capability

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

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