COGENT 2.0

Bidentate C18™
Phenyl Hydride™
Diol™

Analyzing a Mixture of Heroin, MDMA, Morphine, Hydromorphone and 6-MAM

Extended Application Note

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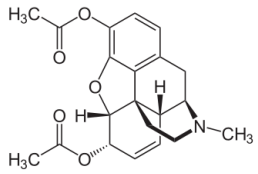
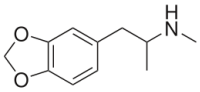
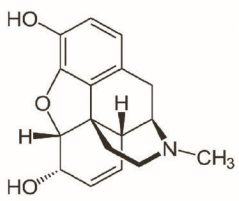


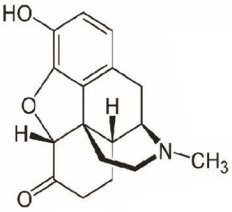
Heroin



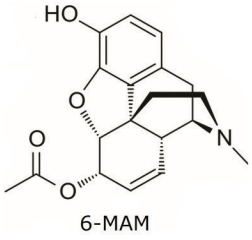
MDMA



Morphine



Hydromorphone



6-MAM

Figure 1

Method 1:

A: DI H_2O/ 0.1% formic acid
B: Acetone/ 0.1% formic acid

Time (min.)	%B
0	90
3	30
5	30
6	90

Introduction

Analysis of drugs of abuse encompasses a wide variety of compounds, including controlled substances, prescription drugs, and even over-the-counter formulations. These drugs can be problematic to analyze due a number of problems. First, many have similar structures (e.g. morphine and hydromorphone), making adequate selectivity difficult to obtain in a chromatographic separation. Second, a good number of these contain amine functionalities (MDMA, heroin, etc.) which can present peak shape issues. Basic molecules can sometimes interact with residual silanols on the column, resulting in tailing. Finally, the range of polarity encountered in a plasma or urine sample may require one column for reversed phase and another for the polar compounds in drugs of abuse.

Stationary phase materials based on TYPE-C Silica™ can address these issues. Each column can be used in either the reversed phase or aqueous normal phase (ANP) mode to retain compounds of a wide polarity range. Hence, one column can be used where ordinarily two may be required. Along these lines, the versatility of retention mode means that superior selectivity may be achieved; if two compounds co-elute in reversed phase, an ANP approach may be able to separate them. As for problems associated with tailing, the TYPE-C Silica™ columns can be advantageous here too. The stationary phase surface is virtually free of silanols, which means tailing for basic compounds is reduced.

Here, we investigated the use of different TYPE-C Silica™ columns for the analysis of various drugs of abuse. In addition to standard separations, urine and plasma-based samples were also analyzed. Figure 1 shows a selection of the analytes that were studied.

Experimental

Materials

Formic acid LC-MS ultra-grade, Heroin, MDMA, hydromorphone, 6-MAM, and morphine were from Sigma–Aldrich (St. Louis, MO, USA). UTAK 4308 Level 2 Control urine samples were obtained from UTAK Laboratories, INC (Valencia, CA, USA). Deionized water (DI H_2O) was prepared on a Milli-Q™ purification system from Millipore (Bedford, MA, USA). Acetone and acetonitrile (HPLC grades) were obtained from GFS Chemicals, Inc. (Powell, OH, USA).

Instrumentation

An Agilent (Little Falls, DE, USA) 1200SL Series LC system, including degasser, binary pump, temperature-controlled autosampler, and temperature-controlled column compartment was used. The mass spectrometer system was an Agilent (Santa Clara, CA, USA) Model 6210 MSD TOF with a dual sprayer electrospray source (ESI). The analytical columns were Diol 2.0™ and Bidentate C18 2.0™ stationary phases, 2.1 x 50 mm, 2.2µm, 120Å. A third Phenyl Hydride™ column was also used, 2.1 x 50 mm, 4µm, 100Å. Different gradients were used (see Tables). In all cases, the flow rate was 0.4 mL/min and the injection volume was 1µL.

Sample Preparation

Methods 1 & 2: Stock solutions of each analyte were prepared at 1mg/mL concentrations using a methanol diluent. Working solutions were then prepared from the stock solutions at concentrations of 1µg/mL. All solutions were stored at -20°C. Solutions used for spiking were prepared at 0.500µg/mL concentrations. For blood samples, 0.2 mL blood in a 2 mL plastic tube was mixed with 0.2 mL methanol and 0.2 mL spiking solution.

Method 2:

A: DI H_2O/ 0.1% formic acid
B: Acetone/ 0.1% formic acid

Time (min.)	%B
0	30
3	90
5	90
6	30

Method 3:

A: DI H_2O/ 0.1% formic acid
B: Acetonitrile/ 0.1% formic acid

Time (min.)	%B
0	5
3	50
5	90
6	90
7	5

Method 4:

A: DI H_2O/ 0.1% formic acid
B: Acetonitrile/ 0.1% formic acid

Time (min.)	%B
0	5
3	5
7	80
8	5

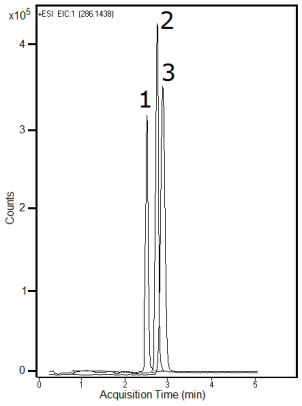


Figure 2

1. Heroin
2. MDMA
3 Morphine
4. Warfarin

The samples were vortexed for 1 min and centrifuged for 10 min at 13,000 rpm. The final solutions were prepared by diluting 0.2 mL supernatant with 0.5 mL water + 0.1% formic acid.

Method 3: To a spiked plasma sample (1 mL), 1 mL of an ammonia solution (0.1% v/v) was added and vortex-mixed for 30 seconds to alkalize the plasma. Then, the alkalized plasma sample was extracted with two 4 mL ethyl acetate aliquots by vortex for 5 min, and centrifuged at 4000 rpm for 8 min at room temperature. The supernatant was separated and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted with 200 microL mobile phase, and a 1 microL aliquot of the reconstituted solution was injected into the RP-HPLC–ESI-MS for analysis.

Method 4: The urine samples were prepared with a solid phase extraction (SPE) step. They were directly injected onto SPE cartiridge I. The analyte that was retained in the SPE column was eluted with 1mL of acetonitrile, 2-propanol, ammonia (89/20/2). The prepared samples were injected into the HPLC column for analysis.

Results and Discussion

The Cogent™ Diol 2.ō™ column is highly versatile and may be suited to both reversed phase and aqueous normal phase (ANP) analyses. In the ANP mode, three controlled substances are separated on this column (Fig. 2, Method 1). Morphine and heroin are structurally similar, both being opioids. Many controlled substances belong to the same class of compounds and have similar structural features. This can make them difficult to distinguish chromatographically. However, excellent selectivity is obtained here for these two compounds. The MDMA peak partially overlaps with morphine, but these peaks can be readily isolated in the EICs.

Warfarin™ is a hydrophobic compound and would be best suited to retention by reversed phase. The Cogent Diol 2.ō™ column can be used in this mode as well, as shown in the data in Figure 3 (Method 2). Here, strong retention and good peak shape are observed. Together with ANP separations, this column can retain the full polarity range in samples which may be encountered in the field of controlled substance analysis.

The Bidentate C18™ column is also available in a near-UHPLC format (2.ō). In a separation of morphine (1), hydromorphone (2), and 6-MAM (3), baseline resolution was achieved (see Figure 4). A reversed phase gradient was used

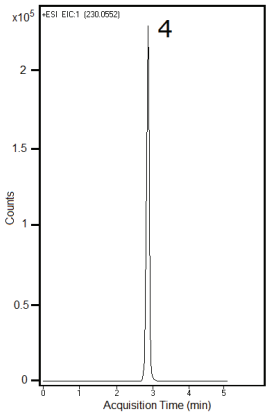


Figure 3

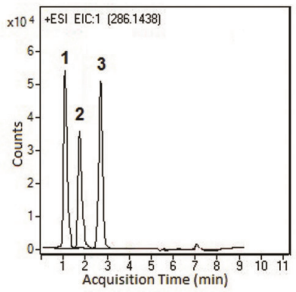


Figure 4

1. Alpha-Hydroxyalprazolam
2. d-Amphetamine
3 d-Methamphetamine
4. Phentermine
5. (±)-3,4-Methylenedioxyamphetamine
6. Ephedrine
7. Pseudoephedrine
8. ±)-3,4- Methylenedioxymethamphetamine
9. Benzoylecgonine
10. Ecgonine Methyl Ester
11. Codeine
12. Diazepam
13. Nordiazepam
14. 6-Monoacetylmorphine
15. Oxazepam
16. Temazepam
17. (l)-9-Carboxy-11-Nor-Delta-9-THC

to separate the three compounds (see Method 3). Subtle differences in structure may make it difficult to obtain adequate selectivity using conventional columns. The sample is spiked plasma so the column can be used in real-world matrices without interference from contaminants.

The Phenyl Hydride™ column has shown excellent promise for drugs of abuse. A drug screening sample was analyzed (Figure 5, Method 4) and good selectivity was observed in the EICs. A variety of drugs of abuse were separated. Furthermore, the peak shapes showed minimal tailing.

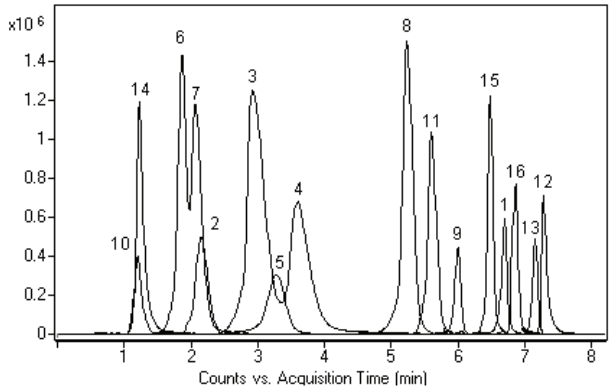


Figure 5

Conclusion

The TYPE-C Silica™ based columns are an excellent choice for analyses of drugs of abuse. Separation of closely related compounds can be achieved with good peak shape. The analyses can be performed with standard 4µm columns and with near-UHPLC (2.2µm). The Bidentate C18™ is excellent for reversed phase analysis while the Cogent Diol™ has been shown to be versatile in both RP and ANP approaches. The Phenyl Hydride™ column provides good selectivity via π-π interactions.

COGENT 2.ō

Bidentate C18™
Phenyl Hydride™
Diol™

Cat. No.	Description
40260-02P-2	Cogent Diol 2.ō HPLC column 120Å 2.2µm 2 x 2 mm. 1 each.
40260-05P-2	Cogent Diol 2.ō HPLC column 120Å 2.2µm 50 x 2.1 mm ID. 1 each.
40260-10P-2	Cogent Diol 2.ō HPLC column 120Å 2.2µm 100 x 2.1 mm ID. 1 each.
40260-15P-2	Cogent Diol 2.ō HPLC column 120Å 2.2µm 150 x 2.1 mm ID. 1 each.
40260-75P-2	Cogent Diol 2.ō HPLC column 120Å 2.2µm 75 x 2.1 mm ID. 1 each.
40260-HG1	Cogent Replacement Guard Columns Kit with Diol 2.o 120Å 2.2µm. Includes 5 each Hichrom Hardware 2.0 x 10 mm Guard Columns in individual cases.
40260-HG2	Cogent Guard Column Kit with Diol 2.o 120Å 2.2µm. Includes one Universal Holder and 5 each Hichrom Hardware 2.0 x 10 mm Guard Columns.
40260-HG3	Cogent Guard Column Diol 2.o 120Å 2.2µm. Holder required. Hichrom Hardware 2.0 x 10 mm Guard Column. 1 each.
40218-02P-2	Cogent Bidentate C18 2.ō HPLC column 120Å 2.2µm 20 x 2.1 mm. 1 each.
40218-03P-2	Cogent Bidentate C18 2.ō HPLC column 120Å 2.2µm 30 x 2.1 mm. 1 each.
40218-05P-2	Cogent Bidentate C18 2.ō HPLC column 120Å 2.2µm 50 x 2.1 mm. 1 each.
40218-10P-2	Cogent Bidentate C18 2.ō HPLC column 120Å 2.2µm 100 x 2.1 mm. 1 each.
40218-15P-2	Cogent Bidentate C18 2.ō HPLC column 120Å 2.2µm 150 x 2.1 mm. 1 each.
40218-75P-2	Cogent Bidentate C18 2.ō HPLC column 120Å 2.2µm 75 x 2.1 mm. 1 each.
40218-HG1	Cogent Replacement Guard Columns Kit with Bidentate C18 2.o 120Å 2.2µm. Includes 5 each Hichrom Hardware 2.0 x 10 mm guard columns in individual cases.
40218-HG2	Cogent Guard Column Kit with Bidentate C18 2.o 120Å 2.2µm. Includes one Universal Holder and 5 each Hichrom Hardware 2.0 x 10 mm Guard Columns.
40218-HG3	Cogent Mini-Column/Guard Column with Bidentate C18 2.o 120Å 2.2µm. Hichrom 2.0 x 10 mm Guard Column. 1 each.
69020-02P-2	Cogent Phenyl Hydride HPLC column 100Å 4µm 2.1 x 20 mm
69020-03P-2	Cogent Phenyl Hydride HPLC column 100Å 4µm 2.1 x 30 mm.
69020-05P	Cogent Phenyl Hydride HPLC column 100Å, 4µm, 4.6 x 50 mm.
69020-05P-2	Cogent Phenyl Hydride HPLC column 100Å, 4µm, 2.1 x 50 mm.
69020-10P	Cogent Phenyl Hydride HPLC column 100Å, 4µm, 4.6 x 100 mm.
69020-10P-2	Cogent Phenyl Hydride HPLC column 100Å, 4µm, 2.1 x 100 mm.
69020-15P	Cogent Phenyl Hydride HPLC column 100Å, 4µm, 4.6 x 150 mm.
69020-15P-2	Cogent Phenyl Hydride HPLC column 100Å, 4µm, 2.1 x 150 mm.
69020-25P	Cogent Phenyl Hydride HPLC column 100Å, 4µm, 4.6 x 250 mm.
69020-25P-2	Cogent Phenyl Hydride HPLC column 100Å, 4µm, 250 x 2.1 mm.
69020-75P	Cogent Phenyl Hydride HPLC column 100Å 4µm 4.6 x 75 mm
69020-HG1	Cogent Guard Columns Kit Phenyl Hydride Replacements 100Å 4µm. Includes 5 each Hichrom 2.0 x 10 mm Guard Columns in individual cases.
69020-HG2	Cogent Guard Columns Kit 100Å Phenyl Hydride replacements 4µm. Includes 5 each Hichrom 4.0 x 10 mm Guard Columns in individual cases.
69020-HG3	Cogent Guard Column Kit with Phenyl Hydride 4µm. Includes one Universal Holder and 5 each Hichrom 4.0 x 10 mm Guard Columns.
69020-HG4	Cogent Guard Column Kit with Phenyl Hydride 100Å 4µm. Includes one Universal Holder and 5 each Hichrom 2.0 x 10 mm Guard Columns.
69020-HG5	Cogent Guard Column Phenyl Hydride 100Å 4µm. Holder required. 2.0 x 10 mm Guard Column. 1 each
69020-HG6	Cogent Guard Column Phenyl Hydride 100Å 4µm. Holder required. 4.0 x 10 mm Guard Column. 1 each

For more information visit www.mtc-usa.com

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