



**DEVELOPMENT OF A NOVEL CLASS OF ULTRA-STABLE
COVALENTLY BONDED CARBON-BASED HPLC PHASES
WITH UNIQUE CHROMATOGRAPHIC SELECTIVITY
FOR USE IN PHARMACEUTICAL ANALYSIS**

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ZirChrom Separations, Inc

DIAMOND BOND™
HPLC Columns



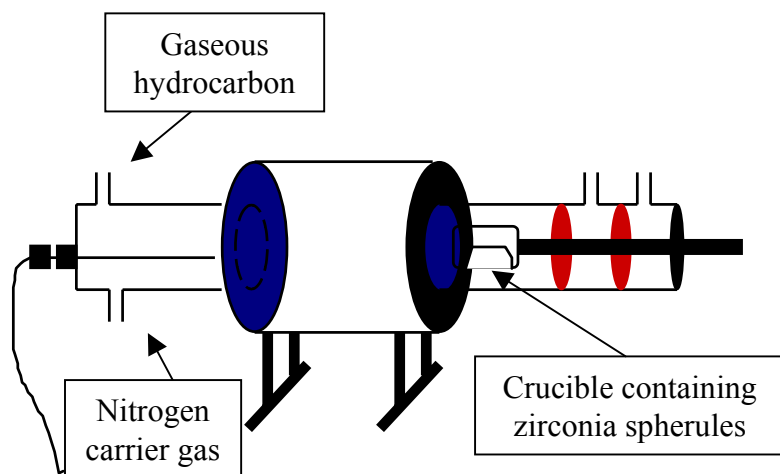
Talk Outline

- Background
 - DiamondBond Bonding Chemistry
 - ZirChrom-CARB Particle Properties
 - Non-Electrolyte Solute Probes
- Selectivity
 - Alkyl Ligand Type and Ligand Density Effects
 - Polar-Embedded-Group
 - Typical Applications
- Stability
 - Base Stability Data
 - High pH / High Temperature Applications
 - LC/MS Bleed Data
 - Pharmaceutical LC/MS/MS

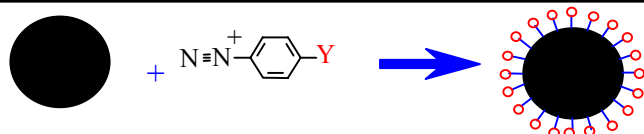
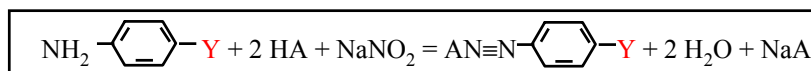


DiamondBond™ - A New Class of Stationary Phase Media

Synthesis of Carbon Clad Zirconia Substrate

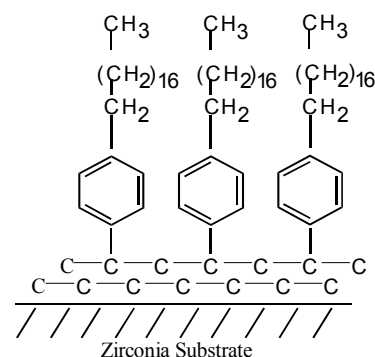


Bonding Reaction on Carbon Clad Zirconia

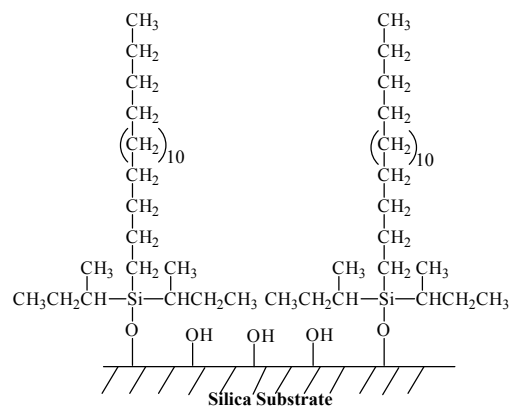


Carbon Clad Zirconia Diazonium Salt Modified Carbon Clad Zirconia

Comparison of Chemical Structure DiamondBond™-C18 vs. ODS-Silica



DiamondBond™-C18

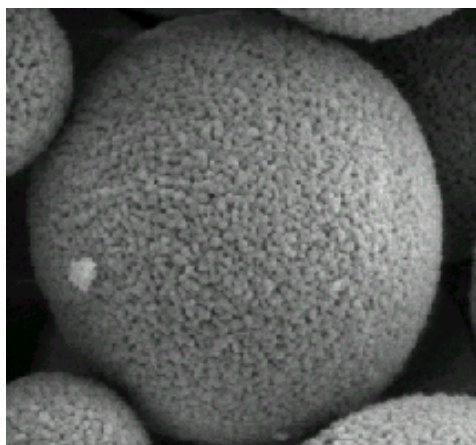
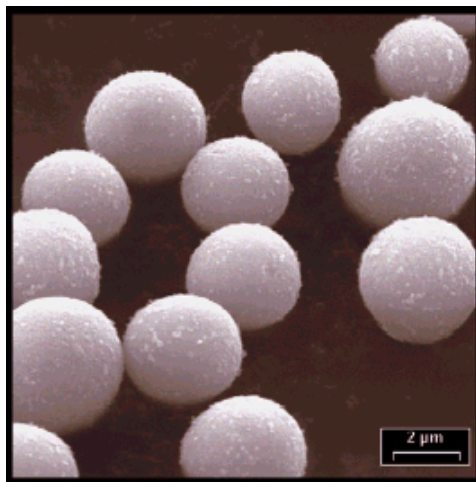


ODS-Silica

DIAMOND BOND™
HPLC Columns



Base CARB Particle Properties

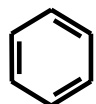


1 μm 25000X

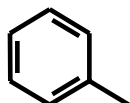
<u>Characteristic</u>	<u>Property</u>
Surface area (m ² /g)	22
Pore volume (cc/g)	0.13
Pore diameter (Å)	250-300
Porosity	0.45
Density (gm/cc)	5.8 (2.5x silica)
Particle diameter (μm)	3.0

22 Non-Electrolyte Solutes

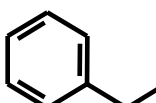
Nonpolar



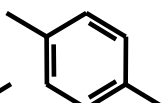
Benzene



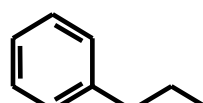
Toluene



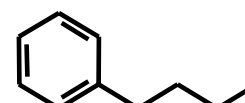
Ethylbenzene



p-xylene

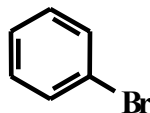


Propylbenzene

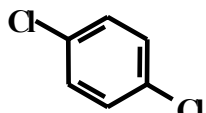


Butylbenzene

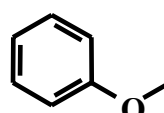
Polar



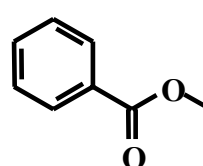
Bromobenzene



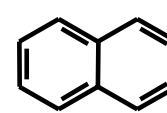
p-Dichlorobenzene



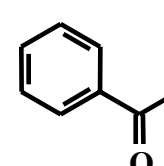
Anisole



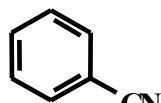
Methylbenzoate



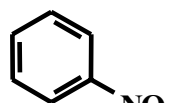
Naphthalene



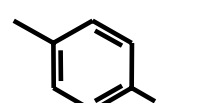
Acetophenone



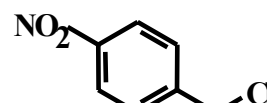
Benzonitrile



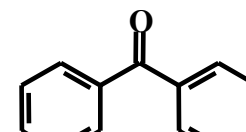
Nitrobenzene



p-Nitrotoluene

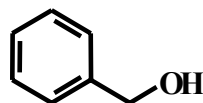


p-Nitrobenzyl Chloride

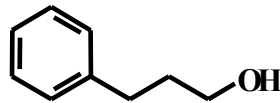


Benzophenone

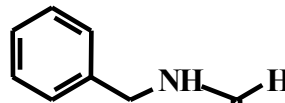
HB Donor



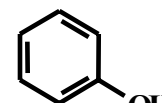
Benzylalcohol



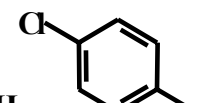
3-Phenyl Propanol



N-Benzyl Formamide



Phenol



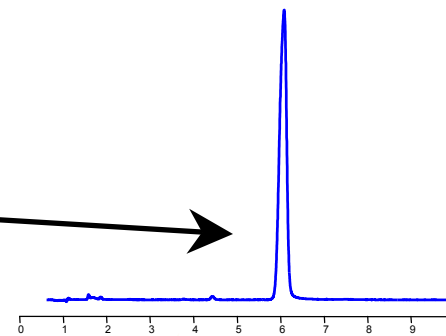
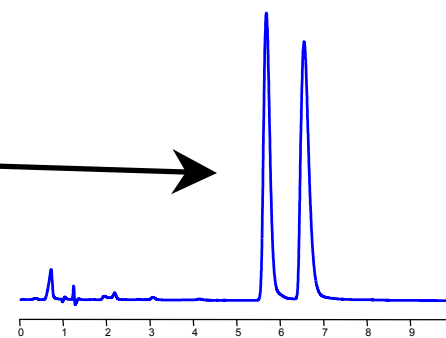
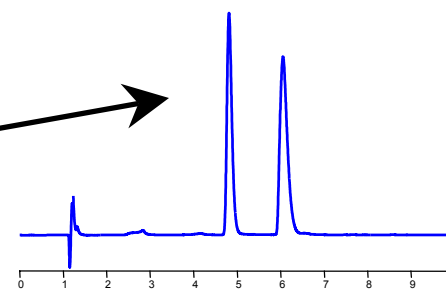
p-Chlorophenol

Mobile phase, 40/60 Acetonitrile/Water; Flow rate, 1.0 ml/min.;
Temperature, 30 °C; Detection at 254nm; 5µl Injection volume.



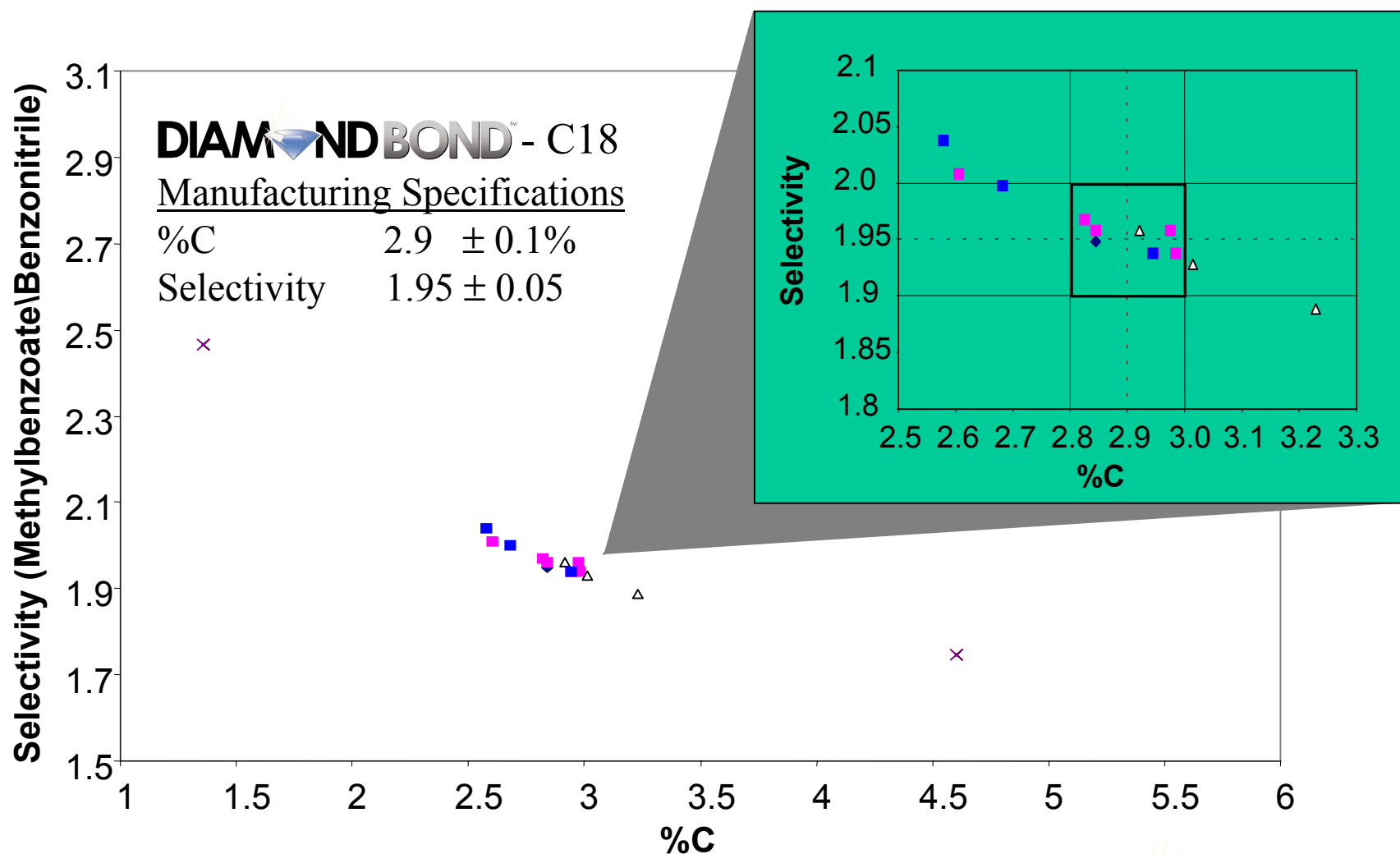
Selectivity Comparison - Carbon Character

Phase	Selectivity (Naphthalene/Benzene)	Selectivity (p-Xylene/Ethylbenzene)	
CARB	48.6	1.63	Carbon Based
C8-1	33.9	1.40	
C8-2	31.7	1.38	
C8-3	24.0	1.32	
C16RP-1	14.3	1.29	
DIAMOND BOND-C18	16.3	1.22	
C18-2	9.4	1.14	
C16RP-2	6.7	1.12	Non-carbon Based
Polymer	4.0	1.08	
C16RP/S	3.3	1.07	
ODS-3	3.3	1.05	
PBD	3.3	1.03	
ODS-1	3.1	1.02	
ODS-2	2.3	0.95	





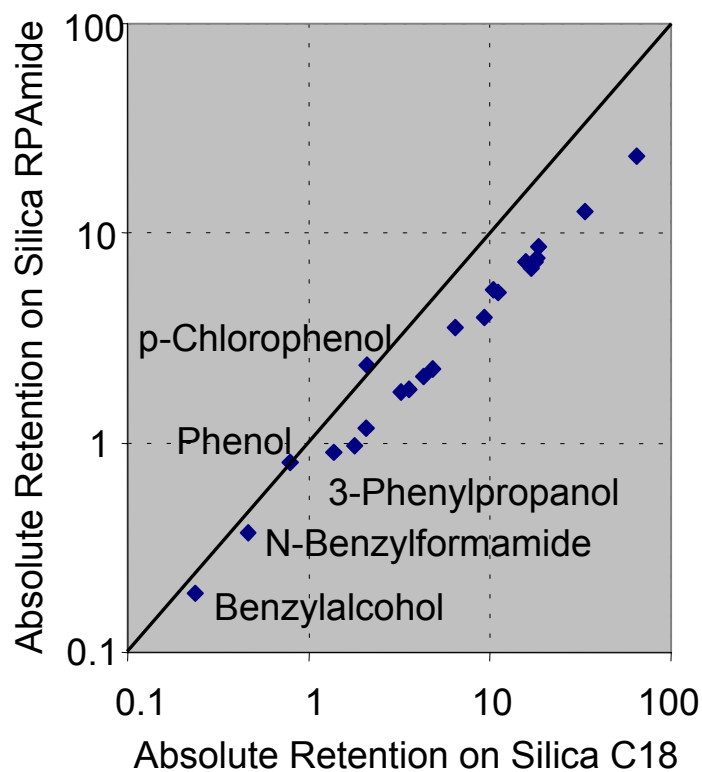
Selectivity versus Ligand Density



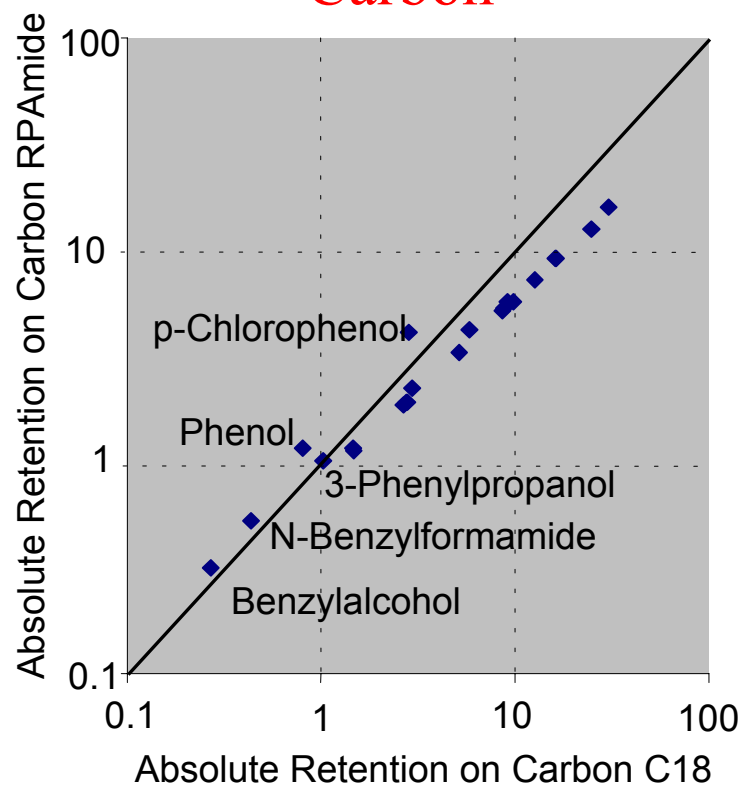


Effect of Polar-Embedded-Group

Silica



Carbon

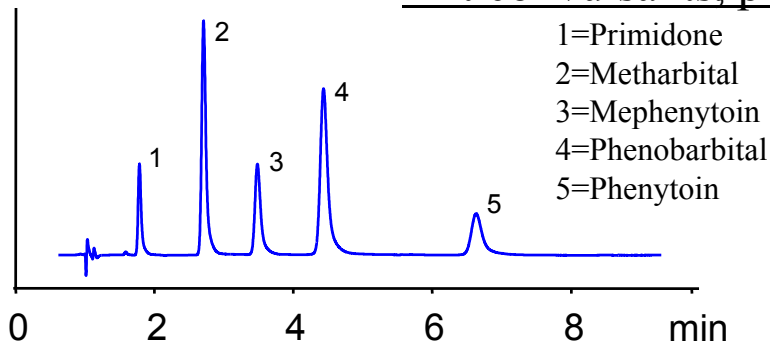


RPAmide shows increased retention of HB Donors, regardless of silanols



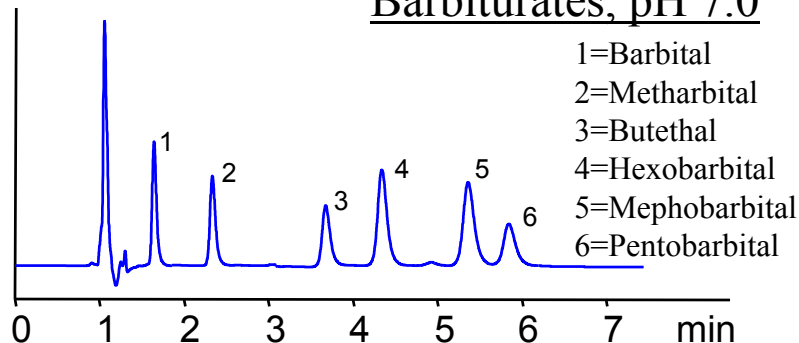
Typical Applications

Anticonvulsants, pH 7.0



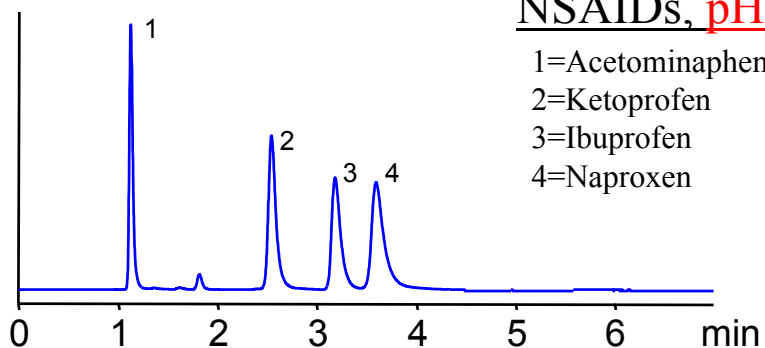
25/75 THF/50mM Ammonium phosphate, pH 7.0;
Flow rate 1.0 ml/min.; Temp. 30 °C; Injection vol. 0.5 ul; 220nm;

Barbiturates, pH 7.0



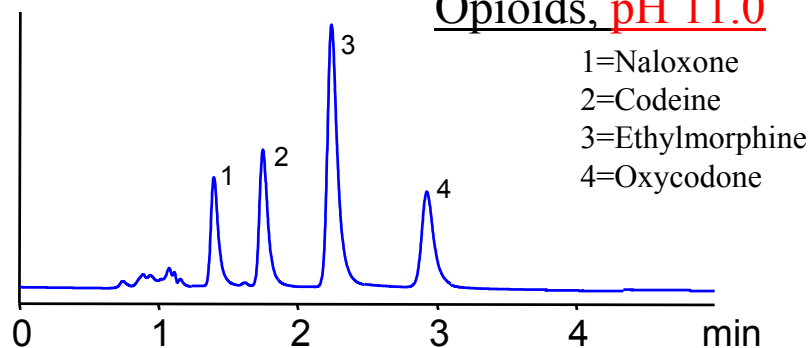
10/15/75 THF/ACN/20 mM Ammonium Phosphate, pH 7.0
Flow Rate 1.0 mL/min.; Temp. 30 °C Injection vol. 5 ul; 254 nm

NSAIDs, pH 1.75



50/50 ACN/50mM Phosphoric acid, pH 1.75;
Flow rate 1.0 ml/min.; Temp. 65 °C; Injection vol. 1.0 ul; 254nm;

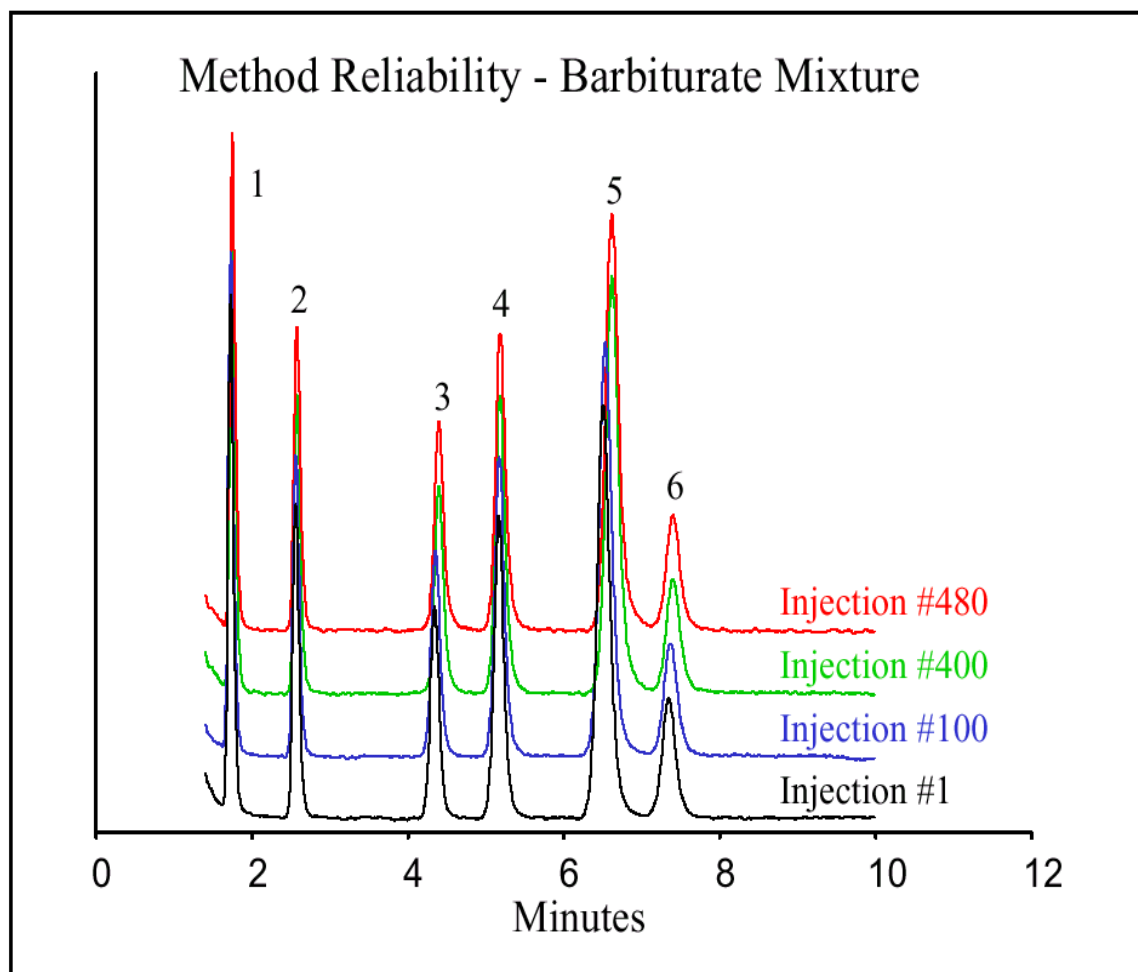
Opioids, pH 11.0



26.5/73.5 THF/20mM Ammonium phosphate, pH 11.0;
Flow rate 1.0 ml/min.; Temp. 40 °C; Injection vol, 1.0 ul; 220nm;



Robust Method - Barbiturates

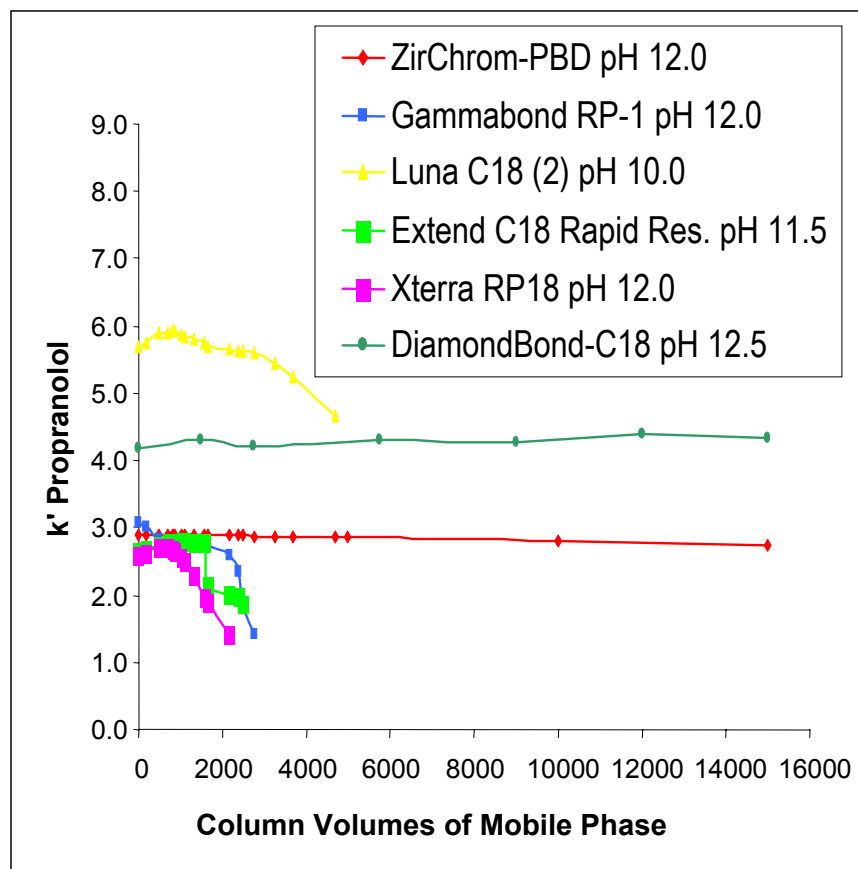


- Simple Pharmaceutical Example, pH 7.0

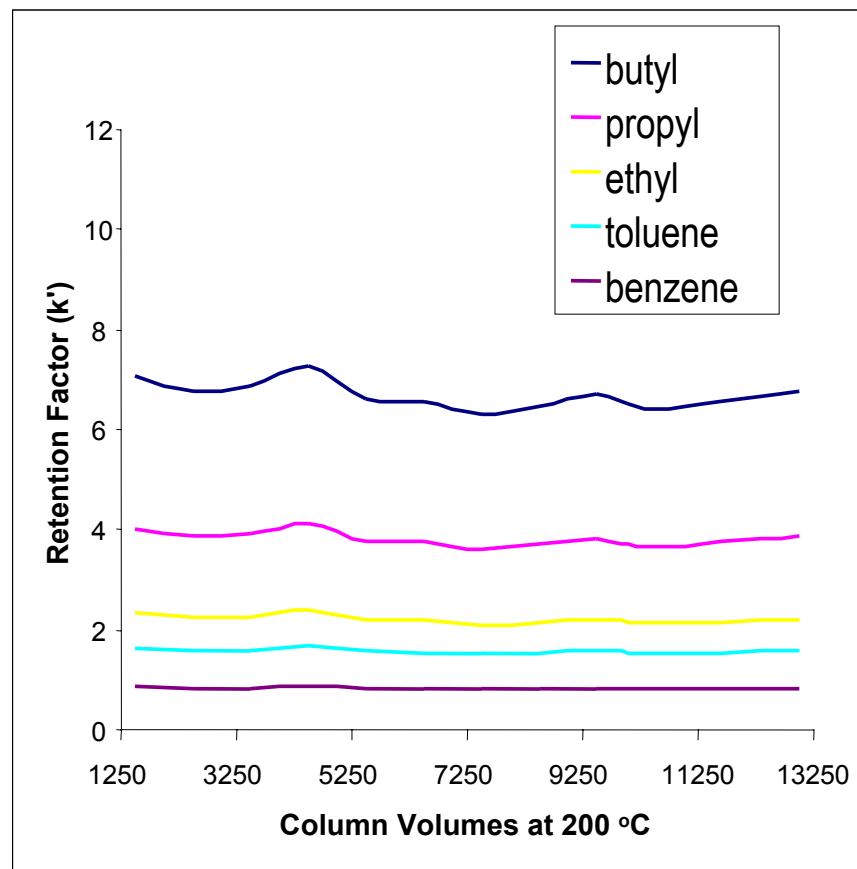


Bonding Stability*

DB-C18 at pH 12.5



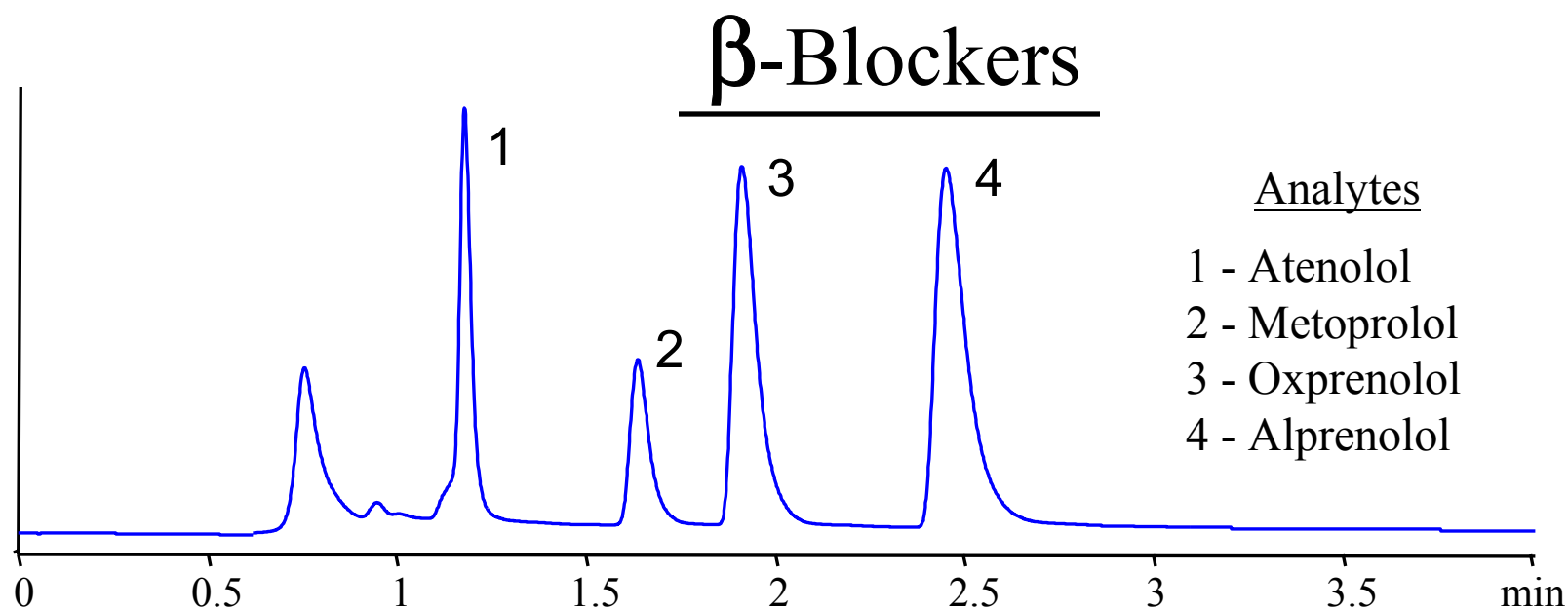
DB-C18 at 200 °C



* Column names are the trademarks of their respective manufacturers.



High pH Application



20/20/60 ACN/THF/200 mM TMAH and 200 mM NaCl, pH 13.3

Flow Rate: 1 mL/min.

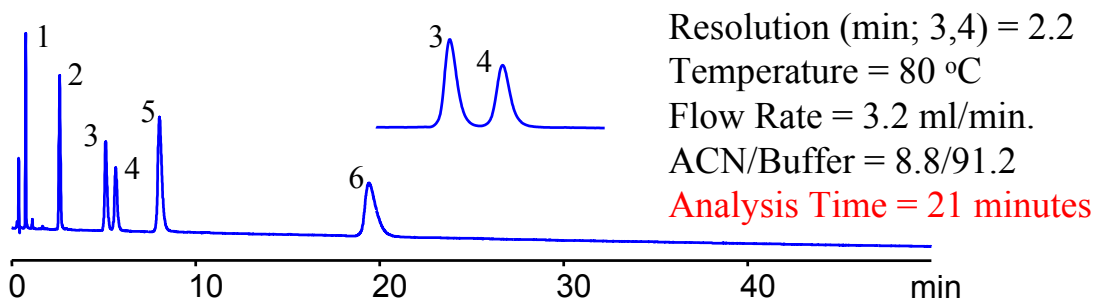
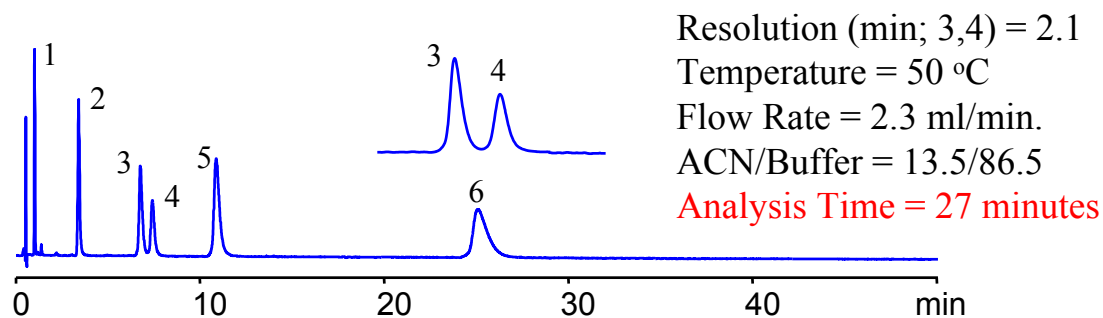
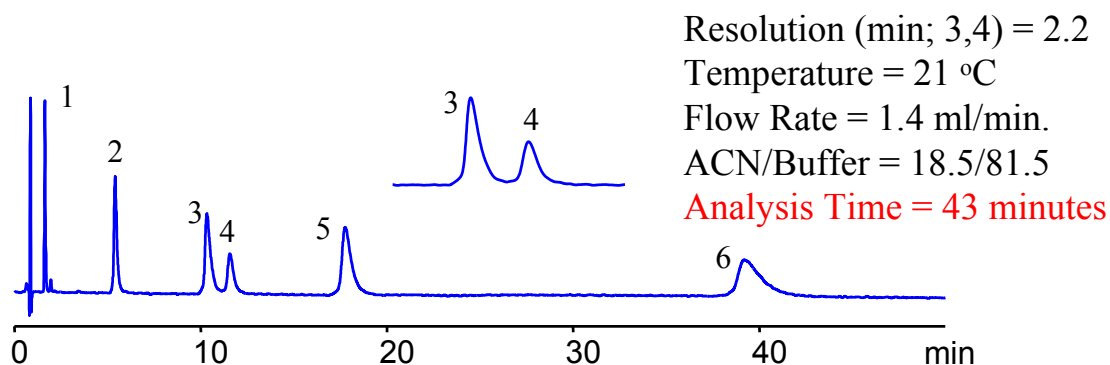
Temperature: 75 °C

Injection Volume: 5 μ L

Detection: 254 nm



High Temperature Application



Analytes:

- 1 = Barbitol
- 2 = Butabarbital
- 3 = Pentobarbital
- 4 = Carbromal
- 5 = Secobarbital
- 6 = Methohexital

Mobile Phase:

ACN/5mM Ammonium phosphate, pH 7.0

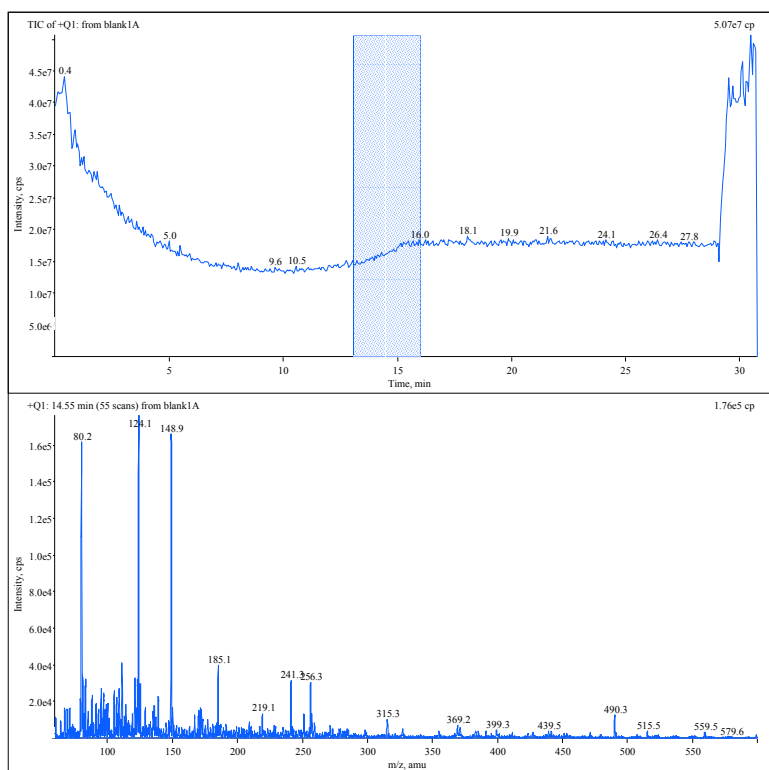
Pressure drop = 195 bar



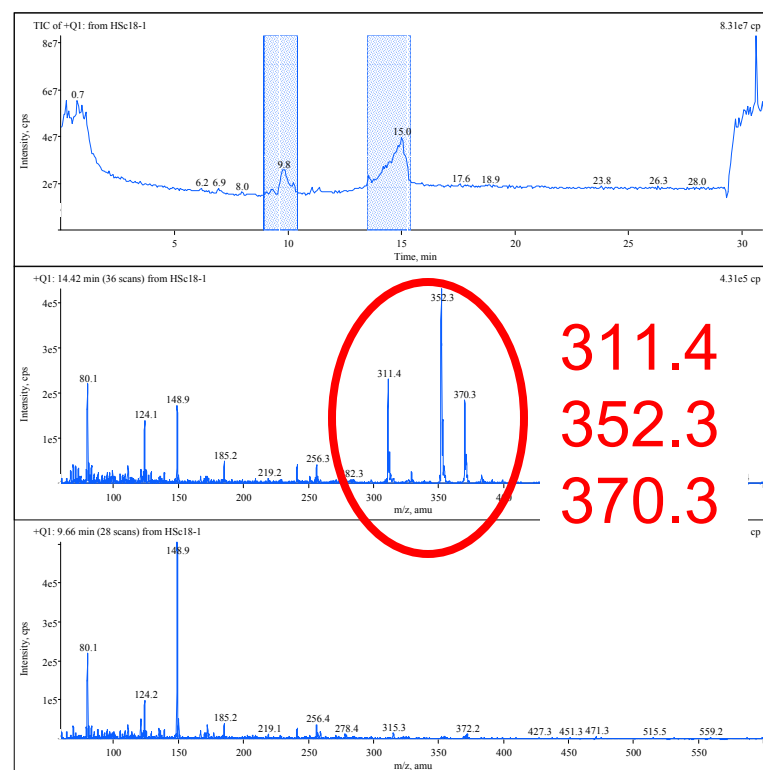
Bleed Results - “No-Bleed” ODS Column

Results at room temp, pH 7.0, ACN/Buffer Gradient

Blank (No Column)



ODS Silica Column



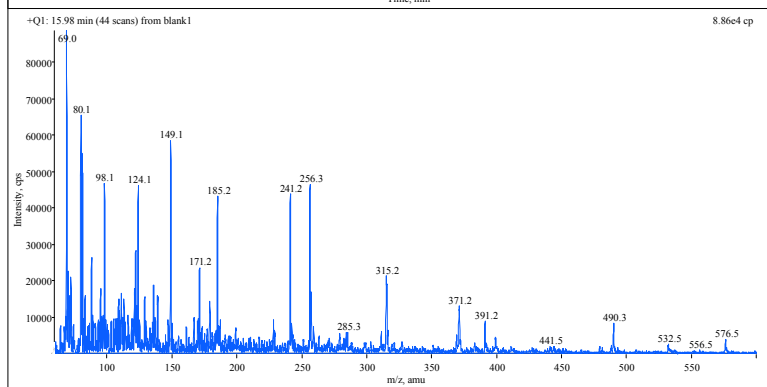
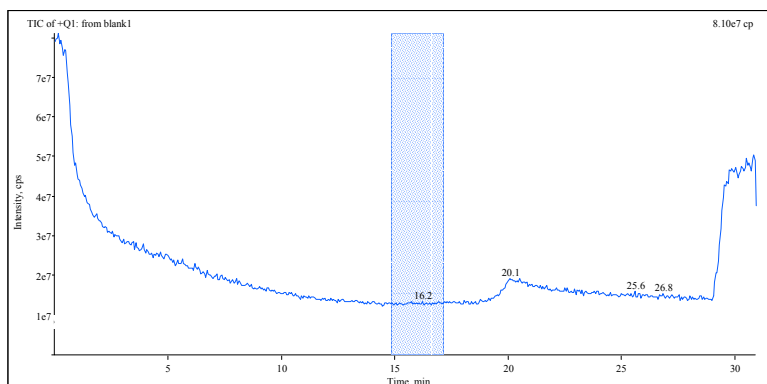
Conclusion : “No-Bleed” columns bleed



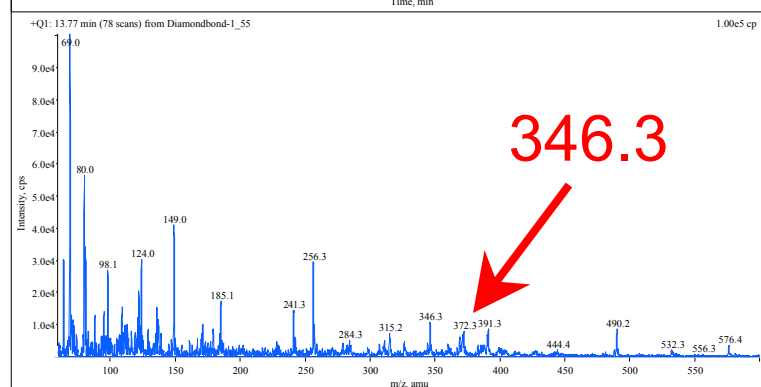
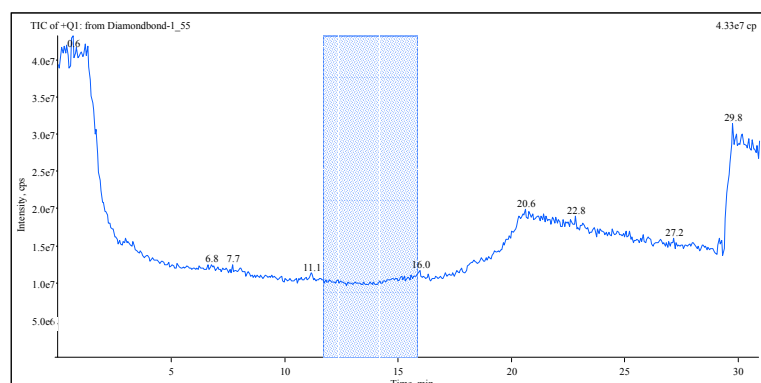
Bleed Results - DB-C18

Results at 55 °C, pH 9.0, ACN/Buffer Gradient

Blank (No Column)



DiamondBond-C18 Column

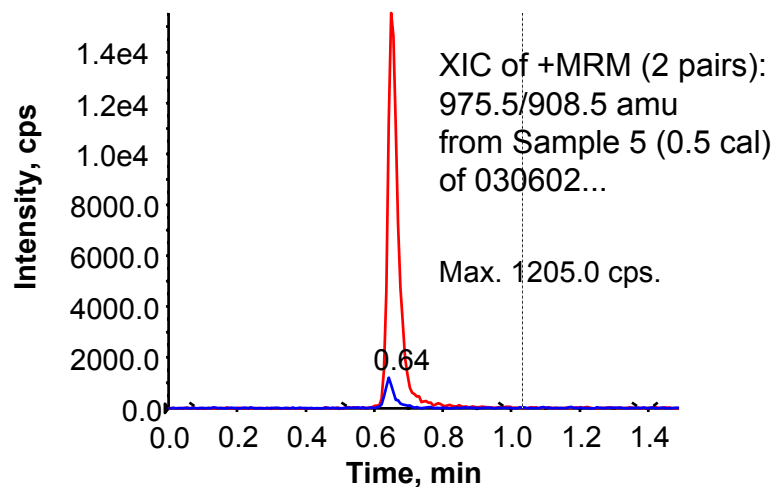
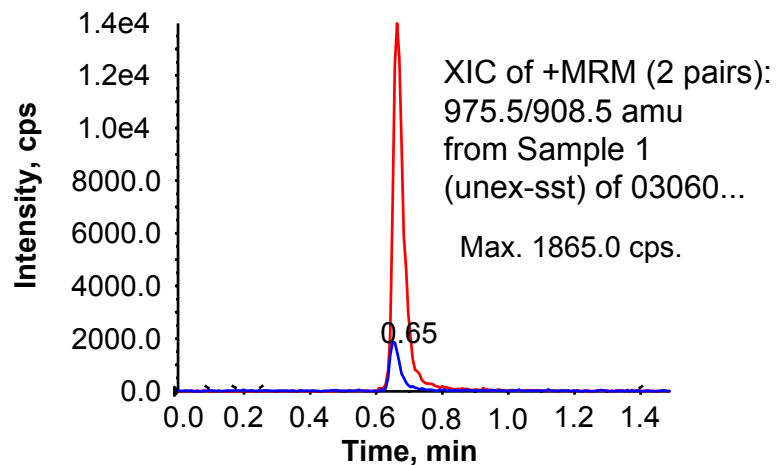


Conclusion : DBC18 doesn't bleed





Pharmaceutical LC/MS/MS



Immunosuppressant Drugs by LC/MS/MS[†]

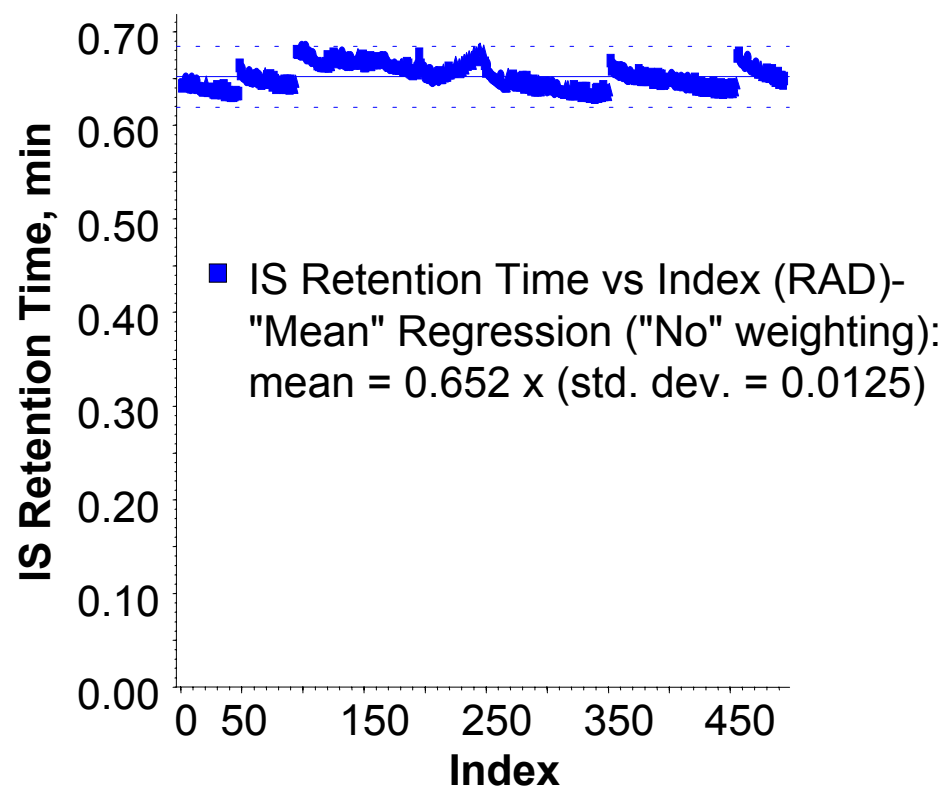
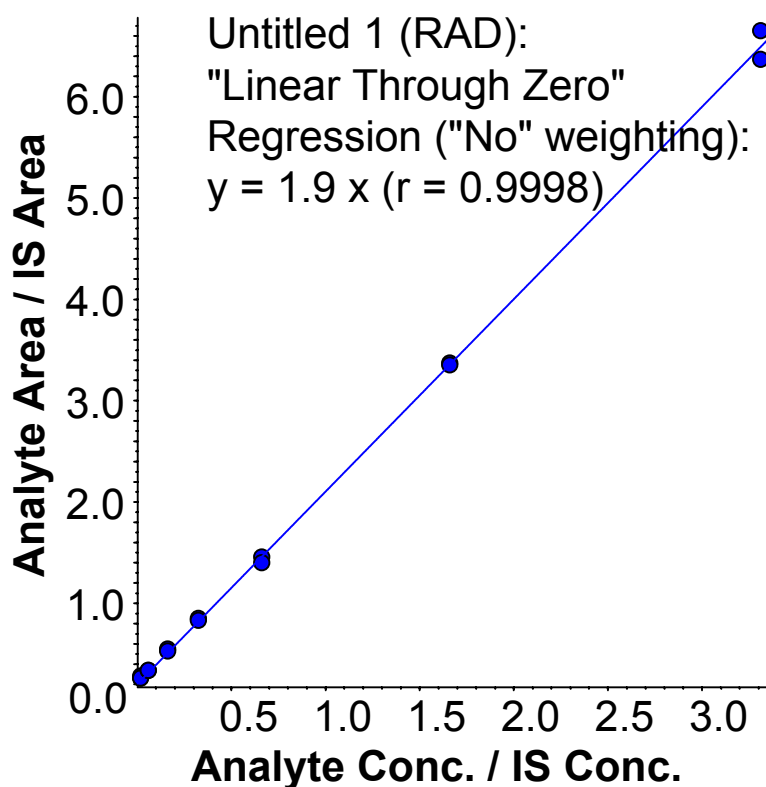
- **Blue** – Certican (Everolimus)
- **Red** – Internal Standard
- Column– 50mm X 4.6mm DBC18
 - 80° C @ 1.5 mL/min
 - Solvent A – 20mM NH₄CH₃CO₂ in 70% Acetonitrile, 30% aqueous (0.1% acetic acid, pH 4.5)
 - Solvent B – Acetonitrile
- Isocratic 35% A, 65%B – 20 uL injection

[†] Data Courtesy R. Clouette - Clinical Reference Laboratories



Linearity and Reproducibility

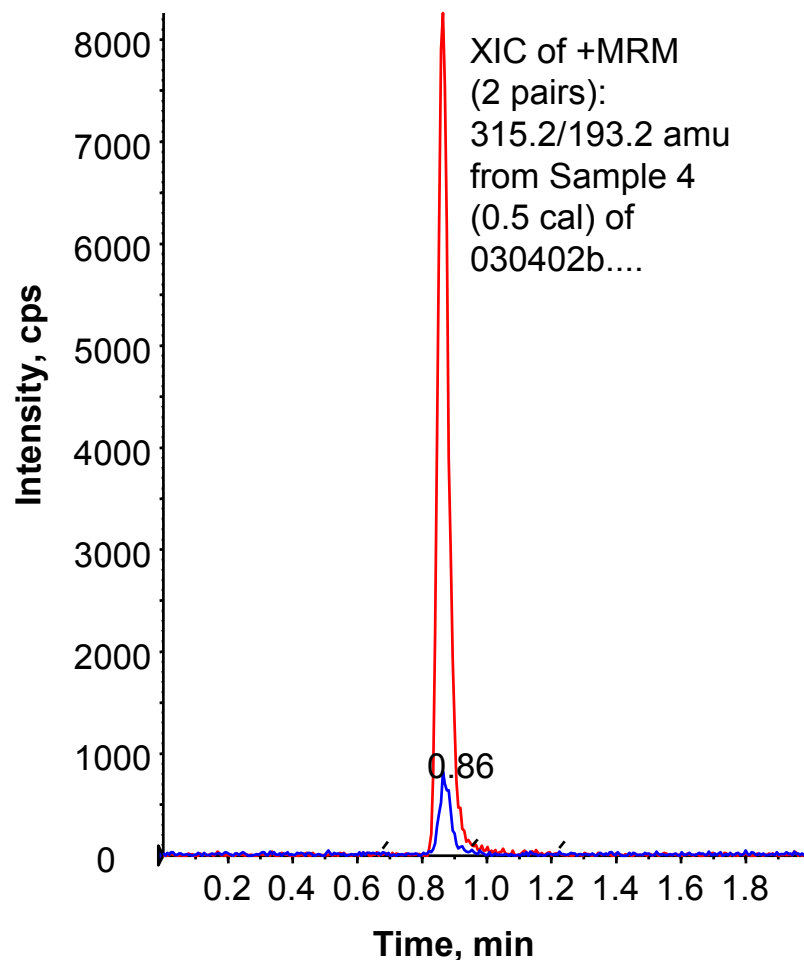
Immunosuppressant (Certican) by LC/MS/MS[†]



[†] Data Courtesy R. Clouette - Clinical Reference Laboratories



Pharmaceutical LC/MS/MS



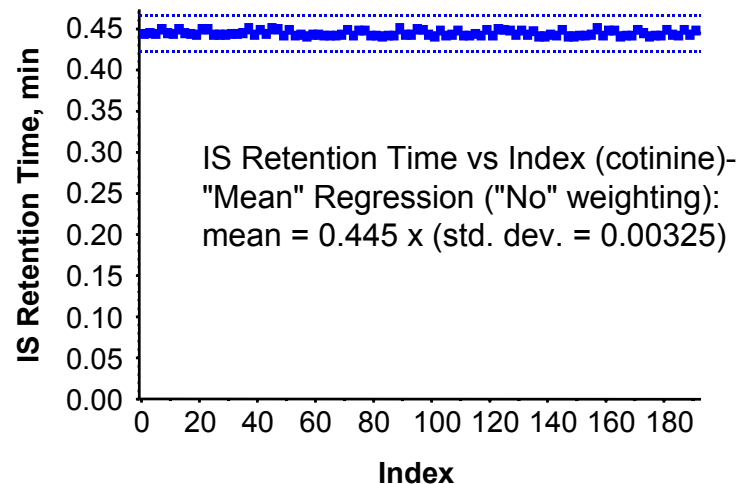
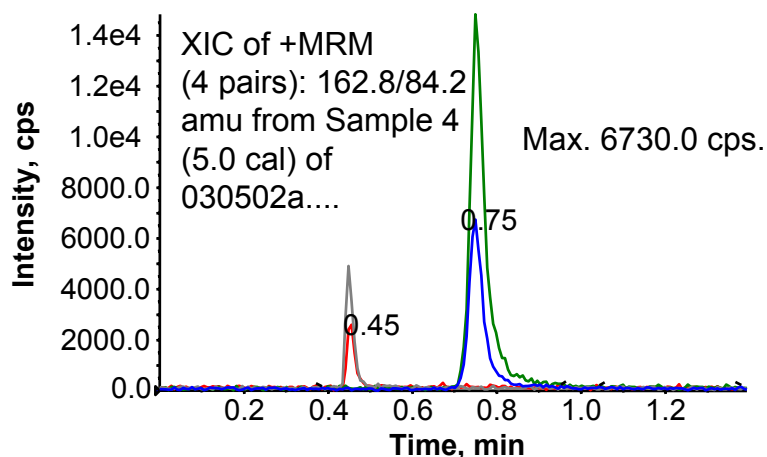
THC in Saliva by LC/MS/MS[†]

- **Blue** – THC (tetrahydrocannabinol parent drug)
- **Red** – D3 THC (Internal Standard)
- Column – 50mm X 4.6mm DBC18
 - 80° C @ 1.5 mL/min
 - Solvent A – 20mM NH₄CH₃CO₂ in 70% Acetonitrile, 30% aqueous (0.1% acetic acid, pH 4.5)
 - Solvent B – Acetonitrile
- Isocratic 35% A, 65%B – 25 uL injection

[†] Data Courtesy R. Clouette - Clinical Reference Laboratories



Pharmaceutical LC/MS/MS



Cotinine and Nicotine in Saliva by LC/MS/MS[†]

- Grey – Cotinine Internal Standard
- Red – Cotinine
- Green – Nicotine Internal Standard
- Blue – Nicotine
- Column– 50mm X 4.6mm DBC18
 - 80° C @ 1.5 mL/min
 - Solvent A – 20mM NH₄CH₃CO₂ in 70% Acetonitrile, 30% aqueous (0.1% acetic acid, pH 4.5)
 - Solvent B – Acetonitrile
- Isocratic 35% A, 65%B – 10uL injection

[†] Data Courtesy R. Clouette - Clinical Reference Laboratories



Conclusions

- Bonded carbon phases behave as expected with respect to ligand type and ligand density
 - Polar Embedded Group has same effect as on silica
- The carbon-carbon attachment bond is extremely stable
 - Low pH and High pH applications, High Temperature / Fast HPLC
 - No bleed in LC/MS, even at high pH
- Both “normal” and high pH, high temperature applications are possible on these new materials
 - LC/MS/MS pharmaceutical applications enabled by this technology



Acknowledgements



ZirChrom (Booth #3036)

- Pete Carr
- Clayton McNeff
- Dwight Stoll
- Bingwen Yan
- Alturas Analytics
 - Shane Needham
- Clinical Reference Labs
 - Randy Clouette

• Cabot Group

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- Elena Khmel'nitskaia
- Lynn Toomey
- Feng Gu
- Sean Sullivan
- Kelly Belmont