ZirChrom®

Characterization of an Efficient *No-Bleed* *Carbon-Based HPLC* *Support for Pharmaceutical Separations*

by

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Dwight R. Stoll, Danielle R. Hawker,
Angelos Kyrlidis (Cabot), Greg Gaudet (Cabot)

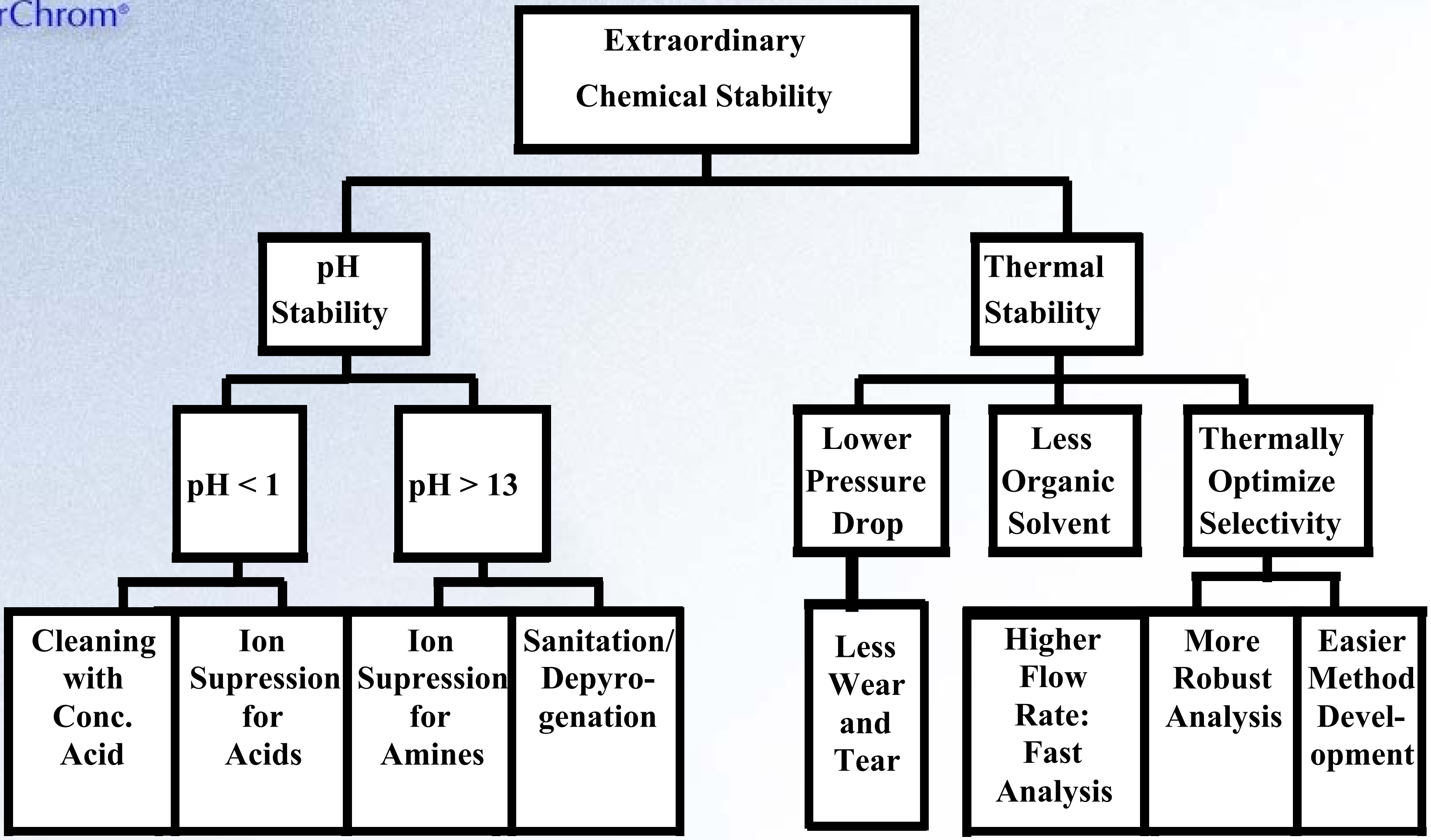
and

Shane Needham (Alturas Analytics).

OUTLINE

- Analytical Advantages of Column Stability.
- Production of ZirChrom®-CARB.
- Selectivity Comparison of ZirChrom®-CARB, Hypercarb and Other RP-Zirconias.
- MS Column Bleed Comparison: ZirChrom®-CARB and Leading Silica Columns.
- Conclusions (CARB shows ultra-low bleed).

Analytical Advantages of Column Stability



NO COLUMN BLEED

Why is Bleed Bad?

- At same mass and elution time it can obscure peak of interest and make quantitation difficult, increases L.O.D. and L.O.Q.
- At same mass and elution time it can cause a chase for "ghost" impurities in sample.
- At same elution time but **different mass** can cause ionization problems for analyte of interest.

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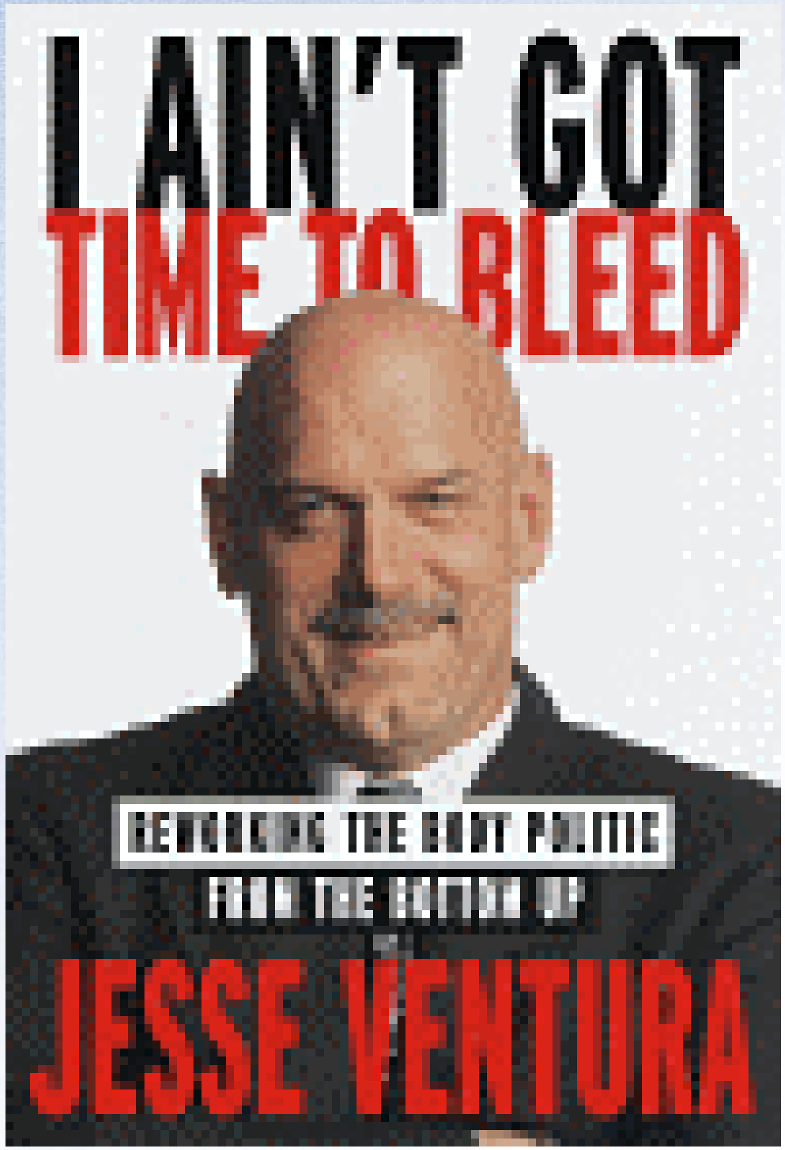
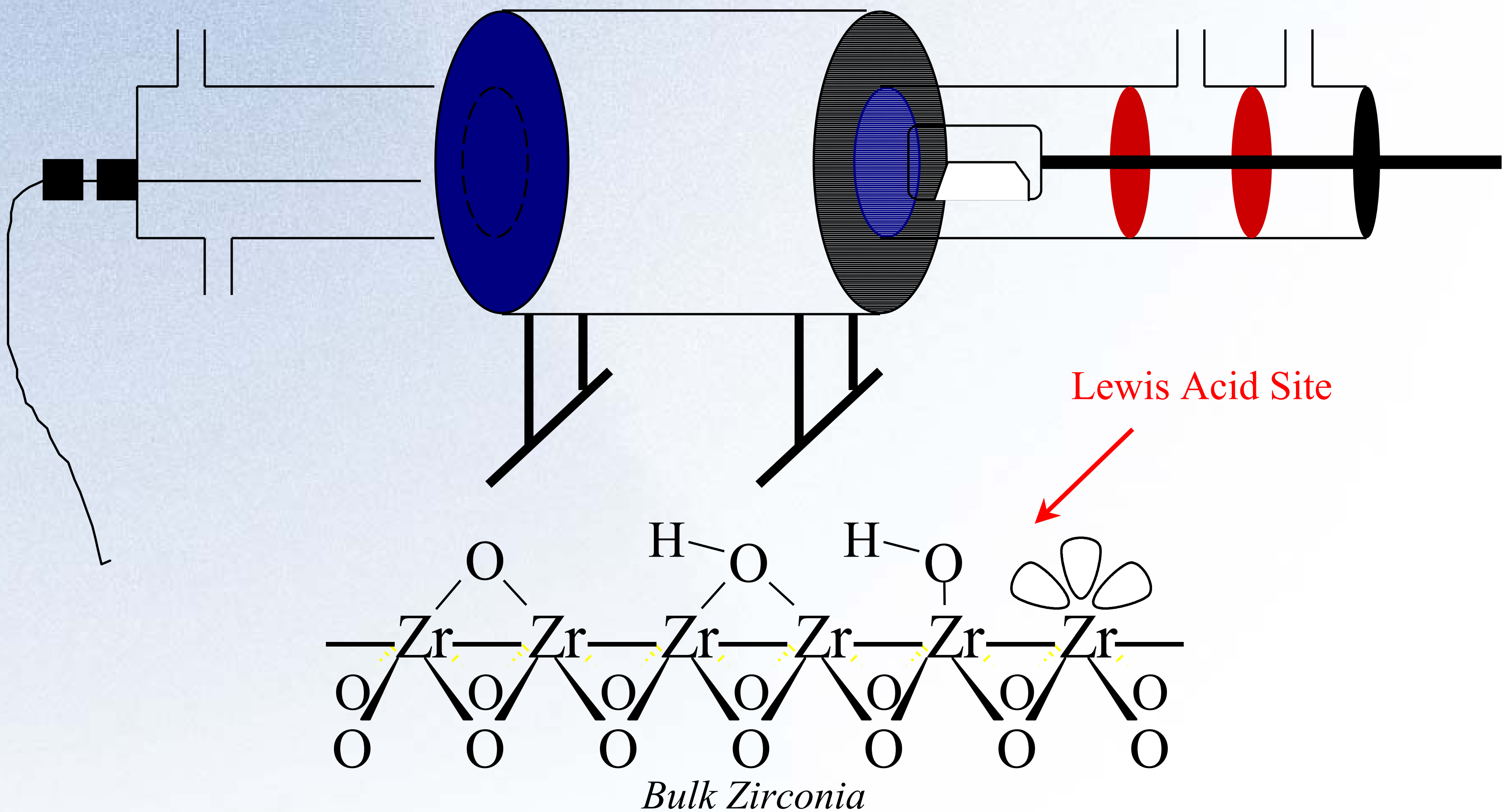


Proprietary Synthesis of Carbon-Clad Zirconia



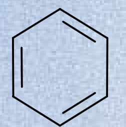
Comparison of ZirChrom CARB and Hypercarb

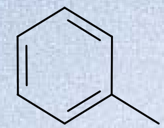
CHARACTERISTIC	HYPERCARB	ZirChrom CARB
Pore Diameter (A)	270	240
Pore Volume (cc/g)	0.7	0.14
Particle Porosity	0.61	0.43
Area/Vol. Mobile Phase (m^2/g)	88	69
k' (toluene) [a]	7.8	2.7
N (toluene) [a]	41,000	92,000

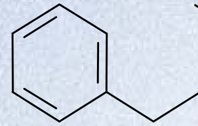
a. 40/60 ACN-water 30 °C

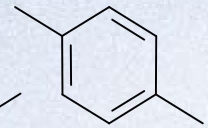
ZirChrom®

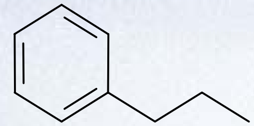
22 Non-Electrolyte Probe Solutes

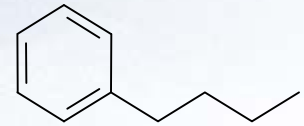

Benzene

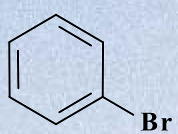

Toluene


Ethylbenzene

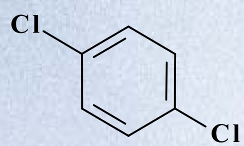

p-xylene


Propylbenzene

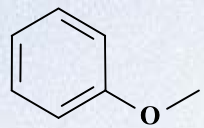

Butylbenzene



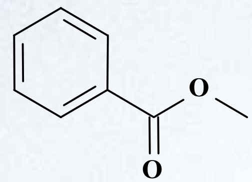
Bromobenzene



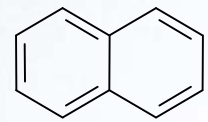
p-Dichlorobenzene

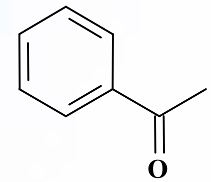


Anisole

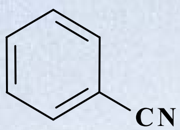


Methylbenzoate

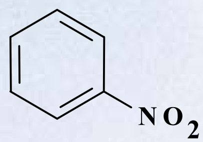

Napthalene



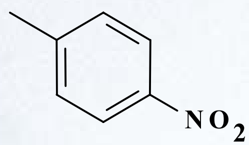
Acetophenone



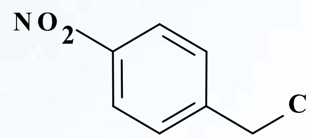
Benzonitrile



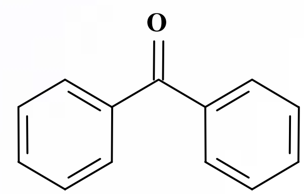
Nitrobenzene



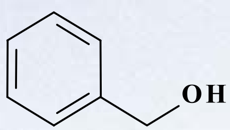
p-Nitrotoluene



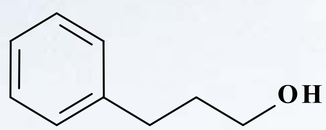
p-Nitrobenzyl Chloride



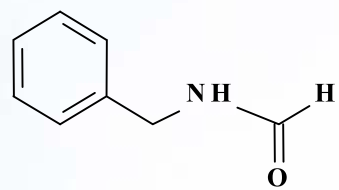
Benzophenone



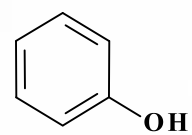
Benzylalcohol



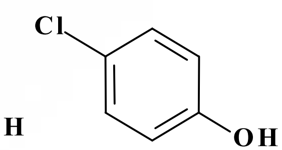
3-Phenyl Propanol



N-Benzyl Formamide

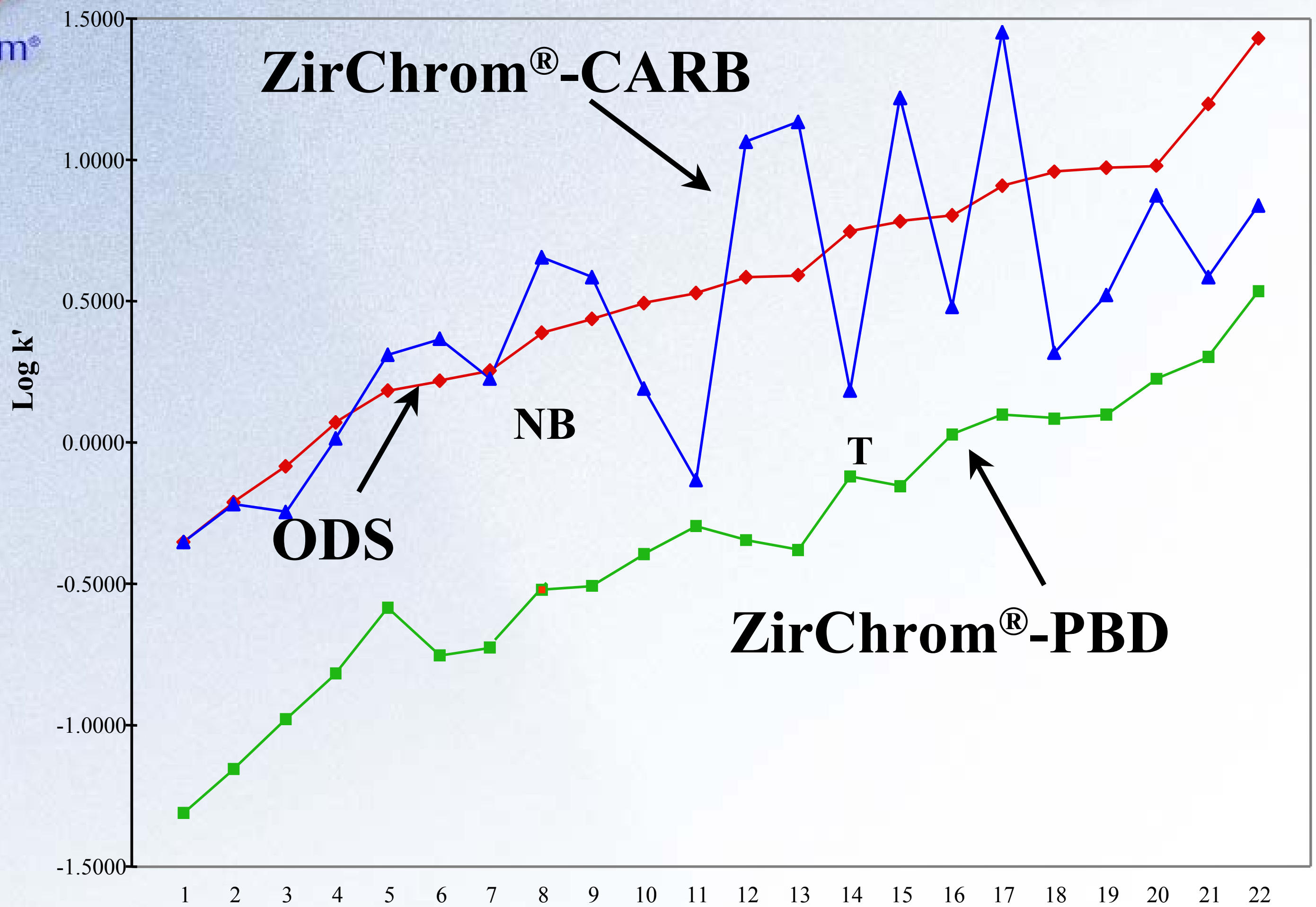


Phenol



p-Chlorophenol

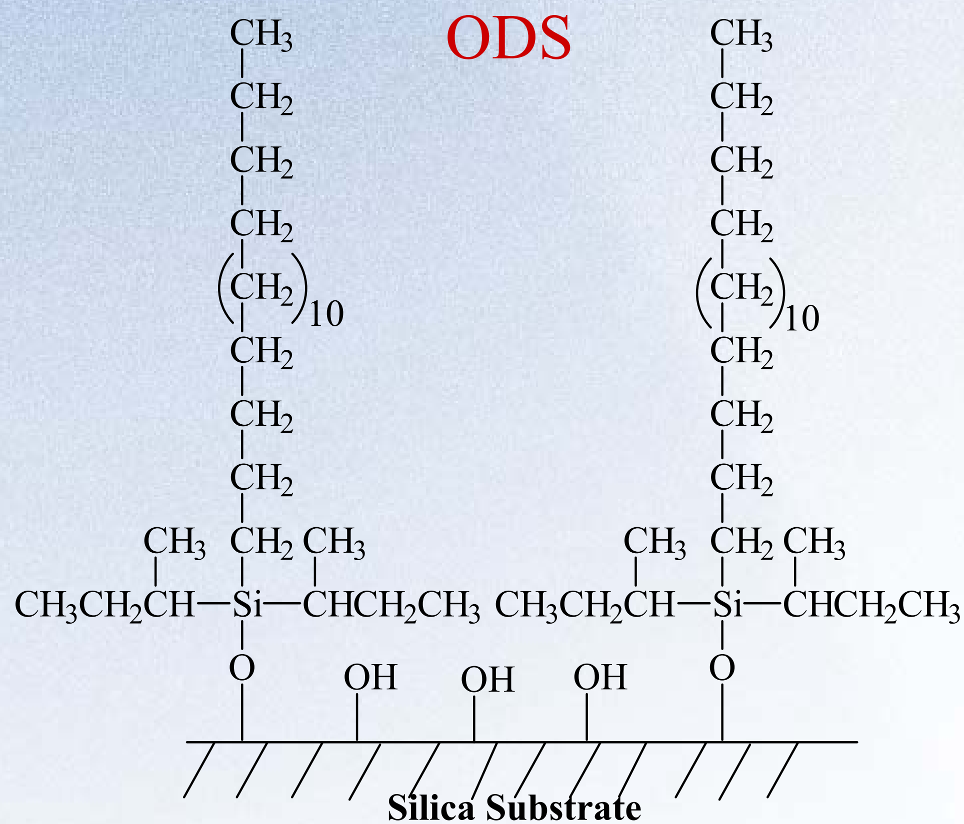
Comparison of Selectivity of ODS, ZirChrom®-PBD &-CARB



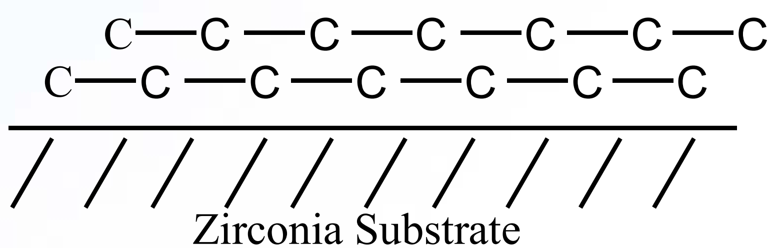
1. N-benzyl formamide
2. Benzylalcohol
3. Phenol
4. 3-phenyl propanol
5. p-chlorophenol
6. Acetophenone
7. Benzonitrile
8. Nitrobenzene
9. methyl benzoate
10. Anisole
11. Benzene
12. p-chlorotoluene
13. p-nitrobenzyl chloride
14. Toluene
15. Benzophenone
16. Bromobenzene
17. Naphthalene
18. Ethylbenzene
19. p-xylene
20. p-dichlorobenzene
21. Propylbenzene
22. n-butylbenzene

Chemical Structure of ZirChrom®-CARB

ODS



ZirChrom®-CARB



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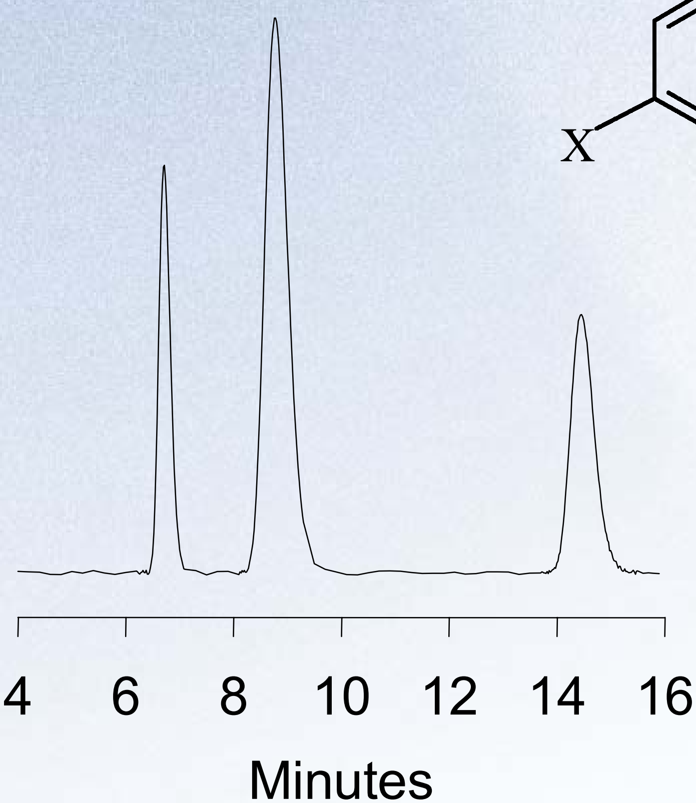
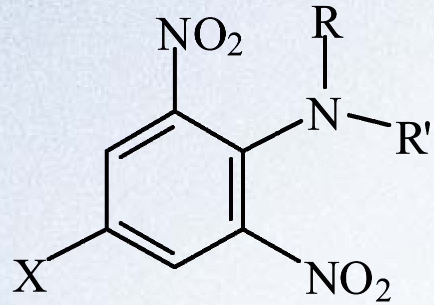
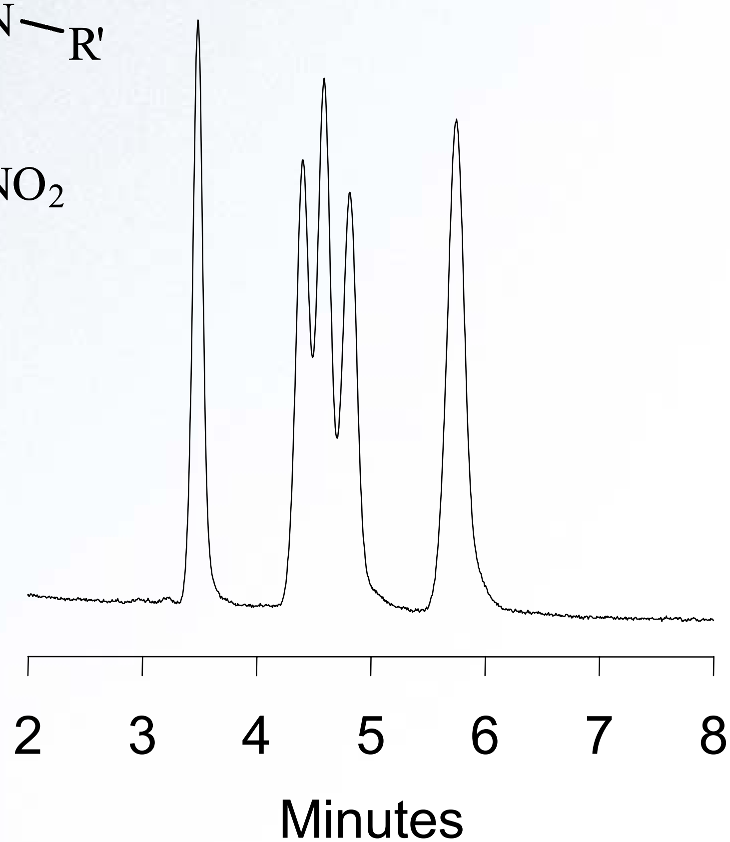
Dinitroaniline Herbicide Separation (EPA Method 627)

ODS





Carbon

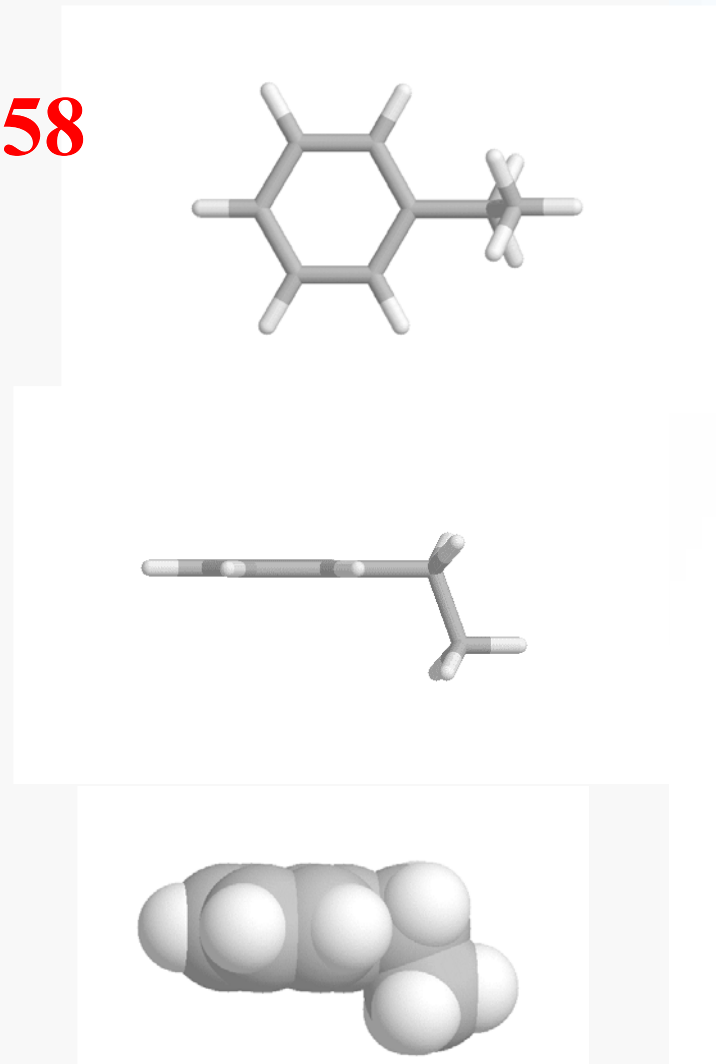


p-xylene

α_{ODS}=1.03

ethylbenzene

$\alpha_{C\text{-}Zr}$=1.58



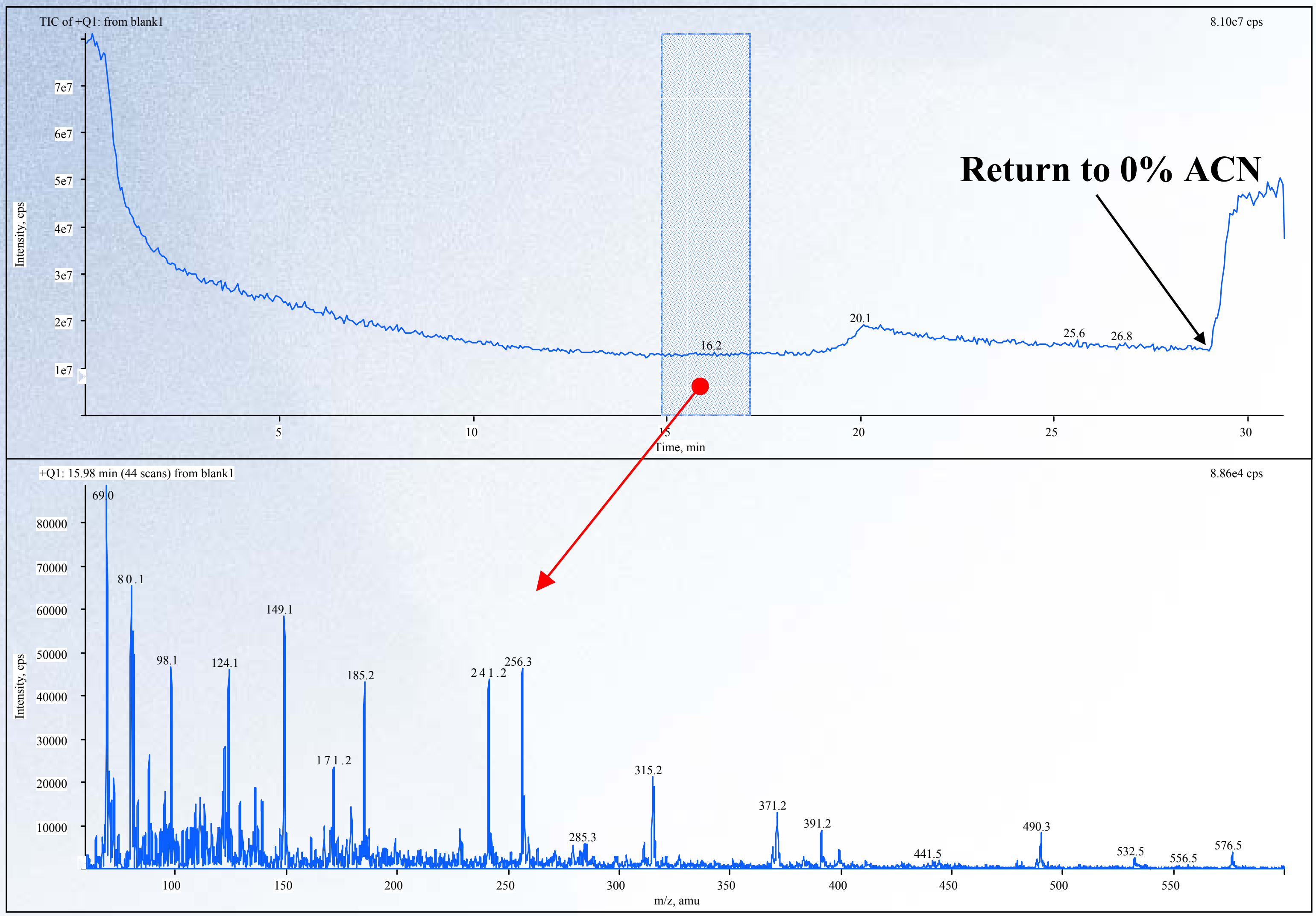
C-C-C-C-C-C-C-C-C-C-C-C

C-C-C-C-C-C-C-C-C-C-C-C

General Conditions of M.S. Bleed Study

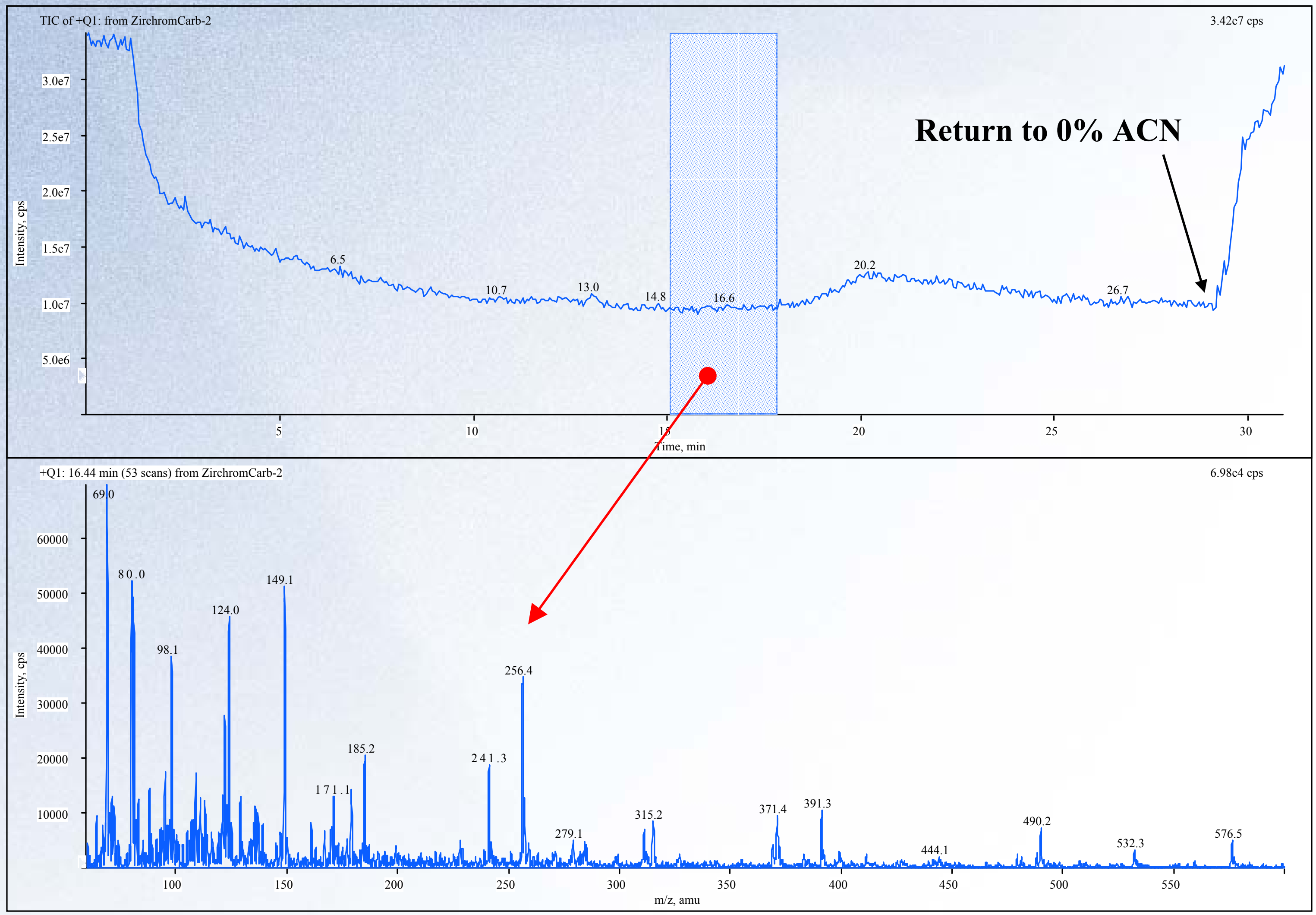
- SCIEX APCI 3000 triple quad in single quad mode at 400 ^{o}C.
- Total ion current 60-600 amu.
- Spectra is sum of 30-35 scans in time window.
- Mobile phase gradient 0-100% ACN/buffer in 15 min. plus 14 min. hold at 100%.
- pH 7 = water only.
- pH 9 = 2mM NH_4Ac + NH_4OH.
- Note pump-column-MS. No injector used.

Gradient Blank at pH 9 and 55 °C



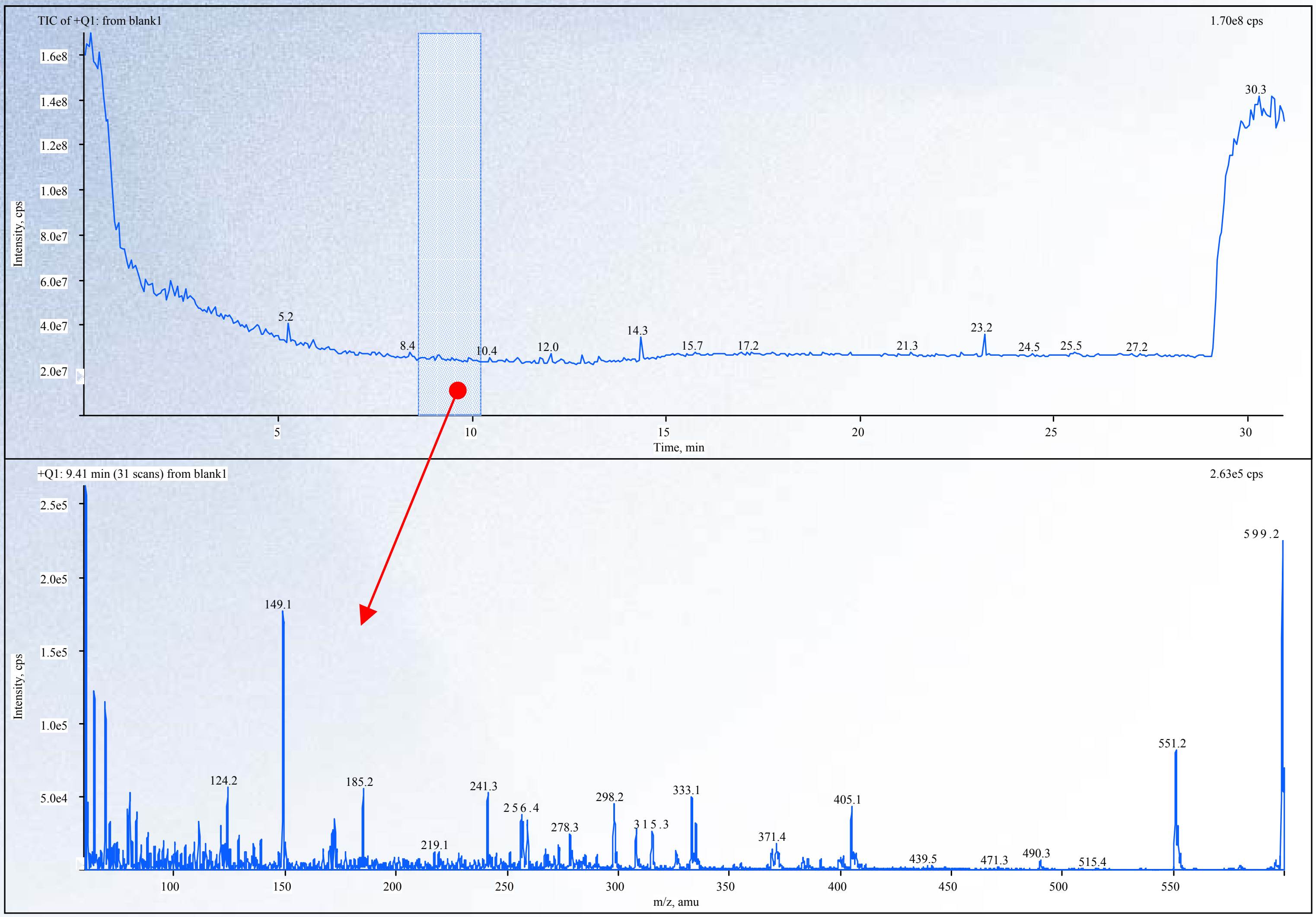
M.S. shows typical numerous, ubiquitous phthalate contaminants.Spectra are same from start to finish of gradient.

ZirChrom-CARB at pH 9 and 55 ^{o}C



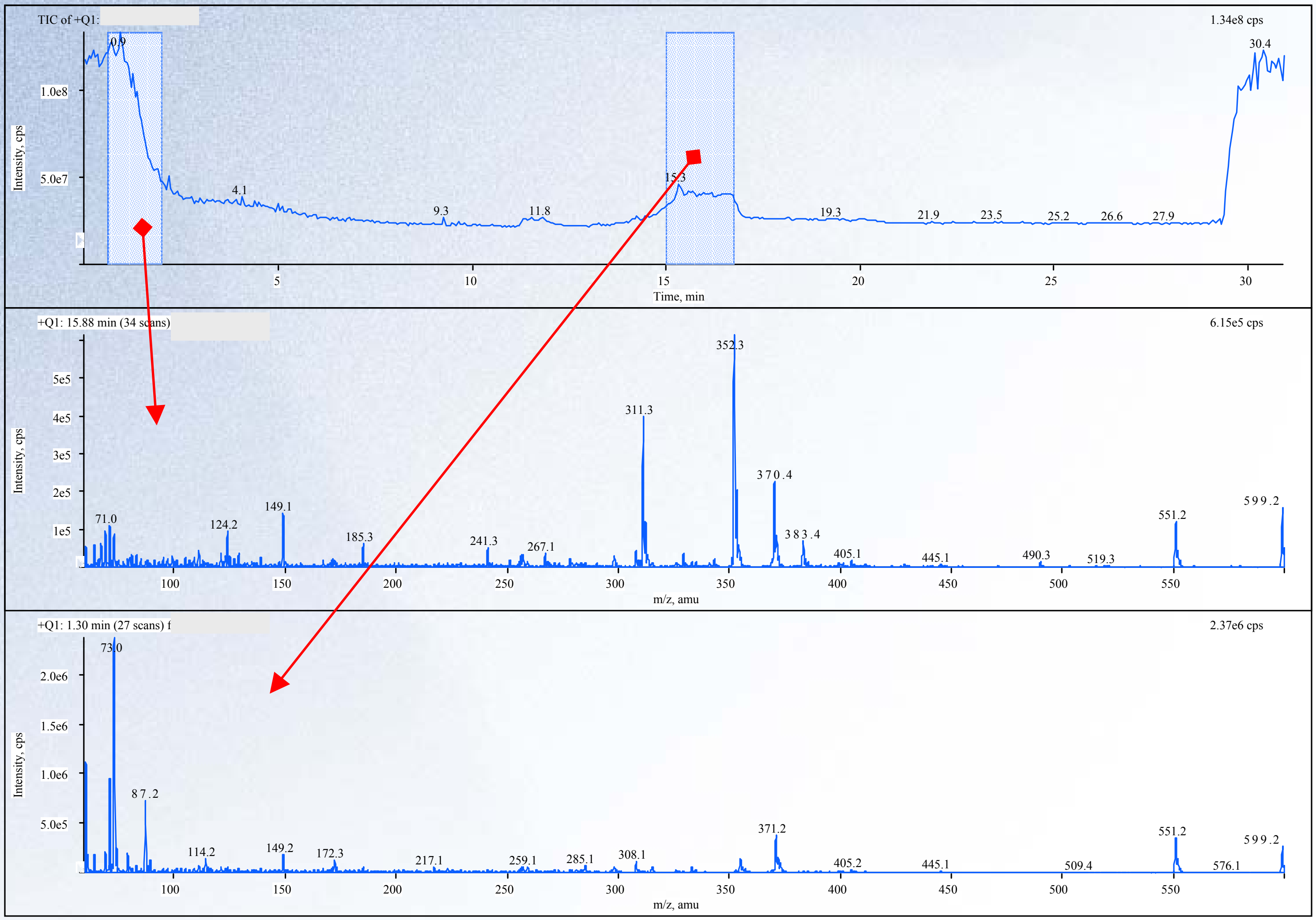
Total ion chromatogram and mass spectra with ZirChrom CARB nearly identical to blank gradient. No new discernible masses or peaks due to column.

Blank Gradient at pH 7 & Ambient Temp.



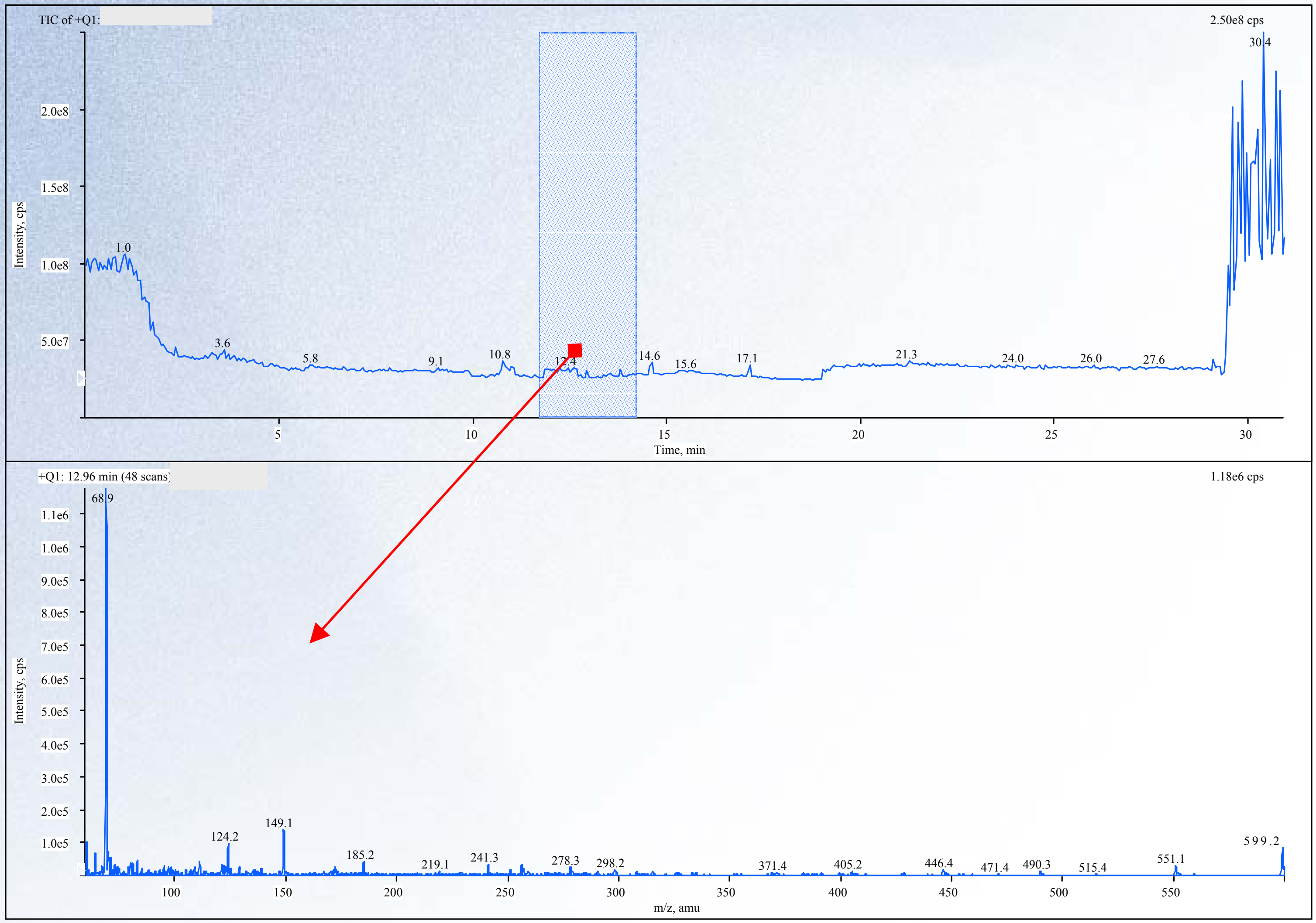
Total ion current appears cleaner and m.s. looks different than blank at pH 9 but this is due to changes in ionization (no NH_4^+).

M.S. Grade SiO_2 (A.) at pH 7 and Ambient Temperature



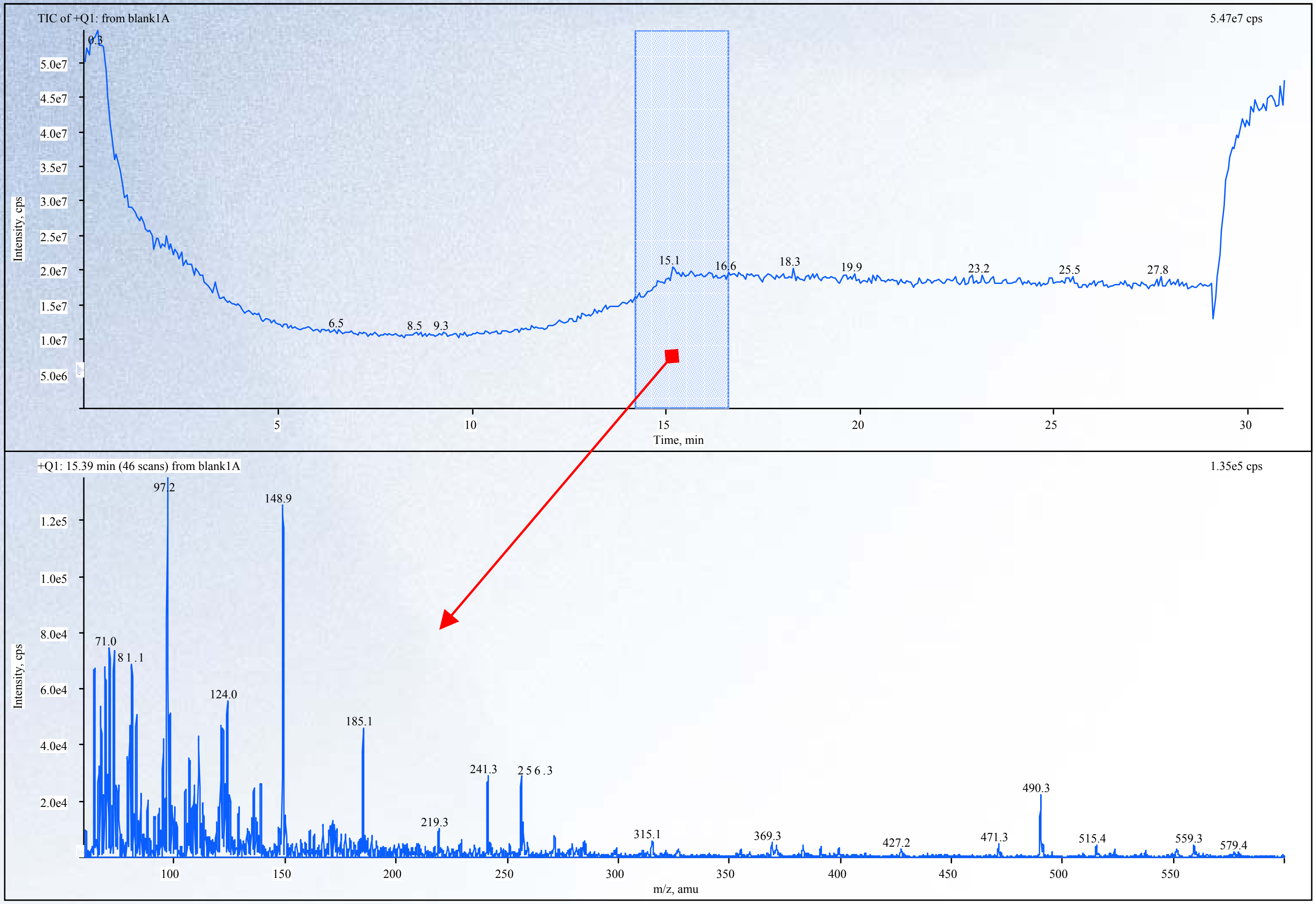
New "humps" in total ion chromatogram and new masses are evident in spectra.

M.S. Grade SiO_2 (B.) at pH 7 and Ambient Temperature



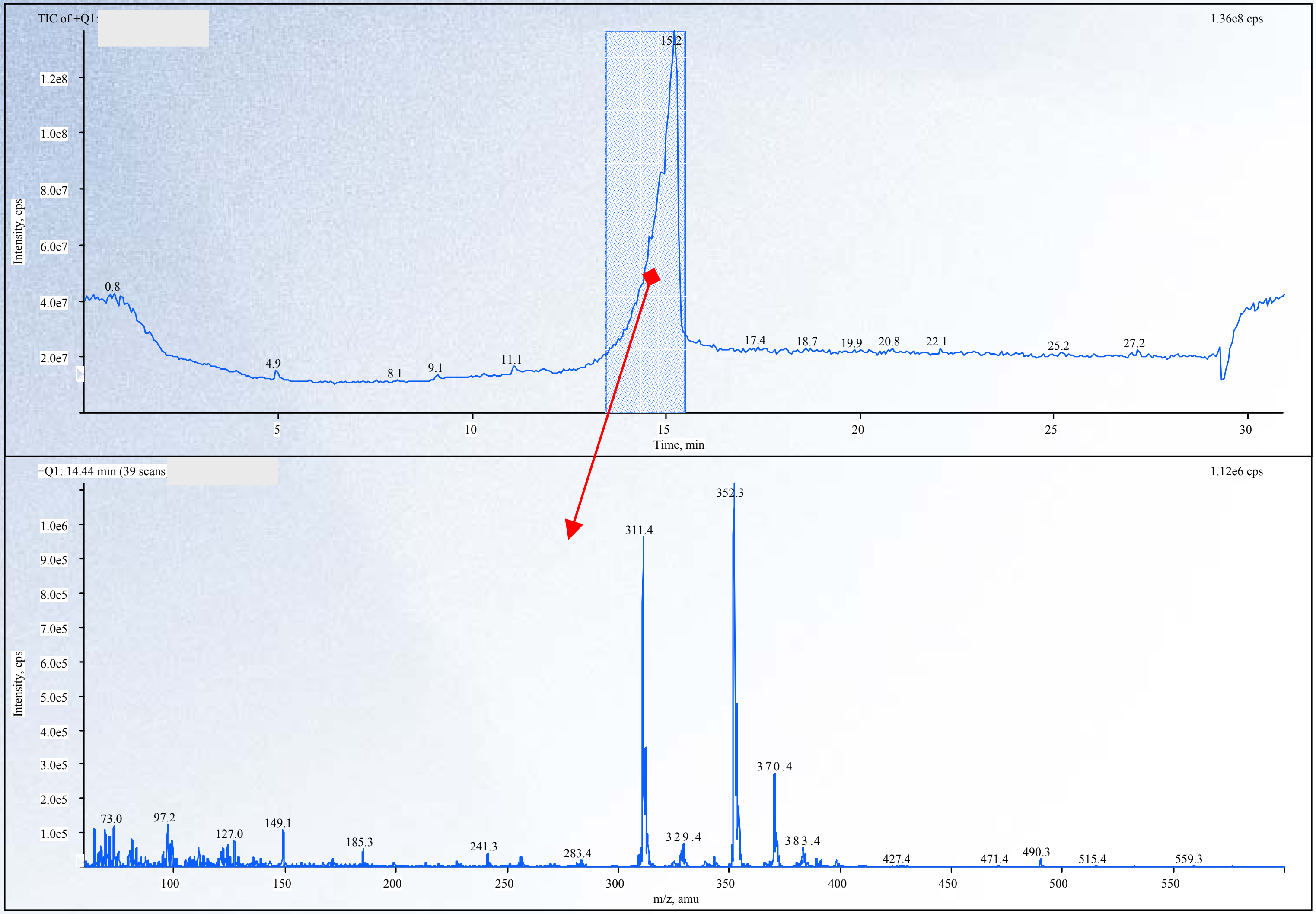
Note huge increase in mass 69 (identity unknown).

Blank Gradient at pH 9 and 55 ^{o}C



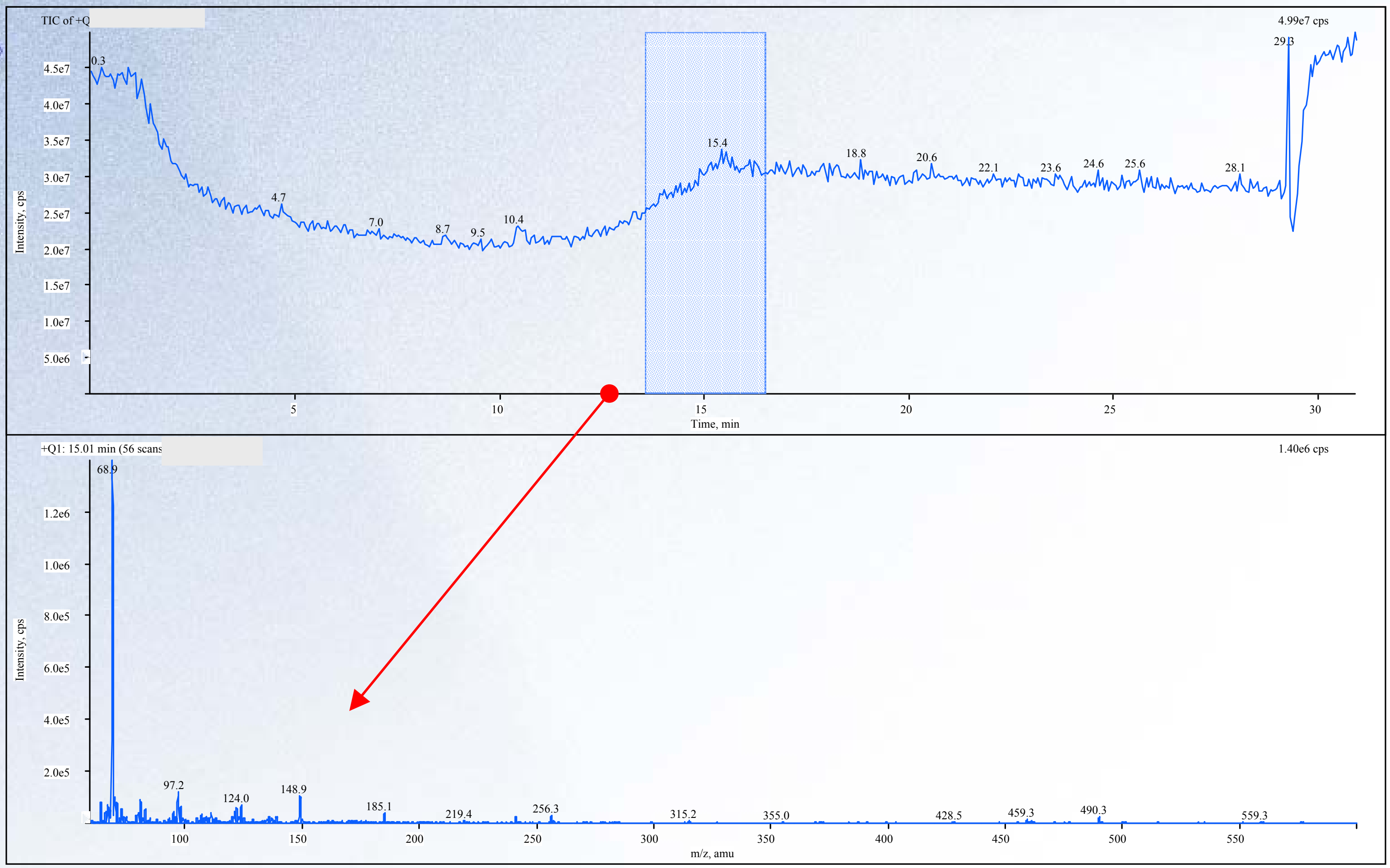
M.S. different than blank gradient at pH 7 probably due to ionization differences.

M.S. Grade SiO_2 (A.) at pH 9 and 55 °C



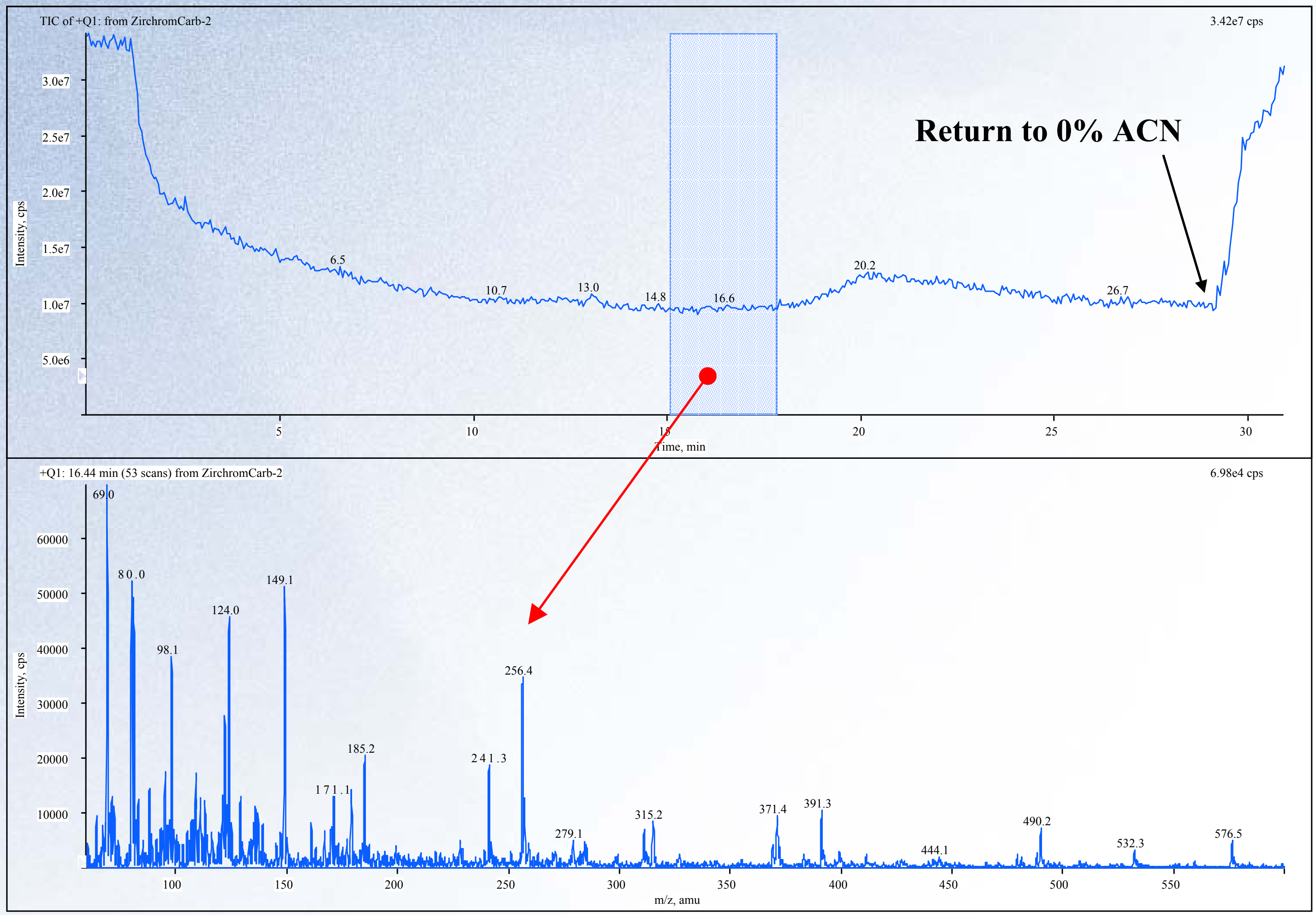
Note big peak at 12-15 min. in total ion chromatogram and new peaks in mass spec.

M.S. Grade SiO_2 (B.) at pH 9 and 55 ^oC



Note new peak in total ion chromatogram and increase in mass 69.

ZirChrom-CARB at pH 9 and 55 °C



Total ion chromatogram and mass spectra with ZirChrom CARB nearly identical to blank gradient. No new discernible masses or peaks due to column.

Conclusions

ZirChrom®

- **ZirChrom-CARB** is ***ultra-durable*** and ***efficient***.
- **ZirChrom-CARB** is stable at the ***extremes of pH*** and at ***200 ^{o}C***.
- **ZirChrom-CARB** has ***radically different selectivity***.
- **ZirChrom-CARB's** durability allows for ***ultrafast*** (< 1 min.) separations at elevated temperatures.
- **ZirChrom-CARB** shows "***No-Bleed***" by MS under highly aggressive LC conditions (pH 9, 55 ^{o}C).

ZirChrom Technical Presentations and Posters

Time	Date	Room #	Title	Who	Program #
8:50-9:10 am	March 18 (Monday)	245	Chromatographic Characterization of an Efficient No-Bleed Carbon-based HPLC Support for Pharmaceutical Separations	**Clayton V. McNeff**, Dwight Stoll, Peter Carr, Bingwen Yan	160
9:30-9:50 am	March 18 (Monday)	245	Development of a Novel Class of Ultra-stable Covalently Bonded Carbon-Based HPLC Phases with Unique Chromatographic Selectivity for Use in Pharmaceutical Analysis	**Gregory Gaudet**, Peter Carr, Clayton V. McNeff, Dwight Stoll, Danielle Hawker, Angelos Kyrlidis	162
9:10-9:30 am	March 19 (Tuesday)	243	Development of Carbon Based Chiral Separation HPLC Column	**Feng Gu**, Angelos Kyrlidis, Clayton V. McNeff, Steve Ponto, Peter W. Carr, John A. Blackwell	523
10:00-12:30 pm	March 19 (Tuesday)	Aisle 100 Exhibit Floor	A New Generation of Covalently Bonded Stationary Phases on Carbon Clad Nonpourous Zirconia for Fast High Temperature Liquid Chromatography	**Clayton V. Mc Neff**, Gregory Gaudet, Angelos Kyrlidis, Peter Carr, Bingwen Yan, Alan McCormick	1989P
8:30-8:50 am	March 20 (Wednesday)	244	Ultra-fast Liquid Chromatography: The Importance of System Temperature	**Jon D. Thompson**, Peter W. Carr	760
8:50-9:10 am	March 20 (Wednesday)	244	A Novel Approach to Column Heaters for Application to Ultra-fast High Temperature (UFHTLC) and Thermally Tuned Tandem Column (T3C) Liquid Chromatography.	**Peter W. Carr**, Jon D. Thompson, Yun Mao, Carl W. Sims, Yuri E. Gerner, Tom Thielen	761
9:30-9:50 am	March 21 (Thursday)	235-236	Optimizing the Separations of Basic Compounds in Mixed-Mode Separations by Understanding the Role of Lewis Acidity of Zirconia Based Phases	**Xiqin Yang**, Peter Carr	1089
10:00-12:30 pm	March 21 (Thursday)	Aisle 100 Exhibit Floor	One-Step Monoclonal Antibody Purification Method Using Preparative Porous Ethylenediamine-N, N'-tetra(methylphosphonic) Acid Modified Zirconia	**Clayton V. McNeff**, Dwight Stoll, Peter Carr, Danielle Hawker, Anuradha Subramanian	2233P
10:00-12:30 pm	March 21 (Thursday)	Aisle 100 Exhibit Floor	Stable RPLC Phases: Are They Really Stable? A Comparison of the Stability, Symmetry, Selectivity and Efficiency of Leading Stable Phases at Elevated pH	**Clayton V. McNeff**, Dwight Stoll, Peter Carr, Danielle Hawker	2323P

Thanks *very much* for listening!

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ZirChrom Separations & Cabot Corporation

Partners in Chromatography

For more information and web access to the free **Buffer Wizard:** www.zirchrom.com

Selectivity Comparison of ZirChrom-CARB to Other RP Zirconia Phases

- **LC Conditions**: Mobile phase, 40/60 Acetonitrile/50mM phosphate at pH 3.2), Flow rate, 1.0 ml/min., Temperature = 30 ^{o}C, Detection at 254nm, 5µl Injection volume.
  (LC-GC, Vol 13, No. 9, September 1995, 720-726.)
- **Columns Tested**: DiamondBond™-C18, Luna® C18, ZirChrom®-PBD, Gammabond™, Xterra™, Hamilton PLRP™

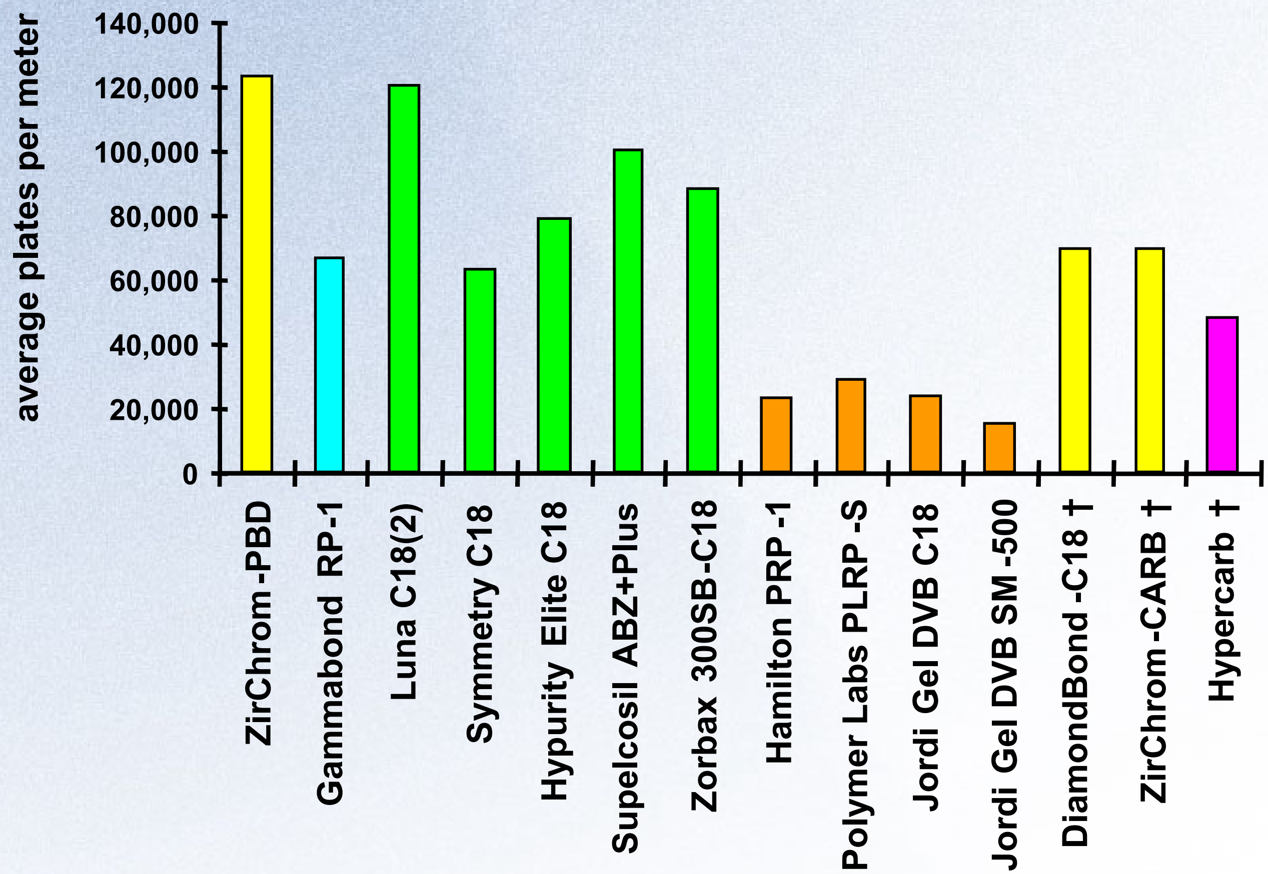
Phase Comparison by Covariance of log k'-log k' Plots*

	CARB	DB-C18	PBD	RP18	C18 (2)	PLRP-S	RP-1
ZirChrom-CARB	1						
DiamondBond-C18	0.80	1					
ZirChrom-PBD	0.51	0.90	1				
Xterra RP18	0.53	0.85	0.90	1			
Luna C18 (2)	0.53	0.86	0.93	0.97	1		
PLRP-S	0.60	0.90	0.93	0.92	0.96	1	
Gammabond RP-1	0.52	0.88	0.96	0.97	0.98	0.95	1

* Column names are the trademarks of their respective manufacturers.

- ZirChrom-CARB most different from ODS.

Efficiency Comparison of Leading HPLC Columns*



LC Conditions: Mobile Phase, 65/35 Acetonitrile/50mM Potassium phosphate buffer, pH 3.2; Flow Rate, 1.0 mL/min.; Injection volume, 1 ul; 254 nm detection; Column Temperature, 21°C. Solutes: uracil, phenol, pyridine, 4-butylbenzoic acid, N,N – dimethylaniline, toluene.

† Mobile Phase: 45/5/50 Acetonitrile/THF/50mM Potassium phosphate buffer, pH 7.0; Column Temperature: 30°C; all other conditions are identical.