



*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).

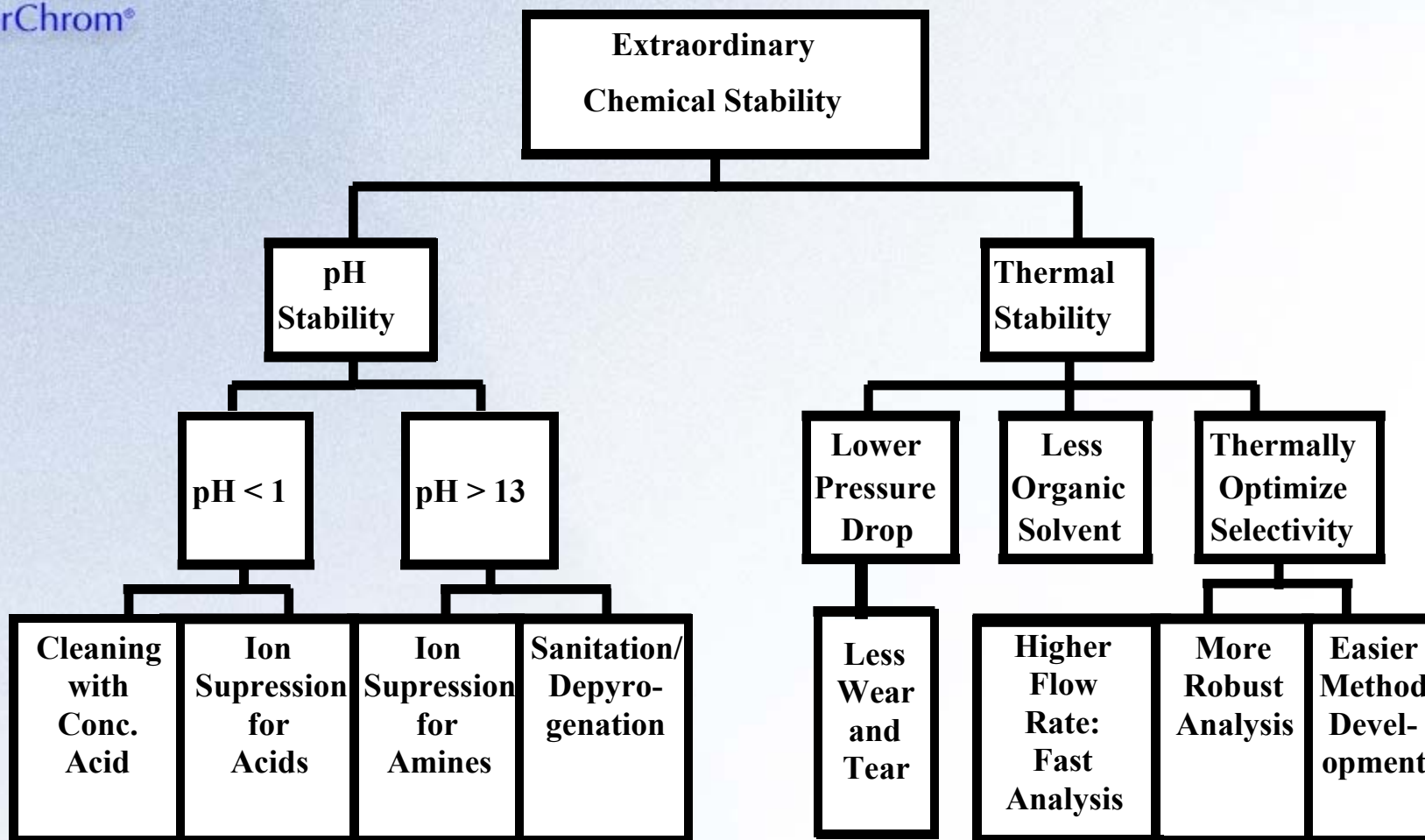


# OUTLINE

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



# Analytical Advantages of Column Stability



**NO COLUMN BLEED**



# Why is Bleed Bad?

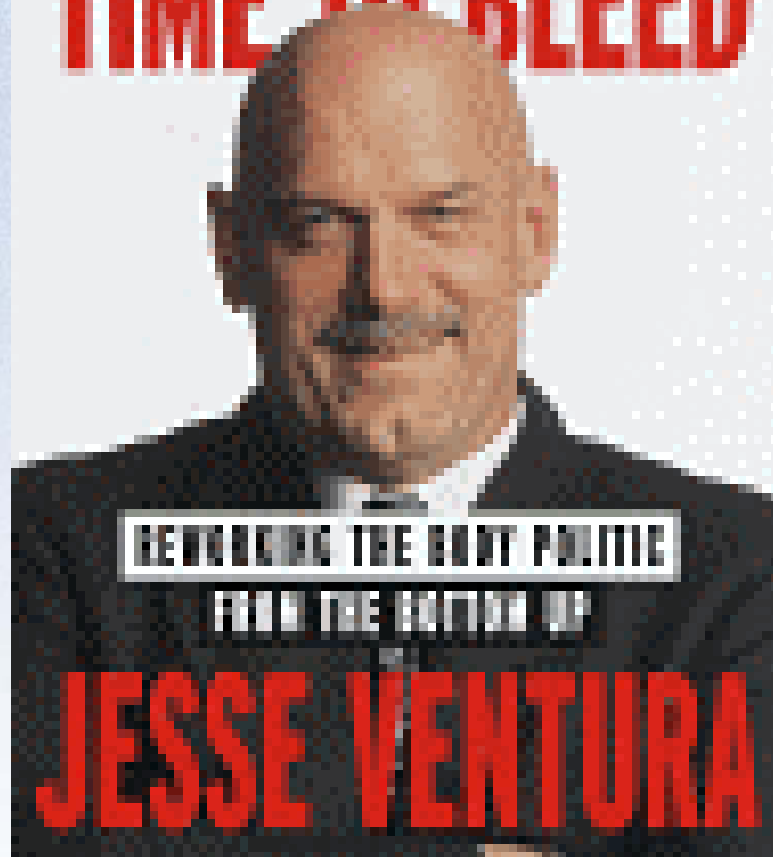
- ◆ At same mass and elution time it can obscure peak of interest and make quantitation difficult, increases L.O.D. and L.O.Q.
- ◆ At same mass and elution time it can cause a chase for “ghost” impurities in sample.
- ◆ At same elution time but **different mass** can cause ionization problems for analyte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





ZirChrom®

**I AIN'T GOT  
TIME TO BLEED**



**REWORKING THE BODY POLITIC**

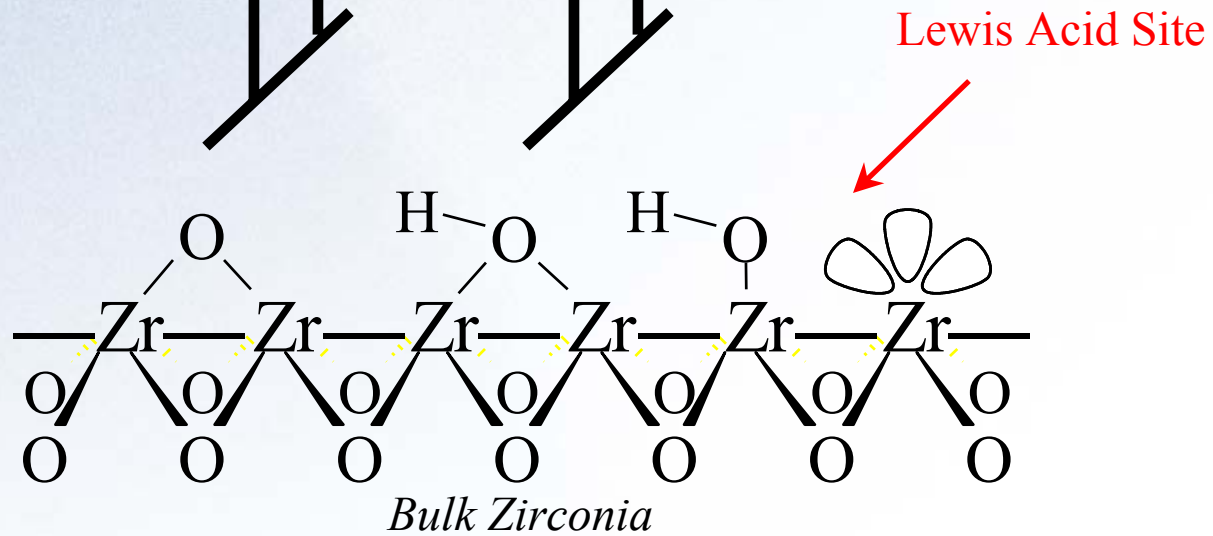
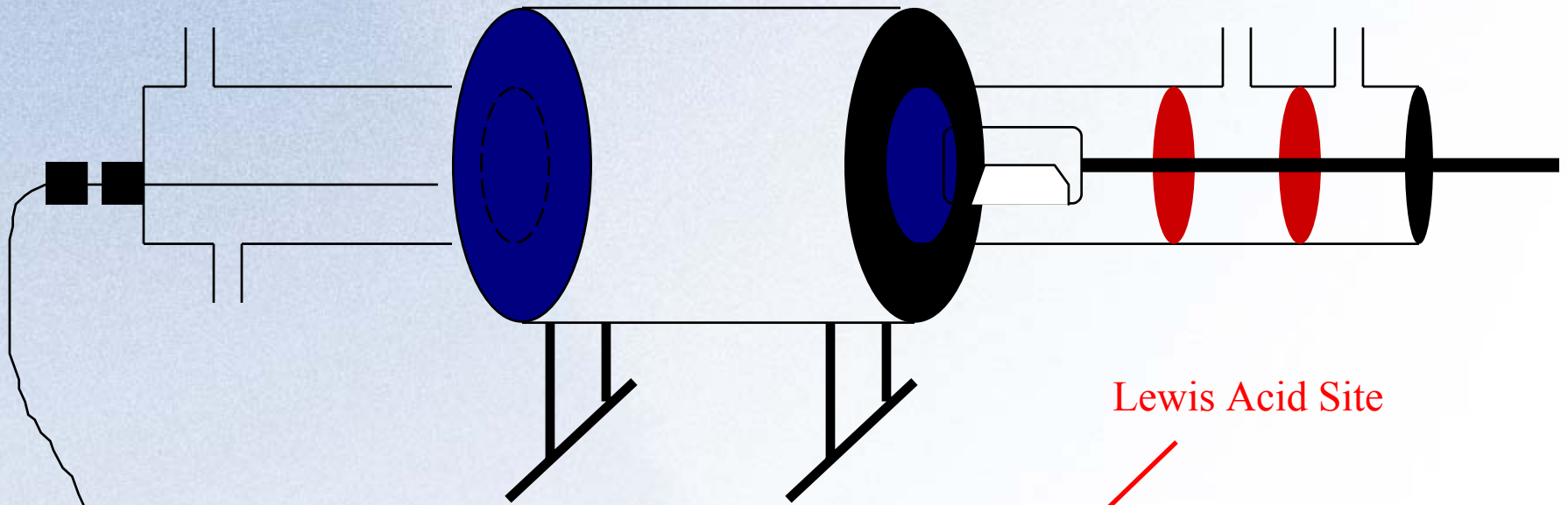
**FROM THE BOTTOM UP**

**JESSE VENTURA**



ZirChrom®

# Proprietary Synthesis of Carbon-Clad Zirconia





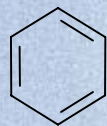
# Comparison of ZirChrom CARB and Hypercarb

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

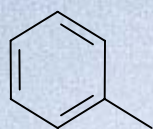
a. 40/60 ACN-water 30 °C



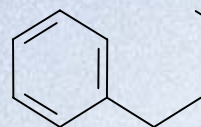
# 22 Non-Electrolyte Probe Solutes



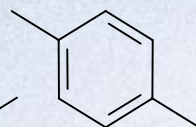
Benzene



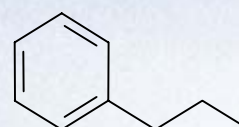
Toluene



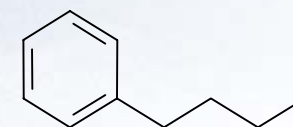
Ethylbenzene



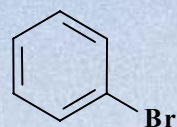
*p*-xylene



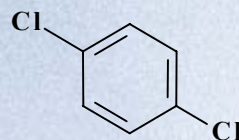
Propylbenzene



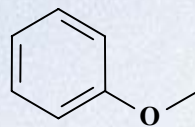
Butylbenzene



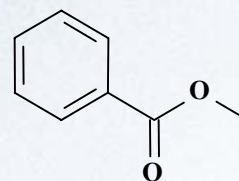
Bromobenzene



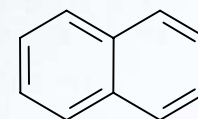
*p*-Dichlorobenzene



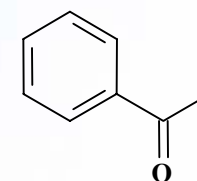
Anisole



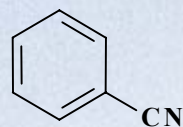
Methylbenzoate



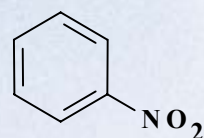
Napthalene



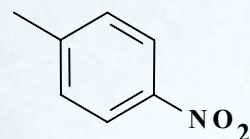
Acetophenone



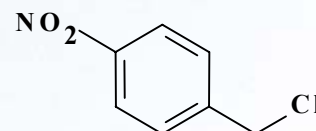
Benzonitrile



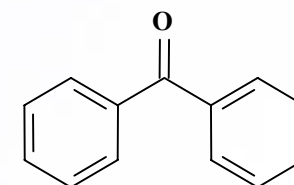
Nitrobenzene



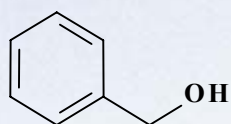
*p*-Nitrotoluene



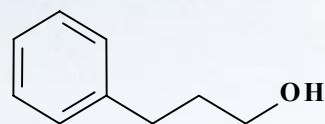
*p*-Nitrobenzyl Chloride



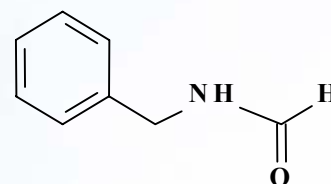
Benzophenone



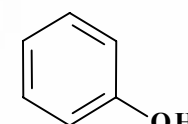
Benzylalcohol



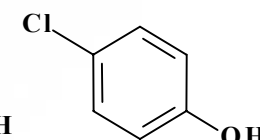
3-Phenyl Propanol



N-Benzyl Formamide



Phenol

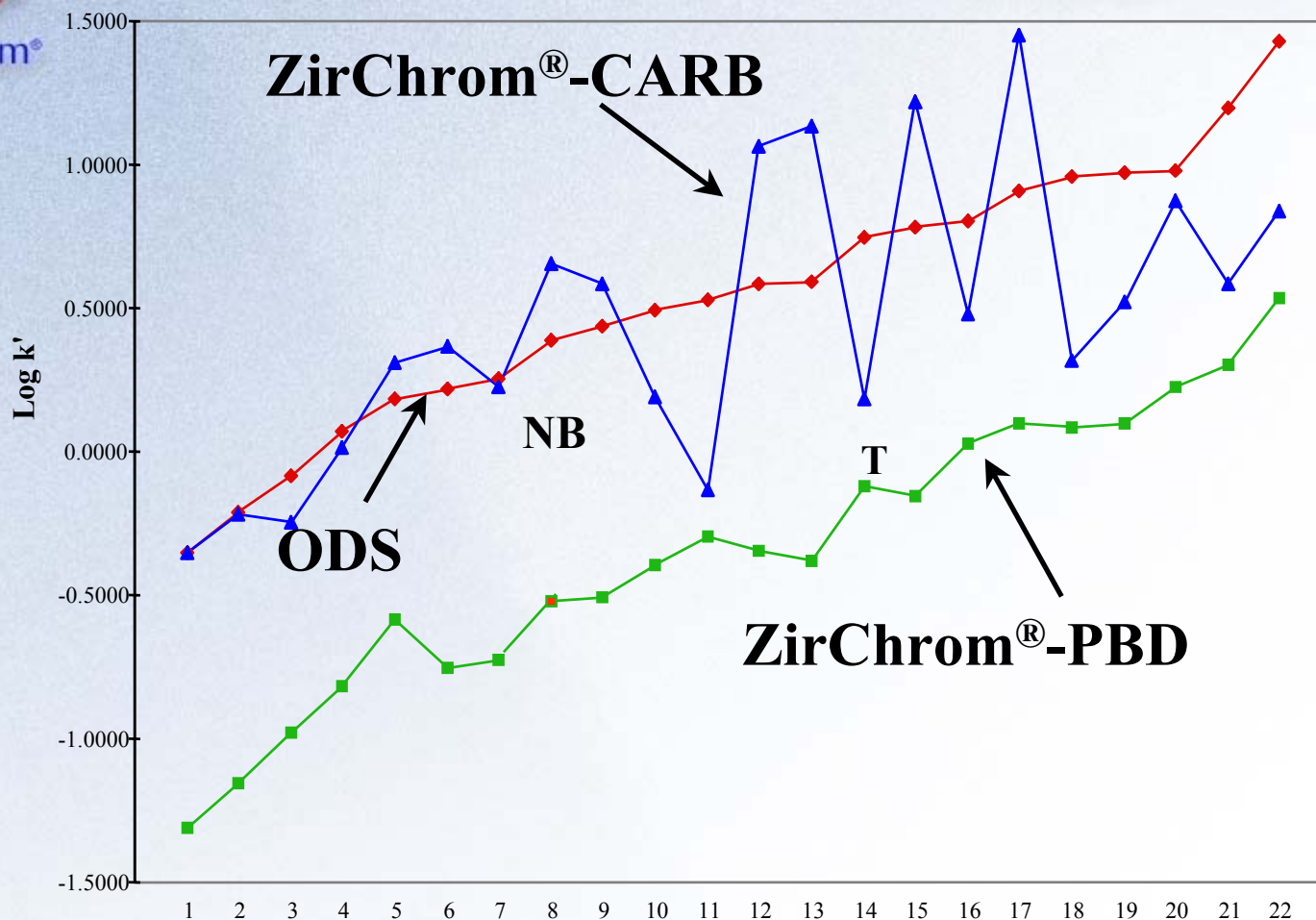


*p*-Chlorophenol





# Comparison of Selectivity of ODS, ZirChrom<sup>®</sup>-PBD &-CARB

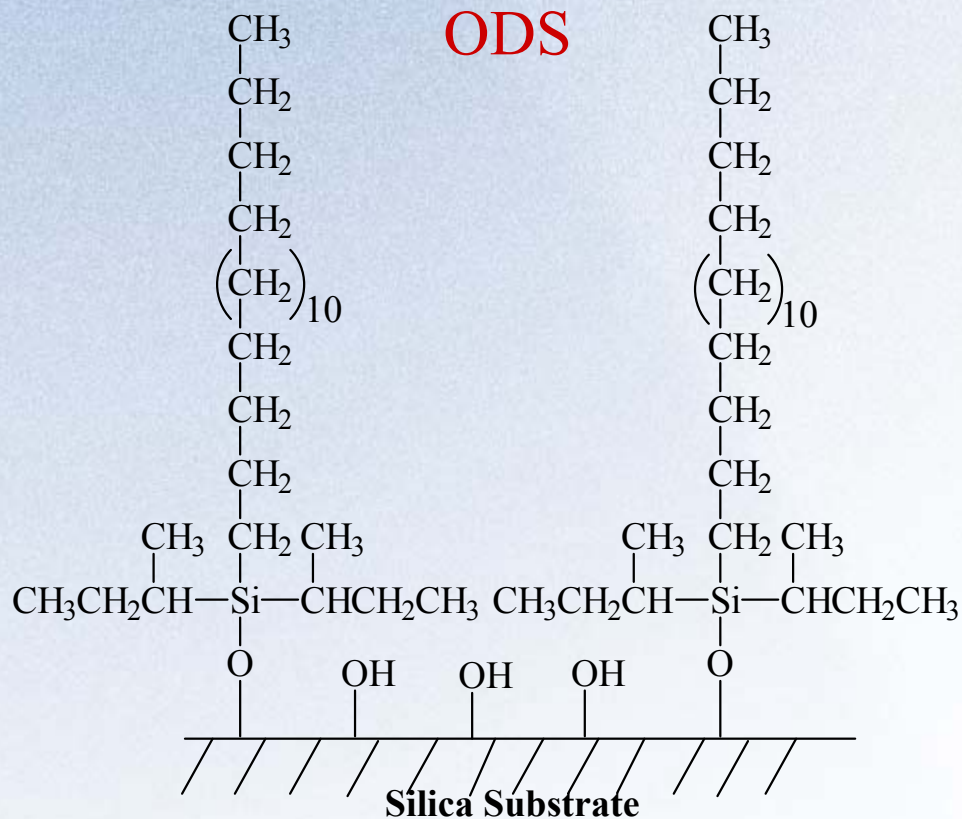


- |                       |                    |                            |                       |                    |
|-----------------------|--------------------|----------------------------|-----------------------|--------------------|
| 1. N-benzyl formamide | 6. Acetophenone    | 11. Benzene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

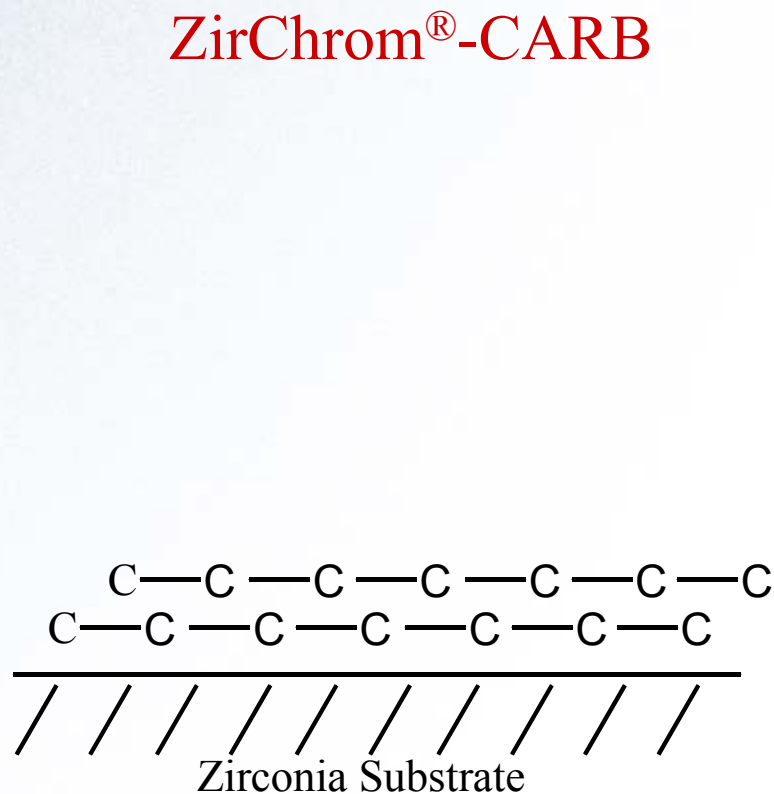


# Chemical Structure of ZirChrom<sup>®</sup>-CARB

ODS



ZirChrom<sup>®</sup>-CARB



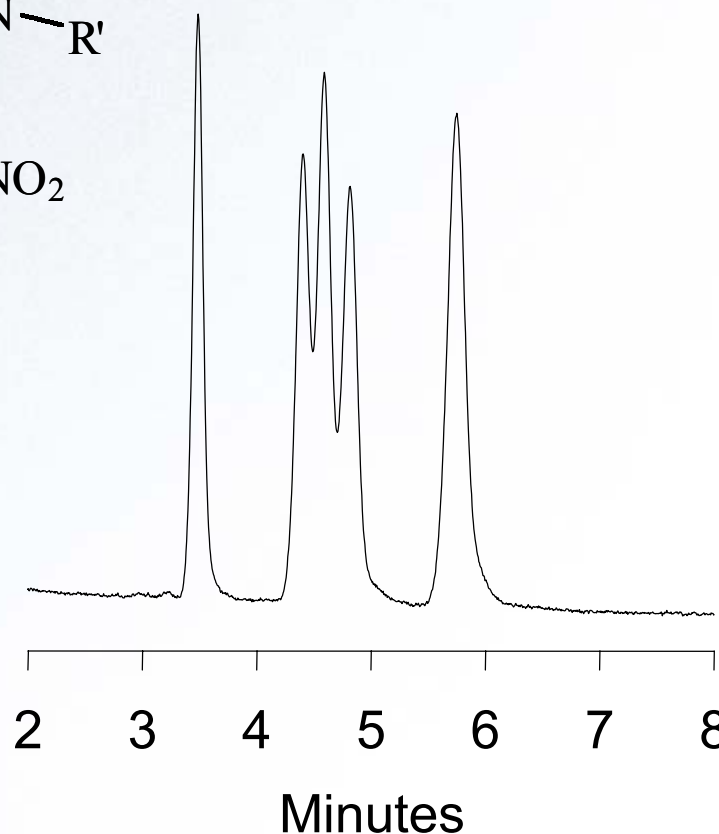
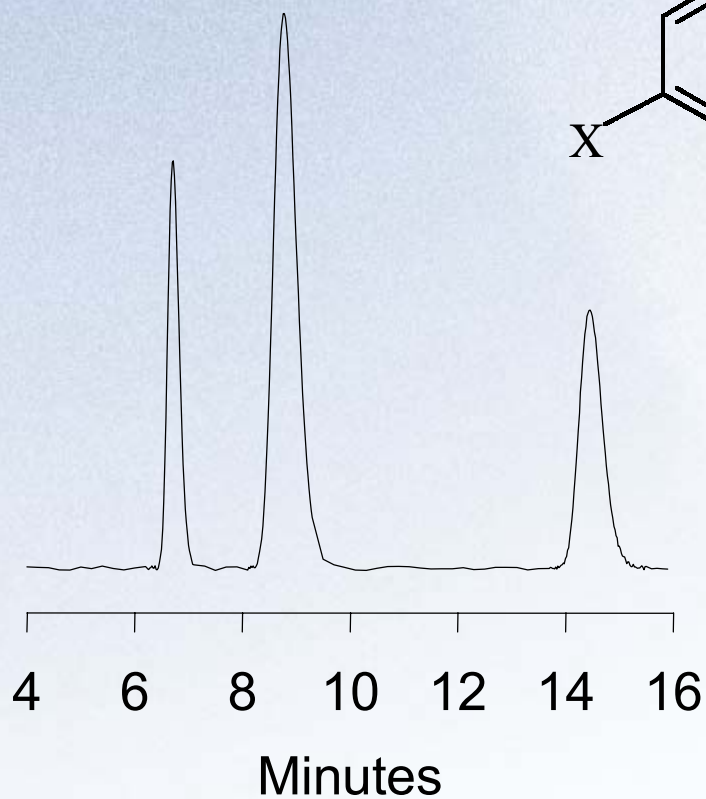
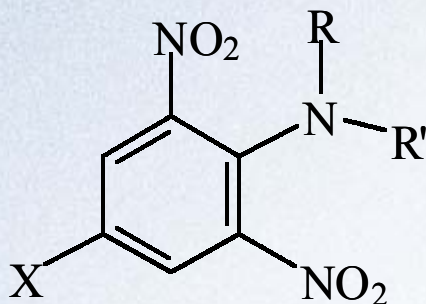


ZirChrom®

# Dinitroaniline Herbicide Separation (EPA Method 627)

ODS

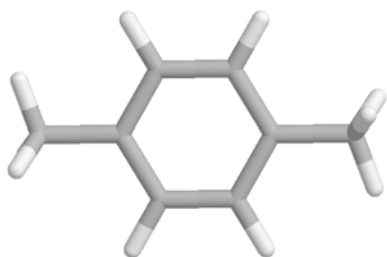
Carbon



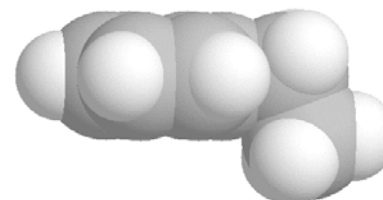
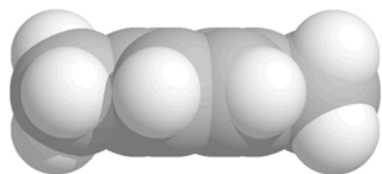
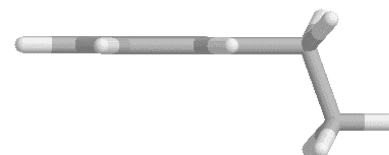
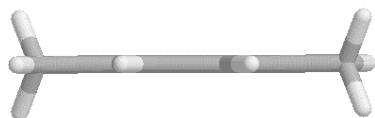
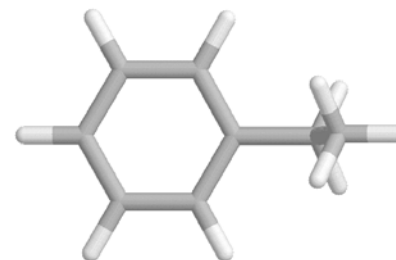
p-xylene

$$\alpha_{\text{ODS}}=1.03$$

ethylbenzene



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



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

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



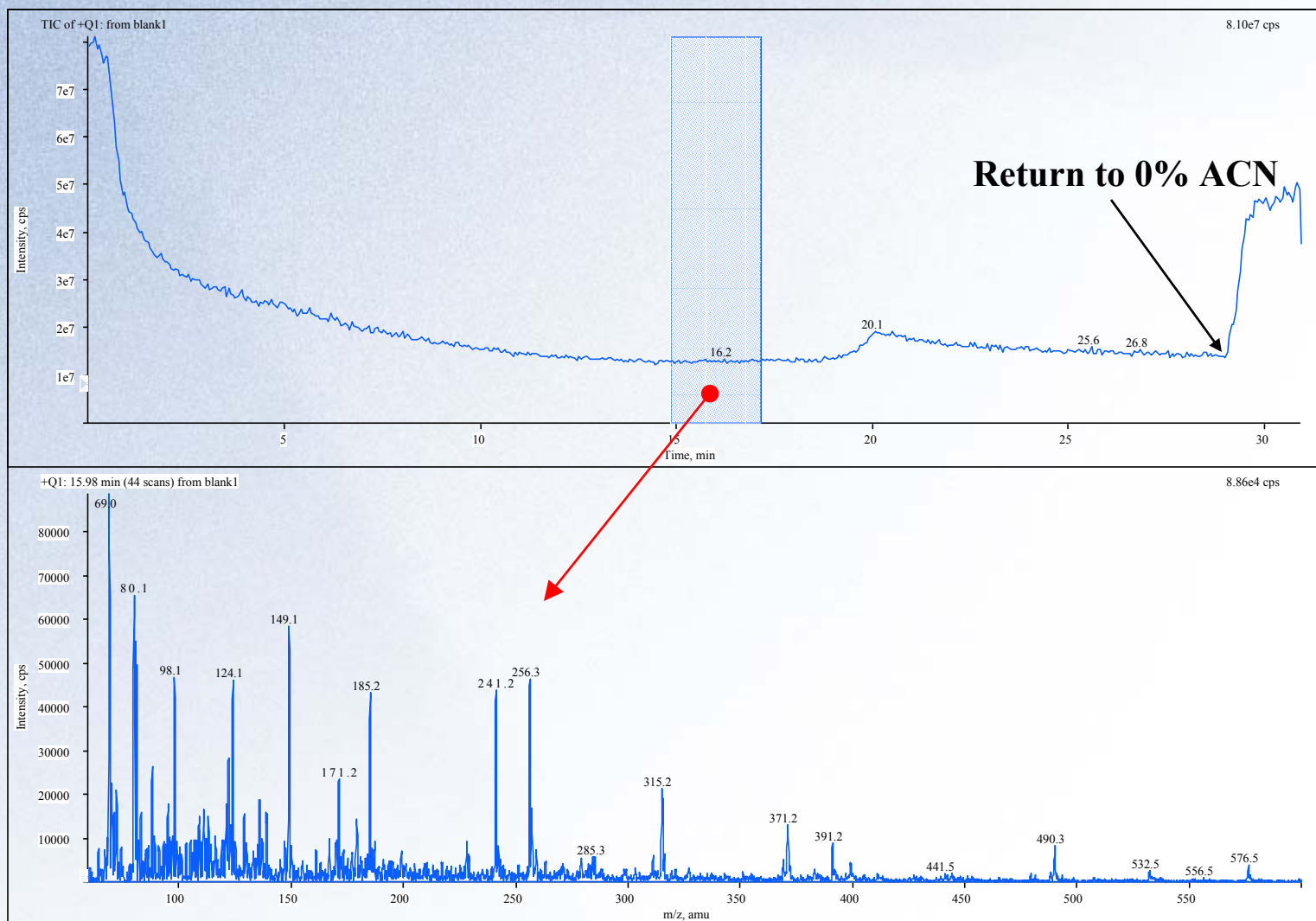


# General Conditions of M.S. Bleed Study

- ◆ SCIEX APCI 3000 triple quad in single quad mode at 400 °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  $\text{NH}_4\text{Ac}$  +  $\text{NH}_4\text{OH}$ .
- ◆ Note pump-column-MS. No injector used.



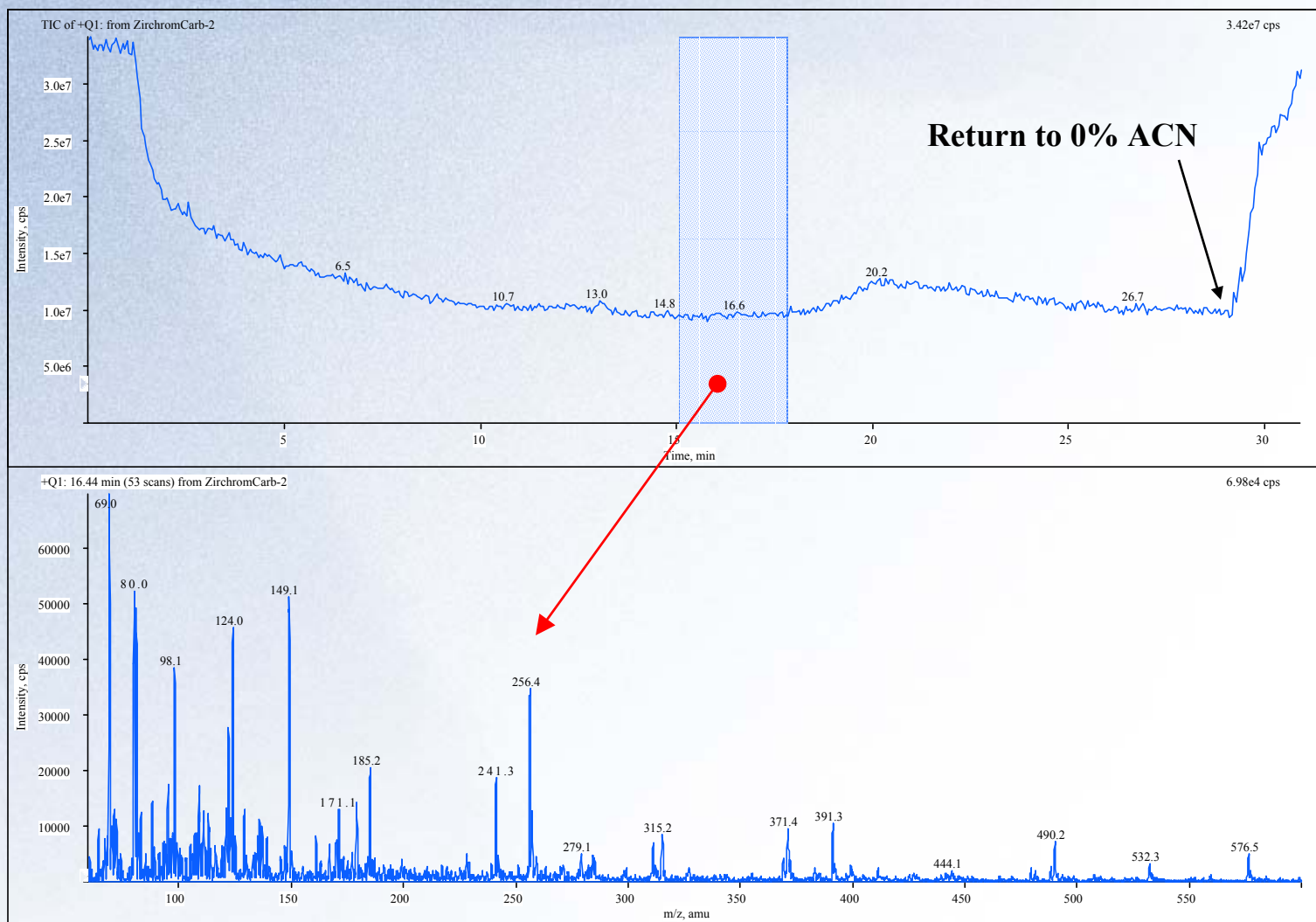
# Gradient Blank at pH 9 and 55 °C



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



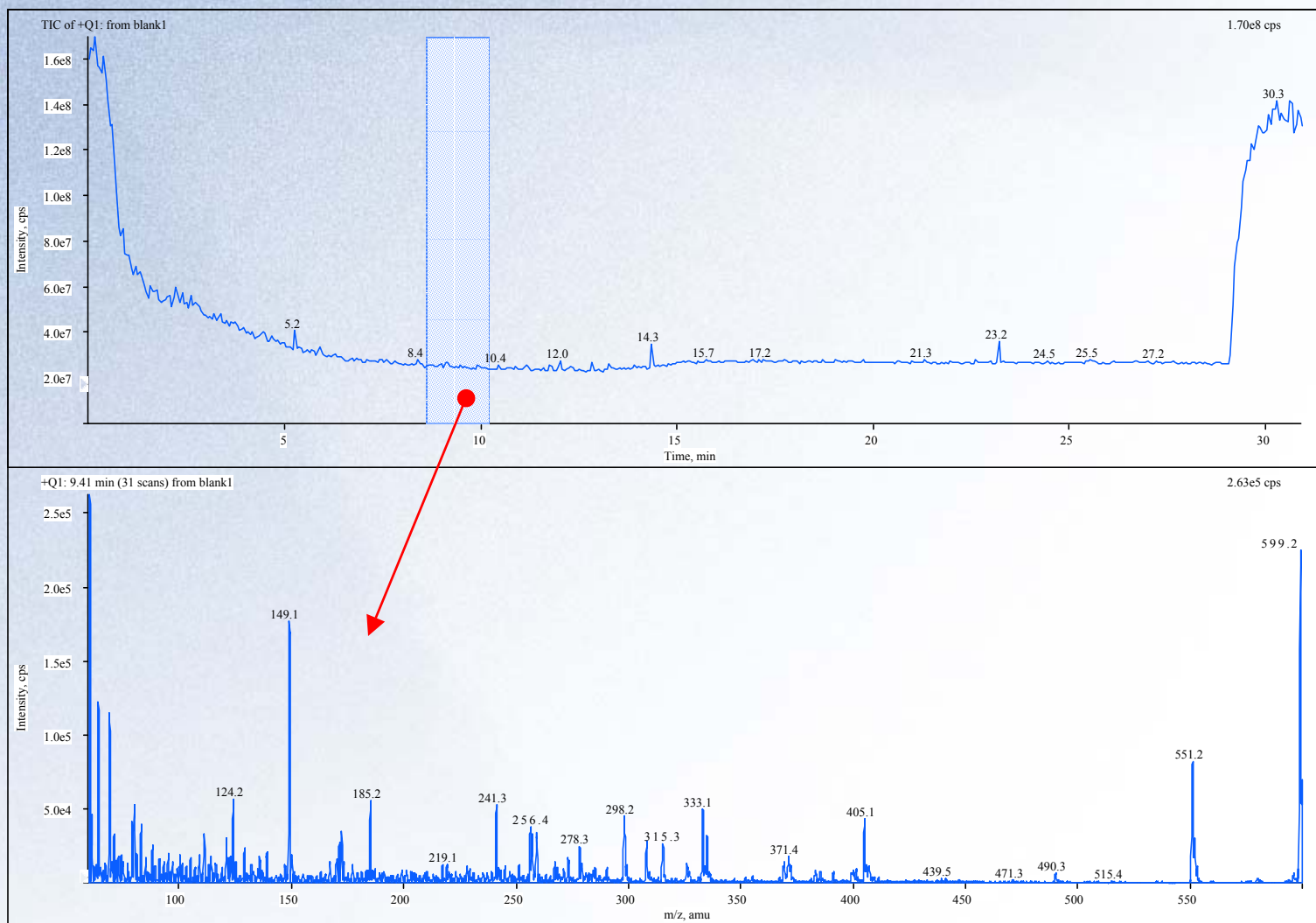
# ZirChrom-CARB at pH 9 and 55 °C



**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.

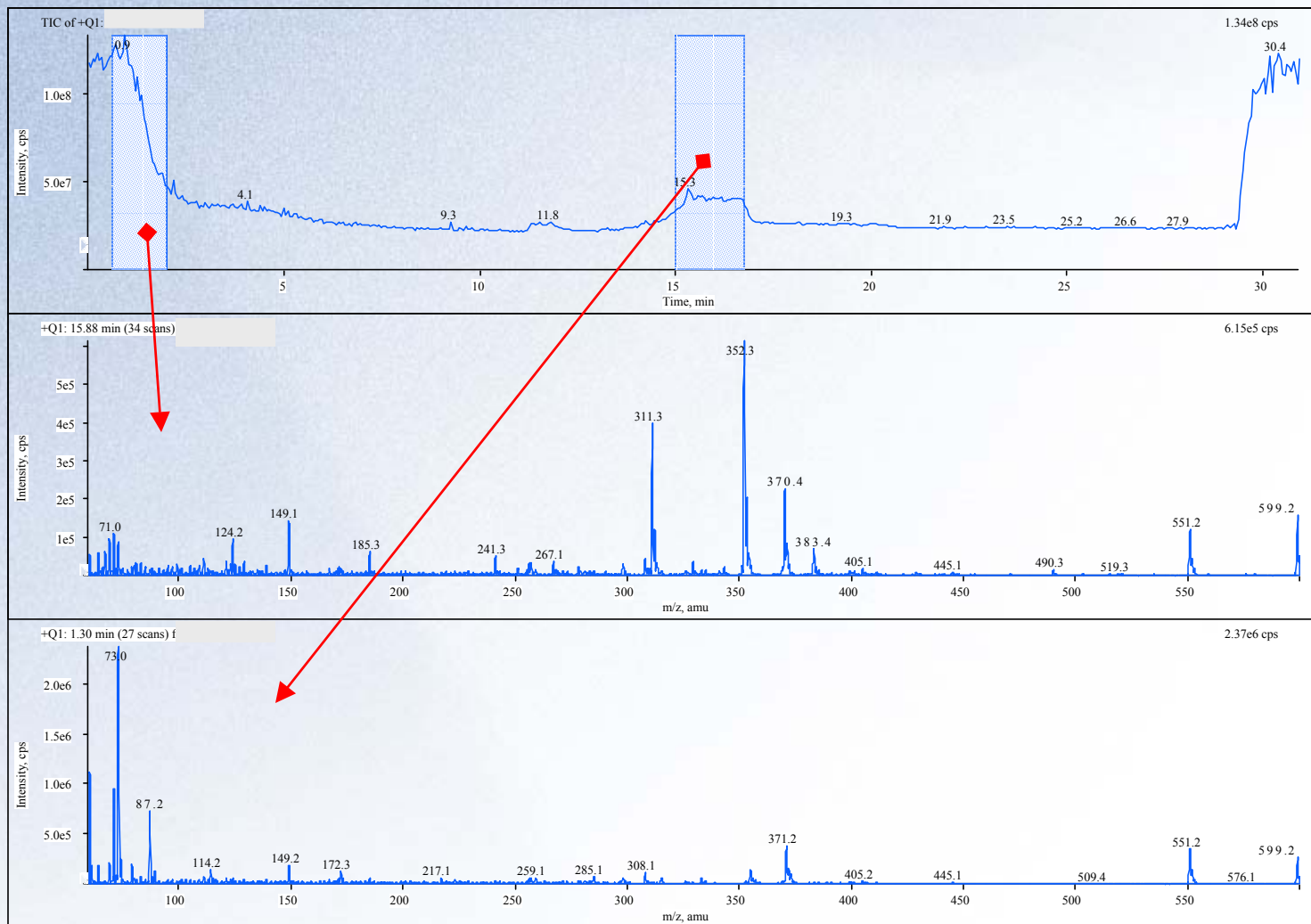


**Total ion current appears cleaner and m.s. looks different than blank at pH 9 but this is due to changes in ionization (no  $\text{NH}_4^+$ ).**





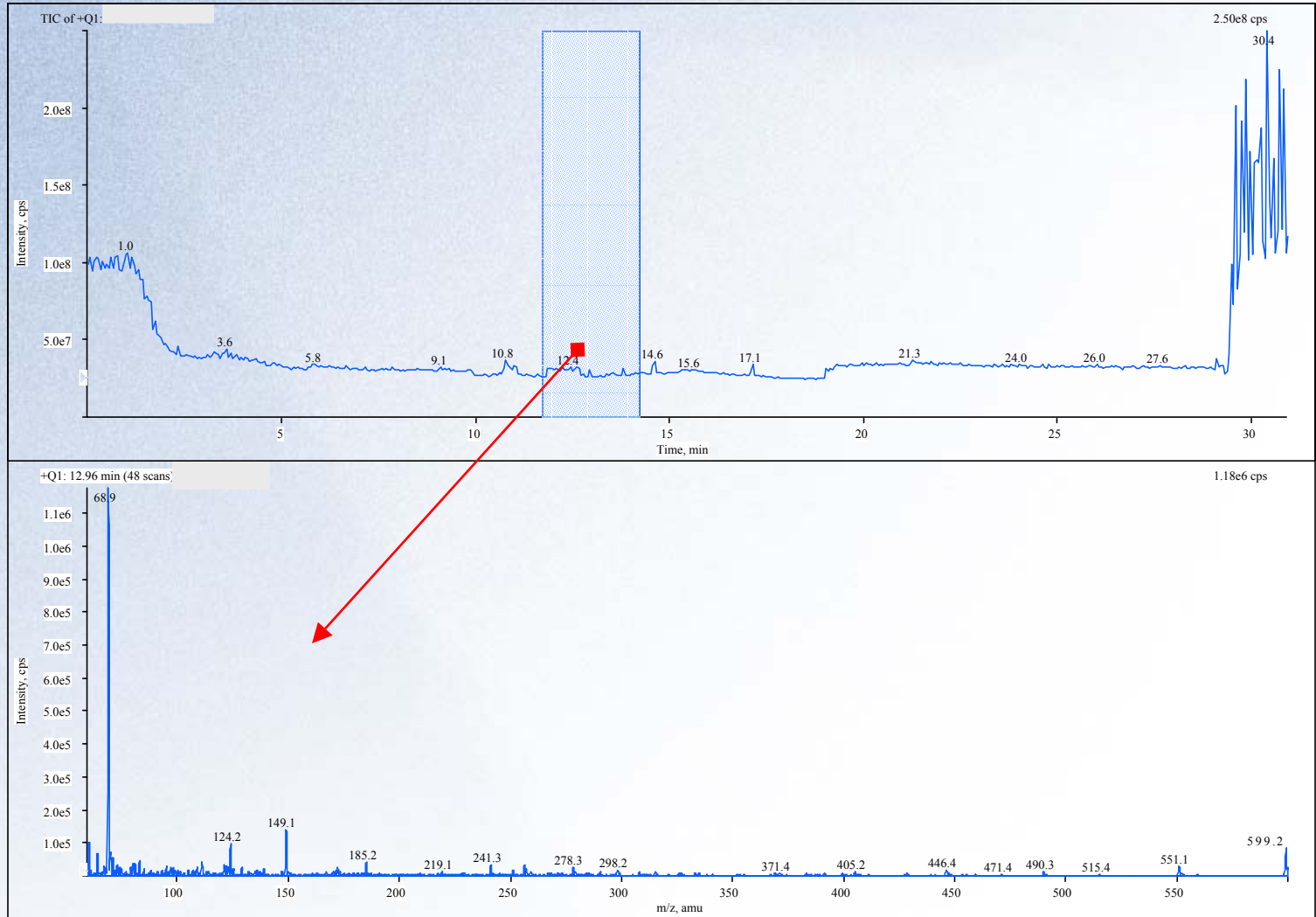
# M.S. Grade SiO<sub>2</sub> (A.) at pH 7 and Ambient Temperature



**New “humps” in total ion chromatogram and new masses are evident in spectra.**



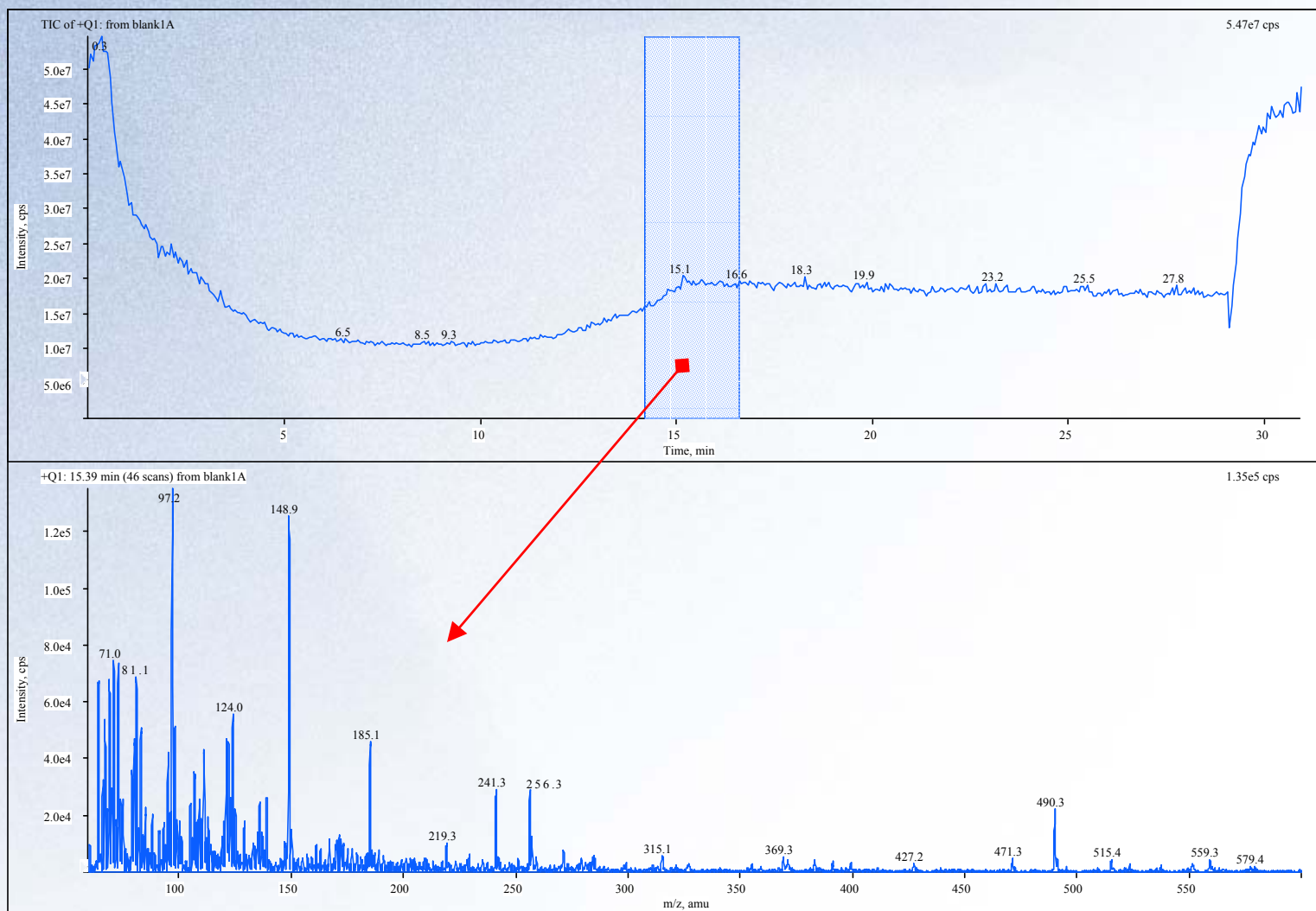
# M.S. Grade SiO<sub>2</sub> (B.) at pH 7 and Ambient Temperature



**Note huge increase in mass 69 (identity unknown).**



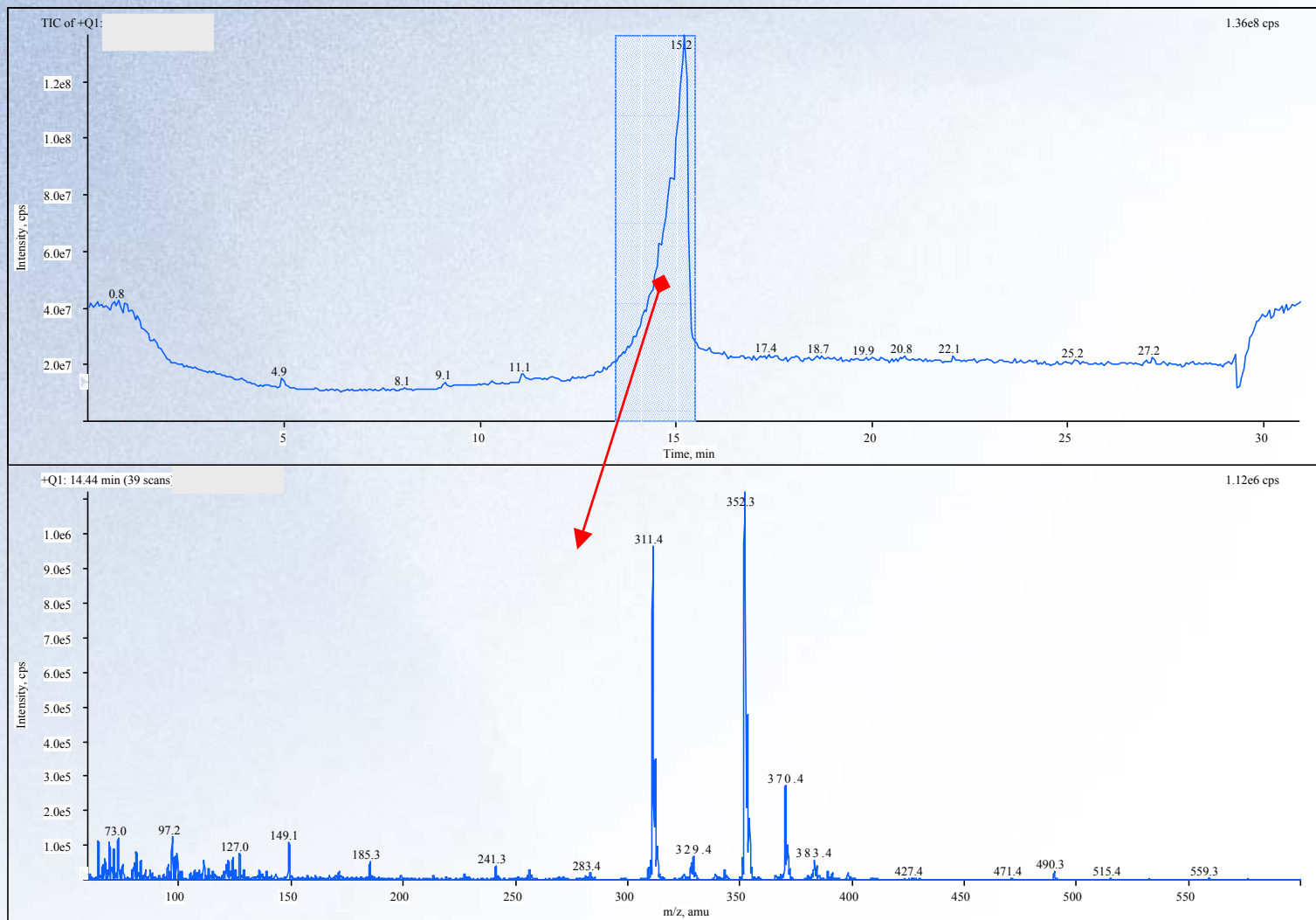
# Blank Gradient at pH 9 and 55 °C



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



# M.S. Grade SiO<sub>2</sub> (A.) at pH 9 and 55 °C

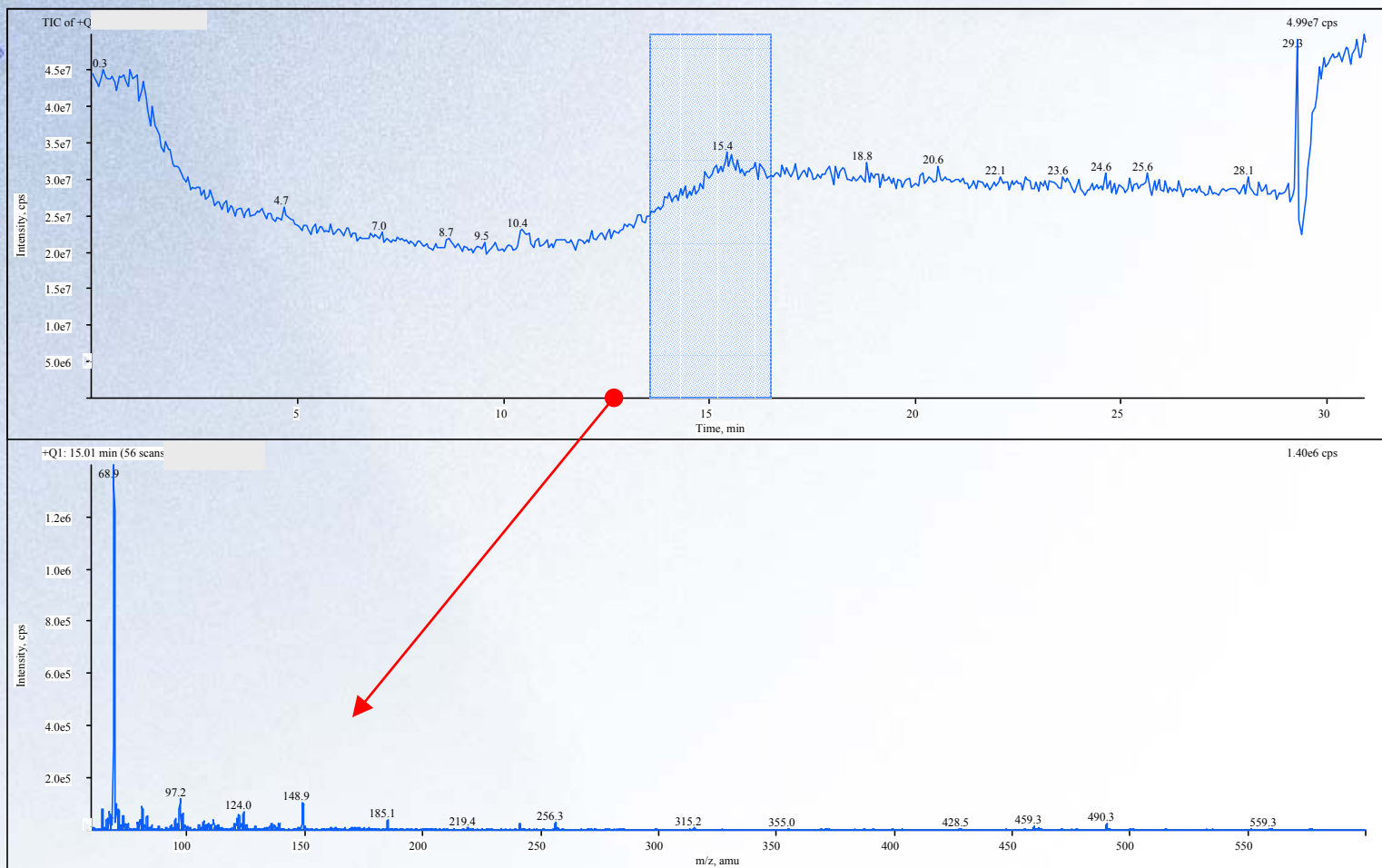


**Note big peak at 12-15 min. in total ion chromatogram and new peaks in mass spec.**





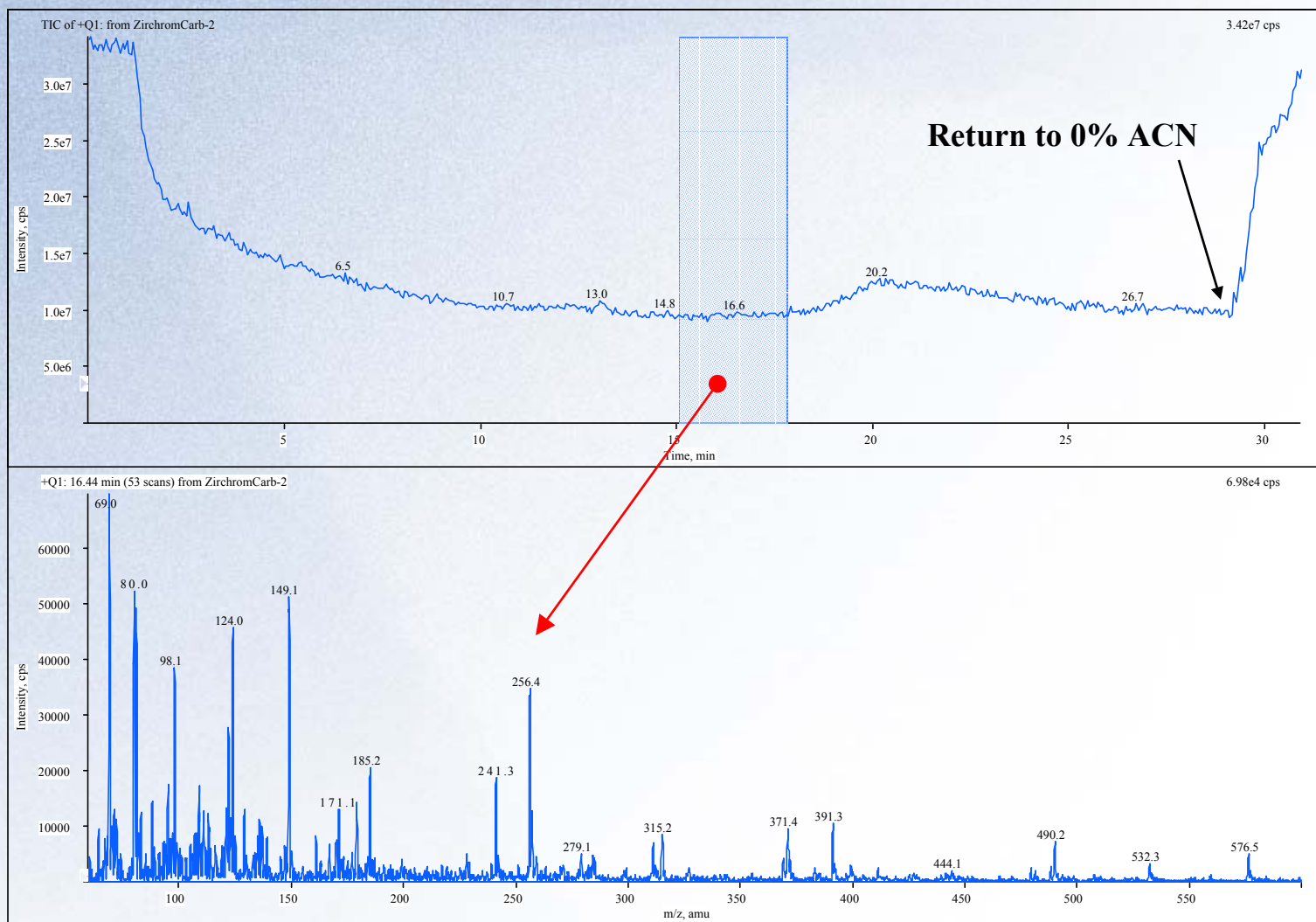
# M.S. Grade SiO<sub>2</sub> (B.) at pH 9 and 55 °C



**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-CARB** is *ultra-durable* and *efficient*.
- **ZirChrom-CARB** is stable at the *extremes of pH* and at *200 °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 °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	<b>Clayton V. McNeff</b> , 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	<b>Gregory Gaudet</b> , Peter Carr, Clayton V. McNeff, Dwight Stoll, Danielle Hawker, Angelos Kyrilidis	162
9:10-9:30 am	March 19 (Tuesday)	243	Development of Carbon Based Chiral Separation HPLC Column	<b>Feng Gu</b> , Angelos Kyrilidis, 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 Nonporous Zirconia for Fast High Temperature Liquid Chromatography	<b>Clayton V. Mc Neff</b> , Gregory Gaudet, Angelos Kyrilidis, 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	<b>Jon D. Thompson</b> , 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.	<b>Peter W. Carr</b> , 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	<b>Xiqin Yang</b> , 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	<b>Clayton V. McNeff</b> , 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	<b>Clayton V. McNeff</b> , Dwight Stoll, Peter Carr, Danielle Hawker	2323P





Thanks *very much*  
for listening!

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free **Buffer Wizard**: [www.zirchrom.com](http://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 °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\*

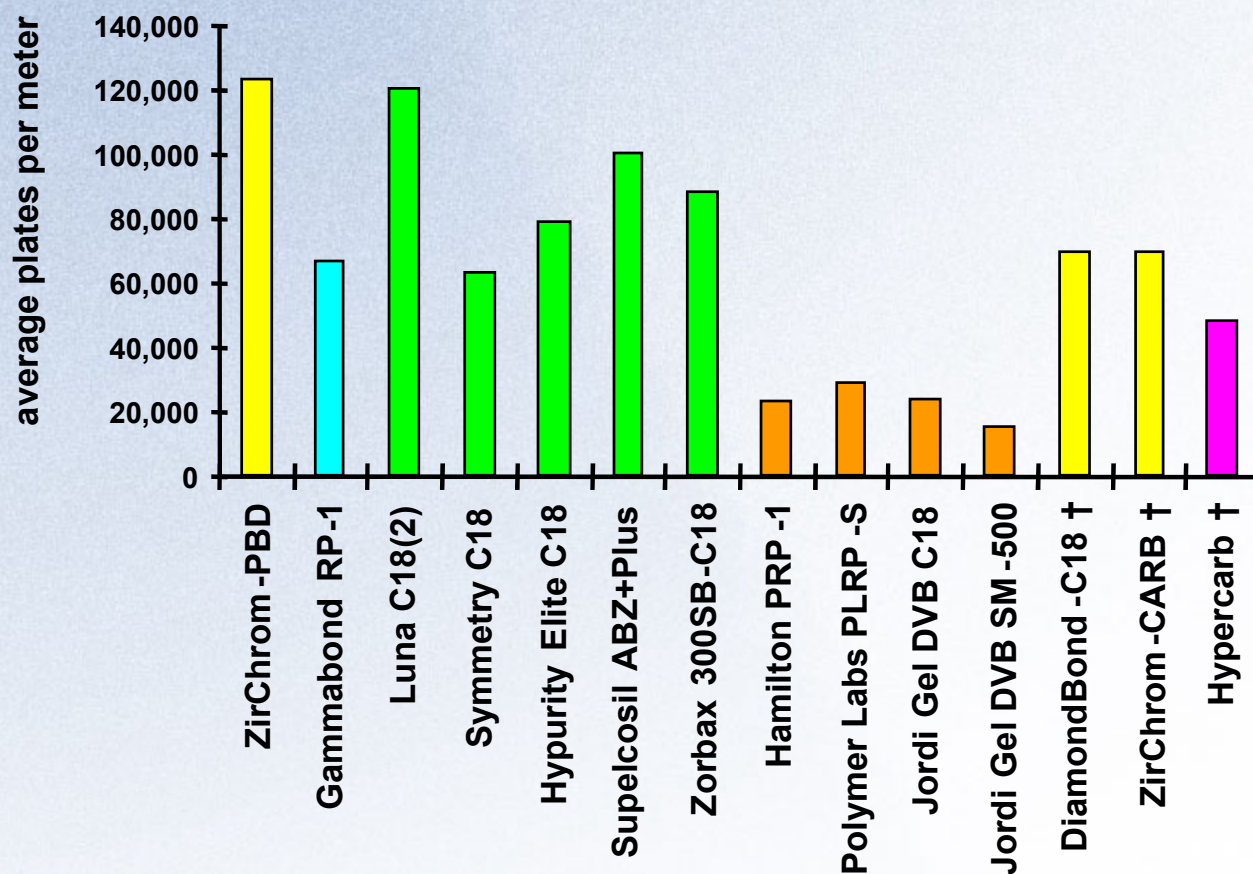
	CARB	DB-C18	PBD	RP18	C18 (2)	PLRP-S	RP-1
ZirChrom-CARB	1						
DiamondBond-C18	0.80	1					
ZirChrom-PBD	0.51	0.90	1				
Xterra RP18	0.53	0.85	0.90	1			
Luna C18 (2)	0.53	0.86	0.93	0.97	1		
PLRP-S	0.60	0.90	0.93	0.92	0.96	1	
Gammabond RP-1	0.52	0.88	0.96	0.97	0.98	0.95	1

\* 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.

\* Column names are the trademarks of their respective manufacturers.