ZirChrom®

Developing Better HPLC Methods
"New Zirconia-Based Phases of Improved Performance in LC/MS"

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ZirChrom®

1-866-STABLE-1
www.zirchrom.com

... For Peak Performance

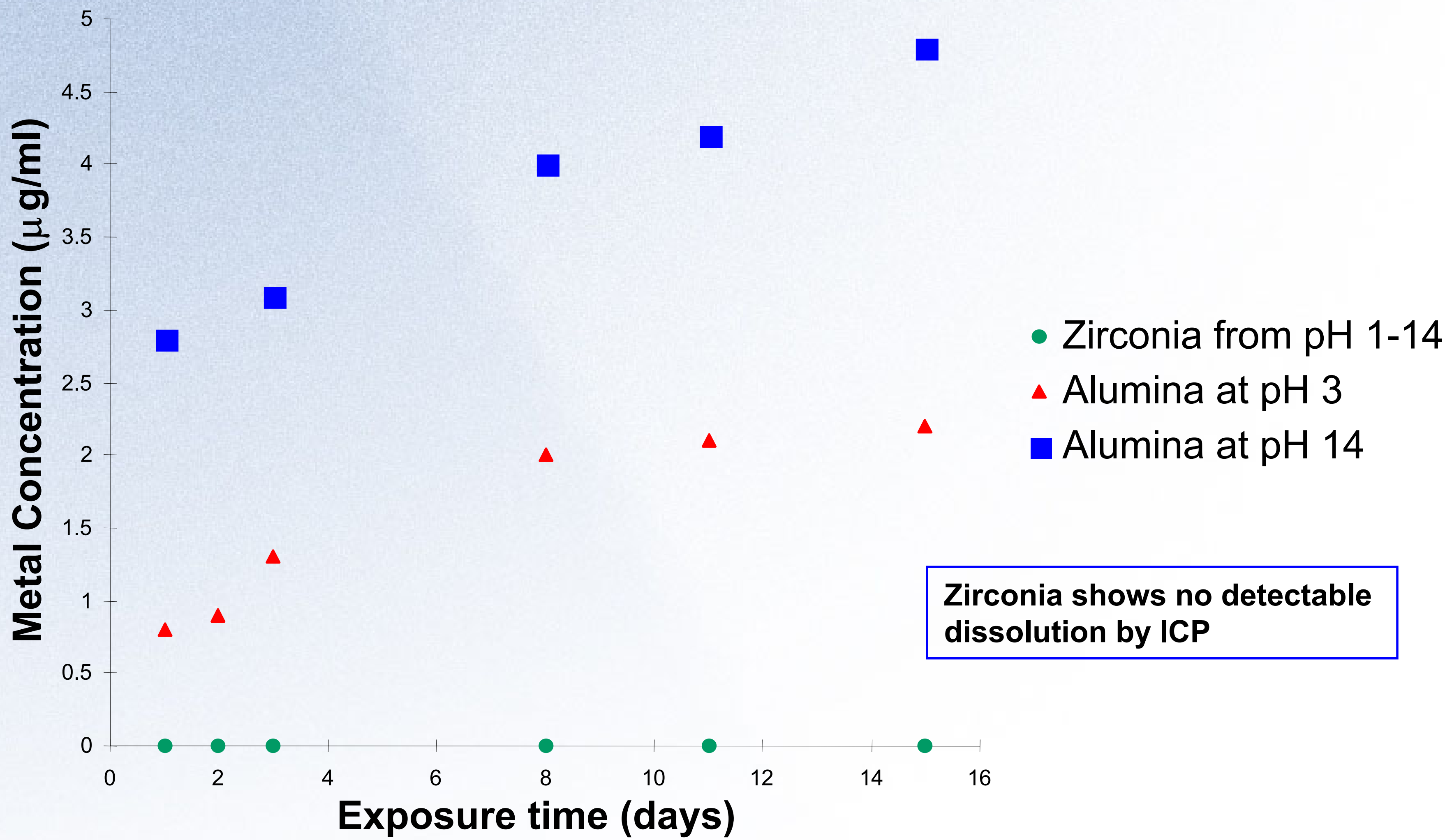
Specialists in High Efficiency, Ultra-Stable Phases for HPLC

Outline

1.) Substrate Zirconia Properties
2.) ZirChrom-CARB, PBD and MS Columns
3.) Synthesis of RP Zirconia Phases
4.) Chromatographic Selectivity Studies
5.) Developing Robust HPLC Separations Using Zirconia
6.) Column Bleed Study on ZirChrom-CARB and Silica C18
7.) Example Method Development Case Studies

Conclusions: New Polymer and Carbon coated zirconia based HPLC Stationary phases provide different chromatographic selectivity than silica C18 and are ideal for MS detection.

Chemical Stability of Zirconia



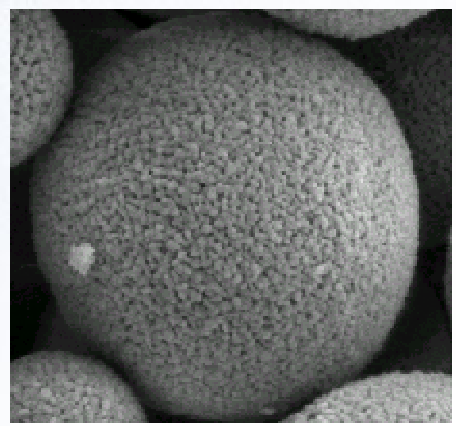
Zirconia shows no detectable dissolution by ICP

1.) ZirChrom® Particle Properties

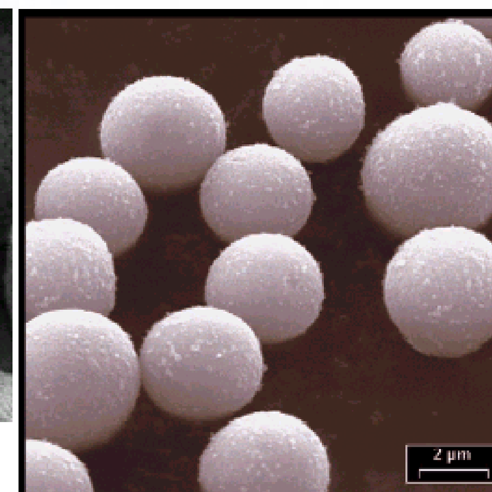
ZirChrom®-PBD and **ZirChrom®-MS** are prepared by coating with a layer of highly crosslinked polymer

ZirChrom®-Carb particles are prepared by coating base particles with a thin layer of carbon using a chemical vapor deposition process

Characteristic	Property
Surface Area (m^2/g)	22
Pore Volume (cc/g)	0.13
Pore Diameter (Å)	250-300
Porosity	0.45
Density (g/cc)	5.8 (2.5x silica)
Particle Diameters (µ)	3.0, 5.0, 10, 25



1µm 25000X



2.) ZirChrom Product List

ZirChrom®

Part #	Product Name	Chromatographic Mode and Uses
ZR01	ZirChrom®-CARB	Reversed-Phase
ZR02	ZirChrom®-PHASE	Normal Phase and SEC
ZR03	ZirChrom®-PBD	Reversed-Phase
ZR04	ZirChrom®-WCX	Weak Cation-Exchanger
ZR05	ZirChrom®-WAX	Weak Anion-Exchanger and Sugar Analysis
ZR06	ZirChrom®-SAX	Strong Anion-Exchanger
ZR07	ZirChrom®-SHAX	Strong Hydrophilic Anion-Exchanger
ZR08	ZirChrom®-PEZ	Cation-Exchanger for Proteins
DB01	DiamondBond™-C18	Reversed-Phase
ZR09	ZirChrom®-PS	Reversed-Phase
EZ01	ZirChrom®-EZ	Reversed-Phase (Lewis acid deactivated)
MS01	ZirChrom®-MS	Reversed-Phase (Lewis acid deactivated)

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Zirconia vs. Silica

The difference is the surface chemistry.

Surface Chemistry of Zirconia-Based Supports for HPLC

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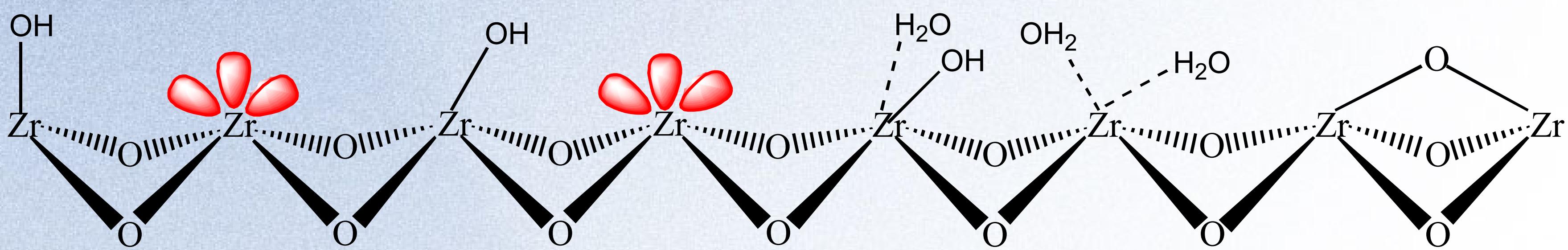
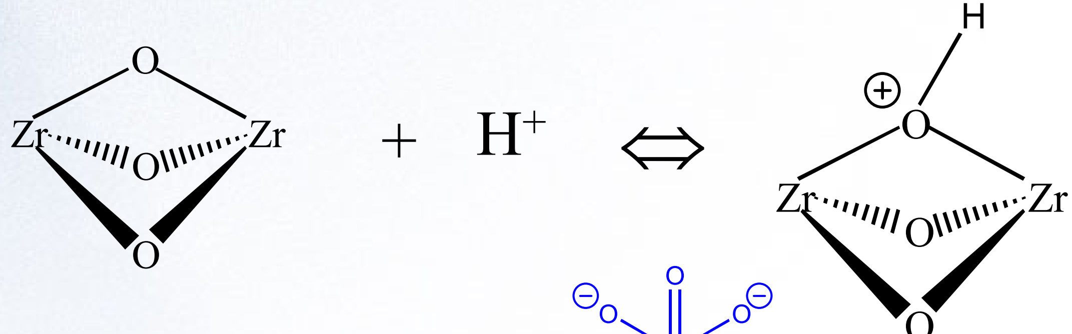
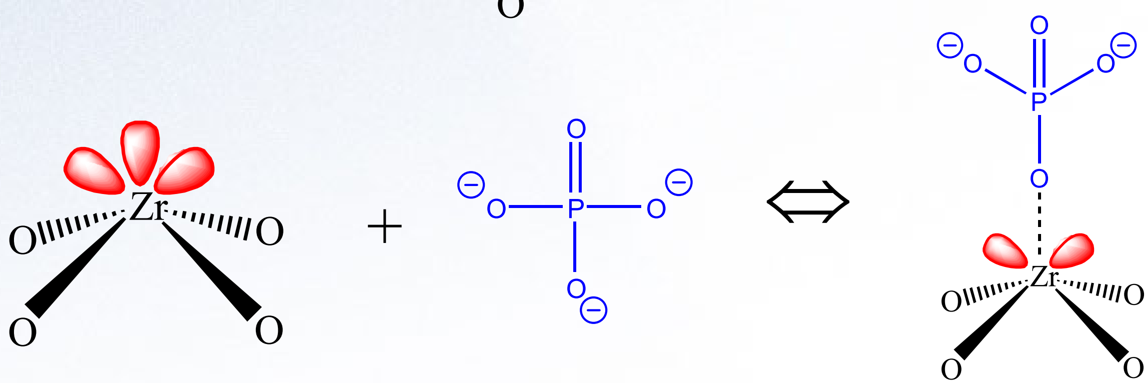


Brönsted Acid: $$ZrOH + OH^- \Leftrightarrow ZrO^- + H_2O$$

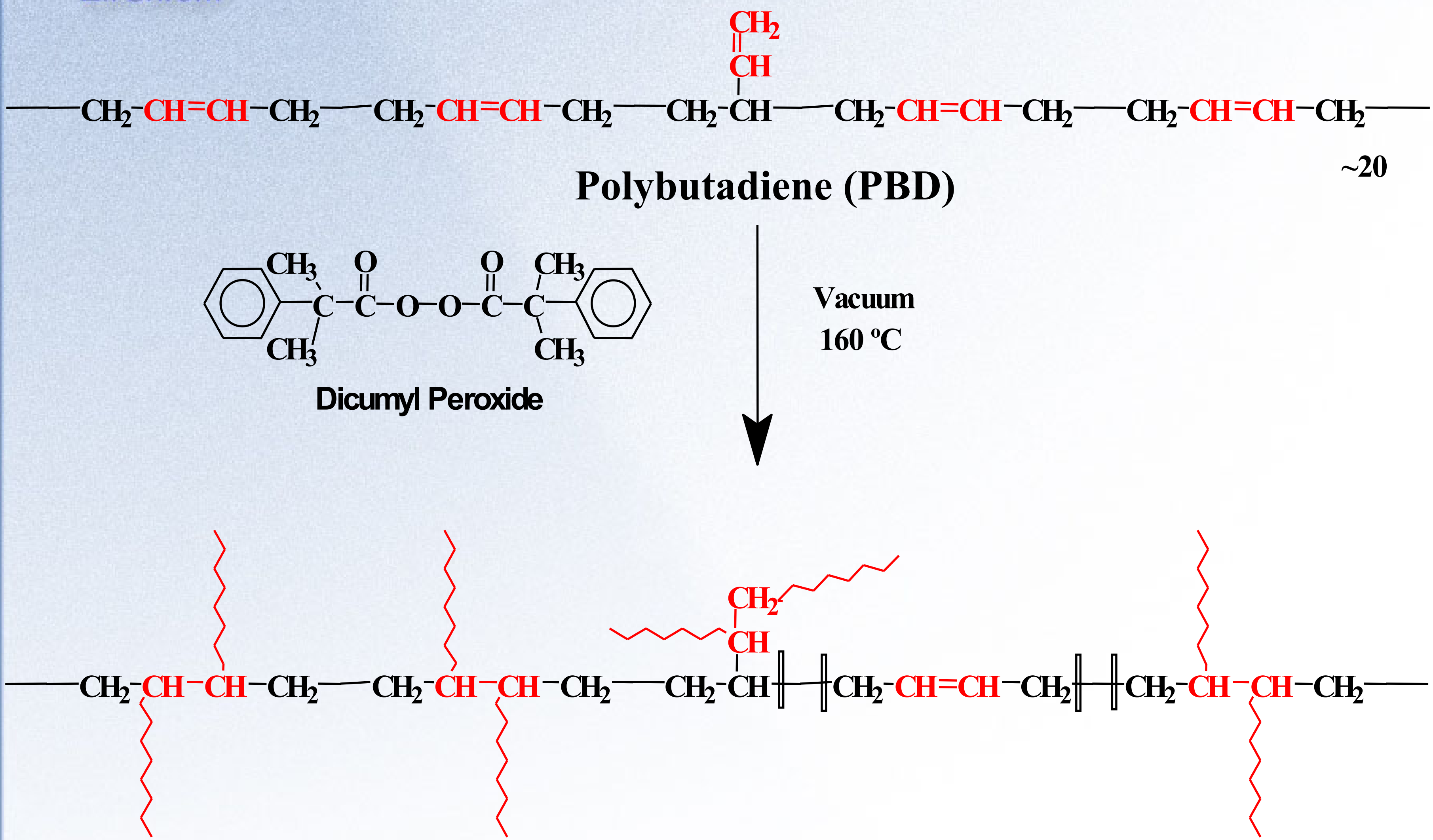
Brönsted Base:



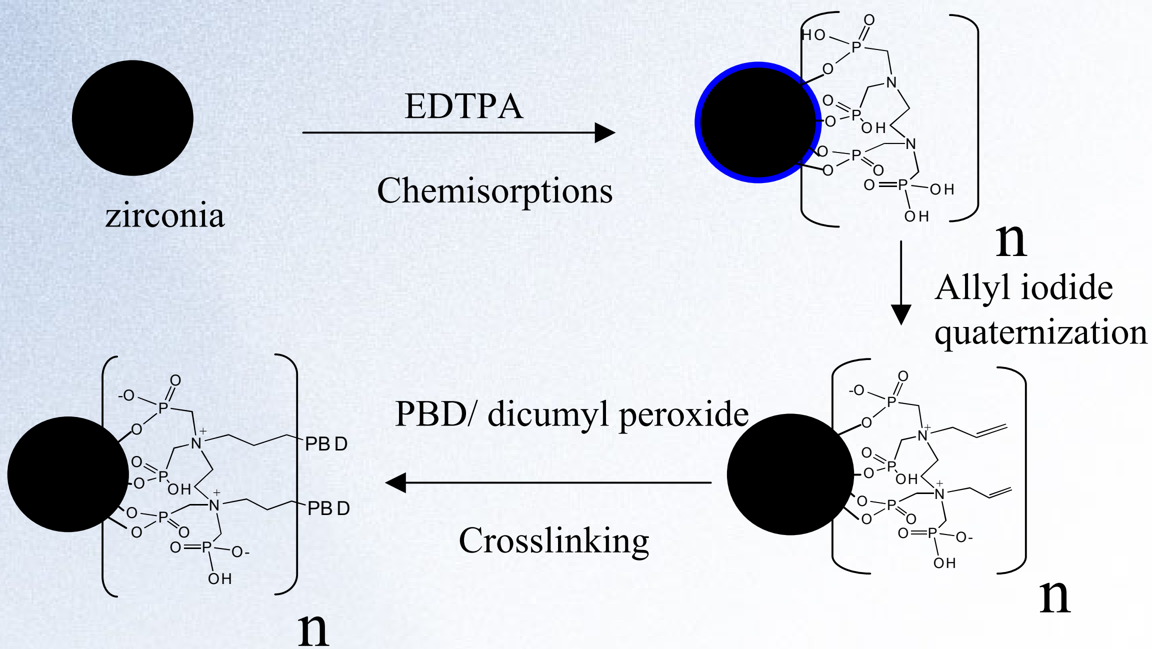
Lewis Acid:



ZirChrom®-PBD Synthesis



Synthetic Strategy for ZirChrom-MS

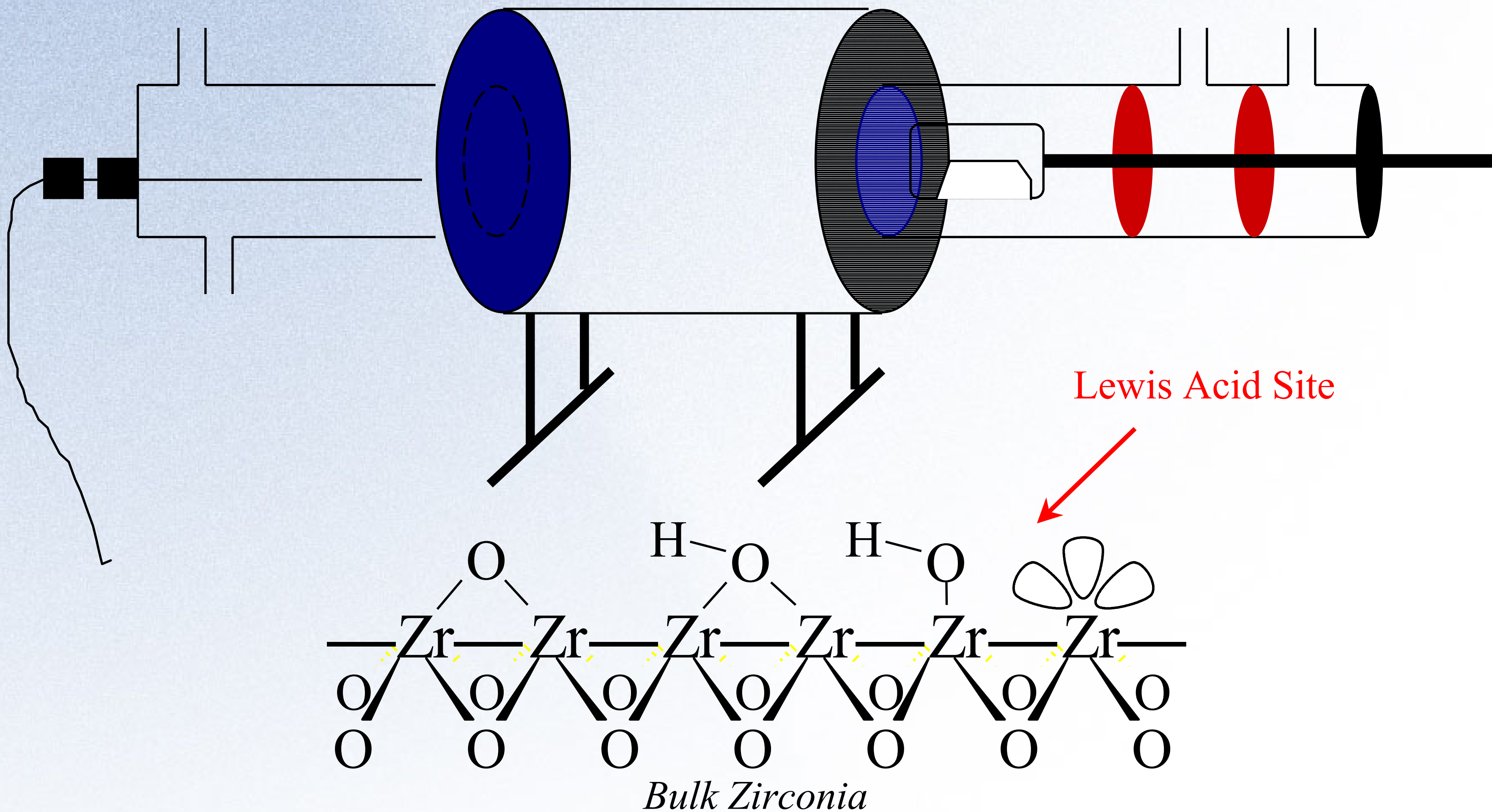


1 **Chemisorb Ethylenediamine N,N,N’,N’-tetra(methylenephosphonic)acid (EDTPA) to the zirconia surface.**

2 **Quaternize amines on the zirconia surface with allyl iodide**

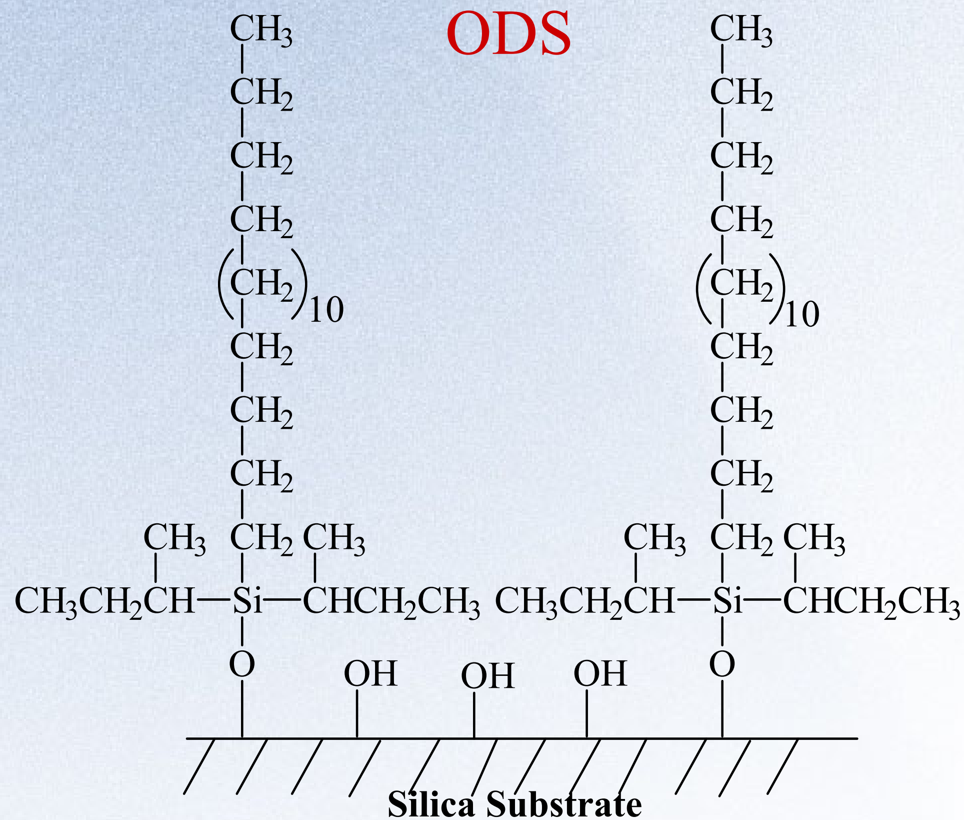
3 **Coat polybutadiene (PBD) on the chelator-modified zirconia surface and crosslink PBD with allyl group and PBD itself using dicumyl peroxide as initiator**

Synthesis of ZirChrom®-CARB - Carbon Clad Zirconia

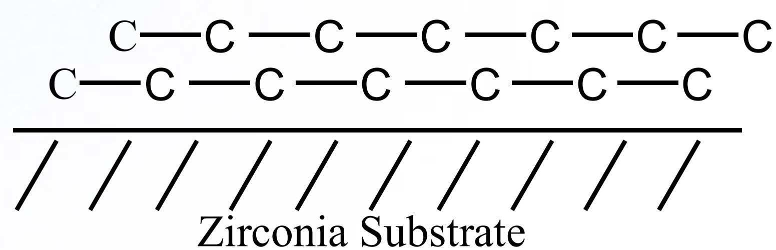


Chemical Structure of ZirChrom®-CARB

ODS



ZirChrom®-CARB



3.) Selectivity: The Key to Success

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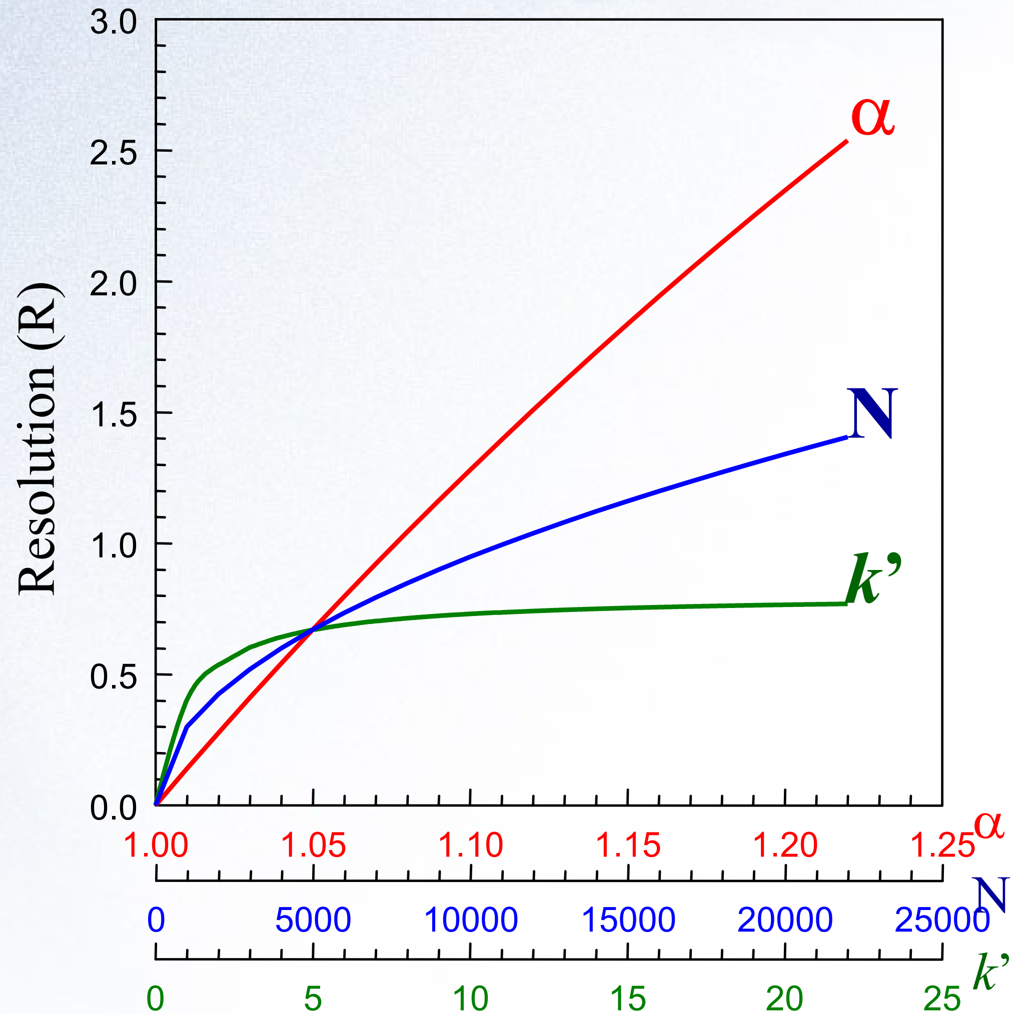
Efficiency → Retention → Selectivity

$$R = \frac{\sqrt{N}}{4} \; \frac{k'}{k'+1} \; \frac{\alpha - 1}{\alpha}$$

$$\alpha = \frac{k_j'}{k_i'}$$



➢ **Selectivity (α) has the greatest impact on improving resolution.**

4.) Selectivity Comparison Solutes

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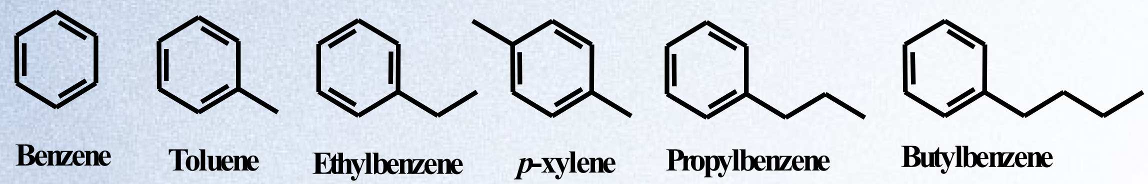
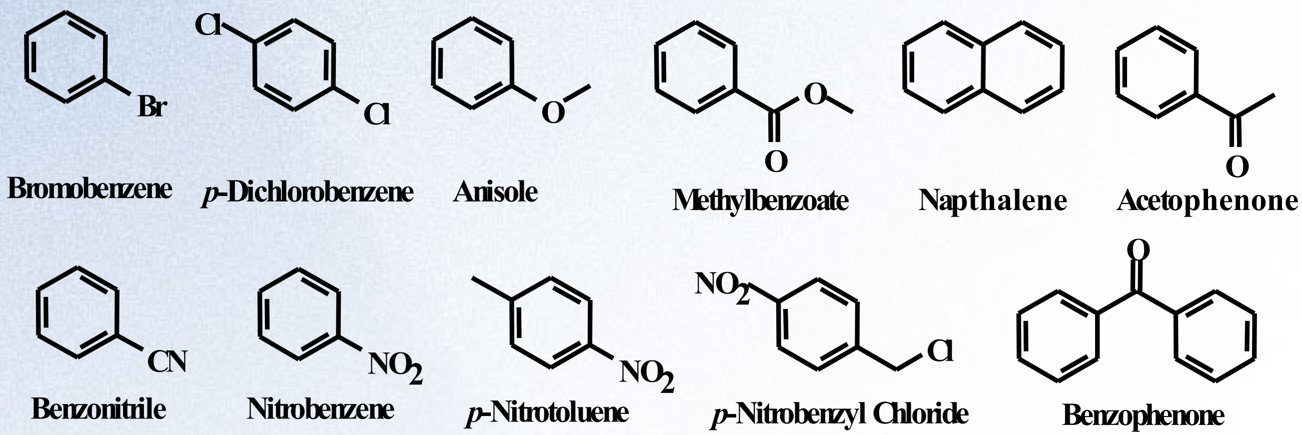
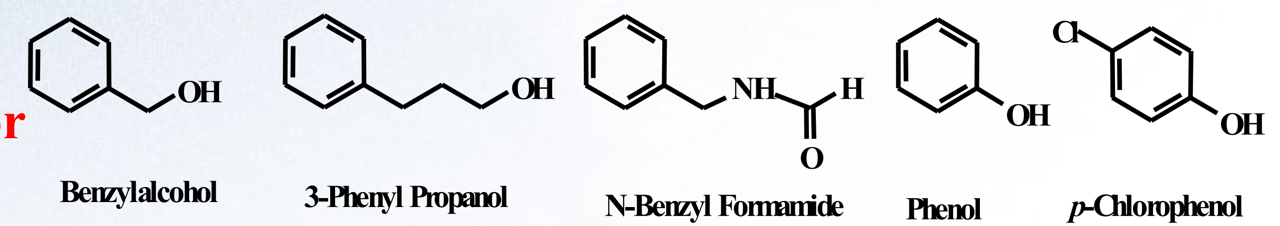
Nonpolar



Polar

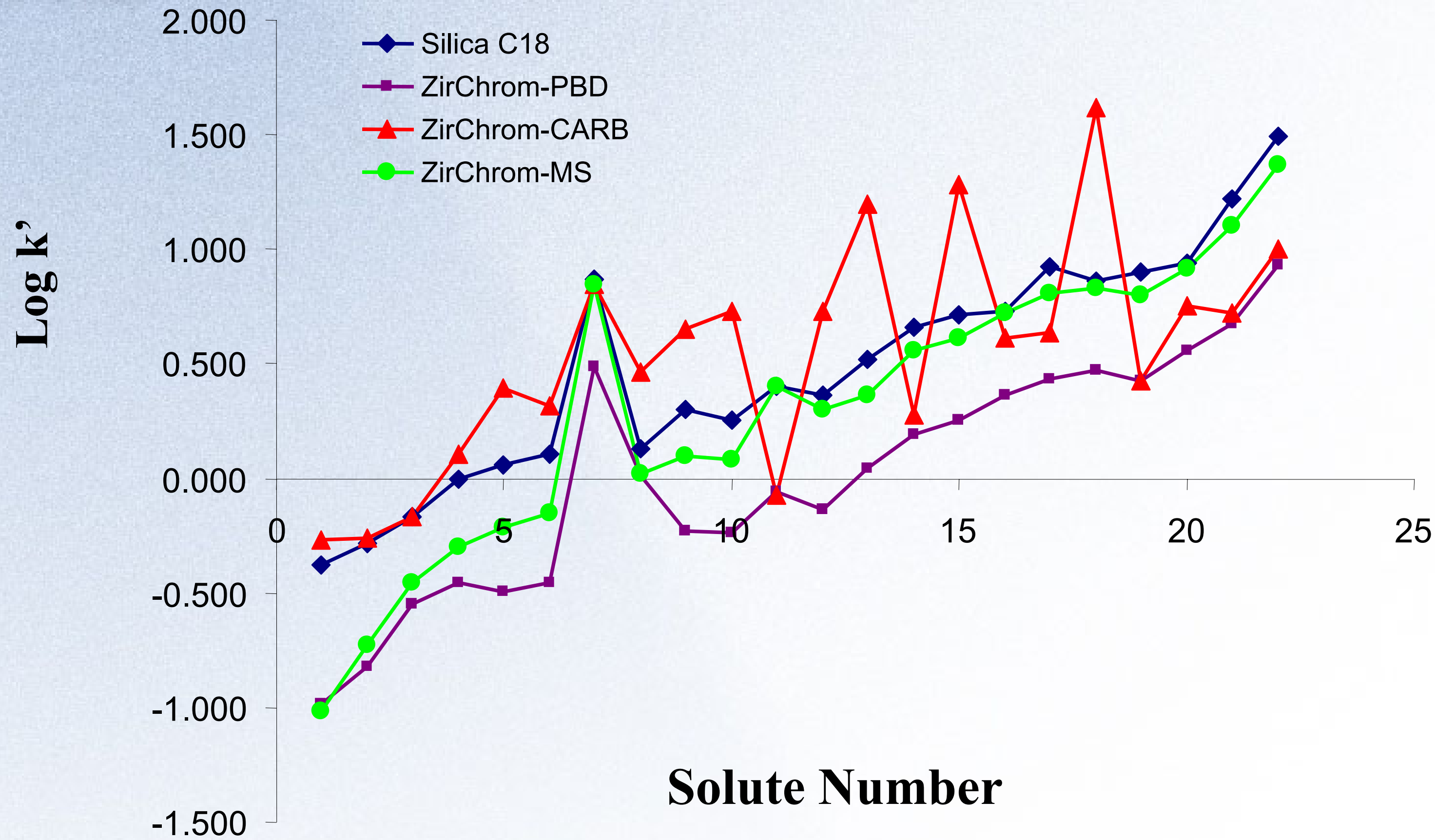


HB Donor



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

Comparison of Selectivity of ODS, ZirChrom®-PBD, -CARB and -MS



1.) benzyl formamide 2.) benzyl alcohol 3.) phenol 4.) 3-phenyl propanol 5.) p-chlorophenol 6.) acetophenone 7.) benzonitrile 8.) nitrobenzene 9.) methylbenzoate 10.) anisole 11.) benzene 12.) p-chlorotoluene 13.) p-nitrobenzyl chloride 14.) toluene 15.) benzophenone 16.) bromobenzene 17.) naphthalene 18.) ethyl benzene 19.) p-xylene 20.) p-dichlorobenzene 21.) propyl benzene 22.) butyl benzene

Selectivity Matrix

Correlation Coefficient	Waters Xterra (RP18)	Luna	PLRP	Gammabond	ZirChrom-PBD	ZirChrom-CARB	DB-C18	Hypercarb	Discovery BIO Wide Pore C18	ZirChrom-EZ	ZirChrom-MS
Waters Xterra (RP18)	1	0.99	0.96	0.98	0.95	0.71	0.94	0.77	0.96	0.96	0.96
Luna		1	0.98	0.99	0.95	0.70	0.94	0.77	0.96	0.96	0.97
PLRP			1	0.98	0.97	0.70	0.95	0.76	0.98	0.98	0.98
Gammabond				1	0.97	0.70	0.95	0.76	0.98	0.98	0.98
ZirChrom-PBD					1	0.69	0.97	0.77	0.98	0.99	0.99
ZirChrom-CARB						1	0.84	0.97	0.68	0.70	0.70
DB-C18							1	0.90	0.95	0.97	0.97
Hypercarb								1	0.76	0.78	0.77
BIO Wide Pore C18									1	0.99	0.99
ZirChrom-EZ										1	0.998
ZirChrom-MS											1

Conclusion: All CARBON-BASED Columns have different selectivity for nonelectrolytes. All other column retention is very highly correlated.

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

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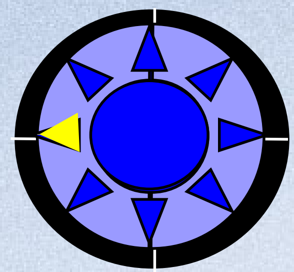
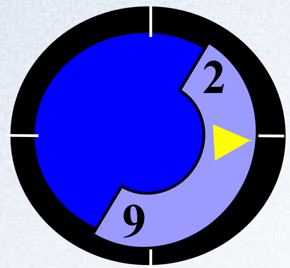
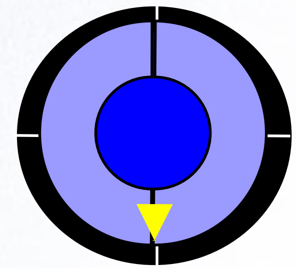
5.) Method Development Variables for Silica RP Phases



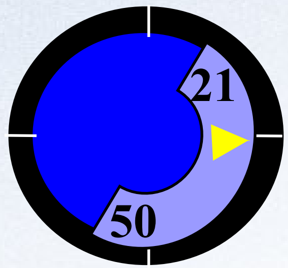
Selectivity



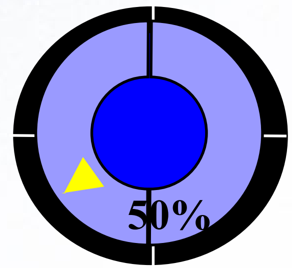
pH (2-9)



Organic Modifier Type (many)

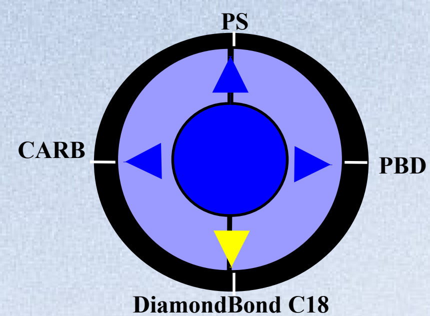


Temperature up to 50°C

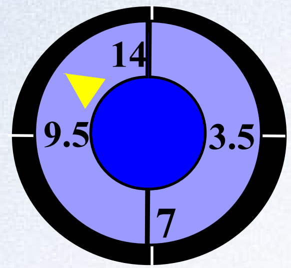


Percent Organic Modifier

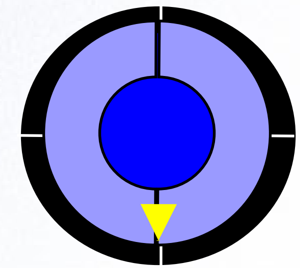
Method Development Variables on Zirconia RP Phases



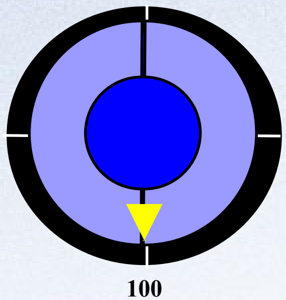
Selectivity



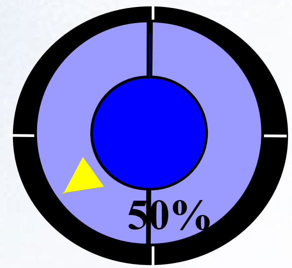
pH (1-14)



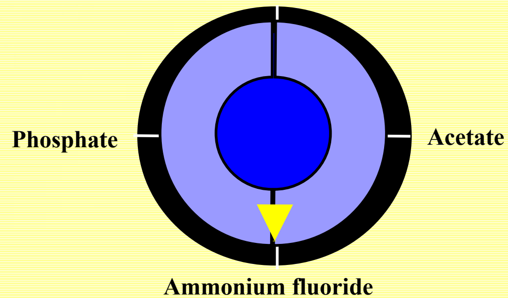
Organic Modifier Type (many)



Temperature up to 200°C



Percent Organic Modifier



Buffer or Additive - Type and Concentration*

* Except for ZirChrom EZ and MS

6.) Why is Column Bleed Bad in LCMS?

- 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.

General Conditions of M.S. Bleed Study

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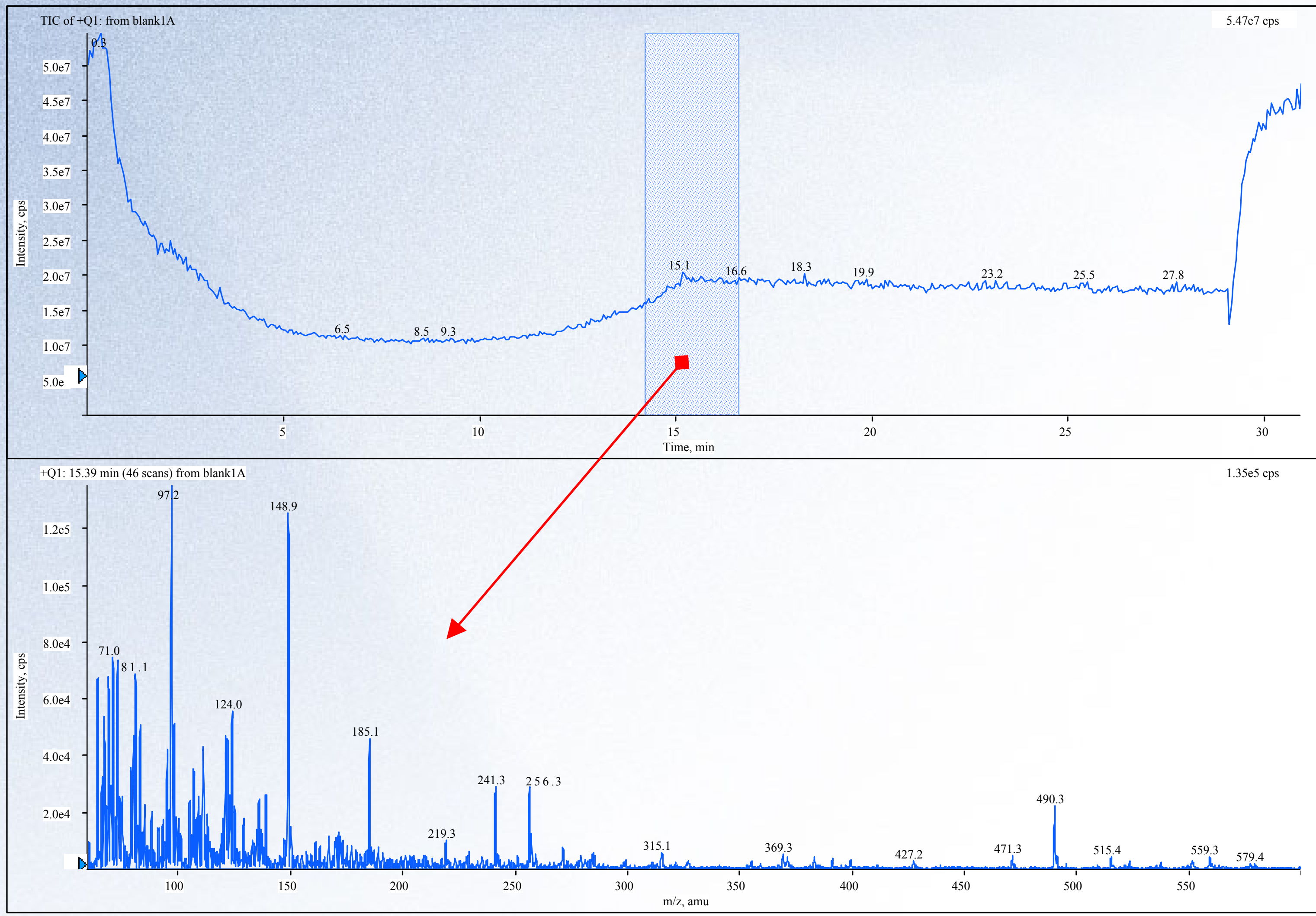
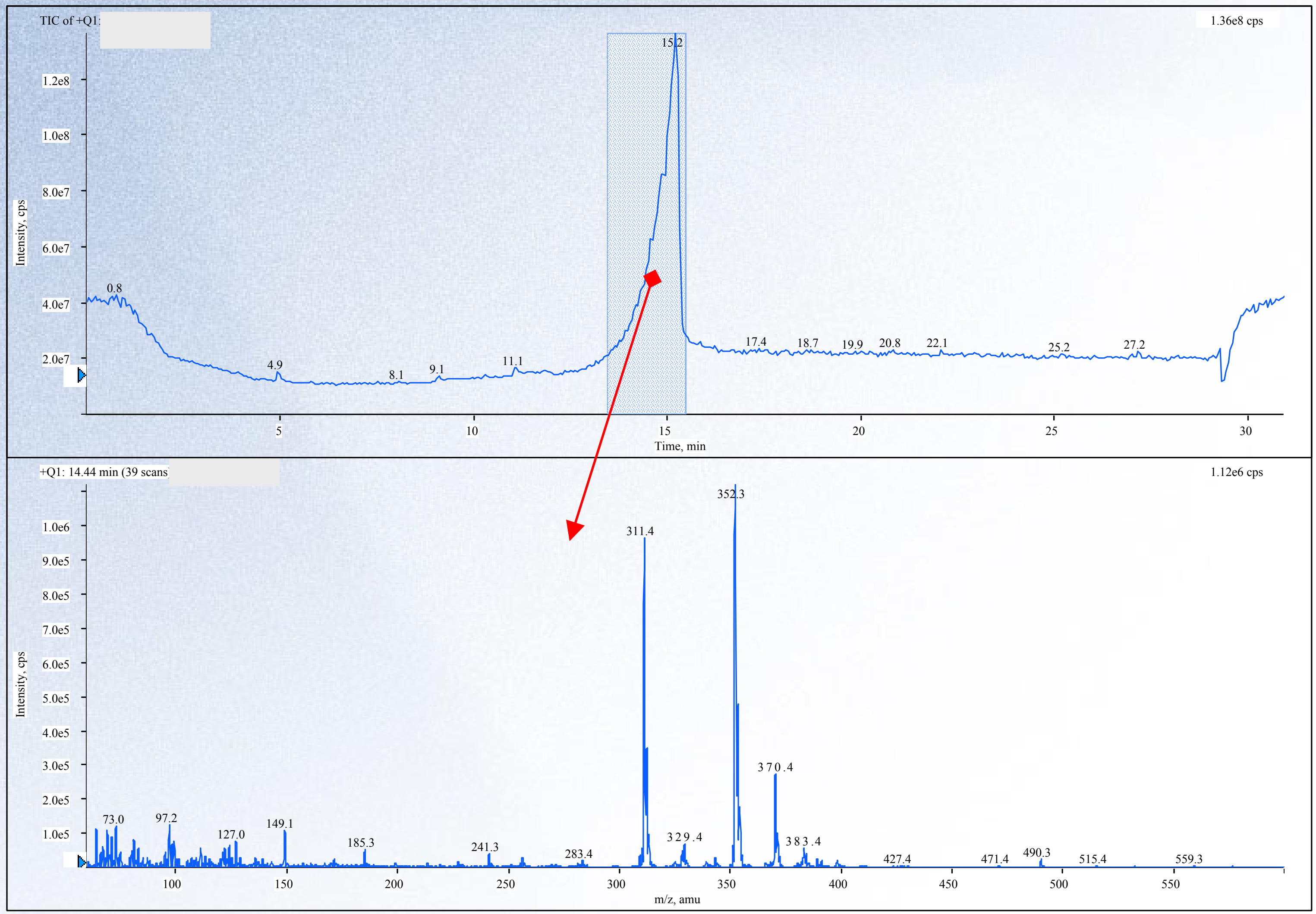
- SCIEX APCI 3000 triple quad in single quad mode at 400 ^{o}C.
- Total ion current 60-600 amu.
- Spectra is sum of 30-35 scans in time window.
- Mobile phase gradient 0-100% ACN/buffer in 15 min. plus 14 min. hold at 100%.
- pH 9 = 2mM NH_4Ac + NH_4OH.
- Note pump-column-MS. No injector used.

Blank Gradient at pH 9 and 55 ºC



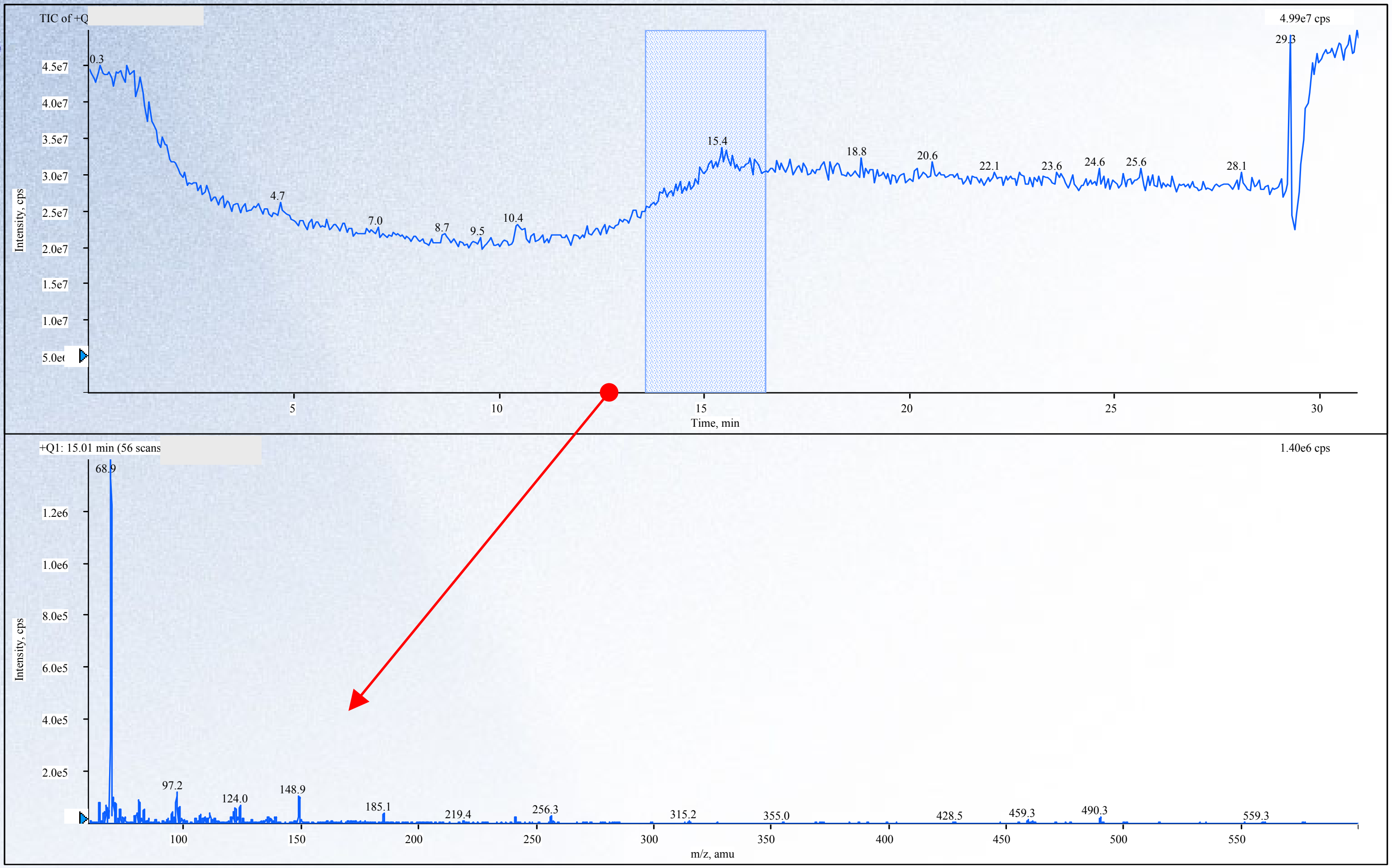
M.S. different than blank gradient at pH 7 probably due to ionization differences.

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



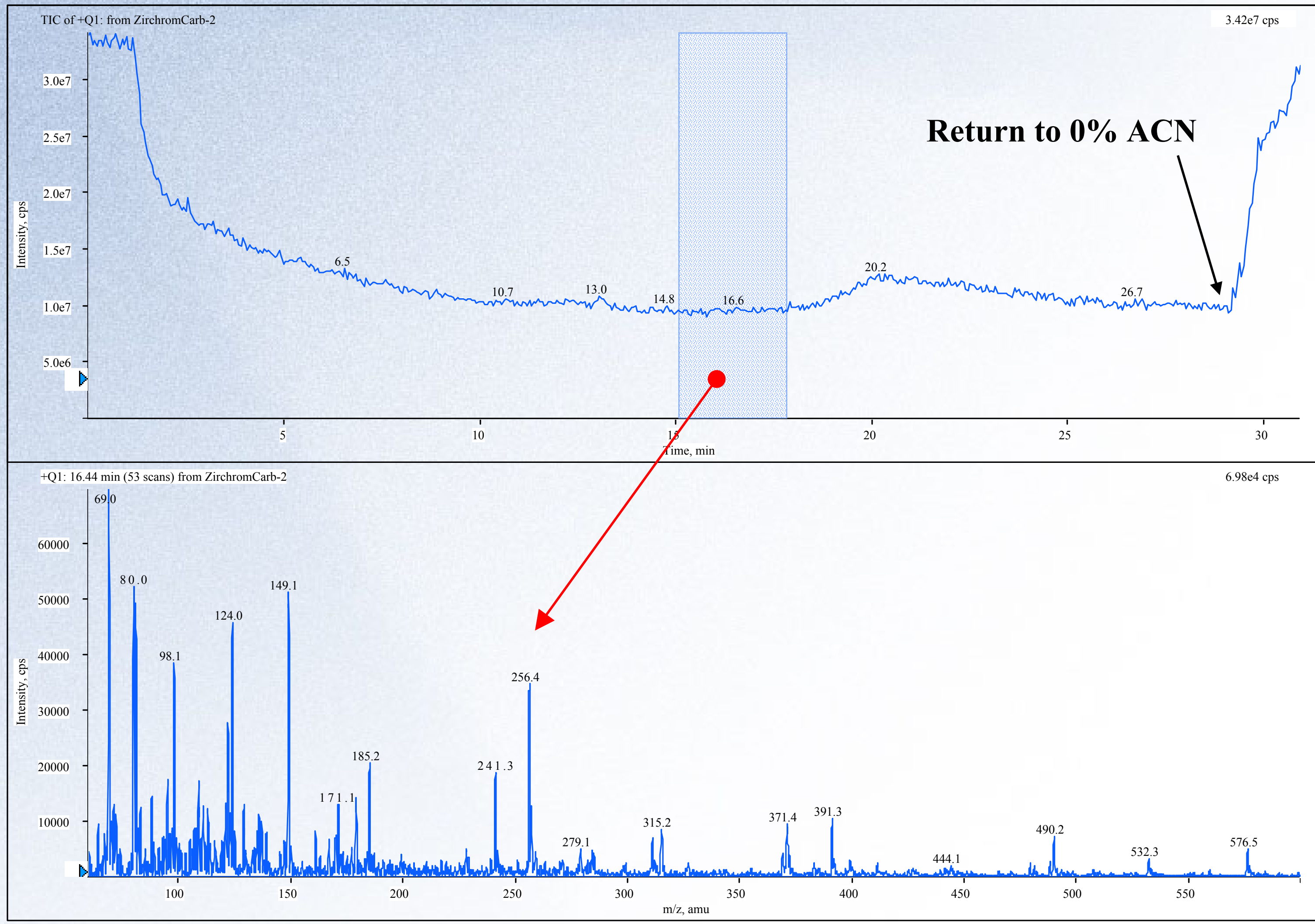
Note big peak at 12-15 min. in total ion chromatogram and new peaks in mass spec.

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 ^{o}C

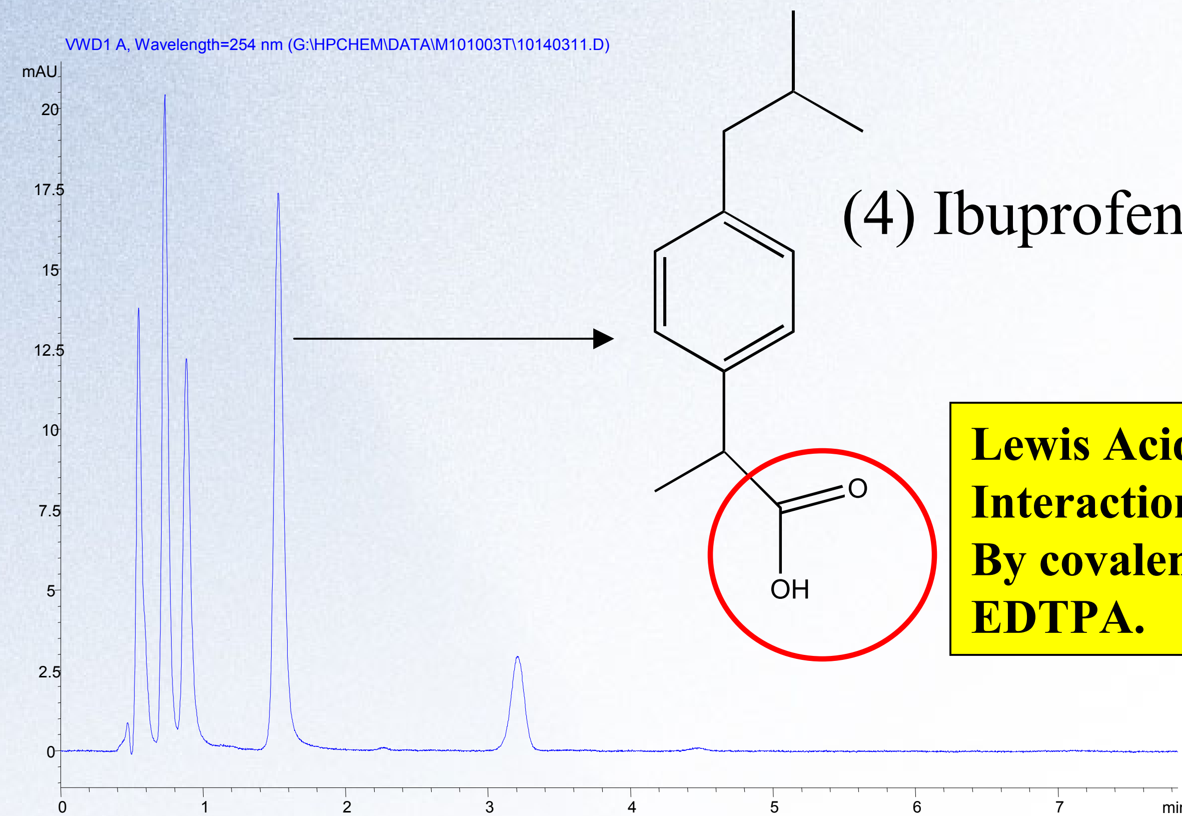


Total ion chromatogram and mass spectra with ZirChrom CARB nearly identical to blank gradient. No new discernible masses or peaks due to column.

7.) Example Method Development Case Studies

a.) ZirChrom-MS
b.) ZirChrom-PBD
c.) ZirChrom-CARB

Separation of Acidic Compounds on ZirChrom-MS



(4) Ibuprofen

Lewis Acid-Base Interaction "blocked" By covalently attached EDTPA.

Chromatographic Conditions: Column Dimension: 50X4.6 MS101003T; Mobile phase, Machine-mixed 40/60 ACN/10 mM ammonium acetate pH=5. Flow rate:1 ml/min, Temperature, 35^{o} C; Injection volume: 5 µl; Solutes eluted in order, (1) Acetaminophen, (2) Ketoprofen, (3) Naproxen, (4) Ibuprofen, (5) Impurity; Detection, 254 nm. Pressure drop, 68 bar.

Separation of β-Blockers on ZirChrom-MS

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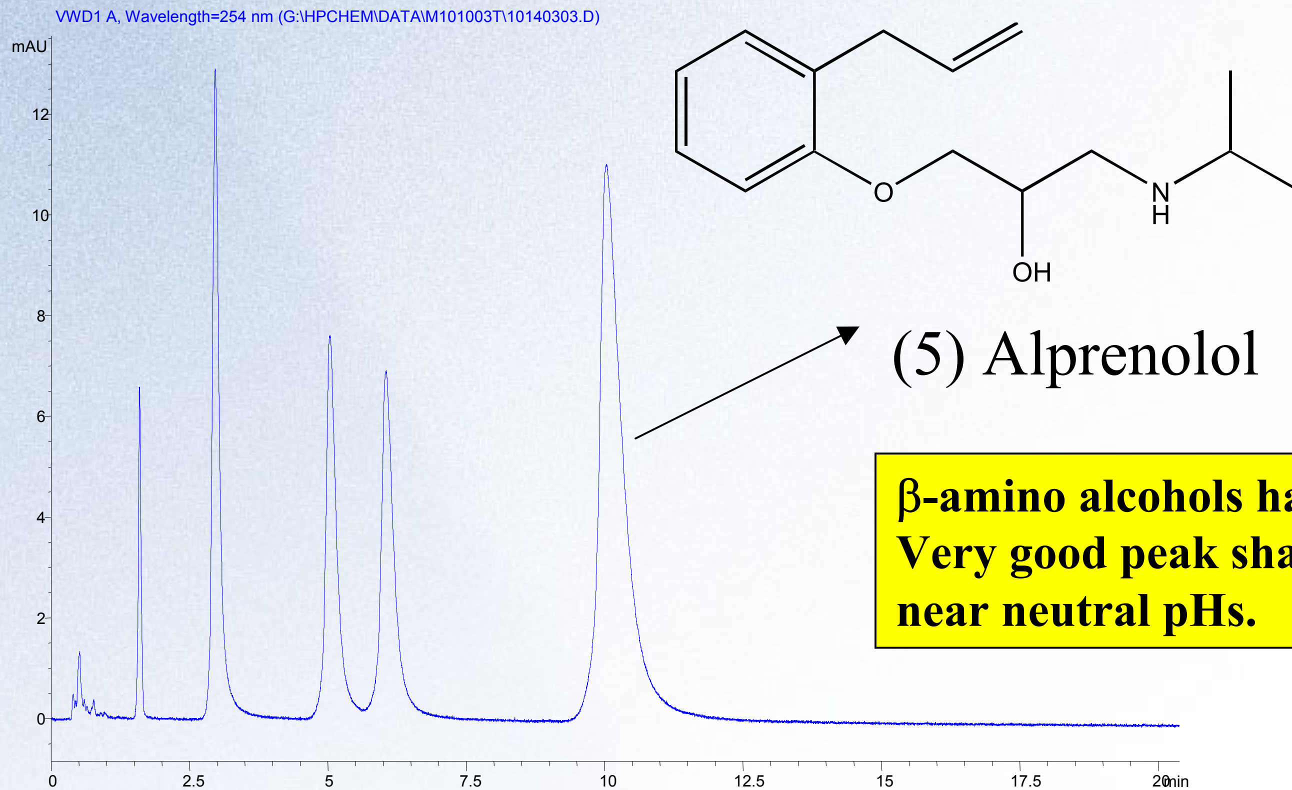


(5) Alprenolol

β-amino alcohols have Very good peak shape at near neutral pHs.

Chromatographic Conditions: Column Dimension: 50X4.6 MS101003T; Mobile phase: Machine-mixed 65/35 ACN/10 mM ammonium acetate pH=5; Flow rate:1 ml/min; Temperature, 35^{o} C; Injection volume: 5 µl. Solutes eluted in order: (1) Lidocaine, (2) Atenolol, (3) Metoprolol, (4) Oxprenolol, (5) Alprenolol
Detection: 254 nm; Pressure drop, 59 bar.

Fast Analysis of Glyburide for LC/MS on ZirChrom-PBD

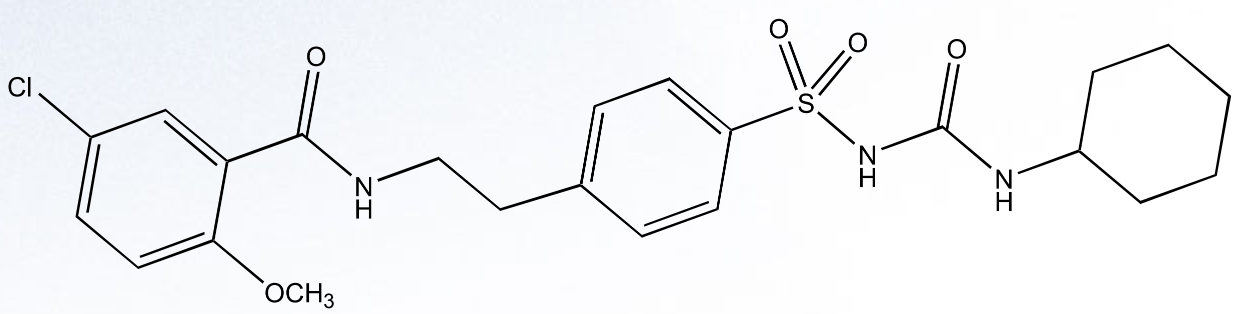
Current method: Isocratic elution in acetic acid buffer on C18-silica

Problem: Analysis time is too long

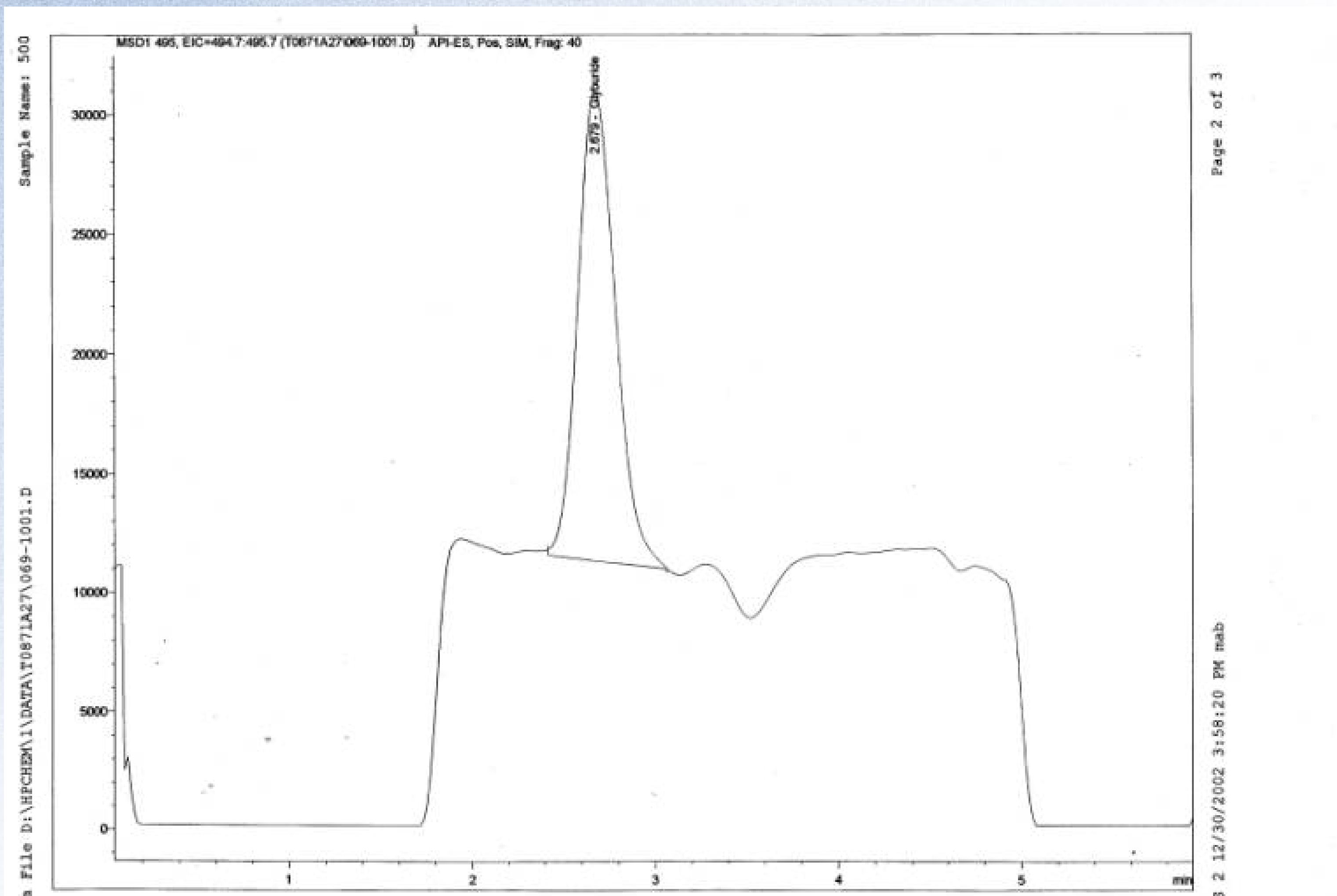
Customer Goal: Reduce analysis time by a factor of 2

Starting Conditions

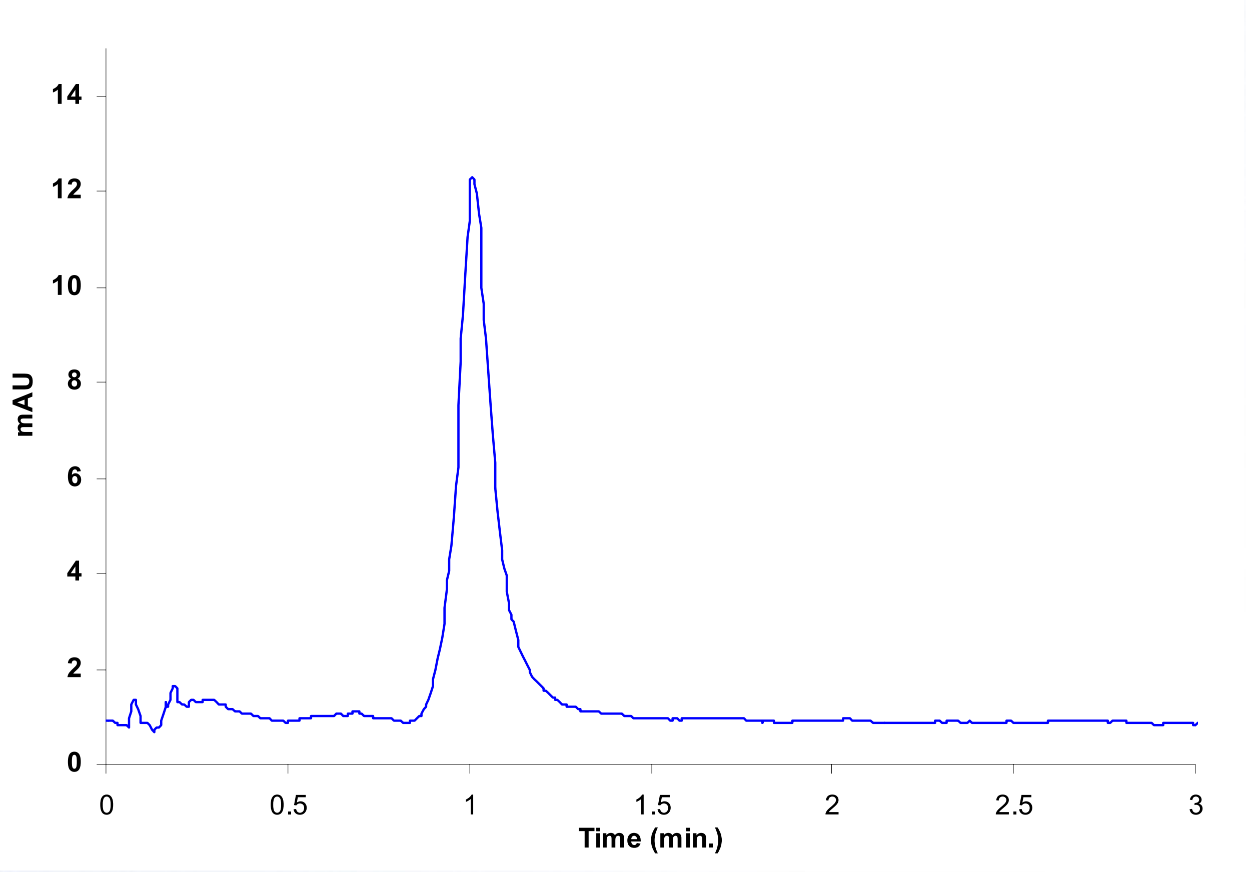
Mobile phase:	ACN/Acetic acid, pH 3.3
Flow rate:	0.8 ml/min.
Temperature:	75 ^{o}C
Detection:	254 nm



C18-Silica Method



Final Glyburide Separation



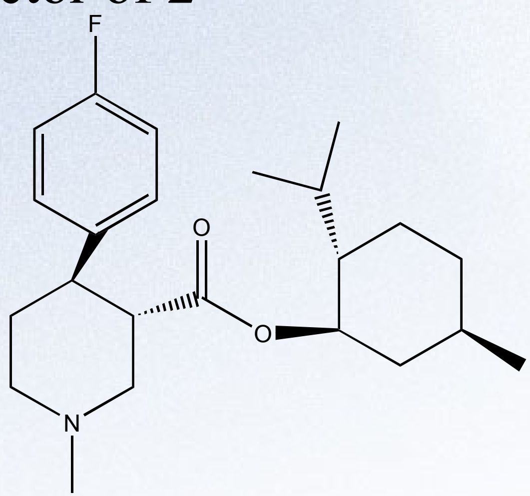
LC Conditions: Mobile phase, 30/70 ACN/20mM Acetic acid, pH 3.3; Flow rate, 0.80 ml/min.; **Temperature, 70 °C**; Injection volume, 1 µl; Detection at 240 nm; Column, 50mm x 2.1mm i.d. ZirChrom®-PBD, backpressure = 126 bar

Fast Diastereomer Separation on ZirChrom-CARB

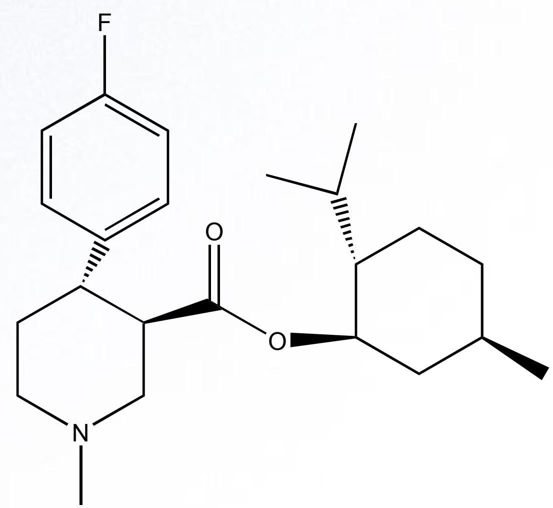
Current method: Methanol gradient with phosphate buffer at pH 3.5 on a Zorbax SB-CN column

Problem: Very little resolution, even with a run time of 20 minutes

Customer Goal: Improve resolution of diastereomers and reduce analysis time by a factor of 2



Diastereomer #1



Diastereomer #2

Carbon Has Unique Selectivity

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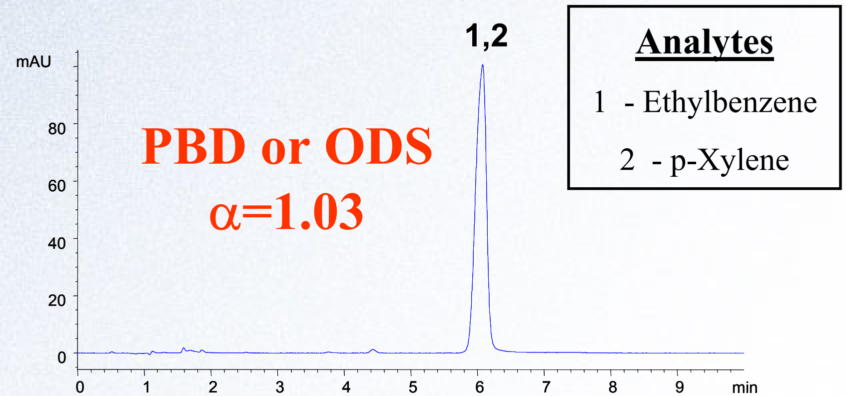
LC Conditions

Column: ZirChrom®-PBD, 100 x 4.6 mm

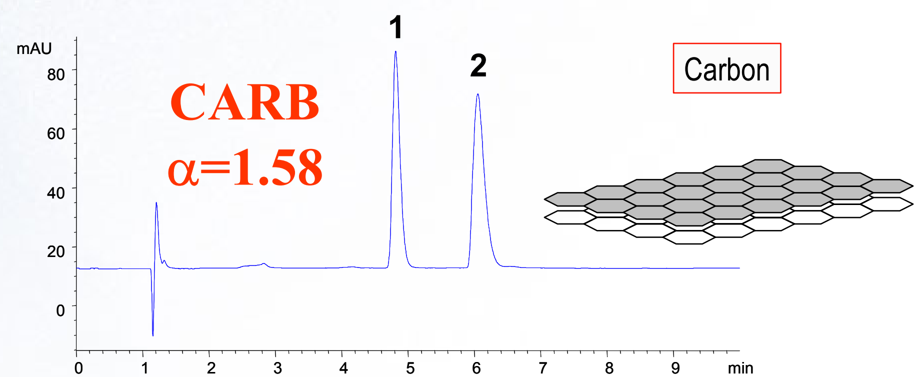
Mobile phase:		Flow rate:	1.0 mL/min.
	35/65 A/B	Temperature:	30 °C
A:	ACN	Injection volume:	5 µL
B:	Water	Detection:	254 nm



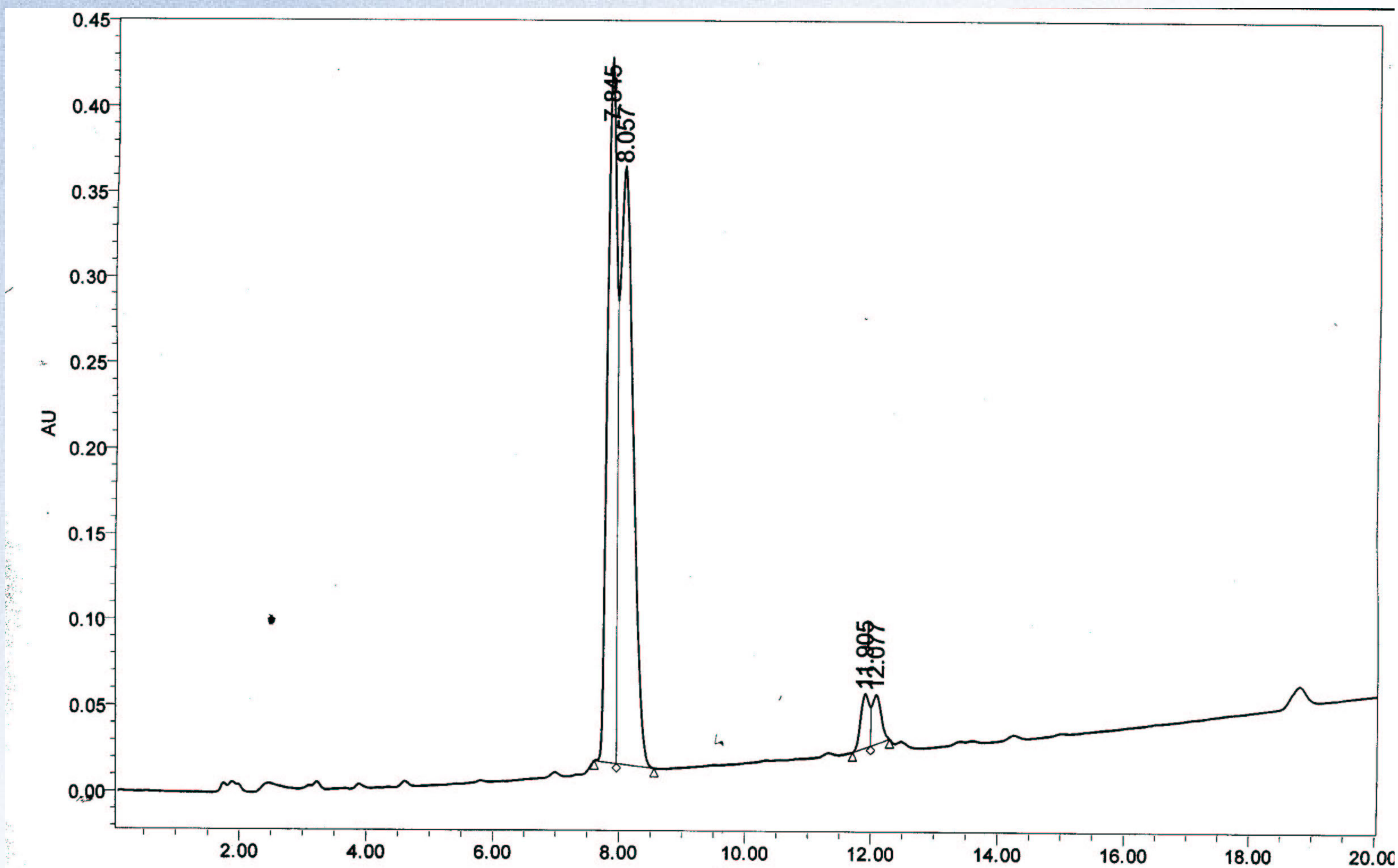
LC Conditions

Column: ZirChrom®-CARB, 100 x 4.6 mm

Mobile phase:		Flow rate:	1.0 mL/min.
	32.5/67.5 A/B	Temperature:	60 °C
A:	ACN	Injection volume:	5 µL
B:	Water	Detection:	254 nm



C18-Silica Method



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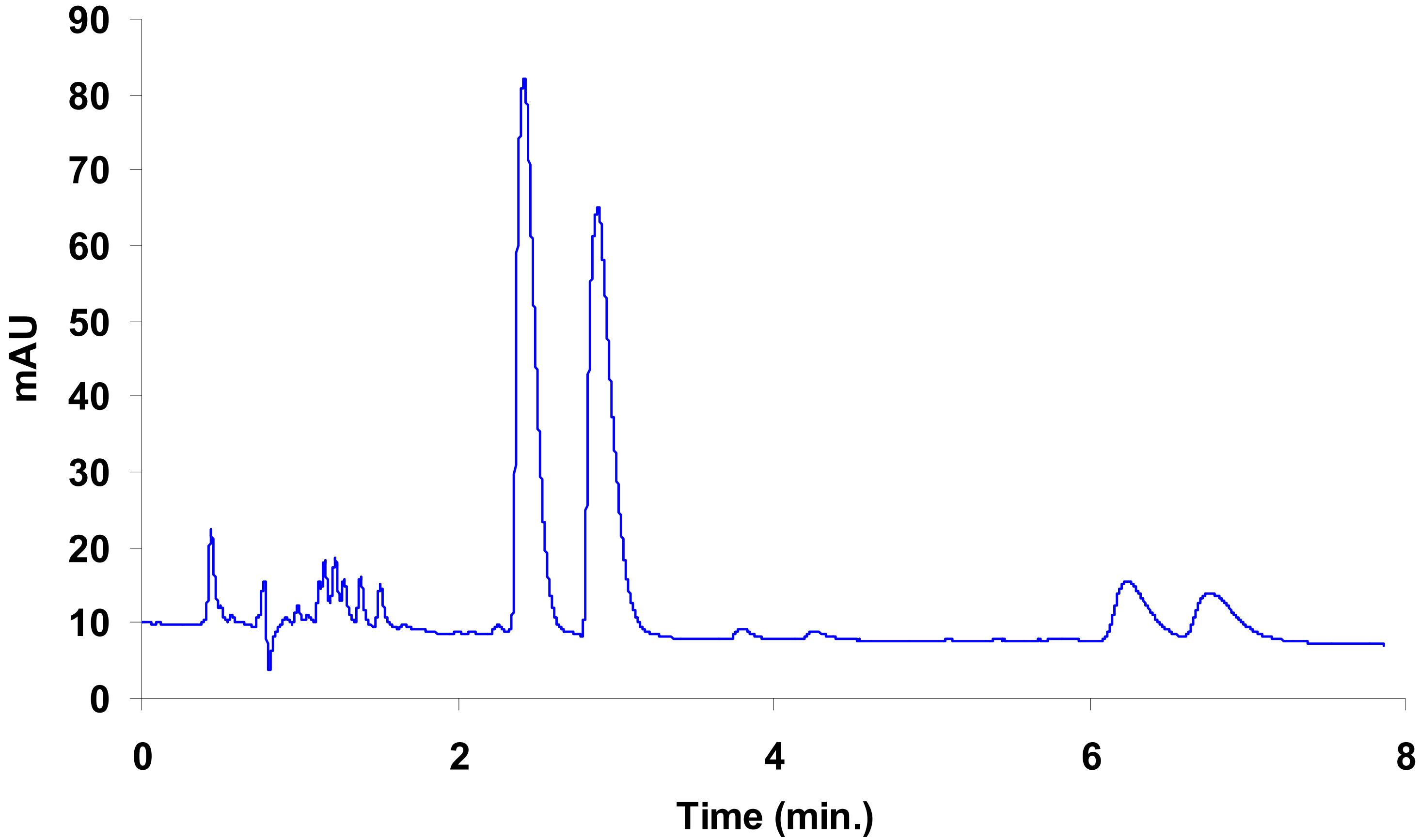
Method Development Strategy

1. ZirChrom-CARB will provide the best selectivity for diastereomers
2. Elevated temperature and strong solvents can be used to improve peak shape
3. A high pH buffer can be used to deprotonate the amine functionalities

Starting Conditions:

Column:	ZirChrom-CARB	Temperature:	80 ^{o}C
Mobile phase:	Gradient elution from 10-90% B	Detection:	240 nm
	A: Water	Injection vol.:	5 µl
	B: ACN		

The Final Separation



LC Conditions: Column, 150 mm x 4.6 mm i.d. ZirChrom-CARB; Mobile phase, 35/35/30 ACN/Butanol/10mM Diethylamine, pH 11.2; Flow rate, 2.00 ml/min.; Temperature, 80 ^{o}C; Injection volume, 5 µl; Detection at 265 nm.

Advantages of the Zirconia-Based Solution

- This method capitalizes on the unique selectivity of ZirChrom-CARB to give a fast separation with excellent resolution for these structurally similar analytes.
- The chemical stability of ZirChrom-CARB allows the use of high pH and high temperatures to improve peak shape and reduce analysis time.
- The customer, a senior analytical chemist in method development, was particularly impressed with the good resolution of these diastereomers.

9 Nitrosamines Analysis

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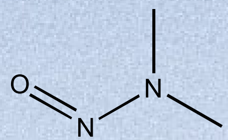
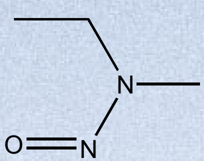
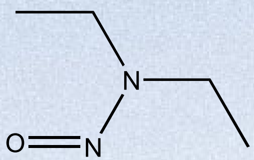


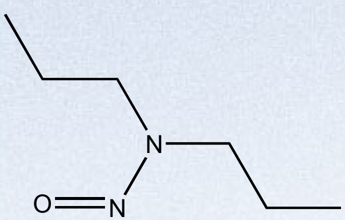
N-nitrosodimethylamine



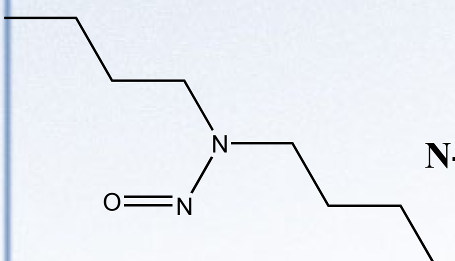
N-nitroso-n-ethylmethylamine



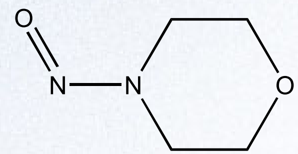
N-nitrosodimethylamine



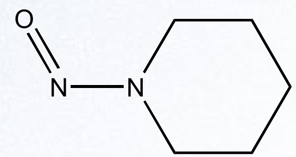
N-nitrosodi-n-propylamine



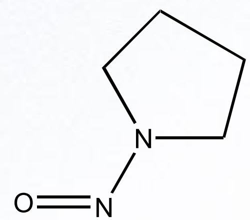
N-nitrosodi-n-butylamine



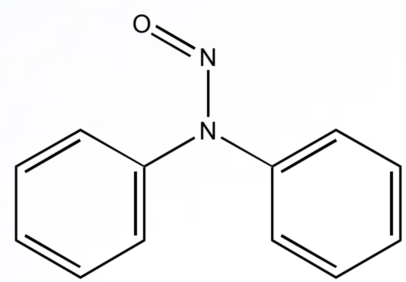
N-nitrosomorpholine



N-nitrosopiperidine



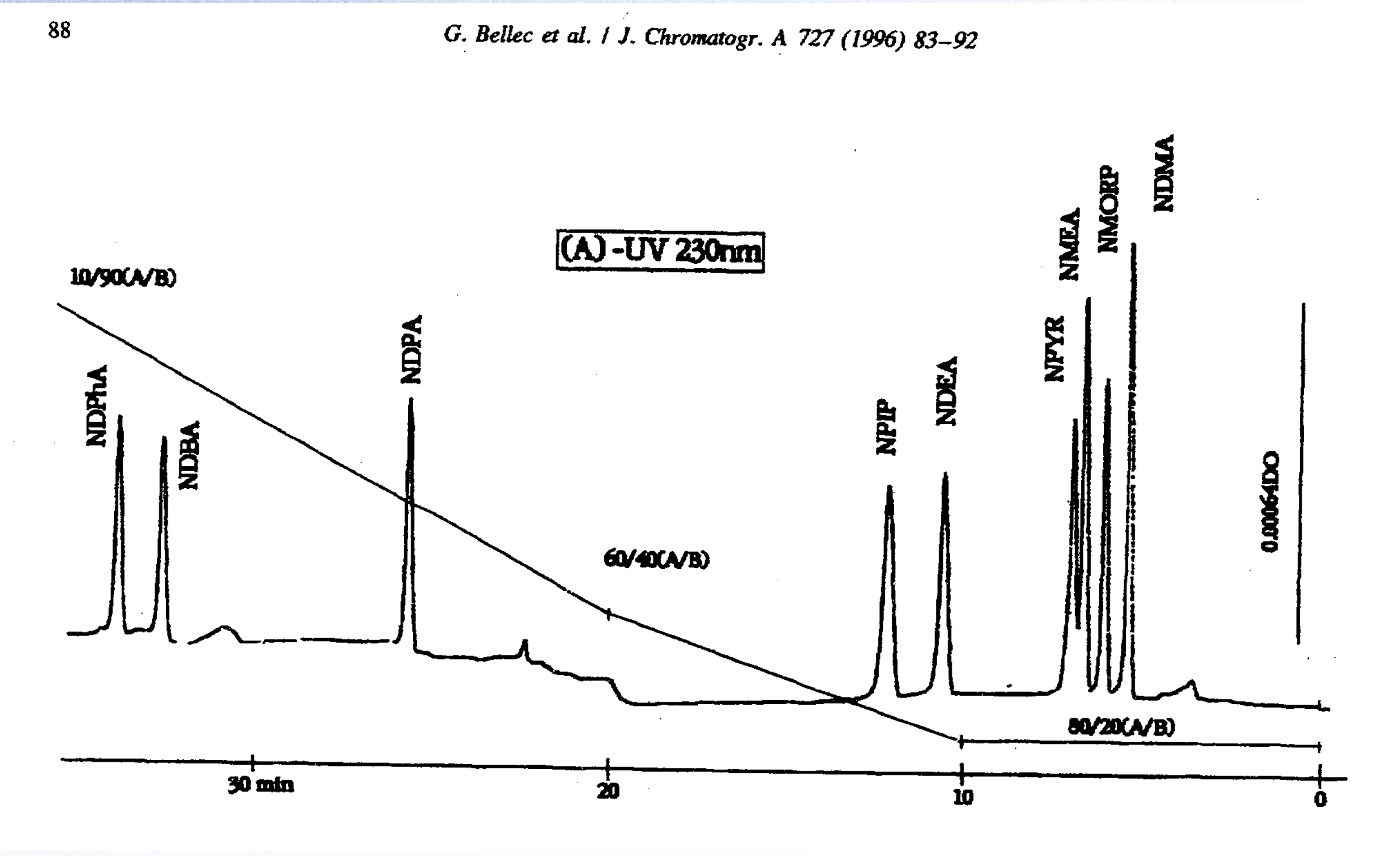
N-nitrosopyrrolidine



N-nitrosodiphenylamine

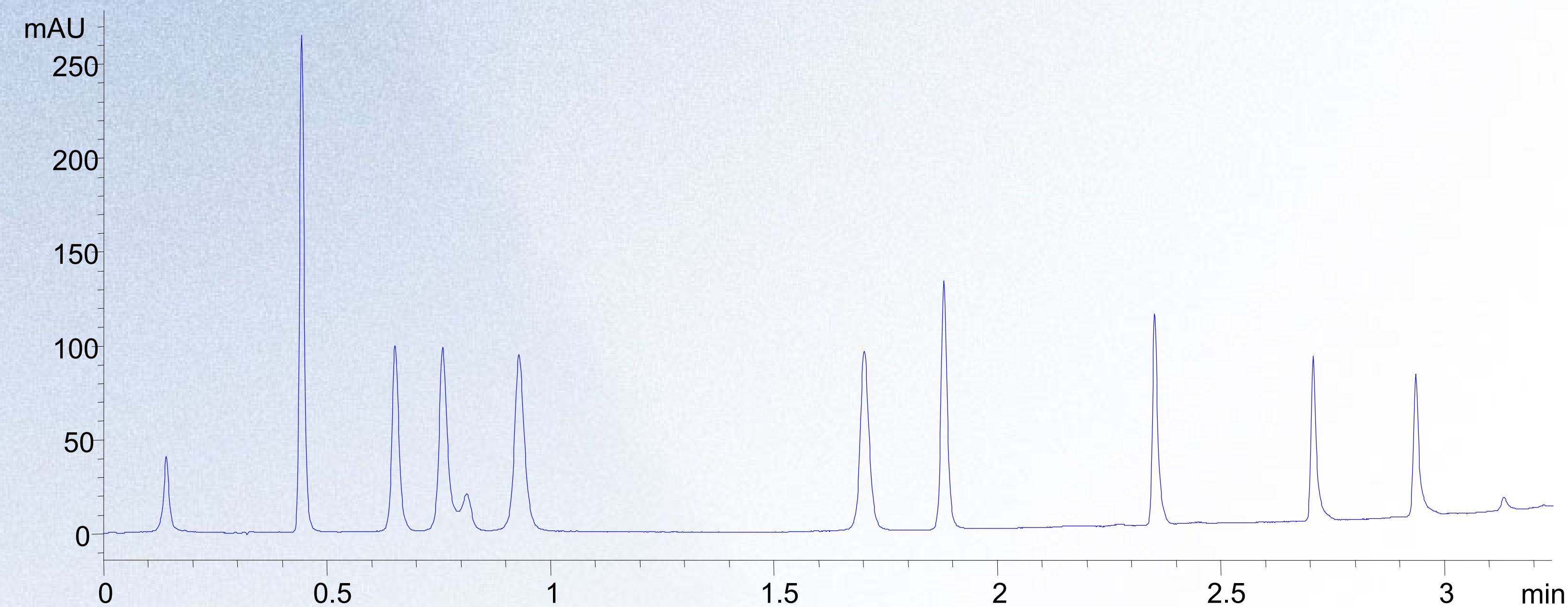
Nitrosamines (Supelco Test Mix)

Peer-reviewed Silica-based RPLC Method



A *Fast* Zirconia-based Method at pH 9.5

ZirChrom®



Column: DiamondBond C18, 100 × 4.6 mm
Mobile Phase: 2.5-90%B from 1-3 minutes
A: 10mM Ammonium hydroxide, pH 9.5
B: 100% Acetonitrile

Flow rate: 4.0 mL/min.
Temperature: 75 ºC
Injection volume: 1.0 µL
Detection: 230 nm
Back Pressure: 200 bar

Conclusions

- **Zirconia based columns are *chemically and thermally robust*.**
- **Zirconia-based columns often have *very different chromatographic selectivity* than silica C18, which is extremely useful for method development.**
- **ZirChrom-CARB shows "*No-Bleed*" by MS under highly aggressive LC conditions (pH 9, 55 ^{o}C).**
- **ZirChrom-CARB has *excellent selectivity* for diastereomeric compounds.**
- **The *new* ZirChrom-MS column is a stable zirconia-based column designed for HPLC MS applications.**

Acknowledgements

The author wishes to thank the following:

- **The research group under Professor Peter W. Carr at the University of Minnesota**
- **The Research Team at ZirChrom Separations led by Dr. Bingwen Yan**

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