ZirChrom®

# Improving Method Development through Stationary Phase Optimization


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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

- **Surface Chemistry**
- **Lewis Base Additives**
- **Selectivity**
- **pH Stability**
- **Organic Modifiers**
- **Carbon Phase Considerations**
- **Buffer Choice**

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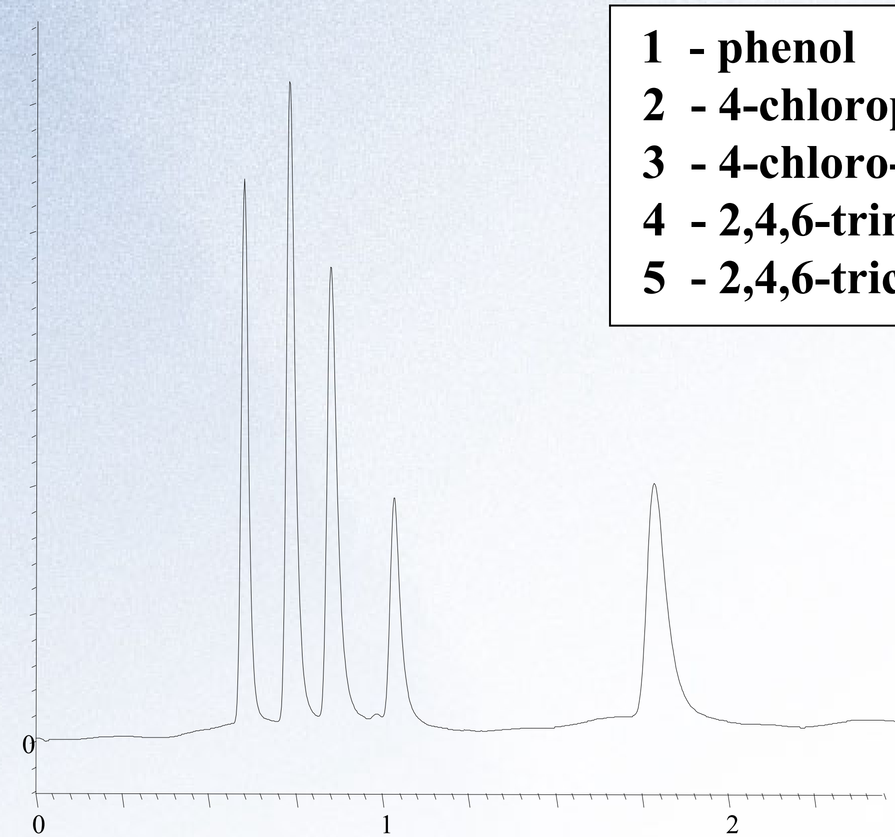
# *Zirconia*
# the "*Un-Silica*".
# The difference is the surface chemistry.

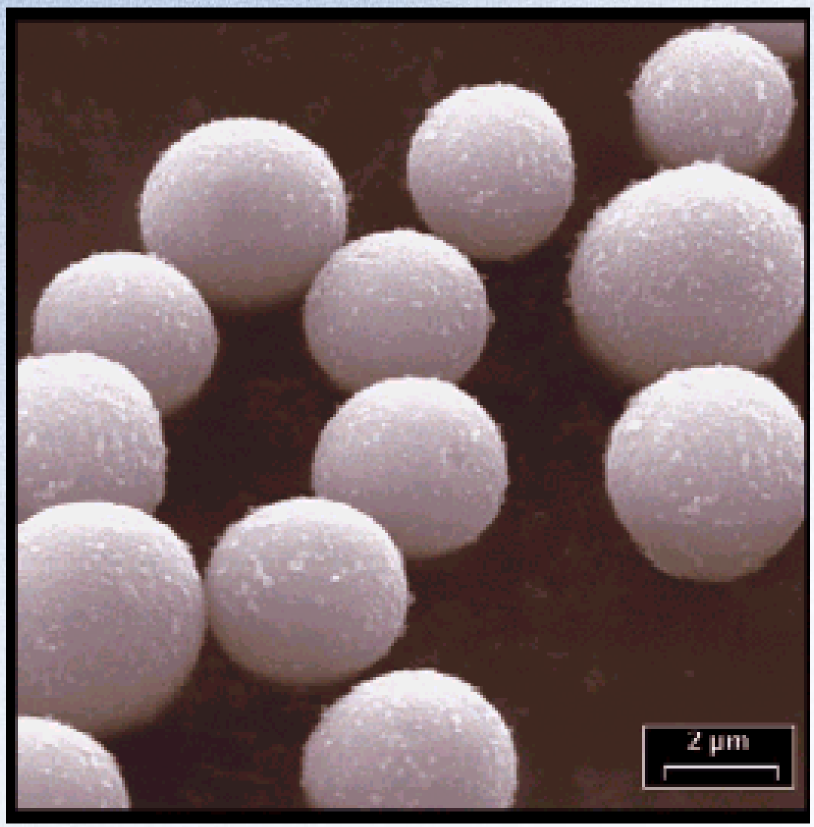
# *Don't try this with Silica*

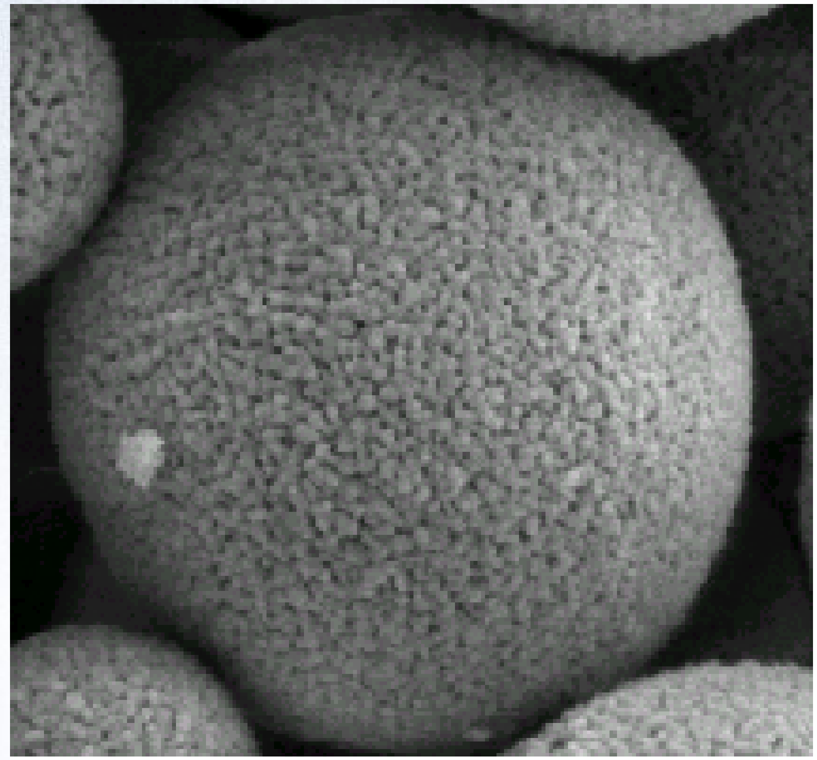



**1 - phenol**
**2 - 4-chlorophenol**
**3 - 4-chloro-3-methyl phenol**
**4 - 2,4,6-trimethylphenol**
**5 - 2,4,6-trichlorophenol**

Column: ZirChrom®-PBD, 150 x 4.6 mm, Mobile Phase: 100% water, Flow rate = 1 mL/min. Flow rate: 3.0 ml/min, **Temperature: 200 ºC**, Detection: 254 nm.

# Analytical Diameter Porous Zirconia Particles



1μm 25000X

# Properties of Porous Analytical Zirconia

| Characteristic | Property |
|---|---|
| Surface area ($m^2/g$) | 22 |
| Pore volume (cc/g) | 0.13 |
| Pore diameter (Å) | 250-300 |
| Porosity | 0.45 (silica 0.48) |
| Density (gm/cc) | 2.6 (2.5x silica) |
| Particle size(μm) | 3.0 (130,000 p/m) |

Prep-scale particles also available

# Surface Chemistry of Zirconia

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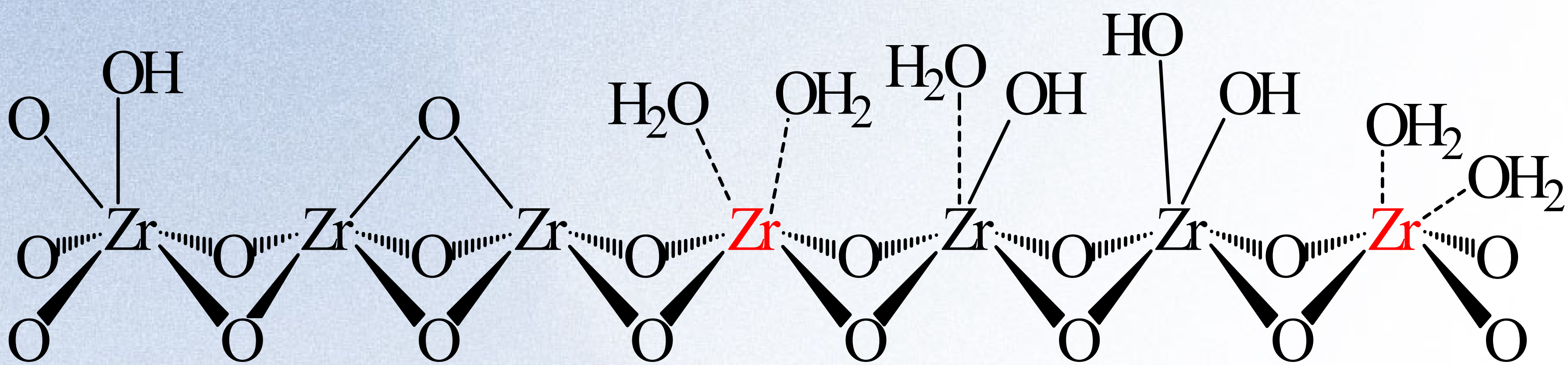


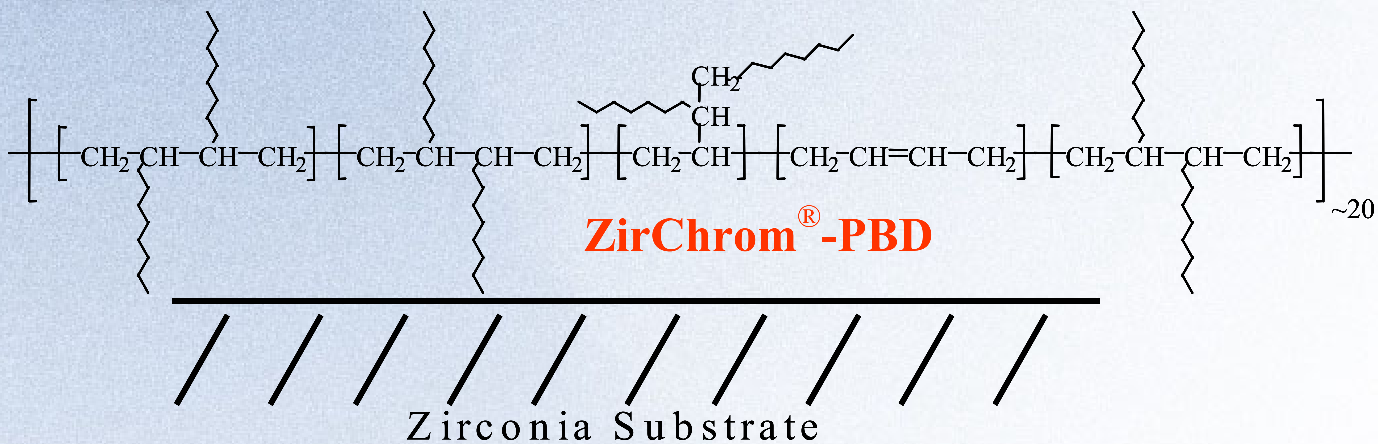
**Brönsted Acid:** $ZrOH + {}^{-}OH \rightleftharpoons ZrO^{-} + H_2O$

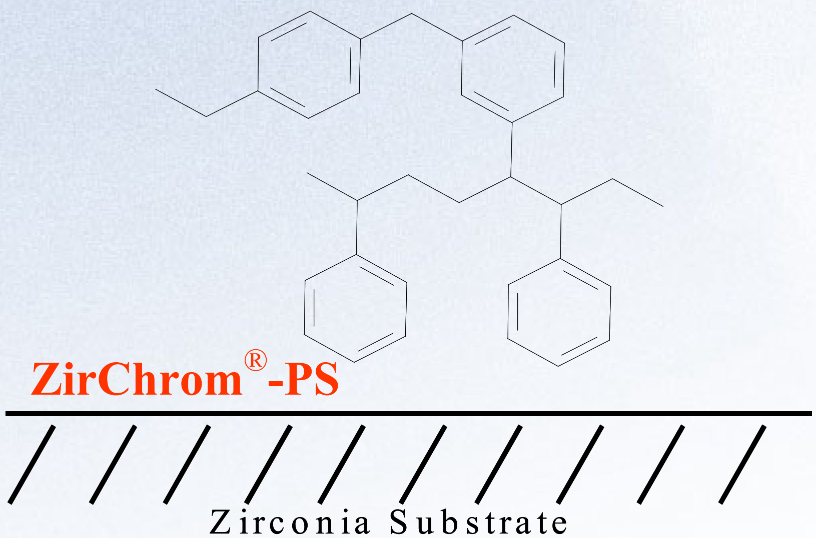
**Brönsted Base:** $Zr{-}O{-}Zr + H^{+} \rightleftharpoons Zr{-}O(H^{+}){-}Zr$

**Lewis Acid:** $\mathbf{Zr^{4+}\!: H_2O + R\text{-}COO^{-} \rightleftharpoons Zr^{4+}\!:{}^{-}OOC\text{-}R + H_2O}$

**Ligand Exchange Chromatography**

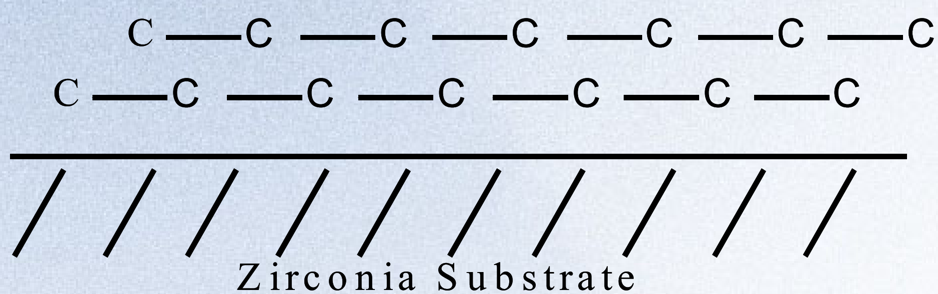
# ZirChrom®-PBD and ZirChrom®-PS



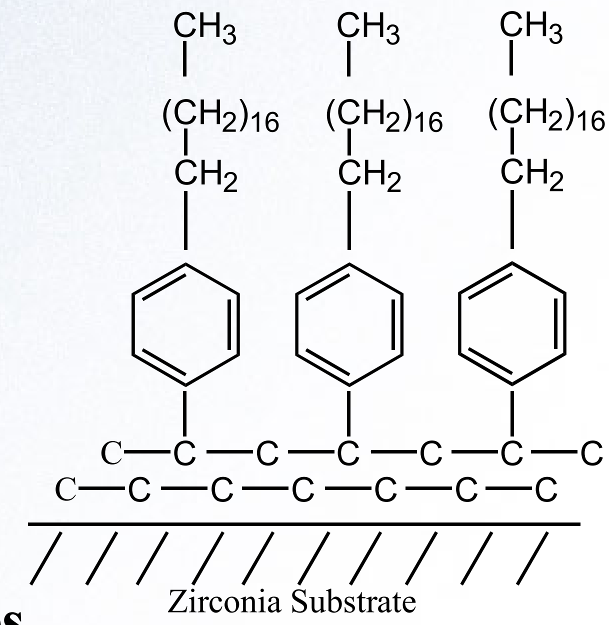




- **Thin layer of polymer**
- **Closest selectivity to silica phases**
- **Mixed-mode (RP and IEX)**
- **Tunable selectivity**
- **pH and thermally stable**

# ZirChrom®-CARB and DiamondBond®-C18



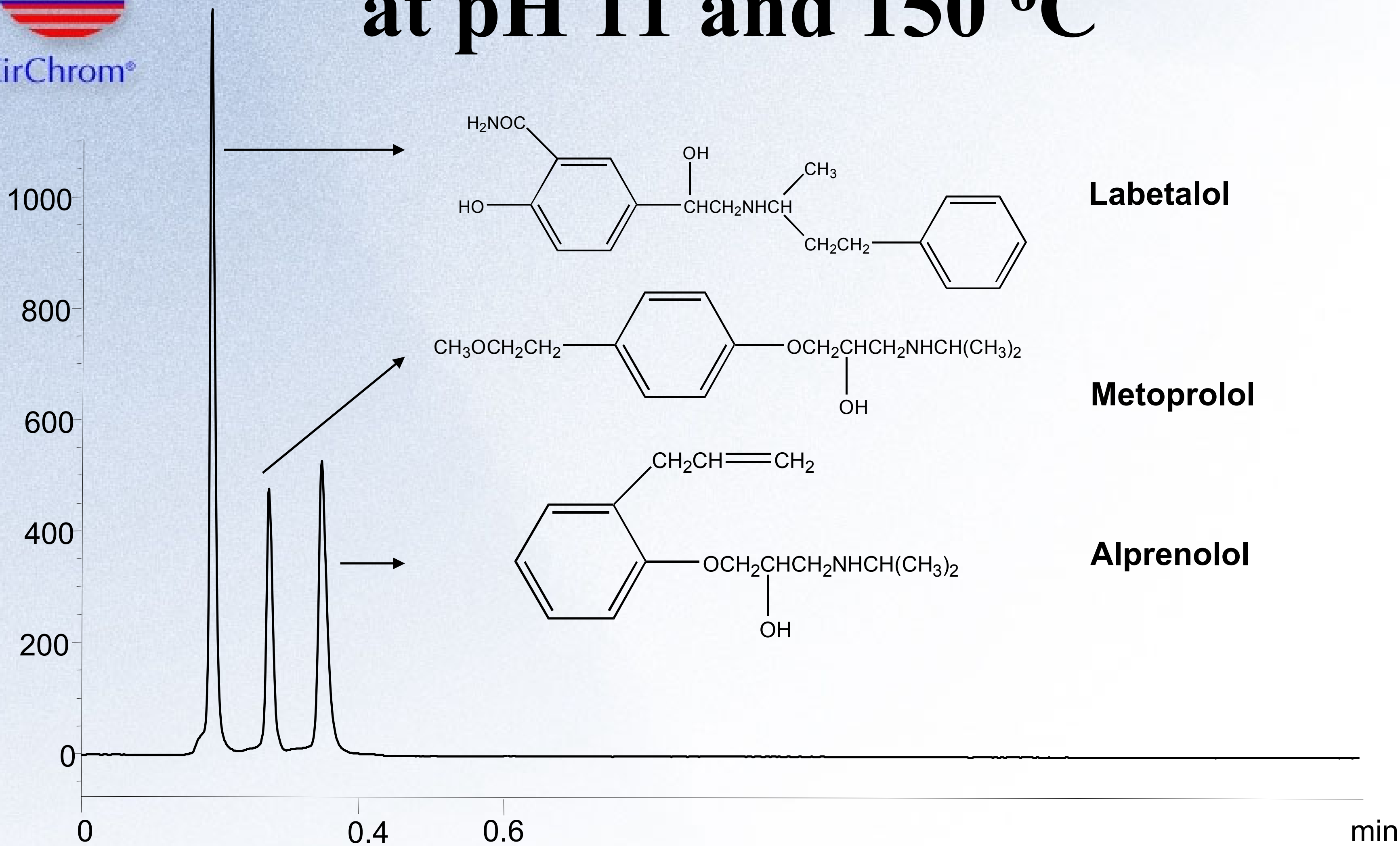

**ZirChrom®-CARB**




**DiamondBond™-C18**

- **Thin layer of carbon**
- **Very different selectivity to silica phases**
- **Mixed-mode (RP and IEX)**
- **Tunable selectivity**
- **pH and thermally stable**
- **Great for LC/MS**

# Fast Pharmaceutical Separation at pH 11 and 150 °C




LC Conditions: Column, 50 x 4.6 Diamondbond-C18, OD0121601A; Mobile phase, 45/55 ACN/20mM Ammonium Phosphate pH11.0; Flow rate, 3.0 ml/min; Temperature, 150 °C; Injection volume, 1.0 ul; Detection at 210 nm; Solutes, 1=Labetalol, 2=Metoprolol, 3=Alprenolol

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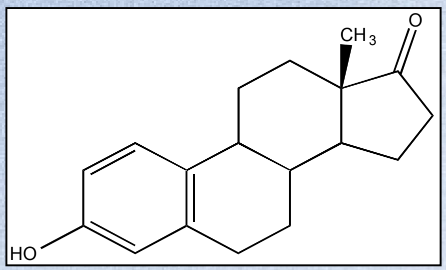
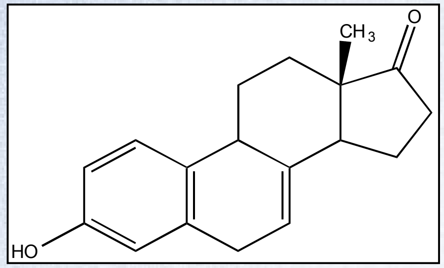
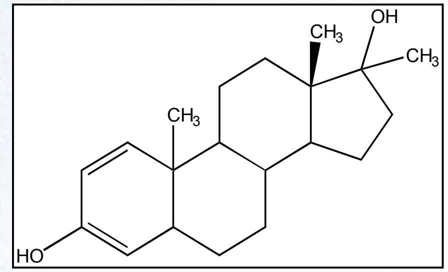
# Case Study I – Analysis of Free Steroids and Sulfate Conjugates



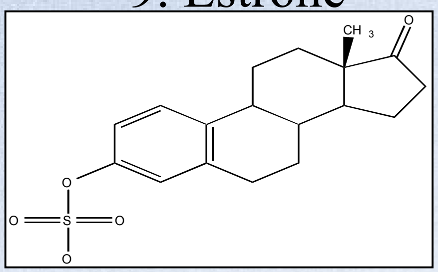

9. Estrone



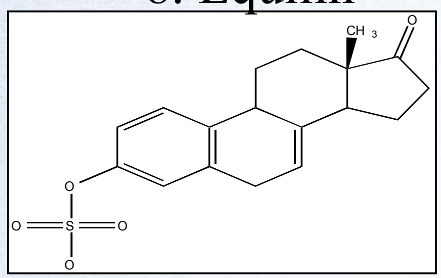

8. Equilin



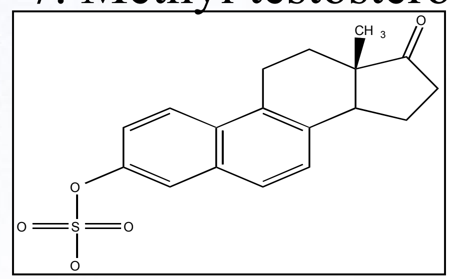

7. Methyl testosterone



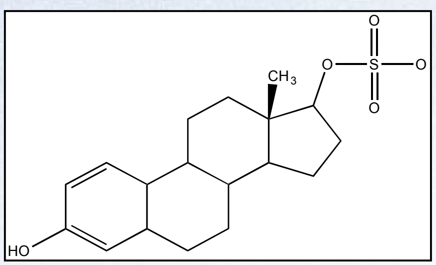

4. Estrone sulfate



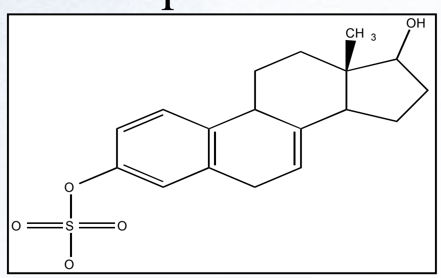

3. Equilin sulfate



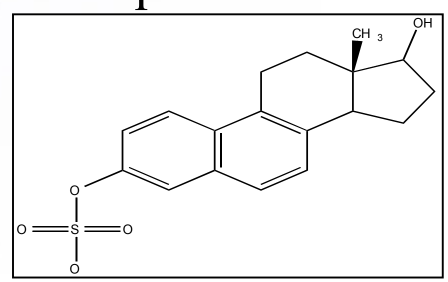

5. Equilenin sulfate




1. Estradiol sulfate

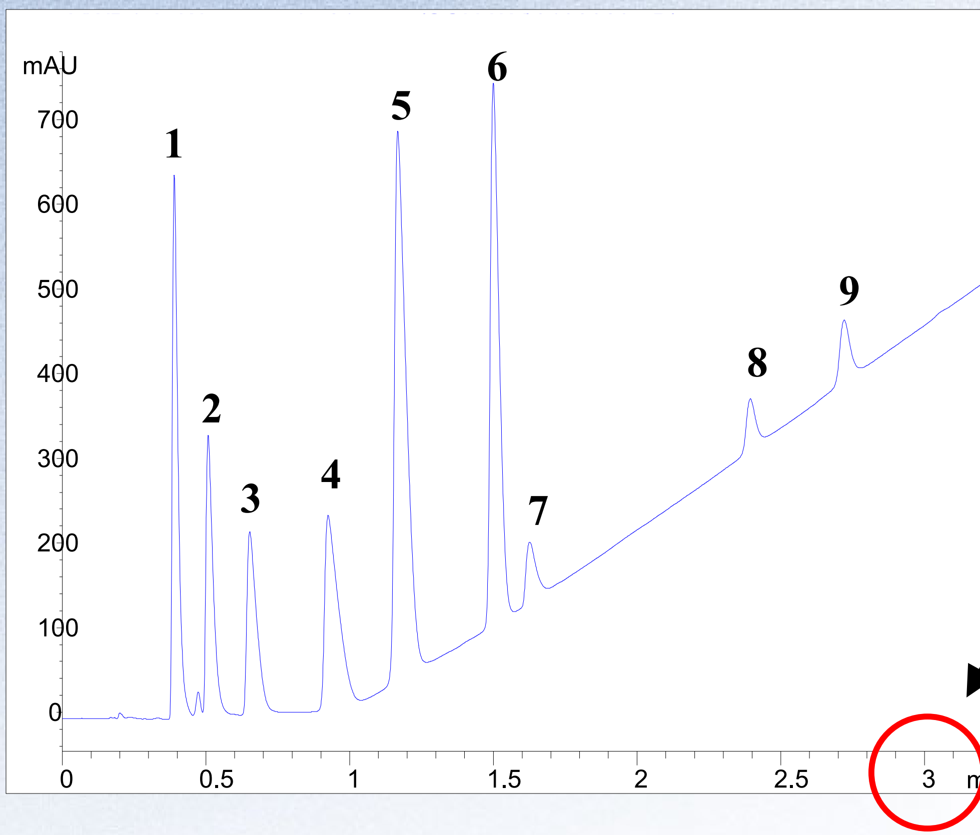



2. Dihydroequilin sulfate




6. Dihydroequilenin sulfate

# *Selectivity and Speed*




**LC Conditions**:

Column, 100 x 3.0 ZirChrom-CARB

2-90% B from 0.3-3.9 minutes

A = 40/60 ACN/25mM Ammonium fluoride, pH 5.6

B = 40/60 ACN/THF

**Flow rate: 2.5 ml/min.**

Temperature, 125 $^{o}$C

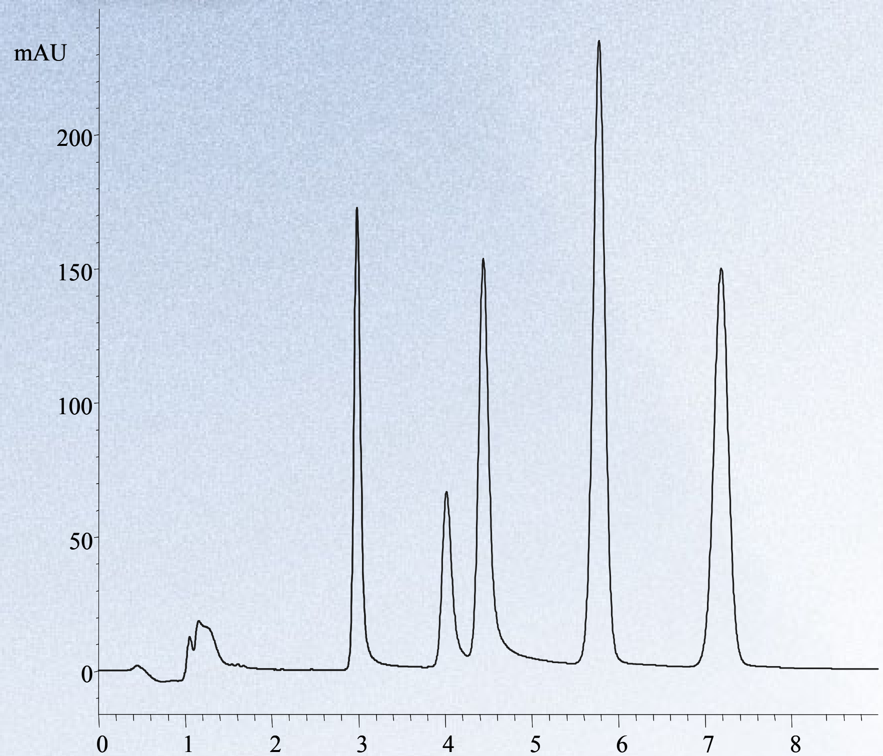
Injection volume, 2 µl

Detection at 215 nm

**Solutes**: 1=Estradiol sulfate, 2=Dihydroequilin sulfate, 3=Equilin sulfate, 4=Estrone sulfate, 5=Equilenin sulfate, 6=Dihydroequilenin sulfate, 7=Methyl testosterone, 8=Equilin, 9=Estrone

# *Excellent Peak Shapes*

## Tricyclic antidepressants on ZirChrom-PBD




1 – Nordoxepin
2 – Protriptyline
3 – Nortriptyline
4 – Imipramine
5 - Amitriptyline

Column: (A) ZirChrom®-PBD, 150 × 4.6 mm
Mobile Phase: (A) 45/55 A/B
A: Acetonitrile
B: 20 mM potassium phosphate at **pH 12.0**

Flow rate: 1.0 mL/min.
Temperature: 30 ºC
Detection: 254 nm

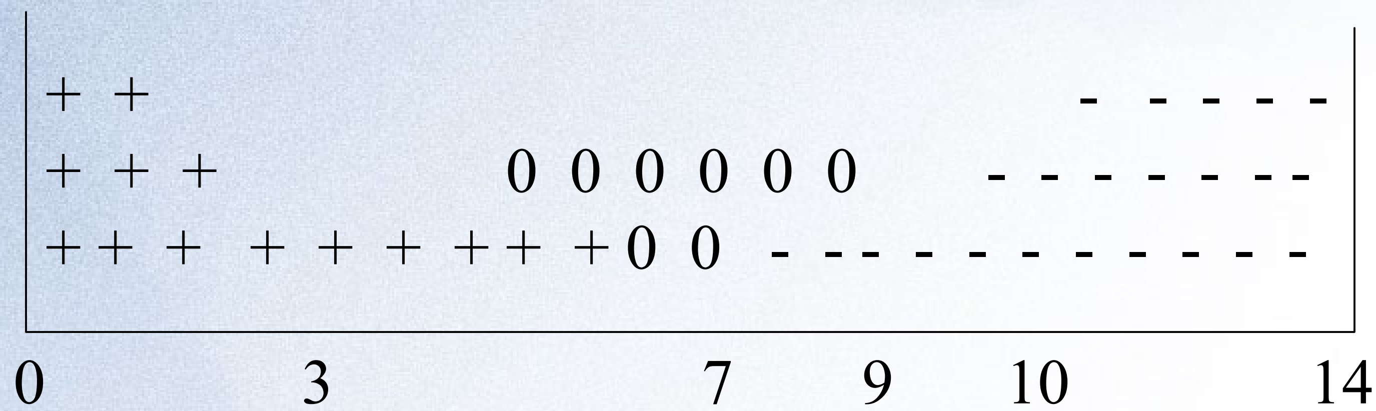
# Interaction Strength of Lewis Bases with Lewis Acid (Zr(IV)) Sites on Zirconia

| Interaction Strength | Lewis Base (A) |
|---|---|
| Strongest | Hydroxide |
| ↓ | Phosphate |
| | Fluoride |
| | Citrate |
| | Sulfate |
| | Acetate |
| | Formate |
| Weakest | Chloride |

- Small Lewis bases with higher electron density and lower polarizability interact more strongly with zirconia.

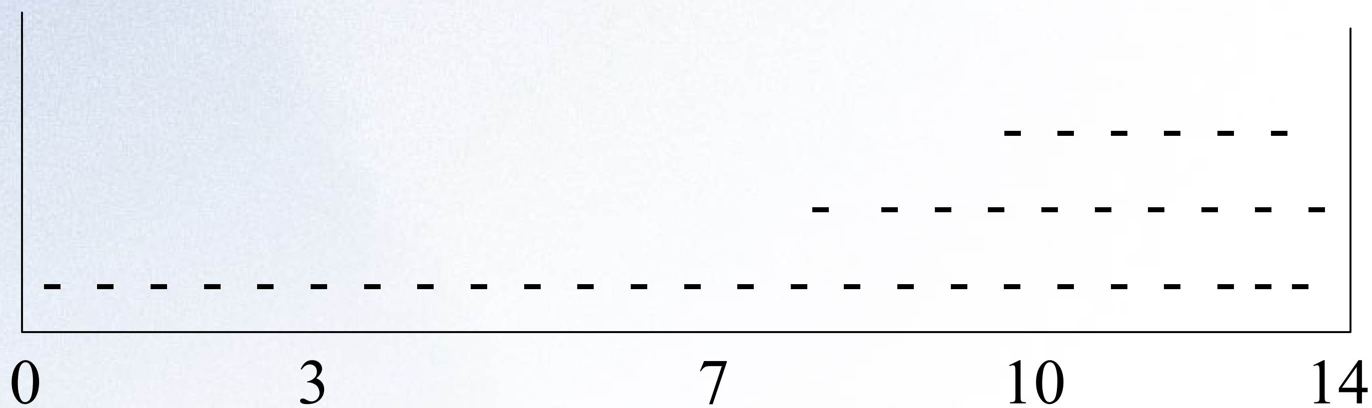
# Net Surface Charge State of Zirconia

No Modifier




With Lewis Base Modifier

(Phosphate)




*The higher on the Lewis base scale the modifier is the larger the negative charge imparted on the zirconia surface.*

# $ZrO_2$ / $SiO_2$ Surface Chemistry

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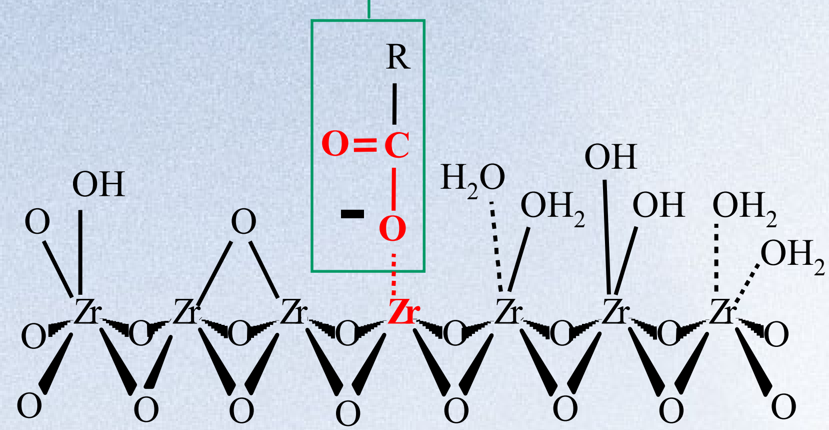
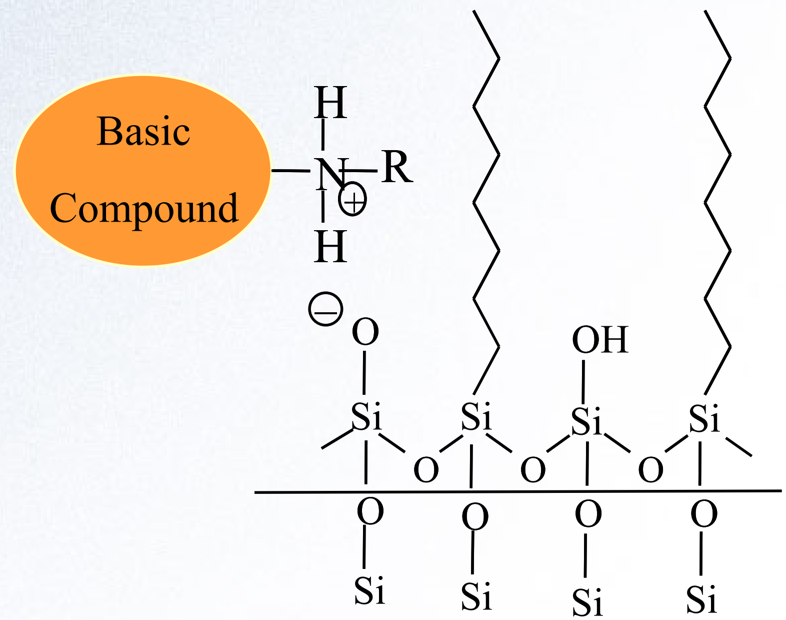
**Lewis base:**
**$PO_4^{3-}$, $F^-$, $RCOO^-$**




**Lewis Acid Site**

**Active Silanol**

Correct buffering can eliminate unwanted residual surface interactions (or enhance desirable residual surface interactions)

# Separation of Antiarrythmic Drugs

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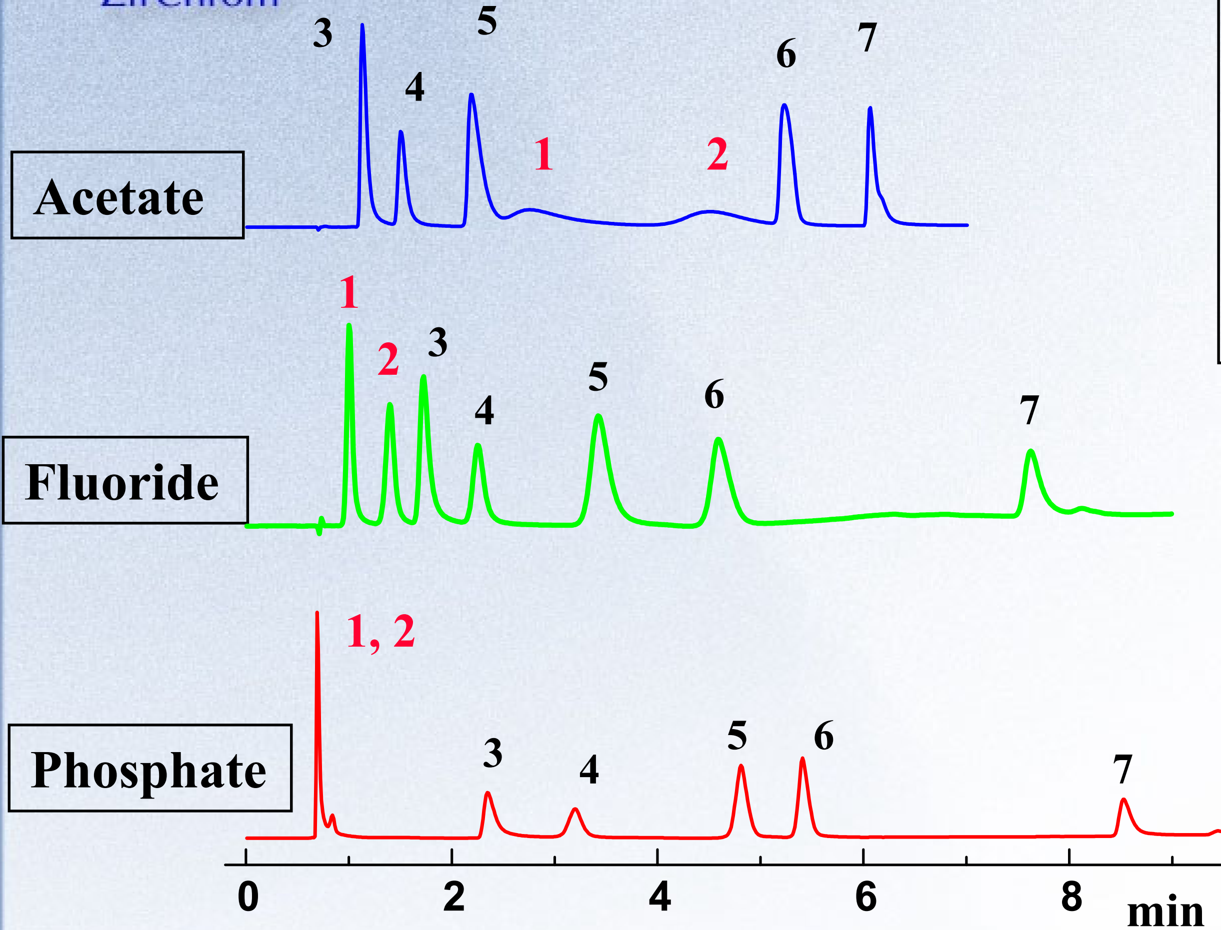
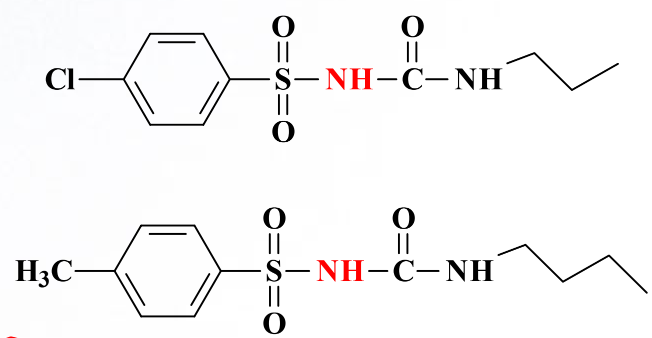



1. Chlorpropamide
2. Tolbutamide
3. Procainamide
4. Acetylprocainamide
5. Propionylprocainamide
6. Lidocaine
7. Quinidine

(1) $pK_a = 4.9$




(2) $pK_a = 4.9$

**Gradient elution; 30mM Additive, 15 mM TRIZMA , pH 7.5; 0.8 mL/min; 40 °C**

# Selectivity Matrix*

| | 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-PBD is the most similar to ODS for non-ionic analytes**
- **ZirChrom-CARB is the most different to ODS**
- **DiamondBond-C18 is ODS-like but with some CARB selectivity (generally better peak shapes than CARB)**

# Comparison of "Stable" Phases

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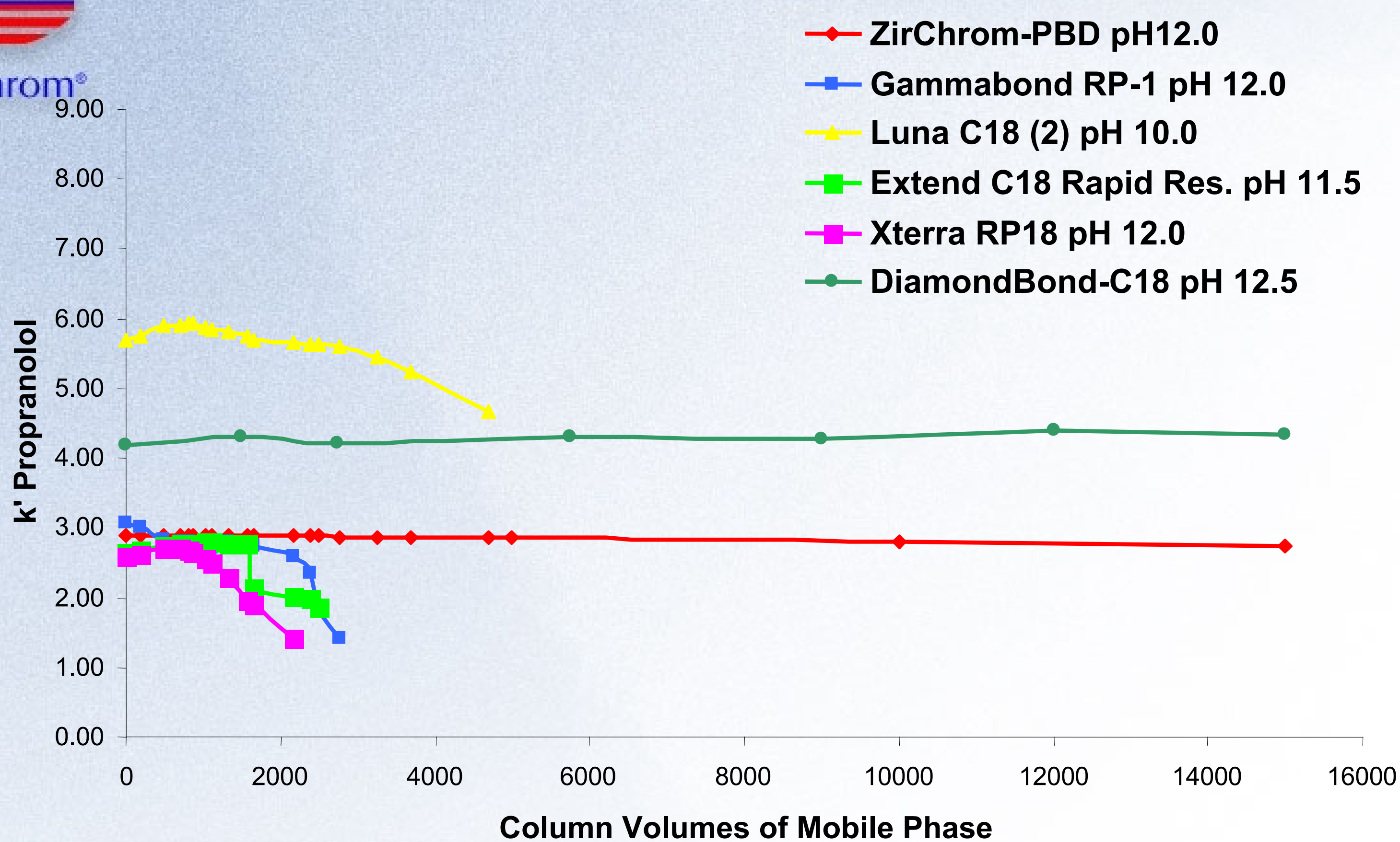
| CHARACTERISTIC | $ZrO_2$ | $SiO_2$ | Polymer |
|---|---|---|---|
| Stable pH > 11 | YES | NO | YES |
| Stable pH < 2 | YES | NO | YES |
| Stable T > 60 °C | YES | NO | YES |
| Stable to many solvents | YES | YES | NO |
| High efficiency | YES | YES | NO |
| Good mass xfer in pores | YES | YES | NO |
| Tunable selectivity for amines | YES | NO | NO |
| High loadability of amines | YES | NO | ? |
| One product covers all pHs | YES | NO!!! | YES |
| Low pressure drop | YES | Some | NO |

# High pH Stability Comparison*




**<u>Exposure Conditions</u>:** Mobile phase, ACN/50mM Potassium phosphate buffer at indicated pH; Temperature, 30 °C.

**<u>LC Conditions</u>:** Mobile phase, ACN (or THF)/50mM Potassium phosphate buffer at indicated pH; Flow Rate, 1.0 mL/min.; Temperature, 30 °C; Injection Volume, 5 uL; Detection, 254nm.

* Column names are the trademarks of their respective manufacturers.

# Organic Modifiers

- **What organic modifier should I start with?**
  - **Acetonitrile best for general purpose**
  - **Methanol may not fully wet carbon-based phases**
  - **Tetrahydrofuran, Methyl tert-butyl ether, Isopropanol, methylenechloride may be needed for carbon-based phases**
- **Are there any organic modifiers I can't use?**

# Carbon Phase Considerations

- **Temperature**
  - More efficient peaks at higher temperatures
- **Organic Modifiers**
  - Often times THF or MTBE can be used to “wet” the carbon phase. Improves peak shape.
- **Analytes with Multiple Aromatic Rings**
  - Very Strongly Retained
- **Selectivity**
  - Very Sensitive to Small Differences in Molecule Shape
  - Radically Different Selectivity
  - More Retentive than ZirChrom-PBD

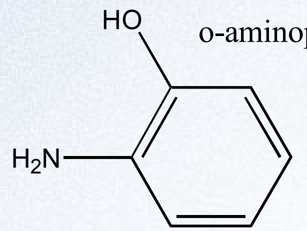
# Buffer Choice and Preparation

- **Buffer Preparation**
  - Filtering
  - Analytical Grade Reagents
- **Buffer Choice**
  - Why do I need a buffer?
    - *To control Analyte/Surface Charge*
    - *To compensate for Analyte Lewis Basicity*
  - pK range/buffering capacity
  - Buffer Concentration
    - *Avoid concentrations below 5 mM (slow equilibration and tailing peaks) or above 100 mM (precipitation with organics)*
  - Lewis Base Strength

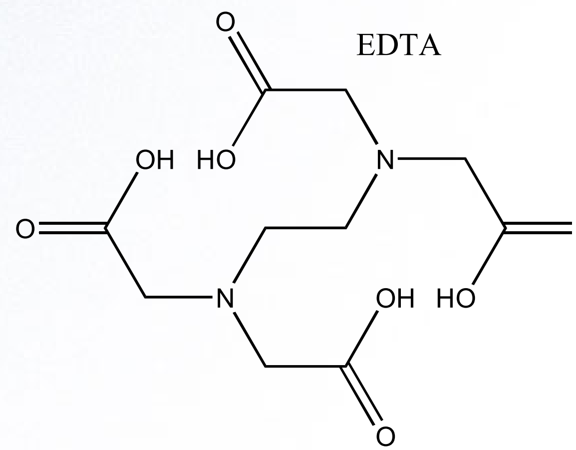
# Sample Considerations

- **Proteins**
  - Strongly Retained on Zirconia Reversed Phases
- **Chelators**
  - ETDA
  - o-aminophenol

o-aminophenol

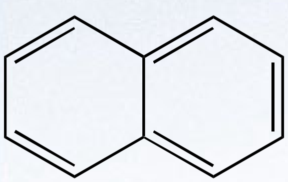



EDTA




- **Lewis Bases**
  - Must use a competing Lewis base with a high affinity for the zirconia surface.

Naphthalene



- **Conjugated Aromatic Rings on Carbon Phases**
  - Naphthalene – Strongly Retained under ambient conditions

# Buffer Changes

- **Switching Lewis Base Modifiers**
  - Dedicated columns
  - Regenerate when moving from a strong Lewis base to a weak Lewis base
- **Switching Columns**
  - Flush system thoroughly
    - *Residual strong acids/bases can irreparably harm silica, and phosphates commonly used with silica can strongly affect zirconia*
- **Solubility Issues and Precipitation**
  - Flush system thoroughly with a mobile phase that is miscible and soluble in both the condition used last and the one planned next

# Method Development Guide

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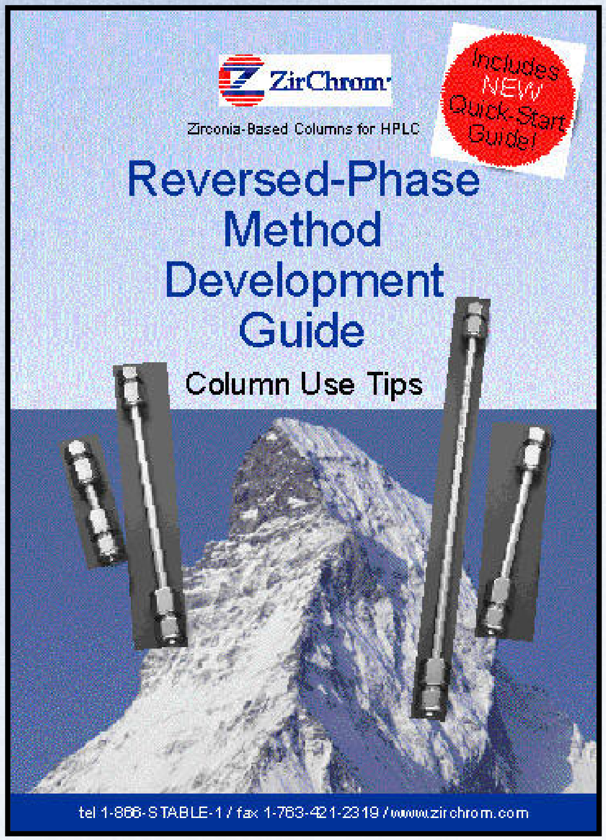


- **Summarizes Information Presented**
- **Read this document Before Using a Zirconia Column.**
- **Important for First Time Zirconia users.**

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# Technical Support

- **Technical Support**
  - Phone Support
  - Method Development
  - On Site Visits

**1-866-STABLE-1 (1-866-782-2531)**

# Summary

- Zirconia phases provide an ultra stable highly efficient alternative to traditional silica phases.
- Temperature and pH stability allows for a new possibilities in separation optimization and method development.
- ZirChrom strives to provide superior product literature and technical support to keep your method development team one step ahead.
- The versatility of the surface chemistry of Zirconia allows for the tunable selectivity of ionic compounds.
- Zirconia's unique stability allows for very thorough regeneration and cleaning.