# Application of Zirconia Phases for Structurally Similar Compounds and other Difficult HPLC Separations


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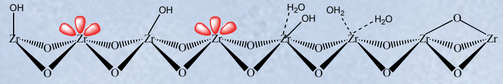
## Why Zirconia?

- Bigger Method Development Toolbox
  - Buffer Type and Concentration can "Tune" Selectivity
  - Stable Over a Wide Range of pH (1-14)
  - Stable at Temperatures Up to 200 °C
- Selectivity
  - Unique Multimodal Selectivity

## Zirconia Surface Chemistry



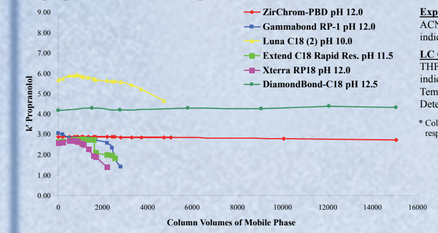
**Weak Brönsted Acid:** $ZrOH + OH^- \Leftrightarrow ZrO^- + H_2O$

**Weak Brönsted Base:** $+ H^+ \Leftrightarrow$

**Strong Lewis Acid:** $+ \Leftrightarrow$

**Ligand Exchange Interaction**

## High pH Stability Comparison*



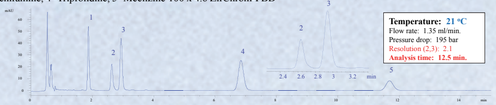

**Exposure Conditions:** Mobile phase, ACN/50 mM Potassium phosphate buffer at indicated pH; Temperature, 30 °C.

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

* Column names are the trademarks of their respective manufacturers.

## Elevated Temperature – Enables Fast Separation Without Switching Columns or Loss of Resolution

LC Conditions: Mobile Phase, 29/71 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 1.35 mL/min.; Injection volume, 0.5 µl; 254 nm detection; Column Temperature, 21°C; Pressure drop = 195 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine 100 x 4.6 ZirChrom-PBD




LC Conditions: Mobile Phase, 26.5/73.5 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 3.00 mL/min.; Injection volume, 0.2 µl; 254 nm detection; Column Temperature, 80°C; Pressure drop = 195 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine 100 x 4.6 ZirChrom-PBD


## Choosing Buffer Type

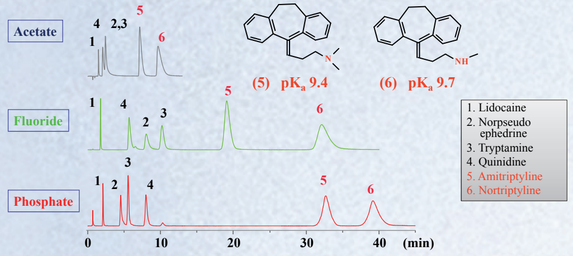
| Interaction Strength | Lewis Base Additive (A) | Retention |
|---|---|---|
| Strongest | Hydroxide | More Retention |
| | Phosphate | |
| | Fluoride | |
| | Citrate | |
| | Sulfate | |
| | Acetate | |
| | Formate | |
| Weakest | Chloride | Less Retention |

A stronger interaction strength means a greater charge on the surface, producing more retention for cations.

Anions would be affected in the opposite way, and neutrals are not affected.

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

## Effect of Buffer Type & Concentration on Selectivity

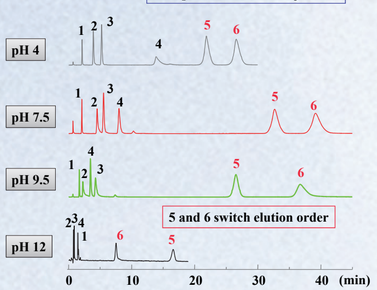



1. Lidocaine
2. Norpseudo ephedrine
3. Tryptamine
4. Quinidine
5. Amitriptyline
6. Nortriptyline

**20% ACN, 20 mM Lewis base additive, pH 7.5; 0.8 mL/min; 30 °C**

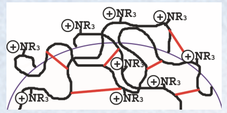
## Effect of pH on Selectivity

$RNH_2 + H^+ <---> RNH_3^+$



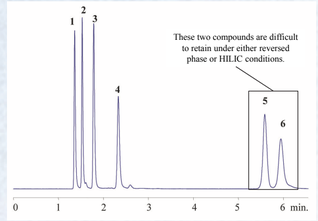

| Charge Analytes (Surface) | Mode |
|---|---|
| Cations (Slightly Negative) | Cation Exchange + RP |
| Cations (Negative) | Cation Exchange + RP |
| Near Neutral (More Negative) | Cation Exchange + RP |
| All Neutral (Most Negative) | RP |

## ZirChrom®-SAX



ZirChrom®-SAX is a zirconia particle coated with polyethyleneimine polymer and then crosslinked using a hydrophobic crosslinker. The crosslinker modifies the phase with a bit of reversed phase character, giving the ZirChrom-SAX phase a unique, multimodal selectivity.

## Water Soluble Vitamins on ZirChrom®-SAX

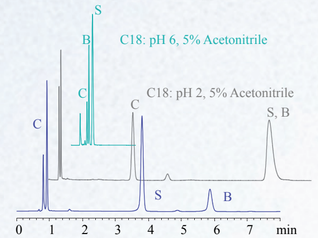



1=Thiamine (Vit. $B_1$), 2=Pyridoxine (Vit. $B_6$), 3= Nicotinamide (form of Vit. $B_3$), 4=Riboflavin (Vit. $B_2$), 5=Nicotinic acid (form of Vit. $B_3$), 6=Ascorbic acid (Vit. C)

Six water soluble vitamins are easily separated under isocratic conditions.

Column: ZirChrom®-SAX, 150 x 4.6 mm i.d., 3 µm
Mobile Phase: 50 mM Ammoniumdihydrogenphosphate, pH 4.5
Flow rate: 1.0 ml/min.
Temperature: 30 °C
Injection Vol.: 5.0 µl
Detection: UV at 254 nm

## "Green" Analysis of Energy Drink

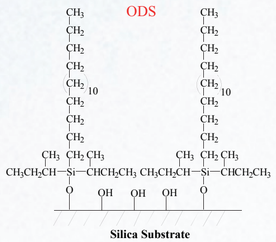



Columns: Leading C18 column, ZirChrom®-SAX, 100 x 4.6 mm i.d., 3 µm
Mobile Phase: 10 mM $NH_4H_2PO_4$ + 5 mM $NH_4CO_3$
Flow rate: 1.5 ml/min
Temperature: 50 °C
Injection Vol.: 5 µL
Detection: UV at 230 nm

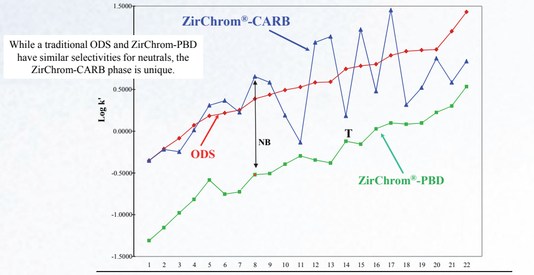
Traditional C18 phases do not readily separate benzoate and sorbate (preservatives in energy drinks). The unique multimodal selectivity of Zirchrom-SAX can take advantage of differences between the ionized forms, producing excellent selectivity and retention, resulting in 6 minute baseline resolution of caffeine (C), benzoate (B) and sorbate (S).

"Green" Mobile Phase

## Chemical Structure of ZirChrom®-CARB

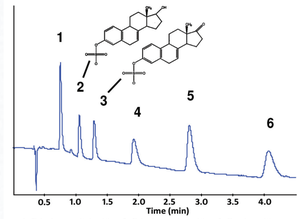



## Comparison of Selectivity of ODS, ZirChrom®-PBD &-CARB




| | | | | |
|---|---|---|---|---|
| 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 (NB) | 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 | |

## Structurally Similar Sulfated Steroids on ZirChrom®-CARB




1=Dihydroestradiol sulfate, 2=Dihydroequilin sulfate, 3=Equilin sulfate, 4=Estrone sulfate, 5=Equilenin sulfate, 6=Dihydroequilenin sulfate

This degree of selectivity is not possible on a traditional reversed phase column.

Column: 4.6 mm x 100 mm, ZirChrom-CARB
Mobile Phase: Gradient elution from 55/5/40 to 90/5/5 A/B/C from 0 to 4.5 minutes.
A: Acetonitrile
B: Tetrahydrofuran
C: 25 mM Ammonium fluoride, 10 mM Ammonium acetate, pH 5.6
Temperature: 80 °C
Injection Vol.: 10 µl
Flow rate: 3.0 ml/min.
Pressure Drop: 195 bar
Detection: UV at 270 nm

## Conclusions

- Zirconia-based stationary phases have very different selectivity from silica-based phases.
  - ZirChrom®-SAX has a unique multimodal selectivity – ionic and hydrophobic.
  - Zirconia phases have selectivity that can be tuned by the addition of different buffers.
- Zirconia reversed phases, such as ZirChrom®-CARB offer excellent chemical and thermal stability:
  - Faster separations at high temperature.
  - Better selectivity for structurally similar compounds.


## Acknowledgements

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