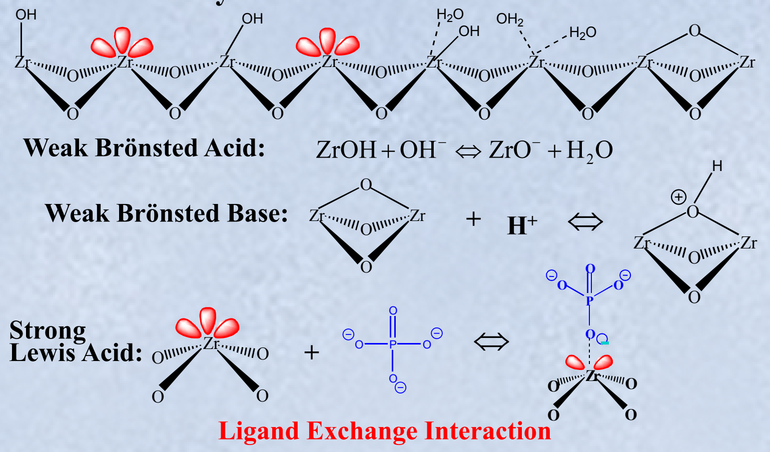
Unique Properties of Zirconia Phases for Structurally Similar Compounds and Other Difficult HPLC Separations

Kelly S. Johnson[1], Merlin Bicking[2], and Richard A. Henry[3]
[1] ZirChrom Separations, 617 Pierce St, Anoka, MN 55303
[2] ACCTA, Inc., 3534 Jessie Ct, Saint Paul, MN 55125
[3] Consultant, Supelco, Div. of Sigma Aldrich

Why Zirconia? - Bigger Method Development Toolbox

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

Zirconia Surface Chemistry

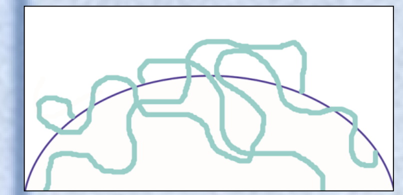


Choosing Buffer Type

Interaction Strength	Lewis Base Additive (A)	Retention
Strongest	Hydroxide	More Retention
	Phosphate	A stronger interaction strength means a greater charge on the surface, producing more retention for cations.
	Fluoride	
	Citrate	
	Sulfate	Anions would be affected in the opposite way, and neutrals are not affected.
	Acetate	
	Formate	
Weakest	Chloride	Less Retention

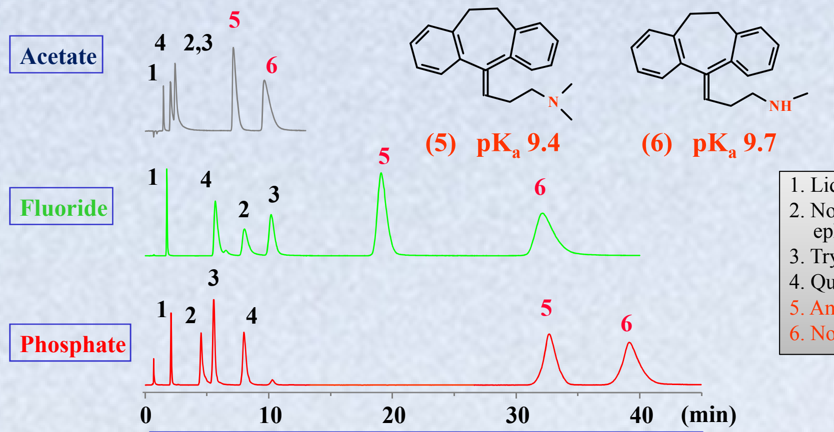
- Small Lewis bases with higher electron density and lower polarizability interact more strongly with zirconia.

ZirChrom®-PBD



ZirChrom®-PBD is a zirconia particle coated with polybutadiene polymer and then crosslinked. The unique surface chemistry coupled with the polymer coating enables a superior, multi-modal selectivity.

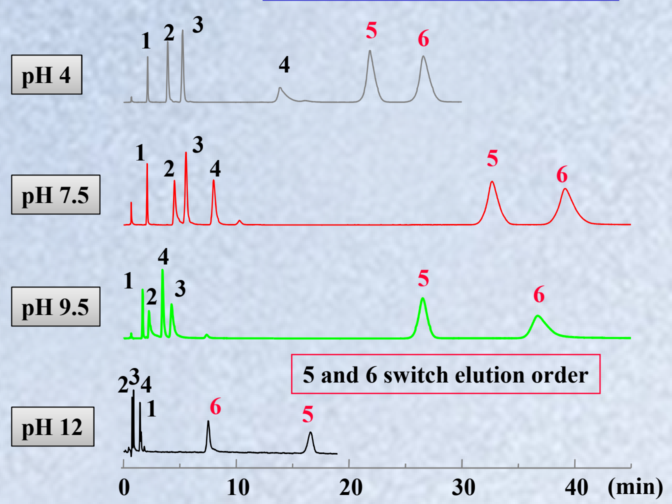
Effect of Buffer Type & Concentration on Selectivity of ZirChrom-PBD



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

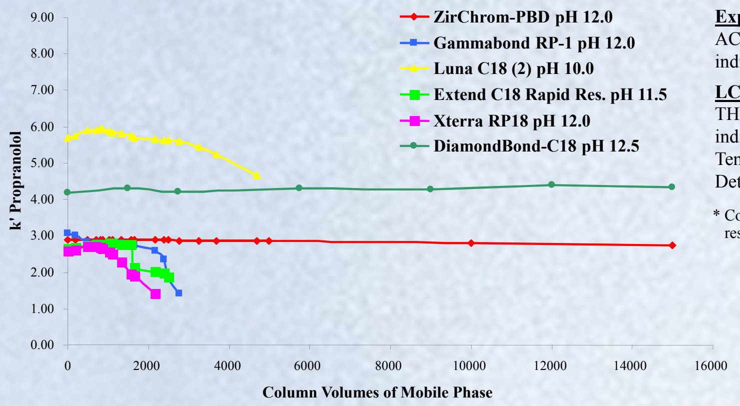
Effect of pH on Selectivity of ZirChrom-PBD

$RNH_2 + H^+ \Longleftrightarrow RNH_3^+$



Analyte Charge	Surface Charge	Separation Mode
Cations	Slightly Negative	Cation Exchange + RP
Cations	Negative	Cation Exchange + RP
Near Neutral	More Negative	Cation Exchange + RP
All Neutral	Most Negative	RP

High pH Stability Comparison*



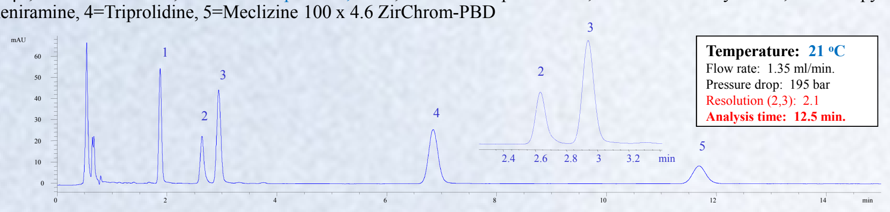
Exposure Conditions: Mobile phase, ACN/50 mM Potassium phosphate buffer at indicated pH; Temperature, 30 °C.

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.

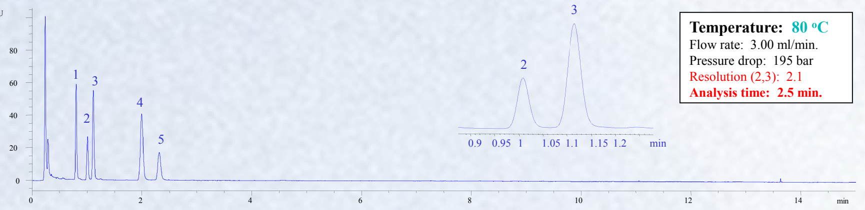
* Column names are the trademarks of their respective manufacturers.

Elevated Temperature Enables Fast Separation Without Switching Columns or Losing Resolution

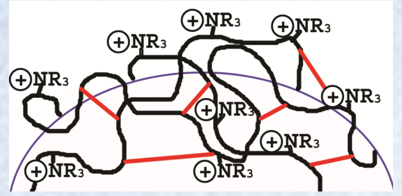
LC Conditions: Mobile Phase, 29/71 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 1.35 mL/min.; Injection volume, 0.5 µl; 254 nm detection; Column Temperature, 21°C; Pressure drop = 195 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine 100 x 4.6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

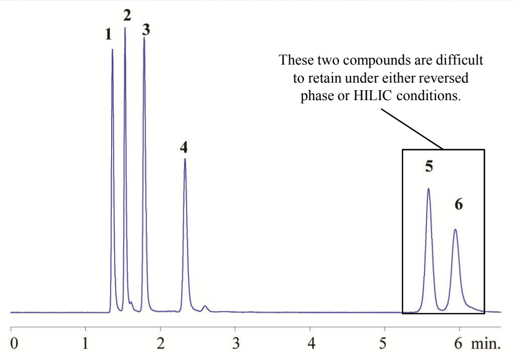


ZirChrom®-SAX



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

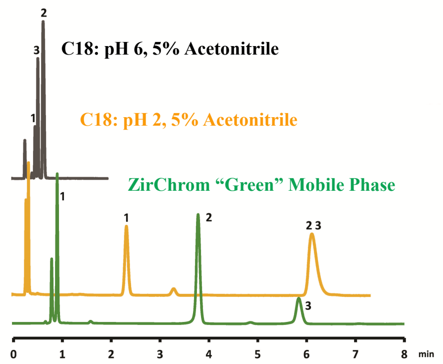


1=Thiamine (Vit. B$_1$), 2=Pyridoxine (Vit. B$_6$), 3= Nicotinamide (form of Vit. B$_3$), 4=Riboflavin (Vit. B$_2$), 5=Nicotinic acid (form of Vit. B$_3$), 6=Ascorbic acid (Vit. C)

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

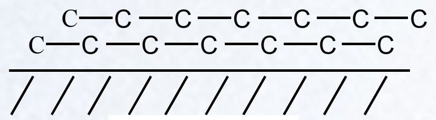
"Green" Analysis of Energy Drink on ZirChrom®-SAX



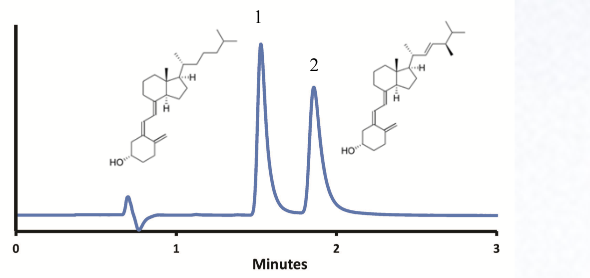
Columns: Leading C18 column, ZirChrom®-SAX, 100 x 4.6 mm i.d., 3 µm
Mobile Phase: 10 mM $NH_4H_2PO_4$ + 5 mM NH_4CO_3
Flow rate: 1.5 mL/min
Temperature: 50 °C
Injection Vol.: 5 µL
Detection: UV at 230 nm

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 (1), sorbate (2), and benzoate (3) .

ZirChrom®-CARB



Fast Separation of Vitamin D on ZirChrom®-CARB

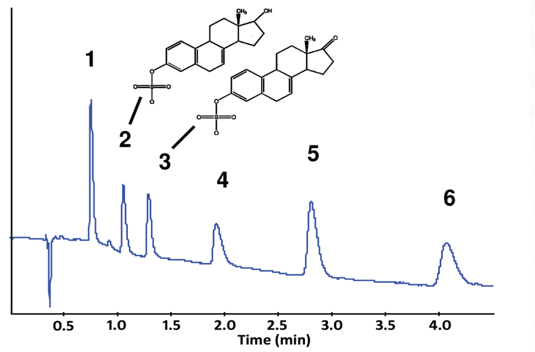


1=Cholecalciferol (vitamin D$_3$), 2= Ergocalciferol (vitamin D$_2$)

Unique selectivity allows separation using an isocratic UV detection method

Column: 4.6 mm x 50 mm, 3 µm, ZirChrom-CARB
Mobile Phase: Isocratic elution from 50/50 A/B
A: Acetonitrile/Isopropyl Alcohol
B: Tetrahydrofuran
Temperature: 70 °C
Injection Vol.: 5 µL
Flow rate: 1.5 mL/min.
Pressure Drop: 74 bar
Detection: UV at 275 nm

Structurally Similar Sulfated Steroids on ZirChrom®-CARB

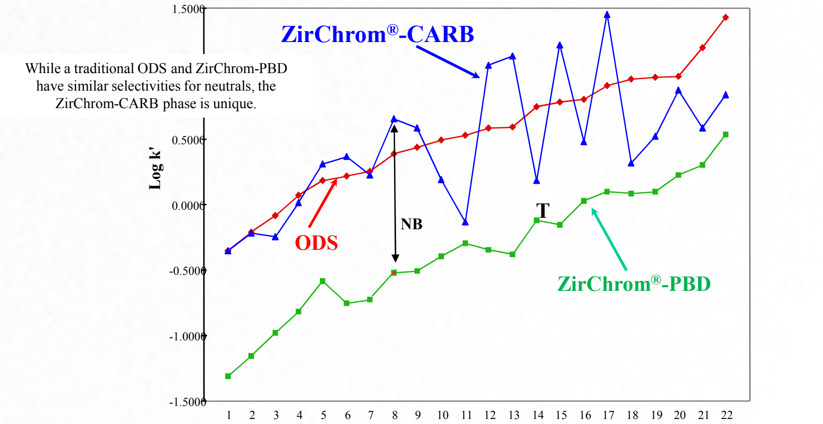


1=Dihydroestradiol sulfate, 2=Dihydroequilin sulfate, 3=Equilin sulfate, 4=Estrone sulfate, 5=Equilenin sulfate, 6=Dihydroequilenin sulfate

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

Comparison of Selectivity on Silica 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 (NB)
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

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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®-PBD &-CARB offer excellent chemical and thermal stability:
  - Faster separations at high temperature.
  - Better selectivity of ZirChrom®-CARB for structurally similar compounds.

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