Characteristics and Advantages of Zirconia-Based Stationary Phases for Use in Multi-Dimensional HPLC

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ZirChrom Separations, Inc.

Multi-Dimensional Chromatography Workshop

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Outline

1. Review of theory and requirements – Why bother with multi-dimensional chromatography?
2. Why use zirconia for two-dimensional chromatography?
3. ZirChrom®-CARB and DiamondBond-C18™– Very unique phases for RPLC
4. ZirChrom®-PBD, ZirChrom®-EZ and ZirChrom®-MS – Phases with mixed mode retention characteristics for ionizable analytes
5. Selectivity comparisons using ZirChrom®-CARB
6. Selectivity comparisons using ZirChrom®-PBD
7. An example two-dimensional HPLC separation of ten triazine herbicides using ZirChrom®-PBD and ZirChrom®-CARB
8. Conclusions

A Common Problem in HPLC

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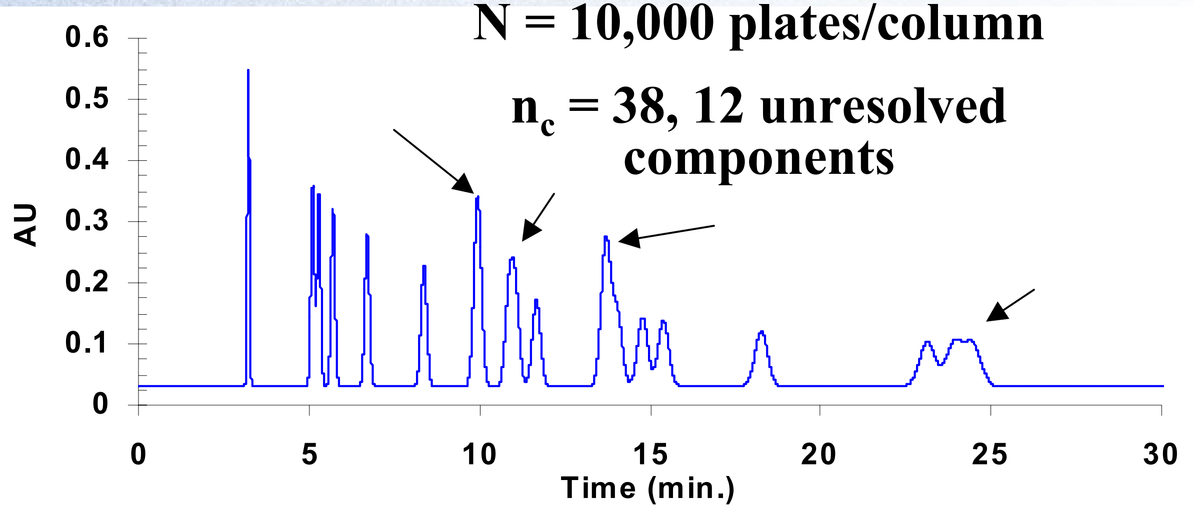
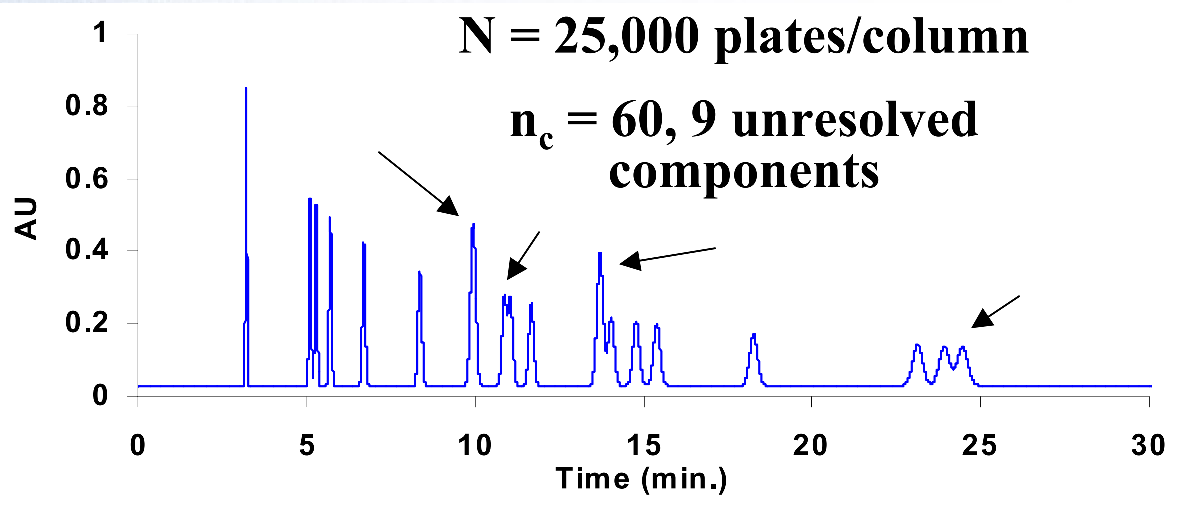
Sample composed of 20 components with randomly distributed k' values

150 mm x 4.6 mm i.d. column





Even with state-of-the-art HPLC, only 50% of the components in this sample can be resolved !!!

Requirements and Advantages in Two-Dimensional HPLC

ZirChrom®

*Two conditions must be met for the technique to be considered “two-dimensional”

1. **Orthogonality of separation mechanisms – This is a requirement imposed on the stationary phase chemistry**
2. Separation gained in one dimension cannot be diminished by separation in the other dimension

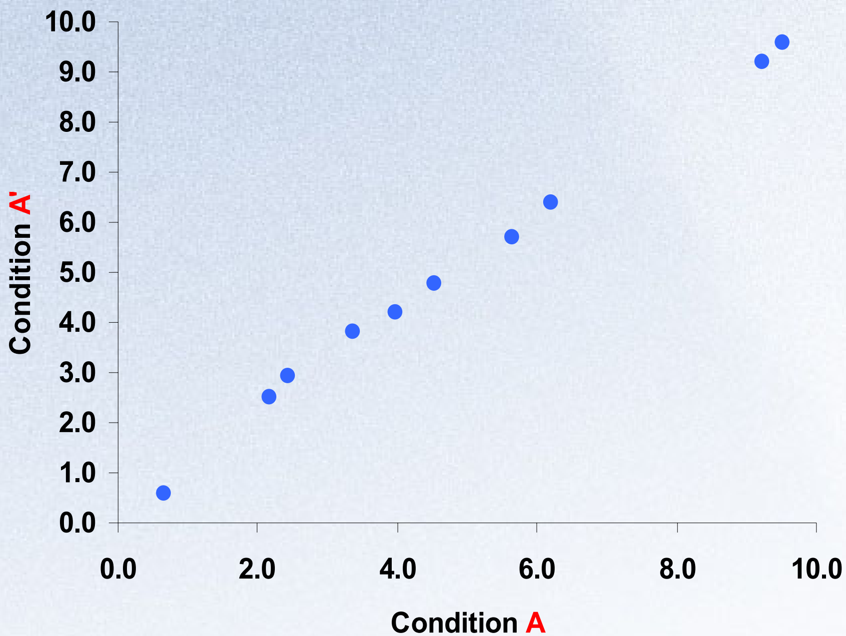
If these two conditions are satisfied, the maximum total peak capacity of the two-dimensional system is:

$$n_{cTotal} = n_{c1} \times n_{c2}$$

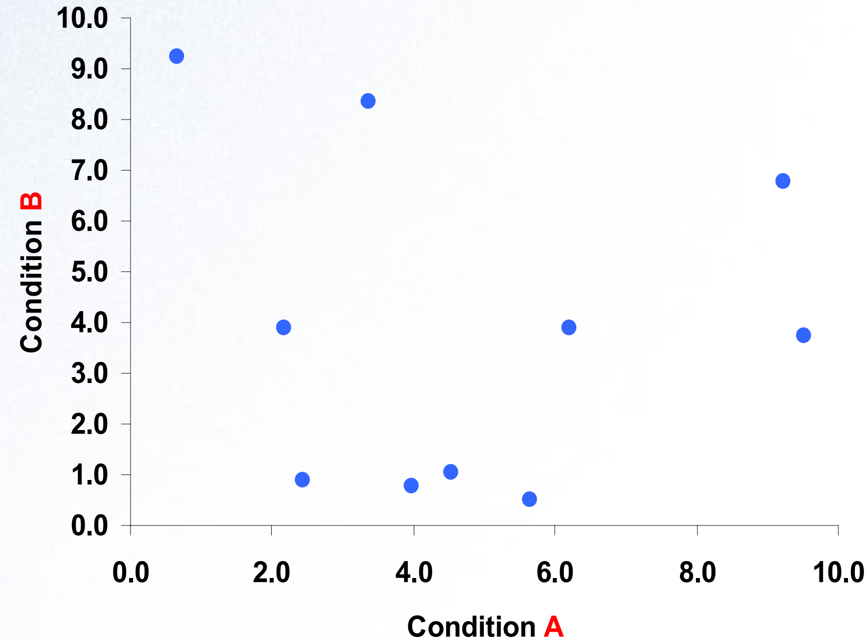
Giddings, J. C. *Multidimensional Chromatography: Techniques and Applications*; Marcel Dekker: New York, 1990

What Can We Expect From a Two-Dimensional Separation Based on Known One-Dimensional Data?

Condition A' is the same as Condition A except that the retention has been varied randomly by 5%



Condition B assumes no relationship to Condition A



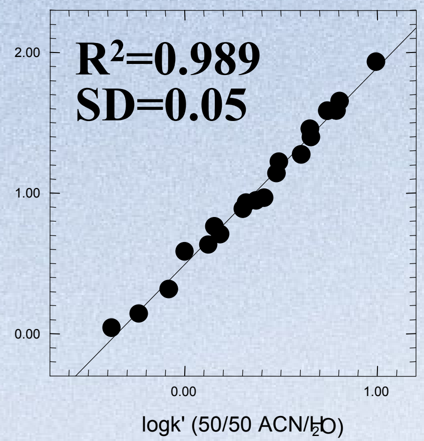
This scenario is ineffective in two-dimensional HPLC

This scenario has a higher probability of success in two-dimensional HPLC

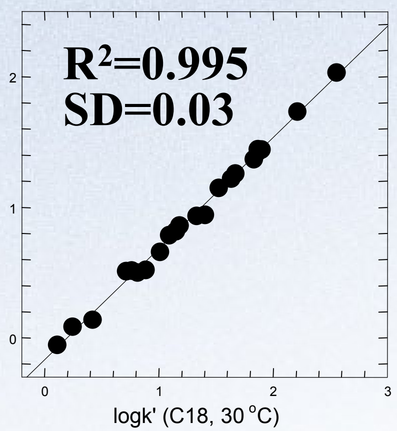
Comparison of Variables Affecting Selectivity

ZirChrom®

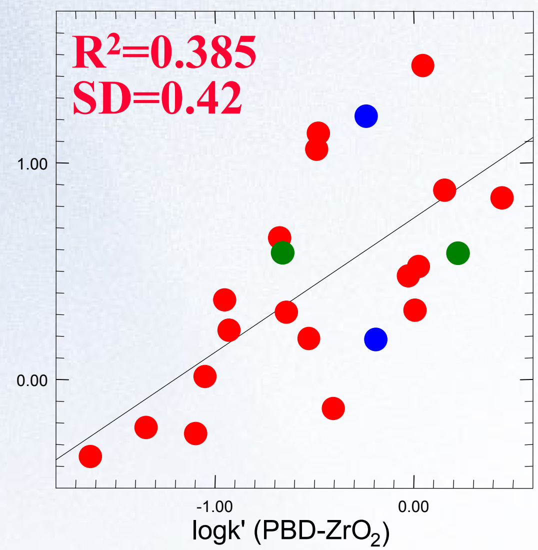
30% ACN vs. 50% ACN



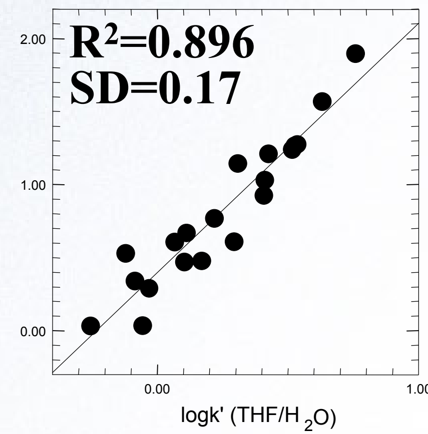
80°C vs. 30°C



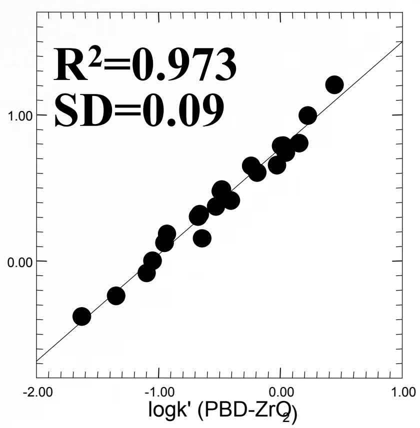
ZirChrom®-CARB vs. ZirChrom®-PBD



MeOH vs. THF



ODS vs. ZirChrom®-PBD



- **Stationary phase type can have a very large effect on selectivity**

Why Zirconia-Based Phases - Advantages for Multi-Dimensional RPLC

1. Stability - Enables the use of otherwise extreme conditions for adjustment of selectivity

2. Stationary phase chemistry – Allows the user to explore a wide range of chemistry to obtain the largest changes in selectivity

   A. Carbon-clad zirconia phases
   B. Polymer coated phases with mixed mode charateristics

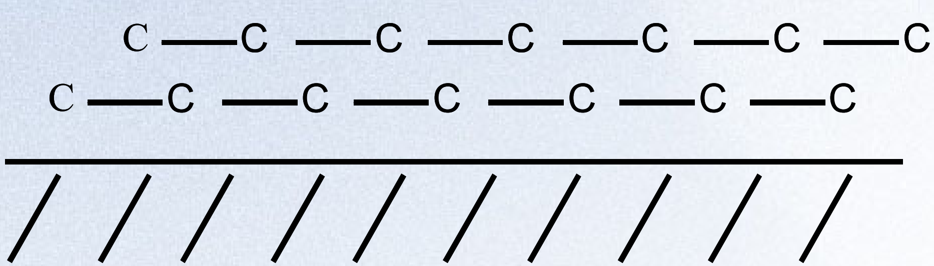
      I. Reversed-phase
      II. Ion-exchange

3. Speed – Thermal stability allows for faster multi-dimensional separations

ZirChrom HPLC Columns

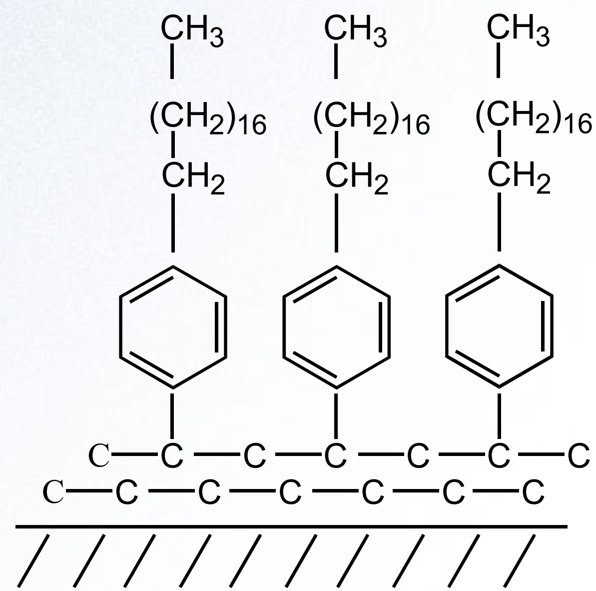
Part #	Packing	Mode
DB01	DiamondBond®-C18	Reversed-Phase
EZ01	ZirChrom®-EZ	Reversed-Phase (Lewis Acid Deactivated)
MS01	ZirChrom®-MS	Reversed-Phase (Lewis Acid Deactivated)
ZR01	ZirChrom®-CARB	Reversed-Phase
ZR02	ZirChrom®-PHASE	Normal Phase and SEC
ZR03	ZirChrom®-PBD	Reversed-Phase
ZR04	ZirChrom®-WCX	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
ZR09	ZirChrom®-PS	Reversed-Phase

ZirChrom®-CARB and DiamondBond®-C18



Zirconia Substrate

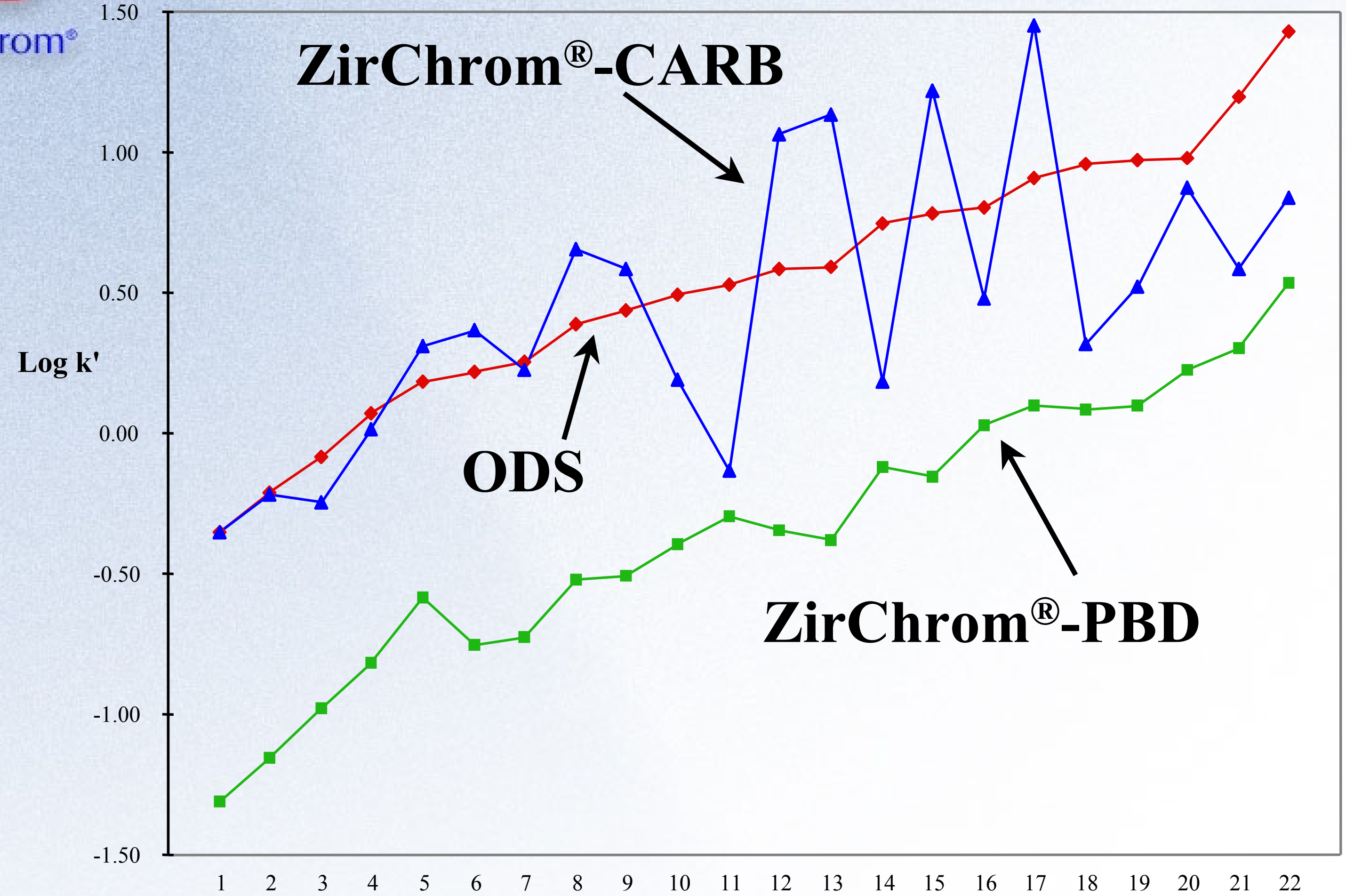
ZirChrom®-CARB



Zirconia Substrate

DiamondBond-C18™

Retention of Different Solutes on ODS, ZirChrom®-PBD and ZirChrom®-CARB



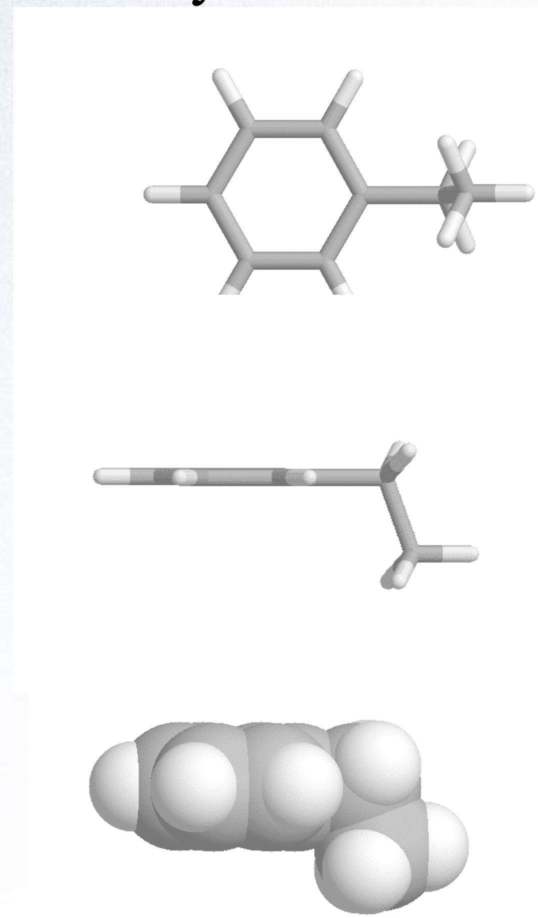
1. N-benzyl formamide
2. Benzylalcohol
3. Phenol
4. 3-phenyl propanol
5. p-chlorophenol
6. Acetophenone
7. Benzonitrile
8. Nitrobenzene
9. methyl benzoate
10. Anisole
11. Benzene
12. p-chlorotoluene
13. p-nitrobenzyl chloride
14. Toluene
15. Benzophenone
16. Bromobenzene
17. Naphthalene
18. Ethylbenzene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

Selectivity and Shape: Isomeric Analytes

ZirChrom®

p-xylene

ethylbenzene



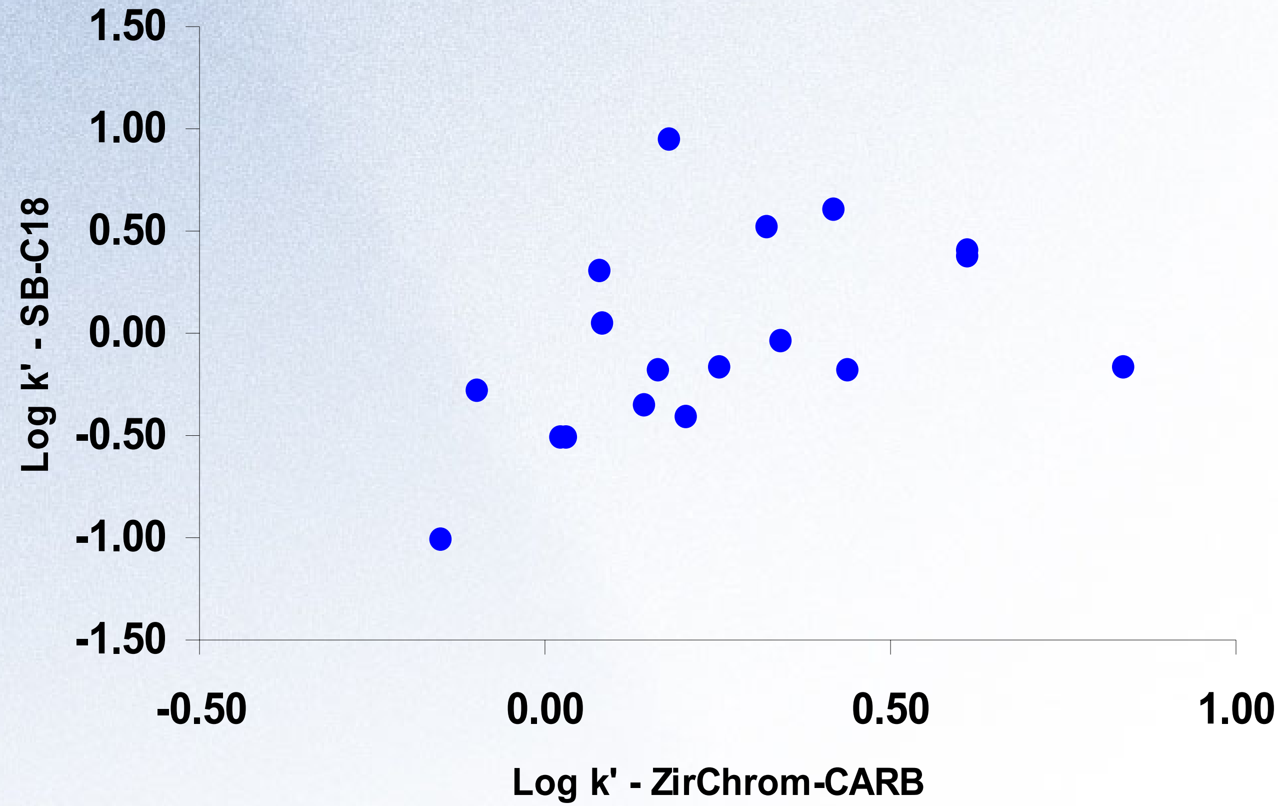
α_{ODS}=1.03

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

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

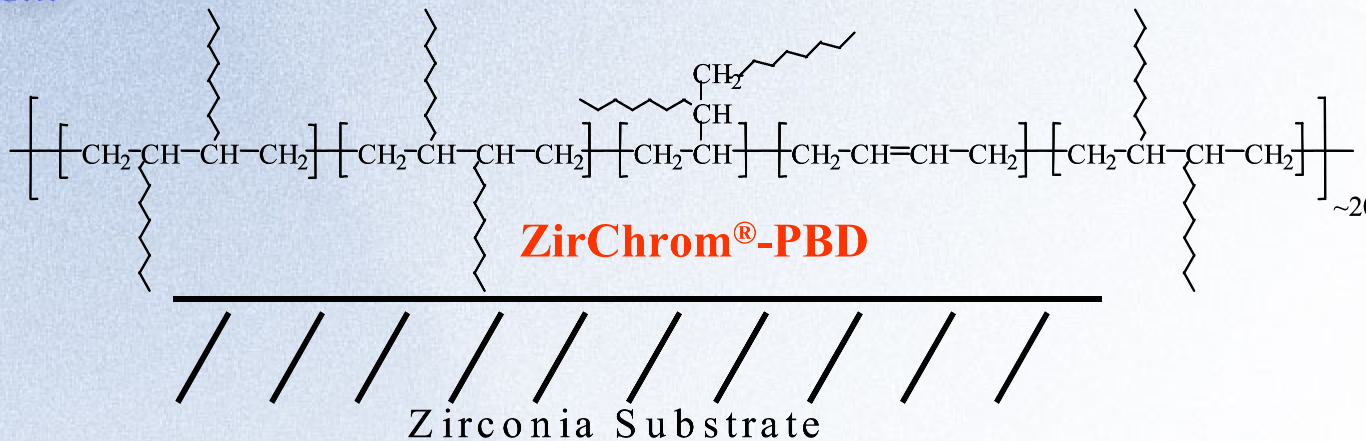
C-C-C-C-C-C-C-C-C-C-C-C

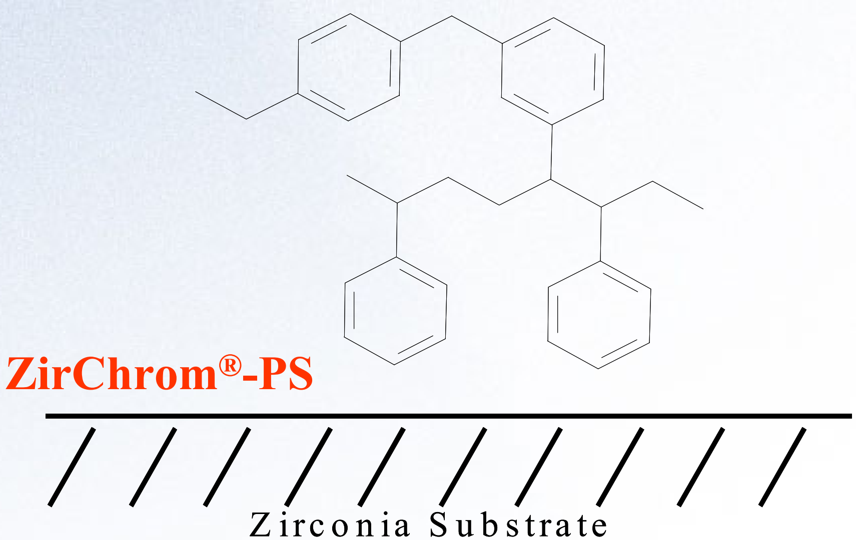
Selectivity of ZirChrom®-CARB and SB-C18 for 18 Substituted Phenols



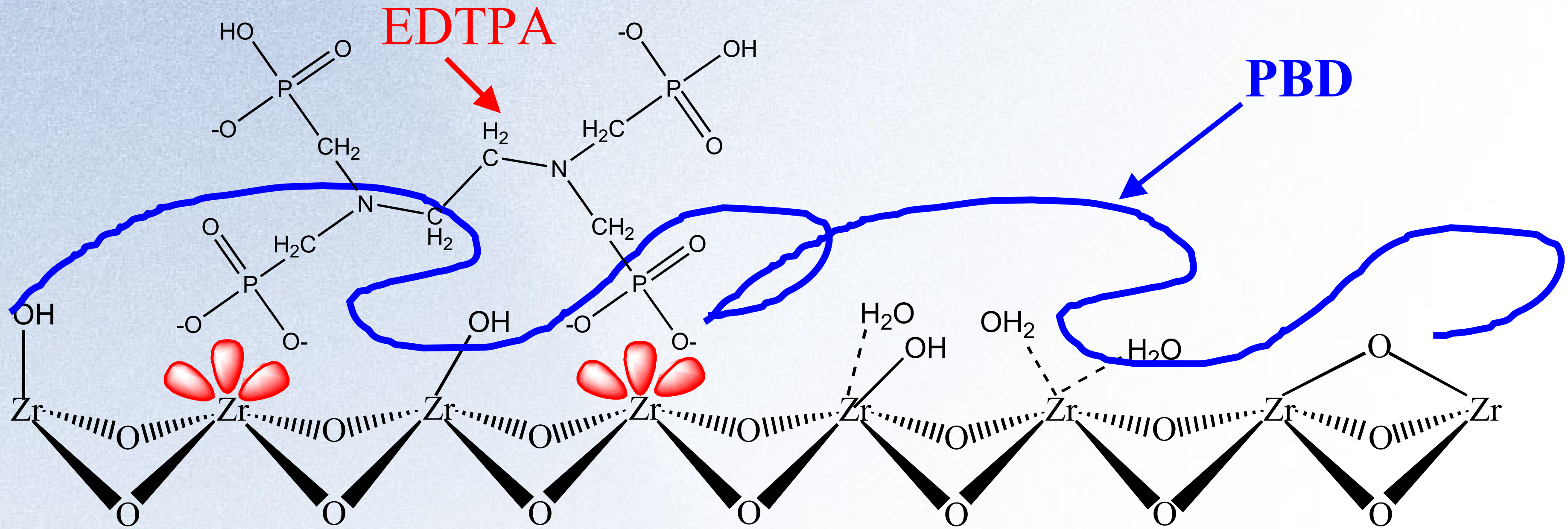
LC Conditions: Mobile phase, 45/55 ACN/10mM phosphoric acid, pH 2.4; Flow rate, 2.0 ml/min.; Temperature, 40 ^{o}C – Data courtesy of Adam Schellinger

ZirChrom®-PBD and ZirChrom®-PS





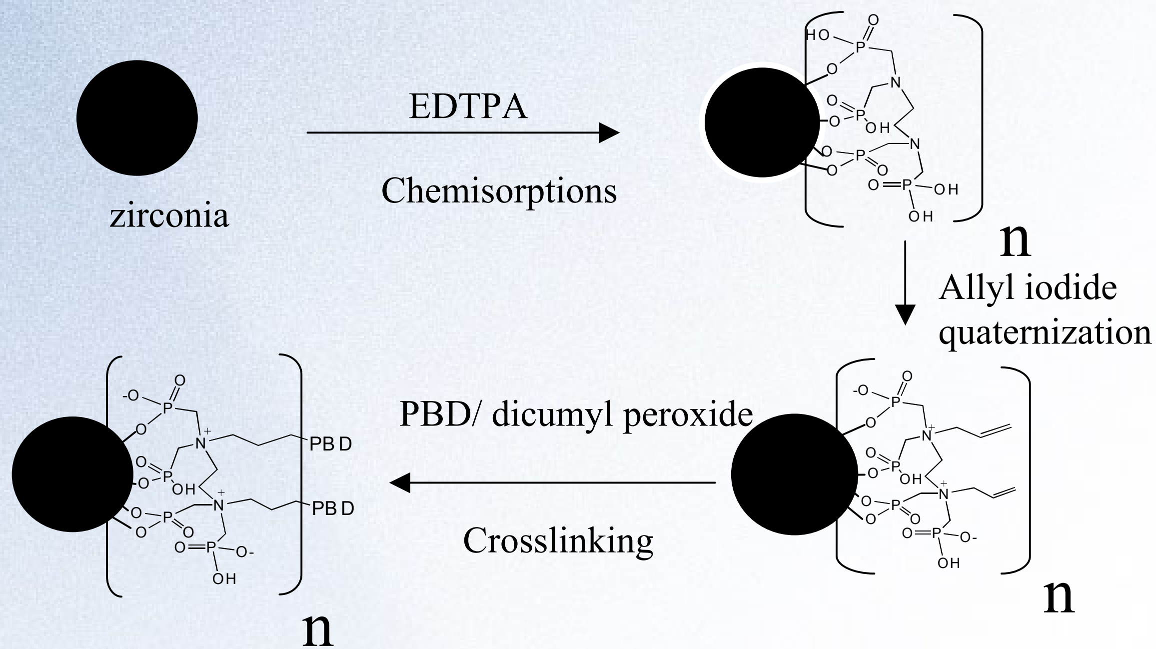
ZirChrom®-EZ



1. Coat bare zirconia with polybutadiene (PBD)[1]
2. Crosslink PBD chains together using dicumyl peroxide as initiator
3. Reflux PBD-ZrO_2 in Ethylenediamine-N,N,N',N'-tetra(methylenephosphonic)acid (EDTPA) solution
4. Wash to remove residual EDTPA

1) Li, J. W.; Reeder, D. H.; McCormick, A. V.; Carr, P. W. *Journal of Chromatography A* **1998**, *791*, 45-52

ZirChrom®-MS

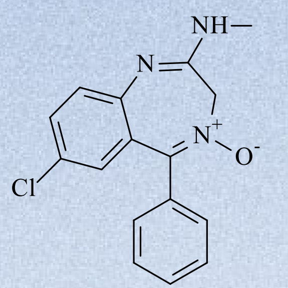


1 **Chemisorb Ethylenediamine N,N,N',N'-tetra(methylenephosphonic)acid (EDTPA) to the zirconia surface.**

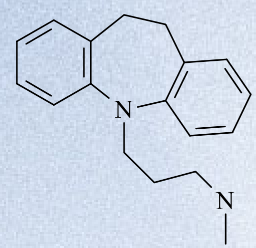
2 **Quaternize amines on the zirconia surface with allyl iodide.**

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

Selectivity Study of Eleven Antidepressants

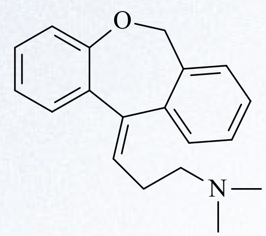


Chlordiazepoxide

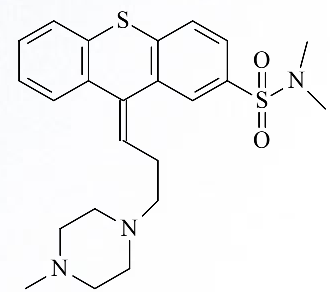


Desipramine

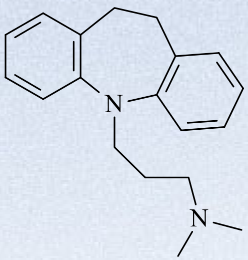
Nortriptyline

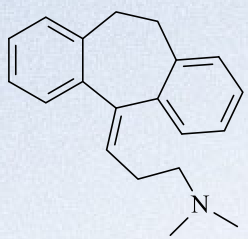


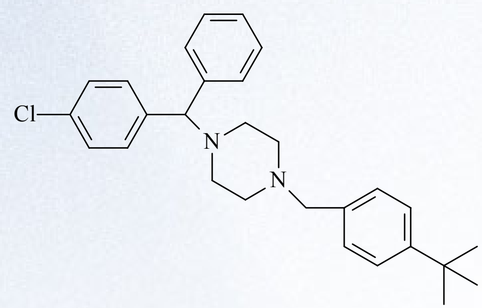
Doxepin

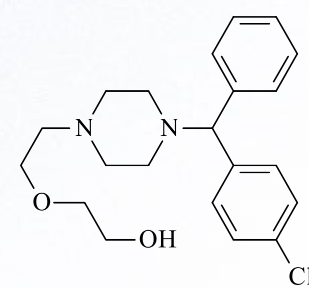


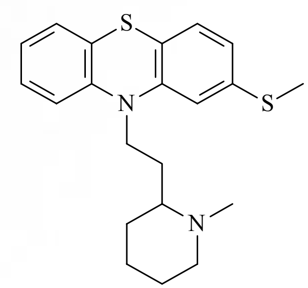
Imipramine









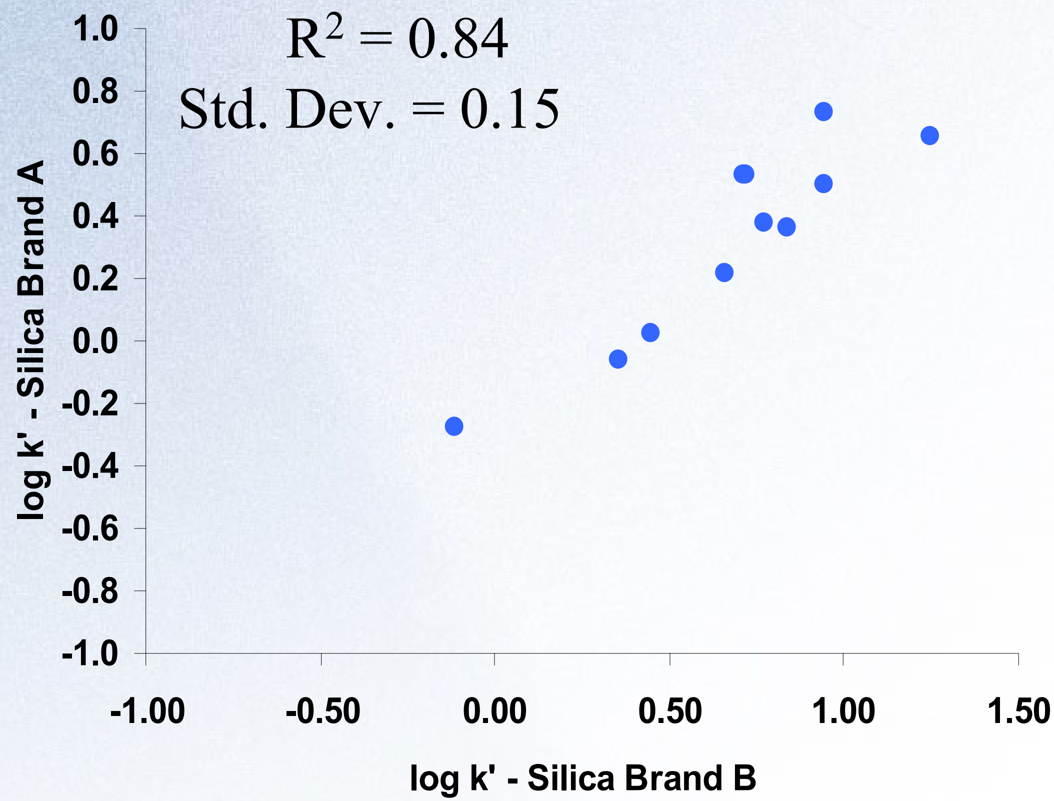


Amitriptyline   Buclizine   Hydroxyzine   Thioridazine   Perphenazine

Dai, J.; Yang, X.; Carr, P. W. *Journal of Chromatography, A* **2003**, *1005*, 63-82

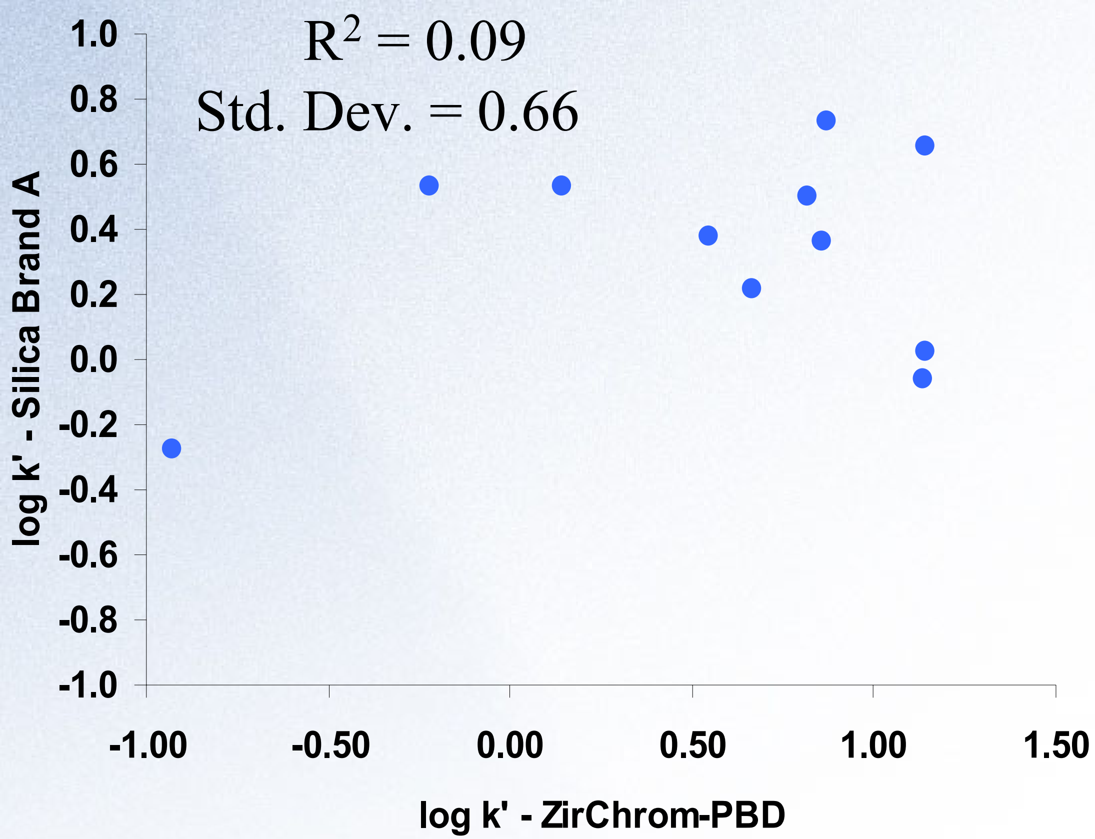
Selectivity for Antidepressant Compounds on ODS Brand A vs. Brand B

ZirChrom®



LC Conditions: Mobile phase, 72/28 MeOH/25 mM ammonium phosphate, pH 6.0; Flow rate, 1.0 mL/min; Temperature, 35 °C; UV detection at 254 nm.

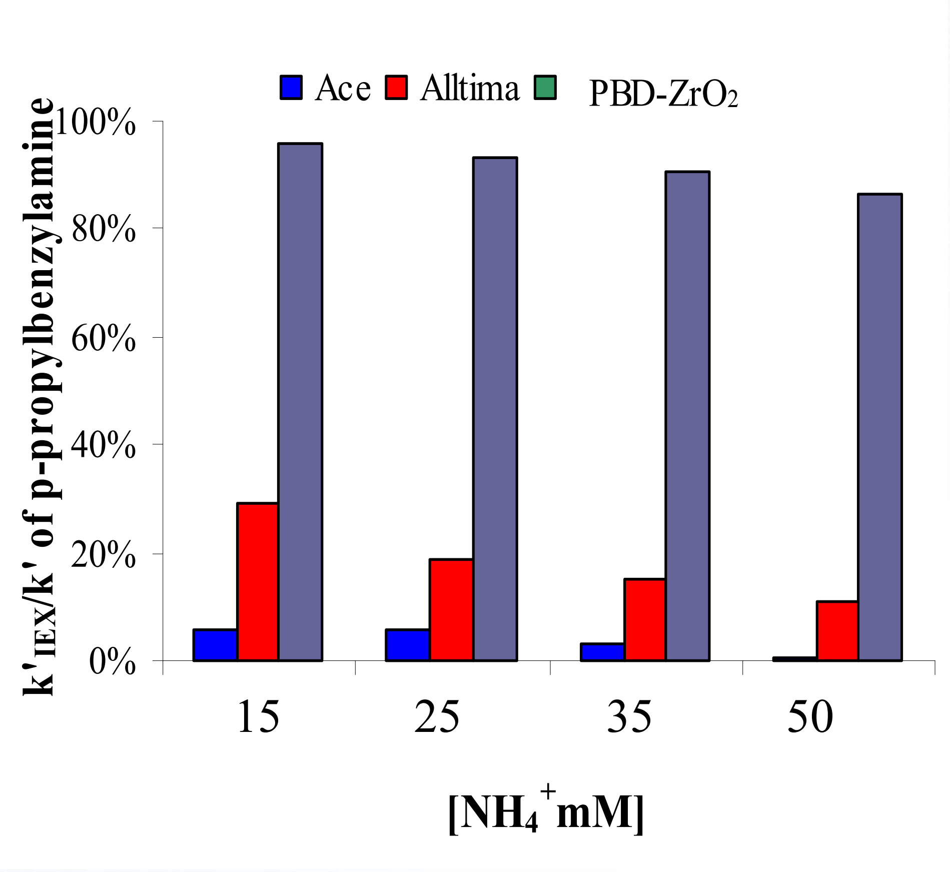
Selectivity for Antidepressant Compounds on ZirChrom®-PBD vs. ODS



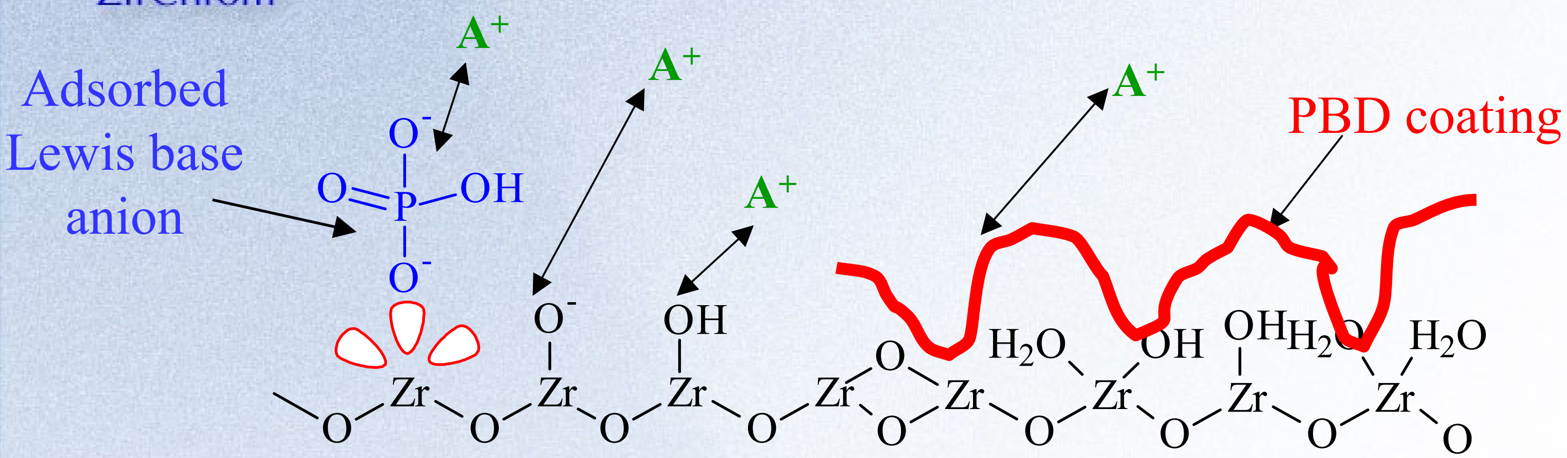
LC Conditions: Mobile phase, 72/28 MeOH/25 mM ammonium phosphate, pH 6.0; Flow rate, 1.0 mL/min; Temperature, 35 °C; UV detection at 254 nm.

Significantly Higher Ion-Exchange Retention of Amines on ZirChrom®-PBD Leads To Selectivity Differences

$$k'_{IEX} = k' - k'_{RP}$$



Retention of Basic Analytes on ZirChrom®-PBD



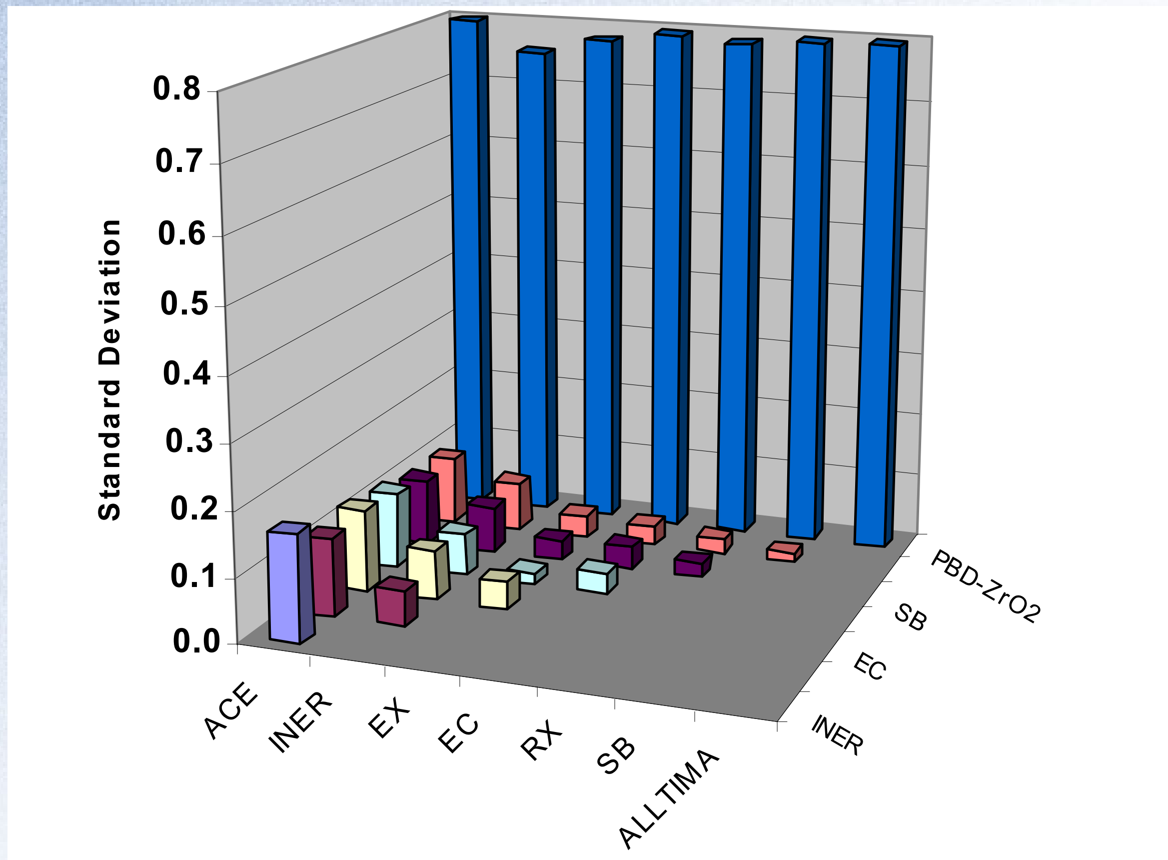
- PBD Coating — Reversed-Phase (RP) Moieties
- Lewis Base Anions — Ion-Exchange (IEX) Sites

$$Zr\text{-}L^{-}:X^{+} + A^{+} = Zr\text{-}L^{-}:A^{+} + X^{+}$$

$$Zr\text{-}O^{-}:X^{+} + A^{+} = Zr\text{-}O^{-}:A^{+} + X^{+}$$

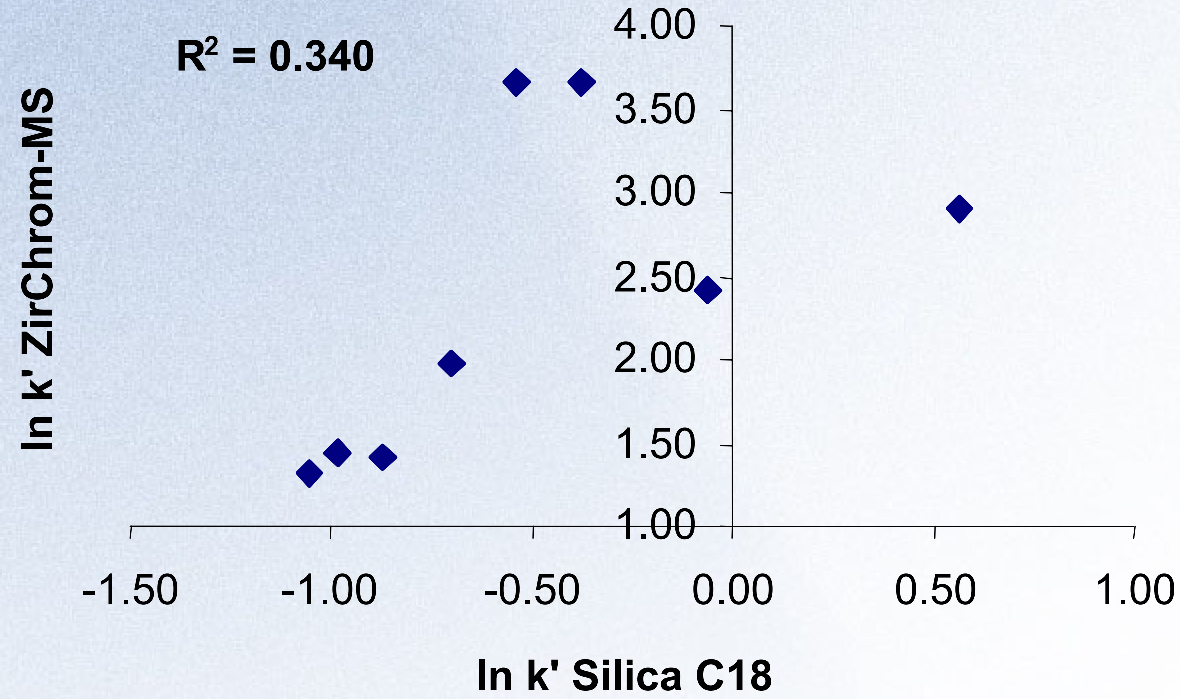
A^{+}: analyte cation, X^{+}: counterion, L^{-}: adsorbed Lewis base anion.

ZirChrom®-PBD is Very Different Compared to All ODS Phases



The very large s.d. for ZirChrom®-PBD vs. all other phases indicates a dramatic difference in selectivity from ODS (Antidepressant solute set)

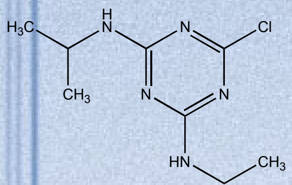
ZirChrom®-MS Compared to ODS



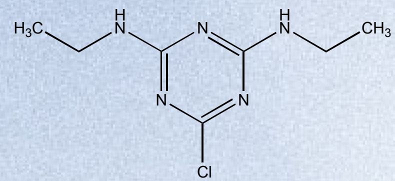
Basic Compounds are much more retained on ZirChrom-MS than on Silica C18 and have very different chromatographic selectivity.

LC Conditions: Mobile Phase, 72/28 MeOH/25mM Ammonium phosphate, pH 6.0; Flow Rate, 1.0 ml/min.; Temperature, 35 °C; Injection Volume, 5 µl; Detection by UV at 254 nm; Solutes from left to right: Methapyrilene, Pyrilamine, Tripelennamine, Brompheniramine, Desipramine, Nortryptyline, Doxepin, and Amitryptyline.

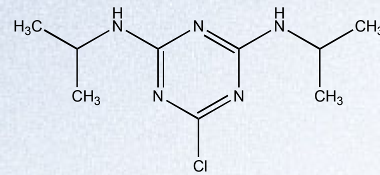
An Example 2DLC Separation - Ten Triazine Herbicides



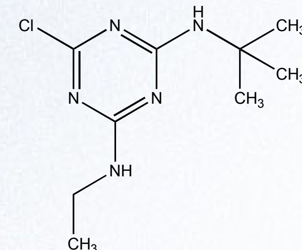
Atrazine



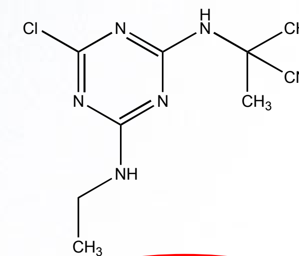
Simazine



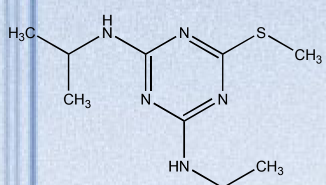
Propazine



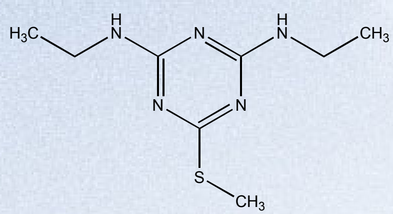
Terbuthylazine



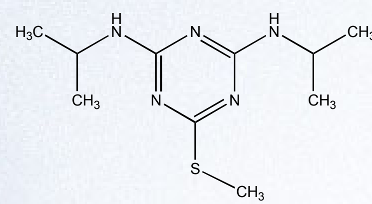
Cyanazine



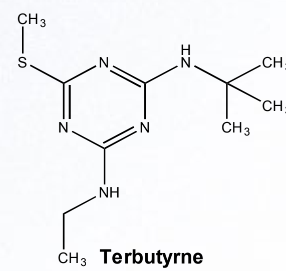
Ametryne



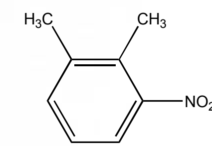
Simetryne



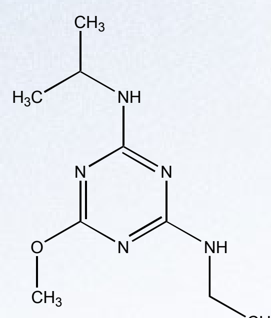
Prometryne



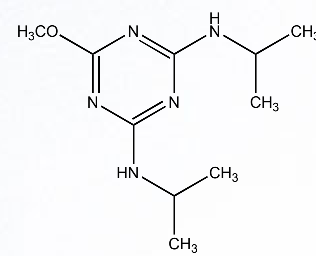
Terbutyrne



2-nitroxylene

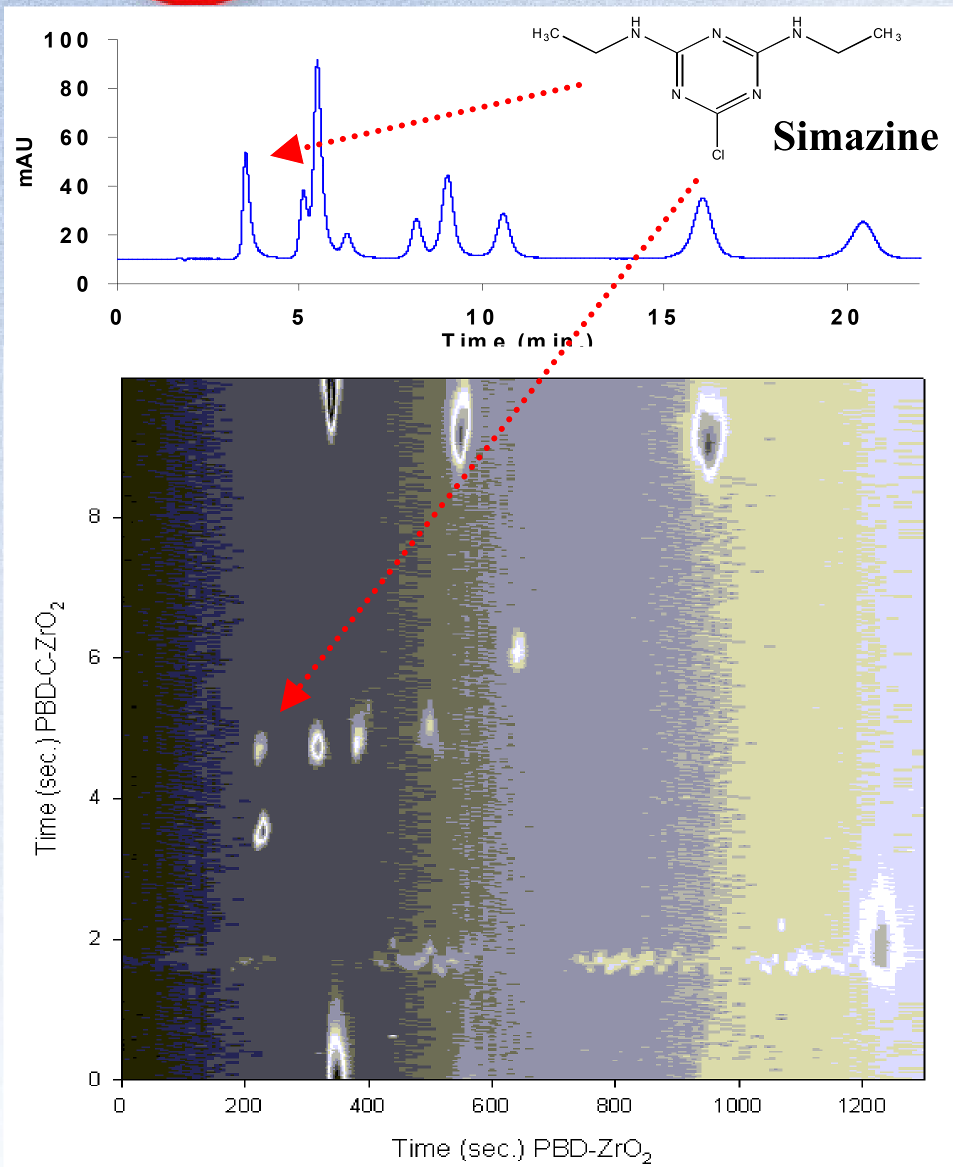


Atraton



Prometon

2DLC Separation of Ten Triazine Herbicides



1st Dimension Conditions: Column, 50 mm x 2.1 mm i.d. ZirChrom®-PBD; Mobile phase, 20/80 ACN/Water; Flow rate, 0.08 ml/min.; Injection volume, 20 µl; Temperature, 40 °C

2nd Dimension Conditions: Column, 50 mm x 2.1 mm i.d. ZirChrom®-CARB; Mobile phase, 20/80 ACN/Water; Flow rate, 7.0 ml/min.; Injection volume, 15 µl; Temperature, 150 °C; 1st dimension sampling frequency, 0.1 Hz

Conclusions

1. The importance of differences in selectivity between conditions selected for different dimensions in multi-dimensional chromatography cannot be emphasized enough.
2. The most dramatic changes in selectivity are most easily brought about by changing the stationary phase.
3. Zirconia-based reversed phases (there are 5 of them) offer dramatically different selectivity relative to conventional silica-based phases for several classes of analytes

Acknowledgements

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