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

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Multi-Dimensional Chromatography Workshop

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Outline

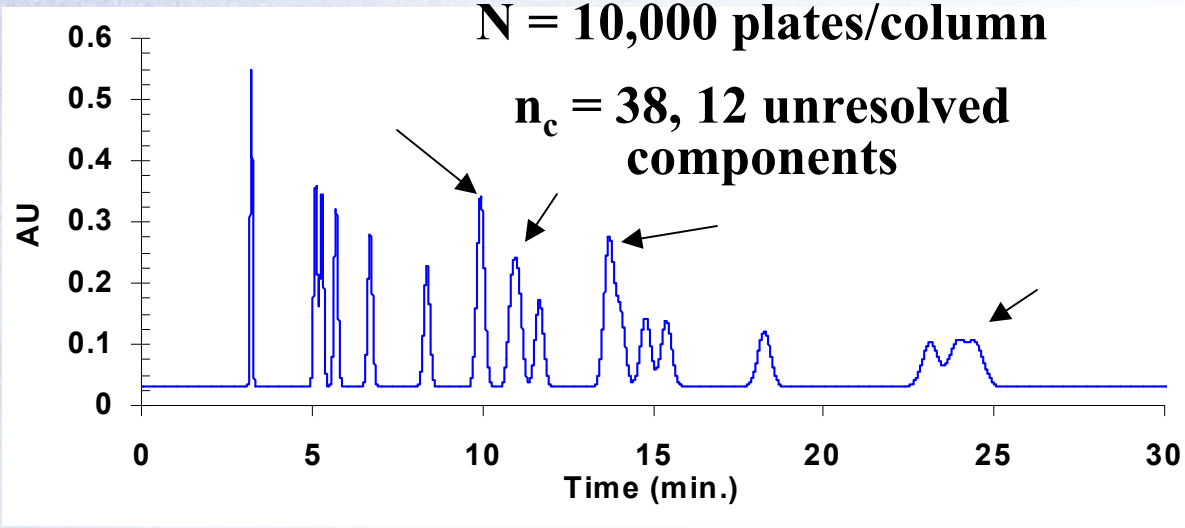
1. Review of theory and requirements – Why bother with multi-dimensional chromatography?
2. Why use zirconia for two-dimensional chromatography?
3. ZirChrom®-CARB and DiamondBond-C18™– Very unique phases for RPLC
4. ZirChrom®-PBD, ZirChrom®-EZ and ZirChrom®-MS – Phases with mixed mode retention characteristics 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

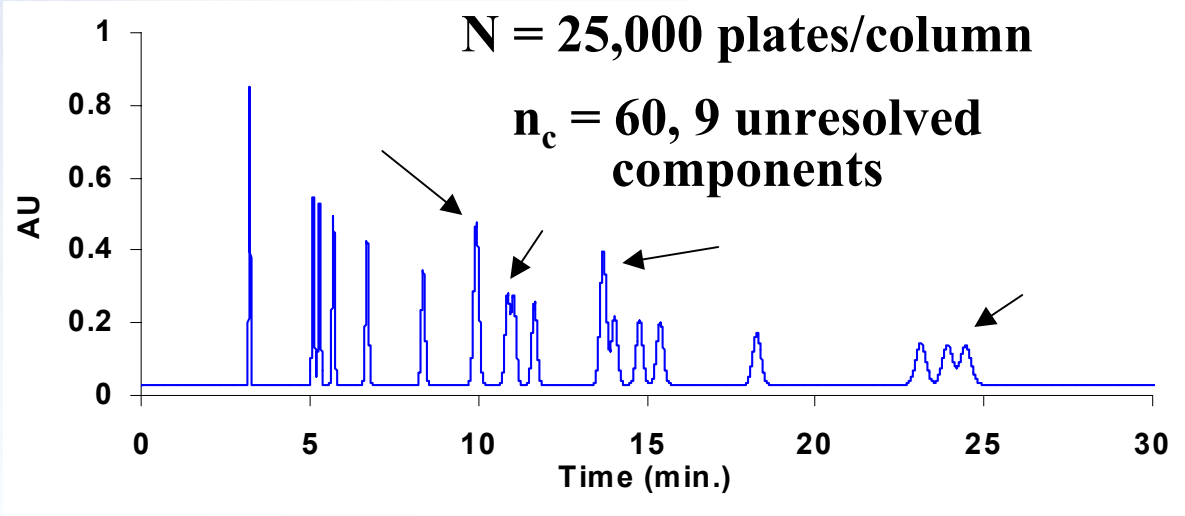
A Common Problem in HPLC

ZirChrom®

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

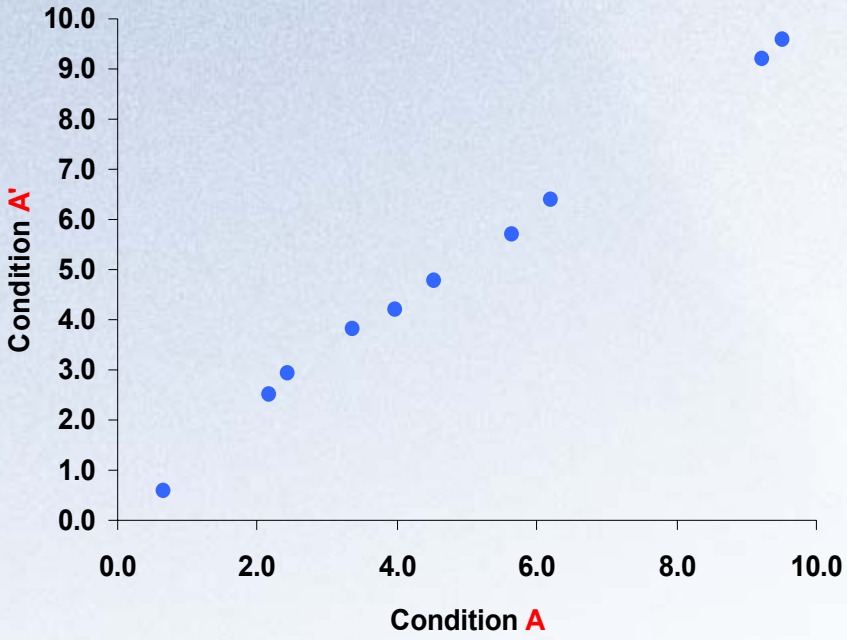
If these two conditions are satisfied, the maximum total peak capacity of the two-dimensional system is:

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

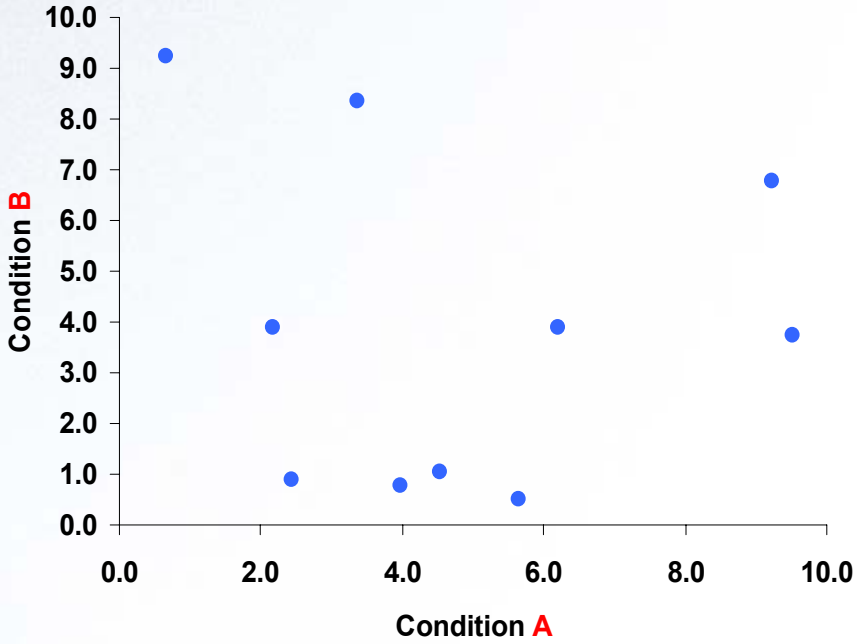
Giddings, J. C. *Multidimensional Chromatography: Techniques and Applications*; Marcel Dekker: New York, 1990

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



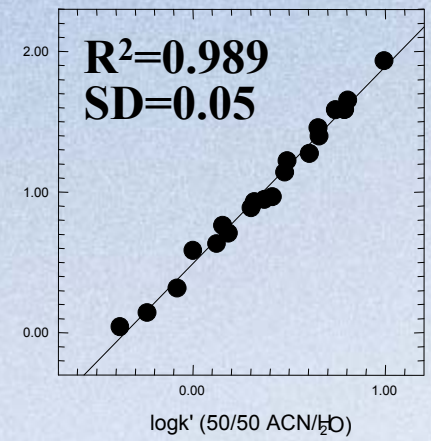
This scenario is ineffective in two-dimensional HPLC

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

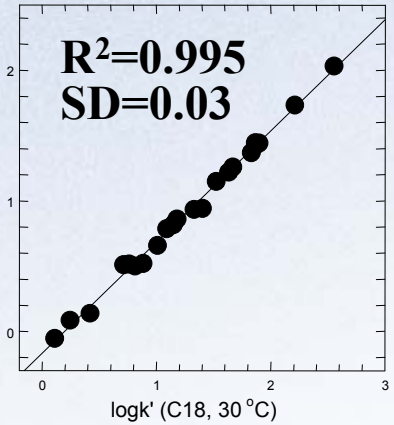
Comparison of Variables Affecting Selectivity

ZirChrom®

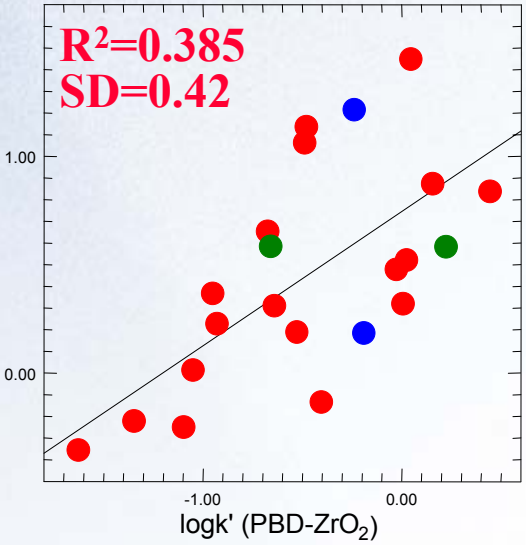
30% ACN vs. 50% ACN



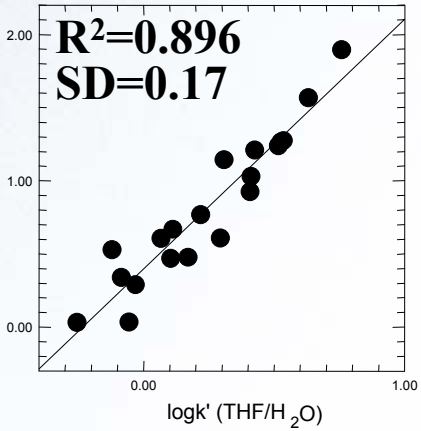
80°C vs. 30°C



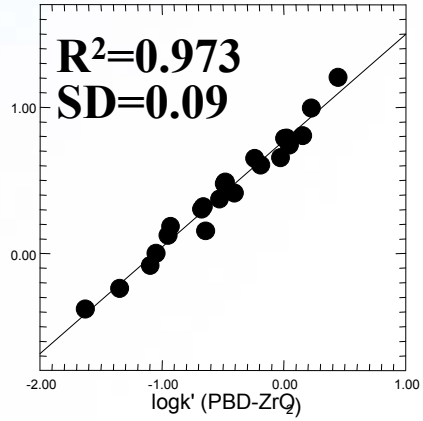
ZirChrom®-CARB vs. ZirChrom®-PBD



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

ZirChrom®

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

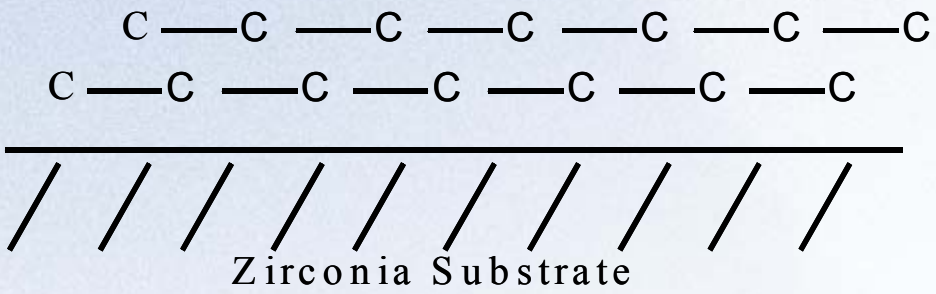
        I. Reversed-phase
        II. Ion-exchange

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

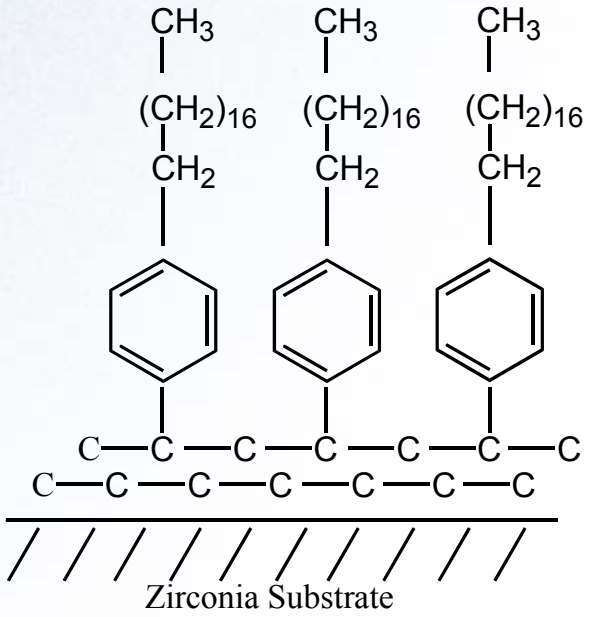
ZirChrom HPLC Columns

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

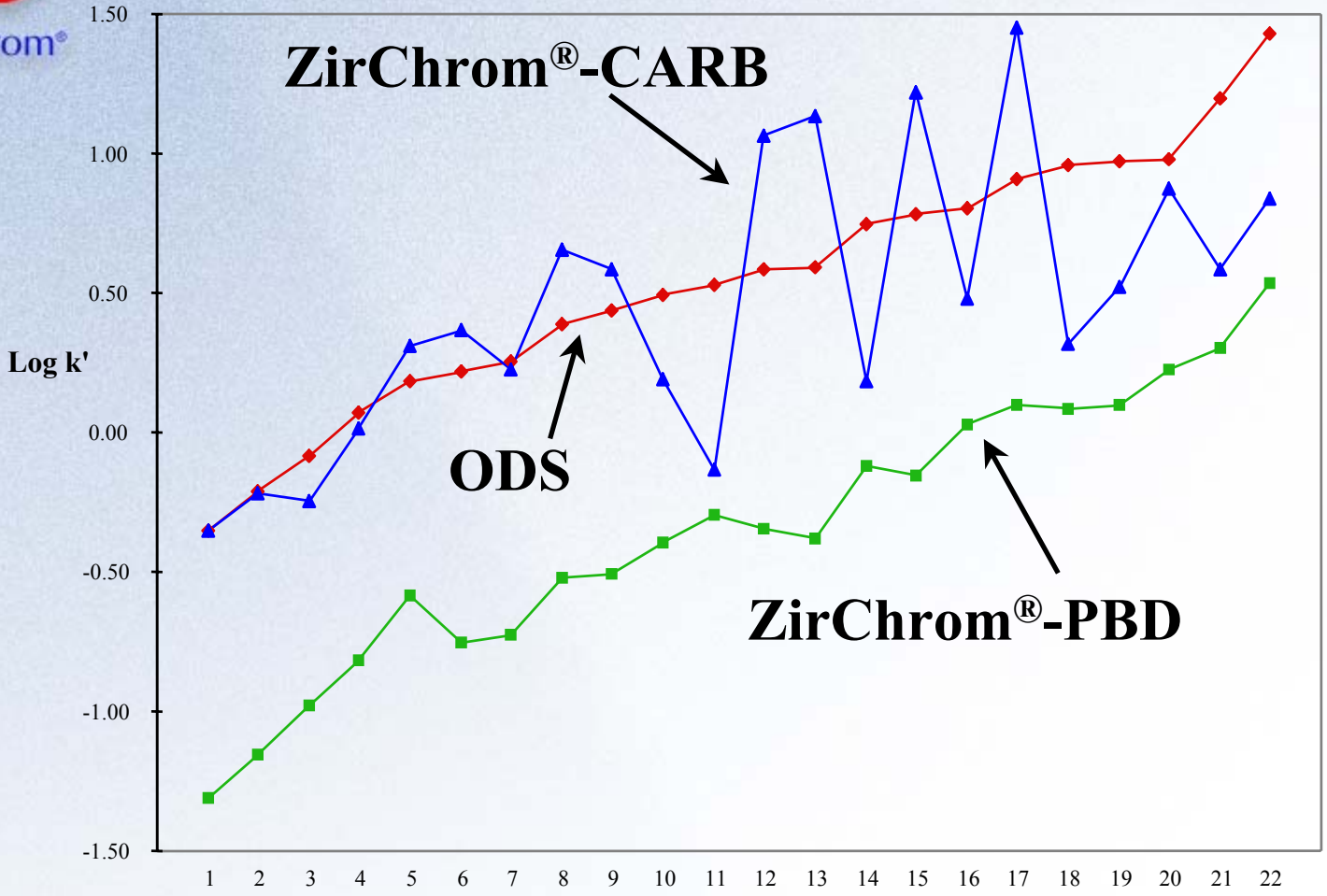


ZirChrom®-CARB



DiamondBond-C18™

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

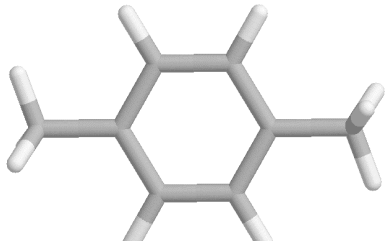


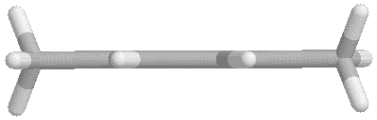
1. N-benzyl formamide
2. Benzylalcohol
3. Phenol
4. 3-phenyl propanol
5. p-chlorophenol
6. Acetophenone
7. Benzonitrile
8. Nitrobenzene
9. methyl benzoate
10. Anisole
11. Benzene
12. p-chlorotoluene
13. p-nitrobenzyl chloride
14. Toluene
15. Benzophenone
16. Bromobenzene
17. Naphthalene
18. Ethylbenzene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

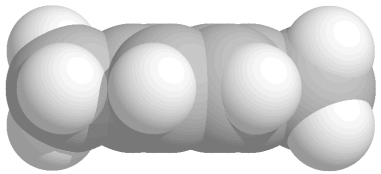
Selectivity and Shape: Isomeric Analytes

ZirChrom®

p-xylene



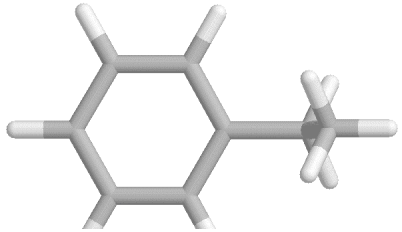


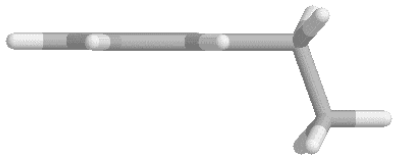


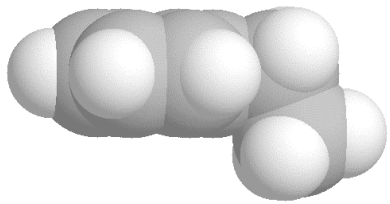
α_{ODS}=1.03

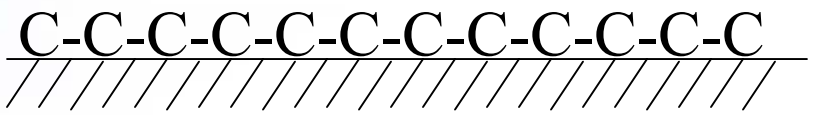
$\alpha_{C\text{-}Zr}$=1.58

ethylbenzene

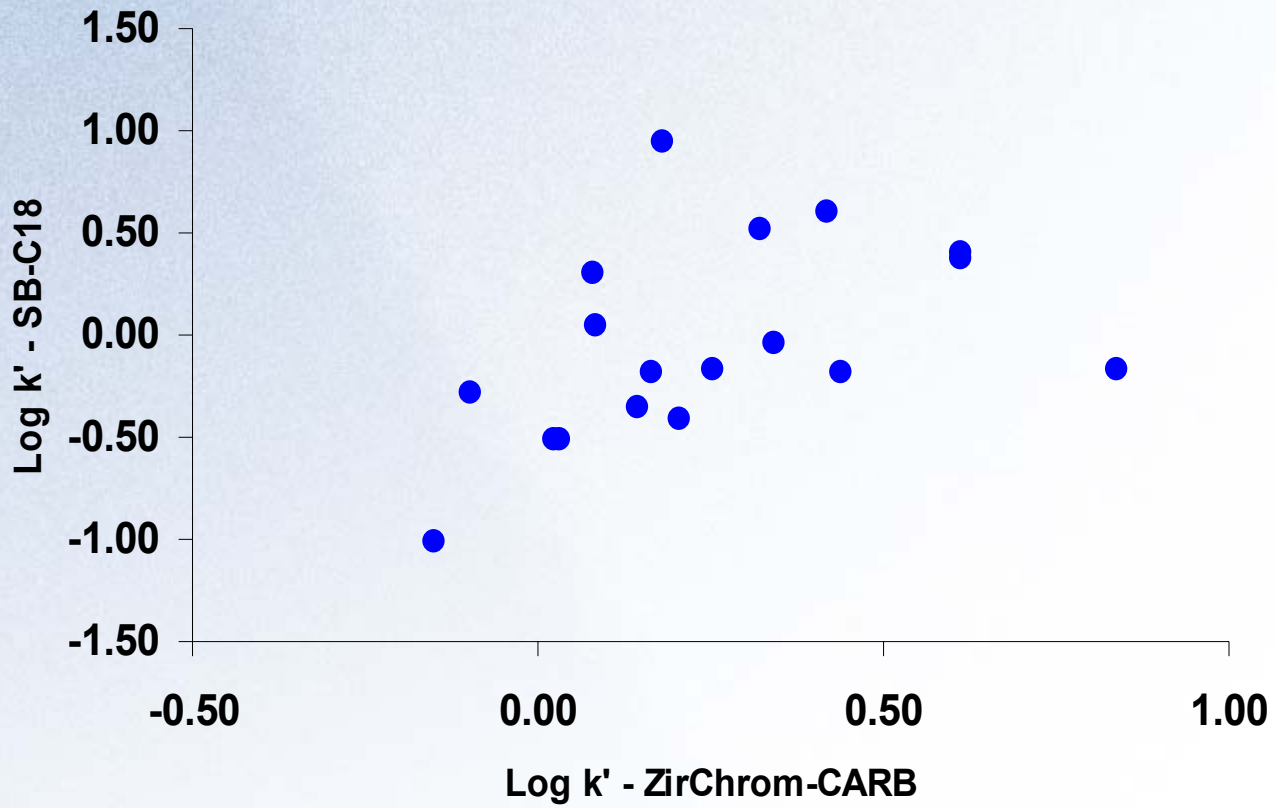






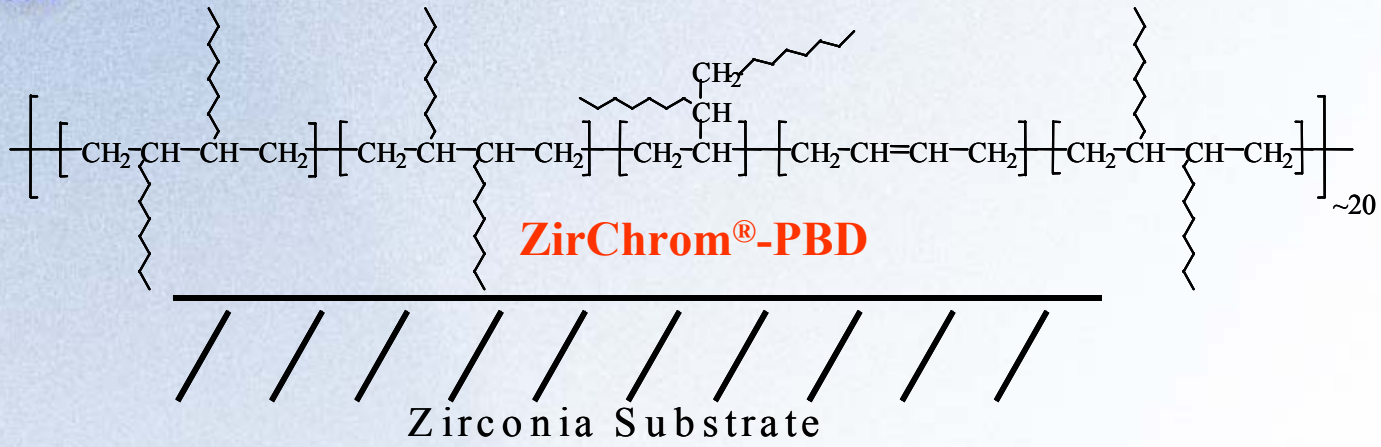


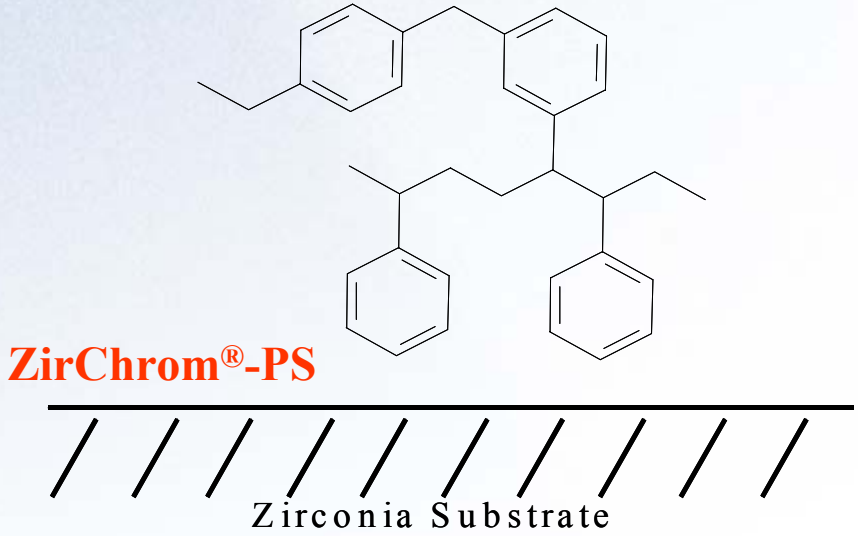
Selectivity of ZirChrom®-CARB and SB-C18 for 18 Substituted Phenols



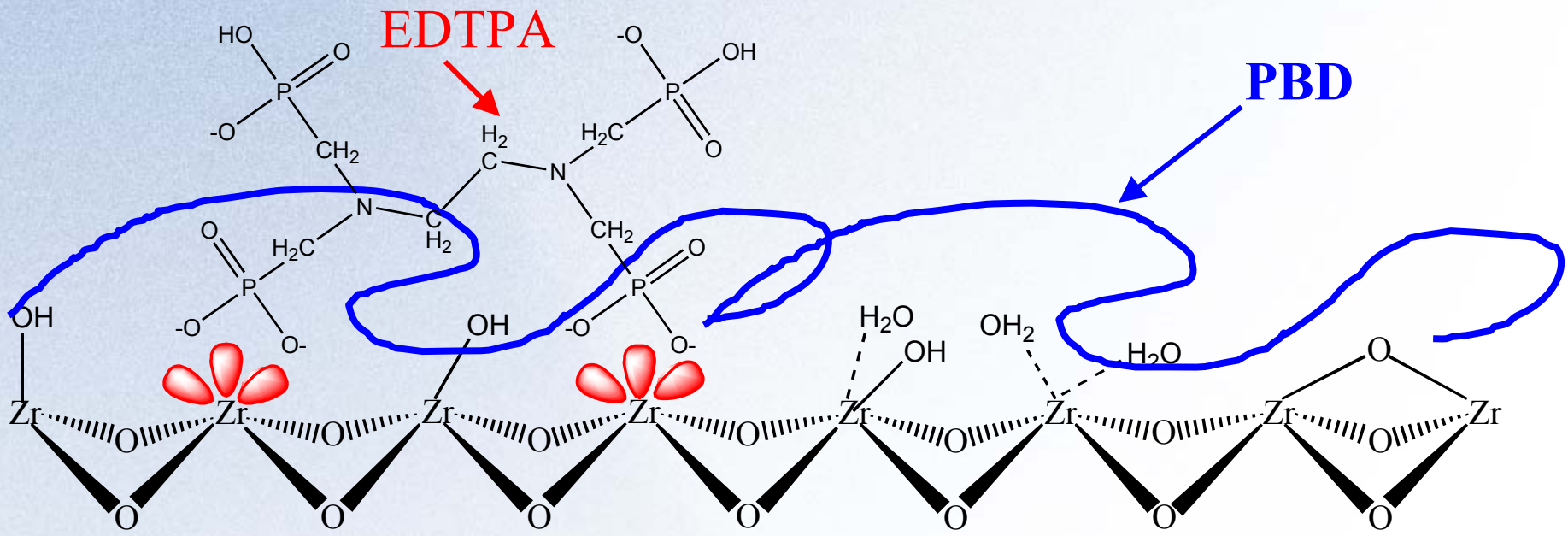
LC Conditions: Mobile phase, 45/55 ACN/10mM phosphoric acid, pH 2.4; Flow rate, 2.0 ml/min.; Temperature, 40 °C – Data courtesy of Adam Schellinger

ZirChrom®-PBD and ZirChrom®-PS





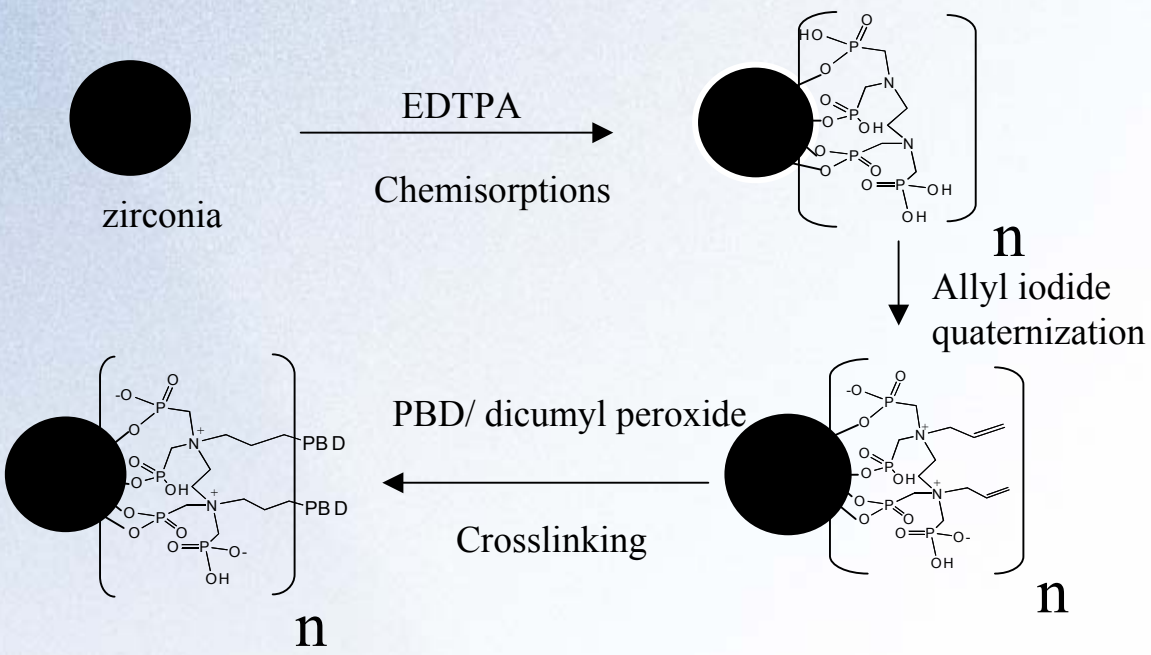
ZirChrom®-EZ



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

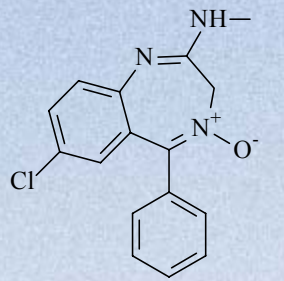


1 **Chemisorb Ethylenediamine N,N,N',N'-tetra(methylenephosphonic)acid (EDTPA) to the zirconia surface.**

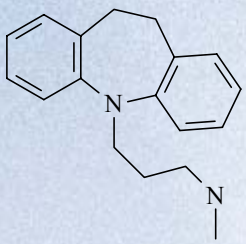
2 **Quaternize amines on the zirconia surface with allyl iodide.**

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

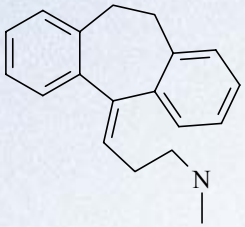
Selectivity Study of Eleven Antidepressants



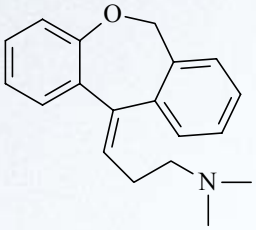
Chlordiazepoxide



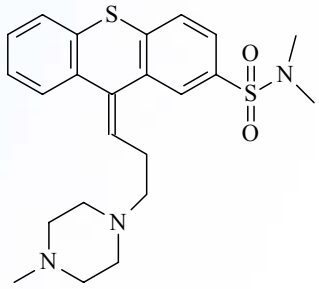
Desipramine



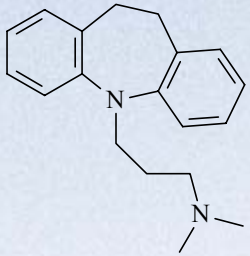
Nortriptyline



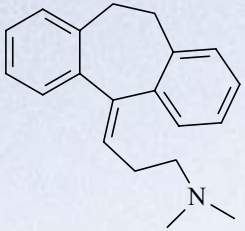
Doxepin



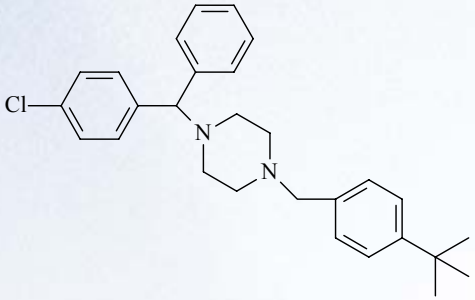
Imipramine



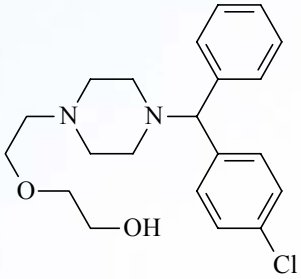
Amitriptyline



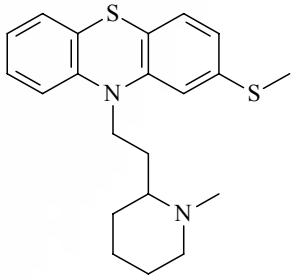
Buclizine



Hydroxyzine



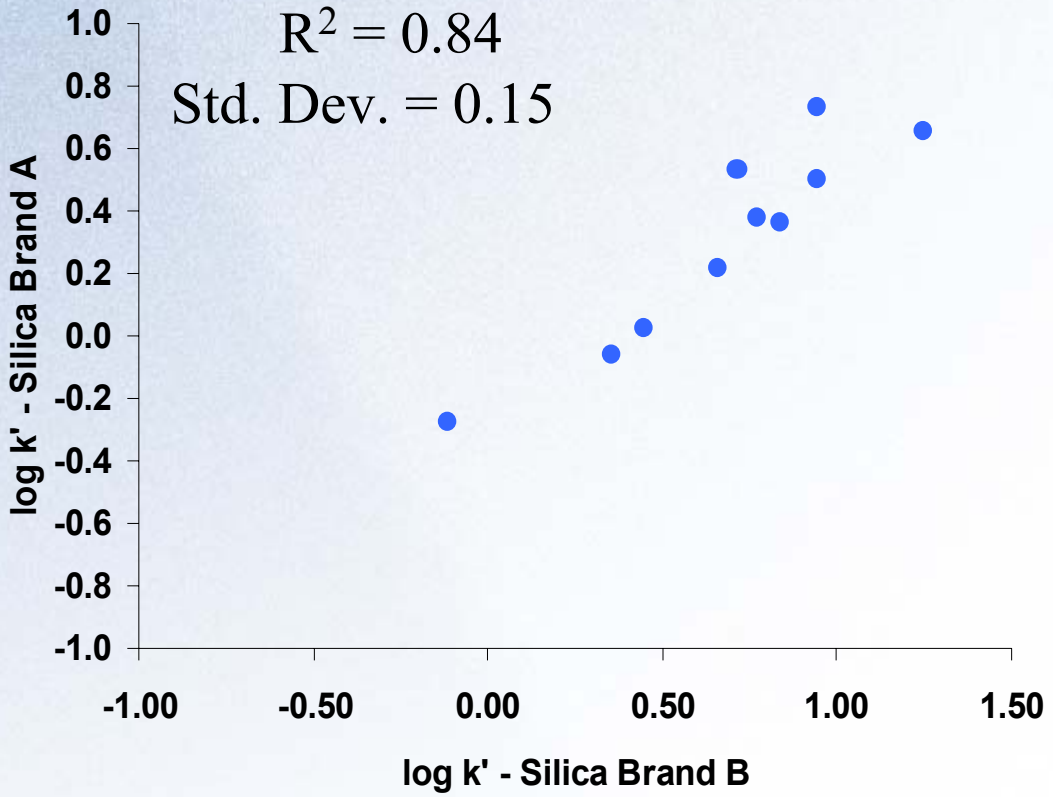
Thioridazine



Perphenazine

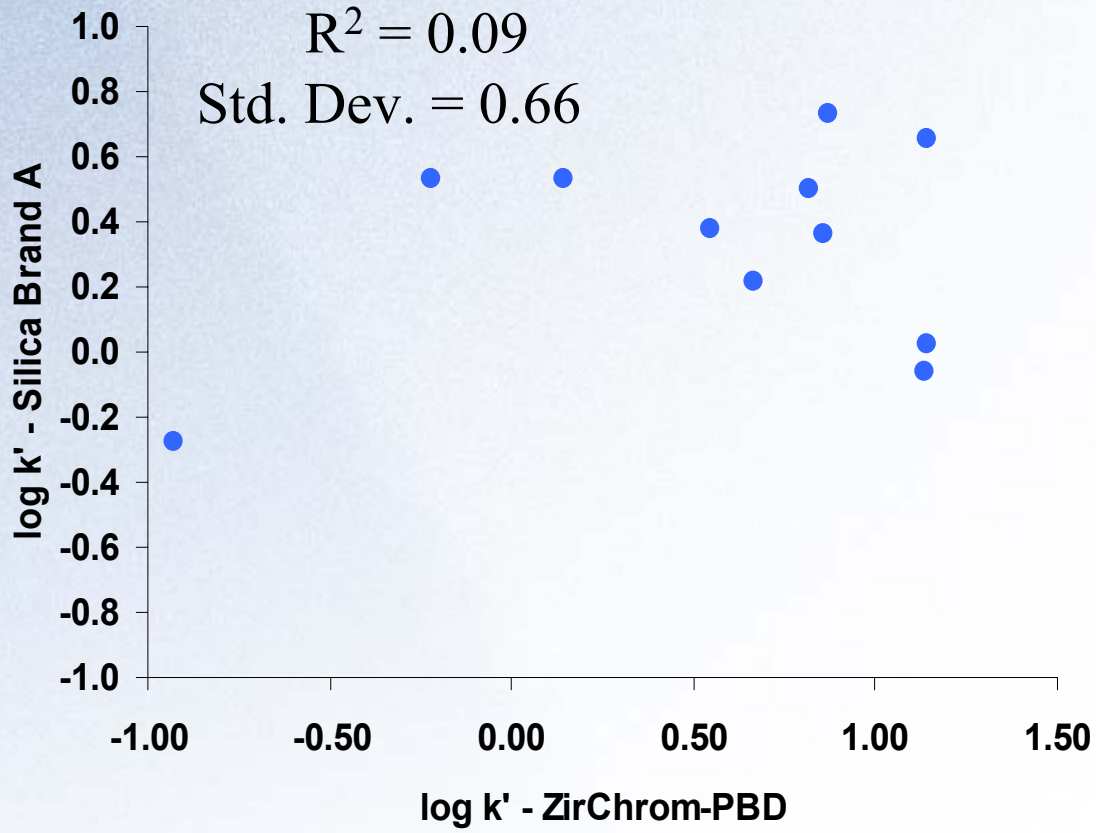
Dai, J.; Yang, X.; Carr, P. W. *Journal of Chromatography, A* **2003**, *1005*, 63-82

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



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.

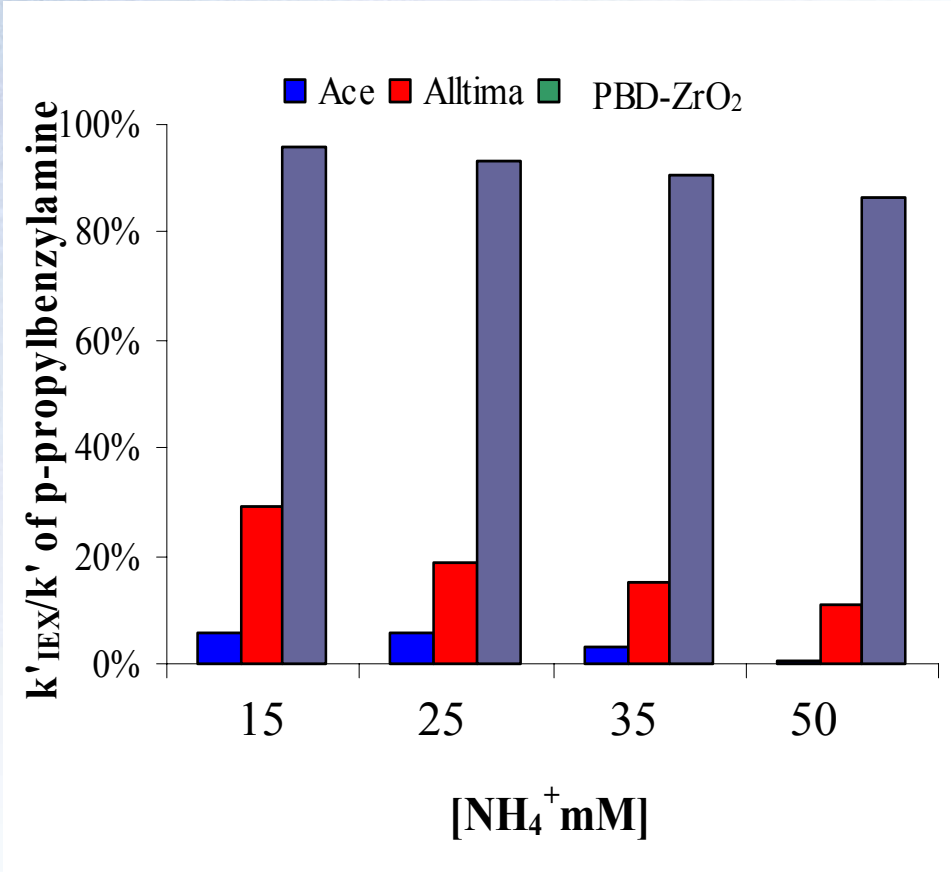
Selectivity for Antidepressant Compounds on ZirChrom®-PBD vs. ODS



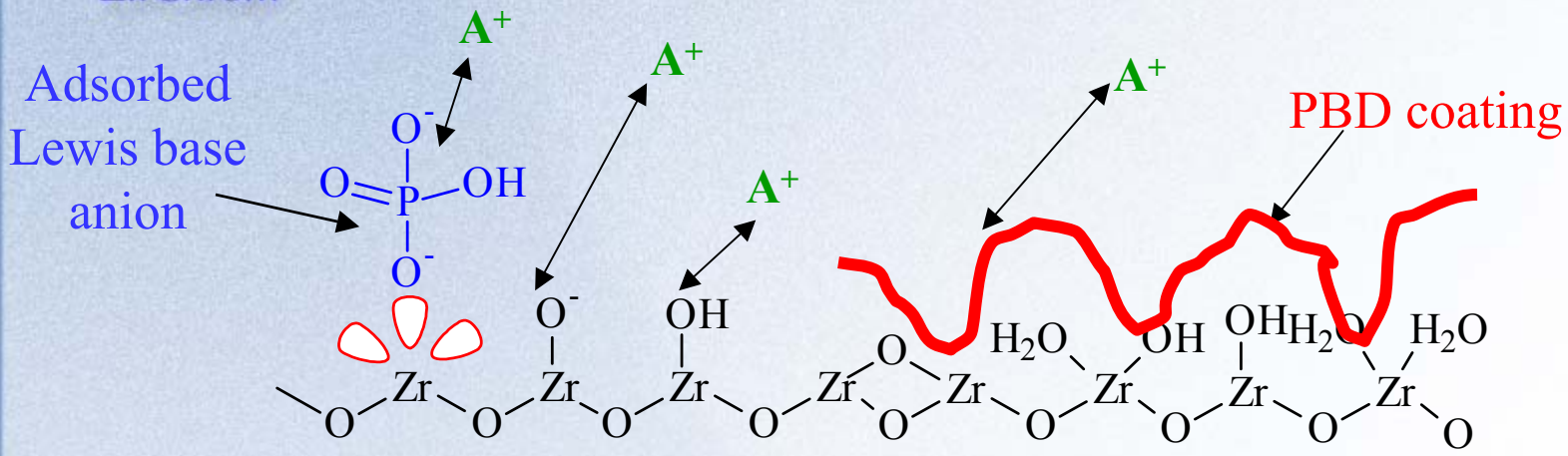
LC Conditions: Mobile phase, 72/28 MeOH/25 mM ammonium phosphate, pH 6.0; Flow rate, 1.0 mL/min; Temperature, 35 °C; UV detection at 254 nm.

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



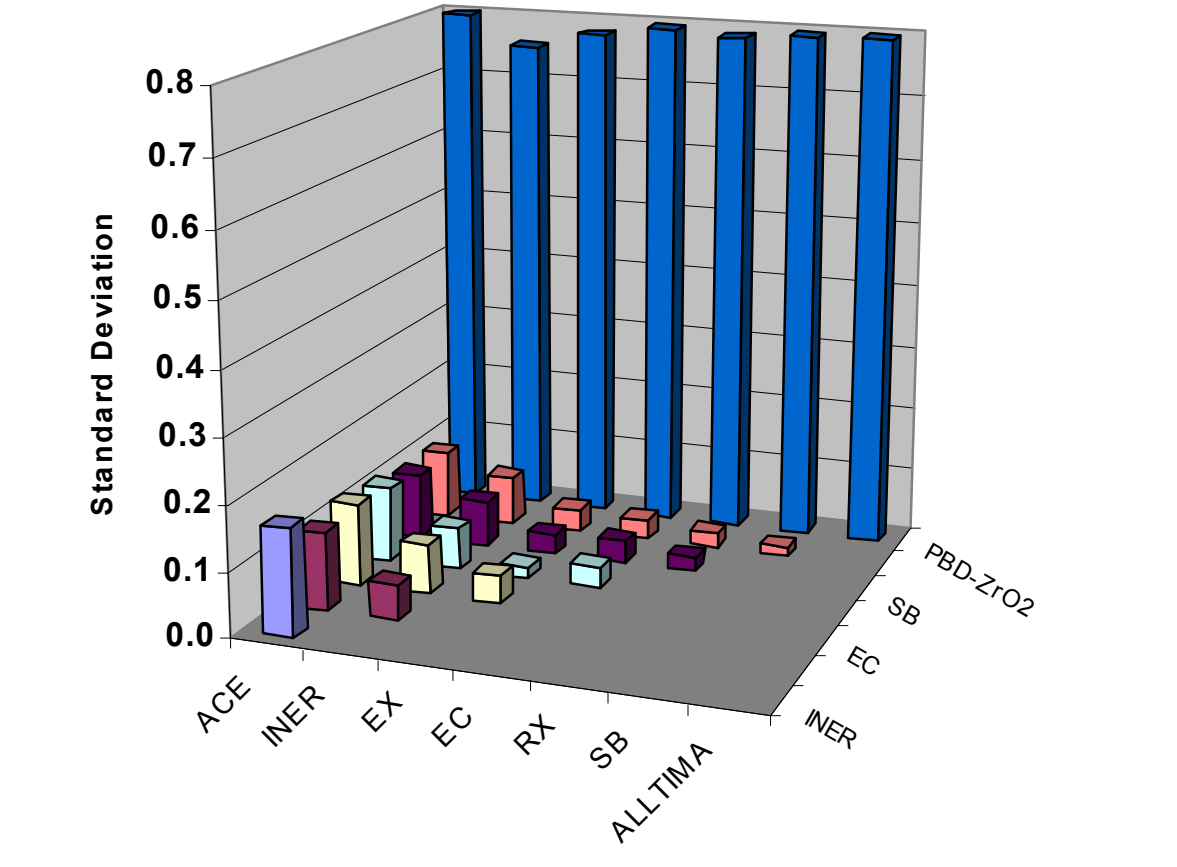
- PBD Coating — Reversed-Phase (RP) Moieties
- Lewis Base Anions — Ion-Exchange (IE) Sites

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

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

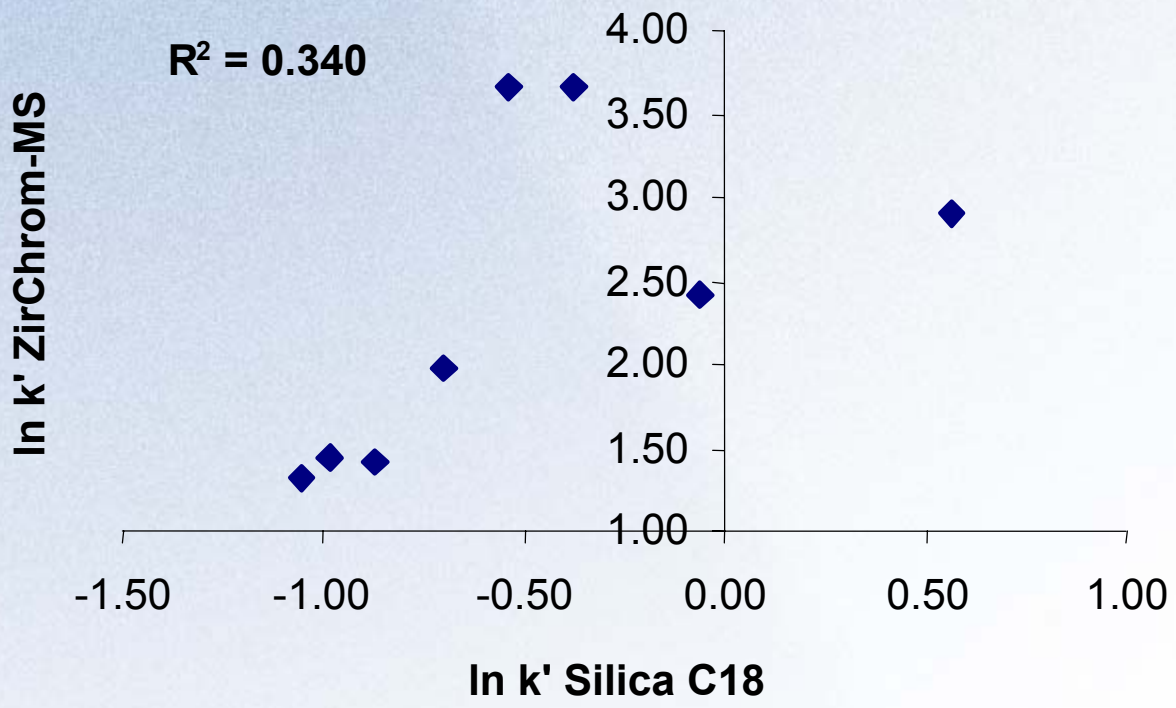
A^{+}: analyte cation, X^{+}: counterion, L^{-}: adsorbed Lewis base anion.

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

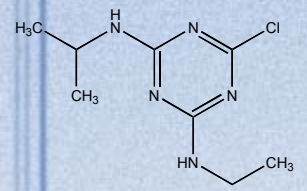


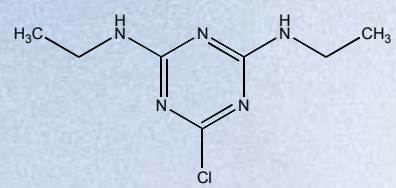
Basic Compounds are much more retained on ZirChrom-MS than on Silica C18 and have very different chromatographic selectivity.

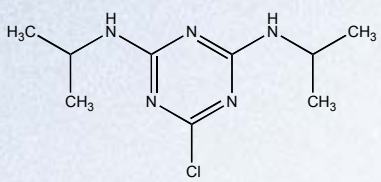
LC Conditions: Mobile Phase, 72/28 MeOH/25mM Ammonium phosphate, pH 6.0; Flow Rate, 1.0 ml/min.; Temperature, 35 °C; Injection Volume, 5 µl; Detection by UV at 254 nm; Solutes from left to right: Methapyrilene, Pyrilamine, Tripelennamine, Brompheniramine, Desipramine, Nortryptyline, Doxepin, and Amitryptyline.

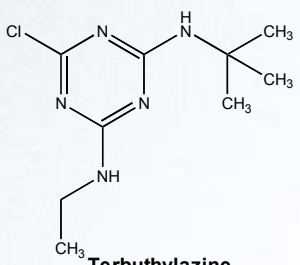
An Example 2DLC Separation - Ten Triazine Herbicides

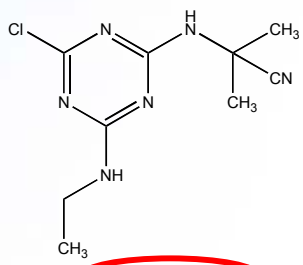
ZirChrom®

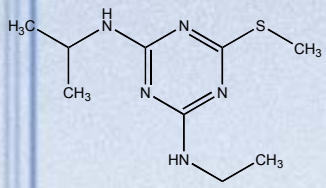

Atrazine

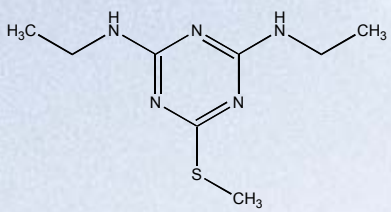

Simazine


Propazine


Terbuthylazine


Cyanazine


Ametryne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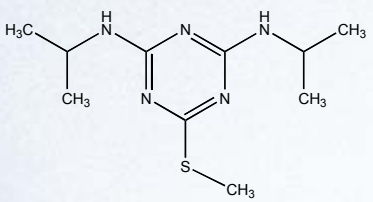
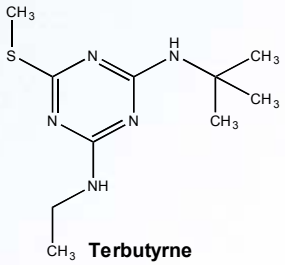
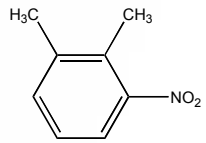
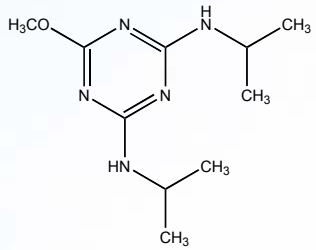

Prometryne

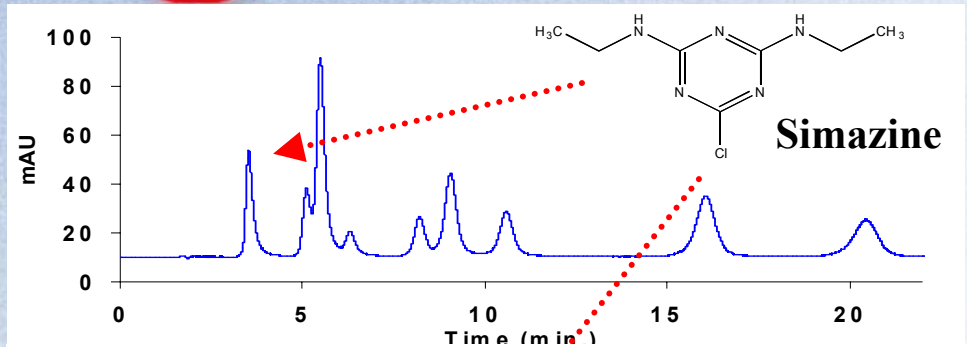

Terbutyrne

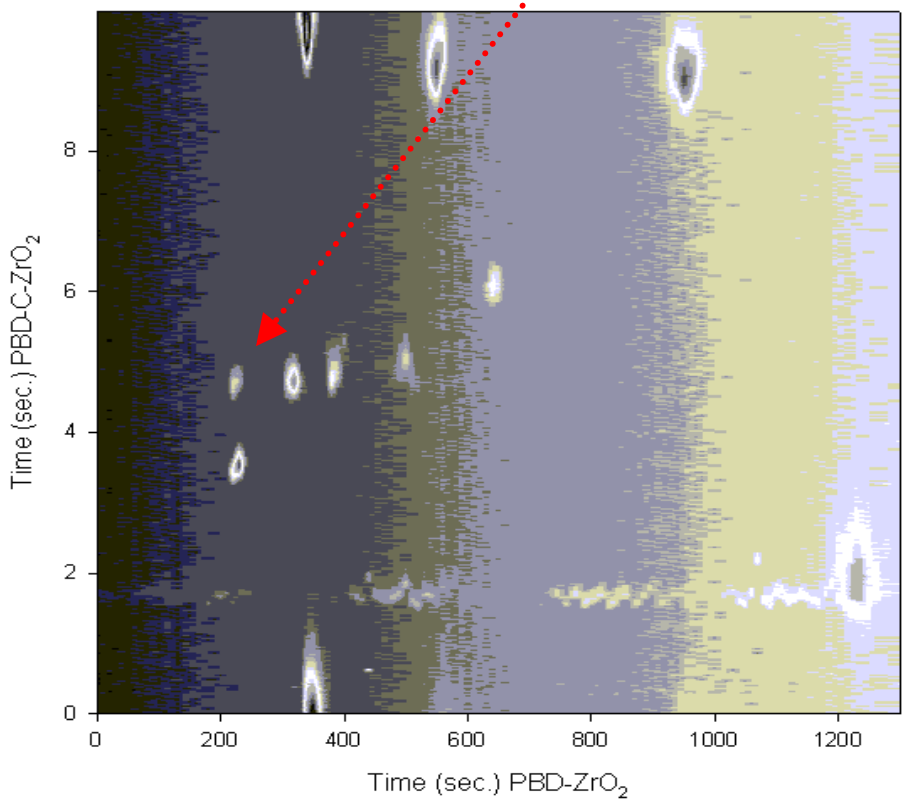

2-nitroxylene


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

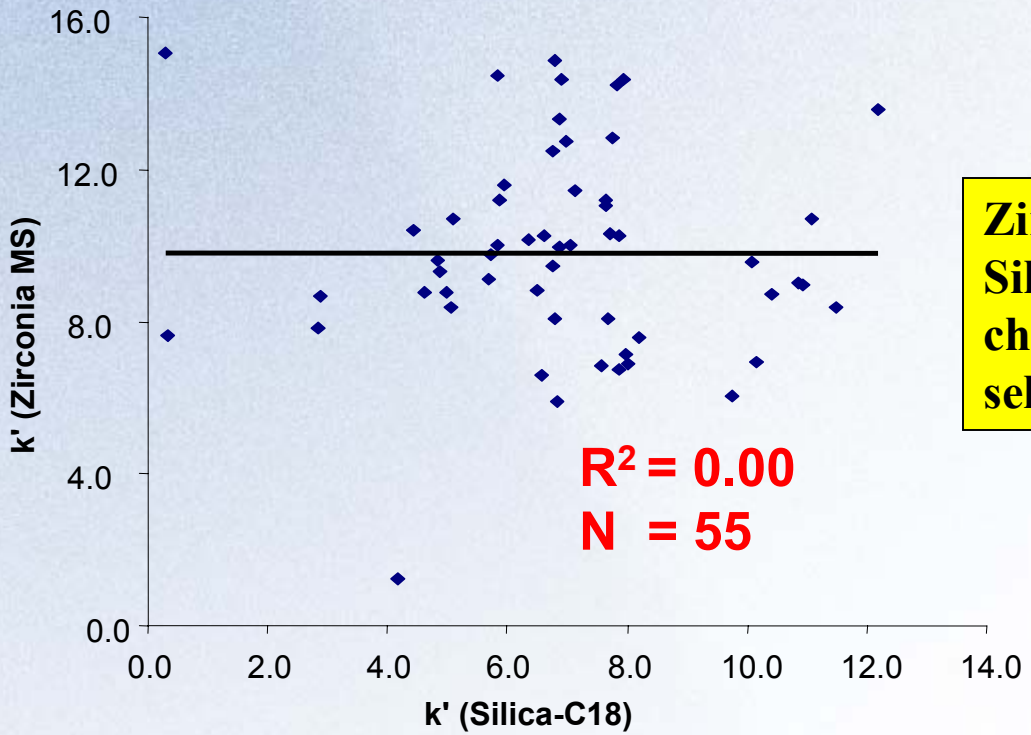
Selectivity Comparison of 55 Pharmaceuticals

ZirChrom®

1	cotinine	20	bretyllium	39	pindolol
2	piroxicam	21	labetalol	40	oxyphenonium
3	progesterone	22	tryptophan	41	metoprolol
4	enalopril	23	simvastatin	42	sildenafil
5	hydrocortisone acetate	24	lidocaine	43	diphenhydramine
6	nitrazepam	25	scopolamine	44	ritalin
7	cortisone acetate	26	isopropramide	45	chlorpheniramine
8	tadalafil	27	morphine	46	triprolidine
9	warfarin	28	naltrexone	47	hydroxyzine
10	diclofenac	29	acebutolol	48	brompheniramine
11	nicotine	30	berberine	49	meclizine
12	atenolol	31	fentanyl	50	amitriptyline
13	chlordiazepoxide	32	tramadol	51	fluoxetine
14	prednisone	33	deprenyl	52	alprenolol
15	methylscopolamine	34	mepenzolate	53	hydroxypropranolol (blue)
16	ipratropium	35	methoxyverapamil	54	propranolol
17	cimetidine	36	verapamil	55	terbutaline
18	lovastatin	37	codeine		
19	hydroxymetoprolol	38	vardenafil		

Note: number indicates elution order on the ZirChrom-MS column.

k-k Plot for 55 Ionizable Compounds

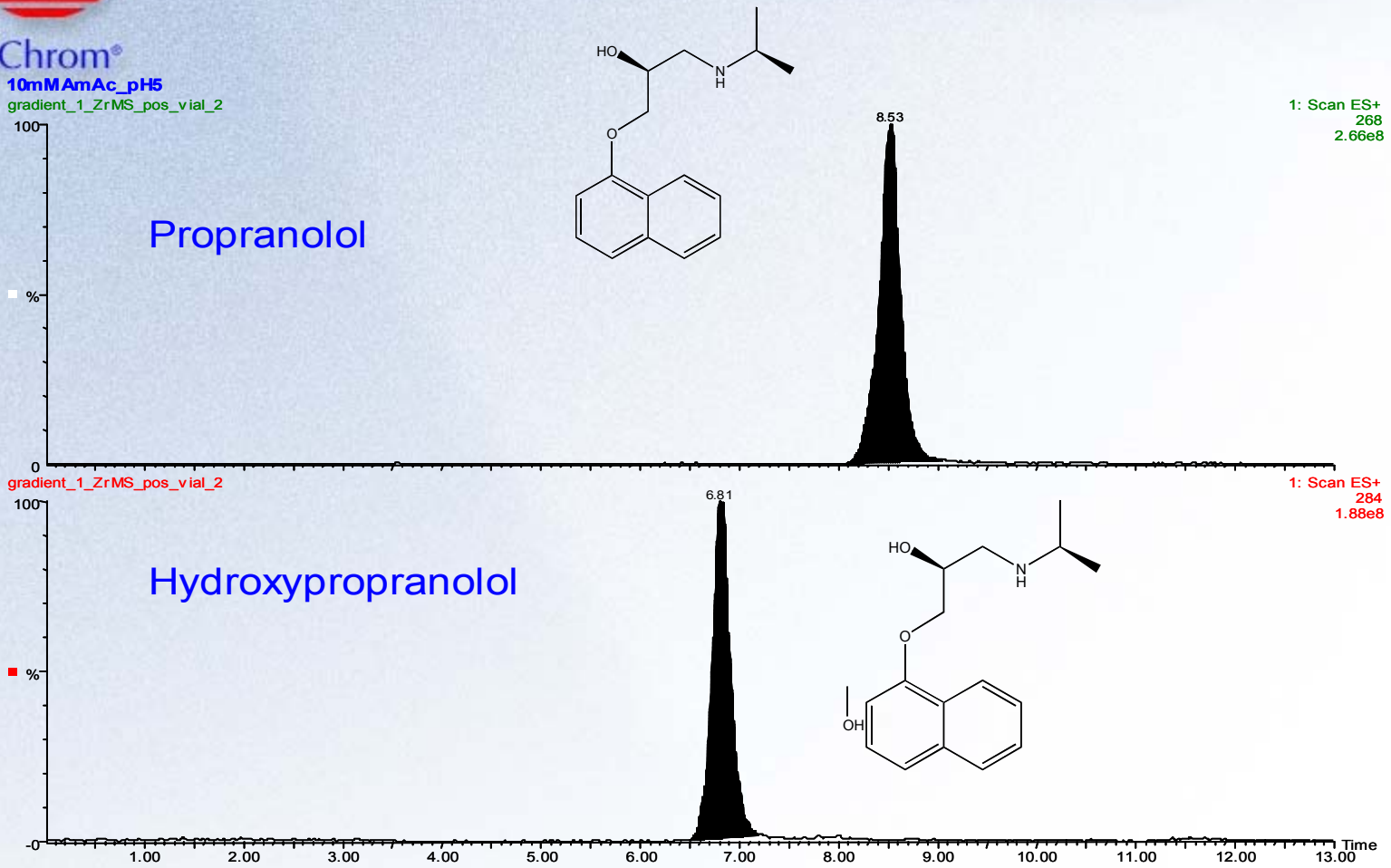


ZirChrom®-MS and C18 Silica have very different chromatographic selectivity for ionic drugs.

LC Conditions: Machine-mixed 80/20 ACN/10 mM ammonium acetate pH=6.7 without pH adjustment; Flow rate, 1.0 ml/min.; Injection volume 0.1 µl; Temperature, 35 °C; Detection at 254 nm; Columns, ZirChrom®-MS, 50 x 4.6 mm i.d. (3um particles), S/N:MS020204T; Silica-C18 150 x 4.6 mm i.d., (3.5 um particles).

LC-MS of Basic Pharmaceuticals

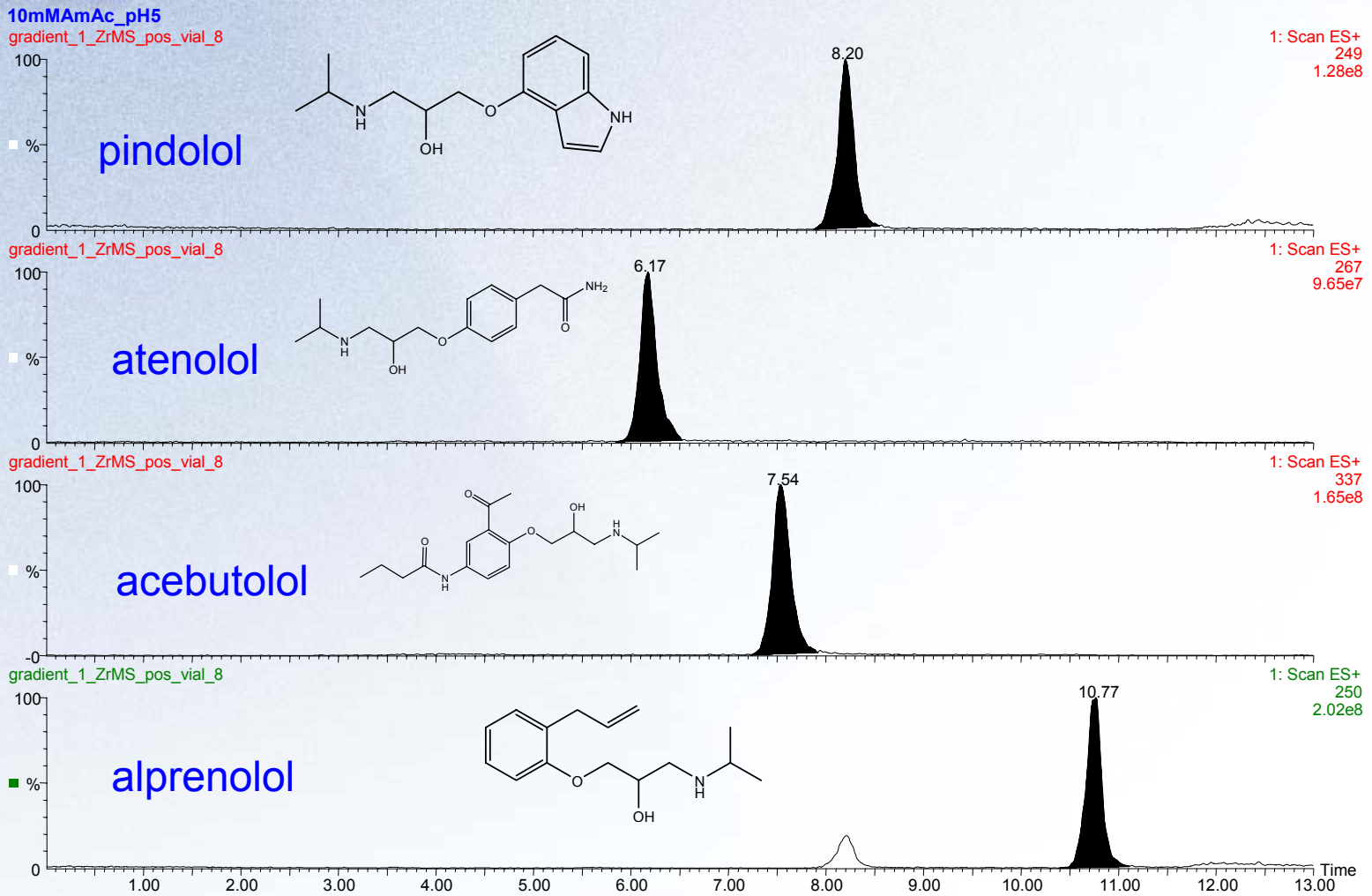
ZirChrom®



LC Conditions: Column, ZirChrom®-MS, 5 x 2.1 mm i.d. (3 micron particles). Waters Alliance 2795 LC, Flow rate, 0.2mL/min, Mobile phases channel C=10mM ammonium acetate at pH 5, channel D=10mM ammonium acetate at pH 5:acetonitrile (10:90, v/v) , Linear gradient 5% D to 100% D in 6 minutes, hold 100% 6-7.4 min, 100 to 5% D 7.4-8.1min, hold 5% D 8.1-13.0 min. Temperature, 35°C. Waters/Micromass ZQ single quadrupole interfaced with the LC using an electrospray ionization (ESI) interface. Positive ion mode (XIC) from full scan acquisitions from m/z 120-700. Solute concentrations = 10μg/mL, 2μL injections.

HPLC-MS of Beta-Blockers*

ZirChrom®



*HPLC Conditions are the same as the receding slide.

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

ZirChrom - Dr. Bingwen Yan

Supelco - Dr. David Bell