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SYNTHESIS OF A NEW CHEMICALLY STABLE LEWIS-ACID DEACTIVATED REVERSED-PHASE ZIRCONIA STATIONARY PHASE FOR LC-MS

RICHARD HENRY[1], DAVE BELL[2], CLAYTON MCNEFF, BINGWEN YAN, ZIRCHROM SEPARATIONS, INC., 617 Pierce St. , Anoka, MN 55303

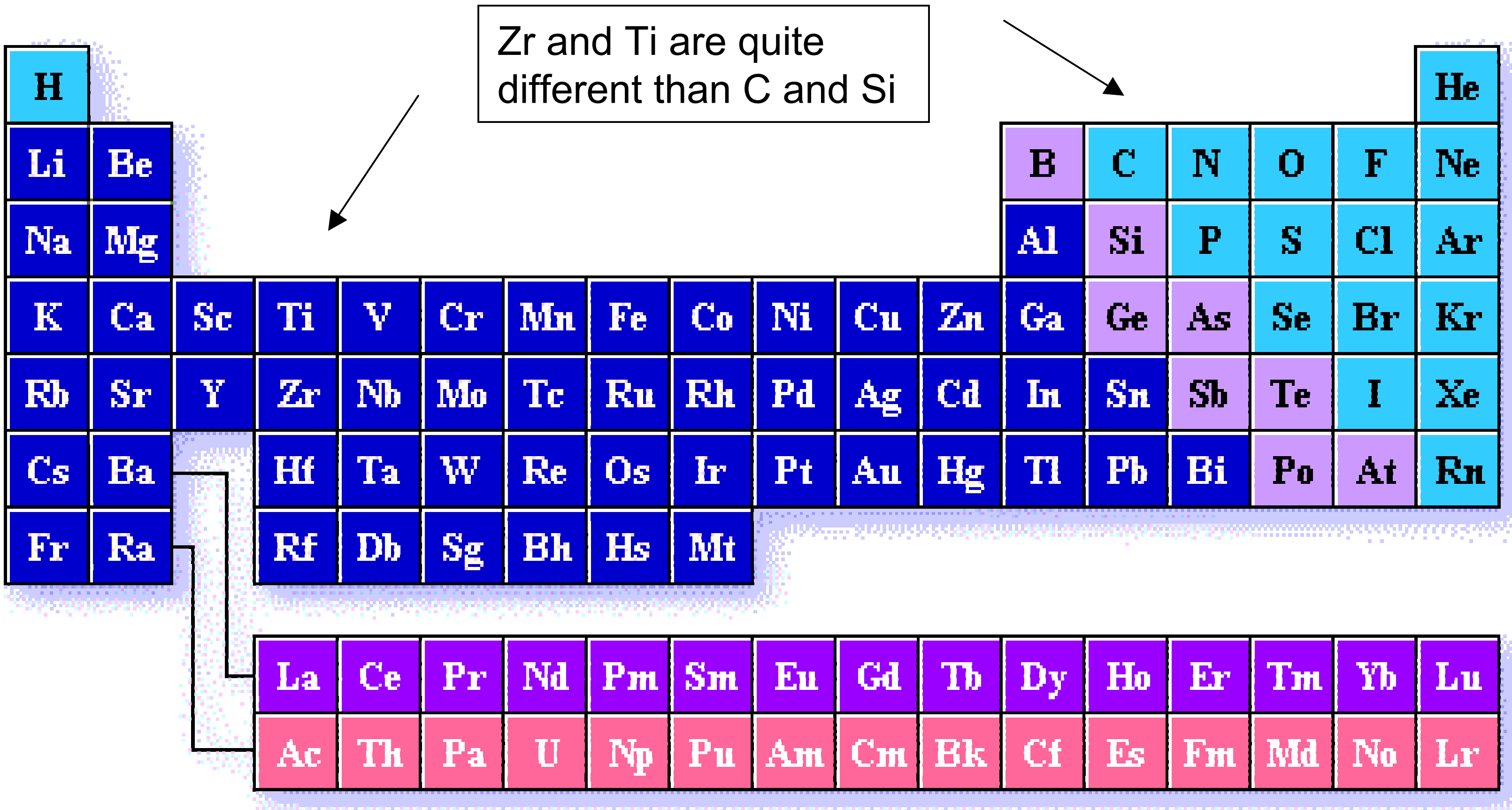
1. Consultant

2. Supelco Division of Sigma Aldrich

Outline

- Advantages and surface chemistry of zirconia-based supports for HPLC
- Synthesis of internally deactivated zirconia reversed-phase packings
- Chromatographic characterization
    - Chromatography of Lewis-base analytes
    - Reversed-phase characteristics
    - Ion-exchange characteristics
    - Comparison to C18 silica
- Pharmaceutical LC-UV and LC-MS applications

Periodic Table of Elements



Source: University of Kentucky, Chemistry Dept. web site
http://www.uky.edu/Projects/Chemcomics/

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Element Electronic Structures

- Silicon (element 14; 2.3 g/cc; Si^{4+} ionic radius 0.400 Å)
  - Ne$3s^2 3p^2$
- Titanium (element 22; 4.5 g/cc; Ti^{4+} ionic radius 0.605 Å)
  - Ar$3d^2 4s^2$
- Zirconium (element 40; 6.5 g/cc; Zr^{4+} ionic radius 0.720 Å)
  - Kr$4d^2 5s^2$

All have four valence electrons so some chemistry is similar, but presence of d orbitals and very electropositive nature allow Ti and Zr (metals) to form strong electron donor-acceptor complexes (coordination chemistry).

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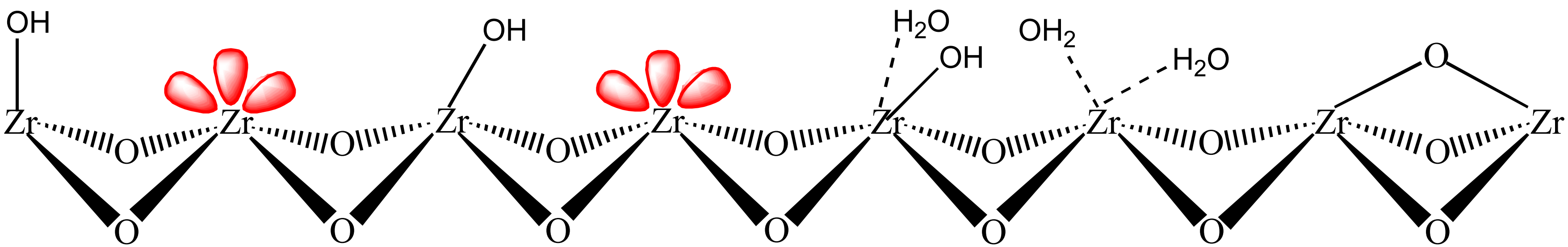
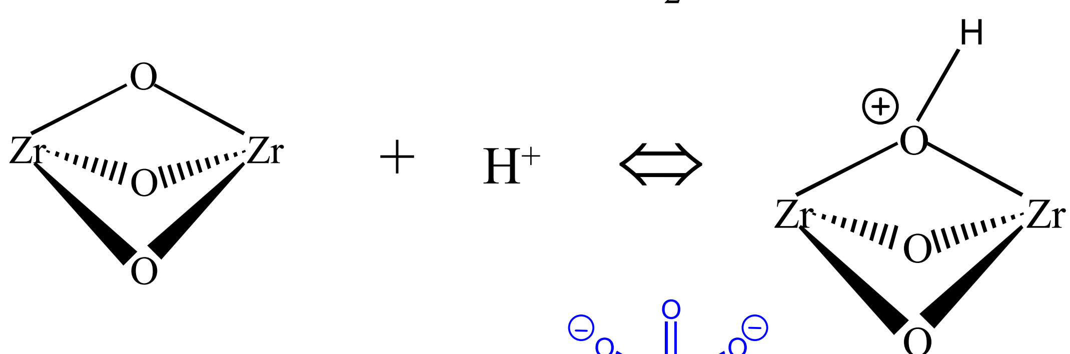
Surface Chemistry of Zirconia-Based Supports for HPLC

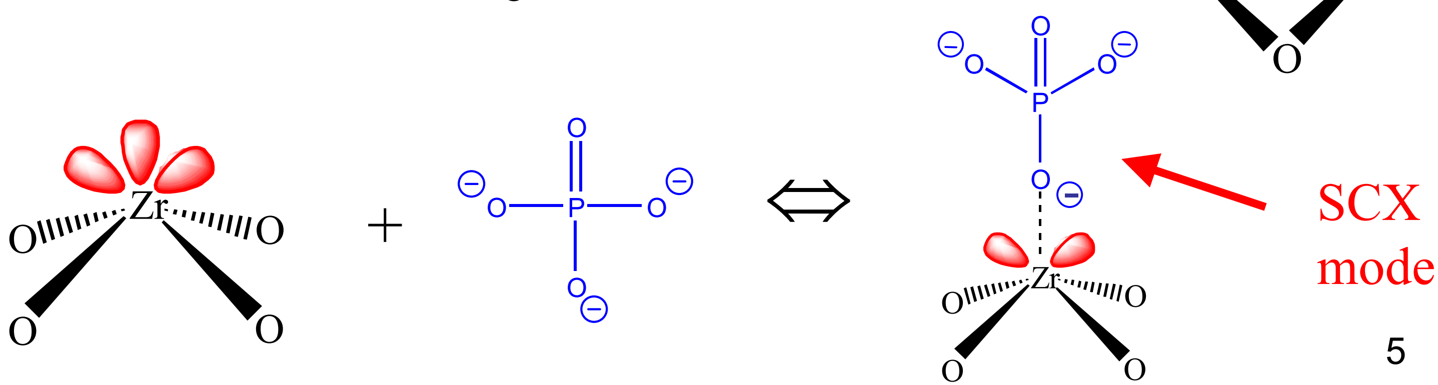


Weak Brönsted Acid: $ZrOH + OH^{-} \Leftrightarrow ZrO^{-} + H_2O$

Weak Brönsted Base:

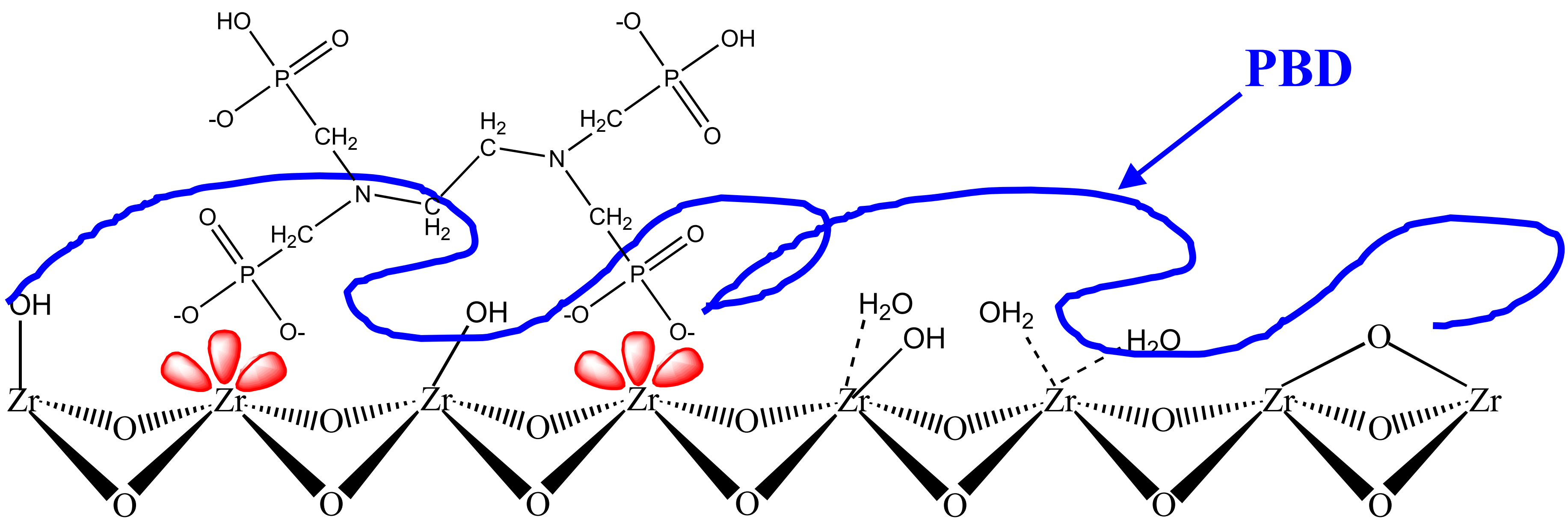


Strong Lewis Acid:



Synthesis of a Deactivated Reversed-Phase LC Packing

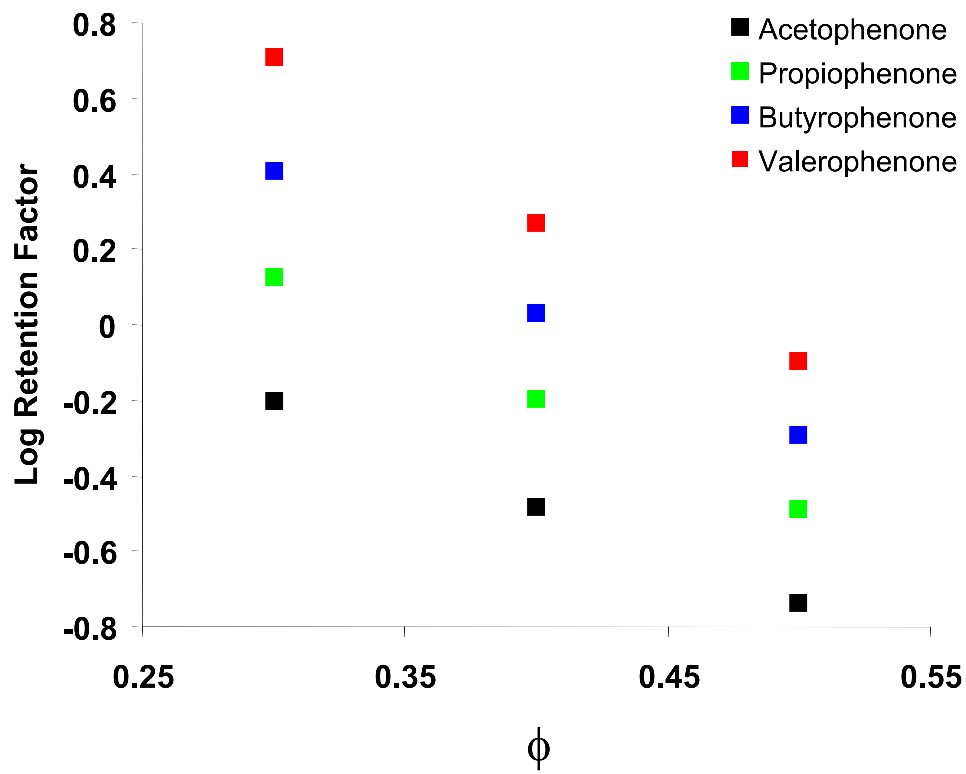
EDTPA (Strong Lewis Base)



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)
4. Wash to remove residual EDTPA

1. P. W. Carr, et. al., *Journal of Chromatography A* **1998**, *791*, 45-52

Zirchrom®-EZ Reversed-Phase Characteristics



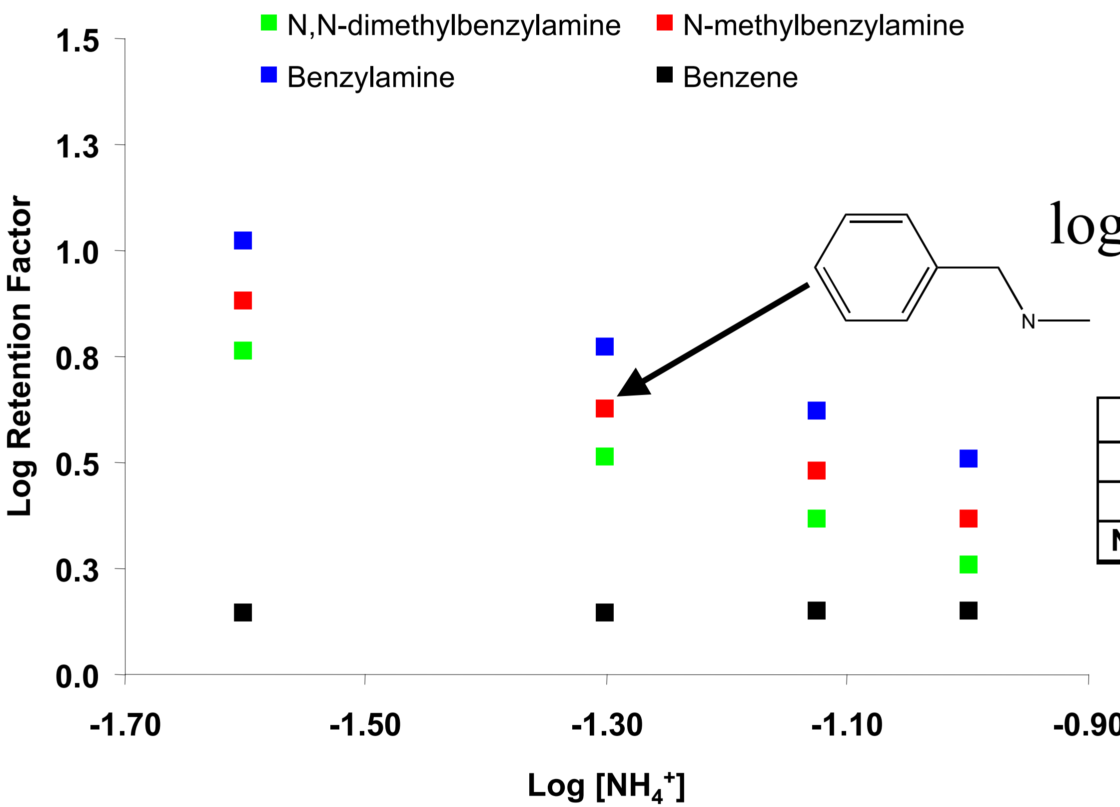
Same RP behavior as ZirChrom-PBD

$$\log k'_{RP} = \log k_w - S\phi$$

Solute	Slope	R^2
Acetophenone	-2.67	0.999
Propiophenone	-3.06	0.999
Butyrophenone	-3.51	0.998
Valerophenone	-4.03	0.997

LC Conditions: Mobile phase, indicated composition of ACN/Water; Flow rate, 2.0 ml/min.; Temperature, 35 °C; Injection volume, 5 μl; Detection at 254 nm; Column, 50 mm x 4.6 mm i.d. ZirChrom®-EZ

ZirChrom®-EZ Ion-Exchange Characteristics



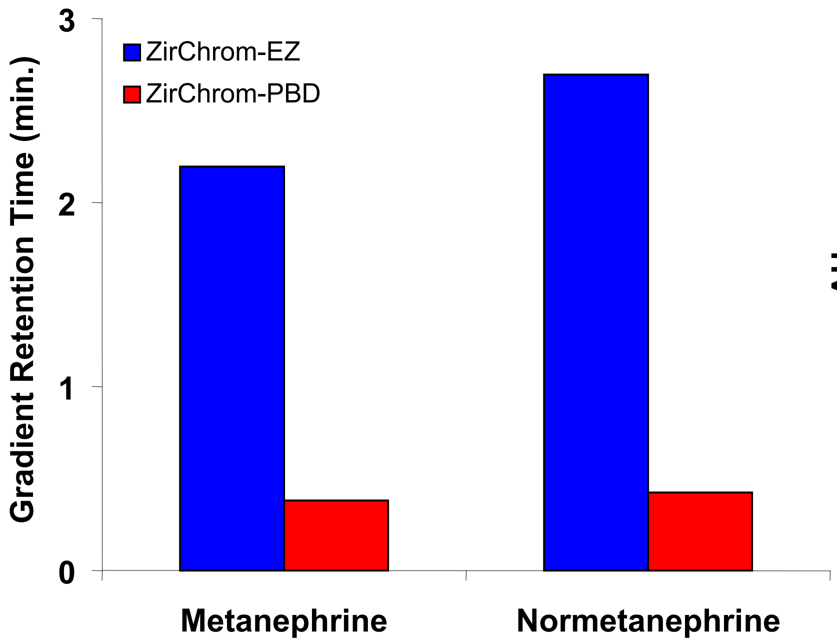
More SCX behavior than ZirChrom-PBD

$$\log k'_{IEX} = \text{Constant} - \log [C^+]_m$$

	Slope	R^2
Benzylamine	-0.846	0.999
N-methylbenzyamine	-0.847	0.999
N,N-dimethylbenzylamine	-0.840	0.999

LC Conditions: Mobile phase, 15/85 ACN/5mM MES, 25-100mM Ammonium acetate, pH 6.0; Flow rate, 2.0 ml/min.; Temperature, 35 °C; Injection volume, 5 µl; Detection at 254 nm; Column, 50 mm x 4.6 mm i.d. ZirChrom®-EZ

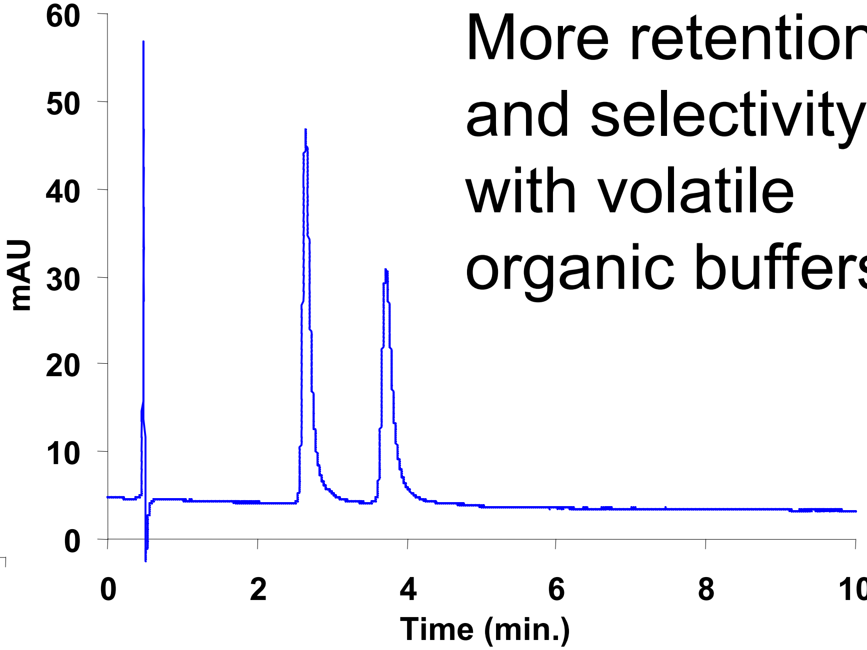
Chromatographic Characteization-Basic Analytes



Time (min.)	%A	%B
0	90	10
5	10	90

A: 20mM Ammonium acetate, pH 6.0

B: Acetonitrile

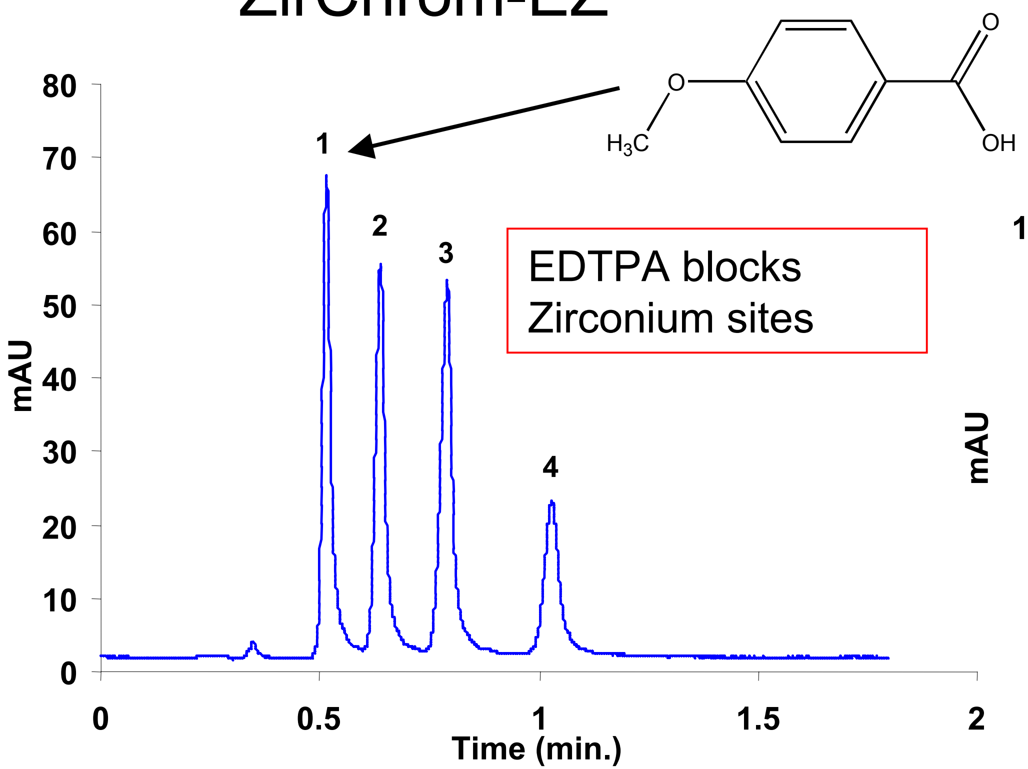


More retention and selectivity with volatile organic buffers.

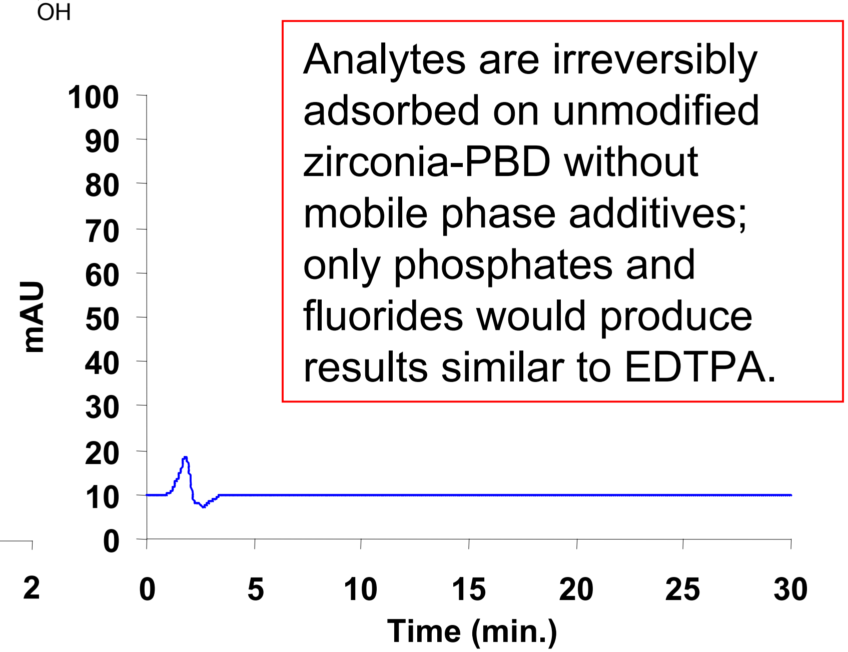
LC Conditions: Column, 50 mm x 4.6 mm i.d. ZirChrom®-EZ; Mobile phase, 25/75 ACN/20mM ammonium acetate, pH 6.0; Flow rate, 1.20 ml/min.; Temperature, 35 ^{o}C; Injection volume, 10 μl; Detection at 254 nm.

Chromatographic Characterization – Acidic Analytes

ZirChrom-EZ



ZirChrom-PBD



LC Conditions: Mobile phase, 40/60 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 1 µl; Detection at 254 nm; Solutes: 1. methoxybenzoic acid, 2. ethoxybenzoic acid, 3. propoxybenzoic acid, 4. butoxybenzoic acid; Column, 50 mm x 4.6 mm i.d. ZirChrom®-EZ

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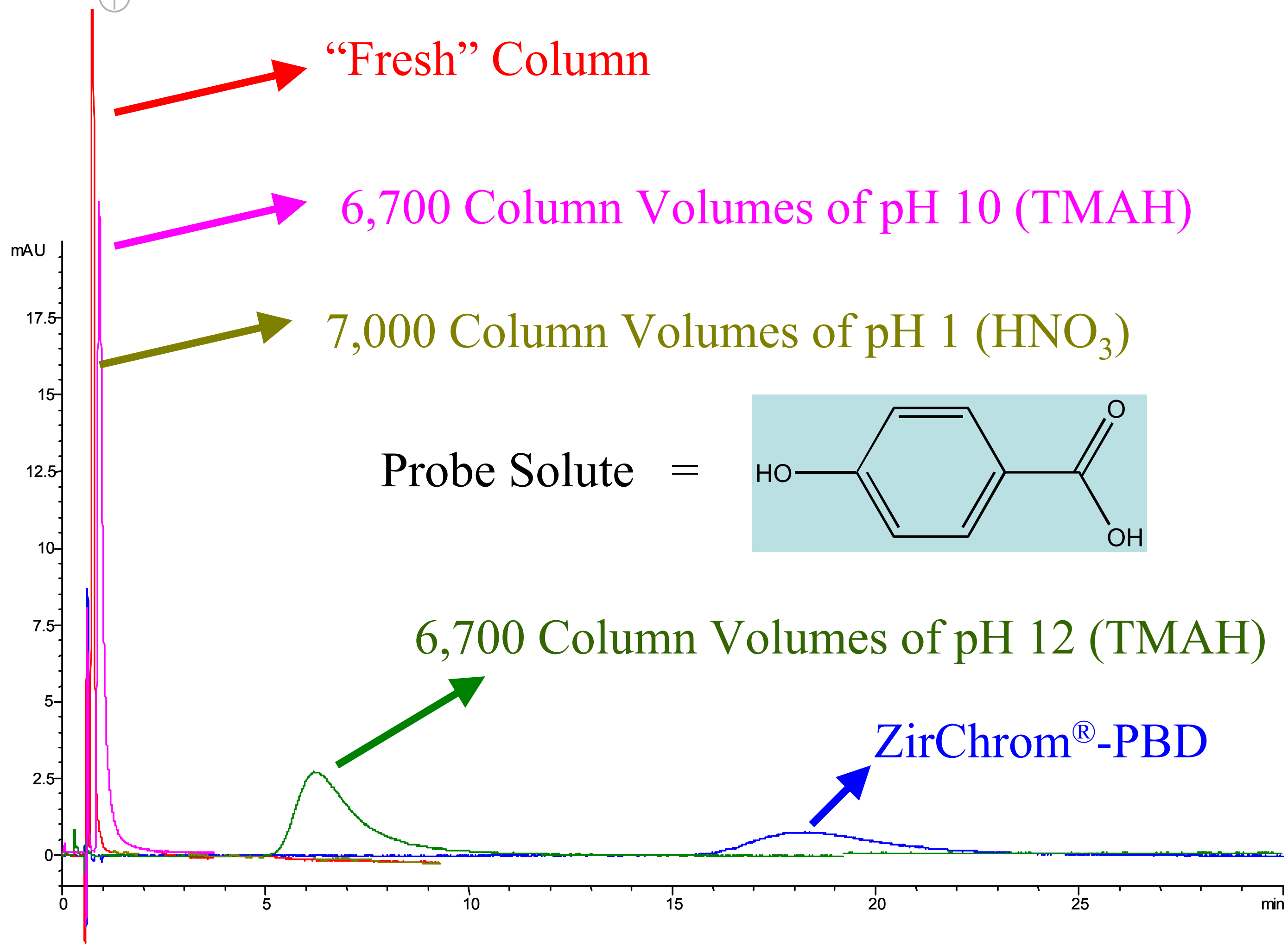
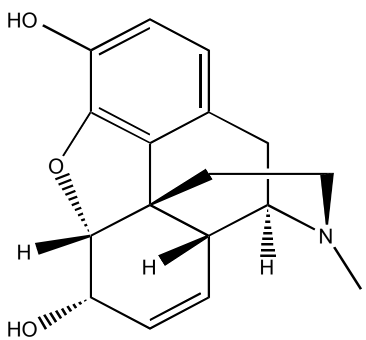
ZirChrom® EZ Stability Testing

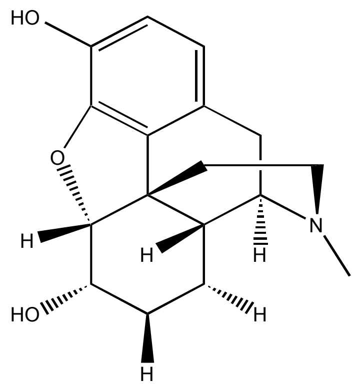


Flushing Conditions: Mobile phase, 15/85 ACN/indicated buffer; Temperature, 30 °C; **Evaluation Conditions**: Mobile phase, 15/85 ACN/20 mM ammonium acetate, pH 4.0; Temperature, 30 °C. Injection volume, 5 µl; Detection at 254 nm; Solute: Hydroxybenzoic acid.

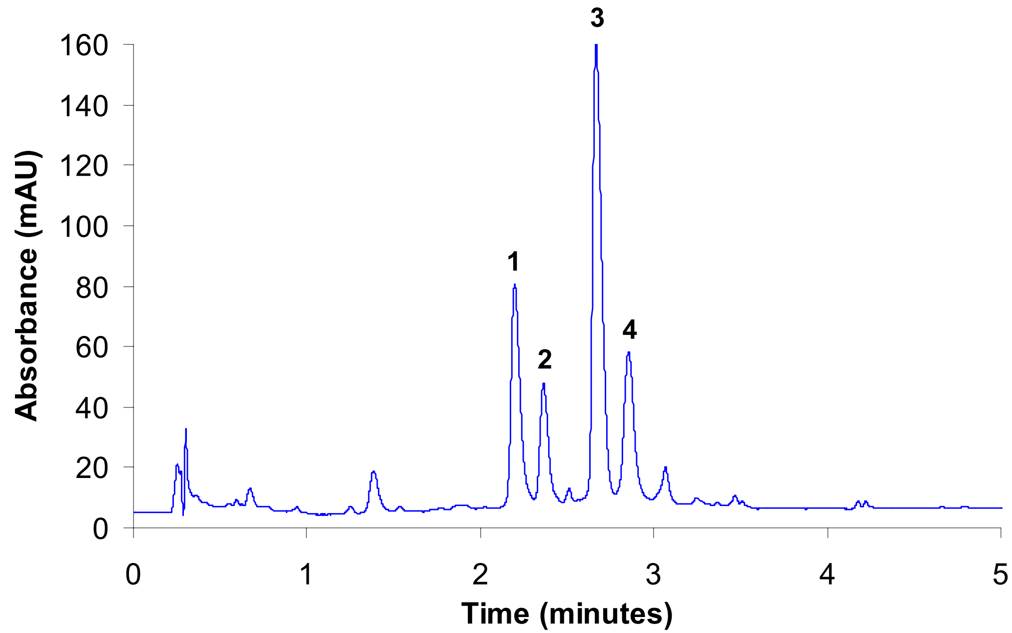
Separation of Opioid Isomers

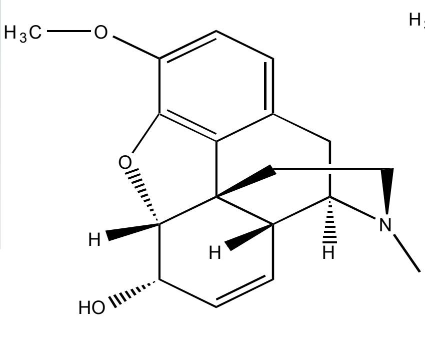


Morphine
M.W. 285.33

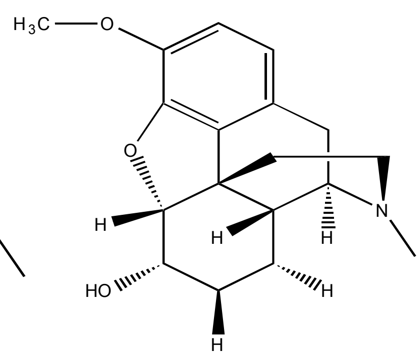


Hydromorphone
M.W. 285.33





Codeine
M.W. 299.36

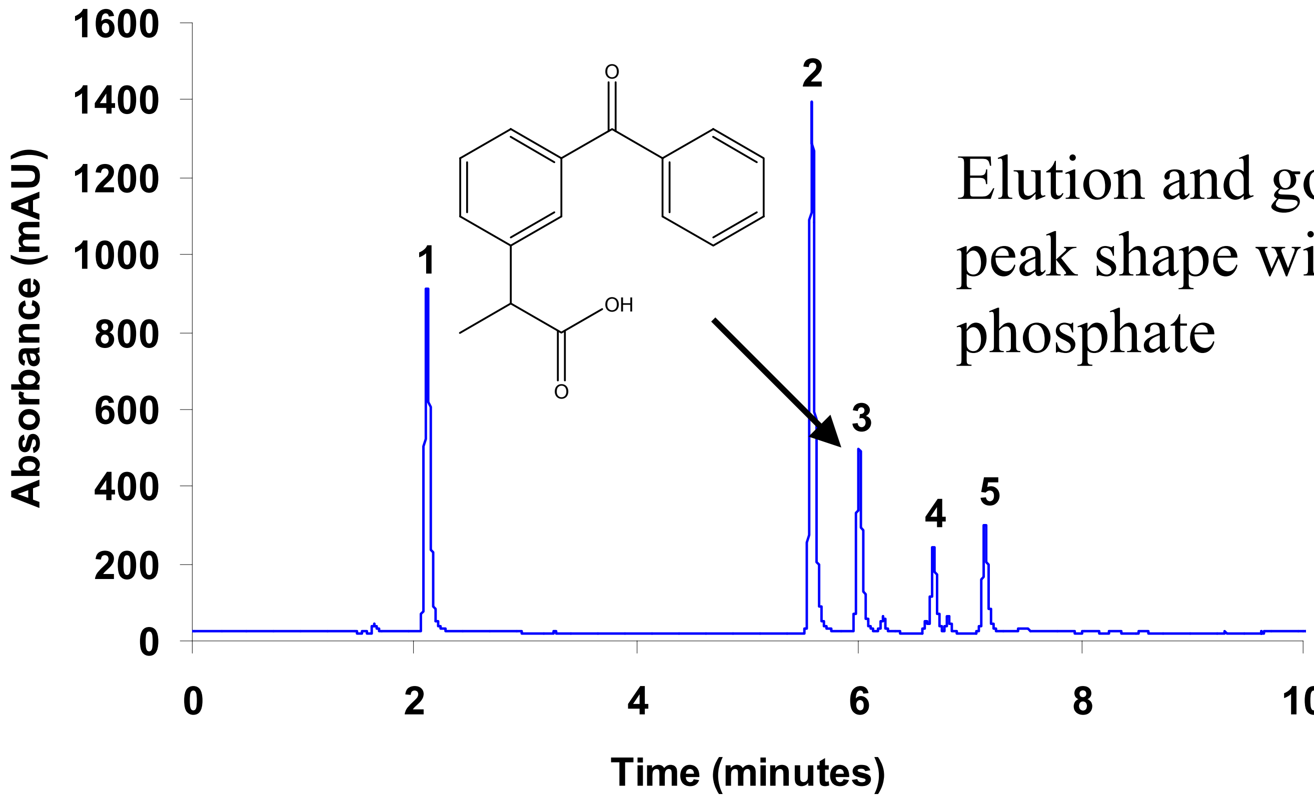


Hydrocodone
M.W. 299.36

Time (min.)	%A	%B
0	90	10
5	10	90

LC Conditions: Column, 50 mm x 4.6 mm i.d. ZirChrom-EZ; Mobile phase, A = 20mM ammonium acetate, pH 6.0, B = ACN; Flow rate, 2.00 ml/min.; Temperature, 35 ^{o}C; Injection volume, 10 µl; Detection at 254 nm.; Solutes: 1=Morphine, 2=Hydromorphone, 3=Codeine, 4=Hydrocodone

Separation of Acidic Pharmaceuticals



Elution and good peak shape without phosphate

Time (min.)	%A	%B
0	90	10
10	10	90

LC Conditions: Column, 150 mm x 4.6 mm i.d. ZirChrom®-EZ; Mobile phase, A = 20mM ammonium acetate, pH 5.0, B = ACN; Flow rate, 1.0 ml/min.; Temperature, 35 ^{o}C; Injection volume, 10 µl; Detection at 254 nm.; Solutes: 1=Acetaminophen, 2=Naproxen, 3=Ketoprofen, 4=Fenoprofen, 5=Indomethacin

Advantages of ZirChrom®-EZ

Advantages over **silica** reversed-phases…

- Stable from pH 1-10, with similar temperature stability (50 °C).
- Increased retention and loading for cationic compounds.
- Very different selectivity, particularly for cationic compounds.

Advantages over other **zirconia** reversed-phases…

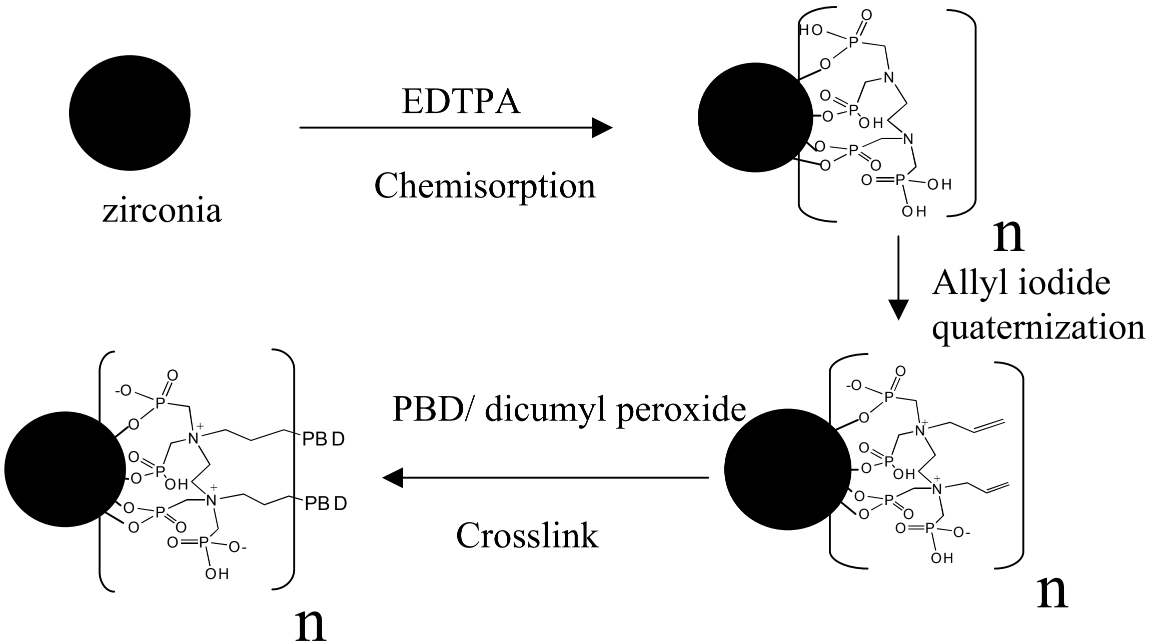
- Does not require inorganic additives (phosphate, fluoride) for elution of Lewis base analytes.
- Increased retention and selectivity for cationic compounds with volatile organic buffers.

Development of a Special Zirconia Phase for LC-MS

- Goals:
  - Internally deactivated like Zirchrom-EZ.
  - Large cation exchange retention and selectivity similar to Zirchrom-EZ.
  - More RP retention, similar to C18 silica phases.

- Strategy:
  - EDTPA treatment of base zirconia followed by application of heavier-than-normal loading of PBD polymer coating.

Synthetic Strategy for 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

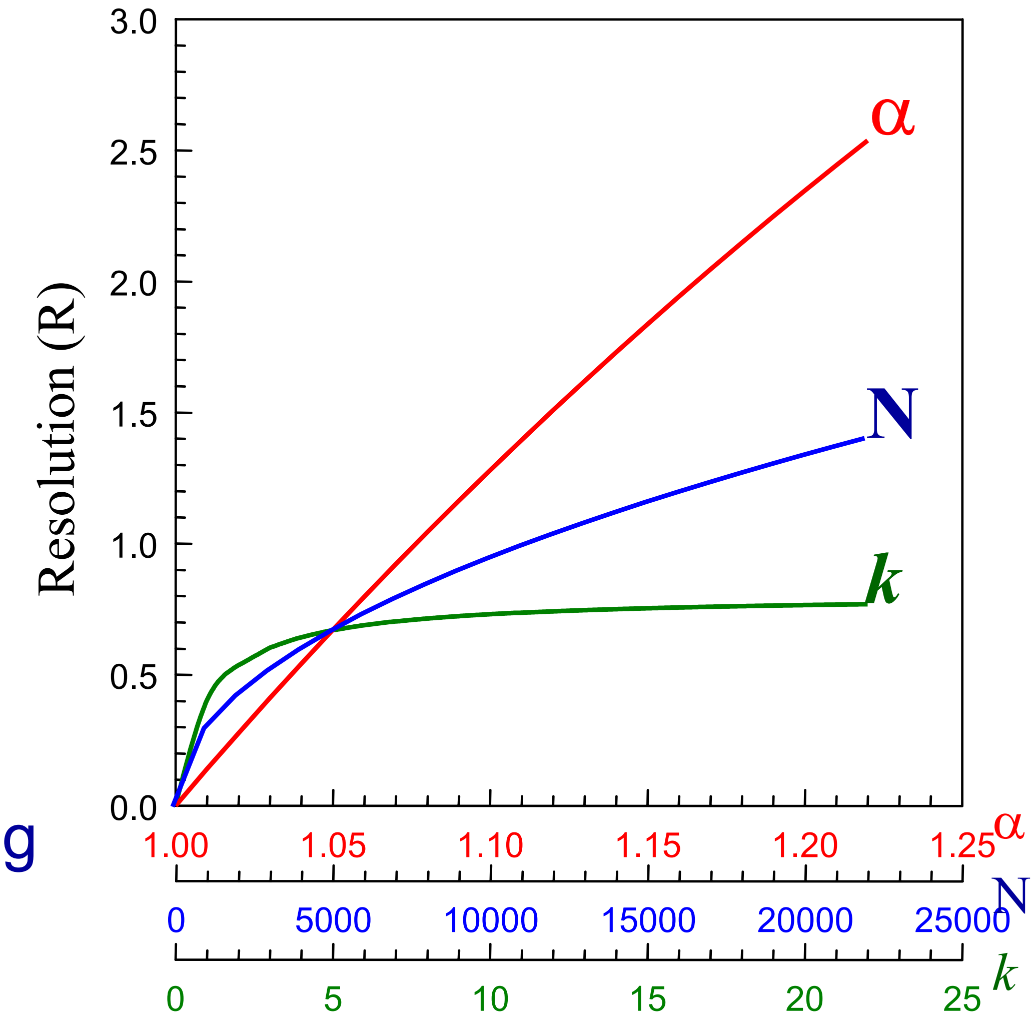
3 Coat polybutadiene (PBD) on the chelator-modified zirconia surface and crosslink PBD with allyl group and PBD itself using dicumyl peroxide as initiator

Selectivity: The Key to Resolution

Efficiency Retention Selectivity

$$R = \frac{\sqrt{N}}{4} \; \frac{k}{k+1} \; \frac{\alpha - 1}{\alpha}$$

$$\alpha = \frac{k_j}{k_i}$$



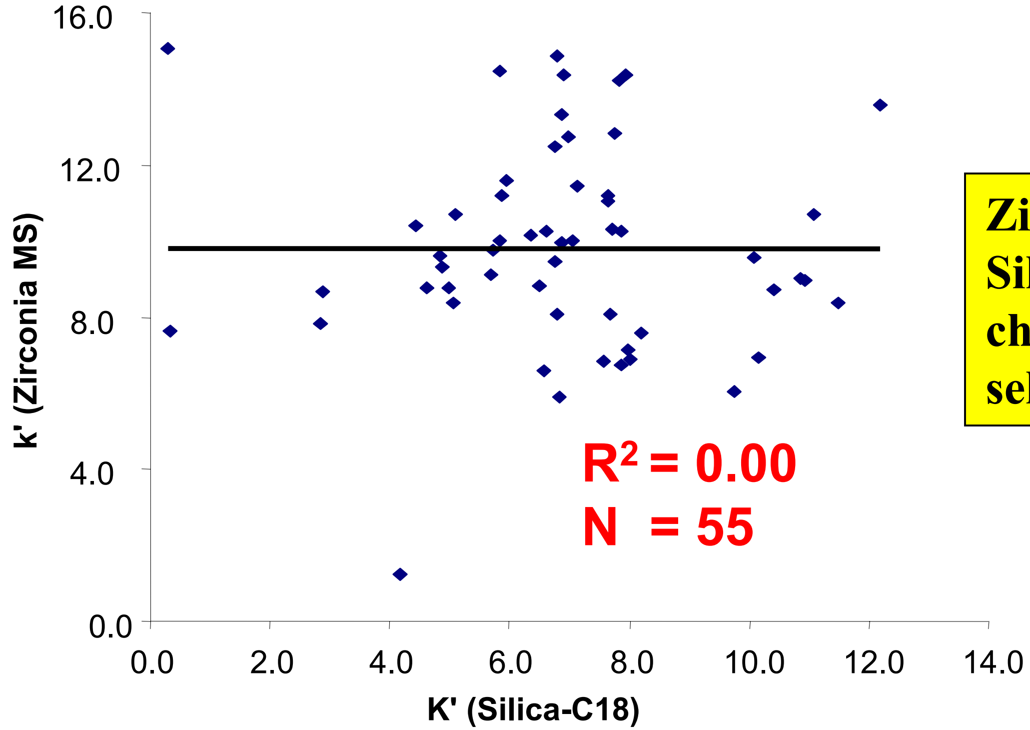
- Selectivity (α) has the greatest impact on improving resolution.

Selectivity Comparison of 55 Pharmaceuticals

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 Zr-MS column.

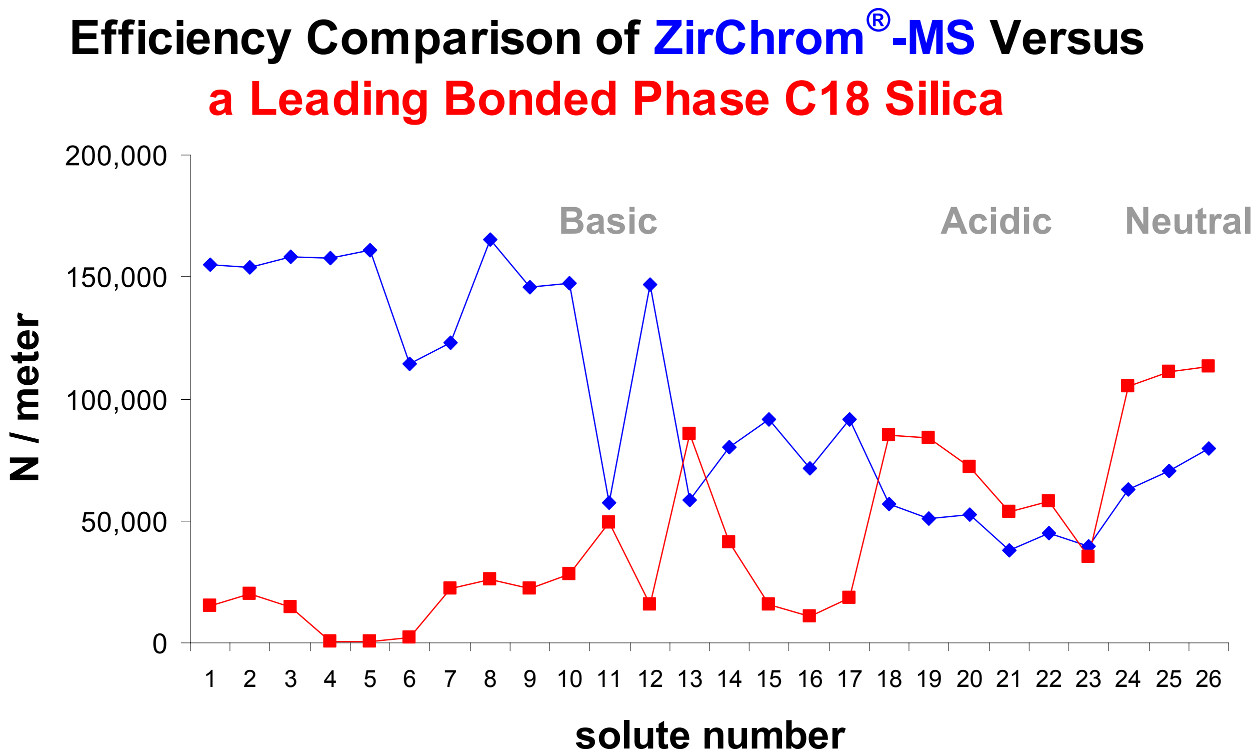
k-k Plot for 55 Ionizable Compounds



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).

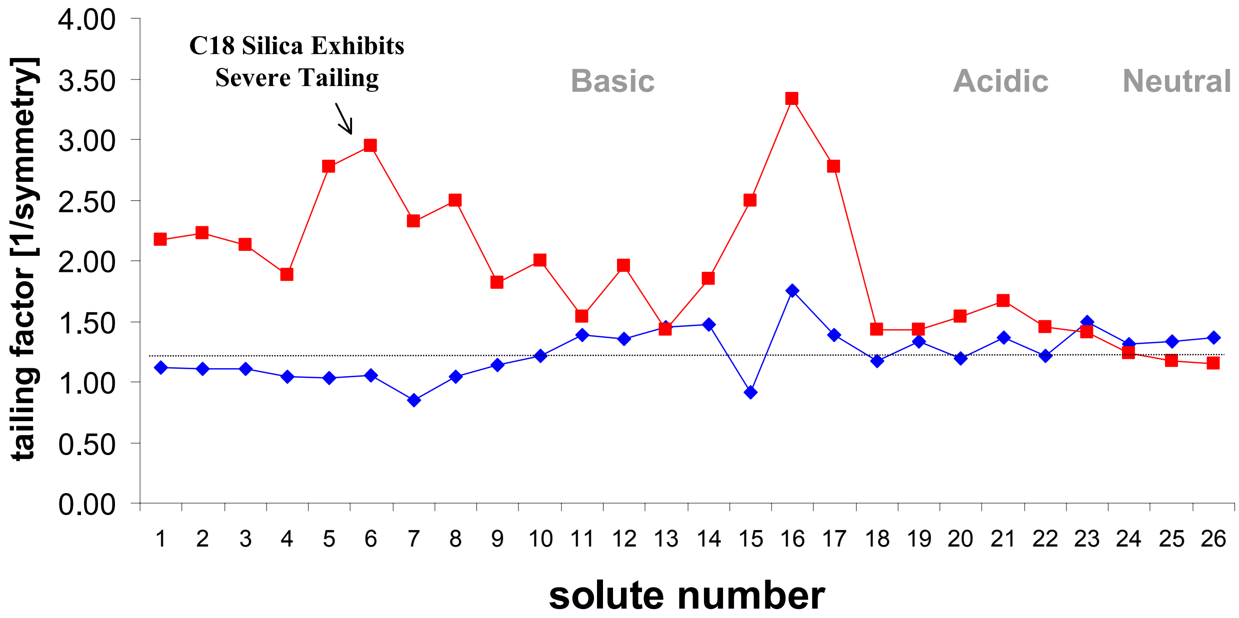
Efficiency Comparison on 26 Solutes



For details, see ZirChrom Technical Bulletin 303.

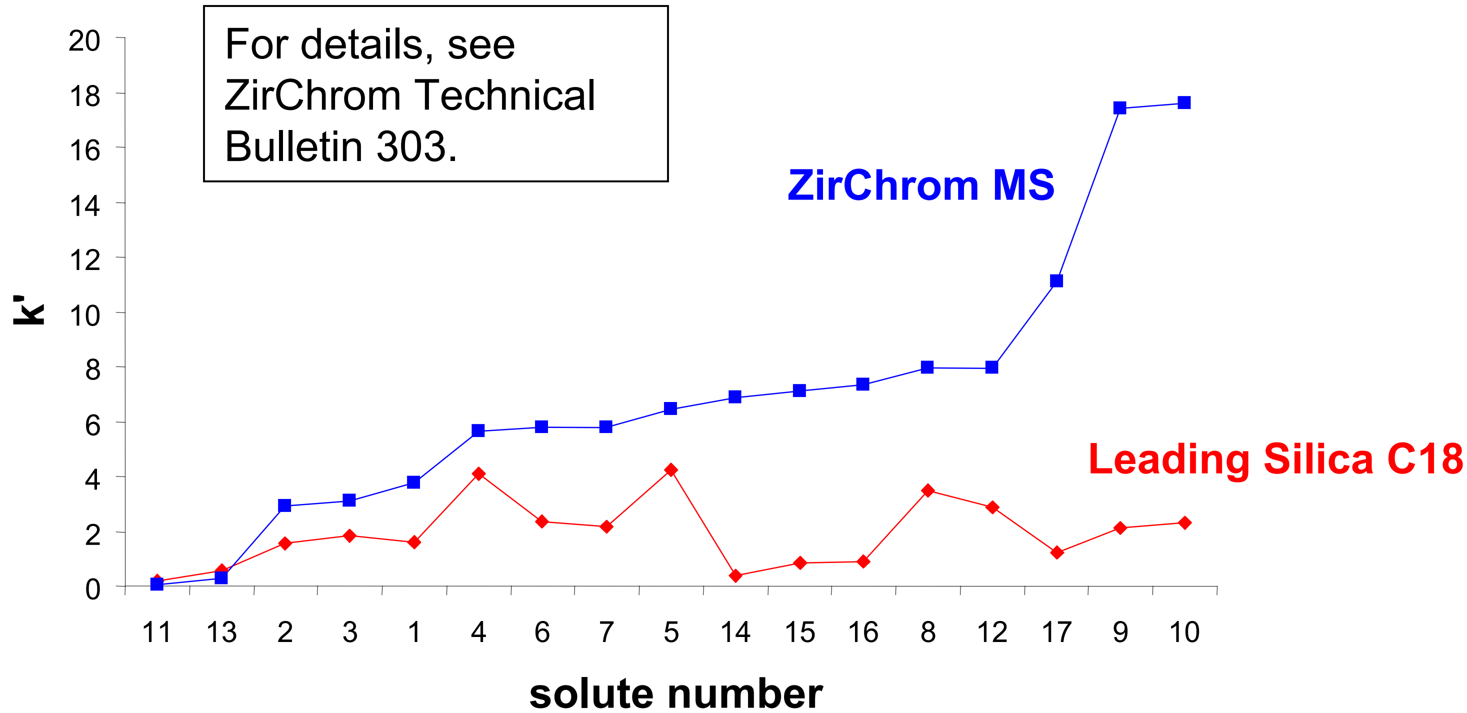
Symmetry Comparison

Symmetry Comparison of ZirChrom®-MS Versus a Leading Bonded Phase C18 Silica



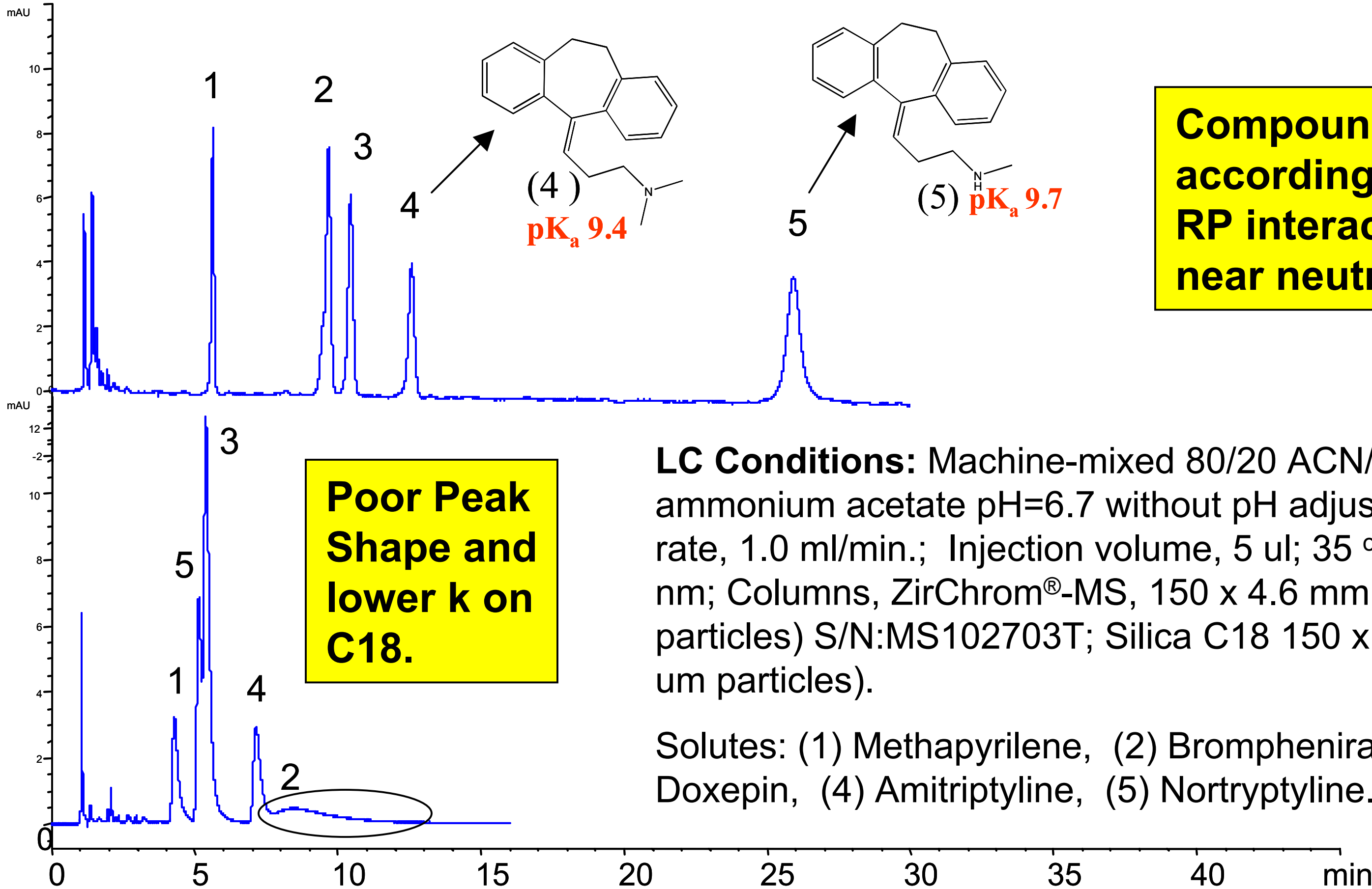
For details, see ZirChrom Technical Bulletin 303.

Retention Comparison for Bases



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 oC; 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).

Separation of Basic Pharmaceuticals



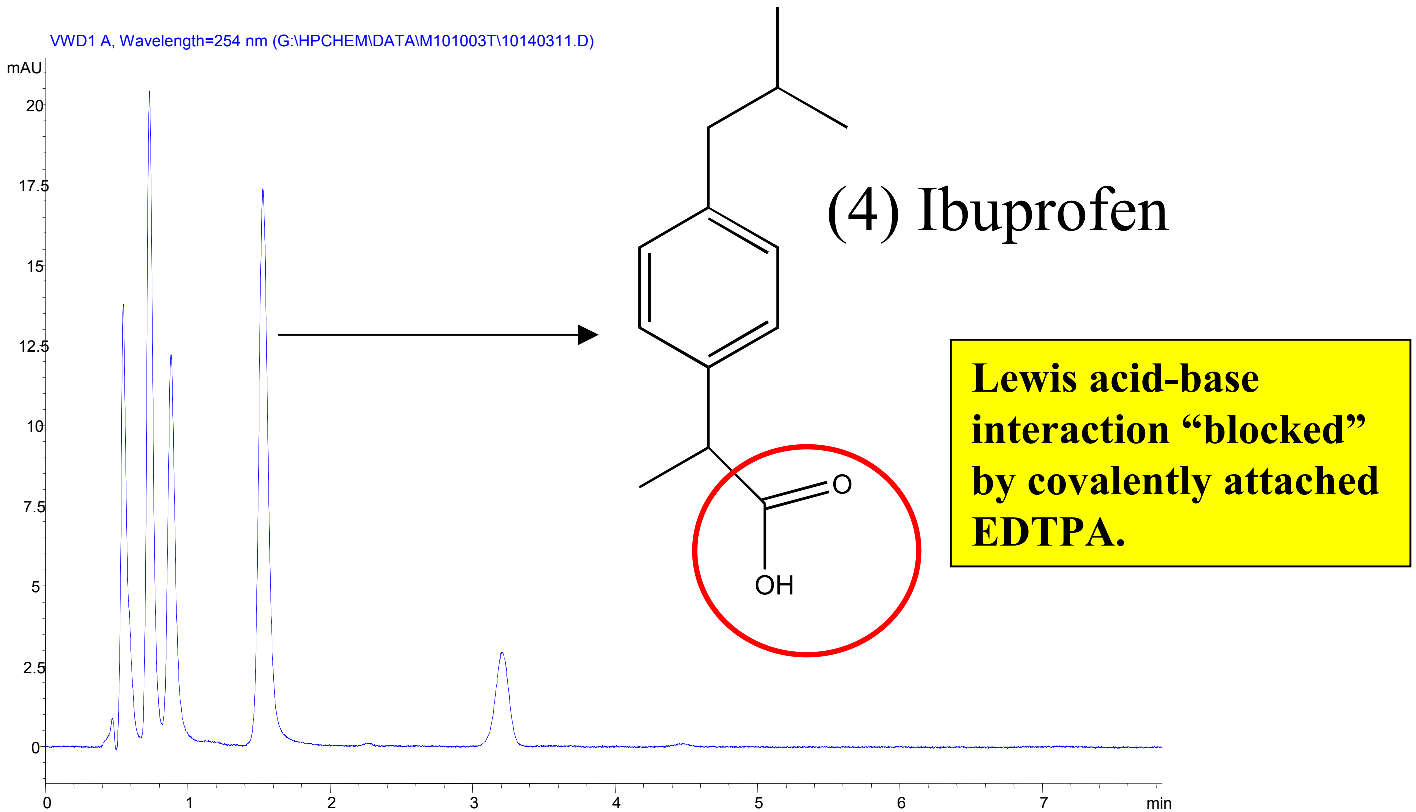
Compounds elute according to IEX, not RP interactions at near neutral pHs.

Poor Peak Shape and lower k on C18.

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, 5 ul; 35 °C; UV @ 254 nm; Columns, ZirChrom®-MS, 150 x 4.6 mm i.d. (3 um particles) S/N:MS102703T; Silica C18 150 x 4.6 mm i.d., (3.5 um particles).

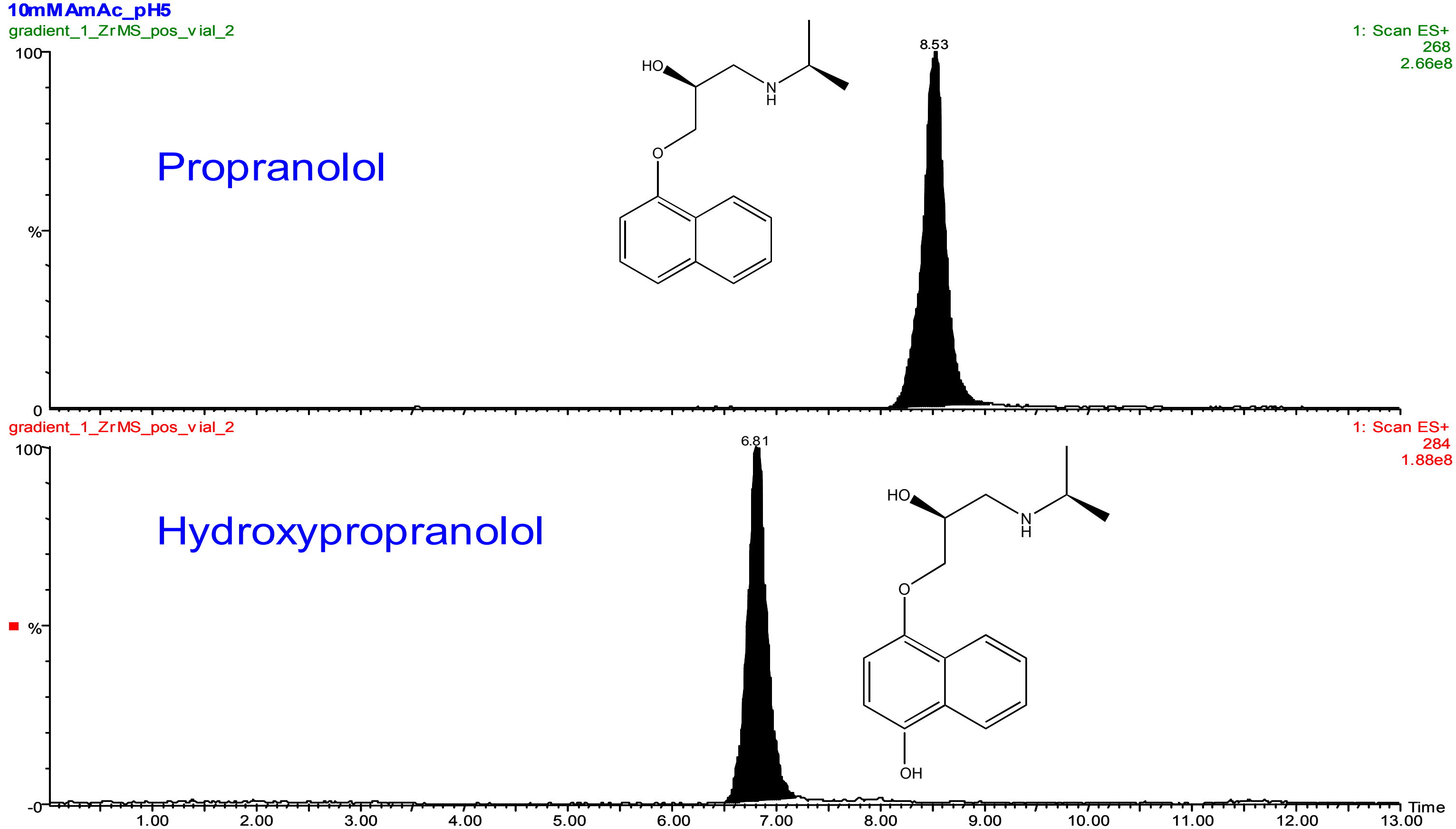
Solutes: (1) Methapyrilene, (2) Brompheniramine, (3) Doxepin, (4) Amitriptyline, (5) Nortryptyline.

Separation of Acidic Pharmaceuticals



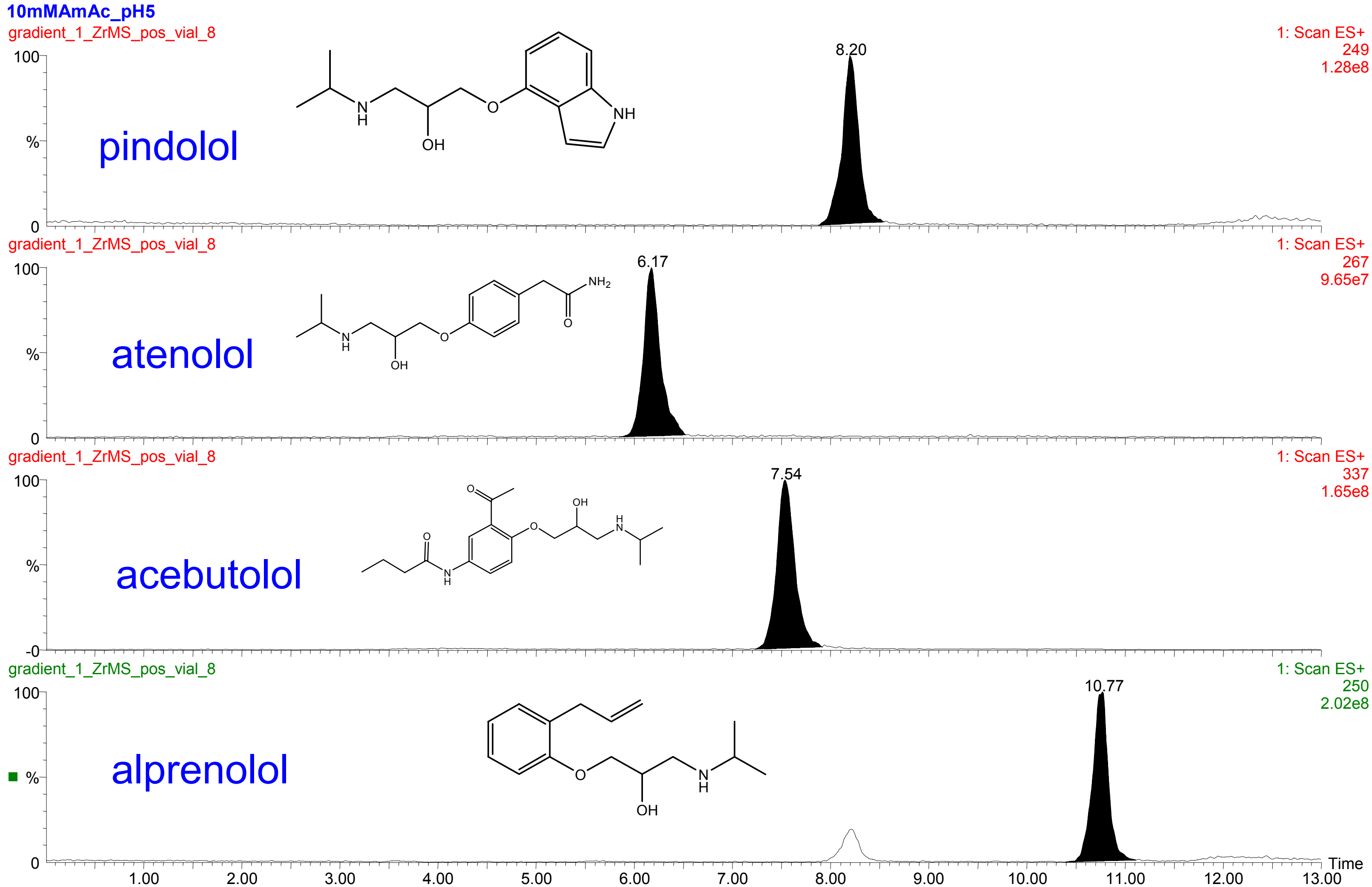
LC Conditions: Column, ZirChrom®-MS, 50 x 4.6 mm i.d. (MS101003T); Mobile phase, Machine-mixed 40/60 ACN/10 mM ammonium acetate pH=5. Flow rate:1 ml/min, Temperature, 35° C; Injection volume: 5 µl; Solutes eluted in order, (1) Acetaminophen, (2) Ketoprofen, (3) Naproxen, (4) Ibuprofen, (5) Impurity; Detection, 254 nm. Pressure drop, 68 bar.

LC-MS of Basic Pharmaceuticals

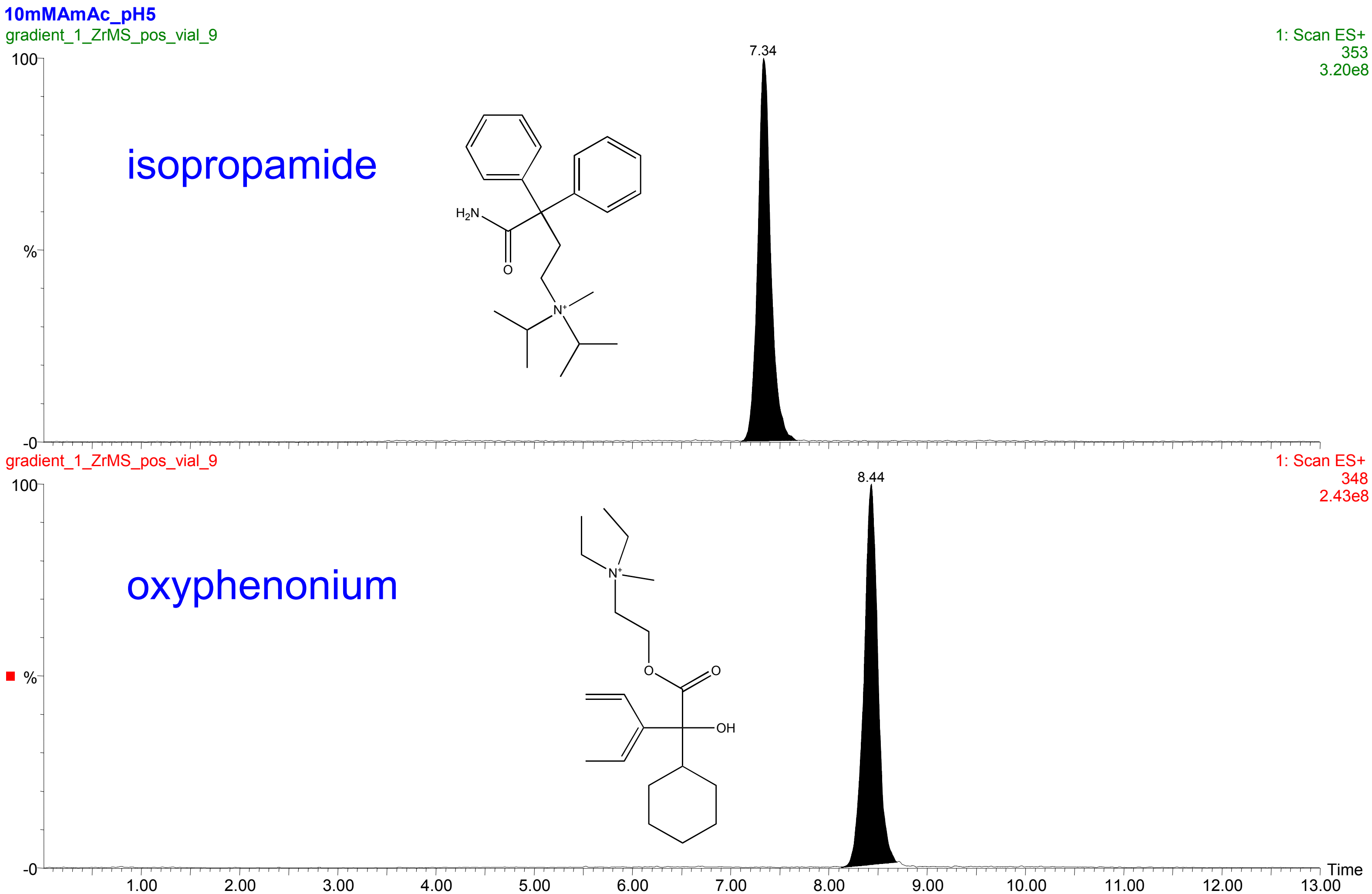


LC Conditions: Column, ZirChrom®-MS, 5 x 2.1 mm i.d. (3 micron particles). Waters Alliance 2795 LC, Flow rate, 0.2mL/min, Mobile phases channel C=10mM ammonium acetate at pH 5, channel D=10mM ammonium acetate at pH 5:acetonitrile (10:90, v/v) , Linear gradient 5% D to 100% D in 6 minutes, hold 100% 6-7.4 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 = 10mg/mL, 2μL injections.

LC-MS of Beta-Blockers



LC-MS of Quaternary Amine Drugs



Conditions that Favor Optimum Retention of Ionizable Analytes

- Hydrophobic or Reversed Phase Mode
  - Smaller organic mole fraction, Φ_{org} = 0 - 0.30
  - Neutral analyte: pH << pK_a (acids); pH >> pK_a (bases)

- Hydrophilic or Normal Phase Mode
  - Larger organic mole fraction, Φ_{org} = 0.70 – 1.0
  - Neutral analyte[1]: pH << pK_a (acids); pH >> pK_a (bases)

- Ionic or Ion Exchange Mode (packing and analyte oppositely charged)
  - Organic mole fraction not very important
  - Lower ionic strength (favors LC-MS solute ionization)
  - Ionic analyte: pH >> pK_a (acids); pH << pK_a (bases)

1. More data on effect of solute ionization on retention under HILIC conditions is needed.

Observations when Ionic Mode Dominates Retention

- Analytes elute approximately in order of pK_a value (often opposite to reversed phase order).
- Elution order can shift slightly with changes in organic mole fraction, but average retention varies only slightly.
- Presence of groups with multiple charges or more than one charged group usually enhances retention.
- Most effective gradient will be ionic strength, not organic mole fraction.

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ZirChrom-EZ Conclusions

- ZirChrom®-EZ phase is a novel zirconia-based RP column ***designed for use with very low or no phosphate***.
  - internally ***Lewis acid site deactivated***.
  - similar selectivity to silica C18 for neutral compounds, but less retention.
  - ***very different selectivity*** than silica C18 for pharmaceuticals that have a cationic form.
  - very similar to Zirchrom-PBD in RP behavior, but has increased cation exchange retention.
  - ***chemically stable*** from pH 1-10 and at 50 °C.

ZirChrom-MS Conclusions

- ZirChrom®-MS is a novel zirconia-based RP column ***designed for use with MS***.
  - internally ***Lewis acid site deactivated***.
  - similar selectivity ***and retention*** behavior to silica-C18 for neutral compounds.
  - ***very different selectivity*** than silica C18 for pharmaceuticals that form cations.
  - ***slightly more cation-exchange behavior and significantly more RP retention*** than Zirchrom-EZ.
  - chemically stable from pH 1-10 and at 50 °C.

Acknowledgements

- The author would like to thank both ZirChrom and Supelco for assistance in generating experimental data and supporting this presentation. LC-MS data was provided by David Bell of Supelco.

- For additional zirconia information, contact the companies at ZirChrom.com or Sial.com. The author may be reached at rhenry@psualum.com.