COMPARISON BETWEEN LEWIS-ACID DEACTIVATED REVERSED-PHASE ZIRCONIA- AND SILICA-BASED STATIONARY PHASE in LC/MS

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Abstract

We report here the development of a novel zirconia-based reversed-phase high performance liquid chromatographic (HPLC) stationary phase achieved by covalently crosslinking a metal chelator into a polybutadiene coated zirconia in order to make a thermally and chemically stable Lewis acid "deactivated" reversed-phase zirconia stationary phase. We also demonstrated the feasibility of doing separations on this new stationary phase using no special strong Lewis base additives (acetate, fluoride, or phosphate), which are absolutely essential for the separation of low MW hard Lewis bases on other "unprotected" commercial reversed-phase zirconia-based HPLC columns. This new stationary phase is stable over a wide pH and temperature range, namely pH 1 to 10 and at temperatures as high as 80 degrees Celsius. The selectivity for neutral compounds is close to that of bonded phase C-18 silica, while the basic and acidic samples have significantly different chromatographic selectivity as compared by retention data via κ–κ plots. Elution data for a number of probe solutes, including many pharmaceuticals, under volatile LC/MS-compatible conditions is also shown.

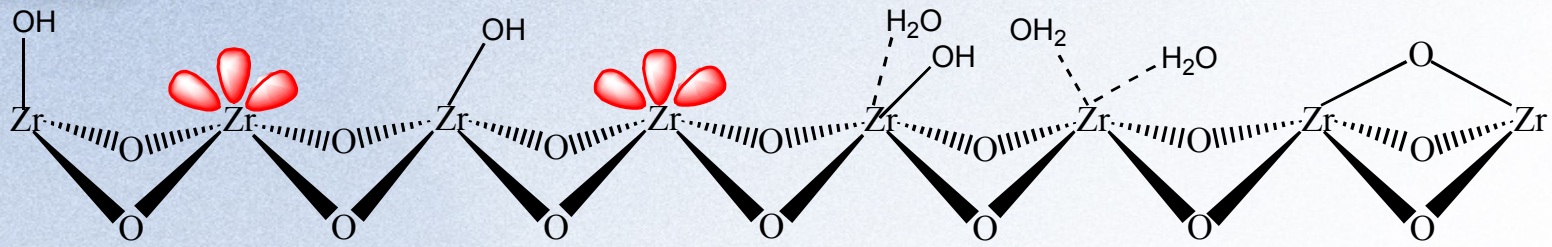
Comparison of Element Electronic Structures for Silica, Titania and Zirconia

- Silicon (element 14; 2.3 g/cc; Si^{4+} ionic radius 0.400 Å)
  - $Ne3s^23p^2$
- Titanium (element 22; 4.5 g/cc; Ti^{4+} ionic radius 0.605 Å)
  - $Ar3d^24s^2$
- Zirconium (element 40; 6.5 g/cc; Zr^{4+} ionic radius 0.720 Å)
  - $Kr4d^25s^2$

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).

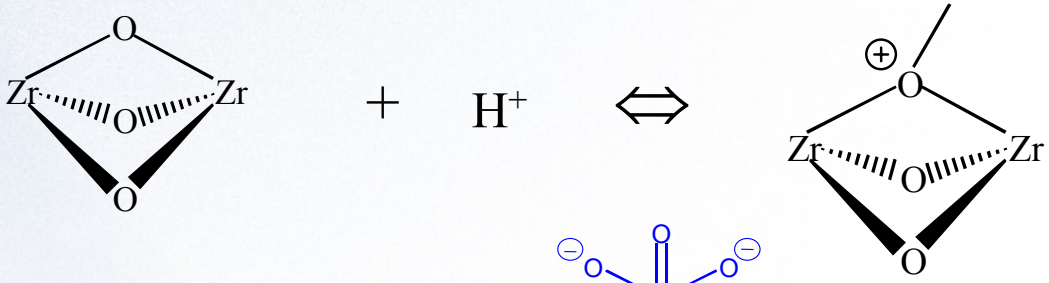
Surface Chemistry of Zirconia-Based Supports for HPLC

ZirChrom®

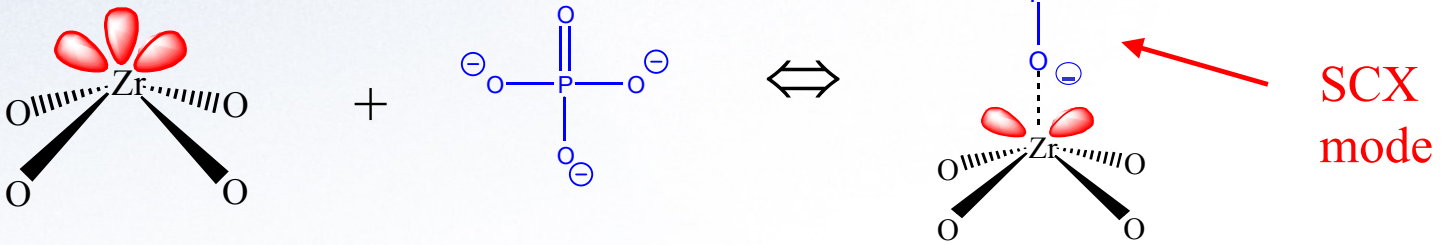


Weak Brönsted Acid: $ZrOH + OH^- \Leftrightarrow ZrO^- + H_2O$

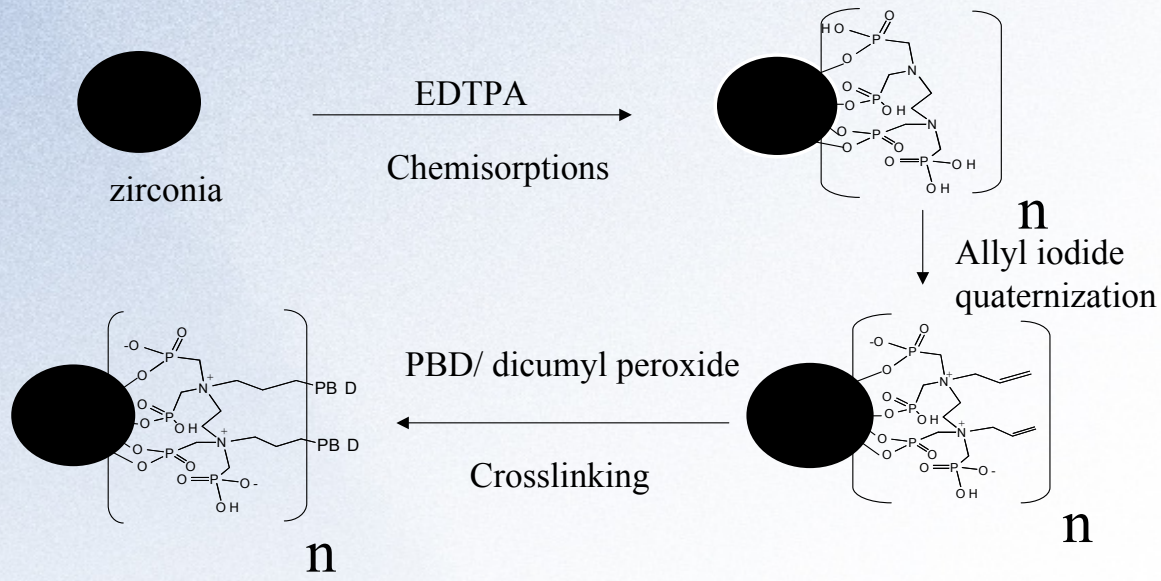
Weak Brönsted Base:



Strong Lewis Acid:



New Stationary Phase Strategy



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.**

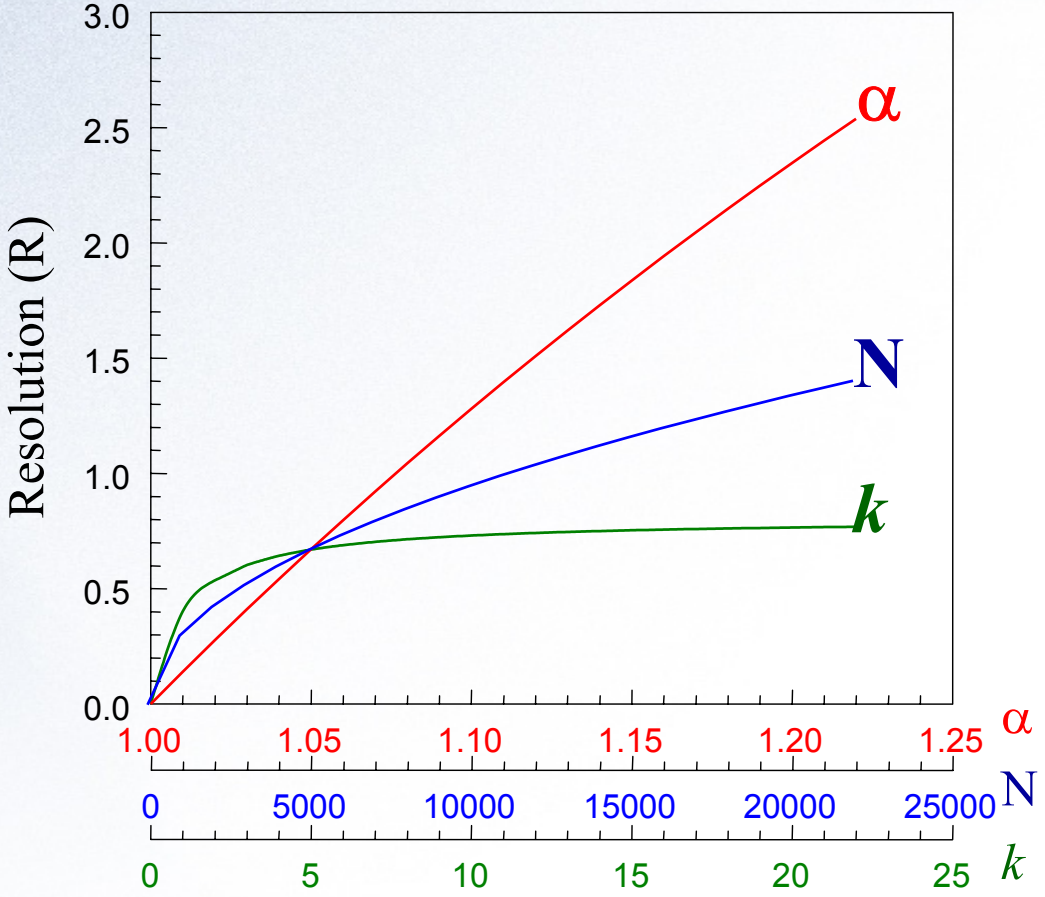
Selectivity: The Key to Resolution

ZirChrom®

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.

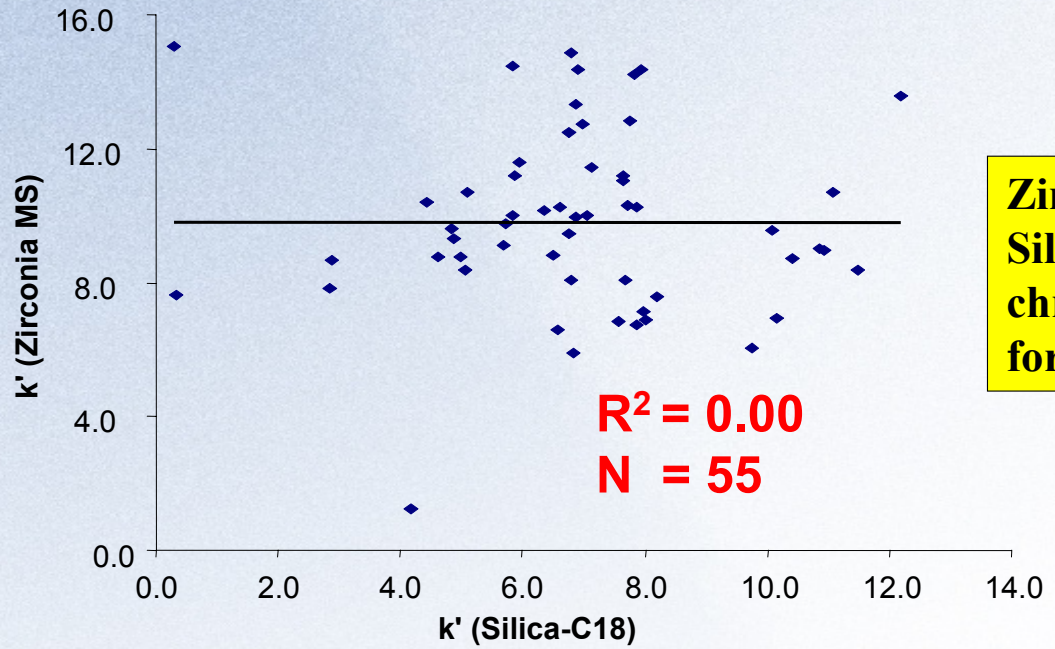


Selectivity Comparison of 55 Pharmaceuticals

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 ZirChrom-MS column.

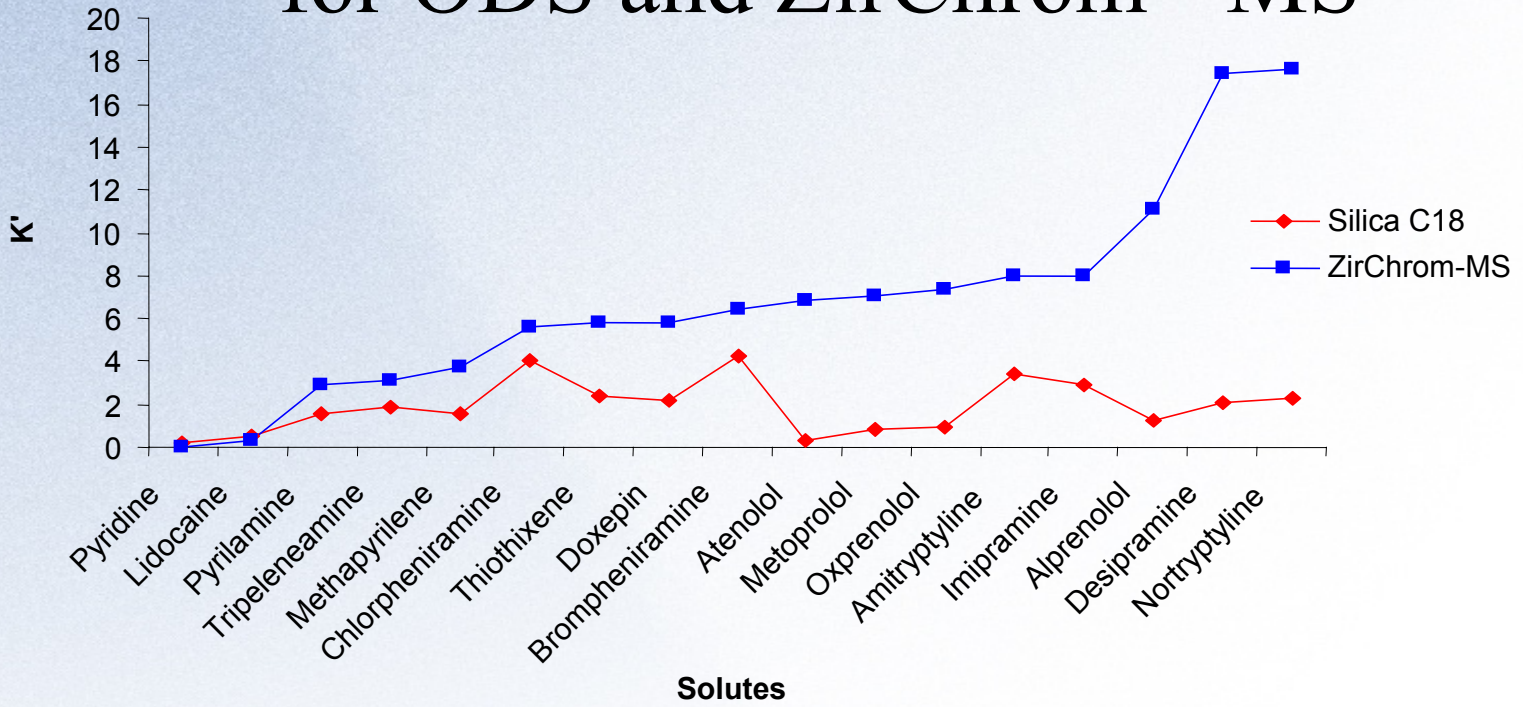
k-k Plot for 55 Ionizable Compounds



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).

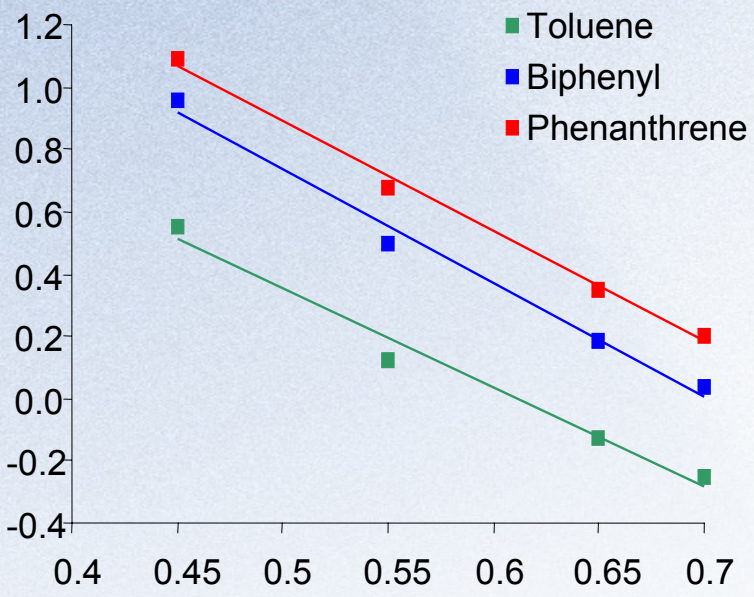
Comparison of Retention of Basic Pharmaceuticals for ODS and ZirChrom®-MS



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 ul; 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).

Reversed-Phase Characteristics

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



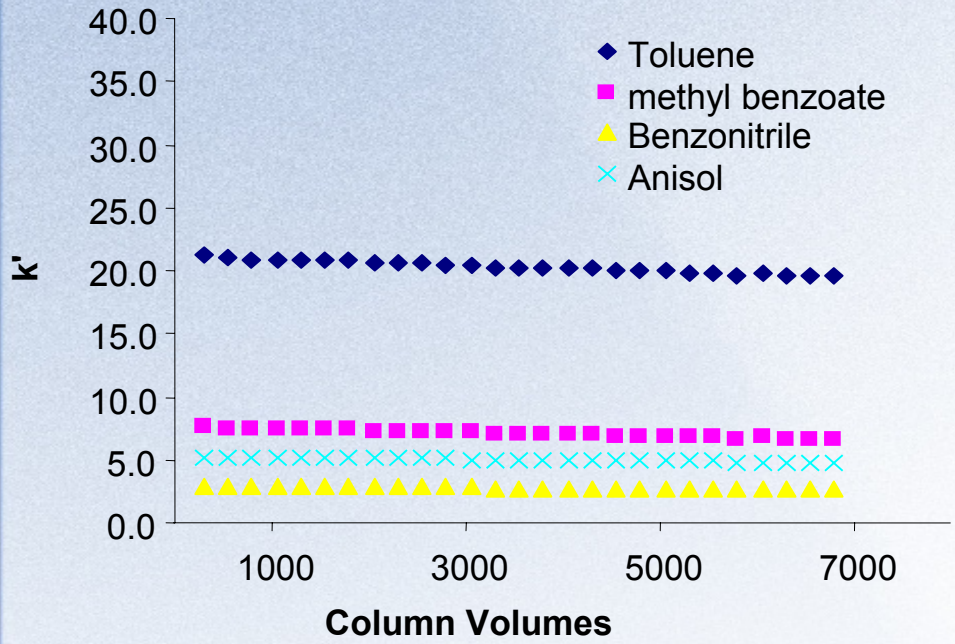
	Toluene	Biphenyl	Phenanthrene
logkw	2.06	2.67	2.75
S*	3.41	3.86	3.71
R^2	0.980	0.990	0.990

* Typical value for S for butylbenzene on silica C18 is 3.4 and intercept of 3.0.
(Jianhong Zhao and Peter W. Carr, Anal Chem. Vol. 71 (1999) 5217-5224.)

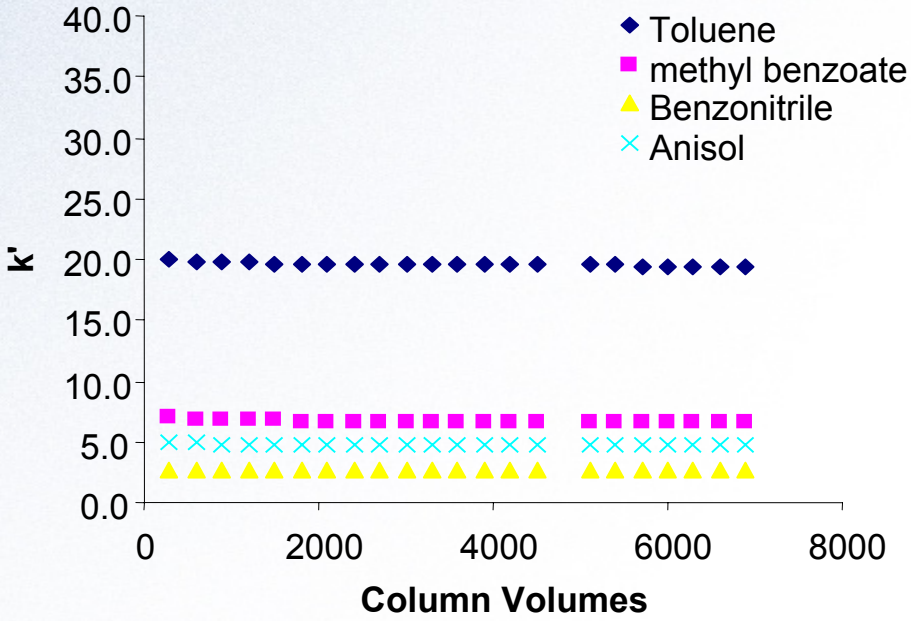
ZirChrom®-MS has very similar RP behavior to Silica C18.

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®-MS.

pH 1 and 10 Stability Testing

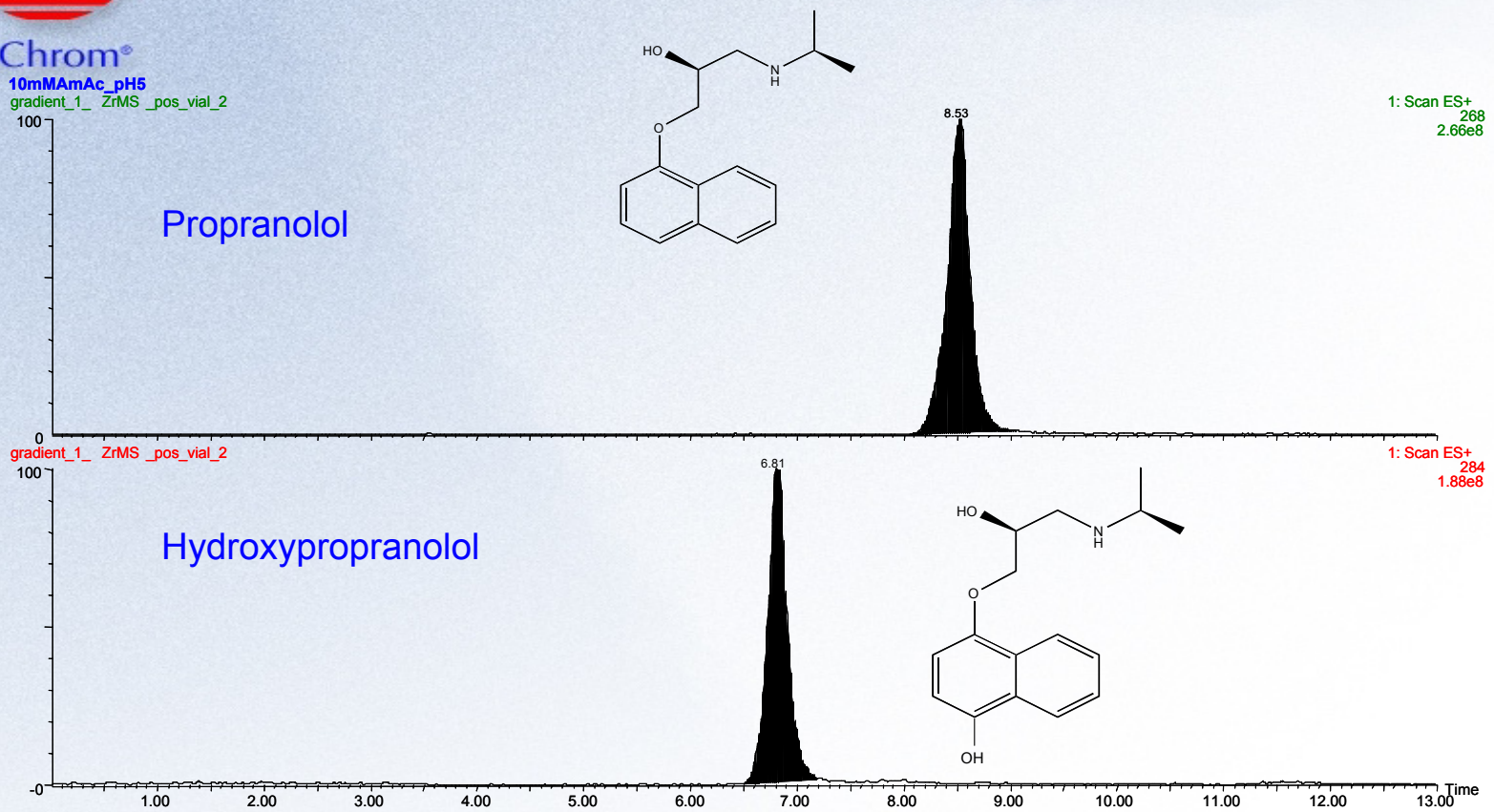


ZirChrom®-MS, S/N: MS0082903X; Mobile phase, 15/85 ACN/pH=1 nitric acid, Temperature: 30 °C; Injection volume: 5 µl; UV, 254 nm.



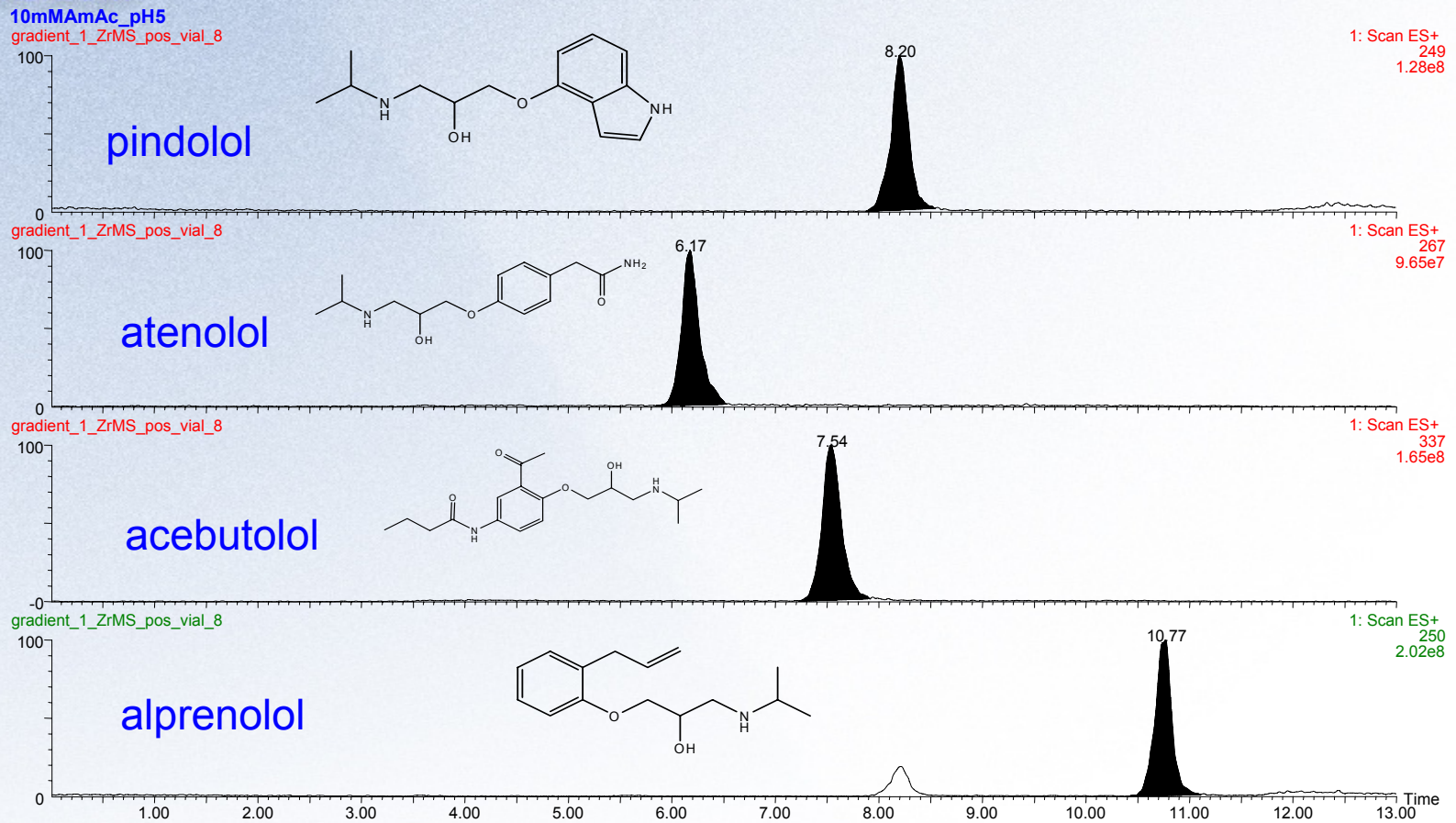
ZirChrom®-MS, S/N: MS0082903X; Mobile phase, 15/85 ACN/pH=10 with tetramethylammonia hydroxide, Temperature: 30 °C; Injection volume: 5 µl; UV, 254 nm.

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 = 10μg/mL, 2μL injections.

HPLC-MS of Beta-Blockers*



*HPLC Conditions are the same as the receding slide.

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)

Conclusions

- The ZirChrom®-MS phase is a novel zirconia-based RP column ***designed for use with MS***.
- The ZirChrom®-MS phase is ***Lewis acid site deactivated***.
- The ZirChrom®-MS phase has ***very different selectivity compared to to silica C18 for pharmaceuticals due to IEX character***.
- ZirChrom®-MS ***is chemically stable*** from pH 1-10.
- ZirChrom®-MS ***has similar reversed-phase behavior*** to silica C18 ***for small neutral organic compounds***.

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