LC/MS APPLICATIONS OF A NEW CHEMICALLY AND THERMALLY STABLE, LEWIS-ACID-DEACTIVATED, REVERSED-PHASE ZIRCONIA STATIONARY PHASE

HPLC 2004

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Outline

- The Goal -To produce a new MS compatible Reversed-Phase Zirconia Stationary Phase that has unique selectivity for basic pharmaceuticals.

- Chromatographic Data
  - ***Selectivity*** Comparison between Silica C18 and the ***new ZirChrom®-MS***
  - ***Stability*** Testing
  - ***Applications and MS testing***

- Conclusion – The new ZirChrom®-MS column is thermally and pH stable over a wide range and has ***very different chromatographic selectivity for basic compounds compared to silica C18***. The column also performs well under MS-compatible conditions.

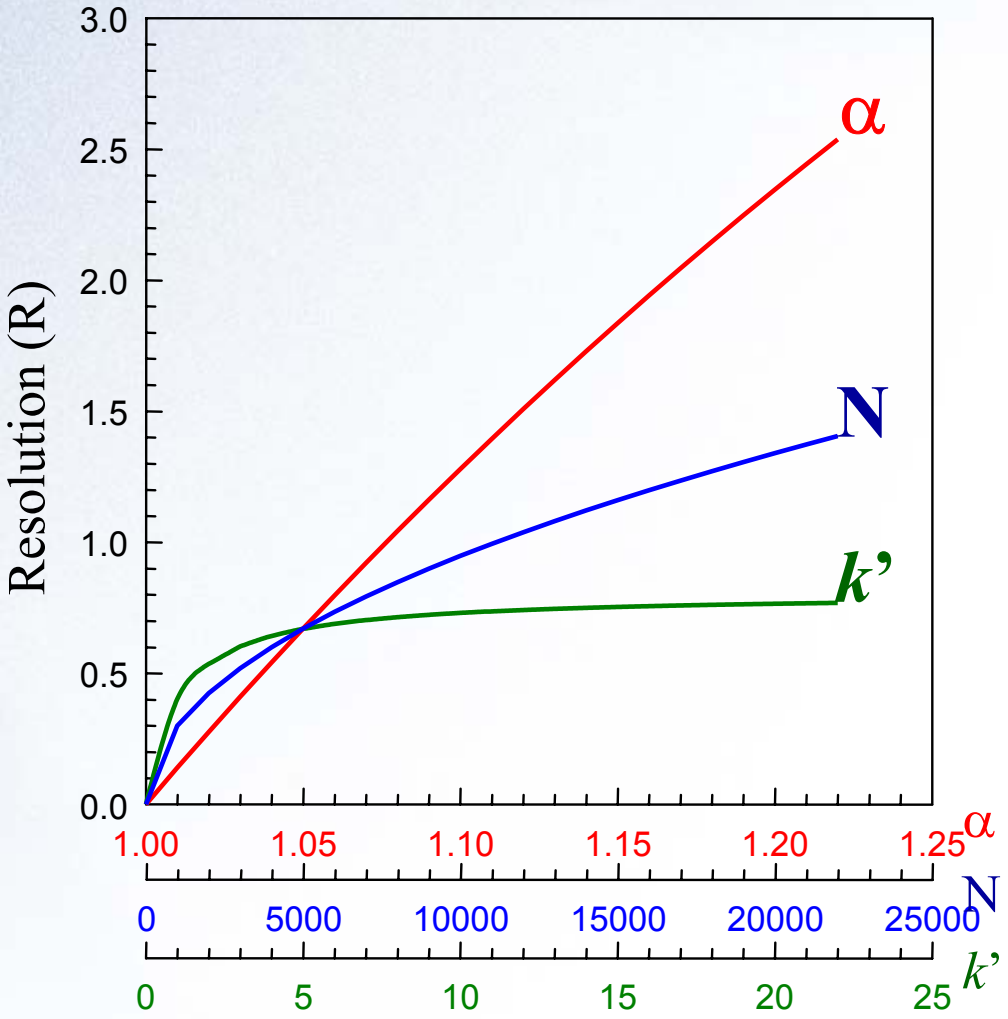
Selectivity: The Key to Success

ZirChrom®

Efficiency ↘ Retention ↓ Selectivity ↙

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

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

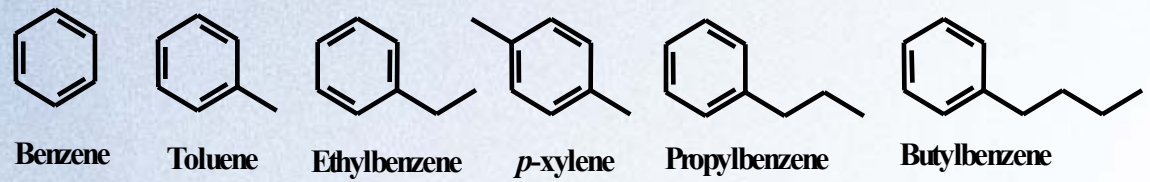


➢ **Selectivity (α) has the greatest impact on improving resolution.**

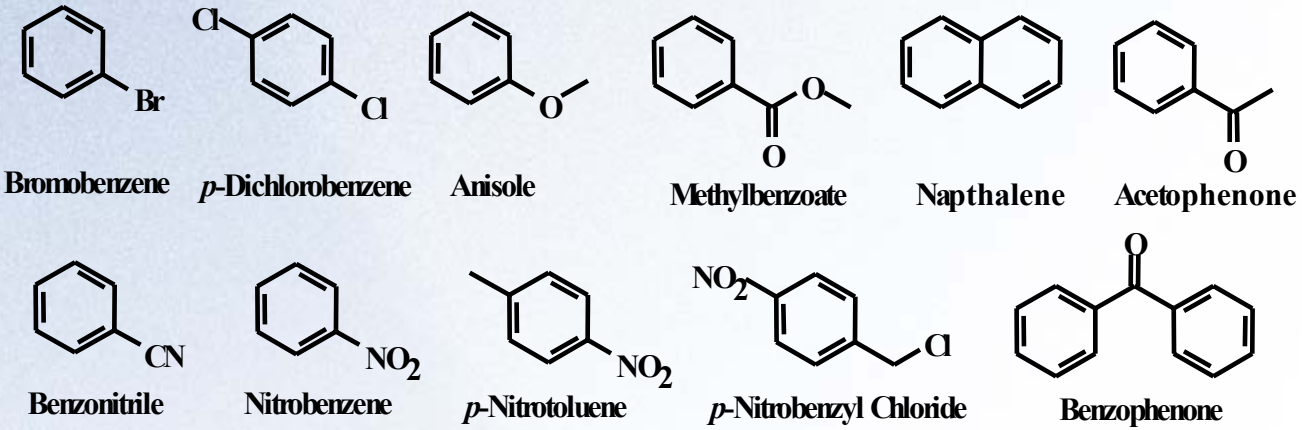
Selectivity Comparison Solutes

ZirChrom®

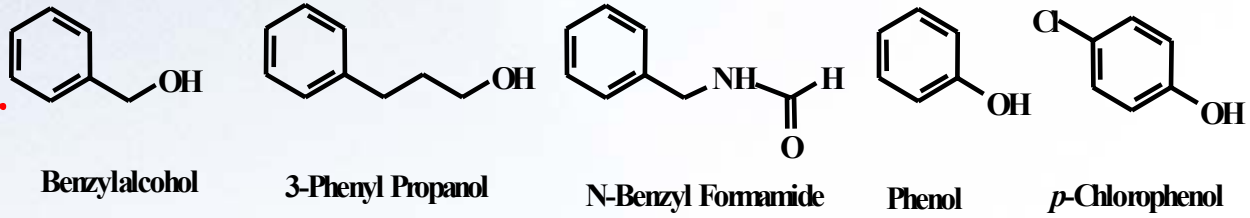
Nonpolar



Polar



HB Donor



Mobile phase, 40/60 Acetonitrile/Water; Flow rate, 1.0 ml/min.;
Temperature, 30 °C; Detection at 254nm; 5µl Injection volume.

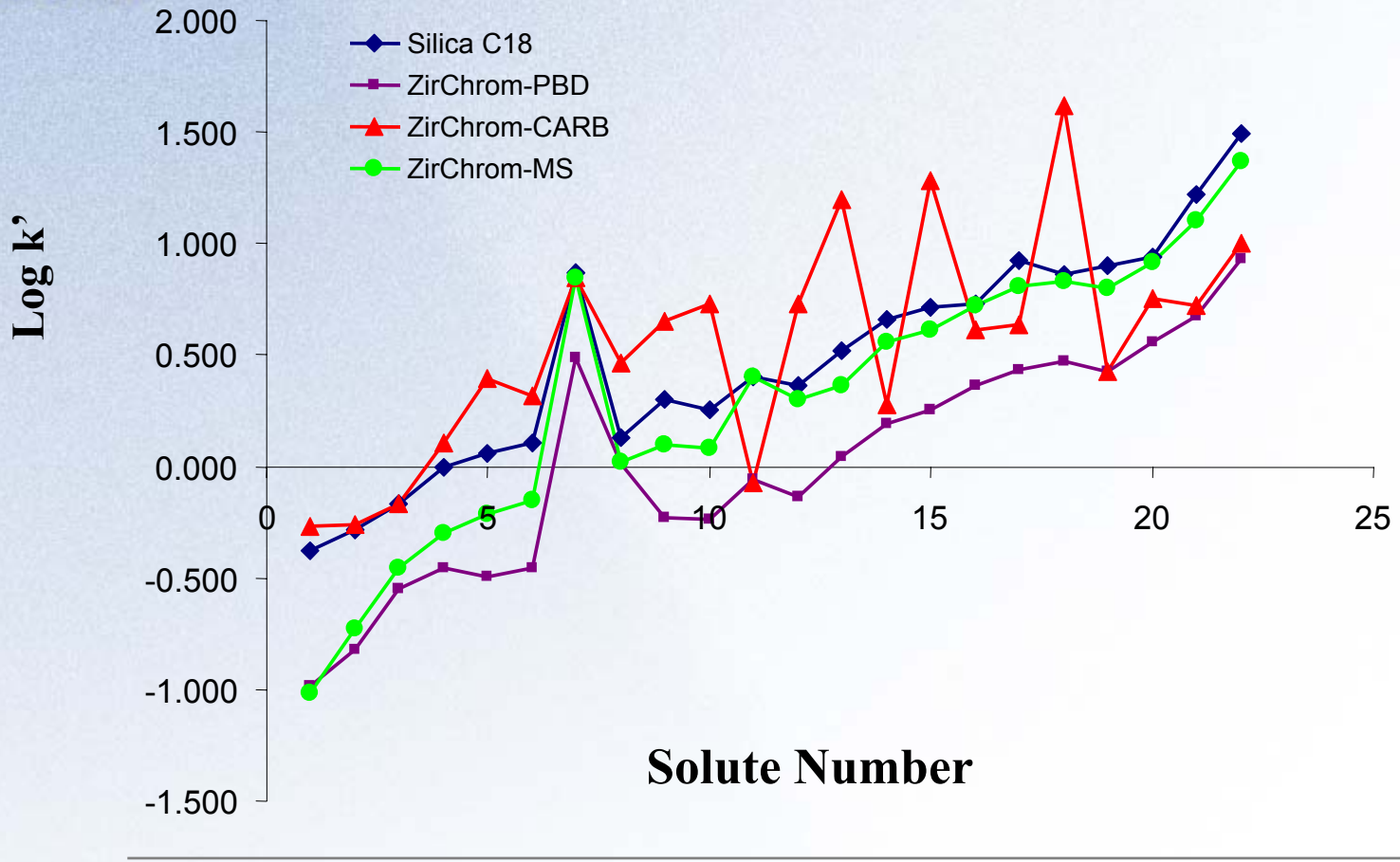
Selectivity Matrix for Nonelectrolytes

Correlation Coefficient	Waters Xterra (RP18)	Luna	PLRP	Gammabond	ZirChrom-PBD	ZirChrom-CARB	DB-C18	Hypercarb	Discovery BIO Wide Pore C18	ZirChrom-EZ	ZirChrom-MS
Waters Xterra (RP18)	1	0.99	0.96	0.98	0.95	0.71	0.94	0.77	0.96	0.96	0.96
Luna		1	0.98	0.99	0.95	0.70	0.94	0.77	0.96	0.96	0.97
PLRP			1	0.98	0.97	0.70	0.95	0.76	0.98	0.98	0.98
Gammabond				1	0.97	0.70	0.95	0.76	0.98	0.98	0.98
ZirChrom-PBD					1	0.69	0.97	0.77	0.98	0.99	0.99
ZirChrom-CARB						1	0.84	0.97	0.68	0.70	0.70
DB-C18							1	0.90	0.95	0.97	0.97
Hypercarb								1	0.76	0.78	0.77
BIO Wide Pore C18									1	0.99	0.99
ZirChrom-EZ										1	0.998
ZirChrom-MS											1

<u>Summary</u>: All CARBON-BASED Columns have different selectivity for nonelectrolytes. All other column retention is very highly correlated.

LC Conditions: Mobile phase, 40/60 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5 µl; Detection at 254 nm.

Comparison of Selectivity for ODS, ZirChrom®-PBD, -CARB and -MS



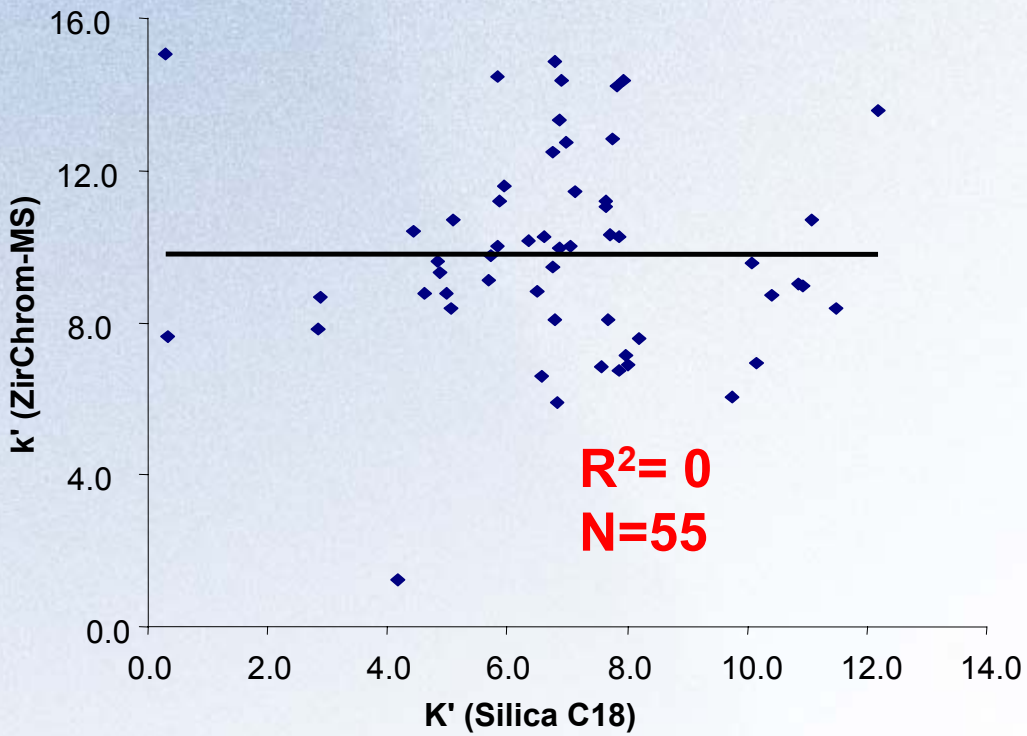
1.) benzyl formamide 2.) benzyl alcohol 3.) phenol 4.) 3-phenyl propanol 5.) p-chlorophenol 6.) acetophenone 7.) benzonitrile 8.) nitrobenzene 9.) methylbenzoate 10.) anisole 11.) benzene 12.) p-chlorotoluene 13.) p-nitrobenzyl chloride 14.) toluene 15.) benzophenone 16.) bromobenzene 17.) naphthalene 18.) ethyl benzene 19.) p-xylene 20.) p-dichlorobenzene 21.) propyl benzene 22.) butyl benzene

Selectivity Comparison of 55 Drugs

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 sequence indicates the elution order on MS column under MS conditions.

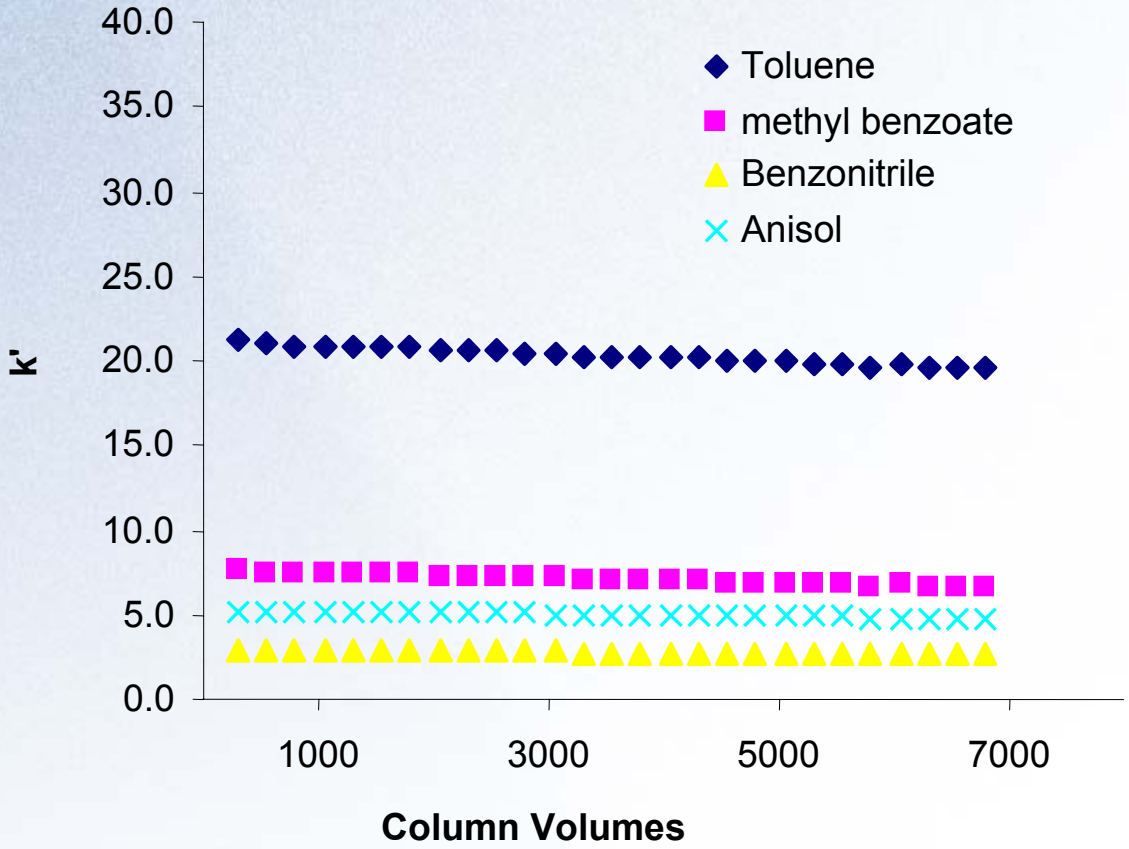
K–K Plot for 55 Pharmaceuticals on ZirChrom®-MS and Silica C18



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

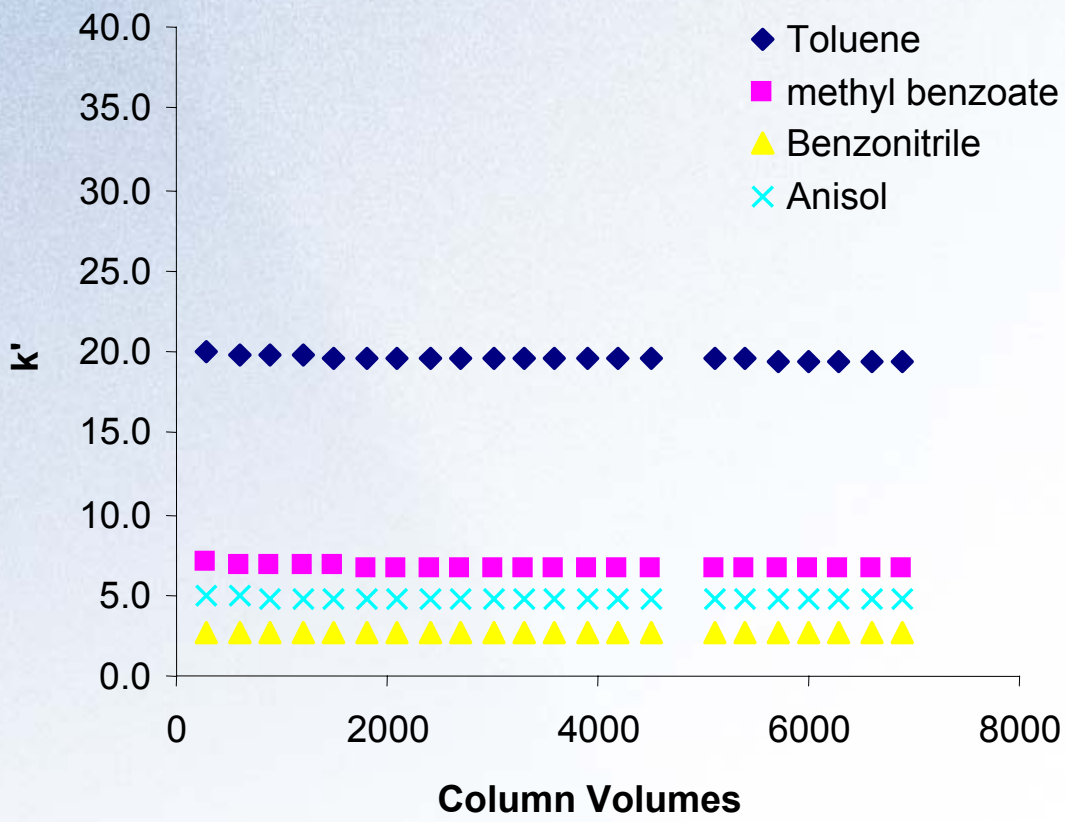
LC Conditions: Columns, 5 x 2.1 mm i.d. ZirChrom®-MS (3 micron particles), and Silica C18 (5 micron particles). Waters Alliance 2795 LC, Flow rate, 0.2mL/min, Mobile phases channel C=10mM ammonium acetate at pH 5, channel D=10mM ammonium acetate at pH 5:acetonitrile (10:90, v/v) , Linear gradient 5% 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.

pH 1 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; Solutes (see figure).

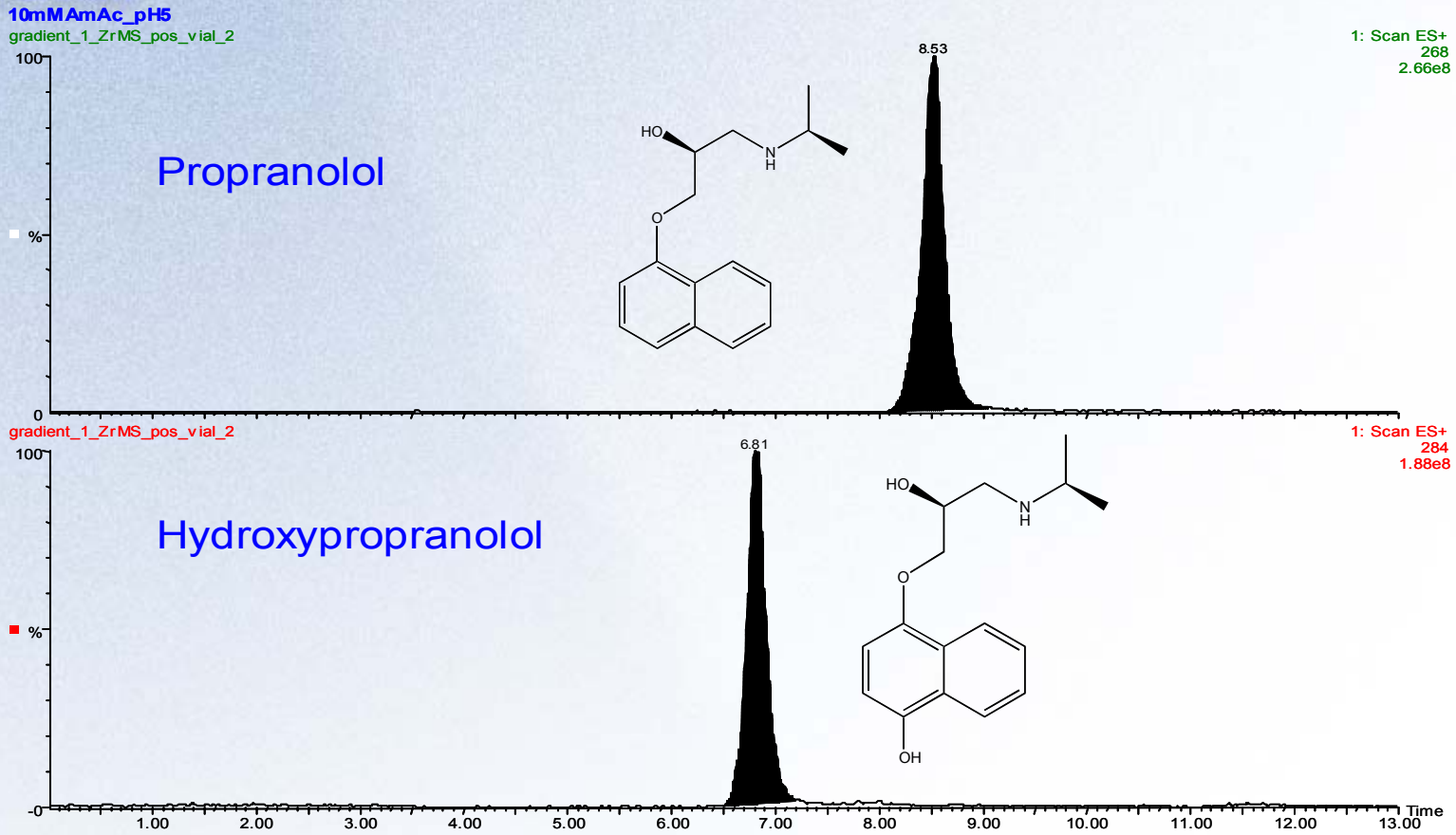
pH 10 Stability Testing



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; Solutes (see figure).

HPLC-MS of Basic Pharmaceuticals

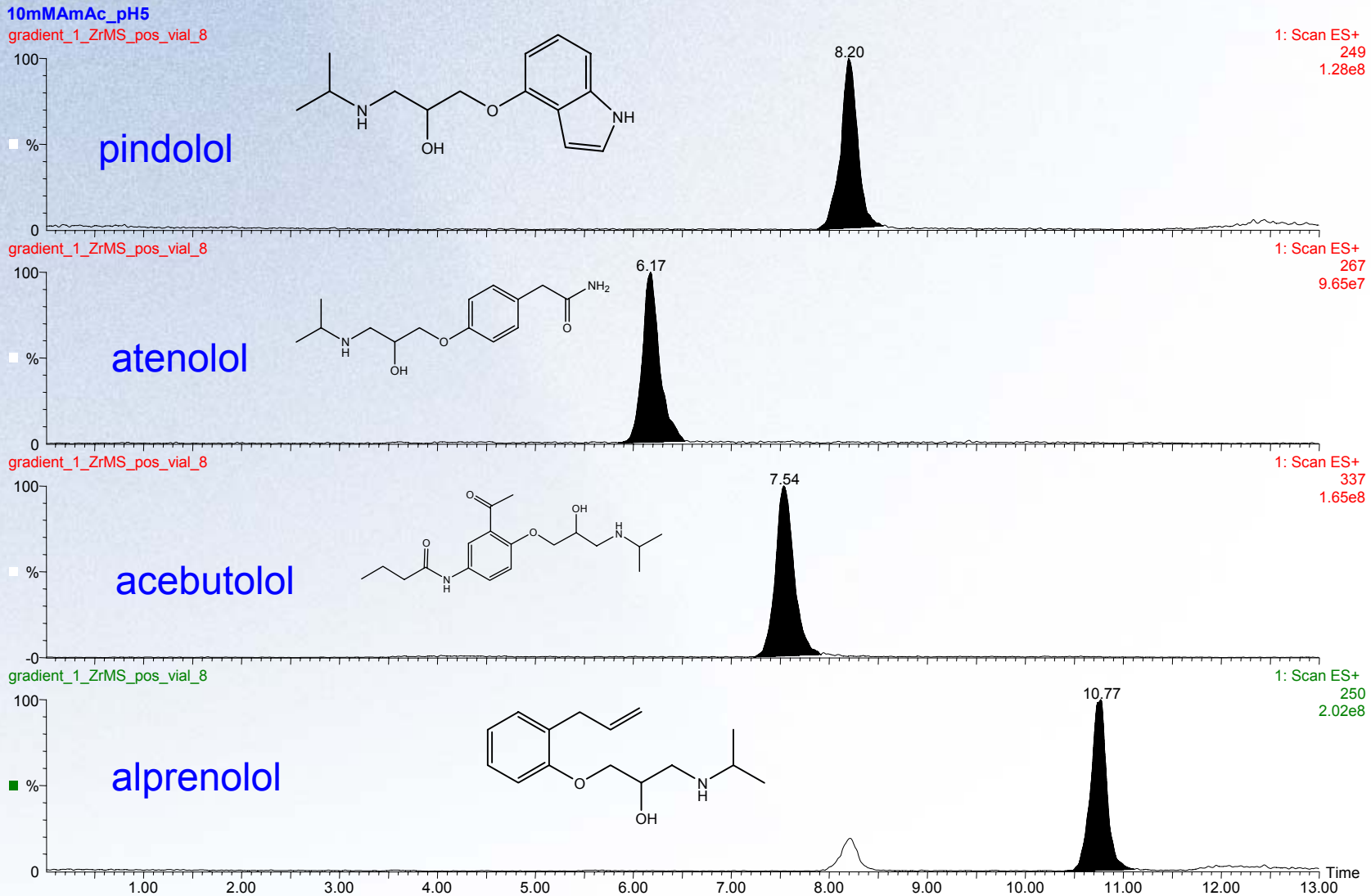
ZirChrom®



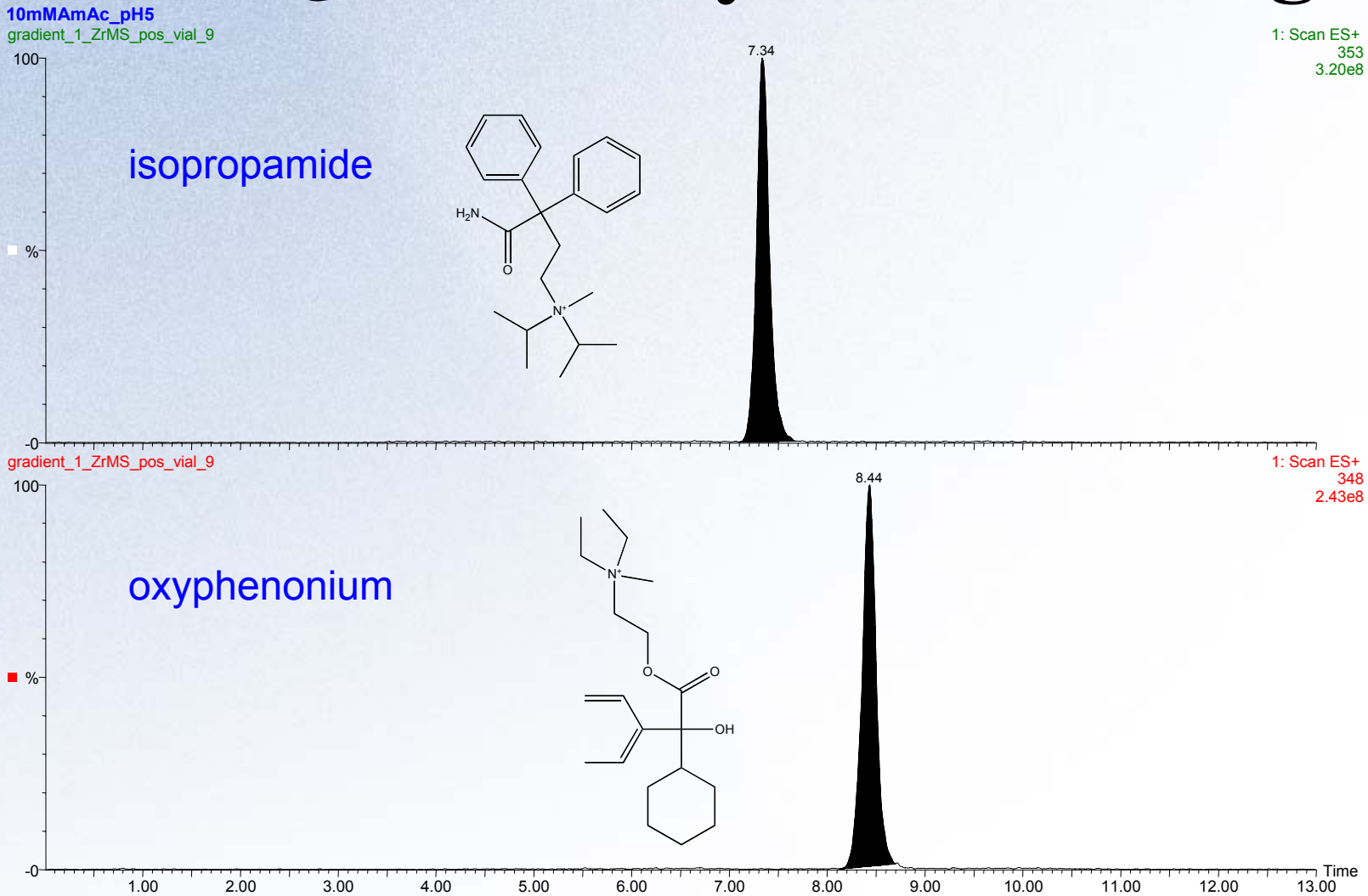
LC Conditions: Column, ZirChrom®-MS, 5 x 2.1 mm i.d. (3 micron particles). Waters Alliance 2795 LC, Flow rate, 0.2mL/min, Mobile phases channel C=10mM ammonium acetate at pH 5, channel D=10mM ammonium acetate at pH 5:acetonitrile (10:90, v/v) , Linear gradient 5% D to 100% D in 6 minutes, hold 100% 6-7.4 min, 100 to 5% D 7.4-8.1min, hold 5% D 8.1-13.0 min. Temperature, 35ºC. 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

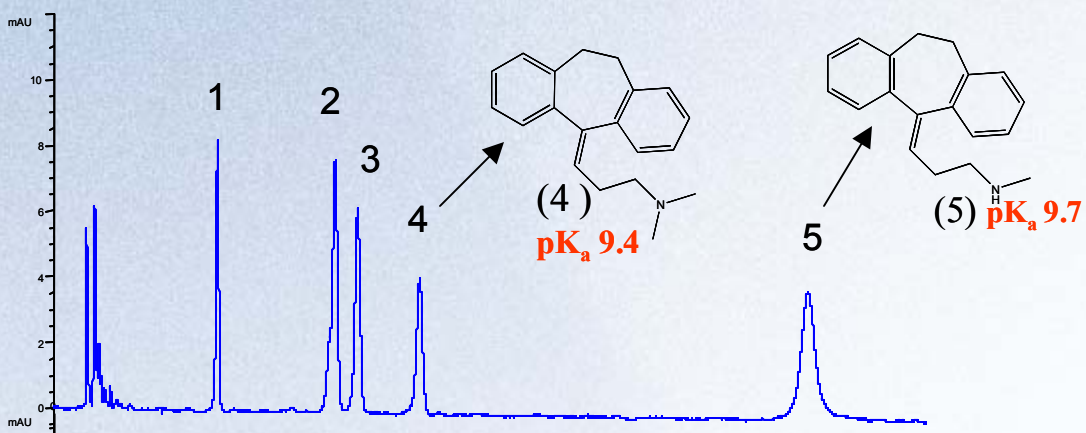
ZirChrom®



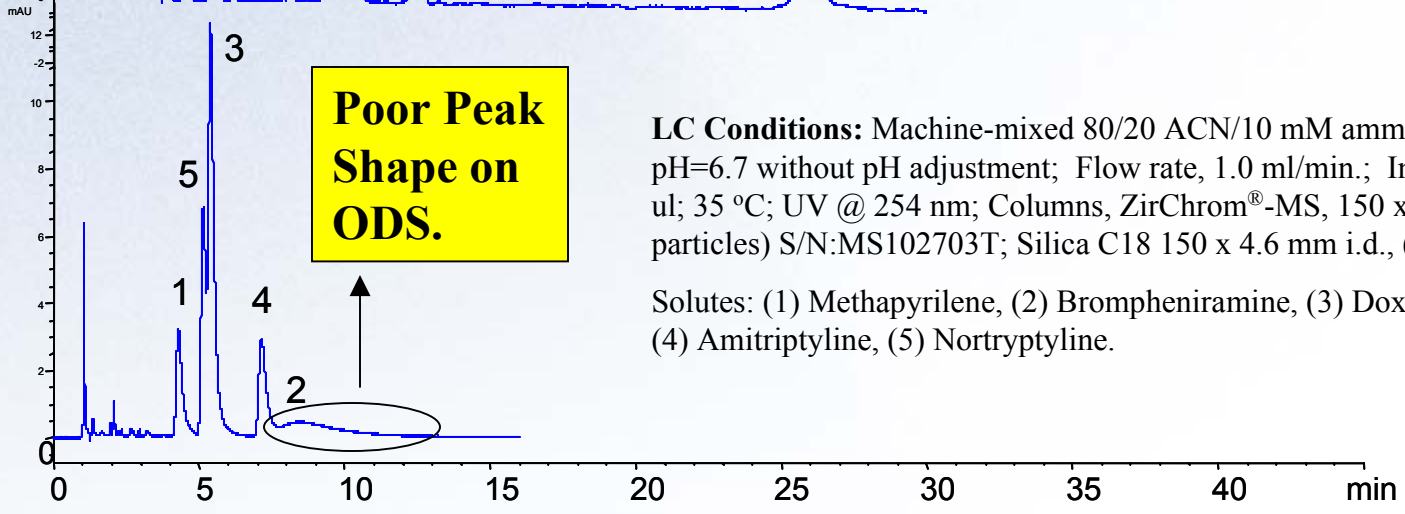
HPLC-MS of Quaternary Amine Drugs



Separation Comparison of Basic Pharmaceuticals on ZirChrom®-MS and ODS



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

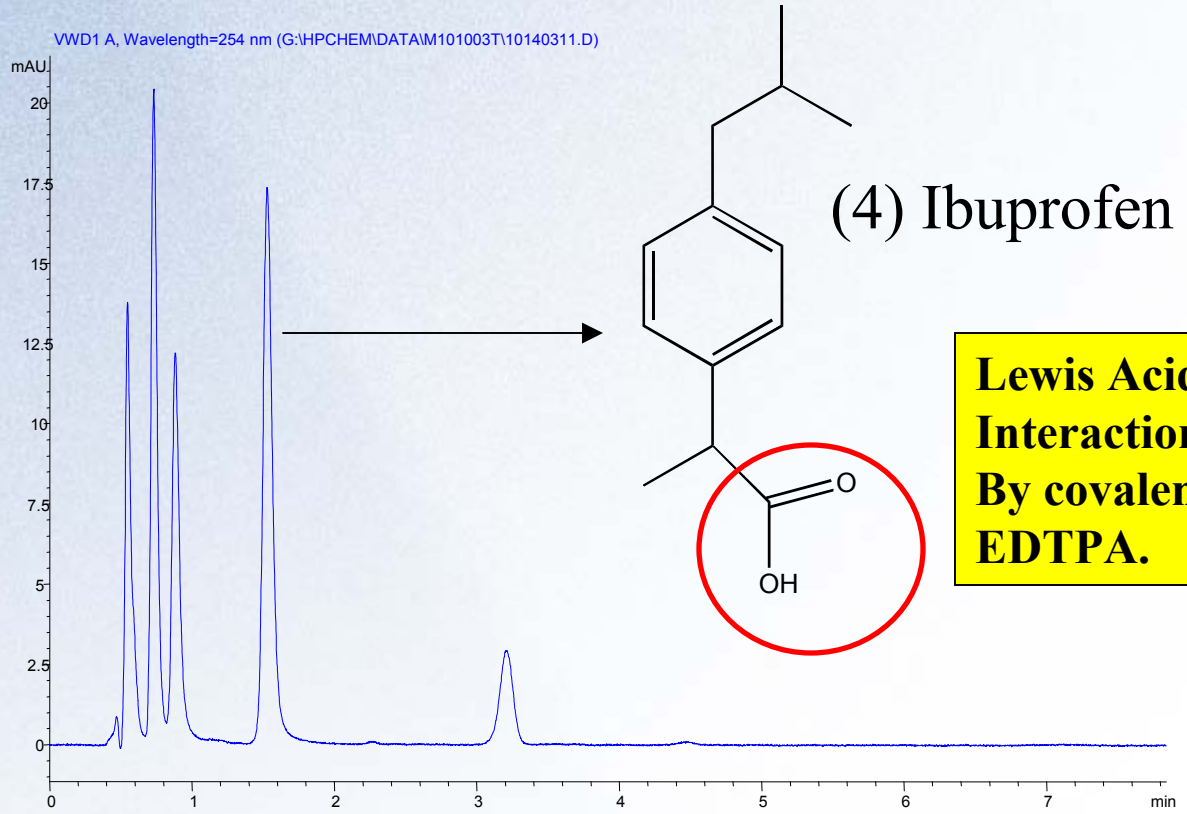


Poor Peak Shape on ODS.

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 ºC; 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



(4) Ibuprofen

Lewis Acid-Base Interaction "blocked" By covalently attached EDTPA.

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.

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 ***similar selectivity*** and RP behavior to silica C18 ***for neutral compounds***.
- ZirChrom®-MS ***is chemically stable*** from pH 1-10.
- ZirChrom®-MS ***has very different selectivity*** than silica C18 ***for pharmaceuticals***.