



*Stable RPLC Phases – How Stable Are They?
A Comparison of the Stability and Selectivity
of “Stable Phases” at Elevated pH*

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ZirChrom®

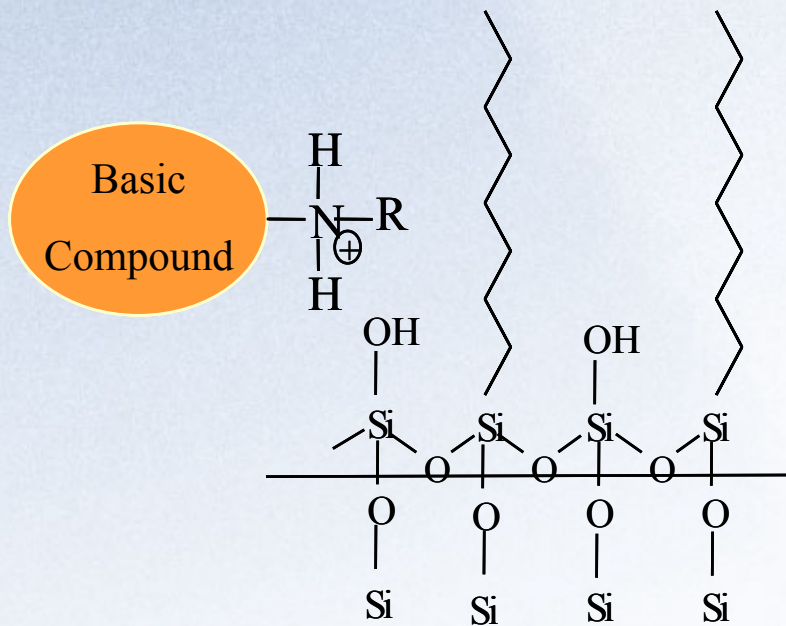
OUTLINE

- Analytical Advantages of Column Stability
- Synthesis of DiamondBond-C18 and ZirChrom-PBD
- Comparison of Seven Stable RPLC Columns
- Selectivity Study of Selected Model Compounds
- High pH Column Stability Study
- Improved Separations of Basic Pharmaceuticals at High pH
- Fast Separations at High pH and High Temperature
- Conclusions

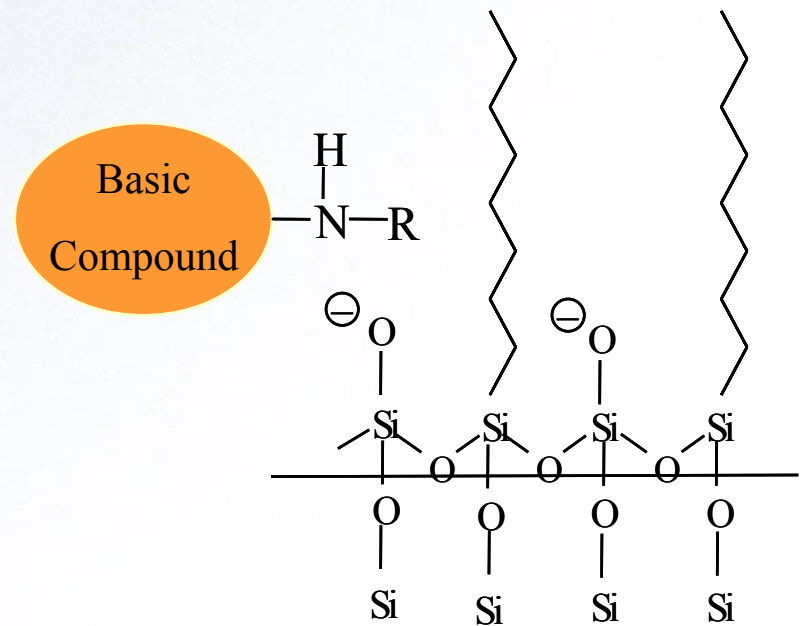


Analytical Advantages of High and Low pH Column Stability

Low pH



High pH

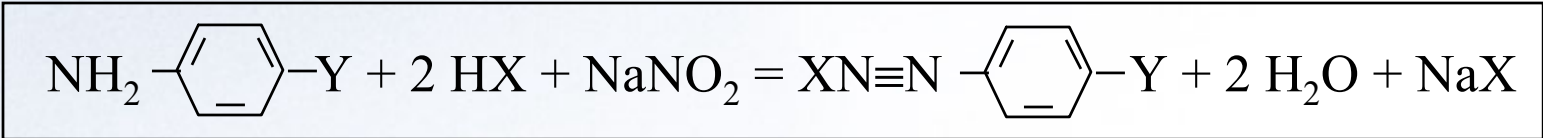
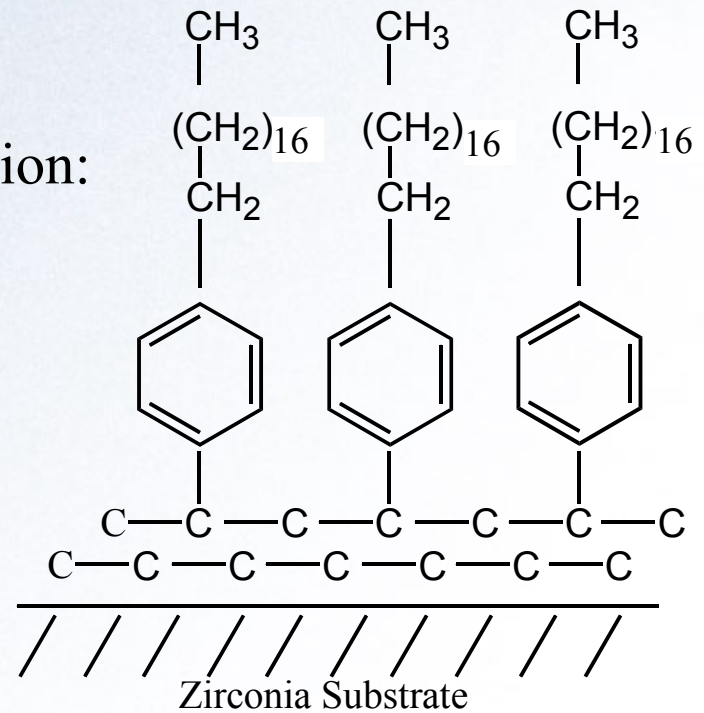




Synthesis DiamondBond-C18

■ General approach - Cabot Corporation:

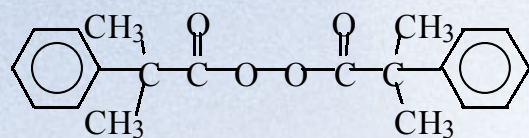
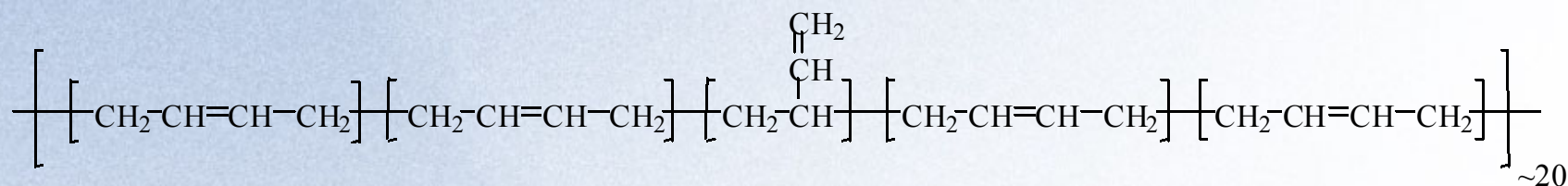
- Functionalizing agent **X-R-Y**
- **X** reacts with surface
- **Y** = functional group





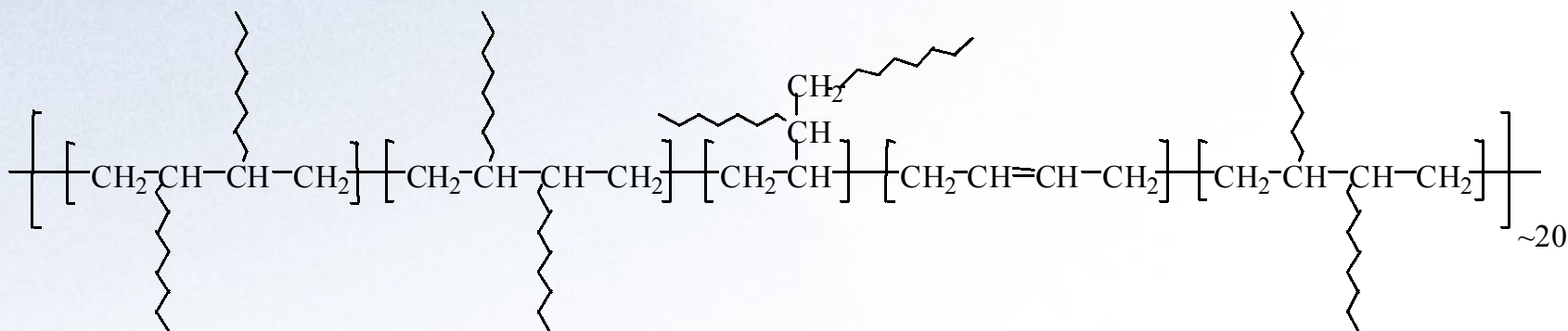
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Synthesis of ZirChrom-PBD



Dicumyl Peroxide

Vacuum
160 °C





Comparison of Seven Stable RPLC Columns*

<i>Manufacturer</i>	ZirChrom	ZirChrom	Polymer Labs	
<i>Description</i>	DiamondBond-C18	ZirChrom-PBD	PLRP-S	
<i>Particle Size</i>	3	3	5	
<i>Pore Size</i>	300	300	100	
<i>Dimensions (mm)</i>	50 x 4.6	150 x 4.6	150 x 4.6	
<i>Price</i>	\$675.00	\$595.00	\$480.00	
Low pH	1	1	1	
High pH	14	13	14	
High Temp Limit (°C)	200	150	150	
<i>Manufacturer</i>	ES Industries	Phenomenex	Waters	Zorbax
<i>Description</i>	GammaBond RP-1	Luna C18 (2)	Xterra RP18	Extend SB300-C18
<i>Particle Size</i>	5	3	3.5	3.5
<i>Pore Size</i>	80	100	130	80
<i>Dimensions (mm)</i>	150 x 4.6	150 x 4.6	150 x 4.6	150 x 4.6
<i>Price</i>	\$695.00	\$435.00	\$425.00	\$540.00
Low pH	1.3	1.5	1	2
High pH	12	10	12	11.5
High Temp Limit (°C)	NA	NA	80	60

* Column names are the trademarks of their respective manufacturers.



Selected Model Compound Comparison

1	<i>N</i> -benzyl formamide
2	benzyl alcohol
3	phenol
4	3-phenyl propanol
5	<i>p</i> -chlorophenol
6	acetophenone
7	benzotrile
8	nitrobenzene
9	methyl benzoate
10	anisole
11	benzene

12	<i>p</i> -chlorotoluene
13	<i>p</i> -nitrobenzyl chloride
14	toluene
15	benzophenone
16	bromobenzene
17	naphthalene
18	ethylbenzene
19	<i>p</i> -xylene
20	<i>p</i> -dichlorobenzene
21	propylbenzene
22	<i>n</i> -butylbenzene

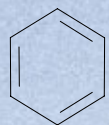
LC Conditions: Mobile phase, 40/60 Acetonitrile/50mM phosphate at pH 3.2; Flow rate, 1.0 ml/min.; Temperature = 30 °C; Detection at 254nm; 5µl Injection volume. *LC-GC*, Vol 13, No. 9, September 1995, 720-726.



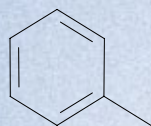
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Nonpolar

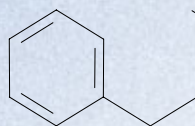
22 Non-electrolyte Solutes



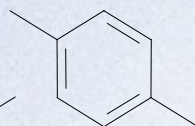
Benzene



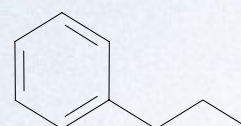
Toluene



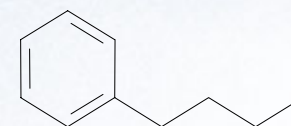
Ethylbenzene



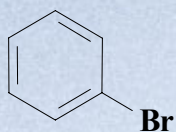
p-xylene



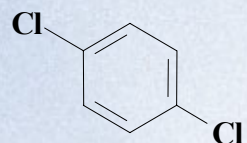
Propylbenzene



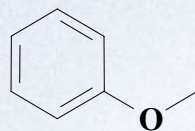
Butylbenzene



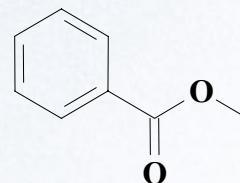
Bromobenzene



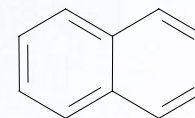
p-Dichlorobenzene



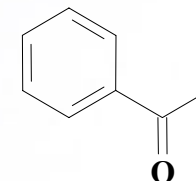
Anisole



Methylbenzoate

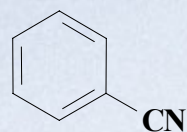


Naphthalene

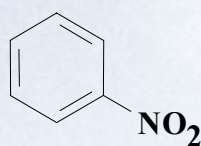


Acetophenone

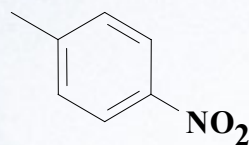
Polar



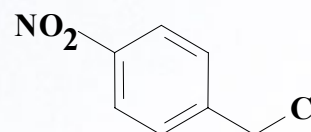
Benzonitrile



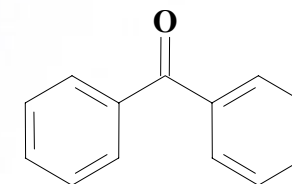
Nitrobenzene



p-Nitrotoluene

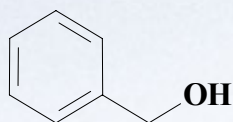


p-Nitrobenzyl Chloride

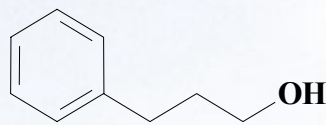


Benzophenone

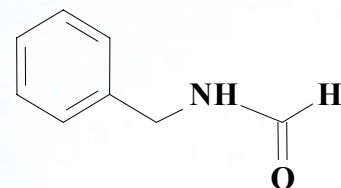
HB Donor



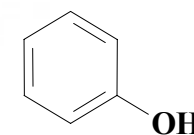
Benzylalcohol



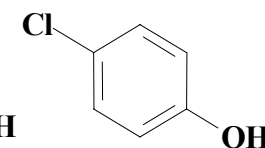
3-Phenyl Propanol



N-Benzyl Formamide



Phenol



p-Chlorophenol



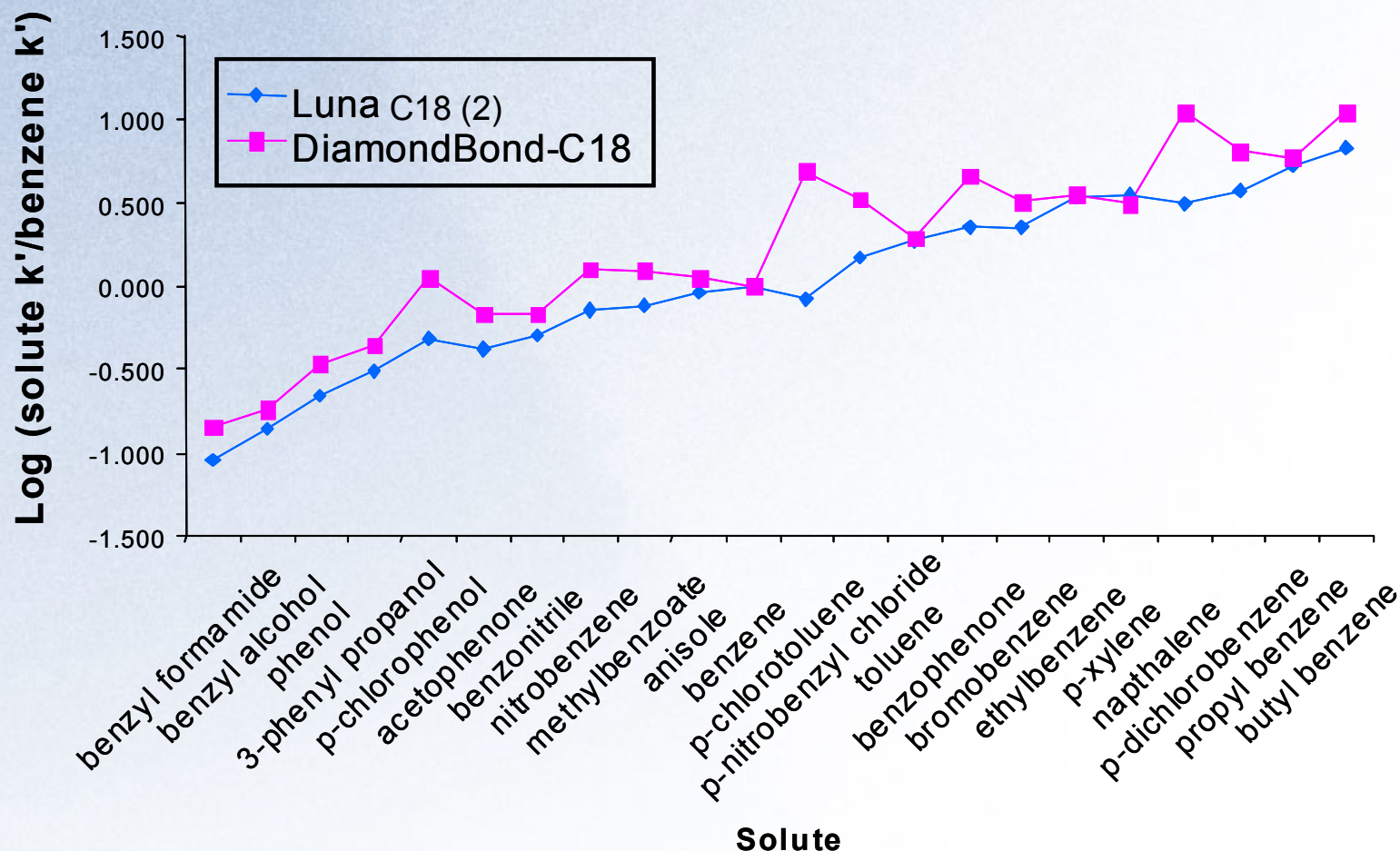
Regression Data from $\log k'$ vs. $\log k'$ Plots for All Columns versus Luna C18 (2)*

<i>Column vs. Luna C18 (2)</i>	<i>R²</i>
ZirChrom-PBD	0.985
Gammabond RP-1	0.981
Xterra RP18	0.972
Polymer Labs PLRP-S	0.963
DiamondBond-C18	0.889

* Column names are the trademarks of their respective manufacturers.



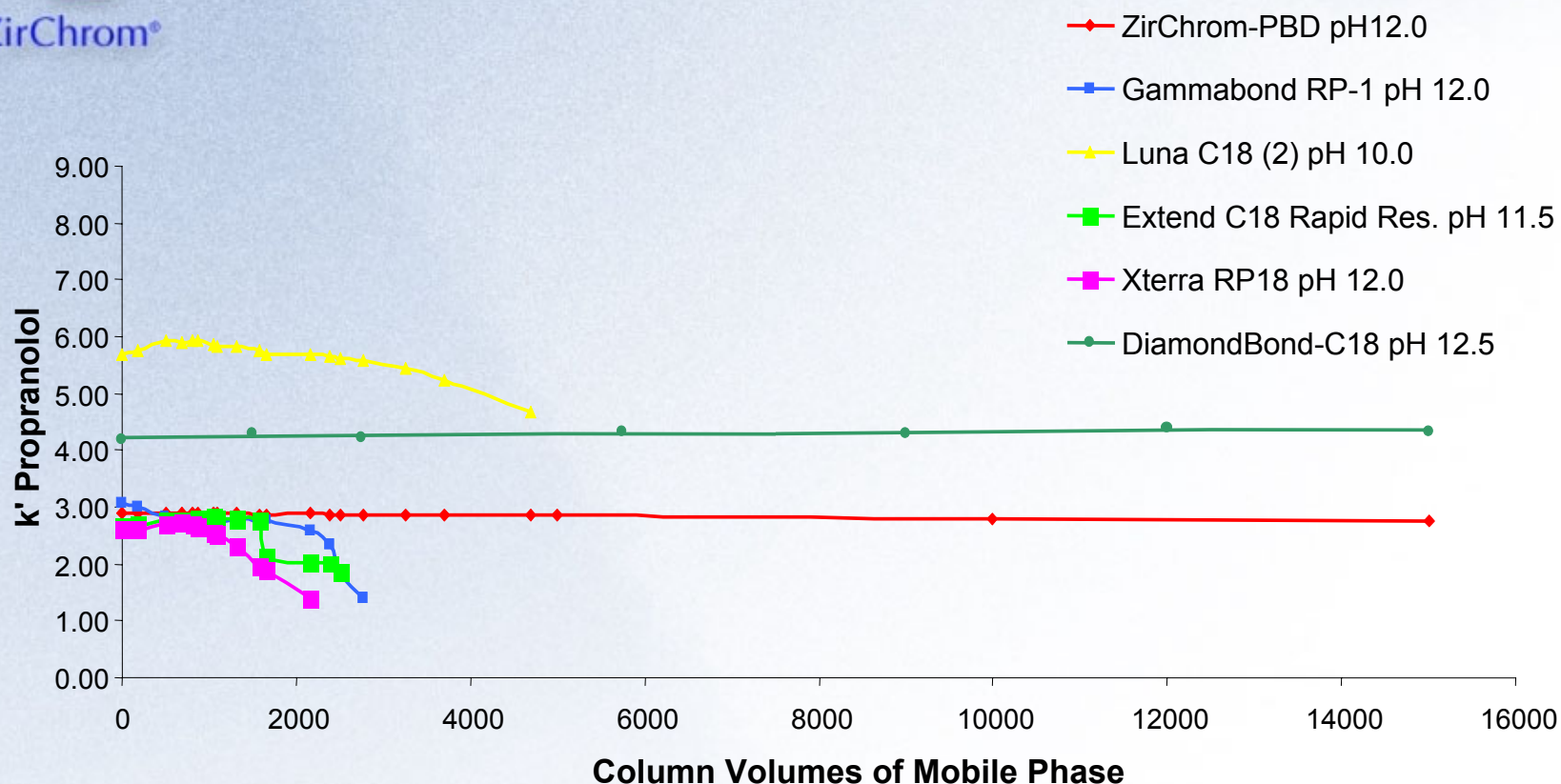
Normalized Selectivity Comparison*: DiamondBond-C18 and Luna C18 (2)



* Column names are the trademarks of their respective manufacturers.



High pH Stability Comparison*



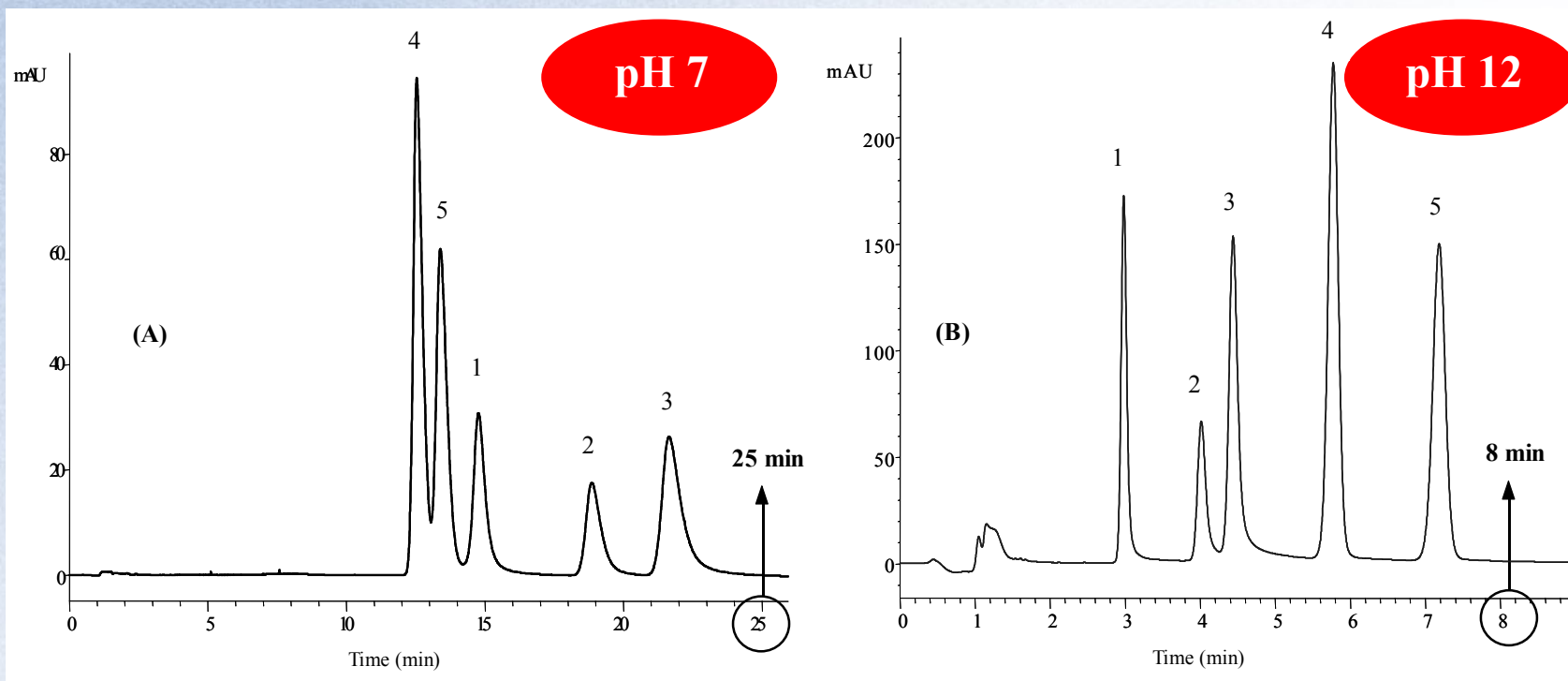
Exposure Conditions: Mobile phase, 35/65 ACN/50mM Potassium phosphate buffer at indicated pH; Temperature, 30 °C.

LC Conditions: Mobile phase, ACN (or THF)/50mM Potassium phosphate buffer at indicated pH; Flow Rate, 1.0 mL/min.; Temperature, 30 °C; Injection Volume, 5 uL; Detection, 254nm.

* Column names are the trademarks of their respective manufacturers.



Applications: Separation Improvement at High pH



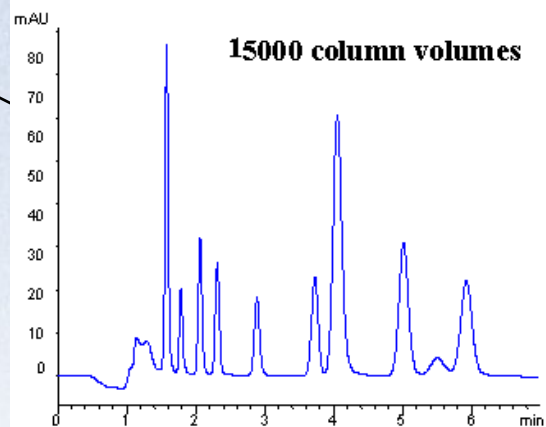
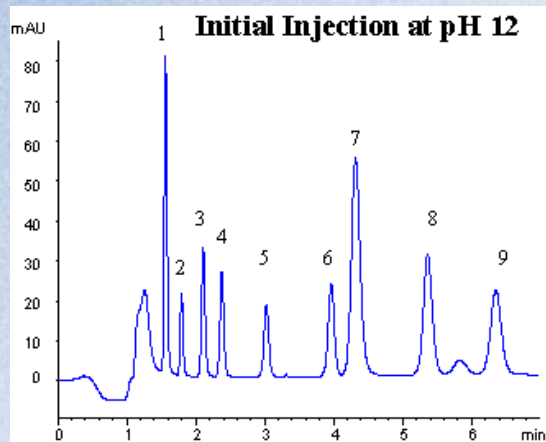
LC Conditions: (A) ZirChrom-PBD, 150 x 4.6mm I.d.; Mobile phase, 58/42 Acetonitrile/20mM Potassium phosphate, pH 7.0; Flow rate, 1.0ml/min.; Detection at 254nm; Temperature, 30°C; (B) Same conditions as in (A) except 45/55 Acetonitrile/20mM Potassium phosphate, pH 12.0; Solutes: 1=Nordoxepin, 2=Protryptiline, 3=Nortryptiline, 4=Imipramine, 5=Amitryptiline.



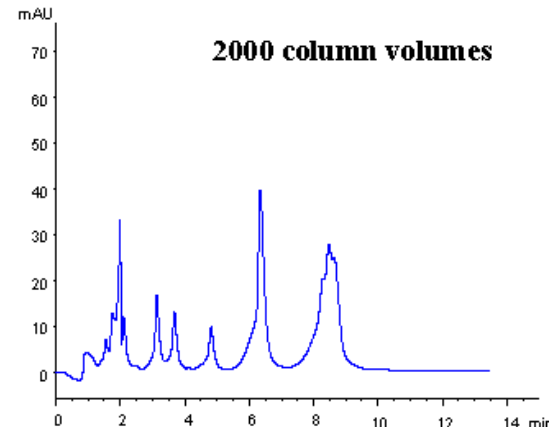
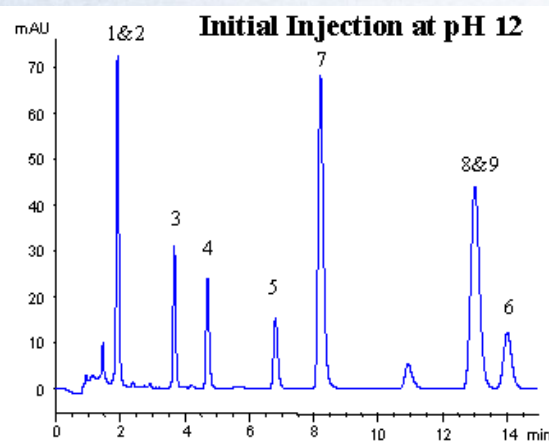
ZirChrom®

Selectivity Comparison for Basic Pharmaceuticals at pH 12

ZirChrom®-PBD



LC Conditions:
 ZirChrom®-PBD; Mobile Phase, 28/72 acetonitrile/
 20 mM potassium phosphate at pH=12.0; Flow Rate,
 1.0 mL/min.; Temperature, 30°C; Detection, 254 nm.
 Solutes: 1=Labetalol, 2=Atenolol, 3=Acebutolol,
 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine,
 7=Quinidine, 8=Alprenolol, 9=Propranolol.



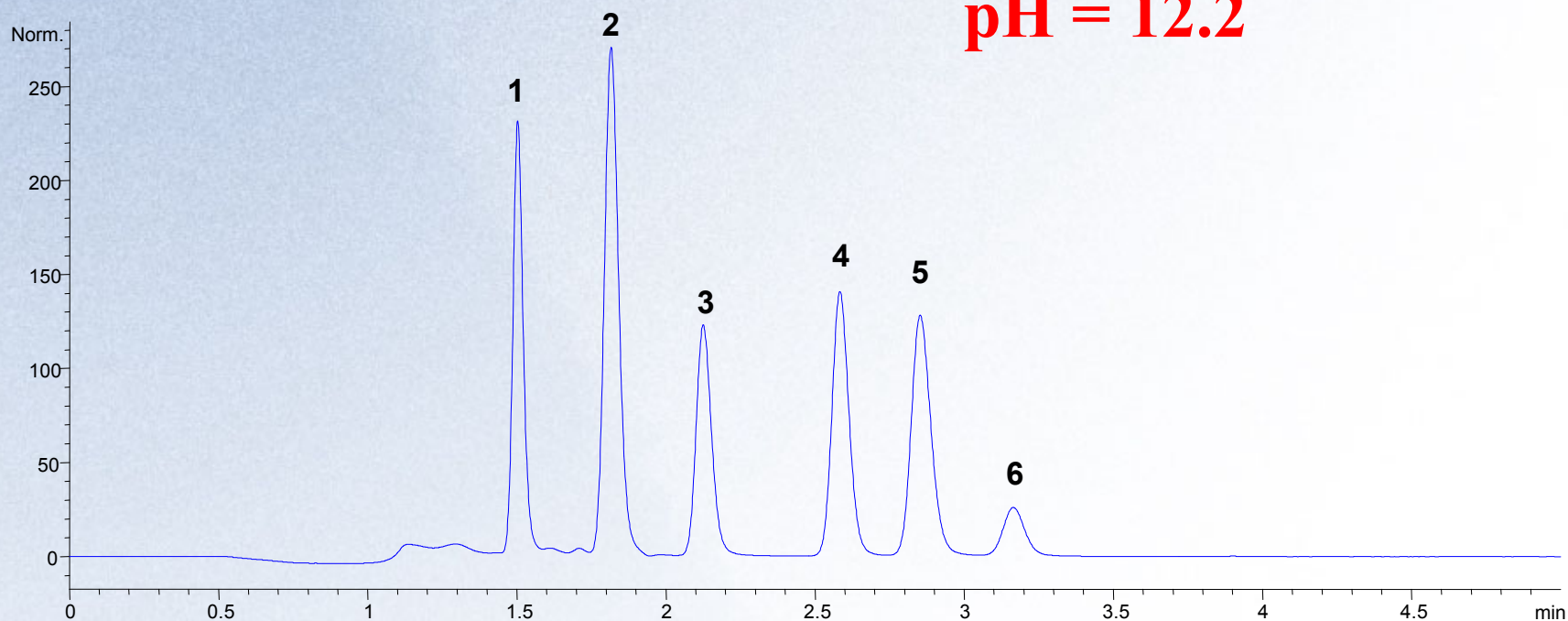
LC Conditions:
 Waters Xterra™ RP18; Mobile Phase, 35/65 acetonitrile/
 20 mM potassium phosphate at pH=12.0; Flow Rate,
 1.0 mL/min.; Temperature, 30°C; Detection, 254 nm.
 Solutes: 1=Labetalol, 2=Atenolol, 3=Acebutolol,
 4=Metoprolol, 5=Oxprenolol, 6=Lidocaine,
 7=Quinidine, 8=Alprenolol, 9=Propranolol.

Xterra® RP18



Separation of β -Blockers with a MS-Friendly Mobile Phase

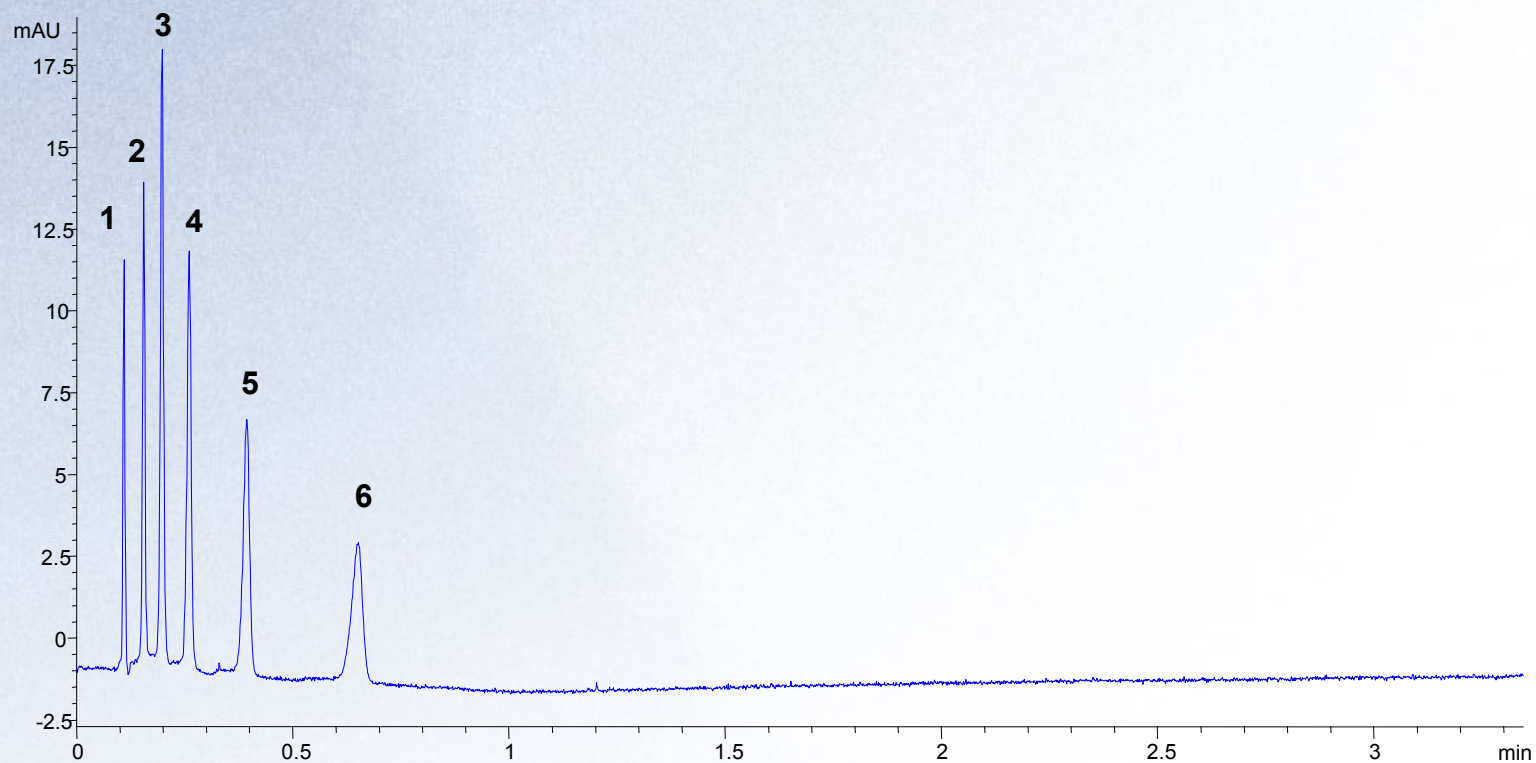
pH = 12.2



LC Conditions: Column: ZirChrom-PBD 150 x 4.6; Mobile phase: 35/65 ACN/10mM Triethylamine, 50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate: 1.0 ml/min.; Temperature: 30 °C; Detection: 254 nm; Injection volume, 5 ul; Solutes: 1=Labetalol, 2=Acebutolol, 3=Oxprenolol, 4=Lidocaine, 5=Alprenolol, 6=Propranolol



Fast Separation of Parabens at 150°C



LC Conditions: Column, 50 x 4.6 Diamondbond-C18 (phase A), OD021601A; Mobile phase, 20/80 ACN/40mM Phosphoric acid, pH 2.3; Flow rate, **5.5 ml/min.**; Temperature, 150 °C; Injection volume, 1ul; Detection at 254nm; Solutes, 1=Uracil, 2=Phenol, 3=Methylparaben, 4=Ethylparaben, 5=Propylparaben, 6=Butylparaben.



Conclusions

- Silica-based and hybrid columns *are not stable in phosphate media at high pH.*
- **ZirChrom[®]-PBD** and **DiamondBond[™]-C18** are the most *chemically (pH 12, phosphate) and thermally (>150°C) stable.*
- *Differences in selectivity* are observed between columns for mixtures of basic pharmaceuticals.
- **DiamondBond[™]-C18** shows the *greatest differences in selectivity compared to ODS* for the 22 selected non-electrolytes.
- **Zirconia** *can be used at high pH and high column temperature* for fast separations.



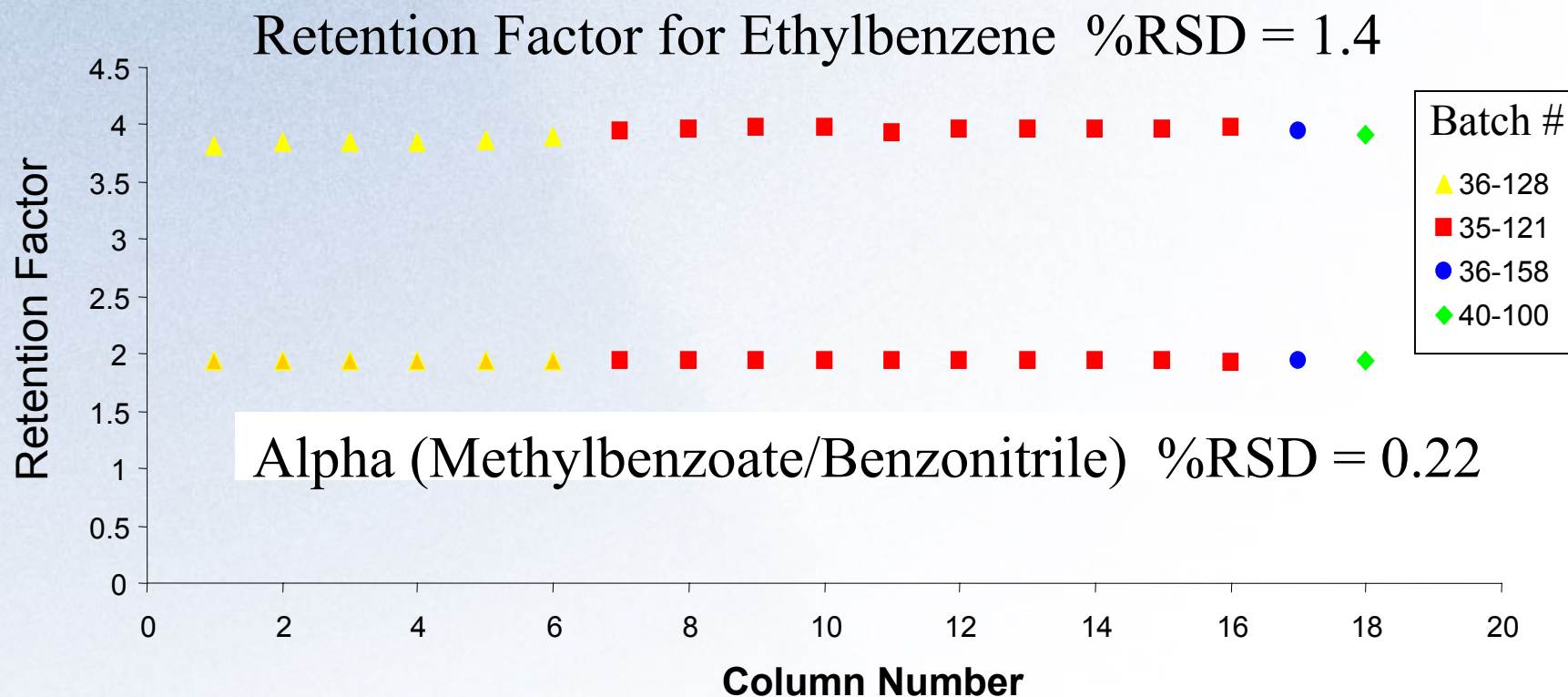
*Stable RPLC Phases – How Stable Are They?
A Comparison of the Stability, Symmetry,
Selectivity and Efficiency of Stable Phases at
Elevated pH*

Dr. Clayton McNeff
e-mail: mcneff@zirchrom.com





Reproducibility Data for DiamondBond™C18 Columns



LC Conditions: Column 100 x 4.6 mm id; Mobile phase, 37.5/5/57.5 ACN/THF/Water; Flow rate, 1.0 ml/min.; Temperature, 60 °C; Injection volume, 5 ul; Detection at 254 nm



Reproducibility Data for DiamondBond™C18 Columns

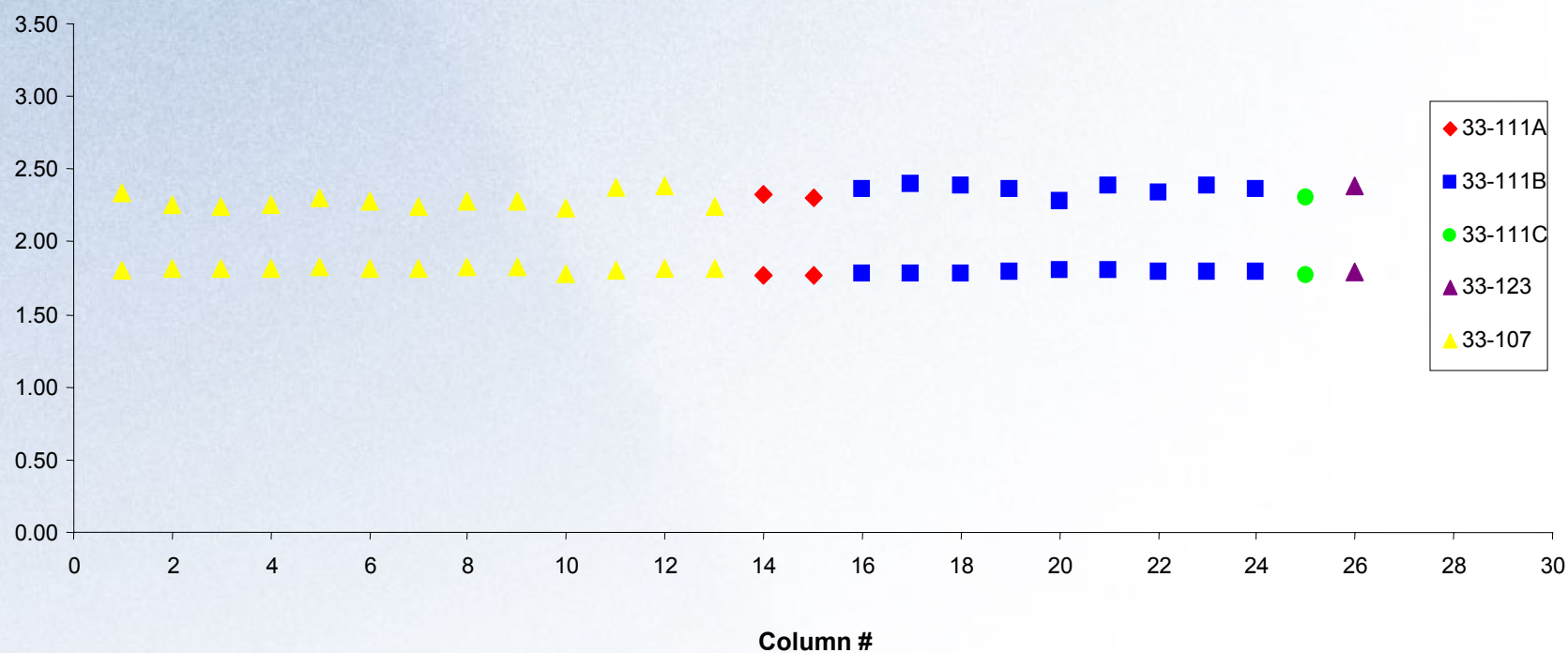
Parameter	Average	%RSD
k' (Ethylbenzene)	3.92	1.4%
α (Methylbenzoate /Benzonitrile)	1.94	0.2%
N (plates/m)	106,789	13%
Symmetry	0.77	7.3%

LC Conditions: Mobile phase, 37.5/5/57.5 ACN/THF/Water; Flow rate, 1.0 ml/min.;
Temperature, 60 °C; Injection volume, 5 μ l; Detection at 254 nm



Reproducibility Data for ZirChrom[®]-PBD Columns

Reproducibility of ZirChrom-PBD 150 x 4.6 Columns



LC Conditions: Mobile phase, 35/65 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5 µl; Detection at 254 nm



Reproducibility Data for ZirChrom[®]-PBD Columns

Parameter	Average	%RSD
k' (Toluene)	2.32	2.4%
α (Methylbenzoate/ Benzonitrile)	1.80	1.1%
N (plates/m)	151,000	6.8%
Symmetry	0.93	7.2%

LC Conditions: Mobile phase, 35/65 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5 μ l; Detection at 254 nm