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Synthesis of a New Lewis Acid Deactivated Reversed-Phase Zirconia Stationary Phase for HPLC

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Outline

- Background – Advantages and surface chemistry of zirconia-based supports for HPLC
- Synthesis of Lewis acid deactivated reversed-phase support
- Chromatographic characterization
  - Chromatography of Lewis-base analytes
  - Reversed-phase characteristics
  - Ion-exchange characteristics
  - Chemical stability testing
  - LC/MS column bleed study
- Pharmaceutical applications
- Conclusion – ZirChrom®-EZ allows the use of LC/MS compatible mobile phases for the analysis of both acidic and basic analytes not previously possible on other zirconia-based reversed-phases

Advantages of Zirconia-Based Supports for HPLC

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	Silica	Zirconia	Polymeric phase
Pore structure	+ +	+ +	+
Particle size	+ +	+ +	+ +
Chemical flexibility	+ +	+	+
Surface homogeneity	- -	- -	+
Mechanical stability	+ +	+ +	+
Swellibility	+ +	+ +	- -
Chemical stability	- -	+ +	+ +
Thermal stability	+	+ +	- -
Column efficiency	++	++	- -

++ excellent; + good; -- fair.

Surface Chemistry of Zirconia-Based Supports for HPLC

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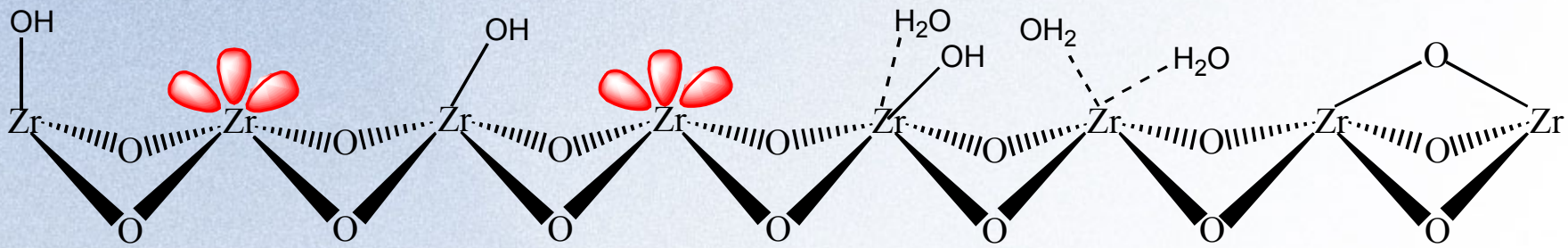
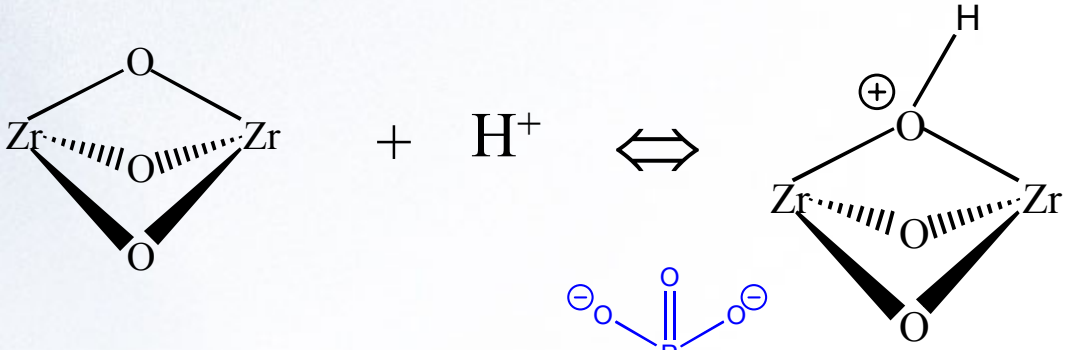
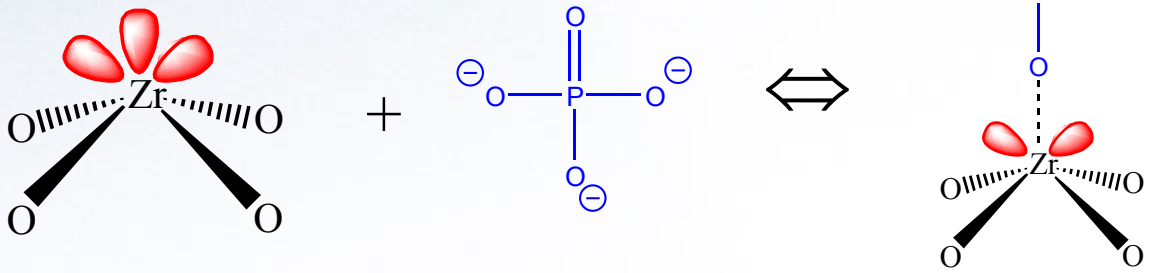


Brönsted Acid: $ZrOH + OH^- \Leftrightarrow ZrO^- + H_2O$

Brönsted Base:



Lewis Acid:



Synthesis of a Lewis-Acid Deactivated Reversed-Phase Support

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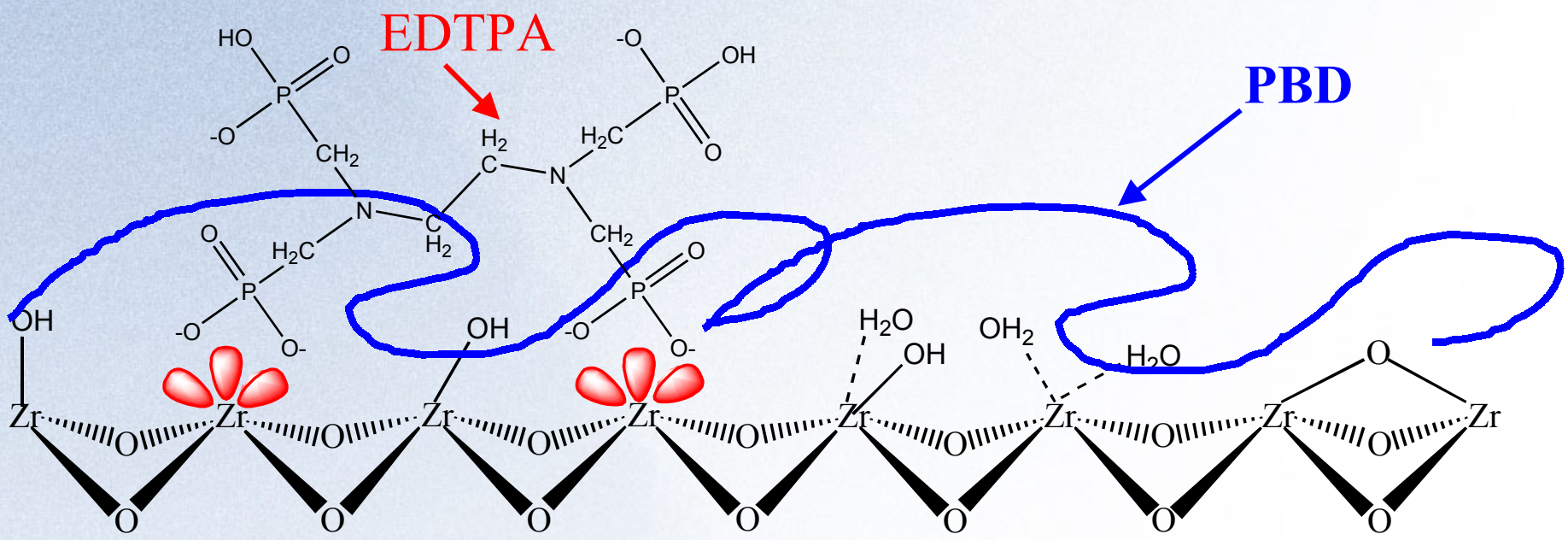
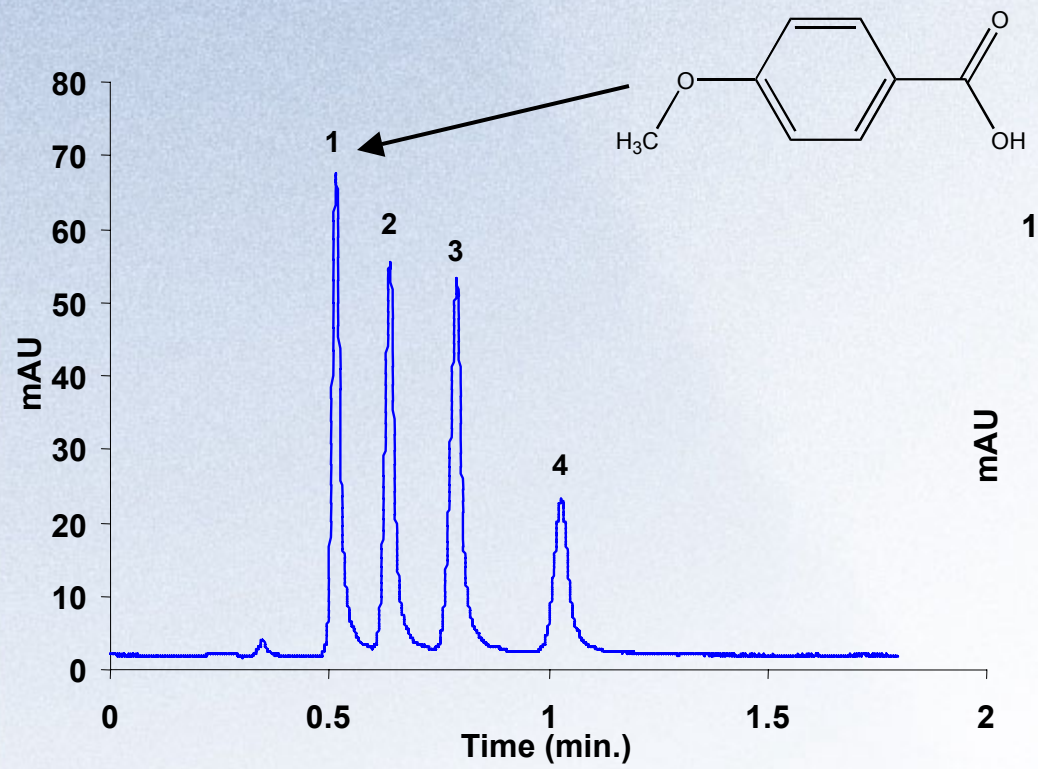
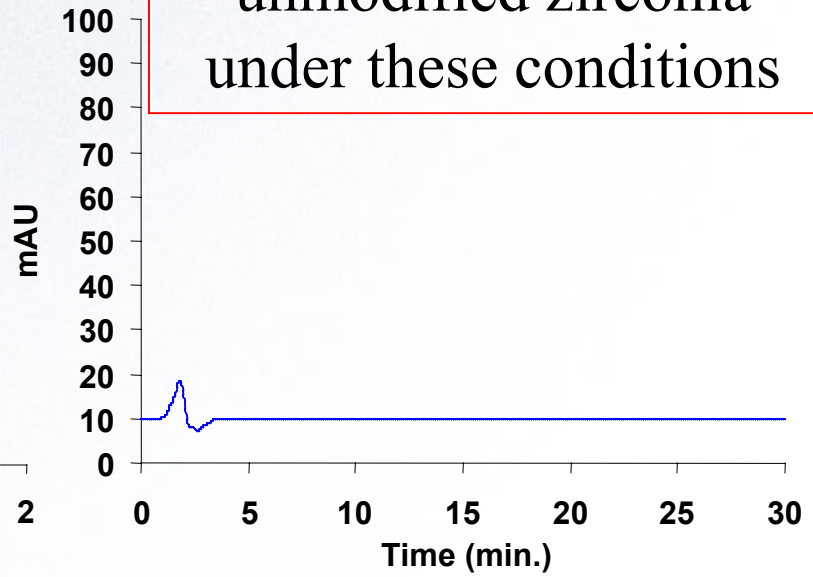


1. Coat bare zirconia with polybutadiene (PBD)[1]
2. Crosslink PBD chains together using dicumyl peroxide as initiator
3. Reflux PBD-ZrO_2 in Ethylenediamine-N,N,N',N'-tetra(methylenephosphonic)acid (EDTPA) solution
4. Wash to remove residual EDTPA

1) Li, J. W.; Reeder, D. H.; McCormick, A. V.; Carr, P. W. *Journal of Chromatography A* **1998**, *791*, 45-52

Chromatographic Characterization – Chromatography of Lewis Base Analytes

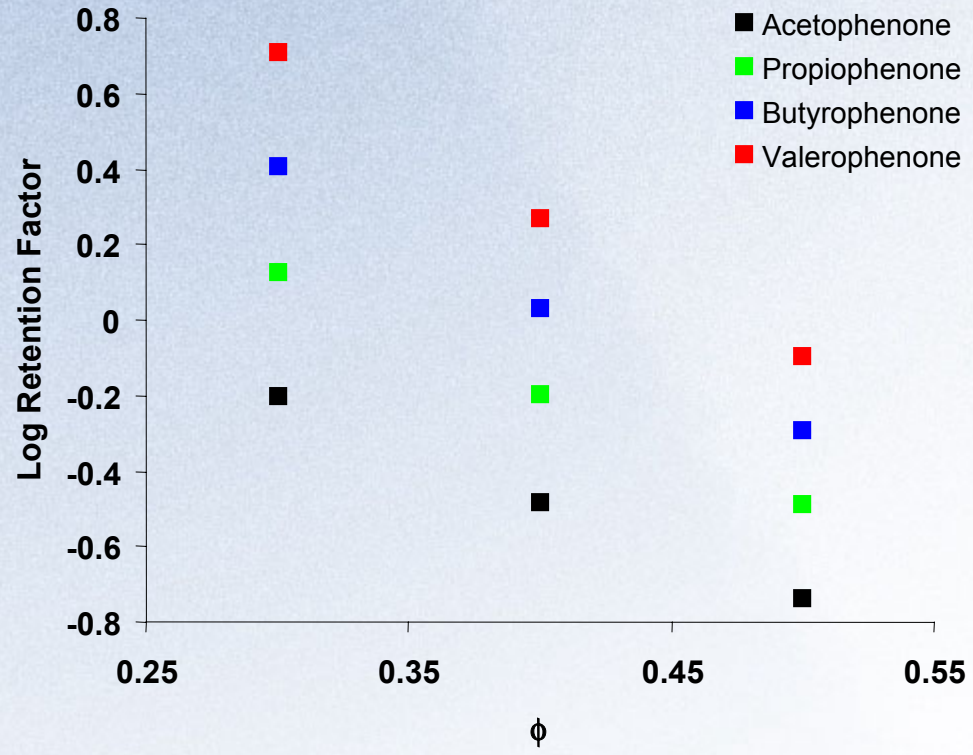




These analytes are irreversibly adsorbed on unmodified zirconia under these conditions

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

Reversed-Phase Characteristics

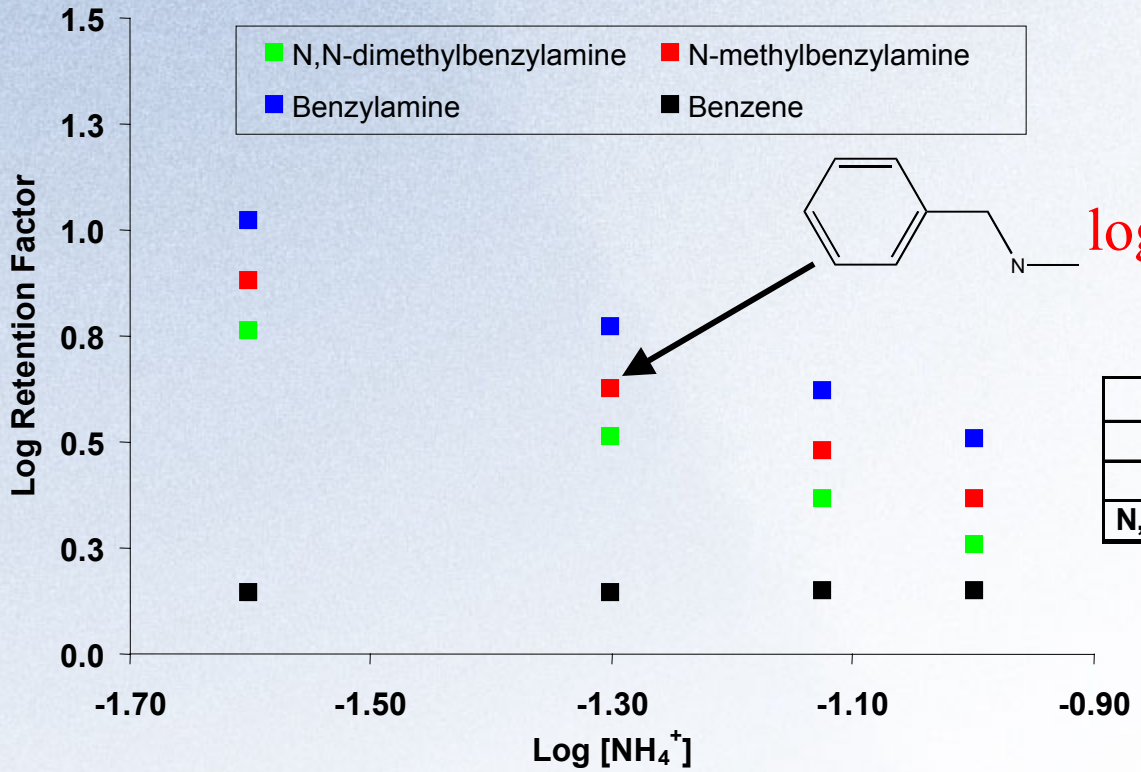


$$\log k'_{RP} = \log k_w - S\phi$$

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

Ion-Exchange Characteristics



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

	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

Chemical Stability

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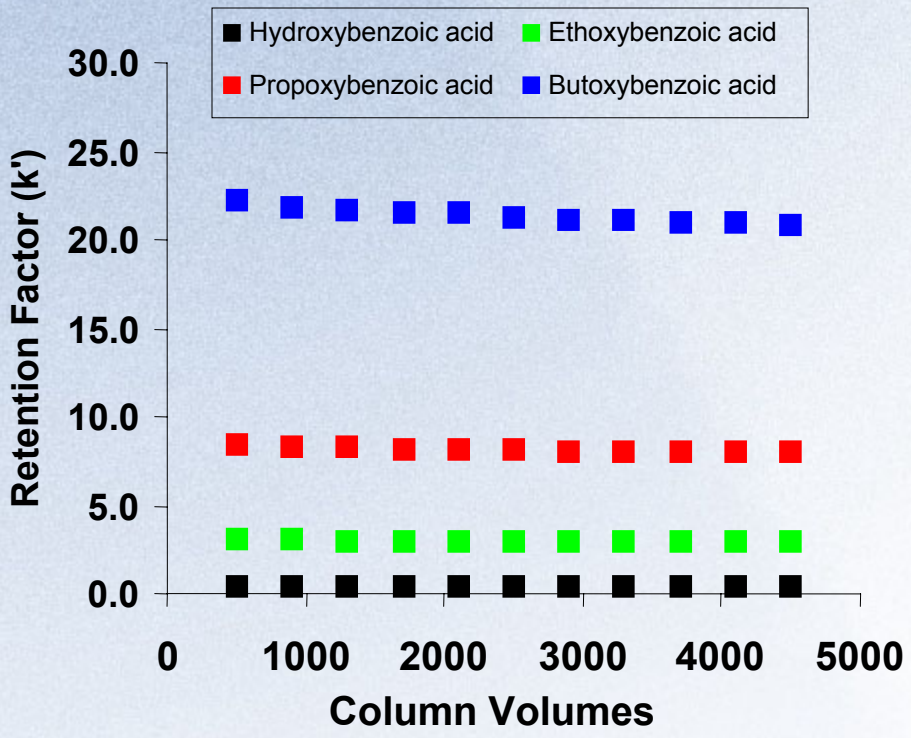
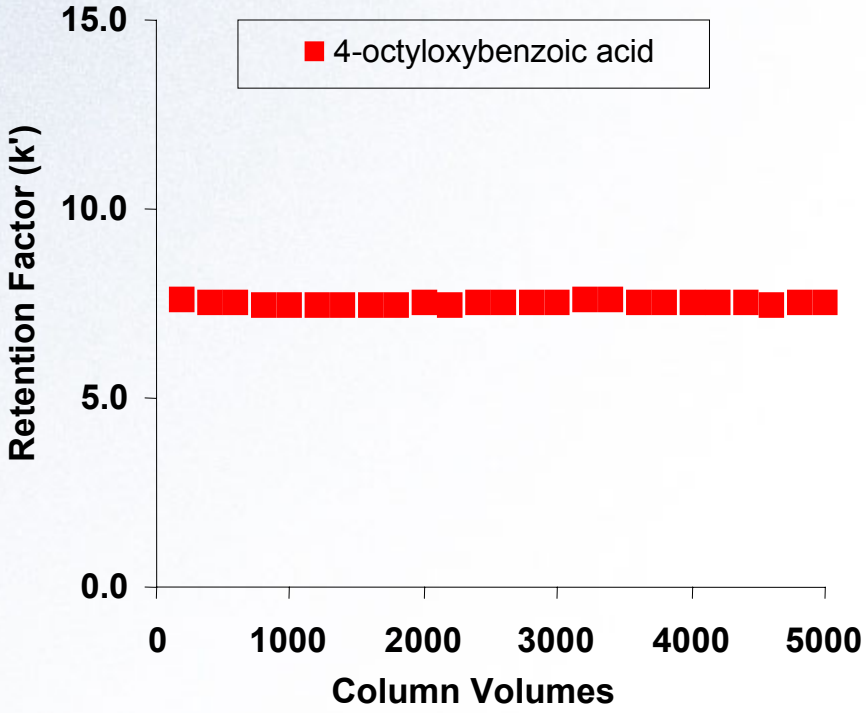
pH 1.0



pH 10.0



Exposure and Evaluation Conditions: Mobile phase, 15/85 ACN/0.1M Nitric acid, pH 1.0, or 0.1M Ammonium hydroxide, pH 10.0; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5 µl; Detection at 254 nm; Column, 50 mm x 4.6 mm i.d. ZirChrom®-EZ

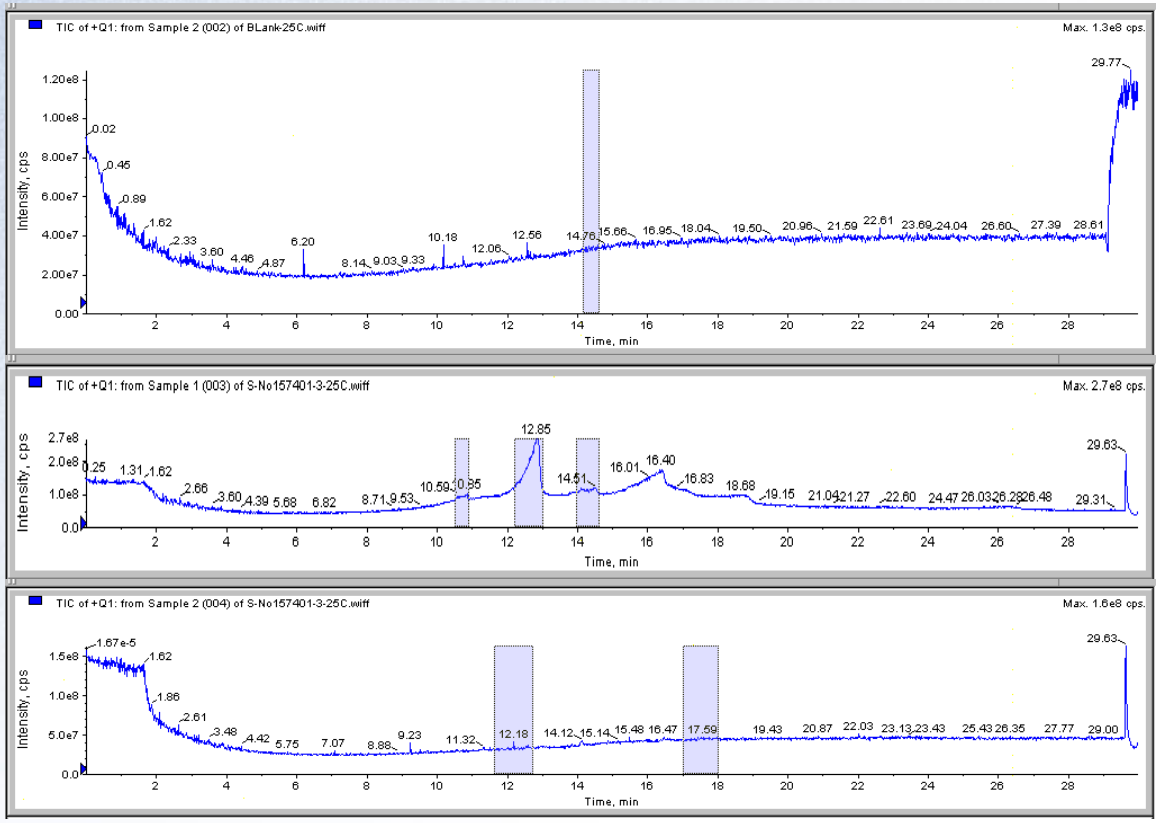
LC/MS Bleed Study – TIC's From Gradient Elution

LC Conditions: Mobile phase, 0-100% ACN from 0-30 minutes; Flow rate, 0.80 ml/min.; Temperature, 25 ºC; Detection by ESI-MS.

Blank gradient – No column installed

Gradient #1

Gradient #2



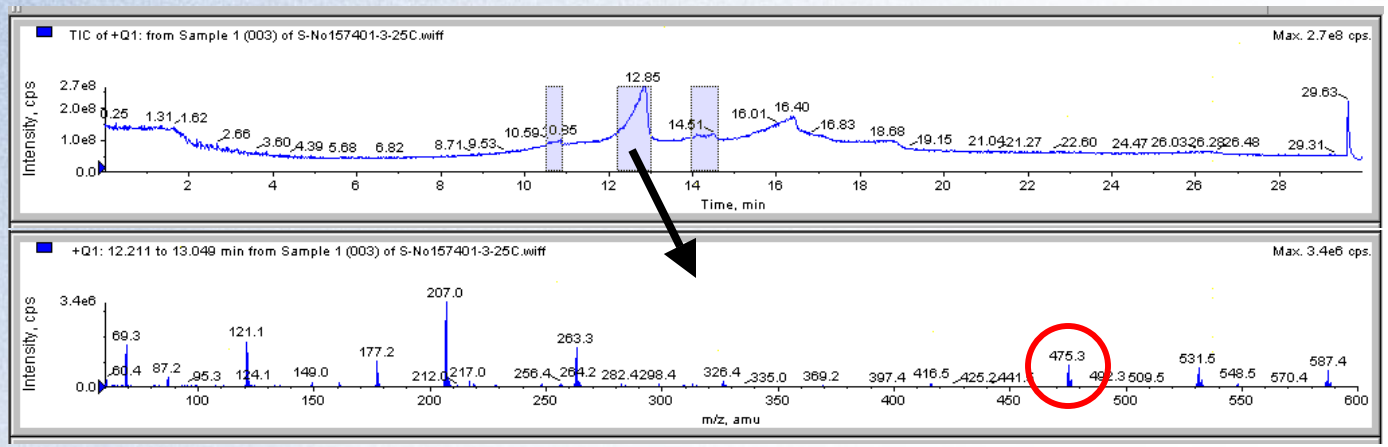
Extracted Spectra

ZirChrom®

Gradient #1

TIC

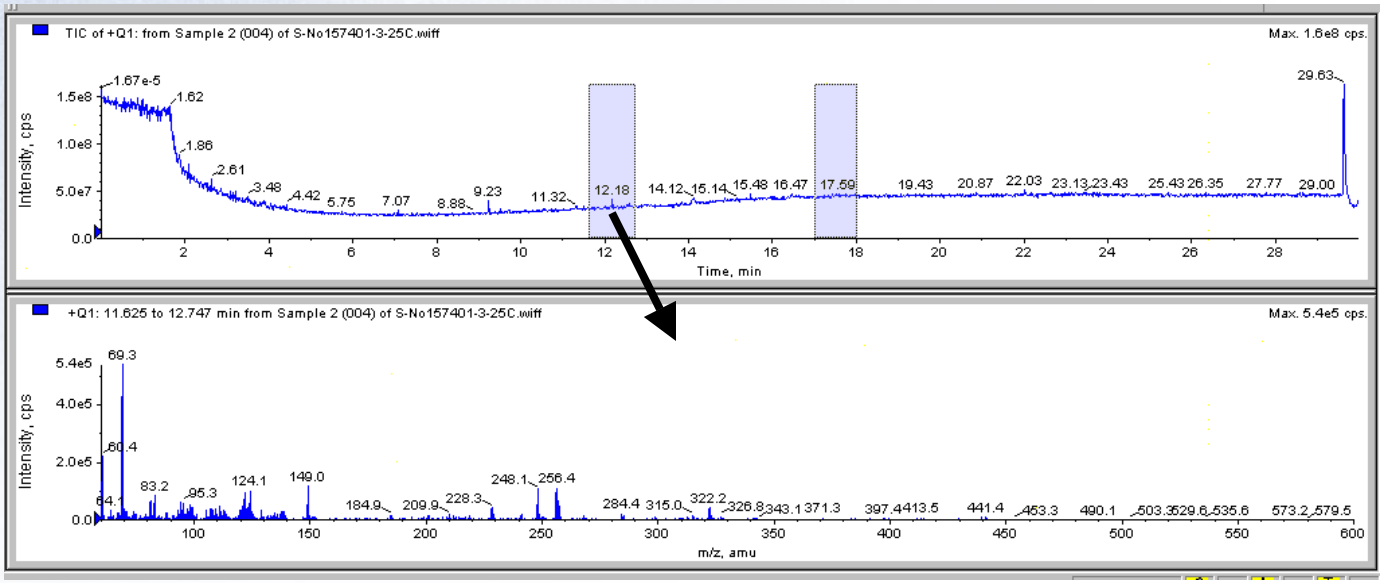
Scan



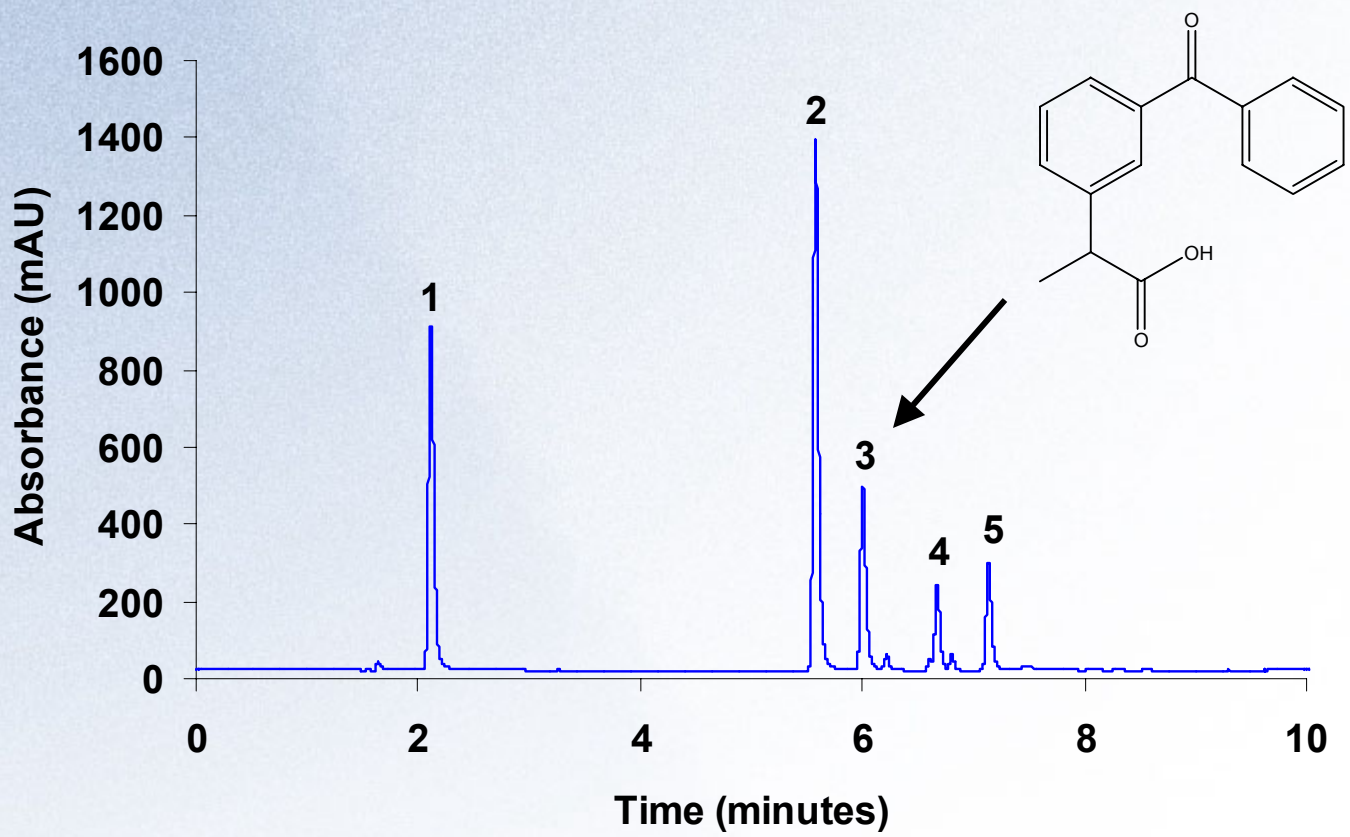
Gradient #2

TIC

Scan



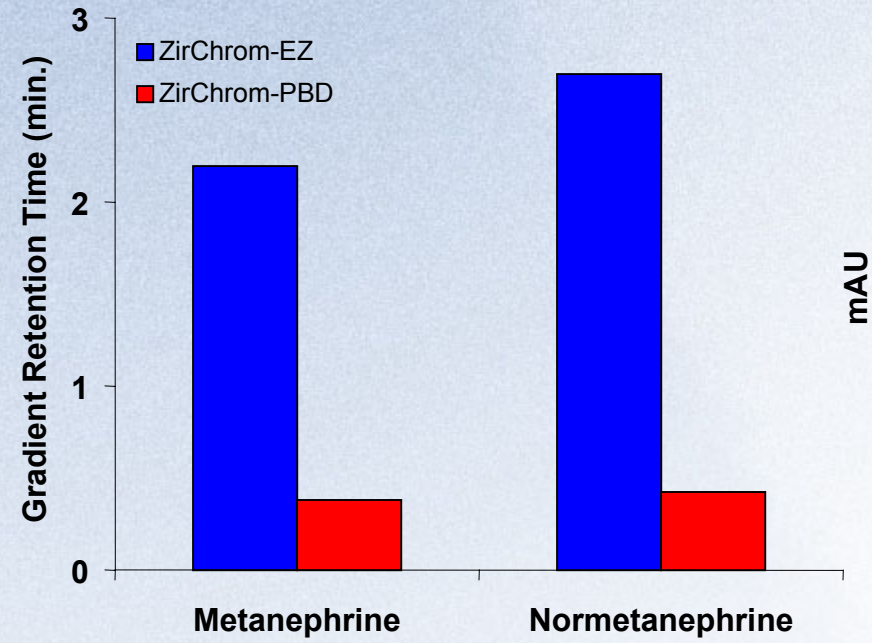
Separation of Acidic Drugs with LC/MS-Friendly Mobile Phase

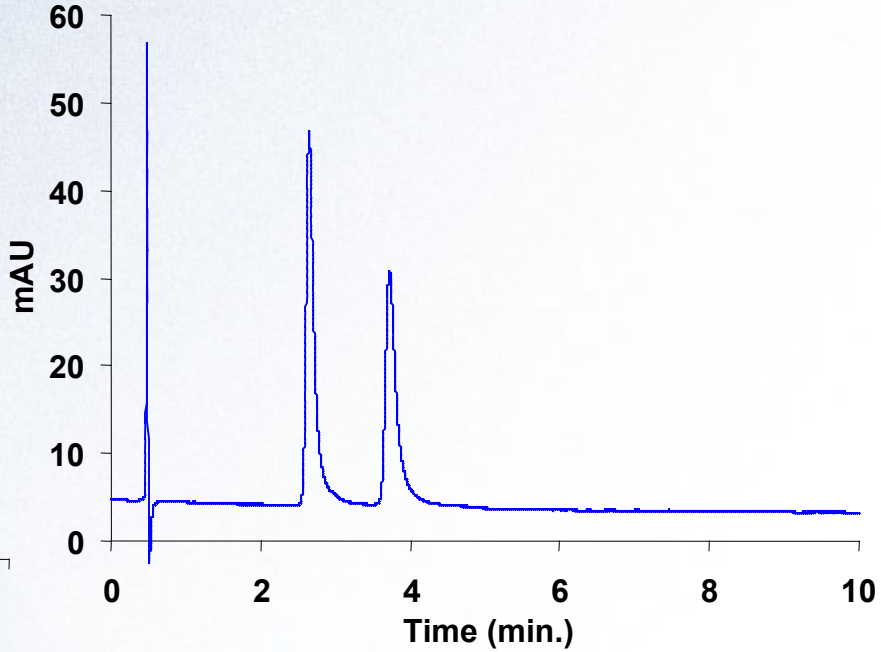


Time (min.)	%A	%B
0	90	10
10	10	90

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 °C; Injection volume, 10 µl; Detection at 254 nm.; Solutes: 1=Acetaminophen, 2=Naproxen, 3=Ketoprofen, 4=Fenoprofen, 5=Indomethacin

ZirChrom®-PBD vs. ZirChrom®-EZ for Metanephrine by LC-MS





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

A: 20mM Ammonium acetate, pH 6.0

B: Acetonitrile

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 °C; Injection volume, 10 µl; Detection at 254 nm.

Advantages of ZirChrom®-EZ Over Silica- and Other Zirconia-Based Phases

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Advantages over ***silica*** reversed-phases…

- Stable from pH 1-10, with similar temperature stability
- Increased retention and loading for cationic compounds
- Very different selectivity, particularly for cationic compounds

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

- Does not require non-volatile buffers for Lewis base analytes
- Increased retention for cationic compounds, particularly at low pH

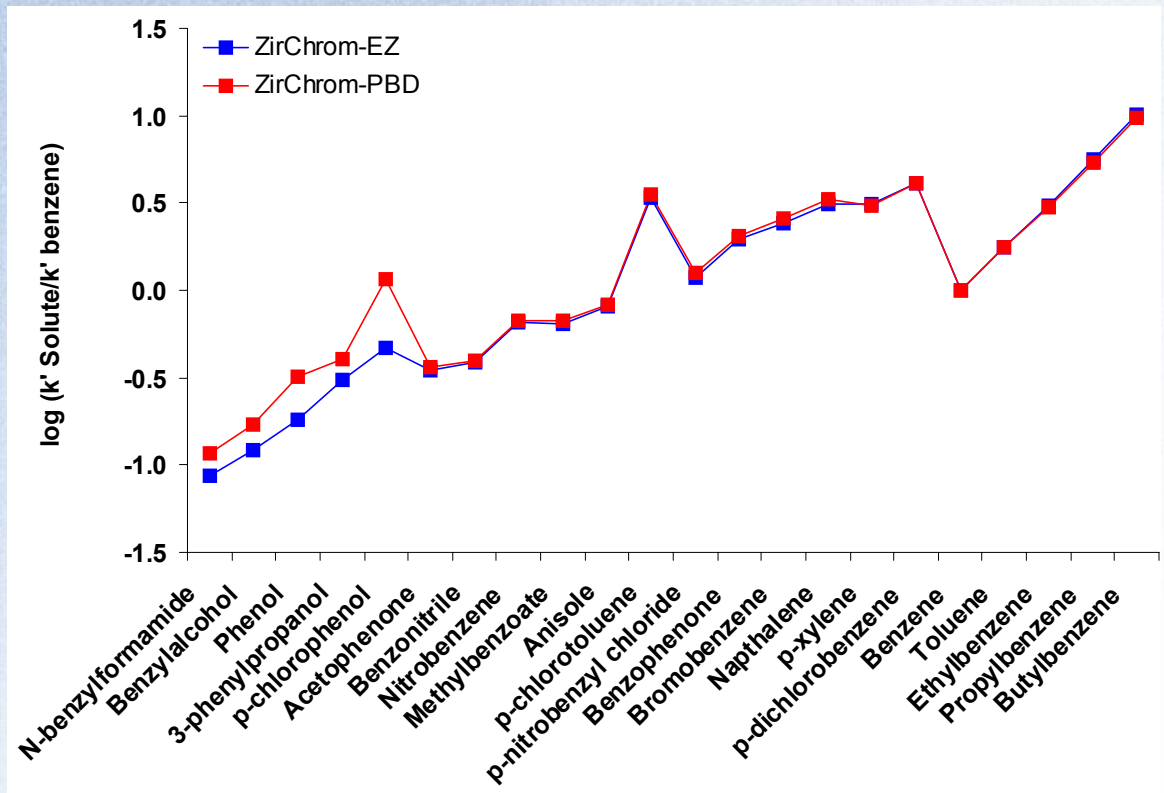
Conclusion – ZirChrom®-EZ allows the use of LC/MS compatible mobile phases for the analysis of both acidic and basic analytes not previously possible on other zirconia-based reversed-phases

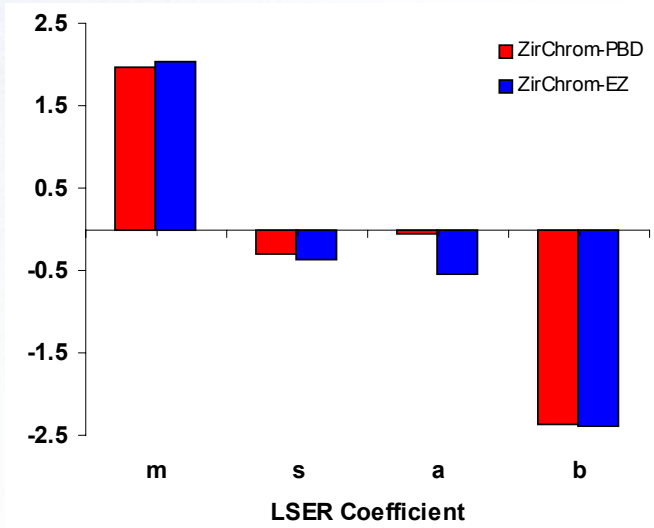
Acknowledgements

Cabot Corporation

LSER Comparison of ZirChrom-PBD and ZirChrom-EZ

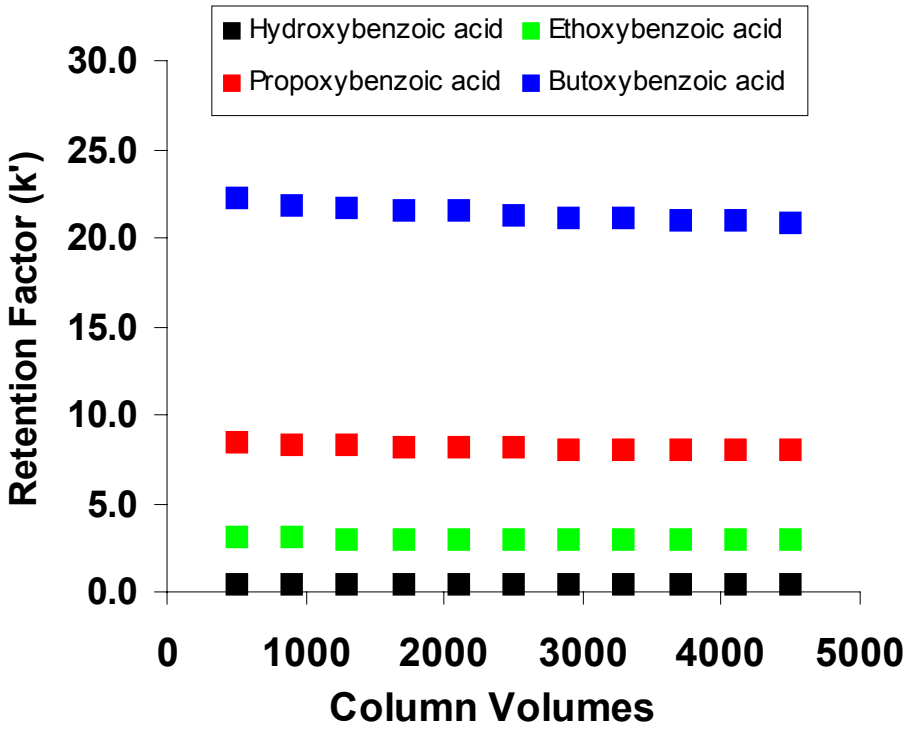
$$\log k' = \log k'_0 + mV_x + s\pi^*_2 + a\,\Sigma\,\alpha_2 + b\,\Sigma\,\beta_2$$

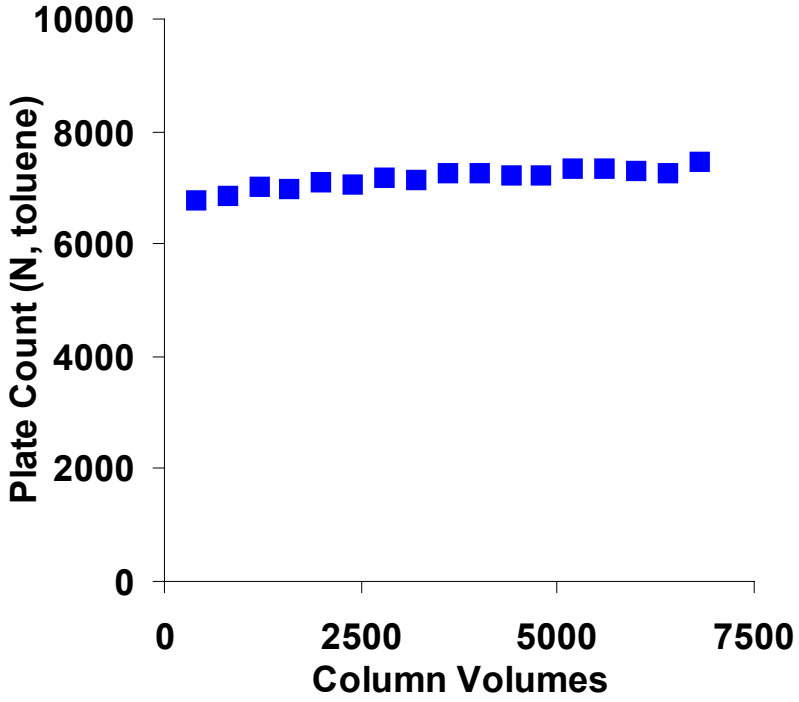




LC Conditions: Mobile phase, 40/60 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5 µl; Detection at 254 nm; where mV_x represents cavity formation and dispersion interactions, $s\pi^*_2$ represents polar and dipolar interactions, a $\Sigma\,\alpha_2$ represents hydrogen bond acidity, b $\Sigma\,\beta_2$ represents hydrogen bond basicity, and $\log k'_0$ is the intercept term.

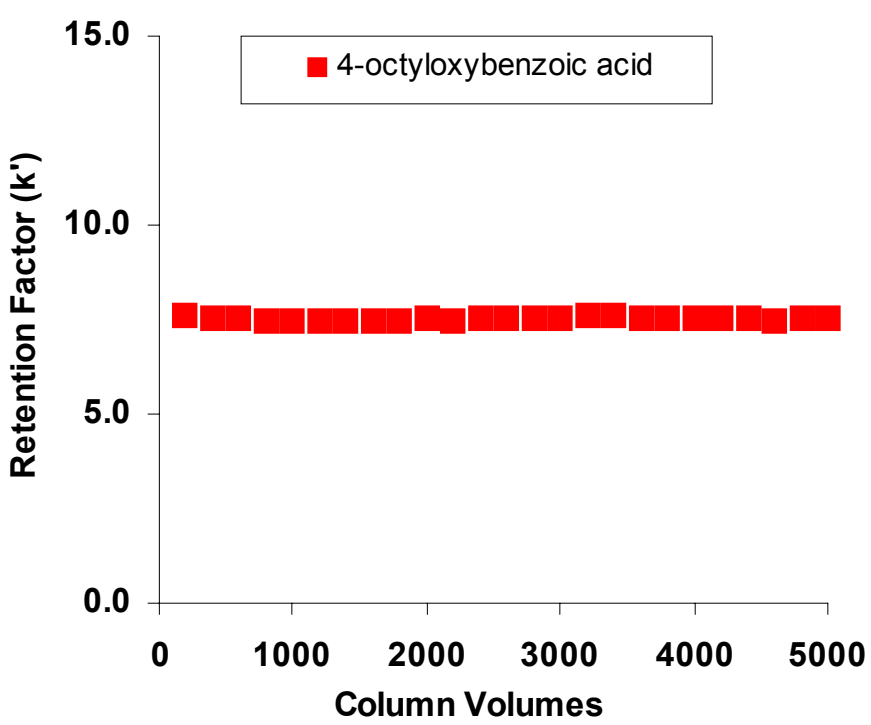
Chemical Stability – pH 1.0

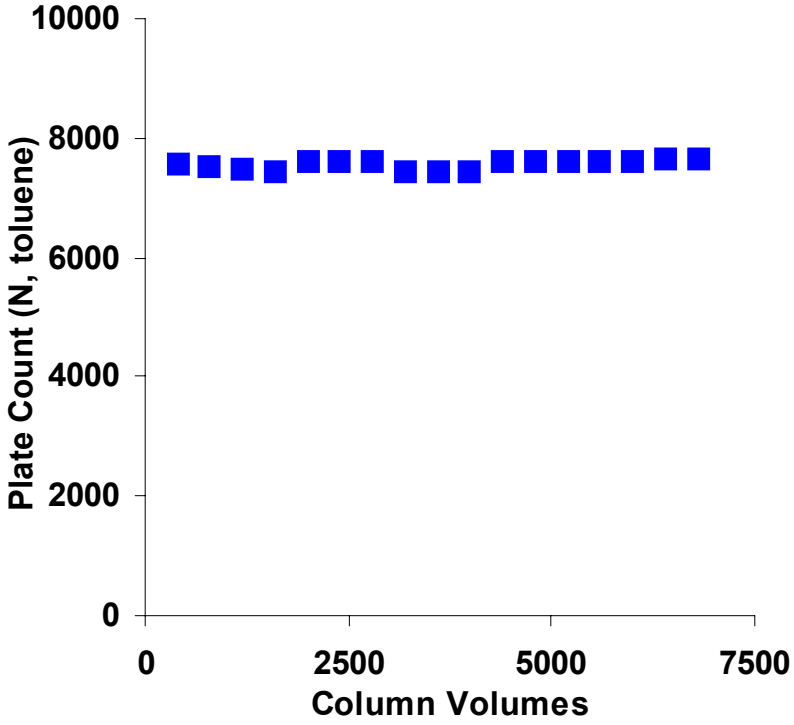




Exposure and Evaluation Conditions: Mobile phase, 15/85 ACN/0.1M Nitric acid, pH 1.0; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5 µl; Detection at 254 nm; Column, 50 mm x 4.6 mm i.d. ZirChrom-EZ

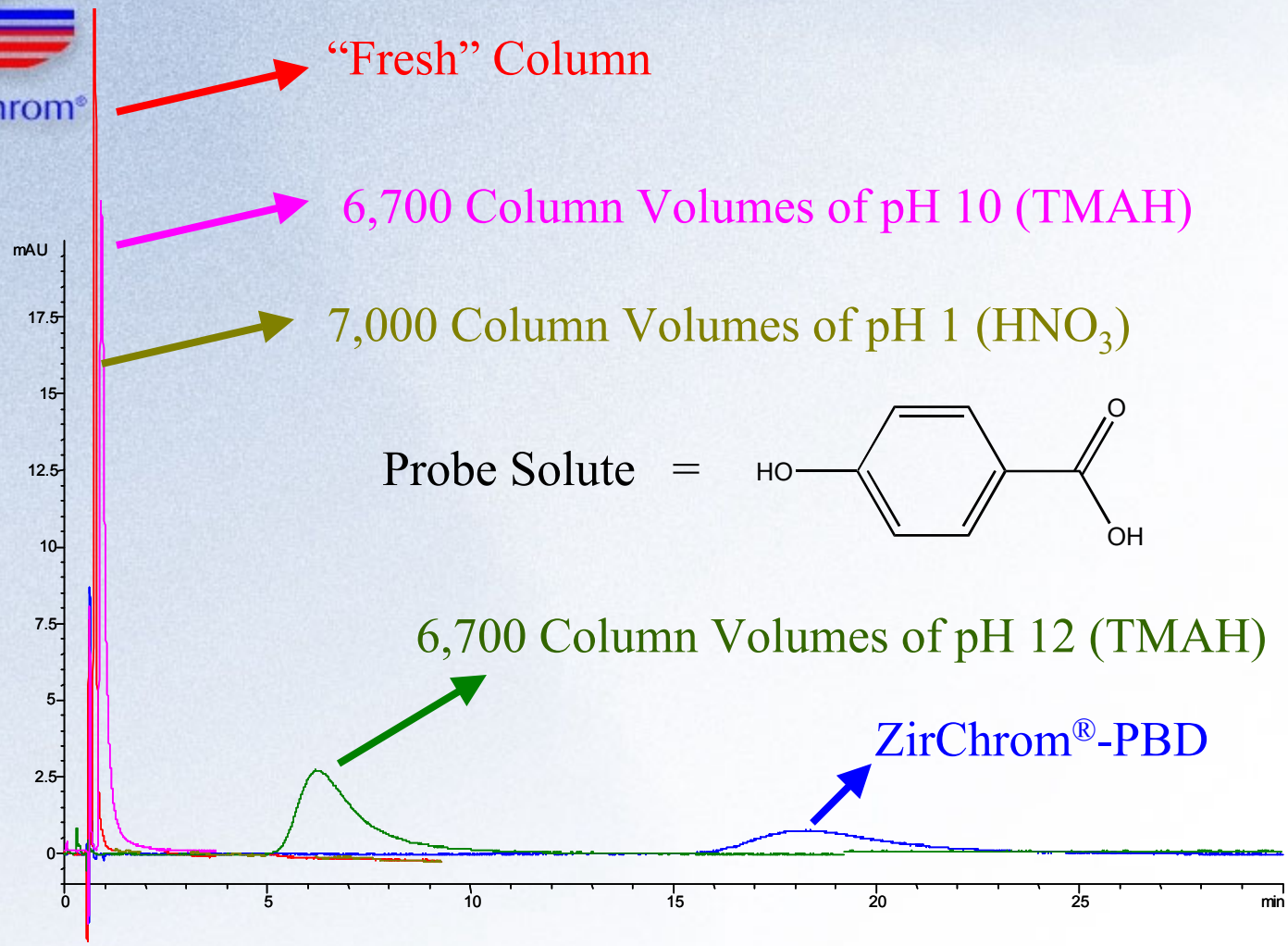
Chemical Stability – pH 10.0





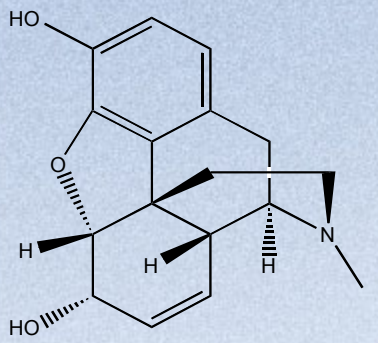
Exposure and Evaluation Conditions: Mobile phase, 15/85 ACN/0.1M Ammonium hydroxide, pH 10.0; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5 µl; Detection at 254 nm; Column, 50 mm x 4.6 mm i.d. ZirChrom-EZ

Summary of Stability Testing

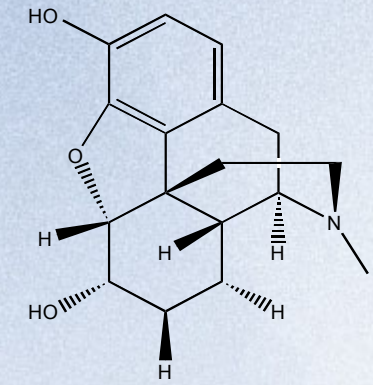


Flushing Conditions: Mobile phase, 15/85 ACN/indicated buffer; Temperature, 30 °C; **Evaluation Conditions**: Mobile phase, 15/85 ACN/20 mM ammonium acetate, pH 4.0; Temperature, 30 °C. Injection volume, 5 µl; Detection at 254 nm; Solute: Hydroxybenzoic acid.

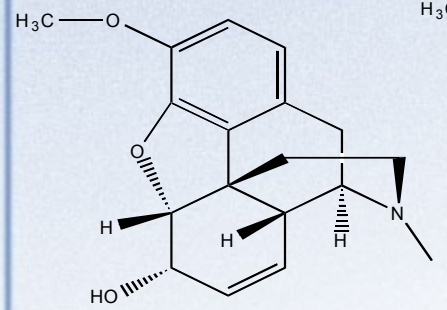
Pharmaceutical Applications – Opioid Isomers



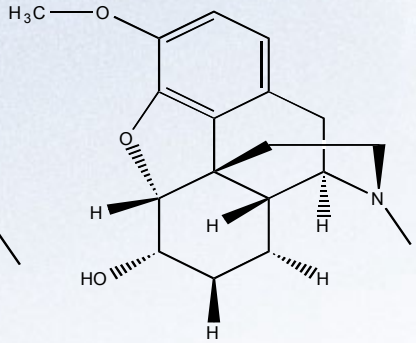
Morphine
M.W. 285.33



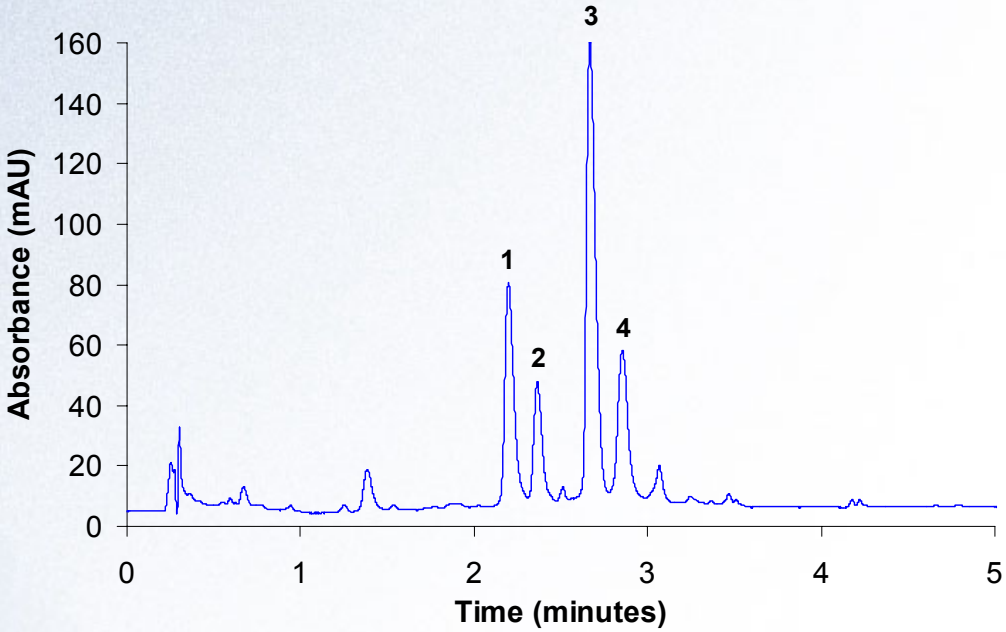
Hydromorphone
M.W. 285.33



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 °C; Injection volume, 10 µl; Detection at 254 nm.; Solutes: 1=Morphine, 2=Hydromorphone, 3=Codeine, 4=Hydrocodone