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

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Pittcon 2004

# 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

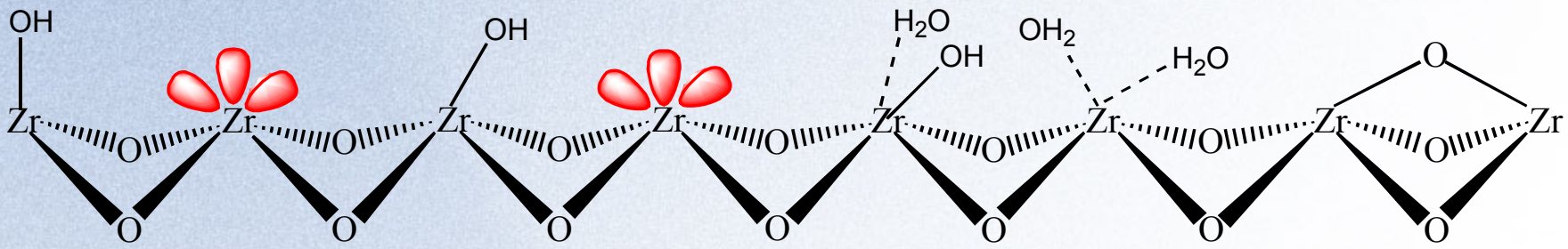
ZirChrom®

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

++ excellent; + good; -- fair.

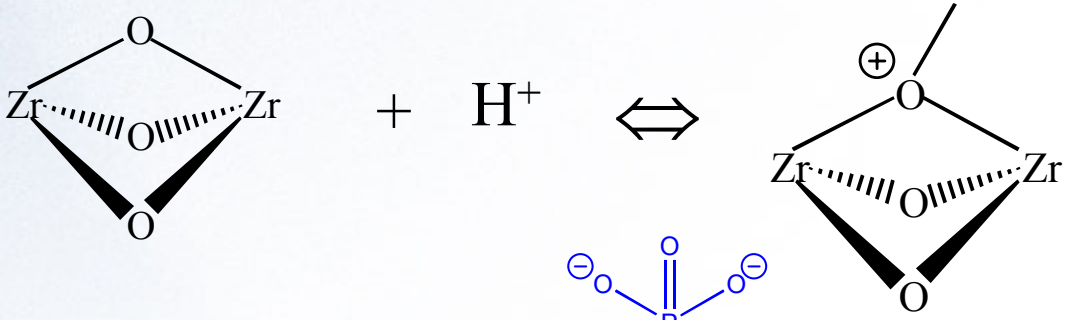
# Surface Chemistry of Zirconia-Based Supports for HPLC

ZirChrom®

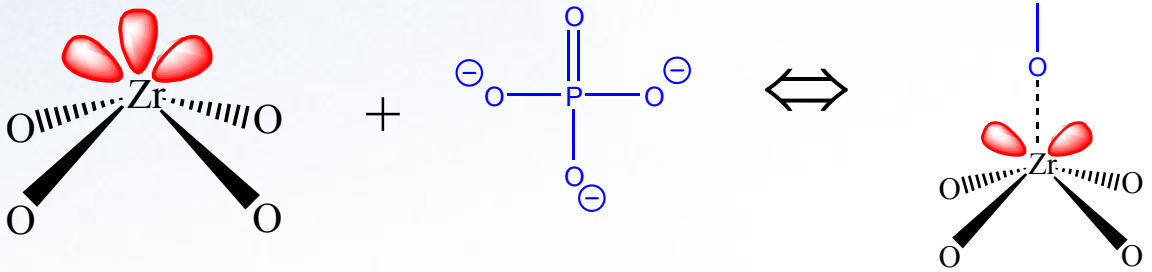



**Brönsted Acid:** $ZrOH + OH^- \Leftrightarrow ZrO^- + H_2O$

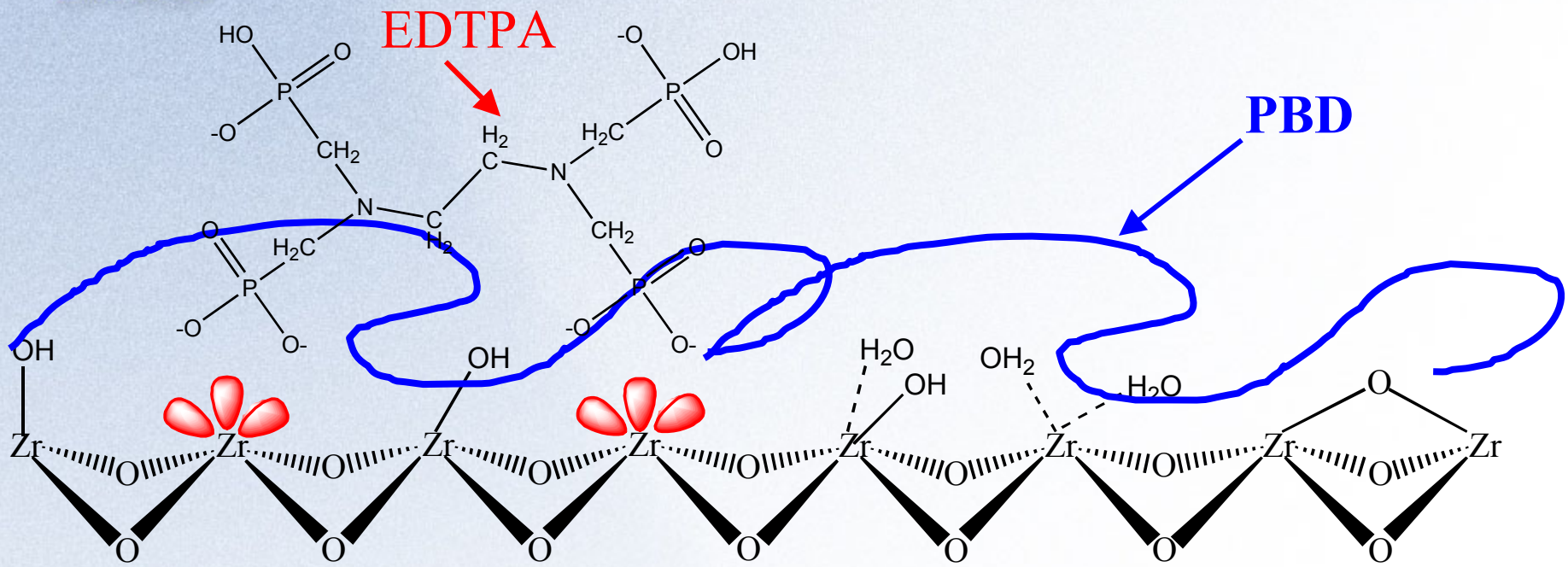
**Brönsted Base:**




**Lewis Acid:**



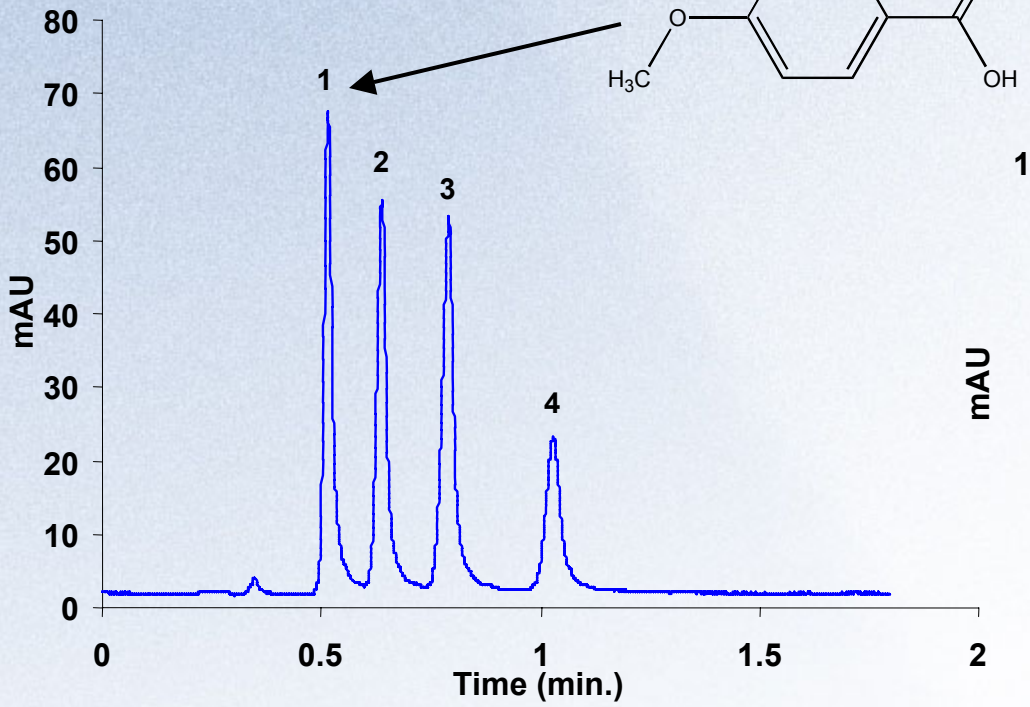
# Synthesis of a Lewis-Acid Deactivated Reversed-Phase Support

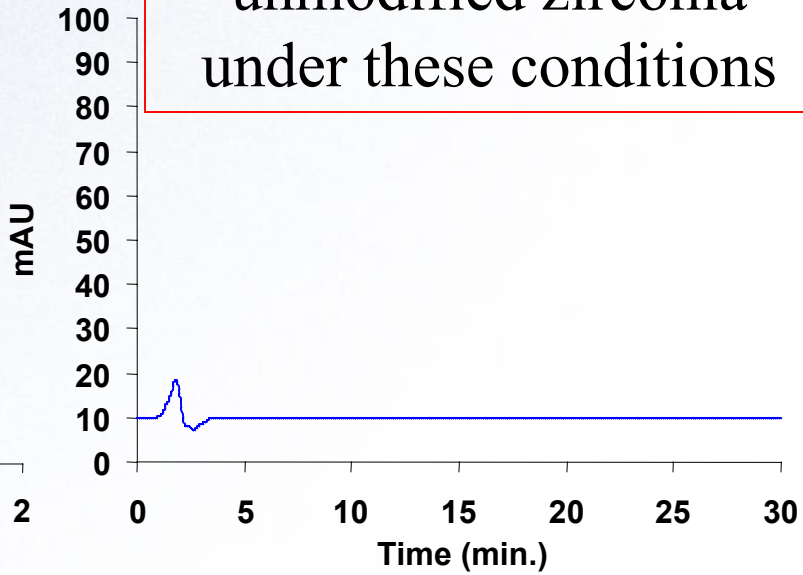



1. Coat bare zirconia with polybutadiene (PBD)[1]
2. Crosslink PBD chains together using dicumyl peroxide as initiator
3. Reflux PBD-$ZrO_2$ in Ethylenediamine-N,N,N',N'-tetra(methylenephosphonic)acid (EDTPA) solution
4. Wash to remove residual EDTPA


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



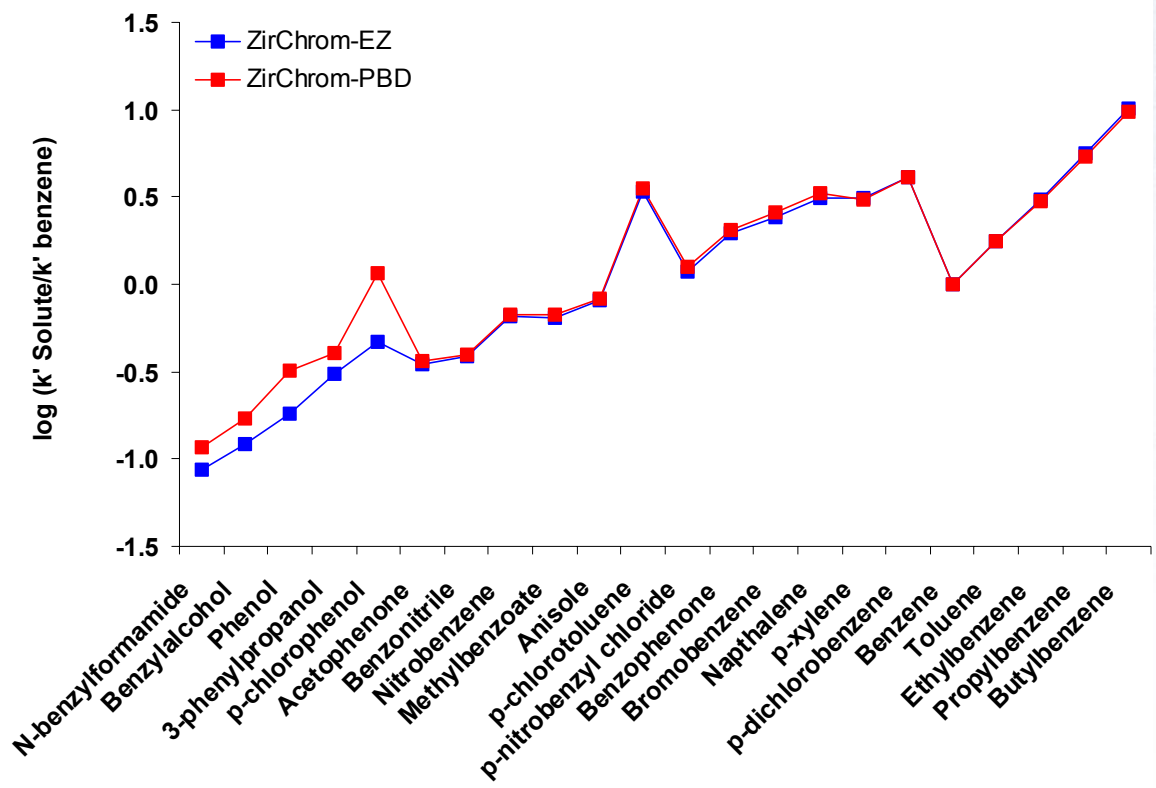



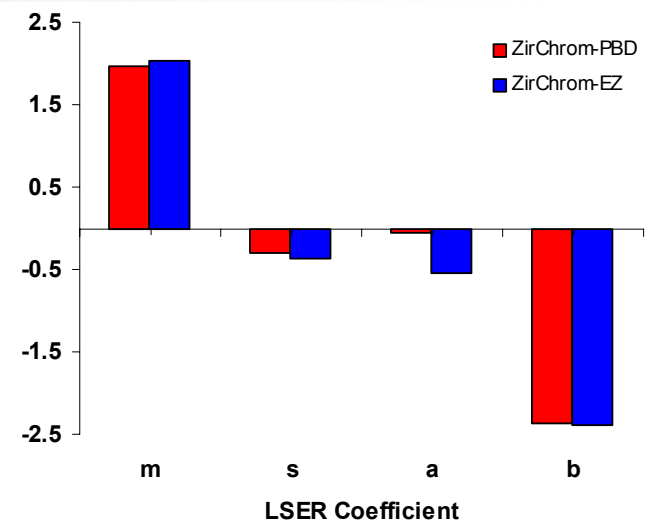

These analytes are irreversibly adsorbed on unmodified zirconia under these conditions

**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

# LSER Comparison of ZirChrom®-PBD and ZirChrom®-EZ

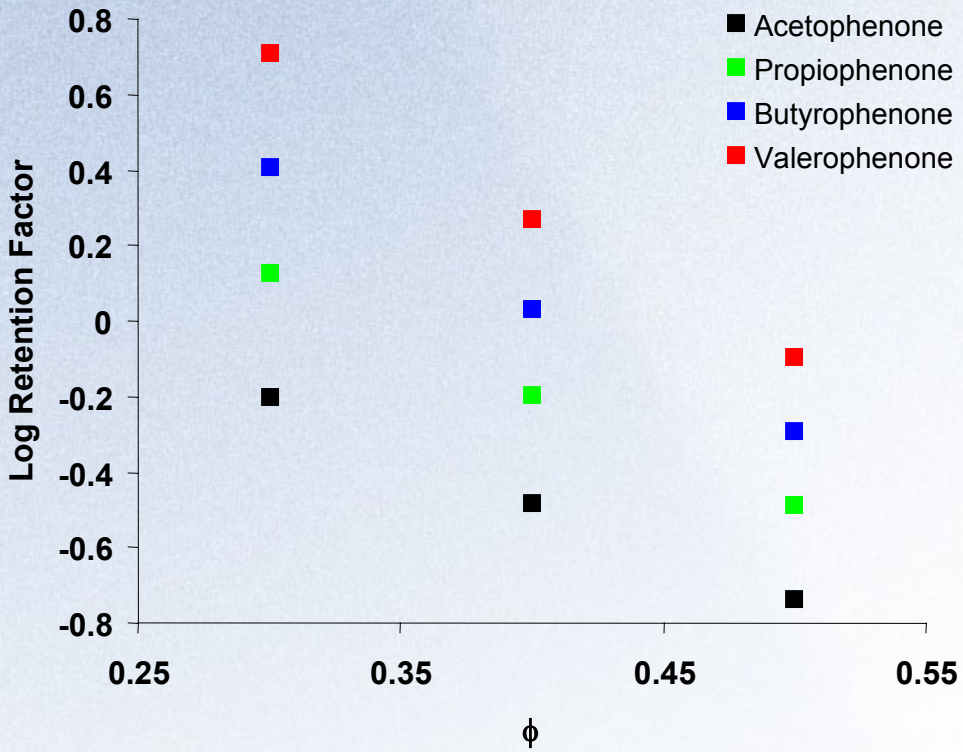
$$\log k' = \log k'_0 + mV_x + s\pi^*_2 + a\,\Sigma\alpha_2 + b\,\Sigma\beta_2$$




**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.

# Reversed-Phase Characteristics

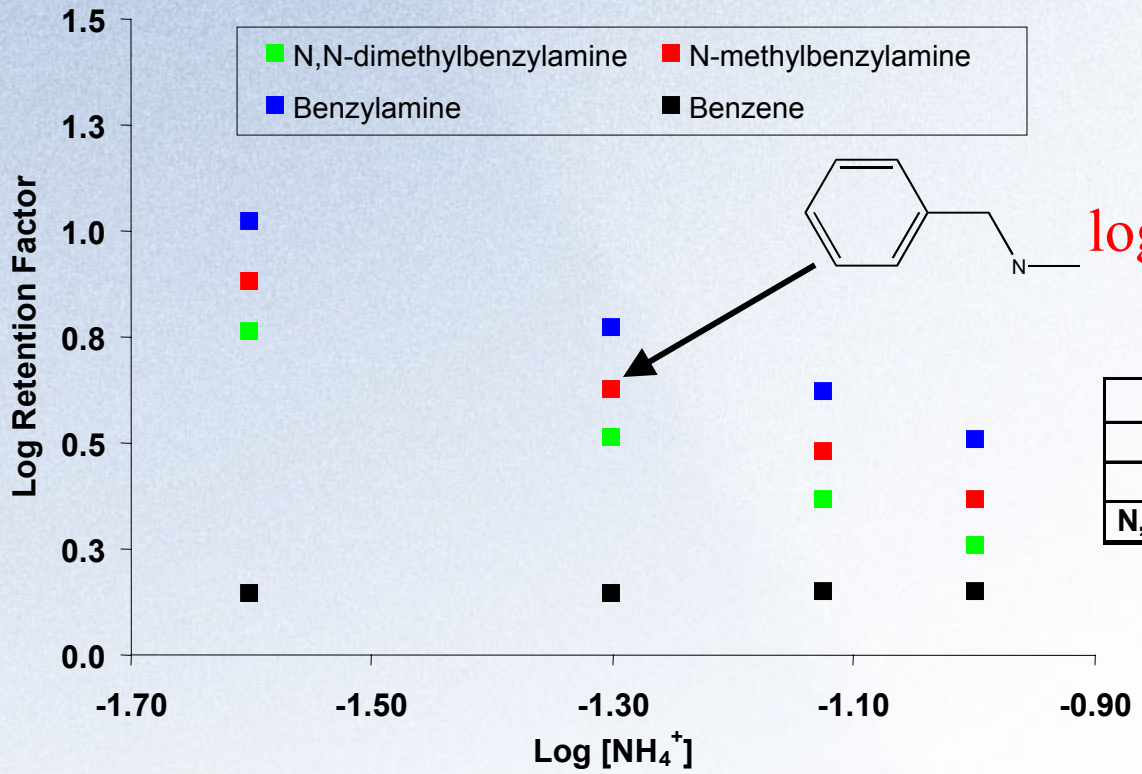



$$\log k'_{RP} = \log k_w - S\phi$$

| 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






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

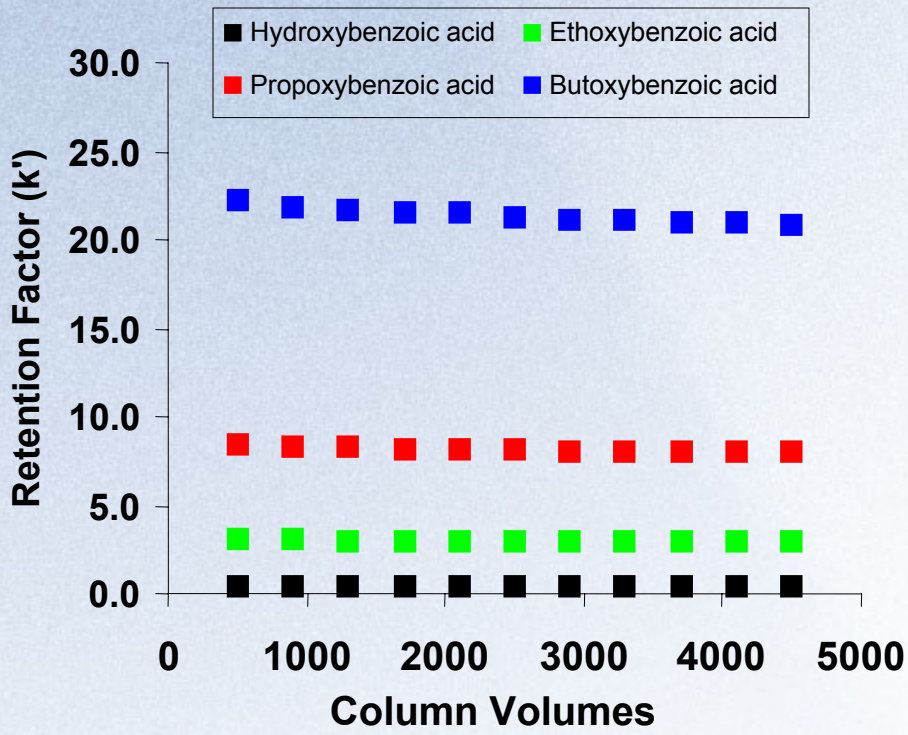
| | Slope | $R^2$ |
|---|---|---|
| Benzylamine | -0.846 | 0.999 |
| N-methylbenzyamine | -0.847 | 0.999 |
| N,N-dimethylbenzylamine | -0.840 | 0.999 |

**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

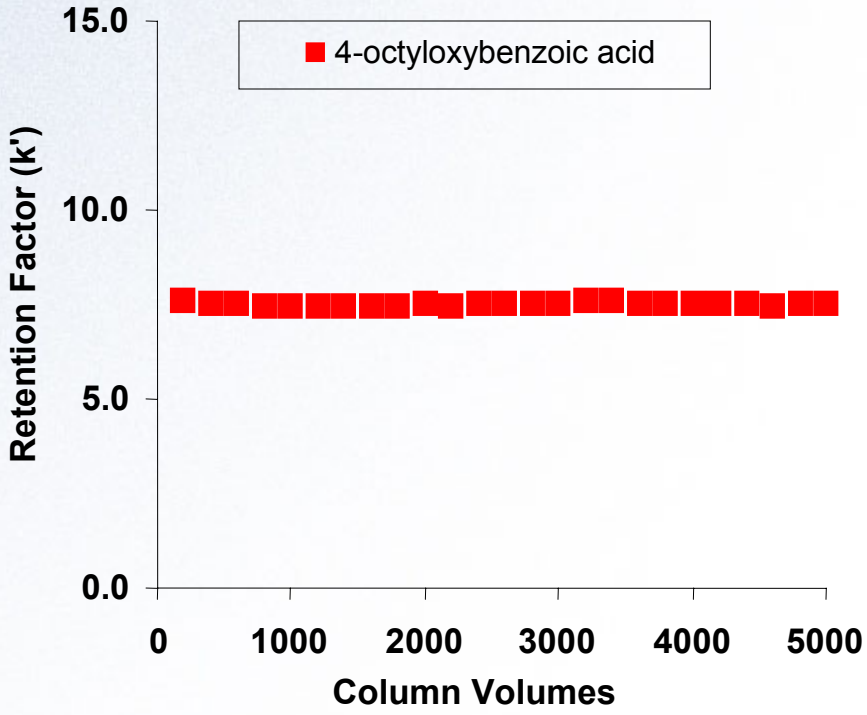
# Chemical Stability

ZirChrom®

## 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

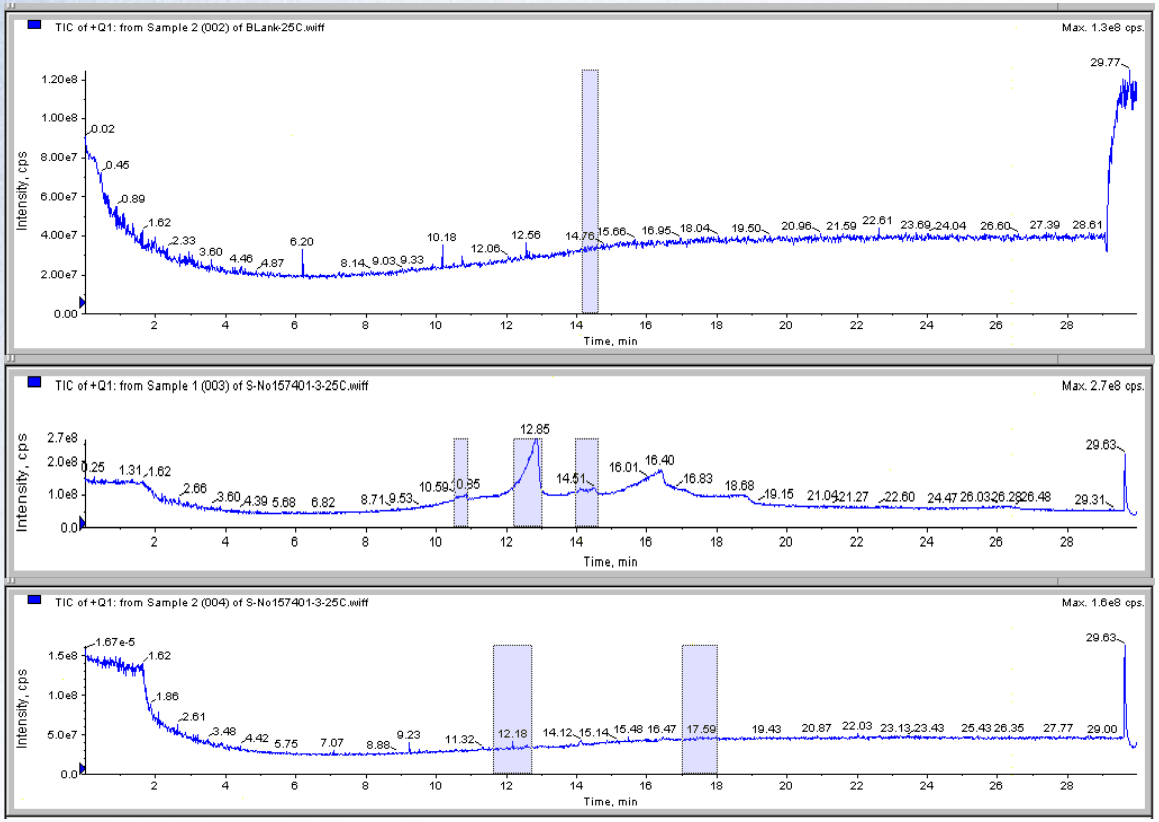
# LC/MS Bleed Study – TIC's From Gradient Elution

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

Blank gradient – No column installed

Gradient #1

Gradient #2

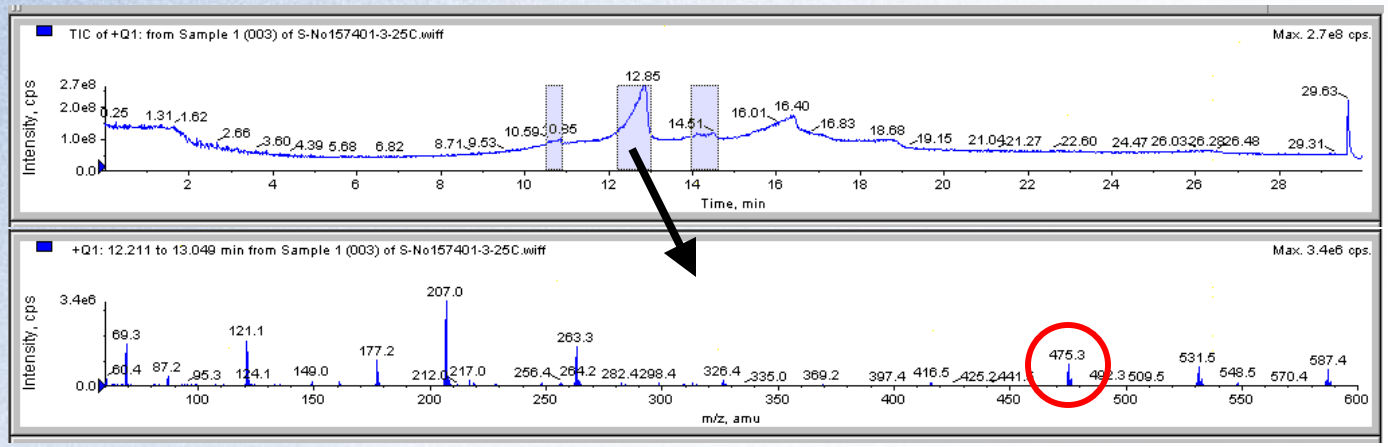


# Extracted Spectra

**Gradient #1**

TIC

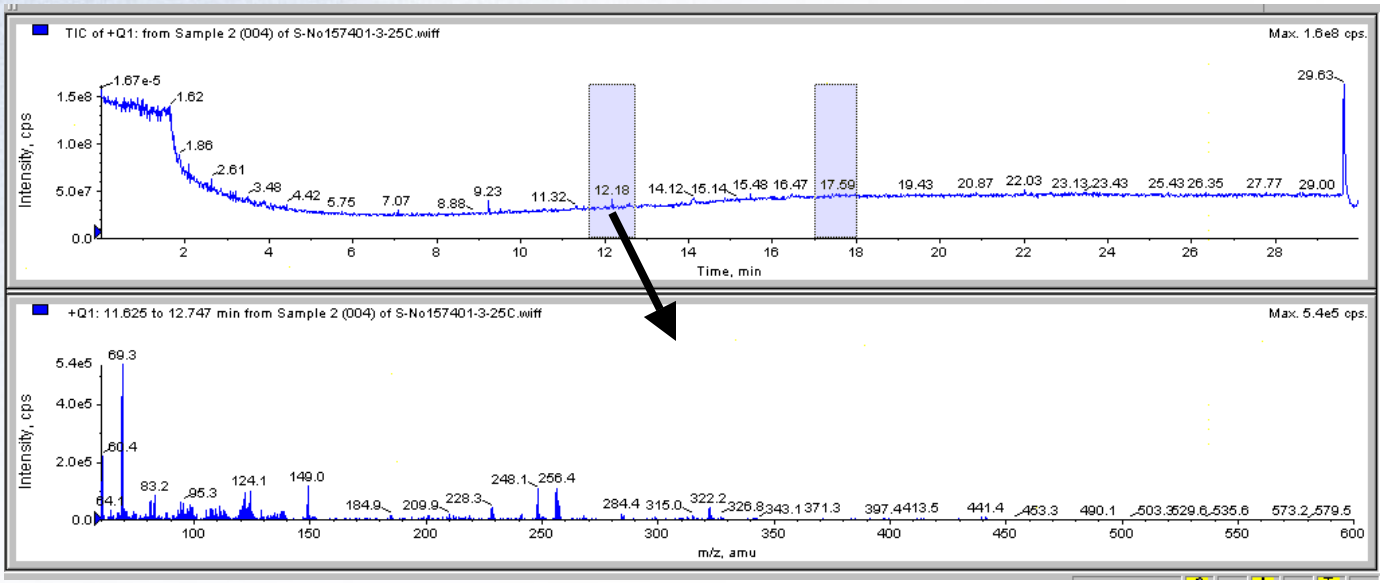
Scan



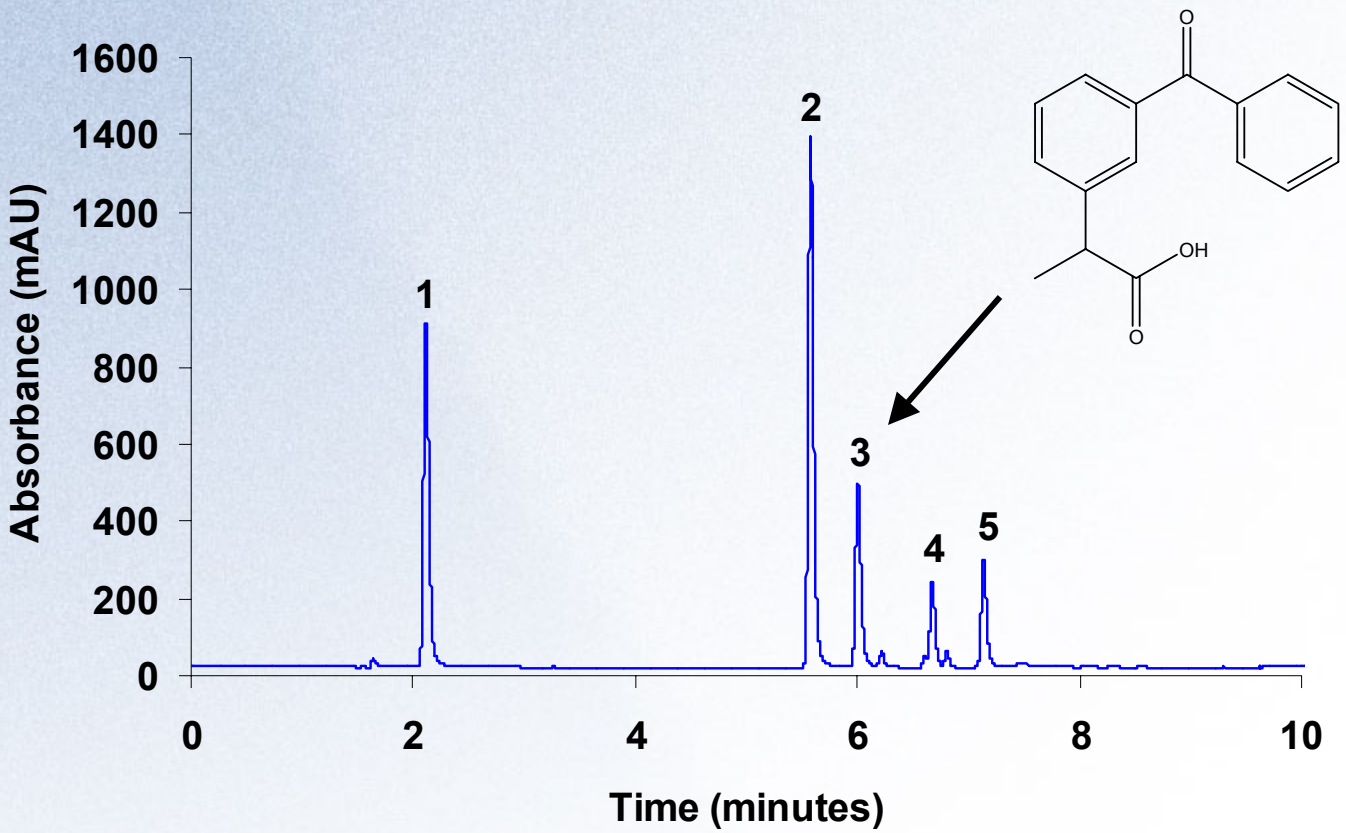

**Gradient #2**

TIC

Scan



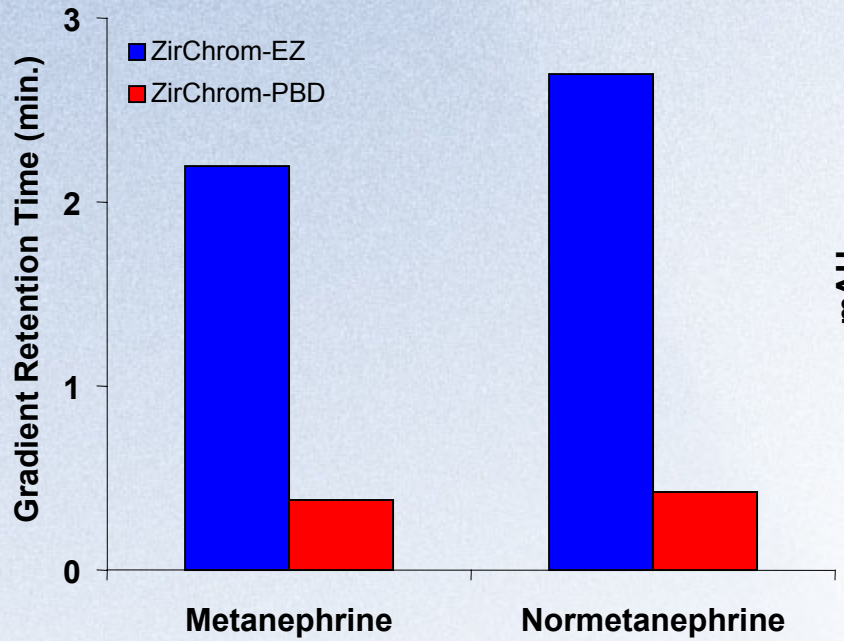
# Separation of Acidic Drugs with LC/MS-Friendly Mobile Phase

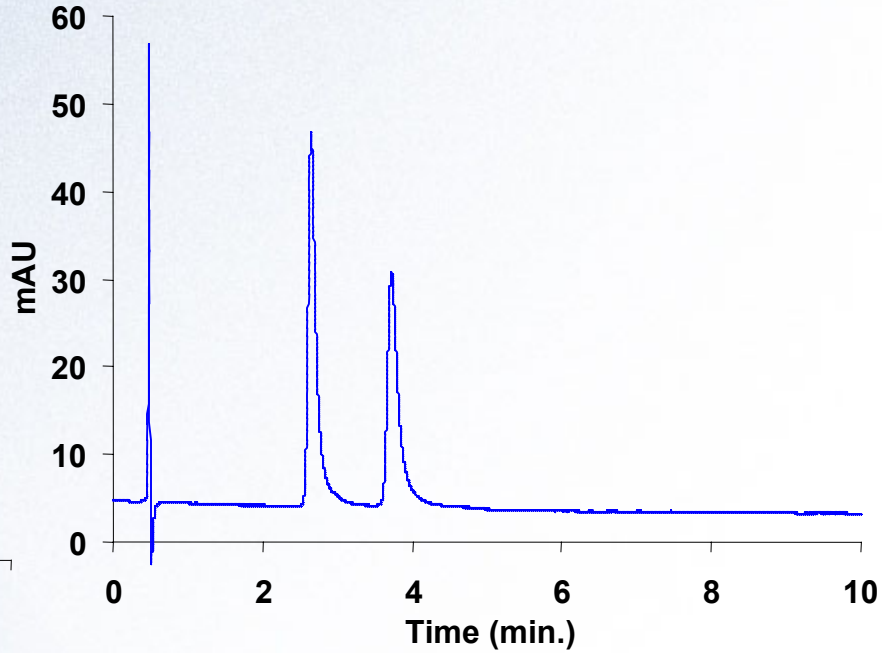



| Time (min.) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 10 | 10 | 90 |

**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



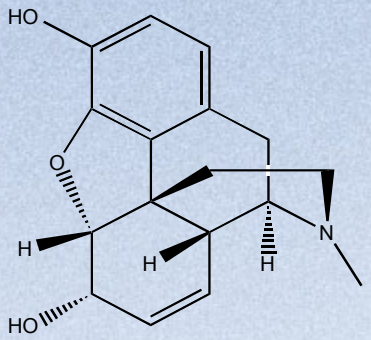

| Time (min.) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 5 | 10 | 90 |

**A: 20mM Ammonium acetate, pH 6.0**

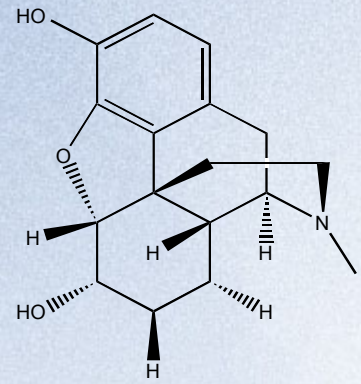
**B: Acetonitrile**

**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.

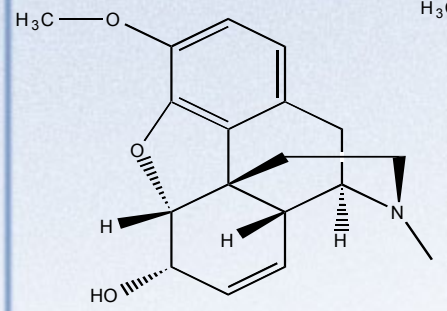
# Pharmaceutical Applications – Opioid Isomers



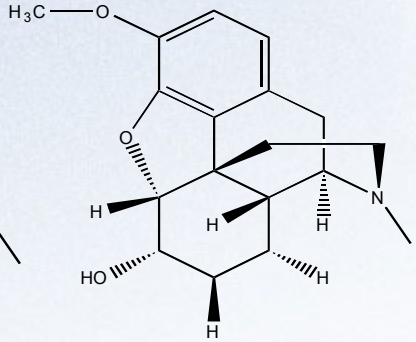
**Morphine**
M.W. 285.33



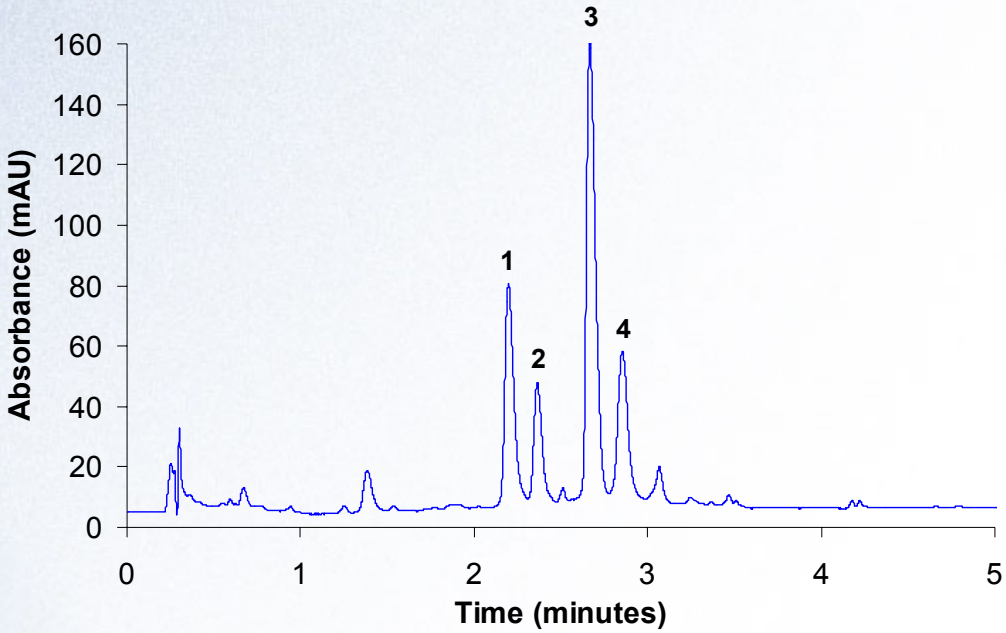
**Hydromorphone**
M.W. 285.33



**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

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

ZirChrom®

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