Carbonaceous RPLC Stationary Phases Based on Porous Zirconia – STABILITY is the Name and SELECTIVITY is the Game

Paul T. Jackson

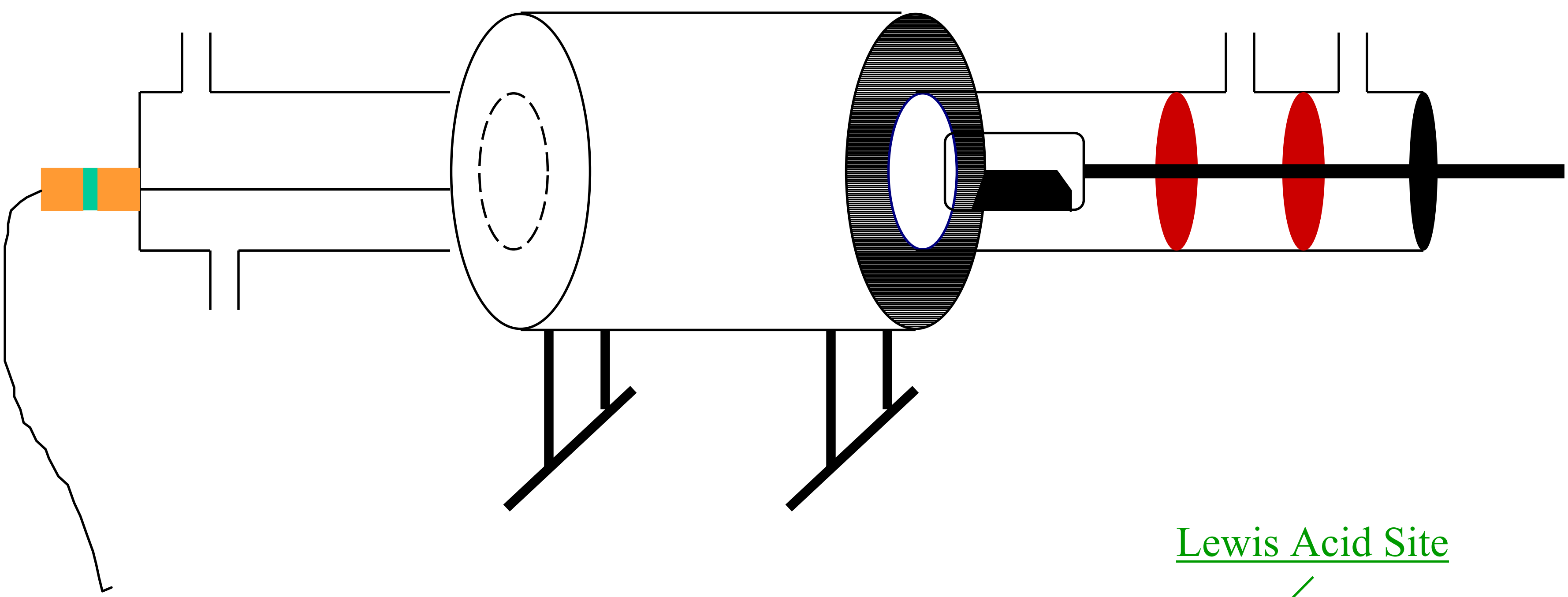
Chemistry Department, St. Olaf College
1520 St. Olaf Avenue
Northfield, MN 55057 USA
jackson@stolaf.edu

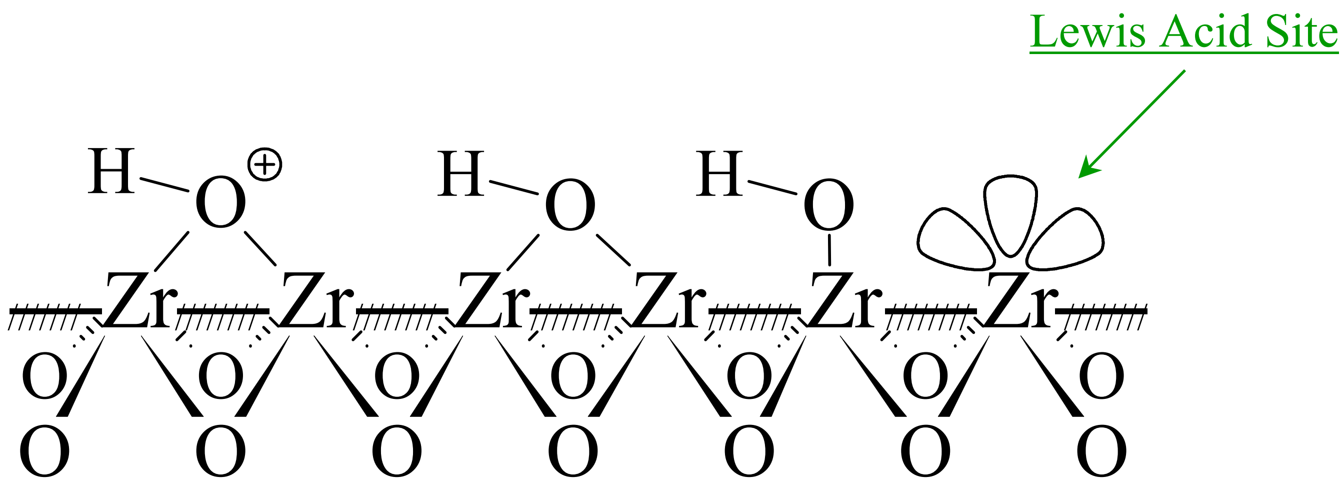
ST·OLAF COLLEGE

Presentation Summary

- Carbon coated porous zirconium oxide (C/ZrO_2)
  - ✓ Material synthesis
  - ✓ General retention characteristics
- Selectivity and Isomeric Separations
  - ✓ Constitutional and positional isomers
  - ✓ Diastereomers - amino acid esters, pharmaceuticals
- Selectivity and Chemical/Thermal Stability
  - ✓ PAHs and chlorinated phenols — T^3C
  - ✓ 100% Water mobile phases — 2D Separations
- Carbon Modification
- Crystal Ball Gazing - the Future of Carbon Separations

Carbon Modified Porous Zirconia C/ZrO_2

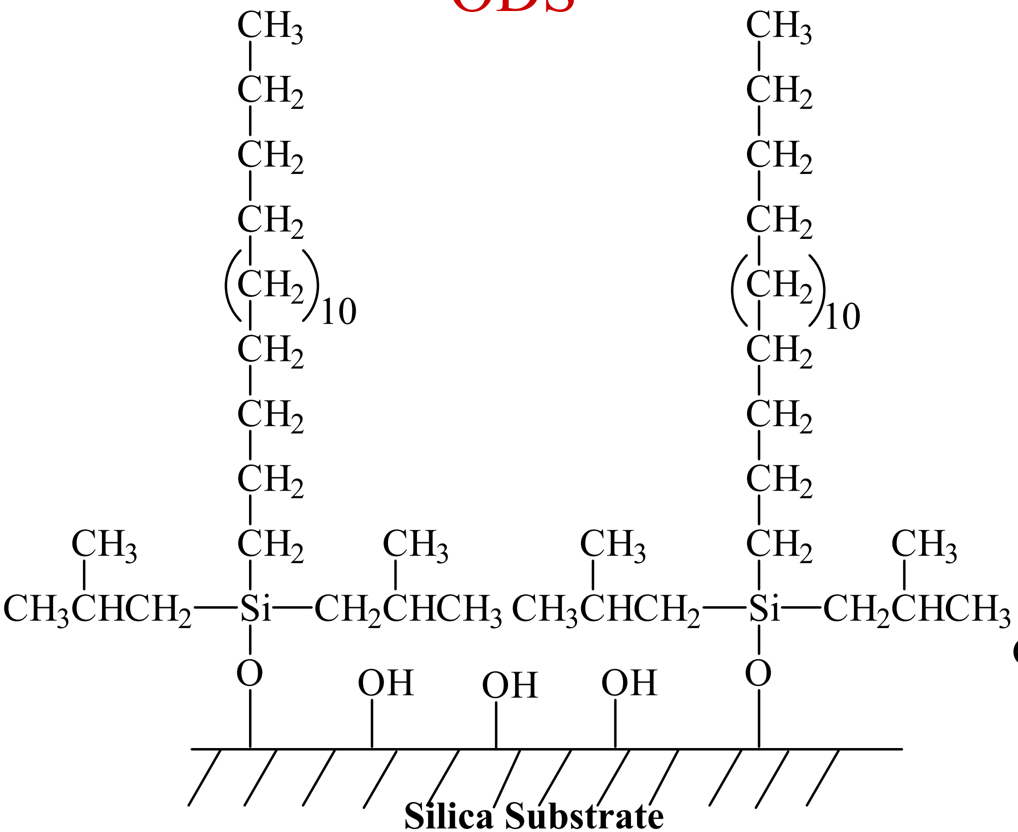




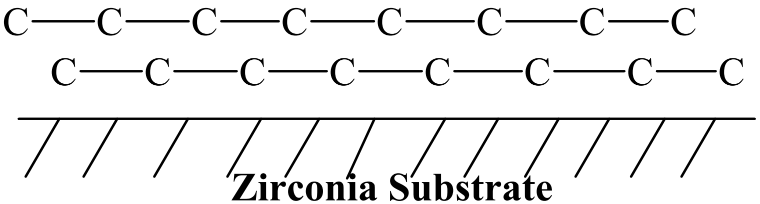
Bulk Zirconia

Liquid Chromatographic Stationary Phases

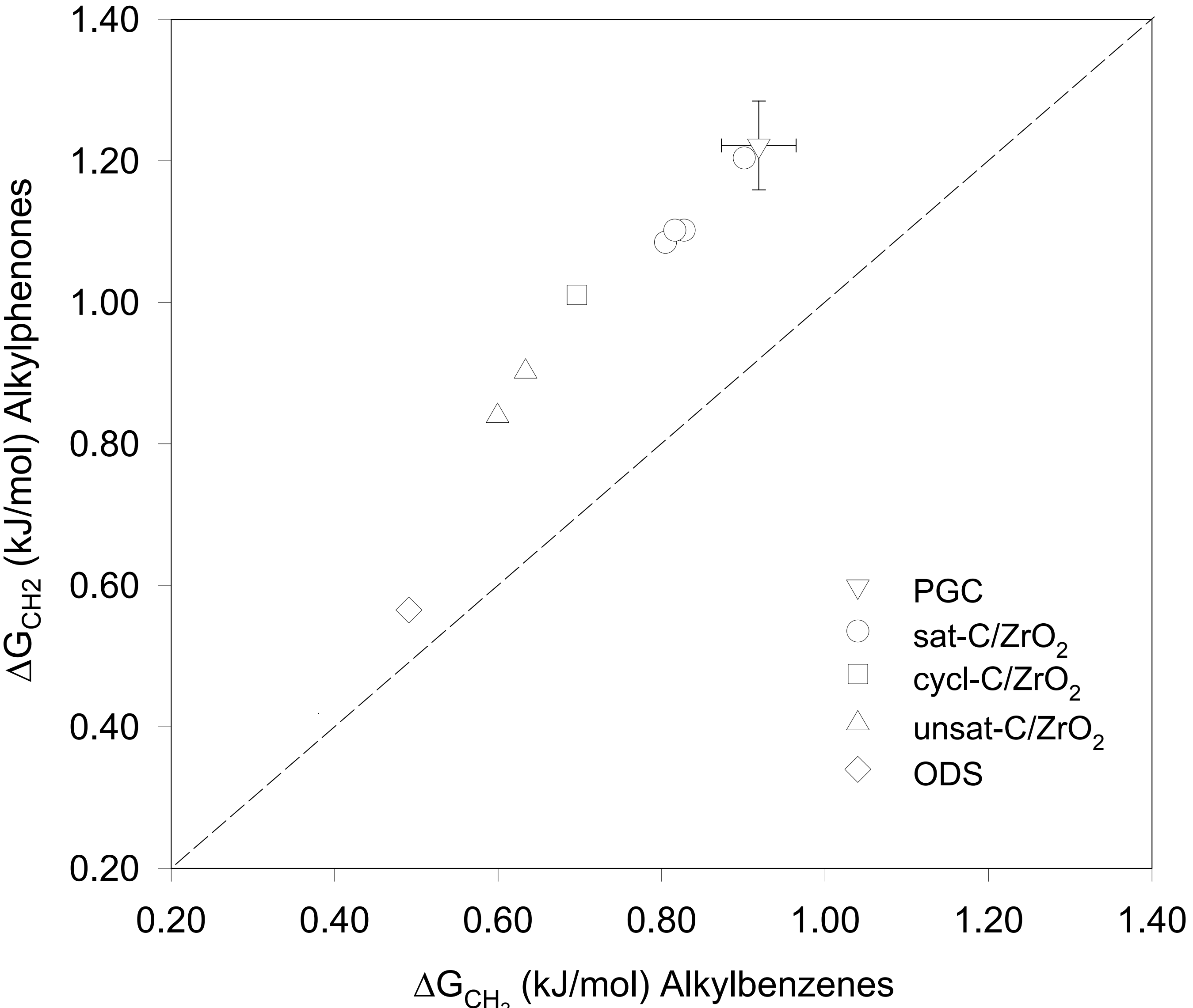
ODS



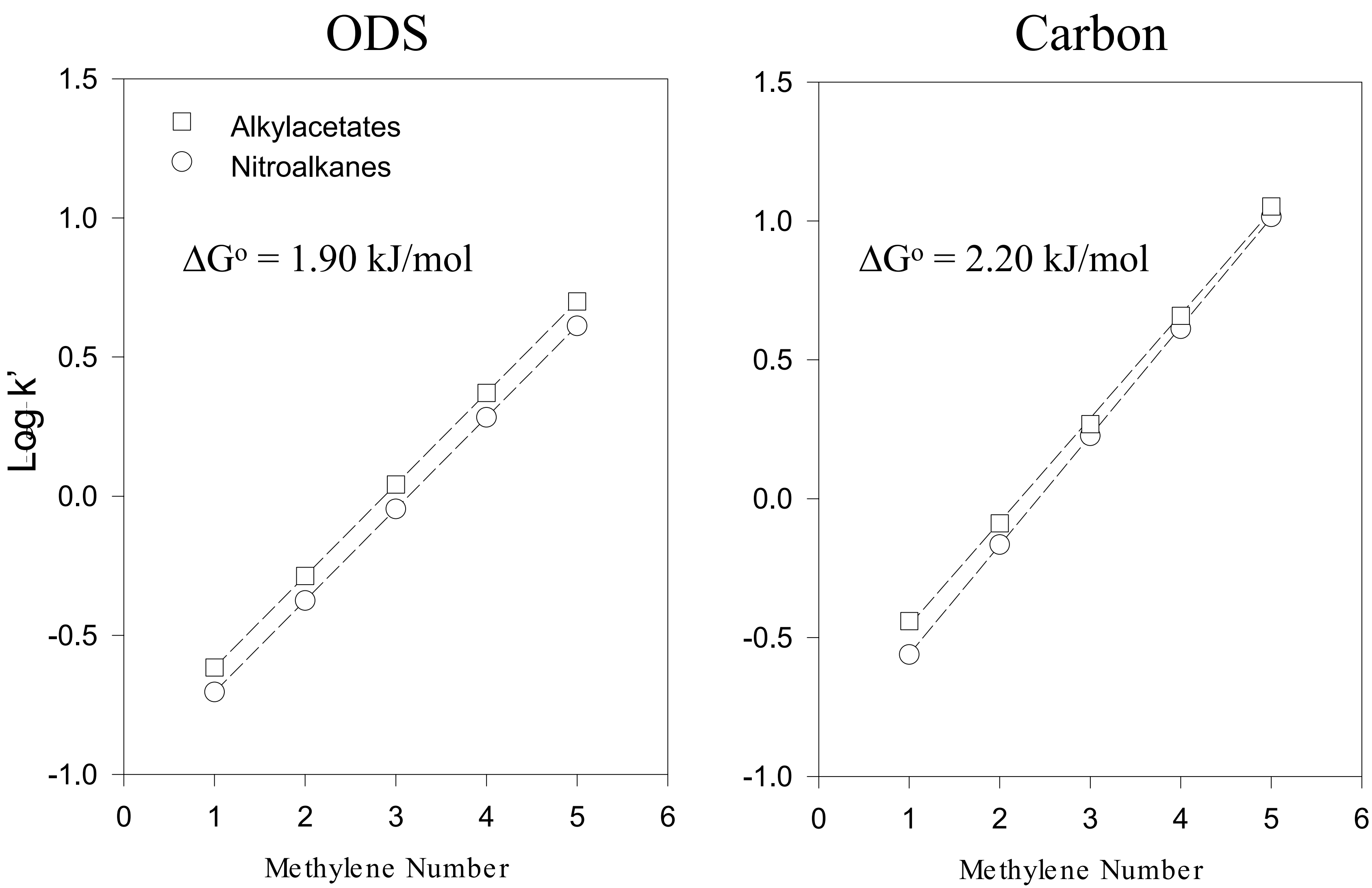
Carbon



Carbon Phase Classification



Methylene Selectivity of Different Homolog Series



Linear Solvation Energy Relationships

$$\Delta G_X = \Delta G_O + \mathbf{m}\, V_X + \mathbf{s}\, \pi^*_2 + \mathbf{a}\, \alpha_2^H + \mathbf{b}\, \beta_2^H + \mathbf{r}\, R_2$$

SOLUTE PROPERTIES			SYSTEM PROPERTIES	
V_X	molecular volume	⟷	m	cavity formation/ dispersion interactions
π^*_2	dipolarity/polarizability	⟷	s	dipolarity/polarizability
α_2^H	hydrogen bond acidity	⟷	a	hydrogen bond basicity
β_2^H	hydrogen bond basicity	⟷	b	hydrogen bond acidity
R_2	excess molar refraction	⟷	r	polarizability correction

Intermolecular Interactions Involved in Solute Retention

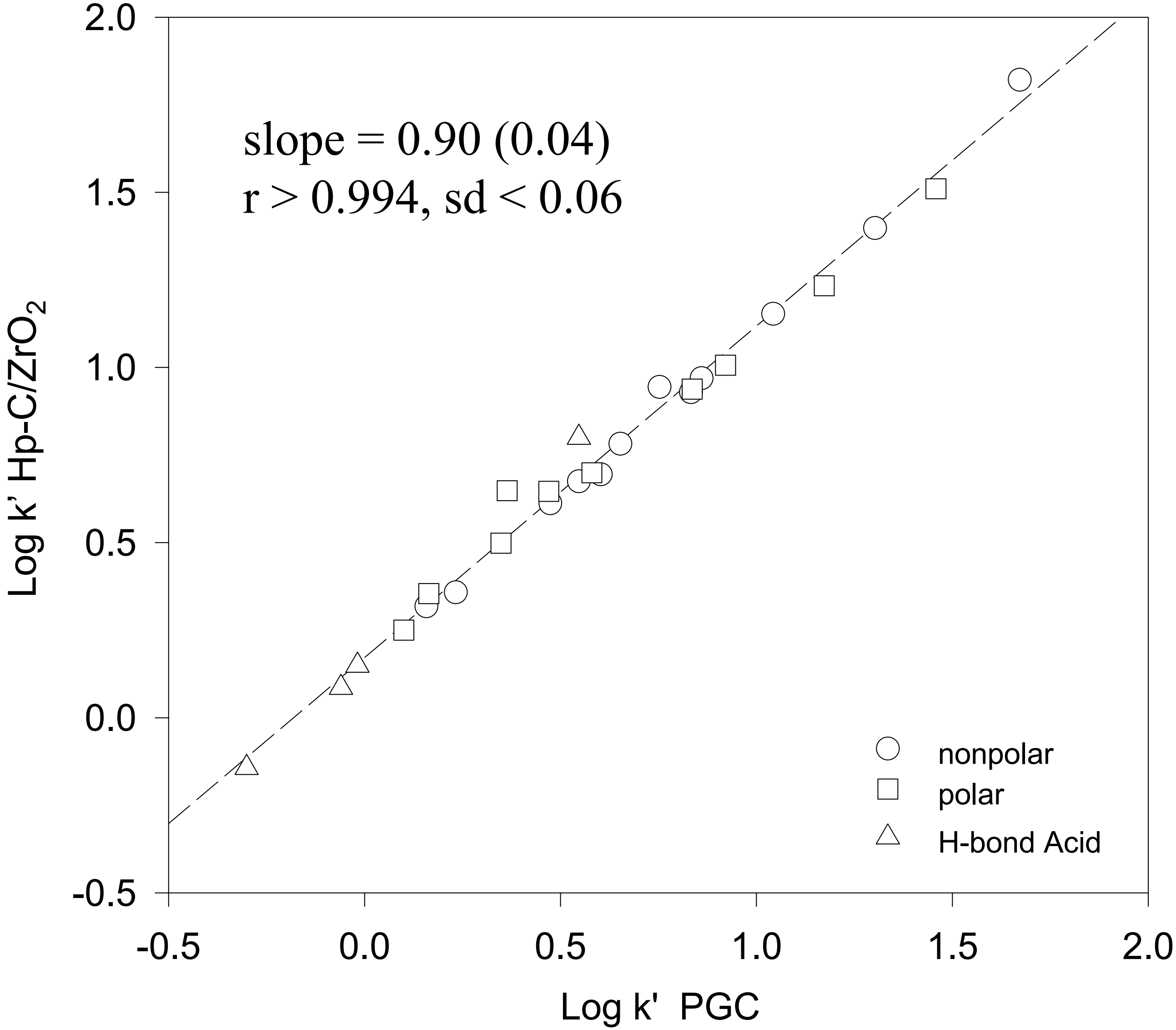
$$\log k' = SP_o + \boldsymbol{m}V_x + \boldsymbol{s}\pi^*_2 + \boldsymbol{a}\Sigma a_2 + \boldsymbol{b}\Sigma b_2$$

- Differences in Dipolarity/Polarizability
- C/ZrO_2 and PGC phases show similar response

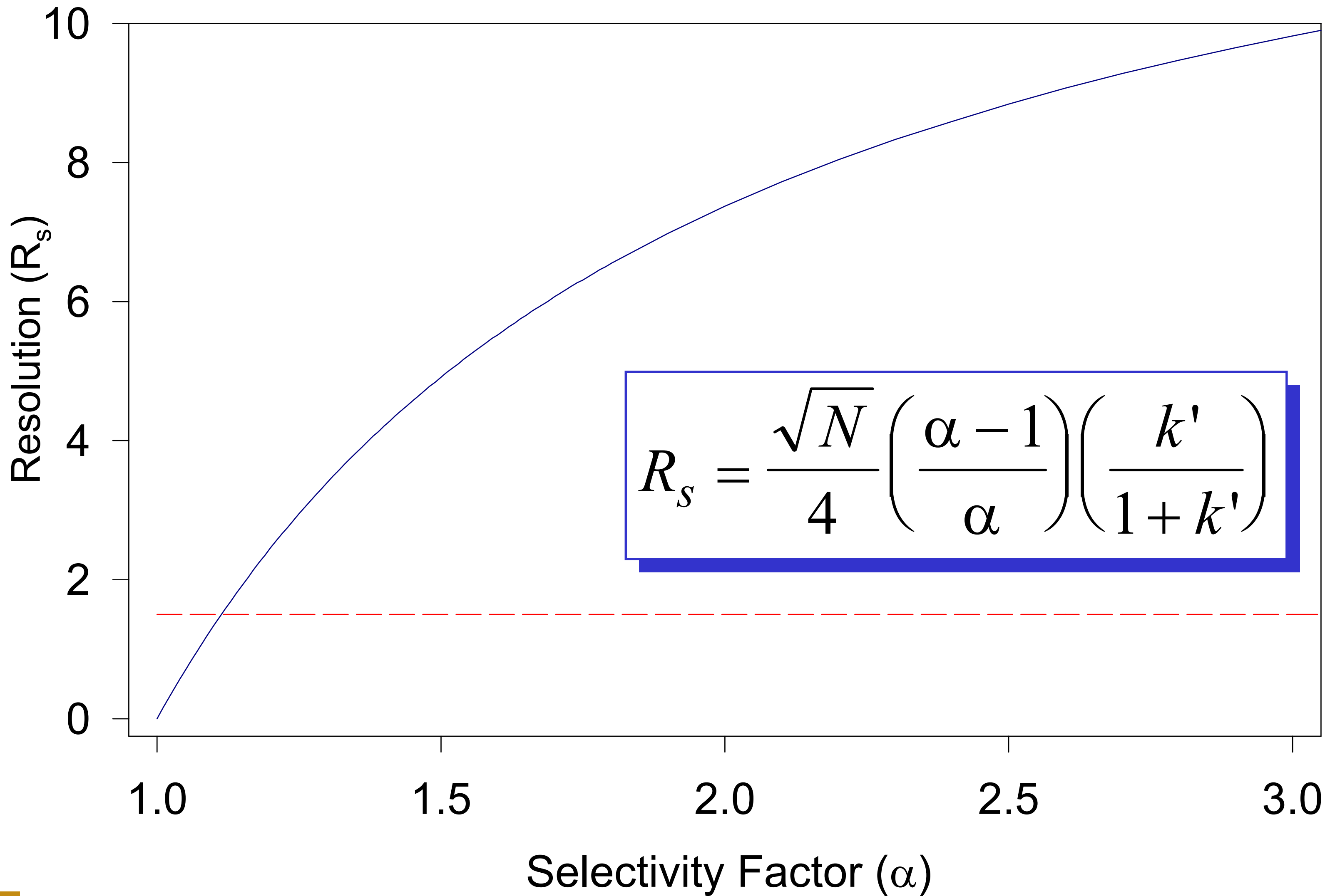
		Carbon	**ODS**
$\mathbf{mV_x}$	cavity formation, dispersion	++	++
$\mathbf{s\pi^*_2}$	dipolarity, polarizability	**++**	**-**
$\mathbf{a\Sigma\alpha_2}$	hydrogen bond acidity	**-**	**-**
$\mathbf{b\Sigma\beta_2}$	hydrogen bond basicity	**--**	**--**

Jackson, P.T.; et al., *Anal. Chem.* **1997**, *69*, 416-425

Retention Correlation between Carbon RPLC Media

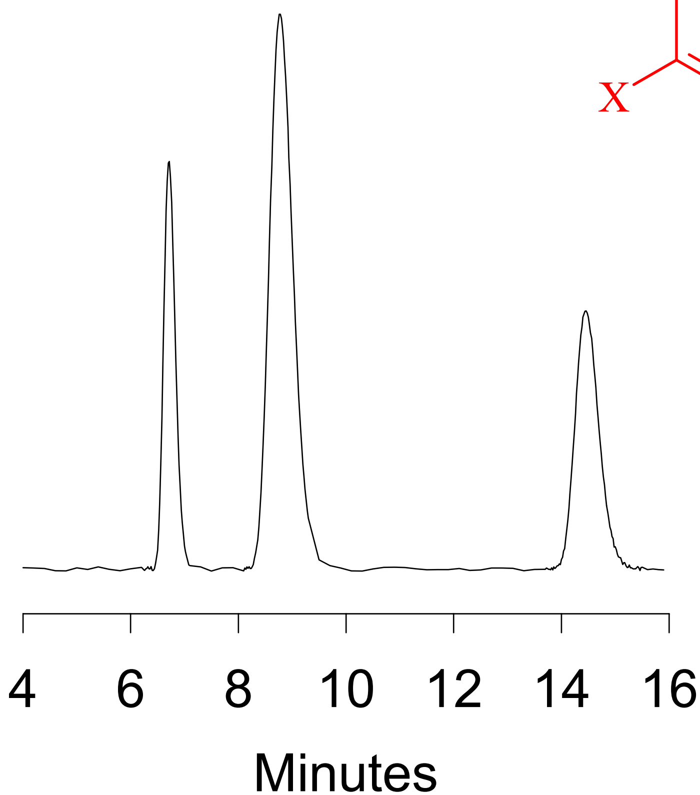


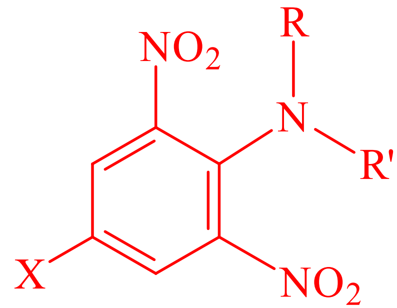
Selectivity Impacts Resolution



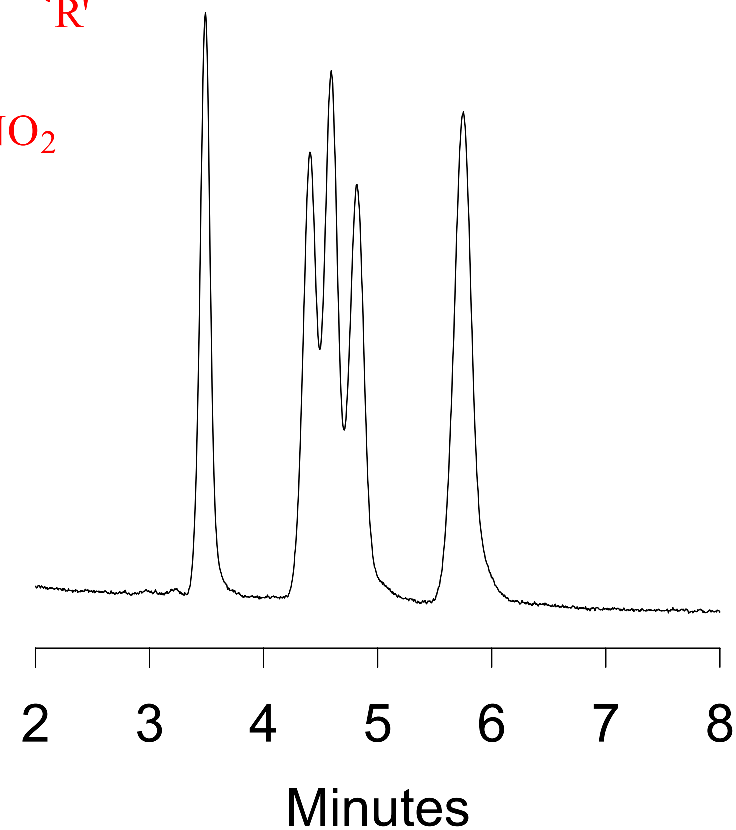
Fluralin Herbicide Separation (EPA Method 627)

ODS



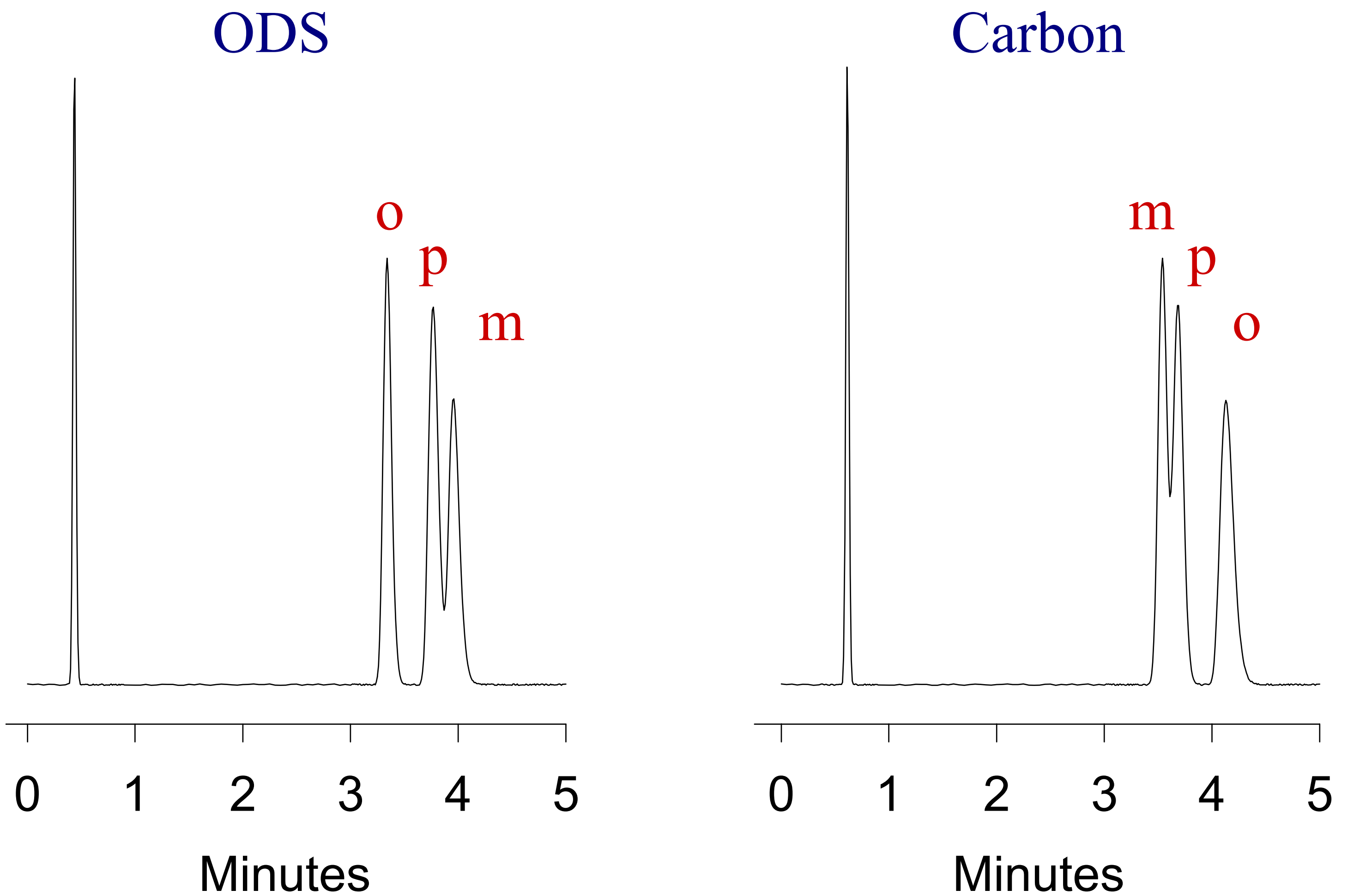


Carbon



50 x 4.6 mm, 60:40 ACN:water, 1 mL/min, 30 °C,
5 µL inj. [50 µg/mL], 222 nm detection. Elution order:
Ethalfluralin, Trifluralin, Benfluralin, Profluralin, Isopropalin

Optimized Separation of Dibromobenzenes



50 x 4.6 mm, 1 mL/min, 30 °C, 2 μL inj, 254 nm detection,
ODS 60:40 ACN:water, CARB 80:20 ACN:water.

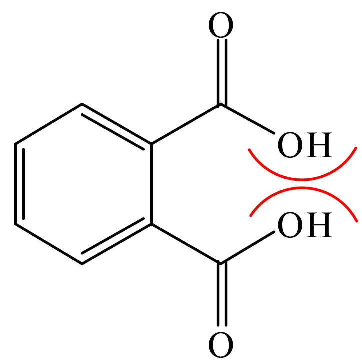
ST · OLAF COLLEGE

PTJ

Jackson, P.T.; Carr, P.W. *J. Chromatogr. A* **2002**, *958*, 121-129.

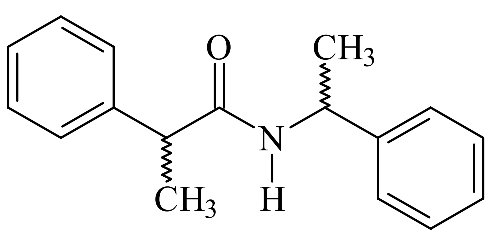
Elution Order of Constitutional Isomers

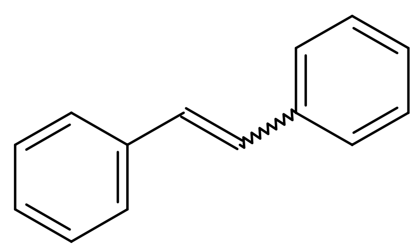
Substituent	ODS	Carbon
dimethyl	o < m = p	m < p < o
dichloro	o < p < m	m < p < o
dibromo	o < p < m	m < p < o
dialdehyde	o < m < p	o < m < p
dinitro	o < m < p	o << m < p
dicarboxylate	o < m < p	o << m < p



Selectivity and Isomeric Analytes

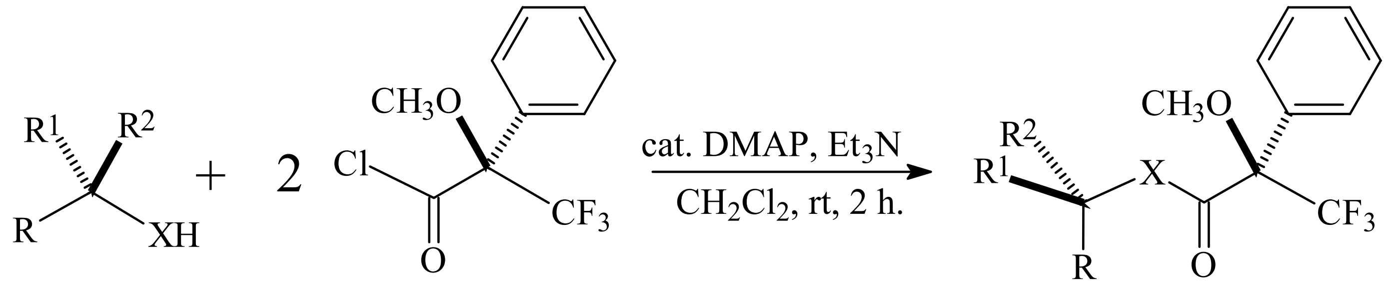
Compound	ODS	Carbon
m/o-dinitro	1.04	4.50
m/o-dicarboxylate	1.77	9.60
di(phenethyl)amide	1.19	1.20
cis-/trans-stilbene	~2	22





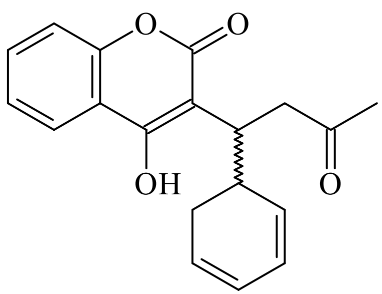
cis-/trans-stilbene

Mosher Reaction and Compound Structures

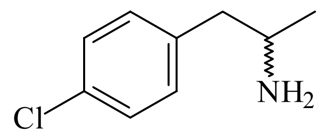


X = O, NH

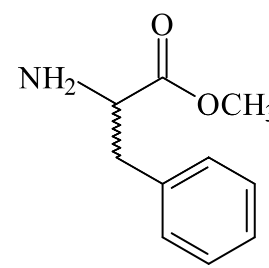
Solutes:



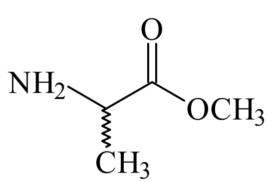
Warfarin



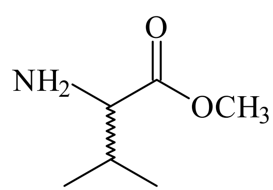
4-Chloroamphetamine



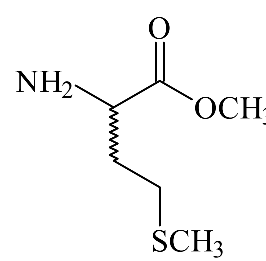
Phenylalanine



Alanine

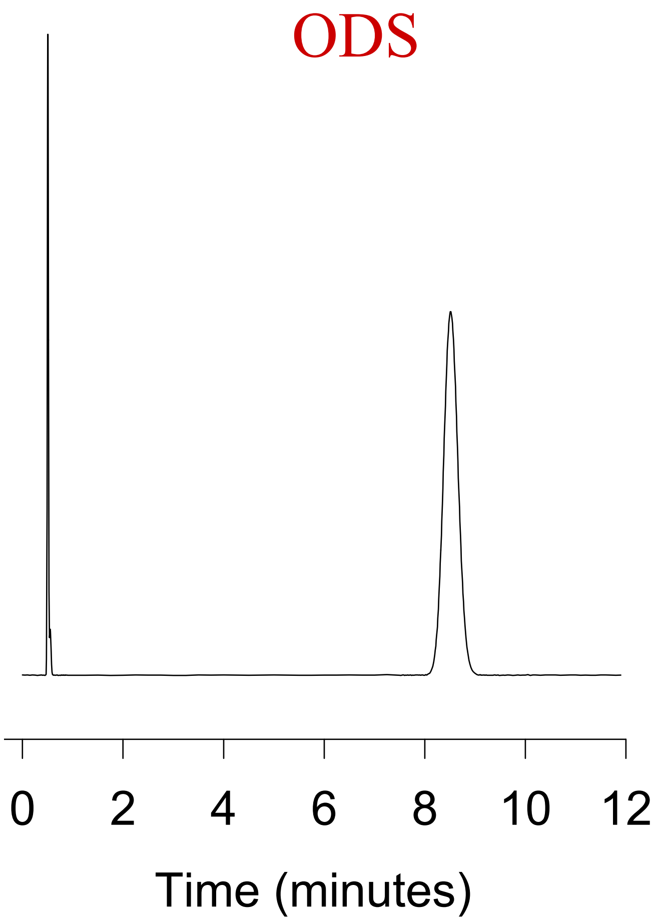


Valine

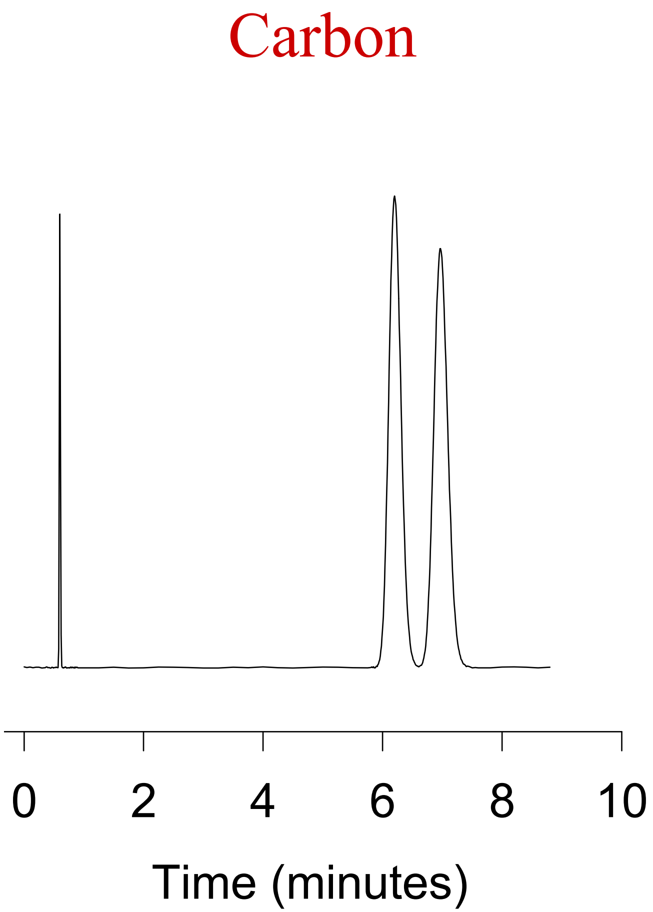


Methionine

Separation of (R)-Mosher-(+)-Warfarin Derivative



40/60 THF/water



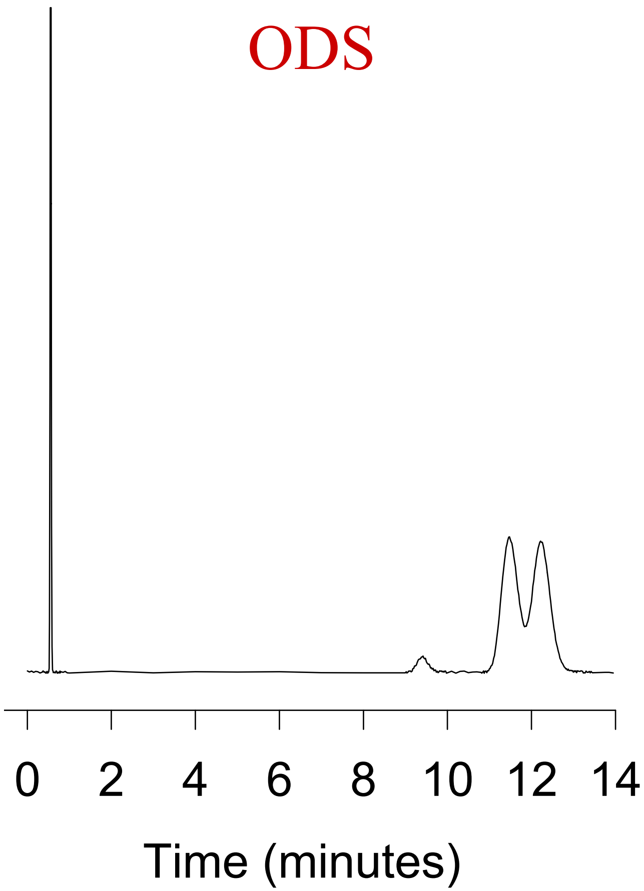
45/55 THF/water

50 x 4.6 mm, 1 mL/min, 30 ^{o}C, 1.5 µL injection, 254 nm detection

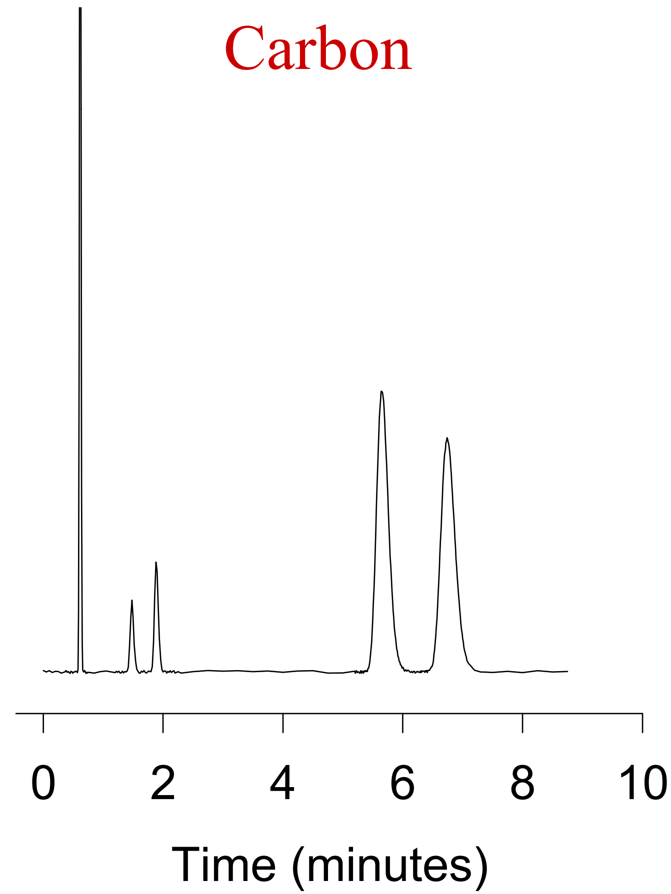
ST · OLAF COLLEGE

PTJ Jackson, P.T.; Kim, T-Y.; Carr, P.W. *Anal. Chem.* **1997**, *69*, 5011-5017.

(R)-Mosher-dl-Phenylalanine Derivative



35/65 THF/water

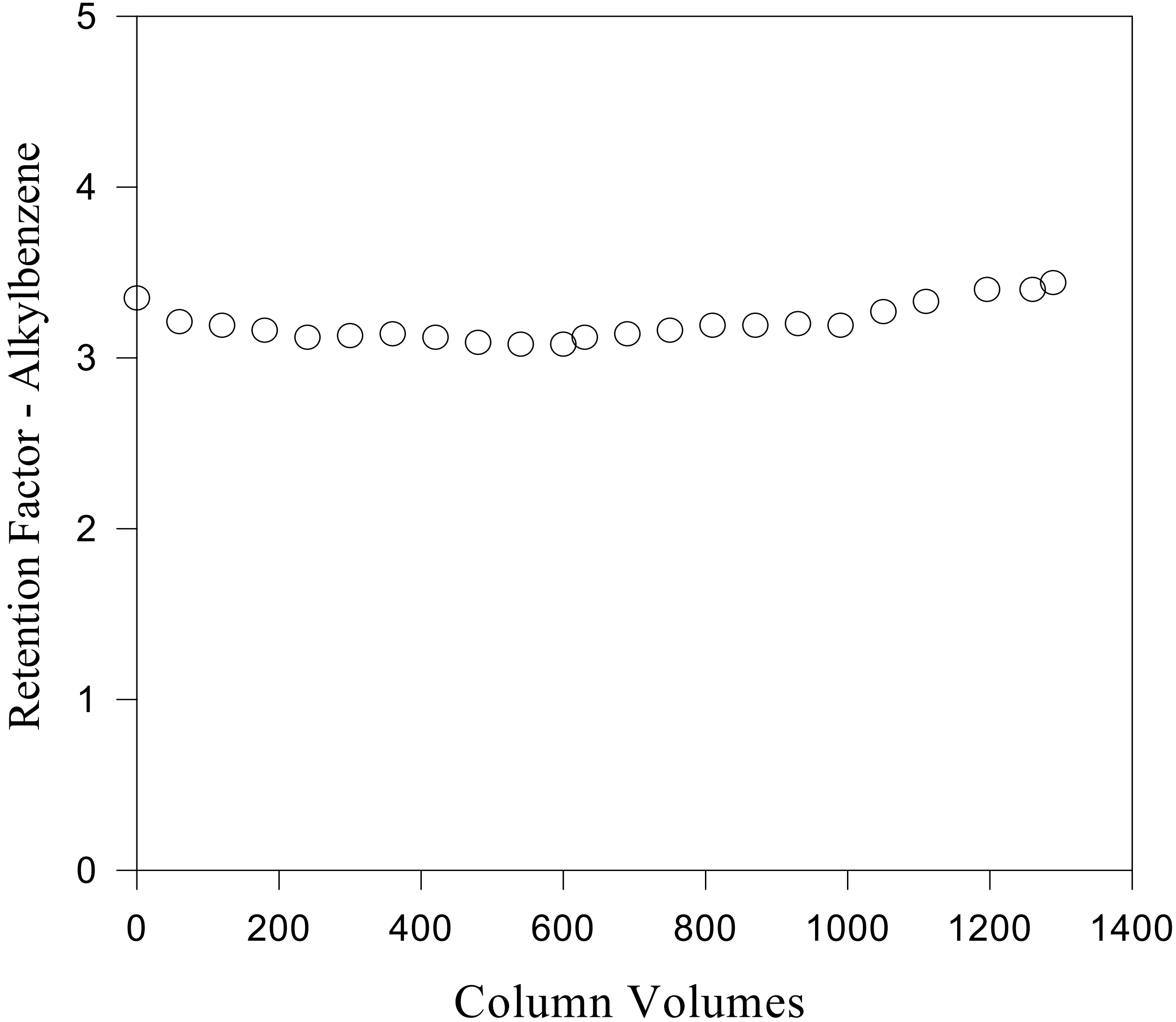


40/60 THF/water

Selectivity & Resolution of Mosher Derivatives

Derivative	ODS		Carbon	
	α	R_s	α	R_s
Warfarin	1.00	0.00	1.14	1.40
4-Clamphet	1.45	1.48	1.25	2.27
Phenylalanine	1.07	0.80	1.22	2.06
Valine	1.08	0.89	1.05	0.70
Methionine	1.07	0.87	1.07	0.79
Alanine	1.12	1.30	1.11	1.30

Chemical (pH) Stability of Toluene-C/ZrO2

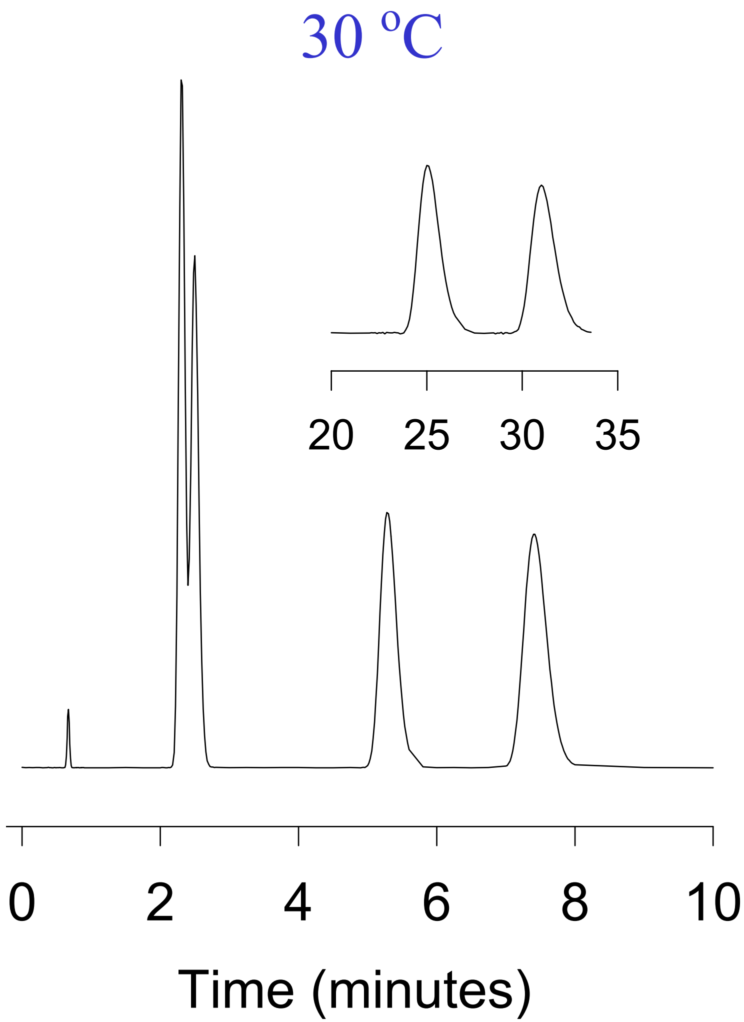


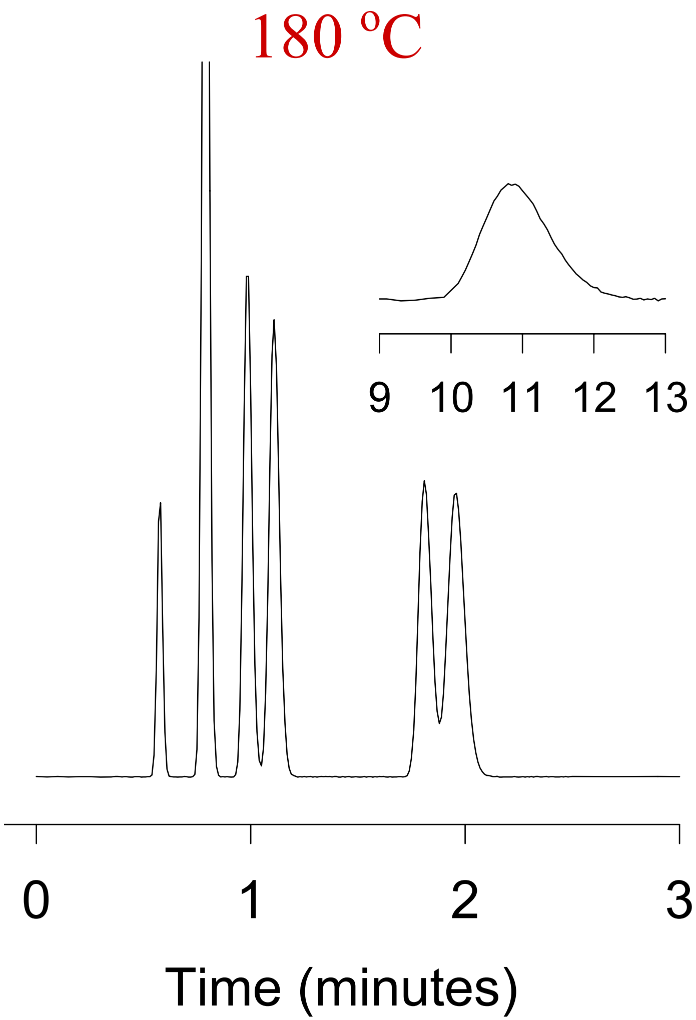
ST · OLAF COLLEGE

PTJ

50/50 MeOH/Buffer pH 12, 80 ^{o}C

Chemical & Thermal Stability: Chlorinated Phenols



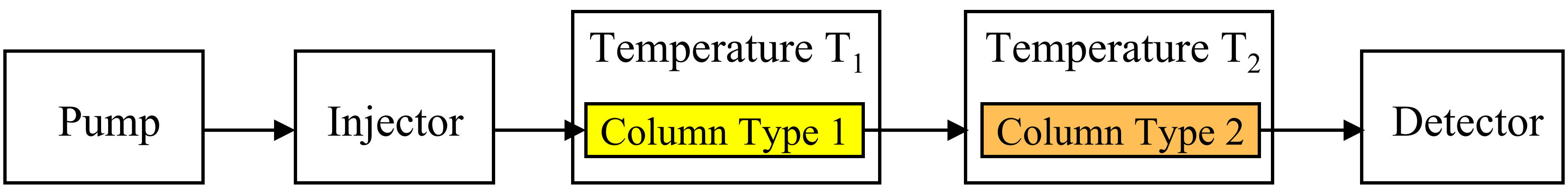


Hx-C/ZrO_2 50 x 4.6 mm, 1 mL/min, 65:35
ACN:water+1% TFA, 2 μL injection, 254 nm detection.

PTJ

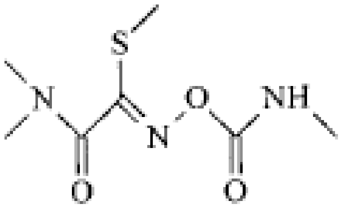
2D Separations: Thermally Tuned Tandem Concept - T^3C

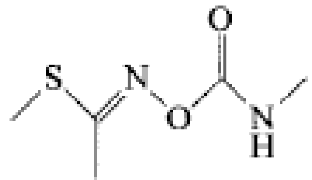
- Advantages
  - ✓ C/ZrO_2 thermal stability
  - ✓ Selectivity change w/temp
  - ✓ Programming temperature in addition to mobile phase
- Requires orthogonal stationary phases (retention properties)
- Adapt standard instrumentation

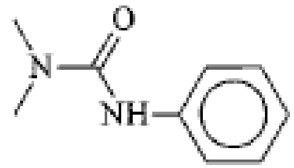


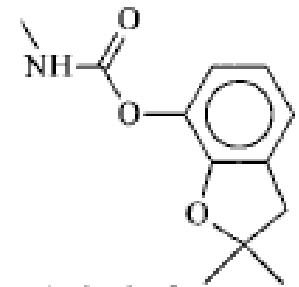
Mao and Carr, *Anal. Chem.* **2001**, *73*, 1821-1830.

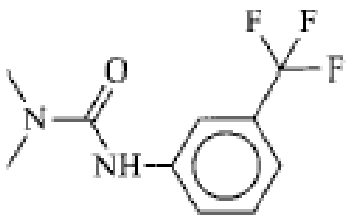
Urea & Carbamate Herbicides

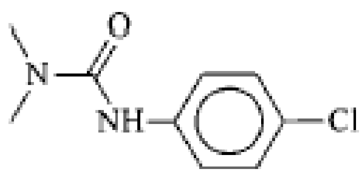

1.Oxamyl

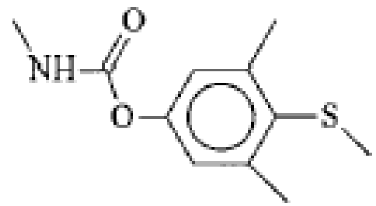

2. Methomyl

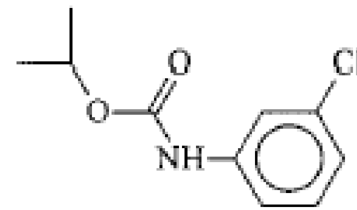

3. Fenuron

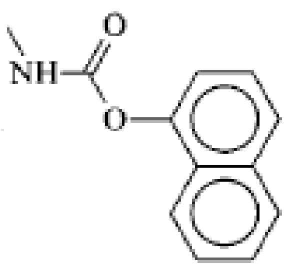

4. Carbofuran

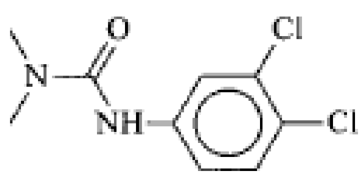

5. Fluometuron

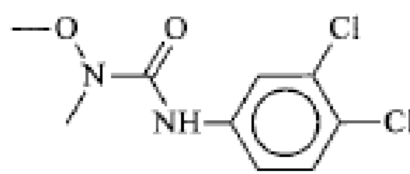

6. Monuron

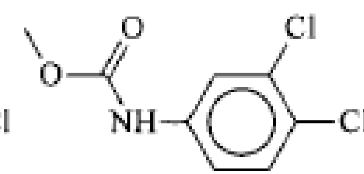

7. Methiocarb


8. Chloropropham

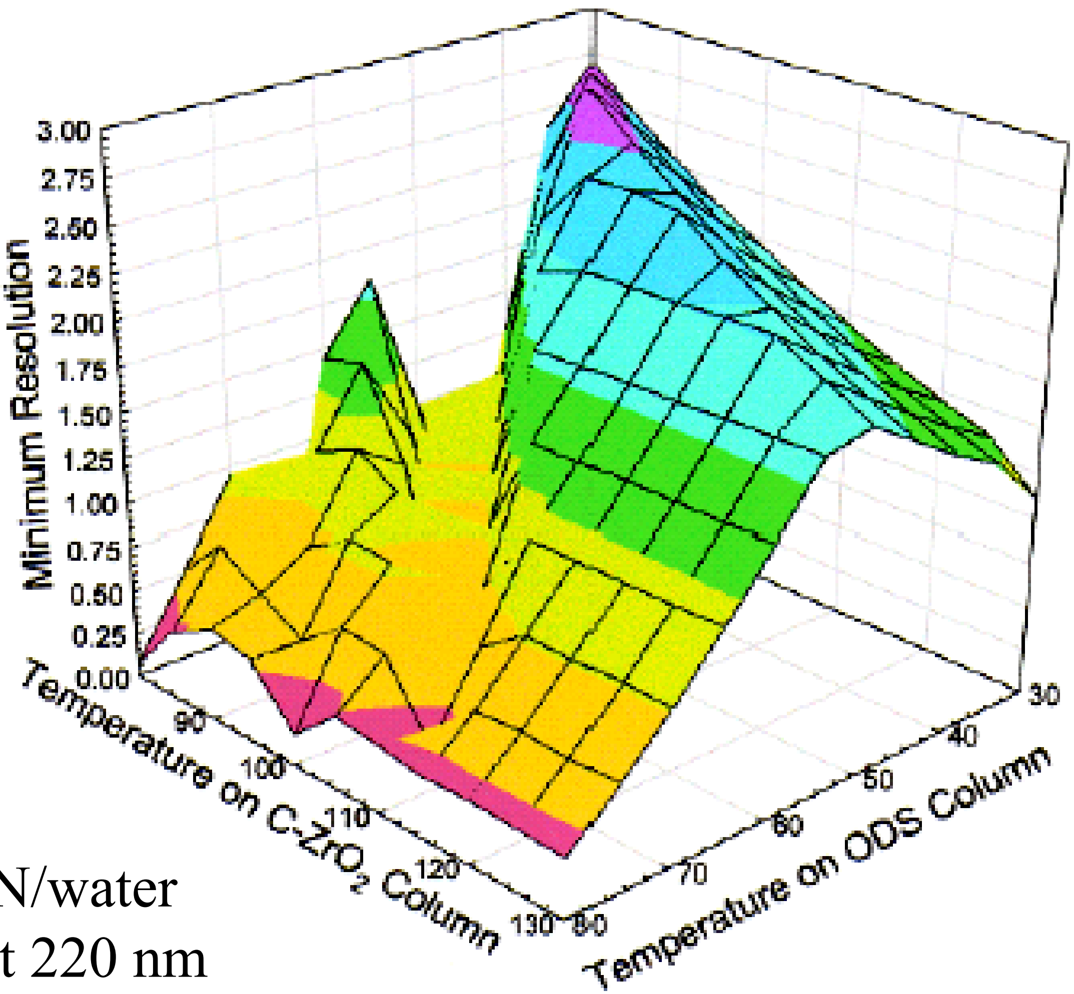

9. Carbaryl


10. Diuron


11. Linuron


12. Swep

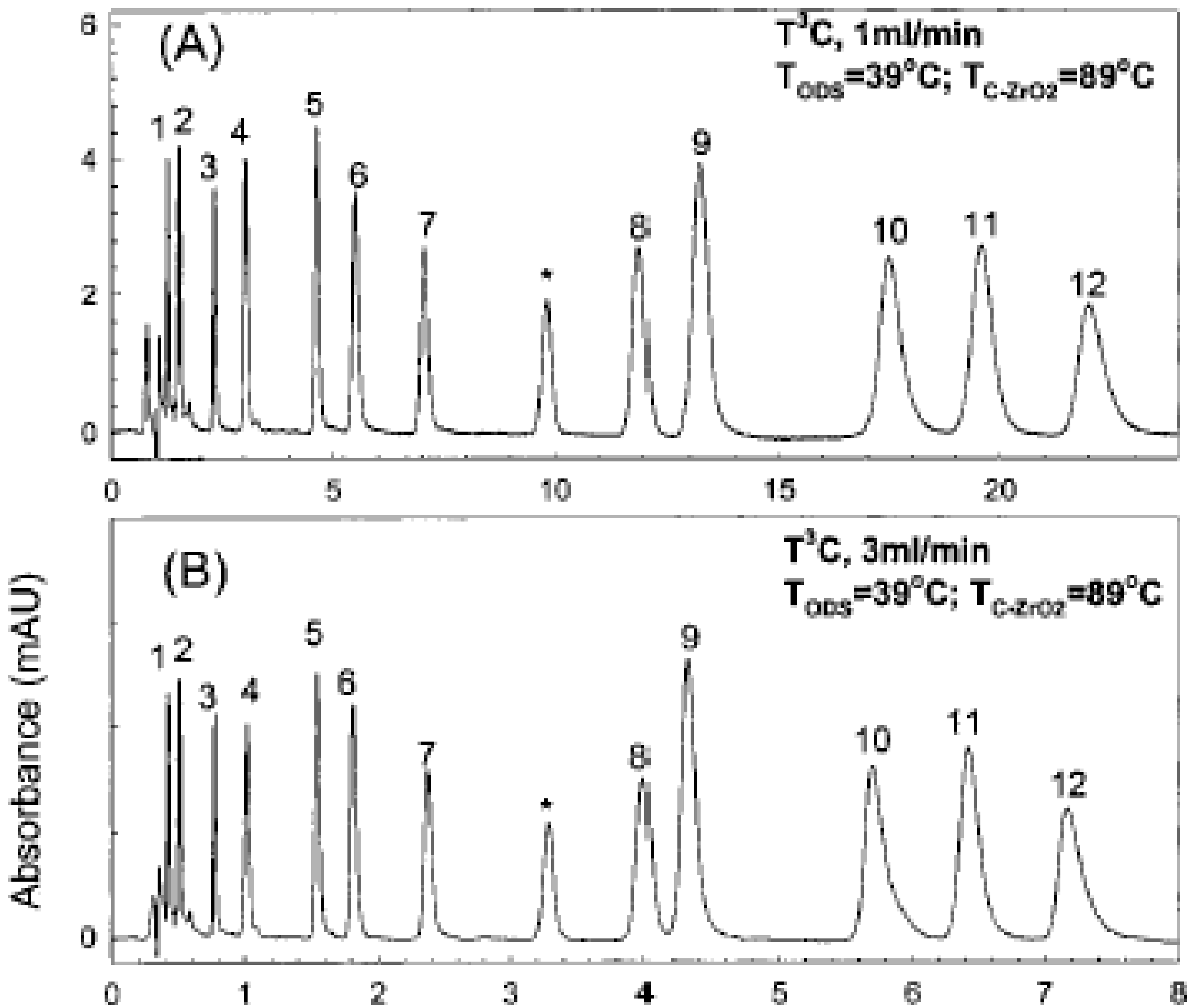
R_s Window Diagram for Urea & Carbamate Herbicides



40/60 ACN/water
detection at 220 nm

Mao and Carr, *Anal. Chem.* **2000**, *72*, 2788-2796.

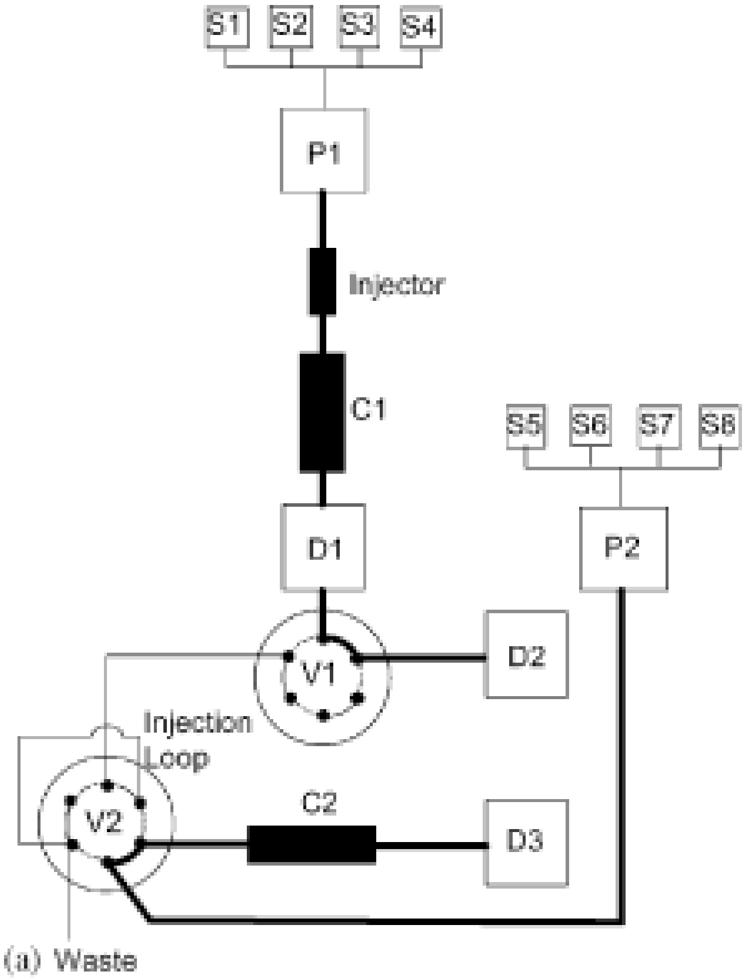
T^3C of Urea & Carbamate Herbicides



PTJ

Mao and Carr, *Anal. Chem.* **2000**, *72*, 2788-2796.

2D HPLC Configuration for Heart-Cut Oligostyrenes



P1–P2: Low pressure quaternary solvent delivery systems

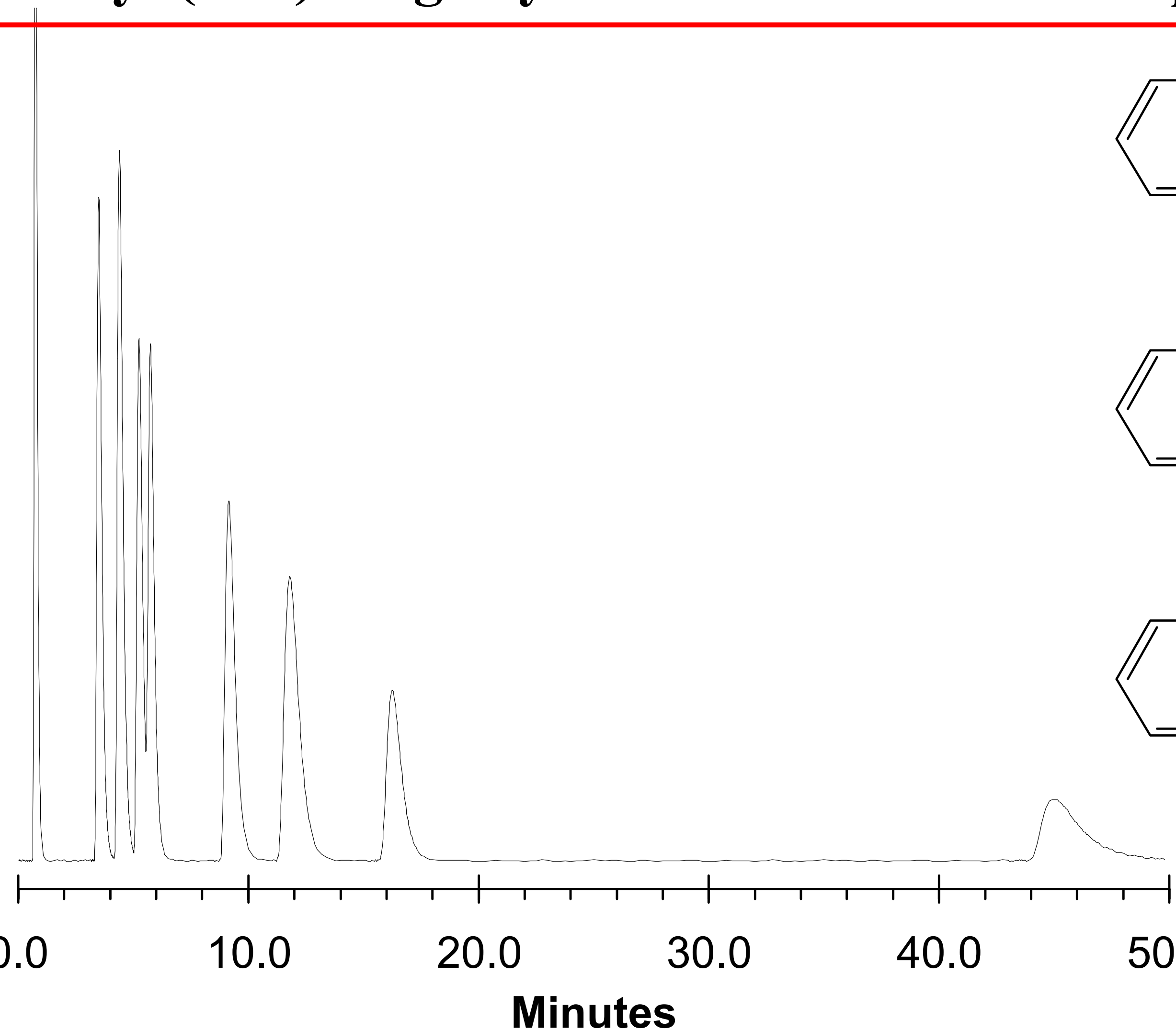
V1–V2: six-port two-position switching valves

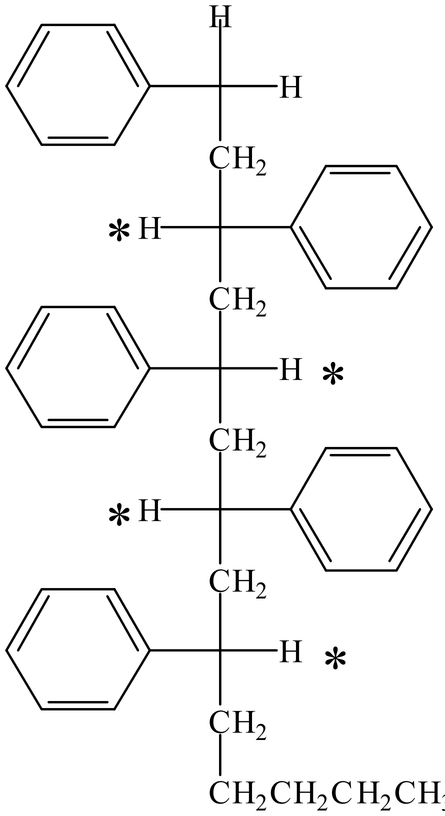
C1: column in first separation dimension, Activon-C18 250 x 4.6 mm, dp = 5 μm.

C2: column in second separation dimension, ZirChrom-CARB 30 x 4.6 mm, dp = 3 μm.

Gray, M., et al., *J. Chromatogr. A,* **2004**, *1028*, 247-257.

n-Butyl (n=5) Oligostyrenes: Diastereomer Separation



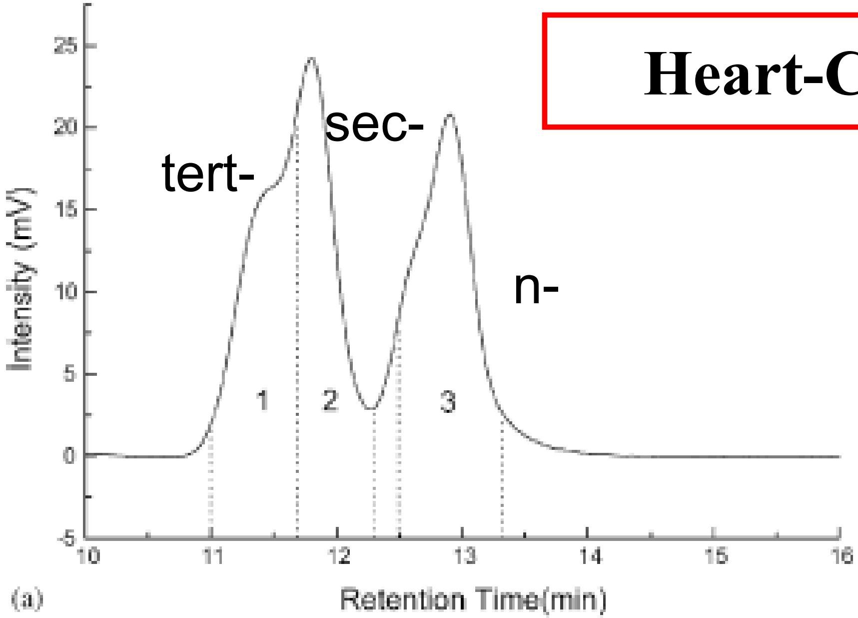


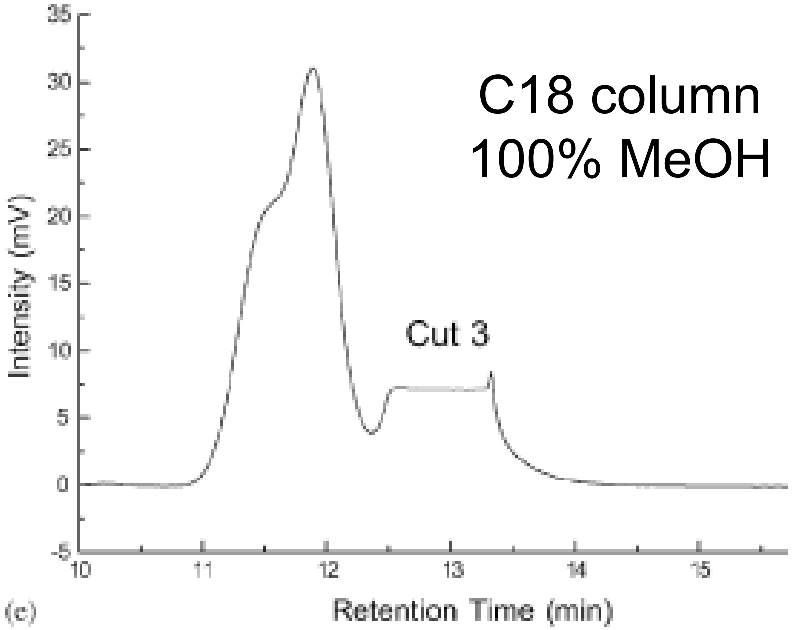
ZirChrom-CARB 50 x 4.6 mm, dp = 3 µm, 100% ACN, 1 mL/min, 10 µL inj., 20 °C, 262 nm.

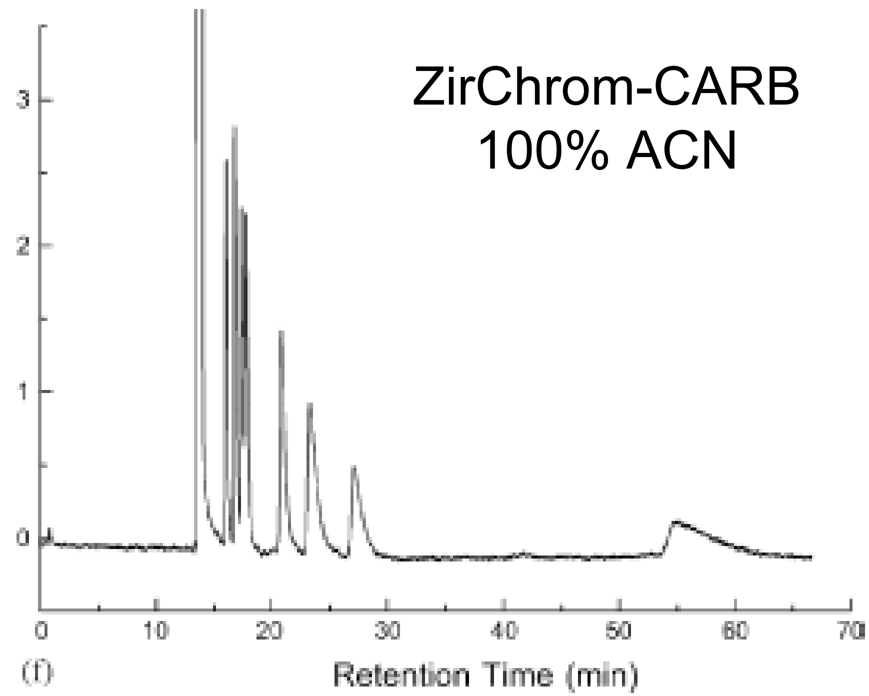
PTJ

Gray, M., et al., *J. Chromatogr. A,* **2002**, *975*, 285–297

Heart-Cut of Oligomeric Polystyrenes

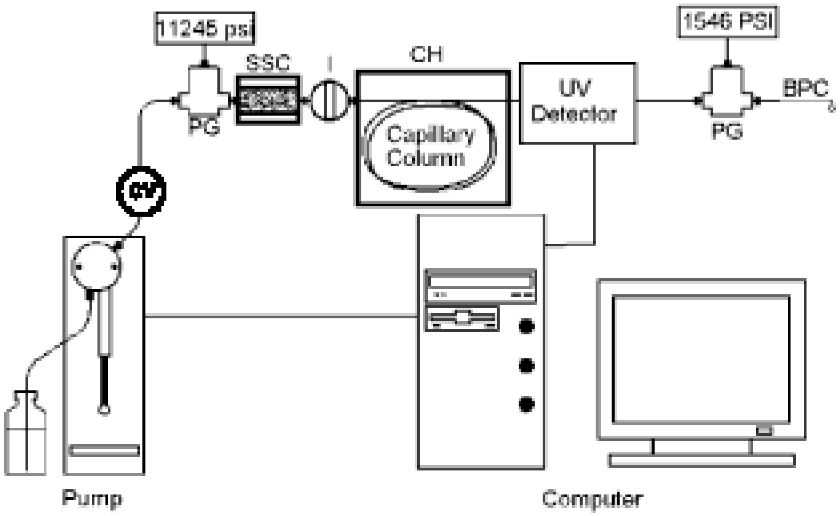


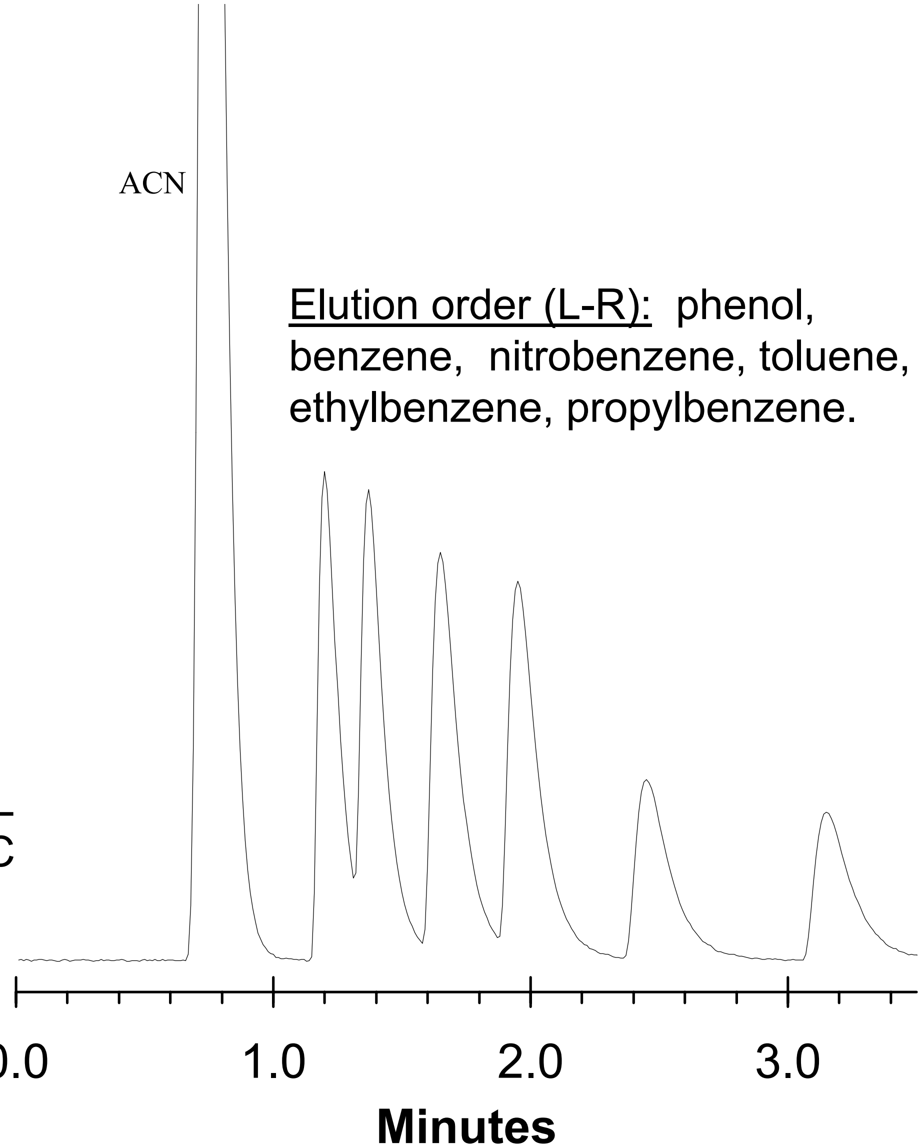




Gray, M., et al., *J. Chromatogr. A,* **2004**, *1028*, 247-257.

Water-Based Temperature Programmed Separations



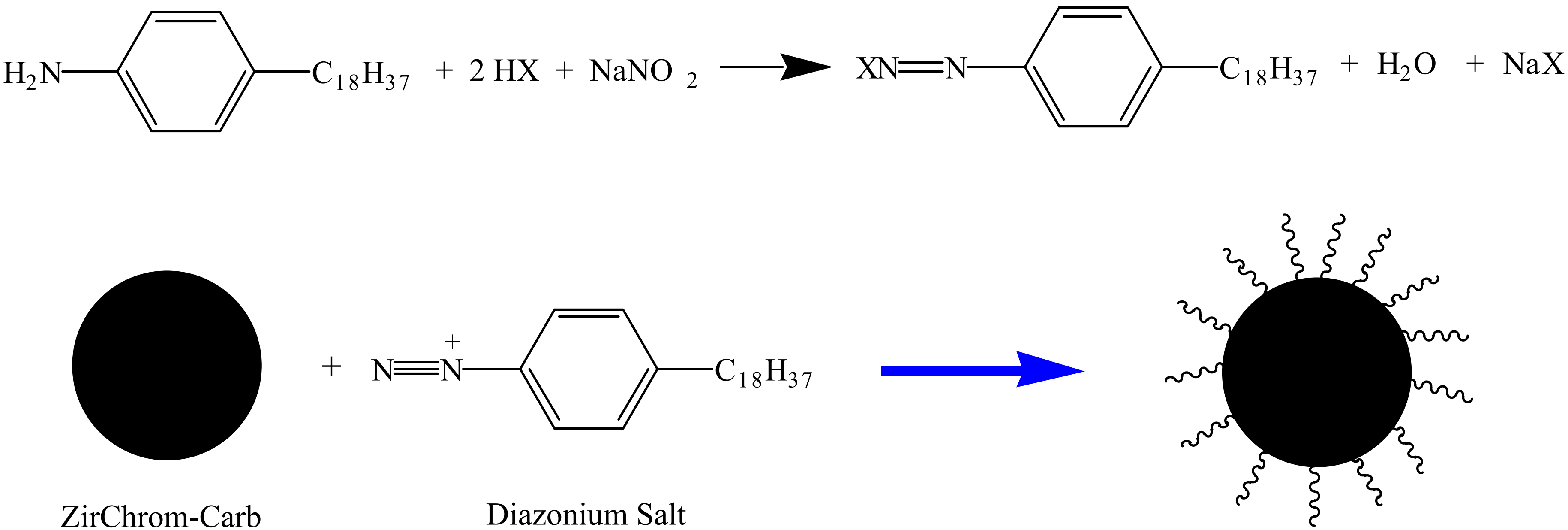


Elution order (L-R): phenol, benzene, nitrobenzene, toluene, ethylbenzene, propylbenzene.

Conditions: 3 μm ZirChrom–CARB, 180 μm id x 13 cm silica capillary, 100% water, 8.6 μL min^{-1}. Temperature gradient 100 °C to 250 °C at 50 °C min^{-1}.

Kephart and Dasgupta, *Talanta*, **2002**, *56*, 977-987

Carbon Surface Modification

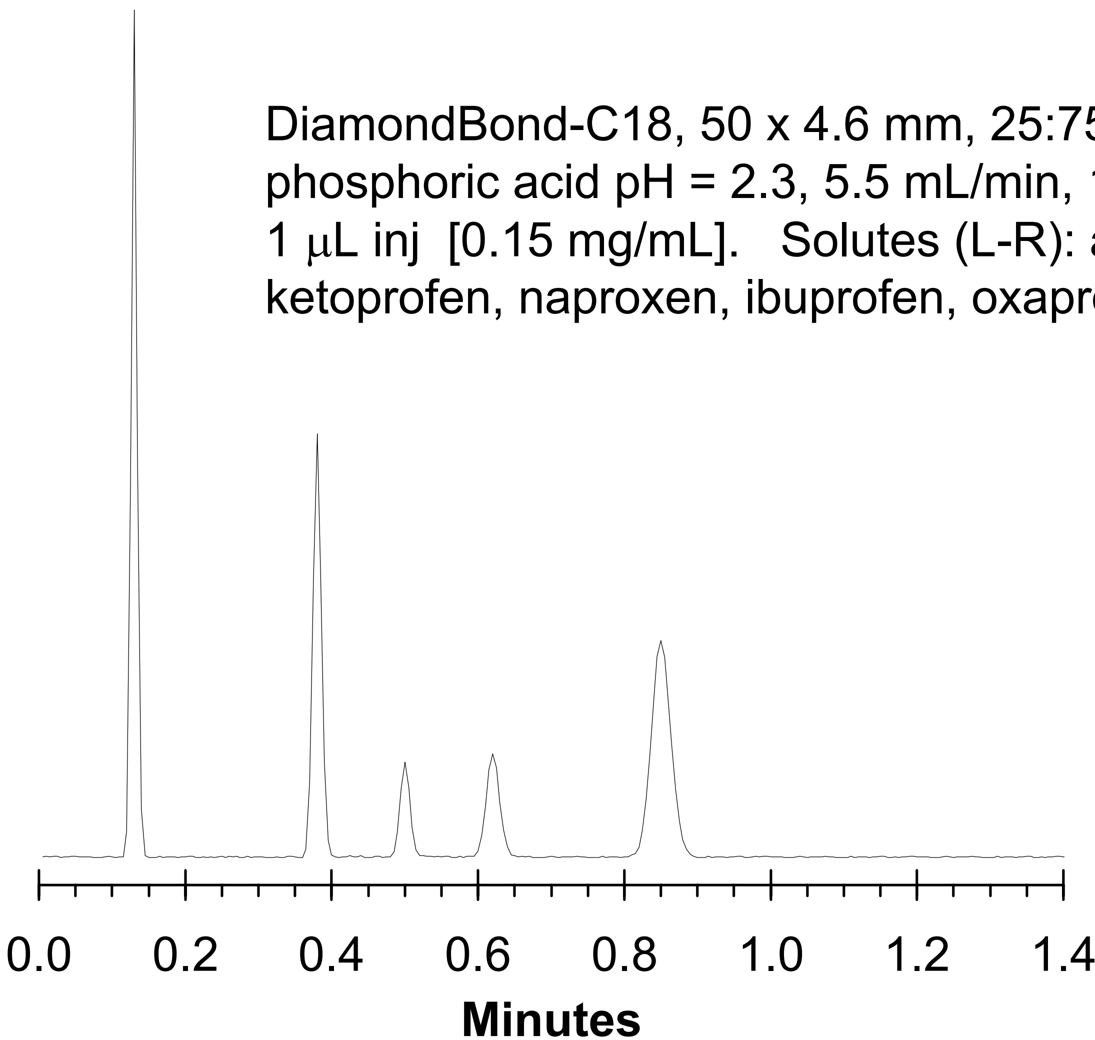


ZirChrom-Carb

Diazonium Salt

Derivatized Carbon Particle

NSAIDs on Aliphatic Modified Carbon



DiamondBond-C18, 50 x 4.6 mm, 25:75 ACN:40 mM phosphoric acid pH = 2.3, 5.5 mL/min, 150 °C, 254 nm, 1 μL inj [0.15 mg/mL]. Solutes (L-R): acetaminophen, ketoprofen, naproxen, ibuprofen, oxaprofen.

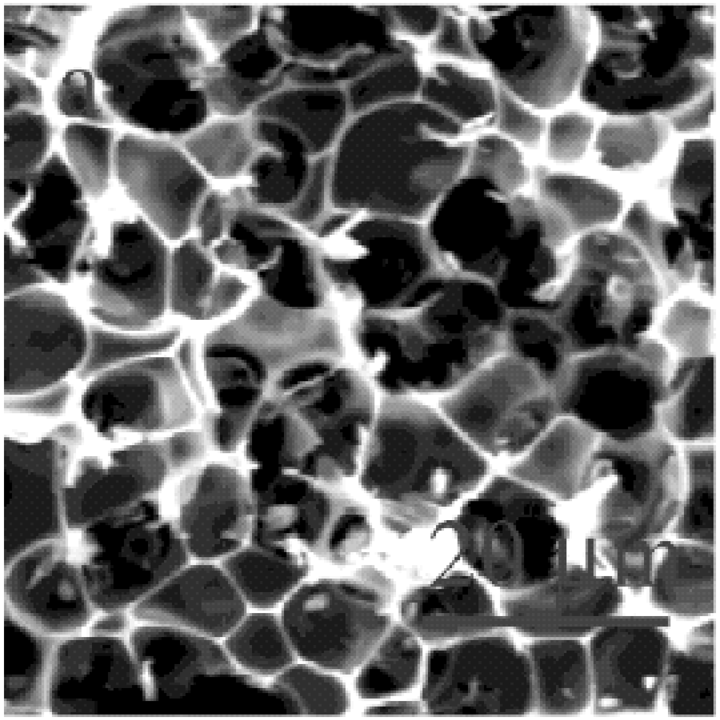
Nawrocki, J., et al., *J Chromatogr. A*, **2004**, *1028*, 31-62.

Future Directions of Carbon or C/ZrO_2

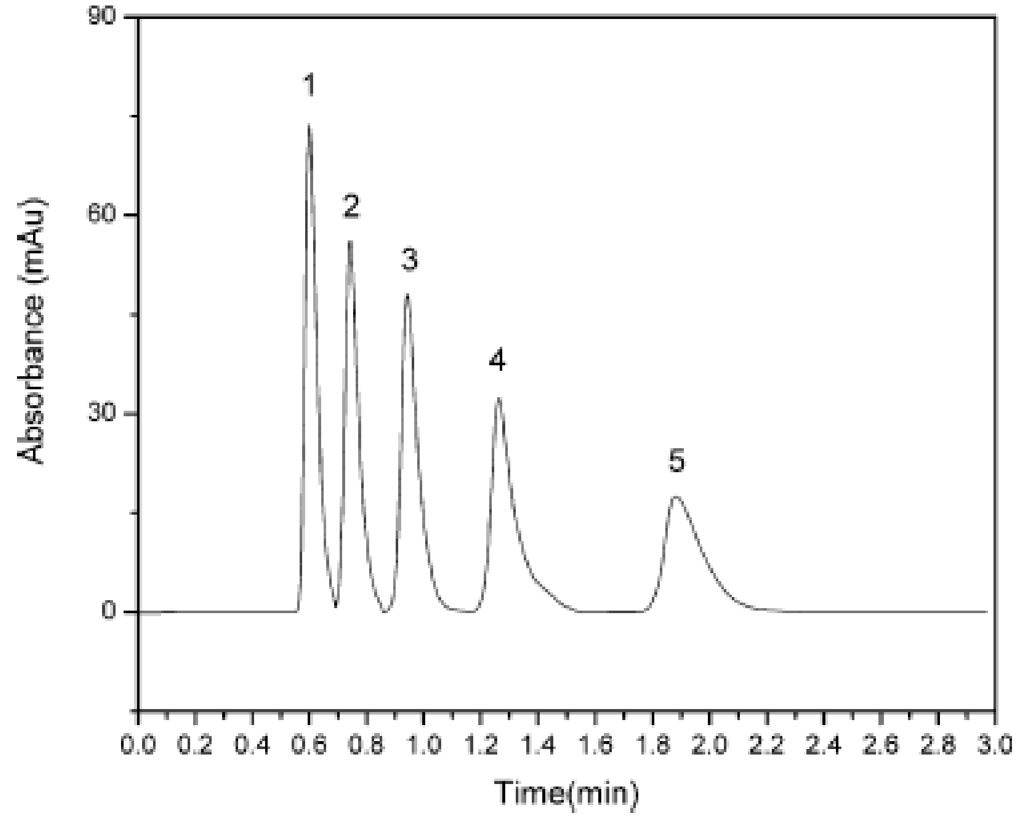
- Separations routinely conducted at elevated temperatures and with temperature programming on stable media.
- ODS-Carb, Polymer-Carb, IEX-Carb 2D separations of complex mixtures.
- C/ZrO_2 in microbore and capillary column format.
- Expansion of on-line sample preconcentration with carbon media.
- Chemically and thermally stable chiral stationary phases created via chemical bonding to the carbon surface.

Future Directions of Carbon or C/ZrO_2

Monolithic Carbon Separations Media



Mesopores in Monolith
Iron-Resorcinol-Silica Synthesis

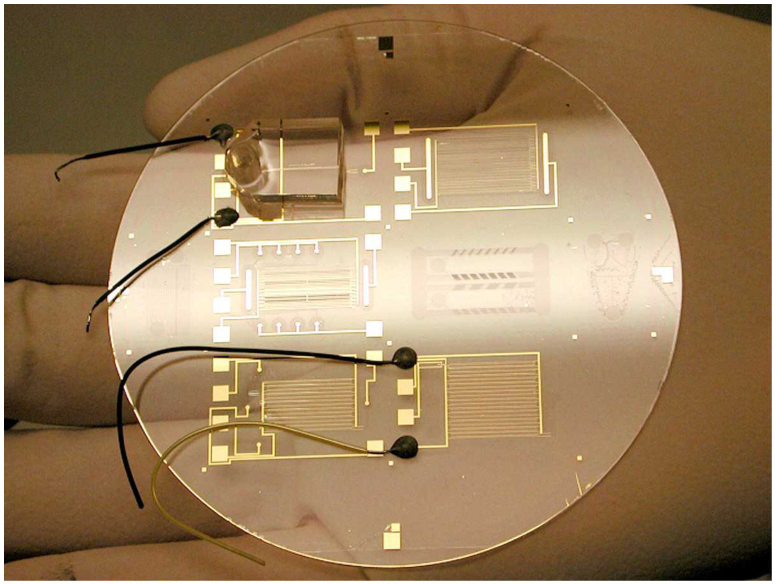


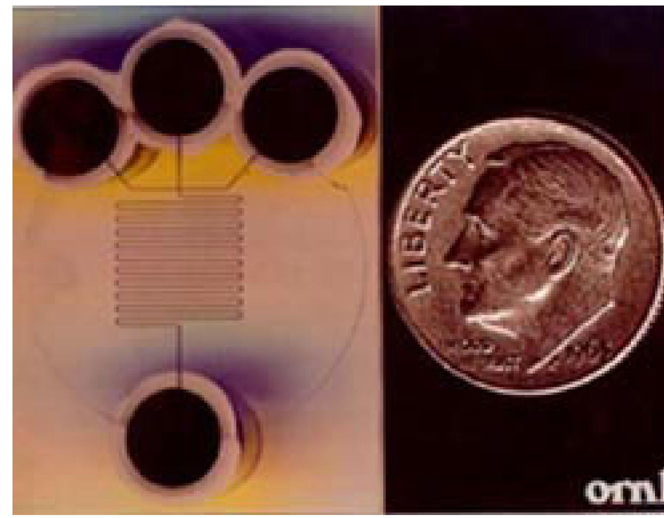
Alkylbenzene Separation on Carbon Monolith
30:69:1 MeOH:CH_2Cl_2:hexane

Liang, Dai, Guiochon *Anal. Chem.* **2003**, *75(18)*, 4904-4912

Future Directions of Carbon or C/ZrO_2

Microfluidic Separations Devices: Lab-on-a-Chip or μ-TAS





- Controlled carbon deposition on surfaces
- Fluid flow - electrophoretic or electrochemically modulated

Images from: U Washington & Oak Ridge National Lab

The Carbon (C/ZrO_2) Advantage

- ☆ Exhibit RPLC character with a liquid-solid interface.
  - Orthogonal retention compared to bonded phases.
  - Solute structure and dipolarity/polarizability significantly influences retention, selectivity and resolution.
- ☆ Demonstrate acid-base stability.
- ☆ Show stability at high temperatures (200 ^{o}C).
- ☆ Stationary phase structure provides geometric selectivity useful in resolving isomeric analytes, especially diastereomers.
- ☆ Aromatic platforms provide a means to modify the carbon surface.

Chromatographic selectivity impacts resolution.
The retention mechanism controls the selectivity.

Acknowledgments

National Institutes of Health

National Institute of Occupational Safety & Health

Camille and Henry Dreyfus Foundation

St. Olaf College Chemistry Department

University of Minnesota Chemistry Department

ZirChrom Separations, Inc.