The Effect of Temperature on Selectivity in HPLC

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The Use of Temperature in HPLC

Temperature is considered to be the overlooked or forgotten optimization parameter in HPLC by many of the experts.

"***Although nearly all of the physical parameters that play a role in liquid chromatographic separation are a function of temperature, temperature has not yet been adequately explored as a parameter to tune separation and shorten analysis times in LC .***" *

* Nebojsa M. Djordjevic, Patrick W.J. Fowler, Fabice Houdiere *J. Microcolumn Separations* **11**(6) (1999) 403-413

Typical Retentive Behavior

- Retention uniformly decreases with temperature
- Linear van't Hoff plots – log k vs. 1/T
- A 1% methanol increase is equivalent to ~4°C temperature increase

Change in Retention with Temperature

$$k_1' / k_2' = \exp(\Delta H(T_2 - T_1) / (RT_1T_2)$$

- Effect of temperature on retention factor depends on enthalpy of the solute. The larger the enthalpy, the greater the change in k'

F. Houdiere, P.W.J. Fowler, N.M. Djordjevic, *Anal. Chem.*, **69,** 2589-2593 (1997)

Analyte Families Giving Reversed Retention with Increasing Temperature

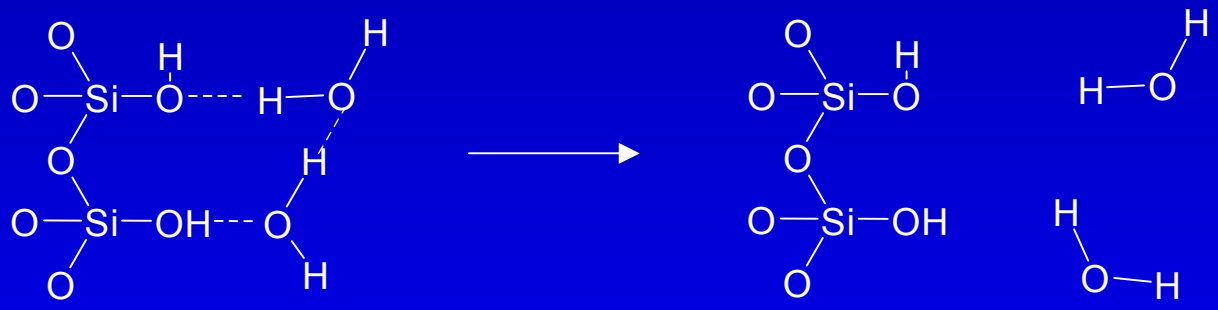
- Polyethers
  - Reverse retention due to apparent decreasing polarity due to hydrogen bond strength reduction
- Some dipeptides
  - Decreasing polarity with conformational change

Factors Affecting Retention That Are Influenced by Temperature

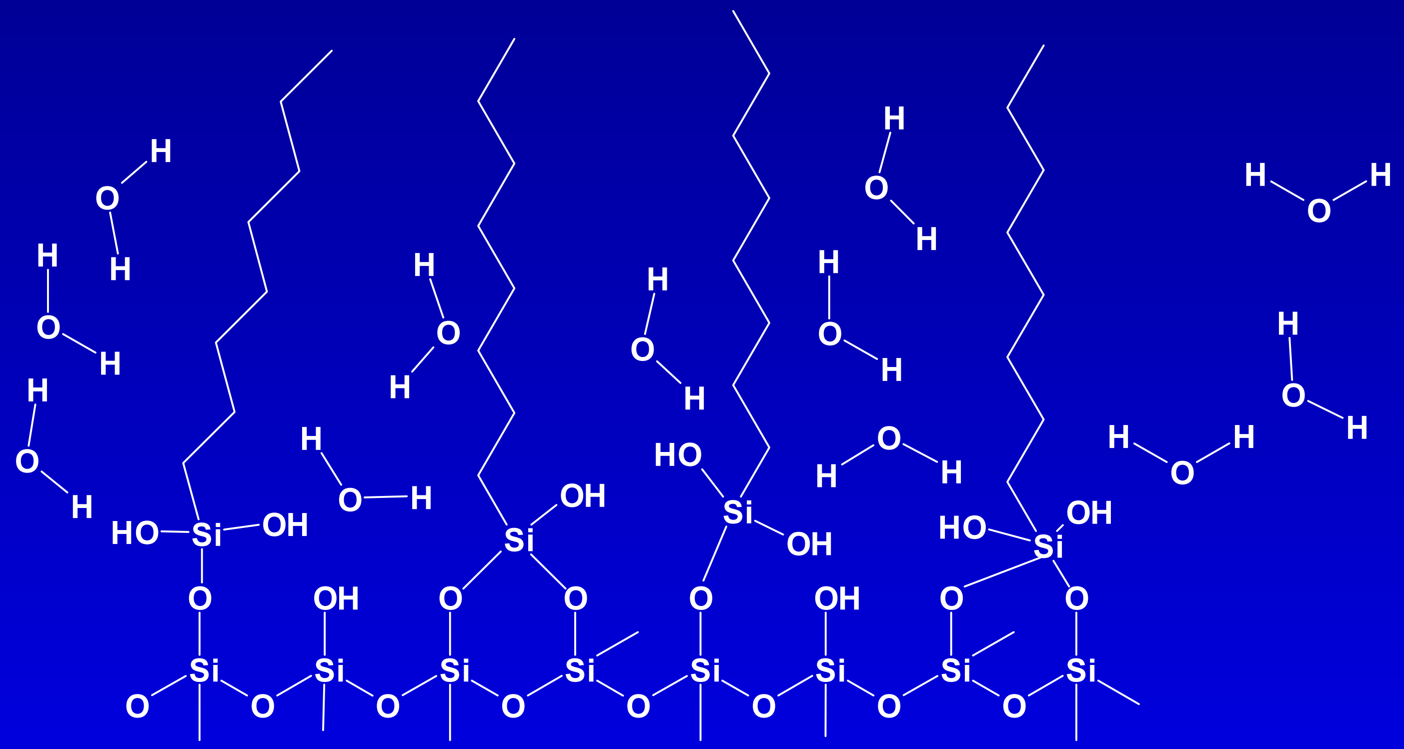
- Hydrogen bonding
- Solvation sphere around analytes
- Hydration extent of column surface
- Functional group interaction
- Ordering, shape and hydration transitions
  - Conformation changes
- Dielectric constant of mobile phase

Temperature Affects Hydrogen Bonding

- Increasing temperature-
  - Increases intermolecular distance
  - Weakens hydrogen bonds

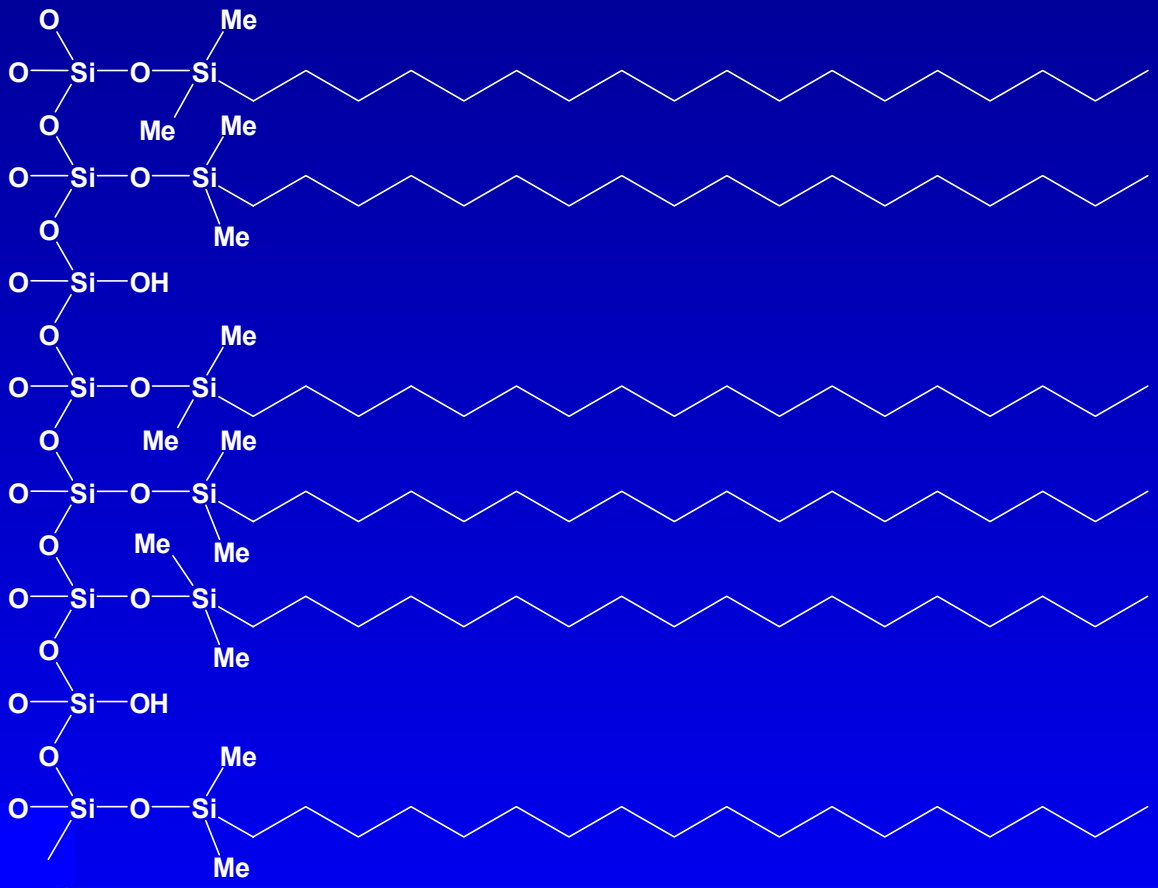


Column Surface Hydration



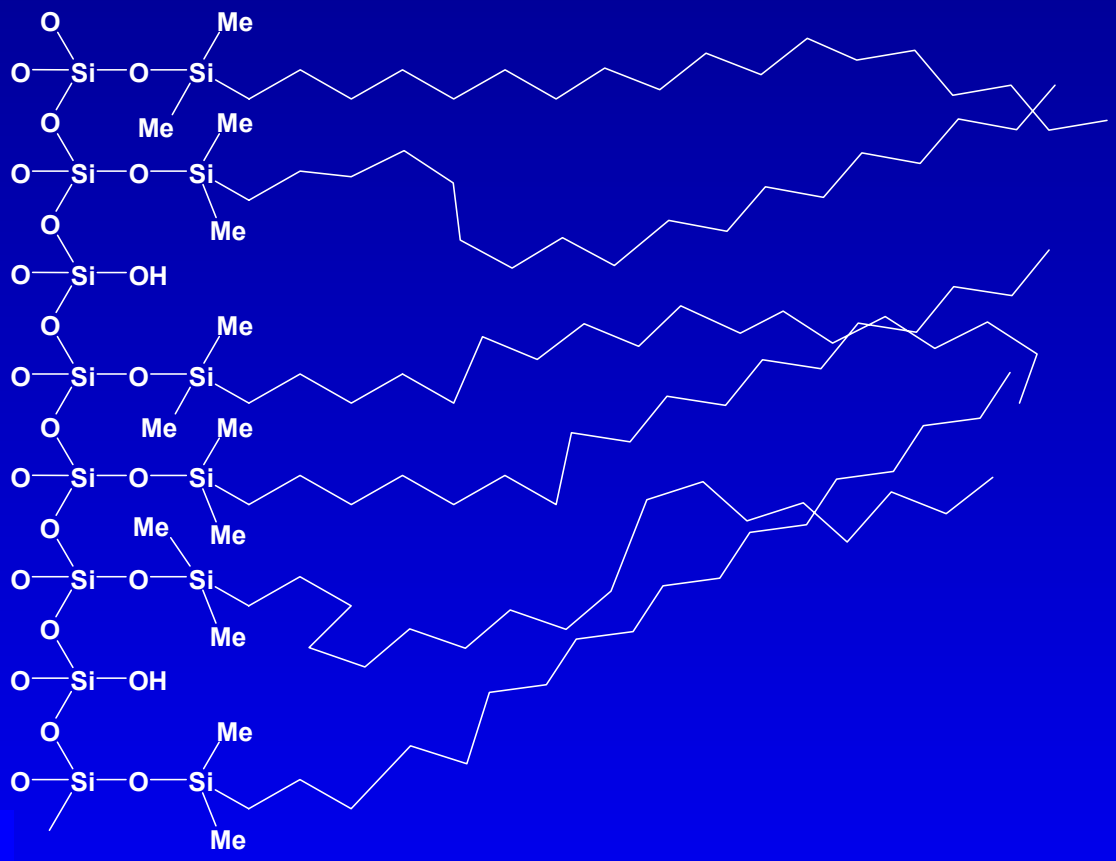
Functional Group Conformation

Octadecyl ordering at low temperatures



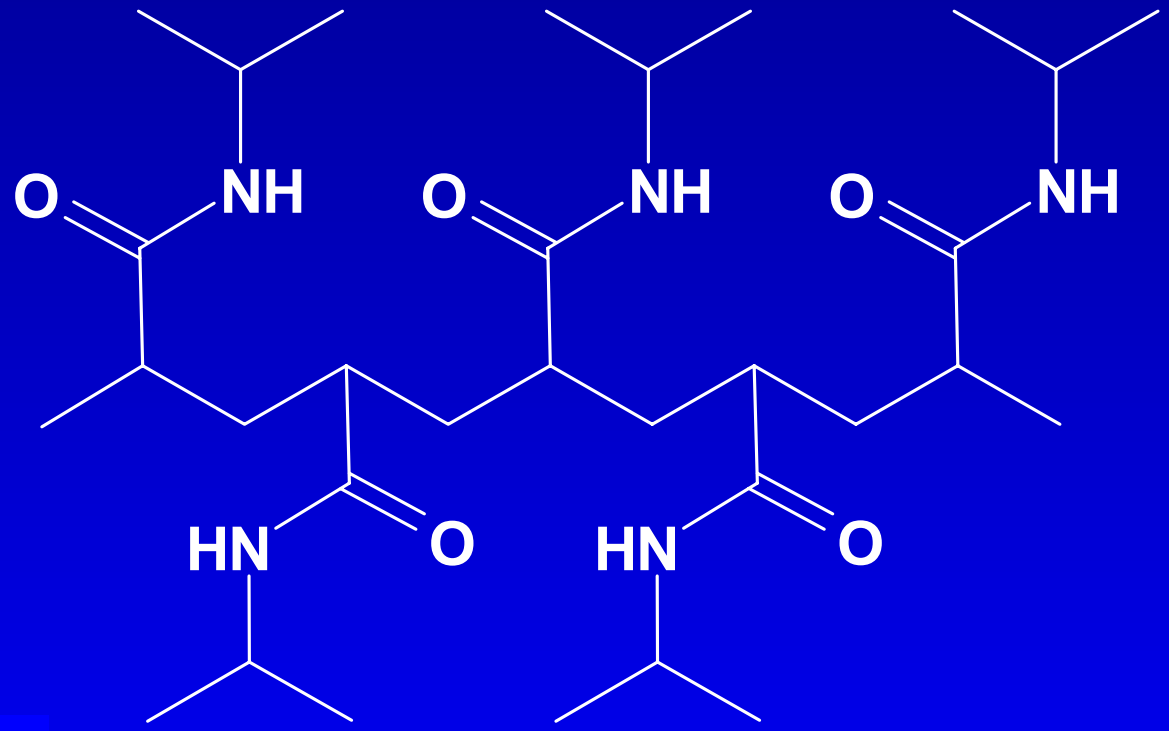
Functional Group Conformation

Octadecyl disorder at higher temperatures



Functional Group Phase Transitions

- *N*-Isopropylacrylamide coated silica

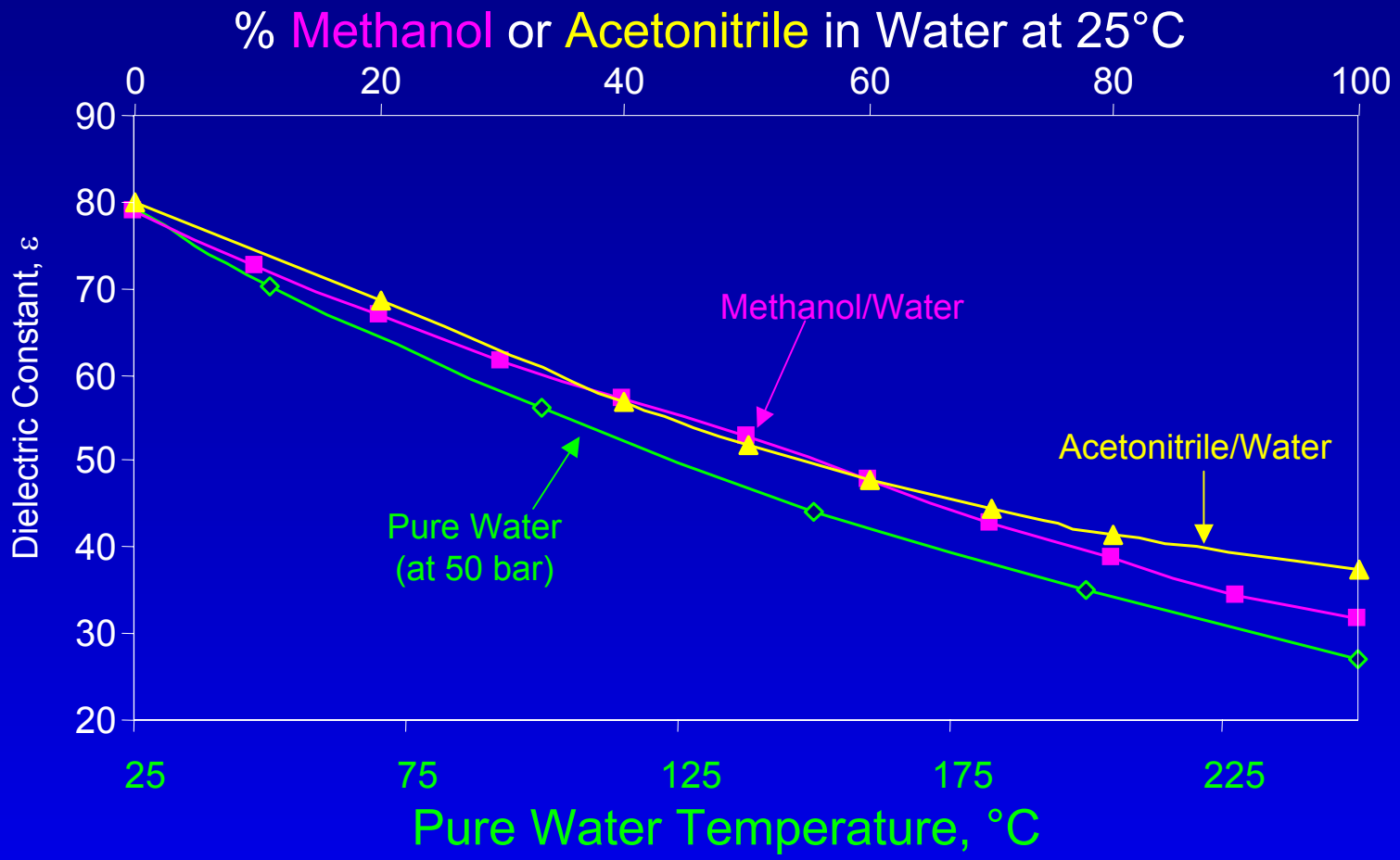


Analyte Conformational Changes

- Heteroduplex and homoduplex oligonucleotides separable by HPLC with temperature gradients
  - Axial or spatial gradients
  - Temperature programming of the entire column

From R.E. Gerber and R.G. Hatch in US Patent 6,486,309

Solvent Polarity as a Function of Temperature



Data from Y. Yang et al. *J. Chromatogr. A* **810** (1998) 149.

High Temperature Liquid Chromatography Advantages

- The advantages of using temperature to optimize separations in HPLC are well documented in the literature.
- The major advantages are
  - **Increased speed**
  - **Higher efficiencies and resolution**
  - **Ability to tune selectivity with temperature**
  - **Decreased organic solvent usage**
    - **Use less organic in solvent ratio**
    - **Perform isocratic separations and recycle solvent**

Increased Diffusivity

- Increasing the temperature increases the enthalpy of solute transfer from mobile phase to stationary phase*
  - Improves efficiency, particularly for large analytes
  - Allows operation at higher flow rates without penalty

*F.D. Antia and Cs. Horvath, *J. Chromatogr.* **435** (1988) 1-15.

*B. Yan, J. Zhao, J.S. Brown, J. Blackwell, P. W. Carr, *Anal. Chem.* **72** (2000) 1253-1262

Decreased Viscosity

- As the temperature increases the viscosity of the eluent decreases thus lowering the system back pressure
  - Perform analysis at higher flow rates without over-pressurizing the pump
  - Use smaller size packing materials in columns increasing efficiency

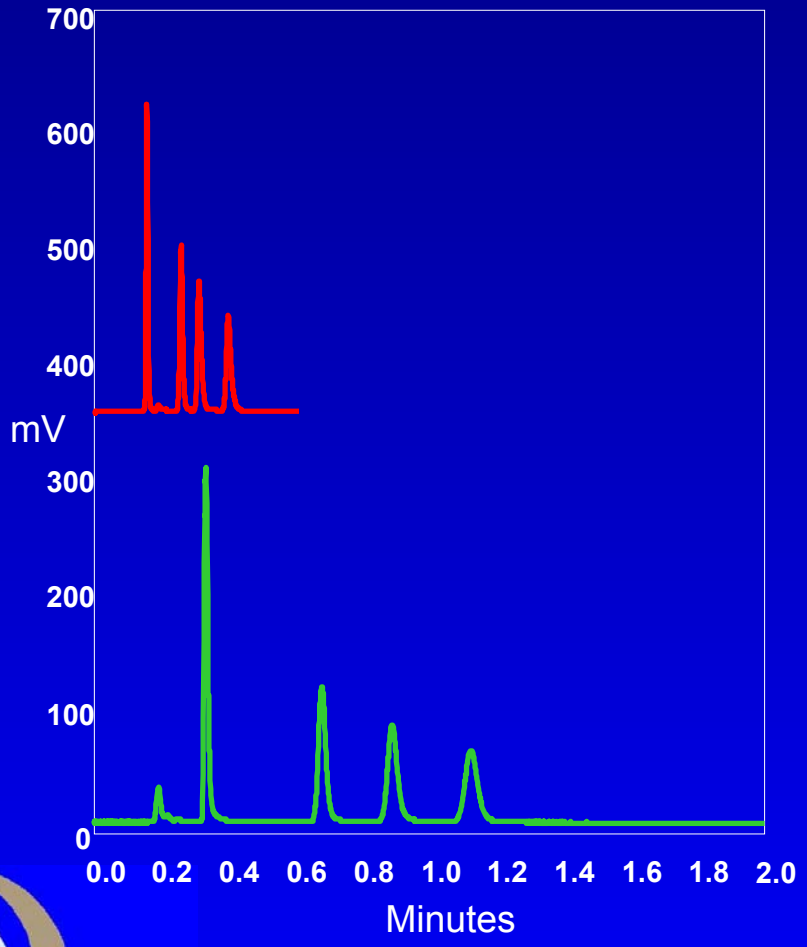
Temperature and Elution Strength

- Increasing temperature 4 to 5 ºC is comparable to increasing the methanol or acetonitrile concentration by 1% in a reversed phase system
- Viscosity is reduced 1 to 2 % per ºC increase
  - Result – a significant reduction in back pressure

$$P = \eta\mu L/d_p^2$$

T. Greibrokk, et al,, Chapter 8 in ACS Symp. Ser., **748** (ed. Parcher and Chester), 2000, page 121

Elution Strength Illustration: Separation of Steroids Using Water as the Mobile Phase



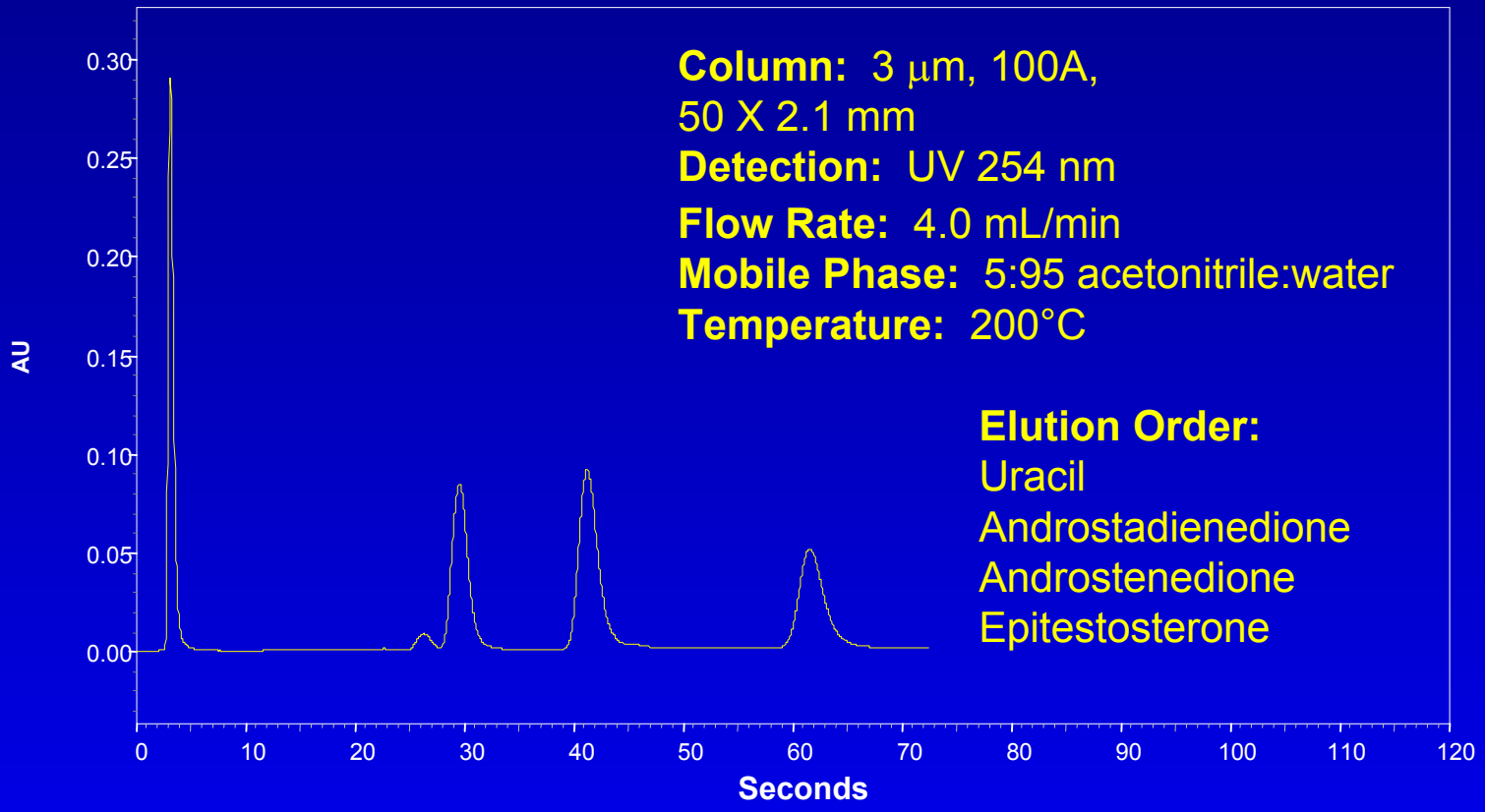
Column: ZirChrom PBD, 3 μm
100 X 4.6 mm
Detection: UV 254 nm

Flow Rate: 6.0 mL/min
Mobile Phase: Water
Temperature: 200°C

Flow Rate: 3.0 mL/min
Mobile Phase: 25:75 acetonitrile:water
Temperature: 50°C

Elution Order:
Uracil
Androstadienedione
Androstenedione
Epitestosterone

Prototype C_{18} Silica Column Separation of Steroids

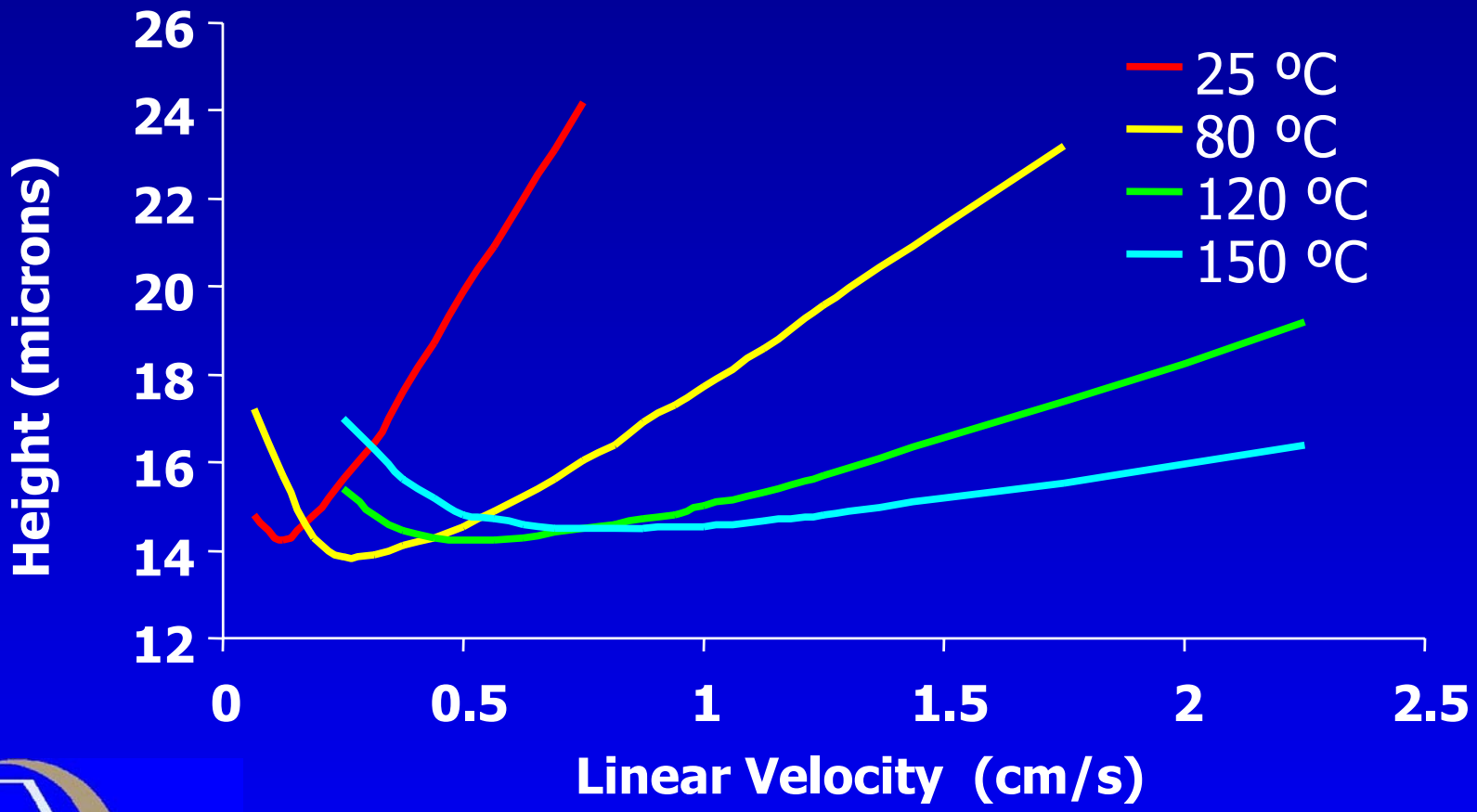


Column: 3 μm, 100A, 50 X 2.1 mm
Detection: UV 254 nm
Flow Rate: 4.0 mL/min
Mobile Phase: 5:95 acetonitrile:water
Temperature: 200°C

Elution Order:
Uracil
Androstadienedione
Androstenedione
Epitestosterone

Efficiency with High Speed

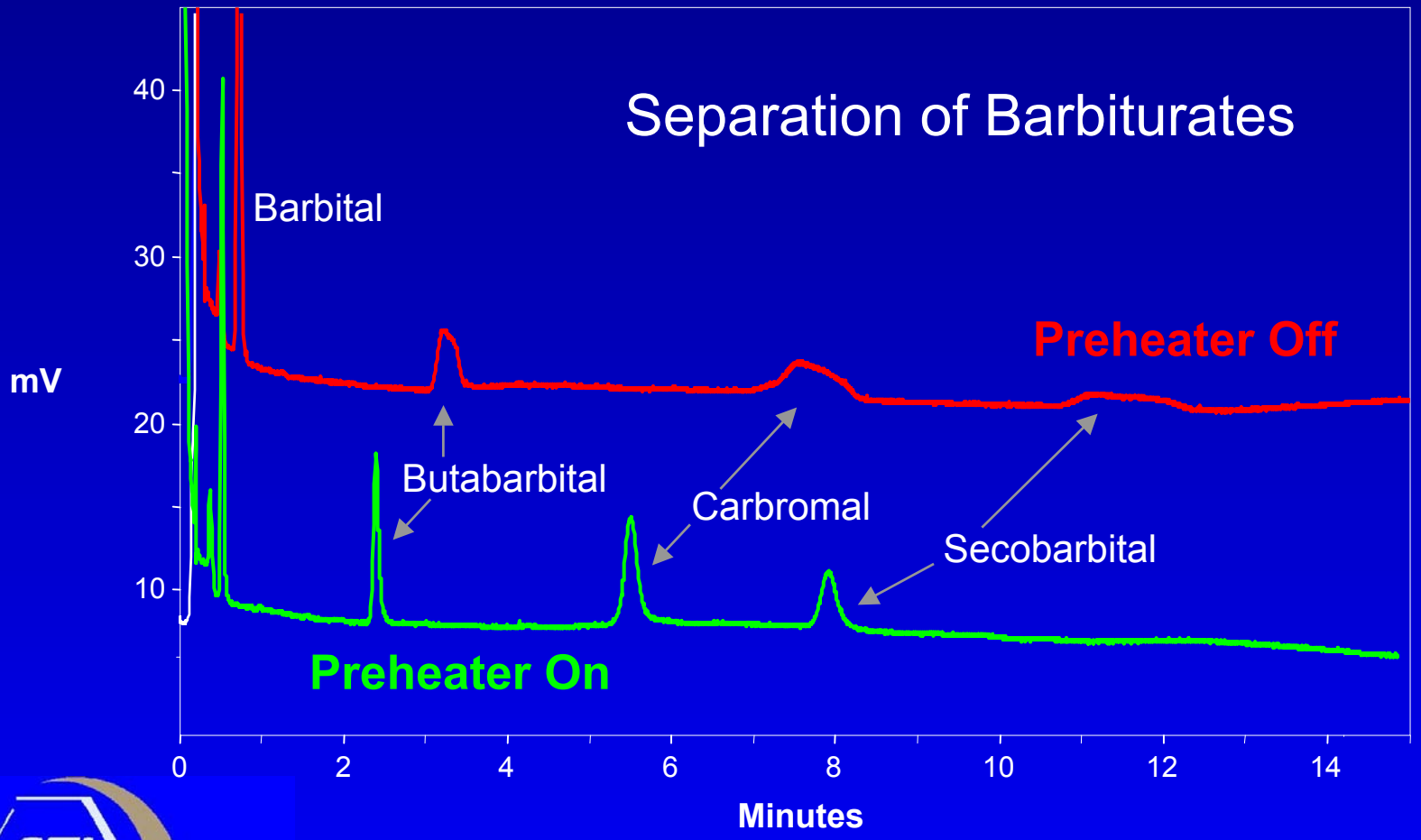
- Temperature effect on plate height



Historical Obstacles in the Pathway of Extended Range HPLC

- Most commonly listed reasons why temperature has not been utilized as an optimization tool in reversed phase
  - Poor temperature stability of silica based columns
  - Lack of adequate column heating system
  - Mobile phase composition is easily adjusted and provides the flexibility to handle a wide range of samples

Mobile Phase Pre-heating

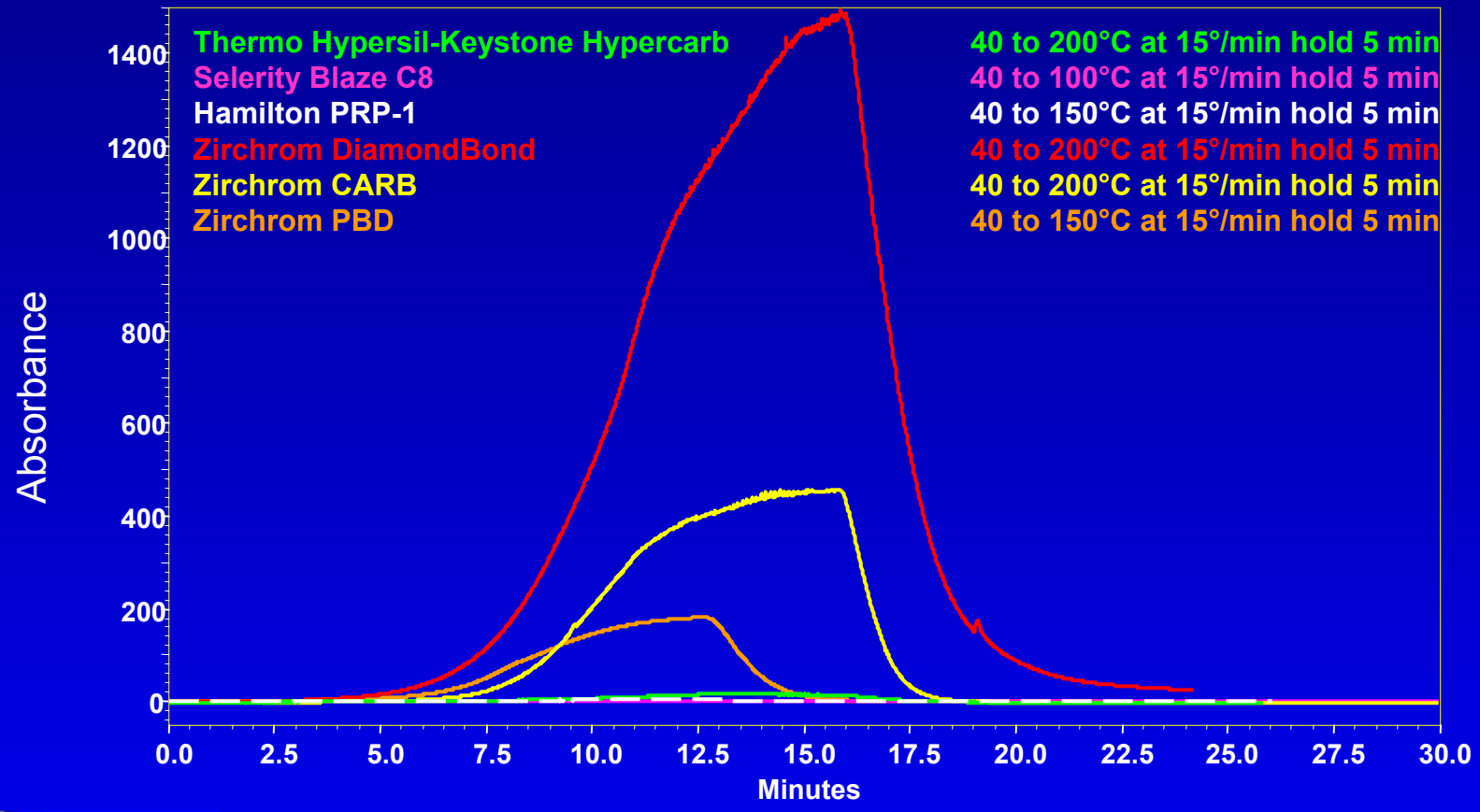


Stable Column Phases

- Silica (Selerity Blaze C_8)
- Zirconia* (ZirChrom PBD, DiamondBond, CARB)
- Carbon (Thermo Hypercarb)
- Polymeric (Jordi, Hamilton with SS fittings)

* Not recommended for temperature programmed conditions because of severe column bleed

Blank Runs with Thermal Gradients



50:50 Acetonitrile:Water at 254 nm

Polaratherm ™ Design

- Temperatures from sub-zero to 200 °C
- Forced air circulation
- Isothermal and thermal gradient operation
- Integrated solvent preheating
- Effluent temperature control
- Flammable vapor sensor
- Compatible with any HPLC system

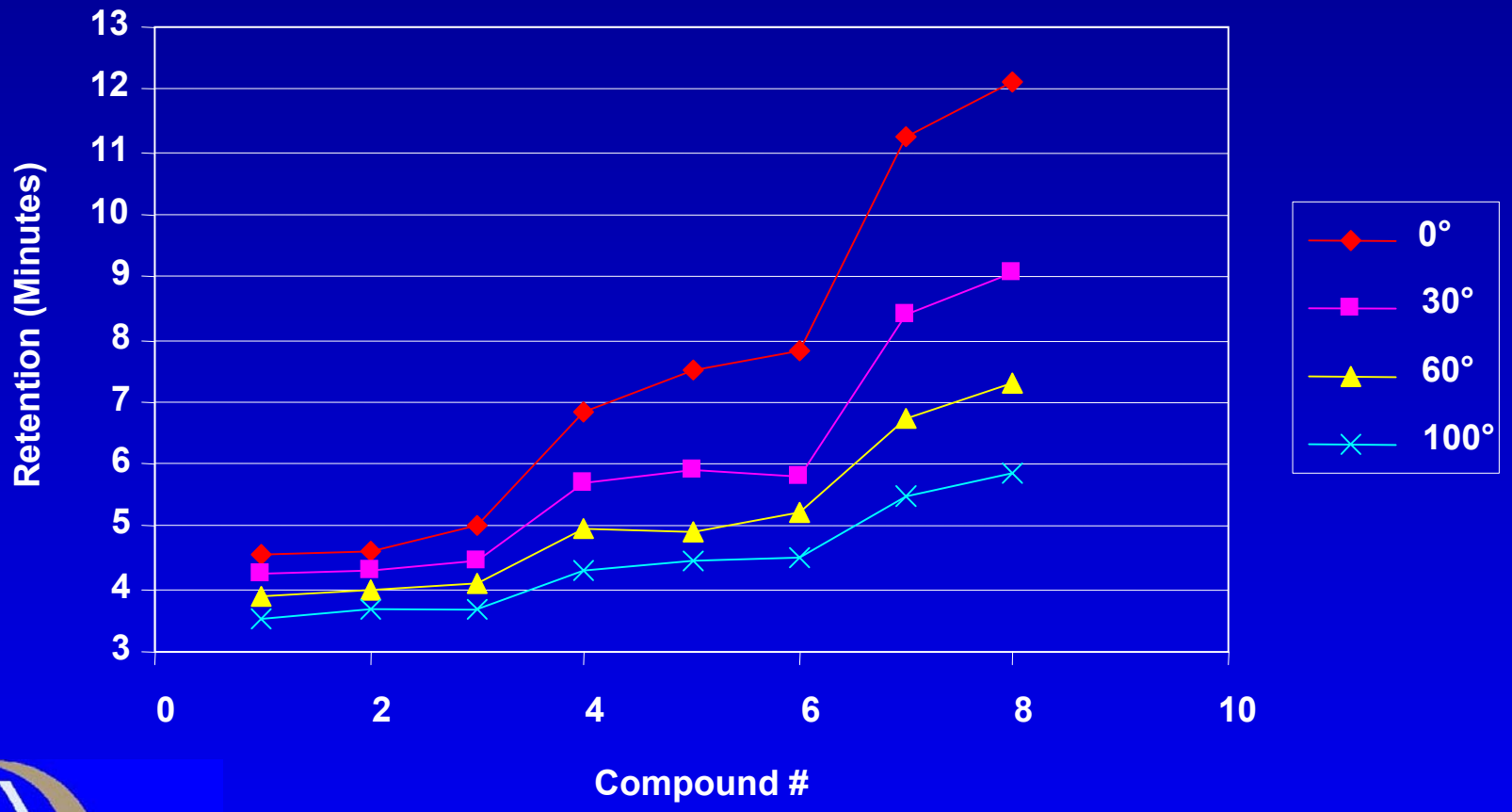
Diesel Range Aromatics - Columns

- Partisil Amino-Cyano (PAC) column
  - 5μm, 250 x 4.6 mm
- All stainless steel hardware
- Hexane mobile phase

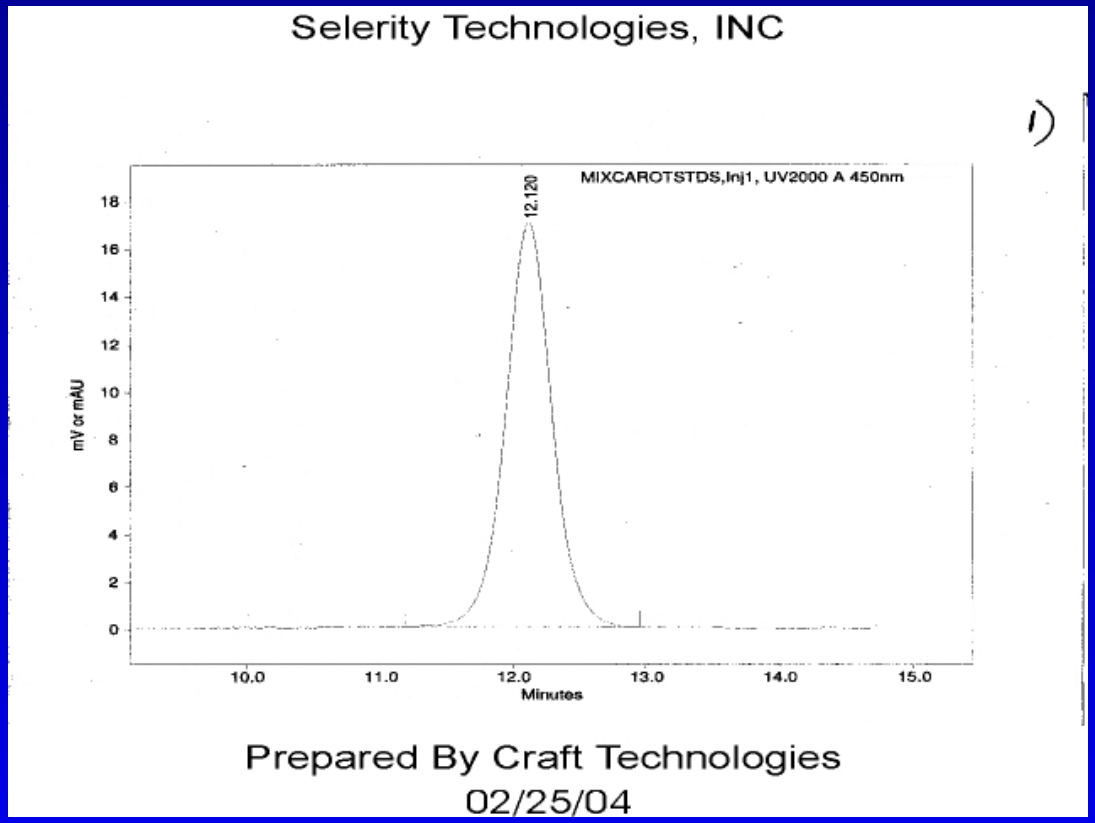
Retention Data for PAC Column Isothermal Conditions

Analyte	Retention Time			
	0°C	30°C	60°C	100°C
toluene	4.56	4.23	3.89	3.54
tetralin	4.59	4.29	4.00	3.69
thiophene	5.02	4.46	4.08	3.69
naphthalene	6.81	5.69	4.99	4.32
acenaphthene	7.49	5.89	4.92	4.47
benzothiophene	7.81	5.82	5.22	4.49
dibenzothiophene	11.25	8.38	6.73	5.47
anthracene	12.14	9.04	7.30	5.86

Retention Data for PAC Column Isothermal Conditions



β-Carotene and Isomers at 24 ºC



ACN/THF 90:10
Isocratic
1 mL/min
Column: Genesis C_{18}

β-Carotene and Isomers at 10 ºC

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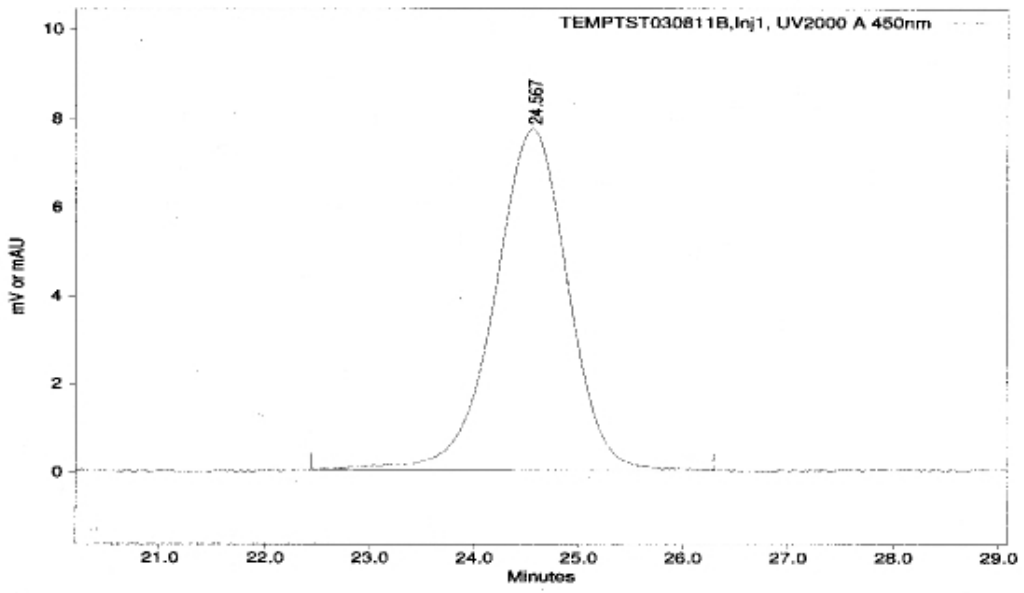
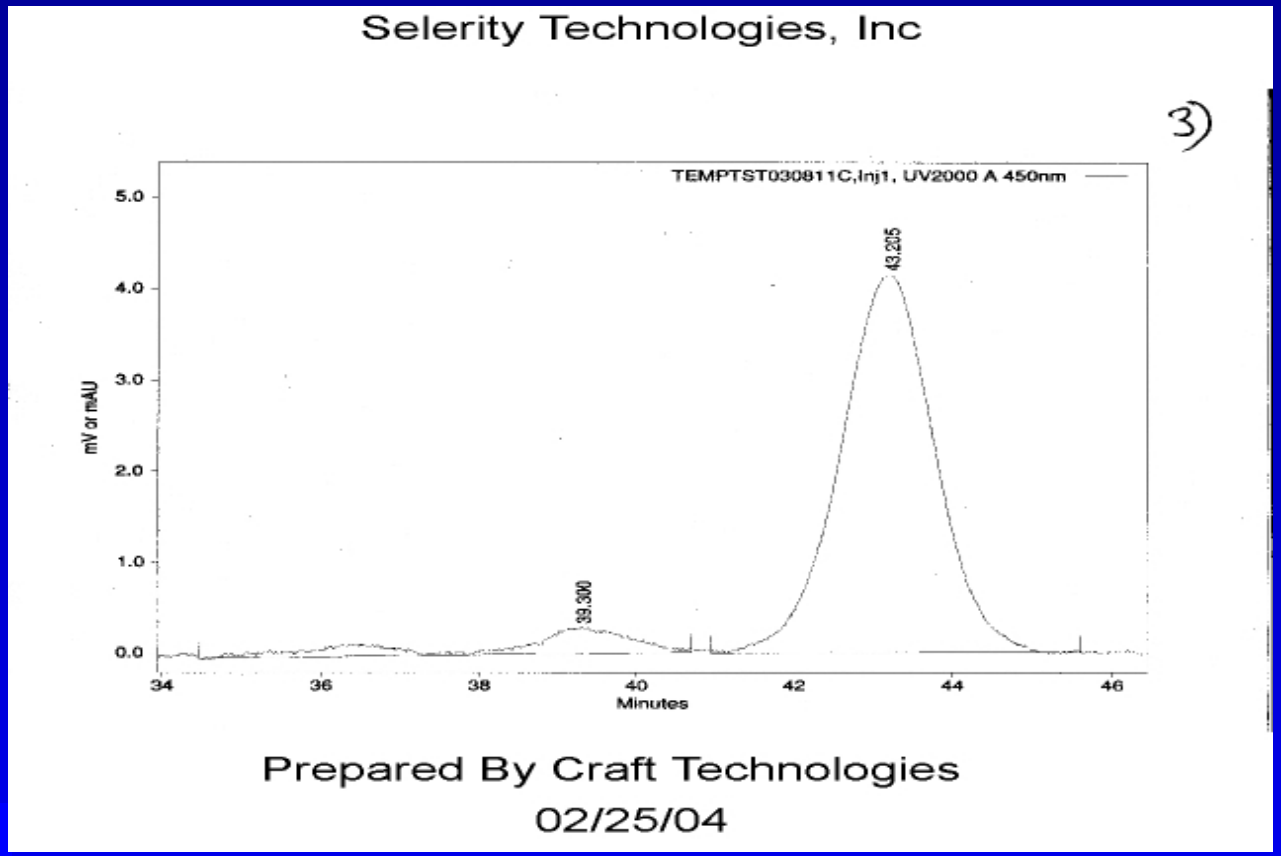


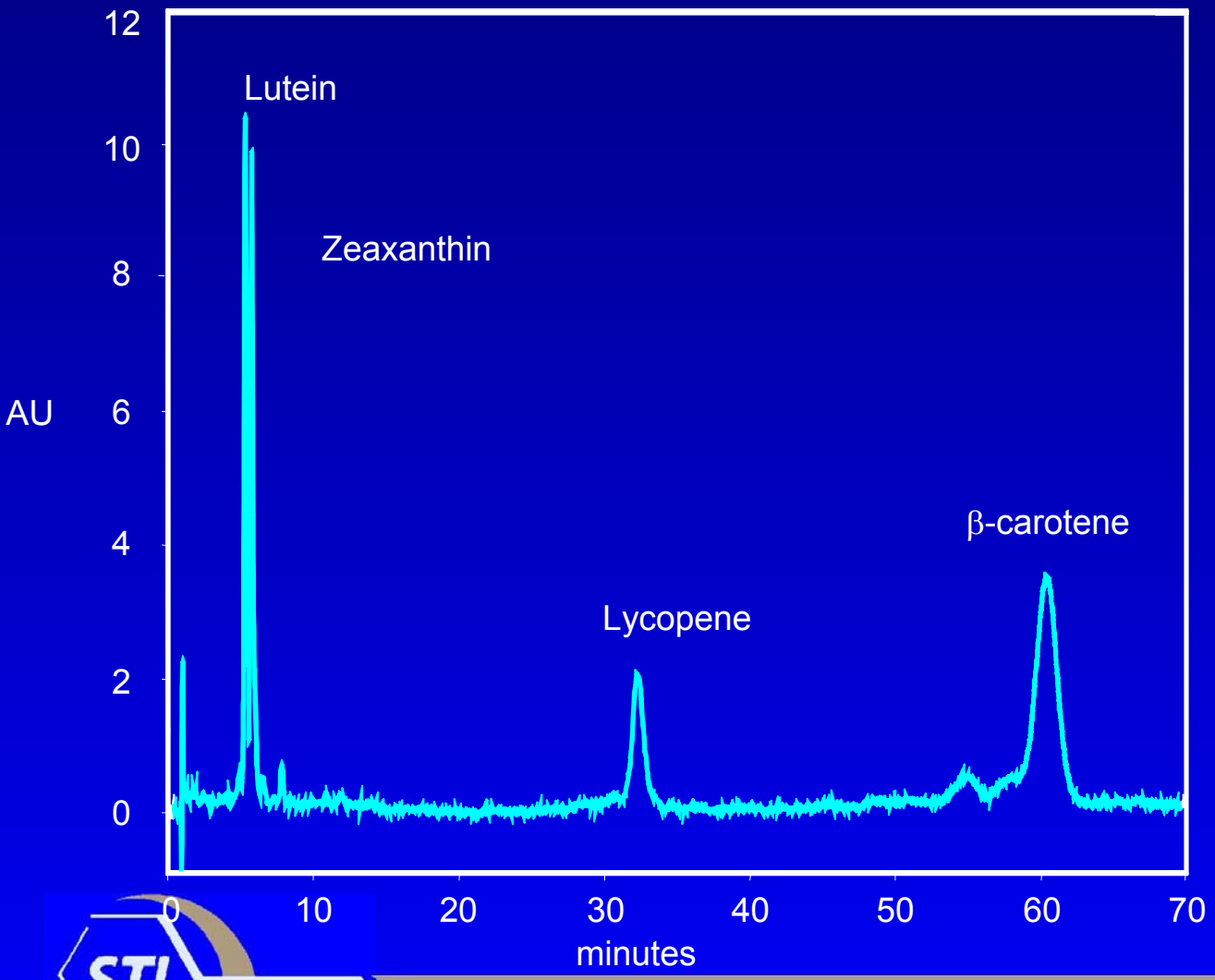
Prepared By Craft Technologies

02/25/04

β-Carotene and Isomers at 0 ºC



Separation of Carotenoid Mixture at 15°C



Column: Jones Chromatography Genesis C_{18}, 150 x 4.6 mm

Mobile Phase: 1.25% THF in acetonitrile

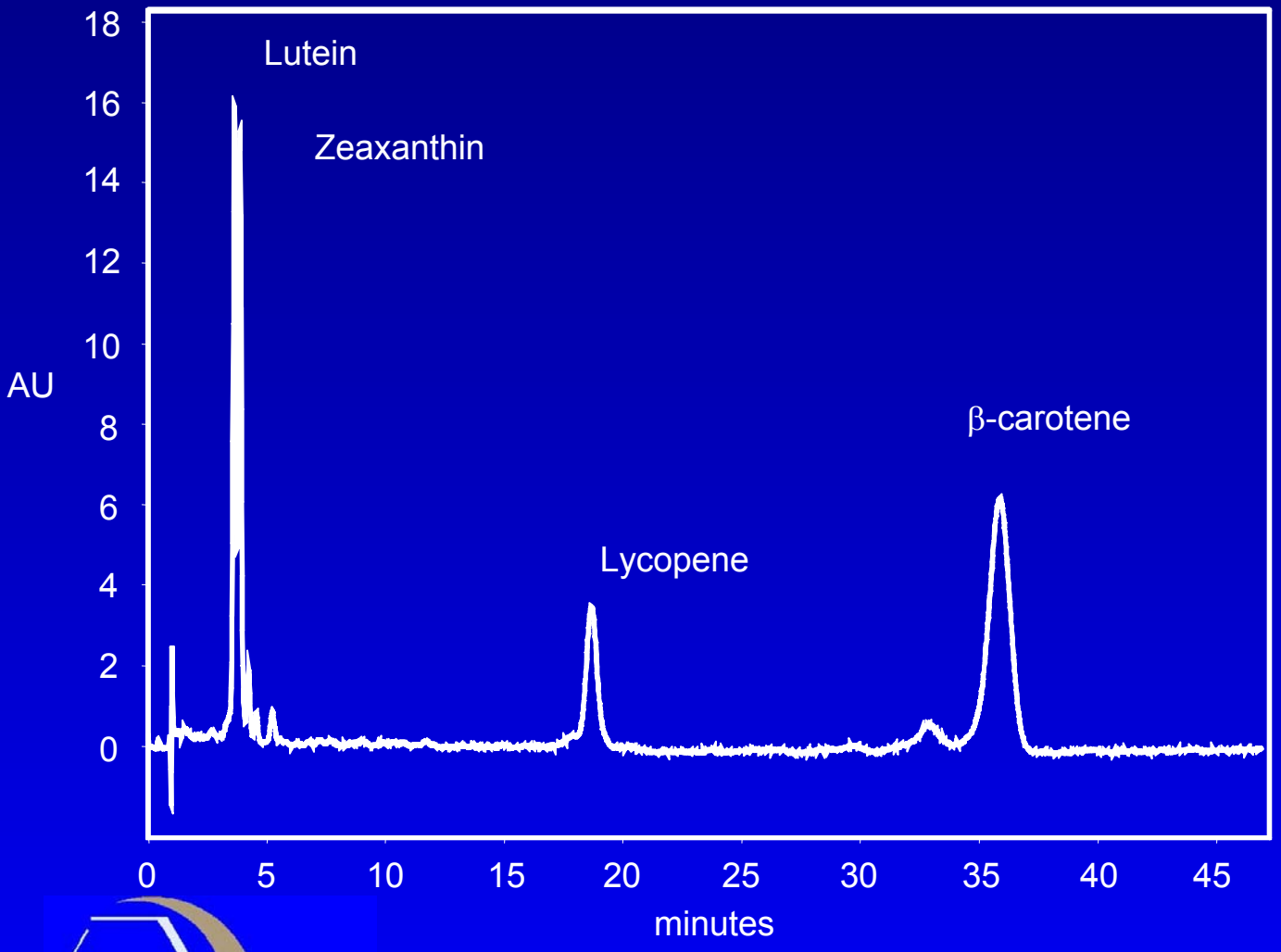
Flow Rate: 1.0 mL/min

Detection: UV 450 nm

Temperature: 15°C, isothermal

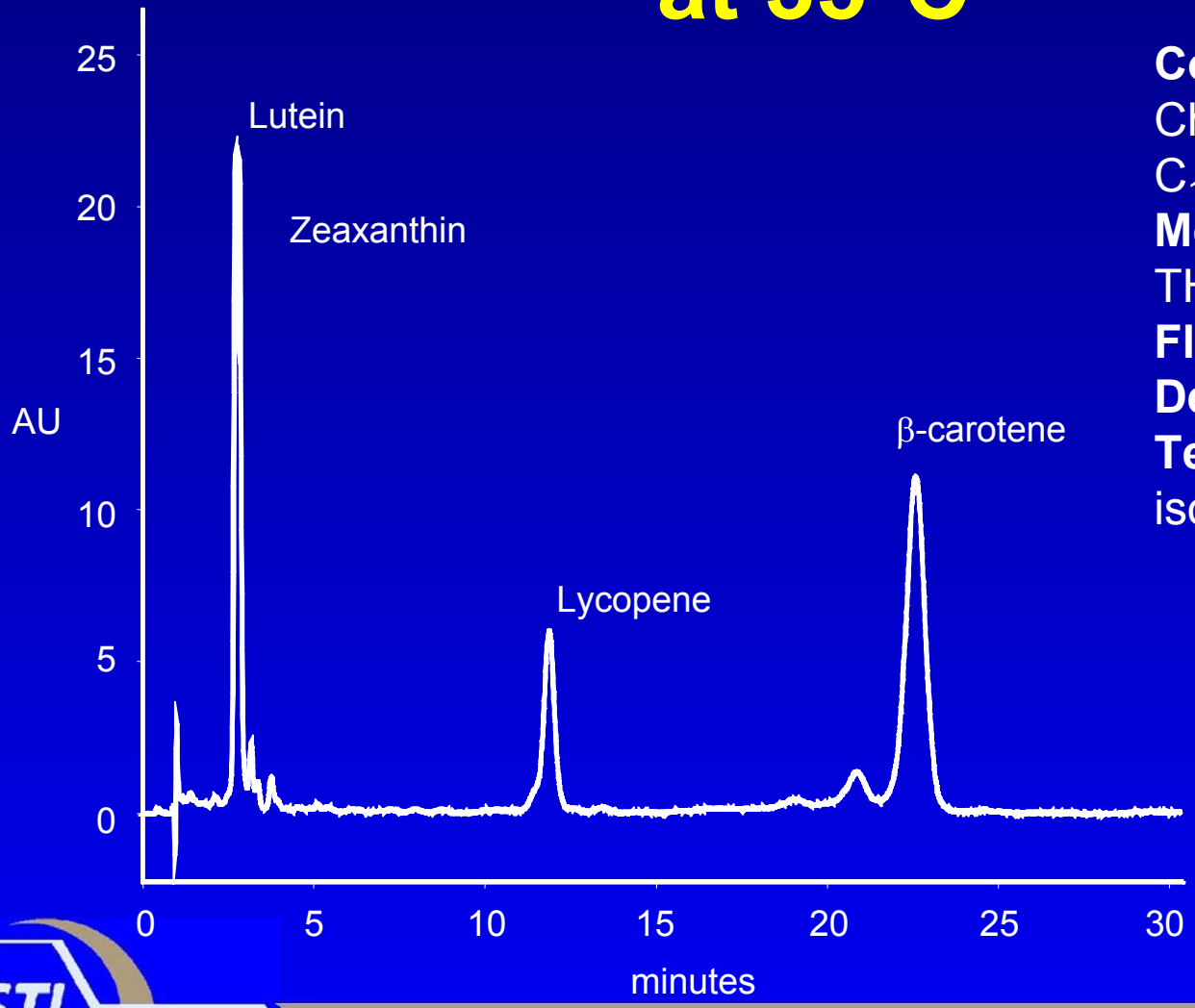
70 minute run time

Separation of Carotenoid Mixture at 25°C



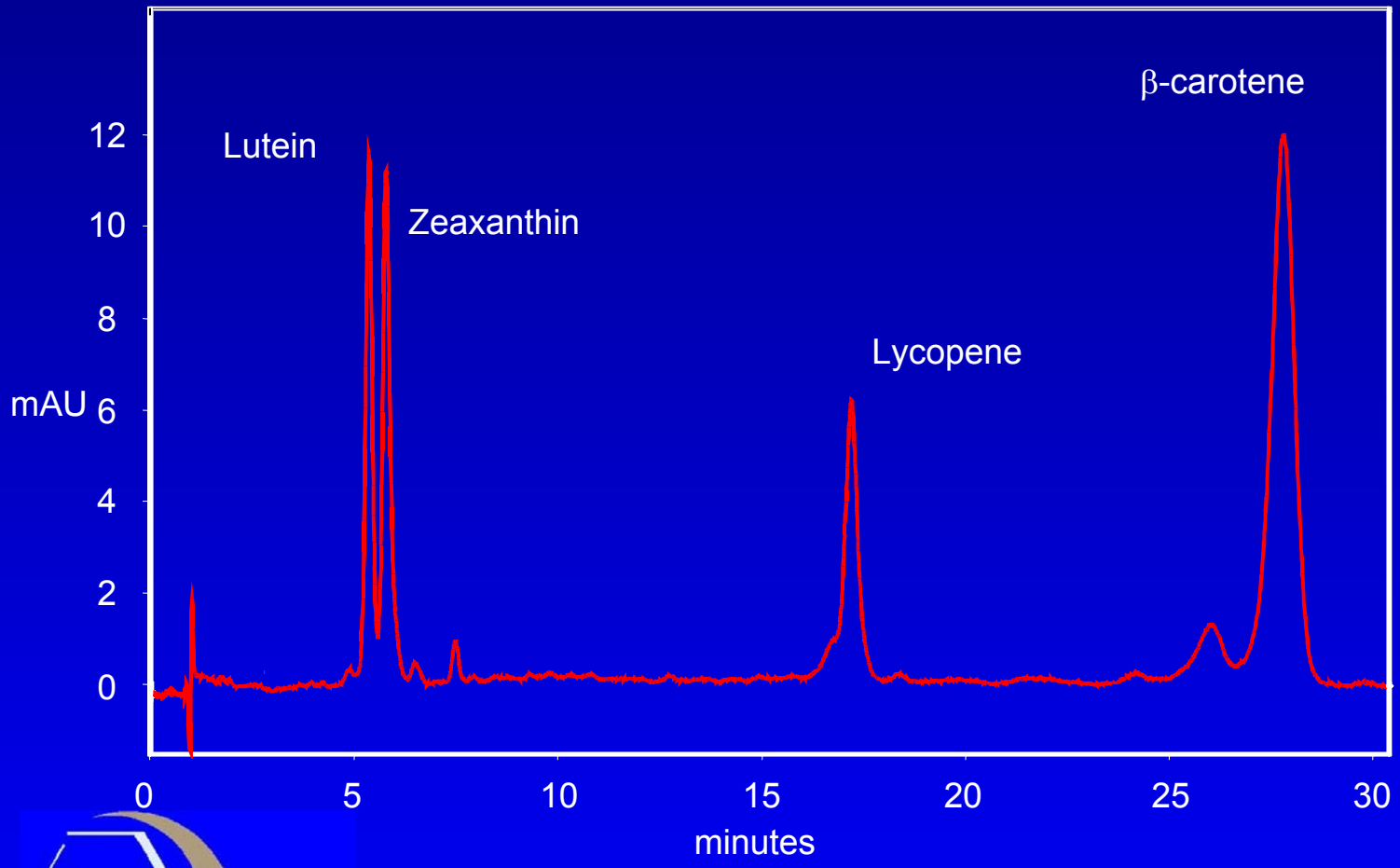
Column: Jones Chromatography Genesis C_{18}, 150 x 4.6 mm
Mobile Phase: 1.25% THF in acetonitrile
Flow Rate: 1.0 mL/min
Detection: UV 450 nm
Temperature: 25°C, isothermal

Separation of Carotenoid Mixture at 35°C



Column: Jones Chromatography Genesis C_{18}, 150 x 4.6 mm
Mobile Phase: 1.25% THF in acetonitrile
Flow Rate: 1.0 mL/min
Detection: UV 450 nm
Temperature: 35°C, isothermal

Separation of Carotenoid Mixture Using a Temperature Program



Column: Jones Chromatography Genesis C_{18}, 150 x 4.6 mm

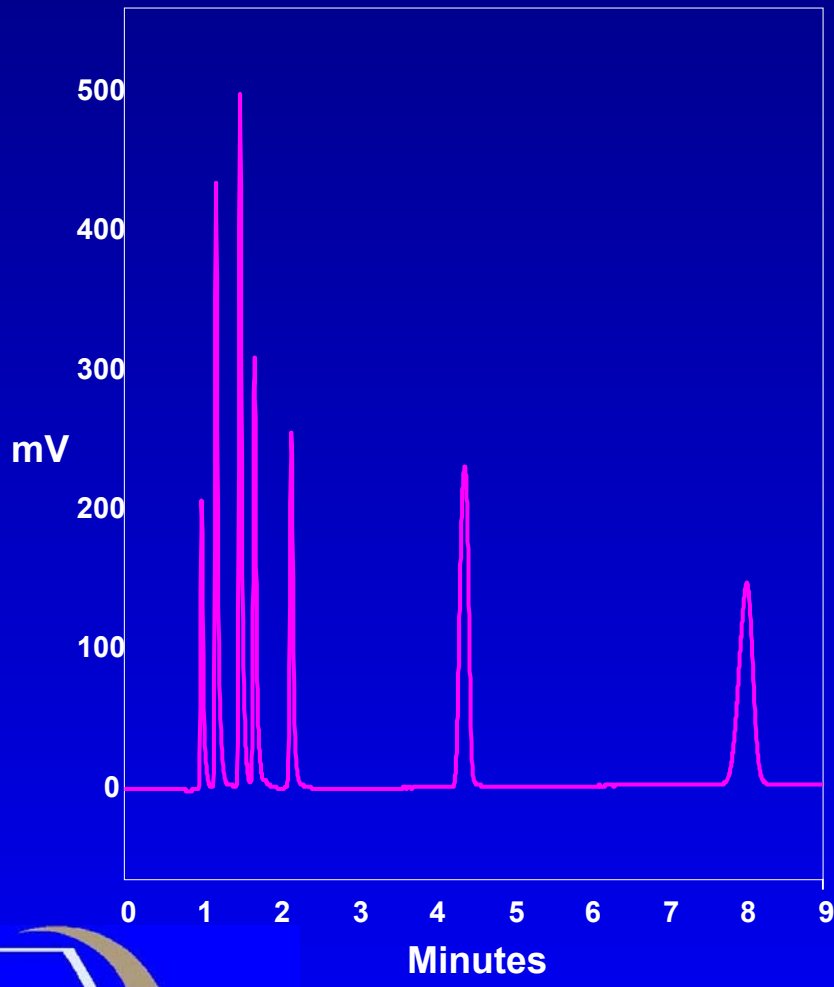
Mobile Phase: 1.25% THF in acetonitrile

Flow Rate: 1.0 mL/min

Detection: UV 450 nm

Temperature Program: hold at 15°C for five minutes, ramp to 35°C over two minutes, hold 15 minutes.

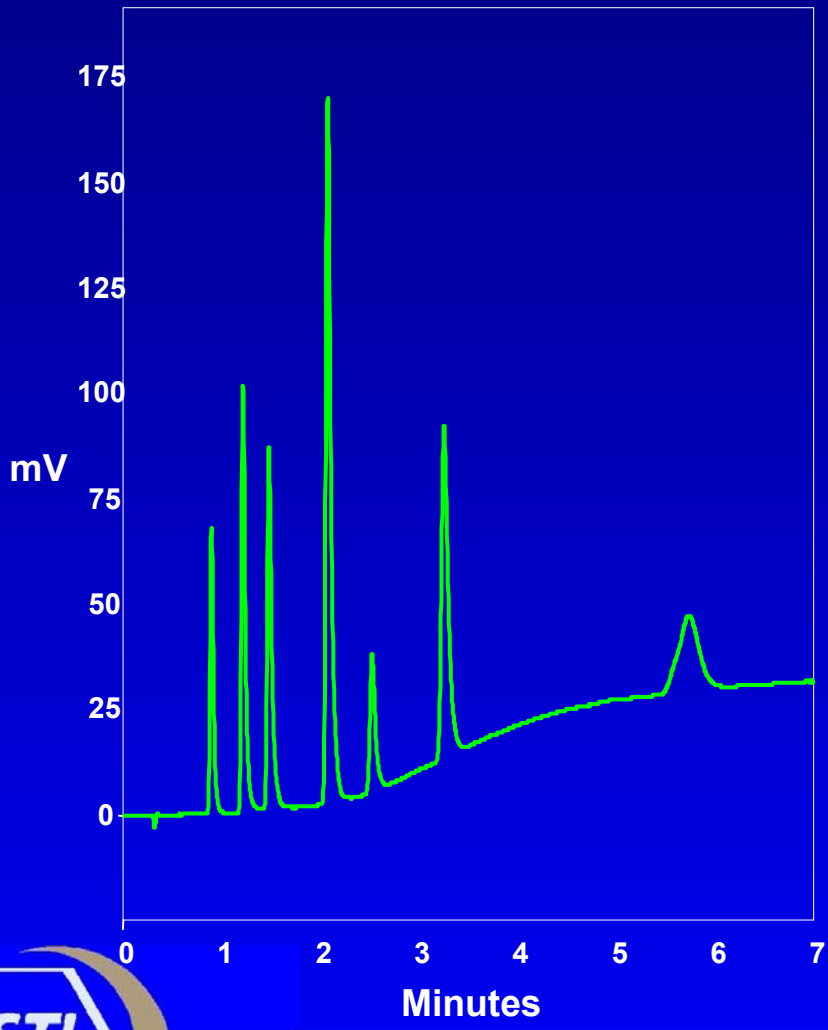
Separation of Analgesics on a Selerity Blaze™ C_8 Using a Thermal Gradient



Column: Selerity Blaze C_8, 3 μm 100 x 4.6 mm
Mobile Phase: 40:60 acetonitrile:water with 0.1%TFA
Flow Rate: 1.5 mL/min
Detection: UV 220 nm
Temperature Program: hold at 50°C for one minute, ramp to 100°C at 30°C/min, hold six min.

Elution Order:
Acetaminophen
Caffeine
Salicylamide
Aspirin
Salicylic acid
Ibuprofen
Naproxen

Analgesics Using a Hypercarb® Column and a Thermal Gradient

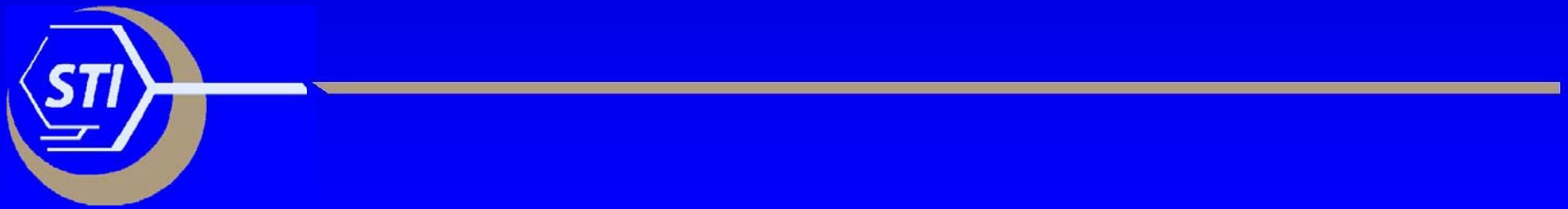


Column: Thermo Electron Hypercarb®, 7 μm, 100 x 4.6 mm
Mobile Phase: 35:65 acetonitrile:water with 0.1% TFA
Flow Rate: 4.0 mL/min
Detection: UV 220 nm
Temperature Program: thermal gradient from 125° to 200°C at 30°/min, hold five min.

Elution Order:
Caffeine
Aspirin
Salicylic Acid
Ibuprofen
Phenacetin
Acetaminophen
Naproxen

Conclusions

- Temperature is powerful but under-used tool for selectivity tuning.
- Increasing the temperature reduces the amount of organic modifier needed and generally speeds elution.
- Temperature adjustment can be a fast way to optimize a separation.
- Shorter analysis times with better efficiency usually result from higher temperatures.



Acknowledgements

The authors thank Thermo Electron and Hamilton Company for providing columns for this work.

Appreciation is also given to Craft Technologies for the carotenoid data.

Turn up the Heat!