Dramatic Increases in Efficiency and Resolution Using High Temperature Liquid Chromatography

Stephanie J. Marin, Dale Felix, Brian Jones and Jody Clark

Selerity Technologies

Salt Lake City, UY

www.selerity.com

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

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 particles to increase efficiency
  - Extend column length to add plates

Temperature Programming Benefits

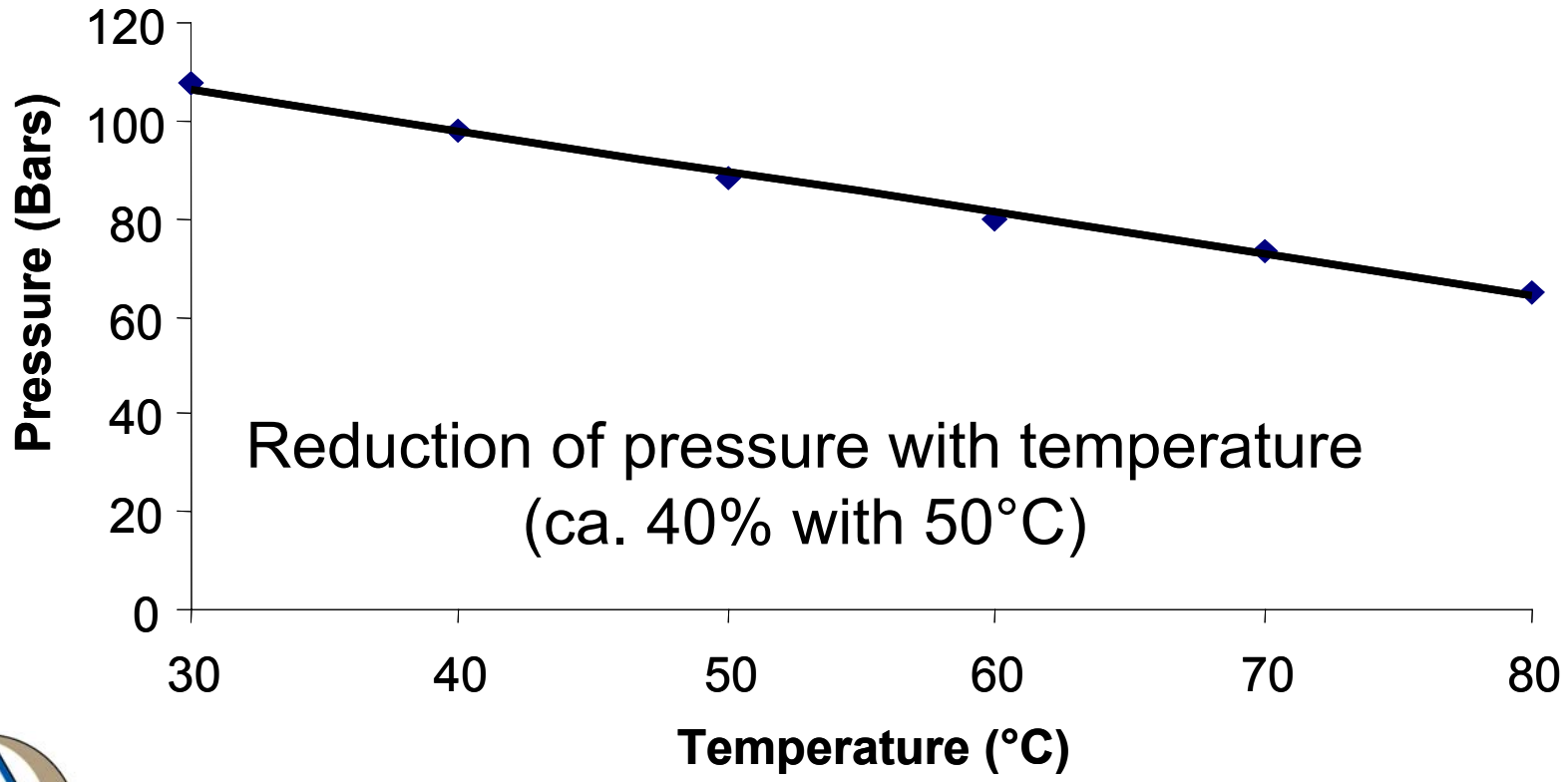
- Allows increased plate count by adding column length
- Increases speed by increasing flow rate as viscosity decreases at elevated temperatures
- Changes selectivity as function of temperature and column phase
- Reduces organic solvent content – “Green Chemistry”

Selerity Polaratherm™ Series 9000 Total Temperature Controller

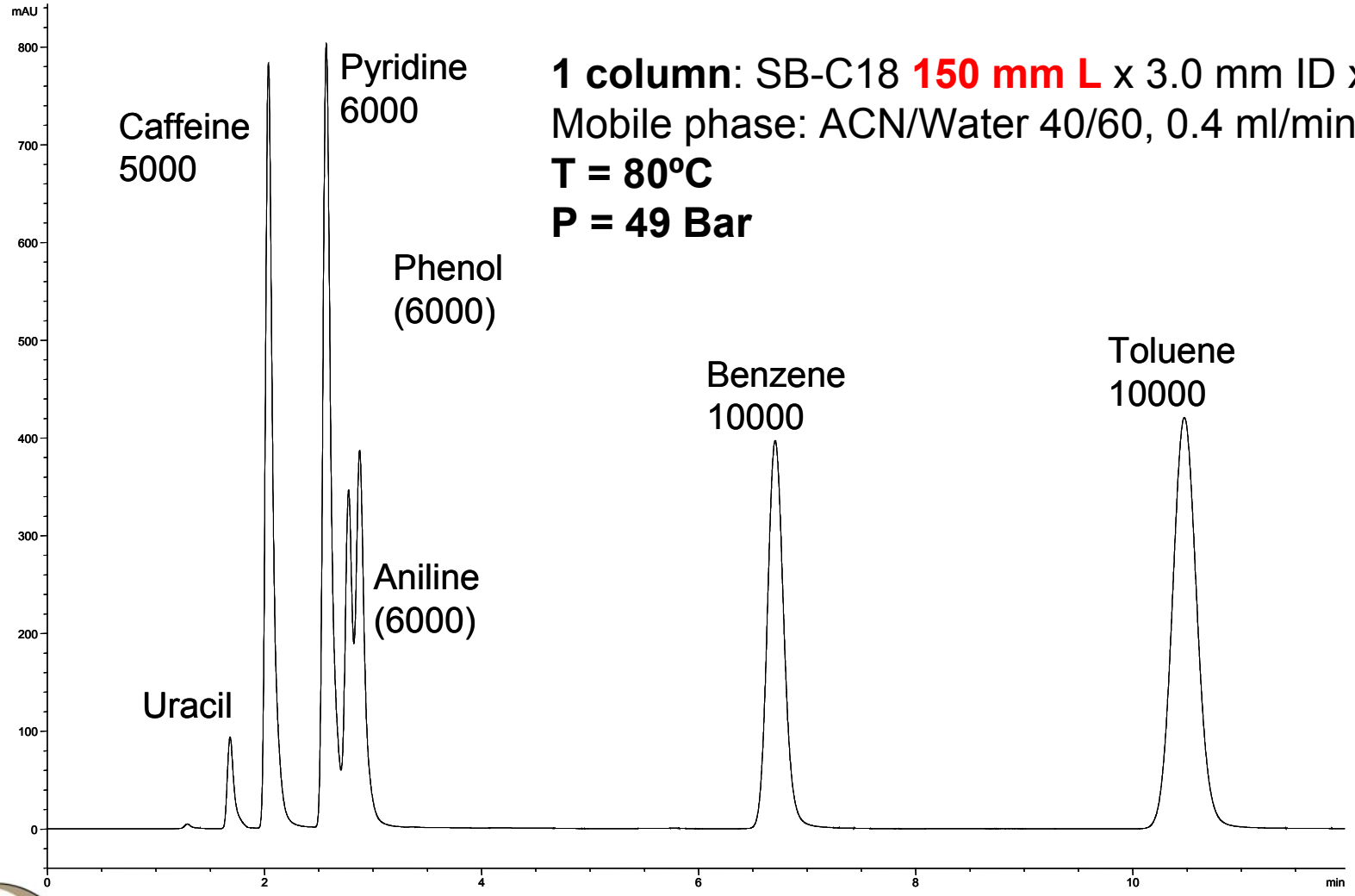
- Used in this study except where noted
- Forced air oven and chiller
- Isothermal and thermal gradient operation
  - –Sub-zero to 200°C
  - –Thermal gradients up to 30°C/min
- Mobile phase preheating and pre-cooling
- Peltier effluent temperature control
- Vapor sensor
- Compatible with any HPLC system
- Integrated software control with Waters, Agilent, and EZChrom

High efficiency analyses

Utilization of multiple columns to improve efficiency



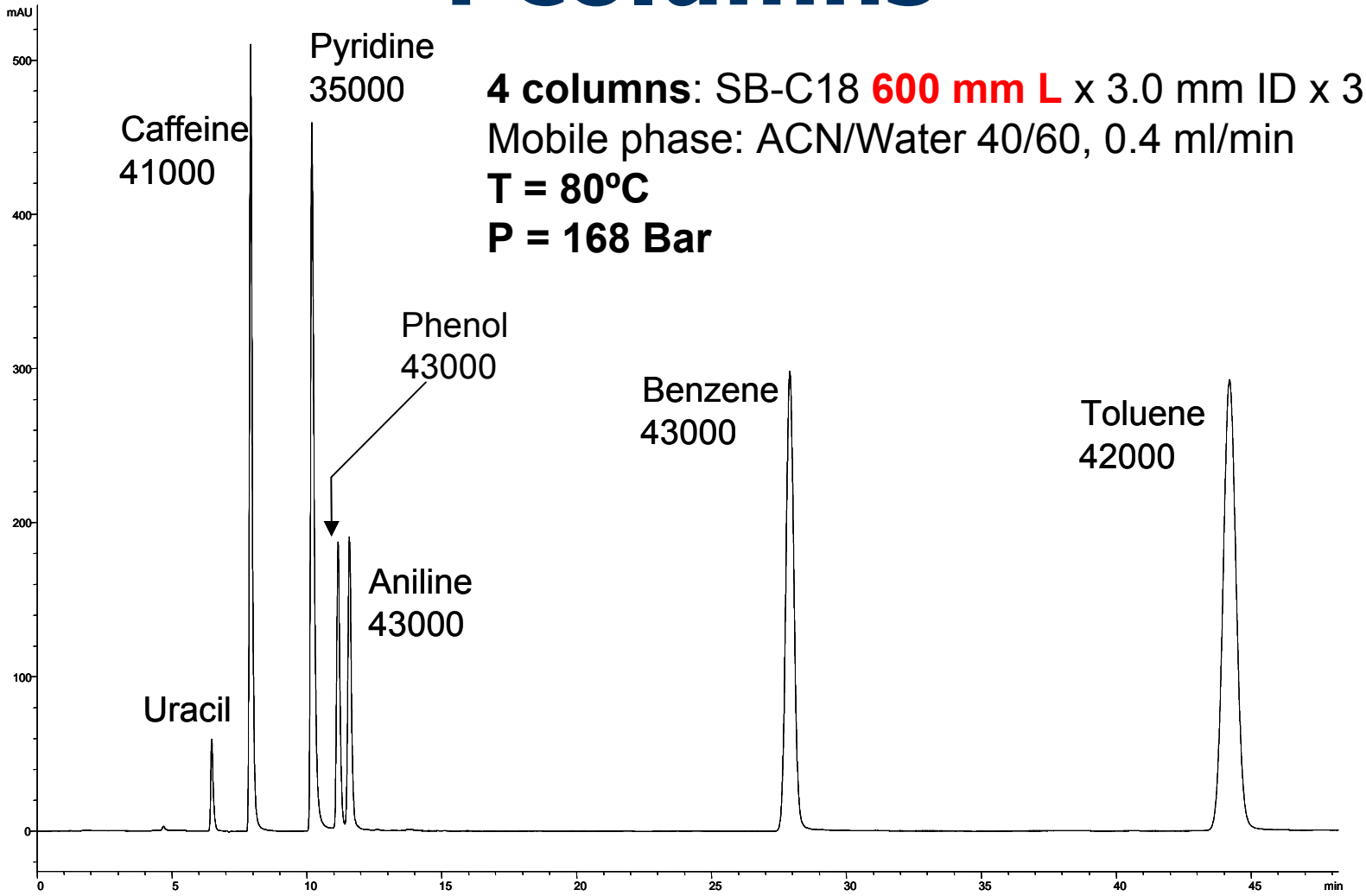
1 column



1 column: SB-C18 **150 mm L** x 3.0 mm ID x 3.5 µm
Mobile phase: ACN/Water 40/60, 0.4 ml/min
T = 80ºC
P = 49 Bar

Chromatogram courtesy of Dr. Pat Sandra, Research Institute for Chromatography

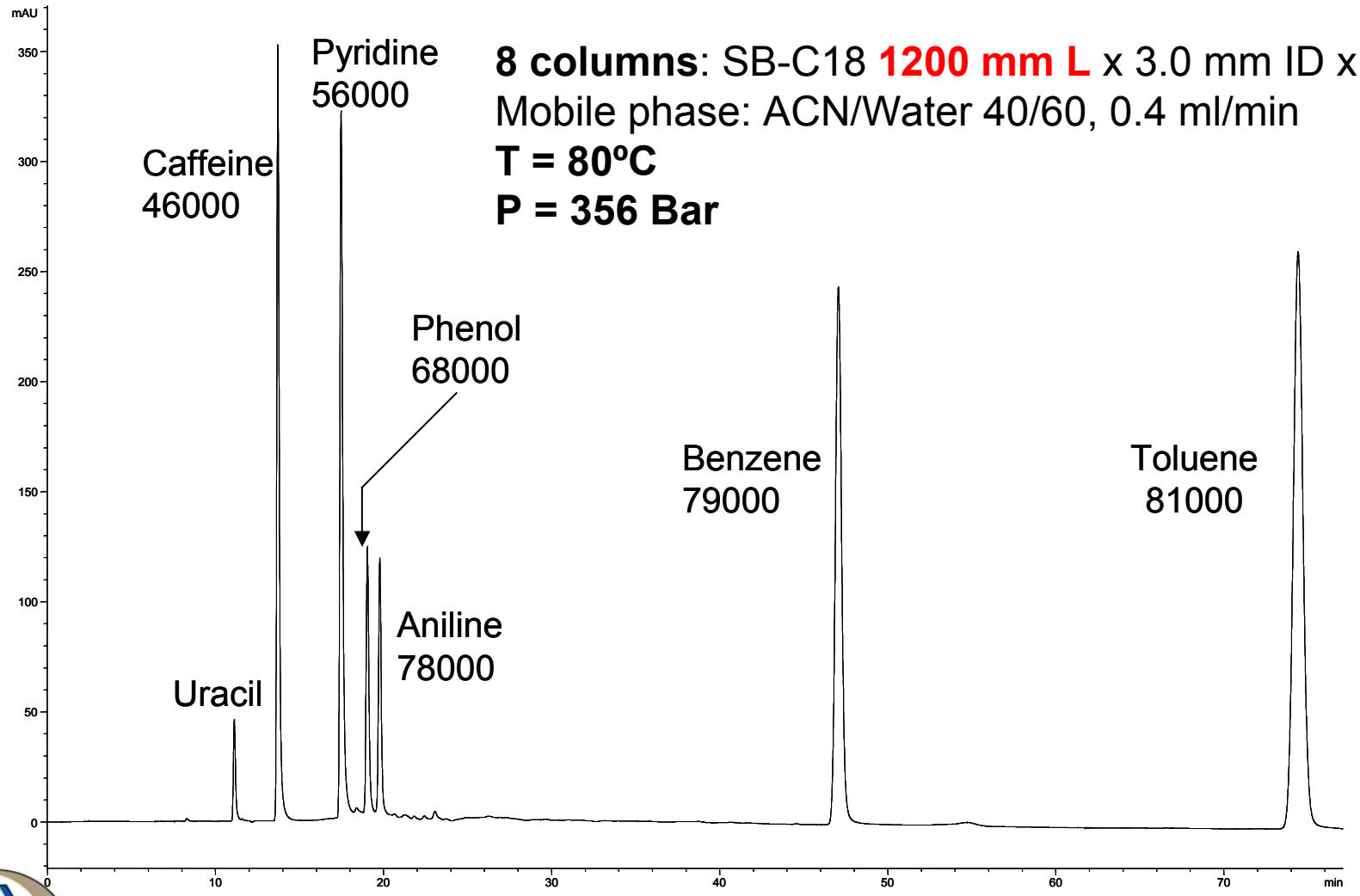
4 columns



4 columns: SB-C18 **600 mm L** x 3.0 mm ID x 3.5 µm
Mobile phase: ACN/Water 40/60, 0.4 ml/min
T = 80ºC
P = 168 Bar

Chromatogram courtesy of Dr. Pat Sandra, Research Institute for Chromatography

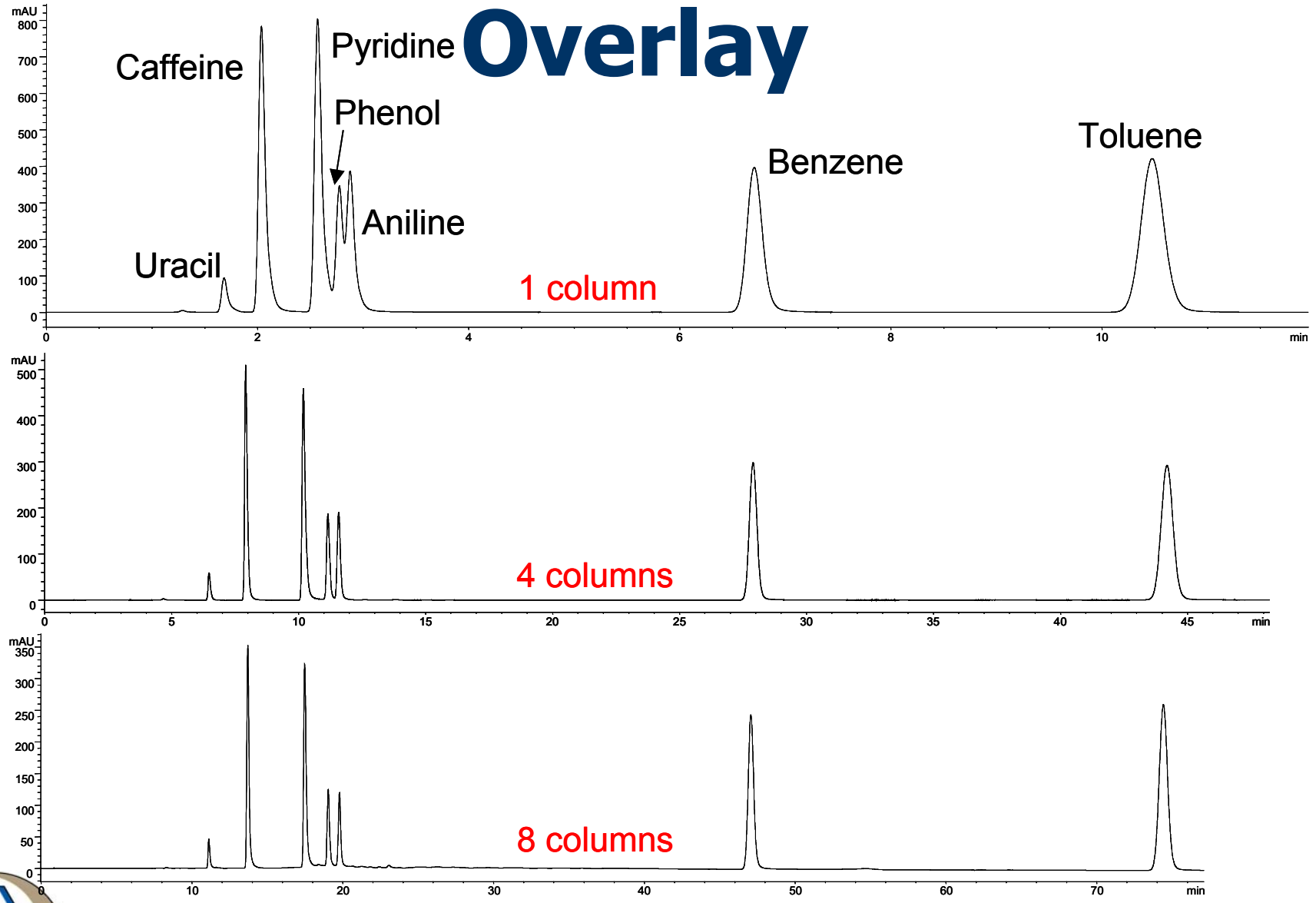
8 columns



8 columns: SB-C18 **1200 mm L** x 3.0 mm ID x 3.5 µm
Mobile phase: ACN/Water 40/60, 0.4 ml/min
T = 80ºC
P = 356 Bar

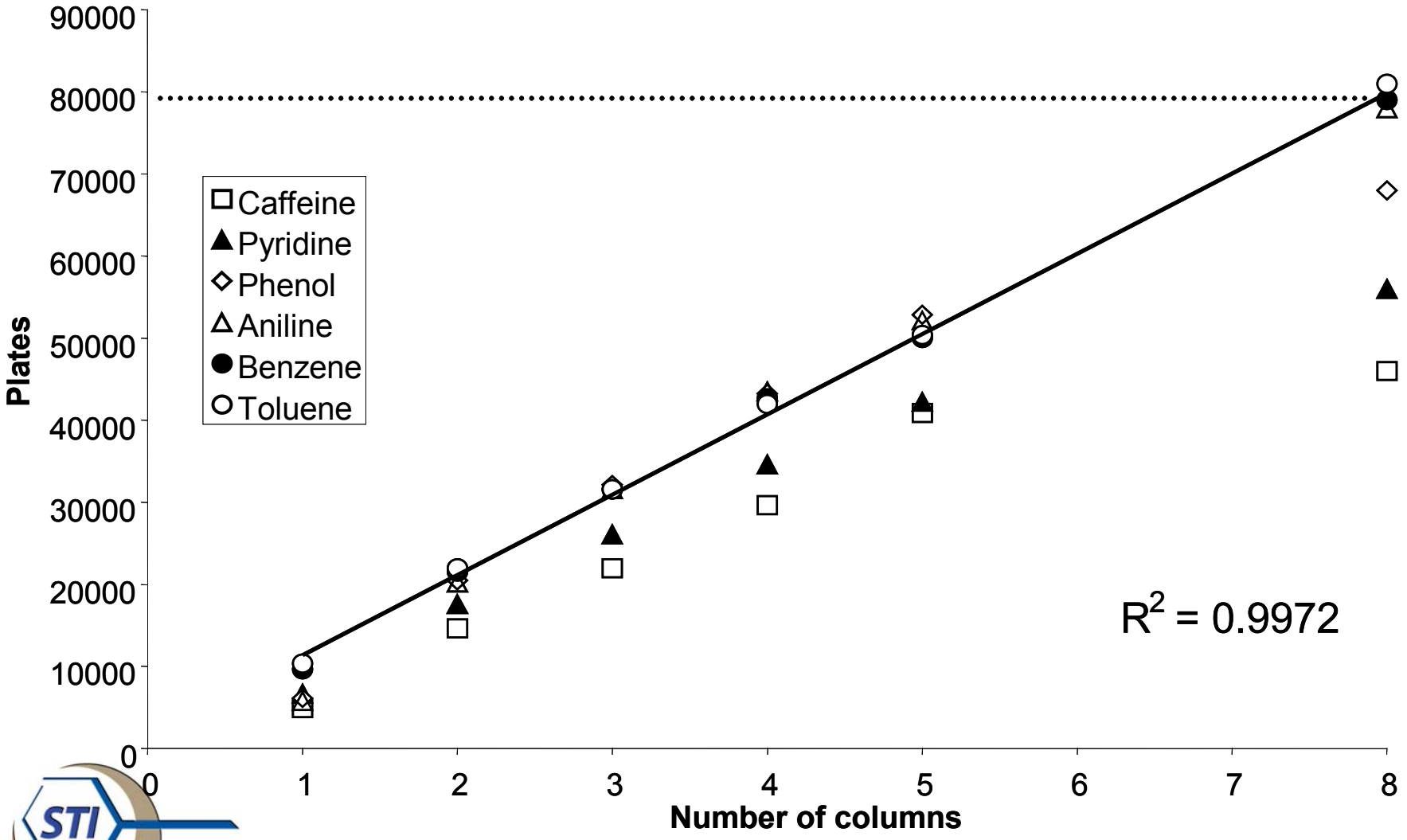
Chromatogram courtesy of Dr. Pat Sandra, Research Institute for Chromatography

Overlay



Chromatogram courtesy of Dr. Pat Sandra, Research Institute for Chromatography

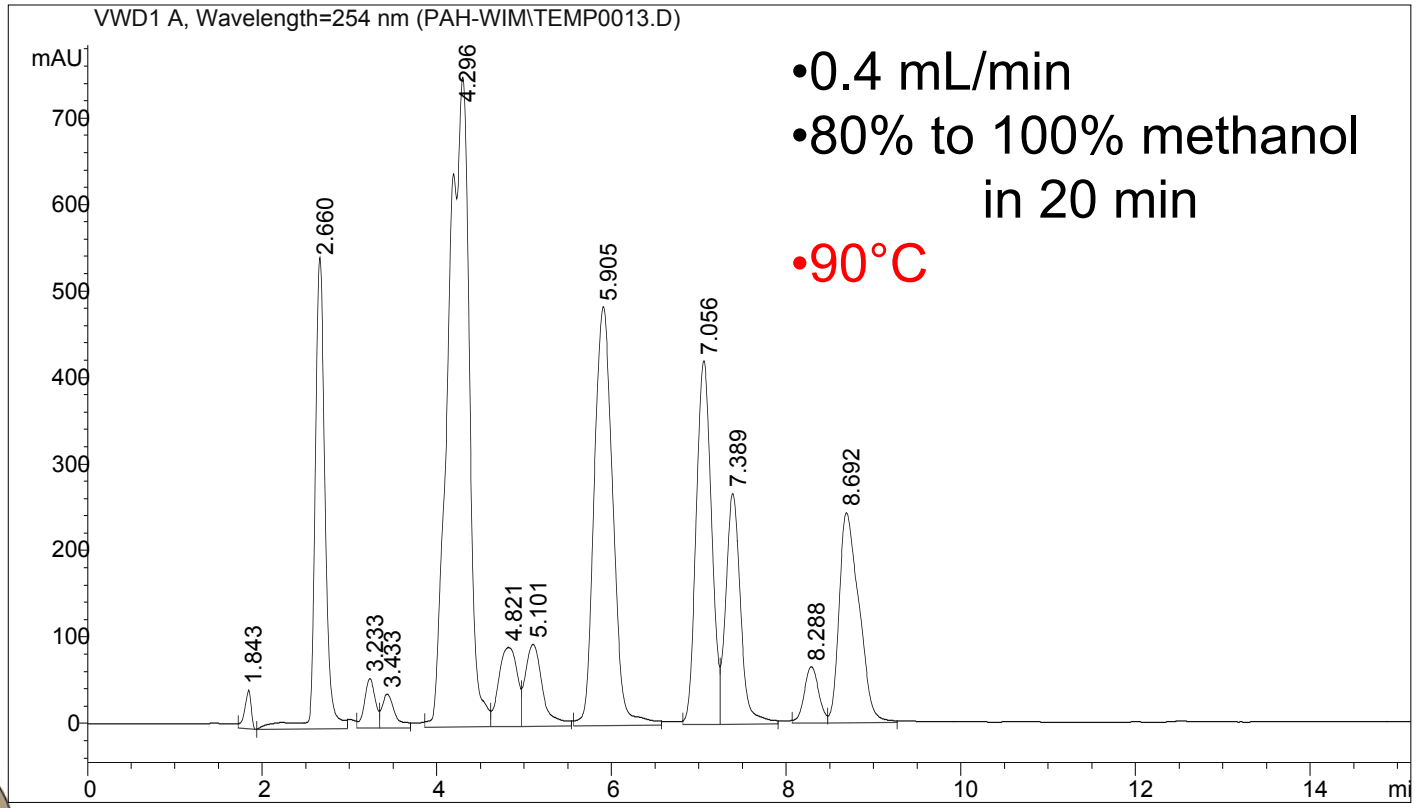
Plate numbers vs columns



Analysis of PAHs on ODS column

- Column:

  Zorbax SB-C18 150 mm L x 3.0 mm ID x 3.5 µm d_p

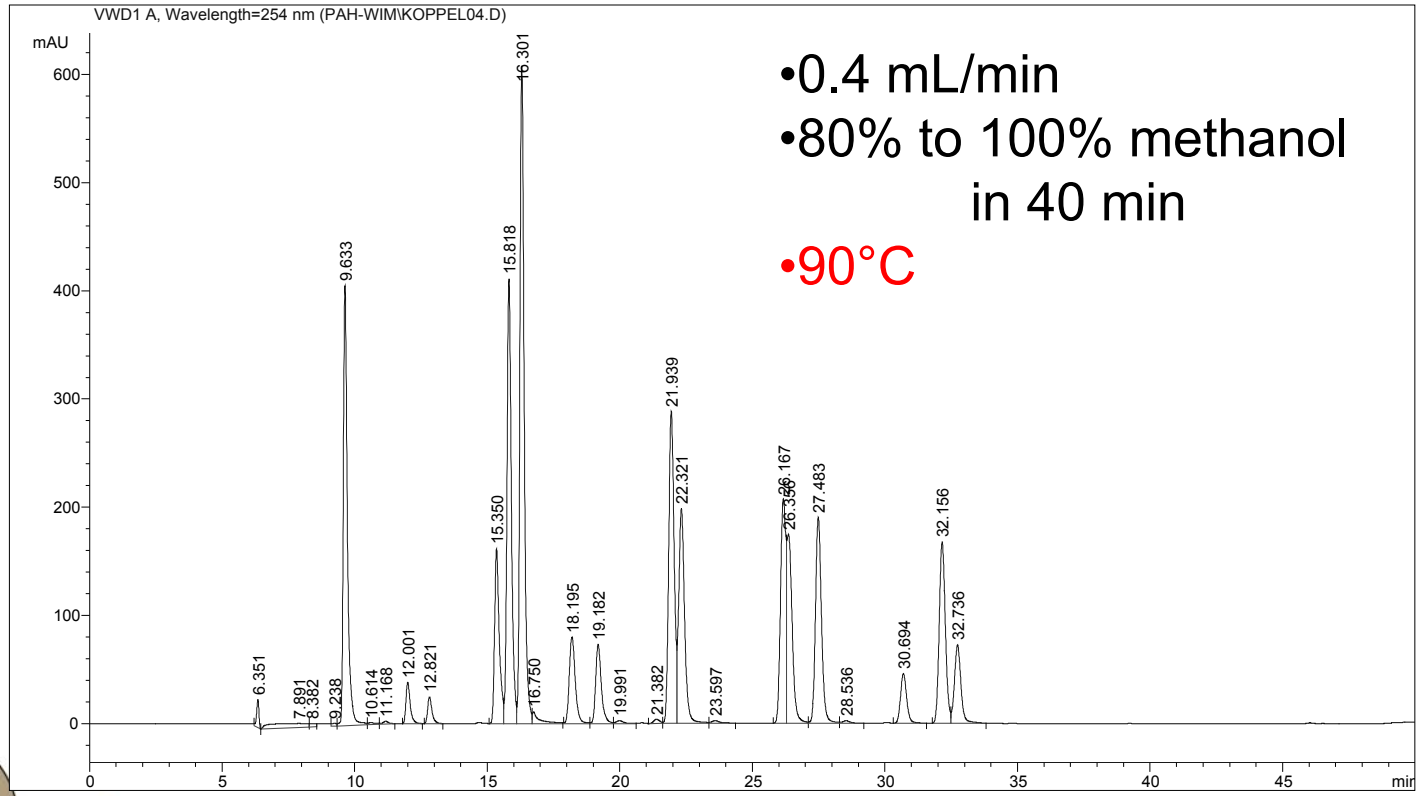


Chromatogram courtesy of Dr. Pat Sandra, Research Institute for Chromatography

Analysis of PAHs on ODS column

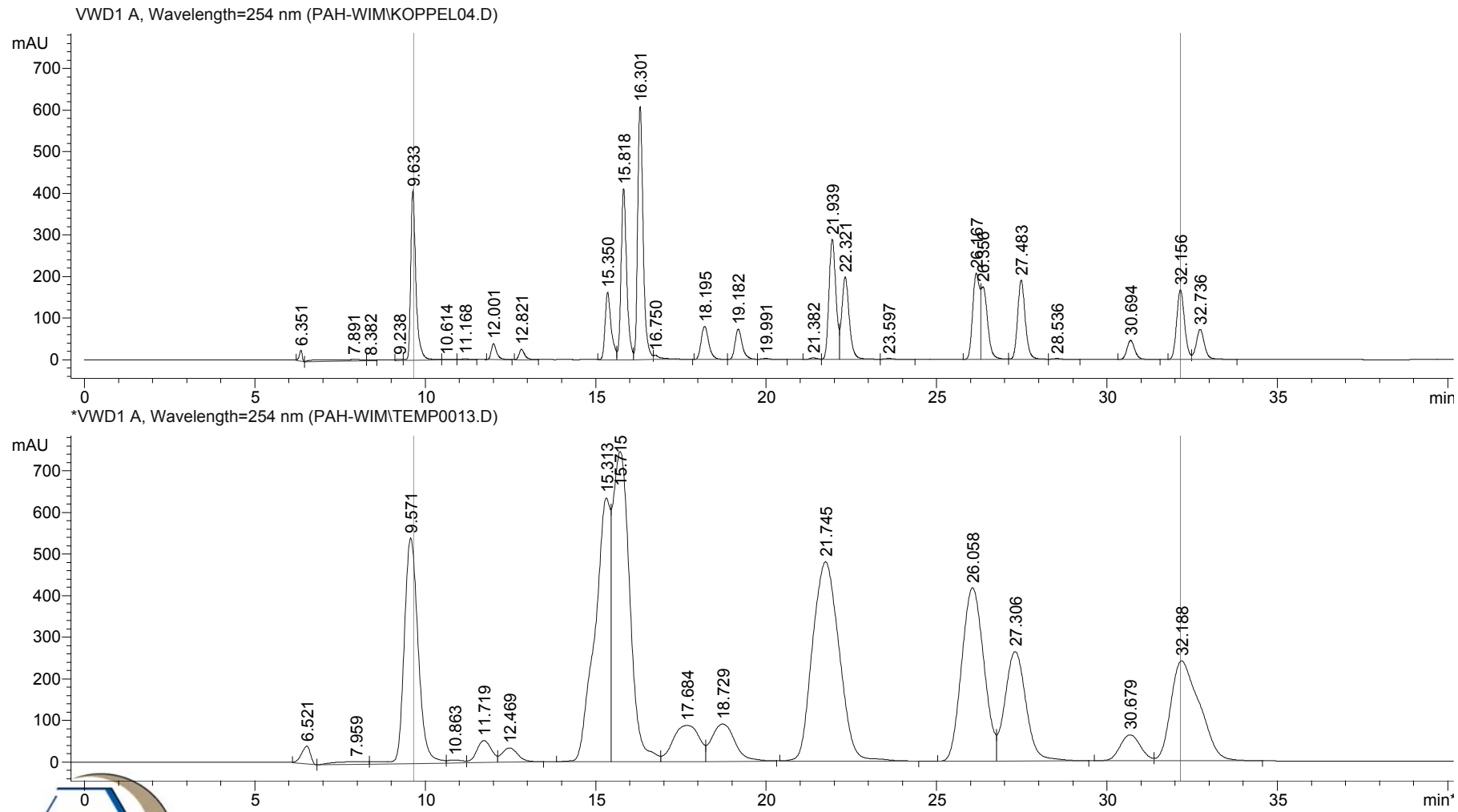
- Column: **60 cm**

  4x Zorbax SB-C18 150 mm L x 3.0 mm ID x 3.5 µm d_p

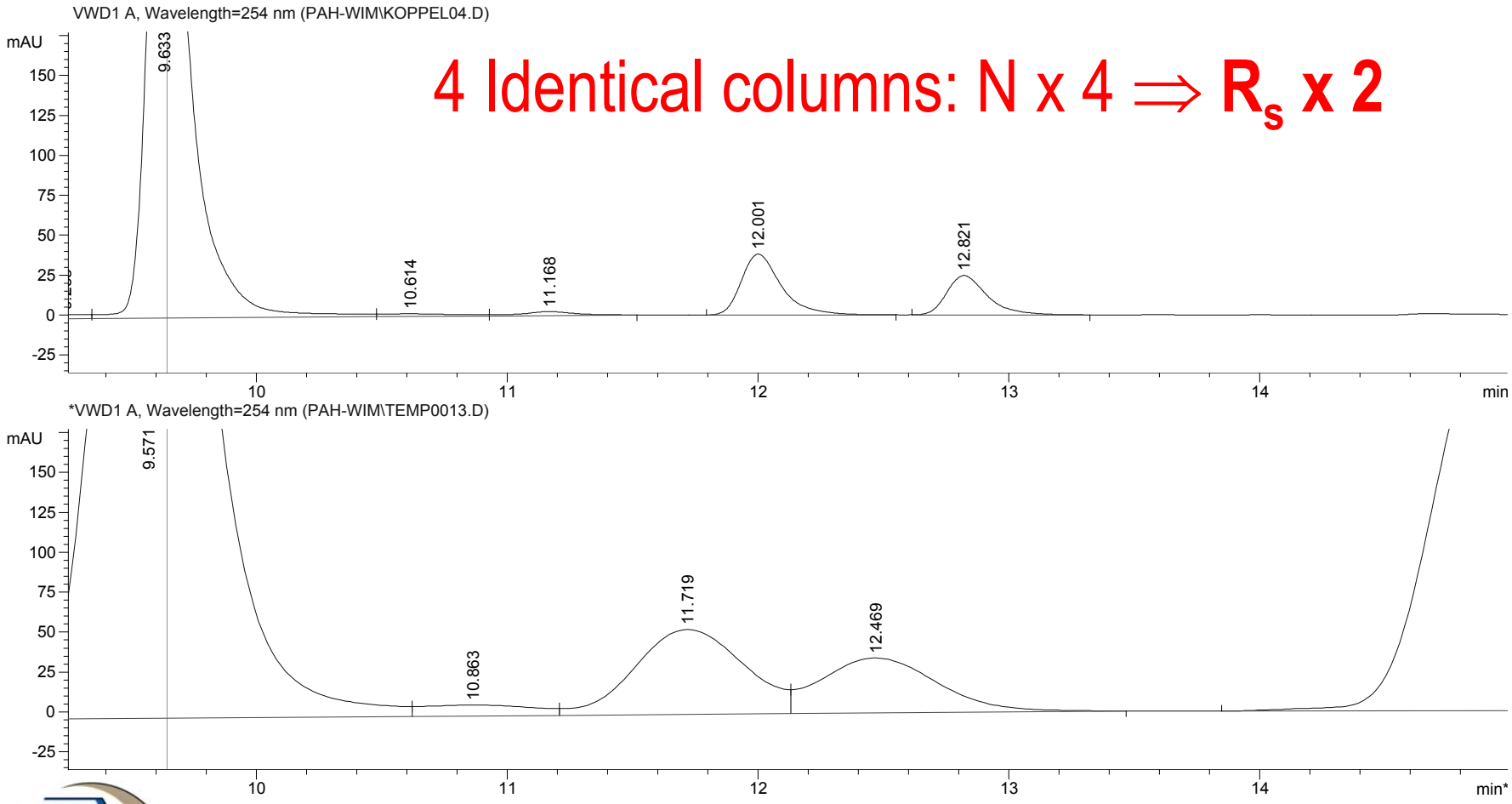


Chromatogram courtesy of Dr. Pat Sandra, Research Institute for Chromatography

Aligned chromatograms



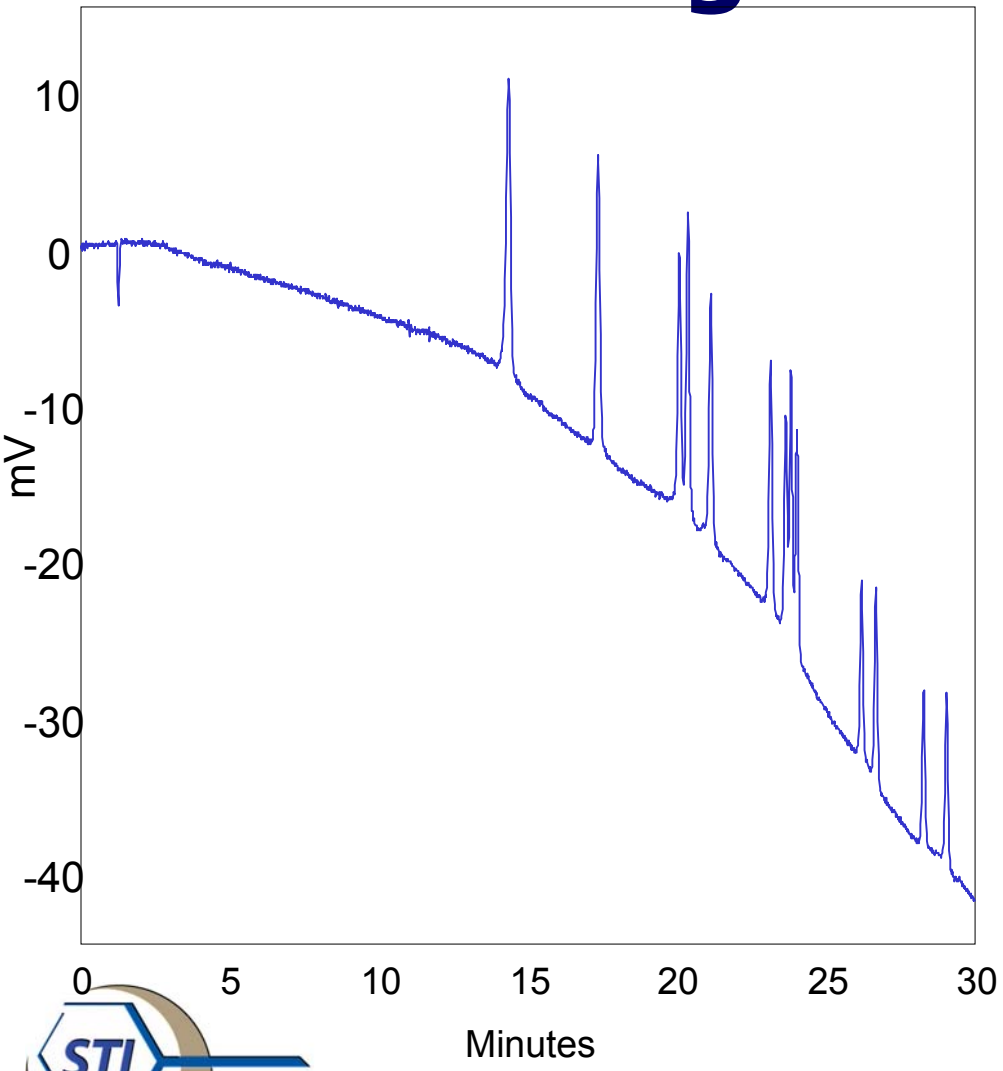
Comparison resolution



4 Identical columns: N x 4 $\Rightarrow$ **R_s x 2**

Chromatogram courtesy of Dr. Pat Sandra, Research Institute for Chromatography

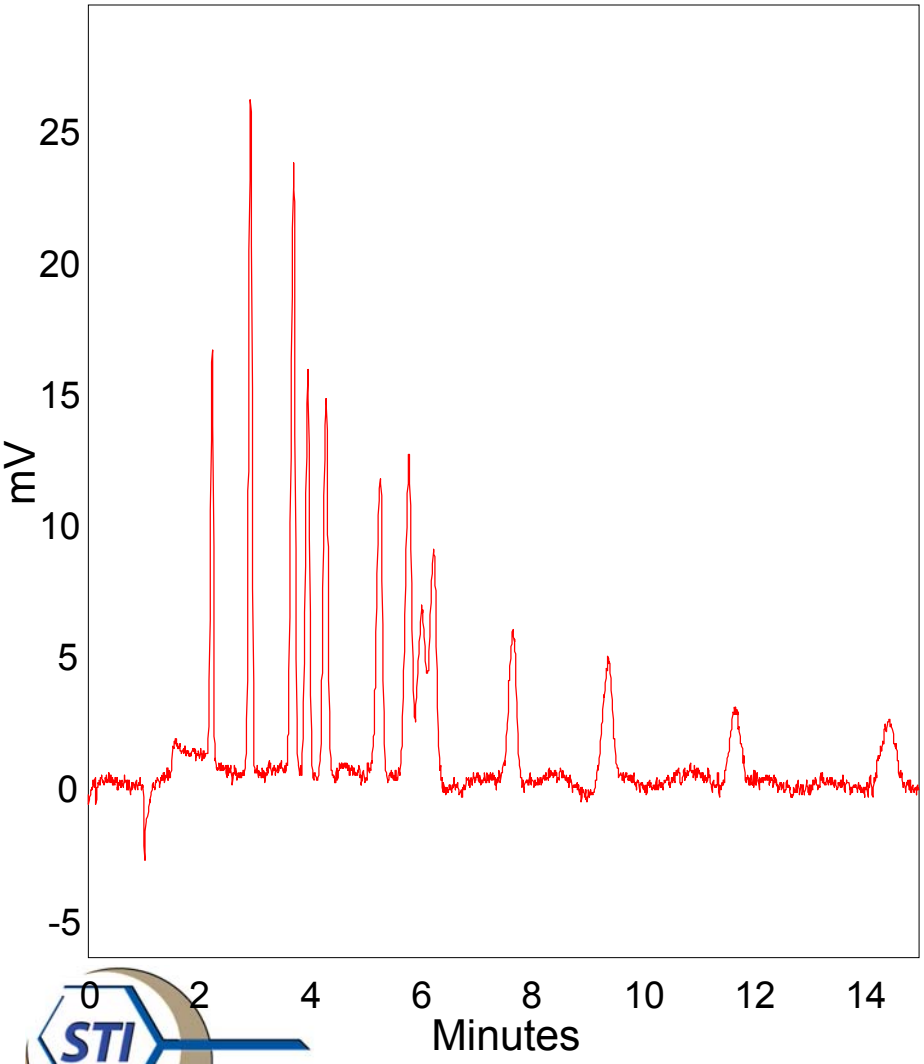
Aldehydes and Ketones of CARB 1004 using a solvent gradient



Column: Selerity Blaze$_{200}$ C_{18}, 3 μm
2 150 x 4.6 mm columns
Mobile Phase: 10:90 acetonitrile:water, 10 to 60% ACN over 30 minutes.
Flow Rate: 1.5 mL/min
Detection: UV-VIS 365 nm

Elution Order:
Formaldehyde-DNPH
Acetaldehyde-DNPH
Acetone-DNPH
Acrolein-DNPH
Propionaldehyde-DNPH
Crotonaldehyde-DNPH
MEK-DNPH
Methacrolein-DNPH
Butyraldehyde-DNPH
Benzaldehyde-DNPH
Valeraldehyde-DNPH
Tolualdehyde-DNPH
Hexanaldehyde-DNPH

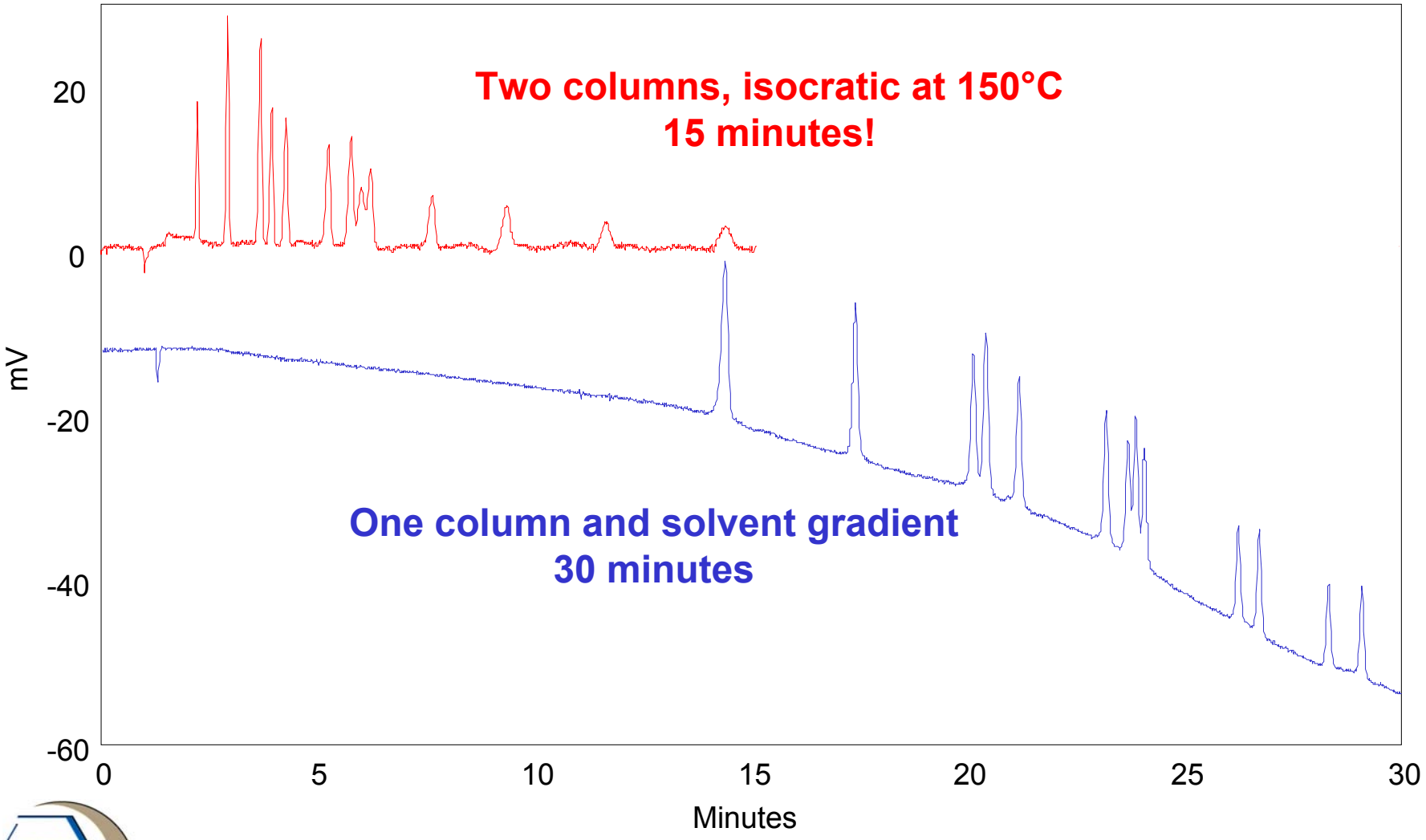
Aldehydes and Ketones of CARB 1004 at 150°C



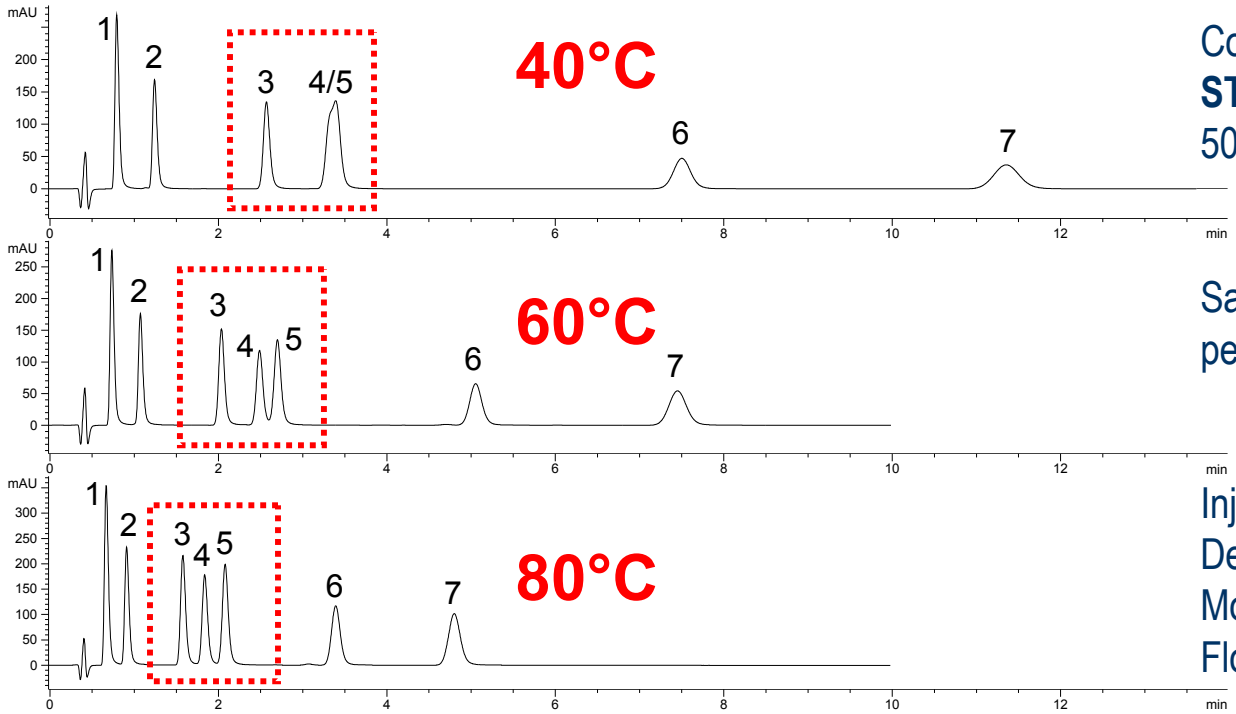
Column: Selerity Blaze$_{200}$ C_{18}, 3 μm
2 150 x 4.6 mm columns
Mobile Phase: 25:75 acetonitrile:water
Flow Rate: 3.0 mL/min
Detection: UV-VIS 365 nm
Temperature: 150°C isothermal

Elution Order:
Formaldehyde-DNPH
Acetaldehyde-DNPH
Acetone-DNPH
Acrolein-DNPH
Propionaldehyde-DNPH
Crotonaldehyde-DNPH
Methacrolein-DNPH
MEK-DNPH
Butyraldehyde-DNPH
Benzaldehyde-DNPH
Valeraldehyde-DNPH
Tolualdehyde-DNPH
Hexanaldehyde-DNPH

Aligned chromatograms



Phenylurea pesticides - Selectivity



Column:
STM Zorbax StableBond C18,
50 mm L x 2.1 mm ID x 1.8 µm particles

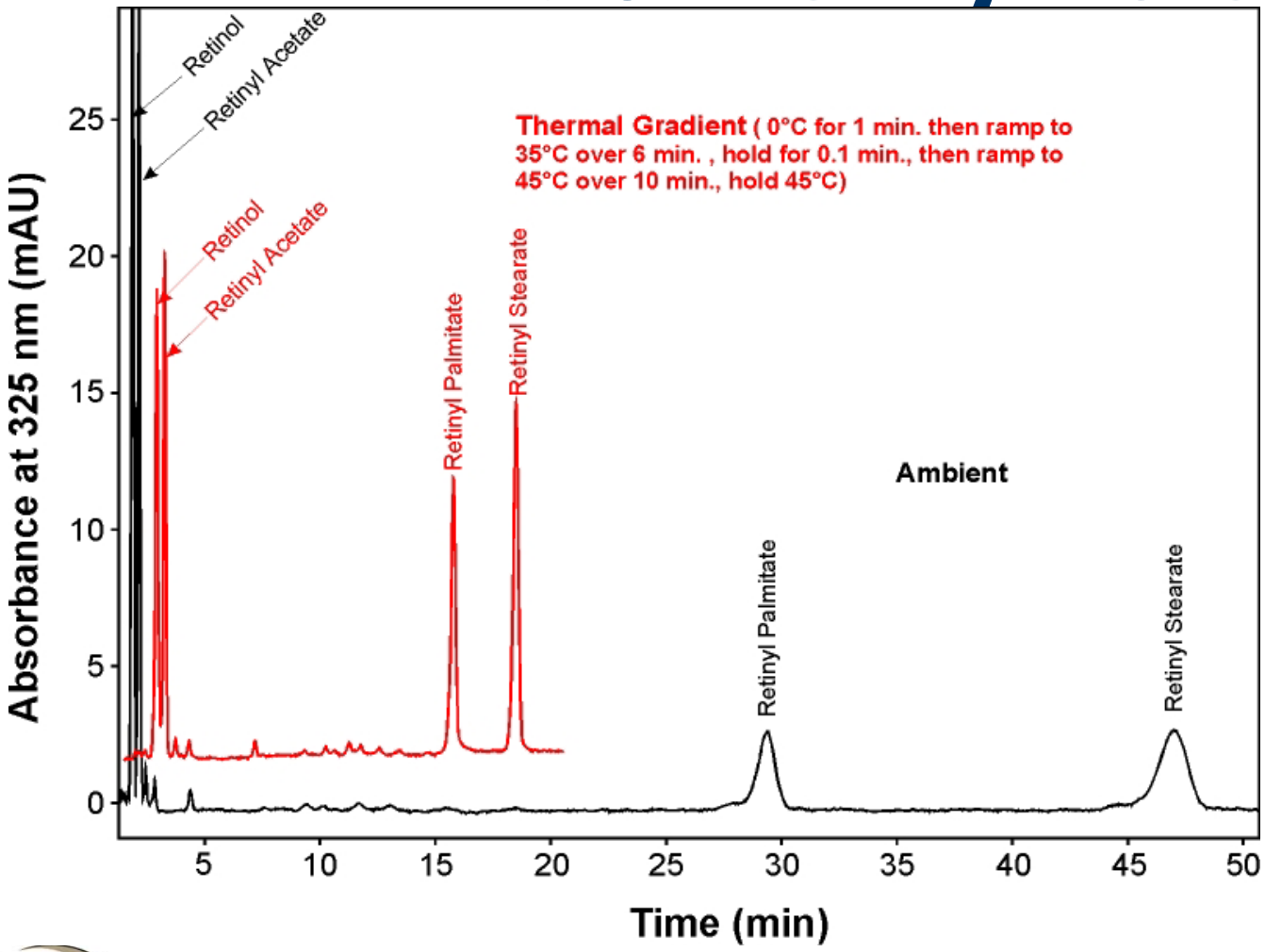
Sample: Standard solution Phenylurea pesticides (100 ppm each)

Injection: 1 µl
Detection: UV, 245 nm
Mobile phase: ACN/Water 30/70
Flow-rate: 0.35 ml/min

1. Fenuron, 2. Metoxuron, 3. Chlortoluron, 4. Diuoron,
5. Isoproturon, 6. Linuron, 7. Chloroxuron

Chromatogram courtesy of Dr. Pat Sandra, Research Institute for Chromatography

Low Temperature HPLC of Retinyl Esters



Chromatographic conditions:

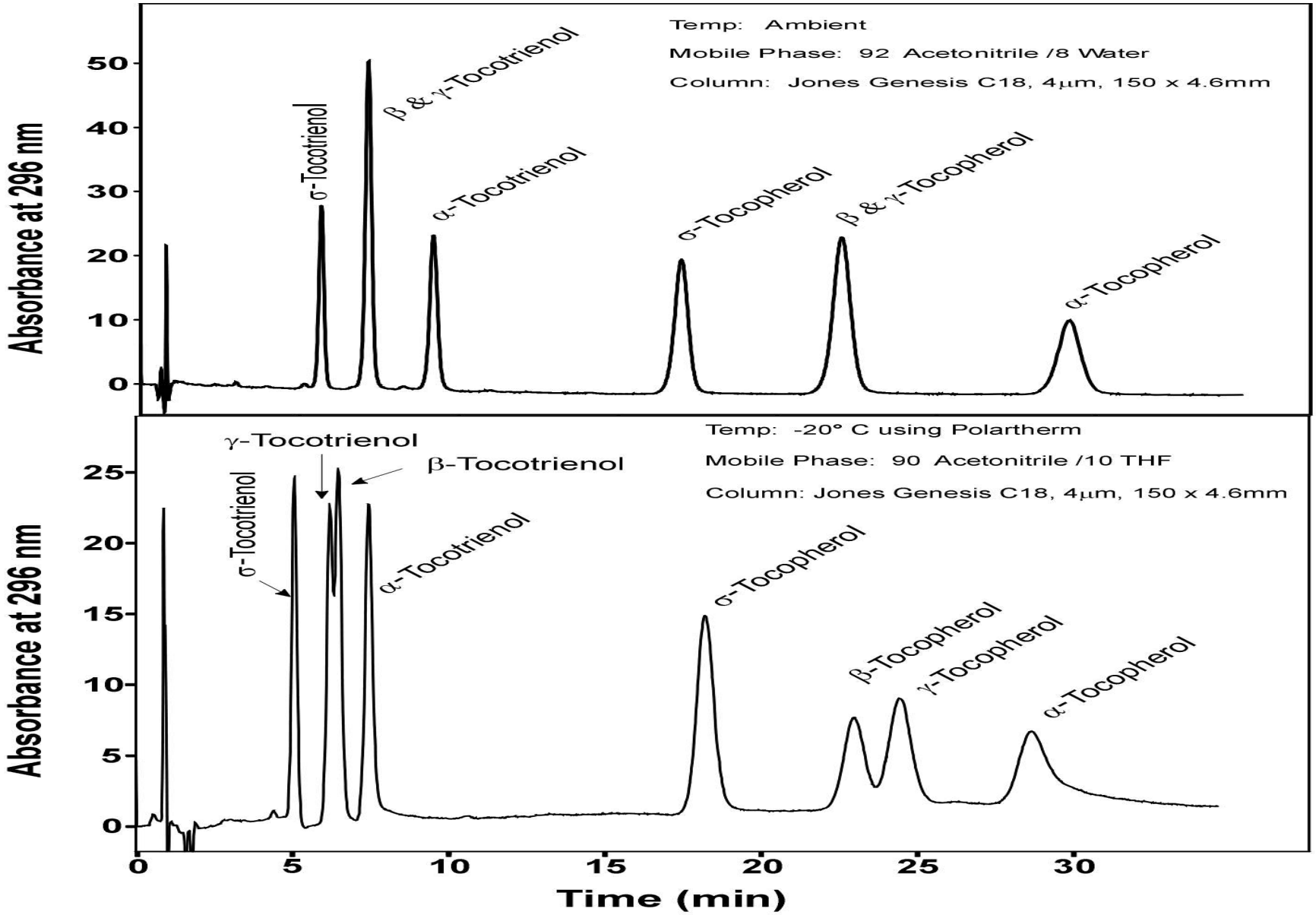
Column: Genesis C18 column (4um particle size, 4.6 mm x 150 mm length; Jones Chromatography).

Mobile phase: 97.5% acetonitrile / 2.5% tetrahydrofuran, 1.0 mL/min

Detection: Absorbance at 325 nm

Courtesy of Craft Technologies

Low Temperature HPLC of Tocopherols



Conclusions

Acknowledgements

Dr. Pat Sandra, RIC

Jeffrey Loo, GM

John Estes and Neal Craft, Craft Technologies