Temperature vs Pressure - Achieving High Resolution, Highly Efficient HPLC Separations Using Elevated Temperature In Place of High Pressure

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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

Benefits of High Temperature 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

Benefits of High Temperature 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

Benefits of Temperature Programming

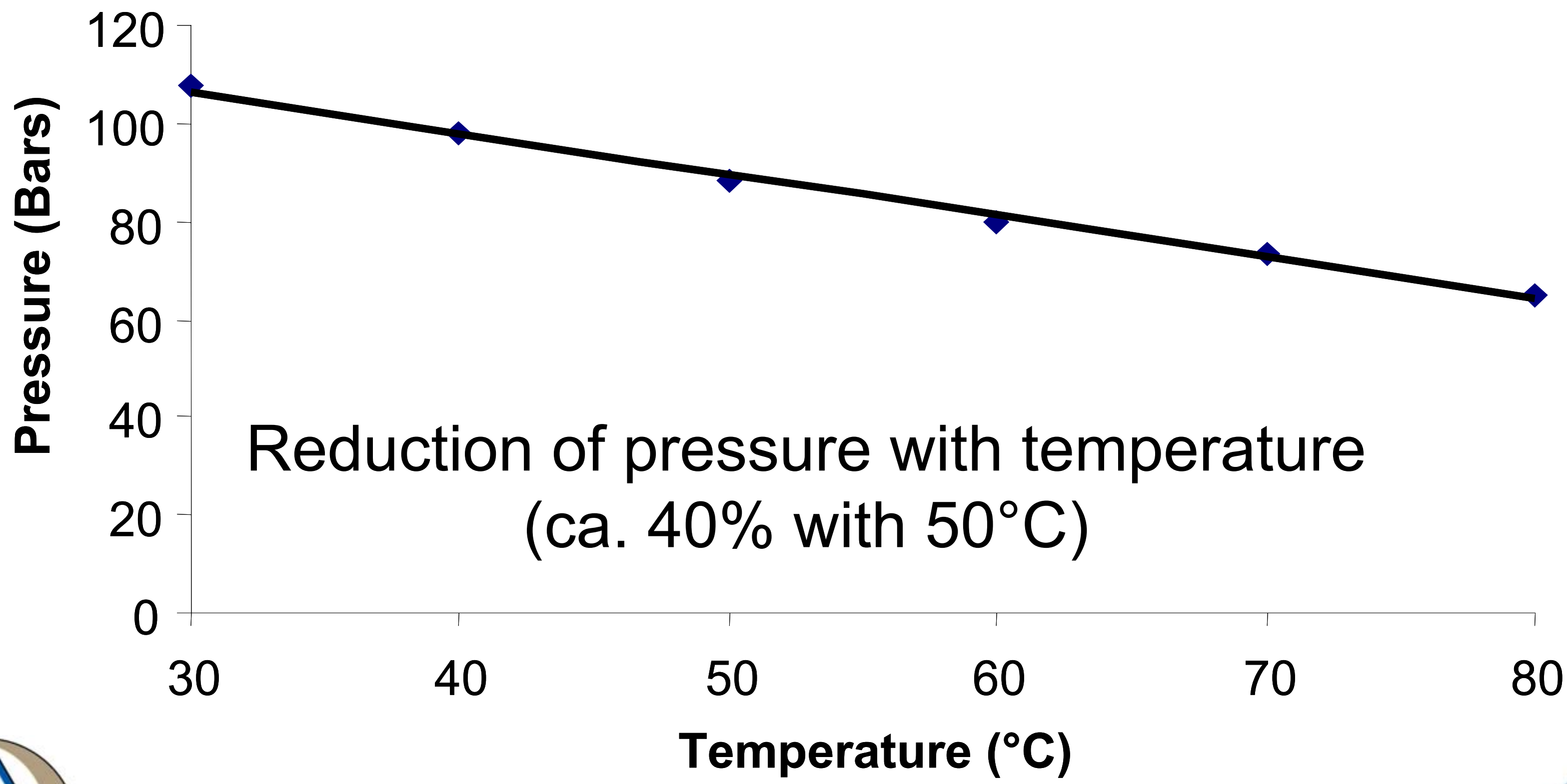
- Allows increased plate count by adding column length
- Increases speed by increasing flow rate as viscosity decreases at elevated temperatures
- Changes selectivity as function of temperature and column phase
- Reduces organic solvent content – “Green Chemistry”

Selerity Polaratherm™ Series 9000 Total Temperature Controller

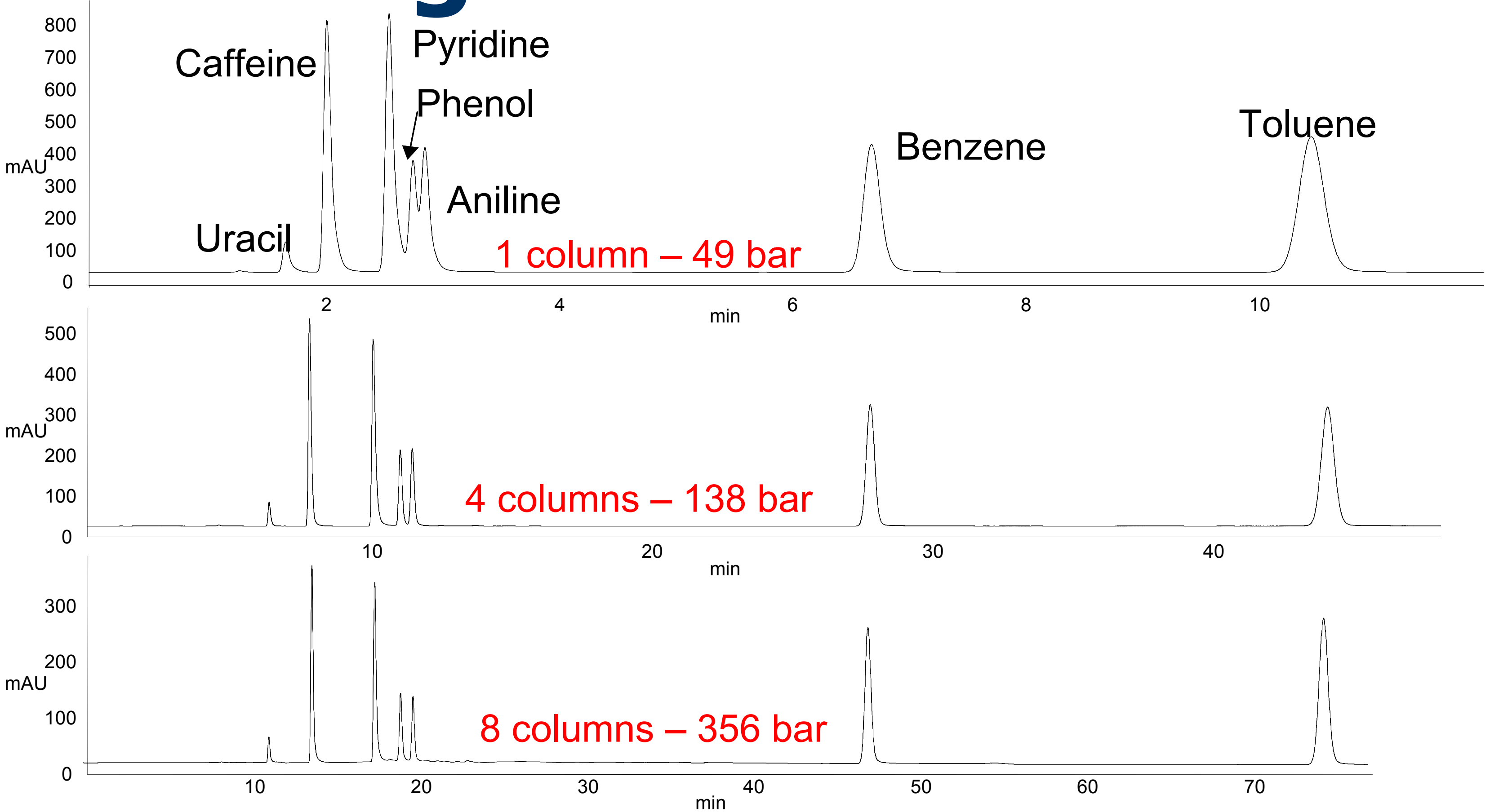
- ❑Forced air oven and chiller
- ❑Isothermal and thermal gradient operation
  - ▪Sub-zero to 200°C
  - ▪Thermal gradients up to 30°C/min
- ❑Mobile phase preheating and pre-cooling
- ❑Peltier effluent temperature control
- ❑Vapor sensor
- ❑Compatible with any HPLC system
- ❑Integrated software control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

High efficiency analyses

Utilization of multiple columns to improve efficiency



Increase Resolution by Increasing Number of columns



Column: Agilent SB-C18 150 x 3.0 mm, 3.5 µm
Mobile phase: ACN:Water 40:60
Flow Rate: 0.4 mL/min
Temperature = 80ºC

Efficiency for analytes using one, four and eight colums

Analyte	1 column	4 columns	8 columns
caffeine	5,000	41,000	46,000
pyridine	6,000	35,000	56,000
phenol	6,000	43,000	68,000
aniline	6,000	43,000	78,000
benzene	10,000	43,000	79,000
toluene	10,000	42,000	81,000

Column: Agilent SB-C18 150 x 3.0 mm, 3.5 µm
Mobile phase: ACN:Water 40:60
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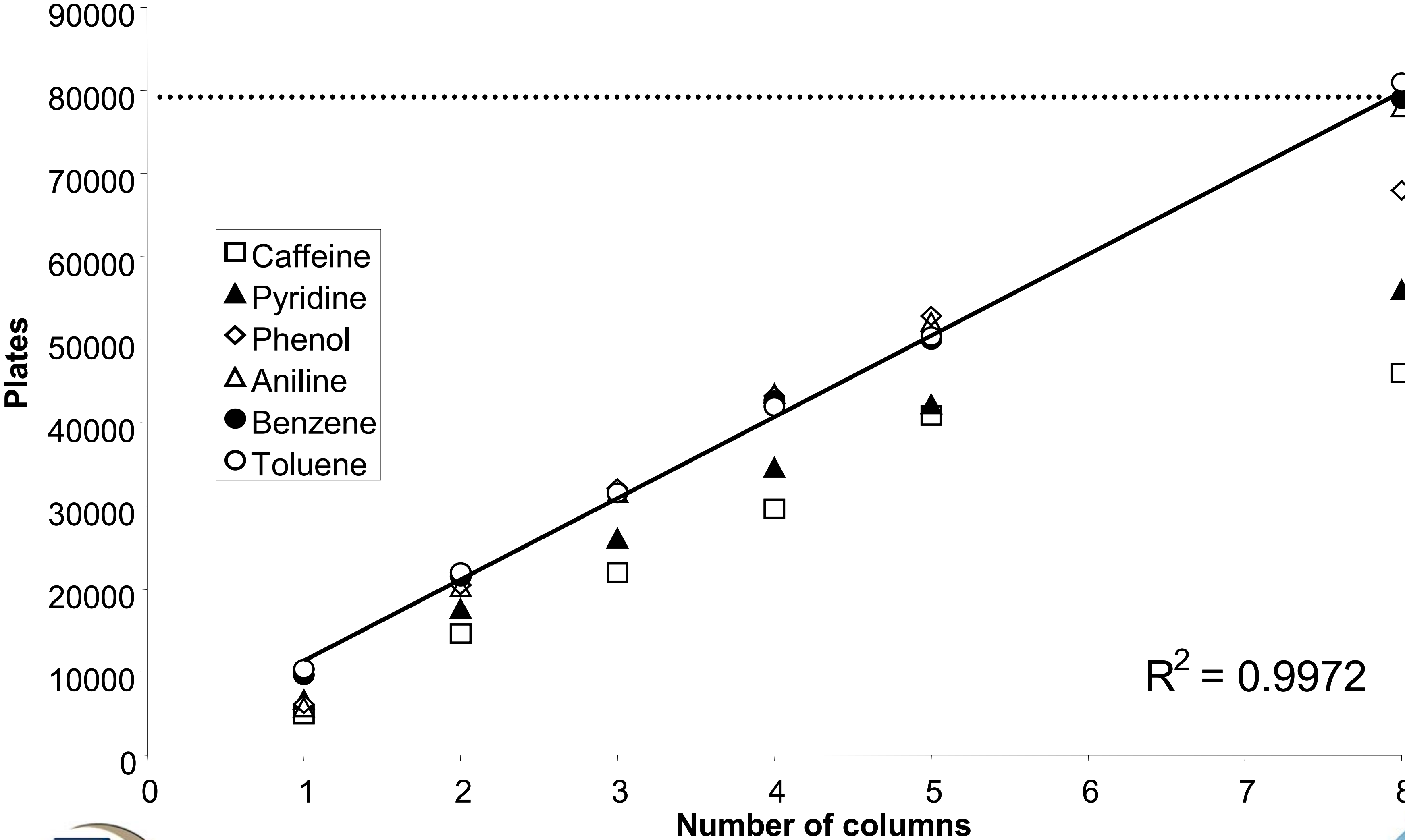
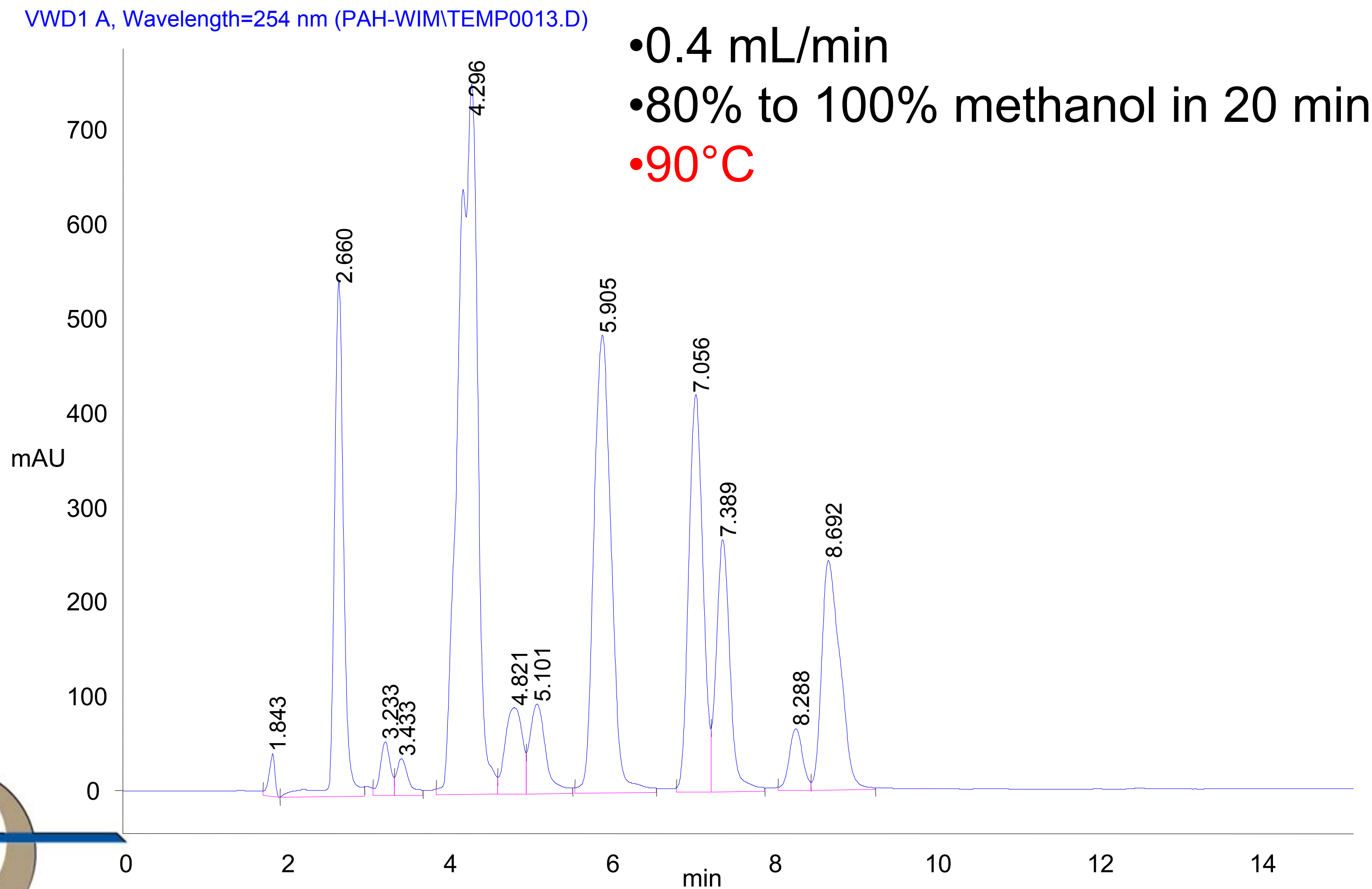
Plate numbers vs columns



Analysis of PAHs on ODS column

- Column: **15 cm**
  Zorbax SB-C18 150 x 3.0 mm, 3.5 µm d_p

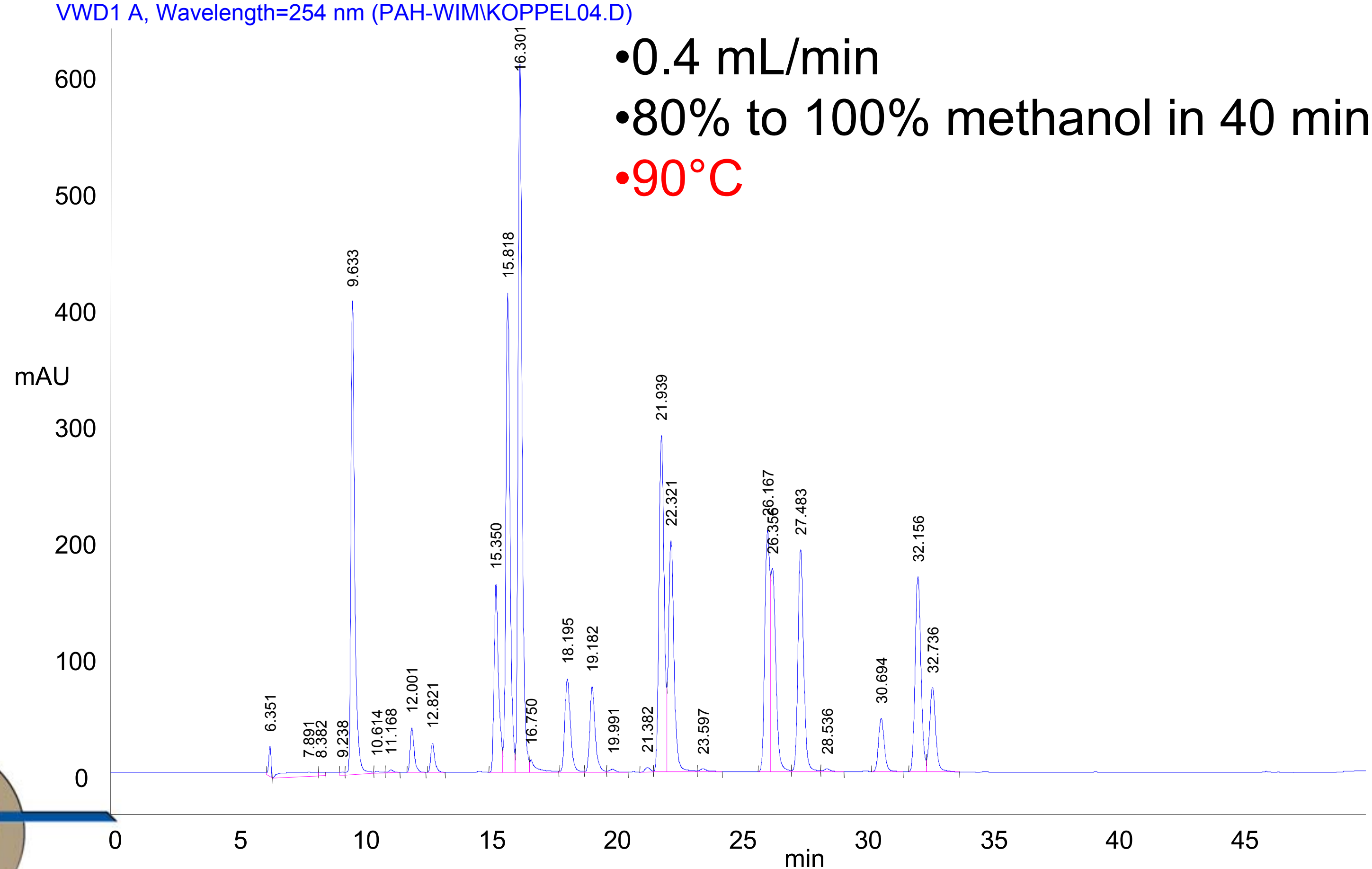


- 0.4 mL/min
- 80% to 100% methanol in 20 min
- 90°C

Analysis of PAHs using four ODS Columns

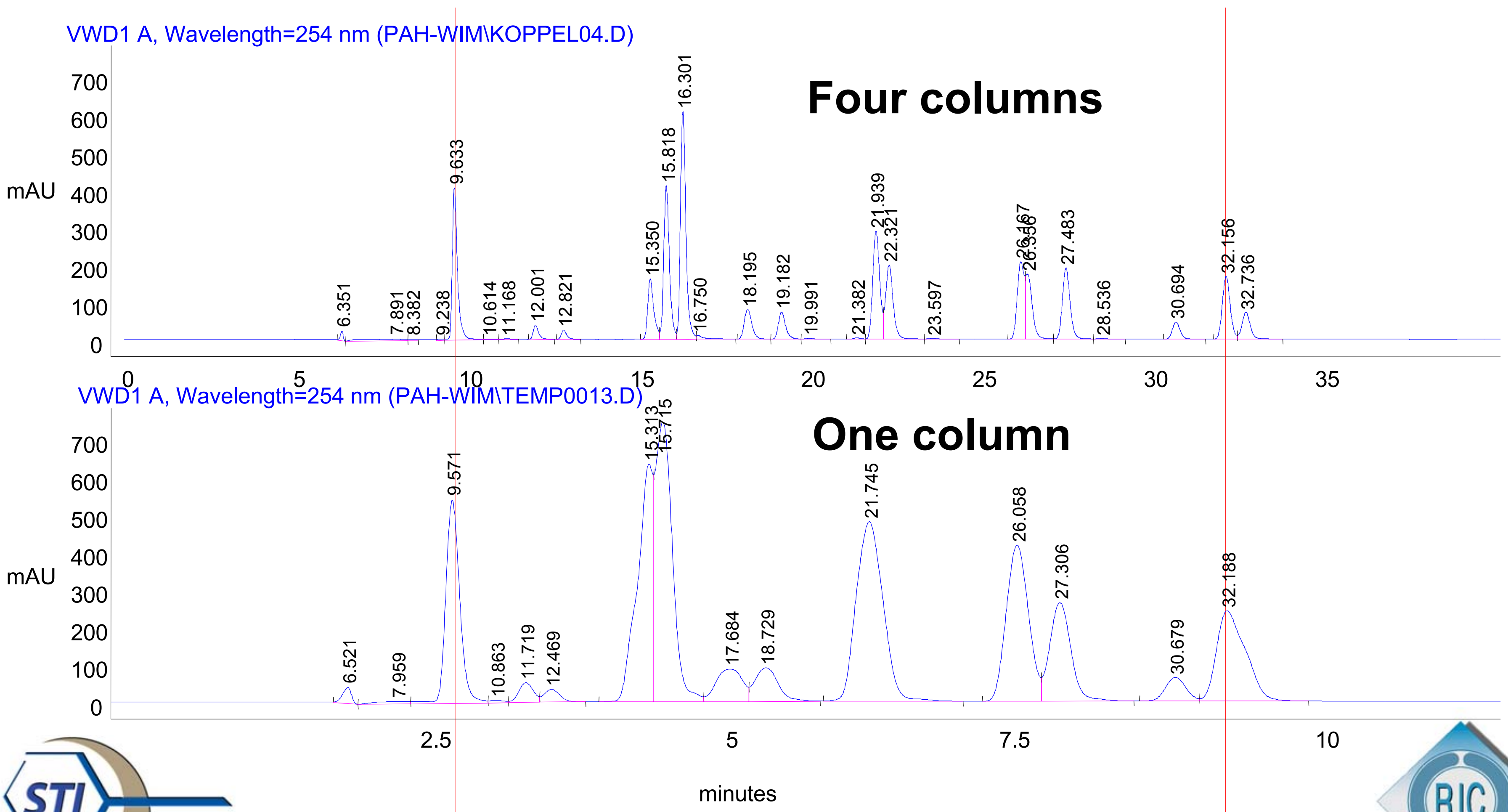
- Column: **60 cm**

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



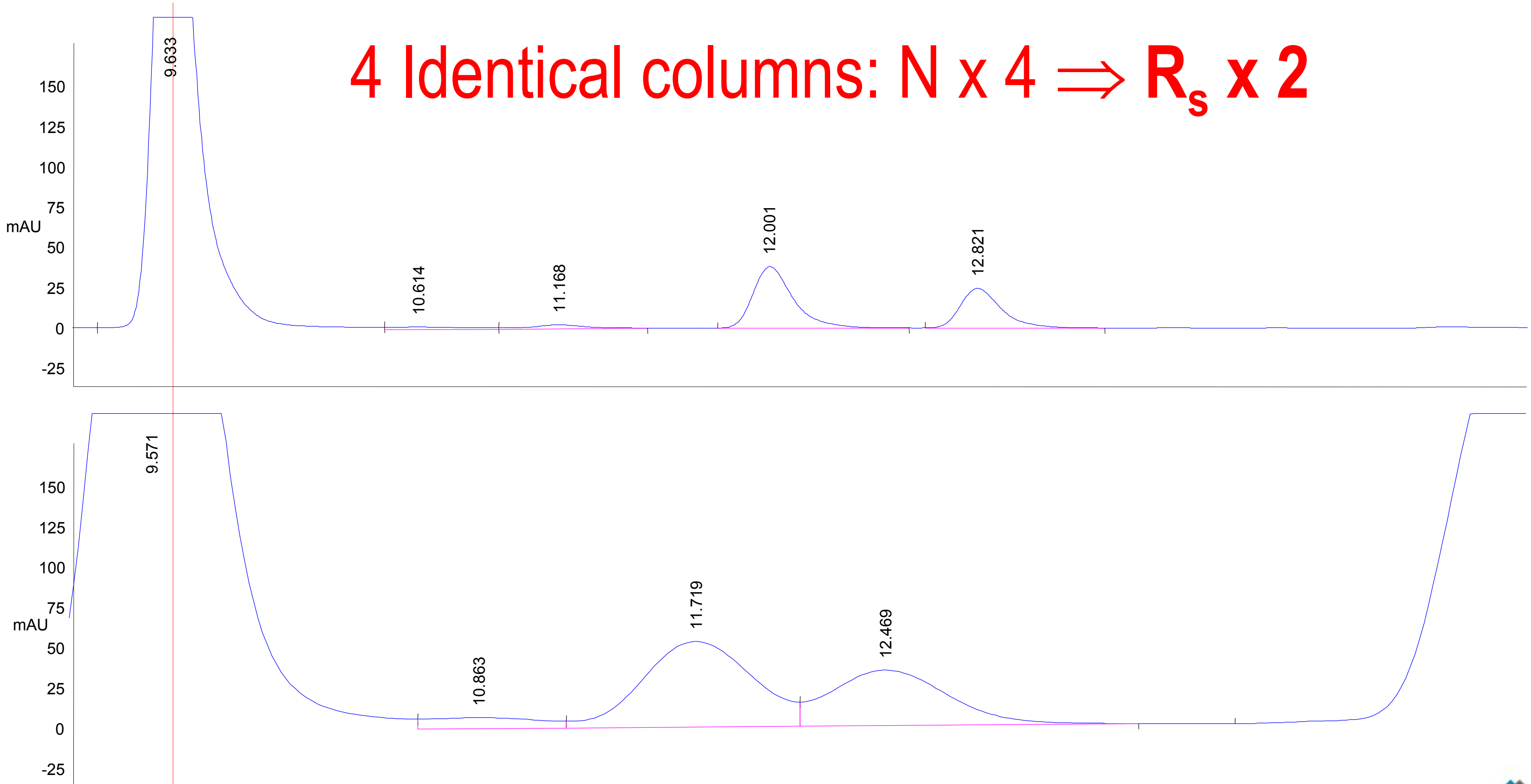
- 0.4 mL/min
- 80% to 100% methanol in 40 min
- 90°C

Analysis of PAHs
Aligned Chromatograms

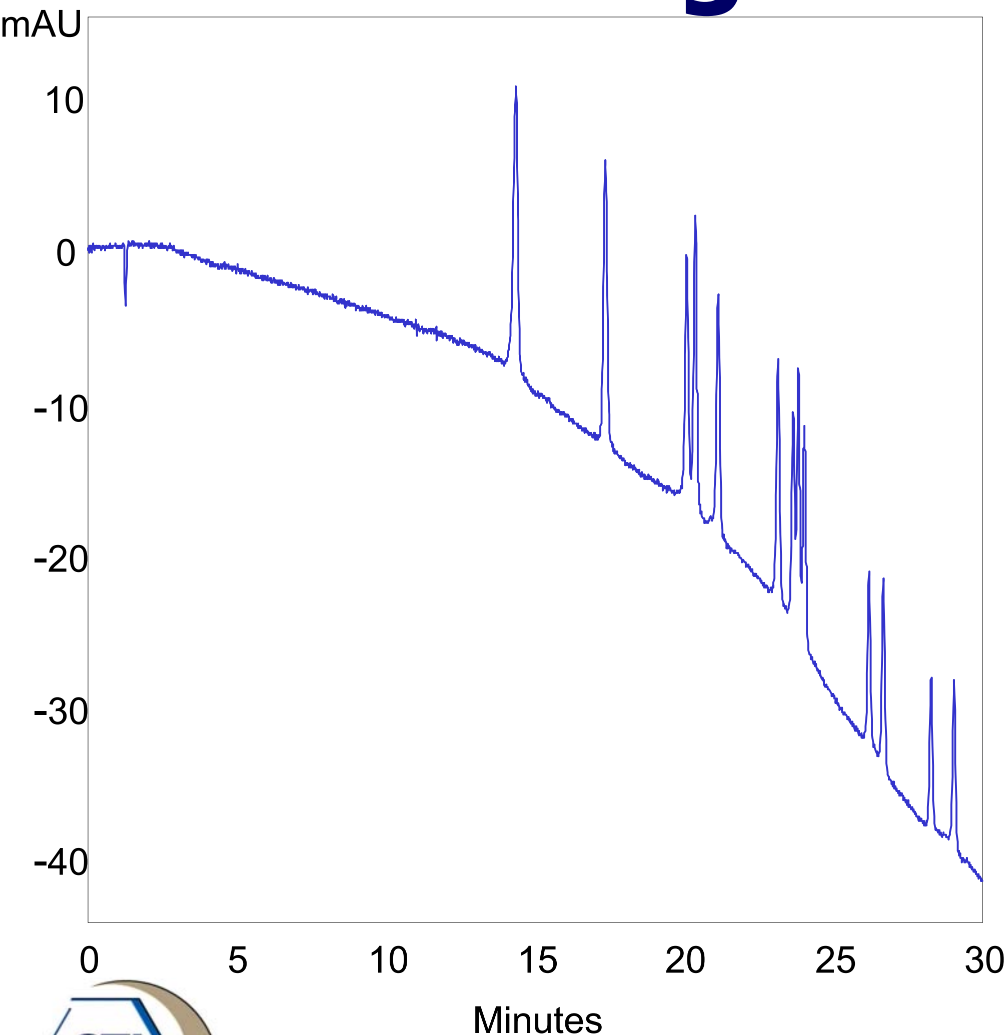


Analysis of PAHs Resolution Comparison

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



Aldehydes and Ketones of CARB 1004 using a solvent gradient



Column: Selerity Blaze$_{200}$ C_{18}, 3 mm 150 x 4.6 mm columns

Mobile Phase: 10:90 acetonitrile:water, 10 to 60% ACN over 30 minutes.

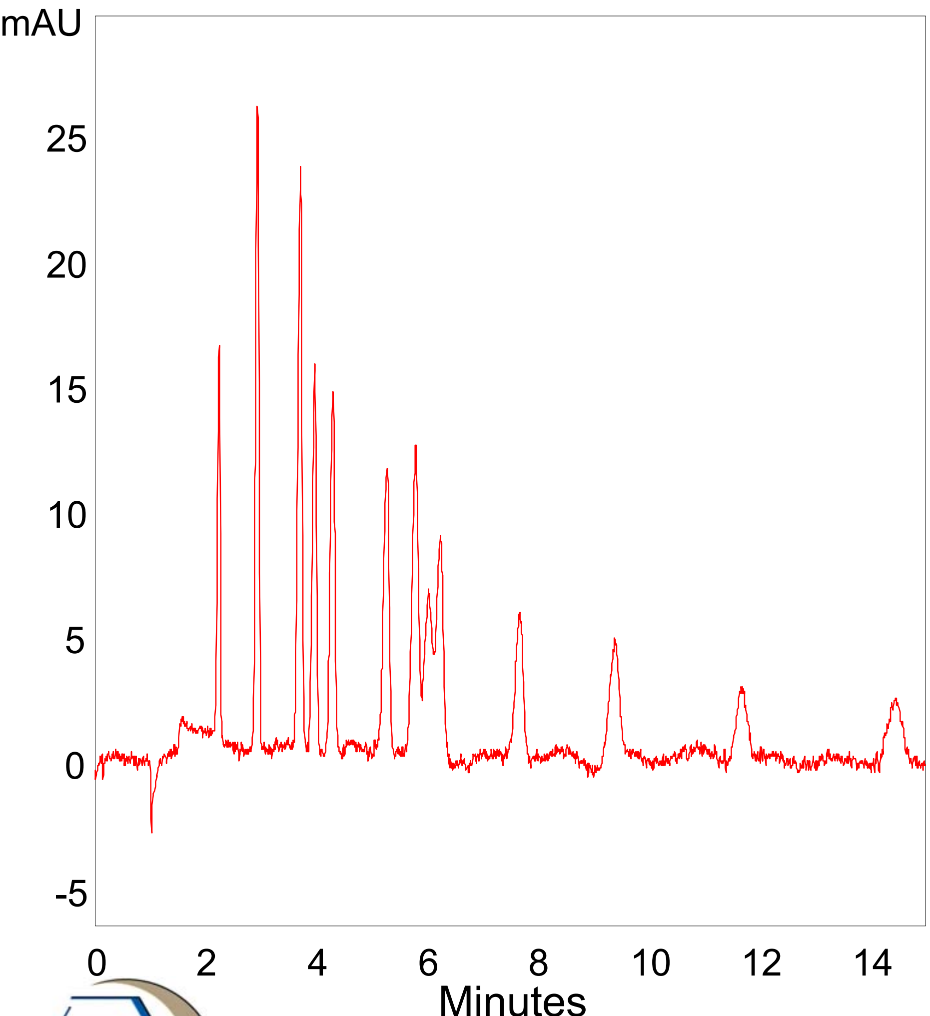
Flow Rate: 1.5 mL/min

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

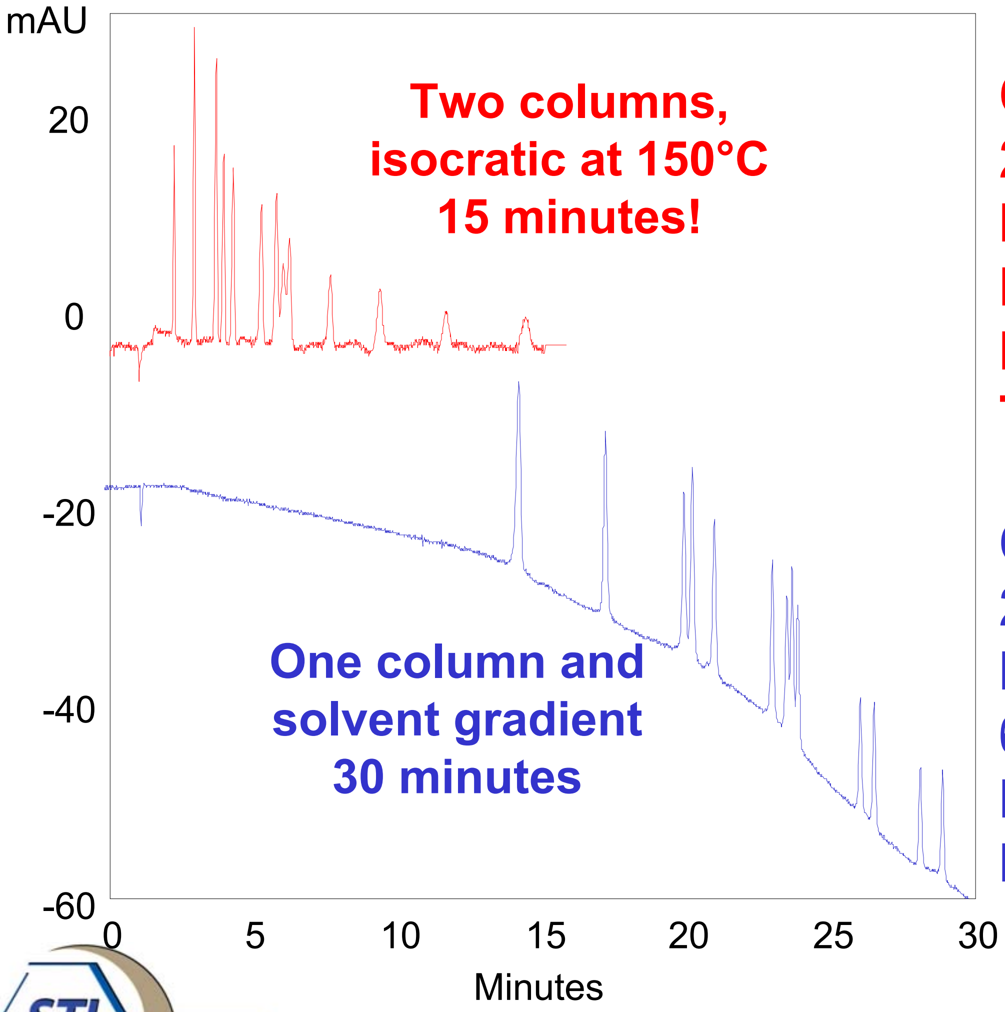
Aldehydes and Ketones of CARB 1004 at 150°C



Column: Selerity Blaze$_{200}$ C_{18}, 3 mm
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

Aldehydes and Ketones of CARB 1004 at 150°C



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Column: Selerity Blaze$_{200}$ C_{18}, 3 mm
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Detection: UV-VIS 365 nm

Conclusions

- High resolution separations can be achieved by increasing temperature.
- Lower mobile phase viscosity and system back pressure allow the use of longer columns to increase efficiency and resolution at elevated temperature.

Acknowledgements

Dr. Jeffrey Loo, GM

Turn up the Heat!

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