Natural Product Analysis Using Temperature Programmed HPLC: Sub-Ambient through 200 ºC

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Temperature - the overlooked optimization parameter in HPLC

- ***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

The Use of Temperature in HPLC

- Temperature Programming
- Speed
- Efficiency
- Decreased organic solvent usage
  - ✓Use less organic in solvent ratio
  - ✓Perform isocratic separations and recycle solvent

Temperature Programming

- Temperature Programming in HTLC provides added benefits
  - Water becomes more like methanol as temperature increases so less organic solvent is required
  - Even faster analysis times*
  - Improves detectability for highly retained solutes*
  - Offers an alternative to gradient elution*

*Jian Chen, Dirk Steenackers, Andrei Medvedovici, Pat Sandra J. *High Res. Chromatogr.* Vol 16 (1993) 605-608

Faster and More Efficient Separations

- **Speed**
  - Flatter van Deemter curves allow operation at flow rates many times optimal velocity
- **Higher efficiency - better resolution**
  - Increased diffusion rates provide lower plate heights at higher flow rates
  - Lower viscosity and back pressure permits higher flow rates with smaller particle size packings

Change in Retention with Temperature

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

Effect of T on retention factor depends on enthalpy of the solute. The larger the enthalpy, the greater the change in k'

F. Houdiere, PWJ Fowler, NM Djordjevic, Anal. Chem., **69,** 2589-2593 (1997)

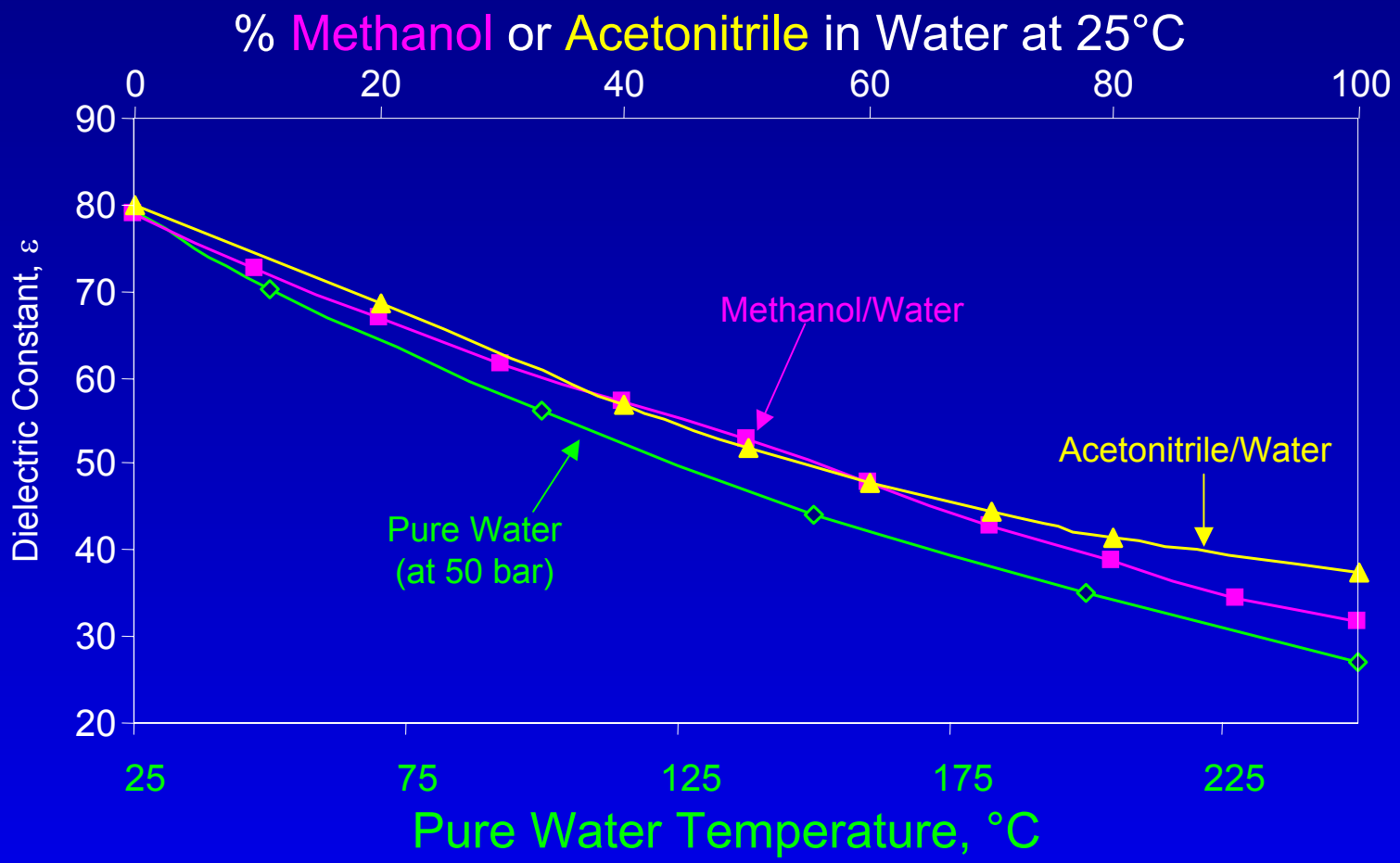
Temperature and Elution Strength

- 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

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

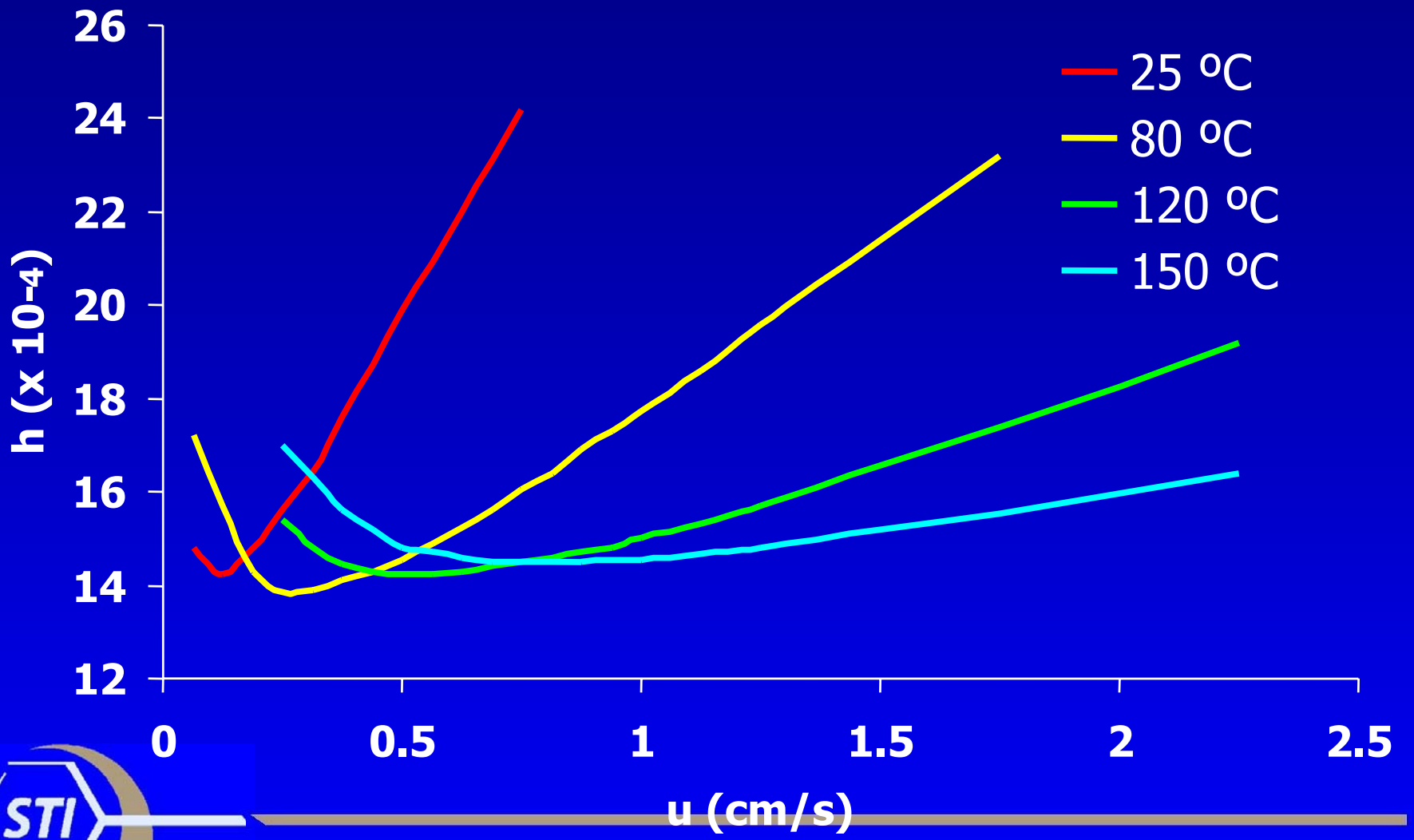
T. Greibrokk, et al,, Chapter 8 in ACS Symp. Ser., **748** (ed. Parcher and Chester), 2000, page 121

Solvent Polarity as a Function of Temperature



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

Temperature Effects on Plate Height



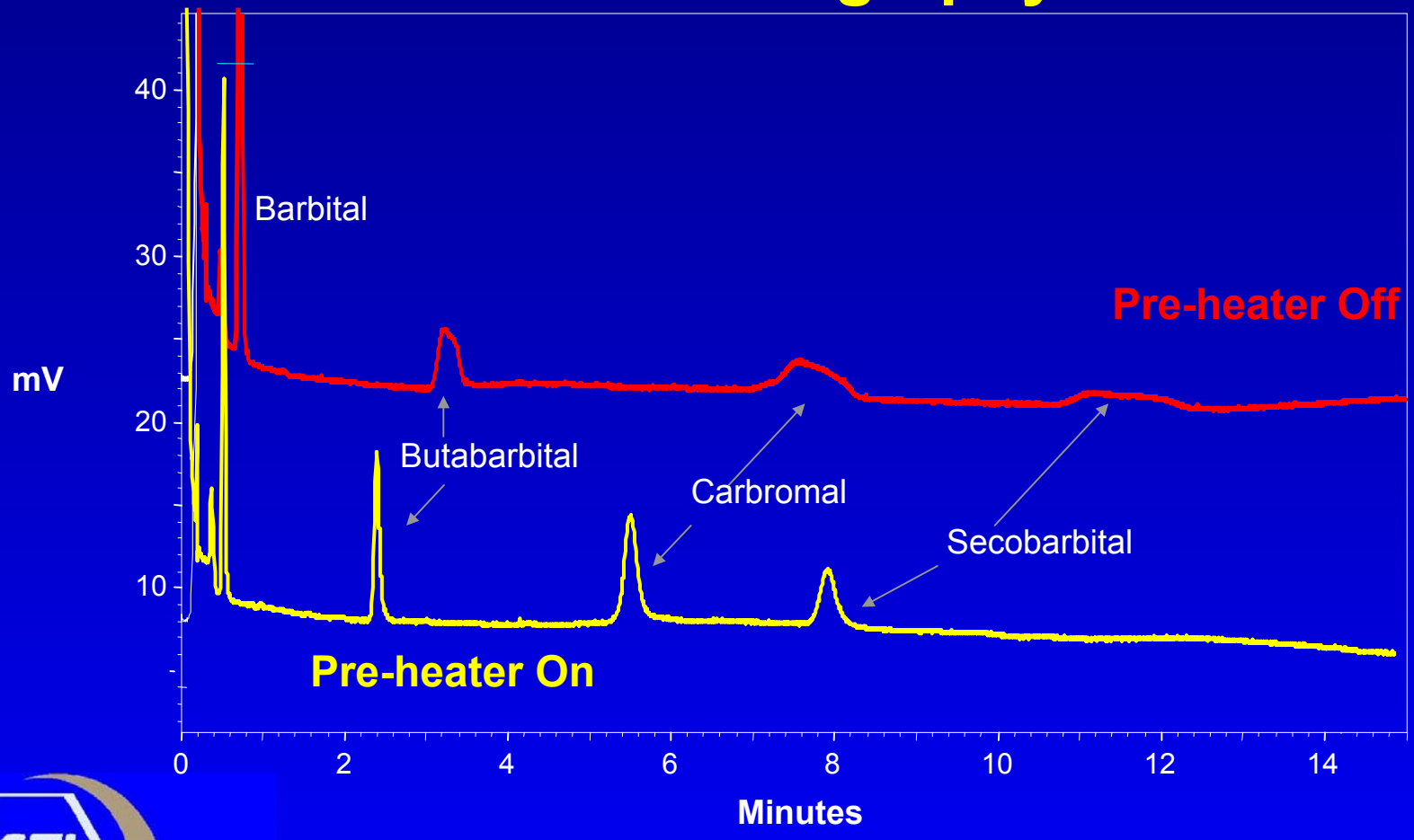
Column Efficiency

- Efficiency increases with increasing temperature
  - Increased diffusivity with increasing temperature
  - Reduction of C term in vanDeemter equation

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

Separation of Barbiturates
Mobile Phase Preheating Improves Chromatography

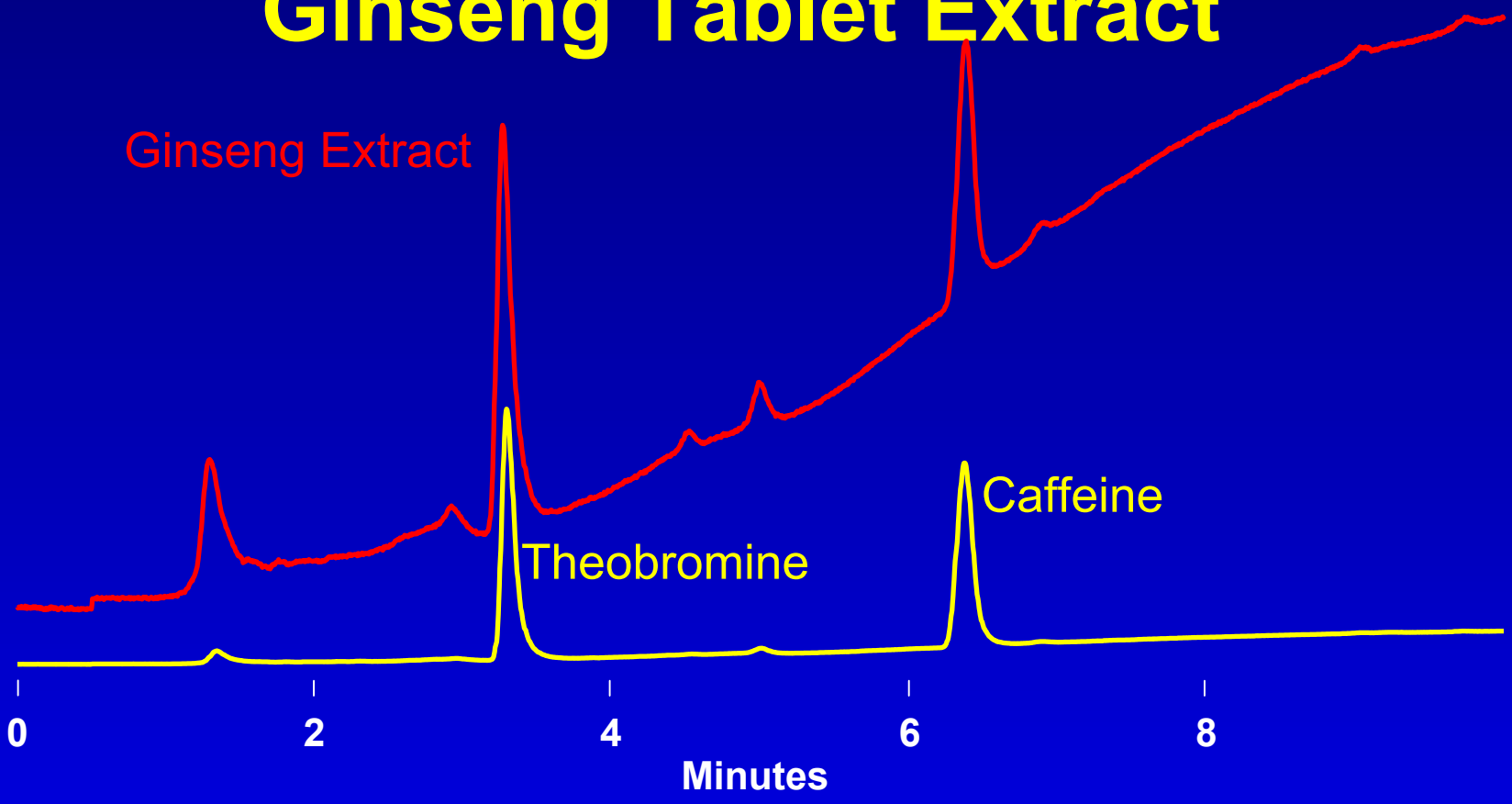


Zirchrom PBD, 80°C

Fast temperature programming

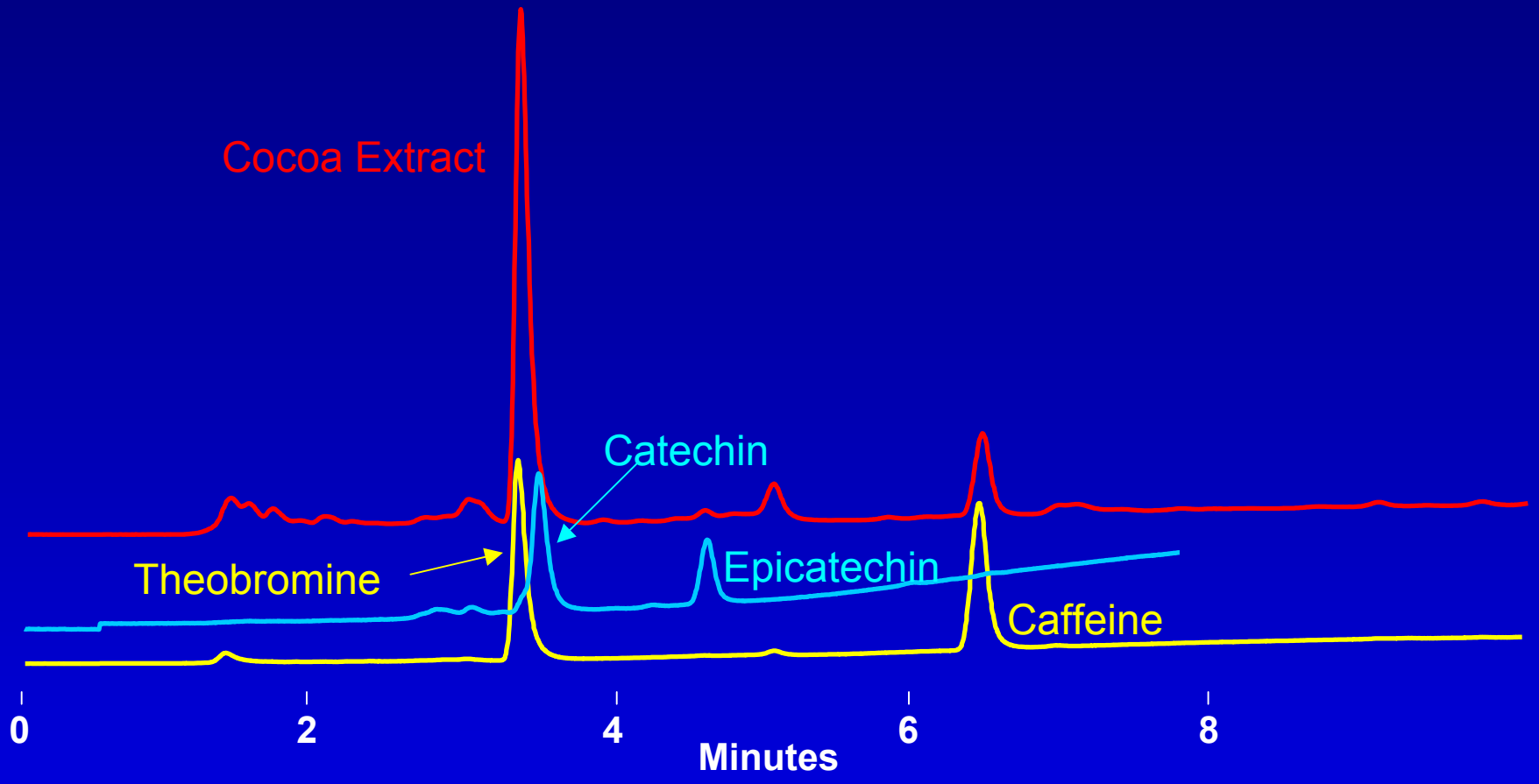
- Obtain unique selectivities
- Replace solvent gradients with temperature gradients
- Simplifies the method development process

Natural Products
Ginseng Tablet Extract



Column: Selerity Blaze® C_8, 3 µm, 100 x 4.6 mm
Mobile Phase: 80:20 Acidic water:MeOH
Flow Rate: 0.70 mL/min
Detector: UV at 254
Temperature Program: 40 to 90 °C at 10°/min

Natural Products - Cocoa Extract



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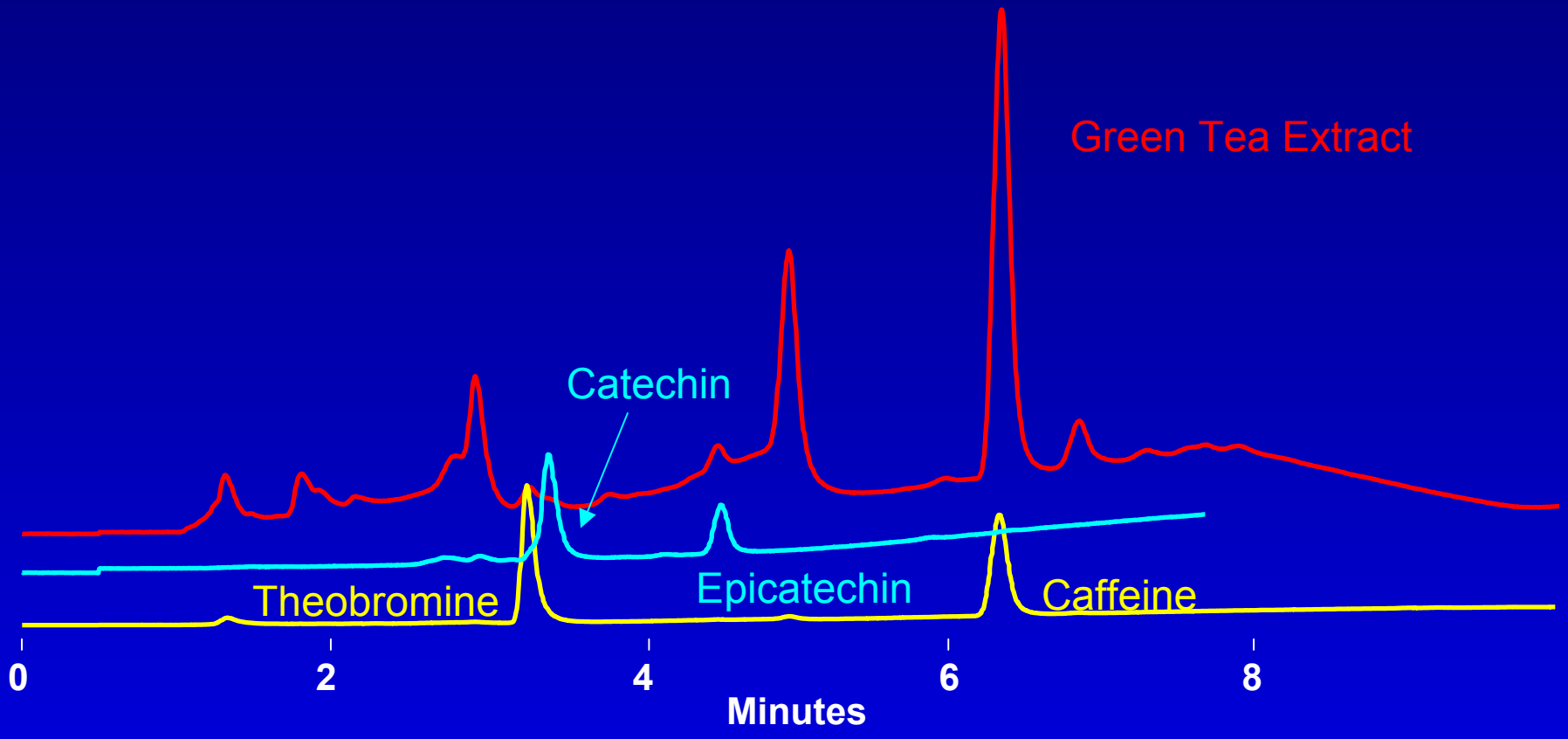
Mobile Phase: 80:20 Acidic water:MeOH
Flow Rate: 0.70 mL/min
Detector: UV at 254
Temperature Program: 40 to 90 °C at 10°/min

Natural Products - Green Tea Extract

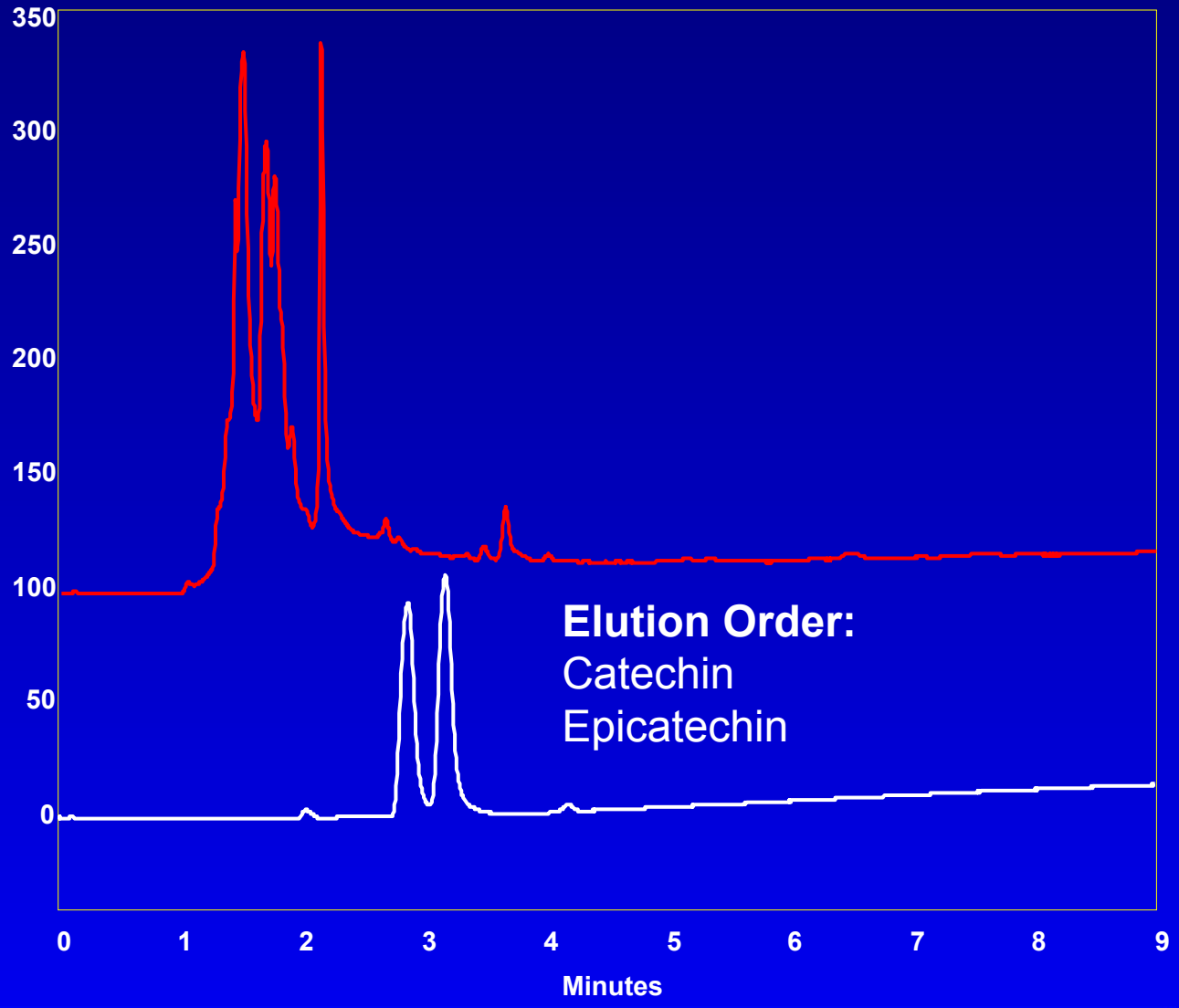


Column: Selerity Blaze® C_8, 3 µm, 100 x 4.6 mm
Mobile Phase: 80:20 Acidic water:MeOH
Flow Rate: 0.70 mL/min
Detector: UV at 254
Temperature Program: 40 to 90 °C at 10°/min

Separation of Spotted Knapweed Extract

Column: Blaze C8, 150 x 4.6 mm **Mobile Phase:** 20:80 ACN/Water with 0.1%TFA
Flow Rate: 1.0 mL/min
Detection: UV 254 nm
Temperature Program: hold at 40°C 2 min, ramp to 100 at 30°/min, hold five min

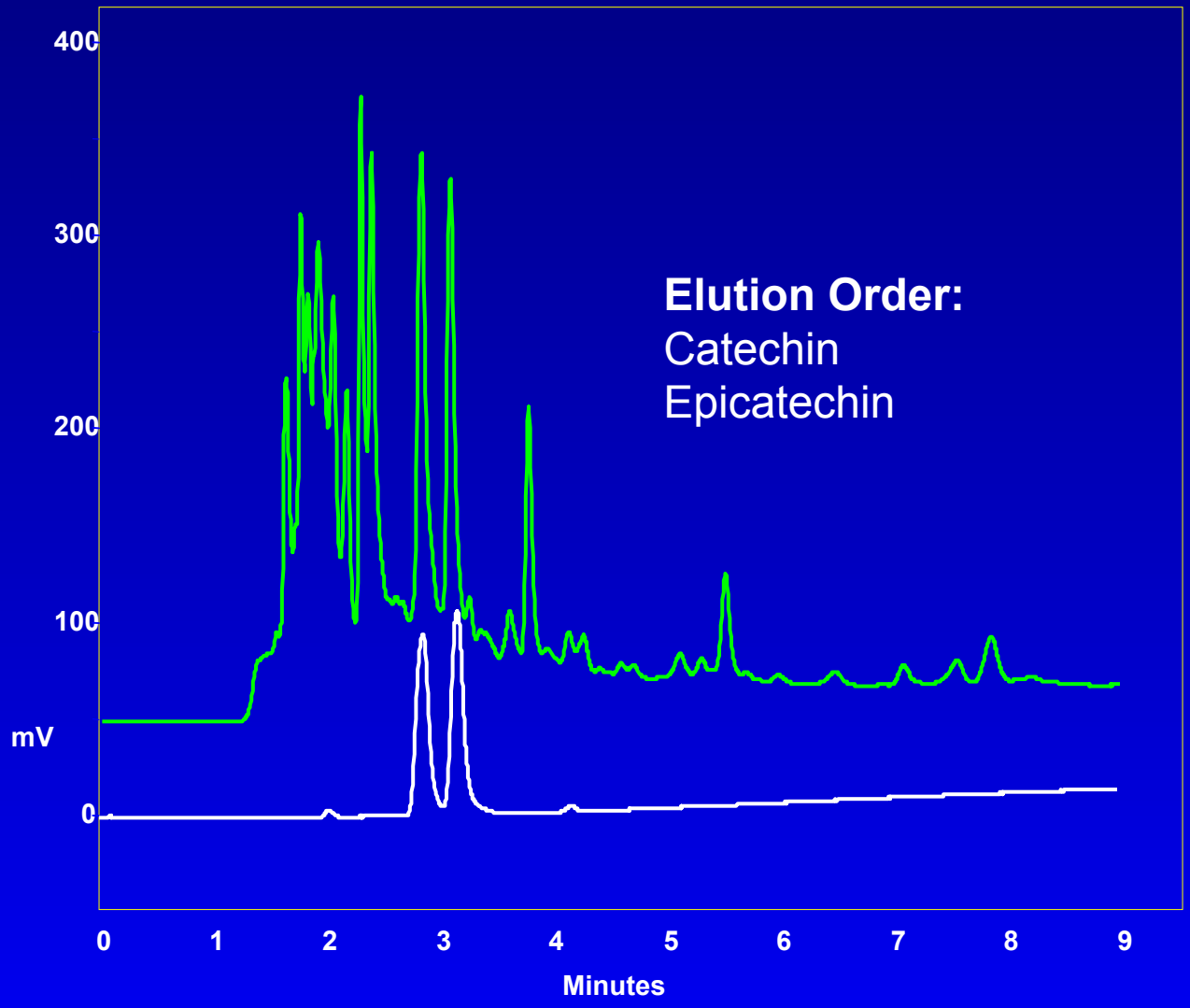
Sample Prep:
5 g weed extracted with 100 mL water (100 °C)



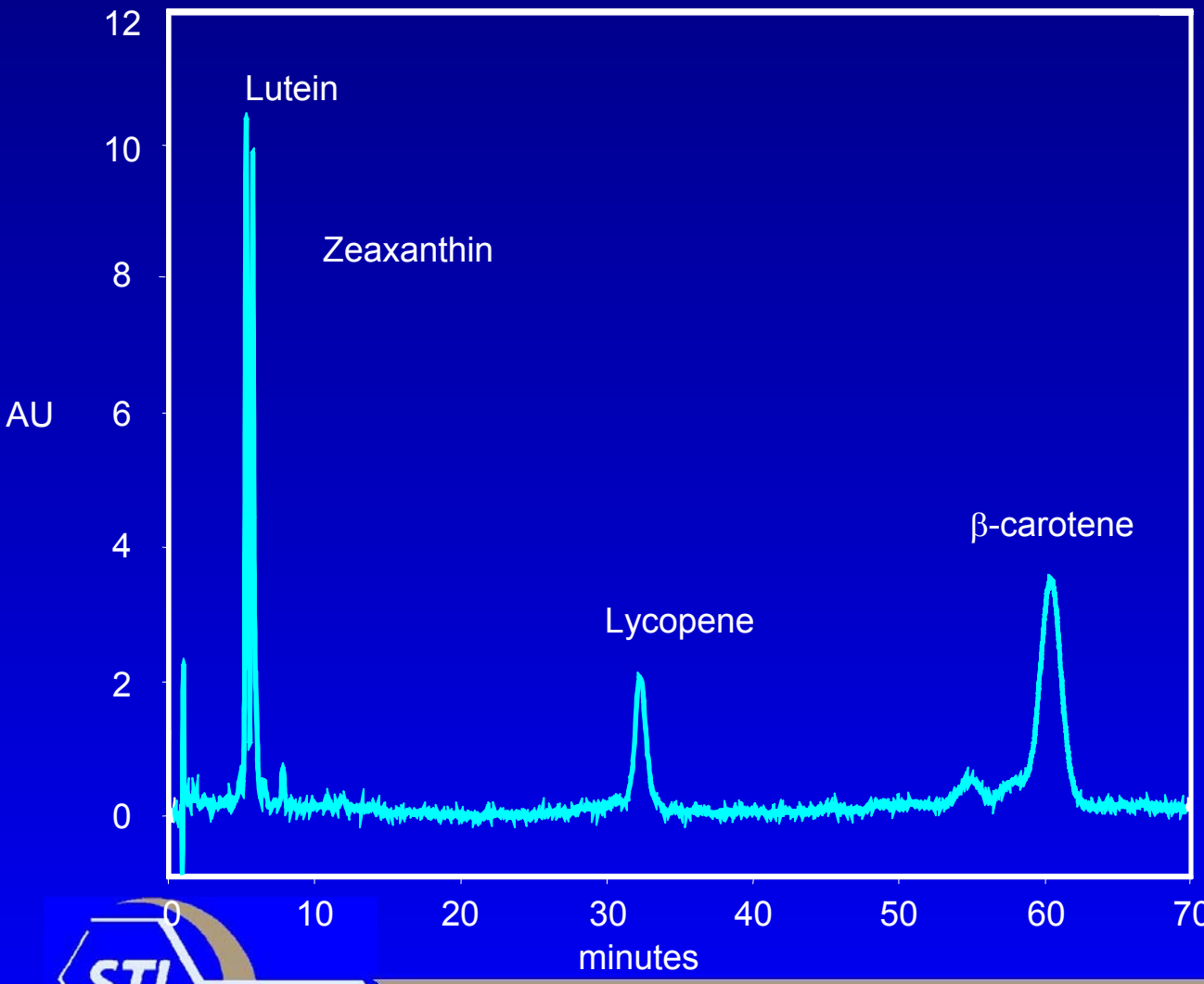
Separation of Russian Knapweed

Column: Blaze C8, 150 x 4.6 mm **Mobile Phase:** 20:80 ACN/Water with 0.1%TFA
Flow Rate: 1.0 mL/min
Detection: UV 254 nm
Temperature Program: hold at 40°C 2 min, ramp to 100 at 30°/min, hold five min

Sample Prep:
5 g weed extracted with 100 mL water (100 °C)



Separation of Carotenoid Mixture at 15°C

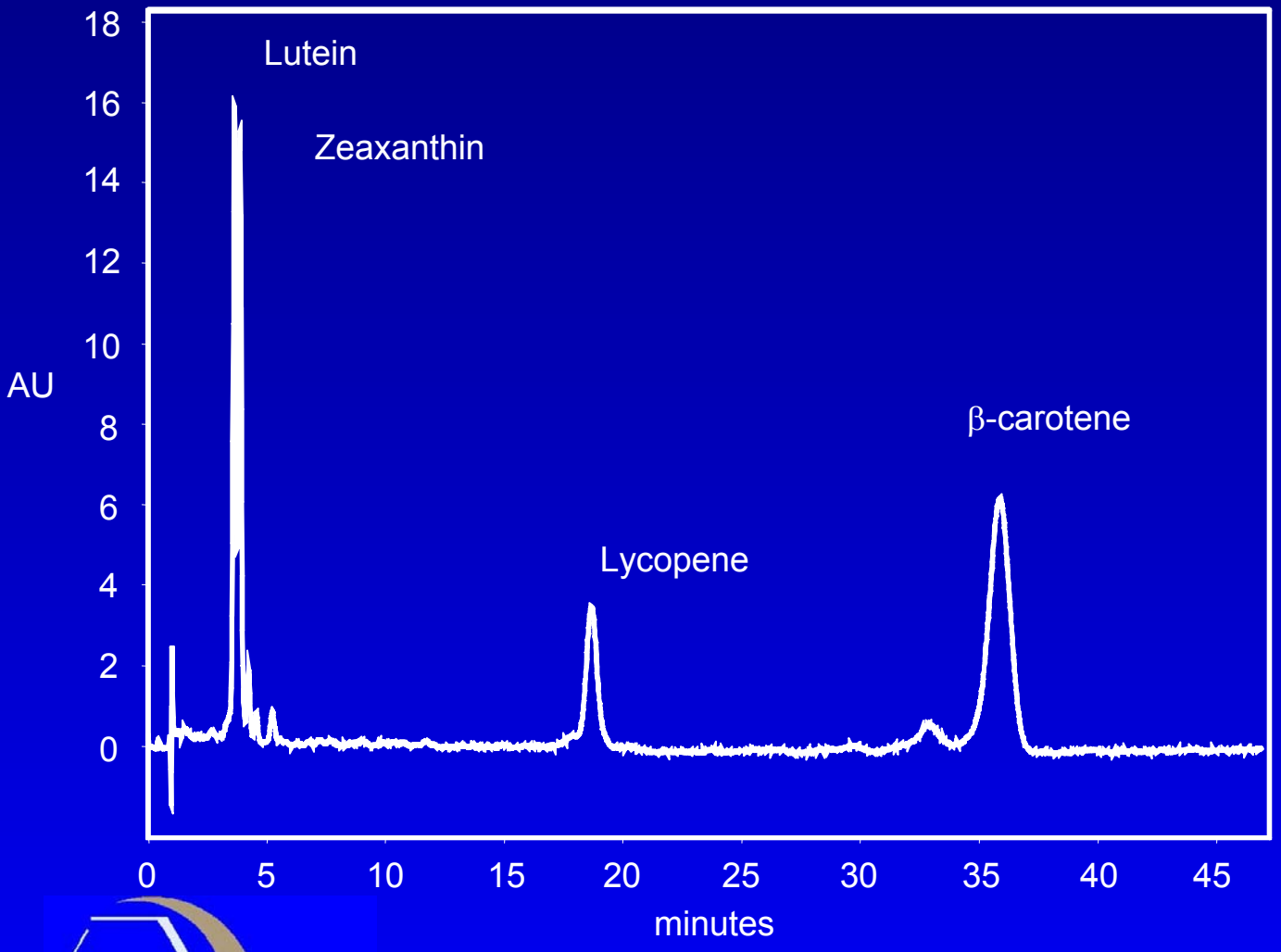


Column: Jones Chromatography Genesis C_{18}, 150 x 4.6 mm
Mobile Phase: 1.25% THF in acetonitrile
Flow Rate: 1.0 mL/min
Detection: UV 450 nm
Temperature: 15°C, isothermal

70 minute run!

Elution Order:
Lutein
Zeaxanthin
Lycopene
β-carotene

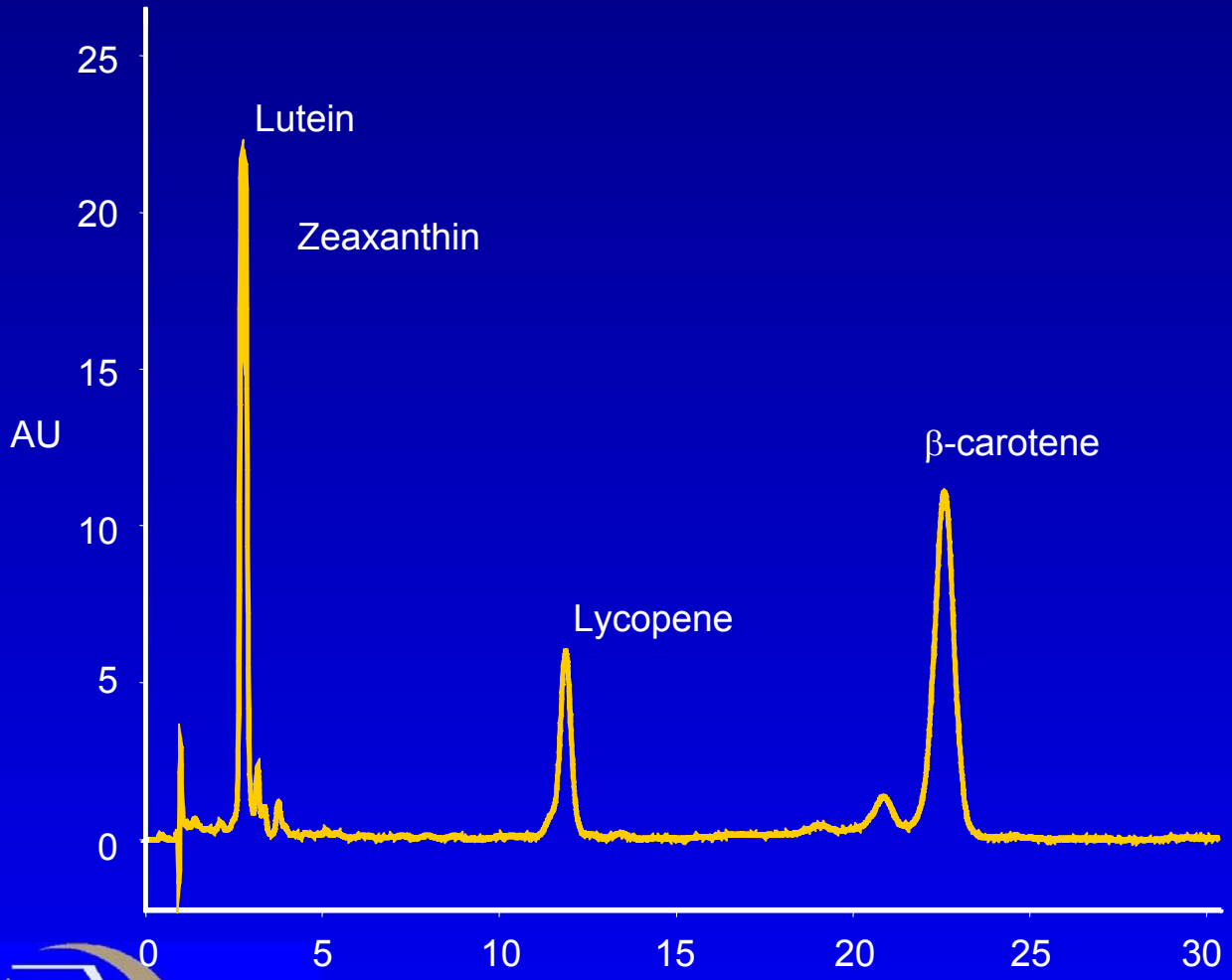
Separation of Carotenoid Mixture at 25°C



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

Elution Order:
Lutein
Zeaxanthin
Lycopene
β-carotene

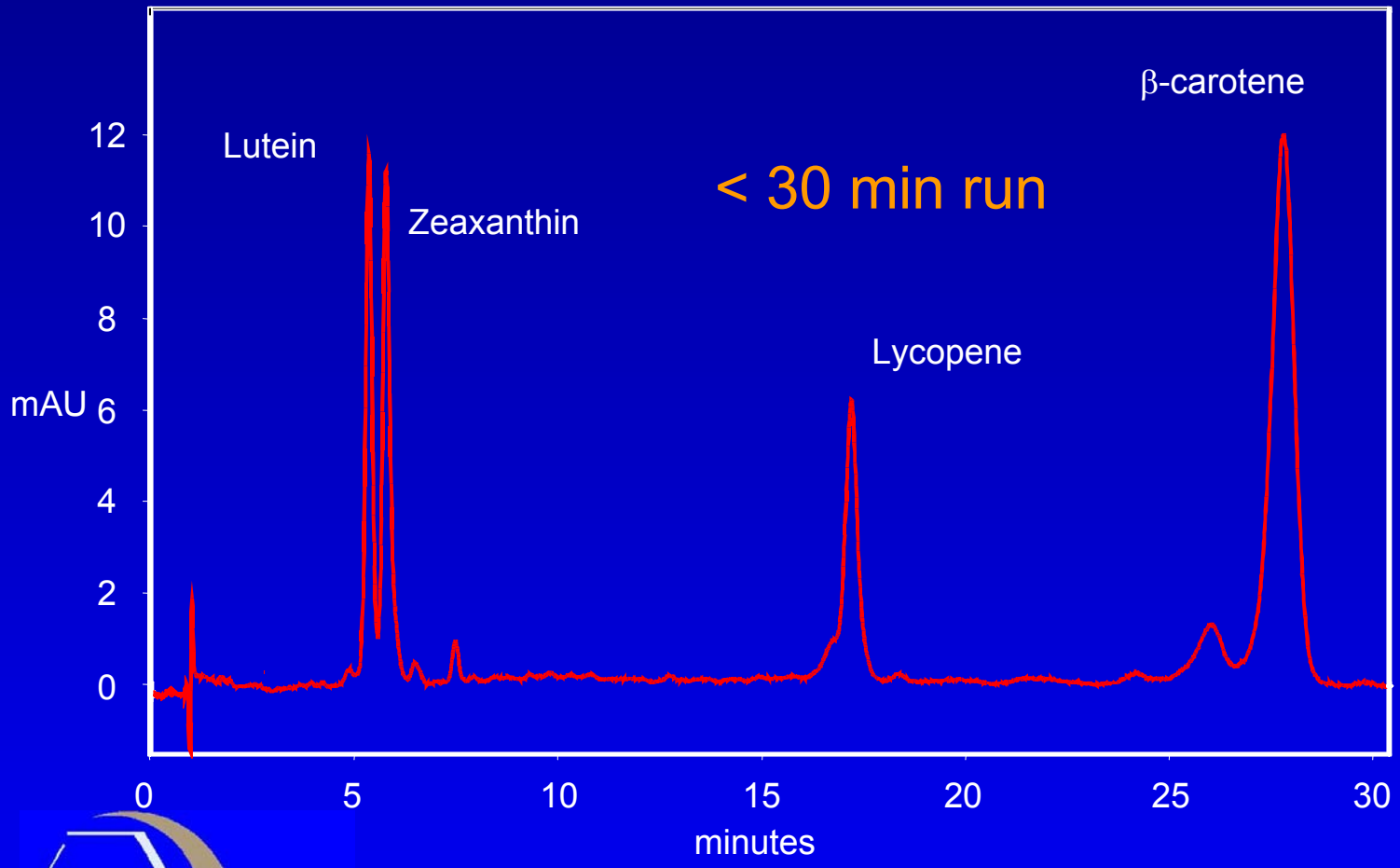
Separation of Carotenoid Mixture at 35°C



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

Elution Order:
Lutein
Zeaxanthin
Lycopene
β-carotene

Separation of Carotenoid Mixture By Temperature Programming



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.

Conclusions

- Thermal gradients can replace solvent gradients for analysis of many natural products
- Increasing the temperature reduces the amount of organic modifier needed
- Temperature programming greatly simplifies method development for complex natural product separations

Acknowledgements

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Steven Burningham, Utah State Dept of Agriculture

Turn up the Heat!

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