

# Improving Pharmaceutical Analyses with Temperature Programmed HPLC

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# The High Temperature HPLC Advantage

- **Temperature Programming**
- **Speed**
- **Efficiency**



# Better Chromatography with Temperature Gradient Programming

- **Change retention through temperature gradient programming**
  - Replace solvent gradients with temperature gradients
  - Water less polar and more like methanol so less organic modifier needed

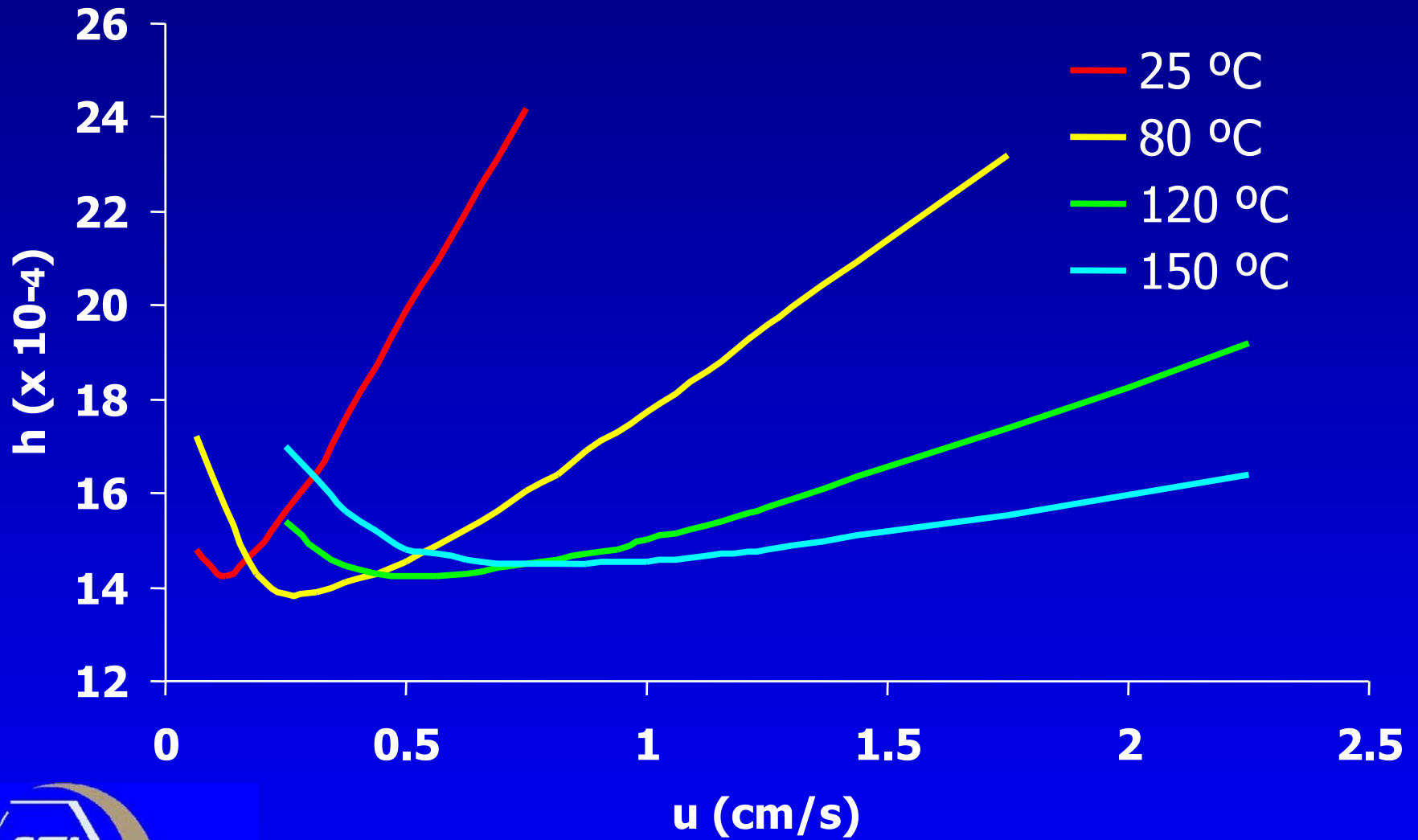


# Faster and More Efficient Separations

- **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



# Temperature Effects on Plate Height



# Obstacles to High Temperature HPLC

- **Need a fully programmable easy-to-use oven capable of fast response**
- **Must eliminate thermal mismatch which causes band-broadening**
- **Need columns stable at elevated temperatures**
- **Must correct for refractive index differences when mobile phase is heated**



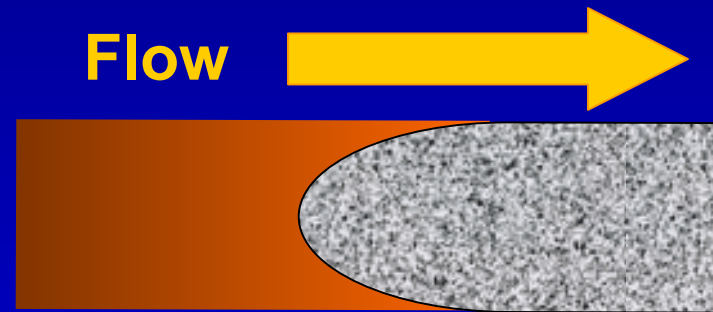
# The Selerity Series 9000 Total Temperature Controller

- Forced air oven and chiller
- Isothermal and thermal gradient operation
  - Sub-zero to 200°C
  - Flow rates up to 10.0 mL/min
  - Thermal gradient up to 30°C/min
- Mobile phase pre-heating
- Peltier effluent cooling
- Vapor sensor
- Compatible with any HPLC system



# Why is Solvent Pre-heating so Important?

No Pre-heating

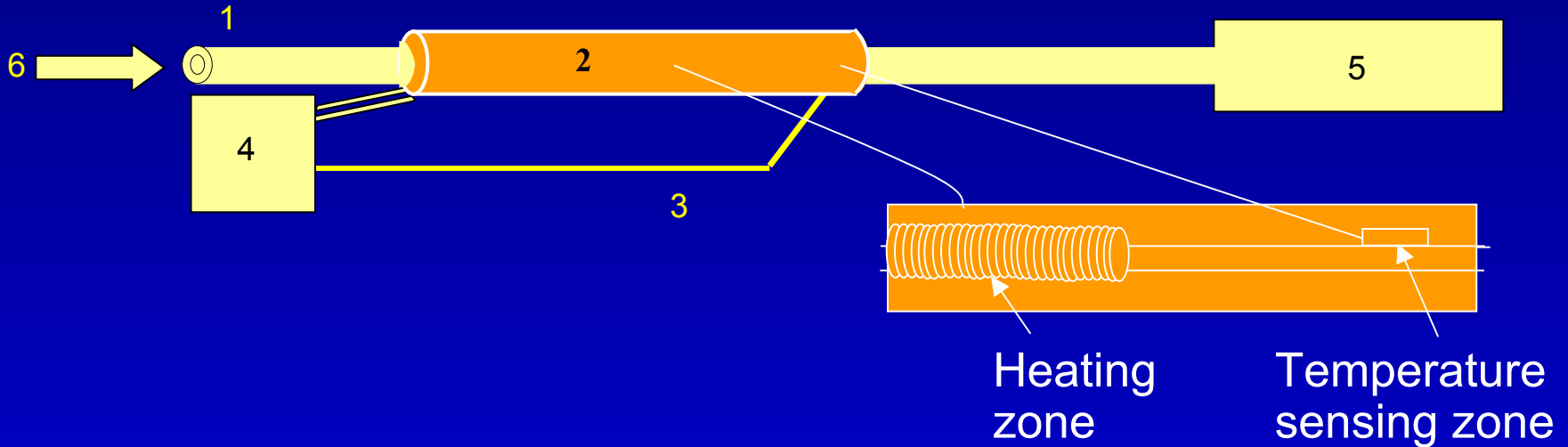


With Pre-heating





# Solvent Pre-heater Design



**(1) stainless steel tubing, (2) heater, (3) thermocouple sensor, (4) temperature controller, (5) column, (6) from pump**

**Patent pending Selerity Technologies, Inc.**



# Solvent Pre-heater

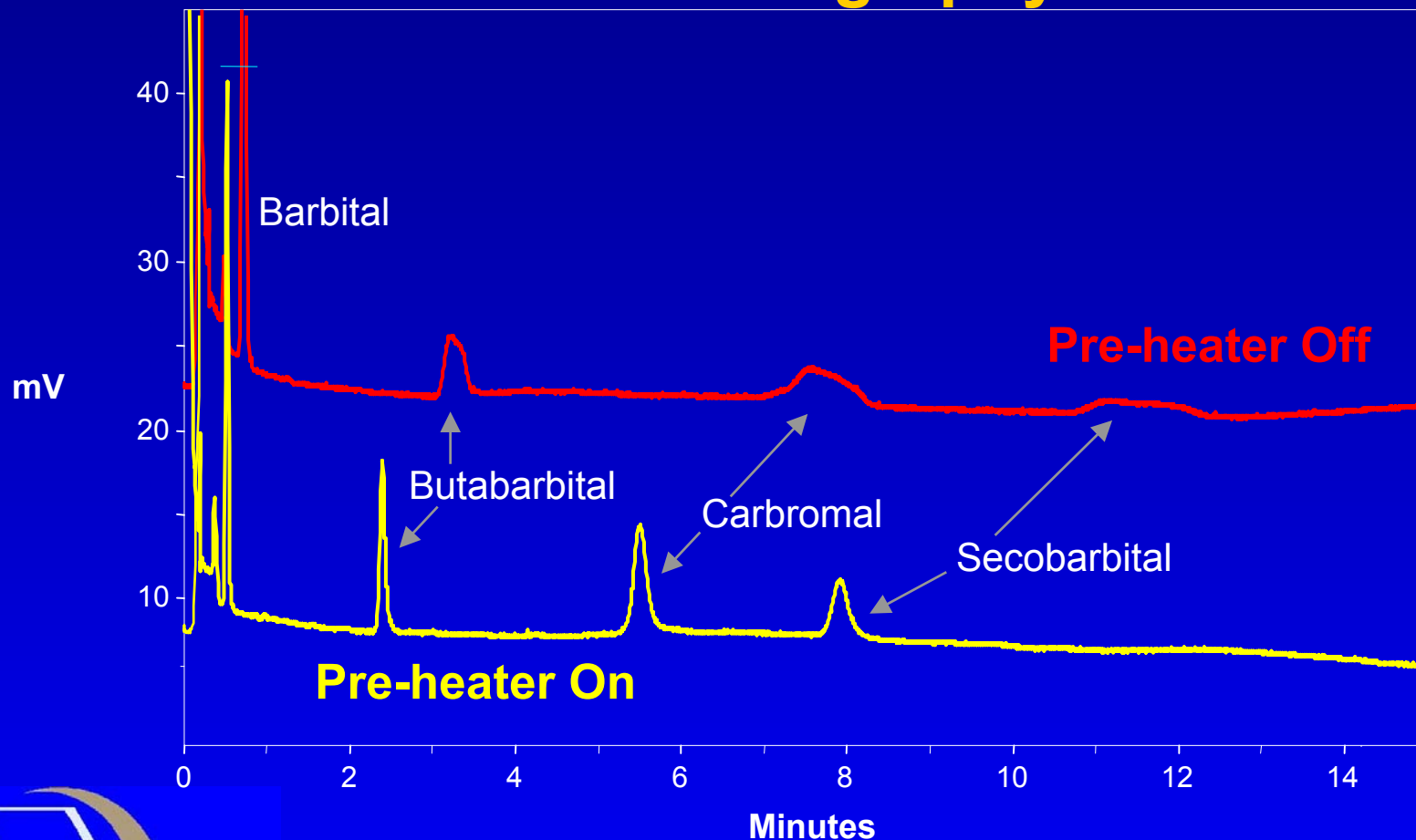


- **Very responsive and non-invasive**
- **Low-mass and low-volume: <2 grams mass (including the tubing), <1  $\mu\text{L}$  totally swept volume**
- **0.005", 0.007" and 0.010" ID available**



# Separation of Barbiturates

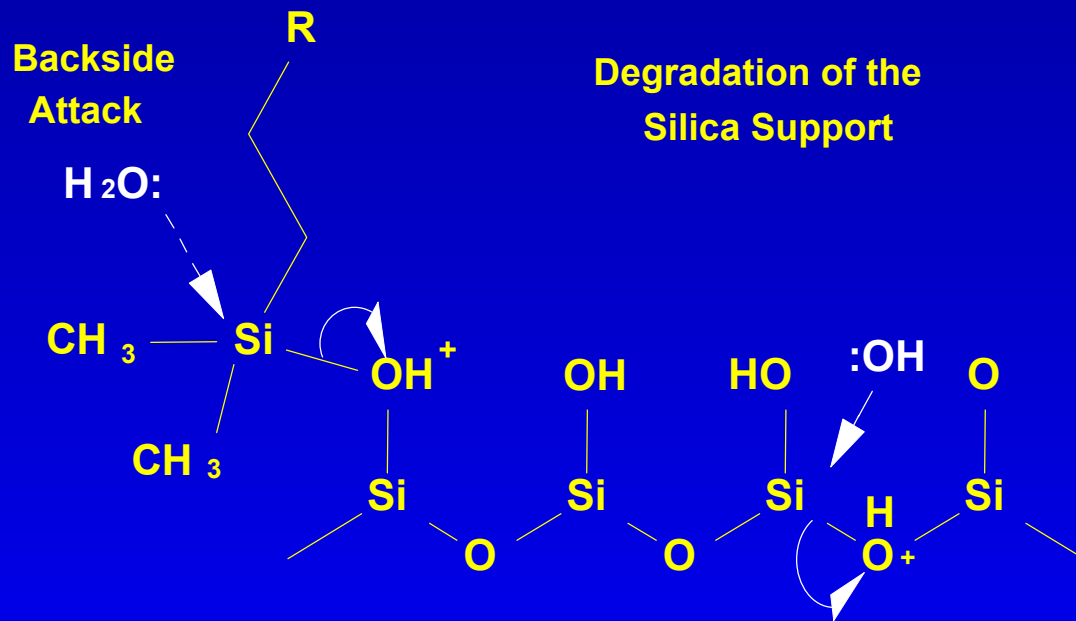
## Mobile Phase Preheating Improves Chromatography



Zirchrom PBD, 80°C

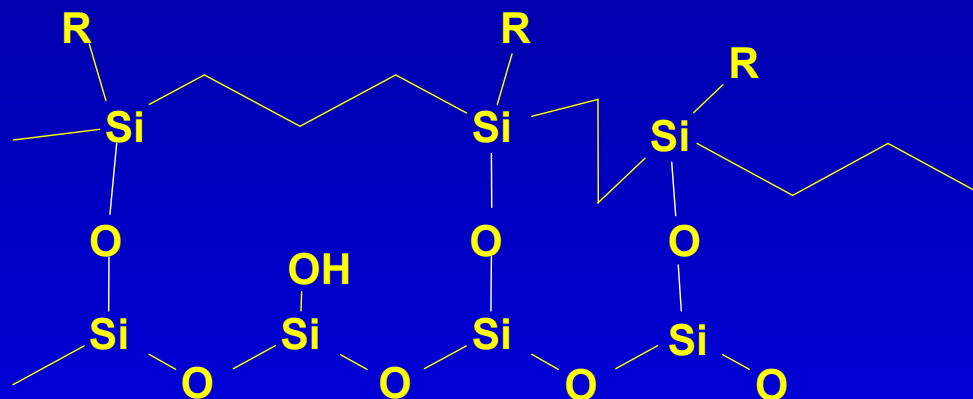
# Traditional Silica Columns Can't Take the Heat

Water attacks siloxane bond or behind point of phase attachment



# The Selerity **Blaze** Silica Column Can Take the Heat

- Selerity polydentate phase protects the silanol groups
- Polymer attaches to backbone at several points



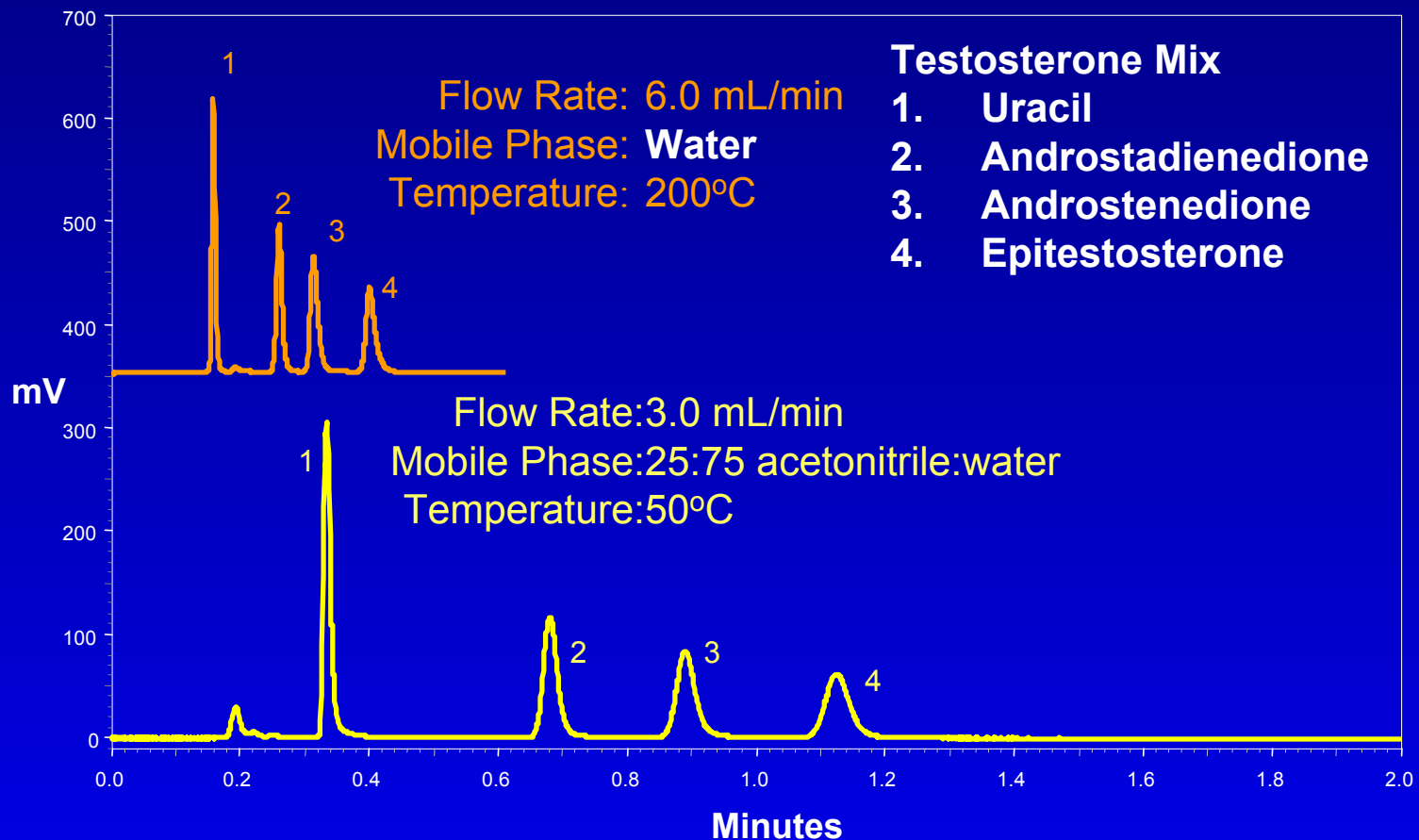
# Other Columns That Can Take the Heat

**Columns stable at high temperatures**

- **Hamilton PRP-1<sup>®</sup> columns**
- **Thermo Hypersil-Keystone Hypercarb<sup>®</sup>**
- **ZirChrom<sup>™</sup> stationary phases**
- **Other columns under evaluation**

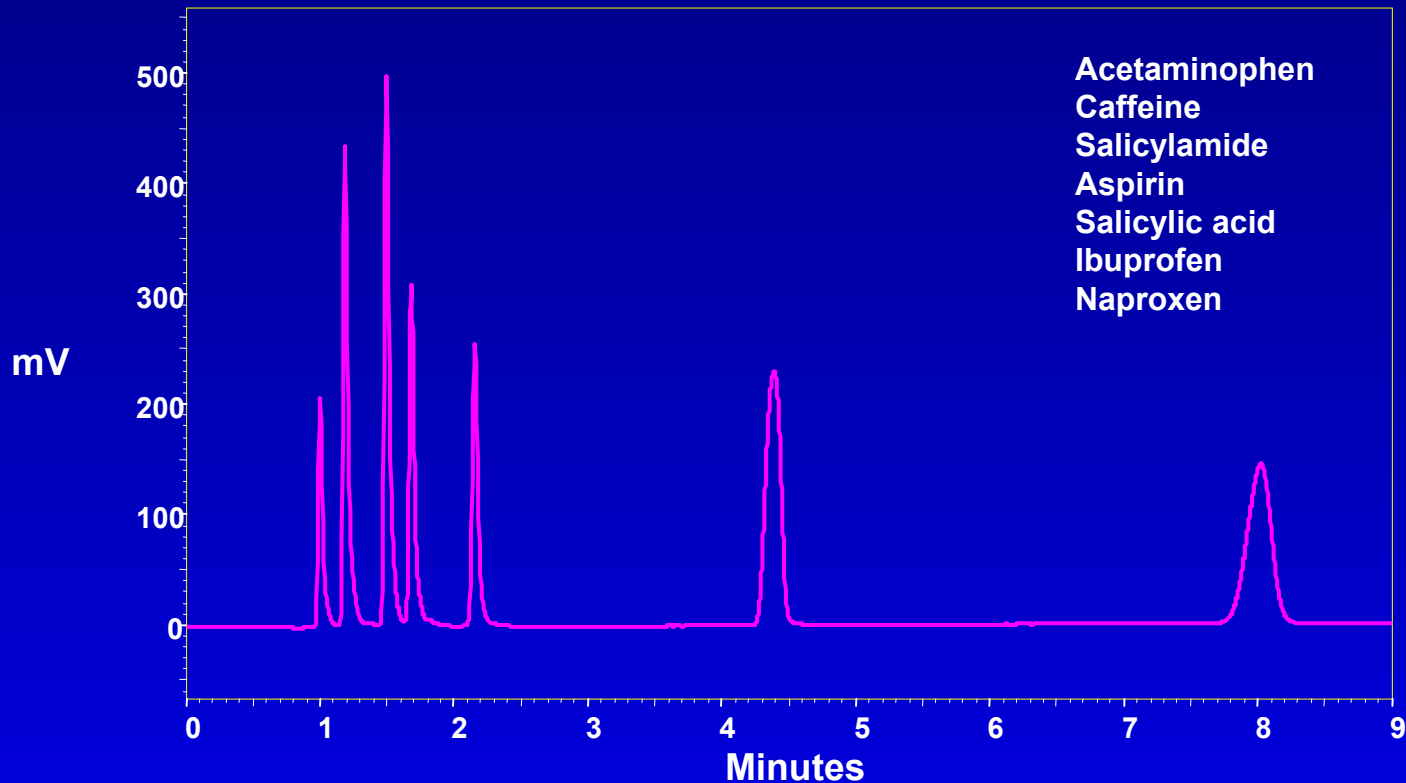


# Separation of Steroids Using Water as a Mobile Phase



Zirchrom PBD 100 X 4.6 mm column, UV 254 nm

# Separation of Analgesics on a Selerity Blaze C8 Using a Thermal Gradient



Column: Selerity Blaze C8, 3  $\mu\text{m}$ , 100 x 4.6 mm

Mobile Phase: 40:60 acetonitrile:water with 0.1%TFA

Flow Rate: 1.5 mL/min

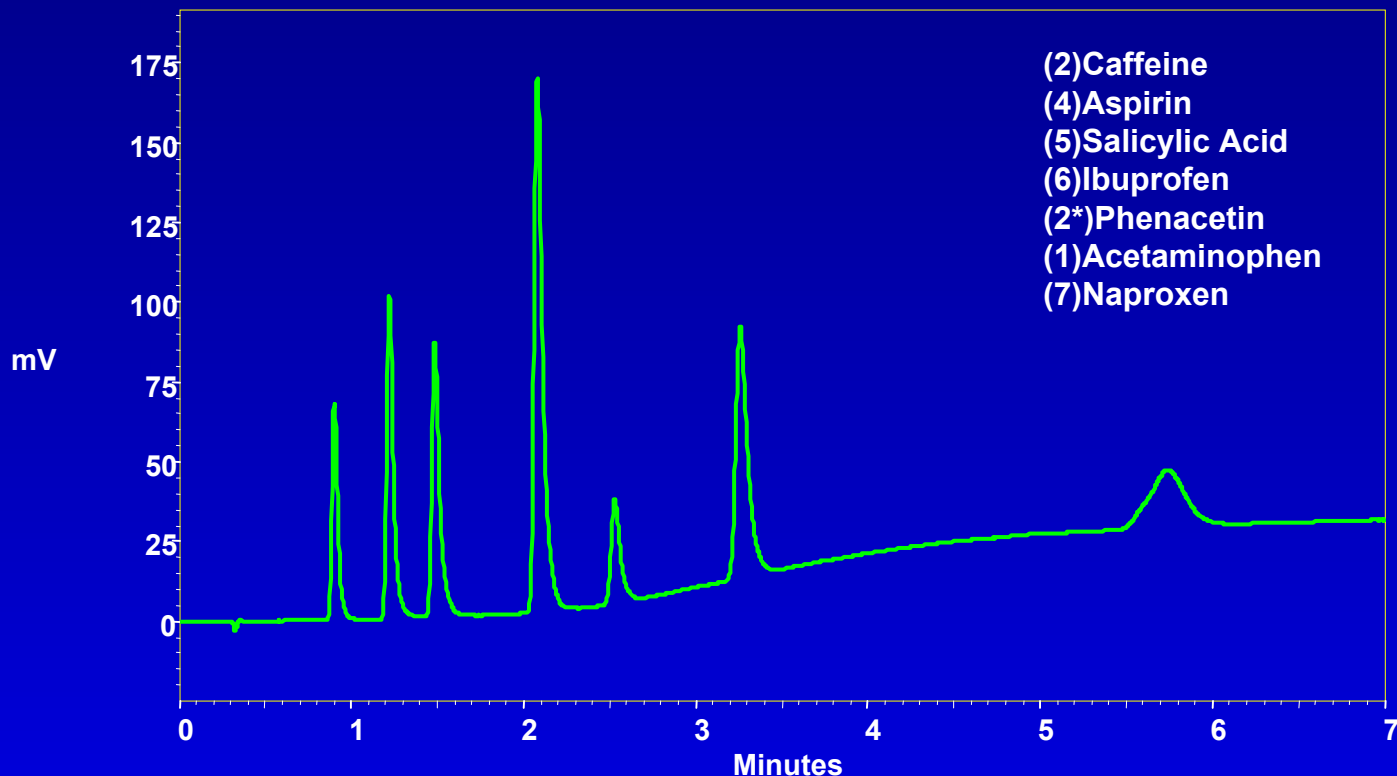
Detection: UV 220 nm

Temperature Program: hold at 50°C for one minute, ramp to 100°C at 30°C/min, hold six min.





# Separation of Analgesics using a Hypercarb<sup>®</sup> Column and a Thermal Gradient



Column: Thermo Hypersil-Keystone Hypercarb<sup>®</sup>, 7  $\mu\text{m}$ , 100 x 4.6 mm

Mobile Phase: 35:65 acetonitrile:water with 0.1% TFA

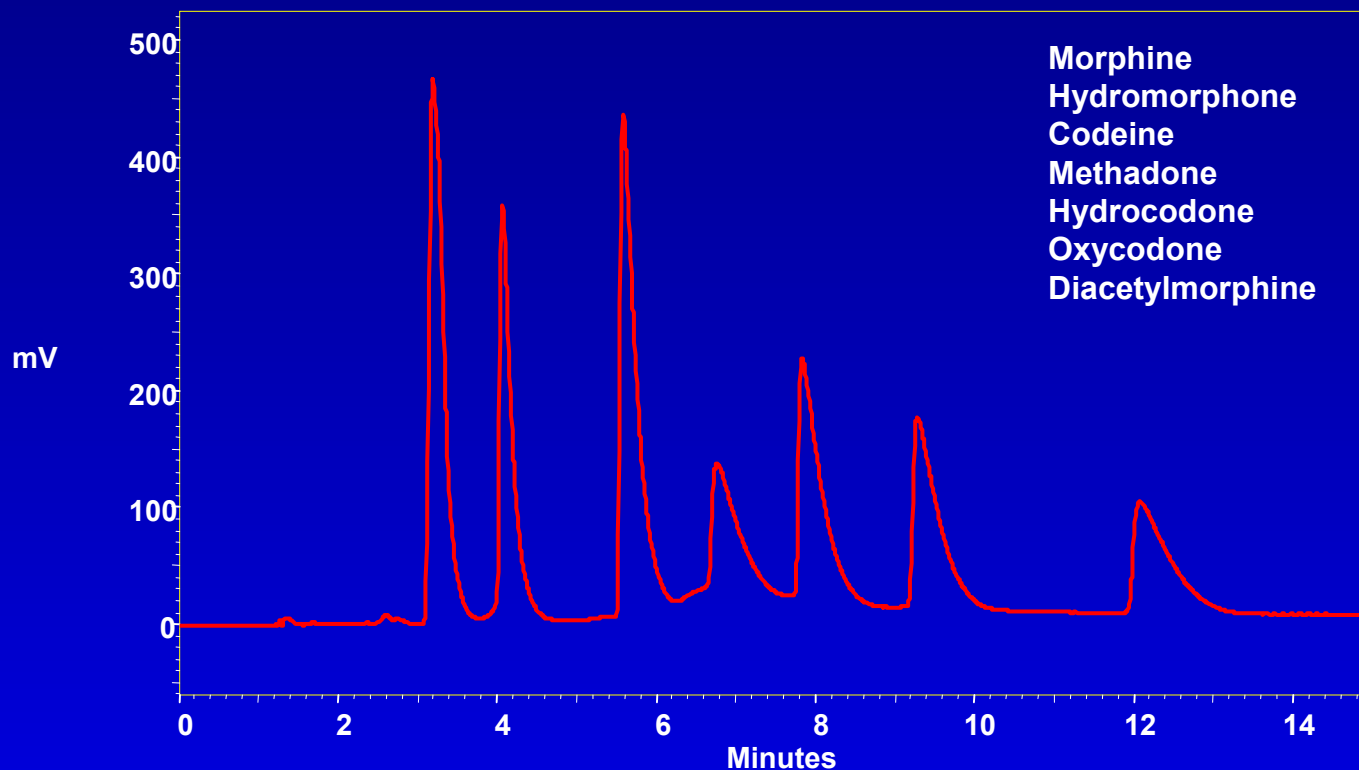
Flow Rate: 4.0 mL/min

Detection: UV 220 nm

Temperature Program: thermal gradient from 125° to 200°C at 30°/min, hold five min.



# Separation of Narcotics on Hypercarb<sup>®</sup> Column Using a Thermal Gradient



Column: Thermo Hypersil-Keystone Hypercarb<sup>®</sup>, 7  $\mu$ m, 100 x 4.6 mm

Mobile Phase: 50:50 acetonitrile:50 mM ammonium acetate pH 9.0

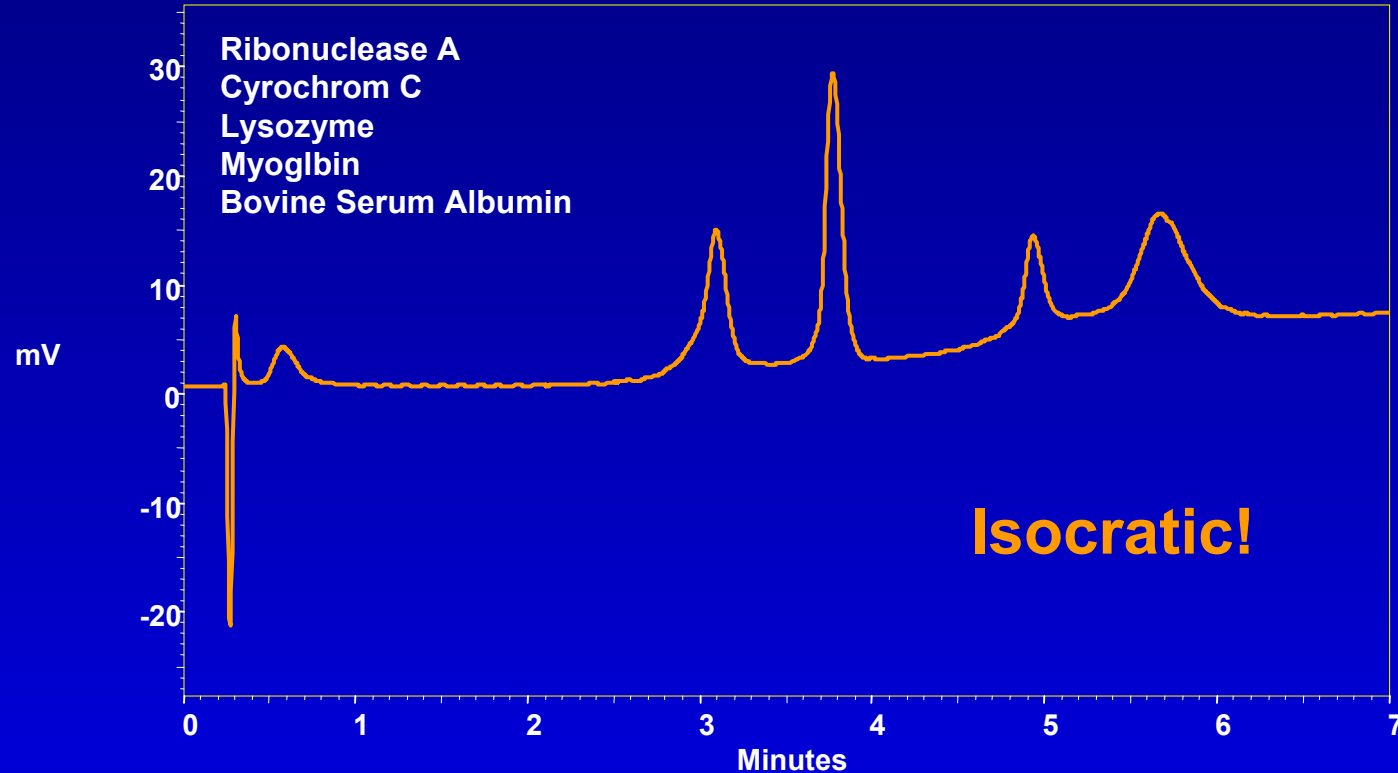
Flow Rate: 1.0 mL/min

Detection: UV 220 nm

Temperature Program: hold at 50°C for two minutes, ramp to 150°C at 30°/min, hold ten min.



# Separation of Proteins Using a Hamilton PRP-3<sup>®</sup> Column and a Thermal Gradient



Column: Hamilton PRP-3<sup>®</sup>, 3  $\mu$ m, 100 x 2.1 mm  
Mobile Phase: 25:75 acetonitrile:water with 0.1% TFA  
Flow Rate: 1.0 mL/min  
Detection: UV 215 nm  
Temperature Program: 50°C to 150°C at 30°/min, hold five min.



# Sample Prep for Sildenafil Analysis In 100 mg Viagra and Offshore Tablet

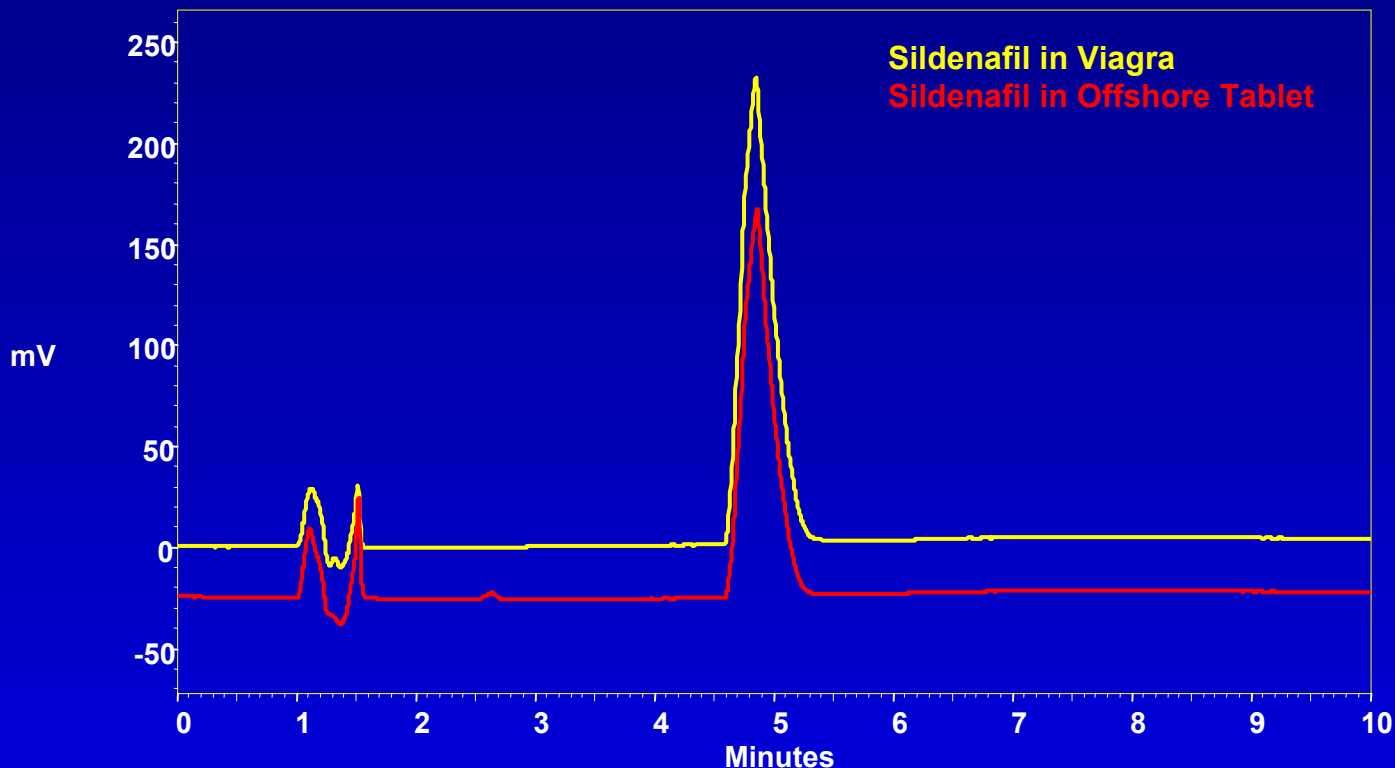
- Ground tablet in mortar and pestle
- Extracted with 30 mL 50:50 methanol:water by sonicating for 20 minutes
- Centrifuged at 3000 rpm for 15 minutes
- Diluted supernatant to 100 mL with 50:50 methanol:water (rinsed three times)
- Filtered through 0.45 $\mu$ m nylon prior to injection



Tseng and Lin, *J. of Food and Drug Analysis*, 10, 112-119 (2002)



# Analysis of Sildenafil in Viagra and Offshore Tablet Using a Selerity Blaze C8



Column: Selerity Blaze C8 100 x 4.6 mm, 3 $\mu$ m

Mobile Phase: 35:65 acetonitrile:water with 0.1% TFA.

Flow Rate: 1.0 mL/min

Detection: UV 220 nm

Temperature Program: hold at 40°C for two minutes, ramp to 100°C at 15°/min, hold for six min.



# Analysis of Sildenafil in Viagra and Offshore Tablet Using a Selerity Blaze C8

- Assume Pfizer Viagra tablet contains **100 mg** of Sildenafil
- Compare peak areas
- Correct for amount of tablet used
- Offshore tablet contains about **80 mg** of Sildenafil



# Conclusions

- Thermal gradients can replace solvent gradients for analysis of many pharmaceuticals
- Increasing the temperature reduces the amount of organic modifier needed
- Shorter analysis times with better efficiency results from higher temperatures
- Use authentic Pfizer Viagra





# Acknowledgements

- **Thermo Hypersil-Keystone**
  - Steve Kozel and Rick Ludwig
- **Hamilton Company**
  - Mike Benning and Dan Lee





# Turn up the Heat!



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