Using High Temperature HPLC for Improved Analysis in the Pharmaceutical Industry

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The High Temperature HPLC Advantage

- Temperature Programming
- Speed
- Efficiency

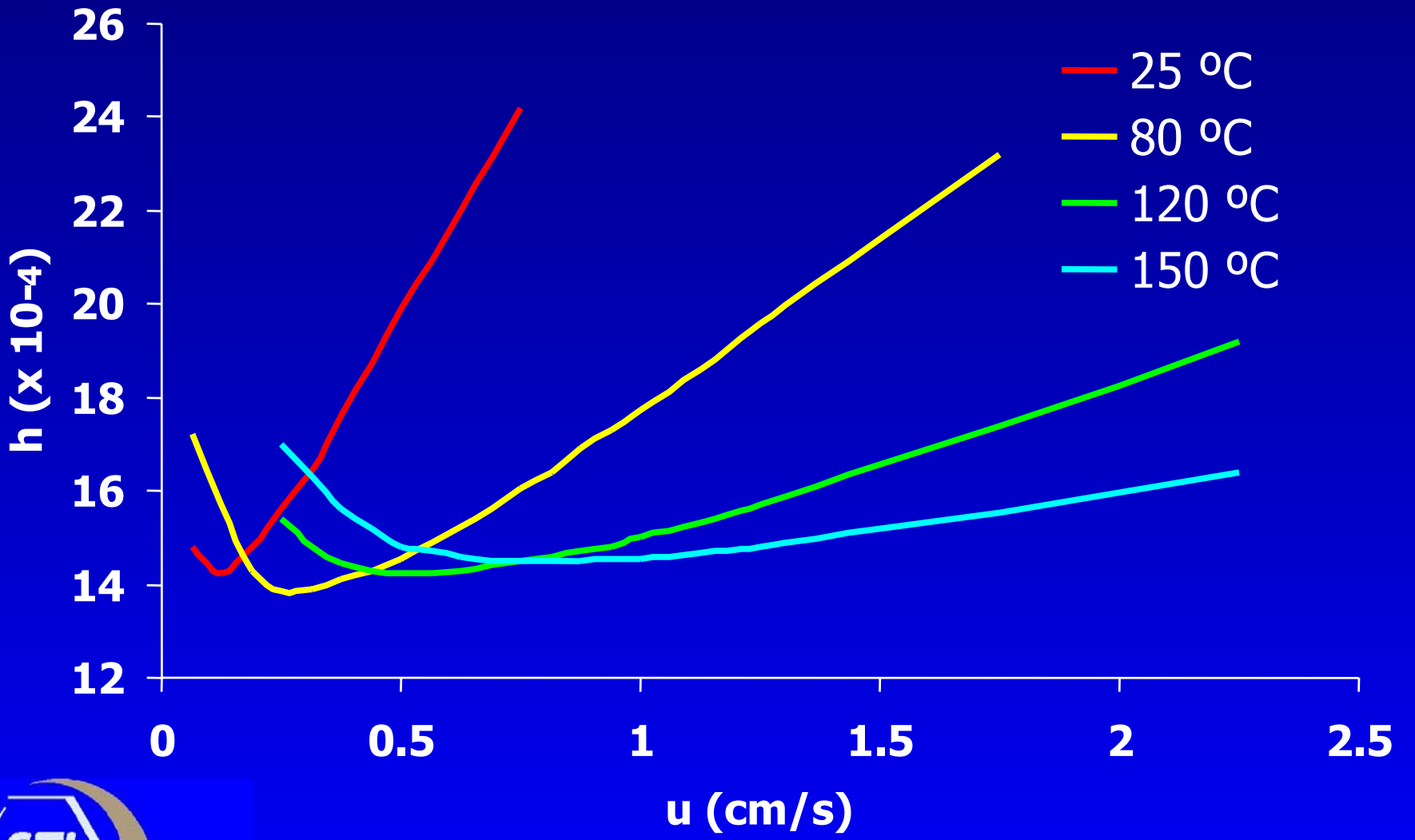
Better Chromatography with Temperature Gradient Programming

- **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

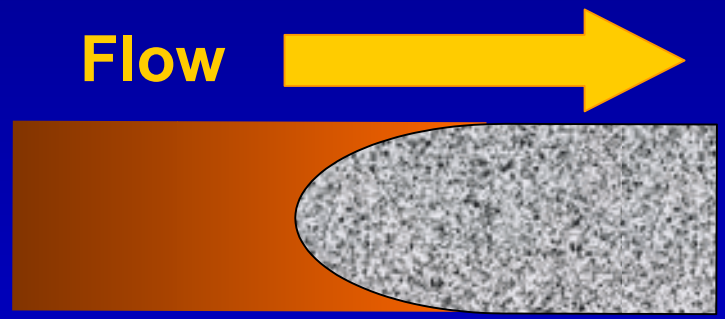
- Need a fully programmable easy-to-use oven capable of fast response
- Must eliminate thermal mismatch which causes band-broadening
- Need columns stable at elevated temperatures
- Must correct for refractive index differences when mobile phase is heated

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 and pre-cooling
- Peltier effluent cooling
- Vapor sensor
- Compatible with any HPLC system

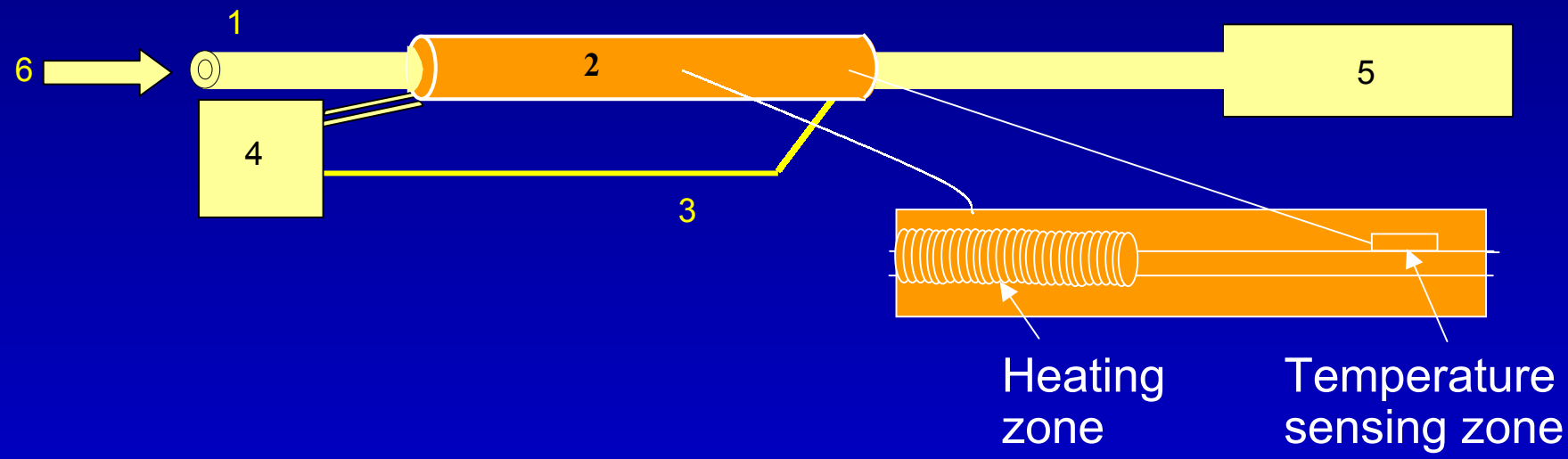
Why is Solvent Pre-heating so Important?

No Pre-heating



With Pre-heating

Solvent Pre-heater Design



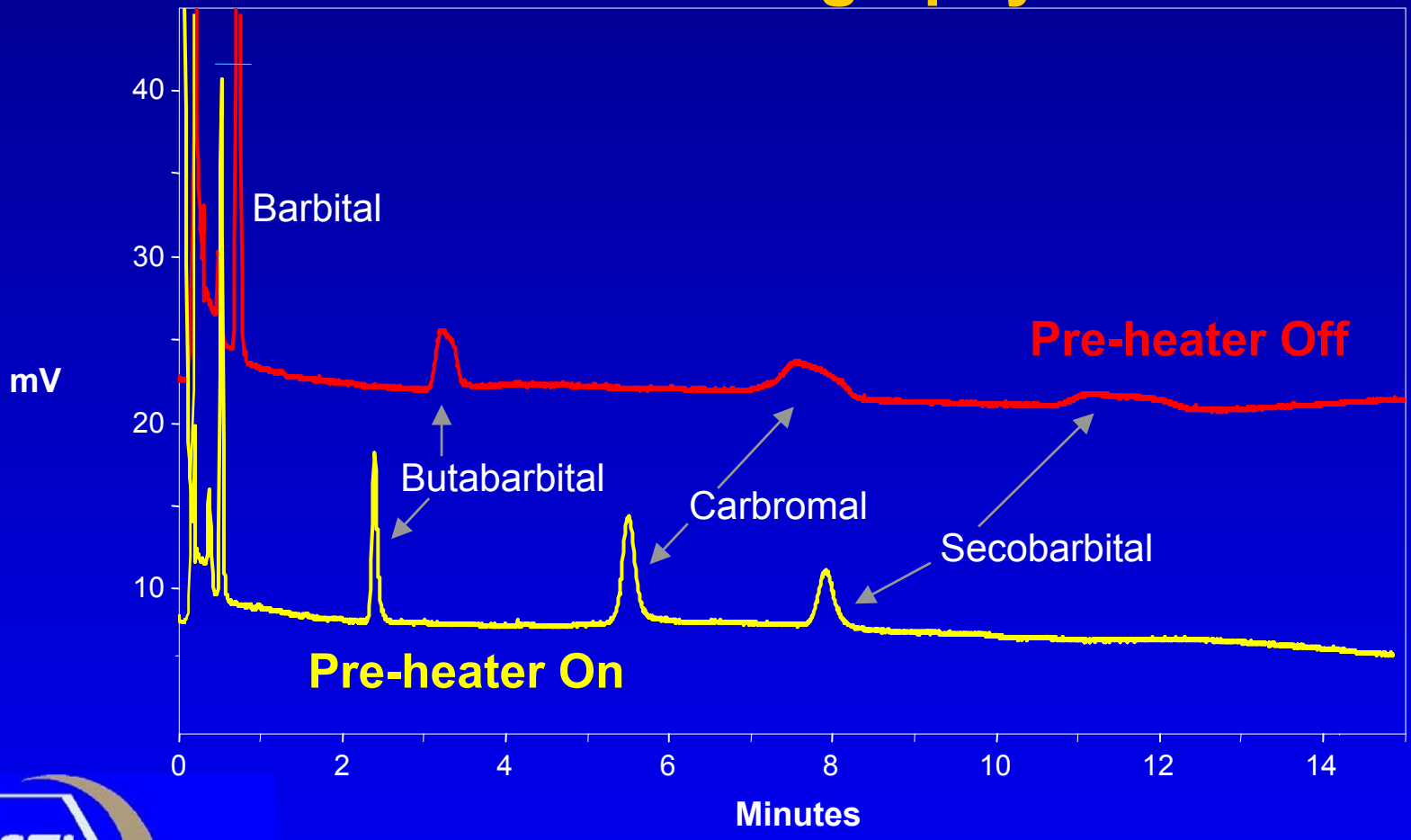
(1) stainless steel tubing, (2) heater, (3) thermocouple sensor, (4) temperature controller, (5) column, (6) from pump

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 μL totally swept volume
- 0.005”, 0.007” and 0.010” ID available

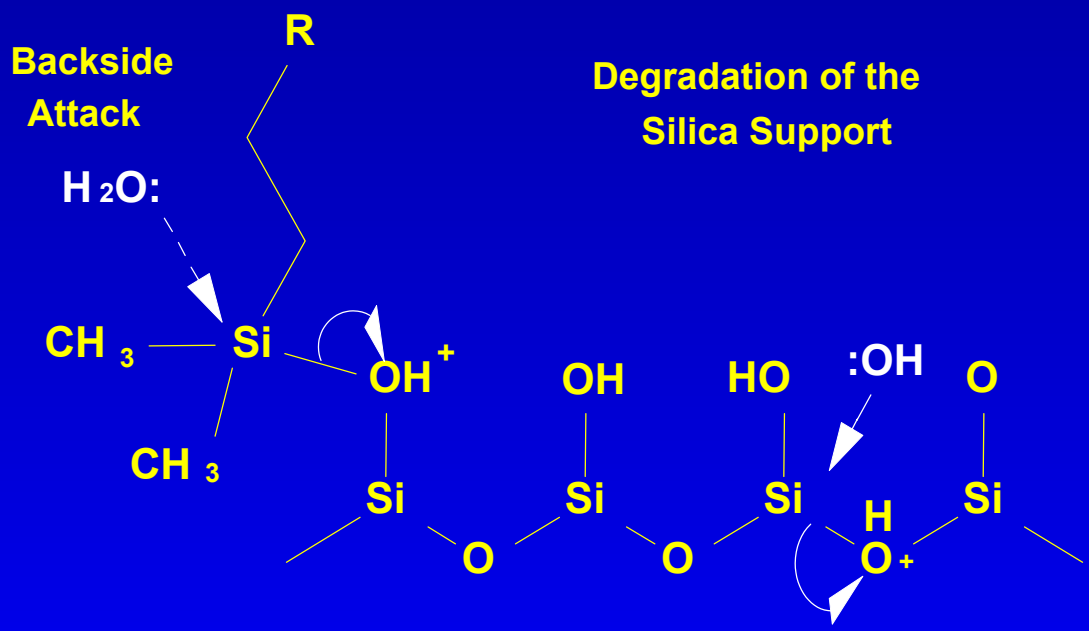
Separation of Barbiturates
Mobile Phase Preheating Improves Chromatography



Zirchrom PBD, 80°C

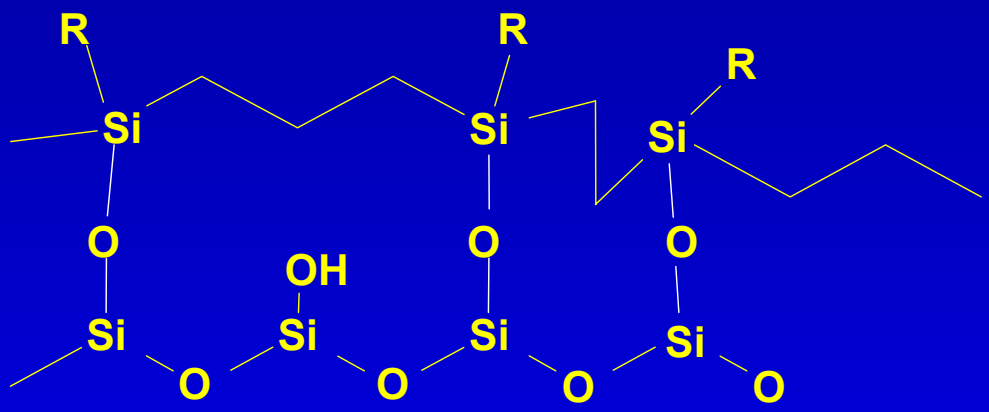
Traditional Silica Columns Can't Take the Heat

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

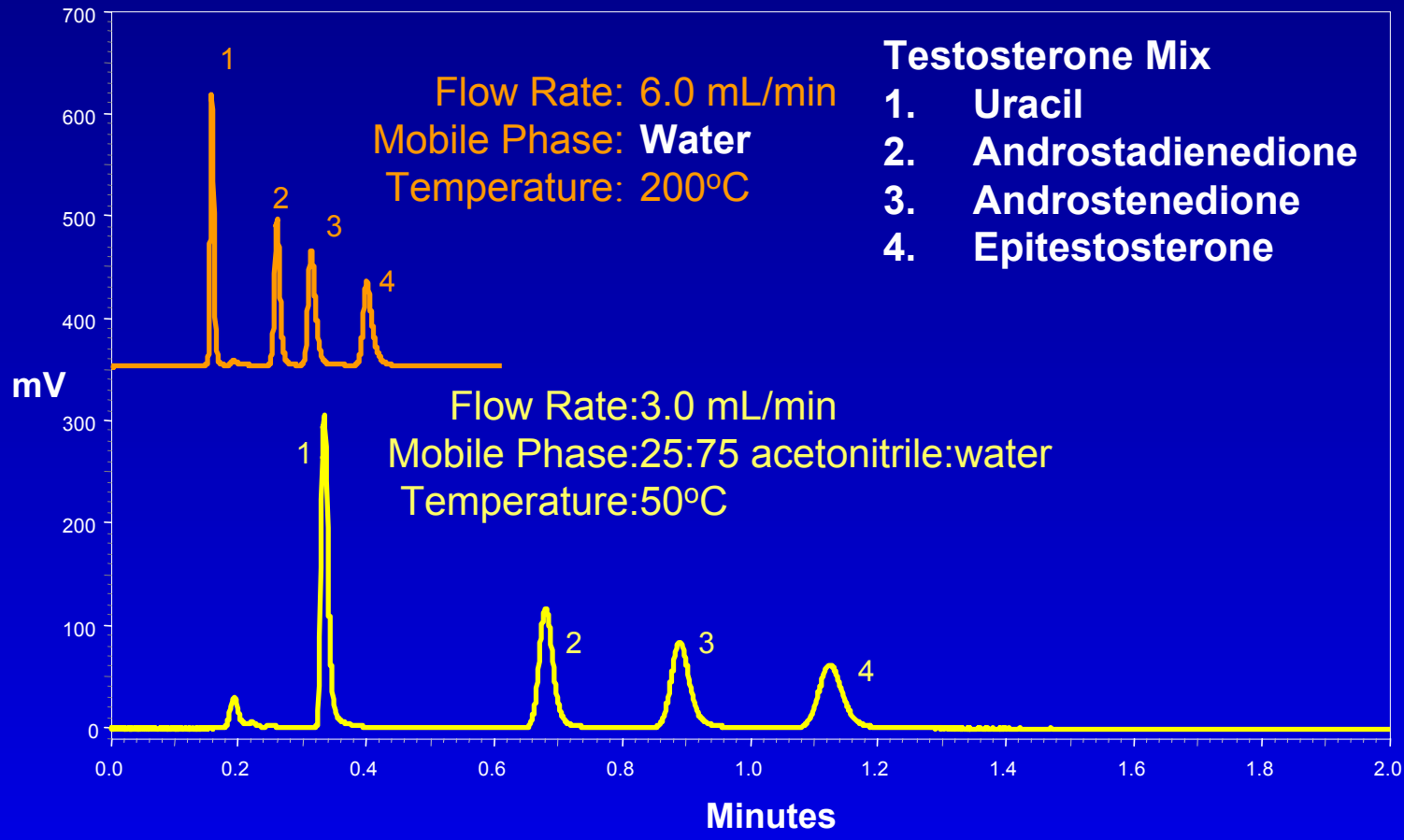


Other Columns That Can Take the Heat

Columns stable at high temperatures

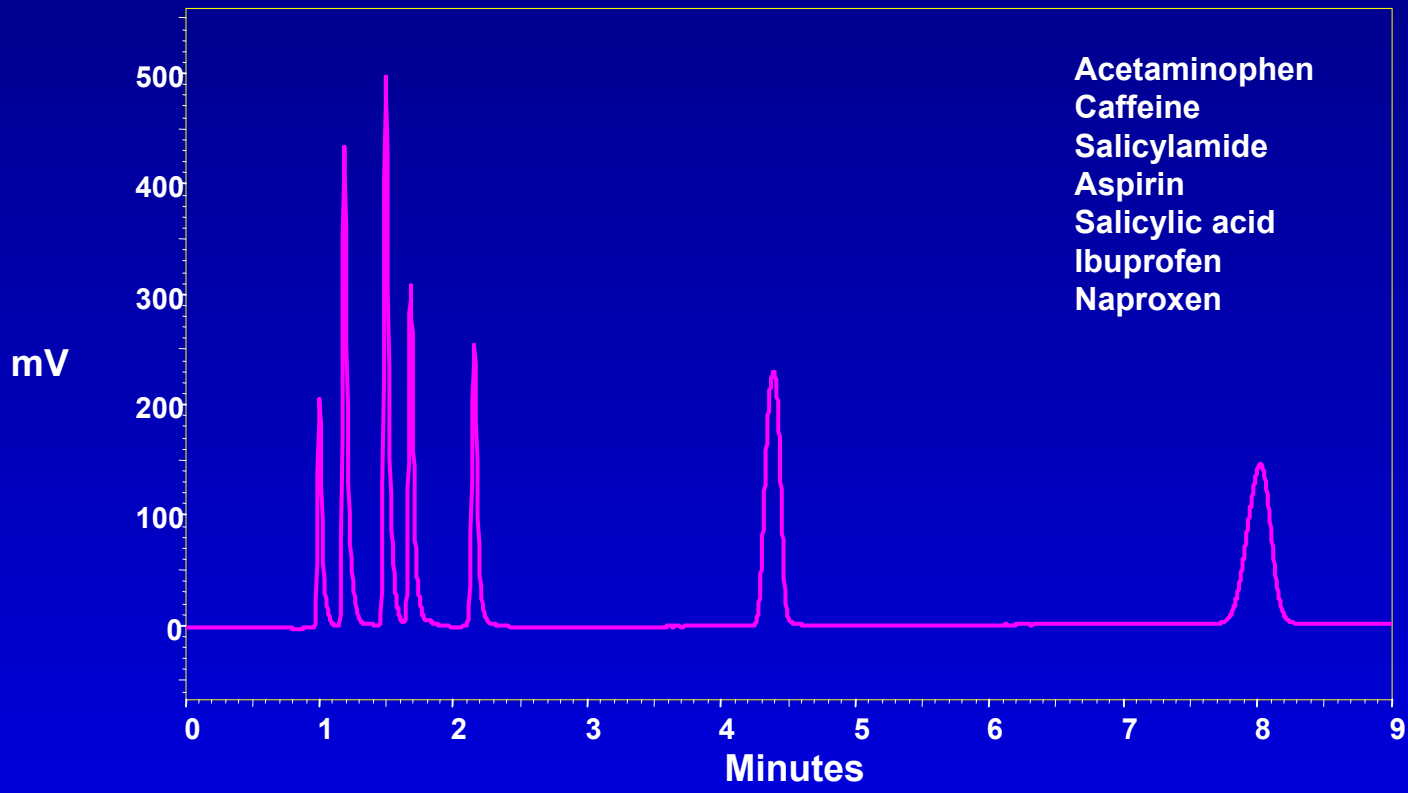
- Hamilton PRP-1® columns
- Thermo Hypersil-Keystone Hypercarb®
- ZirChrom™ stationary phases
- Other columns under evaluation

Separation of Steroids Using Water as a Mobile Phase



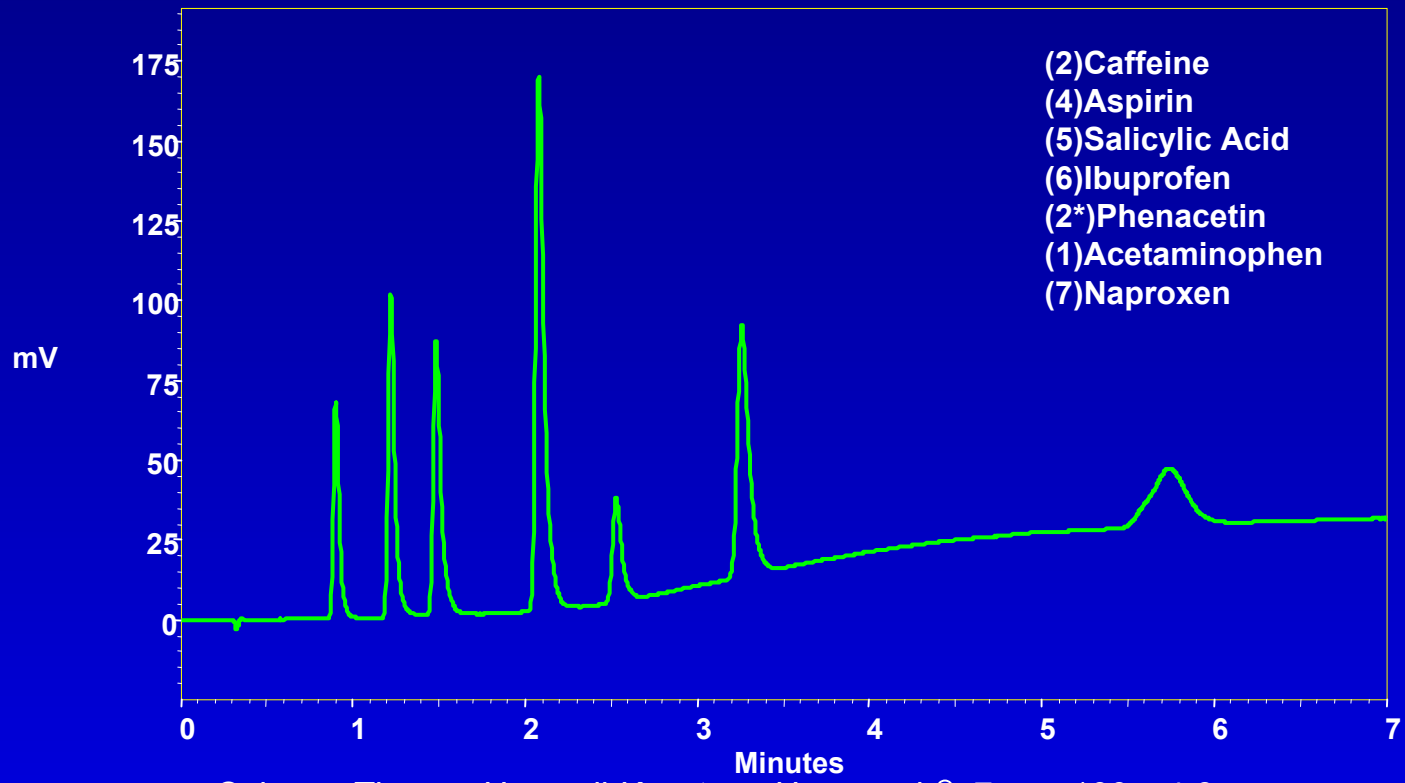
Zirchrom PBD 100 X 4.6 mm column, UV 254 nm

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



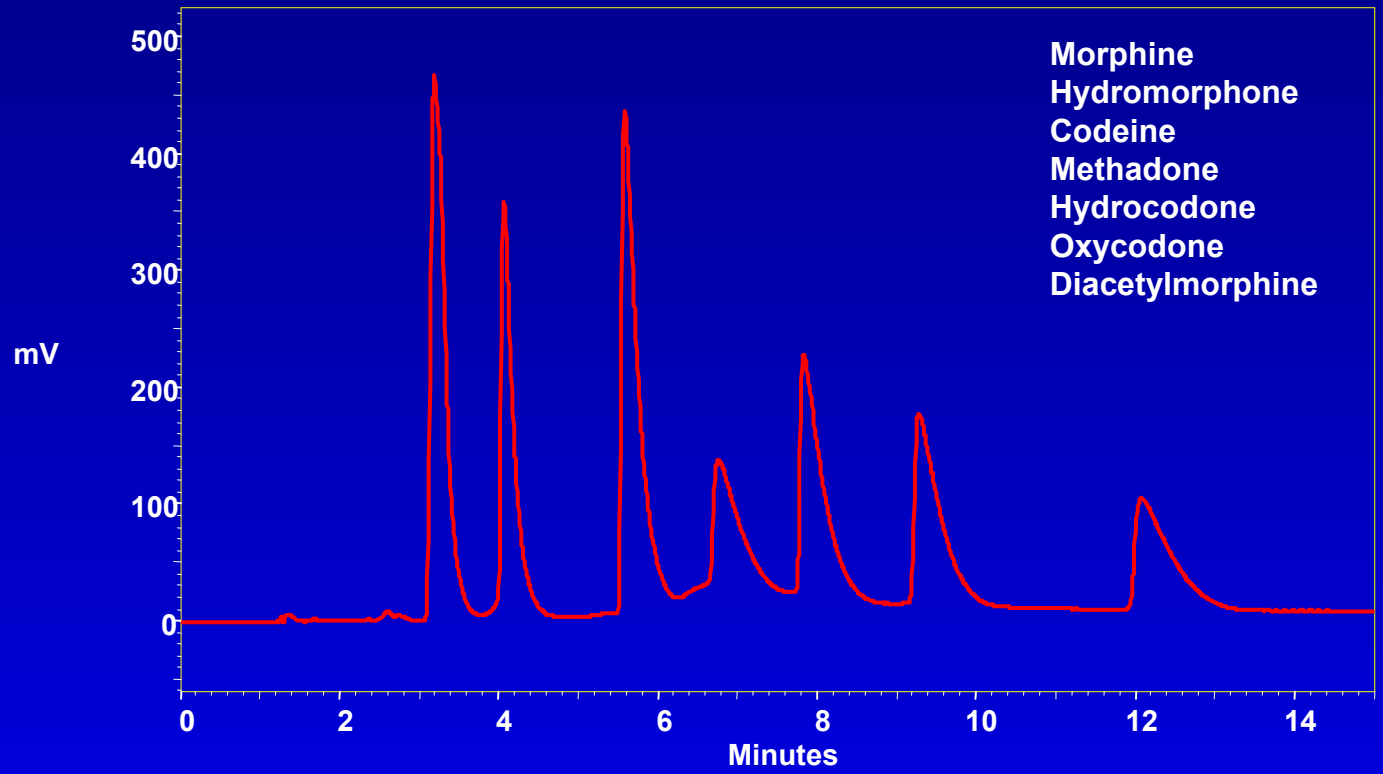
Column: Selerity Blaze C8, 3 µm, 100 x 4.6 mm
Mobile Phase: 40:60 acetonitrile:water with 0.1%TFA
Flow Rate: 1.5 mL/min
Detection: UV 220 nm
Temperature Program: hold at 50°C for one minute, ramp to 100°C at 30°C/min, hold six min.

Separation of Analgesics using a Hypercarb® Column and a Thermal Gradient



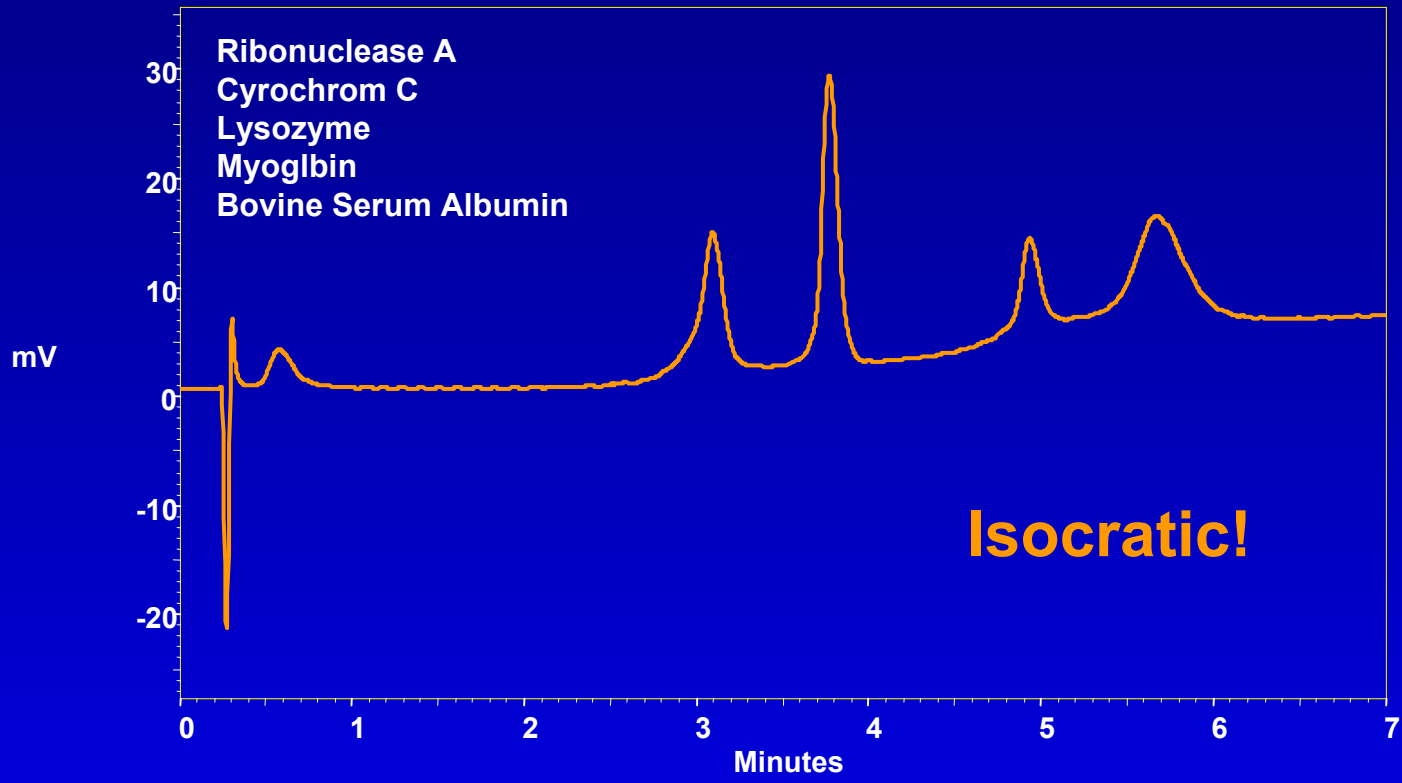
Column: Thermo Hypersil-Keystone Hypercarb®, 7 µm, 100 x 4.6 mm
Mobile Phase: 35:65 acetonitrile:water with 0.1% TFA
Flow Rate: 4.0 mL/min
Detection: UV 220 nm
Temperature Program: thermal gradient from 125° to 200°C at 30°/min, hold five min.

Separation of Narcotics on Hypercarb® Column Using a Thermal Gradient



Column: Thermo Hypersil-Keystone Hypercarb®, 7 µm, 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® Column and a Thermal Gradient



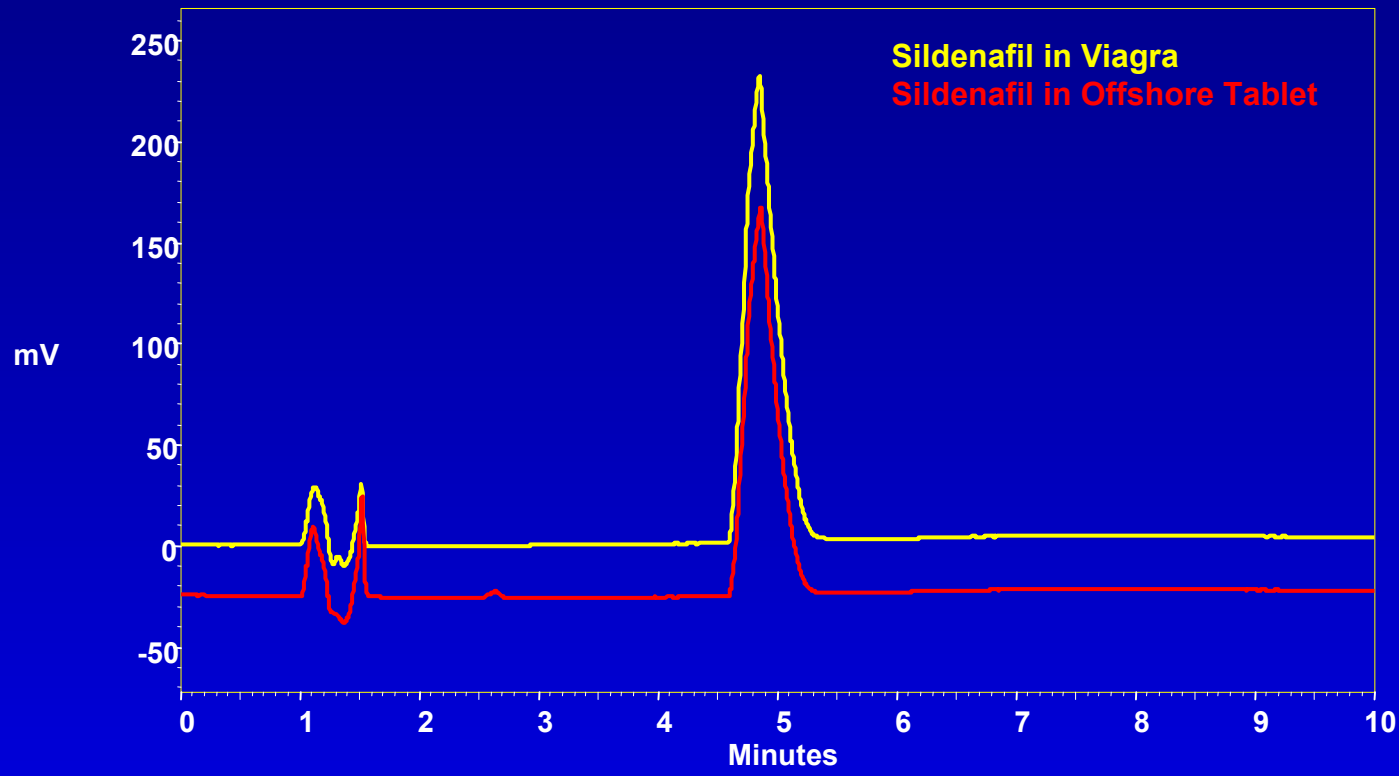
Column: Hamilton PRP-3®, 3 µm, 100 x 2.1 mm
Mobile Phase: 25:75 acetonitrile:water with 0.1% TFA
Flow Rate: 1.0 mL/min
Detection: UV 215 nm
Temperature Program: 50°C to 150°C at 30°/min, hold five min.

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 0.45μm 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, 3µm
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

- No evidence of degradation of API
- 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

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Turn up the Heat!

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