

Enhancing your HPLC Separations using Temperature Programming

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

Selerity Technologies, Inc.

2484 W. Custer Road

Salt Lake City, UT 84104

www.selerity.com



The High Temperature HPLC Advantage

- **Speed**
- **Efficiency**
- **Selectivity**
- **Gradient elution**



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



Better Chromatography with Temperature Gradient Programming

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



Obstacles to High Temperature HPLC

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



The Selerity Series 9000 Total Temperature Controller

- Small footprint
- Sub-zero to 200°C
- Mobile phase preheating or precooling
- Precision mobile phase outlet temperature control
- Flammable vapor sensor
- Variable low voltage mobile phase preheater
- Integral high speed microcomputer control



Small and Versatile Temperature Programmable Column Heater/Chiller



Vapor Sensor

- Provides operator safety when working with flammable or toxic mobile phases at elevated temperatures
- Automatically cools and ventilates oven and sends a stop signal to the HPLC pump



Multiple Point Mobile Phase Temperature Monitoring

- Preheater (integral)
- Outlet temperature controller
- Auxiliary for optional user placement

Electrically isolated thermocouples and RTD

All can be internally calibrated to the column oven, which is itself calibrated with a NIST traceable reference standard



Cryo Capability with Mobile Phase Pre-Cooling

- Solid state Peltier electronic cooling
- Cryo fluid option for both mobile phase and oven (nitrogen and CO₂)



Precision Mobile Phase Temperature Conditioning



The hazards and detector instability problems of hot exiting mobile phases are eliminated



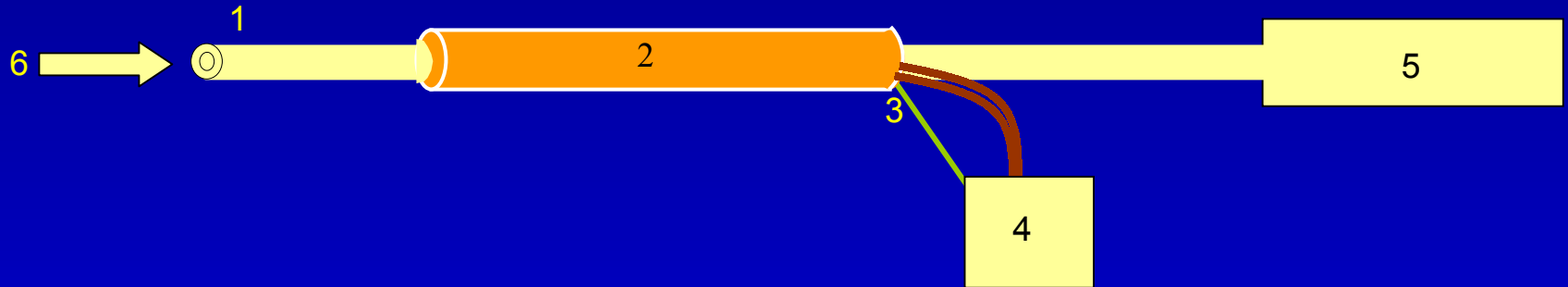
Fast Temperature Programming, Fast Cool-down

- Temperature gradients to $+30^{\circ}\text{C}/\text{min}$
- Cool-down from 200°C in 2 minutes

--High Analysis Throughput--



Solvent Pre-heater Design



(1) connection tubing from injector, (2) heater, (3) temperature sensor, (4) microprocessor controller, (5) column, (6) mobile phase flow

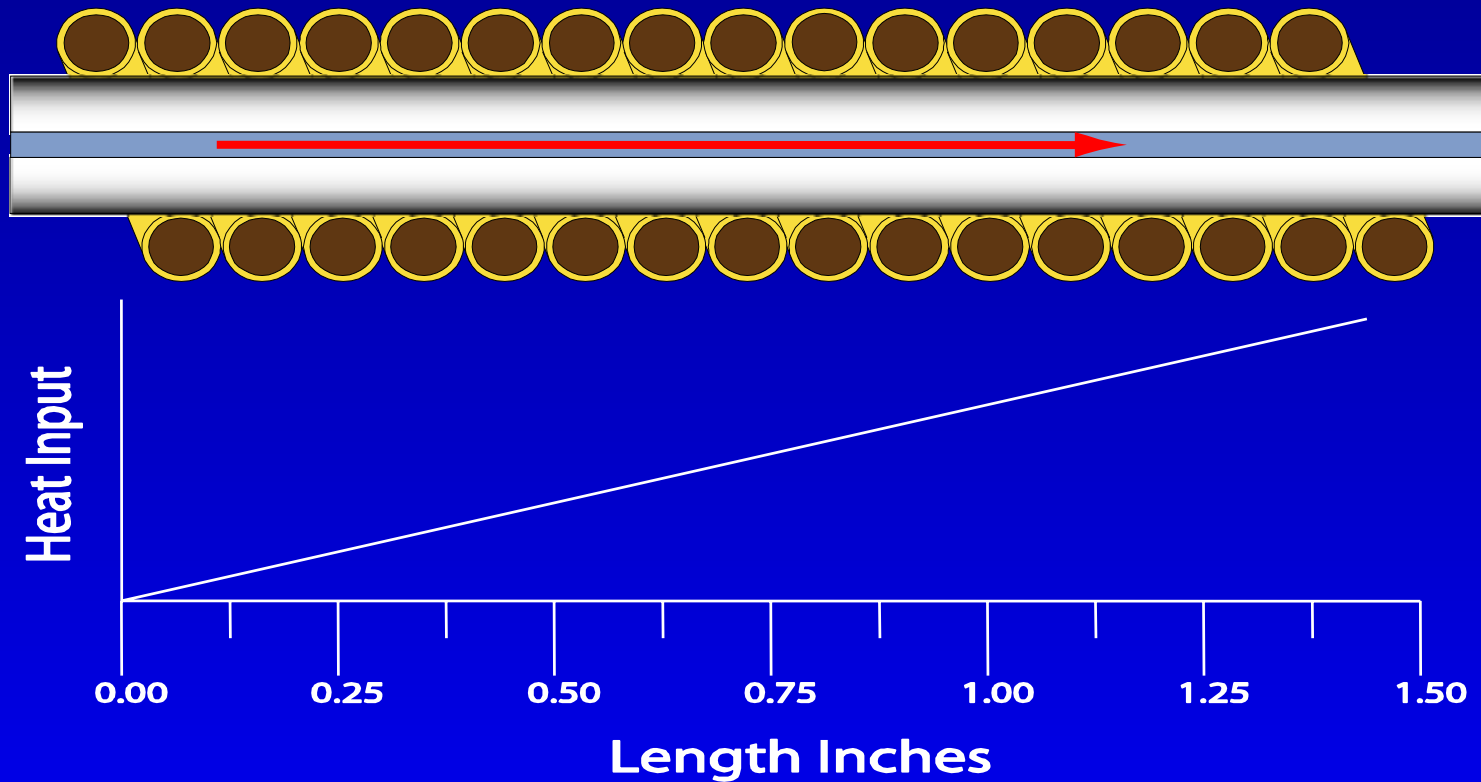


Mobile Phase Pre-heater

- Very fast response and non-invasive
- Low-mass and low-volume, $<1 \mu\text{L}$
- 0.005", 0.007" and 0.010" ID available
- Adds no dead volume



Axial Heat Profile



Variable Low Voltage Pre-heater

- Automatically steps power to the pre-heater in 60 mV DC increments
- Safe low voltage operation
- Typical voltage feeding the heater at 1 ml/min mobile phase flow brought to 150°C is only 6 volts



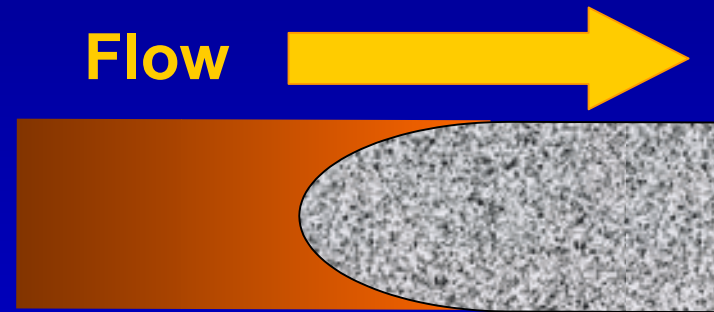
Variable Low Voltage Pre-heater

- Can lead, match, or lag oven temperature program for analyte focusing
- Automatically responds to changing mobile phase heat capacity through composition gradients or flow programming
- Convenient plug-in access in the front door



Why is Solvent Pre-heating so Important?

No Pre-heating

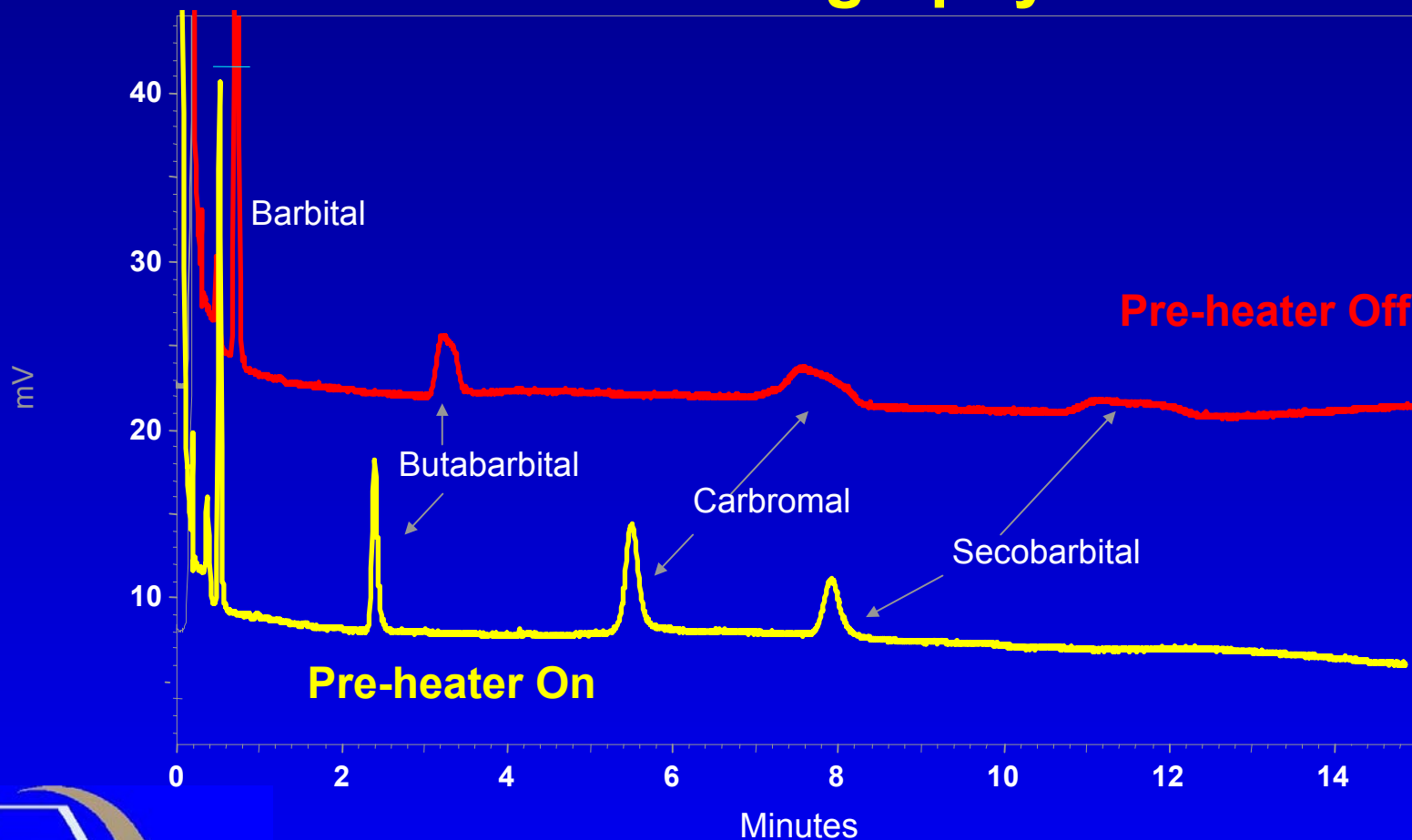


With Pre-heating



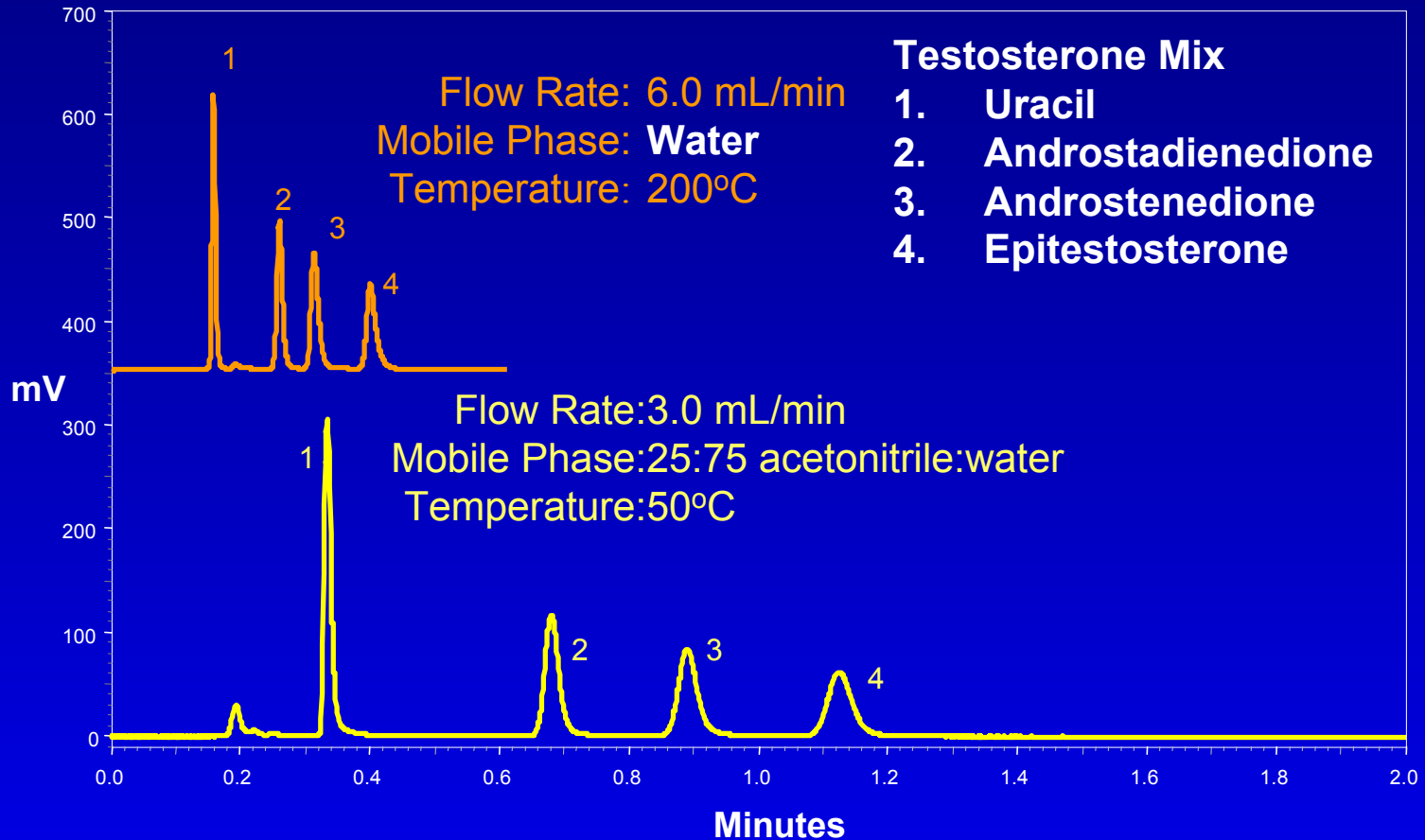
Separation of Barbiturates

Mobile Phase Pre-heating Improves Chromatography



Zirchrom PBD, 80°C

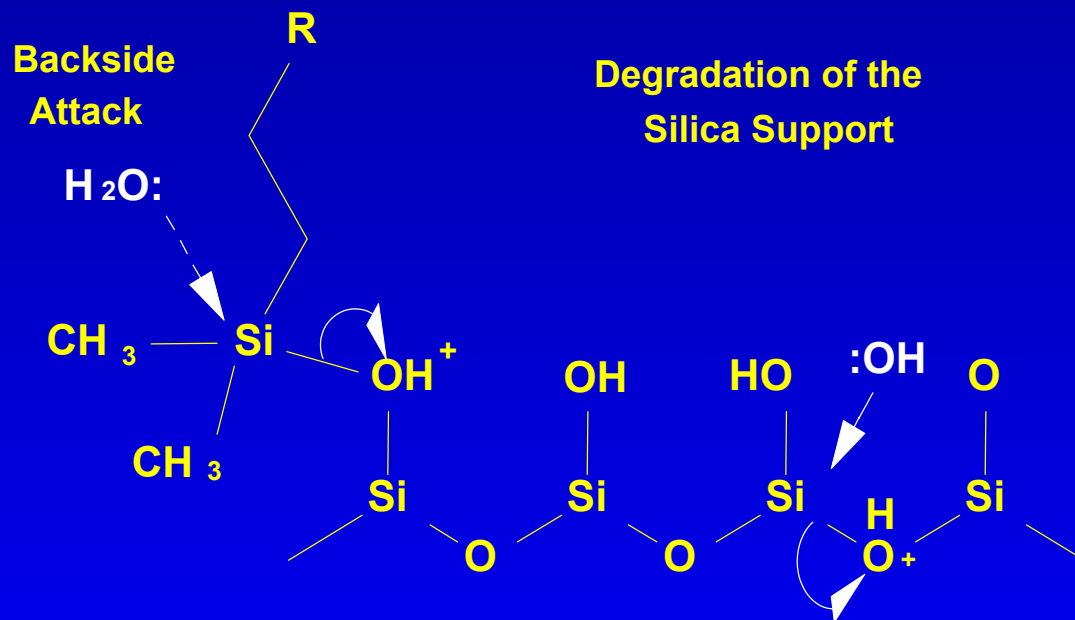
Separation of Steroids Using Water as a Mobile Phase



Zirchrom PBD 100 X 4.6 mm column, UV 254 nm

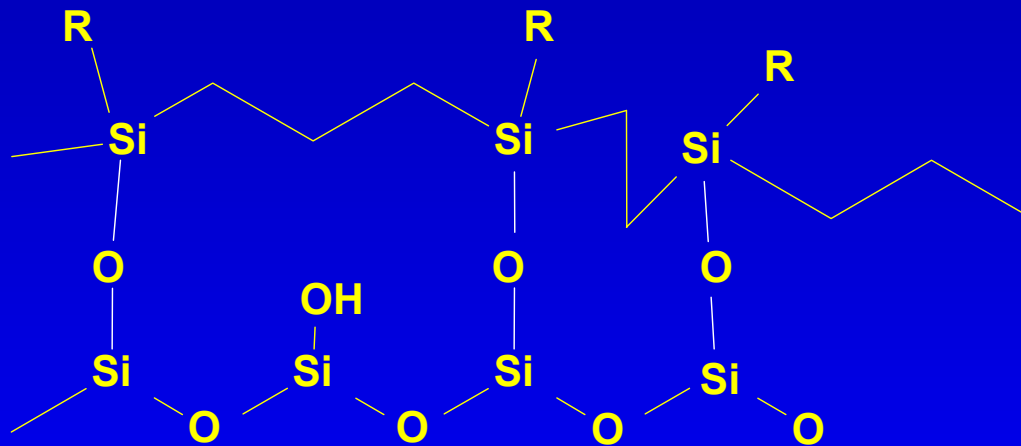
Traditional Silica Columns Can't Take the Heat

Water attacks siloxane bond or behind point of
phase attachment

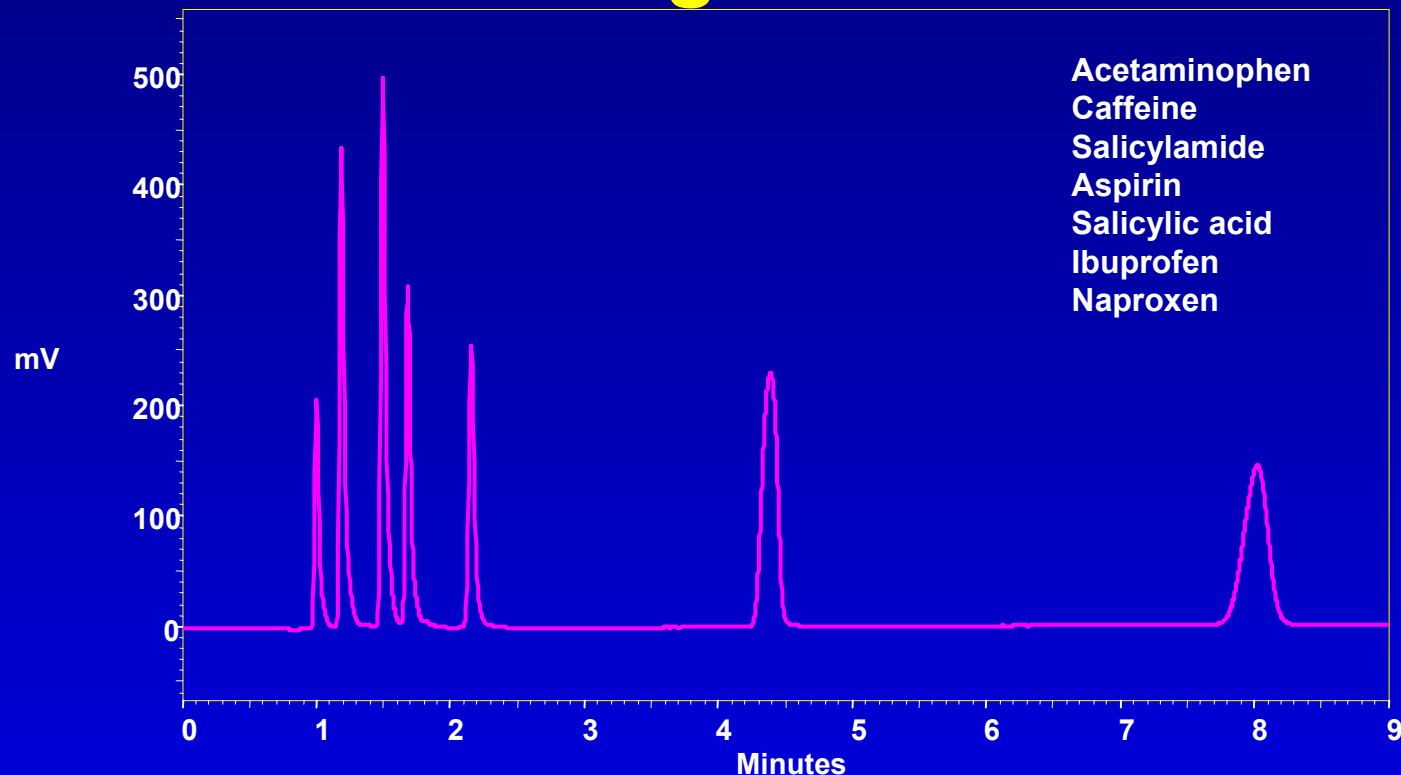


The Selerity *Blaze* Silica Column Can Take the Heat

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



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



Column: Selerity Blaze C8, 3 μ 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.



Other Columns That Can Take the Heat

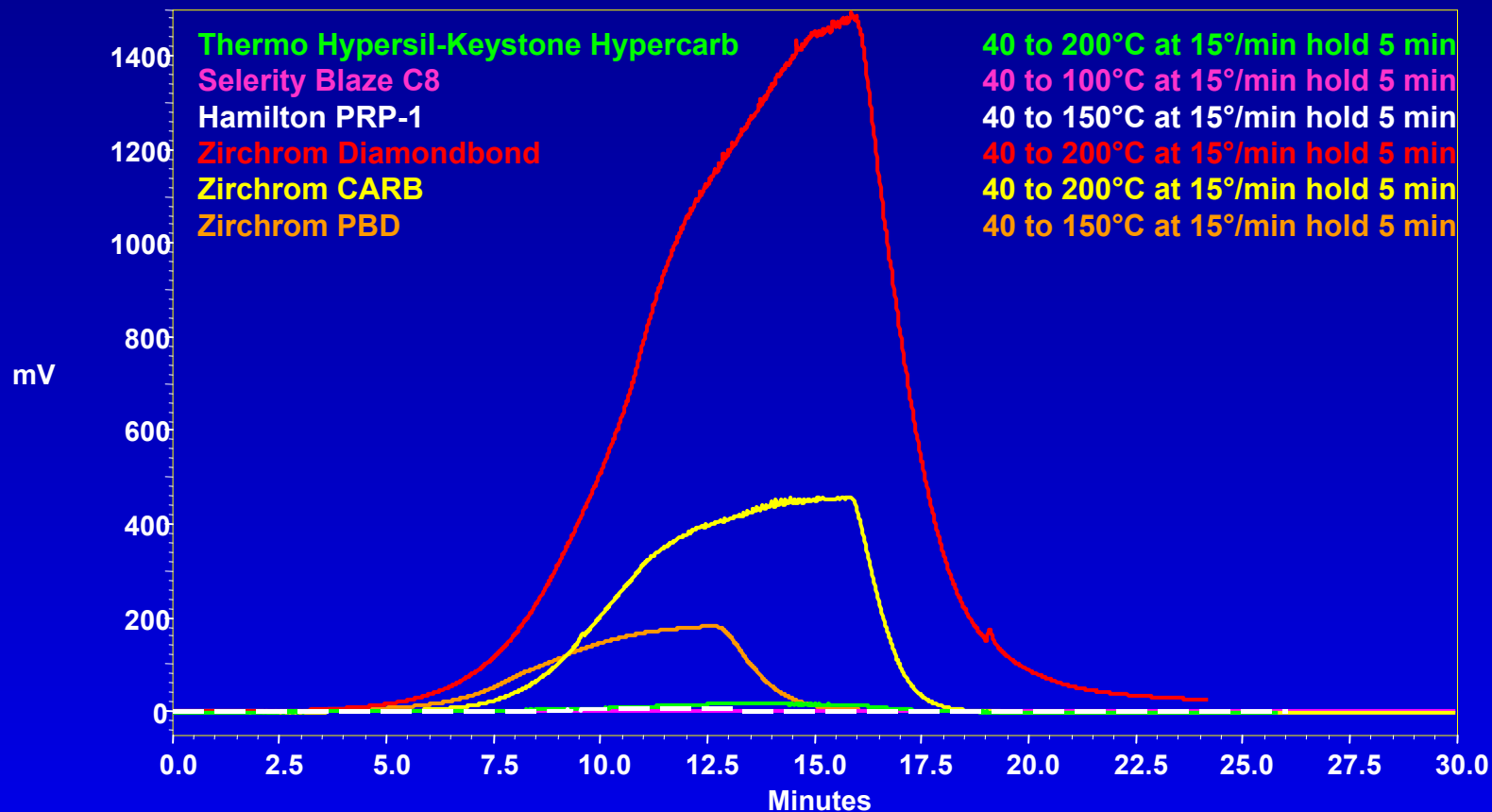
Columns stable at high temperatures

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

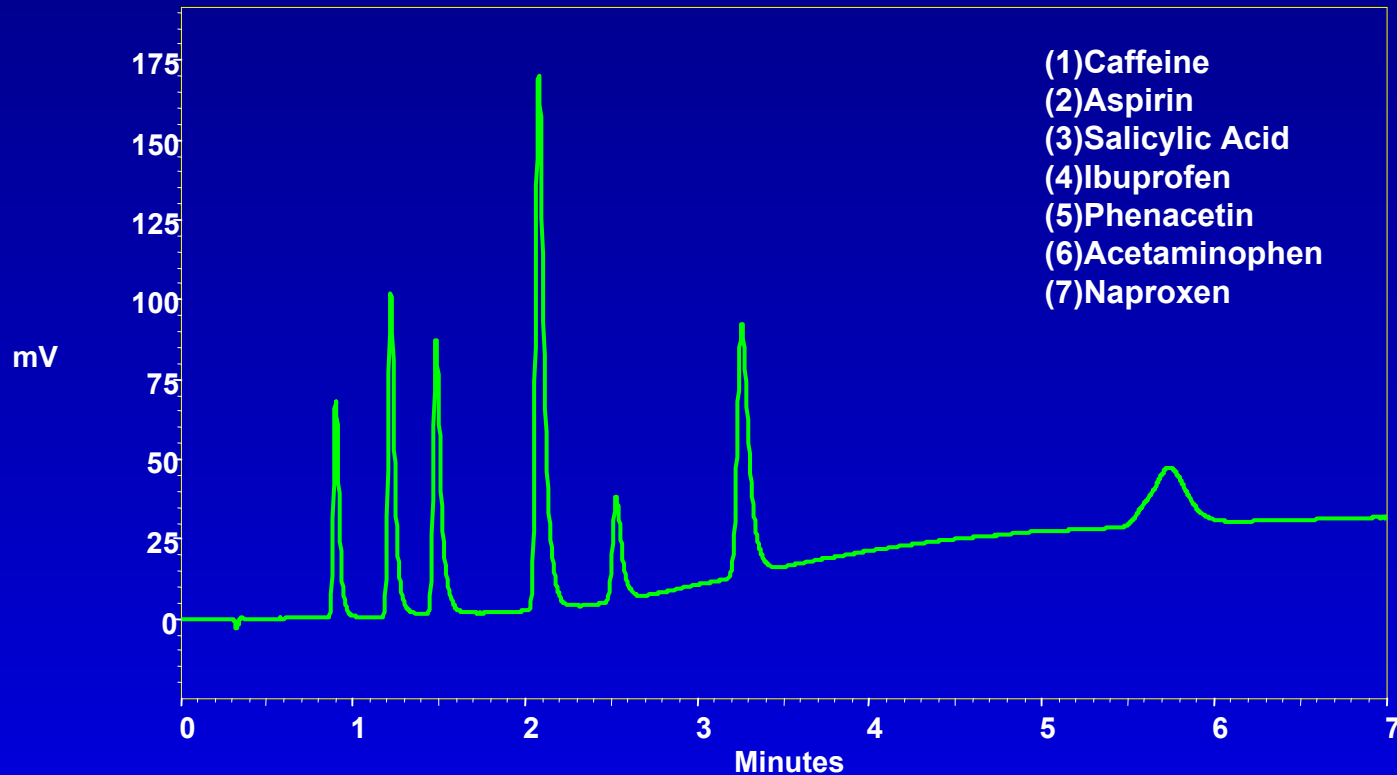


Blank Thermal Gradients

50:50 Acetonitrile:Water at 254 nm



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



Column: Thermo Hypersil-Keystone Hypercarb[®], 7 μ m, 100 x 4.6 mm

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

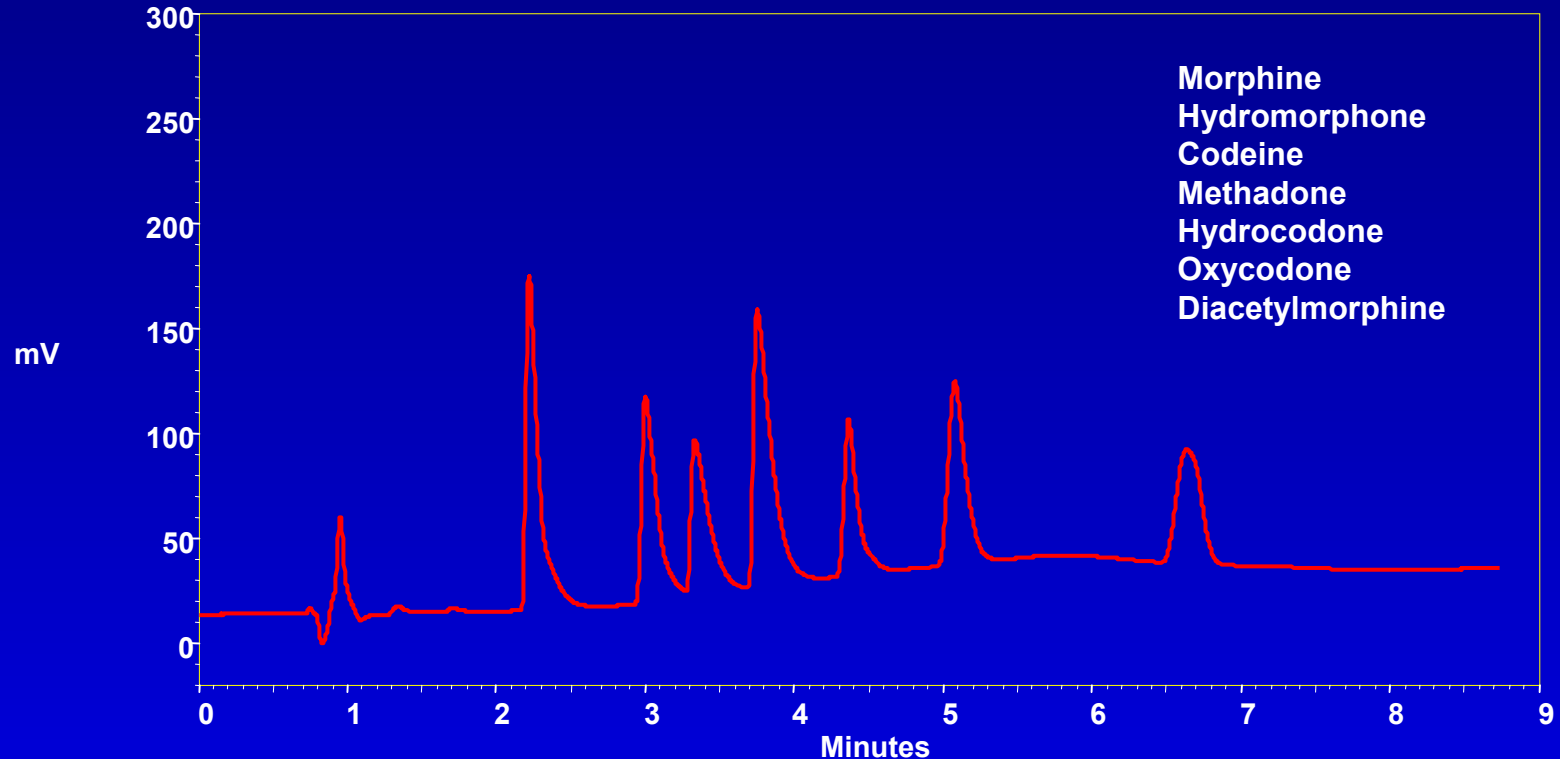
Flow Rate: 4.0 mL/min

Detection: UV 220 nm

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



Separation of Narcotics on Hypercarb® 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

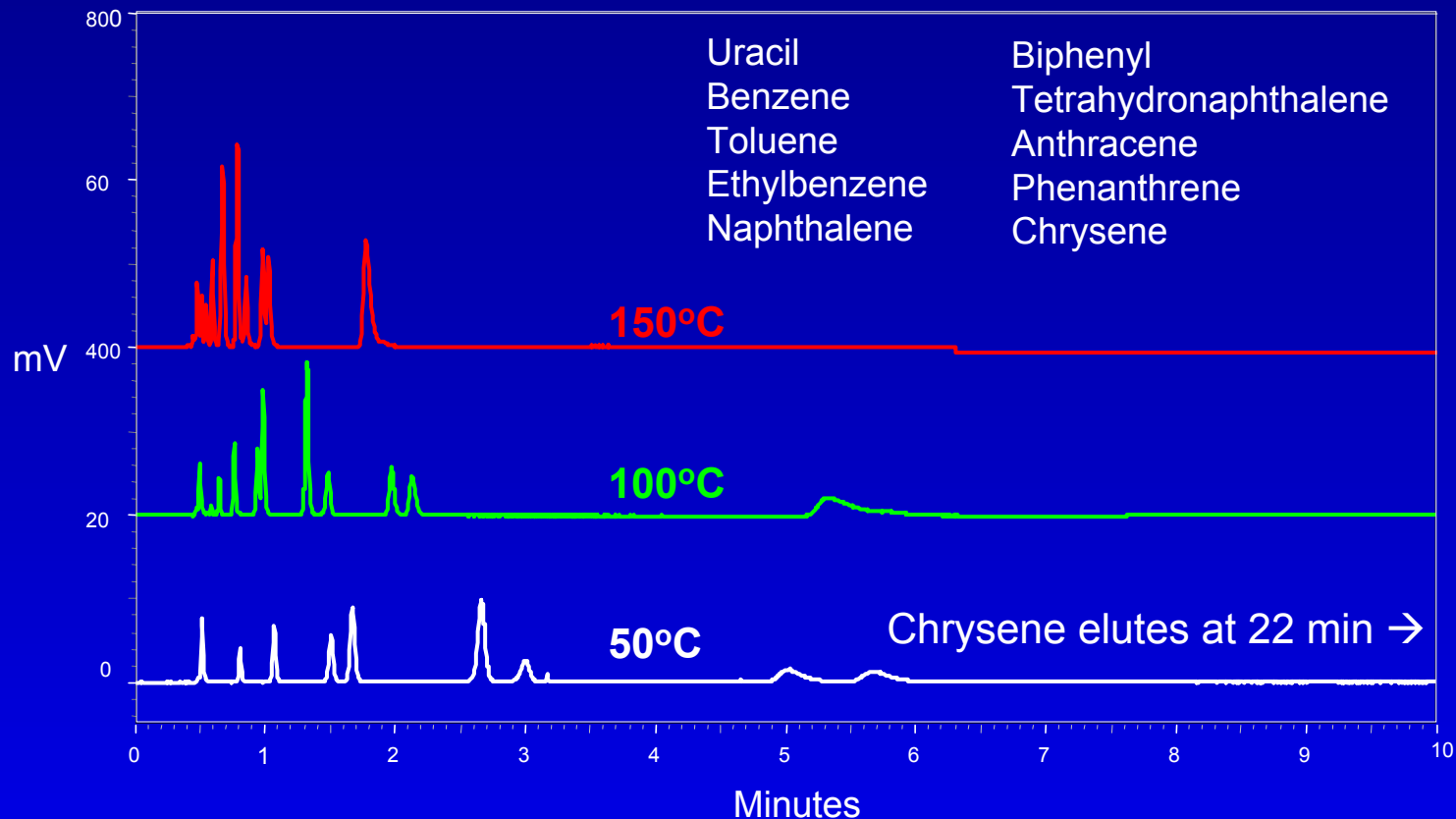
Flow Rate: 1.0 mL/min

Detection: UV 220 nm

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



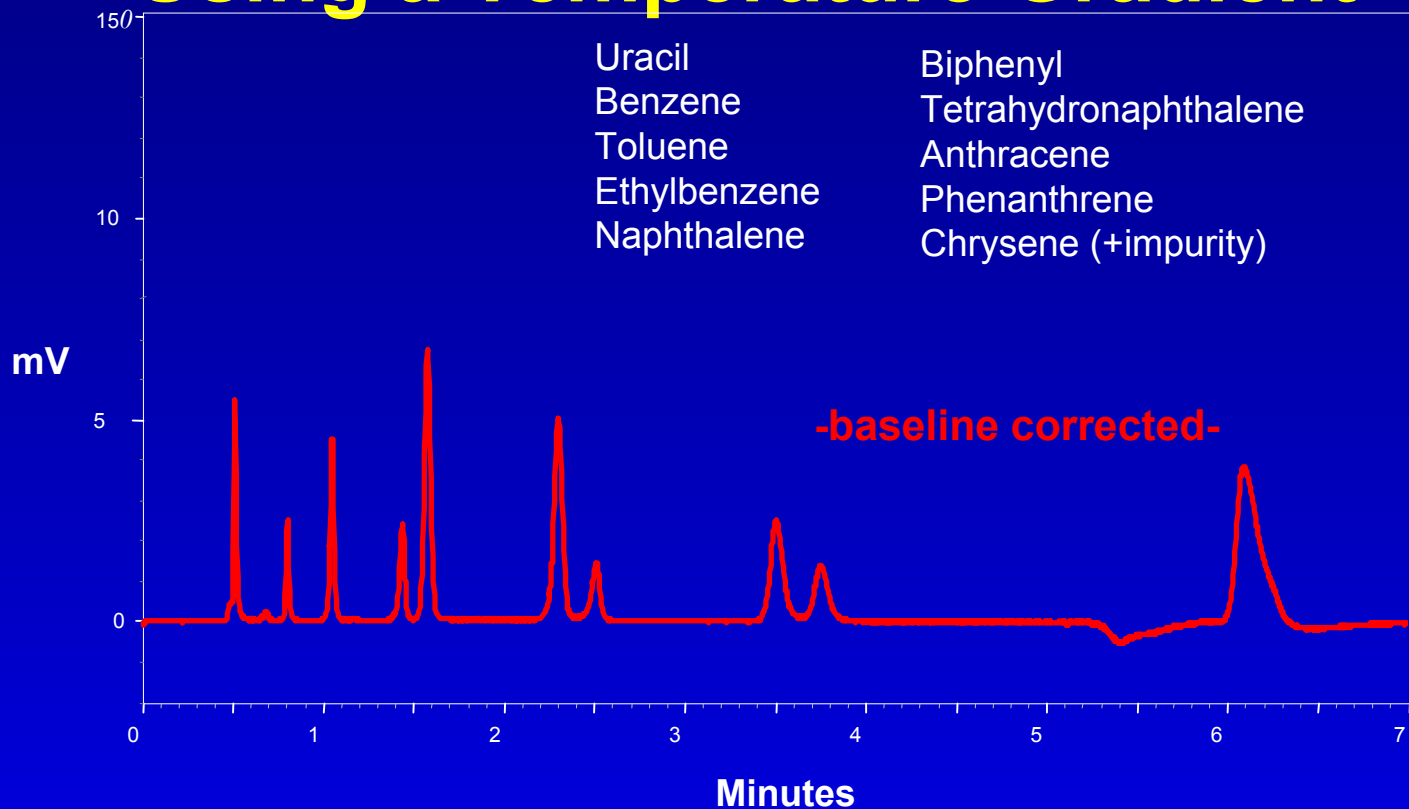
Aromatic Hydrocarbons at Three Temperatures



ZirChrom PBD 100 x 4.6 mm, UV 254 nm,
35:65 Acetonitrile:Water, 2.0 mL/min



Aromatic Hydrocarbons on PBD Column Using a Temperature Gradient



Column: Zirchrom PBD 100 x 4.6 mm

Mobile Phase: 35:65 Acetonitrile:Water

Flow Rate: 2.0 mL/min

Detection: UV 254 nm

Temperature Program: 50°C to 150°C at 20°/min



Conclusions

- Temperature programming can be used successfully with 4.6 mm ID columns
- Independent mobile phase pre-heater control allows for improvement in peak efficiency
- The benefits of elevated temperature HPLC are now available for routine use



Future Work

- Explore the new capability of precision mobile phase temperature matching in subambient HPLC
- Determine optimal preheater program profiles as a function of column dimensions and oven ramp rates



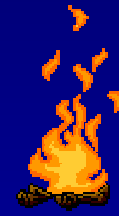
Acknowledgements

- Thermo Hypersil-Keystone
- Hamilton Company
- ZirChrom Separations





Turn up the Heat



BRING ON THE COLD

Selerity Technologies Inc.

2484 W. Custer Rd.

Salt Lake City, UT 84121

801-978-2295

hotlc@selerity.com

www.selerity.com

Patent applications have been filed relative to the new technologies presented in this work.

