

Temperature Programmed HPLC

Series 9

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*Selerity
Technologies Inc.*



Outline

- **The HTLC Advantage**
- **Obstacles to HTLC**
- **Design of the Series 9000 HTLC**
- **Mobile Phase Preheating and precooling**
- **Effluent Cooling**
- **Applications and Examples**



Programmed Temperature Advantages for HPLC

- **Replace solvent gradients with temperature gradients**
- **Dramatically increase speed**
- **Gain efficiency**
- **Completely control temperature from below 0 °C to 200 °C**



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



The High Temperature HPLC Advantage

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

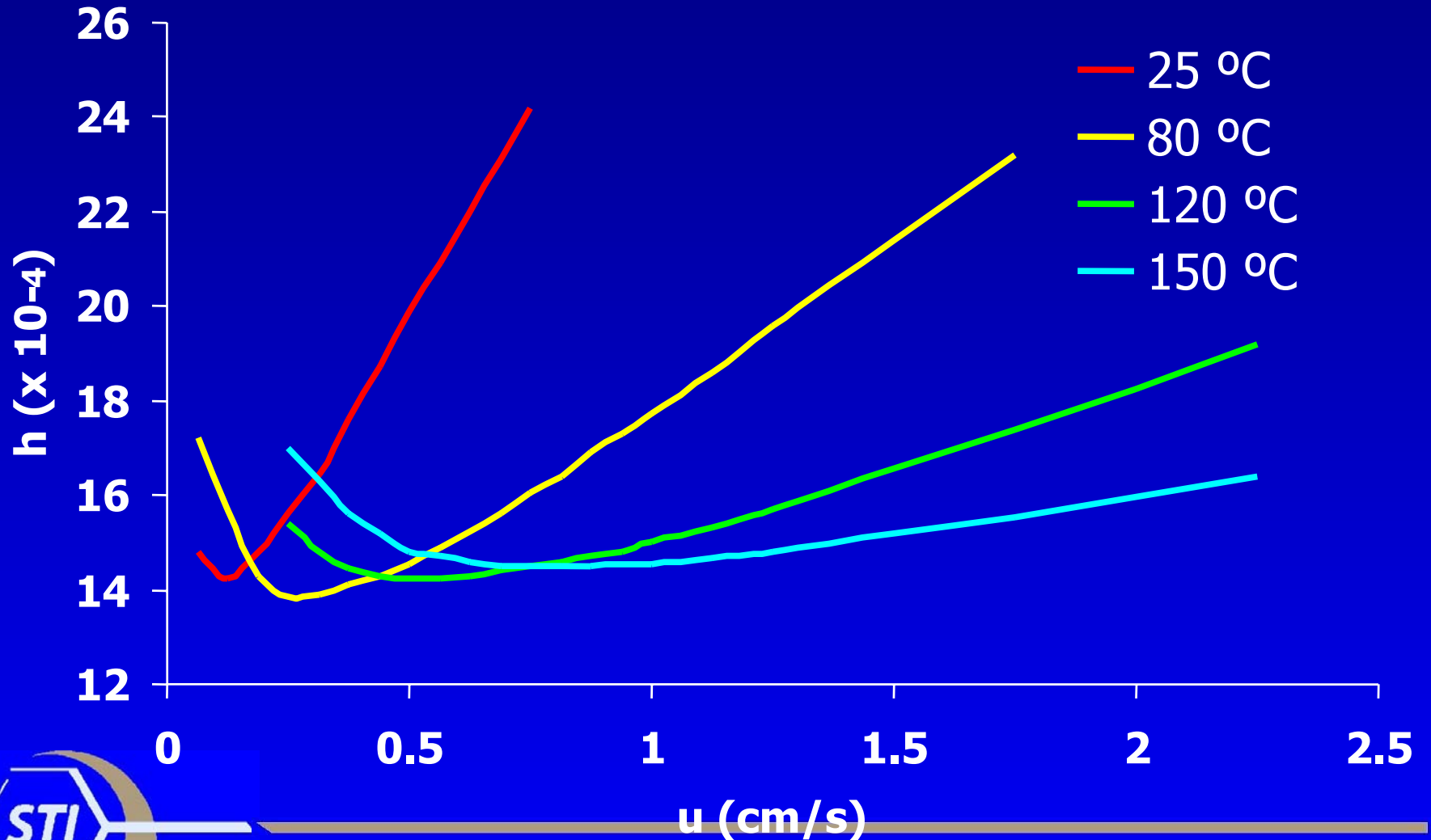


Faster and More Efficient Separations

- **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



The Series 9000 Total Temperature Controller

- Forced air oven
- Temperatures from sub-zero through 200 °C
- Solvent pre-heating and pre-cooling
- Effluent temperature stabilization to pre-set temperature
- Vapor sensor for complete safety
- Isothermal and thermal gradient operation
- Compatible with any HPLC system



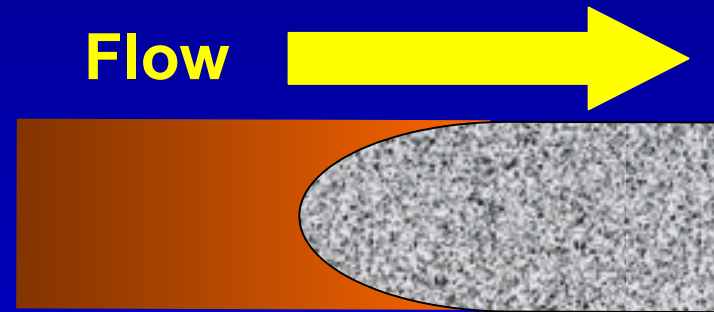
The Series 9000 Programmable Total Temperature Controller

- High capacity forced air compartment
 - Accurate temperature control at flow rates up to 10 mL/min
 - Accommodates a variety of column lengths and diameters
 - Rapid heating and cool-down
 - Temperature gradients up to 30°C per minute
- Solvent preheating and precooling independently controlled from oven



Why is Solvent Pre-heating so Important?

No Pre-heating



With Pre-heating



Solvent Pre-heater Design

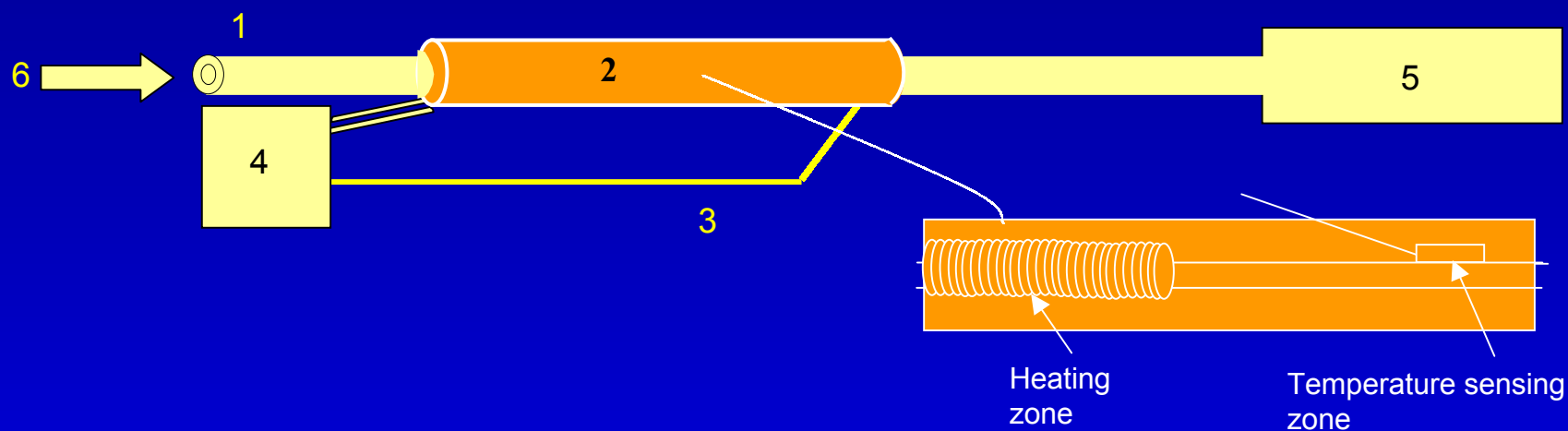


Fig. 1. Schematic of mobile phase preheater: (1) 1/16" OD stainless steel tubing, (2) heater and sensing zone, (3) thermocouple sensor, (4) temperature controller, (5) HPLC column, (6) flow direction from pump

Patent Pending, Selerity Technologies



Solvent Pre-heater

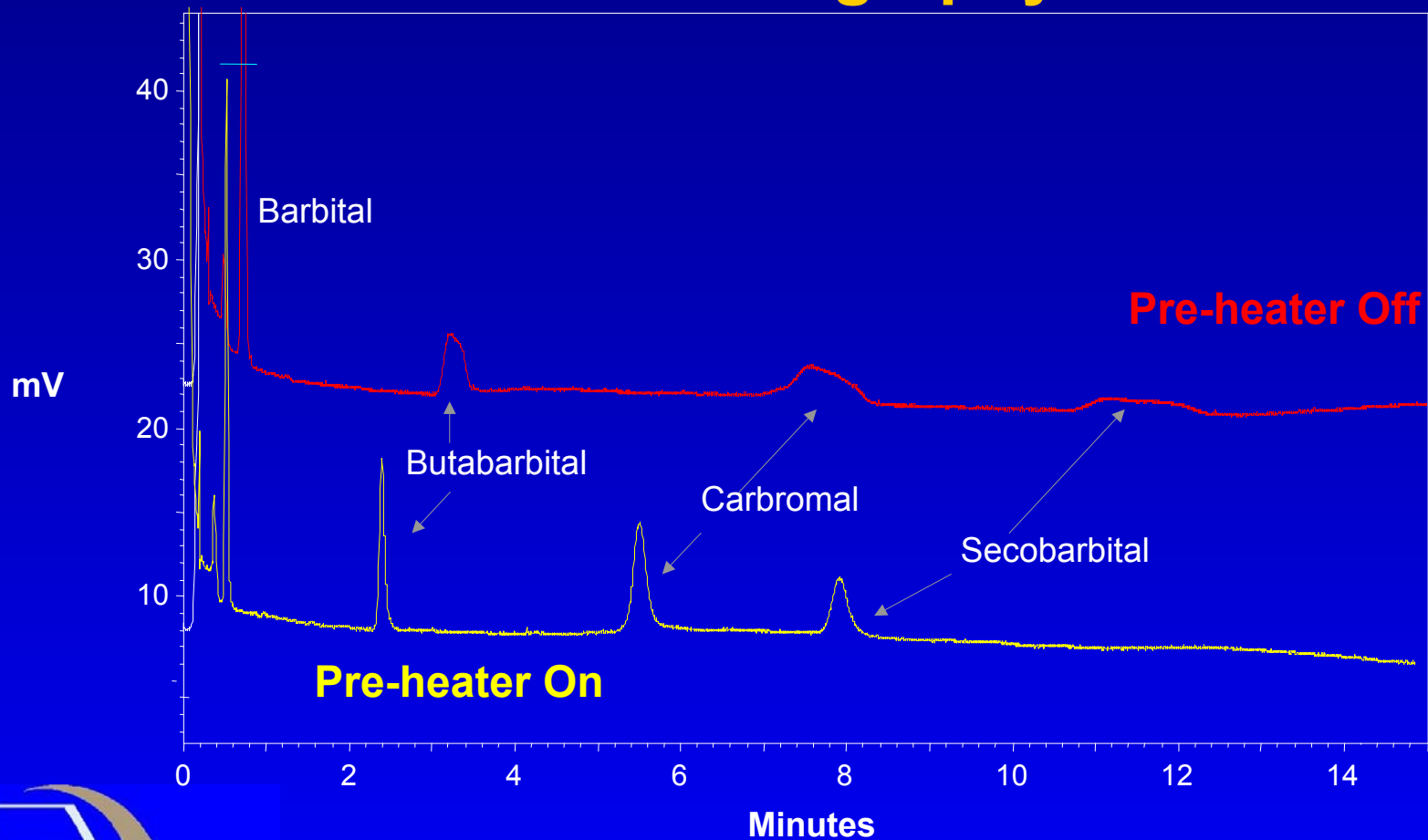
Compact and versatile

- 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



Separation of Barbiturates

Mobile Phase Preheating Improves Chromatography



Zirchrom PBD, 80°C

Solvent Pre-heater

Corrects thermal mismatch

- Makes temperature programming possible with 4.6 mm ID columns
- Heats incoming mobile phase at flow rates up to 10 mL/min
- Cools mobile phase to sub-zero temperatures at high flow rates
- ✓ Better peak shape at high temperatures
- ✓ Sub-ambient temperatures for chiral separations



Column Effluent Cooling

Stable baselines with most HPLC detectors

- **Thermoelectric cooling of effluent to pre-set temperature**
- **Eliminates baseline drift from refractive index differences**
- **Prevents damage to detector cells**
- ✓ **Compatible with a variety of detectors**



Safety Considerations

Vapor sensor and thermal runaway prevention

- Initiates oven shut-down on flammable vapor detection
 - Initiates oven shut-down if large disparity between pre-heater and oven cavity exists
- ✓ Work at high temperatures safely!



Columns That Can Take the Heat

- **Traditional silica columns break down at high temperatures**
- **Polycarbosilane columns withstand significantly higher temperatures.**
- **Selerity's polycarbosilane stationary phases resist hydrolytic breakdown.**



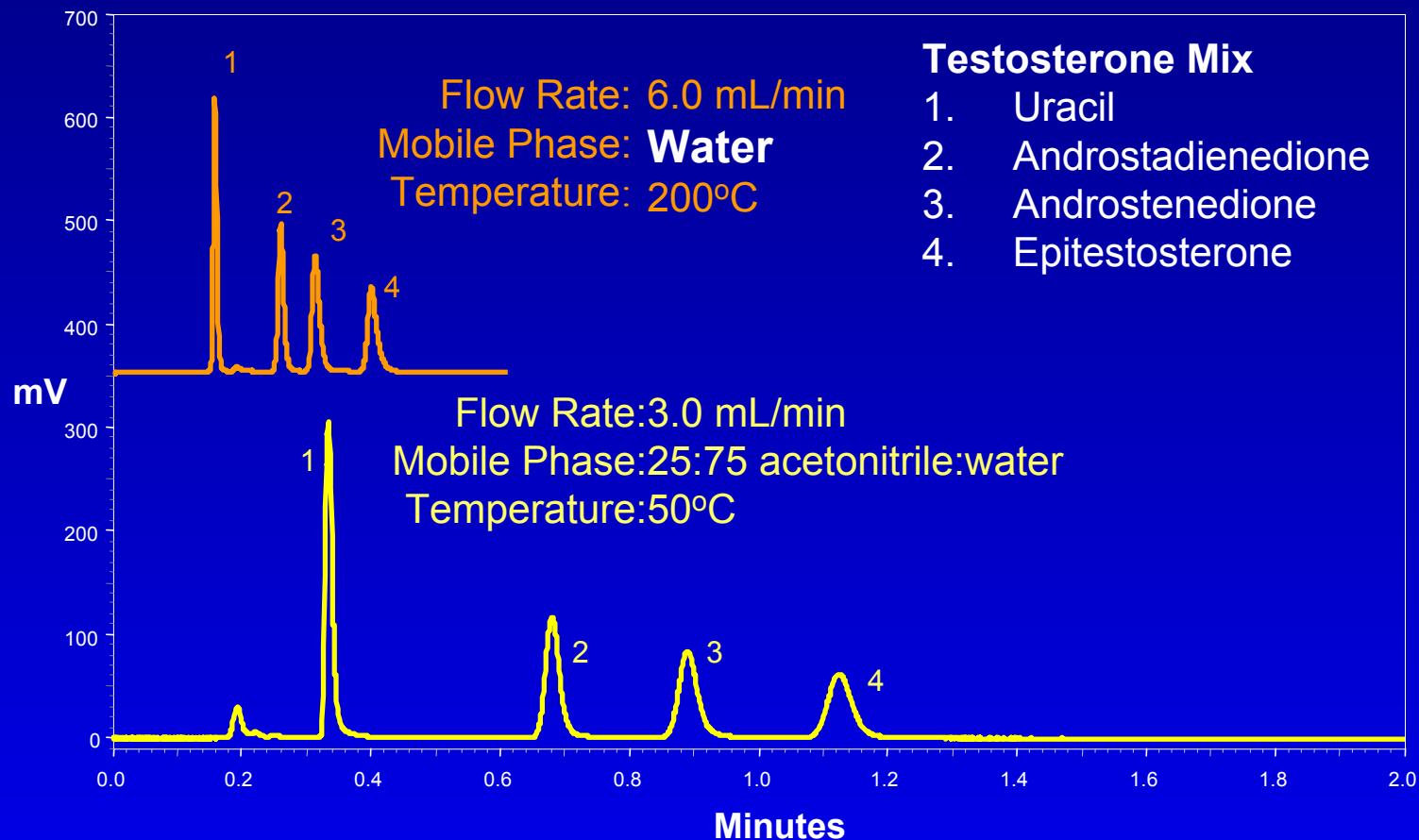
Columns are now available that can take the heat

Columns stable at high temperatures

- Hamilton PRP-1[®] and PRP-3[®] columns
- Selerity **Blaze** silica columns
- Thermo Hypersil-Keystone Hypercarb
- ZirChrom[™] stationary phases
 - C18, PBD and Carb

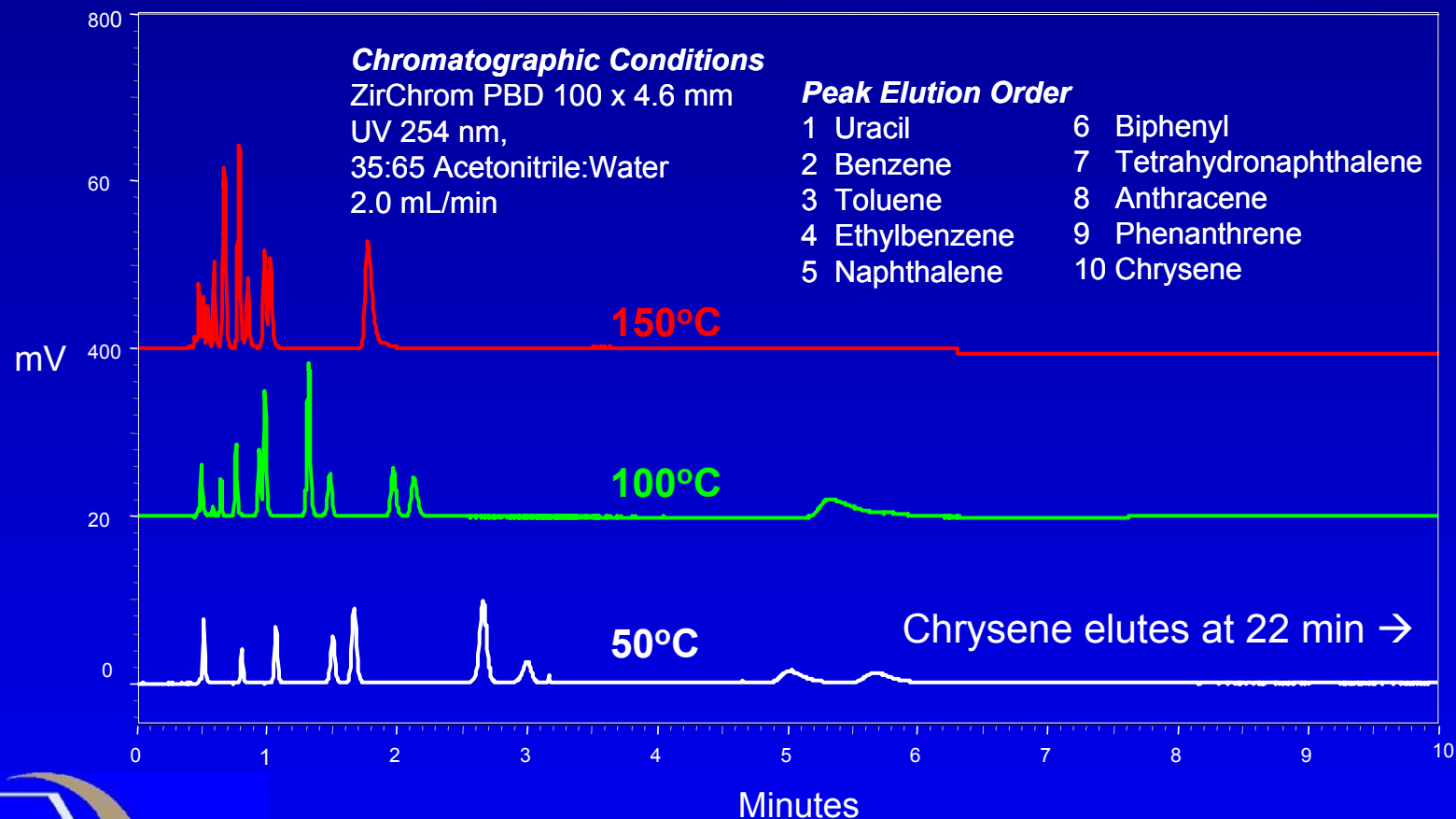


Water as a Mobile Phase

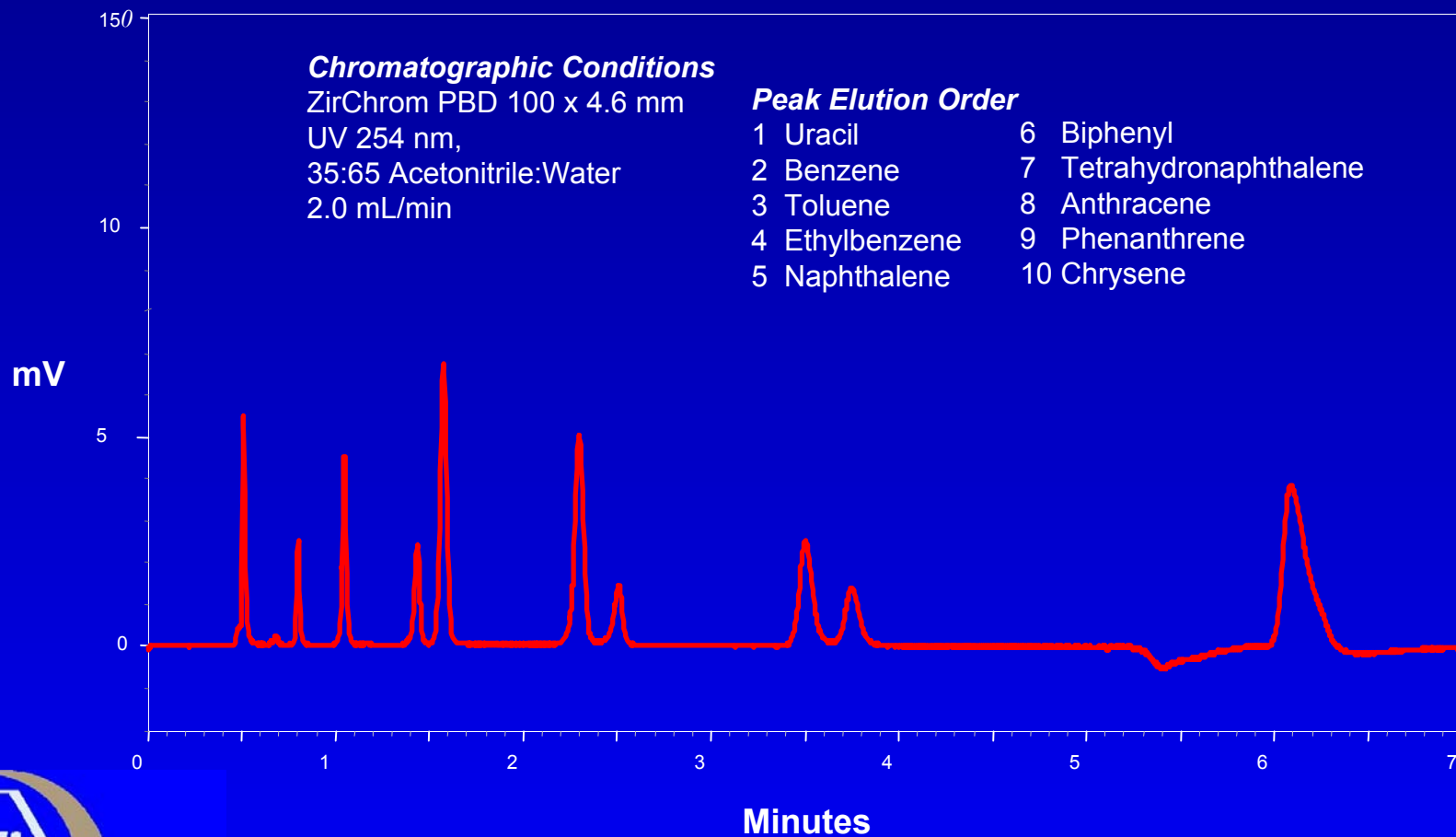


Zirchrom PBD 100 X 4.6 mm column, UV 254 nm

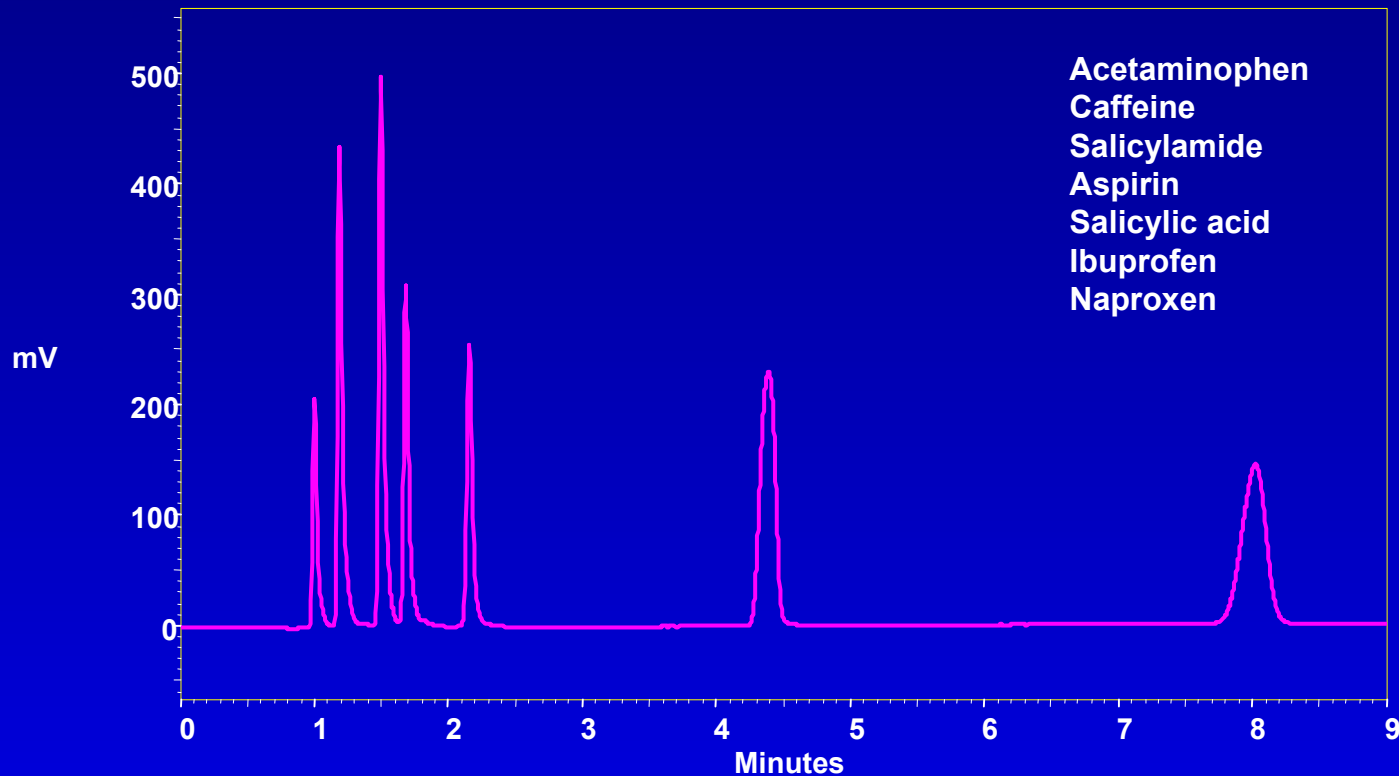
Aromatic Hydrocarbons at Three Temperatures



Aromatic Hydrocarbons Using a Temperature Gradient



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.



Cocoa Extract

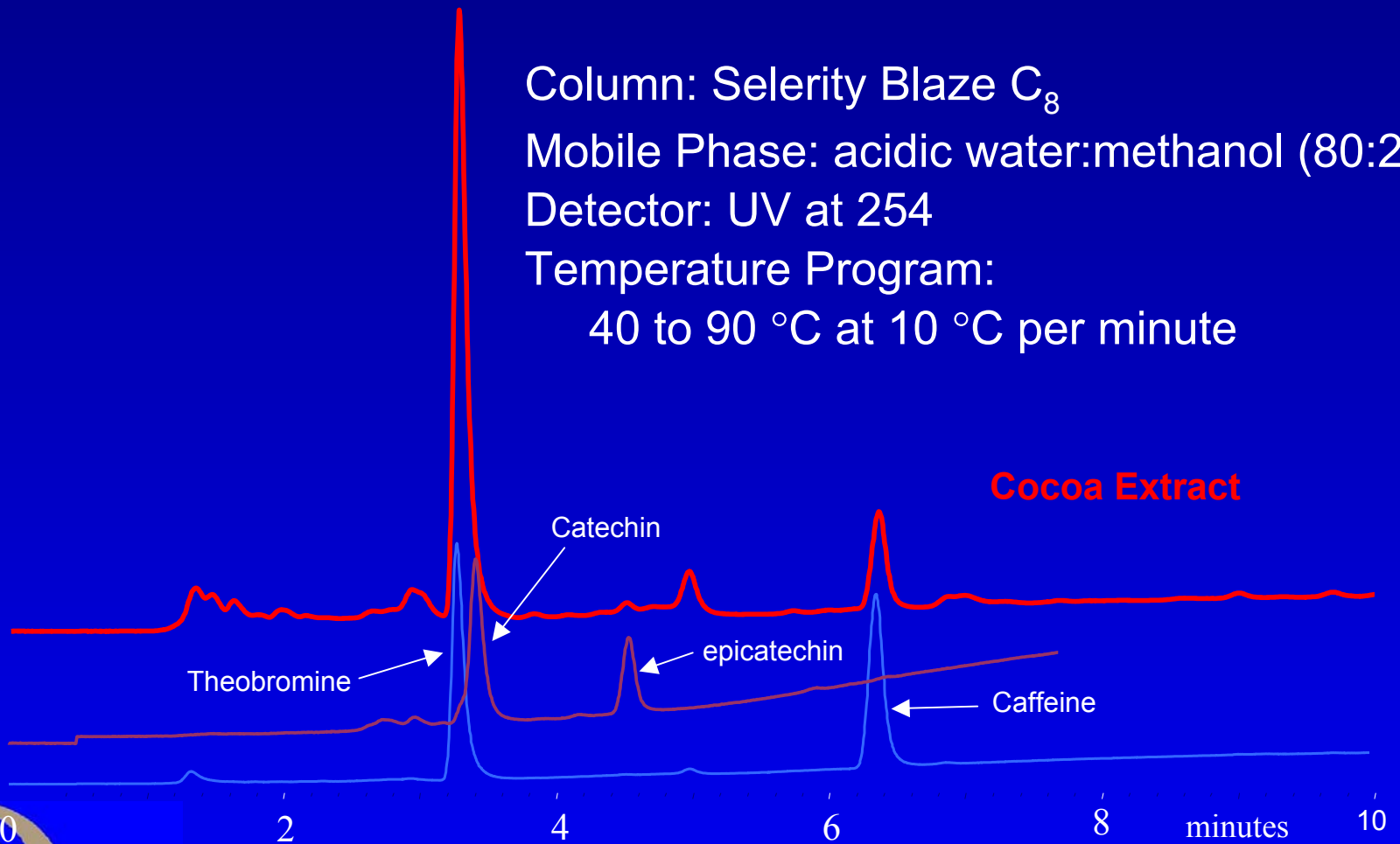
Column: Selerity Blaze C₈

Mobile Phase: acidic water:methanol (80:20)

Detector: UV at 254

Temperature Program:

40 to 90 °C at 10 °C per minute



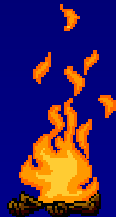
Temperature Offers a New Dimension in HPLC

- Faster, more efficient separations
- Mobile phase preheating and precooling
- Water as a mobile phase
- Temperature gradient programming
- Sub-zero to 200 °C
- Effluent cooling

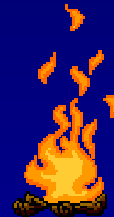


Selerity Technologies has applied for patent protection on several of the new technologies presented here.





Turn up the Heat!



Enhancing HPLC with the Series 9000 Total Temperature Controller

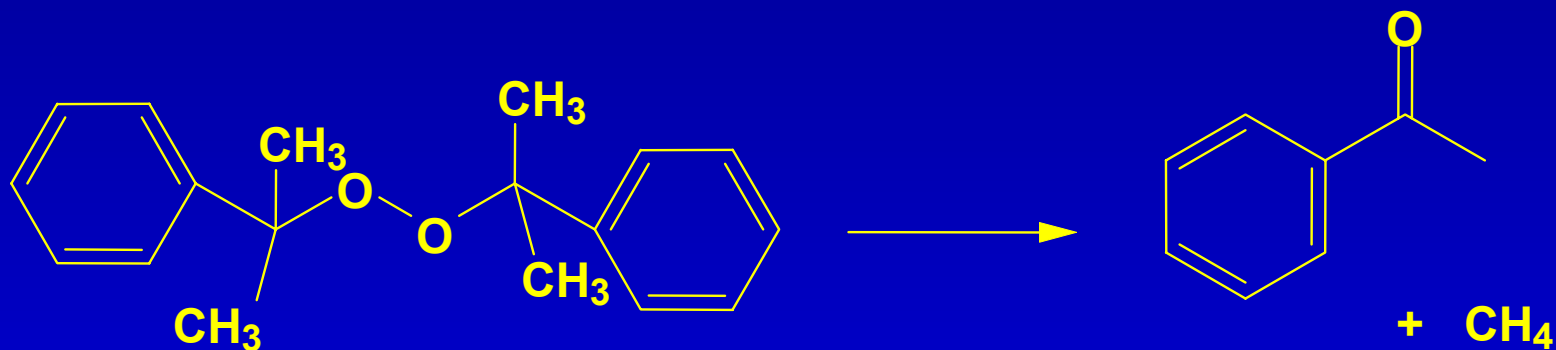
- Faster, more efficient separations with minimal method development time
- Replace solvent gradients with temperature gradients
- Use less organic modifier
- Affordable and easy to use
- Sub-zero to elevated temperature control



Bring on the Cold!



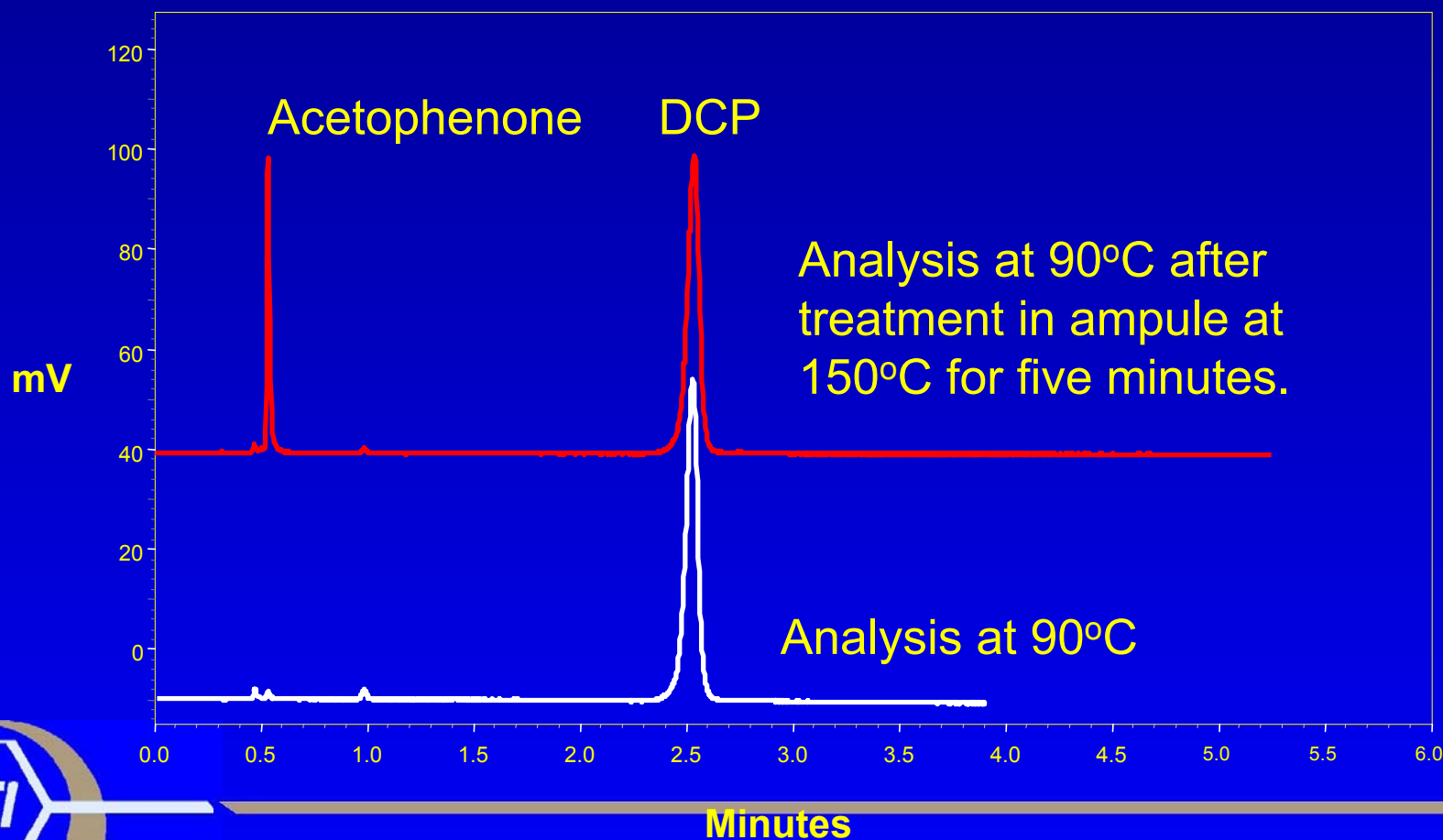
Thermal Decomposition of Analytes



Dicumyl Peroxide



Decomposition of Dicumyl Peroxide



Analysis Conditions for Dicumyl Peroxide

Column: Zirchrom PBD 100 x 4.6 mm

Mobile Phase: 40:60 acetonitrile:water,
isocratic

Detection: UV@ 254 nm

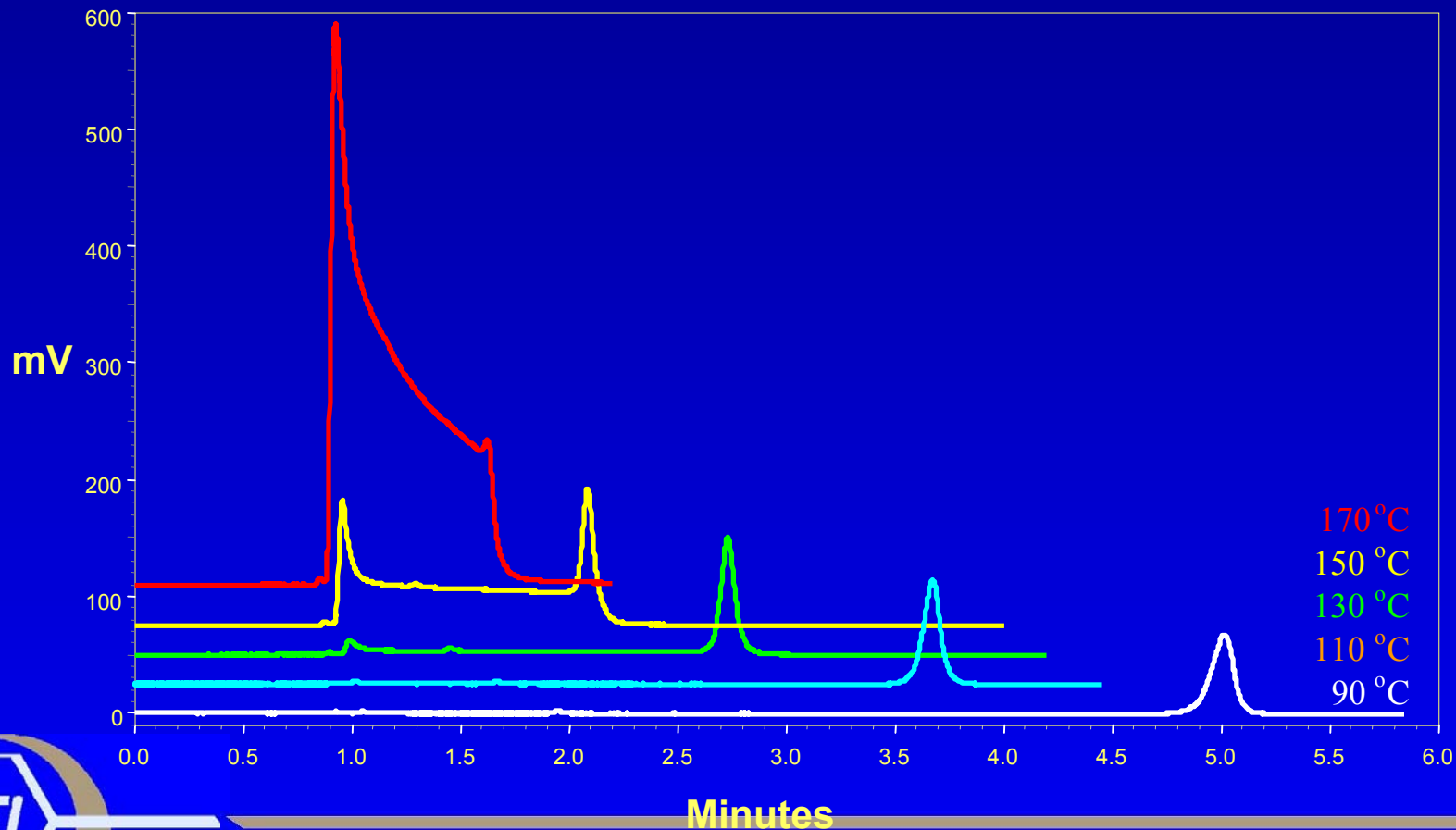
Injection: full loop (2.5 uL)

Flow Rates: 1.0, 2.0, 4.0 mL/min

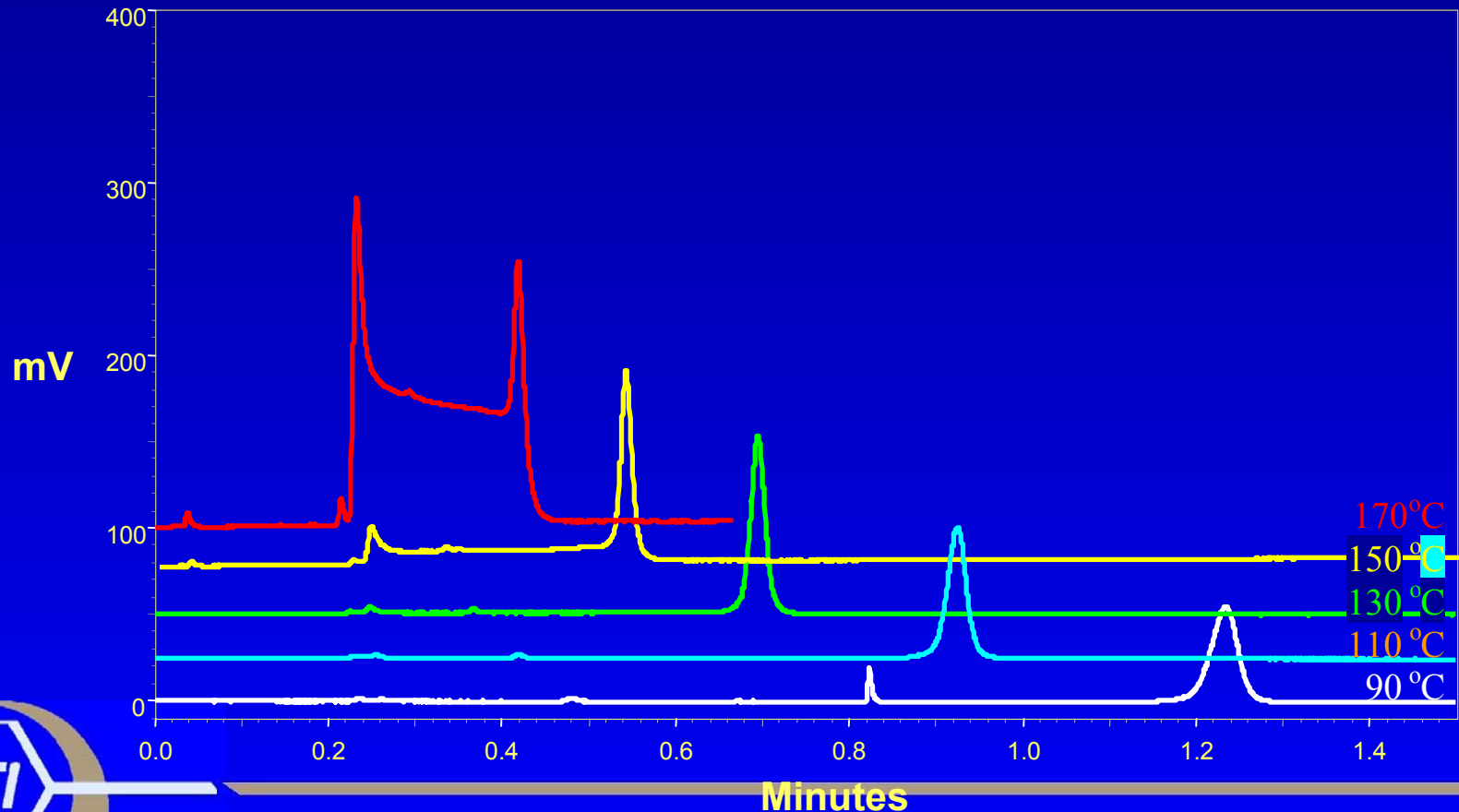
Temperatures: 90 °C, 110 °C, 130 °C, 150 °C,
170 °C, isothermal



Dicumyl Peroxide at Five Temperatures at 1.0 mL/min



Dicumyl Peroxide at Five Temperatures at 4.0 mL/min



Normalized Percent of Dicumyl Peroxide Remaining

Temp.	Flow Rate		
	1.0 mL/min	2.0 mL/min	4.0 mL/min
90 °C	100%	100%	100%
110 °C	100%	99%	98%
130 °C	88%	87%	98%
150 °C	71%	77%	77%
170 °C	6%	23%	45%

