

~~Augmenting~~ *Replacing* Solvent Gradient HPLC 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

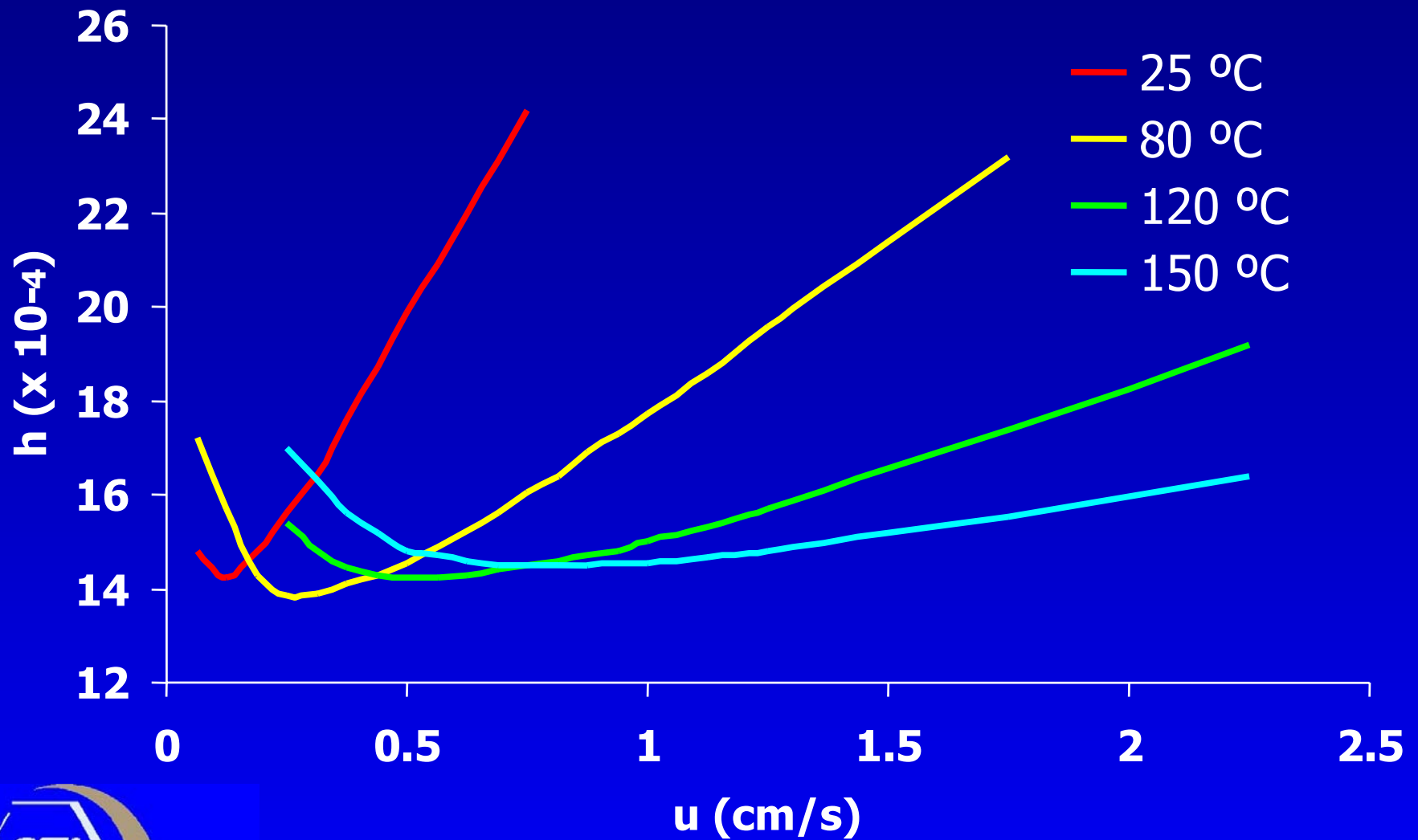


Faster and More Efficient Separations

- **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



Columns That Can Take the Heat

Columns stable at high temperatures

- Selerity **Blaze** C8 polydentate silica column – 100°C
- Hamilton PRP-1[®] columns – 150°C
- Thermo Hypersil-Keystone Hypercarb[®] - 200°C
- ZirChrom[™] stationary phases – 150 to 200°C
 - bleed is a problem during temperature programming with Zirchrom columns



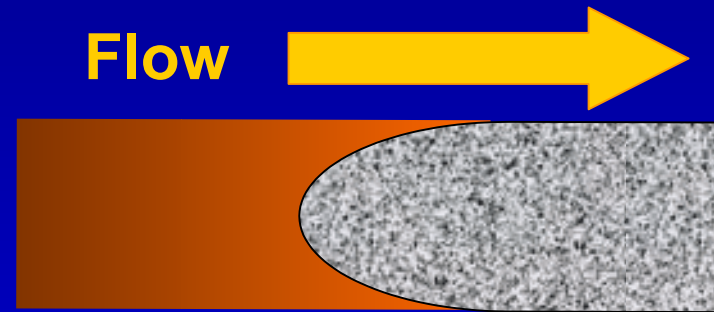
The Selerity Series 9000 Total Temperature Controller

- 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 40°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?

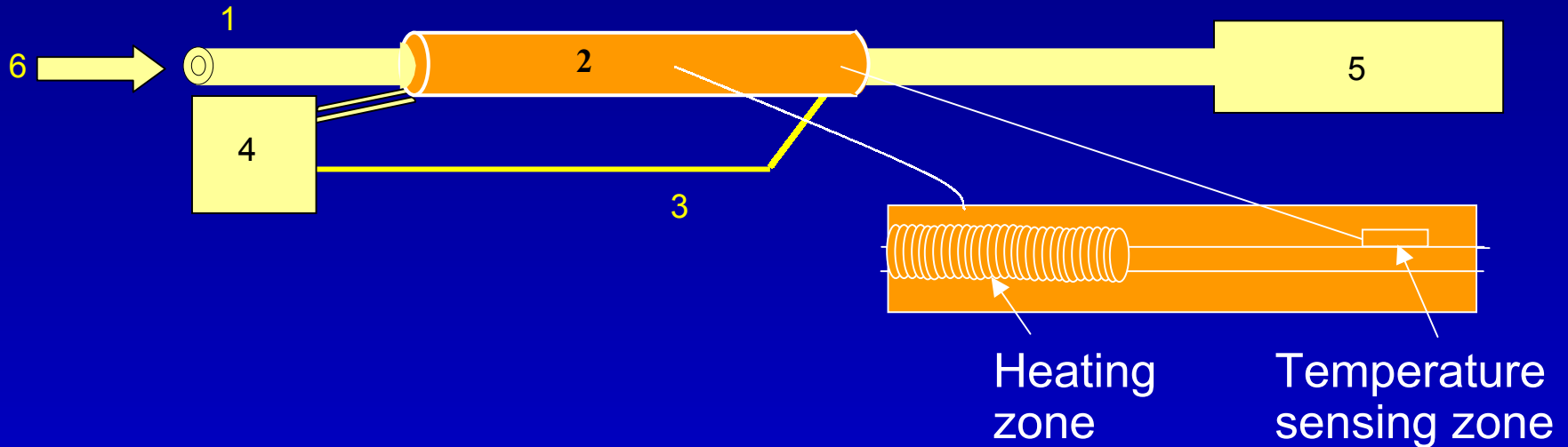
No Pre-heating



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.



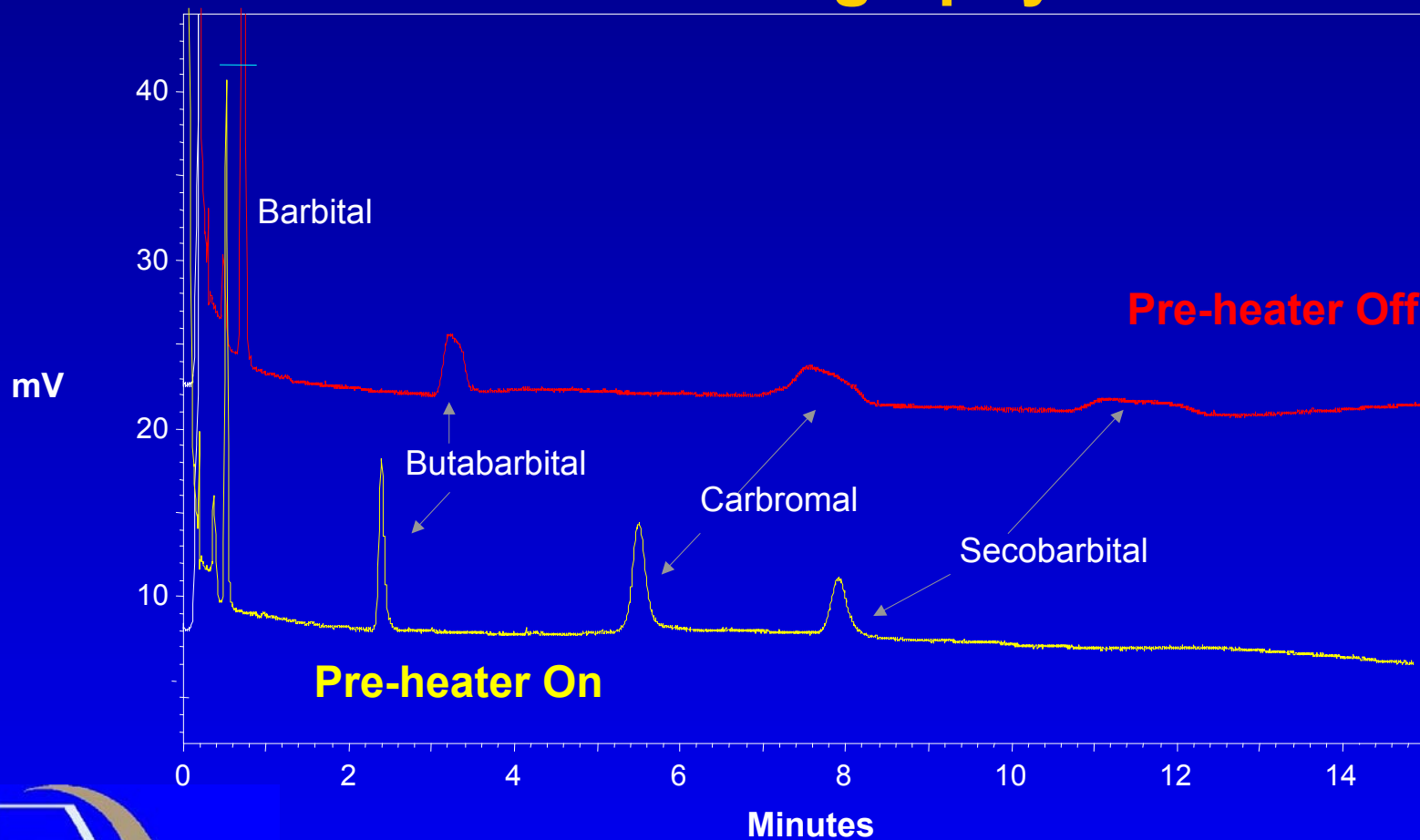
Solvent Pre-heater

- **Very fast response and non-invasive**
- **Low-mass and low-volume**
- **0.005", 0.007" and 0.010" ID available**
- **Adds no dead volume**



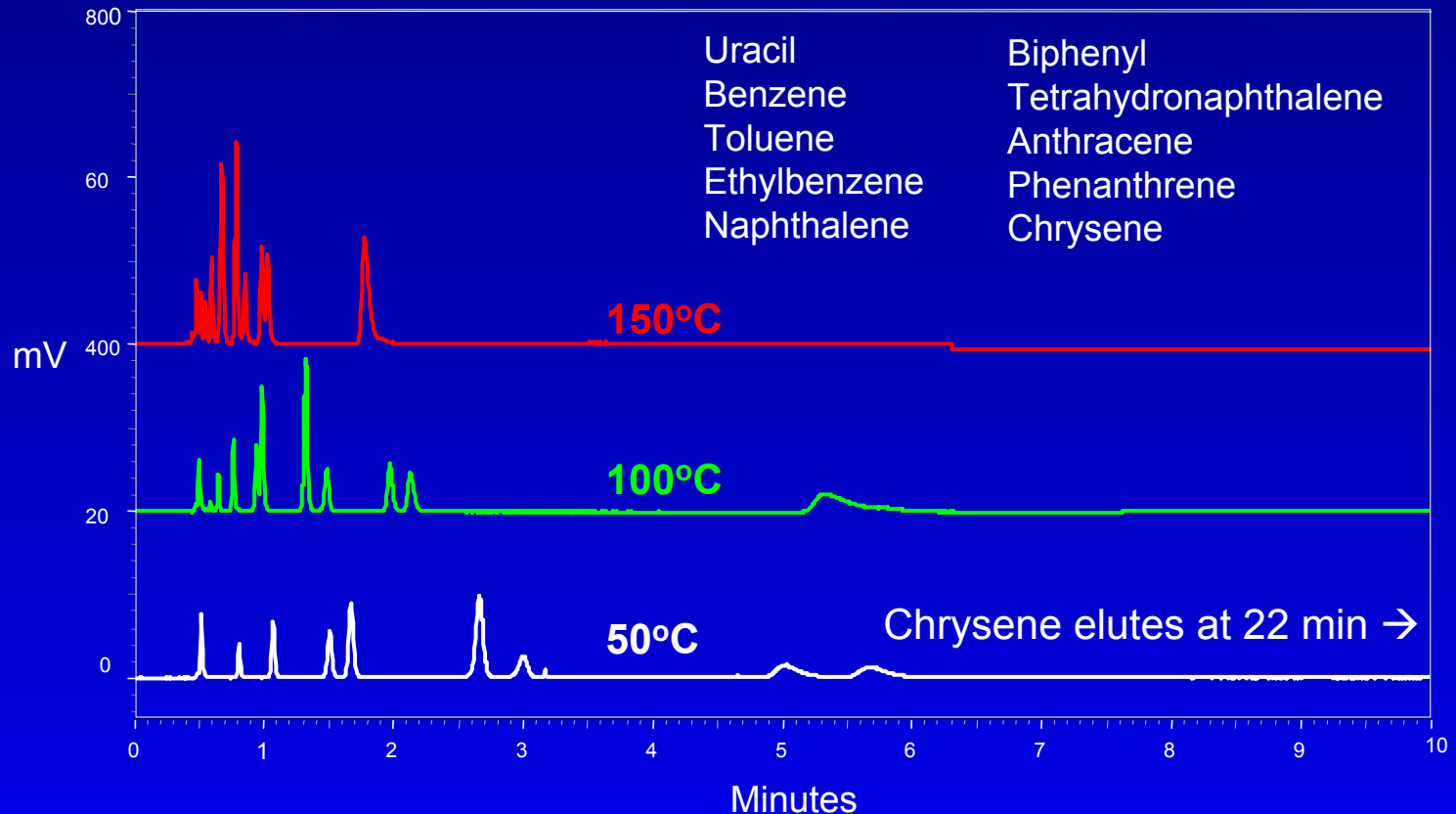
Separation of Barbiturates

Mobile Phase Preheating Improves Chromatography



Zirchrom PBD, 80°C

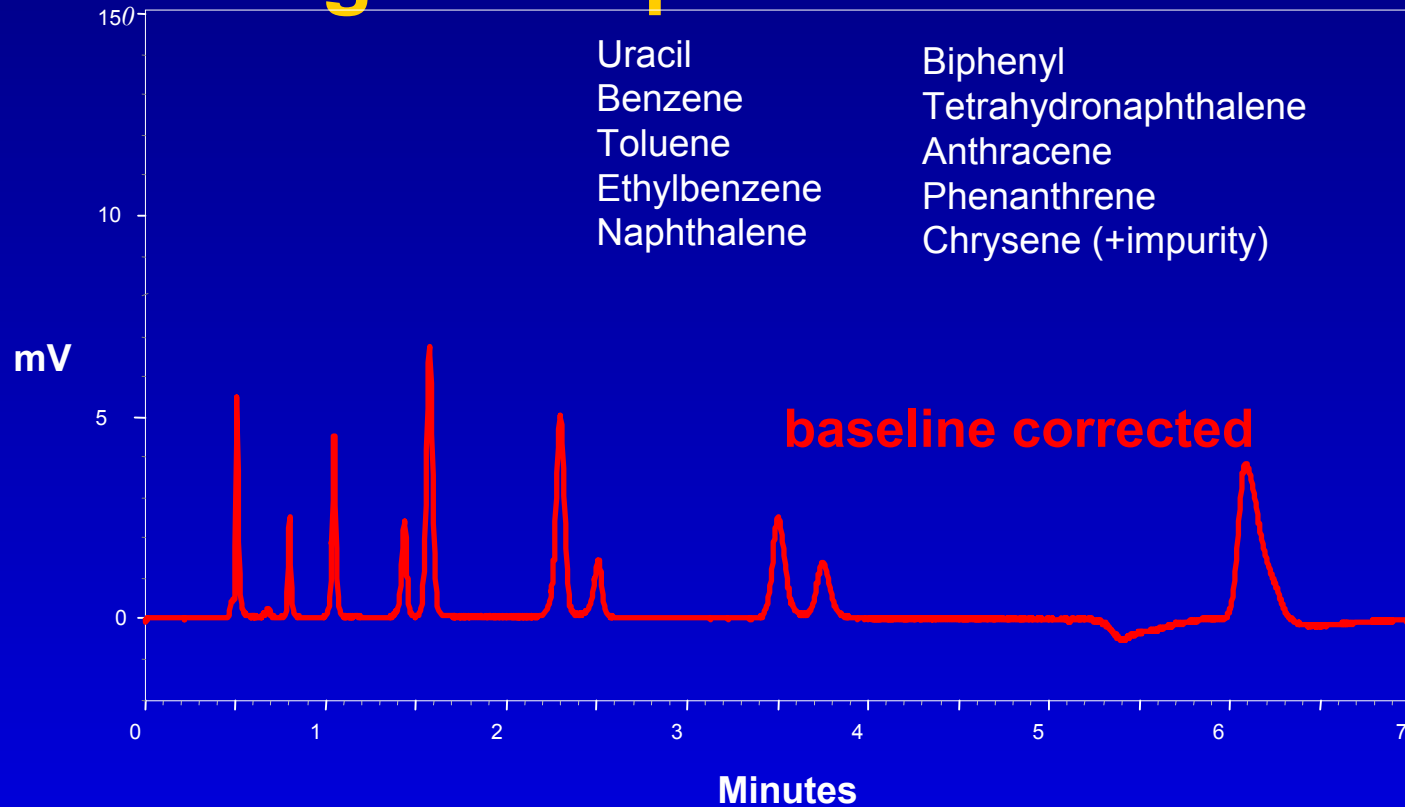
Aromatic Hydrocarbons at Three Temperatures



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

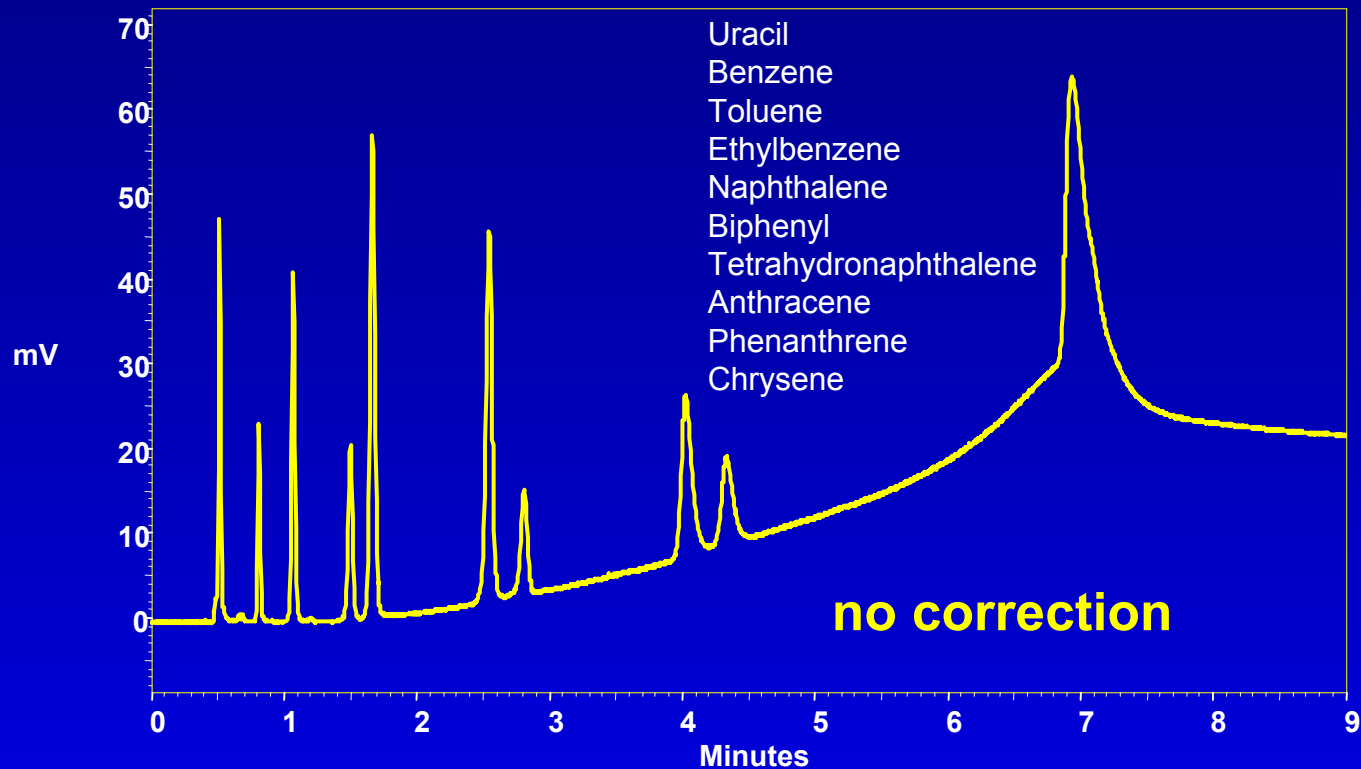
Flow Rate: 2.0 mL/min

Detection: UV 254 nm

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



Aromatic Hydrocarbons on PBD Column Using a Temperature Gradient



Column: Zirchrom PBD 100 x 4.6 mm

Mobile Phase: 35:65 Acetonitrile:Water

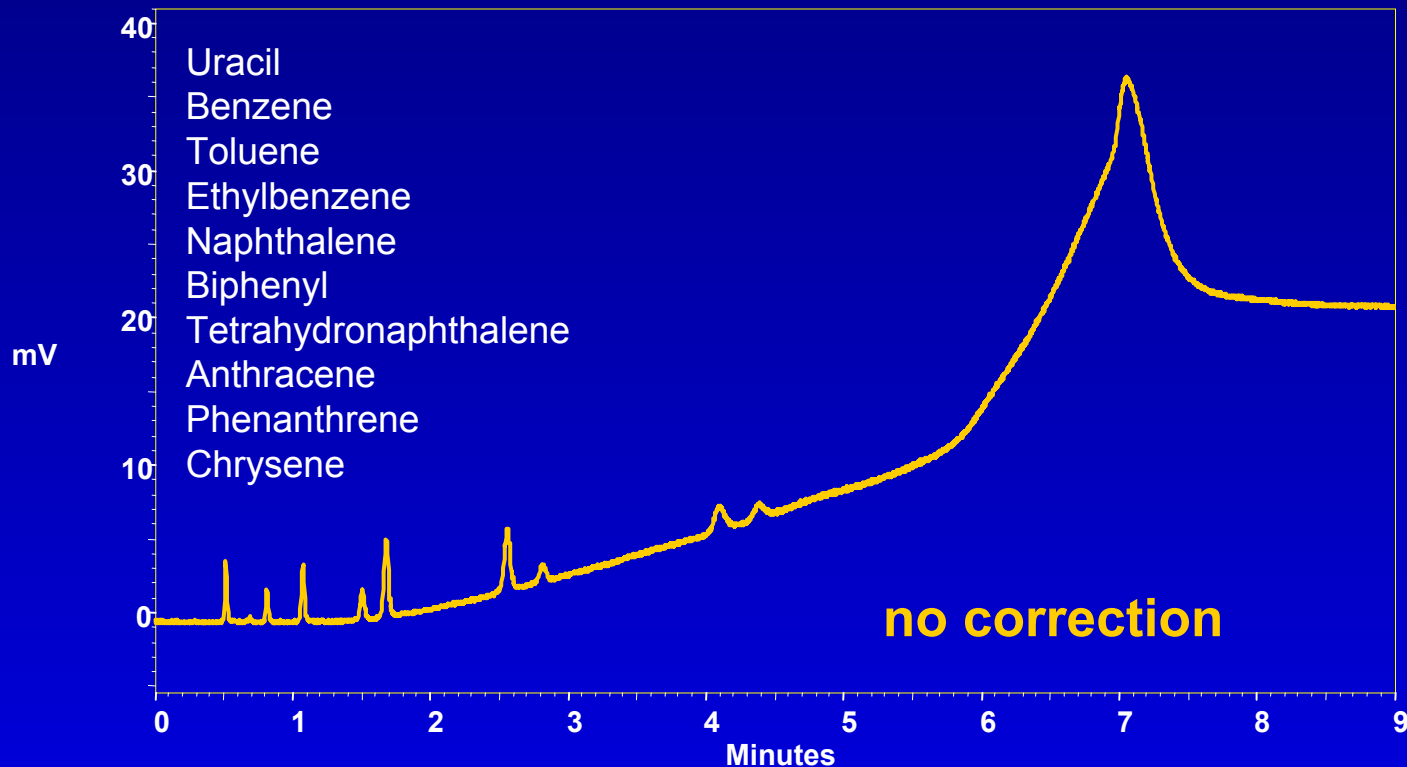
Flow Rate: 2.0 mL/min

Detection: UV 254 nm

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



Low-level Aromatic Hydrocarbons on PBD Column Using a Temperature Gradient



Column: Zirchrom PBD 100 x 4.6 mm

Mobile Phase: 35:65 Acetonitrile:Water

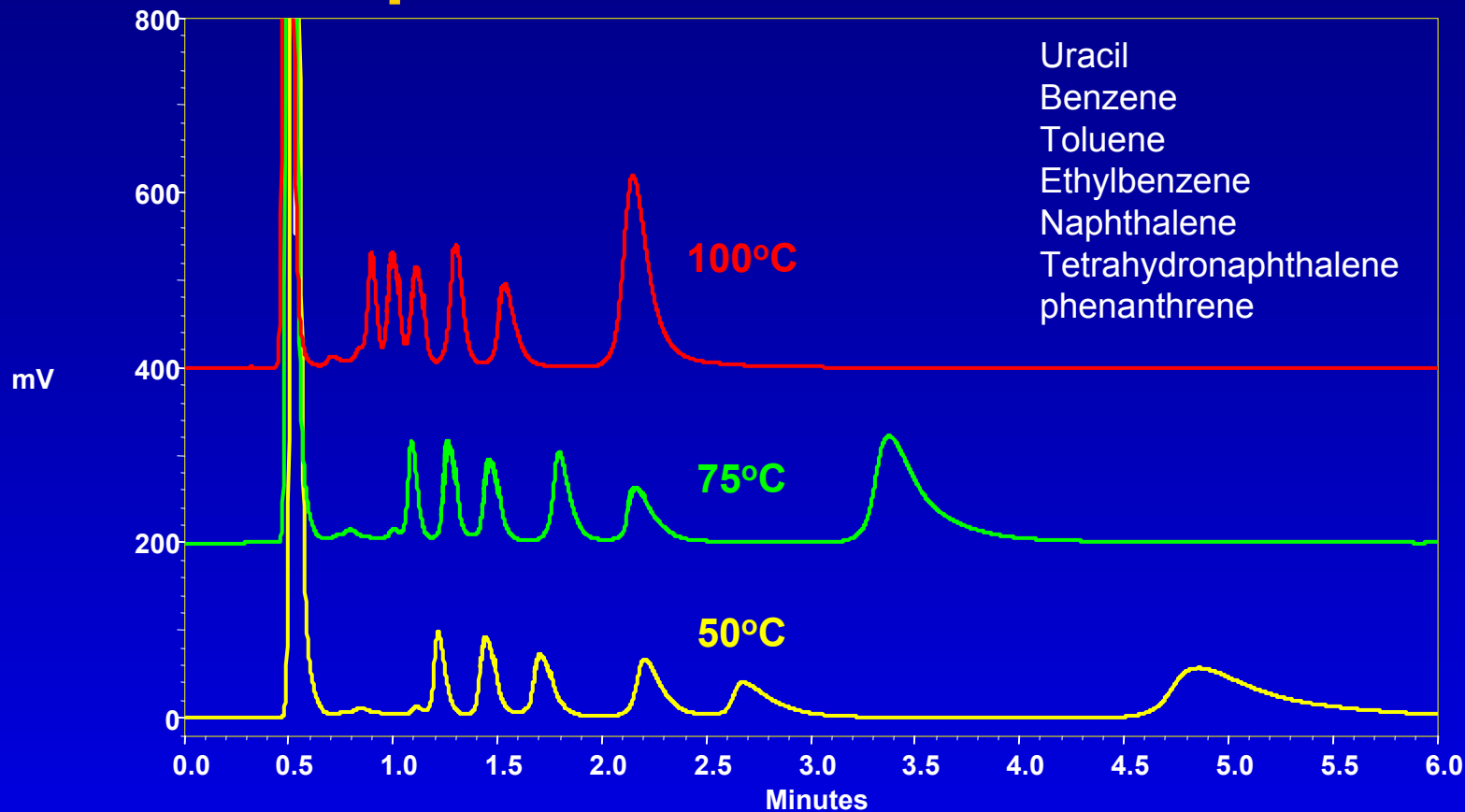
Flow Rate: 2.0 mL/min

Detection: UV 254 nm

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



Aromatic Separation on PRP-1[®] at Three Temperatures with a Solvent Gradient



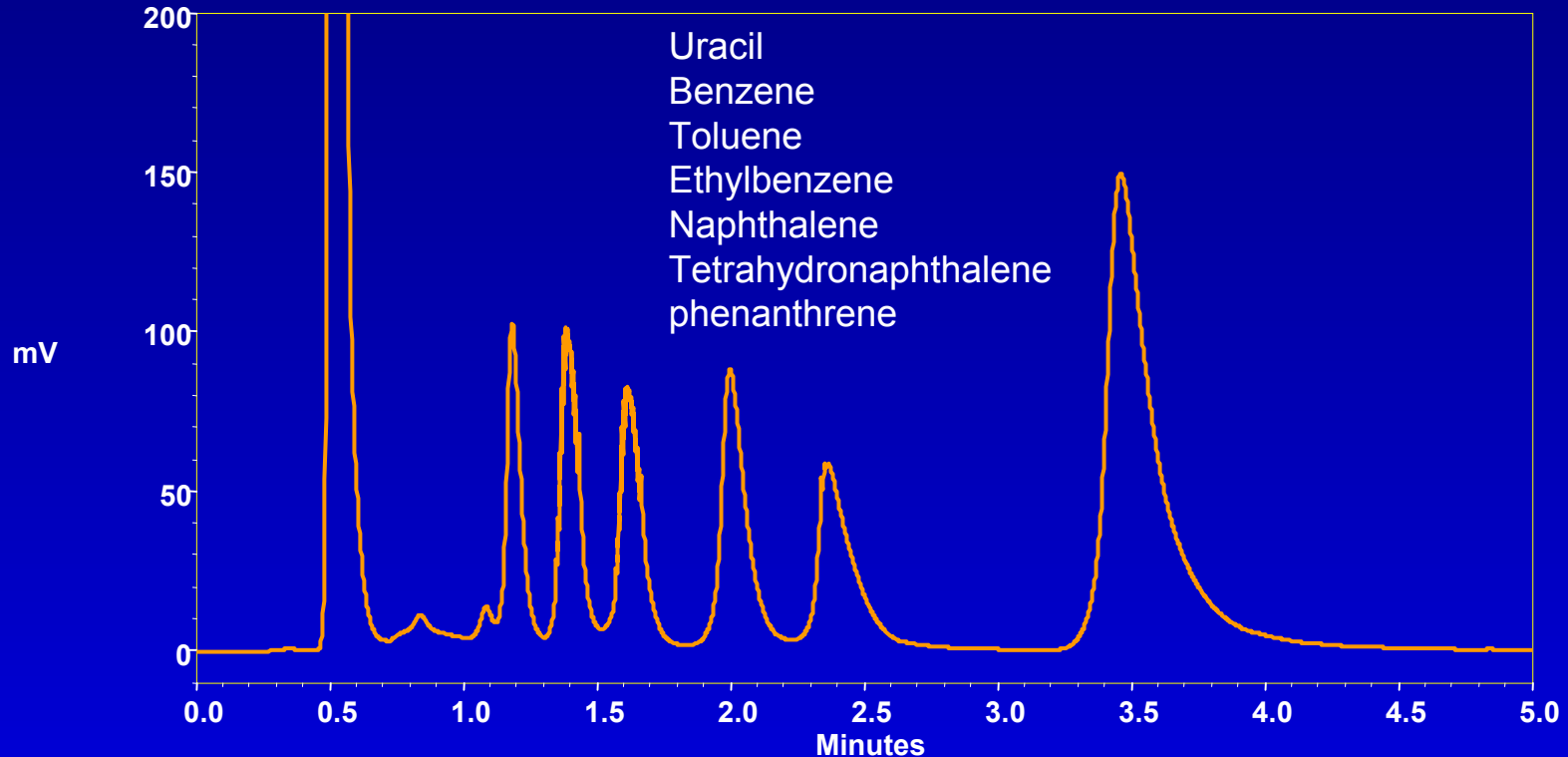
Column: PRP-1 3 μ m, 100 x 2.0 mm

Mobile 75:25 acetonitrile:water, solvent gradient 75-90%ACN over five minutes, hold two minutes.

Flow Rate: 0.5 mL/min



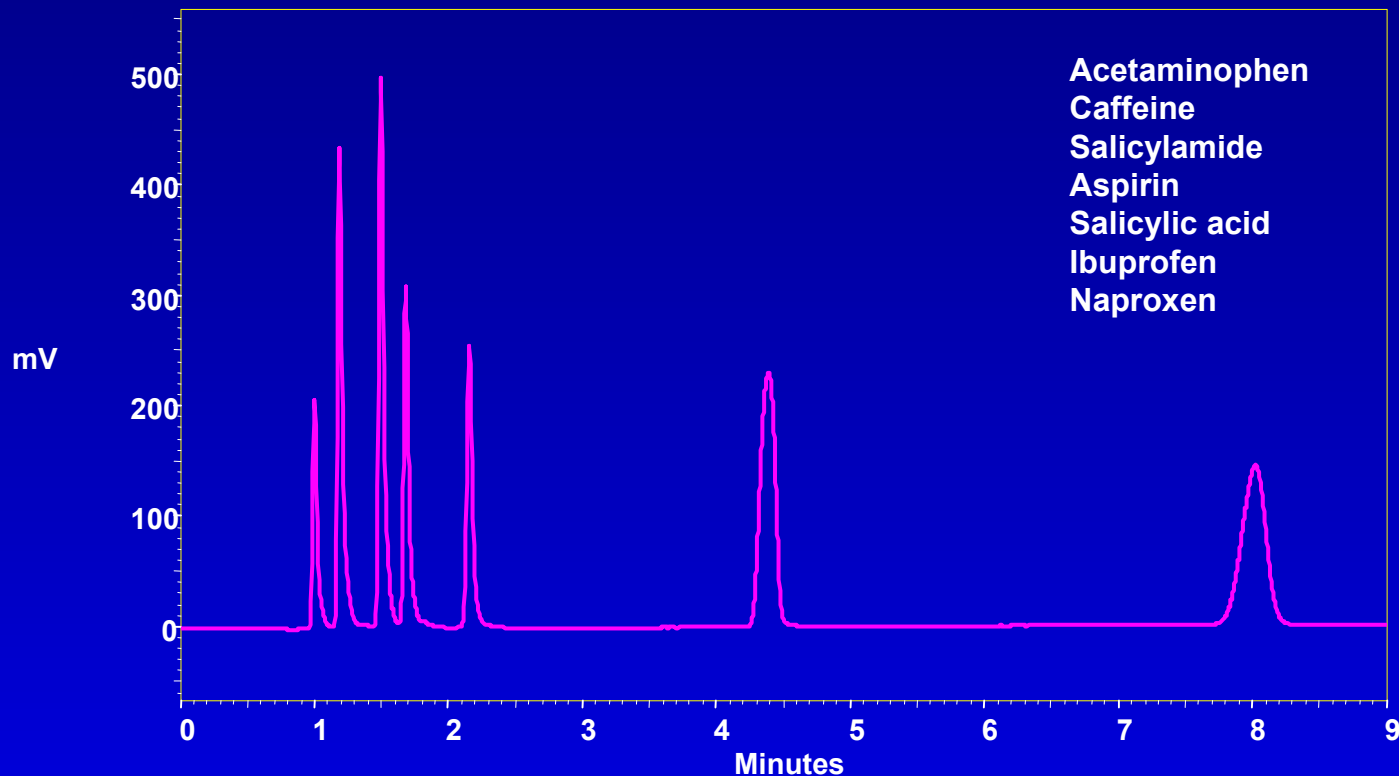
Aromatic Separation on PRP-1[®] Using Thermal Gradient at 20°C/min



Column: PRP-1 3 μ m, 100 x 2.0 mm
Mobile 75:25 acetonitrile:water, isocratic
Flow Rate: 0.5 mL/min
Detection: UV 254 nm
Temperature Program: 50°C to 150°C at 20°/min, hold two minutes



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

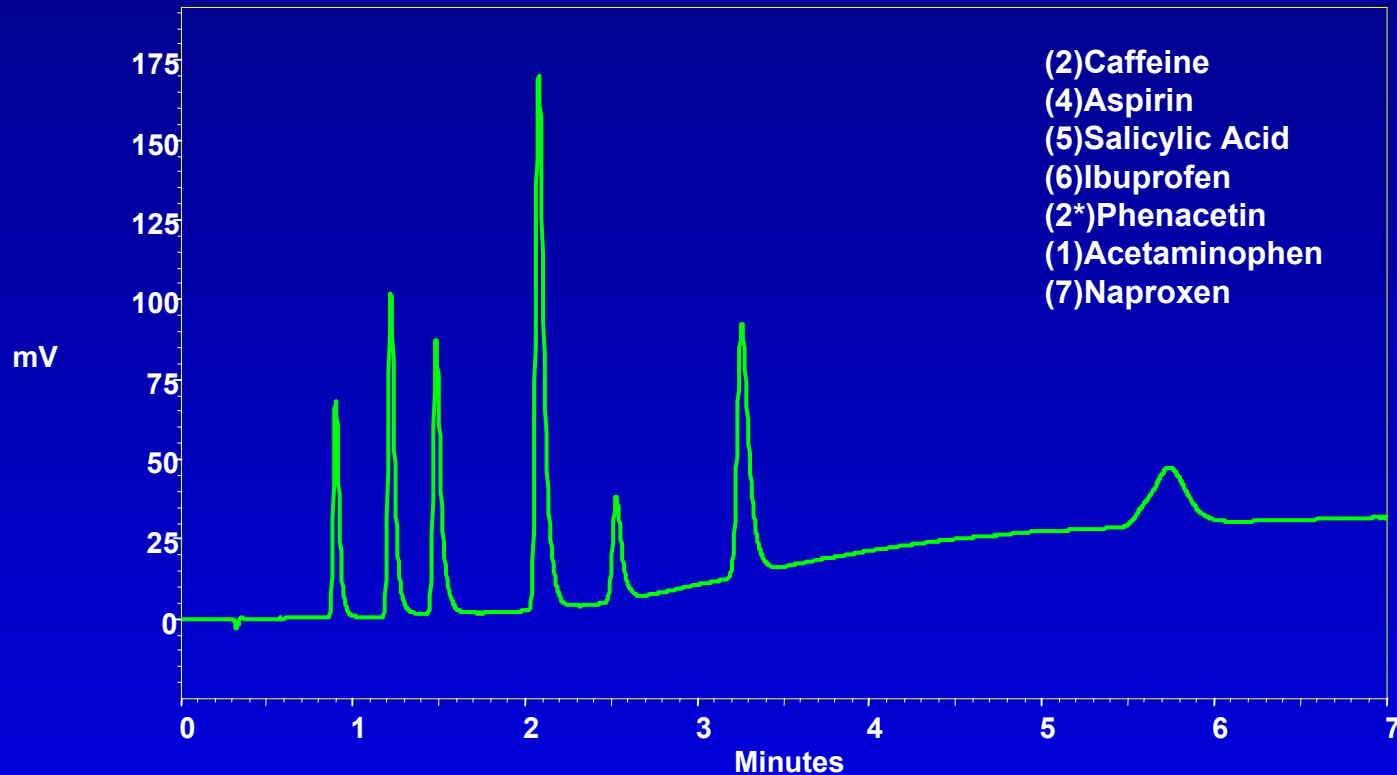
Flow Rate: 1.5 mL/min

Detection: UV 220 nm

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



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

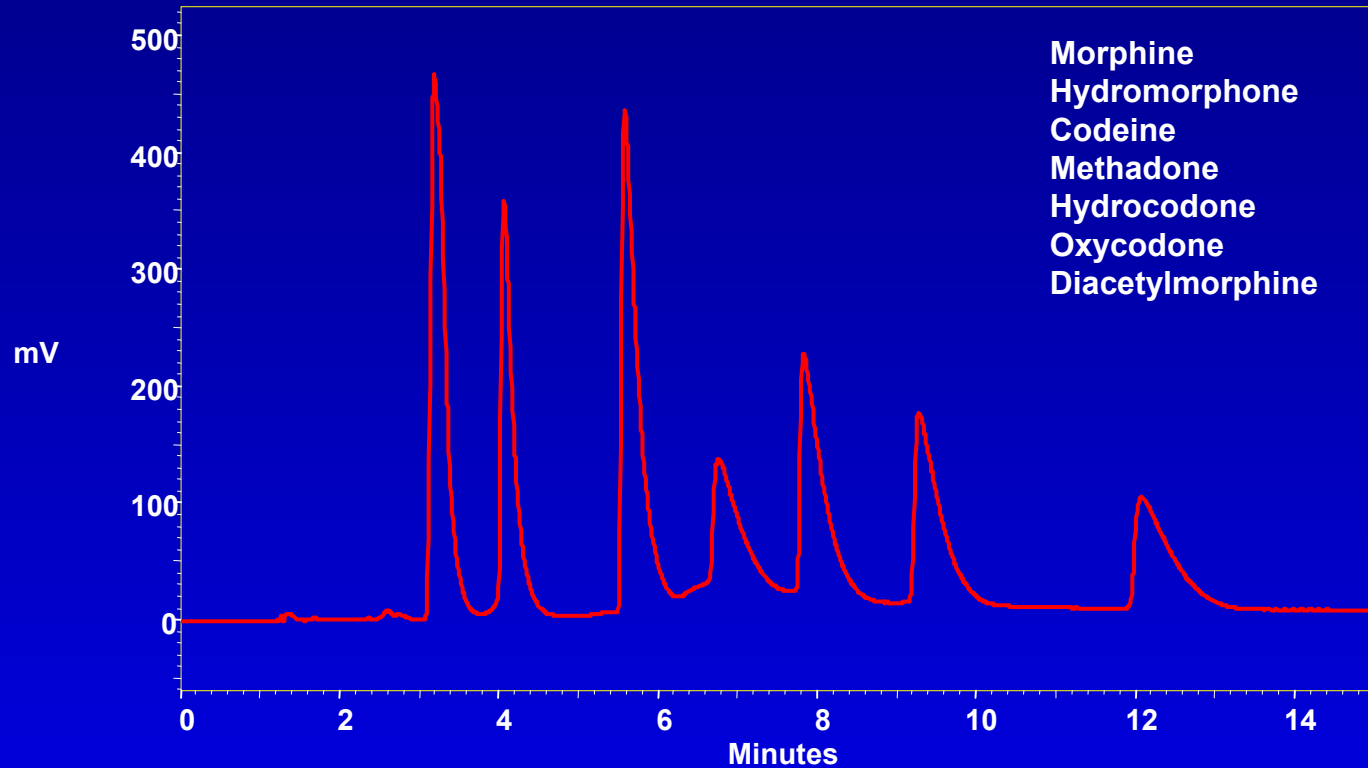
Flow Rate: 4.0 mL/min

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 μ m, 100 x 4.6 mm

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

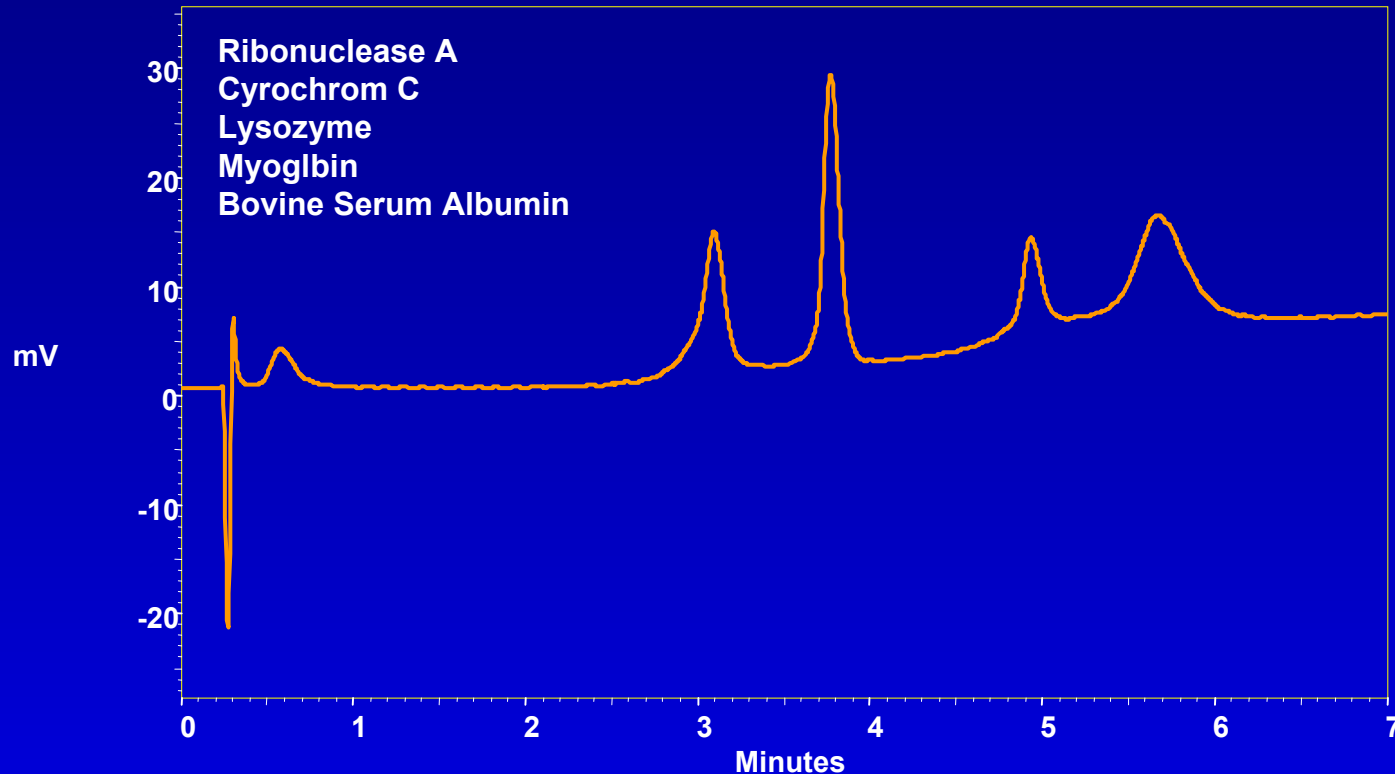
Flow Rate: 1.0 mL/min

Detection: UV 220 nm

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



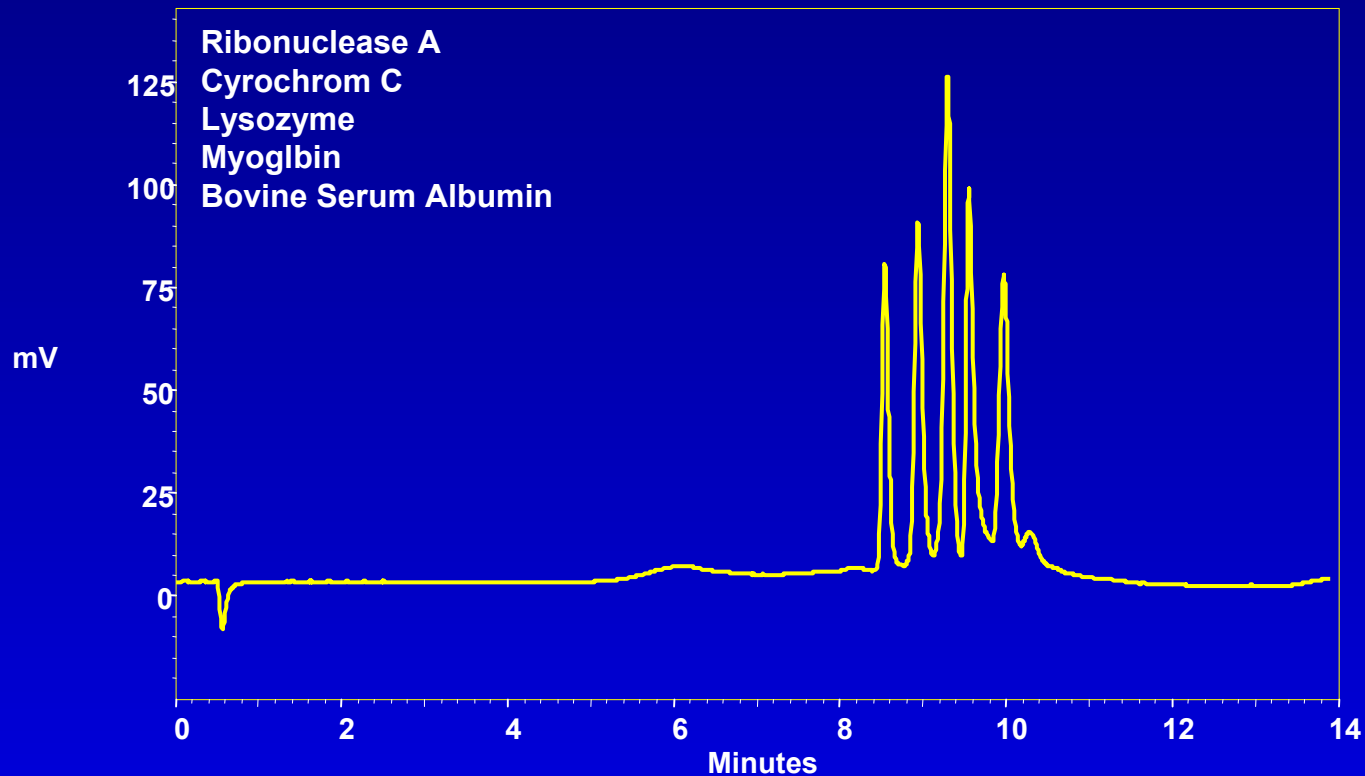
Separation of Proteins Using a Hamilton PRP-3[®] Column and a Thermal Gradient



Column: Hamilton PRP-3[®], 3 μ 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.



Separation of Proteins Using a Hamilton PRP-3[®] Column and a Solvent Gradient



Column: Hamilton PRP-3[®], 3 μ m, 100 x 2.1 mm
Mobile Phase: acetonitrile:water with 0.1% TFA. Solvent gradient from zero to 100% Acetonitrile over ten minutes, hold ten minutes.
Flow Rate: 0.5 mL/min
Detection: UV 215 nm
Temperature Program: 35°C isothermal



Conclusion

No more solvent gradients!



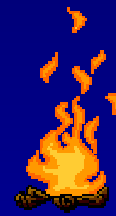
Acknowledgements

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





Turn up the Heat!



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