HPLC Peak Focusing Facilitated by Independent Mobile Phase Pre-heating

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

Selerity Technologies, Inc.
2484 W. Custer Road
Salt Lake City, UT 84104
www.selerity.com

Temperature in HPLC

- Speed
- Efficiency
- Selectivity
- Peak focusing with temperature gradients

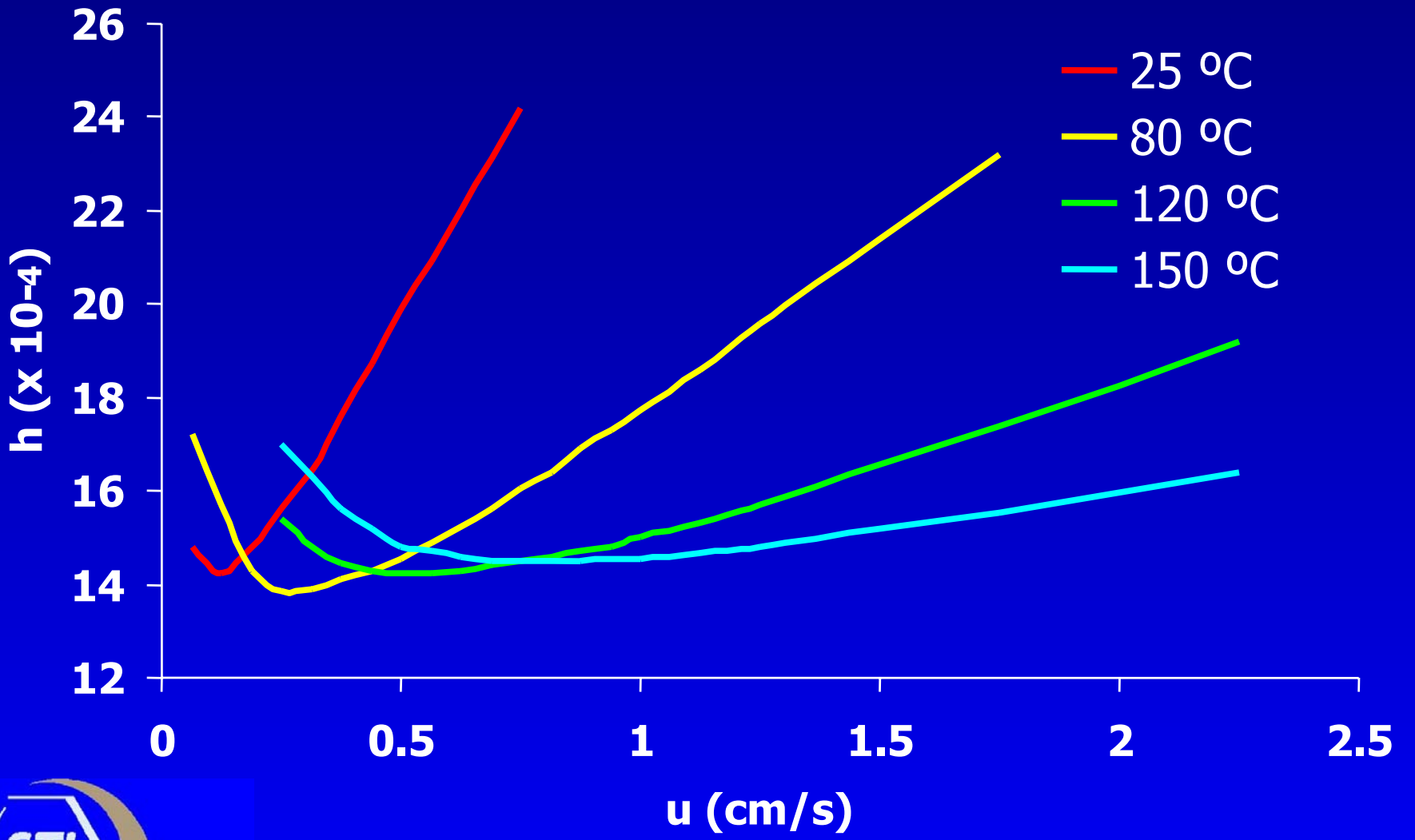
Faster and More Efficient Separations

- **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 like methanol so less organic modifier needed

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 to reduce band-broadening
- Need columns stable at elevated temperatures
- Must correct for refractive index differences when mobile phase is heated

Selerity Series 9000 Total Temperature Controller

Small and Versatile Temperature Programmable Column Heater/Chiller

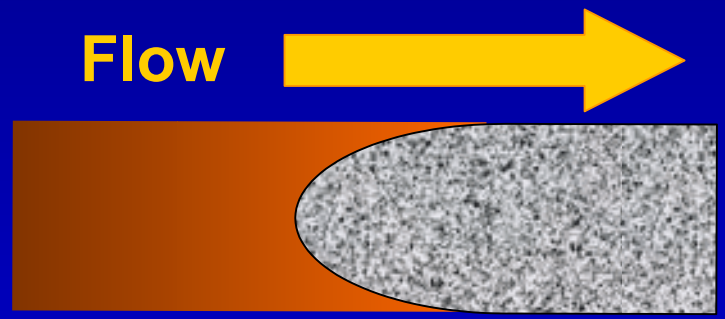
- Small footprint
- Sub-zero to 200°C
- Mobile phase preheating or precooling
- Precision mobile phase outlet temperature control
- Flammable vapor sensor
- Variable low voltage mobile phase preheater
- Integral high speed microcomputer control
- Accommodates columns up to 25 cm in length

Vapor Sensing

- The need for vapor sensing is not limited to forced air ovens
- Leaks can produce an explosive and toxic vapor cloud
- All Selerity column heaters incorporate continuous flammable vapor sensing

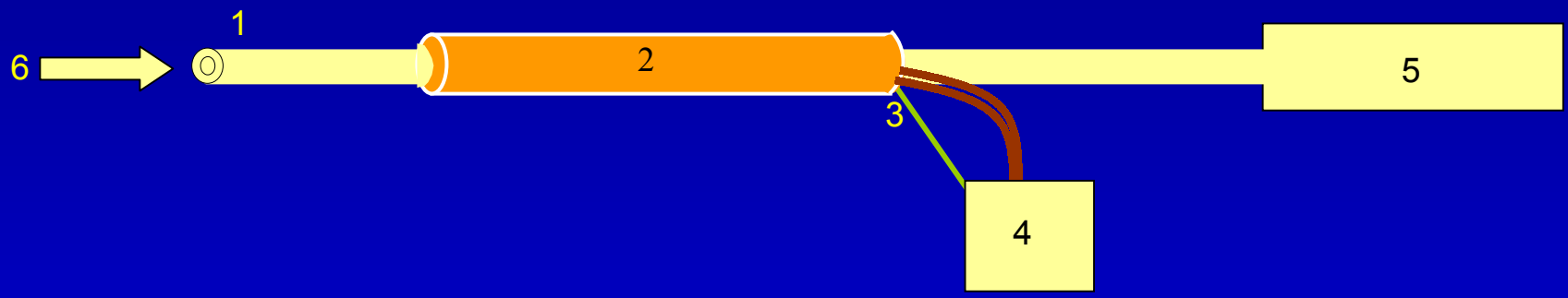
Why is Solvent Pre-heating so Important?

No Pre-heating



With Pre-heating

Solvent Pre-heater Design



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

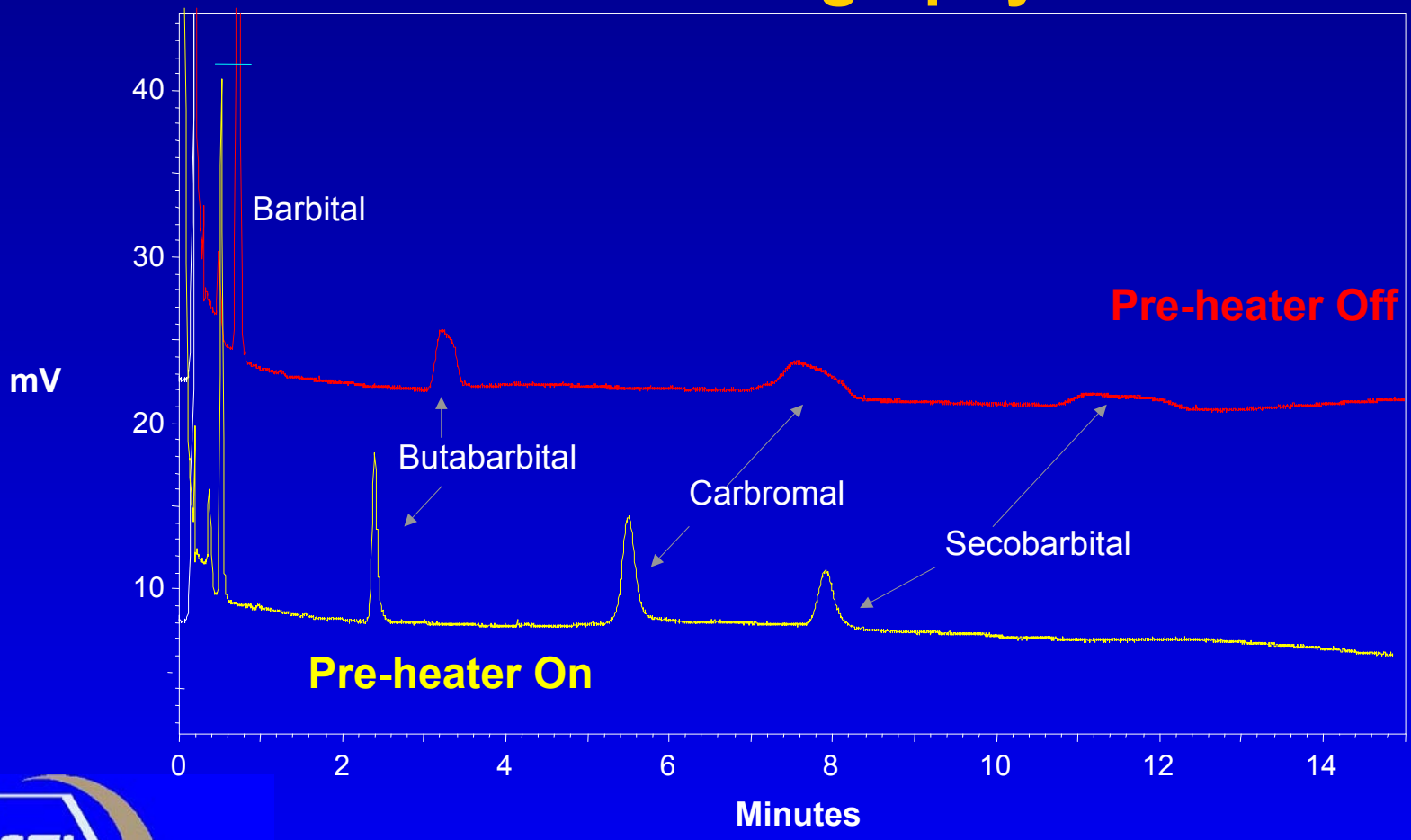
Solvent Pre-heater

- 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

Low Voltage Pre-heater Design

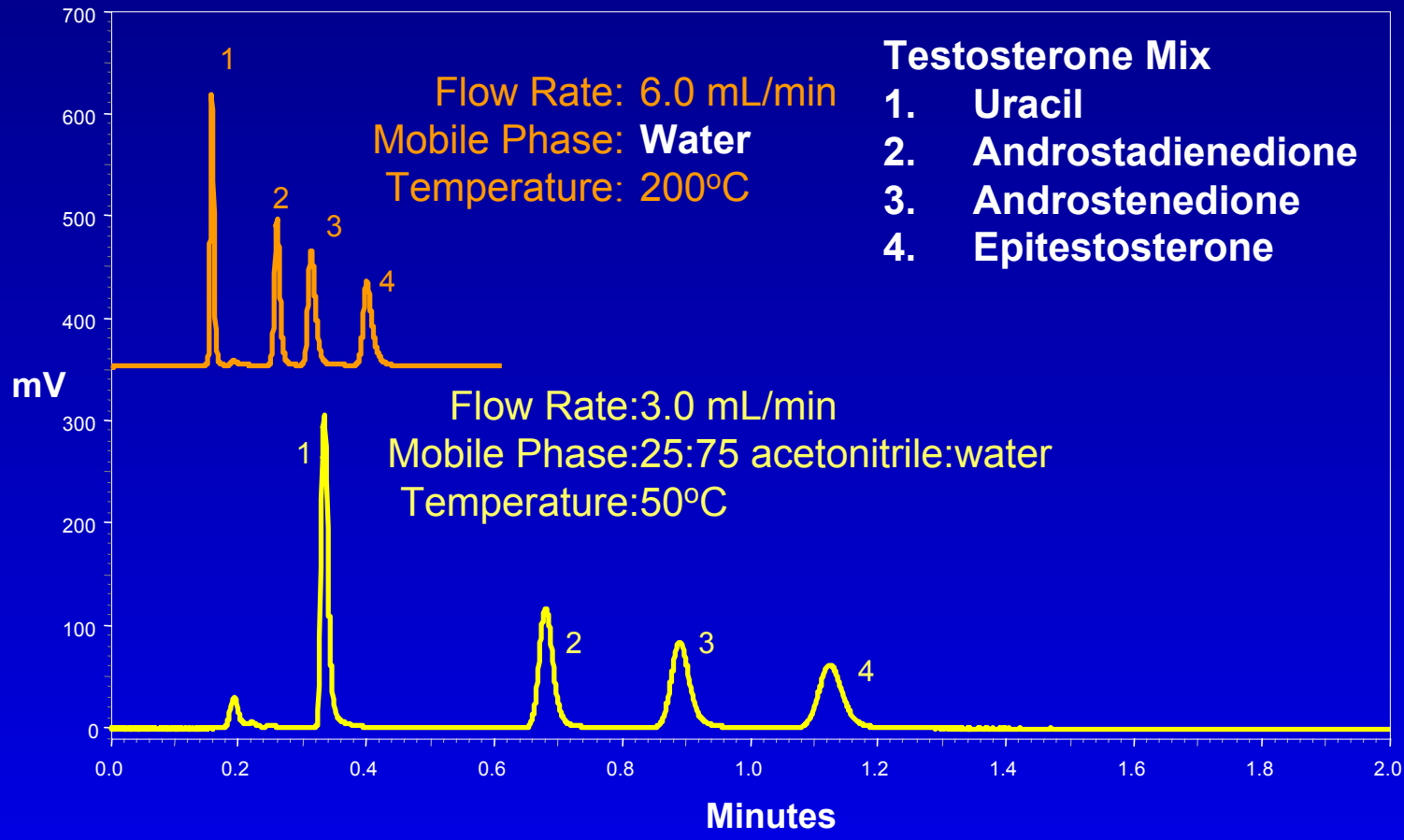
- Voltage from 60 mV to 22 V in 4000 increments under continuous microprocessor control
- Demand is only 6 V for 1 ml/min mobile phase flows heated to 150°C

Separation of Barbiturates
Mobile Phase Preheating Improves Chromatography



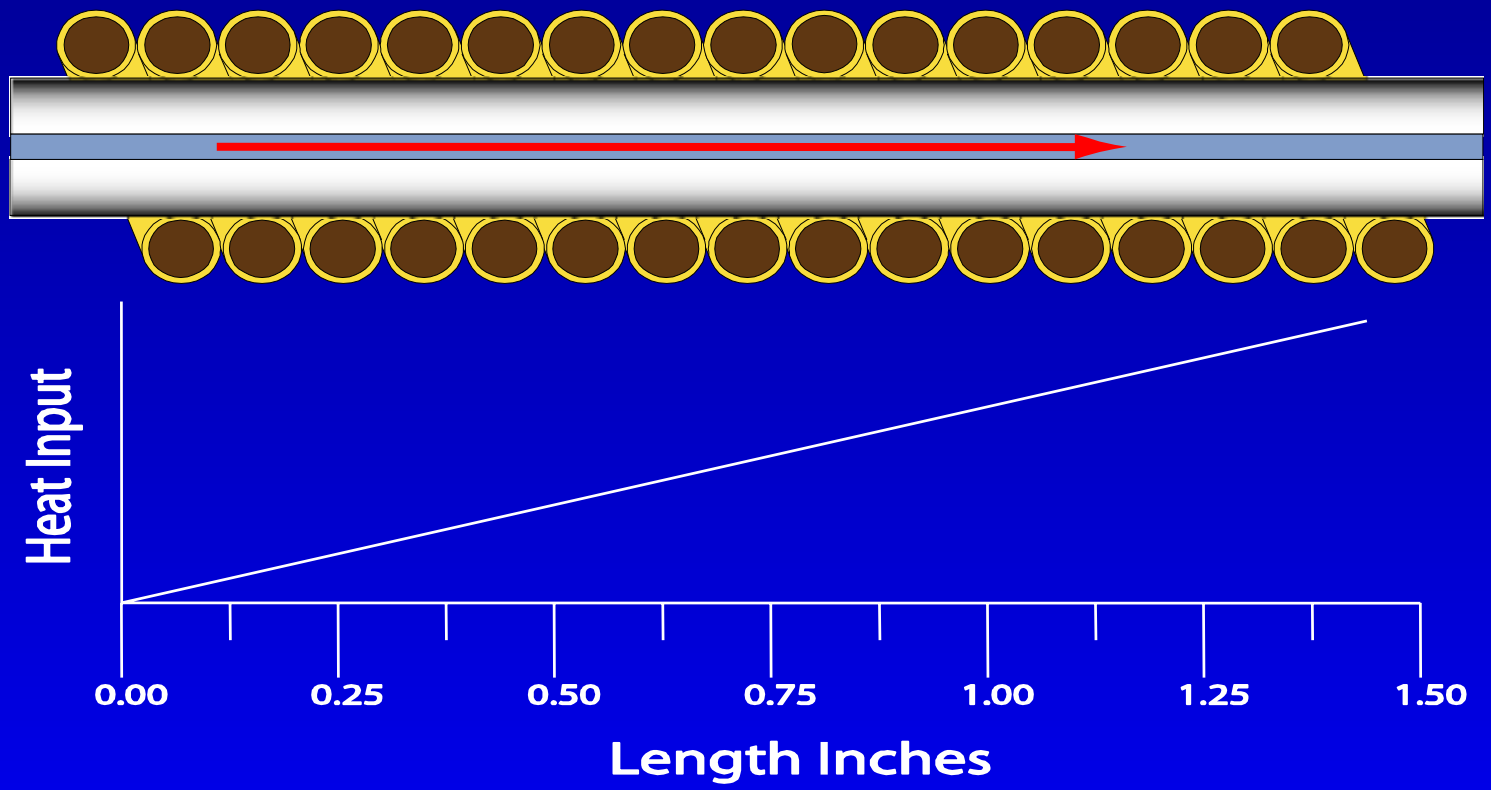
Zirchrom PBD, 80°C

Separation of Steroids Using Water as a Mobile Phase

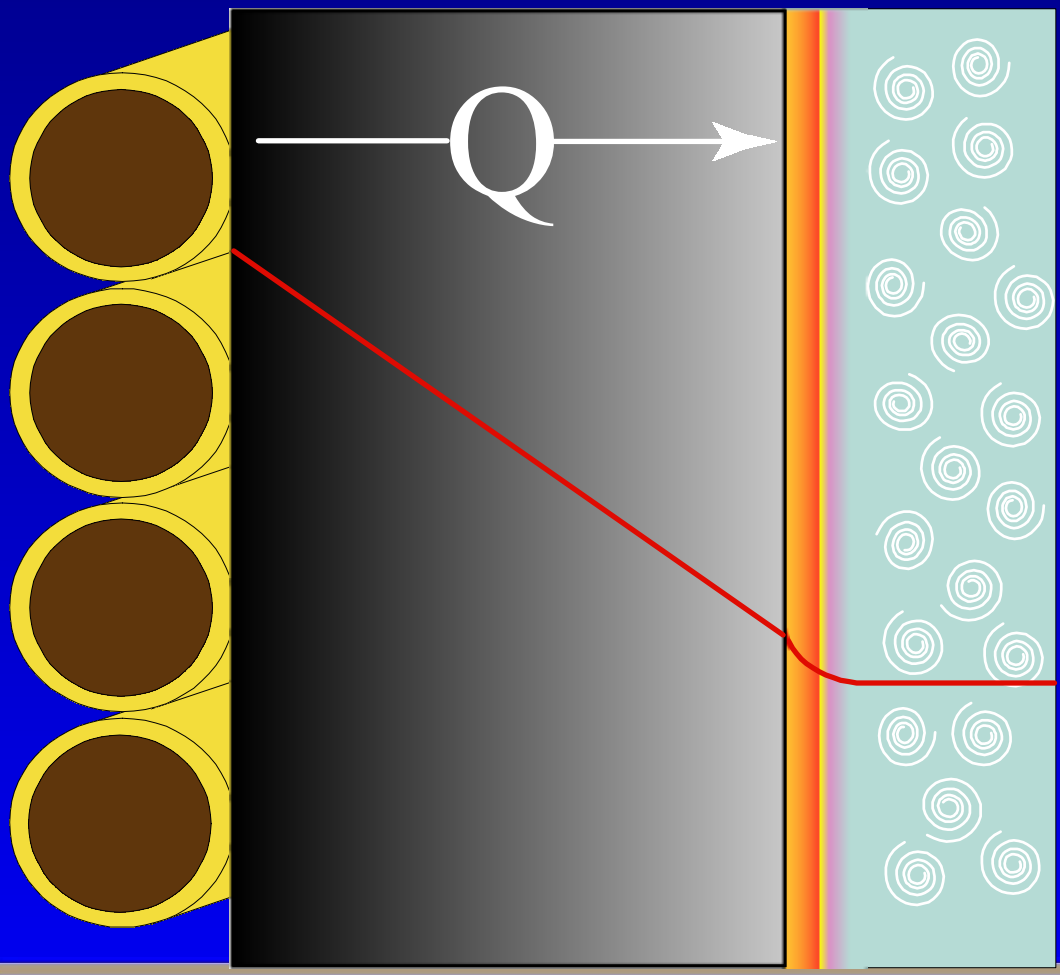


Zirchrom PBD 100 X 4.6 mm column, UV 254 nm

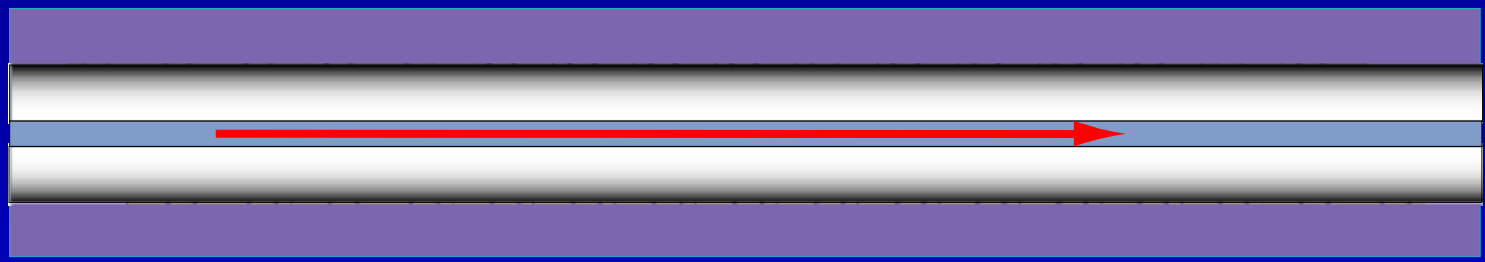
Axial Heat Profile

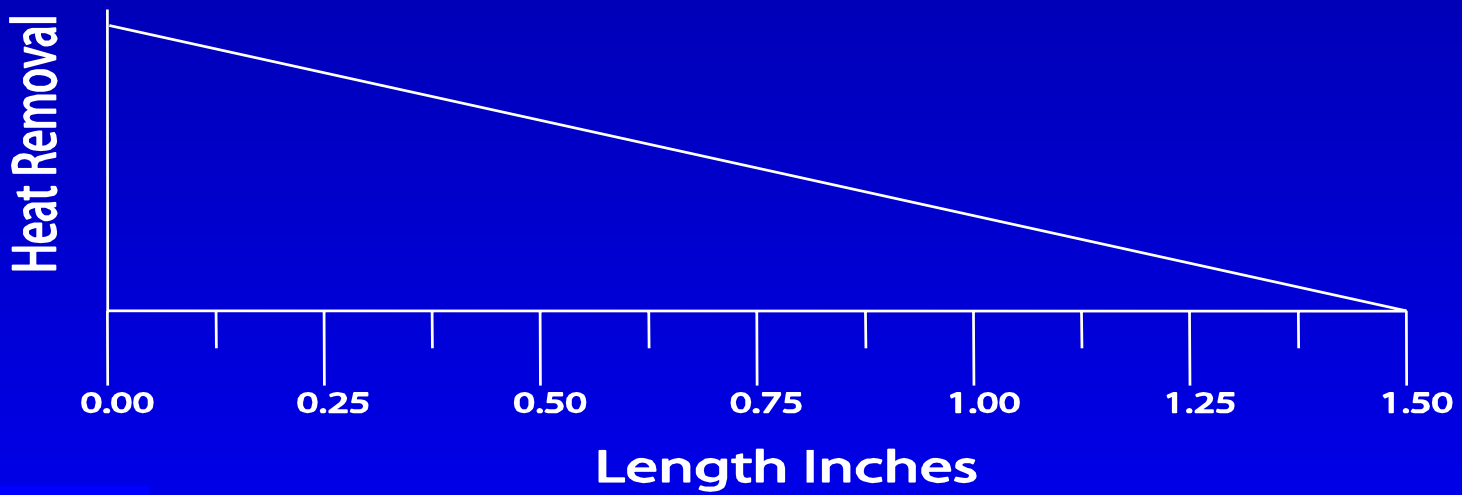


Radial Heat Flow Into the Mobile Phase

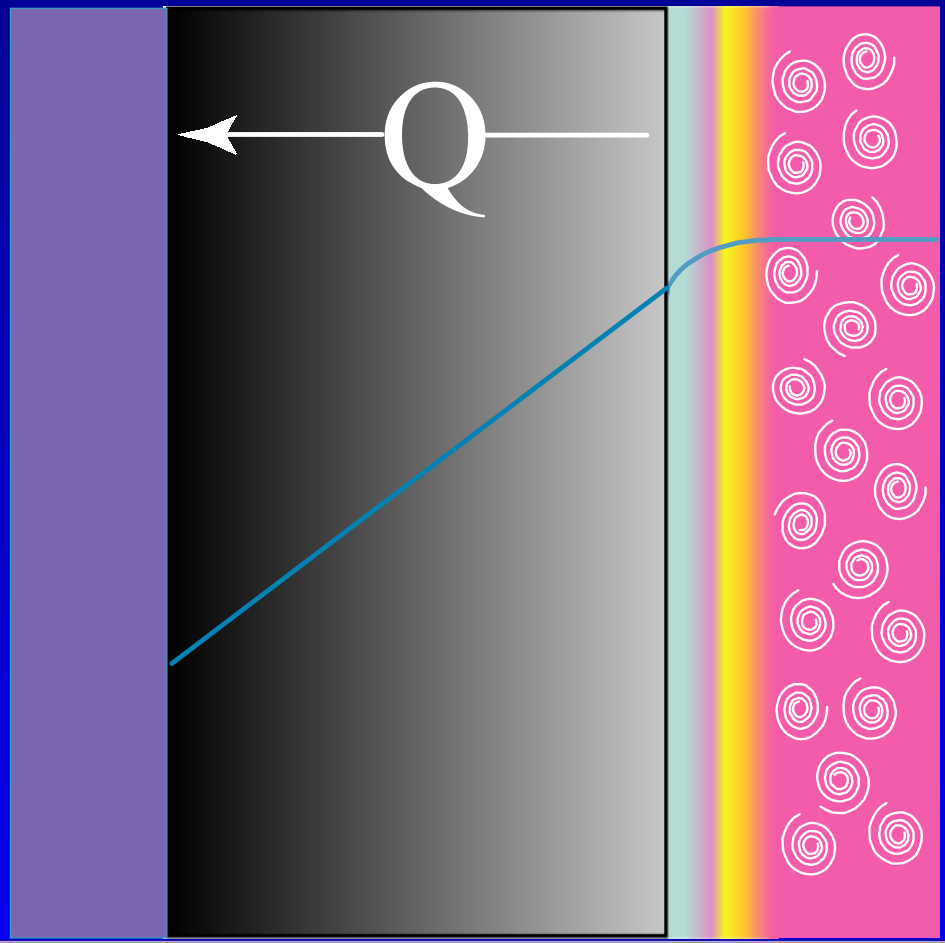


Axial Cooling Profile

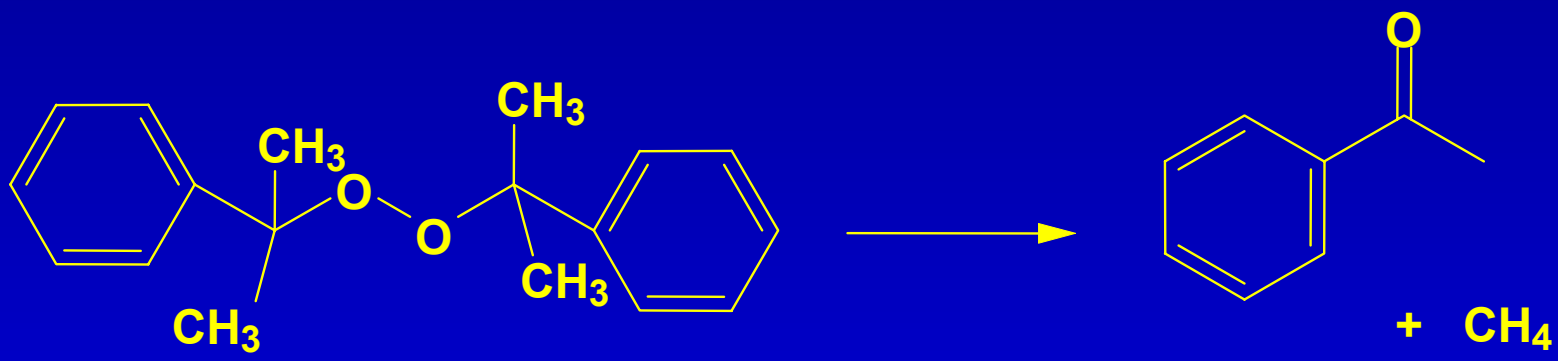




Radial Heat Flow out of the Mobile Phase



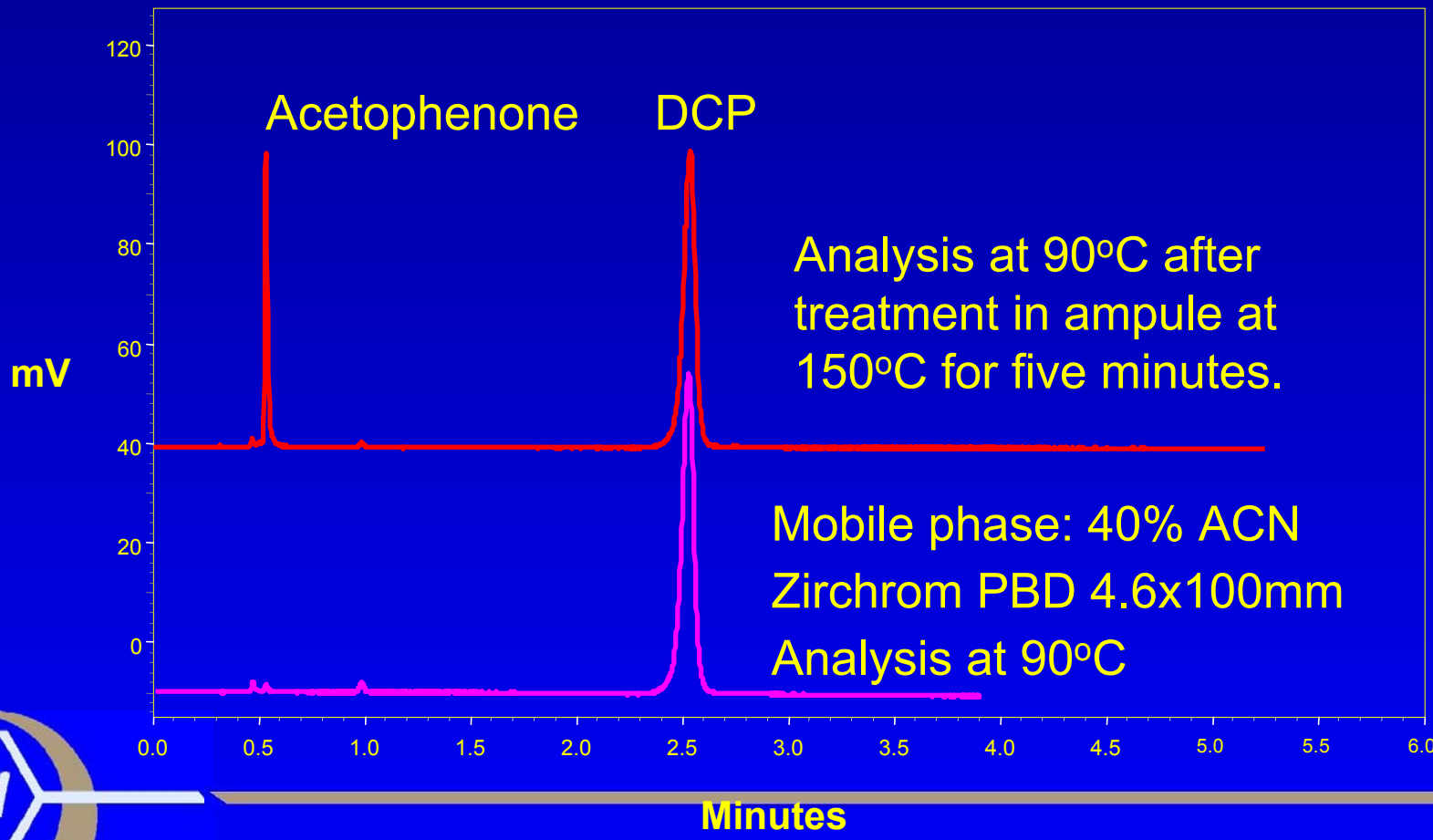
Thermal Decomposition of a Labile Compound



Dicumylperoxide

Acetophenone

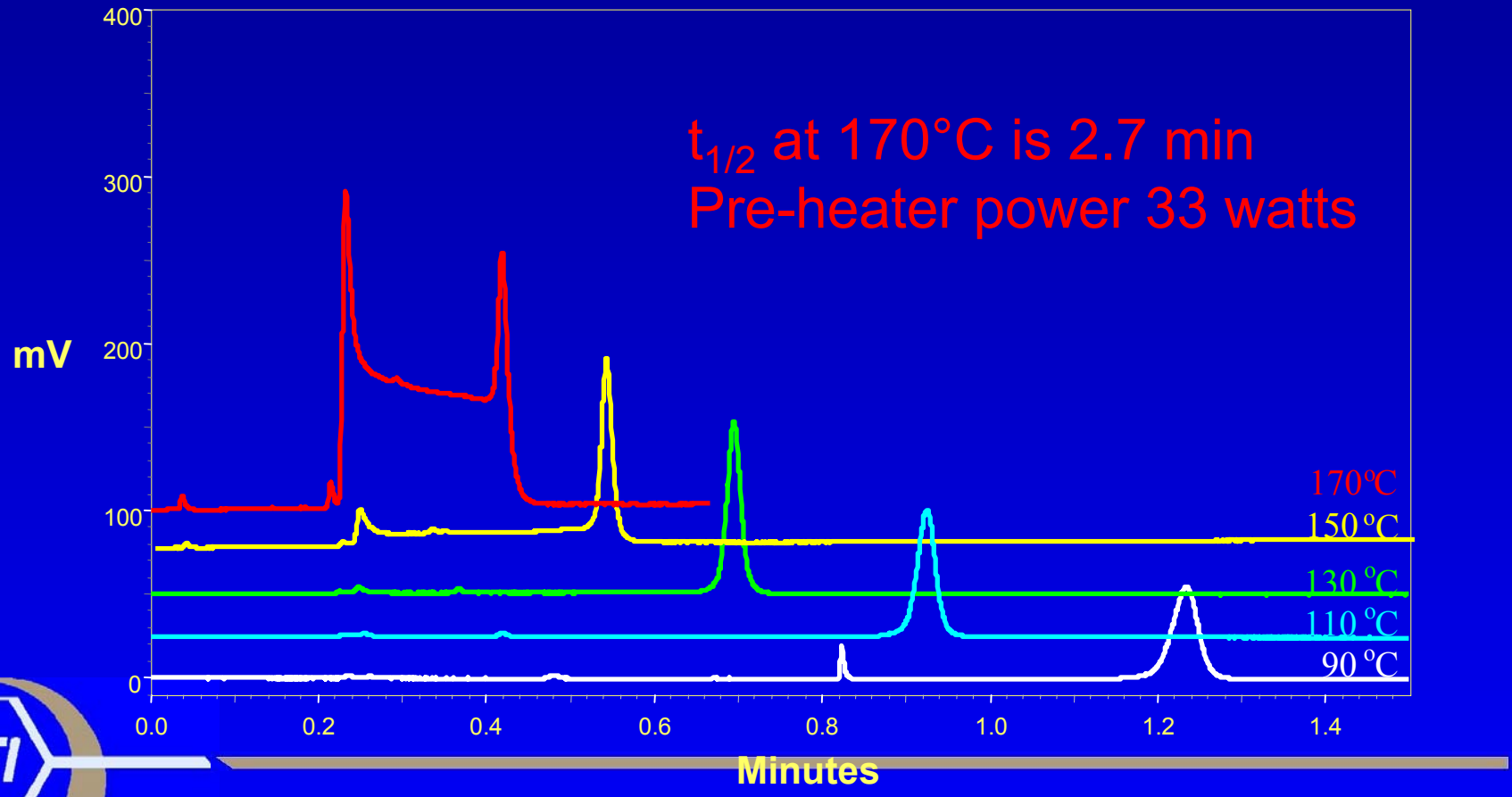
Decomposition of Dicumyl Peroxide



Normalized Percent of Dicumyl Peroxide Remaining

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

Dicumyl Peroxide at Five Temperatures at 4.0 mL/min



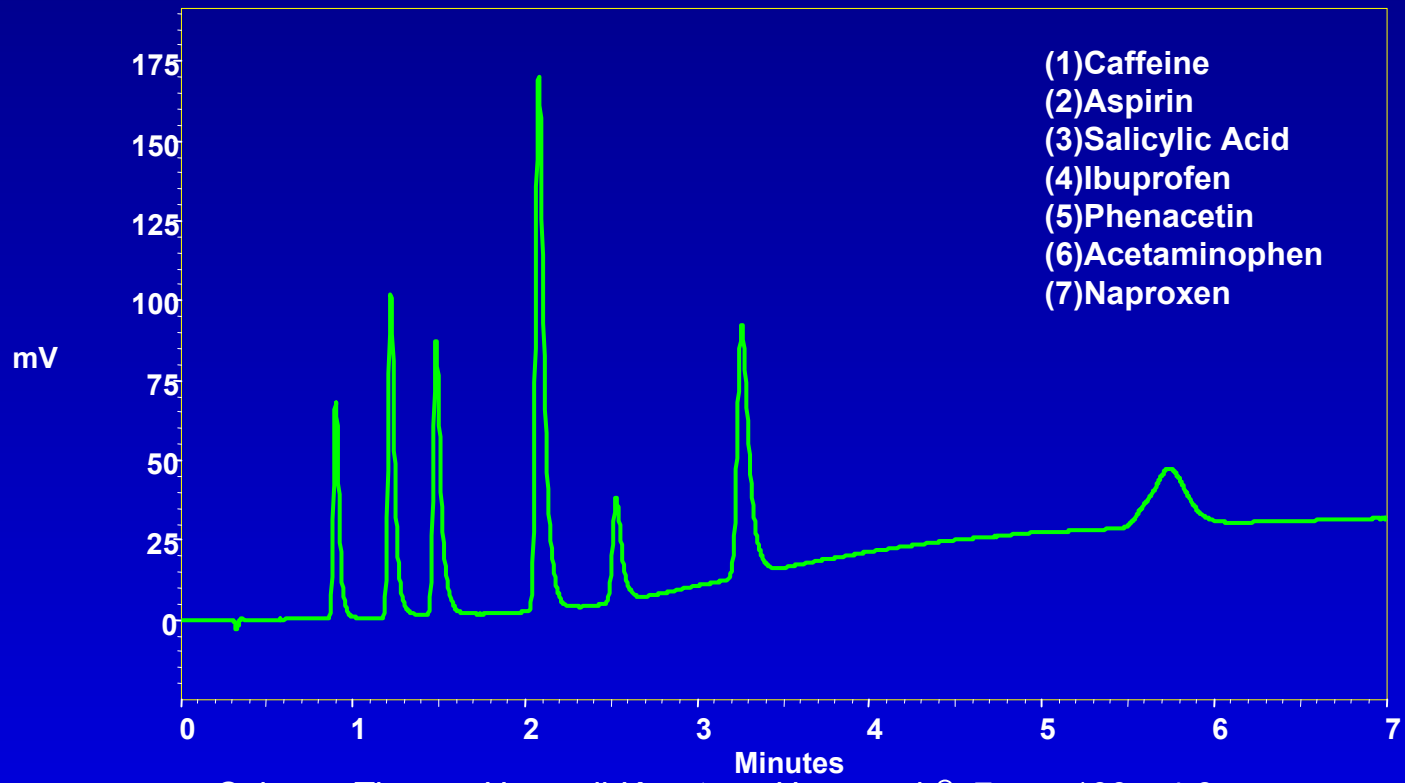
Decomposition Distribution

- Approximately 6% of the breakdown occurred from the pre-heater position to the column inlet, with the balance taking place within the body of the column
- About 1/3 of this value is due to residence time in tubing and column at 170°C after the pre-heater
- Drastic superheating of the mobile phase in the pre-heater would have shifted the decomposition distribution

Mobile Phase Temperature Offsetting

- Independent pre-heater control allows mobile phase temperature matching with internal column temperature
- Useful in programmed runs for peak focusing

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



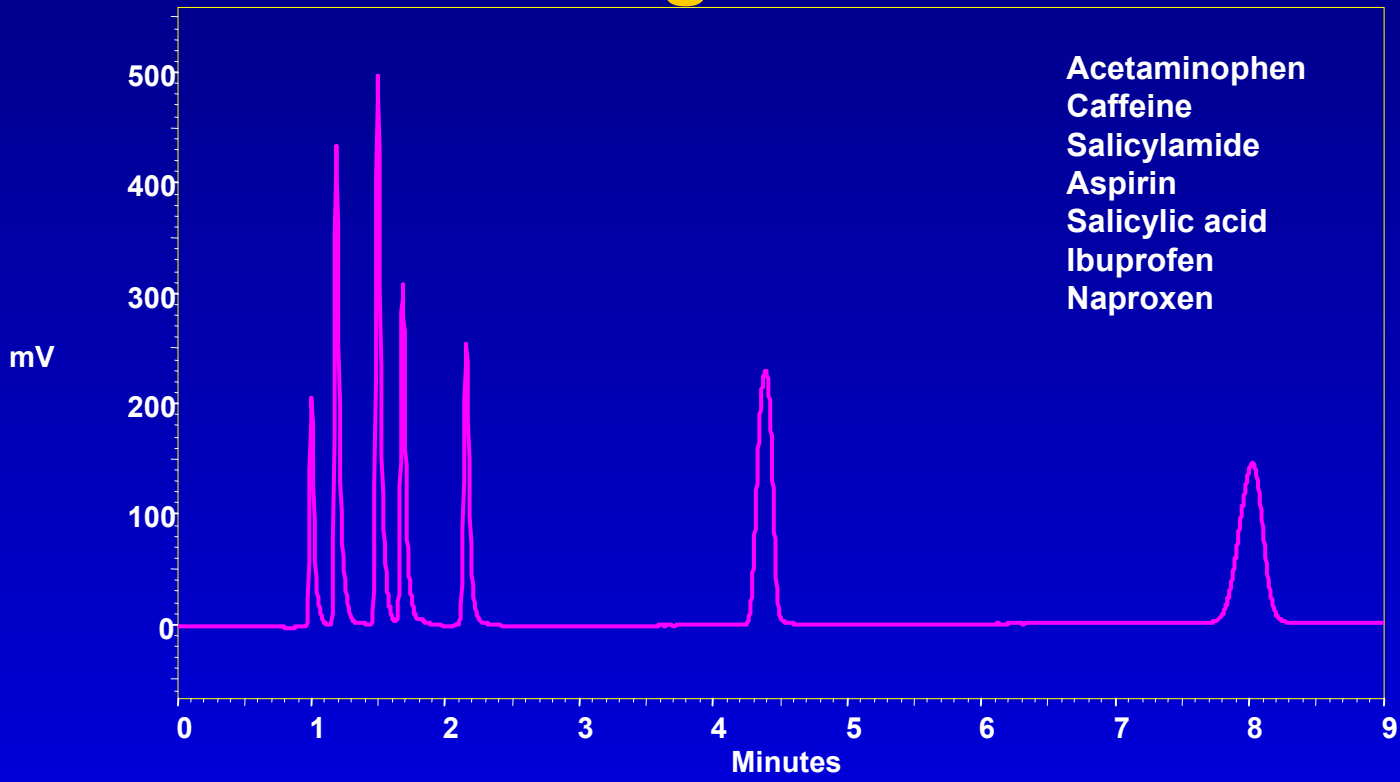
Column: Thermo Hypersil-Keystone Hypercarb®, 7 µm, 100 x 4.6 mm
Mobile Phase: 35:65 acetonitrile:water with 0.1% TFA
Flow Rate: 4.0 mL/min
Detection: UV 220 nm
Temperature Program: thermal gradient from 125° to 200°C at 30°/min, hold five min.

Analysis of Acetaminophen on a Hypercarb Column

7µm Graphitic Carbon 4.6x100mm, 35:65 ACN:H_20 w/0.1%TFA, 125-200°C(6 min) 30°/min

Flow Rate (ml/min)	Pre-heater Temperature	Retention Time	Peak Width	Asymmetry
1	+10°C	7.99	0.243	1.423
1	0	8.00	0.239	1.443
1	-10	8.11	0.244	1.399
2	+10	4.49	0.109	1.727
2	0	4.63	0.115	1.575
2	-10	4.73	0.117	1.541
4	+10	2.84	0.061	1.621
4	0	3.03	0.065	1.505
4	-10	3.08	0.064	1.468

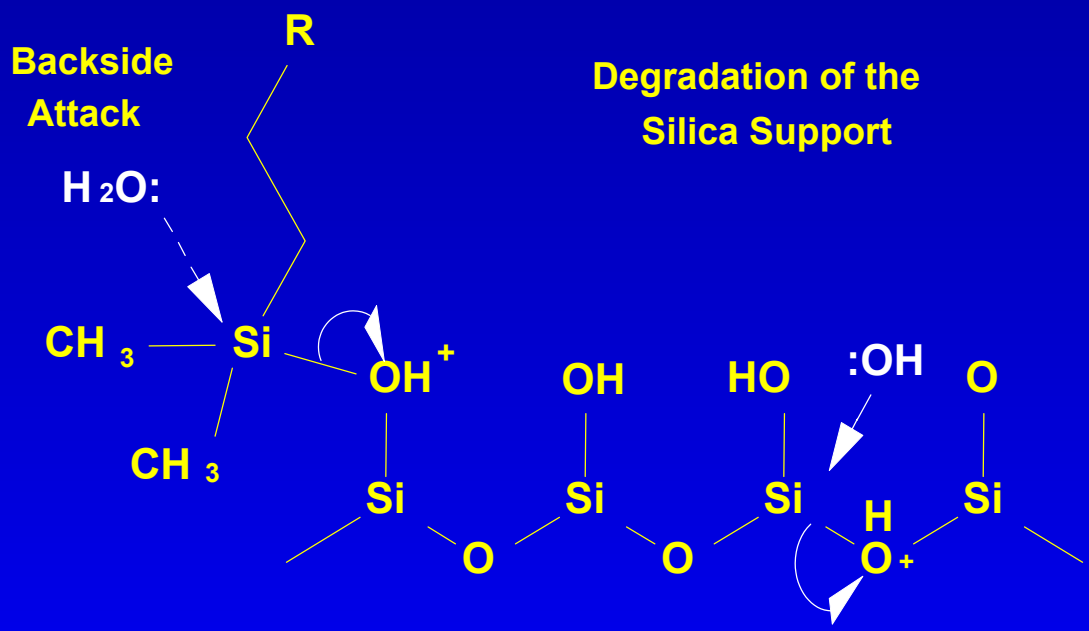
Separation of Analgesics on a Selerity Blaze C8 Using a Thermal Gradient



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.

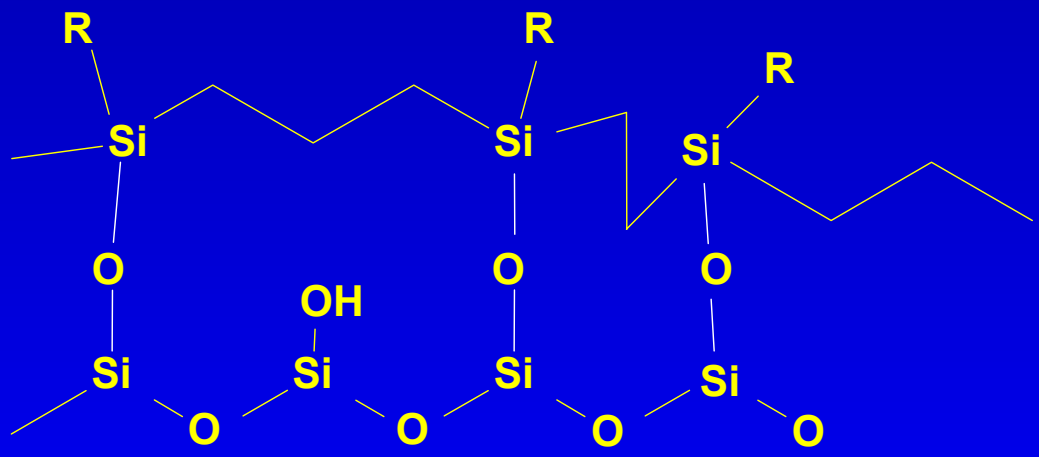
Traditional Silica Columns Can't Take the Heat

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

Conclusions

- The Selerity low mass mobile phase pre-heater is effective in controlling power distribution without overheating damage to sensitive analytes
- It is independently controllable and can be used to focus solutes and improve peak shape
- It enables the extension of elevated temperature HPLC beyond the isothermal range, allowing temperature gradient separations in large bore columns

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
- 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.