

Water Only HPLC With FID Detection

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Introduction

Utilizing elevated temperatures in HPLC reduces run time, improves resolution and efficiency, and permits the use of a temperature program in place of a solvent gradient. Because the polarity of water is reduced as its temperature is increased, performing separations at higher temperatures mimics adding organic modifier to the mobile phase. This opens the door for water only reversed phase separations if elevated temperatures are used.



Water as a Mobile Phase

- Inexpensive
- Readily available
- Non-polluting
- Transparent to most detectors including UV, FID, and NMR (D_2O)
- Elution strength can be controlled by varying temperature



Change in Retention with Temperature

$$k_1' / k_2' = \exp(\Delta H(T_2 - T_1) / (RT_1T_2))$$

- Effect of temperature on retention factor depends on enthalpy of the solute. The larger the enthalpy, the greater the change in k'



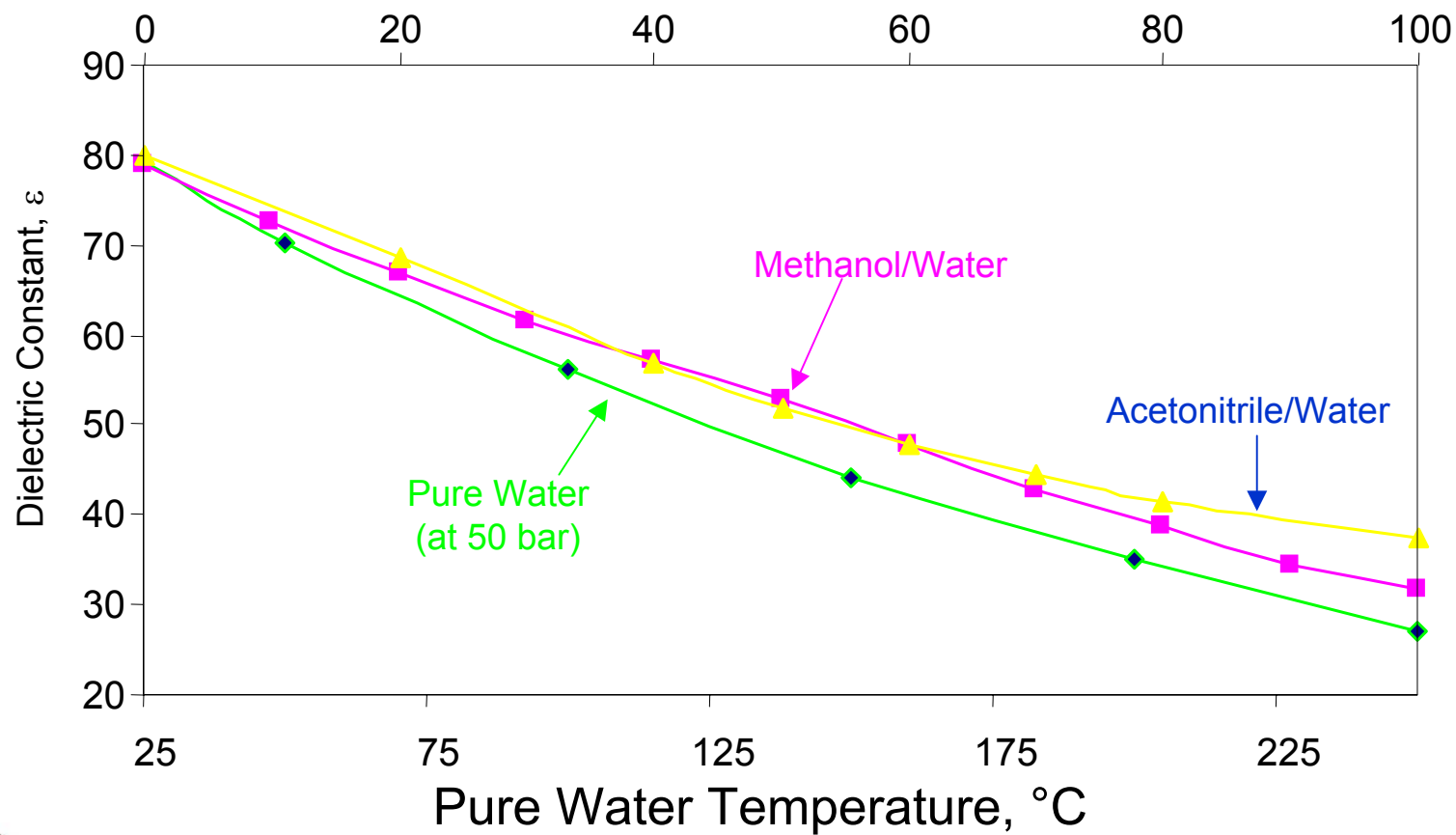
Factors Affecting Retention That Are Influenced by Temperature

- Hydrogen bonding
 - Increasing temperature increases intermolecular distance and weakens hydrogen bonds
- Solvation sphere around analytes
- Hydration extent of column surface
- Functional group interaction
- Ordering, shape and hydration transitions
 - Conformation changes
- Dielectric constant of mobile phase



Solvent Polarity as a Function of Temperature

% Methanol or Acetonitrile in Water at 25°C



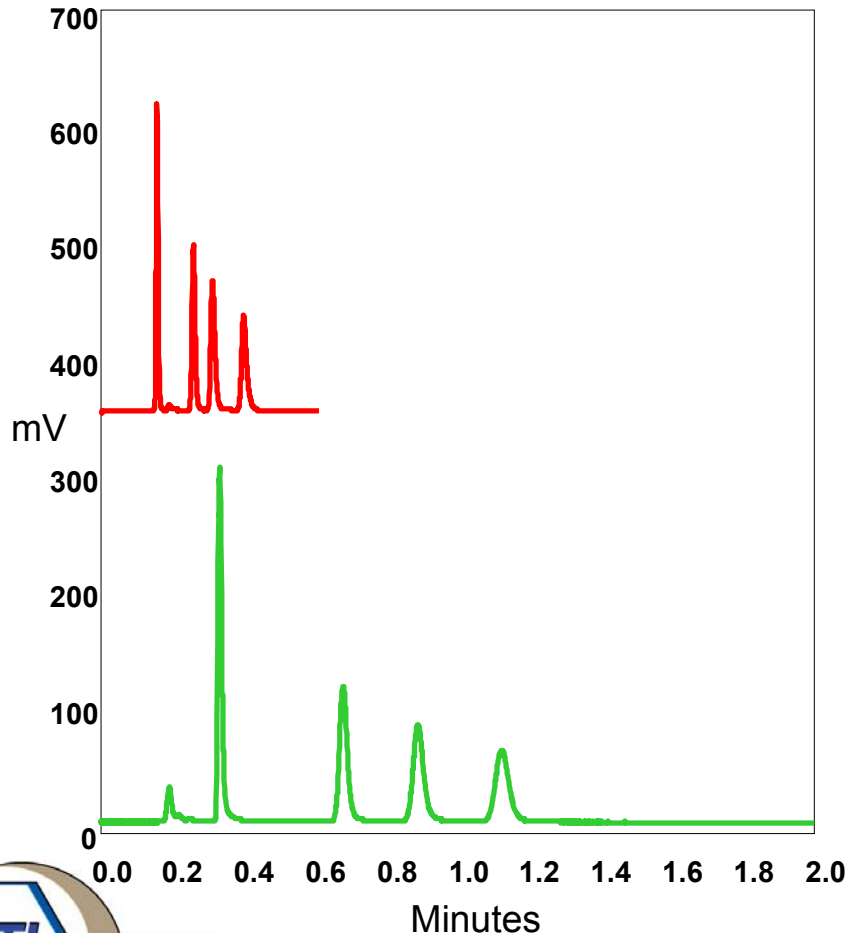
Temperature and Elution Strength

- Increasing temperature 4 to 5 °C is comparable to increasing the methanol or acetonitrile concentration by 1% in a reversed phase system
- Viscosity is reduced 1 to 2 % per °C increase
 - Result – a significant reduction in back pressure

$$P = \eta \mu L / d_p^2$$



Elution Strength Illustration: Separation of Steroids Using Water as the Mobile Phase



Instrument: Polaratherm™ Total
Temperature Controller

Column: ZirChrom PBD, 3 μm
100 X 4.6 mm

Detection: UV 254 nm

Flow Rate: 6.0 mL/min

Mobile Phase: Water

Temperature: 200°C

Flow Rate: 3.0 mL/min

Mobile Phase: 25:75 acetonitrile:water

Temperature: 50°C

Elution Order:

Uracil

Androstadienedione

Androstenedione

Epitestosterone



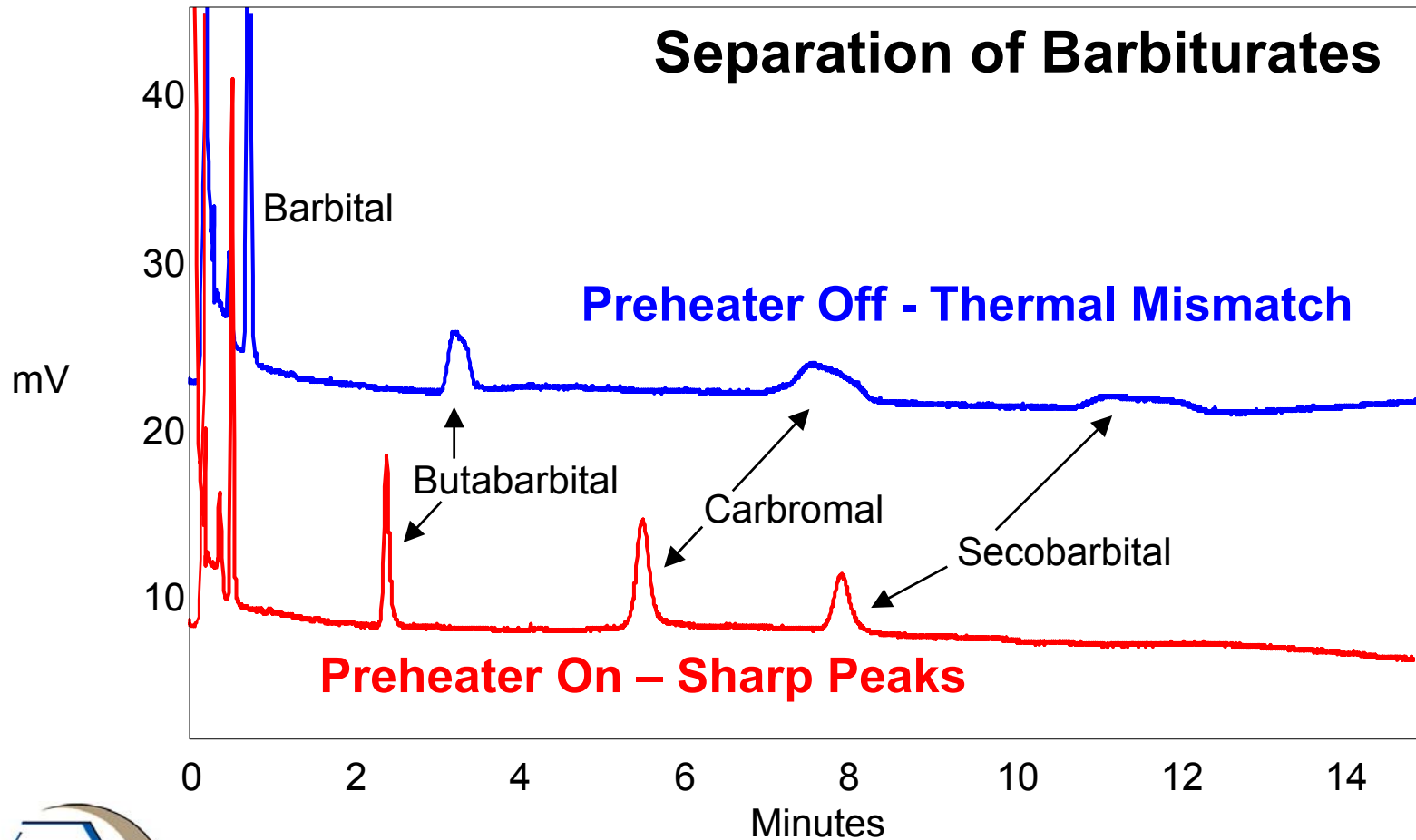
Preheating Avoids Thermal Mismatch



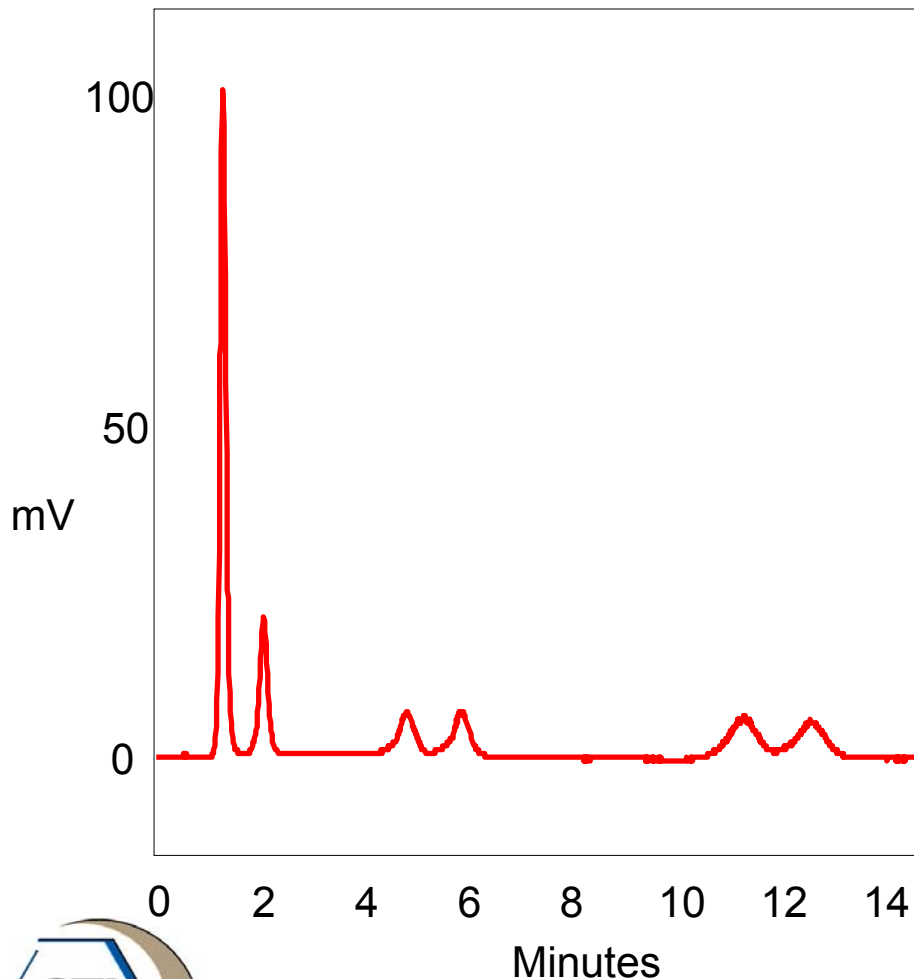
- Highly responsive and non-invasive
- Low-mass: <2 grams (including the tubing)
- Low-volume: <1 μL totally swept volume

Mobile Phase Preheating

Essential for good chromatography at elevated temperatures



Separation of Alcohols Using Water and FID Detection



Instrument: Aquachrom™ Green Machine

Oven profile: 50°C ramp to 135°C at 10°C/min hold 10 min.

Column: Jordi DVB, 2.1 X100 mm, 5µm

Mobile phase: 0.025%TFA

Split flow: 250 psi back pressure

Pump flow: 0.500 mL/min

Detection: FID @ 400°C

Elution Order:

Methanol

Ethanol

2-Propanol

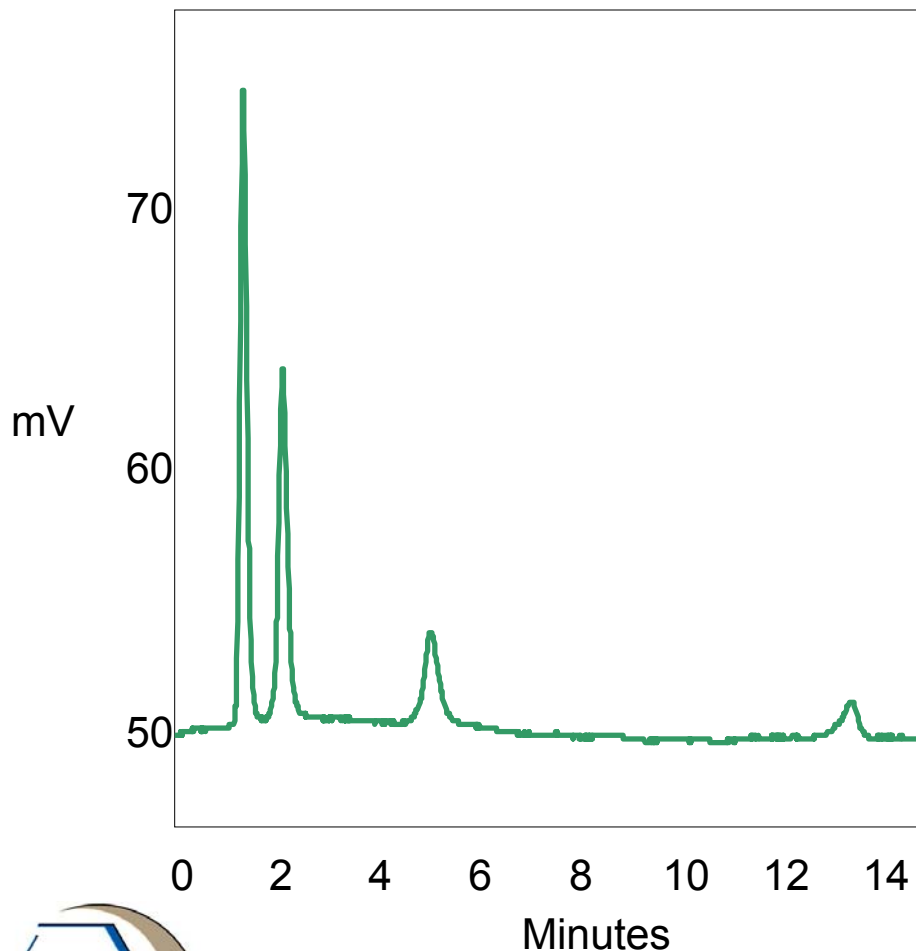
1-Propanol

2-Butanol

1- Butanol



Separation of Glycols Using Water and FID Detection



Instrument: Aquachrom™ Green Machine

Oven profile: 50°C ramp to 135°C at 10°C/min hold 10 min.

Column: Jordi DVB, 2.1 X100 mm, 5µm

Mobile phase: 0.025%TFA

Split flow: 250 psi back pressure

Pump flow: 0.500 mL/min

Detection: FID @ 400°C

Elution Order:

Ethylene Glycol

Diethylene Glycol

Triethylene Glycol

Styrene Glycol

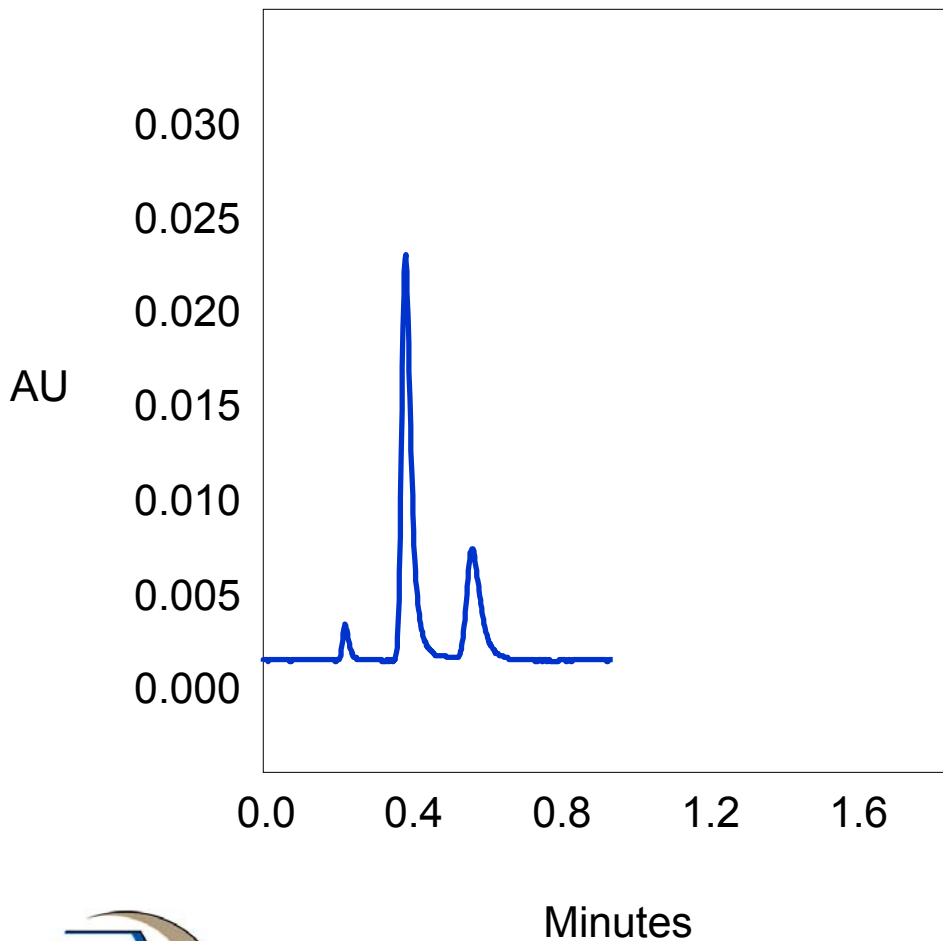


Aquachrom™ Instrument

- Temperature programmable HPLC oven
- FID for universal detection
- Compatibility with any LC system and detector



Separation of Amino Acids Using Water as the Mobile Phase



Instrument: Polaratherm™ Total Temperature Controller
Temperature: 75°C
Column: Experimental C18 Silica, 2.1 X100 mm, 5µm (used as high as 200°C)
Mobile phase: Water
Pump flow: 1.0 mL/min
Detection: UV @ 254nm

Elution Order:
Tyrosine
Phenylalanine
Tryptophan



Conclusions

- Pure water is useful as an LC mobile phase, and is compatible with the FID.
- Water only separations are possible when temperature is used to alter retention and selectivity instead of organic modifier in the mobile phase
- Temperature programming can focus peaks and elute compounds over a wider polarity range than isothermal methods.



Acknowledgements

Many thanks to Jordi FLP who provided packing material used in this work

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Turn up the Heat!

