# Water Only HPLC Separations with Universal Flame Ionization Detection Using the Aquachrom™ Green Machine

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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

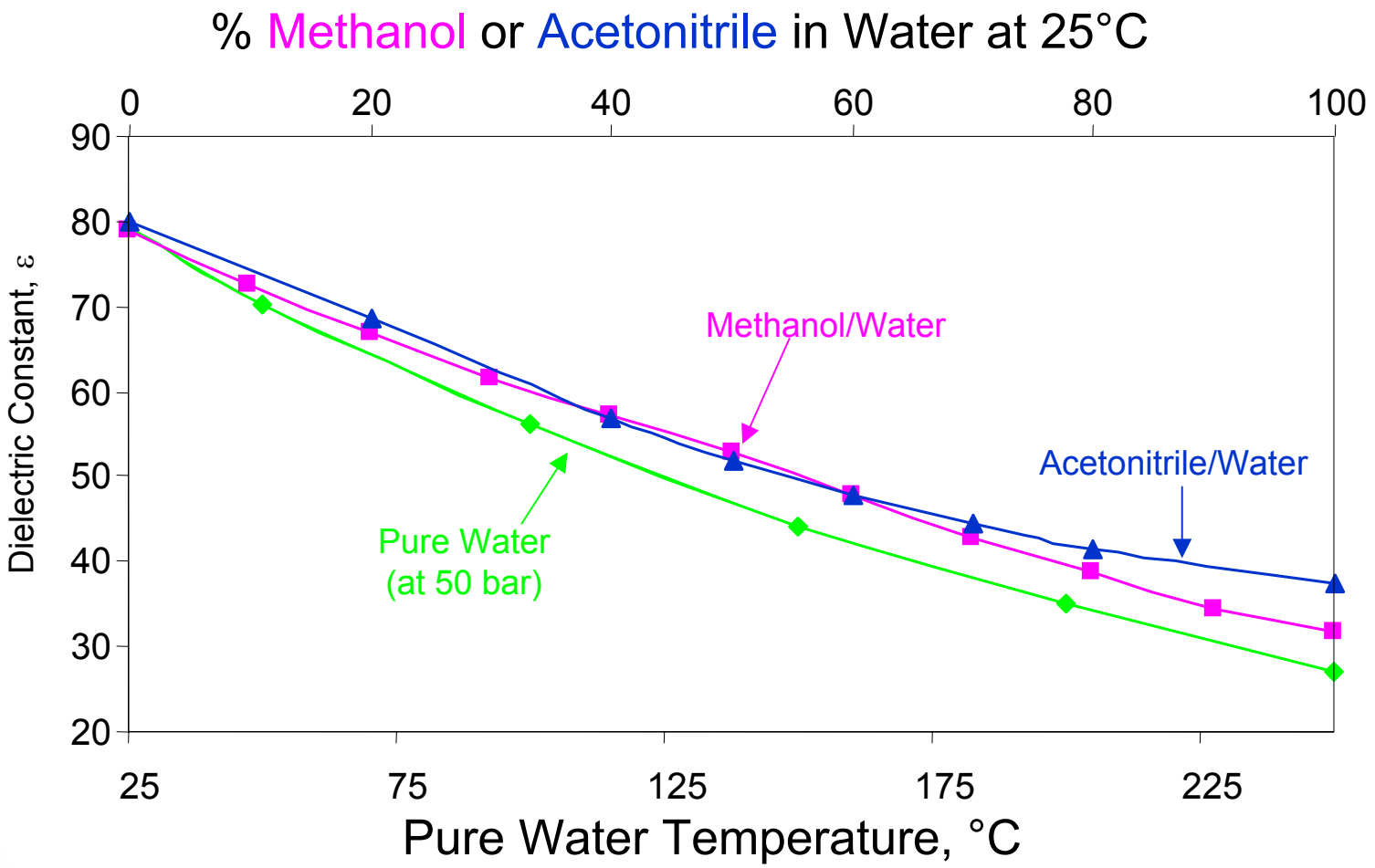
$$k_1' / k_2' = \exp(\Delta H(T_2 - T_1) / (RT_1T_2)$$

- 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



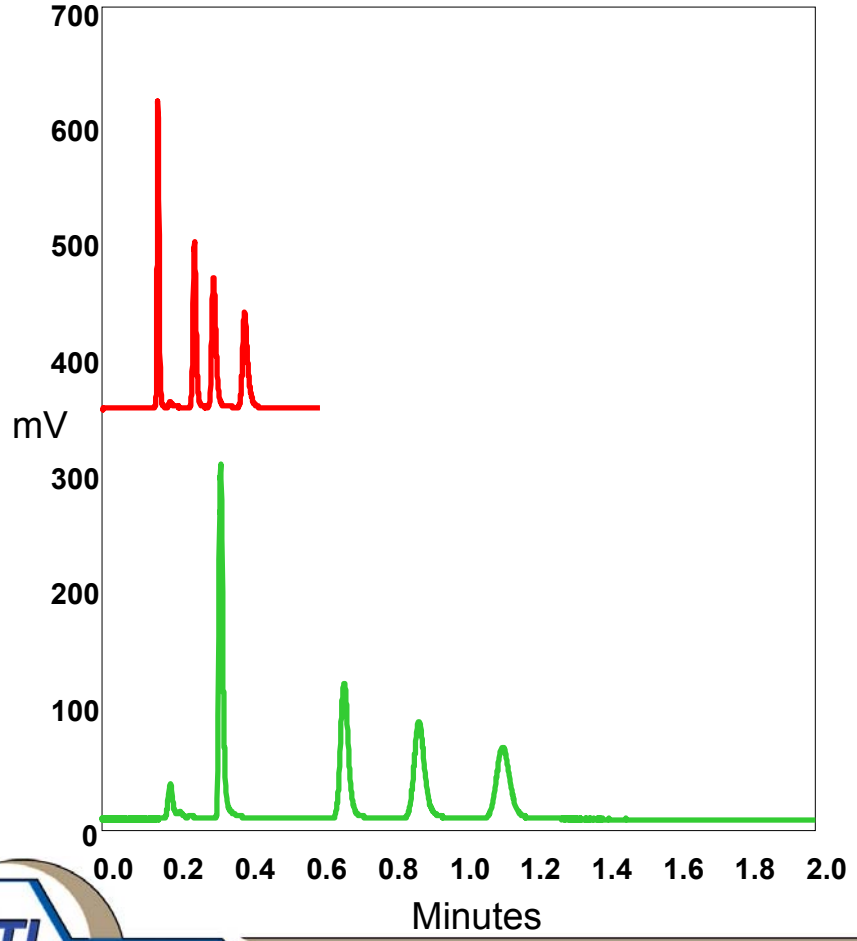

Data from Y. Yang et al. *J. Chromatogr. A* **810** (1998) 149.

# Temperature and Elution Strength

- Increasing temperature 4 to 5 ºC is comparable to increasing the methanol or acetonitrile concentration by 1% in a reversed phase system
- Viscosity is reduced 1 to 2 % per ºC 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

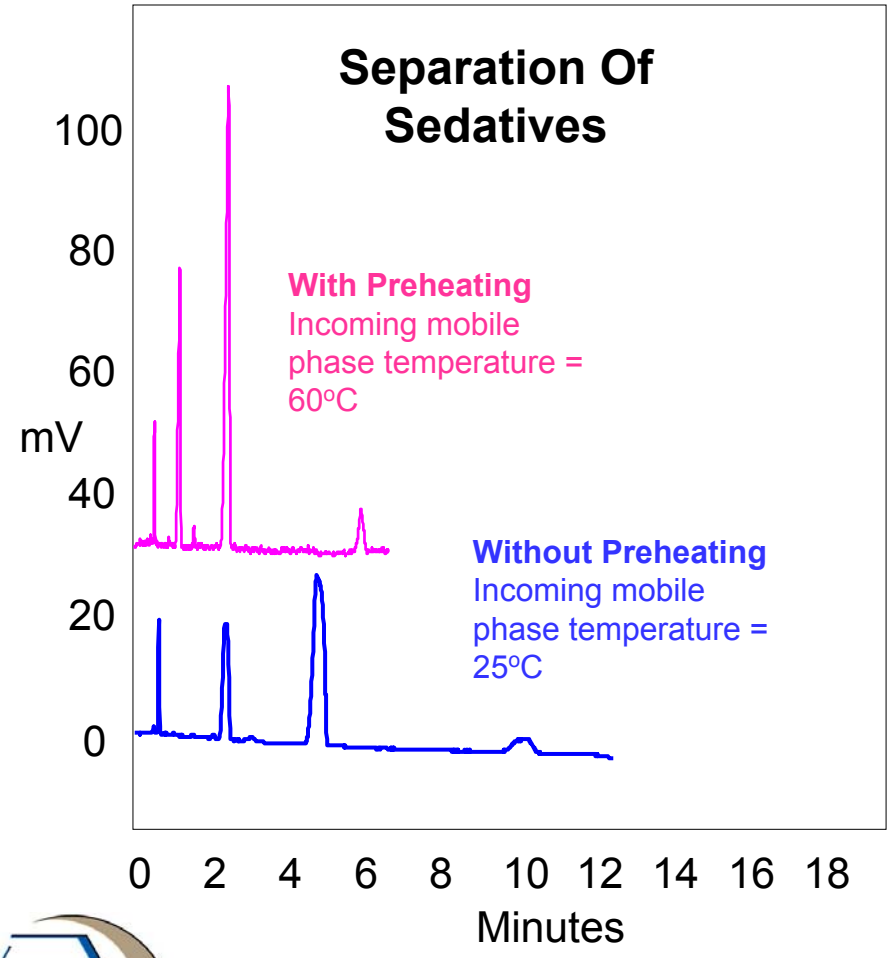
**Elution Order:**
Uracil
Androstadienedione
Androstenedione
Epitestosterone

# 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

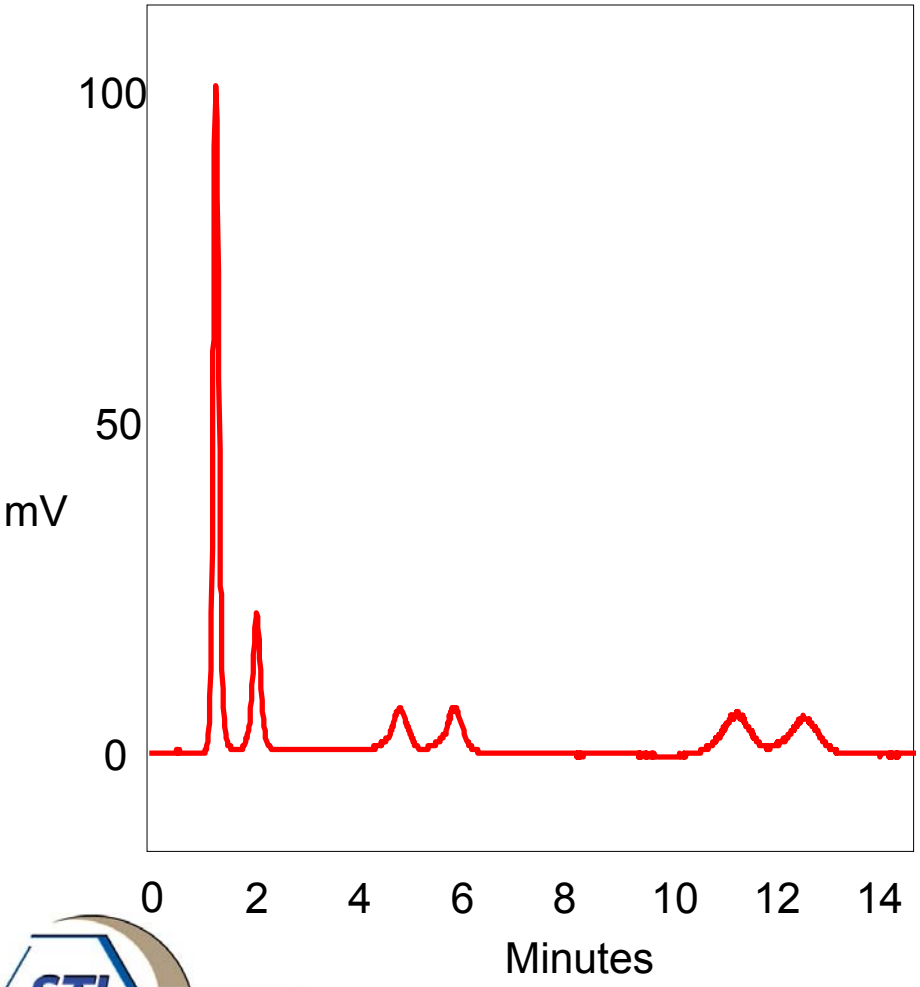
## Essential for good chromatography at elevated temperatures




**Instrument:** Polaratherm Total Temperature Controller
**Oven profile:** 60°C isothermal
**Column:** Zobax SB-C18 150 x 4.6 mm
**Mobile phase:** 20:80 Acetonitrile:3.0 mM Phosphate pH 7.0
**Flow:** 3.0 mL/min
**Detection:** UV

**Elution Order:**
Uracil
Butalbarbital
Phenobarbital
Carbromal

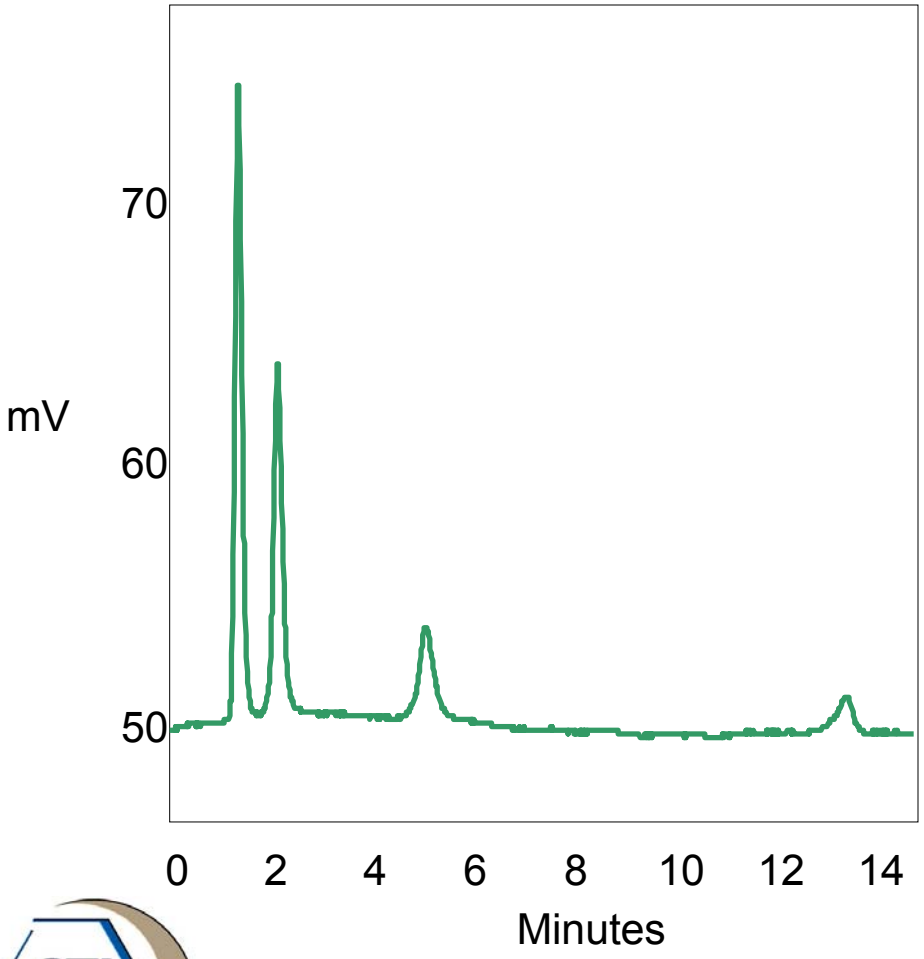
# Separation of Alcohols Using Water and FID Detection




**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

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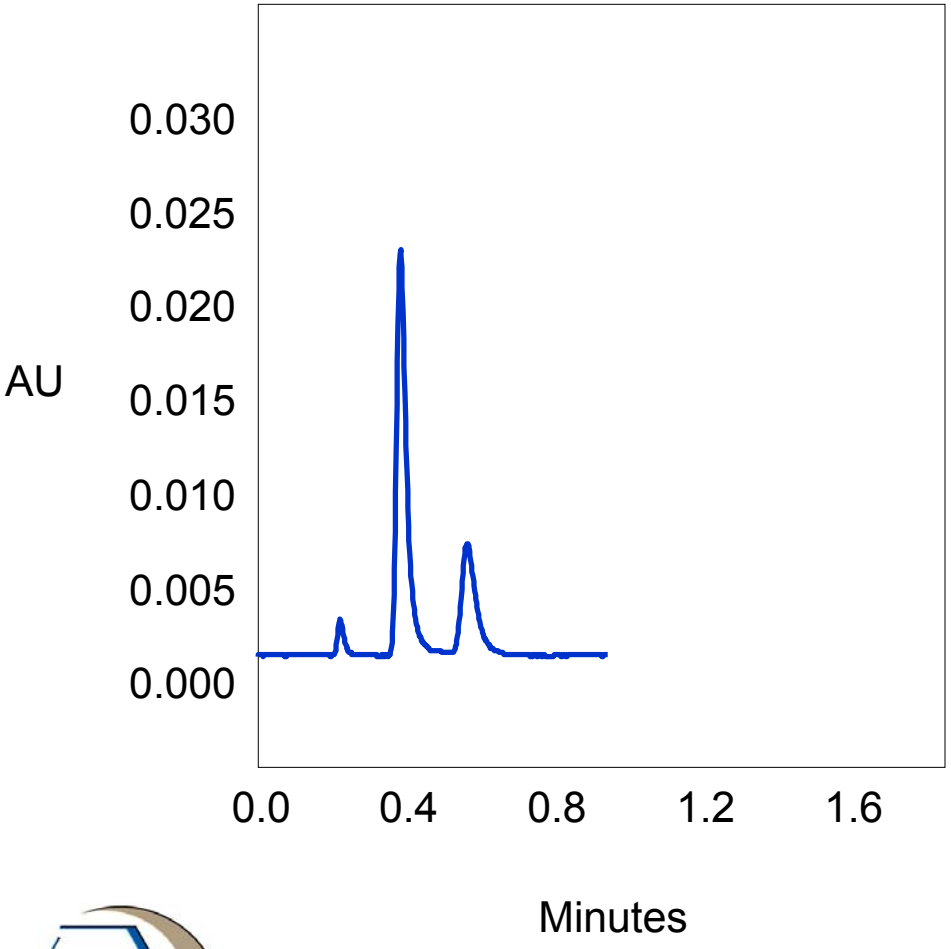
**Column:** Jordi DVB, 2.1 X100 mm, 5µm
**Mobile phase**: 0.025%TFA
Split flow: 250 psi back pressure
**Pump flow**: 0.500 mL/min
**Detection**: FID @ 400°C

**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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