Use and Practicality of Water-Only HPLC Separations

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Introduction

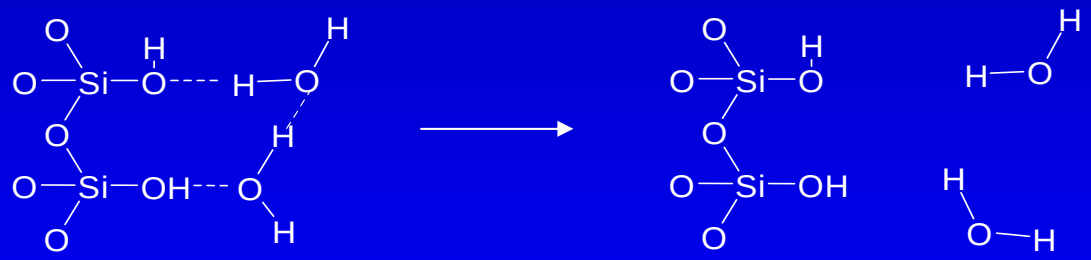
- Properties of water
- Advantages of using temperature
- Rules of thumb for determining the feasibility of using pure water as the mobile phase for separations
- Practical applications

Why Use 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

Unique Properties of Water are Primarily Due to Hydrogen Bonding

- Increasing temperature
  - Increases intermolecular distance
  - Weakens hydrogen bonds



- Results in
  - Decreasing density and viscosity

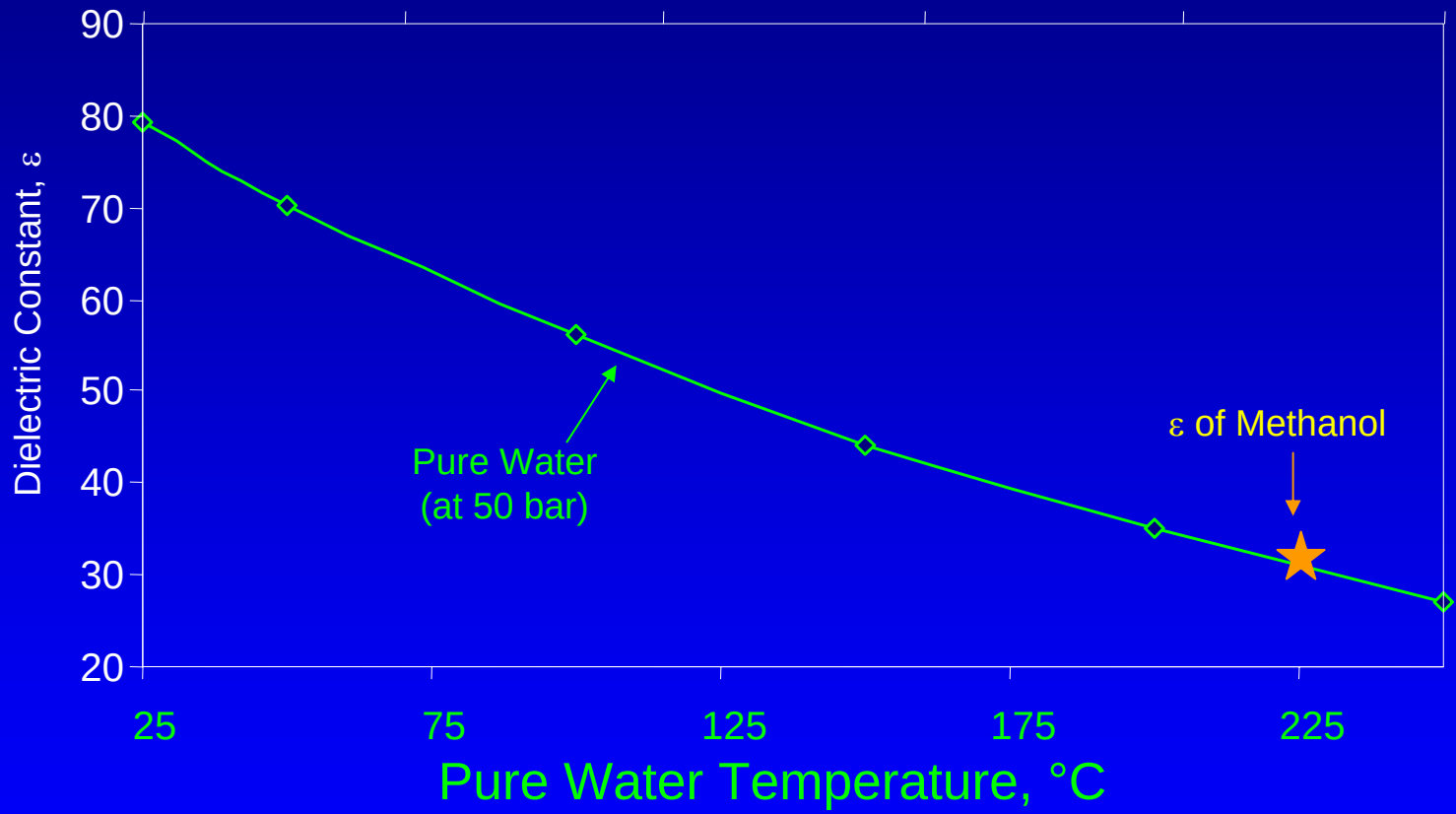
Structure of High Temperature Water (HTW)

- HTW is structurally different from ambient liquid water
  - An infinite percolating network of H-bonds exist in ambient water
  - Small clusters of H-bonded water molecules exist in HTW
  - As the temperature increases and the density decreases the average cluster size decreases
  - The breaking of the H-bond network reduces the barrier for translational and rotational motion

Changes in the Dielectric Constant

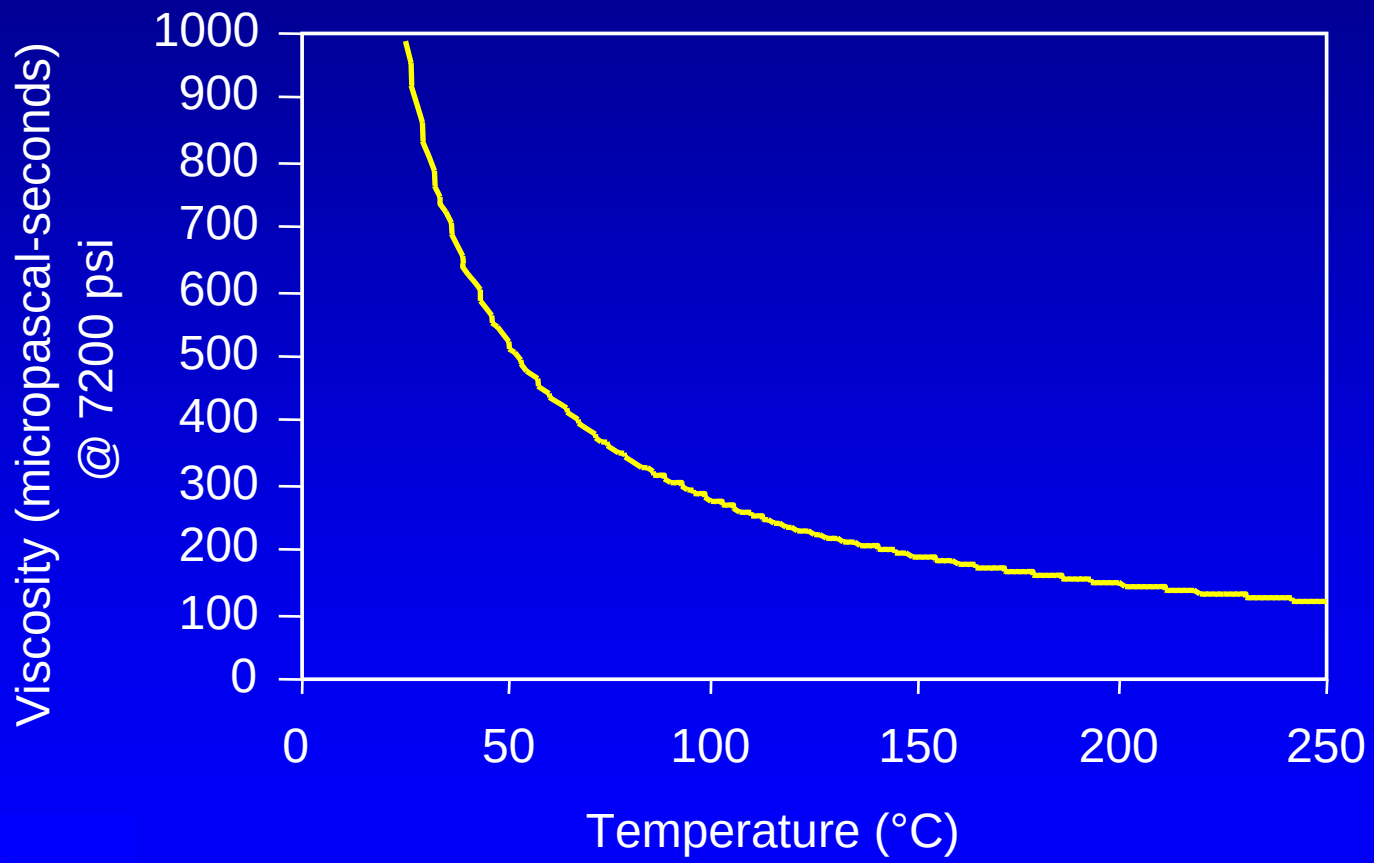
- Changes in the extent of hydrogen bonding are accompanied by corresponding changes in the dielectric constant
  - With increasing temperature and decreasing density, the dielectric constant of water decreases
  - HTW behaves more like polar organic solvents
  - Small organic compounds are highly soluble in HTW

Solvent Polarity as a Function of Temperature



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

Viscosity of Water vs. Temperature

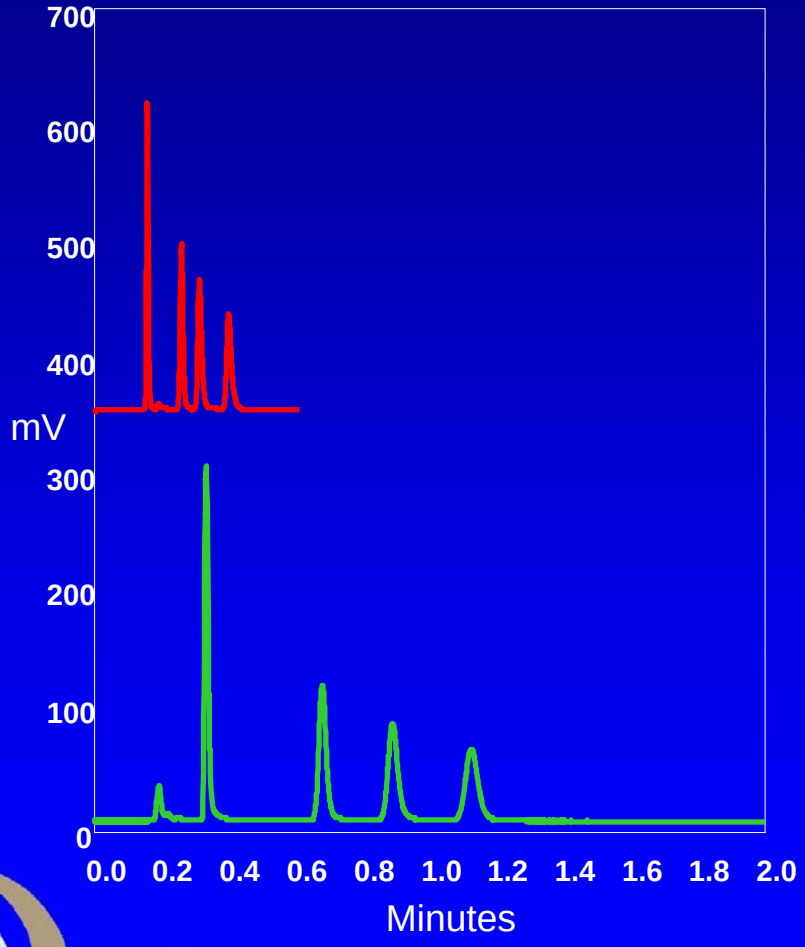


--T. S. Kephart and P. K. Dasgupta, *Talanta* **56** (2002) 977-987

High Temperature Liquid Chromatography (HTLC) Advantages

- Decreasing analysis time
  - Due to ease of increasing linear velocity
  - Due to a generally decreasing retention for most compounds
- Increasing efficiencies and resolution
- Selectivity tuning
- Decreasing organic solvent usage

Chromatographic Example of Using High Temperature



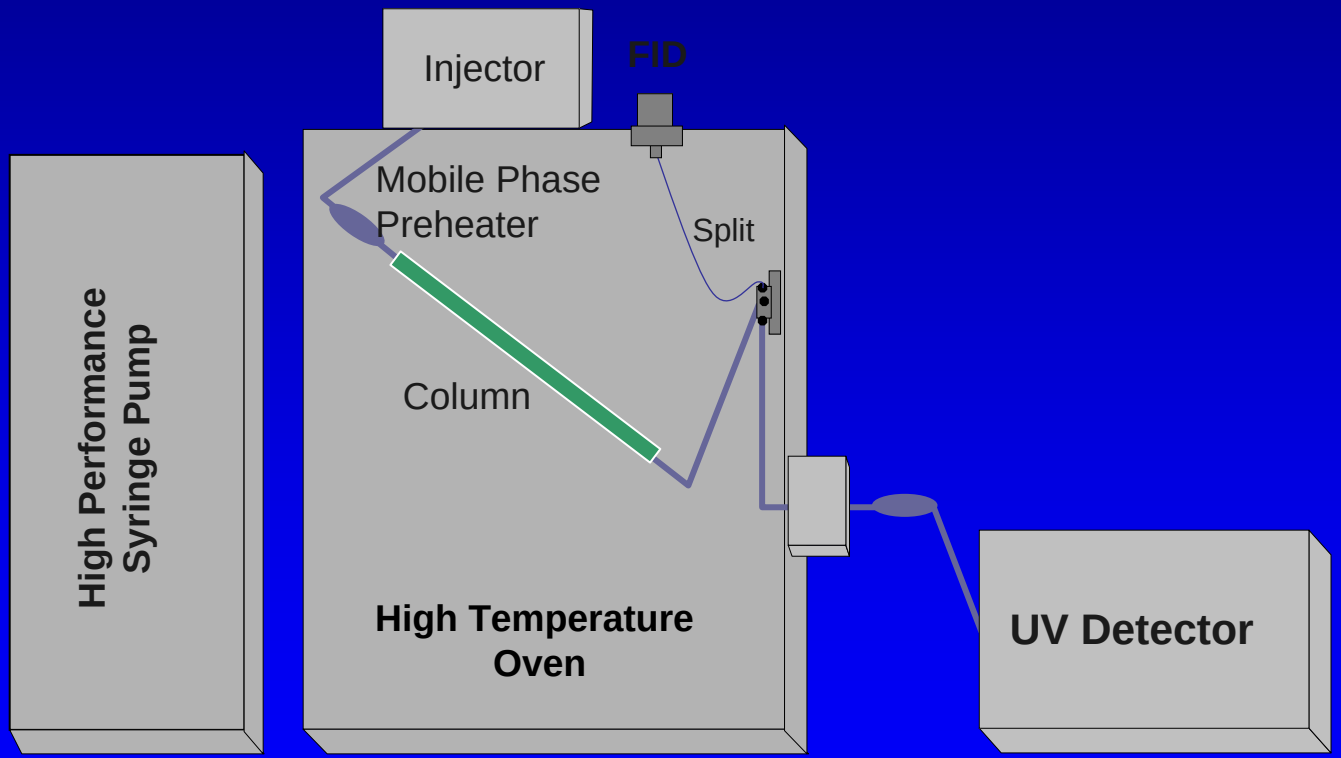
Column: ZirChrom PBD, 3 µm
100 X 4.6 mm
Detection: UV 254 nm

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

Instrument Diagram



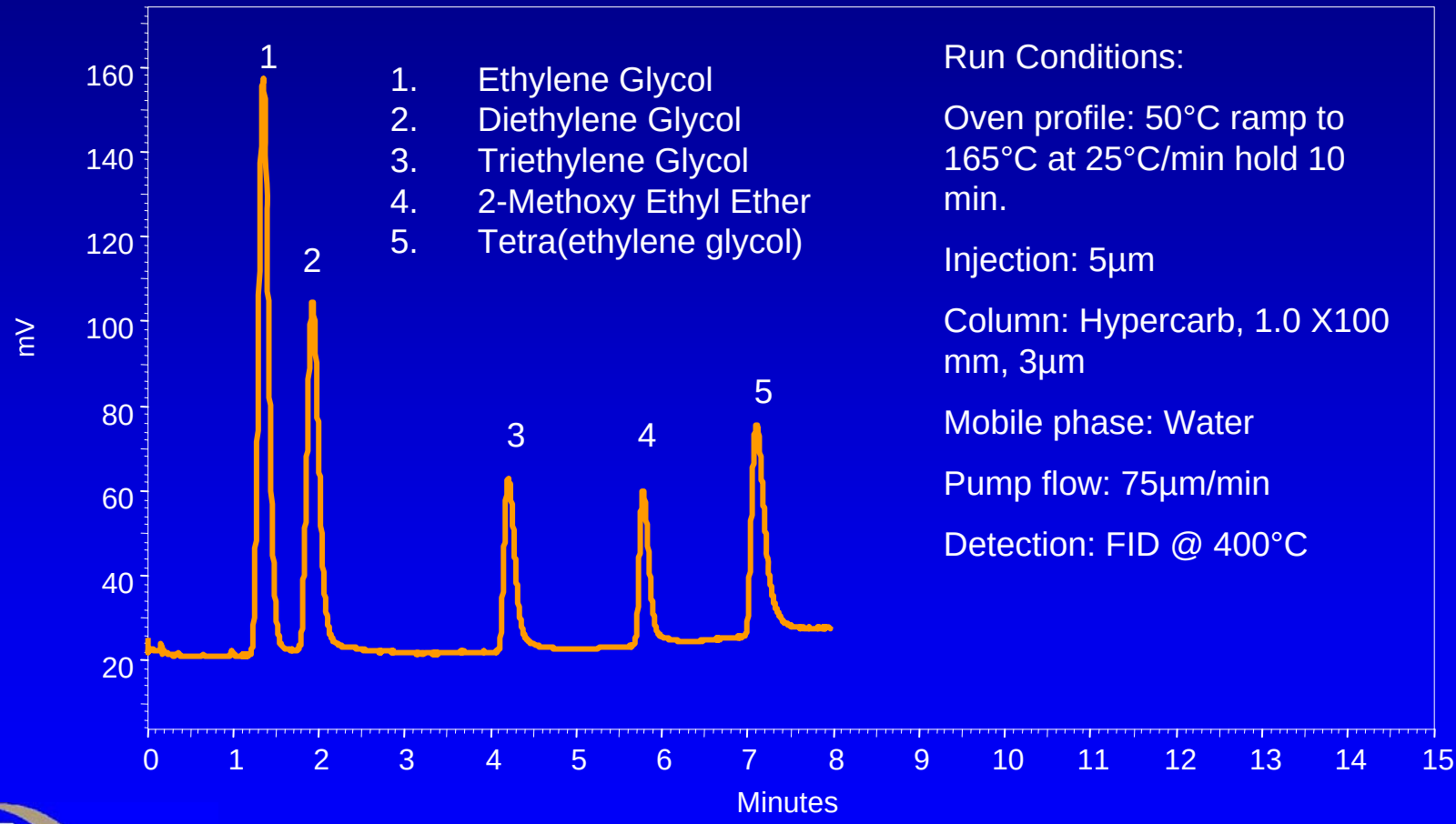
Columns Available for Water Separations

- Polymer (temp. limit: 150-175°C)
- Graphitic Carbon (temp. limit: above 200°C)
- Bridged Ethyl Hybrid (temp. limit: 200°C)
- Selected Silica-highly cross linked (temp. limit: 100-150°C)

Rules of Thumb

- Analytes that are good candidates for using water as the mobile phase
  - Soluble in water
  - Slightly soluble in water
  - Insoluble in water but soluble in alcohols, glycerin, and acidified water

Glycols on 1.0mm ID Hypercarb™



1. Ethylene Glycol
2. Diethylene Glycol
3. Triethylene Glycol
4. 2-Methoxy Ethyl Ether
5. Tetra(ethylene glycol)

Run Conditions:

Oven profile: 50°C ramp to 165°C at 25°C/min hold 10 min.

Injection: 5µm

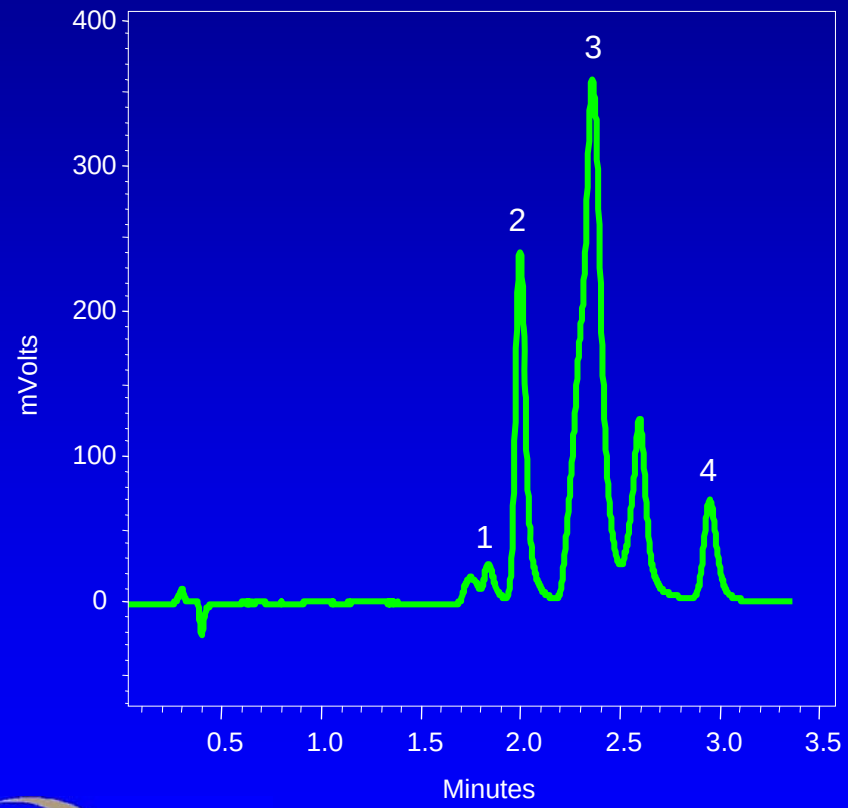
Column: Hypercarb, 1.0 X100 mm, 3µm

Mobile phase: Water

Pump flow: 75µm/min

Detection: FID @ 400°C

Analytes Soluble in Water (found in diet cola)

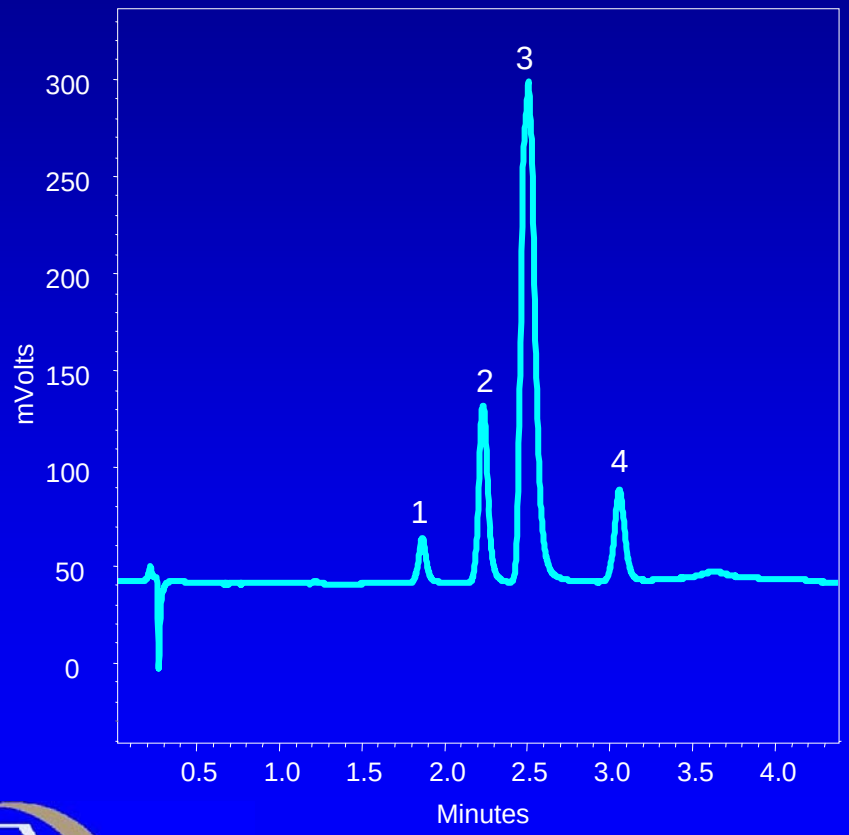


Column: Xbridge C8, 3.5 µm
150 X 2.1 mm
Detection: UV 214 nm

Flow Rate: 1.5 mL/min
Mobile Phase: Water
Temperature: 110°C

Elution Order:
Aspartame
Caffeine
Sodium Benzoate
Acesulfame K

Addition of ~0.005% Formic Acid and Temperature Programming

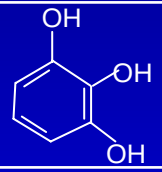
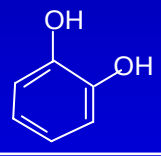
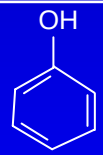
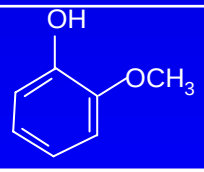


Column: Xbridge C8, 3.5 μm
150 X 2.1 mm
Detection: UV 214 nm

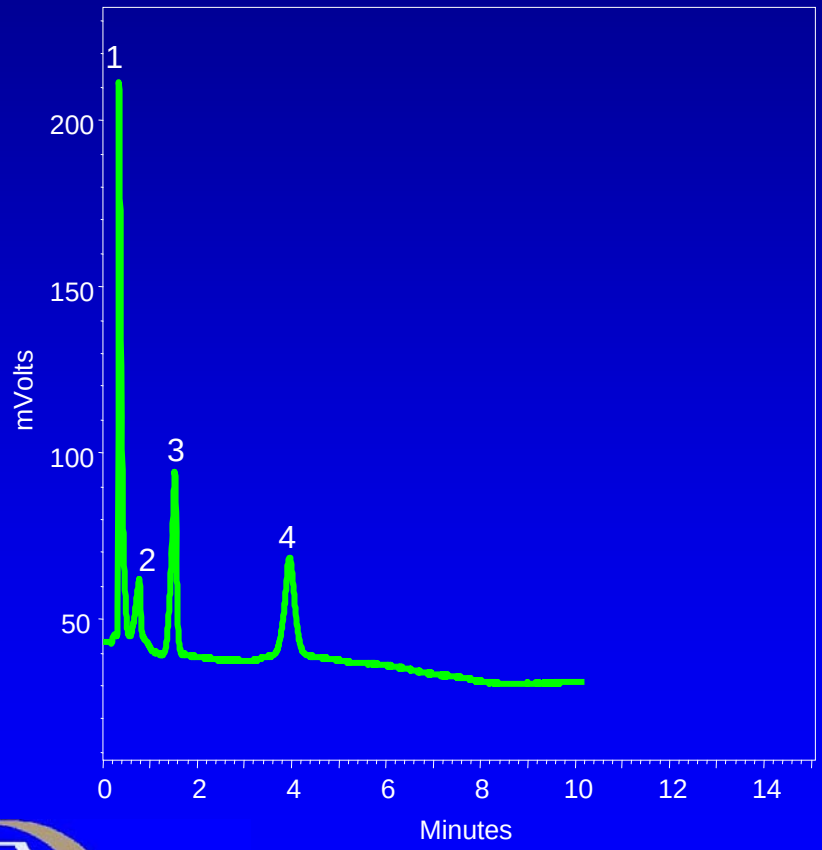
Flow Rate: 1.5 mL/min
Mobile Phase: 0.005% formic acid in water
Temperature: 95°C
hold 2 min.
ramp to 110°C @ 10°C/min.

Elution Order:
Aspartame
Caffeine
Sodium Benzoate
Acesulfame K

Compounds Slightly Soluble

Name	Structure	Solubility
Pyrogallol		~590 g/L
Catechol		~450 g/L
Phenol		~80 g/L
Guaiacol		~16 g/L

Hydroxyphenols Eluting in Order of Analyte Affinity for Water

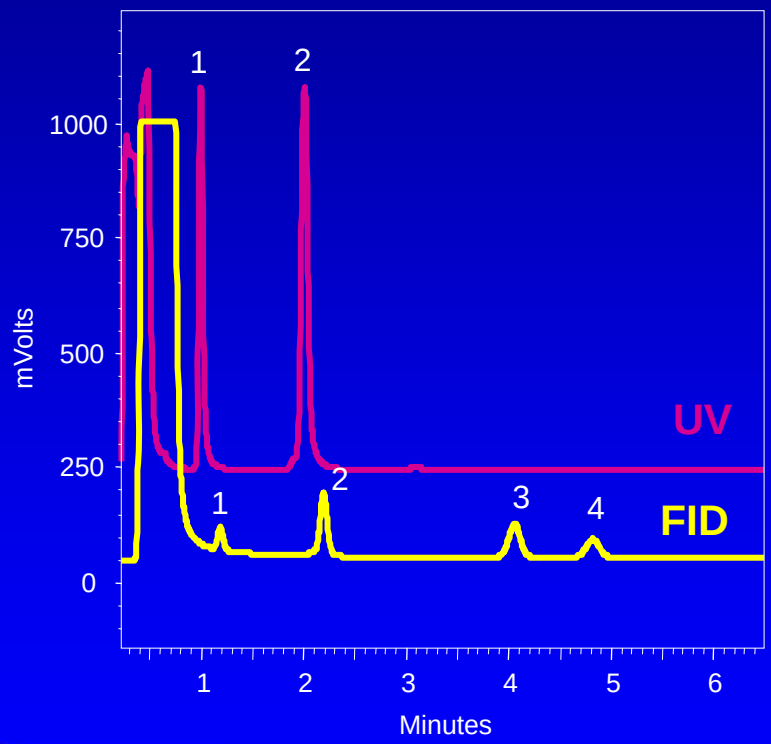


Column: Polymer Labs PLRP-S, 3.0 μm 150 X 2.1 mm
Detection: UV 254 nm

Flow Rate: 1.5 mL/min
Mobile Phase: **Water**
Temperature: 175°C

Elution Order:
Pyrogallol
Catechol
Phenol
Guaiacol

Alcohol Based Mouthwash

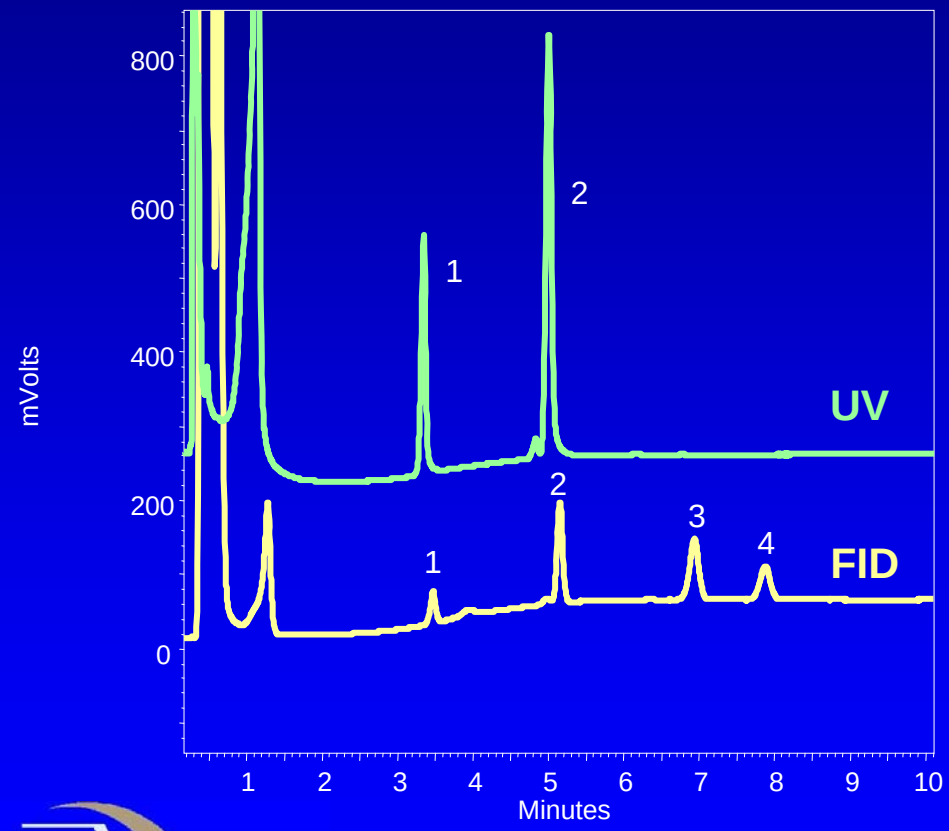


Column: Xbridge C18, 3.5 μm
150 X 2.1 mm
Detection: UV 254 nm
FID @ 400°C

Flow Rate: 1.0 mL/min
Mobile Phase: Water
Temperature: 200°C
Split Ratio: 9:1 with 0.1 mL/min into FID

Elution Order:
Methyl Salicylate
Thymol
Eucalyptol
Menthol

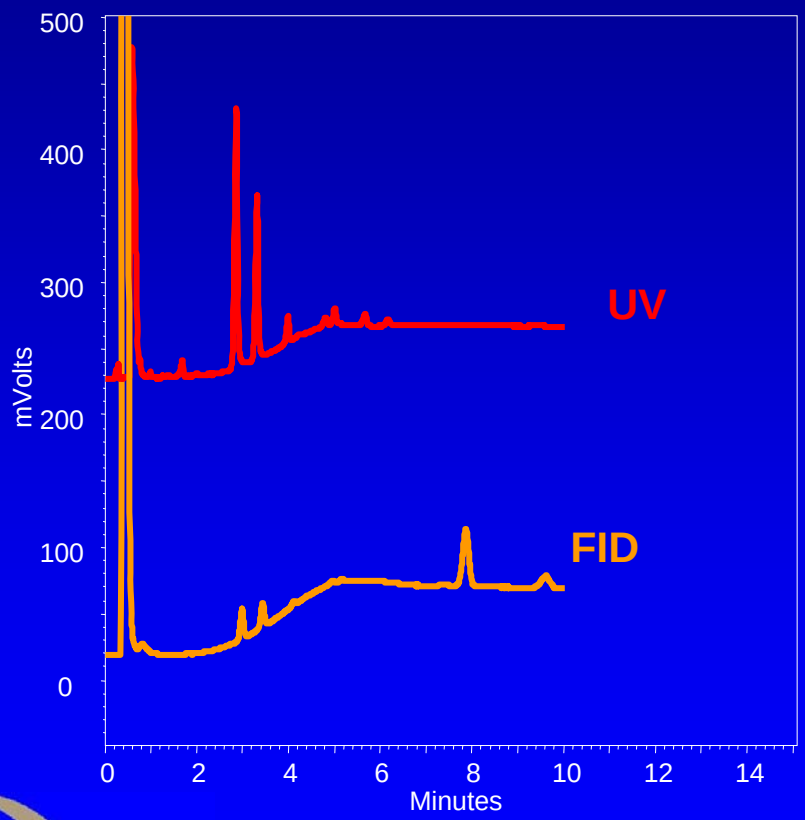
Flexibility with Temperature Programming (Alcohol Based Mouthwash)



Column: Xbridge C18, 3.5 μm
150 X 2.1 mm
Detection: UV 254 nm
FID @ 400°C
Flow Rate: 1.5 mL/min
Mobile Phase: Water
Temperature: 125°C
hold 1 min.
ramp to 200°C @ 25°C/min.
Split Ratio: 14:1 with 0.1 mL/min into FID

Elution Order:
Methyl Salicylate
Thymol
Eucalyptol
Menthol

Glycerin Based Mouthwash



Column: Xbridge C18, 3.5 μm
150 X 2.1 mm
Detection: UV 254 nm
FID @ 400°C
Flow Rate: 1.5 mL/min
Mobile Phase: Water
Temperature: 135°C
hold 1 min.
ramp to 200°C @ 25°C/min.
Split Ratio: 14:1 with 0.1 mL/min into FID

Most Practical Use of Using Water as the Mobile Phase

- Gain use of the flame ionization detector (FID)
  - Mass sensitive detector
  - Response is proportional to the number of carbon atoms being burned
  - Wide linear range, about 10^8
  - Responds with high sensitivity to organic compounds

Conclusion

- The use of water as a mobile phase is possible when used with temperature for compounds that are soluble in water, alcohol and glycerin
- Great choice when analyzing compounds that do not contain chromophores

Acknowledgements

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