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$_2$O)
• 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


Dielectric Constant, $\varepsilon$

Pure Water (at 50 bar)

$\varepsilon$ of Methanol

Pure Water Temperature, °C

25 75 125 175 225
Viscosity of Water vs. Temperature

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
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:**
1. Aspartame
2. Caffeine
3. Sodium Benzoate
4. 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

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrogallol</td>
<td><img src="image" alt="Pyrogallol Structure" /></td>
<td>~590 g/L</td>
</tr>
<tr>
<td>Catechol</td>
<td><img src="image" alt="Catechol Structure" /></td>
<td>~450 g/L</td>
</tr>
<tr>
<td>Phenol</td>
<td><img src="image" alt="Phenol Structure" /></td>
<td>~80 g/L</td>
</tr>
<tr>
<td>Guaiacol</td>
<td><img src="image" alt="Guaiacol Structure" /></td>
<td>~16 g/L</td>
</tr>
</tbody>
</table>
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

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[Graph showing UV and FID detection with peaks labeled 1, 2, 3, 4 and corresponding mVolts and Minutes axes.]
Glycerin Based Mouthwash

**Flow Rate:** 1.5 mL/min

**Mobile Phase:** Water

**Temperature:**
- Hold 1 min.
- Ramp to 200°C @ 25°C/min.

**Column:** Xbridge C18, 3.5 μm 150 X 2.1 mm

**Detection:**
- UV 254 nm
- FID @ 400°C

**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.
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