At-Column Dilution for Increased Purification Performance

Uwe D. Neue
Thomas E. Wheat
Cecilia B. Mazza
Jie Y. Cavanaugh
Waters Corporation, 34 Maple St
Milford, MA 01757, USA

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Outline

- At-Column-Dilution Techniques
  - Sample in organic solvent
  - Sample in ionic form
- Reason for Difficulties with DMSO
- Conclusion
At-Column-Dilution Techniques

- Sample in organic solvent
- Sample in ionic form

Reason for Difficulties with DMSO

Conclusion
Purpose of At-Column Dilution

The problems

- Sample solubility in the mobile phase – especially in gradient chromatography
- Can be improved by using a sample solvent that is a strong eluent (e.g. DMSO)
- A sample in the salt form is highly soluble in water, but the salt form has the disadvantage of low retention for preparative chromatography

The solution:

- At-Column Dilution
Supplementary Techniques: At-Column-Dilution

Standard System

At-Column-Dilution System

- **Autosampler**
- **Gradient Pump**: 30mL/min 95:5
- **Column**
- **Loading Pump**: 1.5 mL/min 100% CH₃CN

- **Autosampler**
- **Gradient Pump**: 28.5 mL/min gradient mobile phase
- **Tee**: 30mL/min 95:5
- **Column**
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Solving Sample Problems: Sample Dissolved in DMSO

- Sample pump feeds MeCN at 1/20\textsuperscript{th} of the total flow
- Gradient system delivers gradient from 0\% to 90\% at 19/20\textsuperscript{th} of the total flow
- No peak distortion due to DMSO - high load of 10 \textit{mg/g} possible!
Sample in DMSO

Sample: 20 mg/mL\(^{-1}\) each of diphenhydramine, oxybutynin and terfenadine in 2 mL of DMSO
Column: XTerraPrep\textsuperscript{®} MS C\textsubscript{18} 19 mm x 30 mm with 19 mm x 10 mm guard cartridge

Same sample, at-column dilution

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Synthesis of XTerra® Particles

Tetraethoxysilane

Organofunctional triethoxysilane

Polyethoxyoligosiloxane Polymer

Porous Hybrid Particles

First generation Hybrid, $R = \text{CH}_3$

Characterized by %C, SEM, TGA, BET, NMR

Patent Pending

$SA = 140 - 330$ m$^2$/g

$TPV = 0.4 - 1.0$ cc/g

$MPD = 90 - 300$ Å
At-Column-Dilution Example 3: under Basic Conditions

Diphenhydramine 20 mg/mL, Load 40 mg
Column: XTerra® MS C18 19 X 50 mm
Monitor: 254 nm

With At-Column Dilution

Diphenhydramine 20 mg/mL, Load 40 mg
Column: XTerraPrep® MS C18 19 X 50 mm
Monitor: 254 nm

Buffers
A 100% H2O Flow rate: 30 ml/min
B Acetonitrile
C 100 mM NH4HCO3, pH 10

Gradient
<table>
<thead>
<tr>
<th>Time</th>
<th>Flow</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30</td>
<td>90</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>2.5</td>
<td>30</td>
<td>90</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>2.6</td>
<td>30</td>
<td>55</td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>5</td>
<td>90</td>
<td>5</td>
</tr>
<tr>
<td>6.3</td>
<td>30</td>
<td>5</td>
<td>90</td>
<td>5</td>
</tr>
<tr>
<td>6.6</td>
<td>30</td>
<td>90</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
DMSO is often the preferred solvent
DMSO is a strong eluent in RPLC
Loading in the solvent used at the beginning of the gradient is preferred
At-column dilution makes this possible
Significant increases in loadability (5- to 10-fold) are possible
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Solving sample problems: Polar ionic sample in salt form

- 50-100x higher load if sample is not ionic
- Sample pump feeds sample in ionic form
- Sample is converted to non-ionic form at a high buffer concentration at the beginning of the gradient
- Reversed-phase gradient is executed at low buffer concentration
Loadability Improvement at High pH (800 mg Load)

Column: XTerra® Prep MS C_{18} 19 x 50 mm, 5 µm
Gradient: Equilibrated for 5 min at 5% ACN, then gradient \( tg = 5 \text{ min, } 5 \text{ to } 90 \% \text{ ACN, and hold at } 90\% \text{ ACN for } 1 \text{ min,} \) The mobile phases contain 10 mM NH_{4}HCO_{3}, pH 10.0.
Flow Rate: 30 mL/min.
Analyte: Diphenhydramine (800 mg) dissolved in H_{2}O.

With At-Column Dilution:
200 mM NH_{4}HCO_{3}, pH 10

Standard Loading from Water

Diphenhydramine
Hydrochloride salts of basic compounds give low retention in reversed-phase HPLC due to the ionization of the sample.

The solubility of this sample at high pH is low, since the sample is non-ionic.

However, using the at-column-dilution methodology, loading the sample with water and doing chromatography at high pH eliminates the precipitation and we take advantage of high loadability of bases at high pH.
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Propyl Gallate
Sample dissolved in DMSO
Chromatography run at pH 3.8

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At-Column Dilution Improves Impurity Isolation

5.6X increase in mass load by using DMSO as sample solvent and at-column-dilution method

Sample: Propyl Gallate dissolved in DMSO

XTerra® MS C$_{18}$ 4.6 x 50 mm
Load: 1.8 mg

XTerra® MS C$_{18}$ 19 x 50 mm
At-Column Dilution
Load: 30 mg
Injection volume: 0.3 mL
Comparison of Sample Solvents

- Acetonitrile
- Dimethylsulfoxide
- Isopropanol
- Methanol
- Tetrahydrofuran

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### Viscosity Maxima of Mixtures of Water with Solvent

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Viscosity (Neat Solvent) [cP]</th>
<th>Viscosity Maximum [cP]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>0.37</td>
<td>1.1</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>2.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>2.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>0.46</td>
<td>1.9</td>
</tr>
</tbody>
</table>

The **hydrophobicity** of the solvent is only part of the problem. The high **viscosity** of DMSO/water mixtures is the primary cause of the difficulties with DMSO!!!
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At-Column Dilution Conclusions

- Successfully demonstrated the use of at-column-dilution with drugs dissolved in DMSO
- Successfully demonstrated the use of at-column-dilution with bases dissolved in water and carried out the chromatography at high pH.
- Generally, at-column dilution solves the conflict between good sample solubility and good chromatography
- The primary reason for the difficulties with DMSO is the viscosity of the DMSO/water mixture
Diagram of At-Column Dilution

Water with a low buffer concentration feeds the sample.

Gradient starts at a high buffer concentration to load sample onto column in a non-ionic form at the beginning of the gradient.
At-Column Dilution: Example 1

- Samples: Sulfadruugs
- Evaluation of Injection Conditions
- Large Sample Load: 800 mg
- Conditions:

  Column: Symmetry® C₁₈, 19 mm x 50 mm, 5 µm
  Gradient: A: Water; B: Acetonitrile; C: 1% Formic Acid
      0 - 0.5 min: 85% A, 5% B, 10% C
      5.5 min: 0% A, 90% B, 10% C
  Flow Rate: 30 mL/min
  Samples: Sulfanilamide, Sulfathiazine and Sulfasoxazole
  Sample dissolved in DMSO
At-Column Dilution:
Monitor All Components

800 mg Total Load in 2000 µL DMSO on a 19 mm x 50 mm 5 µm Symmetry® C₁₈ Column

Standard Injection

At-Column Dilution

Wheat
Sample Dissolved in DMSO: Single-Ion Chromatogram of Peak 3

Standard Injection

Same Compound !!!

At-Column Dilution

800 mg Total Load in 2000 µL DMSO on a 19 mm x 50 mm, 5 µm Symmetry® C₁₈ Column

Wheat