Tips and tricks to increase HPLC column lifetime

Dr. Frank Michel
Overview

How to influence HPLC column life time?
- HPLC method
- Helpful accessories
- Best practice
HPLC Method – Mobile Phase

Mobile phase is impacting HPLC column lifetime most strongly:

- Concentration of organic modifier
- pH value
- Buffer salts
HPLC Method – Mobile Phase
Concentration of organic modifier

Higher concentration of organic modifier are more gentle to stationary phase
Advantages of acetonitrile compared to MeOH
Acetonitrile (and its mixtures with water) are less viscous compared to MeOH
HPLC Method – Mobile Phase

pH value

pH > 8: degradation of silica
pH < 2: hydrolysis of siloxane bonding of stationary phase

**Alternatives:** zirconia-based HPLC columns (Discovery Zr, etc.), HPLC columns based on polymers (e.g. apHera C18 or NH$_2$), ion pair reagents
Phosphate is most often applied as buffer

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pKa (25°C)</th>
<th>Max. Buffer range</th>
<th>UV Cutoff (nm)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trifluoroacetic Acid</td>
<td>0.3</td>
<td>---</td>
<td>210 (0.1%)</td>
<td>* not really a buffer</td>
</tr>
<tr>
<td>Oxalate 1</td>
<td>1.27</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Phosphate 1</td>
<td>2.1</td>
<td>1.1-3.1</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Citrate 1</td>
<td>3.1</td>
<td>2.1-4.1</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>Formate</td>
<td>3.8</td>
<td>2.8-4.8</td>
<td>210 (10mM)</td>
<td>* Volatile buffer</td>
</tr>
<tr>
<td>Oxalate 2</td>
<td>4.27</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Citrate 2</td>
<td>4.7</td>
<td>3.7-5.7</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Acetate 1</td>
<td>4.8</td>
<td>3.8-5.8</td>
<td>210 (10mM)</td>
<td>* Volatile buffer</td>
</tr>
<tr>
<td>Citrate 3</td>
<td>5.4</td>
<td>4.4-6.4</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Phosphate 2</td>
<td>7.2</td>
<td>6.2-8.2</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Tris</td>
<td>8.3</td>
<td>7.3-9.3</td>
<td>205</td>
<td>*Tris(hydroxymethyl)aminomethane</td>
</tr>
<tr>
<td>Borate 1</td>
<td>9.24</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Ammonia</td>
<td>9.25</td>
<td>8.3-10.3</td>
<td>210 (10mM)</td>
<td>* not really a buffer</td>
</tr>
<tr>
<td>Ammonium Bicarbonate</td>
<td>9.25</td>
<td>8.25-10.25</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Ammonium Bicarbonate</td>
<td>10.25</td>
<td>9.25-11.25</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Triethylamine*</td>
<td>10.7</td>
<td>9.7-11.7</td>
<td>200</td>
<td>* Volatile buffer, not really a buffer</td>
</tr>
<tr>
<td>Pyrrolidine</td>
<td>11.3</td>
<td>10.3-12.3</td>
<td>200</td>
<td>* not really a buffer</td>
</tr>
<tr>
<td>Phosphate 3</td>
<td>12.3</td>
<td>11.3-13.3</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Borate 2</td>
<td>12.7</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Borate 3</td>
<td>13.8</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>
HPLC Method – Mobile Phase

Buffer salts

Counter ion of buffer salt impacts degradation of silica, too

pH = 10
Buffer (0.1M):MeOH 1:1
Column: C18
Temp.: 25 °C

Lit: H.A. Classens,
M.A. van Straten, J.J. Kirkland, J. Chrom. A,
728, (1996) 259-270
HPLC Method – Mobile Phase
Buffer salts

Organic buffers (TRIS, citrate, glycine, ...) are more gentle than phosphate
Lower concentrations of buffer salts are more gentle
Impact of counter ion on degradation is quite low compared to impact of different buffers
Filtration after preparation of buffers
Addition of sodium azide
Maximum life time of buffers and aqueous mobile phases:
  • 3 days without preservative
  • < 15% org. solvent: 1 Month
  • > 15% org. solvent: 3 Months
Stationary phases based on type A silica are degraded faster than phases based on type B silica.
Non-polar stationary phases generally exhibit a longer lifetime than polar ones.
Phases with endcapping are more durable than non-endcapped.
Special phases (zirconia-based, polymer-based)
HPLC Method – Temperature

The lower, the better
Helpful accessories - Filter

Particles in mobile phase from following sources:

- Solvent
- Additives
- Precipitates
- Sample
- Abrasive wear from pump & injection valve
Helpful accessories - Filter

Particles in mobile phase from following sources:

- Solvent
- Additives
- Precipitates
- Sample
- Abrasive wear from pump & injection valve
Helpful accessories – High pH value and matrix loaded samples

If extreme pH values can not be avoided:
- Saturation column

Highly matrix loaded samples:
- Sample preparation
- Guard columns
Helpful accessories – Guard columns

Low dead volume
Direct-Connect

Stand-Alone Hardware

Direct-Connect Hardware
Helpful accessories – Guard columns

Column: SUPELCOSIL LC-8 (58220-U), 15cm x 4.6mm, 5μm particles (Figure F1), with Supelguard™ LC-8 guard column (59554), 2cm, 5μm packing (Figure F2)
Temp.: ambient
Mobile Phase: methanol:water (60:40)
Flow Rate: 1mL/min
Chart Speed: 1cm/min for 6 min., then to 10cm/min
Sample: 10μL of test mixture (Cat. No. 58278)

F1 — Analytical Column
1. Uracil
2. Acetophenone
3. Benzene
4. Toluene

F2 — Analytical Column plus 5μm Guard Column

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# Helpful accessories – Guard columns

<table>
<thead>
<tr>
<th>Flow Rate</th>
<th>Column Alone</th>
<th>Column + Guard Column</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>AF</td>
</tr>
<tr>
<td>1mL/min</td>
<td>10525</td>
<td>0.98</td>
</tr>
<tr>
<td>2mL/min</td>
<td>8310</td>
<td>1.01</td>
</tr>
<tr>
<td>3mL/min</td>
<td>6740</td>
<td>1.03</td>
</tr>
</tbody>
</table>

Column: SUPELCOSIL LC-8 (58220-U), 15cm x 4.6mm, 5μm particles, with Supelguard LC-8 guard column (59562), 2cm, 5μm packing

Temp.: ambient
Flow Rate: 1, 2, or 3mL/min
Mobile Phase: methanol:water (60:40)
Sample: 10μL test mix (Cat. No. 58278)

Data represent mean for 3 measurements.
Helpful accessories – Guard columns
Maintaining high efficiency

Modern HPLC particle columns (Fused Core or sub-2 µm) have higher requirements on guard columns:
- Physical protection (more easily clogging, higher cost)
- Chemical protection (higher cost)
- Maintaining high efficiency/performance
- Easy handling for high-pressure systems

Ascentis® Express Guard Columns:
Best practice – Column testing, control cards, logbook

Column testing on a regular basis and documentation
Control cards for easily recognizing changes/issues
Columns logbook (date, column, mobile phase, samples, number of injections, pressure, ...)

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Summary – mobile phase

High content of organic modifier
Preference on acetonitrile instead of methanol
Recommended pH range for silica columns: 2-7
Alternative buffers than phosphate
Buffer concentration as low as possible
Temperature as low as possible
Use of modern HPLC phases (Type B silica)
Summary – helpful accessories & best practice

Filtration of samples prior injection
Filtration of mobile phase
Inlet filter for mobile phase
pH of mobile phase in the range of 2-7, if out of that range: saturation column
Inline filter remove wear & tear from pumps & injection valves (additionally particles from mobile phase)
Guard columns

Column testing on regular basis, control cards, logbook
Dziękuję za uwagę!