ZORBAX Poroshell technology is designed to facilitate extremely rapid HPLC separations at high relative flow rates (high linear velocities). Poroshell particles consist of a solid silica core surrounded by a very thin (0.25 µm) porous silica crust. High linear velocities of mobile phase are tolerated because of the short diffusion distance for molecules moving into and out of this thin crust. Typical flow rates are five times that used for a comparable column packed with totally porous material.

ZORBAX Poroshell 300Extend-C18 is made using the same, reliable, Extend-C18 bonding used for ZORBAX totally porous packings; column lifetime at high pH is extended. Enhanced lifetime of the column at high pH makes the columns useful for atmospheric pressure ionization-electrospray (API-ES) mass spectrometric (MS) detection of peptides and small proteins using NH₄OH in the mobile phase. There are advantages in signal-to-noise (S/N) and in charge state distribution when using NH₄OH at high pH rather than trifluoroacetic acid (TFA) or formic acid at low pH. The following positive-mode spectra demonstrate the effect of the mobile-phase modifier on the MS signal.

### Conditions

<table>
<thead>
<tr>
<th>Column</th>
<th>See column info in figure (operation at ambient temperature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>330-ng Angiotensin I, 1-µL injection</td>
</tr>
<tr>
<td>Gradient</td>
<td>0%–100% B in 2 min, F = 0.5 mL/min (see mobile phase at right)</td>
</tr>
<tr>
<td>Detection</td>
<td>Agilent 1100 Series MSD, API-ES Pos Scan Mode, 200–1500 m/z, 0.52 s/cycle, Capillary = 4500V, Frag = 70V, Nebulizer 35 psi, Drying gas 12 L/min @325 °C</td>
</tr>
</tbody>
</table>

### Highlights

- ZORBAX Poroshell technology facilitates very rapid HPLC separations at flow rates, up to 5x that used for totally porous packing.
- ZORBAX Poroshell 300Extend-C18 allows for MS detection of peptides and small proteins using NH₄OH in the mobile phase, which can lead to reduced background noise and a higher S/N ratio.
- Reliable use of NH₄OH with ZORBAX Poroshell 300Extend-C18 provides a means of altering charge-state distribution.

### Mobile phase (pH 1.8)

| A | 0.1% TFA in H₂O |
| B | 0.085% TFA in ACN |

<table>
<thead>
<tr>
<th>Mobile phase (pH 10)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10 mM NH₄OH in H₂O</td>
</tr>
<tr>
<td>B</td>
<td>10 mM NH₄OH in ACN</td>
</tr>
</tbody>
</table>
The figure demonstrates the difference in S/N in the MS ion spectrum from angiotensin I analyzed with TFA in the mobile phase (A) or with NH₄OH in the mobile phase (B). For speed of analysis, ZORBAX Poroshell packings were used in each case - the 300SB-C18 version for stability under low-pH operation and the 300Extend-C18 version for stability under high-pH operation. Relative to the spectrum obtained using TFA, the spectrum obtained using NH₄OH shows decreased background noise, especially within the 200–400 m/z range. The molecular ion appears at an m/z of 1296, with the +2 and +3 ions appearing at 648.7 and 432.9, respectively. There is a shift from +2 being the dominant ion in TFA, to the +3 being the dominant ion in NH₄OH. Note that the abundance is similar for these ion species at low pH with TFA and at high pH with NH₄OH even though the detector is set to positive mode. While the abundance is slightly less (5000 vs. 4000) this makes use of NH₄OH with ZORBAX Poroshell 300Extend-C18 a useful technique. Not only may background noise be reduced and the charge distribution shifted, but also the column can be expected to perform reliably at high pH to produce very rapid analyses. Please refer to the following Agilent application notes for relevant information: Comparison of ZORBAX Poroshell 300Extend-C18 to totally porous packing for rapid separations at high pH, 5989-0675EN [1]. Use of low and high pH to achieve unique selectivity, 5989-0645EN and 5989-0676EN [2, 3].

The angiotensin peptides are part of a system that controls hypertension and cardiovascular structure. Angiotensin II, the principal peptide, is derived from angiotensin I by cleavage of the C-terminal dipeptide his-leu. For reference, the sequence and molecular weights of angiotensins I, II, and III are shown in Table 1.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Peptide sequence</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin I</td>
<td>asp-arg-val-tyr-ile-his-pro-phe-his-leu</td>
<td>1296.5</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>asp-arg-val-tyr-ile-his-pro-phe</td>
<td>1046.2</td>
</tr>
<tr>
<td>Angiotensin III</td>
<td>arg-val-tyr-ile-his-pro-phe</td>
<td>931.1</td>
</tr>
</tbody>
</table>

References


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