**Evaluation of new high pH stable, superficially-porous particle columns for the reversed-phase separation of oligonucleotides**

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**Introduction**

Synthetic oligonucleotides have emerged as promising therapeutic agents. They are synthesized using a multi-step process. Although coupling efficiencies are high, the overall yield decreases as the cycles increase with failure coupling with single (H-H) and double (H-N) deletions so the major impurities. Therefore, fast, high resolution analyses are needed. HPLC is usually done in basic pH mobile phases at high temperatures; thus, requiring chemically stable columns. Totally porous hybrid particles are commonly used but the mass transfer for the larger size oligos is not ideal. In this work, we evaluate the uses of new high pH stable, superficially-porous particles for oligo separations for fast and high resolution analysis, and compare them with totally porous particles.

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**Experimental**

**Materials**

**Description**

<table>
<thead>
<tr>
<th>Description</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdvancedBio Oligonucleotide 2.1 x 50 mm, 2.7 µm</td>
<td>689750-702</td>
</tr>
<tr>
<td>AdvancedBio Oligonucleotide 2.1 x 100 mm, 2.7 µm</td>
<td>685750-702</td>
</tr>
<tr>
<td>AdvancedBio Oligonucleotide 2.1 x 150 mm, 2.7 µm</td>
<td>685750-702</td>
</tr>
<tr>
<td>AdvancedBio Oligonucleotide 2.1 mm Fast Guard</td>
<td>281725-921</td>
</tr>
<tr>
<td>AdvancedBio Oligonucleotide 4.6 x 50 mm, 2.7 µm</td>
<td>685950-702</td>
</tr>
<tr>
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<td>685950-702</td>
</tr>
<tr>
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<td>685950-702</td>
</tr>
<tr>
<td>AdvancedBio Oligonucleotide 4.6 mm Fast Guard</td>
<td>820750-921</td>
</tr>
</tbody>
</table>

**Column characteristics**

<table>
<thead>
<tr>
<th>Bonded Phase</th>
<th>Pore Size</th>
<th>Temp. Limits</th>
<th>pH Range</th>
<th>End Capped</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18 100A</td>
<td>65°C</td>
<td>3.0 - 11.0</td>
<td>Double</td>
<td></td>
</tr>
</tbody>
</table>

**Acetonitrile, methanol, TEAA, HFP, TEA (Sigma-Aldrich)**

**Method**

**Column:**

| Column: AdvancedBio Oligonucleotide, 2.1 x 50 mm (p/n 689750-702) |
|------------------|------------------|

**Options**

1. **Option 1**

   - Mobile phase A: 100 mM TEAA in water
   - Mobile phase B: 100 mM TEAA in acetonitrile

2. **Option 2 (LC/MS friendly)**

   - Mobile phase A: HFP,TEA (400 mM/15 mM) in water
   - Mobile phase B: Methanol : mobile phase A (50:50)

**Gradient:**

- See chromatogram

**Stop time:**

- See chromatogram

**Post run:**

- 5 min

**Flow rate:**

- 0.6 mL/min (or other flow rates)

**Col. temp.**

- 65°C

**Sample:**

- See figures

**Injection:**

- See figures

**Detection:**

- UV at 260 nm

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**Results and Discussion**

**Resolution of N and N-1 Oligonucleotides**

**Separation of RNA-oligonucleotides**

**Comparison**

**Separation of 23-mer RNA-oligonucleotide (2.1 x 50 mm)**

The peak width of the superficially-porous column was slightly narrower than the totally porous material 1. Thus, this supports the fact that there is shorter distances required for diffusion into/out of the superficially-porous stationary phase. This faster mass transfer results in higher resolution capability. With 2.7 um particle size, AdvancedBio Oligonucleotide column operates at low backpressure compare to 1.7 um particle size column, it is compatible with 600 bar HPLC system as well as 1200 bar HPLC system.

**Column stability (2.1 x 50 mm) - 300 consecutive injections of 25-mer DNA-oligo**

Column stability of 300 consecutive injections of 25-mer (RNA-oligo) showed that though out 300 injections, the retention time of main peak and its impurities were highly reproducible. Column still maintains its performance after those 300 injections.

**Comparison of column stability (2.1 x 50 mm)**

The peak retention time from 400 injections of 25-mer which was generated by AdvancedBio Oligonucleotide column (high pH stable superficially-porous particles) showed to be as stable as the totally porous hybrid particles. This data also indicated that the AdvancedBio Oligonucleotide column has a long lifetime.

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**Conclusions**

- AdvancedBio Oligonucleotide columns are designed for separating RNA and DNA-oligonucleotides of different sizes with fast and high resolution.
- RNA and DNA-oligonucleotides can be successfully analyzed by AdvancedBio Oligonucleotide columns using both LC/UV and LC/MS mobile phases.
- High numbers of injections - column stability data generated with TEAA (>pH 8.0) indicated that columns packed with high pH stable superficially-porous particles have long life time.
- AdvancedBio Oligonucleotide columns are packed with 2.7 um superficially-porous particles - low backpressure columns - can be compatible with 600 bar and 1200 bar HPLC systems.

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**LC instruments**

Compatible with

- 1260 Infinity Quaternary
- 1290 Infinity Binary

Note: Conventional (green) capillaries are 0.17 mm ID can be used. However, red capillaries, 0.12 mm ID, will reduce connector volumes by 50%. Black capillaries (0.07 mm ID) can also be used but be careful of increased back pressure.