Separation of Short and Long Chain Oligonucleotides through the use of a Large Pore SPP Column

Peter Pellegrinelli, Stephanie Schuster, Conner McHale
Advanced Materials Technology Inc., Wilmington, DE

Introduction

Oligonucleotides are becoming increasingly important in medical research and chromatography. The use of siRNA, aptamers, and other oligomers in treatments is widening. These treatments have begun to aid people suffering from genetic diseases like Duchenne muscular dystrophy, acute porphyria, and familial amyloid neuropathy. Successful UHPLC of nucleotides has become critical for these treatments to advance. The chemical structure and molecular weight of oligos varies widely. These variables make oligonucleotides difficult to separate with good resolution.

Experimental

20/100 Oligonucleotide Ladder

The oligonucleotide ladder samples were purchased through IDT as a dried sample. The 10µg sample was first centrifuged to eliminate loss of sample. Next the sample was reconstituted in 200µL of 10mM Tris-EDTA solution at pH 8.0 (Sigma-Aldrich). The sample was injected after reconstitution.

Instrumentation

All samples were analyzed on a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA).

LC Gradient

<table>
<thead>
<tr>
<th>Time</th>
<th>% B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>40</td>
</tr>
<tr>
<td>20.00</td>
<td>62.5</td>
</tr>
<tr>
<td>22.00</td>
<td>100</td>
</tr>
<tr>
<td>23.00</td>
<td>100</td>
</tr>
<tr>
<td>23.10</td>
<td>40</td>
</tr>
<tr>
<td>28.00</td>
<td>40</td>
</tr>
</tbody>
</table>

Mobile Phase A: 100mM TEAA Buffer pH 7.0 Mobile Phase B: 80% A, 20% ACN

Flow Rate: 0.2 mL/min Temp: 60°C

Column

A 1000 Å, 2.7 µm, ES-C18, 2.1x150mm HALO® (Advanced Materials Technology, Inc. Wilmington, DE) column was used for the initial separation.

Separation of Oligonucleotide Ladder on 1000 Å ES-C18

The 1000 Å ES-C18 shows excellent separation of the larger oligonucleotides. The 160 Å pore size allows for better peak shape of the larger oligonucleotides which elute after the 6 minute mark. The ladder being separated shows 10 peaks with the extra peak potentially being an impurity or intermediate oligonucleotide.

*We are currently in the process of determining the peak identities of the ladder as well as any impurities or potential extra oligonucleotides within the sample.

1000 Å ES-C18 Stability

A 1000 Å ES-C18 column shows excellent stability using an oligonucleotide ladder ranging from 20 to 100 bases in length. No change in back pressure was observed and retention time and peak shape were constant over the course of 10,000 column volumes.

Stability Test Conditions:

- Column: HALO-1000 Å ES-C18, 2.7µm, 2.1x150mm
- Part Number: 92712-312
- Mobile Phase A: 100mM TEAA Buffer pH 7.0
- Mobile Phase B: Methanol
- Flow Rate: 0.25 mL/min (Sample Injection) 0.80 mL/min (Stability Gradient)
- Temperature: 50°C
- Detection: UV, 254 nm
- Initial Back Pressure: 49 bar
- Final Back Pressure: 49 bar
- Injection Volume: 1.0µL
- Sample: 20/100 Ladder (IDT)

Conclusions

Over the past few years, with the emergence of Covid-19, oligonucleotide research and characterization has become a topic of increasing interest. The current knowledge surrounding oligonucleotides is developing and successful synthesis of these nucleotides requires a strong understanding. In order for scientists and corporations to confirm the synthesis for lab and drug ready oligonucleotides, robust chromatography methods are necessary. Chromatographers can take advantage of multiple large pore size column chemistries in order to obtain improved chromatographic results when running oligonucleotides. As we learn more, we may be able to increase the successful synthesis of larger oligonucleotides, expanding the possibilities of these drugs potentially saving many lives in the process. In order to confirm the synthesis of the larger oligonucleotides, large pore size column chemistries can be crucial for the successful separations of the oligonucleotides.

HALO® is a registered trademark of Advanced Materials Technology, Inc.