Impurity Analysis of a Crude 90-mer ssDNA using the New HALO 1000 Å Oligo Column

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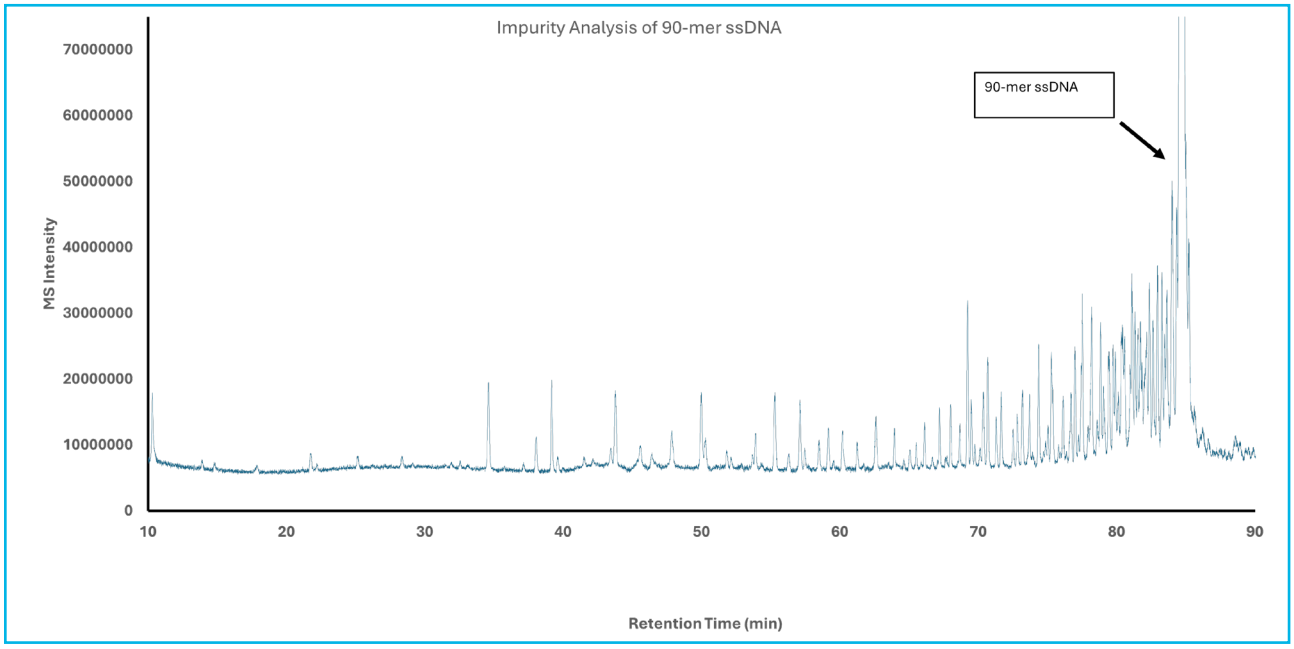

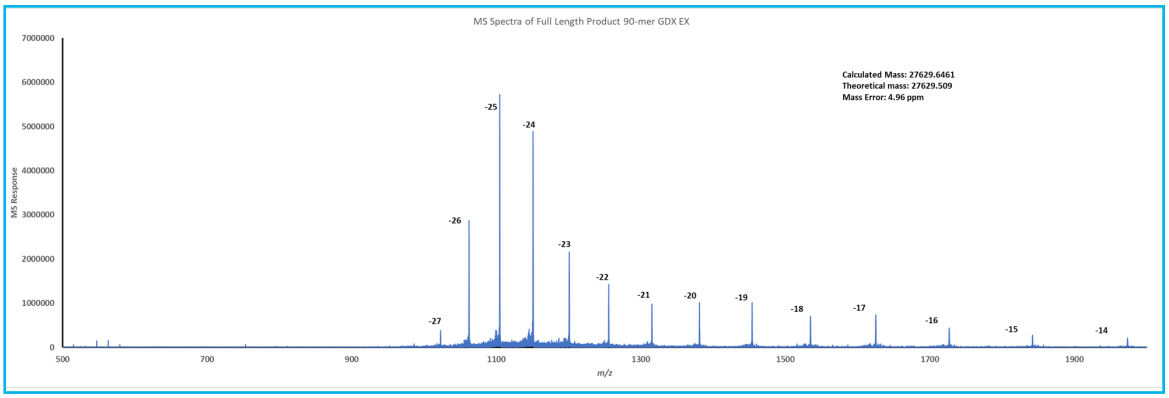

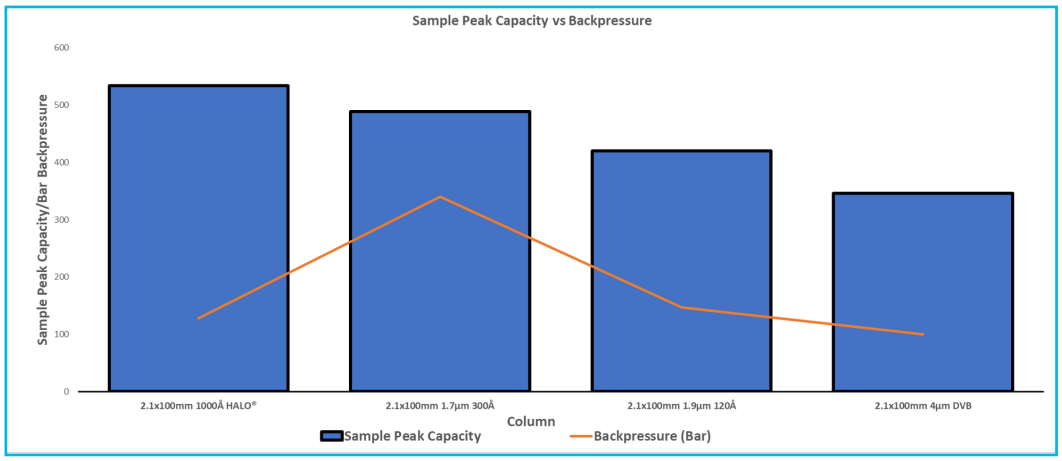

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BIOPHARMACEUTICALS

TEST CONDITIONS:

Column: HALO 1000 Å OLIGO C18, 2.7µm, 2.1 x 150 mm
Part Number: P2762-702
Mobile Phase A: 10mM Diisopropyl-amine(DiBA)/100mM Heptafluoroisopropanol (HFIP)/5% Methanol/5% Acetonitrile
Mobile Phase B: 10mM DiBA/100mM HFIP/5% Methanol/50% Acetonitrile

Gradient:

Time	%B
0.00	0
120	32
125	50
125.1	0
130	0

Flow Rate: 0.2 ml/min
Pressure: 150 bar
Temperature: 60 °C
Detection: High Resolution MS
Injection Volume: 10 µl (Sample diluted 1:100 in Mobile Phase A
Sample Solvent: 90-mer crude ssDNA in nuclease-free water
LC System: Shimadzu Nexera X2

PEAK IDENTITIES

1. 90-mer ssDNA

MS CONDITIONS:

Mass Spectrometer: Thermo Q-Exactive HF
Ion mode: Negative Electrospray
MS1 Scan Mode:
Sheath Gas Flow Rate: 40
Aux Gas Flow Rate: 20
Sweep Gas Flow Rate: 3
Spray Voltage: 3000 V
Capillary Temp: 350°C
S-Lens RF: 60V
Aux Gas Heater Temp: 400°C
MS1 Resolution 120,000
AGC Target: 3.00E+06
Max IT: 200ms
Scan Range: m/z 450-2000

Solid phase synthesis of oligonucleotides has become a highly reliable and reproducible process such that generation of modified DNA and RNA strands >100bp have become commonplace for a variety of applications such as the growing CRISPR therapeutics space. However, there is a pressing need for analytical techniques that can evaluate and characterize these long oligos for QA/QC purposes.

LCMS has emerged as a useful tool for characterization of oligonucleotides but the chromatographic separation via HPLC has often been limited to oligonucleotides up to 60-70bp due to difficulties in separating long chain impurities from the full length product (FLP).

In this note, we utilize the new HALO 1000 Å OLIGO C18 column to perform an impurity analysis of a crude 90-mer ssDNA. The HALO 1000 Å OLIGO C18 column combines several technologies including our Fused-Core© silica particle technology, surface passivated column hardware, and our proprietary Elevate coating process for high pH and temperature stability to create an HPLC column that can reliably analyze oligonucleotides up to 100bp in length and beyond.

The Total Ion Chromatogram in the figure above shows the impurity profile of the 90-mer ssDNA. We have characterized over 100 impurities thus far with excellent separation of impurities of similar molecular weight to the FLP.

To compare the performance of the HALO 1000 Å OLIGO C18 column to other commercial oligonucleotide columns, we re-ran the crude 90-mer ssDNA using the same methods on several 2.1 x 100 mm commercial oligonucleotide columns and calculated Sample Peak Capacity using 22 repeatably identifiable impurities across the retention time space. The 2.1 x 100 mm HALO 1000 Å OLIGO C18 column outperformed all other commercial columns compared. Additionally the use of the Fused-Core© silica allows for very managable backpressures, even up to 250 mm in length.

The new HALO 1000 Å OLIGO C18 column represents new opportunities to simplify analytical characterization of long oligonucleotides in the therapeutic space.

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