HALO®

1000 Å OLIGO C18

GO BEYOND to the next level of performance for long-chain oligonucleotide analysis

MEET THE NEW: HALO® 1000 Å OLIGO C18

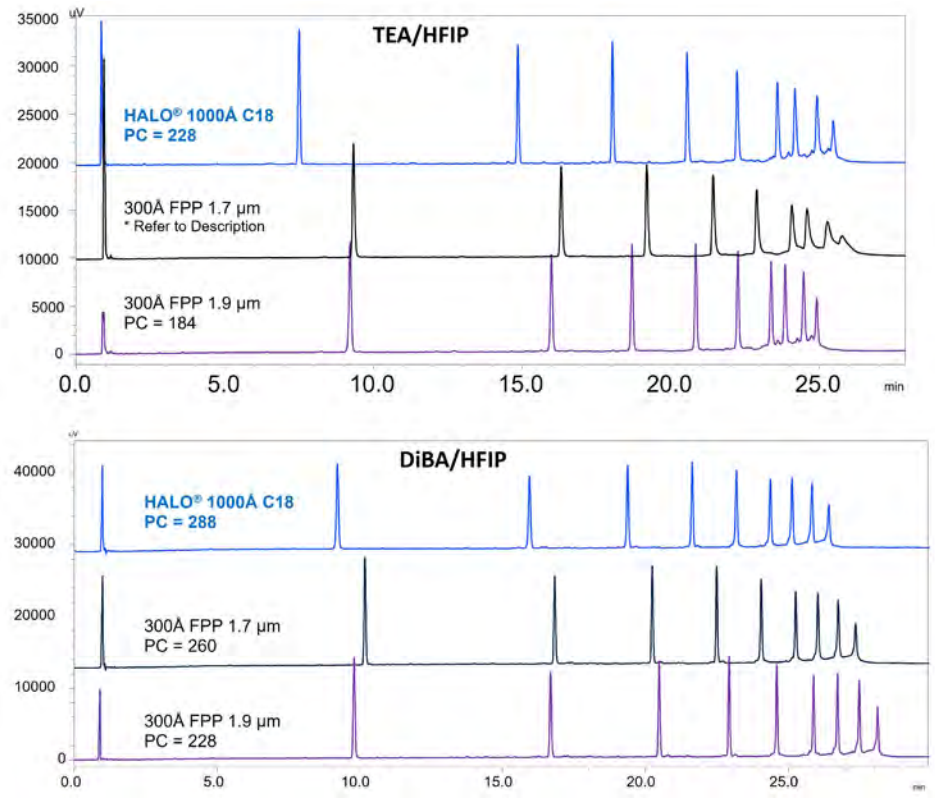
The new HALO 1000Å OLIGO C18 columns deliver superior separation and impurity profiling for long-chain oligonucleotides, with higher peak capacity and lower backpressure than competitor columns. Their robust design supports high pH and temperature conditions, scalable column lengths, and reliable performance for advanced applications.

FEATURES OF HALO® OLIGO C18

- **1000 Å Large Pore Size:** Purpose-built for efficient separation of long oligonucleotides (up to and beyond 100 nucleotides), overcoming the limitations of conventional columns.
  - **Ideal for advanced applications like CRISPR and therapeutic development.**
  - **Outperforms competitor columns with improved peak widths and capacity, delivering more accurate results for challenging samples.**
- **Proprietary Particle Technology:** Operates robustly under demanding conditions; high pH, elevated temperatures ensuring consistent results and extended column life.
- **Low Backpressure:** Enables scalable workflows and faster analyses, even with longer columns.

ADVANTAGE OF HALO® 1000 Å OLIGO C18 OVER THE COMPETITION

Separations of oligonucleotides using the 1000Å superficially porous particle (SPP) material were compared to those achieved with commercially available wide-pore (300Å) sub-2 µm silica particles. The HALO 1000Å OLIGO C18 SPP demonstrated modest improvements in peak widths under TEA/HFIP conditions and exhibited a greater gradient range (DeltaT) between the 20- and 100-base oligonucleotides, contributing to a higher peak capacity (PC) along with lower backpressure. Across all columns, peak shapes for larger oligonucleotides improved using the more hydrophobic DiBA/HFIP mobile phase. The enhanced peak capacities observed with the 1000Å SPP material were attributed to both narrower peak widths at 50% height and the broader gradient span of the separation.



*Due to the heavily tailed peak 10, peak capacity could not be correctly calculated.

TEST CONDITIONS:

Column: HALO 1000 Å OLIGO C18, 2.7 µm, 2.1 x 100 mm
Mobile Phase:
Plot A
A: 15mM TEA/50mM HFIP pH - 8.9
B: 50/50 Water/ACN
Plot B
A: (90)/5/5 (10mM DiBA/100mM HFIP)/MeOH/ACN
B: 50/50 Water/ACN

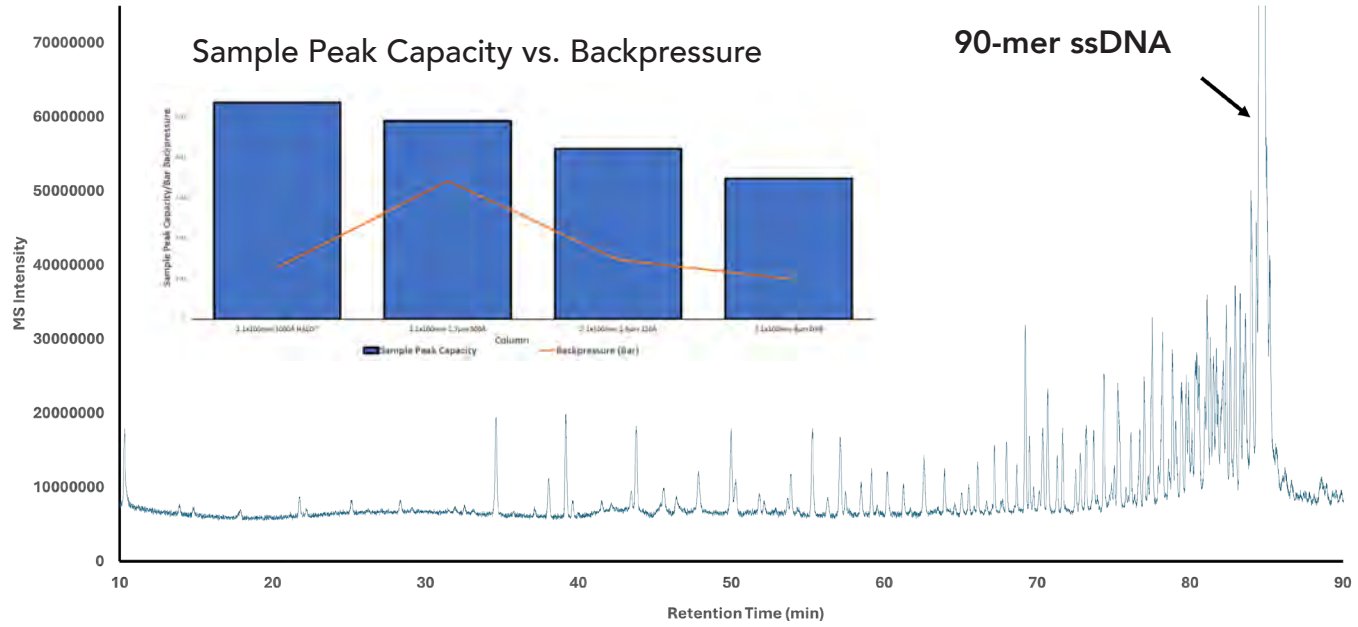
Gradient:	Time	%B
	0.0	5
	30.0	12

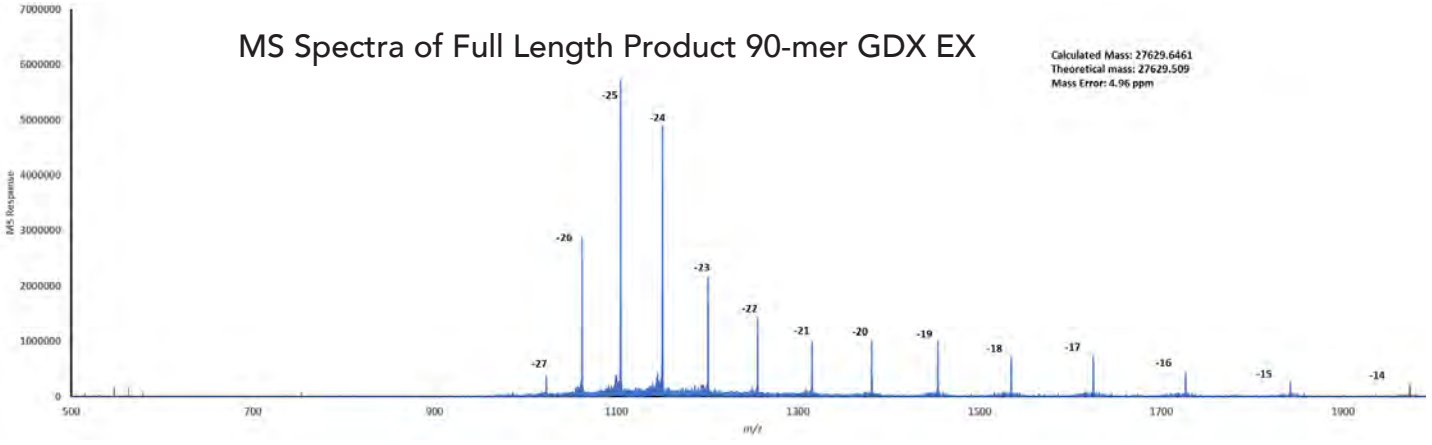
Flow Rate: 0.4mL/min.
Back Pressure: 1000 Å HALO - 241 bar
300 Å FPP 1.7µm - 540 bar
120 Å FPP 1.9µm - 286 bar
Temperature: 60 °C
Injection: 1.0 µL of ssDNA (10µg/mL)
Sample Solvent: 10mM Tris/1mM EDTA
Wavelength: PDA, 260 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2

EXCEPTIONAL IMPURITY CHARACTERIZATION

DISTINGUISH AND QUANTIFY OVER 100 IMPURITIES IN COMPLEX SAMPLES

The Total Ion Chromatogram in the figure above shows the impurity profile of the 90-mer ssDNA. To compare the performance of the HALO® 1000Å OLIGO C18 column to other commercial oligonucleotide columns, we ran a crude 90-mer ssDNA using the same methods on several 2.1 x 100mm commercial oligonucleotide columns and calculated sample peak capacity using 22 repeatably identifiable impurities across the retention time space. The 2.1 x 100mm HALO® 1000Å 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 250mm in length.





TEST CONDITIONS:

Column: HALO 1000Å OLIGO C18, 2.7µm, 2.1 x 150mm
Mobile Phase A: 10mM Diisopropylamine(DiBA)/100mM Hepta-fluoroisopropanol (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

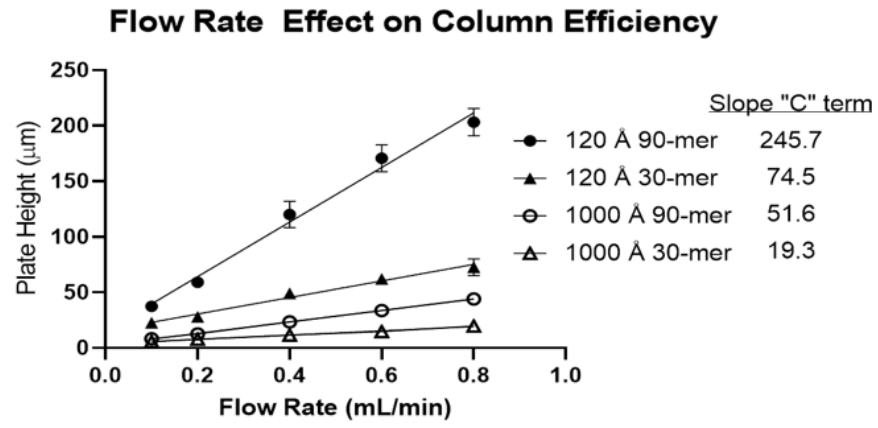
Mass Spectrometer: Thermo Q-Exactive HF
Ion mode: Negative Electrospray
MS1 Scan Mode:
Sheath Gas Flow Rate: 40
Aux Gas Flow Rate: 20
Sweep Gas Flow Rate: 3
Spray Voltage: 3000 V
Capillary Temp: 350°C
S-Lens RF: 60V
Aux Gas Heater Temp: 400°C
MS1 Resolution 120,000
AGC Target: 3.00E+06
Max IT: 200ms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

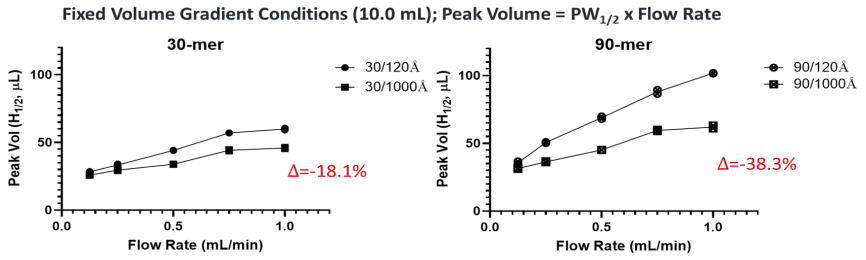
EFFECTS OF PORE SIZE AND COLUMN LENGTH

IMPACT OF PORE SIZE AND FLOW RATE ON OLIGOMER SEPARATIONS

At a constant gradient volume of 10.0 mL, with varied flow rate, band dispersion was compared under TEAA conditions for different pore sizes and oligonucleotide chain lengths in gradient elution with acetonitrile (AcN); notably, band dispersion was lower on the larger pore SPP relative to the smaller pore material, with a more pronounced difference observed for the large oligonucleotide. To address retention pressure dependence, a method similar to that recently described by Stoll et al. (J. Chromatogr. A, 1744 (2025), 465687) can be employed, where column efficiency is evaluated at constant retention by adjusting solvent strength estimated via the linear solvent strength (LSS) relationship.

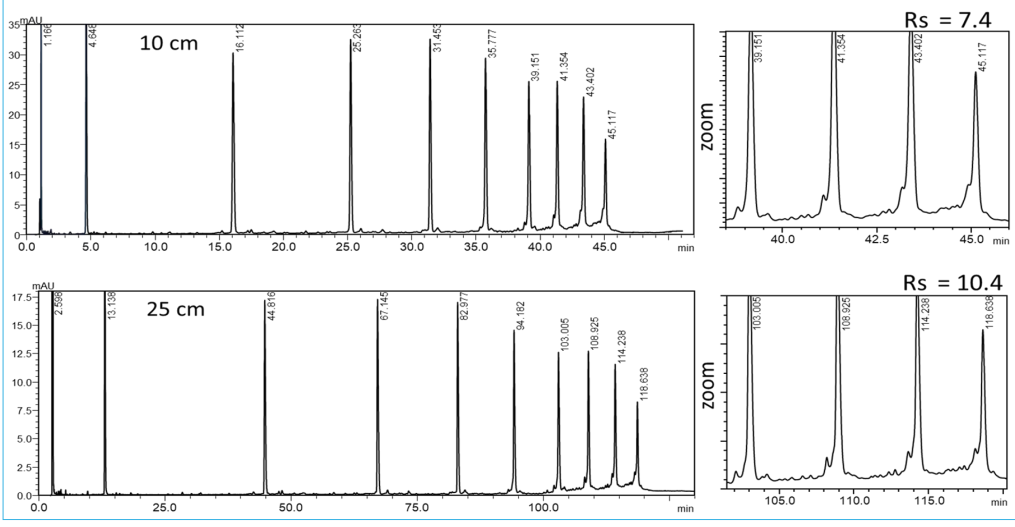
Columns packed with two pore size variants of 2.7 µm particles exhibit very similar permeabilities; however, the larger pore material demonstrates significantly higher efficiency for the 90-mer at high flow rates and improved band width for the 30-mer at lower flow rates compared to the smaller pore packing.





THE IMPACT OF COLUMN LENGTH ON RESOLUTION

The resolution of an oligonucleotide mixture was evaluated using a longer column (25 cm) under a shallow gradient, successfully resolving species up to 100 nucleotides in length. The resolution scaled approximately with the square root of column length (√L), consistent with chromatographic theory when the gradient rate was adjusted proportionally to column length—specifically, comparing 150-minute and 60-minute gradients for the 25 cm and 10 cm columns. The data supports the expected trend that longer columns, when paired with appropriately scaled gradients, enhances resolution for complex oligonucleotide mixtures.



TEST CONDITIONS:

Column: HALO 1000 Å OLIGO C18, 2.7 µm, 2.1 x 100 mm
Column: HALO 1000 Å OLIGO C18, 2.7 µm, 2.1 x 250 mm

Mobile Phase A:
(90)/5/5 (10mM DiBA/100mM HFIP)/MeOH/ACN
Mobile Phase B:
50/50 Water/ACN

Gradient:	Time	%B
10cm	0.0	15
	60.0	75
25cm	0.0	15
	0.0	75

Flow Rate: 0.4mL/min.
Back Pressure: bar
Temperature: 60 °C
Injection: 2.0 µL of ssDNA (10µg/mL)
Sample Solvent: 10mM Tris/1mM EDTA
Wavelength: PDA, 260 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2

PRODUCT CHARACTERISTICS

Ligand: dimethyloctadecylsilane, surface modified
Particle Size: 2.7 µm
Pore Size: 1000 Å

USP Designation: L1
Carbon Load: 2.4%
Surface Area: 22 m^2/g
Endcapped: YES

Low pH Limit: 2
High pH limit*: 9
Temp limit @ low pH: 90 °C
Temp limit @ high pH*: 85 °C

PART NUMBERS

2.7 µm ANALYTICAL COLUMNS	
Dimensions: ID x Length (in mm)	Part Number
2.1 x 30	P2762-302
2.1 x 50	P2762-402
2.1 x 100	P2762-602
2.1 x 150	P2762-702
2.1 x 250	P2762-902
2.1 x 5 (Guard 3pk)	P2762-102

SURFACE PASSIVATED HARDWARE

*Column lifetime will vary depending on the operating temperature and the type and concentration of buffers used. Operation at extreme specifications of temperature and pH may reduce column lifetime. Consult the column Care and Use document for more information.

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HALO®

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