Application note | 004530

Oligo analysis

Comprehensive analysis of nucleic acids across a wide size range using a monodisperse reversed-phase particle platform

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Keywords

Oligonucleotides, reversed-phase chromatography, SurePac Oligo RP MDi column, nucleic acids, resolution, mRNA, siRNA, ASO, Vanquish Neo UHPLC system

Application benefits

- High-resolution separation of small oligonucleotides up to long mRNA
- Improved resolution for short oligonucleotides compared to Thermo Scientific™ DNAPac™ RP Columns
- Consistent lot-to-lot and column-to-column performance

Goal

To evaluate a reversed-phase column (Thermo Scientific™ SurePac™ Oligo RP MDi™ Column) packed with 2.5 µm monodisperse supermacroporous (SMP) particles as a single-solution platform for nucleic acids ranging from 10 to over 1,000 units in length, and to assess its performance relative to DNAPac RP columns and other commercially available columns.

Introduction

Nucleic acid–based therapeutics encompass a wide range of molecular sizes and structures, from short synthetic oligonucleotides to long mRNA constructs. Analytical characterization of these molecules remains challenging, as columns and methods designed for smaller oligonucleotides often lack resolving power for larger species, while columns suitable for larger oligonucleotides can compromise performance for shorter sequences. As a result, multiple chromatographic columns are frequently required to support different analytical workflows across oligonucleotide samples of varying molecular size.

Polymer-based, reversed-phase columns, such as the 4 µm particle DNAPac RP columns, are well established for the analysis of larger oligonucleotides and RNA species. Relative to many silica columns, the polymeric particle confers advantages such as temperature and pH stability; however, the relatively large particle size limits the resolution of smaller analytes. In contrast, sub-2 µm reversed-phase columns, typically silica-based, are commonly used for high-efficiency separation of shorter oligonucleotides. Due to the limitations of current column types, many laboratories often maintain multiple columns to address separation needs for different molecule sizes and analytical requirements.

In this work, we investigate the new SurePac Oligo RP MDi column based on a novel 2.5 µm monodisperse SMP polymeric stationary phase for the analysis of nucleic acids spanning small to large molecular sizes, including siRNA (20–25 nt), $tRNA^{Phe}$ (~76 nt), sgRNA (~100 nt), ssDNA mix (98-mer–100-mer), and mRNA (1,000–3,000 nt). Like DNAPac RP columns, the new SMP stationary phase is designed with a broad, continuous pore size distribution that includes both small and large pores within the same particle architecture, contributing to the utilizable separation surface area. Smaller pores support efficient interaction and high resolving power for short oligonucleotides, while the larger pores provide surface area and good mass transfer for longer nucleic acid species. The balanced continuum of pore sizes allows the column to perform effectively across a wide molecular size range. As a result, the SurePac Oligo RP MDi column addresses the need for improved resolution of small oligonucleotides while maintaining strong performance for larger nucleic acids typically analyzed using DNAPac RP columns. The column body, frits, and end fittings are coated with a mildly hydrophobic inert layer to minimize secondary sample interactions, thereby improving analyte recovery and peak shape from the first injection. Compared to conventional polydisperse particle materials (Figure 1, right image), the monodisperse particles (Figure 1, left image) exhibit consistent size distribution, resulting in improved column manufacturing and lot-to-lot reproducibility. The increase in consistency of the resulting columns supports robust method development for reliable method transfer and long-term analytical use.

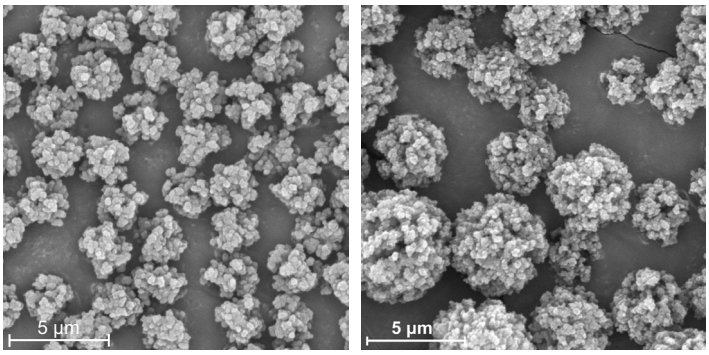


Figure 1. Scanning electron microscopy (SEM) image of monodisperse SurePac Oligo RP MDi column particles (left) vs. polydisperse particles (right). White scale bars are 5 µm in length.

This application note demonstrates that the SurePac Oligo RP MDi column enables straightforward characterization with strong sensitivity, high resolution, and consistent performance across a wide sample size range. The column performance provides confidence in the detection and identification of closely related oligonucleotide impurities, truncated species, and high-molecular-weight RNA variants during late-stage development, process monitoring, purification, and stability studies. As nucleic acid therapeutics continue to increase in structural complexity and size diversity, the SurePac Oligo RP MDi column offers the resolving power and robustness required to meet both characterization and regulatory expectations.

Experiment

Reagents and consumables

Deionized (DI) water, 18.2 MΩ·cm resistivity

Invitrogen™ Nuclease-Free Water (not DEPC-treated) (Part No. 9938)

Glen Research™ 2.0M Triethylamine Acetate (Part No. 60-4110-60)

Glen Research™ 2.0M Hexylammonium Acetate (Part No. 60-4210-57)

Supelco™ 1,1,1,3,3,3-Hexafluoro-2-propanol (Part No. 18127)

Sigma-Aldrich™ Dibutylamine (Part No. 471232)

Fisher Chemical™ Acetonitrile (Part No. A955-4)

Fisher Chemical™ Methanol (Part No. A454-4)

Thermo Scientific™ Pierce™ FlexMix™ Calibration Solution (Part No. A39239)

Invitrogen™ Nonstick, RNase-free Microfuge Tubes, 1.5 mL (Part No. AM12450)

Thermo Scientific™ SureSTART™ 0.4 mL GOLD-Grade Polypropylene Screw Microvials and Cap Kits (Part No. 6PKSV9GCST1)

Samples

Thermo Scientific™ 8-combo ssDNA:

12-mer: $(GACT)_3$, 16-mer: $(GACT)_4$, 20-mer: $(GACT)_5$, 24-mer: $(GACT)_6$, 28-mer: $(GACT)_7$, 32-mer: $(GACT)_8$, 36-mer: $(GACT)_9$, and 40-mer: $(GACT)_{10}$

MilliporeSigma™ MISSION™ siRNA Universal Negative Control #1 (Part No. SIC001)

Integrated DNA Technologies™ Hs.Cas9.ALK.1.AA sgRNA:

mG*mC*mG* rUrArG rArCrA rCrGrG rArArG rArGrC rGrArG rUrUrU rUrArG rArGrC rUrArG rArArA rUrArG rCrArA rGrUrU rArArA rArUrA rArGrG rCrUrA rGrUrC rCrGrU rUrArU rCrArA rCrUrU rGrArA rArArA rGrUrG rGrCrA rCrCrG rArGrU rCrGrG rUrGrC mU*mU*mU* rU

Integrated DNA Technologies™ 98-mer DNA:

GATTGTAGGTTCTCTAACGCTGATTGTAGGTTCTCTAACGC TGATTGTAGGTTCTCTAACGCTGATTGTAGGTTCTCTAACG CTGATTGTAGGTTCTC

Integrated DNA Technologies 99-mer DNA:

GATTGTAGGTTCTCTAACGCTGATTGTAGGTTCTCTAACGCT
GATTGTAGGTTCTCTAACGCTGATTGTAGGTTCTCTAACGCT
GATTGTAGGTTCTCT

Integrated DNA Technologies™ 100-mer DNA:

GATTGTAGGTTCTCTAACGCTGATTGTAGGTTCTCTAACG
CTGATTGTAGGTTCTCTAACGCTGATTGTAGGTTCTCTAA
CGCTGATTGTAGGTTCTCTA

Thermo Scientific™ phiX174 DNA/BsuRI (HaeIII) Marker (Part No. SM0252):

phiX174 DNA/BsuRI (HaeIII) Marker (72bp, 118bp, 194bp, 234bp, 271bp, 281bp, 310bp, 603bp, 872bp, 1078bp, 1353bp)

1960nt mRNA transcript encoding firefly luciferase, containing a poly(A) tail and lacking a 5' cap structure, was synthesized in-house at Thermo Fisher Scientific.

Sigma-Aldrich™ Ribonucleic acid, transfer, phenylalanine specifically from brewer's yeast (Part No. R4018)

Sample preparation

8-combo ssDNA sample was diluted to a final concentration of 5 µM using DI water.

siRNA sample was diluted to a final concentration of 20 µg/mL using DI water.

sgRNA sample was diluted to a final concentration of 5 µM using DI water.

98-mer–100-mer DNA mix sample was diluted to a final concentration of 20, 20, and 40 µg/mL using DI water.

phiX174 DNA/BsuRI (HaeIII) ladder sample was diluted to a final concentration of 100 µg/mL using DI water.

mRNA sample (3.5 mg/mL) was used as received in the original formulation buffer.

$tRNA^{Phe}$ sample was diluted to a final concentration of 400 µg/µL using mobile phase A.

Instruments

Thermo Scientific™ Vanquish™ Horizon UHPLC System, including:

Thermo Scientific™ Vanquish™ System Base (Part No. VF-S01-A-02)

Thermo Scientific™ Vanquish™ Binary Pump H (Part No. VH-P10-A-02)

Thermo Scientific™ Vanquish™ Column Compartment H (Part No. VH-C10-A-03)

Thermo Scientific™ Vanquish™ Active Pre-Heater, MP35N 0.10 x 380 mm (Part No. 6732.0110)

Thermo Scientific™ Vanquish™ Post-Column Cooler, MP35N 0.10 x 445 mm (Part No. 6732.0510)

Thermo Scientific™ Vanquish™ Split Sampler HT (Part No. VH-A10-A-02)

Thermo Scientific™ Vanquish™ Split Sample Loop, 25 µL (Part No. 6850.1911)

Thermo Scientific™ Vanquish™ Diode Array Detector HL (Part No. VH-D10-A)

Thermo Scientific™ Vanquish™ LightPipe™ Flow Cell, 10 mm standard (Part No. 6083.0100)

Thermo Scientific™ Viper™ Fingertight Fitting Systems, MP35N (Part No. 6042.2340)

Columns

SurePac Oligo RP MDi column, 2.5 µm, 2.1 x 50 mm, (Part No. 43712-052132)

SurePac Oligo RP MDi column, 2.5 µm, 0.3 x 50 mm, (Part No. 43712-050332)

Vendor A column 1, C18 1.7 µm, 130Å, 2.1 x 50 mm

Vendor A column 2, C18 1.7 µm, 300 Å, 2.1 x 50 mm

Vendor B column, C18 2.7 µm, 100 Å, 2.1 x 100 mm

DNAPac RP column, 4 µm, 2.1 x 50 mm, (Part No. 088924)

The SurePac Oligo RP MDi column, Vendor A, and Vendor B columns are packed in inert column hardware. The DNAPac RP column is packed in stainless steel hardware.

For clarity, chromatographic conditions and gradients are given with the respective results.

LC-UV data processing

The Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) version 7.2.10 was used for data acquisition and analysis.

Liquid chromatography mass spectrometry

All separations were accomplished on a Thermo Scientific™ Vanquish™ Neo UHPLC System (Part No. VN-S10-A-01). High-resolution accurate mass analysis was performed on a Thermo Scientific™ Orbitrap Ascend Editions Tribrid™ Mass Spectrometer (Part No. FSN06-10000).

Mass spectrometry

The Orbitrap Ascend Tribrid mass spectrometer was operated with Thermo Scientific™ Xcalibur™ Software version 4.7. Instrument calibration was performed using Pierce FlexMix calibration solution. Data acquisition was performed in negative ion mode. Data was analyzed with Thermo Scientific™ FreeStyle™ SP3 software version 1.8 and Thermo Scientific™ BioPharma Finder™ software version 5.3.

The MS method was built in the method editor using the standard MS template provided with the Orbitrap Ascend Tribrid mass spectrometer control software and then modified accordingly. Table 1 lists the scan parameters used in these acquisitions. Thermo Scientific™ OptaMax™ NG Ion Source settings were default for the flow rate used in the experiment.

Table 1. MS parameters used in the analysis.

Global settings	Value
Application mode	Intact protein
Pressure mode	Low pressure
Default charge state	10
Advanced peak determination	TRUE
Full scan	**Value**
Orbitrap resolution	240,000
Scan range (*m/z*)	810–2,500
RF lens (%)	50
AGC target	4e5
Maximum injection time mode	Auto
Maximum injection time (ms)	Auto
Microscans	2
Data type	Profile
Polarity	Negative
MS source settings	**Value**
Sheath gas (Arb)	50
Aux gas (Arb)	10
Sweep gas (Arb)	1
Ion transfer tube temperature (°C)	350
Vaporizer temperature (°C)	320
Spray voltage (V)	3,500

Deconvolution was performed using BioPharma Finder software version 5.3 with Intact Mass Analysis using the Sliding Windows deconvolution algorithm. Sliding Windows Definition used an RT range of only the chromatographic peak, with a target Avg Spectrum Width of 0.17 minutes and a Target Avg Spectrum Offset of 25%. A merge tolerance of 30 ppm with the Legacy Merge Scheme and 1-minute Max RT Gap was used. The parameters used for this analysis are listed in Table 2. Under Identification, Sequence Matching Mass Tolerance was set to 10 ppm with Multi Consensus Component Merge Mass Tolerance at 10 ppm.

Table 2. BioPharma Finder Intact Mass Analysis parameters.

Main parameters	Value
Chromatogram trace type	TIC
m/z range	800–1,800
Output mass range (Da)	24,400–25,050
S/N threshold	3
Relative abundance threshold (%)	5
Charge range	10–35
Minimum number detected charge	3
Isotope table	Nucleotide

Results and discussion

For the following discussions on separations, please reference the Experimental and Samples sections for specific details on analytes used for testing. For each section on the analysis of short, mid-, and large-size nucleic acids, data are presented using standards (i.e., non-functional oligonucleotides) and representative functional oligonucleotides, such as siRNA, sgRNA, tRNA, and mRNA.

Separation of short oligonucleotides

To evaluate the separation of short oligonucleotides, an 8-combo mixture of ssDNA oligonucleotides (12-, 16-, 20-, 24-, 28-, 32-, 36-, 40-mer) and a 21 bp siRNA was used. The 8-combo ssDNA mix was first used to evaluate separation power for short oligonucleotides. Evaluating the 8-combo sample under comparable gradient conditions optimized for each column, the SurePac Oligo RP MDi column produced equivalent or sharper peaks compared to the other evaluated columns (Figure 2). The smaller pore structure of the SurePac Oligo RP MDi column stationary phase enables sharp peak shape and high resolution for short nucleic acid samples improving upon the performance observed with the DNAPac RP column for short oligonucleotides.

The peak spread between the first and last eluting components across the gradient window was broader using the SurePac Oligo RP MDi column, indicating good stationary phase capacity for short oligonucleotides. Additionally, the SurePac Oligo RP MDi column generated significantly lower backpressure compared to columns from Vendor A and Vendor B. This lower operating pressure provides more flexibility in instrument choice and method development, especially if higher flow rates are required for fast, QC-oriented methods.

For the 21 bp siRNA negative control sample analysis, resolution of closely related impurities is critical. The SurePac Oligo RP MDi column demonstrated equivalent or better peak shape, improved separation of minor impurities, and different selectivity compared to other tested columns from different vendors (Figure 3). A higher number of impurity peaks was resolved, and baseline separation between antisense and sense peaks was improved. This higher level of detail is particularly important for process development and stability studies, where small differences in impurity profiles may be significant.

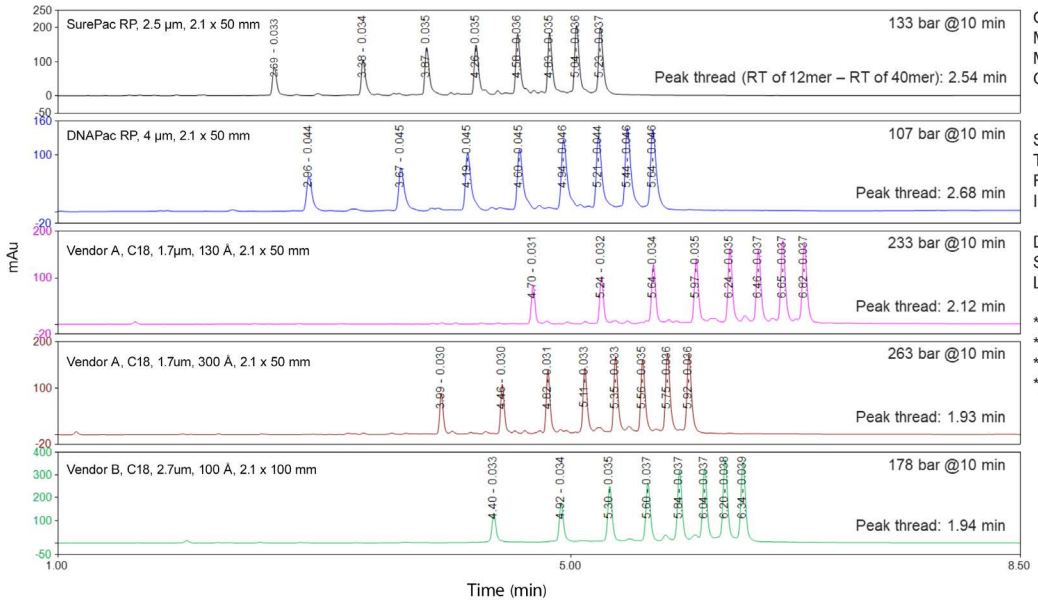


Figure 2. Chromatograms of 8-combo ssDNA using a SurePac Oligo RP MDi column (black), DNAPac RP column (blue), Vendor A column 1 (pink), Vendor A column 2 (brown), and Vendor B column (green). Peak labels reflect retention time and peak width at half height.

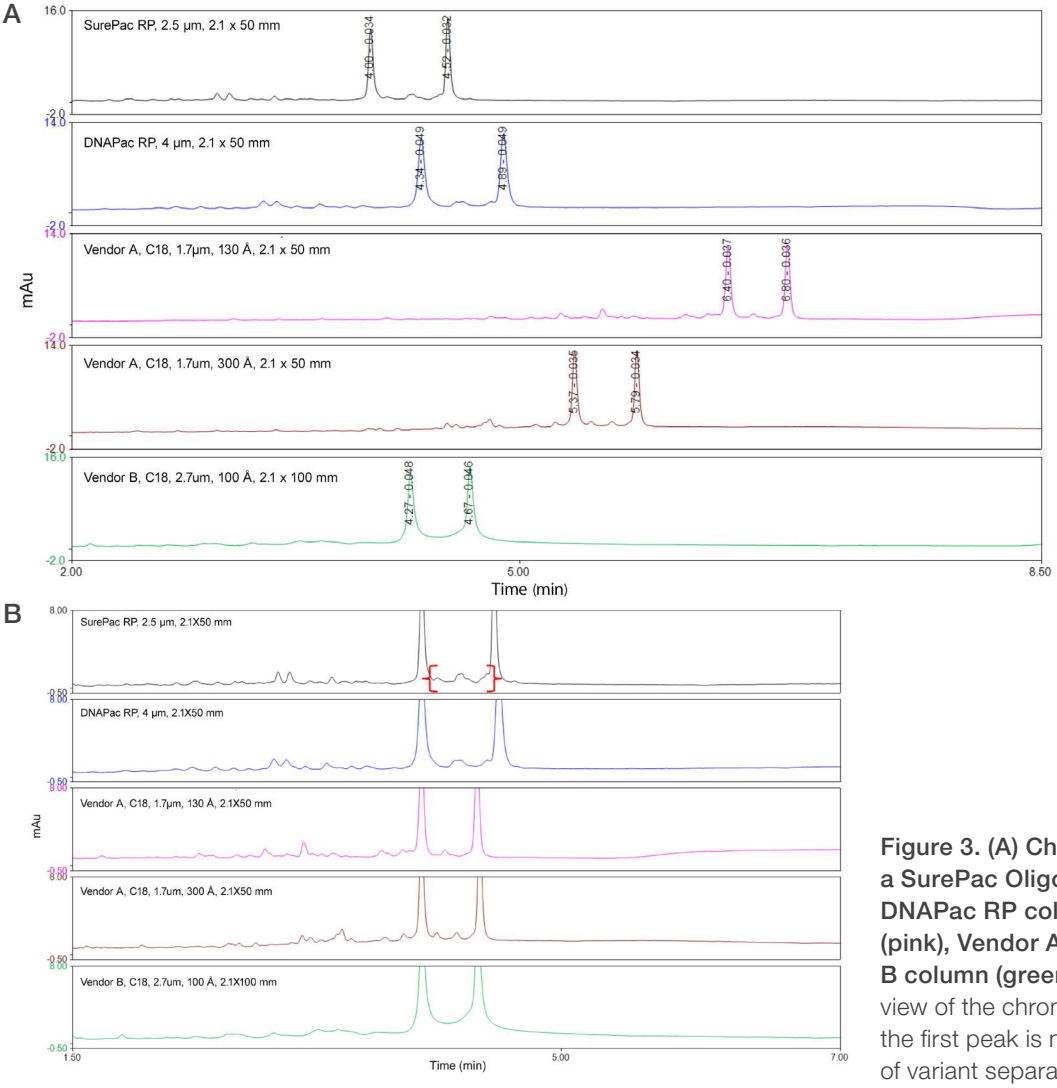


Figure 3. (A) Chromatograms of siRNA using a SurePac Oligo RP MDi column (black), DNAPac RP column (blue), Vendor A column 1 (pink), Vendor A column 2 (brown), and Vendor B column (green) separately. (B) An enlarged view of the chromatograms. The retention time of the first peak is normalized for ease of comparison of variant separation.

Separation of mid-size oligonucleotides (~100 nt)

As the oligonucleotide length increases to approximately 100 nt, pore accessibility becomes a limiting factor for particle and consequently column performance. To evaluate the separation of mid-size oligonucleotides, a 98-, 99-, and 100-mer ssDNA mixture and a 100-mer sgRNA were used. The 98-mer–100-mer ssDNA mix is particularly challenging to evaluate, with the SurePac Oligo RP MDi column providing the best resolution, achieving a clear peak-to-valley for each analyte under the tested conditions. Other tested columns showed reduced or no separation of the analytes. These results highlight the resolving power of the SMP stationary phase for closely related long oligonucleotides differing by a single nucleotide. For applications such as purity assessment or truncated species analysis, this level of resolution is highly beneficial.

For sgRNA (100 nt), which represents an intermediate size range of a therapeutic oligonucleotides sample, the SurePac Oligo RP MDi column was able to provide the most detailed impurity profile among the evaluated columns. The resulting sharper peak shape and improved separation of closely eluting species are likely due to accessible pore structure and selectivity differences.

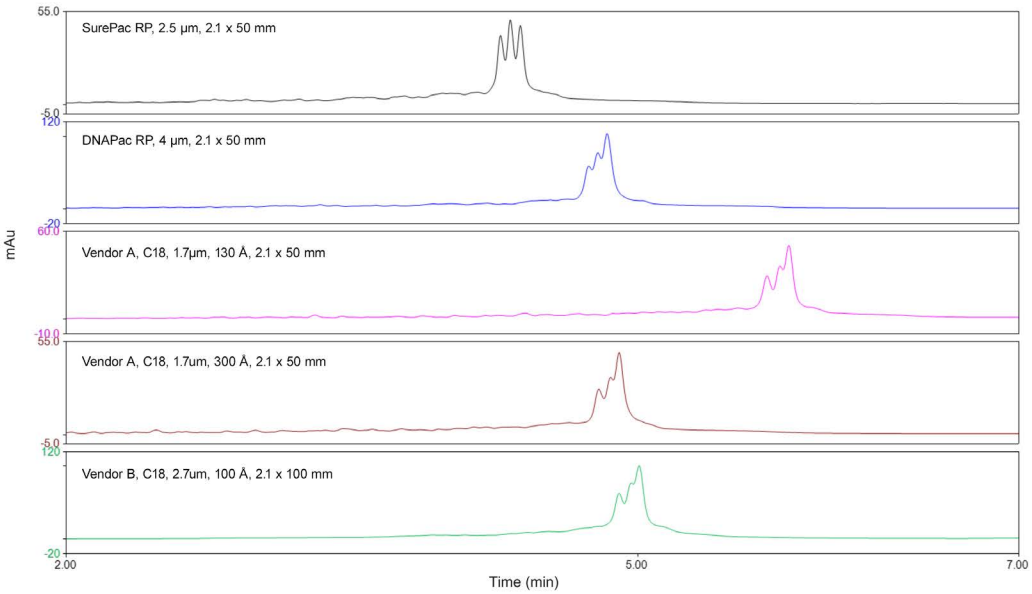


Figure 4. Chromatograms of 98-mer–100-mer ssDNA mix using a SurePac Oligo RP MDi column (black), DNAPac RP column (blue), Vendor A column 1 (pink), Vendor A column 2 (brown), and Vendor B column (green) separately.

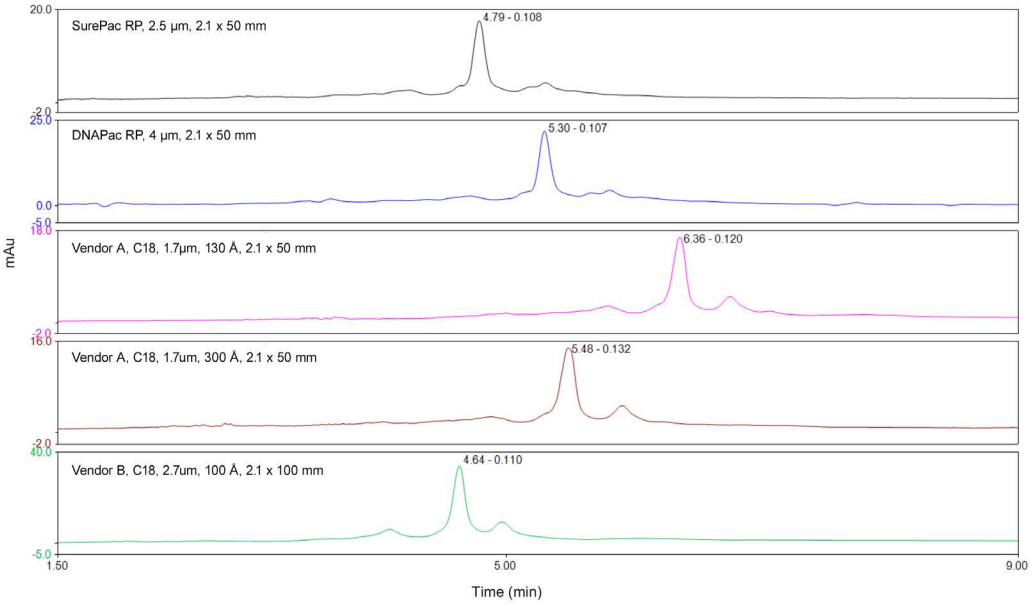


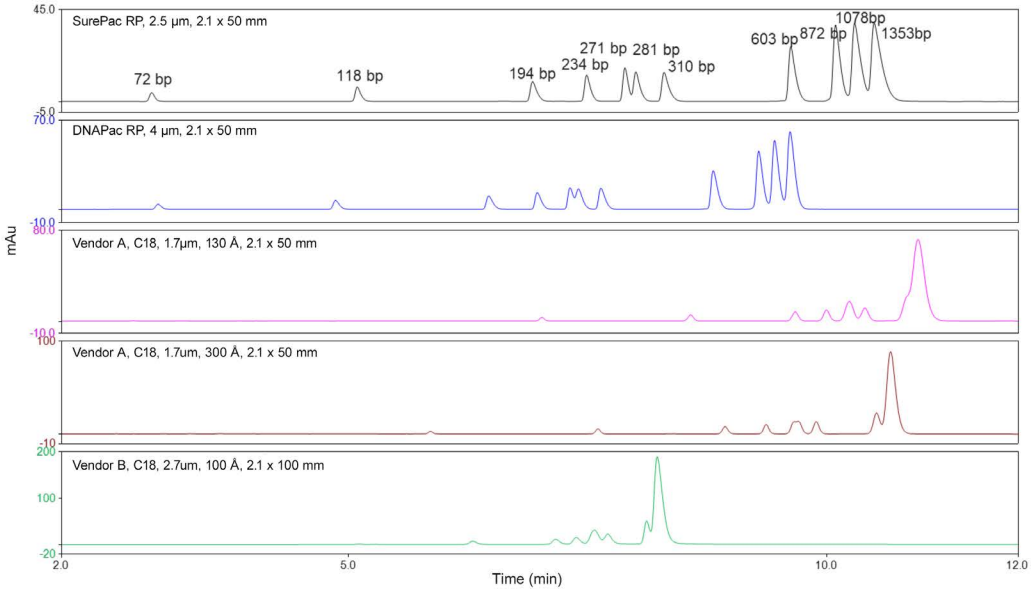
Figure 5. Chromatograms of sgRNA using a SurePac Oligo RP MDi column (black), DNAPac RP column (blue), Vendor A column 1 (pink), Vendor A column 2 (brown), and Vendor B column (green) separately.

Separation of large-size nucleic acids

To evaluate performance with high molecular samples, a double-stranded plasmid DNA digest with fragments from 72–1,353 bp and a 1,960-nucleotide messenger RNA (mRNA) were analyzed. For the dsDNA mix (72–1,353 bp) evaluation, the SurePac Oligo RP MDi column showed a greater peak separation and improved resolution compared to the DNAPac RP column, allowing clearer differentiation of individual components. Under the tested conditions, the other vendors' columns could not effectively separate samples above ~310 bp. These results indicate that the SurePac Oligo RP MDi SMP particles possess accessible large pores similar to the DNAPac RP column for large nucleic acids but with enhanced resolving power.

Large intact mRNA molecules are particularly challenging in separation due to their size and structural heterogeneity. For the analysis of the 1,960 nt mRNA sample, the SurePac Oligo RP MDi column provided improved peak shapes with detection of a proximal early eluting peak. In contrast, the other evaluated vendor columns exhibited sharp peak fronts and significant tailing, indicating limited pore access and limited available surface area (capacity) for large molecule analysis. This result is consistent with the smaller pore size media used for mRNA analysis.

Carryover was assessed by performing a blank run without injection immediately following the mRNA sample injection. The column demonstrated low carryover (~1%), which was comparable to or better than most tested columns. Minimizing carryover is critical in high-throughput environments and for accurate quantitation of low-level impurities, particularly when analyzing high-concentration RNA samples.



Column format:	2.1 x 50 and 100 mm
Mobile phase A:	0.1M TEAA
Mobile phase B:	ACN
Gradient:	9–18% B in 12 min for Vendor B 11–20% B in 12 min for all other columns
System:	Vanquish H
Temperature:	50 °C (PH 50 °C)
Flow rate:	0.4 mL/min
Inj. volume:	5 µL for 2.1 x 50 mm 10 µL for 2.1 x 100 mm
Detection:	UV (260 nm)
Sample:	dsDNA (100 µg/mL)

Figure 6. Chromatograms of dsDNA ladder using a SurePac Oligo RP MDi column (black), DNAPac RP column (blue), Vendor A column 1 (pink), Vendor A column 2 (brown), and Vendor B column (green) separately.

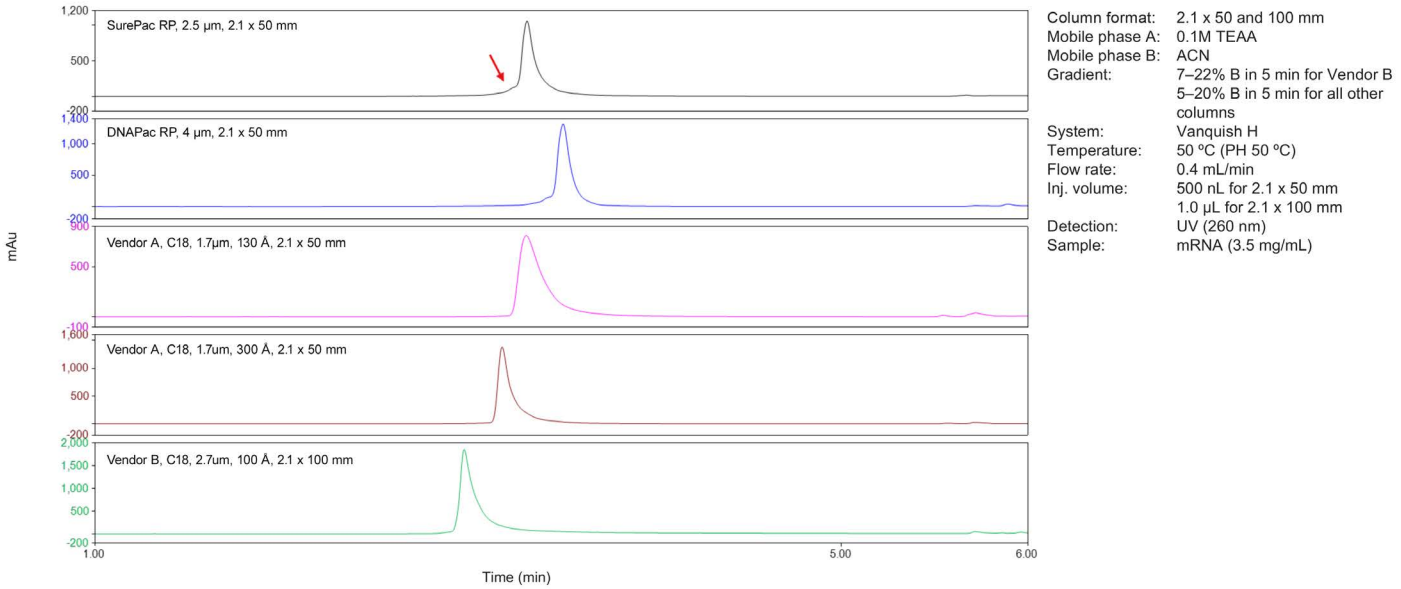
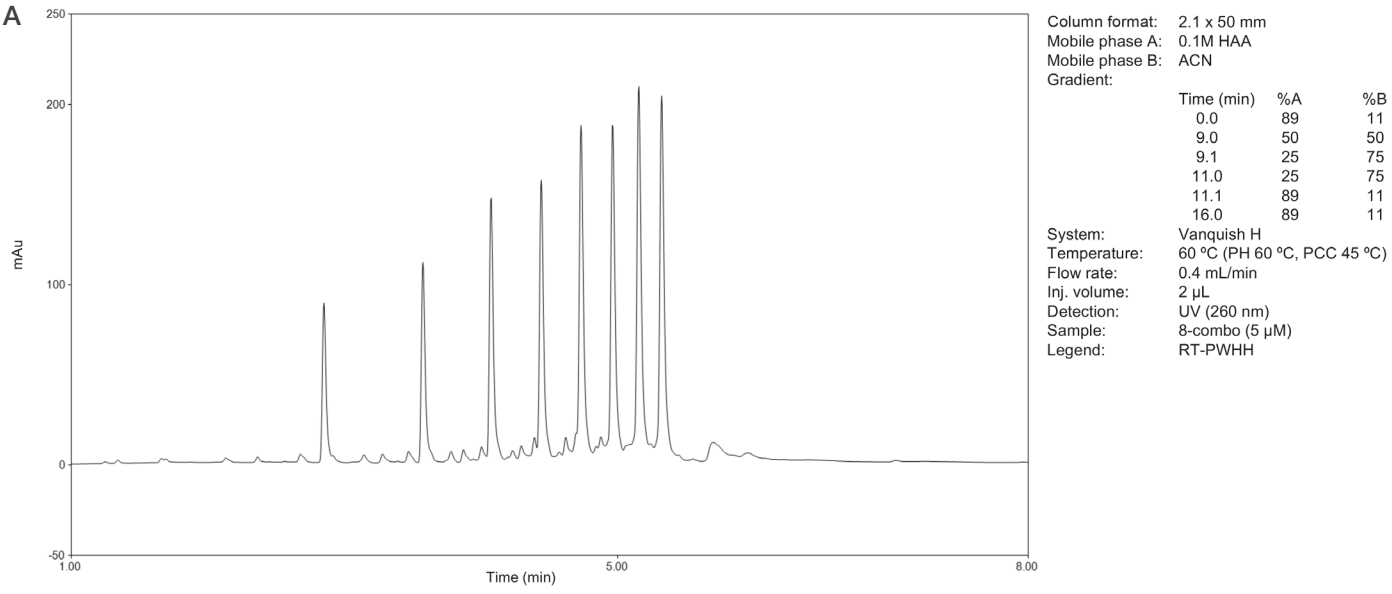


Figure 7. Chromatograms of mRNA using a SurePac Oligo RP MDi column (black), DNAPac RP column (blue), Vendor A column 1 (pink), Vendor A column 2 (brown), and Vendor B column (green) separately.

Evaluation of different mobile phase systems

In addition to the traditional TEAA/ACN mobile phase system, alternative ion-pairing conditions were evaluated on the column to assess flexibility and compatibility with downstream detection methods. Specifically, hexylammonium acetate (HAA) with acetonitrile and a volatile ion-pairing system consisting of HFIP and DBA with methanol were investigated using the 8-component ssDNA mix, 21 bp siRNA, and ~100 nt sgRNA samples.

Using HAA/ACN conditions, the SurePac Oligo RP MDi column had sharper peaks and maintained strong resolving power for short and mid-size oligonucleotides. The overall selectivity, similar to TEAA-based separations with some differences, was observed in the minor variant profile. This mobile phase system provides an alternative option for optimizing the resolution of closely eluting species. Retention behavior was generally consistent and robust, demonstrating that the stationary phase performs well across different ion-pairing reagents without compromising efficiency.



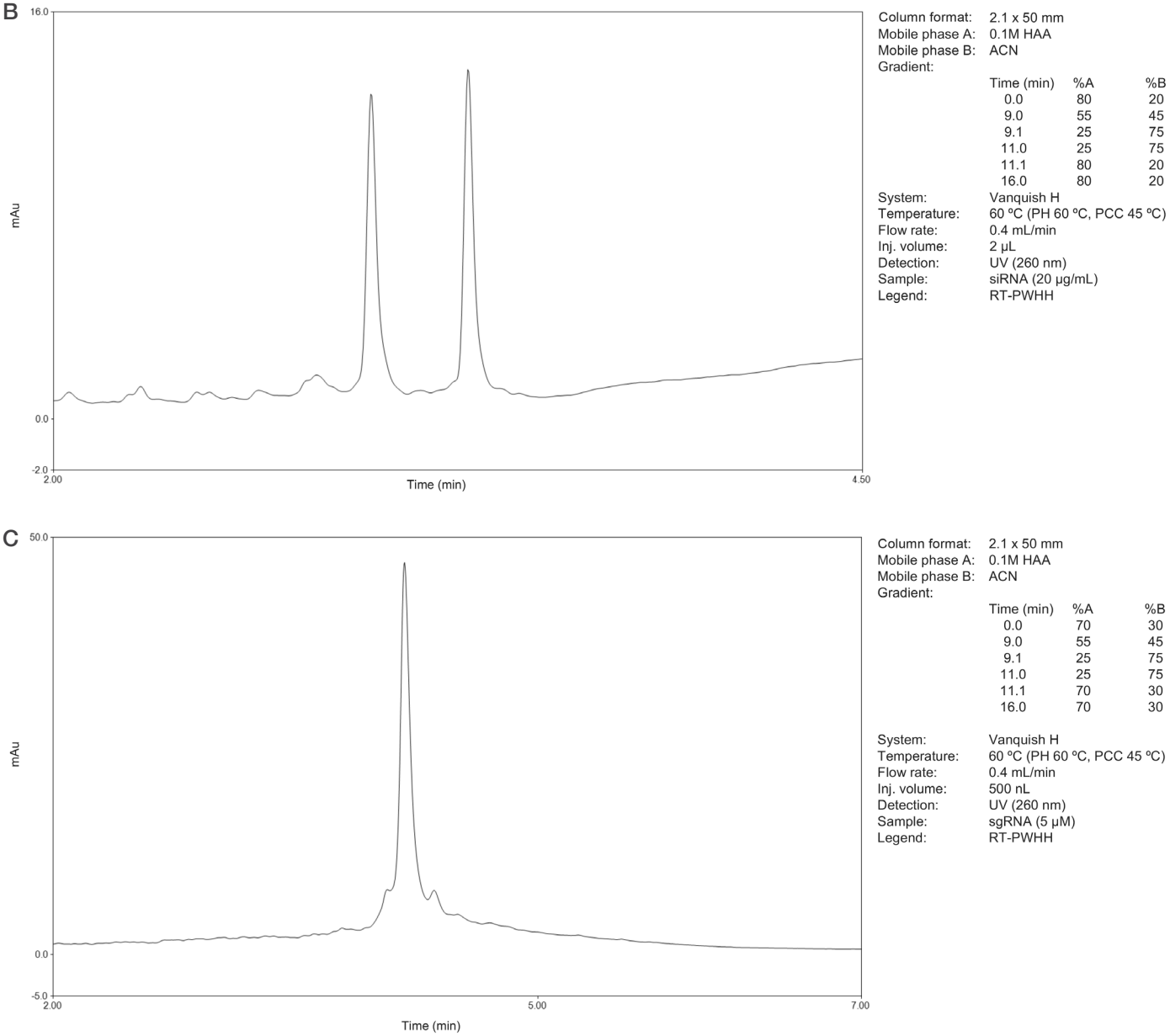
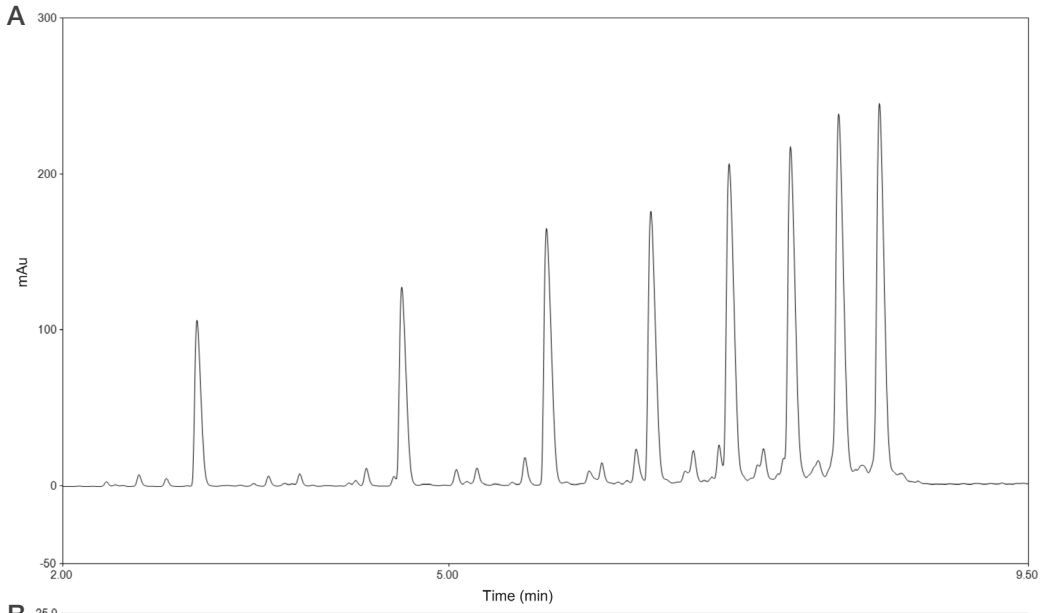


Figure 8. Chromatograms of (A) 8-combo ssDNA, (B) siRNA, and (C) sgRNA using a SurePac Oligo RP MDi column with HAA/ACN mobile phase system.

For MS analysis, volatile mobile phases including ion-pairing agents are required to avoid MS interference and signal suppression. When using the HFIP/DBA/methanol mobile phase system, the SurePac Oligo RP MDi column continued to demonstrate good peak symmetry and impurity resolution, as shown in Figure 9 for 8-combo, siRNA, and sgRNA. For each sample, differences in retention behavior relative to acetate-based systems were observed, reflecting the change in ion-pairing strength and organic modifier. Importantly, the HFIP/DBA system is fully volatile and therefore compatible with mass spectrometry detection. This enables direct coupling with LC-MS workflows for identity confirmation and impurity characterization, without requiring significant modification of chromatographic conditions.

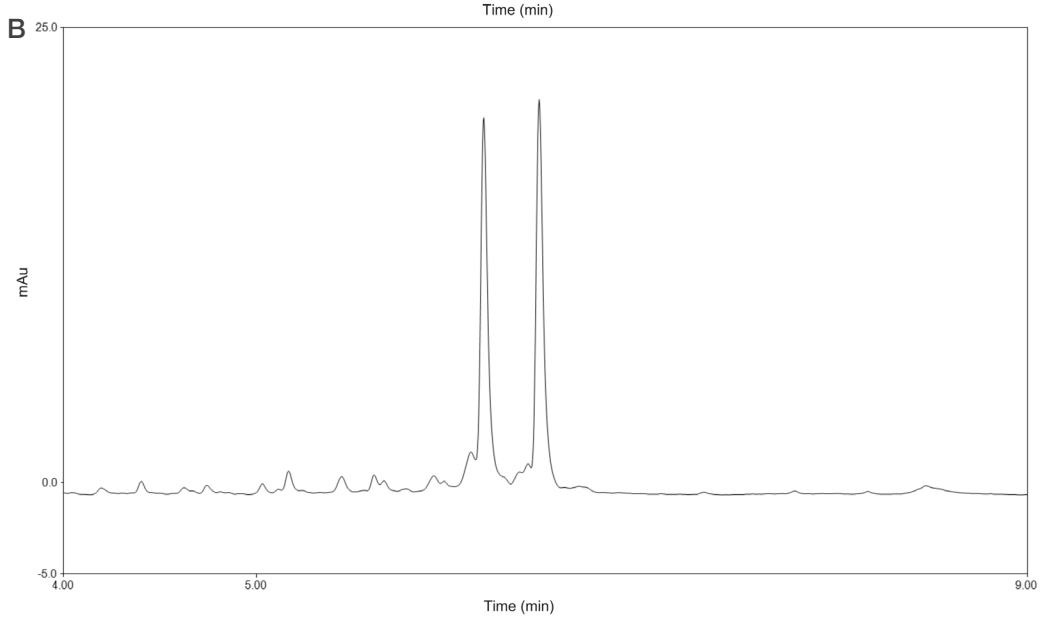
Together, these results demonstrate that the SurePac Oligo RP MDi column is compatible with and performs well with multiple ion-pairing chemistries and organic modifiers, providing flexibility for both UV-based methods and MS-based characterization workflows.



Column format:	2.1 x 50 mm
Mobile phase A:	15 mM DBA, 25 mM HFIP, pH 9.5
Mobile phase B:	Methanol
Gradient:	

Time (min)	%A	%B
0.0	95	5
10.0	60	40
10.0	20	80
13.0	20	80
13.1	95	5
18.0	95	5

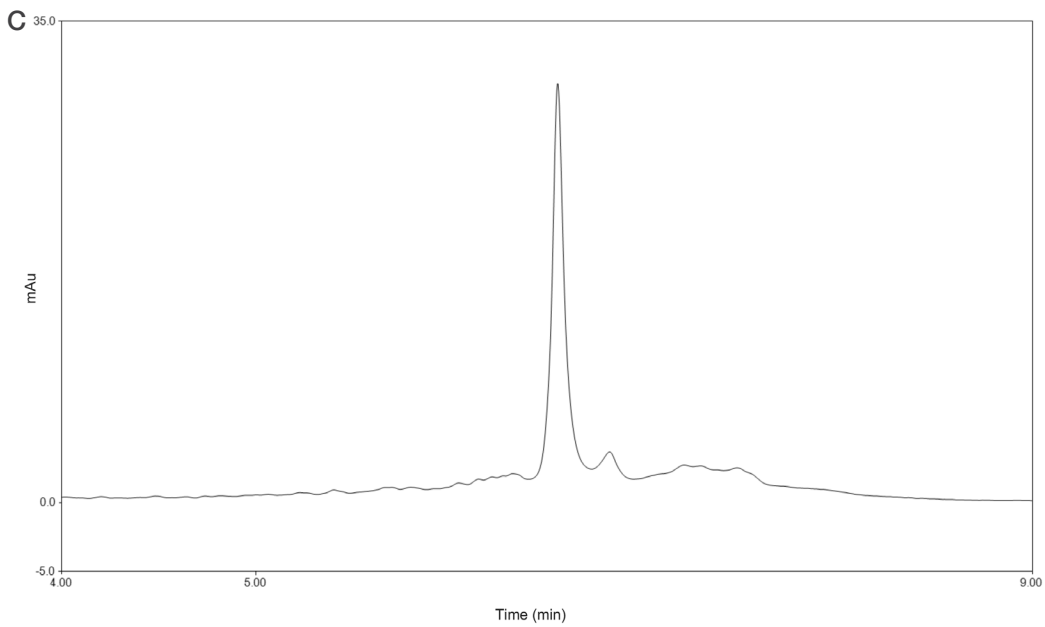
System:	Vanquish H
Temperature:	60 ºC (PH 60 ºC, PCC 45 ºC)
Flow rate:	0.4 mL/min
Inj. volume:	2 µL
Detection:	UV (260 nm)
Sample:	8-combo (5 µM)
Legend:	RT-PWHH



Column format:	2.1 x 50 mm
Mobile phase A:	15 mM DBA, 25 mM HFIP, pH 9.5
Mobile phase B:	Methanol
Gradient:	

Time (min)	%A	%B
0.0	95	5
10.0	60	40
10.0	20	80
13.0	20	80
13.1	95	5
18.0	95	5

System:	Vanquish H
Temperature:	60 ºC (PH 60 ºC, PCC 45 ºC)
Flow rate:	0.4 mL/min
Inj. volume:	2 µL
Detection:	UV (260 nm)
Sample:	siRNA (20 µg/mL)
Legend:	RT-PWHH



Column format:	2.1 x 50 mm
Mobile phase A:	15 mM DBA, 25 mM HFIP, pH 9.5
Mobile phase B:	Methanol
Gradient:	

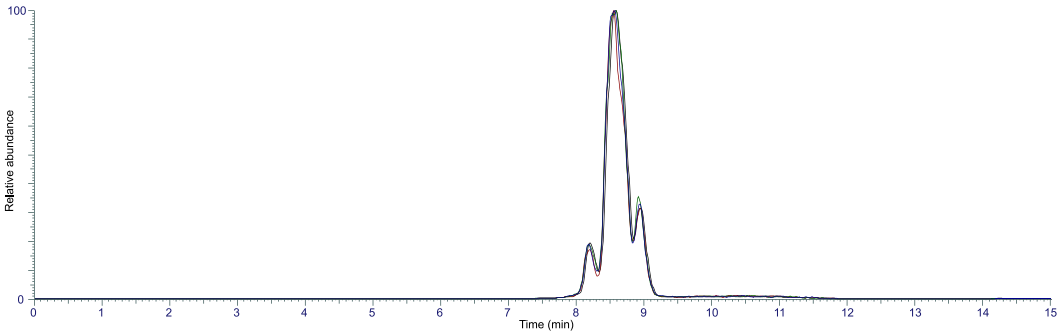
Time (min)	%A	%B
0.0	73	27
10.0	53	47
10.0	20	80
13.0	20	80
13.1	73	27
18.0	73	27

System:	Vanquish H
Temperature:	60 ºC (PH 60 ºC, PCC 45 ºC)
Flow rate:	0.4 mL/min
Inj. volume:	500 nL
Detection:	UV (260 nm)
Sample:	sgRNA (5 µM)
Legend:	RT-PWHH

Figure 9. Chromatograms of (A) 8-combo ssDNA, (B) siRNA, and (C) sgRNA using a SurePac Oligo RP MDi column with HFIP/DBA/methanol mobile phase system.

LC-MS analysis of tRNA^{Phe} sample

To further assess compatibility with LC-MS workflows, intact RNA profiling was performed under HFIP/DBA ion-pairing conditions using high-resolution mass spectrometry. Full-length tRNA^{Phe} (~25 kDa) was analyzed using sliding-window deconvolution (≥5% relative abundance threshold, S/N = 3). Across four replicate injections, 10 intact molecular states were reproducibly detected above the 5% threshold. Injection-to-injection monoisotopic mass accuracy was ≤1 ppm for all major species, with the dominant intact state exhibiting 0.07 ppm CV. Closely related molecular variants differing by 14–16 Da increments were consistently resolved and assigned across replicates, demonstrating stable charge-state distribution and robust neutral mass reconstruction (Figure 10). These results confirm that the SurePac Oligo RP MDi column supports reproducible intact RNA analysis under volatile ion-pairing conditions, enabling reliable detection of modification-state heterogeneity and closely related RNA variants in LC-MS workflows.



Column format:	0.3 × 50 mm
Mobile phase A:	5 mM DBA, 25 mM HFIP
Mobile phase B:	ACN
Gradient:	

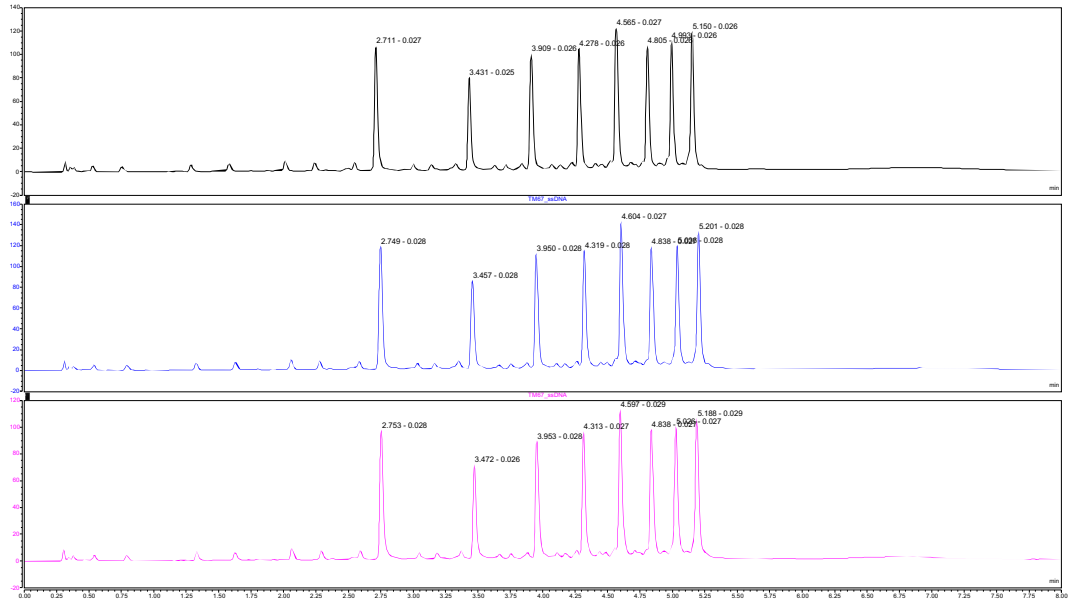
Time (min)	%A	%B
0.0	90	10
0.5	90	10
14.0	72.5	27.5
14.1	10	90
15.0	10	90

System:	Vanquish Neo
Temperature:	50 ºC
Flow rate:	25 µL/min
Inj. volume:	1 µL (400 µg)
Sample:	tRNA^{Phe}

Figure 10. Base peak chromatogram showing overlay of four injections of the tRNA^{Phe} standard with three main variant peaks.

Lot-to-lot reproducibility results

Using the 8-combo ssDNA separation method shown in Figure 8(a), lot-to-lot reproducibility was evaluated across three different lots of the SurePac Oligo RP MDi column stationary phase. Consistent chromatographic performance was observed, with minimal variation in retention time and PWHH for all components. These results demonstrate reliable lot-to-lot reproducibility, providing confidence in method transfer and routine analytical use.



Column format:	2.1 × 50 mm
Mobile phase A:	0.1M HAA
Mobile phase B:	ACN
Gradient:	

Time (min)	%A	%B
0.0	89	11
9.0	50	50
9.1	25	75
11.0	25	75
11.1	89	11
16.0	89	11

Temperature: :	60 ºC (PH 60 ºC, PCC 45 ºC)
Flow rate:	0.4 mL/min
Inj. volume:	2 µL
Detection:	UV (260 nm)
Sample:	8 Combo (5 µM)
Legend:	RT-PWHH

Figure 11. Chromatograms of 8-combo ssDNA using three different lots of SurePac Oligo RP MDi columns.

Conclusions

- The SurePac Oligo RP MDi column provides high-resolution separation across a broad nucleic acid size range from short oligonucleotides to large mRNA molecules.
- Compared to all evaluated columns, the SurePac Oligo RP MDi column demonstrated sharper peak shapes, improved impurity resolution, and robust chromatographic performance across the entire sample size range.
- The SurePac Oligo RP MDi column is compatible with multiple mobile phase systems, including TEAA/acetonitrile, HAA/acetonitrile, and HFIP/DBA/methanol, etc., enabling LC-MS workflows for nucleic acid characterization and impurity analysis.
- Excellent lot-to-lot and column-to-column reproducibility was observed, with consistent retention times and peak shapes, supporting reliable method transfer and routine analytical use.

Learn more at **thermofisher.com/oligoanalysis**