ThermoFisher SCIENTIFIC

(U)HPLC columns

SurePac Oligo RP MDi columns

Product manual

thermo scientific

Contents

Introduction

Introduction to the SurePac Oligo RP MDi column

The Thermo Scientific™ SurePac™ Oligo RP MDi™ (Reversed-Phase Monodisperse and Inert) Column is a chromatography column designed as a single-solution platform for high-resolution separation of nucleic acids varying from tens to over thousands of nucleotides in length. The column is based on a novel 2.5 µm monodisperse supermacroporous (SMP) polymeric stationary phase and is compatible with mass spectrometry-friendly organic solvents, including acetonitrile and methanol, and 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP).

The new monodisperse 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.

The stationary phase is inherently hydrophobic and exhibits broad chemical stability across a wide pH range, along with thermal stability up to 90 °C, supporting flexible and robust method development. In addition, the column hardware is coated with a mildly hydrophobic, inert layer to minimize secondary sample interactions, thereby improving analyte recovery and peak shape from the first injection. Coated hardware parts may exhibit a surface color ranging from grey to orange. Variation in color may also be observed depending on the viewing angle of the hardware.

Compared to conventional polydisperse particle materials (Figure 1, right), the monodisperse particles (Figure 1, left) exhibit a consistent size distribution resulting in improved column manufacturing consistency, and lot-to-lot reproducibility. Improved reproducibility supports easier and more robust method development for reliable method transfer and long-term analytical use.

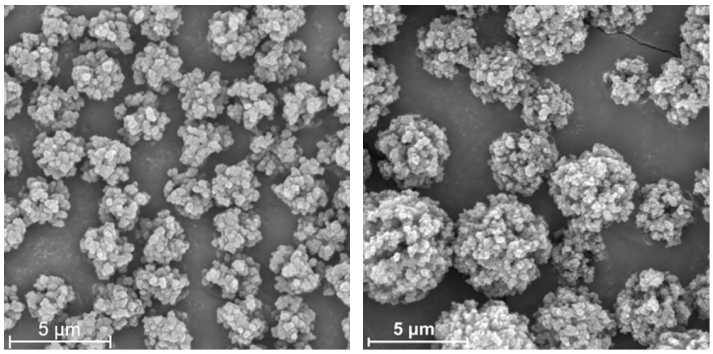


Figure 1. Scanning Electron Microscopy (SEM) image of monodisperse SurePac Oligo RP MDi column particles (left) vs. conventional polydisperse particles (right). White scale bars are 5 µm in length.

Getting started

Thermo Fisher Scientific recommends performing an efficiency test on the SurePac Oligo RP MDi column before use. The purpose of column performance validation is to ensure no damage has occurred during shipping. Steps below outline the necessary process to validate system operation. Test the column using the conditions described on the Certificate of Analysis (CoA) enclosed in the column box. Repeat the test periodically to track the column performance over time. Note that slight variations may be found on two different HPLC systems due to system differences including hardware, plumbing, operating environment, reagent quality, column conditioning, and operator technique.

Step 1: Visually inspect the column

Report any visible damage upon receiving the column to Thermo Fisher Scientific immediately.

Step 2: Prepare mobile phases

To obtain reliable, consistent and accurate results, use High-performance liquid chromatography-mass spectrometry (HPLC-MS) grade solvents and Type 1 reagent grade water with a specific resistance of 18.2 megohm-cm or greater filtered through a 0.22 µm filter.

- Mobile phase A: 0.1 M TEAA, pH 7.0

In a clean 1 L bottle equipped with a stir bar, add 50.65 g of 2.0 M TEAA and 950.0 g of deionized water (18.2 megohm-cm). Stir for 2 minutes.

- Mobile phase B: 100% acetonitrile.

Step 3: Set up the LC system

Use a biocompatible or inert LC system equipped with an LC pump, a column oven, a UV detector (260 nm) and an autosampler. It is highly recommended that the system be optimized for low dead volume; for 2.1 mm ID columns, usage of small internal diameter tubing with Thermo Scientific™ Viper™ TQ UHPLC Fittings (such as 100 µm) and a proper detector flow cell (such as the 2.5 µL semi-micro biocompatible flow cell) is required for best results. The system should be thoroughly primed before use. In addition, temperature gradients across the column will degrade peak shape. Use an eluent preheater between the pump or injection valve and the column.

Note: For increased 0.3 × 50 mm format longevity and result consistency, it is recommended to use relatively fresh or completely new out-of-the box Thermo Scientific™ nanoViper™ Fingertight Fittings in combination with a torque wrench (Part No. 6250.2110) for easy and consistent column attachment. Regularly check for nanoViper fingertight fittings thread deterioration. If thread screw-in resistance increases, a change of nanoViper fingertight fittings is recommended. 20 µm and 50 µm internal diameter viper connections are recommended for the 0.3 × 50 mm column.

Step 4: Condition the column

Set the pressure limit on the pump according to maximum column backpressure specifications given in Table 1. Slowly ramp up the flow rate: a flow ramp rate (mL/min/min) of ~⅓ of the maximum flow rate for the specific column format is recommended. Wash the column with mobile phase for 20 minutes.

Step 5: Verify the performance of the column

Each column is shipped with two Certificates of Analysis (CoA) verifying the resin performance and the column performance. Each CoA provides the test conditions used. These tests can be reproduced to check the performance of your column. The lot qualification tests are performed using a 2.1 × 50 mm column and the gradient and flow rate should be scaled based on column length and diameter, respectively. Note that differences in system configuration may result in differences in retention time and chromatographic performance.

Step 6: Real sample analysis

Once the column performance is satisfactorily confirmed in previous steps, the column is ready for real sample analysis. Equilibrate the column with the desired mobile phase before sample analysis. Note that it is recommended that the column performance test be performed periodically to monitor the condition of the column.

Column care

Column storage and extended care

To maintain the performance of your column between uses, always store the column in mobile phase buffer for short-term storage. For long-term storage (more than 24 hours), it is recommended to store the column in water/acetonitrile (50/50 v/v). Use the plugs the column was shipped with to seal the ends of the column to prevent evaporation of the buffer and drying of the stationary phase.

Operating pH range

Operating pH range for the SurePac Oligo RP MDi columns is 2 to 12 for 2.1 mm I.D. columns, and 2 to 10 for 0.3 mm I.D. columns.

Operating temperature limit

The SurePac Oligo RP MDi columns are stable at high temperature up to 90 °C. The typical operating temperature for nucleic acid separation is between 30 °C and 60 °C. For native analysis of double-stranded nucleic acids, keep the temperature below the melting point (T_m).

Pressure limit

The column pressure should not exceed the limit specified in Table 1. The back pressure of the column is strongly correlated to the column I.D, length, column temperature, flow rate and type of organic solvent used for mobile phase.

Flow rate

Maximum flow rate will depend on the column I.D., length, column temperature and type of organic solvent used for mobile phase. Please refer to operating condition table (Table 1) for recommended flow rate.

Column cleaning and troubleshooting

Column washing procedure: carry-over may occur if the system is not clean or previous sample may not have completely eluted from the column. Wash the system and the column with acetonitrile/0.1M NaOH (90:10 v/v).

For metal contamination (Fe, Cu, etc.) removal, wash the column in the following sequence:

1. 100 mM NH_4OAc for 10 column volumes.
2. 100 mM Pyrophosphate solution for 100 column volumes.
3. 100 mM NH_4OAc for 10 column volumes.
4. ACN/100 mM NH_4OAc (90:10; v/v) for 20 column volumes.
5. ACN/H_2O (50:50; v/v) for 10 column volumes.

Note: Above procedure may need to be combined with the column washing procedure.

Additional requirements for safe column operation

- Always set up the mobile phase flow direction as indicated on the column tag.
- Avoid exposing the column bed to sharp pressure fluctuations that may disrupt the column bed.
- When starting, stopping, or changing the flow rate, a flow ramp rate (mL/min/min) of ~⅓ of the maximum flow rate for the specific column format is recommended.
- Avoid leaving the column in 100% aqueous mobile phase for an extended period of time.

Table 1. Recommend column operating conditions for optimal performance and extending column lifetime

Column dimension (mm)	Recommended flow rate[1] (mL/min)	Max. column pressure drop[2] psi (bar)	Temperature (°C)	pH
0.3 × 50 mm	0.001 – 0.012	8000 (552)	5 - 90	2 - 10
2.1 × 20 mm	0.2 – 1.0	6500 (448)	5 - 90	2 - 12
2.1 × 50 mm	0.2 – 0.6	6500 (448)	5 - 90	2 - 12
2.1 × 100 mm	0.2 – 0.6	6500 (448)	5 - 90	2 - 12
10 × 50 mm	1 – 5	5600 (380)	5 - 90	2 - 12
21.2 × 50 mm	4 – 12	4000 (275)	5 - 90	2 - 12

[1]The maximum usable flow rate is dependent on backpressure. Ensure the column pressure does not exceed the specified pressure limit.

[2]The column pressure drop for a given flow rate is calculated as the pressure of the system with column minus the pressure of system with union in place of column.

Method development and applications

When transferring methods from Thermo Scientific™ DNAPac™ RP Column to the SurePac Oligo RP MDi column, direct transfer may not always give the same results. It is recommended to recheck the injection amount, as differences in stationary phase properties (such as lower surface area) can affect loading, retention, and peak shape. In some cases, mobile phase composition or ion-pairing reagent concentration may also need to be adjusted. DNAPac RP column methods can be used as a starting point, followed by small adjustments to optimize performance.

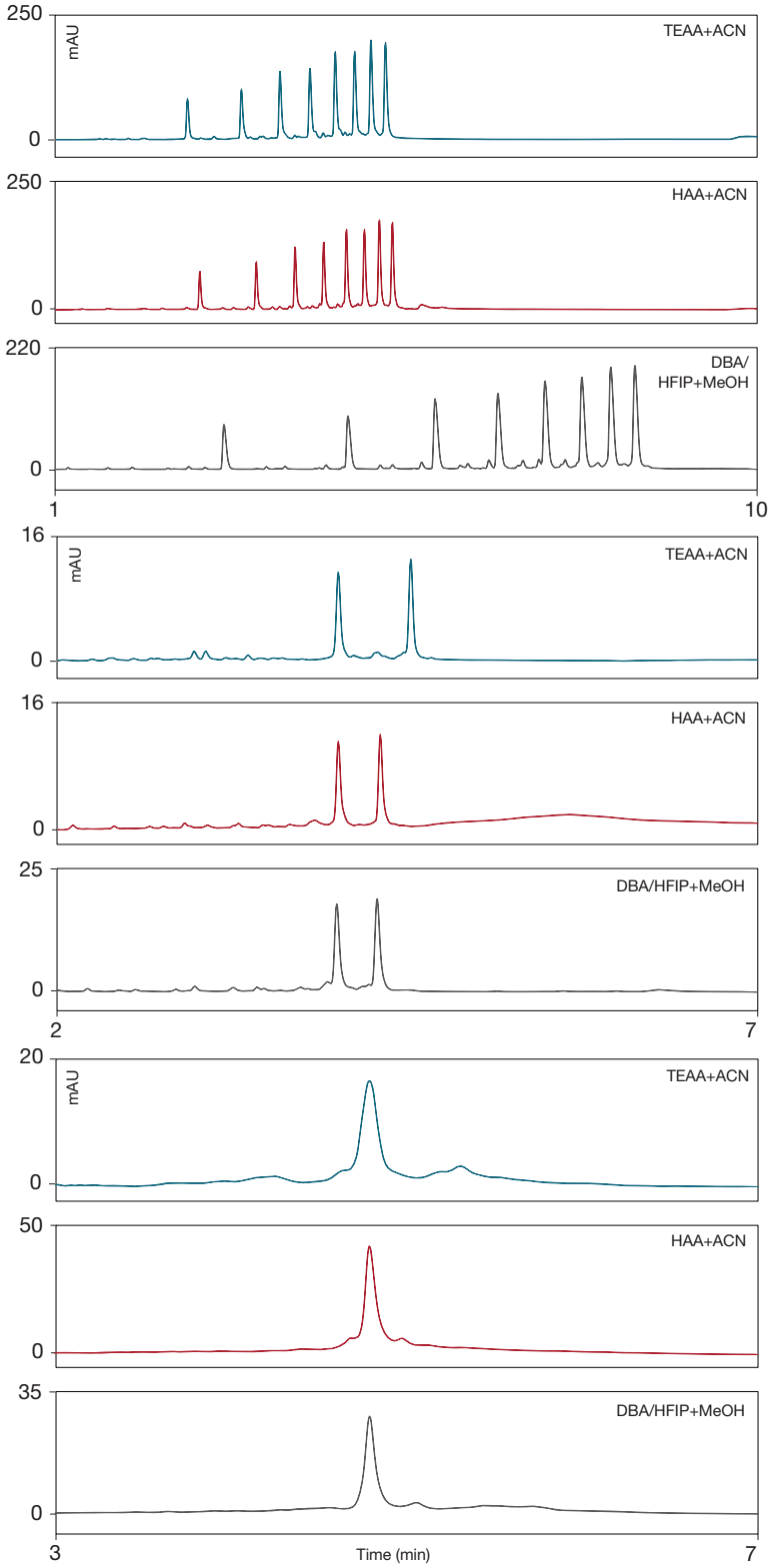
Mobile phase

Selection of mobile phase composition is critical for achieving optimal separation of nucleic acids using the SurePac Oligo RP MDi column. Ion-pairing reagents, organic modifiers, and pH collectively influence retention, selectivity, and resolution.

Figure 2 shows examples of commonly used ion-pairing systems including triethylammonium acetate (TEAA), hexylammonium acetate (HAA), and alkylamine/HFIP systems for MS-compatible workflows. TEAA-based mobile phases are widely used for liquid chromatography with ultraviolet detection (LC-UV) analysis and provide robust and reproducible separations. HAA-based systems may offer improved selectivity for certain oligonucleotide sequences. For liquid chromatography-mass spectrometry (LC-MS) applications, alkylamine (e.g., TEA and DBA) with HFIP enhances ionization efficiency and improves MS sensitivity.

Representative mobile phase systems evaluated with the SurePac Oligo RP MDi column include: TEAA/acetonitrile (ACN), HAA/ACN and Dibutylamine (DBA)/HFIP with methanol (MeOH). These systems support separation of oligonucleotides across a wide size range, from short single-stranded DNA (ssDNA) to longer RNA species such as sgRNA, as shown in Figure 2. Optimization of ion-pairing reagent concentration, pH, and organic solvent composition may further improve resolution depending on sample type and detection mode.

Method development and applications (continued)



Column	SurePac Oligo RP MDi column, 2.1 × 50 mm
Mobile phases	Top: MPA: 0.1 M TEAA; MPB: ACN
	Middle: MPA: 0.1 M HAA; MPB: ACN
	Bottom: MPA: 15 mM DBA, 25 mM HFIP, pH 9.0; MPB: MeOH
Gradient	Top: 3–13% B in 9 min
	Middle: 11–50% B in 9 min
	Bottom: 5–40% B in 10 min
System	Thermo Scientific™ Vanquish™ Horizon System
Temperature	60 °C (PH 60 °C, PCC 45 °C)
Flow rate	0.4 mL/min
Injection volume	2 µL
Detection	UV (260 nm)
Sample	8-combo ssDNA mix

PH-Pre-heater; PCC-Post column cooler

Column	SurePac Oligo RP MDi column, 2.1 × 50 mm
Mobile phases	Top: MPA: 0.1 M TEAA; MPB: ACN
	Middle: MPA: 0.1 M HAA; MPB: ACN
	Bottom: MPA: 15 mM DBA, 25 mM HFIP, pH 9.0; MPB: MeOH
Gradient	Top: 2–12% B in 9 min
	Middle: 20–45% B in 9 min
	Bottom: 5–40% B in 10 min
System	Vanquish Horizon system
Temperature	60 °C (PH 60 °C, PCC 45 °C)
Flow rate	0.4 mL/min
Inj. volume	2 µL
Detection	UV (260 nm)
Sample	siRNA (20 µg/mL)

Column	SurePac Oligo RP MDi column, 2.1 × 50 mm
Mobile phases	Top: MPA: 0.1 M TEAA; MPB: ACN
	Middle: MPA: 0.1 M HAA; MPB: ACN
	Bottom: MPA: 15 mM DBA, 25 mM HFIP, pH 9.0; MPB: MeOH
Gradient	Top: 6–16% B in 9 min
	Middle: 30–45% B in 9 min
	Bottom: 27–47% B in 10 min
System	Vanquish Horizon system
Temperature	60 °C (PH 60 °C, PCC 45 °C)
Flow rate	0.4 mL/min
Inj. volume	500 nL
Detection	UV (260 nm)
Sample	sgRNA (5 µM)

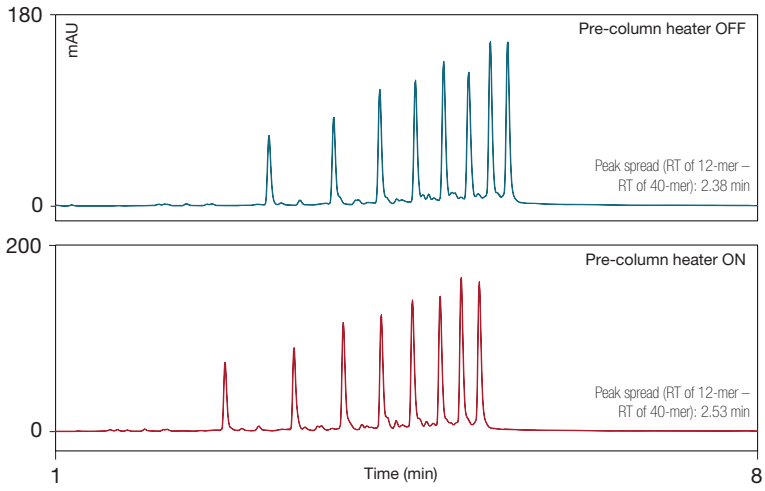
Figure 2. Chromatograms of (top) 8-combo ssDNA mix, (middle) siRNA and (bottom) sgRNA using a SurePac Oligo RP MDi column with TEAA/ACN, HAA/ACN, and HFIP/DBA/methanol mobile phase system. Peak retention time is normalized for ease of comparison.

Method development and applications (continued)

Pre-column heater

Temperature gradients between the mobile phase and the column can lead to band broadening and reduced resolution. Use of a pre-column heater is recommended to ensure thermal equilibration prior to sample entering the column.

Evaluation with the SurePac Oligo RP MDi column as shown in Figure 3 demonstrates that use of a pre-column heater improves separation efficiency and peak spread. For example, the separation spread between 12-mer to 40-mer of the 8-combo ssDNA mix increases (2.53 min vs. 2.36 min) when a pre-column heater is applied, and slightly sharper peaks are also observed.



Column	SurePac Oligo RP MDi column, 2.1 × 50 mm		
Mobile phases	A: 0.1 M TEAA; B: ACN		
Gradient	Time (min)	%A	%B
	0.0	97	3
	9.0	87	13
	9.1	75	25
	11.0	75	25
	11.1	97	3
	16.0	97	3
System	Vanquish Horizon system		
Temperature	60 °C (PCC 45 °C); Top: pre-column heater OFF; bottom: pre-column heater ON		
Flow rate	0.4 mL/min		
Inj. volume	2 µL		
Detection	UV (260 nm)		
Sample	8-combo ssDNA mix		

Figure 3. Effect of pre-column heater on separation of 8-combo ssDNA mix sample.

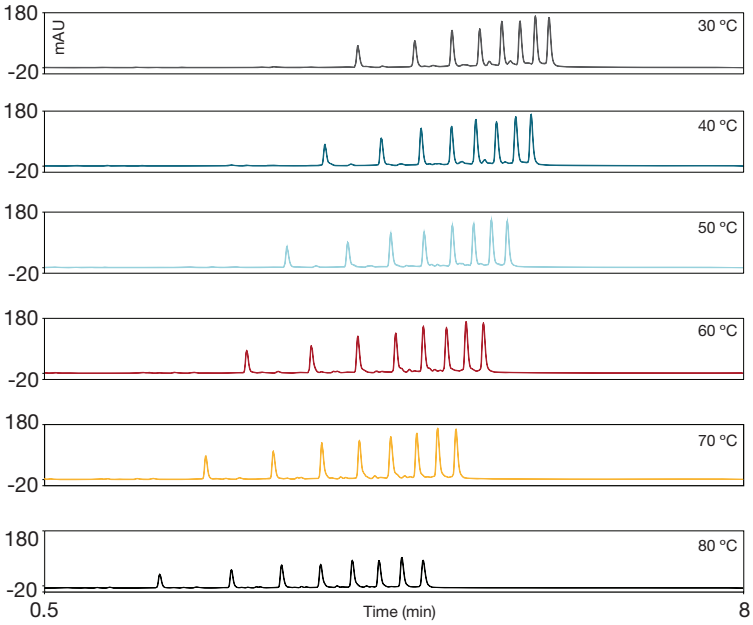
Method development and applications (continued)

Column temperature

Column temperature significantly impacts retention, resolution, and analysis time. Increasing temperature generally reduces mobile phase viscosity, leading to lower back pressure and faster mass transfer. Therefore, oligonucleotide separation at higher temperature requires less organic solvent and supports higher flow rates, increasing throughput.

Higher temperatures improve resolution, particularly for longer oligonucleotides or sequences with secondary structures, by promoting denaturation and reducing intra- or inter-strand interactions. Evaluation across a temperature range (30–80 °C) shows improved peak resolution and reduced retention times at elevated temperatures (Figure 4).

Typical operating temperatures for oligonucleotide separations range from 50 °C to 80 °C. Lower temperatures may be used for native analysis of structured nucleic acids where preservation of conformation is required.



Column	SurePac Oligo RP MDi column, 2.1 × 50 mm		
Mobile phases	A: 0.1 M TEAA; B: ACN		
Gradient	Time (min)	%A	%B
	0.0	97	3
	9.0	87	13
	9.1	75	25
	11.0	75	25
	11.1	97	3
	16.0	97	3
System	Vanquish Horizon system		
Temperature	See chromatograms (PCC 45 °C)		
Flow rate	0.4 mL/min		
Inj. volume	2 µL		
Detection	UV (260 nm)		
Sample	8-combo ssDNA mix		

Figure 4. Effect of column temperature on separation of 8-combo ssDNA mix sample.

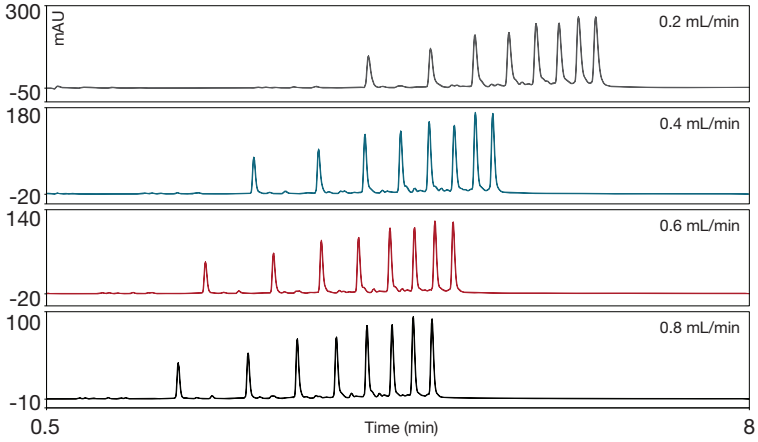
Method development and applications (continued)

Flow rate and gradient time

Flow rate and gradient time are interdependent parameters that together determine separation resolution and throughput. At lower flow rates, higher sensitivity can be achieved due to increased interaction time between analytes and the stationary phase. However, this comes at the expense of longer analysis times. Increasing flow rate reduces run time and improves throughput, but may result in decreased resolution (Figure 5).

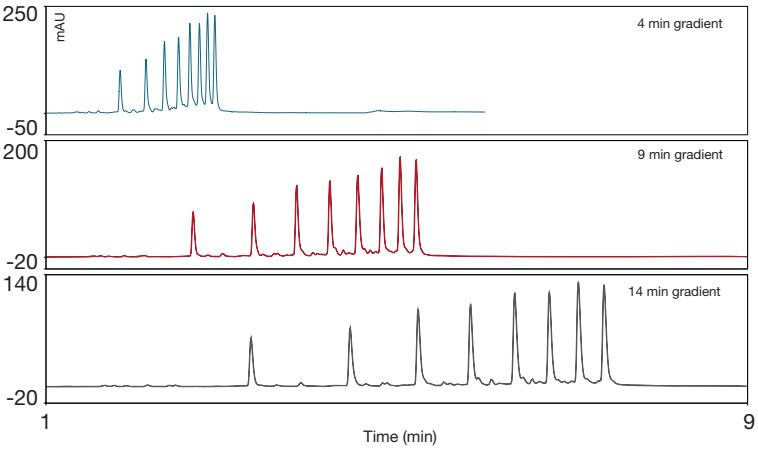
Similarly, longer gradient times improve resolution by allowing more gradual elution of analytes, while shorter gradients enable faster separations with reduced resolving power. For example, gradients ranging from 4 to 14 minutes demonstrate the trade-off between resolution and analysis speed (Figure 6).

Method development typically involves simultaneous optimization of flow rate and gradient slope to achieve the desired balance between resolution and throughput.



Column	SurePac Oligo RP MDi column, 2.1 × 50 mm		
Mobile phases	A: 0.1 M TEAA; B: ACN		
Gradient	Time (min)	%A	%B
	0.0	97	3
	9.0	87	13
	9.1	75	25
	11.0	75	25
	11.1	97	3
	16.0	97	3
System	Vanquish Horizon system		
Temperature	60 °C (PH 60 °C, PCC 45 °C)		
Flow rate	See chromatograms		
Inj. volume	2 µL		
Detection	UV (260 nm)		
Sample	8-combo ssDNA mix		

Figure 5. Effect of flow rate on separation of 8-combo ssDNA mix sample.



Column	SurePac Oligo RP MDi column, 2.1 × 50 mm
Mobile phases	A: 0.1 M TEAA; B: ACN
Gradient	Top: 3–13% B in 4 min Middle: 3–13% B in 9 min Bottom: 3–13% B in 14 min
System	Vanquish Horizon system
Temperature	60 °C (PH 60 °C, PCC 45 °C)
Flow rate	0.4 mL/min
Inj. volume	2 µL
Detection	UV (260 nm)
Sample	8-combo ssDNA mix

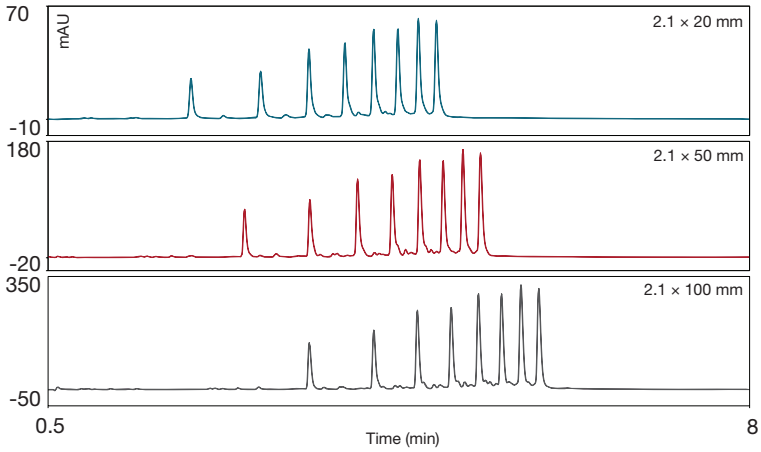
Figure 6. Effect of gradient time on separation of 8-combo ssDNA mix sample.

Method development and applications (continued)

Column format and scaling

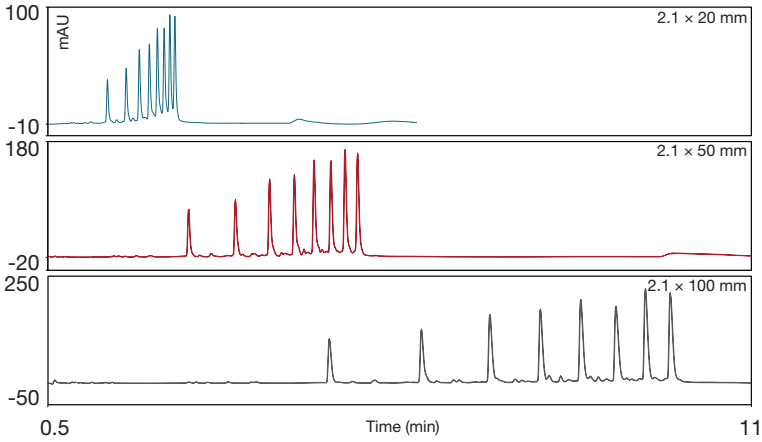
Column dimensions (length and internal diameter) influence resolution, analysis time, and method scalability. When identical gradient conditions are applied across different column lengths (e.g., 2.1 × 20 mm, 50 mm, and 100 mm), similar separation profiles are observed, with comparable selectivity and peak patterns as shown in Figure 7. This indicates that the stationary phase chemistry governs selectivity, while column length primarily affects separation efficiency.

When the gradient is adjusted proportionally to column length (Figure 8), differences in column performance become more apparent. Shorter columns enable faster analysis and higher throughput, while longer columns provide increased resolving power and improved separation of closely related species. This trade-off allows method development to be tailored based on analytical priorities, offering flexibility to optimize for speed, resolution, or a balance of both depending on the application requirements.



Columns	SurePac Oligo RP MDi columns: Top: 2.1 × 20 mm; Middle: 2.1 × 50 mm; Bottom: 2.1 × 100 mm		
Mobile phases	A: 0.1 M TEAA; B: ACN		
Gradient	Time (min)	%A	%B
	0.0	97	3
	9.0	87	13
	9.1	75	25
	11.0	75	25
	11.1	97	3
	16.0	97	3
System	Vanquish Horizon system		
Temperature	60 °C (PH 60 °C, PCC 45 °C)		
Flow rate	0.4 mL/min		
Inj. volume	0.8, 2 and 4 µL		
Detection	UV (260 nm)		
Sample	8-combo ssDNA mix		

Figure 7. Effect of column format on separation of 8-combo ssDNA mix sample using the same gradient.



Columns	SurePac Oligo RP MDi columns: Top: 2.1 × 20 mm; Middle: 2.1 × 50 mm; Bottom: 2.1 × 100 mm
Mobile phases	A: 0.1 M TEAA; B: ACN
Gradient	Top: 3–13% B in 3.6 min Middle: 3–13% B in 9 min Bottom: 3–13% B in 18 min
System	Vanquish Horizon system
Temperature	60 °C (PH 60 °C, PCC 45 °C)
Flow rate	0.4 mL/min
Inj. volume	0.8, 2 and 4 µL
Detection	UV (260 nm)
Sample	8-combo ssDNA mix

Figure 8. Effect of column format on separation of 8-combo ssDNA mix sample using a gradient scaled according to column length.

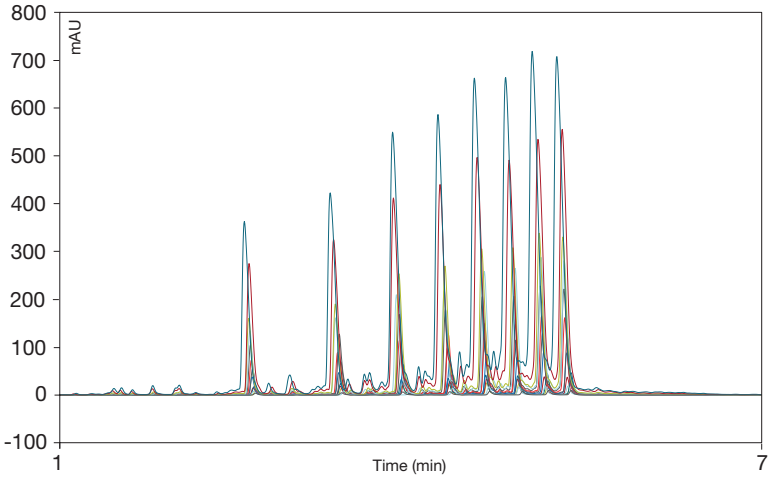
Method development and applications (continued)

Sample loading

Sample loading (injection volume and mass) affects peak shape, resolution, and quantitative performance. In Figure 9, at low injection volumes (0.1–2 µL), peak shapes remain sharp with optimal resolution, indicating operation within the column's optimal dynamic loading range. Increasing sample load can enhance detection sensitivity; however, excessive loading may result in peak broadening and reduced resolution due to column overloading. At higher injection volumes (≥5 µL), a more pronounced increase in peak width is observed, consistent with the onset of overloading effects, which may compromise separation of closely eluting components.

Dynamic loading capacity is influenced by analyte molecular weight and structure. Oligonucleotides with lower molecular weight typically exhibit lower loading capacity due to their higher surface interaction relative to mass. The data presented here may serve as a general reference for other oligonucleotide analytes.

Evaluation across a wide injection volume range (0.5–15 µL) demonstrates that the SurePac Oligo RP MDi column maintains robust performance over a broad loading range. The optimal injection volume should be selected based on sample concentration, target sensitivity, and resolution requirements.



Format	SurePac Oligo RP MDi column, 2.1 × 50 mm		
Mobile phases	A: 0.1 M TEAA; B: ACN		
Gradient	Time (min)	%A	%B
	0.0	97	3
	9.0	87	13
	9.1	75	25
	11.0	75	25
	11.1	97	3
	16.0	97	3
System	Vanquish Horizon system		
Temperature	60 °C (PH 60 °C, PCC 45 °C)		
Flow rate	0.4 mL/min		
Inj. volume	0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 10.0, 15.0 µL		
Detection	UV (260 nm)		
Sample	8-combo ssDNA mix		

Figure 9. Effect of sample loading from 0.1 to 15 µL on separation of 8-combo ssDNA mix sample.

Table 3. PWHH of 12-mer to 40-mer across injection volume from 0.1 to 15 µL

Injection volume (µL)	12-mer	16-mer	20-mer	24-mer	28-mer	32-mer	36-mer	40-mer	Average
0.1	0.032	0.033	0.031	0.032	0.033	0.03	0.031	0.031	0.032
0.2	0.032	0.033	0.03	0.03	0.03	0.03	0.03	0.031	0.031
0.3	0.032	0.031	0.031	0.031	0.031	0.031	0.03	0.031	0.031
0.4	0.032	0.032	0.031	0.031	0.03	0.031	0.031	0.031	0.031
0.5	0.033	0.031	0.031	0.031	0.031	0.032	0.033	0.031	0.032
1	0.032	0.032	0.032	0.032	0.032	0.032	0.033	0.033	0.032
1.5	0.034	0.033	0.034	0.034	0.035	0.035	0.035	0.037	0.035
2	0.034	0.035	0.035	0.037	0.036	0.036	0.037	0.038	0.036
3	0.036	0.038	0.038	0.038	0.04	0.04	0.041	0.042	0.039
4	0.038	0.04	0.043	0.042	0.044	0.042	0.045	0.045	0.042
5	0.04	0.043	0.044	0.044	0.047	0.046	0.048	0.049	0.045
10	0.048	0.055	0.056	0.057	0.06	0.06	0.064	0.061	0.058
15	0.057	0.065	0.065	0.066	0.069	0.069	0.073	0.073	0.067

Method development and applications (continued)

Inert coating performance

The SurePac Oligo RP MDi column hardware is coated with an inert hydrophobic layer to minimize secondary interactions and improve analyte recovery. Evaluation using a nucleotide probe Adenosine 5'-diphosphate (AMPcP) demonstrated consistently high recovery (≥95 %) from the first injection to the tenth injection across five columns, with excellent column-to-column reproducibility (RSD ≤2 %) and consistent peak symmetry. These results indicate that the coating effectively reduces analyte adsorption to metal surfaces, enabling reliable and reproducible performance without the need for extensive system passivation. In contrast, non-coated stainless-steel hardware showed negligible AMPcP recovery, highlighting the importance of the inert coating for accurate quantitation and analysis of sensitive or highly interactive analytes.

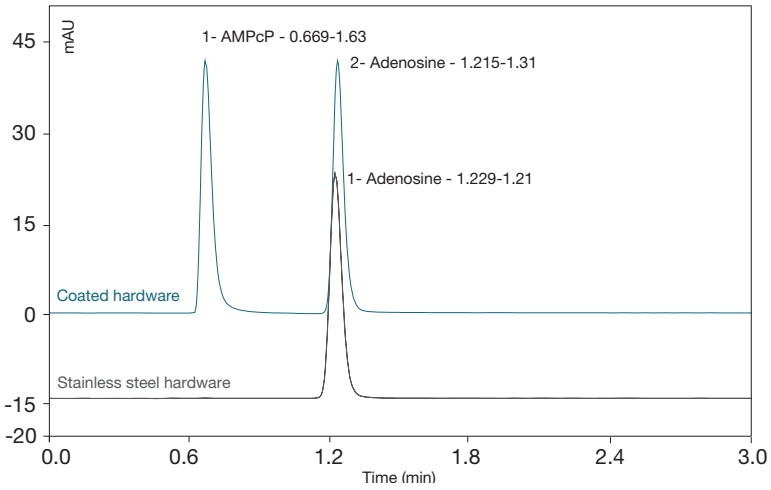
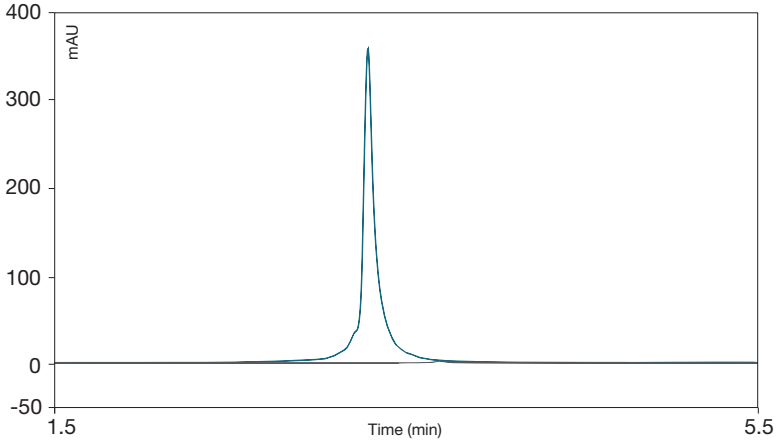


Figure 10. Overlayed chromatograms of the first injection of AMPcP and Adenosine on the SurePac Oligo RP MDi column using coated and stainless-steel hardware. Peak labels are RT and asymmetry at 5%.

Intact mRNA characterization and carryover

Separation of large intact mRNA molecules is particularly challenging due to their high molecular weight and structural heterogeneity. In the analysis of a 1960 nt mRNA sample, the SurePac Oligo RP MDi column provided excellent peak shape and enabled detection of an early eluting proximal peak (Figure 11). Such separations are difficult to achieve with conventional silica-based columns, as their limited pore size restricts accessibility for large mRNA molecules.

Carryover was evaluated by performing a blank run without injection immediately following the mRNA sample analysis. The column demonstrated low carryover (0.48%). Minimizing carryover is critical in high-throughput workflows and for accurate quantitation of low-level impurities, particularly when analyzing high-concentration RNA samples.



Column	SurePac Oligo RP MDi column, 2.1 × 50 mm		
Mobile phases	A: 0.1 M TEAA; B: ACN		
Gradient	Time (min)	%A	%B
	0.0	95	5
	5.0	80	20
	5.1	10	90
	7.0	10	90
	7.1	95	5
	10.0	95	5
System	Vanquish Horizon system		
Temperature	40 °C (PH 40 °C)		
Flow rate	0.4 mL/min		
Inj. volume	100 nL		
Detection	UV (260 nm)		
Sample	1960 nt mRNA (3.5 mg/mL)		

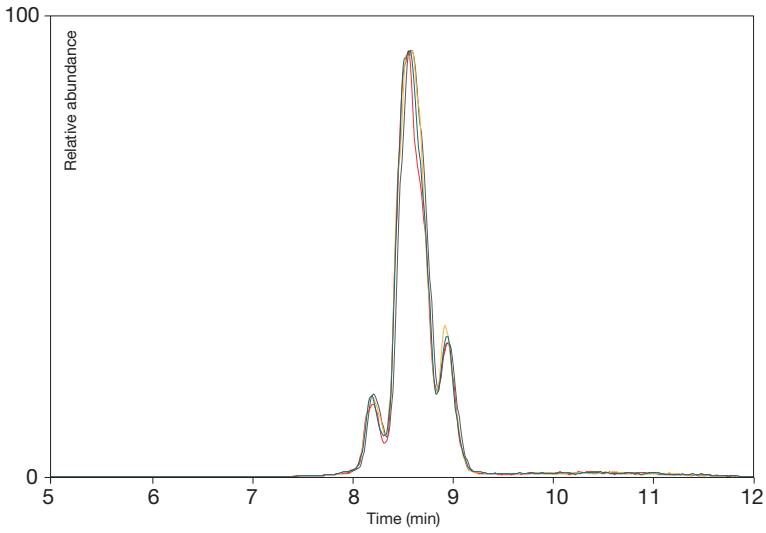
Figure 11. Overlaid chromatogram showing a 0.35 µg injection and elution of an intact 1960 nt mRNA sample on a 2.1 × 50 mm column and the following blank run to measure carryover.

Method development and applications (continued)

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 with a SurePac Oligo RP MDi 0.3 × 50 mm capillary column.
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 an Thermo Scientific™ Orbitrap Ascend Tribrid™ Mass Spectrometer (Part No. FSN06-10000). The mass spectrometer was operated with Thermo Scientific™ Xcalibur™ Software version 4.7. Instrument calibration was performed using Thermo Scientific™ Pierce™ FlexMix™ Calibration Solution. Data acquisition was performed in negative ion mode. Data was analyzed with Thermo Scientific™ Freestyle™ Software version 1.8 and Thermo Scientific™ BioPharma Finder™ Software version 5.3.

Full-length tRNA^{Phe} (~25 kDa) was analyzed using sliding-window deconvolution (≥5% relative abundance threshold, S/N = 3). Across four replicate injections, ten intact molecular states were reproducibly detected above the 5% threshold. Injection-to-injection monoisotopic mass precision 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 12). 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.



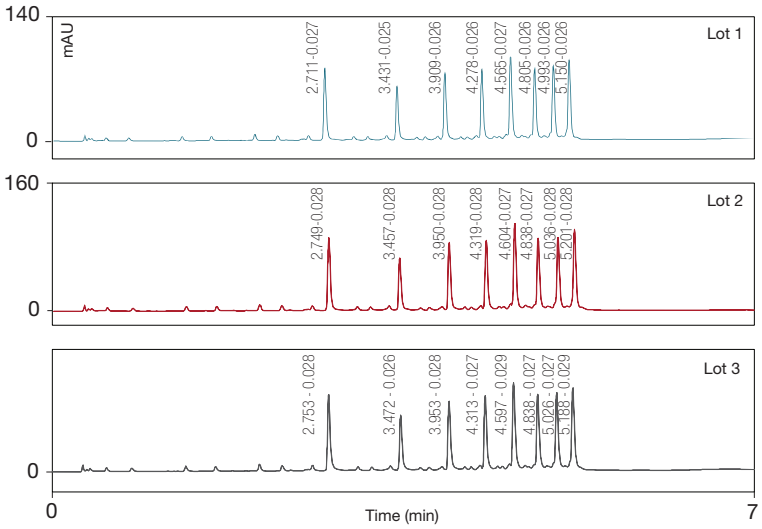
Column	SurePac Oligo RP MDi column, 0.3 × 50 mm		
Mobile phases	A: 5 mM DBA, 25 mM HFIP; B: ACN		
Gradient	Time (min)	%A	%B
	0.0	90	10
	0.5	90	10
	14.0	72.5	27.5
	14.1	10	90
	15.0	10	90
System	Vanquish Neo system		
Temperature	50 °C		
Flow rate	25 µL/min		
Inj. volume	1 µL (400 µg)		
Sample	tRNA^{Phe}		

Figure 12. Base peak chromatogram showing overlay of four injections of the tRNA^{Phe} standard.

Method development and applications (continued)

Lot-to-lot reproducibility results

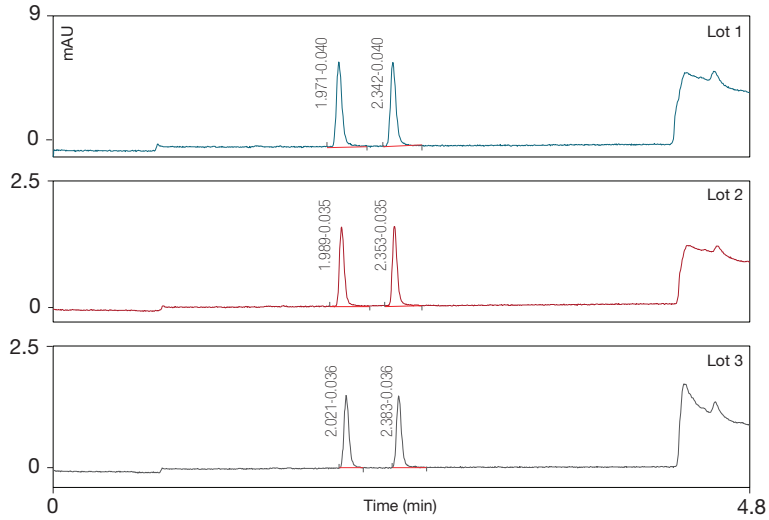
Using the 8-combo ssDNA mix and dT7-8 samples separation method shown in Figure 13 and 14, lot-to-lot reproducibility was evaluated across three different lots of the SurePac Oligo RP MDi column stationary phase for both the analytical and capillary formats. Consistent chromatographic performance was 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	SurePac Oligo RP MDi column, 2.1 × 50 mm		
Mobile phases	A: 0.1 M HAA; 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
System	Vanquish Horizon system		
Temperature	60 °C (PH 60 °C, PCC 45 °C)		
Flow rate	0.4 mL/min		
Inj. volume	2 µL		
Detection	UV (260 nm)		
Sample	8-combo ssDNA mix		
Legend	RT - PWHH		

RT-Retention time; PWHH-Peak width at half height

Figure 13. Chromatograms of 8-combo ssDNA mix sample using three different lots of 2.1 × 50 mm SurePac Oligo RP MDi columns.



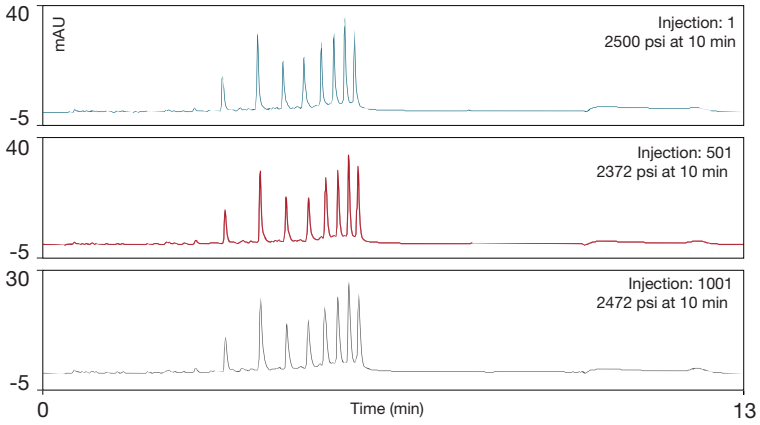
Column	SurePac Oligo RP MDi column, 0.3 × 50 mm		
Mobile phases	A: TEAAc 0.1 M; B: ACN		
Gradient	Time (min)	%A	%B
	0.0	95	5
	0.5	95	5
	3.1	91	9
	3.2	50	50
	4.2	50	50
	4.3	95	5
System	Vanquish Neo system		
Temperature	60 °C		
Flow rate	8 µL/min		
Inj. volume	0.1 µL		
Detection	UV (260 nm)		
Sample	dT 7-8 (10 µg/mL)		
Legend	RT - PWHH		

Figure 14. Chromatograms of dT7-8 using three different lots of 0.3 × 50 mm SurePac Oligo RP MDi columns.

Column ruggedness and operational stability

Column ruggedness and operational stability were evaluated using a SurePac Oligo RP MDi 2.1 × 100 mm column under extended use conditions, including repeated injections of samples and blanks. An 8-combo ssDNA mix sample was analyzed over 1000 injections, demonstrating highly consistent chromatographic performance throughout the study.

Retention time, peak width at half height (PWHH), and system backpressure remained stable with no significant drift observed. These results indicate that the column provides robust performance and maintains separation efficiency under prolonged operation, supporting reliable use in high-throughput and routine analytical workflows.



Column	SurePac Oligo RP MDi column, 2.1 × 50 mm		
Mobile phases	A: 0.1 M HAA; 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
System	Vanquish Horizon system		
Temperature	60 °C (PH 60 °C, PCC 45 °C)		
Flow rate	0.4 µL/min		
Inj. volume	2 µL		
Sample	8-combo ssDNA mix		

Figure 15. Chromatograms of 8-combo ssDNA mix over 1000 injections using a 2.1 × 100 mm SurePac Oligo RP MDi column.

Ordering information

Description	Particle size	Dimension	Quantity	Part No.
SurePac Oligo RP MDi columns	2.5 µm	0.3 × 50 mm	Each	43712-050332
		2.1 × 20 mm	Each	43712-022132
		2.1 × 50 mm	Each	43712-052132
		2.1 × 100 mm	Each	43712-102132
		10 × 50 mm	Each	43712-0510032
		21.2 × 50 mm	Each	43712-0521232

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