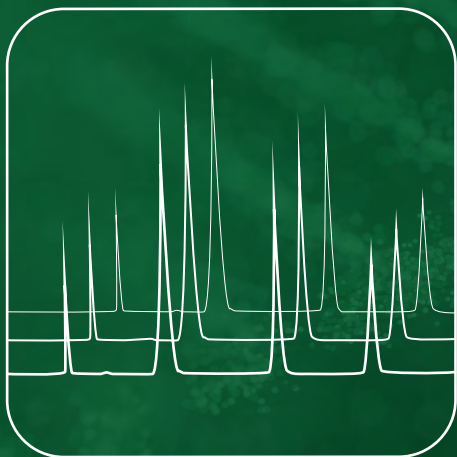


YMC

YMC Oligonucleotide Columns


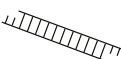
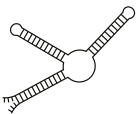
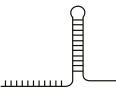
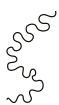
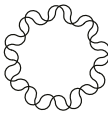


IP-RP
AEX
SEC
HILIC



Nucleic acids

Different types of therapeutic nucleic acids

	Antisense oligonucleotide/ miRNA	siRNA	Aptamer	gRNA	mRNA	Plasmid
Example structure						
Type(s) of nucleic acids	ssDNA, ssRNA	dsRNA	ss/dsRNA, ss/dsDNA	ss/dsRNA	ssRNA	dsDNA
Length (mer)	11–24	19–23	20–100	100	1,000–7,000	4,000–10,000
Mass range (kDa)	5	15	5–30	30	300–2,000	2,000–7,000
Modifications (phosphates, ribose, nucleobase)	Yes	Yes	Yes	Yes	Nucleobases only (5' cap, 3' Poly(A) tail)	No
Chemical conjugates / carrier systems	Yes/ carrier possible	Yes/ carrier possible	Yes/ carrier possible	No/ carrier possible	No/ Lipid nanoparticles	No/ carrier possible
Mode of action	mRNA degradation	mRNA degradation	Protein function modulation	Genome editing	Protein production	Gene expression

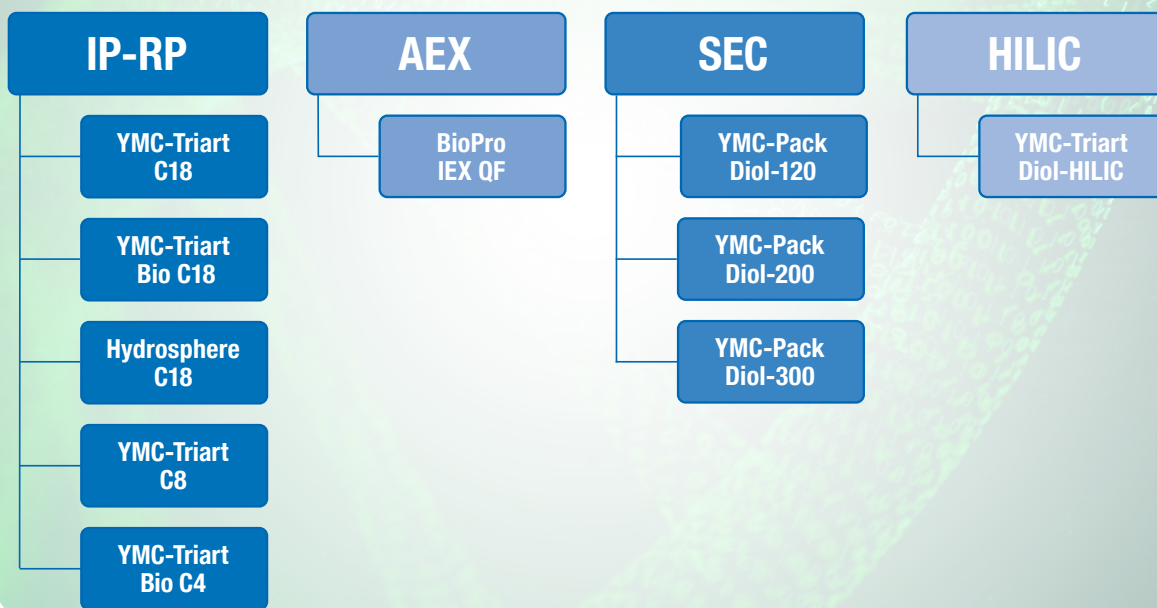
	Page
Oligonucleotide Application Notes	6–10
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AEX Oligonucleotide Columns	22–24
SEC Oligonucleotide Columns.....	26–28
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Phase selection guide

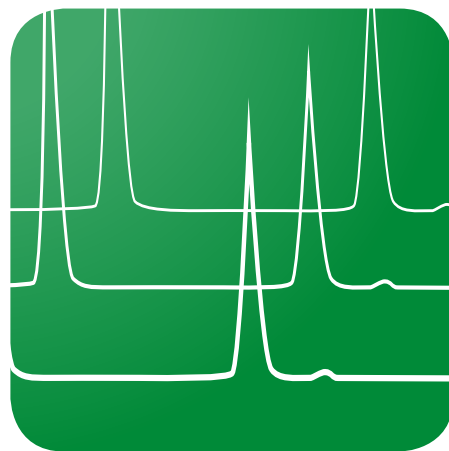
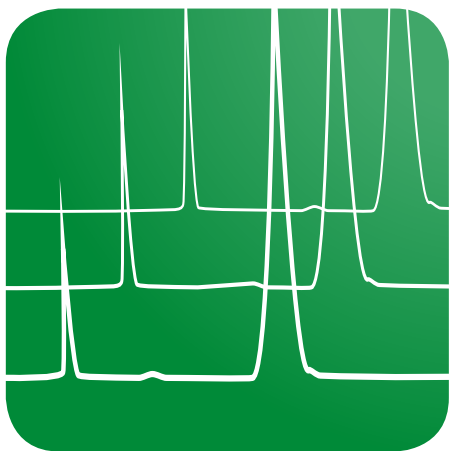


Oligonucleotides / Nucleic Acids





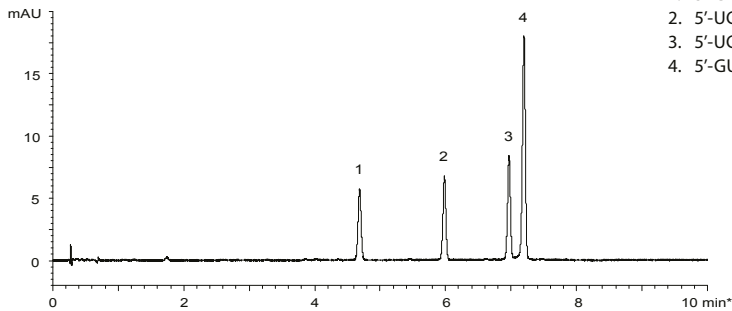
Oligonucleotide Applications



Oligonucleotide applications

Synthetic oligonucleotides

RP



1. 5'-CAC UGA AUA CCA AU-3' (14mer)
2. 5'-UCA CAC UGA AUA CCA AU-3' (17mer)
3. 5'-UCA UCA CAC UGA AUA CCA AU-3' (20mer)
4. 5'-GUC AUC ACA CUG AAU ACC AAU-3' (21mer)

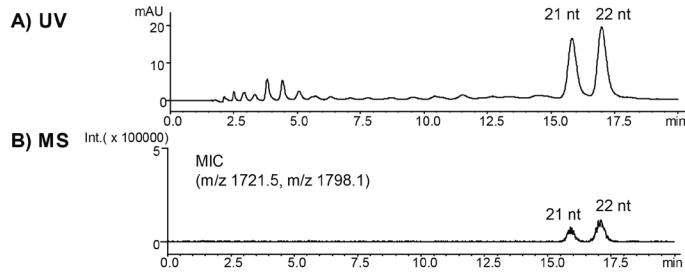
Column: YMC-Triart C18 (1.9 μ m, 12 nm) 50 x 2.1 mm ID
 Part No.: TA12SP9-05Q1PT
 Eluent: A) 200 mM HFIP*·8 mM triethylamine
 B) methanol
 Gradient: 10–20%B (0–10 min)

Flow rate: 0.42 mL/min
 Temperature: 65°C
 Detection: UV at 260 nm
 Injection: 1 μ L (2–4 nmol/mL)

*hexafluoroisopropanol

LC/MS analysis of miRNA

RP



Courtesy of M. Yamada, SHIMADZU CORPORATION

5'-pUGG AGU GUG ACA AUG GUG UUG-3'
 5'-pUGG AGU GUG ACA AUG GUG UUG U-3'

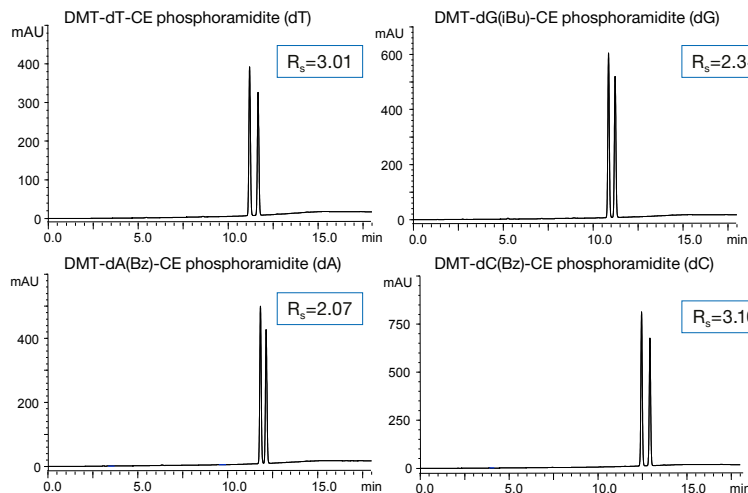
(21mer, MW 6,890.1)
 (22mer, MW 7,196.3)

Column: YMC-Triart C18 (3 μ m, 12 nm) 150 x 2.0 mm ID
 Part No.: TA12S03-1502WT
 Eluent: A) 10 mM di-n-butylamine-acetic acid (pH 7.5)
 B) 10 mM di-n-butylamine-acetic acid (pH 7.5)/acetonitrile (50/50)
 Gradient: 62–72%B (0–20 min)
 Flow rate: 0.2 mL/min

Temperature: 30°C
 Detection: A) UV at 260 nm
 B) ESI-negative mode
 Injection: 4 μ L (5 nmol/mL)
 System: LC) Shimadzu Prominence
 MS) Shimadzu LCMS2020

Analysis of phosphoramidites

RP



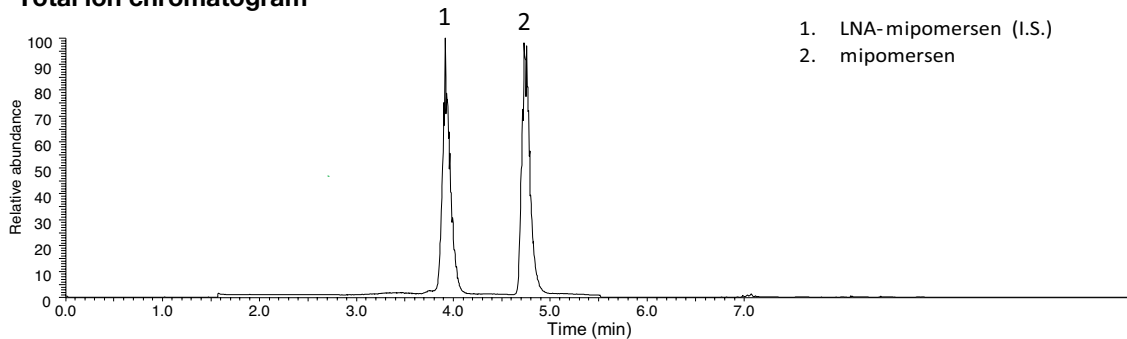
Column: YMC-Triart C18 (5 μ m, 12 nm) 150 x 4.6 mm ID
 Part No.: TA12S05-1546PTH
 Eluent: A) 20 mM triethylammonium acetate (pH 7.0)/acetonitrile (90/10)
 B) acetonitrile
 Gradient: 45–95%B (0–12 min), 95%B (12–17 min)

Flow rate: 1.0 mL/min
 Temperature: 30°C
 Detection: UV at 254 nm
 Injection: 4 μ L (1.0 mg/mL)

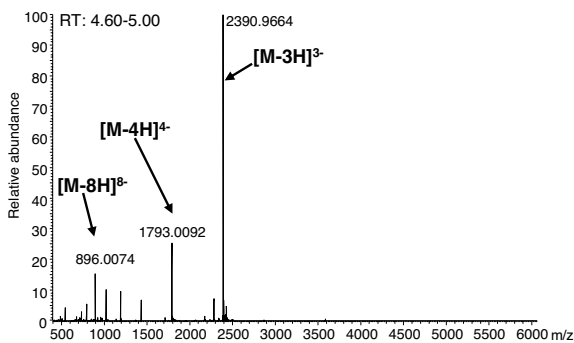
LC-HRMS analysis of the antisense oligonucleotide Mipomersen (Kynamro®)

RP

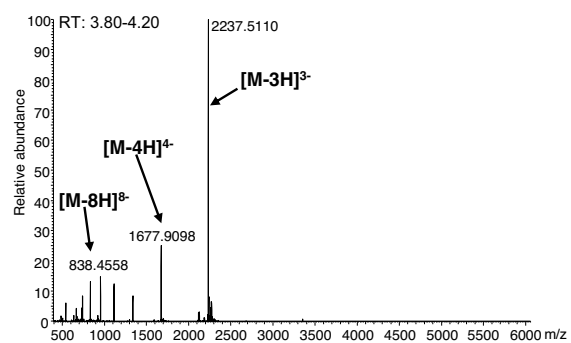
Total ion chromatogram



Mass spectrum of mipomersen



Mass spectrum of LNA-mipomersen



Column: YMC-Triart C8 metal-free PEEK-lined (1.9µm, 12nm)¹ 100 x 2.1 mm ID
 Part No.: TO12SP9-10Q1PTP
 Eluent: A) water/triethylamine/HFIP² (100/0.4/2; triethylamine 28.0mM, HFIP 135.8mM)
 B) methanol/triethylamine/HFIP (100/0.4/2)
 Gradient: [Sample separation step]
 10-40%B (0-5.0 min)
 [Column wash steps]
 40-70%B (5.0-5.1 min), 70%B (5.1-7.0min), 70-10%B (7.0-7.1 min), 10%B (7.1-8.0min), 10-90%B (8.0-8.1 min), 90%B (8.1-9.0min),
 90-10%B (9.0-9.1 min), 10%B (9.1-10.0min), 10-90%B (10.0-10.1 min), 90%B (10.1-11.0min), 90-10%B (11.0-11.1 min)
 Flow rate: 0.3 mL/min
 Temperature: 50°C
 Injection: 10µL (1000ng/mL)
 System: LC) Vanquish Binary Pump H system
 HRMS) Orbitrap HRMS Q Exactive Plus

*1 Prewash the column prior to the first use with water/methanol/phosphoric acid (70/30/0.1) for 1 hour

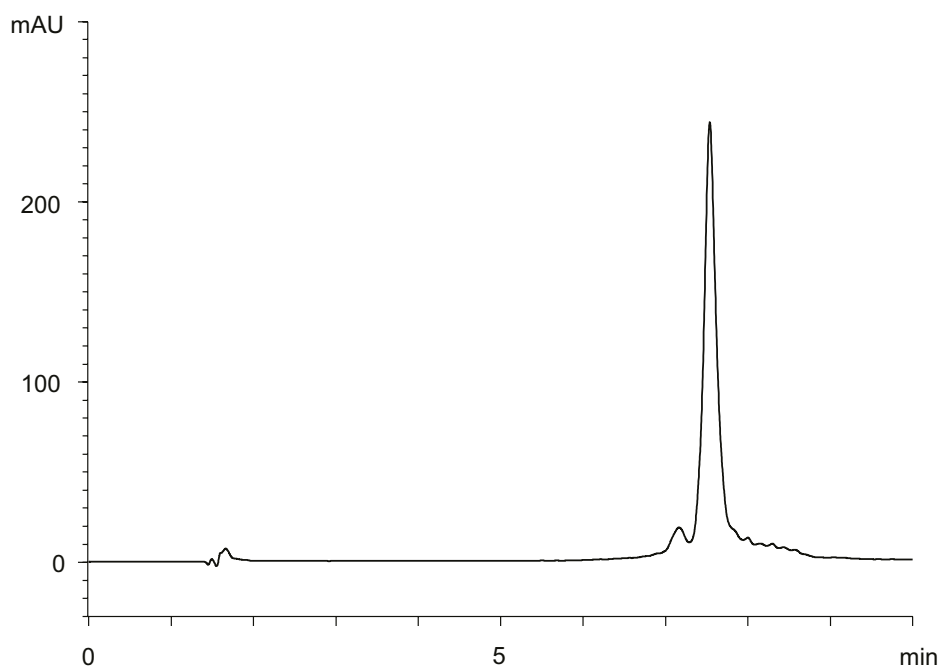
*2 1,1,1,3,3,3-hexafluoro-2-propanol

Reference: Y. Sun et al, Development of a bioanalytical method for an antisense therapeutic using high-resolution mass spectrometry, Bioanalysis, 2020 NOV 26, doi: 10.4155/bio-2020-0225.

Oligonucleotide applications

mRNA coupled to enhanced green fluorescent protein (EGFP)

RP



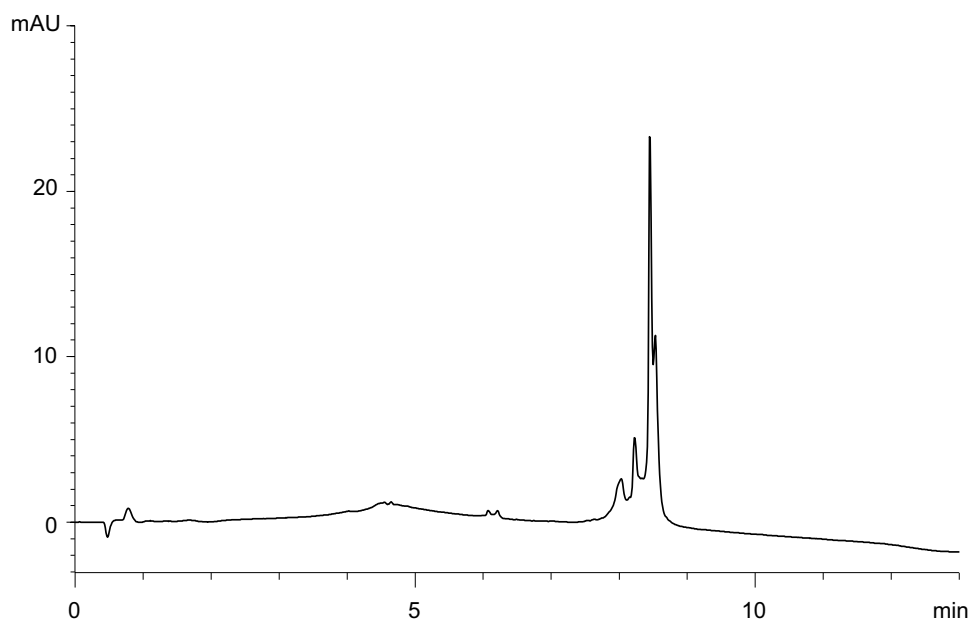
Column: YMC Accura Triart Bio C4 (3µm, 30 nm) 100 x 2.1 mm ID
 Part No. TB30S03-10Q1PTC
 Eluent: A) 50 mM TEAA* (pH 7.0) / acetonitrile (95/5)
 B) acetonitrile
 Gradient: 5–10%B (0–10 min)
 Flow rate: 0.2mL/min

Temperature: 80°C
 Detection: UV at 254 nm
 Injection: 2µL (0.25 mg/mL)
 Sample: CleanCap® EGFP mRNA (5 moU)
 (TriLink Bio Technologies)

*triethylammonium acetate

IEX analysis of EGFP mRNA (996mer)

AEX

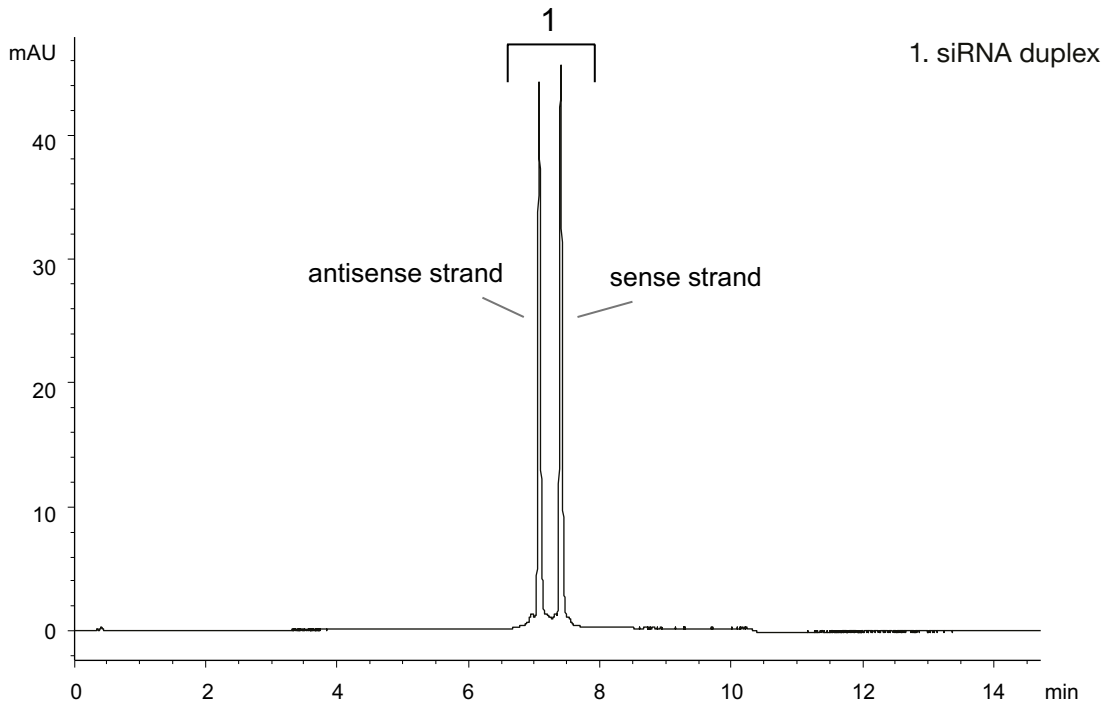


Column: BioPro IEX QF (5 µm) 100 x 4.6 mm ID
 Part No. QF00S05-1046WP
 Eluent: A) 10 mM NaOH (pH 12)
 B) 10 mM NaOH (pH 12) containing 2 M NaCl
 Gradient: 0–100%B (0–9 min), 100%B (9–13 min)
 Flow rate: 1.0mL/min

Temperature: 15°C
 Detection: UV at 260 nm
 Injection: 5µL (0.025 mg/mL)
 Sample: CleanCap® EGFP mRNA (5 moU)
 (TriLink Bio Technologies)

siRNA duplex under denaturing conditions

RP



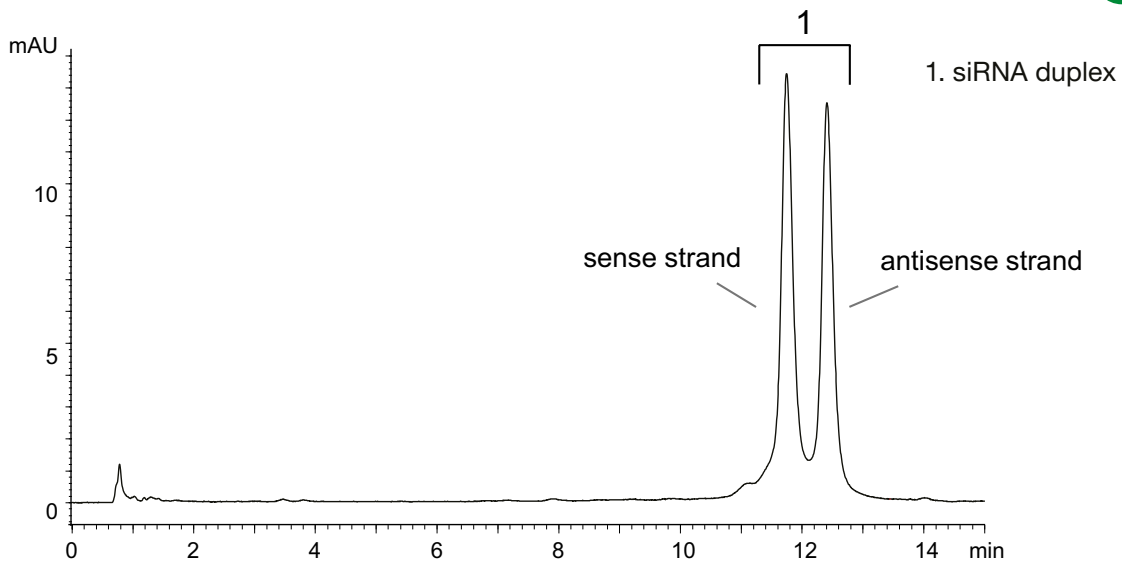
Column: YMC Accura Triart Bio C18 (1.9 μ m, 30 nm) 50 x 2.1 mm ID
 Part No.: TA30SP9-05Q1PTC
 Eluent: A) 15 mM TEAA* (pH 8)
 B) methanol
 Gradient: 5%–20%B (0–15 min)

Flow rate: 0.42 mL/min
 Temperature: 65°C
 Detection: UV at 260 nm
 Injection: 1 μ L (5 nmol/mL)
 Sample: siRNA duplex

*triethylammonium acetate

siRNA duplex under denaturing conditions

AEX



Column: BioPro IEX QF (5 μ m) 100 x 4.6 mm ID
 Part No.: QF00S05-1046WP
 Eluent: A) 10 mM NaOH
 B) 10 mM NaOH containing 1 M NaClO₄
 Gradient: 30%–37%B (0–15 min)

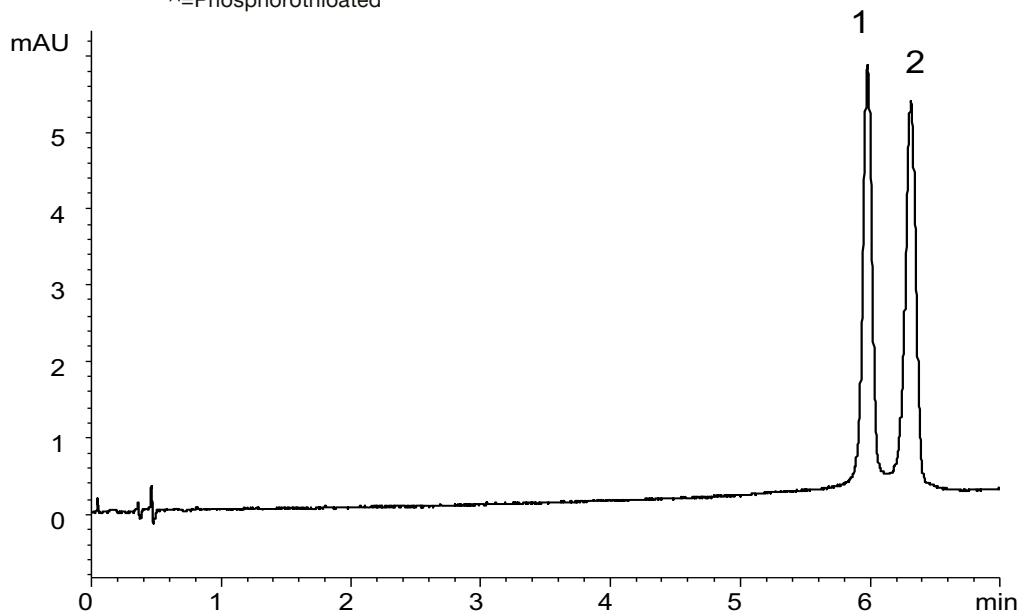
Flow rate: 1.0 mL/min
 Temperature: 25°C
 Detection: UV at 260 nm
 Injection: 4 μ L (5 nmol/mL)
 Sample: siRNA duplex

Oligonucleotide applications

Challenging phosphorothioate oligonucleotides

RP

5'-U[^]C[^]A[^]A[^]U[^]C[^]A[^]C[^]A[^]C[^]A[^]C[^]U[^]G[^]A[^]A[^]U[^]A[^]C[^]C[^]A[^]A[^]U[^]-3' (RNA 20mer)
 5'-G[^]U[^]C[^]A[^]A[^]U[^]C[^]A[^]C[^]A[^]C[^]A[^]C[^]U[^]G[^]A[^]A[^]U[^]A[^]C[^]C[^]A[^]A[^]U[^]-3' (RNA 21mer)
[^]=Phosphorothioated

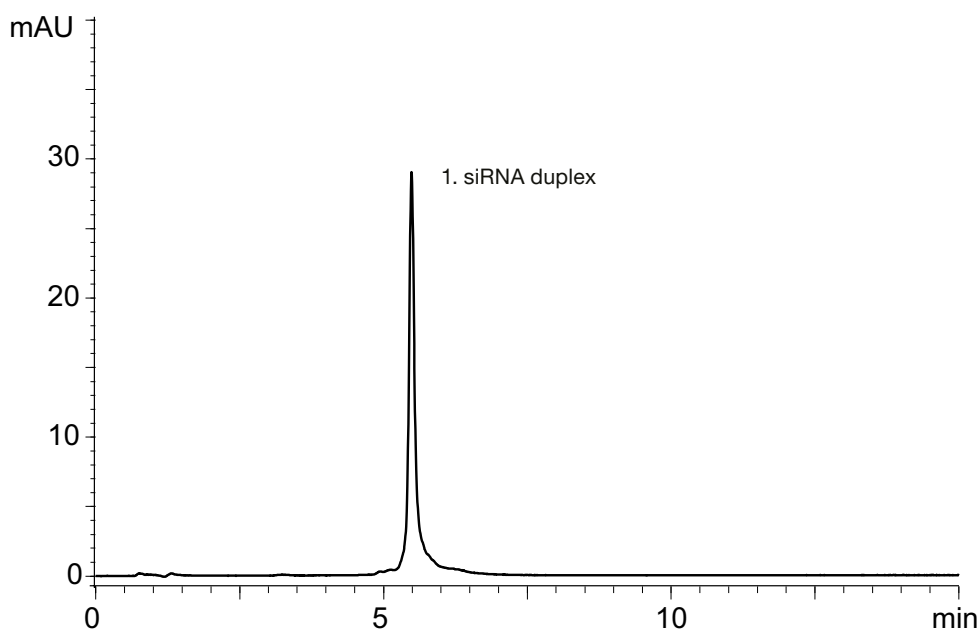


Column:	YMC Accura Triart Bio C18 (1.9µm, 30nm) 50 x 2.1 mm ID	Flow rate:	0.42 mL/min
Part No.:	TA30SP9-05Q1PTC	Temperature:	65°C
Eluent:	A) 15 mM triethylamine - 400 mM HFIP* B) methanol	Detection:	UV at 260 nm
Gradient:	10-20%B (0-10 min)	Injection:	1 µL (each 1.0 nmol/mL)

*1,1,1,3,3,3-hexafluoro-2-propanol

AEX analysis of siRNA duplex under non-denaturing conditions

AEX



Column:	BioPro IEX QF (5µm) 100 x 4.6 mm ID	Flow rate:	1.0 mL/min
Part No.:	QF00S05-1046WP	Temperature:	25°C
Eluent:	A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 1 M NaClO ₄	Detection:	UV at 260 nm
Gradient:	25%-40%B (0-15 min)	Injection:	4 µL (5 nmol/mL)
		Sample:	siRNA duplex



IP-RP



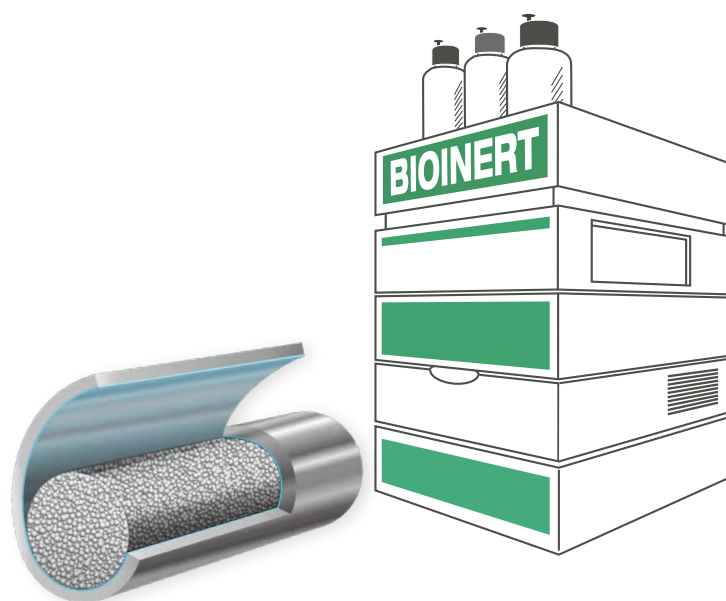
IP-RP – UHPLC/HPLC Selectivities

Features

- Selection of C18, C8 and C4 columns
- For ion-pairing (IP-RP) UHPLC and HPLC
- pH- and temperature stable phases
- Superior reproducibility
- Bioinert coated YMC Accura column hardware

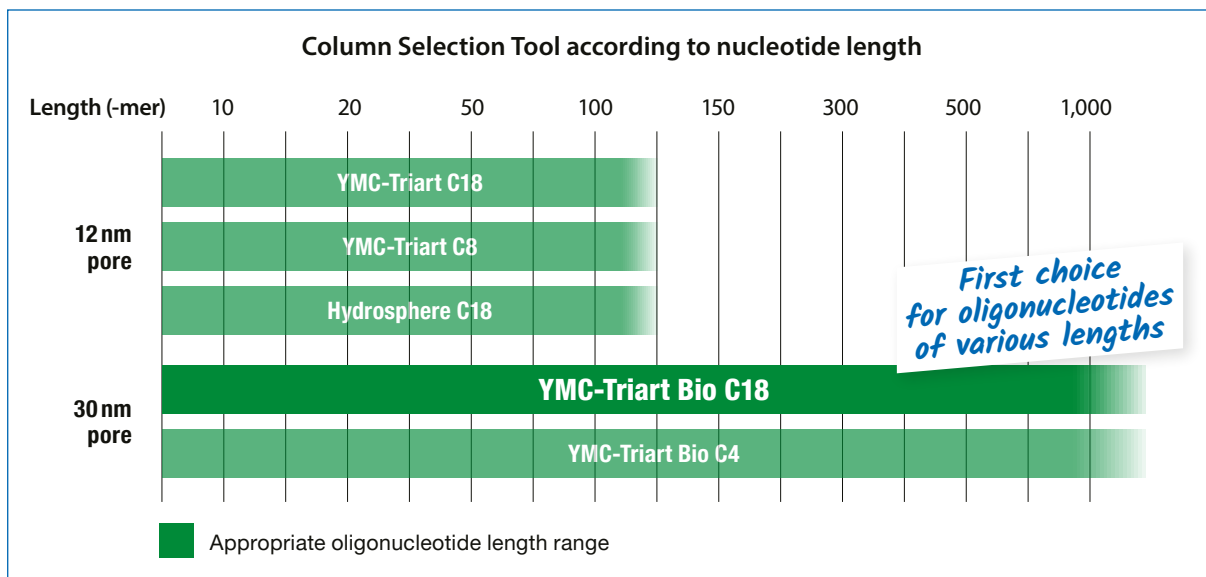
Selectivities for oligonucleotides

	YMC-Triart C18	YMC-Triart Bio C18	YMC-Triart C8	YMC-Triart Bio C4	Hydrosphere C18
Base particle	organic/inorganic hybrid silica				silica
Modification	C18 (USP L1)	C18 (USP L1)	C8 (USP L7)	C4 (USP L26)	C18 (USP L1)
Particle size / μm	1.9, 3, 5	1.9, 3, 5	1.9, 3, 5	1.9, 3, 5	2, 3, 5
Pore size / nm	12	30	12	30	12
pH range	1.0 – 12.0	1.0–12.0	1.0–12.0	1.0–10.0	2.0–8.0
Temperature range	pH < 7: 90°C pH > 7: 50°C	pH < 9: 90°C pH > 9: 50°C	pH < 7: 90°C pH > 7: 50°C	pH < 7: 90°C pH > 7: 50°C	50°C
Column hardware	bioinert coated YMC Accura				stainless-steel

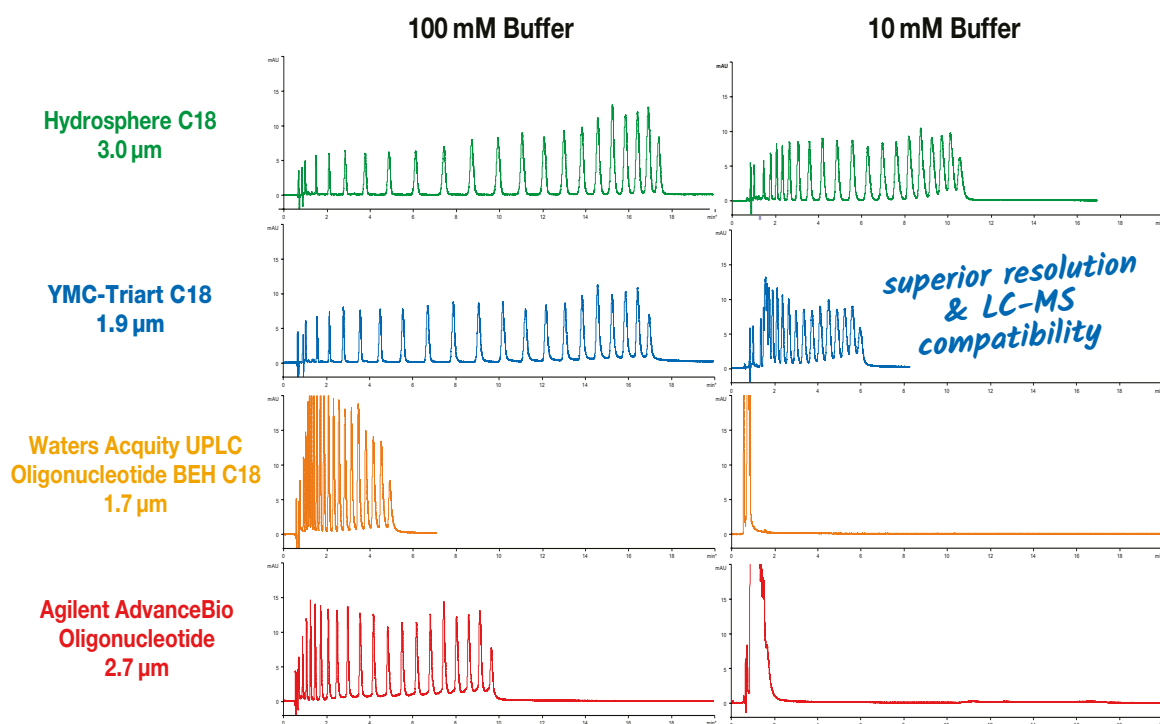


Bioinert hardware available!

Bioinert **YMC Accura Triart columns** are available for improved sensitivity and peak shape of coordinating compounds such as nucleotides or oligonucleotides.



Enhanced retention and resolution even at low buffer concentrations



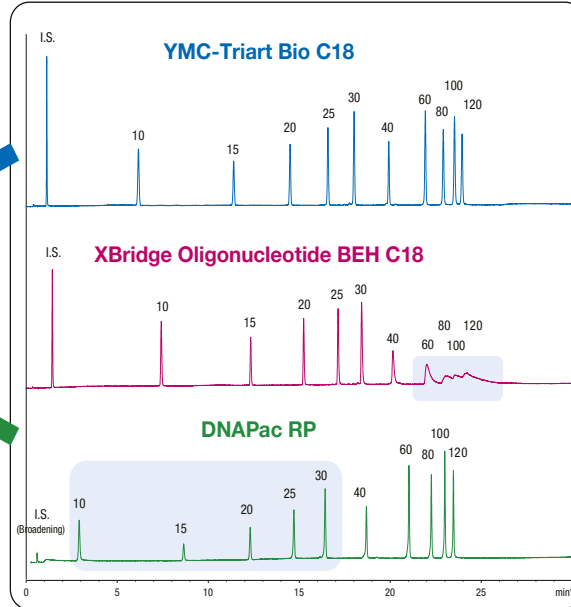
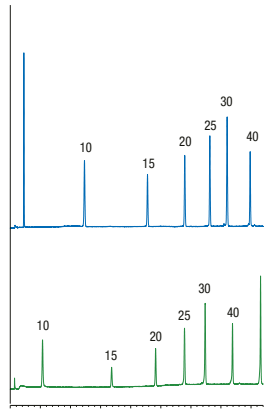
Column:	50 x 2.1 mm ID	Temperature:	35 °C
Eluent:	A) 100 mM or 10 mM triethylamine-acetic acid (pH 6) B) A/acetonitrile (80/20)	Detection:	UV at 260 nm
Gradient:	50–65%B (0–20 min)	Injection:	2 µL (5 nmol/mL)
Flow rate:	0.21 mL/min	Sample:	Oligodeoxythymidylic acid [d(pT)2-20]

The separation of oligo(deoxythymidylic acids), d(pT)2-20, was compared using 100 mM or 10 mM triethylammonium acetate (TEAA) buffer, under the same gradient conditions. Both Hydrosphere C18 and YMC-Triart C18 showed enhanced retention and resolution compared to other commercially available C18 phases designed for oligonucleotide analysis, even at the low ion-pairing buffer concentration such as 10 mM. The higher concentration provides stronger retention and superior resolution of oligonucleotides, although a lower concentration has the advantages of increasing the signal intensity and reducing system contamination in HPLC-MS analysis.

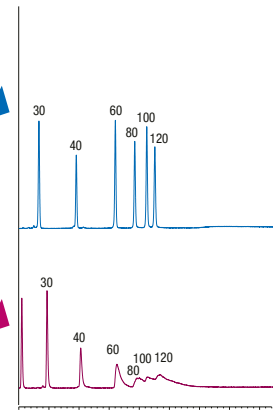
IP-RP – UHPLC/HPLC Selectivities

Optimum resolution for short and long poly(dT) oligonucleotides

Short poly(dT)



Long poly(dT)

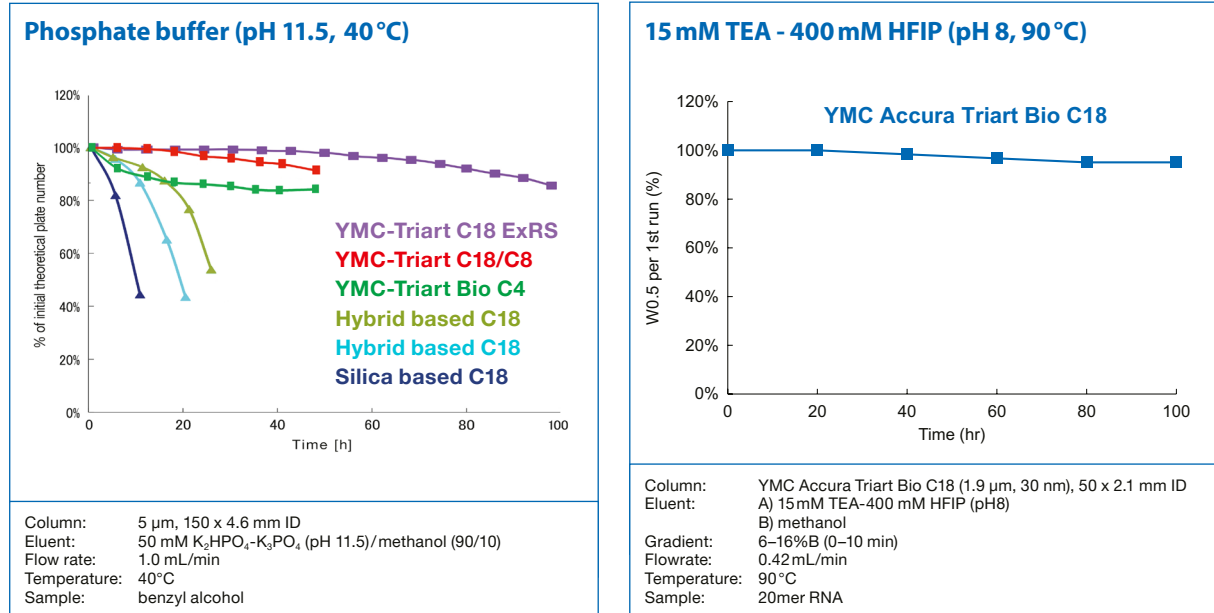


Columns: YMC-Triart Bio C18 (3 μ m, 30 nm) 50 x 2.1 mm ID
 XBridge Oligonucleotide BEH C18 (2.5 μ m, 13 nm) 50 x 2.1 mm ID
 DNAPac RP (4 μ m, proprietary) 50 x 2.1 mm ID
 Part No.: TA30S03-05Q1PTH
 Eluent: A) 4 mM TEA⁺ - 100 mM HFIP**
 B) methanol
 Gradient: 0.5%B/min, initial %B=5
 Flow rate: 0.42 mL/min
 Detection: UV at 260nm
 Temperature: 65 °C
 Injection: 1.0 μ L
 Sample: Poly(dT) oligonucleotides

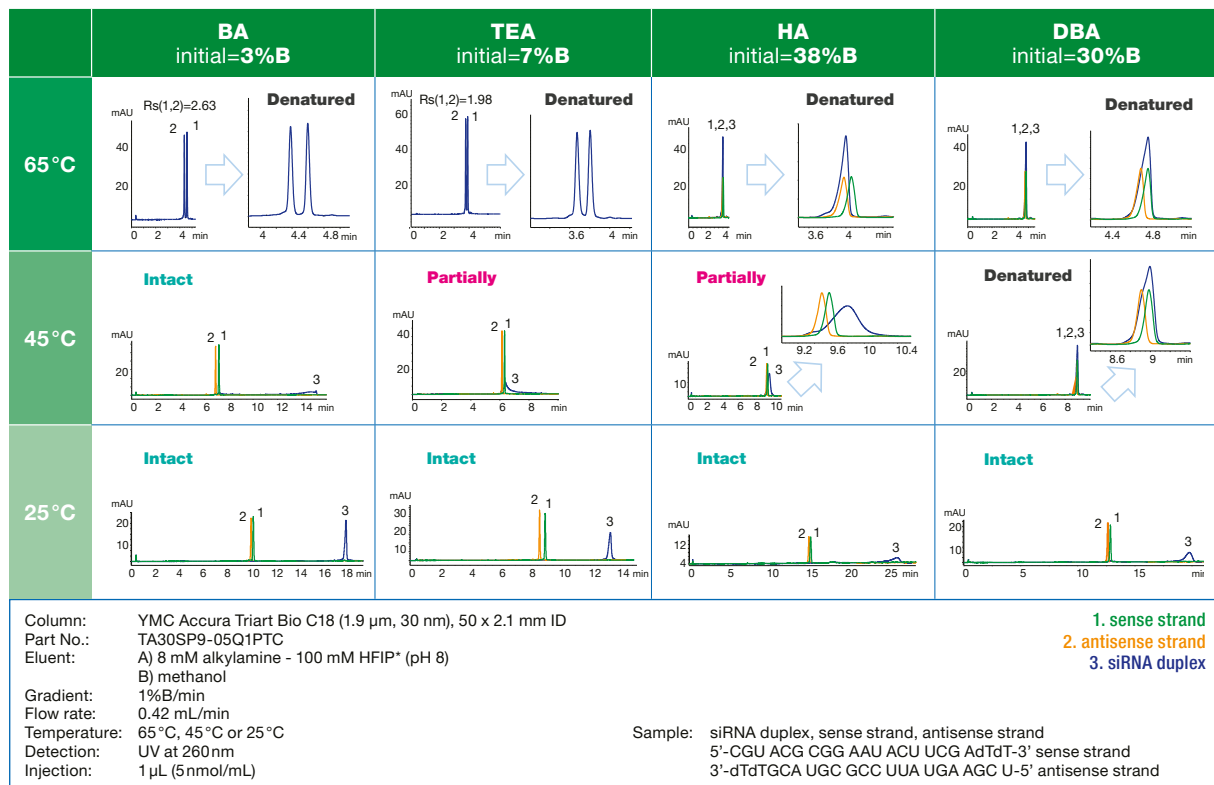
**triethylamine, **1,1,1,3,3,3-hexafluoro-2-propanol

The YMC-Triart Bio C18 column demonstrates a better resolution, higher recovery and reproducibility of poly(dT) oligonucleotides compared to the other two competitor columns. Longer poly(dT) oligonucleotides (60–120mer) were separated poorly by the competitor's hybrid silica based column, whereas YMC-Triart showed high resolution for oligonucleotides of all sizes. Peak areas and therefore recoveries of shorter poly(dT) oligonucleotides (10–40mer) were much smaller when separated using the dedicated DNA competitor column. In addition, YMC-Triart Bio C18 showed reproducible behaviour such as consistent peak areas. This makes YMC-Triart Bio C18 an ideal tool for analysis of poly(dT) oligonucleotides.

Versatile wide pH and temperature stability



Effect of four different ion pair reagents on the denaturation of a siRNA duplex at different temperatures

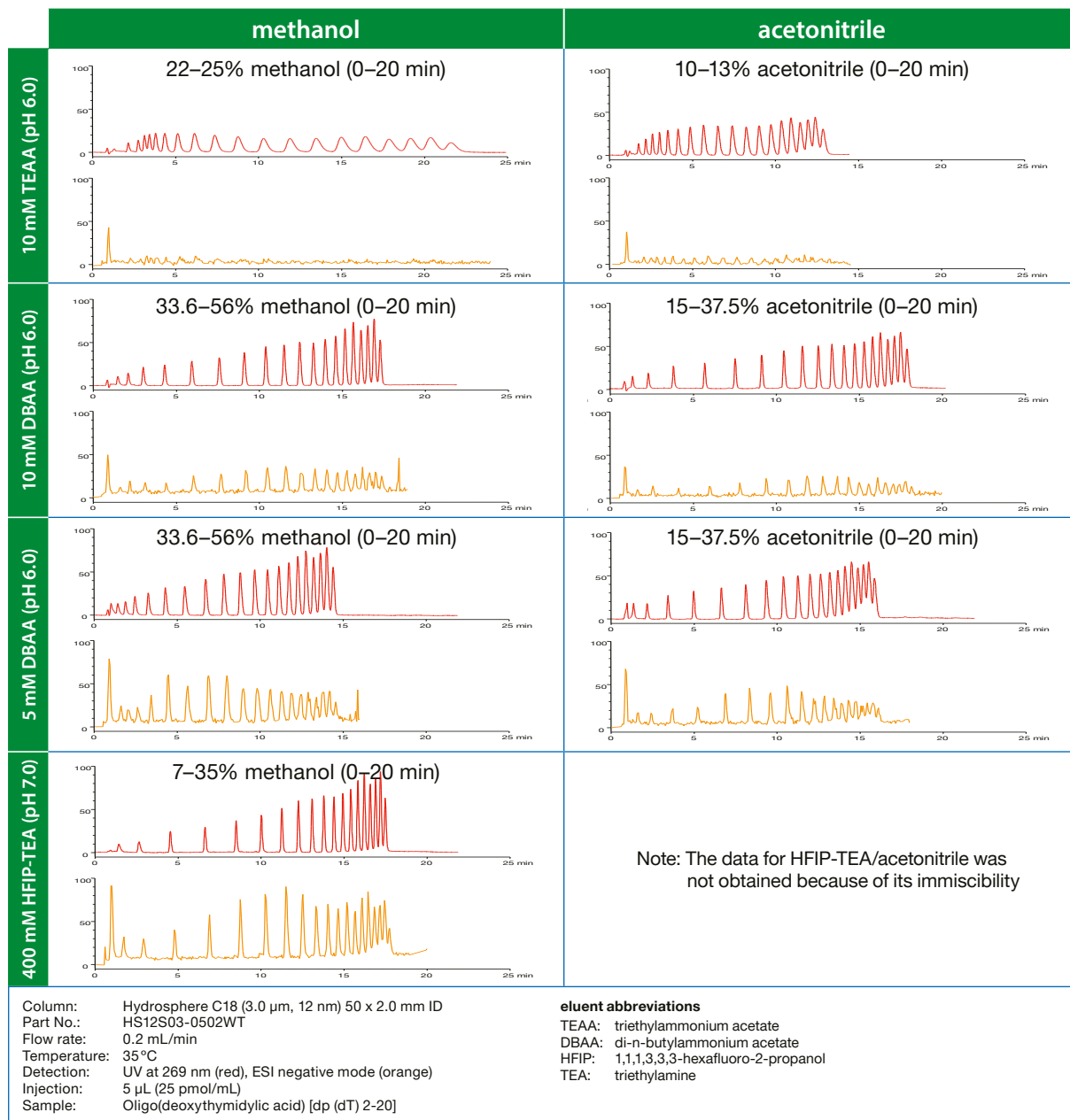


*1,1,1,3,3,3-hexafluoro-2-propanol

IP-RP – Expert Tips: Ion-pairing salts

Effect of composition and salt concentration of ion-pairing mobile phase on the separation and signal intensity

Comparison of separation and ESI-MS signal intensity using different ion-pairing buffers and organic solvents

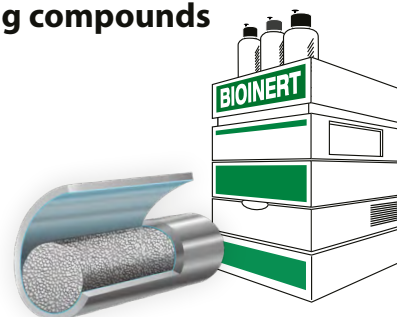


The mobile phase composition has different effects on the separation and signal intensity in electrospray ionisation mass spectrometry (ESI-MS) of oligonucleotides. Using different gradient conditions, acceptable retention and resolution can be achieved (upper UV chromatograms; red trace) for each separation by optimising the gradient slope of the organic solvent regardless of the type of mobile phase. The ESI-MS intensity is significantly influenced by

the type and concentration of ion-pairing buffer as shown in the lower MS chromatograms (orange trace). HFIP-TEA buffer/methanol systems provide the maximum MS intensity. Enhanced retention and MS intensity are obtained using 10 mM DBAA buffer compared to 10 mM TEAA buffer, and the lower DBAA concentration results in approximately 1.5–3 times increase in the intensity without any change in the concentration of organic solvent.

Bioinert columns for bioseparations and coordinating compounds

- Exceptional peak shapes with high sensitivities
- Excellent recoveries without column preconditioning
- Superior reproducibility and no carry-over effects
- Ideal for highly sensitive LC/MS analyses

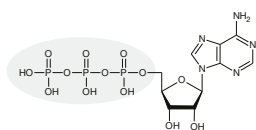


Specifications

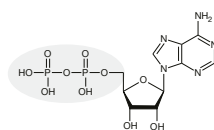
	YMC Accura Triart	YMC Accura BioPro IEX
Modifications	C18, Bio C18, C8, Bio C4, Diol-HILIC	QF
Particle Size	1.9, 3 and 5 μm	3 and 5 μm
Column hardware	stainless-steel with bioinert coating	
Frit hardware	stainless-steel with bioinert coating	
Pressure limit	1.9 μm : 100 MPa (15,000 psi) 3/5 μm : 45 MPa (6,525 psi)	3 μm : 15–20 MPa (2,175–2,900 psi) 5 μm : 10–30 MPa (1,450–4,350 psi)

Improved sensitivity for coordination compounds

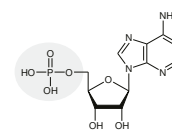
1. ATP



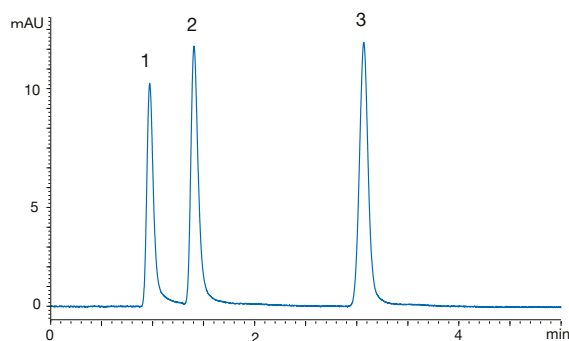
2. ADP



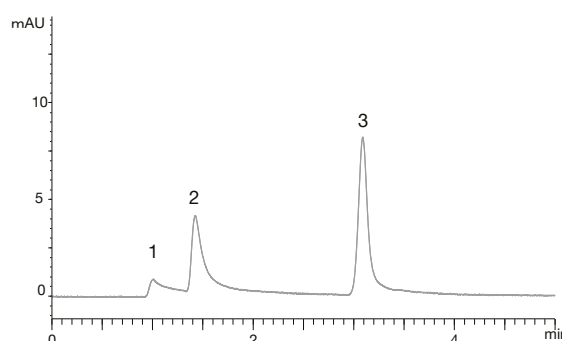
3. AMP



YMC Accura Triart C18



Standard column



Column: YMC Accura Triart C18 (1.9 μm , 12 nm) 50 x 2.1 mm ID (bioinert hardware)
YMC-Triart C18 (1.9 μm , 12 nm) 50 x 2.1 mm ID (standard hardware)
Part Nos.: TA12SP9-05Q1PTC
TA12SP9-05Q1PT
Eluent: 5 mM HCOONH₄

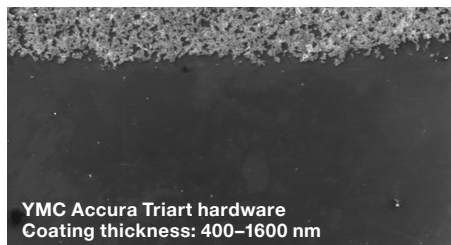
Flow rate: 0.21 mL/min
Temperature: 25 °C
Detection: UV at 265 nm
Injection: 1 μL (10 $\mu\text{g}/\text{mL}$)
System: bioinert/"metal-free" HPLC system

Metal coordinating compounds, which have a phosphate group in their structure, tend to show poor peak shape due to interactions with metals, such as the stainless-steel in column bodies and frits. By using a bioinert column hardware, better peak shapes can be expected.

Nucleotides with phosphate groups also show better peak shapes when compared to the regular column hardware. The bioinert coated YMC Accura column hardware is ideal for highly sensitive analyses using LC/MS.

YMC Accura bioinert hardware

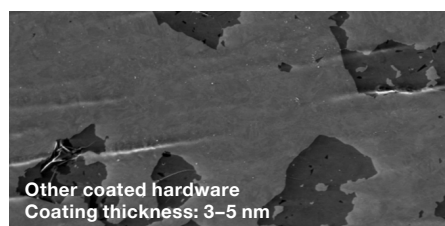
YMC Accura: durable bioinert coating



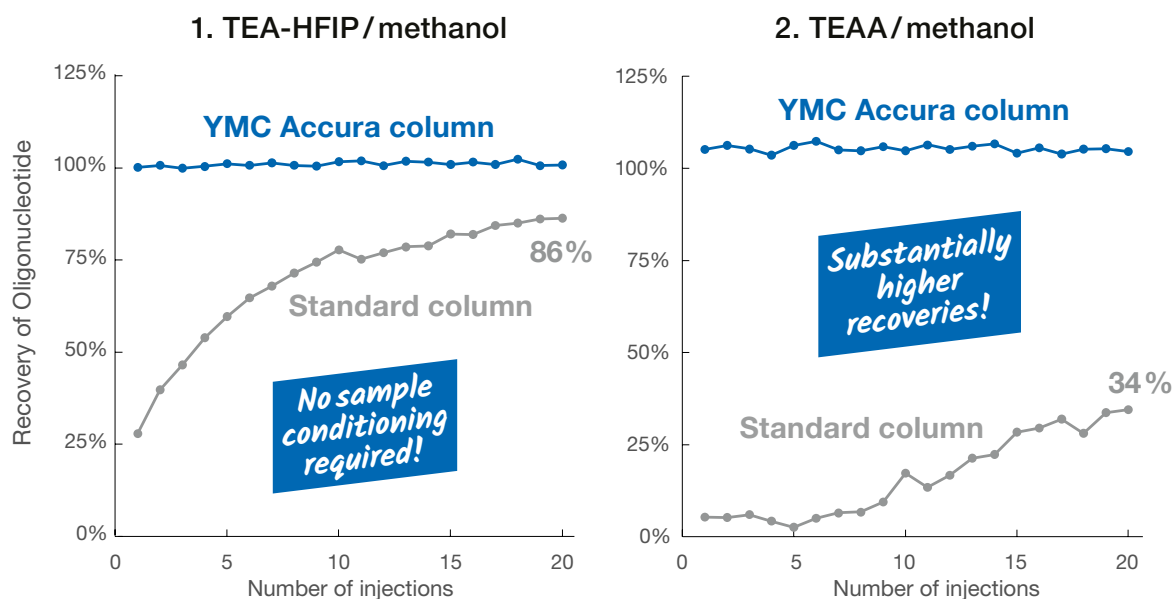
The robust bioinert coating used on YMC Accura hardware is 130 to 320-fold thicker, making it more durable than other similar hardware concepts. A long-term inertness against sensitive substances is ensured. In order to demonstrate its robustness, a YMC Accura column was packed multiple times. Even though this process is hard on the column surface, the coating remains unaffected (SEM* picture: top area is bare steel for comparison).

*Scanning Electron Microscope

Other coated columns can lose their inertness over time. This will again lead to adsorption of sensitive compounds on the uncovered metallic surfaces. Peak tailing, loss of recovery and sample carry-over are typical results of the delamination of the coating. On a competitor's column, most of the coating was already delaminated after merely unpacking the column. (The dark spots in this picture are the remaining coating.)



High surface inertness without any adsorption



Column: Empty YMC Accura (without stationary phase)
 Eluent: 1) 8 mM TEA 200 mM HFIP/methanol (82/18)
 2) 100 mM TEAA/methanol (82/18)
 Flow rate: 0.42 mL/min
 Detection: UV at 260 nm
 Temperature: 65 °C
 Injection: 1 µL
 Sample: All PS RNA 20mer (1) (5'-U[^]C[^]A[^]U[^]C[^]A[^]C[^]A[^]C[^]U[^]G[^]A[^]A[^]U[^]A[^]C[^]A[^]A[^]U[^]-3')

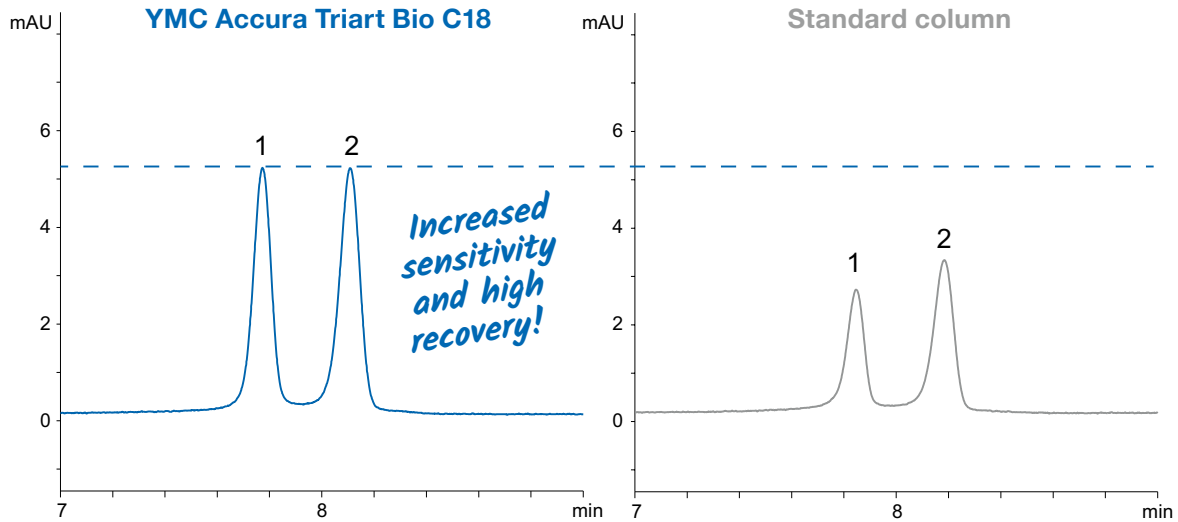
^=Phosphorothioate

The **YMC Accura** hardware with its inert surface area prevents adsorption of oligonucleotides using a range of different buffers. No sample conditioning is required.

YMC Accura columns further provide significantly higher recoveries and sensitivities that cannot be achieved with regular stainless-steel columns – even after conditioning with 20 sample injections. These ready-to-use columns ensure high recovery and reproducibility from the very first use.

Significantly higher sensitivity and recovery

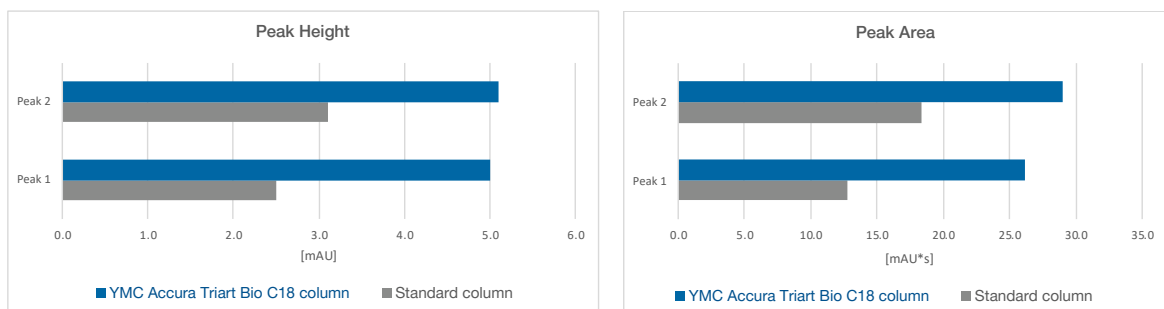
Ideal choice for challenging analytes such as phosphorothioate oligonucleotides



Column: YMC Accura Triart Bio C18 (1.9µm, 30nm) 50 x 2.1 mm ID
 Part No.: TA30SP9-05Q1PTC
 Eluent: A) 15mM triethylamine - 400mM HFIP*
 B) methanol
 Gradient: 8-18%B (0-10 min)
 Flow rate: 0.42mL/min
 Temperature: 65°C
 Detection: UV at 260nm
 Injection: 1 µL
 Sample: All PS RNA 20mer (1) (5'-U^C^A^U^C^A^C^A^C^A^U^G^A^A^U^A^C^C^A^A^U-3')
 All PS RNA 21mer (2) (5'-G^U^C^A^U^C^A^C^A^C^A^U^G^A^A^U^A^C^C^A^A^U-3')
 ^=Phosphorothioate

*1,1,1,3,3,3-hexafluoro-2-propanol

High sensitivity and recovery



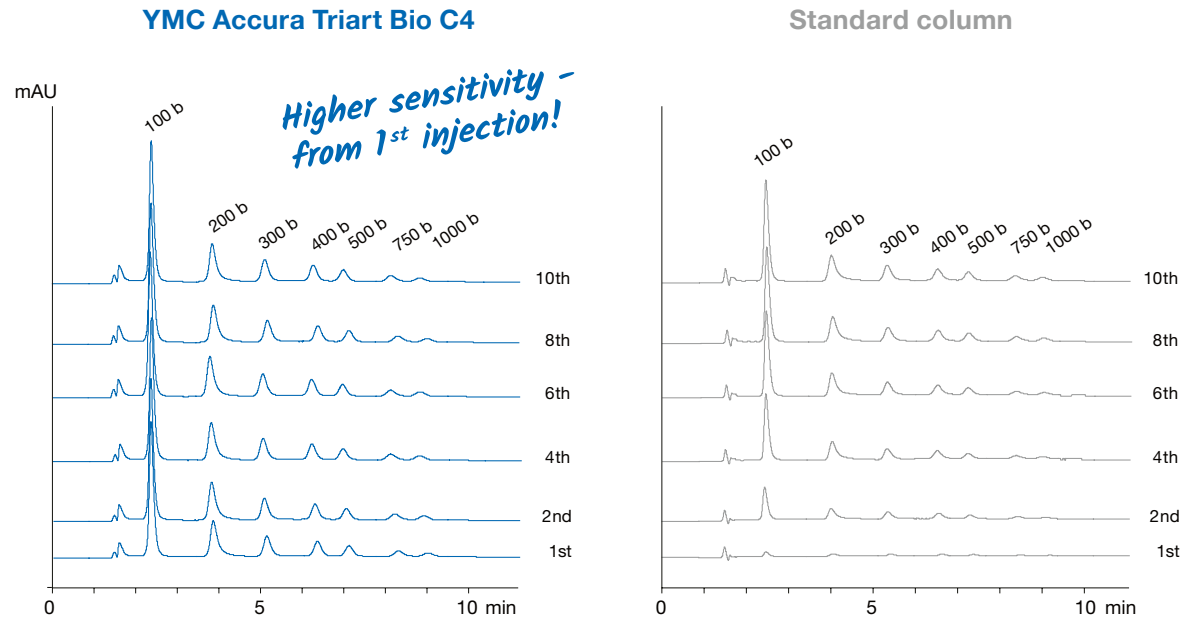
Doubled peak height and area!

The YMC Accura Triart Bio C18 column provides double peak heights and peak areas for the oligonucleotides compared to those for regular stainless-steel columns. YMC Accura Triart columns enhance the analytical sensitivity significantly, thereby avoiding the loss of high-value samples.

YMC Accura bioinert hardware

Reliable results from the first injection

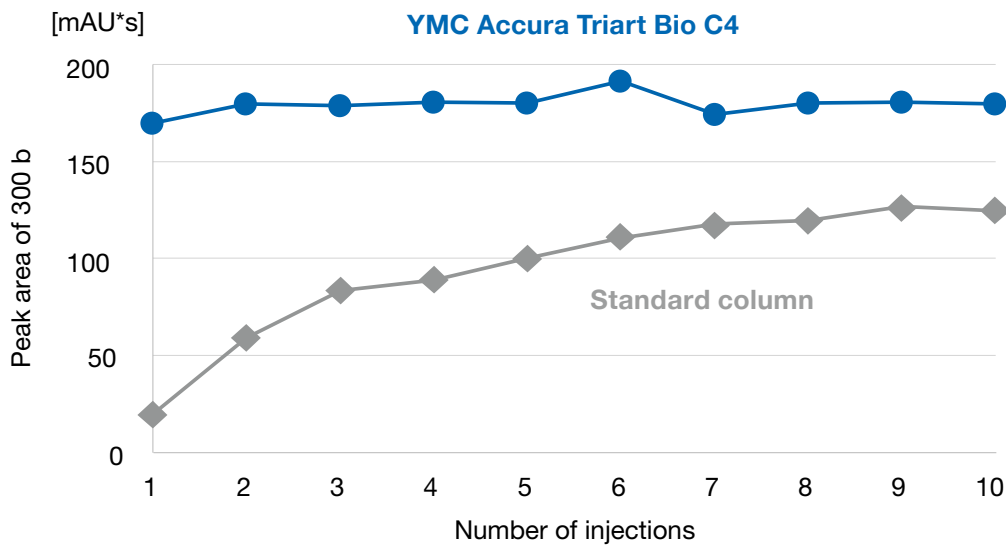
No preconditioning required for reliable results



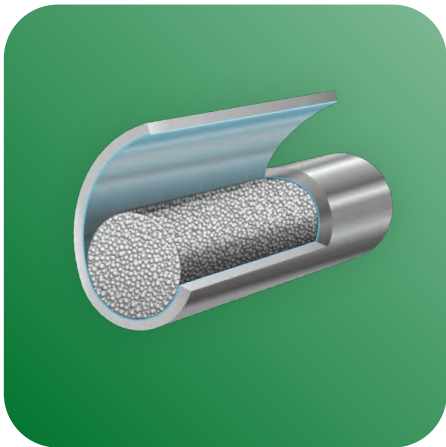
Column:	YMC Accura Triart Bio C4 (3 μ m, 30 nm) 100 x 2.1 mm ID	Flow rate:	0.2 mL/min
Part No.:	TB30S03-10Q1PTC	Temperature:	80°C
Eluent:	A) 50 mM TEAA* (pH 7.0)/acetonitrile (95/5) B) 50 mM TEAA (pH 7.0)/acetonitrile (50/50)	Detection:	UV at 254 nm
Gradient:	9–14%B (0–10 min), 80%B (10–15 min)	Injection:	1 μ L (0.25 mg/mL)
		Sample:	100–1,000 bases (Century™-Plus RNA Markers)

* Triethylammonium acetate

Constantly higher peak areas and therefore recoveries



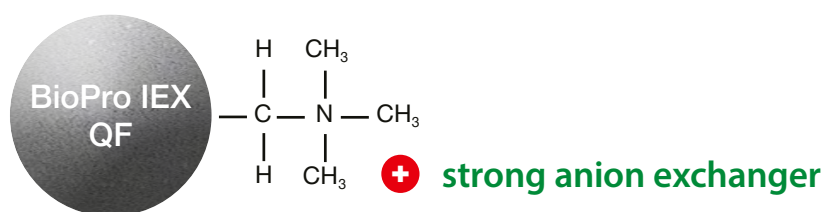
The YMC Accura Triart Bio C4 column shows stable peak areas from the first injection, while the standard stainless-steel column provides only 10% of the peak area (for the 300 base marker) with the first injection. Even after the tenth injection, the peak areas of the stainless-steel column are considerably less than those of the YMC Accura Triart column.

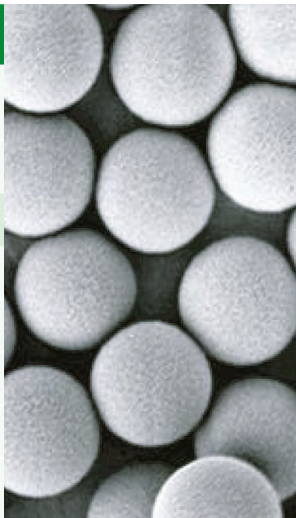


AEX – HPLC Selectivities

Features

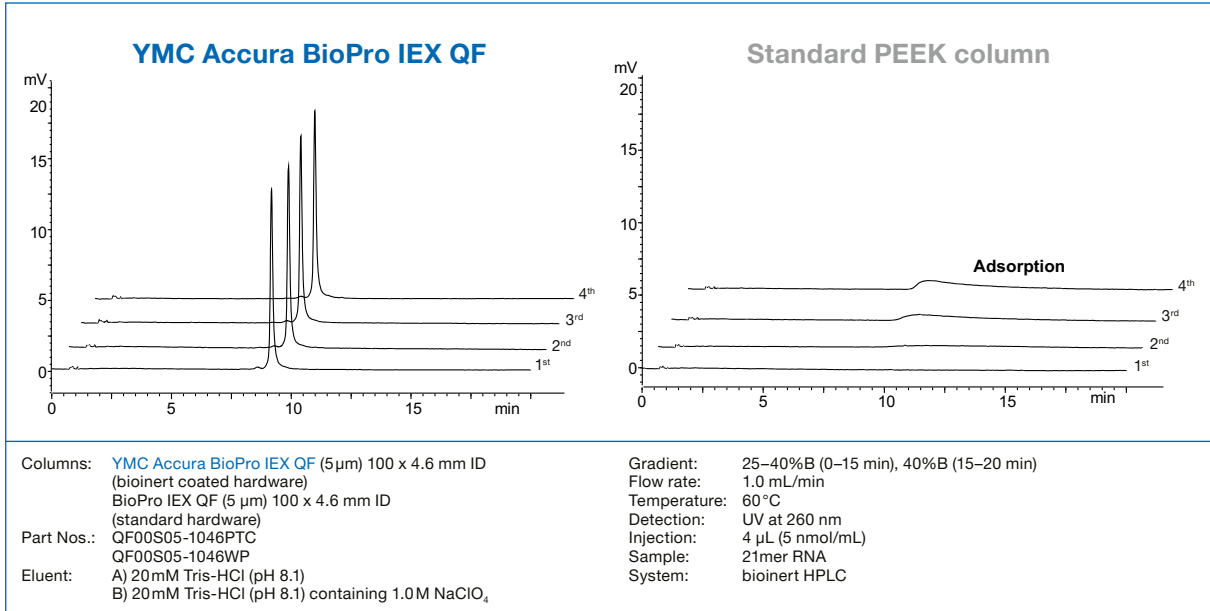
- Non-porous hydrophilic polymers
- High recovery and high resolution
- Low nonspecific adsorption
- Excellent reproducibility
- Bioinert coated YMC Accura column hardware



	BioPro IEX QF	
Matrix	hydrophilic polymer (polymethacrylate)	 <p>Non-porous polymer beads</p>
Particle size / μm	3, 5	
Pore size / nm	non-porous	
Charged group	$-\text{CH}_2\text{N}^+(\text{CH}_3)_3$	
Counter ion	Cl^-	
Available pH range	2.0–12.0	
Temperature range	4–80°C	
Pressure limit	3 μm : 15–20 MPa (2,175–2,900 psi) 5 μm : 10–30 MPa (1,450–4,350 psi)	
Column hardware	bioinert coated YMC Accura	

YMC's anion exchanger (AEX) columns of the BioPro IEX series are available with strong exchanger modification, based on 3 or 5 μm non-porous (QF columns) hydrophilic polymer beads. The non-porous particles offer high efficiency, very high resolution and low operating pressures.

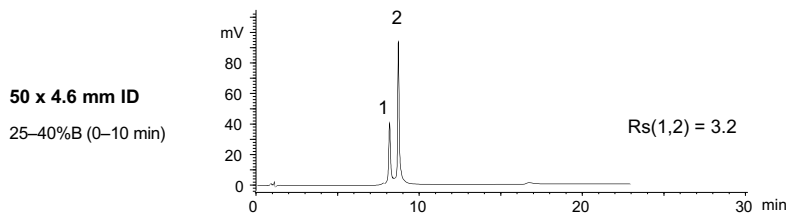
Sharp peaks and reliable recovery with different column dimensions



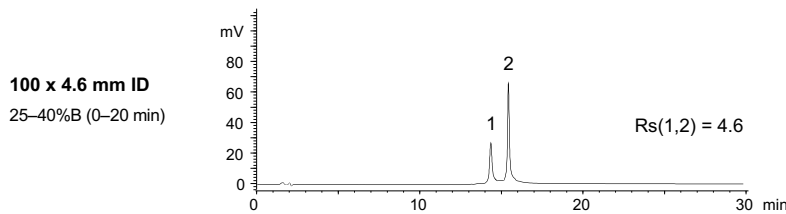
Oligonucleotides generally exhibit poor peak shape and therefore low recovery in AEX analysis, mainly due to adsorption onto the column hardware. YMC Accura BioPro IEX columns provide high recovery and very good peak shapes from the first injection. This makes YMC Accura BioPro IEX QF columns ideal for the analysis of oligonucleotides with reproducible results. The columns show stable peak areas from the 1st injection, so that no preconditioning is required.

Different column lengths for each separation purpose

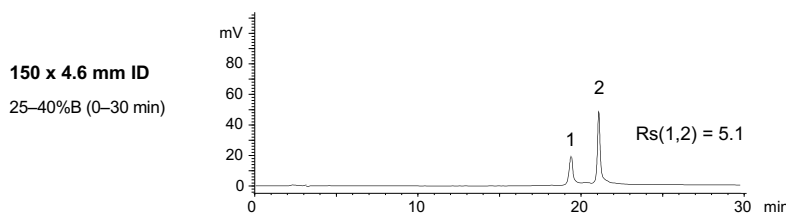
1. antisense strand
2. siRNA duplex



Shortest run time



Best compromise

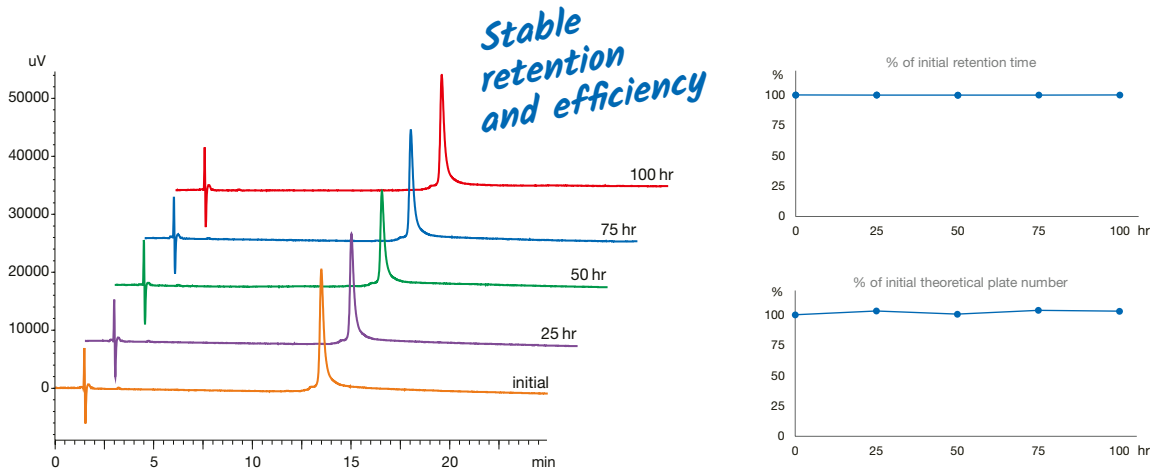


Highest resolution

<p>Columns: YMC Accura BioPro IEX QF (5 µm)</p> <p>Part Nos.: QF00S05-0546PTC QF00S05-1046PTC QF00S05-1546PTC</p> <p>Eluent: A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaClO₄</p>	<p>Flow rate: 0.5 mL/min</p> <p>Temperature: 60 °C</p> <p>Detection: UV at 260 nm</p> <p>Injection: 8 µL (each 5 nmol/mL)</p> <p>System: bioinert HPLC</p>
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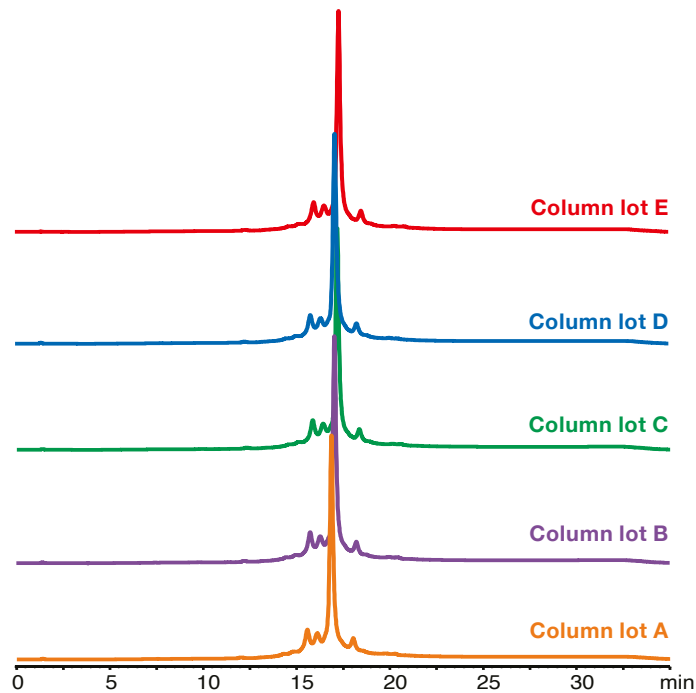
AEX – Stability and Reproducibility

Excellent stability using temperatures up to 80 °C



Column: YMC Accura BioPro IEX QF (5µm) 100 x 4.6 mm ID	Flow rate: 0.5 mL/min
Part No.: QF00S05-1046PTC	Temperature: 80 °C
Eluent: A) 20 mM Tris-HCl (pH 8.1)	Detection: UV at 260nm
B) 20 mM Tris-HCl (pH 8.1) containing 1.0M NaCl	Injection: 4µL (5 nmol/mL)
Gradient: 40–90%B (0–25 min), 90%B (25–35 min), 40%B (35–60 min)	Sample: 21mer RNA

Excellent batch-to-batch reproducibility



Column: BioPro IEX SF (5 µm) 100 x 4.6 mm ID	Flow rate: 0.5 mL/min (180 cm/hr)
Part No.: SF00S05-1046WP	Temperature: 25 °C
Eluent: A) 20 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 6.5)	Detection: UV at 215 nm
B) 20 mM NaH ₂ PO ₄ -Na ₂ HPO ₄ (pH 6.5) containing 0.2 M NaCl	Injection: 10 µL
Gradient: 0–50%B (0.5–30 min)	Sample: monoclonal antibody (IgG1)

BioPro IEX columns exhibit excellent batch-to-batch reproducibility. All gel batches are inspected by rigorous quality control tests, and must meet the required criteria before release. BioPro IEX columns are the best choice for the quality control of biopharmaceuticals such as oligonucleotides or mAbs as in this example.



SEC

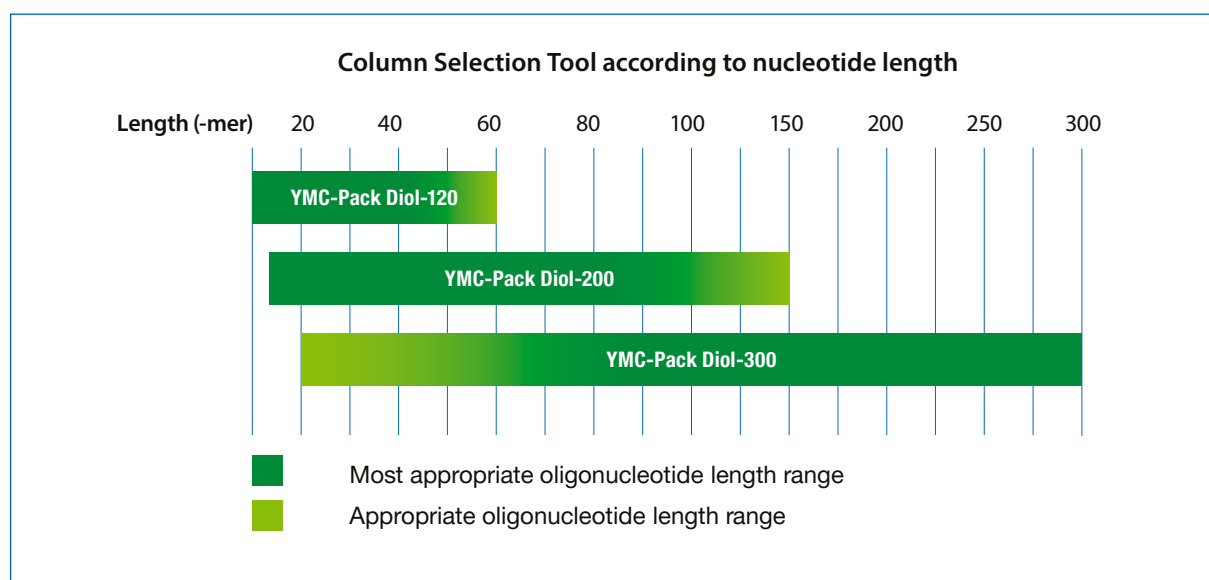


SEC – UHPLC / HPLC Selectivities

Features

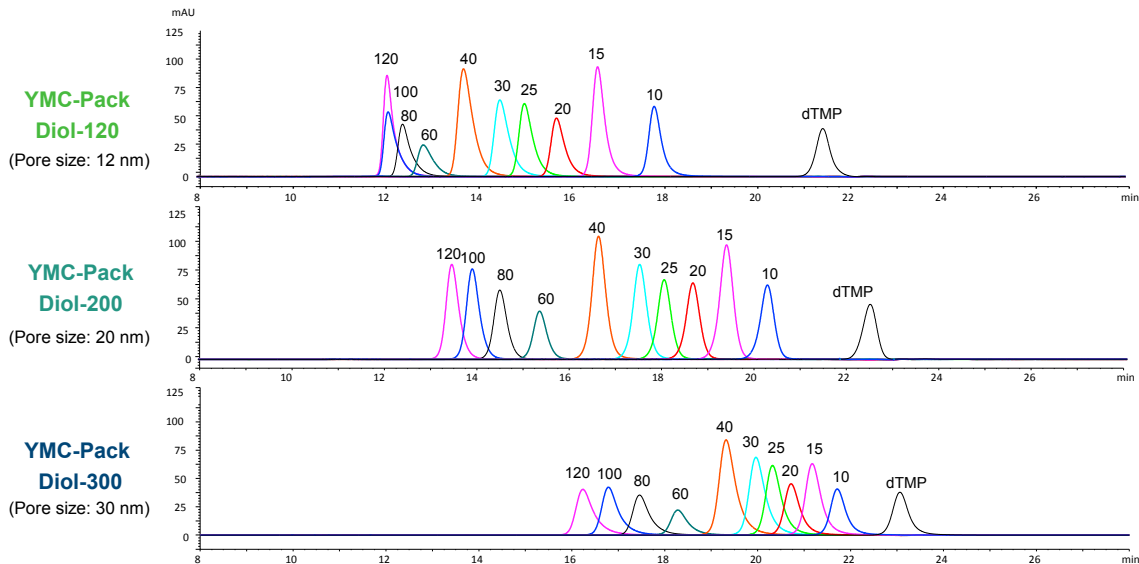
- Excellent reproducibility with minimal secondary interactions
- 2 µm for UHPLC
- Cost-effective

	YMC-Pack Diol-120	YMC-Pack Diol-200	YMC-Pack Diol-300
	For short oligonucleotides	For intermediate oligonucleotides	For longer oligonucleotides
Base particle	silica		
Particle size / µm	3, 5	2, 3, 5	2, 3, 5
Pore size / nm	12	20	30
Modification	dihydroxypropyl		
Temperature range	40°C		
Pressure limit	2 µm: 45 MPa (6,525 psi); 3/5 µm: 20 MPa (3,000 psi)		

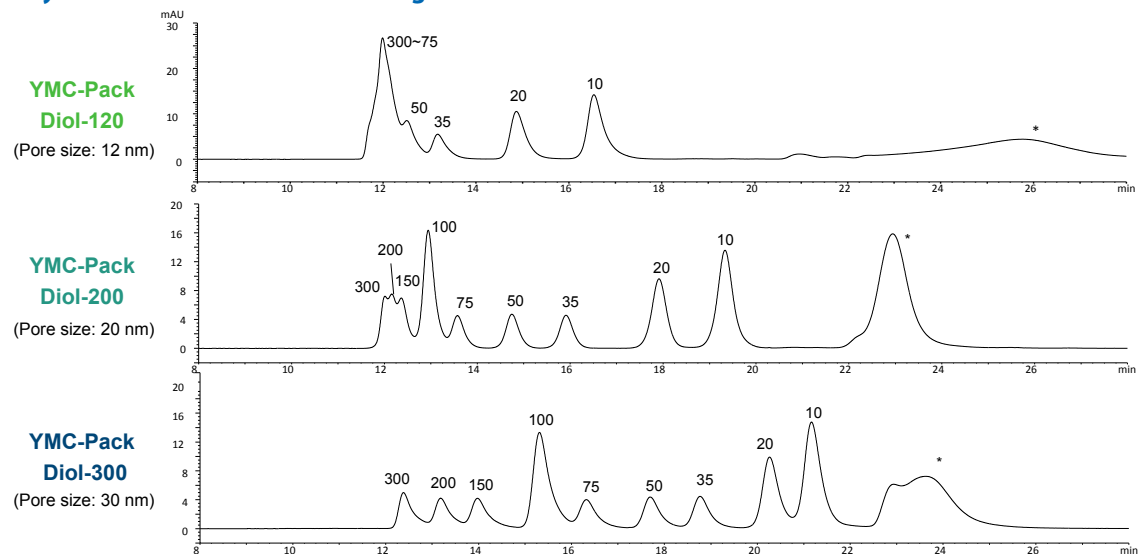


SEC analysis using YMC-Pack Diol columns with different pore sizes

Analysis of ssDNA of 10–120mer length



Analysis of dsDNA of 10–300mer length



Columns: YMC-Pack Diol-120 (5 μ m, 12 nm) 300 x 4.6 mm ID
 YMC-Pack Diol-200 (5 μ m, 20 nm) 300 x 4.6 mm ID
 YMC-Pack Diol-300 (5 μ m, 30 nm) 300 x 4.6 mm ID
 Part Nos.: DL12S05-3046WT
 DL20S05-3046WT
 DL30S05-3046WT
 Eluent: 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl

Flow rate: 0.17 mL/min
 Detection: UV at 260 nm
 Temperature: 25 $^\circ$ C
 Injection: 1.0 μ L (each 5 nmol/mL)
 Samples: ssDNA (10–120mer + dTMP)
 dsDNA (10–300mer)

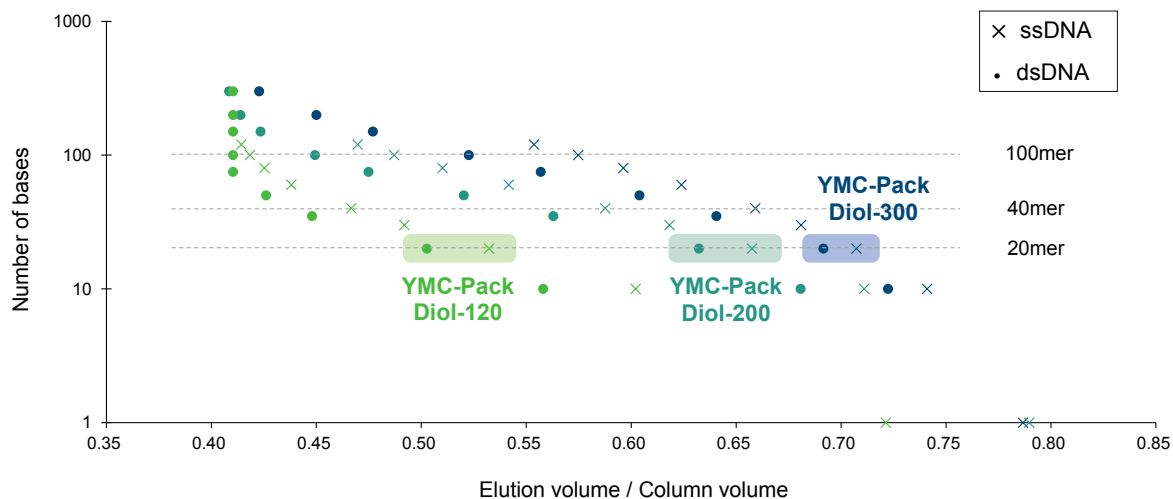
YMC-Pack Diol-120 is best suited for shorter oligonucleotides (10–40mer), whereas YMC-Pack Diol-200 shows the best resolution for oligonucleotides of medium size (30–80mer). Longer oligonucleotides of 60–120mer in length are separated most effectively by YMC-Pack Diol-300.

Similar results are obtained when analysing dsDNA. Small oligonucleotides are separated with higher resolution when smaller pore sizes of 12 and 20 nm are used. Above a length of 50mer, oligonucleotides are unable to penetrate the small pores and elute at the same time. YMC-Pack Diol-200 can resolve oligonucleotides up to a size of 100mer. dsDNA of 150–300mer are only separated by YMC-Pack Diol-300 with the largest pore size of 30 nm. This column also shows the best resolution over a wide range of oligonucleotide lengths.

SEC – Elution volumes

Comparison of the elution volume of ssDNA and dsDNA

Selected corresponding ssDNA and dsDNA pairs of the same number of bases are marked

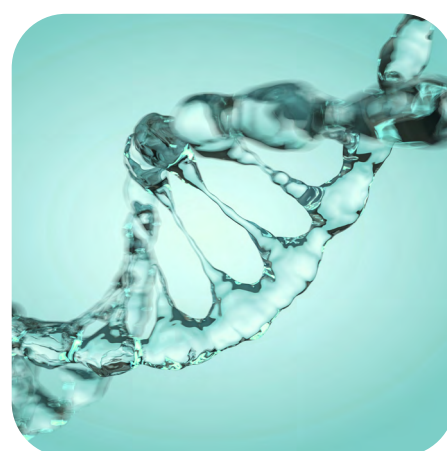


Columns: YMC-Pack Diol-120 (5 μ m, 12 nm) 300 x 4.6 mm ID
 YMC-Pack Diol-200 (5 μ m, 20 nm) 300 x 4.6 mm ID
 YMC-Pack Diol-300 (5 μ m, 30 nm) 300 x 4.6 mm ID
 Part Nos.: DL12S05-3046WT
 DL20S05-3046WT
 DL30S05-3046WT
 Eluent: 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
 Flow rate: 0.17 mL/min
 Detection: UV at 260 nm
 Temperature: 25 $^\circ\text{C}$
 Injection: 1.0 μL (each 5 nmol/mL)
 Samples: ssDNA (10–120 mer +dTMP)
 dsDNA (10–300 mer)

Although dsDNA has the same length as its single-stranded counterpart, the dsDNA elutes at lower elution volumes when separated by SEC. This behaviour is most probably due to the larger hydrodynamic radius of dsDNA compared to ssDNA, which results in faster diffusion through the stationary phase.



HILIC



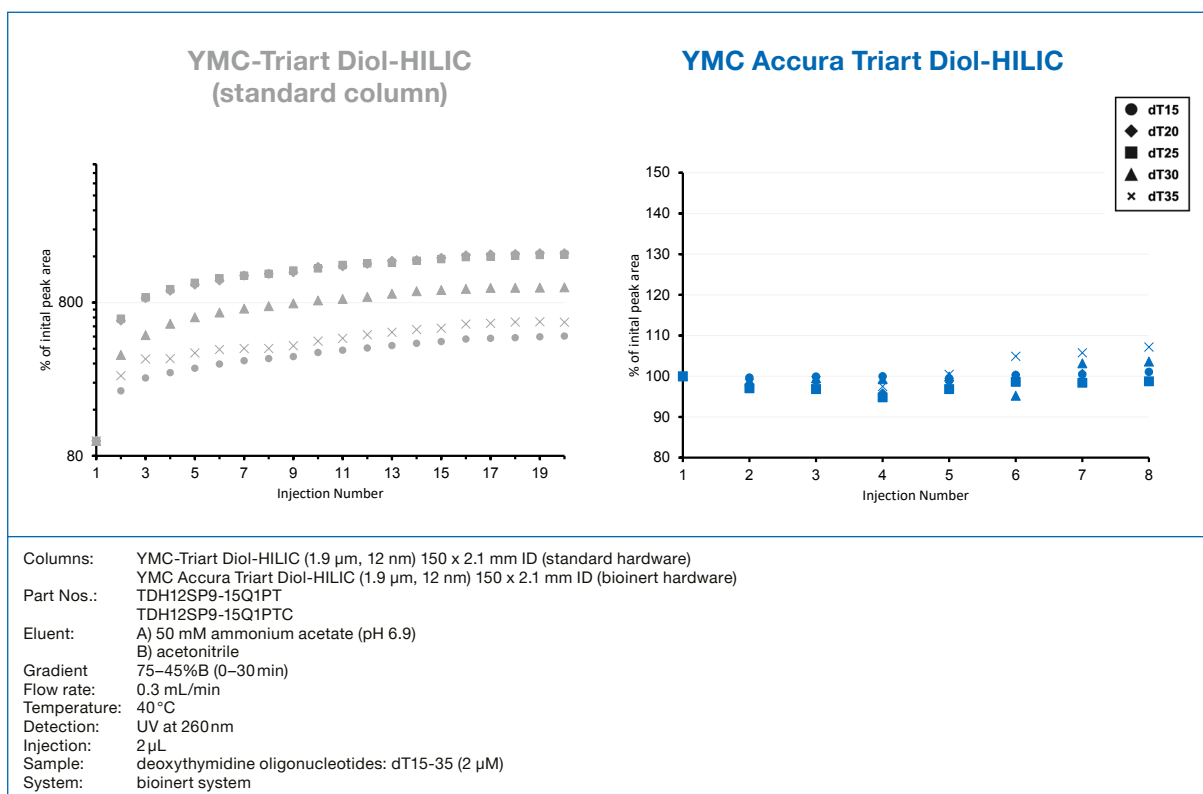
HILIC – UHPLC/HPLC selectivity

Features

- pH- and temperature stable
- Superior reproducibility
- Bioinert coated YMC Accura column hardware

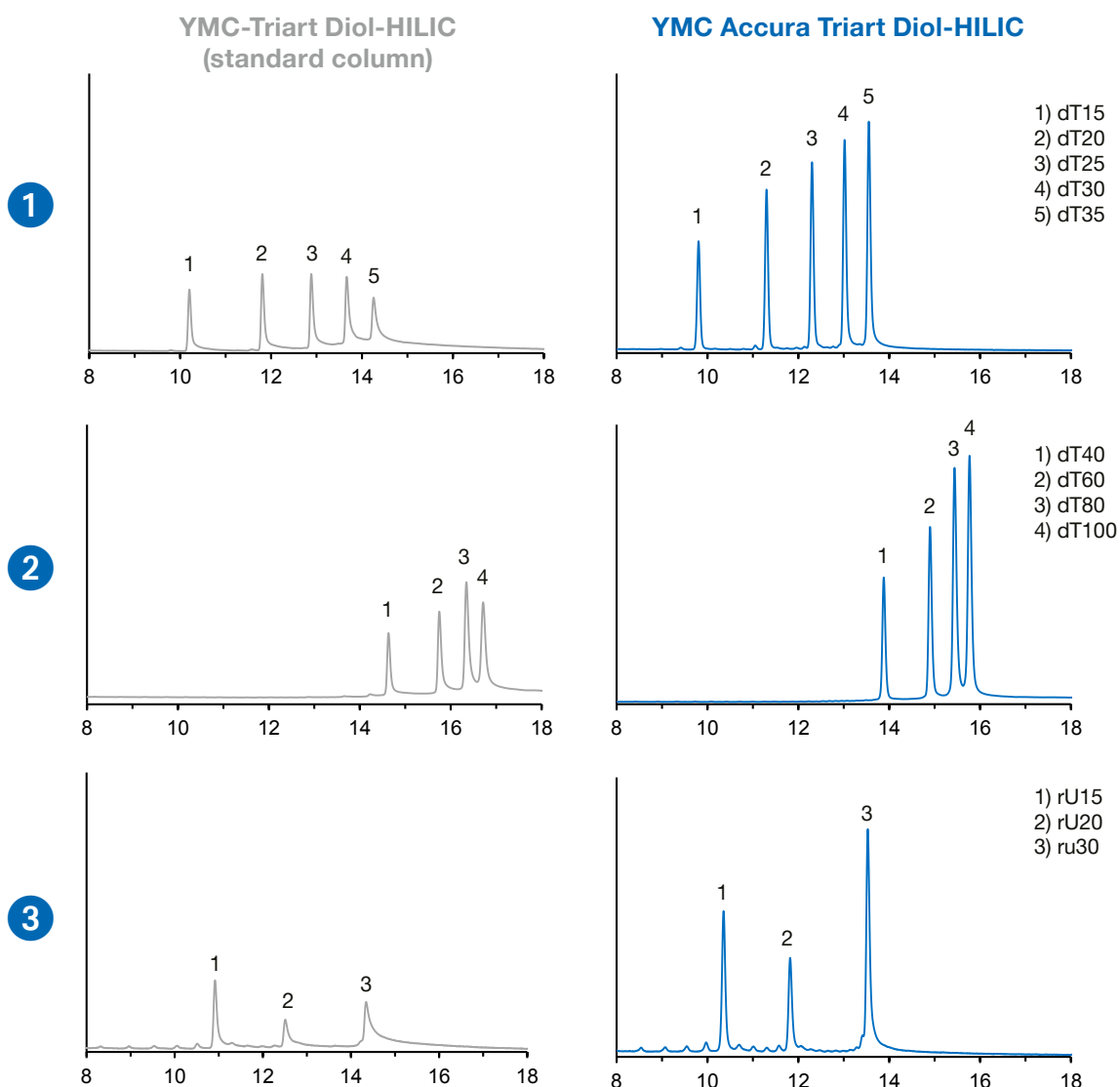
	Base particle	Modification	Particle Size/ µm	Pore Size/ nm	pH range	Temperature range	Column hardware
YMC-Triart Diol-HILIC	organic/inorganic hybrid silica	Diol (USP L20)	1.9, 3, 5	12	2–10	50 °C	bioinert coated YMC Accura

Pre-conditioning of a stainless-steel and a bioinert coated column with short DNA mixture



Pre-conditioning is a typical procedure when working with stainless-steel columns. Using a bioinert column such as YMC Accura Triart usually achieves great performance from the first injection when working with an IP-RP phase. HILIC phases still need some pre-conditioning when a bioinert column is used; however, the number of injections is remarkably reduced. While 20 injections are necessary for the stainless-steel column, the YMC Accura column is already conditioned after 8 injections, with very little difference (less than 10%) between initial and final peak areas.

Improved chromatographic results using bioinert coated YMC Accura Triart column



Columns: YMC-Triart Diol-HILIC (1.9 μm , 12 nm) 150 x 2.1 mm ID (standard hardware)
YMC Accura Triart Diol-HILIC (1.9 μm , 12 nm) 150 x 2.1 mm ID (bioinert hardware)

Part Nos.: TDH12SP9-15Q1PT
TDH12SP9-15Q1PTC

Eluent: A) 50 mM ammonium acetate (pH 6.9)
B) acetonitrile

Gradient: 75–45%B (0–30 min)

Flow rate: 0.3 mL/min

Temperature: 40 °C

Detection: UV at 260 nm

Injection: 2 μL

Sample: deoxythymidine oligonucleotides: dT15-35 (2 μM) and dT40-100 (2 μM)
RNA oligonucleotides: rU15-30 (2 μM)

System: bioinert system

1) dT15–35
2) dT40–100
3) rU15–30

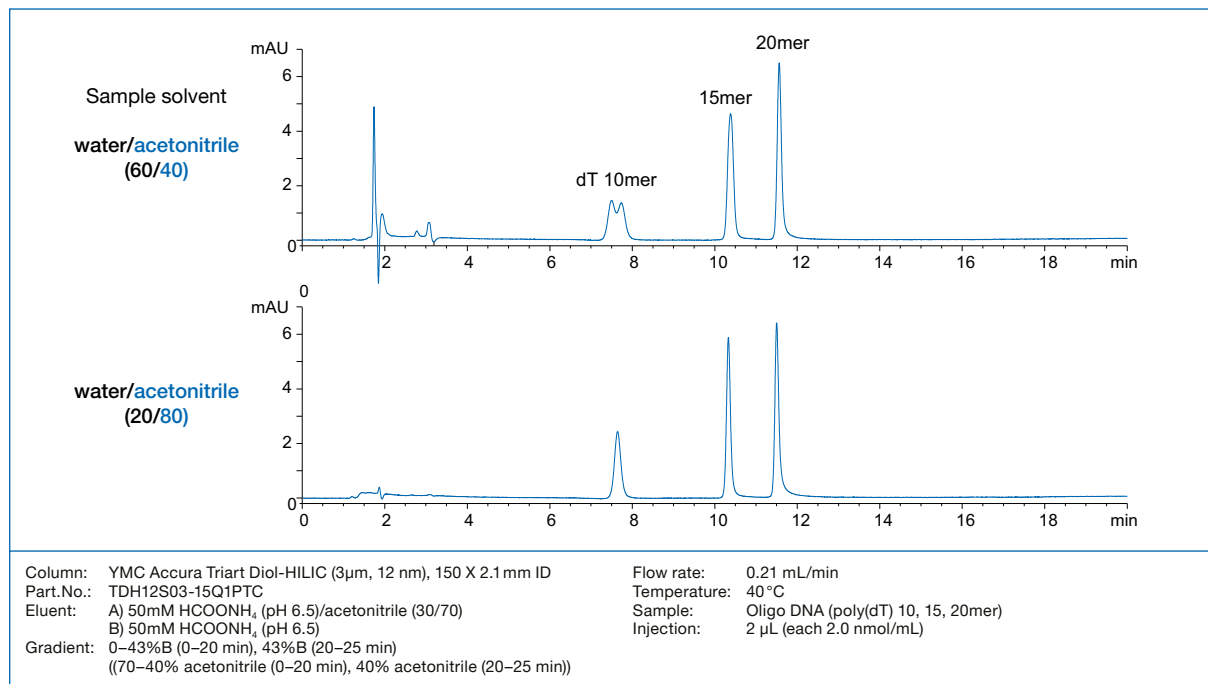
By courtesy of Honorine Lardeux,
Institute of Pharmaceutical Sciences of Western Switzerland
(University of Geneva), Geneva, Switzerland.

Reference: H. Lardeux, A. Goyon, K. Zhang, J.M. Nguyen, M.A. Lauber, D. Guillaume, V. D'Atri, The impact of low adsorption surfaces for the analysis of DNA and RNA oligonucleotides, *J. Chromatogr. A* 1677 (2022) 463324.

After conditioning and analysing the short DNA oligonucleotide mixture of dT15-35, longer DNA oligonucleotides dT40-100 and short RNA oligonucleotides rU15-30 are analysed. Higher sensitivities, peak areas and less tailing are achieved using the bioinert YMC Accura Triart Diol-HILIC column. Non-specific adsorption does not vary according to length, even though the adsorption is usually higher for longer oligonucleotides in IP-RP.

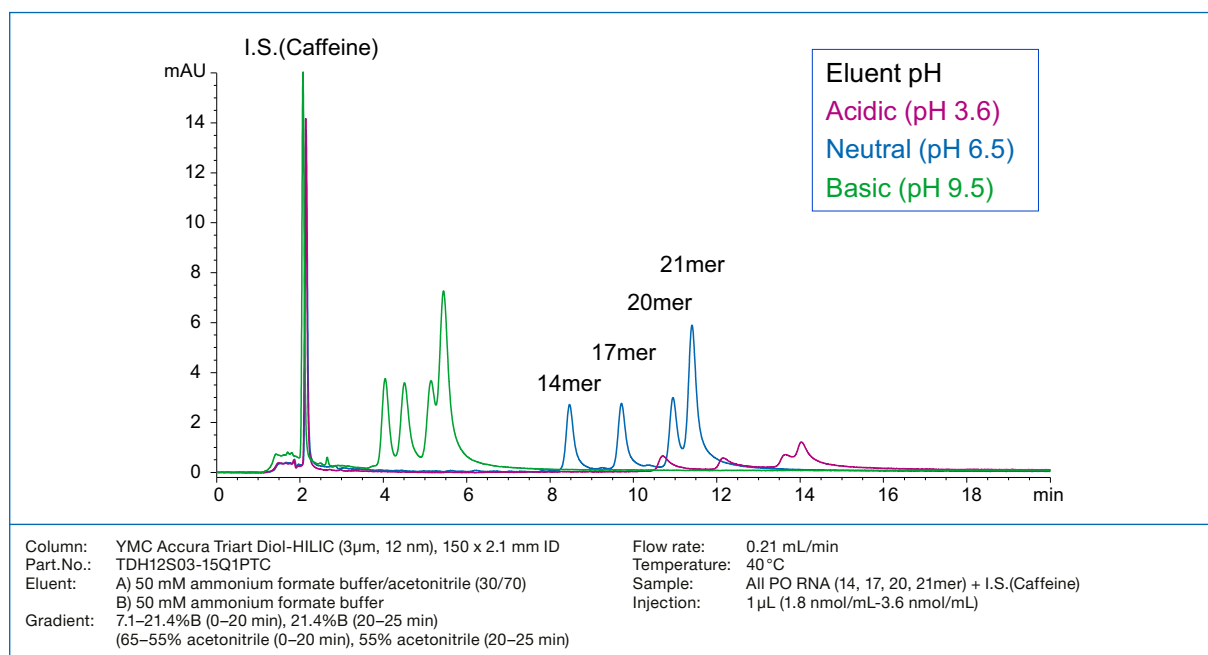
HILIC Expert Tips

Influence of sample solvent

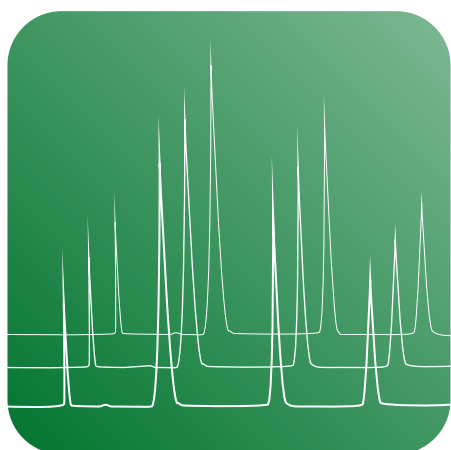


The influence of the sample solvent on the peak shape is significant. The organic composition of the sample solvent must be equal to or higher than the initial gradient composition. A higher water content in the sample solvent leads to massive peak deformation.

Influence of mobile phase pH



The pH of the mobile phase has a massive effect on the recovery and retention of oligonucleotides. Acidic pH results in higher retention, but at the cost of drastically reduced recovery. A neutral-to-basic pH is recommended for the mobile phase. When using a basic pH, a shorter retention time is observed, but also the highest recovery.



Ordering
information



IP-RP – Ordering information

1.9 µm bioinert coated UHPLC columns (max. pressure 100 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC Accura Triart C18	2.1	TA12SP9-05Q1PTC	TA12SP9-100Q1PTC	TA12SP9-150Q1PTC
YMC Accura Triart Bio C18	2.1	TA30SP9-05Q1PTC	TA30SP9-10Q1PTC	TA30SP9-15Q1PTC
YMC Accura Triart C8	2.1	T012SP9-05Q1PTC	T012SP9-10Q1PTC	T012SP9-15Q1PTC
YMC Accura Triart Bio C4	2.1	TB30SP9-05Q1PTC	TB30SP9-10Q1PTC	TB30SP9-15Q1PTC

3 µm bioinert coated HPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC Accura Triart C18	2.1	TA12S03-05Q1PTC	TA12S03-10Q1PTC	TA12S03-15Q1PTC
	4.6	TA12S03-0546PTC	TA12S03-1046PTC	TA12S03-1546PTC
YMC Accura Triart Bio C18	2.1	TA30S03-05Q1PTC	TA30S03-10Q1PTC	TA30S03-15Q1PTC
	4.6	TA30S03-0546PTC	TA30S03-1046PTC	TA30S03-1546PTC
YMC Accura Triart C8	2.1	T012S03-05Q1PTC	T012S03-10Q1PTC	T012S03-15Q1PTC
	4.6	T012S03-0546PTC	T012S03-1046PTC	T012S03-1546PTC
YMC Accura Triart Bio C4	2.1	TB30S03-05Q1PTC	TB30S03-10Q1PTC	TB30S03-15Q1PTC
	4.6	TB30S03-0546PTC	TB30S03-1046PTC	TB30S03-1546PTC

3 µm HPLC columns (max. pressure 25 MPa)

Phase	Column ID [mm]	Column length [mm]						Guard cartridges* with 10 mm length (pack of 5)
		30/33	50	75	100	150	250	
Hydrosphere C18	2.1	HS12S03-03Q1WT	HS12S03-05Q1WT	HS12S03-L5Q1WT	HS12S03-10Q1WT	HS12S03-15Q1WT	HS12S03-25Q1WT	HS12S03-01Q1GC
	3.0	HS12S03-0303WT	HS12S03-0503WT	HS12S03-L503WT	HS12S03-1003WT	HS12S03-1503WT	HS12S03-2503WT	HS12S03-0103GC
	4.6	HS12S03-0346WT	HS12S03-0546WT	HS12S03-L546WT	HS12S03-1046WT	HS12S03-1546WT	HS12S03-2546WT	HS12S03-0104GC

*Guard cartridge holder required, part no. XPGCH-Q1 (for EMEA)/XPGCHP1 (outside EMEA)

Further dimensions and guard cartridges available in regular stainless-steel hardware.

5 µm bioinert coated HPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC Accura Triart C18	2.1	TA12S05-05Q1PTC	TA12S05-10Q1PTC	TA12S05-15Q1PTC
	4.6	TA12S05-0546PTC	TA12S05-1046PTC	TA12S05-1546PTC
	10	–	TA12S05-1010PTC	TA12S05-1510PTC
YMC Accura Triart Bio C18	2.1	TA30S05-05Q1PTC	TA30S05-10Q1PTC	TA30S05-15Q1PTC
	4.6	TA30S05-0546PTC	TA30S05-1046PTC	TA30S05-1546PTC
	10	–	TA30S05-1010PTC	TA30S05-1510PTC
YMC Accura Triart C8	2.1	T012S05-05Q1PTC	T012S05-10Q1PTC	T012S05-15Q1PTC
	4.6	T012S05-0546PTC	T012S05-1046PTC	T012S05-1546PTC
	10	–	T012S05-1010PTC	T012S05-1510PTC
YMC Accura Triart Bio C4	2.1	TB30S05-05Q1PTC	TB30S05-10Q1PTC	TB30S05-15Q1PTC
	4.6	TB30S05-0546PTC	TB30S05-1046PTC	TB30S05-1546PTC
	10	–	TB30S05-1010PTC	TB30S05-1510PTC

5 µm HPLC columns (max. pressure 20–25 MPa)

Phase	Column ID [mm]	Column length [mm]						Guard cartridges* with 10 mm length (pack of 5)
		30/33	50	75	100	150	250	
Hydrosphere C18	2.1	HS12S05-03Q1WT	HS12S05-05Q1WT	HS12S05-L5Q1WT	HS12S05-10Q1WT	HS12S05-15Q1WT	HS12S05-25Q1WT	HS12S05-01Q1GC
	3.0	HS12S05-0303WT	HS12S05-0503WT	HS12S05-L503WT	HS12S05-1003WT	HS12S05-1503WT	HS12S05-2503WT	HS12S05-0103GC
	4.6	HS12S05-0346WT	HS12S05-0546WT	HS12S05-L546WT	HS12S05-1046WT	HS12S05-1546WT	HS12S05-2546WT	HS12S05-0104GC

*Guard cartridge holder required, part no. XPGCH-Q1 (for EMEA)/XPGCHP1 (outside EMEA)

5 µm YMC-Actus high-throughput (semi)preparative columns (max. pressure 20–30 MPa)

Phase	Column ID [mm]	Column length [mm]					Guard cartridges* with 10 mm length (pack of 2)
		50	75	100	150	250	
YMC-Triart C18	20	TA12S05-0520WX	TA12S05-L520WX	TA12S05-1020WX	TA12S05-1520WX	TA12S05-2520WX	TA12S05-0120CCN
	30	TA12S05-0530WX	TA12S05-L530WX	TA12S05-1030WX	TA12S05-1530WX	TA12S05-2530WX	TA12S05-0130CCN
	50	TA12S05-0553DX	–	TA12S05-1053DX	TA12S05-1553DX	TA12S05-2553DX	TA12S05-0553DXG**
YMC-Triart Bio C18	20	TA30S05-0520WX	TA30S05-L520WX	TA30S05-1020WX	TA30S05-1520WX	TA30S05-2520WX	TA30S05-0120CCN
	30	TA30S05-0530WX	TA30S05-L530WX	TA30S05-1030WX	TA30S05-1530WX	TA30S05-2530WX	TA30S05-0130CCN
	50	TA30S05-0553DX	–	TA30S05-1053DX	TA30S05-1553DX	TA30S05-2553DX	TA30S05-0553DXG**
YMC-Triart C8	20	T012S05-0520WX	T012S05-L520WX	T012S05-1020WX	T012S05-1520WX	T012S05-2520WX	T012S05-0120CCN
	30	T012S05-0530WX	T012S05-L530WX	T012S05-1030WX	T012S05-1530WX	T012S05-2530WX	T012S05-0130CCN
	50	T012S05-0553DX	–	T012S05-1053DX	T012S05-1553DX	T012S05-2553DX	T012S05-0553DXG**
YMC-Triart Bio C4	20	TB30S05-0520WX	TB30S05-L520WX	TB30S05-1020WX	TB30S05-1520WX	TB30S05-2520WX	TB30S05-0120CCN
	30	TB30S05-0530WX	TB30S05-L530WX	TB30S05-1030WX	TB30S05-1530WX	TB30S05-2530WX	TB30S05-0130CCN
	50	TB30S05-0553DX	–	TB30S05-1053DX	TB30S05-1553DX	TB30S05-2553DX	TB30S05-0553DXG**
Hydrosphere C18	20	HS12S05-0520WX	HS12S05-L520WX	HS12S05-1020WX	HS12S05-1520WX	HS12S05-2520WX	HS12S05-0120CCN
	30	HS12S05-0530WX	HS12S05-L530WX	HS12S05-1030WX	HS12S05-1530WX	HS12S05-2530WX	HS12S05-0130CCN

*Guard cartridge holder required, part no. XPGHF2P20ID (20 mm ID)
XPGHF2P30ID (30 mm ID)
no holder required for 50 mm

Further dimensions and guard cartridges available in regular stainless-steel hardware.

AEX – Ordering information

3 µm bioinert coated HPLC columns (max. pressure 15–20 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50 (15 MPa)	100 (15 MPa)	150 (20 MPa)
YMC Accura BioPro IEX QF	2.1	QF00S03-05Q1PTC	QF00S03-10Q1PTC	QF00S03-15Q1PTC
	4.6	QF00S03-0546PTC	QF00S03-1046PTC	QF00S03-1546PTC

5 µm bioinert coated HPLC columns (max. pressure 10–30 MPa)

Phase	Column ID [mm]	Column length [mm]			
		50 (10 MPa)	100 (12 MPa)	150 (18 MPa)	250 (30 MPa)
YMC Accura BioPro IEX QF	2.1	QF00S05-05Q1PTC	QF00S05-10Q1PTC	QF00S05-15Q1PTC	–
	4.6	QF00S05-0546PTC	QF00S05-1046PTC	QF00S05-1546PTC	QF00S05-2546PTC

6 µm semiprep. columns, stainless-steel hardware (max. pressure 3–9 MPa)

Phase	Column ID [mm]	Column length [mm]
		100
BioPro IEX QF	10	QF00S06-1010WT
	20	QF00S06-1020WT
	30	QF00S06-1030WT

2 µm UHPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		Guard cartridges* with 10 mm length (pack of 5)
		150	300	
YMC-Pack Diol-200	4.6	DL20S02-1546PTH	DL20S02-3046PTH	DL20S02-0104GC
YMC-Pack Diol-300	4.6	DL30S02-1546PTH	DL30S02-3046PTH	DL30S02-0104GC

*Guard cartridge holder required, part no. XPGCH-Q1 (for EMEA)/XPGCHP1 (outside EMEA)

3 µm HPLC columns (max. pressure 14–20 MPa)

Phase	Column ID [mm]	Column length [mm]			Guard cartridges* with 10/30 mm length (pack of 5)
		150	250	300	
YMC-Pack Diol-120	4.6	DL12S03-1546WT	DL12S03-2546WT	DL12S03-3046WT	DL12S03-0104GC
	6.0	–	–	DL12S03-3006WT	–
	8.0	DL12S03-1508WT	–	DL12S03-3008WT	DL12S03-0308WTG**
YMC-Pack Diol-200	4.6	DL20S03-1546WT	DL20S03-2546WT	DL20S03-3046WT	DL20S03-0104GC
	6.0	–	–	DL20S03-3006WT	–
	8.0	DL20S03-1508WT	–	DL20S03-3008WT	DL20S03-0308WTG**
YMC-Pack Diol-300	4.6	DL30S03-1546WT	DL30S03-2546WT	DL30S03-3046WT	DL30S03-0104GC
	6.0	–	–	DL30S03-3006WT	–
	8.0	DL30S03-1508WT	–	DL30S03-3008WT	DL30S03-0308WTG**

*Guard cartridge holder required, part no. XPGCH-Q1 (for EMEA)/XPGCHP1 (outside EMEA)

**no holder required for 30 x 8 mm ID guard columns (1 piece)
recommended column coupler part no. XRCP1602

5 µm HPLC columns (max. pressure 20 MPa)

Phase	Column ID [mm]	Column length [mm]			Guard cartridges* with 10/30 mm length (pack of 5)
		250	300	500	
YMC-Pack Diol-120	4.6	DL12S05-2546WT	DL12S05-3046WT	–	DL12S05-0104GC
	6.0	DL12S05-2506WT	DL12S05-3006WT	DL12S05-5006WT	–
	8.0	–	DL12S05-3008WT	DL12S05-5008WT	DL12S05-0308WTG**
	10.0	DL12S05-2510WT	DL12S05-3010WT	DL12S05-5010WT	DL12S05-0310WTG**
YMC-Pack Diol-200	4.6	DL20S05-2546WT	DL20S05-3046WT	–	DL20S05-0104GC
	6.0	DL20S05-2506WT	DL20S05-3006WT	DL20S05-5006WT	–
	8.0	–	DL20S05-3008WT	DL20S05-5008WT	DL20S05-0308WTG**
	10.0	DL20S05-2510WT	DL20S05-3010WT	DL20S05-5010WT	DL20S05-0310WTG**
YMC-Pack Diol-300	4.6	DL30S05-2546WT	DL30S05-3046WT	–	DL30S05-0104GC
	6.0	DL30S05-2506WT	DL30S05-3006WT	DL30S05-5006WT	–
	8.0	–	DL30S05-3008WT	DL30S05-5008WT	DL30S05-0308WTG**
	10.0	DL30S05-2510WT	DL30S05-3010WT	DL30S05-5010WT	DL30S05-0310WTG**

*Guard cartridge holder required, part no. XPGCH-Q1 (for EMEA)/XPGCHP1 (outside EMEA)

**no holder required for 30 x 8 mm ID guard columns (1 piece)
recommended column coupler part no. XRCP1602 (for 8mm ID) and XRCP1605 (for 10mm ID)

*Bioinert SEC columns
are available on request.*

HILIC – Ordering Information

1.9 µm bioinert coated UHPLC columns

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC Accura Triart Diol-HILIC	2.1	TDH12SP9-05Q1PTC	TDH12SP9-10Q1PTC	TDH12SP9-15Q1PTC

3 µm bioinert coated HPLC columns

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC Accura Triart Diol-HILIC	2.1	TDH12S03-05Q1PTC	TDH12S03-10Q1PTC	TDH12S03-15Q1PTC
	4.6	TDH12S03-0546PTC	TDH12S03-1046PTC	TDH12S03-1546PTC

5 µm bioinert coated HPLC columns

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC Accura Triart Diol-HILIC	2.1	TDH12S05-05Q1PTC	TDH12S05-10Q1PTC	TDH12S05-15Q1PTC
	4.6	TDH12S05-0546PTC	TDH12S05-1046PTC	TDH12S05-1546PTC

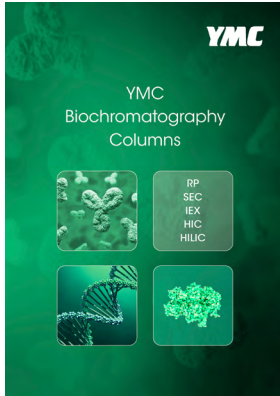
Further dimensions and guard cartridges available in regular stainless-steel hardware.

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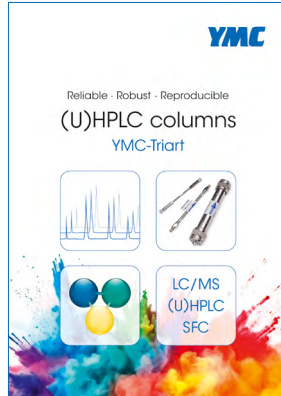
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BROCHURES



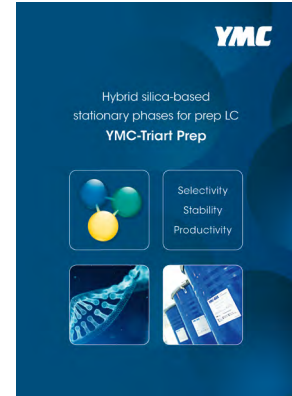
YMC Biochromatography Columns



YMC-Triart



YMC BioPro IEX Resins



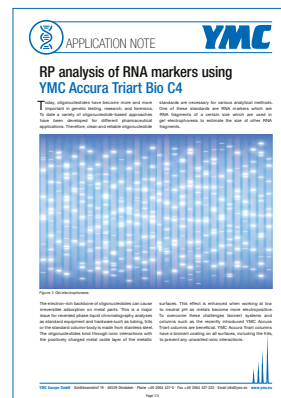
YMC-Triart Prep

WHITE PAPER



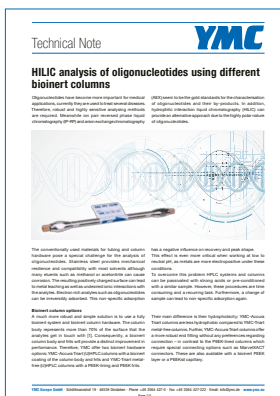
Analysis and Purification of Oligonucleotides by AEX and IP RP

APPLICATION NOTE

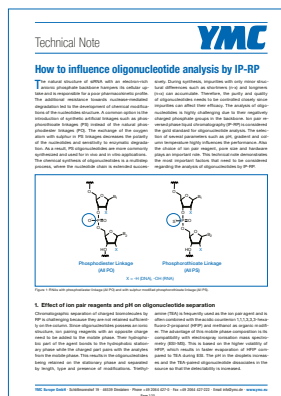


RP analysis of RNA markers using YMC Accura Triart Bio C4

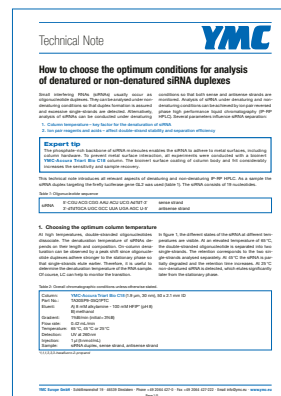
TECHNICAL NOTES



HILIC analysis of oligonucleotides using different bioinert columns



How to influence oligonucleotide analysis by IP-RP



How to choose the optimum conditions for analysis of denatured or non-denatured siRNA duplexes

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