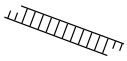
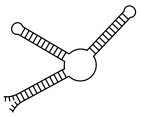
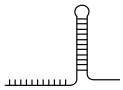
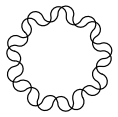
YMC

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

Content

Phase selection guide

Oligonucleotides / Nucleic Acids

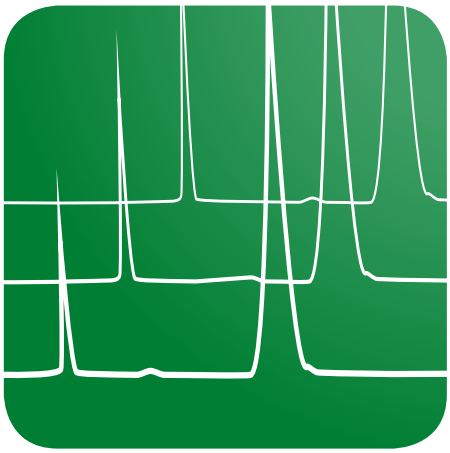
IP-RP
- YMC-Triart C18
- YMC-Triart Bio C18
- Hydrosphere C18
- YMC-Triart C8
- YMC-Triart Bio C4

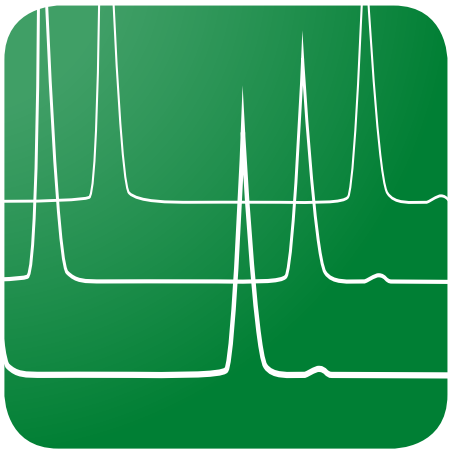
AEX
- BioPro IEX QA
- BioPro IEX QF

SEC
- YMC-Pack Diol-120
- YMC-Pack Diol-200
- YMC-Pack Diol-300

HILIC
- YMC-Triart Diol-HILIC

Oligonucleotide Applications



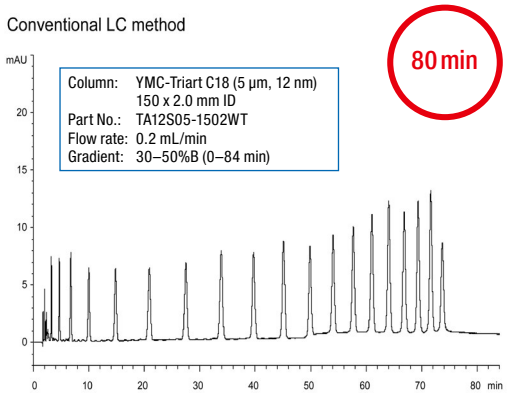


Oligonucleotide applications

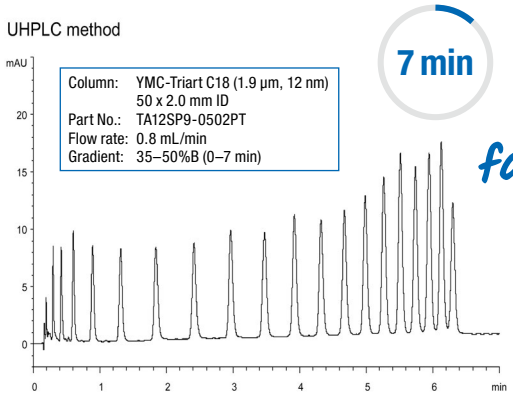
Oligonucleotides d(T)2-20 method transfer from HPLC to UHPLC

RP

Conventional LC method



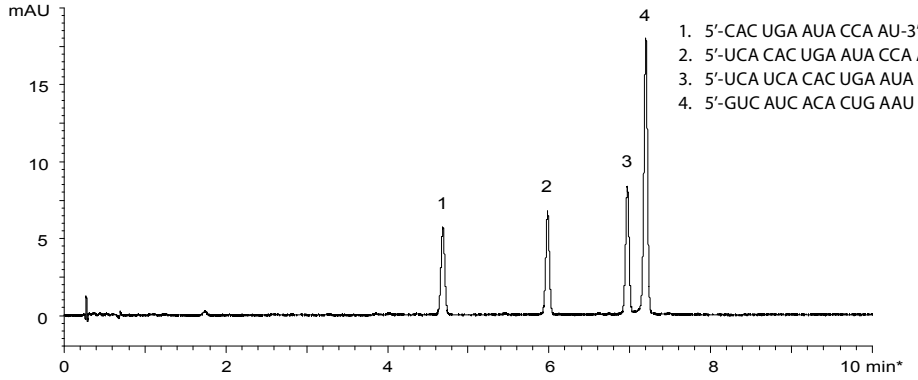
UHPLC method



Eluent:	A) 10 mM di-n-butylamine-acetic acid (pH 6.0) B) methanol	Detection:	UV at 269 nm
Temperature:	37°C	Injection:	1 µL (5 nmol/mL)

Synthetic oligonucleotides

RP



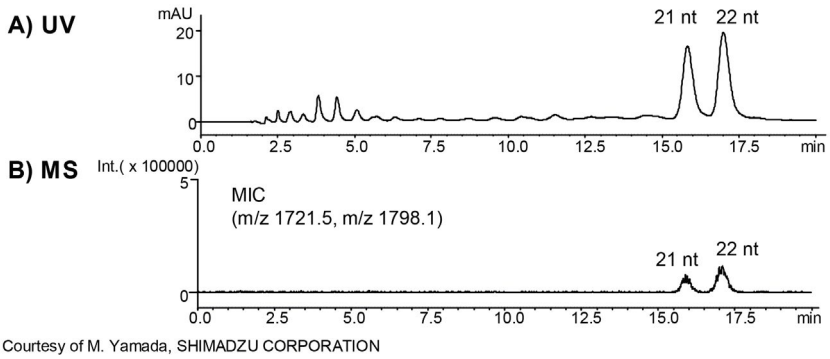
1. 5′-CAC UGA AUA CCA AU-3′ (14mer)
2. 5′-UCA CAC UGA AUA CCA AU-3′ (17mer)
3. 5′-UCA UCA CAC UGA AUA CCA AU-3′ (20mer)
4. 5′-GUC AUC ACA CUG AAU ACC AAU-3′ (21mer)

Column:	YMC-Triart C18 (1.9 µm, 12 nm) 50 x 2.1 mm ID	Flow rate:	0.42 mL/min
Part No.:	TA12SP9-05Q1PT	Temperature:	65°C
Eluent:	A) 200 mM HFIP*-8 mM triethylamine B) methanol	Detection:	UV at 260 nm
		Injection:	1 µL (2–4 nmol/mL)
Gradient:	10–20%B (0–10 min)		

*hexafluoroisopropanol

LC/MS analysis of miRNA

RP



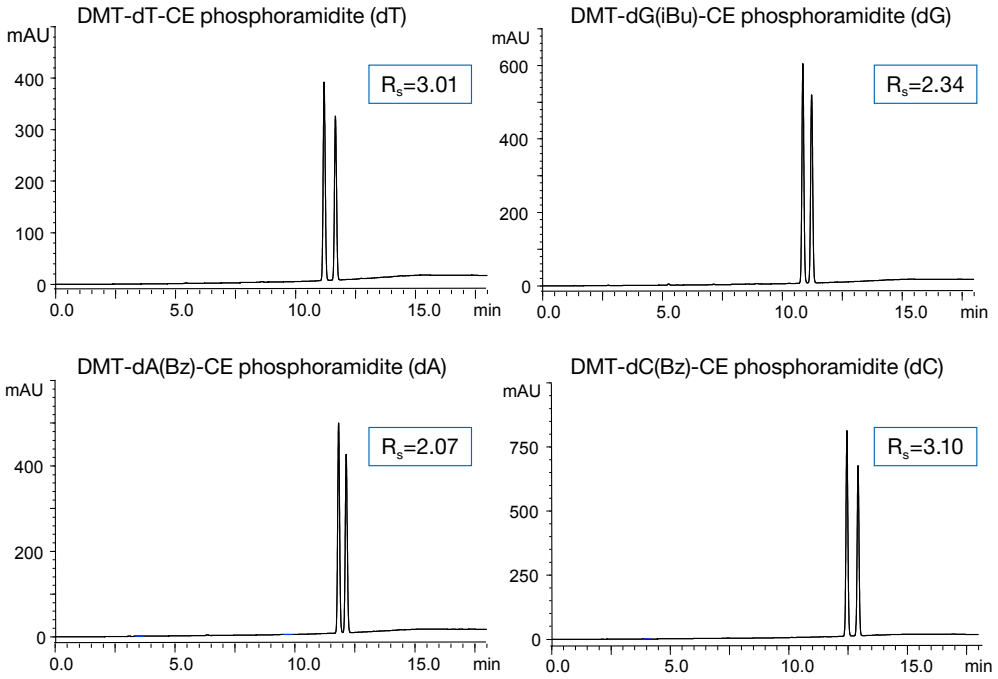
Courtesy of M. Yamada, SHIMADZU CORPORATION

5′-pUGG AGU GUG ACA AUG GUG UUG-3′	(21 nt, MW 6,890.1)
5′-pUGG AGU GUG ACA AUG GUG UUG U-3′	(22 nt, MW 7,196.3)

Column:	YMC-Triart C18 (3 µm, 12 nm) 150 x 2.0 mm ID	Temperature:	30°C
Part No.:	TA12S03-1502WT	Detection:	A) UV at 260 nm
Eluent:	A) 10 mM di-n-butylamine-acetic acid (pH 7.5)		B) ESI-negative mode
	B) 10 mM di-n-butylamine-acetic acid (pH 7.5)/acetonitrile (50/50)	Injection:	4 µL (5 nmol/mL)
Gradient:	62–72%B (0–20 min)	System:	LC) Shimadzu Prominence
Flow rate:	0.2 mL/min		MS) Shimadzu LCMS2020

Analysis of phosphoramidites

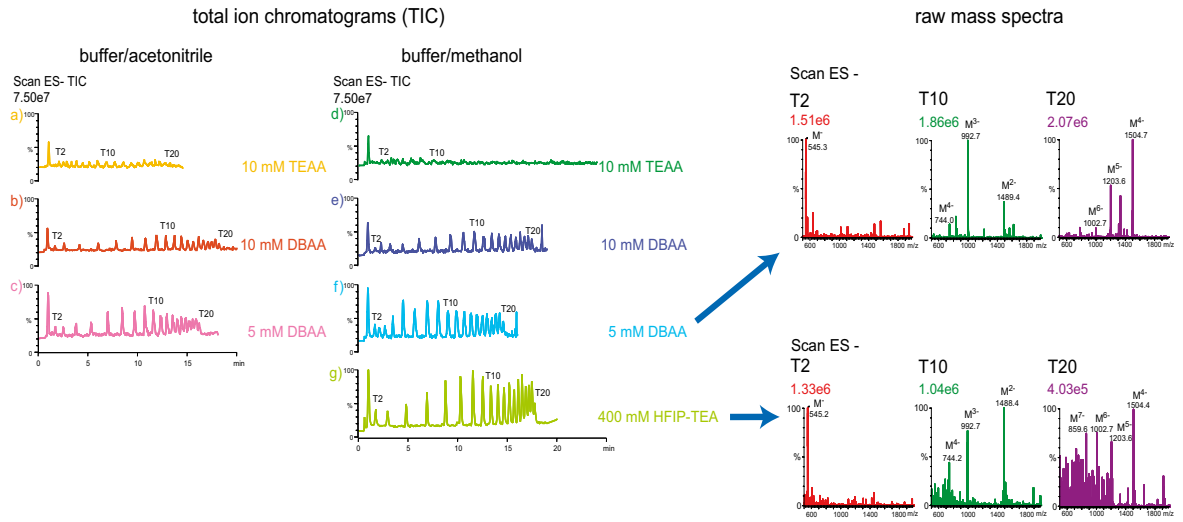
RP



Column:	YMC-Triart C18 (5 µm, 12 nm) 150 x 4.6 mm ID	Flow rate:	1.0 mL/min
Part No.:	TA12S05-1546PTH	Temperature:	30 °C
Eluent:	A) 20 mM triethylammonium acetate (pH 7.0)/acetonitrile (90/10) B) acetonitrile	Detection:	UV at 254 nm
		Injection:	4 µL (1.0 mg/mL)
Gradient:	45–95%B (0–12 min), 95%B (12–17 min)		

Influences of mobile phase conditions on intensity of ESI-MS

RP



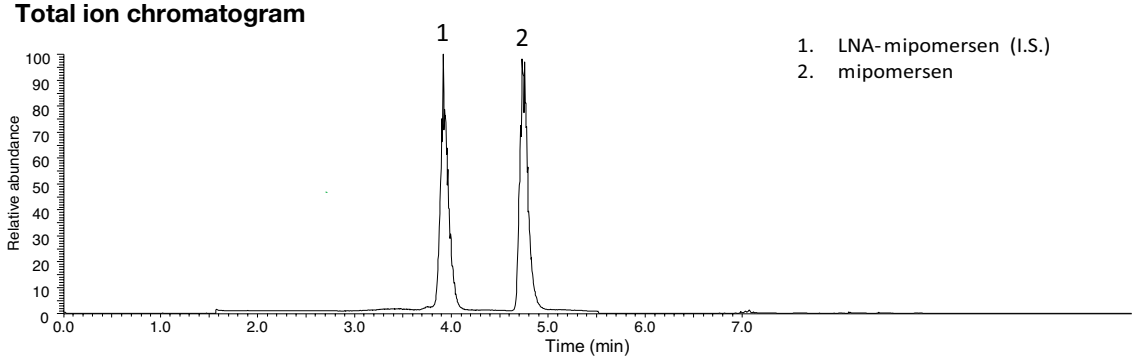
Column:	Hydrosphere C18 (3 µm) 50 x 2.0 mm ID	d)	Eluent: A) 10 mM TEAA buffer (pH 6.0) B) eluent A/methanol (50/50)
Part No.:	HS12S03-0502WT		Gradient: 44–50%B (0–25 min)
Flow rate:	0.2 mL/min	e/f)	Eluent: A) 10/5 mM DBAA buffer (pH 6.0) B) eluent A/methanol (20/80)
Temperature:	35 °C		Gradient: 42–70%B (0–20 min)
Detection:	ESI negative mode	g)	Eluent: A) 400 mM HFIP-TEA buffer (pH 7.0) B) methanol
Injection:	5 µL (25 pmol/component)		Gradient: 7–35%B (0–20 min)
a)	Eluent: A) 10 mM TEAA buffer (pH 6.0) B) eluent A/acetonitrile (80/20)	Sample:	Oligodeoxythymidylic acid [d(pT)$_{2\text{-}20}$]
	Gradient: 50–65%B (0–20 min)		
b/c)	Eluent: A) 10/5 mM DBAA buffer (pH 6.0) B) eluent A/acetonitrile (50/50)		
	Gradient: 30–75%B (0–20 min)		

Oligonucleotide applications

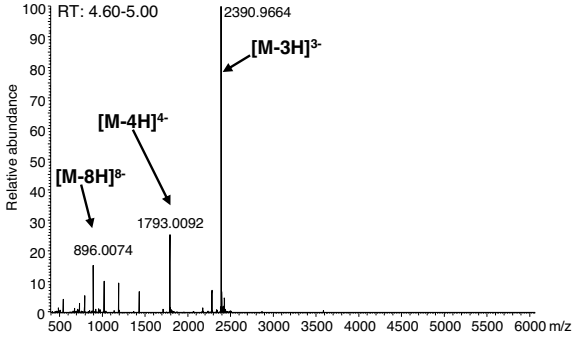
LC-HRMS analysis of the antisense oligonucleotide Mipomersen (Kynamro®)

RP

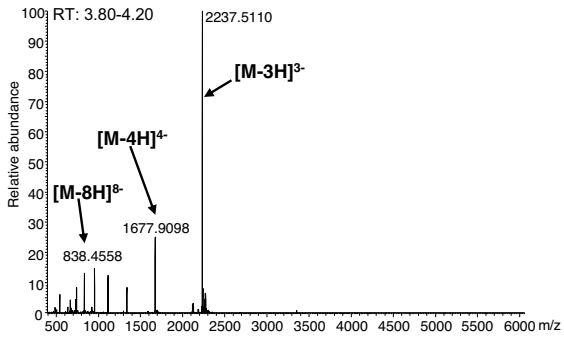
Total ion chromatogram



Mass spectrum of mipomersen



Mass spectrum of LNA-mipomersen



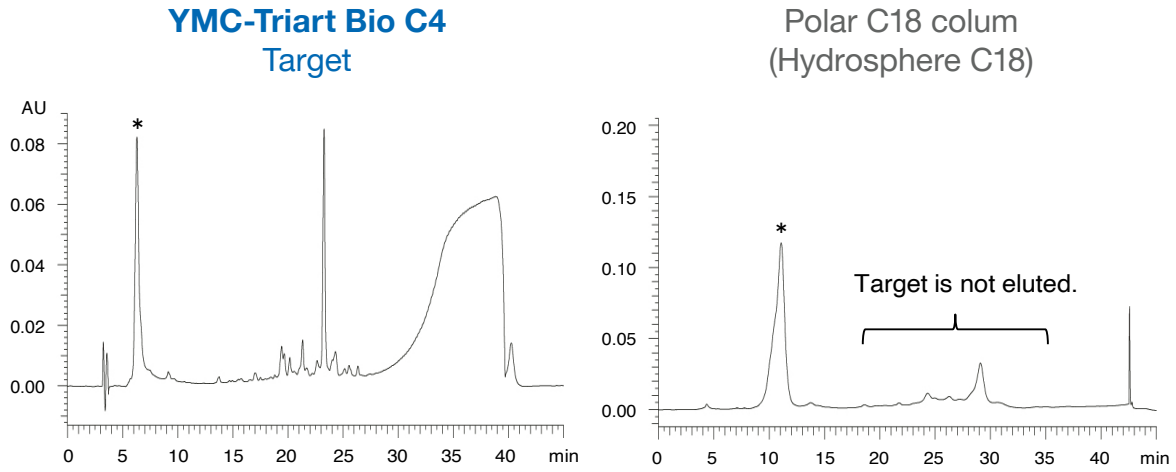
Column:	YMC-Triart C8 **metal-free PEEK-lined** (1.9 µm, 12 nm)*1 100 x 2.1 mm ID
Part No.:	TO12SP9-10Q1PTP
Eluent:	A) water/triethylamine/HFIP*2 (100/0.4/2; triethylamine 28.0 mM, HFIP 135.8 mM) B) methanol/triethylamine/HFIP (100/0.4/2)
Gradient:	[Sample separation step] 10–40%B (0–5.0 min) [Column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

Separation of hydrophobic oligonucleotides modified with disulfides

RP



*phosphorothioate oligonucleotides without disulfide-unit

Column:	5 µm, 250 x 4.6 mm ID	Flow rate:	1 mL/min
Product code:	TB30S05-2546PTH	Temperature:	50 °C
	HS12S05-2546WT	Detection:	UV at 260 nm
Eluent:	A) 50 mM TEAA* (pH 7.0)/acetonitrile (95/5)	Sample:	Crude reaction mixture
	B) acetonitrile		
Gradient:	5–95%B (0-30 min), 95%B (30–35 min), 95–5%B (35–35.1 min), 5%B (35.1–45 min)	By courtesy of Saki Kawaguchi, Chemistry Department, Nagoya University, Japan	

*Triethylammonium acetate

Easy purification of oligonucleotides with YMC-Actus semi prep columns

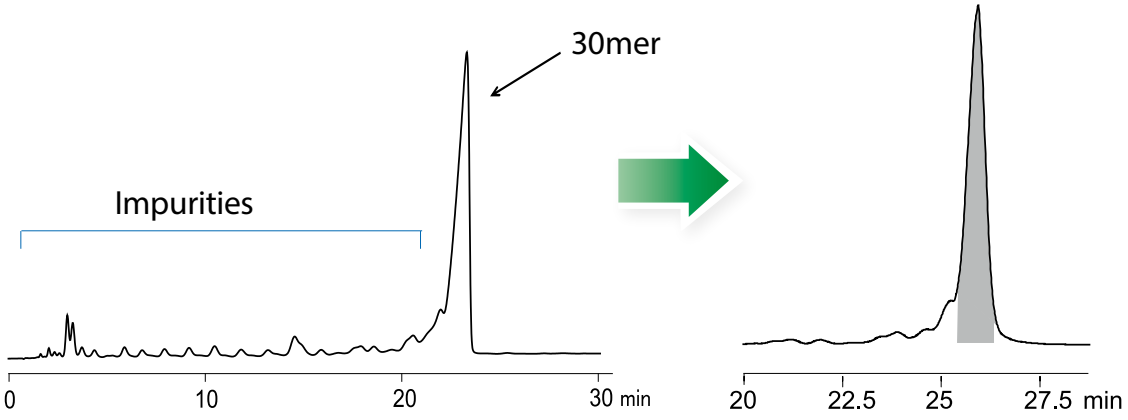
RP

Purification of synthetic 30mer oligonucleotide

Analysis 1.0 mL/min, 5 µL injection
Hydrosphere C18
50 x 4.6 mm ID, 5 µm

Purification 19 mL/min, 100 µL injection
YMC-Actus Hydrosphere C18
50 x 20 mm ID, 5 µm

Recovery 56 %
Purity > 99 %

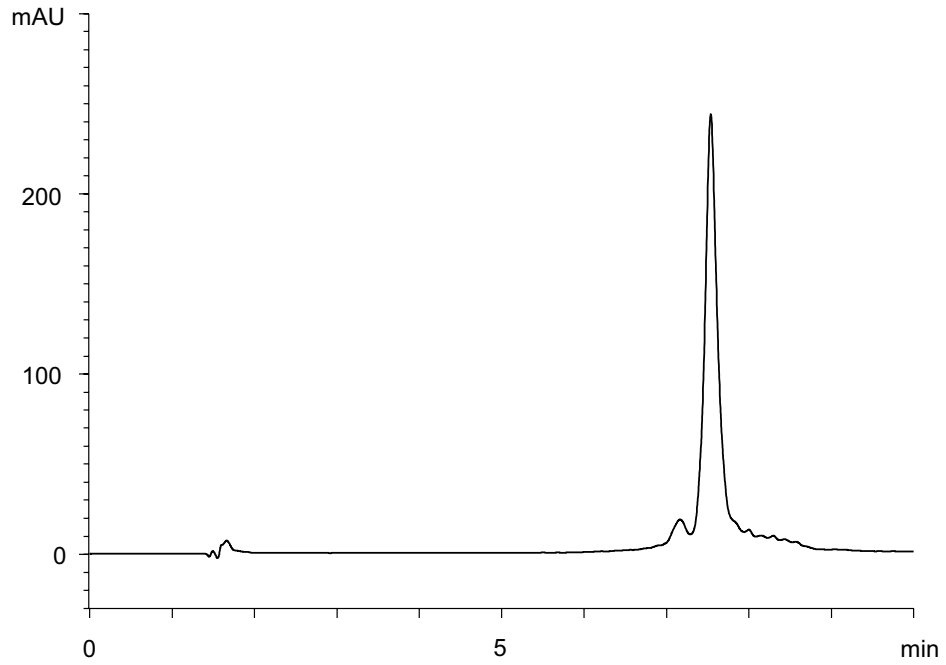


Part Nos.:	HS12S05-0546WT
	HS12S05-0520WX
Eluent:	A) 10 mM DBA-acetic acid (pH 6.0) / methanol (60/40)
	B) 10 mM DBA-acetic acid (pH 6.0) / methanol (20/80)
Gradient:	10%–35%B (0–30 min.)
Temperature:	ambient
Detection:	UV at 269 nm
Sample:	synthetic oligonucleotide (100 µM)

Oligonucleotide applications

mRNA coupled to enhanced green fluorescent protein (EGFP)

RP

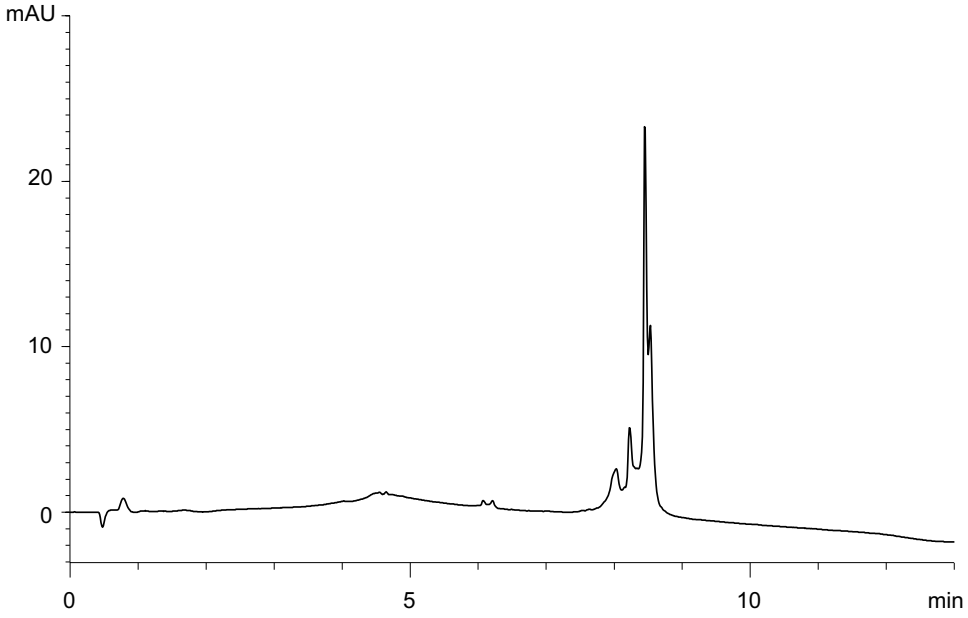


Column:	YMC-Accura Triart Bio C4 (3 µm, 30 nm) 100 x 2.1 mm ID	Temperature:	80 °C
Part number:	TB30S03-10Q1PTC	Detection:	UV at 254 nm
Eluent:	A) 50 mM TEAA* (pH 7.0) / acetonitrile (95/5)	Injection:	2 µL (0.25 mg/mL)
	B) acetonitrile	Sample:	CleanCap® EGFP mRNA (5 moU)
Gradient:	5–10%B (0–10 min)		(TriLink Bio Technologies)
Flow rate:	0.2 mL/min		

**triethylammonium acetate*

IEX analysis of EGFP mRNA (996nt)

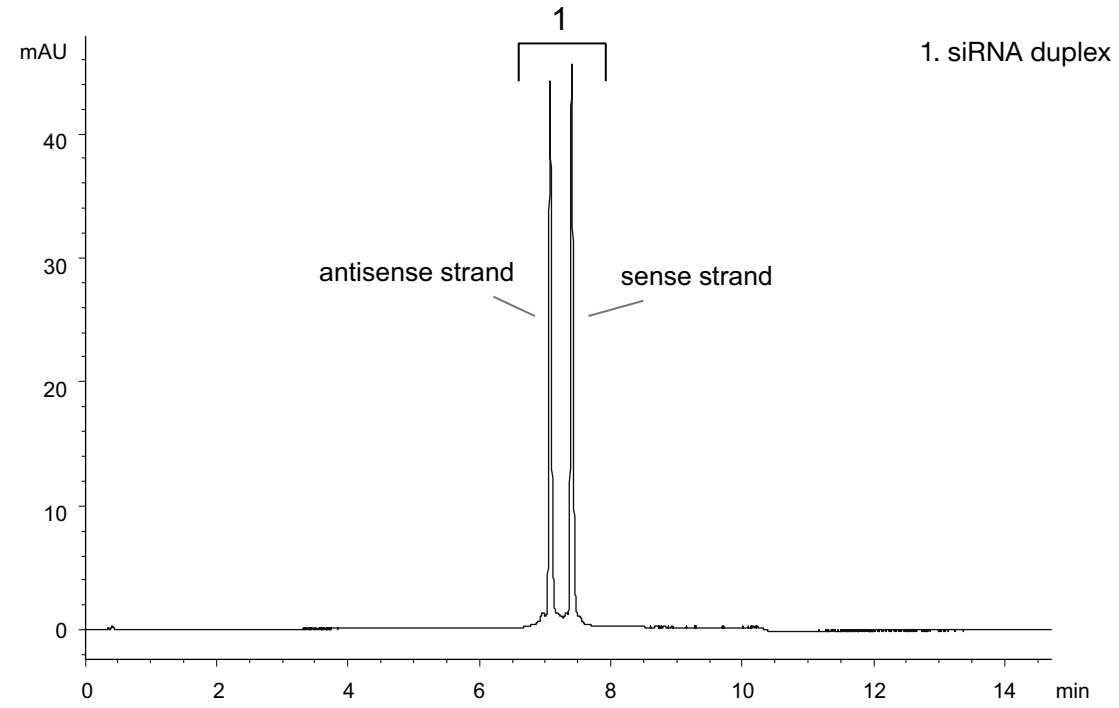
AEX



Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID	Temperature:	15 °C
Part number:	QF00S05-1046WP	Detection:	UV at 260 nm
Eluent:	A) 10 mM NaOH (pH 12)	Injection:	5 µL (0.025 mg/mL)
	B) 10 mM NaOH (pH 12) containing 2 M NaCl	Sample:	CleanCap® EGFP mRNA (5 moU)
Gradient:	0–100%B (0–9 min), 100%B (9–13 min)		(TriLink Bio Technologies)
Flow rate:	1.0 mL/min		

siRNA duplex under denaturing conditions

RP

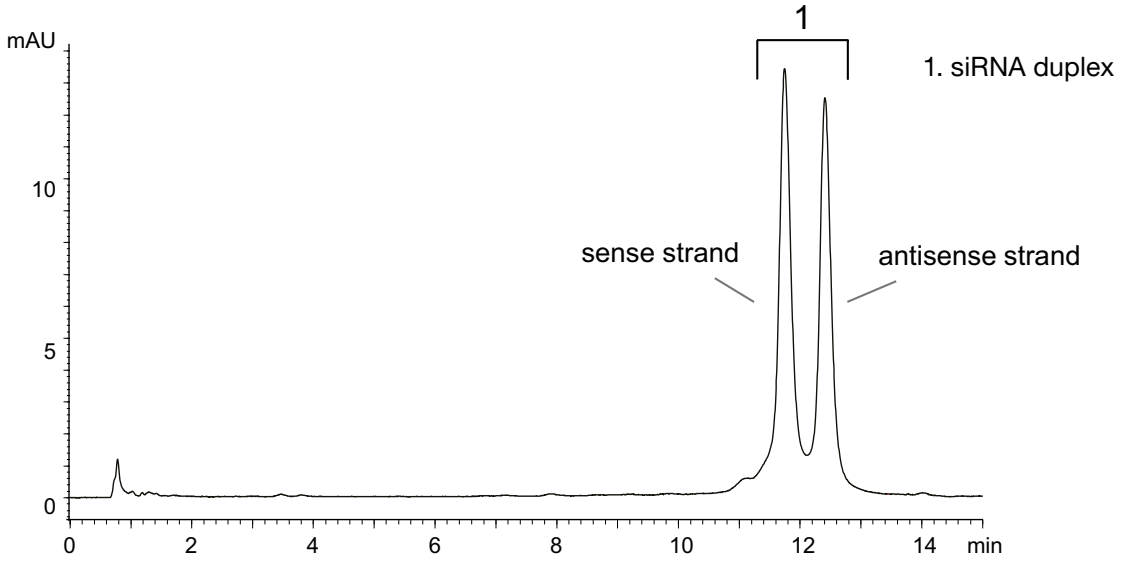


Column:	YMC-Accura Triart Bio C18 (1.9 µm, 30 nm) 50 x 2.1 mm ID	Flow rate:	0.42 ml/min
Part No.:	TA30SP9-05Q1PTC	Temperature:	65 °C
Eluent:	A) 15 mM TEAA* (pH 8)	Detection:	UV at 260 nm
	B) methanol	Injection:	1 µl (5 nmol/ml)
Gradient:	5%–20%B (0–15 min)	Sample:	siRNA duplex

*triethylammonium acetate

siRNA duplex under denaturing conditions

AEX



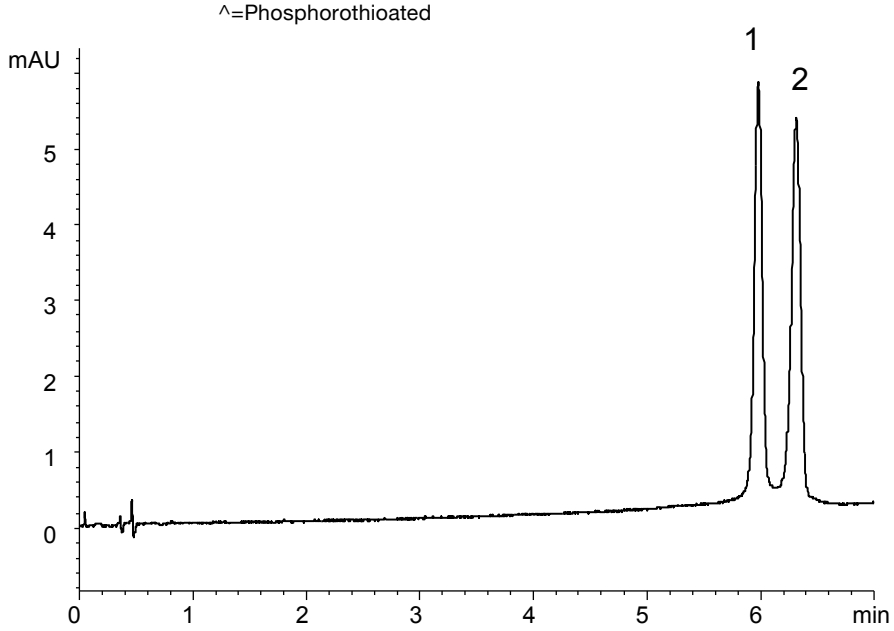
Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID	Flow rate:	1.0 ml/min
Part number:	QF00S05-1046WP	Temperature:	25 °C
Eluent:	A) 10 mM NaOH	Detection:	UV at 260 nm
	B) 10 mM NaOH containing 1 M $NaClO_4$	Injection:	4 µl (5 nmol/ml)
Gradient:	30%–37%B (0–15 min)	Sample:	siRNA duplex

Oligonucleotide applications

Challenging phosphorothioate oligonucleotides

RP

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

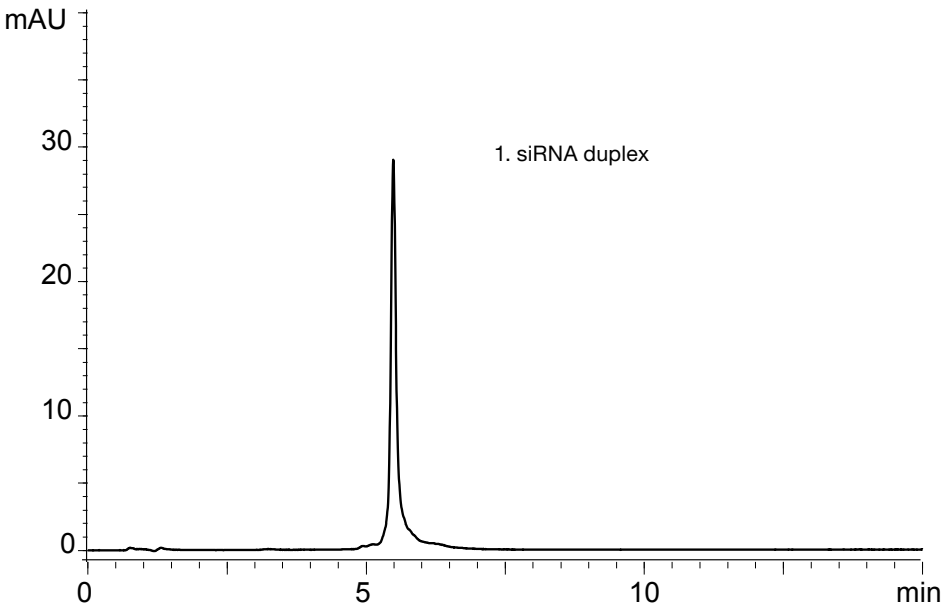


Column:	YMC-Accura Triart Bio C18 (1.9 µm, 30 nm) 50 x 2.1 mm ID	Flow rate:	0.42 mL/min
Part No.:	TA30SP9-05Q1PTC	Temperature:	65 °C
Eluent:	A) 15 mM triethylamine - 400 mM HFIP*	Detection:	UV at 260 nm
	B) methanol	Injection:	1 µL (each 1.0 nmol/mL)
Gradient:	10–20%B (0–10 min)		

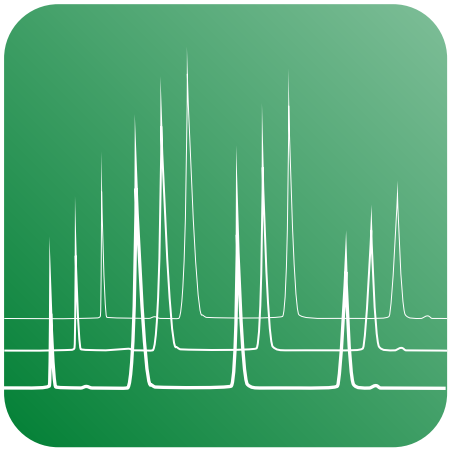
*1,1,1,3,3,3-hexafluoro-2-propanol

AEX analysis of siRNA duplex under non-denaturing conditions

AEX



Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID	Flow rate:	1.0 ml/min
Part number:	QF00S05-1046WP	Temperature:	25 °C
Eluent:	A) 20 mM Tris-HCl (pH 8.1)	Detection:	UV at 260 nm
	B) 20 mM Tris-HCl (pH 8.1) containing 1 M $NaClO_4$	Injection:	4 µl (5 nmol/ml)
Gradient:	25%–40%B (0–15 min)	Sample:	siRNA duplex



IP-RP

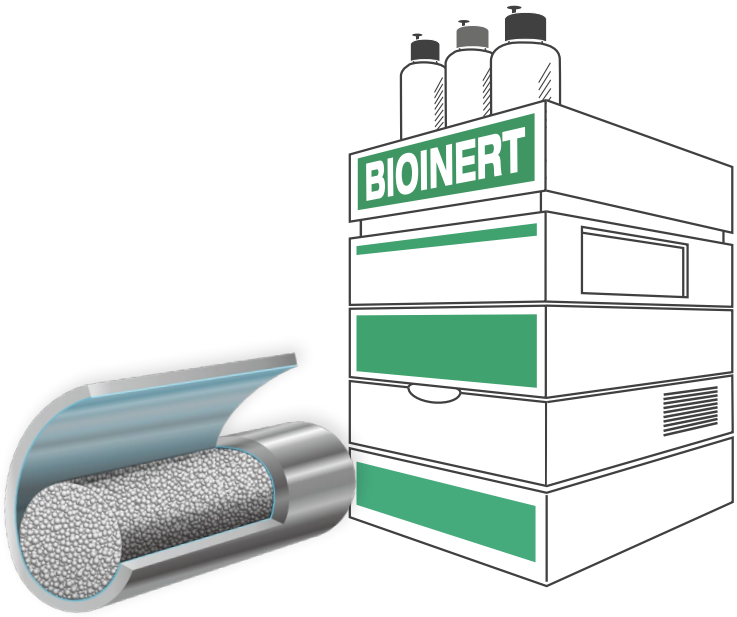
IP-RP – UHPLC/HPLC Selectivities

Features

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

Selectivities for oligonucleotides

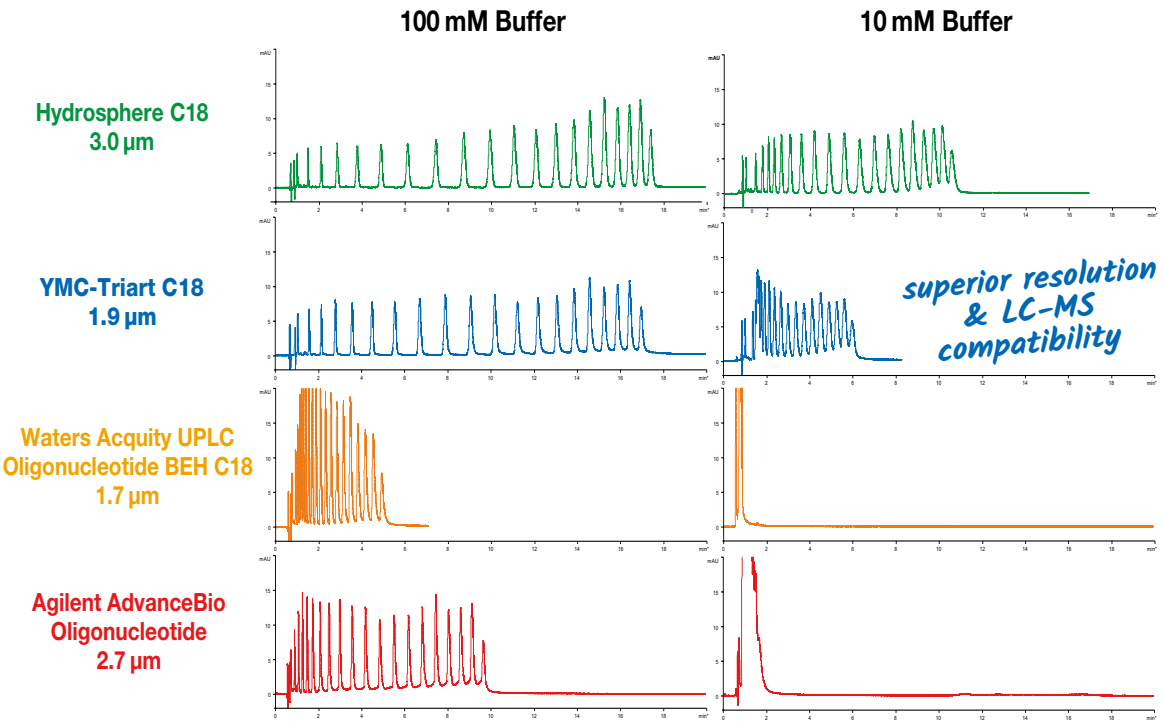
	YMC-Triart C18	YMC-Triart Bio C18	YMC-Triart C8	YMC-Triart Bio C4	Hydrosphere C18
Base particle	organic/inorganic hybrid silica				silica
Modification	C18 (USP L1)	C18 (USP L1)	C8 (USP L7)	C4 (USP L26)	C18 (USP L1)
Particle Size / µm	1.9, 3, 5	1.9, 3, 5	1.9, 3, 5	1.9, 3, 5	2, 3, 5
Pore Size / nm	12	30	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



Bioinert hardware available!

Bioinert YMC-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

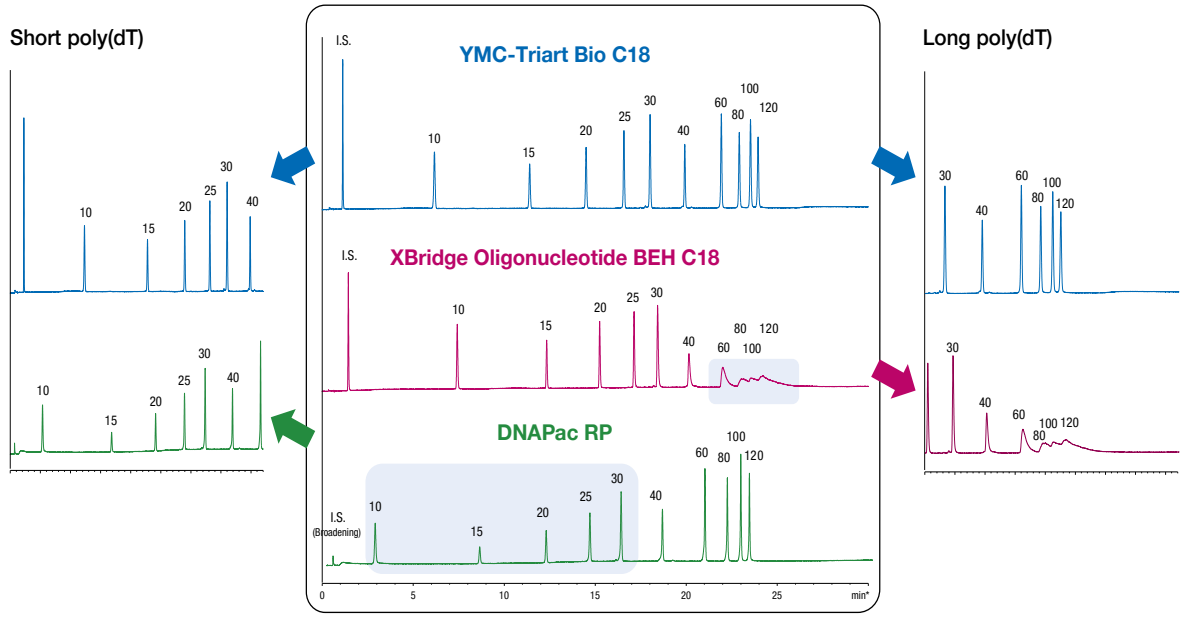


Column:	50 x 2.1 mm ID
Eluent:	A) 100 mM or 10 mM triethylamine-acetic acid (pH 6)
	B) A/acetonitrile (80/20)
Gradient:	50–65%B (0–20 min)
Flow rate:	0.21 mL/min
Temperature:	35 °C
Detection:	UV at 260 nm
Injection:	2 µL (5 nmol/mL)
Sample:	Oligodeoxythymidylic acid [d(pT)2-20]

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



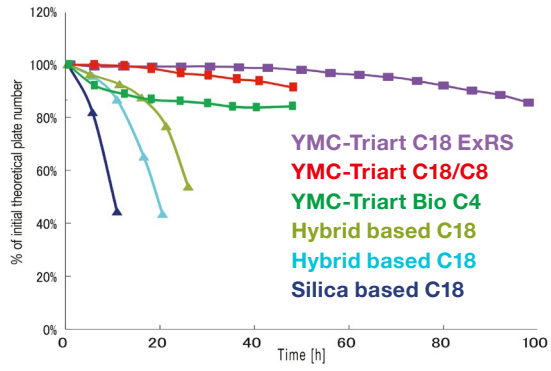
Columns:	YMC-Triart Bio C18 (3 µm, 30 nm) 50 x 2.1 mm ID XBridge Oligonucleotide BEH C18 (2.5 µm, 13 nm) 50 x 2.1 mm ID DNAPac RP (4 µm, proprietary) 50 x 2.1 mm ID
Part No.:	TA30S03-05Q1PTH
Eluent:	A) 4 mM TEA* - 100 mM HFIP** B) methanol
Gradient:	0.5%B/min, initial %B=5
Flow rate:	0.42 mL/min
Detection: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

**trietylamine, **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.

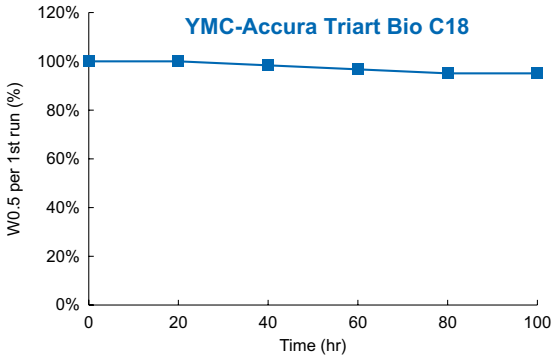
Versatile wide pH and temperature stability

Phosphate buffer (pH 11.5, 40 °C)



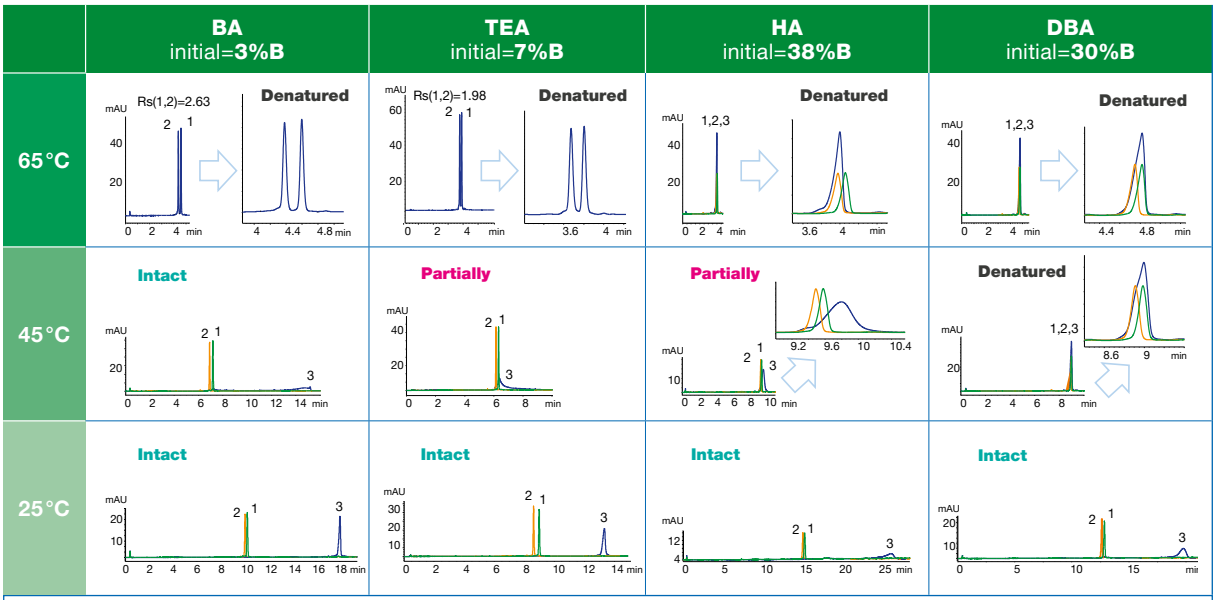
Column: 5 µm, 150 x 4.6 mm ID
Eluent: 50 mM K_2HPO_4-K_3PO_4 (pH 11.5)/methanol (90/10)
Flow rate: 1.0 mL/min
Temperature: 40°C
Sample: benzyl alcohol

15 mM TEA - 400 mM HFIP (pH 8, 90 °C)



Column: YMC-Accura Triart Bio C18 (1.9 µm, 30 nm), 50 x 2.1 mm ID
Eluent: A) 15 mM TEA-400 mM HFIP (pH8)
B) methanol
Gradient: 6–16%B (0–10 min)
Flowrate: 0.42 mL/min
Temperature: 90 °C
Sample: 20mer RNA

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



Column: YMC-Accura Triart Bio C18 (1.9 µm, 30 nm), 50 x 2.1 mm ID
Part No.: TA30SP9-05Q1PTC
Eluent: A) 8 mM alkylamine - 100 mM HFIP* (pH 8)
B) methanol
Gradient: 1%B/min
Flow rate: 0.42 mL/min
Temperature: 65 °C, 45 °C or 25 °C
Detection: UV at 260 nm
Injection: 1 µl (5 nmol/mL)

1. sense strand
2. antisense strand
3. siRNA duplex

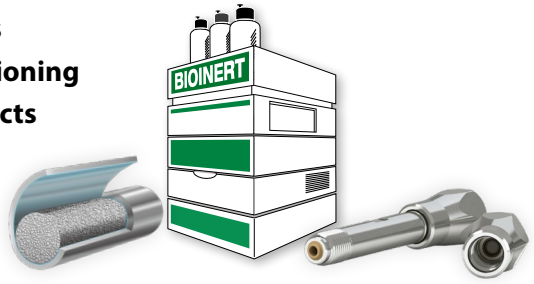
Sample: siRNA duplex, sense strand, antisense strand
5'-CGU ACG CGG AAU ACU UCG AdTdT-3' sense strand
3'-dTdTGCA UGC GCC UUA UGA AGC U-5' antisense strand

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

IP-RP – YMC-Triart: Bioinert hardware

Bioinert columns for bioseparations and coordinating compounds

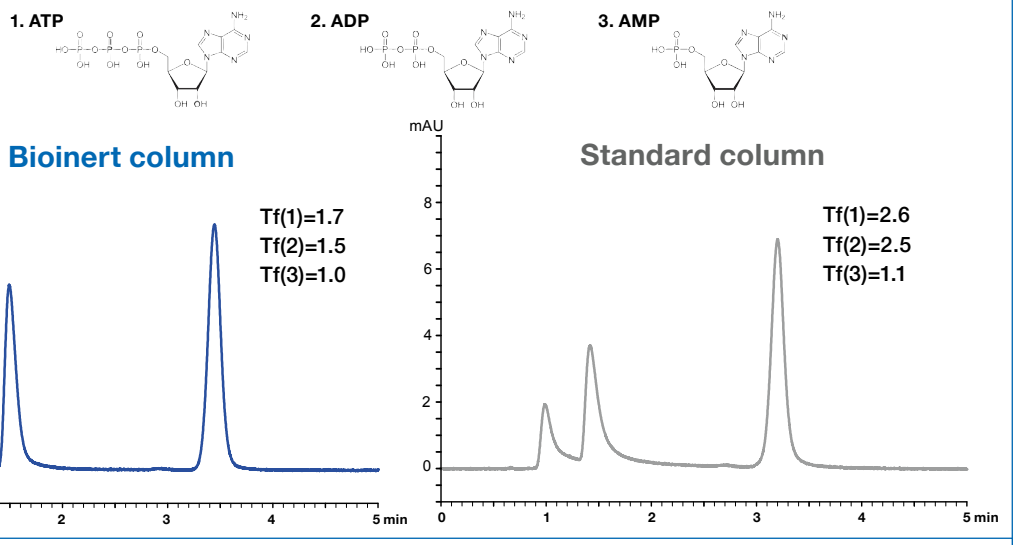
- **Exceptional peak shapes with high sensitivities**
- **Excellent recoveries without column preconditioning**
- **Superior reproducibility and no carry-over effects**
- **Ideal for highly sensitive LC/MS analyses**
- **Different bioinert hardware options**



Specifications

	YMC-Accura Triart	YMC-Triart metal-free PEEK-lined
YMC-Triart modifications	C18, C18 ExRS, Bio C18, C8, Bio C4, Phenyl, PFP, Diol-HILIC	
Particle Size	1.9, 3 and 5 µm	
Column hardware	Stainless steel with bioinert coating	PEEK-lined stainless steel
Frit hardware	Stainless steel with bioinert coating	PEEK
Hardware properties	Less hydrophobic	More hydrophobic
Pressure limit	1.9 µm: 100 MPa (15,000 psi) 3/5 µm: 45 MPa (6,525 psi)	
Column connection	No special connections required	Selected universal connectors such as MarvelXACT™

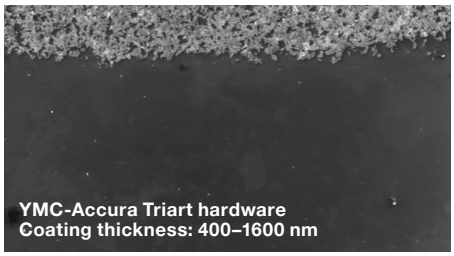
Improved sensitivity for coordination compounds



Column: YMC-Triart C18 (3 µm, 12 nm) 50 x 2.1 mm ID
Part Nos.: TA12S03-05Q1PTP (metal-free PEEK-lined) or TA12S03-05Q1PTH (standard hardware)
Eluent: 5 mM $HCOONH_4$
Flow rate: 0.21 mL/min
Temperature: 25 °C
Detection: UV at 265 nm
Injection: 1 µL (10 µg/mL)
System: bioinert/"metal-free" HPLC system

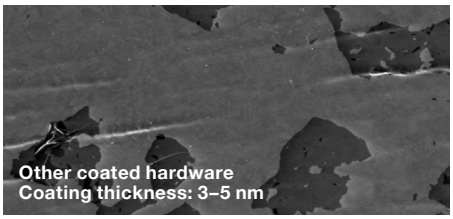
Metal coordinating compounds, which have a phosphate group in their structure, tend to show poor peak shape due to interactions with metals, such as the stainless steel in column bodies and frits. By using a bioinert column hardware, better peak shapes can be expected.
Nucleotides with phosphate groups also show better peak shapes when compared to the regular column hardware. The applied YMC-Triart metal-free PEEK-lined as well as the YMC-Accura Triart column hardware are ideal for highly sensitive analyses using LC/MS.

YMC-Accura Triart: durable bioinert coating

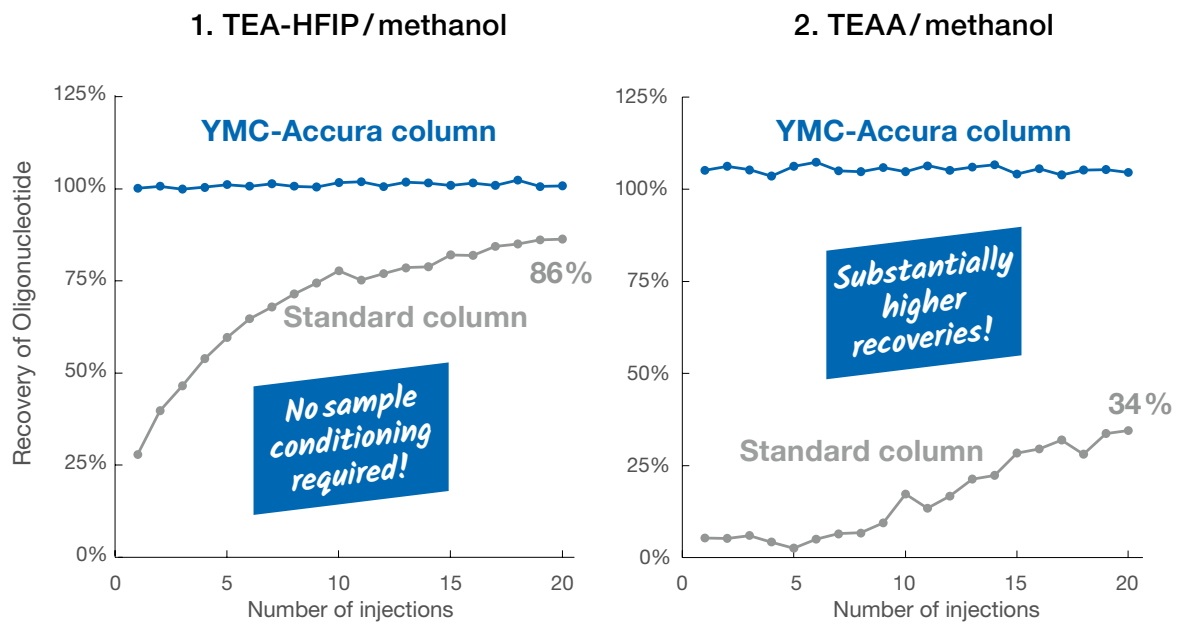


The robust bioinert coating used on YMC-Accura hardware is 130 to 320-fold thicker, making it more durable than other similar hardware concepts. A long-term inertness against sensitive substances is ensured. In order to demonstrate its robustness, a YMC-Accura column was packed multiple times. Even though 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



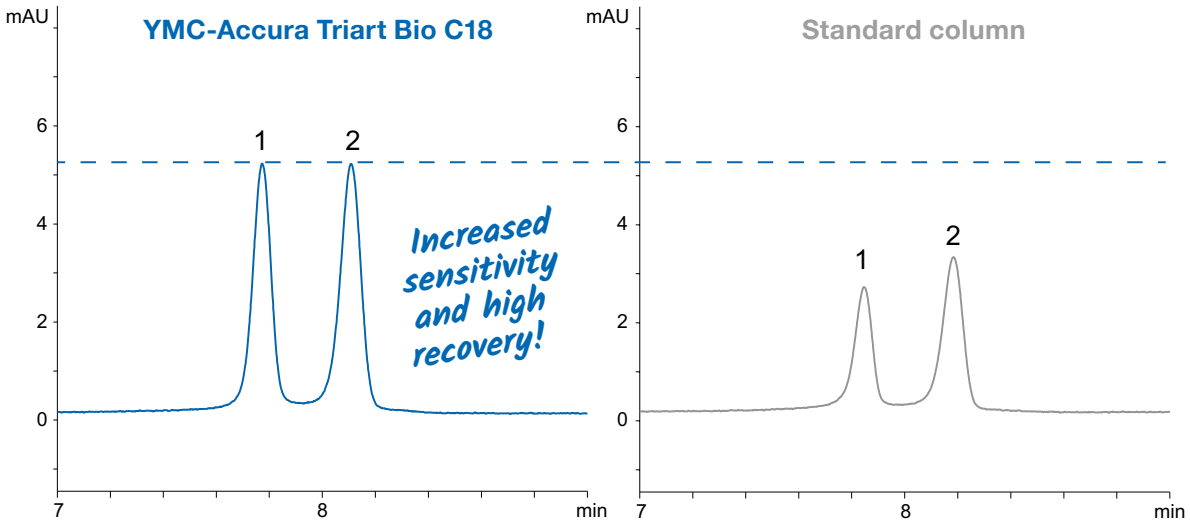
Column:	Empty YMC-Accura (without stationary phase)
Eluent:	1) 8 mM TEA 200 mM HFIP / methanol (82/18) 2) 100 mM TEAA / methanol (82/18)
Flow rate:	0.42 mL/min
Detection:	UV at 260 nm
Temperature:	65 °C
Injection:	1 µL
Sample:	All PS RNA 20mer (1) (5'-U^C^A^U^C^A^C^A^C^U^G^A^A^U^A^C^C^A^A^U-3') ^=Phosphorothioate

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.

IP-RP – YMC-Triart: Bioinert hardware

Significantly higher sensitivity and recovery

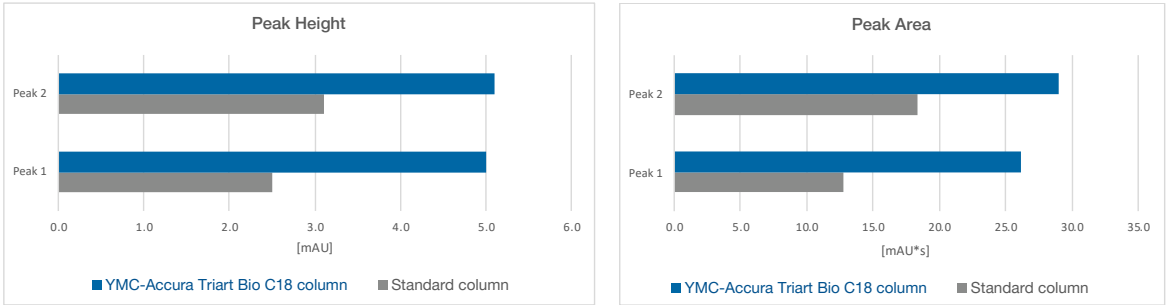
Ideal choice for challenging analytes such as phosphorothioate oligonucleotides



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

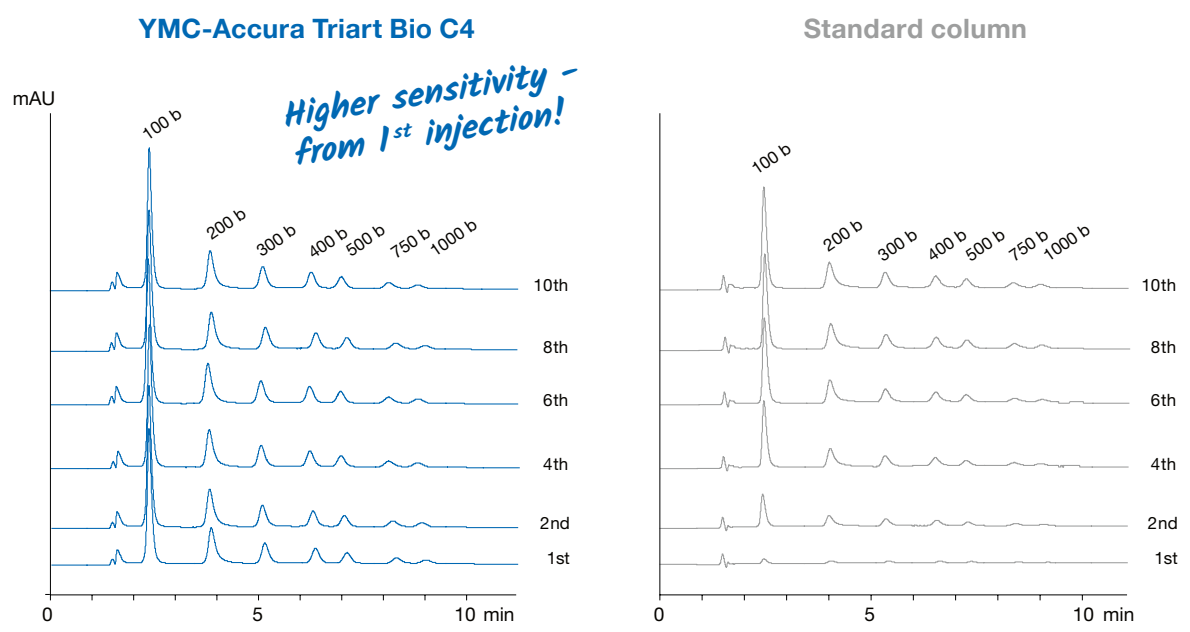


Doubled peak height and area!

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.

Reliable results from the first injection

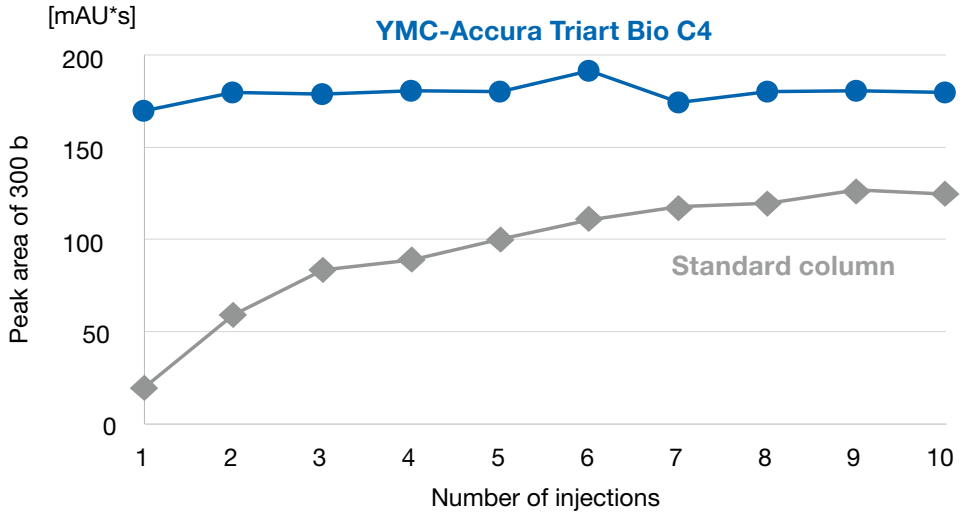
No preconditioning required for reliable results



Column:	YMC-Accura Triart Bio C4 (3 µm, 30 nm) 100 x 2.1 mm ID	Flow rate:	0.2 mL/min
Part No.:	TA30S03-10Q1PTC	Temperature:	80°C
Eluent:	A) 50 mM TEAA* (pH 7.0)/acetonitrile (95/5)	Detection:	UV at 254 nm
	B) 50 mM TEAA (pH 7.0)/acetonitrile (50/50)	Injection:	1 µL (0.25 mg/mL)
Gradient:	9–14%B (0–10 min), 80%B (10–15 min)	Sample:	100–1,000 bases (Century™-Plus RNA Markers)

* Triethylammonium acetate

Constantly higher peak areas and therefore recoveries

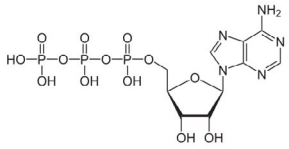


The YMC-Accura Triart Bio C4 column shows stable peak areas from the first injection, while the standard stainless-steel column provides only 10% of the peak area (for the 300 base marker) with the first injection. Even after the tenth injection, the peak areas of the stainless-steel column are considerably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

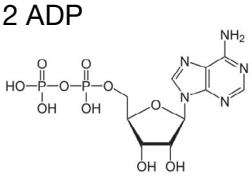
IP-RP – Expert Tips: (Oligo)nucleotides

Influence of system and column hardware on the analysis of nucleotides

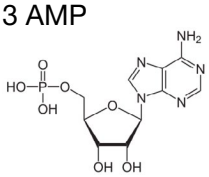
1 ATP



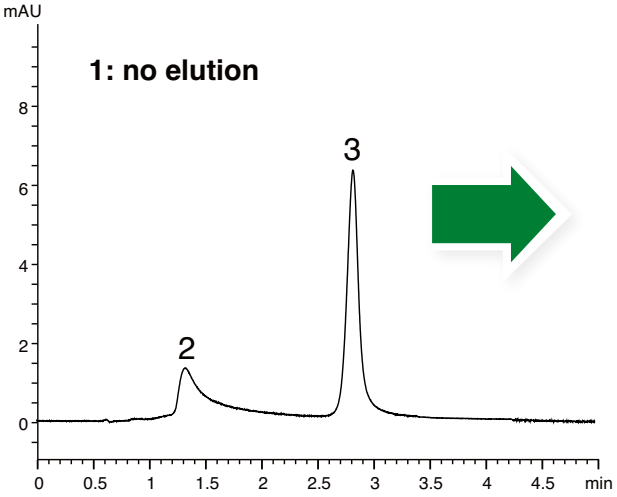
2 ADP



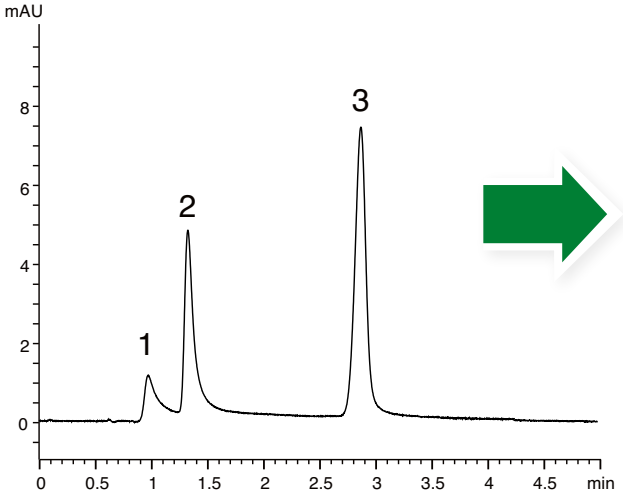
3 AMP

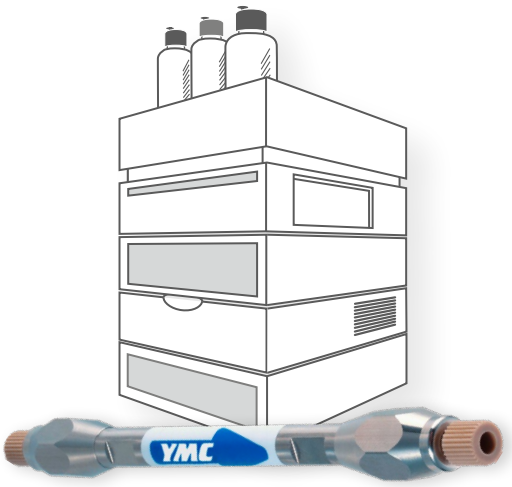


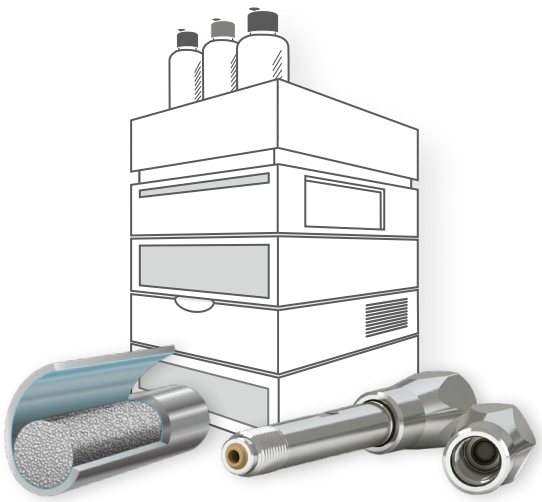
Ordinary HPLC system with standard column



Ordinary HPLC system with bioinert column





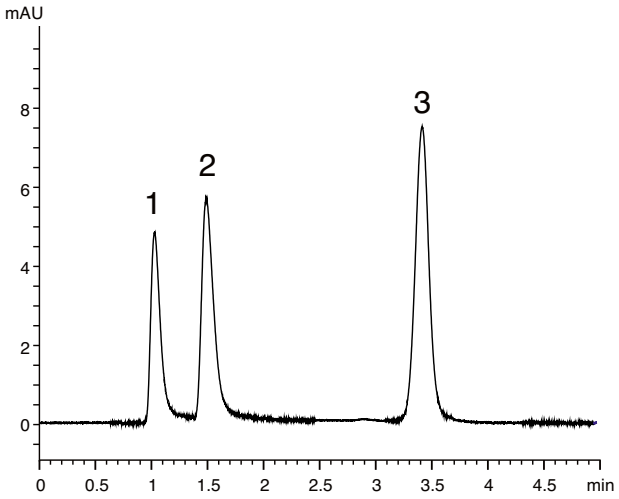


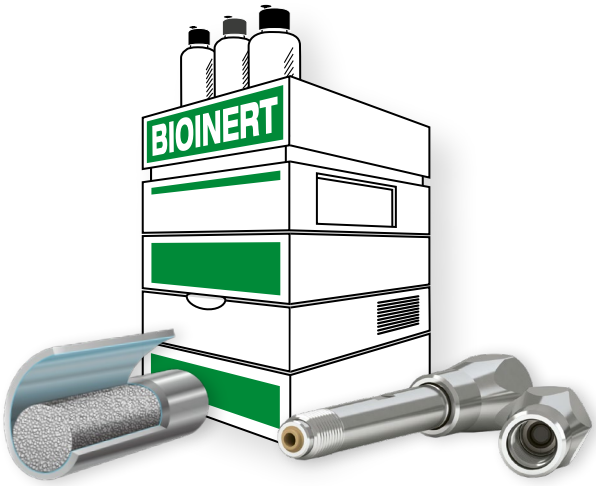
Column: YMC-Triart C18 (3 µm, 12 nm) 50 x 2.1 mm ID
Part Nos: TA12S03-05Q1PT (standard hardware)
TA12S03-05Q1PTP (**bioinert** hardware)
Eluent: 5 mM $HCOONH_4$
Flow rate: 0.21 mL/min
Temperature: 25°C
Detection: UV at 265 nm
Injection: 1 µL (10 µg/mL)

*Bioinert HPLC system: PEEK sample loop, PEEK injector port, and PEEK tubing are used.

ATP peak is detected, and peak shape of ADP is improved as a result of using a bioinert column.

Bioinert HPLC system* with bioinert column





"Metal-free PEEK-lined YMC columns significantly reduce non-specific adsorption phenomena"

"YMC-Triart C18 metal-free PEEK-lined columns significantly reduce non-specific adsorption phenomena during peptides analysis. We use these columns in our laboratory for a specific application. We obtain very good chromatographic resolution and excellent robustness, which is very appreciable during routine analysis."

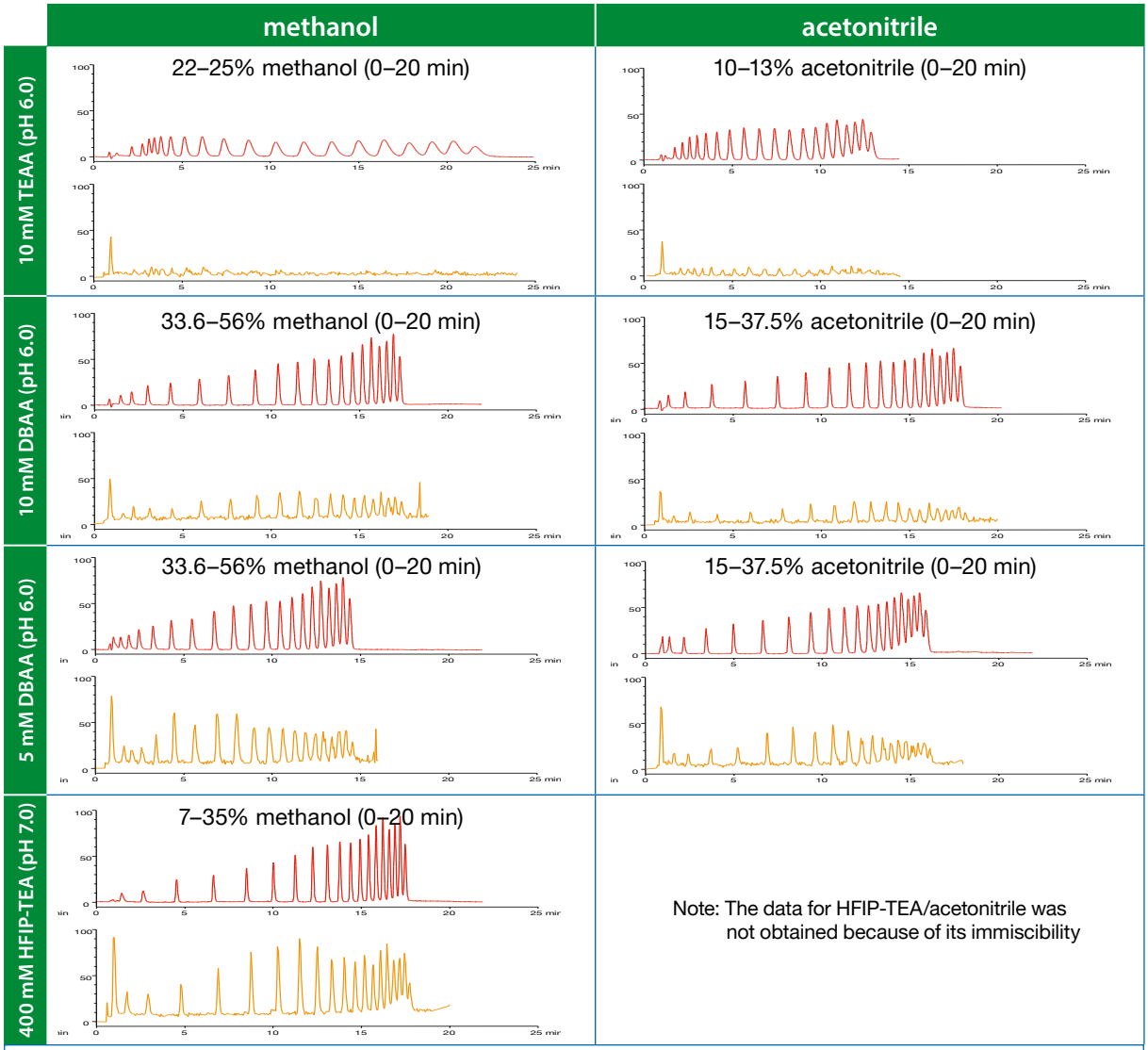
Cynthia Mongongu, LADF,
Laboratoire AntiDopage Français,
Université Paris-Saclay (FR)

Peak shape is greatly improved as a result of using a bioinert HPLC system.

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



Column:	Hydrosphere C18 (3.0 µm, 12 nm) 50 x 2.0 mm ID
Part No.:	HS12S03-0502WT
Flow rate:	0.2 mL/min
Temperature:	35 °C
Detection:	UV at 269 nm (red), ESI negative mode (orange)
Injection:	5 µl (25 pmol/mL)
Sample:	Oligo(deoxythymidylic acid) [dp (dT) 2-20]

eluent abbreviations

TEAA:	triethylammonium acetate
DBAA:	di-n-butylammonium acetate
HFIP:	1,1,1,3,3,3-hexafluoro-2-propanol
TEA:	triethylamine

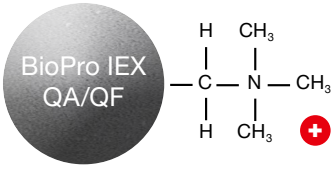
The mobile phase composition has different effects on the separation and signal intensity in electrospray ionisation mass spectrometry (ESI-MS) of oligonucleotides. Using different gradient conditions, acceptable retention and resolution can be achieved (upper UV chromatograms; red trace) for each separation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

AEX

AEX – HPLC Selectivities

Features

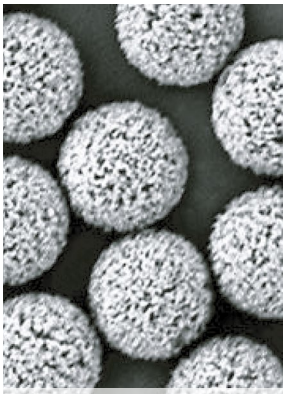
- **Porous or non-porous hydrophilic polymers**
- **High recovery of oligonucleotides**
- **Very high resolution**
- **Low nonspecific adsorption**
- **Excellent reproducibility**



strong anion exchanger

	BioPro IEX QA
Matrix	hydrophilic polymer (polymethacrylate)
Particle size / µm	5
Pore size / nm	100
Charged group	$-CH_2N^+(CH_3)_3$
Counter ion	Cl^-
Available pH range	2.0–12.0
Temperature range	4–60°C
Pressure limit	2.5–3.5 MPa (360–510 psi)
Column hardware	PEEK

Also available in 10, 20, 30 or 75 µm for preparative scale



Porous polymer beads

	BioPro IEX QF
Matrix	hydrophilic polymer (polymethacrylate)
Particle size / µm	3, 5
Pore size / nm	non-porous
Charged group	$-CH_2N^+(CH_3)_3$
Counter ion	Cl^-
Available pH range	2.0–12.0
Temperature range	4–60°C
Pressure limit	3 µm: 25 MPa (3,625 psi) 5 µm: 6–12 MPa (870–1,740 psi)
Column hardware	PEEK

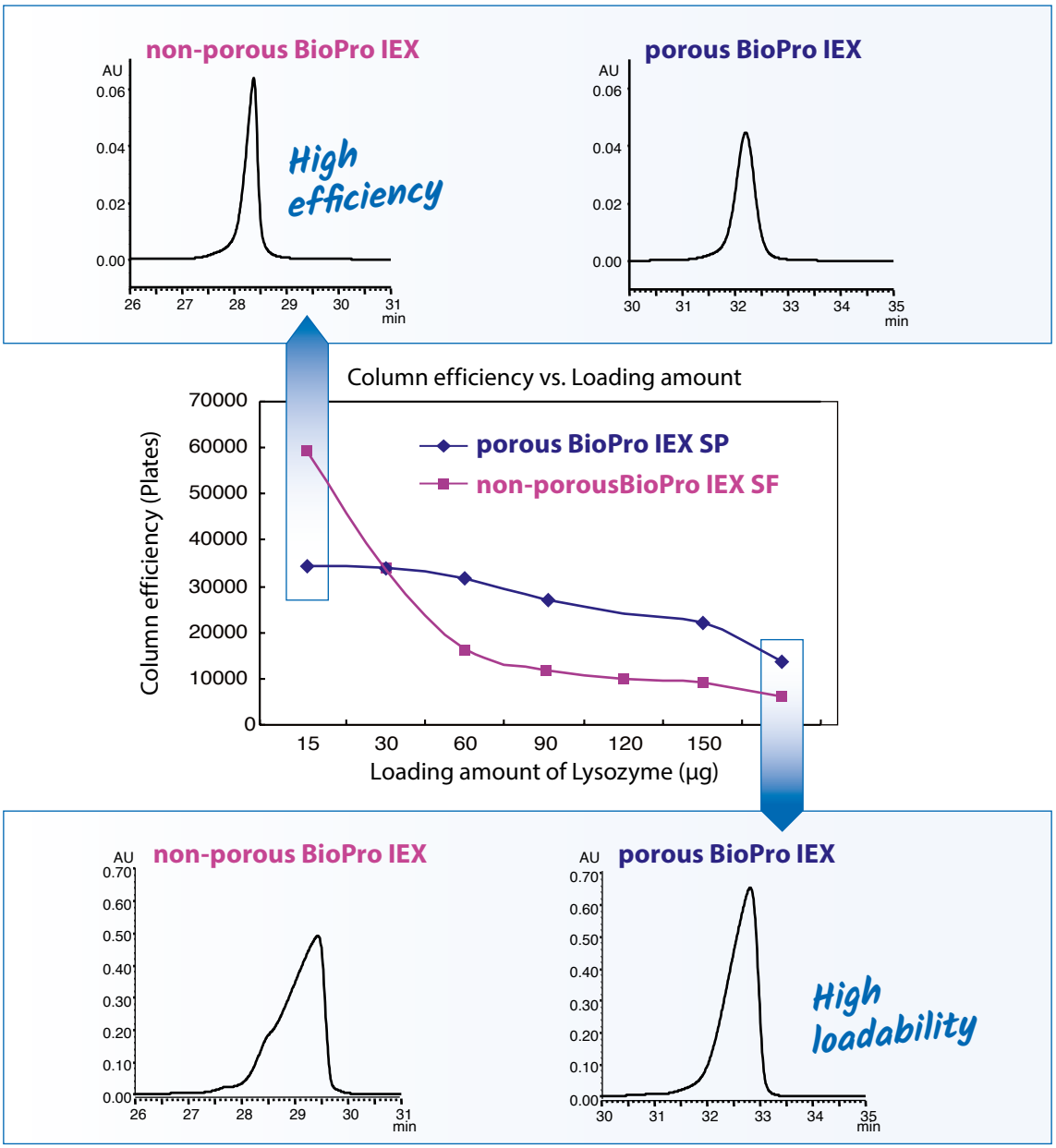
Non-porous polymer beads

YMC´s anion exchanger (AEX) columns of the BioPro IEX series are available with strong exchanger modification, based on 5 µm porous (QA columns) and on 3 or 5 µm non-porous (QF columns) hydrophilic polymer beads.

The porous materials offer excellent binding capacity with exceptionally high efficiency and low operating pressure, whilst the non-porous particles offer high efficiency, very high resolution and low operating pressures.

Column efficiency and loadability

When to use porous and non-porous BioPro IEX

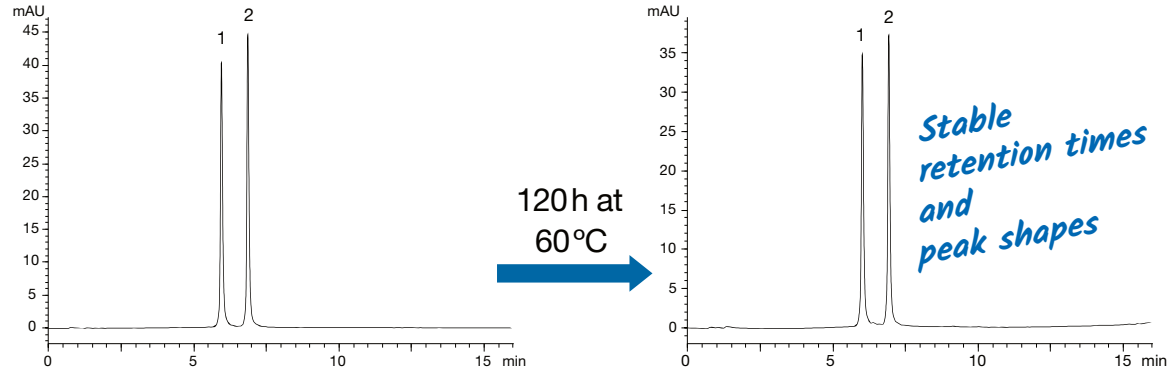


Column:	BioPro IEX SF/SP	Detection:	UV at 280 nm
Eluent:	A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8)	Injection:	100 µL
	B) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8) containing 0.5 M NaCl	Sample:	1. Ribonuclease A
Gradient:	0–100%B (0–60 min)		2. Cytochrome c
Flow rate:	0.5 mL/min		3. Lysozyme
Temperature:	25°C		

Non-porous BioPro IEX columns offer outstanding column efficiency for small sample loading amounts. These columns are especially suitable for microscale analysis, which requires higher resolution. Porous BioPro IEX columns maintain good peak shape even when the loading amount increases. These high-capacity columns are useful for high-load analytical separations and laboratory-scale purification.

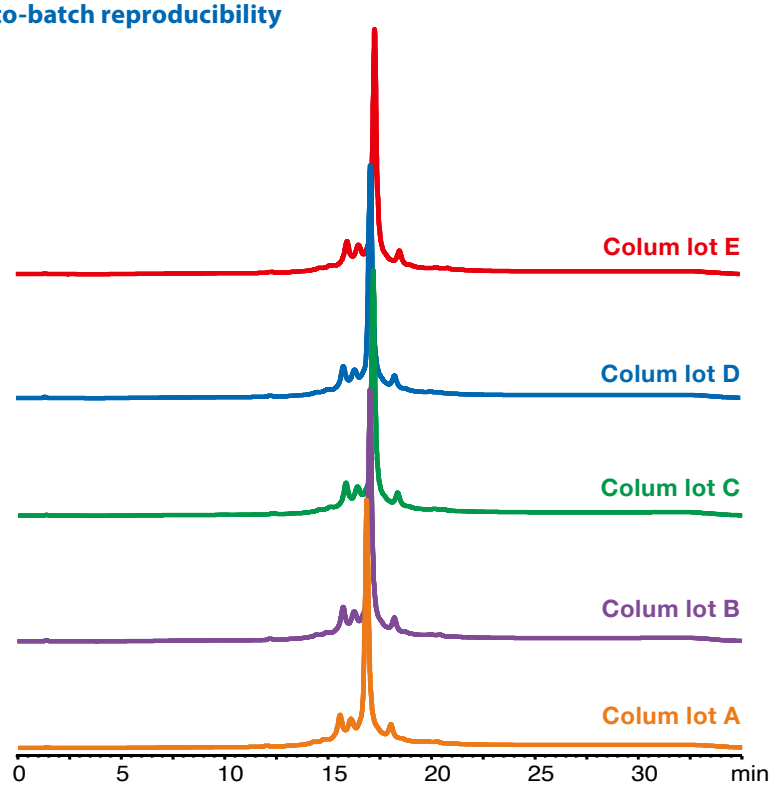
AEX – Stability and Reproducibility

High temperature stability of BioPro IEX columns



Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID	Temperature:	25 °C
Part No.:	QF00S05-1046WP	Detection:	UV at 260 nm
Eluent:	A) 10 mM NaOH	Injection:	4 µl (each 5 nmol/ml)
	B) 10 mM NaOH containing 1.0 M $NaClO_4$	Sample:	1) 5'-TCATCACACTGAATACCAAT-3' (DNA 20mer)
Gradient:	25–55%B (0–15 min), 100%B (15–20 min)		2) 5'-GTCATCACACTGAATACCAAT-3' (DNA 21mer)
Flow rate:	1.0 ml/min		

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_2PO_4-Na_2HPO_4 (pH 6.5)	Detection:	UV at 215 nm
	B) 20 mM NaH_2PO_4-Na_2HPO_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.

Optimisation of oligonucleotide separations on ion exchange chromatography

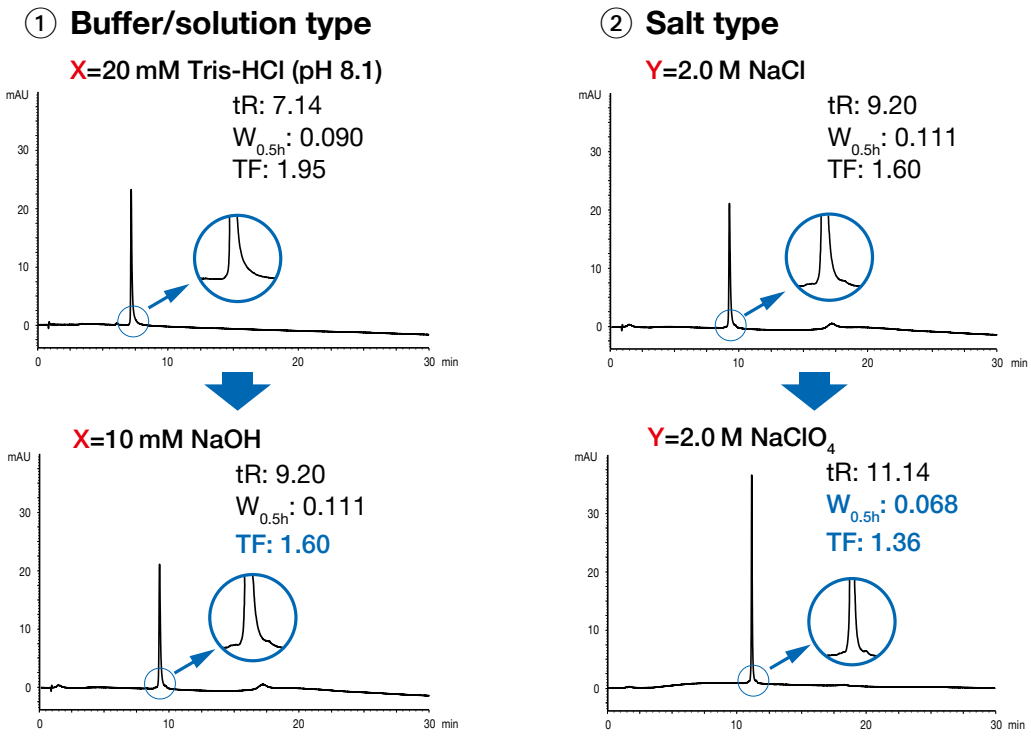
A non-porous anion exchange column is generally suitable for analysis of oligonucleotides. For optimisation of single-stranded DNA and RNA of about 20mer, some conditions, such as type of mobile phase and column temperature, can be changed.

1 Improvement of peak tailing

Sample Group 1 (Phosphodiester oligonucleotides; PO)

Single-stranded RNA (ssRNA)	5′-UCAUCACACUGAAUACCAAU-3′ (RNA 20mer)

By changing the buffer from 20 mM Tris-HCl (pH 8.1) to 10 mM NaOH, the tailing factor for an oligonucleotide is reduced. Furthermore, the peak tailing is further suppressed when $NaClO_4$ was added to 10 mM NaOH instead of NaCl.



Eluent: A) X
B) X containing 2.0 M NaCl
Gradient: 15–100%B (0–30 min)

Eluent: A) 10 mM NaOH
B) 10 mM NaOH containing 2.0 M Y
Gradient: 15–100%B (0–30 min) for NaCl
5–50%B (0-30 min) for $NaClO_4$

Column: BioPro IEX QF (5 µm) 100 x 4.6 mm ID
Part No.: QF00S05-1046WP
Flow rate: 1.0 mL/min
Eluent: A) 20 mM Tris-HCl (pH 8.1) or 10 mM NaOH
B) 20 mM Tris HCl (pH 8.1) or 10 mM NaOH containing 2.0 M NaCl or $NaClO_4$

Gradient: 15–100%B (0–30 min) for NaCl
5–50%B (0–30 min) for $NaClO_4$
Temperatur: 25 °C
Detection: UV at 260 nm
Injection: 2 µL (10 nmol/mL)
Sample: RNA 20mer

2 Improvement of carry-over

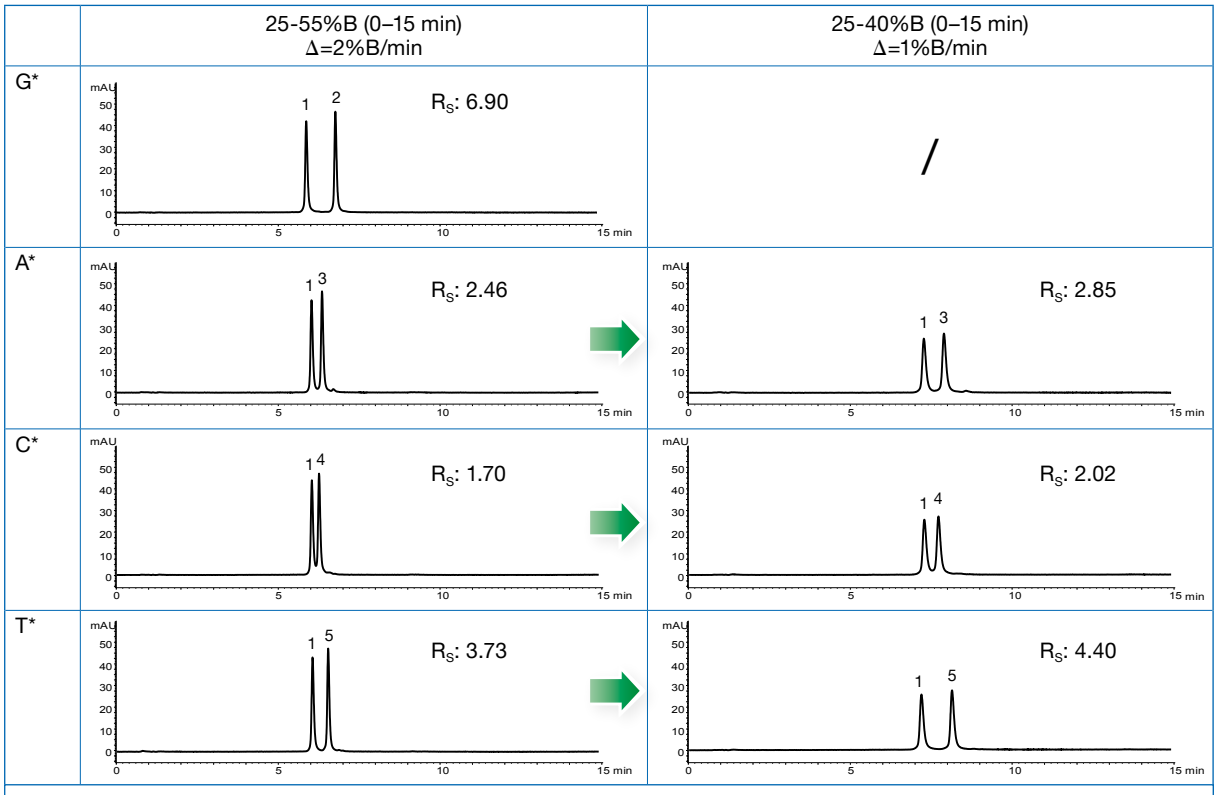
When the initial gradient concentration of NaCl is low (ex. 50 mM), carry-over is observed. By increasing the initial gradient concentration of NaCl up to 400 mM, carry-over can be avoided with good reproducibility.

AEX – Expert Tips: Oligonucleotides

3 Improvement of ssDNA separation with single-base differences (differing in the type of base at the 5′ end of DNA 21mer)

When ssDNAs with single-base differences are analysed, the degree of separation varies depending on the type of base at the 5′ end. If the separation is difficult, it can be improved by making the gradient steeper.

1	Single-stranded DNA	5′-TCATCACACTGAATACCAAT-3′ (DNA 20mer)
2		5′-GTCATCACACTGAATACCAAT-3′ (DNA 21mer)
3		5′-ATCATCACACTGAATACCAAT-3′ (DNA 21mer)
4		5′-CTCATCACACTGAATACCAAT-3′ (DNA 21mer)
5		5′-TTCATCACACTGAATACCAAT-3′ (DNA 21mer)



Column: BioPro IEX QF (5 µm) 100 x 4.6 mm ID
Part No.: QF00S05-1046WP
Eluent: A) 10 mM NaOH
B) 10 mM NaOH containing 1.0 M $NaClO_4$
Flow rate: 1.0 mL/min
Temperature: 25 °C
Detection: UV at 260 nm
Injection: 4 µL (5 nmol/mL each)

*base of 5′end of DNA 21mer

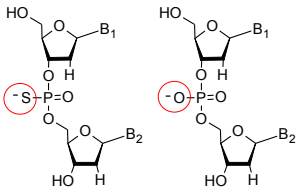
4 Improvement of the separation of phopsphorothioate oligonucleotides with single-base differences in length

Since acidity of all PS is much higher than all PO, a higher salt concentration is required for elution. The peak of all PS is much broader because it is thought that all PS contains as many as 219 (524,288) stereoisomers. A steeper gradient curve, increasing column temperature and adding organic solvent can improve peak separation.

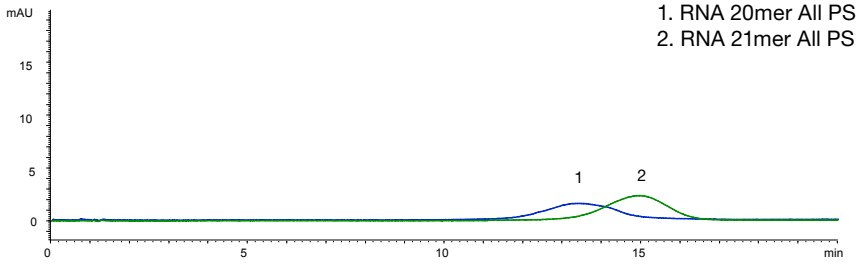
Sample Group 2 (Phosphorothioate oligonucleotides; PS)

1	Single-stranded RNA	5′-U~C~A~U~C~A~C~A~C~U~G~A~A~U~A~C~C~A~A~U-3′ (RNA 20mer All PS)
2		5′-G~U~C~A~U~C~A~C~A~C~U~G~A~A~U~A~C~C~A~A~U-3′ (RNA 21mer All PS)

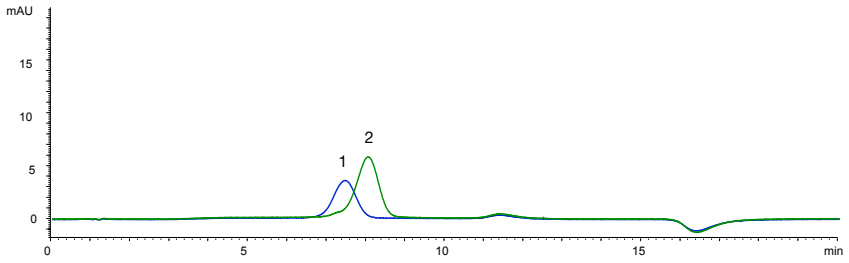
~=Phosphorothioated



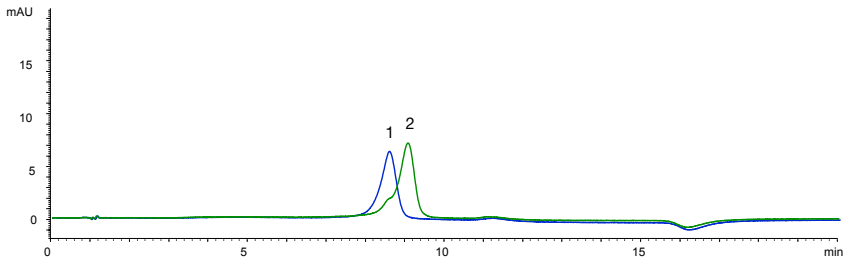
X/Y=100/0
32–80%B (0–24 min)
= Δ20 mM $NaClO_4$/min
25°C



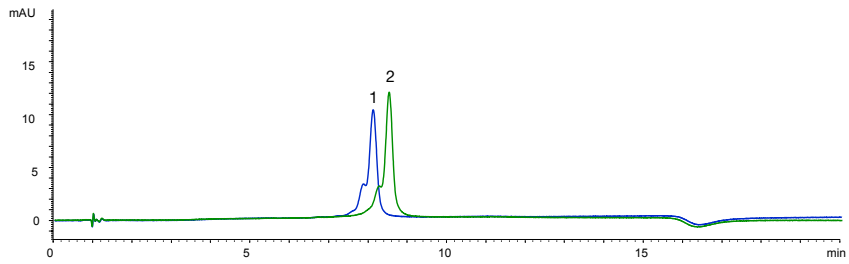
Steep gradient curve
X/Y=100/0
32–80%B (0–8 min)
= Δ60 mM $NaClO_4$/min
25°C



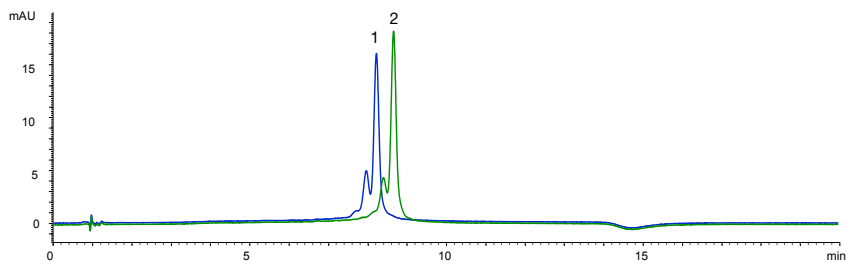
Raising column temperature
X/Y=100/0
32–80%B (0–8 min)
=Δ60 mM $NaClO_4$/min
60 °C



Addition of organic solvent
X/Y=80/20
40–100%B (0–8 min)
= Δ60 mM $NaClO_4$/min
60 °C



Increasing ratio of organic solvent
X/Y=70/30
40–100%B (0–6.3 min)
= Δ60 mM $NaClO_4$/min
60 °C

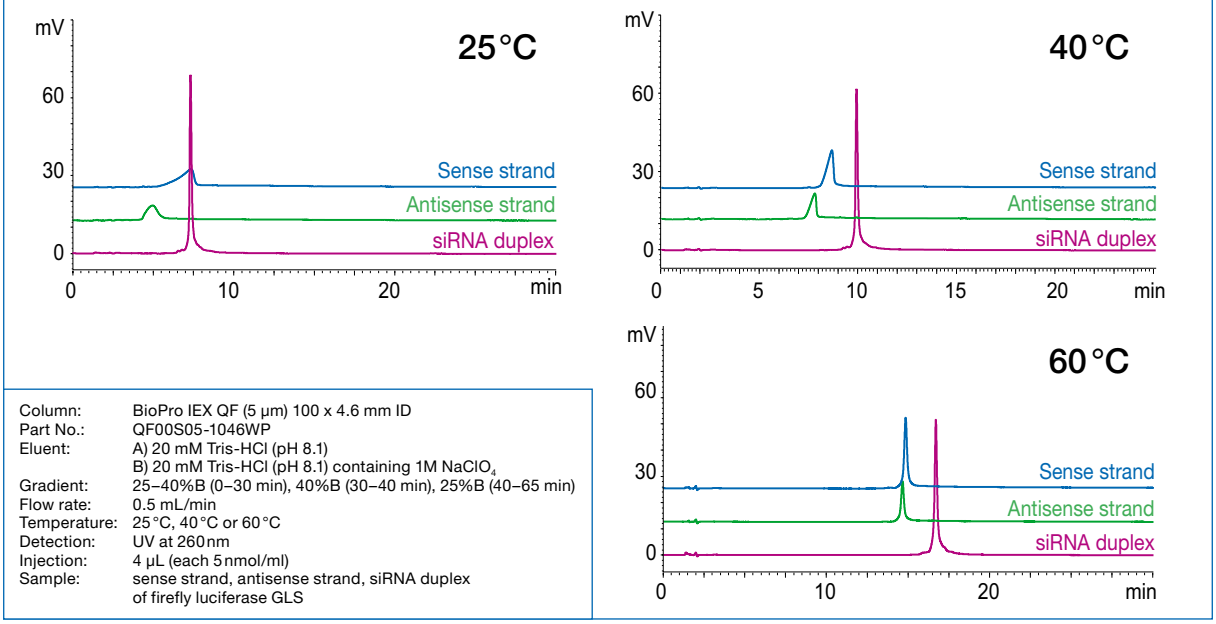


Column: BioPro IEX QF (5 µm) 100 x 4.6 mm ID
Part No.: QF00S05-1046WP
Eluent: A) 10 mM NaOH/methanol (X/Y)
B) 10 mM NaOH containing 1.0 M $NaClO_4$/methanol (X/Y)

Flow rate: 1.0 mL/min
Detection: UV at 260 nm
Injection: 2 µL (10 nmol/mL)

AEX – Expert Tips: Oligonucleotides

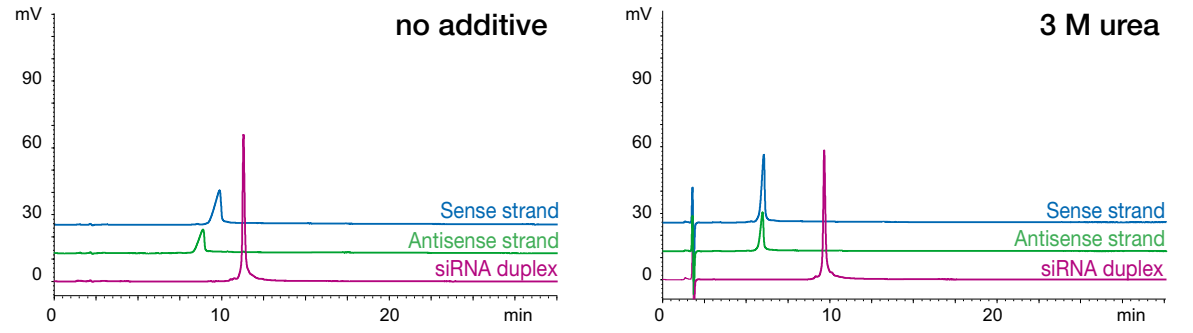
Influence of temperature on the analysis of a non-denaturated siRNA



Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID
Part No.:	QF00S05-1046WP
Eluent:	A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 1M $NaClO_4$
Gradient:	25–40%B (0–30 min), 40%B (30–40 min), 25%B (40–65 min)
Flow rate:	0.5 mL/min
Temperature:	25 °C, 40 °C or 60 °C
Detection:	UV at 260 nm
Injection:	4 µL (each 5 nmol/ml)
Sample:	sense strand, antisense strand, siRNA duplex of firefly luciferase GLS

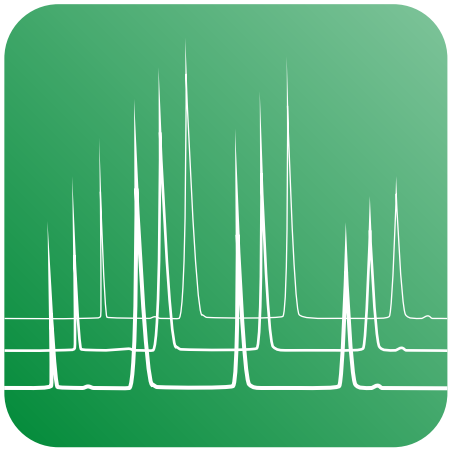
A higher temperature tended to show improved peak shape. Slightly better peak shapes of the ssRNAs were observed at 40 °C, while the dsRNA showed comparable and relatively good peak shape regardless of the temperature. An even higher temperature of 60°C provides better peak shape of the sense and antisense strands. However, peak height of the siRNA duplex decreases due to partial denaturation. It is considered that the higher order structure of ssRNAs is denatured when increasing temperature. The ssRNAs as well as dsRNA retain longer on the stationary phase, as the ion exchange group can access the phosphate groups more easily.

Influence of urea as additive on the analysis of a non-denaturated siRNA



Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID
Part No.:	QF00S05-1046WP
Eluent:	A) 20 mM Tris-HCl (pH 8.1) (containing 3 M Urea) B) 20 mM Tris-HCl (pH 8.1) containing 1 M $NaClO_4$ + (3 M Urea)
Gradient:	25–40%B (0-30 min), 40%B (30–40 min), 25%B (40–65 min)
Flow rate:	0.5 mL/min
Temperature:	40 °C
Detection:	UV at 260 nm
Injection:	4 µL (each 5 nmol/ml)
Sample:	sense strand, antisense strand, siRNA duplex of the firefly luciferase GLS

Addition of 3 M urea to the mobile phase results in better peak shapes of both sense and antisense strands as well as the siRNA duplex. The retention time is reduced for all three analytes and an improvement in resolution of the single strands and the double stranded siRNA is also observed.



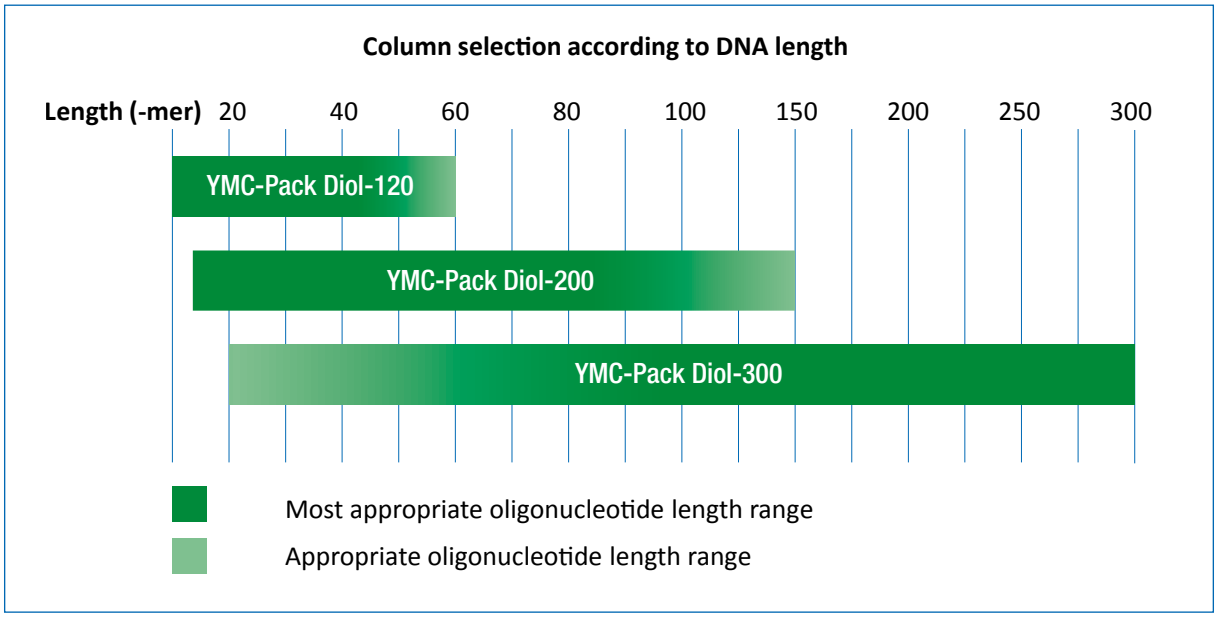
SEC

SEC – UHPLC / HPLC Selectivities

Features

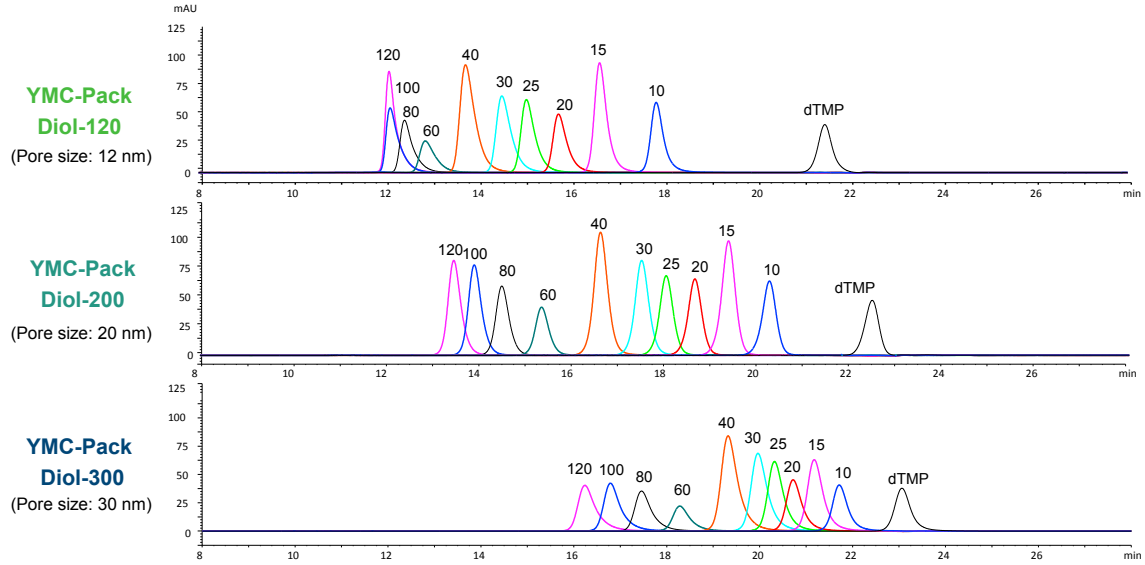
- **Excellent reproducibility with minimal secondary interactions**
- **2 µm for UHPLC**
- **Cost-effective**

	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)		

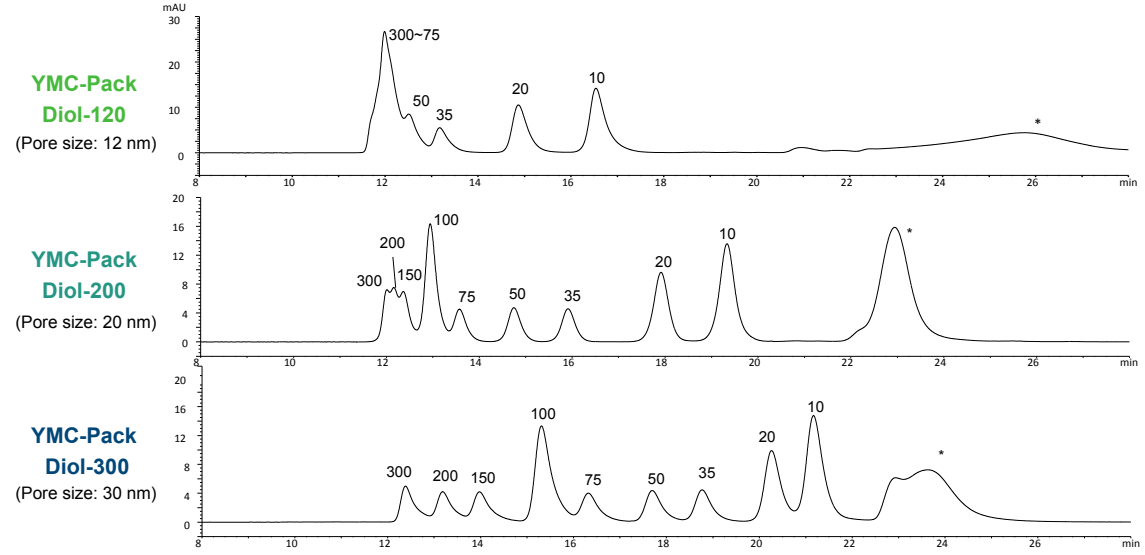


SEC analysis using YMC-Pack Diol columns with different pore sizes

Analysis of ssDNA of 10–120mer length



Analysis of dsDNA of 10–300mer length



Columns: YMC-Pack Diol-120 (5 µm, 12 nm) 300 x 4.6 mm ID
YMC-Pack Diol-200 (5 µm, 20 nm) 300 x 4.6 mm ID
YMC-Pack Diol-300 (5 µm, 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

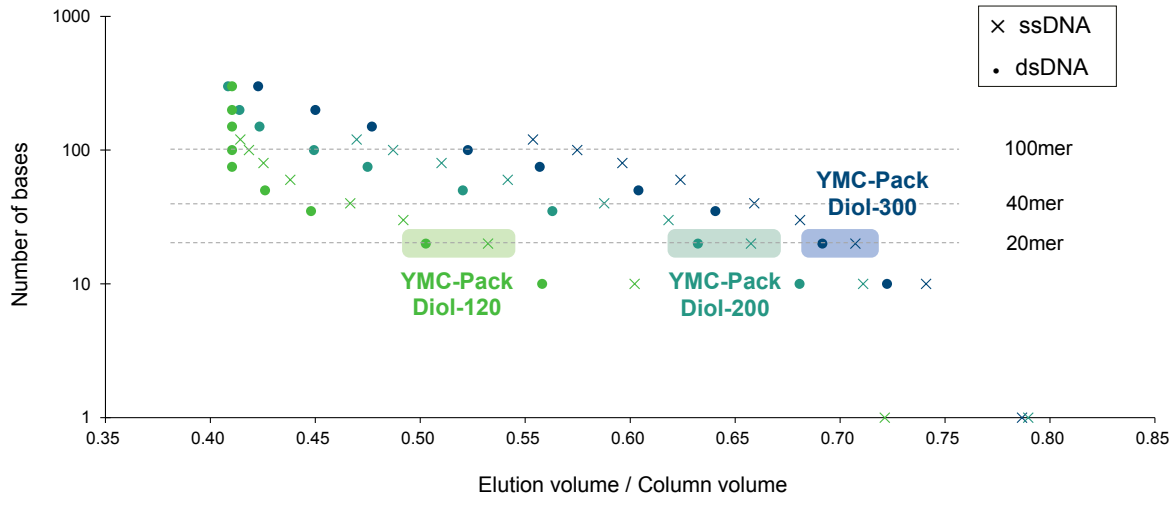
Flow rate: 0.17 mL/min
Detection: UV at 260 nm
Temperature: 25 °C
Injection: 1.0 µL (each 5 nmol/mL)
Samples: ssDNA (10–120 mer +dTMP)
dsDNA (10–300 mer)

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 µm, 12 nm) 300 x 4.6 mm ID YMC-Pack Diol-200 (5 µm, 20 nm) 300 x 4.6 mm ID YMC-Pack Diol-300 (5 µm, 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 °C
Injection:	1.0 µL (each 5 nmol/mL)
Samples:	ssDNA (10–120 mer +dTMP) dsDNA (10–300 mer)

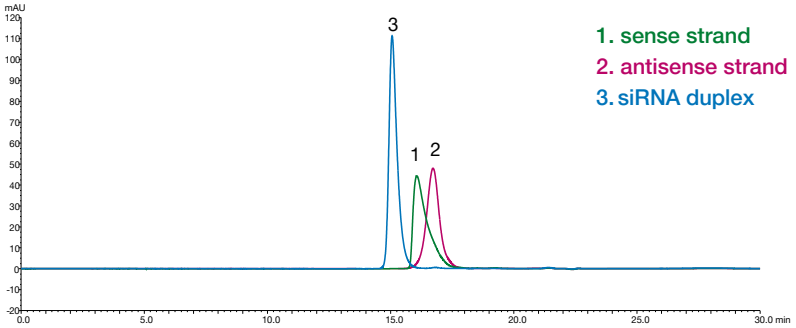
Although dsDNA has the same length as its single-stranded counterpart, the dsDNA elutes at lower elution volumes when separated by SEC. This behaviour is most probably due to the larger hydrodynamic radius of dsDNA compared to ssDNA, which results in faster diffusion through the stationary phase.

Influence of organic modifier on the separation of siRNA duplex and single strands

Effect of acetonitrile on separation of sense strand, antisense strand and siRNA duplex by SEC using YMC-Pack Diol-120

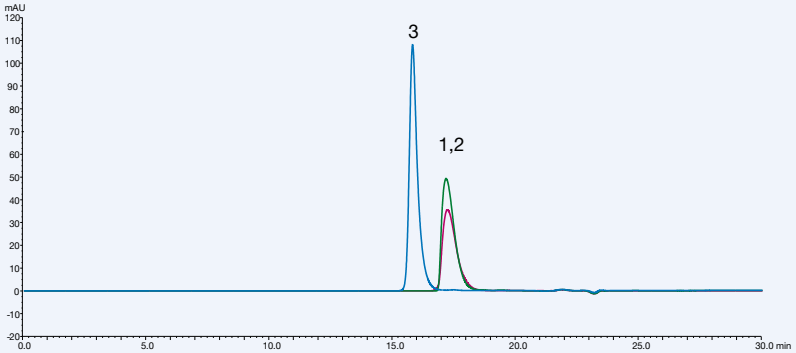
acetonitrile 0%

0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl



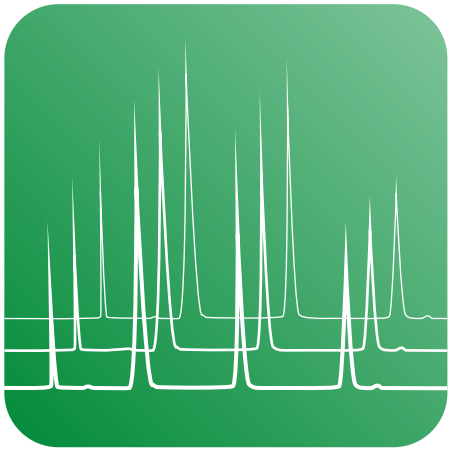
acetonitrile 30%

0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl / acetonitrile (70/30)

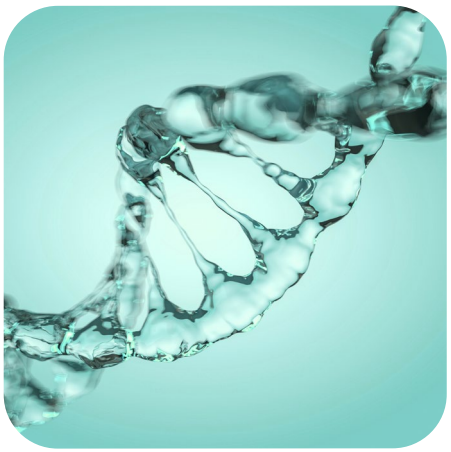


Column:	YMC-Pack Diol-120 (5 µm, 12 nm) 300 x 4.6 mm ID
Part No.:	DL12S05-3046WT
Flow rate:	0.17 mL/min
Detection:	UV at 260 nm
Temperature:	25 °C
Injection:	4.0 µL (each 5 nmol/mL)
Sample:	Sense strand Antisense strand siRNA duplex (Firefly luciferase GL2)

Although the sense and antisense strands have the same molecular weight, their retention times and peak shapes vary slightly. This is probably caused by minor secondary interactions with the stationary phase. If the goal is the separation of siRNA duplex from single strands, these interactions can be overcome with the addition of an organic solvent to the mobile phase.
When 30% acetonitrile is added to the eluent, both single strands elute at the same time and their peak shape is improved.



HILIC



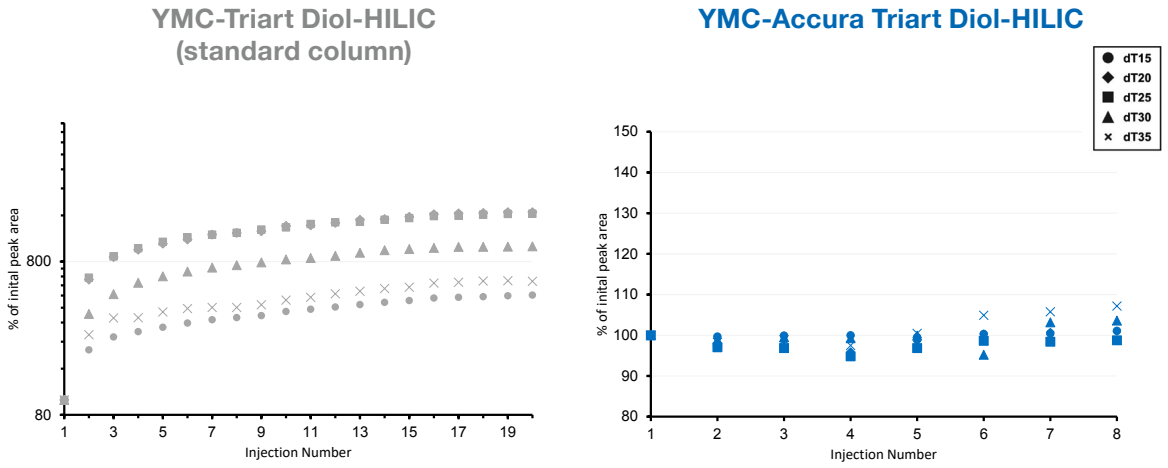
HILIC – UHPLC/HPLC selectivity

Features

- **pH- and temperature stable**
- **Superior reproducibility**
- **Bioinert hardware available**

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

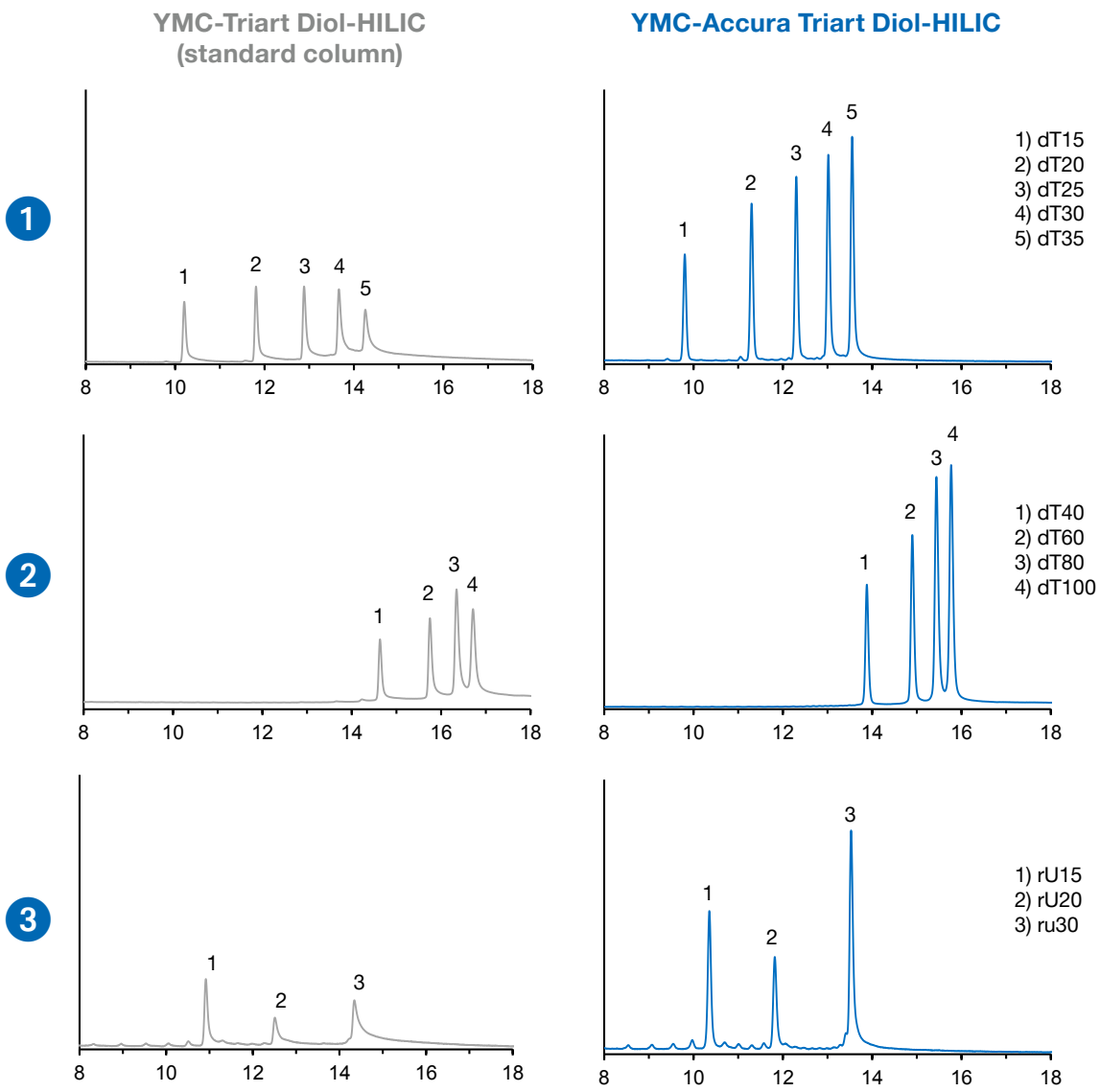
Pre-conditioning of a stainless-steel and a bioinert coated column with short DNA mixture



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)
System:	bioinert system

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



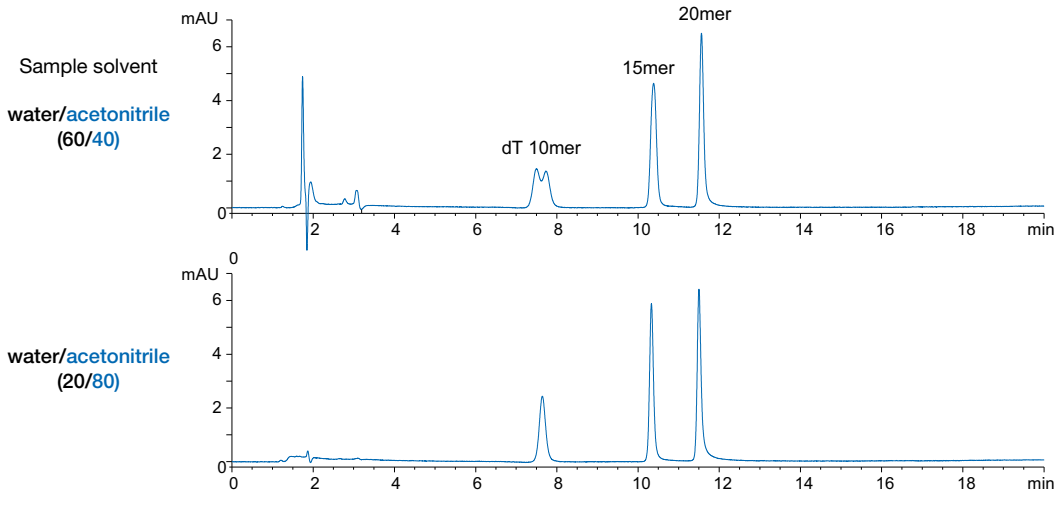
Columns:	YMC-Triart Diol-HILIC (1.9 µm, 12 nm) 150 x 2.1 mm ID (standard hardware) YMC-Accura Triart Diol-HILIC (1.9 µm, 12 nm) 150 x 2.1 mm ID (bioinert hardware)
Part Nos.:	TDH12SP9-15Q1PT TDH12SP9-15Q1PTC
Eluent:	A) 50 mM ammonium acetate (pH 6.9) B) acetonitrile
Gradient	75–45%B (0–30 min)
Flow rate:	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 (2µM) RNA oligonucleotides: rU15-30 (2µM)
System:	bioinert system

dT15-35 1
dT40-100 2
rU15-30 3

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

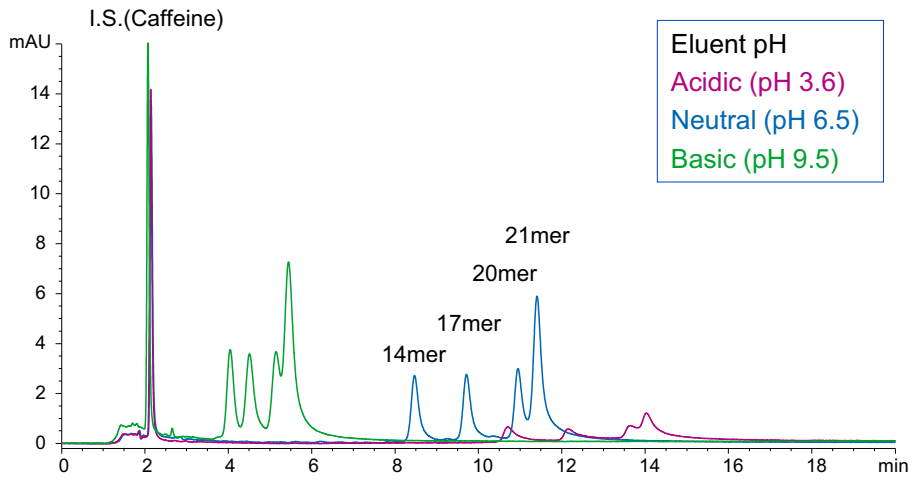
Influence of sample solvent



Column:	YMC-Accura Triart Diol-HILIC (3µm, 12 nm), 150 X 2.1 mm ID	Flow rate:	0.21 mL/min
Part.No.:	TDH12S03-15Q1PTC	Temperature:	40 °C
Eluent:	A) 50mM $HCOONH_4$ (pH 6.5)/acetonitrile (30/70)	Sample:	Oligo DNA (poly(dT) 10, 15, 20mer)
	B) 50mM $HCOONH_4$ (pH 6.5)	Injection:	2 µL (each 2.0 nmol/mL)
Gradient:	0–43%B (0–20 min), 43%B (20–25 min)		
	((70–40% acetonitrile (0–20 min), 40% acetonitrile (20–25 min))		

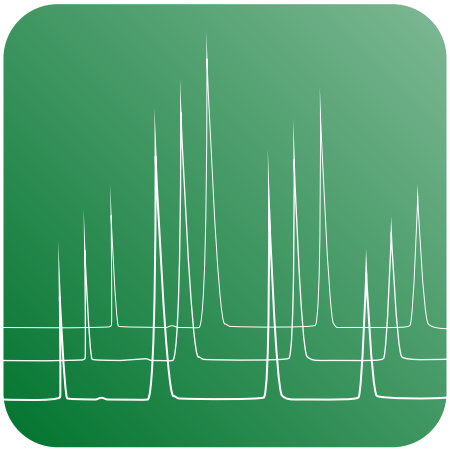
The influence of the sample solvent on the peak shape is significant. The organic composition of the sample solvent must be equal to or higher than the initial gradient composition. A higher water content in the sample solvent leads to massive peak deformation.

Influence of mobile phase pH



Column:	YMC-Accura Triart Diol-HILIC (3µm, 12 nm), 150 x 2.1 mm ID	Flow rate:	0.21 mL/min
Part.No.:	TDH12S03-15Q1PTC	Temperature:	40 °C
Eluent:	A) 50 mM ammonium formate buffer/acetonitrile (30/70)	Sample:	All PO RNA (14, 17, 20, 21mer) + I.S.(Caffeine)
	B) 50 mM ammonium formate buffer	Injection:	1 µL (1.8 nmol/mL-3.6 nmol/mL)
Gradient:	7.1–21.4%B (0–20 min), 21.4%B (20–25 min)		
	(65–55% acetonitrile (0–20 min), 55% acetonitrile (20–25 min)		

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	TA12SP9-15Q1PTC
YMC-Accura Triart Bio C18	2.1	TA30SP9-05Q1PTC	TA30SP9-10Q1PTC	TA30SP9-15Q1PTC
YMC-Accura Triart C8	2.1	TO12SP9-05Q1PTC	TO12SP9-10Q1PTC	TO12SP9-15Q1PTC
YMC-Accura Triart Bio C4	2.1	TB30SP9-05Q1PTC	TB30SP9-10Q1PTC	TB30SP9-15Q1PTC

1.9 µm PEEK-lined UHPLC columns (max. pressure 100 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart C18 metal-free	2.1	TA12SP9-05Q1PTP	TA12SP9-10Q1PTP	TA12SP9-15Q1PTP
YMC-Triart Bio C18 metal-free	2.1	TA30SP9-05Q1PTP	TA30SP9-10Q1PTP	TA30SP9-15Q1PTP
YMC-Triart C8 metal-free	2.1	TO12SP9-05Q1PTP	TO12SP9-10Q1PTP	TO12SP9-15Q1PTP
YMC-Triart Bio C4 metal-free	2.1	TB30SP9-05Q1PTP	TB30SP9-10Q1PTP	TB30SP9-15Q1PTP

Special column connectors required.

Column connectors for metal-free PEEK-lined (U)HPLC columns

Recommendation	✓ ✓		✓	
Ferrule	no		replaceable	
Product	MarvelX™	MarvelXACT™	Handy connector 2	Hand-tight EXP® fitting
Manufacturer	IDEX Health & Science LLC	IDEX Health & Science LLC	YMC Co., Ltd.	Optimize Technologies, Inc.
				
Pressure rating	131 MPa / 1,310 bar	131 MPa / 1,310 bar	42 MPa / 420 bar	137 MPa / 1,370 bar
Product code	e.g. UPFP-6050250	e.g. UPFP-YM7050250	XRP0204	XRHTF-01

MarvelX (ACT) is a registered trademark of IDEX Health & Science LLC · EXP® is a registered trademark of Optimize Technologies, Inc.

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

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	TO12S03-05Q1PTC	TO12S03-10Q1PTC	TO12S03-15Q1PTC
	4.6	TO12S03-0546PTC	TO12S03-1046PTC	TO12S03-1546PTC
YMC-Accura Triart Bio C4	2.1	TB30S03-05Q1PTC	TB30S03-10Q1PTC	TB30S03-15Q1PTC
	4.6	TB30S03-0546PTC	TB30S03-1046PTC	TB30S03-1546PTC

3 µm PEEK-lined HPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart C18 metal-free	2.1	TA12S03-05Q1PTP	TA12S03-10Q1PTP	TA12S03-15Q1PTP
	4.6	TA12S03-0546PTP	TA12S03-1046PTP	TA12S03-1546PTP
YMC-Triart Bio C18 metal-free	2.1	TA30S03-05Q1PTP	TA30S03-10Q1PTP	TA30S03-15Q1PTP
	4.6	TA30S03-0546PTP	TA30S03-1046PTP	TA30S03-1546PTP
YMC-Triart C8 metal-free	2.1	TO12S03-05Q1PTP	TO12S03-10Q1PTP	TO12S03-15Q1PTP
	4.6	TO12S03-0546PTP	TO12S03-1046PTP	TO12S03-1546PTP
YMC-Triart Bio C4 metal-free	2.1	TB30S03-05Q1PTP	TB30S03-10Q1PTP	TB30S03-15Q1PTP
	4.6	TB30S03-0546PTP	TB30S03-1046PTP	TB30S03-1546PTP

Special column connectors required.

3 µm HPLC columns (max. pressure 25 MPa)

Phase	Column ID [mm]	Column length [mm]						Guard cartridges* with 10 mm length
		30/33	50	75	100	150	250	(pack of 5)
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

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

IP-RP – Ordering information

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	TO12S05-05Q1PTC	TO12S05-10Q1PTC	TO12S05-15Q1PTC
	4.6	TO12S05-0546PTC	TO12S05-1046PTC	TO12S05-1546PTC
	10	–	TO12S05-1010PTC	TO12S05-1510PTC
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 PEEK-lined HPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart C18 metal-free	2.1	TA12S05-05Q1PTP	TA12S05-10Q1PTP	TA12S05-15Q1PTP
	4.6	TA12S05-0546PTP	TA12S05-1046PTP	TA12S05-1546PTP
YMC-Triart Bio C18 metal-free	2.1	TA30S05-05Q1PTP	TA30S05-10Q1PTP	TA30S05-15Q1PTP
	4.6	TA30S05-0546PTP	TA30S05-1046PTP	TA30S05-1546PTP
YMC-Triart C8 metal-free	2.1	TO12S05-05Q1PTP	TO12S05-10Q1PTP	TO12S05-15Q1PTP
	4.6	TO12S05-0546PTP	TO12S05-1046PTP	TO12S05-1546PTP
YMC-Triart Bio C4 metal-free	2.1	TB30S05-05Q1PTP	TB30S05-10Q1PTP	TB30S05-15Q1PTP
	4.6	TB30S05-0546PTP	TB30S05-1046PTP	TB30S05-1546PTP

Special column connectors required.

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

Phase	Column ID [mm]	Column length [mm]						Guard cartridges* with 10 mm length
		30/33	50	75	100	150	250	(pack of 5)
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

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

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
		50	75	100	150	250	(pack of 5)
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	TO12S05-0520WX	TO12S05-L520WX	TO12S05-1020WX	TO12S05-1520WX	TO12S05-2520WX	TO12S05-0120CCN
	30	TO12S05-0530WX	TO12S05-L530WX	TO12S05-1030WX	TO12S05-1530WX	TO12S05-2530WX	TO12S05-0130CCN
	50	TO12S05-0553DX	–	TO12S05-1053DX	TO12S05-1553DX	TO12S05-2553DX	TO12S05-0553DXG**
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

AEX – Ordering information

3 µm non-porous analytical columns, PEEK hardware (max. pressure 18–25 MPa)

Phase	Column ID [mm]	Column length [mm]				Precolumn filter 2 µm*
		30 (25 MPa)	50 (25 MPa)	100 (25 MPa)	150 (18 MPa)	(pack of 5)
BioPro IEX QF	4.6	QF00S03-0346WP	QF00S03-0546WP	QF00S03-1046WP	QF00S03-1546WP	XRPRCP25

5 µm non-porous analytical columns, PEEK hardware (max. pressure 6–12 MPa)

Phase	Column ID [mm]	Column length [mm]				Precolumn filter 2 µm*
		30 (6 MPa)	50 (10 MPa)	100 (12 MPa)	150 (12 MPa)	(pack of 5)
BioPro IEX QF	4.6	QF00S05-0346WP	QF00S05-0546WP	QF00S05-1046WP	QF00S05-1546WP	XRPRCP25

5 µm porous analytical columns, PEEK hardware (max. pressure 2.5–3.5 MPa)

Phase	Column ID [mm]	Column length [mm]			Precolumn filter 2 µm*
		30 (2.5 MPa)	50 (3.0 MPa)	100 (3.5 MPa)	(pack of 5)
BioPro IEX QA	4.6	QAA0S05-0346WP	QAA0S05-0546WP	QAA0S05-1046WP	XRPRCP25

* Holder required, part no. XRPRCP02

6 µm non-porous 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

6 µm non-porous bioinert coated semiprep. columns (max. pressure 3–9 MPa)

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

6 µm porous semiprep. columns, stainless-steel hardware (max. pressure 4 MPa)

Phase	Column ID [mm]	Column length [mm]
		100
BioPro IEX QA	10	QAA0S06-1010WT
	20	QAA0S06-1020WT

2 µm UHPLC columns (max. pressure 45 MPa)

Phase	Column ID [mm]	Column length [mm]		Guard cartridges* with 10 mm length
		150	300	(pack of 5)
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

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

Phase	Column ID [mm]	Column length [mm]			Guard cartridges* with 10/30 mm length
		150	250	300	(pack of 5)
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
**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
		250	300	500	(pack of 5)
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
**no holder required for 30 x 8 mm ID guard columns (1 piece)
recommended column coupler part no. XRCP1602 (for 8 mm ID) and XRCP1605 (for 10 mm ID)

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

1.9 µm PEEK-lined UHPLC columns

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart Diol-HILIC metal-free	2.1	TDH12SP9-05Q1PTP	TDH12SP9-10Q1PTP	TDH12SP9-15Q1PTP

Special column connectors required.

3 µm bioinert coated HPLC columns

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

3 µm PEEK-lined HPLC columns

Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart Diol-HILIC metal-free	2.1 4.6	TDH12S03-05Q1PTP TDH12S03-0546PTP	TDH12S03-10Q1PTP TDH12S03-1046PTP	TDH12S03-15Q1PTP TDH12S03-1546PTP

5 µm bioinert coated HPLC columns

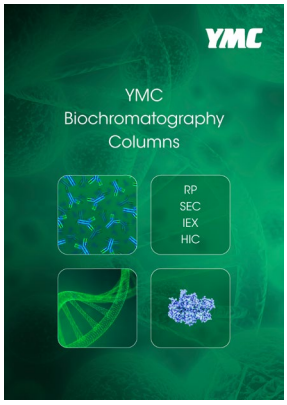
Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Accura Triart Diol-HILIC	2.1 4.6	TDH12S05-05Q1PTC TDH12S05-0546PTC	TDH12S05-10Q1PTC TDH12S05-1046PTC	TDH12S05-15Q1PTC TDH12S05-1546PTC

5 µm PEEK-lined HPLC columns

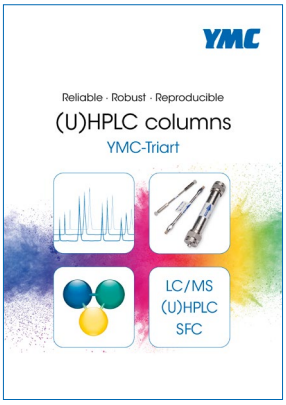
Phase	Column ID [mm]	Column length [mm]		
		50	100	150
YMC-Triart Diol-HILIC metal-free	2.1 4.6	TDH12S05-05Q1PTP TDH12S05-0546PTP	TDH12S05-10Q1PTP TDH12S05-1046PTP	TDH12S05-15Q1PTP TDH12S05-1546PTP

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

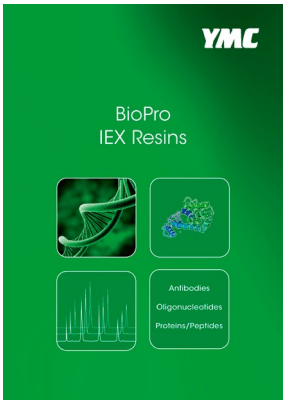
BROCHURES



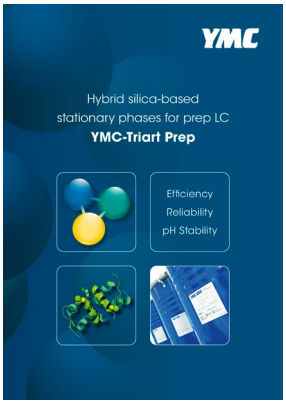
YMC Biochromatography Columns



YMC-Triart



YMC BioPro IEX Resins

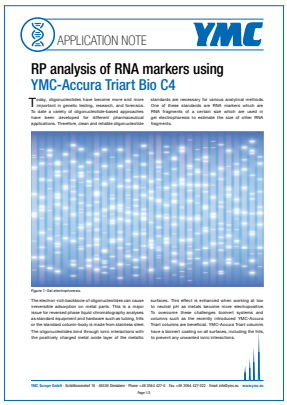


YMC-Triart Prep

WHITE PAPER

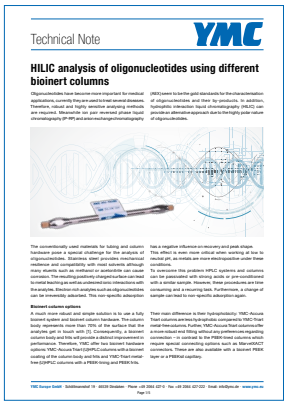
Analysis and Purification of Oligonucleotides by AEX and IP RP

APPLICATION NOTE

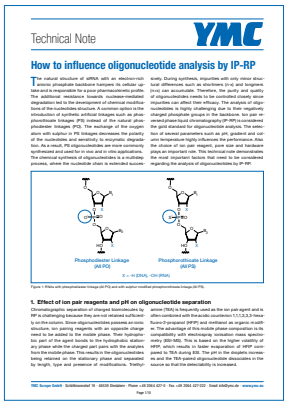


RP analysis of RNA markers using YMC-Accura Triart Bio C4

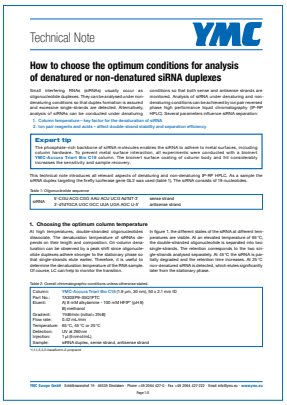
TECHNICAL NOTES



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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www.ymctaiwan.com

YMC Singapore Tradelinks Pte. Ltd.
www.ymc.sg

YMC Oligonucleotide Columns 09/2023_int_V1.0