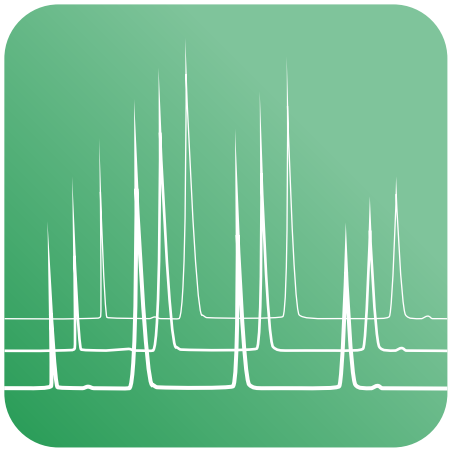
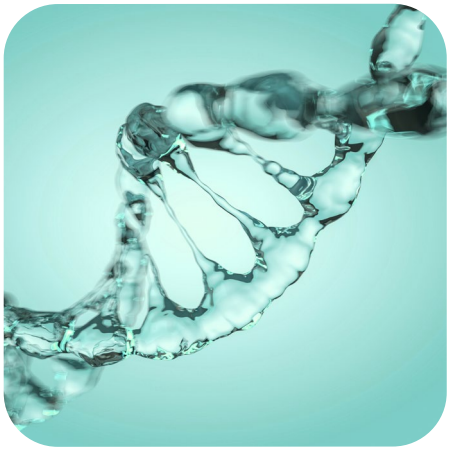


HILIC



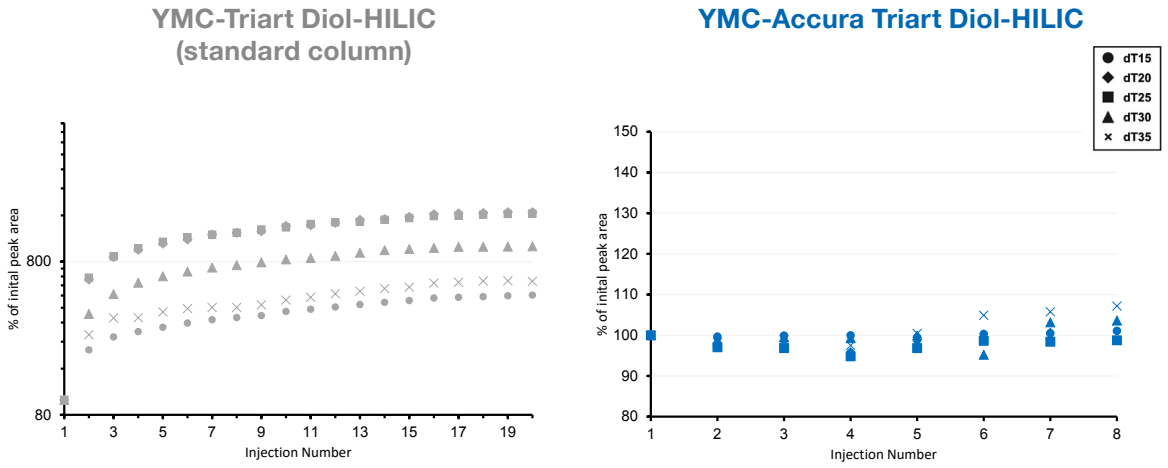
HILIC – UHPLC/HPLC selectivity

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

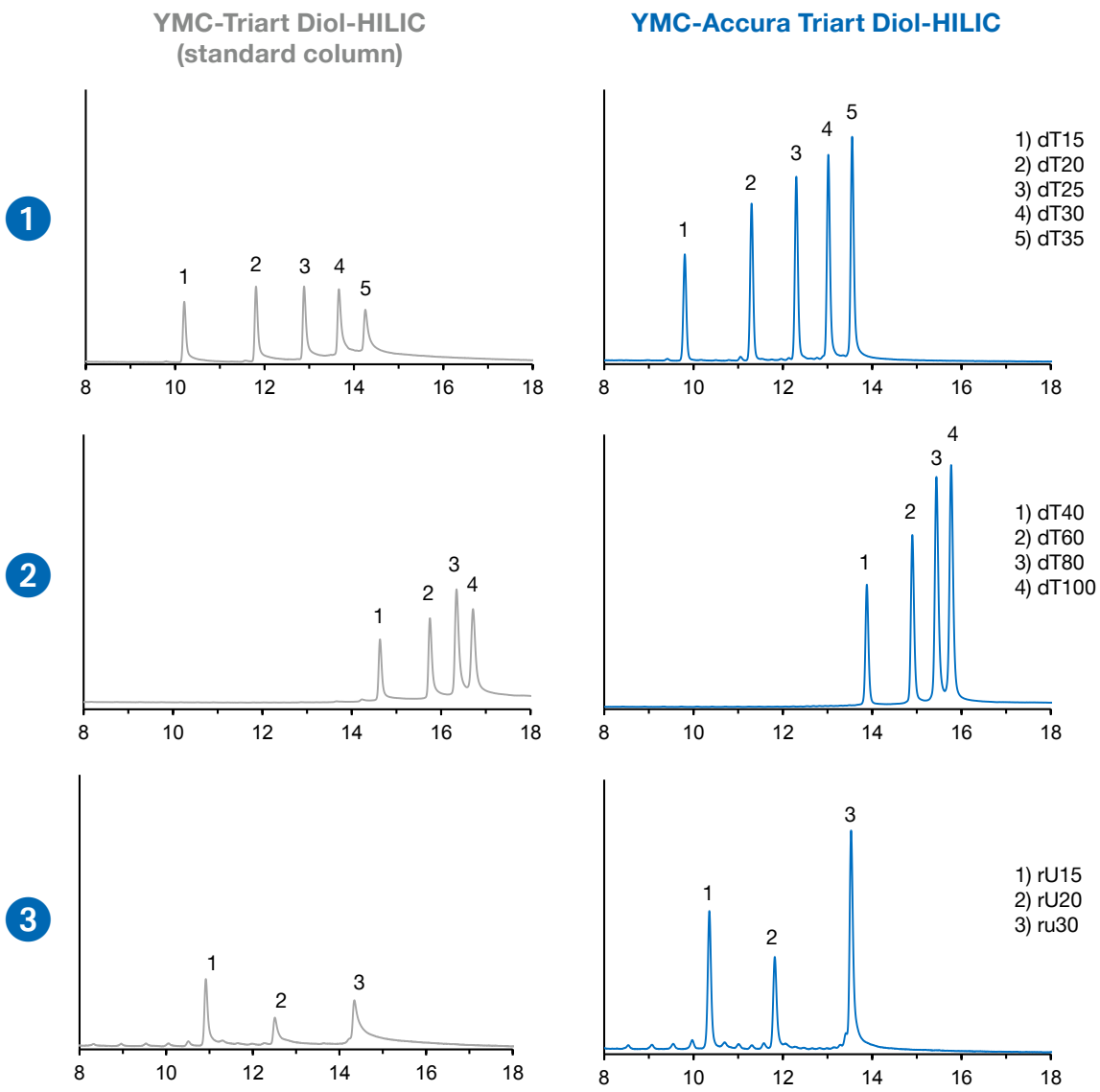
Pre-conditioning of a stainless-steel and a bioinert coated column with short DNA mixture



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



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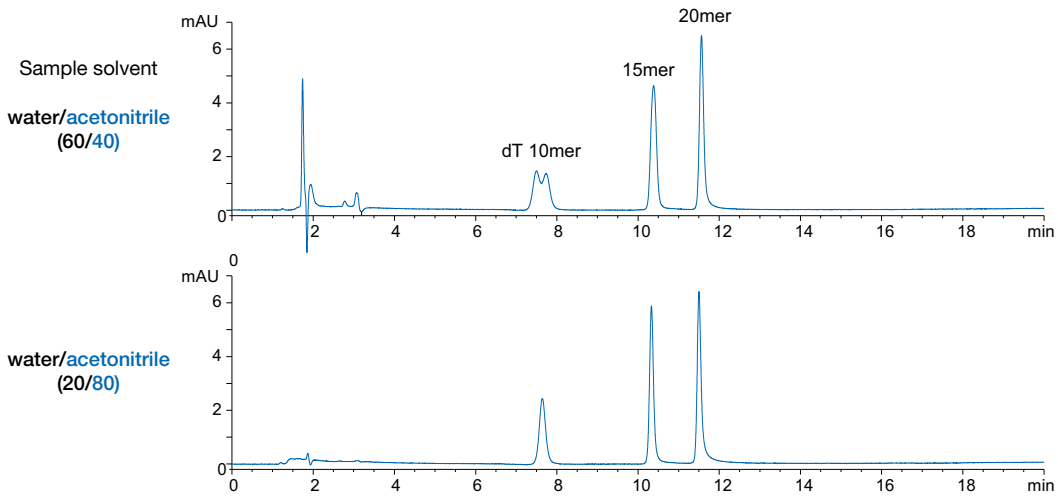
Gradient	75–45%B (0–30 min)
Flow rate:	0.3 mL/min
Temperature:	40 °C
Detection:	UV at 260 nm
Injection:	2 µL
Sample:	deoxythymidine oligonucleotides: dT15-35 (2 µM) and dT40-100 (2µM) RNA oligonucleotides: rU15-30 (2µM)
System:	bioinert system

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

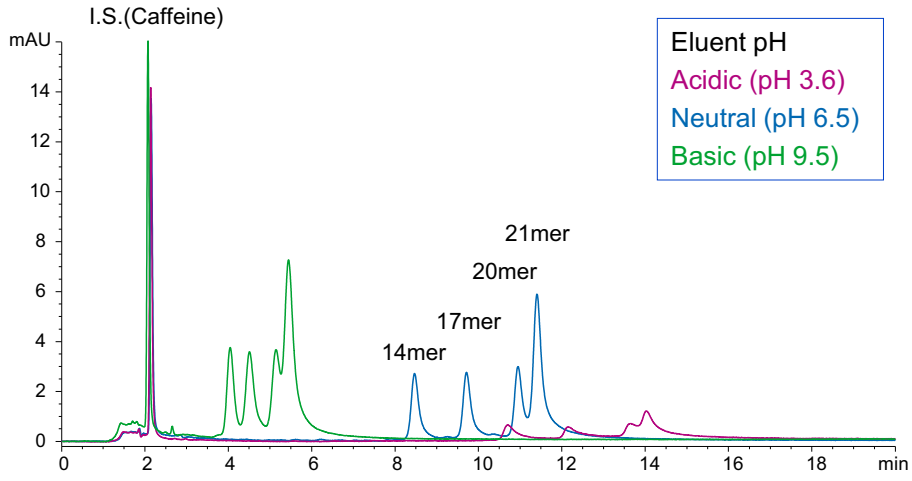
Influence of sample solvent



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.

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