The ideal solution for oligonucleotide analysis – YMC Accura Triart Bio C18 columns

The Vrije Universiteit Brussel (VUB), in collaboration with Janssen Pharmaceutica, confirmed the full benefits of the bioinert YMC Accura Triart Bio C18 column for oligonucleotide analysis. The research results demonstrate that the combination of bioinert coated YMC Accura hardware and the fully porous widepore YMC-Triart Bio C18 stationary phase results in highly reproducible and efficient chromatographic separation of complex biomolecules, such as oligonucleotides.

YMC Accura Triart Bio C18 – the perfect choice for method development

For oligonucleotides, metal ions pose a significant threat to successful analysis, as secondary interactions with these ions can lead to reduced recovery and carryover. The leaching of metal ions from the stainless-steel hardware can be promoted by the mobile phase composition including some organic modifier or specific ion-pairing agents [2]. Therefore, the use of bioinert hardware is a must in oligonucleotide analysis. The YMC Accura hardware with its bioinert coated hardware and frits, prevents metal ion leaching, ensuring efficient and reliable oligonucleotide analysis. Therefore, the YMC Accura Triart Bio C18 column is an ideal choice for method development, as its performance remains unaffected by various organic modifiers and ion-pairing agents, as demonstrated in the publication (see Figure 1 & 2).

The column enables the baseline separation of all oligodeoxythymidines and even its critical n-1 impurity (see Figure 2).

Table 1: Chromatographic conditions

Column:	YMC Accura Triart Bio C18 (1.9 µm) 150 x 2.1 mm ID
Part No.:	TA30SP9-15Q1PTC
Eluent:	A) water containing 10 mM DIPEA*1 and 50 mM HFIP*2
	B) methanol/water (50/50) or acetonitrile/water (50/50) containing 10 mM DIPEA*1 and 50 mM HFIP*2
Gradient:	methanol/water (50/50): 10–60%B (0–15 min)
	acetonitrile/water (50/50): 0–30%B (0–15 min)
Flow rate:	0.5 mL/min
Temperature:	60°C
Detection:	UV at 260 nm
Injection:	1 µL (20 pmol/µL of each oligonucleotide)
Sample:	MassPrep OST Standard with 15, 20, 25, 30, 35mer oligodeoxythymidines
System:	biocompatible UHPLC system

*1 N,N-Diisopropylethylamine, *2 1,1,1,3,3,3-Hexafluoro-2-propanol

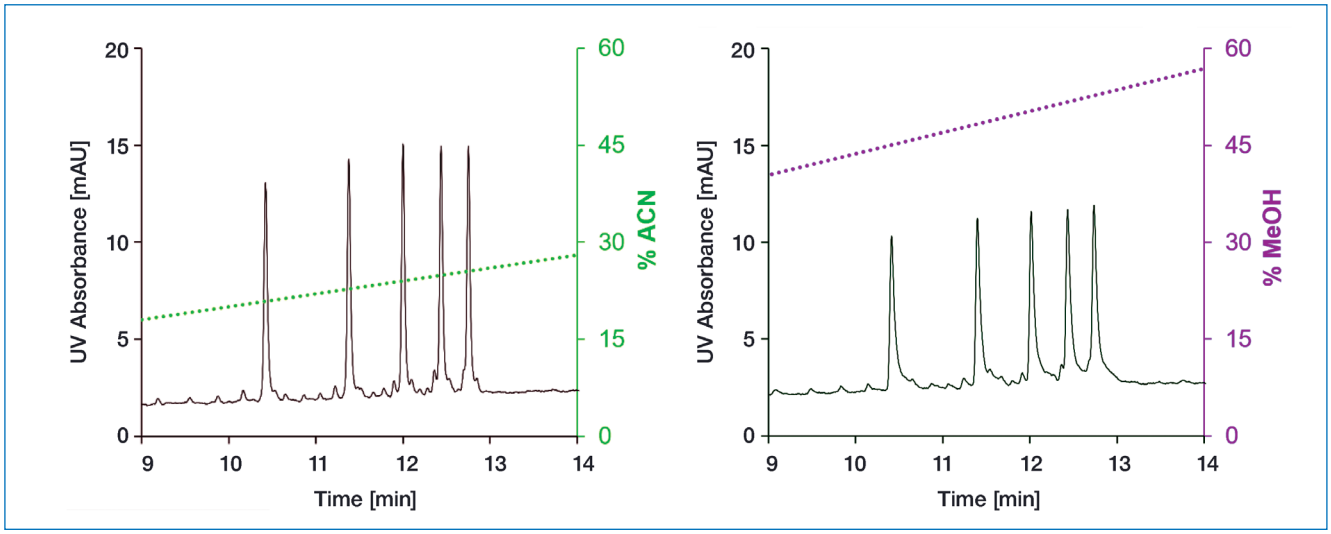

Figure 1: Influence of organic modifier on the separation of poly(dT) oligonucleotides (15, 20, 25, 30, 35mer) using the YMC Accura Triart Bio C18 column.

Table 2: Chromatographic conditions

Columns:	YMC Accura Triart Bio C18 (1.9 µm) 150 x 2.1 mm ID
Part No.:	TA30SP9-15Q1PTC
Eluent:	A) water containing 50 mM IP agent, 10 mM CI
	B) acetonitrile/water (50/50) containing 50 mM IP agent, 10 mM CI
Gradient:	TEA: 9–14.9%B (0–10 min)
	DIPEA: 17–27%B (0–10 min)
	HA: 34.2–52.1%B (0–10 min)
Flow rate:	0.5 mL/min
Temperature:	60°C
Detection:	UV at 260 nm, LC-MS
Injection:	1 µL (20 pmol/µL of each oligonucleotide)
Sample:	MassPrep OST Standard with 15, 20, 25, 30, 35mer oligodeoxythymidines
System:	biocompatible UHPLC system

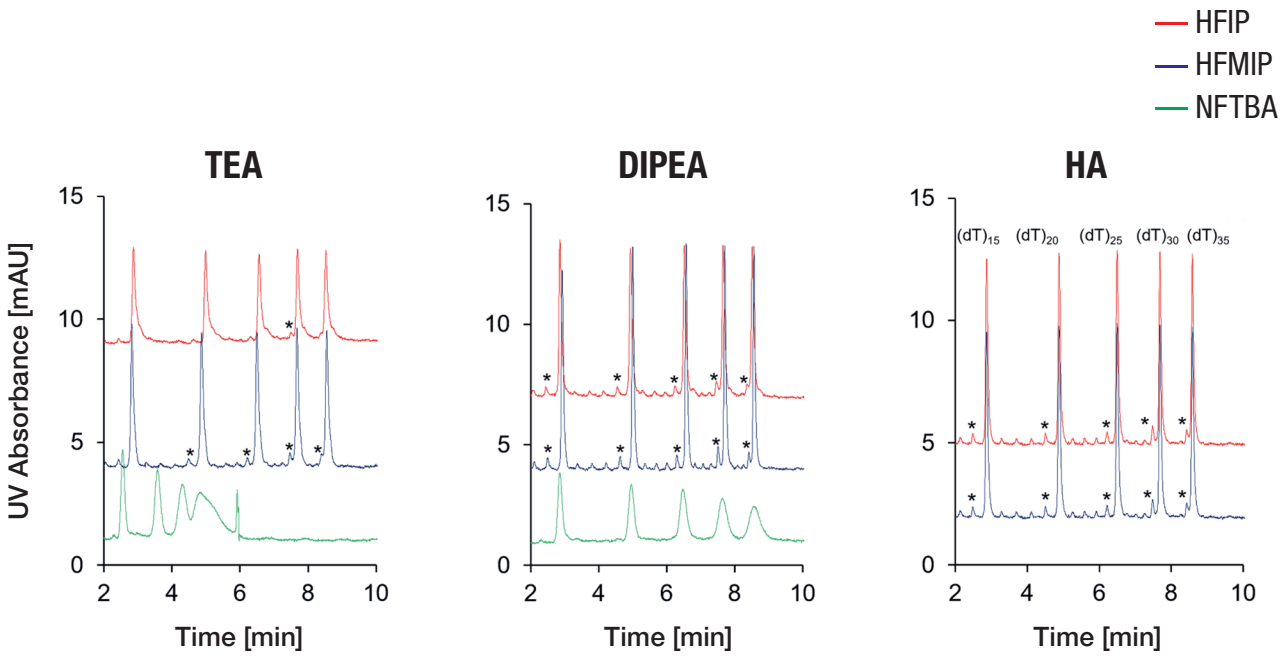

Figure 2: Evaluation of different combinations of ion-pairing agents and counter ions for the analysis of poly(dT) oligonucleotides using the YMC Accura Triart Bio C18.

The decisive advantage of a widepore stationary phase in oligonucleotide analysis

For efficient separation, the pores of the particles must be accessible. If this is not the case, mass transfer is limited, resulting in less efficient analysis and broader peaks. This is clearly demonstrated in the following example: Oligonucleotides of varying lengths (100, 200, 300, 400, 500, 750, 1000mer) were analysed using the YMC Accura Triart Bio C18 widepore column and a core-shell column. Both phases are specifically designed for Bio-LC applications. The YMC Accura Triart Bio C18 column achieved baseline separation of all oligonucleotides within ten minutes (see Figure 3). However, the core-shell column failed to resolve all oligonucleotides. When analysing oligonucleotides of different sizes, the YMC-Triart Bio C18 widepore has a clear advantage compared to core-shell columns.

Table 3: Chromatographic conditions

Columns:	YMC Accura Triart Bio C18 (1.9 µm) 150 x 2.1 mm ID BioZen Oligo (1.7 µm) 150 x 2.1 mm ID, core-shell particle
Part No.:	TA30SP9-15Q1PT
Eluent:	A) water containing 10 mM HA, 50 mM HFIP*1 B) acetonitrile/water (50/50) containing 10 mM HA, 50 mM HFIP*1
Gradient:	YMC Accura Triart Bio C18: 52–58%B (0–10 min) BioZen Oligo: 49–66%B (0–10 min)
Flow rate:	0.5 mL/min
Temperature:	60°C
Detection:	UV at 260 nm, LC-MS
Injection:	1 µL (100 nmol/mL)
Sample:	RNA Century-Plus markers with 100, 200, 300, 400, 500, 750 and 1000mer
System:	biocompatible UHPLC system

2 1,1,1,3,3,3-Hexafluoro-2-propanol

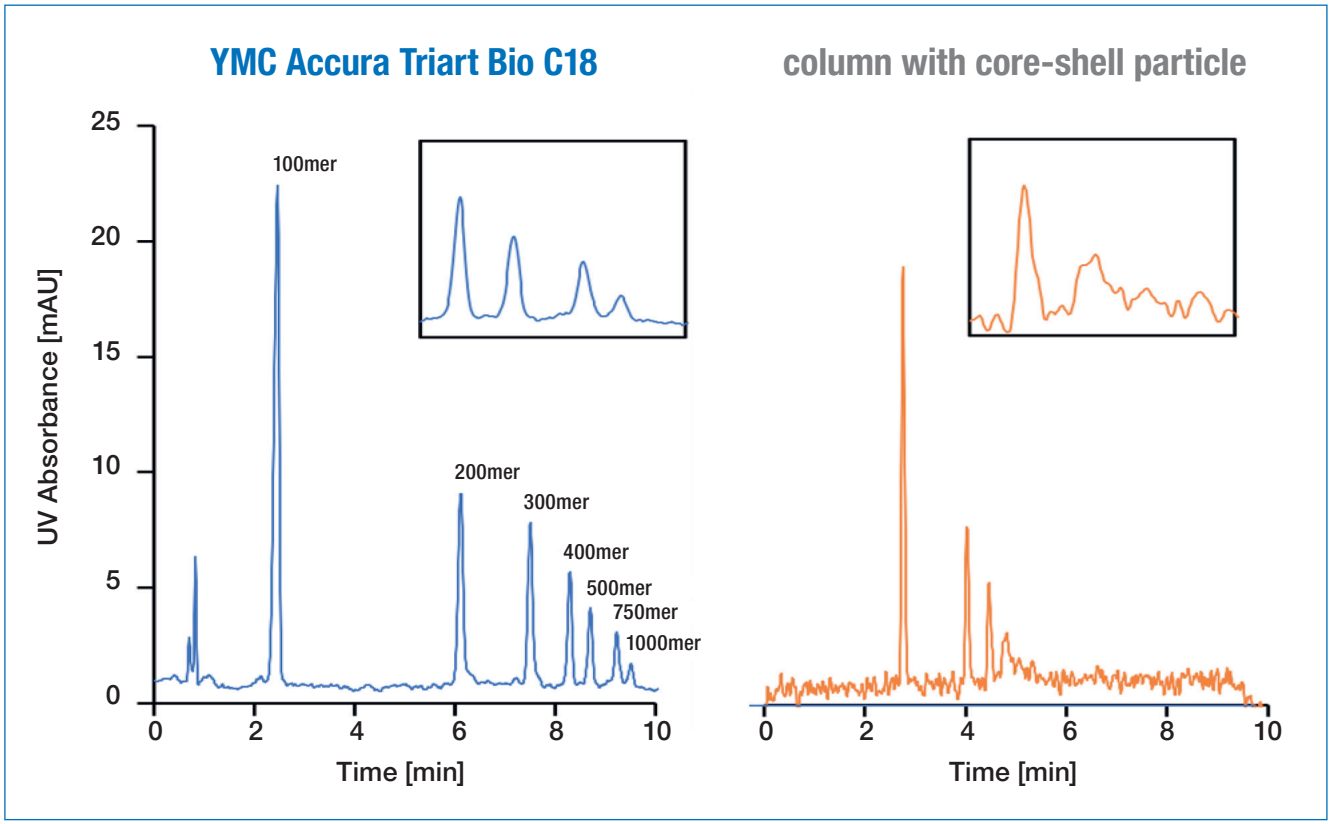

Figure 3: Separation of oligonucleotides of different length using the widepore YMC Accura Triart Bio C18 column compared to a column with core-shell particle.

The key benefits of the YMC Accura Triart Bio C18 in oligonucleotide analysis

- The bioinert coated hardware prevents unwanted interactions under all method conditions, making it an ideal choice for method development.
- High resolution of highly similar oligonucleotides and their impurities.
- Successful simultaneous separation of oligonucleotides of different sizes.

References

[1] Bui Q.D. et al. (2024) Optimization of elution conditions and comparison of emerging biocompatible columns on the resolving power and detection sensitivity of oligonucleotides by ion-pairing reversed-phase liquid chromatography mass spectrometry. J Chromatogr A., 1720:464793

[2] Bischof (2023) Issues with metals in nucleotide based pharmaceutical production, SilcoTek® Corporation