APPLICATION NOTE

Determination of oligonucleotide purity using YMC-Triart C18 UHPLC column

Oligonucleotides are a rising class of drugs which have entered the market as biopharmaceuticals due to their ability to interfere directly with gene expression instead of targeting the protein. The production of oligonucleotides is usually conducted by chemical solid-phase synthesis and use of nucleoside phosphoramidites as building blocks. To prevent undesired side reactions, protecting groups are attached to the nucleosides, for example the 5'-hydroxy group of the sugar moiety is protected with a dimethoxytrityl (DMT) group.

During oligonucleotide synthesis errors accumulate so that impurities and degradation products emerge. The major challenge in oligonucleotide purification is the presence of structurally similar impurities, such as shortmers (n–x) and longmers (n+x), which are hard to separate from the desired product. Therefore, analysis of synthesised oligonucleotides by analytical chromatography is vital to monitor their quality and quantity before and after purification. A robust set-up is needed to achieve reliable and highly reproducible results.

In this application note based on a publication by Fioretti et al. (1) the separation of a crude oligonucleotide sample is demonstrated. A YMC-Triart C18 UHPLC column in ion pairing reversed phase mode is used. Due to an adequately high resolution, the purity of the target, a 20mer single-stranded DNA, could easily be determined (figure 1). The purity was 72.1 %, while two impurities in total could be identified. For further optimization of oligonucleotide analysis, the YMC-Accura Triart Bio C18 column is an ideal choice to achieve even higher recovery and sensitivity.

Table 1: Oligonucleotide Sequence

Single-stranded DNA	5'-ATA CCG ATT AAG CGA AGT TT-3'

Table 2: Chromatographic condition

Column:	YMC-Triart C18 (1.9 µm, 12 nm) 100 x 2.0 mm ID
Part number:	TA12SP-1002PT
Eluent:	A) 4 mM triethylamine - 100 mM HFIP*
	B) methanol
Gradient:	5%–10%B (1–3 min), 10%–20%B (3–20 min), 20%–90%B (20–22 min)
Flow rate:	0.2 ml/min
Temperature:	50 °C
Detection:	UV at 260 nm
Injection:	0.5 µl
Sample:	Crude oligonucleotide

**Hexafluoroisopropanol*

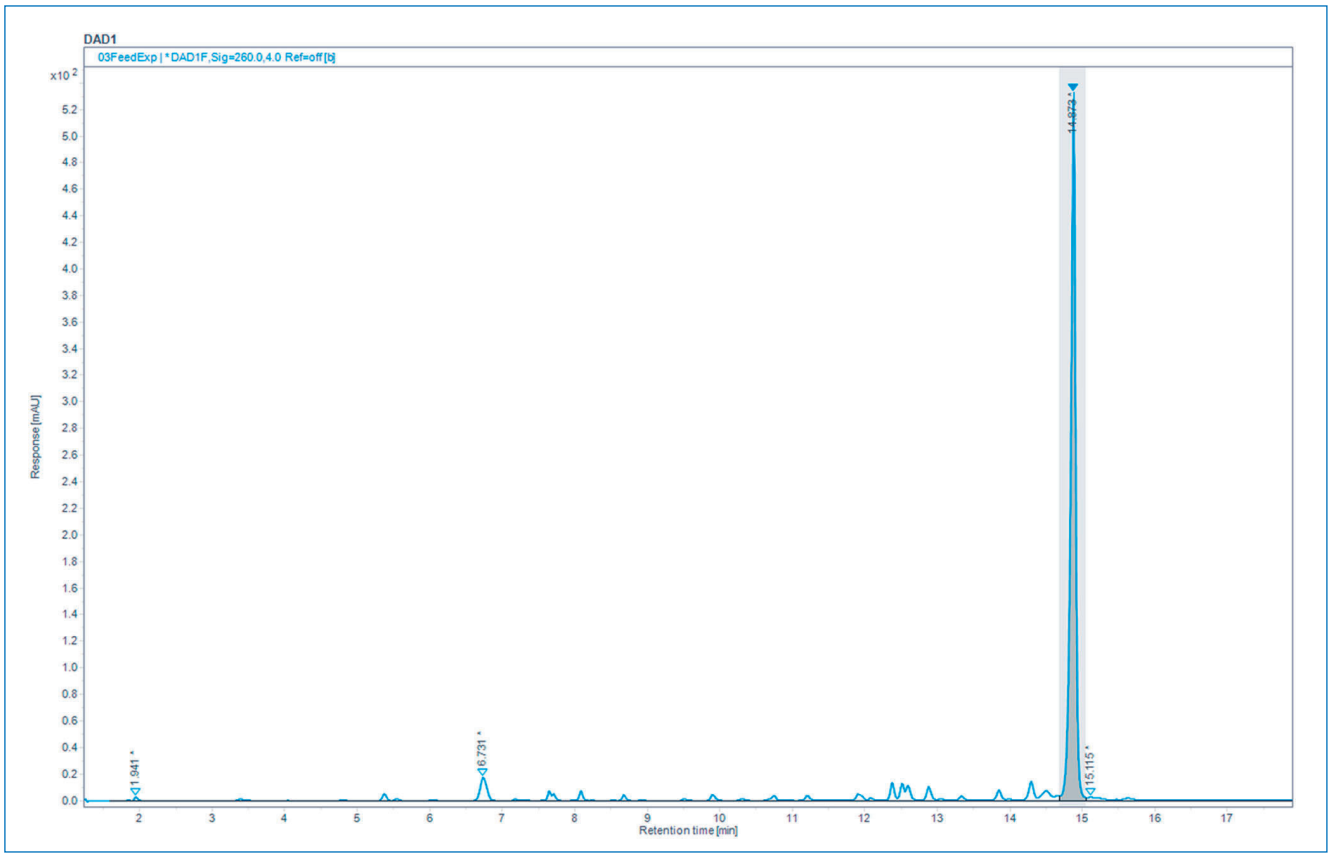

Figure 1: Separation of crude oligonucleotide by ion pairing reversed phase using YMC-Triart C18 UHPLC column.

(1) *Fioretti, I., Müller-Späth, T., Weldon, R., Vogg, S., Morbidelli, M., & Sponchioni, M. (2022). Continuous countercurrent chromatographic twin-column purification of oligonucleotides: The role of the displacement effect. Biotechnology and Bioengineering, 119, 1861– 1872. https://doi.org/10.1002/bit.28093*

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