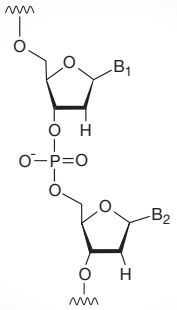
# The best choice for poly(dT) oligonucleotide analysis – YMC-Triart Bio C18

Poly(dT) oligonucleotides are single stranded nucleotide chains, which solely consist of thymine as bases. Due to base pairing of thymine and adenine, poly(dT) oligonucleotides are able to capture polyadenylated messenger RNA (mRNA). This mechanism is widely used during mRNA extraction and purification, for instance in the course of complementary DNA (cDNA) synthesis or mRNA vaccine manufacturing. Therefore, the need for high purity oligonucleotides is of paramount importance. For analysis of oligonucleotides, ion pair reversed phase (IP-RP) high performance liquid chromatography (HPLC) is the most common method used. IP-RP relies on the ionic interaction between the analyte and the ion-pair reagent. The lipophilic alkyl chain of the ion pair reagent has high affinity to the stationary phase, which maximises the retention of analytes on the phase. Due to the high resolution of IP-RP chromatography, oligonucleotides with only minimal size differences can be separated.

In this technical note, YMC-Triart Bio C18 is compared with XBridge Oligonucleotide BEH C18 and the DNAPac columns, which are claimed to be specially designed for the separation of oligonucleotides.

oligo deoxythymidine (dT)




B1, B2, ...= thymine

*Figure 1: Structure of poly(dT) oligonucleotides with thymine as bases.*

*Table 1: Attributes of poly(dT) oligomers and sample conditions used.*

| Name | Length (mer) | Molecular weight (Da) | Sample concentration (µM) | Loading | |
|---|---|---|---|---|---|
| | | | | (1 µL) pmol | (1 µL) ng |
| Caffeine (I.S.) | – | 194 | 25.7 | 25.7 | 5.0 |
| dT 10mer | 10 | 2,980 | 2.0 | 2.0 | 6.0 |
| dT 15mer | 15 | 4,501 | 1.0 | 1.0 | 4.5 |
| dT 20mer | 20 | 6,022 | 1.0 | 1.0 | 6.0 |
| dT 25mer | 25 | 7,543 | 1.0 | 1.0 | 7.5 |
| dT 30mer | 30 | 9,064 | 1.0 | 1.0 | 9.1 |
| dT 40mer | 40 | 12,106 | 0.5 | 0.5 | 6.1 |
| dT 60mer | 60 | 18,190 | 0.5 | 0.5 | 9.1 |
| dT 80mer | 80 | 24,274 | 0.3 | 0.3 | 7.3 |
| dT 100mer | 100 | 30,358 | 0.3 | 0.3 | 9.1 |
| dT 120mer | 120 | 36,442 | 0.2 | 0.2 | 7.3 |

*Table 2: Chromatographic conditions.*

| | |
|---|---|
| Columns: | YMC-Triart Bio C18 (3 µm, 30 nm) 50 x 2.1 mm ID<br>XBridge Oligonucleotide BEH C18 (2.5 µm, 13 nm) 50 x 2.1 mm ID<br>DNAPac RP (4 µm, proprietary) 50 x 2.1 mm ID |
| Part No.: | TA30S03-05Q1PTH |
| Eluent: | A) 4 mM TEA - 100 mM HFIP<br>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 |

The YMC-Triart Bio C18 column demonstrates a better resolution, higher recovery and reproducibility of poly(dT) oligonucleotides compared to XBridge Oligonucleotide BEH C18 and DNAPac RP (figure 2 and 3).

Longer poly(dT) oligonucleotides (60–120mer) were separated poorly by XBridge Oligonucleotide BEH C18, whereas YMC-Triart showed high resolution for oligonucleotides of all sizes (figure 2).

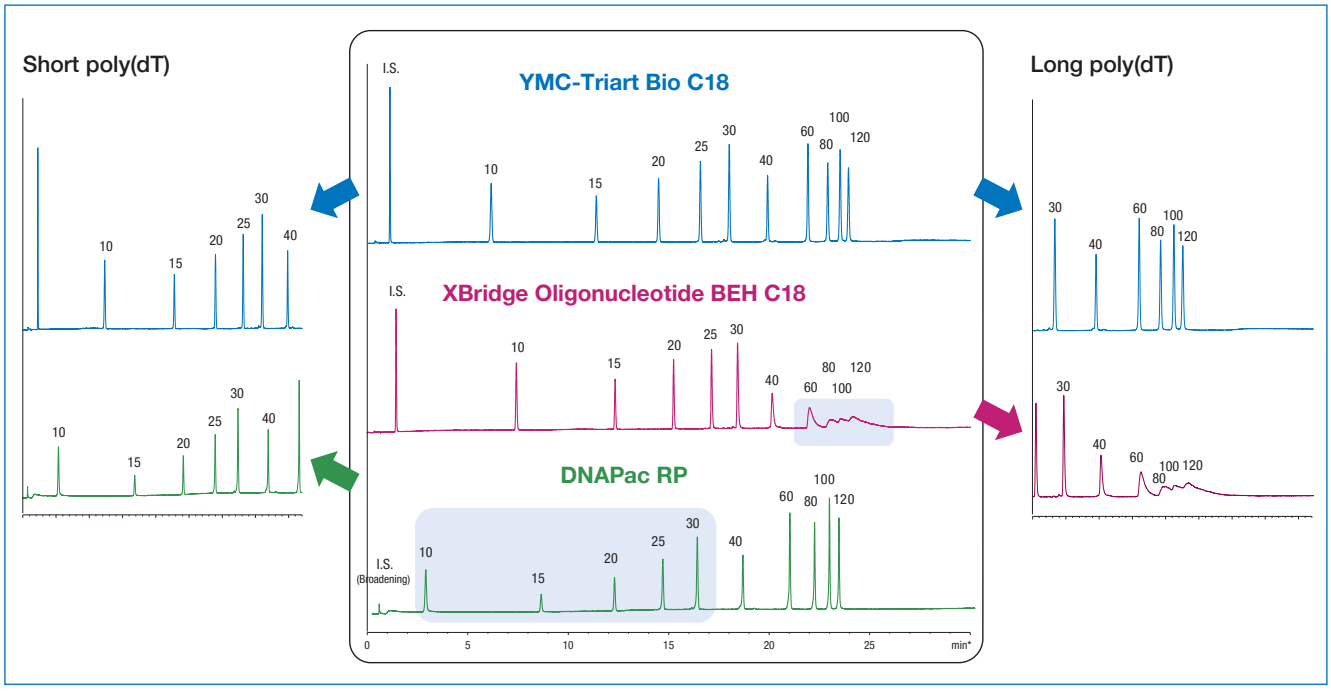



*Figure 2: Comparison of poly(dT) oligonucleotides separation by IP-RP using YMC-Triart Bio C18, XBridge Oligonucleotide BEH C18 and DNAPac RP.*

Peak areas and therefore recoveries of shorter poly(dT) oligonucleotides (10–40mer) were much smaller when separated using DNAPac RP. For instance, the peak area of the 15mer analysed by DNAPac RP was only 43% of the peak area detected when separated by YMC-Triart Bio C18. In addition, YMC-Triart Bio C18 showed reproducible behaviour such as consistent peak areas even after six injections (figure 3). In contrast, DNAPac RP showed a significant decrease in peak areas after only three injections. This makes YMC-Triart C18 an ideal tool for analysis of poly(dT) oligonucleotides.

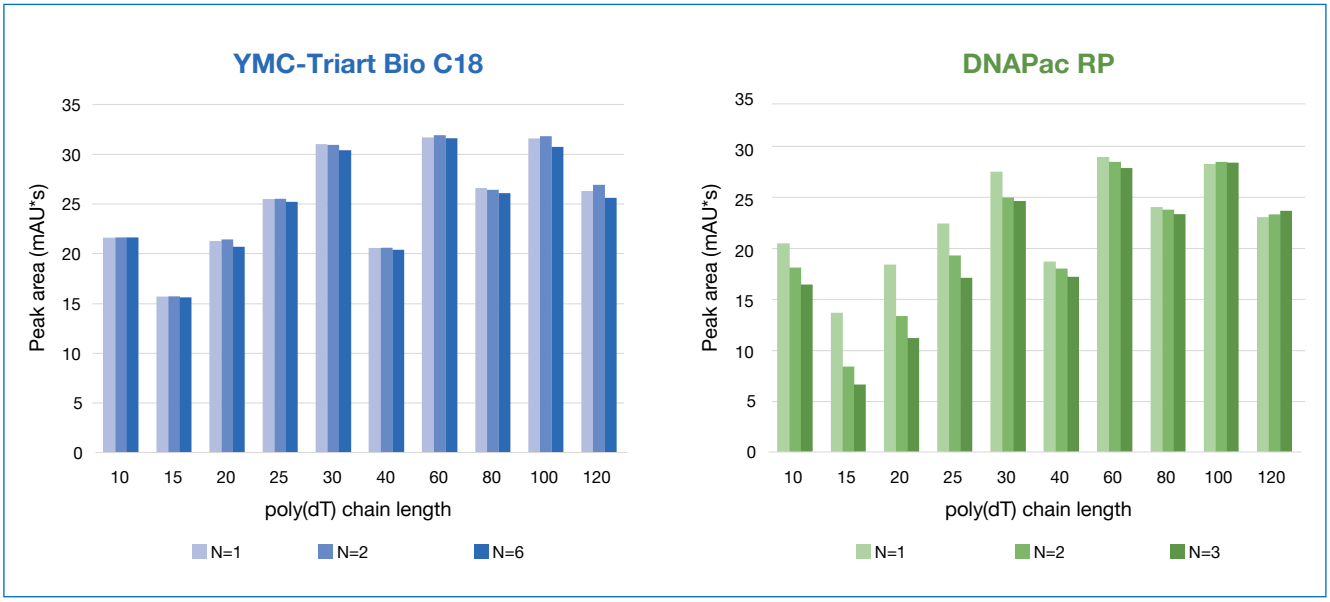



*Figure 3: Peak area of poly(dT) oligonucleotides of up to 6 repeated analyses, separated by IP-RP using YMC-Triart Bio C18 and DNAPac RP from Thermo Fisher Scientific Inc.*

YMC-Triart Bio C18 columns are the ideal choice for poly(dT) oligonucleotides, due to:

- **High resolution for all sizes of oligonucleotides**
- **Higher recoveries of shorter oligonucleotides**
- **Consistent and reproducible separation performance**

*XBridge Oligonucleotide BEH C18 is a trademark of Waters Corp.*
*DNAPac RP is a trademark of Thermo Fisher Scientific Inc.*