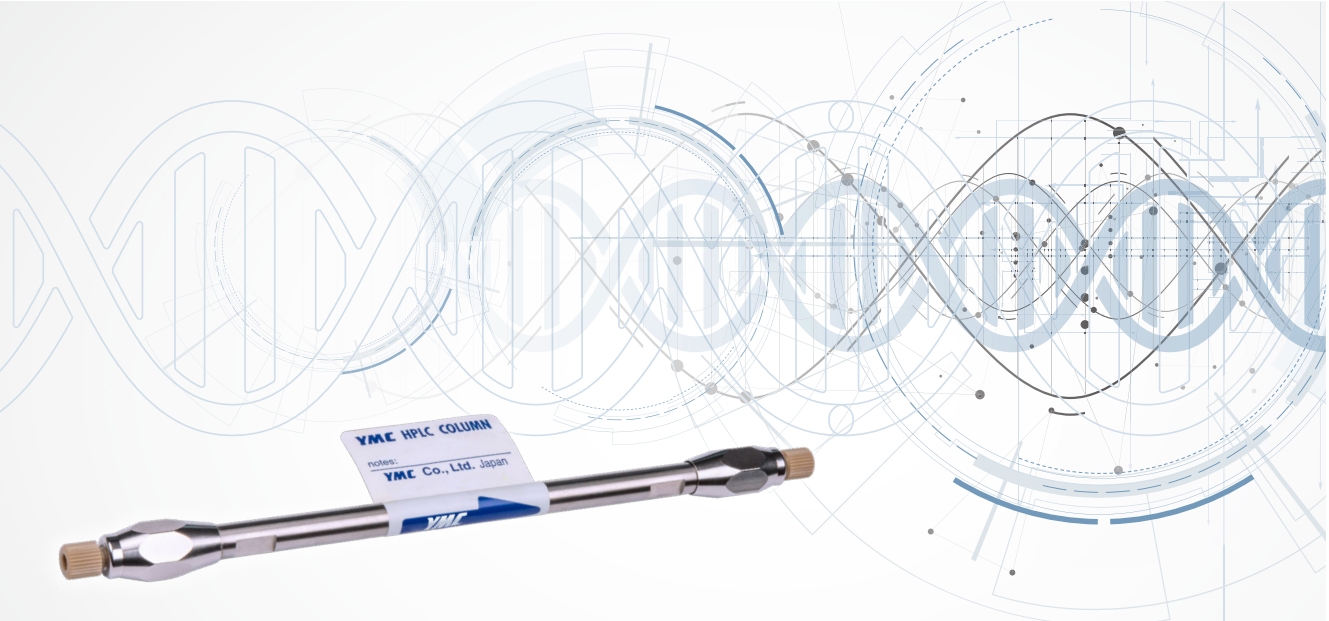
HILIC analysis of oligonucleotides using different bioinert columns

Oligonucleotides have become more important for medical applications, currently they are used to treat several diseases. Therefore, robust and highly sensitive analysing methods are required. Meanwhile ion pair reversed phase liquid chromatography (IP-RP) and anion exchange chromatography (AEX) seem to be the gold standards for the characterisation of oligonucleotides and their by-products. In addition, hydrophilic interaction liquid chromatography (HILIC) can provide an alternative approach due to the highly polar nature of oligonucleotides.



The conventionally used materials for tubing and column hardware pose a special challenge for the analysis of oligonucleotides. Stainless steel provides mechanical resilience and compatibility with most solvents although many eluents such as methanol or acetonitrile can cause corrosion. The resulting positively charged surface can lead to metal leaching as well as undesired ionic interactions with the analytes. Electron rich analytes such as oligonucleotides can be irreversibly adsorbed. This non-specific adsorption has a negative influence on recovery and peak shape.
This effect is even more critical when working at low to neutral pH, as metals are more electropositive under these conditions.
To overcome this problem HPLC systems and columns can be passivated with strong acids or pre-conditioned with a similar sample. However, these procedures are time consuming and a recurring task. Furthermore, a change of sample can lead to non-specific adsorption again.

Bioinert column options

A much more robust and simple solution is to use a fully bioinert system and bioinert column hardware. The column body represents more than 70% of the surface that the analytes get in touch with [1]. Consequently, a bioinert column body and frits will provide a distinct improvement in performance. Therefore, YMC offer two bioinert hardware options: YMC-Accura Triart (U)HPLC columns with a bioinert coating of the column body and frits and YMC-Triart metal-free (U)HPLC columns with a PEEK-lining and PEEK frits.

Their main difference is their hydrophobicity: YMC-Accura Triart columns are less hydrophobic compared to YMC-Triart metal-free columns. Further, YMC-Accura Triart columns offer a more robust end fitting without any preferences regarding connection – in contrast to the PEEK-lined columns which require special connecting options such as MarvelXACT connectors. These are also available with a bioinert PEEK layer or a PEEKsil capillary.

Three different oligonucleotide mixtures were analysed using a YMC-Triart Diol-HILIC column with conventional stainless steel hardware, and the bioinert column options: a YMC-Triart Diol-HILIC metal-free column and a coated YMC-Accura Triart Diol-HILIC column. The comparison demonstrates that using a bioinert column results in higher peak areas, higher intensities, and less tailing especially when using the bioinert YMC-Accura Triart Diol-HILIC column.

Table 1: Chromatographic conditions.

Columns:	YMC-Triart Diol-HILIC (1.9 µm, 12 nm) 150 x 2.1 mm ID (standard hardware) YMC-Triart Diol-HILIC metal-free (1.9 µm, 12 nm) 150 x 2.1 mm ID (PEEK-lined hardware) YMC-Accura Triart Diol-HILIC (1.9 µm, 12 nm) 150 x 2.1 mm ID (bioinert coated hardware)
Part Nos.:	TDH12SP9-15Q1PT TDH12SP9-15Q1PTP TDH12SP9-15Q1PTC
Eluents:	50 mM ammonium acetate (pH 6.9) acetonitrile
Gradient	75–45%B (0–30 min)
Flow rate:	0.3 mL/min
Temperature:	40 °C
Detection:	UV at 260 nm, photodiode array (PDA) detector equipped with 500 nL analytical flow cell (10 mm)
Injection:	2 µL
System:	Bioinert (Acquity H-Class Bio)
Sample:	Deoxythymidine oligonucleotide mixtures: dT15-35 (2 µM) and dT40-100 (2 µM) RNA oligonucleotide mixture: rU15-30 (2 µM)

Pre-conditioning of the stainless steel, PEEK-lined and bioinert coated column

Pre-conditioning is a typical procedure when working with stainless steel columns. Using a bioinert column such as YMC-Accura Triart or YMC-Triart metal-free usually achieves great performance from the first injection at least when working with an IP-RP phase. However, HILIC phases still need some pre-conditioning even when a bioinert column is used. Despite this, the increase of peak area is not as distinct as in IP-RP for the same oligonucleotide samples (up to 440% vs. up to 4200%, respectively) [1].

Figure 1 shows the number of injections of short DNA oligonucleotides needed until a pre-conditioning is achieved. Even though the bioinert columns still require some pre-conditioning, the number of injections is significantly reduced. While 20 injections are necessary for the stainless steel column, 14 are required for the metal-free column.

The YMC-Accura column is already pre-conditioned after only 8 injections, with very little difference (less than 10% between initial and final peak areas).

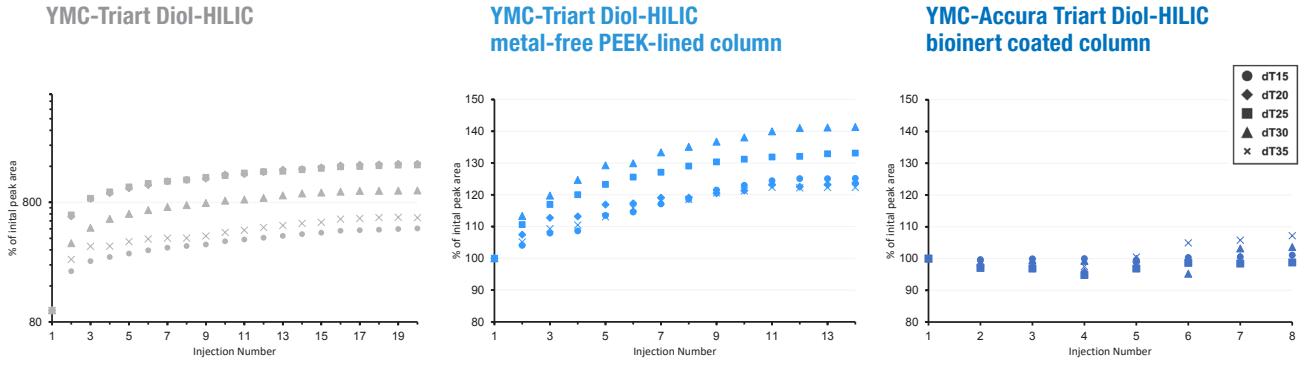


Figure 1: Consecutive injections of mixture dT 15-35 until complete conditioning is achieved using a stainless steel YMC-Triart Diol-HILIC column (left, [1]), a PEEK-lined YMC-Triart Diol-HILIC metal-free column (centre, [1]) and a bioinert coated YMC-Accura Triart Diol-HILIC column (right, [2]). 2 different systems were used: an Acquity Premier system [1] and an Acquity H-Class Bio system.

Comparison of the three hardware types after pre-condioning

After conditioning and analysing the short DNA oligonucleotide mixture dT15-35, longer DNA oligonucleotides dT40-100 and short RNA oligonucleotides rU15-30 were also analysed with all three columns. Figure 2 shows the results of the stainless steel column compared to the metal-free column and the bioinert coated column. Higher sensitivities, peak areas and less peak tailing are achieved using the bioinert column options.

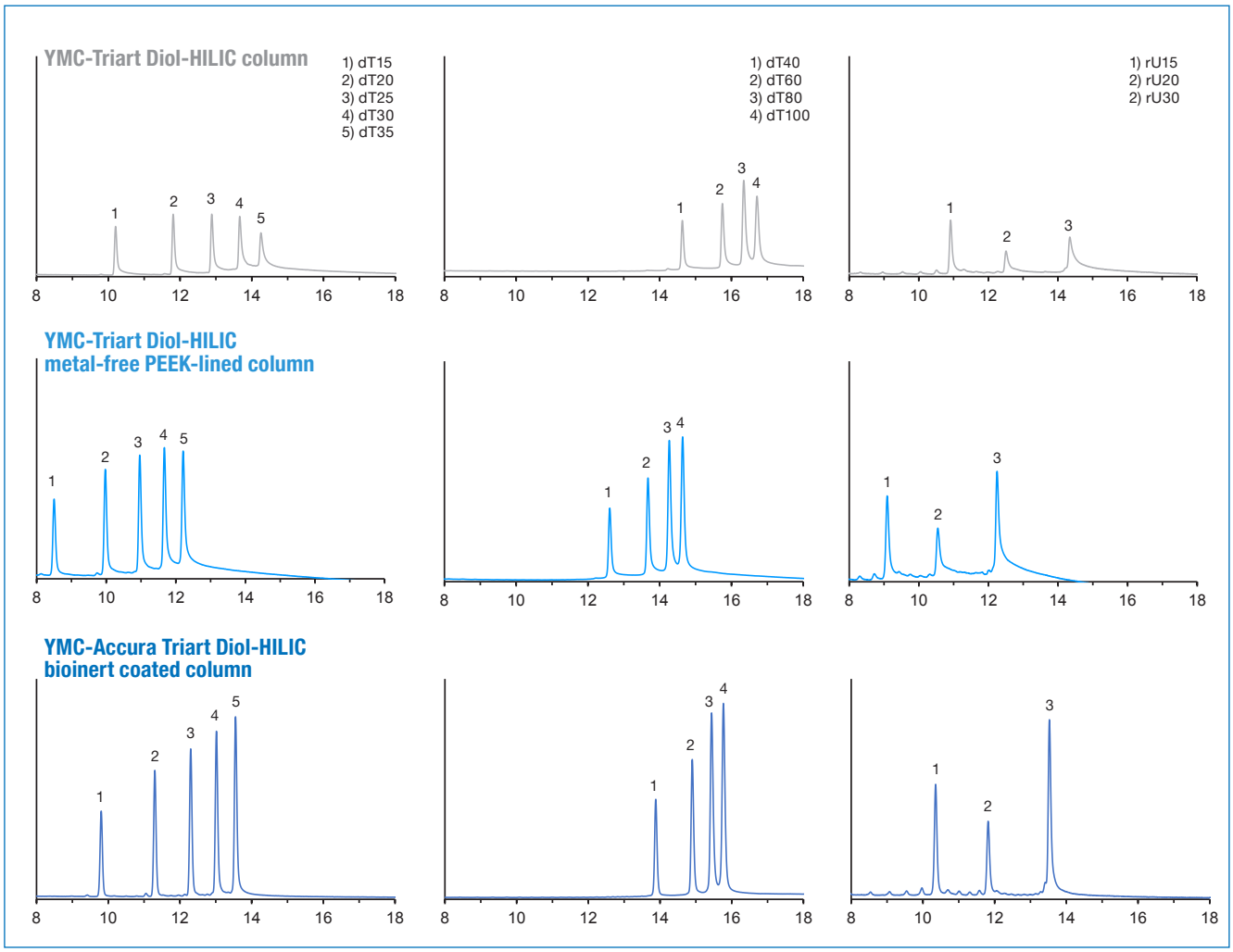


Figure 2: Analysis of dT15-35 (left), dT40-100 (centre) and rU15-30 (right) using a conventional YMC-Triart Diol-HILIC column (top), a YMC-Triart Diol-HILIC metal-free column (middle) and a YMC-Accura Triart Diol-HILIC column (bottom) [2].

Comparing the two bioinert column options the less hydrophobic bioinert coated YMC-Accura Triart Diol-HILIC column shows the best peak shapes. When using the PEEK-lined YMC-Triart Diol-HILIC metal-free column some peak tailing still occurs, while sharp peaks and even higher sensitivities are provided by the bioinert coated YMC-Accura Triart Diol-HILIC column.

The clear retention shift which occurs with the metal-free column and shown in Figure 2, is due to the different nature of column hardware and the different packing procedure. The comparison of the peak areas emphasises that even after pre-conditioning of the stainless steel column a bioinert column is the far better choice. Figure 3 shows the distinct difference of up to 42% less peak area achieved with the conventional column hardware.

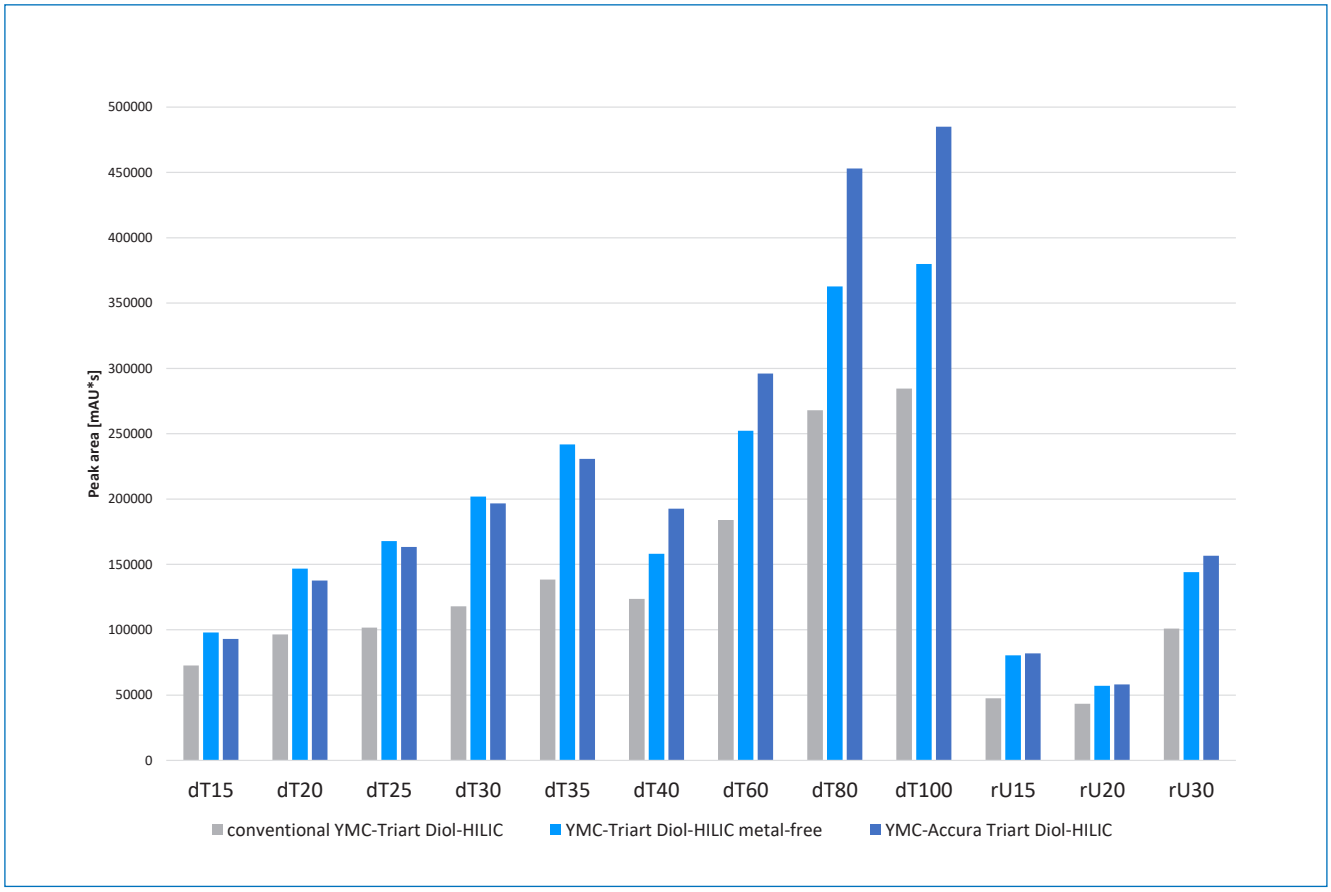


Figure 3: Peak areas achieved with the stainless steel YMC-Triart Diol-HILIC column compared with the PEEK-lined YMC-Triart Diol-HILIC metal-free column and the bioinert coated YMC-Accura Triart Diol-HILIC column [2].

Roughly the same peak areas are achieved when analysing short oligonucleotides using the bioinert column options. Greater differences are observed when analysing longer oligonucleotides. In this case using the bioinert coated YMC-Accura Triart Diol-HILIC column provides significantly larger peak areas.

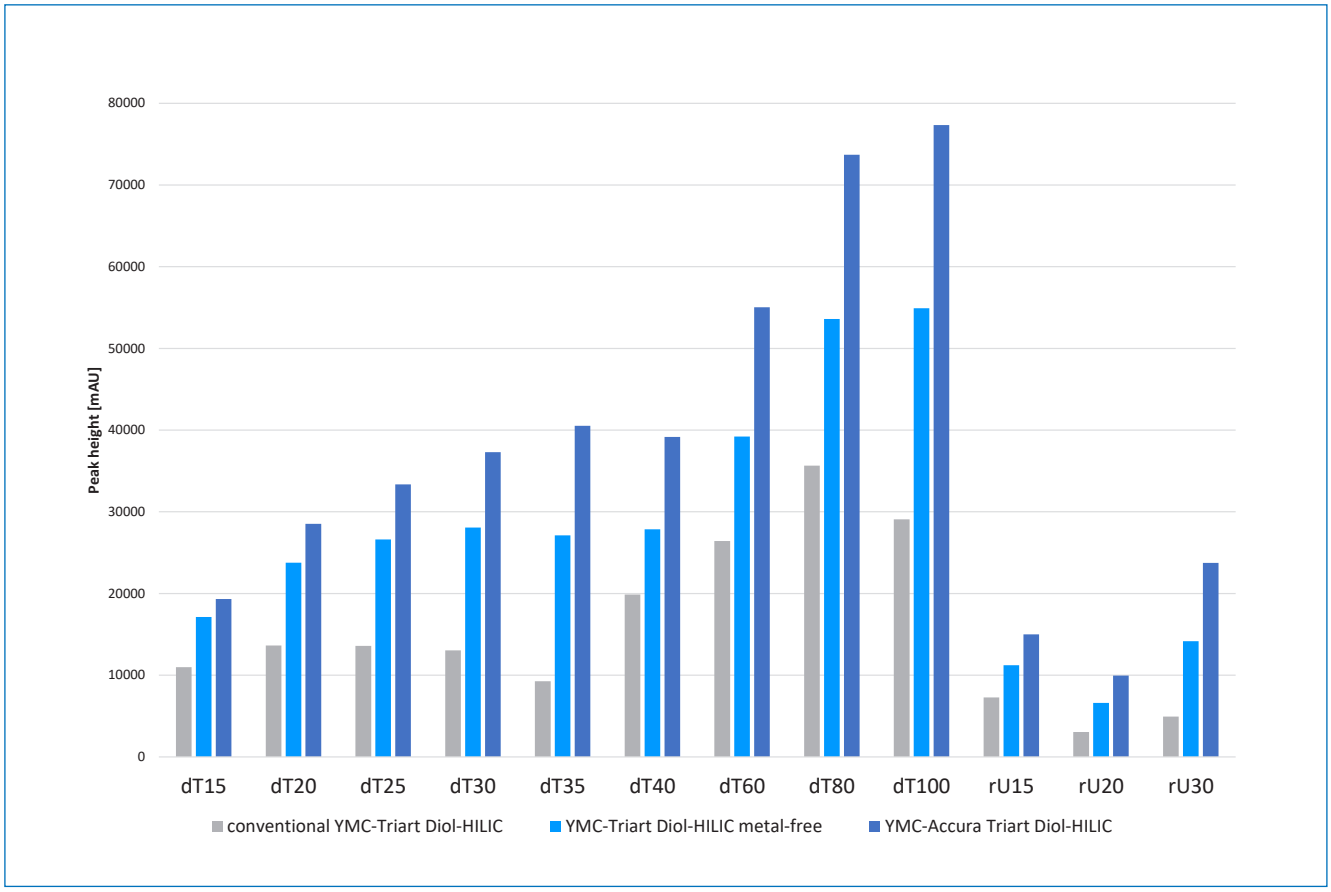


Figure 4: Peak heights achieved with the stainless steel YMC-Triart Diol-HILIC column compared with the PEEK-lined YMC-Triart Diol-HILIC metal-free column and the bioinert coated YMC-Accura Triart Diol-HILIC column [2].

These results are further confirmed regarding the peak heights (see Figure 4). The YMC-Accura Triart Diol-HILIC column provides a much better sensitivity especially for RNA and longer DNA oligonucleotides, also compared to the PEEK-lined YMC-Triart metal-free column.

Therefore, the bioinert coated YMC-Accura Triart Diol-HILIC column is the best choice to obtain excellent peak shapes, sensitivities, and peak areas for the analysis of sensitive substances such as oligonucleotides via HILIC.

References:

[1] H. Lardeux, A. Goyon, K. Zhang, J.M. Nguyen, M.A. Lauber, D. Guillarme, V. D'Atri, The impact of low adsorption surfaces for the analysis of DNA and RNA oligonucleotides, J. Chromatogr. A 1677 (2022) 463324.

[2] Application data by courtesy of Honorine Lardeux, Institute of Pharmaceutical Sciences of Western Switzerland (University of Geneva), Geneva, Switzerland.