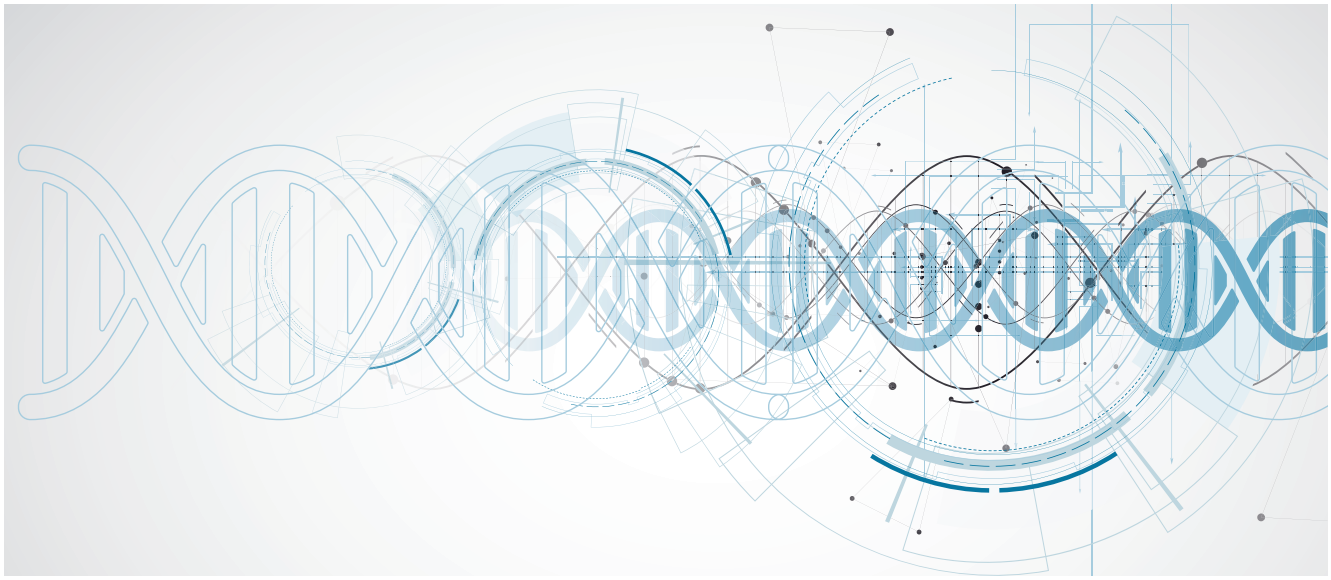
HILIC analysis of oligonucleotides using bioinert columns

Oligonucleotides have become more important for medical applications, currently they are used to treat several diseases. Therefore, robust and highly sensitive analytical methods are required. 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 to AEX, hydrophilic interaction liquid chromatography (HILIC) can provide an alternative approach due to the highly polar nature of oligonucleotides.



The materials conventionally used 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 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 require repeated application. Furthermore, a change of sample can lead to non-specific adsorption again.

A much more robust and simple solution is to use a bioinert system and column hardware. Specifically, the column hardware needs to be paid attention, as it represents more than 70% of the surface that the analytes are in touch with. Consequently, a bioinert column body and frits will bring a distinct improvement in performance. Therefore, YMC provides the bioinert YMC-Accura Triart series of columns which have a bioinert coating on the column body and frits.

In this application note three different oligonucleotide mixtures were analysed using a YMC-Triart Diol HILIC column with conventional stainless steel hardware and the bioinert column option YMC-Accura Triart Diol-HILIC column. The comparison demonstrates that using a bioinert coated column results in higher peak areas, higher intensities and less tailing.

Table 1: Chromatographic conditions.

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) and dT40-100 (2µM) RNA oligonucleotides: rU15-30 (2µM)
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, at least when working with an IP-RP phase. However HILIC phases still need some pre-conditioning even when a bioinert column is used. Figure 1 shows the number of injections of short DNA oligonucleotides needed until full conditioning is achieved. Even though these bioinert columns still need conditioning 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.

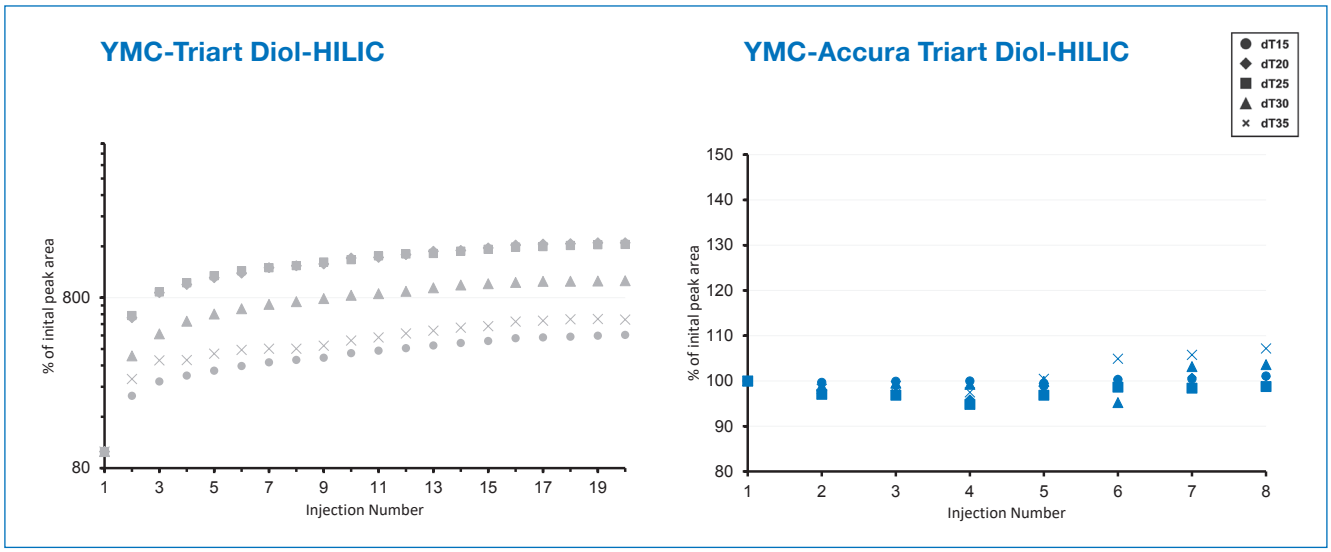


Figure 1: Consecutive injections of a mixture of dT15–35 until conditioning is achieved using a conventional YMC-Triart Diol-HILIC column (left, [1]) and a YMC-Accura Triart Diol-HILIC (right, [2]) and two different bioinert systems.

After conditioning and analysing the short DNA oligonucleotide mixture of dT15-35, longer DNA oligonucleotides dT40-100 and short RNA oligonucleotides rU15-30 were analysed. Figure 2 shows the results of the regular stainless steel YMC-Triart Diol-HILIC column and the YMC-Accura Triart Diol-HILIC column on a bioinert system. Higher sensitivities, peak areas and less tailing are achieved using the bioinert YMC-Accura Triart Diol-HILIC column. Non-specific adsorption did not vary according to length, even though the adsorption is usually higher for longer oligonucleotides in IP-RP.

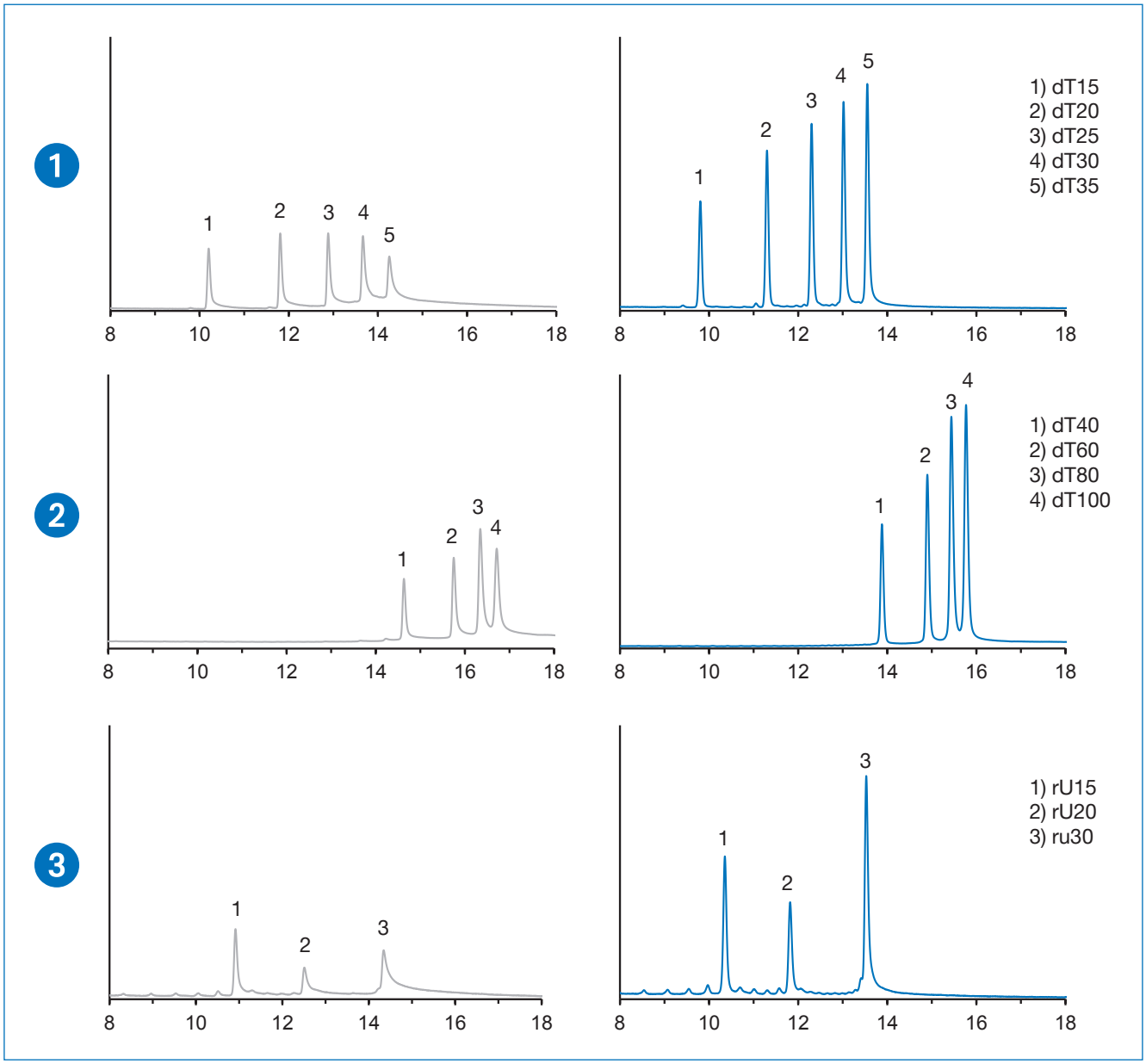


Figure 2: Analysis of dT15-35 (1), dT40-100 (2) and rU15-30 (3) using a regular YMC-Triart Diol-HILIC (left, analogous to [1]) and a bioinert coated YMC-Accura Triart Diol-HILIC (right, [2]) and a bioinert system.

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.