When HILIC is a suitable alternative to IP-RP for the analysis of oligonucleotides

Oligonucleotides have taken a more important role in the market as they cover a broad field of applications ranging from molecular biology to therapy and diagnosis. Therefore, a variety of robust and highly sensitive analytical methods are required. Hydrophilic interaction liquid chromatography (HILIC) represents an orthogonal method to the current standard ion pair reversed phase liquid chromatography (IP-RP) and anion exchange chromatography (AEX). Due to the highly polar nature of oligonucleotides HILIC is ideally suited for their analysis. This expert tip compares the analysis of oligonucleotides of different length, type and chemical modification using HILIC and IP-RP mode.

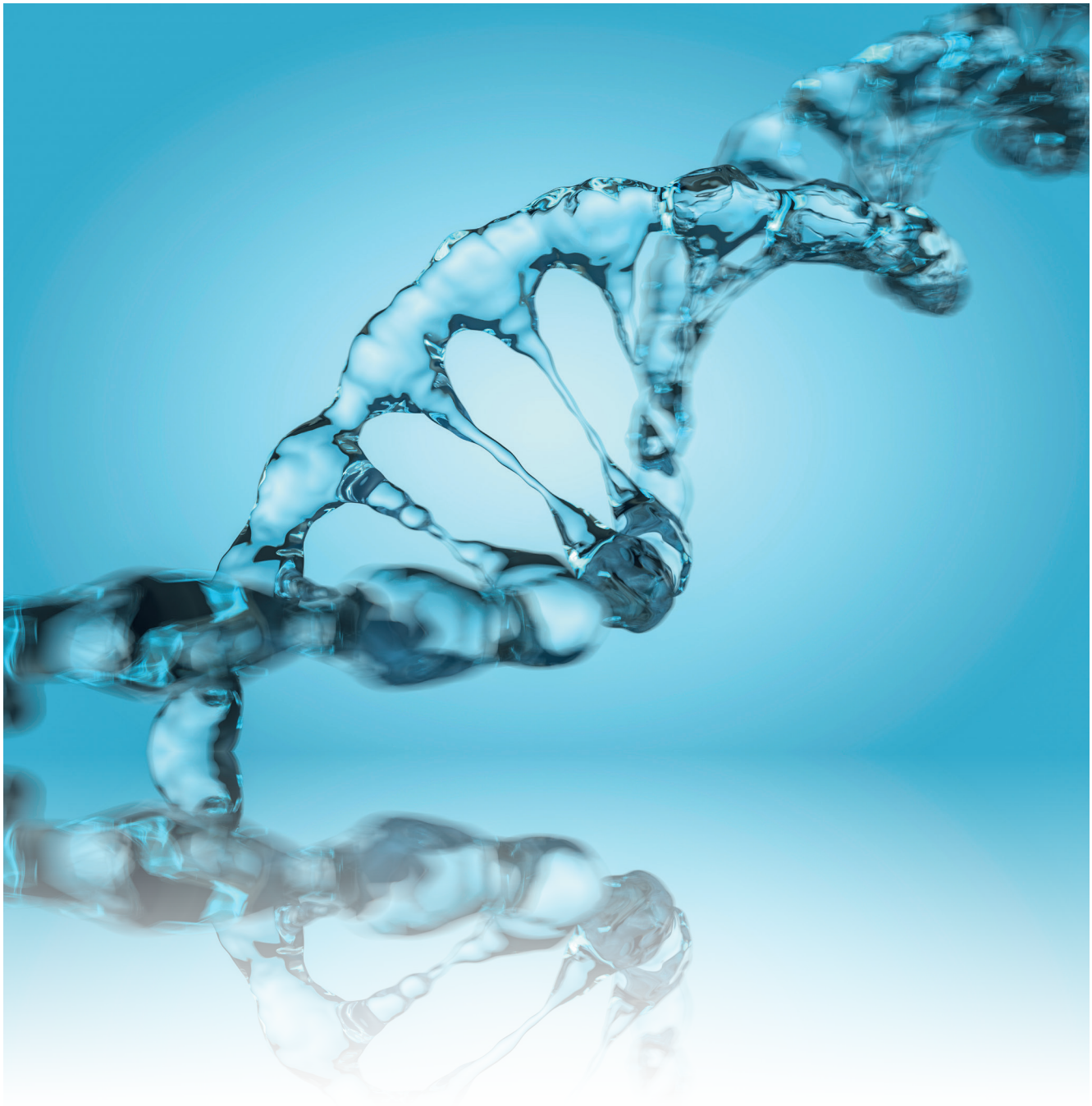

Table 1: HILIC conditions.

Column:	YMC-Accura Triart Diol-HILIC (3 µm, 12 nm) 150 x 2.1 mm ID
Part No.:	TDH12S03-15Q1PTC
Eluent:	A) 50 mM $HCOONH_4$ (pH 6.5)/acetonitrile (30/70)
	B) 50 mM $HCOONH_4$ (pH 6.5)
Gradient:	7.1–21.4%B (0–20 min), 21.4%B (20–25 min)
Flow rate:	0.21 mL/min
Temperature:	40 °C
Injection:	1 µL (1.8 nmol/mL)
	2 µL (dT DNA, each 2.0 nmol/mL)
Detection:	UV at 260 nm

Table 2: IP-RP conditions.

Column:	YMC-Accura Triart Bio C18 (1.9 µm, 3 nm) 50 x 2.1 mm ID
Part No.:	TA30SP9-05Q1PTC
Eluent:	A) 15 mM TEA* - 400 mM HFIP**
	B) methanol
Gradient:	8–23%B (0–20 min), 80%B (20–25 min)
Flow rate:	0.42 mL/min
Temperature:	40 °C
Injection:	1 µL (1.8 nmol/mL)
	1 µL (dT DNA, each 2.0 nmol/mL)
Detection:	UV at 260 nm

**triethylamine **1,1,1,3,3,3-hexafluoropropan-2-ol*

Analysis of different chain length

In a first test deoxythymidine (dT DNA) oligonucleotides of different length (10, 15 and 20mer) were analysed using the conditions described in table 1 and 2. In both HILIC and IP-RP mode the oligonucleotides are separated by length starting with the shortest oligonucleotide (figure 1). Longer oligonucleotides are retained more strongly in both modes, but the increase in retention is greater in IP-RP mode.

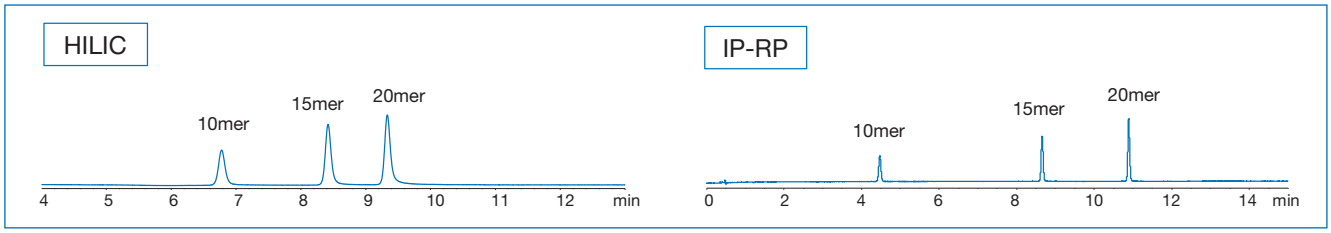

Figure 1: Analysis of dT DNA using HILIC (left) and IP-RP (right).

Analysis of DNA and RNA

Here a 20mer of DNA and RNA was examined. The sequence is identical except for the replacement of thymine by uracil in RNA. In HILIC mode, DNA and RNA show similar retention, while in IP-RP mode, DNA is retained much more than RNA. Therefore, IP-RP is more suitable for the separation of DNA and RNA of similar sequences.

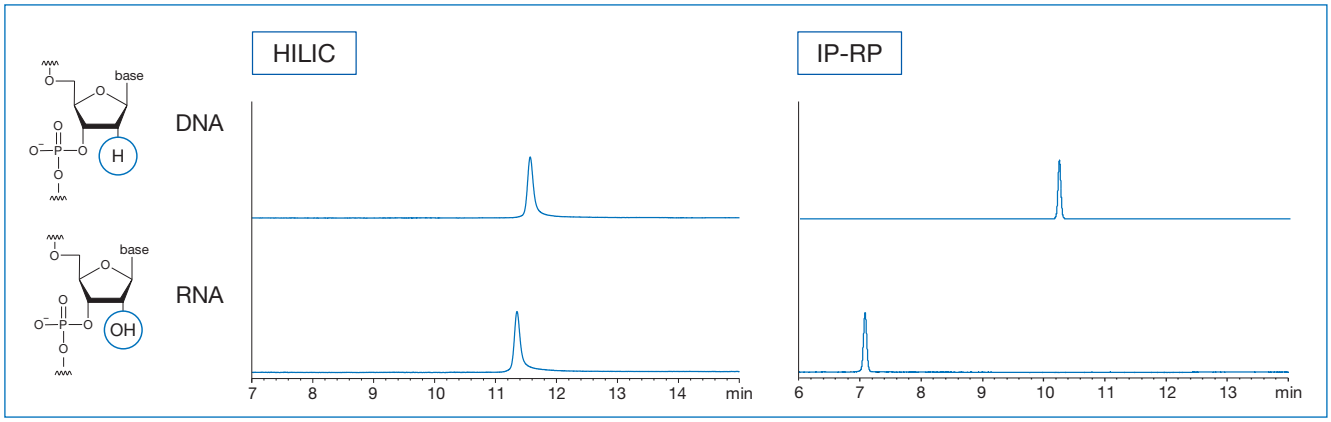

Figure 2: Analysis of DNA (top) and RNA (bottom) with the same sequence using HILIC (left) and IP-RP (right).

Analysis of different chemical modifications

Figure 3 shows the analysis of an RNA (20mer) and its chemical modifications, where all residues are phosphorothioated (PS RNA) or methoxylated (2'-OMe). Both phosphorothioation and methoxylation lead to an increased hydrophobicity of the oligonucleotide, which results in less retention in HILIC mode and more retention in IP-RP mode. In HILIC mode PS RNA shows a stronger shift to lower retention time than 2'-OMe. The additional methyl moieties of 2'-OMe lead to significantly higher retention in IP-RP compared to PS RNA and unmodified RNA.

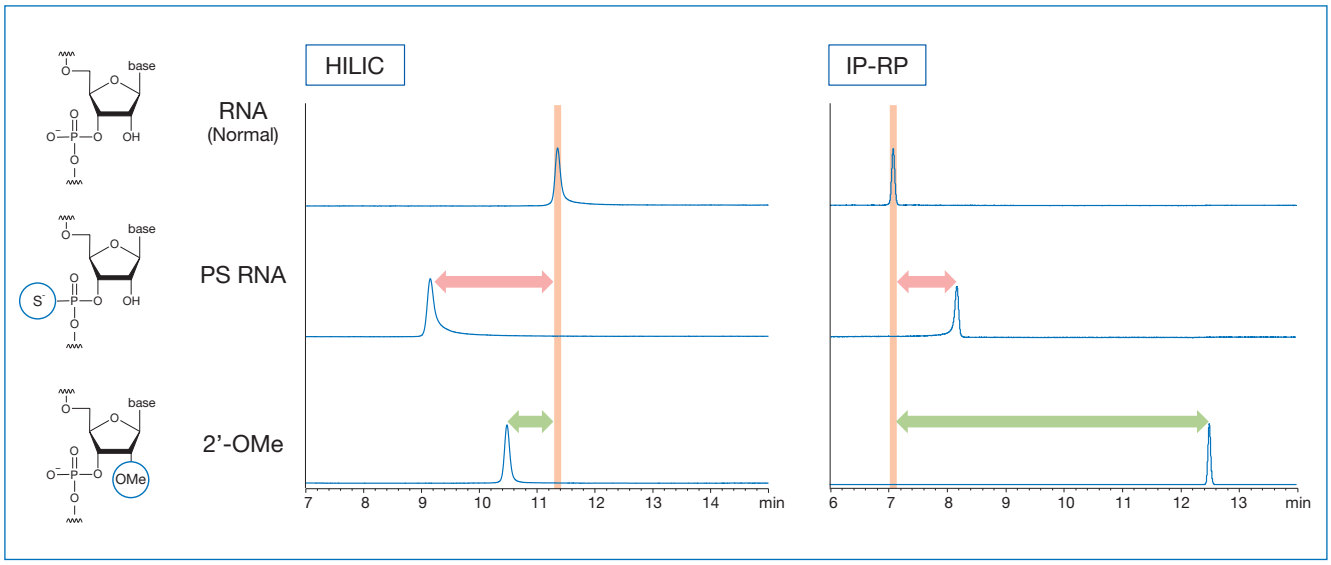

Figure 3: Analysis of RNA (top), phosphorothioated RNA (middle) and methoxylated RNA (bottom) with the same sequence using HILIC (left) and IP-RP mode (right).

Conclusion

Both chromatographic modes are suitable for separating oligonucleotides of different lengths and chemical modifications. HILIC can be an orthogonal method to IP-RP in terms of oligonucleotide analysis. The decision to use one or the other depends on the sample and the goal of the analysis. While IP-RP seems better suited for the separation of DNA and RNA, HILIC is a good alternative for detection using a mass spectrometer.