Chromatography Profile Analysis of Phosphorothiolated Oligos

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Introduction

The ultimate goal of most antisense research is to develop therapeutic agents for diseases such as diabetes, cancer, and HIV/AIDS. Phosphorothiolated oligonucleotides (S-oligos) are often the molecules of choice because of their resistance to cellular nuclease degradation. To avoid or minimize toxicity levels in cell or animal work, S-oligos are usually administered with the highest homogeneity possible.

Purification of S-oligos is not without problems, though. The presence of sulfur creates a stereogenic alpha-phosphorus (**Figure 1**), and the resulting diastereomers have subtly shifted elution times during chromatography[1]. This inherent characteristic of S-oligos complicates their analytical HPLC profiles, which are used to determine purity.

In this note, we illustrate how HPLC profiles are affected by incorporating different numbers of phosphorothiolated bridges into a model oligo sequence. Analytical techniques employed in this study include reverse-phase HPLC (RP-HPLC), ion-exchange HPLC (IE-HPLC), and electrospray ionization mass spectrometry (ESI-MS).

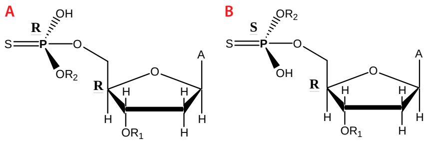

Figure 1. Two diastereomers of an S-oligo with one phophorothiolated bridge

Materials And Methods

Oligo synthesis

A 20mer oligo, CATAAGATCAGTCAGGTTAT, was modified with 0, 1, 2, 5, 10, 15, and 19 phosphorothiolated bridges using standard phosphoramidite chemistry. Following synthesis, crude oligos were purified on reverse phase cartridges, dried, and analyzed.

Quality assessment

All oligos were analyzed by RP-HPLC, IE-HPLC, and ESI-MS using standard QC methods and protocols.

Results And Discussion

The goal of this study was to show how oligo chromatography profiles are affected by the addition of phosphorothiolated bridges. The results are shown in **Table 1**. Although the ESI-MS data indicate that the target product was predominant in all 16 samples, the RP-HPLC and IE-HPLC profiles of each oligo vary greatly. With the incremental addition of phosphorothiolated bridges into the model sequence, chromatography peaks start to split and further broaden

Table 1

#	# Phosphorothiolated Bridges	Name	Sequence (5′-3′)	RP-HPLC Peak Split(s)	IE-HPLC Peak Split(s)
1	0	SO	CATAAGATCAGTCAGGTTAT	1	1
2	1	S1A	cATAAGATCAGTCAGGTTAT	1	2
3	1	S1B	CAtAAGATCAGTCAGGTTAT	2	2
4	1	S1C	CATAAgATCAGTCAGGTTAT	1	1
5	1	S1D	CaTAAGATCAGTCAGGTTAT	1	2
6	1	S1E	CATAAGATCAGTCAGGTtAT	2	2
7	2	S2A	caTAAGATCAGTCAGGTTAT	2	>2
8	2	S2B	CATAAGATCagTCAGGTTAT	1	2
9	2	S2C	CATAAGATCAGTCAGGTtaT	2	1
10	5	S5A	cataaGATCAGTCAGGTTAT	>4	2
11	5	S5B	CATAAGATcagtcAGGTTAT	>3	>3
12	5	S5C	CATAAGATCAGTCAggttaT	broad peak	2
13	10	S10A	cataagatcaGTCAGGTTAT	broad peak	broad peak
14	10	S10B	CATAAGATCagtcaggttaT	broad peak	broad peak
15	15	S15A	CATAagatcagtcaggttaT	broad peak	broad peak
16	19	S19A	cataagatcagtcaggttaT	broad peak	NA*

Table 1. The 16 oligo sequences analyzed.
**Product did not elute possibly due to the hydrophobicity of the sequence.*

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Figure 2, 3, and 4 demonstrate how the RP-HPLC and IE-HPLC profiles differ for S0, S1B, and S10A even though the ESI-MS profiles show that all three were of good purity. S0 had no phosphorothiolated bridges, therefore both the RP-HPLC and IE-HPLC profiles (**Figure 2**) show only one sharp peak.

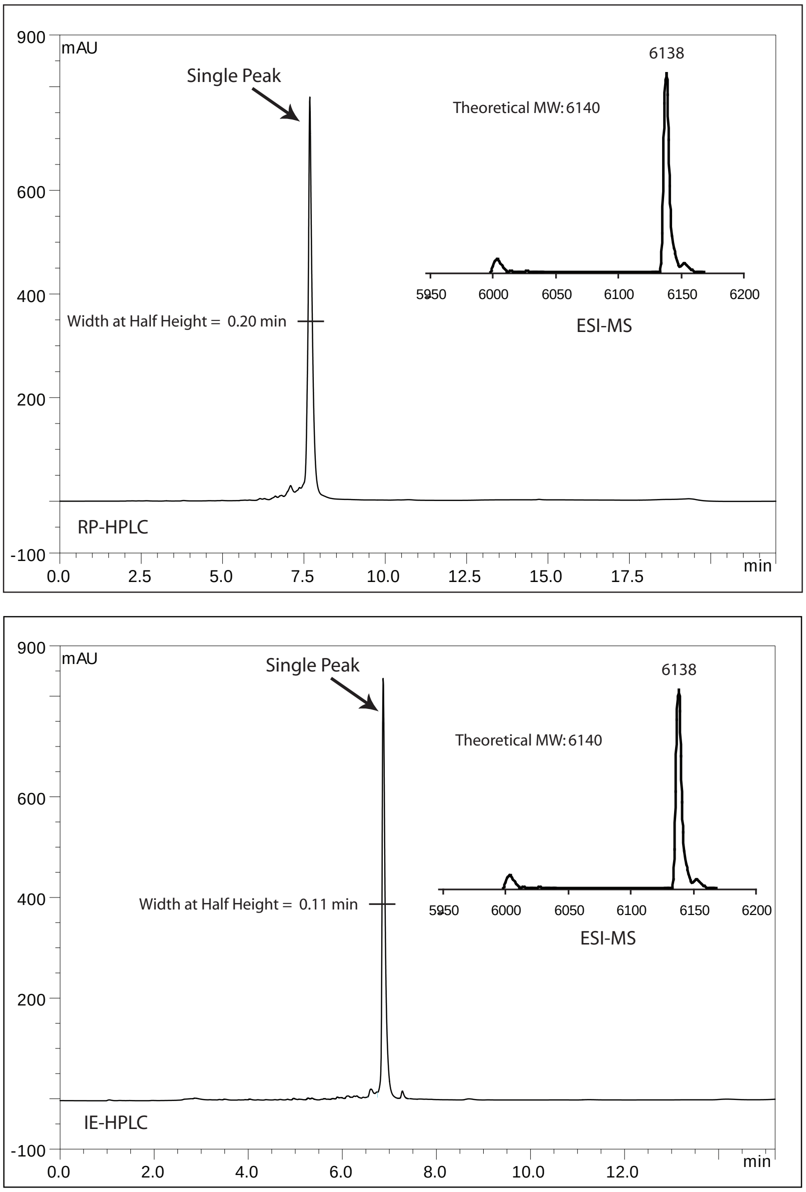

Figure 2. S0 RP-HPLC (top) and IE-HPLC (bottom) data. Inset contains the ESI-MS data for the same sample.

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S1B, which contained one phosphorothiolated bridge, had two possible diastereomers. The RP-HPLC profile shows two partially resolved peaks, which is even more apparent in the IE-HPLC profile (**Figure 3**).

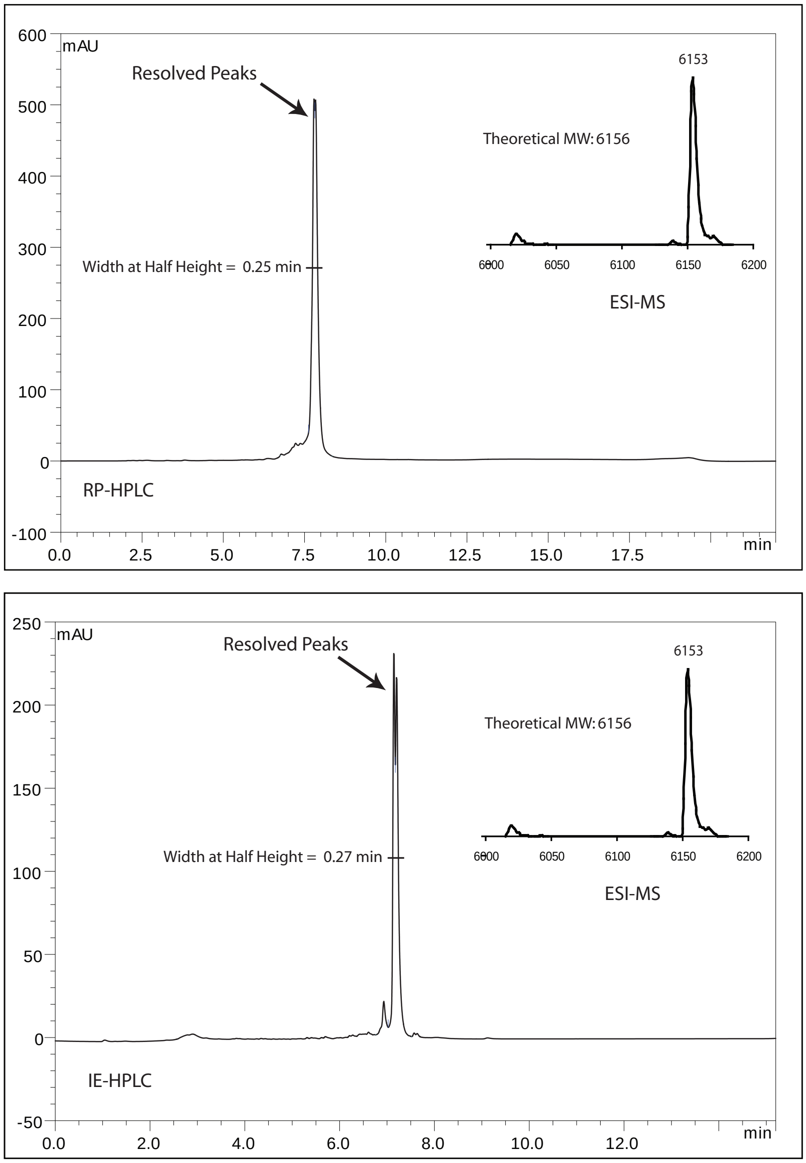

Figure 3. S1B RP-HPLC (top) and IE-HPLC (bottom) data. Inset contains the ESI-MS data for the same sample.

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S10A had ten phosphorothiolated bridges. In theory, it should have had 210 diastereomers present. As observed in (**Figure 4**), the lack of resolution manifests itself in the form of a broad peak in both the RP-HPLC and IE-HPLC profiles. The retention time for S10A likely increased due to the hydrophobicity of the sulfur atom.

Conclusions

Phosphorothiolated bridges affect the resolution of oligo chromatography profiles. Split or broadened product peaks are likely to be observed in analytical RP-HPLC and IE-HPLC profiles due to the presence of diastereomers. In cases like the ones presented in the report, ESI-MS will reveal purity to be equivalent to the area under the observed major HPLC peaks.

References

1. Burgers, P., and Eckstein, F. 1978. PNAS 75:4798–4800.

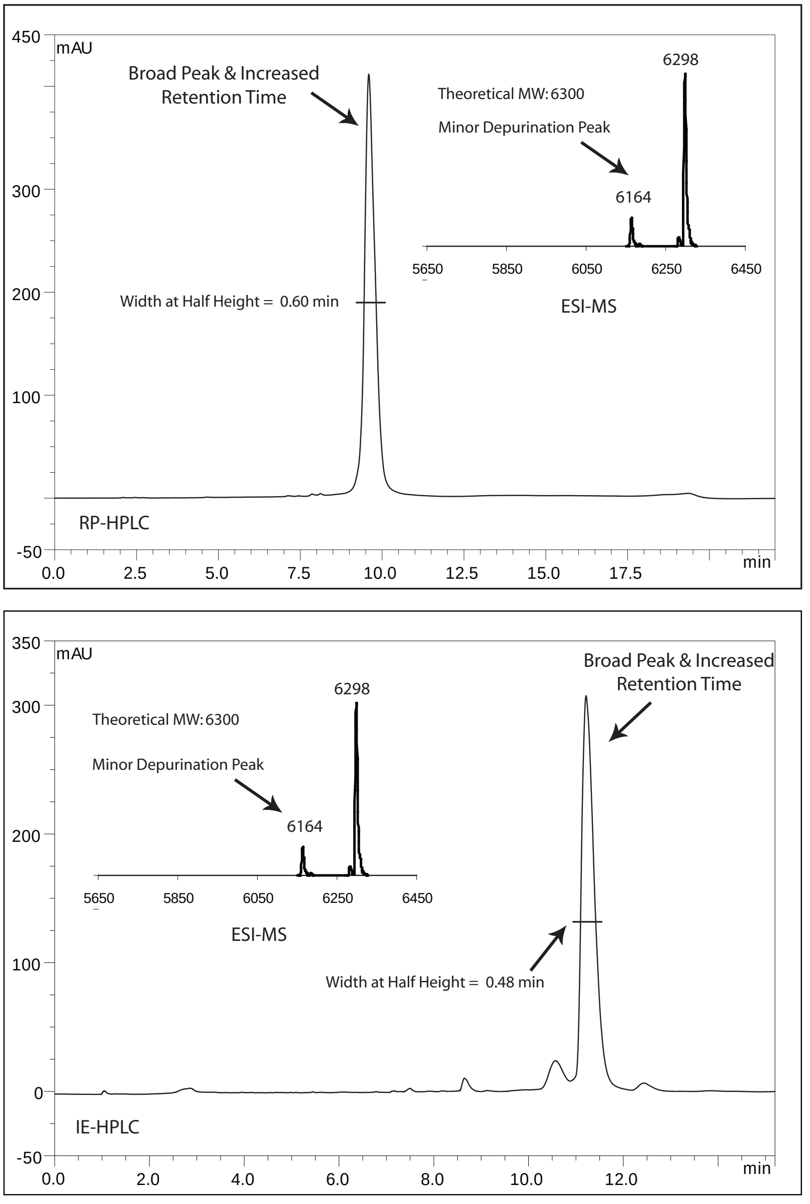

Figure 4. S10A RP-HPLC (top) and IE-HPLC (bottom) data. Inset contains the ESI-MS data for the same sample.

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