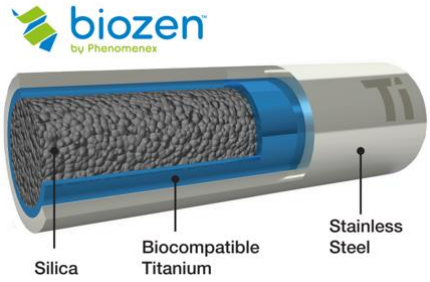

The Effect of Column Hardware on the Analysis of Synthetic Oligonucleotides by LC-MS

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Overview

Therapeutic oligonucleotides represent a recent breakthrough in the pharmaceutical industry. Improved reliability to synthetic routes coupled with new drug delivery technologies has given a heightened level of interest in these clinically interesting targets. Chromatographically, they present a challenge due to their polar nature and phosphodiester backbone. As such, ion-pair reversed phase chromatography (IP-RPLC) is necessary, typically using a mobile phase consisting of an alkylamine ion pair and a perfluorinated alcohol such as Hexafluoro-2-propanol.

Although optimizing the IP-RPLC conditions can improve chromatographic separation, this does not address oligonucleotide propensity to chelate with trace metal in the stainless steel of traditional HPLC column hardware. These non-specific interactions are often associated with analyte loss, poor peak shape, and irreproducibility. In this application note, we demonstrate the positive effect BioTi, a multi-layered titanium tube which provides a bio-inert flow path, has on chromatography and electrospray ionization of two synthetic oligos compared to traditional stainless steel LC columns.

When analyzing a double-stranded RNA by traditional column hardware, presumed nonspecific interactions are observed (**Figure 1**). This results in a bimodal peak for the later eluting anti-sense strand, with full scan and MS/MS data confirming this to be the same analyte. Conversely, the use of bio-inert hardware drastically improves resolution of the sense and anti-sense strand peaks. MS data in **Figure 2** confirms that BioTi hardware mitigates the effects of non-specific binding and subsequently addresses the bimodal chromatographic artifact. We also observe greater sensitivity when run on the BioTi hardware, showing its utility for oligonucleotide characterization using relatively low concentrations of Hexafluoro-2-propanol and ion-pairing reagents.

This improvement in ionization is also observed with single stranded nucleic acids. The analysis of Nusinersen shows a marked improvement with a full order of magnitude in sensitivity when the method was run on a Biozen Oligo column (packed BioTi) compared with a stainless steel column packed to a similar packing efficiency, and with the same batch of media (**Figure 3**). In summary, by minimizing non-specific interactions, the use of BioTi hardware not only serves to improve chromatographic performance and reproducibility of oligonucleotide separations, but also maximizes analyte recovery and sensitivity enabling both consistent quantitation and characterization.

LC Conditions – Figure 1 and 2

Columns: Biozen 2.6 µm Oligo (BioTi™)
Clarity™ 2.6 µm Oligo-XT (stainless steel)
Dimensions: 100 x 2.1 mm
Part No.: 00D-4790-AN (Biozen)
00D-4746-AN (Clarity)
Mobile Phase: A: 4 mM Triethylamine in Water + 12.5 mM Hexafluoro-2-propanol
B: 4 mM Triethylamine in Methanol + 12.5 mM Hexafluoro-2-propanol

Gradient:

Time (min)	%B
0	5
2	5
16	30
16.1	95
20	95
20.1	5

Flow Rate: 0.3 mL/min
Injection Volume: 2 µL (12.5 ng)
Temperature: 55 °C
Instrument: Shimadzu® LC-20A Prominence®
Detection: TOF-MS
Detector: SCIEX® TripleTOF® 6600

LC Conditions – Figure 3

Columns: Biozen 2.6 µm Oligo (BioTi)
Clarity 2.6 µm Oligo-XT (stainless steel)
Dimensions: 100 x 2.1 mm
Part No.: 00D-4790-AN (Biozen)
00D-4746-AN (Clarity)
Mobile Phase: A: 10 mM Hexylamine in Water + 12.5 mM Hexafluoro-2-propanol
B: 10 mM Hexylamine in Methanol + 12.5 mM Hexafluoro-2-propanol

Gradient:

Time (min)	%B
0	25
2	25
16	75
16.1	95
20	95
20.1	25

Flow Rate: 0.3 mL/min
Injection Volume: 2 µL (12.5 ng)
Temperature: 55 °C
Instrument: Shimadzu LC-20A Prominence
Detection: TOF-MS
Detector: SCIEX TripleTOF 6600

Figure 1. siRNA on a Clarity™ 2.6 µm Oligo-XT Column (Standard Stainless Steel Hardware).

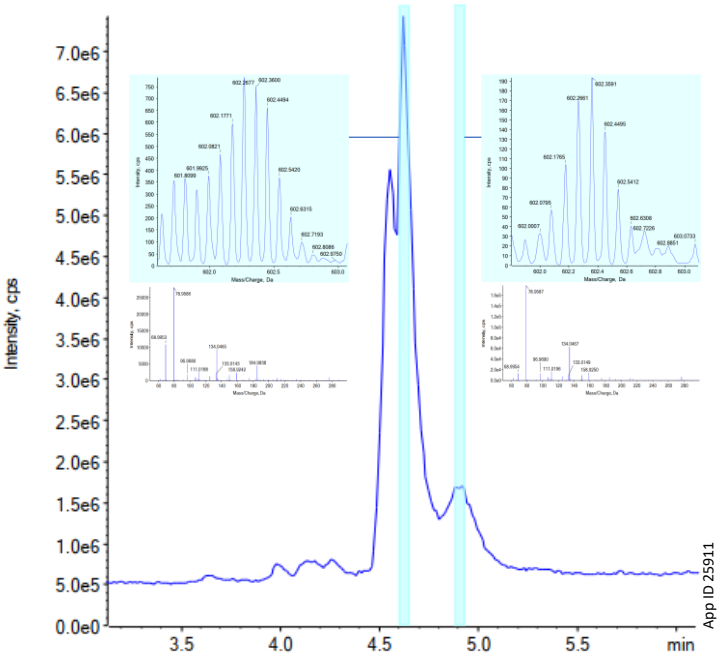


Figure 2. siRNA on a Biozen™ 2.6 µm Oligo Column (BioTi™ Bio-inert Hardware).

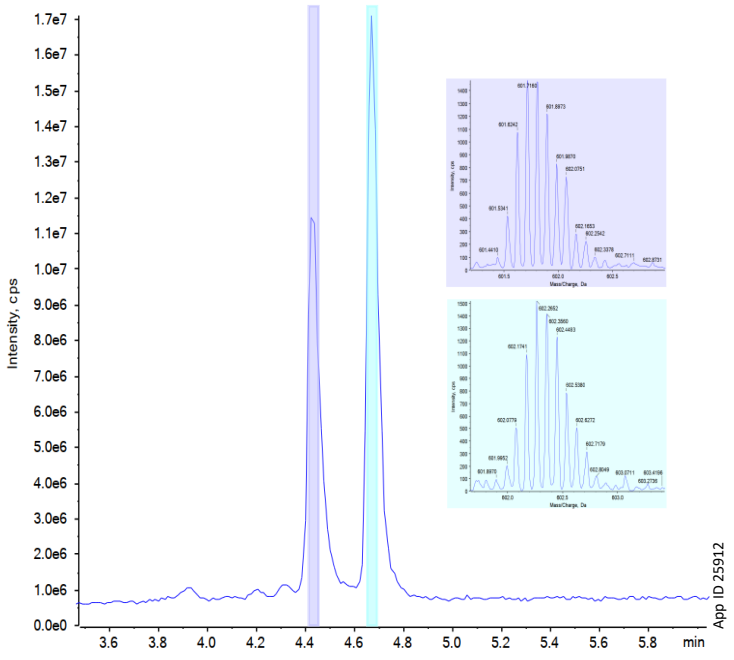
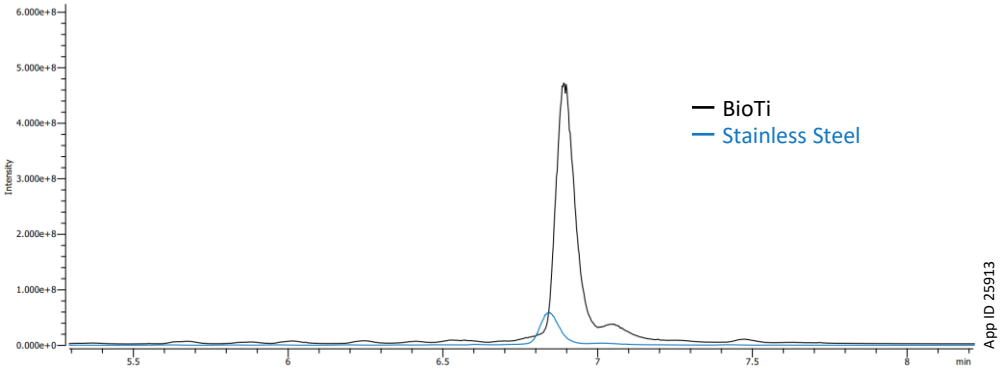


Figure 3. Nusinersen on Biozen 2.6 µm Oligo Column (Bio-inert) Compared to Clarity 2.6 µm Oligo-XT Column (Stainless Steel).



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