

# APPLICATIONS

## 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 mobile phase consisting of an alkylamine ion pair and perfluorinated alcohol such as HFIP.

Although optimizing the IP-RPLC composition can improve chromatographic separation, this does not address oligonucleotide propensity to chelate with trace metal in the stainless steel of traditional HPLC column hardware. Here, we investigate the effect of chromatography and electrospray ionization of two synthetic oligos, comparing traditional stainless steel to BioTi™, a multi-layered titanium tube which provides a bio-inert flow-path.

When analyzing a double-stranded RNA by LC-MS, presumed non-specific interactions are observed, as shown in **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 gives a clear separation of the sense and anti-sense strand and MS data in **Figure 2** confirms little or no effect is seen due to the BioTi hardware. We also observe greater sensitivity, with peak heights and areas improved, when run on the BioTi, showing its utility for oligonucleotide characterization using relatively low concentrations of HFIP and ion-pair.

This improvement in ionization is also observed with single stranded nucleic acids. The analysis of Nusinersen shows a marked improvement in sensitivity with BioTi; here we observed a full order of magnitude improvement in sensitivity when the method was run on bioZen Oligo (packed in BioTi) compared with a stainless steel column packed to the similar packing efficiency and with the same batch of media (**Figure 3**).

In summary, the use of bio-inert hardware can not only improve the chromatographic performance and consistency of oligonucleotides, but also potentially provide improvements in sensitivity, enabling both quantitation and characterization.

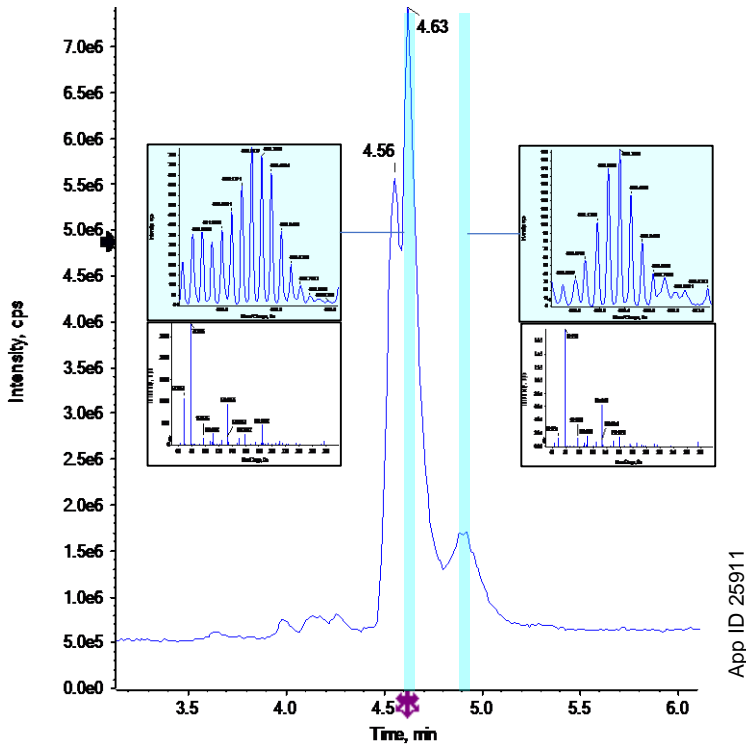
### LC Conditions (Figures 1 and 2)

**Columns:** bioZen™ 2.6 µm Oligo (*BioTi*)  
Clarity® 2.6 µm Oligo-XT (*Stainless Steel*)  
**Dimension:** 100 x 2.1 mm  
**Part No.:** [OOD-4790-AN](#)  
[OOD-4746-AN](#)  
**Mobile Phase:** A: 4 mM TEA in Water; 12.5 mM HFIP  
B: 4 mM TEA in Methanol; 12.5 mM HFIP  
**Gradient:** 5-30 % B in 14 minutes  
**Flow Rate:** 0.3 mL/min  
**Injection:** 12.5 ng  
**Temperature:** 55 °C  
**Detection:** TOF-MS  
**Sample:** siRNA

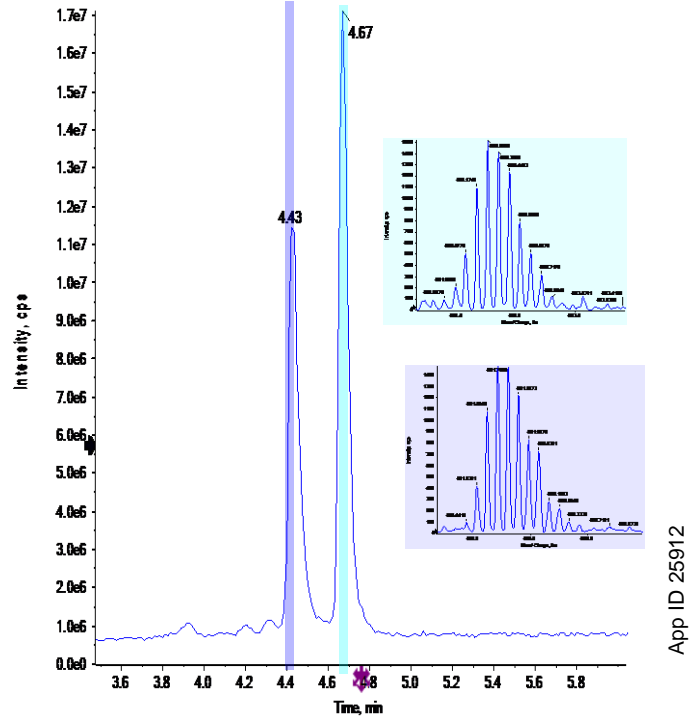
### LC Conditions (Figure 3)

**Columns:** bioZen 2.6 µm Oligo (*BioTi*)  
Clarity 2.6 µm Oligo-XT (*Stainless Steel*)  
**Dimension:** 100 x 2.1 mm  
**Part No.:** [OOD-4790-AN](#)  
[OOD-4746-AN](#)  
**Mobile Phase:** A: 10 mM Hexylamine in Water;  
12.5 mM HFIP  
B: 10 mM Hexylamine in Methanol;  
12.5 mM HFIP  
**Gradient:** 25-75 % B in 14 minutes  
**Flow Rate:** 0.3 mL/min  
**Injection:** 12.5 ng  
**Temperature:** 65 °C  
**Detection:** TOF-MS  
**Sample:** Nusinersen

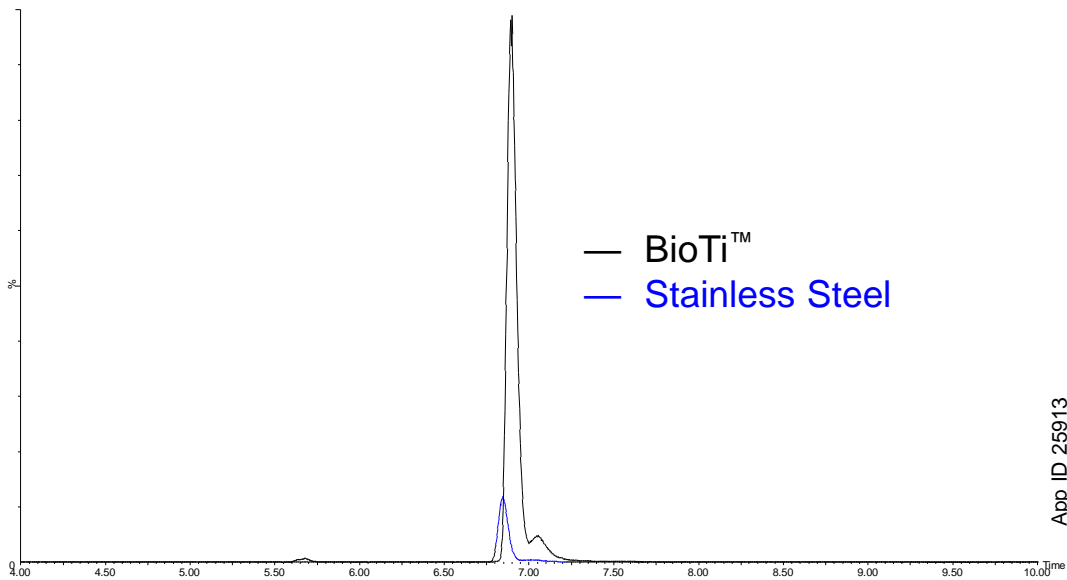
**Figure 1. LC-MS Analysis of siRNA using Standard UHPLC Hardware**



**Figure 2. LC-MS Analysis of siRNA using Bio-inert Hardware**



**Figure 3. Nusinersen Comparison; Stainless Steel vs Bio-Inert**



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