APPLICATIONS

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

Dr. Ivan Lebedev[1], Brian Rivera[1], and Dr. Helen Whitby[2]
[1]Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA
[2]Phenomenex, UK, Macclesfield

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.: 00D-4790-AN
00D-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.: 00D-4790-AN
00D-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

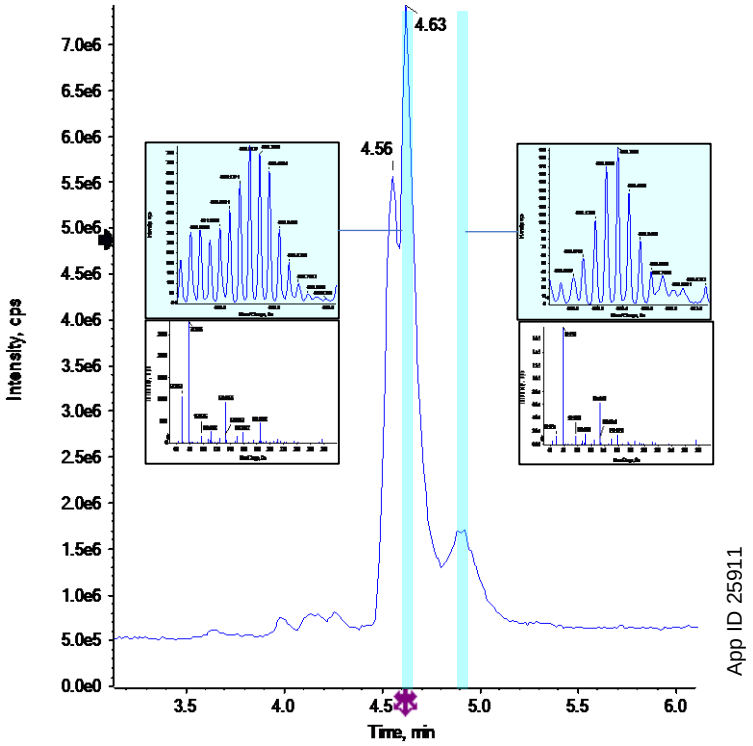


Figure 2. LC-MS Analysis of siRNA using Bio-inert Hardware

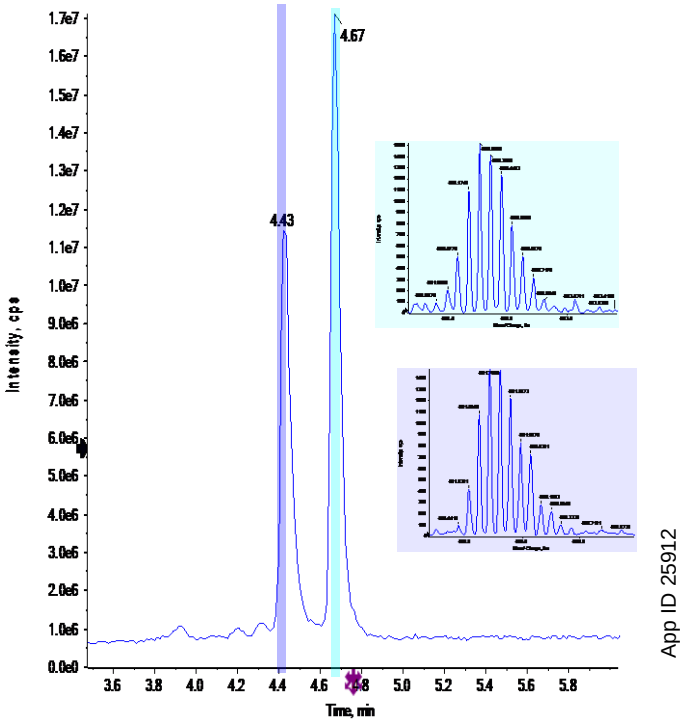
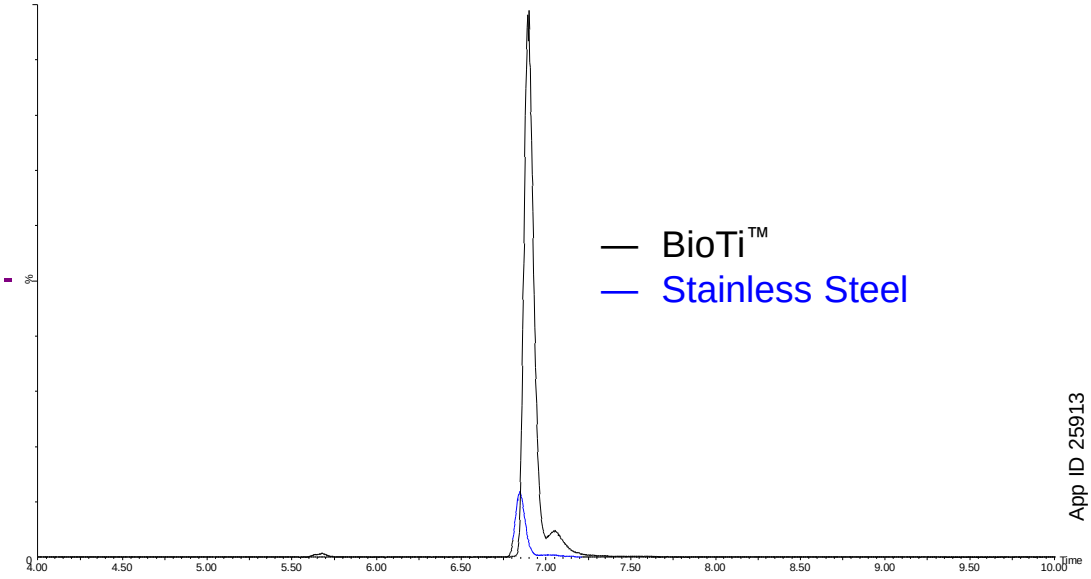


Figure 3. Nusinersen Comparison; Stainless Steel vs Bio-Inert



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t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 (12) 881 0121
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
info@phenomenex.com

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Corporate Office USA
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