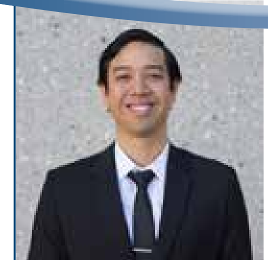
phenomenex ...breaking with tradition

APPLICATIONS



Brian Rivera
In addition to chromatography, Brian also has a passion for ice cream-making, and enjoys experimenting with bold, new flavors.

Analysis of Synthetic Oligonucleotides using Quaternary Amine Functionalized, Non-porous Particles

Brian Rivera, Morgan Kramer
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

Synthetic oligonucleotides (OGNs) are becoming increasingly popular as novel agents for the treatment of disease. The characterization of OGNs is important in the drug development process, and one common technique is strong anion exchange liquid chromatography. This high resolution technique for analysis for OGNs is preferred for many scientists when extensive characterization (i.e. LC-MS) isn't necessary. Another valuable benefit is that ion pair is not necessary in this technique and that n-1 failure sequences can still be separated. Although analysis of so-called "vanilla" or unmodified OGNs can be relatively straightforward, other modified OGNs present challenges. Several different modifications to the nucleic acid can be made to prevent nuclease degradation; these include backbone (e.g. phosphorothioate), ribose/deoxy-ribose sugar (e.g. bridged nucleic acid), and 2'-O substitutions. Additionally, different conjugations can be performed to either the 5' or 3' end of the oligo. Here we present several examples of different oligonucloetides, and how the subsequent mobile phase composition and gradient program may be optimized.

Materials and Methods

Crude, desalted OGNs (poly dT, Amino C12, BNA, and Phosphorothioate) were purchased from Integrated DNA Technologies (Coralville, IA, USA). Sequences, along with modifications, are indicated in **Table 1**, below. Samples were prepared at 30 µg/mL in nuclease free water. Injection volume was 2 µL. Note that mass loads for a non-porous particle will be reduced when compared to other analytical methods. A good starting point for OGNs is 1 µg for a 250 x 4.6 mm ID column.

Table 1. OGNs Sequences and Modifications

Oligo Type	Length	Sequence
Poly (dT)	12-18mer	N/A
Poly (dT)	19-24mer	N/A
Poly (dT)	19/20mer, 39/40mer, 59/60mer	N/A
Bridged Nucleic Acid	19mer	5'-TA/BNA-A/TA/BNA-meC/GT/BNA-T/TA/ BNA-T/AC/BNA-G/CC/BNA-C/A-3'
Amino C12	24mer	5'AmMC125-TCGTGCTTTTGTTGTTTTCGCGTT-3
DNA Phosphorothioate	19mer	5'-ACT*G*A*C*T*G*A*C*T*G*A*C*G*T*A*C*T-3

Experimental Conditions

Each LC method was performed using a Clarity® 5 µm Oligo-SAX column on an Agilent® 1200 (Agilent Technologies, Santa Clara, CA USA) with an upper pressure limit of 600 bar.

Two different mobile phase systems were implemented. Anion exchange relies on the electrostatic interaction between analyte and oppositely charged stationary phase. As such, chloride anions can be used for some methods while strongly adsorbing OGNs may require perchlorate, which has a higher ionic elution strength. Gradients were also optimized depending on the particular OGN. Gradient programs are summarized in **Table 2**.

Column: Clarity 5 µm Oligo-SAX
Dimensions: 250 x 4.6 mm
Part No.: 00G-4749-E0
Mobile Phase: See Table 2
Flow Rate: 1.6 mL/min
Temperature: 30°
LC System: Agilent® 1200
Detection: UV-Vis @ 260 nm

Table 2. Gradient Program

Oligo Type	Mobile Phase A	Mobile Phase B	Gradient Program
Poly (dT) 12-18mer	20 mM Tris, pH 8.0	20 mM Tris + 1.25 M NaCl, pH 8.0	32-46.6 % in 10 min
Poly (dT) 19-24mer	20 mM Tris, pH 8.0	20 mM Tris + 1.25 M NaCl, pH 8.0	32-46.6 % in 10 min
Poly (dT) 19/20mer, 39/40mer, 59/60mer	20 mM Tris, pH 8.0		
Bridged Nucleic Acid	20 mM Tris, pH 8.0	20 mM Tris + 1.0 M $NaOCl_4$, pH 8.0	10-46.6 % B in 10 min
DNA Phosphorothioate	20 mM Tris, pH 8.0	20 mM Tris + 1.0 M $NaOCl_4$, pH 8.0	5-46.6 % in 10 min
Amino C12	20 mM Tris, pH 8.0	20 mM Tris + 1.25 M NaCl	32-46.6 % in 10 min

APPLICATIONS

Figure 1. Poly (dT) 12-18mer

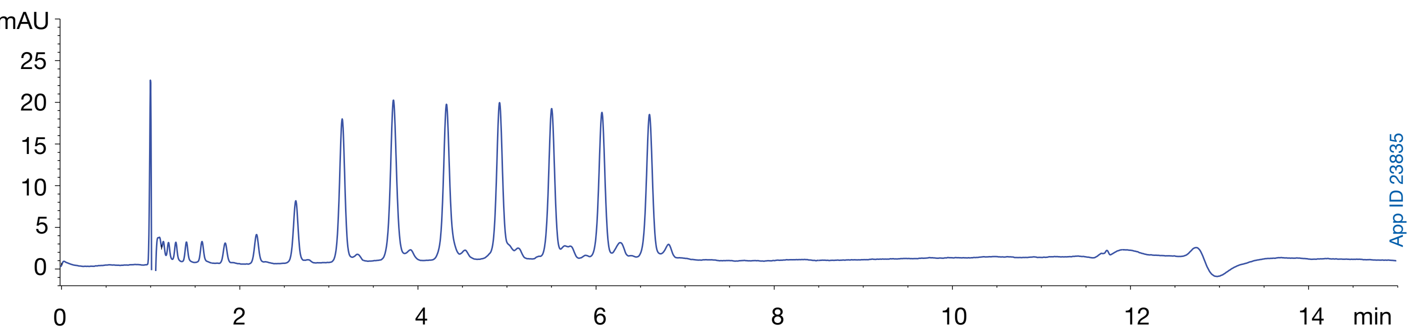


Figure 2. Poly (dT) 19-24mer

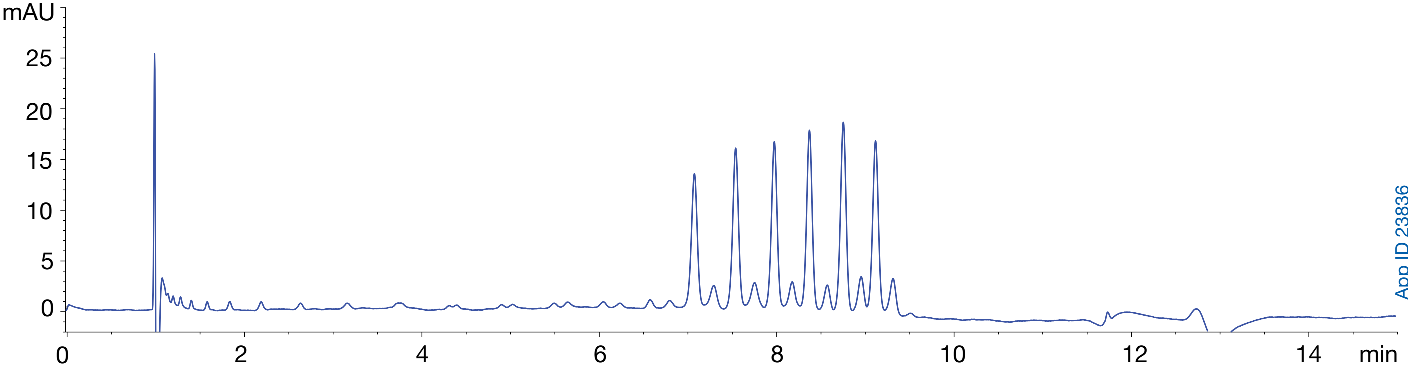


Figure 3. Poly (dT) 19/20mer, 39/40mer, 59/60mer

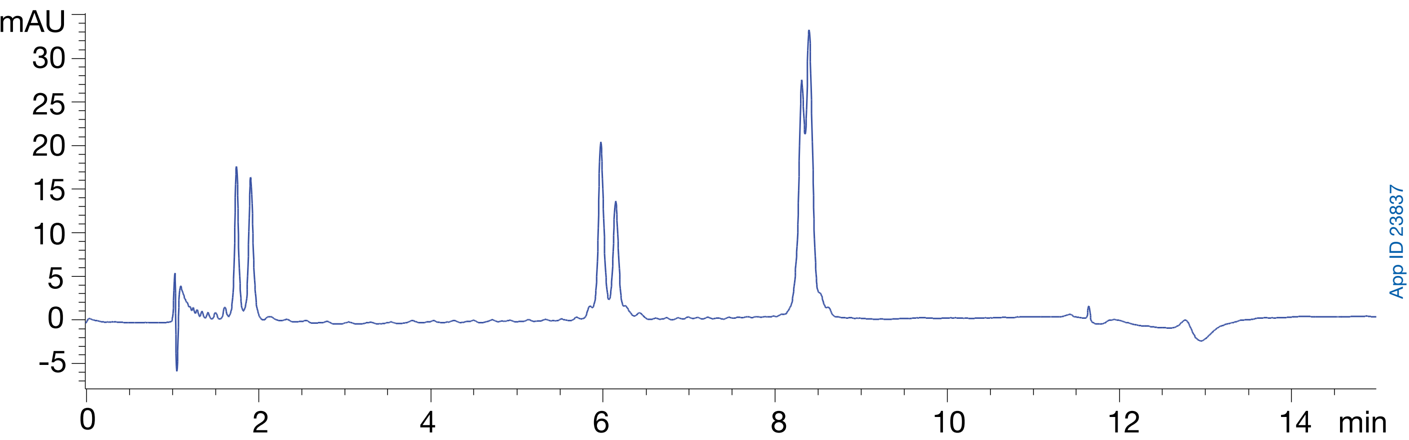


Figure 4. BNA 19mer

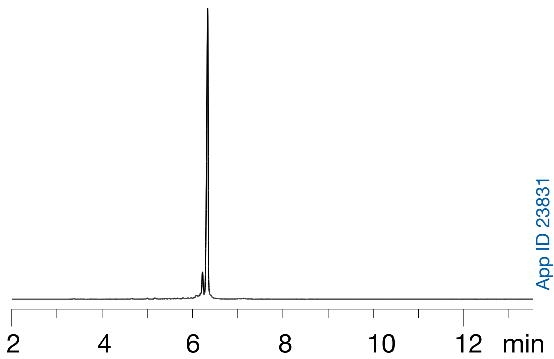


Figure 5. DNA 19mer Phosphorothioate

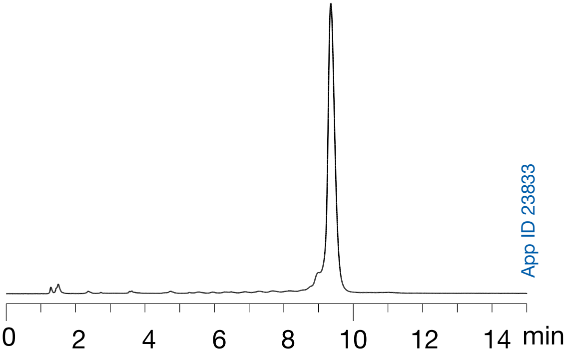
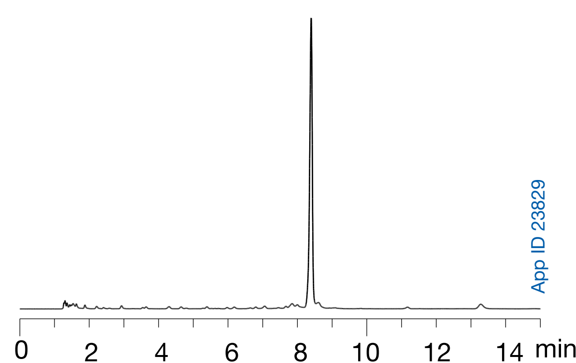


Figure 6. Amino C12 Linker DNA



Results and Discussion

Figures 1 and **2** show the separation of poly dT ladders. This shows OGNs in range of 12-24mer, which are common OGN lengths in both molecular biology and therapeutic applications. Note the separation of synthesis impurities observed between 18-24mer ladder. This extent of characterization allows for separation beyond even n-1 failure sequences.

Longer OGNs typically present challenges in anion exchange. **Figure 3** shows a separation of up to 59 and 60mer. This might apply to aptamers, unique OGNs which bind to protein targets much like antibodies. Aptamers can be anywhere between 22-40mer in length (1), and the optimized chemistry of Clarity Oligo-SAX shows good proof of concept for OGNs exceeding the typically 19-24mer seen in siRNA and antisense applications.

The BNA sample observed in **Figure 4** shows good separation of n-1 failure sequence. BNA and other ribose modified OGNs present challenges since they are rigid in structure and have a higher binding affinity, thus stronger electrostatic effects(2).

Figure 5 shows reasonably good peak shape for an OGN with multiple phosphorothioate modifications. Phosphorothioates typically have higher electrostatic effects, which lend to their binding affinity and thus attractiveness for therapeutic applications (3). As such, again the perchlorate mobile phase is needed to elute the modified thioate OGN. One thing to note is that SAX column chemistries- specifically the hydrophobic coat and quaternary amine used- can have implications for column performance for phosphorothioates. Additionally, phosphorothioates have stereochemistry which contributes to band broadening. That said, the peak shape here, although relatively broad, is more than acceptable for phosphorothioate-modified OGNs.

Finally, **Figure 6** shows good peak shape for an Amino-C12 linker conjugate. A more hydrophobic phase might show slight peak broadening.

Conclusion

With various modifications for OGNs, an optimal column chemistry like the Clarity Oligo-SAX with good ion exchange capacity and minimal secondary interactions is required. Using either standard chloride or perchlorate methods, a variety of different OGNs can be analyzed.

References

1. Sun, Hongguang, Xun Zhu, Patrick Y. Lu, Roberto R. Rosato, Wen Tan, and Youli Zu. "Oligonucleotide Aptamers: New Tools for Targeted Cancer Therapy." Molecular Therapy—Nucleic Acids Mol Ther Nucleic Acids 3.8 (2014): n. pag. Print.
2. Obika, Satoshi, Osamu Nakagawa, Akiko Hiroto, Yoshiyuki Hari, and Takeshi Imanishi. "Synthesis and Properties of a Novel Bridged Nucleic Acid with a P3' → N5' Phosphoramidate Linkage, 5'-amino-2',4'-BNA." Chem. Commun. 17 (2003): 2202-203. Print.
3. Mou, Tung-Chung, and Donald M. Gray. "The High Binding Affinity of Phosphorothioate-Modified Oligomers for Ff Gene 5 Protein Is Moderated by the Addition of C-5 Propyne or 2'-O-Methyl Modifications." Nucleic Acids Research 30.3 (2002): 749–758. Print.

APPLICATIONS

Ordering Information
Clarity Oligo-SAX PEEK Columns

Phases	Analytical 250 x 4.6	Analytical 150 x 4.6	Analytical 100 x 4.6	Guard 50 x 4.6
Oligo-SAX	00G-4749-E0	00F-4749-E0	00D-4749-E0	00B-4749-E0

Australia
t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China
t: +86 (0)20 2282-6668
f: +86 (0)20 2809-8130
chinainfo@phenomenex.com

Denmark
t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain
t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

www.phenomenex.com
Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

If analytical Clarity LC products do not provide at least an equivalent separation as compared to a competing product of the same particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

Terms and Conditions
Subject to Phenomenex Standard Terms and Conditions which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks
Phenomenex and Clarity are registered trademarks of Phenomenex. Agilent is a registered trademark of Agilent Technologies.

Disclaimer
Phenomenex is not affiliated with Agilent Technologies.

© 2016 Phenomenex, Inc. All rights reserved.

TN46020916_W