APPLICATIONS

Optimization of Mobile Phase Composition for the Analysis of Synthetic Oligonucleotides using a Clarity® Oligo-XT Column

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

Mobile phase composition for the analysis of three different synthetic oligonucleotides models (Bridged Nucleic Acid, Antisense RNA, and DNA phosphorothioate) was studied and optimized using a Clarity 2.6µm Oligo-XT C18 column.

Introduction

The characterization of OGNs is important in the drug development process and one common method involves liquid chromatography with detection by either UV (260 nm) or mass spectrometry (LC/MS or LC/MS/MS).

Mobile phase composition is important for the electrospray desorption efficiency and traditional mobile phase composition uses a combination of the alkylamine ion-pairing agent triethylamine (TEA) and the acidic modifier hexafluoroisopropanol (HFIP). Although optimal for LC/MS, TEA and HFIP typically also give better selectivity for OGNs even when UV-Vis is used as the detection method.

Previous studies have shown that a balance of TEA and HFIP are required to ensure both good peak shape and good electrospray ionization efficiency (1). Additionally, work by Chen et al (2) have shown improvements in electrospray desorption efficiency using other alkylamine ion-pairing reagents, including diisopropylethylamine (DIEA).

In this application note, we investigate the use of two mobile phase compositions - TEA HFIP and DIEA HFIP - with Clarity Oligo-XT, a novel core-shell material optimized for synthetic oligonucleotide applications. Three OGN models were investigated: Bridged Nucleic Acid, Antisense RNA, and DNA phosphorothioate. These OGN models were used to be representative of modifications commonly used for therapeutic applications. Methods were developed for optimal peak shape and separation of n-1 failure sequences.

Materials and Methods

Reagents and Chemicals

HFIP, TEA, and DIEA were purchased from Sigma Aldrich (St. Louis, MO, USA).

Crude, desalted OGNs (BNA, 2-MOE Gapmer and Phosphorothioate) were purchased from Integrated DNA Technologies (Coralville, IA, USA). Sequences, along with modifications, are indicated in **Table 1**, above. Samples were prepared at 30 µg/mL in nuclease - free water.

Table 1.
OGNs Sequences and Modifications

Oligo Type	Length	Sequence
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'
2'-MOE Gapmer	20mer	5'- mC mG mA mC mU A T A C G C G C A A mU mA msU mG mG -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

LC method was performed using a Clarity 2.6 µm Oligo-XT C18 column on an Agilent® 1200 (Agilent Technologies, Santa Clara, CA USA) with an upper pressure limit of 600 bar. Because the intent was to assess chromatographic performance with various mobile phases, UV-Vis was used as the detection method.

HPLC conditions were developed for the two methods using DIEA or TEA as ion-pair. Various gradient profiles and mobile phase compositions were investigated with only the optimized methods shown below.

HPLC Conditions

HPLC Method 1: DIEA and HFIP

LC Column: Clarity 2.6 µm Oligo-XT
Dimensions: 50 x 2.1 mm
Part No.: 00B-4746-AN
Mobile Phase: A: 50 mM HFIP & 5 mM DIEA in Water
B: 50 mM HFIP & 5 mM DIEA in Acetonitrile
Gradient: 5 – 20 % B in 15 min to 95 % wash for 5 min
Flow Rate: 0.3 mL/min
Temperature: 60 °C
LC System: Agilent 1200
Detection: UV-Vis @ 260 nm

HPLC Method 2: TEA and HFIP

LC Column: Clarity 2.6 µm Oligo-XT
Dimensions: 50 x 2.1 mm
Part No.: 00B-4746-AN
Mobile Phase: A: 100 mM HFIP & 4 mM TEA in Water
B: 100 mM HFIP & 4 mM TEA in Methanol
Gradient: 10 – 25 % B in 15 min to 95 % wash for 5 min
Flow Rate: 0.3 mL/min
Temperature: 60 °C
LC System: Agilent 1200
Detection: UV-Vis @ 260 nm

Results

Figure 1.
Chromatogram for BNA, TEA-HFIP Mobile Phase

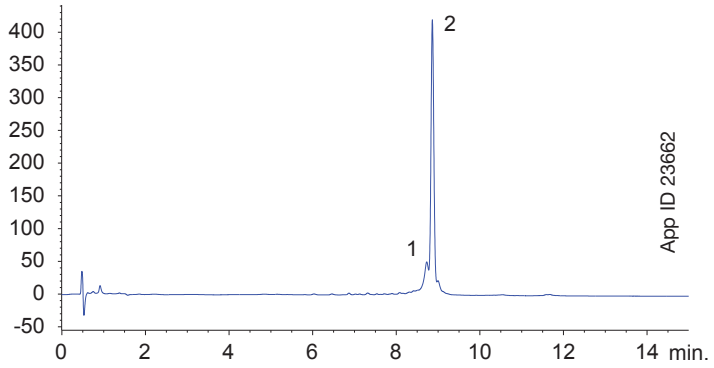


Figure 2.
Chromatogram for BNA, DIEA-HFIP Mobile Phase

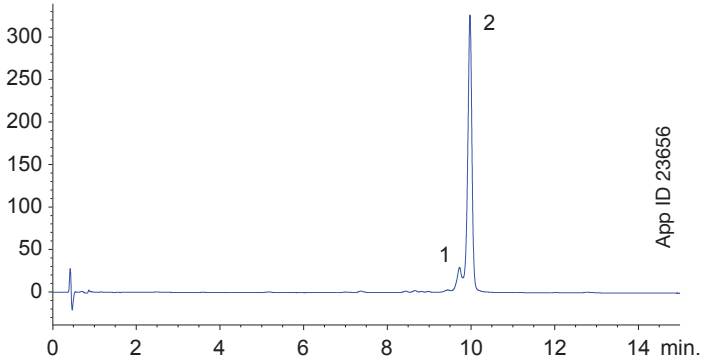


Figure 3.
Chromatogram for DNA Phosphorothioate, TEA-HFIP Mobile Phase

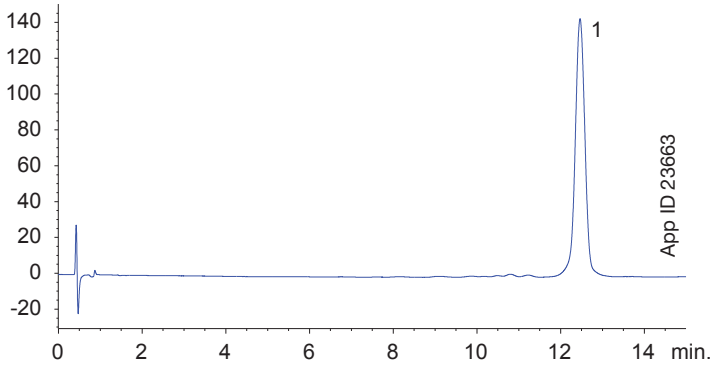


Figure 4.
Chromatogram for DNA Phosphorothioate, DIEA-HFIP Mobile Phase

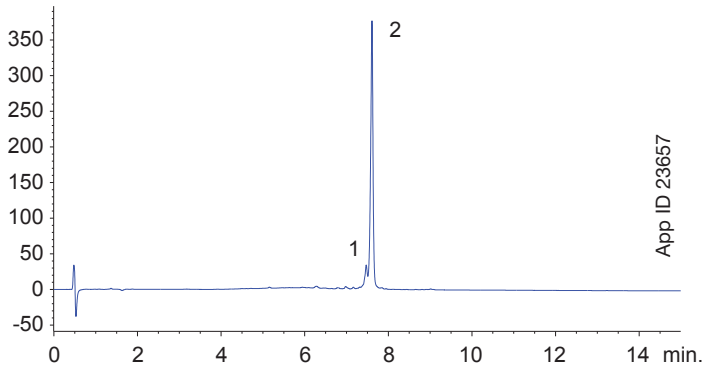


Figure 5.
Chromatogram for 2'-MOE Gapmer, TEA-HFIP Mobile Phase

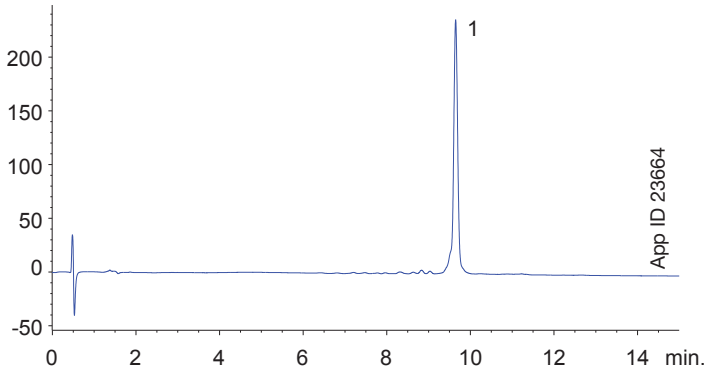
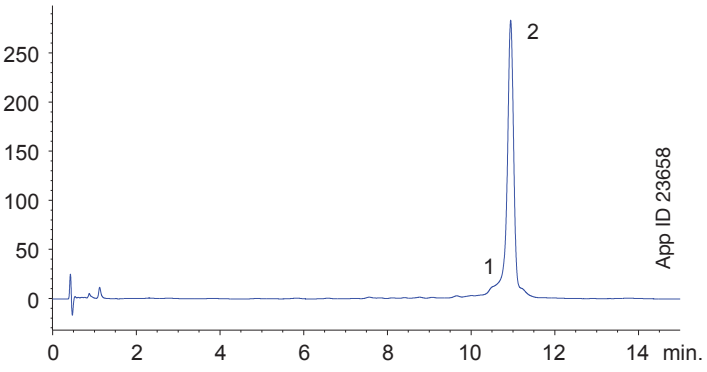


Figure 6.
Chromatogram for 2'-MOE Gapmer, DIEA-HFIP Mobile Phase



Discussion

The scope of method development was optimization of mobile phase composition and gradient profiles for good peak shape and separation on n-1 failure sequences. With all three modified OGNs, the DIEA ion-pairing was optimal.

As seen in **Figures 1 & 2**, TEA and DIEA were relatively similar for BNA. Good peak shape and n-1 separation were observed. However, for the 19mer DNA Phosphorothioate, only DIEA gave partial separation of n-1/n+1 failure sequences (**Figure 4**). Although DIEA did not give n-1 resolution between 2'-MOE Gapmer, it was able to give better peak shape and slight shouldering of the failure sequence (**Figure 6**).

It is important to note that DIEA, being the more hydrophobic alkylamine ion pair, required acetonitrile as the strong solvent since methanol was unable to elute the strongly retaining OGNs. Conversely, TEA performed better with the methanol as the strong solvent. Care should be taken to use the proper ion-pair and strong solvent for good retention and capacity factor.

Finally, only chromatography was assessed; further experiments would need to be determined to investigate the effect of DIEA and TEA in regards to electrospray desorption efficiency.

Conclusions

Mobile phase composition is important when developing methods for characterization of OGNs. Two ion-pairing agents, TEA and DIEA, were investigated, with DIEA giving overall better peak shape and selectivity for failure sequences. Further method development considerations include investigation on the effect of HFIP and DIEA/TEA for electrospray desorption.

References:

1. Rudge, James et al. "Preparation and LC/MS Analysis of Oligonucleotide Therapeutics from Biological Matrices." Chromatography Today. 1 Mar. 2011: 16-20. Print.
2. Chen, Buyun, and Michael G. Bartlett. "Evaluation of Mobile Phase Composition for Enhancing Sensitivity of Targeted Quantification of Oligonucleotides Using Ultra-high Performance Liquid Chromatography and Mass Spectrometry: Application to Phosphorothioate Deoxyribonucleic Acid." Journal of Chromatography A 1288 (2013): 73-81

Ordering Information

Clarity®

1.7 µm Minibore Columns (mm)			SecurityGuard™ ULTRA Cartridges‡
Phase	50 x 2.1	100 x 2.1	3/pk
Oligo-XT	00B-4747-AN	00D-4747-AN	AJ0-9515
			For 2.1 mm ID

2.6 µm Minibore and Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡	
Phase	50 x 2.1	100 x 2.1	50 x 4.6	100 x 4.6	3/pk	3/pk
Oligo-XT	00B-4746-AN	00D-4746-AN	00B-4746-E0	00D-4746-E0	AJ0-9515	AJ0-9514
					For 2.1 mm ID	For 4.6 mm ID

5 µm Minibore and Analytical Columns (mm)			SecurityGuard ULTRA Cartridges‡	
Phase	50 x 2.1	150 x 4.6	3/pk	3/pk
Oligo-XT	00B-4745-AN	00F-4745-E0	AJ0-9515	AJ0-9514
			For 2.1 mm ID	For 4.6 mm ID

5 µm Semi-Preparative Columns (mm)				SecurityGuard SemiPrep Cartridges*
Phase	50 x 10	100 x 10	150 x 10	3/pk
Oligo-XT	00B-4745-N0	00D-4745-N0	00F-4745-N0	AJ0-9516
				For 10 mm ID

5 µm Axia™ Packed Preparative Columns (mm)					SecurityGuard PREP Cartridges**	SecurityGuard PREP Cartridges***
Phase	100 x 21.2	150 x 21.2	250 x 21.2	150 x 30	/ea	/ea
Oligo-XT	00D-4745-P0-AX	00F-4745-P0-AX	00G-4745-P0-AX	00F-4745-U0-AX	AJ0-9517	AJ0-9518
					For 21.2 mm ID	For 30 mm ID

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

* SemiPrep SecurityGuard Cartridges require holder, Part No.: AJ0-9281

** PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8223

*** PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8277

guarantee

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

APPLICATIONS

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