APPLICATIONS

Effect of Perfluorinated Alcohols in Impurity Analysis for Synthetic Oligonucleotides

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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. However, characterization of oligos, specifically by ion-pair reversed phase liquid chromatography (IP-RPLC), can be quite challenging. Oligos are manufactured by solid phase synthesis, where nucleotides are added in a step-wise phase. As such, n-1 and n+1 must be characterized, and this may require extensive method development to optimize. Further, it may be necessary for characterization and quantitation of other closely related impurities like oxidized phosphorothioates.

Typically, mobile phase composition for the analysis of oligos consists of a perfluorinated alcohol such as HFIP, as well as an alkylamine. To demonstrate the effect of changing the concentration of perfluorinated alcohols used in the impurity analysis of synthetic oligonucleotides we evaluated each, a single- and double-stranded oligonucleotide (BNA and siRNA).

HFIP is used as an acidic modifier for oligonucleotide analysis and facilitates ionization leading to a better signal in the mass spec. Previously it was reported that increasing concentrations of HFIP enhances the MS signal for oligonucleotides[1], however the effect to HFIP has not been fully explored for chromatographic performance.

In **Figure 1**, we can see improved separation of sense/anti-sense of an siRNA when decreasing HFIP concentration from 100 mM to 25 mM, with some loss of resolution from 25 to 12.5 mM. Nonetheless, the decreasing of HFIP does not seem to adversely effect chromatography and further can expand the experimental design in method optimization. A nominal effect is observed with BNA, as seen in **Figure 2**, with separation and impurity profiles being relatively similar.

Although the impurity profile of BNA by LC-UV might not show a marked improvement, the differences become much more apparent when the oligos are analyzed by high-resolution mass spectrometry. Decreasing the HFIP improves ionization efficiency 3x, as shown in differences in peak height in **Figure 3**. Interestingly, TICs do show the later eluting peaks with the 12.5 mM HFIP concentration. Spectra reveal to have similar spectral quality as the main peak (data not shown), but they differ enough in physicochemical properties to show sample heterogeneity that the MS could not determine if separation was not obtained.

In summary, chromatographic performance is often improved by decreasing HFIP concentration, with acceptable or even optimal chromatography being obtained at 12.5 mM. Even more compelling is the improved ionization with the decrease in HFIP yielding significantly higher peak heights, facilitating characterization and quantitation.

LC Conditions

Columns:	bioZen™ 2.6 µm Oligo
Dimension:	100 x 2.1 mm
Part No.:	00D-4790-AN
Mobile Phase:	A: 4 mM TEA in Water; HFIP as noted
	B: 4 mM TEA in Methanol; HFIP as noted
Gradient:	5-30 % B in 14 minutes
Flow Rate:	0.3 mL/min
Injection:	200 ng (Figure 1 and 2)
	12.5 ng (Figure 3)
Temperature:	65 °C (Figure 1 and 2)
	55 °C (Figure 3)
Detection:	UV @ 260 nm (Figure 1 and 2)
	TOF-MS (Figure 3)
Sample:	As Noted

1. J Am Soc Mass Spectrum. 2017 Jan; 28(1): 190–199

Figure 1. Effect of HFIP Concentration on siRNA

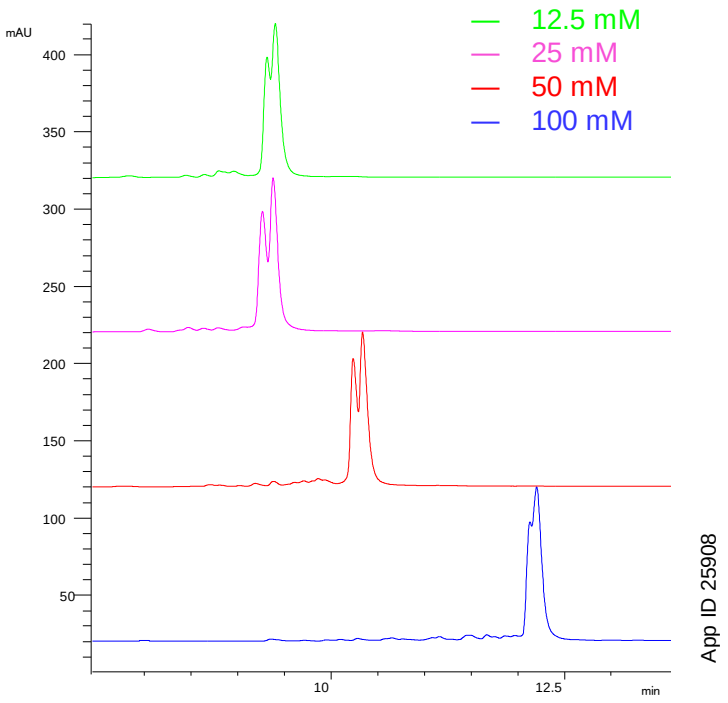


Figure 2. Effect of HFIP Concentration on BNA

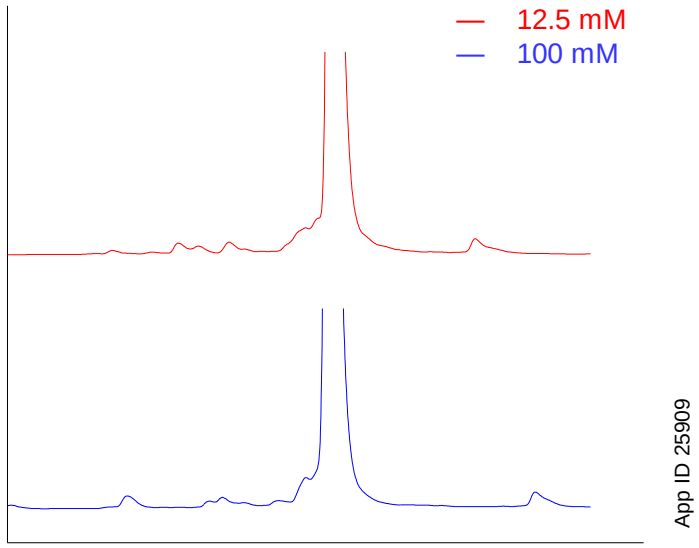
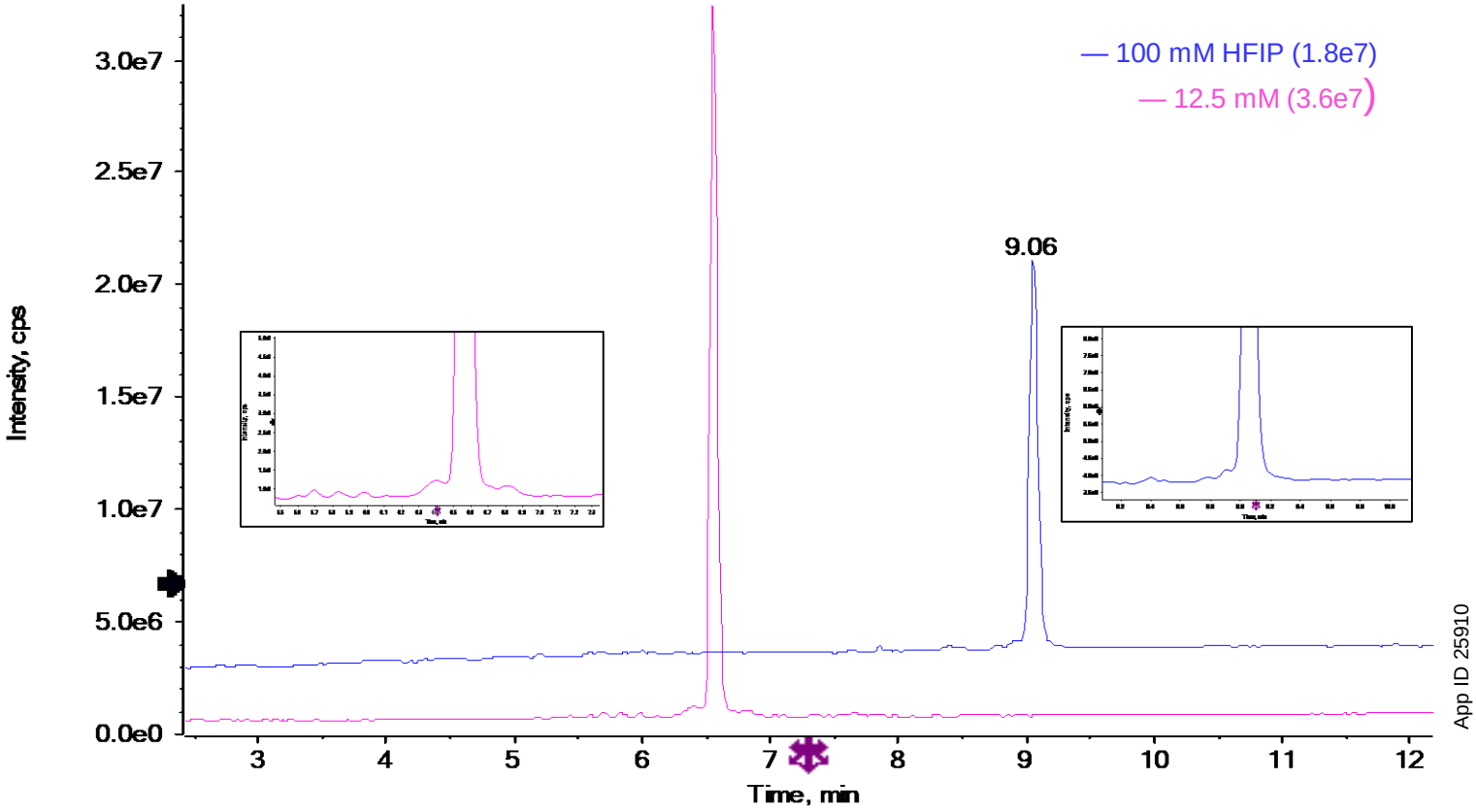


Figure 3. Effect of HFIP on Ionization Efficiency



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