APPLICATIONS

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Comparison of Polymeric and Silica-Based Columns for Oligonucleotide Analysis by Ion-Pair Reversed Phase

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Overview

Therapeutic oligonucleotides represent a recent breakthrough in the pharmaceutical industry. 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 manner. As such, process-related impurities such as n-1 and n+1 must be characterized. Further complicating methods are common mobile phase additives such as perfluorinated alcohols (HFIP) and an alkylamine ion-pair that facility the hydrophobic interaction of the negatively charged oligo. These mobile phase conditions combined with high temperature are often necessary to ensure good peak shapes but result in very harsh conditions that can encourage silica dissolution.

As such, the LC particle is an important consideration for column selection. The particle must be stable at neutral pH when run at moderately high temperature. It might then seem appropriate to run a polymer-based particle such as a polystyrene divinylbenzene to ensure method robustness and column stability. However, polymer-based particles typically do not have the efficiency necessary to separate the closely eluting impurities associated to oligos. Alternatively, superficially porous (core-shell) particles can be used to improve efficiency and resolution. Here, we investigate the chromatographic performance between two PS-DVB particles and the bioZen™ 2.6 µm Oligo column, which uses a pH stable organo-silica superficially porous particle packed in bio-inert hardware.

In comparing the 2.6 µm bioZen Oligo to a 5 µm PS-DVB of similar dimensions in the separation of a 2'-MOE Gapmer, there is a marked difference in chromatographic performance, with the 2.6 µm particle giving superior peak height (**Figure 1**). As such, the increase in chromatographic efficiency offered by the bioZen Oligo improves the separation of different process related impurities. This also may be due to the functionality of the C18 stationary phase that is bonded to the superficially porous particle, which has a better methylene selectivity. A similar performance for an RNA phosphorothioate is also observed, as seen in **Figure 2.** Notably, many short mer failure sequences are not detected using the 5 µm PS-DVB particle platform.

In comparing separation of a 5'-Amino C12 oligonucleotide on the 2.6 µm against a 4 µm PS-DVB column of similar dimensions (**Figure 3),** the superficially porous core-shell particle shows a slightly improved peak height and improved separation. A similar profile is observed with the BNA oligo **(Figure 4)**, though the superficially porous particle yields slightly more peak areas and improved recoveries for some earlier eluting impurities. This improved recovery might be attributed to the bio-inert hardware of the bioZen Oligo, which tends to improve recovery and peak shape for oligos, especially using moderately high HFIP concentrations.

In summary, when selecting a particle for oligo analysis although a PS-DVB particle might seem like an appropriate choice, one may be making concessions for peak shape and efficiency, which might directly effect method sensitivity. Additionally, separation may not be optimal which can impact impurity analysis and characterization. The bioZen Oligo however, affords scientists a versatile combination of performance gains and robustness, along with improved recovery from bio-inert hardware.

LC Conditions

Columns:	bioZen 2.6 µm Oligo (00B-4790-AN) 5 µm PS-DVB 4 µm PS-DVB
Dimension:	50 x 2.1 mm
Mobile Phase:	Figures 1, 2: A: 12.5 mM HFIP, 0.5 mM Hexylamine in Water B: 12.5 mM HFIP, 0.5 mM Hexylamine in Methanol Figures 3, 4: A: 100 mM HFIP, 4 mM TEA in Water B: 100 mM HFIP, 4 mM TEA in Methanol
Gradient:	5-75 % B in 14 minutes (Figures 1,2) 5-30 % B in 14 minutes (Figures 3,4)
Flow Rate:	0.3 mL/min
Injection:	2 µL (1 µg/mL)
Temperature:	65 °C (Figure 1,2) 45 °C (Figure 3,4)
Detection:	UV @ 260 nm
Sample:	As indicated

Figure 1. Comparison of Impurity Profile, 2’-MOE Gapmer

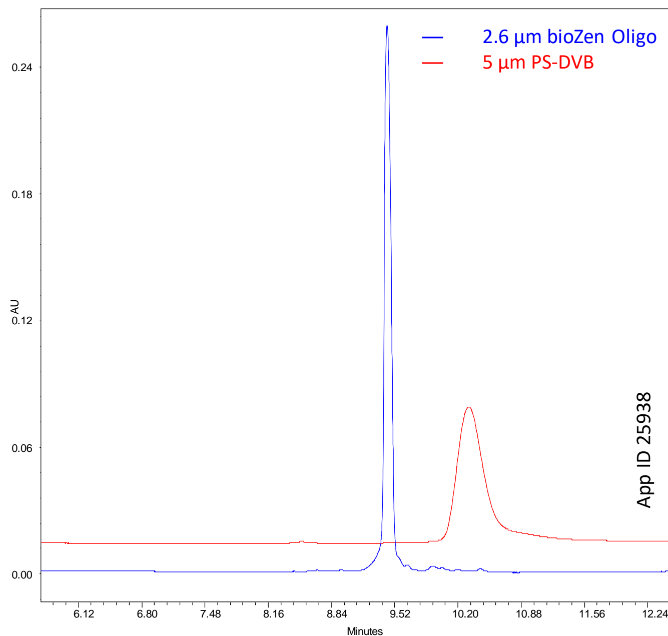


Figure 2. Comparison of Impurity Profile, RNA Phosphorothioate

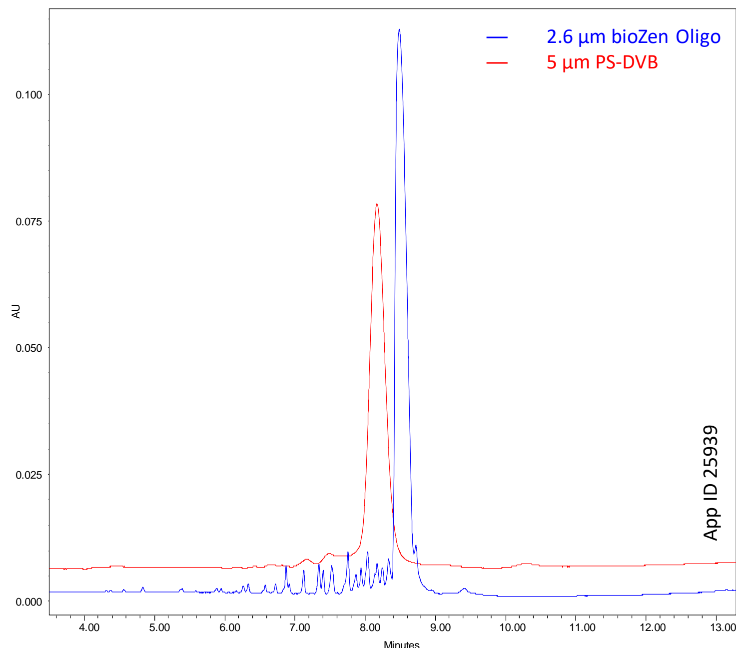


Figure 3. Comparison of Impurity Profile, 5’-Amino C12 Oligonucleotide

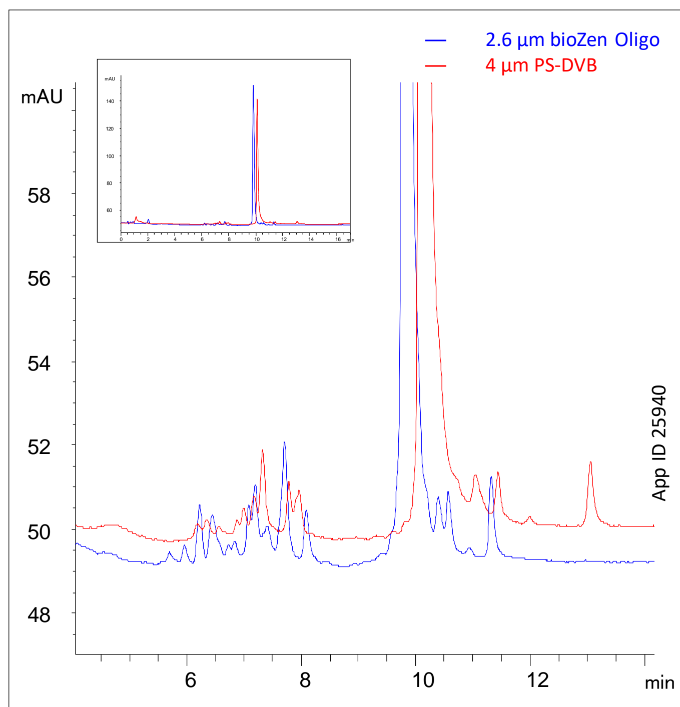
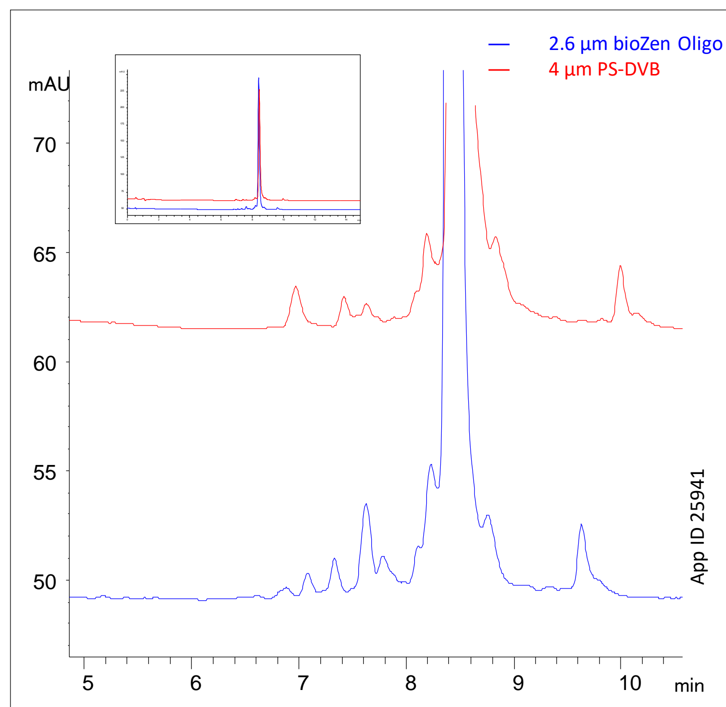


Figure 4. Comparison of Impurity Profile, BNA



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