Comparison of Fully Porous and Superficially Porous Particles for the Analysis of Synthetic Oligonucleotides

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Overview

Superficially porous particle technologies provide a reduced diffusion path in comparison with their fully porous counterparts minimizing the resistance to mass transfer during chromatographic separation. With these particles, one can benefit from their greater efficiency either through improved separation or achieving comparable efficiency with a larger particle size. This enables the method flexibility to increase flow rate. When this is coupled with the benefits of bio-inert hardware, a greater level of separation of oligonucleotide impurities can be observed compared to a sub-2 µm fully porous hybrid silica. The potential to implement a faster flow rate offers the opportunity for shorter analysis times or increased sample throughput; further lower backpressure can contribute to improved column lifetime especially considering the harsh conditions associated to oligo analysis by ion-pairing reversed phase LC. In this application note, we compare the performance of two different particle size core-shell columns (bioZen™ Oligo 2.6 µm and 1.7 µm) with their fully porous alternatives.

With the 2.6 µm bioZen Oligo, we clearly see an improvement in sensitivity over a 2.5 µm fully porous column using the high efficiency core-shell material. **Figure 1** demonstrates the narrower peaks and a greater sensitivity achieved for a 5'-Amino C12 oligo when run using the 2.6 µm bioZen Oligo. This reduction in peak width afforded better resolution and definition of the impurity peaks to be achieved.

In **Figure 2** we compare the separation of a ssDNA using the 1.7 µm bioZen oligo. Using a sub-2 µm core-shell particle, greater level of detail in the impurity profile is observed with when compared to the 1.7 µm fully porous alternative in the same dimensions. For both chemistries running the method at a flow rate slightly higher than is typical (0.5 mL/min) gave better results. It was noted for longer oligonucleotides like the 33mer analyzed here, recovery of shorter impurities is improved with bioZen Oligo, likely a result of the inert bio-inert hardware.

Figure 3 highlights the potential benefits of the high efficiency of a core shell particle at higher flow. The bioZen oligo 2.6 µm when used to analyze an RNA 21mer achieved comparable performance to the 1.7 µm fully porous column however running a significantly lower backpressure. This reduction in backpressure is likely, overtime contribute favorably to improved column lifetime for the 2.6 µm particle compared with sub-2 µm alternatives.

LC Conditions (Figure 1)

Column:	bioZen 2.6 µm Oligo Fully Porous 2.5 µm C18
Dimensions:	50 x 2.1 mm
Mobile Phase A :	10 mM HA in Water; 12.5 mM HFIP
Mobile Phase B:	10 mM HA in MeOH; 12.5 mM HFIP
Gradient:	25-75 % B, 14 minutes
Detection:	UV @ 260 nm
Flow Rate:	0.5 mL/min
Temperature:	65 °C

LC Conditions (Figure 2)

Column:	bioZen 1.7 µm Oligo Fully Porous 1.7 µm C18
Dimensions:	50 x 2.1 mm
Mobile Phase A :	10 mM HA in Water; 12.5 mM HFIP
Mobile Phase B:	10 mM HA in MeOH; 12.5 mM HFIP
Gradient:	25-75 % B, 14 minutes
Detection:	UV @ 260 nm
Flow Rate:	0.5 mL/min
Temperature:	65 °C

LC Conditions (Figure 3)

Column:	bioZen 2.6 µm Oligo Waters® XBridge™ 1.7 µm BEH C18
Dimensions:	50 x 2.1 mm
Mobile Phase A :	10mM HA in Water; 12.5mM HFIP
Mobile Phase B:	10mM HA in MeOH; 12.5mM HFIP
Gradient:	25-75% B, 14 minutes
Detection:	UV@260nm
Flow Rate:	0.5 mL/min
Temperature:	65 °C

Figure 1. bioZen™ Oligo 2.6 µm vs Fully Porous 2.5 µm C18 for a 5'-Amino C12

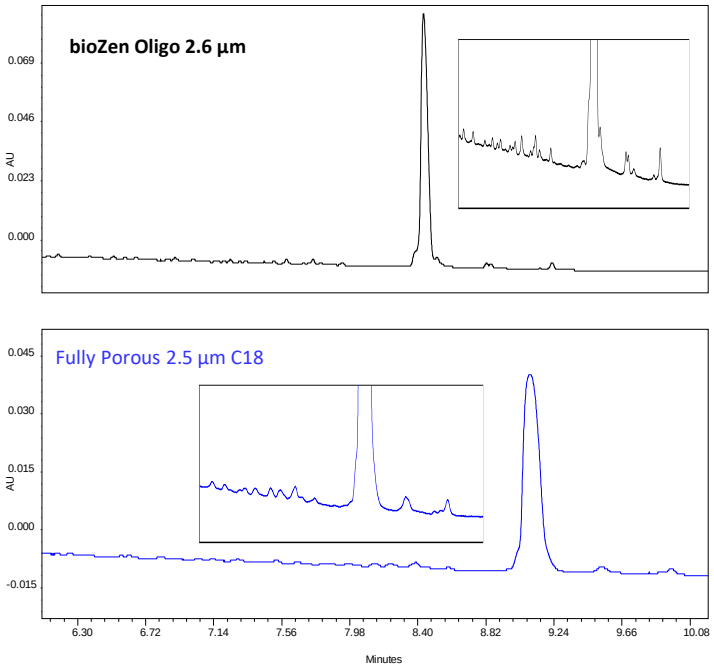


Figure 2. bioZen Oligo 1.7 µm vs Fully Porous 2.5 µm C18 for a ss 33mer.

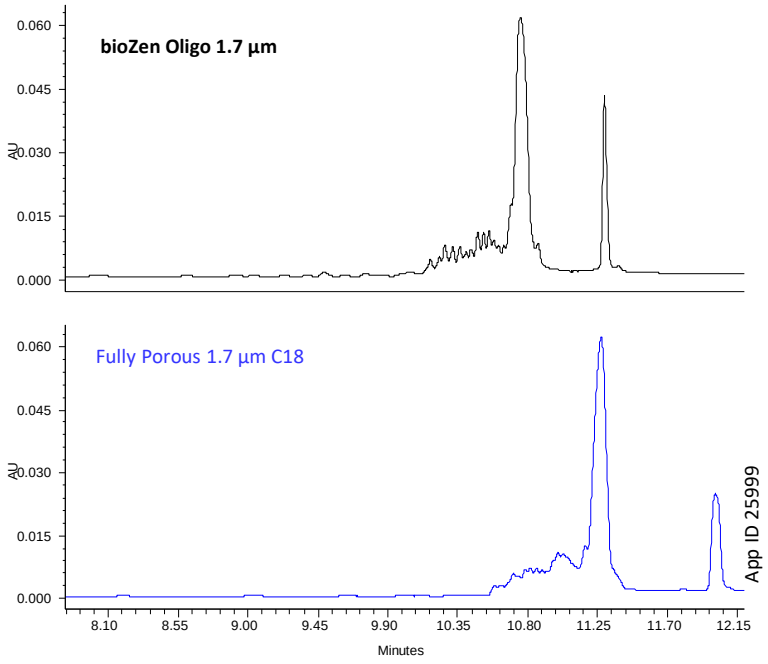
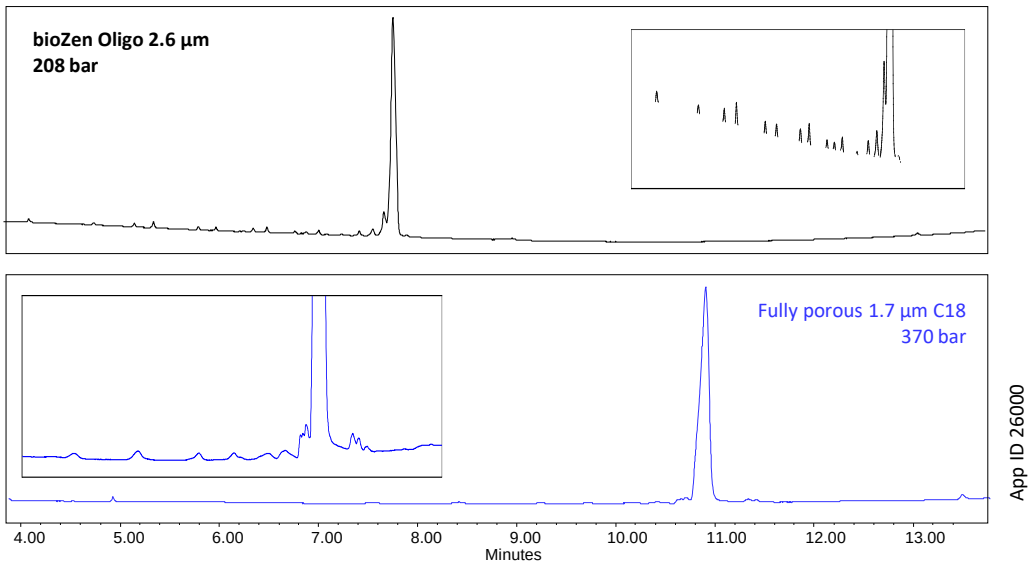


Figure 3. Comparative data for bioZen Oligo 2.6 µm vs Waters® XBridge™ BEH 1.7µm for a ssRNA 21mer



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