Efficient Reversed-Phase Separation of Oligonucleotides by Using High pH Stable Superficially Porous Columns

Xiaoli Wang, Stephen Luke Agilent Technologie, Inc.

HPLC 2016
poster number

Agilent Technologies

Introduction

Synthetic oligonucleotides have emerged as promising therapeutic agents. They are synthesized using a multi-step process. Although coupling efficiencies are high, the overall yield decreases as the cycles increase with failure coupling with single (N-1) and double (N-2) deletions as the major impurities. Therefore, fast, high resolution analyses are needed. HPLC is usually done in basic pH mobile phases at high temperature; thus, requiring chemically stable columns. Totally porous hybrid particles are commonly used but the mass transfer for the larger size oligos is not ideal. In this work, we evaluate the uses of new high pH stable, superficially porous particles for oligo separations for fast and high resolution analysis, and compare them with totally porous particles.

Experimental

Materials

Description	Part Number
AdvanceBio Oligonucleotide 2.1 x 50 mm, 2.7 um	659750-702
Oligonucleotides Resolution Standard	5190-9028
Oligonucleotides Ladder Standard	5190-9029

Column characteristics

Bonded Phase	Pore Size	Temp. Limits	pH Range	End Capped
C18	100Å	65°C	3.0 - 11.0	Double

Acetonitrile, methanol, TEAA, HFIP, TEA (Sigma-Aldrich)

Method

Option 1

Mobile phase A: 100 mM TEAA in water

Mobile phase B: 100 mM TEAA in acetonitrile

Option 2 (LC/MS friendly)

Mobile phase A: HFIP:TEA (400 mM:15 mM) in water

Mobile phase B: Methanol : mobile phase A (50:50)

Gradient: See chromatogram

Stop time: See chromatogram

Post run: 5 min

Flow rate: 0.6 mL/min (or other flow rates)

Col. temp: 65 °C

Sample: See figures

Injection: See figures

Detection: UV at 260 nm

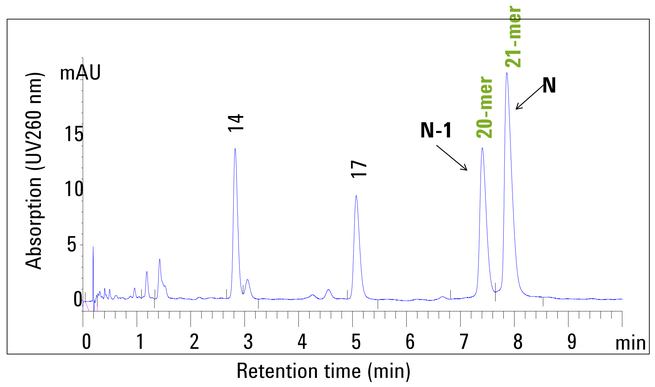
LC instruments

1260 Infinity

1290 Infinity

Results and Discussion

Resolution of N and N-1 Oligonucleotides



Column: AdvanceBio Oligonucleotide, 2.1 x 50 mm (p/n 659750-702)

Mobile phase A: 100 mM TEAA in water

Mobile phase B: 100 mM TEAA in acetonitrile

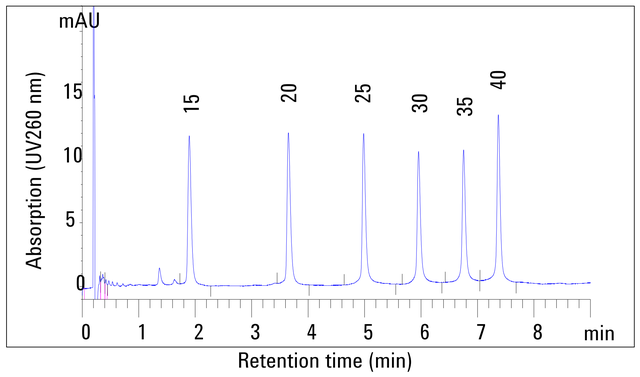
Gradient: 10 to 14%B in 10 min

Sample: 10 µL (Agilent Oligonucleotide Resolution Standard (p/n 5190-9028)

LC: Agilent 1290 Infinity Binary

The AdvanceBio Oligonucleotide column generates sharp peaks and high resolution for RNA-oligos. The N and N-1 RNA-oligonucleotides (21 and 20-mers) were separated close to baseline resolution. This data suggested that column is very capable of resolving a main oligo from impurities.

Separation of DNA-oligonucleotides



Column: AdvanceBio Oligonucleotide, 2.1 x 50 mm

Mobile phase A: 100 mM TEAA in water

Mobile phase B: 100 mM TEAA in acetonitrile

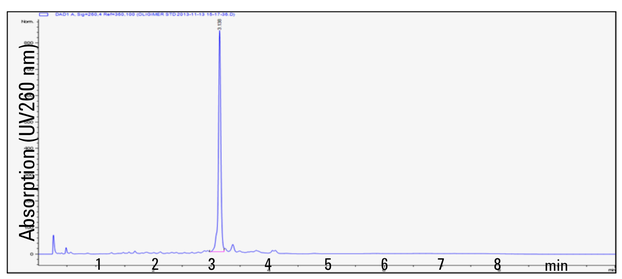
Gradient: 6 to 8% B in 12 min

Sample: 0.5 µL (Agilent Oligonucleotide Ladder Standard (p/n 5190-9029)

LC: Agilent 1290 Infinity Binary

All 6 DNA-oligos were separated with baseline resolution. The separation was completed in less than 10 min. The 15-mer DNA oligo was eluted as soon as 2 min and the average 20 and 25-mers were eluted at 4 and 5 minute, respectively. The data indicated that AdvanceBio Oligonucleotide columns are suitable for high through-put separation of a wide range of oligonucleotide sequence length.

Separation in MS compatible HFIP/TEA mobile phase



Column: AdvanceBio Oligonucleotide, 2.1 x 50 mm

Mobile phase A: HFIP:TEA (400 mM:15 mM) in water

Mobile phase B: Methanol : mobile phase A (50:50)

Gradient: 30-40% B in 0.5 min; 40-70% B in 5 min

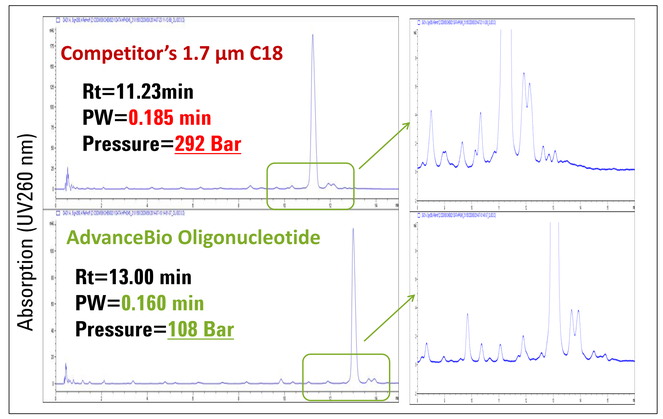
Sample: 1.0 µL (0.5mg/mL 25-mer *DNA-oligo)*

LC: Agilent 1290 Infinity Binary

Results and Discussion

Comparison with other columns

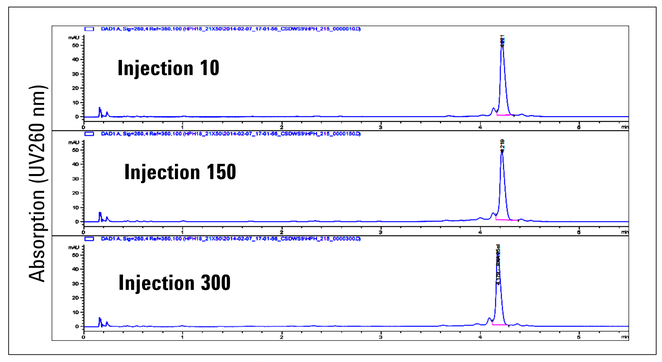
Separation of 23-mer RNA-oligonucleotide (2.1 x 50 mm)



The peak width of the superficially-porous column was slightly narrower than the totally porous material 1.7um, this supports the fact that there is shorter distances required for diffusion into/out of the superficially-porous particle. This faster mass transfer results in higher resolution. With 2.7 um particle size, AdvanceBio Oligonucleotide column operates at low backpressure compare to 1.7 um particle size column, it is compatible with 600 bar HPLC system as well as 1200 bar UHPLC system.

Column chemical stability

300 consecutive injections of 25-mer DNA-oligo (2.1x50mm)



Gradient: 7% B to 11% B in 5 min, 11% B to 80% B in 5.01 min
Hold at 80% B for 5.50 mim, 80% B to 7% B in 5.56 min

Sample: 1uL (0.5mg/mL 25-mer DNA-oligo)

Total run time: 8.5 min

Column stability of 300-consecutive injections of 25-mer (DNA-oligo) showed that through out 300 injections, the retention time of main peak and its impurities were highly reproducible. Column still maintains its performance after these 300 injections.

Conclusions

- AdvanceBio Oligonucleotide columns are designed for separating RNA and DNA-oligonucleotides of different sizes with fast and high resolution.
- RNA and DNA-oligonucleotides can be successfully analyzed by AdvanceBio Oligonucleotides column using both LC/UV and LC/MS mobile phases.
- Large numbers of injections - column stability data generated with TEAA (>pH 8.0)- indicated that columns packed with high pH stable superficially porous particles have long life time.
- AdvanceBio Oligonucleotide columns are packed with 2.7 um superficially porous particles- low backpressure columns- can be compatible with 600 bar and 1200 bar (U)HPLC systems.