Purification of Synthetic Oligonucleotides- Utilizing Denaturing High Performance Liquid Chromatography (dHPLC)

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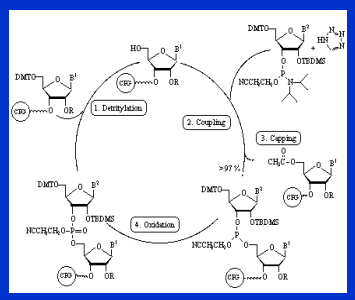
Abstract

The genomics revolution has rapidly increased the demand for highly purified synthetic oligonucleotides for PCR, cloning, genotyping, chip-based technologies, and various applications in the field of pharmacogenetics. Gel electrophoresis and high performance liquid chromatography (HPLC) have previously been the most commonly used techniques for oligo purification. Compared to both gel electrophoresis and typical IP-RP HPLC, dHPLC offers the advantages of higher resolution, larger size range for separation, purification chemistries, reproducibility, automation, and convenient data archiving.

We describe the use of dHPLC for the analysis and purification of synthetic oligonucleotides. The unique separation chemistries of the OligoSEP® cartridges combined with elevated temperatures (80°C) offers rapid and highly efficient separation and purification. The WAVE® OLIGO System is a fully automated dHPLC system specifically designed for the analysis and purification of synthetic oligonucleotides. Using thermally stable polymeric reversed phase columns in the ion-pairing mode with fully denaturing conditions, a broad range of synthetic oligonucleotides can be purified. In addition, by changing the eluent chemistry, separations can be performed based either on oligo length alone, or a combination of length and base pair composition. The purification of unmodified, dye labeled, and biotinylated oligonucleotides has also been accomplished using this technique.

Synthesis of Oligonucleotides Using Phosphoramidites



A sequential and continuous cycle

1. Remove DMT
2. Cap with OAc
3. Couple Amidite
4. Oxidize triester
5. Repeat

Final product deprotected and cleaved from synthesis support using NH_4OH

Purity vs Oligonucleotide Length

Length	Theoretical yields (%) based on coupling efficiency				
	90.0%	95.0%	98.0%	99.0%	99.5%
20 mer	12	36	67	82	90
40 mer	1.5	13	45	67	82
60 mer	0.18	4.6	30	55	74
80 mer	0.02	1.7	20	45	67
100 mer	0.003	0.6	13	37	61

Conclusion: Oligonucleotides directly from a synthesizer always contain significant impurities

WAVE® Analysis and Purification of Oligonucleotides

- The Transgenomic WAVE® System offers high performance separations and purification using rugged polymeric based columns
- Separations are performed under fully denaturing conditions (80°C)
- Automated fragment collection is used in the purification mode
- Fast analysis and purification run times (<15 minutes)
- No desalting required (TEAA)
- Applicable to all classes of oligonucleotides

Wave Oligonucleotide System

Cartridges for Oligonucleotide Analysis and Purification

OligoSep – A 4.6 x 50 mm cartridge containing 2.2μ microporous polystyrene/divinylbenzene particles which are octyldecylated. Primarily used for analytical work

OligoPrep HC – A 7.8 x 50 mm cartridge containing 5.6μ macroporous divinylbenzene particles which are octyldecylated. Used for both analytical and purification. Loading capacity is 1 μmol

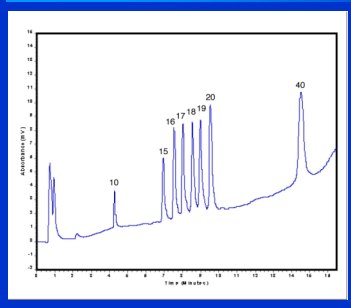
All separations are run at 80°C

Denaturing Separation Chemistries for Oligonucleotide Analysis and Purification

- Triethylammonium acetate
  - Cationic ion pairing reagent which results in separations which are dependent on both length and composition of the oligonucleotide
  - Addition of hydrophobic groups, such as dyes, minimizes compositional differences
- Tetrabutylammonium bromide
  - Cationic ion pairing reagent which results in separations remove are dependent primarily on the length of the oligonucleotide
  - Addition of hydrophobic groups, such as dyes, increase retention time but length dependency is maintained

All separations are run at 80°C

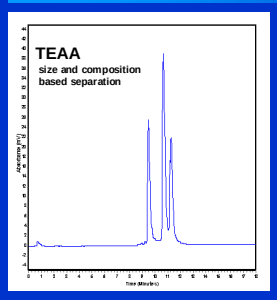
Separation of a Poly dT Size Ladder Using TBAB

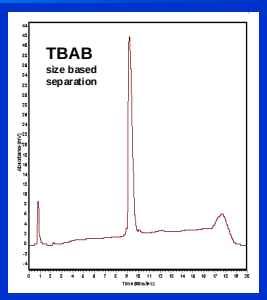


Chromatographic Conditions

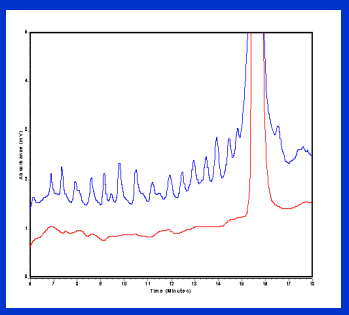
Cartridge: OligoSep
Eluent A: 30 mM TBAB/10% ACN
Eluent B: 30 mM TBAB/65% ACN
Gradient: 34% B-2 to 55% in 2 min, 55% to 68% in 4 min, 68% to 77% in 3 min, 77% to 95% in 5 min
Flow rate: 0.9 mL/min
Sample : 10μL of 1.2 pmol/μL
Temperature: 80°C
Detection: UV@260 nm

Comparison of TEAA and TBAB for the Separation of Five 20 mers





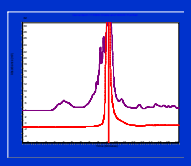
Separation of Failure Sequences for a 26 mer Using TBAB (Crude and Purified)

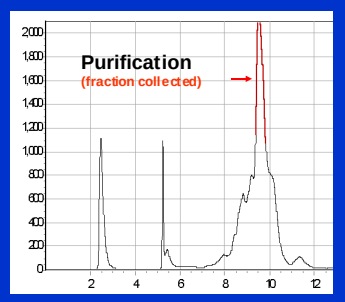


Chromatographic Conditions

Cartridge: OligoPrep HC
Eluent A: 30mM TBAB/10% ACN
Eluent B: 30mM TBAB/80% ACN
Gradient: 10% B for 2 min, 60% to 80% B from 2 to 14 min
Flow rate: 0.9 mL/min
Sample : 50 μL
Temperature: 80°C
Detection: UV@260 nm
Purity by WAVE® assay - 92%

Purification of 1μmol of a 48 mer using the OligoSep Prep HC with TEAA

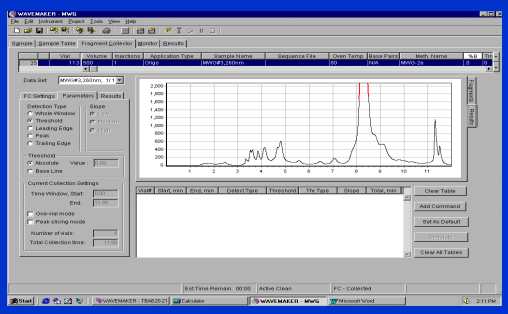




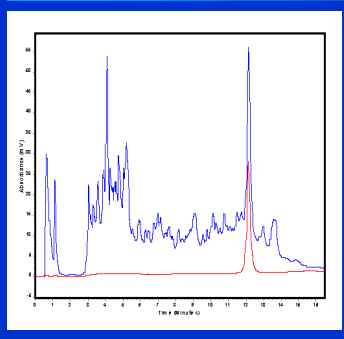
Chromatographic Conditions

Cartridge : OligoPrep HC
Eluent A: 100 mM TEAA
Eluent B: 100 mM TEAA/75% ACN
Gradient 10% to 35% B in 14 min
Flow rate: 0.9 mL/min
Sample : 1500 μL
Temperature: 80°C
Detection: UV@290nm

Wavemaker Fragment Collector Screen



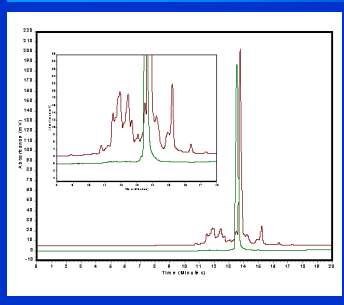
Comparison of Crude and Purified 72 mer



Chromatographic Conditions

Column: OligoPrep HC
Eluent A: 100mM TEAA
Eluent B: 100mM TEAA/25% ACN
Gradient: 5% B for 0.5 min, 20% to 40% B from 0.6 to 15 min
Flow rate: 0.9 mL/min
Sample : 25 μL
Temperature: 80°C
Detection: UV@260 nm

Comparison of a Crude and Purified 36 mer Taqman Probe



Chromatographic Conditions

Cartridge : OligoPrep HC
Eluent A: 100 mM TEAA
Eluent C: 100 mM TEAA/25% ACN
Gradient: 5% to 30% B in 16 min
Flow rate: 1.0 mL/min
Sample : 25 μL
Temperature: 80°C
Detection: UV@300 nm
Purity assay: 93% by anion exchange

Summary

- High resolution assay and purification of synthetic oligonucleotides is achieved using the WAVE® System in the dHPLC mode
- The system is fully automated with preloaded methods for a wide range of oligonucleotides
- Rugged polymeric cartridge operates under fully denaturing conditions to assure high resolution and purification
- Size or size/composition based separations accomplished by changing eluents
- For most applications, no desalting is required with the TEAA chemistry