



Purification and Analysis of Oligonucleotides Using Jupiter™ Proteo

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The widespread use of molecular biology techniques and an ever increasing use of oligonucleotide probes in high-throughput assays have led to a dramatic increase in the demand for custom synthesized oligonucleotides. These high-throughput techniques require that oligonucleotide probes be of high purity to minimize spurious results.

The standard technique for oligonucleotide synthesis is a stepwise chain-extension reaction that generates numerous shorter-length “failure” sequences along with the desired oligonucleotide. Such side reactions result in “typical” oligonucleotide products being only 60-80% pure, thus necessitating a post-reaction purification step. Electrophoresis and ion exchange chromatography (IEX) have traditionally been used to purify and analyze oligonucleotide products. These techniques are slow, inefficient, and not very amenable to automation.

In this application note we show a simple reversed phase HPLC method that can be used for both purification and analysis of crude synthesized oligonucleotides in less than 30 min. The separation is performed using a 50 × 4.6 mm Jupiter™ Proteo HPLC column and is applicable for oligonucleotides of 5 to 30 residues in length.

Instrumentation/Equipment

Analyses were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, California) equipped with a quaternary pump, in-line degasser, multi-wavelength detector, and autosampler. HP Chemstation software (Version A.08.03) was used for the

data analysis. The HPLC column used for the analysis was Jupiter Proteo 50 × 4.6 mm (Phenomenex, Torrance, California). A 12-18mer dT oligonucleotide standard was purchased from Amersham Pharmacia Biotech Inc. (Piscataway, New Jersey) and a crude synthetic 20mer oligonucleotide (sequence: ACGTCATGTCGAGAT-CATCG) was purchased from Integrated DNA Technologies Inc. (Coralville, Iowa).

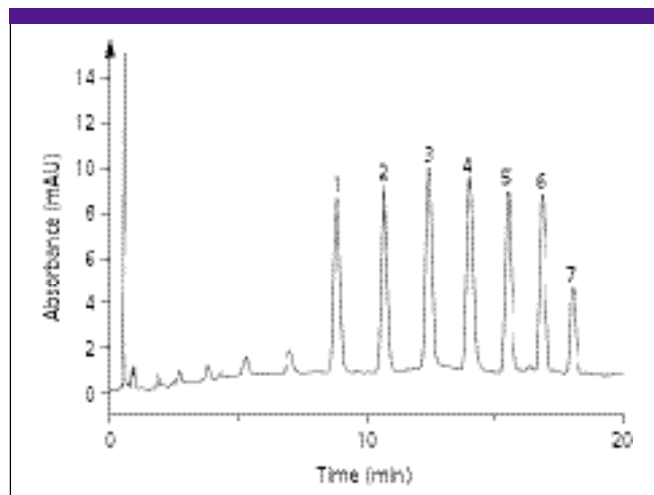


Figure 1: HPLC Chromatogram of an oligonucleotide standard. The standard is a mixture of different poly dT nucleotides between 12 and 18 residues in length. 1 = 12mer, 2 = 13mer, 3 = 14mer, 4 = 15mer, 5 = 16mer, 6 = 17mer, 7 = 18mer.

Experimental Method

Lyophilized samples were reconstituted in 0.1 M Ammonium Acetate, pH 7.0 as per manufacturer's recommendation. Analyses were performed using a simple ion-pair gradient method. The composition of mobile phase A was 0.1 M Triethylamine acetate (TEAA), pH 7.0 in water. Mobile phase B was 100% acetonitrile. The column was initially equilibrated with 97% A and 3% B. An aliquot of 5 μ L of sample was injected for each analysis. Column temperature was maintained at 60 °C and the flow rate was 1.0 mL/min throughout the run. Elution of sample was monitored by UV at 260 nm. The gradient program consisted of a linear ramp from 3% B to 15% B over 20 min. The column was washed with 100% B for 1 min, and re-equilibrated at 3% B for 3 min (3 to 5 column volumes) prior to subsequent injections. The overall cycle time is less than 30 min per sample and can be reduced significantly if shorter cycle time is a requirement.

Results

An injection of a standard 12-18mer poly dT standard was used to demonstrate the ability of the method to separate oligonucleotides that differ by only one base residue (Figure 1). Results show that all of the different length dT oligonucleotides are easily baseline-resolved with excellent peak symmetry. A 7 μ g injection of a crude 20mer hetero-oligonucleotide demonstrates the ability of this method to quickly purify an oligonucleotide from "failed" sequence contaminants (Figure 2). An enlarged view of the chromatogram emphasizes the resolution provided by this method (Figure 3). Additional analyses (data not shown) indicate that this method can be easily adapted for separation of different-sized oligonucleotides (5-30mer) and modified to meet sample purity/cycle time requirements.

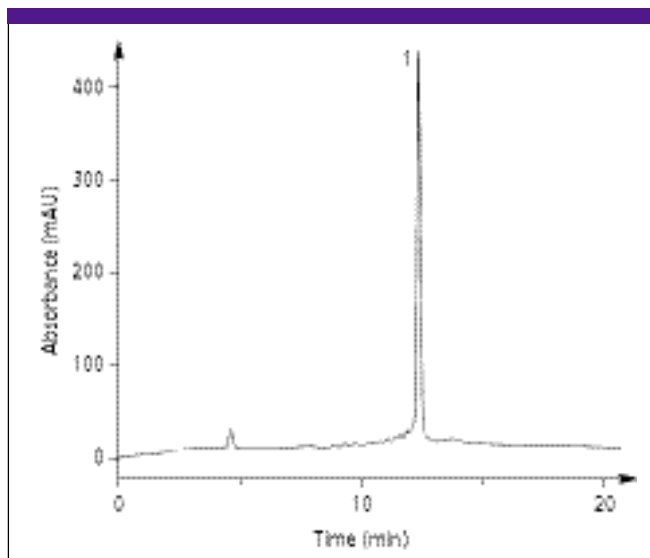


Figure 2: Approximately 7 μ g of a hetero-oligonucleotide was injected on the 50 \times 4.6 mm Jupiter Proteo. 1 = 20mer oligonucleotide.



Figure 3: Detailed zoom-in of Figure 2. Note the separation of 20mer oligonucleotide from "failed" sequence contaminants.

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