Purification and Analysis of Oligonucleotides using Jupiter™ Proteo

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Introduction

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 the oligonucleotide probes used 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 minutes. The separation is performed using a 50 x 4.6mm Jupiter™ Proteo HPLC column and is applicable for oligonucleotides of 5 to 30 residues in length.

Figure 1. rp-HPLC Chromatogram of 12-18mer Poly-dT Oligonucleotide Mixture

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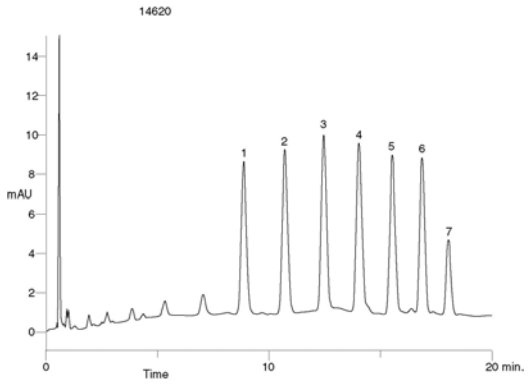

Figure1: HPLC Chromatogram of an oligonucleotide standard. The standard is a mixture of different poly dT nucleotides between 12 and 18 residues in length. 1 = 12mer, 2 = 13mer, 3 = 14mer, 4 = 15mer, 5 = 16mer, 6 = 17mer, 7 = 18mer.

Instrumentation/Equipment

Analyses were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) 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 x 4.6mm (Phenomenex, Torrance, CA, USA). A 12-18mer dT oligonucleotide standard was purchased from Amersham Pharmacia Biotech Inc. (Piscataway, NJ, USA) and a crude synthetic 20mer oligonucleotide (sequence: ACGTCATGTCGAGATCATCG) was purchased from Integrated DNA Technologies Inc. (Coralville, IA, USA).

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Experimental Method

Lyophilized samples were reconstituted in 0.1M 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 0.1M 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 5µL of sample was injected for each analysis. Column temperature was maintained at 60°C and the flow rate was 1.0mL/min throughout the run. Elution of sample was monitored by UV at 260nm. The gradient program consisted of a linear ramp from 3% B to 15% B over 20 minutes. The column was washed with 100% B for one minute, and re-equilibrated at 3% B for three minutes (3 to 5 column volumes) prior to subsequent injections. The overall cycle time is less than 30 minutes per sample and can be reduced significantly if shorter cycle time is a requirement.

Figure 2. rp-HPLC Chromatogram of a Crude 20mer Hetero-Oligonucleotide

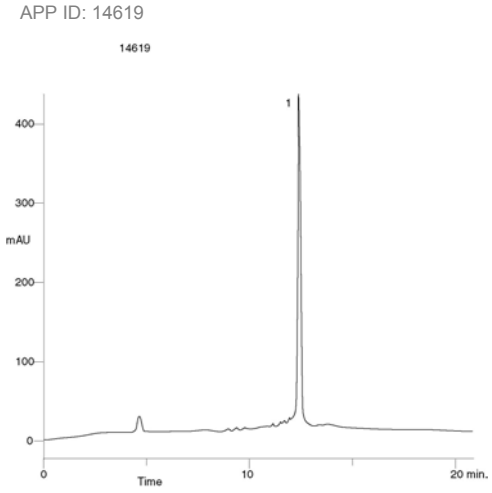

Figure2: Approximately 7µg of a hetero-oligonucleotide was injected on the 50 x 4.6mm Jupiter Proteo. 1 = 20mer oligonucleotide.

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 the different length dT oligonucleotides are easily baseline-resolved with excellent peak symmetry. A 7µg 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 3. 20mer Hetero-Oligonucleotide Chromatogram: Detailed View

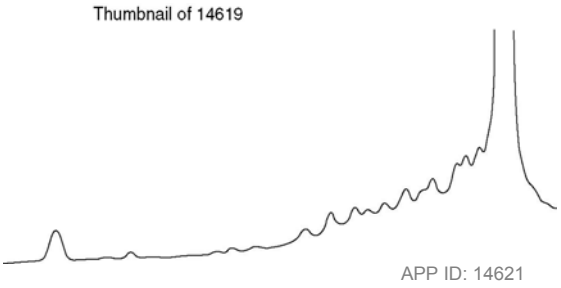

Figure3: Detailed zoom-in of figure 2. Note the separation of 20mer oligonucleotide from "failed" sequence contaminants.

Ordering Information

Order Number	Description
00B-4396-E0	Jupiter 4µ Proteo 90Å 50 x 4.6mm