Purification of Oligonucleotides Using Luna® NH_2, a Weak Anion Exchange Column

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There has been a large increase in the use of synthetic oligonucleotides as techniques such as silencing RNA, antisense DNA, and aptimers have found more widespread use among researchers. With this increase there is a concomitant need for synthetic oligonucleotides to be of the highest purity to meet the stringent demands of many molecular biology techniques.

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-synthesis purification step. Ion Exchange Chromatography (IEX) and reversed phase chromatography are often used either alone or in tandem to purify and characterize oligonucleotide products.

In this application note, we demonstrate an ion exchange HPLC method that can be used for both purification and analysis of crude synthesized oligonucleotides in less than 60 minutes. The separation is performed using a Luna® 5µ NH_2 150 × 4.6 mm HPLC column, a weak anion exchange media, and is applicable for DNA and RNA oligonucleotides of 5 to 30 residues in length.

Instrumentation/Equipment

Analyses were performed using an HP 1100 HPLC system (Agilent Technologies, Palo Alto, California) equipped with a quaternary pump, in-line degasser, multi-wavelength detector, and autosampler.

HP Chemstation software (Version A.09.01) was used for the data analysis. The HPLC column used for the analysis was Luna 5µ NH_2 150 × 4.6 mm (Phenomenex, Torrance, California). A 12–18mer poly dT oligonucleotide standard was purchased from Amersham Pharmacia Biotech Inc. (Piscataway, New Jersey). Crude synthetic 20mer DNA oligonucleotide (sequence: ACGTCATGTCGAGATCATCG) and 19mer siRNA oligonucleotide (sequence: CUGGACUUCCAGAAGAACAdTdT) were purchased from TriLink Biotechnologies (San Diego, California).

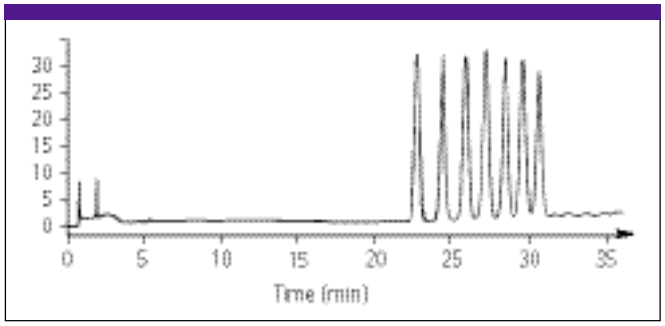

Figure 1: Ion exchange chromatogram of a 12–18mer poly-dT oligonucleotide mixture. The standard is a mixture of different poly dT nucleotides between 12 and 18 residues in length. Larger oligonucleotides demonstrate greater retention by IEX.

Experimental Method

Lyophilized samples were reconstituted in 0.1 M Ammonium acetate, pH 7.0 as per manufacturer's recommendation. Separations of oligonucleotides were performed by ion exchange chromatography with differential elution of analytes being achieved by a linear gradient of increasing salt. An aliquot of 10 µL of the 0.5 mg/mL sample was injected for all analyses. Column temperature was maintained at 40 °C and the flow rate was 1.0 mL/minute throughout the run. Elution of sample was monitored by UV at 260 nm.

For the poly dT standard and crude DNA oligonucleotide the composition of mobile phase A was 20 mM Sodium phosphate pH 7.0 with 10% acetonitrile in water. Mobile phase B was 20 mM Sodium phosphate, 1 M Sodium chloride with 10% acetonitrile in water. The column was equilibrated with 25% B prior to analysis. The gradient program consisted of a linear ramp from 25% B to 95% B over 50 minutes.

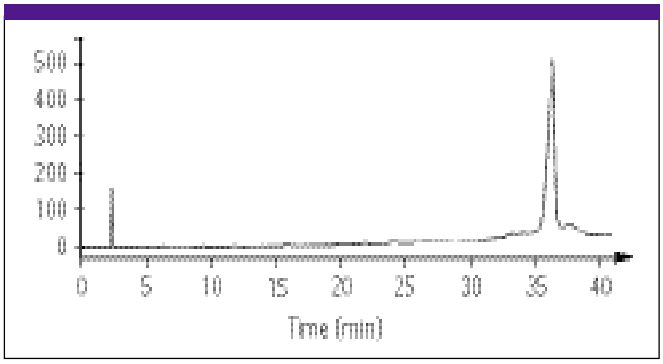

Figure 2: Ion Exchange Chromatogram of a Crude 20mer DNA Oligonucleotide. Approximately 5 μg of a DNA hetero-oligonucleotide was injected on the 150 × 4.6 mm Luna NH_2 column.

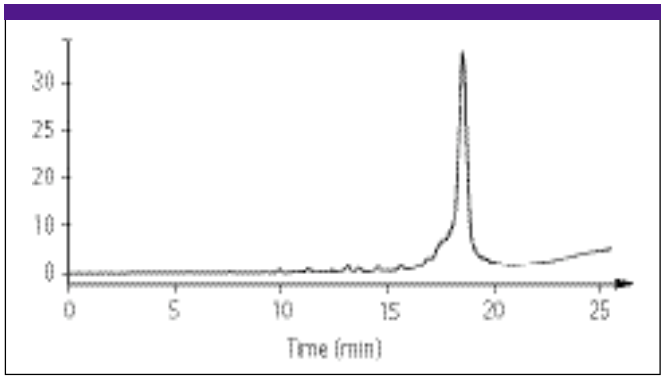

Figure 3: Ion Exchange Chromatogram of a Crude 19mer siRNA Oligonucleotide. Approximately 5 μg of a siRNA hetero-oligonucleotide was injected on the 150 × 4.6 mm Luna NH_2 column.

For purification of the crude siRNA sample mobile phase A was 20 mM Tris, pH 8.0 in water. Mobile phase B was 20 mM Tris pH 8.0, 1 M Sodium chloride in water, and the column was equilibrated with 10% B prior to injection. The gradient program consisted of a linear gradient from 10% B to 100% B over 25 minutes. For all analyses the column was re-equilibrated at initial conditions for three minutes prior to subsequent injections.

Results and Discussion

An injection of a standard 12–18mer poly dT DNA oligonucleotide mixture was used to demonstrate the ability of the method to separate oligonucleotides that differ by only one base in length (Figure 1). Different oligonucleotides from 12 to 18 bases in length are easily resolved and such separation is applicable for both DNA and RNA oligonucleotides up to 30 residues in length. An example of this method being used for the purification of a crude synthesized DNA hetero-oligonucleotide is demonstrated in Figure 2. Different length "failure" sequences are easily separated from the oligonucleotide of interest.

This technique can also be applied for synthetic RNA oligonucleotides. A crude 19mer siRNA hetero-oligonucleotide was injected on column and results are shown in Figure 3. While RNA separations are more challenging, separation between the siRNA oligonucleotide and synthesis contaminants is achieved.

These results demonstrate the use of Luna NH_2 as a weak anion exchange column to either purify or examine the purity of synthesized oligonucleotides. This column can be used in different formats depending on the scale required.

Ordering Information

Order Number	Description
00F-4378-E0	Luna 5μ NH_2 100 Å 150 × 4.6 mm

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