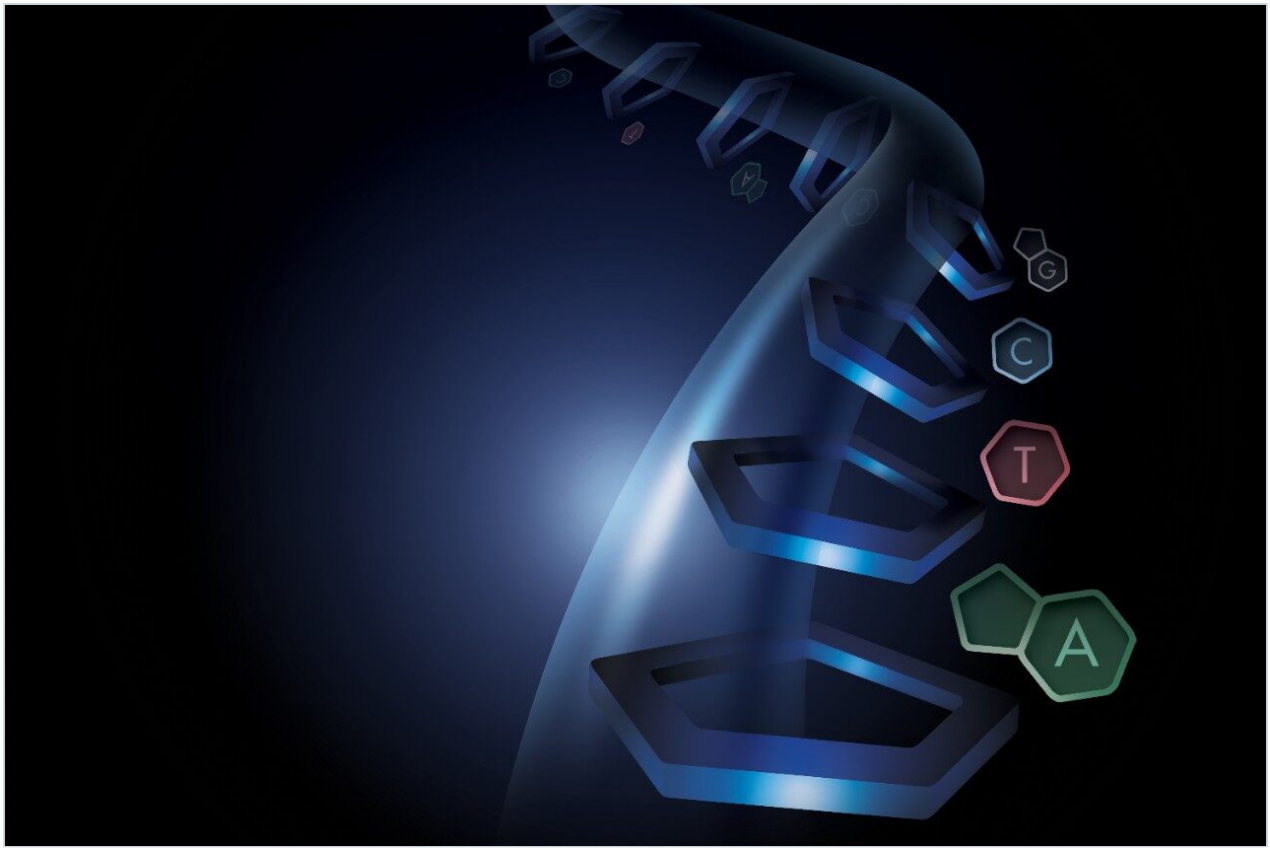
Waters™

Nota de aplicación

HPLC Purification of Long Synthetic Oligonucleotides

Waters Corporation



Abstract

This application note demonstrates HPLC purification of long synthetic oligonucleotides.

Introduction

HPLC Purification of Oligonucleotides

Synthetic oligonucleotides are utilized in a variety of molecular biology applications including polymerase chain reaction (PCR), DNA sequencing, and as antisense drugs. Purification of these oligonucleotides by traditional methods such as polyacrylamide gel electrophoresis (PAGE), can be time consuming, expensive, and more difficult on a larger scale. In addition, the synthesis of oligonucleotides greater than 50 bases in length is challenging, which results in a final product containing many failure impurities (<50% purity). A method has been developed for the reversed-phase high performance liquid chromatography (RP-HPLC) purification of long-chain synthetic oligonucleotides using traditional ionpairing (IP) mobile phases. Using a 4.6 × 50 mm analytical column, up to 0.25 µmole of synthetic DMToff oligonucleotide can be purified in one injection using ultraviolet (UV) detection. The method requires no post-purification desalting or detritylation.

Results and Discussion

Oligonucleotide Separation on XTerra MS C_{18} Columns

XTerra Columns are packed with porous hybrid sorbent with particle sizes as low as 2.5 µm. The sorbent has high mass load capacity needed for semi-preparative and preparative purifications of long-chain synthetic oligonucleotides. The small sorbent particle diameter permits resolution of the target oligonucleotide from closely-eluting impurities (similar retention), even for oligonucleotides greater than 50mer in length. Figure 1 shows analytical scale RP-HPLC separation of a crude synthetic 60mer oligonucleotide under ion-pairing (IP) conditions. Many failure products can be observed in the chromatogram, however, they are all separated from the target product in under 20 minutes. Purity was determined by UV peak area at 260 nm.

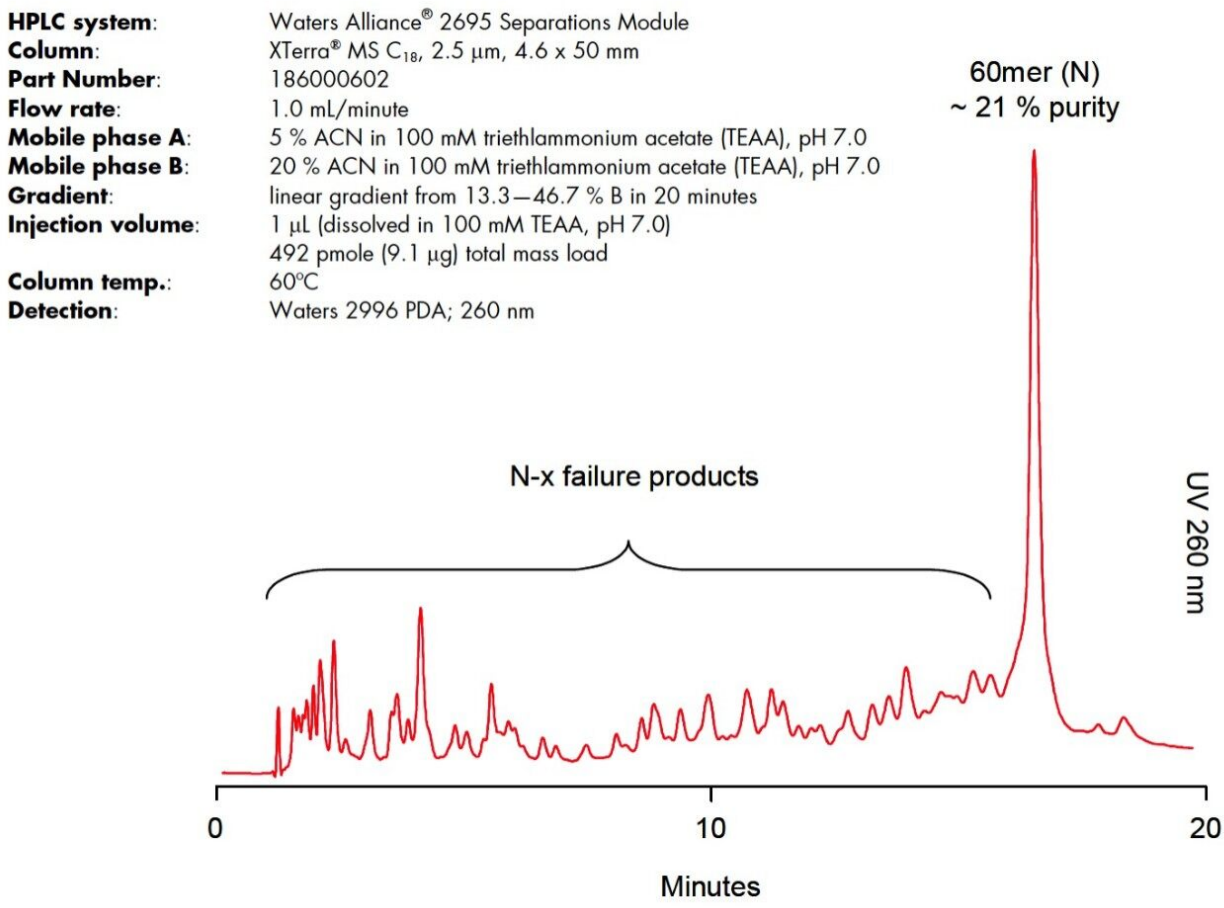


Figure 1. IP-RP-HPLC separation of a crude synthetic 60mer heterooligonucleotide (analytical scale).

Semi-Preparative Purification of Long Oligonucleotides

Figure 2 shows the analytical injection and subsequent purification of a synthetic 59mer oligonucleotide on the 50 nmole scale. The indicated collection window ensures maximum purity of the target fraction (>80%).

HPLC system:	Waters Alliance® 2695 Separations Module
Column:	XTerra® MS C_{18}, 2.5 µm, 4.6 x 50 mm
Part Number:	186000602
Flow rate:	1.0 mL/minute
Mobile phase A:	5 % ACN in 100 mM triethlammonium acetate (TEAA), pH 7.0
Mobile phase B:	15 % ACN in 100 mM triethlammonium acetate (TEAA), pH 7.0
Gradient:	linear gradient from 20—70 % B in 20 minutes
Injection volume:	1—50 µL (dissolved in 100 mM TEAA, pH 7.0) 5, 50, and 250 nmoles total mass load
Column temp.:	60°C
Detection:	Waters 2996 PDA; 260 and/or 290 nm

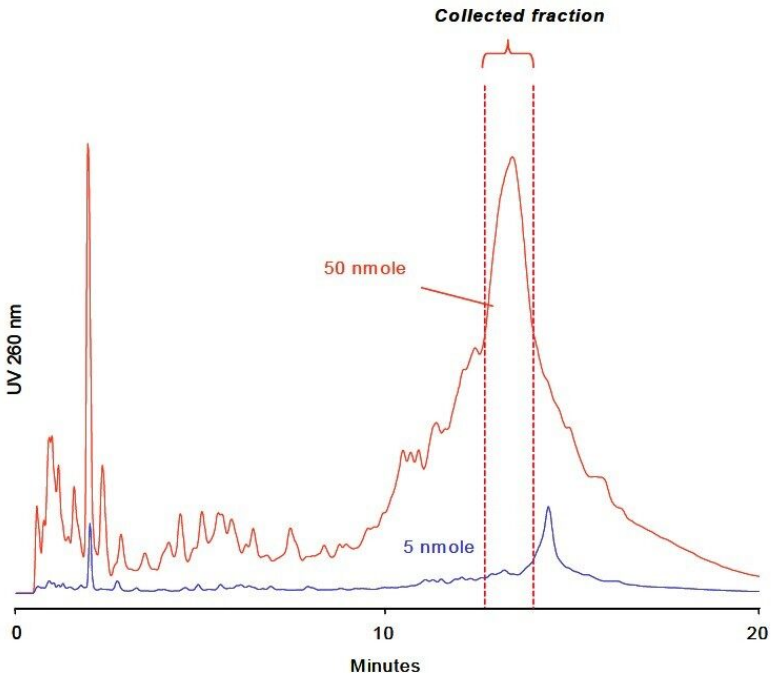


Figure 2. IP-RP-HPLC separation and purification of a 59mer oligonucleotide on the 5 nmole and 50 nmole scales, respectively.

Oligonucleotides synthesized on a 50 nmole–0.25 µmole scale can be purified using a 4.6 × 50 mm XTerra Column. However, it is recommended that a larger column size (i.e. 10 x 50 mm) be used to routinely purify semi-preparative scales (>0.1 µmole). Figure 3 shows the HPLC purification of the same 59mer from Figure 2 on a 0.25 µmole scale. Although the PDA detector is saturated at 260 nm, adjusting the detection wavelength to 290 nm allows one to decide on the appropriate collection window.

Triethylammonium Acetate (TEAA) Buffer Preparation
1 L of 100 mM TEAA, pH 7.0

5.6 mL of glacial acetic acid was placed in ~950 mL of water. While mixing, 13.86 mL of TEA were slowly added; the pH of the resulting solution was typically between 5 and 9. The pH was carefully adjusted to 7 by the addition of either TEA or acetic acid.

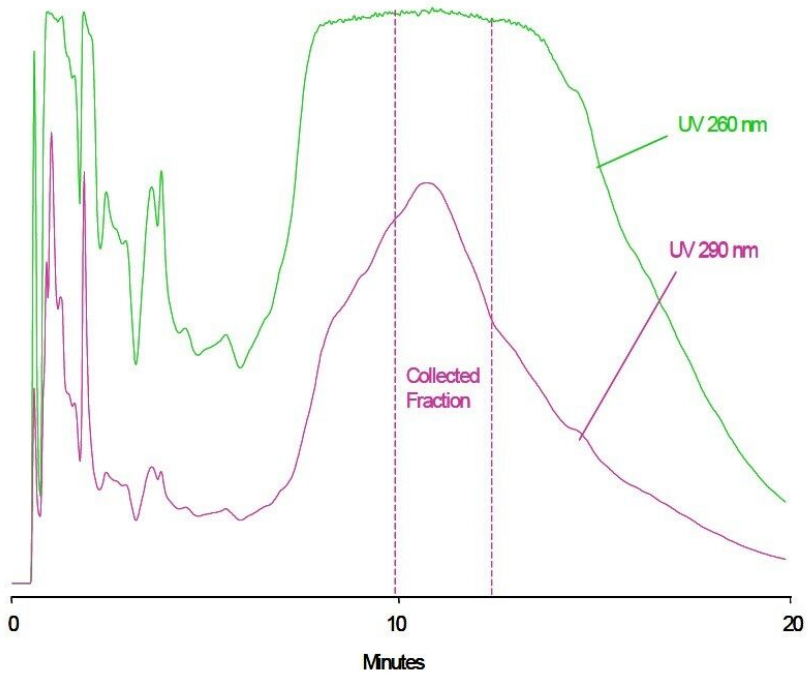


Figure 3. Semi-preparative (0.25 µmole) purification of a 59mer oligonucleotide using UV detection.

Featured Products

Alliance HPLC System <https://www.waters.com/534293>

WA31789, June 2003