

Semi-Preparative Scale Single-Stranded RNA Purification

Sean M. McCarthy, Martin Gilar

Waters Corporation



Abstract

The purification strategy for single-stranded RNA oligonucleotides presented here is rapid, cost

effective, and yields high purity material using the Waters ACQUITY UPLC System with Oligonucleotide Separation Technology (OST) Column chemistry.

Introduction

Oligonucleotide synthesis is a very efficient and high-yielding process. Typical yields of oligonucleotide reactions carried out on solid support range from 98 to 99.5% per coupling step. In a typical multi-step oligonucleotide synthesis, impurities accumulate and the overall yield of even a modest sized 21-mer oligonucleotide can range from 67 to 90%, with longer chain oligonucleotides giving correspondingly lower yields.

For researchers, it is often necessary to work with materials of higher purity than are available from crude synthetic mixtures. For this reason, oligonucleotides used for gene knockout, genotyping, and diagnostic purposes are typically purified following synthesis. Few economically viable solutions exist for lab-scale purification of oligonucleotides, and those that do exist - such as ion-exchange chromatography and polyacrylamide gel electrophoresis - are often cumbersome and/or time-consuming.

In this application note, we describe a cost-effective and rapid method for the purification of modest quantities of material, up to 140 nmoles in a single injection, with final purities of greater than 95% using the Waters ACQUITY UPLC System with Oligonucleotide Separation Technology (OST) Column chemistry. The purification scale presented matches well with typical oligonucleotide synthetic scales (50 to 250 nmol). The method described below allows for the purification of oligonucleotides with high purity products in 15 to 30 minutes.

Experimental

Sample

The RNA oligonucleotide 5' - CCU UGU AAU CGC UUG ACG ATT - 3' was purchased from a vendor and reconstituted in 110 μ L of 0.1 M triethylammonium acetate (TEAA) to yield a solution of approximately 2.8 nmol/ μ L. The sample was prepared immediately prior to use to prevent degradation.

HPLC conditions

The RNA oligonucleotide was purified using a Waters Alliance HPLC Bioseparations System using a Waters XBridge BEH OST C₁₈ 4.6 x 50 mm, 2.5 µm column using ion pair reversed-phase chromatography.¹

LC system:	Waters Alliance HPLC Bioseparations System
Column:	XBridge OST BEH C ₁₈ 4.6 x 50 mm, 2.5 µm
Column temp:	60 °C
Flow rate:	1.0 mL/min
Mobile phase A:	0.1M TEAA, pH 7.5
Mobile phase B:	20% Acetonitrile in A
Gradient:	30 to 52.5% B in 10.0 min (0.15% ACN/min)
Detection:	PDA, 260 nm

UPLC conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC OST C ₁₈ 2.1 x 50 mm, 1.7 µm
Column temp.:	60 °C
Flow rate:	0.2 mL/min
Mobile phase A:	0.1M TEAA, pH 7.5

Mobile phase B:	20% Acetonitrile in A
Gradient:	35 to 85% B in 10.0 min (1% ACN/min)
Detection:	PDA, 260 nm

Results and Discussion

HPLC conditions

Separated products were detected with a Waters PDA detector at 290 nm. The mobile phase A consisted of 0.1 M triethylammonium acetate (TEAA); mobile phase B was 80:20 0.1 M TEAA/acetonitrile. The column temperature was maintained at 60 °C.

As shown in Figure 1, despite the high efficiency of oligonucleotide synthesis, there are many failed sequences present in a 21-mer.

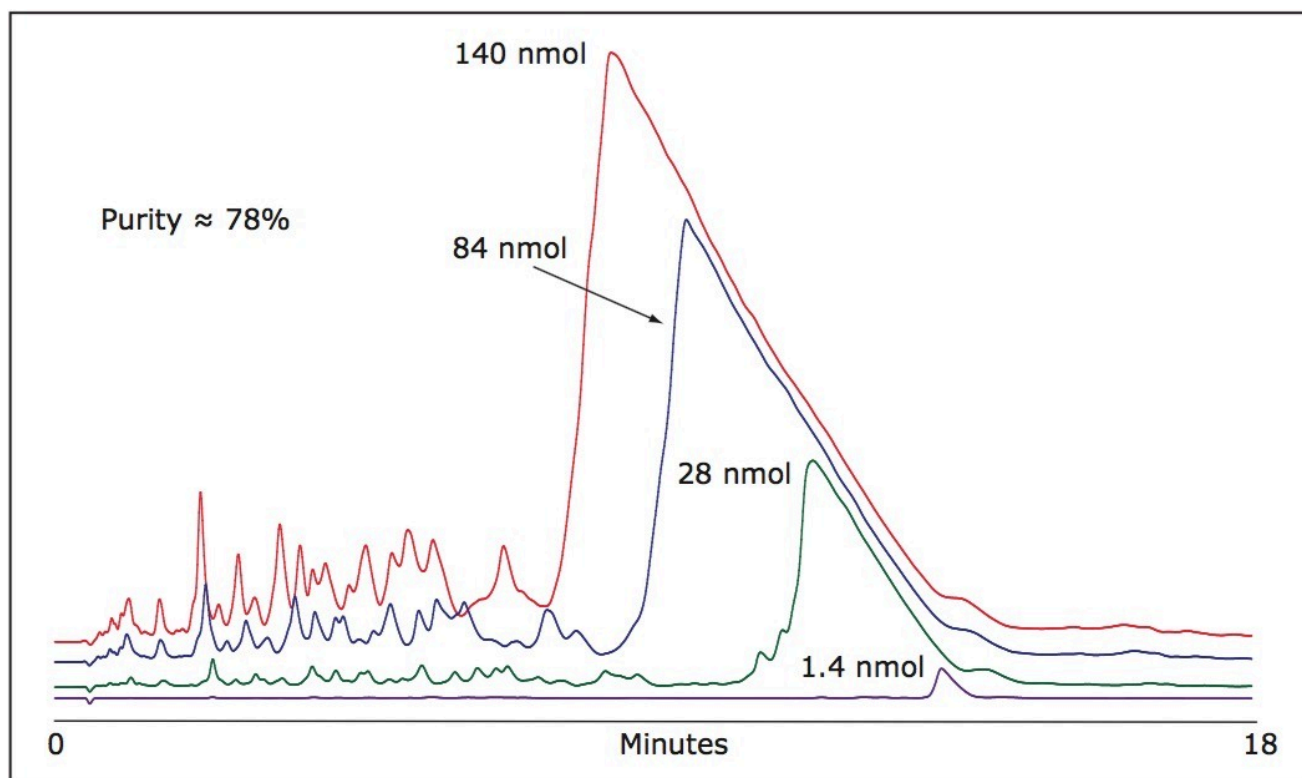


Figure 1. HPLC purification of a synthetic 21-mer oligonucleotide. Sample concentration was 2.8 nmol/ μ L, with on-column loading ranging from 1.4 to 140 nmol.

Although the column is overloaded with greater mass loads, the resolution is maintained with N-1, N-2... impurities eluting at the main peak front. Appropriate hearth-cutting of the main 21-mer oligonucleotide peak yields very high purity product.

The chosen fraction collection windows are indicated in Figure 2 for various mass loads. Following peak collection, samples can be aliquoted as needed and dried for long term storage. The volatility of TEAA allows for an easy removal of ion-pairing buffer components. The purified oligonucleotides after the solvent evaporation are practically salt free.

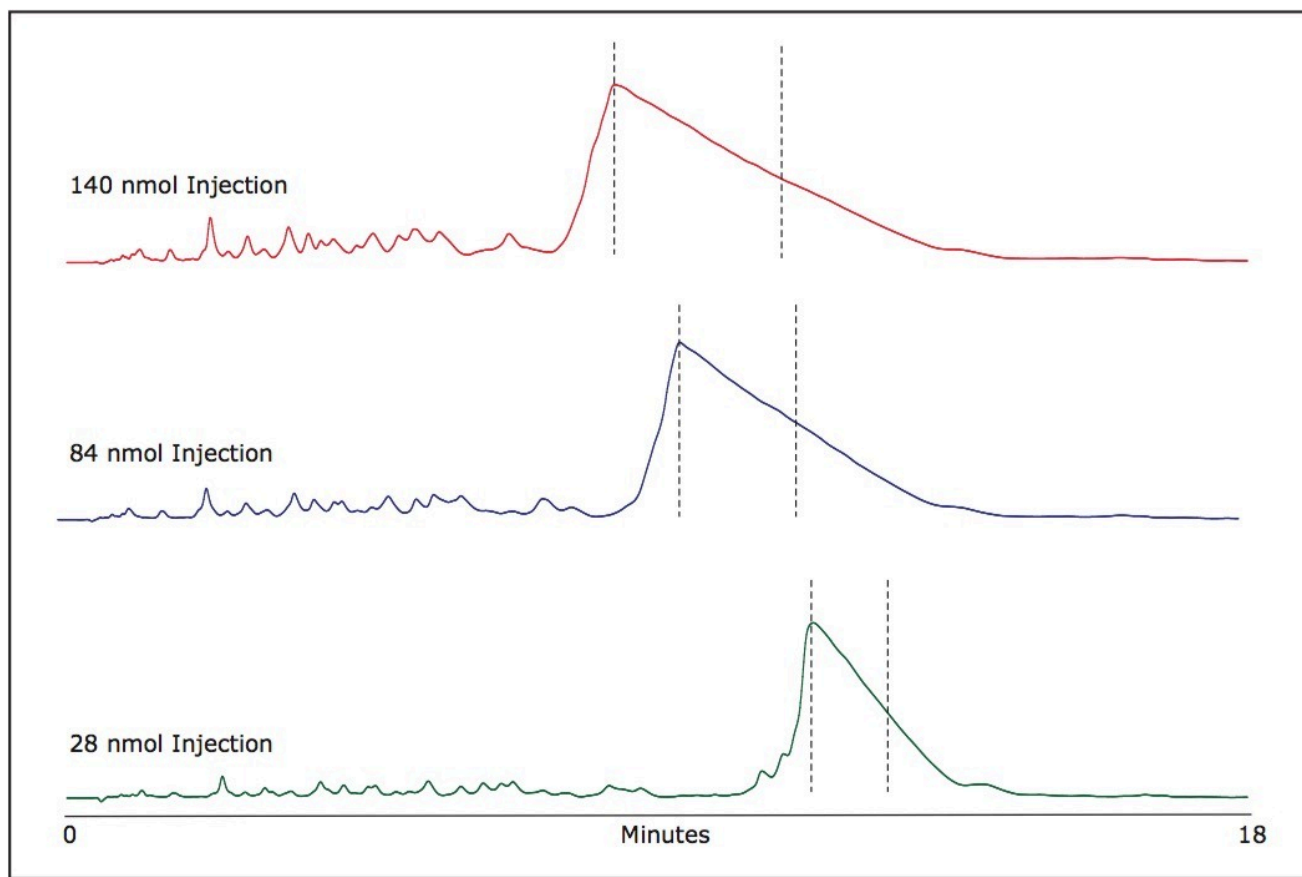


Figure 2. Fraction collection windows. Fractions were collected manually as indicated between dashed lines.

UPLC conditions

The purity of the purified RNA oligonucleotide was verified using the ACQUITY UPLC System. As shown in Figure 3, our purification method efficiently reduces failed sequence impurities and generates an oligonucleotide of superior purity than is available from commercially available oligonucleotides without purification.

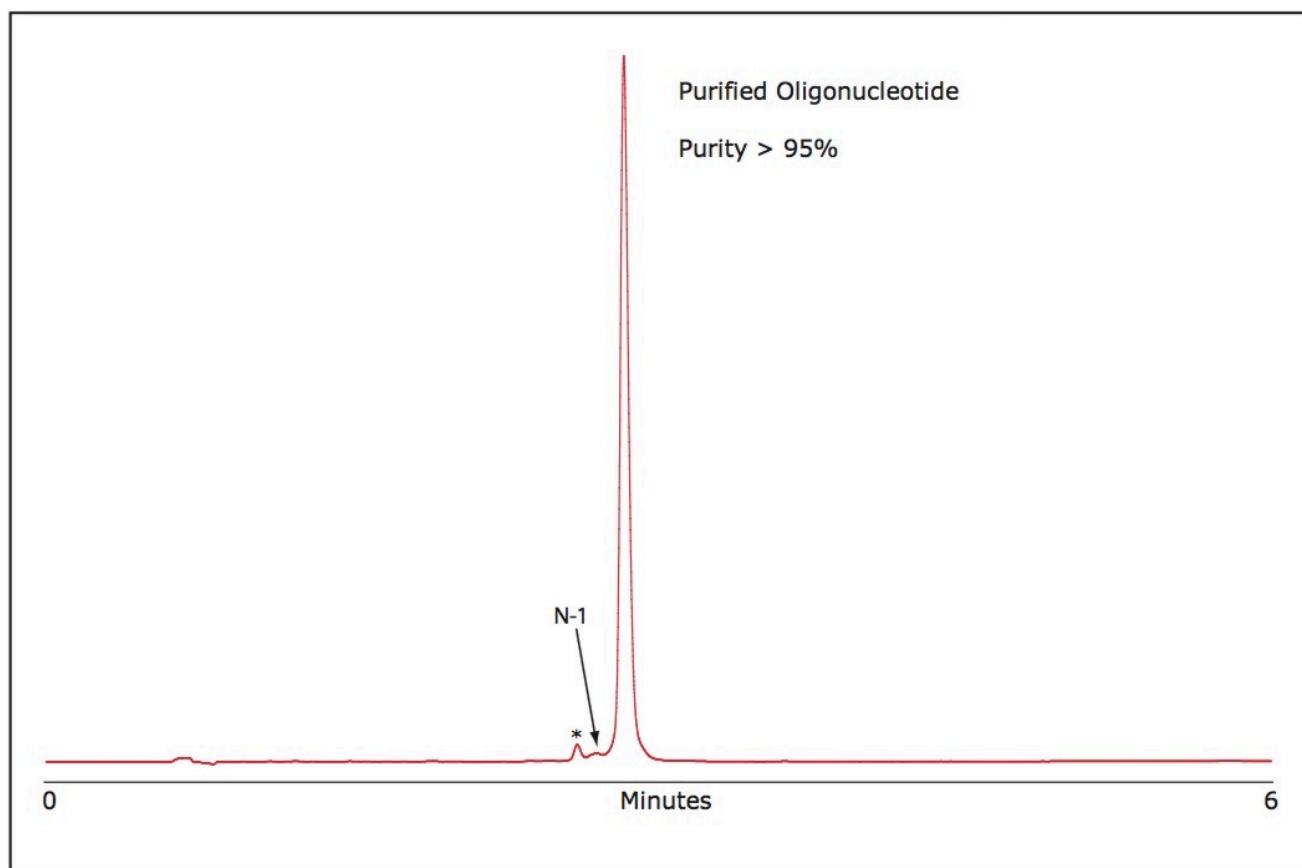


Figure 3. Verification of oligonucleotide purity via UPLC.

(* = non-oligo impurity.)

Conclusion

The purification strategy for single-stranded RNA oligonucleotides presented here is rapid, cost effective, and yields high purity material. In a short time, using OST Column chemistry and the Alliance HPLC Bioseparations System, large quantities of crude single-stranded RNA can be successfully purified yielding material of high purity, ca. 95%, with an estimated yield of 55% based on collected peak area to the total peak area of the sample.

This method is particularly useful for the purification of single-stranded RNA for use in RNAi experiments where assurance of purity, and therefore specificity for the target, are of paramount importance.

Additionally, this strategy allows for storage of purified oligonucleotides in the absence of unwanted salts and other impurities often associated with other purification strategies due to the volatile

nature of TEAA.

Taken together, this strategy offers a comprehensive purification strategy that is superior to those currently available. Furthermore, this purification method is very cost effective when considering the combined cost of time needed for sample purification, reagents, and the long lifetime of Waters XBridge OST columns.

References

1. UPLC Separation of Oligonucleotides: Method Development. Waters Application Note. 2007: 720002383EN.

Featured Products

- [Alliance HPLC System <https://www.waters.com/534293>](https://www.waters.com/534293)
- [ACQUITY UPLC System <https://www.waters.com/514207>](https://www.waters.com/514207)
- [2998 Photodiode Array \(PDA\) Detector <https://www.waters.com/1001362>](https://www.waters.com/1001362)
- [ACQUITY UPLC PDA Detector <https://www.waters.com/514225>](https://www.waters.com/514225)

720002602, April 2008



©2019 Waters Corporation. All Rights Reserved.