



High Performance RNA Oligonucleotide Purification Using Varian TOP-RNA Cartridge

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Introduction

Because of the significant progress achieved in the solid-phase chemical synthesis of oligonucleotides during the past four decades, synthetic oligonucleotides have become readily available and have fuelled the biotechnology revolution that has irreversibly changed biomedical research and the pharmaceutical industry.

Although a powerful technique, solid-phase synthesis has some drawbacks. The main limitation is the need for very high coupling yields in every chain extension step. A consequence of yields of <100% is the accumulation of failure sequences containing deletions. Separation of the full-length product from the shorter failure sequences and especially the n-1 failure sequence is a significant problem.

Trityl-on purification enhances the selectivity between the full length sequence and the truncated failures. Solid phase extraction (SPE) cartridges provide a low cost method for this type of purification. Historically these are capable of performing efficient separations for DNA oligomers of up to 50 bases in length, but have been of limited value for RNA purifications. The reasons for this have been poorly optimised cartridge media and the lack of a robust de-silylation procedure that yields de-silylated trityl-on oligos for purification.

Varian has developed an optimized Trityl-on Oligonucleotide Purification (TOP) cartridge system specifically designed to work for RNA oligomers. The method can purify as few as one, or as many as 96 RNA oligonucleotides in less than 20 minutes using a vacuum procedure.

Materials and Reagents

- TOP-RNA 100 mg tubes, 96/pk (Varian Part No. 7573915C)
- TOP-RNA Quenching Buffer 250 mL (Varian Part No. 75739025)
- TOP™ 2 M triethylammonium acetate (TEAA) (Varian Part No. 79100005)
- Vac Elut™ 20 manifold (Varian Part No. 12234100)
- 1-methyl-2-pyrrolidinone, anhydrous (99.5%)
- Triethylamine (≥99.5%)
- Triethylamine trihydrofluoride (98%)
- Acetonitrile (HPLC grade)

- Trifluoroacetic acid (99%)
- 2% trifluoroacetic acid in deionized water
- Ammonium bicarbonate (≥99.5%)
- 1 M ammonium bicarbonate:acetonitrile (70:30 v/v)

De-silylation Procedure (200 nmol scale preparation)

1. **Cleavage/base deprotection.** The oligomer should be cleaved from the synthesis resin and base deprotected using standard methods (typically concentrated aqueous ammonium hydroxide cleavage, then 55 °C, 16 hours in ammonia solution for base deprotection or 1:1 concentrated aqueous ammonium hydroxide: 40 wt % aqueous methylamine at 65 °C for 10 minutes (the RNA cytosine phosphoramidite must be acetyl protected for this method to work successfully).
2. **Drying of oligonucleotide.** The oligonucleotide is then dried either via vacuum/centrifuge concentration or by freeze-drying. (The bulk of the ammonia is removed prior to freeze-drying by bubbling nitrogen through the sample).
3. **De-silylation.** Add 0.25 mL TEA-3HF de-silylation solution (1.5 mL 1-methyl-2-pyrrolidinone, 0.75 mL triethylamine, 1.0 mL triethylamine trihydrofluoride mixed in that order in a plastic tube) to the dried oligonucleotide. Shake to dissolve the oligonucleotide and place in an oven at 65 °C for 120-150 minutes.
4. **Quench.** Remove from the oven and allow to cool. Add 1.75 mL of TOP-RNA Quenching Buffer to the sample. The sample is now ready for purification.

Method

1. **Condition tube.** With the vacuum on (7 inHg), add 0.5 mL of acetonitrile to the tubes. Ensure a drop rate of approximately 1-2 drops/sec. The vacuum may remain at this setting through step 9.
2. **Equilibrate tube.** As soon as possible, after the acetonitrile has flowed through, add 1 mL of 2 M TEAA to the tubes.
3. **Apply sample.** Add pretreated sample. The sample can be added in 1 mL aliquots.

4. **Wash.** Add 1 mL of 90:10 v/v 2 M TEAA:acetonitrile solution.
5. **Wash.** Add 1 mL of RNase free water.
6. **Detritylate.** Add 2 x 1 mL of 2% trifluoroacetic acid in RNase free water.
7. **Rinse.** Add 2 x 1 mL of water (RNase free).
8. Remove the cover, using the vacuum release valve. Place a tube in the correct position in the rack and replace the cover.
9. **Elute.** Re-apply the vacuum and add 1 mL of 1 M ammonium bicarbonate:acetonitrile (70:30 v/v) to elute product.

The purification should take approximately 10-15 minutes if carried out without any breaks between the additions of the solutions to the cartridge.

Data and Results

A series of Trityl-on RNA oligomer sequences were purified using the above protocol on 100 mg TOP RNA cartridges. A summary of the yield and purity data for the purified products is provided below in Table 1, and HPLC chromatographs in Figures 1, 2, 3 and 4.

Table 1 Purity and yield values from reverse phase chromatograms.

Sequence	Crude Purity	Final Purity	Yield
Figure 1	58%	95%	90%
Figure 2	61%	95%	87%
Figure 3	63%	97%	86%
Figure 4	45%	95%	86%
Figure 5	27%	94%	85%

HPLC Conditions

HPLC analysis of crude and TOP-RNA cartridge-purified RNA.

Column: PLRPS 5 µm, 150 x 4.6 mm
(Varian Part No. PL1111-3500)

Mobile Phase A: 100 mM TEAA, pH 7

Mobile Phase B: Acetonitrile

Gradient: A/B (95:20) from 0-20 min

Flow Rate: 1 mL/min

Detection: UV @ 256 nm, temperature ambient
for 21mer sequences, 60 °C for
63mer

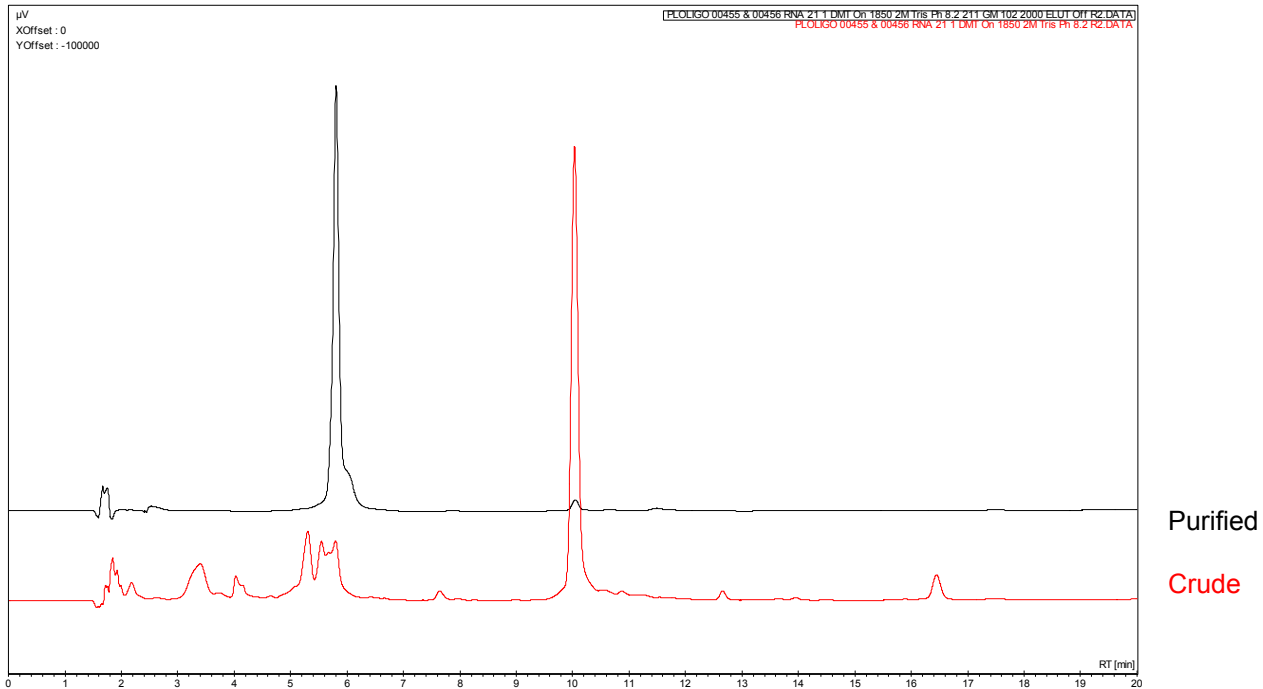
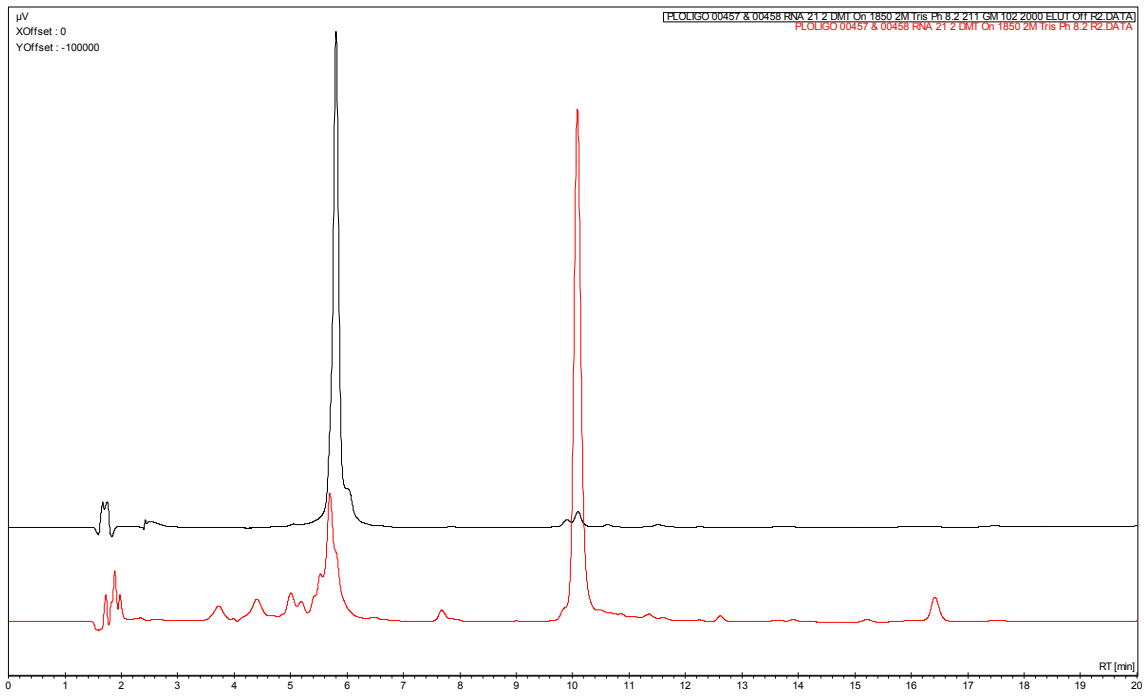


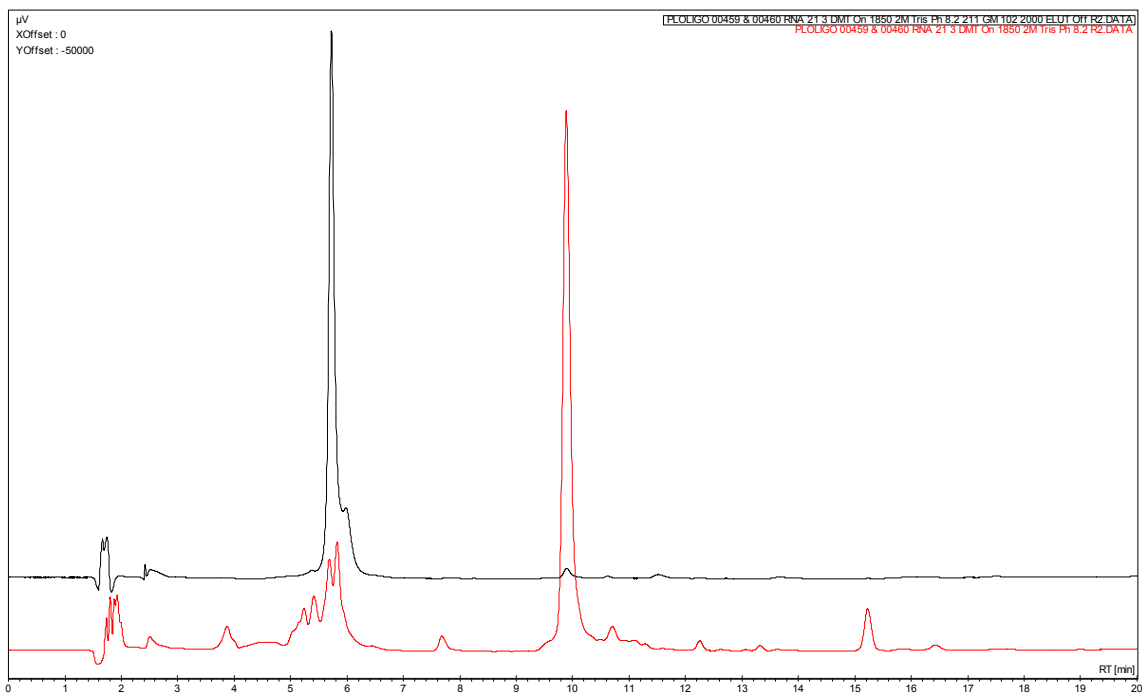
Figure 1 Purification of a 21mer RNA sequence. 5'(DMT) UUG UAC UAC UUU CUG ACG CUU 3'



Purified

Crude

Figure 2 Purification of a 21mer RNA sequence. 5'(DMT) UCC UUU GUG UAU CUC CGU UCA 3'



Purified

Crude

Figure 3 Purification of a 21mer RNA sequence. 5'(DMT) CUC AAC AUU CAU UGC UGU CGG 3'

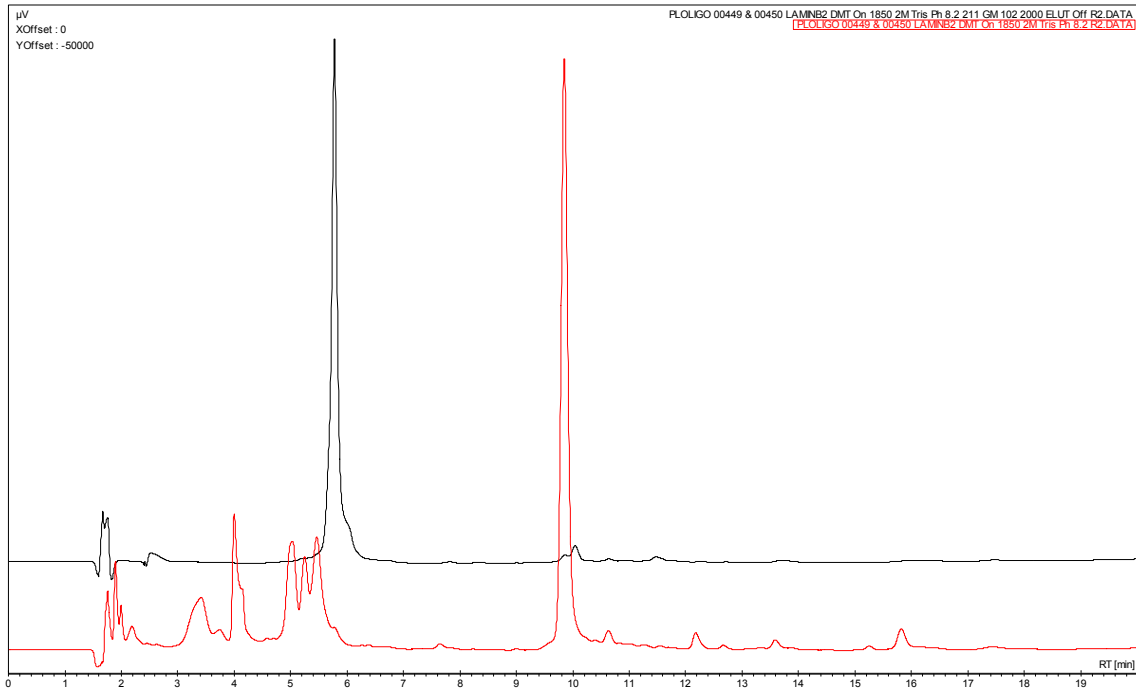


Figure 4 Purification of a 21mer RNA sequence. 5'(DMT) ACU CGG CUU CCU CCU CUU 3'

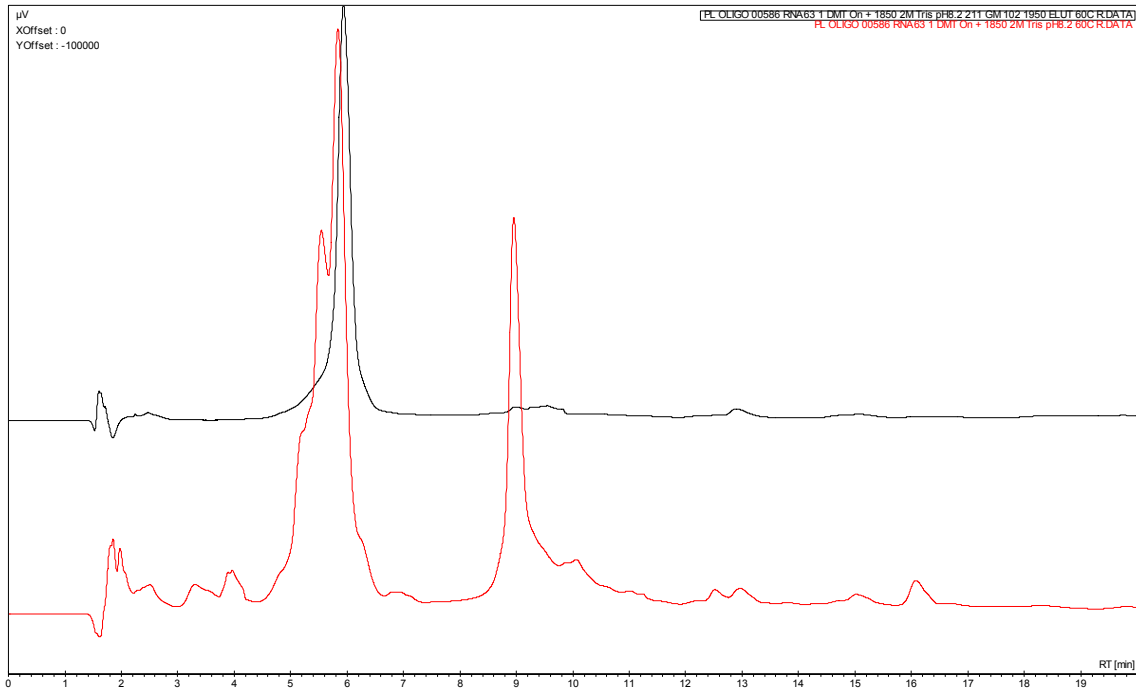


Figure 5 Purification of a 63mer RNA sequence. 5'(DMT) UCC UUU GUG UAU CUC CGU UCA CUC AAC AUU CAU UGC UCU CGG ACU CGG CUU CCU CCU CCU CUU 3'

Conclusion

This protocol, combined with the TOP-RNA high-affinity sorbent, 96-format, and optimized reagents, provides a simple, high throughput process for effective purification of both long and short chain RNA oligonucleotides, providing both high yields (>85%) and high purity were achieved (>90%).

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