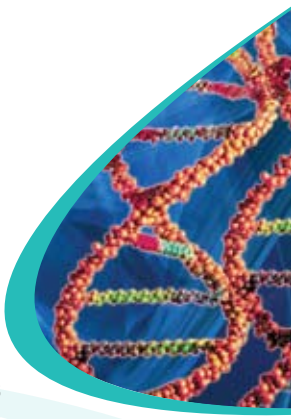
Comparing Performance of High-Throughput, Trityl-on RNA/DNA Purification Products

Author: Greg Scott



Introduction

Clarity® QSP™, which is part of Phenomenex's Clarity® BioSolutions portfolio for synthetic RNA & DNA purification, is a newly developed high-throughput purification (HTP) trityl-on platform developed specifically for modern synthetic oligonucleotides. Designed to alleviate the short falls of reversed phase cartridges (RPC), Clarity® QSP™ offers ease and efficacy. To offer more insight as to the respective differences between QSP™ and a leading RPC product (Varian *TOP™), a direct comparison study was performed. Performance of Varian's *TOP™-DNA & *TOP™-RNA products as well as the QSP™ product was measured by evaluating the finalpurities and recovery yields resulting from the purification of crude synthetic ssDNA and ssRNA samples by each method.

Trityl-on RPC products rely on ion-pairing reversed phase chromatography principles. Offering a bind and elute strategy, the trityl labeled full-length sequence along with aborted sequence fragments are initially retained on a polymeric media through the aid of an ion pairing agent, normally triethylammonium acetate (TEAA). In the RPC technique, a dilute acetonitrile buffer is incorporated to selectively remove the less retentive truncated trityl-off sequence contaminants from the protected full-length sequence. Following detritylation with dilute acid, the purified full-length sequence is eluted free of most synthetic contaminants. Several inherent problems exist with this approach, notably: low recovery yields, retained former trityl-on contaminants and the necessity for downstream purification to remove toxic ion-pairing agents for in vivo studies. The decades old RPC process has encountered mixed reviews by its users as some have praised such RPC products, while others report general dissatisfaction.

Clarity® QSP™ provides an alternative trityl-on approach for separating truncated sequences and synthetic contaminants from the protected full-length sequence. By incorporating a polymeric sorbent with a unique buffer formula, aborted fragments and synthetic contaminants are removed during sample loading offering a departure from the traditional RPC mechanism. Multiple buffers and gradient elutions are eliminated, thereby making Clarity® QSP™ a 3-step product for trityl-on oligonucleotide purification.

Materials and Methods

Materials

Integrated DNA Technologies, Coralville, IA and USC Norris Cancer Center DNA Core Lab, Los Angeles, CA provided 200 nmole synthesis scale of crude trityl-on ssDNA and ssRNA. DNA sequences were retained in concentrated NH_4OH following support cleavage and primary deprotection. For all RNA sequences, a deprotection cocktail of EtOH / NH_4OH was evaporated to dryness prior to 2' deprotection.

Trityl-on purifications were performed using Varian's *TOP™-RNA,*TOP™-DNA products and the Clarity® QSP™ product.

***TOP™ components**
Acetonitrile
2 M TEAA pH 7.0
Water (Nuclease-free)
2 % trifluoroacetic acid (aqueous)
100 mg /mL NaCl solution (DNA)
TOP™-RNA quenching buffer
1 mL Cartridge (150 mg / sorbent)

Clarity® QSP™ components
Methanol
Water (Nuclease-free)
2 % dichloroacetic acid (aqueous)
DNA loading buffer
RNA-TBDMS loading buffer
Na_2CO_3
Sorbent (50 mg / cartridge)

* TOP is a trademark of Varian, Inc. Phenomenex is in no way affiliated with Varian, Inc.

Analysis

Quantitative

Beckman Coulter 700 series Spectrophotometer: OD_{260}

Samples Analyzed

- Crude sample in loading buffer(s): (1:100 dilution)
- Load eluant
- Wash eluant
- 2 % TFA / 2 % DCA eluant
- Water rinse
- Final eluant (1:100 dilution)

Chromatography

HPLC Instrumentation:	Agilent 1100 quaternary system
Column:	IEX column, †DNAPac® PA200 250 x 4.0 mm
Mobile Phase:	A: Water / C: 0.25 M Tris-HCL pH 8/ D: 0.375 $NaClO_4$
Gradient:	A: 80 % C: 10 % D: 10 %, D: 10 % - 65 % in 20 minutes
Flow Rate:	1.2 mL / min
Detection:	UV @ 260 nm

Samples Analyzed

- Crude: (1:10 dilution), 100 µL injection volume
- Load eluant: 100 µL injection volume
- Wash eluant: 100 µL injection volume
- Final elution: (1:10 dilution), 100 µL injection volume

† DNAPac is a registered trademark of Dionex Corporation

DNA Purification - Clarity® QSP™

Sample preparation for Clarity® QSP™ purification required the addition of an equal volume of QSP™ DNA loading buffer to the 300 µL volume of NH_4OH cleavage and deprotection solution. Total sample loading volume was 600 µL.

Performed using a 12-slot manifold with vacuum (3"-5" Hg), QSP™ cartridge purification consisted of the following:

- Sorbent Conditioning: 1 mL Methanol (0.5 mL x 2)
- Sorbent Equilibration: 1 mL Water (0.5 mL x 2)
- Loading vol of Oligo (600 µL)
- Detritylation: 1 mL 2 % aqueous DCA
- Rinse: 1 mL Water (0.5 mL x 2)
- Dried sorbent ~ 10" Hg (~1 min)
- Elute: 1 mL 10 mM Na_2CO_3 / 50 % Methanol

DNA Purification - *TOP™-DNA

Following the *TOP™ user guide, sample preparation required the addition of 1 mL of 100 mg / mL NaCl to 300 µL volume of the NH_4OH cleavage and deprotection solution. Total sample loading volume was 1.3 mL.

Run on a 12-slot manifold with vacuum (5"-7" Hg), purification was performed as follows:

- Cartridge Conditioning: 0.5 mL Acetonitrile
- Equilibration: 1 mL 2 M TEAA pH 7.0
- Sample Applying: Add 1 mL aliquots oligonucleotide solution to cartridge
- Wash: Add 2 mL 100 mg/mL NaCl solution to cartridge (1 mL x 2)
- Detritylation: 2 mL 2 % aqueous TFA (1 mL x 2)
- Wash: 2 mL Water (1 mL x 2)
- Elute: 1 mL 50 % Water / 50 % Acetonitrile

* TOP is a trademark of Varian, Inc. Phenomenex is in no way affiliated with Varian, Inc.

RNA Purification - Clarity® QSP™

Desilylation cocktail

46 % NMP
23 % TEA
31 % TEA.3HF

- EtOH / NH_4OH primary deprotection cocktail was removed via evaporation
- Added 100 µL of desilylation cocktail to RNA pellet and heated @ 65 °C for 1.5 hours
- Cooled for ~2 minutes
- Added 400 µL of 1.5 M NH_4HCO_3 to quench
- Added 500 µL of QSP™ RNA-TBDMS loading buffer (total volume 1 mL)

Protocol

- Conditioned: 1 mL Methanol (0.5 mL x 2)
- Equilibrated: 1 mL Water (0.5 mL x 2)
- Loaded: 1 mL Vol of Oligo
- Wash: 1 mL 60:40 Water / QSP™ RNA-TBDMS Loading Buffer
- Detritylate: 1 mL 2 % DCA
- Rinse: 1 mL Water (0.5 mL x 2)
- Elute: 1 mL 20 mM NH_4HCO_3 / 50 % Methanol

RNA Purification - *TOP™-RNA

Desilylation cocktail

46 % NMP
23 % TEA
31 % TEA.3HF

- EtOH / NH_4OH primary deprotection solution was removed via evaporation
- Added 250 µL of desilylation cocktail to RNA pellet and heated @ 65 °C for 2.5 hours
- Cooled for ~2 minutes
- Added 1.75 mL of the *TOP™-RNA quenching buffer. Total volume: 2 mL

Protocol

- Condition: 0.5 mL Acetonitrile
- Equilibrate: 1 mL 2 M TEAA pH 7.0
- Add 1 mL aliquots of oligoribonucleotide solution to the cartridge
- Add 1 mL 10:90 Acetonitrile / 2 M TEAA
- Add 1 mL water
- Add 1 mL 2 % TFA solution to cartridge and repeat with further 1 mL 2.0 % TFA solution
- Add 1 mL water to the cartridge and repeat with further 1 mL water
- Add 1 mL 1 M NH_4HCO_3 / 30 % acetonitrile to the cartridge

* TOP is a trademark of Varian, Inc. Phenomenex is in no way affiliated with Varian, Inc.

Results and Discussion (DNA)

Quantitative data compiled in **Table 1** and supporting chromatograms in **Figures 1-4** communicate the results of the purification of four crude DNA samples using the *TOP™ and Clarity® QSP™ products. To avoid possible sequence influences, the same DNA sequences were purified for each trityl-on product.

Figure 1 represents the results from the *TOP™-DNA procedure, which incorporates a saturated salt solution for the sample load and wash regimens. Following this procedure, the chromatograms and OD_{260} readings indicated no evidence of effective extraction of the aborted fragment contaminants in either the load or wash fractions. Consequently, the test results indicated that there was virtually no difference between the beginning crude sample and final detritylated purified samples using the *TOP™-DNA product.

In comparison, **Figure 2** represents the results using the QSP™ procedure on the same 30 mer DNA sample as in **Figure 1**. Complete discrimination between the full-length trityl-on sequence and synthetic impurities is pronounced in the load fraction resulting in the recovery of a highly purified detritylated full-length sequence. A similar sequence of length and composition was repeated using both products. As presented in **Table 1**, both quantitative and qualitative data revealed similar results to the prior sequence.

Purity and recovery yields are the essential measurements of nearly every purification product, however, a major concern of trityl-on techniques is the potential to increase purine hydrolysis. Accordingly, the two products were monitored for post purification depurination. Using a lone purine sequence, polyamine hydrolysis was performed after *TOP™-DNA and QSP™ purification. The final elution of each product was incubated at room temperature for 24 hours then examined for apurinic sequences. As shown in **Figure 3**, the *TOP™-DNA procedure, which lacks the necessary pH adjustment, yielded prominent depurination within 24 hours. In contrast, **Figure 4** represents the QSP™ product that elutes in a physiological appropriate medium (pH 7-8) resulted in no detectable depurination during the same period.

Table 1 below highlights the head to head comparison of the *TOP™-DNA and QSP™ products. The data revealed that with fewer steps, components, and volumes, QSP™ provided greater purity and recovery yields of DNA than the available *TOP™-DNA regimen.

DNA Comparison

Table 1

TRITYL-ON PURIFICATION	SEQUENCE	CRUDE OD	LOAD / WASH OD	FINAL OD	RECOVERY YIELD	PURITY
*TOP™-DNA	GTGGATCTGCGCACTTCAGGCTCCTGGGCC	18.8	0.21	15.6	84 %	83 %
QSP™	GTGGATCTGCGCACTTCAGGCTCCTGGGCC	40	5.6	34	88 %	91 %
*TOP™-DNA	GTGGATCTGCGCACTTCAGGCTCCTGGGCT	22.3	0.25	20.6	93 %	80 %
QSP™	GTGGATCTGCGCACTTCAGGCTCCTGGGCT	46	4.5	40.3	97 %	92 %

* TOP is a trademark of Varian, Inc. Phenomenex is in no way affiliated with Varian, Inc. Comparative separations may not be representative of all applications.

Results and Discussion (DNA) *(cont'd)*

Figure 1:
***TOP™-DNA**
Loading buffer: 100 mg/ mL NaCl
Sequence 1. GTGGATCTGCGCACTTCAGGCTCCTGGGCC

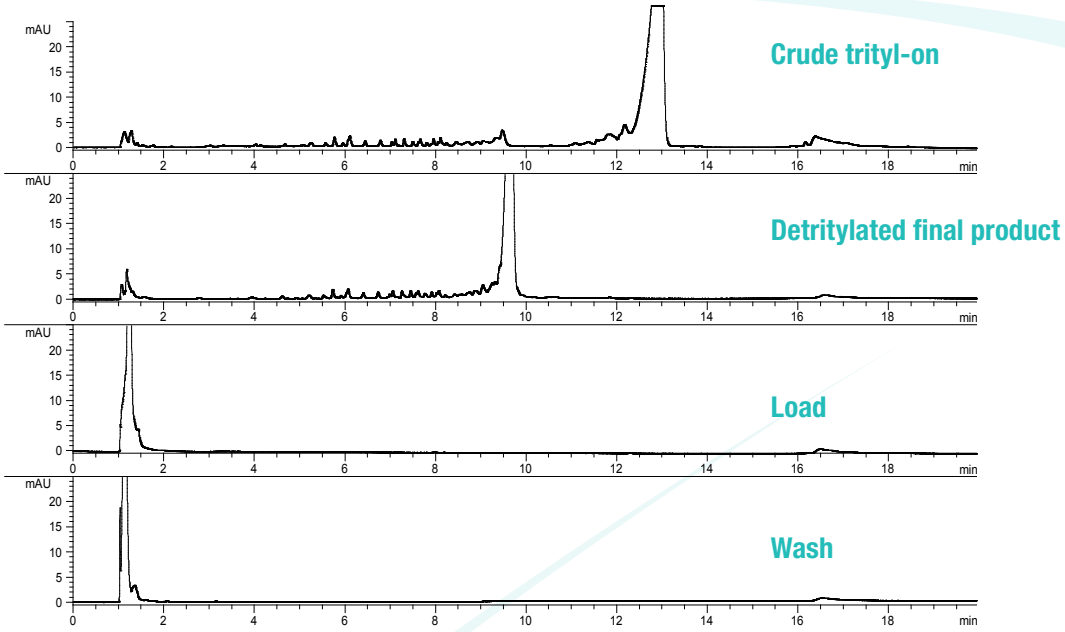
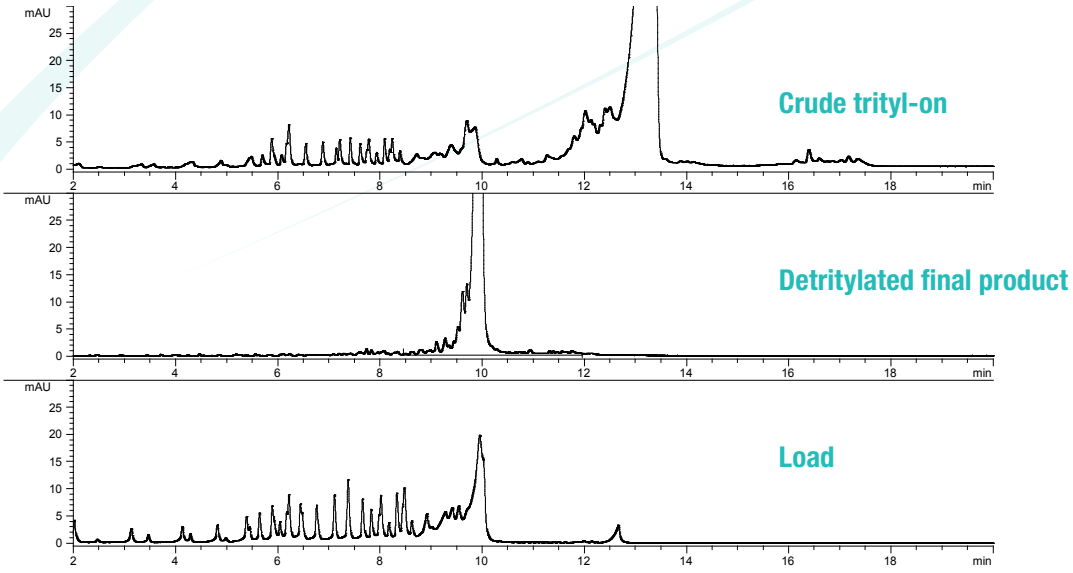


Figure 2:
QSP™ DNA
Sequence 1. GTGGATCTGCGCACTTCAGGCTCCTGGGCC



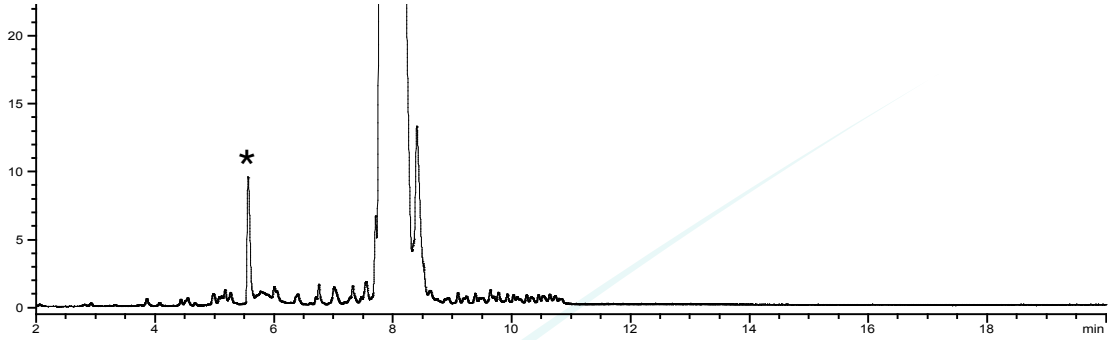
No wash required for DNA purification

* TOP is a trademark of Varian, Inc. Phenomenex is in no way affiliated with Varian, Inc. Comparative separations may not be representative of all applications.

Figure 3:
***TOP™-DNA**
Sequence 2. CTTCTCTCCACTCTCTCTCT

Depurination Protocol

- Following 24 hrs at room temperature, a 200 µL aliquot of the final eluted volume was dried using N_2
- Reconstituted in 200 µL of 50 mM Hepes/1 mM EDTA/2 mM Spermine (pH 8.1)
- Incubated at 37 °C for 1 hr
- Diluted 1:10 injected 50 µL on IEX column

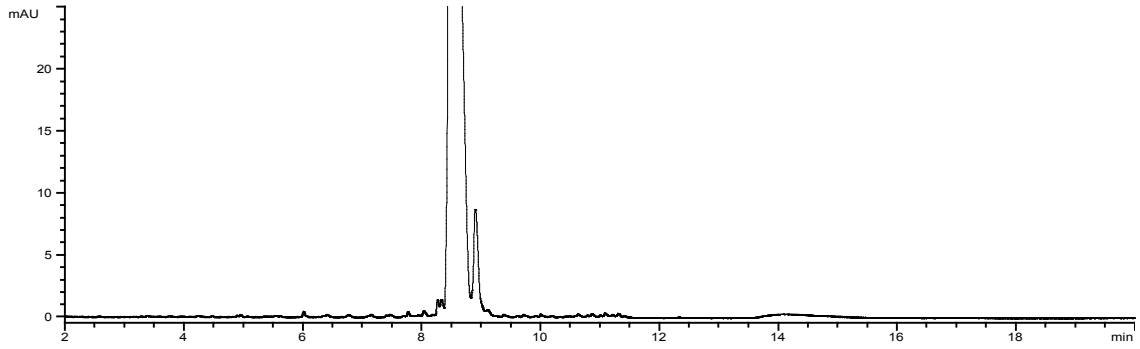


*Depurination peak

Figure 4:
QSP™ DNA
Sequence 2. CTTCTCTCCACTCTCTCTCT

Depurination Protocol

- Following 24 hrs at room temperature, a 200 µL aliquot of the final eluted volume was dried using N_2
- Reconstituted in 200 µL of 50 mM Hepes/1 mM EDTA/2 mM Spermine (pH 8.1)
- Incubated at 37 °C for 1 hr
- Diluted 1:10 injected 50 µL on IEX column



* TOP is a trademark of Varian, Inc. Phenomenex is in no way affiliated with Varian, Inc. Comparative separations may not be representative of all applications.

Results and Discussion (RNA)

In similar fashion with the DNA investigation, results of RNA purification using *TOP™-RNA and QSP™ are presented in quantitative form in **Table 2** and illustrated in **Figures 5-8**. Again, the RNA sequences processed were equivalent between the *TOP™-RNA and QSP™ products as was sample preparation and 2' desilylation, which followed the Wincott method. Unlike DNA purification, the *TOP™-RNA and QSP™ products were quite similar in their RNA purification design. Both products used their respective polymeric sorbent as for DNA purification, and each offered a specific RNA sample-loading/quenching buffer for optimized results.

Figures 5 and 6 provide a comparison of a crude 21 mer RNA purified using the *TOP™-RNA and QSP™ products respectively. The *TOP™-RNA product includes a quenching buffer that is mixed with the desilylation cocktail then directly loaded onto the cartridge. Chromatograms in **Figure 5** demonstrate complete discrimination of aborted fragments in both the sample load and wash fractions with the *TOP™-RNA regimen. Moreover, as shown in **Table 2**, the final product obtained was of a detritylated full-length sequence in moderate recovery yield and with a purity of 90 %. In comparison, the QSP™ product offering a similar optimized loading buffer and sample-prep procedure, also yielded complete discrimination between impurities and the full-length sequence in both the sample load and wash fractions. The final detritylated RNA sequence recovered from the QSP™ product was in good yield and with excellent purity.

Represented in **Figure 7** are the chromatograms following *TOP™-RNA purification of a second crude 21 mer RNA. As with the previous sample, synthetic contaminants were excluded during the sample load and wash steps, delivering RNA in excellent purity and with moderate recovery yields. Similar results following QSP™ purification are shown in **Figure 8**, demonstrating once again that impurities effectively separated in the load and wash regimens and that the full-length sequence was recovered in good yield with excellent purity.

The chromatograms and quantitative data presented for the RNA sequences reveal the similarity in purities between the *TOP™-RNA and QSP™ products for this application. Purity of the six RNA sequences using both *TOP™-RNA and QSP™ were found to be reproducible and approaching HPLC quality. However, the disparity observed between the two products was found in the differences in recovery yields. The QSP™ product consistently recovered higher yields of the detritylated full-length sequences. This can be the attributed to the lack of ion-pairing agents in the QSP™ product, but which are present in *TOP™-RNA. Consequently, the ion-pairing agent (TEAA) used in the *TOP™ product resulted in unwanted retention of the detritylated full-length sequence causing inefficient recovery of the final product.

RNA Comparison

Table 2

TRITYL-ON PURIFICATION	SEQUENCE	CRUDE OD	LOAD / WASH OD	FINAL OD	RECOVERY YIELD	PURITY
*TOP™-RNA	GGAGAACCUGCCCAGCCGCTT	20.7	5.26	9.8	64 %	90 %
QSP™	GGAGAACCUGCCCAGCCGCTT	27	8.88	14.2	78 %	93 %
*TOP™-RNA	GGCUCCCCUCAACAACUUCTT	28.5	3.88	17	69 %	93 %
QSP™	GGCUCCCCUCAACAACUUCTT	29.6	8.78	19.5	94 %	91 %
*TOP™-RNA	CAGUAGUGUAUAAAUUAAATT	26.4	5.45	12.3	59 %	90 %
QSP™	CAGUAGUGUAUAAAUUAAATT	33.5	10.88	19.1	82 %	96 %

* TOP is a trademark of Varian, Inc. Phenomenex is in no way affiliated with Varian, Inc. Comparative separations may not be representative of all applications.

Figure 5:
***TOP™-RNA**
Loading solution: TOP-RNA Quenching Buffer
Sequence 1. GGAGAACCUGCCCAGCCGCTT

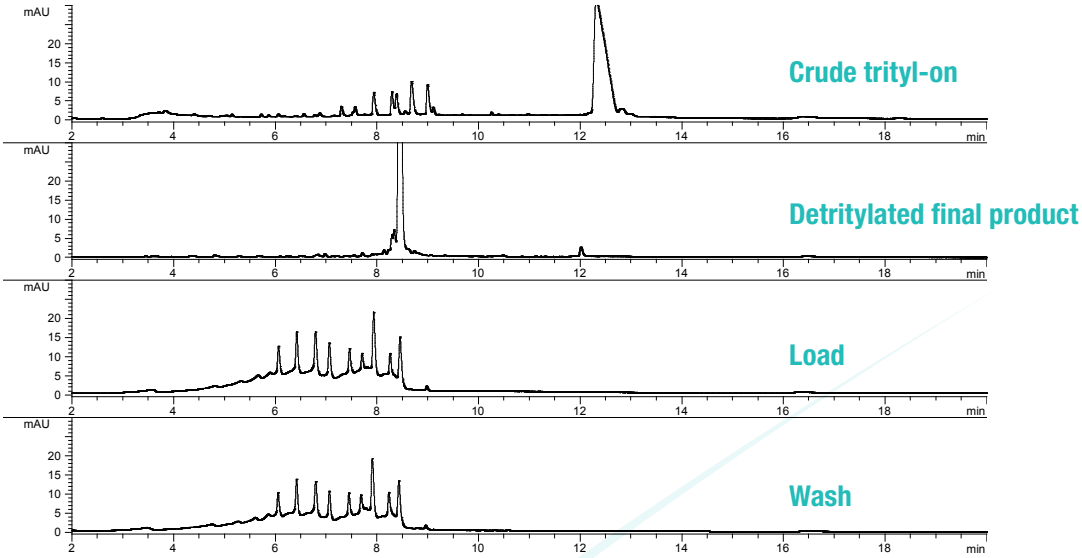
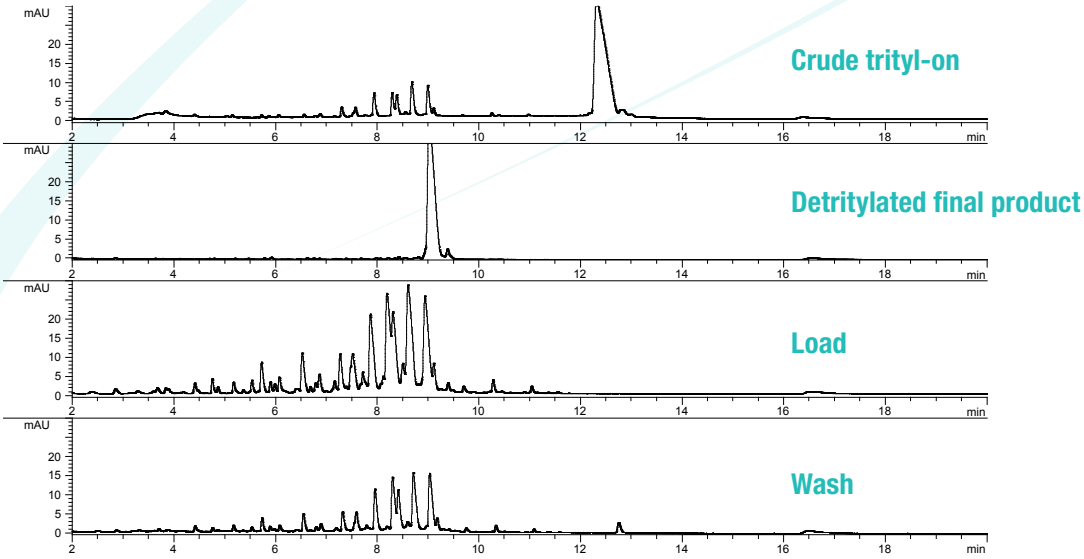


Figure 6:
QSP™ RNA
Sequence 1. GGAGAACCUGCCCAGCCGCTT



* TOP is a trademark of Varian, Inc. Phenomenex is in no way affiliated with Varian, Inc. Comparative separations may not be representative of all applications.

Figure 7:
***TOP™-RNA**
Loading solution: TOP-RNA Quenching Buffer
Sequence 3. CAGUAGUGUAUAAAUUAAATT

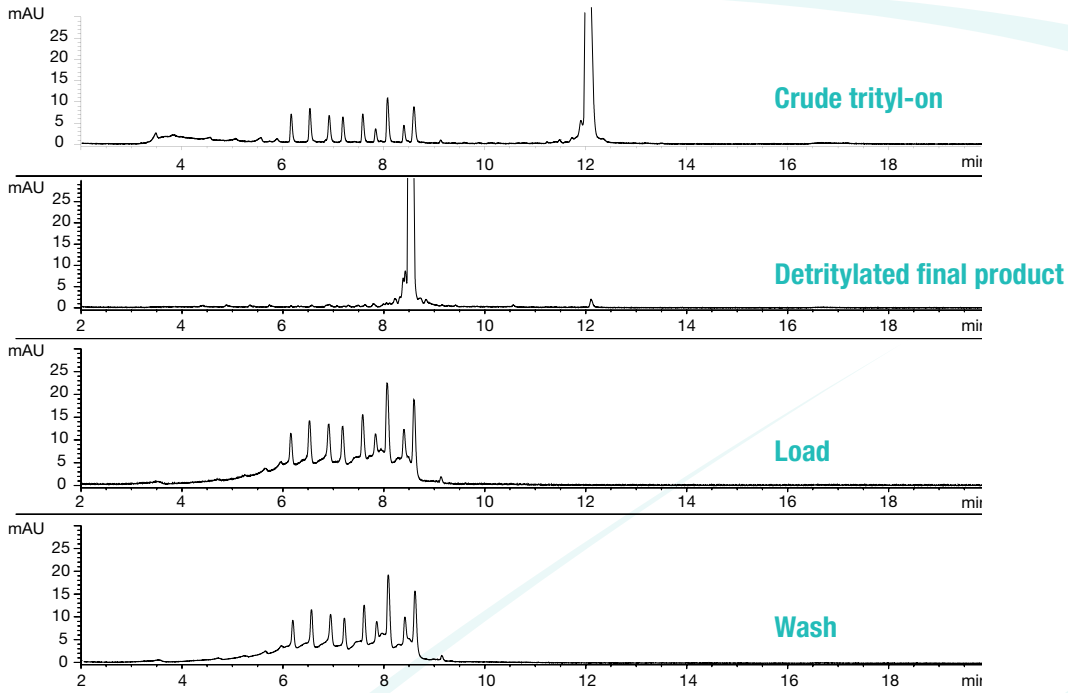
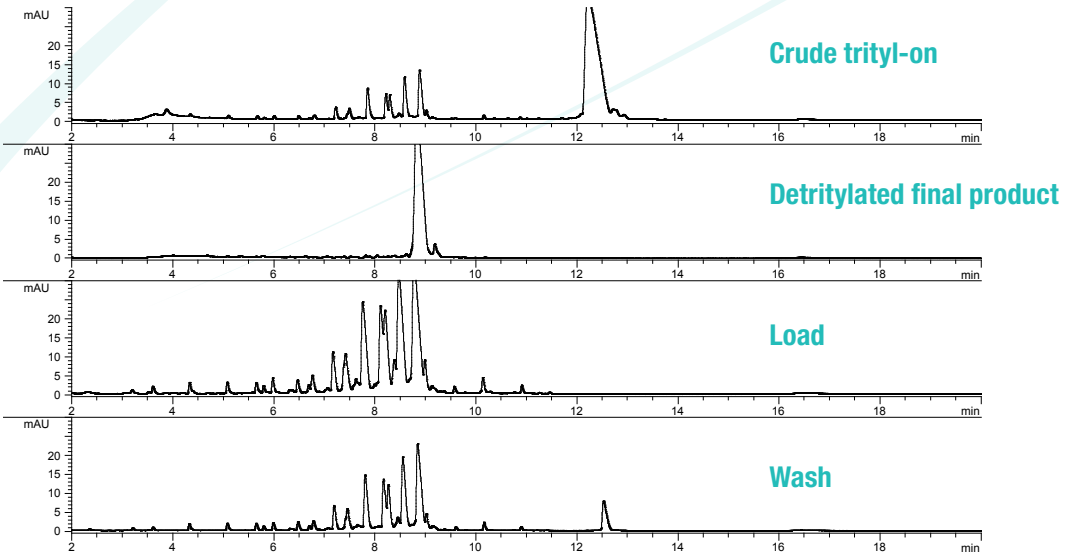


Figure 8:
QSP™ RNA
Sequence 3. CAGUAGUGUAUAAAUUAAATT



* TOP is a trademark of Varian, Inc. Phenomenex is in no way affiliated with Varian, Inc. Comparative separations may not be representative of all applications.

Conclusion

Once recognized as a universal platform, *TOP™ now includes separate buffers and applications for DNA and RNA resulting in the separate *TOP™-DNA and *TOP™-RNA methods. Keeping in line with QSP™, the *TOP™ product has replaced its multi-buffer format with single loading buffers for both DNA and RNA. However, The *TOP™ regimens have retained reversed phase mechanisms (RPC) by maintaining an ion-pairing agent and a reliance on separate wash steps for selective retention and elution. Apart from the recent changes, this investigation clearly demonstrates the advantages of Clarity® QSP™ over *TOP™-DNA and *TOP™-RNA. As highlighted in **Tables 3 and 4**, QSP™ provides ease and efficacy not typically found in competing trityl-on purification platforms, such as the *TOP™ products.

DNA Comparison

Table 3

KEY BENEFITS	QSP™	*TOP™-DNA
Loading / Washing buffers	1	2
Avg purity	95 %	82 %
Avg yield	94 %	89 %
*Total volumes (mL)	6	8.5
Ion-Pairing agent	NO	YES
Depurination	NO	YES
Downstream processing	NONE	TEAA Removal
Available scales	0.02-50 µmole	0.2-1 µmole

*0.2 µmole scale

RNA Comparison

Table 4

KEY BENEFITS	QSP™	*TOP™-DNA
Loading / Washing buffers	1	2
Avg purity	93 %	91 %
Avg yield	85 %	64 %
*Total volumes (mL)	7	10.5
Ion-Pairing agent	NO	YES
Downstream processing	NONE	TEAA Removal
Available scales	0.02-50 µmole	0.2-1 µmole

*0.2 µmole scale

* TOP is a trademark of Varian, Inc. Phenomenex is in no way affiliated with Varian, Inc.

Ordering Information

Formats

Part No.	Description		Unit
8E-S102-DGB	Clarity® QSP™	50 mg/ 96-Well Plate	1/Box
8B-S102-DAK	Clarity® QSP™	50 mg/ 1 mL Cartridge	50/Box
8B-S102-SBJ	Clarity® QSP™	150 mg/ 3 mL Cartridge	50/Box
8B-S042-LFF	Clarity® QSP™	5 g/60 mL Cartridge	16/Box

*Buffer**

Part No.	Description		Unit
AL0-8279	Clarity® QSP™ DNA Loading Buffer	100 mL	Ea
AL0-8280	Clarity® QSP™ DNA Loading Buffer	1 L	Ea
AL0-8281	Clarity® QSP™ RNA-TBDMS Loading Buffer	100 mL	Ea
AL0-8282	Clarity® QSP™ RNA-TBDMS Loading Buffer	1 L	Ea
AH0-7858	Clarity® Nuclease Free Water	1 L	Ea

***NOTE:** *Please contact Phenomenex for Clarity® QSP™ RNA-TOM loading buffer ordering information.*

References
1. Varian's TOP-DNA Trityl-on Purification Protocol: General Procedure 2007
2. Varian's TOP-RNA Trityl-on Purification Protocol: General Procedure 2007
3. Wincott, F., et.al (1995) Nucleic Acid Research. 14 2677-2684