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Application News

DMT-on Purification of Phosphorothioate Oligonucleotide Using SHIMSEN™ Styra HLB SPE Cartridge

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User Benefits

- A simple and straightforward workflow for purification of modified phosphorothioate oligonucleotide.
- Stability of SHIMSEN Styra HLB SPE cartridge at high pH allows direct loading of deprotected oligonucleotide without removal of highly basic deprotecting agent.
- Stability of SHIMSEN Styra HLB SPE cartridge at low pH allows on-cartridge detritylation using acid.

■ Introduction

Oligonucleotide has been used in various applications, including genetic testing (e.g. primers for PCR) and therapeutics (e.g. antisense oligonucleotides and siRNA). These oligonucleotides are usually chemically synthesized using solid-phase synthesis and would require purification to remove impurities such as protecting groups and failure sequences. In this regard, the use of solid-phase extraction (SPE) cartridges offers a simple and efficient strategy for purifying oligonucleotides without the need for complex instruments or extensive processing. In this application, we present the procedure for DMT-on purification of synthetic phosphorothioate oligonucleotide using SHIMSEN Styra HLB SPE cartridge (P/N: 380-00855-10). Analysis of purified oligonucleotide was achieved on Shimadzu Nexera XS inert system using Shim-pack Scepter Claris C18-120 which feature a bioinert coating on the inner surface of the column body, preventing absorption of oligonucleotides to the column surface and increasing analysis sensitivity.

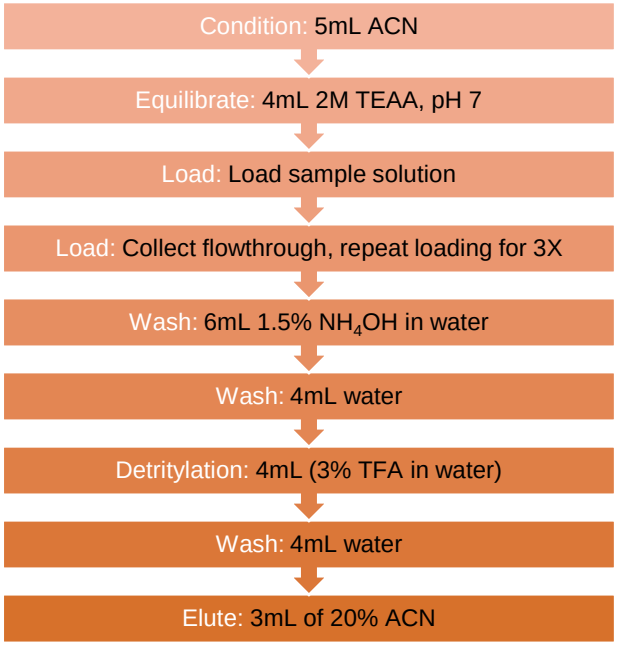

Figure 1. PS Oligonucleotide purification steps.

■ Experimental

The oligonucleotides used in this study are fully phosphorothioate gapmers comprising 10 nucleotides of DNA flanked on both ends by 3 nucleotides of locked nucleic acid (LNA). The oligonucleotides were synthesized and purified by the Phan lab at Nanyang Technological University, Singapore.

Oligonucleotide deprotection:

Oligonucleotides were synthesized on controlled pore glass (CPG) and subsequently cleaved and deprotected in 3 mL of 32% ammonium hydroxide solution at room temperature for 36h. The solution containing the deprotected oligonucleotide was extracted and the CPG was washed once with 1 mL of deionized water followed by twice with 1 mL of 2M TEAA (pH 7). The combined solution was used for purification by SHIMSEN Styra HLB SPE cartridge.

DMT-on oligonucleotide purification by SHIMSEN Styra HLB SPE:

Purification was performed according to procedure detailed in Figure 1 using SHIMSEN Styra HLB, 200mg/6mL (P/N: 380-00855-10). Samples and solvents were allowed to flow through the SPE by gravity. The purified oligonucleotide was analyzed by Nexera™ XS inert UHPLC system. Table 1 list the UHPLC analysis conditions.

Table 1. UHPLC conditions

LC system	:	Shimadzu Nexera™ XS inert
Column	:	Shim-pack Scepter™ Claris C18-120 1.9 µm, 100 × 2.1 mm *1
Column Temp.	:	55 °C
Flow rate	:	0.42 mL/min
Mobile phase A	:	15 mM TEA, 400 mM HFIP
Mobile phase B	:	Acetonitrile
Gradient program	:	10% B (0 min) → 10%B (0.5 min) → 28% B (10.5 min) → 100% B (15.5 min) → 100% B (18.5 min) → 10% B (19.5 min) →10% B (24.5 min)
Injection volume	:	2 µL
Detector	:	PDA, 260 nm

*1 P/N: 227-31209-02

■ Results and Discussion

Automated solid phase oligonucleotide synthesis is typically carried out on solid support such as controlled pore glass (CPG) or polystyrene via phosphoramidite chemistry. Figure 2 shows the typical solid phase oligonucleotide synthetic cycle. Synthesis usually starts from 3′- end, adding a single nucleoside monomer during each cycle, growing towards the 5′- end. During the synthesis, failure sequences (resulted from incomplete coupling) are being capped. Assuming that the capping reaction is efficient, only the full length sequence should contain a dimethoxytrityl (DMT) group on the 5′-end after the completion of the synthesis. Subsequent cleavage and deprotection of the synthetic oligonucleotides are usually performed in highly basic ammonium hydroxide solution. As DMT groups are highly hydrophobic, the full length sequence can be retained on a reversed phase SPE cartridge while the failure sequences are washed off. The DMT group can then be removed on-cartridge under acidic condition and the purified product can be eluted. SHIMSEN Styra HLB cartridges features wide pH stability range of 1-14, allowing purification of deprotected DMT-on oligonucleotide without the need to remove ammonium hydroxide or pH adjustment. At the same time, the stability of the cartridge under low pH allows on-cartridge detritylation reaction under acidic condition using trifluoro acetic acid (TFA) solution.

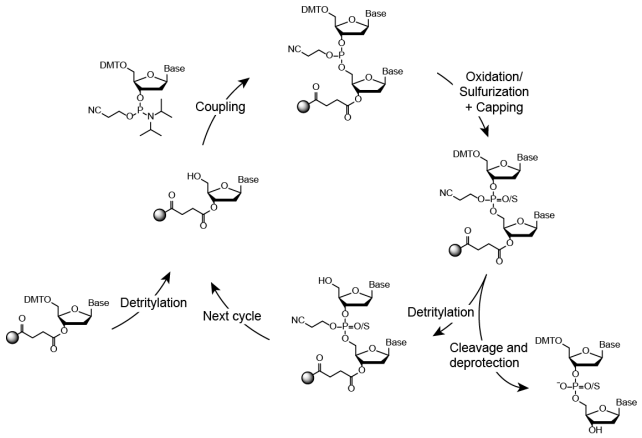

Figure 2. Solid phase oligonucleotide synthesis cycle.

The purification of a 16 nucleotides phosphorothioate gapmer oligonucleotide was successfully achieved using the SHIMSEN Styra HLB SPE cartridge. The SPE cartridge was first conditioned with acetonitrile (ACN), followed by equilibration using a 2M triethylammonium acetate (TEAA) solution. TEAA act as an ion-pairing reagent to aid in retaining the oligonucleotide. The deprotected oligonucleotide could be directly loaded onto the cartridge without the need to remove the basic ammonium hydroxide deprotection solution. The DMT-on oligonucleotide is retained in the cartridge while the impurities elutes in the flowthrough (Figure 3a, 3b).

Next, the cartridge was washed with a 1.5% ammonium hydroxide solution to elute the remaining impurities (Figure 3c), and this is followed by washing with water to remove any remaining ammonium hydroxide as residual ammonium hydroxide will affect the subsequent detritylation reaction. Detritylation was then performed by passing a 3% TFA solution through the cartridge, followed by another water wash to eliminate any residual TFA. Finally, the purified DMT-off oligonucleotide was eluted using 20% ACN and purity was determined by HPLC to be 81% (Figure 3d).

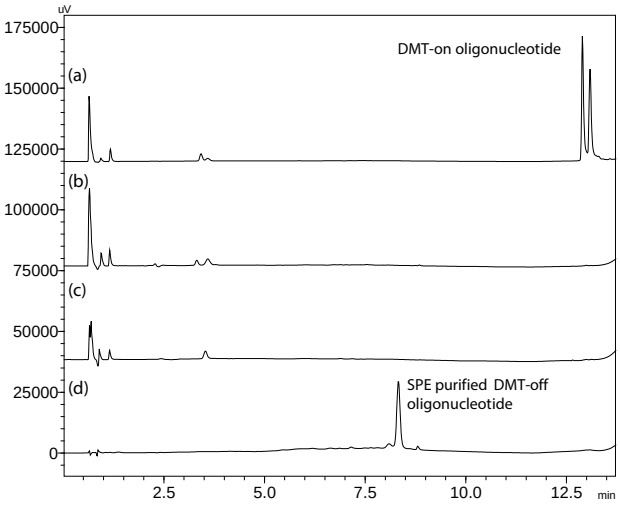

Figure 3. Chromatogram of (a) crude deprotected DMT-on oligonucleotide, (b) flowthrough after loading on SHIMSEN Styra HLB SPE cartridge, (c) flowthrough of 1.5% NH_4OH solution wash, (d) SHIMSEN Styra HLB SPE cartridge purified oligonucleotides.

■ Conclusion

This study showcases a rapid and straightforward approach, employing the SHIMSEN Styra HLB SPE cartridge for purification of synthetic phosphorothioate oligonucleotides by DMT-on strategy.

■ Acknowledgement

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