

Versatility of Polymeric Resins for Oligonucleotide Purification

Exploring the benefits of polymeric resins in challenging purifications across oligonucleotide modalities

Introduction

The development of oligonucleotide therapeutics has been accelerating in recent years as new modalities and chemistries are improving disease targeting and drug stability. DuPont is dedicated to advancing drug manufacturing by providing a broad range of science-based solutions to address oligonucleotide purification challenges. One of the major techniques used in oligonucleotide purification, reverse-phase high-performance liquid chromatography (RP-HPLC), is highlighted here.

Experimental Conditions

Purifications were performed on DuPont™ AmberChrom™ XT20 and XT30 chromatography resins as packed in DuPont™ AmberChrom™ Profile™ 4.6 x 250mm columns on ÄKTA pure™ 25 M or 150 M at 10 – 20 mg oligonucleotide loads per mL of resin at room temperature. Analyses were performed on Agilent™ 1260 Infinity II HPLC system using anion exchange (IEX) (DNAPac™ PA200 column, 8 µm) or ion-pairing reverse phase (IP-RP) chromatography (Xbridge™ column).

Results

Purification of DMT-On antisense oligonucleotide

The widely used phosphoramidite-based oligonucleotide synthesis results in products capped with dimethoxytrityl (DMT) or similar protecting groups. Strong interactions between the hydrophobic DMT group and RP resins lead to a clear separation between the target full-length product (FLP) and hydrophilic impurities. This results in high concentrations and purities under the FLP peak as shown in the chromatographic purification of a 20-mer DMT-On antisense oligonucleotide (ASO) on DuPont™ AmberChrom™ XT30 chromatography resin (Figure 1) and is due to impurities eluting earlier in the gradient, well separated from the DMT-tagged product.

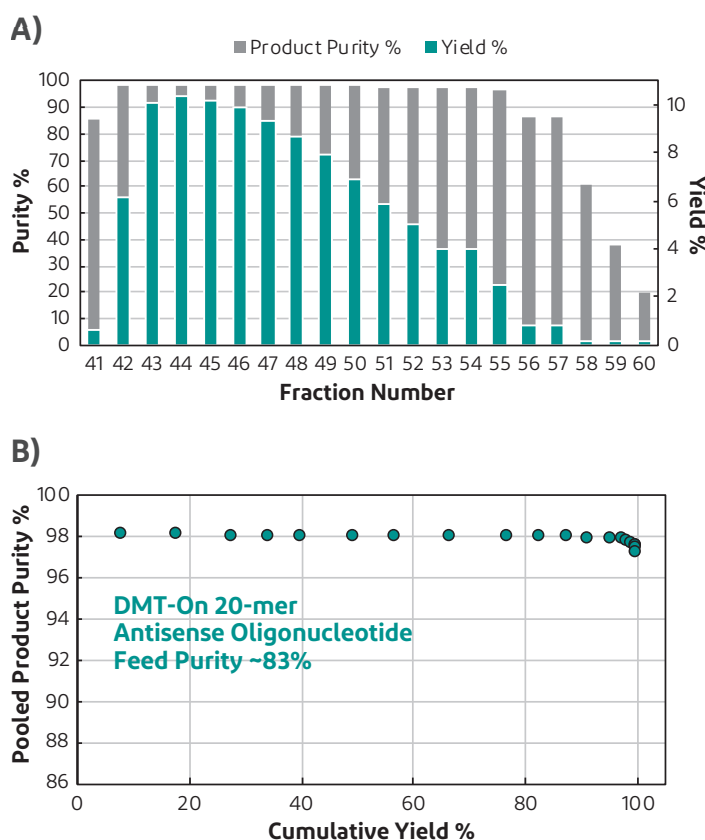


Figure 1: Purification of an antisense oligonucleotide on DuPont™ AmberChrom™ XT30 chromatography resin
A) IEX fraction analysis of a 20-mer oligonucleotide purified on DuPont™ AmberChrom™ XT30 chromatography resin over a linear gradient with 0.2 M sodium acetate in methanol.
B) Pooled purity and cumulative yield analysis based on (A).

Results

Purification of DMT-Off ASO and other modalities

The high chemical resistance and rigidity of polymeric RP resins allow their use at scale in purifications of various oligonucleotide modalities. Table 1 shows the result of the purification of the same ASO feed in DMT-Off mode on DuPont™ AmberChrom™ XT30 chromatography resin. Adding an ion-pairing agent promotes the interaction between the hydrophilic anionic oligonucleotide and the resin in absence of the trityl group while still delivering high purities and yields.

For more challenging, less pure, longer oligonucleotides, the DuPont™ AmberChrom™ XT30 chromatography resins are ideal for DMT-On and DMT-Off purifications. The DuPont™ AmberChrom™ XT20 chromatography resin provides fractions with purities above 96% in a one-step purification of a 91-mer single guide RNA (sgRNA) with crude purity ~30%. Table 1 shows its high recovery at 80% pool purity. This resin is also shown to successfully purify another oligonucleotide modality, a monomethoxytrityl (MMT-On) small interfering RNA (siRNA) feed, with crude purity of ~77%, delivering a pool purity of 91% at 90% yield (Table 1).

Table 1: Pooled purity and cumulative yield analyses for various oligonucleotide modalities

Oligonucleotide Modality	Pool Purity	Yield
DMT-Off ASO (crude purity ~86%)	93%	86%
DMT-On sgRNA (crude purity ~29.8%)	80%	73%
MMT-On siRNA sense strand (crude purity ~76.7%)	91%	90%

Purification details:

- ASO DMT-Off purified with 0.1 M tetraethylammonium acetate in acetonitrile (IEX analysis).
- sgRNA DMT-On purified with 0.1 M triethylammonium acetate in acetonitrile (IP-RP analysis).
- siRNA purified with 0.2 M NaCl with 0.02 M NaOH in 50/50% volume water/acetonitrile (IEX analysis).

Conclusions

Polymeric resins, such as the DuPont™ AmberChrom™ XT20 and XT30 chromatography resins, support DMT-On and DMT-Off purifications of various oligonucleotide modalities, including ASOs, sgRNA, and siRNA. DuPont offers both pre-packed HPLC columns and bulk resin of the DuPont™ AmberChrom™ XT grade chromatography resins to address research and manufacturing needs.

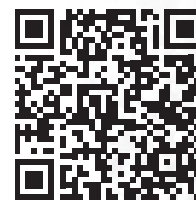
References

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