Single-step purification of a 21-mer oligonucleotide using Capto™ Q ImpRes resin

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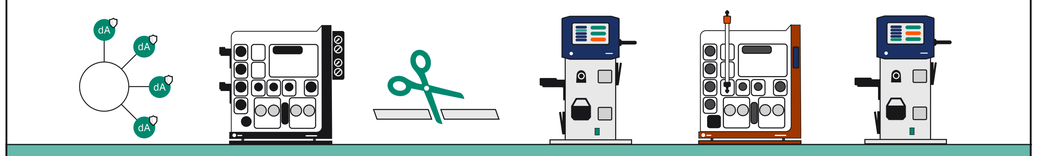
Introduction

The oligonucleotide therapeutics market is growing, and the pipeline is diverse. With more than 2000 oligo therapeutics in development and 20 commercialized oligonucleotide drugs on the market to date, the potential for these highly specific sequences is being realized and is set to continue.

As this pipeline increases, scalability and productivity becomes critical to bring the life-changing siRNA and antisense oligo therapies to a global patient population. Most of these sequences are in the range of 20 to 25 nucleotides, without any natural RNA having 2′-OH and hence are stable at high pH. We have responded with a robust, fast, scalable, and simple purification protocol for such oligonucleotides, using our modern Capto™ Q ImpRes resin.



Starting bases	Synthesis	Cleavage and deprotect	TFF (optional)	Chromatography	TFF
• Amidites • Reagents • Solvents	• ÄKTA oligosynt™ oligonucleotide synthesizer • Column holder, 18113845 • Synthesis columns: 1.2–300 mL range* • Primer Support™ 5G (solid support) * 3 columns recommended	• Batch cleavage • Bottles • For RNA – Resistant bottles (e.g. PTFE)	• ÄKTA flux™ S system • Flat sheet cassettes	• ÄKTA pure™ system • ÄKTA pure T system • ÄKTA avant™ 150 system • Column ID 26 mm • e.g. Capto™ Q ImpRes resin (DMT-off) • e.g. Capto Phenyl ImpRes or Capto PlasmidSelect (DMT-on)	• ÄKTA flux S system • Flat sheet cassettes e.g. OS001T01 for 1KDa TFF (depending on oligo length)

Scaleup anion exchange purification of trityl off D21-mer oligo using Capto Q ImpRes resin shows high purity after single-step purification

Scale up purification on ÄKTA pilot™ 600 system. A total of 27 g of crude oligo diluted 10× in 10 mM NaOH was loaded. The material was eluted using a linear gradient of 7.5%B to 72%B at 15 CV against 10 mM NaOH, 1M NaCl (Fig 2). The eluate was collected in 1 L fractions to a total of fifteen fractions and analyzed on HPLC for full-length product purity. Best recovery at purity of 95% or more was obtained using the green pooling strategy (Fig 3).

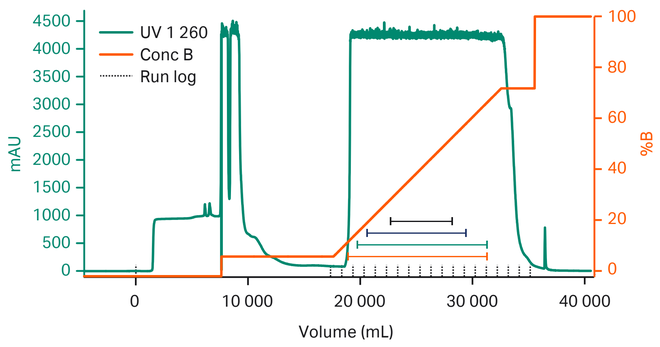


Fig 2. Purification of crude oligonucleotide using an ÄKTA pilot 600. The four different pools of collected fractions are indicated in black (6-12), blue (4-12), green (3-13) and orange (2-14).

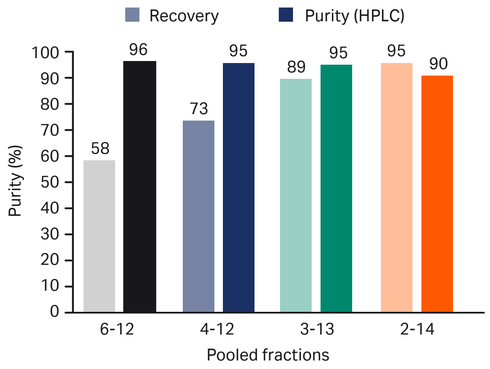


Fig 3. Recovery and purity of pooled fractions for four different pooling strategies.

Synthesis

The DNA oligonucleotide was synthesized on ÄKTA oligosynt™ system at 12 mmol scale using Primer Support™ 5G UnyLinker solid support at 353 µmol/g packed in a FineLINE™ 70 column (8 cm bed height, 308 mL). The synthesis yield was 72% with a concentration of 120 OD/mL.

Anion exchange resin screening shows high purity and DBC

Screening of purity and dynamic binding capacity (DBC) for a D21-mer oligonucleotide on three different anion exchange resins: Capto Q ImpRes, SOURCE™ 30Q and Capto adhere ImpRes (Fig 1, Table 1, and Table 2). Capto Q ImpRes and Capto adhere ImpRes resins showed the highest purities and DBCs. As multimodal resins give different peak shapes dependent on sequence, Capto Q ImpRes resin was therefore selected for a scaleup using a 1 L ReadyToProcess™ column.

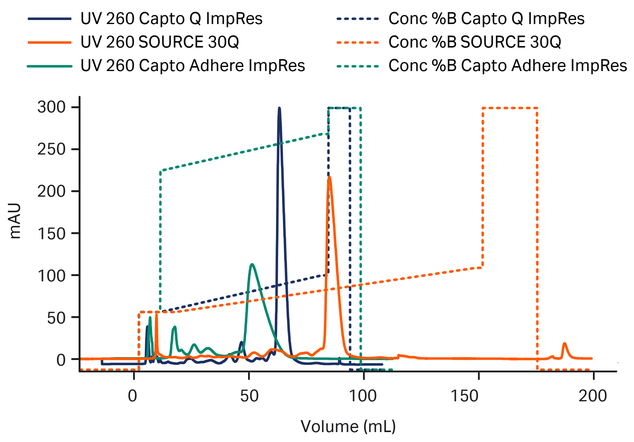


Fig 1. Screening of three different anion exchange resins using ÄKTA pure™ 25 system.

Full-length product purity

Table 1. HPLC purity analysis of eluate fractions from screened resins

Resin	Purity (%)
Capto Q ImpRes	99
SOURCE 30Q	94
Capto adhere ImpRes	97

Dynamic binding capacity of screened resins

Table 2. Dynamic binding capacity for the three screened resins using a residence time of 1.25 min at 10% breakthrough

Resin	DBC, QB10 (mg/mL)
Capto Q ImpRes	34
SOURCE 30Q	20
Capto adhere ImpRes	41

LC-MS analysis confirms high purity

Other than the full-length product, the first eluate fractions also contained small levels of impurities consisting of mainly one nucleotide loses at either the 3′ or 5′ end. Impurities in the end of the eluate peak were comprised of one and two nucleotide losses at the 5′ end (Figs 4 and 5 and Table 3).

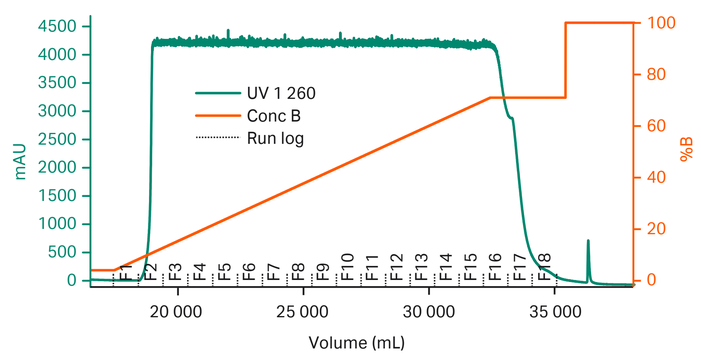


Fig 4. Zoomed eluate chromatogram indicating collected fractions.

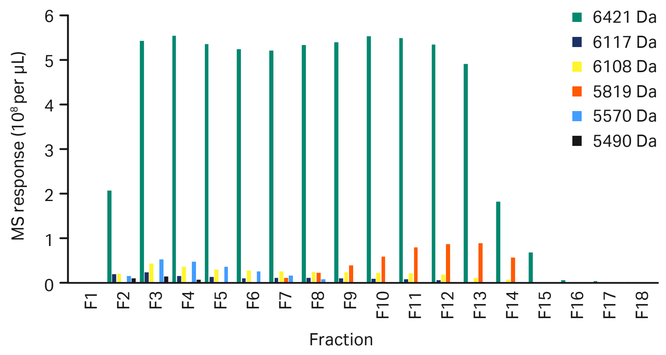


Fig 5. Distribution and identification of impurities detected by LC-MS for each fraction.

Table 3. Detailed sequence identification of each identified impurity

Mw (Da)	Molecule	Sequence
6421 Da	Full-length product (FLP)	ACG TTG CAG ACT CCT AAT GGT
6117 Da	FLP with loss of 3′-T	ACG TTG CAG ACT CCT AAT GG
6108 Da	FLP with loss of 5′-A	CG TTG CAG ACT CCT AAT GGT
5819 Da	FLP with loss of 5′-AC	G TTG CAG ACT CCT AAT GGT
5570 Da	FLP with loss of 5′-ACG (5′-phosphate)	P-TTGCAGACTCCTAATGGT
5490 Da	FLP with loss of 5′-ACG (5′-hydroxyl)	HO-TTGCAGACTCCTAATGGT

High recovery UF/DF using 3 kDa Centramate™ Omega TFF cassette

The Capto Q ImpRes resin eluate (#3–#13) was concentrated first 5 times, then diafiltered 7 times against water, and lastly concentrated an additional 2 times, reaching a total concentration factor of 7. The final product concentration was 6.6 mg/mL (Fig 6 and Table 4). The recovery was 90% with no product loss detected in the permeate stream.

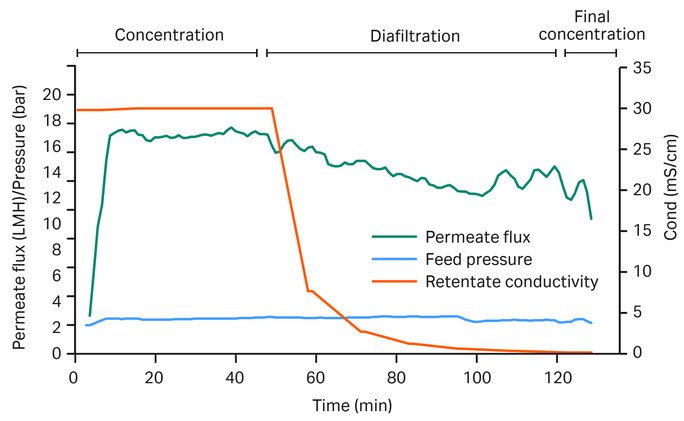


Fig 6. Permeate flux, feed pressure, and conductivity during the process with the initial concentration, diafiltration, and final concentration phases outlined.

Table 4. TFF process parameters

Variable	Value
Sample	Capto Q ImpRes resin eluate
Filter load	12.5 L/m² 19.5 g oligo/m²
Filter area	0.02 m² (OS003T01)
Cutoff	3 kDa
Filter material	Polyethersulfone (PES)
Feed flow	75 mL/min
TMP	0.65 bar
Concentration factor	7×
Diafiltration factor	2×
Recovery	90%

Conclusions

- Scale up of Capto Q ImpRes using in the ReadyToProcess format showed purity of ≥ 95% with recovery of ≥ 89%.
- Oligonucleotides having a length of ~ 20 bases can be successfully purified in a single-use format by a downstream process not requiring stainless steel equipment or organic solvents.
- 3 kDa Centramate Omega cassettes enable efficient buffer exchange and concentration with high recovery for final formulation.

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CY43669-17Apr24-PO