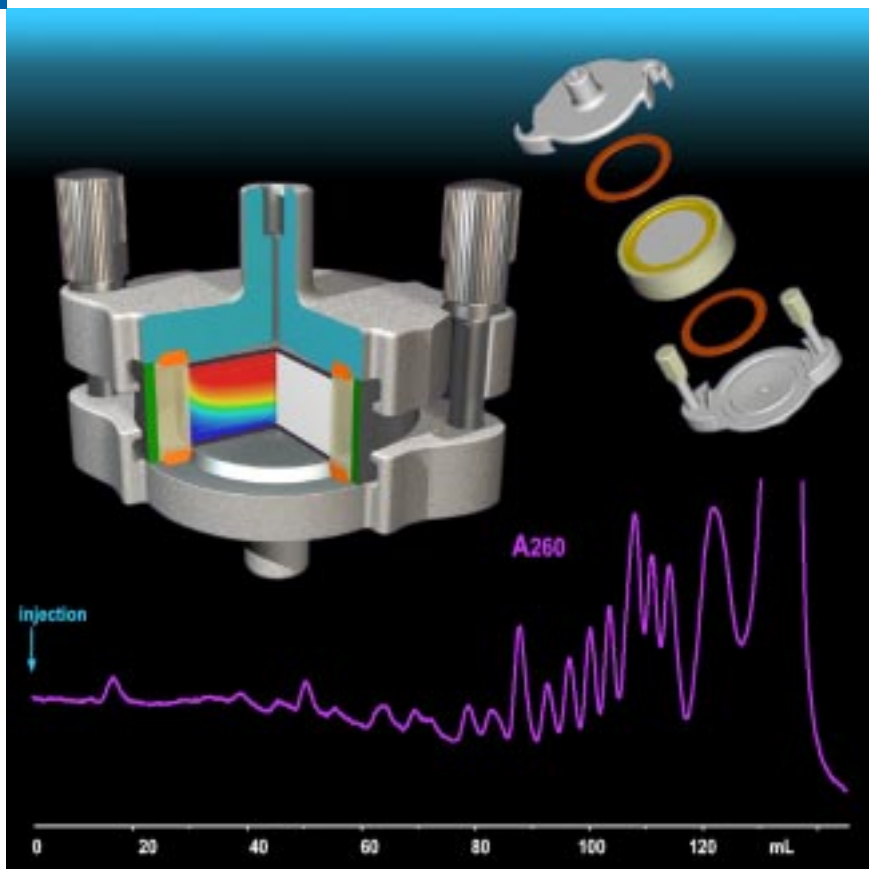
PALL BioPharmaceuticals



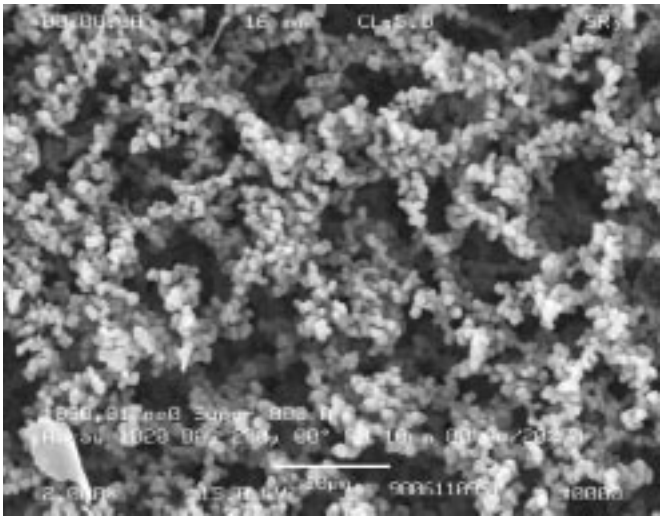
Pall Mustang™ Chromatography for Purification of Oligonucleotides

*Filtration. Separation. Solution.*SM

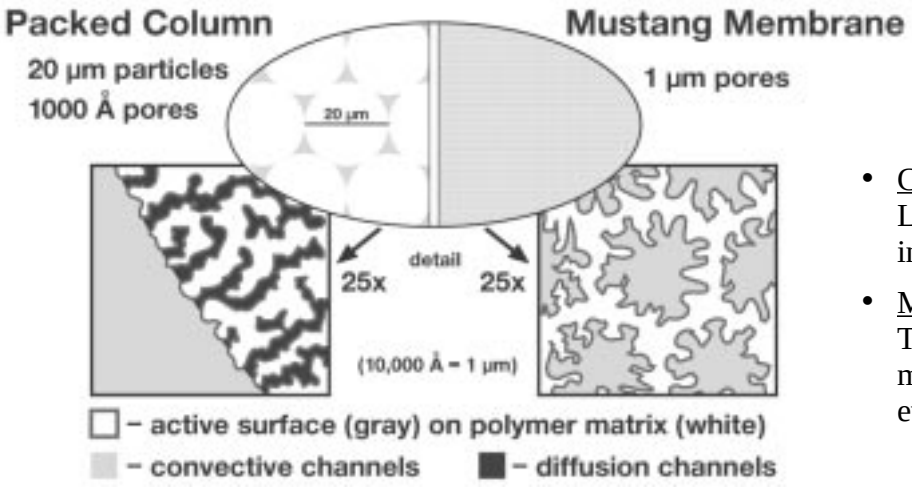
Mustang Membranes

S and Q Ion Exchange

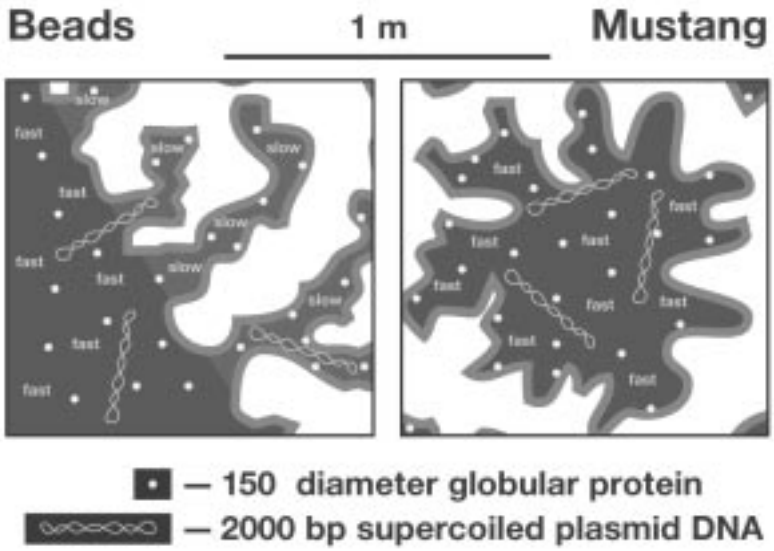
- made by coating polyether sulfone membranes
- 0.8 micron pores for high flow
- nodular structure... accessible surface area for macromolecules



Comparison: Flow Dynamics



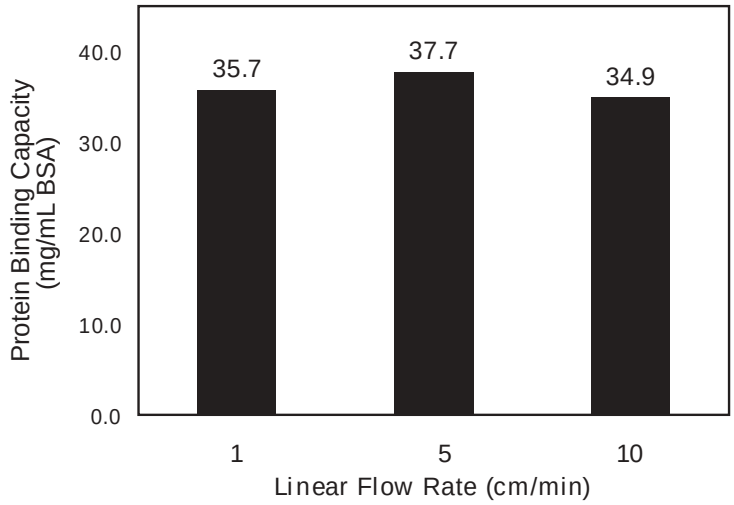
- Conventional Media
  Large spaces between beads result in flow-distribution problems.
- Mustang Media
  Tiny 0.8 micron pores through the membranes give high flow and even distribution.



- Conventional Media
  Diffusion in and out of pores requires lower flow.
- Mustang Media
  Molecules bind to the open surface of membranes without the need to diffuse into pores. Higher flow rates are possible.

Mustang Membranes

Mustang Q: Effect of Flow Rate on Capacity



- Conclusion
  Capacity is independent of flow rate.

Mustang Modules

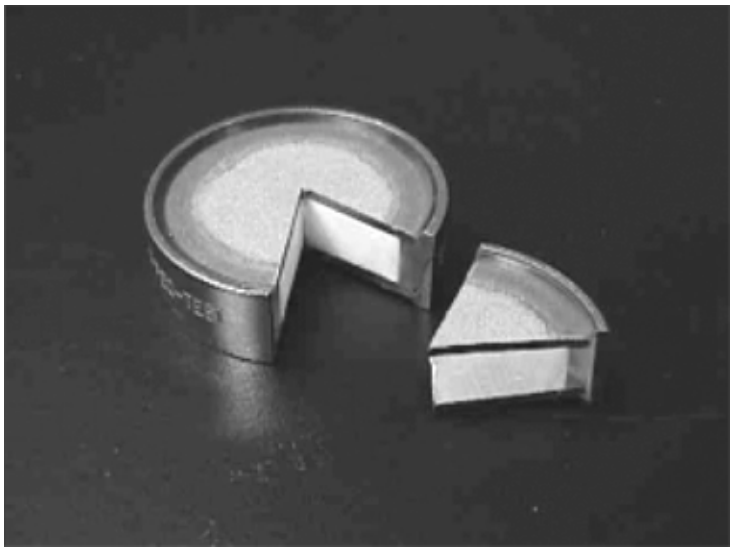
Flow Rate and Pressure Drop

Typical operating conditions:

- Linear flow rate
  1 cm/min (ranging from 0.5 cm/min to 5 cm/min)
- Volumetric flow rate for ~1 cm long module bed
  1 cm/min = 1 CV/min!* = 10 mL/min or 100 mL/min
- Pressure
  at 1 CV/min = 15-25 psi

Flow Path Design

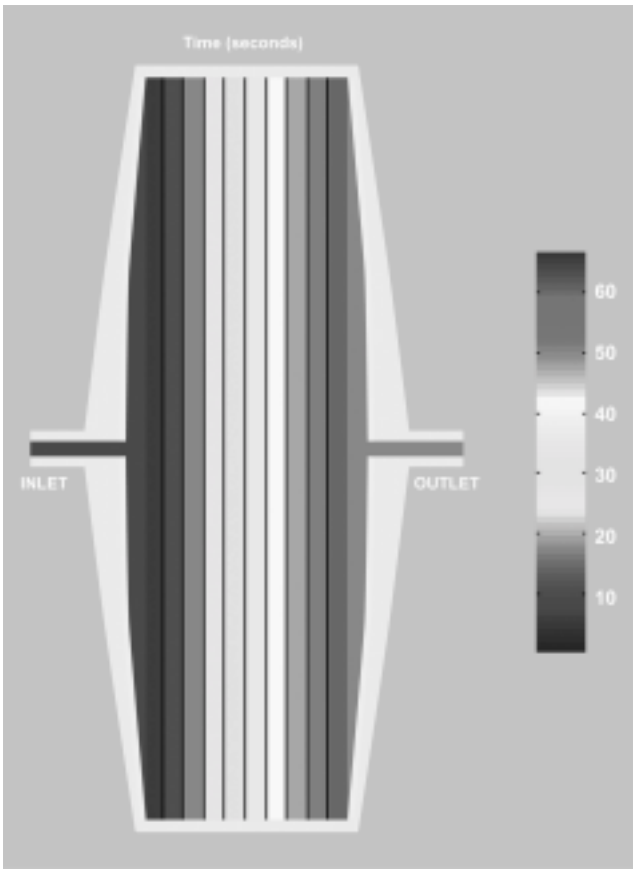
- uniform pore structure
- uniform bed structure
- equal residence time for all flow paths



Mustang Modules

Residence Time

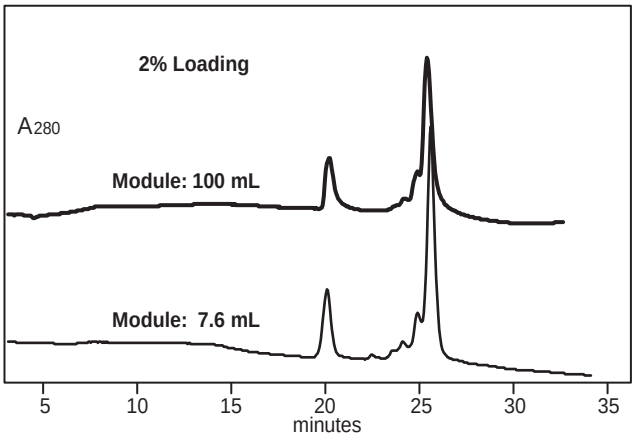
The inlet and outlet of the housing have a hydrodynamically designed tapered gap to distribute plug flow through the module.



10 mL Design

Scalability

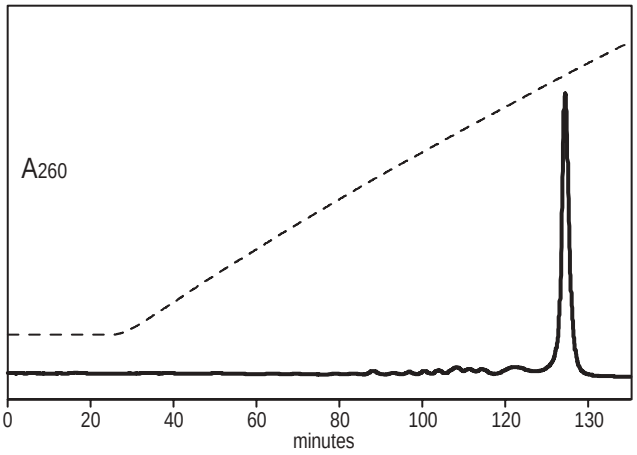
Mustang Modules of different sizes give virtually identical separations when loading and flow rate are adjusted in proportion to column volume.



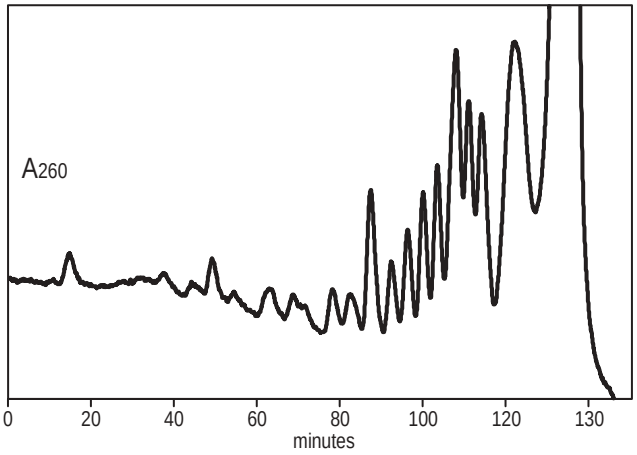
Mustang Q Module

Resolution

Separation of Crude 23-mer DNA Oligomer
Gradient:Linear. 0-1000 mM NaCl over 20 CV
Buffer: 50 mM Tris, pH 8
Flow: 1 CV/min



The same separaration of crude 23-mer DNA oligomer shown with expanded absorbance scale to reveal detail.



Purification of Isis 2302 Oligo on Pall Mustang Q Module

The examples on the following pages are separations of the Isis Pharmaceutical “2302” phosphorothioate oligomer on a 10 mL Mustang Q Module. Special Thanks to the following people for providing the starting material and assistance in this work.

- Yogesh Sanghvi, Ph.D.
  Director of Process Development
- Ranjit Desmukh, Ph.D.*
- Lin Ho, B.S.

  Isis Pharmaceuticals
  2292 Faraday Avenue
  Carlsbad, CA 92008

*Now Senior Scientist II, Process Development, Wyeth-Lederle Vaccine Development, Malvern, PA 19355

Mustang Q Module

Isis 2302 Phosphorothioate Oligo

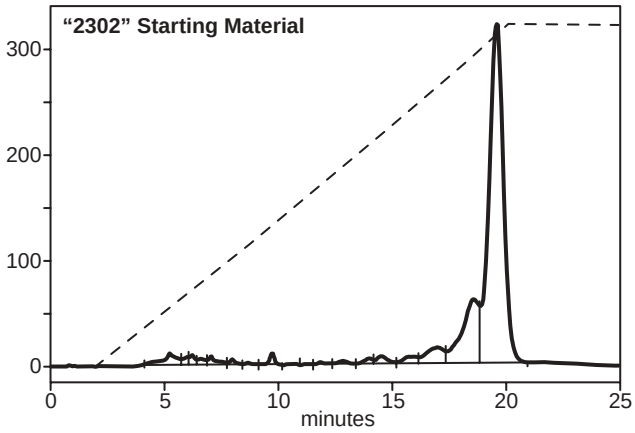
- The Isis 2302 Phosphorothioate Oligo is an antisense olignucleotide that blocks the expression of ICAM1.
- It is currently in Phase II clinical trial for psoriasis and inflamatory bowel disease.
- Its sequence is 5'GCCCAAGCTGGCATCCGTCA3'.
- Its molecular mass is 5089.6 daltons.

Isis SAX Analytical Method

- HPLC Column: Resource Q, Strong Anion Exchange, 1 mL.
- Mobile phase: A = 20 mm NaOH. B = 2.5 M NaCl in A.
- Conditions: Flow at 1 mL/min. Temperature = 70℃. Detection by absorbance at 260 nm.
- Gradient: After a 3 minute hold, linear from 0% to 100% B over 20 minutes.
- Validated for analysis of 2302 Oligo. Separates mono-phosphodiester from phosphorothioate and N-2, N-3 etc. deletion phosphorothioates.
- Final purity (N-1 phosphorothioate) determined by capillary gel electrophoresis (CGE) and mass spectrometry (MS).

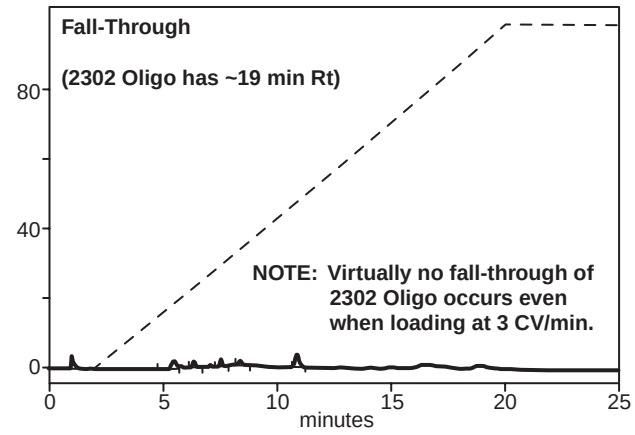
2302 Starting Material

- SAX analytical chromatogram.
- 2302 oligo elutes at approximately 19 minutes.



Sample Application to Mustang Q Module

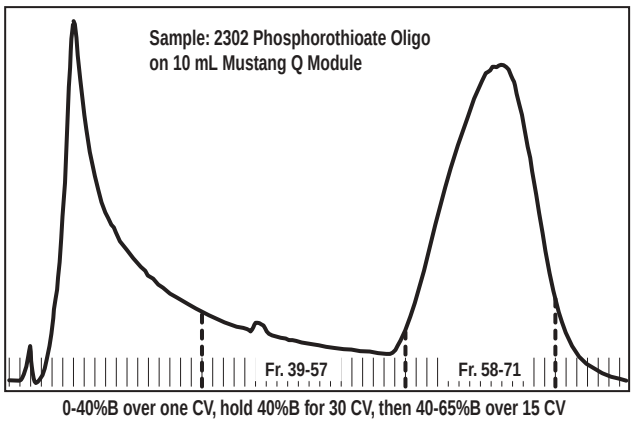
- The loading solution was 1 mg/mL in 20 mm NaOH.
- 200 mg (200 mL) of 2302 starting material were loaded on a 10 mL Mustang Q Module at a flow rate of 3 CV/min.
- The fall-through pool was analyzed using the Isis SAX analytical method.



Mustang Q Module

2302 Oligo Separation on 10 mL Mustang Q Module

- Mobile phase: A = 20 mM NaOH.
  B = 2.5 M NaCl in A.
- Flow: 1 CV/min (or faster). Room temperature. Detection at 260 and 290 nm.
- Initial steep gradient: 0% to 40% B over 1 CV.
- Hold at 40% B to elute N-1 etc. This mobile phase composition was earlier determined to be just below the NaCl concentration required to elute 2302 product. The hold continues until absorbance declines to plateau – in this case, 30 CV.
- Final gradient from 40% to 65% B over 15 CV to elute the full-length 2302 oligo.
- Fractions were collected and pooled as shown to produce an "impure" pool, from the final downslope region of the 40% composition hold and a "pure" pool from the peak eluting during the final gradient.
- Pools can be desalted with 1000 MW cutoff MicroSep centrifugal concentrators for CGE and MS analyses.
- Pools can be concentrated and desalted by tangential flow filtration over 1000 MW cutoff Omega membrane.

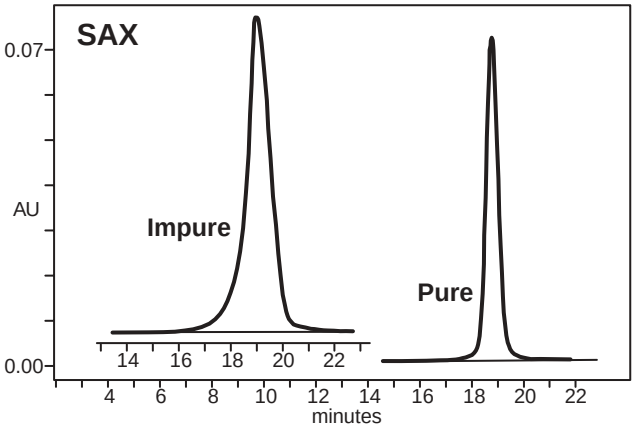


Note: Further method development would include a more detailed fraction-by-fraction analysis of the final peak to optimize the pool selection procedure for highest purity.

Analyses

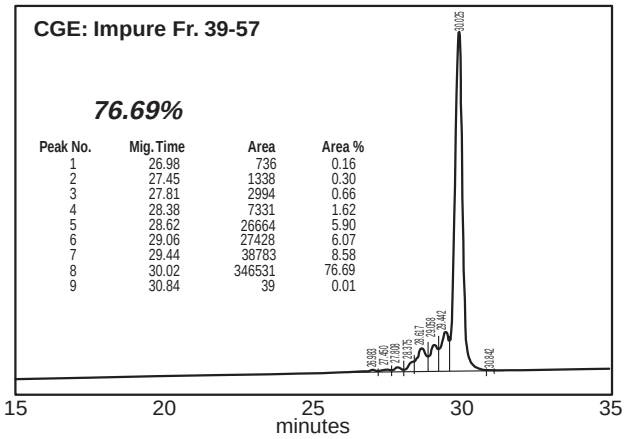
Pooled fractions were initially analyzed by SAX. (Note: It is difficult to assess final purity by this analytical method because N-1 is not completely resolved. Impurity appears as skewing of the single peak in the chromatogram.

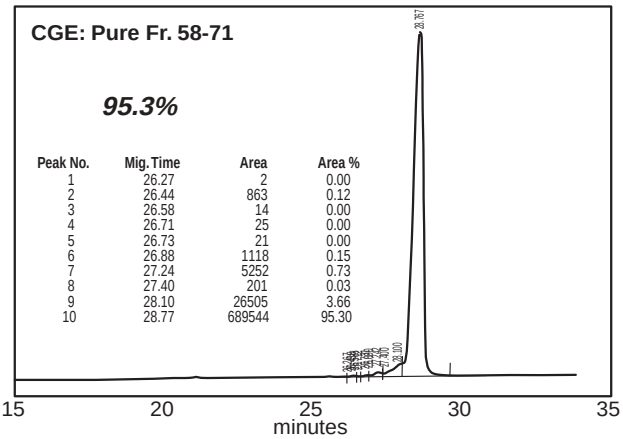
Analyses by CGE and MS, shown on the next page, provide a better indication of product purity.

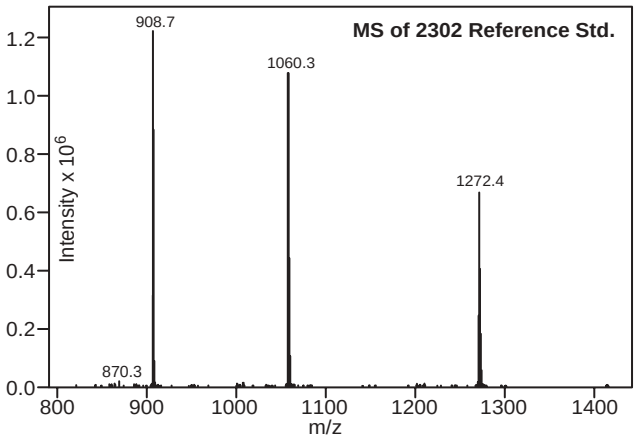


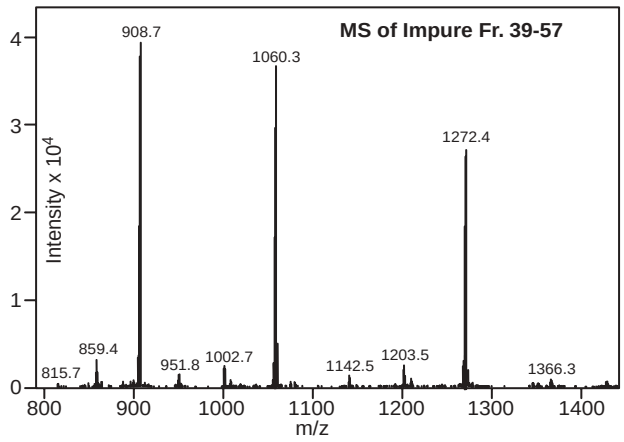
Mustang Q Module

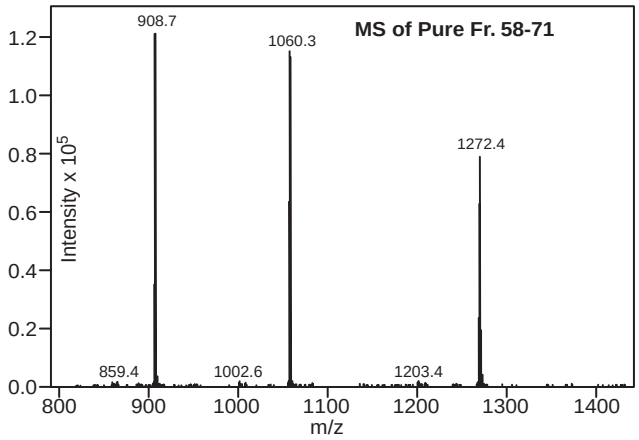
Purity Analyses of 2302 Oligo Pools









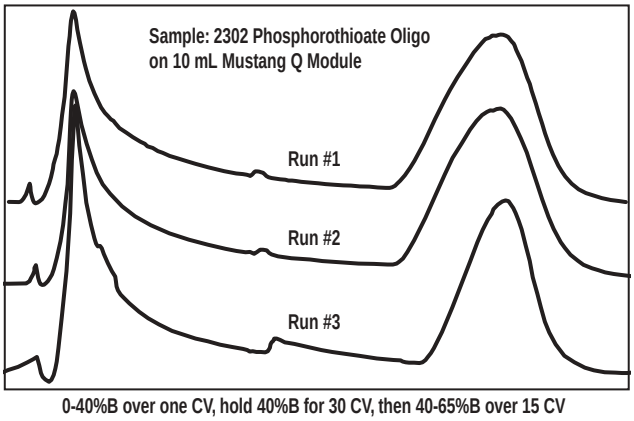


Mass Balance

Calculated from areas of peaks in SAX analytical HPLC for a 10 mL Mustang Q separation of 100 mg 2302 Oligo (not shown), mass recovery of 98% was obtained.

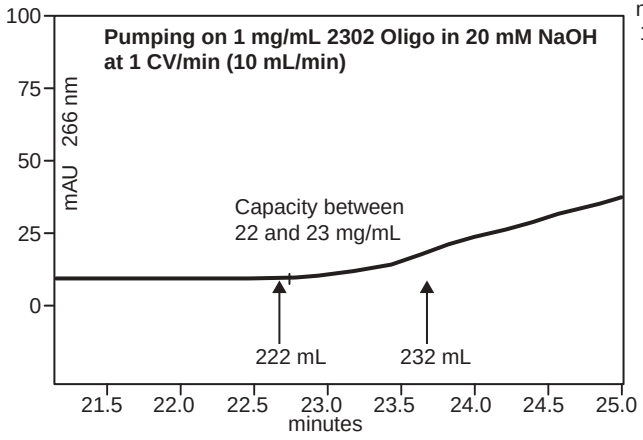
Reproducibility

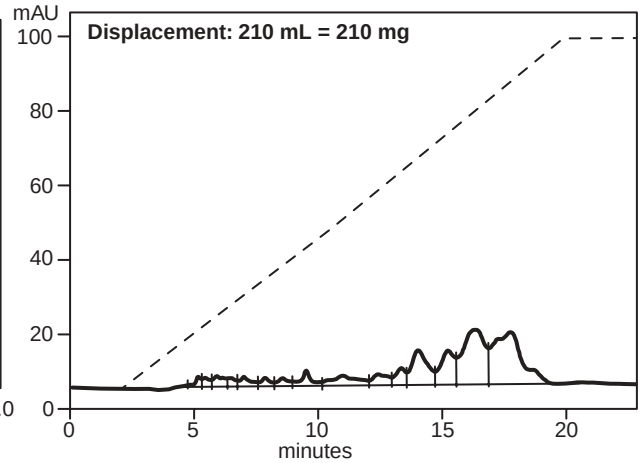
This overlay shows the2302 Oligo purification run previously shown and two replicate runs on the same 10 mL Mustang Q cartridge.



Dynamic Capacity for 2302 Oligo

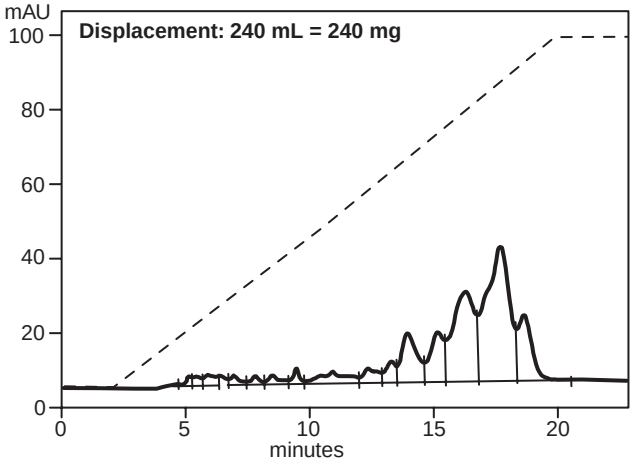
Determination of breakthrough by total absorbance gave a measured capacity between 220 and 230 mg for the 10 mL Mustang Q Module.

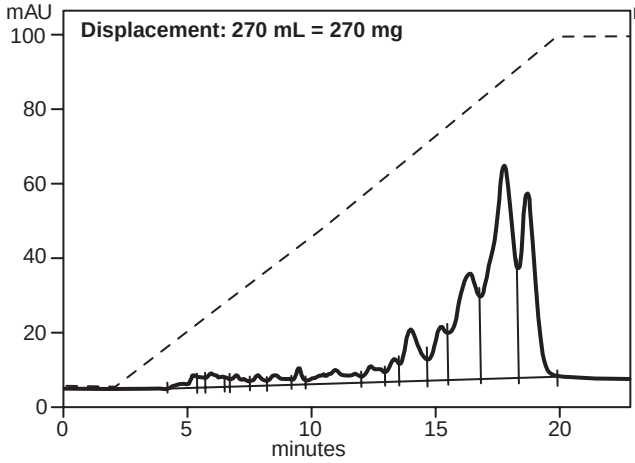


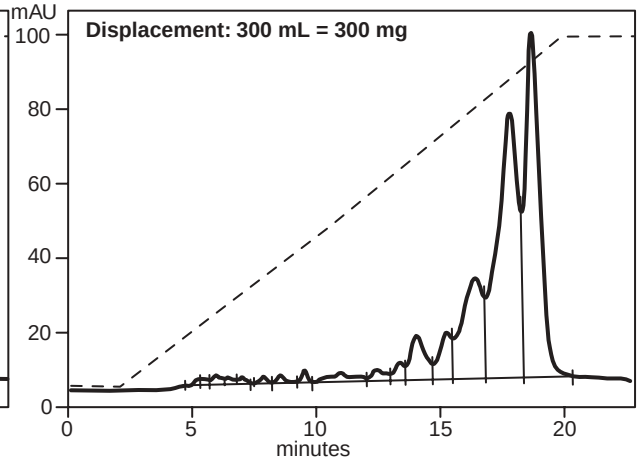


Displacement Analysis

More detailed analysis of displacement fractions using the SAX analytical HPLC method revealed further capacity for 2302 Oligo – up to 300 mg per 10 mL Mustang Q Module. The more strongly retained oligo displaces N-1, N-2 etc. contaminants during sample loading. Loading to full displacement capacity in final developed method would be expected to produce an even higher degree of purification.







Pall Mustang™ Chromatography Products

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