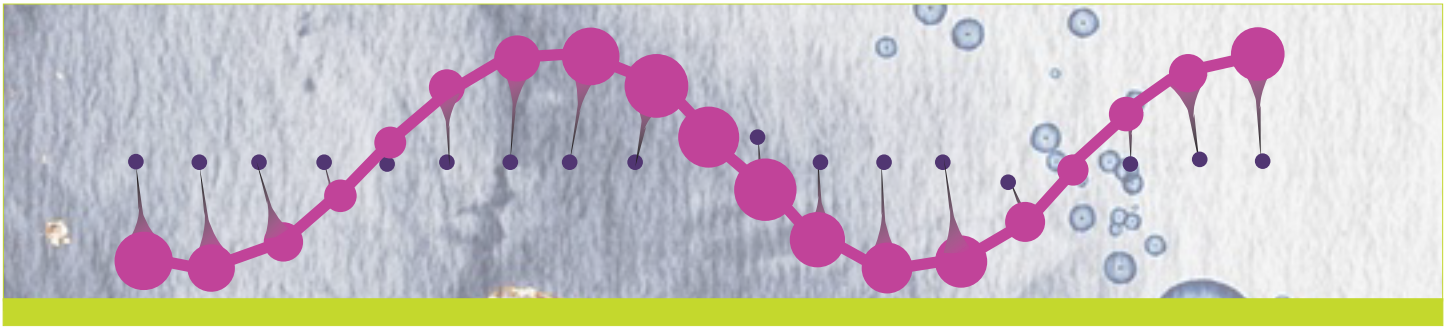
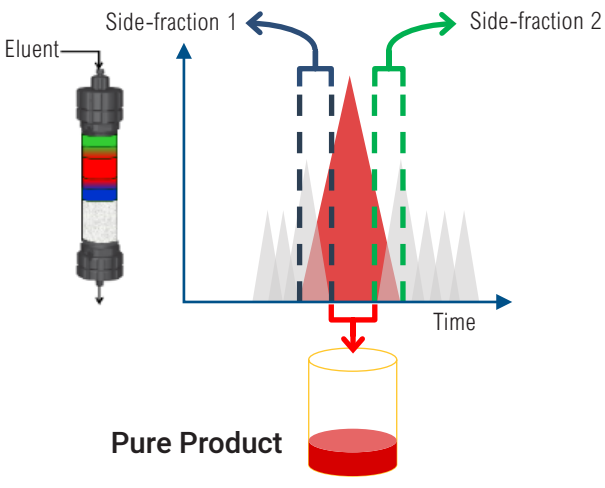
Application Note

Comparison of continuous twin-column technology with single column batch methodology for the purification of an antisense oligonucleotide (ASO)

Antisense Oligonucleotides (ASOs) are short, synthetic, chemically modified chains of nucleotides. They are pharmaceutically active when they bind to specific mRNA sequences, preventing protein production or modulating splicing. There are 13 ASO's approved by the FDA. Spinraza, developed by Ionis and Biogen, is the first-of-its-kind drug for spinal muscular atrophy. In 2023, Spinraza's had the highest global sales for an ASO with $1.7 billion. Most ASO drugs are synthesized with solid-phase oligonucleotide synthesis and use chromatography for final purification. Within the ASO manufacturing process, the chromatography step is a critical step and needs to produce material with sufficient purity and optimal yields. This work describes two AEX purification processes for an ASO. The first process uses a typical single column batch methodology and the second uses twin-column continuous technology. The purity and yield for both these development scale experiments are presented and then used to simulate the results for these processes to produce 100g of the product.

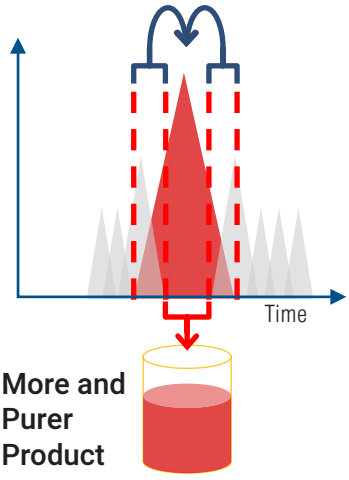


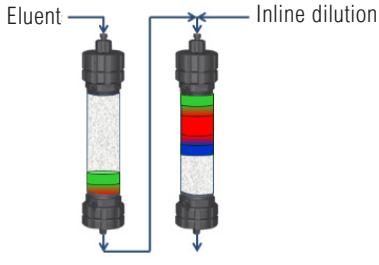
Conventional Batch Chromatography



Count-current Chromatography (MCSGP)

Automatic Internanal Recycling of Side-fractions



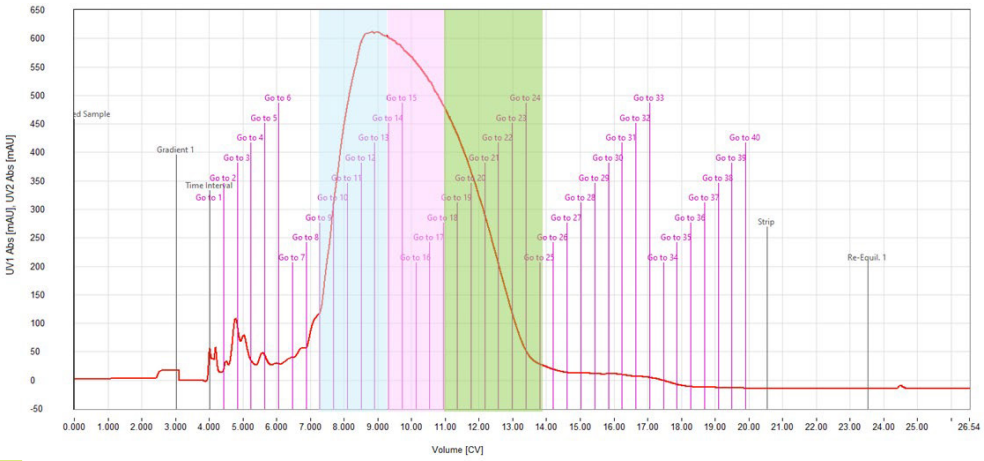


- Center-cut purification
- Linear gradient
- Counter-current
- Automatic recycling of impure side fractions

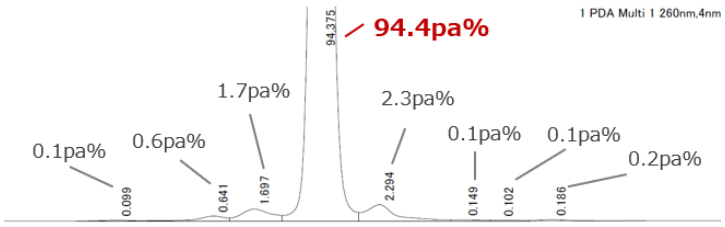
In batch chromatography of peptides and oligos, impure side fractions often have to be discarded to reach a certain product purity. With MCSGP featuring AutoPeak® technology, impure product-containing side fractions are internally (automatically) recycled in a periodic closed-loop process while pure product is continuously extracted. Little product is lost, and the yield of pure product is maximized without any accumulation of impurities while retaining target purity.

Contichrom® CUBE Single Column System

Once the separation is developed, a single column batch run is performed on the CUBE. Fractions are collected across the target peak and assayed analytically. The assay results are used to determine resulting purity and yield results.

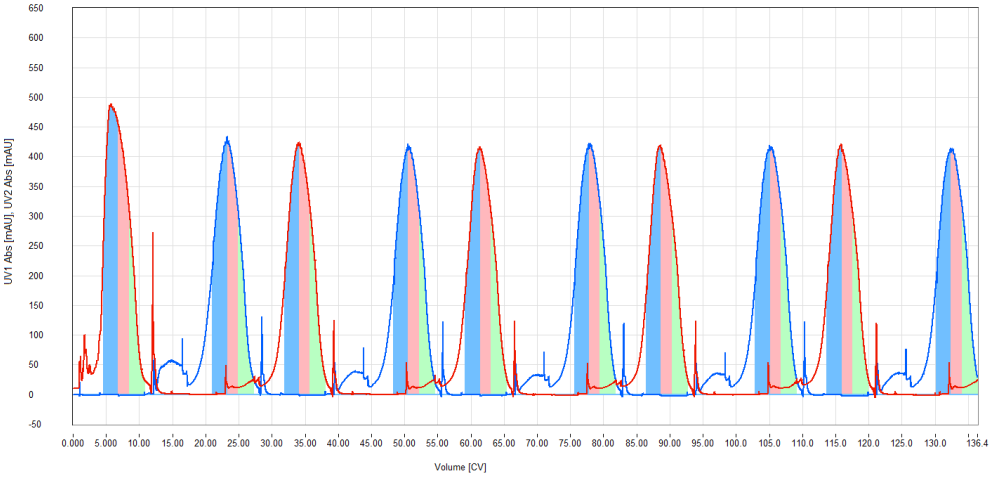


Column:	BioPro IEX SmartSep Q20 150 X 10 mm ID
Eluent:	A) NaOH in water B) NaOH / NaCl in water
Gradient slope:	0.8%B/min
Flow rate:	2.4 mL/min
Detection:	UV at 290 nm
Injection:	8.52 g/L-gel (102 mg)
Sample:	DNA 20 mer All PS
System:	Contichrom CUBE 100

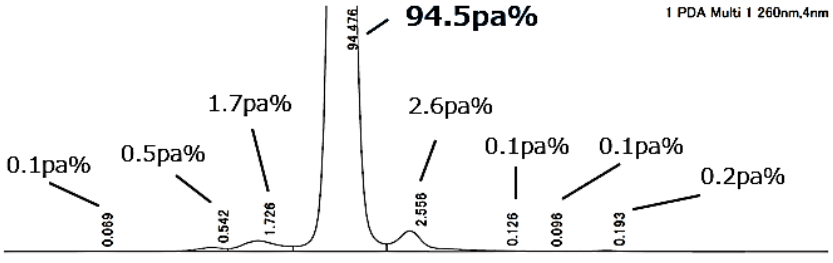


Contichrom® CUBE Twin-Column featuring MCSGP with AutoPeak® Technology

After generating the single column data on the CUBE, the MCSGP Wizard is used to create the MCSGP methodology. The chromatographic trace for 5 cycles is shown here (5 main peaks for each column).

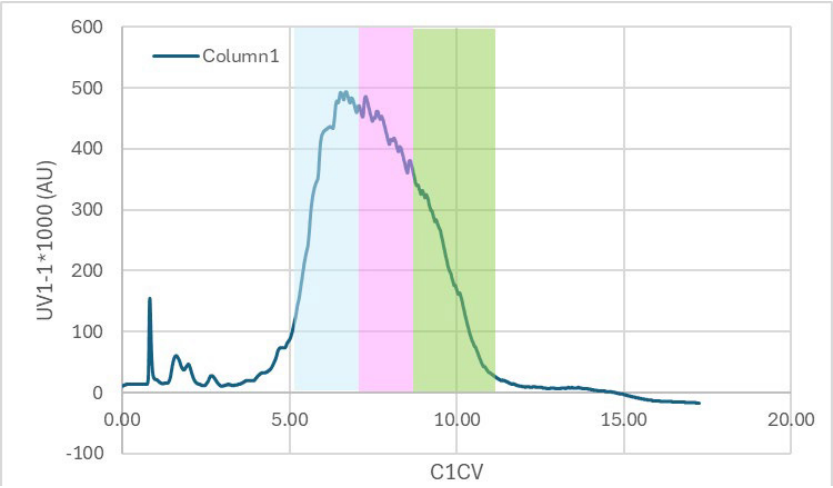


Column:	BioPro IEX SmartSep Q20 150 X 10 mm ID
Eluent:	A) NaOH in water B) NaOH / NaCl in water
Gradient slope:	0.8%B/min
Flow rate:	2.4 mL/min
Detection:	UV at 290 nm
1st Injection:	8.52 g/L-gel (101 mg)
2nd Injection:	3.54 g/L-gel (42 mg)
Sample:	DNA 20mer All PS
System:	Contichrom CUBE100

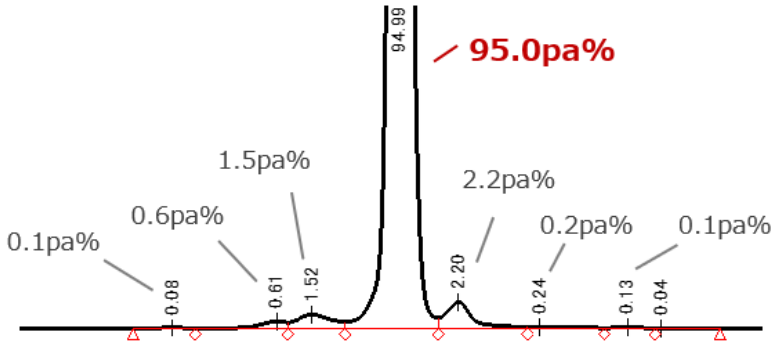


ContichromTWIN System

The CUBE methodology was scaled to the ContinchromTWIN system and a single column batch run was performed. This step is not always necessary for production but was performed in this project for clarity. The Contichrom TWIN MCSGP methodology can be created directly from the Contichrom CUBE MCSGP Method. Three cycles were run and are shown in this chromatographic trace. The data for all 6 injections are summarized in the following table.

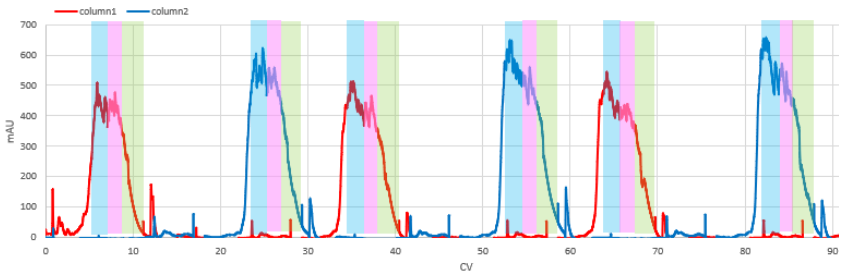


Column:	BioPro IEX SmartSep Q20 150 X 100 mm ID
Eluent:	A) NaOH in water B) NaOH / NaCl in water
Gradient slope:	0.8%B/min
Flow rate:	240 mL/min
Detection:	UV at 290 nm
Injection:	8.52 g/L-gel (10.2 g)
Sample:	DNA 20 mer All PS
System:	Contichrom TWIN 300

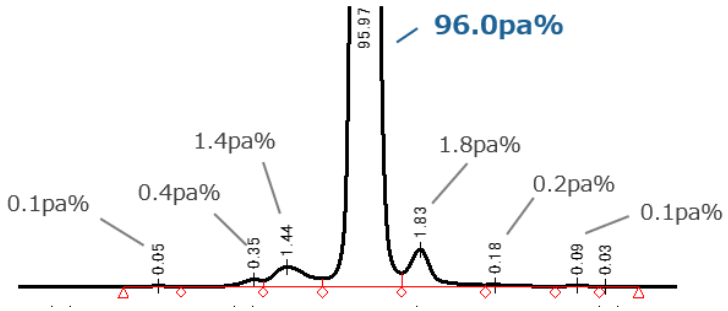


Contrichrom TWIN System featuring MCSGP with AutoPeak® Technology

The TWIN MCSGP methodology was created from the CUBE MCSGP method. Three cycles were run, even though only 3 ½ cycles are shown in the chromatographic trace to the right. The data for all 6 injections are summarized in the table below.



Column:	BioPro IEX SmartSep Q20 150 X 100 mm ID
Eluent:	A) NaOH in water B) NaOH / NaCl in water
Gradient slope:	0.8%B/min
Flow rate:	240 mL/min
Detection:	UV at 290 nm
1st Injection:	8.52 g/L-gel (10.2 g)
2nd Injection:	3.54 g/L-gel (5.0 g)
Sample:	DNA 20mer All PS
System:	Contichrom TWIN 300

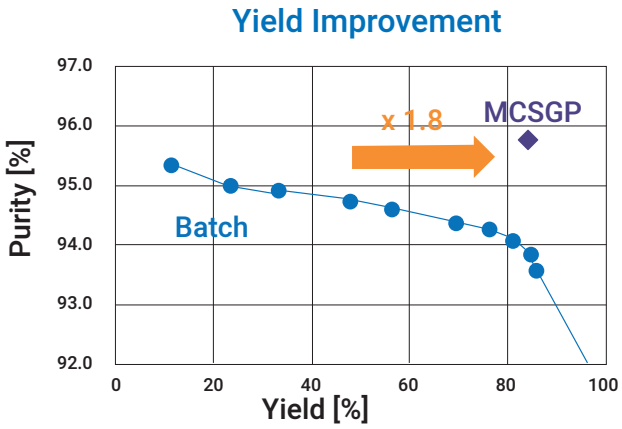


Comparison

The resulting % purity obtained with batch and MCSGP methodology was consistent for both the CUBE and the TWIN instruments. As expected, the % Yield was vastly improved with the MCSGP technique when compared to the results from batch methodology. The CUBE had an increase of 104 % and the TWIN system had 76 % increase.

	Contichrom CUBE system		Contichrom TWIN system	
	Batch	MCSGP	Batch	MCSGP
Column	10 mm ID	10 mm ID	100 mm ID	100 mm ID
Purity (%)	94.6	94.2	94.8	95.8
Yield (%)	42.7	87.1	47.9	84.3

From the Batch data and TWIN MCSGP data, simulations can be generated to obtain 100 g of product from each process. The table below points out the key attributes for these simulations.



Conclusion

The work presented here used an ASO (DNA 20mer All PS) to compare IEX preparative chromatography techniques using YMC BioPro media and equipment at the benchtop scale, with a CUBE100, and the production scale, with a TWIN300. With the BioPro media, in a batch process, both the CUBE and the TWIN systems were able to purify the target compound from an initial purity of 72 %, to a purity that was greater than 94.4 % With the MCSGP technique, the TWIN system and the CUBE system produced comparable levels of purity and yield. At the TWIN scale, MCSGP was able to improve the yield 1.8 times higher than the Batch process. The TWIN MCSGP process used 50 g of crude material to produce 40 g of the target product at the specified purity.

Application by YMC CO., LTD. We gratefully acknowledge the valuable contributions of PeptiStar, Inc. (www.peptistar.com) of which collaboration and insights significantly enhanced the quality of this work.

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