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

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Best Poster Award Participant

Introduction

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.

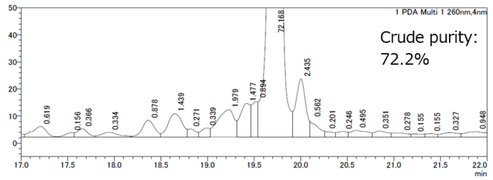
Most ASO drugs are synthesized with solid-phase oligonucleotide synthesis and use chromatography for final purification. Within the ASO manufacturing process, the chromatography step is a critical step and needs to produce material with sufficient purity and optimal yields. This work describes two AEX 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.

Example Oligonucleotide

Target
DNA 20mer All PS
5'-C^T^G^C^T^A^G^C^C^T^C^T^G^G^A^T^T^A^G^A-3'

The target ASO is a DNA 20mer All PS molecule, that was made by solid phase synthesis. The purity of the crude was 72.2% by IP-RP HPLC with HFIP on a Triart C18 column packed in Accura hardware.

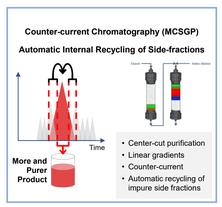
Column	:YMC-Accura Triart C18 (12 nm, 1.9 µm) 150 x 2.1 mmI.D.
Eluent	: A) TEA/HFIP in water B) MeOH in Eluent A
Gradient slope	: 2%B/min
Flow rate	: 0.3 mL/min
Temperature	: 60 °C
Detection	: UV at 260nm
Injection	: 5 µL (Diluted by Eluent A (1:100))



MCSGP Equipment

MCSGP (Multi-column Counter-current Solvent Gradient Purification) is a two-column process that uses the patented AutoPeak® technology. Pure product is continuously extracted while the impure side fractions are internally (automatically) recycled in a periodic closed-loop process. MCSGP delivers higher yields and the same (or better) purity than batch chromatography.

The Contichrom® CUBE is a lab-scale twin-column chromatography platform that improves purification efficiency when compared to traditional single-column chromatography. YMC Contichrom® TWIN HPLC systems are designed to run processes developed with the CUBE at production scale. This two-column continuous process technology, accelerates validation, simplifies maintenance, reduces down-time, and minimizes operating expenses.



Contichrom CUBE

10 mm ID

Contichrom TWIN300

100 mm ID

YMC BioPro IEX Resins

BioPro ion exchange columns are specifically designed for the separation of proteins, peptides and nucleic acids. The hydrophilic polymer beads are completely spherical and monodisperse with low nonspecific adsorption. They also show higher binding capacity and higher recovery of biomolecules compared to conventional ion exchange columns.

Available Ion Exchange Resins:

AEX Resins
- BioPro IEX SmartSep Q10
- BioPro IEX SmartSep Q30
- BioPro IEX SmartSep Q20
- BioPro IEX Q75
- MacroSep IEX Q

CEX Resins
- BioPro IEX SmartSep S10
- BioPro IEX SmartSep S30
- BioPro IEX SmartSep S20
- BioPro IEX S75

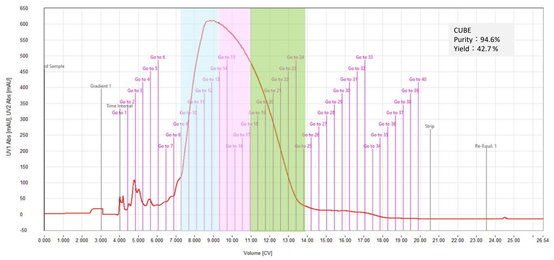
MacroSep IEX Q (30 µm)

Ideal choice for the purification of **large biomolecules and particles** such as AAV, VLPs and large nucleic acids

BioPro IEX (30 µm)

Ideal choice for the purification of **smaller and mid-sized biomolecules** such as proteins, antibodies and oligonucleotides

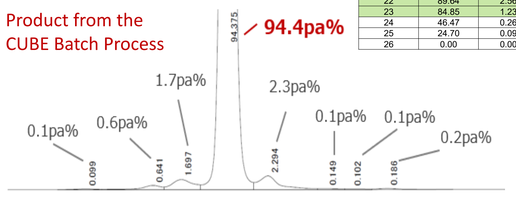
Cube Single Column



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

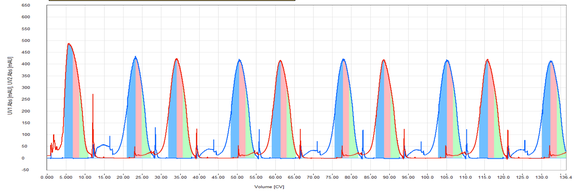
Fr.	Purity %	Yield %
8	0.00	0.00
9	0.00	0.00
10	8.69	0.56
11	33.36	3.28
12	78.53	9.50
13	93.41	11.49
14	94.51	11.91
15	94.57	10.79
16	94.95	10.49
17	94.52	9.51
18	93.99	8.21
19	92.07	7.08
20	91.95	5.67
21	90.54	4.09
22	89.64	2.56
23	84.85	1.23
24	46.47	0.26
25	24.70	0.09
26	0.00	0.00



Cube MCSGP

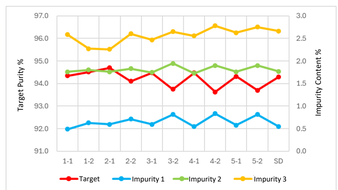
·Column	: BioPro IEX SmartSep Q20 150 X 10 mm ID
·Eluent	: A) NaOH in water B) NaOH / NaCl in water
·Gradient slope	: 0.8%B/min
·Flow rate	: 2.4 mL/min
·Detection	: UV at 290 nm
·1st Injection	: 8.52 g/L-gel (101 mg)
·2nd Injection	: 3.54 g/L-gel (42 mg)
·Sample	: DNA 20mer All PS
·System	: Contichrom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

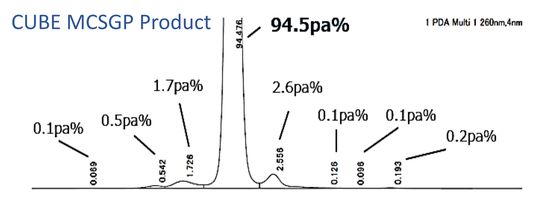
After generating the single column data on the CUBE, the MCSGP Wizard is used to create the MCSGP methodology. Below is the chromatographic trace for 5 cycles (5 for each column).



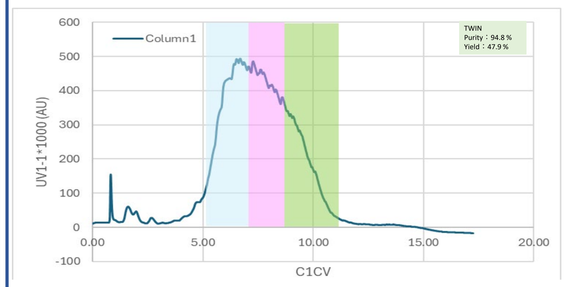
	1-1	1-2	2-1	2-2	3-1	3-2	4-1	4-2	5-1	5-2	S.D.
FLP Yield	37.5%	86.2%	88.7%	86.1%	89.4%	88.2%	88.1%	84.0%	90.2%	84.5%	85.3%
FLP Purity	94.3%	94.5%	94.7%	94.1%	94.5%	93.8%	94.5%	93.6%	94.3%	93.7%	94.3%
Impurity ①	0.5%	0.6%	0.6%	0.7%	0.6%	0.8%	0.5%	0.8%	0.6%	0.8%	0.6%
Impurity ②	1.8%	1.8%	1.8%	1.8%	1.7%	1.9%	1.7%	1.9%	1.8%	1.9%	1.8%
Impurity ③	2.6%	2.3%	2.3%	2.6%	2.5%	2.7%	2.6%	2.8%	2.6%	2.8%	2.7%

- High reproducibility
- Meets purity criteria over 94%
- Consistent results over the 5 different cycles





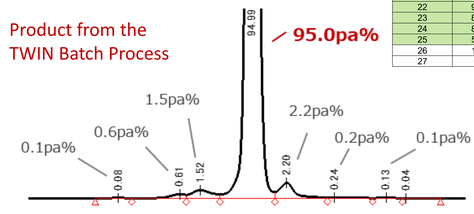
Twin Single Column



The CUBE methodology was scaled to the Twin 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 TWIN MCSGP methodology can be created directly from the CUBE MCSGP Method.

·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

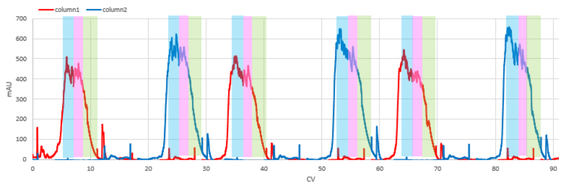
Fr.	Purity %	Yield %
10	0.00	0.00
11	4.02	0.10
12	1.56	0.09
13	16.89	1.66
14	80.19	11.24
15	93.31	13.33
16	94.51	14.69
17	95.32	11.87
18	94.67	11.75
19	94.62	9.58
20	93.82	8.61
21	92.92	6.72
22	91.71	4.85
23	88.12	3.31
24	80.54	1.48
25	56.30	0.46
26	14.99	0.06
27	0.00	0.00



Twin MCSGP

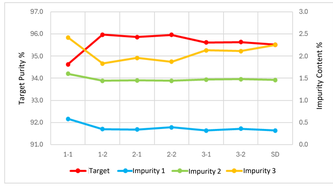
·Column	: BioPro IEX SmartSep Q20 150 X 100 mm ID
·Eluent	: A) NaOH in water B) NaOH / NaCl in water
·Gradient slope	: 0.8%B/min
·Flow rate	: 240 mL/min
·Detection	: UV at 290 nm
·1st Injection	: 8.52 g/L-gel (10.2 g)
·2nd Injection	: 3.54 g/L-gel (5.0 g)
·Sample	: DNA 20mer All PS
·System	: Contichrom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

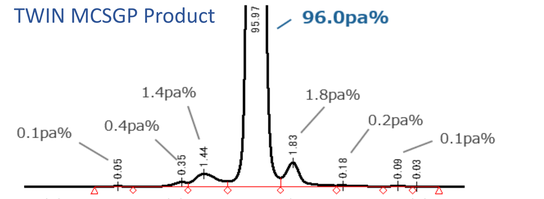
The TWIN MCSGP methodology was created from the CUBE MCSGP method. Three cycles were run, even though only 3 ½ cycles are shown in the chromatographic trace below. The data for all 6 injections are summarized in the table below.



	1-1	1-2	2-1	2-2	3-1	3-2	S.D.
FLP Yield	39.9%	87.7%	83.4%	83.7%	84.1%	84.6%	82.1%
FLP Purity	94.6%	96.0%	95.9%	96.0%	95.6%	95.6%	95.5%
Impurity 1	0.6%	0.4%	0.3%	0.4%	0.3%	0.4%	0.3%
Impurity 2	1.6%	1.4%	1.5%	1.4%	1.5%	1.5%	1.5%
Impurity 3	2.4%	1.8%	2.0%	1.9%	2.1%	2.1%	2.3%

- High reproducibility
- Meets purity criteria over 94%
- Strong recycle should be adjusted to avoid increasing the amount of impurity #3 over the cycles





Compare Cube and Twin

	Contichrom CUBE		Contichrom TWIN	
	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

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.

Sample amount and flow rate applied to TWIN are calculated by multiplying the ratio of cross-sectional area (x (100/10)²)

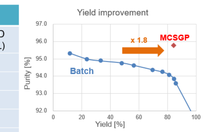
- TWIN has practically the same preparative results as CUBE for both purity and yield
- CUBE/TWIN doubled yield from batch without compromising the purity.

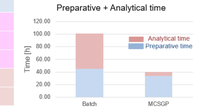
Production Simulation

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

	Batch	MCSGP
Column	150 X 100 mm ID (resin vol. 1.3 L)	150 X 100 mm ID (resin vol. 2.6 L)
FLP Purity [%]	94.8	95.8
FLP Yeild [%]	47.9	x 1.8 → 84.3
Number of batch or cycle	21	12
Required amount of crude [g]	208.8	x 0.6 → 118.6
Required amount of buffer [L]	662	703
Preparative time [h]	45.2	34.2
Productivity [g/L-gel/h]	1.9	1.2
Productivity [g/h]	2.2	x 1.3 → 2.9
Analytical time [h]*	55.6	6.3
Preparative + Analytical time [h]	100.8	x 0.4 → 40.5

* Batch: 5 fractions/batch, MCSGP: 1 fraction/cycle (32 min required for 1 analysis)



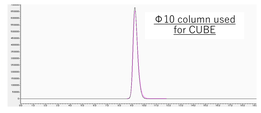


Columns for MCSGP

		BH (cm)	N/m	As
CUBE (ϕ10)	Col. 1	15.0	19,193	1.6
	Col. 2	15.0	18,637	1.6
TWIN (ϕ100)	Col. 1	15.0	9,260	1.9
	Col. 2	15.4	9,656	2.2

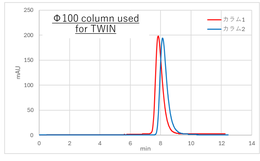
MCSGP requires 2 columns that perform similarly. AutoPeak is an MCSGP feature that uses the detector response to trigger recycling and collection. With AutoPeak, the 2 columns do not need to be the same, as required with SMB. The 2 columns can be evaluated with a lower load of the target component or a well characterized test mix where the bed height, plates/meter and asymmetry are compared.

- Small difference between two columns for both CUBE and TWIN
- The difference of columns for CUBE and TWIN is found, but both have the same separation results in terms of purity >94%



Column Inspection condition

Column I.D.	ϕ10	ϕ100
Eluent	20 mM Tris-HCl aq (pH 8.0)	0.5 M NaCl
Flow rate	1.18 mL/min	118 mL/min
Temperature	ambient	ambient
Detection	UV at 220 nm	UV at 220 nm
Sample	2 µL/mL Formamide	20 µL/mL Formamide
Injection	120 µL	12 mL



Conclusions

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 50g of crude material to produce 40g of the target product at the specified purity.

Acknowledgements

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