ORGANIC PROCESS RESEARCH & DEVELOPMENT
OPR&D

pubs.acs.org/OPRD
Article

Continuous Purification of a Conjugated Short Interfering RNA Therapeutic Using Anion Exchange Twin-Column Chromatography (MCSGP)

Richard Weldon, Tobias Vandermeersch, and Thomas Müller-Späth*

Cite This: Org. Process Res. Dev. 2025, 29, 1400−1410
Read Online

ABSTRACT: Multicolumn Countercurrent Solvent Gradient Purification (MCSGP) process technology improves yields and production efficiency in clinical and commercial manufacturing of therapeutic APIs such as peptides and oligonucleotide drugs (ONDs). In this case study, we developed an MCSGP process for the purification of a sugar-conjugated sense strand of a therapeutic siRNA (short interfering RNA) using anion exchange (AIEX) chromatography and state-of-the-art twin-column chromatography equipment. MCSGP is relatively complex and requires specialized process development tools to optimize and implement. We describe a simplified, software-aided MCSGP method development procedure for fast transfer from batch to continuous-mode chromatography. As expected with MCSGP, the yield improved from 80% to 93% compared to single-column chromatography, allowing for proportional downscaling of steps preceding and including oligonucleotide synthesis. This greatly decreases manufacturing costs and reduces waste (e.g., byproducts, solvents, and reagents). In addition to increasing the OND yield, throughput was increased by 87%. Other generic benefits of implementing MCSGP are also outlined; for example, due to automation, process robustness is improved; column dimensions and resin volumes are downscaled; laborious side-fraction rechromatography is eliminated; and fewer "in-process control" samples are generated, reducing the manufacturing support burden. The automation enabled by MCSGP is a significant advancement in the commercial-scale manufacturing of APIs.

KEYWORDS: *oligonucleotide purification, MCSGP, continuous manufacturing, scale-up, therapeutic siRNA*

1. INTRODUCTION

Oligonucleotide drugs (ONDs) have seen increasing success in recent years, resulting in 15 drug approvals and hundreds of more candidates entering clinical trials.[1−3] ONDs can modulate gene expression in a highly specific manner, creating the possibility of curing previously untreatable conditions. The therapeutic potential of oligonucleotides was greatly improved by recent advancements in synthetic chemistry that have enabled the design and synthesis of analogues with pharmaceutical stability and affinity far beyond native oligonucleotides, thereby improving their bioavailability and specificity.[4,5]

However, due to their chemical complexity, commercial-scale synthesis and purification of modified oligonucleotides have been challenging and expensive. At the time of writing, the maximum commercial GMP scale is limited to 2000 mmol synthesis scale using the Solid Phase Oligonucleotide Synthesis (SPOS) approach. Moreover, losses during downstream purification limit the maximum output scale per batch. In response to an increasing demand for production capacity, the biopharma industry is actively expanding their OND manufacturing capabilities while simultaneously evaluating improved manufacturing techniques to improve efficiency, reduce costs, and reduce environmental impacts.[6,7]

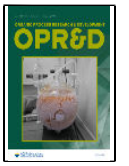
1.1. Oligonucleotide Synthesis and Purification. Oligonucleotide synthesis involves the controlled assembly of short nucleotide sequences. Small, single-stranded DNA or RNA fragments play pivotal roles in various scientific fields such as genetic engineering, gene editing, diagnostics, and therapeutics.

Historically, oligonucleotide synthesis methods have relied on inefficient procedures, limiting both the scale and complexity of the sequences achievable. However, significant advancements in chemical synthesis have catapulted the field forward, allowing for the efficient production of custom oligonucleotides with high yields and purities.[8−10]

Advancements in oligonucleotide production are not limited to nucleotide assembly but have also been made in downstream processing, particularly in the critical purification step. Purification is a fundamental stage in oligonucleotide production, as it directly impacts the yield, quality, and bioactivity of the final product. Significant strides have been made in developing innovative and efficient purification techniques, enabling the production of highly pure oligonucleotides with improved scalability and cost-effectiveness.[6,10]

1.2. The Challenges of Preparative Scale Oligonucleotide Purification. Oligonucleotides are typically manufactured at large scale using solid-phase chemical synthesis

Received: December 2, 2024
Revised: May 15, 2025
Accepted: May 21, 2025
Published: June 2, 2025



followed by chromatographic purification, desalting, and freeze-drying.[9] During synthesis, incomplete chemical coupling at each step of elongation results in a crude mixture of the active ingredient and chemically similar product-related impurities (e.g., shortmers and longmers).[11] Following oligonucleotide synthesis, a preparative chromatographic polishing step is usually essential to achieve clinical-grade purity. The polishing step is normally done using single-column ion exchange or reversed-phase chromatography ("batch" mode) with the pure "center cut" of the chromatogram collected for further processing. Unfortunately, incomplete separation of the target product from chemically similar compounds means that lower purity side-cuts must be reprocessed or discarded because the impurity content is too high. Oligonucleotide synthesis must be increased in scale to compensate for any product lost during purification, which is very costly.

In single-column batch purification, yields are maximized by carefully fractionating and analyzing the elution peak to determine the optimal "center cut" collection boundaries. Multiple "mock" product pools are then generated and sent for further analytical testing and quality control (QC). Unfortunately, these laborious tasks are essential for batch release and represent a significant proportion of the overall manufacturing burden in a GMP environment.

Another common method to increase product recovery in single-column chromatography is by pooling the "out-of-specification" side fractions from multiple batches to perform "re-chromatography." Unfortunately, rechromatography reduces the overall efficiency of the purification process.

1.3. MCSGP as an Alternative for Oligonucleotide Purification. One recent technological advancement in OND and peptide purification is Multicolumn Countercurrent Solvent Gradient Purification (MCSGP), a twin-column continuous chromatography technology that improves production efficiency compared to that of standard batch techniques. In contrast to batch chromatography, MCSGP is a semicontinuous polishing method originally developed using 3−6 columns[12−14] but later simplified to a more operationally flexible twin-column process.[15,16] The major advantage of MCSGP is the ability to internally recycle low-purity side cuts to a second chromatography column, using an in-line dilution to aid readsorbtion. As a result, MCSGP enables considerable yield improvements while making rechromatography unnecessary, and this has been shown for a wide range of biomolecules.[16−24]

The goal of this case study was to convert an established single-column AIEX batch process for the purification of a sugar-conjugated sense strand of a therapeutic siRNA, to continuous mode MCSGP, with a target purity specification of >95%. This study demonstrates the effectiveness of AIEX in MCSGP production, reducing the yield-purity trade-off intrinsic to batch mode chromatography.

1.4. Introduction to the MCSGP Process Principle and Design. MCSGP processes are run using specialized chromatography systems fitted with two identical "twin" columns and a flow path designed to enable continuous operation. MCSGP-enabled systems have approximately double the hardware components compared to conventional batch systems due to the complexity of the flow path required to run the twin-column process. An example MCSGP enabling process development system (Contichrom CUBE, YMC ChromaCon) is shown in Figure 1.

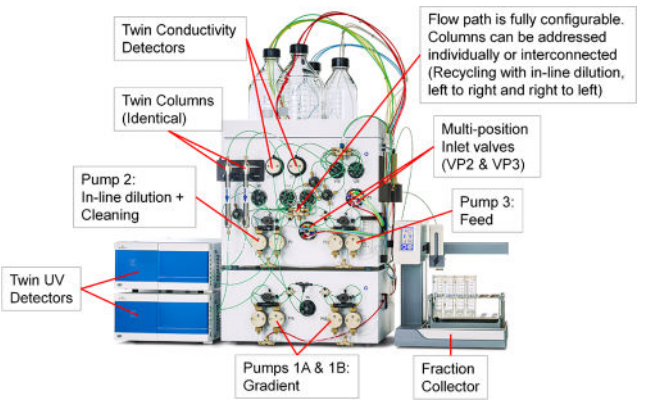


Figure 1. Twin column chromatography system setup for MCSGP (Contichrom CUBE, YMC ChromaCon).

MCSGP requires a "twin-column" chromatography system with a programmable flow path that allows periodic transitions between parallel column operations (for example, cleaning on column 1 with simultaneous elution on column 2 or vice versa) and interconnected column operations for side-cut recycling (left column to right column and vice versa). In addition, the in-line dilution functionality between interconnected columns is essential for rebinding product-containing side-cuts during the recycling steps. Performance is monitored by a pair of UV and conductivity detectors behind each column.

Besides the unique hardware requirements outlined above, the other materials required for running MCSGP are the same as other single-column chromatographic polishing techniques. MCSGP uses the same stationary phases and eluent combinations (ion exchange, reversed phase, HIC); and it works well for isocratic or linear gradient purifications, provided the target compound is fully adsorbed to the stationary phase, and desorbed after the modifier concentration is increased. For this reason, MCSGP can be applied to many existing industrial processes such as polishing of peptides, oligonucleotides, oligosaccharides, conjugated therapeutic proteins, and mAbs.[16−24]

As shown in Figure 2A, a MCSGP process always begins with a short "startup" step that is used to preload one column, so a cyclic steady state is reached quickly. Once the preloading step is complete, there is a seamless transition into the "main" cyclical production phase, where a series of "*n*" number of steps called "switches" alternate between the 2 columns. During each switch, one column undergoes elution while the other column is regenerated and loaded in parallel. Following the completion of one "switch," the same method is repeated, but on the alternate column. An MCSGP "cycle" is defined as the sum of two switches, with one elution from each column. Finally, a "shutdown" step concludes the process, where a final product elution is done, but without loading of the second column. A more detailed theoretical description of the MCSGP process principle is reported elsewhere.[25,26]

Figure 2B shows a schematic representation of a single MCSGP "switch" including 4 phases. Phase 1 initiates a gradient on the first column, while in parallel, cleaning and regeneration steps prepare the second column for loading. As product elution starts, cleaning and regeneration are concluded, and phase 2 is then initiated. Phase 2 is the first internal recycling step where both columns are interconnected and the early eluting, low purity product (side-cut) is transferred from the first column (upstream) to the second

https://doi.org/10.1021/acs.oprd.4c00513
Org. Process Res. Dev. 2025, 29, 1400−1410

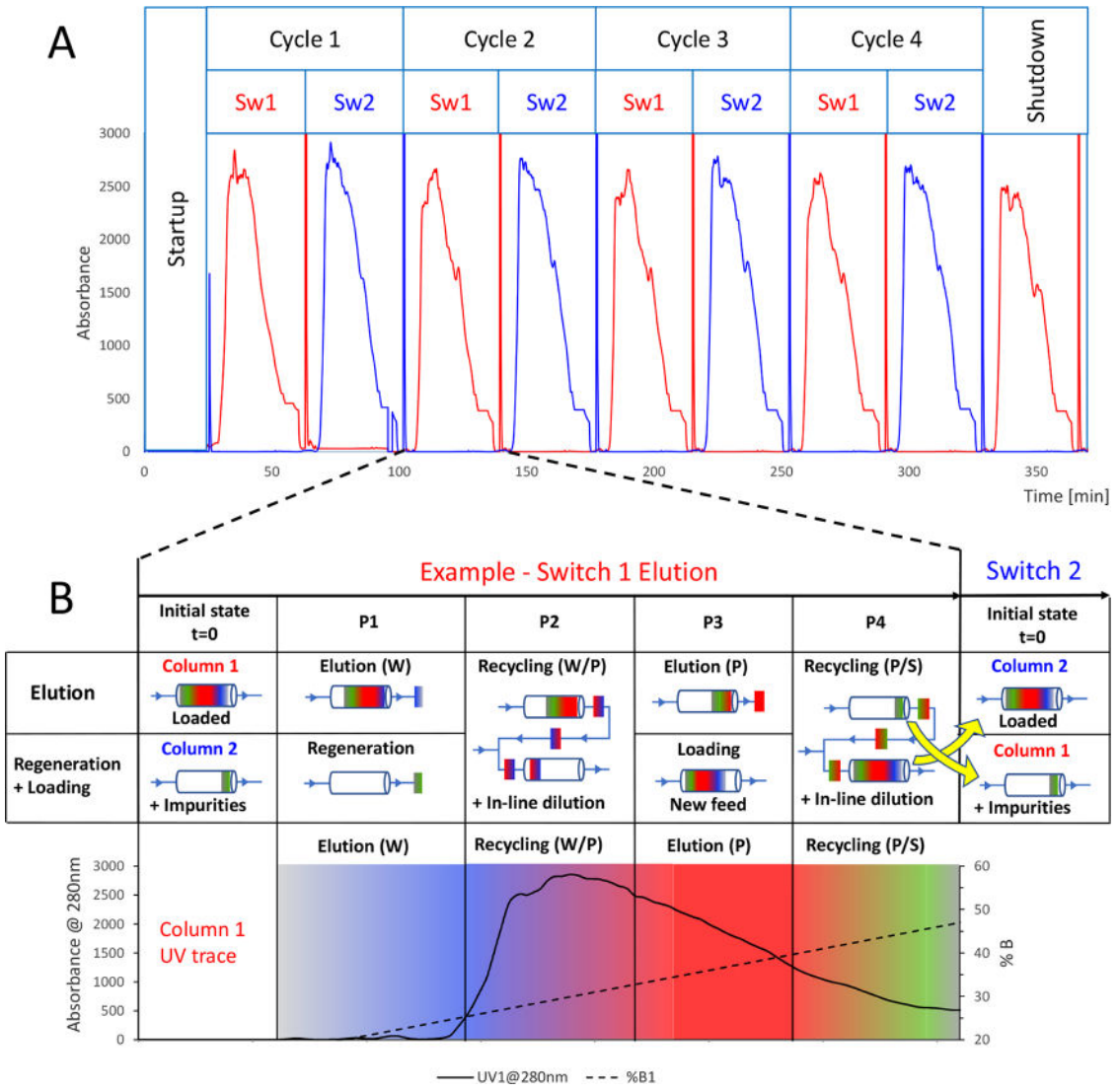


Figure 2. MCSGP process. (A) An MCSGP run with 4 cycles is shown as an example. A short startup step is needed to preload column 1 with feed before the main cyclic phase of MCSGP begins. Each cycle consists of an elution from column 1 (switch 1—red) and an elution from column 2 (switch 2—blue), respectively. Alternating elutions can continue indefinitely for "*n*" cycles until the feed is consumed. To finish the MCSGP run, a shutdown step consisting of a single elution without any refeeding is done. (B) A detailed schematic representation of a single MCSGP "switch" is shown. This consists of four phases: P1 = phase 1 (elution + regeneration); P2 = phase 2 (elution with weak recycling); P3 = phase 3 (product collection + refeeding); and P4 = phase 4 (elution with strong recycling). Columns are operated in parallel during P1 and P3; columns are interconnected for product recycling in P2 and P4. The position of the columns alternates switch to switch, allowing for continuous operation (yellow arrows indicate column interchange between switches). See Section 2.4 for a more complete description of the MCSGP process principle.

column (downstream) until the point where the pure product starts to elute. Readsorption of the product to the second column is enabled by in-line dilution between columns to lower the modifier content of the eluate. In phase 3, the gradient is continued on the first column and the pure product is collected, while in parallel, the second column receives new crude feed material. Finally, in phase 4, the late-eluting, low-purity product is recycled in the same way as in phase 2. The sum of the recycled product plus the new product loaded on the downstream column typically equals the mass of the product loaded during the batch design method. At the end of every switch, the columns change positions, and the same operation begins again. While parallelization of tasks during phases 1 and 3 increases the process efficiency, the major advantage of MCSGP is the ability to interconnect two columns during phases 2 and 4 so that the low-purity product is automatically internally recycled.

1.5. Generic Advantages of MCSGP. *1.5.1. Yield Improvement.* As a result of increased chromatographic yields, MCSGP enables proportional downscaling of the chemical synthesis batches needed for the equivalent output of the product compared to batch chromatography. This results in large economic and raw material savings throughout the process and, thus, is a more sustainable manufacturing option.

1.5.2. Reduced Analytical QC Burden. As mentioned above, oligonucleotide purification done using batch chromatography requires peak fractionation and mock pool analysis before batch release. By contrast, fractionation is not required in MCSGP and a product pool combined from multiple cycles is analyzed for quality. In the best-case scenario, this product pool could consist of the entire MCSGP run, but even if pools

https://doi.org/10.1021/acs.oprd.4c00513
Org. Process Res. Dev. 2025, 29, 1400−1410

Figure 3. Single-column AIEX chromatographic profiles and the MCSGP design. (A) Black line: representative chromatogram of pilot-scale single-column batch production run showing absorbance UV@290 nm vs column volumes (CV) overlaid with %B modifier concentration (purification using the AKTA pure 150 M). The pilot-scale method was downscaled 78.6× and run on the Contichrom CUBE. (B) MCSGP design chromatogram. Absorbance UV@280 nm is overlaid with product purity (area% HPLC-UV). Product purity was measured by HPLC analysis, and each dot represents an analyzed fraction. Phases P1 to P4, as described in Figure 2B, are highlighted showing where MCSGP recycling and product collection boundaries were set. (C) - The MCSGP Wizard v8.1 software simplifies method creation.

include only 5 cycles (10 elutions) each, MCSGP can greatly reduce the analytical burden by generating far fewer samples for QC analysis.

1.5.3. Rechromatography is Eliminated. As mentioned, rechromatography of low-purity side cuts is often done to increase product recovery in processes using batch chromatog-

https://doi.org/10.1021/acs.oprd.4c00513
Org. Process Res. Dev. 2025, 29, 1400−1410

raphy; however, this has multiple negative impacts on a manufacturing operation. First, the side cuts from multiple batch runs are transferred into custom storage bags and temporarily stored in a dedicated walk-in freezer. Side cuts are pooled and undergo diafiltration to adjust the buffer composition and concentration in preparation for reloading the chromatography column. Rechromatography of side cuts results in lower product recovery per run because the feed composition has a higher percentage of key impurities compared to the original feed. While rechromatography does significantly improve the overall yield, the productivity of the overall process decreases. In summary, by eliminating rechromatography in MCSGP, the user effort is minimized, dedicated storage space and consumables are reduced, analytical burden is reduced, and a higher product pool concentration is obtained.

1.5.4. Automation of Purification. Automation in MCSGP is made feasible by the implementation of UV-based dynamic process control (AutoPeak), which compensates for changes in peak retention times caused by variations in eluent preparation, column packing quality, and feed quality.[27] In addition, the possibility to monitor area under the curve (AUC) from cycle-to-cycle during an MCSGP run also allows early intervention if a process drifts from predefined set points. Both of these functions enhance process robustness and allow MCSGP to run for extended periods of time without human intervention.

1.5.5. Reduced Operator Interaction with the Process. There is generally less scope for user error in MCSGP because the user interaction with the process is reduced compared to batch chromatography. For example, tasks related to rechromatography are eliminated, and fewer samples are generated for analysis and storage. In addition, due to the smaller column dimensions needed in MCSGP, column packing procedures can be simpler.

1.5.6. Fewer Highly Trained Operators are Required. Given the automated nature of the MCSGP process, it can be operated with fewer personnel. The reduced labor cost per shift makes it more economically viable to run a process overnight or 24/7 if needed.

1.5.7. Improved Process Mass Intensity (PMI). Due to the continuous operation of MCSGP, PMI can be improved relative to batch chromatography through buffer savings. First, there is a reduction in the number of column storage steps required during a manufacturing campaign, and second, due to increased purification yield, less synthesis solvent is used. A recent comparison of optimized processes shows significantly reduced PMI for oligo manufacturing using an MCSGP-based process.[28]

At the time of writing, MCSGP reached a milestone when the first two GMP scale production systems installed at Bachem AG (Bubendorf, Switzerland) successfully completed their first production runs under GMP conditions (for peptides and oligonucleotides).[29]

2. EXPERIMENTAL SECTION

2.1. Oligonucleotide Synthesis and Crude Preparation. The siRNA sense strand was synthesized using standard phosphoramidite chemistry on the solid phase, with the sugar conjugation performed during the synthesis process. Following this, cleavage and deprotection were carried out in 25% aqueous ammonia and rinsed with ethanol. This solution was then diafiltered with water using a 2 kDa regenerated cellulosic membrane and made available for purification. The solid-phase synthesis was performed using a ÄKTA Oligopilot plus 100.

2.2. Oligonucleotide Quantification. The synthetic crude material was analyzed using a spectrophotometer at an OD of 260 nm. The molar concentration was calculated with the Lambert−Beer law using the molar absorption coefficient of the target molecule. Concentration was converted to (g/L) using the molar mass (main impurities had a similar absorption coefficient and molar mass). The total oligonucleotide concentration (mg/mL) in the chromatographic fractions was measured using a Nanodrop Lite (Thermo Scientific) at an OD of 260 nm. A calibration curve was made to calibrate the Nanodrop readings based on the known feed concentration of the crude starting material. The target compound concentration in the feed and product pools was then calculated as total concentration (mg/mL) × HPLC-UV (area%). The mass (mg) recovered was calculated as concentration (mg/mL) × volume (mL). Product-specific yield% and productivities (g/L resin/h) were then calculated using these HPLC-UV (area%)-corrected concentrations.

2.3. HPLC Analytics. Purity measurements were carried out using analytical HPLC chromatography (HPLC-UV (area %)). This was done using an Agilent 1290 Series at 65 °C. An Acquity UPLC Oligonucleotide BEH C18, 150 × 2.1 mm (1.7 μm particle size, 300 Å pore size) column (Waters, 186003687) was used with a flow rate of 0.25 mL/min. Detection was carried out at 260 nm. Mobile phase A was composed of 60 mM 1,1,1,3,3,3-hexafluoro-2-propanol (Acros, 147541000)/6 mM hexylamine (Acros, 204720010). Mobile phase B was composed of 50 vol % acetonitrile (Merck, 100030)/50 vol % MeOH (Sigma, 34860). Figure S1 details the gradient parameters used in the method. Samples from the batch and MCSGP fractions were prepared for HPLC analysis by diluting 4-fold with 100 vol % water.

2.4. Single-Column Batch Chromatography. As a performance benchmark, a pilot-scale batch purification process was used for comparison to MCSGP. The production batch process was carried out on an AKTA pure 150 M (Cytiva) recorded at Abs 290 nm (Figure 3A (black line). The pilot-scale batch method was then downscaled 78.6× to the Contichrom CUBE 30 and recorded at Abs 280 nm (Figure 3A, blue line). Table 1 summarizes the materials used for chromatographic methods, including the column dimensions, resin type, and mobile phase composition. Table 2 gives an overview of the crude purity, concentration, and loading

Table 1. Materials

Materials	Pilot Scale Production	Downscale—Batch and MCSGP
Chromatographic System	AKTA pure 150 M (Cytiva)	Contichrom CUBE 30 + dynamic mixer (Knauer PN: E0029)
Columns	TOSOH TSKgel SuperQ-5PW (CV = 393 mL, 20 × 5 cm i.d., 20 μm)	TOSOH TSKgel SuperQ-5PW (CV = 5.03 mL, 10 × 0.8 cm i.d., 20 μm)
Mobile phase A	20 mM sodium phosphate (Merck, 1.06585, 1.06345) + 10 vol % acetonitrile (Merck, 1.00030) in water, pH 8.0	
Mobile phase B	20 mM sodium phosphate + 0.9 M NaCl (Merck, 1.06404) + 10 vol % acetonitrile in water, pH 8.0	20 mM sodium phosphate + 1.1 M NaCl (Merck, 1.06404) + 10 vol % acetonitrile in water, pH 8.0
In-line dilution buffer	N/A	Mobile phase A diluted 1:1 with water (like feed preparation)
Feed preparation	The synthesis crude diluted 1:1 with mobile phase A	

https://doi.org/10.1021/acs.oprd.4c00513
Org. Process Res. Dev. 2025, 29, 1400−1410

Table 2. Feed Composition and Loading

Parameters	Units	Batch (pilot scale)	Batch (downscaled)	MCSGP (13 cycles)
Crude purity (HPLC-UV)	(area%)	91.4		91.4
Crude purity (HPLC-UV/MS)	(area%)	85		85
Crude concentration (before 1:1 dilution)	(g/L)	30.9		30.9
	(OD 260 nm/mL)	929		929
Load (1 run vs 1 MCSGP cycle) (Crude)	(g/L resin)	23		15.80
	(OD 260 nm/mL resin)	694		477
Total load (crude)	(g)	9.039	0.115	2.18
	(OD 260 nm)	272,742	3470	65,534
Total load (product)	(g)	7.68	0.098	1.85

parameters. Table 3 shows the process parameters for all methods. Downscaling from a 20 cm × 5 cm i.d. column to a 10 cm × 0.8 cm i.d. column was achieved by keeping the total load (g/L resin), wash volumes (CV), and linear flow rates (cm/h) constant between columns' sizes. In addition, the gradient length (CV) and %B start and %B end values were the same.

Due to the reduced bed height, the residence time during elution for the downscaled batch run was half compared to that of the pilot-scale batch run. One other difference was that the NaCl concentration of mobile phase B was 0.9 M in the pilot experiment compared to 1.1 M in the downscale runs, resulting in a broader peak in the pilot-scale run (see Figure 3A overlay). However, it was expected that any resulting lower resolution and narrower center-cut would be compensated for by the internal recycling capabilities that safeguard a high yield in MCSGP. Finally, a second batch run was carried out with an elevated elution flow rate of 200 cm/h to help maximize MCSGP process performance. The resulting chromatogram (Abs@280 nm) was then overlaid with the product purity determined by HPLC-UV (area%) after fraction analysis (Figure 3B). This chromatogram and purity profile were then used for the MCSGP design procedure described in Section 2.5.

2.5. MCSGP Chromatography. MCSGP method creation was achieved using the MCSGP Wizard version 8.1 (Figure 3C), supplied with the Contichrom CUBE (YMC Chroma-Con). In the top half, the batch "design" method (Figure 3B) was uploaded to the MCSGP wizard as a template for MCSGP design, along with the purity data from the fractionated product peak. The phase boundaries were set by drag-and-drop using the product purity data to optimize the purity of the product collection window. Gradient parameters, feed

Table 3. Process Parameters

				MCSGP		
Step	Buffer	Units	Batch runs	Startup	Main	Shutdown
Run/cycle time		(min)	189 (pilot scale) or 90 (downscale)	20	76 (1 cycle) or 984 (13 cycles)	38
Preload wash	Mobile phase A	(CV)	1	1	1	1
		(cm/h)	150	160	160	
Load	Crude	(g/L resin)	23	11.51	15.8	
		(CV)	1.49	1.025	1.025	
		(cm/h)	75	125	125	
Postload wash	Mobile phase A Mobile phase B	(%B) Start	0	0	0	0
		(%B) End	12	0	0	0
		(CV)	1	1	1	1
		(cm/h)	75	125	125	125
Gradient	Mobile phase A Mobile phase B	(%B) Start	12		12	12
		(%B) End	57		45	45
		(CV)	15		12.8	12.8
		(cm/h)	150		200	200
In-line dilution (weak recycle)	In-line dilution buffer	(CV)			1	
		(cm/h)			245	
In-line dilution (strong recycle)	In-line dilution buffer	(CV)			4	
		(cm/h)			476	
Cleaning 1	Mobile phase B	(CV)	0.5		0.5	0.5
		(cm/h)	150		160	160
Cleaning 2	Mobile phase A	(CV)	0.5		0.5	0.5
		(cm/h)	150		160	160
Cleaning 3	Mobile phase B	(CV)	0.5		0.5	0.5
		(cm/h)	150		160	160
Re-equilibration 1	Mobile phase A	(CV)	1		1	1
		(cm/h)	150		160	160
Re-equilibration 2	In-line dilution buffer	(CV)			0.5	0.5
		(cm/h)			160	160
Wash after strong recycle	Mobile phase A	(CV)		1	1	1
		(cm/h)		250	250	250

https://doi.org/10.1021/acs.oprd.4c00513
Org. Process Res. Dev. 2025, 29, 1400−1410

parameters, and in-line dilution factors were automatically calculated by the wizard using imported batch chromatogram parameters and the relative position of the phase boundaries. In the bottom half, washing, cleaning, and regeneration steps were imported to a template table, and flow rates for elution and inline dilution were set. The MCSGP wizard automatically generated a set of MCSGP methods that were ready-to-run on the Contichrom CUBE hardware (see Tables S2 and S3). MCSGP was then carried out using the Contichrom CUBE 30 with UV absorbance recorded by the UV detectors located at each column outlet (UV1@280 nm and UV2@280 nm, respectively). Table 1summarizes the material required for the MCSGP methods, including the column dimensions, resin type, and mobile phase composition. Table 2 gives an overview of the crude purity, concentration, and loading parameters. Table 3 shows the MCSGP parameters including the mobile phase composition, flow rate, and volume for each chromatographic step, including startup, main, and shutdown methods. Tables S2 and S3 detail specific MCSGP method parameters used to run "startup" and "main" methods on the Contichrom CUBE hardware.

3. RESULTS AND DISCUSSION

3.1. Batch and MCSGP Process Performance Comparisons. For pilot-scale purification of the siRNA sense strand, an AIEX-based preparative batch polishing method was run using a 20 × 5 cm inner diameter column (see Section 2 for details). The chromatographic profile is shown in Figure 3A (black line), and the process performance is summarized in Table 4. The post-synthesis crude was applied at 23 g/L resin with an initial 85.0 area% product purity. After polishing, purity increased to 95 area%, with critical impurity *N*-1 at 1.0 area%. To achieve this target purity, a "center cut" of the batch elution profile was collected, resulting in a product-specific step yield of 80%. This yield is typical for preparative batch chromatography and is the target benchmark we aimed to exceed by implementing MCSGP. The productivity of the batch run, normalized to resin volume, was 5.28 g/L/h.

Table 4. Batch vs MCSGP Process Performance Analysis

Parameters	Units	Feed	Batch (pilot scale)	Batch (downscale)	MCSGP
Product purity (HPLC-UV/MS)	(area%)	85.0	95.0	95.79[a]	95.44
Impurity (*N*-1) (HPLC-UV/MS)	(area%)	0.93	1.0	0.82[a]	0.74
Product yield	(%)	-	80.0	77.32	93.02
Productivity	(g/L resin/h)	-	5.28	10.09	9.88
Buffer cons.	(L/g product)	-	1.25	1.29	1.86
Product concentration	(g/L)	-	2.5	2.91	2.80
Total concentration	(g/L)	-	2.63	3.03	2.93

[a]Downscale batch was evaluated by HPLC-UV only, not MS.

The pilot-scale batch method was first downscaled and run on the Contichrom CUBE 30 using a column with dimensions of 0.8 cm inner diameter × 10 cm bed height (Figure 3A, blue line). There was a difference in peak profile between the pilot and downscaled batch methods primarily due to a difference in mobile phase B, NaCl concentration (0.9 M vs 1.1 M). Despite the different parameters resulting in a narrower peak, process performance was similar after scaling down. Next, a second batch run was carried out with an elevated elution flow rate of 200 cm/h and was overlaid with fraction purity (%area UV-HPLC) (Figure 3B). This batch run was uploaded to the MCSGP wizard software and served as a starting point for MCSGP design, as described in section 3.5.

After phase boundaries were set, the load per switch and the in-line dilution settings were automatically calculated by the MCSGP wizard. The washing and regeneration protocols were then defined as shown in Table 3. The scope of this study did not include evaluation of UV-based dynamic process control (AutoPeak) due to the limited availability of crude material. In GMP-scale production AutoPeak is essential to provide process robustness by compensating for retention time differences caused by variability in column packing, buffer preparation or feed consistency.[27] In the simplest AutoPeak implementation, the "weak" recycling step (P2) can be triggered by a UV threshold at the front of the main peak. This can then be combined with UV-based triggers for the start and stop of product collection. However, the MCSGP method used in this study was fully time-based.

A 13-cycle MCSGP run was carried out, and a chronological view of the resulting chromatogram is shown in Figure 4A. The cycle number was limited only by the feed availability for the study. In total, a series of 27 product elutions are shown (including startup and shutdown steps), leading to a total run time of 17 h. The UV trace (280 nm) for column 1 and column 2 are shown in red and blue respectively, and elution peaks alternate between column positions on a switch-to-switch basis. In Figure 4B all 13 cycles are overlaid with one another. The product collection window is highlighted in red and recycling windows in blue and green. The yellow and green lines show the conductivity traces for column 1 and column 2 respectively. The conductivity traces show highly reproducible elution gradients indicating stable system performance for the whole duration of the run.

A more detailed analysis of the UV traces is shown in Figure 4C,D which shows the total area under the curve (AUC) (calculated as mAU×mL) as well as the AUC for each phase of elution and recycling. The biggest change in the UV profile is between cycle 1 and cycle 2 as the system quickly adjusts to cyclical operation. The AUC for the product collection phase reached a constant value by cycle 5 (Figure 4C), indicating that the mass of product entering vs leaving the system was in steady state.

Figure 4C shows that some accumulation was detectable in the region undergoing recycling of strongly adsorbing impurities. Impurity accumulation in the recycling regions is typical in MCSGP and only becomes problematic if impurities accumulate to the extent that they extend into the product pool, causing the specification to be missed. Although it was not evident in this process, too much accumulation can be counteracted by decreasing the size of the MCSGP recycling windows during the process design and sending more impurities to the waste.

When monitored in real-time, the AUC data as shown in Figure 4D provides important information on the current state of a continuous process. During a manufacturing run, deviations from predefined AUC limits can be used to trigger corrective actions. A run can be adjusted, paused, or aborted

https://doi.org/10.1021/acs.oprd.4c00513
Org. Process Res. Dev. 2025, 29, 1400−1410

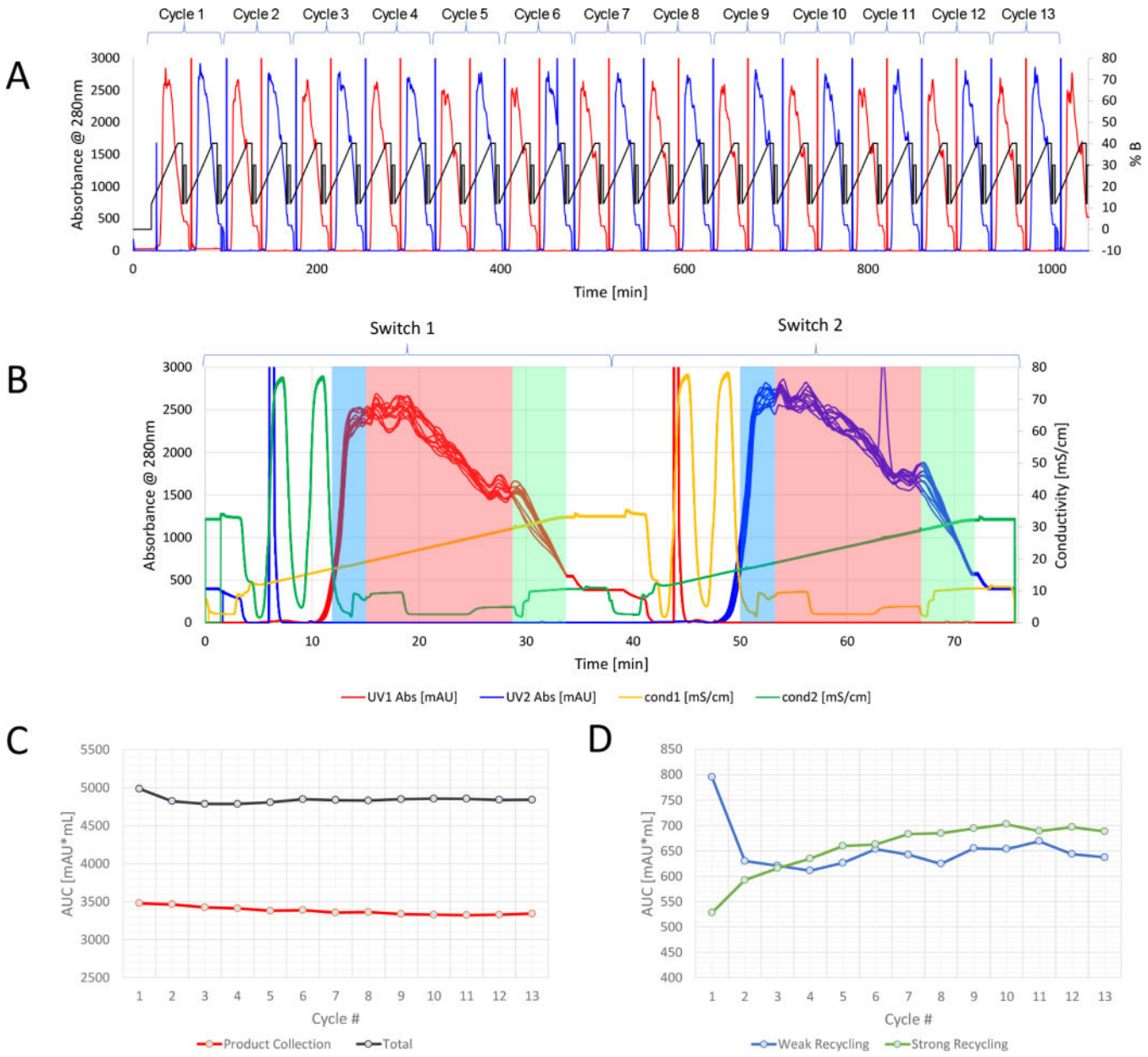


Figure 4. (A, B) MCSGP chromatographic profiles showing absorbance UV@280 nm and %*B* (A) or UV@280 nm and conductivity (mS/cm) (B) plotted vs time (min). (A) Plot showing 13 consecutive MCSGP cycles including startup and shutdown (total of 27 product elutions). (B) Plot showing an overlay of 13 consecutive MCSGP cycles. (C, D) Plots showing the total area under the curve (AUC) in (mAU×mL) vs cycle number. Each dot is the sum of 2 elutions from 2 switches. (C) Black line: total AUC. Red line: AUC for the product collection window. (D) Blue line: AUC for the weak recycling phase. Green line: AUC for the strong recycling phase.

early, avoiding wasted time and material before corrective action is taken.

Figure 5A shows the cycle-by-cycle analysis of product yield (%) and recovery (mg) for the 13 cycles and 1 shutdown. Per cycle yield was steady at ≈94−95%, with the shutdown having a much lower yield (62%) because it was calculated using the load applied in the startup step, which is higher than the load per switch. The overall yield, including shutdown, was 93%. The lower yield of the shutdown step is a normal feature of MCSGP, but its negative impact on the process performance can be minimized by increasing the overall MCSGP cycle number. The recovery of product (mg) per cycle was also stable between 127 and 130 mg/cycle and 62 mg in the shutdown, where just one switch was collected.

Figure 5B shows the cycle-by-cycle product purity and impurity (*N*-1) levels (area% HPLC-UV). As with the AUC analysis, a steady state in product purity was achieved after 5 cycles. Initial purity is 95.6 area% and this declines and stabilizes at ≈95.1 area%. Critical impurity N-1 also starts off at 0.5 area% but increases and stabilizes at ≈0.7 area%, well within the desired specification (<1.0%). The stability of the process between cycles 7 and 13 shows the potential feasibility of running MCSGP with very consistent results for an even larger number of cycles.

Figure 5C shows an analytical HPLC overlay (normalized) comparing the original feed with a single purified MCSGP product pool (13 cycles + shutdown). Most impurity components were significantly reduced, and the final

https://doi.org/10.1021/acs.oprd.4c00513
Org. Process Res. Dev. 2025, 29, 1400−1410

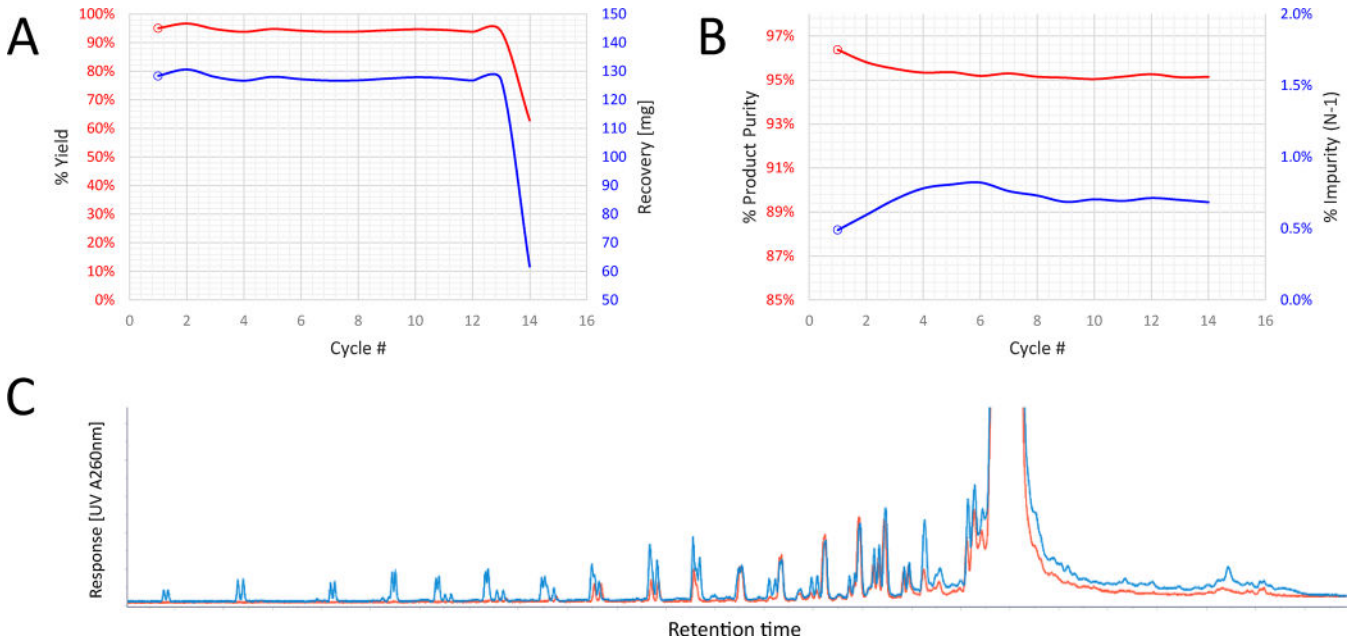


Figure 5. MCSGP product quality evaluation. (A) Plot showing cycle to cycle MCSGP target product yield (%) and mass recovered (mg). (B) Plot showing cycle to cycle product purity HPLC-UV (area%) vs impurity N-1 content HPLC-UV (area%). (C) Analytical HPLC chromatograms (UV Abs at 260 nm, normalized and then zoomed in to show impurities). Blue line is the crude feed material (85 area% purity). Orange line is the 13 cycle MCSGP product pool (95.4 area% purity).

MCSGP product pool exceeded the target purity specification of 95.0 area%.

Table 4 shows MCSGP process performance (including startup and shutdown) compared to the original pilot production method and the downscaled batch method. To make a meaningful comparison between pilot and downscaled processes, productivity is reported in units of (g/L resin/h) and buffer consumption in units (L/g) of the product produced.

When comparing pilot and downscaled batch methods, both had very similar process performances despite the difference in column dimensions and system scale. Product pool purity, yield, and buffer consumption were all comparable. The main difference is that productivity was higher in the downscaled method (10.09 vs 5.28 g/L resin/h) in proportion to the shorter bed height. In practice, this means a process using half the bed height, but with twice the productivity, results in similar overall throughput (throughput = productivity × column volume). This result also shows that the longer 20 cm columns did not greatly improve yield compared to 10 cm columns in this process.

For MCSGP, the total yield was 93%, a significant improvement compared to batch chromatography and in line with many previous MCSGP studies. Productivity (g/L resin/h) was comparable to the downscale batch method with a 10 cm column bed height. However, the overall throughput of an MCSGP system would be 87% higher than a batch system given that there are 2 times the number of columns (assuming identical column diameters). Another way to understand this is the fact that 1 elution in MCSGP is completed in 38 min, whereas 1 elution in batch chromatography from the same 10 cm column takes 90 min. There are three contributing factors to this; first, the yield is higher in MCSGP; second, the flow rates used in MCSGP were higher (200 vs 150 cm/h for elution). There is generally less risk of using higher flow rates in MCSGP because recycling functionality ensures a high yield even if some resolution is lost; third, the "regeneration and cleaning" steps and the "feeding" steps were done in parallel to elution on the second column. This parallel scheduling is a general advantage of MCSGP compared with single column chromatography. Overall, a batch system with larger columns and pumps would be required to match the output of the MCSGP process.

The product concentration in MCSGP was also 12% higher than that in batch chromatography. This is an advantage because it means subsequent desalting and freeze-drying steps are completed more quickly.

Optimization of MCSGP buffer consumption was not within the scope of this study due to a lack of crude starting material. While MCSGP had a higher buffer consumption compared to batch chromatography (1.86 L/g vs 1.25 L/g), it is likely that this can be decreased with specific method optimizations. First, the higher buffer consumption is attributable to in-line dilution, which is essential for MCSGP recycling steps. However, the in-line dilution factor was set conservatively high to ensure rebinding of the recycled product to the column and could be decreased. Second, the "wash after strong recycle" step in MCSGP is optional and may not be required. Third, further load optimization was not attempted for MCSGP in this study. An increase in load may benefit overall MCSGP process performance, including buffer consumption.

Finally, it is notable that the AIEX method we describe (in both batch and MCSGP modes) achieves a product purity of >95%. In our experience, under preparative conditions, NaOH-based AIEX methods typically give a purity of 88−92%. The high purity obtained could be explained by the improved selectivity from the use of a phosphate-buffered mobile phase with 10% acetonitrile (potentially reducing unwanted hydrophobic interactions) but could also be partially due to the use of a crude feed material that had a high starting purity of 85%.

3.2. MCSGP Scale-Up Scenarios. MCSGP processes developed on the Contichrom CUBE are directly scalable to

https://doi.org/10.1021/acs.oprd.4c00513
Org. Process Res. Dev. 2025, 29, 1400−1410

the Contichrom TWIN (YMC America), a large-scale production system capable of operating under GMP. Four MCSGP scale-up scenarios are compared in Table 5.

Table 5. MCSGP Scale-Up Options

Parameters	Units	Twin 300	Twin 500	Twin 1000	Twin 2000
Input material (85 area% purity)	(kg/day)	1.80	4.20	9.50	16.50
Output material (95.4 area % purity)	(kg/day)	1.42	3.32	7.51	13.05
Column volume	(L)	2× 3.0	2× 7.0	2× 15.8	2× 27.5
Column i.d.	(cm)	20	30	45	60
Column bed height	(cm)	10	10	10	10
Max pump flow rate (inc. flow rate factor of 1.3)	(L/h)	196	441	1034	1838
Buffer consumption	(L/day)	2647	6177	13970	24265

Depending on the chosen system, MCSGP can produce between 1.5 and 13 kg of siRNA sense strand product/day per system. The largest system configuration, assuming 24 h of continuous operation, enables multiton-scale production per year.

In terms of resin requirements, for equivalent output, a production-scale single-column batch system requires a total of 86% more resin than MCSGP. However, batch chromatography is not typically run continuously, and thus the resin volumes for equivalent output may need to be further increased to compensate for any system downtime. As a result of the overall larger resin requirement in batch chromatography, and the fact that the resin is divided between 2 smaller columns in MCSGP, means that batch chromatography typically requires much larger column dimensions compared to MCSGP. This is another advantage in favor of MCSGP as small columns are generally less difficult to pack than large ones.

4. CONCLUSIONS

This study shows that MCSGP can be successfully implemented for continuous purification of a synthetic siRNA sense strand using AIEX. A 13-cycle MCSGP run (27 product elutions) was carried out to characterize MCSGP process performance in terms of product quality, yield, productivity, and buffer consumption. Compared to a pilot-scale batch method, MCSGP achieved 13% higher yield and an 87% increase in throughput, while achieving nearly identical 95.4 area% product purity. Buffer consumption was higher in MCSGP, but with some optimization, we would expect this to significantly decrease. In terms of yield, these results are in line with previous studies for the purification of oligonucleotides using MCSGP where yields of >90% were consistently achievable.[24,30]

Given the very high costs involved with oligonucleotide synthesis, the benefit of improving the purification yield by 13% is worth highlighting. For equivalent product output as batch chromatography, MCSGP has 14% lower Cost of Goods (CoG). MCSGP requires fewer raw materials and consumables, fewer custom oligonucleotide intermediates, smaller oligonucleotide synthesis batches, and smaller volumes of chromatography resin. Besides the obvious cost savings, the reduction in waste production and energy usage throughout the process also benefits the environment and can help manufacturers achieve key performance indicators for sustainability.

To understand the full potential of MCSGP to improve process economics in OND manufacturing, one must also discuss the secondary benefits that come through automation. In addition to the directly measureable process improvements reported above, the operational benefits of MCSGP in GMP manufacturing provide additional positive impacts on process economics including: reduced GMP analytical burden due to far fewer "in-process control" samples generated, elimination of rechromatography, improved process robustness, reduced operator interaction with the process, and requirement of fewer highly trained operators.

In conclusion, MCSGP will appeal to both clinical trial and commercial-scale OND manufacturers looking for viable ways to reduce the cost burden of oligonucleotide production. Despite additional time and expertise required to develop the process, the reproducible improvement to purification yields along with many operational advantages makes MCSGP an important advancement for OND manufacturing.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.4c00513.

Table S1: gradient parameters used in the analytical HPLC method described in the Methods section; Tables S2 and S3: specific MCSGP method parameters used to run "Startup" and "Main" methods on the Contichrom CUBE hardware as described in the Methods section (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Thomas Müller-Späth − *ChromaCon AG, Zurich CH-8005, Switzerland*; Email: thomas.mueller-spaeth@chromacon.com

Authors

Richard Weldon − *ChromaCon AG, Zurich CH-8005, Switzerland*; orcid.org/0000-0002-0957-7354

Tobias Vandermeersch − *Johnson and Johnson, Beerse 2340, Belgium*; orcid.org/0000-0001-5143-5387

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.oprd.4c00513

Author Contributions

R.W.: conception or design of the work, data collection, data analysis and interpretation, drafting the article. T.V.: data analysis and interpretation, critical revision of the article. T.M.S.: data analysis and interpretation, critical revision of the article.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Funding and feed material for this study were provided by Johnson & Johnson, for which the authors are grateful.

■ REFERENCES

(1) Xiao, B.; Wang, S.; Pan, Y.; Zhi, W.; Gu, C.; Guo, T.; Zhai, J.; Li, C.; Chen, Y. Q.; Wang, R. Development, opportunities, and

challenges of siRNA nucleic acid drugs. *Mol. Ther. Nucleic Acids* **2025**, *36* (1), 102437.
(2) Xiong, H.; Veedu, R. N.; Diermeier, S. D. Recent Advances in Oligonucleotide Therapeutics in Oncology. *Int. J. Mol. Sci.* **2021**, *22* (7), 3295.
(3) Egli, M.; Manoharan, M. Chemistry, structure and function of approved oligonucleotide therapeutics. *Nucleic Acids Res.* **2023**, *51* (6), 2529−2573.
(4) Wan, W. B.; Seth, P. P. The Medicinal Chemistry of Therapeutic Oligonucleotides. *J. Med. Chem.* **2016**, *59* (21), 9645−9667.
(5) Bege, M.; Borbas, A. The Medicinal Chemistry of Artificial Nucleic Acids and Therapeutic Oligonucleotides. *Pharmaceuticals* **2022**, *15* (8), 909.
(6) Catani, M.; De Luca, C.; Medeiros Garcia Alcantara, J.; Manfredini, N.; Perrone, D.; Marchesi, E.; Weldon, R.; Muller-Spath, T.; Cavazzini, A.; Morbidelli, M.; et al. Oligonucleotides: Current Trends and Innovative Applications in the Synthesis, Characterization, and Purification. *Biotechnol. J.* **2020**, *15* (8), No. e1900226.
(7) Andrews, B. I.; Antia, F. D.; Brueggemeier, S. B.; Diorazio, L. J.; Koenig, S. G.; Kopach, M. E.; Lee, H.; Olbrich, M.; Watson, A. L. Sustainability Challenges and Opportunities in Oligonucleotide Manufacturing. *J. Org. Chem.* **2021**, *86* (1), 49−61.
(8) Lonnberg, H. Solid-phase synthesis of oligonucleotide conjugates useful for delivery and targeting of potential nucleic acid therapeutics. *Bioconjugate Chem.* **2009**, *20* (6), 1065−1094.
(9) Sanghvi, Y. CHAPTER 19. Large-Scale Automated Synthesis Of Therapeutic Oligonucleotides: A Status Update. Drug Discovery Series; Advances in Nucleic Acid Therapeutics, 2019, pp. 453−473, .
(10) Abe, A.; Časar, Z. Overview and Recent Advances in the Purification and Isolation of Therapeutic Oligonucleotides. *Org. Process Res. Dev.* **2025**, *29* (1), 15−33.
(11) Capaldi, D.; Teasdale, A.; Henry, S.; Akhtar, N.; den Besten, C.; Gao-Sheridan, S.; Kretschmer, M.; Sharpe, N.; Andrews, B.; Burm, B.; et al. Impurities in Oligonucleotide Drug Substances and Drug Products. *Nucleic Acid Ther.* **2017**, *27* (6), 309−322.
(12) Aumann, L.; Morbidelli, M. A semicontinuous 3-column countercurrent solvent gradient purification (MCSGP) process. *Biotechnol. Bioeng.* **2008**, *99* (3), 728−733.
(13) Aumann, L.; Stroehlein, G.; Morbidelli, M. Parametric study of a 6-column countercurrent solvent gradient purification (MCSGP) unit. *Biotechnol. Bioeng.* **2007**, *98* (5), 1029−1042.
(14) Strohlein, G.; Aumann, L.; Mazzotti, M.; Morbidelli, M. A continuous, counter-current multi-column chromatographic process incorporating modifier gradients for ternary separations. *J. Chromatogr. A* **2006**, *1126* (1−2), 338−346.
(15) Aumann, L.; Morbidelli, M. A continuous multicolumn countercurrent solvent gradient purification (MCSGP) process. *Biotechnol. Bioeng.* **2007**, *98* (5), 1043−1055.
(16) Muller-Spath, T.; Aumann, L.; Melter, L.; Strohlein, G.; Morbidelli, M. Chromatographic separation of three monoclonal antibody variants using multicolumn countercurrent solvent gradient purification (MCSGP). *Biotechnol. Bioeng.* **2008**, *100* (6), 1166−1177.
(17) Hooshyari Ardakani, M.; Nosengo, C.; Felletti, S.; Catani, M.; Cavazzini, A.; De Luca, C.; Rezadoost, H. Enhancing the purification of crocin-I from saffron through the combination of multicolumn countercurrent chromatography and green solvents. *Anal. Bioanal. Chem.* **2024**, *416* (10), 2553−2564.
(18) Jing, S. Y.; Shi, C.; Gao, D.; Wang, H. B.; Yao, S. J.; Lin, D. Q. Improved process design for monoclonal antibody charge variants separation with multicolumn counter-current solvent gradient purification. *J. Chromatogr. A* **2023**, *1707*, 464292.
(19) Kim, T. K.; Bham, A. A.; Fioretti, I.; Angelo, J.; Xu, X.; Ghose, S.; Morbidelli, M.; Sponchioni, M. Role of the gradient slope during the product internal recycling for the multicolumn countercurrent solvent gradient purification of PEGylated proteins. *J. Chromatogr. A* **2023**, *1692*, 463868.
(20) Kim, T. K.; Sechi, B.; Romero Conde, J. J.; Angelo, J.; Xu, X.; Ghose, S.; Morbidelli, M.; Sponchioni, M. Design and economic investigation of a Multicolumn Countercurrent Solvent Gradient Purification unit for the separation of an industrially relevant PEGylated protein. *J. Chromatogr. A* **2022**, *1681*, 463487.
(21) Luca, C.; Felletti, S.; Lievore, G.; Buratti, A.; Vogg, S.; Morbidelli, M.; Cavazzini, A.; Catani, M.; Macis, M.; Ricci, A.; et al. From batch to continuous chromatographic purification of a therapeutic peptide through multicolumn countercurrent solvent gradient purification. *J. Chromatogr. A* **2020**, *1625*, 461304.
(22) Muller-Spath, T.; Aumann, L.; Strohlein, G.; Kornmann, H.; Valax, P.; Delegrange, L.; Charbaut, E.; Baer, G.; Lamproye, A.; Johnck, M.; et al. Two step capture and purification of IgG2 using multicolumn countercurrent solvent gradient purification (MCSGP). *Biotechnol. Bioeng.* **2010**, *107* (6), 974−984.
(23) Muller-Spath, T.; Krattli, M.; Aumann, L.; Strohlein, G.; Morbidelli, M. Increasing the activity of monoclonal antibody therapeutics by continuous chromatography (MCSGP). *Biotechnol. Bioeng.* **2010**, *107* (4), 652−662.
(24) Weldon, R.; Lill, J.; Olbrich, M.; Schmidt, P.; Muller-Spath, T. Purification of a GalNAc-cluster-conjugated oligonucleotide by reversed-phase twin-column continuous chromatography. *J. Chromatogr. A* **2022**, *1663*, 462734.
(25) Steinebach, F.; Ulmer, N.; Decker, L.; Aumann, L.; Morbidelli, M. Experimental design of a twin-column countercurrent gradient purification process. *J. Chromatogr. A* **2017**, *1492*, 19−26.
(26) Vogg, S.; Ulmer, N.; Souquet, J.; Broly, H.; Morbidelli, M. Experimental Evaluation of the Impact of Intrinsic Process Parameters on the Performance of a Continuous Chromatographic Polishing Unit (MCSGP). *Biotechnol. J.* **2019**, *14* (7), No. e1800732.
(27) Fioretti, I.; Muller-Spath, T.; Aumann, L.; Sponchioni, M. UV-based dynamic control improves the robustness of multicolumn countercurrent solvent gradient purification of oligonucleotides. *Biotechnol. J.* **2024**, *19* (7), No. e2400170.
(28) Minuth, M. https://www.bachem.com/webinar/oligos-nce/going-large-scale-with-manufacturing-of-oligonucleotides-2/. 2024.
(29) Eisenhuth, R. https://www.bachem.com/webinar/generic-api/large-scale-tides-purification-with-mcsgp-under-gmp/.2023.
(30) Fioretti, I.; Muller-Spath, T.; Weldon, R.; Vogg, S.; Morbidelli, M.; Sponchioni, M. Continuous countercurrent chromatographic twin-column purification of oligonucleotides: The role of the displacement effect. *Biotechnol. Bioeng.* **2022**, *119* (7), 1861−1872.

https://doi.org/10.1021/acs.oprd.4c00513
Org. Process Res. Dev. 2025, 29, 1400−1410