

MCSGP Process Development – Part 2:

MCSGP Optimization

MCSGP with AutoPeak® control (Multi-column Counter-current Solvent Gradient Purification) is a twin-column continuous chromatography process that has multiple advantages for oligonucleotide and peptide manufacturing compared to single-column batch chromatography. MCSGP is typically developed using the Contichrom® CUBE system using the built-in “MCSGP Wizard” software feature directly from an input “batch-design” chromatography run. The approach for determining robust input batch conditions is described in a separate application note. This initial design delivers a first MCSGP operating point that is superior to batch chromatography with respect to performance metrics. However, the initial design leaves room for optimization.

This application note presents a systematic approach for optimization of MCSGP with regards to the performance parameters Yield, Productivity and Eluent Consumption, and gives recommendations on the priority of the parameters. The approach is confirmed using simulations.

Introduction

MCSGP is a counter-current chromatographic technique that enables the continuous separation by gradient elution of complex mixtures, such as peptides and oligonucleotides.

MCSGP uses two columns packed with the same stationary phase. The process is based on running linear gradient elutions on the two columns, collecting pure product fractions and internally recycling impure side fractions from one column to the other for re-purification. During recycling, the two columns are interconnected, and inline dilution is performed between them to ensure product re-adsorption on the second column. The MCSGP process is operated in a cyclic manner, with two feed injections, two product elutions (one from each column), and 2 pairs of interconnected phases per cycle (1 pair includes

recycling of side-fractions of the peak front and side fractions of the peak tail) (See Figure 1 in the application note “MCSGP Process Development – Part 1: Gradient Development”).

The MCSGP process reaches a cyclic steady state that is a function of its operating parameters such as load, gradient slope and flow rate, as well as side-fraction cutting borders. In cyclic steady state, the product quality and concentration do not change from cycle to cycle and the process performance is constant.

Chromatography processes, including single column batch chromatography and MCSGP are compared with respect to purity, defined through Critical Quality Attributes (CQAs), and several process performance parameters.

These include Yield, Productivity, Product Concentration and Eluent Consumption. Process optimization aims at maximizing these parameters (except for eluent consumption, which is to be minimized). A comprehensive summary of the process performance parameters is provided in Part 1 of this application note series.

This application note provides a systematic approach for experimental MCSGP optimization.

Flowchart-Guided MCSGP Optimization Procedure

The flowchart shown in Figure 1 is a step-by-step protocol for optimizing MCSGP and is discussed in detail in the following sections.

Before beginning

The flowchart-based MCSGP optimization procedure outlined here can be applied to any existing MCSGP operating point, but the overall process performance is usually better when applied to a MCSGP setpoint designed using a well optimized gradient, where purity requirements **were already met**. Such a gradient can

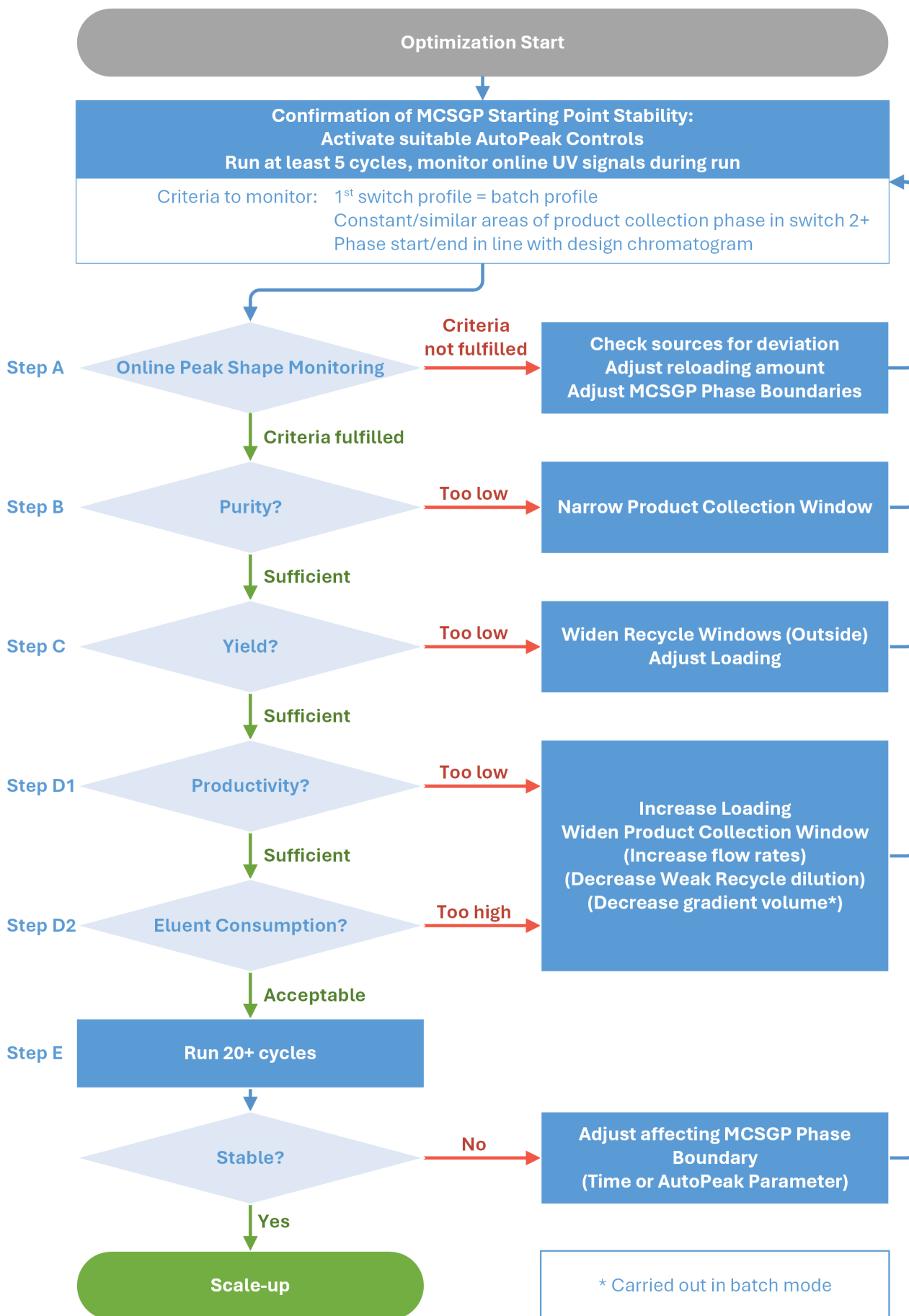


Figure 1 MCSGP experimental optimization procedure.

be developed by following guidelines outlined in Part 1 of this application note series. Note that while MCSGP can improve maximum purity compared to batch chromatography, this is not guaranteed.

Step A: Initial Evaluation of UV Profiles

Before starting MCSGP optimization, the performance of the initial MCSGP method should be evaluated by running the process to cyclic steady state and monitoring cycle-to-cycle UV peak shape.

Important Tip: UV-based process control **AutoPeak should be active** as it is essential for robustness against process and feed variations in manufacturing.

The stability criteria to monitor include:

- UV profile of the 1st switch: The chromatogram should be identical to the design gradient chromatogram. This is because the load and gradient are the same in both the design batch and the 1st switch of the MCSGP process. If the chromatograms are not identical, the feed composition and the eluents should be checked (conductivity trace).
- From the 2nd cycle the overall peak shapes may change compared to the 1st cycle, though not dramatically. In particular, the area under the chromatogram (AUC) during the product collection phase should be consistent, reaching a cyclic steady state by cycle 2 or 3 at the latest. If the AUC during product collection continues to change after cycle 3, the feed amount should be adjusted (reduced if increasing trend is observed, increased if decreasing trend is observed).
- The interconnected and batch phase start/end points should be in line with the design chromatogram. The phase boundaries for the internal recycling and product collection phases of the MCSGP process should be in a similar position as set in the MCSGP wizard during process design. If the chromatograms are not identical, the feed composition and the eluents should be checked (conductivity trace).

When a stable multi-cycle MCSGP run is achieved, we can progress with evaluation of product purity.

Step B: Purity

Before optimizing process performance, ensuring that the product from MCSGP meets purity requirements is imperative. Product from each MCSGP cycle should be collected and analyzed by offline analytics (e.g. HPLC) to verify that the CQAs are within specification. If purity is insufficient, the product collection window in the MCSGP wizard should be narrowed on the side of the

product peak according to the impurities observed in the offline analytics. In other words, if weakly adsorbing impurities are observed in the product pool, the left product collection border should be pushed back (see Figure 2). If strongly adsorbing impurities are observed in the product pool, the right product collection border should be moved forward. Movement of the borders can be done in the MCSGP wizard by adjusting the border times or by changing AutoPeak thresholds values. The advantage of changing the collection borders in the MCSGP wizard is that feed reloading is automatically adjusted to compensate for changes in product removal during product collection.

The new MCSGP operating conditions resulting from movement of the borders, should be confirmed by operating in cyclic steady state for at least 3 consecutive cycles.

Practical Hint: If MCSGP is in operation on the Contichrom CUBE system, a variety of method parameters can be scouted by inserting new MCSGP methods directly after the running one. If necessary, the running method can be cut short “on-the-fly” by reducing the cycle number in the “Online config” tab in ChromIQ®. The current MCSGP method then concludes once the current cycle is complete and the next method is executed. This eliminates the need for start-up & shutdown methods for every new condition. For a reliable transition to a new steady state, the parameters need to be incrementally changed. Larger changes may benefit from a new startup method.

Step C: Yield

If product purity is in specification, the first process performance parameter to check is yield. Due to internal recycling capabilities, MCSGP achieves significantly higher yields than single column

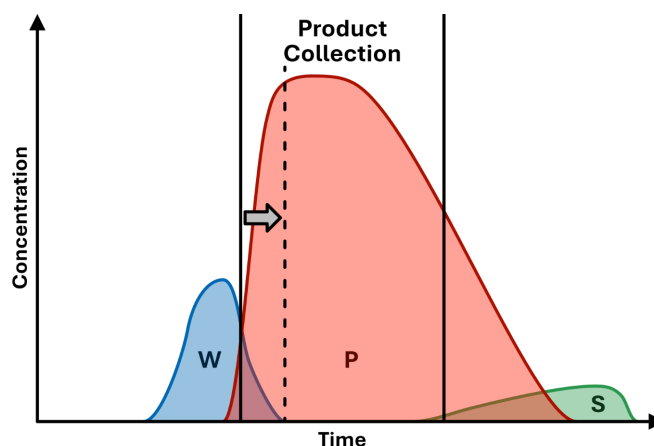


Figure 2 Shifting the left product collection border back in response to W impurities present in the product pool in MCSGP.

chromatography (often >90% yield at target purity). The yield obtained is dependent on the feed purity. Low feed purity leads to lower yield at target purity. Using the gradient development procedure described in Part 1 of this note series increases the likelihood of finding an MCSGP operating point with good yield.

In case a low yield is observed but the product purity is far higher than required, it is possible to enlarge the product collection window in the MCSGP wizard (reverse of what is shown in Figure 2). Essentially, purity can be traded in for yield (Figure 3). However, it is recommended to remain at “safe” distance from the specification limit as variations in the process and the feed material may impact product purity. For example, if product is obtained at 97.0% purity but low yield, and the purity specification is > 95.0%, one may consider broadening the product collection window to increase yield, accepting a decrease in purity to 96.0%. It would be discouraged to aim for an operating point closer to the specification limit as this would reduce robustness against process and feed variations, even if AutoPeak control is employed to balance variations.

As in the previous case, the new MCSGP operating conditions resulting from movement of the borders should be confirmed by operating in cyclic steady state for at least 3 consecutive cycles.

Steps D1 and D2: Productivity and Eluent Consumption

Once product purity and yield have been confirmed to be satisfactory, productivity and eluent consumption can be optimized. Productivity and eluent consumption both depend on the load. While productivity is directly proportional to load, eluent consumption is inversely proportional, so optimizing load automatically optimizes productivity and eluent consumption provided that a high yield can be maintained at target purity.

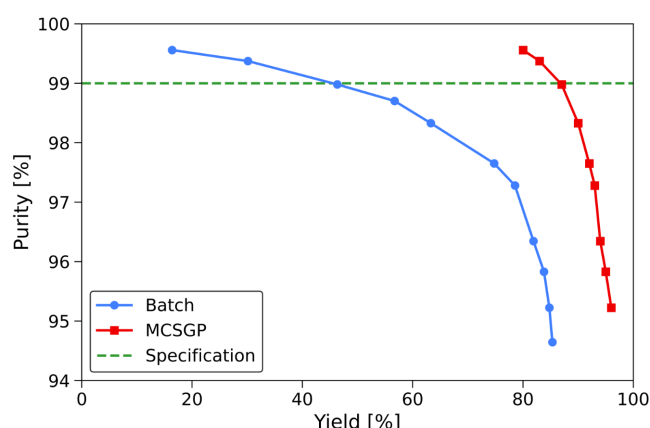


Figure 3 Purity-Yield Trade-off example for MCSGP and Batch chromatography.

Load Increase: The load amount can be simply changed in the MCSGP wizard, however the elevated load may require a widening of the product collection window to prevent accumulation of product inside the process. The direction of the widening of the product collection window should be made dependent on the peak shape. A peak shape that “leans” forward (Langmuirian peak shape) should prompt a widening of the product collection window to the front, while a peak shape that “leans” backward (Anti-Langmuirian peak shape) should prompt a widening of the product collection window to the back. As a guideline, the widening of the window (in percent) should be significantly smaller than the increase of loading (in percent), because most of the product stemming from the additional load will elute readily within the existing product elution window (Figure 4). For example, if load is increased by 40% and the Peak is “leaning” to the front (Langmuirian peak shape), the product collection window could be extended to the front from 5.0 min duration to 6.0 min, corresponding to a 20% increase.

Note that shifting the borders in the MCSGP wizard will make the product collection appear off in the wizard interface since a batch design chromatogram with lower load is still displayed in the interface.

The new MCSGP operating conditions resulting from load increase, should be confirmed by operating in cyclic steady state for at least 3 consecutive cycles.

Productivity may be increased further by method modifications that lead to a shorter cycle time:

- **Increase of flow rates in the parallel phase** of the MCSGP process (batch phase), that are not related to the actual separation process. This includes washing and cleaning steps. However, the properties of the feed/product material must be taken into account as the effectiveness of

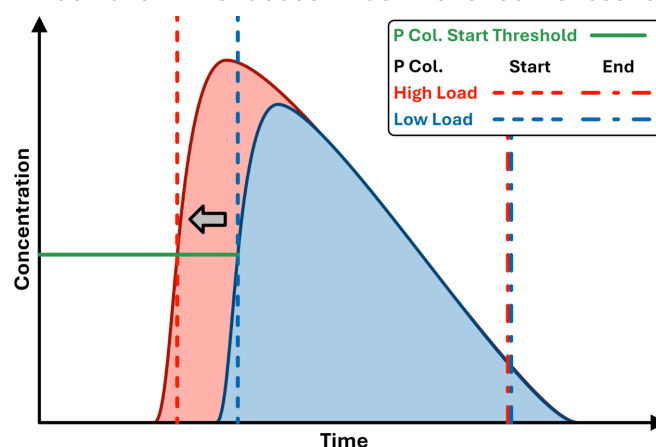


Figure 4 Widening of the product collection window in response to load increase for Langmuirian peak shapes. Note that the product collection end remains at the same position in the peak tail as the increased load does not impact the peak tail.

washing and cleaning may be a function of the exposure time (not only the volume of washing/cleaning solution passing through the column) and is not obvious from online signals. In contrast, equilibration steps can usually be abbreviated without concern as their effectiveness can be directly tracked by monitoring conductivity and/or pH. As a rule of thumb, the flow rate in the parallel (batch) steps in MCSGP can be doubled compared to the flow rate in the interconnected steps as the columns are operated independently and backpressure is proportional to bed height.

- An **increase of the linear gradient flow rate in interconnected mode** improves throughput but is not trivial as the separation would worsen, requiring a new design gradient and restart of the gradient development and MCSGP optimization procedures (see Part 1 of this application note series). The same consideration applies for a decrease of the gradient volume (running a steeper gradient), that would lead to a stronger overlap of product and impurities and a worsening of the separation.
- **Decreasing Weak Recycle Dilution** shortens the time of the interconnected phase that is used to recycle the overlapping part of weakly adsorbing impurities and product (W/P) from one column to the other. Before entering the second column, the stream is adjusted inline to ensure binding of W/P on the column. Depending on the final gradient concentration of the W/P fraction, the MCSGP wizard automatically calculates the inline dilution factor required to reduce the concentration to the gradient starting concentration. As the sum of the flow rates of W/P recycling and inline dilution must not exceed predefined flow rate limits, strong inline adjustment can lead to a long W/P recycling phase because more time is needed to elute the required W/P volume from the first column. In turn, decreasing the inline dilution flow rate will lead to W/P recycling being completed faster. When taking this measure, peak retention time and shape should be carefully monitored as the adsorptive strength of impurities and product is reduced on the downstream column. If peak shape or peak retention change, decreasing recycle dilution should not be considered further for process optimization.

Any of the new MCSGP operating conditions resulting from change of flow rates or gradient, should be confirmed by operating in cyclic steady state for at least 3 consecutive cycles.

Step E: Operating MCSGP for 20+ cycles

After MCSGP cyclic steady state has been confirmed with the new operating parameters identified during the optimization, an MCSGP run of 20 cycles or more should be performed to confirm long-term stability of the MCSGP process. Product of every cycle should be collected and the pool quality checked by offline analysis to confirm the constant product concentration and purity expected in cyclic steady state. In case accumulation during W/P side-fraction recycling is observed in the UV online signals, the W/P recycling start may be pushed back in the MCSGP wizard. Likewise, in case accumulation during P/S side-fraction recycling is observed, the P/S recycling start may be moved forward in the MCSGP wizard to remove W and S impurities, respectively.

Case Study

The optimization procedure was demonstrated for an anion-exchange (AEX) purification of a 20-mer oligonucleotide (5'-ATA CCG ATT AAG CGA AGT TT-3'). The system described in Part 1 of this application note series, further comprised YMC BioPro IEX SmartSep Q30 resin, and NaOH/NaCl-based eluents. Chromatography runs were carried out on a Contichrom CUBE 30 system.

Starting from an operating point designed using the MCSGP wizard, it was first confirmed by online peak shape monitoring and by offline analytics that the wizard design point was operating stably at cyclic steady state and that a purity of 97.3%, was achieved, exceeding the purity specification of > 95% by far. The following optimization was aimed at "trading in" purity for productivity, to increase the throughput of the process. According to the optimization procedure shown in Figure 1, the following modifications were made, which are summarized in Figure 5 and Table 1.

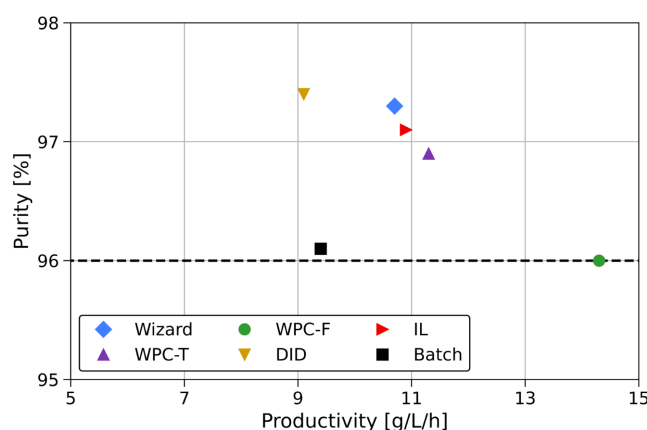


Figure 5 MCSGP Optimization result.

Table 1 Overview of process performance parameters for the different optimization measures.

Parameter		Wizard	WPC-T	WPC-F	DID	IL	Batch
Load	[g/L]	23	23	24	26	28	25
Reload	[g/L]	17.1	17.4	22.0	17.1	18.8	n/a
Purity	[%]	97.3	96.9	96.0	97.4	97.1	96.1
Yield	[%]	98.9	98.9	99.1	79.2	93.7	48.7
Productivity	[g/L/h]	10.7	11.3	14.3	9.1	10.9	9.4
Eluent Consumption	[L/g]	1.5	1.4	1.1	1.8	1.5	1.4
Product Concentration	[g/L]	4.0	3.8	4.2	3.8	4.1	4.3

The performance of the single column batch design batch run is shown as reference.

- **WPC-T:** Starting from the MCSGP Wizard operating point, the window of product collection (WPC) was widened towards the tail (T) of the peak by lowering the peak collection threshold from 20% to 10% in the AutoPeak settings. It was found that this change had little effect on the process performance.
- **WPC-F:** Instead of WPC-T, the window of product collection (WPC) was widened towards the front (F) of the peak (inverse of what is shown in Figure 2), by starting W/P based on time, instead of starting based on UV threshold (AutoPeak settings). This significantly raised productivity by more than 33% (to 14.3 g/L/h), while purity dropped to 96.0%, remaining well above the specification limit.
- **DID:** Next, a decrease of inline dilution (“DID”) was carried out. However, it was found that this measure decreased the yield from 99% to 79% (and thus productivity), which was not acceptable.
- **IL:** Lastly, a load increase (IL) was performed by increasing the reload in the MCSGP wizard from 17.1 g/L to 18.8 g/L (10% increase). With this change, productivity was only increased from 10.7 to 10.9 g/L/h at very similar purity levels of 97.3% and 97.1%, respectively.

In summary, the optimization measure WPC-F was most effective in improving productivity compared to the initial MCSGP wizard design, while remaining well above the specification limit. This can be attributed to a larger portion of the product being recovered (and reloaded) every cycle.

In this study the different measures were not combined but originated from the same “Wizard” starting point. While conceptually, the combination of optimization approaches (e.g. earlier product

collection start (WPF-F) and increased load (IL)) may allow further improvement, in this case study, the purity constraint was violated by such attempts.

MCSGP Scheduling Advantages

Apart from the optimization of MCSGP process performance represented by the performance parameters, MCSGP provides advantages to **process scheduling** that should be considered for scale-up and facility fit calculations and may in themselves be optimized (see Figure 6). These scheduling advantages include:

- **Overnight operation:** MCSGP with AutoPeak dynamic control can be operated at scale overnight without operator monitoring. Consequently, column cleaning and storage steps are minimized. Facility utilization is improved as the purification operates 24 hours a day instead of 12-16 hours.
- **Elimination of re-chromatography:** Due to its high yield, MCSGP eliminates re-chromatography of impure side fractions. Side fraction collection, handling, storage and analysis is avoided.
- **Reduced QC effort:** Due to its controlled steady state operation the number of In-Process-Control (IPC) analyses can be reduced, typically by a factor of 10 or more.
- **Downsizing of neighboring unit operations:** With its capability of internally recycling impure side-fractions, MCSGP generally enables steeper linear elution gradients than batch chromatography. With steeper gradients, product concentration increases. Higher product concentration generally makes downstream operations more efficient. In consequence, for example, freeze-drying and UF/DF can be downsized or completed faster.

These scheduling advantages significantly reduce campaign time and should be considered when setting optimization targets for the process performance parameters.

Importance of AutoPeak Dynamic Process Control

AutoPeak is a UV-based dynamic process control feature that constantly monitors the online UV signals inside the MCSGP process during operation, compares them against threshold values, and triggers start or end of recycling and product elution phases. Thus, AutoPeak automatically adjusts the duration the phases of MCSGP flexibly in response to system perturbations and the consequent peak shifting. AutoPeak can be easily included during MCSGP process design using the MCSGP wizard. With AutoPeak enabled, MCSGP can operate robustly with the process parameter settings identified in the process optimization procedure. AutoPeak is indispensable for operation of MCSGP at large scale to balance process and feed variations.

Further explanation and case studies for AutoPeak are provided in the open access publication [“UV-based dynamic control improves the robustness of multicolumn countercurrent solvent gradient](https://doi.org/10.1002/biot.202400170)

[purification of oligonucleotides” \(doi.org/10.1002/biot.202400170\)](https://doi.org/10.1002/biot.202400170).

Optimization Using Modeling

Mechanistic Modeling is an effective tool to identify global optimal operating points by numerical optimization of MCSGP processes. Moreover, it can be used to investigate the impact of AutoPeak control thresholds, process parameter variations and feed material composition on process performance, i.e. to carry out robustness simulations. Thereby, modeling saves valuable feed material and time. Using mechanistic modeling, MCSGP runs can be simulated rapidly and the global optimum of MCSGP operating parameters can be found. However, calibration of the mechanistic model does require some upfront effort: A set of single column runs must be carried out and analyzed before the model parameters can be determined. Furthermore, profound knowledge of mechanistic modeling and modeling software is required. YMC ChromaCon offers modeling services to support MCSGP process development and optimization.

Conclusion

This application note introduces a procedure for systematic optimization of MCSGP following the gradient development procedure presented in Part 1 of this application note series. The procedure includes the optimization of process performance with regards to Yield, Productivity and Eluent Consumption. Moreover, the application note shows the importance of including scheduling advantages and UV-based process control AutoPeak in the optimization and operation of MCSGP.

Key Recommendations for MCSGP Optimization

- Use the linear gradient development procedure to find a good MCSGP starting point for optimization as presented in Part 1 of this application note series.
- Always confirm cyclic steady state by monitoring online UV and offline analytics.
- Always enable AutoPeak.
- Have fast analytics available to accompany experimental MCSGP optimization.

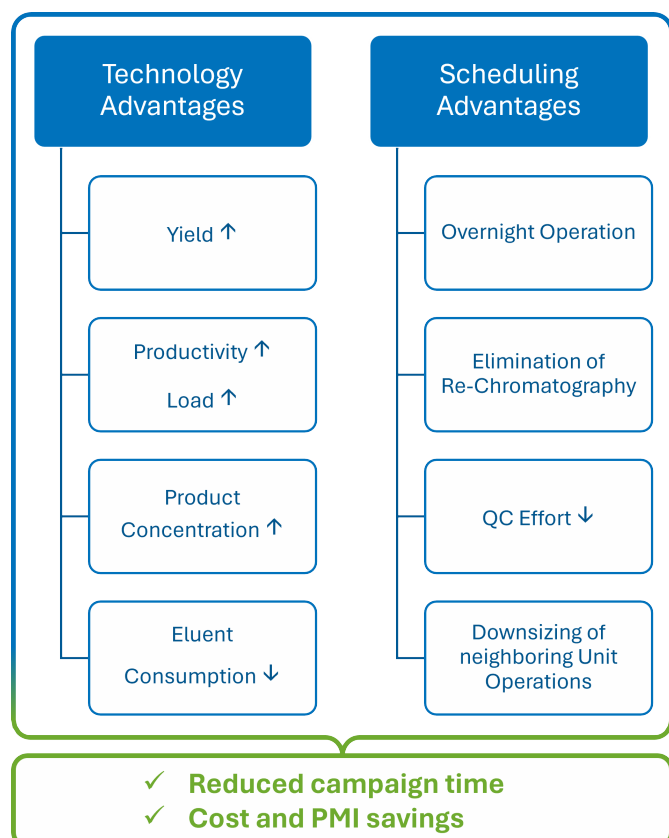


Figure 6 Technology and scheduling advantages of MCSGP.

Table 2 YMC ChromaCon Modeling Service Package

Product	Order #
Model-based MCSGP Feasibility Study Service	700034

YMC ChromaCon Modeling Services

YMC ChromaCon offers a standardized modeling service package for initial MCSGP development based on a predefined customer-supplied experimental data set as well as services for subsequent MCSGP optimization. Additionally, customized modeling services are available upon request.

Contichrom CUBE for Lab-Scale Development

The MCSGP process with AutoPeak control can be operated by all Contichrom CUBE systems. The Contichrom CUBE is a versatile preparative laboratory-scale chromatography system for single- and twin-column processes with 100 bar (1450 psi) pressure rating. ChromIQ, the operating software of Contichrom

Table 3 Contichrom CUBE 30/100 Ordering Information

Product	Order #
Contichrom CUBE 30	220060
Contichrom CUBE 100	220062

systems, contains a wizard for designing and operating the MCSGP process.

Contichrom TWIN HPLC Scale-Up Systems

With the Contichrom TWIN HPLC series from YMC America, MCSGP with AutoPeak control is available for manufacturing under GMP conditions. The twin-column scale-up systems have been co-developed by YMC America and ChromaCon AG to ensure easy process transfer and scale-up.

For inquiries regarding the Modeling Services and Contichrom systems, please visit www.chromacon.com or contact sales@chromacon.com.

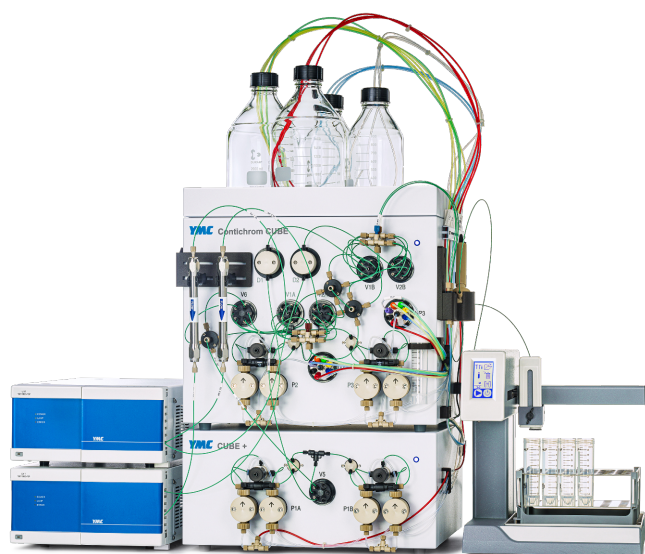


Figure 7 Contichrom CUBE benchtop chromatography system



Figure 8 Contichrom TWIN process scale chromatography system



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