# Dynamic Process Control (MControl®) for Countercurrent Polish Processes (MCSGP)

**The Contichrom® CUBE Combined and Contichrom HPLC systems use batch and continuous periodic countercurrent (PCC) processes for purification of target proteins, peptides, and small molecules. Multicolumn Countercurrent Solvent Gradient Purification (MCSGP) is a unique PCC polishing process that provides superior yield and purity at high productivity. In order to keep the MCSGP process at an optimum, a process control algorithm, MControl®, is employed to offset any variation in conditions that could affect process performance. MControl® thus keeps the process at an optimum.**

**This application note shows how MControl® can be used to adjust for variations in individual column packing, temperature and buffer composition. Dynamic process control is in line with FDA's initiative to improve product quality by employing process analytical technology (PAT).**

## Introduction

Although chromatography is a very powerful separation and purification technique, oftentimes target compounds partially overlap with impurities. Batch chromatography processes have the fundamental disadvantage of then having to choose between getting high purity or high yield. Furthermore, they cannot be run continuously, and thus their productivity and throughput is low. Continuous countercurrent chromatography consists of multiple cyclic sub-processes and alleviates all of these disadvantages. The high productivity of MCSGP processes leads to a reduction in the capital costs, as facilities with smaller footprint can provide the same output as much larger batch process facilities. Optimized continuous processes can also provide high yield and purity at the same time. However, they have to be kept at an optimal set point over the whole process time, which comprises multiple cycles. Thus, a dynamic process control is required.

MCSGP is a unique PCC process that can be used to increase yield and purity (see Fig. 1) and to create tailored product profiles for biopharmaceuticals. Such tailored product profiles are essential for Biosimilars. They need an identical isoform profile compared to an originator product. MCSGP as a continuous process, is controlled by MControl®. MControl® is a powerful algorithm keeping the process at an optimal set point despite variations that could affect the performance.

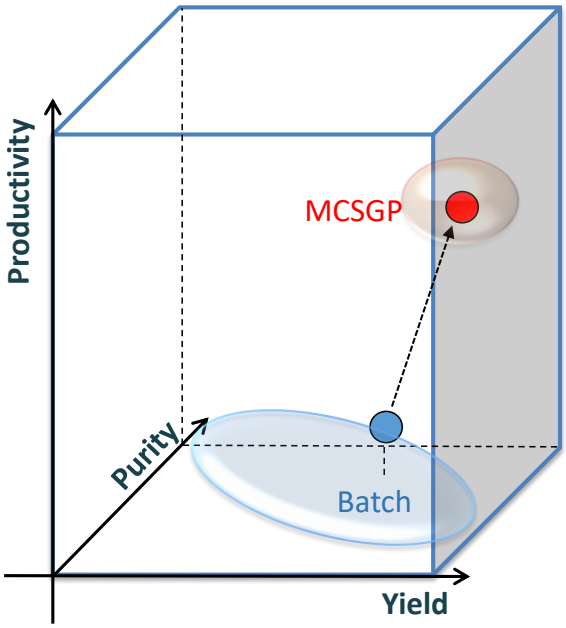


**Fig. 1.** MCSGP compared to batch chromatography. Batch operates in an essentially 2-dimensional continuum between purity and yield with little impact on productivity. MCSGP adds a new dimension with high productivity. Furthermore, both purity and yield remain high with MCSGP with little impact on productivity.

## Principle of MCSGP

MCSGP is a periodic countercurrent (PCC) process. The basic principle of MCSGP consists of internal recycling of product-containing side-fractions (Fig. 2). Impure product-containing eluate is diluted inline and automatically applied to another column. Fresh feed material is applied every cycle. The MCSGP process can employ 2 to 4 chromatography columns to create a continuous polish purification process. Currently only a 2-column configuration is available. With two columns, one pure product can be extracted. With a three- column configuration, two pure products can be extracted simultaneously, albeit with a loss in productivity. MCSGP can be operated with ion exchange (IEX), hydrophobic interaction (HIC) or mixed-mode resins, as well as in reversed phase (RP) mode. MCSGP in

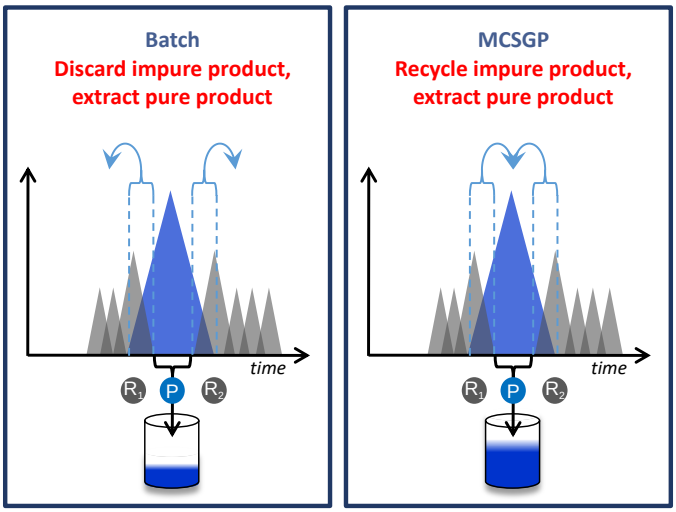


**Fig. 2.** The principles of batch and MCSGP chromatography. The figure shows schematic chromatograms of a product (P, blue) and impurities (grey). The impurities $R_1$ and $R_2$ elute overlapping with the product P. In batch chromatography, the impure product-containing side fractions (between dotted lines) are discarded in order to obtain the product in a high purity. Thus, part of the product is lost. In MCSGP, the impure product fractions are recycled internally and thus essentially all product can be extracted purely.

general provides yield improvements of up to 90%, is up to 10-fold more productive and consumes 70% less solvent (buffer) than a standard batch process. MCSGP can be applied to any purification challenge for small molecules, synthetic peptides and proteins.

## MCSGP using Contichrom® CUBE Combined and Contichrom HPLC systems

The Contichrom® CUBE Combined and Contichrom HPLC systems allow for continuous purification of target molecules. Both product lines are based on the established twin-column Contichrom® platform for chromatography systems and are designed to simplify system interaction and operational handling. The FPLC system is mainly used for protein purification applications, whereas the HPLC is optimized for the purification of smaller proteins, synthetic peptides and small molecule.

Dynamic control functionality for the MCSGP process (MControl®) is included in the ChromIQ® operating software and is a key feature of Contichrom® systems. MControl® compensates process variations and keeps the process at an optimal set point.

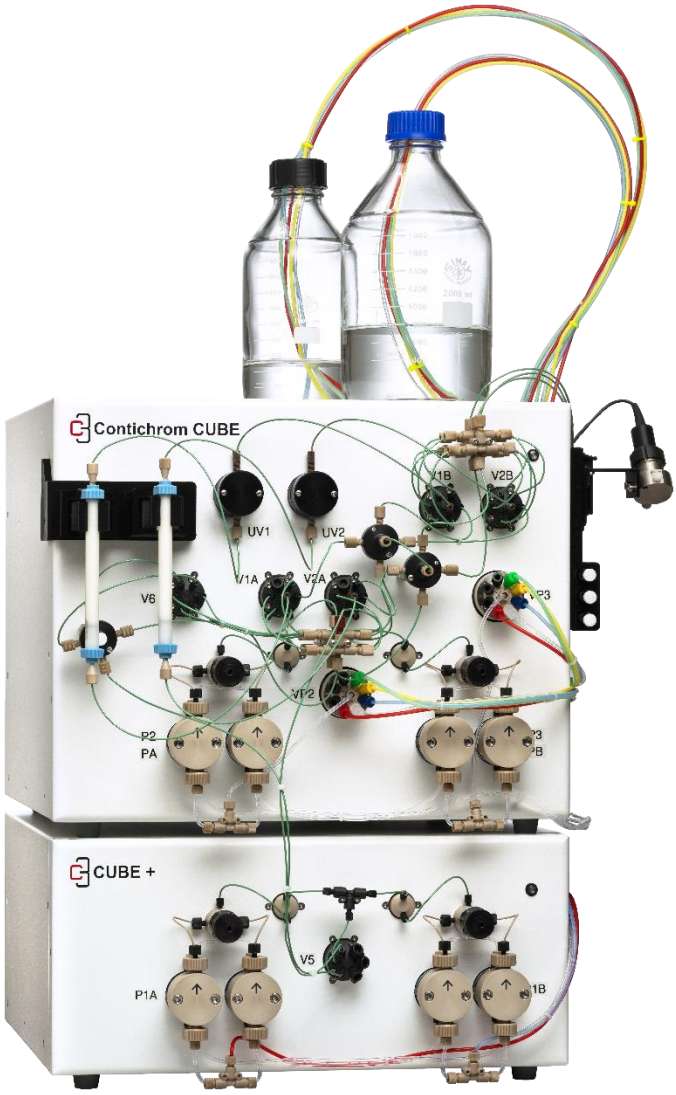


**Fig. 3.** The Contichrom® CUBE Combined consists of the system with two column configuration and the ChromIQ® operating software. The software can operate both batch and countercurrent processes.

## The principle of dynamic process control

Chromatographic RP processes are especially sensitive to temperature variations. Likewise, solvent / buffer variations and differences in column packing may affect performance. These process parameters need to be controlled tightly during continuous operation to avoid peak shifts and variations in product quality:

- Temperature
- Buffer quality
- Conductivity, pH
- Column variability (bed height, aging, packing quality)

Changes in these parameters may cause changes in the retention time of the product peak (Fig. 4). In MCSGP, a fixed product elution time window is set, and thus peak shifts would result in loss of purity and/ or yield. MControl® is thus used to auto-correct the position of the product collection window, concomitantly to peak shifts.

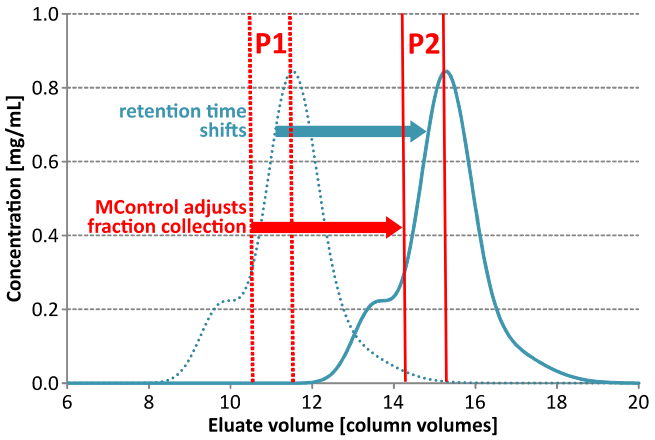


**Fig. 4.** Effect of peak profile shifting during continuous MCSGP process. In the MCSGP process, peak shifts may occur resulting in a different retention time. As the product collection window (P1, red dotted line) is defined by the elution time, for a shifting peak, the product collection window must be corrected (P2, red solid lines). This is done effectively by adjusting the fractionation start point.

## MControl® control concept

MControl® is a tool for process control and keeps the MCSGP process at its set point even if process parameter changes occur. Process changes can be induced for example by changes in temperature, buffer composition, or column quality degradation during prolonged continuous operation. MControl® monitors the elution times of peaks based on the UV signals (see Fig. 5). If the target peak shifts, MControl® compensates for the change by adjusting the fractionation start. In this way a consistent product quality and yield are ensured.

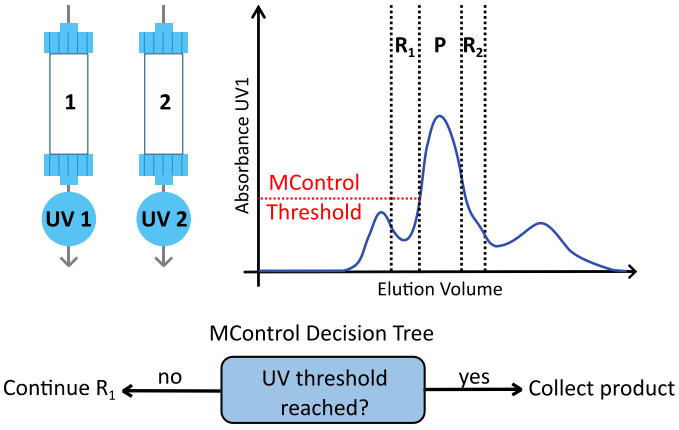


**Fig. 5.** The MControl® principle. The MCSGP process provides continuously UV data at the end of either column. If a certain absorption threshold is reached at the column that is being loaded, the recycling phase $R_1$ is terminated and the product collection phase P is started.

## MControl® process control in operation

In the example shown below, an MCSGP process was perturbed by diluting a buffer by 10%, leading to a peak shift (see Fig. 6). MControl® automatically shifts the product elution window from its original position P1 to the new correct position P2. Without dynamic process control, the product elution window would remain at its original set point P1, collecting no product in later cycles. MControl® thus contributes to process robustness.

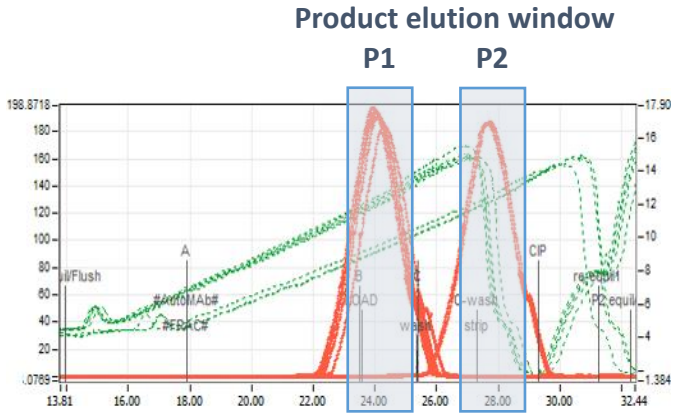


**Fig. 6.** Effect of peak shift due to a change in buffer composition during an MCSGP run. The chromatograms of multiple cycles are shown overlaid (red). Due to the buffer change the peak shifts from the position P1 to P2. The peak shape remains the same. MControl® adjusts the product elution window from P1 to the new peak position P2. The product is thus eluted in consistent quality and yield.

## Is the MCSGP process scalable?

The MCSGP process allows to achieve analytical resolution at preparative scale. Standard protein chromatography resins for batch processes are confined to particle sizes of 30-50 µm particle size due to the high pressure build-up of larger preparative columns. In MCSGP two much shorter columns are used and thus smaller particle sizes can be used providing higher resolution at similar overall backpressure. This means that processes developed with small particle resins of 10-30 µm particle size can only be scaled-up when using MCSGP.

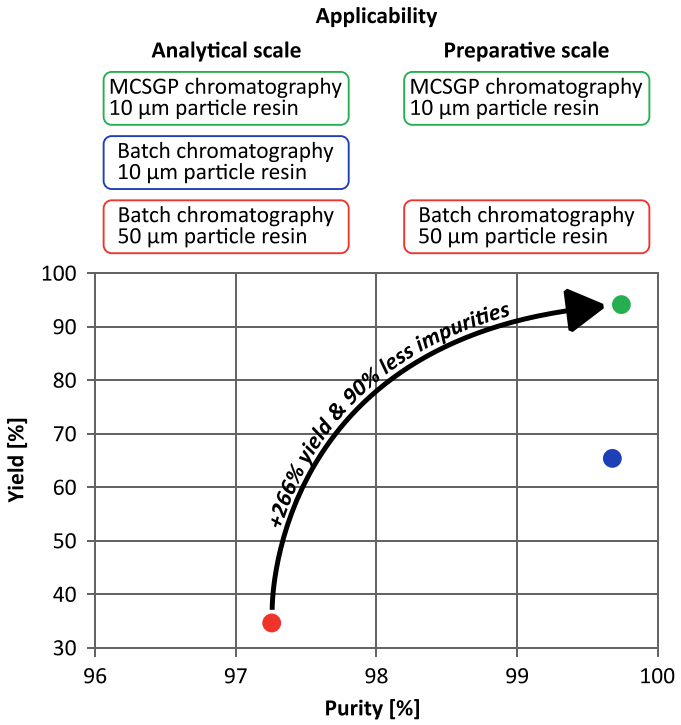


**Fig. 7.** Analytical resolution at preparative scale. With batch processes, the upper limit of particle sizes that can be used for preparative chromatography is 30-50 µm. With MCSGP, 10 µm particles can still be used at preparative scale, because smaller columns are sufficient due to the higher throughput. MCSGP thus enables preparative separations with purities and yields otherwise only achievable at analytical scale.

Due to the up to 10-fold higher productivity of the MCSGP process compared to batch, the overall column and system size will be smaller, providing with the same product output.

The MCSGP process is proven scalable. LEWA, our scale-up partner provides customized GMP FPLC and HPLC systems (Fig. 8). The MCSGP process allows to achieve analytical resolution at preparative scale.


**Fig. 8.** The EcoPrime® Twin GMP scale-up system is custom manufactured for use as FPLC or HPLC system. It is based on the same Contichrom® technology as the Contichrom CUBE and HPLC series with twin-column configuration. The system is manufactured and marketed by LEWA.

## Conclusions

The use of the MControl® dynamic control function enables steady operation of the Contichrom® CUBE Combined / HPLC 30/100 chromatography systems under process conditions where temperature, buffer quality, conductivity, pH, column variability (bed height, aging, packing quality) may vary. MControl® offsets any variation and keeps the MCSGP process at its set point. This results in a constant product quality.

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