The Contichrom® CUBE FPLC Systems

**Twin-Column
Protein Purification Systems
for Batch and Continuous Processing**

ChromaCon

The Contichrom CUBE Platform

Twin-Column Preparative FPLC System

The **Contichrom CUBE** and **CUBE Combined** are flexible modular purification systems for process development of biopharmaceuticals such as monoclonal antibodies.

❑ The **Contichrom CUBE** enables single-column batch and twin-column counter-current capture (CaptureSMB®) processes, typically for monoclonal antibody (mAb) purification.

❑ The **Contichrom CUBE Combined** enables single-column batch several continuous processes, including counter-current capture and polishing. It is an all-in-one process tool for challenging purification tasks for all proteins.

The systems are offered with pump flow rates of up to 36 mL/min or 100 mL/min.

The unique twin column operational design and software offer several process choices for optimal separation and purification of proteins and oligonucleotides, including batch, integrated batch and continuous countercurrent processes.
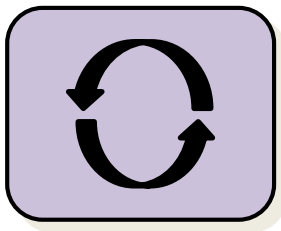
Run batch, integrated batch and continuous processes with ChromIQ automation software.
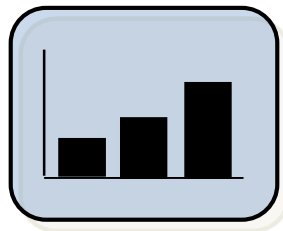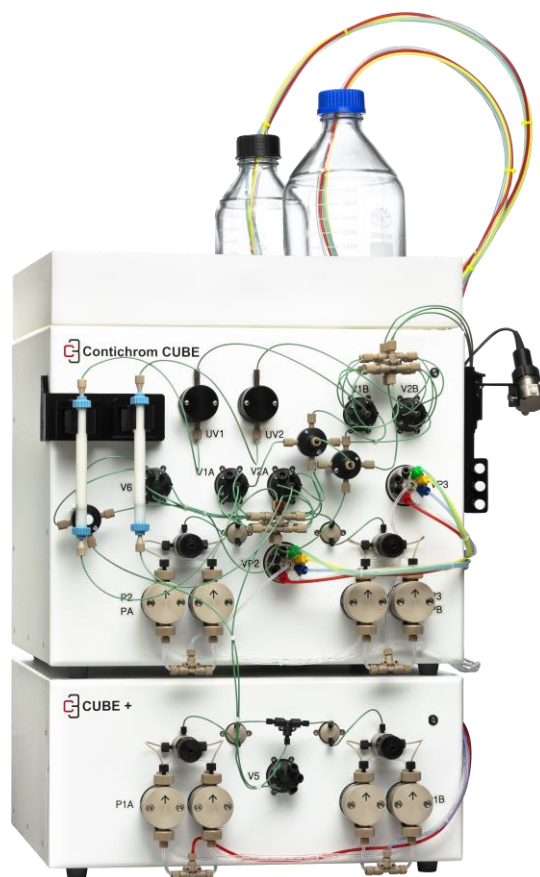
Get 50% more yield with the same target purity and significantly greater throughput.

Counter-current processes save significantly operating costs, including Protein A resin.

The CUBE Systems

The CUBE operates with ChromIQ software having batch and CaptureSMB (optimized 2C-PCC) process capabilities:

An ideal tool for mAb purification.

The AutomAb dynamic process control function keeps the process at an optimum.

The CUBE Combined is an extension consisting of the CUBE and an additional CUBE+ module. The ChromIQ software has all CUBE functions and in addition extended continuous polishing capabilities such as MCSGP, N-Rich, and Flow-2.

MCSGP is supported by MControl, a dynamic process control function keeping the MCSGP process always at an optimum.

The systems are delivered ready-to-use with fully mounted tubing and pre-delivery IQ/OQ testing.

CUBE Process Portfolio

The Contichrom CUBE has batch process capabilities like any FPLC system. Additionally, enhanced continuous process capabilities offer increased performance gains (productivity, yield and throughput).

Twin column capture applications (**CaptureSMB** / optimized 2C-PCC) and the software tool (AutomAb) are useful for automated optimization of the mAb capture process. Twin column capture processes will result in significant cost-of-goods savings at GMP scale-up.

The Contichrom CUBE Combined adds additional twin-column/membrane process capabilities for polishing applications:

- **MCSGP**: **M**ulti-column **C**ounter-current **S**olvent **G**radient **P**urification. A powerful gradient elution process that increases yield by up to 80% while maintaining target purity. MControl, a dynamic process control tool, keeps the MCSGP process at an optimal operating mode.
- **N-Rich**: a process for enriching and isolating minor components from complex mixtures – an ideal tool for fast isolation of product-related impurities for pre-clinical testing.
- **Flow-2**: a robust flow-through process allowing to capture impurities while letting the product pass.
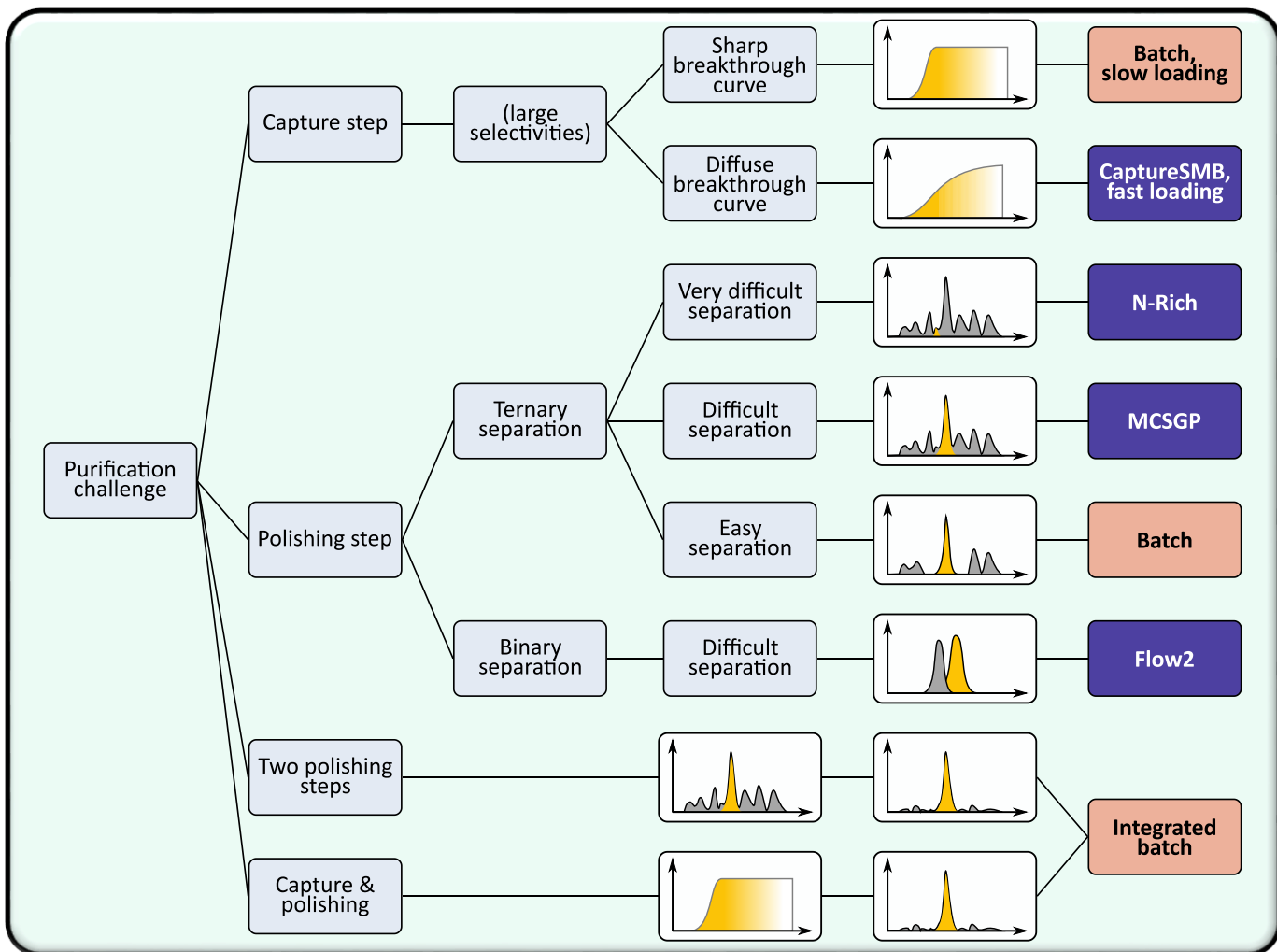
Process Economics

Integrated batch chromatography

The twin-column setup allows to run two process steps consecutively in an integrated way, even with in-line dilution between the first and second column steps, eliminating intermediate hold steps.

CaptureSMB (2C-PCC)

ENABLES
Two-fold faster processing of feed streams preserving product integrity; higher project turnover.

SAVES
30% CAPEX, 30-60% OPEX, 40-60% Protein A consumption, 40-60% buffer consumption.

MCSGP

ENABLES
Isolation of pure components from complex mixtures; 50-90% more yield and higher purity; up to 10x faster processing than batch.

SAVES
Up to 30% CAPEX, 50% OPEX, 70% buffer consumption.

N-Rich

ENABLES
The enrichment of a minor components while simultaneously depleting the large excess of interfering product. It is particularly useful for isolation of product-related impurities.

SAVES
Tedious repetitive analytical separations to isolate the compound of interest. With batch processes, up to several hundred analytical injections are needed to isolate sufficient amounts for further characterizations. With N-Rich, this can be achieved overnight.
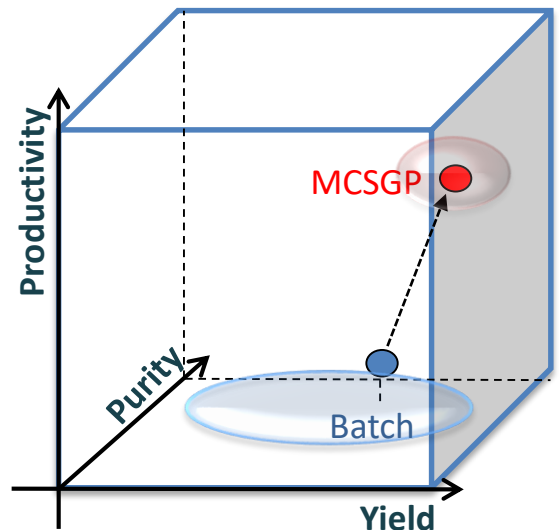
Flow-2

ENABLES
The 100-fold fast processing by capturing impurities and letting the product pass through.

SAVES
Processing time and increases productivity.

ChromIQ Software

The ChromIQ operating software controls the Contichrom CUBE systems. It supports batch and continuous processes and tools for separation and purification with an intuitive, user-friendly interface.

ChromIQ has easy step-by-step wizards to help you design batch chromatography runs and to convert them to more efficient Contichrom Processes. ChromIQ also includes the AutomAb and MControl dynamic process controllers.

ChromIQ includes a number of features that are particularly helpful for continuous processes such as a buffer management system and cycle overlay display options.
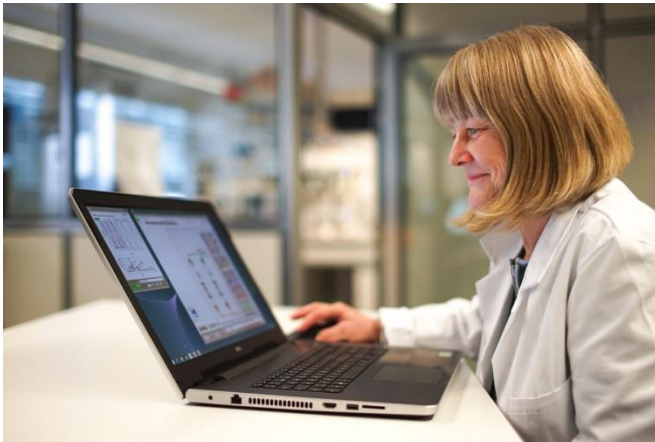
- ✓ Drag-and-drop method creation
- ✓ Wizards for convenient method creation
- ✓ Interactive process picture
- ✓ Single-click evaluation
- ✓ Easy data export (xlsx, csv, jpg)
- ✓ Pre-defined user groups with individual rights management
- ✓ Password protected user accounts
- ✓ Logging with time stamp and user name
- ✓ Electronic signature with check-sum of log and measurement files
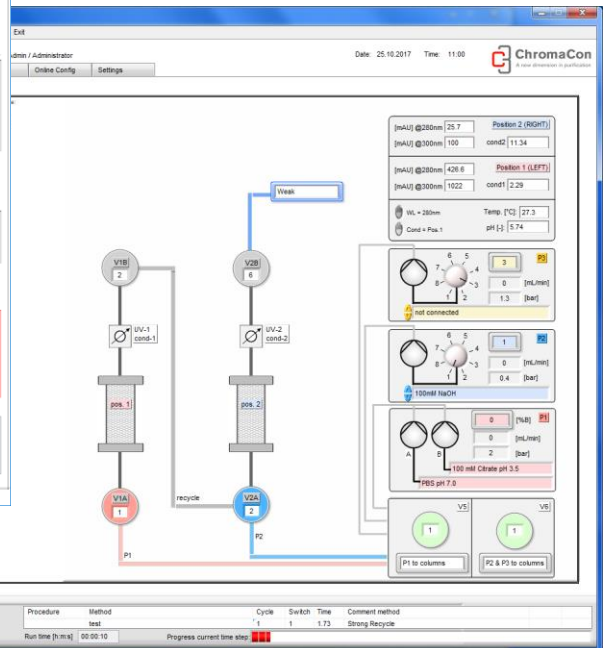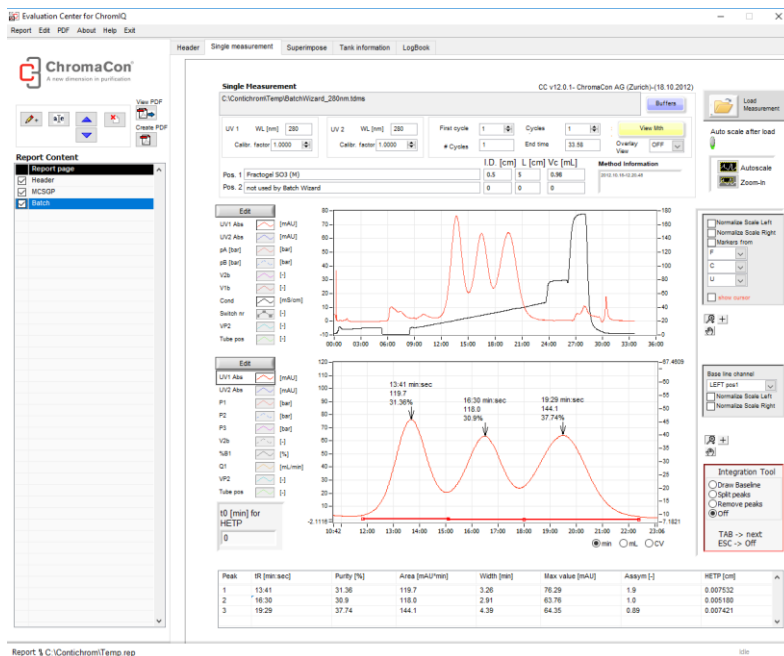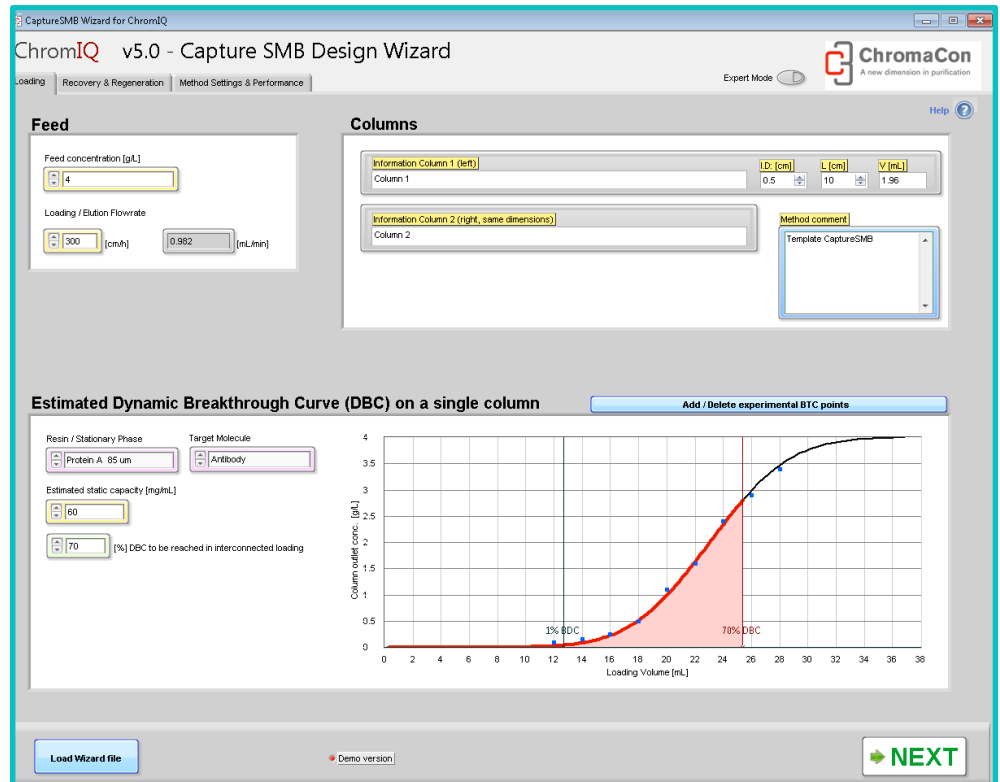
Process Wizard Examples

Load the process wizards from the ChromIQ Software for easy design of processes.

CaptureSMB

STEP 1: Enter feed and column parameters and fit experimental breakthrough curve

STEP 2: Define wash, elution and regeneration steps

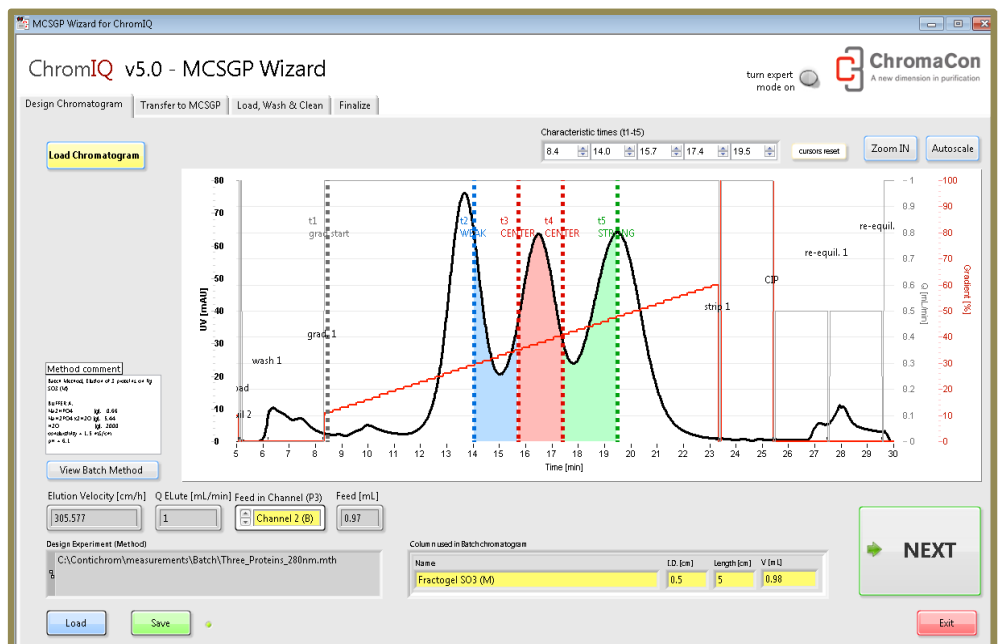**STEP 3:** Activate AutomAb control, auto-generate method and receive performance prediction

MCSGP

STEP 1: Load chromatogram of batch run and select product range and recycling fractions by Drag & Drop

STEP 2: Set column size and feed volume

**STEP 3:** Define washing and regeneration steps

STEP 4: Activate MControl, set number of cycles and fractionation

Dynamic Process Control

AutomAb: Dynamic CaptureSMB process control

AutomAb is a tool that automatically optimizes the CaptureSMB process in terms of resin capacity utilization, throughput, and ensuring steady product quality. AutomAb controls the process and maintains optimal process performance effectively offsetting process changes such as feed titer variations and column aging.
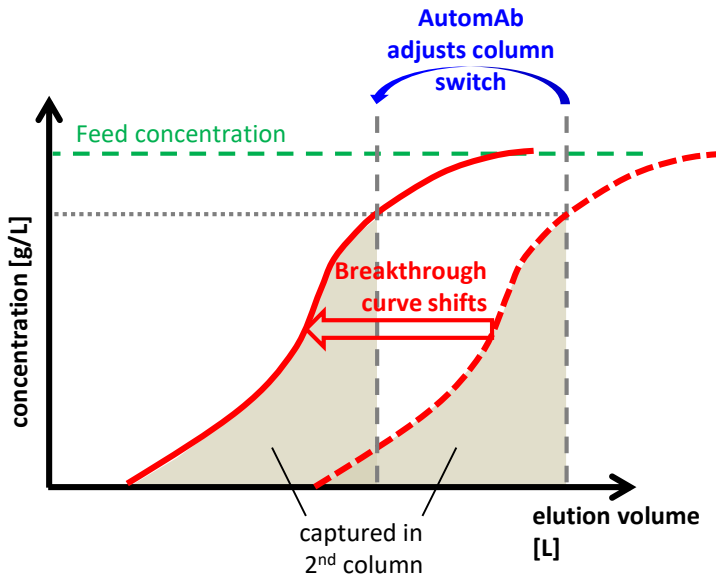

Advantages of AutomAb

- works with minimum process knowledge
- runs fully automatically without intervention
- works with low and high feed titers
- works without feed signal measurement
- works with "dirty" feeds with a high impurity signal and low product feed concentrations
- works without detector calibration

MControl: Dynamic MCSGP process control

The outcome of chromatographic runs can be influenced by various parameters such as temperature, buffer quality, conductivity, pH and quality of the stationary phase (bed height, resin aging, packing variation) leading to variability. To counteract such effects, we have developed a control algorithm allowing to keep the MCSGP runs always at an optimum by compensating for variations. The resulting MCSGP process is very robust and will run at an optimum without sacrificing productivity.
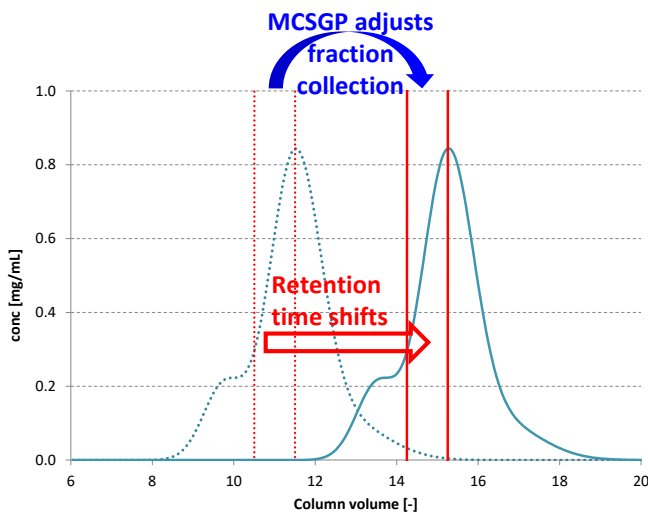
Advantages of MControl

MControl compensates for peak shifts by adjusting the fractionation start

- Always the same product in same fraction
- Always the same product quality
- Perfect control of cyclic continuous processes

Twin-column pilot-process scale

Ecoprime® Twin: Best-in-class GMP skids

Type	Min L/min	Max L/min
Ecoprime 100	0.004	0.6
Ecoprime 250	0.02	2.4
Ecoprime 500	0.06	9

Product features

- Integrated Buffer Inline Dilution (BID)
- Ability to run batch, integrated batch, parallel batch and CaptureSMB. MCSGP will be available soon.
- CIP & SIP
- Drain & blow dry
- Custom designed set up
- Scale-up method conversion
- Allan-Bradley Rockwell or Delta-V operating system
- Compliant with GMP, GAMP, ASTM, 21CFR part 11
- All wetted parts cleanable
- Flow accuracy: better than 0.5% variation.
- Gradient accuracy: better than 0.5% variation
- Pressure rating: 7.5 bar (108 psi)
- Flow path: 316L stainless steel

Customer (BMS) using EcoPrime Twin GMP scale-up system:

James Angelo et al., (**April 2018**), Scale-Up of Twin-Column Periodic Counter-Current Chromatography for MAb Purification, *BioProcess International*.

EcoPrime® is a trademark of LEWA and LEWA Process Technology Ltd. System quotes can be obtained under www.lewaprocesstechnologies.com

Benchtop Cooling Cabinet

Preserving Product Integrity During Purification

Cooling of product feed and of fractions is important for preserving product integrity. We offer a compact cooling chamber that fits on a lab bench and can accommodate a fraction collector (Foxy R-1), feed bottles and also columns allowing for preparative runs under cooled conditions.

Additional useful accessories include a sample loop system for feed loading, an external valve with an injection loop and a stable, re-usable transport box.

CUBE System Accessories

Enhancing System Performance and Convenience

Additional accessories include two external multi-wavelength detectors (190-500 nm), a sample loop system for feed loading, an optional external loading valve with sample injection loops of 500 µL up to 20 mL, a screening valve for column screening addressing up to 6 columns and re-usable transport boxes.

Fraction collectors Foxy R-1 and R-2

Valve system with 6 positions for column screening

Injection valve system with injection loops of 500 µL up to 20 mL allowing to apply different sample volumes
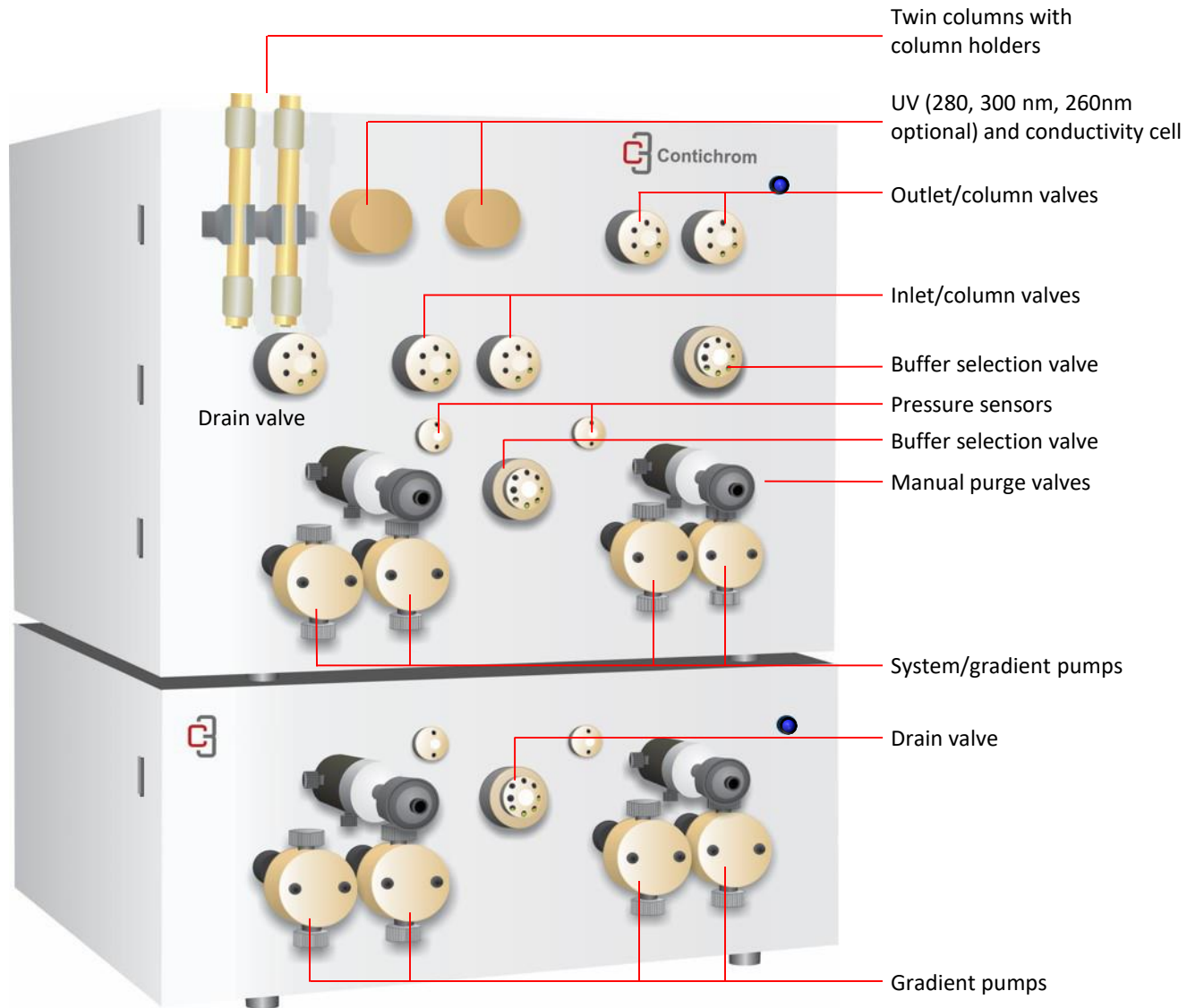
Preparative flow cells in PEEK or steel

External variable wavelength detector (190-500 nm)

External variable multi-wavelength detector (190-700 nm)

Powerful Functionalities

Twin columns with column holders

UV (280, 300 nm, 260nm optional) and conductivity cell

Outlet/column valves

Inlet/column valves

Buffer selection valve

Pressure sensors

Buffer selection valve

Manual purge valves

Drain valve

System/gradient pumps

Drain valve

Gradient pumps

Contichrom

- compact benchtop design
- large buffer tray
- clear easy-to-access interface
- high visibility tubing inlets and outlets
- flexible tubing connections
- high performance pumps (36 or 100 mL/min)
- easy-mount clip-in column supports

- 2 long life LED UV detectors each at 280 nm and 300 nm; 260 nm optional
- easy plug-in CUBE and CUBE+ installation
- pH detector
- 2 conductivity flow cells
- laptop and desktop computer options

Technical Specifications

Contichrom CUBE & CUBE Combined Systems

Process capabilities:	Batch (isocratic, gradient), integrated batch, CaptureSMB, MCSGP, N-Rich, Flow-2
Operating software:	User-friendly operating software with step-by-step wizards to help you to design batch chromatography runs and to convert them into more efficient Contichrom processes, such as MCSGP and N-Rich. ChromIQ also includes dynamic process controllers AutomAb and MControl.
Software compliance:	ChromIQ software with essential elements of 21CFR Part 11 compliance: • Pre-defined user groups, administrators, R&D and production users • Rights management for individual user groups • User accounts are password protected • Logging with time stamp and user name (non-deletable) • Electronic signature with checksum of log and measurement files
Pressure rating:	50 bar (5 MPa)/ 725 psi
Flow rate range:	• 0.1 – 36 mL/min (Contichrom CUBE & CUBE Combined 30) • 0.1 – 100 mL/min (Contichrom CUBE & CUBE Combined 100)
Buffer selection:	16 Inlets (2 x 8-fold buffer selection valve) 4 Outlets
UV, fixed wavelength:	2 Long lifetime LED UV detectors, each 260 & 280 nm recording simultaneously
Conductivity monitoring:	2 Conductivity sensors (1-300 mS/cm)
pH monitoring	1-14
Pump type	High precision double-piston-pumps with active seal wash 2 Pumps (CUBE), 4 pumps (CUBE Combined)
Valves:	4 Reliable multi-position valves 1 Automated drain valve (CUBE), 2 automated drain valves (CUBE combined)
Computer hardware:	Stand-alone laptop computer (Windows, 64 bit, full HD resolution, 1920 x 1080 or higher) with ChromIQ software
Other:	Cold room compatible Large buffer tray Portable & compact Runs resins and membrane stationary phases
Dimensions:	CUBE module: 450 mm x 509 mm x 370 mm (20.0" x 17.7" x 14.6") CUBE+ module: 450 mm x 509 mm x 214 mm (20.0" x 17.7" x 8.43") The modules are stackable.
Weight:	CUBE module: 30 kg (67 lb) CUBE+ module: 17 kg (38 lb)
Materials:	All biocompatible High pressure side capillaries: PEEK Low pressure side tubing: PTFE Fittings: PEEK

Training, Maintenance, and Repair

A reliable and cost-effective service network

Purchasing an FPLC System and operating it is only part of a customer's value proposition. After sales support such as Preventive Maintenance (PM) and total life cycle costs are an important consideration in a system's procurement evaluation.

We offer PM, repair and system validation and qualification support including IQ-OQ and a generic PQ testing scheme. We also offer an annual Software PM package.

We perform on-site and off-site training, webinar-based product support and we organize annual workshops on continuous chromatographic purification.

Our system is designed to have very low maintenance costs: only wear parts from pumps and valves need to be exchanged occasionally in an easy way without disassembling the system.

We offer comprehensive and cost-effective Preventive Maintenance and Repair Service packages.

Worldwide Preventive Maintenance and Repair Service packages. On-site and off-site service with fast turnaround times.

For details please require a quote at your local ChromaCon representative.

Selected Publications

1. J. Angelo et al., "Scale-Up of Twin-Column Periodic Counter-Current Chromatography for MAb Purification," *BioProcess Int.* (2018).

2. F. Steinebach, N. Ulmer, L. Decker, L. Aumann and M. Morbidelli, "Experimental design of a twin-column countercurrent gradient purification process," *J. Chromatogr. A* **1492,** 19–26 (2017).

3. N. Andersson, A. Löfgren, M. Olofsson, A. Sellberg, B. Nilsson and P. Tiainen, "Design and control of integrated chromatography column sequences," *Biotechnol. Prog.* **33,** 923–930 (2017).

4. F. Steinebach, T. Müller-Späth and M. Morbidelli, "Continuous counter-current chromatography for capture and polishing steps in biopharmaceutical production," *Biotechnol. J.* **11,** 1126-1141 (2016).

5. D. Baur, M. Angarita, T. Müller-Späth, F. Steinebach, and M. Morbidelli, "Comparison of batch and continuous multi-column protein A capture processes by optimal design," *Biotechnol. J.* **11,** 920–931 (2016).

6. M. Angarita, D. Baur, T. Muller-Spath, R. Lievrouw, G. Lissens, and M. Morbidelli, "Twin-column CaptureSMB: a novel cyclic process for protein A affinity chromatography," *J. Chromatogr. A* **1389**, 85–95 (2015).

7. N. Ulmer, T. Muller-Spath, L. Aumann, B. Neunstoecklin, M. Bavand, and M. Morbidelli, "Affinity capture of F(ab')2 fragments: using twin-column countercurrent chromatography," *BioProcess Int.* **13**, 22, 24, 26, 28–29 (2015).

8. H.-K. Knutson, M. Max-Hansen, C. Jönsson, N. Borg, and B. Nilsson, "Experimental productivity rate optimization of rare earth element separation through preparative solid phase extraction chromatography," *J. Chromatogr. A* **1348**, 47–51 (2014).

9. M. Krättli, F. Steinebach, and M. Morbidelli, "Online control of the twin-column countercurrent solvent gradient process for biochromatography," *J. Chromatogr. A* **1293**, 51–59 (2013).

10. T. Müller-Späth, M. Angarita, D. Baur, R. Lievrouw, G. Lissens, G. Strohlein, M. Bavand, and M. Morbidelli, "Increasing capacity utilization in protein A chromatography," *BioPharm Int.* **26**, 33-35, 38 (2013).

11. T. Müller-Späth, N. Ulmer, L. Aumann, G. Ströhlein, M. Bavand, L. J. A. Hendriks, J. de Kruif, M. Throsby, and A. B. H. Bakker, "Purifying Common Light-Chain Bispecific Antibodies," *BioProcess Int.* **11**, 36–45 (2013).

12. T. Müller-Späth, G. Ströhlein, L. Aumann, H. Kornmann, P. Valax, L. Delegrange, E. Charbaut, G. Baer, A. Lamproye, M. Jöhnck, M. Schulte, and M. Morbidelli, "Model simulation and experimental verification of a cation-exchange IgG capture step in batch and continuous chromatography," *J. Chromatogr. A* **1218**, 5195–5204 (2011).

13. B. T. Takizawa, *Evaluation of the financial impact of continuous chromatography in the production of biologics*, M.Sc. Thesis, Massachusetts Institute of Technology, 2011.

14. C. Grossmann, G. Ströhlein, M. Morari, and M. Morbidelli, "Optimizing model predictive control of the chromatographic multi-column solvent gradient purification (MCSGP) process," *J. Process Control* **20**, 618–629 (2010).

15. T. Müller-Späth, L. Aumann, G. Ströhlein, H. Kornmann, P. Valax, L. Delegrange, E. Charbaut, G. Baer, A. Lamproye, M. Jöhnck, M. Schulte, and M. Morbidelli, "Two step capture and purification of IgG2 using multicolumn countercurrent solvent gradient purification (MCSGP)," *Biotechnol. Bioeng.* **107**, 974–984 (2010).

16. T. Müller-Späth, M. Krättli, L. Aumann, G. Ströhlein, and M. Morbidelli, "Increasing the activity of monoclonal antibody therapeutics by continuous chromatography (MCSGP)," *Biotechnol. Bioeng.* **107**, 652–662 (2010).

17. T. Müller-Späth, L. Aumann, L. Melter, G. Ströhlein, and M. Morbidelli, "Chromatographic separation of three monoclonal antibody variants using multicolumn countercurrent solvent gradient purification (MCSGP)," *Biotechnol. Bioeng.* **100**, 1166–1177 (2008).

Contact
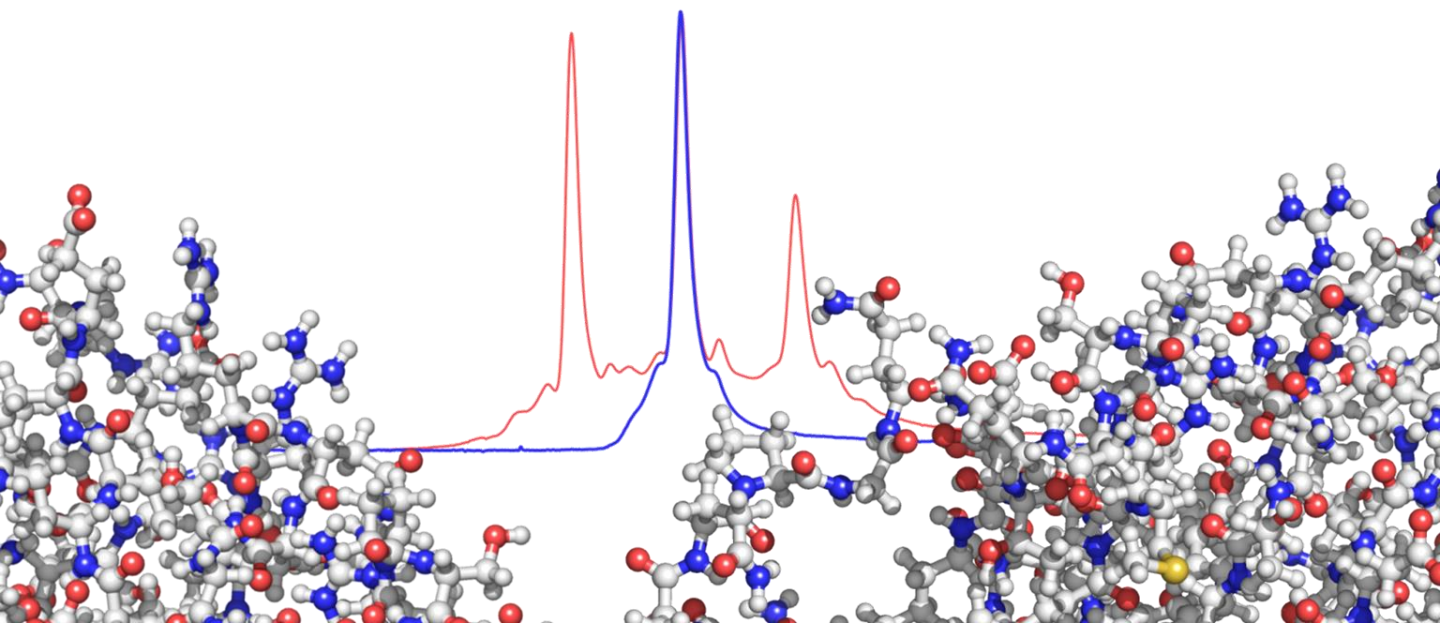
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