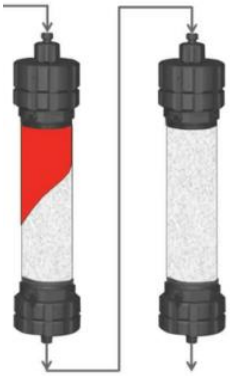
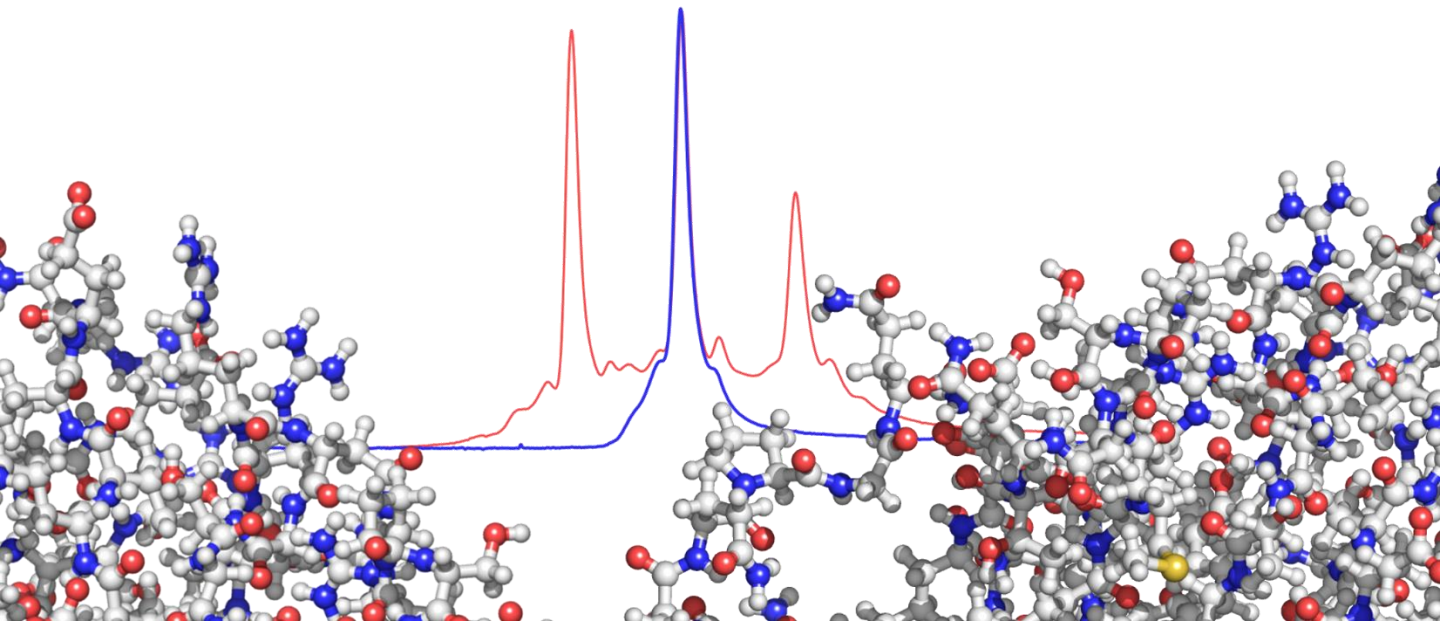
ChromaCon

The Contichrom® Process Portfolio and Software Tools



Superior twin column chromatography processes for chromatographic purification operated by Contichrom® systems



The Contichrom Process Portfolio

The Contichrom process portfolio makes chromatographic separations and purifications easier, faster and more effective.

- ✓ **Single column and integrated multi-step batch operation**
- ✓ **Continuous operation**
- ✓ **Optimized for throughput and low costs**

The Contichrom systems are capable of running standard **single column batch** process with step or gradient elution, including capture and polishing steps.

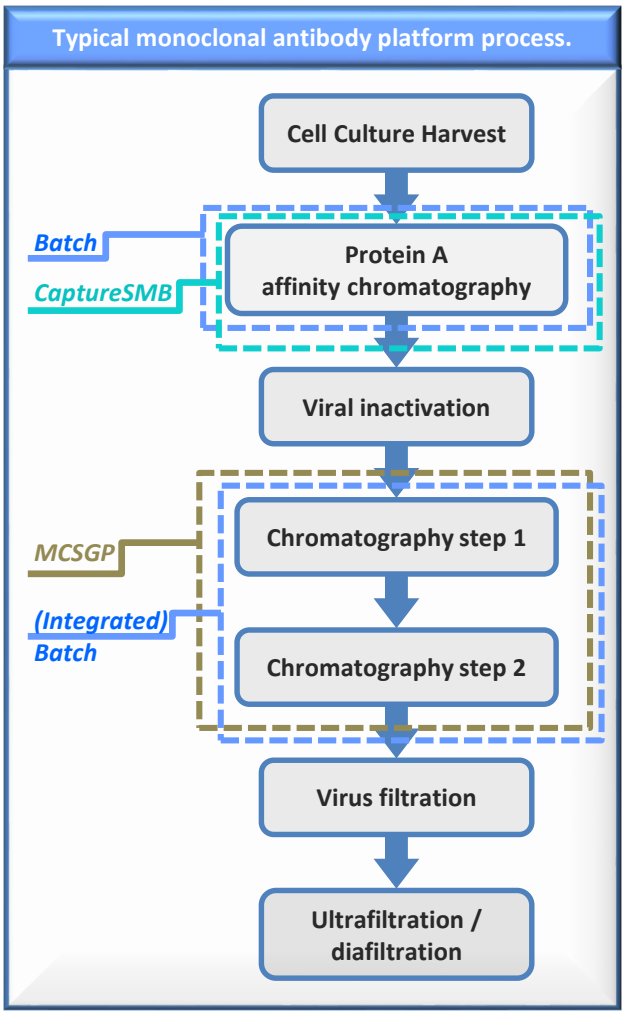
Due to an efficient inline dilution, **integrated two-step batch** processes are possible as well. The eluting product from the first chromatography step is diluted with a fitting buffer and internally transferred to a second chromatography step.

The novel continuous processes are unique in providing higher efficiency than batch processes (see on the right).

CaptureSMB provides more efficient processing for affinity chromatography steps by maximizing resin utilization and increasing throughput with faster loading.

MCSGP increases the yield of pure product while retaining target purity, if there are overlapping impurities. This can even reduce the number of polishing steps required for a given purification task.

N-Rich automatically enriches and isolates minor compounds from complex mixtures, quickly and in required quantity and purity. It thus is an invaluable tool, for example for pre-clinical development.



The Contichrom **twin column chromatography processes** save financial and time resources compared to single column chromatography processes. ChromaCon's novel capture and polish processes are designed for the efficient separation and purification of monoclonal antibodies (mAbs), biosimilars, glycoproteins, oligonucleotides, peptides and small molecules. They are particularly useful for difficult purifications challenges, as they provide higher yield without compromising a chosen target purity when there are overlapping product/impurity fractions. The processes are seamlessly scalable to GMP pilot & production scale.

Batch & Integrated Batch

The twin column Contichrom systems run normal batch capture and polish processes with isocratic, step and gradient elution.
Furthermore, integrated batch with two process steps can be run consecutively, with inline dilution between the first and second step, sparing a time-consuming 'holding' step in between. Orthogonal separation principle can be applied for the two steps. Integrated batch is thus useful for automated high-throughput purification with a combination of a capture step (Protein A, His-tag) and a polishing step (SEC, IEX).

CaptureSMB

A novel capture process using twin columns providing higher processing speed and better use of Protein A.

ENABLES
- two-fold faster processing of feed streams
- 30 – 60% increase in throughput with higher product concentration
- larger project throughput

SAVES
- 30% CAPEX
- 30-60% OPEX
- 30-60% Protein A resin
- 30-60% buffer savings
- Cost of Goods savings of up to US$ 2.5m p.a. for a standard mAb (commercial production)

MCSGP

A novel polish process using twin columns providing higher yield and purity than a batch process.

ENABLES
- isolation of pure compounds from complex mixtures
- 50-90% higher yield and purity
- up to 10x faster processing

SAVES
- 30% CAPEX
- up to 50% OPEX
- up to 70% buffer savings

N - Rich

A novel selective enrichment process using twin columns allowing to isolate minor impurities amidst complex mixtures - overnight

ENABLES
- isolation of minor components and product-related impurities overnight, without manual handling.
- getting compliant to ICH Q3A(R2) more easily

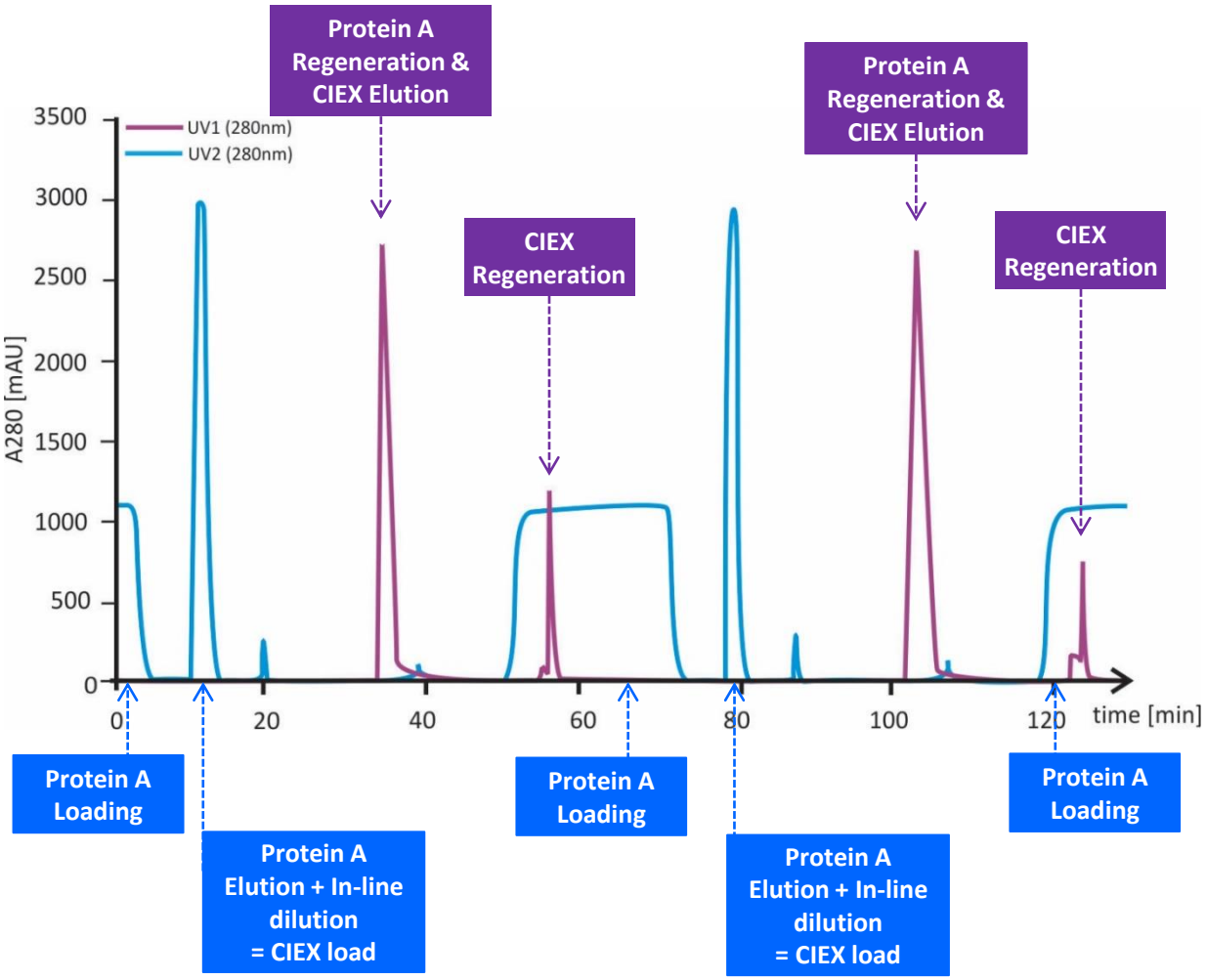
SAVES
- weeks or even months of repetitive work
- Time and resource-consuming sample analyses

Batch & Integrated batch

Contichrom systems provide you with all the batch capture and polishing capabilities you are used to work with, and provides you additional useful functionalities:

- increased flexibility with wizard-based design tools
- facilitated purification train by running two process steps consecutively with inline dilution, eliminating the holding step in between

Integrated two-step batch case study: Protein A and CIEX capture/elution



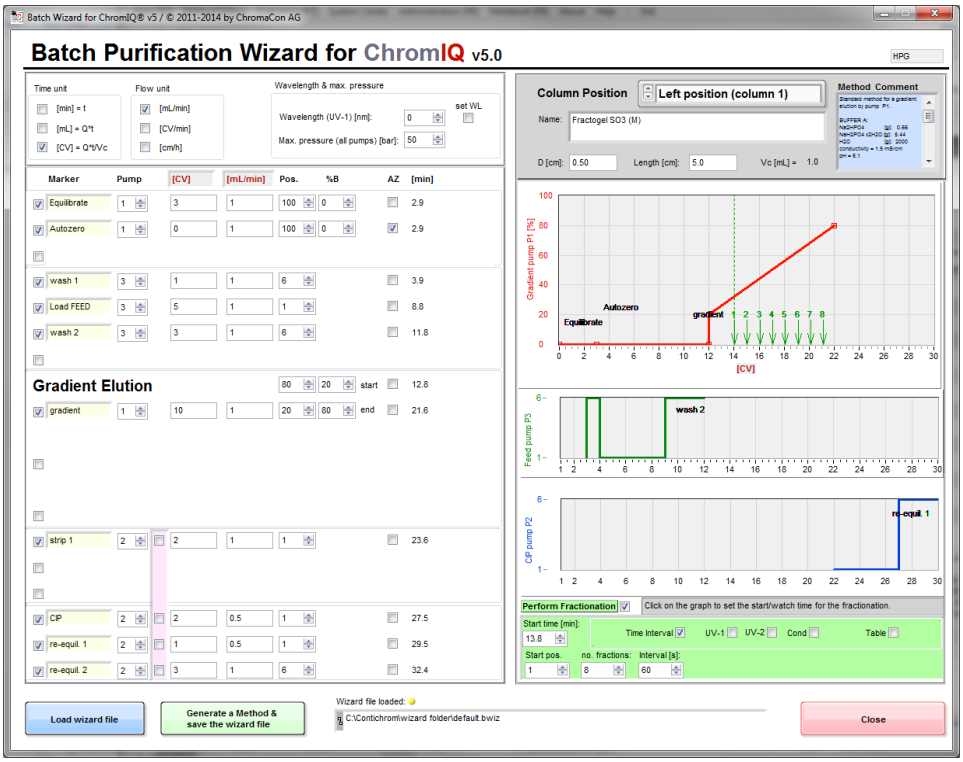
An integrated continuous batch process was run using the Contichrom CUBE for capture and polishing of a monoclonal antibody from a clarified harvest using Protein A and cation exchange chromatography. Inline dilution was applied to condition the Protein A eluate. The online signals from the Contichrom CUBE's ChromIQ control software (shown above) clearly indicate the stages of the experiment for each column. The progress can thus be monitored in real time.

Batch & Integrated batch Software Tools

Visualize your experiment as you design it

The operating software ChromIQ contains user-friendly wizards for all chromatographic processes.

ChromIQ directly generates graphs to visualize the outcome of the ongoing method design process.



The Contichrom CUBE Combined

- Batch, integrated batch and ChromaCon's novel twin column processes all run on the Contichrom CUBE, Contichrom CUBE Combined, and Contichrom HPLC
- Set up batch and integrated batch processes with ease using the Batch Wizard and the Integrated Batch Wizard
- Enable and customize different blocks, e.g. equilibration, feed, washing, elution, strip, cleaning, re-equilibration
- Run standard batch processes just as on any other FPLC or HPLC, including isocratic, step, and gradient elution
- Run two process steps consecutively, with or without inline dilution between first and second column without intermediate holding step

CaptureSMB

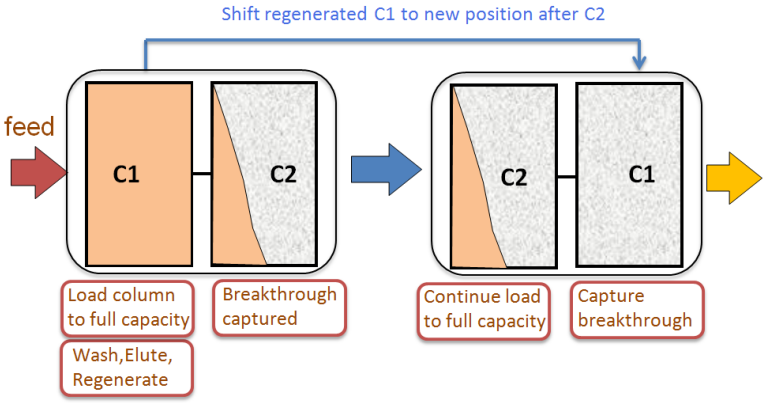
Process Principle

Optimized capture with 2-column periodic countercurrent chromatography (2C-PCC)

Monoclonal antibodies, Fc fusion proteins and otherwise tagged proteins are purified using platform processes involving a affinity capture step. Two factors a especially important for this step: A fast capture step is critical to retain product integrity and allows to process large feed volumes in a reasonable time. Efficient resin utilization is also important, because affinity capture agents are expensive. CaptureSMB can be optimized towards both targets, depending on the weighted importance for the individual project.

CaptureSMB uses a twin column setup to capture the product from a clarified feed stream by using all the available Protein A resin. Breakthrough material is captured in the second column. CaptureSMB thus makes complete use of the affinity resin material, thereby reducing resin costs by typically 40-60%. In the next step, the two columns are alternately loaded and eluted (see below).

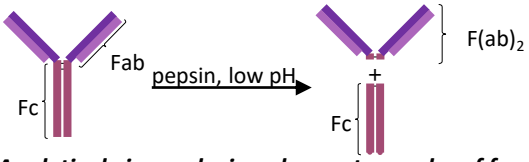
It has been shown that the 2C-PCC CaptureSMB process is superior to 3-column or 4-column processes (3C-PCC, 4C-PCC) in realistic application scenarios (Baur et al. 2016, Biotechnol. J. 11 (7) : 920-931).



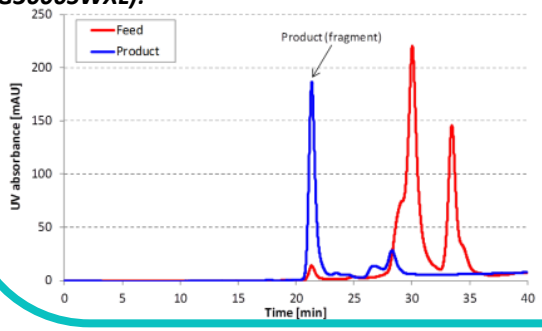
CaptureSMB case study: F(ab)$_2$ capture

The F(ab)$_2$ fragment was separated from a monoclonal antibody (mAb; see below). It was purified with Capto L affinity resin. Batch and CaptureSMB processes were compared.

Synthesis of F(ab)2 fragments:



Analytical size exclusion chromatography of feed and CaptureSMB product fraction (Tosoh TSK Gel G3000SWXL):



Results:

Both processes yielded pure F(ab)$_2$ fragments (see bottom left). CaptureSMB led to significant decreases in both buffer consumption and resin demand. In a production scenario of 100 kg / year, this leads to a total saving of 160'000 L of solvent and resin worth USD 2 mio (40%).

Protein A capture 100 kg/year production	Batch	CaptureSMB
Buffer consumption [L]	390'000	230'000
Resin demand [L]	250	150
Annual resin cost [USD]	5'000'000	3'000'000

CaptureSMB

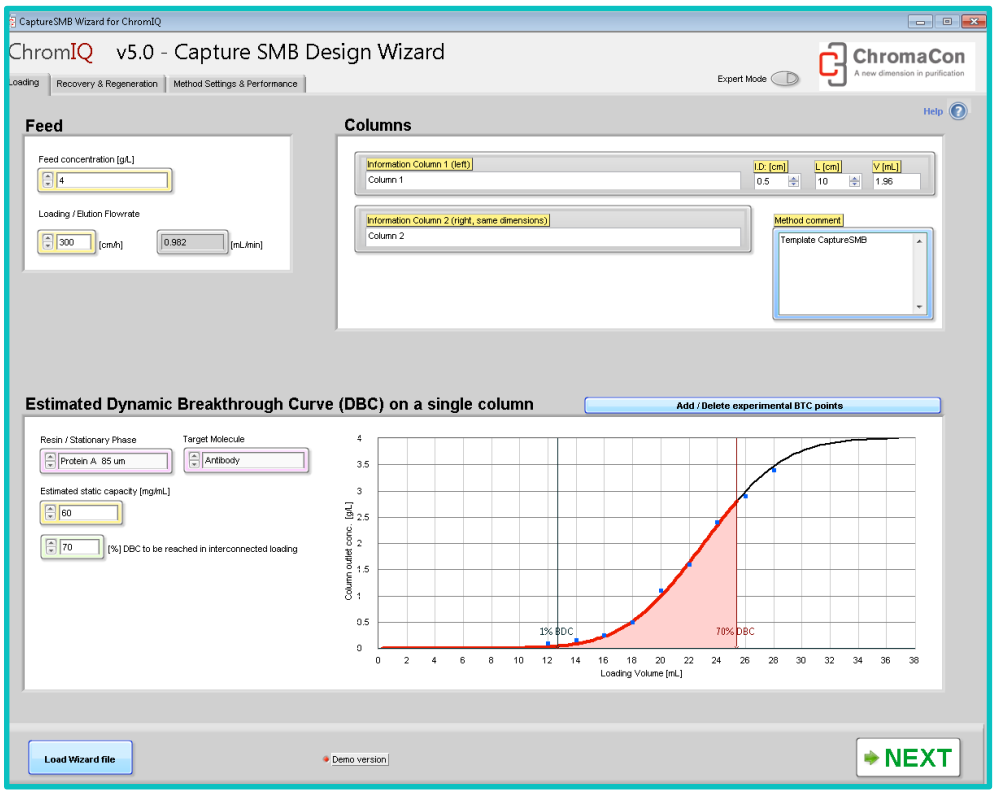
Software Tools

Process design in 3 easy steps with the CaptureSMB Wizard

STEP 1:
Enter feed and column parameters, choose from pre-defined resin types, molecule class or fit experimental breakthrough curve

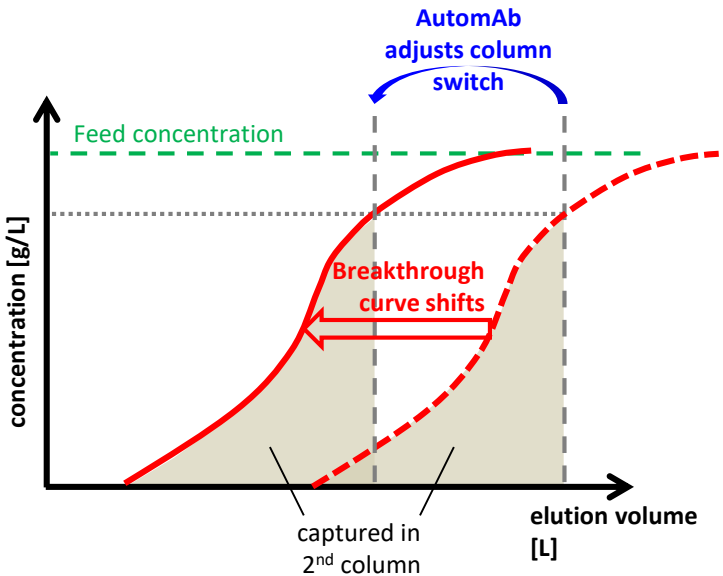
STEP 2:
Define wash steps or choose pre-defined ones

STEP 3:
Setup AutomAb control, auto-generate method and receive performance predict ion



Dynamic process control and process optimization with AutomAb

AutomAb effectively adapts the CaptureSMB process to changes in process conditions such as feed titer or dynamic column capacity. The process is adjusted by AutomAb to maintain an optimal loading level.



Advantages of AutomAb

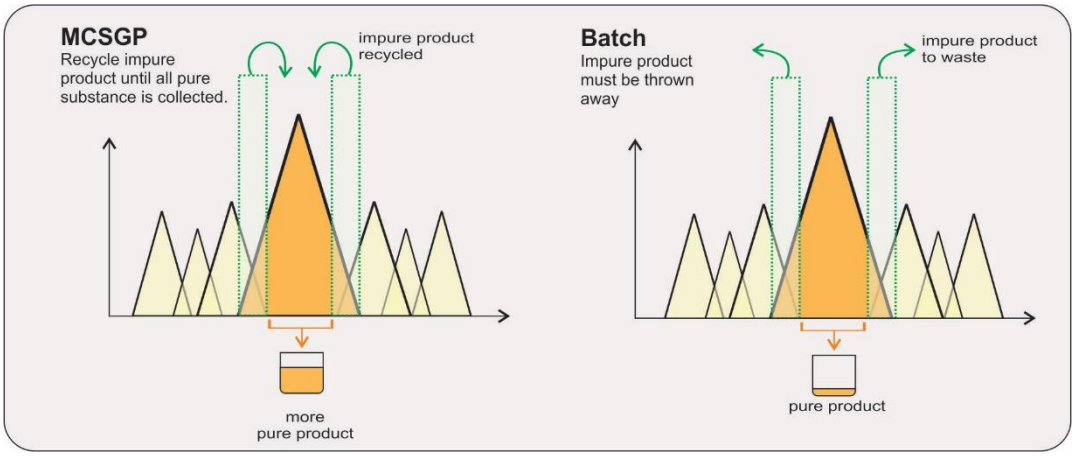
- works with minimum process knowledge
- runs fully automatically without intervention
- works without feed signal measurement
- works with “dirty” feeds with a high impurity signal and low product feed concentrations
- works without detector calibration

MCSGP

Process Principle

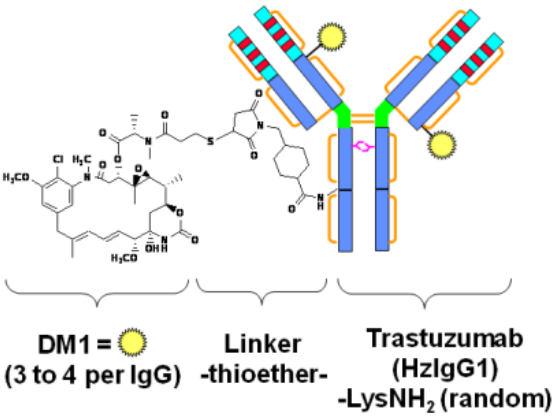
Multicolumn Countercurrent Solvent Gradient Purification: Recycle until it's pure

Polishing steps for peptides, proteins and conjugates such as ADCs can be challenging. Impurities, aggregates, fragments and isoforms are difficult to remove if they overlap with the product. In traditional single column batch chromatography only the pure part of the product peak can be isolated – at the expense of yield. The impure side fractions, containing valuable product, must be discarded. MCSGP internally recycles these side fractions, while continuously loading fresh feed. This not only gives significantly higher yields of pure product, but also enables to process more feed and thereby increase the overall throughput.



MCSGP case study: Antibody-Drug Conjugates (ADCs)

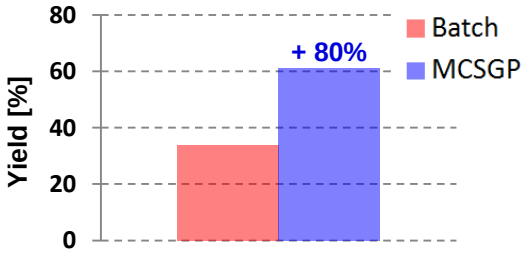
In a model system, ADCs were synthesized with the aim of isolating conjugates with an drug-antibody ratio of 2, from a complex conjugation reaction mixture. Batch and MCSGP processes were evaluated. The MCSGP process was designed based on the optimized batch run within a few minutes using the MCSGP Wizard (see next page).



Results:

MCSGP outperformed the conventional batch chromatography process:

- Yield increase from 34 to 61% for same purity (+80%)
- 80% productivity increase
- 55% reduction in buffer consumption



MCSGP

Software Tools

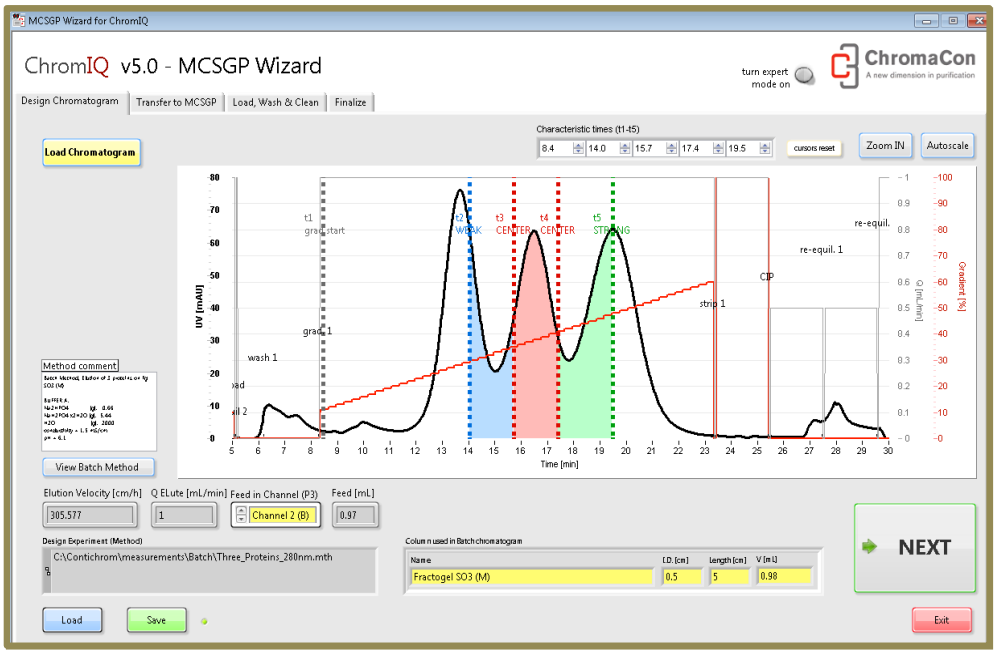
Process design in 4 easy steps with the MCSGP Wizard

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

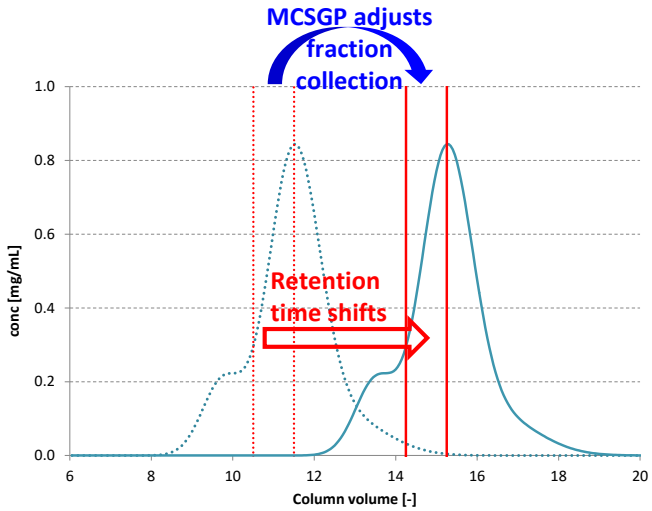
STEP 3:
Set desired washing and cleaning protocol

STEP 4:
Finalize method: Set number of cycles and fractionation



Dynamic process control with MControl

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

N-Rich

Process Principle

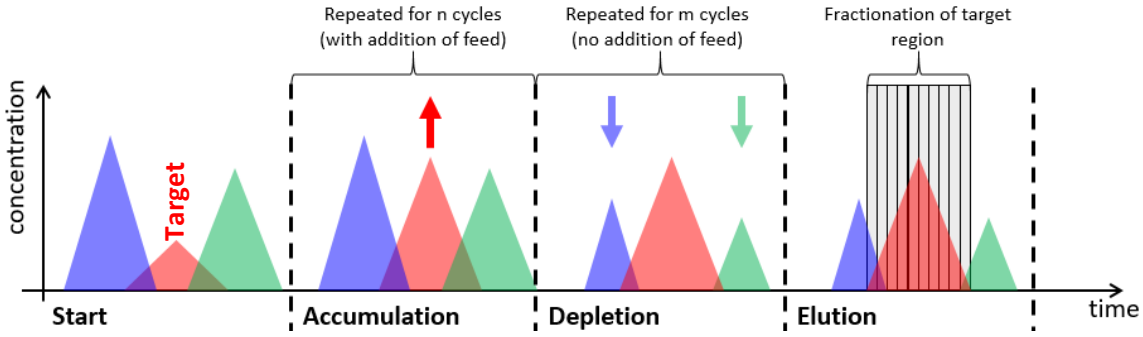
Enrich your target and simultaneously deplete overlapping compounds

The isolation of minor impurities from a drug substance or product can be extremely tedious. Many cycles of high resolution analytical chromatography are typically needed to isolate the target compound in sufficient quantities and purity for further assays and analysis. This can take weeks or months of work, and in the meantime the target compound may be degrading.

N-Rich automatically enriches and isolates initially highly dilute fractions in sufficient quantity and purity, and typically overnight. N-Rich

- enriches the target impurity by accumulating it over several chromatography step cycles
- depletes overlapping contaminants over several chromatography cycles
- and finally separates the enriched impurity by a gradient elution

The number of repeated cycles determines the amount of purified final target and can thus be chosen. N-Rich is an especially useful tool for isolating product-related impurities and in biologics/biosimilar product development.

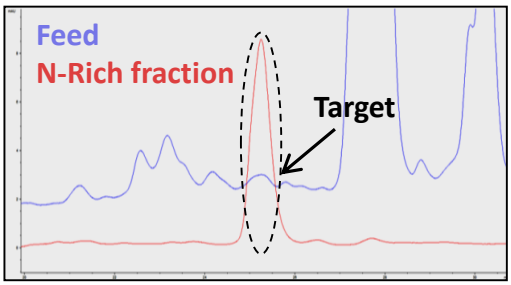


N-Rich case study: Enrichment and isolation of a minor impurity from a peptide

Isolating very small quantities of impurities from synthetic Fibrinopeptide A is tedious. We targeted an impurity that was 1.2% of the feed using N-Rich and batch chromatography.

Using the N-Rich wizard, we ran feed with the twin column Contichrom CUBE Combined system overnight. In the morning we collected the target fraction 600 times enriched. Preparative batch chromatography did not provide sufficient purity and quantity. To obtain a similar amount of compound with same purity by batch we would have had to use much more feed and dozens of injections at high-resolution analytical scale HPLC.

More information on this, and an additional application note on the isolation of antibody isoforms are available upon request from info@chromacon.com.



Process	Purity	Conc. factor	Enrich-ment factor
N-Rich®	> 80%	10x	> 600x
Batch	< 20%	1x	n.a.

N-Rich

Software Tools

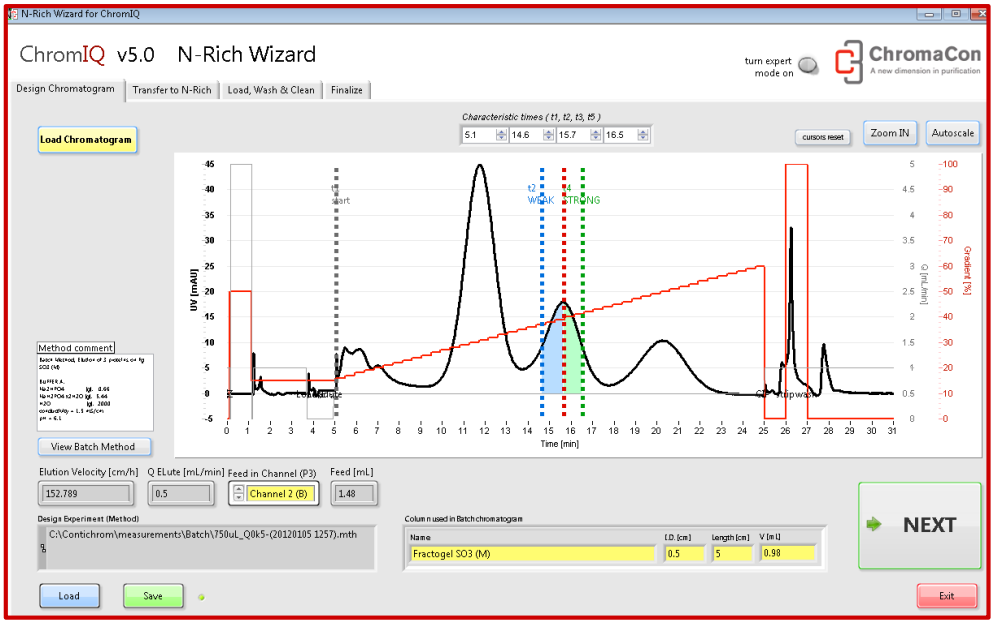
Process design in 4 easy steps with the N-Rich Wizard

STEP 1:
Load chromatogram of batch run and select region to enrich by Drag & Drop

STEP 2:
Set column size

STEP 3:
Set desired washing and cleaning protocol

STEP 4:
Finalize method: Set number of cycles and fractionation



N-Rich for Biopharmaceutical development

Well-characterized biopharmaceuticals are defined during their development by identification and quantification of both process- and product-related impurities. Product-related impurities of the drug substance are molecular variants with properties different from those of the desired product and are formed during manufacturing or storage.

ICH Q6B and ICH Q3A (R2) guidelines require the isolation and characterization of product-related impurities. A long impurity isolation process itself may change the nature of the impurity itself during the isolation process. With N-Rich, a fast overnight run typically provides sufficiently highly enriched material for further analysis on the next day.

The N-Rich process provides unparalleled separation and enrichment of chosen target compounds during chromatographic separation processes.

- ✓ **Isolation of minor compounds from complex feed streams**
- ✓ **Rapid isolation of product-related impurities**
- ✓ **Getting compliant to CMC requirements of ICH Guidelines**

Further N-Rich application examples

- Development: isolation of product-related impurities
- Research: general mining of proteomes or metabolomes: for sample preparation with consecutive MS/MS evaluation
- Research: isolation of biomarkers: for sample preparation with consecutive MS/MS evaluation
- Research: isolation of therapeutic targets for development of bioassays
- Research: mining and isolation of natural products with therapeutic potential
- Research: fractionation and isolation of leads & product-related impurities

ChromIQ

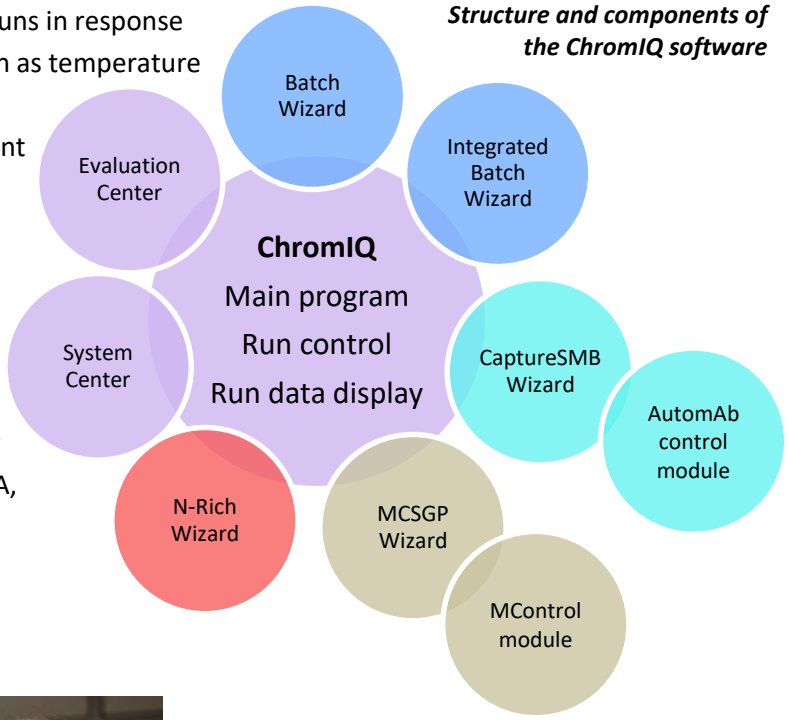
Design Principle

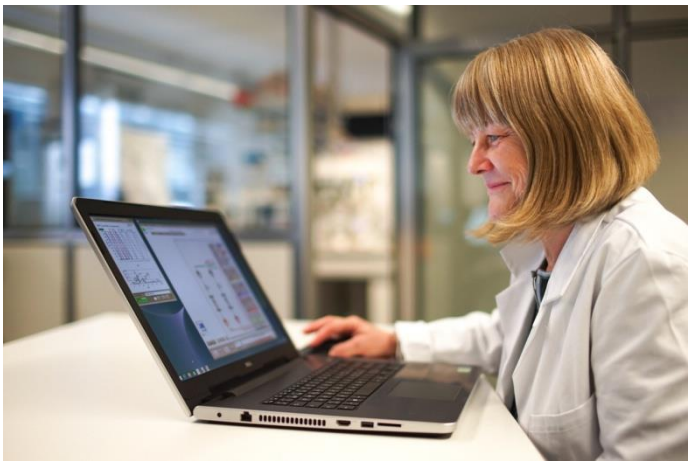
Designed to be intuitive and user-friendly

The ChromIQ software controls the Contichrom CUBE preparative protein chromatography systems through an intuitive, user-friendly interface. It contains all the tools for more efficient separation and purification processes including batch, and twin column processes. Conversion from Batch to multicolumn processes is a simple matter of a few mouse-clicks.

- ChromIQ is navigated through a tab-based structure with different functionalities easily visible
- Wizards and drag-and-drop features make process design easy
- A pool of pre-defined methods is available from the start
- While the experiment is running, you can see exactly what is going on in a real-time flow diagram
- Leaving the run unattended or overnight, the smart buffer management system ensures that you never run out of buffers or reagents
- Dynamic process controllers optimize runs in response to changes in external parameters, such as temperature of buffer quality
- Analyze your data by overlaying different signals and cycles, and configure and print pdf reports
- Alternatively, simply export your data into common external analysis programs including MS Excel
- Data and methods can be transferred seamlessly to the complementary GMP scale-up system from our partner LEWA, the EcoPrime Twin®

Structure and components of the ChromIQ software



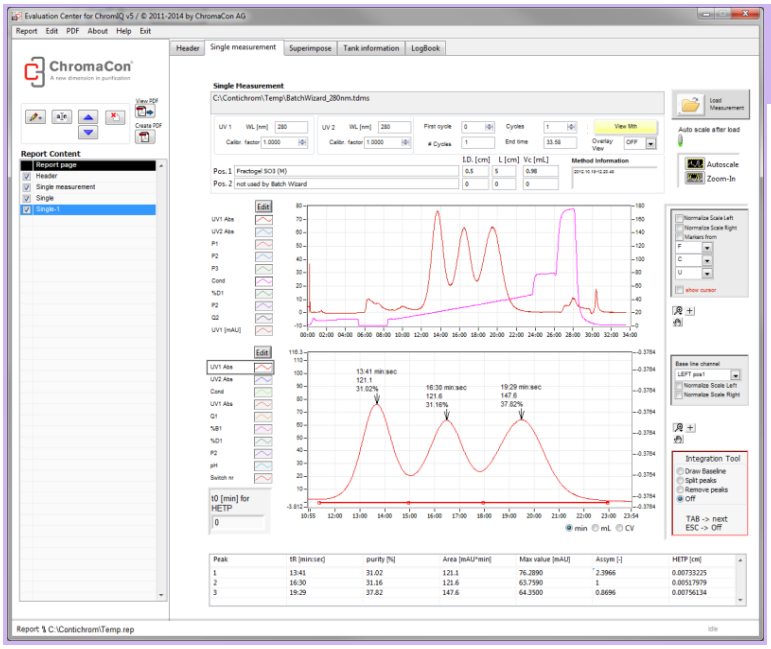


ChromIQ contains essential elements for 21CFR part 11 compliance, including:

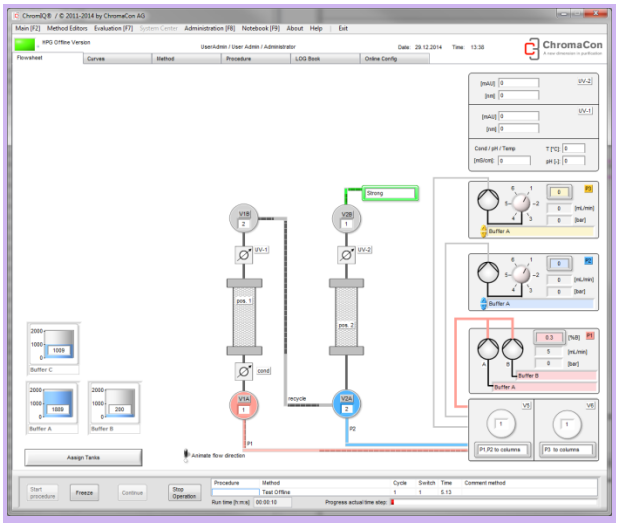
- Pre-defined user groups such as administrator, R&D and production users
- Rights management for individual users
- Password protection of user accounts
- Logging with time stamp and user name
- Electronic signature with checksum of log and measurement files

ChromIQ

User Interface

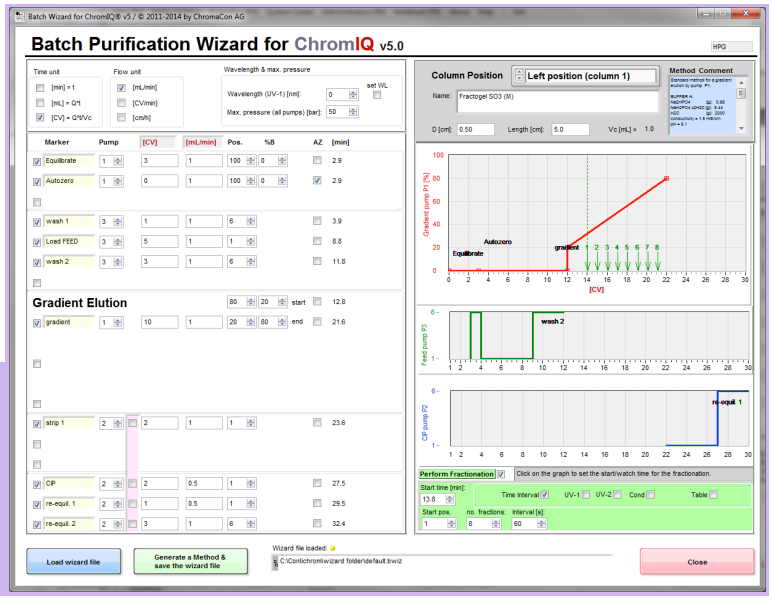


Graph overlays: The Evaluation Center supports the overlay of cycles of a continuous process, and also of entirely different data sets.



Real-time information: The flow diagram shows what is currently happening in the system.

Drag & Drop: The processes can be easily designed and adjusted by dragging vertical guidelines over the chromatogram to define relevant regions.
The picture shows the MCSGP Wizard with regions defined for the product (red) and the recycled side-fractions (blue and green).



Visual process design: During process design the experiments are automatically visualized as graphs. Thus, the results can easily be recognized.

The picture shows the Batch Purification Wizard with the options to customize equilibration, feeding, washing, elution, strip and cleaning sub-procedures. The graphs visualize the overall runs.

Contichrom Systems

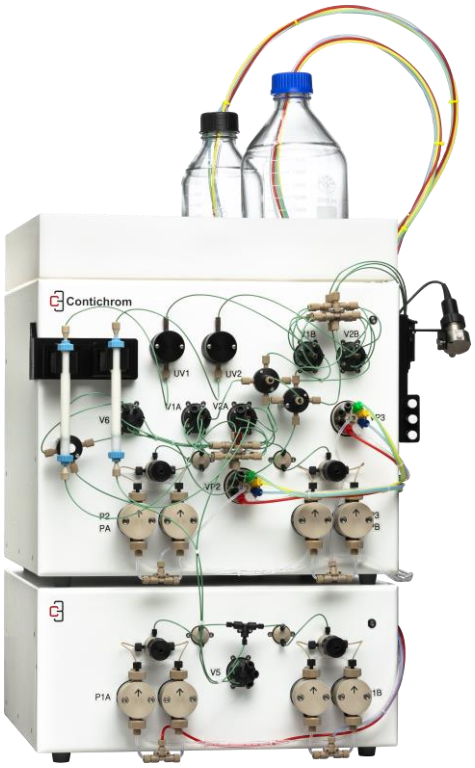
Designed to operate the ChromaCon process portfolio

Contichrom CUBE
Contichrom Discovery

The CUBE – a tool for monoclonal antibody platform purification processes

The Discovery – a tool for fast automated purification of mAbs and His-tagged proteins using standard recipes

- Batch chromatography (isocratic, step gradient or linear gradient)
- CaptureSMB (2C-PCC) (only Contichrom CUBE)
- Capture His-tagged proteins and polish with SEC or IEX
- Capture mAbs and polish with SEC or IEX
- Alternating batch chromatography
  - isocratic or step gradients -
- Integrated batch chromatography
  - isocratic or step gradients -



Contichrom CUBE Combined
Contichrom HPLC

The CUBE Combined – all-in-one process capability

The HPLC – for small molecules and high resolution separation, organic solvent compatible

- Batch chromatography
  - isocratic, step gradient or linear gradient -
- CaptureSMB (2C-PCC) with dynamic AutomAb
- MCSGP with dynamic MControl
- N-Rich : selective enrichment
- Alternating batch chromatography
  - isocratic, step gradient or linear gradient -
- Integrated batch chromatography with gradient and in-line dilution
- Capture His-tagged proteins and polish with SEC or IEX
- Capture mAbs and polish with SEC or IEX

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. O. Ludemann-Hombourger, “The ideal peptide plant,” Spec. Chem. Mag., 30–33 (2013).
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