ChromaCon®
A new dimension in purification

Contichrom® overview

all-in-one purification equipment with a novel process principle

UCB Slough
November 12th 2012

Contichrom® advantages

ENABLES

- the large volume purification of chemicals and biologics
- the generation of lifecycle extensions for marketed biologics

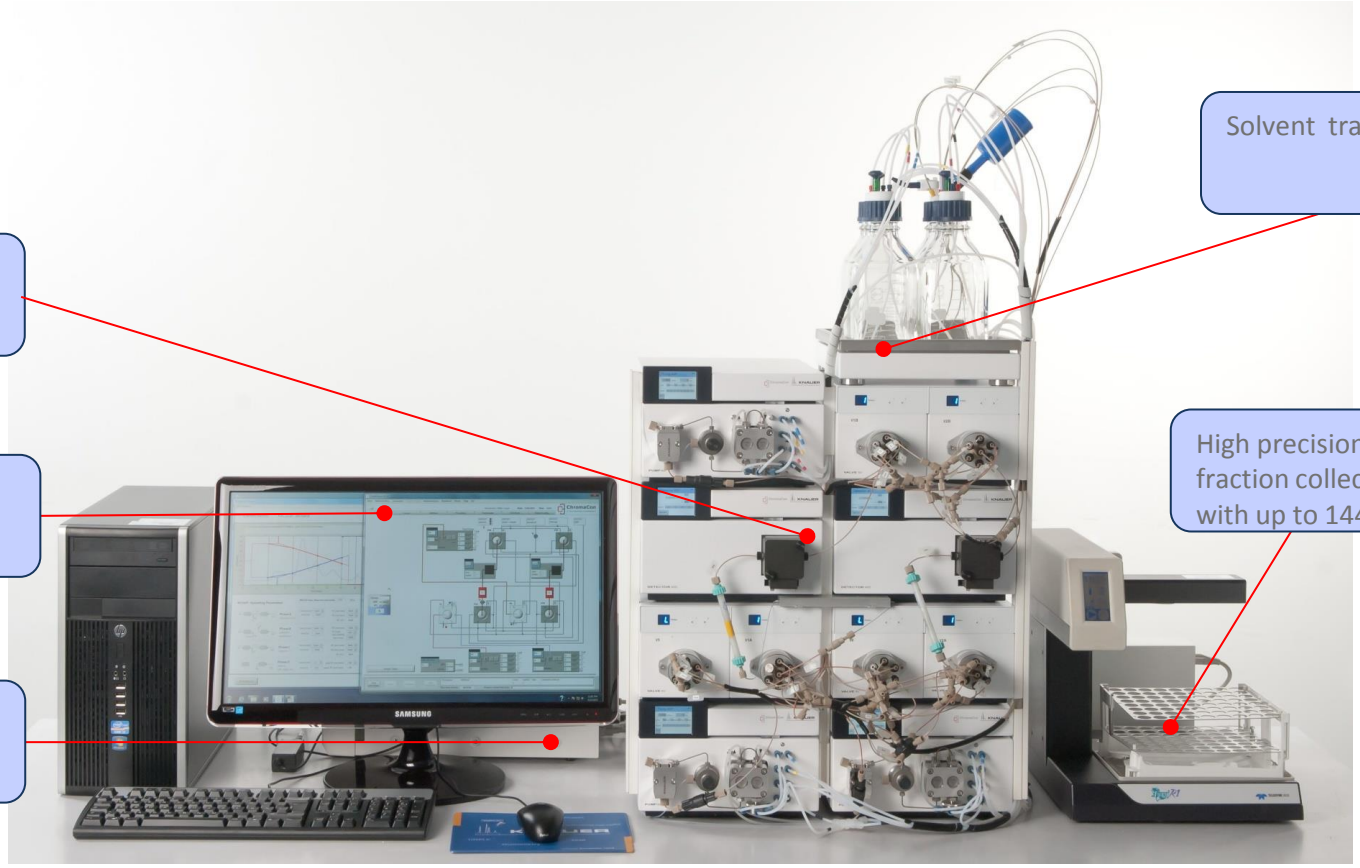
SAVES

- 30% CAPEX & 50% OPEX
- Purity increase by 50%
- Yield increase by 50%
- Throughput increase 10x
- Buffer reduction -75%

ACCELERATES

- Discovery of leads
- Development retaining product profile at upscaling

Contichrom®: Hardware Overview



Contichrom®: Hardware Overview

High-precision sample pump

Two UV-detectors with low hold-up volume

Reliable multi-position valves

Two precise gradient pumps with integrated seal wash

Contichrom®: Equipment Lines

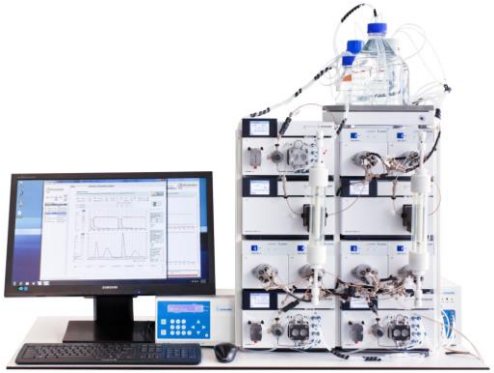
Discovery
- Enabling on-line enrichment of lead targets for enhanced discovery via LC-MS/MS

Lab-scale
- Cost-competitive, all-in-one process capabilities

Pilot-scale (GMP)
- High performance process for preclinical and clinical material

Production-scale (GMP)
- High throughput, reduced CAPEX and COG

(in development)



(**launched world-wide**)

KNAUER

(prototype, **launched**)

(© Novasep)

(in development)

Contichrom® offerings

- Contichrom® product lines:
  - Lab-10: up to 20g/day
  - Prep-100: up to 200g/day
  - Pilot-500: up to 300kg/year
    (for biopharm PhI-III & market,
    launch Q2 2013)
  - Process scale: with large engineering companies,
    dedicated to specific customer needs
- Services:
  - Feasibility studies, process development & optimization
  - Trainings, webinars

Some Reference Customers

- BMS, Lonza, Merck-Serono, Merck KGa, Merus, Novartis, NovoNordisk, Pfizer, Roche, Scinopharm, ETHZ, DSM

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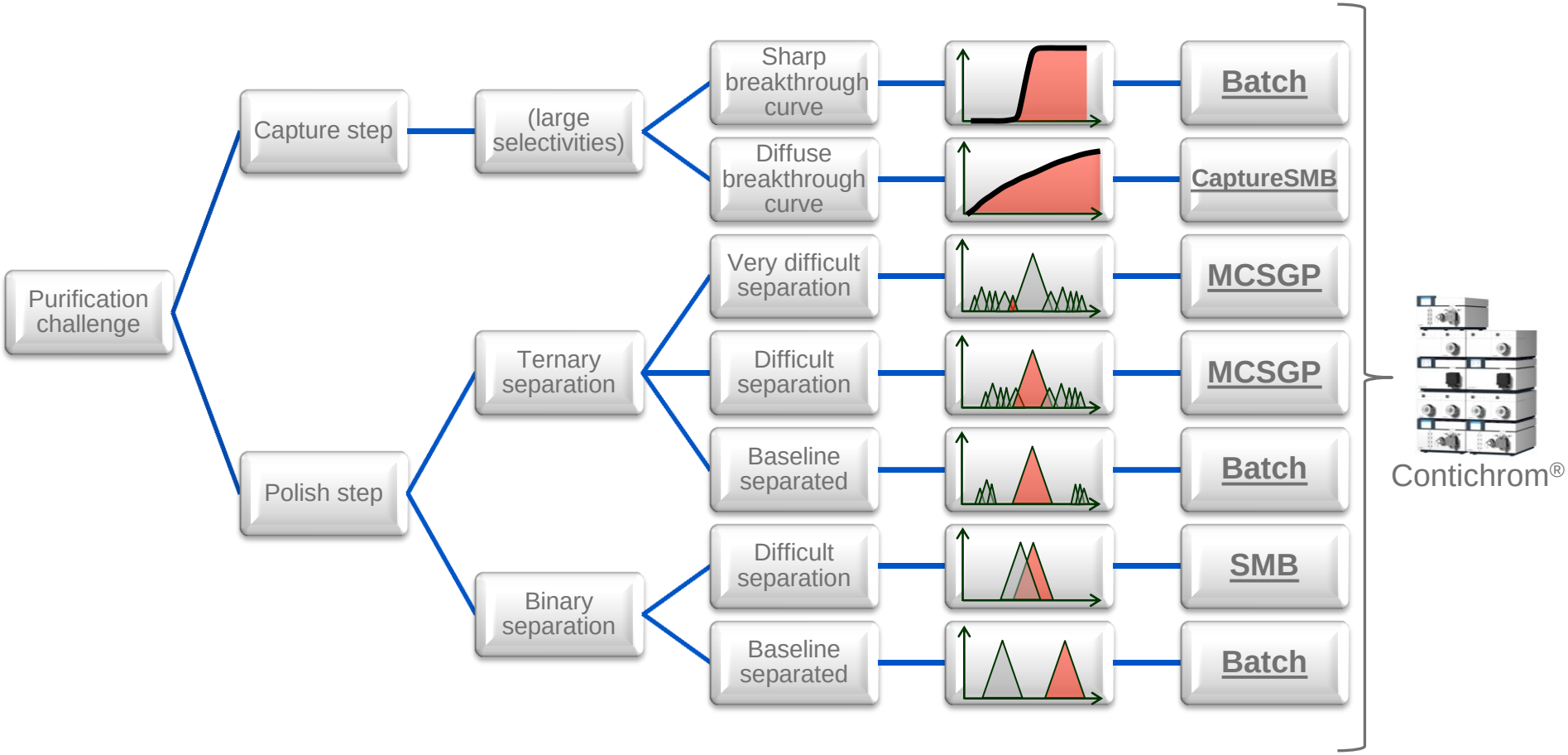
Use of Contichrom®

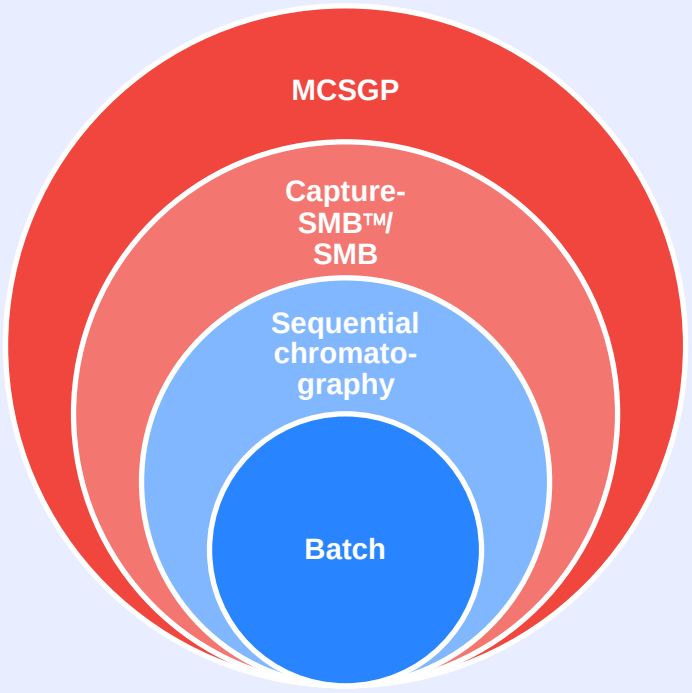
Contichrom®: all-in-one process solutions



Contichrom®: Sales Proposition

All-in-one process capabilities at same price as gold standard

Contichrom®	Preparative HPLC/FPLC



Use of Contichrom®

- **Discovery**
  - Isolation of leads
  - Key driver: speed to lead isolation through enrichment
- **Process development**
  - Isolation of related impurities
  - Fast semi-automated process development
  - Key driver: speed and robust process
- **GMP manufacturing**
  - For clinical trial material and for market supply
  - Key drivers: Compliance, Quality/reliability, CAPEX, OPEX

Application of Contichrom: product classes

Small molecules

- Pharma
  - Synthetic peptides, chiral molecules, macrolides
  - Antibiotics
  - Complex API
- Nutraceuticals/Food
  - Fatty acids, Flavonoids, Polyphenols, Sweeteners
- Industrial biotech
  - Fatty acids, monomers, organic acids
- Chemical intermediates
- Metals (REE)
- Natural extracts

Proteins

- Recombinant bio-pharmaceuticals
- Monoclonal antibodies (mAbs)
  - Antibody capture with CaptureSMB
  - Antibody polish with MCSGP
  - Aggregate removal
- 2nd generation products
  - Biosimilars
  - Antibody isoforms
  - Bispecific antibodies
  - PEGylated and conjugated proteins
- Blood plasma products

Compatibility of Contichrom®

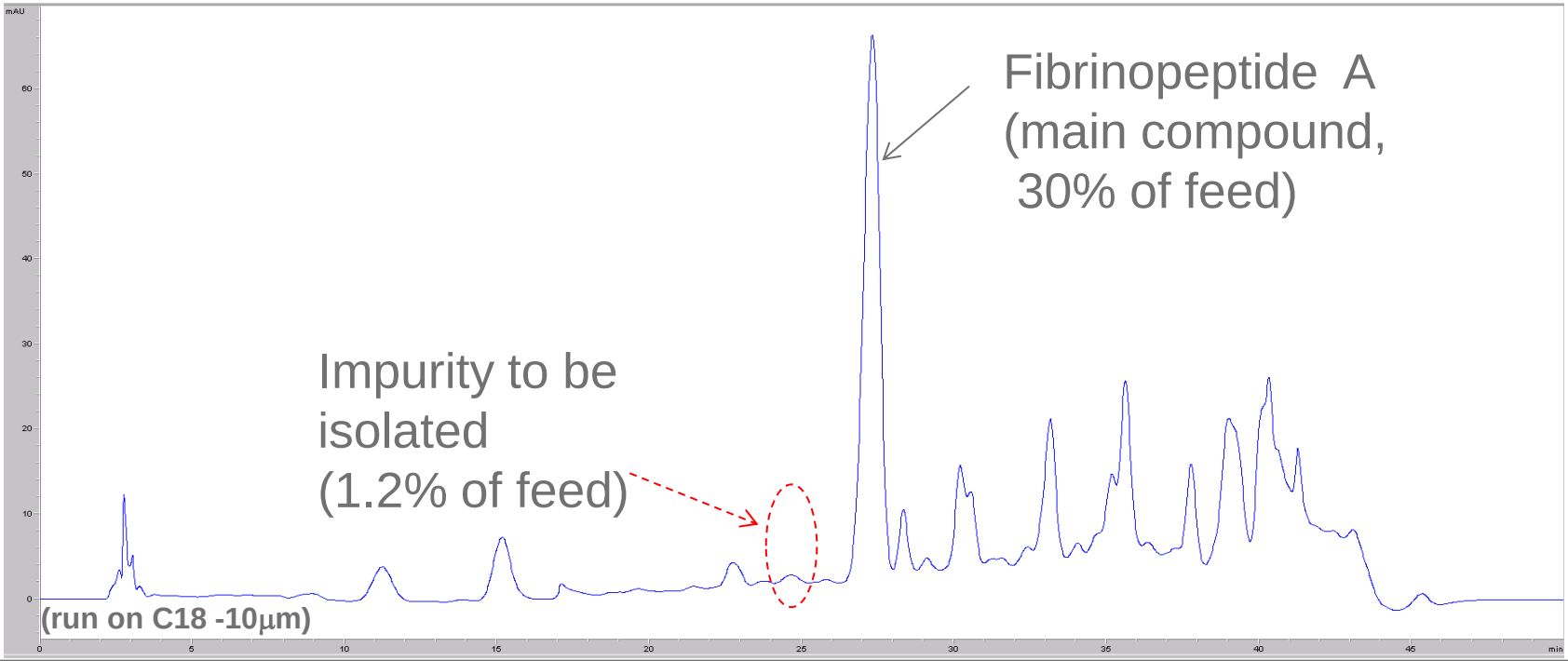
- Contichrom® can be used with all chromatographic modes, e.g.
  - RP
  - CIEX
  - AIEX
  - HIC
  - mixed-mode
  - SEC
  - Affinity
  - …

Contichrom® for discovery

- Applications in Discovery and in the isolation of product-related impurities:
  - The MCSGP process allows selective enrichment of a defined region of the chromatogram
  - The enriched fraction can be isolated for functional characterization or analyzed directly online using mass spectrometry analysis

Impurity isolation using Contichrom® (MCSGP)

- Aim: Isolate weakly adsorbing impurity of Fibrinopeptide A using preparative RP chromatography
- Options:
  - Batch chromatography process
  - MCSGP process



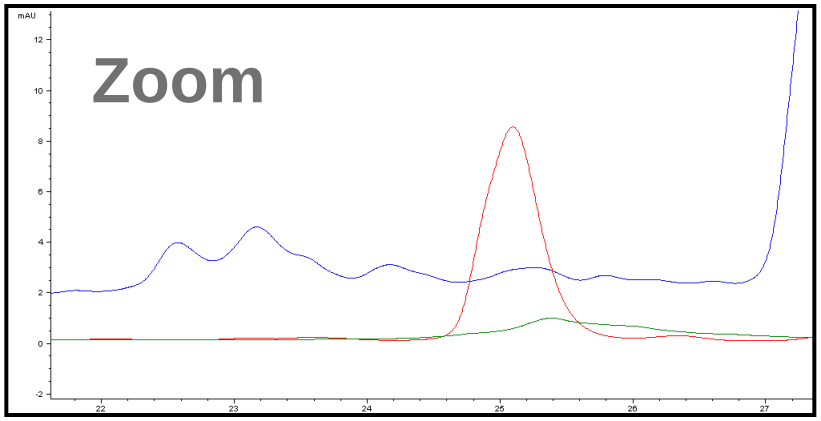
Impurity isolation using Contichrom® (MCSGP)

- Fibrinopeptide A: Analytical chromatograms showing feed, purest side component fractions of batch and MCSGP process

Impurity isolation using Contichrom® (MCSGP)

- Process performance (Fibrinopeptide A case)

Process	Purity	Concentration factor	Enrichment factor (w.r. to main compound)
MCSGP	> 80%	10x	**>600x**
Batch	< 20%	1x	n.a. (purity too low)



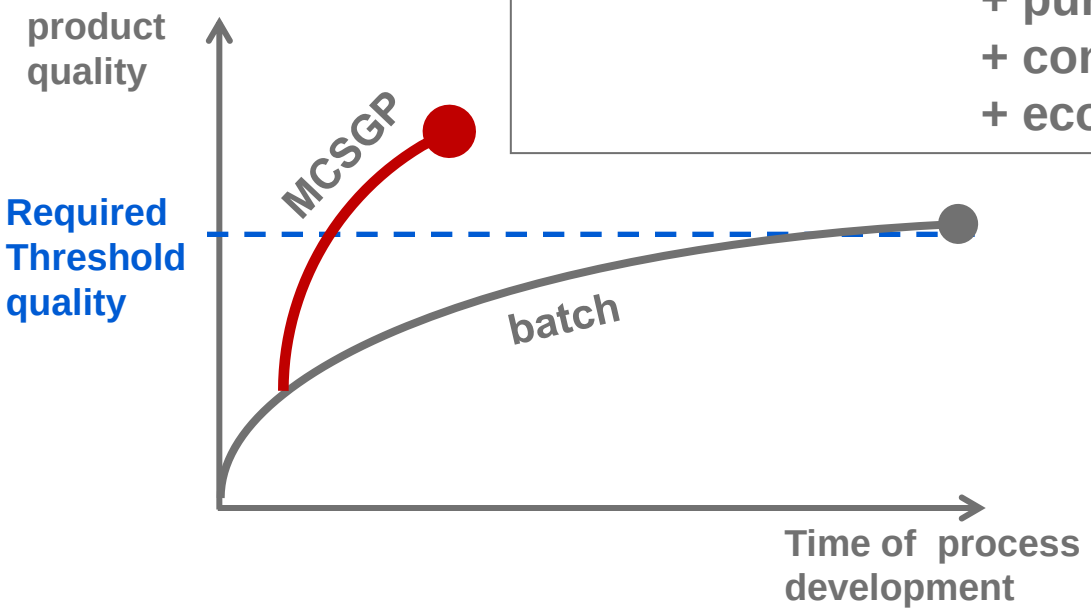
Blue: Feed
Red: Side component by MCSGP
Green: Side component by batch

Contichrom® in Process Development

- Process development is done with the aim of developing fast a robust process that is scalable
- With Contichrom®, PD can be shortened:
  - No need for time- consuming screening as the starting point is a simple non-optimized batch column step that is inherently optimized in the MCSGP mode
  - Automated process conversion from batch to MCSGP yielding superior **process quality features**
  - **The MCSGP process** is easily scalable and robust and can level out upstream process variability by providing a constant product profile
  - Small particle size resins can be employed for higher resolution from small scale to large scale useful for separating aggregates and related impurities

Contichrom® process development

Threshold quality = scalable process
+ purity
+ controlled impurities
+ economic yield



In order to achieve a required threshold quality with an optimized batch process, extensive process development has to be performed. Switching to MCSGP from a simple, non-optimized batch process yields a superior product quality in a shorter time

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Case study:

CaptureSMB® with Protein A on Contichrom®

Executive summary

Using a twin column process (CaptureSMB ®) with Contichrom ® equipment instead of a single column process for Protein A capture steps has significant advantages:

- Higher loading velocities shortening the transit time for the capture step by 200%
- Optimal use of Protein A resin capacity leading to a resin cost saving of 50%

Abstract

- A twin column CaptureSMB® process with JSR's Amsphere™ Protein A media for a cell culture supernatant was successfully executed
- Amsphere™ Protein A is a small particle, pressure resistant material, suitable for high-velocity applications
- Contichrom® equipment was used with a feed flow rate of 300 cm/h
- CaptureSMB® has improved resin capacity utilization by more than 200%
- Excellent yield of mAb: 99%
- Buffer consumption reduced by more than 50%
- Significant productivity advantages of CaptureSMB® expected at higher loading flow velocities

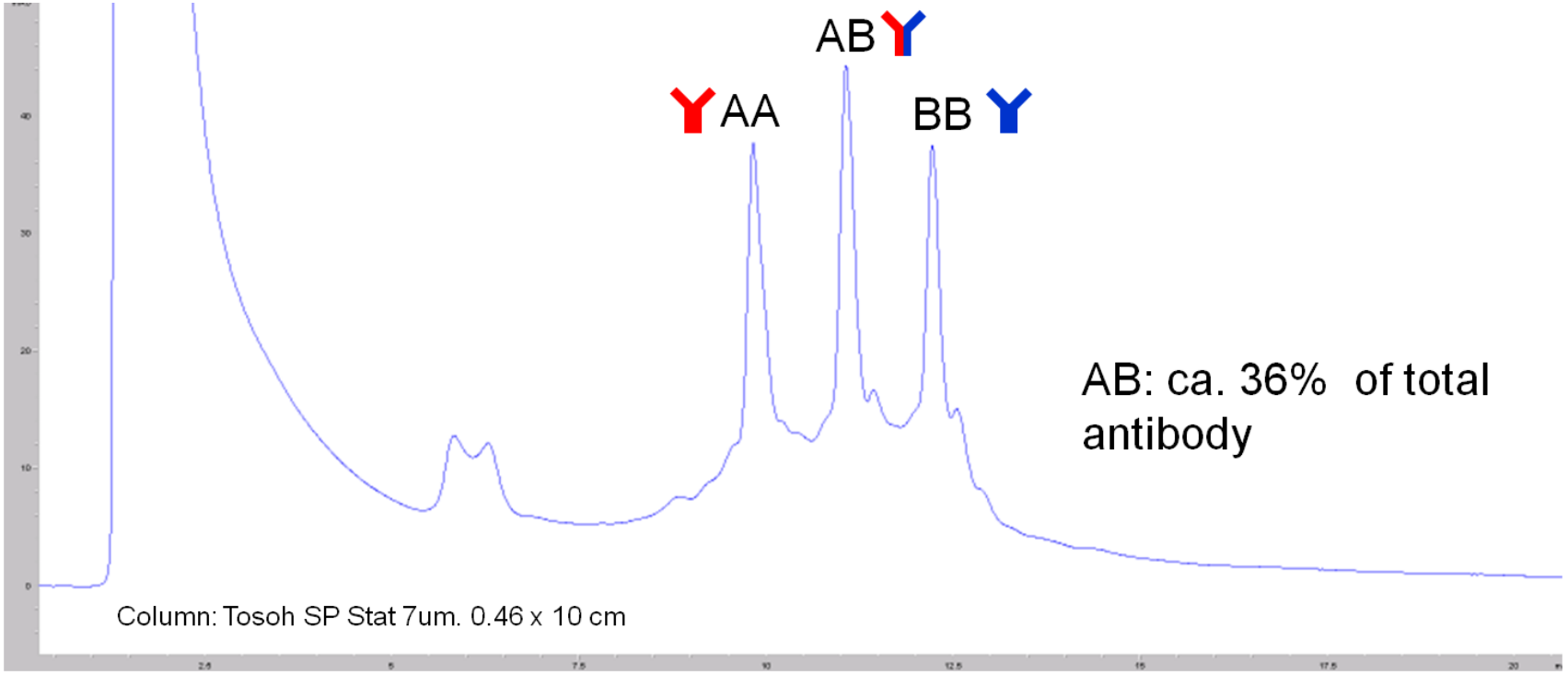
Process	mAb Pool Concentration	Load per column volume	Ratio of load to static capacity	Buffer consumption
	[mg/mL]	[mg/mL]	[%]	[L/g]
CaptureSMB®	7.3	36.5	90%	0.29
Batch	3.3	16.3	40%	0.65

Bi-specific mAb purification

- Partner: merus

- Purification of a common light chain bispecific antibody using Contichrom (MCSGP)

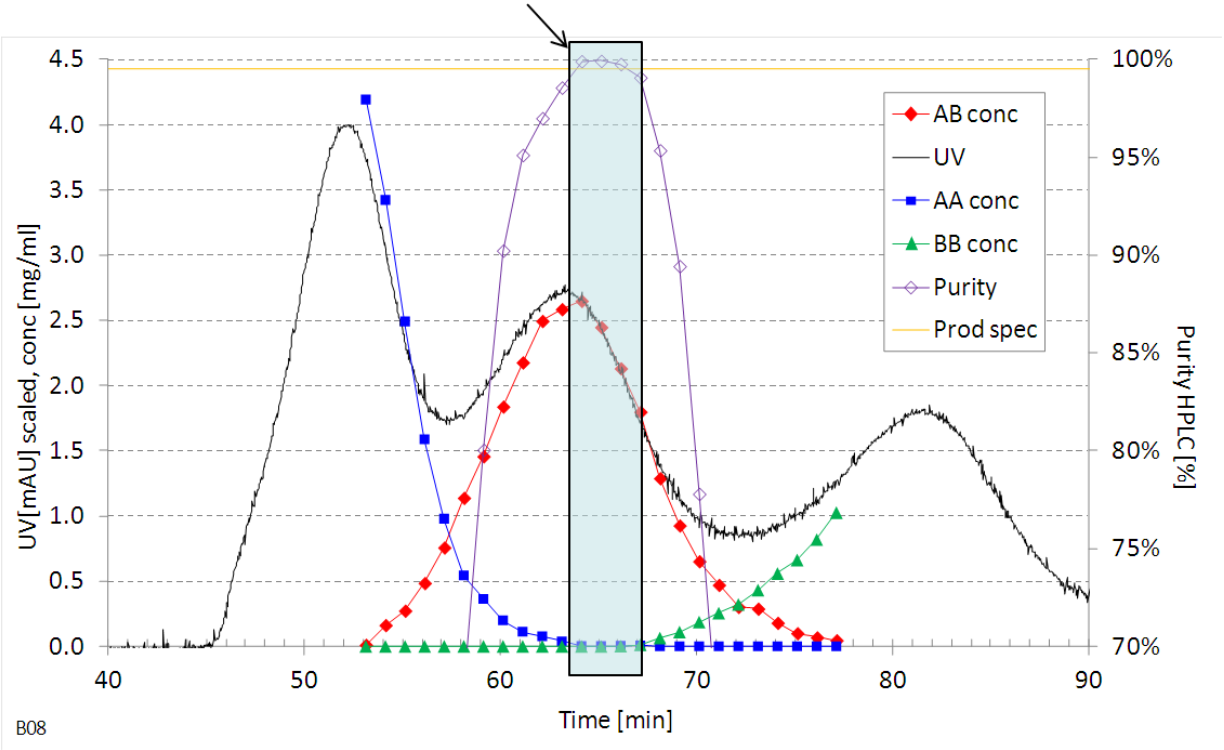
Purification challenge



(Representative analytical chromatogram (CIEX) of the clarified harvest)

Purification challenge

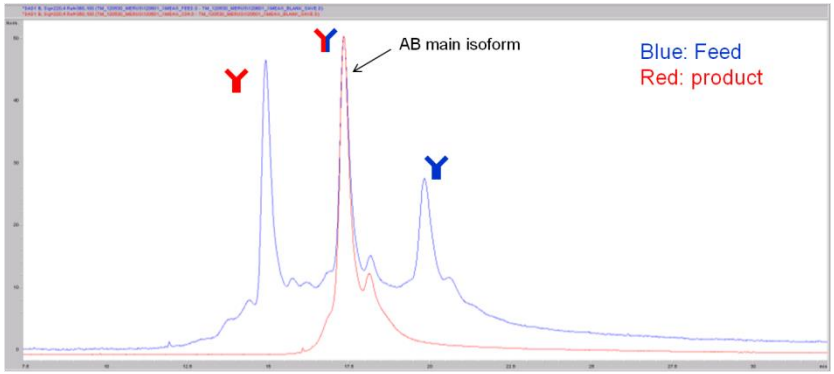
- Preparative overloaded run (0.5 x 15 cm column, Poros 50HS)
- Only a small fraction of the product is in specification!



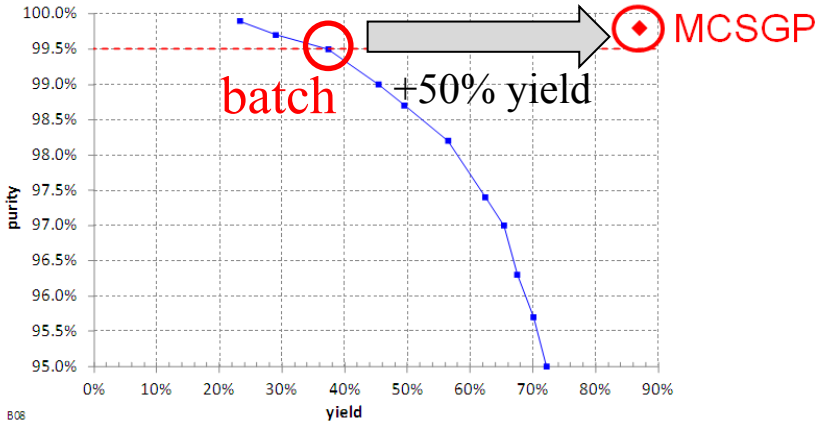
Contichrom® (MCSGP) performance

Contichrom® (MCSGP):

- delivers high purity >99.5%



- increases yield by 50%
  - batch yield: 37%
  - MCSGP yield: 87%

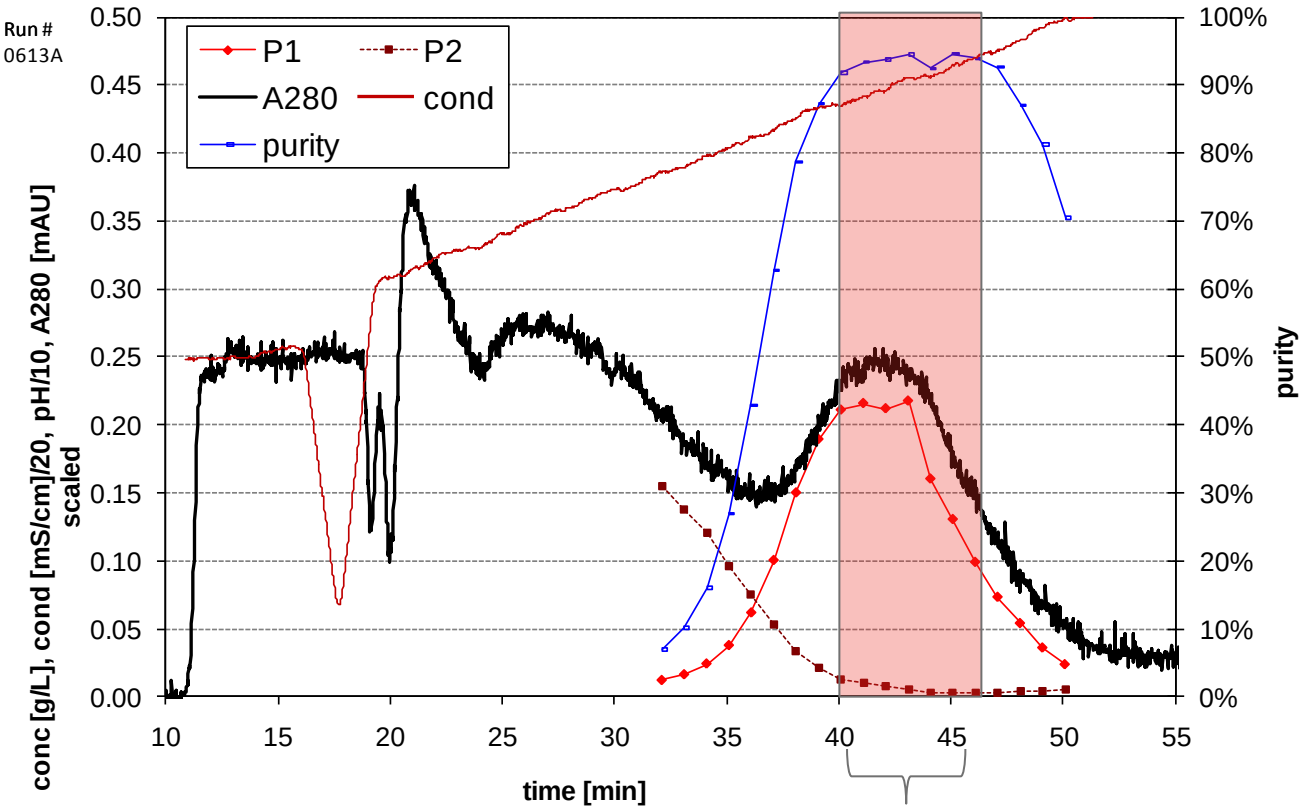


Purification of PEGylated proteins

- Preparative separation with AIEX
- Separation of mono-PEGylated protein from multi- and un-PEGylated protein
- Model system

Preparative AIEX Separation – batch

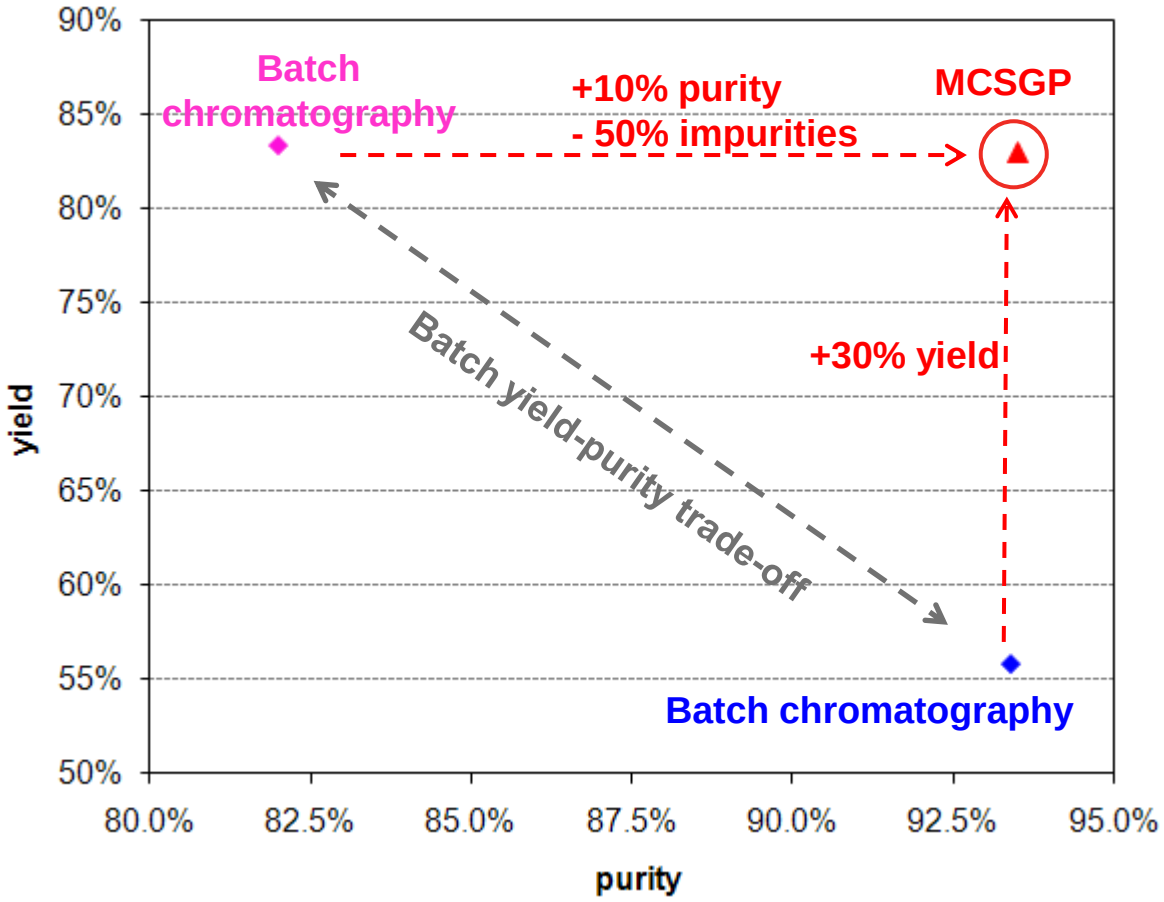
- Linear gradient elution, single column, load 4.3 g/L



Pool fraction

Preparative AIEX Separation – comparison

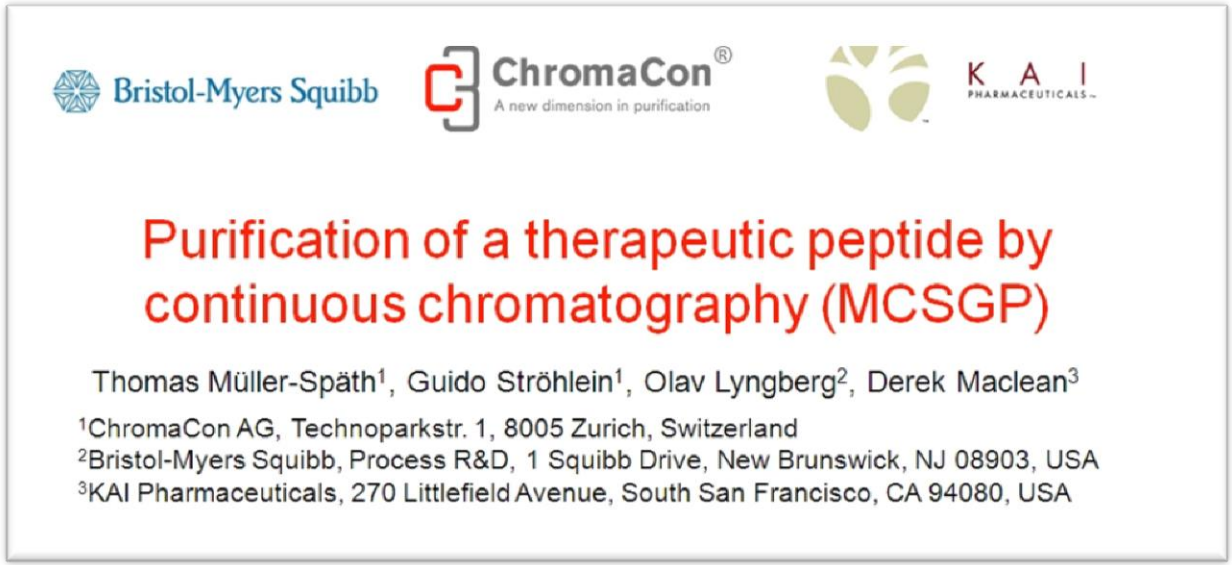
- Contichrom® significantly increases the yield at high purity



Application

Peptide Purification

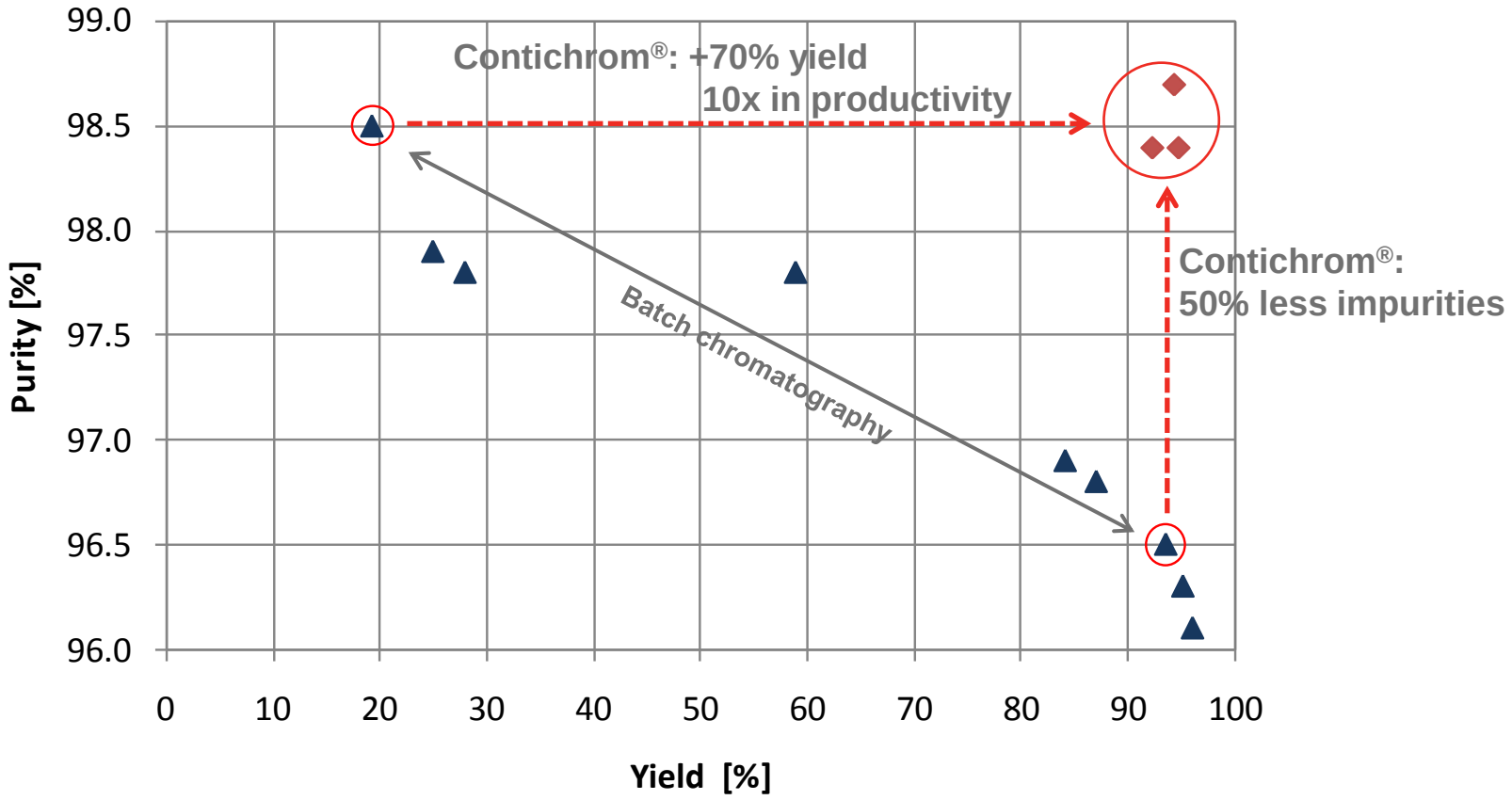
- Acknowledgments:



- Aim of project: Purify a peptide from chemical synthesis with high yield and high purity

Comparison: Batch and MCSGP

- Overview of results



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Appendix

Comparison of multi-column, lab-scale system

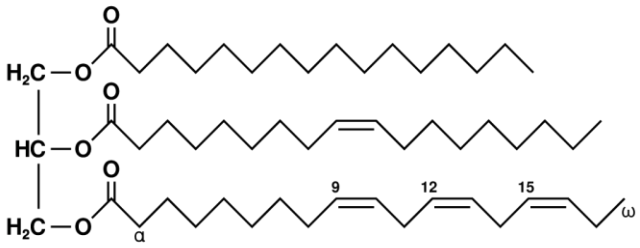
	Contichrom® all-in-one (ChromaCon)	Octave® (Semba Bioscience)	BioSMB® (Tarpon Biosystems)	
No. of columns	2	4 (max. 8)	6 (max. 16)	>90% of applications require only 2 columns
No. of valves	8	72	128	Less hardware increases robustness
Process modes:	• CaptureSMB/SMB • MCSGP • Batch • Seq. flowthrough	• CaptureSMB/SMB	• CaptureSMB/SMB	Contichrom® has all-in-one process capabilities
Misc.	• 1 gradient pumps • no need for utilities	• no gradient pumps • requires pressurized N_2	• no gradient pumps • requires pressurized air	more flexibility

ω-3 fatty acid separation

- Fatty acids are useful in for many applications, including in pharmaceuticals, nutraceuticals, food supplements and chemical intermediates
- Often large volumes (multi-tons) at high purity (>80%) are required for the applications
- Classical large-scale purification techniques such as distillation and precipiation are not capable of providing high purity fatty acids
- Comparing available purification technologies, Contichrom® with MCSGP seems to be the only available production technology capable of producing high volume, high purity fatty acid products at reasonable COG

Introduction: Fat, Oil

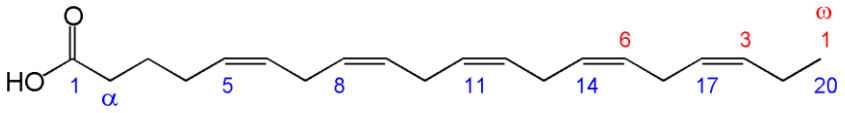
- Oil (liquid at room temperature), fat (solid at room temperature):



Example of an unsaturated fat triglyceride. Left part: glycerol, right part from top to bottom: palmitic acid, oleic acid, alpha-linolenic acid,

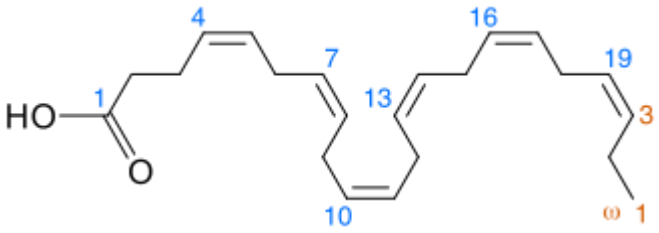
Source: Wikipedia

- Major ω-3 fatty acids:
- Eicosapentaenoic acid (EPA): C20:5



The first double bond is located at the third carbon from the methyl end of the fatty acid chain, known as the *n* end. Thus, EPA acid is a polyunsaturated *n*−3 (omega-3) fatty acid

- Docosahexaenoic acid (DHA): C22:6



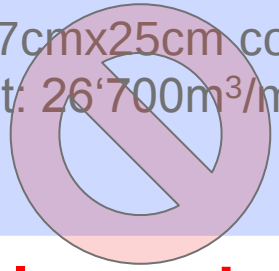
Experimental verification

- Performance summary:

	MCSGP (20 µm resin)	Batch (15 µm resin)	Improvement by MCSGP
Purity [%]	>97%	>97%	
Yield [%]	90%	36%	+ 250%
Productivity (Throughput) [(g product)/(L resin)/(hr operation time)]	65	11	+ 590%
Solvent Consumption [L solvent/g product]	0.8	3.2	- 75%

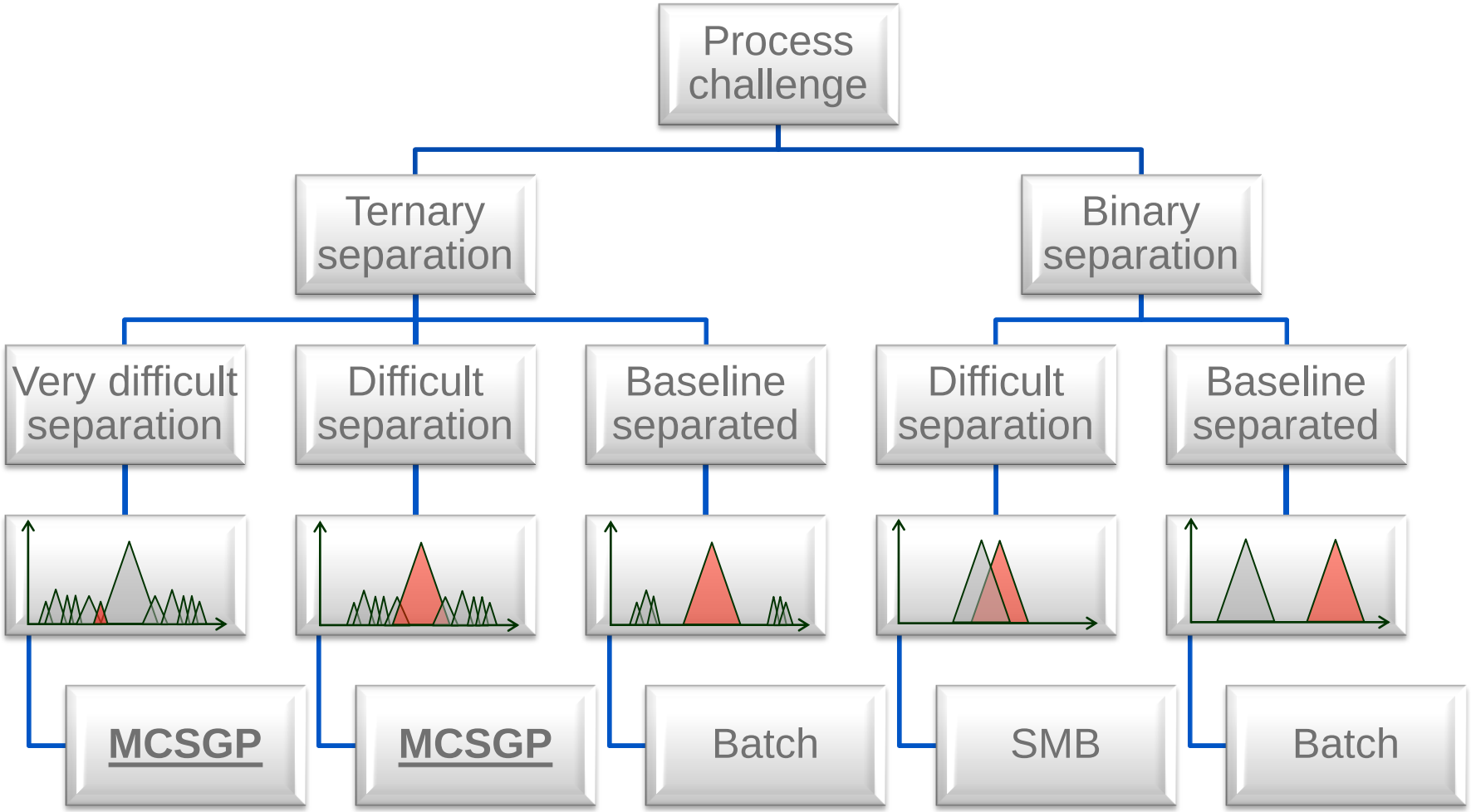
Purification of EPA: Scale-Up modelling

Contichrom® (2 column MCSGP)		Batch
Lab/prep scale (for feasibility)	i.d. 4.5cmx15cm columns, 200kg/year	
Pilot-scale (2 t/year)	i.d. 15cmx15cm columns, solvent: 133m^3/month	i.d. 45cmx25cm column, solvent: 530m^3/month
Production-scale (100 t/year)	i.d. 100cmx15cm columns, solvent: 6‘700m^3/month	i.d. 317cmx25cm column, solvent: 26‘700m^3/month

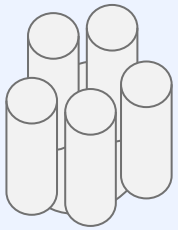
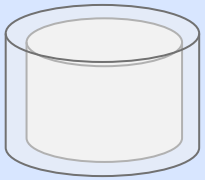


Column size not feasible in batch

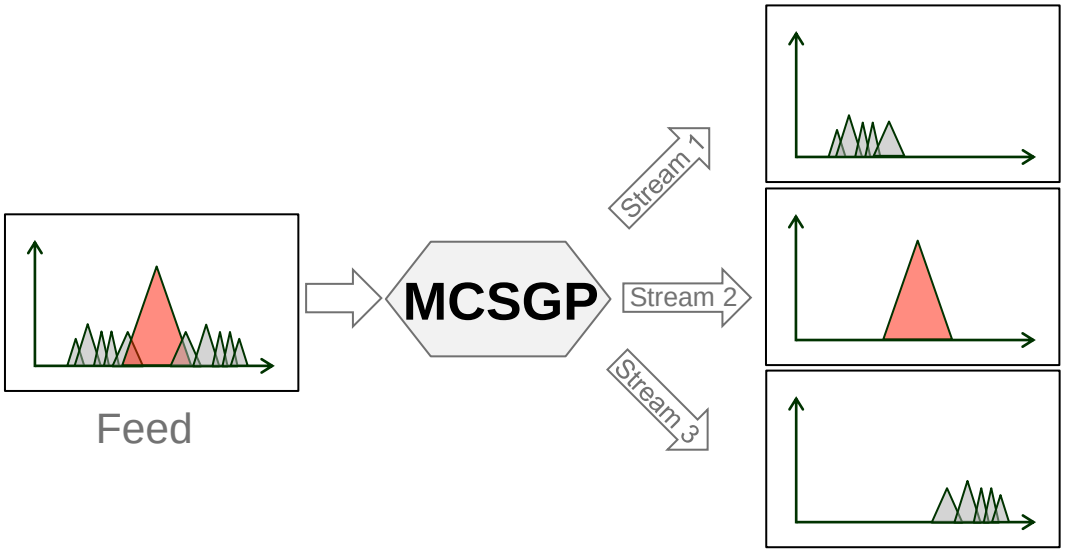
Contichrom®: all-in-one process solutions



Other processes for complex separations

Process	Performance comparison with respect to MCSGP
Batch chromatography 	Relativ to MCSGP, process has • 2-3x higher buffer consumption • 10x higher stationary phase volume • lower yields and purities
Caroussel 	
Annular chromatography 	

MCSGP can purify complex mixtures in a single step



- Two SMB units needed for the same task: ⇨ low throughput, high buffer consumption, large equipment effort