

Breaking the yield-purity trade-off in chromatography purification of peptides and oligonucleotides

Thomas Müller-Späth¹, Massimo Morbidelli²

¹ChromaCon AG / YMC, Technoparkstrasse 1, 8005 Zurich, Switzerland

²ETH Zurich, Institute for Chemical and Bioengineering, Vladimir-Prelog-Weg 1, 8093 Zurich, Switzerland

Summary

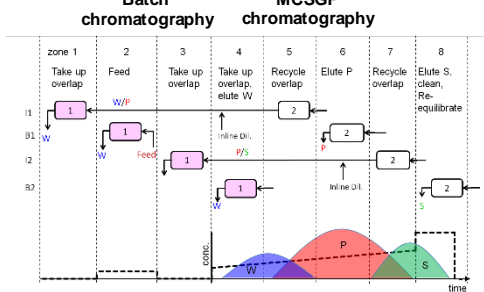
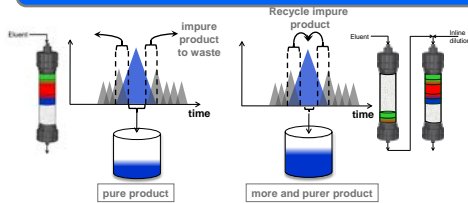
- MCSGP (Multicolumn Countercurrent Solvent Gradient Purification) simultaneously achieves high yield and purity in difficult peptide and oligo purifications

The increased yield of MCSGP ...

- allows downscaling of the upstream chemical synthesis steps
- improves productivity of the downstream process leading to smaller columns required
- reduces solvent consumption
- eliminates the need for re-chromatography
- avoids generation of side-fractions to be stored and analyzed (reduction of analytical burden)

- Economic analysis:** Savings for an annual production amount of 10 kg peptide (synthesis, chromatography and re-chromatography) from US\$ 0.6 million to US\$ 2.1 million expected, in comparison to the single column reference process

MCSGP counter-current chromatography



MCSGP (Multicolumn Countercurrent Solvent Gradient Purification) is a scalable twin-column chromatographic purification process based on the internal automatic recycling of partially pure side-fractions to obtain high yield / high purity simultaneously

Twin-column MCSGP is a process for continuous center-cut purification. The process outperforms traditional batch chromatography by achieving high purity and yield at the same time

The process uses automated internal recycling to recover the product contained in the impure side fractions

Suitable for removal of product-related impurities such as side-compounds and impurities from chemical synthesis, fragments, aggregates

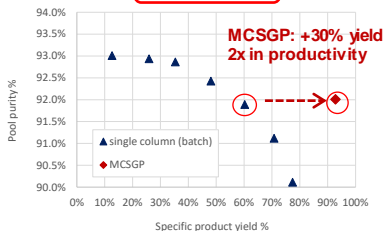
MCSGP operates with only two columns with the same packing material, offering the full benefits of multicolumn chromatography while minimizing equipment complexity

Process schematic showing the tasks of the two columns of the MCSGP process based on the single column design chromatogram

Case studies Oligos / Peptides

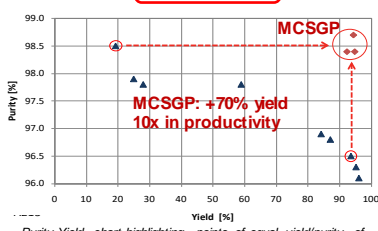
Comparison of batch and MCSGP chromatography for the purification of oligonucleotides and peptides showing improvements in yield/purity through MCSGP

Oligo case



Purity-Yield chart highlighting points of equal purity of MCSGP and batch chromatography

Peptide case



Purity-Yield chart highlighting points of equal yield/purity of MCSGP and batch chromatography

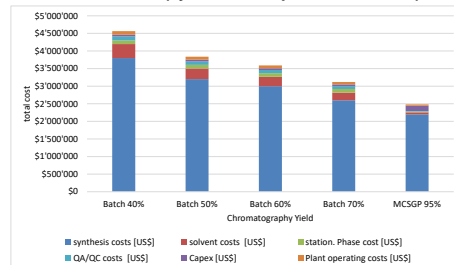
Publication: Chemistry Today - vol. 31(5), pp. 56-60

Economic evaluation (Peptide)

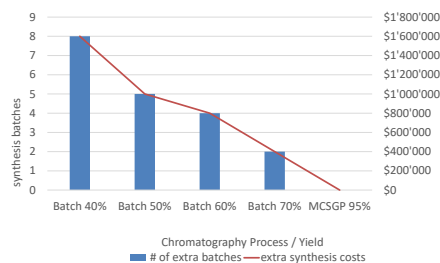
Modeling Scenario (10 kg peptide per year)

- Batch chromatography has varying yields of 40, 50, 60, 70% representing varying impurity content
- The different yields reflect the difficulty of purification for longer peptides (15, 20, 25-mer): longer peptides → more complex synthesis → more impurities → lower yield in chromatography
- The two-column MCSGP process has 95% yield
- The load for all runs was assumed to be 10 g/L

Total cost (synthesis and purification costs)



Number of extra synthesis batches required and associated synthesis costs in dependence of the yield (10 kg peptide p.a.)



Twin-column equipment

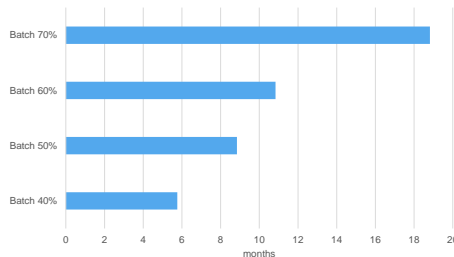


Contichrom HPLC equipment for peptide and oligo purification (ChromaCon)

- Operates with 2 (twin) columns (0.5 – 5.0 cm i.d.)
- Flow rates 0.1-36 mL/min or 0.1-100 mL/min
- pressure rating 100 bar, 1450 psi
- Compatible with all stationary phases, aqueous buffers and common RP solvents, linear gradient capabilities
- ChromIQ operating software for process automation
- Process capabilities:
 - Batch and integrated two-step batch chromatography
 - MCSGP (Multicolumn Countercurrent Solvent Gradient purification, polishing)
 - N-Rich process (purification of product-related impurities)

Economic evaluation (Peptide)

Payback period of MCSGP GMP system compared to single column batch process with X% yield → Investment payback in 6 months to 19 months



Downsizing of columns and equipment

- Batch column diameter reduced from 60 cm i.d. to 2x 30 cm i.d.
- Total column packing volume reduced from 70.7 L to 14.2 L
- Pump flow rate on skid reduced from 8.5 L/min (510 L/h) to 3.2 L/min (190 L/h)

	Batch 1	Batch 2	Batch 3	Batch 4	MCSGP
Yield [%]	40	50	60	70	95
Column inner diameter [cm]	60	60	60	60	30
Column volume [L]	70.7	70.7	70.7	70.7	2x 7.1
Required pump size on skid [L/min]	8.5	8.5	8.5	8.5	3.2



MCSGP GMP system (YMC)
Up to 20 L/min

Solvent consumption of MCSGP compared to batch with X% yield

