Towards Green and Sustainable Peptide Purification using YMC Triart Resin and Twin-column Continuous Chromatography

Best Poster Award Participant

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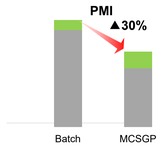
Summary

- A batch purification method for a dual GLP-1/GIP RA has been developed and optimized as
  Stationary phase : YMC Triart Prep C8-XP
  pH: pH 6.7 (ammonium acetate)
  Load: 5.6% (w/w)
- The pH and load amount have a significant impact on the distribution of impurities and the target. Impurities at RRT 0.31, 0.86, and 1.05 are main contributors affecting target purity.
- Retention factor was also optimized by balancing PMI and productivity. Bigger k' leads to higher yield but longer run time.
- MCSGP further increased yield from the optimized batch process, improving high productivity and PMI at the same time.
- AutoPeak controls the process by UV, making it easier to obtain high level quality of fractions in a robust way.



Introduction

Among the various production processes of pharmaceuticals proposed in recent years, SPPS is commonly used in the synthesis process but often needs more buffer and creates many impurities difficult to remove, resulting in higher PMI and lower productivity. To achieve greener and more sustainable peptide production, optimized batch purification method and following continuous purification technology found the reduced PMI and increased productivity from a conventional way.

Following parameters are compared in this study

✓ **Productivity (g/L/h) =** $\frac{\text{Yield of Target}}{\text{Packing material volume} \times \text{Process time}}$

✓ **PMI (process mass intensity) =** $\frac{\text{Total solvent and sample volume}}{\text{Yield of Target}}$

Sustainable process conditions
- ✓ *High Productivity*
- ✓ *Low PMI*

Synthesis PMI and purification PMI[1]

The PMI of the peptide consisting of 39 amino acid residues is estimated to be 34,585, with the majority of PMI attributed to synthesis and purification. In phase 3 and commercial stage peptide case, synthesis and purification accounts for 52.6% and 46.3% of PMI, respectively.

Target Compound

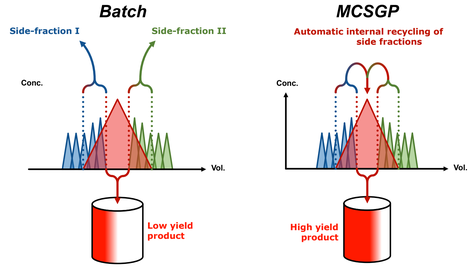
Dual GLP-1 and GIP receptor agonist

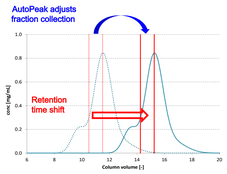
The sample used in this study is a glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) receptor agonist, one of the most promising drugs approved for the treatment of type 2 diabetes and obesity. It controls blood glucose levels, stimulates insulin release, suppresses appetite, and weight loss.

- Molecular weight: Between 4500 and 5000 Da
- Structure: 39 AA sequence

MCSGP Process Principle

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





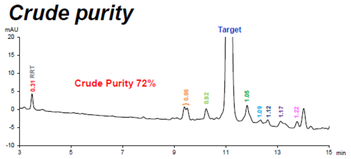
AutoPeak monitors UV behaviors to adjust each recycling and collection phase in MCSGP. It compensates variations of sample lot, resin, buffer and temperature, which allows more robust runs on a production scale.

1) Kekessie Ivy, *et al.*, "Process Mass Intensity(PMI): A Holistic Analysis of Current Peptide Manufacturing Processes Informs Sustainability in Peptide Synthesis", *The Journal of Organic Chemistry*, **89**, 4261-4282, (2024)

2) Ismaele Fioretti, *et al.*, "UV-based dynamic control improves the robustness of multicolumn countercurrent solvent gradient purification of oligonucleotides", *Biotechnology Journal*, **19** (7), (2024)

Process Development Strategy

Crude purity



(Analytical conditions)

Column	: Accura Triart Bio C18 (1.9 µm, 30 mm) 100 X 2.1 mm i.d.
Eluent	: A) 20 mM CH_3COONH_4/acetonitrile (95/5) : B) 20 mM CH_3COONH_4/acetonitrile (5/95) 34.4→52.2%B (0-32 min), 100%B (32-36 min), 34.4%B (36-46 min)
Flow rate	: 0.20 mL/min
Temperature	: 60℃
Detection	: UV at 220 nm
Injection	: 2 µL
Sample	: Target crude (2.0 mg/mL)

Preparative purification parameters

Common conditions Flow rate: 0.5 mL/min (equilibration, gradient), 1 mL/min (strip, re-equilibration), Temperature: ambient, Detection: 260 nm, Sample: 30 mg/mL (target in initial solvent)

Stationary phase X **pH** X **Load** X **Retention Factor**

- ☐ Triart Prep C18-S
- ☐ Triart Prep Phenyl-S
- ☐ Triart Prep C8-S
- ☐ Triart Prep Bio200 C8
- ☐ YMC Triart Prep C8-XP

Column size: 250 X 4.6 mm i. d.
Particle Size: 10 um

Eluent A
- ☐ pH 2.0 (TFA)
- ☐ pH 6.7 (ammonium acetate)
- ☐ pH 8.0 (ammonium bicarbonate)

- ☐ 1.4% (w/w)
- ☐ 4.2% (w/w)
- ☐ 5.6% (w/w)
- ☐ 9.7% (w/w)

=Target Crude [g] / gel [g]

Further study was conducted to apply the best-case scenario to MCSGP, aiming to reduce **process time** and **amount of solvent used** to achieve greener and more sustainable purification process.

Column size: 150 X 4.6 mm i. d.
Particle Size: 10 um

Batch purification methods are optimized by changing parameters listed above

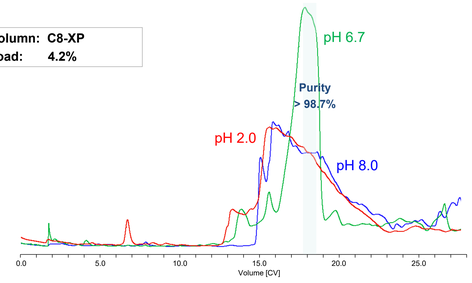
Each PMI and productivity was calculated from fractions of > 98.7% purity.

Results and Discussion

Batch

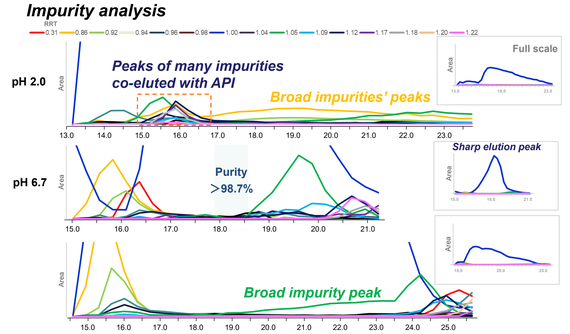
pH

Column: C8-XP
Load: 4.2%



No fraction of > 98.7% purity was obtained under acidic or basic conditions

Impurity analysis

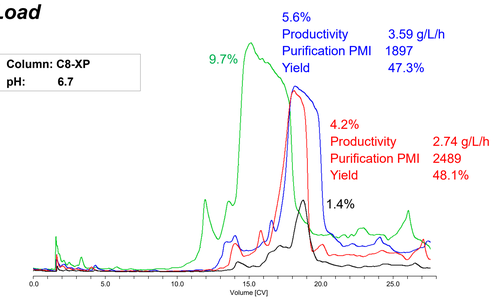


Load

Column: C8-XP
pH: 6.7

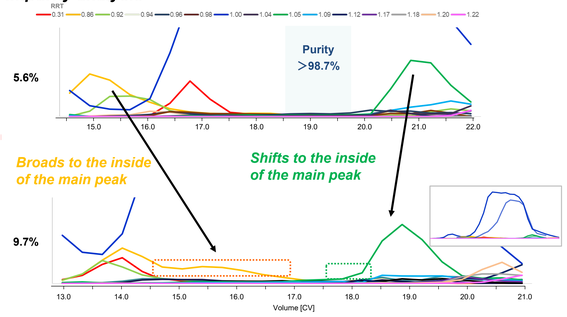
5.6%
Productivity 3.59 g/L/h
Purification PMI 1897
Yield 47.3%

4.2%
Productivity 2.74 g/L/h
Purification PMI 2489
Yield 48.1%



Increasing amount of load improves yield,
while too much load leads to no fraction meeting purity criterion

Impurity analysis



Loading 9.7% crude against resin in weight shifts peaks of some impurities towards the center of the target main peak

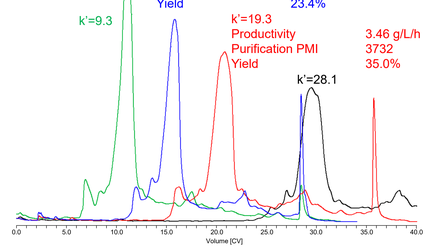
k'

Column bed height was shortened from 25 cm to 15 cm to apply the batch condition to MCSGP to minimize the run time.

Column: C8-XP
Load : 4.2%
pH: 6.7

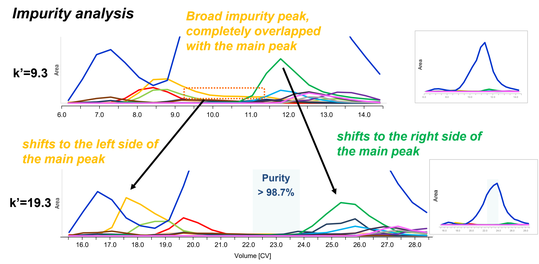
k'=14.2
Productivity 2.75 g/L/h
Purification PMI 4803
Yield 23.4%

k'=19.3
Productivity 3.46 g/L/h
Purification PMI 3732
Yield 35.0%



Bigger k' improves separation, leading to higher yield but this also causes longer run time.

Impurity analysis



Optimized condition of Batch

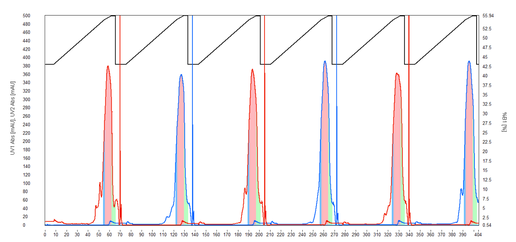
C8-XP × pH 6.7 × 5.6% × k'=19.3

Productivity 3.59 g/L/h
Purification PMI 1897

Optimized method increased yield, maximizing the productivity and minimizing PMI at the same time.

MCSGP

Above mentioned condition k'=14.2 was applied to MCSGP, considering its shorter run time compared to optimized condition k'=19.3. MCSGP improves yield from batch without compromising its high purity, which minimizes the required amount of crude from SPPS. Throughout the 3 cycles using AutoPeak, almost identical peaks of fractions within the same range of high purity were obtained, suggesting a robust production possibility.



Cycle	Purity (%)	Yield (%)
1	98.7	39.7
2	98.9	80.0
3	98.8	76.4
2-3	98.8	78.2

Due to the increased yield from batch, MCSGP decreased PMI by 30% and slightly increased productivity.