Increased Yield

# PEC vs. Flash

A collaborated study of Bachem and Belyntic sheds light on the recoveries of the purification of peptides by both Peptide Easy Clean (PEC) technology and reversed-phase flash chromatography. In total, eight peptides were purified with both methods and investigated. The results reveal significant advantages of PEC to improve the yield efficiency of peptide purification.

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## 1. Introduction

In an ideal scenario of peptide production, a single purification step delivers the desired purity without considerable losses of material. This mission, however, is often difficult to achieve because of the diversity of peptides.[1]
Reversed-phase high-pressure liquid chromatography (RP-HPLC) is a common and well-known technique used for peptide purification. Reversed-phase flash (flash), ion exchange, and size exclusion chromatography are some other purification methods with certain benefits.
Regularly, a combination of two complementary (orthogonal) methods is required and of advantage. In every case, the outcome yields remain a critical issue. High recoveries of each purification step are crucial to avoid high losses of the product while improving the economic and ecological efficiency for the subsequent stage.
Belyntic has added the Peptide Easy Clean (PEC) as a new tool to the repertoire of peptide manufacturing technologies. PEC relies on chemo-selective isolation of the target peptide using catch & release principles.
In this case study, we highlight aspects of purity and recovery of the PEC technology in comparison to flash by the example of eight peptides.

## 2. Method

Peptides (Table 1) were synthesized by Bachem (UK) in a 200 µmol scale on 2-chlorotritylchloride resin. Routine capping using acetic anhydride after each coupling step ensured the selective coupling of the PEC-Linker only on the full-length peptide. The synthetic resin was split into two parts, of which 100 µmol were kindly provided for PEC purification.

The basic cleavable PEC-Linker BL (4.75 eq.) was employed and coupled using Oxyma (8.75 eq.) and DIPEA (8.5 eq.) in DMF for 2 h.
Additionally, the peptides #1, # 3, #8 and #9 were synthesized by Belyntic in a 100 µmol scale on Rink Amide AM resin. The PEC-Linker RC (4 eq.) was employed here, and coupled using Oxyma (6 eq.) and DIPEA (6 eq.) in DMF for 2 h. TFA cleavage for all peptides was performed for 2 h using Reagent K (10 mL cleavage cocktail per 100 µmol peptide).

*Table 1: Peptides used in this study*

| ID | name | Sequence |
|---|---|---|
| #1 | Amyloid-β (21-40) | H-AEDVG SNKGA IIGLM VGGVV-OH |
| #2 | Amyloid-β (1-20) | H-DAEFR HDSGY EVHHQ KLVFF-OH |
| #3 | Prepro Atrial Natriuretic Factor (104-123) (human) | H-SSDRS ALLKS KLRAL LTAPR-OH |
| #4 | PTH Related Protein (67-86) | H-TRYQA KPVNR STPIS TGKEG-OH |
| #5 | T-20-G | H-YLTQE TNKVE TYKEQ PLKTP-NH2 |
| #6 | R-20-L | H-RTGKL APSFN GKSSQ TREIL-OH |
| #7 | Miraculin (1-20) | H-DSAPN PVLDI DGEKL RTGTN-OH |
| #8 | E-20-A | H-ERTGN PIKEQ SSDFQ EEAGA-OH |

PEC purification. The crude material was dissolved in 5 mL citric acid buffer (pH 4.5). The dissolved peptide was added to a pre-conditioned suspension (3 mL) of activated filter material, and the immobilization was performed for 60-90 min before washing off impurities.
Cleavage of PEC-Linker BL: A 0.2 M solution of ethanolamine at pH 11.0 was added in six bead volumes for 60 min to release the peptide. After collecting the filtrate, an additional release-step was performed with the same portion of the alkaline solution for 30 min.
Cleavage of PEC-Linker RC: Per peptide, 500 mg of $PPh_3$ in 9 mL MeCN/H2O/AcOH (90:5:5) were added to reduce the linker (15 min reaction time). The activated filter material was washed to remove excess $PPh_3$. The acidic PEC-Linker cleavage was initiated by adding TFA/$H_2O$ (2:3). After 60 min, 2 ml TFA was added, and the filtrate was collected in 15 ml tubes. Each sample was washed/eluted twice with 2 ml 95% aq. TFA. The peptide precipitated in chilled diethyl for direct use.

Flash chromatography. Flash purification was carried out using a Teledyne ISCO CombiFlash instrument on Daiso C18 20 µm media with a gradient of 10-100% B in 30 minutes (A: 0.1% heptafluorobutyric acid (HFBA) in $H_2O$, B: 0.1% HFBA in 60/40 MeCN/$H_2O$).

Analytics. UPLC-UV and mass spectra were recorded with an analytical Acquity H-Class UPLC-ESI-MS system from Waters on a C-18 column (1.7 µm, 2.1 x 500 mm). As the mobile phase, mixtures of water (A) and MeCN (B) with 0.1% TFA were used.

## 3. Discussion

Choice of peptides. The peptides cover common types of peptides within the research environment. The first two peptides represent difficult/hydrophobic candidates from the amyloid-β family. Two peptides from the catalog portfolio by Bachem were chosen as "easy" candidates. The choice for T-20-G and R-20-L origins in the fact that both show side-reactions in alkaline conditions. Namely, the N-terminal threonine on the T-20-G would undergo a base-induced side reaction to form an oxazolidinone, and the R-20-L bears an Arg-Glu sequence which forms citrulline during basic treatment. The reductively cleavable PEC-Linker, therefore, offers remedy due to side-reaction free release. Finally, peptide #7 and #8 were chosen as acidic candidates to address solubility and investigate aspartimide formation.

*Equation 1: Calculation of the recovery*

$$\frac{weight_{purified}\ x\ purity_{purified}\ /\ MW_{peptide}}{weight_{crude}\ x\ synthetic\ scale}$$

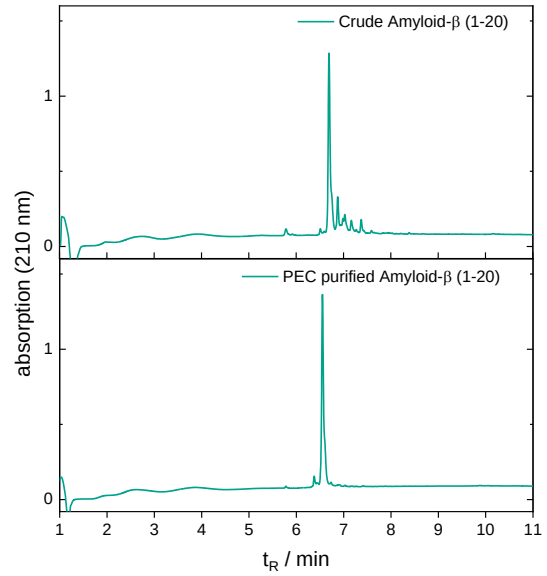
Comparison to flash. We compared the purity and recovery of the peptides by both the PEC process and flash chromatography. Table 2 provides the details. The recovery was calculated according to Equation 1.

*Table 2: Comparison between PEC and flash*

| ID | crude purity / % | purity flash / % | purity PEC / % | recovery flash / % | recovery PEC / % |
|---|---|---|---|---|---|
| #1 | 13[a]/23[b] | 68 | 77 | 83 | 32 |
| #2 | 79[a]/58[b] | 86 | 90 | 43 | 45 |
| #3 | 75 | 80 | 89 | 33 | 36 |
| #4 | 79 | 80 | 91 | 51 | 38 |
| #5 | 72[a]/71[b] | 75 | 96 | 57 | 82 |
| #6 | 49[a]/49[b] | 76 | 87 | 47 | 89 |
| #7 | 82 | 94 | 91 | 57 | 76 |
| #8 | 51 | 81 | 82 | 47 | 101 |
| av. | 58[a]/61[b] | 80 | 88 | 52 | 62 |

The tested peptides showed an overall purity gain of 22% after flash chromatography, whereas the PEC process achieved an average of 27% purity increase. PEC enables a mean recovery of 62%, which was 10% higher than the mean recovery with flash chromatography.
More than half of the peptides (55%) contained one or more Met, which tends to oxidize during SPPS. The addition of TMSBr and EDT at the end of the TFA cleavage successfully reduced any existing Met=O.
Also, looking at the individual sequences, PEC showed better purities except for the miraculin (94% vs. 91%). Auspicious results were obtained even for the aggregation-prone peptide Amyloid-ß (1-20), which was purified using the standard protocol with a final purity of 90% and a recovery of 45% (Figure 1).

*Figure 1: Chromatograms for crude and PEC purified Amyloid-β.*




## 4. Results at a glance

- purify a wide range of peptides including hydrophobic and acidic peptides in a routine procedure
- achieve better purities in a single step using PEC in comparison to low-pressure chromatography
- improve the manufacturing economy with higher recoveries

## 5. References

[1] Anderson L.; Blomberg L.; Flegel M.; Lepsa L.; Nilsson B.; Verlander M. *Biopolymers* 2000, 55, 277 – 250.

## About Belyntic

Belyntic GmbH is a chemistry-for-healthcare enterprise focusing on the manufacturing of biopolymers, especially peptides. Belyntic offers the world's first broadly applicable peptide purification kits as well as development and implementation services of its proprietary Peptide Easy Clean (PEC) technology.

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