Improve ecological efficiency

Less is more

Due to its repetitive cycles of deprotection, washing, and coupling, SPPS is a poorly atom efficient process, resulting in the consumption of a considerable amount of solvents and reagents. Furthermore, typical peptide purification with RP-HPLC also consumes a high amount of organic solvents. In this study, we compare the solvent consumption and waste generation for peptide manufacturing between RP-HPLC and the PEC purification technology.

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1. Introduction

Lowering the overall solvent consumption and waste generation - the latter being the first of the 12 principles of green chemistry [1] – can significantly improve the ecological efficiency for the manufacturing of peptides via solid-phase peptide synthesis (SPPS).
Even though the solvent consumption of this method decreased over time, the overall solvent economy of the process is still unsatisfactory. Moreover, subsequent purification is mainly carried out by chromatographic techniques such as reversed-phase high-pressure liquid chromatography (RP-HPLC). Due to the elution of the product under high pressure, RP-HPLC consumes a considerable amount of solvents and produces lots of aqueous solvent waste.
Belyntic's Peptide Easy Clean (PEC) technology helps to reduce solvent consumption and waste generation substantially. Capping during SPPS ensures that only the full-length peptide is accessible for modification with a cleavable purification linker (PEC-Linker) after the synthesis. Routine capping or capping after known difficult couplings are generally advisable since it eases purification by chromatographic methods, as shown by Bachem.[2] However, the capping adds additional organic solvent consumption and waste to the total sum during synthesis.
In this case study, we compare the consumption of organic solvents, as well as the waste generation between RP-HPLC or PEC purification of Histone H3 (1-15) peptide, and show, despite the additional capping step, how PEC engages green chemistry in peptide manufacturing.

2. Method

Synthesis. The Histone H3 (1-15) peptide was 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 PEC-Linker RC (4 eq.) was employed and coupled using Oxyma (6 eq.) and DIPEA (6 eq.) in DMF for 2 h. TFA cleavage was performed for 2 h using Reagent K (10 mL cleavage cocktail per 100 µmol peptide).

Table 1: Peptides used in this study

name	sequence
Histone H3 (1-15)	H-ARTKQ TARKS TGGKA-OH

PEC Purification. The crude material was first dissolved in 4.5 mL DMSO. Then, 500 µL citric acid/GdmCl buffer (0.1 M/7M; pH 4.5) was added. The dissolved peptide was added to 3 mL pre-conditioned suspension of activated filter material (150 µmol aldehyde content), and the immobilization was performed for 60-90 min before washing off impurities.
Per peptide, 500 mg of PPh_3 in 9 mL MeCN/H_2O/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 50 ml tubes. Each sample was washed/eluted twice with 2 ml 95% aq. TFA. The peptide precipitated in chilled diethyl for direct use.

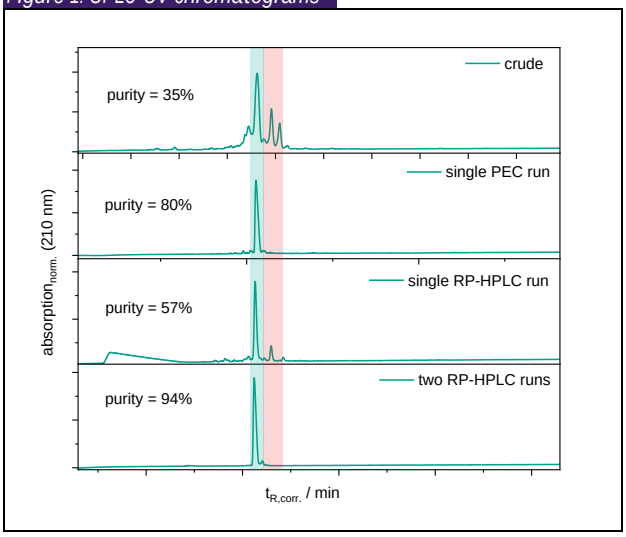
RP-HPLC purification. The chromatographic purification of Histone H3 (1-15) was conducted in a two-dimensional approach using a C18 column with heptafluorobutyric acid (HFBA) applied in the first dimension and TFA in the second. The first run with acidic HFBA modifier (RP-HPLC HFBA: solvent A: 0.1% HFBA in 3% MeCN/H O, solvent B: 2 0.1% HFBA in 60% MeCN/H O) was required to remove critical co-eluting impurities. The second run (RP-HPLC TFA: solvent A: 0.1% TFA in H O, solvent B: 0.1% TFA in 60% 2 MeCN/H O) was performed for purification and conversion to the TFA salt form of the peptide. Both runs were performed on a Varian ProStar prep HPLC instrument.

Analysis. 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

Purity. Figure 1 shows the chromatograms of the crude and the three purified peptides. The PEC purification efficiently removes the critical impurities (highlighted within the red bar) in a single run. The overall purity of the target peptide (highlighted in the green bar) was determined to 80% after PEC purification.

Figure 1: UPLC-UV chromatograms



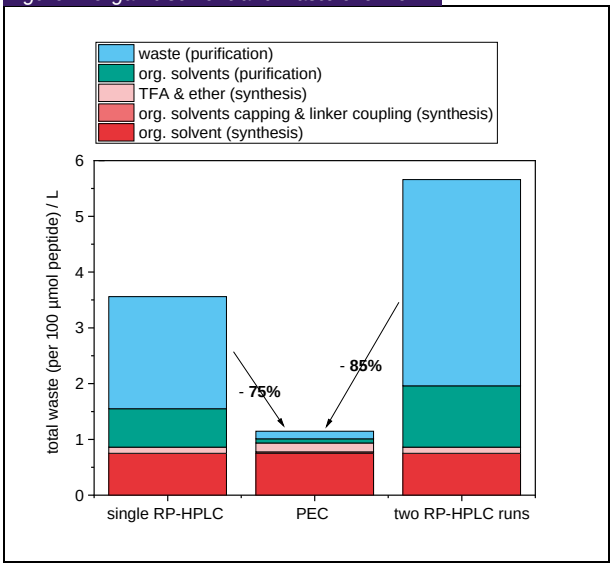
The remaining side products are mostly smaller impurities (e.g., t-Butyl re-addition, insertions, all below 2%). Accordingly, this PEC-purified sample is perfectly suitable for research purposes.
The first-dimension RP-HPLC run shows only moderate purity increase to 57%, with one of the close eluting impurities still present in higher amounts. A second-dimension purification was therefore required to obtain satisfactory purities.

Solvent economy. Figure 2 compares all consumed solvents for peptide synthesis and purification (100 µmol scales). The following assumptions were made:

- Organic solvents during the synthesis of a single peptide are calculated from a parallel synthesis of six peptides using an Intavis RSi synthesizer.
- The amount of solvent consumed during synthesis is equal to the amount of waste produced.
- More TFA and ether are required for the PEC purification since they are used during the release step to cleave the PEC-Linker, elute and precipitate the peptide.

The results reveal the immense potential of PEC to reduce solvent consumption and waste. Overall, the relative contribution of purification to the whole manufacturing process is reduced to 25% (from 80% using single RP-HPLC, or 90% for two RP-HPLC runs).

Figure 2: Organic solvent and waste overview



Moreover, PEC saves 75% to 85% of the total waste load, depending on the number of RP-HPLC purifications. Note that other synthesizers and/or methods would show slightly different numbers. The bottom line, however, remains the same: According to the first principle of green chemistry, PEC can be considered a promising approach towards greener peptide manufacturing.

4. Results at a glance

- reduce the total waste generation of single run purifications by up to 75% to 85%
- purify highly hydrophilic peptides such as histone fragments efficiently in a single step
- apply capping to get high-quality crude and final product purity

5. References

[1] Hofmann F.; Sambeth G.; Dettner F.; Schönleber R. Bachem AG 2017, Poster
[2] Antas P. T.; Warner J. C. *Green Chemistry: theory and practice*, New York, 1998.

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 the development and implementation services of its proprietary Peptide Easy Clean (PEC) technology.

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