1. Introduction

Solid-phase peptide synthesis (SPPS) has revolutionized chemical peptide manufacturing since its invention by Merrifield in 1963.[1] Today, very long and complex natural and non-natural sequences can be obtained via SPPS. Even though the solvent consumption of this method has been reduced over time, the overall solvent economy of the process is still unsatisfactory. Subsequent purification is mainly carried out by chromatographic methods, that separate the crude mixture by sorting the molecules according to their adherence to the column material (stationary phase). Reversed-phase high-pressure liquid chromatography (RP-HPLC) is probably the most commonly used method to purify peptides. RP-HPLC relies on the use of aqueous mixtures with organic solvents (e.g. acetonitrile (MeCN) or methanol) containing an ion-pair reagent, such as trifluoroacetic acid (TFA), that forms hard ions with basic residues on the peptide. The elution under high pressure hereby consumes a large amount of solvent and waste for peptide manufacturing is compared between RP-HPLC and Belyntic's PEC purification technology.

Due to its repetitive cycles of deprotection, washing and coupling, SPPS is a poorly atom-efficient process. This results 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, the amount of solvents and waste for peptide manufacturing is compared between RP-HPLC and Belyntic's PEC purification technology. This results in the consumption of minimal amounts of washing solutions are needed to remove impurities.

In the following, we compare the consumption of organic solvents, as well as the waste generation between RP-HPLC or PEC purification of Histone H3 (1-15) peptides and how green chemistry can be approached with PEC.

2. Methods

Synthesis. The Histone H3 (1-15) peptide was synthesized in a total scale of 200 µmol by Bachem UK (St. Helens). 100 µmol were kindly provided for PEC purification.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Mol. weight / Da</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histone H3 (1-15)</td>
<td>H-ARTKQTARKSTGGKA-OH</td>
<td>1560.88</td>
</tr>
</tbody>
</table>

PEC purification. The PEC-Linker RC+ was used for PEC purification and the standard PEC procedure was applied.

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/H₂O, solvent B: 0.1% HFBA in 60% MeCN/H₂O) was required to remove critical co-eluting impurities. The second run (RP-HPLC TFA: solvent A: 0.1% TFA in H₂O, solvent B: 0.1% TFA in 60% MeCN/H₂O) 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 Agilent H-Class UPLC-ESI-MS system from Waters on a C18 column (1.7 µm, 2.1 x 500 mm) by Belyntic. A mobile phase, mixtures of H₂O (A) and MeCN (B) with 0.1% TFA were used.

Oliver Reimann¹, Robert Zitterbart¹, Gavin Noble²

¹Belyntic GmbH, Berlin, Germany
²Bachem (UK) Ltd., St. Helens, United Kingdom

#myPECcase
3. Discussion

### Choice of peptide

The Histone H3 (1-15) peptide represents an ideal candidate for this study. SPPS difficulty and the hydrophilic nature of the peptide resulted in a complex crude mixture with various co- or close-eluting deletion sequences. This scenario serves well to showcase the systematic differences of PEC and chromatography, while highlighting the large potential to save organic solvents and waste during purification.

### 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%.

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 run was therefore required to obtain satisfactory purities.

### Solvent economy

Figure 2 compares all consumed solvents for peptide synthesis and purification (100 µmol scale). 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 clearly reveal the large potential of PEC to dramatically reduce solvent consumption and waste. Overall, the relative contribution of purification is reduced to 25% (from 80% using single RP-HPLC, or 90% for two RP-HPLC runs).

Looking into the total waste generation, PEC saves approximately 75% to 85% of the 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

- PEC purification of hydrophilic Histone H3 (1-15) delivers a high-quality product in a single step.
- Capping is negligible in the overall solvent economy.
- PEC reduces the total waste generation by 75% to 85%.

5. References


About Belyntic

Belyntic GmbH is a chemistry-for-healthcare enterprise focusing on purification 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.

Get in touch for more information

Email: support@belyntic.com
Phone: +49 30 8104-1113
or visit us online at belyntic.com