New Technologies Enabling Parallel Purification of Peptide Libraries

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Introduction

Peptide drug discovery efforts are increasing as unbiased screening technologies have improved and strategies to evaluate passive cell permeability have been defined. As a result, the demand for synthetic peptide libraries has increased significantly. Chemists tasked with delivering peptide libraries for evaluation, particularly in plate-based formats, encounter significant practical handling challenges not common during larger scale, lower throughput projects. Moreover, the purification bottleneck demands new technologies that enable high-throughput purification ahead of library evaluation.

Herein, we review the latest innovations from Biotage designed specifically to improve plate-based synthesis, purification, and general downstream processing of peptide libraries from start to finish.

Experimental protocol

Peptide Synthesis and Analysis

Peptides were synthesized using standard methods on a Syro II peptide synthesizer. All peptides were analyzed for purity with an Agilent 1260 Infinity series HPLC equipped with a Restek Raptor™ ARC-18 (2.1 x 50 mm) column. Peptide library purification in parallel was performed with a Biotage® Extrahera™ sample preparation workstation equipped with a Biotage® PeptiRen-C18 96-well plate or Biotage® PeptiPEC-96 High-Throughput Purification Kit.

Results and discussion

Eliminating Ether Precipitation from the Traditional Workflow

Significant modifications to the traditional ether precipitation procedure must be made in plate format:

- Cleavage cocktail volume must be reduced to accommodate ether addition in a proportion that maintains miscibility of the two solutions
- Visual inspection of each well to confirm precipitated peptide is difficult and may not be sufficient, resulting in product loss
- Peptide pellets are difficult to pack tightly with centrifugation, requiring alternative strategies to remove the ether supernatant

Solid Phase Extraction (SPE), Figure 1, has been growing in popularity with high-throughput synthesis groups.[1,2,3]

Proof of concept experiments demonstrated a clear chromatographic delineation between peptidic and non-peptidic

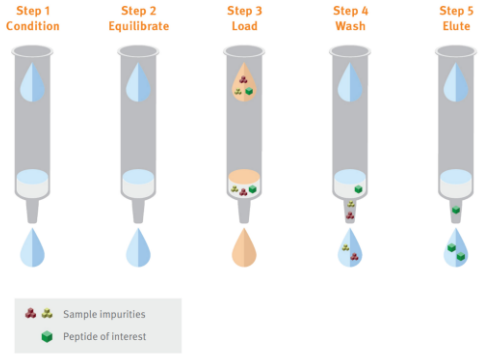


Figure 1. *Schematic representation of a typical solid phase extraction protocol*

components that remain after evaporation of cleavage cocktail during a simple reversed-phase SPE protocol, Figure 2.

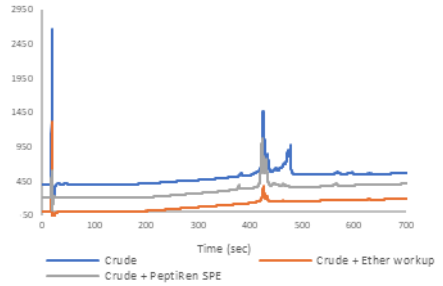


Figure 2. *Analytical chromatograms for a single crude peptide evaporated completely after cleavage (blue), precipitated in ether (orange), or crude peptide dried and treated on Biotage® PeptiRen-96 SPE media (grey).*

These data suggest that reversed-phase SPE treatment of crude peptides is a viable alternative for traditional ether precipitation workup. Careful selection of the solvent modifier may also remove harmful TFA counterions left behind, effectively eliminating the need for a second "workup" step to exchange counterions. Importantly, SPE methods can be automated, reducing hands-on impact even further.

Media Choice Impacts SPE Efficiency

SPE protocols implemented in a workflow must comply with current prediction tools to prevent significant time investment for analytical characterization both prior to and after the workup. Work by Biotage and others[4] has indicated that a reversed-phase media is the most general and predictable chromatographic strategy for synthetic peptide libraries.

Regardless of the type of chromatography, the parameters of the media selected (pore size and particle size specifically) play a role in the success or failure of the purification. To ensure SPE parameters could be predictable and enable high sample recovery percentages, three types of reversed-phase media were compared using an SPE

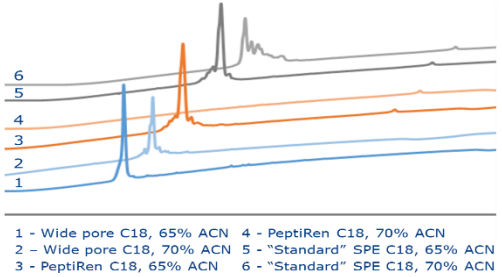


Figure 3. *Comparison of elution efficiency for three commercially available reversed-phase SPE medias.*

protocol, Figure 3.

This representative peptide was expected to elute complete in the 65% Acn fraction, based on previous analytical HPLC studies. Therefore, any peptide present in the 70% Acn fraction would have been lost during processing. This data demonstrates clearly that not all C18 medias enable the same recovery of synthetic peptides. Only the C18 media contained in Biotage® PeptiRen-96 well plates enabled complete elution of the peptide in the appropriate fraction.

Covalent Catch-and-Release Purification Delivers Higher Purity

Peptide libraries produced for secondary screening often require minimal purity for conclusive validation assays. However, some assay types require higher purities[5] which coupled with an increasing number of unnatural amino acids, drives the need for some degree of purification. In recognition of this technology gap, Biotage®, in partnership with Gyros Protein Technologies, moved to automate PEC™ purification using the Biotage® Extrahera platform, Figure 4.

To demonstrate the efficacy of this platform, the Biotage® Extrahera workstation equipped with Biotage® PeptiPEC-96 High-Throughput Kits were employed to expedite delivery of a peptide library for immunological testing. This workflow enabled delivery of 192 compounds within two weeks. Delivered peptides were required to have >70% purity and >0.5 mg/peptide to complete the order. In the first attempt 188/192 met purity requirements and 182/192 met the

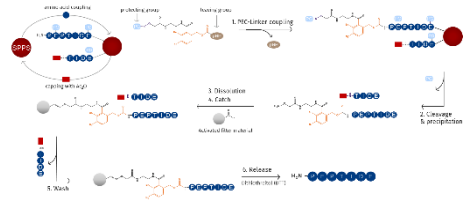
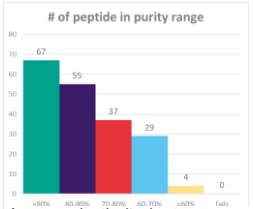


Figure 4. *Chemical details outlining PEC™ catch-and-release purification.*

quantity requirements. Figure 6 shows a distribution of the 192 peptides delivered, Figure 5.



Library Details

- 15-18 amino acids
- Average library purity - 83%
- Average quantity – 3.4 mg

Figure 5. *Purity distribution for a 192-peptide library purified with PeptiPEC-96 High-Throughput Kits and Biotage® Extrahera sample prep workstation.*

Conclusions

Demand for peptide libraries has increased dramatically recently and is expected to continue in the future. Producing a library of peptides introduces different challenges than typically encountered for synthesis of fewer number of peptides. Herein we highlighted synthesis tools and purification strategies that mitigated the significant manual intervention steps required to produce a library of peptides. Combining these tools in a single workflow reduces production time significantly, Figure 6.

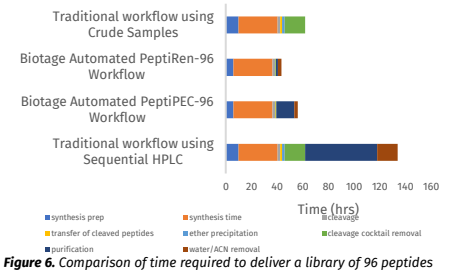


Figure 6. *Comparison of time required to deliver a library of 96 peptides utilizing different workflow strategies and those described herein.*

[1] Cepeda, D. S. I. et al. Molecules **2019**, 24, 1215-1223.

[2] Ardila-Chantre, N. et al. RSC Adv. **2020**, 10, 29580-29586.

[3] Schelleter, L. *et al. Rap. Comm. Mass Spec.* **2021**, *35*, e8873

[4] Bennett, R. et al Anal. Chim. A. **2020**, 1142, 10-18.

[5] S. Putta et al., Structure **2022**, 30, p. 1

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P282