# An Unparalleled Peptide Library Workflow: Library Production Efficiency Improvements Aided by Technology

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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

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

### Peptide synthesizers with complementary pre- and post-synthesis tools improve synthesis efficiency

General setup is one of the most tedious tasks associated with automated peptide synthesis. Dispensing resin is particularly onerous given the extremely large number of repetitions and small quantity per reactor vial. To alleviate this, Biotage® created resin dispensing tools, Figure 1, that facilitate resin transfer into many reactor vials simultaneously with a high degree of precision.


***Figure 1.*** *Resin dispensing tools transfer resin into 96 syringe-like reactor vials (left) or 96 pipette tip-like reactor vials (right) simultaneously.*

Synthesizer configuration flexibility that maintains high-throughput capacity while improving synthetic yield and purity . Systematic design improvements to the Syro II peptide synthesizer enable reaction heating with high throughput capacity which decreases synthesis time and improves crude purity for peptide libraries, Table 1.

***Table 1.*** *Comparison of synthetic strategies available on a single Syro II platform for a representative 96-peptide library.*

| Synthesis strategy | Reactor vial type | Resin amount (mg) | Synthesis time (h) | Total system solvent (mL) | Average Library Crude Purity |
|---|---|---|---|---|---|
| Single coupling, *in situ* activation, r.t. | 0.4 mL Tip | 8 | 34.6 | 2865.2 | 44.4 ± 8.5% |
| Double coupling, pre-activation, r.t. | 0.4 mL Tip | 8 | 56.6 | 4785.4 | 54.6 ± 8.8 |
| Single coupling, *in situ* activation, 75°C | 2 mL | 8 | 32.1 | 2641.5 | 59.03 ± 13.2% |

Once synthesis is complete, the next step is to remove each reactor vial and filter the resin from the cleaved peptide solution, manually. For a library of peptides composed of potentially hundreds of compounds, this poses safety risks to the chemist, introduces opportunities for compound mishandling, and is extremely time consuming.

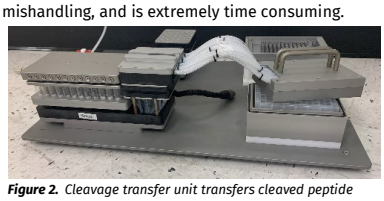


***Figure 2.*** *Cleavage transfer unit transfers cleaved peptide solution from 96 syringe-like reactor vials in to a deep-well 96-well plate simultaneously.*

Cleavage transfer units facilitate filtration of the cleaved peptide solution into a collection vessel using positive pressure, Figure 2. For high-throughput applications, simultaneous transfer of the cleaved peptides from their synthesis position on the reactor block to the corresponding position in a deep-well 96-well plate in parallel improves the safety profile of this filtration step, removes any compound management concerns that may arise and significantly reduces chemists' time investment.

### Eliminating Ether Precipitation from the Peptide Library Workflow

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

- the solution volume must be reduced to accommodate ether addition and 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 not well packed after centrifugation, requiring alternative strategies to remove the ether supernatant

Solid Phase Extraction (SPE) 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 components that remain after evaporation of cleavage cocktail during a simple reversed-phase SPE protocol, Figure 3.

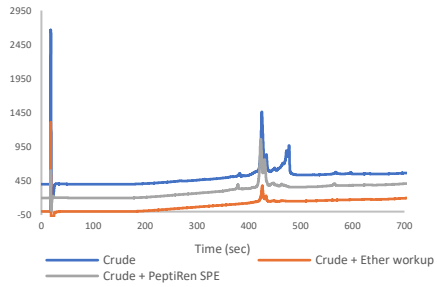



***Figure 3.*** *Analytical chromatograms for a single crude peptide evaporated completely after cleavage (blue), precipitated in ether (orange), or crude peptide dried and treated on Biotage® PeptiRen-96 SPE media (grey).*

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. Importantly, SPE methods can be automated, reducing hands-on impact even further.

Work by Biotage® and others[4] has indicated that a reversed-phase SPE approach is the most general and predictable chromatographic strategy for peptide libraries. Further investigation, it was determined that the specific media parameters impacts the behavior of peptides during reversed-

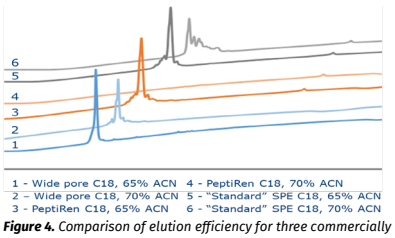



***Figure 4.*** *Comparison of elution efficiency for three commercially available reversed-phase SPE medias.*

phase SPE, Figure 4.

This example peptide was expected to elute 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. Only the media contained in Biotage® PeptiRen-96 well plates enabled complete elution of the peptide in the appropriate fraction.

### Orthogonal Plate-Based Catch and Release Eliminates Need for HPLC Purification of Peptide Libraries

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.

Biotage®, in collaboration with GYROS PROTEIN Technologies, optimized a plate-based, orthogonal catch and release purification strategy, Figure 5.

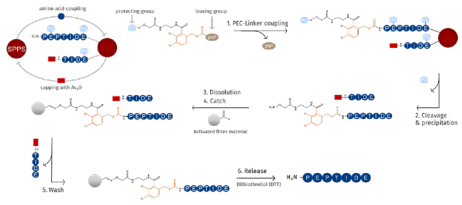
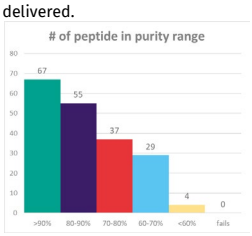



***Figure 5.*** *Chemical details outlining orthogonal catch and release purification.*

Coupling the Biotage® Extrahera with PeptiPEC-96 High-Throughput Kits, chemists were able to deliver 192 compounds destined for immunological testing 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 meeting the quantity requirements. Figure 6 shows a distribution of the 192 peptides delivered.




Library Details

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

***Figure 6.*** *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 highlight synthesis tools and purification strategies that mitigate the significant manual intervention required to produce a library of peptides. Combining these tools in a single workflow reduces production time significantly, Figure 7.

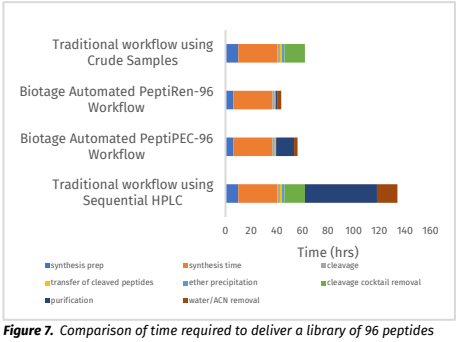



***Figure 7.*** *Comparison of time required to deliver a library of 96 peptides utilizing different workflow strategies and those described herein.*

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Part Number: P261