Flash Purification Methodology for Synthetic Peptides

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Statement of Work

Presented here is a rapid 2-step process for purifying crude synthetic peptide material. First, the material is evaluated with a nonspecific HPLC gradient method. Second, the result from the generic gradient method is used to create a flash chromatography method that can purify the material.

This 2-step process is demonstrated with a 22 amino acid crude synthetic peptide sample. The initial crude had a purity of 77.3 % and depending on how the fractions were pooled, the purity could have been upgraded to 94.0 % (yield of 45.1 %) or 90.4 % (yield of 84.6 %). This 2-step process took approximately 45 minutes to complete.

Introduction

Synthetic peptides are utilized for a wide range of functions including vaccines, control of diabetes and as affinity ligands in protein purification. Most common peptides are comprised of no more than 50 amino acids. Due to this they can be synthesized with ease and at a low cost. The commonality and low cost of production drives the need for a quick, simple and cost effective purification method.

Flash chromatography is often used for quick purifications during intermediate steps of pharmaceutical product synthesis. These intermediate purifications allow for higher yields of subsequent steps and ultimately more end product. Unlike LC columns, flash cartridges are considered disposable. They require less cleaning and maintenance since they are often disposed of after 1-3 purification runs. Additionally, flash systems typically only have basic functions, including low pressure pumps, a fraction collector and a UV-Vis detector. This simple setup allows for easier maintenance and a lower purchase cost. Likewise, flash cartridges are available in many common stationary phase chemistries suitable for reversed phase, normal phase and even HILIC separations.

This poster builds on these ideas with a rapid 2-step process for purifying crude synthetic peptide material. HPLC is used to determine the chromatographic behavior of the sample by screening the sample with a gradient method. From these LC results, a flash method is created and the material is processed. A 22aa crude synthetic peptide sample is used to demonstrate this concept.

Experimental

Methodology Basic Concept

- Screen by HPLC
  - Determine % Acetonitrile for elution of the desired component
- Purify with Flash
  - Focus conditions based on HPLC screen
  - Collect multiple fractions
- Fraction analysis by HPLC
- Determine pool options and calculate final purity

HPLC Conditions

Column: Luna® 3 µm C18(2)
Dimensions: 50 x 4.6 mm
Part No.: 00B-4251-E0
Mobile Phase: A: 0.1 % TFA in Water
B: Acetonitrile
Gradient:

Time (min)	% B
0.0	15
10	45
11	15
13	15

Flow Rate: 1.0 mL/min
Injection Volume: 5 µL
Concentration: See figure
Temperature: Ambient
Detector: UV @ 210 nm

Flash Conditions

Column: Claricep™ Spherical Silica C18
Description: 20-35 µm, 100 Å, 40g
Part No.: S0230040-0
Mobile Phase: A: 0.1 % TFA in Water
B: Acetonitrile
Gradient:

Time (min)	% B
0.0	15
10	30
18	40

Flow Rate: 60 mL/min
Injection Volume: 2 mL
Concentration: 100 mg/mL
Temperature: Ambient
Detector: UV @ 230 nm
Monitor: UV @ 214 nm

FIGURE 1.
HPLC Screen

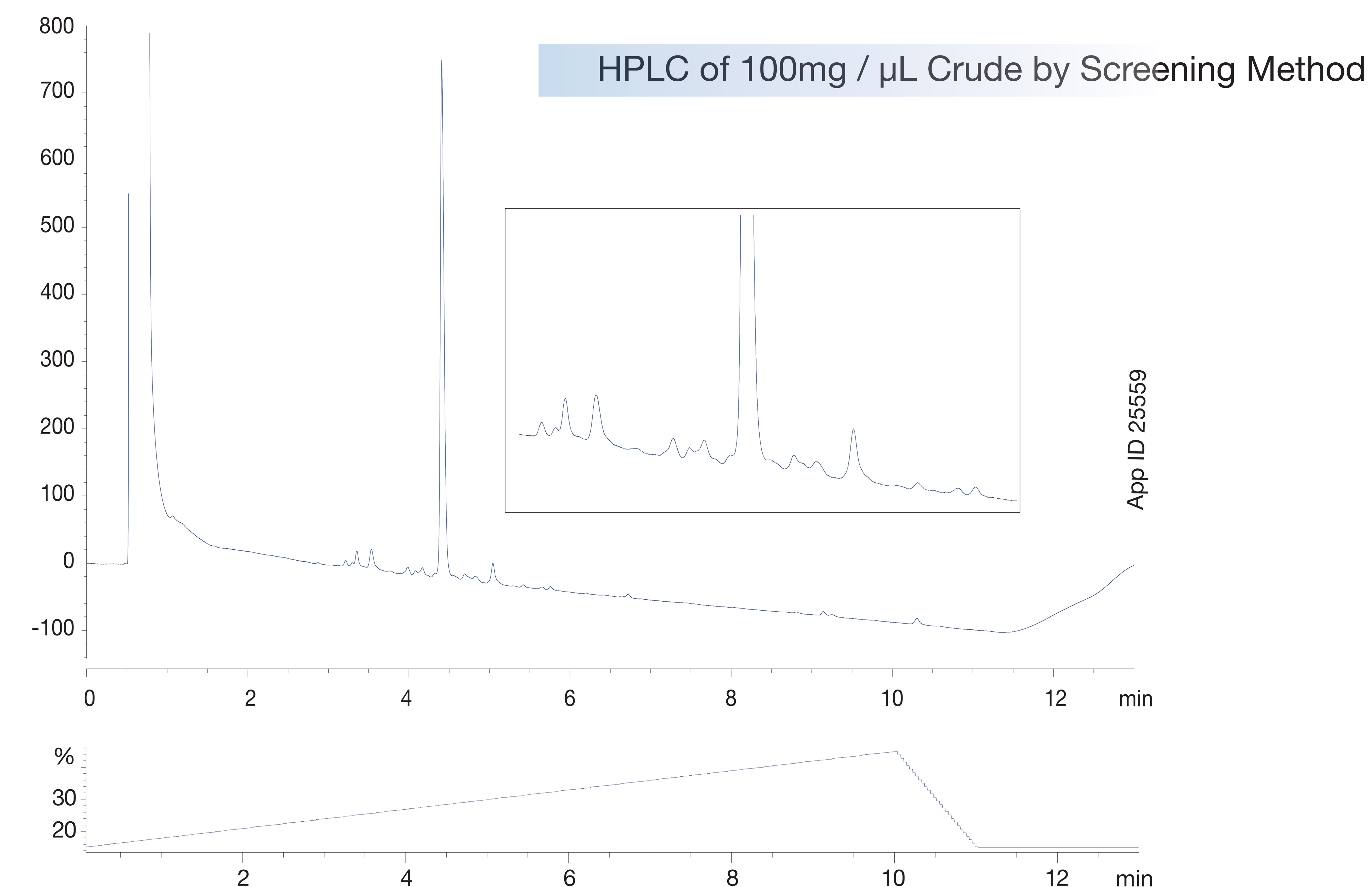

FIGURE 2.
Peptide Purification on Flash

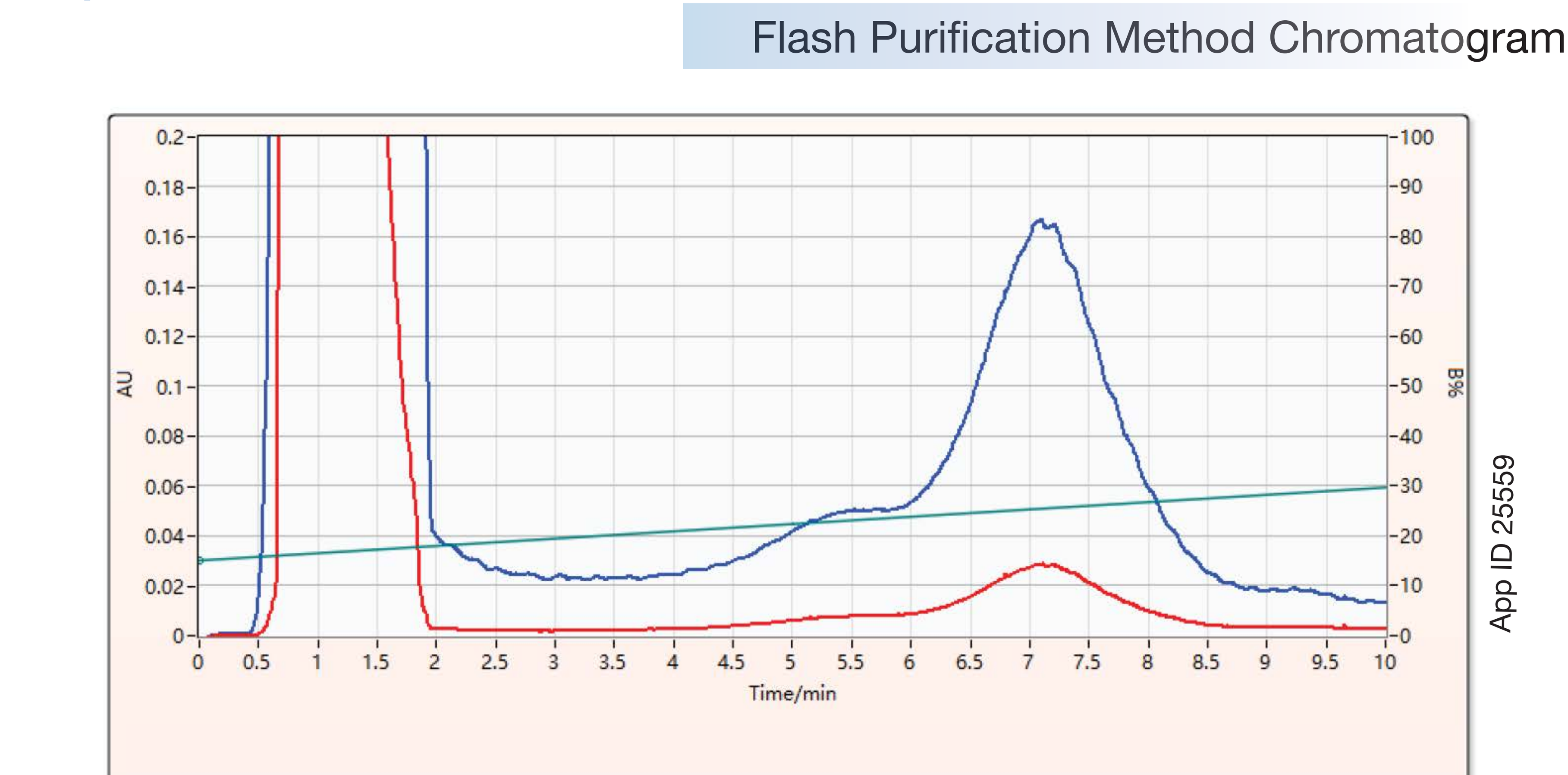

FIGURE 3.
Fraction Evaluation by HPLC

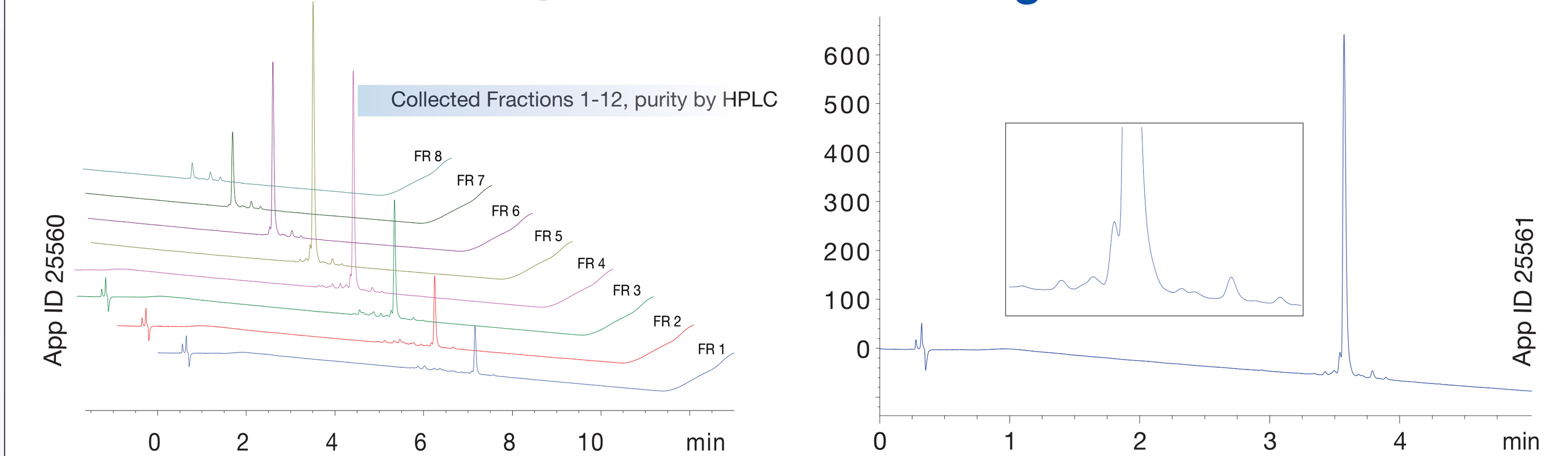

FIGURE 4.
Best Single Fraction Result

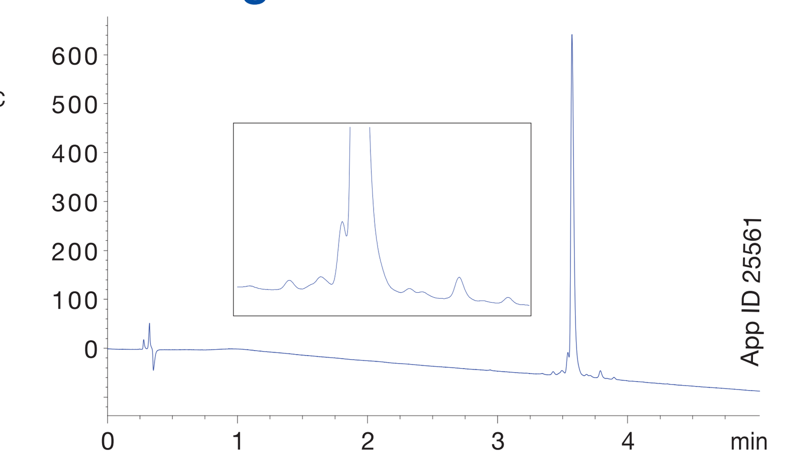

TABLE 1.
Fraction Analysis and Possible Fraction Pooling Results

Desired		
Fraction #	Peak Area	PA %
1	182.0	45.7
2	271.4	52.3
3	371.6	67.1
4	682.7	79.8
5	1535.3	89.7
6	1986.7	94.8
7	1185.1	92.8
8	559.9	87.0
9	210.1	64.0
10	50.6	31.9

Pooled		
Fractions	% Yield	% Purity
6	28.2	94.8
6+7	45.1	94.0
5+6+7	66.9	92.6
5+6+7+8	74.9	91.9
4+5+6+7+8	84.6	90.4

Results and Discussion

A crude synthetic peptide with 77.3 % purity was obtained from a collaborating lab and used to demonstrate the purification method development protocol presented in this poster. While the peptide's amino acid sequence was unknown, it was determined to be hydrophobic from its resulting retention time in the HPLC screen, **Figure 1**. This methodology can be used for synthetic peptides of unknown amino acid sequence, since the scouting gradient on HPLC is used to decide the conditions used for the purification by flash chromatography.

The analytical HPLC scouting gradient method was performed using a Luna 3 µm C18(2) 50 x 4.6 mm column with 0.1 % TFA and acetonitrile as the eluent. The gradient went from 15 % acetonitrile to 45 % over 10 minutes. From the screening chromatogram, it was observed that the peptide eluted at approximately 28 % acetonitrile.

A flash chromatography method was then scaled directly from the analytical HPLC result. A Claricep spherical silica C18 20-35 mm 40 g cartridge was chosen for the purification of this crude as it provides a similar retention profile to that obtained with the Luna C18(2) used in the HPLC screening methodology. The 40 g size was chosen since the sample was approximately 200 mg, resulting in a loading of approximately 0.5 % of the bed mass. No changes were made to the eluent constituents. Based on the desired component eluting at 28 % in the screening methodology, the gradient was adjusted to 15 % acetonitrile to 30 % acetonitrile over 10 minutes. Since a peptide typically has a particular eluent composition where it will elute, a narrower gradient method is typically chosen for the purification of synthetic peptide.

An Agela CHEETAH™ purification system was used for the flash purification work because of it's ease of use and ability to program 2 component gradients. The CHEETAH is also capable of collecting fractions based on UV detection, which allows for a rapid identification of the desired fractions. This cuts down on the total number of fractions needed to be run by analytical HPLC.

Ultimately a 2 mL injection of a 100 mg/mL solution of the crude peptide in DMSO was loaded onto the Claricep 40 g C18 spherical 20-35 mm cartridge. A total of 13 fractions were collected with the method. Fractions were then evaluated on the analytical HPLC using the same Luna 3 µm C18(2) column, solvents and method as used in the screening step to determine the purity of each fraction. From the fraction analysis, several possible results were calculated (**Table 1**) depending on how the fractions are pooled. The purity could be upgraded to 94.0 % (yield of 45.1 %) or 90.4 % (yield of 84.6 %) depending on the needs of the project. This 2-step process took approximately 45 minutes to complete.

Conclusion

A 2-step process for the purification of synthetic peptides using HPLC and flash chromatography was successfully demonstrated. **Step 1** screens the sample with HPLC. **Step 2** utilizes a flash chromatography method based on the previous HPLC result. The collected flash fractions are then analyzed by HPLC. The analytical results are used to determine how the fractions are to be pooled and the final results.

The synthetic peptide example presented here, started with a 77.3 % purity crude that could be upgraded to 94.0 % (yield of 45.1 %) or 90.4 % (yield of 84.6 %) depending on the needs of the project. The 2-step purification process took approximately 45 minutes to complete. The Claricep C18 40 g cartridge was effective in the purification of this synthetic peptide.

Flash chromatography provides a quick and inexpensive way to purify peptides. Methods developed on HPLC can be translated over to flash chromatography directly and with great ease. Final fractions can be confirmed by HPLC and then pooled together. This synthetic peptide purification method development process was designed to minimize the required time and overall cost of the process while being able to achieve the desired purity of the final peptide product.

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