Peptide Purification with Biotage® SNAP Bio Cartridges: Achieving High Purity and High Recovery

This work demonstrates that peptide purification efficiency by flash chromatography is improved with Biotage® SNAP Bio cartridges containing a spherical wide pore stationary under a wide variety of purification conditions.

Introduction

Peptide drug discovery efforts increase as the rules governing cytosolic delivery and improved pharmacokinetic stability are further elucidated. Progress has certainly been aided by advances in coupling reagents and synthetic methodologies, thus enabling chemical synthesis of peptides with greater lengths and higher crude purities. Despite these improvements, crude peptides are still subjected to laborious purification protocols. Reversed-phase flash chromatography presents itself as an attractive tool for peptide purification due to its high sample loading capacities and expedient chromatographic methods.

Many questions arise when considering a new technique for such an important component of the peptide drug discovery process though. Herein we present purification efficiency and recovery results to address some of these critical concerns.

Experimental

Materials

All materials were obtained from commercial suppliers and used without further purification. Sigma-Aldrich (diisopropyl carbodiimide (DIC), LC-MS grade water, LC-MS grade acetonitrile, triisopropyl silane (TIS)), Fisher Scientific (Fmoc-amino acids, Oxyma Pure, trifluoroacetic acid (TFA)), Reagents, Inc. (dimethylformamide (DMF), acetonitrile, dimethylsulfoxide (DMSO), PCAS BioMatrix (Rink Amide ChemMatrix® resin) were the principle suppliers.

Synthesis

Peptides were synthesized using Fmoc-based solid phase peptide synthesis on a Biotage® Initiator+ Alstra™ automated microwave assisted peptide synthesizer using default deprotection and DIC-mediated coupling methods on Rink Amide ChemMatrix® resin. Fmoc removal proceeded at room temperature twice, the first for 3 minutes, the second for 10 minutes with piperidine-DMF solution (1:4). Coupling reactions proceeded with 4 eq amino acid in DMF (0.5M), 4 eq DIC in DMF (2M), and 4 eq. Oxyma Pure in DMF (2 M) at 75 °C for 5 minutes. Upon synthesis completion, the resin was washed thoroughly with DCM (x3) and diethyl ether (x3), repeated twice, and dried thoroughly under vacuum.

The peptides were cleaved from the resin with TFA·H_2O·TIS (95:2.5:2.5) for 3 hours at room temperature. The cleaved resin was evaporated with a Biotage® V-10 evaporation system, rinsed with cold diethyl ether (x2) and 50% acetonitrile (aq) twice before final drying for purification.

Analytical HPLC-MS was performed with an Agilent 1260 coupled to an ESI-MS (AB Sciex 4000 qTrap) running Analyst software. The peptide was analyzed on a Biotage® Resolux 300 Å C18 column (5 µm, 150 x 4.6 mm) with a flow rate of 0.5 mL/min. The following solvent system was utilized: solvent A, water containing 0.1% formic acid; solvent B, acetonitrile containing 0.1% formic acid. The column was eluted using a linear gradient from 10%–90% solvent B. Sample purity was calculated by UV peak integration (compiling absorbances from wavelength 215–220 nm) with MultiQuant2.0.

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Purification

Crude peptides were purified with an Isolera™ Dalton equipped with a Biotage® SNAP Ultra C18 or Biotage® SNAP Bio C18 cartridge. Crude peptides were dissolved in DMSO for purification. Peptides were eluted using the following solvent systems:

- Solvent A, water + 0.1% TFA
- Solvent B, acetonitrile + 0.1% TFA
- Solvent C, water + 0.1% NH_4OH
- Solvent D, acetonitrile + 0.1% NH_4OH

Fractionation was triggered by UV absorbance greater than 100 mAU.

Results and Discussion

Synthesis of ACP Peptide

The ACP (65-74) fragment peptide was successfully synthesized on 0.66 mmol scale using the Biotage® Initiator+ Alstra™. The large scale synthesis was necessary to supply sufficient crude material for a truly comparative loading and recovery analysis from a single synthesis. The principle impurities are a des-Val deletion sequence and residual protecting groups, Figure 1. While there are very few peptidic impurities from the synthesis, the strongly absorbing residual protecting groups substantially decrease the crude synthesis to only 36% purity.

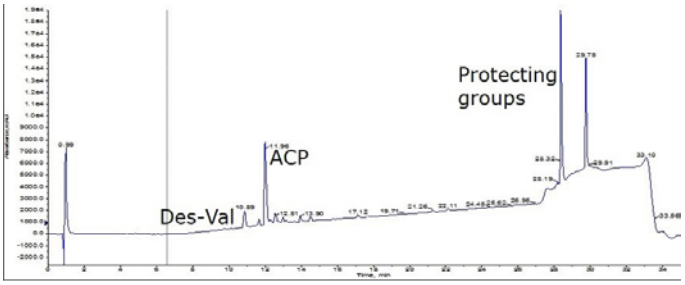

Figure 1. Crude analytical HPLC of ACP (65-74) synthesized with the Biotage® Initator+ Alstra™. The desired peptide is the majority product with only a small percentage of des-Val peptidic contaminant. A significant UV signal due to the presence of residual protecting groups decreases overall crude purity.

Peptide Purification

Purification with Biotage® SNAP Ultra C18

For the first injection, approximately 50 mg of crude peptide was dissolved in 300 µL DMSO and directly injected onto a pre-equilibrated 12 g SNAP Ultra C18 cartridge. The peptide was eluted using an optimized linear gradient of solvents A and B, standard fractionation parameters and fraction volumes, Figure 2. The asymmetrical peak shape suggests that the des-Val deletion sequence co-elutes with the desired product under these conditions, later confirmed with analytical HPLC-MS of the recovered fractions. In a second purification, approximately 100 mg of crude peptide was dissolved in DMSO and purified as above. The des-Val impurity peak is more resolved, enabling distinct fractionation between the two product peaks without any adjustments to the peak detection parameters, Figure 3. While the separation was more effective at the larger loading level, it also resulted in a lower product recovery, Table 1.

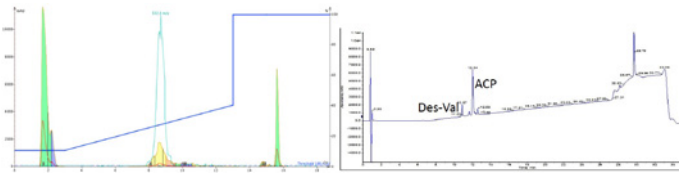

Figure 2. ACP (65-74) purification with Biotage® SNAP Ultra C18 and Isolera™ Dalton. The purification efficiency was evaluated with an approximately 50 mg loading level (left). The analytical HPLC (right) confirms that the purification was not effective.

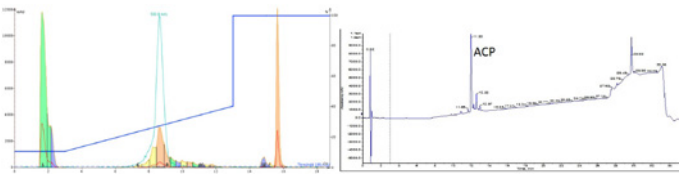

Figure 3. ACP purification with Biotage® SNAP Ultra C18 and Isolera™ Dalton. Increasing the loading to approximately 100 mg crude ACP enabled sufficient resolution of the des-Val deletion product (left), increasing the overall final purity (right).

Peptide	Cartridge Type	Crude Purity (%)	Sample Load (mg)	Expected Recovery (mg)	Final % Purity	Recovered Peptide (mg)	% Recovery
ACP	SNAP Ultra C18	36	47.6	17.1	77	16.4	95.91
ACP	SNAP Ultra C18	36	99.8	35.9	86	22.3	62.12
ACP	SNAP Bio C18	36	48.1	17.3	92	14.5	83.82
ACP	SNAP Bio C18	36	95.9	34.5	85	32.9	95.36
GLP-1	SNAP Bio C18	26	147.5	38.35	87	33.7	87.87

Table 1. Calculated purity and peptide recovery after flash purification.

Purification with Biotage® SNAP Bio C18

The stationary phase packed into a Biotage® SNAP Bio C18 cartridge is differs from that in the Biotage® SNAP Ultra C18 cartridge in two key areas:

1. The BioSphere™ stationary phase is slightly smaller (20 µm vs. 25 µm).
2. The pore size is significantly large (300 Å vs. 100 Å).

This difference is expected to have dramatic implications for both final purity and peptide recovery, hence motivating the comparative evaluation. In a manner identical to that described above, crude ACP was purified in two injections, the first loading of approximately 50 mg and the second loading of approximately 100 mg dissolved in DMSO using a pre-equilibrated 10 g SNAP Bio cartridge. Even at the lower loading level, improvements in resolution are immediately apparent and confirmed via analytical HPLC-MS, Figure 4. The overall improvement in purity is likely the result of greater peptide accessibility to the C18 stationary phase due to the wider pore size. Both purifications yielded peptide with a higher level of purity and recovery than the SNAP Ultra cartridge at equivalent loading, Table 1.

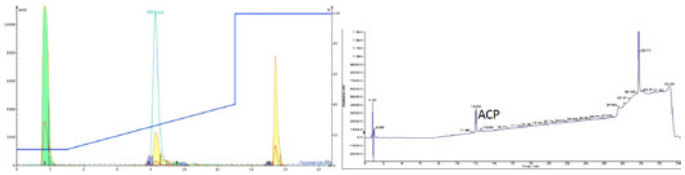

Figure 4. Purification of approximately 50 mg crude ACP with Biotage® SNAP Bio C18 and Isolera™ Dalton (left). The des-Val contaminant is sufficiently resolved such that fractionation was automatically triggered, leading to higher final purity, as confirmed by analytical HPLC-MS (right).

Purifying GLP-1 with Biotage® SNAP Bio C18

The ACP purification data suggests that flash chromatography can yield highly pure peptides with high peptide recoveries, particularly when starting with relatively pure crude materials. To further evaluate the purification and recovery efficiency of SNAP Bio C18 cartridges, crude GLP-1 (26% pure) was then purified using a basic mobile phase. Altering the mobile phase pH can negatively impact the stability of the alkyl chain conjugation to the sorbent, therefore compromising the purification and recovery of peptide samples.

Approximately 150 mg of crude GLP-1 was dissolved in 800 µL DMSO and injected onto a pre-equilibrated 25 g SNAP Bio C18 cartridge. The peptide was eluted with a linear gradient of solvents C and D, using standard UV fractionation parameters, fraction volumes and monitored by mass spectrometry, Figure 5. Using the mass spectrum as guidance, fractions 23–31 were combined, concentrated and evaluated for final purity and recovery, Table 1. Despite the significant difference in mobile phase pH as well as general peptide properties, high purity and recovery were both achieved.

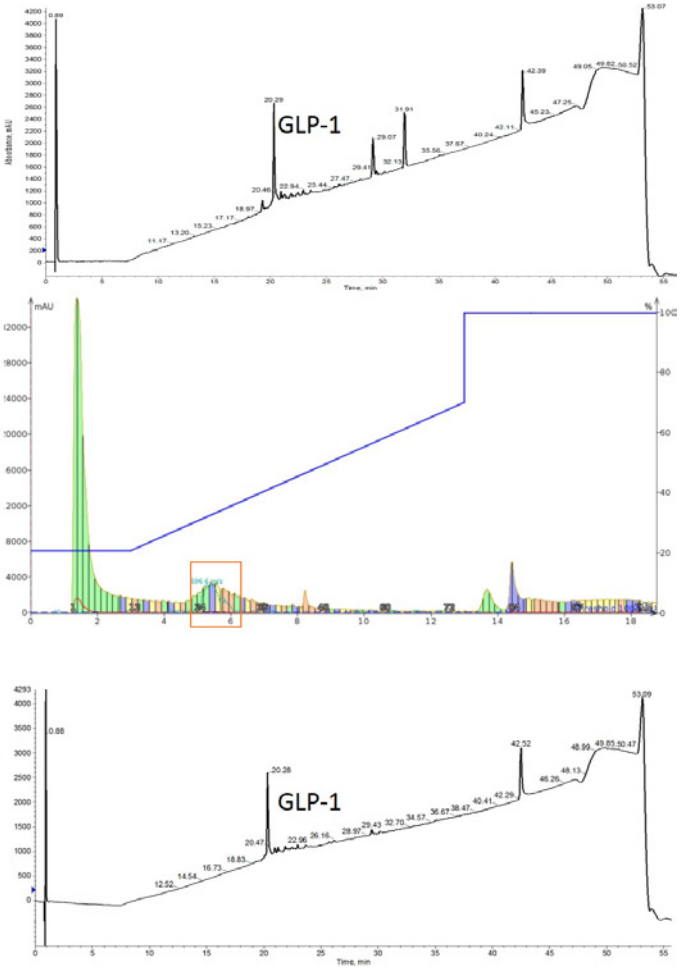

Figure 5. Crude GLP-1 (top) purified using an Isolera™ Dalton equipped with a 25 g Biotage® SNAP Bio C18 cartridge (middle) to greater than 87% purity as determined by analytical HPLC (bottom). The peptide is purified using a mobile phase modified with 0.1% NH_4OH to further evaluate the cartridge performance.

Conclusions

Herein we demonstrate high performance flash column chromatography as a tool for expediting the purification of synthetic peptides. The differences in resolution, but also loading capacity and recovery potential for two Biotage RP C18 cartridges are presented using crude synthetic ACP (65-74), purified with a gradient that requires only 10 minutes to complete. The work is then expanded to the purification of crude GLP-1, a 37 amino acid peptide with only 26% crude purity. This work demonstrates that peptides can in fact be purified to a high level of purity and with high recovery rates under a wide variety of conditions using SNAP Bio cartridges and flash purification.

Part Number: AN114

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