Purification of a Peptide ACE Inhibitor

using the ACCQPrep HP125

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Chromatography Application Note AN106

Abstract

Peptide synthesis and purification is becoming increasingly important. Peptides are used as active site models in drug discovery and are also increasingly being used as Active Pharmaceutical Ingredients (APIs). The increased use of peptides necessitates improved purification techniques. Impurities in synthesized peptides often come from impurities in the protected amino acid reagents used, and incomplete reactions as the peptide chain is lengthened.

The peptide HNWYPAAPH, synthesized by a local peptide synthesis lab, was studied as an ACE inhibiter[1].

Experimental and Results

Crude peptide was purchased. The method development screen of the ACCQPrep HP125 was used to verify the identity of the peptide and to confirm that the default ionization settings would allow mass spectral detection of the peptide (Figure 1).

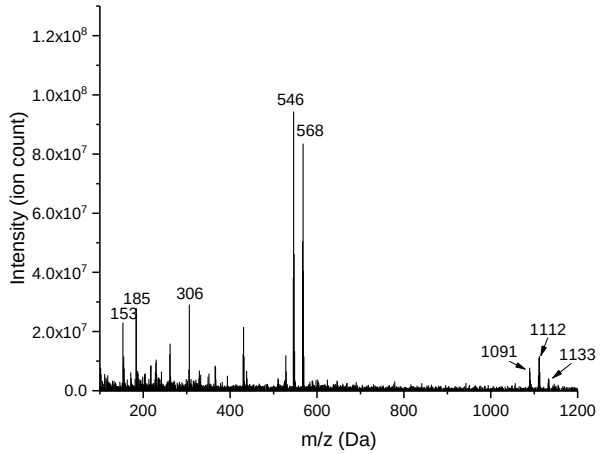

Figure 1: Verification of purchased HNWYPAAPH peptide

The mass was found to compare well with the expected mass (monoisotopic mass 1091.5 Da). Peaks visible include the $[M+H]^+$ (1091 Da); $[M+Na]^+$ (1112 Da); and the doubly-charged $[M+2H]^{2+}$ ion (546 Da).

Method Development

Method development was performed on an Agilent 1290 UHPLC system equipped with an experimental Teledyne Isco C18 column (4.6x50 mm, 2 µ particles, 1.0 mL/min) with a packing that matched the RediSep® Prep C18 column used for purification. The UHPLC was also equipped with a mass spectrometer set to monitor for the $[M+2H]^{2+}$ ion at 546 Da (Figure 2). The gradient was programmed to be 5 to 100% acetonitrile in water, both containing 0.1% trifluoroacetic acid (TFA).

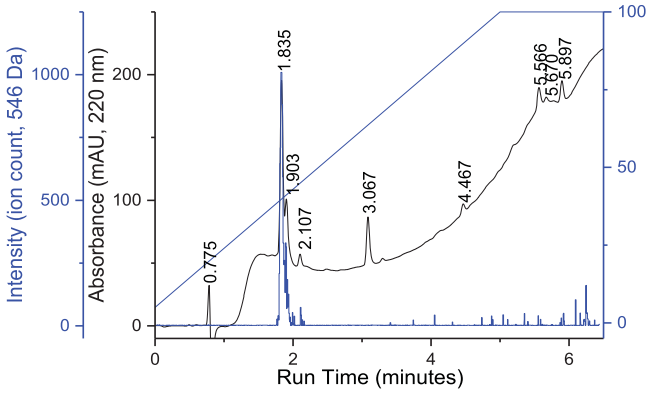

Figure 2: Analytical run of crude HNWYPAAPH

The UHPLC trace indicated the peptide (eluting at 1.843 minutes) was impure. The retention time suggested a gradient from 11 to 21 %B would offer the optimum separation of the target peptide from the closely eluting impurities.

Purification

The peptide (12.9 mg) was purified on a 20x150 mm RediSep Prep C18 column (P/N 69-2203-810) with a gradient from 11 to 21 %B (water/acetonitrile both containing 0.1% TFA) with an ACCQPrep HP125 (PN 68-5230-035) using the CombiFlash® PurIon Mass Spectrometer (P/N 68-5237-084) for detection (Figure 3). The sample was dissolved in 0.5 mL dimethyl sulfoxide (DMSO) with 0.1 mL water added. A time window was used to collect only the fractions containing the desired peptide.

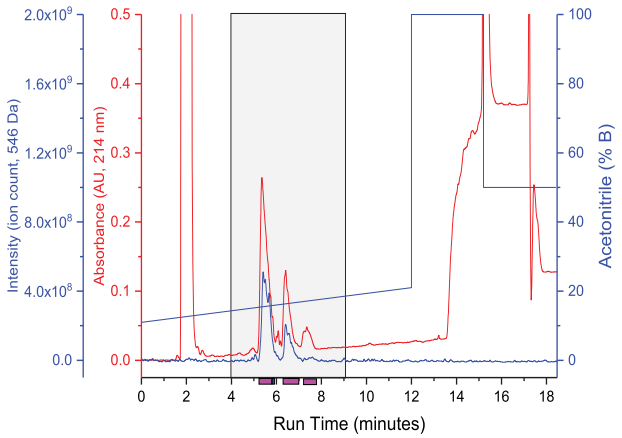

Figure 3: ACCQPrep run of crude HNWYPAAPH

The peak eluting at ~5.5 minutes matched the mass spectrum of the desired peptide. This material was rotary evaporated to remove acetonitrile and freeze-dried to yield 8.1 mg of material. This purified fraction was analyzed on an analytical UHPLC (Figure 4). A different column was used in case there were closely eluting impurities; using a different C18 column chemistry would help to expose those impurities.

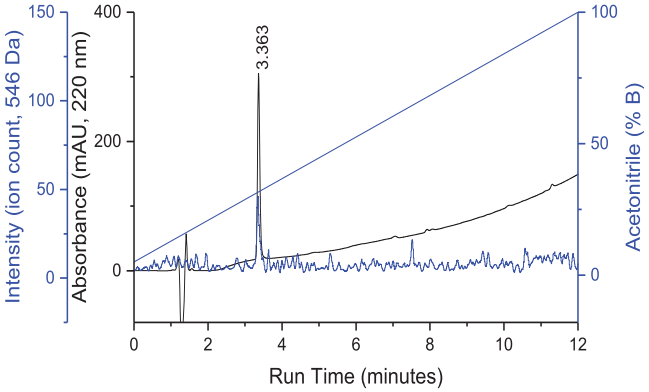

Figure 4: Analytical run of purified HNWYPAAPH

Conclusion

The ACCQPrep HP125 system coupled with the CombiFlash Purlon Mass Spectrometer is an excellent platform for purifying peptides. For fully automated purification, the AutoSampler option can be used for unattended multiple injections of the same sample if larger quantities of materials are required. The patented active solvent management prevents the loss of sample by stopping the pumps automatically when the solvent gets low in either solvent container.

[1]Lee,S-J; Kim, Y-S; Kim, S-E; Kim, E-K; Hwang, J-W; Park, T-K; Kim, B.K; Moon, S-H; Jeon, B-T; Jeon, Y-J; Ahn, C-B; Je, J-Y, Park, P-J. Purification and Characterization of a Novel Angiotensin I-Converting Enzyme Inhibitory Peptide Derived from an Enzymatic Hydrolysate of Duck Skin Byproducts. *J. Agric. Food Chem.* 2012, **60**, 10035-10040

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