Save Time and Money by Purifying Peptides Yourself

TELEDYNE ISCO
Everywhereyoulook™

Chromatography Application Note AN103

Overview

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 come from impurities in the protected amino acid reagents used and incomplete reactions as the peptide chain is grown.

Many research labs purchase their custom peptides from labs that specialize in the synthesis of these compounds. This reduces the required space in the lab for peptide synthesis equipment. These synthesis labs also offer purification services for the synthesized peptides at an additional cost and longer lead time. The Combi*Flash*® EZ Prep system with PurIon Model L mass spectrometer (P/N 68-5230-025 and 68-5237-084) and Redi*Sep*® Prep columns allows a research lab to save time and money by purifying the crude peptides received from the outside synthesis lab themselves.

Figure 1: EZ Prep System with Mass Spectrometer and Redi*Sep* Columns

The peptide HNWYPAAPH was studied as an ACE inhibiter[1] and was synthesized by a local peptide synthesis and purification lab, with the delivery times and prices quoted below[2].

Table 1: Time and Cost to Synthesize and Purify 1000mg HNWYPAAPH

Purity Level	Lead Time	Cost (per gram)
Crude	1 week	$2000 USD
95% purity	3 weeks	$2320 USD
99% purity	4 weeks	$2800 USD

Crude peptide was purchased. The method development screen of the Combi*Flash* EZ Prep system was used to verify the identity of the peptide and to confirm that the default ionization settings would detect the peptide (Figure 2).

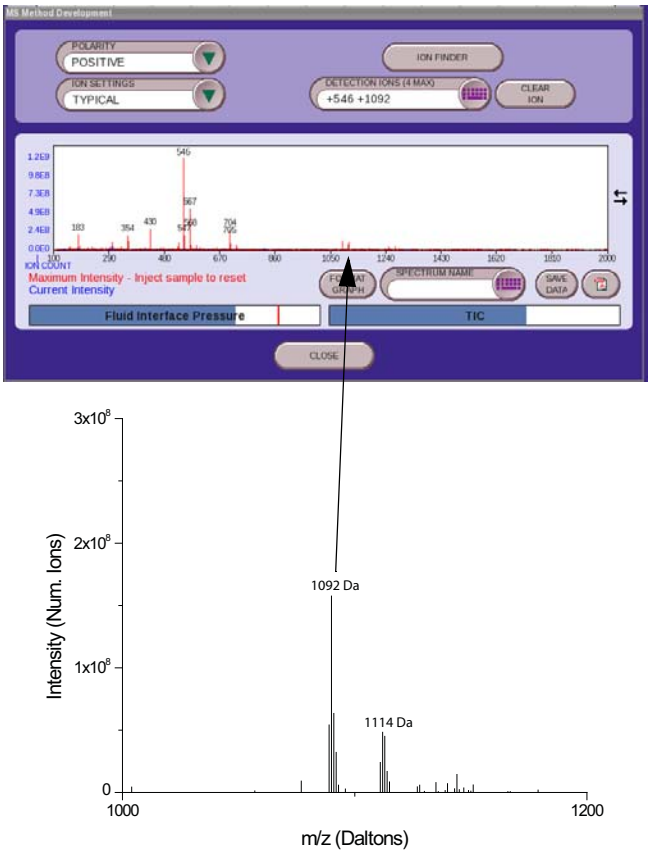

Figure 2: Verification of purchased HNWYPAAPH crude peptide

The mass was found to compare well with the expected mass (monoisotopic mass 1091.5 Da). Peaks visible include the $[M+H]^+$ (1092 Da); $[M+Na]^+$ (1114 Da); and the doubly-charged $[M+2H]^{2+}$ ion (546 Da). The 546 and 1092 Da peaks were selected as the detection ions in the Method Development window (Figure 2) and these values were automatically transferred to the run method for the column (Figure 3).

For an initial study, 10.2 mg of the sample was purified on a 10 mm x 150 mm Redi*Sep* Prep C18 column (P/N 69-2203-808). A water/acetonitrile gradient, both containing 0.1% trifluoroacetic acid (TFA), at 5.0 mL/min was used to elute the peptide.

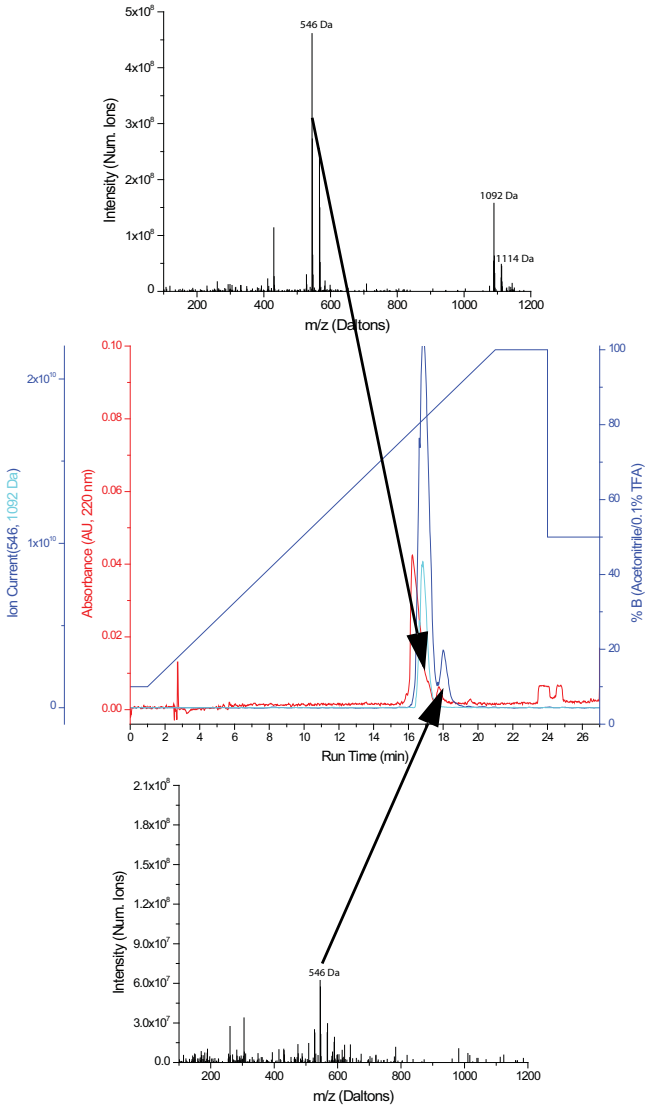

Figure 3: Purification of HNWYPAAPH on Combi*Flash* EZ Prep

Of the sample loaded, the major peak collected between 16.3 and 17.6 minutes yielded 7.8 mg (76.4% yield) at greater than 99% purity.

The use of the Combi*Flash* EZ Prep coupled to a PurIon Model L mass spectrometer allowed work on this peptide to begin three weeks earlier than would be otherwise possible if the peptide synthesis lab were to purify the material. An additional savings of $800 was also realized. By purifying the peptide as needed, a continuous supply of fresh peptide is always available as peptides can degrade over time during storage. Purifying the peptide only when needed always ensures there will be a supply of pure material for experiments and eliminates the possibility that degradation products could interfere with the experiments.

References

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.
2. Quoted 26 May 2015.

Teledyne ISCO
P.O. Box 82531, Lincoln, Nebraska, 68501 USA
Toll-free: (800) 228-4373 • Phone: (402) 464-0231 • Fax: (402) 465-3091
www.teledyneisco.com