Flash Peptide Purification and Method Development: AS48

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Chromatography Application Note AN126

Abstract

This application note continues the exploration of purification of peptides with flash chromatography. In our previous Application Notes, AN124 and AN125, different columns showed selectivity changes that could be exploited to improve purification. A C8 flash column showed good purification during column screening for this example.

Background

Peptides, especially those with longer chain lengths, are traditionally purified on preparative HPLC systems. Until now, flash chromatography traditionally didn't have the resolution needed to resolve these compounds, especially as the peptides become larger and the proportion of impurities is increased. Single amino acid deletions are similar to the desired compound, and there are more of them as the size of the peptide increases. The peptide AS-48 (sequence H2N-VVEAGGWVTTIVSILTAVGSGGLSLLAAAGRESIKAYLKKEIKKKGKRAVIAWMAKEFGIPAAVAGTVLNHV KKK-OH) was chosen for these experiments because it is a reasonably large peptide.

Experimental and Results

Method Development

Method development consists of two parts: determining the best column and determining the elution conditions. The pI of this peptide was estimated to be 9.52, so the solvent system was buffered to pH 3.5 with 50 mMol ammonium formate. The organic solvent, methanol, contained no additives. Ammonium formate is volatile, and so may be used with a mass spectrometer; the fluid interface reduces the buffer concentration to a level compatible with the mass spectrometer. 15.5 g Redi*Sep*® columns were used for scouting runs.

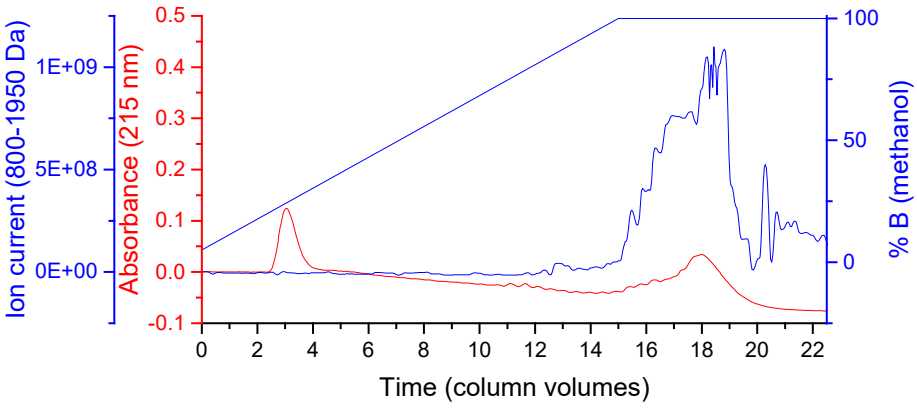

Figure 1—Peptide scouting run on a 15.5 g RediSep Gold® C18 flash column.

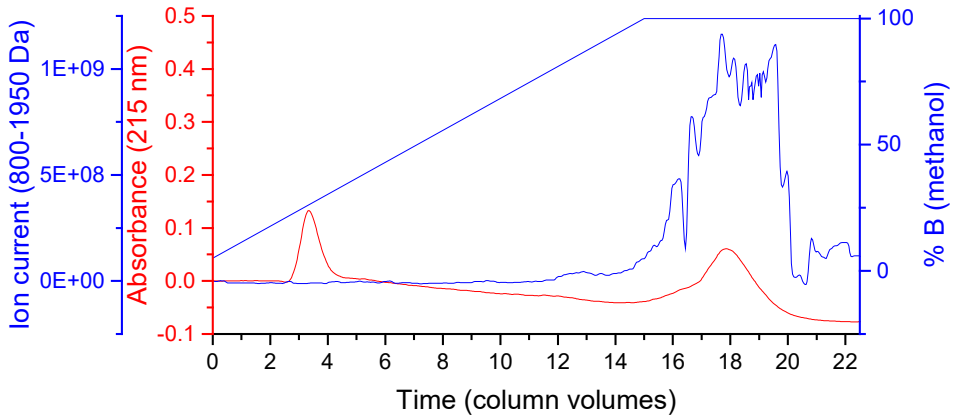

Figure 2—Peptide scouting run on a 15.5 g RediSep Gold C18AQ flash column.

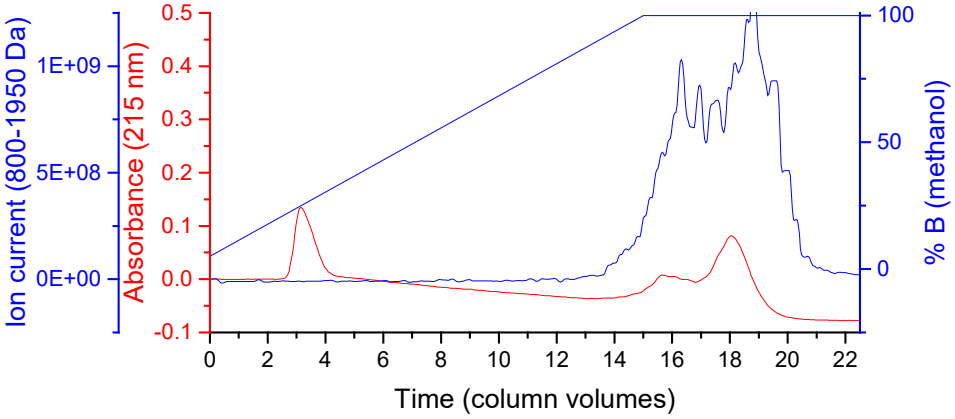

Figure 3—Peptide scouting run on a 15.5 g RediSep Gold C8 flash column.

The runs all used the same scouting gradient, solvent, and sample. Only 15 milligrams of sample was injected for each run. Neat trifluoroacetic acid (0.2 mL) was used to dissolve 15 mg of the sample. A larger mass of peptide was used because of its large molecular weight (7784 Da). Although all columns had similar retention, the C8 column showed improved resolution from earlier eluting materials at ~18.1 column volumes (CV).

The identity of the desired peak was further identified by extracting the mass data from the text file. Traces for the multiply charged ions at 1113 $[M+7]^{7+}$, 974 $[M+8]^{8+}$, 866 $[M+9]^{9+}$, and 780 $[M+10]^{10+}$ Daltons were extracted and summed to reduce noise and to better isolate the peptide peak.

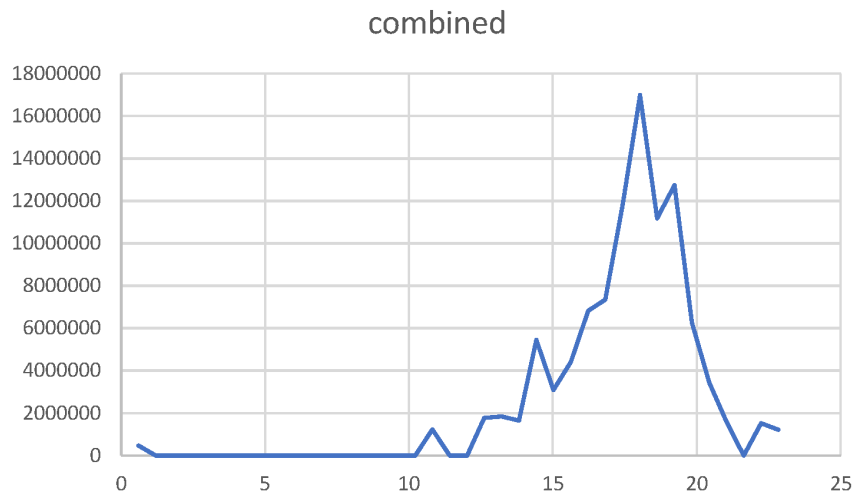

Figure 4—Combined mass traces to identify AS-48 elution.

The calculated gradient suggested the peptide was very non-polar, so scouting runs using acetonitrile were tested. The peptide eluted earlier, but the resolution using methanol was better. The PeakTrak Flash Focus Gradient Generator was used to calculate the gradient for purification (see Chromatography Technical Note 65).

Purification

The solvents for the purification were the same as those used in the scouting gradients. The focused gradient ran from 79% to 99% methanol on a 50 g column. The focused gradient was calculated using the "Time-on-Target" algorithm from the scouting run. The larger column was used to improve resolution. Rather than a step, the gradient went to 100% methanol to reduce the tailing and to wash out late-eluting compounds. Mass detection (PurIon L mass spectrometer with fluid interface) was used for these runs.

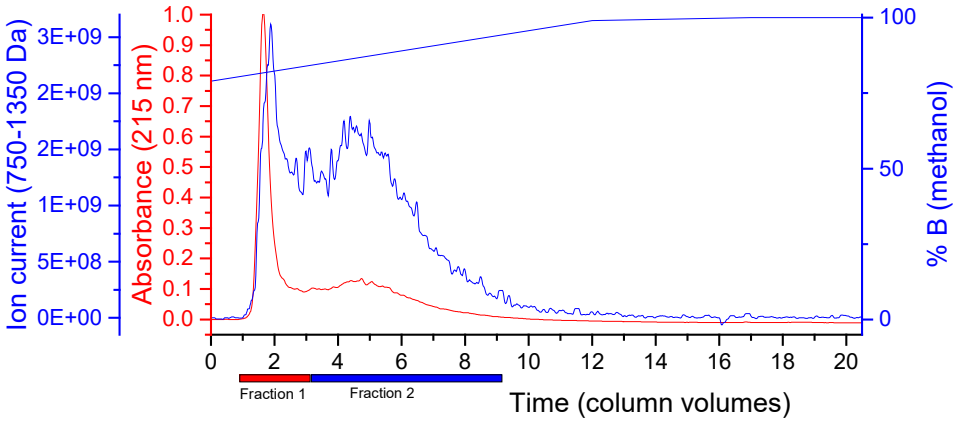

Figure 5—Flash focused gradient on a RediSep Gold C8 column; fractions combined as per the colored bars.

Based on the data from the scouting runs, only the C8 column was run. AS-48 was loaded (300 mg) to yield 61 mg of pure peptide. This low yield is expected because such a large peptide allows many chances for deletion errors during the synthesis.

Analytical HPLC

Analytical HPLC was run on an Agilent 1290 with a Redi*Sep* Prep 2 x 50 mm column in water/acetonitrile, both containing 0.1% TFA. The fractions were combined and analyzed prior to removal of methanol and freeze-drying. The peptide was very clean, although it was "only" purified with a flash column.

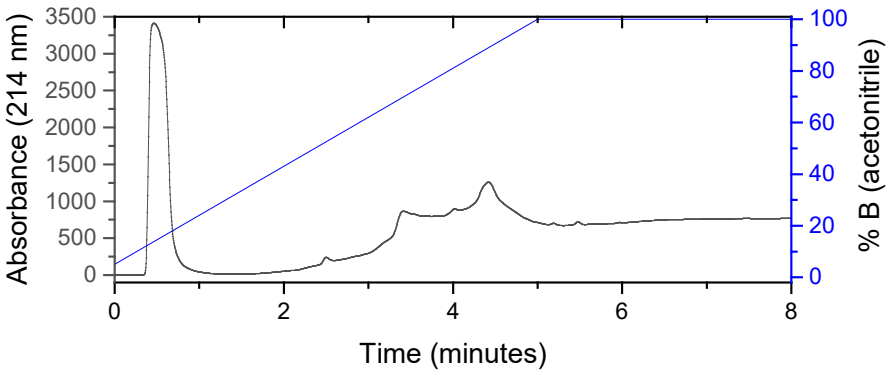

Figure 6—Analytical run of the crude peptide.

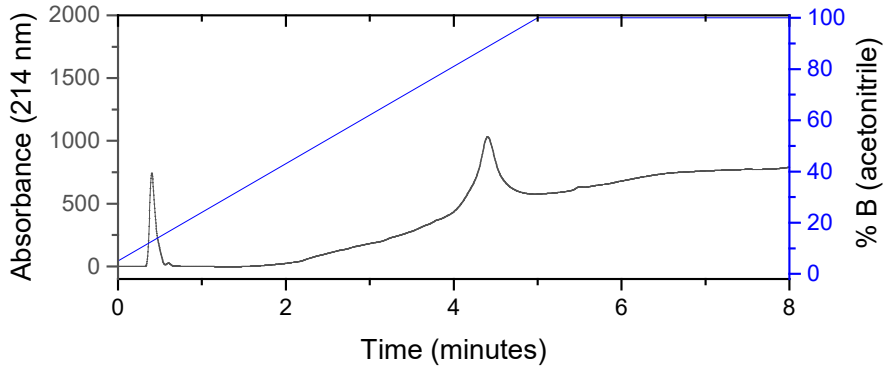

Figure 7—Peptide after purification.

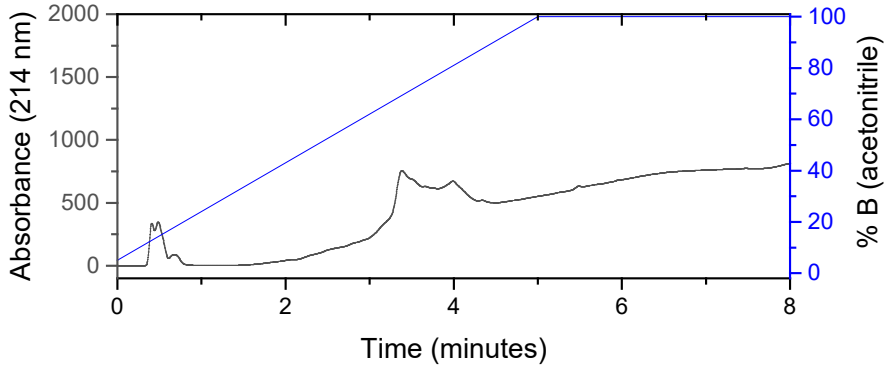

Figure 8—Early eluting compounds (first combined fractions) showing nearly complete removal of the peak eluting at 4.2 minutes in the top run.

Conclusions

Flash chromatography works very well to purify peptides with a molecular weight of ~8000 Daltons. The use of the PurIon L mass spectrometer allowed confirmation of the eluting peak from impurities. The flash chromatography total run time was about 9 minutes, with the desired peptide eluting within 5 minutes, so the purification time was very fast.

[1] *Silver, J. Overview of Analytical-to-Preparative Liquid Chromatography Method Development.* ACS Combinatorial Science ***2019***, 21 (9), *609–613.*

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