Purify lots of peptide with RediSep® 8.6 kilogram reverse phase columns

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Chromatography Application Note AN136

Abstract

Peptides are typically purified with high performance liquid chromatography (HPLC). HPLC columns are often limited in the mass of peptides than can be purified at one time due to a limited injection loop volume. Peptides are liquid loaded with syringes or pumps so that large volumes of diluted samples may be injected and focused at the top of the column. The limited solubility and loading amount for the purification of peptides may necessitate the need for larger columns as scale increases. Flash chromatography can purify peptides very well as described in Application Notes 124, 125, and 126. The gradient methods in those application notes can be scaled to purify grams to tens of grams of peptides in a single run. An extension from these Application Notes is described to show the utility of purifying peptides at larger scales using the RediSep Gold® C18, C18Aq or C8 columns.

Results and discussion

Scale-up

The run depicted in Figure 1 is from Application Note 124, where the Teledyne ISCO Focus Gradient Generator was used to create a preparative gradient on a 15.5 g RediSep C18 column, with 100 mg loading in a water/methanol gradient (26-36% methanol); the water contained 50 mM ammonium formate buffer (pH 3.8).

The preparative method was transferred to the CombiFlash Torrent® and the 8.6 kg RediSep Gold C18 column. As the focused preparative method proposed on the NextGen is in column volumes (CV), the same gradient length (in CVs) and the same starting and ending solvent composition can be used on the Torrent as on the CombiFlash® NextGen for the 40 g column. Using column volumes makes it easy to scale-up the method to larger columns independently of size or flow rate.

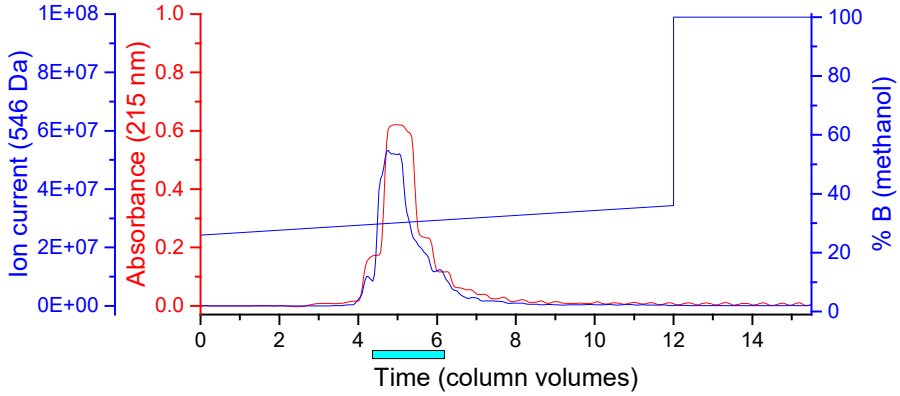

Figure 1. Crude EGFRvii peptide (100 mg) run on a 15.5 g RediSep Gold C18 column in water (50 mM ammonium formate, pH 3.8) and methanol as the strong solvent.

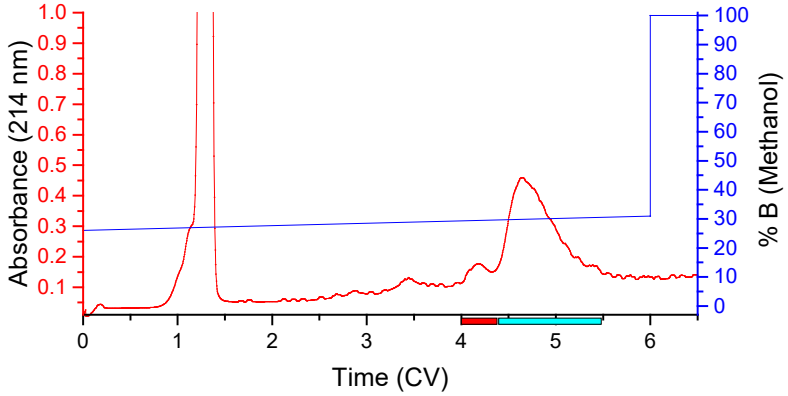

Figure 2. Crude EGFRvii peptide (100 mg) run on a 8.6 g RediSep Gold C18 column in water (50 mM ammonium formate, pH 3.8) and methanol.

The run in Figure 2 is the same peptide (> 5 g) run on a 8.6 kg RediSep Gold C18 column using the CombiFlash Torrent. The gradient method was initially the same as the run in Figure 1, but the run was truncated after 6 CV, as the peptide had completely eluted to save solvent. The peak at 4.45 CV was determined to be the purified peptide using the Method Development feature on the PurIon mass spectrometer coupled to the CombiFlash NextGen.

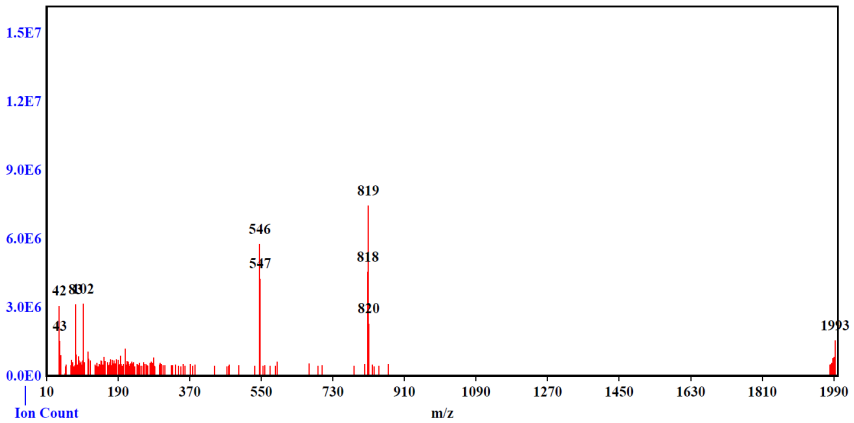

Figure 3. Mass spectrum of purified fraction showing (M+2H)2+ and (M+3H)3+ ions.

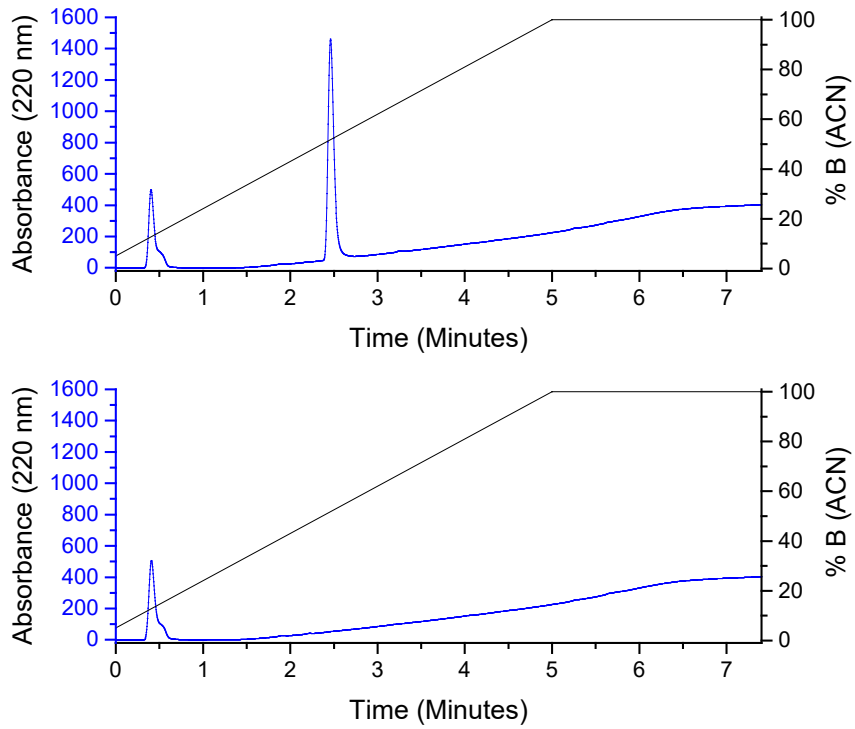

Figure 4. UHPLC of fraction 2 shown in Figure 2 (upper chromatogram) and solvent blank demonstrating the purity of a peptide purified via large scale flash chromatography.

Conclusion

Peptides can be purified using flash chromatography on a larger scale than HPLC while still providing pure fractions. Redi*Sep* columns scale up and deliver consistent results no matter the size. Coupled with the flash Focus Gradient Generator, scaling up has never been easier or as reliable.

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