The Effect of Reverse Phase Chain Length on Peptide Purification

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Chromatography Application Note AN109

Abstract

Peptide synthesis and purification continues to increase in importance. Peptides are used as active site models in drug discovery and are increasingly being used as Active Pharmaceutical Ingredients (APIs). Earlier chromatographic work focused on the analytical chemistry of peptides, but there have been few studies that looked at the relationship of alkyl chain length and its impact on larger scale purification of peptides where resolution and loading are both important. This application note compares the purification of several different sized peptides where the reverse phase stationary phase alkyl chain length is changed to determine its effect on the resolution and loading capacity of peptides.

Overview

Peptides vary widely in size causing them to behave differently during purification, even on the same media. Larger peptides tend to lay across the alkyl chains while smaller molecules are able to interact within the space between the alkyl chains. The smaller alkyl chains also increase the effective pore size of the stationary phase, improving access for larger peptides.

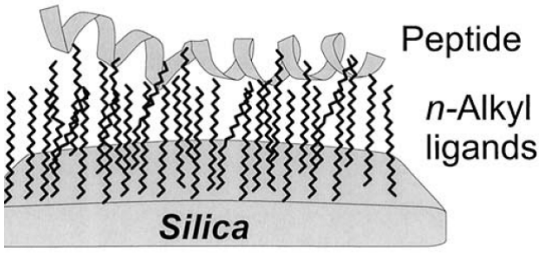


Figure 1: Peptide adsorbs with reverse phase by interacting with the ends of the alkyl chains

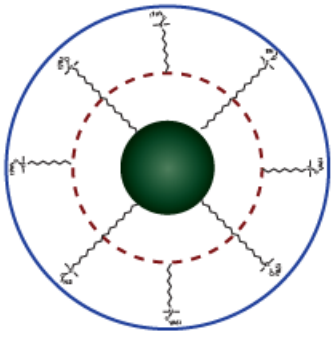


Figure 2: Smaller alkyl chains increase the effective pore size in reverse phase

Experimental and Results

Three peptides were chosen due to their sizes. The peptides used were EGFRvii (1635 g/mol), Thymosin (3066 g/mol), and AS-48 (7788 g/mol).

Analytical Runs

All peptide UHPLC experiments were run on an Agilent system with LC-MS grade water and acetonitrile containing 0.1% TFA (Midland Scientific, Omaha, NE, USA). Columns were heated to 70 °C for these runs. The columns were 150 x 2.1 mm, run at 0.400 mL/min.

Small Molecules

Experiments were run on columns using a mixture of parabens (methyl, ethyl, propyl, and butyl parabens) on C18, C8, and C4 columns with pore size of 200 Å prior to surface modification in a water/methanol gradient. There was a slight decrease in retention when the chain length was reduced to C8, and a greater change when chain length was further reduced to C4.

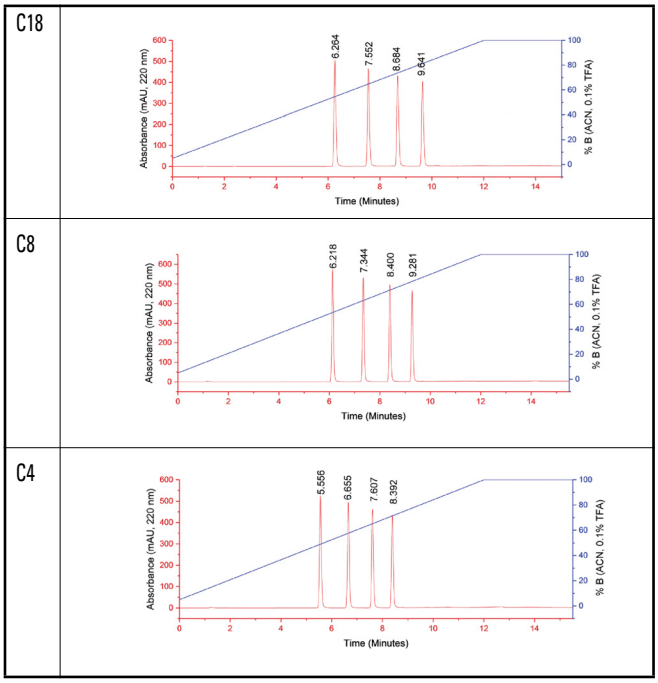


Table 1: Decreased alkyl chain length reduces retention time for small molecules

Peptides

Unlike the parabens, the peptides showed a small increase in retention as the alkyl chain length was reduced. This supports the idea that the peptides interact mainly with the end of the alkyl chain. The greatest increase in retention was observed with the AS-48 compound, possibly due to increased accessibility into pores in the stationary phase from the shorter chain length.

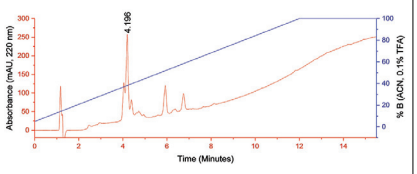
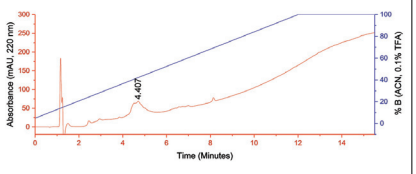
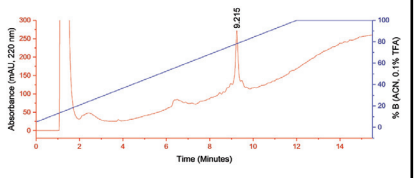
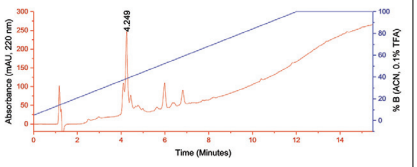
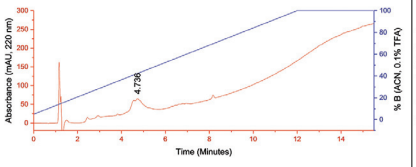
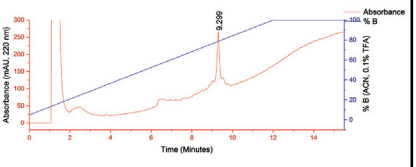
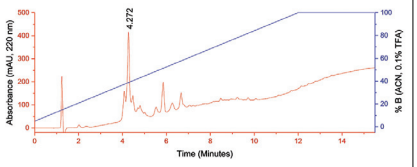
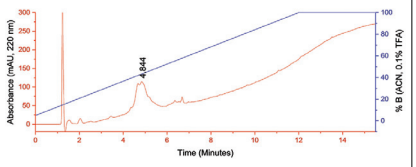
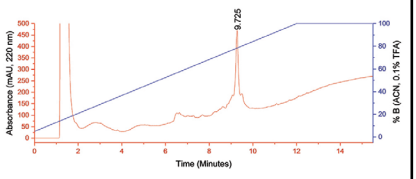
	EGFRvii	Thymosin	AS-48
200 Å C18			
200 Å C8			
200 Å C4			

Table 2: Peptides with scouting gradients

Preparative Runs

Peptides were run in 20 x 150 mm C18 columns at 18.9 mL/min in water/acetonitrile, both containing 0.1% TFA on an ACCQ*Prep* HP125 (PN 68-5230-033) with column switcher and auto sampler A solvent heater, in conjunction with a separate column heater was also used at 70 °C.

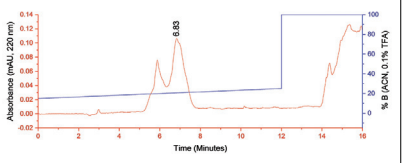
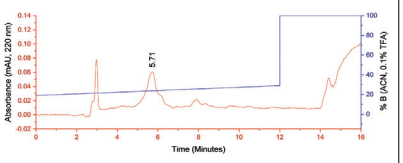
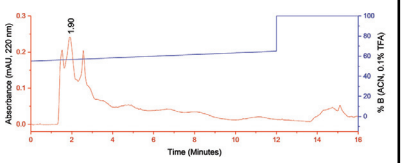
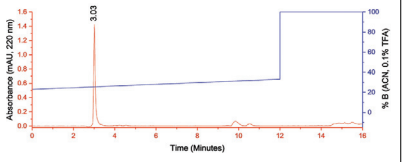
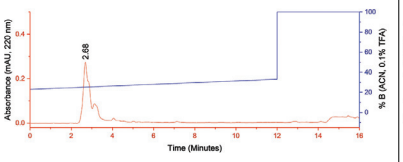
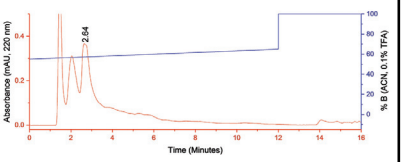
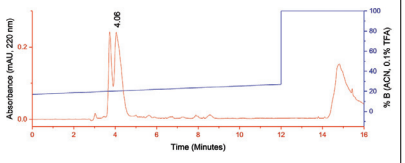
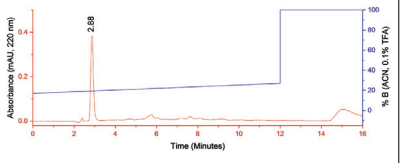
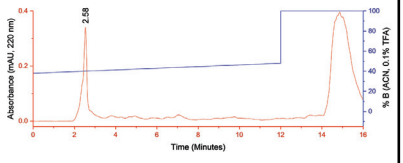
	EGFRvii	Thymosin	AS-48
200 Å C18			
200 Å C8			
200 Å C4			

Table 3: Preparative peptide runs on C18

EGFRvii ran best with the C18 based on resolution. Both Thymosin and AS-48 gave better results with the C8 material than either the C18 or C4 in terms of retention and resolution from impurities.

Conclusion

The C18 works best results for shorter peptides, smaller than 2000 g/mol, while the range from 2000 to 10,000 g/mol seem to run better with shorter carbon chains such as C8. The smaller peptides behave more like small molecules that have a better interaction with the C18 chains. The C8 chains are long enough to reduce secondary interactions with the base silica while having good interaction with the peptide.

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