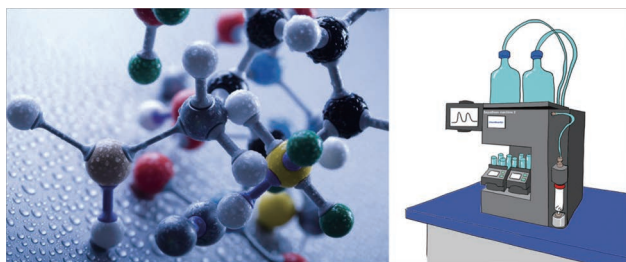


The Application of C18AQ Columns in the Purification of Strong Polar Peptides

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Introduction

A peptide is a compound composed of amino acids, each of which has unique physical and chemical properties due to the different types and order of amino acid residues constituting its sequence. With the development of solid phase chemical synthesis, the chemical synthesis of various active peptides has made great progress. However, due to the complicated composition of the peptide obtained by solid phase synthesis, the final product should be purified by reliable separation methods. The commonly used purification methods for peptides include ion exchange chromatography (IEC) and reversed-phase high performance liquid chromatography (RP-HPLC), which have the disadvantages of low sample loading capacity, high cost of separation media, complicated and costly separation equipment, etc. For the rapid purification of small molecule peptides (MW < 1 kDa), a successful application case was previously published by Santai Technologies, in which a SepaFlash™ RP C18 cartridge was utilized for the rapid purification of thymopentin (TP-5) and the target product meeting the requirements was obtained.

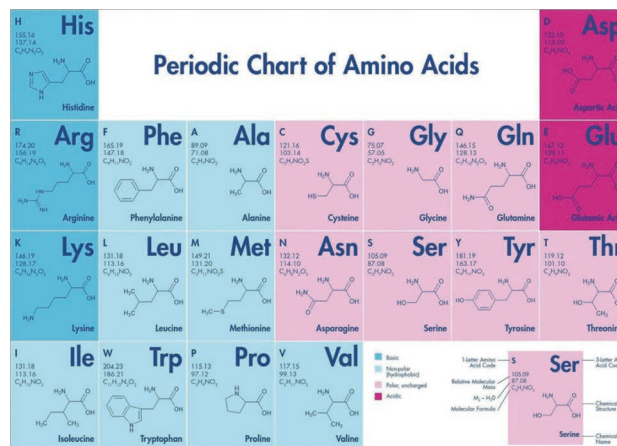


Figure 1. 20 common amino acids (reproduced from www.bachem.com).

There are 20 kinds of amino acids that are common in the composition of peptides. These amino acids can be divided into the following groups according to their polarity and acid-base property: non-polar (hydrophobic), polar (uncharged), acidic or basic (as shown in Figure 1). In a peptide sequence, if the amino acids constituting the sequence are mostly polar ones (as marked in pink color in Figure 1), such as Cysteine, Glutamine, Asparagine, Serine, Threonine, Tyrosine, etc. then this peptide might have a strong polarity and be highly soluble in water. During the purification procedure for these strong polar peptide samples by reversed-phase chromatography, a phenomenon called hydrophobic phase collapse will occur (refer to a previously published application note by Santai Technologies: Hydrophobic Phase Collapse, AQ Reversed Phase Chromatography Columns and Their Applications). Compared with the regular C18 columns, the improved C18AQ columns are most suitable for the purification of strong polar or hydrophilic samples. In this post, a strong polar peptide was utilized as the sample and purified by a C18AQ column. As a result, the target product meeting the requirements was obtained and could be used in the following research and development.

Experimental Section

The sample used in the experiment was a synthetic peptide, which was kindly provided by a customer laboratory. The peptide was about 1 kDa in MW and has strong polarity due to multiple polar amino acid residues in its sequence. The purity of the raw sample is about 80%. To prepare the

sample solution, 60 mg white powdery crude sample was dissolved in 5 mL pure water and then ultrasonicated in order to make it become a completely clear solution. The sample solution was then injected into the flash column by an injector. The experimental setup of the flash purification is listed in the Table 1.

Instrument	SepaBean™ machine 2			
Cartridges	12 g SepaFlash™ C18 RP flash cartridge (spherical silica, 20 - 45 µm, 100 Å, Order number:SW-5222-012-SP)		12 g SepaFlash™ C18AQ RP flash cartridge (spherical silica, 20 - 45 µm, 100 Å, Order number:SW-5222-012-SP(AQ))	
	Wavelength		214 nm	
Mobile phase	Solvent A: Water Solvent B: Acetonitrile			
Flow rate	15 mL/min		20 mL/min	
Sample loading	30 mg			
Gradient	Time (CV)	Solvent B (%)	Time (min)	Solvent B (%)
	0	0	0	4
	1.0	0	1.0	4
	10.0	6	7.5	18
	12.5	6	13.0	18
	16.5	10	14.0	22
	19.0	41	15.5	22
	21.0	41	18.0	38
	/	/	20.0	38
	/	/	22.0	87
/	/	29.0	87	

Table 1. The experimental setup for flash purification.

Results and Discussion

To compare the purification performance for the polar peptide sample between regular C18 column and C18AQ column, we utilized a regular C18 column for the flash purification of the sample as a start. As shown in Figure 2, due to the hydrophobic phase collapse of the C18 chains caused by high aqueous ratio, the sample was barely retained on the regular C18 cartridge and was directly eluted out by the mobile phase. As a result, the sample was not effectively separated and purified.

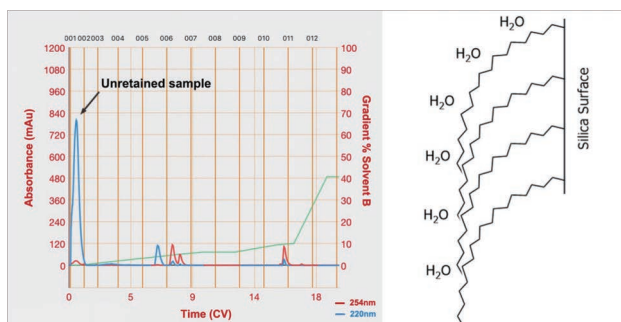


Figure 2. The flash chromatogram of the sample on a regular C18 cartridge.

Next, we used a C18AQ column for the flash purification of the sample. As shown in Figure 3, the peptide was effectively retained on the column and then eluted out. The target product was separated from the impurities in the raw sample and collected. After lyophilization and then analyzed by HPLC, the purified product has a purity of 98.2% and could be further utilized for next step research and development.



Figure 3. The flash chromatogram of the sample on a C18AQ cartridge.

In conclusion, SepaFlash™ C18AQ RP flash cartridge combined with the flash chromatography system SepaBean™ machine could offer a fast and effective solution for the purification of strong polar or hydrophilic samples.

About the SepaFlash™ C18AQ RP flash cartridges

There are a series of the SepaFlash™ C18AQ RP flash cartridges with different specifications from Santai Technology (as shown in Table 2).

Item Number	Column Size	Flow Rate (mL/min)	Max. Pressure (psi/bar)
SW-5222-004-SP(AQ)	5.4 g	5-15	400/27.5
SW-5222-012-SP(AQ)	20 g	10-25	400/27.5
SW-5222-025-SP(AQ)	33 g	10-25	400/27.5
SW-5222-040-SP(AQ)	48 g	15-30	400/27.5
SW-5222-080-SP(AQ)	105 g	25-50	350/24.0
SW-5222-120-SP(AQ)	155 g	30-60	300/20.7
SW-5222-220-SP(AQ)	300 g	40-80	300/20.7
SW-5222-330-SP(AQ)	420 g	40-80	250/17.2

Table 2. SepaFlash™ C18AQ RP flash cartridges. Packing materials: High-efficiency spherical C18(AQ)-bonded silica, 20 - 45 µm, 100 Å.



For further information on detailed specifications of SepaBean™ machine, or the ordering information on SepaFlash™ series flash cartridges, please visit our website:

<http://www.santaitech.com/en/>.

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