Peptide Separations Using Reverse Phase Redi*Sep*® Columns

Application Overview

A peptide can be defined as a short polymer of amino acids. The characteristics of an individual peptide will of course depend on its particular makeup and arrangement of amino acids. Peptides are relatively polar and their binding characteristics are fairly consistent with that of other proteins.

Peptide mixtures may be separated into individual peptide species. If the separate and more specific peptides can be contained in a single fraction, this can greatly expedite many scientific processes.

The following is a generalized approach to separating peptide mixtures into fractions on reusable reversed phase Redi*Sep*® C18 columns.

Generally it is best to start with the 4.3 gram Redi*Sep* reversed phase column. Less peptide sample and solvent will be used in discovering good parameters for the particular peptide. Once elution parameters are established the process can be scaled up to suit. It may be beneficial to review the reversed phase theory applications note AN10.

For the particular peptide mixture used in this reference, the parameters are listed in Table 1. Parameters will vary with peptide structure.

Table 1: General Method Parameters

Instrumentation	Isco CombiFlash SQ16x	
Wavelength	210 nm	
Mobile phase	Solvent A: Purified Water/0.1% TFA in A Solvent B: Acetonitrile/0.1% TFA in B	
Flow Rate	17 mL/minute	
Equilibration Volume	20 mL	
Inject Volume	0.5 mL	
Gradient	% Solvent B	Minutes
	5	Initial
	25	1.0
	95	8.0
Run time	9.0 minutes, not including equilibration	

General Method Wavelength

The ideal wavelength will vary according to peptide structure. It is desirable to choose the wavelength with the greatest sensitivity without picking up too much noise or missing certain compounds. If 210 nm is too noisy then 214 nm will generally offer a cleaner baseline. Ring structures absorb best at 280 nm, double bonds at 254 nm, and single bonds at 210 nm. The recommended general-purpose wavelength for proteins and peptides is 210 nm.

Counterion

To improve peptide and column performance a counterion is commonly used. This helps to provide a more uniform separation and thus improves column performance. A commonly used counterion is 0.1% v/v triflouroacetic acid (TFA). This is added to the A and B mobile phase in equal amounts. Keeping the counterion concentration throughout the separation will result in a more consistent baseline. One mL in one Liter of mobile phase (0.1% v/v) is commonly used. TFA is pipetted directly into the mobile phase and mixed thoroughly.

Column Loading

This parameter varies greatly with the makeup of the peptide. For a 4.3 gram Redi*Sep* reversed phase column loading 0.5 mg should be a good starting point.

Column construction

The column itself consists of three major parts:
1. The supporting medium is the solid surface on which the stationary phase is bound.
2. The stationary phase is the active coating attached to the supporting medium, *i.e.* C18.
3. The mobile phase is the fluid that passes over the supporting medium in order to expose the active portions of the peptides to the stationary phase.

Mobile phase or solvent system

Many peptides adhere strongly to C18 and will generally require a strong gradient to elute. Typically, water is used as solvent A and acetonitrile as solvent B for eluting peptides.

Gradient

When establishing a new method it is generally recommended to create a gradient to ramp very slowly from 0 to 95% of the B solvent to ensure that all peaks are eluted and the column has been cleaned.

After the initial 0–95% gradient has been run and the elution profile roughly established, the gradient can be modified to suit a particular peptide. By altering the

slope of the gradient at particular retention times, separation time and peak shape can be optimized.

Analytical Results

A reference peptide chromatogram is provided in Figure 1. The particular peptide was separated using the parameters listed in Table 1. Parameters will vary with peptide structure.
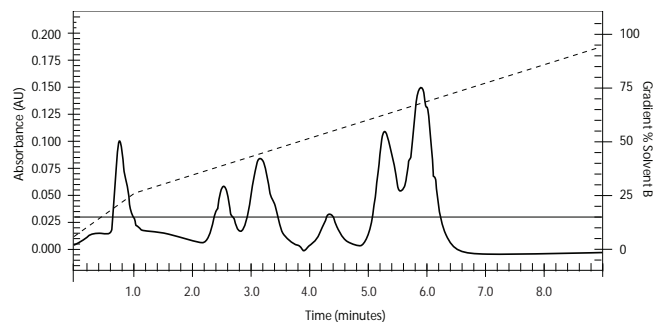

Figure 1: Sample Peptide Chromatogram

Summary

Peptide purification methods can be established on smaller columns then scaled up to suit desired sample load.

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