Improve Characterization of Complex Protein Digests

Using Viva Wide Pore HPLC Columns

by Julie Kowalski, Innovations Chemist

- Superior resolution—many peaks contain one or two peptides, not three or more.
- Excellent results with highly aqueous mobile phases, compatible with digest matrices.
- Restek-manufactured silica in Restek-manufactured columns.

Protein analyses often incorporate a combination of liquid chromatography and electrospray mass spectrometry. Typically, a protein sample is chemically or enzymatically digested to produce peptides, HPLC/MS is used to resolve and identify the peptides, and this information is used to search protein databases to identify the protein of interest. This type of analysis is now used in many fields, including the bioanalytical and pharmaceutical disciplines.

We tailored Viva silica specifically to provide superior chromatography for peptides and other large molecules, and we highly recommend Viva columns for analyses of protein digests. Featuring the largest available surface area in 250-350 Angstrom pores, packings prepared from Viva silica allow longer interaction between peptides and the stationary phase, affording greater resolution.

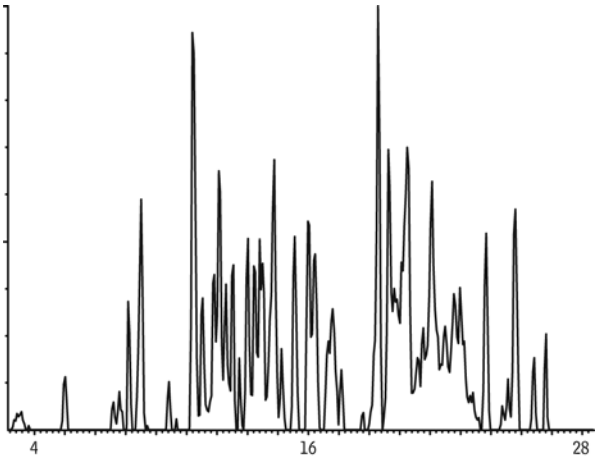
For an example analysis, we prepared a trypsin digest of bovine serum albumin (BSA).[1] We used a 150mm x 1mm ID Viva C18 column (5µm particles, cat# 9514561) to separate the peptides, which number approximately 70, and identified them through manual data analysis.

Figure 1 is a TIC chromatogram for the BSA trypsin digest. Close observation reveals the Viva C18 column has provided outstanding separation, based on the large number of discrete peaks representing only one or two peptides. In contrast, in typical results from other "wide pore" columns it is common to see three or more peptides per peak; this can reduce the number of peptides that are identified. The large number of discrete peaks in Figure 1 also indicates that peptide interaction with the Viva C18 stationary phase, rather than with one another, is the primary retention/separation mechanism.

Figure 1 A Viva C18 column resolves a BSA tryptic digest into many 1-2 peptide peaks, for more reliable identification.

for **more** info

For details on this analysis, please visit our website: **www.restek.com/bioanalytical**



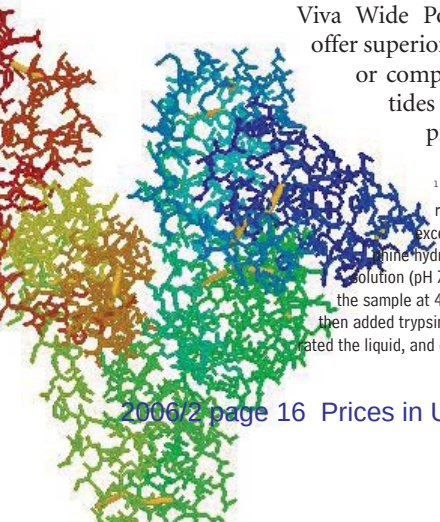
Sample:
Inj.: 15µL
Conc.: bovine serum albumin tryptic digest, 16pmol/µL
Sample diluent: water/0.15% formic acid (v/v)

Column: **Viva C18** (cat. # 9514561)
Dimensions: 150mm x 1mm
Particle size: 5µm
Pore size: 300Å

Conditions:
Mobile Phase: A: water/0.15% formic acid (v/v)
B: acetonitrile/0.15% formic acid (v/v)

Time:	%B
0.0	5
4.5	5
64.0	65

Flow: 0.2mL/min.
Temp.: ambient
Det.: Micromass Quattro II



Viva Wide Pore HPLC Columns offer superior resolution of simple or complex mixtures of peptides - a critical factor in protein identifications.

[1] BSA disulfide bonds were reduced by adding a molar excess of tris(2-carboxyethyl)phosphine hydrochloride (TCEP) to a buffered solution (pH 7) containing BSA. We stored the sample at 40°C for one hour, under argon, then added trypsin to digest the protein, evaporated the liquid, and dissolved the digest in water.

Viva C18 Columns

Physical Characteristics:

particle size: 3µm or 5µm, spherical
pore size: 300Å
carbon load: 9%
endcap: yes
pH range: 2.5 to 10
temperature limit: 80°C

	1.0mm ID		2.1mm ID		3.2mm ID		4.6mm ID	
Length	cat.#	price	cat.#	price	cat.#	price	cat.#	price
5µm Columns								
30mm	9514531	$328	9514532	$308	9514533	$308	9514535	$308
50mm	9514551	$328	9514552	$308	9514553	$308	9514555	$308
100mm	9514511	$354	9514512	$334	9514513	$334	9514515	$334
150mm	9514561	$380	9514562	$360	9514563	$360	9514565	$360
200mm	9514521	$407	9514522	$386	9514523	$386	9514525	$386
250mm	9514571	$432	9514572	$412	9514573	$412	9514575	$412