RESTEK
Turning Visions into Reality
Chromatography Products

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.

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

RESTEK PRODUCTS

- **HPLC Column**
  Viva C18 Wide Pore Columns

FOOTNOTE

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.

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

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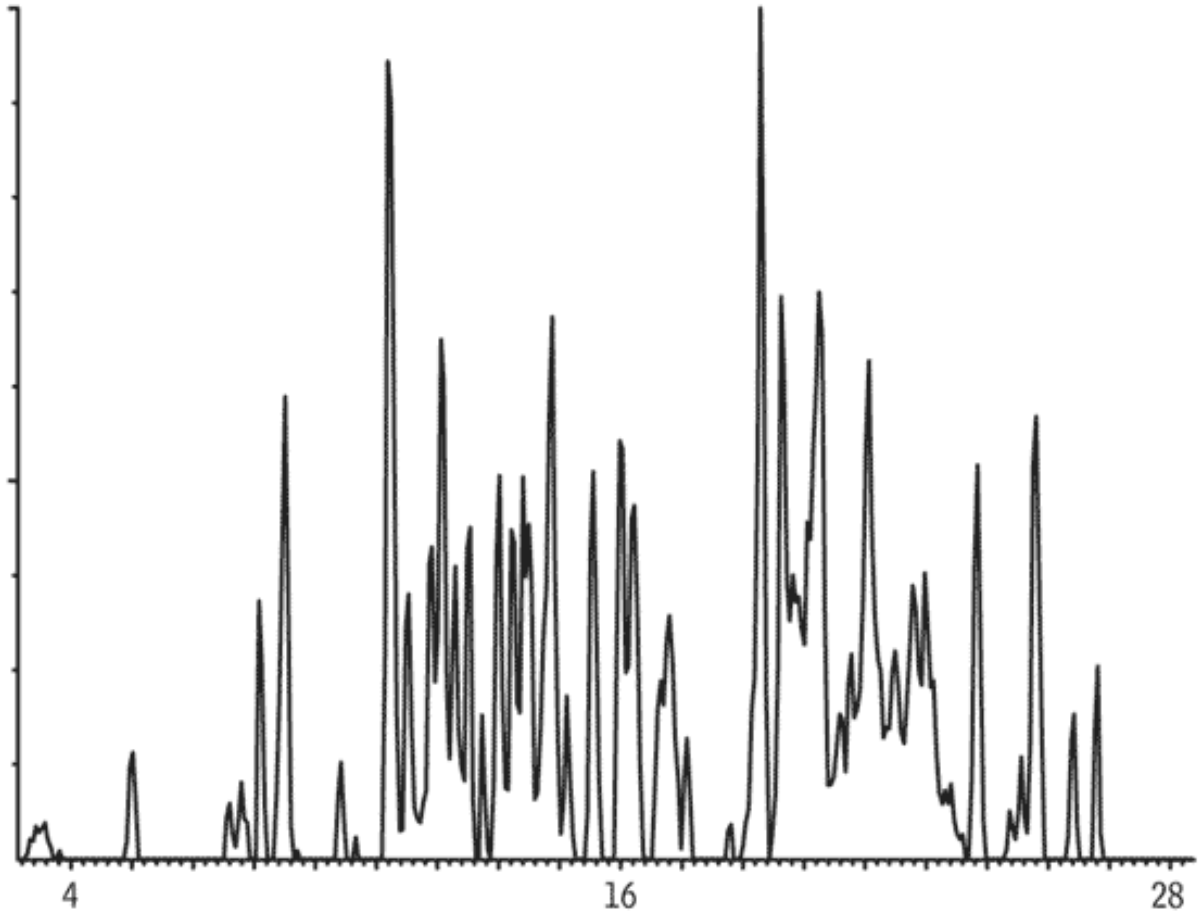

Sample:

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

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