

PROTO™ 200 More Selectivity and Resolution for Peptides

200

Phases	C4 and C18
Particle Sizes	4.5 and 10µm
Pore Size	200Å
Pore Volume	1.1 mL/gm
Surface Area	200m ² /gm
%Carbon (w/w)	C4. = 4.5%, C18= 14%
Phase type	Monofunctional & fully endcapped
Silica Class	Type B
USP Class	L26 PROTO 200 C4 L1 PROTO 200 C18

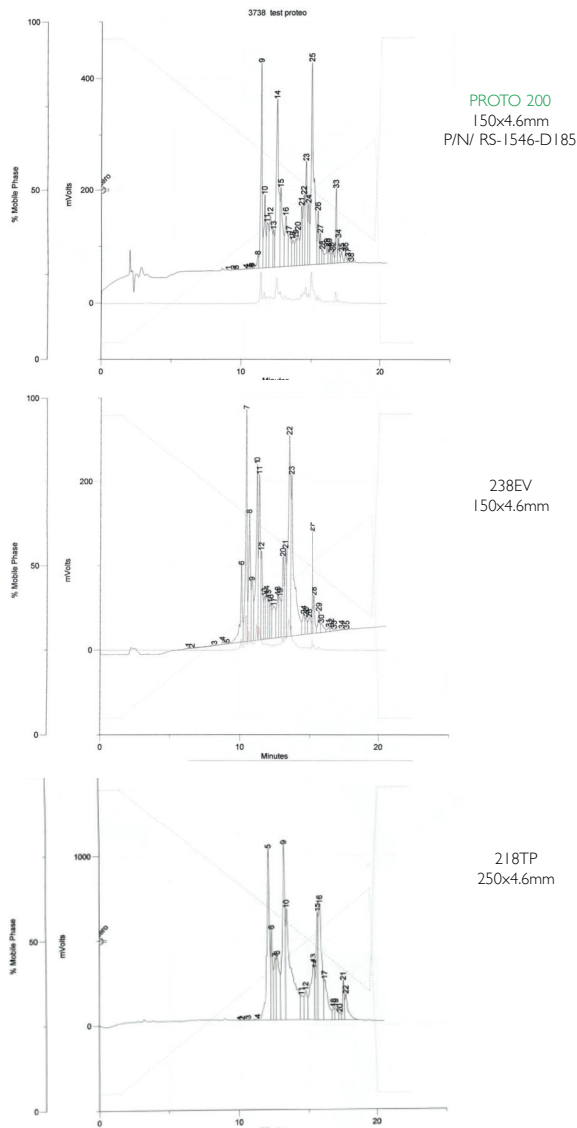
Applications

300Å wide pore HPLC columns are traditionally used for biomolecule analysis since the molecular radii of large peptides and proteins are better matched than with smaller pore size columns (80 - 120Å) typically used for small molecule analysis. 300Å sorbents, however, have reduced surface area, thus less capacity and selectivity than would be had with smaller pore size materials. Higgins Analytical's new PROTO™ 200 presents a powerful alternative for the analysis of peptides of ~19kD and less.

Guide to PROTO 200 Part Numbers

Rx-xxxx-D045 PROTO 200 C4 5µm
 Rx-xxxx-D185 PROTO 200 C18 5µm
 See Page 23 for complete Part Number information

Comparison of Phosphopeptide Purity Analysis



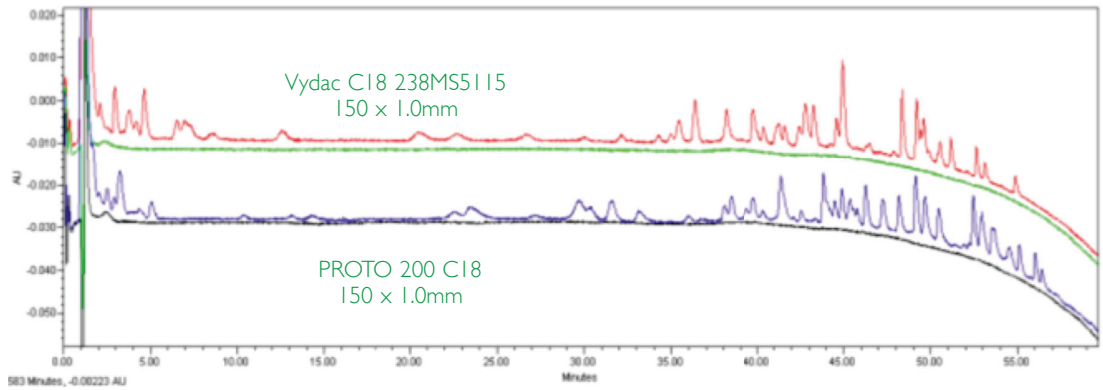
Phosphopeptide Purity Analysis

Peptide HPLC column performance, using the same gradient conditions, was evaluated on the PROTO™ 200 C18, a new peptide analysis column obtained from The Nest Group, Inc.. Against the industry standard, Grace/Vydac® 218TP C18, and their newer peptide chemistry, Everest® C18, the data show that the PROTO™ 200 C18 column has superior resolution to the 218TP chemistry and better resolution, especially for the more hydrophobic peptides, than the Everest® C18. In addition, the PROTO 200 columns can be run without TFA, which allows better detection when utilizing LC-MS.

Previously, the Everest chemistry had been shown by Grace/Vydac to be superior to all other wide pore columns, especially for the more hydrophobic peptides. However, the ready availability of the PROTO™ 200 peptide columns, in either C4 or C18 chemistries, and in capillary through preparative sizes, now allows researchers their first opportunity to save both time and money on a wider variety of applications, compared to the Grace/Vydac columns, and by extension to all other peptide columns.

200

Proto™ 200 C18 provides greater selectivity than the popular 300Å choice



Tryptic Digest of Carbamidomethylated BSA (16h, 20 pMols on column).

Column Size:
150 × 1.0mm

Column Brand:

Upper Chromatogram:	Vydac 300Å	238MS C18 5µm	P/N 238MS5115
Lower Chromatogram:	Higgins 200Å	Proto 200 C18 5µm	P/N RS-1501-D185

Conditions:
 A = 0.1% formic acid containing 0.01% TFA
 B = 0.085% formic acid in CH₃CN containing 0.01% TFA
 Initial 5 min isocratic hold at 5% B, then 60 min hyperbolic gradient to 65%B
 Flow 0.15 ml/min, Green & Black curves: Blank gradient profile

Higgins Analytical thanks Amos Heckendorf of The Nest Group (www.nestgrp.com) for these Proto 200 data.