

PROTO™ 200 More Selectivity and Resolution for Peptides

200

Phases	C4 and C18
Particle Sizes	4.5 and 10µm
Pore Size	200Å
Pore Volume	1.1mL/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

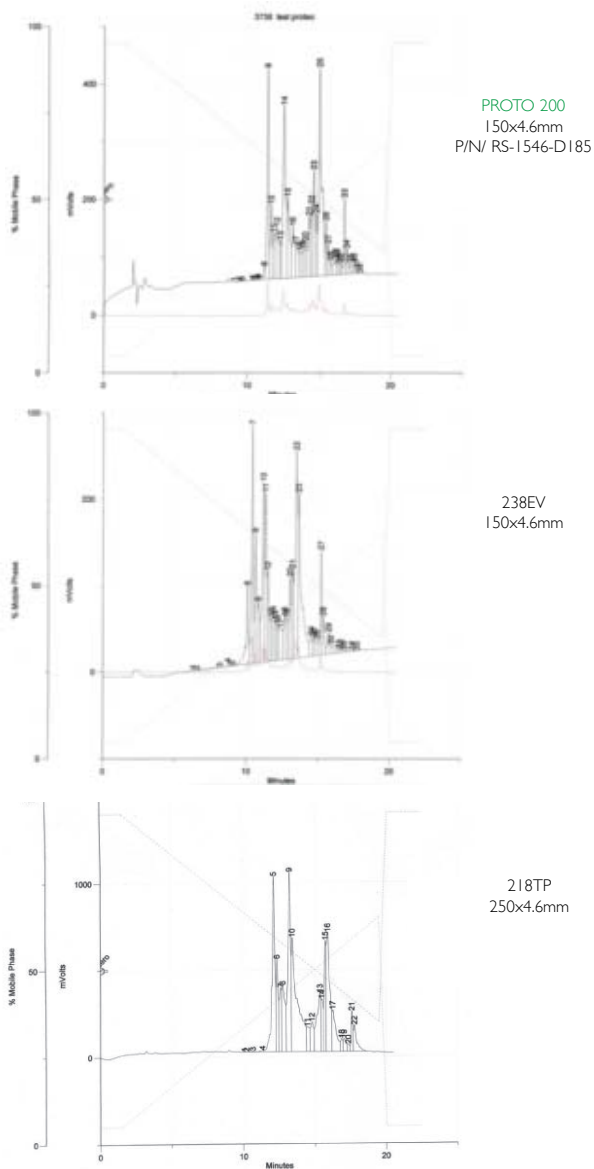
Applications

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

Comparison of Phosphopeptide Purity Analysis

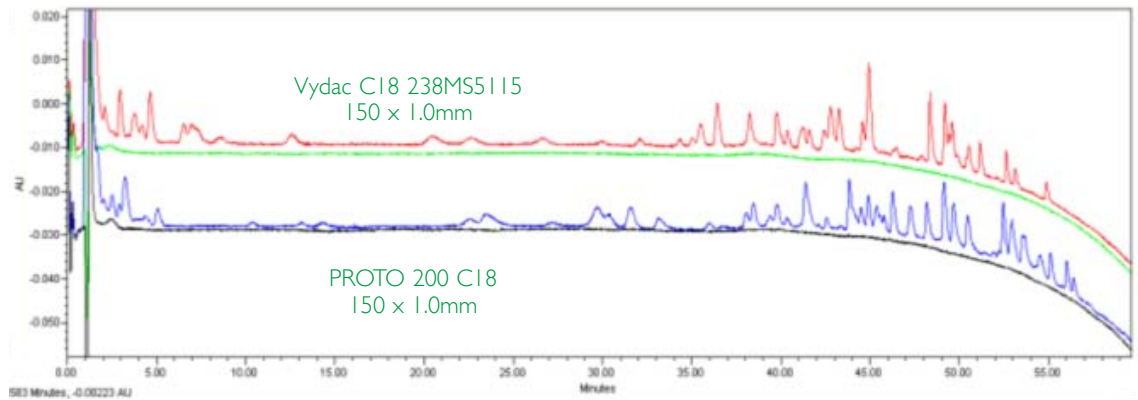


Phosphopeptide Purity Analysis

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.

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



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

Column Size:
150 x 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

PROTO™ 300 Robust Bioseparations



Phases	C4 and C18
Particle Sizes	4.5 and 10µm
Pore Size	300Å
Pore Volume	0.9mL/gm
Surface Area	100m ² /gm
%Carbon (w/w)	C4 = 3%, C18= 8%
Phase type	Monofunctional & fully endcapped
Silica Class	Type B
USP Class	L26 PROTO 300 C4 L1 PROTO 300 C18

Applications

Low pH operating conditions and high pH column washing steps are typical bioprocessing operating conditions that can severely limit the lifetime and performance of widepore columns typically used for protein and peptide analysis. Higgins Analytical's new PROTO300 has high capacity for enhanced resolution and is robust enough to withstand prolonged use at extreme pH (1.5 - 10).

Guide to PROTO 300 Part Numbers

Rx-xxxx-W045 PROTO 300 C4 5µm
 Rx-xxxx-W185 PROTO 300 C18 5µm

Stability Study Under Extremely Alkaline Conditions

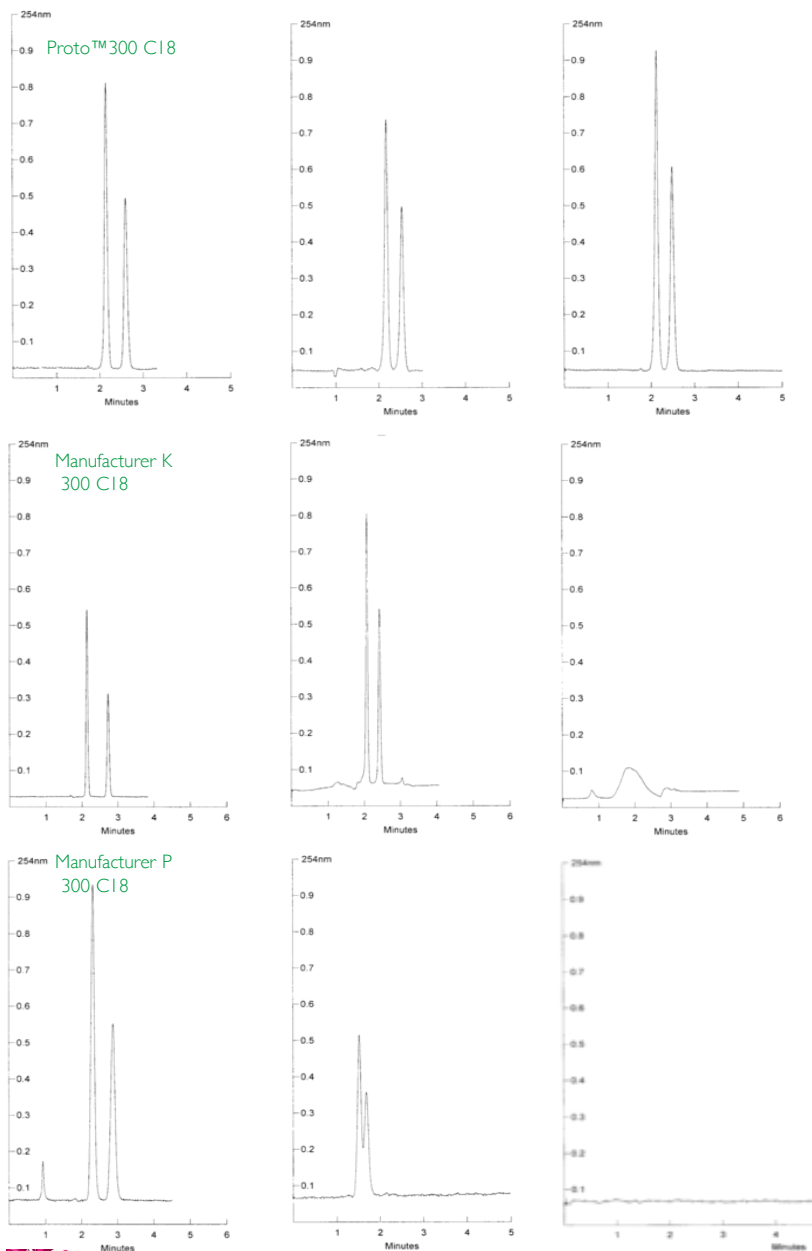
Experimental:
 150 x 4.6mm columns were studied. All experiments were conducted on duplicate columns. Solutes used for performance measurement were dimethylphthalate and fluorene.

Step One:
 Column stability and measurement precision was established for each column with multiple injections over 18hrs under neutral conditions (70% MeCN/water; 1mL/min). All columns were very stable. The chromatograms of a column from each manufacturer at the end of the 18 hour period are shown on the left hand side.

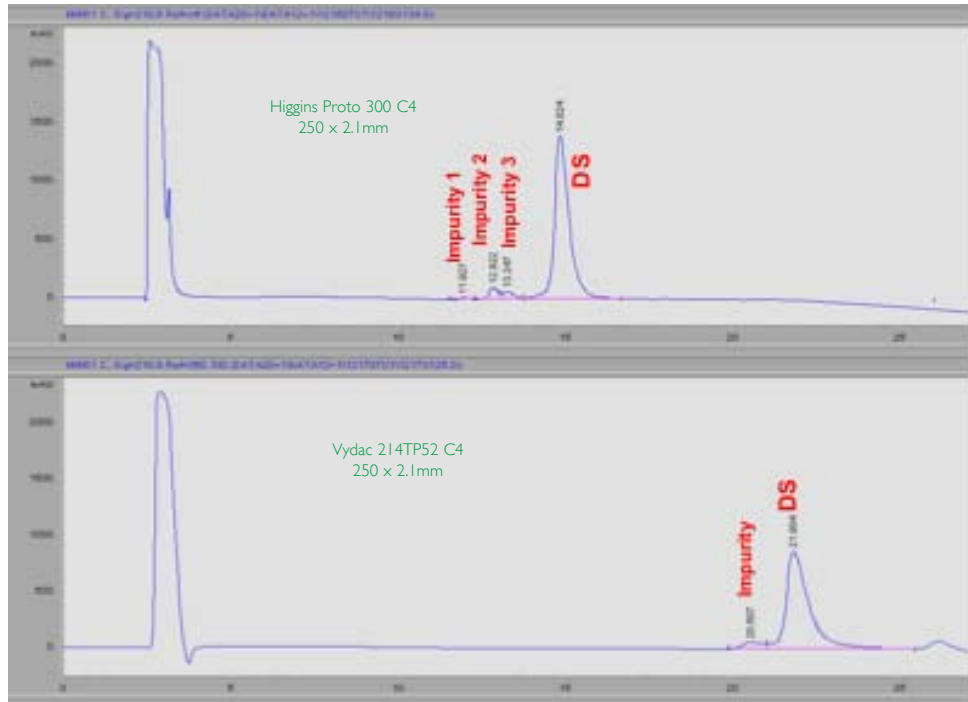
Step Two:
 Each column was flushed with 70% MeCN/0.5N NaOH solution at 1mL/min for one hour (23°C). The columns were then purged and equilibrated under the standard neutral conditions then retested. The center chromatograms are the results of the first test after neutral equilibration

Step Three:
 After the second step, the neutral test conditions were maintained for one hour and a repeat injection was made. The results are illustrated in the chromatograms on the right hand side of the figure.

While Higgins Analytical does not recommend flushing any silica-based HPLC column under such aggressive conditions, these data show the superior alkaline stability of Proto™ 300 C18.

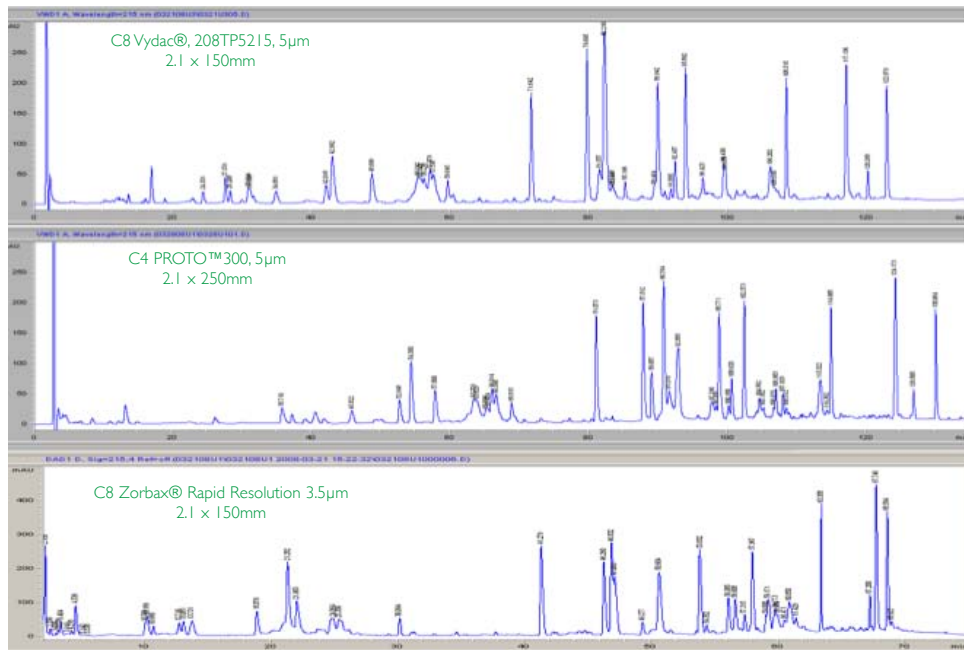


PROTO™ 300 C4 for Protein Analysis



Column: 250 × 2.1mm Higgins P/N RS-2521-W045 (5µm, 300Å)
 Vydac P/N 214TP52 (5µm, 300Å)
 Mobile Phase: (A) 0.1%TFA in water; (B) 0.08%TFA in ACN, Flow Rate: 0.3min
 Start Conditions: 25%B, Linear Gradient Run Time 34min

Superior Resolution with PROTO™300 C4 for Protein Tryptic Digests



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

