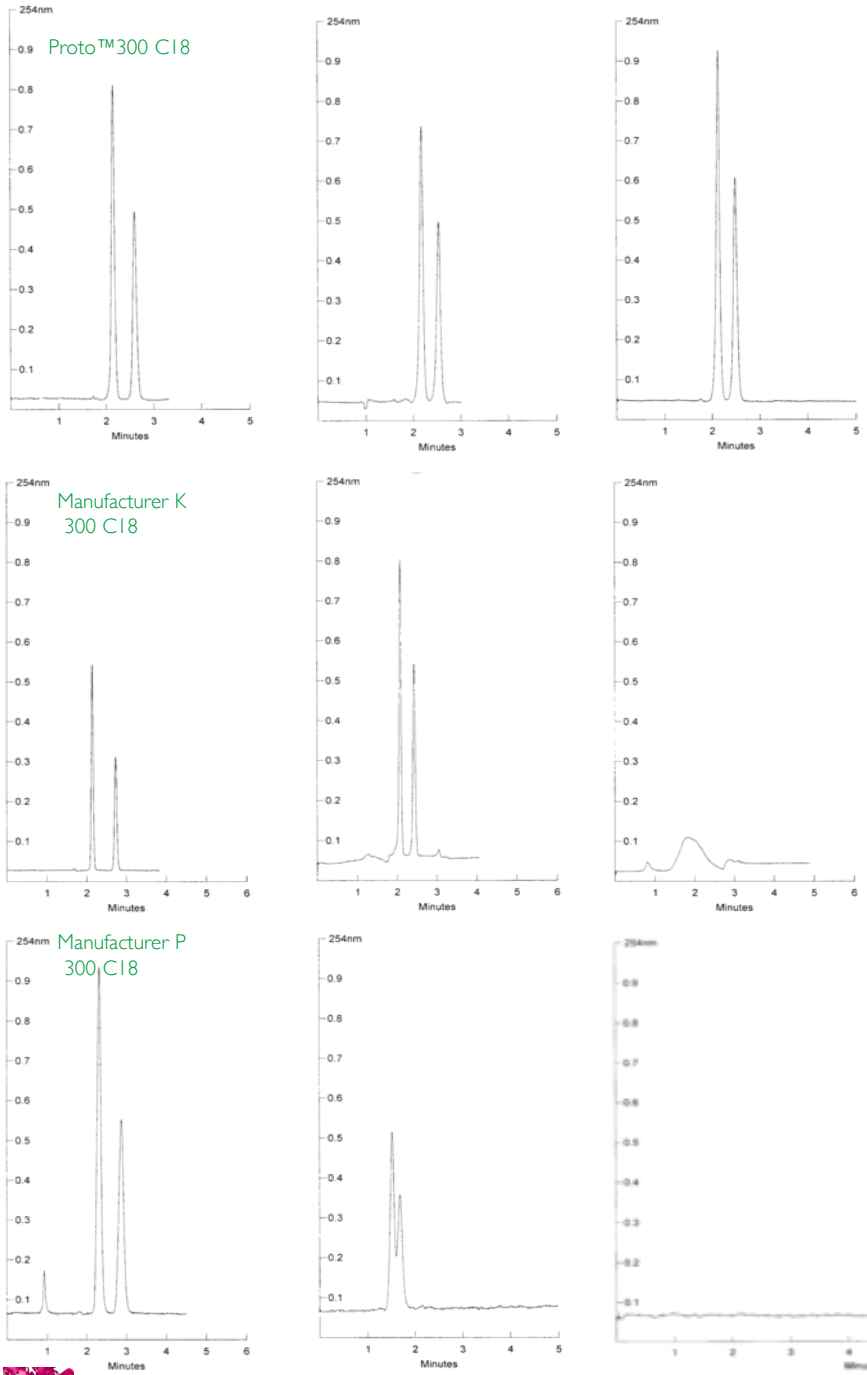
PROTO™300 *Robust Bioseparations*

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
See Page 23 for complete Part Number information

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 akaline stability of Proto™ 300 C18.