

TSKgel® Protein C4-300 Columns

Reversed phase columns for protein separations with high resolution and excellent recovery

TSKgel
PRODUCT OVERVIEW

Introduction:

Reversed phase chromatography is one of the most frequently used chromatographic modes for analytical separations. It is often used for the analysis of small molecular weight compounds, but there are also various standard applications for the separation of biomolecules, such as proteins. Conventional reversed phase HPLC packing materials with 80-140 Å pore sizes are not generally suitable for the analysis of large intact proteins, as the analytes are not able to access the surface area within these pores. Silica-based TSKgel TMS-250, with 250 Å pores, and polymer-based TSKgel Octadecyl-4PW (500 Å) and Phenyl-5PW RP (1000 Å) have been widely applied for the reversed phase separation of intact proteins. However, though the polymer RPC columns are stable at alkaline pH, limitations in pressure, lower mass transfer and swelling make the polymer columns less favorable for RPC protein separation. Now a wide pore 300 Å, silica-based butyl (C4) column, the TSKgel Protein C4-300, is available from Tosoh which is optimized for the separation of large biomolecules such as proteins.

TSKgel Protein C4-300 columns are available in 2.0 and 4.6 mm inner diameter and 5, 10 and 15 cm column length. The optimized pore size of 300 Å is ideal for the separation of proteins, resulting in higher resolution because of high accessibility into the pores. A particle size of 3 µm yields high theoretical plate counts. Optimized ligand density and alkyl length in the stationary phase result in lower adsorption of the protein, providing better peak shape compared to other leading RP-C4 HPLC phases. Latest surface modification techniques and endcapping of residual silanol groups reduce undesirable secondary interactions and peak tailing.

The silica-based, wide pore TSKgel Protein C4-300 HPLC columns are suitable for highly-efficient, reversed phase separations of proteins such as recombinant proteins, antibody fragments or PEGylated proteins.

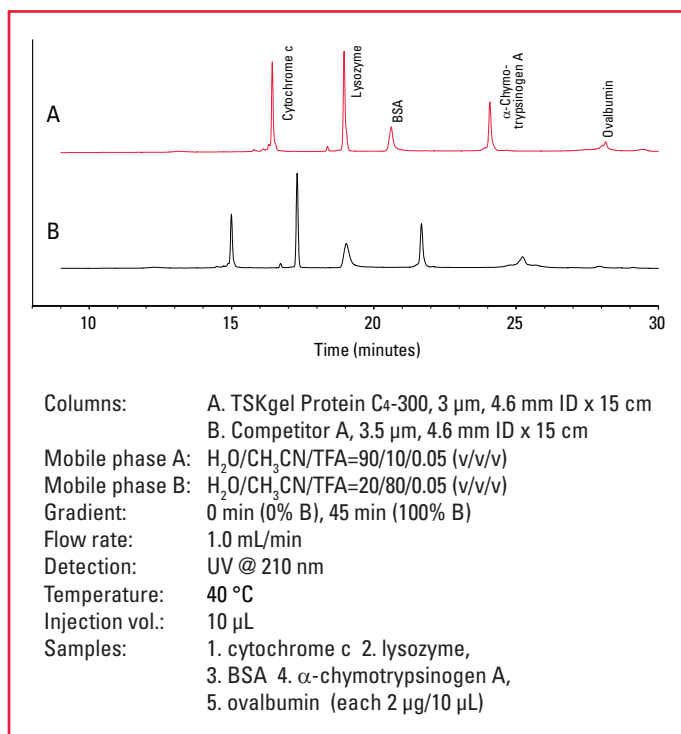
Product Highlights:

- The C4 short alkyl chain ligand and its controlled bonding density provide moderate hydrophobicity to the stationary phase, which results in protein separations with high recovery and less peak tailing.
- The large pore size, allowing macromolecules to enter the interior of the pore, provides higher peak capacities than reversed phase columns with 100 Å pore size.

Applications:

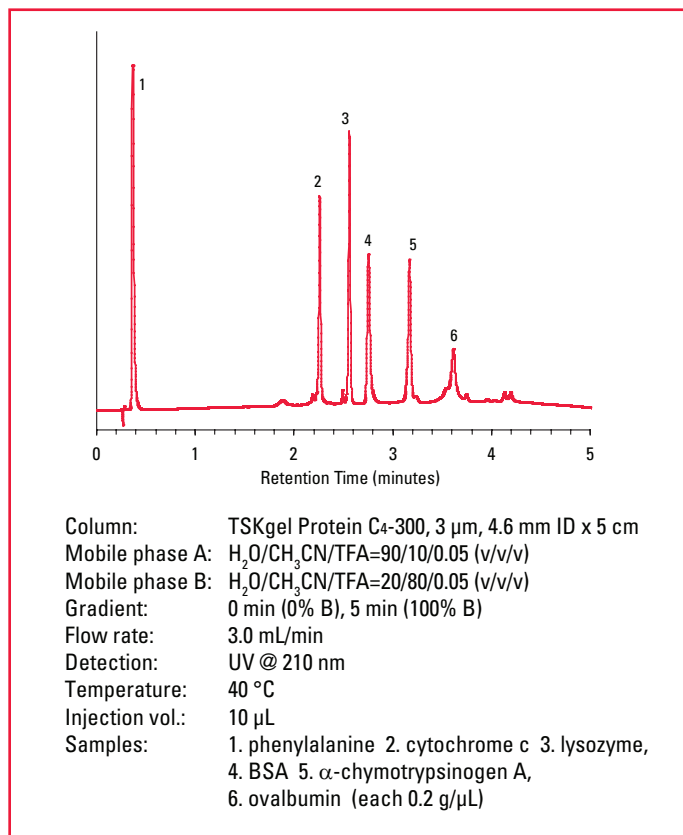
The chromatogram below (Figure 1) shows the separation of a mixture of standard proteins on the new TSKgel Protein C4-300 column compared to a competitor column with 3.5 µm particle size. The resolution between cytochrome c and lysozymes reaches 24.8 on the TSKgel Protein C4-300 column compared to 18.6 on the competitor C4 column. Furthermore, the TSKgel column shows higher theoretical plates and less peak tailing, especially for BSA (Peak 3), and also a better resolution of minor peaks.

Figure 1.



For high speed separations, the analysis time can be reduced by more than eighty percent when using the short 5 cm long column and increasing the flow rate to 3 mL/min (see Figure 2). The backpressure remains below 150 bars, allowing the use of standard HPLC systems. The long term stability of the new C4 phase in acidic solution was tested by flushing the column with 30% acetonitrile, 0.2% TFA (4 times the standard TFA concentration) at 40 °C. There was no change in theoretical plates even after 1,000 hours of run time under this chromatographic condition. Also retention times of standard proteins didn't have significant loss when compared to the initial values.

Figure 2.



Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
22830	TSKgel Protein C4-300, 3 µm	Silica	Stainless Steel	2.0	5
22831		Silica	Stainless Steel	2.0	10
22832		Silica	Stainless Steel	2.0	15
22827		Silica	Stainless Steel	4.6	5
22828		Silica	Stainless Steel	4.6	10
22829		Silica	Stainless Steel	4.6	15
22834	TSKgel Guard Cartridge for 2.0 mm ID columns, 3pk	Silica	Stainless Steel	2.0	1.0
19308	Guard Cartridge Holder for 2.0 mm ID cartridges		Stainless Steel	2.0	1.0
22833	TSKgel Guard Cartridge for 4.6 mm ID columns, 3pk	Silica	Stainless Steel	3.2	1.5
19018	Guard Cartridge Holder for 3.2 mm ID cartridges		Stainless Steel	3.2	1.5



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