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#437

TSKgel® Protein c4-300

INTRODUCTION

Reversed phase (RP) chromatography is one of the most frequently used chromatographic modes for analytical separations. Conventional reversed phase HPLC packing materials with 80-140 Å pore sizes are not generally suitable for 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 (1000 Å) have been widely applied for the reversed phase separation of intact proteins. Now a wide pore silica based butyl/C4 column, the TSKgel Protein C4-300, completes the existing range of TSKgel reversed phase protein columns.

PRODUCT HIGHLIGHTS

- Polymerically bonded butyl phase on wide pore silica
- High resolution, sample recovery and acid stability
- Perfect peak shape
- Excellent batch-to-batch reproducibility

The new TSKgel C4-300 expands the range of reversed phase solutions for protein analysis and address the current needs for increased throughput in bioanalysis. It consists of a polymerically bonded butyl (C4) phase on wide pore ultrapure silica. The pore size of 300 Å is ideally suited for the HPLC and LC/MS analysis of intact proteins such as antibodies, recombinant proteins and PEGylated proteins. Latest surface modification techniques and endcapping of residual silanol groups reduce undesirable secondary interactions and peak tailing. Hence, peak shape, acid stability and sample recoveries are excellent. The use of a 3 micron silica particle combined with optimized ligand density and alkyl chain length results in better protein and peptide resolution compared to other leading C4 phases. TSKgel Protein C4-300 columns are available in 2 and 4.6 mm ID and 5, 10 and 15 cm length.

HIGH RESOLUTION

The small particle size increases separation efficiency and resolution. Figure 1 shows the separation of a mixture of standard proteins on a TSKgel Protein C4-300 column. The resolution between cytochrome c and lysozymes reaches 24.8 on the TSKgel C4 column compared to 18.6 on the competitor C4 column. Further, the TSKgel column shows higher theoretical plates and less peak tailing, especially for BSA (Peak 4), and also a better resolution of minor peaks.

FAST PROTEIN SEPARATION

For high speed separations the analysis time can be reduced by more than eighty percent when using the short 5 cm column and increasing the flow rate to 3 mL/min (Figure 2). The backpressure remains below 150 bars allowing the use of standard HPLC systems.

SEPARATION OF STANDARD PROTEINS

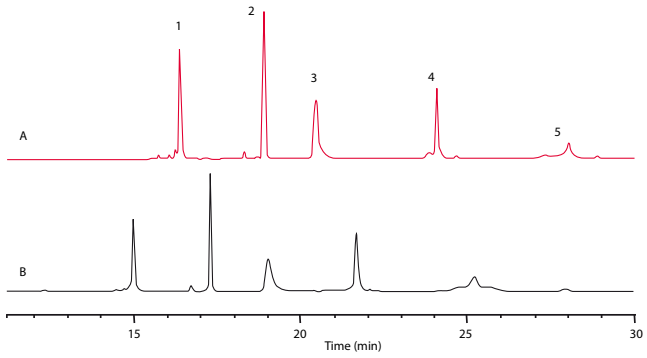

Figure 1

Column A: TSKgel Protein C4-300 3 µm (4.6 mm ID x 15 cm L); B: Competitor C4 3.5 µm (4.6 mm ID x 15 cm L);
Sample: [1] cytochrome c, [2] lysozyme, [3] BSA, [4] α-chymotrypsinogen A, [5] ovalbumin; Mobile phase: A: H_2O/ACN/TFA=90/10/0.05 (v/v/v); B: H_2O/ACN/TFA=20/80/0.05 (v/v/v); Gradient: 0% - 100% B in 45 min; Flow rate: 1 mL/min; Temp.: 40°C; Detection: UV@210nm

FAST SEPARATION OF STANDARD PROTEINS

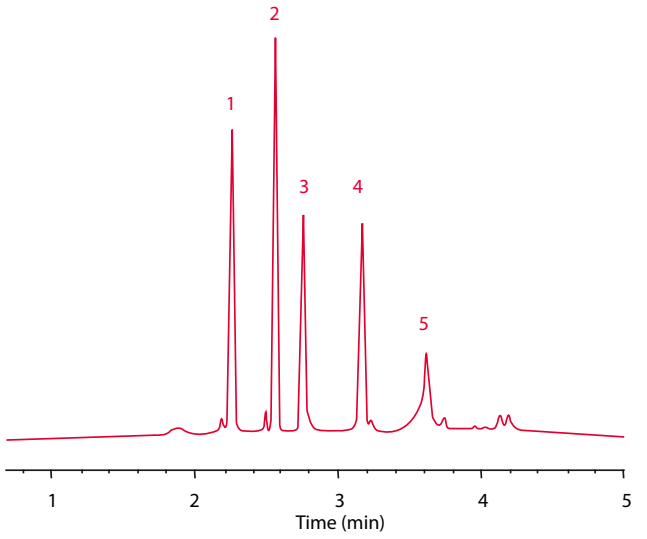

FIGURE 2

Column A: TSKgel Protein C4-300 3 µm (4.6 mm ID x 5 cm L); Sample [1] cytochrome c, [2] lysozyme, [3] BSA, [4] α-chymotrypsinogen A, [5] ovalbumin; Mobile phase: A: H_2O/ACN/TFA=90/10/0.05 (v/v/v); B: H_2O/ACN/TFA=20/80/0.05 (v/v/v); Gradient: 0% - 100% B in 5 min; Flow rate: 3 mL/min; Temp.: 40°C; Detection: UV@210nm

TOSOH

BATCH-TO-BATCH REPRODUCIBILITY

Carefully controlled manufacturing and column packing processes are leading to high batch-to-batch reproducibility. Figure 3 shows a comparison of 5 different batches of TSKgel Protein C_4-300. The thorough endcapping of residual silanol groups improves the acid stability of the silica phase. The long time 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. After 1,000 hours the number of theoretical plates did not change at all and also retention time of standard proteins only slightly decreased when compared to the initial separation.

LOT-TO-LOT REPRODUCIBILITY

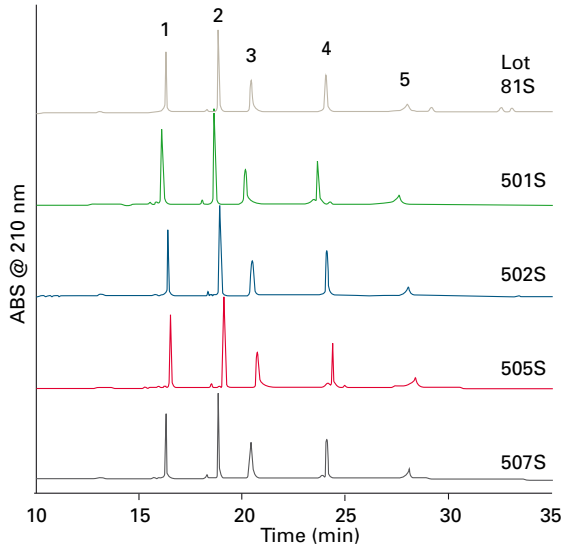

FIGURE 3

Columns: TSKgel Protein C_4-300, 4.6 mm ID x 15 cm L; Eluent A: H_2O/ACN/TFA = 90/10/0.05 (v/v/v); Eluent B: H_2O/ACN/TFA = 20/80/0.05 (v/v/v); Gradient: 0% - 100% B in 45 min; Detect.: UV@210nm; Temp.: 40°C; Injection vol.: 10 µL; Samples: [1] cytochrome c (Equine), [2] lysozyme, [3] BSA, [4] α-chymotrypsinogen A, 5. ovalbumin (2 µg/10 µL each)

APPLICATIONS

The silica based wide pore TSKgel Protein C_4-300 is ideally suited for the analysis of peptides, protein fragments, and intact proteins, such as antibodies, recombinant proteins, or PEGylated proteins. Figure 4 shows the analysis of heavy and light chains of two antibodies, IgG-A and IgG-B. The samples were reduced with dithiothreitol to dissociate into heavy chain and light chain, and then applied to the TSKgel Protein C_4-300 RPC column. The chromatograms show small differences in hydrophobicity between IgG-A and IgG-B. In addition, the chains of IgG-B are eluted as broader peak with shoulder, indicating IgG-B is heterogeneous, and a hydrophilic variant is present.

SEPARATION OF HEAVY AND LIGHT CHAINS OF IgG

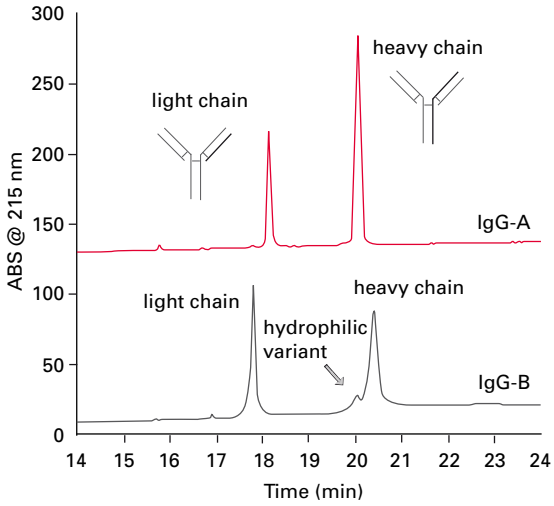

FIGURE 4

Column: TSKgel Protein C_4-300, 4.6 mm ID x 15 cm L; Mobile phase A: H_2O/ACN/TFA = 90/10/0.05 (v/v/v); Mobile phase B: H_2O/ACN/TFA = 20/80/0.05 (v/v/v); Gradient: 0% - 100% B in 45 min; Temperature: 50°C; Detection: UV@215nm; Inj. vol.: 100 µL; Samples: [1] IgG-A (mouse monoclonal), [2] IgG-B (mouse monoclonal), each reduced with dithiothreitol

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Ordering information

Part-No	Description	Matrix	Housing	Dimensions
22827	TSKgel Protein C_4-300	Silica	Stainless steel	4.6 mm ID x 5.0 cm L
22828	TSKgel Protein C_4-300	Silica	Stainless steel	4.6 mm ID x 10.0 cm L
22829	TSKgel Protein C_4-300	Silica	Stainless steel	4.6 mm ID x 15.0 cm L
22830	TSKgel Protein C_4-300	Silica	Stainless steel	2.0 mm ID x 5.0 cm L
22831	TSKgel Protein C_4-300	Silica	Stainless steel	2.0 mm ID x 10.0 cm L
22832	TSKgel Protein C_4-300	Silica	Stainless steel	2.0 mm ID x 15.0 cm L
22833	TSKgel Guard Cartridge for 4.6 mm ID, 3 p	Silica	Stainless steel	3.2 mm ID x 1.5 cm L
22834	TSKgel Guard Cartridge for 2 mm ID, 3 p	Silica	Stainless steel	2.0 mm ID x 1.0 cm L
19018	Cartridge holder for 3.2 mm ID x 1.5 cm L column			
19308	Cartridge holder for 2.0 mm ID x 1.0 cm L column			

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