

Increased Resolution and Reproducibility with TSKgel[®] UP-SW3000 Columns Versus a Competitive SEC Column

TSKgel
APPLICATION NOTE

Introduction

TSKgel UP-SW3000 columns are 2 μm SEC columns designed for the analysis of monoclonal antibodies and other biopharma products and can be used on both HPLC and UHPLC systems. The columns are packed with silica-based beads shielded with a hydrophilic diol-type bonded phase that prevents the silica surface from interacting with protein samples.

TSKgel UP-SW3000 columns are available in 4.6 mm ID with 15 or 30 cm length. Higher resolution can be achieved for the separation of antibody monomers, dimers and higher order aggregates with a TSKgel UP-SW3000 column compared to a competitor UHPLC column. In addition, a TSKgel UP-SW3000 column yielded very low percent relative standard deviation (%RSD) for peak parameters including retention times, peak asymmetry, and efficiency, demonstrating the exceptional reproducibility of this column versus a competitor UHPLC column.

Experimental Conditions

Columns: TSKgel UP-SW3000, 2 μm , 4.6 mm ID \times 15 cm (Figure 1) \times 30 cm (Figure 2)
Competitor column, 1.7 μm , 4.6 mm ID \times 15 cm (Figure 1) \times 30 cm (Figure 2)

Mobile phase: 100 mmol/L sodium phosphate buffer, pH 6.7, + 100mmol/L Na_2SO_4 + 0.05% NaN_3

Gradient: Isocratic

Flow rate: 0.35 mL/min

Detection: UV @ 280 nm

Temperature: 25 $^\circ\text{C}$

Injection vol.: 5 μL

Samples: QC standard protein test mixture:
thyroglobulin, 640 kDa, 0.5 g/L
 γ -globulin, 155 kDa, 1 g/L
ovalbumin, 47 kDa, 1 g/L
ribonuclease A, 13,700 Da, 1.5 g/L
p-aminobenzoic acid, 137 Da, 0.01 g/L

Results and Discussion

The separation of a QC standard protein mixture on a 15 cm TSKgel UP-SW3000 column compared to a competitor UHPLC column of the same length is depicted in Figures 1A and 1B. Clearly the TSKgel UP-SW3000 column yielded better separation of thyroglobulin on the high molecular weight (HMW) side of the monoclonal antibody monomer of 150 kDa, as indicated by the brackets in Figure 1B. In addition, the resolution between thyroglobulin and γ -globulin peaks and the γ -globulin and ovalbumin peaks also have higher resolution in the case of the TSKgel UP-SW3000 column although particle size is slightly larger than in the competitor column.

The number of theoretical plates for the competitor column was 22,618, while 27,082 was obtained for the TSKgel UP-SW3000 column. In the case of γ -globulin (150 kDa), the TSKgel UP-SW3000 column also yielded a larger number of theoretical plates (2,738) compared to 2,675 for the competitor column. The difference in intensity of the proteins comes from two different preparations containing the same amount of pAba.

Figure 1A. Comparison of QC Standard Protein Mixture Using TSKgel UP-SW3000 and Competitor Column

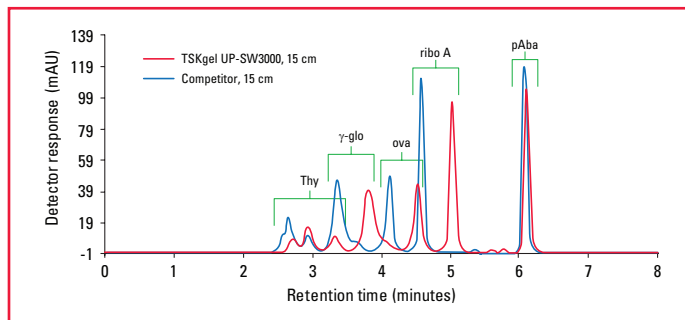
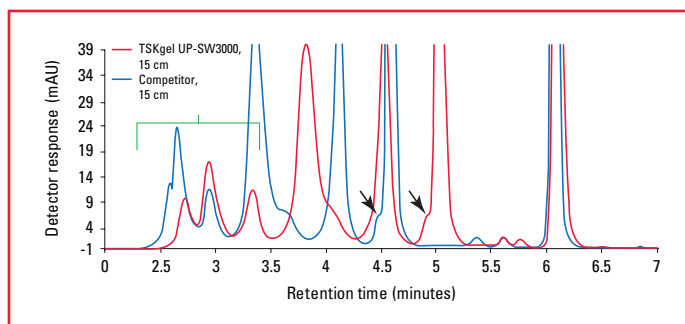


Figure 1B. Comparison of QC Standard Protein Mixture Using TSKgel UP-SW3000 and Competitor Column – Zoomed in View



A monoclonal antibody was analyzed using a TSKgel UP-SW3000, 30 cm column and a 30 cm competitor UHPLC column, as shown in Figures 2A and 2B. The TSKgel UP-SW3000 column provided excellent reproducibility for the peak parameters of retention time, asymmetry, and column efficiency. Injection-to-injection reproducibility was superior to the competitor column as demonstrated by the %RSD values in Table 1.

Figure 2A. mAb Analysis Using TSKgel UP-SW3000 Column

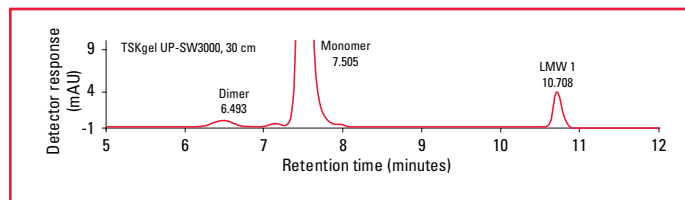
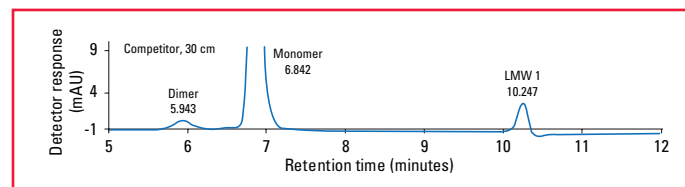


Figure 2B. mAb Analysis Using Competitor UHPLC Column



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Table 1. Comparative Analysis of Peak Parameters of TSKgel UP-SW3000 and Competitor Column

TSKgel UP-SW3000			
	RT (min)	As	N
Run 1	7.505	1.23	12982
Run 2	7.503	1.23	13015
Run 3	7.507	1.22	12992
Avg	7.505	1.227	12996
SD	0.002	0.006	16.921
%RSD	0.027	0.471	0.130

Competitor			
	RT (min)	As	N
Run 1	6.738	1.23	12177
Run 2	6.735	1.22	12300
Run 3	6.725	1.21	12315
Avg	6.732667	1.22	12264
SD	0.006807	0.01	75.71658
%RSD	0.101102	0.819672	0.617389

Conclusion

TSKgel UP-SW3000 columns are 2 μm SEC columns designed for the analysis of monoclonal antibodies and other biopharma products. Higher resolution can be achieved for the separation of antibody monomers, dimers and higher order aggregates with a TSKgel UP-SW3000 column compared to a competitor UHPLC column. The TSKgel UP-SW3000 column provided excellent reproducibility for the peak parameters of retention time, asymmetry, and column efficiency. As demonstrated by the %RSD values, injection-to-injection reproducibility was superior to the competitor column.

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