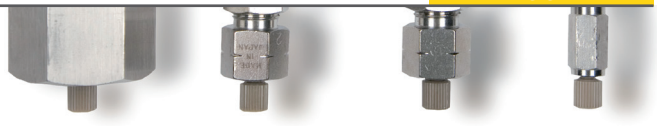

TOSOH

Increased Monoclonal Antibody Resolution with TSKgel® UP-SW3000 Columns

INTRODUCTION

The antibody therapeutics market is enjoying high growth rates, the major areas of therapeutic application being cancer and immune/inflammation-related disorders including arthritis and multiple sclerosis. In 2013, six of the top ten best-selling global drug brands were monoclonal antibodies (mAbs) and more than 400 mAbs were in clinical trials. The characterization of these complex biomolecules is a major challenge in process monitoring and quality control. The main product characteristics to be monitored are aggregate and fragment content, glycosylation pattern and charged isoforms.

The standard method used in biopharmaceutical QC for mAb aggregate and fragment analysis is size exclusion chromatography (SEC). TSKgel G3000SWXL columns have been the industry standard for quality control of mAbs by SEC for decades. With the introduction of TSKgel UP-SW3000, 2 µm silica-based UHPLC/HPLC columns, increased speed and higher resolution can be achieved for the separation of antibody fragments, monomers, and dimers. These columns feature the same pore size (25 nm) as the renowned TSKgel G3000SWXL columns while improving resolution through a smaller particle size.

RESULTS AND DISCUSSION

Figure 1 demonstrates the advantages of the TSKgel UP-SW3000 column for mAb analysis versus the use of a TSKgel G3000SWXL column. The TSKgel UP-SW3000 column offers higher resolution of both the high molecular weight (HMW) species and the Fab/c on the low molecular weight side. In addition, the analysis was completed in half the run time since the TSKgel UP-SW3000 column was used on a UHPLC system.

COMPARISON OF mAb ANALYSIS USING TSKgel G3000SWXL AND UP-SW3000 COLUMNS

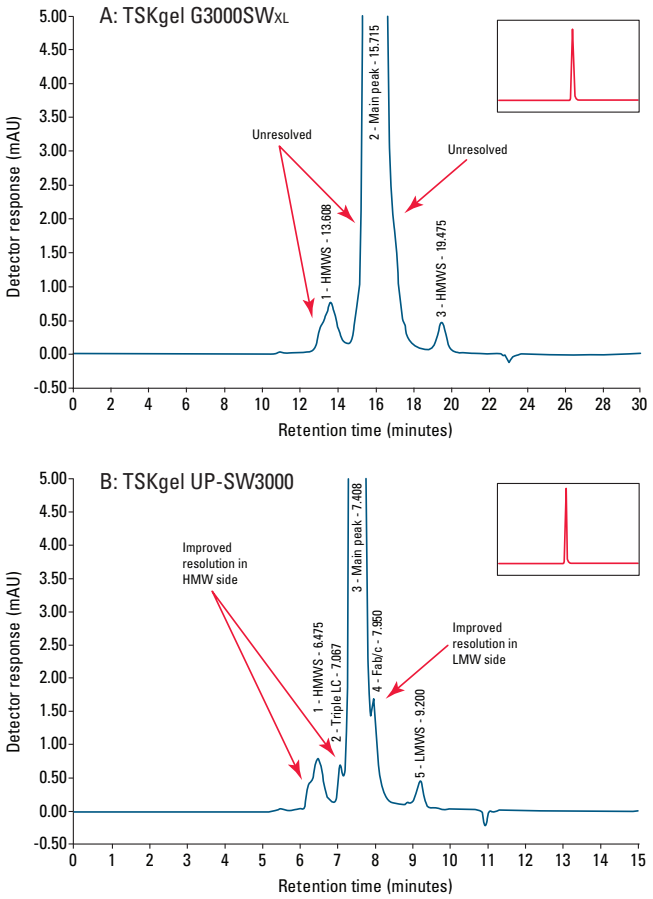

Figure 1

Columns: A. TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm
B. TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm
Instruments: A. Dionex UltiMate® 3000RS UHPLC System
B. Agilent 1260
Mobile phase: 0.2 mol/L potassium phosphate/0.25 mol/L KCl, pH 6.2
Flow rate: A. 0.35 mL/min, B. 0.5 mL/min
Detection: UV @ 280 nm
Temperature: A. 40 °C, B. 25 °C
Injection vol.: A. 10 µL, B. 50 µL

The TSKgel UP-SW3000 column is suited for the separation of antibody dimer, monomer, and fragments in one run with ultra-high resolution, as shown in Figure 2. One TSKgel UP-SW3000 achieves even higher resolution than two TSKgel G3000SWXL columns connected in series.

COMPARISON OF mAb ANALYSIS USING TWO TSKgel G3000SWXL COLUMNS VERSUS ONE UP-SW3000 COLUMN

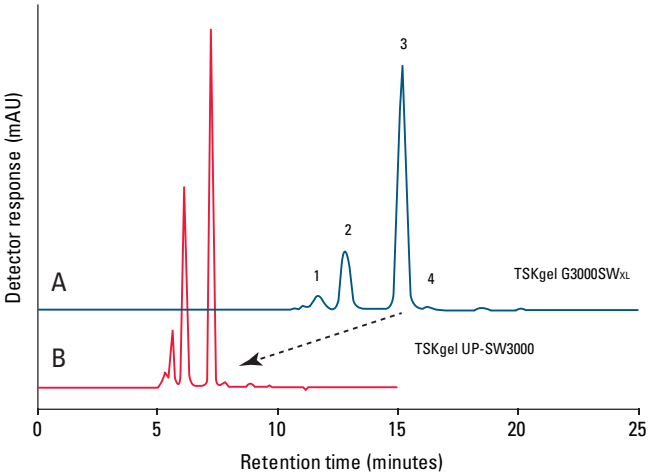

Figure 3

Column	Rs (peak 1/2)	Rs (peak 2/3)	Rs (peak 3/4)
Myoglobin	1.60	3.63	1.77
Ribonuclease	2.16	5.02	2.56

Columns: A. TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm × 2
B. TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm
Mobile phase: 100 mmol/L phosphate buffer + 100 mmol/L sodium sulfate + 0.05% sodium azide, pH 6.7
Flow rate: A. 1.0 mL/min, B. 0.35 mL/min
Detection: UV @ 280 nm
Temperature: 25 °C
Injection vol.: 10 µL
Samples: mouse-human chimeric IgG, monoclonal
1. trimer, 2. dimer, 3. monomer, 4. fragment

CONCLUSION

The TSKgel UP-SW3000 column is ideally suited for the analysis of aggregate and fragment contents of antibody preparations. It features the same pore size as the renowned TSKgel G3000SWXL column while improving resolution through a smaller particle size.