TOSOH

TSKgel® UP-SW3000 UHPLC COLUMNS

TSKgel UP-SW3000 columns packed with 2 µm silica based particles are the latest addition to the popular TSKgel SW series, the gold standard for QC analysis of antibody therapeutics. The new silica-based UHPLC columns are based on the proven proprietary surface technology of the renowned TSKgel SW series and facilitate the transfer of existing HPLC methods to UHPLC systems.

Aqueous size exclusion chromatography (SEC) is the method of choice for the analysis of proteins fragments, monomers, and aggregates under non-denaturing conditions. Based on the flow of the sample through a porous stationary phase SEC separates molecules according to their size, or more precisely, their hydrodynamic volume. In aqueous elution systems SEC is also referred to as gel filtration chromatography (GFC). TSKgel G3000SWXL columns have been the industry's standard for quality control of monoclonals by SEC for decades.

HIGHLIGHTS

- Proven TSKgel SW SEC quality
- Virtual absence of nonspecific interaction
- Easy transfer of existing HPLC methods
- Optimized for mAb quality control

The new columns can be used with modern HPLC and UHPLC systems and are available with 15 or 30 cm length. The short one enables short analysis times; the long one provides higher resolution for mAb analysis. The lifetime of the columns can be improved when using the corresponding guard columns. A "direct connect" (DC) guard column allows minimizing extra column dead volume.

COMPARISON OF CALIBRATION CURVES

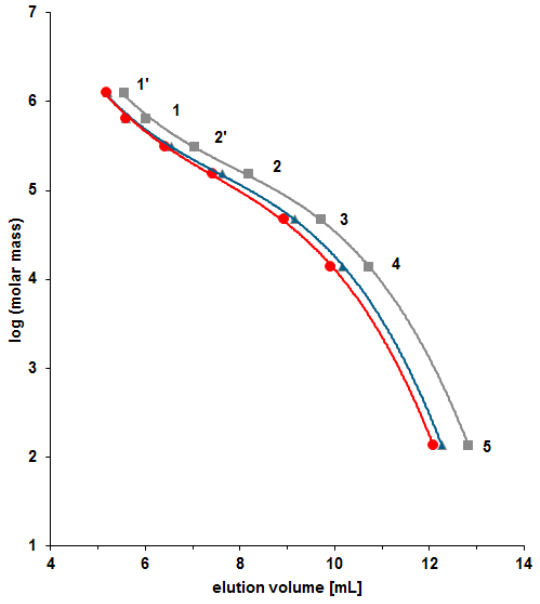

Figure 1

Columns: A: TSKgel UP-SW3000 (4.6 mm ID × 30 cm, red)
B: TSKgel SuperSW3000 (4.6 mm ID × 30 cm, blue)
C: TSKgel G3000SWXL (7.8 mm ID × 30 cm, grey)
Mobile phase: 100 mmol/L phosphate buffer (pH 6.7) + 100 mmol/L sodium sulfate + 0.05% NaN_3
Flow rate: A & B: 0.35 mL/min; C: 1.0 mL/min
Temperature: 25°C; Detection: UV @ 280 nm
Injection vol.: 10 µL
Samples: 1. thyroglobulin (640,000 Da); (1' thyroglobulin aggregate);
2. γ-globulin (155,000 Da); (2' γ-globulin dimer);
3. ovalbumin (47,000 Da);
4. ribonuclease A (13,700 Da);
5. p-aminobenzoic acid (137 Da)

COMPARISON OF TSKgel SW COLUMN SERIES

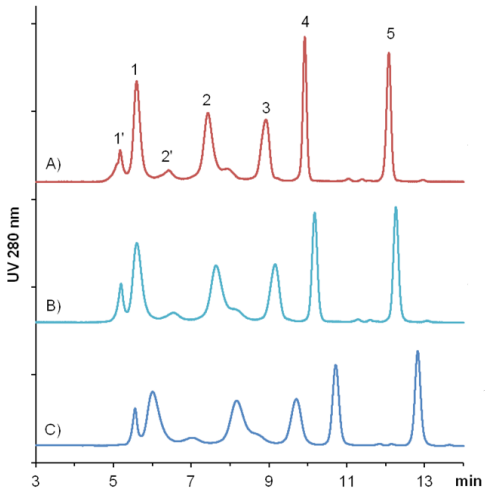

Figure 2

Column	Particle size	N (peak 4)	AS (peak 4)
A: TSKgel UP-SW3000	2 µm	45,625	0.95
B: TSKgel SuperSW3000	4 µm	24,419	1.02
C: TSKgel G3000SWXL	5 µm	18,325	1.05

Columns: A: TSKgel UP-SW3000, 2 µm, 4.6 mm ID x 30 cm
B: TSKgel SuperSW3000, 4 µm, 4.6 mm ID x 30 cm
C: TSKgel G3000SWXL, 5 µm, 7.8 mm ID x 30 cm
Mobile phase: 100 mmol/L phosphate buffer (pH 6.7) + 100 mmol/L sodium sulfate + 0.05 % NaN3
Flow rate: A, B: 0.35 mL/min, C: 1.0 mL/min
Temperature: 25 °C
Detect.: UV @ 280 nm (A,B: micro flow cell, C: standard flow cell)
Injection vol.: 10 µL
Sample: 1. thyroglobulin, 640,000 Da (1' thyroglobulin dimer)
2. γ-globulin, 155,000 Da (2' γ-globulin dimer)
3. ovalbumin, 47,000 Da; 4. ribonuclease A, 13,700 Da
5. p-aminobenzoic acid, 137 Da

MASS RANGE

Figure 1 shows the calibration curve and the molecular weight range of the new 2 µm TSKgel UP-SW3000 compared to those of 5 micron TSKgel G3000SWXL and 4 micron TSKgel SuperSW3000. Calibration curves and mass ranges are almost identical which facilitates transfer of existing methods.

TSKgel UP-SW3000 has the same molecular mass separation range as the equivalent grades of conventional TSKgel SW-type columns but much higher column efficiency: Figure 2 shows the increase in resolution achieved by reducing the particle size from 5 (respectivly 4) micron to 2 micron.

REPRODUCIBLE PERFORMANCE

TSKgel SW SEC columns are known for their outstanding quality and reproducibility. 40 years of expertise in development and production of gel filtration columns have paved the road to UHP-SEC. The good lot-to-lot reproducibility of TSKgel UP-SW3000 is proved in Figure 3.

APPLICATION

TSKgel UP-SW3000 is suited for the separation of antibody dimer, monomer, and fragments in one run with ultra-high resolution (Figure 4). One TSKgel UP-SW3000 achieves even higher resolution than two TSKgel G3000SWXL columns connected in series.

BATCH-TO-BATCH REPRODUCIBILITY

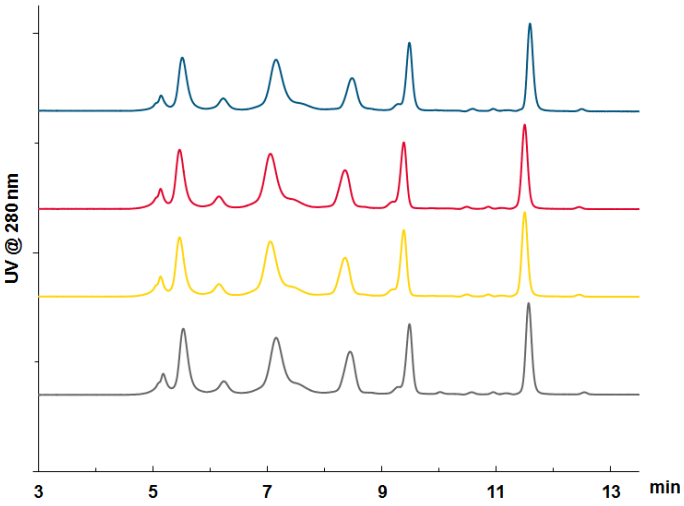

Figure 3

Column: TSKgel UP-SW3000 4.6 mm ID x 30 cm from 4 different batches
Condition and samples: see Figure 1 A

ANALYSIS OF MONOCLONAL ANTIBODIES

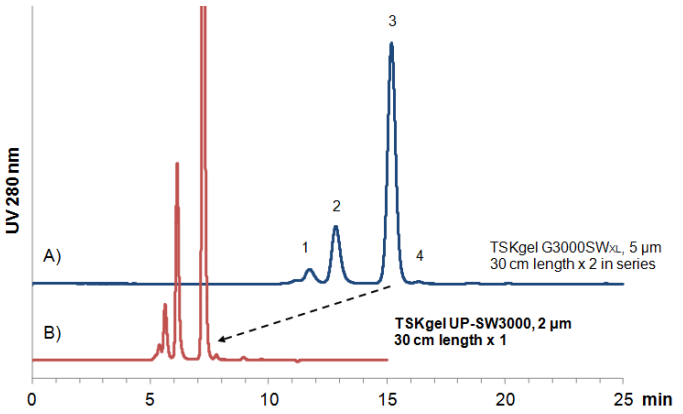

Figure 4

Column	Rs (peak 1/2)	Rs (peak 2/3)	Rs (peak 3/4)
A: TSKgel G3000SWXL x2	1.60	3.63	1.77
B: TSKgel UP-SW3000	2.16	5.02	2.56

Columns: A) TSKgel G3000SWXL, 5 µm, 7.8 mm ID x 30 cm x 2;
B) TSKgel UP-SW3000, 2 µm, 4.6 mm ID x 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; Temperature: 25°C
Detection: UV @ 280 nm; Injection vol.: 10 µL
Sample: mouse-human chimeric IgG, monoclonal
1 trimer, 2 dimer, 3 monomer, 4 fragment

Ordering information

Part-No	Description	Matrix	Housing	Dimensions
0023449	TSKgel UP-SW3000, 2 µm	Silica	Stainless steel	4.6 mm ID x 15.0 cm L
0023448	TSKgel UP-SW3000, 2 µm	Silica	Stainless steel	4.6 mm ID x 30.0 cm L
0023450	TSKgel Guardcolumn UP-SW	Silica	Stainless steel	4.6 mm ID x 2.0 cm L
0023451	TSKgel Guardcolumn UP-SW DC	Silica	Stainless steel	4.6 mm ID x 2.0 cm L