

TSKgel® UP-SW3000, 2 µm UHPLC/HPLC SEC Columns

Uniting Technologies for Method Continuity in Quality and Expertise with TSKgel Columns

Introduction

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. These new silica-based UHPLC/HPLC columns are based on the same proven proprietary surface technology of the renowned TSKgel SW series. The surface of the particles has been shielded from interacting with proteins by derivatization with ligands containing diol functional groups.

TSKgel UP-SW3000 columns feature the same pore size as the well-established TSKgel G3000SW_{XL} columns. Hence methods developed using TSKgel G3000SW_{XL} columns can easily be transferred to TSKgel UP-SW3000 columns on conventional HPLC systems as well as on UHPLC systems. TSKgel UP-SW3000 columns are available in 4.6 mm ID with 15 or 30 cm length. The 15 cm column offers a shortened analysis time with improved efficiency versus the TSKgel G3000SW_{XL} column. The 30 cm column delivers dramatically increased peak parameters such as efficiency, asymmetry, and resolution between fragments, monomers, and aggregates compared to the TSKgel G3000SW_{XL} column.

The lifetime of the TSKgel UP-SW3000 columns are superior and can be maintained and further improved when using the corresponding guard columns. A “direct connect” (DC) guard column allows minimizing extra column dead volume.

Highlights

- Proven TSKgel SW SEC quality
- Easy method transfer from HPLC to HPLC and UHPLC systems
- Increased throughput, improved efficiency with short columns
- Superior resolution for aggregate analysis
- Ideal MW range for mAb quality control and analysis
- High precision reproducibility from injection-to injection and across lot-to-lot, column-to-column

Easy Method Transfer of TSKgel G3000SW_{XL} to UP-SW3000 on HPLC Systems

A 2 µm TSKgel UP-SW3000 column easily replaces an existing method on a conventional HPLC system using a 5 µm TSKgel G3000SW_{XL} column. The TSKgel UP-SW3000 column offers several advantages versus the TSKgel G3000SW_{XL} column, as shown in *Figure 1* comparing the analysis of QC protein standards at the same concentrations. The TSKgel UP-SW3000 column offers higher sensitivity, with better peak shape, higher resolution and slightly shorter retention time. No change in the mobile phase composition is required; only an adjustment to a lower flow rate is necessary.

Table 1 lists the peak resolutions of the TSKgel UP-SW3000 and TSKgel G3000SW_{XL} columns for the analysis of QC protein standards using a conventional HPLC system. This data demonstrates the improved resolution gained with the effortless transfer of a method developed using a TSKgel G3000SW_{XL} column to a TSKgel UP-SW3000 column.

Figure 1. Analysis of QC Protein Standards using TSKgel G3000SW_{XL} and UP-SW3000 Columns on a Conventional HPLC System

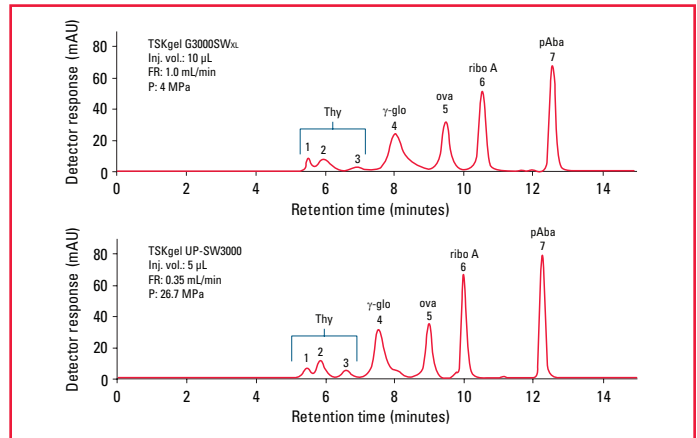


Table 1. Peak Resolution Comparison using TSKgel G3000SW_{XL} and UP-SW3000 Columns

Peak resolutions	Rs 1-2	Rs 2-3	Rs 3-4	Rs 4-5	Rs 5-6	Rs 6-7
TSKgel UP-SW3000	0.89	1.68	1.94	3.32	3.35	8.75
TSKgel G3000SW _{XL}	0.98	1.46	1.56	2.52	2.71	6.41

Easy Method Transfer of UP-SW3000 from HPLC to UHPLC Systems

A method developed on a conventional HPLC system using a TSKgel UP-SW3000, 2 µm column is smoothly transferrable to a UHPLC system. Two separation profiles of a QC protein standard mixture using a TSKgel UP-SW3000 column from a HPLC and a UHPLC system are overlaid and shown in *Figure 2* below.

The analysis was carried out under identical chromatographic conditions at a flow rate of 0.35 mL/min. The two sets of three consecutive runs were carried out with the following criteria: two different instruments (Agilent 1100 HPLC and Dionex UltiMate® 3000RS UHPLC), on two different days, using two columns from two different lots, using two different batches of QC buffer, and two different preparations of the QC protein standard mixture.

The experimental plan provided all the different variables for testing reproducibility. The two overlaid chromatograms clearly show that the method is robust, reproducible and transferrable. As seen in *Table 2*, upon transferring the method from HPLC to UHPLC, the resolution between the peaks increased over the entire range of the separation.

The TSKgel UP-SW3000 column yielded similar back pressure of 26 MPa in the Agilent 1100 HPLC compared to a back pressure of 24 MPa in the Dionex UltiMate 3000RS UHPLC system. Considering the maximum pressure limit of 40 MPa of the Agilent 1100 system and a maximum pressure of 34.2 MPa of the TSKgel UP-SW3000 column, there is still room for further reducing the run time by increasing the flow rate.

Figure 2. Analysis of QC Protein Standards using a TSKgel UP-SW3000 Column on a HPLC and UHPLC System

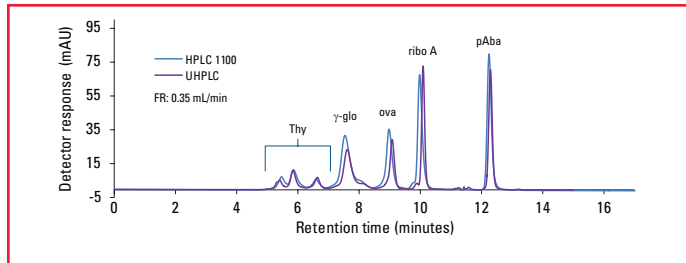


Table 2. Peak Resolution Comparison using a TSKgel UP-SW3000 Column on a HPLC and UHPLC System

	Rs (Thy-γ-Glo)	Rs (γ-Glo-Ova)	Rs (Ribo A-pAba)
Agilent 1100 HPLC	3.52	3.22	10.23
Dionex UltiMate 3000RS UHPLC	4.46	3.85	11.61

Increase in Resolution with Use of the TSKgel UP-SW3000 Column

TSKgel UP-SW3000 columns have the same molecular mass separation range as the TSKgel G3000SW_{XL} and SuperSW3000 columns while demonstrating much higher column efficiency. *Figure 3* shows the increase in resolution achieved by the reduction in particle size from the 5 μm, 7.8 mm ID × 30 cm TSKgel G3000SW_{XL} and 4 μm, 4.6 mm ID × 30 cm TSKgel SuperSW3000 columns to the 2 μm, 4.6 mm ID × 30 cm TSKgel UP-SW3000 column.

Table 3 lists the peak resolutions and asymmetry of peak 4 of the TSKgel UP-SW3000, G3000SW_{XL} and SuperSW3000 columns for the analysis of QC protein standards. This data demonstrates the improved resolution gained with the use of a TSKgel UP-SW3000 column.

Ordering Information

Part#	Description	Matrix	Housing	ID (mm)	Length (cm)
23449	TSKgel UP-SW3000, 2 μm	Silica	Stainless Steel	4.6	15
23448	TSKgel UP-SW3000, 2 μm	Silica	Stainless Steel	4.6	30
23450	TSKgel guard column UP-SW3000	Silica	Stainless Steel	4.6	2
23451	TSKgel guard column UP-SW3000 DC*	Silica	Stainless Steel	4.6	2

*The guard column can be directly connected to the analytical column without tubing between the two columns. A male-type outlet endfitting on the guard column enables the direct connection to the screw-type endfitting of the analytical column.

Figure 3. TSKgel SW Column Comparisons

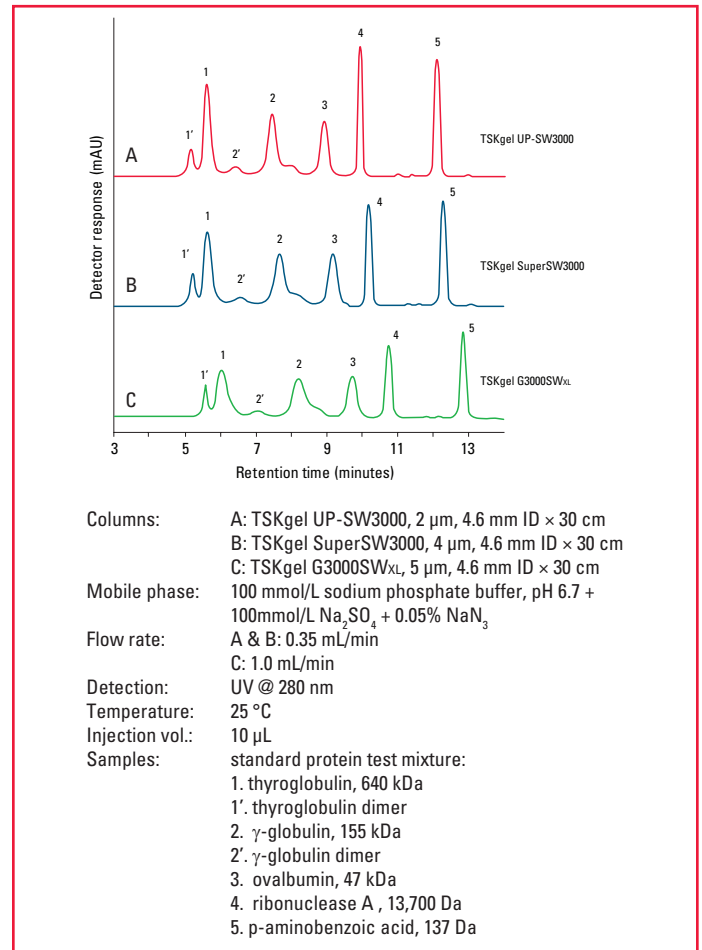


Table 3. TSKgel SW Column Peak Resolution and Asymmetry Comparison

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 G3000SW _{XL}	5 μm	18,325	1.05

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