TSKgel® UP-SW2000 UHPLC COLUMNS

TSKgel UP-SW2000 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 proteins. The new TSKgel UP-SW2000 UHPLC columns with 12.5 nm pore size expand the existing UP-SW series with a smaller pore size version. They are based on the proven proprietary surface technology of the renowned TSKgel SW series and facilitate the transfer of existing HPLC methods from TSKgel G2000SW/SWXL to UHPLC systems.

Aqueous size exclusion chromatography (SEC) is the method of choice for the analysis of peptides and proteins 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 SWXL columns have been the industry's standard for quality control of protein biotherapeutics by SEC for decades.

HIGHLIGHTS

- Proven TSKgel SW SEC quality
- Virtual absence of nonspecific interaction
- Easy transfer of existing HPLC methods
- Optimized for peptides, small proteins, ænd oligonucleotides

TSKgel UP-SW series columns can be used with modern HPLC and UHPLC systems and are available with 15 or 30 cm length. The short version enables short analysis times; the long version provides higher resolution and maximum peak capacity. 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 12.5 nm TSKgel SW COLUMNS

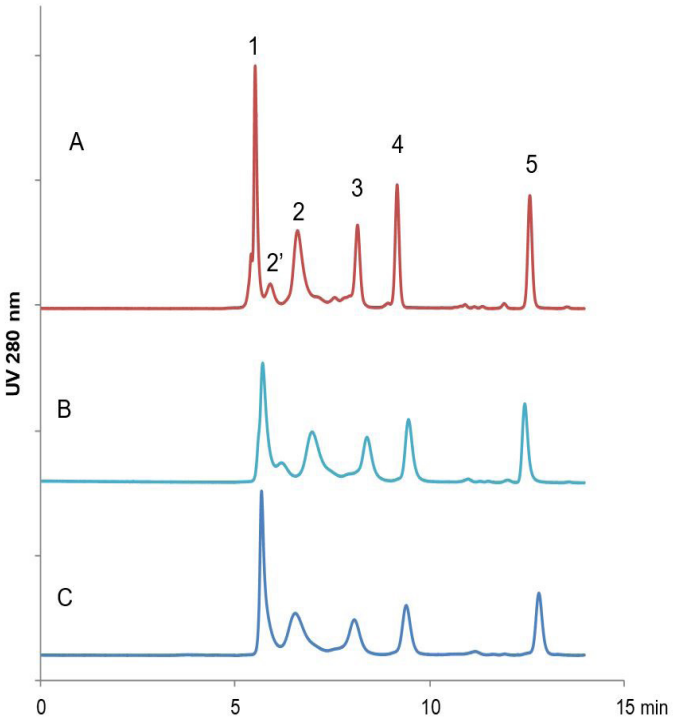

Figure 1

Columns: A) TSKgel UP-SW2000 , 2 µm, 4.6 mm ID x 30 cm L
B) TSKgel SuperSW2000, 4 µm, 4.6 mm ID x 30 cm L
C) TSKgel G2000SWXL, 5 µm, 7.8 mm ID x 30 cm L
Mobile phase: 100 mmol/L phosphate buffer (pH 6.7)
+ 100 mmol/L sodium sulfate + 0.05 % sodium azide
Flow rate: A), B) 0.35 mL/min, C) 1.0 mL/min
Temperature: 25 °C
Detection: UV @ 280 nm
Injection vol.: 10 µL
Sample: 1. thyroglobulin (640,000 Da);
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

The molecular weight range (1-150 kDa) of the 2 µm TSKgel UP-SW2000 is identical to those of 5 micron TSKgel G2000SWXL and 4 micron TSKgel SuperSW2000, which facilitates transfer of existing methods. While providing the same molecular mass separation range the new column has much higher column efficiency: Figure 1 shows the similarity of the separation range between the 12.5 nm pore size TSKgel SW column portfolio and the increase in resolution achieved by reducing the particle size from 5 (respectively 4) micron to 2 micron.

APPLICATIONS

The separation range of TSKgel UP-SW2000 is ideally suited to analyze peptides and small molecular weight proteins. Figure 2 shows the analysis of recombinant human insulin with a molecular weight of 5800 Da using the new two mikron column in comparison with the analysis using a conventional five mikron TSKgel G2000SWXL column. Mobile phase conditions were chosen according to the Ph. Eur. monograph 838.

A conventional HPLC system which has been optimized with regard to extra column dead volume was used for both analyzes. There is no need to use a high pressure UHPLC system for TSKgel UP-SW2000, although for any SEC analysis UHPLC systems typically provide ideal technical conditions with regard to system volume, injector and detector technology. Besides a shorter run time the analysis using TSKgel UP-SW2000 delivers sharper peaks.

The increasing importance of oligonucleotides used for therapeutic purposes rises the demand for suitable size exclusion columns. Figure 3 shows the analysis of a mixture of N and N+1 synthetic oligonucleotides (19 mer and 20 mer) using two TSKgel UP-SW2000 column connected in line. This method achieved a very good separation of the two oligonucleotides.

ANALYSIS OF RECOMBINANT HUMAN INSULIN

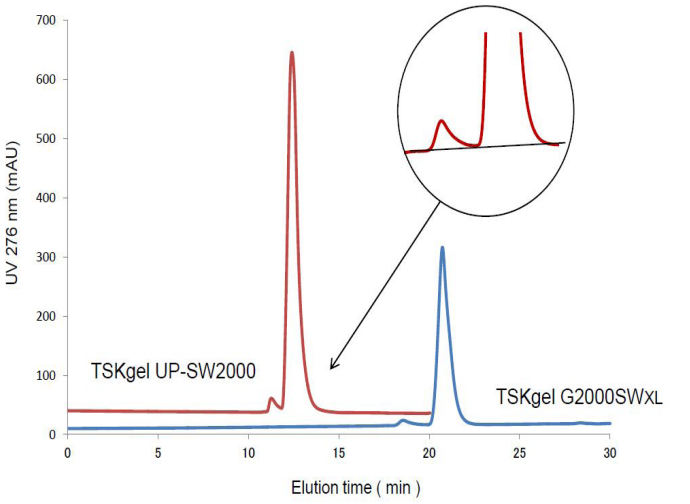

Figure 2

Columns: TSKgel UP-SW2000, 2 µm, 4.6 mm ID x 30 cm L
TSKgel G2000SWXL, 5 µm, 7.8 mm ID x 30 cm L
Mobile phase: 0.1 % L-arginine/acetonitrile/acetic acid =65/20/15
Flow rate: 0.2 mL/min (TSKgel UP-SW2000)
0.5 mL/min (TSKgel G2000SWXL)
Detection: UV@276 nm
Sample: rec. human insulin

ANALYSIS OF OLIGONUCLEOTIDES

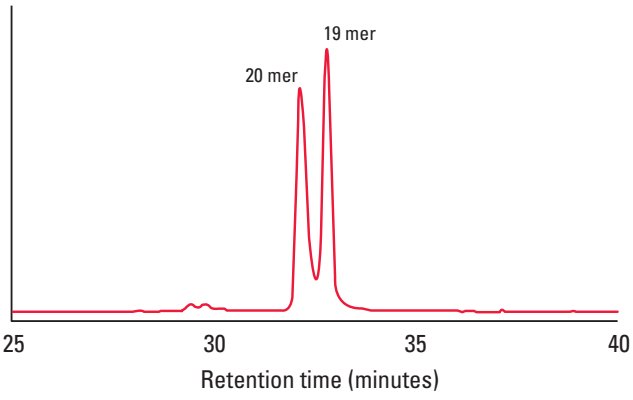

Figure 3

Column: TSKgel UP-SW2000, 2 µm, 4.6 mm ID × 30 cm L × 2
Mobile phase: 0.05% NaN3 and 0.3 mol/L NaCl in 0.05 mol/L phosphate, pH 6.7
Flow rate: 0.2 mL/min
Detection: UV @ 260 nm
Temperature: 25 °C
Sample: 20 mer: 5′-GAATTCATCGGTTCAGAGAC-3′
19 mer: 5′-AATTCATCGGTTCAGAGAC-3′

Ordering information

Part-No	Description	Matrix	Housing	Dimensions
0023515	TSKgel UP-SW2000, 2 µm	Silica	Stainless steel	4.6 mm ID x 15.0 cm L
0023514	TSKgel UP-SW2000, 2 µm	Silica	Stainless steel	4.6 mm ID x 30.0 cm L
0023516	TSKgel Guardcolumn UP-SW2000	Silica	Stainless steel	4.6 mm ID x 2.0 cm L
0023517	TSKgel Guardcolumn UP-SW2000 DC	Silica	Stainless steel	4.6 mm ID x 2.0 cm L

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OPERATING CONDITIONS and SPECIFICATIONS

TSKgel ® UP-SW2000 Products

Part Numbers:	0023515	4.6 mm ID x 15.0 cm L	**TSKgel** UP-SW2000	**2.0 µm**
	0023514	4.6 mm ID x 30.0 cm L	**TSKgel** UP-SW2000	**2.0 µm**
Guardcolumn:	0023517	4.6 mm ID x 2.0 cm L	For **TSKgel** Guard Column DC*	**2.0 µm**
	0023516	4.6 mm ID x 2.0 cm L	For **TSKgel** Guard Column	**2.0 µm**

Both guard columns can be connected to either analytical column

*The DC 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.

This sheet contains the recommended operating conditions and the specifications for **TSKgel** UP-SW2000 columns and guard columns. Installation instructions and column care information are described in a separate Instruction Manual.

A. OPERATING CONDITIONS

1.	Shipping Solvent:		0.05% NaN_3 and 0.1 mol/L Na_2SO_4 in 0.1 mol/L phosphate buffer, pH 6.7
2.	Standard Flow Rate:	0.10 - 0.35	mL/min
3.	Max. Flow Rate:	0.50	mL/min 15 cm Length
		0.35	mL/min 30 cm Length
4.	Max. Pressure:	25	MPa 15 cm Length
		34	MPa 30 cm Length
5.	Temperature:	10 – 30 °C	Reduce flow rate when operating below 10 °C
6.	pH Range:	2.5 – 7.5	
7.	Organic Conc.:	0 - 100%	for aqueous soluble organic solvents. Make gradual solvent changes using a shallow gradient at low flow rate.
8.	Cleaning Solvents:		1. To remove basic substances (Ionic adsorption): a. Increase the salt concentration of the mobile phase to an appropriate ionic strength (normally around 0.5 mol/L) and pass this through the column to clean. b. Clean the column by passing through an acidic aqueous solution (phosphate buffer solution pH 2.5). 2. To remove adsorbed hydrophobic substances (Hydrophobic adsorption): Add an aqueous organic solvent (around 10 to 20%) such as methanol or acetonitrile, etc., to the mobile phase, and pass this through the column to clean (exercise caution regarding buffer solution and salt precipitation). 3. Using an eluent containing added urea or surfactant (To remove poorly soluble proteins such as membrane proteins, etc.): Use 6 to 8 mol/L urea or 0.2 to 0.3% neutral surfactant (such as Triton, Tween, Brij, etc.) in the mobile phase, and pass this through the column to clean (residual urea and surfactant can remain in the column). **Note:** Use the solvent replacement flow rate (<0,17mL/min) during cleaning and when replacing with the shipping solvent. Clean the columns with 5 to 10 column volume of cleaning solvents
9.	Storage:		1. Procedure: a. Replace the column contents with the shipping solvent, disconnect the column from the instrument, seal both ends with the end plugs, and store. b. After disconnecting the column from the instrument, wash the instrument tubing with distilled water or ion exchange water. **Note:** Use the solvent replacement flow rate (<0,17mL/min) during cleaning and when replacing with the shipping solvent. 2. Storage temperature: 15 to 30°C

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and e-mail, techsupport.tbg@tosoh.com

DS 1244 / April 19 / VN

10. Column Protection:

The use of guard columns is recommended to prolong the life of the analytical column. Guard column life depends greatly on various factors, including sample properties, sample loading, solvents, etc. As a general rule, guard columns should be replaced when there is an increase in pressure to some extent, when the peaks become excessively wide or when the peaks show splitting.

B. SPECIFICATIONS

The performance of **TSKgel UP-SW2000**columns is tested under the conditions described in the Data Sheet. All columns have passed the following quality control specifications

Number of Theoretical Plates (N):	≥	25,000	4.6 mm ID x 15.0 cm L
	≥	45,000	4.6 mm ID x 30.0 cm L
Asymmetry Factor (AF):		0.90 – 1.40	4.6 mm ID x 15.0 cm L
		0.90 – 1.40	4.6 mm ID x 30.0 cm L