



Characterization of New 2 μ m Particle Size, 25 nm Pore Size Analytical Size Exclusion Chromatography Column With Larger Exclusion Limit

***Useful For The Separation Of Biomolecules
Using UHPLC And HPLC***

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Introduction

Here we introduce TSKgel® UP-SW3000 columns which were developed and optimized as HPLC-UHPLC SEC columns for biomolecules.

Advantages of these columns include:

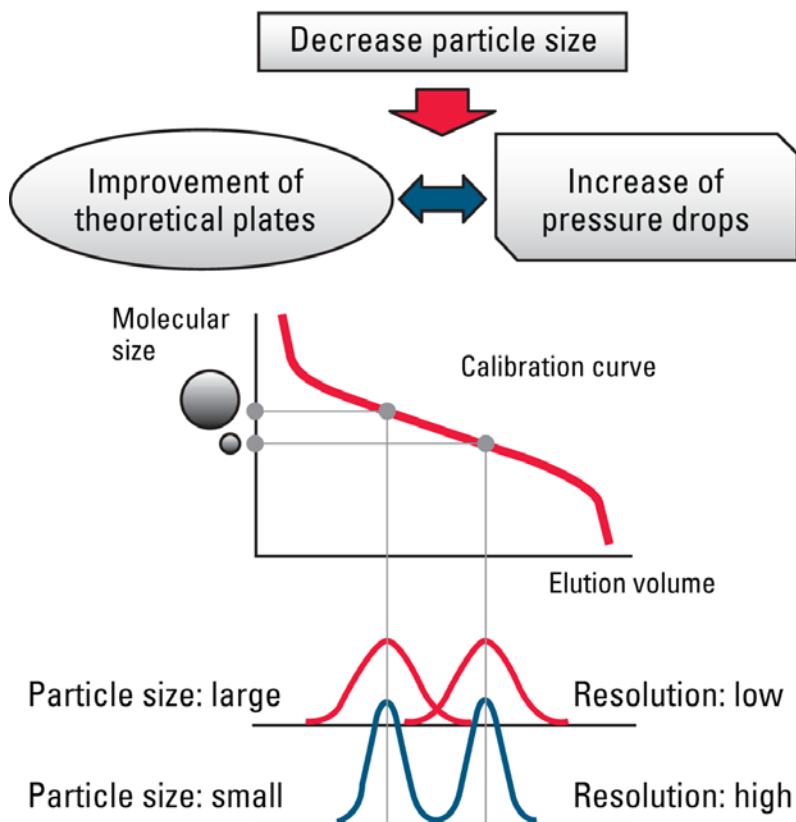
- Easy method transfer of TSKgel G3000SW_{XL} to UP-SW3000 on HPLC systems, as well as transfer of TSKgel UP-SW3000 from HPLC to UHPLC systems
- Useful for the detection of heterogeneity in proteins, particularly in monoclonal antibodies (mAb) and Antibody Drug Conjugates (ADC)
- Excellent reproducibility and lifetime, with both standard proteins and monoclonal antibodies

Material and Methods

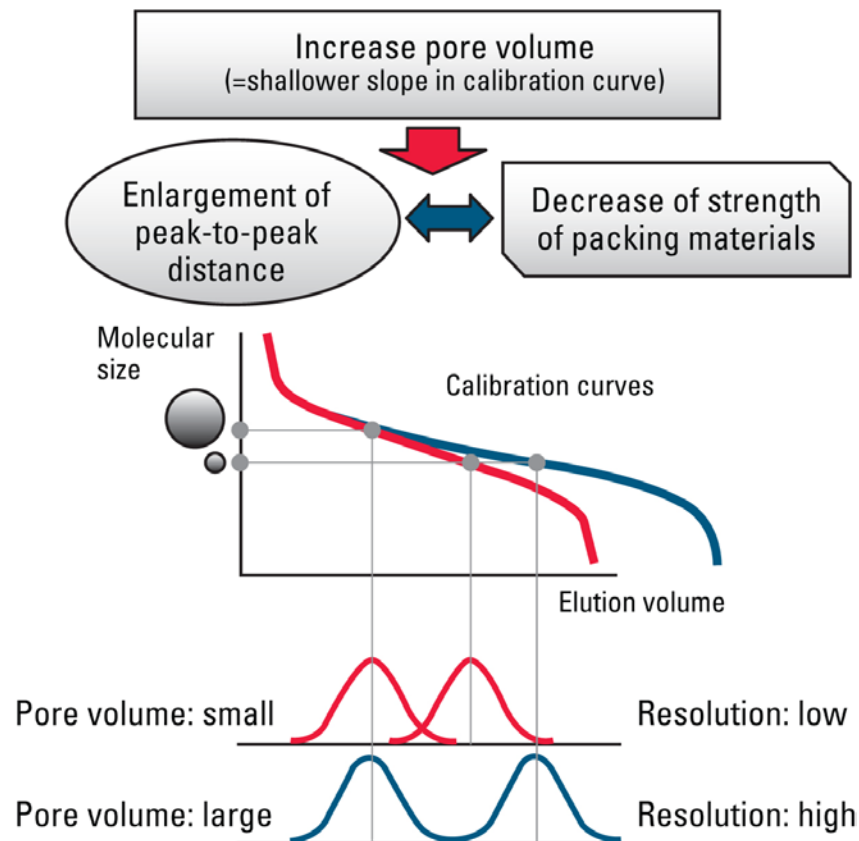
- Instrument: All analyses were carried out using an Ultimate[®] 3000 UHPLC system run by Chromeleon[®] (ver 7.2) (unless mentioned otherwise)
- Column: TSKgel UP-SW3000, 2 μ m, 4.6 mm ID x 30 cm
TSKgel UP-SW3000, 2 μ m, 4.6 mm ID x 15 cm
- Mobile phase: 100 mmol/L KH_2PO_4 / Na_2HPO_4 , pH 6.7, 100 mmol/L Na_2SO_4 , 0.05 % NaN_3
- Flow rate: 0.35 mL/min
- Pressure: 30 cm: 27.2 MPa; 15 cm: 21.2 MPa
- Detection: UV @ 280 nm
- Temperature: 25 °C
- Injection vol.: 5 μ L

Optimization of Packing Materials

1. Particle size of packing materials



2. Pore characteristics of packing materials





Introduction of TSKgel UP-SW3000 SEC Columns

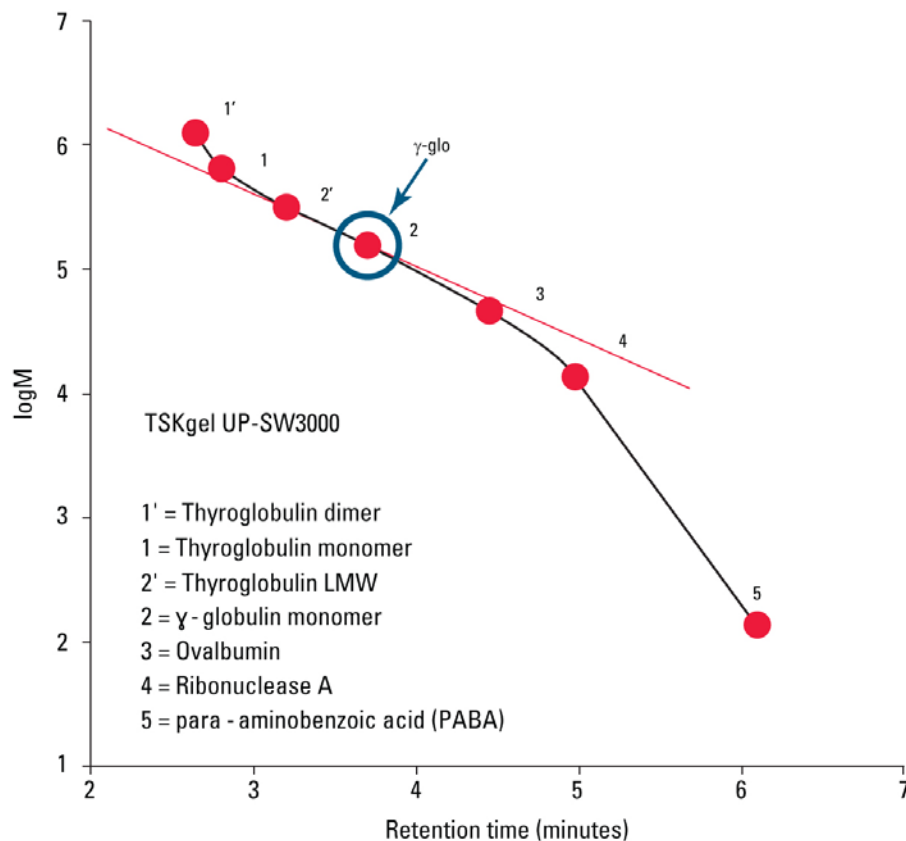
TSKgel column	TSKgel UP-SW3000	
Column size	4.6 mm ID × 15 cm	4.6 mm ID × 30 cm
Base material	Silica	
Stationary phase	Diol	
Particle size	2 µm	
Pore size	25 nm	
Exclusion limit (Proteins)	800 kDa	
Separation range (Proteins)	10 - 500 kDa	
Applications	High-speed analysis of antibody (dimer/monomer)	High-resolution analysis of antibody (dimer/monomer/fragment)



Features of TSKgel UP-SW3000 Columns

- The columns are compatible to both UHPLC and HPLC.
 - Use semi-micro LC system, i.e. low dead volume LC system to further optimize the column performance.
- Easy transfer of existing HPLC methods
- Useful for the detection of heterogeneity in proteins particularly in monoclonal antibody (mAb) or Antibody Drug Conjugate (ADC)
- Excellent reproducibility
- Excellent reproducibility in separating mAb monomer as well as the species on both on HMW side and LMW side of the monomer
- Excellent lifetime with protein standards as well as antibodies

Calibration Curve for Standard Proteins



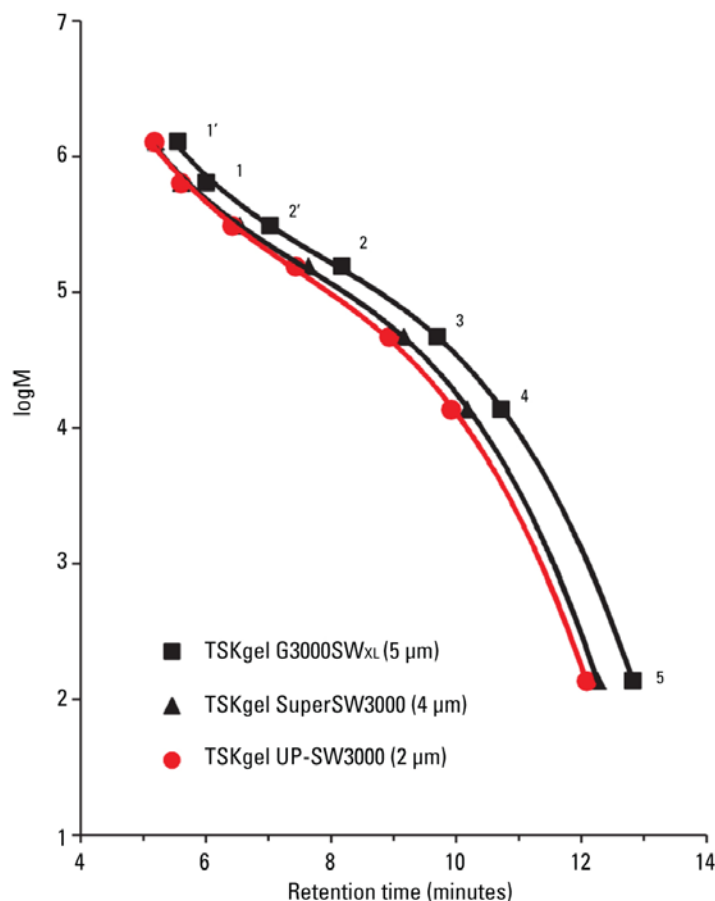
- The TSKgel UP-SW3000 column showed shallower slope around MW region of γ -globulin.
- The TSKgel UP-SW3000 column has an optimized pore size for the separation of proteins.



Comparison with TSKgel G3000SW_{XL}

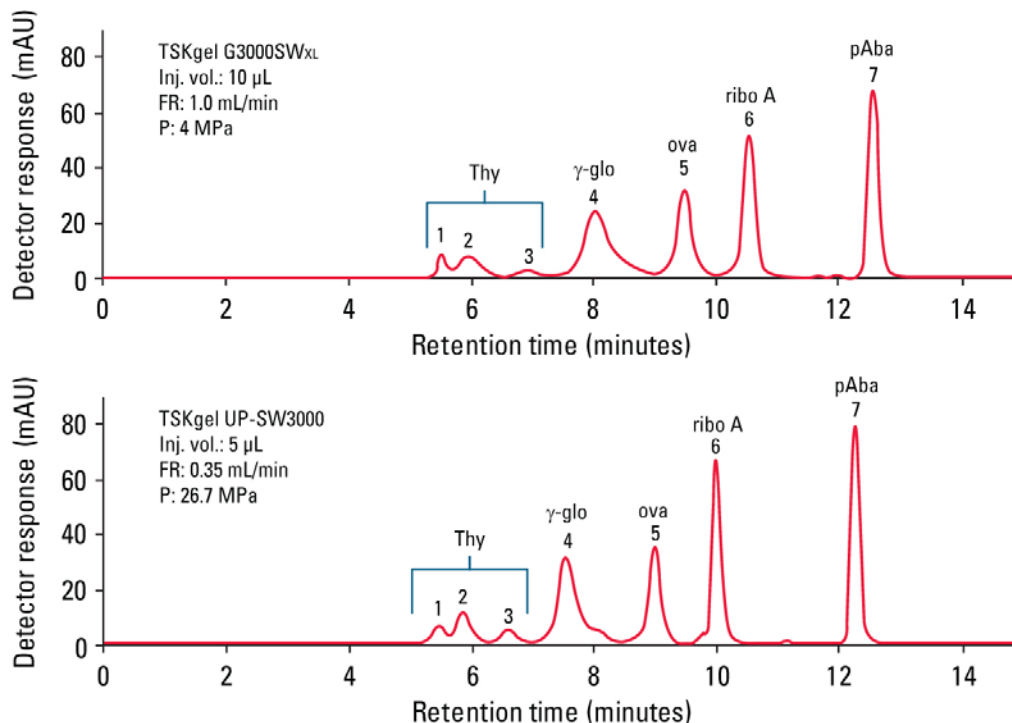
TSKgel column	TSKgel UP-SW3000		TSKgel G3000SW _{XL}
Column size	4.6 mm ID × 15 cm	4.6 mm ID × 30 cm	7.8 mm ID × 30 cm
Base material	Silica		
Stationary phase	Diol		
Particle size	2 μm		5 μm
Pore size	25 nm		
Exclusion limit (Proteins)	800 kDa		
Separation range (Proteins)	10 - 500 kDa		
Applications	High-speed analysis of antibody (dimer/monomer)	High-resolution analysis of antibody (dimer/monomer/fragment)	Separation of general proteins, high resolution analysis of antibody (dimer/monomer)

Calibration Curves for Standard Proteins



- The TSKgel UP-SW3000 and TSKgel G3000SW_{XL} column matrices have the same pore characteristics.
- The same calibration curve trends are followed in these SW columns.

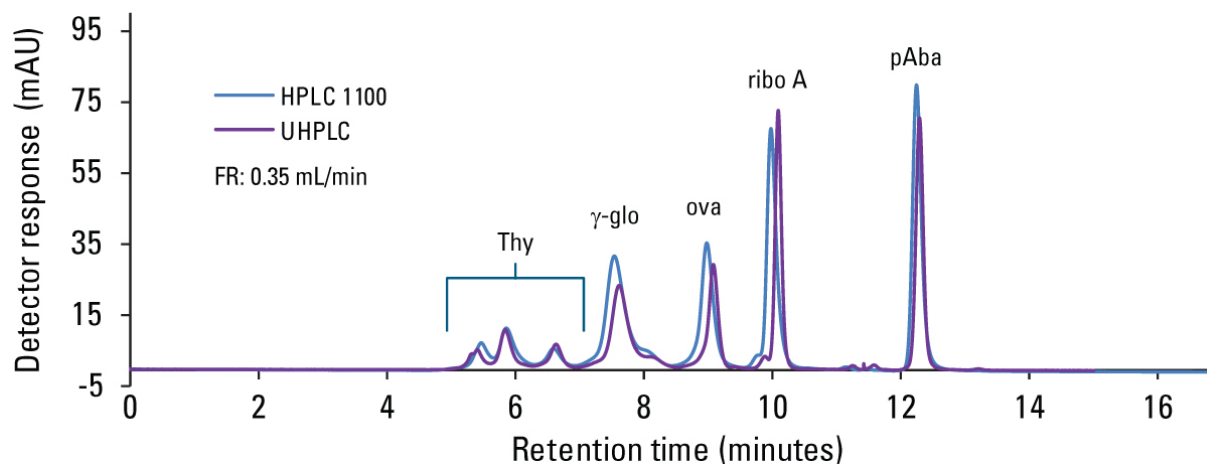
Method Transfer of TSKgel G3000SW to UP-SW3000



- TSKgel UP-SW3000 maintains RT consistency and separation at lower flow rate.
- TSKgel UP-SW3000 has higher sensitivity.
- TSKgel UP-SW3000 has backpressure acceptable for use in both HPLC and UHPLC.

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 from HPLC TO UHPLC

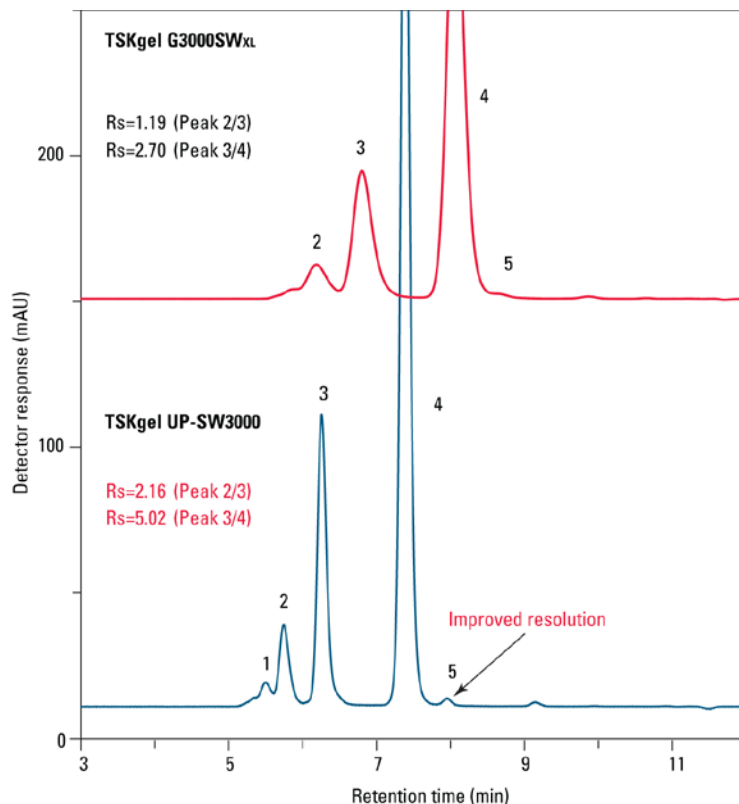


	Rs (Thy-γ-Glo)	Rs (γ-Glo-Ova)	Rs (Ribo A-pAba)
Agilent 1100 HPLC	3.52	3.22	10.23
Dionex UltiMate 3000RS UHPLC	4.46	3.85	11.61

Method can be transferred directly from HPLC to UHPLC, without any change in conditions



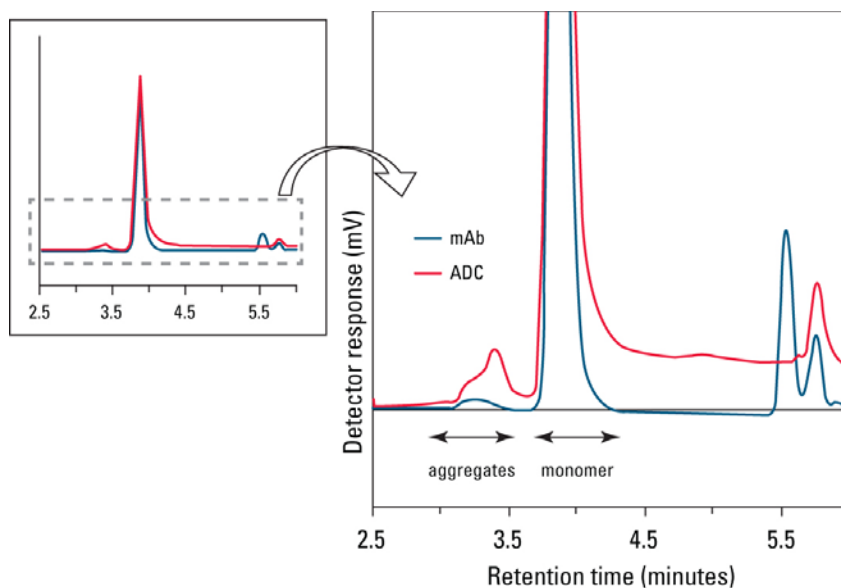
Comparison to TSKgel G3000SWXL Column: Separation of Therapeutic mAb



Column: A: TSKgel G3000SWXL, 5 μ m, 7.8 mm ID \times 30 cm
B: TSKgel UP-SW3000, 2 μ m, 4.6 mm ID \times 30 cm
Mobile phase: 100 mmol/L phosphate buffer, pH 6.7 + 100 mmol/L Na₂SO₄ + 0.05% NaN₃
Flow rate: A: 1.0 mL/min
B: 0.35 mL/min
Detection: UV @ 280 nm
A: normal flow cell
B: micro flow cell
Temperature: 25 $^{\circ}$ C
Injection vol.: 10 μ L
Sample: therapeutic mAb (mouse-human chimeric)
Peak 1: tetramer
2: trimer
3: dimer
4: monomer
5: fragment

- Fragment and monomer of mAb were separated completely by one column of TSKgel UP-SW3000.
- Trimer/dimer/monomer were separated with better resolution than TSKgel G3000SWXL column.
- In addition, tetramer was separated clearly by TSKgel UP-SW3000 column.

Detection of Protein Heterogeneity



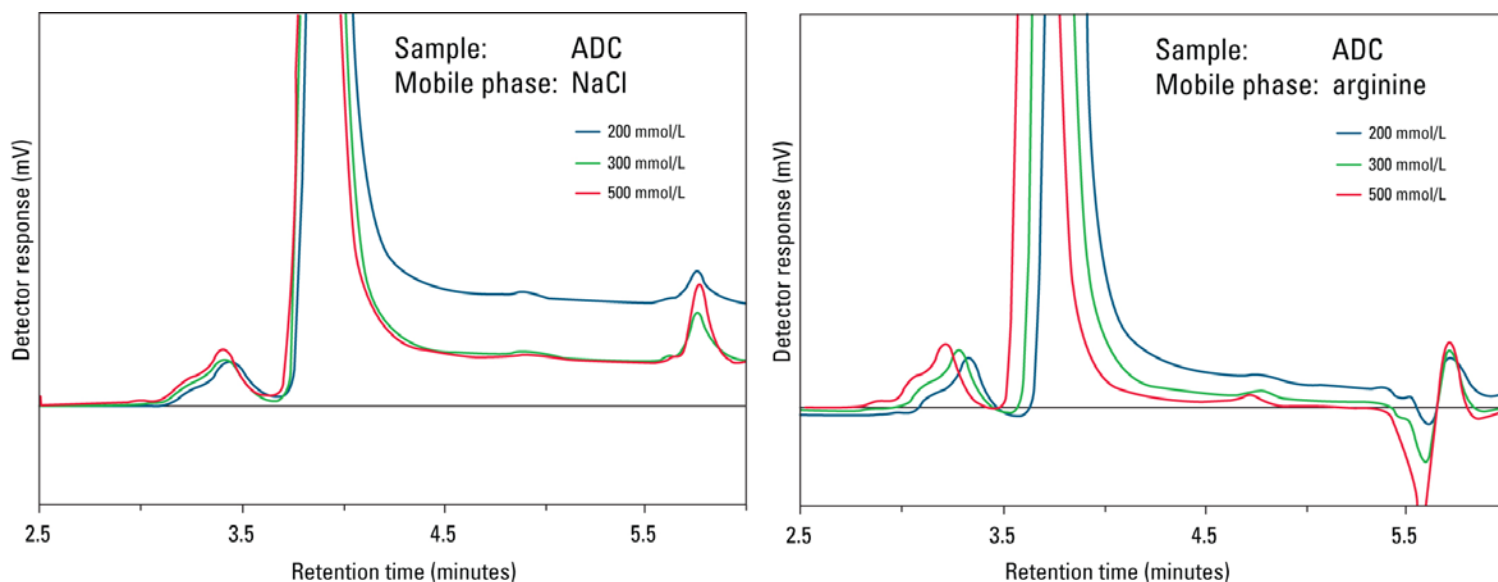
Column: TSKgel UP-SW3000, 2 μ m, 4.6 mm ID \times 15 cm
 Mobile phase: 50 mmol/L phosphate buffer + 500 mmol/L sodium chloride, pH 6.8
 Flow rate: 0.35 mL/min
 Detection: UV @ 280 nm
 Temperature: ambient
 Injection vol.: 5 μ L
 Sample: trastuzumab (mAb)
 trastuzumab-vcMMAE conjugate (ADC)

ADCs with strong hydrophobic character were selected to assess TSKgel UP-SW3000 column ruggedness when exposed to common additives.



Detection of Heterogeneity in Antibody Drug Conjugate (ADC)

The analysis of ADC using TSKgel UP-SW3000 at different concentrations of NaCl and arginine.

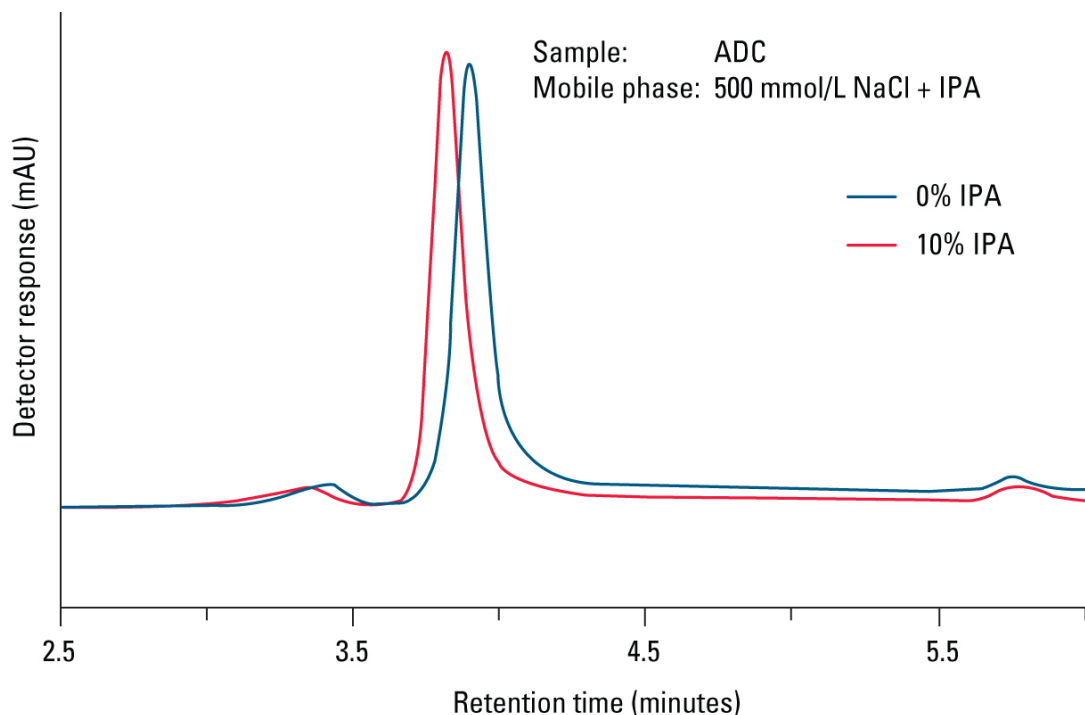


The TSKgel UP-SW3000 column is useful in assessing additive efficacy in improving ADC tailing factor, even with conditions as high as 500 mmol/L NaCl and 500 mmol/L arginine, for this very hydrophobic ADC.



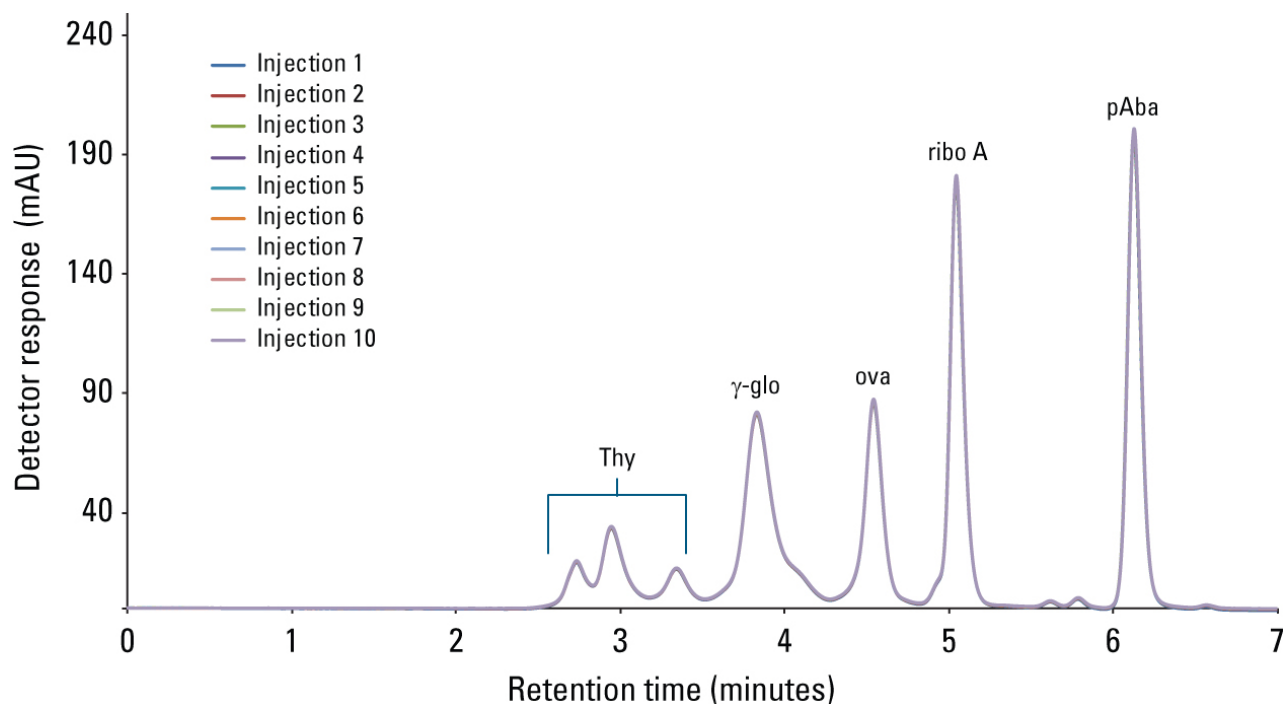
Detection of Heterogeneity in Antibody Drug Conjugate (ADC)

The effect of isopropanol on the analysis of ADC using TSKgel UP-SW3000.



The TSKgel UP-SW3000 column is useful in assessing IPA efficacy in improving ADC tailing factor, however optimal concentration is sample dependent and should be chosen to avoid sample denaturation.

Reproducibility



- The TSKgel UP-SW3000 15 cm column provides results in half the time of the 30 cm column, with nearly unchanged resolution profile.
- Excellent reproducibility in separation over 10 consecutive injections

Ref: AN 89

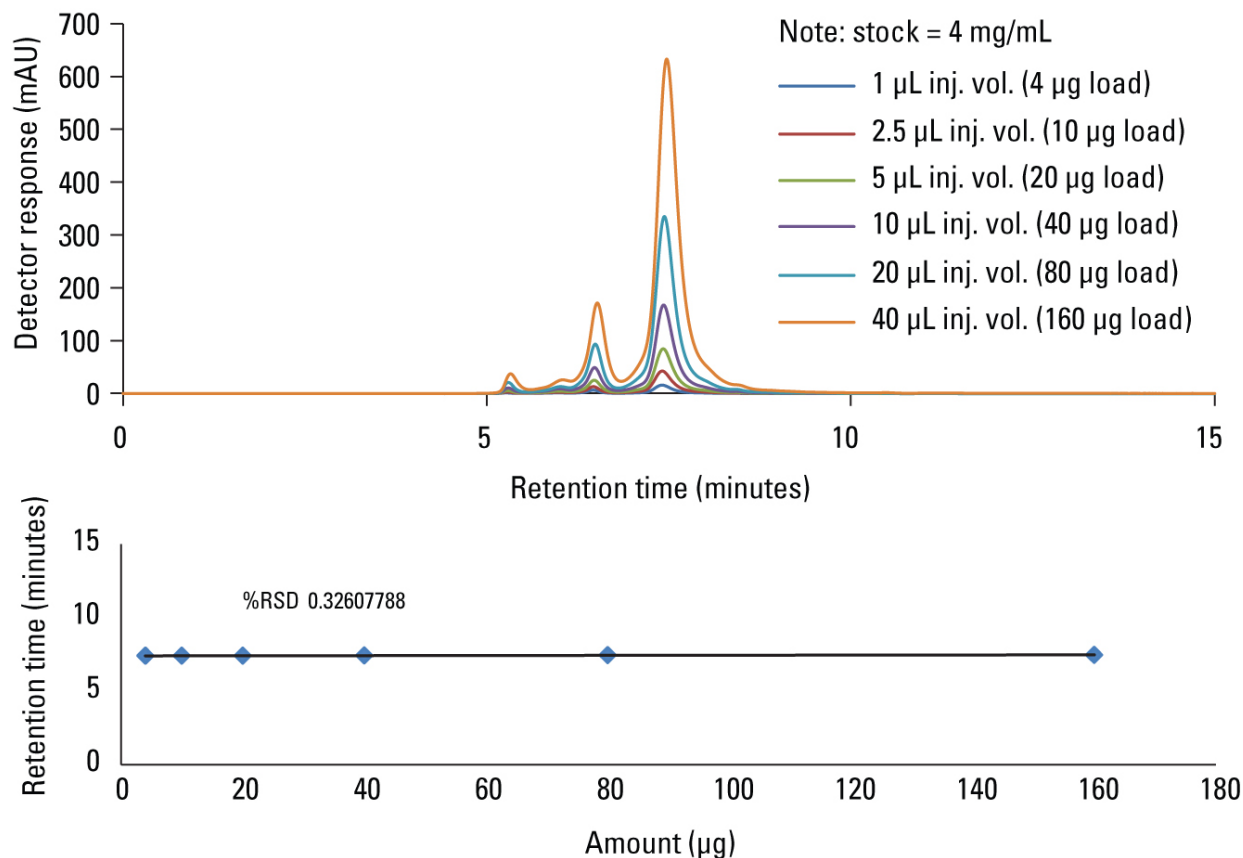
Reproducibility

Injection #	pAba-theoretical plates (N)	γ -globulin-theoretical plates (N)	Thyroglobulin-theoretical plates (N)
1	252743	2533	2260
2	25300	2541	2278
3	25397	2541	2281
4	25519	2555	2299
5	25558	2557	2306
6	25567	2554	2302
7	25550	2556	2304
8	25529	2561	2304
9	25521	2558	2303
10	25502	2554	2307
Average	25468.6	2551.0	2297.4
%RSD	0.451	0.362	0.691

Excellent reproducibility illustrated by low % RSD with 10 consecutive injections



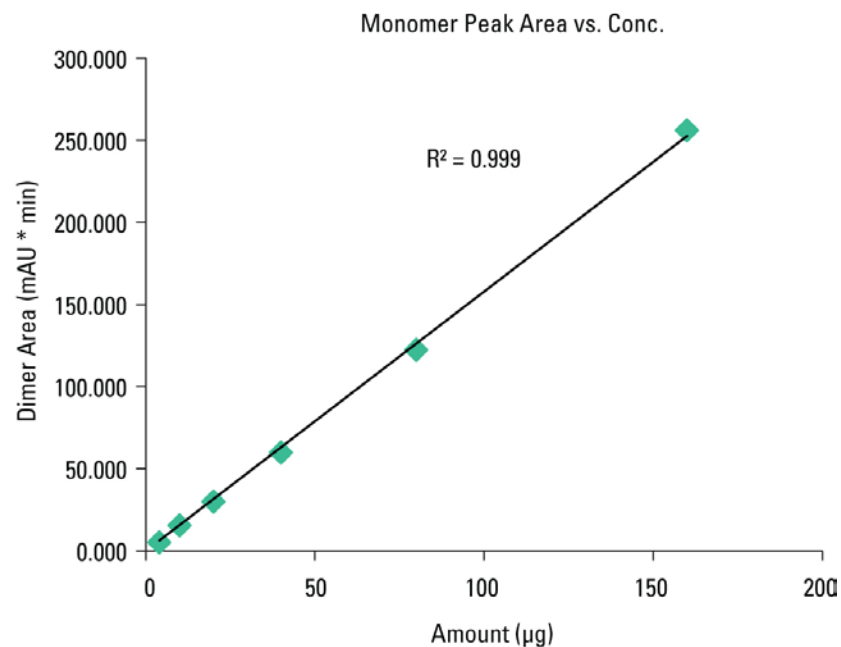
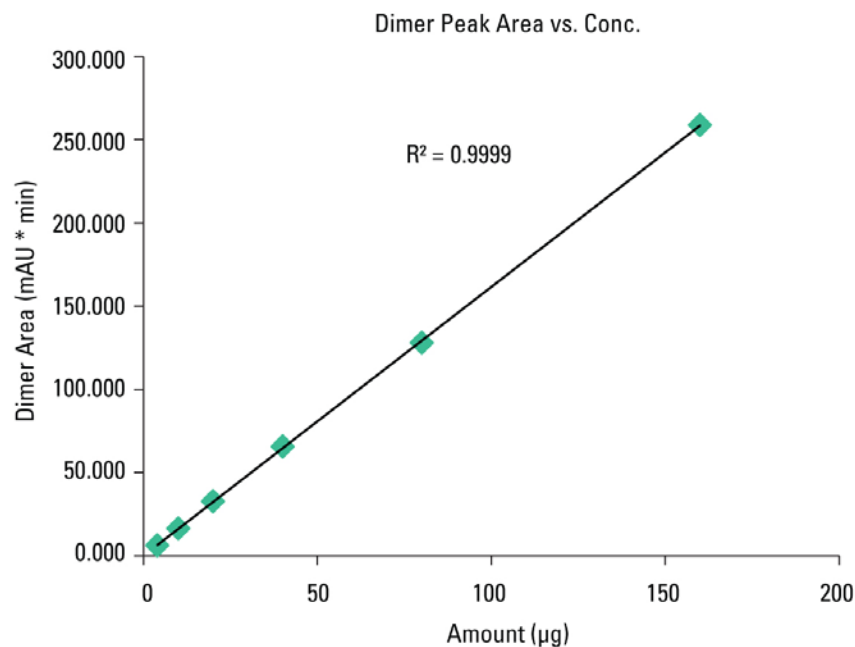
Loading Study of γ -globulin using the TSKgel UP-SW3000 Column



IgG retention time remains nearly unchanged over varying load concentrations.



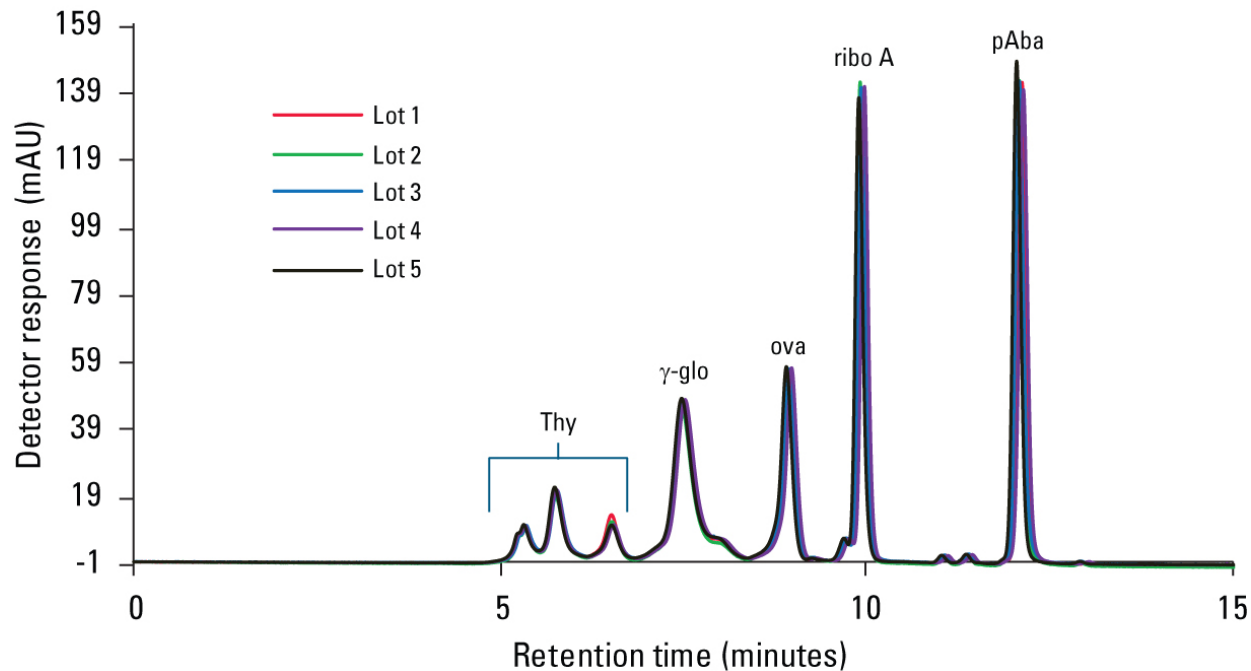
Loading Study of γ -globulin using the 30 cm TSKgel UP-SW3000 Column



- Excellent linearity of both monomer and dimer peak areas versus total concentration
- This demonstrates the sensitivity of detection of the TSKgel UP-SW3000 column.



Lot-to-Lot Variability - 30 cm TSKgel UP-SW3000 Column



Excellent Reproducibility: Column-to-Column, Day-to-Day, Analyst-to-Analyst



Conclusions

- The TSKgel UP-SW3000 column was developed and optimized as an HPLC-UHPLC SEC column for biomolecules.
- Easy Method Transfer of TSKgel G3000SW_{XL} to UP-SW3000 on HPLC Systems, as well as transfer of TSKgel UP-SW3000 from HPLC to UHPLC Systems
- Useful for the Detection of Heterogeneity in proteins particularly in monoclonal antibodies (mAb) and Antibody Drug Conjugates (ADC)
- Excellent reproducibility and lifetime, with both standard proteins and monoclonal antibodies



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- Tosoh Research Center, Japan
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