



High Throughput and Highly Reproducible Sub-4 Minute Separation of Proteins and Antibodies using Size Exclusion Chromatography

Justin Steve and Atis Chakrabarti, Ph.D.
Tosoh Bioscience LLC, King of Prussia, PA



Introduction

- Aqueous Size Exclusion Chromatography (SEC), popularly known as Gel Filtration Chromatography, is a powerful analytical tool in the separation of protein species of differing size, molar mass variants and impurities.
- Traditionally, GFC columns with a dimension of 7.8 mm ID × 30 cm are used for analytical purposes.
- The longer column dimension leads to longer run times and sample dilution, as well as substantial solvent waste.
- Alternatively, a 4.6 mm ID × 15 cm column may give high throughput separation with shorter run times, high resolution and minimal solvent waste using a conventional HPLC system.
- Here we show the use of a 4.6 mm ID × 15 cm TSKgel SuperSW mAb HTP SEC column for the highly reproducible separation of proteins and antibodies in less than 3.5 minutes by using a moderate flow rate of 0.75 mL/min.
- This study illustrates adequate stability of the TSKgel SuperSW mAb HTP column for high speed, sub-4 minute separations of monoclonal antibodies.



TSKgel SuperSW mAb HTP

- The TSKgel SuperSW mAb HTP SEC column is a 4.6 mm ID × 15 cm column designed for the high throughput, highly reproducible separation of proteins and antibodies in half the time of conventional SEC separations.
- The 4 μm particle size and 25 nm pore size are optimally suited for high resolution of monoclonal antibody monomers and dimers.

Base material:	Silica
Particle size (mean):	4 μm
Pore size (mean):	25 nm
Functional group:	Diol
pH stability:	2.5-7.5
Calibration range:	10,000-500,000 Da (globular proteins)

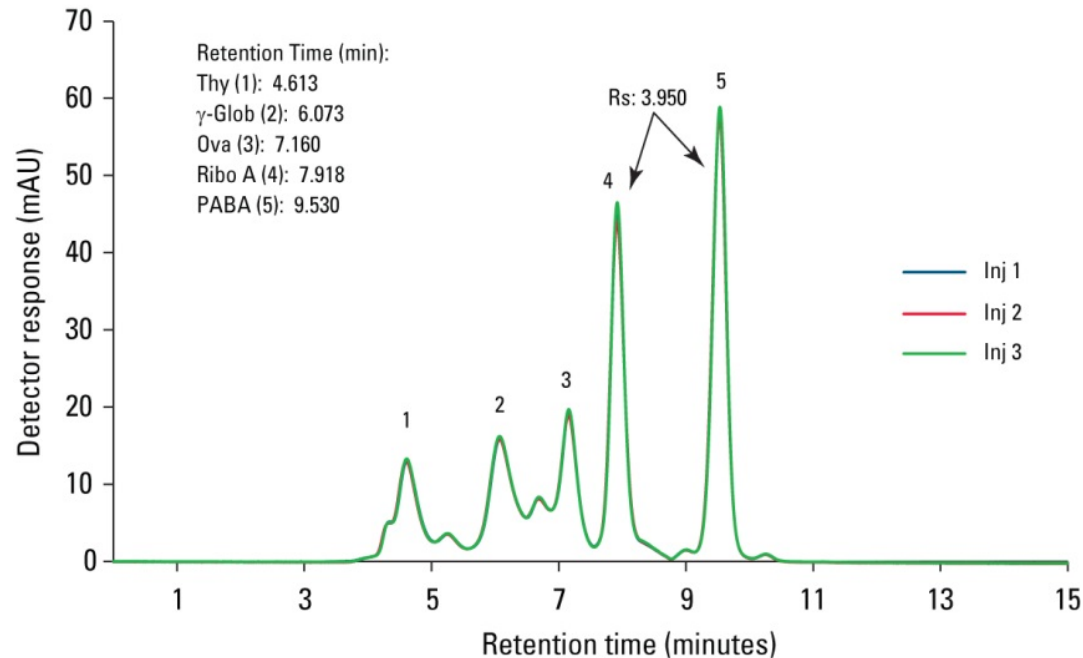


Materials/Methods

Column:	TSKgel SuperSW mAb HTP, 4 μ m, 4.6 mm ID \times 15 cm
Instrument:	Agilent 1100 series HPLC
Mobile phase:	100 mmol/L phosphate/100 mmol/L sulfate buffer, pH 6.7 + 0.05% NaN ₃
Flow rate:	as noted in chromatograms
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	5 μ L
Samples:	thyroglobulin, 0.58 mg/mL gamma-globulin, 1.02 mg/mL ovalbumin, 1.08 mg/mL ribonuclease A, 1.53 mg/mL PABA, 0.01 mg/mL mAb 01, 4.6 mg/mL mAb 02, 4.6 mg/mL human IgG, 4.6 mg/mL mouse IgG, 4.6 mg/mL



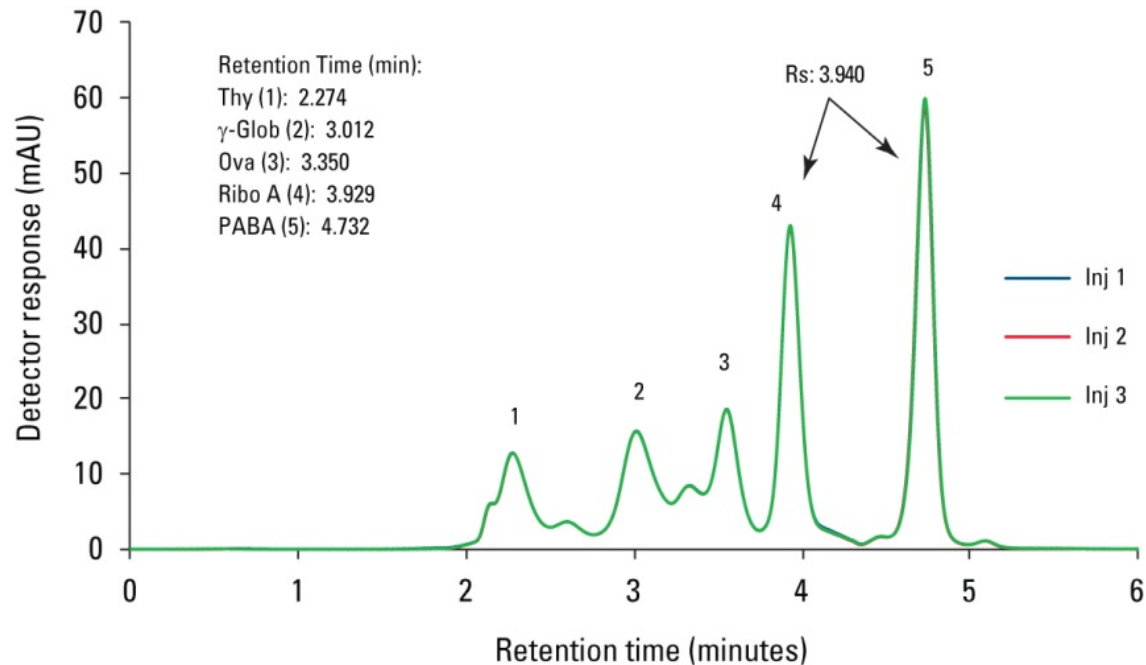
Separation of Protein Standard on TSKgel SuperSW mAb HTP Column



- With a flow rate of 0.25 mL/min, the protein standard was well separated within 10 minutes.
- High resolution of 3.950 between ribonuclease A and PABA was observed.



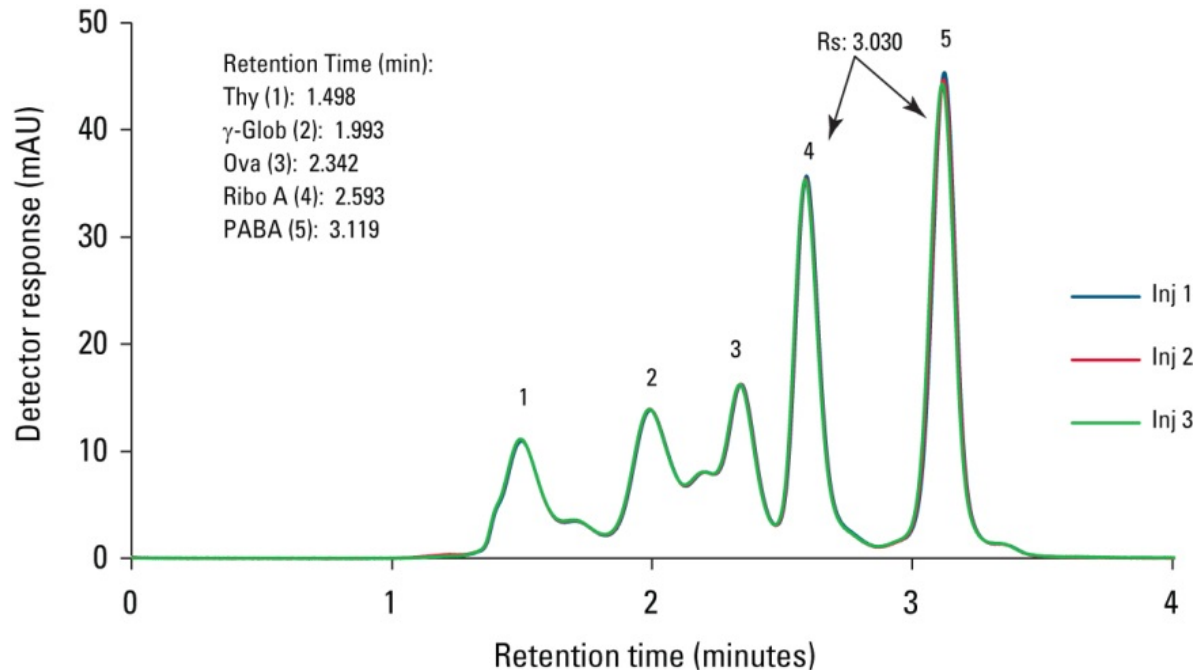
Separation of Protein Standard on TSKgel SuperSW mAb HTP Column



- Doubling of the flow rate to 0.50 mL/min reduced the run time by a factor of 2.
- Even at the elevated flow rate, high resolution between ribonuclease A and PABA was maintained.
- Additionally, no sacrifice in resolution between all other peaks was observed.



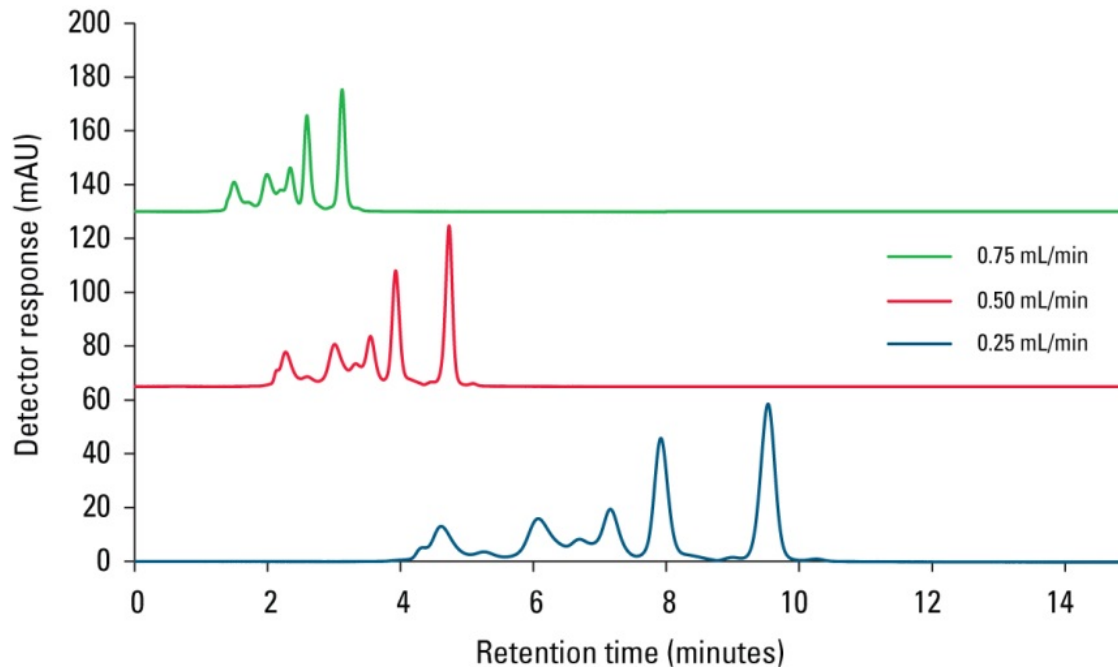
Separation of Protein Standard on TSKgel SuperSW mAb HTP Column



- Increasing the flow rate to 0.75 mL/min further reduced the run time to less than 4 minutes.
- Under these conditions, all protein species remain well separated and resolution between ribonuclease A and PABA is maintained.



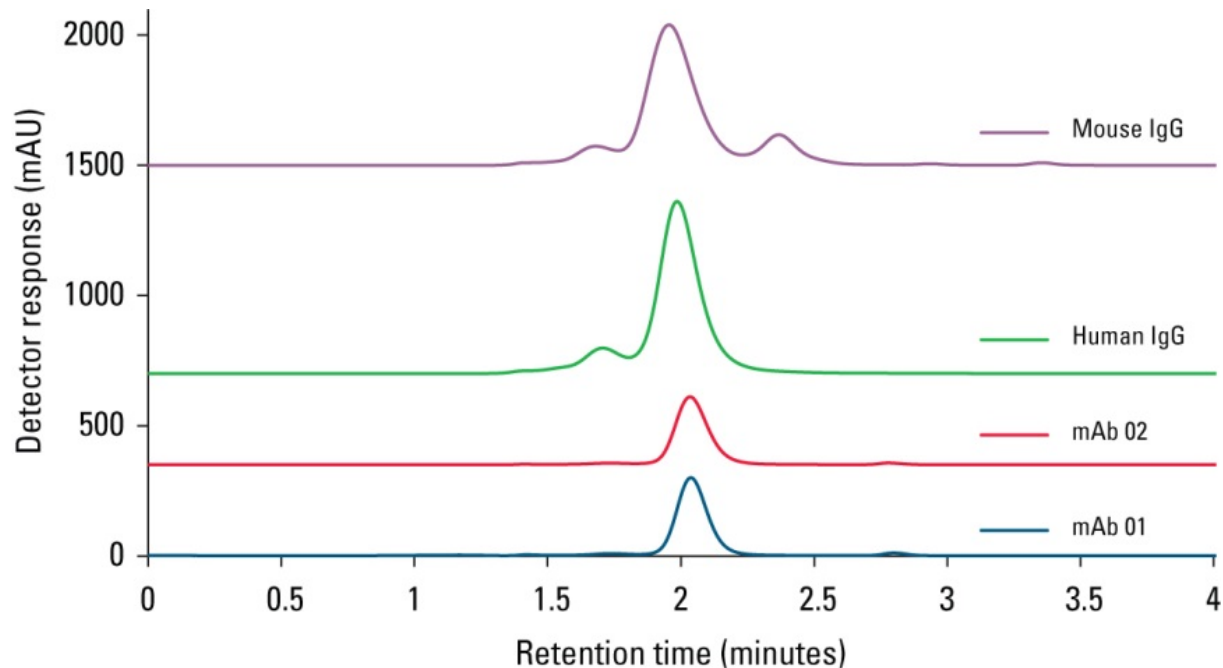
Comparative Profile of the Separation of Protein Standard on TSKgel SuperSW mAb HTP Column



- As shown, using a flow rate of 0.75 mL/min allows for extremely fast separation of 5 species in less than 4 minutes.
- The use of the TSKgel SuperSW mAb HTP column at elevated flow rates can yield separations in slightly over $\frac{1}{4}$ of the time required for conventional (7.8 mm ID \times 30 cm) SEC columns.



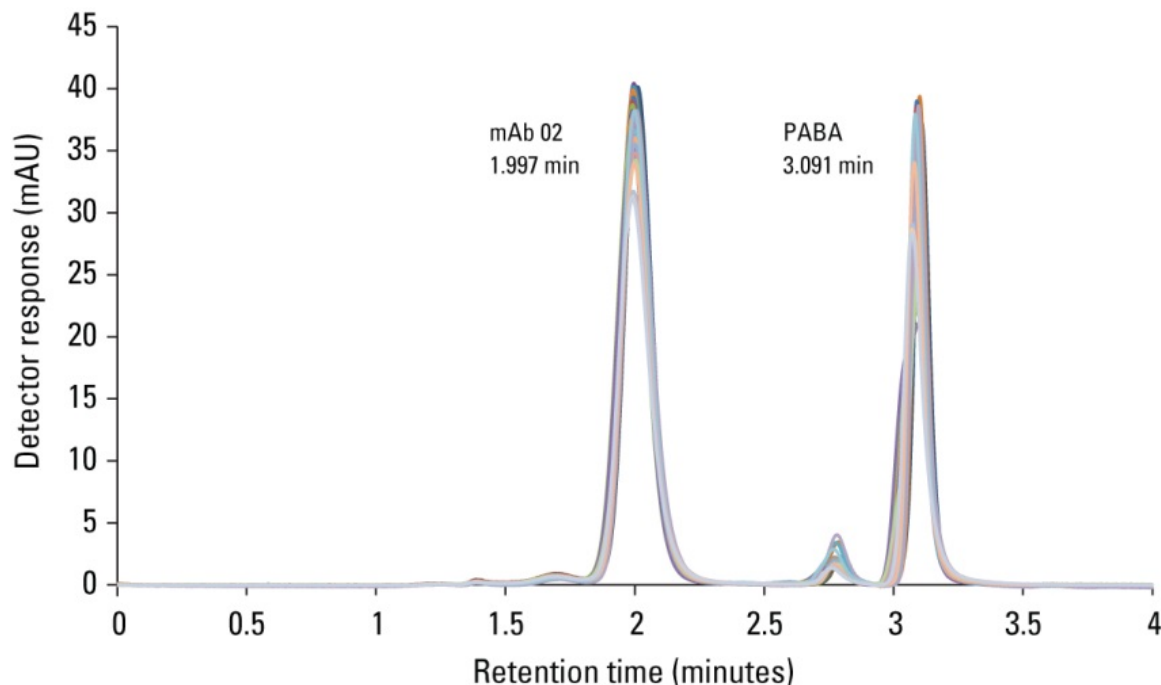
Separation of Monoclonal Antibodies on TSKgel SuperSW mAb HTP Column under High Flow Conditions



- Separation of 4 different monoclonal antibodies in less than 3 minutes was performed using the TSKgel SuperSW mAb HTP column at a flow rate of 0.75 mL/min.
- High resolution separation of the monomer, dimer, and fragment peaks of the mouse IgG sample are clearly shown under these conditions.



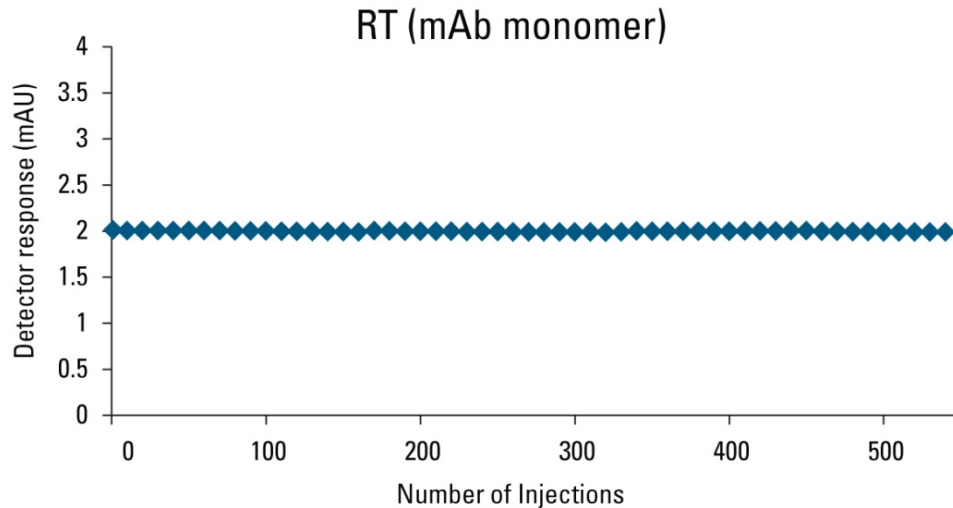
Retention Time Reproducibility of the TSKgel SuperSW mAb HTP Column under High Flow Conditions



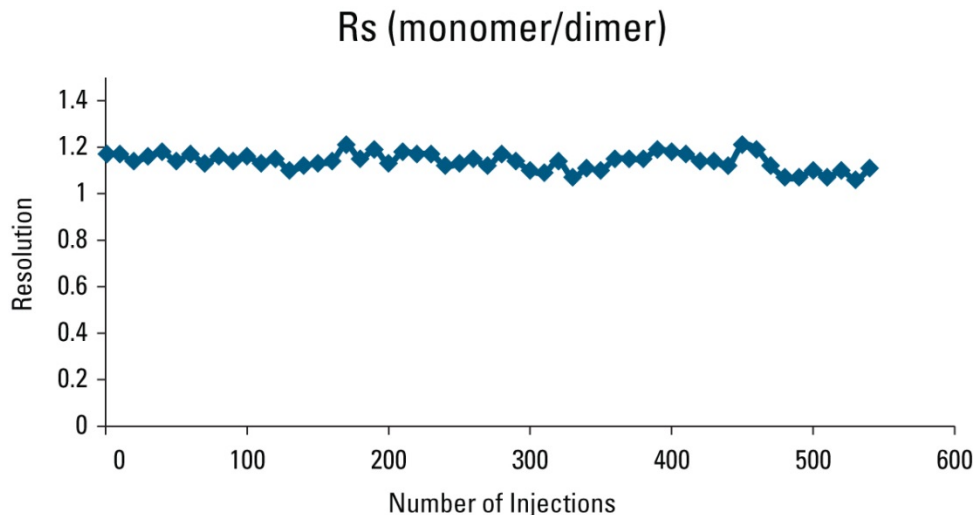
- Sustained pressure from operation at elevated flow rates can lead to voids within the column, generating poor peak shapes and drifting retention time.
- As shown in the figure, 540 consecutive injections of mAb 02 and PABA separated on the TSKgel SuperSW mAb HTP column at 0.75 mL/min show good reproducibility with no discernible drift in retention.



Retention Time and Resolution Stability of the TSKgel SuperSW mAb HTP Column under High Flow Conditions



- Highly stable retention of the mAb monomer over 540 consecutive injections yielded a %RSD = 0.28.



- Additionally, no significant loss in resolution between the mAb monomer and dimer was observed on the TSKgel SuperSW mAb HTP column operated at 0.75 mL/min, yielding a %RSD < 3.



Conclusions

- The purpose of this study was to evaluate the feasibility and reproducibility of sub-4 minute protein separations using the TSKgel SuperSW mAb HTP SEC column.
- Separation performed at 0.75 mL/min yielded highly reproducible results with high resolution and moderate back pressure of the standard protein mixture in less than 3.5 minutes.
- Likewise, IgG-based proteins were effectively separated within 3 minutes using the identical chromatographic conditions. This corresponds to a 3.75-fold decrease in analysis time relative to traditional SEC methodology.
- Additionally, due to the smaller dimension of the TSKgel SuperSW mAb HTP column, minimal solvent waste is observed even at increased flow rates, making this a cost effective and “green” method for protein separations when compared to that of traditional, 7.8 mm ID × 30 cm SEC columns.



Conclusions

- The TSKgel SuperSW mAb HTP column operated at 0.75 mL/min was able to withstand nearly 550 consecutive injections of monoclonal antibody prior to complete column failure while maintaining excellent reproducibility with regards to peak retention time and peak area.
- Despite the observed column longevity at a flow rate of 0.75 mL/min, 0.5 mL/min is the generally recommended flow rate, as mentioned in the operational conditions and specification (OCS) sheet for this column.
- It is expected that the use of a guard column would yield extended stability of the peak parameters, less drifting to lower efficiency and a greater number of injections in between column cleanings.
- These results show that the TSKgel SuperSW mAb HTP, 4 μm , 4.6 mm ID \times 15 cm column can clearly have a competitive advantage in fast assay and high throughput analysis of proteins and antibodies using a conventional HPLC system.