High Throughput Sub-4 Minute Separation of Antibodies using Size Exclusion Chromatography

TSKgel
APPLICATION NOTE

Introduction

Gel Filtration Chromatography (GFC) is a powerful analytical tool in the separation of antibodies. Traditionally, GFC columns with a dimension of 7.8 mm ID × 30 cm are used for analytical purposes. The longer column dimension, however, leads to longer run times and sample dilution, as well as substantial solvent waste. Alternatively, a column of smaller dimension provides high throughput separation with shorter run times, high resolution and minimal solvent waste when used on a conventional HPLC system. This Application Note demonstrates the use of a 4.6 mm ID × 15 cm TSKgel® SuperSW mAb HTP SEC column for the highly reproducible separation of antibodies in less than 3.5 minutes by using a moderate flow rate of 0.75 mL/min. The SuperSW mAb HTP column provides excellent stability for high speed, sub-4 minute separations of monoclonal antibodies.

Materials and Methods

Column:	TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm
Instrument:	Agilent 1100 HPLC system
Mobile phase:	100 mmol/L phosphate/100 mmol/L sulfate buffer, pH 6.7 + 0.05% NaN_3
Flow rate:	0.75 mL/min
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	5 µL
Samples:	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

Results and Discussion

Figure 1 shows the separation of four different monoclonal antibodies in less than three minutes 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. The separation of these IgG-based proteins within 3 minutes using the TSKgel SuperSW mAb HTP column corresponds to a 3.75-fold decrease in analysis time relative to conventional (7.8 mm ID × 30 cm) SEC columns.

Sustained pressure from operating at elevated flow rates can lead to voids within the column, generating poor peak shapes and drifting retention time. As shown in *Figure 2*, 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. 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 *(Figure 3)*.

Figure 1. Separation of Monoclonal Antibodies on TSKgel SuperSW mAb HTP Column under High Flow Conditions

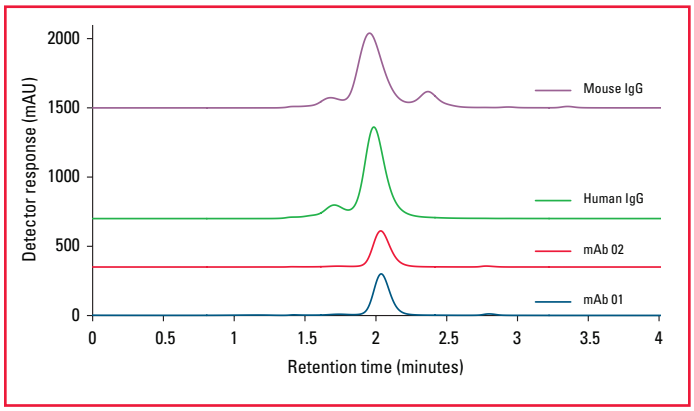


Figure 2. Retention Time Reproducibility of the TSKgel SuperSW mAb HTP Column under High Flow Conditions

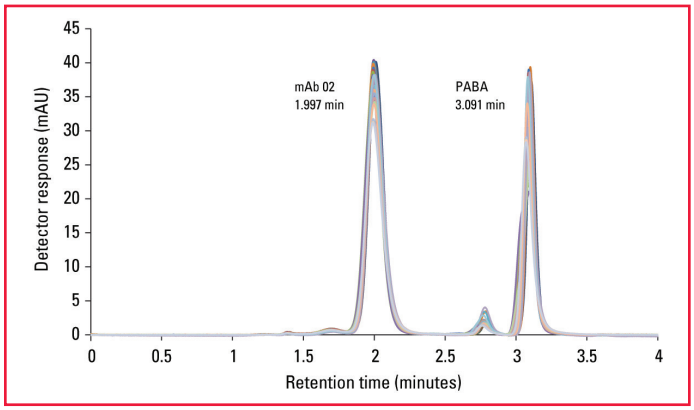
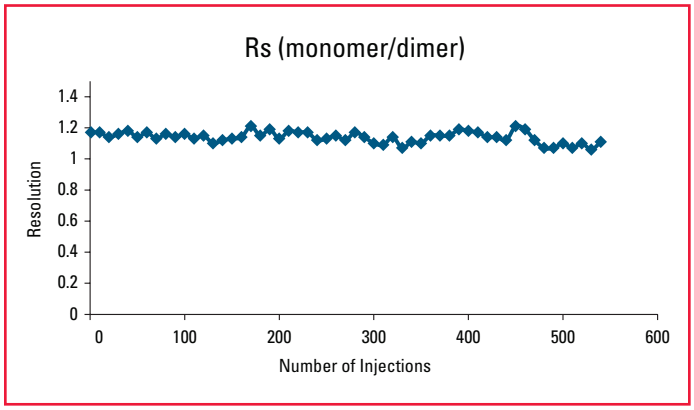


Figure 3. Resolution Stability of the TSKgel SuperSW mAb HTP Column under High Flow Conditions



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Conclusions

Separations of IgG-based proteins using the TSKgel SuperSW mAb HTP column at 0.75 mL/min yielded highly reproducible results with high resolution and moderate back pressure within 3 minutes. 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. The TSKgel SuperSW mAb HTP column operated at 0.75 mL/min for 540 injections of monoclonal antibody showed no drift in retention and good reproducibility. These results demonstrate that the TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm column clearly has a competitive advantage in fast assay and high throughput analysis of antibodies using a conventional HPLC system.

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