

# Improved SEC Separations using TSKgel® Columns on a UHPLC System

TSKgel  
APPLICATION NOTE

## Introduction

Ultra High Performance Liquid Chromatography (UHPLC) continues to grow in popularity due to highly efficient columns and fast run times, which result in excellent resolution and high throughput. UHPLC instrumentation is an optimized HPLC with low dead volume and has the ability to maintain constant flow rate against the higher back pressure generated by columns packed with small particles.

Recently introduced by Tosoh Bioscience, the TSKgel SuperSW mAb HTP column is able to be used with a UHPLC system, taking advantage of this column's smaller particle size and optimized dimensions. The end result is fast run times with a traditional HPLC column on a UHPLC system.

This note details the rapid analysis of an aggregated monoclonal antibody.

## Experimental Conditions

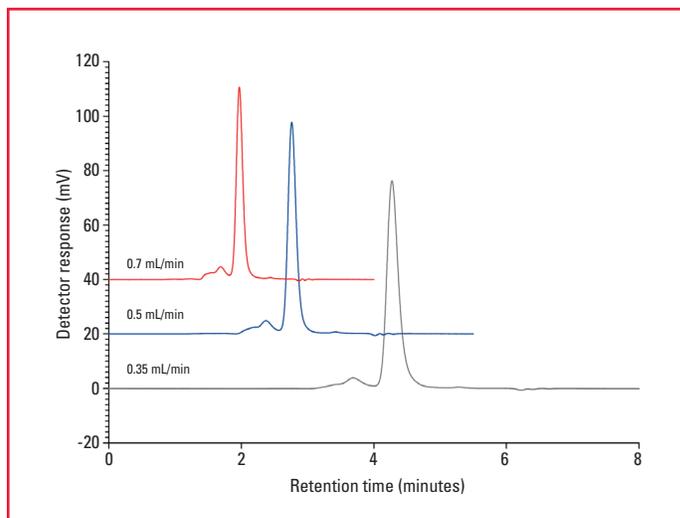
Column: TSKgel SuperSW mAb HTP, 4  $\mu$ m, 4.6 mm ID  $\times$  15 cm  
System: Dionex UltiMate® 3000RS UHPLC System  
Mobile phase: 0.1 mol/L sodium phosphate, pH 6.7 + 0.1 mol/L sodium sulfate  
Flow rate: 0.35 mL/min  
0.5 mL/min  
0.7 mL/min  
Detection: UV @ 280 nm  
Injection vol.: 5  $\mu$ L  
Sample: aggregated monoclonal antibody

## Results and Discussion

*Figure 1* shows what is possible with a short gel filtration column made of HPLC column hardware packed with 4  $\mu$ m particles when used on a UHPLC system. The new TSKgel SuperSW mAb HTP (4.6 mm ID  $\times$  15 cm) is optimized for high throughput applications and is compatible on UHPLC systems because of its ability to withstand high flow rates and low pressure drops.

It is possible to shorten analysis time of antibody aggregates from 15 minutes, using a conventional 30 cm column with 5  $\mu$ m particles, down to 4 minutes when using a 15 cm column with 4  $\mu$ m silica particles. The particle size is still large enough to keep the pressure in a moderate range, avoiding frictional heating inside the column, which might cause further aggregation or fragmentation during the analysis. These kinds of artifacts can be misinterpreted as an indication of a higher degree of impurity than actually exists within the sample vial.

**Figure 1.** Aggregated mAb Sample Analyzed on a TSKgel SuperSW mAb HTP Column



## Conclusions

The results of the analysis of a monoclonal antibody using the new TSKgel SuperSW mAb size exclusion column show a fast and high resolution separation using a UHPLC system. The TSKgel SuperSW mAb HTP column can withstand high flow rates and low pressure drops.

The use of HPLC columns with UHPLC systems offers the advantages of cost and time savings over having to purchase and develop new methods with UHPLC columns. Whether you are working with a UHPLC system or a conventional system, TSKgel columns are the first choice for bioseparations.

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