

How a column facilitates combination of SEC with MS



**Analysis of
biotherapeutics**

Your Challenge

- ▶ You wish to characterize biotherapeutics by SEC and mass spectrometry
- ▶ You suffer from long equilibration times and quickly decreasing ionization efficiency

Our Solution

TSKgel® UP-SW3000-LS

- ▶ A SEC column dedicated for MS applications

What was done?

- ▶ Shedding and ionization efficiency of a mAb was compared between TSKgel and competitive columns

What was the result?

- ▶ UP-SW3000-LS column performed best in shedding, equilibration times and ionization efficiency

The TSKgel UP-SW3000-LS simplifies the combination of SEC analysis of biotherapeutics combined with MS by delivering superior performance in shedding, equilibration time, and ionization efficiency.

Your Benefit

Save time for SEC-MS analyses by reducing shedding and ion source cleanings

TOSOH BIOSCIENCE

<https://www.separations.eu.tosohbioscience.com/solutions/hplc-products/size-exclusion/aqueous-size-exclusion-gfc/tskgel-up-sw3000-ls>



Selecting the Optimal Column for Native SEC-MS of Monoclonal Antibodies

Characterization of monoclonal antibodies (mAbs) is essential to product safety and efficacy. Determining the purity and characterizing the impurities such as dimers or fragments are two critical quality parameters. Size exclusion chromatography (SEC) coupled with mass spectrometry (MS) is increasingly used to identify the accurate molecular mass of mAbs. However, traditional SEC typically generates high particle shedding, which decreases ionization efficiency over time, even when operating in high molecular weight (HMW) m/z ranges. To avoid shedding for MS and multi-angle light scattering (MALS) applications, Tosoh Bioscience developed TSKgel® UP-SW3000-LS U/HPLC size-exclusion columns.

In this application note, a TSKgel UP-SW3000-LS column was coupled with an MS instrument for the analysis of a mAb standard. Data demonstrates that the TSKgel UP-SW3000-LS column surpasses competitive UHPLC columns and a dedicated low shedding column for SEC of proteins in terms of particle shedding observed by MS. Moreover, this column helps maintain ionization efficiency in the electrospray ionization (ESI) source >90% compared to the initial injection over >50 injections.

Experimental Conditions

| | |
|---------------------|--|
| Columns: | TSKgel UP-SW3000-LS, 4.6 mm ID x 15 cm, 2 μ m (P/N 0023547) UHPLC column for SEC of proteins, 4.6 mm ID x 15 cm, 1.7 μ m Dedicated light scattering column, 4.6 mm ID x 15 cm, 3 μ m |
| Instruments: | |
| UHPLC instrument : | Shimadzu Nexera® XR |
| MS instrument: | SCIEX X500B QTOF |
| Ionization mode: | ESI, positive mode |
| MS mode: | Scanning, TOF MS m/z 4000-8000 |
| Ion source gas 1: | 50 psi |
| Ion source gas 2: | 50 psi |
| Curtain gas: | 30 psi |

Method and Mobile phase:

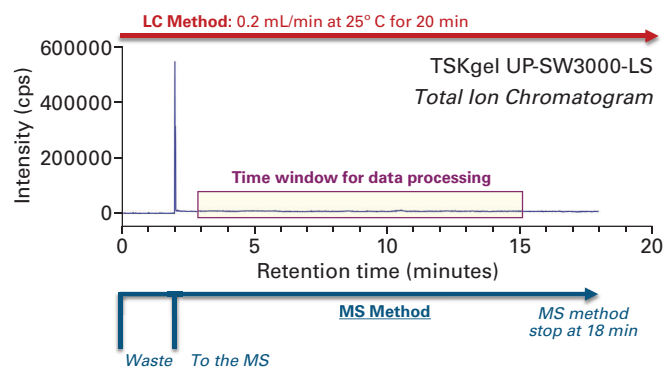
| | |
|-------------------------|--|
| Mobile phase: | 100 mmol/L ammonium formate, pH 6.8 |
| Flow rate: | 0.2 mL/min |
| Detection: | UV @ 280 nm |
| Temperature: | 25° C |
| Injection vol.: | 10 μ L |
| CAD gas: | 7 psi |
| Spray voltage: | 5500 V |
| Source temperature: | 450° C |
| Declustering potential: | 275 V \pm 20 V |
| Collision energy: | 5 V |
| Accumulation time: | 0.5 s |
| Time bins to sum: | 80 |
| Script of Intact | |
| Protein Mode: | ON |
| Q1 transmission window: | 100% at 2250 Da |

Results and Discussion

Reduced particle shedding in SEC-MS using TSKgel UP-SW3000-LS

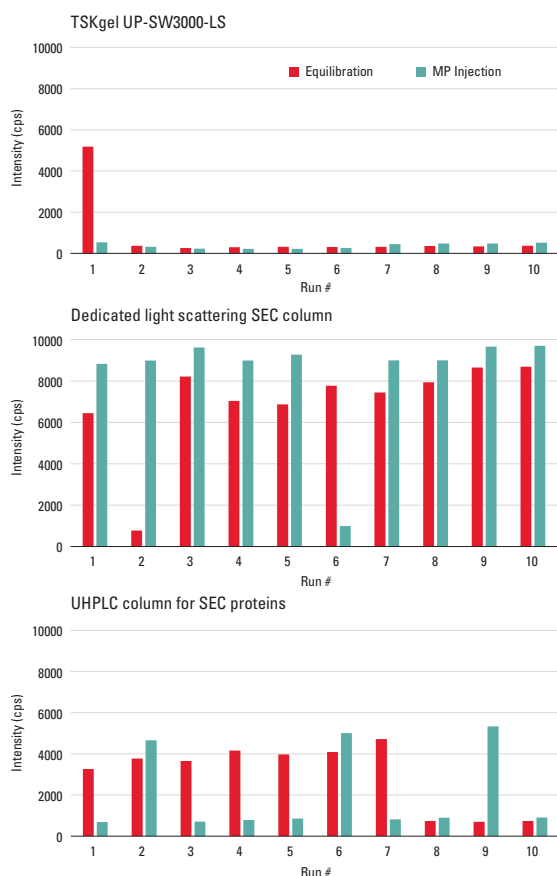
Based on our first observations, particle shedding does not occur at a specific retention time. It is a continuous process that occurs throughout the elution process. Therefore, we decided to collect the data accumulated between 3 and 15 min elution time to quantify the amount of column shedding occurring during one SEC run (see [Figure 1](#)).

➤ **Figure 1.** Experimental design to measure SEC particle shedding using LC-MS.



We quantified column shedding throughout multiple SEC runs by counting the measured intensities of the various molecular ions stemming from each column. The analysis sequence consisted of 10 equilibration runs (no injections), followed by 10 runs where mobile phase blanks were injected onto the column. The results from the quantification of particle shedding are presented in **Figure 2**, in which we plotted the intensity or count per second (cps) of particle shedding as a function of the run number.

Figure 2. Quantification of SEC particle shedding using LC-MS.



The TSKgel UP-SW3000-LS column exhibited almost no particle shedding after the first equilibration run and surpassed both the UHPLC and dedicated light scattering columns in terms of particle shedding. As shedding is not measurable after the first equilibration run, users do not have to condition the SEC column for a long time before starting their analysis.

Increased ionization efficiency in SEC-MS using TSKgel UP-SW3000-LS

We measured the area under the curve of the 28+ peak to quantify the MS ionization efficiency of the eluted NIST mAb monomer peak for the various columns tested over 50 consecutive injections (see **Figures 3 and 4**). We observed a correlation between the decrease in ionization efficiency and the high particle shedding.

The TSKgel UP-SW3000-LS column, which exhibited the lowest high particle shedding, maintained high ionization efficiency (above 90%) for more than 50 injections.

Figure 3. SEC-MS analysis of a mAb

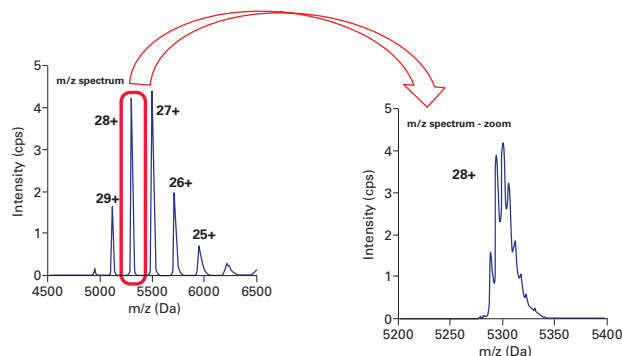
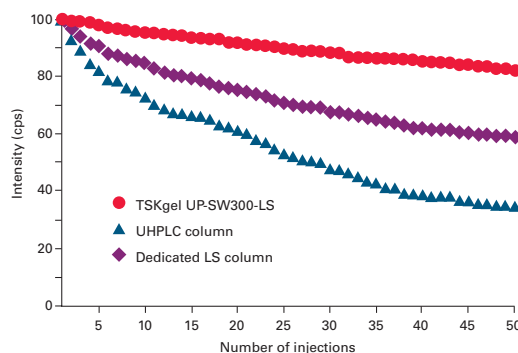


Figure 4. Electrospray ionization efficiency of a mAb.



Conclusion

Using the TSKgel UP-SW3000-LS column for SEC-MS analysis helps limit column shedding, which significantly improves the overall analysis performance. It also helps maintain the instrument's uptime with less frequent cleaning of the ESI source compared with competitor columns. As a consequence, SEC-MS analyzes are more reproducible and reliable while being done at a higher throughput when using the TSKgel UP-SW3000-LS column.

Featured Products

| Part # | Description | Column dimensions |
|---------|--------------------------------|----------------------|
| 0023547 | TSKgel UP-SW3000-LS | 4.6 mm ID x 15 cm L |
| 0023546 | TSKgel UP-SW3000-LS | 4.6 mm ID x 30 cm L |
| 0023548 | TSKgel guardcolumn UP-SW-LS | 4.6 mm ID x 2.0 cm L |
| 0023549 | TSKgel guardcolumn UP-SW-LS DC | 4.6 mm ID x 2.0 cm L |

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