TOSOH

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Application Note

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 µm (P/N 0023547)
UHPLC column for SEC of proteins, 4.6 mm ID x 15 cm, 1.7 µm
Dedicated light scattering column, 4.6 mm ID x 15 cm, 3 µm

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:

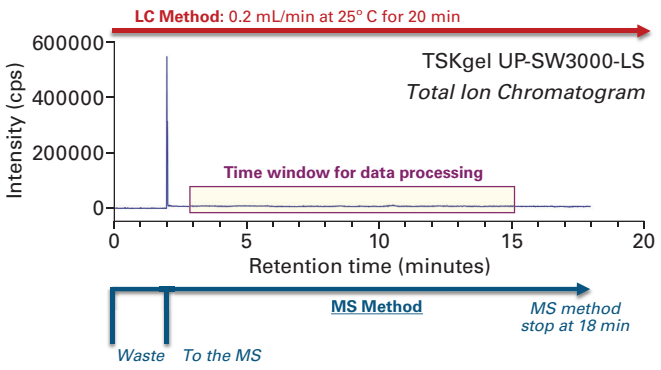
Mobile phase:	100 mmol/L ammonium formate, pH 6.8
Flow rate:	0.2 mL/min
Detection:	UV @ 280 nm
Temperature:	25° C
Injection vol.:	10 µL
CAD gas:	7 psi
Spray voltage:	5500 V
Source temperature:	450° C
Declustering potential:	275 V ± 20 V
Collision energy:	5 V
Accumulation time:	0.5 s
Time bins to sum:	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

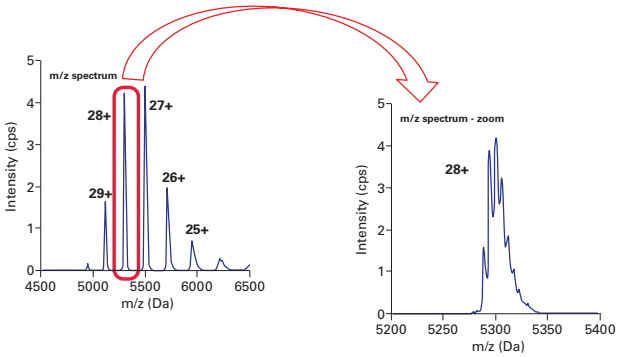
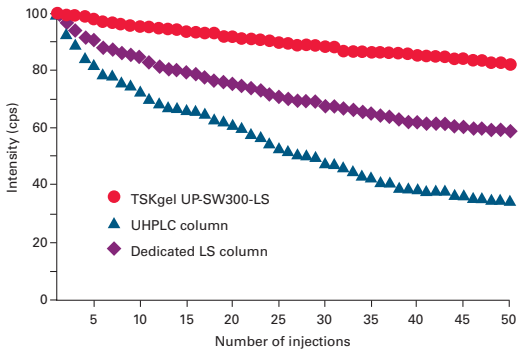


Figure 4. Electrospray ionization efficiency of a mAb.



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