The Usage of Isopropyl Alcohol in SEC for Monoclonal Antibody Separation

INTRODUCTION

Size exclusion chromatography (SEC) is widely used to quantitate monomers, dimers, aggregates, and fragments in antibody analysis. Due to a high demand for better resolution and faster analysis time, more well-designed SEC columns have been introduced. These are 2 µm and sub-2 µm particle size SEC columns with the appropriate pore size for analyzing antibodies with optimized particle chemistry. Despite this improvement, nonspecific absorption of antibodies onto the column gel matrix poses a challenge, with some newly engineered antibodies possessing a high degree of hydrophobicity. The use of organic solvents such as isopropyl alcohol (IPA) or salts can decrease this interaction as reported by many scientists. However, the additives may alter the diffusion of these molecules, which results in retention time shift and poor peak resolution that did not occur in a typical aqueous buffer system, such as sodium phosphate buffer at neutral pH.

In this application note, a TSKgel UP-SW3000, 2 µm SEC column was used for analyzing monoclonal antibodies (mAbs) with the addition of 15% IPA in sodium phosphate buffer, pH 6.7. As demonstrated, peak resolution and retention time were not impacted.

EXPERIMENTAL HPLC CONDITIONS

Column:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID x 30 cm (PN 0023448)
Instrument:	UltiMate® 3000 UHPLC system run by Chromeleon® (ver 7.2)
Mobile phase:	15% IPA in 100 mmol/L KH_2PO_4/ Na_2HPO_4 pH 6.7, 100 mmol/L Na_2SO_4, 0.05% NaN_3
Flow rate:	0.30 mL/min
Detection:	UV @ 280 nm
Temperature:	30 ˚C
Pressure:	22 MPa (maximum column pressure is 34 MPa)
Injection vol.:	5 µL, 4 mg/mL
Sample:	USP mAb standard

RESULTS AND DISCUSSION

The excellent reproducibility of injection to injection of the USP mAb standard onto the TSKgel UP-SW3000, with a typical sodium phosphate buffer, pH 6.7, is shown in Figure 1. This figure is an overlay of 14 consecutive injections of the USP mAb standard sample at the flow rate of 0.3 mL/min. The retention times of monomer, dimer, aggregate, and fragment peaks are nearly unchanged. Peak width and peak shape are very consistent from injection to injection.

SEPARATION OF USP mAb STANDARD

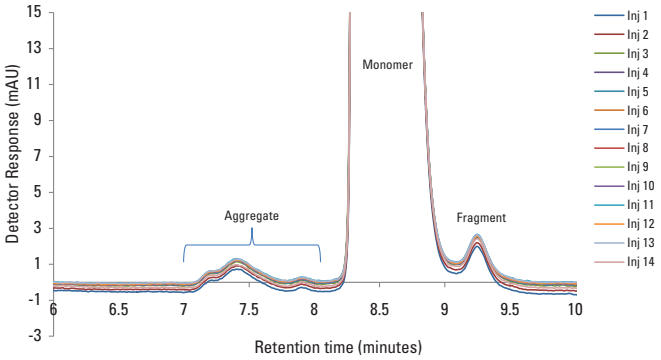

Figure 1

% RSD OF MONOMER AND DIMER PEAK OF 14 INJECTIONS: SEPARATION OF USP mAb STANDARD

	MONOMER PEAK		DIMER PEAK	
INJECTION	Ret. time (min)	Area (mAU* min)	Ret. time (min)	Area (mAU* min)
1	8.370	99.300	7.403	0.380
2	8.370	99.290	7.400	0.400
3	8.367	99.250	7.407	0.400
4	8.367	99.270	7.433	0.390
5	8.367	99.260	7.423	0.400
6	8.367	99.270	7.413	0.390
7	8.367	99.260	7.403	0.400
8	8.367	99.260	7.403	0.400
9	8.367	99.090	7.420	0.390
10	8.367	99.270	7.427	0.390
11	8.367	99.260	7.400	0.400
12	8.367	99.250	7.407	0.400
13	8.367	99.250	7.397	0.400
14	8.367	99.260	7.403	0.390
Average	**8.367**	**99.253**	**7.410**	**0.395**
Std Dev	**0.001**	**0.049**	**0.011**	**0.007**
%RSD	**0.013**	**0.049**	**0.153**	**1.647**

Table 1

Table 1 consolidates the recorded calculated data from the monomer and dimer peaks of the 14 injections from Figure 1 with the % RSD of retention time and percent relative area below the allowance from the USP monograph guidance.

Figure 2 shows the overlay of 15 injections of the USP mAb standard sample onto the TSKgel UP-SW3000 column with the addition of 15% IPA. These injections are performed after the column is subjected to 15 injections of the USP mAb standard sample with sodium phosphate buffer, pH 6.7, without IPA. The baseline of the first injection (as shown in blue) indicates that the column takes only one to two injections to be stabilized. After that all subsequent injections are overlaid perfectly.

Table 2 lists the calculated data from the monomer and dimer peaks of the 15 injections from Fig. 2 with the % RSD of retention time and percent relative area. As shown, the % RSD is below the allowance from the USP monograph guidance.

The retention times and peak areas from the injections without IPA added are very similar to the retention times and peak areas with IPA added (compare Table 1 to Table 2).

At 0.3 mL/min, the pressure of the column is slightly higher when IPA is added to the mobile phase compared to when the column is operated without IPA. However, the pressure is only at 22 MPa with the IPA added. It is still far below the allowance of the maximum pressure of 34 MPa of the column's rating. With this low operating pressure, the TSKgel UP-SW3000 column can be operated with both HPLC and UHPLC systems. As the chromatograms indicate, all runs are completed within 15 minutes.

Figure 3 is an overlay of injections with and without IPA added to the mobile phase. The overlay indicates the similarities of peak retention times, peak width and peak height of dimer, monomer, aggregate and fragment peaks between the two different conditions.

CONCLUSION

An appropriate percentage of organic solvent such as isopropyl alcohol (IPA) does not alter the diffusion of mAb molecules using a TSKgel UP-SW3000 column. As demonstrated, this column can be successfully operated with the addition of 15% IPA. Data indicates that the column's particle chemistry and packing are optimized so that with the addition of an appropriate amount of selected organic solvents, there is no alteration of peak retention time or poor peak resolution.

SEPARATION OF USP mAb STANDARD WITH 15% IPA ADDED

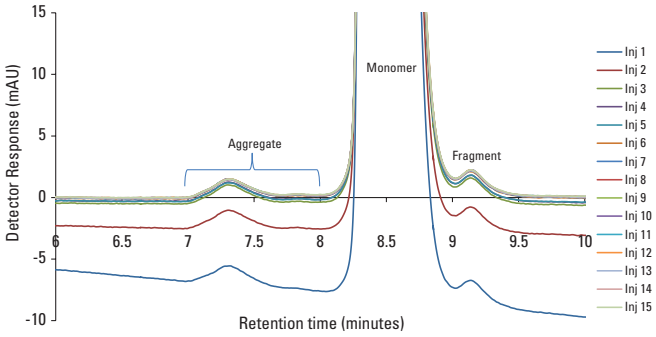

Figure 2

SEPARATION OF USP mAb STANDARD WITH AND WITHOUT 15% IPA ADDED

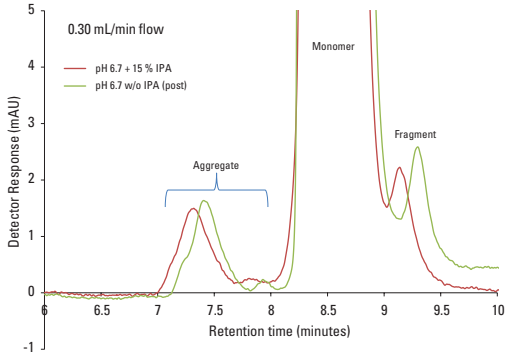

Figure 3

% RSD OF MONOMER AND DIMER PEAK OF 15 INJECTIONS: SEPARATION OF USP mAb STANDARD WITH 15% IPA ADDED

	MONOMER PEAK		DIMER PEAK	
INJECTION	Ret. time (min)	Area (mAU* min)	Ret. time (min)	Area (mAU* min)
1	8.340	97.110	7.417	0.470
2	8.340	98.280	7.410	0.460
3	8.340	98.420	7.410	0.470
4	8.340	98.400	7.407	0.490
5	8.340	98.440	7.417	0.470
6	8.340	97.940	7.413	0.500
7	8.337	98.010	7.420	0.470
8	8.337	98.030	7.437	0.470
9	8.337	98.110	7.407	0.470
10	8.337	98.110	7.423	0.470
11	8.337	98.120	7.413	0.460
12	8.337	98.220	7.417	0.470
13	8.337	98.130	7.420	0.480
14	8.337	98.220	7.413	0.460
15	8.367	98.260	7.413	0.390
Average	**8.338**	**98.120**	**7.416**	**0.471**
Std Dev	**0.002**	**0.317**	**0.008**	**0.012**
%RSD	**0.018**	**0.323**	**0.102**	**2.598**

Table 2