Superior Reproducibility of TSKgel® UP-SW3000 Columns

Introduction

Particle mechanical stability, and thus overall column stability, plays a major role in reproducibility in HPLC analysis. This is true in size exclusion chromatography (SEC) to a greater extent than any other mode. This is for the simple reason that the pore volume per unit column volume of SEC columns is maximized to provide optimal mass resolution, and the higher the pore volume, the more fragile the particle. Also contributing to column stability is the consistency in the availability of this pore volume, which is the sole separation mechanism in SEC. It is therefore critical that lot-to-lot reproducibility of the packing material be as tightly controlled as possible. The benefit of increased particle porosity must be balanced with the need to maintain the structural integrity of individual resin particles as well as the column as a whole.

Most variability in peak retention time using SEC is attributable to the factors mentioned above. Good control over the particle size distribution, pore size distribution, coating chemistry, and packing procedure ensures delivery of columns with minimal lot- to-lot differences as measured by peak percent relative standard deviation (%RSD). Retention time precision is extremely important in being able to accurately compare results. Instrument characteristics, such as such as consistency in flow rate and pressure, as well as column factors mentioned before, all play major roles in influencing retention time.

TSKgel UP-SW3000 columns are 2 µm SEC columns designed for the analysis of monoclonal antibodies and other biopharma products and can be used on both HPLC and UHPLC systems. The columns are packed with silica-based beads shielded with a hydrophilic diol-type bonded phase that prevents the silica surface from interacting with protein samples. TSKgel UP-SW3000 columns are packed in a tightly controlled manufacturing process that minimizes any potential variance in bonding chemistry of the diol groups. The robust column packing procedure minimizes any potential variance in column asymmetry and theoretical plate count.

TSKgel UP-SW3000 columns offer superior reproducibility injection-to-injection, from column-to-column within the same lot and from lot-to-lot. %RSD values for peak parameters including retention times, area peak height, peak asymmetry, and theoretical plate count demonstrate the exceptional reproducibility of these columns.

Experimental Conditions/Results

Column:	TSKgel UP-SW3000, 2 µm, 4.6 mm ID × 30 cm
Instrument:	Dionex UltiMate® 3000RS UHPLC System
Mobile phase:	100 mmol/L sodium phosphate buffer, pH 6.7, + 100mmol/L Na_2SO_4 + 0.05% NaN_3
Gradient:	Isocratic
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	5 µL
Samples:	QC protein standard test mixture: thyroglobulin, 640 kDa, 0.5 g/L γ-globulin, 155 kDa, 1 g/L ovalbumin, 47 kDa, 1 g/L ribonuclease A , 13,700 Da, 1.5 g/L p-aminobenzoic acid, 137 Da, 0.01 g/L

Results and Discussion

Figure 1 demonstrates the superior reproducibility of a TSKgel UP-SW3000 column for three consecutive injections when analyzing a QC protein standard mixture. As shown in *Table 1*, the column yielded very low %RSD injection-to-injection for retention time and theoretical plate count for all of the peaks. The calculation of the peak area percentages and the reproducibility of percentage peak area is very important since it doesn't require any prior calibration and does not depend upon the amount of sample injected within the limits of the detector. No response factors are used in this calculation. Reproducibility of the peak area and height also yielded very low %RSD (data not shown). Low %RSD in the consecutive injections provide confidence about the method and the column performance.

Figure 1. TSKgel UP-SW3000 Column Injection-to-Injection Reproducibility

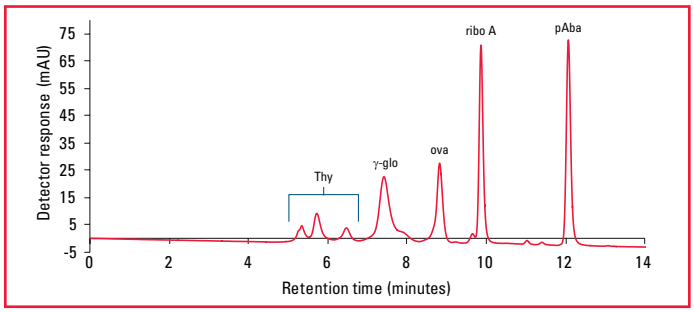


Table 1. TSKgel UP-SW3000 Column Injection-to-Injection Reproducibility

	pAba		Thyroglobulin		γ-globulin		Ovalbumin		Ribonuclease-A	
Injection	**RT (min)**	**N**	**RT (min)**	**N**	**RT (min)**	**N**	**RT (min)**	**N**	**RT (min)**	**N**
1	12.058	50871	5.730	4015	7.415	3785	8.850	16045	9.850	39461
2	12.060	50774	5.730	3995	7.417	3774	8.850	15970	9.852	39351
3	12.067	50752	5.735	3998	7.420	3764	8.855	15976	9.857	39413
Avg	12.062	50799	5.732	4003	7.417	3774	8.852	15997	9.853	39408
SD	0.005	63.31666	0.003	10.785793	0.003	10.50397	0.003	41.677332	0.004	55.14828
%RSD	0.039	0.124642	0.050	0.269465	0.034	0.2798	0.033	0.2605	0.037	0.1399

Figure 2 demonstrates the superior column-to-column reproducibility when analyzing a QC protein standard mixture of three consecutive injections for each individual TSKgel UP-SW3000 column.

Figure 2. TSKgel UP-SW3000 Column-to-Column Reproducibility

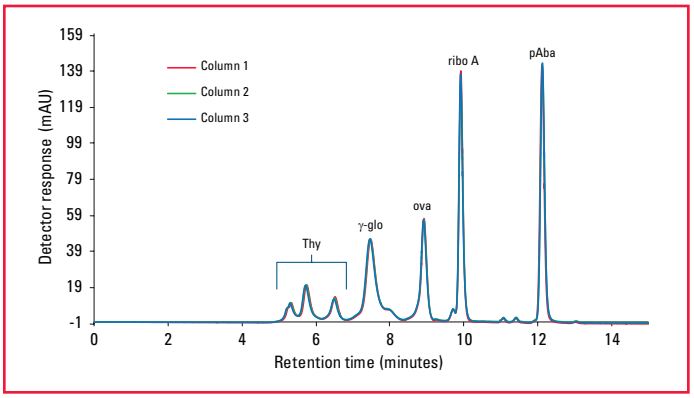
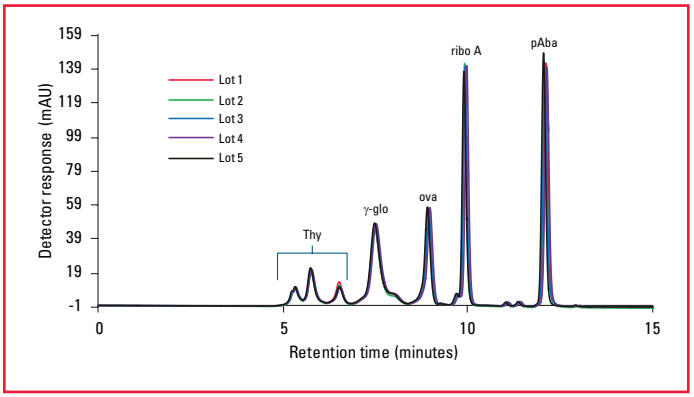


Figure 3 demonstrates the superior lot-to-lot reproducibility when analyzing a protein standard mixture of three consecutive injections for each individual TSKgel UP-SW3000 column. Though it is very close to the exclusion volume, the large protein molecule peak, thyroglobulin, is also reproducibly resolved.

Figure 3. TSKgel UP-SW3000 Lot-to-Lot Reproducibility



Conclusions

TSKgel UP-SW3000, 2 µm columns are packed with silica-based beads shielded with a hydrophilic diol-type bonded phase that prevents the silica surface from interacting with protein samples. The columns offer excellent reproducibility injection-to-injection, from column-to-column and from lot-to-lot. TSKgel UP-SW3000 columns yield very low %RSD values for peak parameters such as retention times, area peak height, peak asymmetry, and theoretical plates. Though they are very close to the exclusion volume, the large protein molecule peaks, thyroglobulin, are also reproducibly resolved.

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