Evaluating the Column Lifetime of TSKgel SuperSW mAb HTP

TSKgel
APPLICATION NOTE

Introduction

Column lifetime is one of the most critical parameters of column selection. Although a variety of other chromatographic conditions and other sources, in addition to sample composition, play a critical role in how long a column will deliver acceptable performance, the use of lifetime studies performed in a controlled environment allow column manufacturers to better inform chromatographers of what they can expect from a column under relatively general conditions. This "benchmark" information allows chromatographers to better assess column performance prior to committing to a purchase.

This Application Note demonstrates the excellent performance stability of the TSKgel SuperSW mAb HTP column when separating protein standards under chromatographic conditions as suggested by the Inspection Data Sheet (IDS). The TSKgel SuperSW mAb HTP is a 4.6 mm ID × 15 cm size exclusion UHPLC-compatible column designed with pore-controlled technology specialized for monoclonal antibody analysis. Its short length allows for fast run times, while its performance stability after as many as 1,000 injections shows its excellent column lifetime.

Materials and Methods

Column:	TSKgel SuperSW mAb HTP, 4 µm, 4.6 mm ID × 15 cm
Instrument:	Agilent 1200 HPLC system run by Chemstation® (ver B.04.02)
Mobile phase:	100 mmol/L phosphate /100 mmol/L sodium sulfate, pH 6.7 + 0.05% NaN_3
Gradient:	isocratic
Flow rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	5 µL (21.1 µg total protein load)
Samples:	protein standard: thyroglobulin, 0.58 mg/mL, γ-globulin, 1.02 mg/mL, ovalbumin, 1.08 mg/mL, ribonuclease, 1.53 mg/mL, PABA, 0.01 mg/mL

Results and Discussion

The TSKgel SuperSW mAb HTP column demonstrates highly reproducible performance over a significant number of injections of protein standard, as shown in *Figure 1*. The column yielded less than a 10% loss in column efficiency over 1,000 consecutive injections. Additionally, the packing integrity of the column is extremely high since even in the reverse flow orientation a nearly identical chromatographic trace to that of normal flow orientation was obtained (data not shown). During this study a guard column was not used, the analytical column was not cleaned/back flushed, and the mobile phase and sample were not filtered, to give additional stress to the analytical column. Implementation of such protective measures can be expected to yield extended column lifetime.

Figure 1. Performance Stability of the TSKgel SuperSW mAb HTP Column over 1,000 Consecutive Injections of Protein Standard

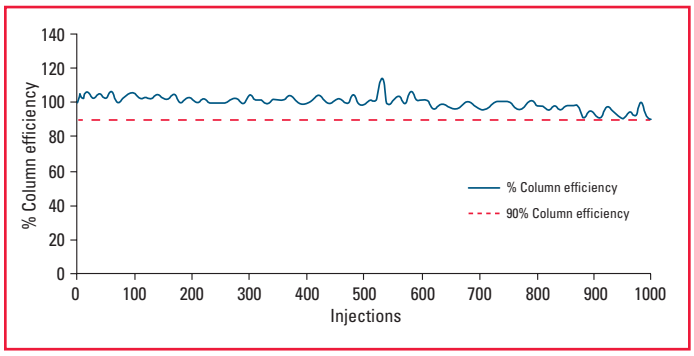

Table 1 gives the averaged peak parameters of 1,000 injections of para-aminobenzoic acid (PABA) separated on the TSKgel SuperSW mAb HTP column for this study. All peak parameters illustrate an extremely high level of reproducibility throughout the study. These results indicate the highly stable performance of the TSKgel SuperSW mAb HTP column, despite exposure to approximately 21.1 mg of total protein during the extent of this study.

Table 1. Averaged Peak Parameters for γ-Globulin over 1,000 Consecutive Injections of Protein Standard Separated on the TSKgel SuperSW mAb HTP Column

	γ-Globulin						
	Retention time	**Area**	**Height**	**As**	**Width**	**N**	**Rs**
Average	4.10	210.76	14.56	1.15	0.22	1,850.67	1.73
Std. Dev	0.00	0.55	0.04	0.00	0.00	10.84	0.00
%RSD	0.05	0.26	0.30	0.00	0.34	0.59	0.27

Conclusions

The TSKgel SuperSW mAb HTP column demonstrates highly reproducible performance over 1,000 consecutive injections of protein standard, yielding less than a 10% loss in column efficiency. The importance of lifetime as a criteria for column selection cannot be overstated. The TSKgel SuperSW mAb HTP column is extremely stable, even under less than ideal conditions, including the lack of a guard column. This stability, coupled with its ability to deliver high resolution at fast run times, makes the TSKgel SuperSW mAb HTP an excellent choice for the analysis of monoclonal antibodies.

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