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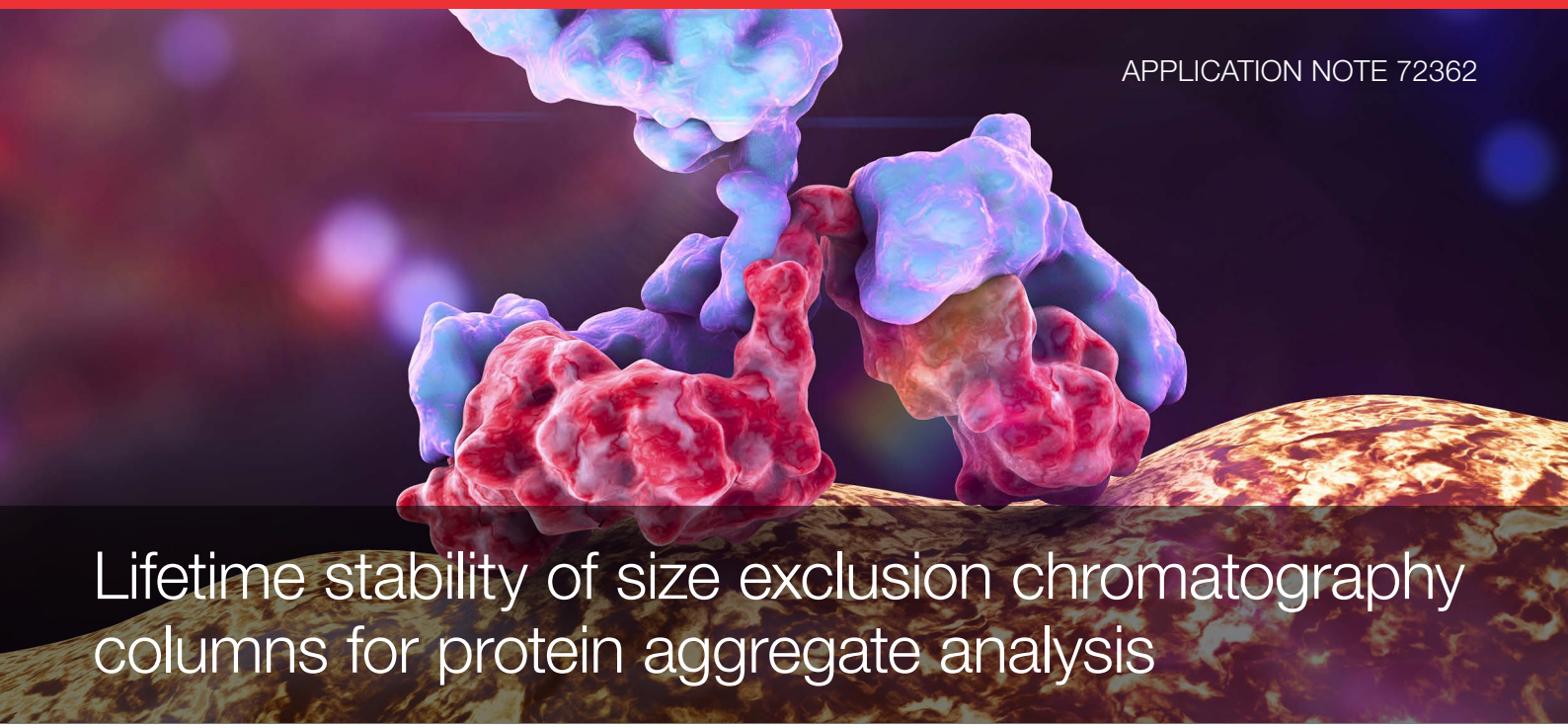
APPLICATION NOTE 72362



Lifetime stability of size exclusion chromatography columns for protein aggregate analysis

Authors

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Keywords

MAbPac SEC-1, Monoclonal Antibody, mAb, Protein Aggregation Analysis, Size Exclusion Chromatography, Biotherapeutics, Critical Quality Attributes, Vanquish Flex UHPLC

Application benefits

- Columns for analysis of mAb aggregates with exceptionally long lifetime
- A biocompatible UHPLC system capable of operating with high salt content and potentially corrosive mobile phases
- A UHPLC solution for mAb aggregate analysis that operates continuously for several weeks

Goal

To demonstrate the long-term stability of the Thermo Scientific™ MAbPac™ SEC-1 column for monoclonal antibody aggregate analysis using the Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system.

Introduction

Monoclonal antibodies (mAbs) are currently the dominant class of protein therapeutics in the biopharmaceutical industry due to their high specificity to target antigens, long serum half-life in humans, and capabilities for use in the treatment of a wide range of ailments such as inflammatory diseases and cancer. During product expression and purification from host

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cells, formulation, and storage, mAbs may undergo various degradation processes, which may alter the safety, efficacy, and quality profile of the drug product. Consequently, a number of critical quality attributes (CQAs) must be monitored throughout drug development and production to ensure biotherapeutic substances are suitable for clinical use.

Aggregation is a common degradation process for therapeutic proteins that can result from partial unfolding or other types of conformational changes in protein structure to form dimers, trimers, and other higher order structures. Protein aggregation is considered a CQA as aggregates may reduce product efficiency by lowering the effective concentration of the product and have been found to result in immunogenic effects in patients. Hence, it is a regulatory requirement to monitor the aggregation profile of therapeutic proteins.

Size exclusion chromatography (SEC) is the most commonly applied method for protein aggregate analysis. In SEC, sample molecules are passed through a column containing porous polymer or silica beads to facilitate separation of species based on their size. For protein therapeutics, a pore size of 300 Å is used, which allows smaller species to penetrate into the porous beads (e.g. fragments, monomers) while larger molecules (e.g. dimers, trimers) are more excluded from the pores and therefore elute more quickly from the column. Most frequently, SEC columns with widths of 7.8 mm are used for aggregate analysis in industry. However, use of low dispersion, biocompatible UHPLC systems and narrow internal diameter pre-column tubing has been shown to improve peak shape in chromatograms generated using columns of reduced widths.[1] Narrow columns have advantages such as small sample volume requirements and reduced mobile phase consumption.

Due to high costs associated with drug development and production and the potential for mAb-based biosimilars resulting from patent expiry for some of the top-selling mAb therapeutics, reliable, long-use consumables and equipment for CQA evaluation is needed. Suppliers of SEC columns have illustrated column stability lifetimes of approximately 550 injections (without a column guard) and up to 902 injections (with a column guard). In this study, a MAbPac SEC-1 column (4 × 300 mm, 5 µm, 300 Å) and a Vanquish Flex Quaternary UHPLC system were applied to assess the long-term stability of the column based on protein aggregation monitoring of the commercial drug product bevacizumab, a humanized monoclonal IgG1 antibody produced from a Chinese hamster ovary mammalian cell expression system. Use of pre-column tubing with internal diameter of 75 µm ensured that excellent peak shape was achieved using a SEC column with dimensions of 4 × 300 mm. However, as SEC columns with widths of 7.8 mm are most commonly utilized in the biopharmaceutical industry, the column lifetime stability of an MAbPac SEC-1 column (7.8 × 300 mm, 5 µm, 300 Å) was also evaluated.

Experimental

Recommended consumables

- Deionized water, 18.2 MΩ·cm resistivity
- Thermo Scientific MAbPac SEC-1, 5 µm, 300 Å, 4.0 × 300 mm (P/N 074696)
- Thermo Scientific MAbPac SEC-1, 5 µm, 300 Å, 7.8 × 300 mm (P/N 0088460)
- Fisher Scientific™ sodium phosphate dibasic anhydrous (P/N 010440481)
- Fisher Scientific™ sodium phosphate monobasic anhydrous (P/N 010751135)
- Fisher Scientific™ sodium chloride (P/N 011964051)
- Thermo Scientific™ Virtuoso™ Vial Identification System (P/N 060180-VT100)
- Virtuoso 9 mm Wide Opening SureStop Screw Thread Vial Convenience Kit (P/N 060180-VT405)

Sample preparation

Bevacizumab (25 mg/mL) was diluted 1:10 in 100 mM sodium phosphate, pH 6.8, 300 mM NaCl (for MAbPac SEC-1 4.0 × 300 mm column) or 50 mM sodium phosphate, pH 6.8, 300 mM NaCl (for MAbPac SEC-1 7.8 × 300 mm column). Diluted samples were aliquoted and stored at -20 °C. On each day of analysis, a fresh aliquot of diluted bevacizumab sample was removed from the freezer for analysis.

Buffer preparation

MAbPac SEC-1 4.0 × 300 mm column: 100 mM sodium phosphate, pH 6.8 in 300 mM NaCl was used as mobile phase. Buffers were filtered through a 0.2 µm filter membrane before use.

MAbPac SEC-1 7.8 × 300 mm column: 50 mM sodium phosphate, pH 6.8 in 300 mM NaCl was used as mobile phase. Buffers were filtered through a 0.2 µm filter membrane before use.

Separation conditions

Instrumentation

Vanquish Flex UHPLC system, including:

- Quaternary Pump (P/N VF-P20-A)
- Column Compartment H (P/N VH-C10-A)
- Split Sampler FT (P/N VF-A10-A) with 25 µL Sample Loop
- Diode Array Detector HL (P/N VH-D10-A) with LightPipe 10 mm Standard Flow Cell (P/N 6083.0100)
- System Base Vanquish Flex (P/N VF-S01-A)

Flow rate

- MAbPac SEC-1 4.0 × 300 mm column: 0.250 mL/min
- MAbPac SEC-1 7.8 × 300 mm column: 0.8 mL/min

Column temperature

- 30 °C

Injection details

- MAbPac SEC-1 4.0 × 300 mm column: 2.0 µL of 2.5 µg/µL bevacizumab, diluted in mobile phase.
- MAbPac SEC-1 7.8 × 300 mm column: 0.5 µL of 2.5 µg/µL bevacizumab, diluted in mobile phase.

Detector settings

- MAbPac SEC-1 4.0 × 300 mm column: Data was collected at 280 nm and 214 nm on a DAD detector.
- MAbPac SEC-1 7.8 × 300 mm column: Data was collected at 280 nm on a DAD detector.

Data processing

The Thermo Scientific™ Chromeleon™ 7.2 SR4 Chromatography Data System was used for data acquisition and analysis.

Results and discussion

To evaluate the stability of a MAbPac SEC-1 analytical column for aggregate analysis of therapeutic monoclonal antibodies, repeated injections of bevacizumab drug substance were performed using a Vanquish Flex UHPLC system with Chromeleon software version 7.2. The MAbPac SEC-1 column is a silica-based SEC column covalently modified with a proprietary diol hydrophilic layer to prevent secondary interactions of analytes to the stationary phase. A new and unused column was employed for the study and was conditioned by performing ten injections of mAb until a constant peak area and peak height were observed as per the manufacturer's guidelines. Each day of analysis and for each new buffer preparation, blank injections were performed prior to mAb determination to ensure column equilibration.

The industry-standard SEC column dimension for protein aggregate analysis is 7.8 mm internal diameter (i.d.) using flow rates of 1 mL/min. It has previously been shown that pre-column dispersion often associated with reduced i.d. SEC columns (4 mm) and low flow rates (<0.3 mL/min) may be overcome using a low-dispersion, biocompatible UHPLC, such as the Vanquish Flex UHPLC, with smaller injection volumes and fitted with narrow i.d. pre-column transfer tubing (75 µm i.d. tubing).[1] Using 75 µm pre-column transfer tubing, a 4 mm i.d. MAbPac SEC-1 column was evaluated. Over the course of the study excellent resolution of aggregates and fragment peaks from the main mAb monomer peak was observed as shown in Figure 1.

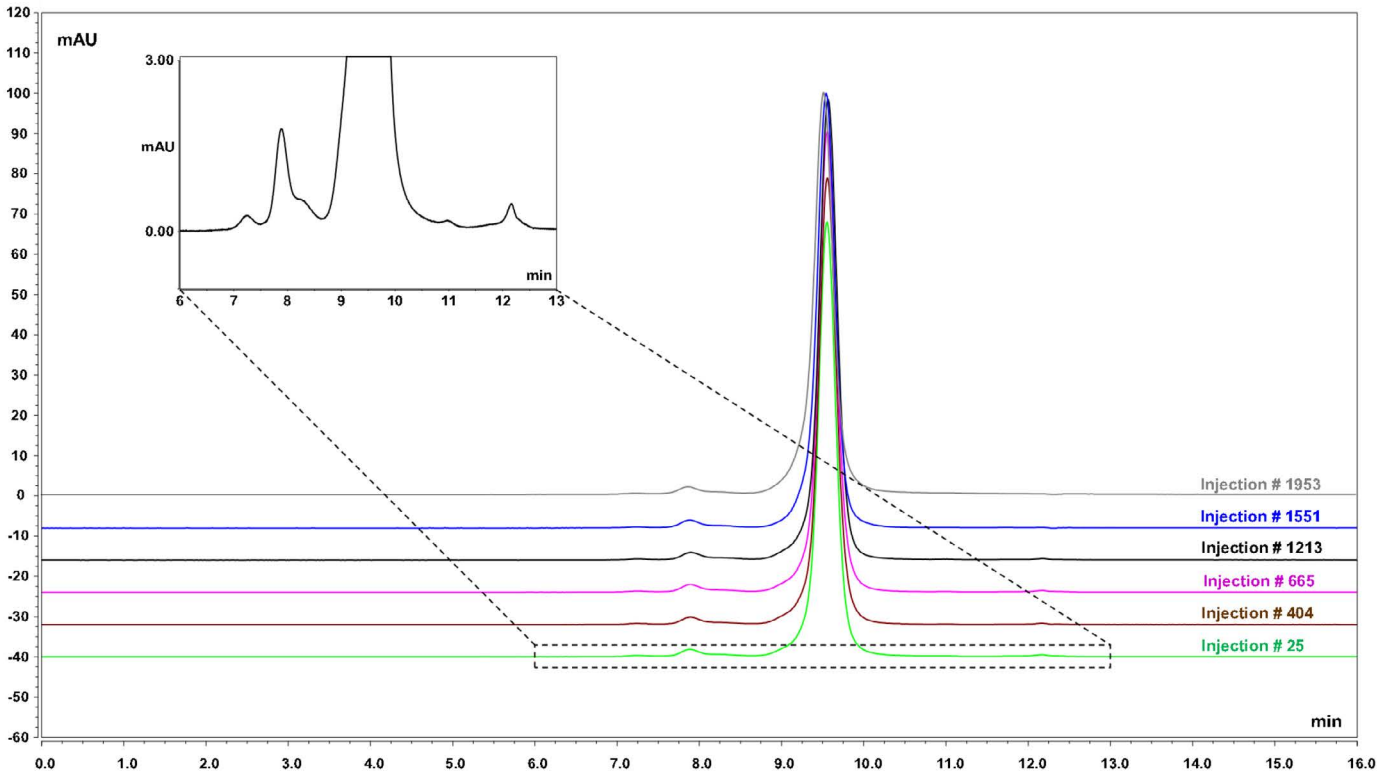

Figure 1. Aggregate analysis of bevacizumab using a MAbPac SEC-1 column.

As Table 1 shows, consistent retention times were observed for the monomer peak over the course of more than 1950 injections, with a retention time difference of just 0.043 minutes between injection numbers 25 and 1953; this illustrates the absence of chemical or electrostatic interactions with the column stationary phase following extensive use. Similarly, excellent peak symmetry was detected throughout the column lifetime stability study (asymmetry range 0.88 to 0.93), further demonstrating a lack of secondary interactions with the column packing material and hardware across the lifetime of the column. Column efficiency, based on European Pharmacopoeia theoretical plate count, was found to be greater than 85% of initial efficiency following 1953 injections on the column (equivalent to 1866 injections of mAb). After 1866 injections of bevacizumab, a loss in column performance was observed as shown in Figure 2. The loss in column performance (<85% theoretical plate count) did not appear to result in a change to the aggregation profile of bevacizumab.

Table 1. Monomer peak information acquired following aggregate analysis of bevacizumab using a MAbPac SEC-1 analytical column.

On Column Injection #	Retention Time (min)	Monomer Relative Peak Area (%)	Monomer Peak Width @ 50% Height (min)	Asymmetry (EP)	Theoretical Plates (EP)
25	9.554	96.96	0.237	0.92	9032
404	9.558	97.21	0.240	0.92	8797
665	9.558	96.96	0.241	0.93	8736
1213	9.567	97.27	0.243	0.89	8604
1551	9.544	96.99	0.244	0.88	8460
1953	9.524	96.30	0.255	0.89	7737

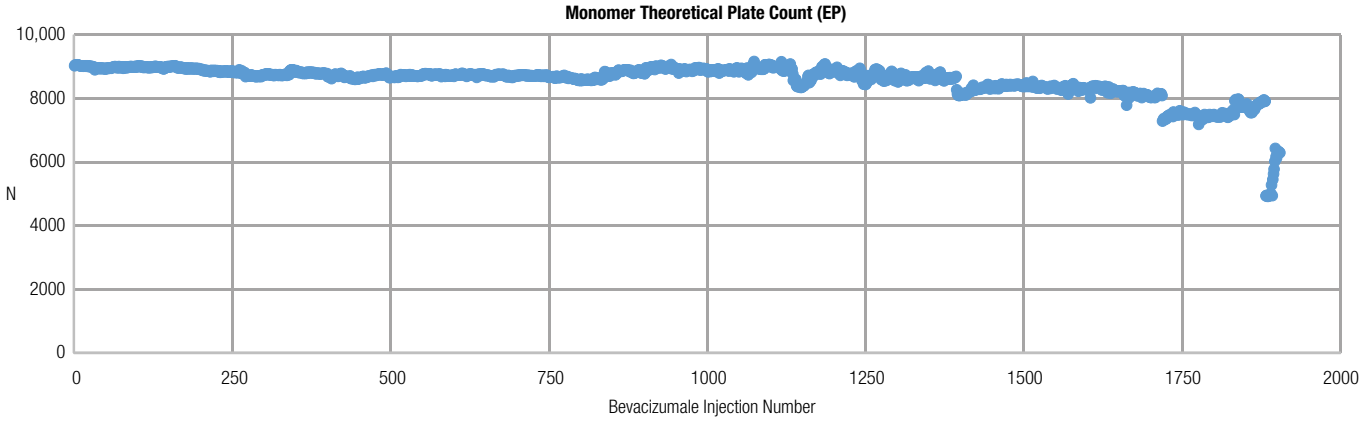

Figure 2. Theoretical plate count for the mAb monomer in each injection using the MAbPac SEC-1, 4.0 × 300 mm column. Greater than 85% of initial column efficiency was preserved following 1866 injections of bevacizumab (corresponding to 1953 on column injections). A loss of column performance was observed after 1866 injections of bevacizumab.

Because SEC columns with internal diameters of 7.8 mm are most often applied for aggregate analysis in the biopharmaceutical industry, the column lifetime stability of a MAbPac SEC-1, 5 µm, 300 Å, 7.8 × 300 mm column was also evaluated. In this case, a new and unused column was also employed for the study and was conditioned prior to use following recommended guidelines. During analysis the column was injected ten times with bevacizumab, followed by two injections of a protein check standard. This injection cycle was repeated until column degradation was observed.

Table 2 shows highlighted chromatography data for bevacizumab analyzed using a MAbPac SEC-1, 7.8 × 300 mm column. The final line on the table displays information for the final injection that was above the column efficiency specification for monoclonal antibodies, namely injection number 1292 (>6300 theoretical plates (EP) for the monomer peak). Like the MAbPac SEC-1, 4.0 × 300 mm column, the MAbPac SEC-1, 7.8 × 300 mm column displayed relatively consistent retention time and peak symmetry and excellent column efficiency over the lifetime of the column (Figure 3). Despite exhibiting reduced column lifetime stability in comparison to the MAbPac SEC-1, 4.0 × 300 mm column, the MAbPac SEC-1, 7.8 × 300 mm column showed extended column lifetime stability when compared to the lifetime stability previously demonstrated for alternative SEC columns. Greater than 55% of initial column efficiency was preserved following 1077 injections of bevacizumab (corresponding to 1292 on column injections). A loss of column performance was observed after 1077 injections of bevacizumab. The efficiency drops observed between 300 and 500 injections were not attributed to column degradation. It was observed that sample aging negatively affected peak width. When sample was replaced, high efficiency values were restored. The root cause for this behaviour could not be found. When the column degraded, the replacement of aged sample with fresh did not have any beneficial effect on peak width.

Table 2. Monomer peak information acquired following aggregate analysis of bevacizumab using a MAbPac SEC-1, 7.8 × 300 mm analytical column.

On Column Injection #	Retention Time (min)	Monomer Relative Peak Area (%)	Monomer Peak Width @ 50% Height (min)	Asymmetry (EP)	Theoretical Plates (EP)
30	11.192	97.56	0.247	1.08	11349
236	11.183	97.30	0.252	1.11	10870
555	11.192	97.74	0.252	1.11	10918
769	11.200	97.95	0.253	1.06	10876
975	11.225	97.92	0.262	0.93	10158
1292	11.225	97.40	0.333	1.08	6301

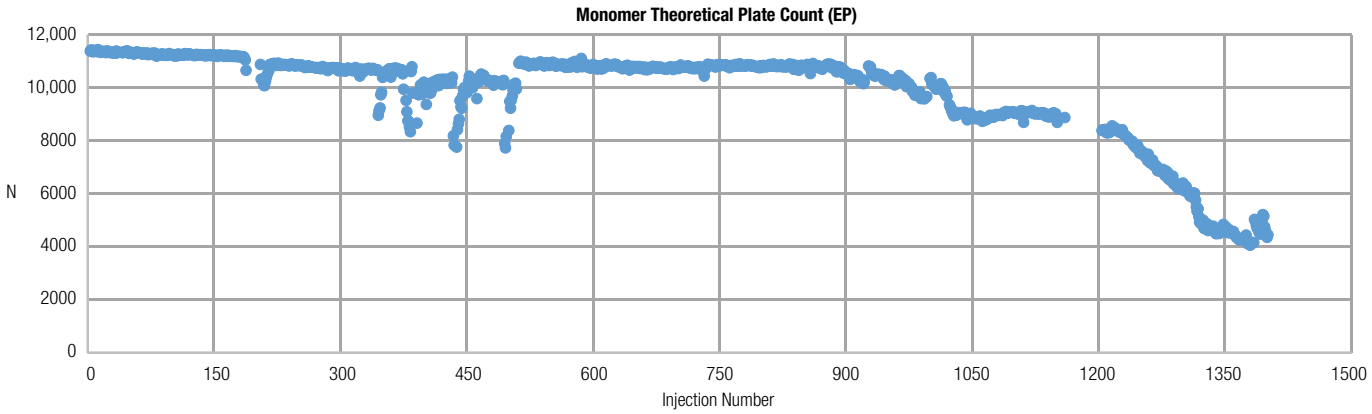

Figure 3. Theoretical plate count for the mAb monomer in each injection using the MAbPac SEC-1, 7.8 × 300 mm column.

Conclusion

- The MAbPac SEC-1 column coupled to a Vanquish Flex Quaternary UHPLC system is a robust platform for aggregate analysis of mAbs.
- Consistent retention time, excellent peak symmetry, and exceptional column efficiency were observed over the course of 1953 on-column injections for the MAbPac SEC-1, 4.0 × 300 mm column and for 1292 on-column injections on the MAbPac SEC-1, 7.8 × 300 mm column.
- To our knowledge, the MAbPac SEC-1 column lifetime stability determined far exceeds other commercially available SEC columns, without requirements for guard columns.

Reference

1. Thermo Scientific Application Note AN21602: The importance of correct UHPLC instrument set-up for protein aggregate analysis by size-exclusion chromatography, 2016.

Find out more at **thermofisher.com/proteinaggregates**

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