

Characterization of IgG: Repeatability and Long-Term Performance of the Agilent MAB Column

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Abstract

In this application brief, the long-term stability and repeatability of the Agilent MAB column during the GPC/SEC analysis of IgG is described.

Introduction

In recent years, monoclonal antibody-mediated (mAb-mediated) therapy has revolutionized the treatment of cancer and several other human disorders. MABs have been found to be less immunogenic, more effective, and produce fewer side effects than conventional treatments.¹

Size exclusion chromatography is increasingly applied for biopolymer characterization in its native and degraded form. MAB 3 μm columns were specifically developed for GPC/SEC analysis of antibodies and are available in analytical (8 \times 300 mm) or micro dimensions (4.6 \times 250 mm).

GPC/SEC is typically used for aggregate and fragment analysis of antibodies. The long-term stability and reproducibility of this analysis is crucial to producing reliable results.

Experimental

See Table 1.

Results and discussion

Repeatability was tested in combination with the recovery rate by injecting the same native IgG sample 250 times into the system, using the analytical MAB 3 μm column for separation. Figure 1 shows an overlay of every 10th injection of IgG.

The injected sample was fully recovered from the column and the overlaid chromatograms of all 250 injections did not exhibit any significant inconsistencies.

Table 1. Instrument and sample conditions.

	Conditions
Pump	Isocratic pump Flow rate: 1.00 mL/min Mobile phase: aqueous 34 mM phosphate buffer, pH 6.4, 0.3 M NaCl
Injection System	Autosampler Injection volume: 20 μL (IgG concentration: 1.5 g/L)
Columns	MAB 3 μm , 8 \times 300 mm (p/n MAA083003MC)
Detectors	Variable wavelength UV-Vis detector (VWD) at $\lambda = 280 \text{ nm}$ Refractive index (RI) detector Multi-angle light scattering detector (MALLS) at $\lambda = 660 \text{ nm}$
Software	Agilent WinGPC

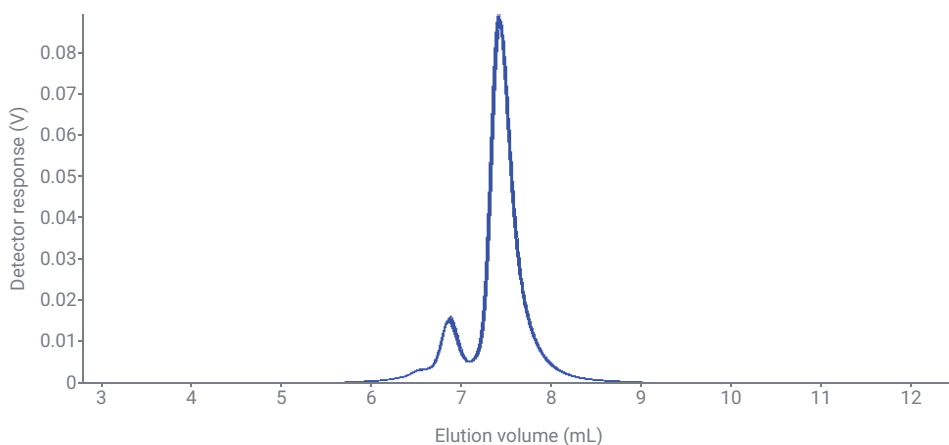


Figure 1. Overlay of every 10th injection of IgG (UV at 280 nm trace) with an Agilent MAB 3 μm column.

Conclusion

The Agilent MAB column showed high long-term stability and reproducibility during repeatability measurements of IgG, which was demonstrated with an overlay of multiple injections.

Reference

1. Klein, C. Monoclonal Antibodies, MDPI books, **2018**.

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