Rapid IgM Quantification in Cell Culture Production and Purification Process Monitoring Using the Agilent Bio-Monolith QA Column

Application Note

Biopharmaceutical

Abstract

A number of IgM monoclonal antibodies are currently in development for the treatment of autoimmune disease, infectious disease, and cancer. Growing interest in these molecules has created a need for an accurate, rapid, simple analytical method to measure IgM levels in cell culture supernatants, and to document the distribution of IgM and protein contaminants in chromatography fractions. High-performance protein A columns are used for this application with IgG monoclonals, but because IgMs are easily denatured by the harsh conditions required for elution of most affinity ligands, other options must be explored. IgM monoclonal antibodies often exhibit strong retention on either cation exchangers, anion exchangers, or both, making ion exchange chromatography a strong candidate for this application. The Agilent Bio-Monolith QA column (strong anion exchange) can be used to monitor the IgM purification process and to quantify IgM concentrations from cell culture supernatants.

Agilent Technologies

Introduction

High-velocity separations using the Agilent Bio-Monolith HPLC columns demonstrate the utility of monolithic assays for monitoring product and contaminant distribution in chromatography fractions.

Data documenting that the IgM purification process is operating within specifications can be obtained much more rapidly using the Agilent Bio-Monolith HPLC columns than by PAGE polyacrylamide gel electrophoresis or immunological assays. Such data may also be valuable for purification process characterization during early development, and later for process validation (Figure 1). The sensitivity, linearity, accuracy, and speed of the separation are also well suited for IgM quantitation of cell culture supernatants (Figure 2).

Experimental

Conditions

Column	Agilent Bio-Monolith QA Part number 5069-3635 5.2 mm id × 4.95 mm
Mobile phase A	10 mM sodium phosphate, pH 7.0
Mobile phase B	250 mM sodium phosphate, pH 7.0
Gradient	Linear gradient from A to B within 10 min (20 mL)
Detection	UV at 280 nm
Flow rate	2 mL/min

Agilent Bio-Monolith HPLC column (left), Bio-Monolith column housing and monolith disc (right).

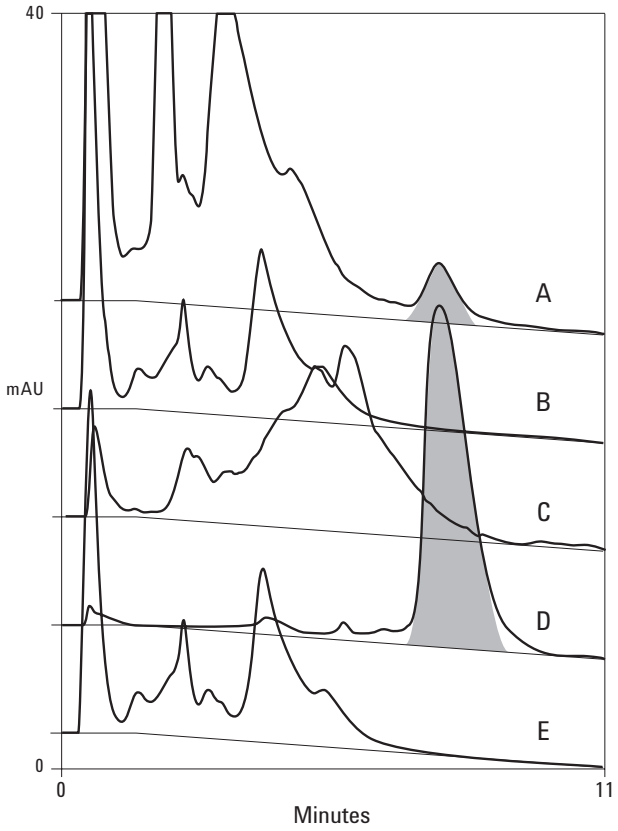

Figure 1. A series of hydroxyapatite chromatography fractions (A through E) that were collected and analyzed using the Agilent Bio-Monolith QA analytical column.

Fraction A:	Cell culture supernatant
Fraction B:	Flow-through from sample load
Fraction C:	Pre-elution wash
Fraction D:	IgM elution peak
Fraction E:	Post-elution cleaning

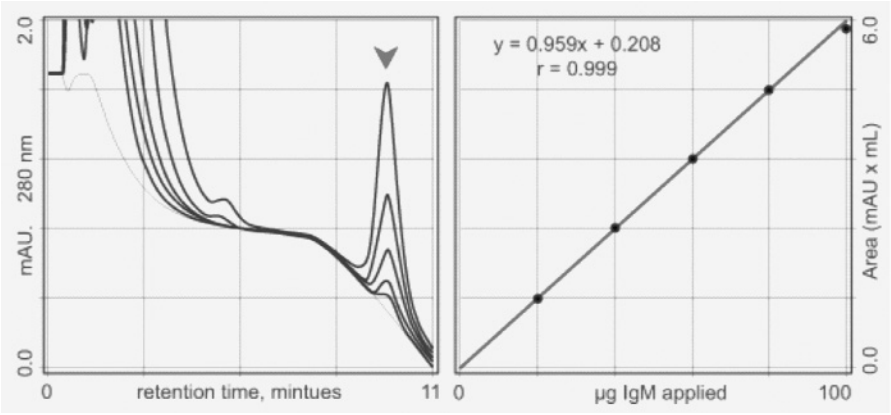

Figure 2. Results from a two-fold dilution series from cell culture supernatant; peaks were integrated and plotted.

Results and Discussion

Ceramic hydroxyapatite CHT type II (Bio-Rad Laboratories, Hercules, CA) was used to purify IgM. The Bio-Monolith QA column was used to monitor five fractions from this CHT type II purification step. The use of CHT type II for this purification step and the Agilent Bio-Monolith QA column for the purification monitoring decreases IgM exposure to the harsh buffer conditions used in Protein A preparative and analytical separations.

The results shown in Figure 1 confirm that all of the IgM was bound to the hydroxyapatite column (fraction B), that it remained bound during the wash (fraction C), and that it was fully eluted in the gradient (fractions D and E). Additional chromatograms from this series were used to determine which fractions to pool, based on the proportion of IgM to contaminating proteins. The buffer conditions used for this evaluation are suitable for anion and cation exchange screening of most IgMs. Results quickly confirmed that the IgM was present in the fractions where it was expected, and absent from other fractions. The ability to perform this assay with the same instrument and buffers used for process development allows developers to obtain immediate feedback, without the delay of sending samples for analysis by PAGE or ELISA. The Bio-Monolith QA column was also used to create an IgM calibration curve that could be used to determine IgM concentrations from cell culture supernatants. Figure 2 represents results from a two-fold dilution series of IgM from cell culture supernatant. The IgM concentration was estimated by ELISA to be about 100 µg/mL. IgM peak areas were integrated and plotted. The linear range of the assay easily covers the production ranges necessary to accommodate developmental and manufacturing cell cultures.

Conclusions

The Agilent Bio-Monolith QA column can be effectively used for monitoring product purification and contaminant distribution in chromatography fractions. The resulting analysis ensures that the purification process is operating within specifications and can be obtained much more rapidly than by PAGE or immunological assays. This data can be used throughout early development of the purification process and later for process monitoring and quality control purposes. The Agilent Bio-Monolith QA column provides rapid separations and accurate quantification of IgM antibodies from cell culture supernatants. This quantitative analysis can be used throughout the IgM production and purification processes.

Acknowledgment

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