Rapid Human Polyclonal IgG Quantification Using the Agilent Bio-Monolith Protein A HPLC Column

Application Note

Biopharmaceutical

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Abstract

Protein A is an immunoglobulin-Fc (IgG) receptor of *Staphylococcus aureus*, which has strong affinity for polyclonal and monoclonal IgG. Chromatography media with an immobilized Protein A receptor is commonly used for the preparative and process scale purifications of IgG. Due to its high selectivity and high recovery, IgG separations from complex mixtures can be completed in a single step. For the same reasons, high selectivity and recovery, Protein A chromatography can be exploited at the analytical scale. The Bio-Monolith Protein A HPLC column can be used for fast analytical separations and quantification of IgGs in complex mixtures or pure samples. Here we present a brief method that demonstrates the applicability of the Bio-Monolith Protein A column for the analytical separation and quantification of polyclonal IgG from human plasma. The method presented can also be used to monitor fermentation titers throughout large scale IgG production.

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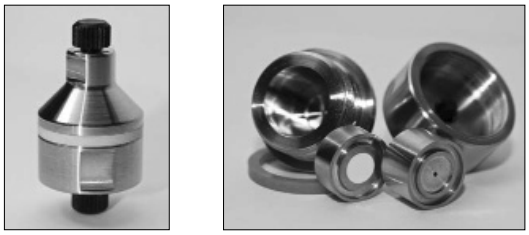
Introduction

The Bio-Monolith Protein A column combines high selectivity and high recovery with flow rate independent separations. The column contains a short monolith disc, which has continuous channels, no pores, and no void volumes. This monolithic structure eliminates diffusion and offers rapid mass transfer, especially for larger bio-molecules.

The rapid separation and quantification of IgG is critical to many decisions that must be made throughout fermentation and process scale purifications. Data documenting that IgG products conform to safety and purity specifications and that the IgG purification process is operating with the highest efficiency are critical. This data can be obtained much more rapidly using the Bio-Monolith Protein A column than by PAGE (polyacrylamide gel electrophoresis) or immunological assays. The sensitivity, linearity, accuracy, and speed of the separation is well suited for IgG quantification from complex samples (human plasma or cell cultures).



Agilent Bio-Monolith Protein A Column (left), Bio-Monolith column housing and monolith disc (right)

Experimental

Conditions

Column	Agilent Bio-Monolith Protein A Part number 5069-3639 5.2 mm id × 4.95 mm
Mobile phase A	PBS buffer, pH 7.4
Mobile phase B	0. 5 M acetic acid, pH 2.6
Gradient	Stepwise gradient: 100 % buffer A-100 % buffer B-100 % buffer A (0.5 min each step)
Detection	UV at 280 nm
Flow rate	1 mL/min
HPLC system	A high-pressure gradient HPLC system, Agilent 1200

Results and Discussion

The Bio-Monolith Protein A column was used to separate polyclonal IgG from human serum. Two flow rates were applied to demonstrate how separations using the Bio-Monolith columns are independent of flow rate. Figure 1 demonstrates how the IgG elution profile is maintained despite a change in the flow rate from 1 mL/min and 2 mL/min, which correspond to 10 column volumes/min and 20 column volumes/min, respectively. The presence and the concentration of IgG in the sample can be reliably obtained within minutes.

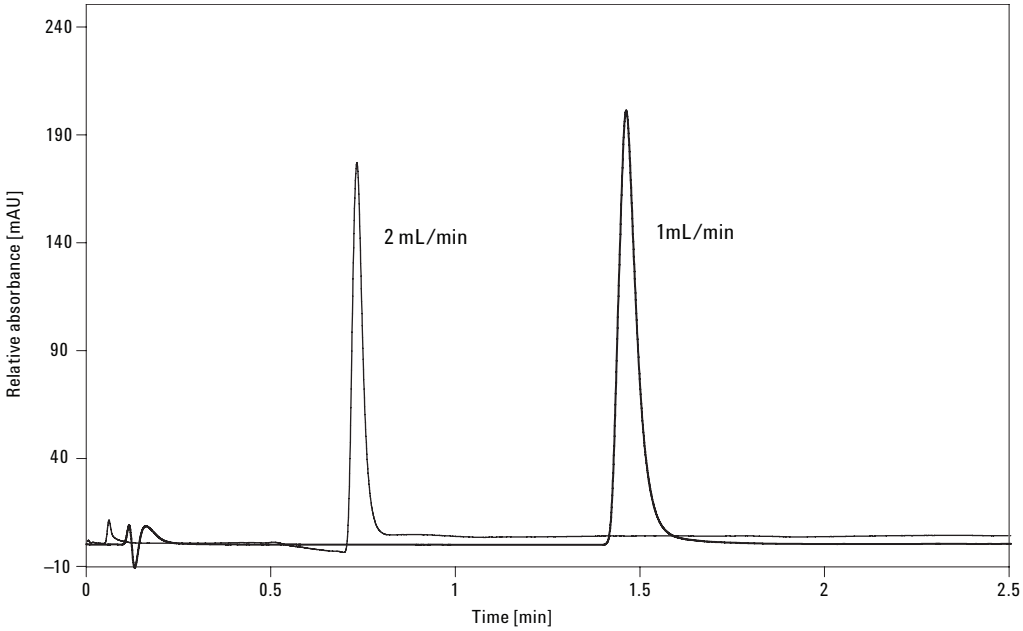


Figure 1. The independence of the IgG elution profile from the Bio-Monolith Protein A column from the applied flow rate.

Selectivity and recovery are critical for both analytical and process scale IgG separations. The Bio-Monolith Protein A column selectively captures IgG from complex mixtures. Figure 2 shows the separation of IgG from a complex sample, human plasma, using the Bio-Monolith Protein A column. Human plasma was diluted 10 times with the binding buffer (PBS buffer, pH 7.4) and 10 µL of the sample was injected onto the Protein A column at the flow rate of 1 mL/min. A step gradient was applied, 100 percent of buffer A switching to 100 percent buffer B, eluting the IgG in a single peak. Figure 3 represents the SDS PAGE analysis of the whole human plasma fractions from the separation in Figure 2. IgG is present in the whole serum prior to the separation (lane 1), in a purified IgG standard (lane 2), and in the elution fraction or peak 2 in Figure 2 (lane 4). The flow-through fraction, peak 1 in Figure 2 (lane 3) contains no IgG1 or IgG2, only IgG3 remains as it does not bind to protein A. The recovery (capture) of IgG from human plasma was determined by spiking known concentrations of IgG, creating a calibration curve. The concentration of IgG in the plasma sample was determined and compared to the published data. IgG bound to the column, while all other plasma proteins remained in the flow through. The recovery was determined to be 95 to 100 percent.

Determining the concentration of IgG in cell culture supernatants is extremely important. In order to achieve maximum IgG yields (high titers) from cell-based production, a rapid and reliable analytical method is required. The Bio-Monolith Protein A column was used to create a six-point calibration curve that could be used to determine the IgG concentration in a complex mixture. Figure 4 illustrates the results from a two-fold dilution series of human IgG concentrate using an initial IgG concentration of 2 mg/mL; IgG

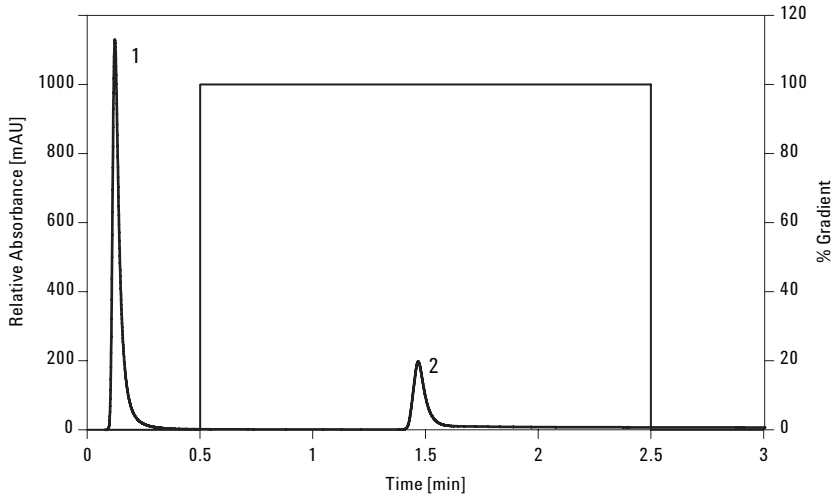


Figure 2. The selectivity of the Bio-Monolith Protein A column for the IgG from human plasma. IgG binds to protein A, a 100% buffer B step gradient is applied, and IgG elutes at 1.5 minutes.

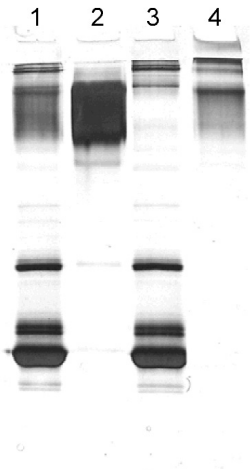


Figure 3. SDS PAGE analysis of fractions from the separation in Figure 2.

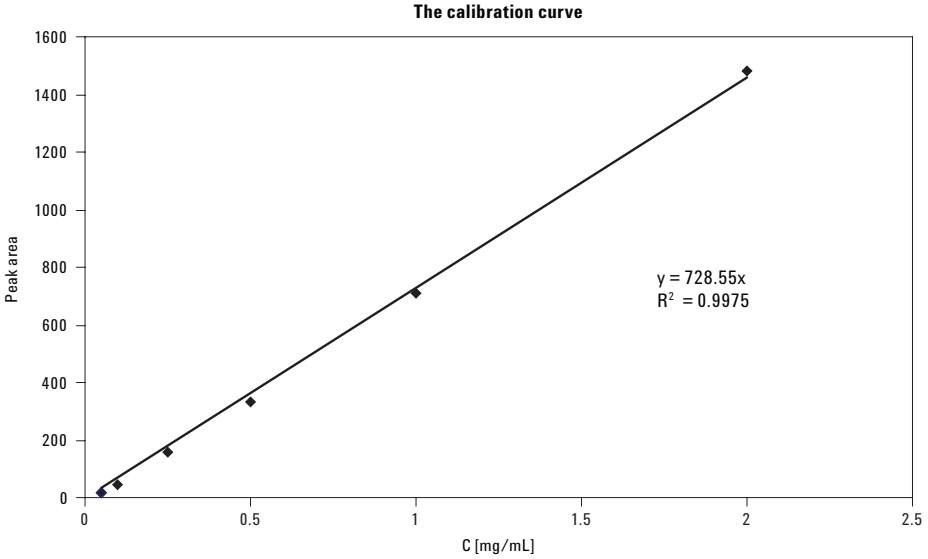


Figure 4. Results from a two-fold dilution series of human IgG concentrate.

peak areas were then integrated and plotted. The linear range of the assay easily covers the production ranges necessary to accommodate IgG development and manufacturing cell cultures.

Conclusions

The Bio-Monolith Protein A column can effectively be used to monitor IgG purification and verify product purity. 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. The Bio-Monolith Protein A separates IgG in 1 to 2 minutes, while offering high recovery (95 to100 percent). The sensitivity, linearity, accuracy, and speed of the separation are also well suited for IgG quantitation. This method can easily be used for the analytical separation and quantification of a variety of complex samples.

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