Reducing Cycle Time for Quantification of Human IgG Using the Agilent Bio-Monolith Protein A HPLC Column

Alternating Column Regeneration Using an Agilent 1200 Infinity Quick-Change Bio-inert 2-position/10-port Valve and the 1290 Infinity Flexible Cube

Application Note

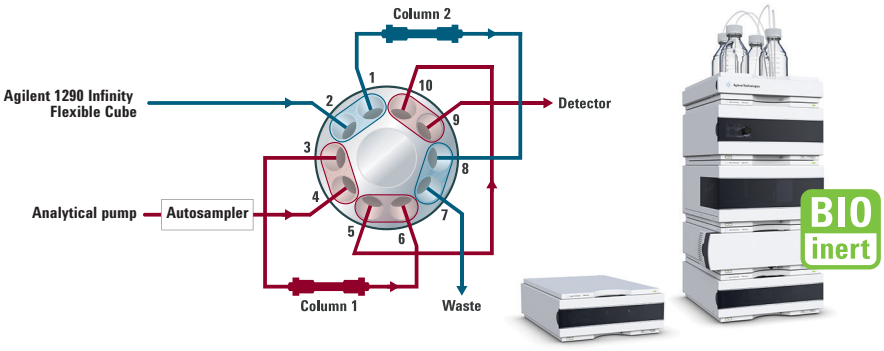
Biotherapeutics & Biosimilars

Authors

Sonja Schneider and Bettina Schuhn
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

This Application Note describes the quantification of human IgG with the Agilent Bio-Monolith Protein A HPLC Column using the Agilent 1260 Infinity Bio-inert Quaternary LC. Alternating column regeneration using the Agilent 1290 Infinity Flexible Cube in combination with an Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve reduced the total cycle time by approximately 20 %. The employment of a complete bio-inert flow path ensures a high reproducibility as metal leaching and corrosion, caused by high salt/low pH buffers, is avoided. With this setup, good intra- and inter-column precisions were achieved for the IgG quantification procedure.



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Introduction

Protein A is an immunoglobulin-Fc (IgG) receptor found in the cell wall of *Staphylococcus aureus.* It has strong affinity for polyclonal and monoclonal IgGs like human IgG 1, IgG 2, and IgG 4, in addition to IgGs from some other species such as rabbits and some mouse IgGs. Immobilized Protein A is commonly used for preparative and process scale purifications of IgG. At the analytical scale, the Agilent Bio-Monolith Protein A HPLC Column can be used for fast quantification of IgGs in complex mixtures or pure samples[1,2].

To reduce the total cycle time, two Agilent Bio-Monolith Protein A HPLC columns were installed in the Agilent 1290 Infinity Flexible Cube. The 1290 Infinity Flexible Cube houses a low-pressure bio-inert piston pump (up to 60 bar and 4 mL/min), one Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve, and an Agilent 1200 Infinity Series Quick-Change Solvent Selection valve. This system can select from up to three solvents. The piston pump of the 1290 Infinity Flexible Cube can be used to simultaneously regenerate one Bio-Monolith Protein A HPLC column while the other column is connected to the analytical pump and is performing an IgG quantification run. Due to the low backpressure of the Bio-Monolith Protein A HPLC Column (~ 35 bar at 1 mL), the 1290 Infinity Flexible Cube is able to regenerate the second column with the included piston pump. This means that a second much more expensive HPLC pump is not required.

Because of high salt and low pH buffers used in the capturing and elution procedure with the Bio-Monolith Protein A HPLC column, the use of an inert system to avoid problems arising from stainless steel systems, such as metal leaching or corrosion of the system, is recommended.

Figure 1 shows the valve schematics for the Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve with the IgG affinity procedure running on Bio-Monolith Protein A HPLC Column 1 and simultaneous regeneration of the second Bio-Monolith Protein A HPLC column.

After every run, the position of the valves switches so that the columns are running alternately (Figure 2).

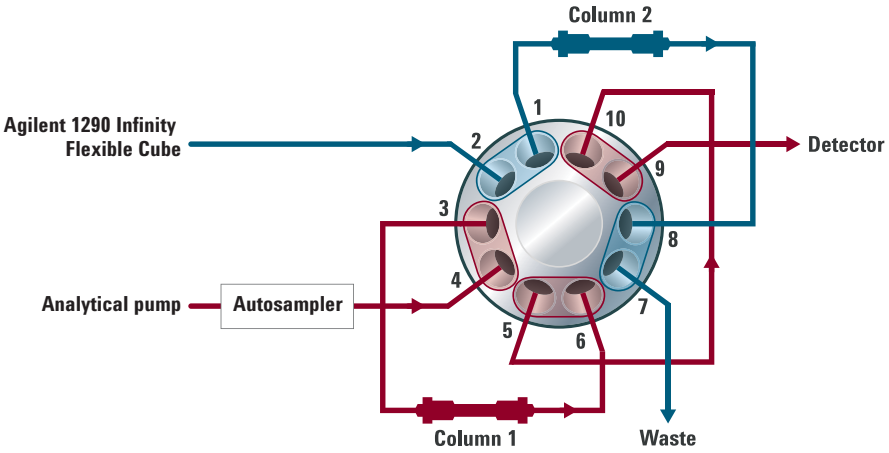

Figure 1. Flow scheme for Column 1 running and Column 2 being regenerated simultaneously (valve position 1).

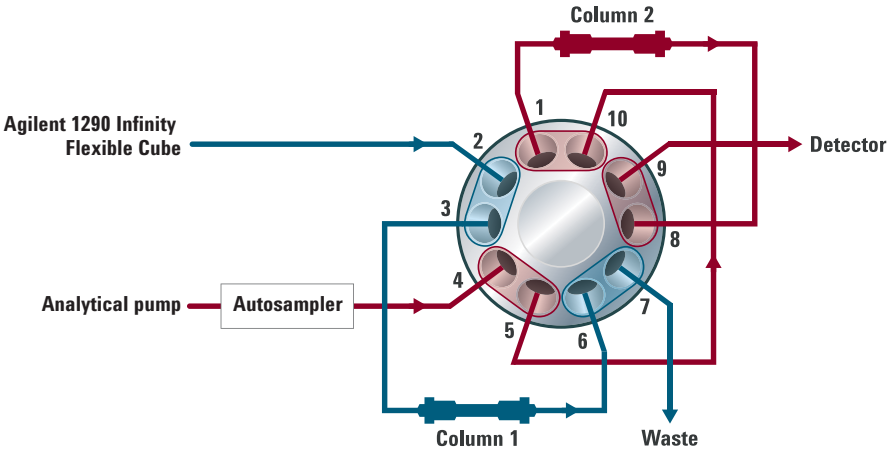

Figure 2. Flow scheme with Column 2 running, Column 1 being regenerated (valve position 2).

Experimental

The Agilent 1260 Infinity Bio-inert Quaternary LC system consisted of the following modules:

- Agilent 1260 Infinity Bio-inert Quaternary Pump (G5611A)
- Agilent 1260 Infinity Bio-inert High-Performance Autosampler (G5667A)
- Agilent 1290 Infinity Thermostat (G1330B) for sample cooling
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C) with bio-inert solvent heat exchangers (G5616-81000)
- Agilent 1260 Infinity DAD VL (G1315D with 10-mm bio-inert standard flow cell)
- Agilent 1290 Infinity Flexible Cube (G4227A)
- Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve, 600 bar (G5632A)

Columns

2 × Agilent Bio-Monolith Protein A HPLC Column (p/n 5069-3639)

Software

Agilent OpenLAB CDS, ChemStation Edition Rev. C.01.05 [38]

Solvents and samples

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak). Human IgG, phosphate buffered saline (PBS) tablets, and acetic acid were purchased from Sigma-Aldrich, St. Louis, USA.

Chromatographic conditions

Table 1. Chromatographic conditions.

Gradient		
Buffer A		PBS, pH 7.4
Buffer B		0.5 M Acetic Acid, pH 2.6
Time (min)	% B	Flow rate (mL/min)
0.00	0.00	1
0.50	0.00	1
0.51	100.00	1
1.50	100.00	1
1.51	0.00	1
1.52	0.00	1.5
Stop time	2.50 minutes	
Agilent 1290 Infinity Flexible Cube		
Time	Parameter	
0.00	Pump volume → Pump 2.49 mL, Flow: 1 mL/min, Channel B: B2	
2.50	Left valve change → Increase valve position	
Injection volume		3 µL
Agilent 1290 Infinity Thermostat Autosampler		4 °C
Temperature TCC		RT
DAD		280 nm/4 nm, Ref.: OFF
Peak width		> 0.025 minutes (0.5 seconds response time) (10 Hz)

Results and Discussion

Figure 3 shows an overlay of 20 subsequent chromatograms showing IgG affinity runs with alternating column regeneration, representing 10 individual runs per column.

For sample loading, 100 % Buffer A (PBS) was used at a flow rate of 1 mL/min. After 0.51 minutes, the solvent was switched to 100 % Buffer B (0.5 M acetic acid) to elute the IgG from the column. After 1.51 minutes, the solvent was switched back to 100 % Buffer A. The flow rate was enhanced to 1.5 mL/min to ensure that the flow path from pump to column was filled completely with Buffer A to prevent the injected IgG from immediate elution due to residual Buffer B in the capillaries. The delay volume of the Agilent 1260 Infinity Bio-inert Quaternary Pump in combination with the Agilent 1260 Infinity Bio-inert High-Performance Autosampler should be maximally 1 mL, which is an important part to consider when using alternate column regeneration.

Good precision of retention time and area was achieved over 10 injections for Bio-Monolith Protein A HPLC Column 1. The intra-column precision (10 runs on Column 1) of retention time and area was < 0.495 % and < 2.15 % relative standard deviation (RSD), respectively. With respect to both columns used, the inter-column precision (20 runs in total) of retention time and area was < 0.481 % and < 2.21 % RSD, respectively.

Using this setup, 0.5 minutes of post-time can be saved, resulting in a total of time-savings of approximately 20 %, compared to the normal gradient with a one-column setup. This results in a savings of 0.5 minutes for one sample, and, for 500 samples, it saves more than 4 hours.

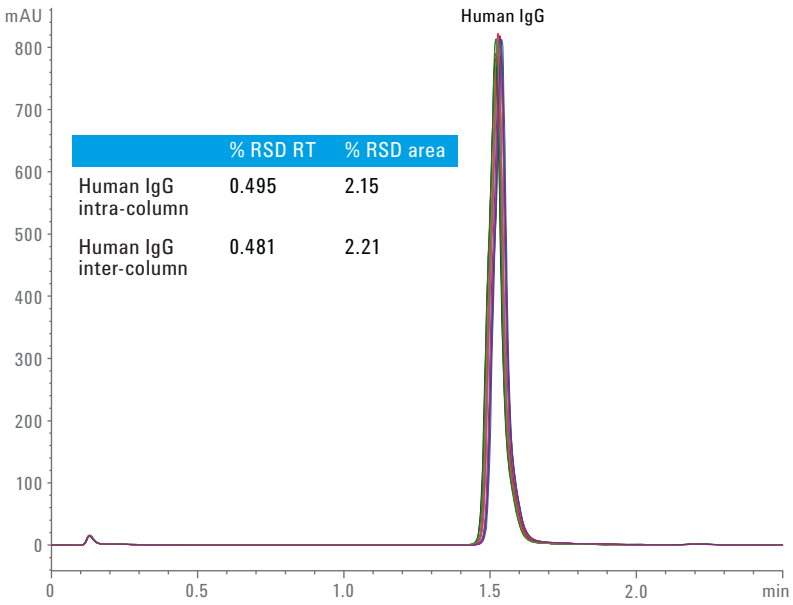

Figure 3. Overlay of 20 subsequent chromatograms showing affinity runs for IgG on Bio-Monolith Protein A HPLC Columns 1 and 2.

Conclusions

To save run time and, thus, increase throughput, two Agilent Bio-Monolith Protein A HPLC columns can be alternately used on the Agilent 1260 Infinity Bio-inert Quaternary LC in combination with the Agilent 1290 Infinity Flexible Cube equipped with an Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve. While one Bio-Monolith Protein A HPLC column performs an IgG quantification run, the other could be regenerated in alternation using the low-pressure piston pump of the 1290 Infinity Flexible Cube due to the low backpressure of the Bio-Monolith Protein A HPLC columns. Great intra- and inter-column precision could be observed with this setup. The combination of the Agilent 1260 Infinity Bio-inert Quaternary LC system and the 1290 Infinity Flexible Cube, containing a bio-inert valve, ensured an absolute bio-inert flow path for highest reproducibility.

References

1. Rapid Human Polyclonal IgG Quantification Using the Agilent Bio-Monolith Protein A HPLC Column, *Agilent Technologies Application Note*, publication number 5989-9733EN, **2008**.

2. Agilent Bio-Monolith Protein A Monitors Monoclonal Antibody Titer from Cell Cultures, *Agilent Technologies Application Note*, publication number 5991-2990EN, **2014**.

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