Reducing Cycle Time for Affinity Removal of High-Abundant Proteins in Human Plasma

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

Application Note

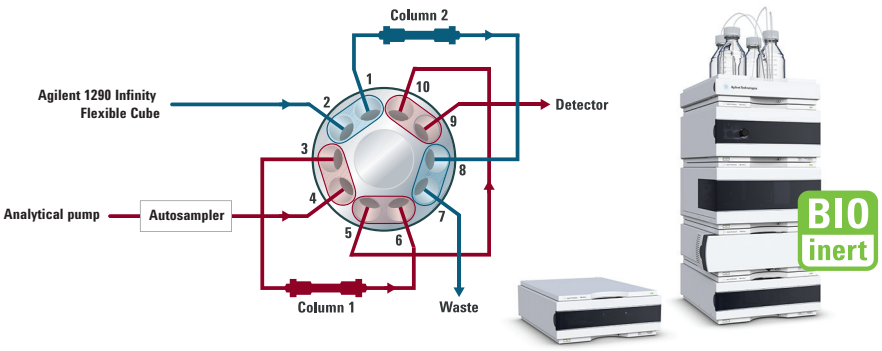
Proteomics & Protein Sciences

Authors

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

Abstract

This Application Note describes the depletion of the Top 14 high abundant proteins from human plasma using the Agilent Multiple Affinity Removal System together with 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 32 %. 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, excellent intra- and inter-column precision was achieved for the depletion procedure.



Agilent Technologies

Introduction

The plasma proteome is the most complex human-derived proteome, containing proteins present at concentrations that can differ by more than 10 orders of magnitude[1]. In proteomic studies, the employment of mass spectrometric analysis plays an essential role. However, mass spectrometry based assays are particularly affected by ionization suppression. To obtain limits of detection (LOD) and limits of quantification (LOQ) in the relevant range, either an enrichment of the low abundant proteins or a depletion of the high abundant proteins must be performed.

The Agilent Human 14 Multiple Affinity Removal System (MARS) can achieve a depletion of the Top 14 high abundant proteins with an efficiency of up to 94 %[2]. It is specifically designed to fractionate 14 high-abundant proteins from human biological fluids such as plasma, serum, and cerebral spinal fluid. Due to the high salt and low pH buffers used in the depletion procedure with the MARS 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[3].

Due to the relatively long time needed for regeneration (9 minutes, 36 % of total cycle time), it is desirable to work with two columns, that is, to regenerate one column in parallel with the depletion run on the second column to save total cycle time. The Agilent 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, to select between up to three solvents. Here, the piston pump of the 1290 Infinity Flexible Cube can be used to simultaneously regenerate one MARS column while the other column is connected to the analytical pump and is performing a depletion run. Due to the low backpressure of the MARS columns (~30 bar at 1 mL for the 4.6 × 50 mm column), the piston pump of the 1290 Infinity Flexible Cube is sufficient to easily perform alternating column regeneration.

Figure 1 shows the valve schematics for the 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve with the depletion procedure running on MARS Column 1, and simultaneous regeneration of MARS Column 2.

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

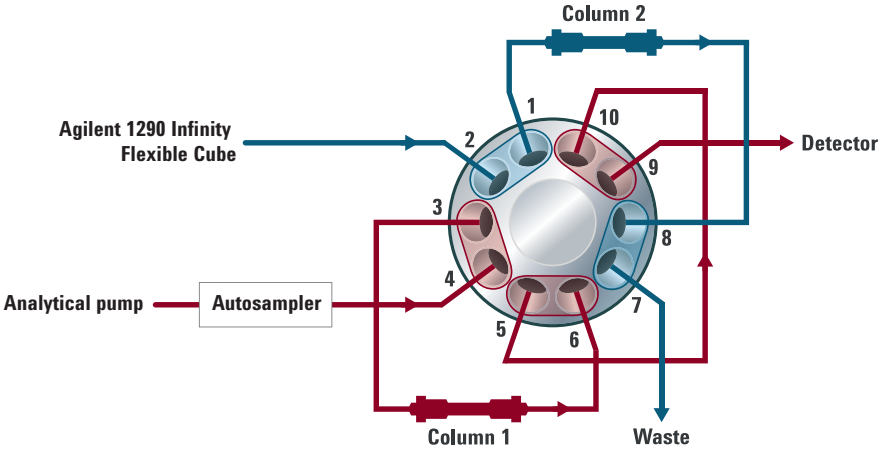


Figure 1. Flow scheme for Column 1 running and Column 2 being regenerated simultaneously (valve position 1).

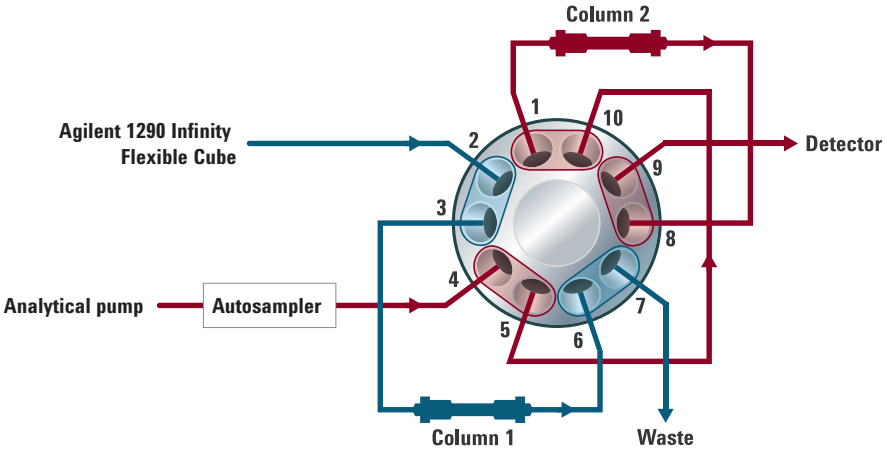


Figure 2. Flow scheme with Column 2 running, Column 1 being regenerated (valve position 2).

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 Thermostat (G1330B) for fraction cooling
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C) with bio-inert solvent heat exchangers (G5616-81000)
- Agilent 1260 Infinity DAD VL (G1315D with bio-inert standard flow cell, 10 mm)
- Agilent 1260 Infinity Bio-inert Analytical-scale Fraction Collector (G5664A)
- Agilent 1290 Infinity Flexible Cube (G4227A)
- Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve, 600 bar, (G5632A)

Software

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

Agilent Multiple Removal System

The Agilent Multiple Affinity Removal System consists of the following:

- Two affinity columns (here, 4.6 × 50 mm dimensions were used with a capacity of 20 µL human plasma/serum (p/n 5188-6557))
- Agilent Starter Reagent Kit (5185-5986), which includes:
  - Buffer A for sample loading
  - Buffer B for eluting bound proteins
  - Spin filters 0.22-µm for sample cleanup before loading column
  - Concentrators, 5 kDa, MWCO, for concentrating flow-through fractions

Human plasma and phosphate buffered saline (PBS) tablets were purchased from Sigma-Aldrich, St. Louis, USA. The plasma was diluted according to the MARS protocol.

Chromatographic conditions

Sample processing and fractionation were performed according to the protocol provided with the Agilent Multiple Affinity Removal System. Table 1 shows the chromatographic conditions.

Table 1. Chromatographic conditions.

Gradient		
Time (min)	%B	Flow rate
0.00	0.00	0.125
9.50	0.00	0.125
9.51	0.00	1.000
11.50	0.00	1.000
11.51	100.00	1.000
16.00	100.00	1.000
16.01	0.00	1.500
Stop time 17.00 minutes		

Flexible Cube	
Time	Parameter
0.00	Pump volume → Pump 4.5 mL, Flow: 1 mL/min, Channel B: B2
12.00	Pump volume → Pump 4.9 mL, Flow: 1 mL/min, Channel B: B2
17.00	Left valve change → Increase valve position

Injection volume	80 µL
Thermostat Autosampler and FC	4 °C
Temperature TCC	RT
DAD	280 nm/4 nm, Ref.: OFF
Peak width	> 0.05 minutes (1.0 seconds response time) (5 Hz)
Fraction collection	Peak-based with a threshold at 17.5 mAU

Results and Discussion

Figure 3 shows an overlay of seven subsequent depletion runs on MARS Column 1 for the removal of the Top 14 high abundant proteins from human plasma. For sample loading, a slow flow rate of 0.125 mL/min was used to ensure optimal antibody-antigen recognition and binding. In contrast to the binding step, the flow rate was then elevated to 1 mL/min for elution. The flow-through fraction peak from minute 5 to 7 represents the low abundant proteins, which are not binding to the affinity column. After changing the loading buffer to the elution buffer at 11.51 minutes, the peak between minute 13 and 14 represents the eluted 14 high abundant proteins, which were bound to the column. The green and red line, respectively, represent the fraction collection start and end of both peaks. After 16.01 minutes, the solvent was switched back to 100 % buffer A. The flow rate was enhanced to 1.5 mL/min for 1 minute before switching the valve position to ensure that the flow path from pump to the column was filled completely with buffer A to prevent the injected sample from immediate elution due to residuals of 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. This is an important point to consider when using alternate column regeneration.

High precision of retention time and area was achieved over seven injections for MARS Column 1. The precision of retention time and area was < 0.080 % and < 0.856 % relative standard deviation (RSD), respectively, for both peaks.

Figure 4 shows the overlay of 14 subsequent runs on both columns (alternating runs). Although the depletion runs were run on two different columns, high precision of retention time and area was observed. The precision of retention time and area was < 0.13 % and < 1.043 % RSD, respectively, for both peaks.

	% RSD RT	% RSD area
Low abundant	0.077	0.856
High abundant	0.079	0.463

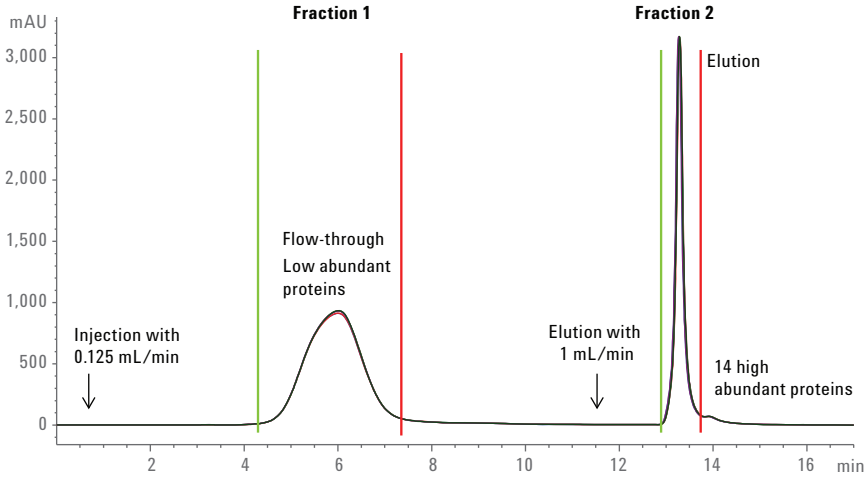


Figure 3. Overlay of seven subsequent depletion runs on an Agilent Human 14 Multiple Affinity Removal System Column 1.

	% RSD RT	% RSD area
Low abundant	0.127	1.042
High abundant	0.065	0.442

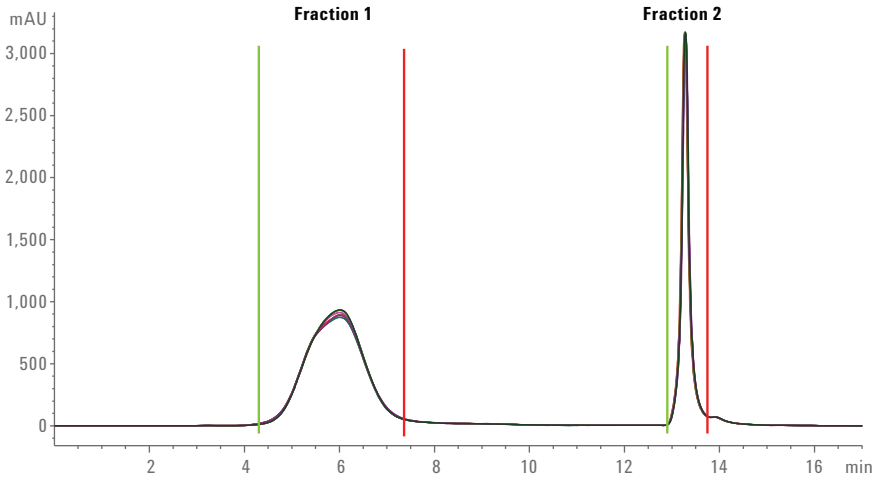


Figure 4. Overlay of the 14 subsequent runs on an Agilent Human 14 Multiple Affinity Removal System Columns 1 and 2, both columns alternating.

Eight minutes of post-time can be saved using this setup, resulting in a total of time saving of 32 % compared to a one-column setup with sequential regeneration (Figure 5).

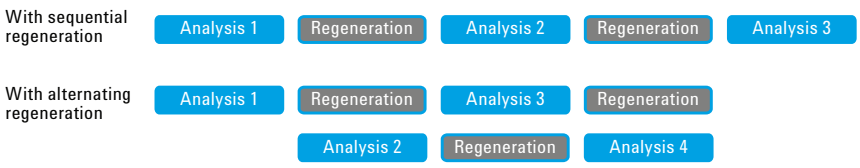


Figure 5. Alternating column regeneration increases analysis throughput.

Conclusions

To save run time significantly, two columns of the Agilent Multiple Affinity Removal System 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 MARS column performed a depletion run, the other could be re-equilibrated simultaneously using the low-pressure piston pump of the 1290 Infinity Flexible Cube due to the low backpressure of the MARS columns, resulting in a total time saving of 32 %. Excellent intra- and inter-column precisions could be observed with this setup. The combination of the 1260 Infinity Bio-inert Quaternary LC System with the 1290 Infinity Flexible Cube, containing a bio-inert valve, ensured an absolute bio-inert flow path for highest reproducibility.

References

1. Anderson, L. Candidate-based proteomics in the search for biomarkers of cardiovascular disease. *The Journal of Physiology* **2005**, *563* (Pt 1), pp 36-60.

2. Human Serum and Plasma Protein Depletion – Novel High-Capacity Affinity Column for the Removal of the "Top 14" Abundant Proteins, *Agilent Technologies Application Note*, publication number 5989-7839EN, **2008**.

3. Affinity removal of the 14 most abundant proteins in human plasma using the Agilent 1260 Infinity Bio-inert LC System, *Agilent Technologies Application Note*, publication number 5991-3207EN, **2013**.

www.agilent.com/chem

Published in the USA, June 1, 2014
5991-4721EN

Agilent Technologies