Reducing Cycle Time for Charge Variant Analysis of Monoclonal Antibodies

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

Application Note

Biotherapeutics & Biosimilars

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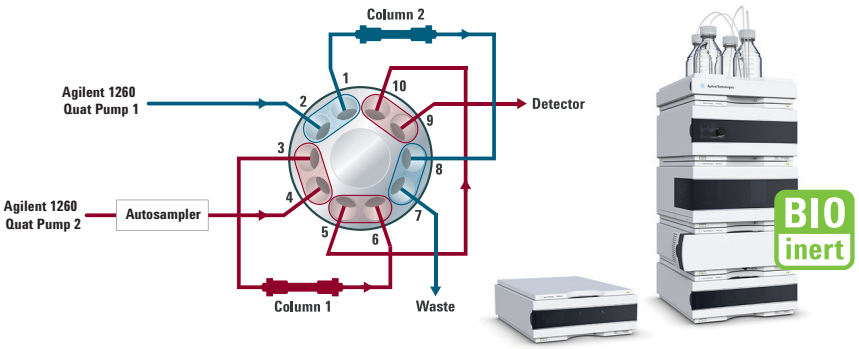
Abstract

This Application Note describes the charge variant analysis of monoclonal antibodies with pH gradients using the Agilent 1260 Infinity Bio-inert Quaternary LC. Alternating column regeneration using an Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve in combination with a second 1260 Infinity Bio-inert Quaternary pump reduced the total cycle time by 44 %, and thus enabled nearly doubled throughput. With this setup, good intra- and inter-column precisions were achieved.



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Introduction

Charge variant analysis of pharmaceutical biomolecules is an important part of product stability monitoring during development and production. Post translational modifications of the proteins such as glycosylation, phosphorylation, deamidation, and many others lead to charge heterogeneity of the protein. To ensure safety and efficacy, it is essential to characterize and quantify the charge variant profile of the protein.

The charge heterogeneity of biotherapeutic proteins is usually analyzed using ion-exchange chromatography (IEX), separating molecules according to their net surface charge. The comparison of salt with pH gradients showed higher separation efficiency due to narrower band focusing during the pH-gradient elution, which results in higher resolution[1]. With the Agilent 1260 Infinity Bio-inert Quaternary LC system and the Agilent Buffer Advisor software, highly linear pH gradients can be performed[2].

Column regeneration after salt or pH gradients can be time-consuming due to the quite long equilibration time needed, especially using long columns such as 250-mm columns. For reproducible ion-exchange separation, the column equilibration and cleanup phases of the gradient are critical.

To reduce the total cycle time, two Agilent 1260 Infinity Bio-inert Quaternary pumps were used for regeneration of a second Agilent Bio mAb PEEK column.

Figure 1 shows the valve schematics for the Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve with the charge variant analysis running on Agilent Bio mAb PEEK Column 1, and simultaneous regeneration on the second Agilent Bio mAb PEEK column.

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

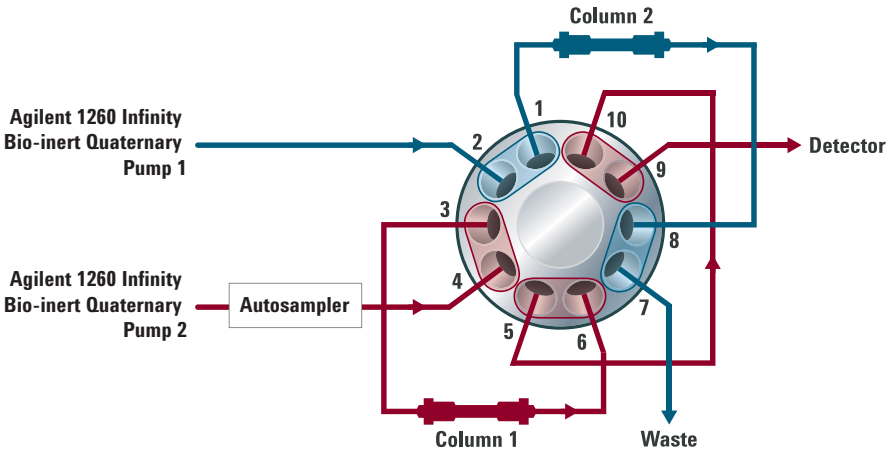

Figure 1. Flow scheme for Column 1 running and Column 2 being regenerated simultaneously (valve position 1).

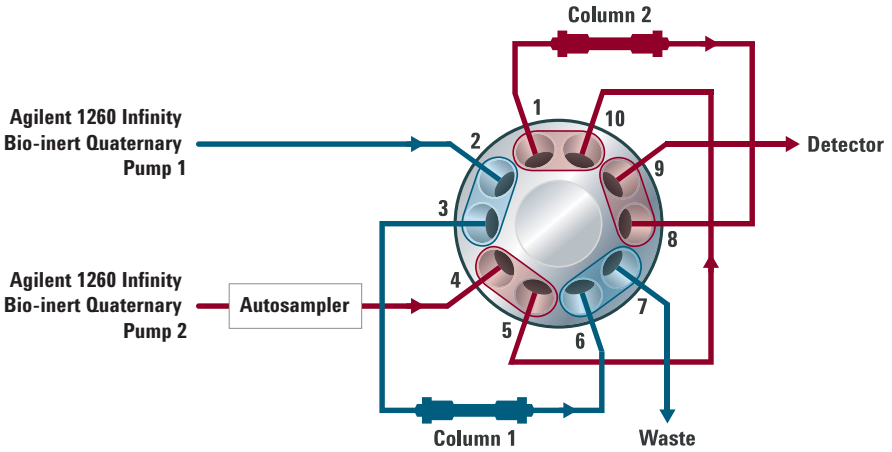

Figure 2. Flow scheme with Column 2 running, Column 1 being regenerated (valve position 2).

Experimental

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

- 2 x 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 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve, 600 bar (G5632A)
- Agilent 1260 Infinity DAD VL (G1315D with a 10-mm bio-inert standard flow cell)

Columns

2 × Agilent Bio MAb PEEK, 2.1 × 250 mm, 5 µm (p/n 5190-2411)

Software

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

Agilent Buffer Advisor Software, revision A.01.01

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). Phosphate monobasic and dibasic and sodium chloride were purchased from Sigma-Aldrich, St. Louis, USA.

Monoclonal Antibody (mAb): RAT Anti-DYKDDDDK Tag Antibody

Chromatographic conditions

Table 1. Chromatographic conditions

Pump 1				
pH gradient 6.4–7, 30 mM Sodium phosphate buffer, gradient was calculated from the Agilent Buffer Advisor				
Time (min)	A) Water	B) 500 mM NaCl	C) NaH_2PO_4 (55 mM)	D) Na_2HPO_4 (49 mM)
0.00	44.00	0.00	42.30	13.70
19.00	41.80	0.00	24.50	33.70
19.50	44.00	0.00	42.30	13.70
Stop time: 25.00				
Pump 2				
Column regeneration using a high salt step with subsequent re-equilibration, gradient was calculated with the Agilent Buffer Advisor				
Time (min)	A) Water	B) 500 mM NaCl	C) NaH_2PO_4 (55 mM)	D) Na_2HPO_4 (49 mM)
0.00	44.00	0.00	42.30	13.70
1.00	11.30	29.40	16.00	43.30
4.00	11.30	29.40	16.00	43.30
5.00	44.00	0.00	42.30	13.70
Stop	time:	25.00		
Flow rate		0.3 mL/min		
Injection volume		7 µL		
Thermostat autosampler and FC		6 °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

Charge variant analysis of the mAb RAT Anti-DYKDDDDK Tag was performed on two Agilent Bio MAb PEEK, (2.1 × 250 mm, 5 µm) columns with alternating column regeneration using an Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve with a second 1260 Infinity Bio-inert Quaternary pump. While the pH gradient for charge variant analysis is running on Column 1, the second column could first be flushed with a high-salt step to remove any residual protein from the column, then subsequently regenerated for about 20 minutes.

Figure 3 shows charge variant separation of the used mAb on Bio MAb PEEK Column 1. With pH gradient elution from pH 6.4 to 7, it was possible to resolve six different charge variants.

Good precision of retention time and area was achieved over six injections for Bio mAb PEEK Column 1, and for 12 injections on Columns 1 and 2. The intra-column (six runs) precision of retention time and area was < 0.31 % and < 2.8 % relative standard deviation (RSD), respectively, except for peak CV4. With respect to both columns used, the inter-column (12 runs) precision of retention time and area was < 0.29 % and < 3.70 % RSD, respectively, except for peak CV4 (Table 2).

Alternating column regeneration, using a 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve with a second 1260 Infinity Bio-inert Quaternary pump reduced the run time by a total of 44 % compared to a run time of 25 minutes and a post-time of 20 minutes in a one-column setup. Here, the total cycle time was 25 minutes before valve switching, resulting in an almost doubled run time speed-up, shown in Figure 4.

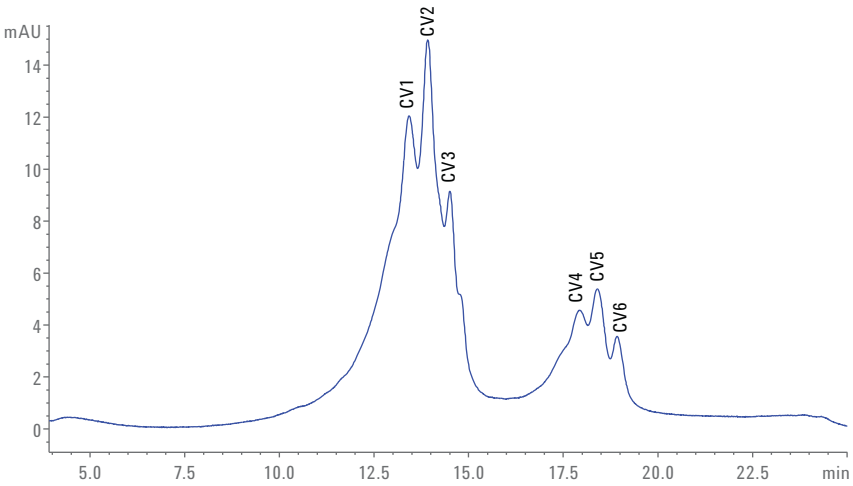

Figure 3. Charge variant analysis of RAT Anti-DYKDDDDK on Agilent Bio MAb PEEK Column 1.

Table 2. Intra- (n = 6) and inter-column (n = 12) precision of retention time and area.

	Intra-column % RSD RT	Intra-column % RSD area	Inter-column % RSD RT	Inter-column % RSD area
CV1	0.205	2.50	0.247	3.69
CV2	0.183	1.91	0.218	1.63
CV3	0.247	1.13	0.277	2.56
CV4	0.302	6.73	0.286	6.67
CV5	0.301	1.63	0.255	1.41
CV6	0.252	2.78	0.213	2.93

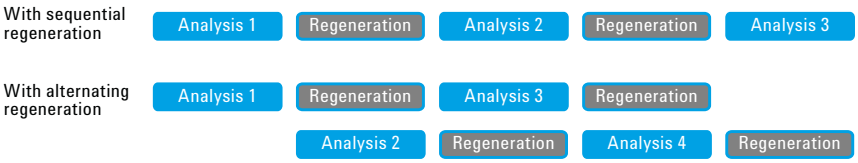

Figure 4. Alternating column regeneration using a 2-position/10-port valve increases analysis throughput by a factor of almost 2.

Conclusions

Two Agilent Bio MAb PEEK columns can be alternately run on the Agilent 1260 Infinity Bio-inert Quaternary LC using an Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port valve with a second Agilent 1260 Infinity Bio-inert Quaternary pump. While on the first Bio mAb PEEK Column, charge variant analysis of a mAb is performed using pH gradient elution, the second column could be regenerated using the second 1260 Infinity Bio-inert Quaternary pump. Good intra- and also inter-column precision could be observed with this setup.

With this solution, it was possible to speed up the analysis almost to half the time, resulting in a total cycle time savings of 44 % and an almost two-fold increase in sample throughput.

References

1. Simple Method Optimization in mAb Charge Variant Analysis using pH Gradients Generated from Buffer Advisor with Online pH and Conductivity Monitoring, *Agilent Technologies Application Note*, publication number 5991-3365EN, **2013**.

2. Protein Separation with pH Gradients Using Composite Buffer Systems Calculated by the Agilent Buffer Advisor Software, *Agilent Technologies Application Note*, publication number 5991-1408EN, **2012**.

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