Application Note

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Two-In-One Bioprocess Analytics

Combining mAb titer determination and spent media analysis by internal valve switching using the Agilent 1260 Infinity II Bio-Inert LC and InfinityLab LC/MSD iQ

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Abstract

The analysis of critical process parameters (CPP) and critical quality attributes (CQA) plays an important role in monitoring and ensuring optimal process yields and product quality during the production of biopharmaceuticals. HPLC methods are often used for the analysis of these parameters. However, cost, lab space, and workload are crucial for the implementation of such tools. This application note demonstrates the use of an Agilent 1260 Infinity II Bio-Inert LC System coupled to an Agilent InfinityLab LC/MSD iQ with an automated column selection valve for spent media analysis and protein A-based titer determination. By applying hydrophilic interaction liquid chromatography (HILIC) with smart MS detection, excellent linearity, sensitivity, and selectivity for important cell culture medium components were achieved. The quaternary pump used in the 1260 Infinity II Bio-Inert LC System enabled the subsequential analysis of the monoclonal antibody (mAb) product with UV detection, without manual reconfiguration of the instrument. All necessary maintenance operations such as column switching, washing, or equilibration could be performed using Agilent OpenLab CDS 2 software, which can be fully qualified to meet the requirements of the FDA regarding 21 CFR part 11 compliance.

Introduction

The production of biopharmaceuticals such as mAbs is mainly performed by bioreactor cultivations of mammalian cell lines such as Chinese hamster ovary (CHO) cells. These production hosts require numerous nutrients such as sugars, amino acids, vitamins, and growth factors for optimal growth and yields.[1] The analysis of the process and product is encouraged by the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) through concepts such as quality by design (QbD), which aims to build quality into the process instead of testing the quality of the final product.[2] Various analytical methods can be used for upstream/downstream processes or quality control as online, at-line, or offline tools. Important parameters for monitoring are nutrient consumption, product formation, and product quality (CQA).[1] Since lab space is limited, and the preparation or switching of analytical methods can be time-consuming, there is a need for advanced methods to analyze multiple parameters at the same time. This application note presents two methods: HILIC for polar medium compounds, and protein A affinity chromatography for product titer determination. Both methods were run on a single 1260 Infinity II Bio-Inert LC System coupled to an LC/MSD iQ with an automated column selection valve.

Experimental

Equipment

The 1260 Infinity II Bio-Inert LC System coupled to the LC/MSD iQ comprised the following modules:

- Agilent 1260 Infinity II Bio-Inert pump (G5654A)
- Agilent 1260 Infinity II Bio-Inert multisampler (G5668A) equipped with Agilent InfinityLab sample thermostat (G5668A#101) and the multiwash option (G5668A#112)
- Agilent 1260 Infinity II multicolumn thermostat (G7116A) equipped with a bio-inert 4-position/10-port column selection valve (G5639A) and divider assembly for use with different temperature zones (G7116-60006)
- Agilent 1260 Infinity II diode array detector WR (G7115A) with bio-inert standard flow cell, 10 mm (G4212-60007)
- Agilent InfinityLab LC/MSD iQ (G6160AA)
- Agilent InfinityLab Flex Bench MS (G6015B)

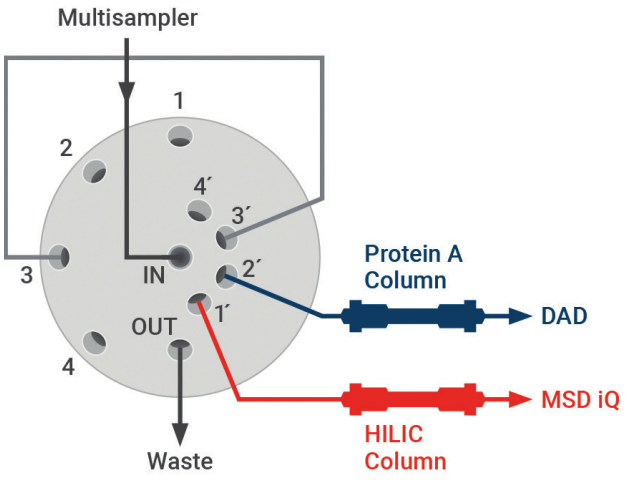

Figure 1. Schematic presentation of the configured bio-inert 4-position/10-port column selection valve.

Software

Agilent OpenLab CDS Version 2.4

Columns

- Agilent InfinityLab Poroshell 120 HILIC-OH5, 2.1 × 100 mm, 2.7 µm (p/n 685775-601)
- Agilent Bio-Monolith protein A, 4.95 × 5.2 mm (p/n 5069-3639)

Valve configuration

A bio-inert 4-position/10-port column selection valve was installed and configured according to Figure 1, to achieve spent media analysis and mAb titer analysis on a single LC system without reconfiguration between runs. The additional bypass capillary on position 3 enables the flushing of the LC system upstream of the column selection valve, avoiding contact between the mobile phases used for the two methods and potential precipitation of salts.

Chemicals

LC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak, Merck-Millipore, Billerica, MA, USA). D-glucose, L-glutamic acid, L-glutamine, L-lactic acid, and L-alanyl-L-glutamine were purchased from Sigma-Aldrich (Steinheim, Germany). Additionally, a premixed amino acid standard was used (p/n 5061-3330, 1 nmol/µL). Ammonium formate and formic acid were obtained from VWR (Darmstadt, Germany).

Standards and sample preparation

Stock solutions of all analytes were prepared in water as reference standards with a concentration of 10 mM. Aqueous standards or cultivation supernatants (provided by the Institute of Biochemical Engineering, University of Stuttgart) were either injected directly after centrifugation (12.100 × g, 2 minutes) for titer analysis via protein A chromatography or diluted 1:10 with water, centrifuged, and adjusted to 60% (v/v) acetonitrile and 30 mM ammonium formate (pH 3.0) for HILIC chromatography. The sample predilution with organic solvent and buffer was required to improve the chromatographic performance of the media compounds.

Cultivation

Bioreactor cultivations of an IgG_1 monoclonal antibody (anti-IL-8) producing CHO DP12 cell line were conducted in batch mode with a starting volume of 1.0 L. The medium was chemically defined (TC42, Xell AG, Germany), comprising L-glutamine, L-alanyl-L-glutamine, D-glucose, growth factors, and various other amino acids. Samples were taken daily and filtered through a 0.2 µm PES syringe filter. Resulting supernatants were stored at –70 °C until analysis.

Table 1. HILIC method for spent media analysis.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 HILIC-OH5, 2.1 × 100 mm, 2.7 µm
Solvent	A) 85% acetonitrile / 5% 2-propanol / 10% water + 30 mM ammonium formate, pH 3 B) 10% acetonitrile / 5% 2-propanol / 85% water + 30 mM ammonium formate, pH 3
Gradient	0.00 min – 100% A/0% B 1.00 min – 100% A/0% B 11.00 min – 90% A/10% B 12.00 min – 0% A/100% B 12.01 min – 100% A/0% B 20.00 min – 100% A/0% B
Flow rate	0.600 mL/min
Temperature	20 °C
MS Detection	Auto Acquire mode / SIM (see Table 3)
Injection	Injection volume: 1 µL Sample temperature: 4 °C Multiwash: 5 s in 90% acetonitrile / 10% water (S1, needle wash)

Table 2. Protein A method for mAb titer analysis.

Parameter	Value
Column	Agilent Bio-Monolith protein A, 4.95 × 5.2 mm
Solvent	C) 50 mM sodium phosphate buffer pH 7.4 D) 500 mM acetic acid, pH 2.6
Gradient	0.00 min – 100% C/0% D 0.50 min – 100% C/0% D 0.60 min – 0% C/100% D 1.70 min – 0% C/100% D 1.80 min – 100% C/0% D 3.5 min – 100% C/0% D
Flow Rate	1 mL/min
Temperature	30 °C
UV detection	280 nm/4 nm, reference 360 nm/100 nm, 20 Hz
Injection	Injection volume: 50 µL Sample temperature: 4 °C Multiwash: 5 s in 100% water (S2, needle wash)

Results and discussion

Chemically defined cell culture media for the cultivation of CHO cells comprise several polar chemicals that can be analyzed without prior derivatization on HILIC columns. Since most of these compounds lack a chromophore, standard UV detection is not suitable. In this case, easy-to-use mass detection, as offered by the LC/MSD iQ, provides increased specificity and selectivity. By using the Auto Acquire mode, ion source parameters and fragmentor voltages were automatically adjusted based on the configured LC flow rate and ion mass of the compound. Only appropriate SIM channels were manually configured. For separation, an InfinityLab Poroshell 120 HILIC-OH5 column was used with ammonium formate as buffer at low pH (Table 1). In addition, 5% (v/v) 2-propanol was added as an organic modifier to mobile phases A and B to optimize peak shapes in the HILIC separation. Figure 2 shows the separation of 16 polar compounds typically present in a cell culture medium comprising organic acids, amino acids, and dipeptides.

To demonstrate the ability of the LC/MSD iQ for external quantification, a 10-point calibration curve for every compound with the following levels was prepared: 1, 2, 5, 10, 20, 50, 75, 100, 150, 200 µM. Additionally, the limit of detection (LOD, S/N = 3) was determined with an automated signal-to-noise (S/N) calculation (P2P) in OpenLab CDS for the average of the three lowest levels for every compound. Table 3 shows good linearity (R^2 >0.99) and LODs below one pmol on-column for most analytes, demonstrating that the LC/MSD iQ is a great choice for user-friendly quantification with a small lab footprint.[3]

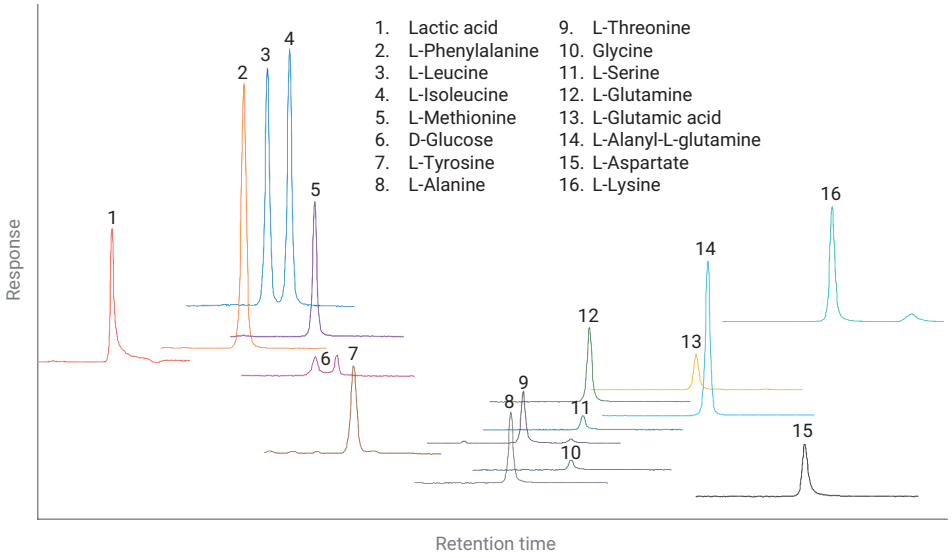

Figure 2. Chromatograms of cell culture medium compounds, analyzed as standards (200 µM) with the HILIC method and Agilent InfinityLab LC/MSD iQ as a detector.

Table 3. MS and quantification parameters for each compound standard analyzed in the HILIC spent media analysis.

Compounds	SIM (*m/z*)/ Polarity	Fragmentor (V)	Retention Time (min)	Calibration Range (µM)	R^2	LOD (pmol On-Column)
Lactic acid	89 / –	90	1.734	1 to 200	0.9979	0.63
L-Phenylalanine	166 / +	100	3.611	1 to 200	0.9956	0.41
L-Leucine	132 / +	90	3.951	1 to 200	0.9964	0.86
L-Isoleucine	132 / +	90	4.274	1 to 200	0.9964	0.67
L-Methionine	150 / +	100	4.615	1 to 200	0.9966	0.23
D-Glucose	179 /–	100	4.917	20 to 200	0.9934	12.24
L-Tyrosine	182 / +	100	5.134	2 to 200	0.9997	1.09
L-Alanine	90 / +	90	7.358	1 to 200	0.9980	0.88
L-Threonine	120 / +	90	7.534	1 to 200	0.9984	0.90
Glycine	76 / +	90	8.218	5 to 200	0.9991	3.72
L-Serine	106 / +	90	8.387	5 to 200	0.9982	2.80
L-Glutamine	147 / +	90	8.512	2 to 200	0.9989	1.72
L-Glutamic acid	148 / +	90	10.100	2 to 200	0.9996	1.46
L-Alanyl-L-glutamine	218 / +	100	10.158	1 to 200	0.9952	0.54
L-Aspartate	132 /–	90	11.537	2 to 200	0.9958	1.19
L-Lysine	147 / +	90	11.969	1 to 150	0.9901	0.29

Two CHO cell cultivations (A+B) were performed, and bioreactors were sampled daily to show the applicability of the latter HILIC method for spent media analysis. The dynamic profiles of the nutrients are shown in Figure 3. On the left side, all compounds are depicted as having decreasing concentrations during the cultivation. Compounds with increasing concentrations are shown on the right side of the diagram. Overall, the medium components indicate a typical behavior, for example, the consumption of D-glucose (carbon source) and L-glutamine/L-alanyl-L-glutamine (nitrogen source) or the formation of lactic acid as a side product during overflow metabolism. Slight differences in the compound formation or consumption rate demonstrate the different performances of CHO cell cultivations (A versus B). By applying this HILIC method during the cultivation, in-process measures could be taken to control the process and optimize product yield at the end of cultivation.

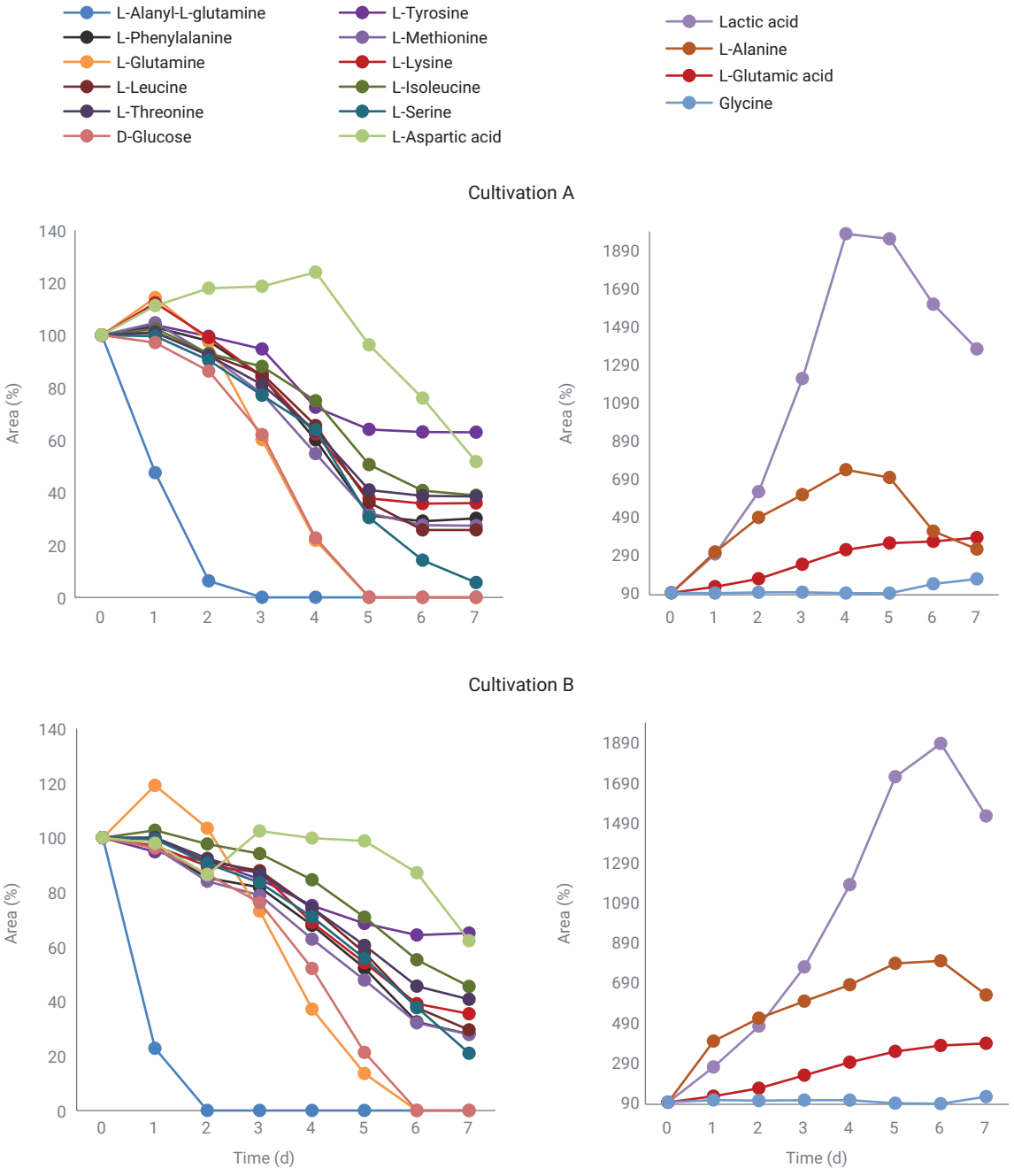

Figure 3. Profiles of HILIC-analyzed media compounds during cell culture cultivations.

To further monitor process variables, protein A affinity chromatography was applied subsequently on the same 1260 Infinity II Bio-Inert LC System, which features a fully bio-inert flow path, especially suited for sticky biomolecules and metabolites. Using all four available solvent channels (A + B for HILIC, C + D for protein A), both methods could be run without manual solvent switching. Additionally, both columns were installed at the same time, controlled at different temperatures, and could be switched by an internal column selection valve, further decreasing manual operations. The developed and optimized protein A method is depicted in Table 2. Figure 4 shows UV chromatograms for the protein A method of cultivation A over six cultivation days. The mAb titer reached a maximum at day six and decreased afterward, probably due to thermal or enzymatic degradation of the Fc region of the mAb, which binds to protein A.

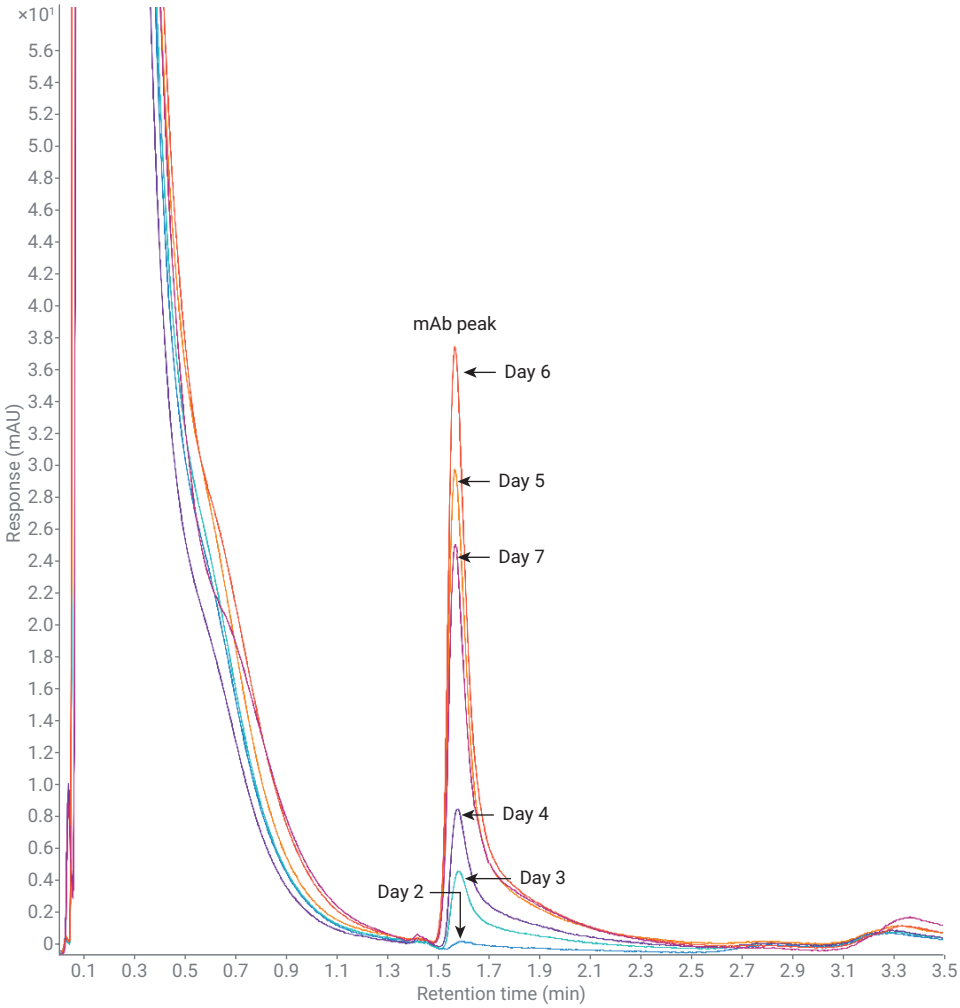

Figure 4. Chromatograms of supernatant samples of cultivation A over the course of six fermentation days, analyzed by the protein A method coupled to the Agilent 1260 Infinity II diode array detector (at 280 nm).

After the integration of mAb peaks, OpenLab offers a feature called Peak Explorer. Figure 5A shows a snapshot of the Peak Explorer view, where the Y-axis shows the different injections and the X-axis the corresponding retention times. The area of the integrated mAb peak is depicted as a circle, where the diameter of the circle is proportional to the amount of mAb. This Peak Explorer view gives a quick qualitative and straightforward display of the dynamic mAb production. When comparing the dynamic mAb accumulation of cultivation A and B, significant differences in the increase of mAb became obvious (Figure 5B), which could again be a signal for in-process adjustments. Combining both methods presents an excellent toolbox for employing at-line process analysis in a production or process development environment.

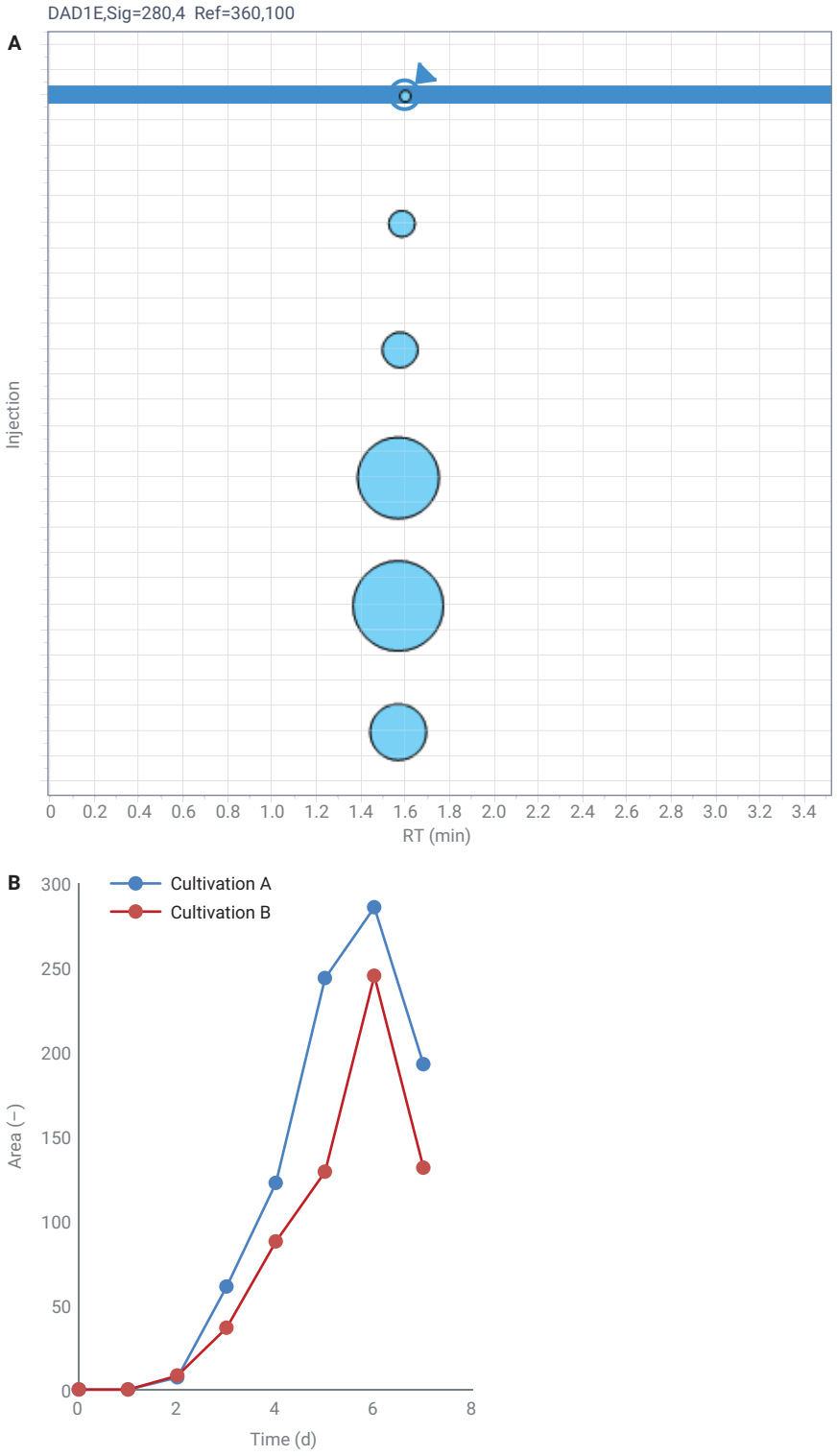

Figure 5. A snapshot of the Peak Explorer view for cultivation A in Agilent OpenLab CDS (A) and mAb production during the cultivation period (B).

Conclusion

Process analytical technology (PAT) is a powerful tool to adjust and improve bioprocess parameters such as growth rate, product yield, and product quality during the production process. As a prerequisite, we showed the application of HPLC measurements as a means for process control in this application note. The presented HILIC analysis with the LC/MSD iQ as detector showed excellent linearity, sensitivity, and selectivity of key cell culture nutrients with the easy-to-use Auto Acquire mode. By combining spent media analysis and protein A affinity chromatography on a 1260 Infinity II Bio-Inert LC coupled to an LC/MSD IQ with automated column switching, manual operations were significantly reduced. Paired with OpenLab CDS 2 software, which can be fully qualified and is available with all necessary services to meet the requirements of the FDA regarding 21CFR Part 11, this at-line HPLC solution is fully suited to be part of a modern QbD environment.

References

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