Application Note

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Online LC Monitoring of Downstream Processing in the Production of Therapeutic mAbs

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Abstract

This application note demonstrates the use of the Agilent 1260 Infinity II Prime Online LC System in the downstream processing of biopharmaceutical products. The shown example will demonstrate online HPLC monitoring of a process purification system for a mAb. The real-time monitoring increases process understanding and can improve yield and purity of the desired product by enabling optimization of the loading and pooling criteria of processing steps. By applying fast quantitative online SEC-UV-UHPLC methods, optimal main product concentrations and minimized by-products, namely aggregates, could be achieved. The real-time online monitoring of purification processes facilitates decision making for improved product quality, offering savings in time and cost, and removes manual sample handling requirements. It also allows for real-time decision making via automated process feedback. The complete monitoring process is orchestrated by the Agilent Online LC Monitoring Software, which facilitates sampling and sample analysis and enables on-the-fly real-time monitoring via trending plots.

Introduction

In the modern biopharmaceutical industry, the production of an active pharmaceutical ingredient (API) is differentiated into upstream and downstream processing. Upstream processing (USP) comprises the aspects pertaining to the fermentation process of, e.g., engineered mammalian cells to produce a desired biomolecule. This includes steps related to the growth of the micro-organisms and the cell culture under controlled conditions.

The downstream process (DSP) starts with the separation of the cells from the solution, known as cell separation and harvesting. The desired API can be obtained directly from the filtration solution and further processed, or in some cases, after extra processing steps to release the API from the cells into the solution. The desired API is typically purified with several chromatographic steps based on charge, size, hydrophobicity, or affinity. Several adjustment, polishing, and conditioning steps lead to the final API (Figure 1).

Just as the cell culture in the bioreactor must be monitored for the desired product, by-products, properties of the cell growth medium, and product quality over the subsequent purification and processing steps must also be monitored. Given that biologics are complex molecules, sensitive analytical tools such as (U)HPLC are required to closely monitor critical quality attributes (CQAs) and critical process parameters (CPPs).

This application note demonstrates the use of the Agilent 1260 Infinity II Prime Online LC System for monitoring the chromatographic purification of a biopharmaceutical API in near real time. This enables fast decision-making for pooling and further processing requirements depending on purity.

Experimental

Instrumentation

- Two Agilent 1290 Infinity II High Speed Pumps (G7120A)
- Agilent 1260 Infinity II Online Sample Manager Set (G3167AA): Agilent 1260 Infinity II Online Sample Manager (G3167A) clustered with external valve (part number 5067-6680) located at the Agilent 1290 Infinity Valve Drive (G1170A) and Agilent Online LC Monitoring Software
- Thermostat for 1260 Infinity II Online Sample Manager (G7167-60005)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) with standard heat exchanger (G7116-60051) and 2-position/10-port valve (part number 5067-4283).
- Agilent 1290 Infinity II DAD, G7117B, equipped with the standard Max-Light Cartridge Cell (1 µL, 10 mm; G4212-60008)

Columns

Polymer-based UHPLC SEC column, 4.6 × 150 mm, 200 Å, 1.7 µm

Software

- Agilent OpenLab CDS, version 2.6
- Agilent Online LC Monitoring Software, version 1.0

Sampling method from purification system

- Online sampling from the purification eluent flow with an in-house developed GMP-compliant, aseptic FPLC solution equipped with a NovaSeptum transfer unit (Merck) and fractionation (Äkta Go, Cytiva)[1]
- **Flow sampled from the process and directed to the Online LC:** 2.2 mL/min
- **Sampling interval:** 4.25 minutes
- **Sampling speed for Online LC:** Setting 2 (draw speed: 100 µL/min)

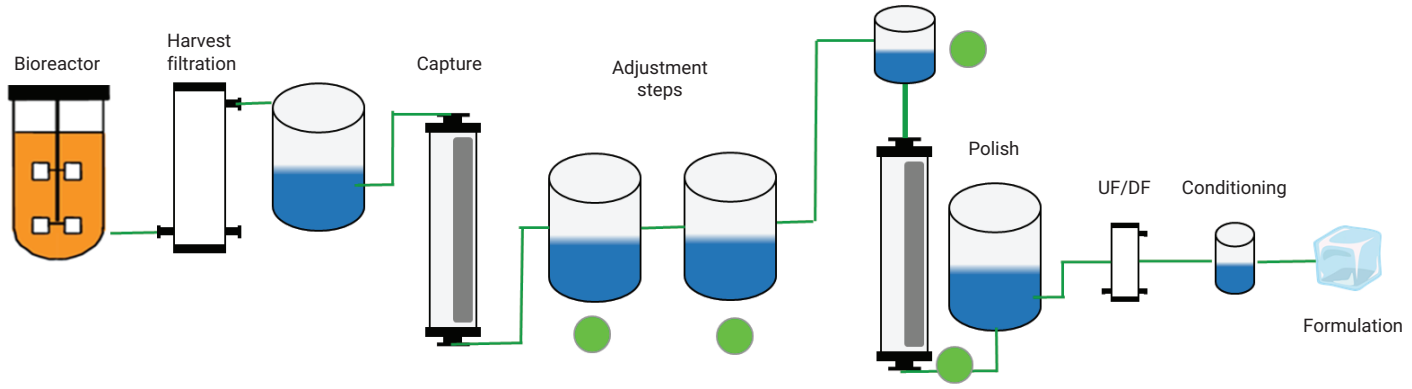

Figure 1. Downstream processing of a biopharmaceutical API. The green dots indicate access points for online LC monitoring of the product quality by determination of the percentage of higher molecular weight aggregates in a monoclonal antibody process (%HMWs mAb aggregates).

Analytical method

Parameter	Value
Solvent	100 mM $CH_3CO_2NH_4$, pH 5.5
Analytical Flow Rate	0.6 mL/min
Gradient Mode	Isocratic
Stop Time	3 minutes
Column Temperature	40 °C
Injection Mode	**Feed mode** **Feed speed:** Adaptive (80% of Pump Flow) **Flush out mode:** Automatic (12.44 µL)
Sample Injection Volume	4 µL
Needle Wash	**Outer wash mode:** Standard - 5 seconds, S1 100% H_2O **Inner wash mode:** Off - Reconditioning with S2 (Mobile Phase) only
Sampling	See sampling method
Diode Array Detector	**Wavelengths:** 280 ±4 nm and 260 ±4 nm, Ref. 360 (100 nm) **Data rate:** 10 Hz

Note: As backup, an identical second column was connected via a 2-position/10-port valve and configured with a second high-speed pump for regeneration.

Data processing

Automated integration, raw data export, and result report export (CSV and PDF data formats and via method-based OPC UA interface), software beta-version.

Sample

Proprietary mAb, eluting from purification system.

Solvents and chemicals

- All solvents were purchased from Merck, Germany.
- Chemicals were purchased from VWR, Germany.
- Fresh ultrapure water was obtained from a Milli-Q Q-POD system equipped with a 0.22 µm Millipak membrane point of use cartridge.

Results and discussion

In the downstream processing of the mAb produced from mammalian cells, product quality of the elution from an AEX chromatographic polishing step was verified by Online LC (SEC-UV) (Figure 2). The Agilent 1260 Infinity II Prime Online LC was connected to the effluent line coming out of the applied purification system with a GMP-compliant aseptic FPLC solution with a NovaSeptum sampling connection. A sample was drawn by the Online LC system about every four minutes and analyzed on an SEC column by direct injection in a three minute run. A second pump and a 2-position/10-port valve in the MCT were incorporated in the configuration to enable column switching, providing an immediately available alternative solution in case of column issues. The obtained SEC chromatograms provided information about the concentration of the desired mAb and the percentage of higher molecular weight aggregates.

In the Online LC Monitoring Software, which orchestrates the sampling and analysis schedule, the data can be reviewed in near real time (when each individual run is finalized). The fast quantitative SEC-UV method provided mAb and area %HMW aggregate composition every four minutes (Figure 3). The trending plot shows the area percentage of the HMW aggregates over the DSP process.

One HMW aggregate (SEC RT: 1.67 minutes) is observed to increase from 0.1 to 1.68% from 24 to 56 minutes, with a following decline to 0.47% at 76 minutes.

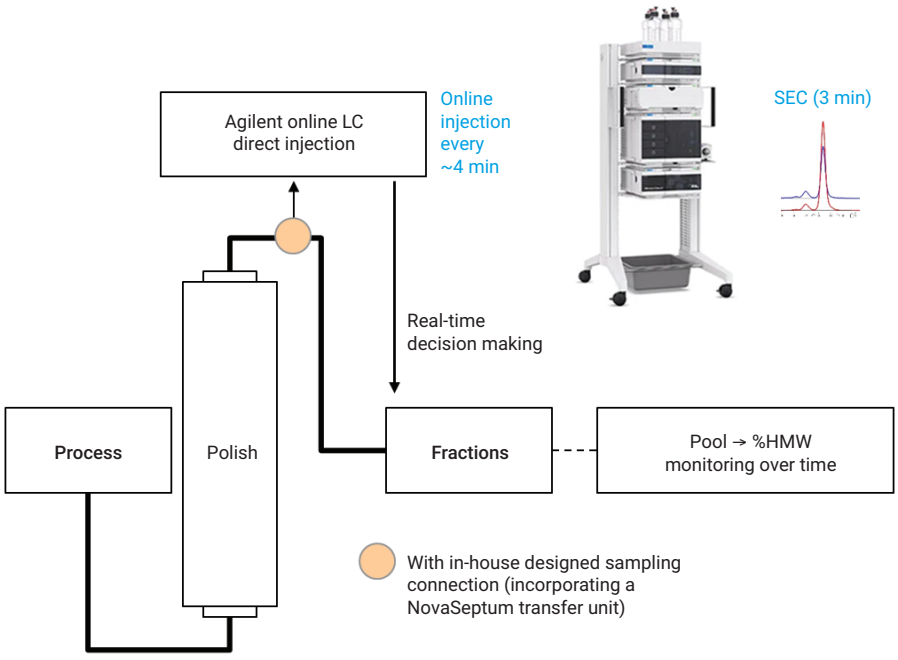

Figure 2. Schematic set up of the Agilent 1260 Infinity II Online LC in the downstream processing of a biopharmaceutical mAb.

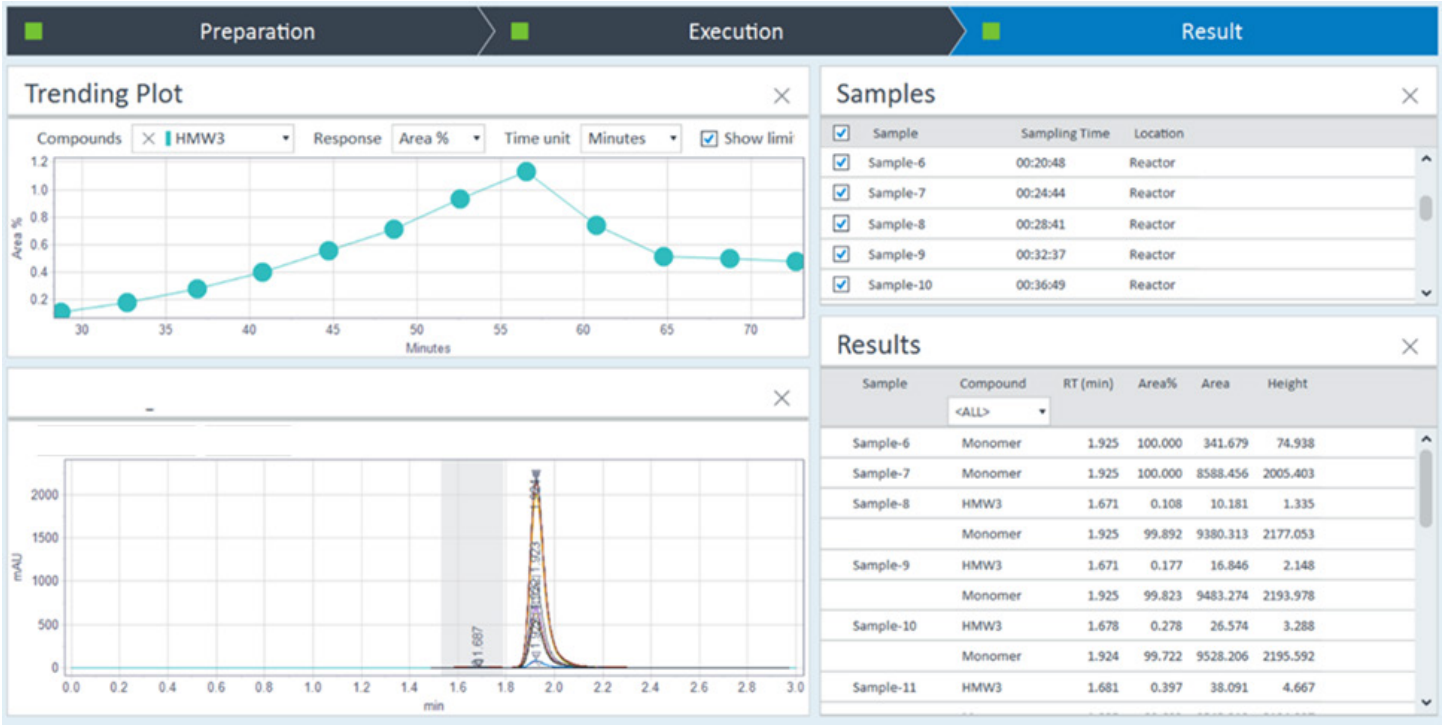

Figure 3. Overview of results displayed in the Agilent Online LC Monitoring Software. The trending plot shows the area percentage of the occurring high molecular weight (HMW) aggregate of the mAb (green). In the Samples tab, the sample number, sampling time, and sample source can be seen. The Results tab displays the detected compounds and analytical results of the samples. The chromatogram displays an overlay of the selected (checked) samples.

The area percentage of the main product, the desired mAb, is displayed in the trending plot in Figure 4A. This plot provides an overview of the content of main product in the samples in contrast to the plot in Figure 3, which shows the area% of the HMW aggregate. The mAb starts to elute from the purification system after 20.48 minutes (sample 6) and the HMW aggregate was detected for the first time after 28.41 minutes (sample 8) with a relation of 99.892 to 0.108% of mAb monomer and HMW aggregate, respectively. A trending plot of the real peak areas displays the elution of the main amount on monomer from 24.44 minutes (sample 7) to 72.40 minutes (sample 19) (Figure 4B).

The Online LC Monitoring Software supports two modes: a controller mode, where it actively dictates the sampling schedule set up by the user, and a receiver mode, where it waits for an external trigger/start signal. In both modes, the data generated by the Online LC system can be used for feedback-looping.

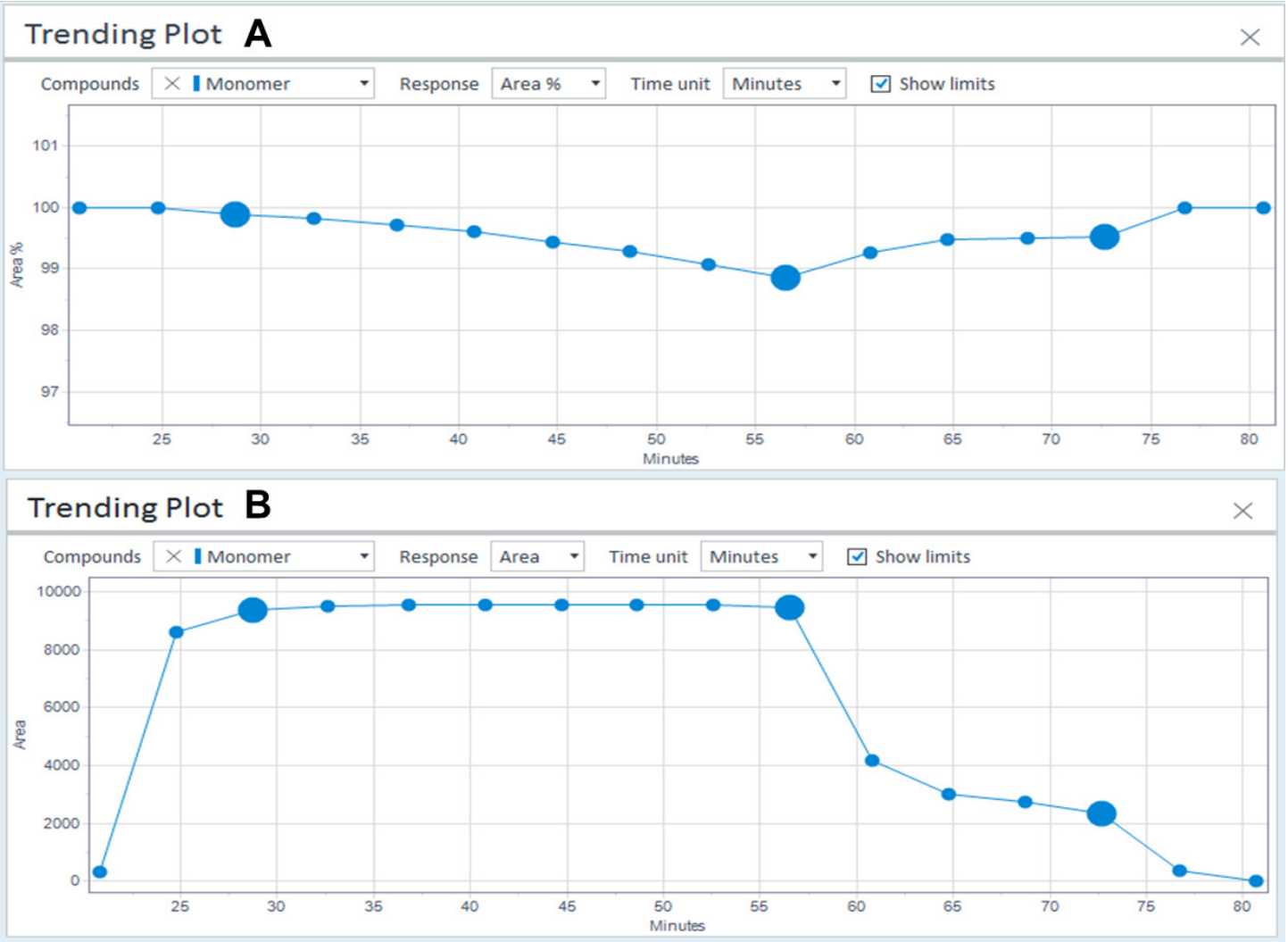

Figure 4. The trending plot A shows the area percentage of the mAb in the collected fractions (blue). Trending plot B shows the peak area of the main peak occurring in the drawn sample and drawn fractions.

Conclusion

This application note demonstrates the use of the Agilent 1260 Infinity II Online LC with the Agilent Online LC Monitoring Software in the downstream processing of a therapeutic mAb. The resultant data, obtained in near real time, can be used to aid pooling decisions to achieve predefined accepted maximum threshold limits of product impurities, such as in the HMW aggregates example presented here. This online monitoring approach saves time and reduces costs when compared to offline approaches, and aids in achieving improved product quality and process confidence.

References

1. Scheidecker, B.; Braaz, R.; Vinnemeier, J. Fluid Dynamic Sampling Site Characterization Improves Process Correlation During Continuous Online Sampling. *J. Pharm. Innov.* **2020**. https://doi.org/10.1007/s12247-020-09458-w

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