Application Note

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Multiple Critical Quality Attributes Assessment of mAbs for Process Control

Agilent InfinityLab Online LC Solution for automated heart-cutting 2D-LC experiments

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Abstract

This application note demonstrates the capability of the Agilent Online LC Monitoring Software to orchestrate heart-cutting 2D-LC experiments. These experiments use the Agilent 1290 Infinity II 2D-LC System as an online LC for process control in heart-cutting mode. As an example, the analysis of a monoclonal antibody (mAb) for titer determination in the first dimension (^{1}D) will be shown. And, in the second dimension (^{2}D), aggregates by size exclusion chromatography (SEC) as well as charge variants by ion-exchange chromatography (IEC) will be shown. The obtained results will be analyzed and displayed directly in the Agilent Online LC Monitoring Software.

Introduction

Monoclonal antibodies (mAbs) belong to one of the most important biopharmaceutical drug classes. A monoclonal antibody is uniform on a molecular level, but during production, purification, and formulation, changes might happen, which can compromise their efficacy and safety. These changes may include the formation of higher molecular weight aggregates like dimers or trimers or modifications resulting in charge variants (for example, through deamidation). These modifications are two of the most important critical quality attributes (CQAs). Therefore, it is important to control the CQAs of an mAb during production (upstream) as well as during and after purification and formulation (downstream).

The Agilent InfinityLab Online LC Solution is a versatile tool for the automated control of the product quality of mAbs and is capable to analyze the CQAs during the production or the purification process.[1] For the analysis of mAb CQAs, a two-dimensional heart-cutting LC analysis can be applied with online analytics. The combination of Protein A affinity chromatography in the first dimension and a method for the determination of a quality attribute in the second dimension enhances efficiency in contrast to separated workflows. The combined automated workflow reduces analysis time and cost especially if used in process analytical technology (PAT) for up- and/or downstream product quality assessment.[2]

The protein A affinity chromatography in the first dimension is applied for titer determination and/or purification for the subsequent analyses. The effluent from the Protein A column is collected in a loop and transferred to the second-dimension column to separate the mAb on, for example, a size exclusion or ion-exchange column.

This setup allows the determination of titer and aggregates or titer and charge variants in one method at a time. For the determination of more than one CQA, the LC can be equipped with a column switching valve in the second dimension to combine different separation mechanism columns with the first-dimension Protein A affinity chromatography. This approach has already been shown for the determination of three CQAs by three different columns in the second dimension.[3]

The present application note demonstrates the use of the Agilent Online LC Monitoring Software for online process control by an Agilent Online LC applied for a two-dimensional heart-cutting experiment for the determination of two CQAs of an mAb. This setup enables complete automation of up- and downstream online process monitoring of mAb production for their CQAs in an economic and time saving fashion and enables automated intact multi-attribute method (MAM) analysis during the bioprocess.

Experimental

Instrument

- Two Agilent 1290 Infinity II High-Speed Pumps (G7120A)
- Agilent 1260 Infinity II Online Sample Manager, G3167A, clustered with external valve, 5067-6680, in the Agilent 1290 Infinity Valve Drive (G1170A)
- Thermostat for 1260 Infinity II Online Sample Manager (G7167-60005)
- Agilent 1290 Infinity II Multicolumn Thermostat (MCT, G7116B) equipped with an Agilent InfinityLab Quick Change 4-position/10-port Valve, 800 bar (G4237A)
- First-dimension DAD: Agilent 1290 Infinity II DAD (G7117B) equipped with an Agilent InfinityLab Max-Light Cartridge Cell, 3.7 mm (G4212-60032)
- Second-dimension DAD: Agilent 1290 Infinity II DAD (G7117B) equipped with an Agilent InfinityLab Max-Light Cartridge Cell, 10 mm (G4212-60008)
- Agilent 1290 Infinity Valve Drive (G1170A) with Agilent InfinityLab 2D-LC Valve (G4236A)

Software

- Agilent OpenLab CDS, version 2.6
- Agilent 2D-LC Software for OpenLab CDS, version 1.0
- Agilent Online LC Monitoring Software, version 1.0

Columns

- Bio-Monolith Protein A Column, 5.2 × 5 mm (part number 5069-3639)
- Agilent AdvanceBio SEC 300Å, 7.8 × 300 mm, 2.7 µm (part number PL1180-5301)
- Bio MAb NP5, 2.1 × 250 mm, 5 µm, PEEK (part number 5190-2411)

Agilent InfinityLab 2D-LC ProtA-SEC Kit[4]

The InfinityLab 2D-LC ProtA-SEC Kit (G4245A) includes columns for both dimensions (Protein A and SEC), tailored sample loops, methods on a memory stick, documentation, and a NISTmAb standard for demonstrating and practicing this workflow.

Instrumental setup

The 2D-LC instrument was set up in recommended standard configuration for single heart-cutting experiments with one 180 µL loop (5004-0036) installed to the 2D-LC valve. To use the 2D-LC instrument for online LC experiments with the Agilent Online LC Monitoring Software, the Agilent 1260 Infinity II Online Sample Manager was introduced.

For the use of two different columns in the second dimension, a four-column selection valve was used in the MCT. The Online LC Sample Manager was connected to the sample delivery pump or device. If a bioreactor is connected, the sample delivery device must draw sample in an aseptic manner and filter out cells before the reactor solution is transported to the online sample manager.

Methods

- The method for the combination of first-dimension Protein A chromatography and second-dimension size exclusion chromatography is described in detail in the Agilent InfinityLab 2D-LC ProtA-SEC Kit.[4]
- The method for the combination of first-dimension Protein A and second-dimension ion-exchange chromatography is described in an earlier application note.[1]
- Blank runs were applied before each 2D-LC analysis to flush the system and the columns with the according starting conditions.

Sampling

Samples were drawn by direct injection mode from a solution of rituximab (7 g/L) connected to the reactor interface valve, which was controlled by the Online LC Monitoring Software. The applied injection volume was 10 µL using a draw speed of 100 µL/min.

Scheduling

Before the analysis of the combinations of Protein A/SEC and Protein A/IEC, an appropriate blank was run. The complete set of blank and analytical 2D-LC runs was repeated 12 times in three-hour intervals. The complete experiment took 1 day and 10 hours (Table 1).

Samples

- Rituximab stock solution (10 g/L)
- Rituximab sample solution, 7 g/L, dilution in phosphate buffered saline (PBS) solution
- Calibration: 0.2, 1.0, 2.0, 10.0 g/L Rituximab, diluted in PBS solution

Solvents and chemicals

- Di- and monobasic sodium phosphate, sodium chloride, and acetic acid were used.
- PBS was prepared as follows: one tablet dissolved in 200 mL of de-ionized water yields 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride, pH 7.4, at 25 °C.
- All solvents were purchased from Merck, Germany.
- Chemicals were purchased from VWR, Germany.
- Fresh ultrapure water was obtained from a Milli-Q Integral Water Purification System equipped with LC-Pak polisher and a 0.22 µm membrane point-of-use cartridge (Millipak).

Results and discussion

To run the online 2D-LC heart-cutting experiment for the determination of multiple CQAs of mAbs, two methods were developed. Both methods use a Protein A column for enrichment of the mAb and determination of the titer in the first dimension. The mAb was retained on the Protein A column with binding buffer, and after flushing out all impurities, the mAb was released completely and detected by DAD. The effluent was collected in a 180 µL loop and transferred later to the second-dimension separation. The transfer was done for subsequent SEC and IEC chromatography, accessible through a column selection valve in the MCT. The sampling and scheduling of the individual runs were orchestrated by the Agilent Online LC Monitoring Software. The obtained data could be monitored in real time during acquisition. To mimic an upstream bioreactor scenario, a pure solution of the mAb rituximab was used directly connected with a flask to the reactor interface valve. The sample was drawn in direct injection mode. Alternatively, a dilution or undiluted sampling is also possible. In practice, a bioreactor would be connected to the online LC by a specialized sampling device for drawing sample, filtration, and transportation in an aseptic manner.

Table 1. Scheduling of blank and analytical runs in the Agilent Online LC Monitoring Software (Direct Injection Setting 01: Protein A/SEC, Direct Injection Setting 02: Protein A/IEC).

Type	Setting	Start time	Interval	Count	Start last action
Blank Sample	BlankSampleSetting 01	00 d 00 h 00 m	00 d 03 h 00 m	12	01 d 09 h 00 m
Direct injection	DirectInjectionSetting 01	00 d 00 h 20 m	00 d 03 h 00 m	12	01 d 09 h 20 m
Blank Sample	BlankSampleSetting 02	00 d 00 h 40 m	00 d 03 h 00 m	12	01 d 09 h 40 m
Direct injection	DirectInjectionSetting 02	00 d 01 h 15 m	00 d 03 h 00 m	12	01 d 10 h 15 m

To determine the titer of the rituximab solution from the flask during the first dimension, the Protein A column was previously calibrated in the range of 0.2 to 10.0 g/L. The calibration was done by the first-dimension method and the data acquired with the first-dimension DAD. The connected test solution of rituximab had a concentration of 7 g/L. The mAb rituximab purified on the Protein A column eluted at 0.821 minutes and was transferred to the second dimension using a single time-based heart cut from 0.75 to 0.95 minutes (Figure 1).

To monitor the measured concentrations of the first-dimension experiment, the results were monitored by the Online LC Monitoring Software's trending plot for both methods independently (Figure 2). The results for the determined mAb titer showed a concentration of 7.13 g/L (1.15% RSD) for the method set with SEC separation in the second dimension and a titer of 7.17 g/L (0.94% RSD) for the method set with IEC in the second dimension.

In the connected results table, it is possible to select the values obtained for the first-dimension Protein A purification (Table 2). The table shows the uneven sample numbers, which connected the first-dimension Protein A column to the second dimension applying SEC. As can be seen from the table, the incoming mAb was already clean with about 96 area%.

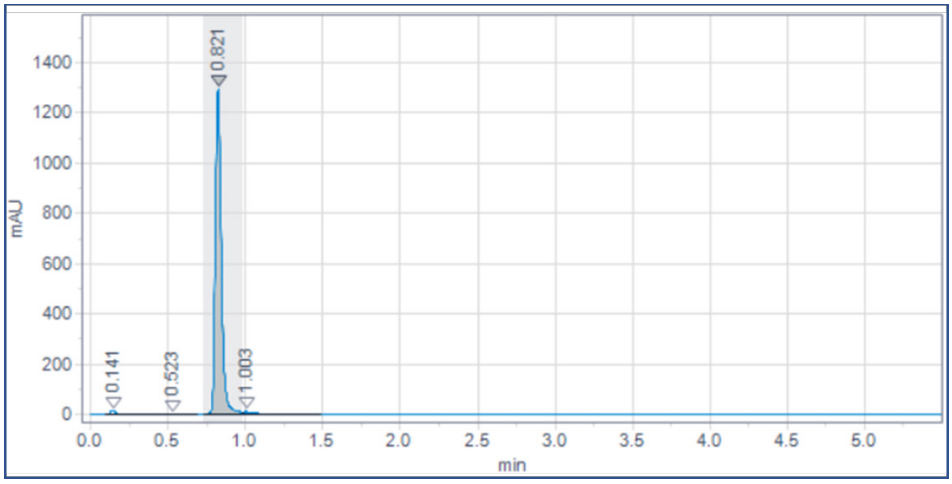

Figure 1. Chromatogram obtained for the elution of the mAb from the Protein A column at 0.821 minutes. The time-based heart cut was done between 0.75 and 0.95 minutes for the transfer to the second dimension.

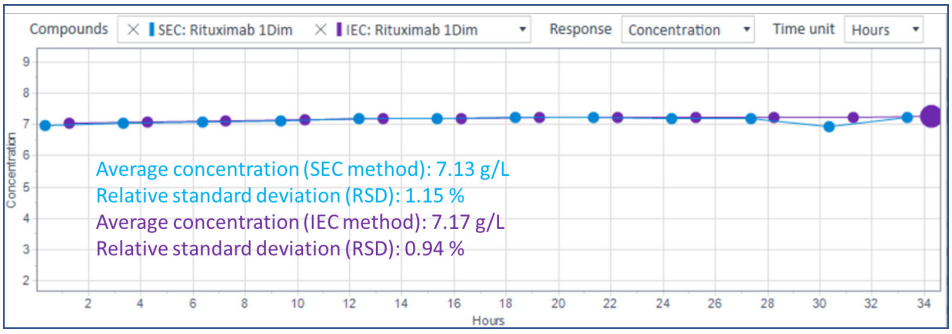

Figure 2. Agilent Online LC Monitoring Software trending plot for the titer determined by Protein A column binding for both applied method sets. SEC: Rituximab 1Dim: Protein A binding separation in the first dimension (blue) followed by SEC in the second dimension. IEC: Rituximab 1Dim: Protein A binding separation in the first dimension (violet) followed by IEC in the second dimension.

Table 2. Values obtained from the selected first-dimension Protein A purification.

Sample	Compound	RT (min)	Area%	Area	Height	Concentration
	SEC: Rituximab 1Dim					
Sample-1	SEC: Rituximab 1Dim	0.821	96.433	3540.456	1292.056	6.967 g/L
Sample-3	SEC: Rituximab 1Dim	0.830	96.248	3577.720	1309.917	7.041 g/L
Sample-5	SEC: Rituximab 1Dim	0.825	96.459	3599.814	1312.146	7.084 g/L
Sample-7	SEC: Rituximab 1Dim	0.829	96.420	3623.753	1322.340	7.131 g/L
Sample-9	SEC: Rituximab 1Dim	0.827	96.534	3650.420	1326.742	7.183 g/L
Sample-11	SEC: Rituximab 1Dim	0.821	96.760	3650.717	1331.793	7.184 g/L

In the connected second dimension, the size exclusion chromatography (SEC) determines the content of earlier eluting mAb aggregates of higher molecular weight (HMW), monomer mAb, and a later eluting lower molecular weight buffer-related compound (Figure 3). The detailed results for the second dimension SEC can also be displayed in the results table (Table 3). The table shows the results obtained for sample 1. The main compound is eluting at 7.930 minutes with a content of 97.714 area%. A higher molecular weight aggregate elutes at 7.063 minutes with 0.160 area%, and a lower molecular weight buffer-related compound elutes at 11.011 minutes with 2.127 area%.

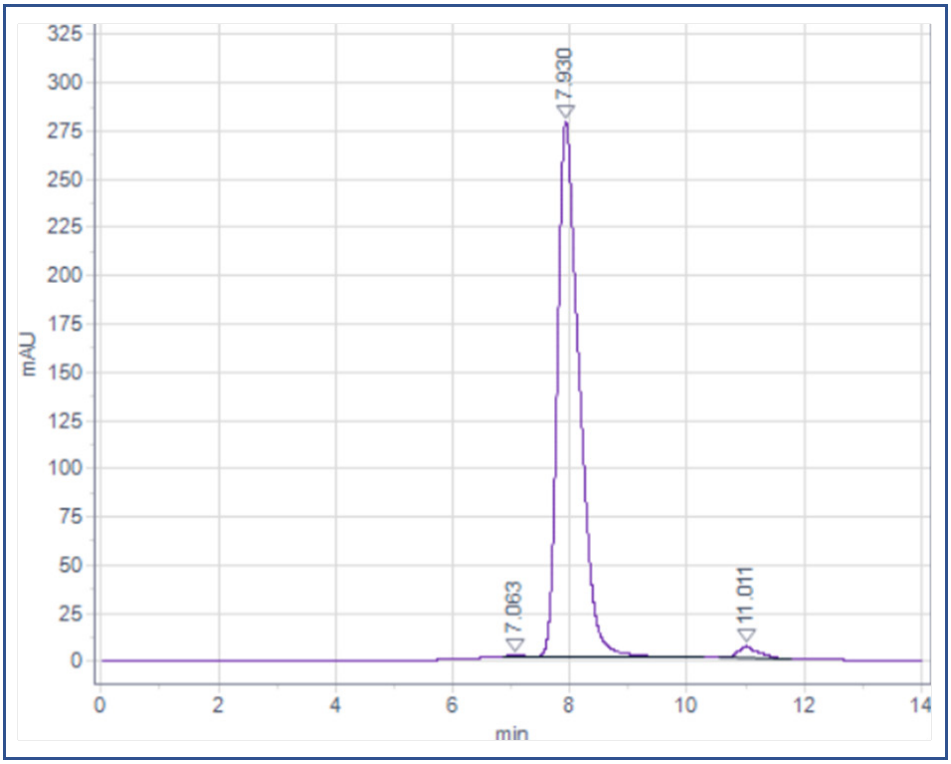

Figure 3. Second-dimension SEC analysis of the mAb purified from the first-dimension Protein A column.

Table 3. Second-dimension SEC determined from the second-dimension DAD used for the analysis of the cut from the Protein A elution.

Sample	Signal	RT (min)	Area%	Area	Height
	DAD2A Cut 1				
Sample-1	DAD2A Cut 1	7.063	0.160	11.674	0.541
	DAD2A Cut 1	7.930	97.714	7146.342	277.839
	DAD2A Cut 1	11.011	2.127	155.548	6.247

For the even sample numbers, the first-dimension Protein A purification was connected to ion-exchange chromatography in the second dimension by the column selection valve in the MCT. The elution from the ion-exchange column shows the eluting mAb at 24.889 minutes and the charge variants of the mAb with earlier eluting acidic and later eluting basic variants (Figure 4). The main peak has an area percentage of 83.945. The remaining is allotted to earlier and later eluting variants (Table 4).

An overview of the second-dimension SEC and IEC could be displayed by the trending plot of the obtained area percentage of the mAb monomer from SEC and the main charge variant from IEC (Figure 5). This view enables an at-a-glance overview of the obtained product quality for all analyzed CQAs. Here, the analyzed mAb has a monomer purity of about 98% and about 84% of charge variant purity.

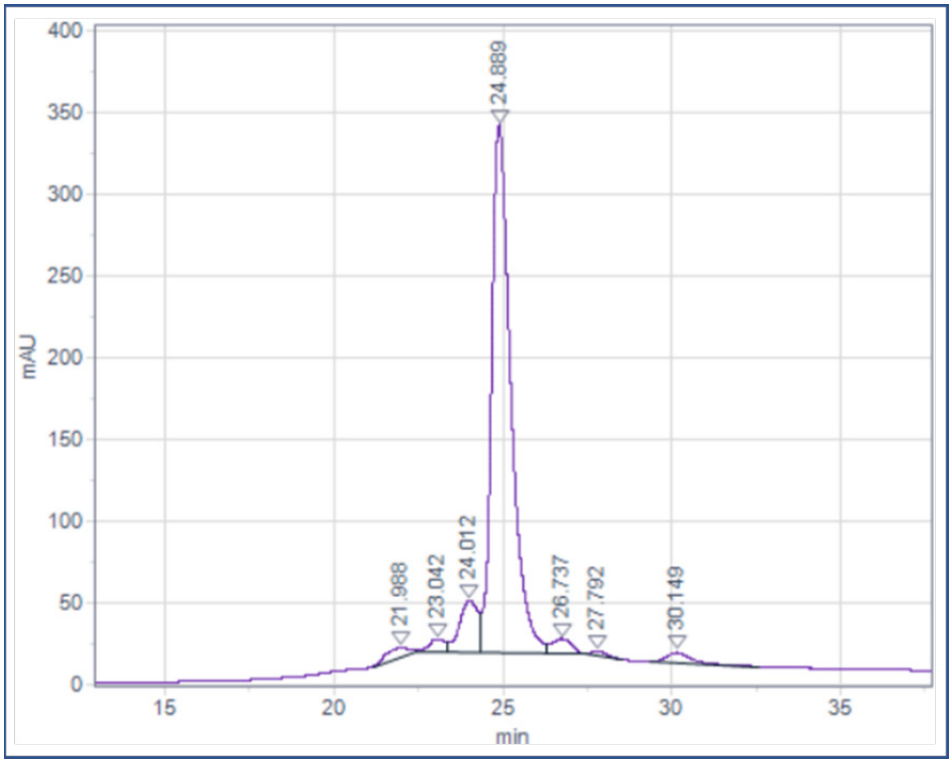

Figure 4. Second-dimension IEC analysis of the mAb purified from the first-dimension Protein A column.

Table 4. Second-dimension IEC determined from the second-dimension DAD used for the analysis of the cut from the Protein A elution.

Sample	Signal	RT (min)	Area%	Area	Height
	DAD2A Cut 1				
Sample-2	DAD2A Cut 1	21.988	1.659	253.556	5.269
	DAD2A Cut 1	23.042	1.564	239.065	7.400
	DAD2A Cut 1	24.012	7.682	1174.180	31.442
	DAD2A Cut 1	24.889	83.945	12830.116	322.719
	DAD2A Cut 1	26.737	2.523	385.579	9.093
	DAD2A Cut 1	27.792	0.620	94.708	3.075
	DAD2A Cut 1	30.149	2.007	306.694	6.253

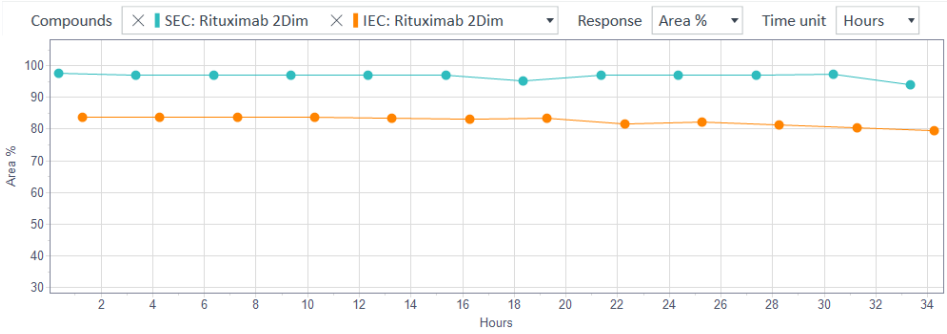

Figure 5. Trending plot of the area percentage obtained for the mAb monomer from second-dimension SEC (turquoise) and the main charge variant from second dimension IEC over all collected samples.

Conclusion

This application note demonstrates the use of the Agilent Online LC Monitoring Software to control an automated heart-cutting 2D-LC experiment by the Agilent InfinityLab Online LC Solution for online process control in the production or purification of mAbs. The first-dimension Protein A separation was used to quantitatively determine the titer of the mAb. In the second dimension, a column switching valve was used to switch between an SEC and an IEC column for the determination of HMW aggregates and the charge variant distribution of the mAb. All results from the first and second dimension can be displayed at-a-glance in the Agilent Online LC Monitoring Software in near real time. This setup enables time and cost savings during the production of mAbs and provides a tool to perform intact MAM analysis of CQAs directly during upstream or downstream bioprocessing.

References

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