Application Note

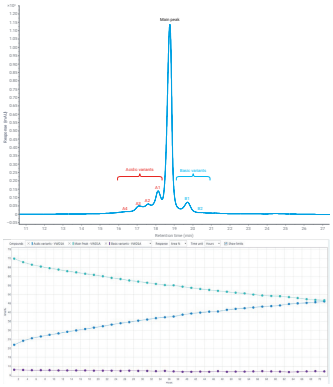
Biopharma/Pharma

Agilent

Trusted Answers

# Online Monitoring of Monoclonal Antibody Degradation

## Process analytics with the Agilent 1290 Infinity II Bio Online LC System




**Authors**

Adem Cakar and
Edgar Naegele
Agilent Technologies, Inc.



## Abstract

This application note demonstrates the capability of monitoring the degradation of a monoclonal antibody (mAb) during production in a bioreactor. The Agilent 1290 Infinity II Bio Online LC System and Agilent Online LC Monitoring Software are used during the process.

Degradation of mAbs can have a negative impact on product quality, safety, and efficacy and must therefore be recognized and controlled when it occurs. Here, the analysis of a forced deamidation and the monitoring of the consequential degradation over three days using charge variant analysis is shown.

## Introduction

MAbs can change their properties through certain chemical degradation processes such as oxidation, deamidation, isomerization, and fragmentation, which influences charge variants, heterogeneity and, accordingly, the isoelectric point.[1] This can negatively affect product quality, safety, and efficacy and must be detected when it occurs.[2] Therefore, a correspondingly fast and automated quality check is indispensable.

Modification such as deamidation leads to an increased negative charge on the mAb and can cause a decrease in isoelectric pH (pI) values, which can lead to acidic variants.[1] Alternatively, modification such as oxidation leads to an increased positive charge on the mAb and can increase the pI value, which can lead to basic variants.[1] These modifications can be induced by harsh conditions, for example, increasing pH value and temperature to generate deamidation or by adding hydrogen peroxide to generate oxidation modifications.[1] This method is also known as forced degradation and is often used for method development, qualification and transfer, critical quality attributes (CQA) evaluation, and the identification of product variants.[2]

This application note demonstrates the use of the 1290 Infinity II Bio Online LC to monitor the product quality of an mAb during the manufacturing process. For this purpose, a forced deamidation with increased pH value and increased temperature was carried out in a vial that serves as a substitute for a bioreactor. This degradation was determined by a charge variant analysis method, which was developed with Agilent Buffer Advisor Software.

## Experimental

### Equipment

The 1290 Infinity II Bio Online LC comprised the following modules:

- Agilent 1290 Infinity II Bio Flexible Pump (G7131A)
- Agilent 1290 Infinity II Bio Online Sample Manager (G3167B) clustered with an Agilent 1290 Infinity Valve Drive (G1170A), featuring a reactor valve pod (part number 5067-6680) and Online LC Monitoring Software
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) equipped with an Agilent Quick Connect Bio Heat Exchanger Standard Flow (G7116-60071)
- Agilent 1290 Infinity II Variable Wavelength Detector (VWD) (G7114B), equipped with a Bio Micro Flow Cell VWD, 3 mm, 2 µL, RFID.

### Software

- Agilent OpenLab CDS version 2.6 or later versions
- Agilent Online LC Monitoring Software version 1.2 or later versions
- Agilent Buffer Advisor version 1.01.01 or later versions

### Columns

Agilent Bio MAb, NP5, 2.1 × 250 mm, 5 µm, PEEK (part number 5190-2411)

### Chemicals

Ammonia solution and sodium chloride were purchased from Merck (Darmstadt, Germany). Sodium phosphate monobasic and sodium phosphate dibasic were purchased from Sigma-Aldrich (Steinheim, 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).

### Sample

MabThera (rituximab) was purchased from Roche (Penzberg, Germany).

### Sample preparation

To obtain a final concentration of 5 mg/L rituximab with a pH value between 9 and 10, 100 µL of rituximab (10 mg/L) was diluted with 100 µL of ammonia solution (600 µM). The sample was heated to 37 °C and the temperature was maintained throughout the experiment. High pH and temperature were necessary to induce deamidation.

## Methods

**Table 1.** Chromatographic conditions.

<table>
<tr><th>Parameter</th><th>Value</th></tr>
<tr><td>Column</td><td>Agilent Bio MAb, NP5, 2.1 × 250 mm, 5 µm, PEEK</td></tr>
<tr><td>Solvent</td><td>A) Water<br>B) 1.7 M NaCl<br>C) 3.9 mM $NaH_2PO_4$<br>D) 21.5 mM $Na_2HPO_4$</td></tr>
<tr><td>Gradient</td><td>
<table>
<tr><th rowspan="2">Time (min)</th><th rowspan="2">Buffer (mM)</th><th rowspan="2">NaCl (mM)</th><th rowspan="2">pH</th><th colspan="4">Software-Calculated Mobile Phase Compositions</th></tr>
<tr><th>A%</th><th>B%</th><th>C%</th><th>D%</th></tr>
<tr><td>0</td><td>10</td><td>10</td><td>7.65</td><td>16.7</td><td>0.6</td><td>44.2</td><td>38.5</td></tr>
<tr><td>20</td><td>10</td><td>90</td><td>7.65</td><td>22.1</td><td>5.3</td><td>31.9</td><td>40.7</td></tr>
<tr><td>20.01</td><td>10</td><td>500</td><td>7.65</td><td>11.2</td><td>29.4</td><td>15.7</td><td>43.7</td></tr>
<tr><td>30</td><td>10</td><td>500</td><td>7.65</td><td>11.2</td><td>29.4</td><td>15.7</td><td>43.7</td></tr>
<tr><td>30.01</td><td>10</td><td>10</td><td>7.65</td><td>16.7</td><td>0.6</td><td>44.2</td><td>38.5</td></tr>
<tr><td>40</td><td>10</td><td>10</td><td>7.65</td><td>16.7</td><td>0.6</td><td>44.2</td><td>38.5</td></tr>
</table>
Stop time: 40 min<br>Post-time: 0 min
</td></tr>
<tr><td>Flow Rate</td><td>0.200 mL/min</td></tr>
<tr><td>Temperature</td><td>30 °C</td></tr>
<tr><td>Detection</td><td>VWD: 280 nm, 10 Hz</td></tr>
<tr><td>Injection</td><td>Injection volume: 4 µL<br>Sample temperature: 37 °C<br>Wash: 3 s in water (flush port)</td></tr>
</table>

**Table 2.** Online LC Monitoring Software conditions.

<table>
<tr><th>Parameter</th><th>Value</th></tr>
<tr><td>Sampling</td><td>Direct injection</td></tr>
<tr><td>Sampling Source</td><td>Vial</td></tr>
<tr><td>Sampling Speed</td><td>Setting 1<br>Draw speed: 130 µL/min</td></tr>
<tr><td>Schedule</td><td>
<table>
<tr><th>Type</th><th>Setting</th><th>Start Time</th><th>Interval</th><th>Count</th><th>Start Last Action</th></tr>
<tr><td>Blank sample</td><td>Blank no injection</td><td>00 d 00 h 00 m</td><td>00 d 00 h 00 m</td><td>1</td><td>00 d 00 h 00 m</td></tr>
<tr><td>Direct injection</td><td>Rituximab</td><td>00 d 01 h 00 m</td><td>00 d 02 h 00 m</td><td>36</td><td>02 d 23 h 00 m</td></tr>
</table>
</td></tr>
</table>

## Results and discussion

To monitor the degradation process of the mAb with the Bio Online LC, it was necessary to induce a degradation. The mAb was treated with ammonia solution to obtain a high pH, and the temperature was increased in the Bio Online Sample Manager to force deamidation modifications, leading to an increase in acidic variants and a decrease in the main peak. In this experiment, the sample vial was used as a replacement for a bioreactor. Because of this, a method was required to separate the charge variants of the mAb. For this purpose, a cation exchange method was used, including a standard-flow biocompatible heat exchanger with a Bio MAb, NP5, 2.1 × 250 mm, 5 µm, PEEK column at 30 °C.

The forced deamidation of the mAb was necessary to demonstrate the capability of the 1290 Infinity II Bio Online LC to monitor the entire process of deamidation. Analysis was done over three days with sample injection every two hours to ensure that the deamidation process was traceable over a longer period. To simplify data analysis and facilitate visualization, several acidic peaks were combined into one acidic variant, and basic peaks were treated in the same way (see Figure 1).

The sample initially shows higher levels of acidic variants, as is usual for a therapeutic mAb. Long storage in the refrigerator at 2 to 8 °C after opening and resealing the sample several times could be the reason for this.

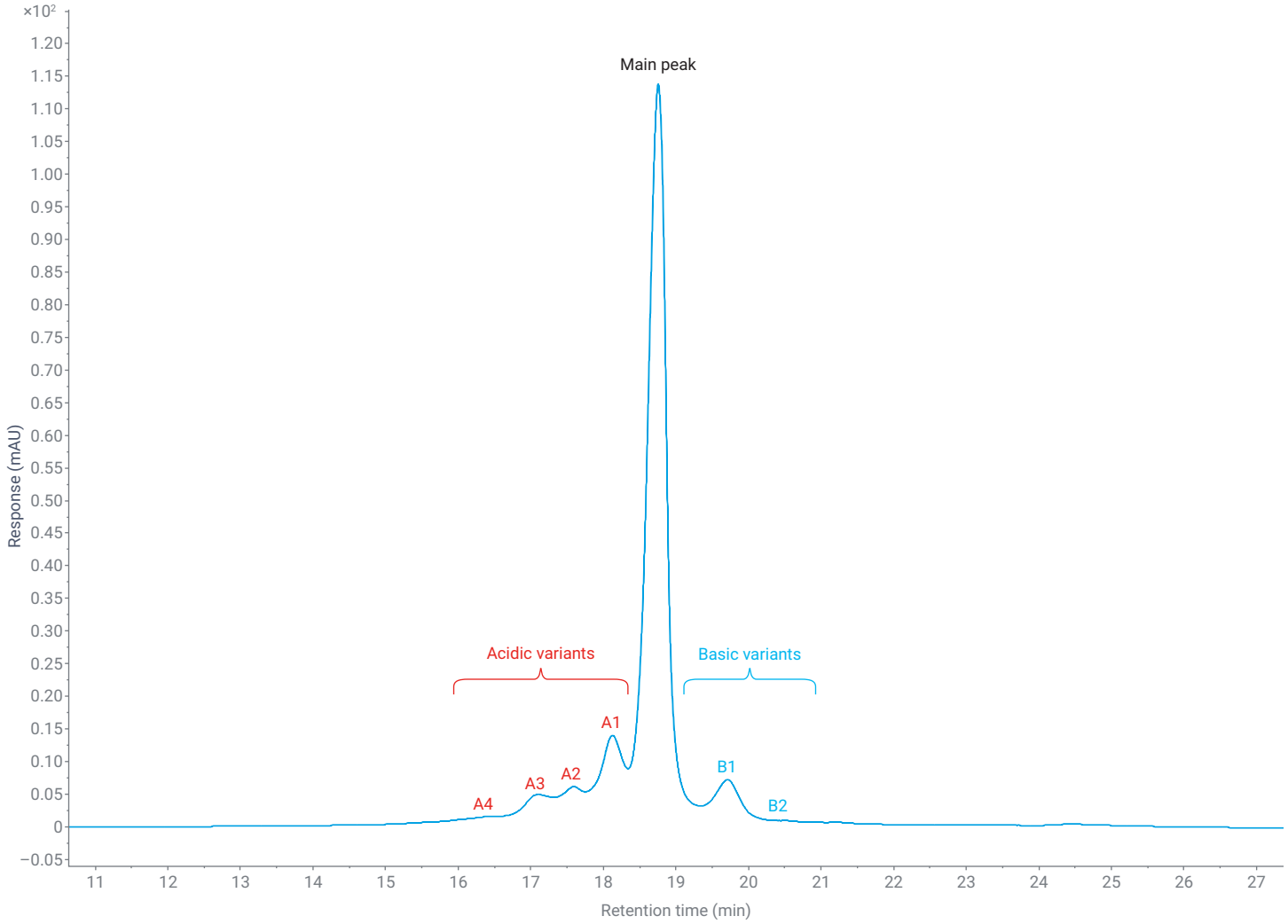



**Figure 1.** Cation exchange chromatogram of rituximab separated by an Agilent 1290 Infinity II Bio Online LC with an Agilent Bio MAb, NP5, 2.1 × 250 mm, 5 µm, PEEK column. The peaks are summarized as acidic variants, main peak, and basic variants for better comparison and visualization.

To set up the experiment, the Online Monitoring Software was used. This software can schedule a time-based sample analysis, which enables monitoring of the reaction or, in this case, a degradation of a mAb. The Online LC Monitoring Software is versatile and offers numerous options to customize experiments in terms of handling and quantifying samples. The specific features of the Online LC Monitoring Software have already been published.[3]

Figure 2 shows an overlay of charge variant analysis of rituximab at different time points after adding ammonia solution to the sample. The main peak decreases sharply over time, and the basic variants decrease slightly. The acidic variants increase sharply as a result. To ensure a good overview, only four data points were shown in Figure 2.

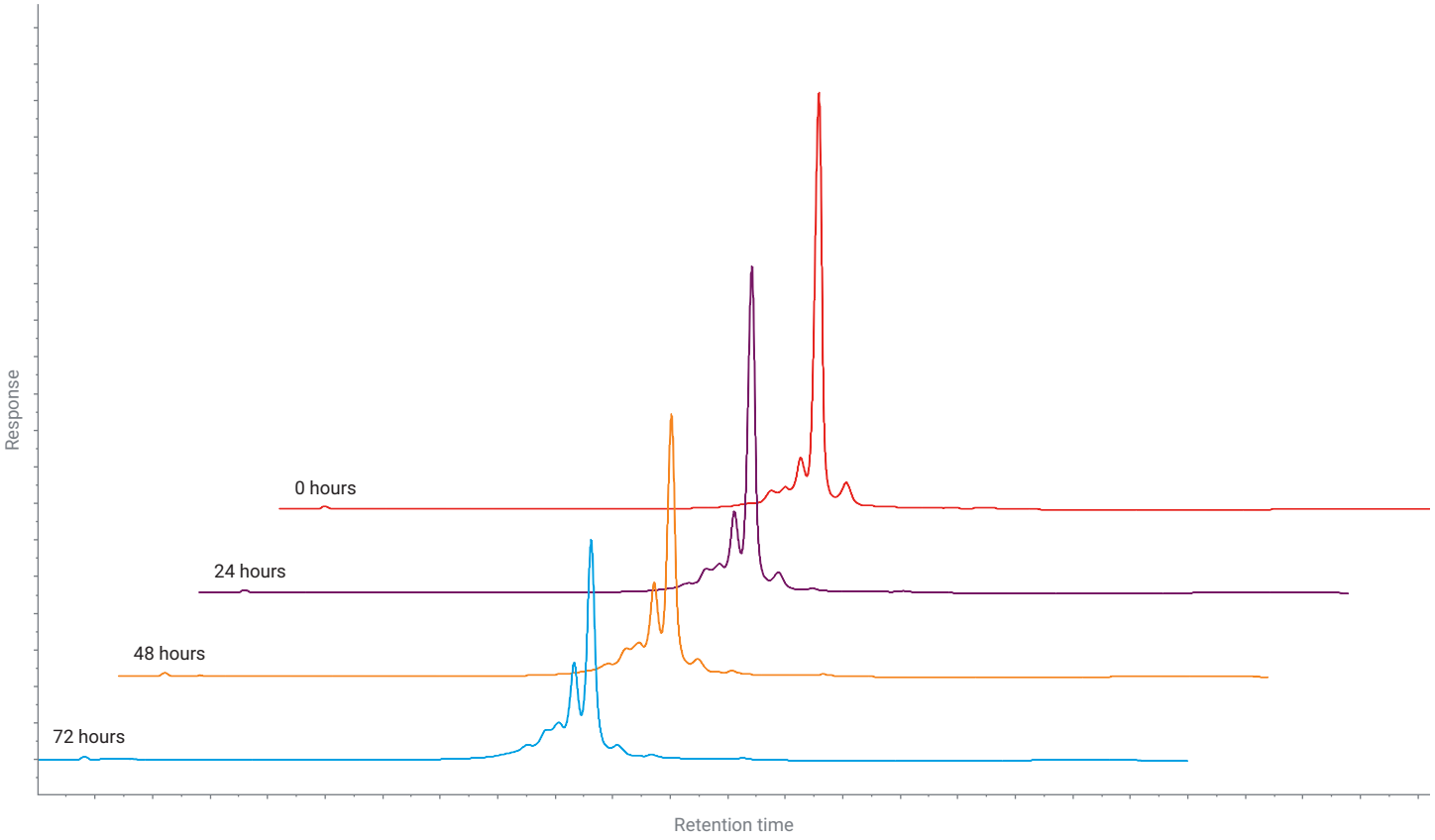



**Figure 2.** Comparison of charge variants analysis of rituximab at high pH and 37 °C at different time points.

The Online LC Monitoring Software is also capable of processing and displaying data in real time. Figure 3 shows a trending plot with the main peak decrease, which is depicted in green. The increase of acidic variants is depicted in blue, and the slight decrease of basic variants is shown in purple.

Furthermore, the 1290 Infinity II Bio Online LC shows generally excellent precision in terms of retention time for the respective acidic peaks, basic peaks, and the main peak over three days, all with RSD values below 0.2% (Table 3). The first injection was excluded because the equilibration of the column was insufficient.

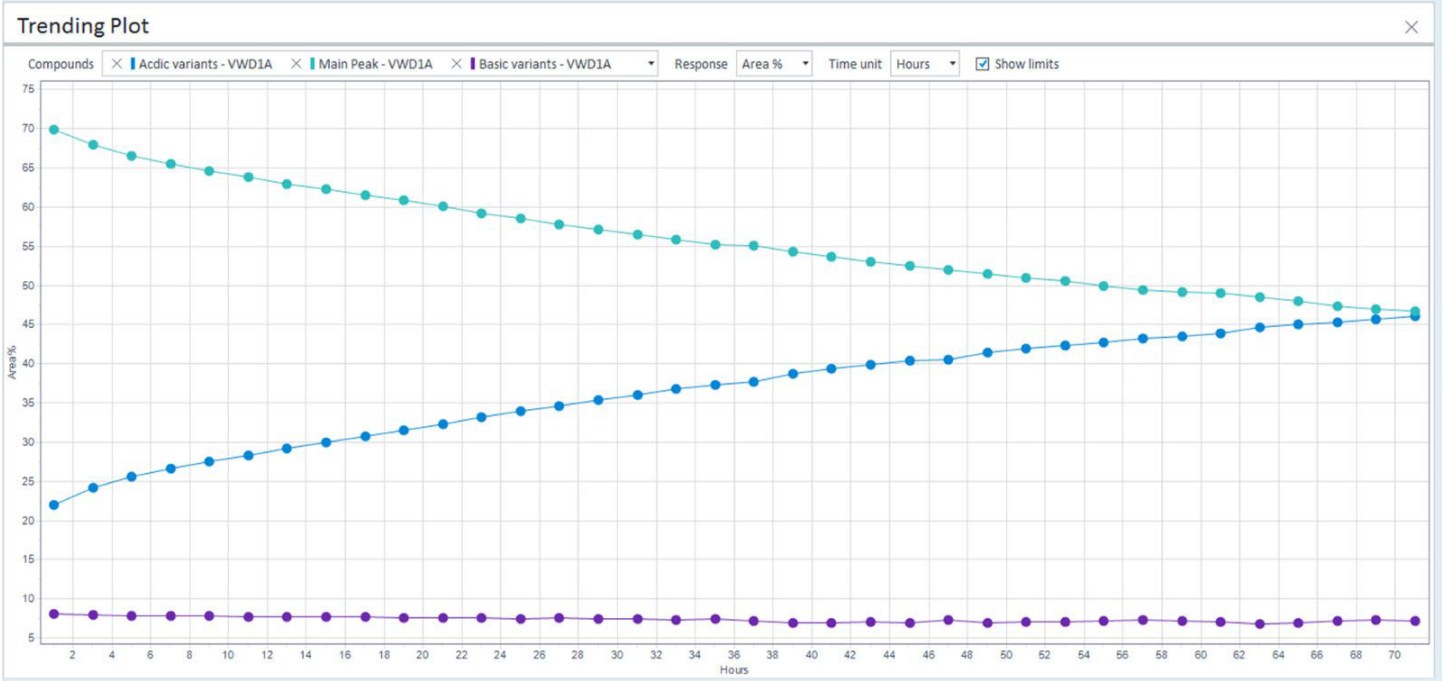



**Figure 3.** Visualization of a forced deamidation of a monoclonal antibody using an Agilent 1290 Infinity II Bio Online LC and Agilent Online LC Monitoring Software. The trending plot shows the decrease of the main peak (green), the slight decrease of basic variants (purple), and the increase of the acidic variants (blue).

**Table 3.** Relative standard deviation (RSD) values of retention time (RT) of acidic peaks, basic peaks, and the main peak over three days (n = 35).

| RT RSD (%) | | | | | | |
|---|---|---|---|---|---|---|
| **A4** | **A3** | **A2** | **A1** | **Main Peak** | **B1** | **B3** |
| 0.19 | 0.12 | 0.16 | 0.12 | 0.13 | 0.14 | 0.19 |

## Conclusion

This application note demonstrates the capability of the Agilent 1290 Infinity II Bio Online LC in combination with Agilent Online LC Monitoring Software to monitor the quality of a monoclonal antibody in a bioreactor over a period of three days. The increase in acidic variants and decrease of the main peak during the experiment was traceable even if there were only minor differences. The system also shows robust retention time stability and separation efficiency over three days of analysis.

## References


1. Khawli, L.; *et al.* Charge Variants in IgG1. *MAbs* **2010**, *2(6)*, 613–624.
2. Nowak, C.; *et al.* Forced Degradation of Recombinant Monoclonal Antibodies: A Practical Guide. *MAbs* **2017**, *9(8)*, 1217–1230.
3. Naegele, E.; Kutscher, D. Online Reaction Monitoring by the Agilent InfinityLab Online LC Solutions. *Agilent Technologies application note*, publication number 5994-3528, **2021**.


**www.agilent.com**


DE02414427





Printed in the USA, January 25, 2024
5994-7091EN


Agilent
Trusted Answers