Biologics quality control

Affinity titer analysis to enable scalable mAb and bispecific production

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Purpose

To monitor critical to quality attributes (CQA) during antibody (mAb) production and provide timely feedback.

Introduction

Timely, robust analytical data is essential for effective mAb process development. While antibody titer is a key metric for monitoring productivity and guiding downstream operations, it does not capture changes in product quality attributes such as charge heterogeneity.

Protein A affinity chromatography enables rapid, selective titer determination directly from complex samples, while also providing a clean input for further analysis. In this work, a two-dimensional (2D) LC approach combines Protein A affinity chromatography with strong cation exchange (SCX) or size exclusion chromatography (SEC) to enable simultaneous measurement of antibody concentration and charge variant distribution.

By linking productivity with product quality in a single workflow, this approach supports faster, data-driven process optimization and helps ensure that improvements in titer do not compromise product quality.

Materials and methods

Sample preparation

A biosimilar version of trastuzumAb (Herceptin™) was produced from Chinese Hamster Ovary (CHO-K1) cells. Samples were aseptically extracted and the culture harvests were clarified by centrifugation at 5000 rpm for 5 minutes. The supernatant was then transferred to autosampler vials and injected directly. IgG standards were prepared by serial dilution using purified IgG and centrifuged as above prior to injection.

System configuration

A Thermo Scientific™ Vanquish™ Flex Simple Switch™ 2D-LC System for loop heart-cutting was used, enabling the transfer of a fraction from a 1D separation to a 2D separation.

Data analysis

Data analysis was performed using Themo Scientific™ Chromeleon™ Chromatography Data System.

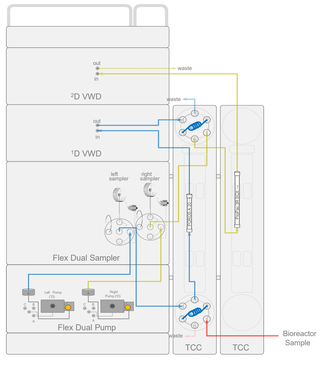

Figure 1: Fluidic scheme of a 2D-LC setup that supports multiple CQA analysis

Methods

First dimension: Affinity

Column: Thermo Scientific™ POROS™ A 20µm HPLC column (PN: 2100100); 2.1 x 30 mm; Inj. Vol.: 20µL; Flow rate: 0.5mL/min (unless otherwise noted); Buffer A: 50 mM ammonium acetate pH 7; Buffer B: 300mM acetic acid, pH 2.6; Gradient: 100% A for 4 min, 100% B for 4 min; 100% A 2 mins; Carry over monitoring 2 min cycles

Second dimension (A): Strong Cation Exchange (SCX)

Column: Thermo Scientific™ ProPac™ 3R SCX 3µm (PN: 43103-054068); 4 x 50 mm, 150 µL via heart cut; Flow rate: 0.3 mL/min; Buffer A: Thermo Scientific™ CX-1 pH Gradient Buffer A, pH 5.6 (PN 303274; Buffer B: Thermo Scientific™ CX-1 pH Gradient Buffer B, pH 10.2 (PN 303275); Gradient: 0% B for 10 mins, 0-100% B in 20 mins, 100% B for 2 min

Second dimension (B): Size exclusion

Column: Thermo Scientific™ MabPac SEC-1 (PN: 088460); 7.8 x 300 mm, Inj. Vol.: 150 µL via heart cut; Flow rate: 0.76 mL/min; Isocratic Buffer: 100 mM Ammonium Acetate pH 5

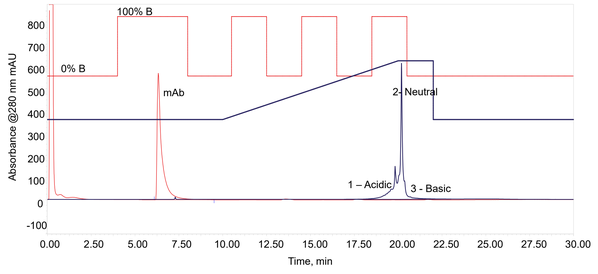

Figure 2. Example overlay of channels monitoring antibody titer via affinity and charge variants via SCX in a single run. Sample taken at day 14

During upstream process development, bioreactor performance is monitored to evaluate cell growth, viability and recombinant protein production. In parallel with standard cell culture parameters (VCD, viability, pH, dissolved oxygen, metabolite profiles), product titer was quantified using a Protein A affinity method.

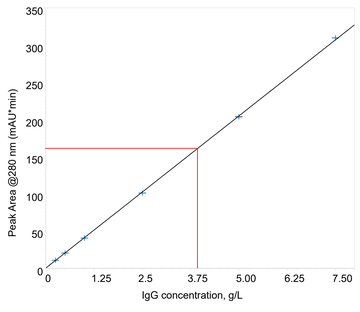

Figure 3. Antibody calibration using purified IgG. Flow rate: 0.5 mL/min

Process monitoring

Protein A HPLC monitoring of product titer

Protein A HPLC enabled rapid, selective mAb titer monitoring directly from bioreactor samples, minimizing matrix interference. Titers exceeded 7 g/L over 14 days. The method supports reproducible quantitation and integration into 2D workflows for simultaneous titer and product characterization.

The POROS A 20 protein A method was tested at both 0.5 and 2 mL/min (calibration data not shown) and samples quantified at both flow rates. The 2 mL/min method enables quantitation in less than 2 mins, whereas 0.5 mL/min method is more compatible with the flow rates required for the SCX and SEC methods. Quantitation was equivalent using both methods showing through can be significantly increased without impact to data quality.

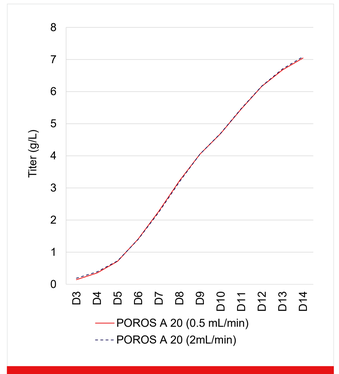

Figure 4: Antibody titer measured during the cell culture process

Product quality monitoring

Charge variant analysis using SCX

SCX separates mAb charge variants to monitor product heterogeneity and quality. Increasing acidic species observed over the 14-day period indicates modifications impacting stability and potency. Tracking these trends informs process optimization and supports CQA monitoring to ensure consistent product quality and regulatory compliance.

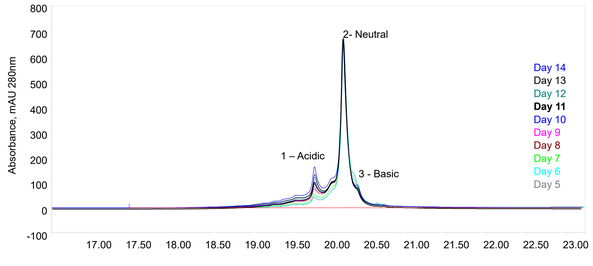

Figure 5. Acid, neutral and basic variants from day 5 to day 14. Signal normalized to the neutral variant

Analysis of aggregation using SEC

Switching from SCX to SEC allows for monitoring mAb aggregation, a critical quality attribute impacting stability, efficacy, and immunogenicity. Increased high molecular weight species after day 8 indicates process-related stress or extended residence time. Tracking aggregation upstream enables timely process adjustments and supports CQA monitoring to facilitate consistent product quality and downstream purification efficiency.

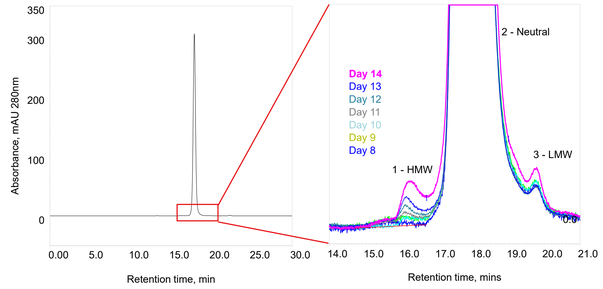

Figure 6: High and low molecular weight specifies monitored from day 5 to day 14. Signal normalized to the main IgG peak. Data prior to day 8 not shown

Conclusions

- **Integrated analytics enable better decisions:** Combining Protein A titer with SCX and SEC facilitates simultaneous insight into productivity and product quality from the same sample
- **Upstream conditions directly impact CQAs:** Increases in acidic variants and aggregates over time highlight the importance of monitoring culture duration and conditions to control product quality.
- **Monitoring supports process control and compliance:** These methods support data-driven optimization and serve as CQA monitoring tools to assess product consistency, safety, and quality throughout development and manufacturing.

References

- **ICH Q11** – *Development and Manufacture of Drug Substances*
- **ICH Q6B** – *Specifications: Test Procedures and Acceptance Criteria for Biotechnological Products*

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