HaLCon, a Fit for Purpose, Platform Method for Rapid Antibody Concentration Measurements from Cell-Free Culture Media

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Abstract

In this study, we utilized HaLCon, a fit-for-purpose protein A liquid chromatography system that generates protein (IgG antibody) titer information for biologic drugs directly from a cell-free sample from a bioreactor. This study will demonstrate the rapid time to result, wide dynamic range, comparative data with HPLC and BLI, and the additional information provided by tracking antibody concentration alongside cell viability. HaLCon is a plug-and-play system that requires no method optimization, automates all washing and elution steps, and provides g/L protein concentration measurements in less than 5 minutes from sample injection. HaLCon can be utilized as a stand-alone system providing a protein titer measurement in less than 5 minutes, or connected to an on-line autosampler for hands-free aseptic sampling at user defined intervals, or connected to a traditional autosampler enabling hands-free batch analysis of cell free culture samples.

Figure 1. Simple workflow associated with measuring a single cell free culture sample, minimal training and no chromatography expertise required.

Introduction

RedShift BioAnalytics is a forward-thinking technology company providing novel life science analytical platforms, reagents, software, and services to leading biopharmaceutical companies and research laboratories. RedShiftBio has recently launched the HaLCon Protein Analyzer, which is a completely automated liquid chromatography system, designed to be a simple and user-friendly tool for measuring antibody titers in process development and manufacturing environments. HaLCon removes the bottlenecks associated with HPLC set-up time, HPLC training/expertise, sending samples to an analytical core facility, and provides a less than 5-minute sample load to antibody concentration measurement with great correlation to the gold standard HPLC protein A chromatography. Data will be presented monitoring protein titer across an Adalimumab manufacturing run.

A

B

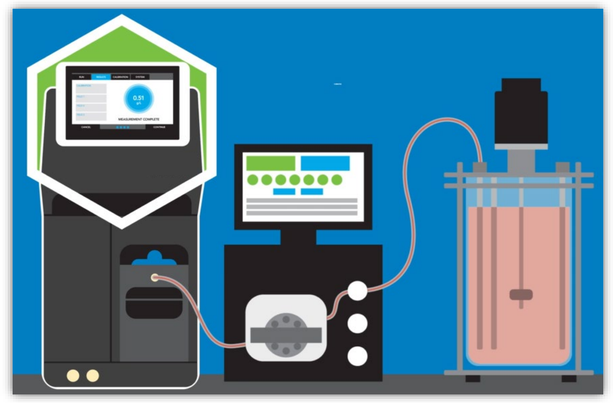


Figure 2. A Standalone HaLCon instrument with integrated computer touchscreen interface. Dedicated analysis module and reagent pack made for a simple user experience. **B** HaLCon can integrate with an external autosampler for automated in-line antibody concentration measurements

Results

Calibration Curve for Human IgG + Reproducibility

Figure 3 illustrates the wide dynamic range and linearity, from less than 0.1g/L to 5g/L with an R^2 of 0.999. The true upper limit of quantitation is 10g/L, but CHO cell line used in this study was not expected to produce antibody concentrations >5g/L. HaLCon can store multiple standard curves, and users can either generate a standard curve with the antibody of interest or use a more general calibrant and apply the curve to multiple antibody products. The standard curve is valid for the life of the analysis module which is 3 months or 1000 samples. **Figure 4** displays triplicate measurements which show strong repeatability.

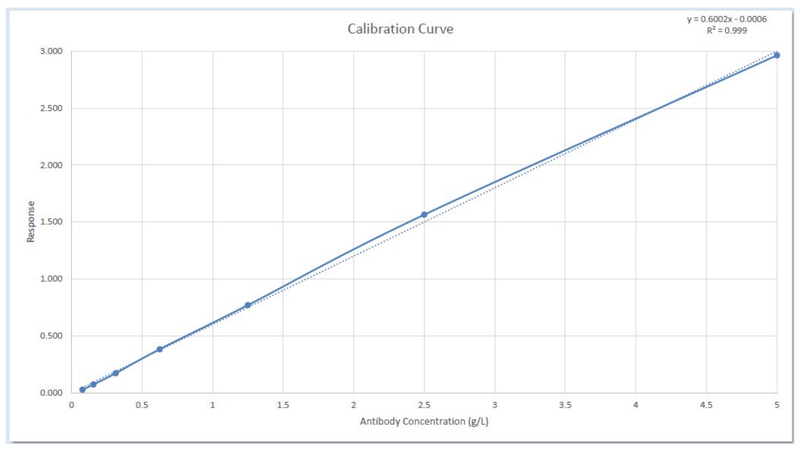


Figure 3. Calibration curve for a well characterized human IgG from 5g/L to 0.07g/L

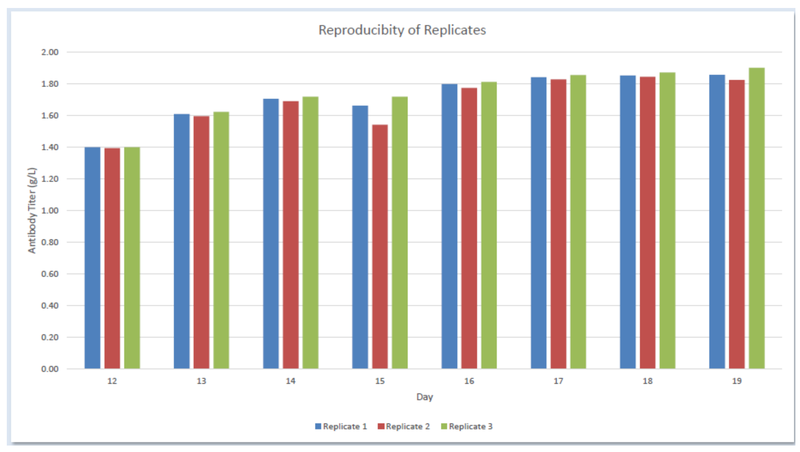
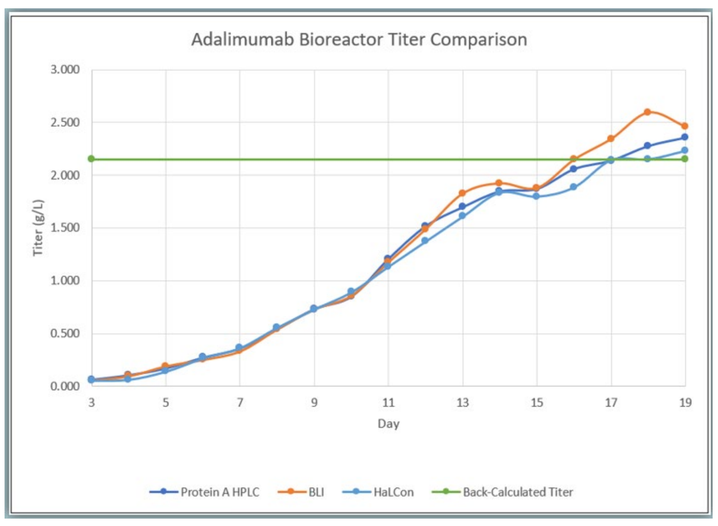


Figure 4. Triplicate measurements for titer samples from days 12 - 19

Comparability Between Techniques



HaLCon vs. ProA HPLC	94.8%
HaLCon vs. Back-Calculated	103.8%

Figure 5. Titer comparison as measured by HaLCon, BLI, and protein A HPLC of daily samples

Figure 5 (left) highlights the similarity in Adalimumab titer measurements between HaLCon and HPLC protein A. The two measurements track very closely through the entire bioreactor run and the ending values are within +/-5% of each other. The BLI data correlates but has larger differences overall from the other two techniques. Additionally, the samples required multiple dilutions before BLI analysis, potentially contributing to the larger discrepancies between BLI, HaLCon, and HPLC protein A.

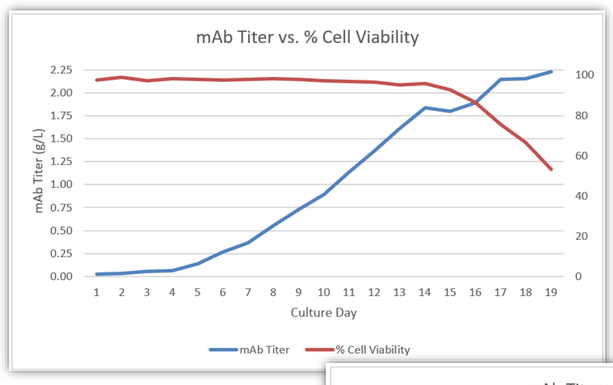
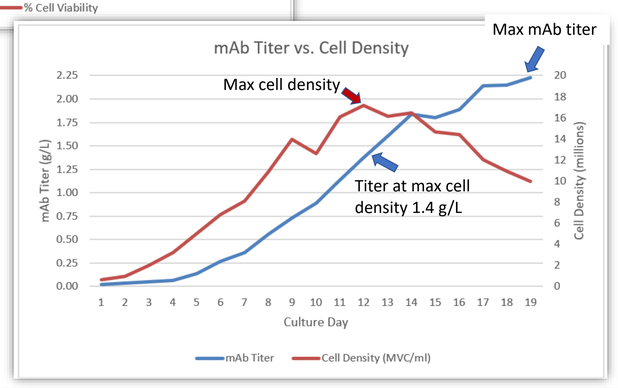


Figure 6 (left). Titer vs Cell Viability graph, for entire 19-day culture.

Figure 7 (below). Titer vs Cell Density graph for the same 19-day culture as figure 6.



Figures 6 & 7 (right). Cell viability and cell density are critical parameters for understanding the health of the cells in the bioreactor, and if changes need to be made to the culture conditions. The plots tracking cell viability or cell density alongside antibody titer highlight that antibody titer continues to increase even after the cell viability and cell density have begun to decline. Only monitoring cell health would have potentially led to erroneous conclusions regarding the ideal time to harvest the cells and begin the downstream purification process, sacrificing total potential antibody yield.

Conclusions

- HaLCon is a fit for purpose, simple, liquid chromatography system, capable of delivering reproducible antibody concentrations from a bioreactor with a dynamic range of 0.1 to 10g/L in an automated fashion with no manual interventions and minimal instrument training required.
- Dedicated consumables make the system fast and easy to set-up, with no column or media preparations required before each run. The stored calibration curve saves time and allows for very rapid sample analysis, while retaining high accuracy and precision for reported antibody concentrations.
- The antibody concentrations agree very well with the gold standard technique of protein A HPLC.
- Antibody concentration measurements provide essential and useful information on top of cell density and viability measurements. Cell density and viability are crucial measurements with respect to the health of the culture, but are not directly reflective of the current or future trend in the antibody concentration.