

Automating Titer Measurement

Tech Note

Automating At-Line Titer Measurement with a Novel Fit-For-Purpose Chromatographic Device

Abstract

A number of process analytic technologies (PAT) have been developed for optimizing cell attributes and culture media components, but increasing viable cell density (VCD) does not always produce a commensurate increase in product titer¹. Therefore, the measurement of antibody titers in CHO cell cultures is a critical process parameter (CPP) for optimizing cell culture conditions. Titer measurement must be rapid, robust, and the assay must have a wide dynamic range to monitor product yields throughout a bioreactor run.

Here, we describe a novel, compact device, the HaLCon analyzer, capable of rapid, automated at-line measurement of antibody titers from stirred bioreactors using an aseptic sampling device. Assay linearity and accuracy were evaluated over a measurement range of 0.1 to 6.5 g/L using a simulated cell culture titer curve spiked with bovine serum albumin (BSA) and human IgG. Manual and automated aseptic sampling measurements using the HaLCon analyzer compared very favorably with a commercial Protein A HPLC system. Excellent intra-assay precision (CV < 3%) was demonstrated for both off-line and at-line measurements with the HaLCon analyzer. Measurement results were also unaffected by BSA levels up to 2.5% (v/v).

1. *Appl Microbiol Biotechnol* (2015) 99:4645–4657.

Simulated Titer Curve

- Samples Spiked with BSA and Purified HuIgG to Simulate an 18-day bioreactor campaign

| Day | IgG (g/L) | % BSA |
|-----|-----------|-------|
| 2 | 0.10 | 0.10 |
| 4 | 0.20 | 0.15 |
| 6 | 0.75 | 0.23 |
| 8 | 1.75 | 0.34 |
| 10 | 3.00 | 0.51 |
| 12 | 4.75 | 0.76 |
| 14 | 5.75 | 1.14 |
| 16 | 6.25 | 1.71 |
| 18 | 6.50 | 2.56 |

Analytical Conditions

Instrumentation

- Agilent 1100 HPLC system with autosampler and 2.1 x 30 mm Protein A column for off-line titer measurement.
- Flownamics Seg-Flow® 1200 with F-Series FISP probe for automated aseptic at-line sampling with HaLCon analyzer.
- RedShiftBio HaLCon analyzer with proprietary titer module for manual and automated at-line analyses.



Compact Footprint



Automated At-Line Titer Measurement



Correlates to Off-Line HPLC



redshiftbio.com/products/halcon

Real Time Antibody Titer

- The HaLCon analyzer is a self-contained system with pumps, valves, analytical module, detector, and reagent pack, built into a small footprint:

Height: 21" (53.3 cm)
 Width: 10" (25.4 cm)
 Depth: 17" (43.2 cm)
 Weight: 37 pounds (17 kg)*
 *not including full reagent pack

- Measurement range from 0.1 to 10 g/L without sample dilution or recalibration.
- Titer results are automatically displayed on integrated monitor in under 5 minutes.
- Reagent pack and analytical module provide up to 1,000 analyses.



Evaluation Results

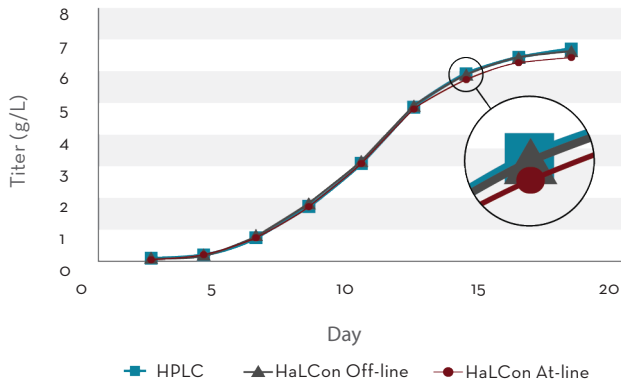
Comparing At-Line and Off-Line Titer Measurements with Off-Line Protein A HPLC

- Antibody titer at each level was calculated using the average of three measurements for all data sets.
- Accurate at-line and off-line titer measurements up to 6.5 g/L were obtained using the HaLCon analyzer without sample dilution using linear calibration curves.

Accuracy Table

- HaLCon measurement errors calculated relative to Protein A HPLC titer values.
- Relative errors < 10% were obtained for HaLCon off-line
- Excellent intra-assay precision (CV < 3%) demonstrated for both off-line and at-line measurements

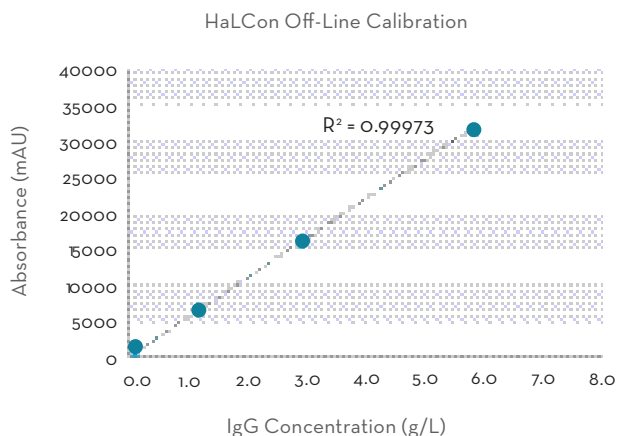
Simulated Cell Culture Titer



| Day | ProA HPLC Titer (g/L) | HaLCon Off-Line Error | HaLCon At-Line Error |
|-----|-----------------------|-----------------------|----------------------|
| 2 | 0.10 | 1.99% | 9.21% |
| 4 | 0.21 | 2.79% | 3.08% |
| 6 | 0.76 | 7.31% | 4.31% |
| 8 | 1.75 | 5.90% | 1.54% |
| 10 | 3.09 | 2.70% | 1.30% |
| 12 | 4.86 | 1.86% | 0.62% |
| 14 | 5.92 | 0.21% | 2.62% |
| 16 | 6.44 | 0.37% | 2.41% |
| 18 | 6.68 | 0.56% | 3.74% |

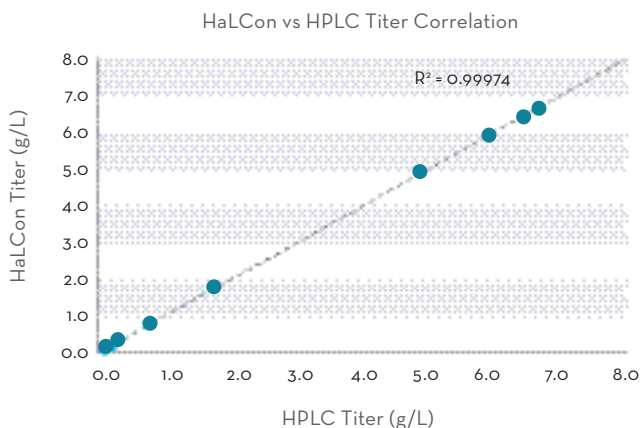
Calibration Curves Generated Using a Human IgG Isotype Control Standard

- Standard measurements at 0.1, 1.0, 2.5, and 5.0 g/L used for generation of HaLCon and HPLC calibration curves.
- Excellent fit (R^2) of linear regression across calibration range.
- Extended dynamic range allows extrapolation to higher titer values.



Correlation of HaLCon and Protein A HPLC Titer Measurements

- Plot of HaLCon vs. HPLC titer values shows excellent agreement across titer range.
- Measurement results unaffected by changing BSA levels up to 2.5% (v/v).
- Demonstrates successful application of a fit-for-purpose device for antibody titer.



Summary

- A novel fit-for-purpose chromatographic device was successfully integrated with a Flownamics Seg-Flow unit for automated at-line titer measurement of a simulated cell culture.
- Linear response of the HaLCon analyzer was demonstrated from 0.1 to 6.5 g/L without recalibration or sample dilution.
- Automated at-line and off-line titer measurements from the HaLCon analyzer were within 10% of the titer values from off-line Protein A HPLC.
- Excellent intra-assay precision ($CV < 3\%$) was obtained for both off-line and at-line measurements using the HaLCon analyzer.
- Titer results were obtained in under 5 minutes for off-line measurements, and under 10 minutes for automated at-line measurements using the HaLCon analyzer.