Automated protein quantification in bioprocess development and pharmacokinetic studies: Improving assay performance

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Introduction

Gyrolab Bioaffy® – Single system solution

Gyros offers a unique set of analytical tools to address applications within biotherapeutic development.

The automated solution facilitates protein quantification with a measurement range from micrograms to grams per liter, minimizing the need for pre-assay dilution.

Examples of customer applications using Gyrolab Bioaffy:

- Quantification of drug product
- Quantification of host cell proteins
- Quantification of drug molecule in serum and plasma
- Quantification of anti-drug antibodies (ADAs)

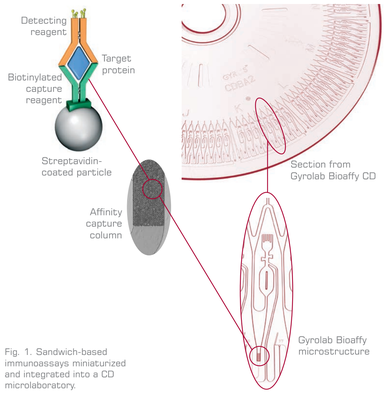

Fig. 1. Sandwich-based immunoassays miniaturized and integrated into a CD microlaboratory.

Assay methods

The principles for miniaturizing and integrating assays on Gyrolab® Bioaffy CD microlaboratories are illustrated in Figure 1. A fully automated instrument is able to process different CD microlaboratories:

- **Gyrolab Bioaffy 20 HC** for quantification up to the g/L range
- **Gyrolab Bioaffy 200** for high sensitivity assays

The open design principle underlying the Gyrolab Bioaffy format facilitates quantification of any target protein for which an immunoassay can be developed and allows experimental set-up to be designed on a day-to-day basis. Each CD contains individual microstructures in which samples are analyzed in parallel. Each microstructure contains a column (15 nL) pre-packed with streptavidin coated beads. Biotinylated capture reagents are then bound to the beads creating protein specific columns.

Experimental

CD microlaboratories are run in Gyrolab® Workstation LIF (see Figure 2). Samples and reagents are transferred sequentially to specific inlets on the CD by a robotic arm. Liquid enters the microstructures by capillary action and the required volumes are defined within each microstructure. Samples and reagents are moved through the microstructure by spinning the CD at pre-programmed intervals and speeds to create the desired flow rates. Detection begins as soon as the reactions are completed using a laser-induced fluorescence detector integrated in the workstation. Thus, an image of the localized fluorescence intensity of each individual column is created. The total integrated fluorescence of each column corresponds to the total protein bound to the column (see Figure 3 a,b). Each CD takes approximately 50 minutes to process and generates 112 data points.

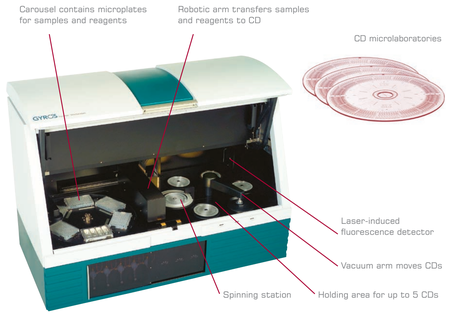

Fig. 2. Gyrolab Workstation LIF.

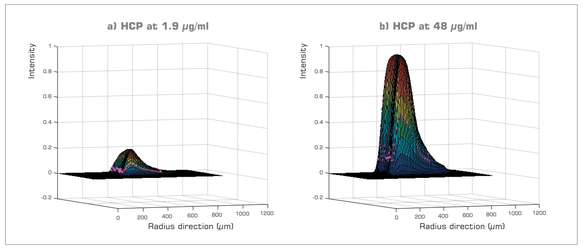

Fig. 3. Graphical representation of individual binding responses. Profiles of total fluorescence reflect the total amount of protein bound to each column. Liquid flows from left to right so that the fluorescent signal has its highest intensity where protein concentration is highest, i.e. at the top of the column. Sample protein: HCP at concentrations 1.9 µg/ml (a) and 48 µg/ml (b).

Bioprocess development

The results presented in this section were generated in collaboration with AstraZeneca, Södertälje, Sweden and with GE Healthcare, Uppsala, Sweden.

Conclusions

- Broader measurement range:
  - Quantification of monoclonal IgG and HCP at concentrations relevant for the entire purification process from cell supernatant to purified product
  - Minimizes need for pre-assay dilution
- The open platform makes it possible to adjust assay conditions, such as CD type and reagents, to suit analytical requirements
- Fully automated, easy-to-use solution reduces operator 'hands-on' time
- Increased throughput with 112 data points generated in < 60 minutes

Fast and accurate quantification of drug product and contaminants is essential for efficient bioprocess development and optimization. Current technologies, such as ELISA and HPLC, are able to deliver good quality results but have limitations in terms of throughput and measurement range. See Figure 4 for an illustration of how the two Gyrolab Bioaffy CD microlaboratories provide analytical support in the different stages of biotherapeutic process development.

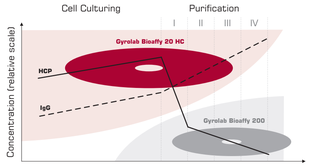

Fig. 4. Illustration of the analytical support for biotherapeutic process development offered by the combination of Gyrolab Bioaffy 20 HC and Gyrolab Bioaffy 200. In the early stages, Gyrolab Bioaffy 20 HC can be used for simultaneous quantification of IgG and HCP. As HCP concentrations decrease high sensitivity assays are achieved using Gyrolab Bioaffy 200.

IgG quantification

Quantification of monoclonal IgG was performed using Gyrolab Bioaffy 20 HC, designed to facilitate quantification of high-concentration samples. As shown in Figure 5, the combination of Gyrolab Bioaffy 20 HC and the selected reagents results in a measurement range covering three orders of magnitude, allowing for quantification of monoclonal IgG up to the g/L range.

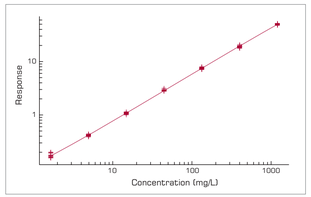

Fig. 5. Standard curve for monoclonal IgG using Gyrolab Bioaffy 20 HC.

Excellent correlation with HPLC and ELISA

Samples were analyzed with regards to content of monoclonal IgG using Gyrolab Bioaffy 20 HC. Samples were run undiluted or diluted 1:2 or 1:4 in Gyros Standard Diluent (Gyros AB). All samples provided by AstraZeneca had previously been analyzed using affinity HPLC absorbance measurements at 280 nm and the correlation between the methods is shown in Figure 6. Correlation between Gyrolab Bioaffy 20 HC and ELISA data generated at GE Healthcare is shown in Figure 7. The limited measurement range of the ELISA required up to 6000 times dilution of sample to perform the analysis that was then performed with undiluted samples using Gyrolab Bioaffy 20 HC.

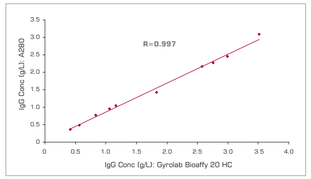

Fig. 6. Correlation between Gyrolab Bioaffy 20 HC and HPLC absorbance measurements at 280 nm when quantifying monoclonal IgG in samples provided by AstraZeneca.

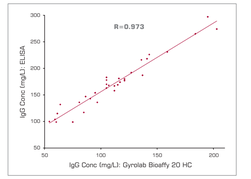

Fig. 7. Correlation beween Gyrolab Bioaffy 20 HC and ELISA data when quantifying monoclonal IgG in cell supernatant samples provided by GE Healthcare.

HCP quantification

To achieve a high-sensitivity assay, quantification of HCP was performed on Gyrolab Bioaffy 200. To expand the measurement range to higher concentrations, quantification was performed using Gyrolab Bioaffy 20 HC. Through this simple change of CD, the upper limit of quantification was increased by a factor of 50, as illustrated in Figure 8, allowing for quantification of HCP in crude cell supernatants.

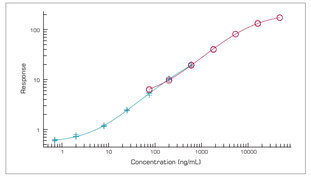

Fig. 8. Extension of measurement range for HCP quantification, using Gyrolab Bioaffy 200 (crosses) and Gyrolab Bioaffy 20 HC (circles) respectively. Assay for Chinese Hamster Ovary HCP (Cygnus Technologies, Southport, USA) transferred to Gyrolab Bioaffy.

Excellent reproducibility

Figure 9 presents reproducibility data from IgG and HCP quantification in the GE Healthcare samples. Samples were run in duplicate and concentrations ranged between 100 and 300 µg/mL of IgG and between 10 and 250 ng/mL of HCP. The coefficient of variation (CV) concentration was well below 10% in all cases.

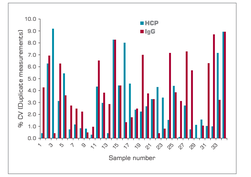

Fig. 9. Precision (% CV) when quantifying HCP and IgG in cell supernatant samples (duplicate measurements).

Pharmacokinetics of therapeutic antibodies

Conclusions

- Assays for studying the pharmacokinetics of monoclonal IgGs in serum were easily adapted to Gyrolab Bioaffy
- Flow-through assay principle minimized matrix effects
- Assay measurement range was increased to better fit analytical needs and recovery values were significantly improved compared to ELISA

The results presented in this section were generated in collaboration with a research group performing pharmacokinetic studies of therapeutic antibodies.

The group was using an in-house developed ELISA to quantify a drug molecule in cynomolgus monkey serum and were looking to improve assay performance by increasing measurement range and reducing interference from the sample matrix.

An assay for quantifying a human monoclonal antibody drug molecule was transferred from ELISA format to Gyrolab Bioaffy. Utilizing the antigen binding properties of the human monoclonal antibody, labeled target protein was used as both capture and detection reagent in a bridging immunoassay format.

Quantification in cynomolgus monkey serum

Figure 10 shows the resulting standard curves for the transferred assay when diluting the drug molecule in pooled cynomolgus monkey serum, undiluted and diluted 1:4 in Gyros Standard Diluent respectively. When comparing the two curves there is no evidence of matrix effects from the serum components.

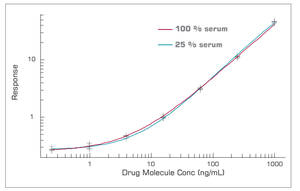

Fig. 10. Standard curves for quantification of drug molecule generated with undiluted serum (red) and serum diluted 1:4 (blue) respectively.

Five individual cynomolgus monkey serum samples, diluted 1:4 in Gyros standard diluent, were spiked with drug molecules in concentrations ranging from 3.9–1000 ng/mL. The resulting recovery values are shown in Figure 11.

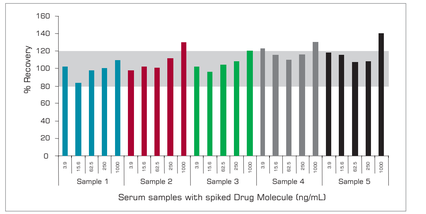

Fig. 11. Recovery of drug molecule spiked into serum samples diluted 1:4.

Gyros would like to express their thanks to AstraZeneca, Södertälje, Sweden, (www.astrazeneca.com) and GE Healthcare Bio-Sciences AB, Uppsala, Sweden, (www.gehealthcare.com) for permission to show their results.