

## Conclusions

- Five CHO HCP kits were evaluated for their suitability in analyzing samples from a bioprocess and six pharmaceutical grade drug products, all produced in CHO cells.
- Based on several criteria (response level, calculated concentration and dilution linearity), three of the five kits were shown to be the best option for at least one of the six drugs.
- Kit 2 and Kit 5 demonstrated somewhat better capability to detect CHO HCP in process samples.
- Four lots of Drug D were analyzed for presence of CHO HCP using the five kits. One of the kits consistently generated CHO HCP concentrations exceeding 100 ppm for this drug.
- Size-exclusion chromatography of Drug D followed by analysis of fractions for CHO HCP revealed that the two types of molecules are effectively separated with HCP responses corresponding to molecular weights < 100 kDa, without indications of aggregates of IgG and CHO HCP proteins.
- Access to several CHO HCP kits increases the probability of selecting a generic CHO HCP kit that is suitable for a given process/drug product.

## Material and methods

### Reagents

Antigen affinity purified antibodies directed against CHO HCP were purchased from leading suppliers of immunoreagents for HCP analysis. Standard preparations were used as recommended by the respective antibody supplier.

All antibodies were labeled with:

- Biotin (Cat no. 21327, Life Technologies)
- Alexa Fluor® 647 (Cat.no. 20186, Life Technologies)

### Samples

A 12-day cell culture at 2 L scale of a CHO cell line producing a recombinant human IgG1 antibody was initiated. Filtered harvest material was purified using MabSelect SuRe LX. Eluted material was subjected to low pH virus inactivation and further polished using anion exchange chromatography in flow through mode. Finally, the product was concentrated to approximately 4.1 mg IgG1 per mL.

In addition, six pharmaceutical grade drug products (Drugs A-F, produced in CHO cells) were analyzed for CHO HCP content in the linear range of the assays. As controls, several drug products produced in NS0 and SP2/0 were included and analyzed in several dilutions without generating significant responses (not reported).

To verify accuracy of analyses, four lots of Drug D were re-analyzed using the five kits. CHO HCP was related to the contents of IgG using an immunoassay specific for human IgG and expressed in ppm (CHO HCP in relation to IgG).

### CHO HCP analysis

Analyses were performed in a CD with a 1000 nL sample volume and containing 96 microstructures, each including a 15 nL packed column of a porous particle covalently coupled with saturating amounts of streptavidin. Biotinylated/fluorescently labelled antibodies used for a certain kit originated from the same antibody preparation. The analysis procedure was performed completely automatically in a three-step immunoassay. On-column fluorescence detection was performed in the instrument and the results reported. Total analysis time for a full CD generating 96 data points was approximately 75 min.

### Analytical size-exclusion chromatography

An ÄKTA™ system was used for analytical size exclusion chromatography on a Superdex™ 200 Increase (3.2/300 mm) column (GE Healthcare LifeSciences). Twenty-five µL of Drug D at 10 mg/mL were separated on the column and fractions of 50 µL were collected. Fractions were analyzed for the presence of CHO HCP antigen using Kit 2 and Kit 3 after appropriate dilution of fractions.

## References

- Zhu-Shimoni J, Yu C, Nishihara J, Wong RM, Gunawan F, Lin M, Krawitz D, Liu P, Sandoval W, Vanderlaan M. Host cell protein testing by ELISAs and the use of orthogonal methods. *Biotechnol. & Bioeng.* 2014, **111**, 2367-79

## Introduction

Biologic drugs are produced in recombinant host cells and development requires attention to many factors in order to fulfil qualitative and quantitative requirements. Besides the recombinant product, host cells also produce proteins that must be removed from the product during downstream purification. Several analytical approaches are used to follow purification, among them immunoassays to collectively measure total Host Cell Protein (HCP).

The development of HCP immunoassays is inherently difficult. Assay performance is dependent on antibody specificity and coverage

of potential HCP impurities generated during cell culture, and how these impurities are eliminated or enriched in subsequent downstream processing. Antibody preparations used for impurity testing should preferably react with all potential HCP impurities. However, it is very difficult to achieve the goal of 'one assay fits all'.

The immune response can be broadened by fractionating the collective HCP pool prior to immunizing animals used to generate polyclonal antibodies. This approach increases the probability of generating antibody response against low abundant or weakly immunogenic HCPs. Accordingly, assays

based on different sets of antibodies, produced using different procedures, may potentially have different utility for different downstream processes or products.

We have developed five kits for Chinese Hamster Ovary (CHO) HCP impurities based on different commercially available antibody preparations, for application in Gyrolab. We have evaluated the five kits for technical performance using samples from a small scale bioprocess of a CHO cell line including downstream processing of a human IgG1 antibody. The five kits were also evaluated for technical performance using pharmaceutical grade drug products produced in CHO cells.

## Results

### CHO HCP assay performance

The five kits were validated according to conventional performance parameters including LOD, LLOQ, ULOQ, precision, linearity and spike recovery. All kits demonstrated LLOQ in the range of 1-3 ng/mL and the ULOQ was 8 000 ng/mL of CHO HCP. The precision in the mid-range of the curves as assessed from duplicate determinations was < 5 %CV.

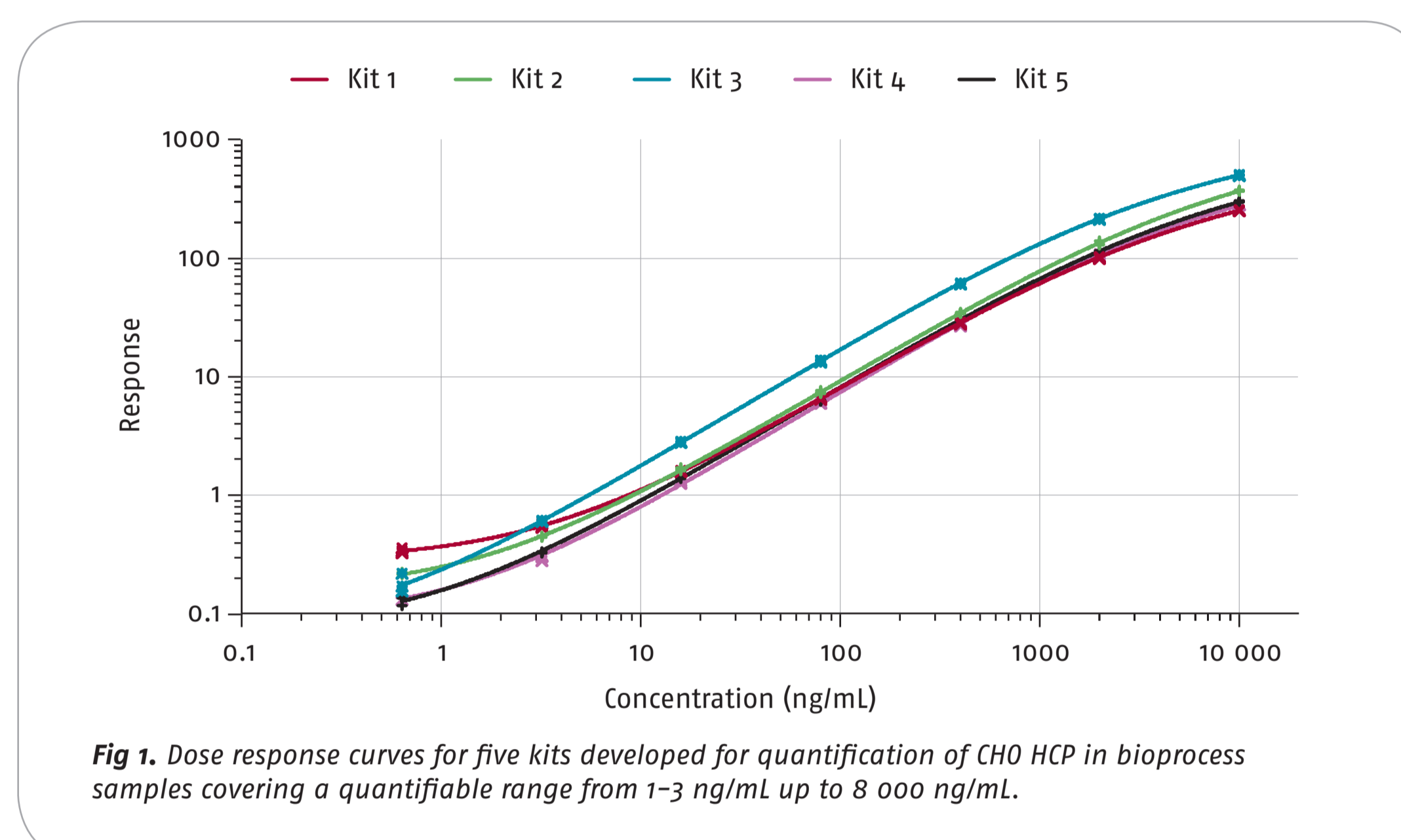


Fig 1. Dose response curves for five kits developed for quantification of CHO HCP in bioprocess samples covering a quantifiable range from 1-3 ng/mL up to 8 000 ng/mL.

### Process samples

Three samples from downstream processing of CHO cell culture supernatant were analyzed for CHO HCP using five kits. The results are reported in Figure 2. The results indicate CHO HCP concentrations from 60 to 130 µg/mL in the harvest sample, approximately 2-16 µg/mL after protein A elution, and 230-300 ng/mL in the final product, depending on kit; this represents a reduction of CHO HCP of approximately 500-fold during the entire purification process. The concentration of the remaining CHO HCP corresponded to approximately 56-73 ppm depending on kit, a level in accordance with regulatory requirements for therapeutic products (1).

When ranking kit based on the performance of all bioprocess samples, all five kits gave comparable results for the final product. Considering the recommended criteria for evaluating assay performance:

- Reduction of quantified HCP for each additional chromatographic step
- Quantity of CHO HCP in samples
- Dilution linearity

it appears that Kit 2 and Kit 5 are most suitable for detecting CHO HCP in harvest and the post-protein A sample compared to the other kits. All kits showed similar reduction of HCP in the chromatographic steps and dilutional linearity.

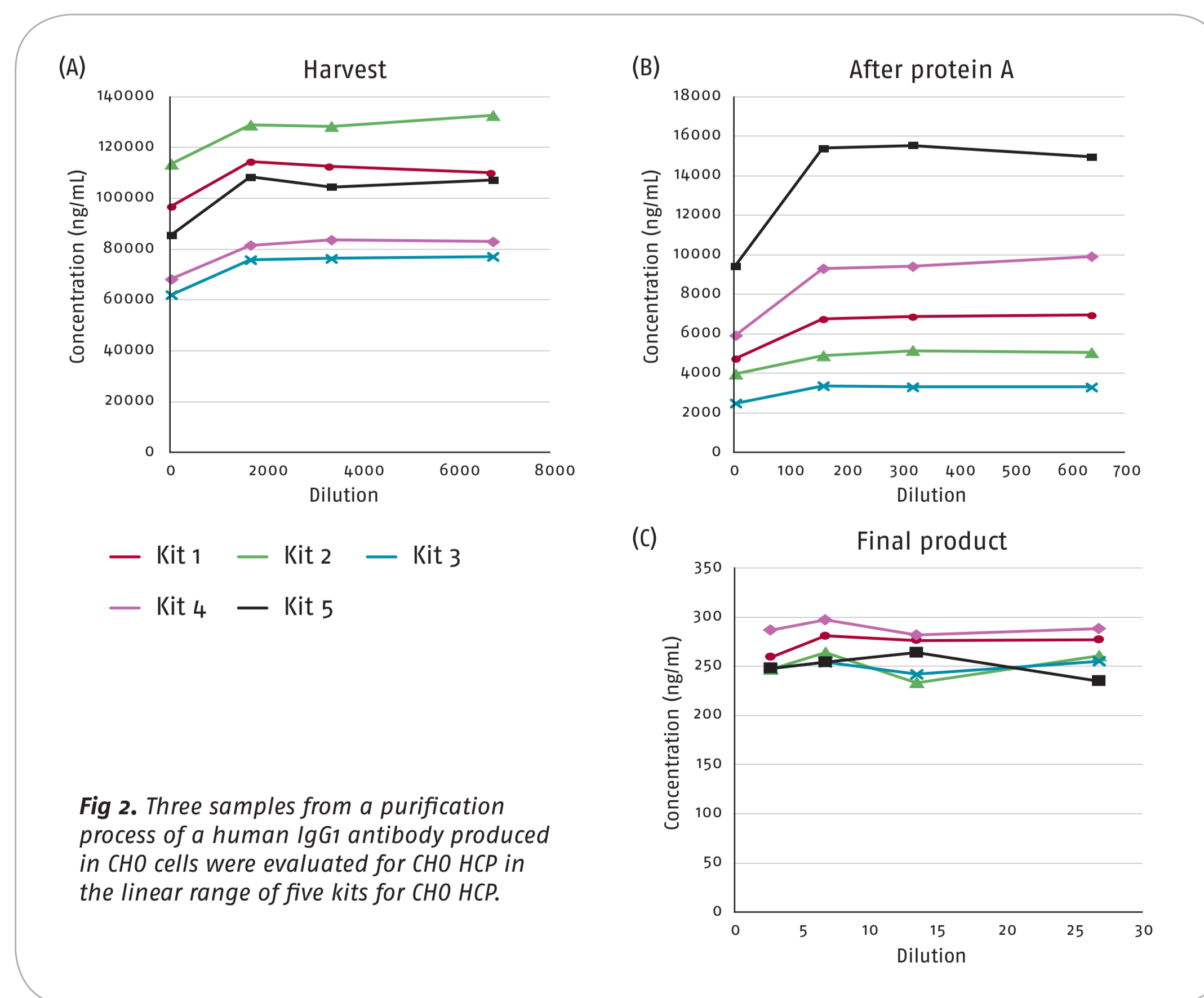


Fig 2. Three samples from a purification process of a human IgG1 antibody produced in CHO cells were evaluated for CHO HCP in the linear range of five kits for CHO HCP.

### Pharmaceutical grade drug products

Six drug products manufactured in CHO cells were evaluated for the presence of CHO HCP antigen using five kits. Three drug products contained low concentrations of CHO HCP (< 1 ppm). The remaining three drug products contained more substantial amounts of CHO HCP, as indicated in all five CHO HCP kits. Different kits, however, demonstrated different abilities to reproducibly quantify CHO HCP. In one case the CHO HCP concentrations determined differed by a factor of approximately 30 when using the different kits. When using Kit 2 the CHO HCP impurity level greatly exceeded the acceptable level of 100 ppm (1).

Ranking kits based on the outcome of the three drug products that contained significant HCP levels showed that each drug product has its own best kit. Kit 5 is best for Drug B, Kit 2 is best for Drug D, and Kit 3 is best for Drug E. The results are summarized in Figure 3 and Table 1.

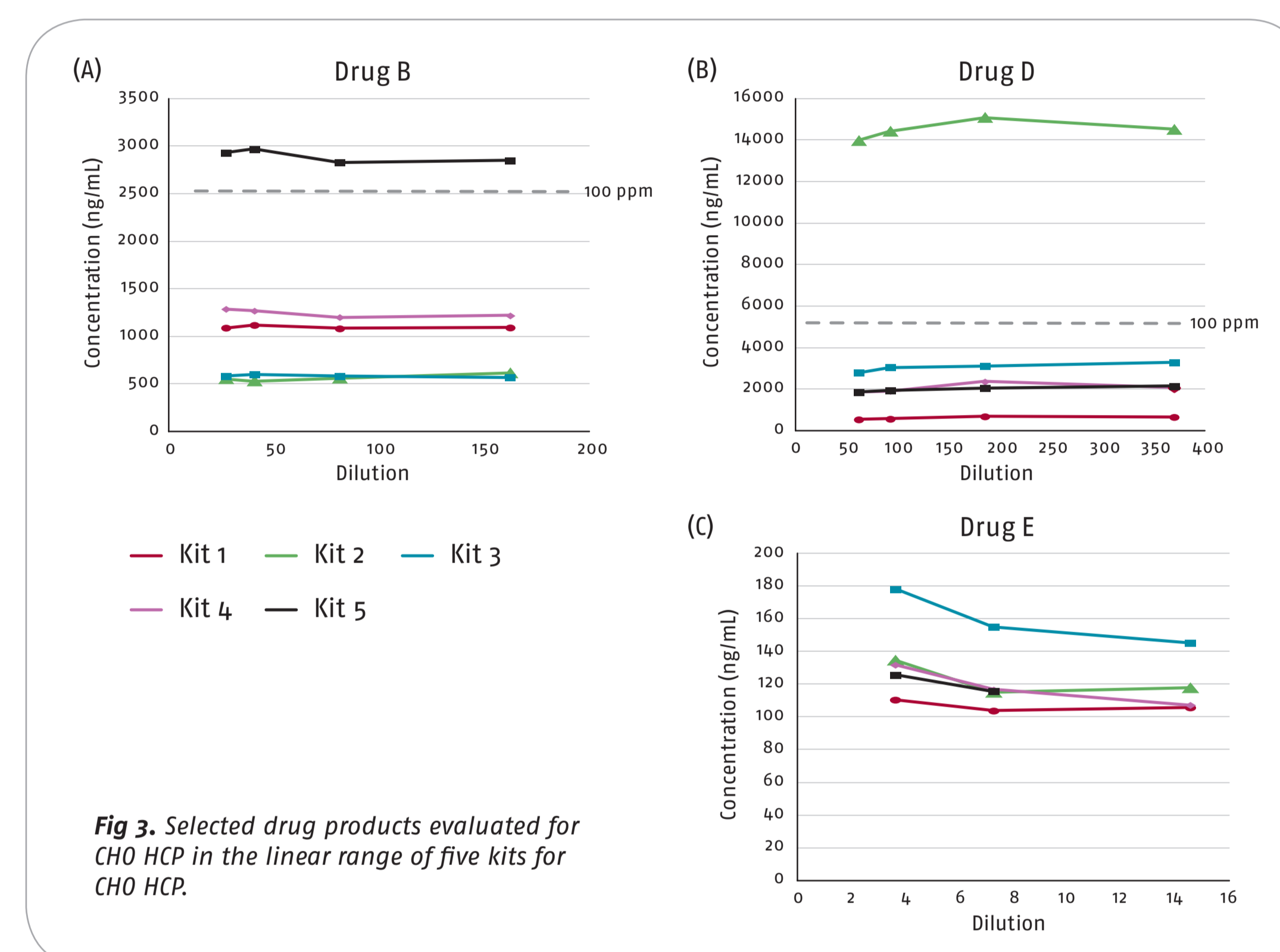


Fig 3. Selected drug products evaluated for CHO HCP in the linear range of five kits for CHO HCP.

Table 1. Ranking of five CHO HCP kits with regards to utility for six pharmaceutical grade drug products. Samples were diluted to linearity in CHO HCP kits (See also Fig 3)

Ranking of kits for different products					
	Kit 1	Kit 2	Kit 3	Kit 4	Kit 5
DRUG A	*	*	*	*	*
DRUG B	Intermediate	Less good	Less good	Intermediate	Best
DRUG C	*	*	*	*	*
DRUG D	Less good	Best	Intermediate	Intermediate	Intermediate
DRUG E	Less good	Intermediate	Best	Intermediate	Intermediate
DRUG F	*	*	*	*	*

\* Data inconclusive due to too low levels of HCP

### Lot variation

Lots of Drug D were tested using five CHO HCP kits. CHO HCP concentrations were related to the contents of IgG and expressed in ppm (Fig 4).

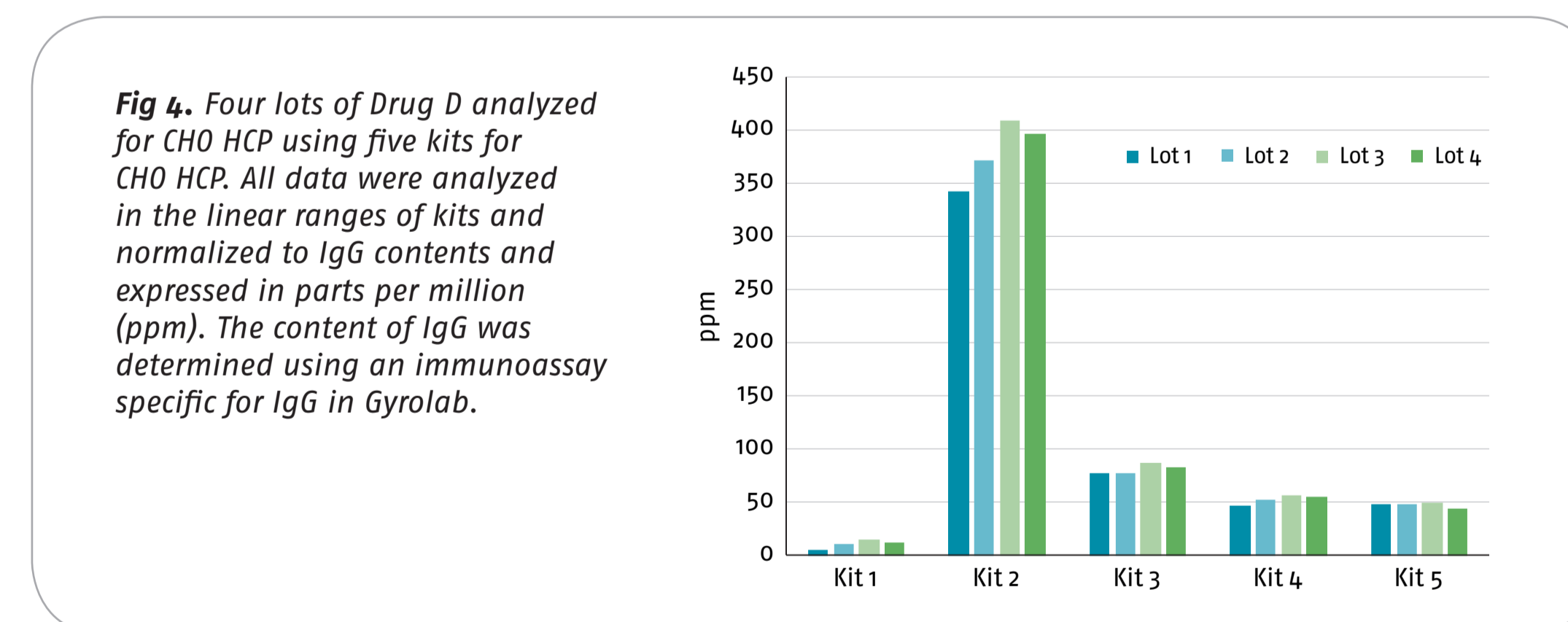


Fig 4. Four lots of Drug D analyzed for CHO HCP using five kits for CHO HCP. All data were analyzed in the linear ranges of kits and normalized to IgG contents and expressed in parts per million (ppm). The content of IgG was determined using an immunoassay specific for IgG in Gyrolab.

### Further characterization of Drug D with respect to CHO HCP impurities

Drug D displayed a high concentration of CHO HCP as determined in Kit 2 and was further characterized by analytical size exclusion chromatography on a Superdex Increase column. Fractions were analyzed for CHO HCP using Kit 2 and Kit 3 (Fig 5).

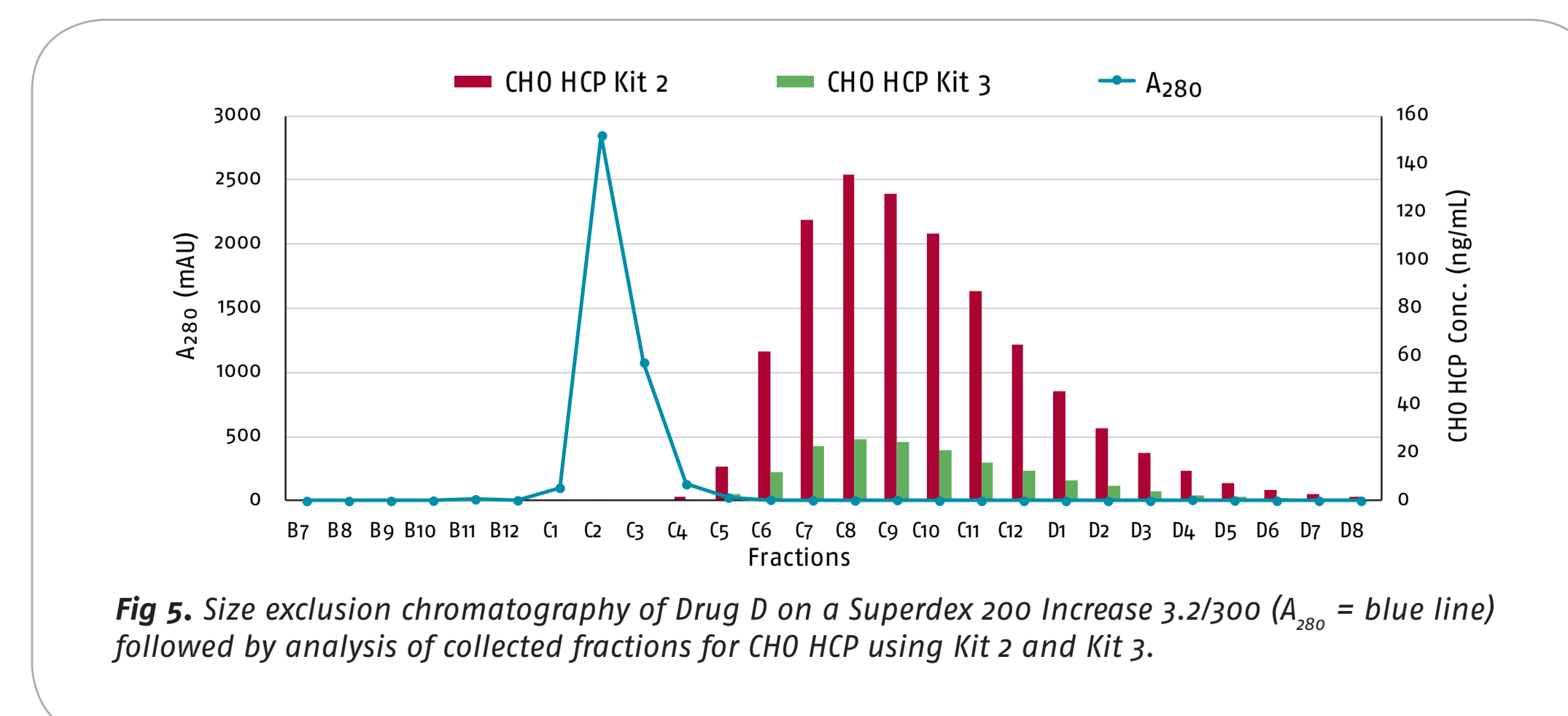


Fig 5. Size exclusion chromatography of Drug D on a Superdex 200 Increase 3.2/300 (A<sub>280</sub> = blue line) followed by analysis of collected fractions for CHO HCP using Kit 2 and Kit 3.