Quantification of Host Cell Proteins (HCPs) by 2D–DIGE
A validated and FDA submitted new method

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Introduction

Host cell proteins (HCPs) comprise the majority of protein contaminants associated with biopharmaceuticals, produced by recombinant DNA-technologies. HCPs are often immunogenic, causing allergies or altering the therapeutic efficacy of a drug and therefore, significantly may affect patients´ safety.
Therefore, detection and quantification of host cell proteins is a crucial precondition for all recombinant proteins provided for clinical trials or for any use in human beings.
FDA and other legal authorities demand validated methods, demonstrating that the HCP level is minimized in the product. HCPs also can lead to significant cost increases during drug development and manufacturing. Failure to identify, quantify and sufficiently remove contaminants early in drug development can delay or even kill a promising drug candidate.
State of the art in HCP analysis is a combination of a Coomassie-Blue stained SDS-PAGE, an immunological method (immunoassay / western blot) and an IEF-PAGE. Establishing and validating these three methods is cumbersome and expensive. For the immunological method specific antibodies have to be developed. Moreover, all of those techniques display crucial deficits (see table 1) for identification and quantification of HCPs.

Here a new method is introduced which can substitute the common used HCP determination procedures showing increased sensitivity and specificity without the need of antibodies.

Table 1: Deficits of the current HCP analytic

SDS - PAGE Coomassie stained	**Immunoassay** Elisa	**IEF - PAGE** Coomassie stained
Less sensitive ~ 50-80 ng / band	Not quantitative	Limited resolution
Limited separation capacity Separation distance ~ 8cm	Results depending of the samples state of denaturation	Computer - densitometrical quantitation depending on the software to be applied
Critical HCP quantification due to a mostly saturated main compound (recomb. protein)	Sterically hindered epitopes, cross-reactivity to the recombinant protein possible	Difficult to correlate with the SDS – PAGE data
Quantification depending on software, equipment and applied parameter	Critical end point detection	Reproducibility depending on performance (Ampholytes / Immobilines)
Recombinant protein mostly in its saturation, deficits in quantification, normalisation	Epitope tagging to thousands of various cellular proteins not realistic	Limit of detection (LOD) only about 150ng / band in Polyacrylamidgels
Internal markers are difficult to verify (absolute quantities, quality control)	Measures only total HCP content	Internal markers are difficult to verify (absolute quantities, quality control)

Experiments

The HCP content (%) of a monoclonal antibody preparation was determined following two different purification methods (MAP and Protein A).
The quantification data derived from the Coomassie-Blue stained SDS-PAGE (Fig.1) as the currently used HCP quantification method were compared to the new developed 2D-DIGE (Fig.2) method for HCP analysis.

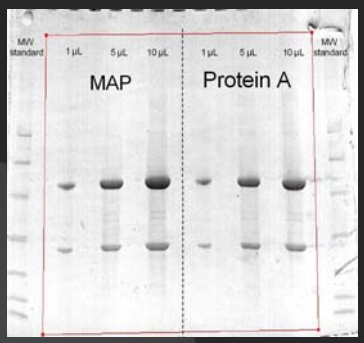

Fig. 1: Coomassie stained SDS – PAGE (three dilutions) Reduced monoclonal antibody, purified by MAP or Protein A

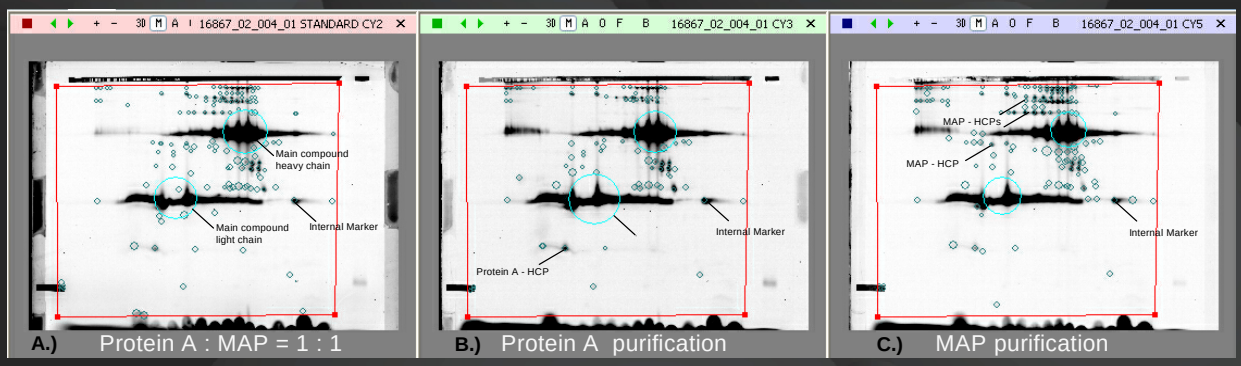

Fig.2: 2D-DIGE HCP analysis of a reduced monoclonal antibody, purified by MAP or Protein A
A.) internal standard (CY2), B.) Protein A purified antibody (Cy3), C.) MAP purified antibody (Cy5)

Table 2: Comparison of the protein quantification using SDS-PAGE and 2D-DIGE HCP analysis

		Number of HCPs detected	Quantity of the internal standard	Calculated % of the HCP content
SDS - PAGE	**MAP**	11	Not detected	1.3%
	Protein A	11	Not detected	1.2%
2D-DIGE HCPA	**MAP**	124	92.21 u	3.8%
	Protein A	88	106.92 u	3.2%

- 10 fold more HCP proteins were detected by the new method
- internal marker was not detectable in the SDS PAGE, probably due to an overlap with the antibody's light chain band
- increased HCP content (%) is detected by the new method, proving increased sensitivity
- normalisation of the SDS PAGE quantification is difficult due to the saturated antibody bands
- normalisation in the 2D DIGE approach was performed using the internal marker, the saturated light and heavy chain spots were not included in the normalisation and quantification procedure

Summary

- Only one method for the whole HCP-determination procedure, no antibody necessary
- Method validated according to ICH Q2(R1) criteria
- Possibility to compare up to three batches on one 2D gel
- Limit of detection (LOD) ~ 4 ppm
- Linearity from 4 – 2048 ppm
- Limit of quantitation (LOQ) <8 ppm
- Highly suitable tool for characterising impurities during the purification process (indicating pI, MW, quantity, and protein identity)
- No interference of the main compound (recombinant protein) in the HCP quantification procedure since quantification is performed using internal markers
- Verified robustness and reproducibility by validating within one gel and different readouts using strong denaturating conditions
- High specificity due to lysine-labeling for visualisation of all proteins

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