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Host cell protein analysis by microflow-LC high resolution SWATH-MS of vaccine samples under development

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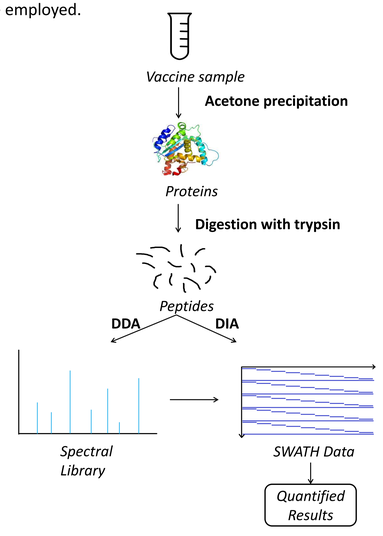
Introduction

A large part of today's biopharmaceuticals consist of recombinant proteins, expressed in a non-human cell line. The initial sample consists of raw harvest media, and is subjected to several purification steps, in order to yield the final product. The product is however still likely to contain some contaminants. The non-drug-protein contaminants usually include DNA, lipids, connective tissue and proteins. The protein contaminants are referred to as host cell proteins (HCPs) and are of special interest since they may contribute decreased drug-protein stability or a series of adverse effects, such as unwanted immune responses in the recipient. Antibody based strategies, such as ELISA assays are usually employed for characterization of the HCP content. Mass spectrometry (MS) is however emerging as a complementary technique. In this project we used Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra (SWATH-MS) to monitor the HCPs of an antigenic target in development, through six purification steps. The antigenic target used in this study, was provided by Statens Seruminstitut (SSI, Copenhagen). This work was supported by Innovation Fund Denmark

Experimental

Samples were subjected to acetone precipitation, followed by reduction, alkylation and tryptic digestion. Each sample was analysed in both a data dependent and data independent manner. The DDA results were combined to form a spectral library, which was combined with the DIA data to yield the quantified results. All samples were separated across a 60 minute gradient using an Eksigent HPLC system and a 100mm*300µm YMC C18 column, running at 5µL/min. Samples were anlysed using a 6600 TripleTOF Mass spectrometer from Sciex. For each SWATH cycle, 50 variable SWATH windows were employed.

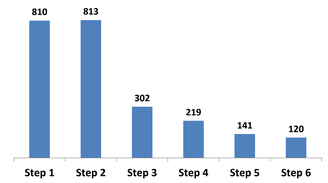


General workflow describing how the quantified HCP data was obtained.

Results and Discussion

Number of Identified HCPs

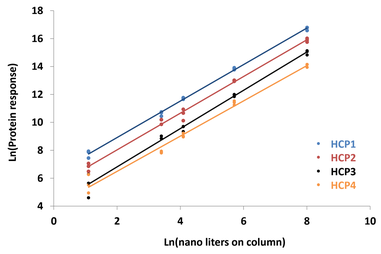
The DDA analyses identified a total of 876 proteins. It was determined that 92 % of all identified HCPs were found in the initial purification step. However, the remaining 8 % are likely to be of interest, as these are the proteins carried through the purification process and thus purified along with the product. 589 proteins could be quantified across all purification steps.



Number of HCPs identified in different purification steps.

Linearity

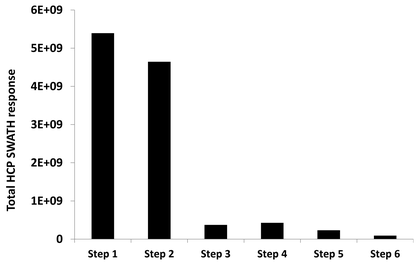
The initial sample was subjected to a series of dilutions from 1:1 to 1:1000 and analysed by SWATH MS. The undiluted sample consisted of 3 µL from the initial purification step. The HCP responses were found to be linear across three orders of magnitude, and well within the values observed from the different purification steps. This means that the responses could be used to measure the relative amount of each individual HCP.



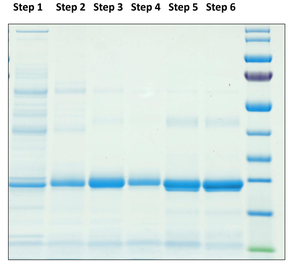
Linearity of selected HCPs across four dilution steps.

SWATH Analyses

The collected responses from the 589 HCPs were measured and compared between the different purification steps. It was determined that the total HCP response, measured by peak area, in the final sample constituted 1.7 % of the HCP response in the initial sample. When comparing with SDS-Gel results, it can be seen that a large part of the HCP bands are cleared throughout the process. The most significant decrease in HCP is observed between step 2 and 3, which is supported by both SWATH-MS and SDS-gel analysis.



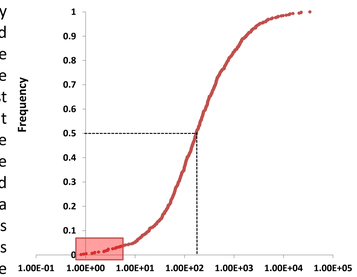
Collected HCP responses obtained with SWATH-MS for the six different purification steps.



SDS-PAGE of the six different purification steps, increasing in purity from left to right. A fixed volume was loaded onto the gel.

Clearance of HCPs

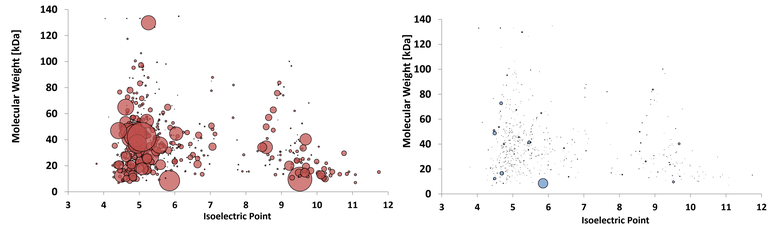
The clearance of the individual HCPs are measured by the ratio of the responses from the initial sample and the final sample (Initial response / Final response). The figure to the right shows the actual frequency of the clearance of each HCP. The frequency is plotted against the clearance (Higher number = higher clearance). It can be observed that 50 % of the HCPs have a clearance rate ≤ 172 (meaning the final response is 1/172 of the initial response). Proteins which are not being cleared throughout the process can be identified by having a low clearance (Marked by the red box). These proteins might either have the same physiochemical properties as the drug-protein, and be carried through the purification process, or be so-called *hitchhiker proteins,* which interact with the drug-protein. When these proteins have been identified, the purification process may be altered in order to clear such proteins in a higher rate.



Actual frequencies showing the clearance of HCPs, by comparing the initial and final purification steps.

Visualisation of HCPs

The HCPs can be visualised using a bubble plot, using the MW and pI as y- and x-axes, and the response as bubble area. The clearance of HCPs with different physiochemical properties can thus be monitored, and this may assist in evaluating the purification methods.



Bubble plots showing the MW (y-axis), pI (x-axis) and SWATH responses (bubble area) of identified HCPs. The red plot (left) shows HCPs from step 1, and the blue plot (right) shows HCPs from step 6.

Conclusion

More than 500 HCPs from a commercial antigenic target could be identified. The proteins could furthermore be quantified across six purification steps. SWATH-MS is a promising tool in monitoring HCP content, with considerably lower assay development costs than antibody based strategies. Results were in agreement with SDS-PAGE results. It is likely that LC-MS based workflows will be a crucial component for future HCP characterizations.

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