Multiplex quantitation of critical host cell proteins (HCPs) using a targeted mass spectrometry assay peptide panel

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ABSTRACT

Purpose: To develop a targeted assay to quantitate endogenous HCPs by using critical HCPs heavy peptide mixture.

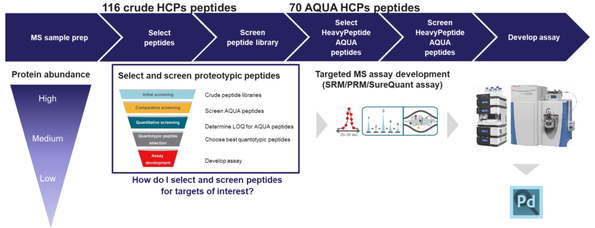
Methods: The HCP digest samples were prepared by either Thermo Scientific™ EasyPep™ or traditional method. The known amount of the critical HCPs heavy peptides mixture was spiked in HCPs digest samples. The PRM data was acquired for the absolute quantification in LCMS instrument. The Skyline software was used for the data analysis.

Results: We were able to quantify unknown endogenous HCPs by spiking critical HCPs heavy peptide mixture into HCPs digest sample. We found that double number of HCPs peptides were quantifiable in the Herceptin mAb digest sample prepared by the EasyPep™ kit compared to in the sample prepared by the traditional sample prep method.

INTRODUCTION

HCPs are a heterogeneous mixture of impurities found in recombinant biotherapeutics. During process development of biopharmaceuticals, monitoring and controlling HCPs contaminants is crucial for ensuring product safety, stability, and efficacy. Therefore, it is not only important to globally monitor HCPs levels throughout the process, but also quantitate individual HCPs proteins. ELISA is the current gold standard method used to assess total HCPs contamination; however, ELISA measures all the HCPs in a sample based on polyclonal pan-HCPs antibody and does not adequately measure individual HCPs. Here we have developed critical HCPs AQUA™ heavy peptide mixture for targeted mass spectrometry (MS) assay to measure absolute quantitation of critical HCPs with high accuracy, precision, and specificity.

Figure 1. Targeted assay development workflow



MATERIALS AND METHODS

Sample Preparation

The EasyPep MS sample prep kit (Cat. No. A40006) was used to denature, reduce, alkylate, digest, and clean up the HCPs sample protein digest. Traditional sample preparation method includes Guanidine-HCl based denaturation, reduction, alkylation, and trypsin digestion. The modified traditional sample preparation method is the same as the traditional method except that desalting step with performed using Thermo Scientific™ Pierce™ polyacrylamide spin desalting column (Cat. No. 89849).

Test Method(s)

200 fmol of the critical HCPs AQUA heavy peptide mixture was spiked in 0.5 µg of each digest sample or Pierce™ 6 Protein Digest (Cat. No. 88342) and Pierce™ Peptide Retention Time Calibration Mixture (Cat. No. 88320). For discovery and targeted PRM analysis, the HCPs digest samples with heavy peptide mixture were analyzed by a Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLCnano System coupled to a Thermo Scientific™ Q Exactive™ plus or Q Exactive™ HF Hybrid Quadrupole-Orbitrap Mass Spectrometer.

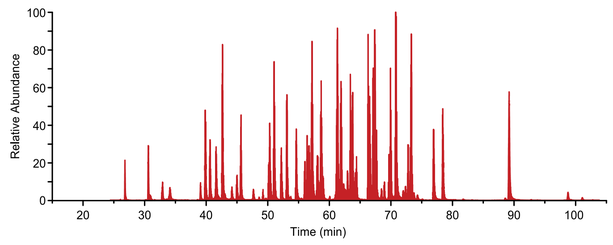
Data Analysis

Thermo Scientific™ Proteome Discoverer™ software was used to search discovery MS data. For targeted data analysis, Skyline software (University of Washington) was used.

RESULTS

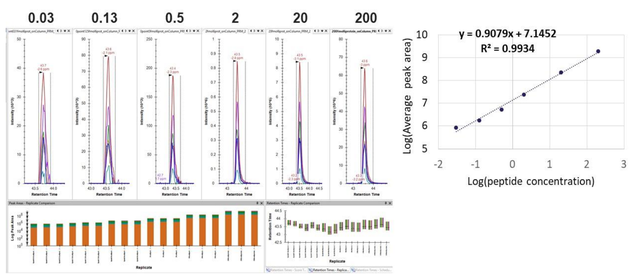
The CHO HCPs standard proteins were digested with two different sample prep methods. Proteome Discoverer data processing generated >3000 HCPs proteins and >20,000 peptides. We created a HCPs peptide spectral library in Skyline software and selected 28 critical HCPs[1]. We selected five unique peptides per protein from spectral library dataset and synthesized 116 crude peptides. For the final assay development, the 70 best-performing peptides from 28 critical HCPs were selected for the AQUA grade heavy peptide synthesis (Figure 2) .

Figure 2. Critical HCP heavy peptide mixture analysis in the 6 protein digest matrix analyzed by PRM



We created the critical HCPs heavy AQUA peptide mixture by mixing equal amount of each peptide. A 200 fmol of each peptide in the mixture was spiked into 2 pmol of 6 protein digest and analyzed by DDA and PRM method (Figure 2). All 70 AQUA heavy peptides were verified by both DDA and PRM analysis.

Figure 3. Skyline PRM analysis and Generation of 6 point standard curve



The 70 critical HCPs AQUA peptide mix was used to generate extracted ion chromatograms of fragment ions of each AQUA heavy peptide and 6 points standard curve spanning from 0.03 to 200 femtomole on column (Figure 3). The targeted MS assays allowed linear quantitation and 3 orders of magnitude dynamic range. Most target peptides were quantified with lower limit of quantitation of 0.03 or 0.13 femtomole as shown here in bar graph (Figure 4).

Figure 4. Lower Limit of Quantification (LLOQ)

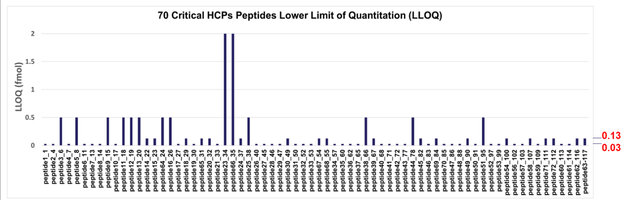
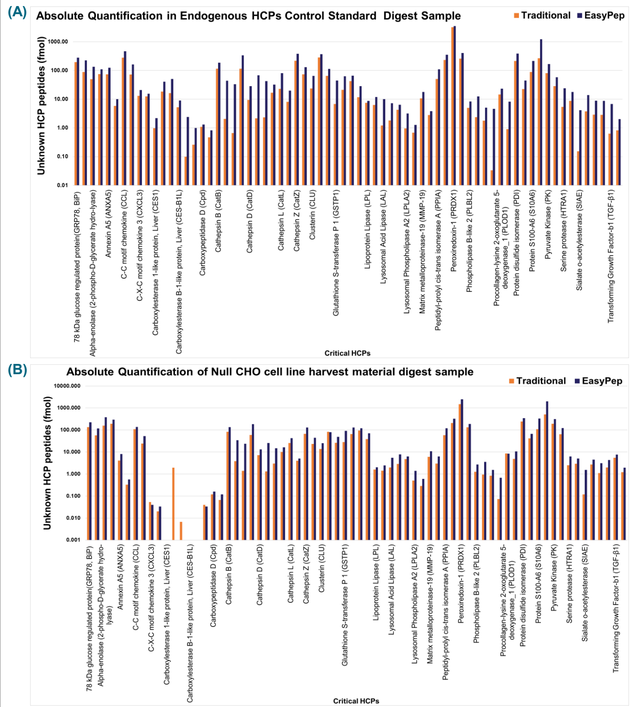
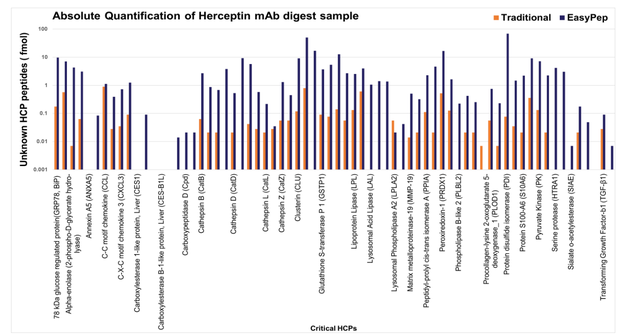


Figure 5. Quantification of unknown HCPs in the null CHO cell line harvest material



The known concentration of critical HCPs heavy peptides mixture (200 fmol) was spiked in the HCPs control standard digest (Figure 5A) or null CHO cell line harvest material sample (Figure 5B) prepared by either traditional or EasyPep method. Heavy and endogenous light peptides are both measured by targeted PRM method. The absolute concentration of the endogenous peptides is then determined by the light-to-heavy peak area ratio to quantitate endogenous HCPs levels. Figure 5A and 5B show that both sample prep methods produce comparable HCPs quantification.

Figure 6. Quantification of unknown HCPs in Herceptin mAb sample



However, there was significant difference in HCPs quantification in Herceptin mAb digest sample prepared by either traditional or EasyPep sample (Figure 6). The EasyPep sample prep method generates 62 quantifiable peptides whereas only 33 quantifiable peptides from the traditional prep sample.

CONCLUSIONS

- A critical HCP heavy peptide mixture was created and used to quantitate unknown endogenous HCPs level in different types of samples.
- Most HCP target peptides were quantified with lower limit of quantitation of 0.03 femtomole or greater with a 4 order of magnitude dynamic range of linearity.
- The HCPs digest sample prepared by EasyPep™ generated more quantifiable peptides than samples prepared by traditional method.

REFERENCES

1. Jones et al (2021): “High-risk” host cell proteins (HCPs): A multi-company collaborative view. Biotechnol Bioeng. 2021 Aug;118(8):2870-2885

TRADEMARKS/LICENSING

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