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

Jae Choi1, Bhavin Patel1, Terry Hicks2, Matthew Daniels2, Sarah Baron3, Kam Shams Ud Doha1, Paul Guilde1, Scott Peterman2, Nikk Jarrett1, Kay Opperman1, Ryan Bomgarden1
1Thermo Fisher Scientific, Rockford, IL; 2Thermo Fisher Scientific, St. Louis, MO; 3Thermo Fisher Scientific, Grand Island, NY; 1Thermo Fisher Scientific, San Jose, CA

ABSTRACT

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

METHODS: The HCP target peptide mixtures were prepared by either Thermo Scientific™ EasyPRM™ 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 quantitation 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 peptide were quantifiable in the Heracpin mAb digest sample prepared by the EasyPRM™ kit compared to 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 contamination is crucial for ensuring product safety, stability, and efficacy. Therefor, it is not only important to globally monitor HCPs levels throughout the process, but also quantify 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 a polyclonal 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 quantitation of critical HCPs with high accuracy, precision, and specificity.

RESULTS

The CHO HCPs standard proteins were digested with two different sample prep methods. Proteome Discoverer data processing generated 3000 HCPs protein and 50,000 peptides. We created a HCPs peptide spectral library in Skyline software and selected 28 critical HCPs. The selected unique peptides per protein from spectral library dataset and searched 118 critical peptides. For the final assay development, the 70 top 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 5 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 TO critical HCPs AQUA™ peptide mixture was used to generate extracted ion chromatograms of fragment ions of each AQUA heavy peptide and 5 points standard curve spanning from 0.03 to 71 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 to 0.13 femtomole as shown here in bar graph (Figure 4).

Figure 4. Lower Limit of Quantitation (LLOQ)

We found the critical HCPs heavy peptides mixture (200 fmol) was spiked in the HCPs control standard digest (Figure 5A) or nul CHO cell line harvest material (Figure 5B) prepared by either traditional or EasyPRM 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 calculate endogenous HCPs levels. Figure 5A and B show that both sample prep methods produce comparable HCPs quantitation.

Figure 5. Quantification of unknown HCPs in Heracpin mAb sample

CONCLUSIONS

• A critical HCP heavy peptide mixture was created and used to quantify unknown endogenous HCPs levels.

• 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 EasyPRM™ generated more quantifiable peptides than samples prepared by traditional method.

REFERENCES


TRADEMARKS/LICENSING

Research Use Only. Not for use in diagnostic procedures or protocols. © 2023 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.

PO223-711EN