MS-Based HCP Identification and Quantitation for Drug Substance Analysis

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PURPOSE
- Biological drugs produced from host cell expression systems inevitably contain host cell protein (HCP) impurities whose identity, presence, and levels may determine whether or not the drug is accepted by regulatory agencies.
- Conventional HCP analysis relies on immunosassays based on polyclonal antibodies raised against the host cell system, which provide an aggregate measurement of total HCP. However, deeper characterization of HCPs using orthogonal approaches is increasingly expected by regulatory authorities.
- MS-based proteomic platforms are routinely used to analyze complex matrices with a wide dynamic range of protein abundance. Non-targeted MS enables the comprehensive identification and profiling of proteins. Targeted MS is used for the relative or absolute quantitation of specific proteins of interest.

The purpose of this study was to use MS-based assays to obtain deeper characterization of the residual HCP content in in-process, Drug Substance or Drug Product (DS/DP) samples.

APPLICATIONS:
- Monitoring of purification on process
- Demonstration of HCP clearance
- Comparison of culture media, process improvements, and changes
- Evaluation of batch reproducibility and scale up
- Characterization of mock immunogen content for immunosassay

OBJECTIVES
- HCP IDENTIFICATION: To use non-targeted MS to comprehensively assay HCP content (identification and relative quantitation) in in-process samples and in formulated bulk DS.
- HCP QUANTITATION: To develop a targeted MS-based assay for HCPs of interest (e.g. problematic HCPs) based on their protein sequences, and perform sample analysis in absolute quantitation mode to track the HCP levels in ppm, at key clearance steps and in the DP.

METHODS
HCP IDENTIFICATION: For each study sample (harvested cell culture fluid, in-process samples, DS), a starting amount of up to 500 µg was digested with trypsin enzyme to generate peptides for analysis. For selected samples, an additional peptide prefractionation step was performed using SCX and HPPR to enhance the detection and coverage of low-abundance HCPs. Peptides were analyzed on a nanoAcquity UPLC (Waters) coupled to a high-resolution absolute mass Q Exactive mass spectrometer (Thermo). The equivalent of 2.5 µg protein was used per injection. The acquisition method alternated between LC-MS for peptide ion detection and LC-MS/MS to obtain sequence information from peptide fragments. A process-specific protein sequence database was built based on all sequences known or assumed to be present in the study samples (e.g. DS, host proteome, process-related protein additives, etc.). The acquired MS/MS data were searched against the custom database to match the acquired peptide sequence data to corresponding database sequences, and any expected or unexpected post-translational protein modifications were also identified. Three database search engines (Mascot, OMSSA, and X!Tandem) were used to leverage the strengths of each search algorithm and maximize the number of high confidence peptide identification. High confidence peptides were clustered by sequence information from peptide fragments. A process-specific LC-MS for peptide ion detection and LC-MS/MS to obtain absolute quantitation of HCPs of interest for development (e.g. HCPs with known or potential product modifying capabilities, efficient or inefficient clearance profiles, etc.) The intended purpose of the assay was for the multiplexed monitoring of the HCP levels in individual batches of in-process and DS/DP samples, using absolute quantitation.

MULTIplexed LC-MS/MS ASSAY DEVELOPMENT

Bioinformatic analysis of the target protein sequences and empirical evidence from the HCP identification study were used to identify ≤5 surrogate peptides per target protein. The corresponding synthetic peptide standards were obtained in ‘light’ non-labeled, and ‘heavy’ stable-isotope labeled (SIL) form. The standards were HPLC-purified and quantified for accurate weight-for-weight, for use in assay development and as reference material. LC-MS/MS assay conditions were developed using the synthetic peptide standards and optimized in sample matrix, using a nanoAcquity UPLC (Waters) coupled to a QTRAP (AB Sciex) mass spectrometer. Optimized parameters included the chromatography and retention time, the collision energy for peptide fragmentation, the best peptide fragments for monitoring each target peptide, and various assay performance characteristics were evaluated (e.g. linearity, precision and accuracy, etc.).

RESULTS

A test sample mimicking a DS with protein impurities in the ~1 ppm to 600 ppm range was analyzed in triplicate, using LC-MS/MS either with or without peptide prefractionation. The test sample consisted of SiLuLight Lite Mab Universal Standard and was spiked with the UPS2 dynamic range protein standard set (Sigma), using a ratio of 500:1. Results show a protein-dependent assay sensitivity of ~1 to 15 ppm for the different HCP impurities (UPS2 proteins).

CONCLUSION

MS-based HCP assays enable direct identification and quantitation of total and individual HCP impurities, with an assay sensitivity in the ~1 to 15 ppm range (DS/DP dependent). Identification and accurate quantitation of HCPs provides critical information for biosimilar vs originator comparisons, process development, optimization and monitoring, as well as for patient and product risk assessments.