Table 1: Host Cell Proteins: Sample Preparation, Methods, Instruments, Software, And Validation

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SCOPE:
Host cell proteins (HCPs) are residual protein impurities expressed along with the desired recombinant protein therapeutic. They are unique to their host cell types, and are often difficult to remove completely. Failure to remove residual HCPs sufficiently during downstream processing can affect efficacy and safety of the therapeutic drug, or even induce adverse effects to patients. Typically, biopharmaceutical companies utilize an enzyme-linked immunosorbent assay (ELISA) to monitor total levels of HCPs at each step of the purification process and in the final drug substance. Recently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as an orthogonal HCP analysis method. This approach can obtain both qualitative and quantitative information on individual HCPs. In this roundtable discussion, we would like to focus on the best practices of HCP analysis using LC-MS/MS including digestion methods, instrumentation, system suitability, quantitation methods, and bioinformatics tools. Discussion of the different MS approaches to HCP analysis, as well as method performance (i.e., sensitivity, robustness, etc.) also is in scope. The goal is to achieve a better understanding of HCP analysis across the industry and share lessons learned.

DISCUSSION QUESTIONS:
1. Basics for MS
   • Instrument (QTof vs. Orbi vs. Triple-Quad)
   • Method (digestion method, instrument method, number of replicates, etc)
   • What is the testing point regarding HCP analysis (DS vs. in-process samples, clone selection, manufacturing batches, process validation, final DS, or commercial lots)?
   • System suitability study (i.e., samples, criteria to define a “good” run)
   • Performance validation (i.e., generate consistent/reproducible results)

2. Identification and Quantitation
   • Limit of detection/quantitation
   • Different quantitation method (i.e., label-free vs. labeling techniques)
   • Software packages for data analysis

3. Next Gen HCP analysis?
   • What is the future?
   • Are we doing too less or too much?

Notes: Each roundtable had about 8-9 participants from industry and vendor companies.

Synopsis: The focus of this roundtable discussion was to discuss host cell protein analysis by LC-MS/MS as an orthogonal technique to ELISA. Most of industry seems to be analyzing HCPs by using a label free approach. Everyone is using similar instrumentation and software for data analysis.

Sample Preparation: Everyone agrees that sample preparation is a critical factor. Some of the industry is using reduction/alkylation/desalting with trypsin and some are using Lys-C/trypsin. Most people are doing manual sample preparation and not using automation. Sample preparation seems to not be as successful using
automation due to condensation and samples not properly mixing. An online column for proteolytic digestion (Lys-C or Trypsin) is needed from vendors. Those using a trypsin column overload the column to gain more sensitivity. Concentration of samples: How low is too low and what are people using to concentrate their low concentrated samples? Some people are getting in-process samples at 0.1 mg/mL. Most people are using molecular cutoff spin columns to concentrate. We learned about S-Traps from PROTIFI. One easy-to-use spin column combines sample concentration, clean up and digestion all in one. Does anyone see solubility/or digestion issues after concentration of samples? **Solution:** run a 1D gel to see if digestion is complete or not.

**Instruments:** What mass spectrometer instruments are people using? The majority is using Waters: QTOFS, Thermo: Orbitraps, and Sciex: TripleTOFS and Triple Quads. Are people using 1D or 2D HPLC’s for HCPs? Some are using 2D-LC/MS, but most are not as it is tedious and time-consuming.

**Quantitation/Detection:** Depending on dynamic range of instrument (use the Hi3 method vs. Mid3 method). Some companies are removing the mAb to gain more sensitivity of HCPs. A lot of people are using MRM for quantitation of HCPs and Orbitraps and/or QTOFs for identification. Some are using a Sciex TripleTOF for identification and a Sciex Triple Quad for quantitation. For relative quantitation for HCPs, some people use abundance obtained during HCP identification analysis and for absolute HCP quantitation, they use a quantitation through a targeted MRM.

**Concern:** Protein grouping: Most software used for HCP quantitation has a black box with regard to protein grouping. This is a concern for most people. We do not know how they assign unique proteins.

**Bioinformatic Tools:** Analyst 2, Progenesis QI, Proteome Discoverer, ProSightPD, GeneData, and Protein Metrics.

**Databases searched:** NCBI, Uniprot, and Swissprot (*Cricetulus griseus* specific)

**Replicates:** Does anyone worry about carryover? Yes, methanol can be used in-between the samples. Some people do run replicates for statistical analysis and some do not.

**System Suitability/Performance validation:** Run monoclonal antibodies that you know has an HCP present, or take a monoclonal antibody and spike in a low level host cell protein. For Triple TOF instruments run a Beta Galactose digest for auto calibration.

**Sample Types Analyzed:** In-process controls, drug substance batches, pilot batches, lots representative to reference material. Most people are running the whole process train: the cleanest samples (drug substance) and all corresponding in-process samples.

**Data Acquisition:** Some people are using DIA (SWATH), but most people are using DDA for acquisitions.

**Question:** Coverage for ELISA: Do companies perform western blots to quantify ELISA coverage? Most of the industry is not doing this.

**What is the future?** Do we think mass spectrometry will replace ELISA in the future? Or will we use it less? Most agree that we need both because they are very powerful together. How do we prove the robustness of LC-MS/MS to the FDA if all industry is using different instruments, samples preparation, and software? Another reason it may be hard to align HCP analysis by LC-MS/MS is because there are so many different modalities: Gene therapy, monoclonal antibodies, bispecifics, vaccines, and fusion proteins. Everybody agrees that there’s a prompt need for FDA to provide guidance for HCP analysis.