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SOLUTIONS FOR BIOANALYSIS

A universal analytical tool for protein identification and individual quantification in gene or cell therapies development

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INTRODUCTION

Through the development of new therapies such as gene or cell therapies, the need to identify and quantify residual proteins considered as impurities for final product is critical. Such proteins can present a safety risk to patients. These proteins can be come from the production of the viral vector and known as Host Cell Proteins or Host Related Proteins but they could also come from reagents used in the full cell therapy process (feeders or any serum or proteins added during the process).

Protein impurities must be monitored for consistency purpose. In order to develop a generic approach, mass spectrometry coupled to liquid chromatography appears as an interesting tool.

A global LC-MS based approach for accurate assessment of individual protein quantities was developed. This strategy consists in performing protein identification and individual quantification evaluation within the same analysis for a control of residual proteins in batch-to-batch comparison analysis.

WORKFLOW

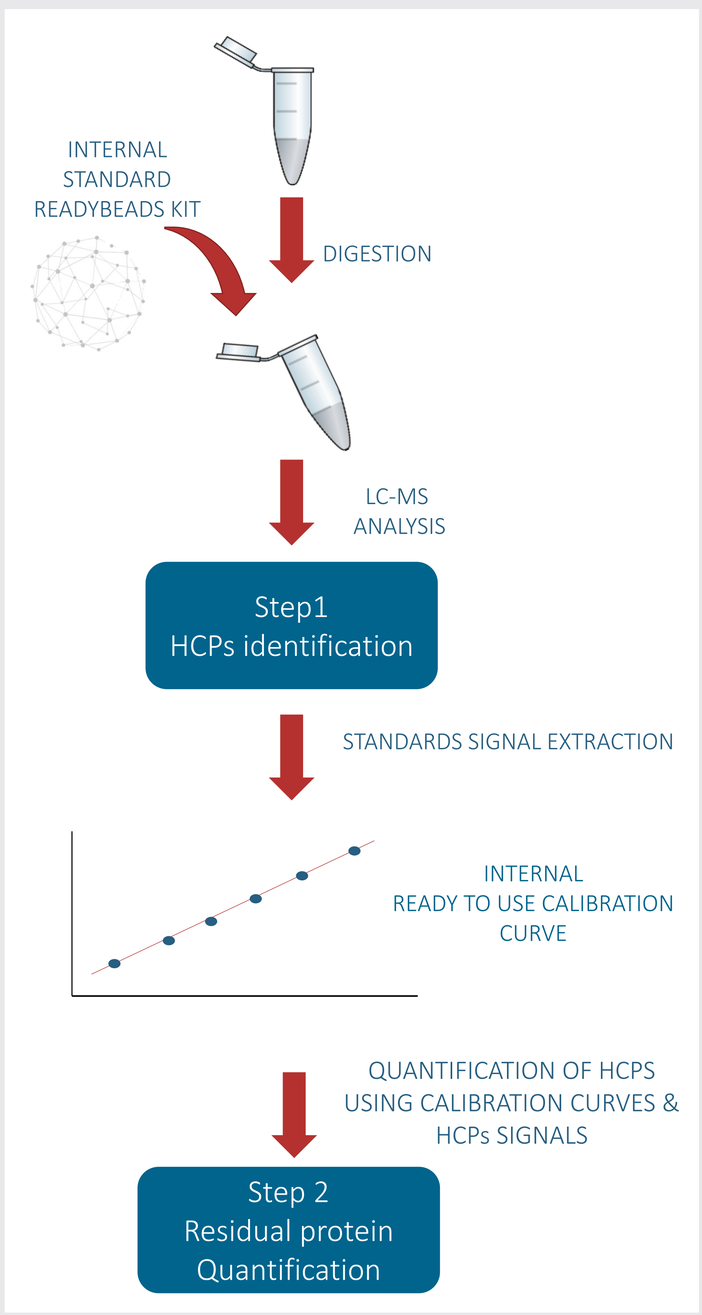

Figure 1: analysis pipeline with universal strategy.

In order to characterize the Drug Product (DP) or Drug Substance (DS) biopharma companies need to compare the identified Host Cell Proteins (HCPs) and their estimated amount in different production batches of their drug product.

Figure 1 presents the global workflow for protein identification and quantification in DS or DP samples.

The first step is the protein Identification of several dozen of proteins with the peptide mapping LC-MS method. Protein names, Isoelectric point and molecular weigh are generated for each detected protein.

Once proteins are identified, the internal standard is used for the quantification evaluation of individual proteins.

Protein quantification allow batch-to-batch comparison and data completeness analysis.

PROTEIN TAXONOMY IDENTIFICATION

Most of the Cell and Gene therapy processes involve several taxonomies such as human, bovine and even sometimes bacteria (E Coli). For protein identification, LC-MS correlates experimental data with proteome database. As soon as detected proteins have specific sequences, LC-MS can differentiate proteins coming from different taxonomies. Here, proteins from human, bovine and E.Coli have been identified and differentiated. Figure 2 presents pie charts of the 59 detected proteins and their related taxonomies. On the right hand side, the pie chart presents that the 45 human protein represents 90% of the total amount of protein whilethe 12 bovine and the 2 E Coli ones represent respectively 9 and 1%.

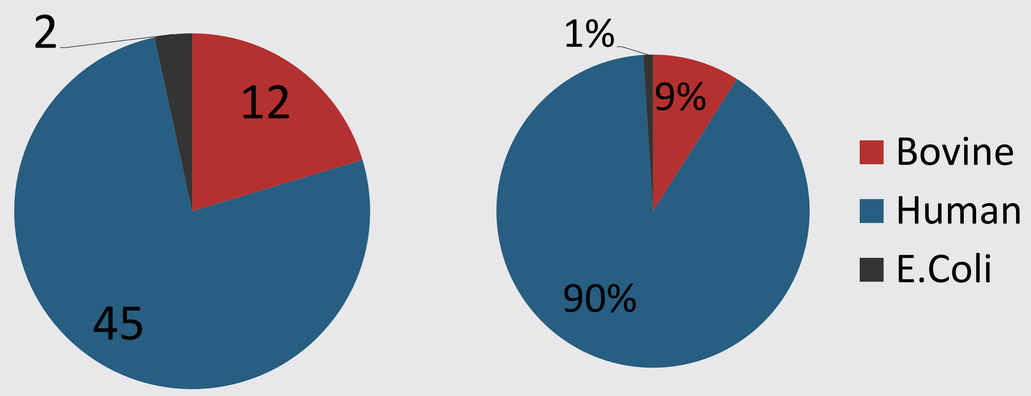

Figure 2: On the left hand side, pie chart with numbers of identified proteins and their relative taxonomies. On the right hand side, their relative abundance in the sample.

BATCH-TO-BATCH COMPARISON

Following protein identification and quantification, two different batches have been compared. Most of proteins have been detected in both samples and only slight differences have been detected. Figure 3 shows a graphical representation of all detected and quantified proteins in the 2 batches. The size of the plot is directly correlated to protein quantification. Most of detected proteins have molecular weight closed to 40 000 Da and isoelectric point between 4 to 6. This information can be useful for bio-manufacturing optimization.

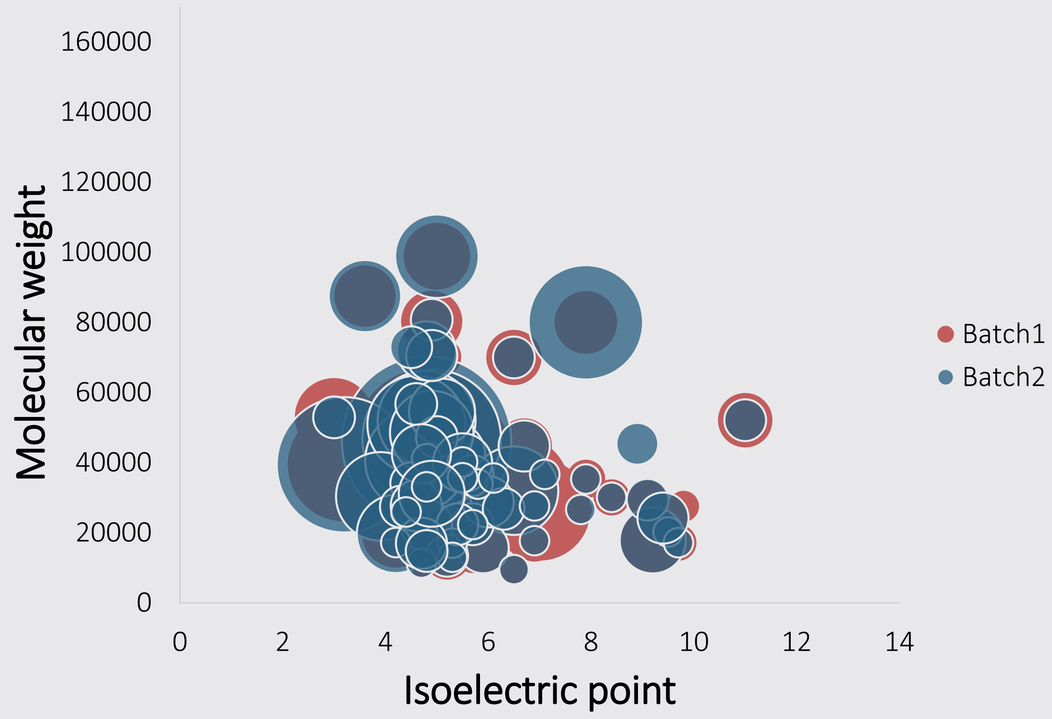

Figure 3: Batch-to-batch comparison of detected and quantified proteins in 2 batches.

CONCLUSIONS

Thanks to this analytical tool, completely independent from any biological reagents, residual proteins can be detected and individual quantities accurately assessed to have full data completeness through the whole R&D and manufacturing development. LC-MS appears as the method of choice for supporting data in HCPs analysis for regulatory approval process. This tool has been used for several purpose such as batch-to-bacth comparison, cell line comparison and specific HCPs detection.