Host Cell Protein Analysis of Biopharmaceuticals Using Automatic Offline Fractionation and LC/MS

Introduction

Most biopharmaceuticals such as monoclonal antibodies today are produced from genetically modified host cell systems. A large number of low level (1-100 ppb) host cell proteins (HCPs) can still remain in the final products even after multiple purification steps. Since HCPs can induce an immune response, regulatory agencies require identification and quantification of HCPs and their fragments in the final product. LC/MS is a powerful technique to provide more in-depth characterization of HCPs throughout the biopharmaceuticals production process. In this study, we evaluated different offline fractionation methods and LC/MS was done as the MS1 and MS-MS analysis. Both high pH reverse phase (HPRP) fractionation and strong cation exchange (SCX) fractionation were evaluated followed by low pH reverse phase LC/MS peptide mapping analysis. These results were also compared to 1D LC/MS analysis without offline fractionation.

Experimental

Assay/MAP Sample Preparation
A human IgG1 mAb produced from CHO cells in a hollow fiber bioreactor was thawed with 70% ACN, diluted with 30% milliQ water, and concentrated using Vivaspin 10K.

LC Conditions

Aptabio 1200 Infinity UHPLC with an Agilent Eclipse Plus C18 column (1.8 µm, 2.1 mm x 100 mm, 959758-902)

MS Conditions

0.1% formic acid in Acetonitrile

Conclusions

- A Host cell protein analysis workflow in demonstrated that AssayMAP for automated offline fractionation followed by reverse phase LC/MS.
- With the same injection amount, high pH fractionation with LC/MS provided the most host cell proteins identified, compared to SCX fractionation with RP LC/MS and 1D LC/MS analysis.
- Aptabio AssayMAP system provides a superior dynamic range (2-4 orders) allowing detection and identification of low amount HCP co-eluting with extremely intense mAb peptide peaks.

References


For Research Use Only. Not for Use in Diagnostic Procedures.