nibrt
National Institute for Bioprocessing Research and Training

Contract Research

CASE STUDY

A mass spectrometry approach to identify problematic Host Cell Proteins (HCPs)

CASE STUDY

A mass spectrometry approach to identify problematic Host Cell Proteins (HCPs)

Challenge

A client requested the support of NIBRT Contract Research to identify Host Cell Proteins (HCPs) in drug substance samples. Client data from stability studies showed that for one particular batch a truncated variant of the therapeutic could be detected which increased in abundance over time. The cause of this truncated variant was unknown however it was hypothesised that a peptidase HCP may be present in the problematic batch. HCPs are a major class of process related impurities that pose a considerable risk to drug efficacy and patient safety. The majority of HCPs are removed during downstream purification steps. However, some may still be present at very low concentrations in the final drug product, and can affect the product potency and/or stability, and potentially compromise patient safety.

Solution

A 2D-LC-MS/MS peptide mapping approach was employed to identify HCPs. The workflow incorporated high pH reversed-phase chromatography (RP-LC) in the first dimension to fractionate the trypsin digested sample prior to low pH RP-LC-MS/MS in the second dimension. Rabbit phosphorylase b Hi3 peptide standards were spiked into samples prior to RP-LC-MS/MS analysis allowing for quantitation of HCPs using the Hi3 methodology which is based on peptide peak intensity. Resultant mass spectrometric data was analysed using the HCP workflow in the Byos® software package from Protein Metrics for identification and quantitation of HCPs within the samples. Raw data were searched against a proteome database of the proprietary host cell line. This allowed for a comparison of HCP profiles of the failing batch alongside the reference batch. Following this, a targeted search was performed using a smaller database of peptidases which may be present in the host. The database was manually compiled using an online peptidase database.

Outcome

Two comprehensive reports were provided to the client, detailing the results of the host proteome search (report 1) and the targeted peptidase database search (report 2). The data compared HCP profiles of the failing batch and reference batch. Data from the targeted search identified a specific peptidase in the failing batch that may be responsible for the observed truncated product.

Project Process

nibrt
National Institute for Bioprocessing Research and Training

Contact Us

Tel: +353 1 215 8100 Email: contract.research@nibrt.ie
Web: www.nibrt.ie/contract-research /
Foster Avenue, Mount Merrion, Blackrock, Co. Dublin, A94 X099, Ireland

REGISTERED scientist.com SUPPLIER