AN HS-MRM ASSAY FOR QUANTIFICATION OF HOST CELL PROTEINS IN THERAPEUTIC MONOCLONAL ANTIBODIES

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OVERVIEW

An HS-MRM assay was developed for quantification of low abundance peptide standards spiked in a monoclonal antibody (mAb) digest and demonstrated that it has the required sensitivity and dynamic range (at least 3 orders of magnitude) for a typical HCP monitoring assay.

INTRODUCTION

- Analysis of low-levels (1-100 ppm) protein impurities (e.g. host cell proteins-HCPs) in protein therapeutics is a challenging assay requiring high sensitivity and a wide dynamic range.
- Mass spectrometry based quantification assays for proteins typically involve protein digestion followed by selective reaction monitoring/multiple reaction monitoring (SRM/MMR) quantification of peptides using a low-resolution (6-1000) tandem quadrupole mass spectrometer. One of the limitations of this approach is the interference phenomenon observed when the peptide of interest has the same precursor and fragment mass (in terms of m/z values) as another co-eluting peptide present in the sample (within 1 Da window). To avoid this phenomenon, we propose an alternative mass spectrometric approach, a high selectivity (HS) MRM assay, which combines on-nucleus separation of peptides precursors with high-resolution (60-40,000) MSdetection of peptide fragments.
- We explored the capabilities of this approach for quantification of low abundance peptide standards spiked in a monoclonal antibody (mAb) digest and demonstrated that it has the sensitivity and dynamic range (at least 3 orders of magnitude) required for HCP analysis. Four peptide standards were detected at concentrations as low as 0.1 nM (1 femtomole loaded on a 2.1 mm ID chromatographic column) in the presence of a high-abundance peptide background (2 µg of a mAb digest loaded on-column).

RESULTS

Sample Preparation
- A herring whale larval (HWA, 15 mg) was derivatized with 0.875 M Acetic anhydride (Millipore Sigma, St. Louis, MO) and digested with 25 µL (2000 U) of trypsin (Promega, Madison, WI) at 37°C with 0.1 M NaHCO3 (10 mM) and digested with a mixture of Lys-C and trypsin (Promega, Madison, WI) overnight. A MassPREP digest standard (Waters P/N 186006011) spiked in the HWA digest. The most abundant precursors from each peptide of HS-MRM chromatograms were selected for quantification.
- LC Conditions
  - ACQUITY UPLC (Waters) HPLE systems equipped with a C18 (charged-air free) UNIFI Scientific Information System 1.8.1 was used for data acquisition and processing.
- MS conditions
  - Data-independent acquisitions (DIA) following precursor level ion mobility separation (HDMS E) were performed on a VION IMS QTof mass spectrometer. The precursor ions were isolated using the quadrupole and high mass resolution (>40,000) electron transfer dissociation (ETD) was performed by the ion mobility fragment of each peptide.
- Informatics
  - UNIFI Scientific Information System 1.8 was used for data analysis and processing.

Workflow (4 steps)
- Unmodified peptides (2-10 Da) were detected at concentrations as low as 0.1 nM (1 femtomole loaded on a 2.1 mm ID chromatographic column) in the presence of a high-abundance peptide background (2 µg of a mAb digest loaded on-column).

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