Increasing sensitivity of high throughput host protein analysis on a novel high-resolution accurate mass platform

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ABSTRACT
Purpose: Evaluate the performance of the new Thermo Scientific™ Orbitrap™ Astri™ mass spectrometer for fast and sensitive identification of low level host cell proteins (HCPs) in biopharmaceuticals using a combination of non-denaturing tryptic digestion, fast chromatographic separation, sensitive and fast HRMMS detection, and advanced data processing to reduce the dynamic range challenge and maximize the identification rate.

Methods: (1) Chromatographic separation of non-depleted vs. depleted samples. (2) data interpretation algorithms (DIA) using native isolation windows (35%L) and (3) data processing with Proteome Discoverer software using the ThermoChrom algorithm. Results: Demonstrate excellent performance of the new Orbitrap Astri™ mass spectrometer for identification of HCPs at sub ppm level with increased throughput.

INTRODUCTION
Recent advances in LC/MS/MS analysis software have improved the overall sensitivity of detection by reducing the dynamic range. However, the separation of the host cell proteins from the therapeutic protein of interest is essential for successful identification. While convenient methods for improving sensitivity, especially when a small amount of sample is available, are preferred, the separation process may pose a challenge to detect low concentrations of HCPs in biopharmaceuticals. Herein, we demonstrate the use of a novel HRMMS platform to improve the dynamic range identification for HCPs at sub ppm level with increased throughput.

MATERIALS AND METHODS
Proteolytic NIST/MBig digests were generated upon digestion with the Thermal Scisensor™ SMART Digest (produced from methylated trypsin) for 30 min at 37°C; and b) for 3 hrs at 37°C (Clyton et al., Huang et al.) and removal of undigested protein via precipitation at 4°C for 15 min. Each sample was digested for 4 min (Fig. 1). Recovered supernatant from each digest was Redigested with DTT solution (final concentration of 30 mM) for 45 min at 57°C. 100-500 mg of peptides were separated by reverse phase UPLC with a 100 μm x 15 mm Thermo Scientific™ EASY-Spray™ PepMap300™ Basic column with a 20 μm gradient on an Thermo Scientific™ Vanquish® Nexsys system operated in Trap + Elite mode at a flow rate of 0.8 g/min. The new mass spectrometer was operated in data independent acquisition (DIA) mode using 7 IS isolation windows and acquisition spread up to 200 m/z (Fig. 2). Data analysis was performed with Proteome Discoverer 3.1 software with Chromatitis algorithm and Uniprot MIA mouse database.

RESULTS
Qualitative analysis of trace level HCPs in NISTMaBio
Detection of trace level HCPs impalpable to biochemical, which present themselves in low levels sub ppm to 100-200 ppm, is a common nuisance due to the wide dynamic range of the biopharmaceuticals. Highly abundant multi-protein HCPs and background noise challenges both the chromatographic separation, as well as the MIA software’s ability to detect their signal at the noise level. Herein developed non-denaturing proteolysis facilitates detection of HCPs and improves detection limit by exploiting the resolution of native protein structure in proteomics and therefore reducing the dynamic range of peptides present in the sample.

Figure 3. Base peak chromatograms obtained from digests (A) under non-denaturing conditions (37°C, 3 h) (B) and (C) after HCP depletion and following crystallization and centrifugation.

Figure 4. (A) Base peak chromatograms of triplicate injections of NISTMaBio peptides obtained under digests without non-denaturing conditions using a 20 ppm gradient (B) and (C) HCP depletion chromatogram showing peak representing peptides associated to light and heavy chains demonstrating incomplete sequence coverage.

CONCLUSIONS
In this work, we demonstrated outstanding performance of the new Orbitrap Astri™ mass spectrometer for fast and sensitive host cell proteins analysis.

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REFERENCES

TRADEMARKS/LICENSING
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Table 1. HCPs identified in this study and not reported by Beaumel et al. (A) and (B)

Table 2. Subset of 40 HCPs identified in this study matched against measured ng of HCP per mg of mAb shown by Beaumel et al.

Figure 5. High confidence NISTMaBio HCP identifications: (A) number of unique peptides (B) and (C) number of PSMs per HCP (B).

Figure 6. Overlay of host cell proteins identified by DIA in Beaumel et al. (2023) (blue) vs. our study (red) (A) and (B) by Beaumel et al. study (DIA, PABA, DIA, DIA and GFF) vs. our study.

Figure 7. Examples demonstrating MS1 spectral quality for HCPs of low and high abundance

Figure 8. Representative MS2 spectrum of HCPs identified by HCD and data-dependent acquisition (Acc. P-2DMS) identified in this study.

Figure 9. Representative MS2 spectrum of peptides identified by HCD and data-dependent acquisition (Acc. P-2DMS) identified in this study.