Fast Identification and Quantification Assay for Host Cell Proteins using Data Independent Acquisition Mass Spectrometric Analysis

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INTRODUCTION

- Here we investigated the capabilities of LC/MS for HCP analysis, based on a single dimension chromatographic separation coupled with high-resolution (R > 30,000) MS-detection for both precursors and fragments.
- Four protein digests were spiked at two different concentration levels in two Inflectra digests and the concentration changes of the proteins were monitored using Progenesis QI for Proteomics.
- These results indicate that the 1D LC/MS assays can be used for identification and quantification of HCP impurities across multiple samples in a high-throughput fashion.
- The LC/MS assay achieved comparable sensitivity to traditional ELISA assays, while offering the unique advantage in providing unambiguous HCP identification.

METHODS

Sample preparation:

A licensed biosimilar (Inflectra, 10 mg/mL) was denatured with RapiGest surfactant (60°C, 15 min), DTT reduced (60°C, 1h), alkylated with IAM (RT, 30 min) and digested with a mixture of Lys-C and porcine trypsin (Promega) overnight.

Five protein digest standards (ADH—yeast alcohol dehydrogenase, BSA - bovine serum albumin, CYC - horse cytochrome C, ENL - yeast enolase, PHO - rabbit phosphorylase b) were spiked post-digestion in two Inflectra digests, as indicated in Table II. The amounts loaded on-column with a 10-uL injection were in the range of 10-500 fmoles on-column for each of the spiked proteins (or 12-490 in terms of ppm concentrations in the sample), while the total amount of mAb digest loaded every time was 10 µg.

LC Conditions:

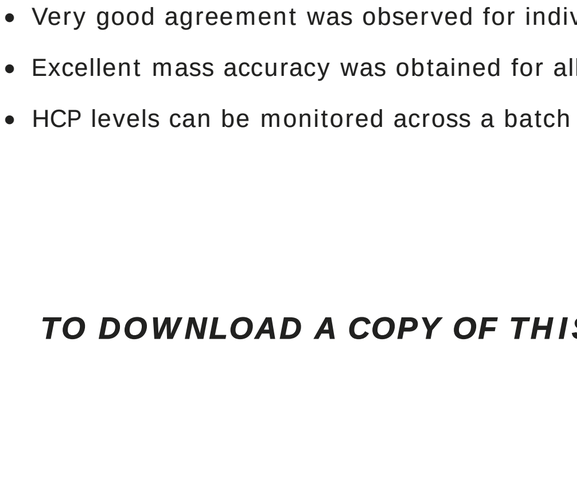
The LC/MS assay was also performed on an ACQUITY™ UPLC® H-class Bio system equipped with a CSH (charged-surface hybrid) C18 column (2.1 x 150 mm, 1.7 µm particles, P/N 186005298). Peptide separations were performed at a flow rate of 0.2 mL/min with a gradient of from 0% to 40% Solvent B in 30 min, at a column temperature of 60 deg C. The total LC runtime was 45 min.
The mobile phases were: 0.1% FA (formic acid) in DI water (Solvent A) and 0.1% FA in acetonitrile (Solvent B).

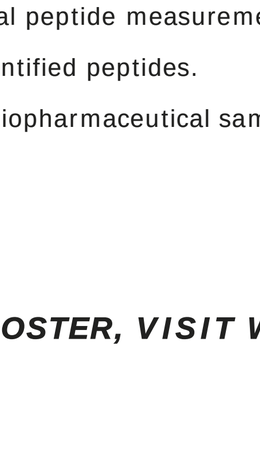
Mass Spectrometry:

Data-independent acquisitions were performed on two instruments, a Vion IMS QTof and a Xevo G2-XS mass spectrometer, over the m/z range of 100-2000 with an acquisition time of 0.3 sec. Low-energy scans were acquired with a CE of 6 eV, while the high-energy fragmentation scans used CE ramping from 15 to 40 V.

Data processing:

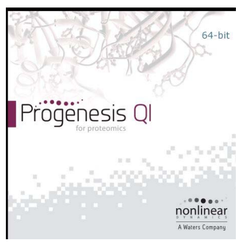
Progenesis QI for Proteomics (QIP) software was used for data processing.



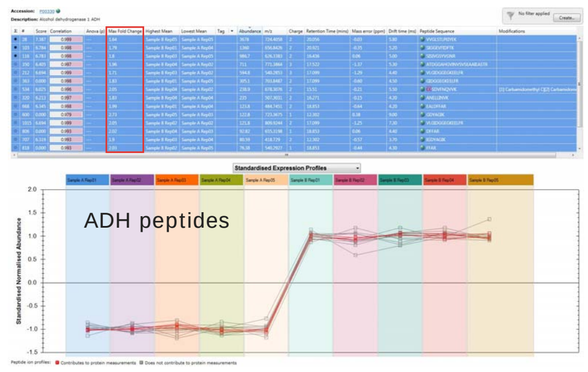


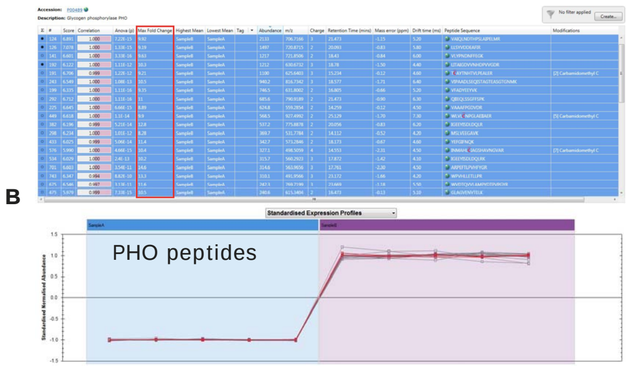
VION IMS QTof MS and Xevo G2-XS systems can be used for HCP monitoring assays in conjunction with Progenesis QIP software.

RESULTS

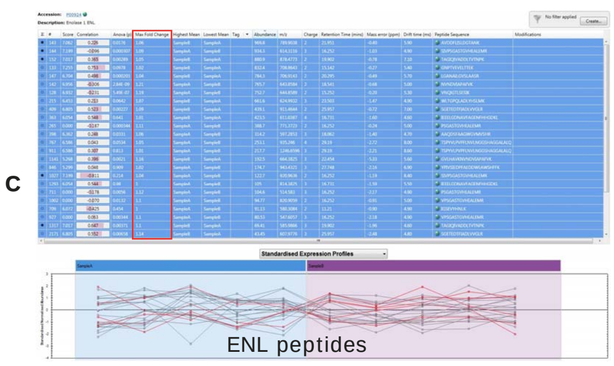


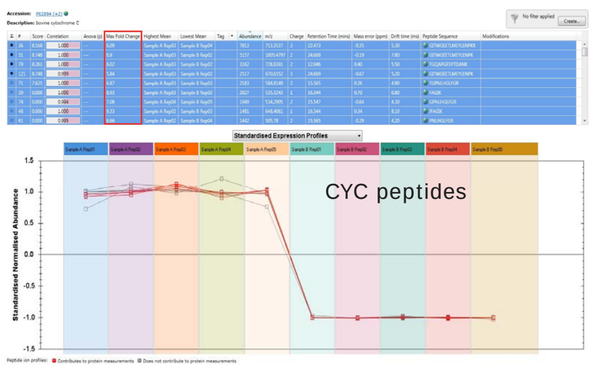
Progenesis QIP was used for tracing all five spiked proteins across 10 LC/MS injections:





		Sample A	Sample B
Sample matrix:	Inflectra tryptic digest amount	10 µg	10 µg
Internal standard:	ENL tryptic digest concentration (ppm)	117	117
Spiked protein digests:	ADH tryptic digest concentration (ppm)	37	74
	BSA tryptic digest concentration (ppm)	130	13
	CYC tryptic digest concentration (ppm)	60	12
	PHO tryptic digest concentration (ppm)	49	490





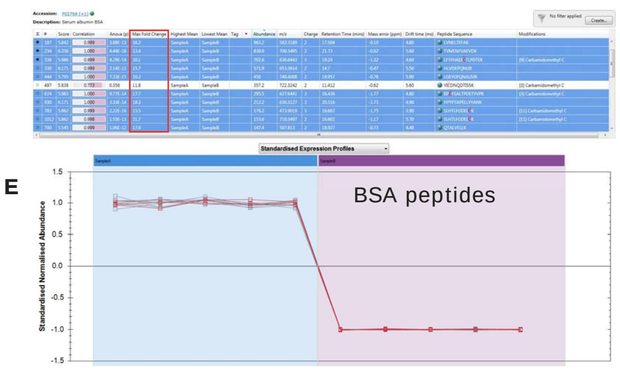


Table I. Results of the HCP monitoring assay. Four protein standards (ADH, BSA, CYC and PHO) were spiked at two different concentration levels in two Inflectra digests and one protein digest (ENL) was spiked at the same level in both samples (see Table II for protein concentrations). The five panels shown above display the Progenesis QIP trend plots observed for each spiked protein across 5 replicate injections from each sample: (A) - ADH digest; (B) - PHO digest; (C) - ENL digest; (D) - CYC digest and (E) - BSA digest. Each panel shows a list of peptides identified from each spiked protein. In addition, the individual peptide measurements obtained for each spiked protein are highlighted by a red rectangle.

Protein ID	Spiked protein concentration (ppm)			Measured fold change	No of peptide ions used for the measurement	RSD (%)
	Sample A	Sample B	Fold change			
ADH	37	74	2.0	1.9	14	7.2
BSA	130	13	10.0	15.7	10	21.5
CYC	60	12	5.0	7.1	9	14.9
ENL	117	117	1.0	1.1	24	5.4
PHO	49	490	10.0	10.6	19	15.1

Table II. Results of the HCP monitoring assay: 4 protein digest standards were spiked in two Inflectra digest at two different concentrations in the ranges listed in the second column, while one protein (ENL) was spiked at the same concentration in both samples.
As shown, the measured fold changes between samples A and B correlate very well with the expected values.

CONCLUSIONS

- Sensitive HCP identification and quantification down to 10 ppm are facilitated by using a CSH C18 column for peptide separation.
- Very good agreement was observed for individual peptide measurements for each of the spiked proteins, as highlighted by the trend plots.
- Excellent mass accuracy was obtained for all identified peptides.
- HCP levels can be monitored across a batch of biopharmaceutical samples in a high-throughput fashion using Progenesis QIP software.

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