AN LC/ESI-IM-MS/MS ASSAY FOR IDENTIFICATION AND QUANTIFICATION OF HOST CELL PROTEINS IN THERAPEUTIC MONOCLONAL ANTIBODIES

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OVERVIEW

Two assays based on single dimension LC/MS analysis were developed for identification and quantification of HCPs in therapeutic mAbs: one assay was able to detect protein impurities down to 10 ppm, and the other was able to accurately track HCPs across multiple batches of biopharmaceuticals

INTRODUCTION

- Low levels (1-100 ppm) of host cell proteins (HCPs) remaining in the final purified biopharmaceuticals cause safety and stability concerns because HCPs can sometimes elicit an immunogenic response or can affect the drug stability.
- Although ELISA assay for HCP quantification enjoys several advantages including high sensitivity, high-throughput, ease of use and low cost per sample, ELISA assay do not provide individual HCP identifications and is difficult to prove its HCP proteome coverage.
- Several mass spectrometry-based assays have been recently developed to overcome these limitations [1-5].
- Here we investigated the capabilities of LC/MS methodology for HCP analysis (1D LC/MS HCP assay), based on a single dimension chromatographic separation, optimized for sample overloading conditions, coupled with ion mobility separation of peptide precursors and followed by high-resolution (>30,000) MS-detection.
- The 1D LC/MS HCP assay was able to detect all 4 protein digest standards spiked in an Inflectra digest down to concentrations as low as 10 ppm.
- In a separate experiment, the 4 protein digests were spiked at 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 assay achieves 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. Four protein digest standards (ADH, BDS, ENL, PHO) were spiked post-digestion such that the amounts loaded on-column for a 250-uL injection were: 5,000 fmoles ADH (yeast alcohol dehydrogenase), 1,000 fmoles PHO (rabbit phosphorylase b), 250 fmoles BSA (bovine serum albumin) and 50 fmoles ENL (yeast enolase).

LC Conditions

HCP Discovery Assay (90 min gradient)
An ACQUITY™ UPLC® I-class system equipped with a CSH (charged-surface hybrid) C18 column (2.1 x 150 mm, 1.7 µm particles) was used to separate the mAb digest at a flow rate of 200 µL/min and a column temperature of 60 °C. Gradient elution was performed from 0% to 40% mobile phase B in 90 min and the total LC runtime was 105 min.

HCP Monitoring Assay (30 min gradient)
The assay was also performed on an ACQUITY™ UPLC® H-class system with the same CSH column (P/N 186005298). Peptide separation was undertaken at the same flow rate and temperature with a gradient of from 0% to 40% B in 30 min. For monitoring assay, the total LC runtime was 45 min.

The same mobile phases were used for both assays, Solvent A: 0.1% FA (formic acid) and Solvent B: 0.1% FA in acetonitrile.

MS conditions

HCP Discovery Assay (90 min gradient)
Data-independent acquisition following precursor level ion mobility separations (HDMS^E) were performed on a SYNAPT G2-Si mass spectrometer, over the m/z range of 100-2000 with an acquisition time of 0.5 sec. Low-energy HDMS^E scans were acquired with a CE of 5 eV, while the high-energy fragmentation scans used mobility synchronized CE ramping.

HCP Monitoring Assay (30 min gradient)
Data-independent acquisition following precursor level ion mobility separations HDMS^E) were performed on a Vion IMS QTof mass spectrometer, over the m/z range of 100-2000 with an acquisition time of 0.3 sec. Low-energy HDMS^E scans were acquired with a CE of 5 eV, while the high-energy fragmentation scans used mobility synchronized CE ramping.

Informatics

Unifi Scientific Information System was used for data collection on Vion IMS QTof. Progenesis QI for Proteomics software was used for data processing.

VION IMS QTof MS system was used for the HCP monitoring assay in conjunction with Progenesis QI for Proteomics software

RESULTS

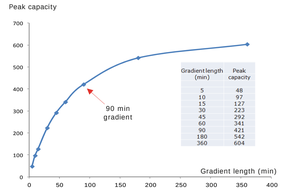

Figure 1. Dependence of peak capacity on the gradient length for peptide separations. Column: C18 CSH, 2.1 x 150 mm.

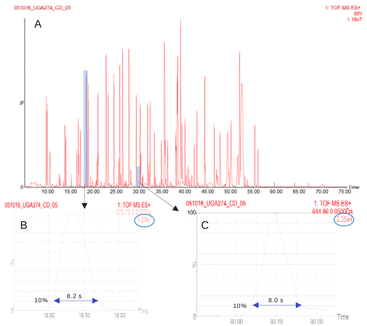

Figure 2. (A) Base peak chromatogram showing the separation of the Inflectra digest spiked with MIX-4 protein digest standards; (B) mass chromatogram of a high-abundance peptide (Inflectra HC T12: TEDTGVYYCSR (m/z = 675.78, +2); (C) mass chromatogram of T43 peptide (VNQGITLSESIG, m/z = 644.86, +2) from the ENL digest spiked at 10 ppm. The two peptides have very similar chromatographic peak widths, even though the Inflectra peptide is present at much higher concentration.

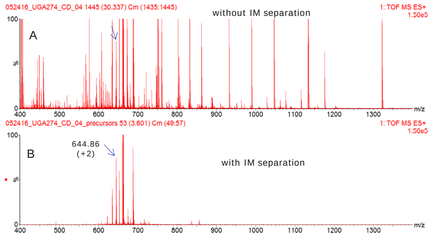

Figure 3. The role of precursor level ion mobility (IM) separation in reducing spectral complexity: (A) ESI-MS spectrum generated from 10 combined scans recorded during the elution of a low-abundance peptide (VNQIGTLSESIK, T43 from the ENL digest, spiked at 10 ppm), without IM separation; (B) ESI-IM-MS spectrum recorded for the same peptide after combining 9 mobility bins.

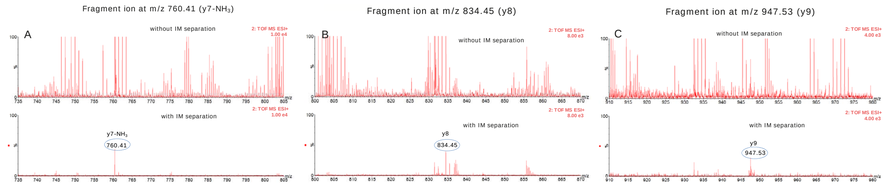

Figure 4. Comparison of high-energy fragmentation spectra obtained with/without ion mobility separation. Each panel displays a portion of the fragmentation spectrum obtained without the IM separation (top spectrum) compared against the spectrum obtained following precursor level IM separation (bottom spectrum). All spectra are centered around the three most abundant fragment ions of the ENL T43 peptide: (A) y7-NH$_3$; (B) y8 and (C) y9.

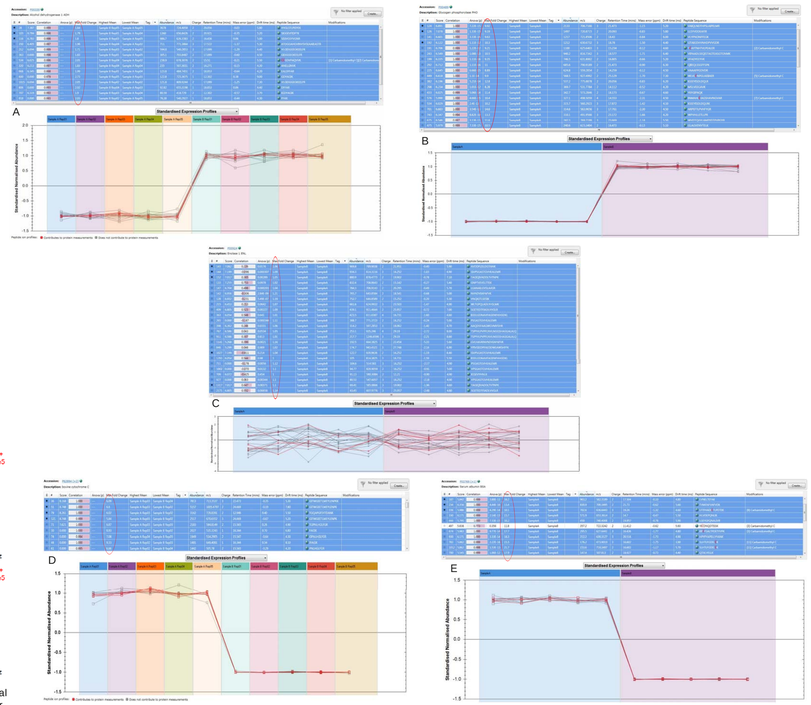

Figure 5. Results of the HCP monitoring assay. Four protein standards (ADH, BSA, CYC and PHO) were spiked at different concentration levels in two Inflectra digests and one protein digest (ENL) was spiked at the same level in both samples (see Table II for protein concentrations). Panels (A-E) display the Progenesis QI for Proteomics trend plots observed for each spiked protein across 5 replicate injections from each sample.

Evaluating the Detection Limit of the HCP Discovery Assay

Protein ID	Uniprot accession no	Average MW (kDa)	Amount on-column femtomoles	Amount on-column nanograms	Protein concentration ng/mL	Protein concentration ppm	RSD (%)
PHO	P00489	97.1	1000	97.1	3884.0	388.4	-
ADH	P00330	36.7	575	21.1	844.1	84.4	8.1
BSA	P02769	66.3	267	17.7	708.1	70.8	9.0
ENL	P00924	46.6	46	2.1	85.7	8.6	16.1

Table I. Results of the HCP discovery assay: 4 protein standards were spiked at different concentration levels in the Inflectra digest. The amounts loaded on-column were 1,000 fmoles PHO, 500 fmoles ADH, 250 fmoles BSA and 50 fmoles ENL. The lowest spiked protein (ENL) was detected at 8.6 ppm.

HCP Monitoring Assay

Protein ID	Concentration range (ppm)	Expected fold change	Measured fold change	No of peptide ions used for the measurement	RSD (%)
ADH	37 - 74	2.0	1.9	14	7.2
BSA	13 - 130	10.0	15.7	10	21.5
CYC	12 - 60	5.0	7.1	9	14.9
ENL	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.

CONCLUSIONS

- The use of ion mobility separation on high-resolution QTOF instruments reduces the interference of mAb-related ions and enables the identification and monitoring of HCPs at lower concentration levels.
- Sensitive HCP identification and quantification down to 10 ppm are facilitated by using a CSH C18 column for peptide separation, providing an increased sample loading capacity.
- HCP levels can be monitored across a batch of biopharmaceutical samples in a high-throughput fashion using Progenesis QI for Proteomics software.

References

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