An Efficient Workflow for Identification and Monitoring of Host Cell Proteins During Monoclonal Antibody Bioprocessing

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INTRODUCTION

- In recent years, the LC-MS assays have been adopted as orthogonal techniques to ELISA for HCP analysis due to their flexibility and potential for full proteome-wide applications. Here we describe an efficient analytical scale LC/MS workflow that allows the identification and quantification of HCPs during mAb purification in a CHO cell line.
- The first step of the HCP identification and quantification workflow is the HCP Discovery Assay, employing data-independent MS^E acquisition using 90 min peptide separations.
- In the second step, samples are analyzed by higher–throughput HCP Monitoring Assays using MS^E acquisitions with 30 min peptide separations.
- The data for both assays was acquired in UNIFI 1.9.4, exported in the RAW file format and processed with Progenesis QI for proteomics 4.0 for HCP identification and monitoring.
- The HCP workflow described here was applied to identification and monitoring of HCPs from five different mAb preparations.

1D LC-MS platform used for HCP identification and quantification in conjunction with Progenesis QI for proteomics software

METHODS

Sample preparation:

A well characterized mAb (NIST mAb) in terms of the HCP content [1,2], was digested using a previously reported protocol designed to deplete a significant amount of the mAb-derived peptides [2]. The NIST mAb was diluted into 50 mM ammonium bicarbonate to a protein concentration of 25 mg/mL and digested overnight with a mixture of Lys-C and porcine trypsin (Promega). Next morning, the undigested mAb was denatured with RapiGest surfactant (60°C, 15 min), reduced with DTT (60°C, 1h) and alkylated with IAM (RT, 30 min) to achieve the precipitation of the undigested mAb. Three MassPREP protein digest standards (ADH—yeast alcohol dehydrogenase, PHO - rabbit phosphorylase b, and BSA - bovine serum albumin) were spiked at different concentration levels (See Table I) post-digestion.

Five preparations of a different mAb were digested with the same digestion protocol and spiked with the same protein digests.

LC Conditions:

Discovery HCP Assays were performed on an ACQUITY™ UPLC® I-class Plus 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 45% Solvent B in 90 min, at a column temperature of 60 deg C. The mobile phases were: 0.1% FA (formic acid) in DI water (Solvent A) and 0.1% FA in acetonitrile (Solvent B).
Monitoring HCP Assays were performed on the same LC-MS system using shorter, 30 min gradient separations.

Mass Spectrometry:

Data-independent acquisitions were performed in MS^E mode on a Xevo® G2-XS QTof mass spectrometer operated by UNIFI 1.9.4. data acquisition software. For the Discovery HCP Assays, the data was acquired with 0.5 s scans over a mass range of 100-2000 Da. For the HCP Monitoring Assays, the data was acquired using 0.3 s scans over the same mass range. For both acquisition modes, low-energy scans were acquired with a CE of 4 eV, while the high-energy fragmentation scans used CE ramping from 15 to 45 V.

Data processing:

Progenesis QI for proteomics 4.0 software was used for data processing. The MS^E datasets produced by the Discovery Assays were searched against a mouse protein database containing 16,644 entries (for the NIST mAb sample) or against the CHO proteome (25,485 proteins) for the other five mAb preparations. The HCPs identified were compiled into a spectral library for easier, faster identification in the subsequent HCP Monitoring Assays.

RESULTS

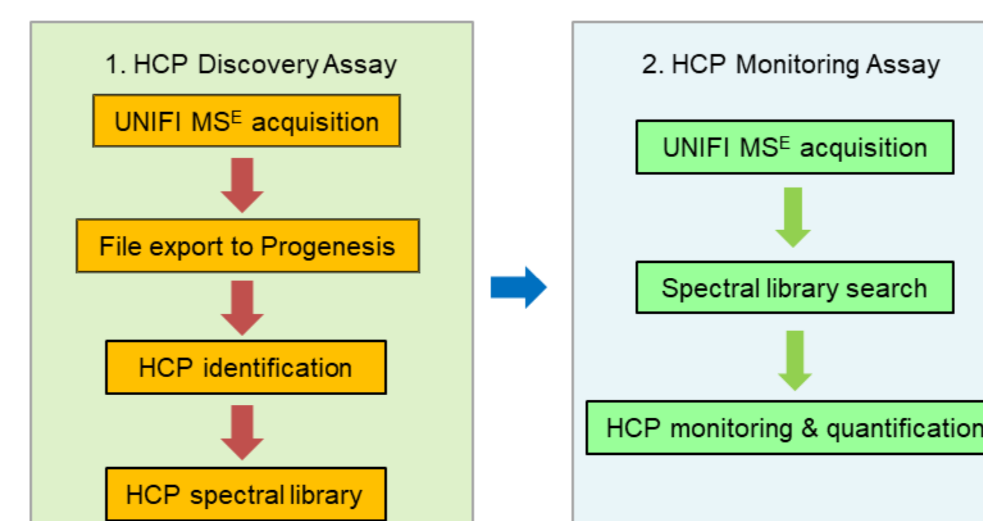

Figure 1. Flow-chart describing the two-step workflow for HCP identification, quantification and monitoring.

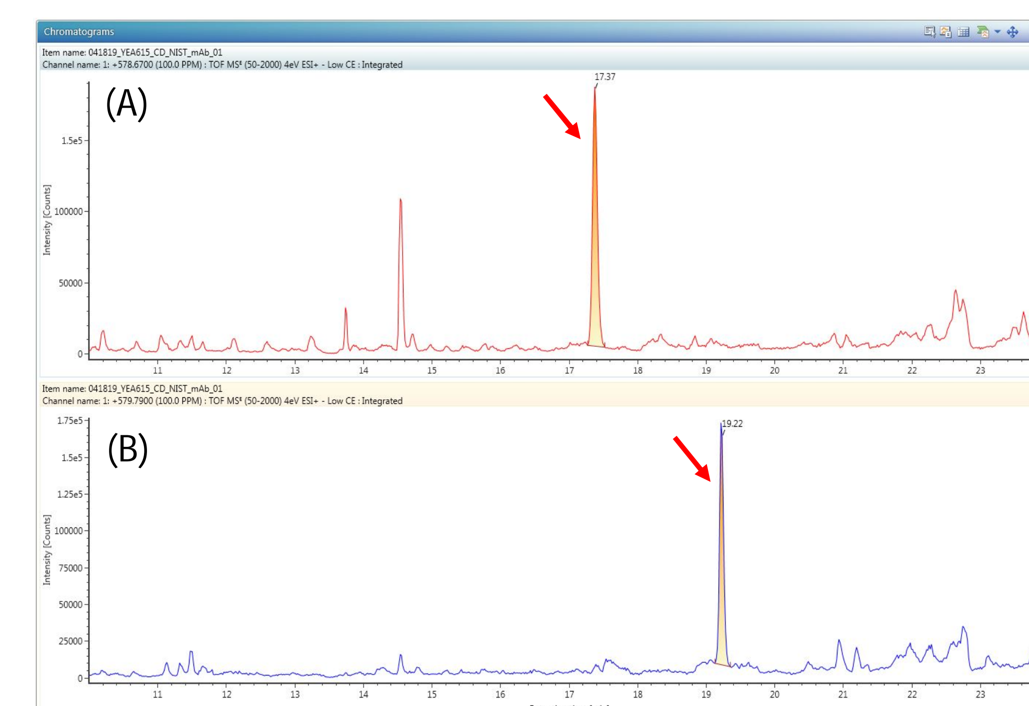

Figure 2. Extracted mass chromatograms of two low-abundance HCP peptides identified in the NIST mAb digest using the Discovery Assay performed with UNIFI acquisition:
(A) peptide TLHQSKPVTITVQGPK (precursor 578.66, +3);
(B) peptide SQVQASYTFK peptide (precursor 579.79, +2).
Both peptides belong to an HCP identified as the low affinity IgG gamma Fc region receptor (UniProt accession no P08101) present at 16 ppm in the NIST mAb.

No crt	UniProt Number	Protein Description	Sequence Coverage (%)	Average MW (kDa)	Amount on column fmoles	Amount on column ng	Concentration ng/mL	Concentration ppm	RSD (%)
1	P00330	Alcohol dehydrogenase yeast (ADH) - 5000 fmoles	57.4	36.7	4533	166	26618	266	18.4
2	P05064	Fructose biphosphate aldolase A isoform	62.3	39.3	2614	103	16437	164	14.3
3	P00489	Glycogen phosphorylase rabbit (PHO) - 1000 fmoles	35.3	97.1	1000	97	15536	155	0.0
4	P05063	Fructose biphosphate aldolase C isoform	29.4	39.4	1529	60	9639	96	8.7
5	P02769	Bovine serum albumin (BSA) - 250 fmoles	30.7	66.3	232	15	2461	25	11.3
6	P08101	Low affinity immunoglobulin gamma Fc region receptor	15.3	36.7	265	10	1556	16	16.8
7	P06745	Glucose 6-phosphate isomerase	8.3	62.7	118	7	1184	12	19.4
8	P01887	Beta-2-microglobulin	16.0	13.8	451	6	996	10	18.4
9	Q8BL97	Serine/arginine-rich splicing factor 7	6.4	30.8	111	3	547	5	15.7

Table I. HCPs identified and quantified in the NIST mAb using the Discovery Assay performed with UNIFI acquisition. Three spiked proteins (ADH, PHO and BSA) along with 6 HCPs (highlighted in red) were identified in 3 replicate injections. The detection limit of the assay was **5 ppm**.

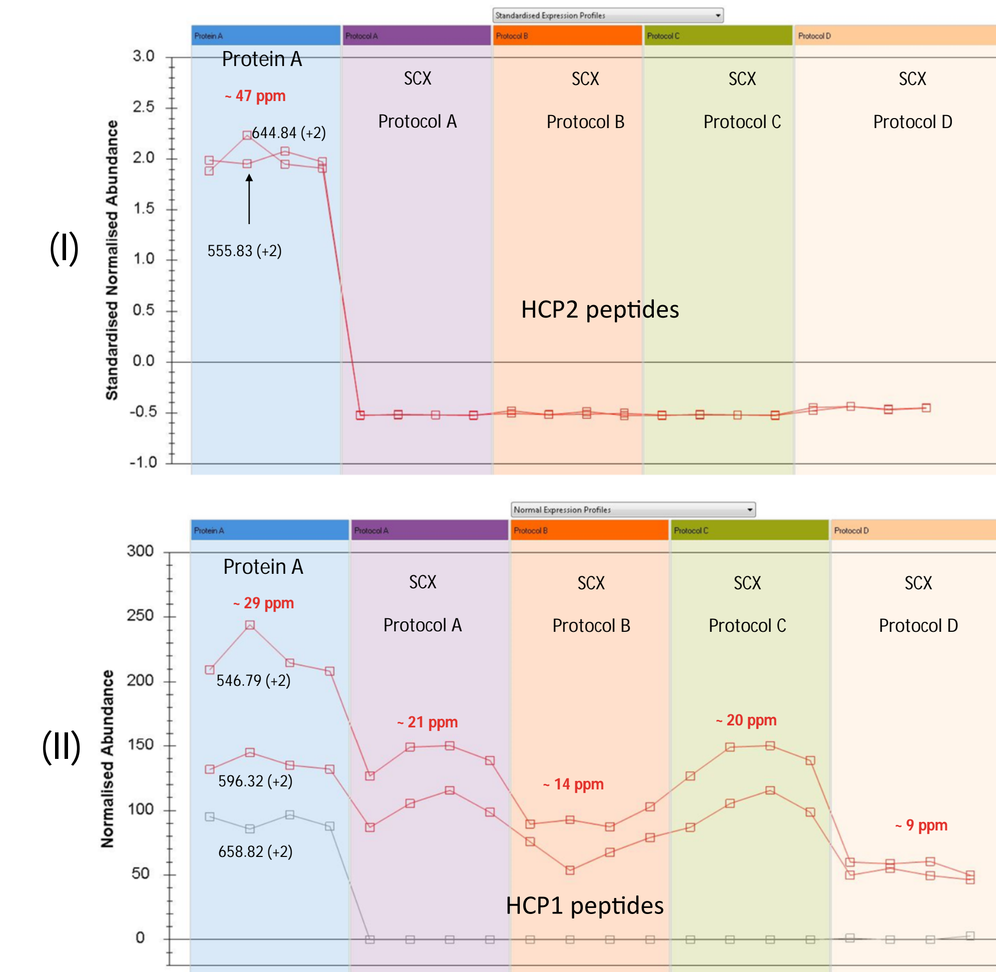

Table II

Protein ID	Peptide precursor m/z	Charge state	RT (min) 30 min gradient	Average peak area	Peak area RSD (%)
HCP1	**546.7878**	**2**	**8.7**	**348**	**7.3**
HCP1	**596.3220**	**2**	**8.9**	**461**	**10.3**
HCP1	537.7805	2	9.5	256	12.7
HCP1	379.7240	2	10.3	555	6.7
HCP1	493.7613	2	11.5	563	5.8
HCP1	**658.8221**	**2**	**15.1**	**203**	**11.4**
HCP2	461.2391	2	10.7	194	8.9
HCP2	**644.8408**	**2**	**11.1**	**2059**	**9.6**
HCP2	**555.8295**	**2**	**17.0**	**551**	**6.3**

Figure 3. Peptide level monitoring of two HCPs (identified as HCP1 and HCP2 in Table II) across five mAb preparations (one Protein A eluate and 4 SCX (strong cation exchange) chromatographic purifications using four different protocols (A-D). Panels I and II display the trend plots and ppm concentrations for three HCP1 peptides and two HCP2 peptides. The HCP monitoring data was acquired in MS^E mode and searched against a spectral library. Table II displays the monoisotopic peaks, charge states, retention times, peak areas and peak area RSDs for 9 HCP peptides identified in the Protein A eluate. As illustrated here, Protocol D provided the best results.

CONCLUSIONS

- Three spiked proteins and 6 HCPs were identified by the Discovery Assay with a detection limit of **5 ppm**.
- For several mAb preparations, HCPs can be quickly and robustly identified, quantified and monitored by a 1D LC-MS workflow using UNIFI acquisition and Progenesis QI data processing.

References:

1. Doneanu et al. Anal Chem, 2015, 87, 10283.
2. Huang et al. Anal Chem, 2017, 89, 5436.

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