IDENTIFICATION AND QUANTIFICATION OF LOW-ABUNDANCE HOST CELL PROTEINS IN HIGH-PURITY PROTEIN THERAPEUTICS USING ION MOBILITY MASS SPECTROMETRY

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OVERVIEW

Identification and quantification of low abundance HCPs (1-200ppm) in a highly-purified mAb was achieved with a 2D-LC/MS approach enabled by ion mobility separation on a high performance Synapt G2-S platform.

INTRODUCTION

- Regulatory guidelines mandate the establishment of suitable analytical methods to ensure accurate determination of residual HCP and to demonstrate that HCP contaminant levels are minimized during the bioprocess development of therapeutics.

- Sensitive detection and quantification of residual HCPs during bioprocess development is critical in the design of robust and well-controlled manufacturing processes that meet the HCP regulatory guidelines and yield high quality biotherapeutics, therefore protecting patient safety.

- LC/MS-based methods are becoming a routine approach for HCP analysis where residual HCPs can be detected, identified, and quantified directly [1-4].

- The 2D-LC/MS assay employs proteolytic digestion, 2D chromatographic separation (high pH/low pH) by reversed-phase UPLC, high-resolution mass spectrometry coupled with ion-mobility separation (IMS), and database searching to confidently identify and quantify potential HCPs.

- Our inter-laboratory study explores the 2D-LC/MS approach for identification and quantification of residual HCPs from a highly-purified mAb derived from a murine cell line.

Synapt G2-S HDMS system coupled to an M-class Acquity system with 2D technology.

Sample Preparation
A monoclonal antibody (mAb) expressed in a murine cell culture and extensively purified (100mg/mL) was obtained from NIST. The sample was denatured with RapiGest, reduced with DTT, alkylated with IAM and digested with trypsin. Four protein digest standards used for HCP quantification were spiked post-digestion.

METHODS

LC Conditions
An M-class ACQUITY™ UPLC® system with 2D technology was used for peptide separations. A RP/RP 2DLC method was developed that uses the pH of the mobile phases to change the selectivity of a peptide separation in two separate dimensions:

- **1st Dimension pH=10**: 1.0 x 50 mm, XBridge BEH300 C_{18}, 5 µm column, 10 µL/min flow rate. Mobile phase: 20 mM NH4COOH in water and ACN
- **Trap column**: 0.3 x25 mm, 5-µm Symmetry C_{18}
- **2nd Dimension pH=2.4**: 0.3 mm x 150 mm, CSH C_{18} 1.7 µm column, kept at 60 °C and operated at 10 µL/min.

Fractions were eluted from the first dimension column in 10 steps and each fraction was mixed in a 1:10 ratio with 0.1% TFA in water (pH=2.1) before trapping. A 40 min gradient from 3-40% ACN in 0.1% FA was used for the 2nd dimension separation.

MS Conditions
- Fixed CE values were applied to the transfer cell of SYNAPT G2-S for peptide fragmentation after IM separations. CE values were correlated with the mobility of each peptide precursor ion (UDMS^E mode [5]).

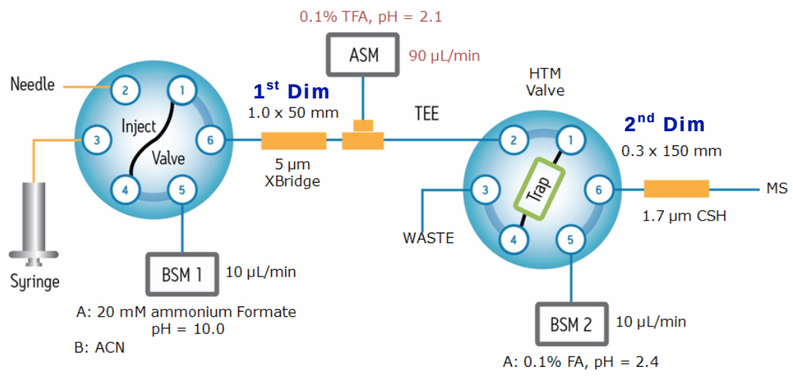

Figure 1. Fluidic configuration for the two-dimensional high pH/ low pH RP/RP chromatographic setup employing on-line dilution.

- For HCP validation, several peptide precursors, belonging to very low-abundance HCPs were isolated using the quadrupole filter, separated from other co-eluting precursors using ion mobility and fragmented with a fixed CE in the transfer cell.

RESULTS

Inter Lab Comparison

No crt	Accesssion Number	Protein Description	Average MW (kDa)	Measured HCP Concentration (ppm) Lab I	Lab II	Lab III	Average
1	P00489	Glycogen phosphorylase rabbit (PHO) - 1000 fmoles	97.1	155	155	155	155
2	P00330	Alcohol dehydrogenase yeast (ADH) - 5000 fmoles	36.7	106	125	164	132
3	P05064	Fructose biphosphate aldolase A isoform	39.3	113	126	109	116
4	P05063	Fructose biphosphate aldolase C isoform	39.4	106	93	91	97
5	P02769	Bovine serum albumin (BSA) - 250 fmoles	66.3	35	28	20	28
6	Q922R8	Protein disulfide isomerase A6	48.1	-	40	8	24
7	Q99KN9	Clathrin interactor	68.5	22	19	7	16
8	P08101	Low affinity immunoglobulin gamma Fc region receptor	36.7	10	28	5	14
9	Q60864	Stress-induced phosphoprotein 1	62.5	10	16	13	13
10	P06745	Glucose-6-phosphate isomerase	62.7	7	15	13	12
11	P01887	Beta-2-microglobulin ←	13.8	5	14	1	7
12	Q9CZ44	NSFL1 cofactor p47	40.7	7	10	5	7
13	P00924	Enolase 1 yeast (ENL) - 50 fmoles	46.6	7	7	3	6
14	Q8BL97	Serine/arginine-rich splicing factor 7	30.8	6	7	3	5
15	P40142	Transketolase	67.6	5	4	6	5
16	Q9WTP6	Adenylate kinase 2	26.5	4	6	2	4
17	Q91YR9	Prostaglandin reductase 1	35.5	4	3	3	3
18	Q923D2	Flavin reductase (NADPH) ←	22.2	1	3	1	2
19	Q9D2M8	Ubiquitin conjugating enzyme E2 variant	16.4	-	2	1	2
20	P99029	Peroxiredoxin 5	21.8	1	2	1	1

3X more HCPs identified with IMS !

Lowest HCP conc measured: 1 ppm !

Table I. Inter-Laboratory comparison study: list of 14 common HCPs identified in the NIST mAb by all three independent laboratories performing the 2DLC/UDMS^E experiment, employing peptide precursor separation by ion mobility. Individual HCP concentrations (in the range of 1—200 ppm) were calculated by each laboratory from 3 replicate injections based on the signals produced by the PHO protein standard (spiked in the NIST mAb post-digestion).

No crt	Accesssion Number	Protein Description	Average MW (kDa)	Amount on column fmoles	ng	Concentration ng/mL	ppm
1	P00330	Alcohol dehydrogenase yeast (ADH) - 2000 fmoles	36.7	1512	55	22196	222
2	P05064	Fructose biphosphate aldolase A isoform	39.3	914	36	14368	144
3	P00489	Glycogen phosphorylase rabbit (PHO) - 400 fmoles	97.1	400	39	15536	155
4	P05063	Fructose biphosphate aldolase C isoform	39.3	584	23	9180	92
5	Q922R8	Protein disulfide isomerase A6	48.1	260	13	5002	50
6	P02769	Bovine serum albumin (BSA) - 100 fmoles	66.3	173	11	4588	46
7	P08101	Low affinity IgG gamma Fc region receptor	36.7	304	11	4463	45
8	Q9D2M8	Uniquitin conjugating enzyme E2 variant	16.3	260	4	1695	17
9	P00924	Yeast enolase 1 (ENL) - 20 fmoles	46.6	20	1	373	4

Table II. HCPs identified in the NIST mAb after 2DLC 10-step fractionation and MS^E data acquisition. The dataset was acquired in triplicate WITHOUT ion mobility separation of peptide precursors and only 5 HCPs were identified.

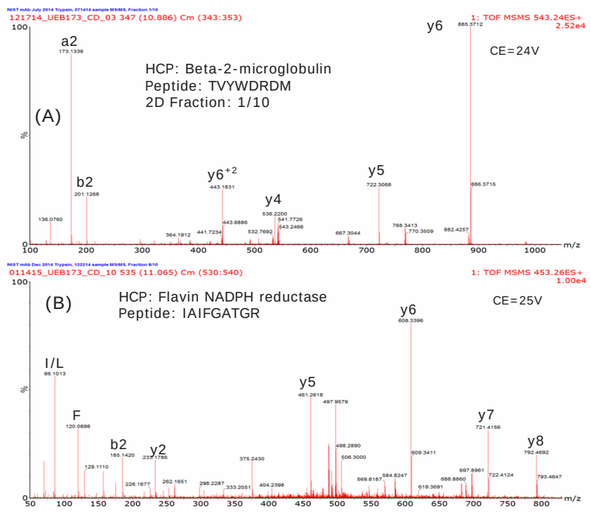

Figure 2A-B. HCP validation: 11 low-abundance (1-30 ppm) proteins identified by the UDMS^E approach were confirmed by "pure" MS/MS fragmentation spectra.

CONCLUSIONS

- A generic 2D-LC/UDMS^E assay has been developed for quantitative HCP analysis in biopharmaceutical industry.
- The 2D RP/RP separations with or without ion-mobility separations (IMS) were compared for the identification and quantification of HCPs in a high-purity NIST mAb: 14 HCPs were identified with IM separations, while only 5 HCPs were identified without IM separation.
- Ion mobility separation extends the capabilities of the 2D-LC/MS^E assay by an order of magnitude, allowing for the identification and quantification of HCPs down to concentrations of 1 ppm.
- In comparison with ELISA assay, the MS-based assay is able to provide the identifications for individual HCP.

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