Global HCP Profiling and Quantification by inSPECt™ LC-MS Assay

Timothy Licknack, Ph.D., Stephen Stahlschmidt, BSc, Johanna Barnhill, MS, Brinkley Thornton, MS, and Jared Isaac, Ph.D.

Research & Development Department, Chromatography and Mass Spectrometry Group

CYGNUS TECHNOLOGIES

part of Maravai LifeSciences

Abstract

Liquid Chromatography-Mass Spectrometry (LC-MS) is a powerful tool for identifying host cell proteins (HCPs), but most high-throughput workflows often yield inaccurate results. A new LC-MS workflow was developed and qualified to identify and quantify up to hundreds of HCPs in downstream biopharmaceutical samples, called the inSPECt MS Assay. inSPECt utilizes native digestion for sample preparation and Data-Independent Acquisition using the state-of-the-art Astral Zoom Mass Spectrometer (Thermo Fisher Scientific). To evaluate the analytical limits of this method, calibration curves with spike-in protein standards were generated; subsequently, a unique blend of problematic HCPs were spiked into a representative Drug Substance (DS) sample to evaluate method performance. Our results indicate that HCP inSPECt is an accurate and precise assay for identification and quantification of HCPs in process samples.

Background

The large dynamic range between HCPs and biotherapeutic proteins in DS samples leads to poor identification and quantification. Stochastic elements exist in LC-MS workflows which make low abundance HCPs particularly difficult to survey. One element of stochasticity is precursor selection for fragmentation in traditional data dependent acquisition (DDA) assays. To alleviate this, data independent acquisition (DIA) is used to fragment all precursors, however, MS/MS scanning speed still limits sensitivity. Here, we utilize an ultra-fast mass spectrometer (Astral Zoom MS; ThermoFisher Scientific) to provide sensitive, precise, and accurate HCP analytics by LC-MS.

Methods & Materials

Sample Preparation: NISTmAb (Agilent; PN 5191-5744) was digested with Trypsin/Lys-C without reduction/alkylation prior to desalting, and solubilization.

Calibration Curve: The Cygnus Protein Standard (CPS) was spiked into NISTmAb prior to sample preparation at the following concentrations: 0, 10, 25, 50, 75, 100 ppm. This was done three times on three separate days.

Spike and Recovery: High risk, recombinant HCPs (rHCPs) were spiked into NISTmAb prior to sample preparation at the following concentrations: 0, 10, 25, 50, 75, and 100 ppm.

Data Analysis: Raw data were searched by CHIMERYS (MSAID) in a ProteomeDiscoverer (ThermoFisher Scientific) workflow including protein quantification via the Top3 algorithm. Microsoft Excel was used to generate statistics, Tables, and Figures.

Results

- Between 10 – 100 parts per million (ppm):
  - inSPECt MS calibration curves display high linearity ($R^2 > 0.94$) and precision (%coefficient of variance (CV) < 25%)
  - Back-calculated CPS concentrations are highly accurate

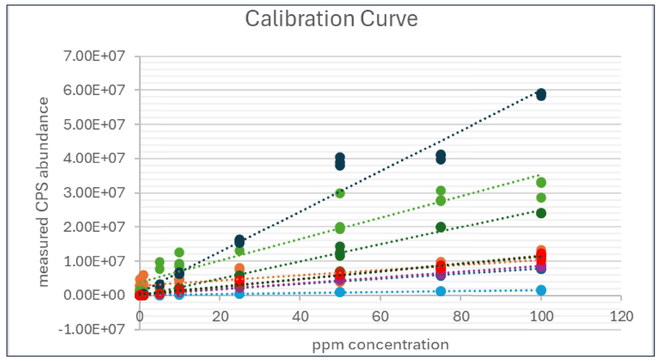

Figure 1. Calibration Curve. A representative CPS calibration curve was generated by plotting measured CPS abundance (y-axis) vs known ppm concentration (x-axis). The median points are shown in red.

	Intra Assay		Inter Assay	
Known ppm	Average (ppm)	%CV	Average (ppm)	%CV
0	3.49	346%	1.0	804%
	4.15	24%		
	-4.87	-17%		
1	1.34	205%	0.4	918%
	4.36	14%		
	-4.37	-3%		
5	9.83	49%	4.5	125%
	5.58	8%		
	-1.81	-7%		
10	12.96	15%	11.4	15%
	11.26	9%		
	10.04	3%		
25	20.99	25%	25.5	32%
	19.92	4%		
	35.64	2%		
50	41.47	8%	47.8	11%
	52.73	1%		
	49.32	4%		
75	75.06	4%	74.4	7%
	78.05	3%		
	70.16	9%		
100	104.68	2%	101.3	6%
	97.46	2%		
	101.65	11%		

Table 1. Intra- and Inter-assay Precision. %CV values were measured for three calibration curves of CPS between 1 – 100 ppm. Intra-assay precision was evaluated with each curve's triplicate injections, while inter-assay precision was evaluated with all nine observations. The average value at each level is shown within and between replicate curves.

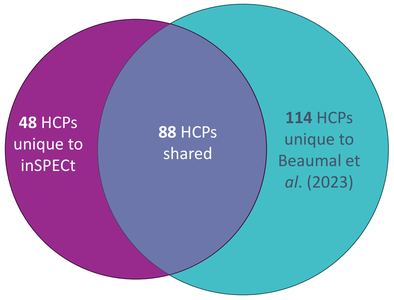

Figure 2. HCP Overlap. 136 HCPs were measured in this study, while 202 were measured by Beaumal et al. (2023). 88 HCPs were found in both studies.

- 136 unique HCPs measured in NIST mAb
  - 88 HCP overlap with Beaumal et *al*. (2023)
    - ~65% shared
- rHCP spike and recovery demonstrated high accuracy, precision, and linearity for 4 problematic HCPs
  - %CV < 25%

HCP	0 (ppm)	10 (ppm)	25 (ppm)	50 (ppm)	75 (ppm)	100 (ppm)	R^2
GSTP1	7	17	39	58	89	153	0.95
PLA2G15	ND	11	22	37	61	75	0.99
CTSA	8	16	31	56	72	89	0.99
CLU	ND	15	27	54	65	77	0.97

Table 2. rHCP Spike and Recovery. Four recombinant HCPs were spiked into NISTmAb between 10 – 100 ppm. Their measured concentration at each level is shown here as well as their linearity (R^2). GSTP1 = Glutathione S-transferase P 1; PLA2G15 = Lysosomal phospholipase A and acyltransferase; CTSA = Carboxypeptidase; CLU = Clusterin.

Conclusions

- inSPECt MS has a quantitative range of 10 – 100 ppm according to back-calculated CPS concentrations
- HCP identification in NISTmAb shows high agreement with the literature
- Novel spike and recovery experiment demonstrates assay accuracy in diverse rHCPs of known concentrations

References

1. Han, Ying. (2024). Residual Host Cell Protein Measurement in Biopharmaceuticals by Liquid Chromatography-Mass Spectrometry. Pharmacopeial Forum 49(3). USP-NF <1132.1>
2. Beaumal, Corentin, et al. "Advanced mass spectrometry workflows for accurate quantification of trace-level host cell proteins in drug products: Benefits of FAIMS separation and gas-phase fractionation DIA." *Proteomics* 23.16 (2023): 2300172.

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