Mass Spectrometry for Host Cell Protein Identification and Quantitation

November 7, 2018

Stéphane Parent

Caprion Biosciences

Session Description and Objectives

- Biological drugs contains host cell protein (HCP) impurities
- Immunoassays are the traditional method to monitor HCP
- Mass spectrometry-based platforms were developed for unbiased profiling and targeted quantitation of HCP
- MS-based HCP assays enable direct identification and quantitation with a sensitivity in the ~1-10 ppm range

Contact Information

Stéphane Parent
Senior Principal Scientist
Caprion Biosciences
Montreal, Canada
sparent@caprion.com

Experience in HCP Analysis

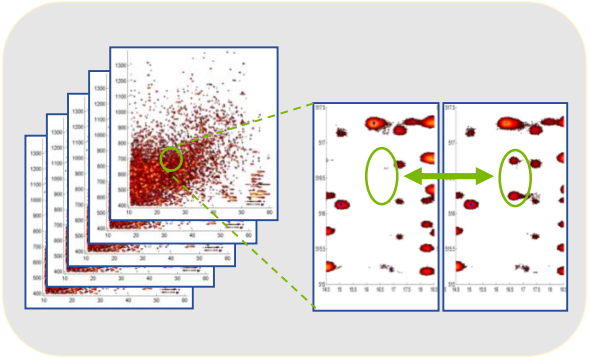
- **30+ projects**
- **20+ different DS (and their in-process samples)**
  - mAbs, recombinant proteins, vaccines, peptides
  - Mammalian, E.coli, yeast production systems
- **Variety of study goals**
  - ❑ Monitoring of purification process to improve the quality target product profile
  - ❑ Demonstration of HCP clearance
  - ❑ Process improvements e.g. comparison of culture media or
  - ❑ Evaluation of batch reproducibility and scale up
  - ❑ Biosimilar vs Originator comparison
  - ❑ Determination of mock immunogen content for immunoassay characterization

Why Mass Spec for HCP Analysis?

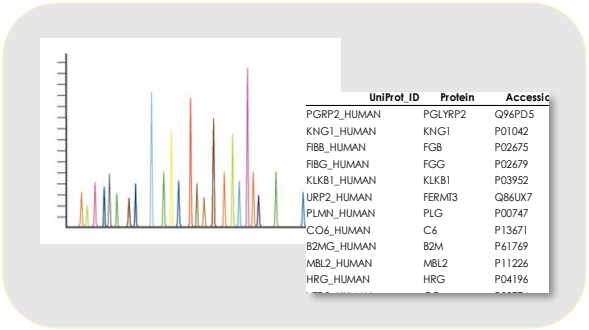
- Biological drugs produced from host cell expression systems inevitably contain host cell protein (HCP) impurities whose identity, presence and levels may determine whether or not the drug is accepted by regulatory agencies
- Process-related impurities are considered critical quality attributes that should be monitored early in process development
- Existing methods rely almost exclusively on polyclonal antibodies raised against the host cell system, but there are well-recognized gaps:
  - Little is known about the individual HCPs
  - Some proteins may be missed
- Mass spectrometry methods are playing an increasing role in HCP characterization
  - USP 1132 – “Immunoassay and (increasingly) mass spectrometry are highly complementary and the most powerful methods for monitoring residual HCP levels in samples and confirming their absence in final DSs.”

Overview: MS-Based Approaches

- **Screening Phase (Unbiased LC-MS/MS)**
  - Identification of HCPs present in samples (1 to 1000s)
  - Provides an estimated conc. for each HCP identified
  - LID ~10 ppm (no-fractionation); ~1ppm (fractionation)
  - No assay development time required



- **Confirmatory Phase (Targeted LC-MRM/MS)**
  - Precise and accurate quantitation of specific HCPs
  - Highly multiplexed assays:
    - Relative quantitation ≤ 325 HCP
    - Absolute quantitation ≤ 10-15 HCP
  - Single-digit ppm sensitivity (no fractionation)
  - Assays can be validated per regulatory requirements

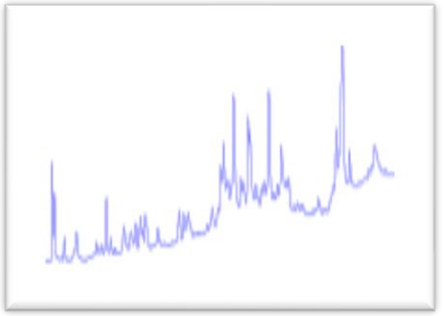


HCP Profiling Workflow (Identification)

Drug Substance
(DS)

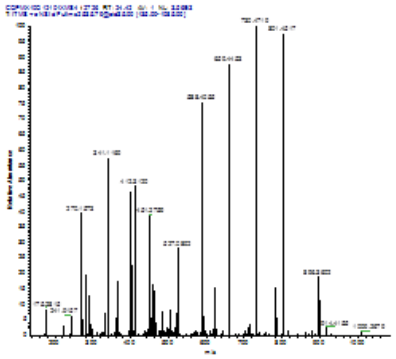
Denaturation/ Digestion

Fractionation (Optional)
(SCX+HPRP)



Desalting

LC-MS/MS



BI Analysis

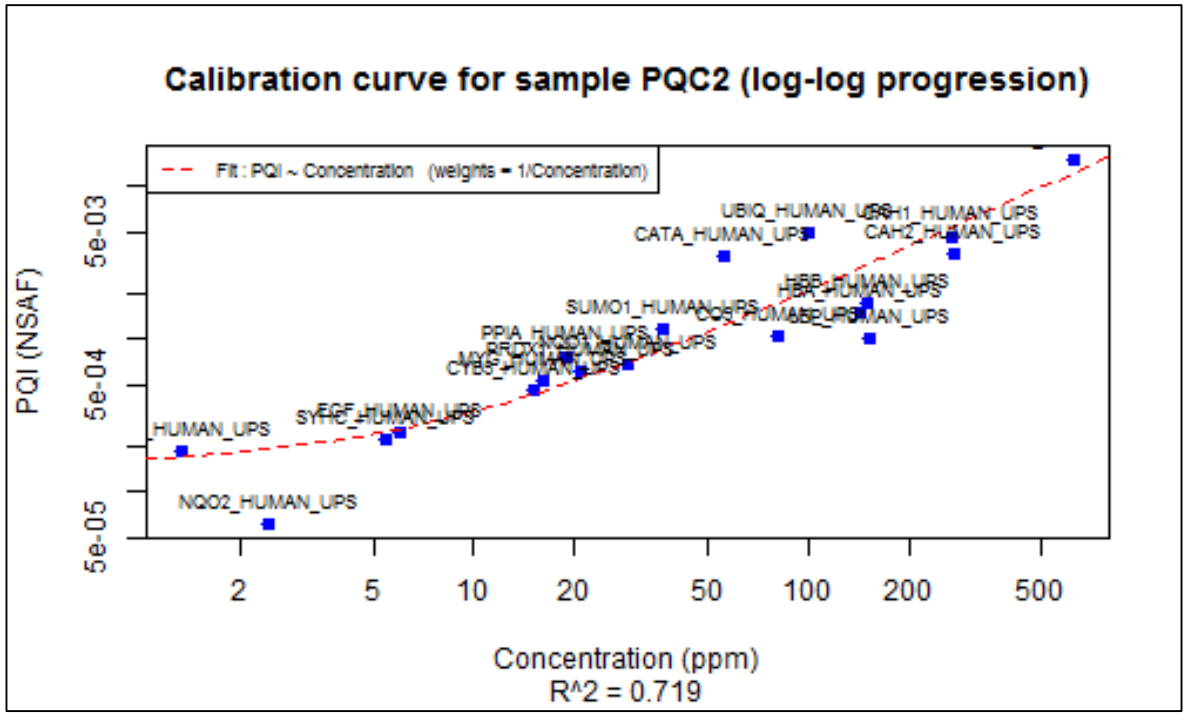
1. Peak Alignment
2. MS1 peak detection
3. Protein ID (3 search engines)
4. Assignment Validation
5. Curation of Data
6. Calculation HCP ppm estimation
7. Electronic Report Generation

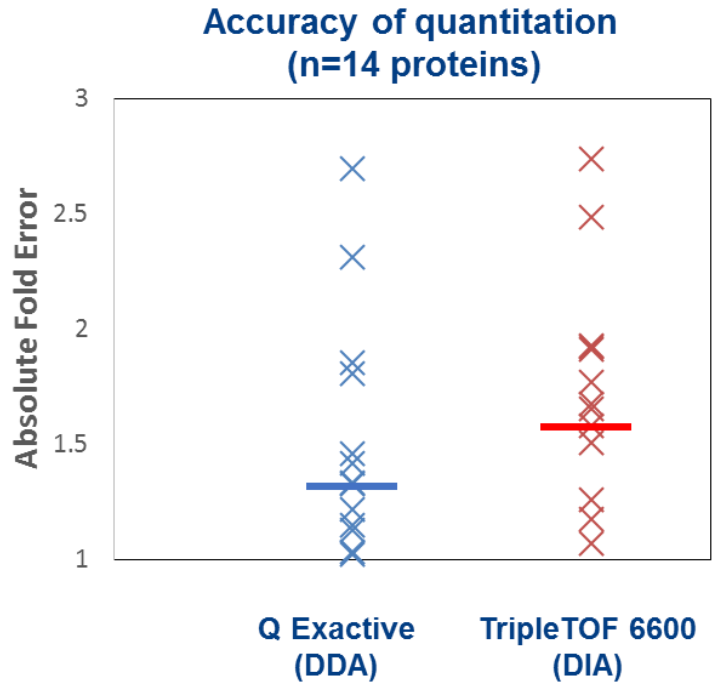
Sensitivity of Unbiased LC-MS/MS Approach

UPS2 (Protein_ID)	Spiked Level (ppm)	No fractionation		Fractionation
		Q Exactive (DDA)	TripleTOF6600 (DIA, SWATH)	Q Exactive (DDA)
ALBU_HUMAN	626.0	Yes	Yes	Yes
CAH2_HUMAN	274.7	Yes	Yes	Yes
CAH1_HUMAN	271.1	Yes	Yes	Yes
LEP_HUMAN	152.4	Yes	Yes	Yes
HBB_HUMAN	149.7	Yes	Yes	Yes
HBA_HUMAN	142.7	Yes	Yes	Yes
UBIQ_HUMAN	100.0	Yes	Yes	Yes
CO5_HUMAN	80.8	Yes	No	Yes
CATA_HUMAN	56.3	Yes	Yes	Yes
SUMO1_HUMAN	36.6	Yes	Yes	Yes
NQO1_HUMAN	29.0	Yes	Yes	Yes
PRDX1_HUMAN	20.7	Yes	Yes	Yes
PPIA_HUMAN	19.0	Yes	Yes	Yes
MYG_HUMAN	16.1	Yes	Yes	Yes
CYB5_HUMAN	15.1	Yes	Yes	Yes
EGF_HUMAN	6.0	No	No	Yes
SYHC_HUMAN	5.5	No	No	Yes
KCRM_HUMAN	4.1	No	No	Yes
NQO2_HUMAN	2.4	No	No	Yes
RETBP_HUMAN	2.0	No	No	No
UBC9_HUMAN	1.7	No	No	Yes
LYSC_HUMAN	1.4	No	No	Yes
LALBA_HUMAN	1.3	No	No	Yes
NEDD8_HUMAN	0.9	No	No	Yes

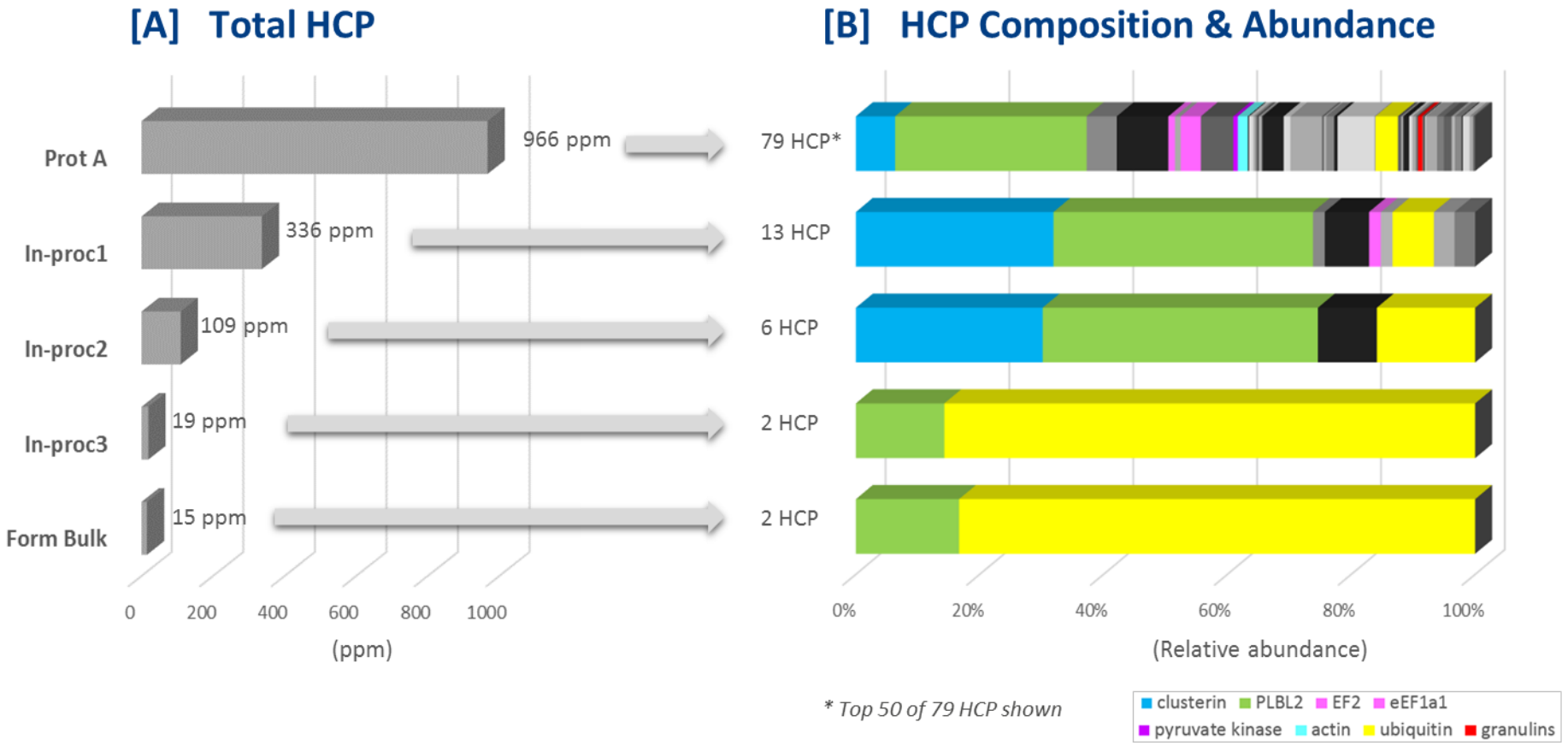
Estimation of Concentration of HCPs

- PQC2 sample = UPS2 spiked into DS; detected UPS2 proteins are used to generate a calibration curve based on either spectral counts or peak intensities
- HCP concentrations are *estimated* by back-calculating its spectral count or peak intensities against the calibration curve (UPS2 proteins)

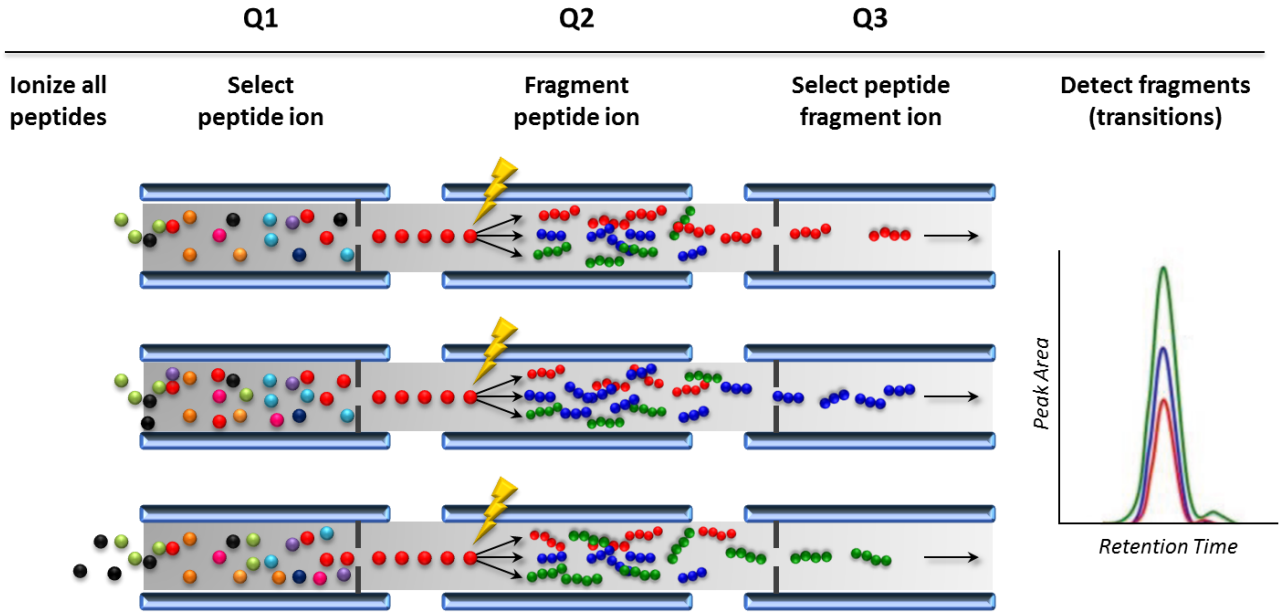




Case Study:
Comprehensive HCP Analysis of In-Process Samples



Multiple Reaction Monitoring (MRM)



Technology:

- Highly multiplexed
- Selective monitoring of peptides/ fragment ions (transitions)
- Linear dynamic range: 5-log
- Relative or absolute quantitation
- Stable isotope labeled (SIL) peptides are used as internal standards

Assay Development:

- Prioritize list of HCPs and select ≤5 signature peptides per HCP
- Use SIL peptides to develop LC-MRM/MS assay conditions

Precision & Accuracy

- High precision & accuracy; single digit ppm sensitivity

TIAQDYGVLK_554.31_893.5	*Standard concentrations (pg/µg Protein; ppm)*									
	STD1	**STD2**	**STD3**	**STD4**	**STD5**	**STD6**	**STD7**	**STD8**	**STD9**	**STD10**
	0.664	**2.014**	**6.064**	**13.456**	**26.913**	**67.281**	**336.406**	**470.969**	**605.532**	**672.813**
rep 1	0.727	2.247	5.655	12.401	26.519	63.783	327.053	442.009	607.429	697.271
rep 2	0.715	2.111	5.952	13.138	25.670	65.916	337.365	481.028	597.379	689.859
rep 3										
Mean	0.72	2.18	5.80	12.77	26.09	64.85	332.21	461.52	602.40	693.56
STDEV	0.01	0.10	0.21	0.52	0.60	1.51	7.29	27.59	7.11	5.24
CV (%)	**1.2%**	**4.4%**	**3.6%**	**4.1%**	**2.3%**	**2.3%**	**2.2%**	**6.0%**	**1.2%**	**0.8%**
Bias (%)	**8.6%**	**8.2%**	**-4.3%**	**-5.1%**	**-3.0%**	**-3.6%**	**-1.2%**	**-2.0%**	**-0.5%**	**3.1%**

	BSA QC samples (pg/µg Protein; ppm)					
	QC1	**QC2**	**QC3**	**QC4**	**QC5**	**QC6**
	0.664	**2.014**	**6.064**	**18.170**	**269.125**	**538.250**
	0.749	1.677	5.995	17.559	273.361	566.661
	0.702	2.256	5.676	17.085	258.082	521.136
	0.638	2.297	5.918	16.919	262.450	508.010
Mean	0.73	1.97	5.84	17.32	265.72	543.90
STDEV	0.03	0.41	0.23	0.34	10.80	32.19
CV (%)	**8.1%**	**16.7%**	**2.8%**	**1.9%**	**3.0%**	**5.8%**
Bias (%)	**4.9%**	**3.1%**	**-3.3%**	**-5.4%**	**-1.7%**	**-1.2%**

Matrix QC samples (pg/µg Protein; ppm) corrected for endogenous						
QC1	**QC2**	**QC3**	**QC4**	**QC5**	**QC6**
6.396	**7.746**	**11.796**	**23.903**	**274.857**	**543.982**
6.737	7.879	11.497	22.487	277.997	548.668
6.157	7.465	10.953	25.429	288.135	523.758
6.065	7.730	11.051	23.676	262.799	536.034
6.45	7.67	11.23	23.96	283.07	536.21
0.41	0.29	0.38	2.08	7.17	17.61
5.8%	**2.7%**	**2.6%**	**6.2%**	**4.6%**	**2.3%**
-1.2%	**-0.7%**	**-5.3%**	**-0.2%**	**0.5%**	**-1.4%**

Summary

Unbiased shotgun LC-MS/MS Assay

- Provides a list of individual HCP present in your sample (incl non-immunogenic & unknown)
- Direct HCP measurement and provides an estimated concentration of each identified HCP (ppm)
- Identification of HCP down to 10 ppm sensitivity (without fractionation) and single digit ppm range with fractionation

Targeted LC-MRM/MS Assay

- Absolute or relative quantitation of specific HCP
- Sensitivity down to the single digit ppm range
- Quick assay development time requiring protein sequence(s) only, no antibodies
- Assays can be validated

► **MS is a powerful technology which can be used as an orthogonal approach to confirm and supplement HCP-ELISA**

Questions

Stéphane Parent
Senior Principal Scientist, Caprion Biosciences
sparent@caprion.com
caprion.com
LinkedIn | Twitter | Facebook

CAPRION
ACCELERATING PRECISION MEDICINE