

# Use of Mass Spectrometry for High Coverage Process Specific HCP Identification and Quantitation



CAPRION

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## BACKGROUND

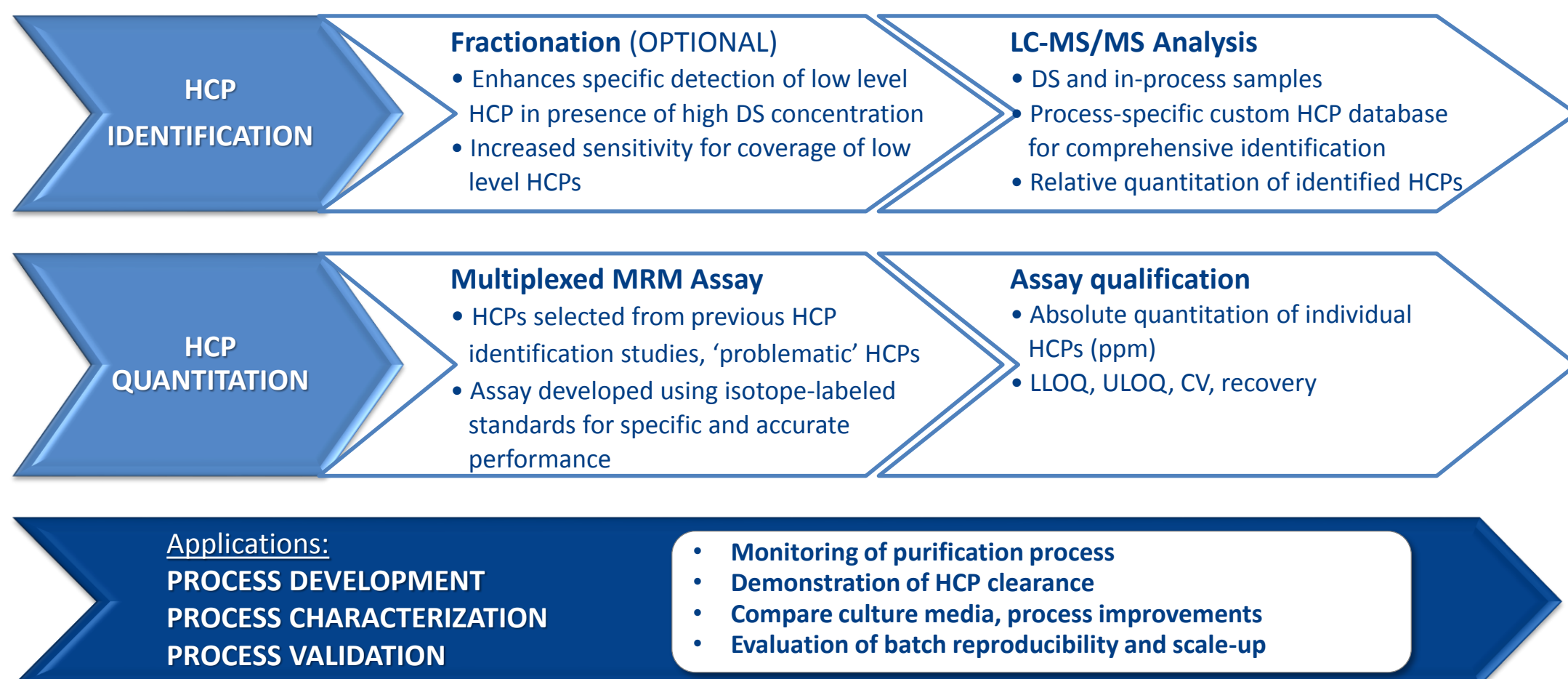
### Regulatory trend towards increasingly deeper HCP characterization

- Regulatory framework: 42 USC 262, ICH Q6B, ICH Q8
- Current practice of using immunoassays has well-recognized gaps; little is known about individual HCPs
- Post-market commitments for development of an HCP assay with improved coverage was required for 3 of 8 BLAs approved in 2014 due to insufficient characterization of HCPs

### Mass spectrometry is playing a greater role in characterization of HCPs

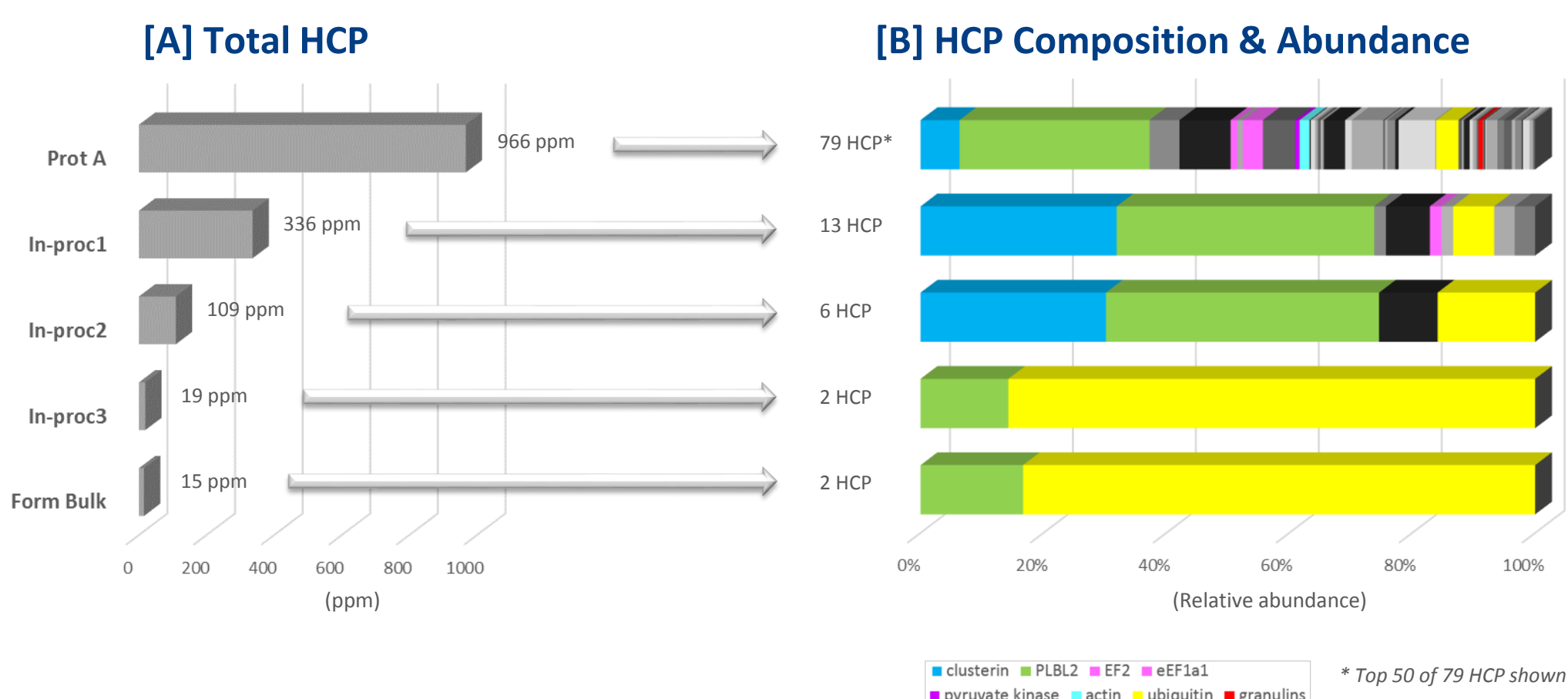
- "Immunoassay and (increasingly) mass spectrometry are highly complementary and the most powerful methods for monitoring residual HCP levels in samples and confirming their absence in final DSs." - USP 1132

### Mass Spectrometry Platform Features and Applications for HCP



## LC-MS/MS Enables Identification and Relative Quantitation of Individual HCPs

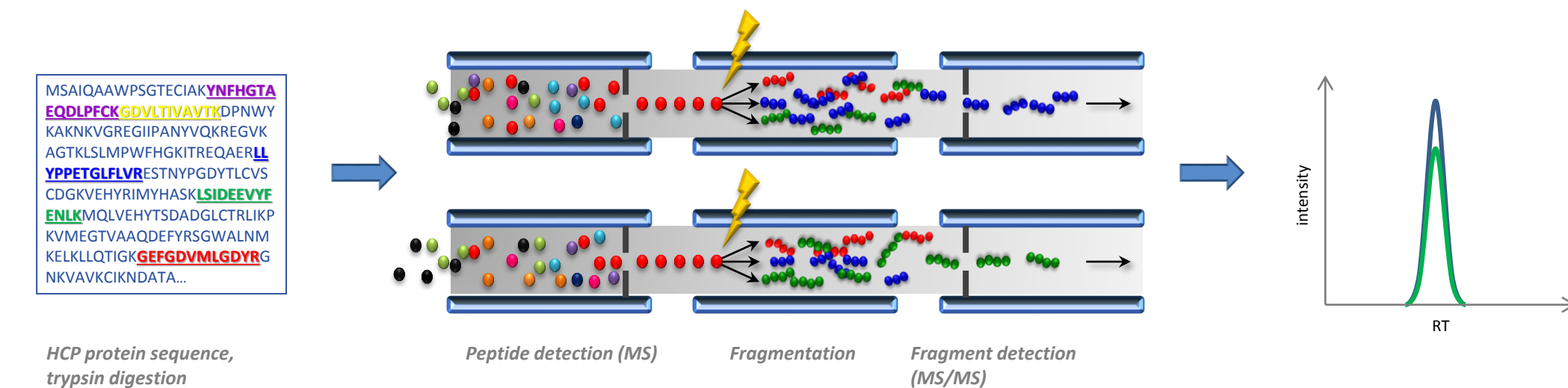
- A comparative analysis of in-process samples and formulated bulk DS with wide dynamic range in composition and abundance. Data show: [A] total HCP readout in ppm (similar to ELISA) via *direct* measurement of constituent HCPs, and [B] identification of individual HCPs, their relative abundance and clearance thereof.



## HCP QUANTITATION (ABSOLUTE)

### Targeted Multiplexed LC-MRM/MS Assay Development

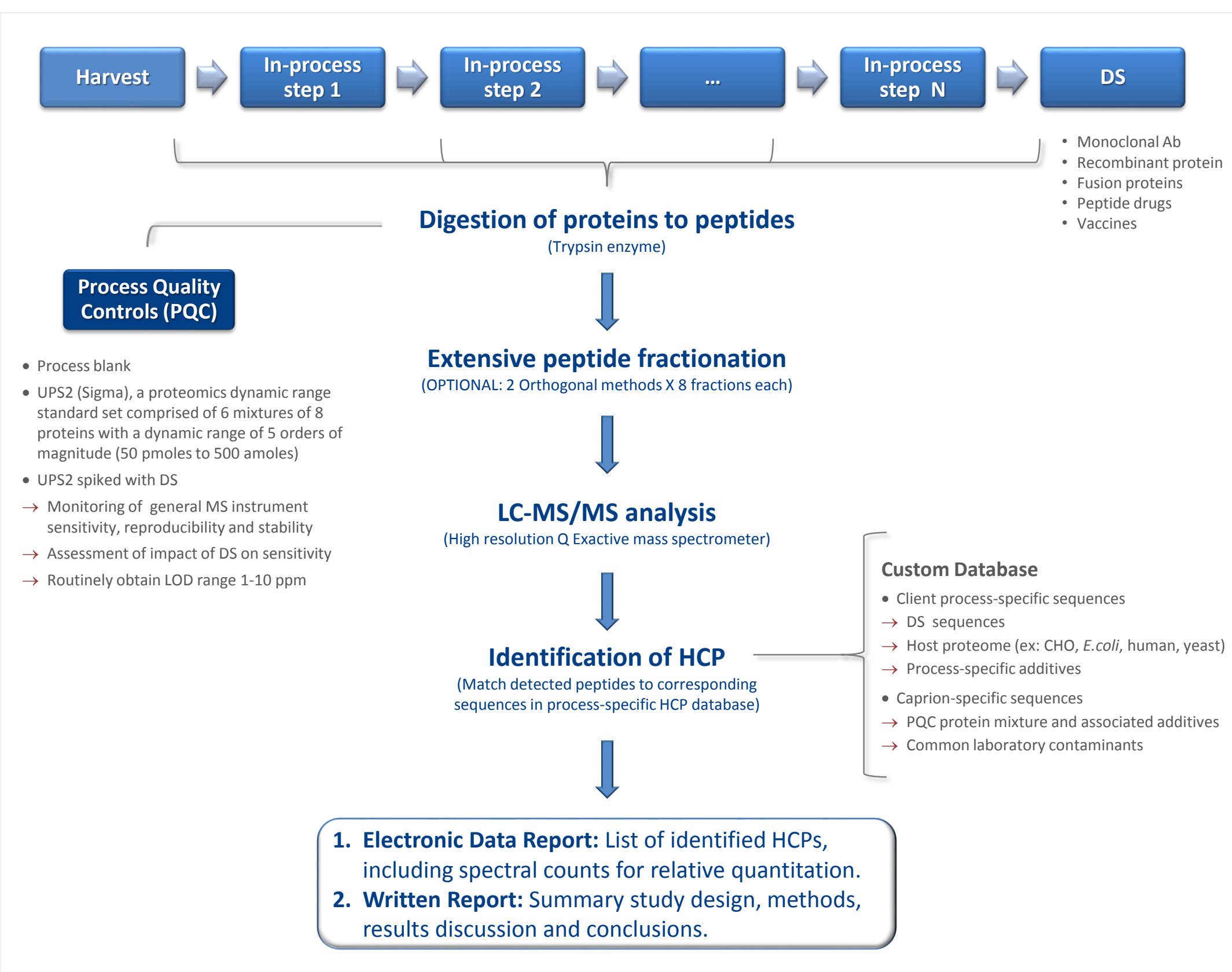
- Prioritize list of HCPs
- Select ≤5 surrogate peptides per HCP
- Use synthetic isotope-labeled peptides to develop assay conditions
- Final optimized assay monitors 2 fragments (transitions) per peptide



## HCP IDENTIFICATION

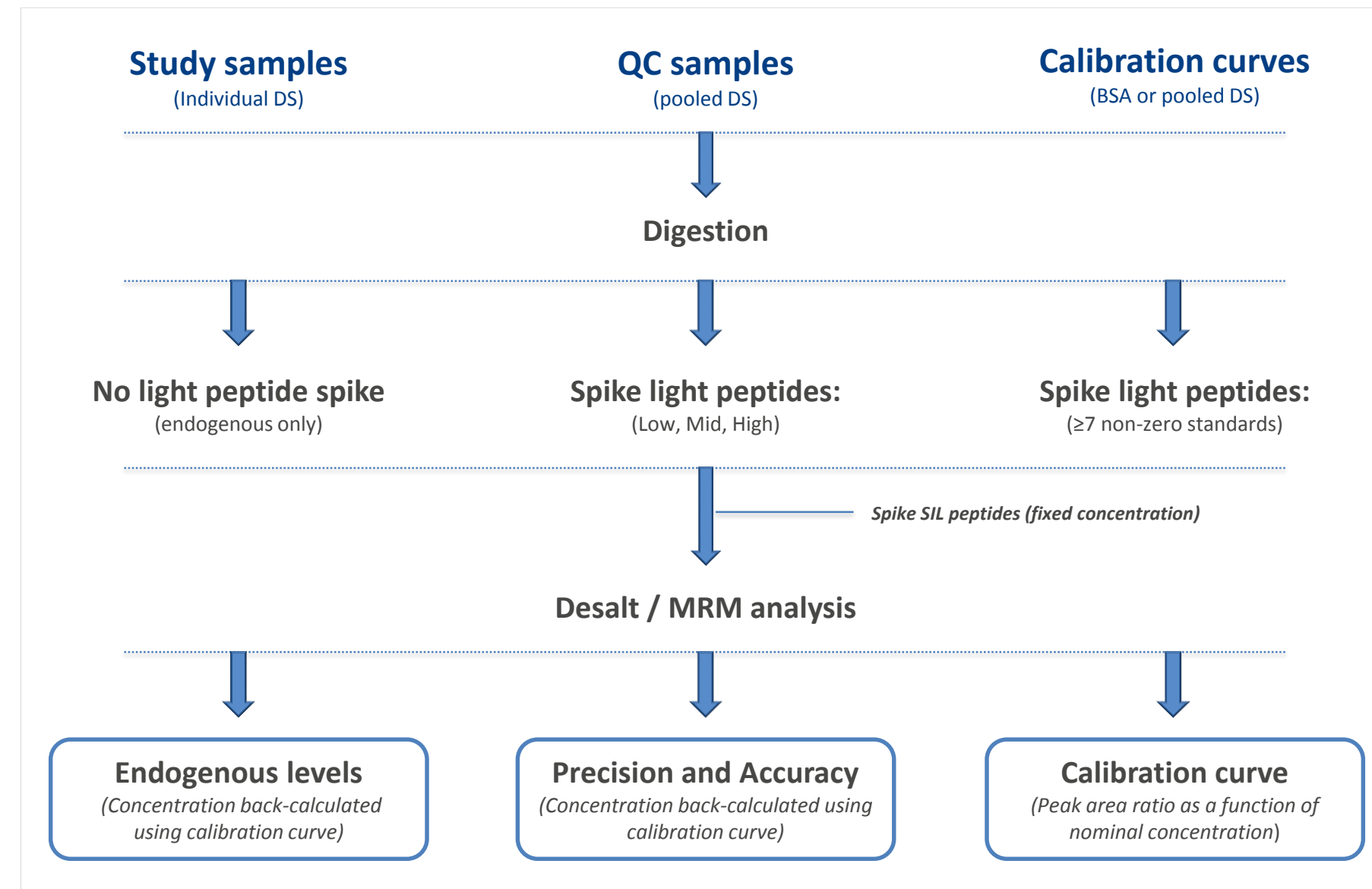
### LC-MS/MS Identification & Relative Quantitation of HCP

- Experimental workflow



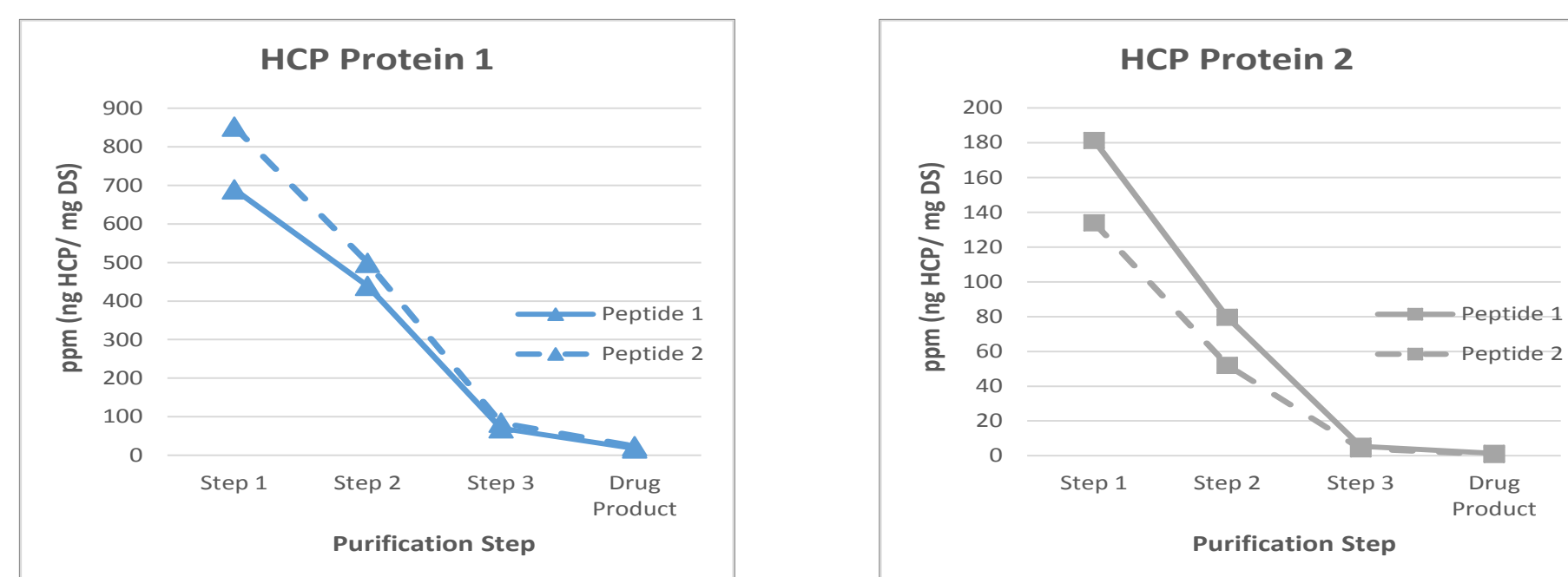
### Absolute Quantitation of HCP using LC-MRM/MS

- Experimental workflow



### Quantitative Assessment of HCP Clearance

- Data show successful clearance of two individual HCPs across the purification process
- Two peptides from the same protein yield similar results



### Sensitivity of HCP Identification Using LC-MS/MS

- Mock DS sample consisting of SILu™ Lite SigmaMab Universal Antibody Standard (Sigma) spiked 500:1 with UPS2 dynamic range protein standard set, injected on a high resolution Q Exactive in triplicate

UPS2 (Protein_ID)	Spiked Level (ppm)	No peptide fractionation	Optional peptide fractionation
ALBU_HUMAN	626.01	Yes	Yes
CAH2_HUMAN	274.67	Yes	Yes
CAH1_HUMAN	271.11	Yes	Yes
LEP_HUMAN	152.43	Yes	Yes
HBB_HUMAN	149.69	Yes	Yes
HBA_HUMAN	142.70	Yes	Yes
UBIQ_HUMAN	99.97	Yes	Yes
COS_HUMAN	80.78	Yes	Yes
CATA_HUMAN	56.25	Yes	Yes
SUMO1_HUMAN	36.62	Yes	Yes
NQO1_HUMAN	29.00	Yes	Yes
PRDX1_HUMAN	20.73	Yes	Yes
PPIA_HUMAN	19.03	Yes	Yes
MYG_HUMAN	16.09	Yes	Yes
CYB5_HUMAN	15.12	Yes	Yes
EGF_HUMAN	5.99	No	Yes
SYHC_HUMAN	5.49	No	Yes
KCRM_HUMAN	4.07	No	Yes
NQO2_HUMAN	2.44	No	Yes
RETBP_HUMAN	1.99	No	No
UBC9_HUMAN	1.70	No	Yes
LYSC_HUMAN	1.39	No	Yes
LALBA_HUMAN	1.33	No	Yes
NEED8_HUMAN	0.86	No	Yes

## SUMMARY

- Mass spectrometry provides additional HCP identification and characterization information to complement & supplement immunoassays.
- Targeted mass spectrometry assays enable absolute quantitation of specific HCPs with a high level of analytical rigor, without need for antibodies.
- Caprion's mass spectrometry-based platforms for HCP identification and quantitation routinely provide 1-10 ppm range sensitivity.
- Examples of client studies analyzed on Caprion's platforms:
  - Monitoring / identification of key clearance steps
  - HCP profiling of Originator vs. Biosimilar
  - Characterization of process validation run reproducibility
  - Purification process optimization / resin re-use
  - Characterization of HCP in clinical vs. Commercial process
  - Vaccine HCP characterization