

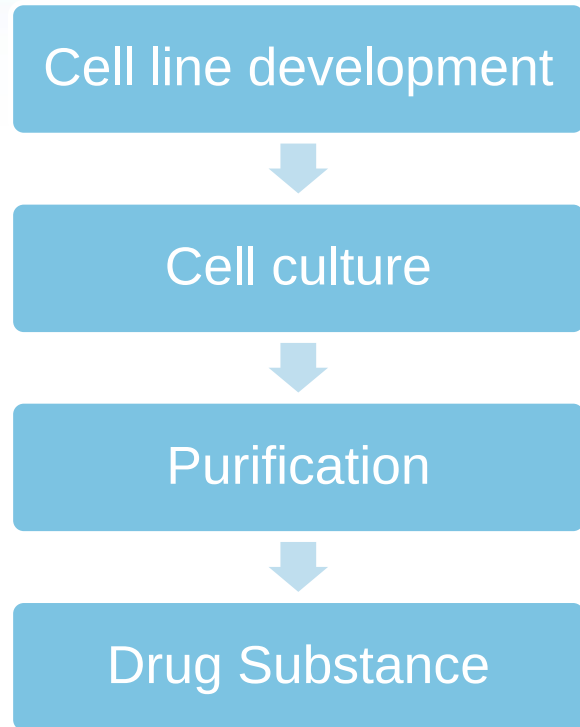


Monitoring Clearance of Lipase Host Cell Proteins in mAb Manufacturing using an LC-MS/MS Quantitation Method

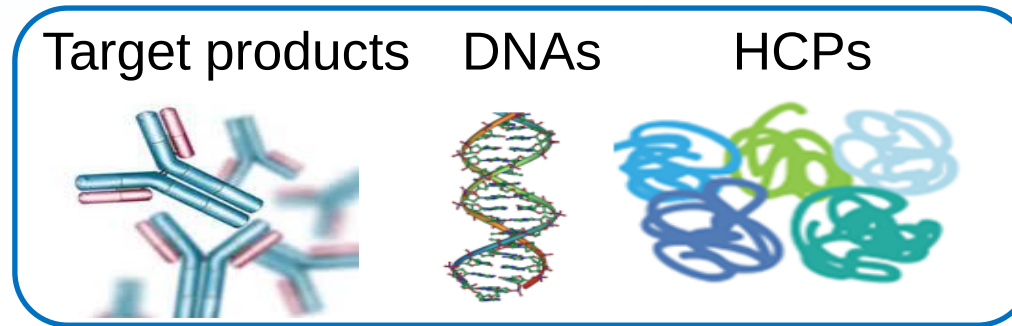
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Host Cell Protein (HCP) Impurities



Harvest cell culture fluid

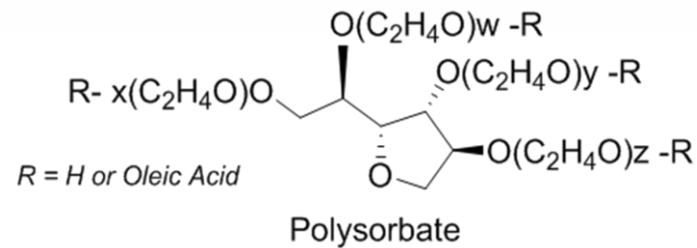


Host cell proteins (HCPs)

- Secreted or from cell lysis
- CHO, HEK, NS0: 20k+ genes
- Potentially immunogenic
- Enzymatic activity: affect product and excipient stability

Lipase host cell proteins (HCPs) and Polysorbate Degradation

- Residual lipase HCPs can cause polysorbate degradation



Lipase and other
hydrolytic enzymes

at low- or sub-ppm level

- Degraded PS
- Particle formation
- Protein stability issue, etc.

- Control strategy for lipase HCP**
 - Modify process for better lipase clearance
 - Better detection of lipases

Reported PS hydrolytic enzymes:

- Putative phospholipase B like 2 (PLBL2)
- Lipoprotein lipase (LPL)
- Group XV lysosomal phospholipase A2 Isomer X1 (LPLA2)
- phospholipase A2 group VII (PLA2G7)
- liver carboxylesterase

Chiu J, et al. *Biotechnol Bioeng* 2017; 114(5), 1006.
Dixit N, et al. *J. Pharm. Sci.* 2016; 105(5), 1657.
Hall T, et al. *J. Pharm. Sci.* 2016; 105(5), 1633.
Li X, et al. *Anal. Chem.* 2021; 93(23), 8161.
Zhang S, et al. *J. Pharm. Sci.* 2020.

Methods for Lipase Quantitation

Non-targeted Methods

Total HCP ELISA

- Quantify total HCP
- Not specific for lipase

HCP proteomics

- Profiling of total HCP population
- Sensitivity can be boosted by affinity enrichment

Targeted Methods

Lipase-specific ELISA

- Specific to one lipase
- Sensitive
- Method development can be time consuming and expensive

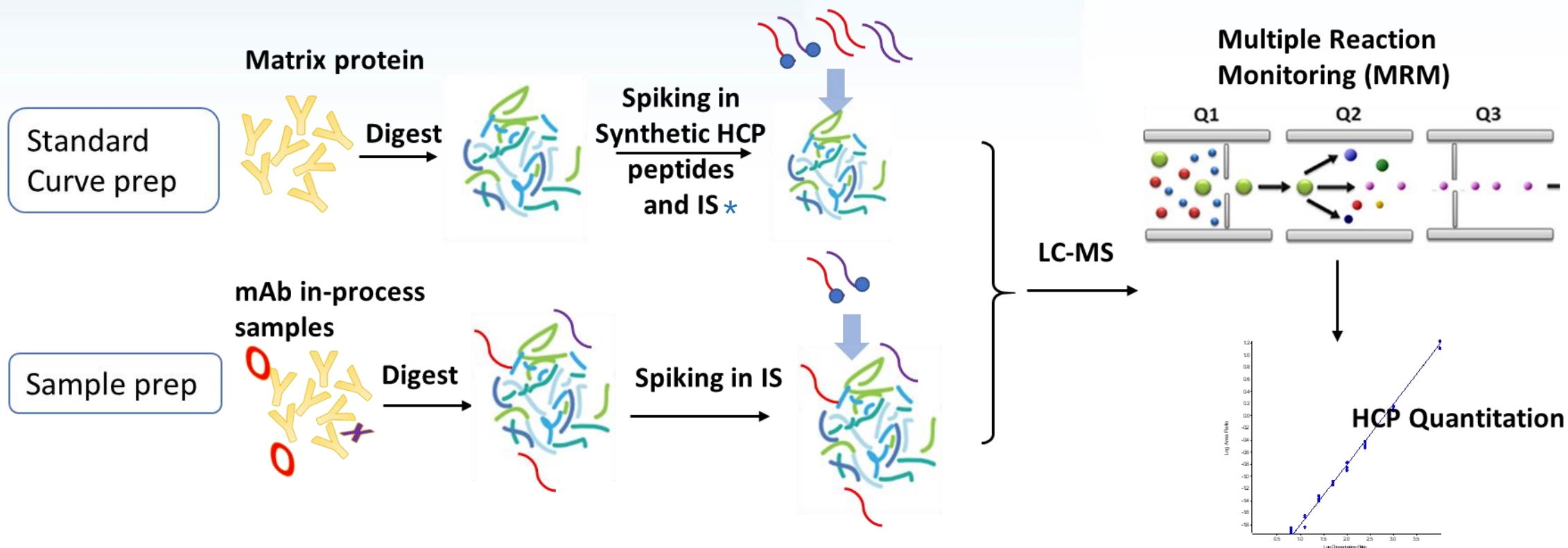
Targeted LC- MS/MS

- Specific to one or more lipases
- Sensitive
- Monitor multiple HCPs simultaneously
- Fast development timeline

**Sensitivity
requirement:
single digit to sub-
ppm**

Other methods: lipase activity-based method, etc.

LC-MS/MS Based Targeted Quantitation Scheme

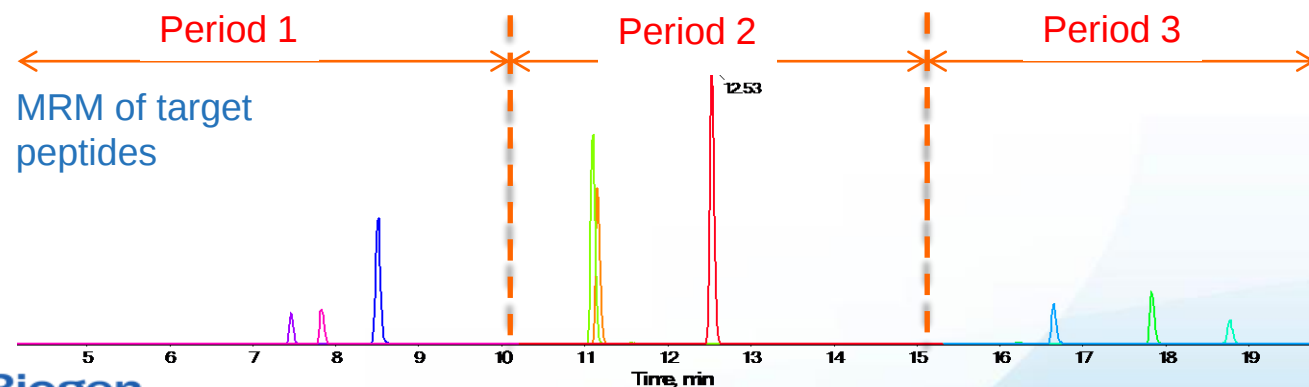


*IS: internal standard; heavy isotope labeled peptides

Three Lipase HCPs for Targeted Quantitation

Three lipase HCPs identified through shot-gun proteomics

Lipase Name	Size (kDa)	Peptides for Quantitation
Lipoprotein lipase	52.8	GLGDVDQLVK
		LSPDDADFVDVLHTFTR
		SIHLFIDSLNEENPSK
Lysosomal acid lipase	45.6	WPEVIIEDLFGHK
		VNVYTSHSPAGTSVQNLR
		GNTWSLK
Putative phospholipase B-like 2	65.4	LTLLQLK
		SVLLDAASGQLR
		AFIPNGPSPGSR



- 20 min RPLC gradient
- High resolution MRM using Sciex TripleTOF MS
- MRM scans scheduled according to retention time

Method Limit of Quantitation (LOQ) and Accuracy

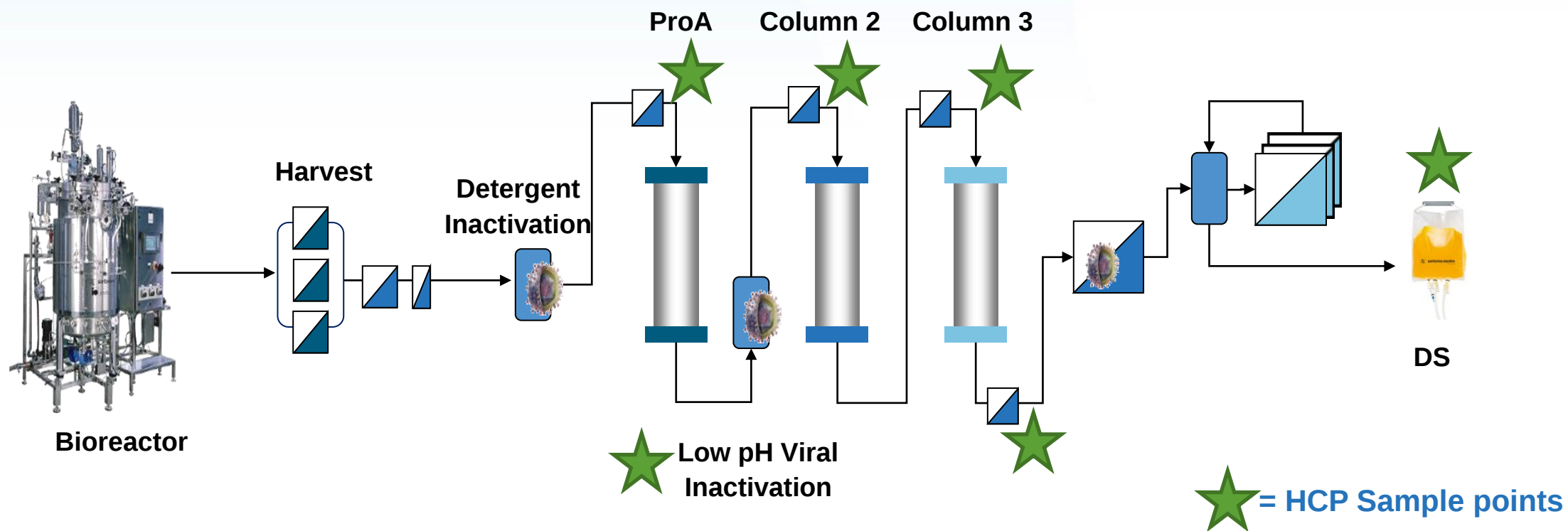
Protein	Peptide LOQ (pM)	Linear range (pM)	LOQ of HCP (ng/mL)
Lipoprotein lipase	12.5	12.5-2500	0.7
Lysosomal acid lipase	25	25-2500	1.1
putative phospholipase B-like 2	6.25	6.25-2500	0.4

- Close or lower than 1 ng/mL of lipase HCP can be quantified
- Linear range spanning three orders of magnitude
- Accuracy and reproducibility demonstrated

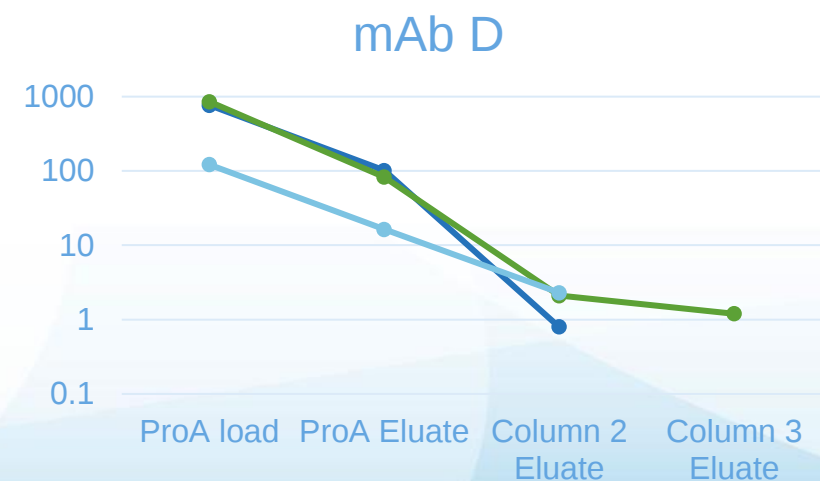
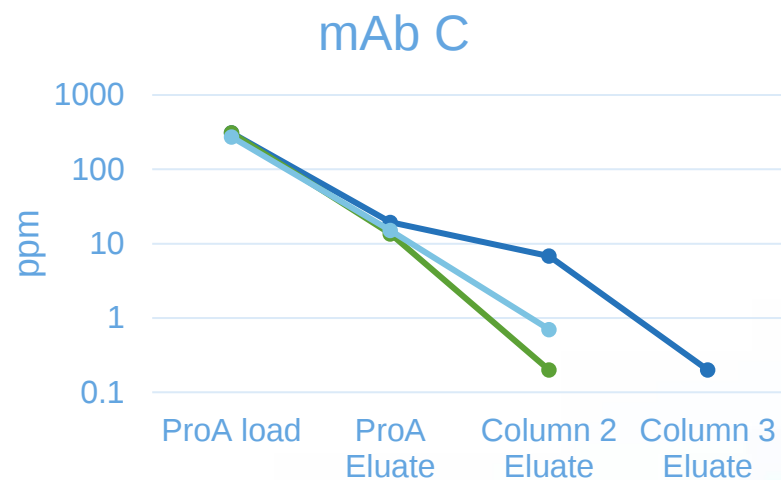
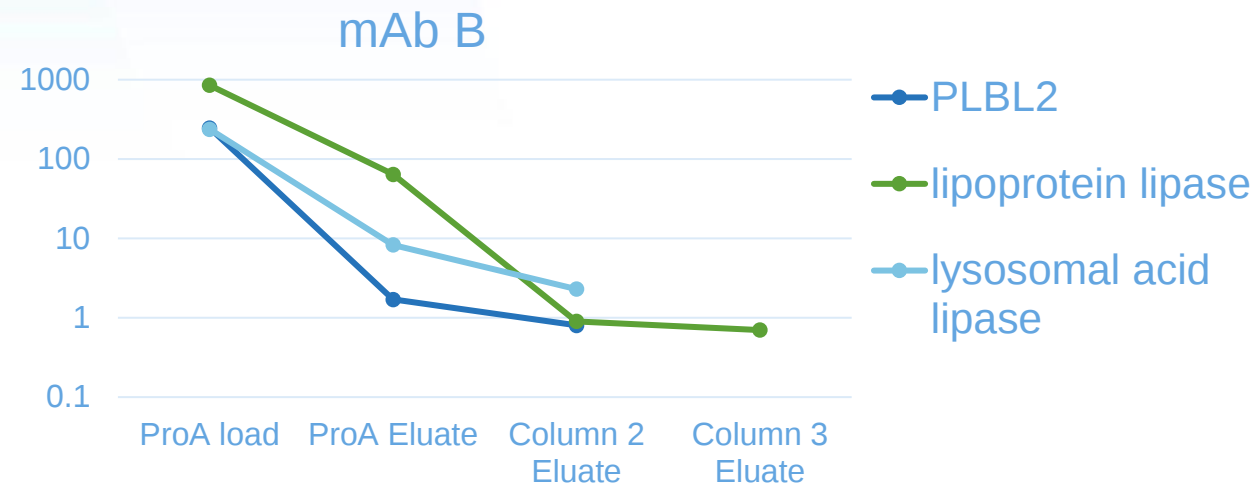
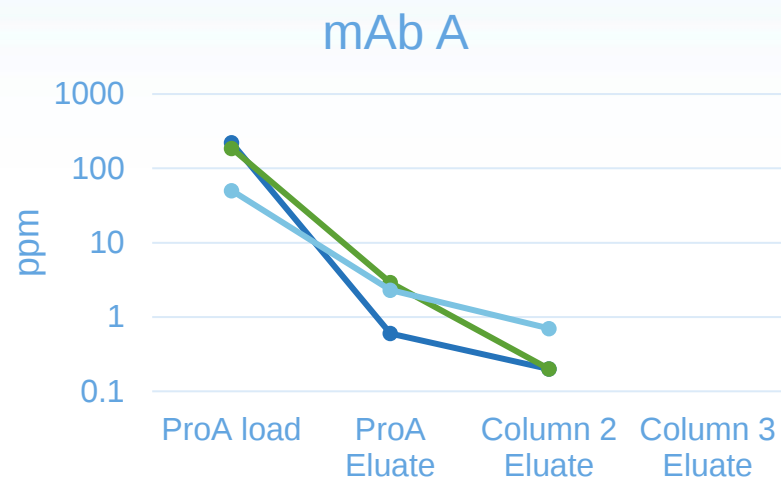
Accuracy assessment by spiking in recombinant PLBL2

Spike-in amount (ppm)	Calculated (ppm)	%CV (N=3)	%Accuracy
1	1.0	6%	103%
5	6.1	12%	121%
10	11.3	4%	113%
100	117.0	6%	117%

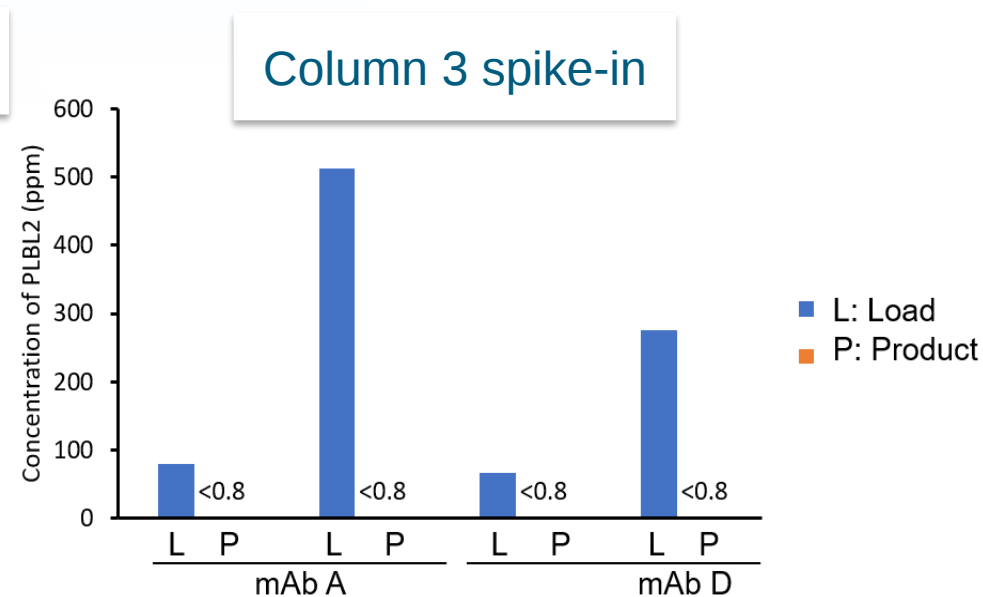
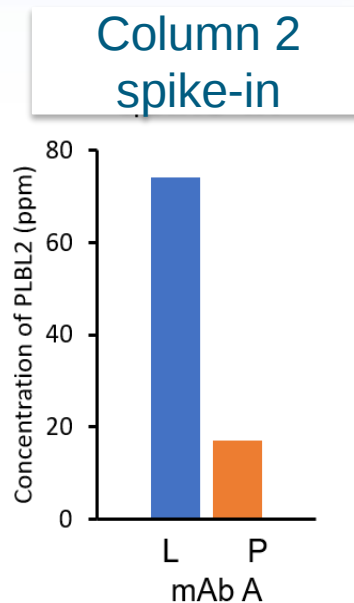
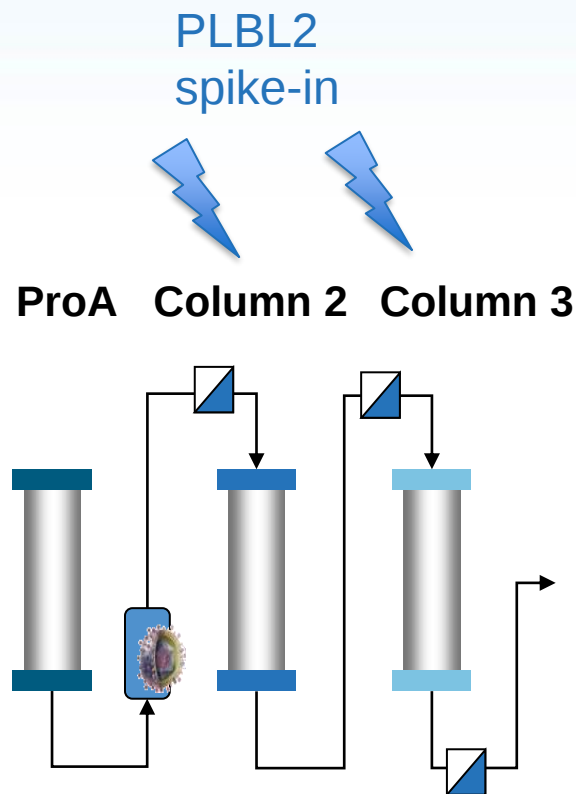
Applications: HCP Analysis Throughout Downstream Process



Monitoring Lipase Clearance in Downstream Process

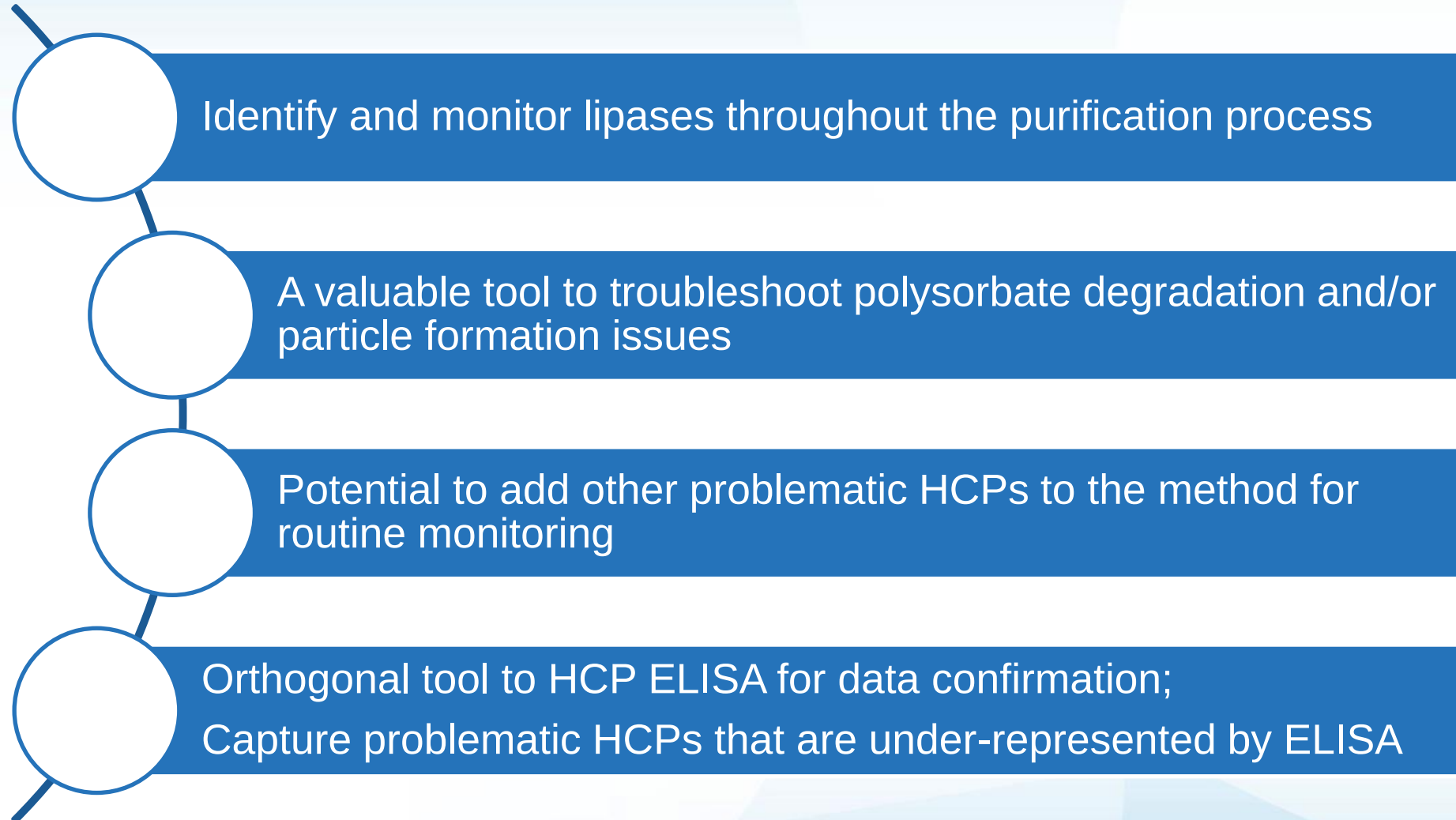


Understanding the Capacity of Lipase Clearance for Each Column



- Column 2 has limited capacity for PLBL2 clearance
- Column 3 was able to clear PLBL2 down to <LOQ for two mAbs

Applications of the Lipase Quantitation Method



Acknowledgment

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Thank you!
Questions?