Absolute Quantification of Problematic CHO Lipases by PRM-MS – Case Studies

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

Research & Development Department, Chromatography and Mass Spectrometry Group

CYGNUS TECHNOLOGIES

part of Maravai LifeSciences

Abstract

Presence of host cell-derived lipases in biologic drug substances has been linked to polysorbate degradation, which can compromise drug stability, shorten shelf life, and lead to costly manufacturing deviations or regulatory concerns. While untargeted Liquid Chromatography-Mass Spectrometry (LC-MS) permits the identification of such host cell proteins (HCPs) with high precision, quantitative data is often inaccurate. Here, we present a parallel reaction monitoring (PRM) assay for highly sensitive lipase identification and quantification using stable isotope labeled (SIL) peptides. Assay qualification with synthetic, unlabeled (UL) peptides demonstrates assay parameters, while a case study with two unique drug substance (DS) samples demonstrates how the CHO 6x Lipase (C6XL) MS Assay enables manufacturers to better assess drug substance quality.

Background

Cygnus qualified this PRM assay to measure the following lipases: 1) Phospholipase A2 Group XV lysosomal (GXVP_A2), 2) Lysosomal Acid Lipase (LAL_A), 3) Lipoprotein Lipase (LPL), 4) Phosphoinositide phospholipase C (PIPL_C), 5) Phospholipase A1 (PL_A1), 6) Phospholipase B like 2 (PLBL2).

Two similar Drug Samples (DS) were submitted by the same company for lipase quantification.

Methods & Materials

Sample Preparation: Qualification Samples and Customer Samples were solubilized, reduced, alkylated, digested with Trypsin/Lys-C, desalted, and concentrated.

Qualification Calibration Curve: Purified peptides (>97% purity) were spiked into the protein digest such that the stable isotope labeled (SIL) peptide concentration was fixed and the unlabeled peptide concentration varied across three orders of magnitude.

Sample SIL Spike IN: Purified peptides (>97% purity) were spiked into the protein digest, and assay quality controls and injected on a Vanquish Neo UHPLC coupled to an Orbitrap Eclipse Tribrid Mass Spectrometer.

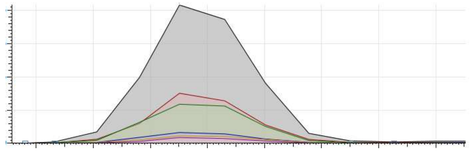

Figure 1. Representative SIL peak (PLBL2_1) with fragment ions overlayed. Peak height on y-axis and retention time on x-axis.

Results - CHO 6xLipase™ Qualification

- SIL peptide peak areas are highly similar between different calibration curve standards where concentration was fixed.
- UL:SIL ratios generate a linear response curve for 2 – 6 peptide pairs per HCP.
- Most R^2 values were 0.99 - 1.0, and at least two peptides per protein met acceptance criteria for precision (< 25%) and accuracy (+/- 35%) based on the expected UL:SIL ratio.

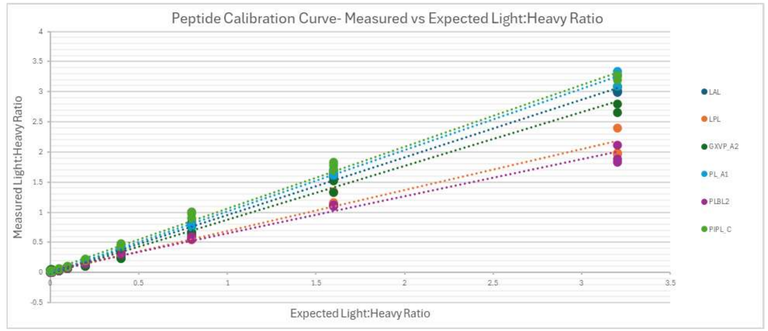

Figure 2. Assay Qualification: Peptide calibration curves. The measured UL:SIL ratio is on the y-axis and expected ratio on the x-axis.

Peptides	LOD (ppm)	HCP MW (Daltons)	LLOQ (ppm)	ULOQ (ppm)
LAL_1	0.15	45635	9.13	292.06
LAL_2	4.39		18.25	73.02
LPL_1	0.31	50522	1.01	323.34
LPL_2	0.08		10.10	323.34
GXVP_A2_1	1.84	47236	4.72	302.31
GXVP_A2_2	0.03		4.72	302.31
PL_A1_1	0.38	48698	4.87	311.67
PL_A1_2	0.12		0.49	311.67
PLBL2_1	0.86	65541	1.31	419.46
PLBL2_2	0.53		0.66	209.73
PIPL_C_1	0.08	139074	445.04	890.07
PIPL_C_2	2.86		13.91	890.07

Table 1. Assay Qualification: LOD, lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) for two peptides corresponding to each HCP.

Results - Customer Samples

- The first DS reported Group XV Lysosomal, Lysosomal Acid Lipase, Lipoprotein Lipase, and Phospholipase A1 under 10ng/mL, while the second DS only reported Phospholipase B Like 2 under 10ng/mL (Table 2).

Protein	DS #1		DS #2	
	Estimated concentration (ppm)	%CV	Estimated concentration (ppm)	%CV
Lysosomal Acid Lipase	55.76	9.6%	<LOD	NA
Lipoprotein Lipase	140.20	2.3%	<LOD	NA
Group XV lysosomal	23.23	5.2%	<LOD	NA
Phospholipase A1	4.65	3.2%	<LOD	NA
Phospholipase B like 2	<LLOQ	N/A	61.81	3.2%
Phosphoinositide phospholipase C	<LOD	NA	<LOD	NA

Table 2. Two DS analyzed for six CHO lipases using SIL to accurately determine the ng/mL concentration with reported precision values (CV%).

Conclusions

- PRM acquisition with internally-spiked SIL is effective at estimating the absolute concentration of HCPs.
- Precision and accuracy extend across several orders of magnitude (peptide molarity) which corresponds to sub- and low-ppm levels.
- CHO lipase quantification was successfully completed for two customer drug substance (DS) samples, each with distinct CHO lipase panels. The assay reliably identified and quantified individual lipase species in both samples, demonstrating its applicability across diverse process platforms and supporting product quality and residual HCP risk assessment.