HCP Profiling and Prediction of Polysorbate Stability in mAb Formulation

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Outline

- LC/MS Method Development for HCP Monitoring
  - Novel sample preparation
- LC/MS Method Qualification for HCP Monitoring
  - Qualitative evaluation
  - Quantitative evaluation
- Application to Monitor Ultra Low HCPs
  - Phospholipase monitoring in biopharmaceutical products
- Prediction of Polysorbate Stability in mAbs

Methods for Identification of HCPs in Bioproducts by MS

- Band identification of 1D or 2D SDS-PAGE
- 1D LC/MS/MS or CE-MS/MS with DDA or DIA
- 2D UPLC/MS^E or 2D-UPLC/IM-MS^E
- HCP enrichment before LC/MS identification
  - Immobilized mAb for co-purification with null strain
  - Removing mAb or enriching HCPs with immuno-capturing
  - 100K filter to separate HCPs from mAb

- General procedure of sample preparation for shotgun proteomics
  - Enzymatic digest following denaturation, reduction, ± alkylation

Challenges in HCP Analysis by MS

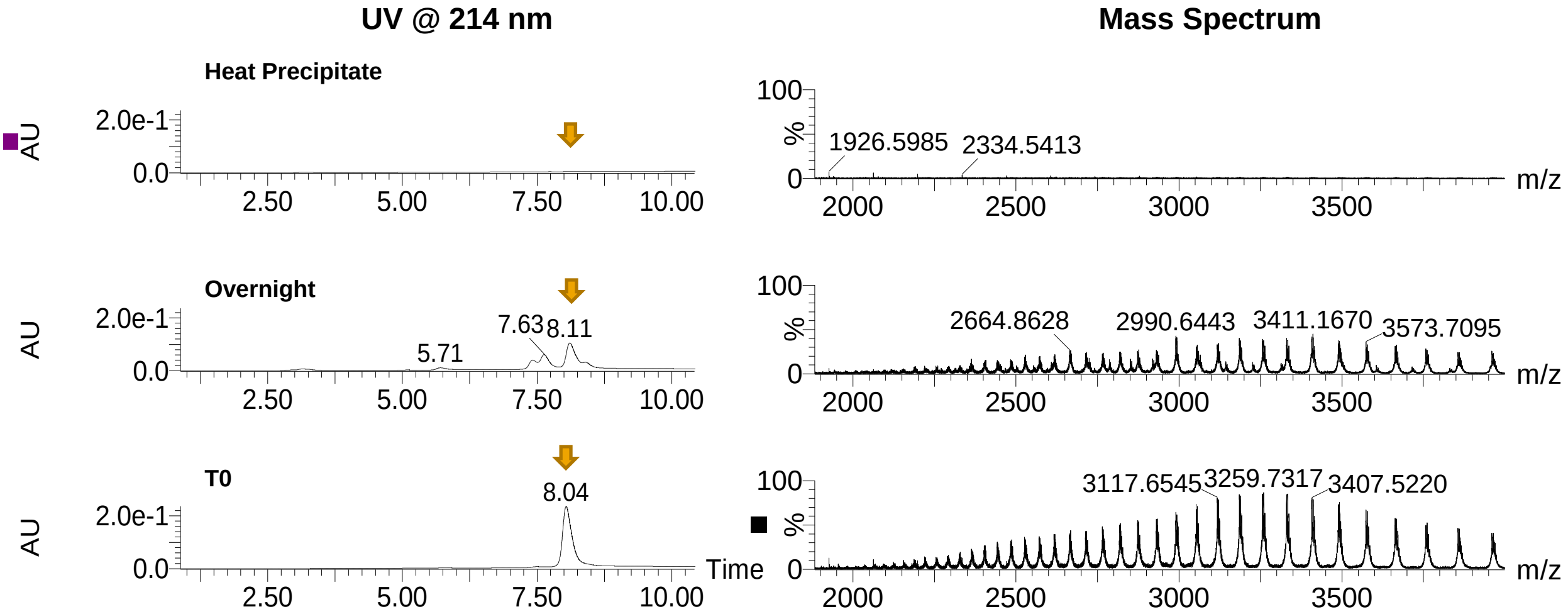
- Detection
  - HCPs can be present at extremely low levels
    - Typically ppm concentration (Relative to Biotherapeutic)
    - Common industry spec limits: 10-100 ppm
    - 10^5-10^6 dynamic range requirement for individual HCP
    - Specific HCPs, such as, lipases: < 0.1 ppm individual HCP, i.e. >10^7 dynamic range
  - MS dynamic range for detection
    - 3 to 4 (<5) orders of magnitude
- Quantitation
  - No appropriate standard

Methods to Overcome Dynamic Range Issue

- Resolving co-eluted peptides
  - Long separation time
  - Additional dimension separation
    - Such as 2D-HPLC
- HCP enrichment
- Using better mass spectrometers

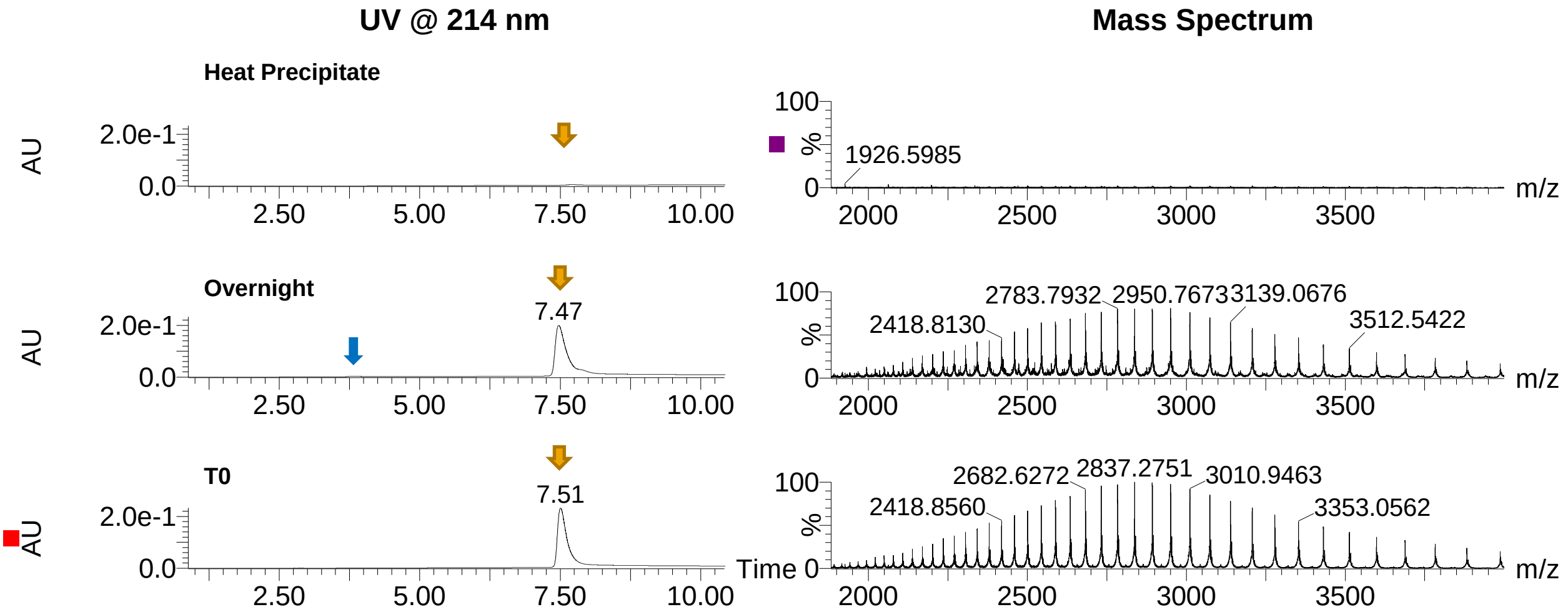
- Our Approach
  - Making minimal mAb to be digested while HCPs are digested
  - Removing undigested mAb (optional)

IgG1 Directly Treated with Trypsin at 37 °C



Huang et al. *Anal Chem*. 2017

IgG4 Directly Treated with Trypsin at 37 °C



Huang et al. *Anal Chem*. 2017

Comparison of Sample Preparation Procedures

- HCPs from five null strain cells
  - HCP material was treated with trypsin overnight after reduction/alkylation
  - HCP material was directly treated with trypsin, then reduced with DTT
  - LC/MS/MS with DDA

Null Strain	HCPs Detected		For Top 500 HCPs, Unique HCPs with	
	Traditional	Novel	Traditional	Novel
NS 1	1159	1199	18	7
NS 2	1179	1165	19	3
NS 3	1147	1211	20	7
NS 4	1113	1049	18	4
NS 5	1077	1134	24	13
NS 5, 10% Injected	871	959	33	18
NS 5, 20% Injected	1084	1074	26	11
NS 5, 50% Injected	1175	1176	15	11

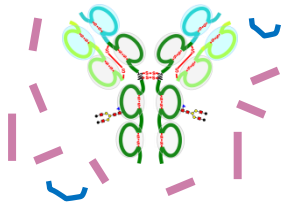
Workflow for LC-MS Determination of HCP

mAb Sample

API or in-Process +
hPCSK9 & ADH1

Trypsin

Overnight



HCP Peptides

DTT, Heat

Spin

mAb

LC-MS/MS

Data Processing

CHO K1
Database Search

FDR ≤ 1%

Criteria

HCP ID

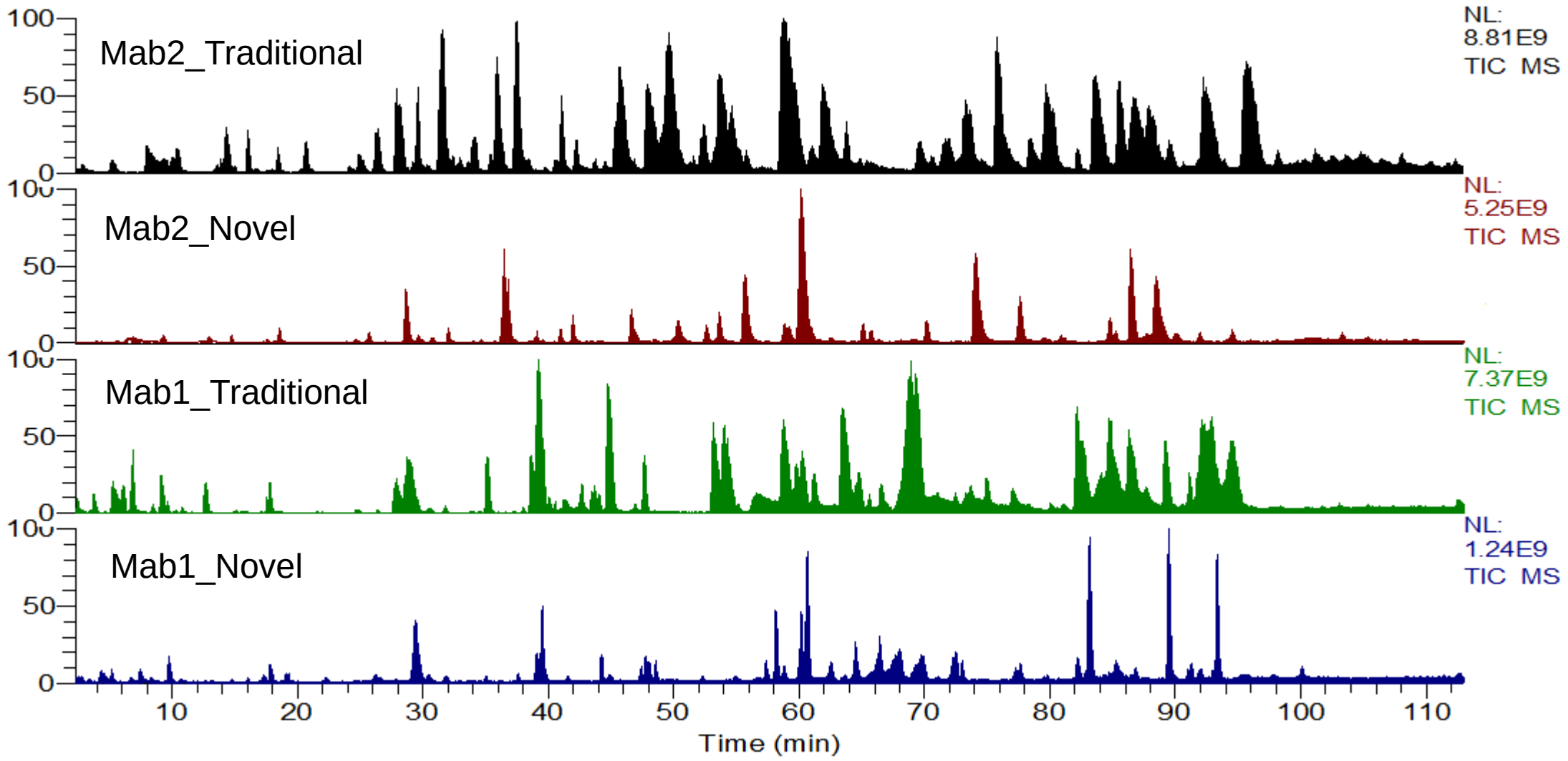
HCP Quantification

Top 3 peptides

Spiked Proteins

Protein Intensity

TIC of LC/MS/MS Analysis



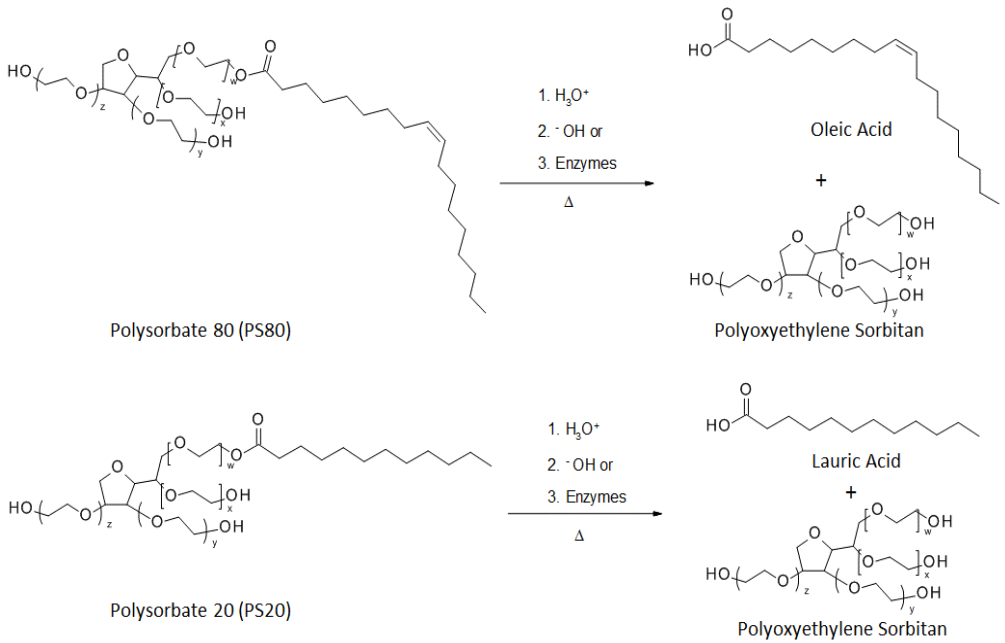
Summary of Method Evaluation

- Null strain material with or without IgG
  - Detected similar number of HCPs and quantity
- IgG1 and IgG4 spiked with five CHO HCPs
  - Linear between 1 to 100 ppm
- Robustness
  - Control samples for over two years and > 150 analyses
    - < 25% RSD for total HCO
    - < 40% for each individual HCP when it > 10 ppm
- NIST mAb Reference Standard
  - >100 HCPs
  - ≥ 60 HCPs with ≥ unique peptides (MSMS data) per HCP

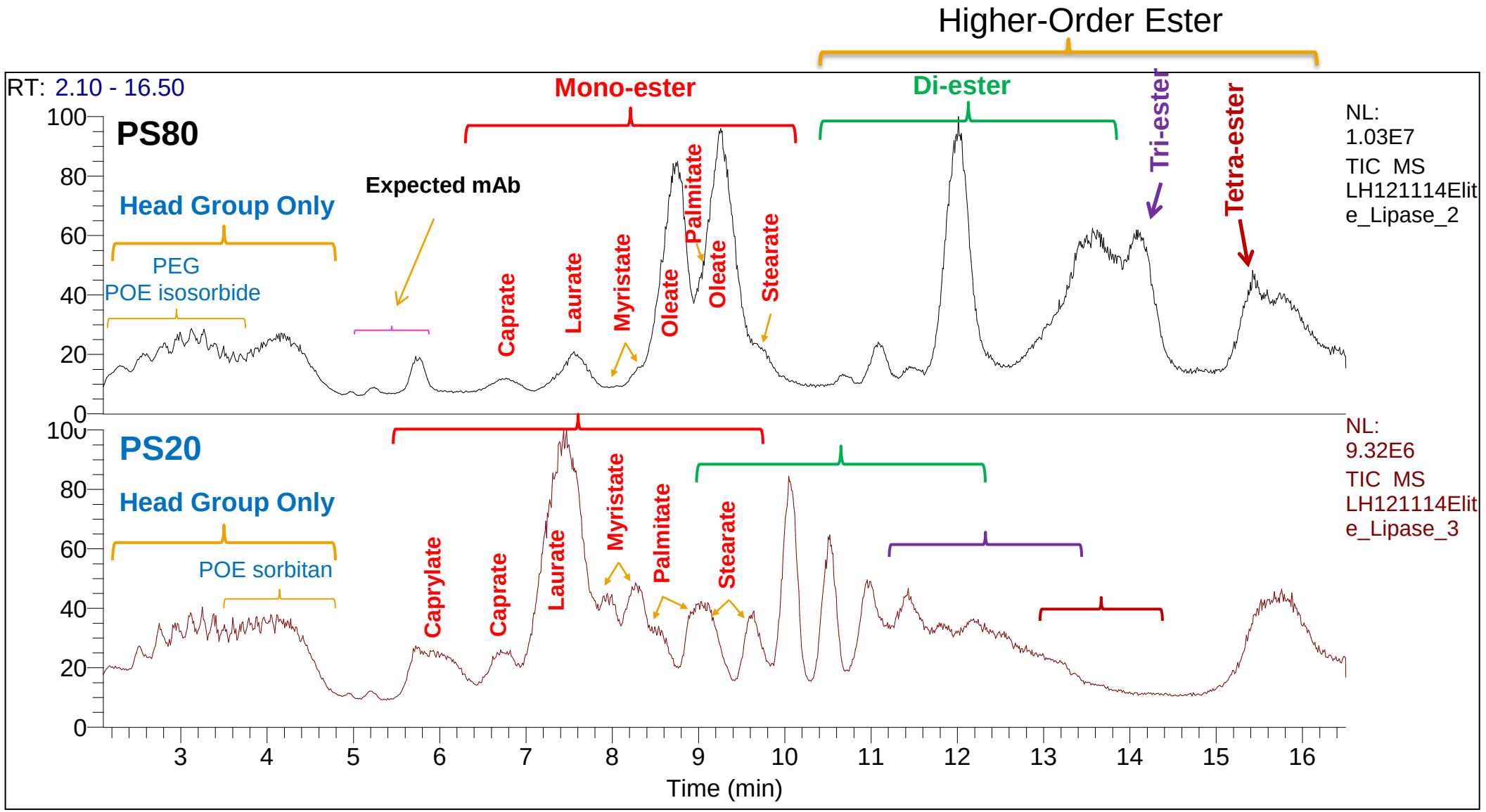
Huang et al. *Anal Chem*. 2017

Polysorbate Hydrolysis

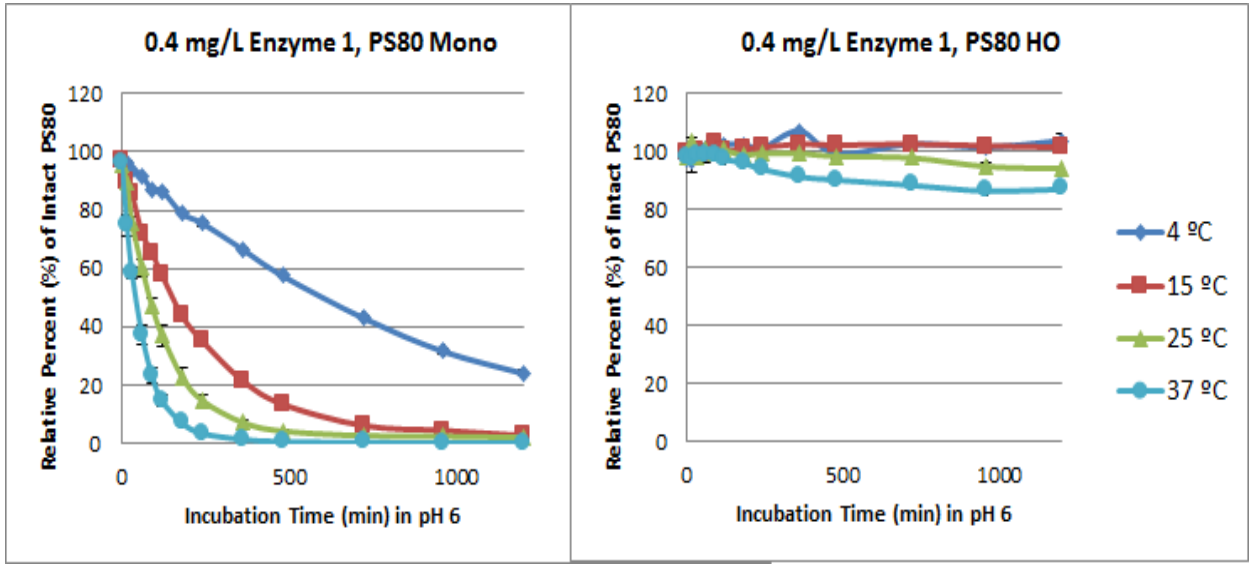
- PS20 or 80
  - Important excipient for stabilizing high concentration mAb formulations
- PS20 or 80 degradations
  - Auto-oxidation
  - Hydrolysis
    - pH and heat
    - Enzymatic (residual HCPs) hydrolysis
      - PLA2, Hall T. et al. *J Pharm Sci.* 2016;105:1633-1642.
      - PLBL2, Dixit N et al. *J Pharm Sci*. 2016;105:1657-1666.
      - LPL, Chiu J. et al. *Biotechnol Bioeng.* 2017;114:1006-1015.
      - LAL, Huang et al. BEPBA Conference on HCP, May 17-19, 2016, Lisbon, Portugal

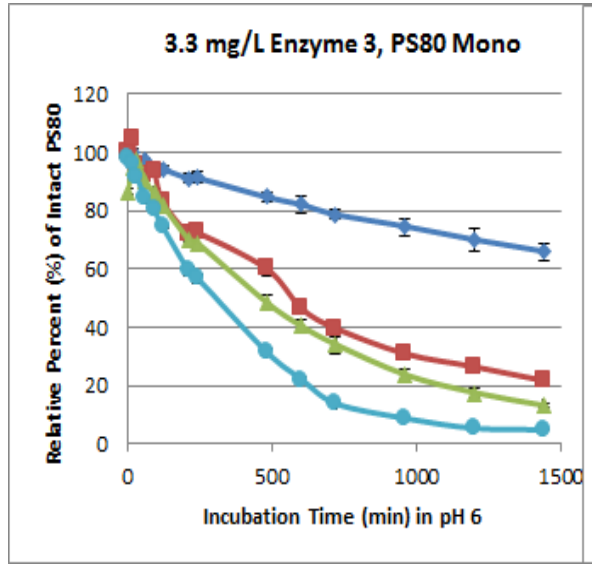


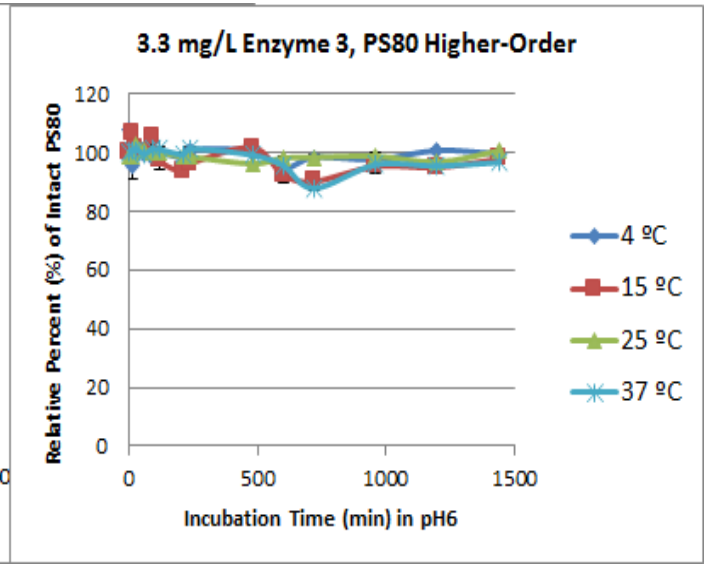
LC/MS Analysis for PS20 and PS80

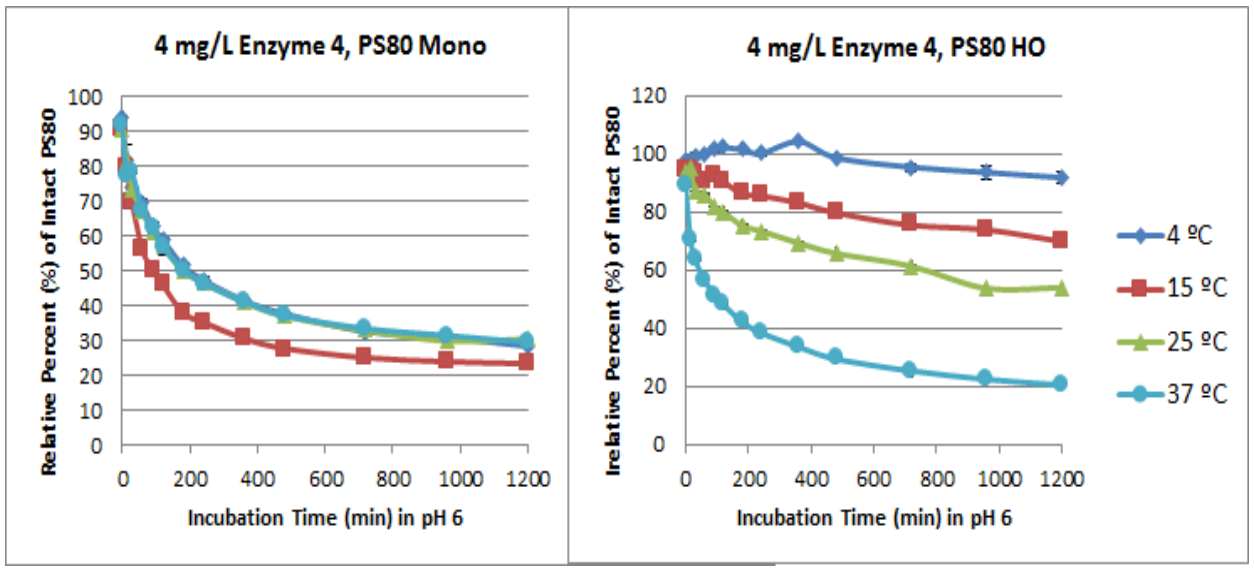


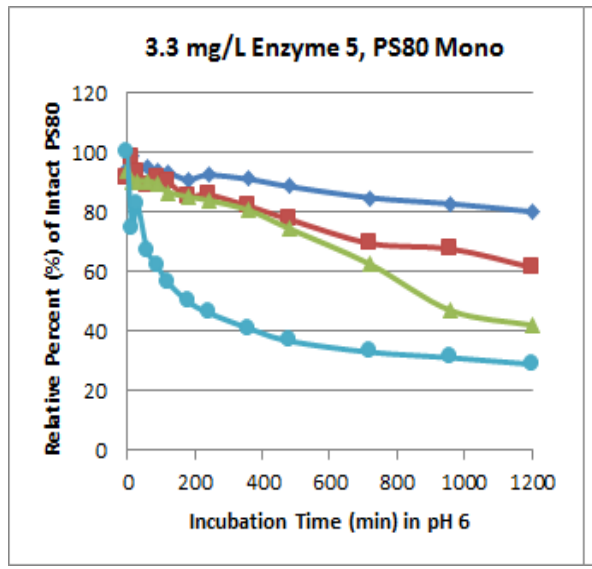
PS80 Hydrolysis Profile with Enzymes

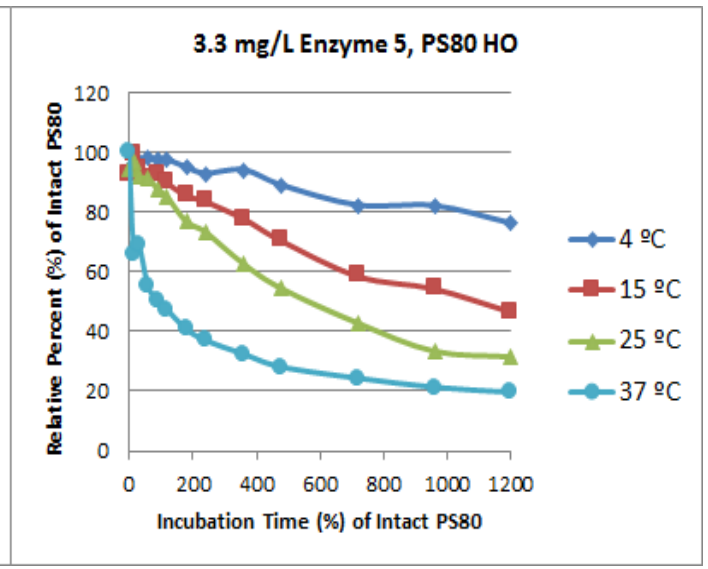




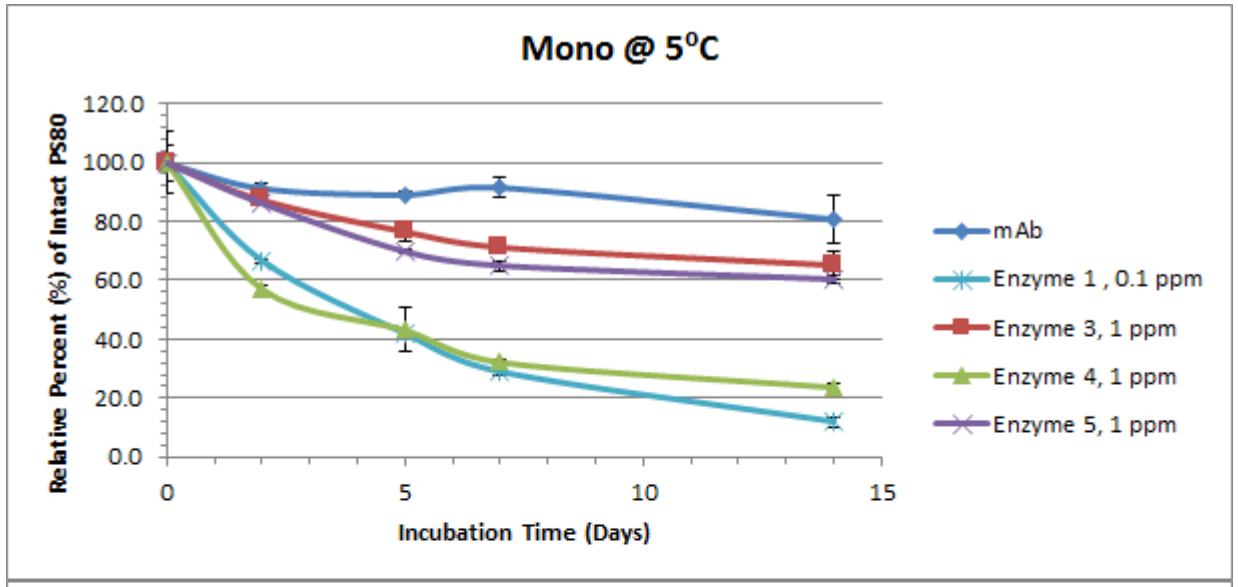


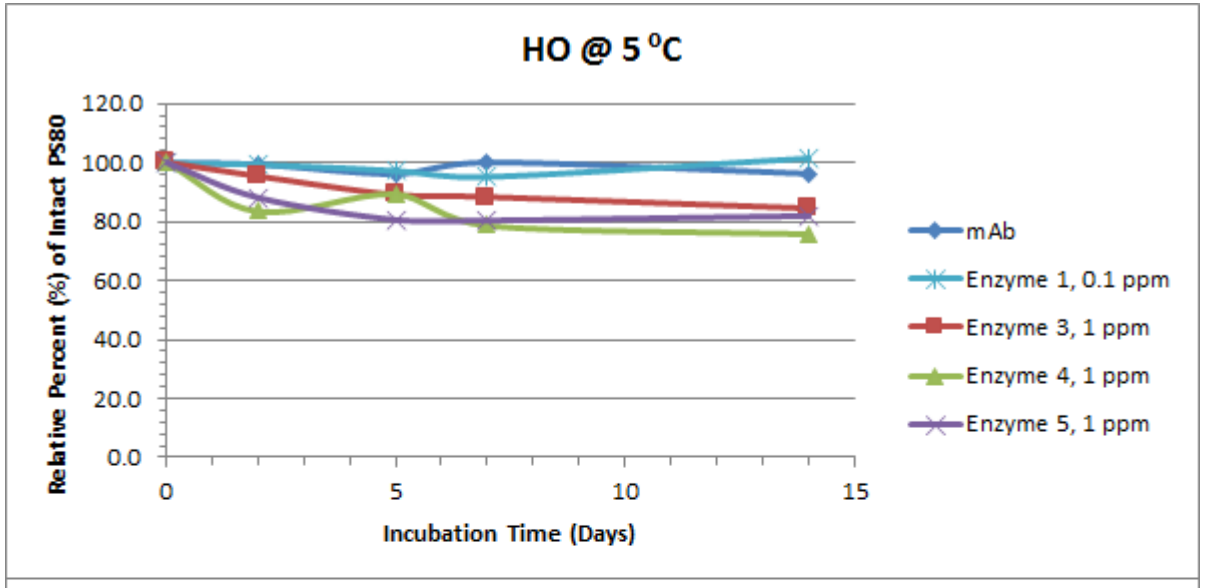


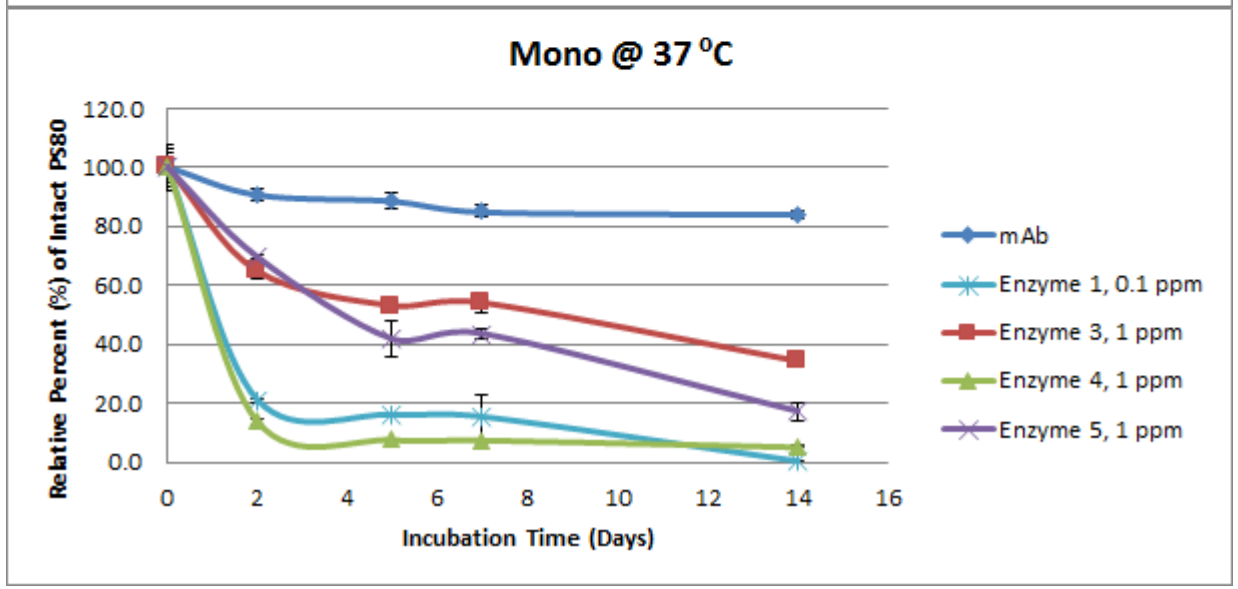


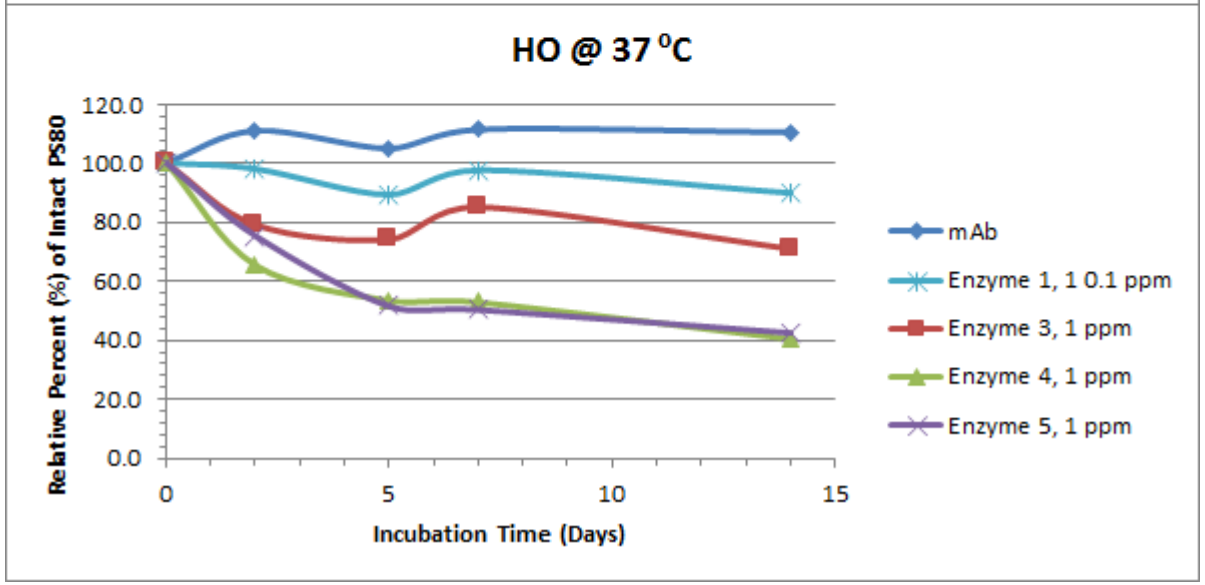


PS80 Hydrolysis Profile in mAb Spiked with Enzymes

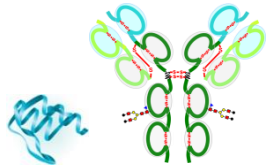








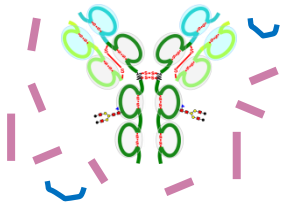
Schematic Diagram of PRM for Specific HCPs



mAb

Spiked with 0, 0.01, 0.02, 0.05, 0.1, 0.2, 1.0 ppm of each of six Enzymes

Trypsin



HCP Peptides

Heat

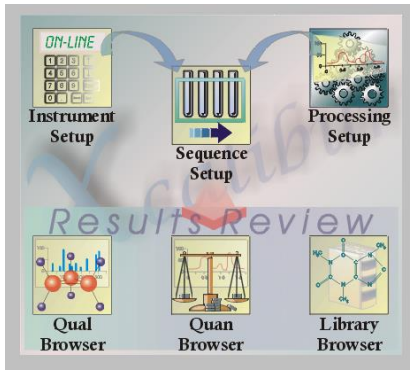
Spin

mAb

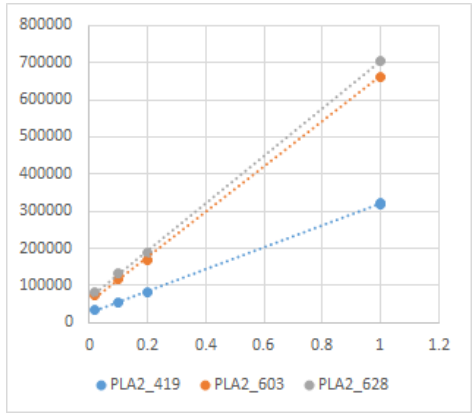
LC-MS/MS, Target MS/MS

Data Processing

Xcalibur Quan Browser



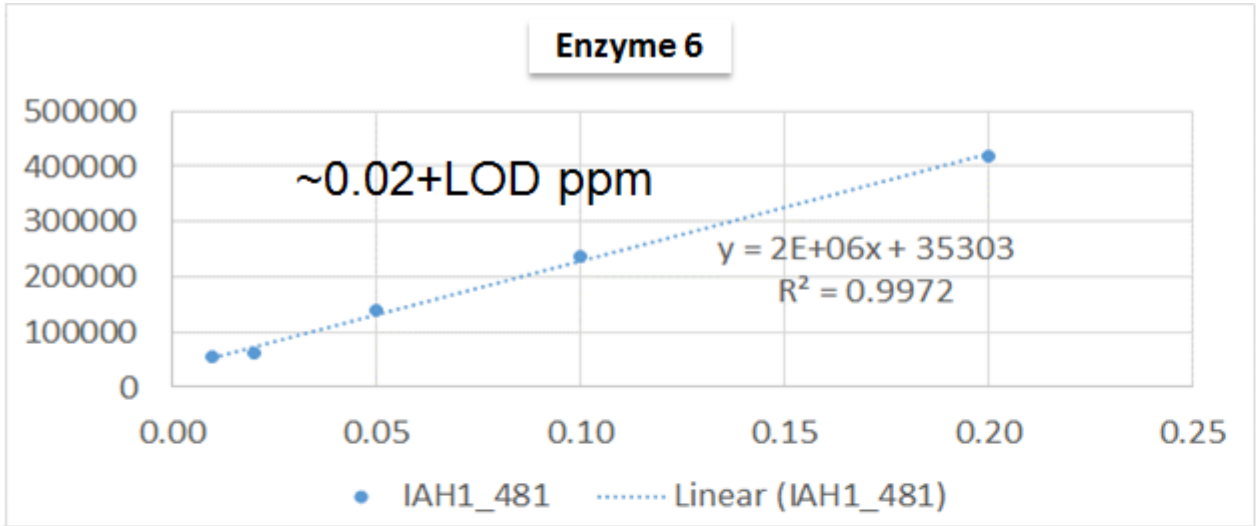
Plots for Target Peptides

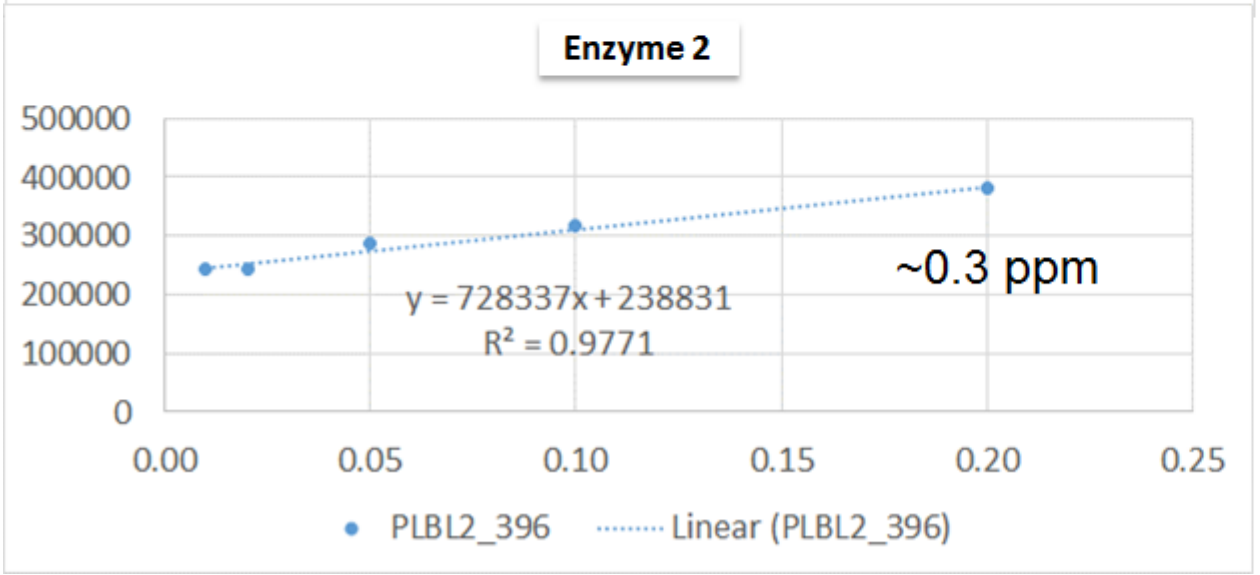


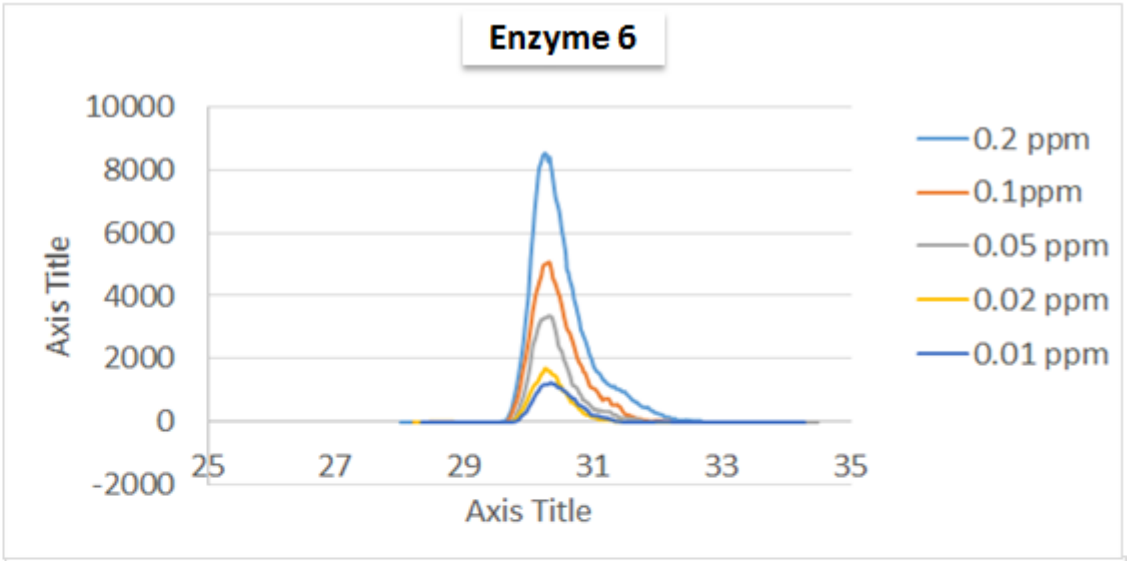
Slope Intercept

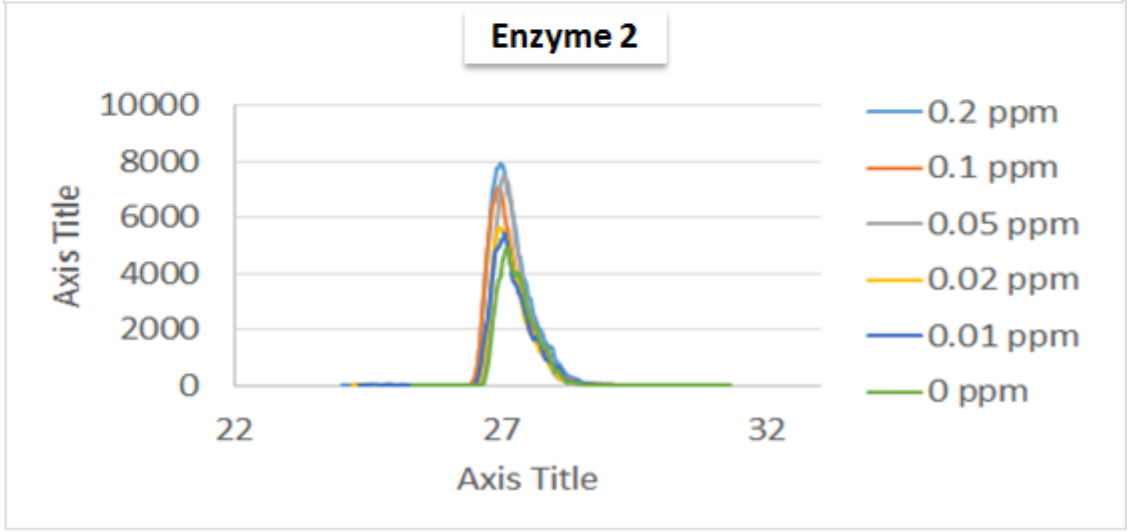
Trace Amount of **Enzymes**

Determination of Enzymes in mAb Sample (> LOD)

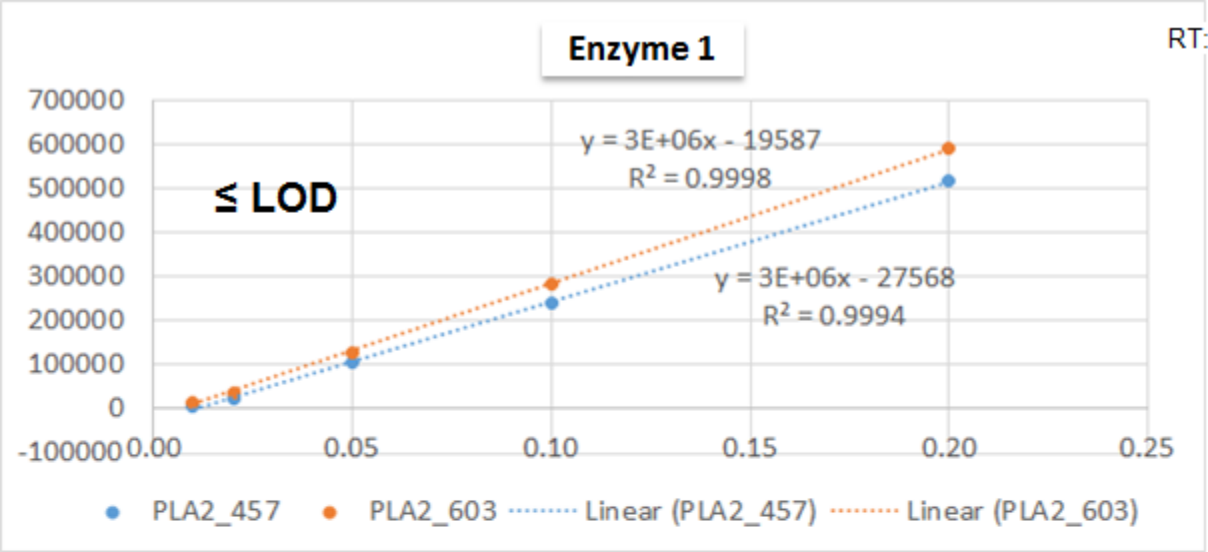


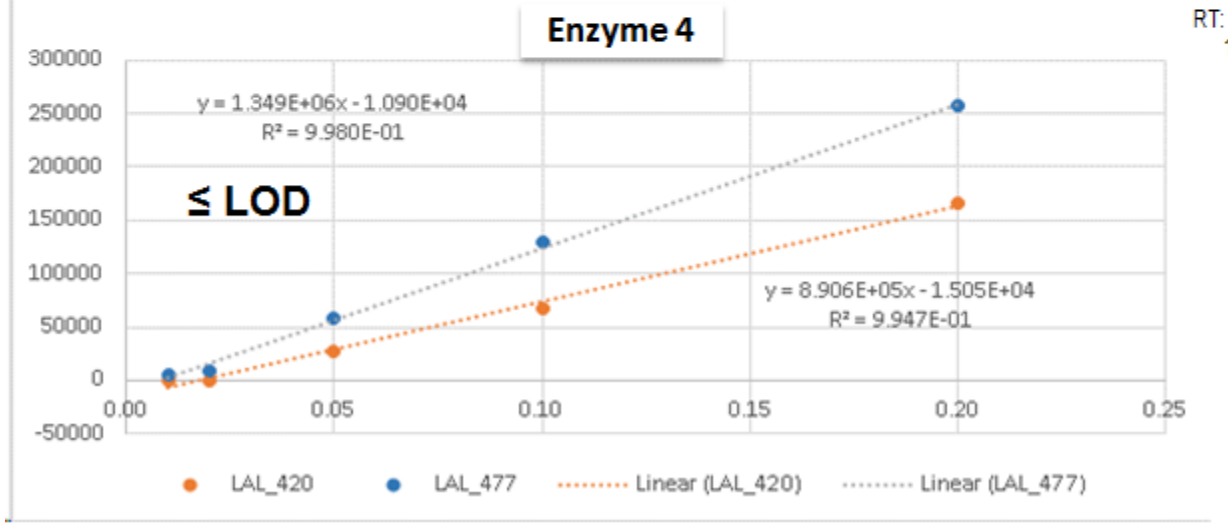


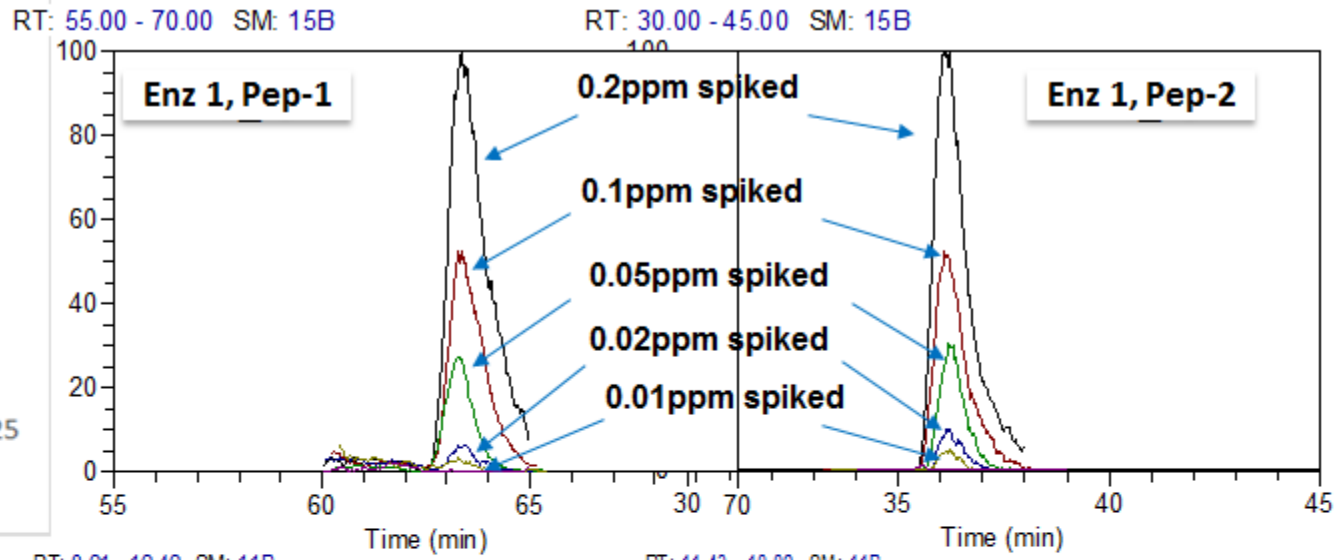


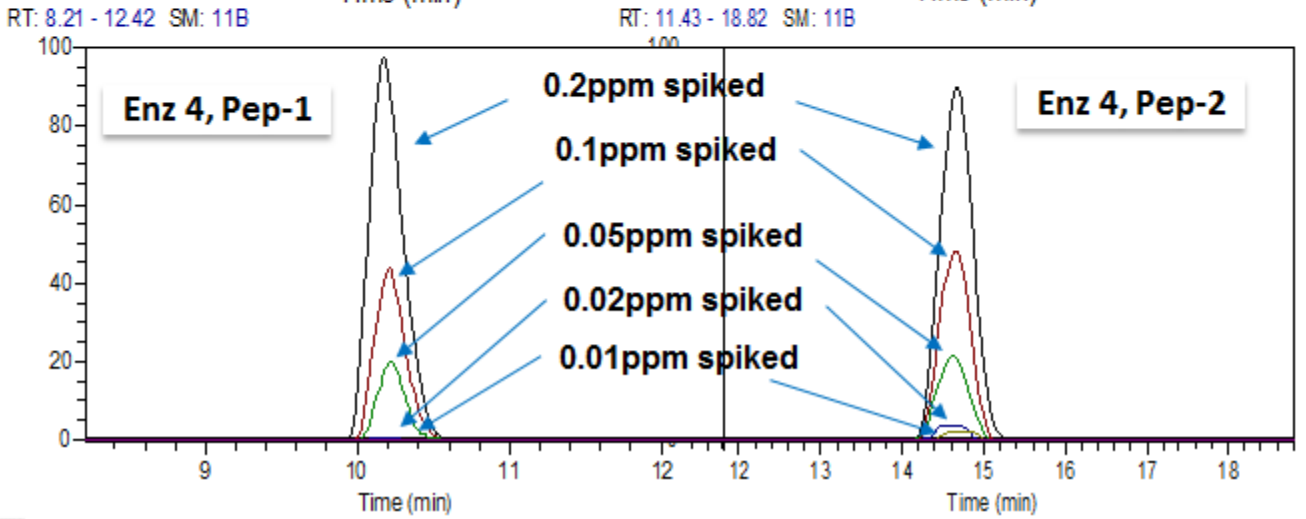


Determination of Lipases in mAb Sample (< LOD)



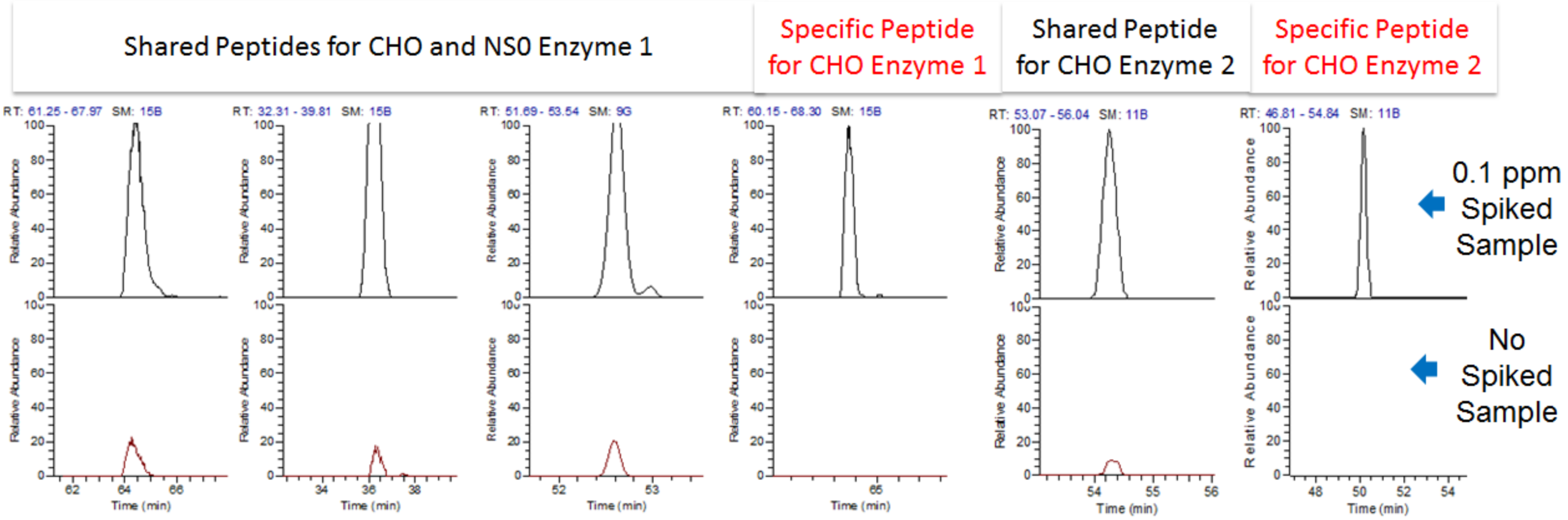




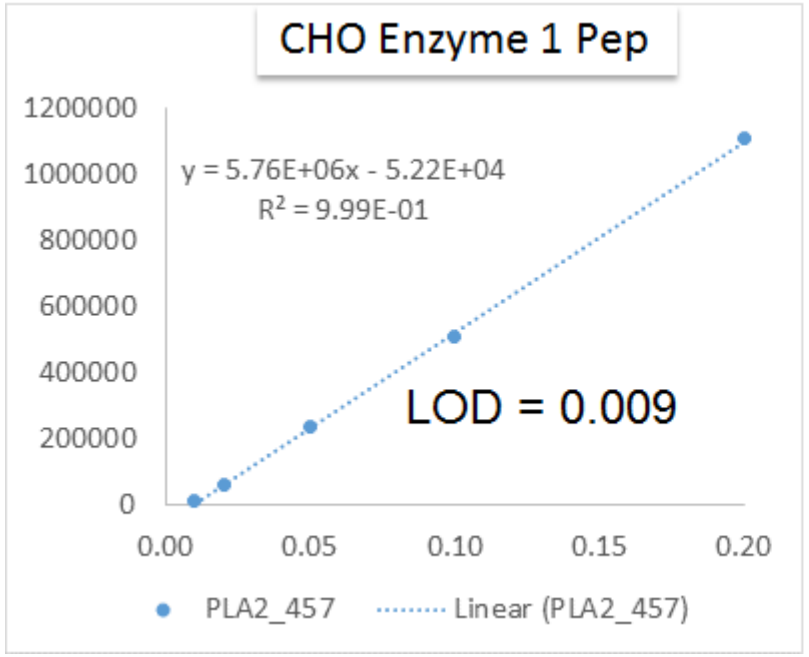


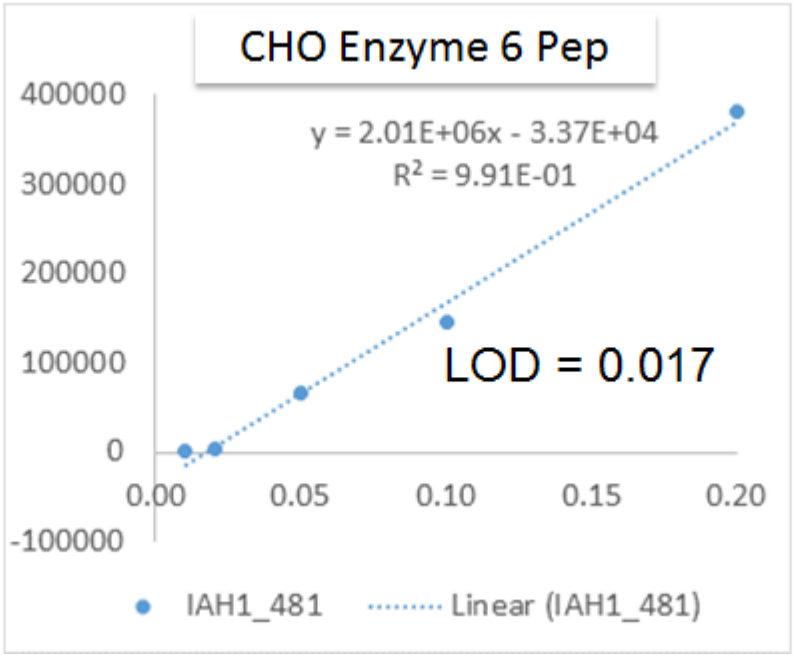
How to Determine LOD

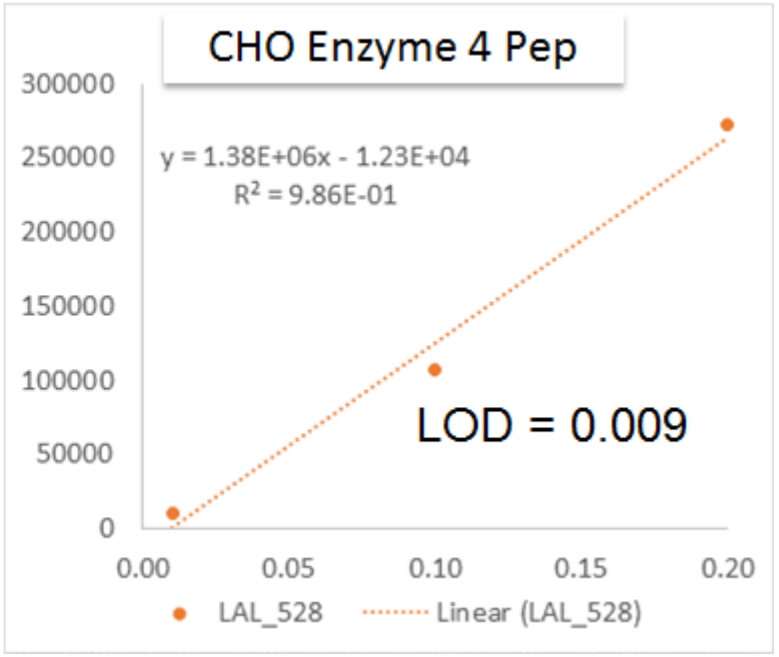
- mAb expressed in NS0 spiked with CHO lipases and esterase
- Target MSMS (or PRM) analysis for specific CHO or both peptides
- Plots for specific CHO peptides of lipases and esterase



Limit of Detection (LOD)



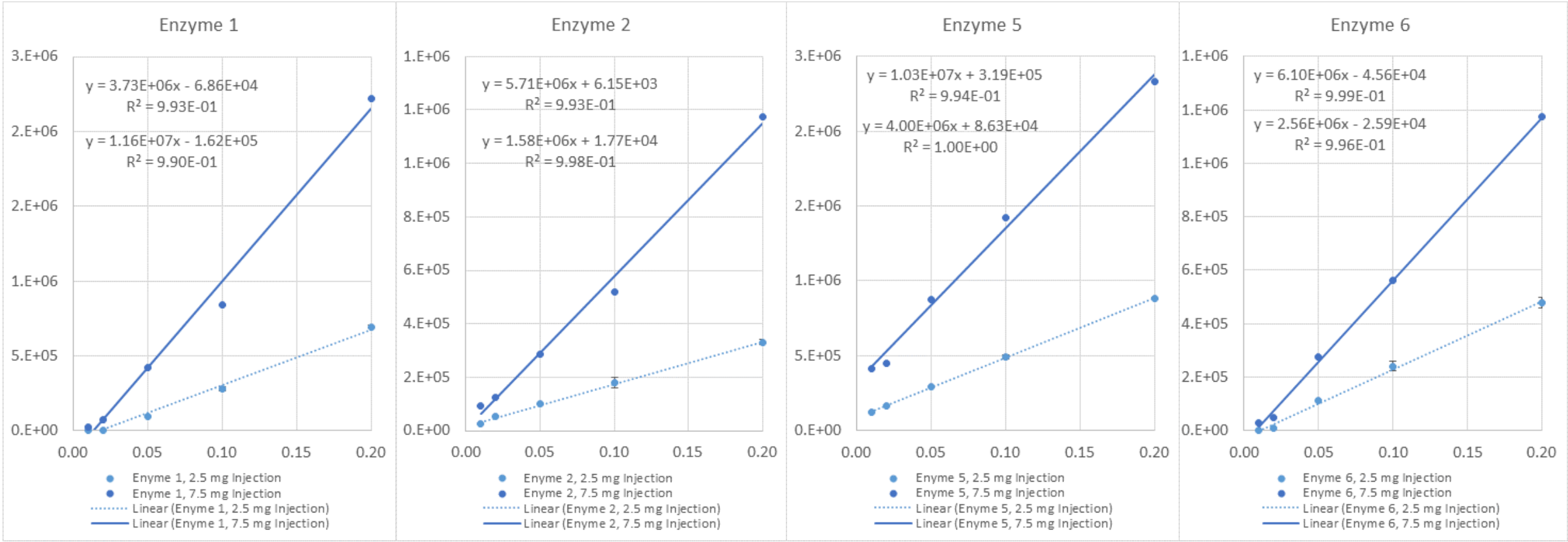




Spiked CHO HCP	Enzyme 1	Enzyme 2	Enzyme 4	Enzyme 6
LOD (ppm or ng/mg mAb)	0.01	0.05	0.01	0.02

Plots of the Spiked Samples with Different Injections

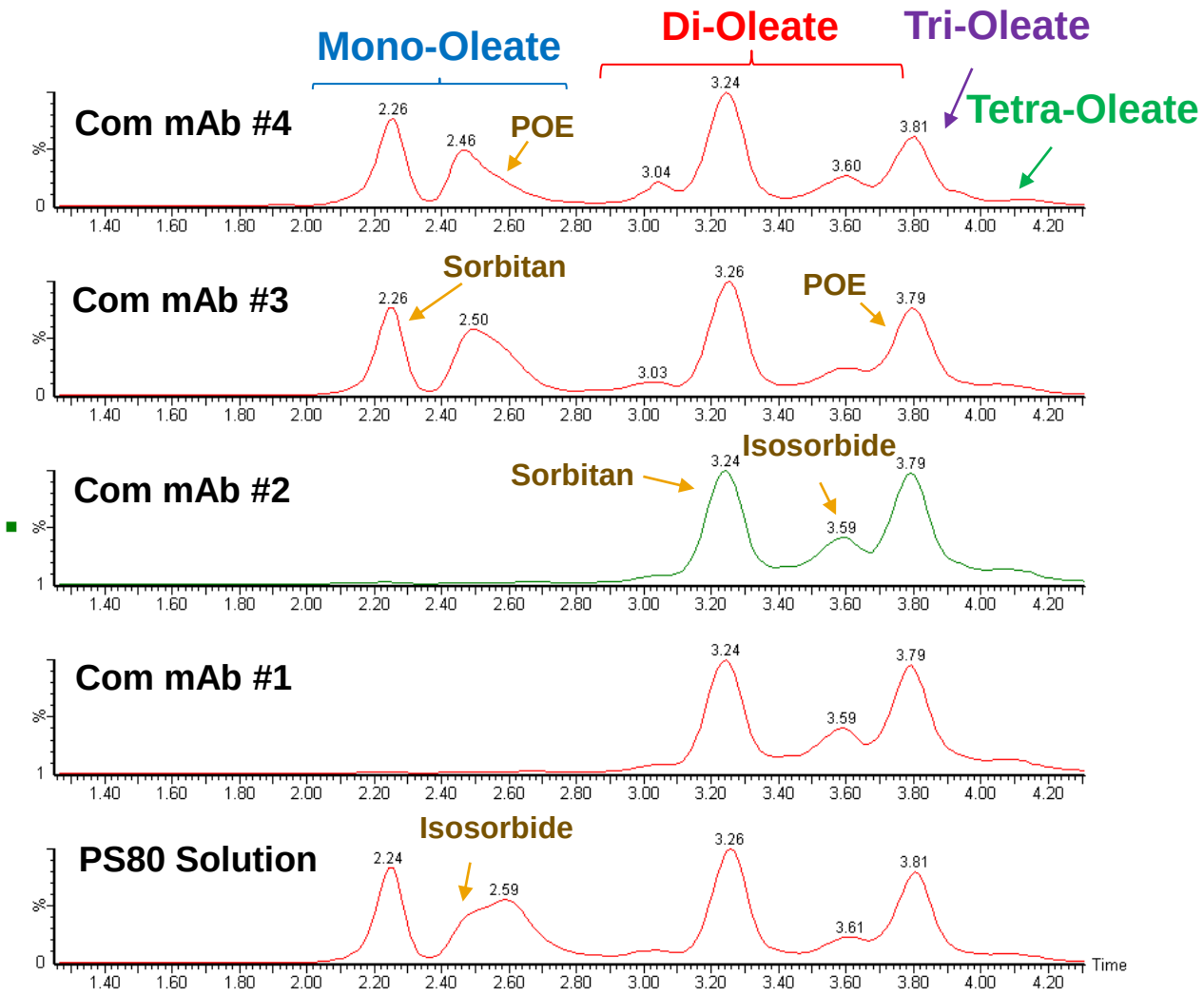
The tryptic digests of 5 mg/mL mAb spiked with lipases/esterase with 2.5 or 7.5 μL/injection.



LOD = 3.3×σ/slope
LOD = 10×σ/slope

Enzyme 1		Enzyme 2		Enzyme 5		Enzyme 6	
LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
0.01	0.03	0.02	0.06	0.01	0.02	0.02	0.07

Polysorbate Stability in Commercial mAbs



Commercial mAb	Polysorbate		HCP ppm
	Type	Stability	
1	PS80	No	5
2	PS80	No	< 1
3	PS80	Yes	> 500
4	PS80	Yes	< 1
5	PS80	No	27
6	PS80	Yes	3
7	PS20	Yes	2
8	PS20	Yes	17
9	Not Polysorbate		35

Lipase and Esterase Measurement in Commercial mAbs

Commercial mAb	Polysorbate		HCP ppm	ppm or ng/mg mAb				
	Type	Stability		Enzyme 1	Enzyme 2	Enzyme 3	Enzyme 4	Enzyme 5
1	PS80	No	5	0.03	< LOD	< LOD	< LOD	0.11
2	PS80	No	< 1	0.01	0.02	< LOD	< LOD	0.07
3	PS80	Yes	> 500	< LOD	0.02	< LOD	< LOD	< LOD
4	PS80	Yes	< 1	< LOD	< LOD	< LOD	< LOD	< LOD
5	PS80	No	27	0.02	0.19	< LOD	< LOD	< LOD
6	PS80	Yes	3	< LOD	< LOD	< LOD	< LOD	< LOD
7	PS20	Yes	2	< LOD	< LOD	< LOD	< LOD	< LOD
8	PS20	Yes	17	< LOD	< LOD	< LOD	< LOD	< LOD
9	Not Polysorbate		35	< LOD	1.6	< LOD	< LOD	33

Is it possible to predict mAb formulation stability?

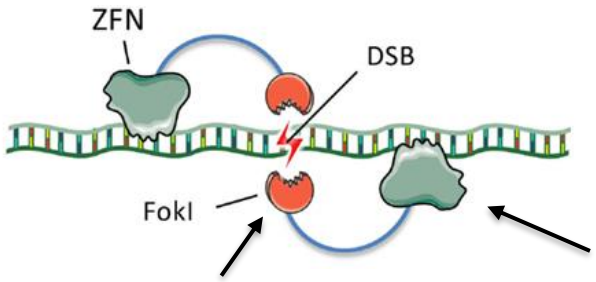
Commercial mAb

- No detection for enzymes 1, 3, 4, 5
  - No degradation of PS20 or PS80

Lilly development mAb

- No detection for enzymes 1, 3, 4, 5
  - General no degradation of PS20 or PS80
- Detection for ≥ one of enzymes 1, 3, 4, 5
  - Detection of PS20 or PS80 degradation

PoC Experimental Flow Scheme



Cell Line Engineering

Parental Cell Lines

- KO (4 enzymes)
- HCP profiling

Generate CDCLs

- Fc Fusion
- mAb (IgG4)
- Bispecific (heteromAb)

Screening & Confirmation

- Shake-Flask Expression

CDCLs = clonally-derived cell lines

DS Manufacture & Formulation

Scale-up (36 L) – mimic historical process

Purification – mimic historical processes

DP set-up:

- Historical API control
- In-expt. control (w.t. cell line) API
- Engineered cell line API

Characterization

HCP profiling

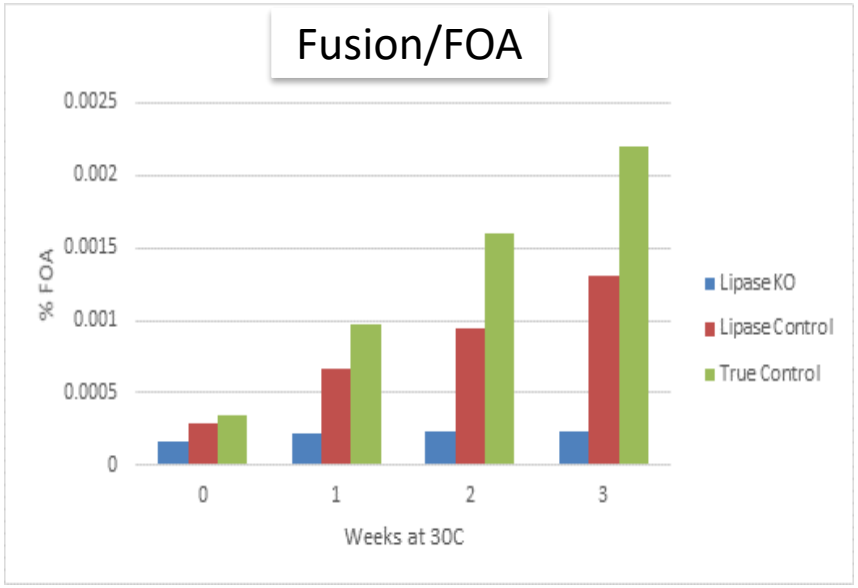
- Cell-Free Medium
- TFF

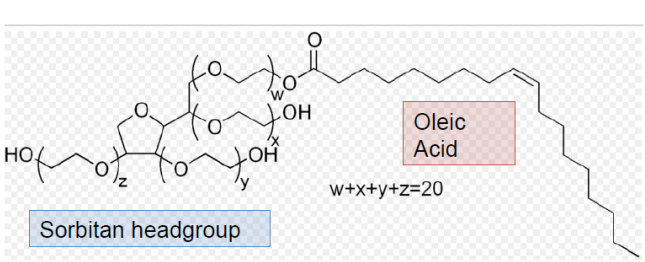
PS-80 analyses

- Cell-Free Medium (Intact)
- DP (Intact and FOA)

Cell Line Engineering Impact on Polysorbate Stability

- Stability samples analyzed for free oleic acid (UPLC-based method)
- PoC Example:





Lipase KO: Engineered cell line API
Lipase control: Non-engineered cell line API (in-experiment control)
True control: Historical API (Historical Production Cell Line)

- **<u>PoC Data</u>:**
  - No polysorbate hydrolysis at 30 ºC at 8 weeks for fusion molecule
  - No hydrolysis observed at 25 ºC at 6 months for bispecific (heteromAb)
  - 7-fold reduction in hydrolysis for IgG4 antibody
- **<u>Platform Data</u>:**
  - <10% hydrolysis observed for > 10 programs since implementation of engineered cell line

Engineered Cell Line Performance

- Engineered cell line size is similar to WT parental cell line
- Engineered cell line doubling time is shorter than parental cell line
- Broader HCP profiles not significantly different between engineered and WT parental cell line
- Engineering did not negatively impact productivity of selected bulk cultures
- Highly-Productive CDCLs for Fc fusion protein (>7 g/L/14d) and mAb (>10 g/L/14d)
- Comparable product quality profiles
- Performance demonstrated on IgG1, IgG4, bispecifics, Fc fusions and non-mAb scaffold therapeutic proteins

Summary

- A simple and powerful methodology for HCP monitoring has been developed with a novel sample preparation.
- Methodology is sensitive and robust.
- The novel sample preparation makes possible detection of very low (< 0.1 ppm) level residual HCPs with PRM.
- Extremely low level of lipases or/and esterase was detected in commercial mAbs with corresponding PS80 instability.
- It is generally possible to predict polysorbate stability in mAb formulation based on lipase and esterase detection.
- Polysorbate is generally stability in molecules from the engineered cell line (Lipase KO)

Acknowledgements

HCP Characterization

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Vince Corvari

Andrew Werner

Kevin Duffy

Richard Irvin

Members of Protein Characterization Team