Identification and Quantification of Polysorbate-degrading Host Cell Protein Impurities in Biopharmaceuticals using Advanced Mass Spectrometry-based Techniques

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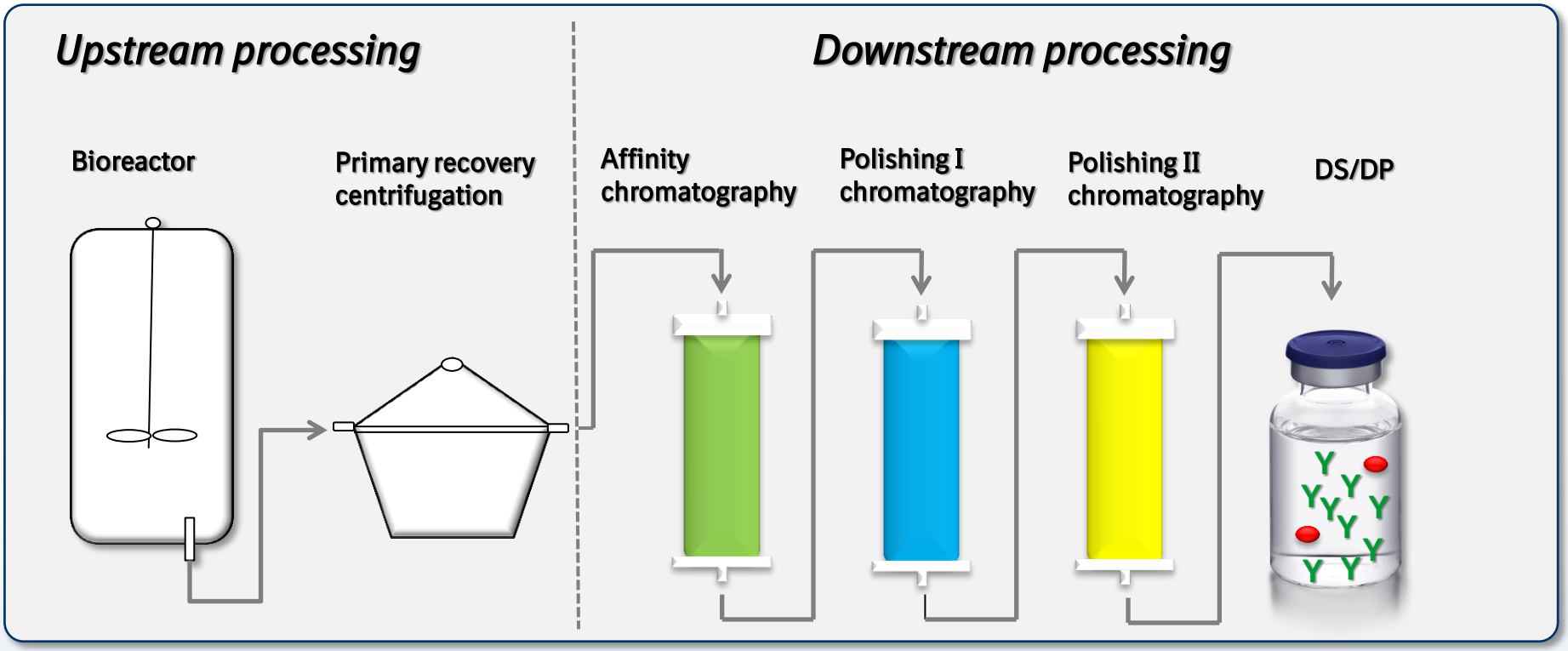
Outline

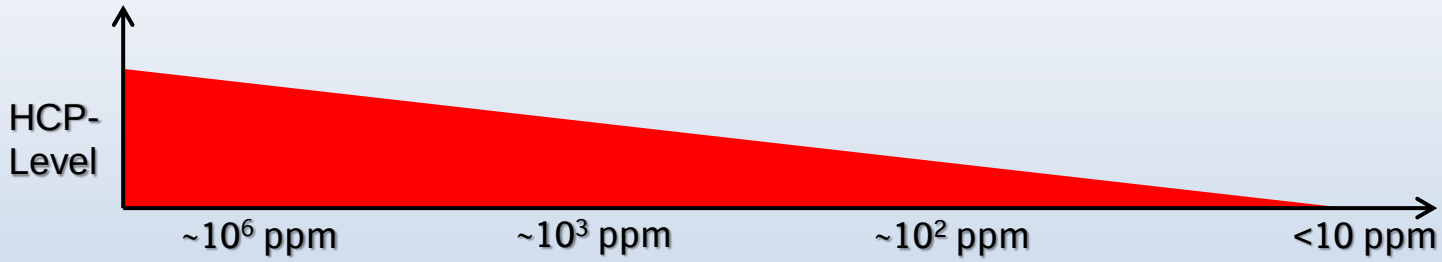
- Introduction to process-related protein impurities
  - ➢ Bioprocess and host cell proteins (HCPs)
  - ➢ Polysorbate-degradation through lipolytic enzyme activity

- Advanced sample preparation strategies for sensitive identification and quantification of HCPs in bulk drug substance via LC-MS
  - I. Quantification of target lipases in polysorbate-free drug substance
  - II. Hexapeptide-based HCP-enrichment and isotope dilution mass spectrometry for relative quantification of lipases
  - III. Establishment of an activity-based probes (ABP) proteomic workflow for lipase profiling

- Summary

Process-related Protein Impurities





Walker et al., 'A modular and adaptive mass spectrometry-based platform for support of bioprocess development toward optimal host cell protein clearance,, *mAbs* Volume 9, 2017

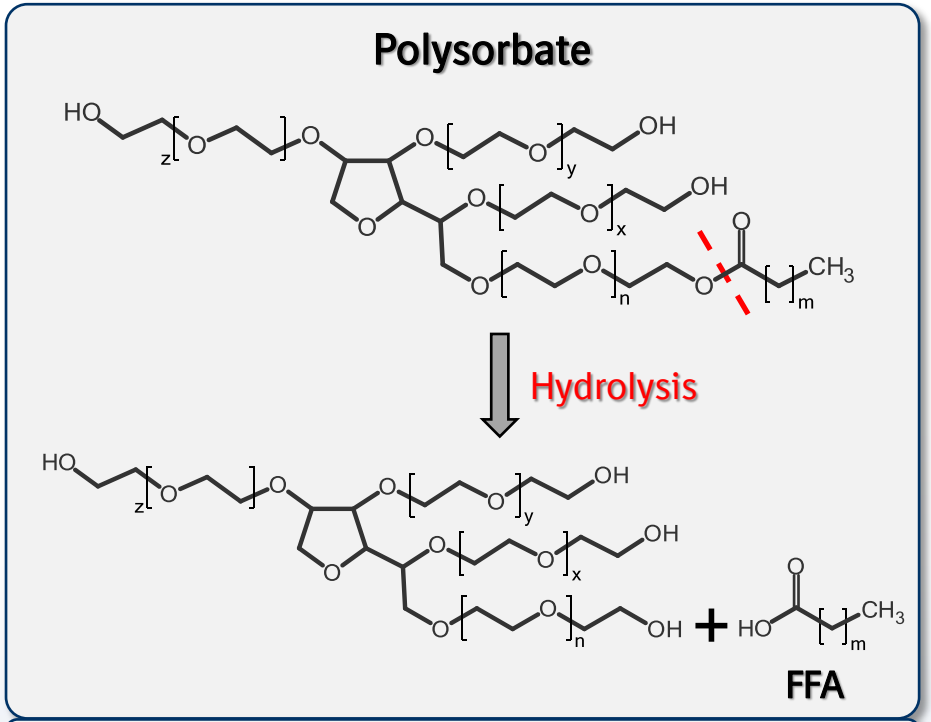
Impact of Process-related Protein Impurities

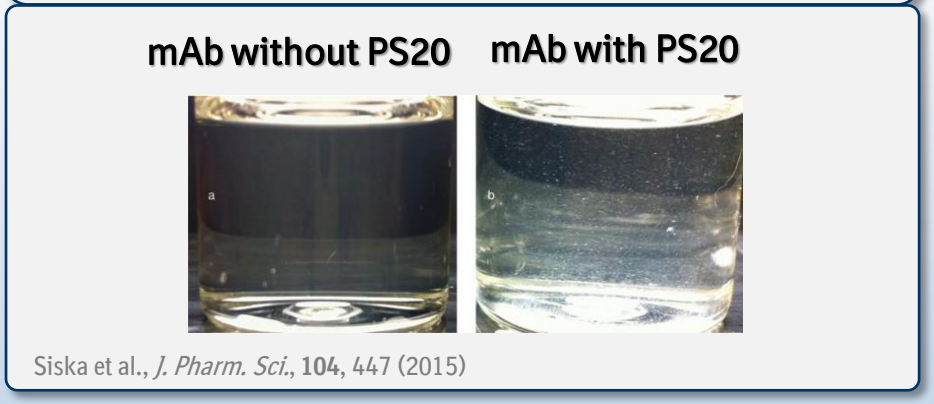
Potential to impact product quality, efficacy and patient safety

- May potentially induce immune response in patients
- Impact of API potency by blockage of mAb paratope
- Undesired API variants due to presence of catalytic activity of proteases and disulfide reductases
- Polysorbate degradation by lipolytic enzymes

Impact of Process-related Protein Impurities on Excipients Stability

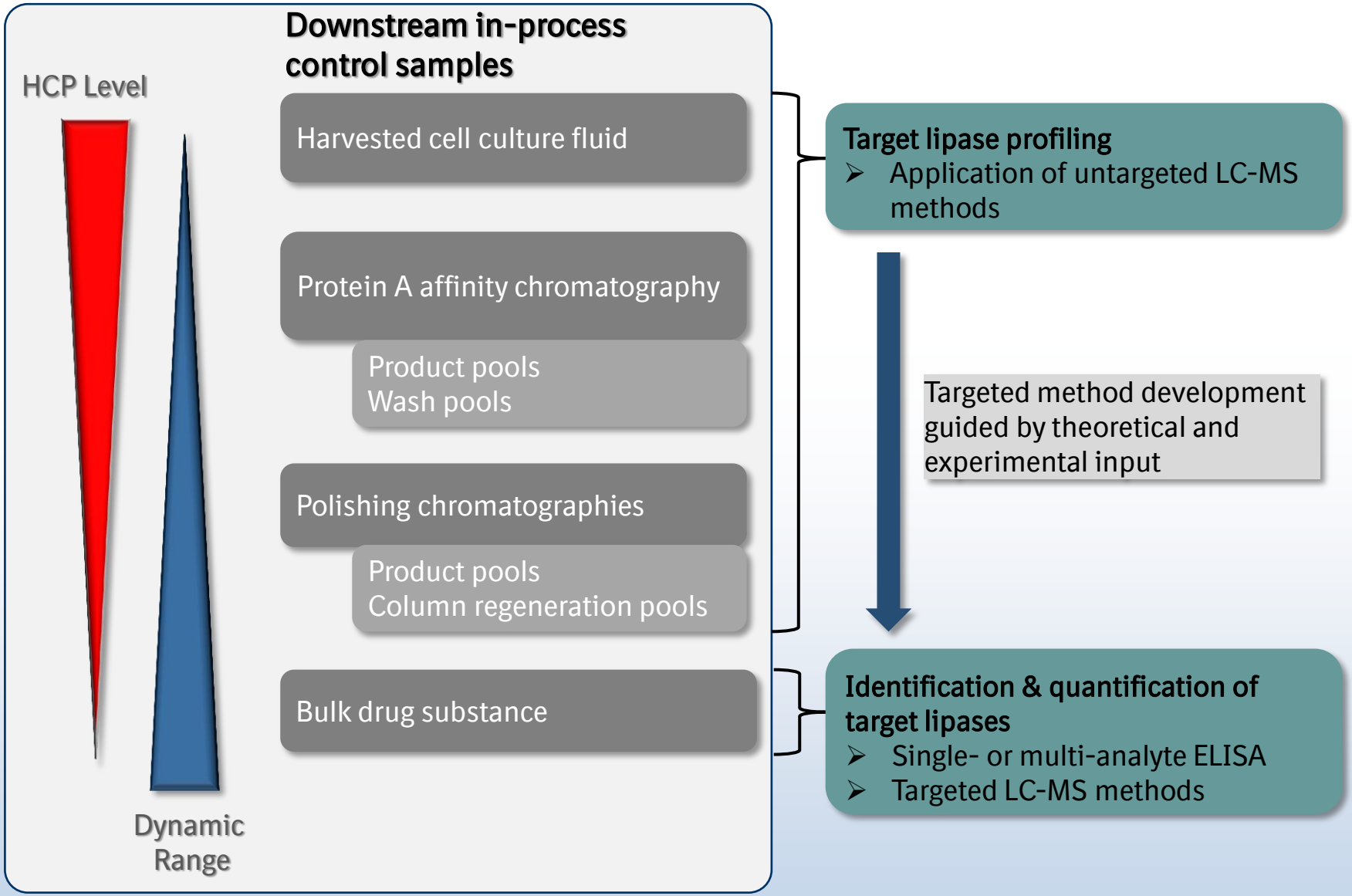
- Polysorbates commonly used to improve stability of API
- Present in majority of biopharmaceuticals
- Prone to degradation by hydrolysis and auto-oxidation
- Susceptible to enzymatic cleavage of ester bond because of structural similarity to triglycerides





Siska et al., *J. Pharm. Sci.*, **104**, 447 (2015)

Strategy for Identification and Quantification of Process-related Protein Impurities



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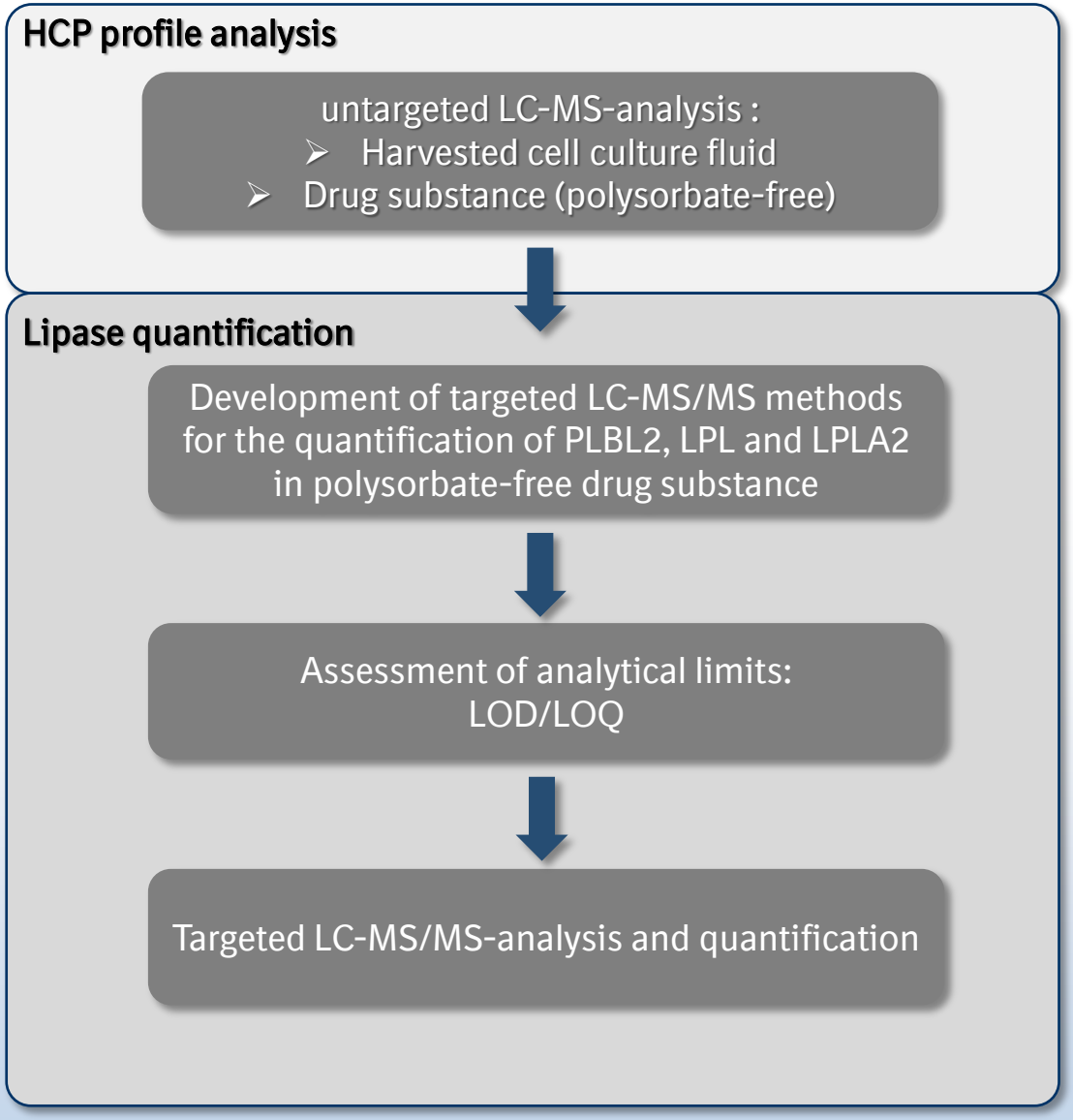
Quantification of Lipases in Polysorbate-free Drug Substance



LOD and LOQ Assessment for LPL, PLBL2, and LPLA2

Sample preparation:

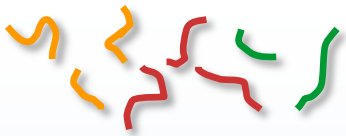
- **recombinant PLBL2**, **LPLA2** and **LPL** proteins were spiked into polysorbate-free drug substance at 0, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 ppm
- **Native digestion*** and removal of incompletely digested mAb by heating and centrifugation

LC-MS instrumental setup and data processing

- Waters Acquity Arc HPLC (**85 min gradient-run**) coupled to Thermo Q Exactive Plus
- Parallel reaction monitoring data analysis software: **Skyline 4.1**

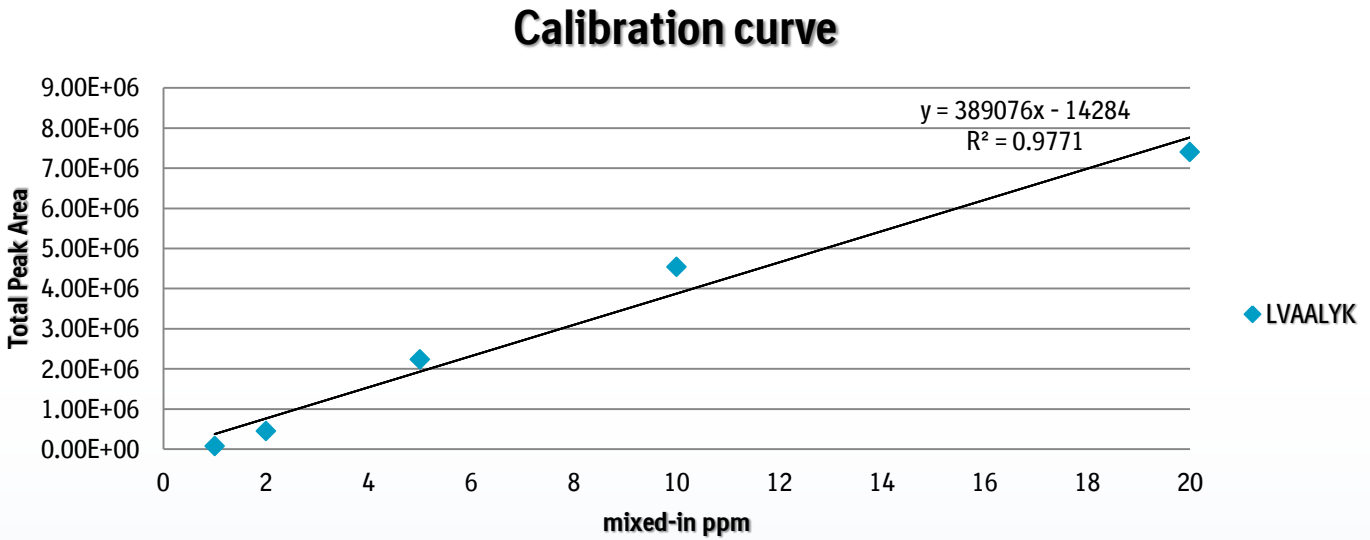
Spiking of recombinant lipases

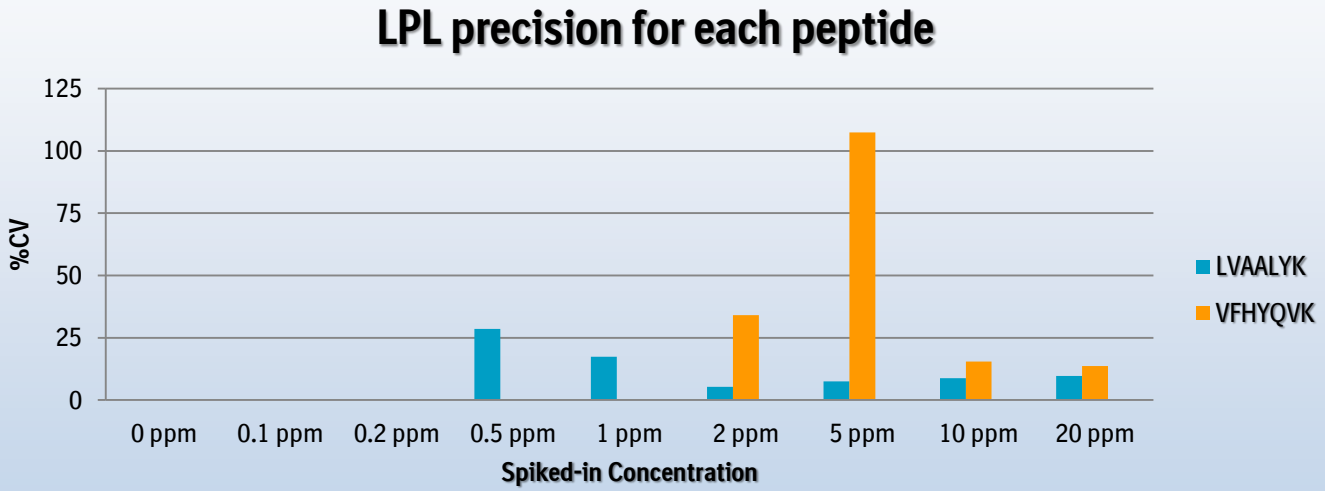
Peptide mixture



*Huang, et. Al. Anal. Chem., 2017, 89 (10), pp 5436-5444

Limit of Quantification Assessment for LPL





Limit of Quantification Assessment for LPL

LPL can be confidently detected at 5 ppm spiking level.

The recovery was determined by comparing the theoretical spiking level and the readout from the standard curve by peptide **LVAALYK.** The LOQ for LPL is determined as 10 ppm.

	Readout from standard curve (ppm)								
LVAALYK	-	-	-	-	0.2	1.2	5.8	11.7	19.1
Spiked-in value	0.0	0.1	0.2	0.5	1.0	2.0	5.0	10.0	20.0
Spiked-in recovery [%]	-	-	-	-	23.1	60.3	115.7	117.1	95.3

Limit of Detection and Limit of Quantification for PLBL2, LPL and LPLA2

Lipase	Limit of detection	Limit of quantification
PLBL2*	<2 ppm	<2 ppm
LPLA2	1 ppm	1 ppm
LPL	5 ppm	10 ppm

*PLBL2 exists in the respective drug substance.

- LOD determination criteria:
  The MS^2 detectible level

- LOQ determination criteria:
  Spiked-in recovery= 75-125%, and precision CV% for each peptide measurement ≤25%

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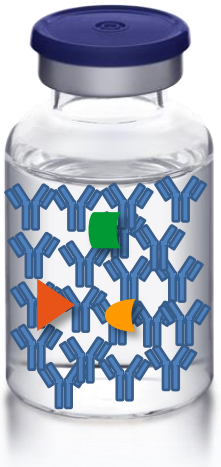
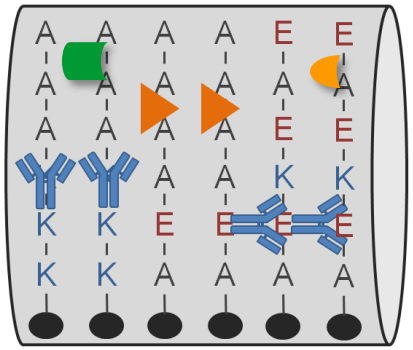
Mechanism of HCP Enrichment by ProteoMiner™ Kit

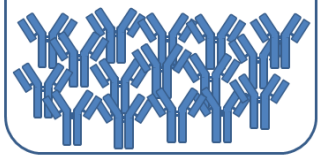


Load



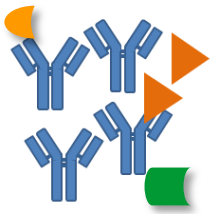
Combinatorial hexapeptide library – highly complex and a binding partner should exist for every protein

Flow-through



Elution of HCPs with MS-compatible solvents

Eluate



LC-MS analysis

- **Majority of highly abundant API is depleted while low abundant HCPs are retained**

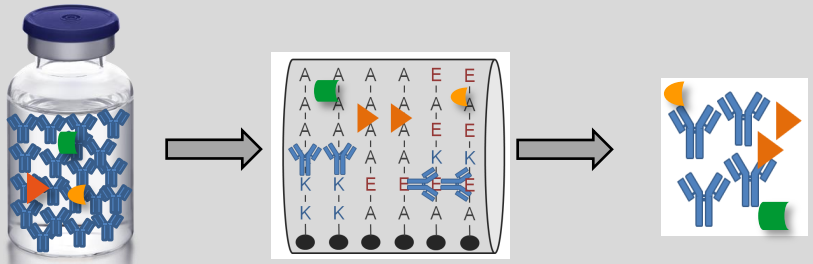
Relative Quantification of Lipase Level in Drug Substance via Isotope Dilution Mass Spectrometry

Investigation of HCP profile

Untargeted LC-MS-analysis of harvested cell culture fluid

Targeted LC-MS/MS-analysis

Hexapeptide-based enrichment of HCPs from bulk drug substance



Tryptic digestion and spiking of stable isotope labeled synthetic (SIS)-peptides

LC-MS/MS-analysis

Relative quantification of lipases

Relative Quantification of Lipase Level in Drug Substance via Isotope Dilution Mass Spectrometry

Bulk drug substance batch	**I**	**II**
Lipase I level	100%	98%
Lipase II level	100%	467%
Observation of PS20 degradation	-	+

→ **Relative quantification of lipase levels in BDS indicates Lipase II as the potential lipolytic enzyme causing PS20-degradation**

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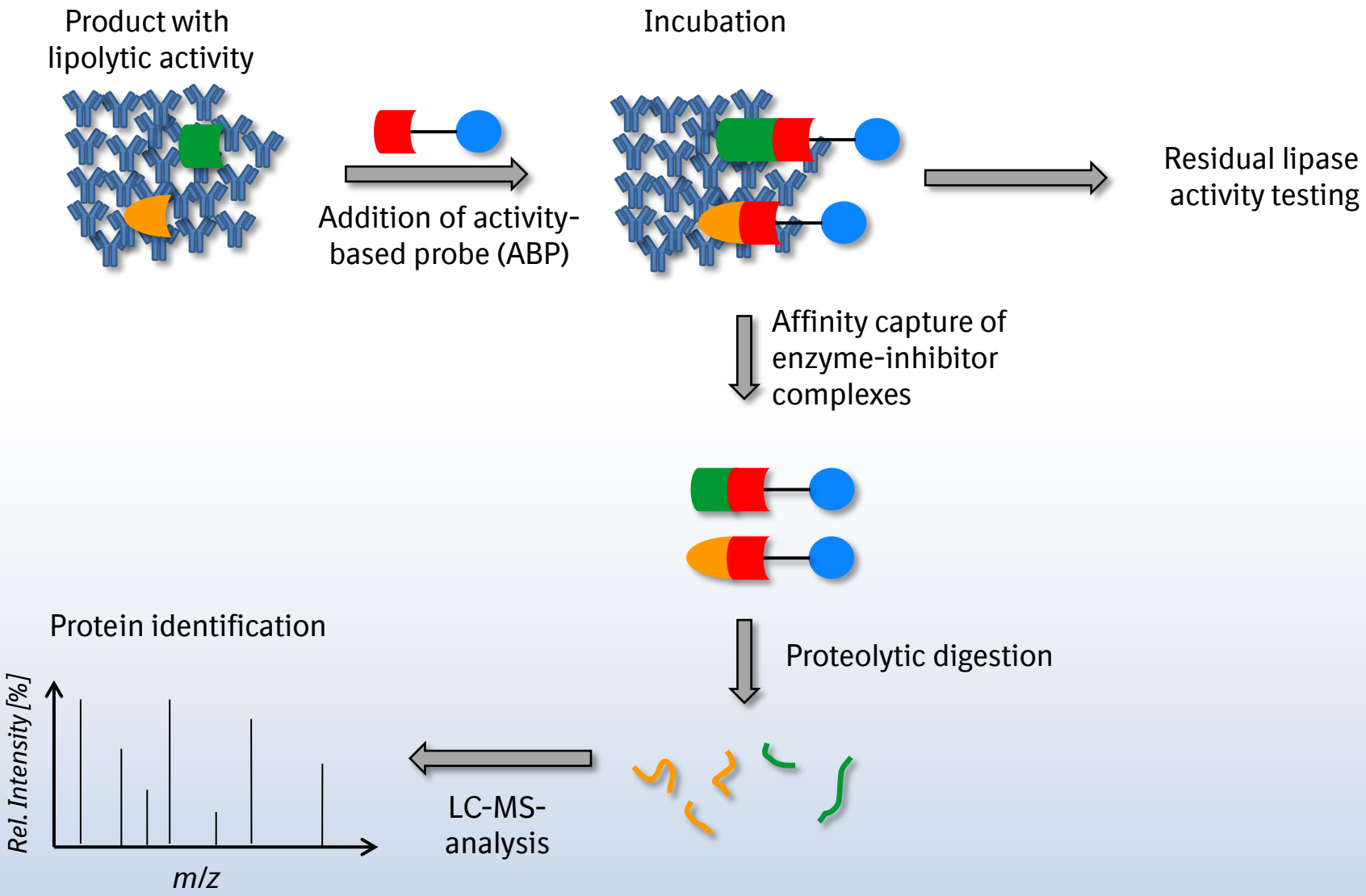
Activity-based Probes for Profiling lipolytic Enzymes



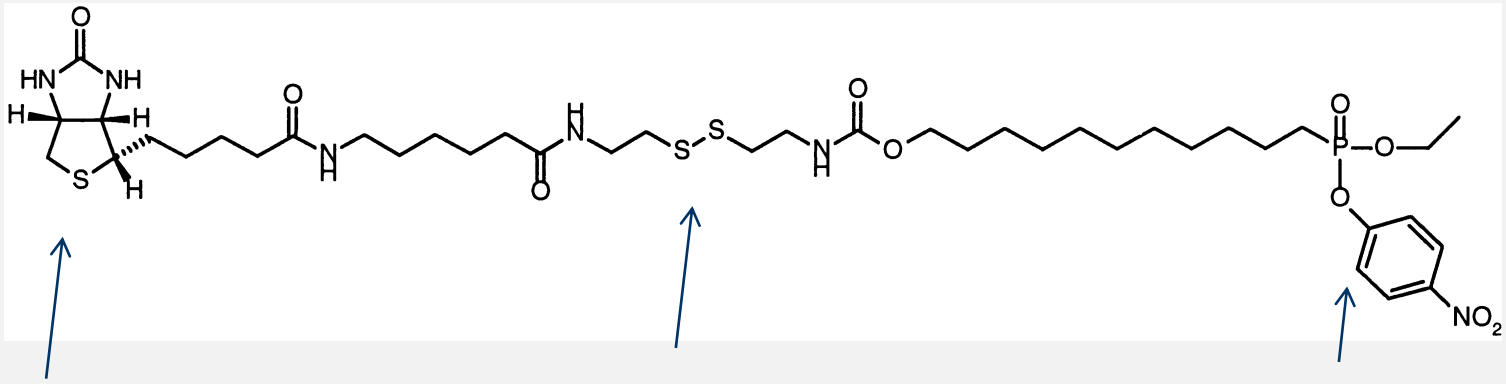
Activity-based Probes for Profiling lipolytic Enzymes

A Novel Biotinylated Suicide Inhibitor for Directed Molecular Evolution of Lipolytic Enzymes

H.-J. Deussen,* S. Danielsen, J. Breinholt and T. V. Borchert

Protein Discovery, Novo Nordisk A/S, Novo Allé, 2880 Bagsværd, Denmark

Received 7 July 1999; accepted 11 October 1999



Biotin - affinity tag

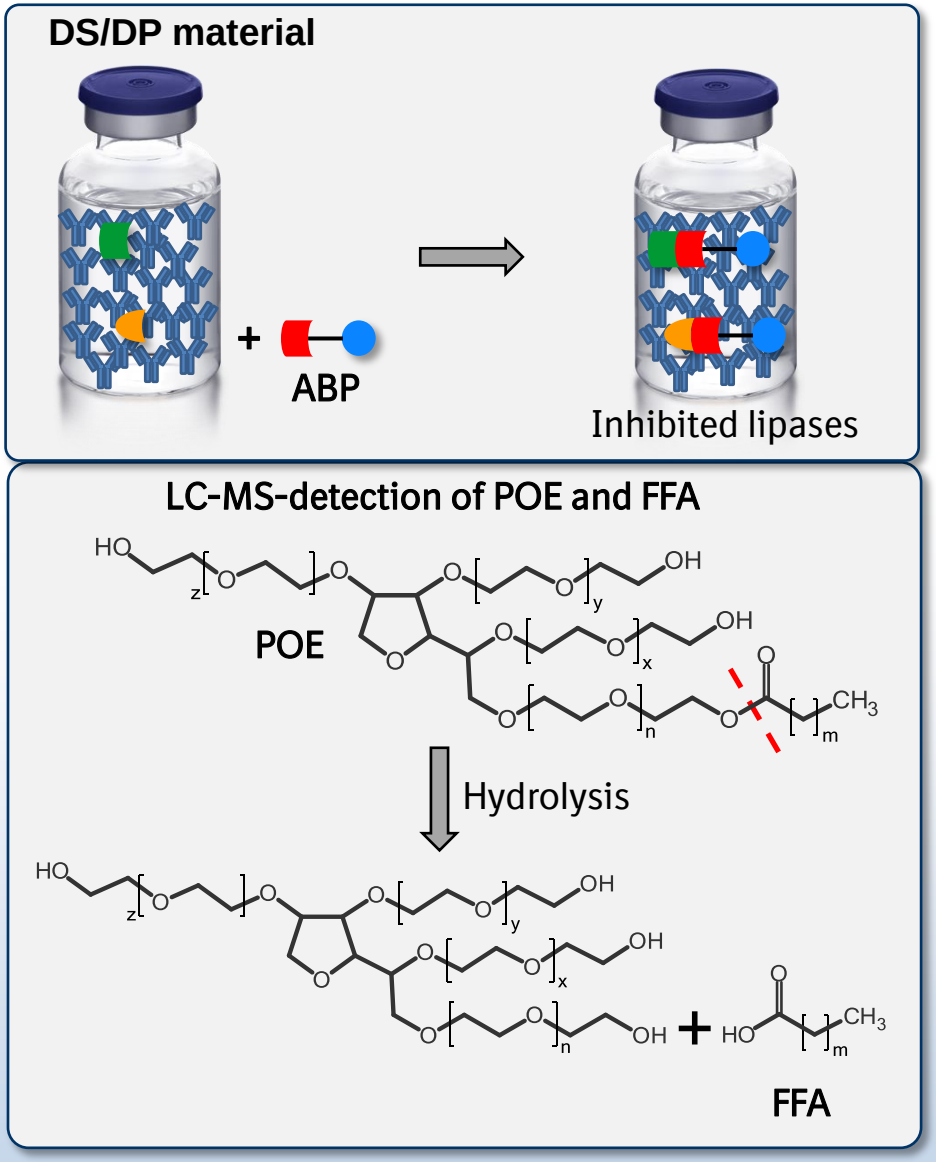
Reductive cleavage site

4-nitrophenyl activated phosphonate

Bioorg Med Chem. 2000 Mar;8(3):507-13.

Screening for effective Lipase-Inhibitors

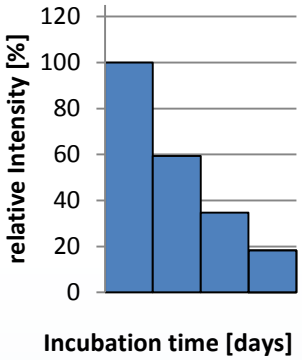
- PS20 stability testing in DS/DP material in presence of lipase-inhibitor
- Incubation for several days at 25°C
- LC-MS-monitoring of intact Polysorbate-esters
  - e. g. isosorbide C12
- LC-MS-monitoring of free fatty acids
  - e. g. lauric acid



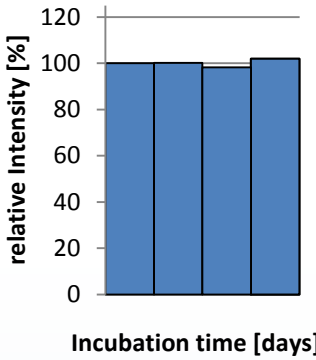
Activity-based Probes for Profiling lipolytic Enzymes

LC-MS-analysis of POE content

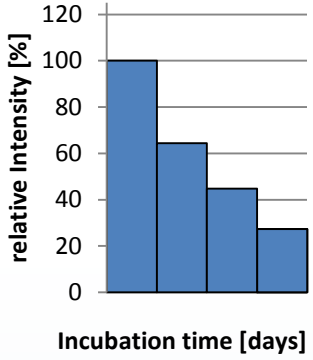
Isosorbide C12 Drug Substance



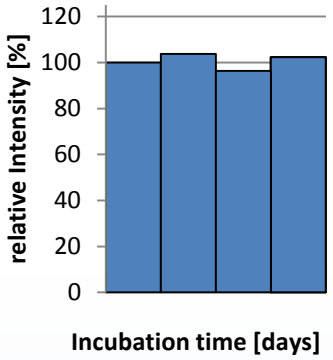
Isosorbide C12 Placebo



Isosorbide C12 Inhibitor I

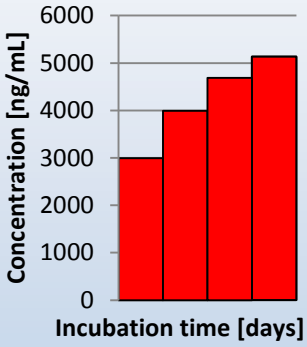


Isosorbide C12 Inhibitor II

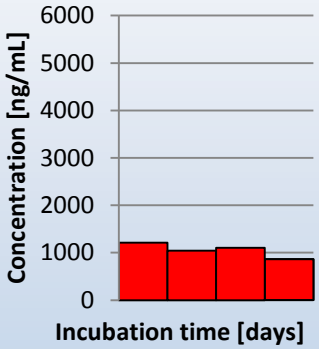


LC-MS-analysis of FFA content

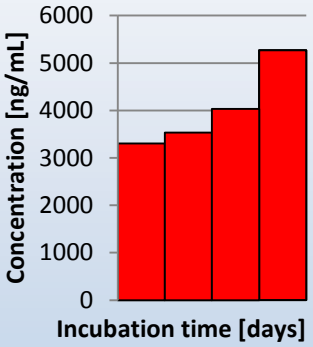
Lauric Acid Drug Substance



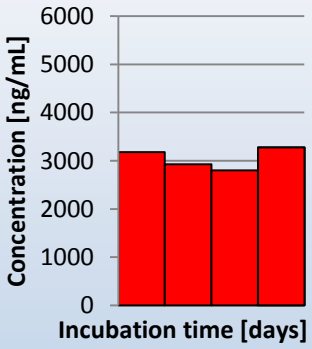
Lauric Acid Placebo



Lauric Acid Inhibitor I



Lauric Acid Inhibitor II



Summary

- Strategy for the identification & quantifiifcation of polysorbate-degrading enzymes in biopharmaceutical products

- Native digestion and LC-MS-analysis of polysorbate-free drug substance allows quantification of LPL, PLBL2 and LPLA2 $\leq$ 10 ppm

- Hexapeptide-based HCP-enrichment and isotope-dilution mass spectrometry are advantageous for relative lipase quantification in bulk drug substance material

- Establishment of activity-based probes for profiling of lipolytically active enzymes

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Thank you for your Attention

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DEV ADB & RES MedChem, Biberach

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