Case Examples of CMC Surprises

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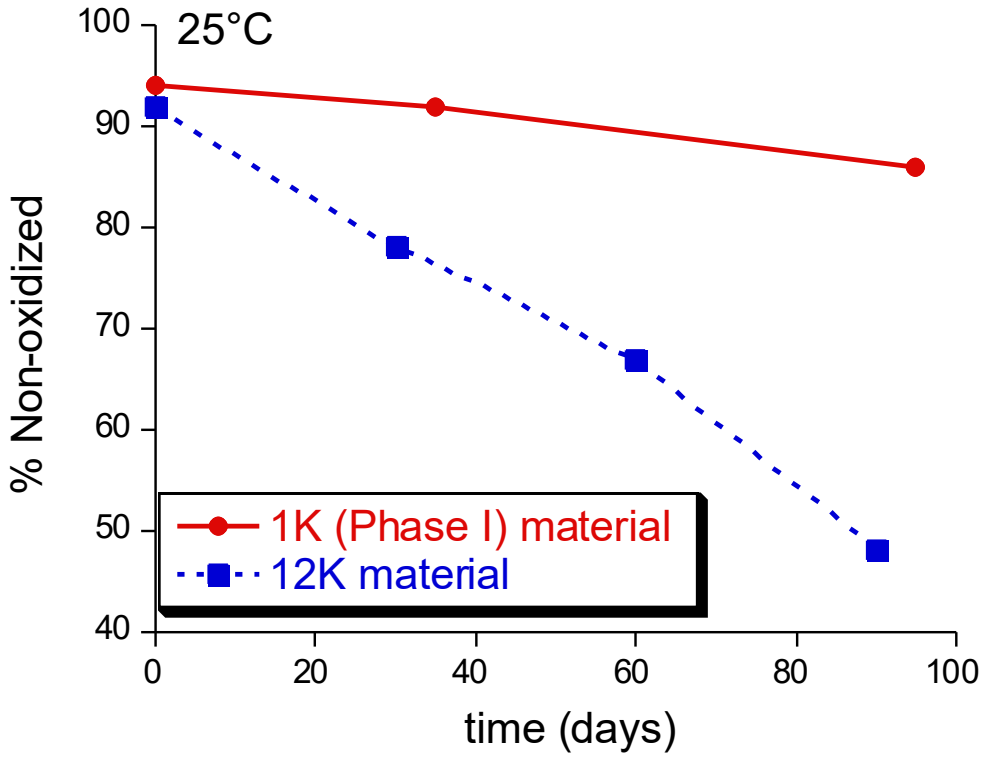
Case 1 –Formulation and Manufacturing Process Co-dependency

- Incidence: A stable liquid formulation throughout Phase I clinical phase showed significant oxidation upon process scale up for Phase III.
- Triggered GMP Investigation
- Findings:
  - Phase I material (pH 5.0, isotonic NaCl in glass containers) stable for 18 months at 2-8C
  - Minimal change in manufacturing process (scale up from 1K to 10K)
  - No changes in excipient raw materials
  - Material made by 10K and 1K processes in pilot plant showed similar degradation profile and kinetics

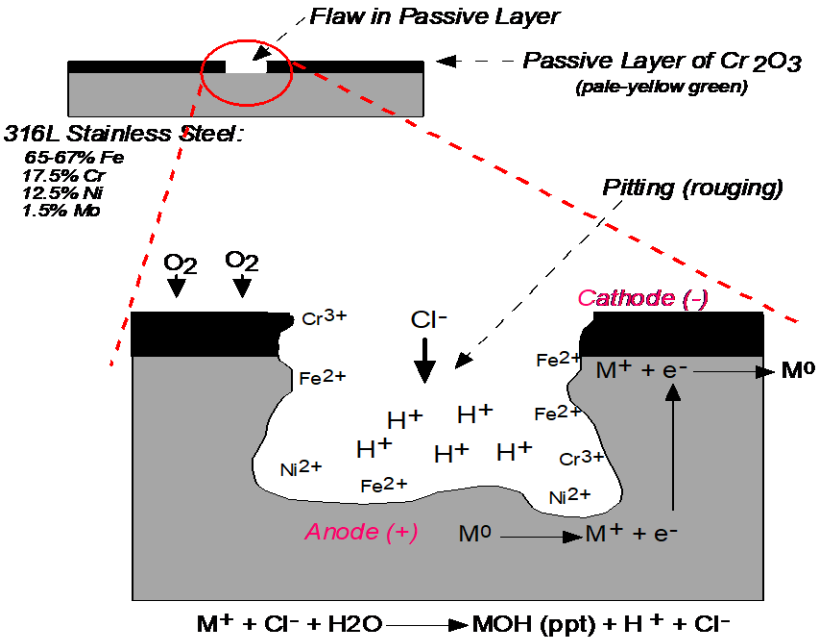
Case 1 –Formulation and Manufacturing Process Co-dependency

- Findings ctd.
  - 10K material (made in GMP manufacturing site) had faster degradation kinetics



Case 1 –Formulation and Manufacturing Process Co-dependency

- Root Cause: Scaled up material stored in large (old) stainless steel tanks for longer periods, leading to corrosion (even buffers stored in the tanks caused oxidation of 1K material)
- Corrective Action: Change formulation to exclude chloride in low pH (pH had to be kept low for this protein's stability)

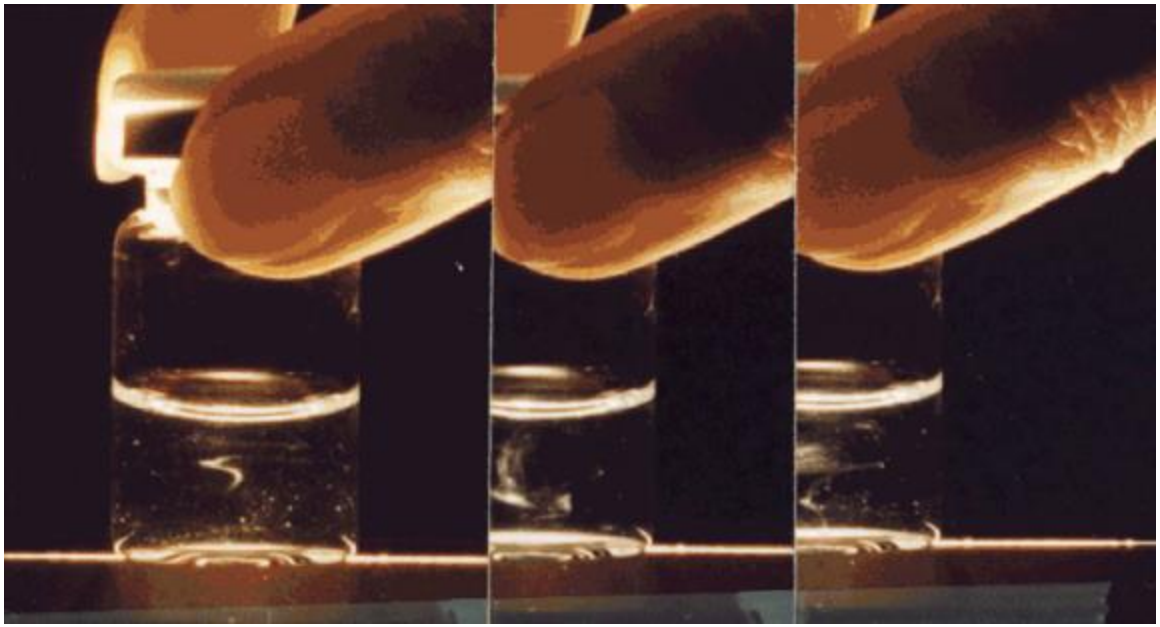


Acid and chloride formulations corrode stainless steel: In flawed steel regions (e.g. welds), the exposed raw metal is dissolved by acid, and in the presence of chloride reacts further to metal hydroxide. Inside the corrosion front (pit) becomes anodic and the surface becomes cathodic, further perpetuating the reaction.
The microenvironment inside the pit has high metal ion and acid content that degrades proteins.

- *$1.5 mil product trashed*
- *6 months delay in the program investigating and finding a solution, which required comparability studies and refiling*

Case 2 –Beware of your process and formulation excipients

- Incidence: Fine (micron-sized) particles (tornado-like) noted visually in Quality Control unit (as part of routine Appearance monitoring) in a clinical product lot placed on stability after 6 months of storage at 2-8C (failed subvisible particle specs)
- Triggered GMP Investigation



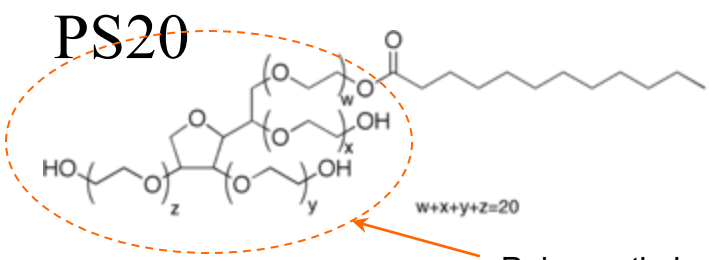
Case 2 –Particulates: Excipient Dependency

- Findings:
  - Not attributed to bacterial contamination
  - No loss in protein concentration in supernatant
  - No change in product by SEC, RP, SDS-PAGE, Potency assays
  - Similar observation in another lot at 9 months (not an isolated case) – but not observed in earlier clinical lots (different process)
  - Particles collected on filters and analyzed by GC-MS →were fatty acids
  - Particles not observed in unformulated Drug Substance (no polysorbate)
- Root Cause: Polysorbate hydrolysis released fatty acids which have low aqueous solubility
- Corrective Action: in filter (0.2 μ) prior to IV administration
  - Consider alternative nonionic surfactant without fatty acid ester (e.g. poloxamer)

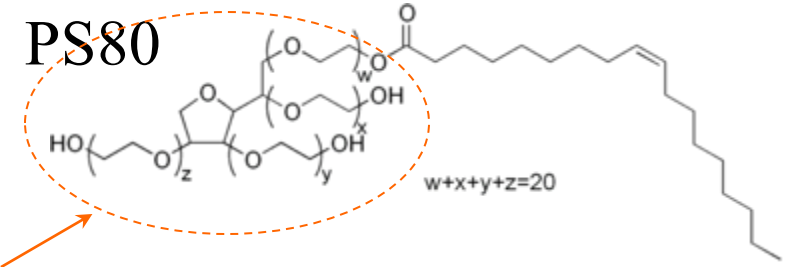
Case 2 –HCPs and Polysorbate-Derived Particulates

- Years later……several publications reported residual host cell lipases and esterases in bulk DS (HCPs) that result in hydrolysis of polysorbates
  - Rate of hydrolysis dependent on the level of HCP, type of HCP, the acyl chain of the polysorbate, the purity of polysorbate, temperature and pH

PS20



PS80



Polyoxyethylene (20) sorbitan

Lauric (C12:0) 40-60%
Palmitic acid (C16:0) 7-15%
Myristic acid (C14:0) 14-25%
Capric acid (10:0) ≤ 10%
Caprylic acid (C10:1) ≤ 10%
Oleic acid (C18:1) ≤ 11%
Stearic acid (C18:0) ≤ 11%
Linoleic (C18:2, omega 3) ⩽3%
Caproic acid (C10:0) ≤ 1%

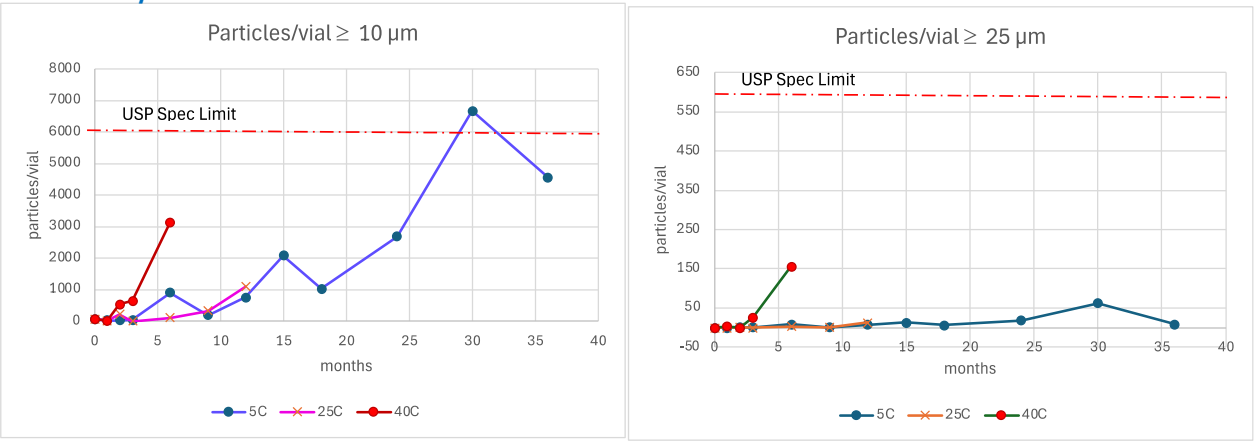
Oleic (C18:1) ⩾ 58%
Linoleic (C18:2, omega 3) ⩽ 18%
Palmitic (C16:0) ⩽ 16%
Palmitoleic acid (C16:1) ⩽8.0%
Myristic acid (C14:0) ⩽5.0%
Stearic acid (C18:0) ⩽6.0%,
a-Linolenic acid (omega-6) ⩽4.0%

Oxidation products of oleic acid

- Esterases hydrolyze shorter chain fatty acids (C12, C14) more
- Shorter chain fatty acids have lower solubility, hence more particulates are seen with PS20 than PS80, and among different sources of PS20 or PS80, the more homogeneous P20 (all monolaureate) and PS80 (>98% oleic acid) gives less particulates.

Case 2 –PS Hydrolysis Induced Particulates May Take Years to Detect

- The previous case was detected in 6 months, below example failed subvisible particle spec at 30 mo/2-8C



- Generic HCP ELISA kits cannot tell the presence of such enzymes
  - Low ppm HCP level does not mean you won't have particulate problem later in refrigerated products containing polysorbates
- Accelerated stability not always predictive
  - By the time PS-stability indicating assay shows hydrolysis it may be late to change the process
- For a refrigerated formulation, best method for early detection/**PREVENTION** of such enzymes is mass spec identification of HCPs during process development combined with stress studies using stability indicating polysorbate assays

Case 3: When Compendial Assay Didn't Predict Pyrogenicity in Humans

- A small company developed an enzyme for treatment of acute cardiovascular indications
- Phase I process development and manufacturing conducted at a clinical stage CDMO
- Shortly after IND submission, a subset of patients developed mild pyrogenic response (fever)
  - Program placed on hold
- Investigation by CDMO did not reveal any obvious root cause
  - Endotoxin was tested by Chromogenic kinetic LAL assay (Charles River Endosafe-PTS) < 0.15 EU/mg (below detection limit)

why didn't this assay detect pyrogenicity

Case 3. Deep Dive into the situation

- *E. coli* produced enzyme
- Inclusion body (IB) requiring refolding and centrifugal separation
- Three orthogonal chromatography purification steps
- Very limited in-process analytics: enzyme activity and SDS-PAGE (Coomassie)
- Very little process-product understanding
- Final Product Purity tests:
  - by RP-HPLC 98%
  - by SDS-PAGE (Coomassie) ”conforms to RS” (major band at expected MW and 5 minor bands)
  - by SEC 100%

Case 3. Further investigation revealed product positive in rabbit pyrogen test

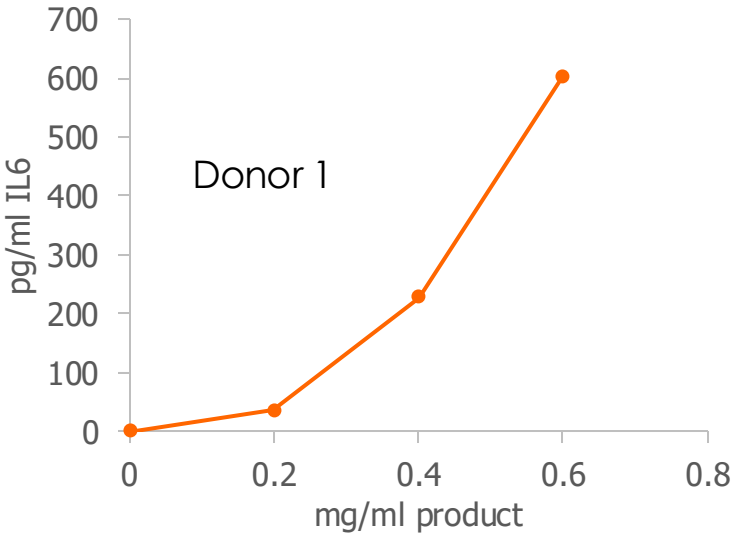
- STC was engaged to develop/use *in vitro* tools for analysis of endotoxin in this product and potentially optimize the process to remove the pyrogen
  - started with simple endotoxin assays and evaluated spike/recovery
  - biological assays of endotoxin response
    - Monocyte Activation Test (MAT): where cytokine release is measured as a result of endotoxin stimulation of monocytes in human blood
    - TLR4 Activation Cell Based Assay: where cytokine release is measured as a result of endotoxin stimulation of engineered cells expressing TLR4.
    - Developed a targeted ELISA assay that ultimately helped with process development /optimization

Case 3. Endotoxin measured by compendial assays at STC

- Product had low response in kinetic chromogenic LAL assay and suppressed CSE signal:
  - 1 EU/ml CSE was suppressed by 4-fold
  - Spike recovery of 1 EU/ml was 24%
- Product had no response in gel clot LAL assay and suppressed CSE signal:
  - 1 EU/ml spiked CSE became undetectable
  - Even 20 EU/ml became undetectable!
- Extensive efforts at Cape Cod Associates could not improve CSE spike/recovery beyond 20% in this product
- Commercial LPS ELISA kit showed no dose response

Product is a great endotoxin removal reagent!!!

Case 3. Endotoxin measured by MAT at STC



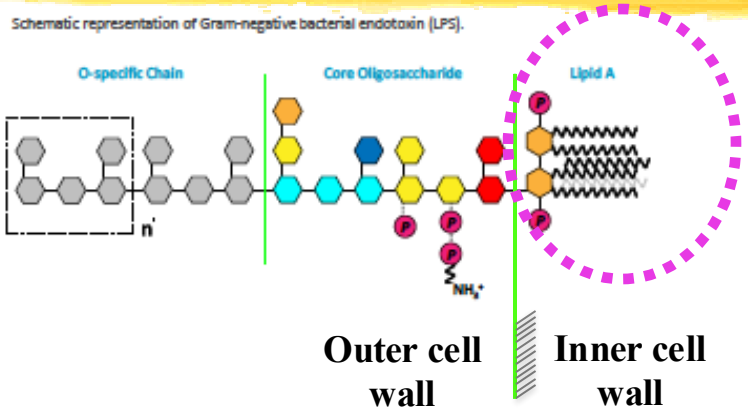
Product was added to human volunteer blood cells in the MAT assay. Supernatant was tested at serial dilutions in binding buffer (PBS/1%BSA) by hIL6 ELISA.

Product dilution	Donor 2	Donor 3	Donor 4
none	>ULD	114	>ULD
2x	>ULD	128	>ULD
4x	1185	162	1131
8x	1399	228	932
16x	1666	21*	1004
32x	2232	~LOQ	

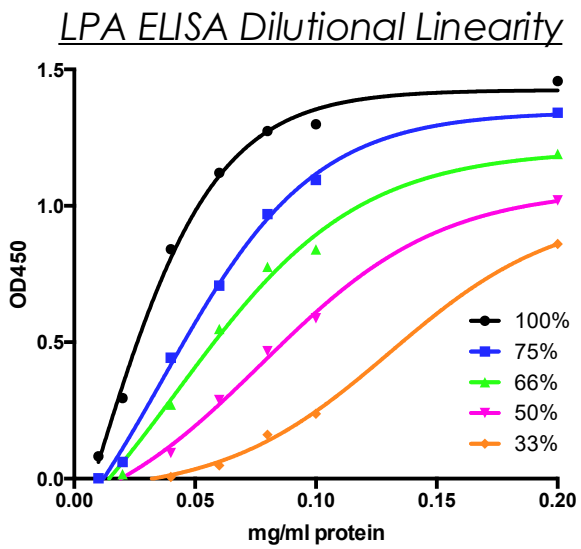
	Product (ave 0.25-1 mg/ml)	1 EU/ml CSE	Product +1 EU/ml CSE
IL6 (pg/ml) (Donor 5)	886	545	2086 (67% spike-recovery)

- Positive response observed for product
  - ~67% recovery of spiked 1EU/ml was observed
- However, no consistent dose response, high donor variability in IL6 response
- The Phase I product showed ≤0.5 EU/ml CSE equivalent in 6 out of 18 donors (33%)

Case 3. STC Developed Direct "LPA ELISA" which enabled process improvement



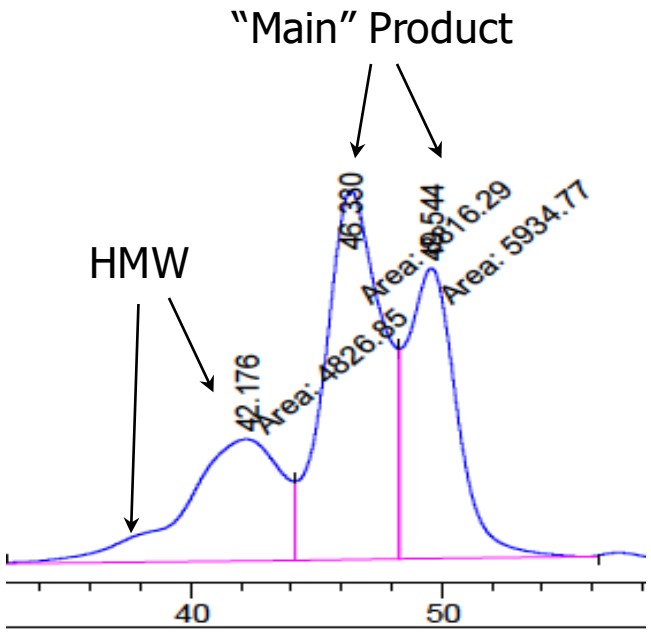
- LPA ELISA: coat product, add anti-LPA IgG
  - High specificity and sensitivity
  - Dilutional linearity
  - Interfering reagents are washed off and do not mask the endotoxin
  - The assay was qualified



%P expected	Interpolated Conc	%P calculated	%Spike Recovery
100%	0.2	NA	
75%	0.100	50%	67%
67%	0.088	44%	66%
50%	0.056	28%	56%
33%	0.040	20%	59%

Case 3. Improved SEC Method Revealed High HMW Levels in Product

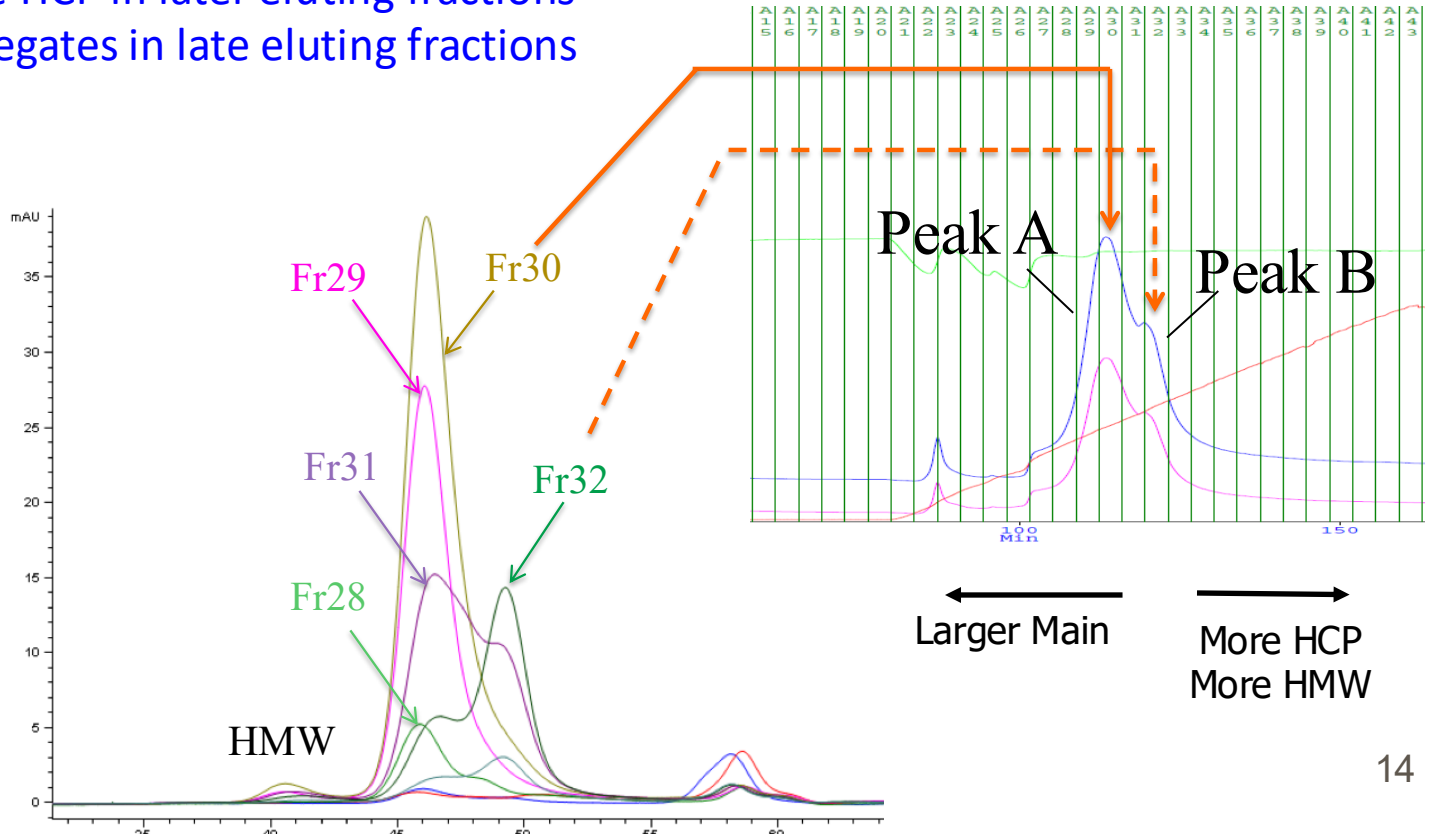
- SEC Profile of Phase I DP reported 100% purity
- Improved SEC method developed at STC showed that product actually had 25% HMW species and the 'main" species was bimodal



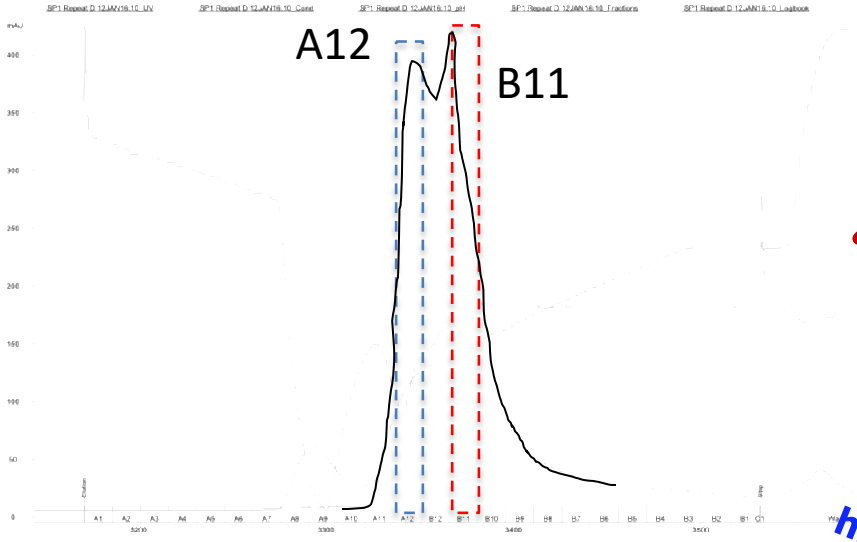
Improved methods enabled product-process understanding and improving product quality

Case 3. CEX Purification Step Characterization Enabled Lowering HMW

- The earlier eluting peak A of the CEX column is the “larger” MW peak on SEC and the later eluting peak B is the “smaller” MW peak.
  - Both have same enzyme activity
  - Both have similar SDS-PAGE purity
  - More HCP in later eluting fractions
  - Aggregates in late eluting fractions



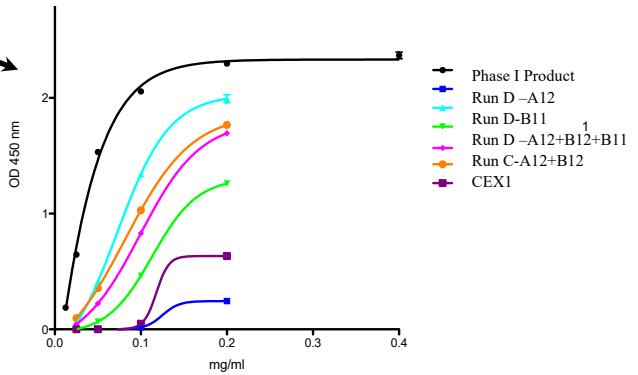
Case 3. Leading CEX Peak Enriched in Endotoxin seen only by LPS ELISA



while LAL assay showed no endotoxin in fractions

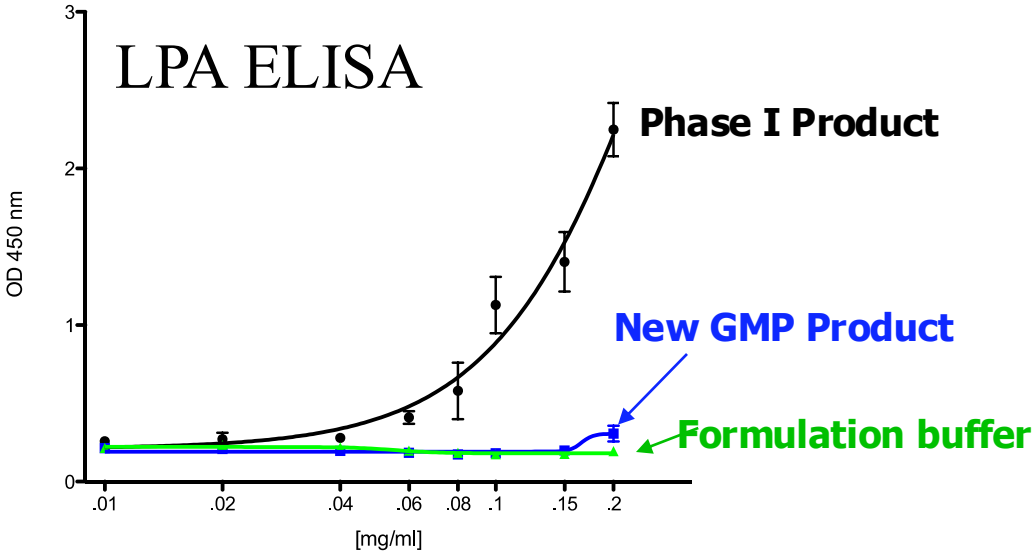
	SP Fractions	interpolated data (EU/ml)	EU/mg
Leading Edge	A85	0.000	
	A90	0.000	
	B3	0.000	
	B7	0.000	
	B11	0.000	
	B15	0.000	
	B19	0.000	
	B23	0.000	
	B27	0.000	
	B30	0.001	
Main Peak	B48	0.009	0.0043
	B54	0.013	0.0061
	B60	0.017	0.0085
Trailing Edge	A(2) 39	0.009	0.0143
	A(2) 42	0.007	0.0119
	A(2) 46	0.004	0.0070
	A(2) 50	0.002	0.0061
	A(2) 54	0.001	
	A(2) 58	0.000	
	breakthough	0.003	0.0108
LOD is ~0.01 EU/ml			

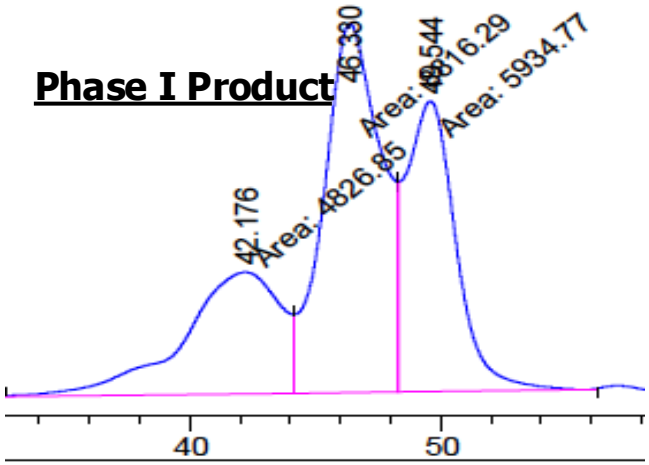
LPA ELISA showed higher LPS in leading shoulder

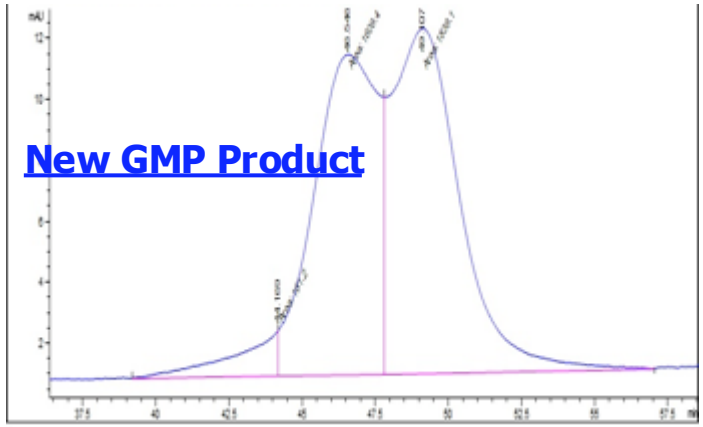


Case 3. Good analytics as the "eyes" to process enabled a 'clean" product that went back to clinic

- A number of changes to upstream and downstream process steps and parameters led to a "clean" product
- Improved process had minimal *in vitro* pyrogen response and <5% aggregates
- Product went back to the clinic after significant cost and time!!!







Acknowledging my industry colleagues and regulatory reviewers who work hard together to make good medicines possible…

Scios-Nova, Genentech, TKT-Shire, STC Biologics and Clients…