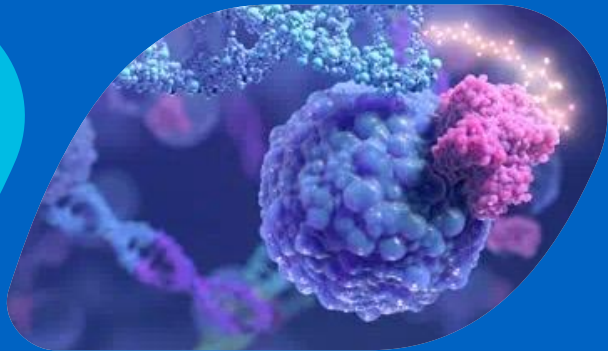


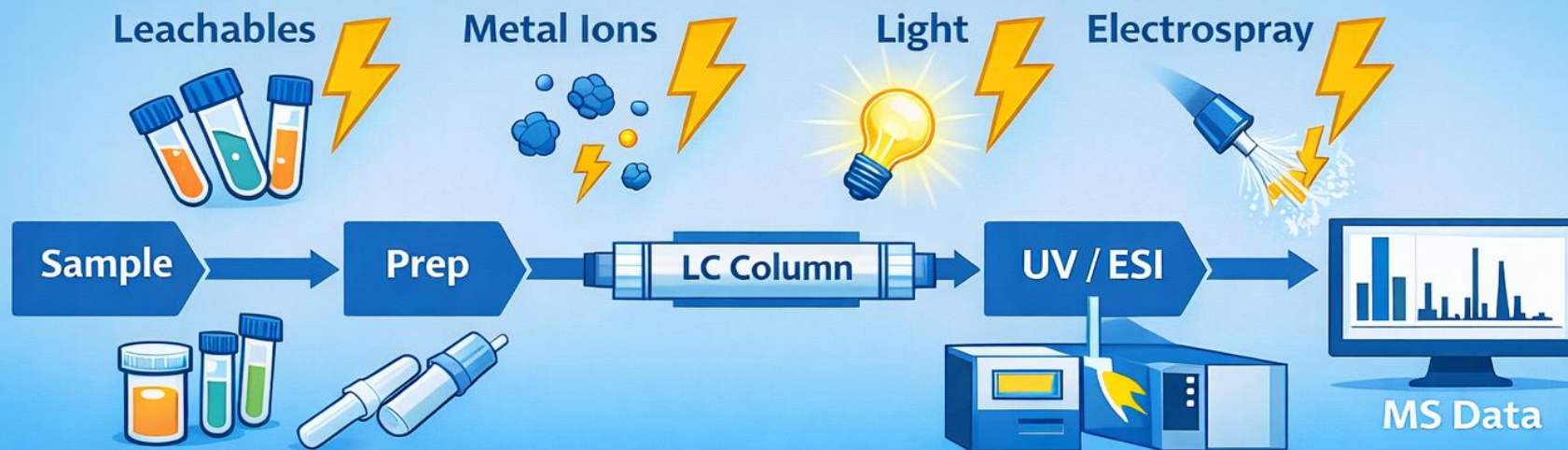


ARTIFICIAL OXIDATION: A MAJOR CHALLENGE FOR MAM IMPLEMENTATION AND LONG-TERM QC TESTING

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CASSS MS 2026

AMGEN

Artificial Oxidation Challenges in MAM



Communication

Artificial Oxidation: A Major Challenge in Implementing Multi-Attribute Methods for Therapeutic Protein Analysis

Yaokai Duan¹, Michael Lanzillotti², Dylan L. Riggs², Albana Nito³, Junnichi Mijares¹, Amanda Helms² , Carl Ly², Kevin Millea², Xingwen Li², Hao Zhang² and Zhongqi Zhang^{1,*} 

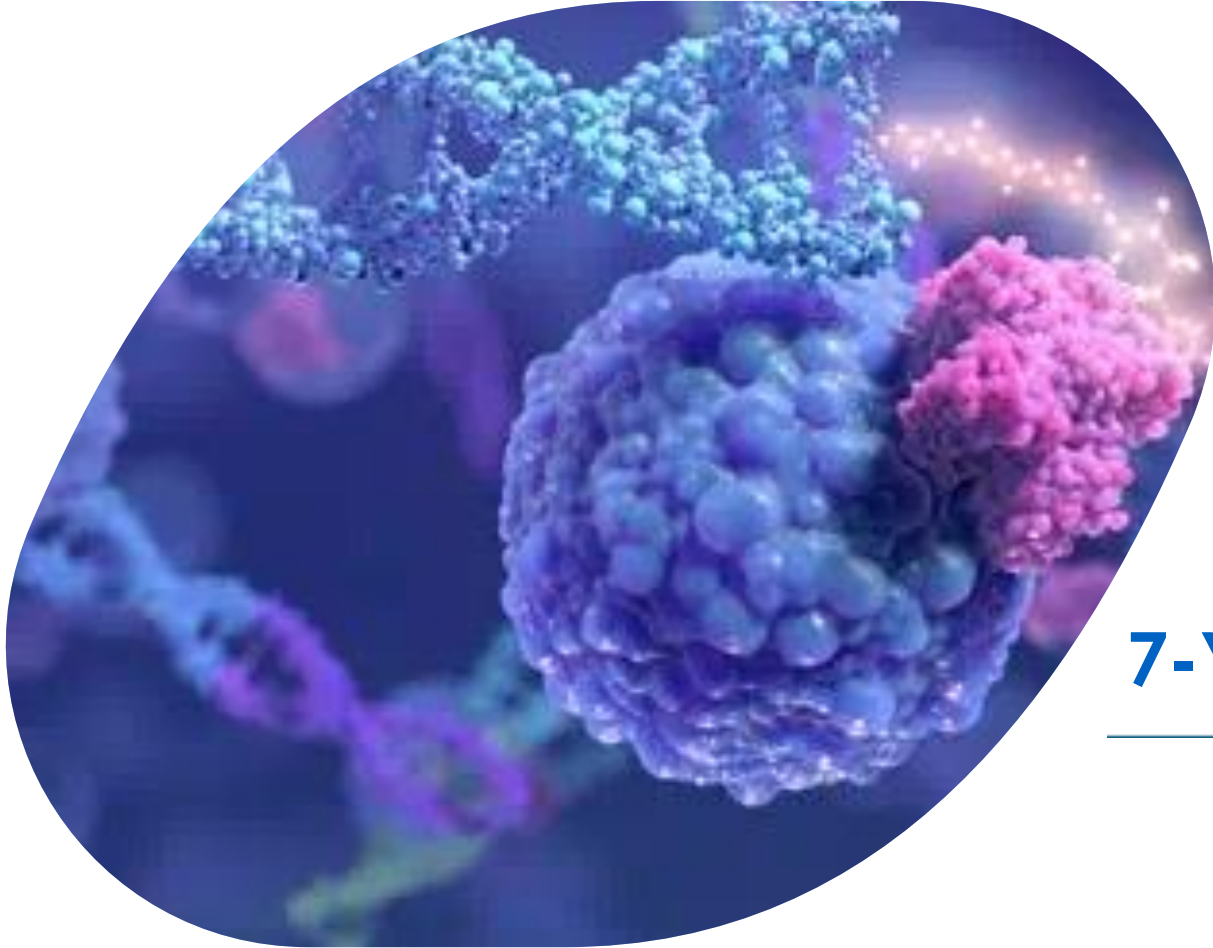
Central Thesis

- MAM has the potential to transform therapeutic protein analysis by enabling near-comprehensive attribute monitoring in a single extensible assay.

Problem Statement

- Artificial oxidation is an unusual and unpredictable analytical artifact. We propose that it may be the most significant unresolved technical obstacle to implementing MAM in regulated QC laboratories.

Selected Case Studies



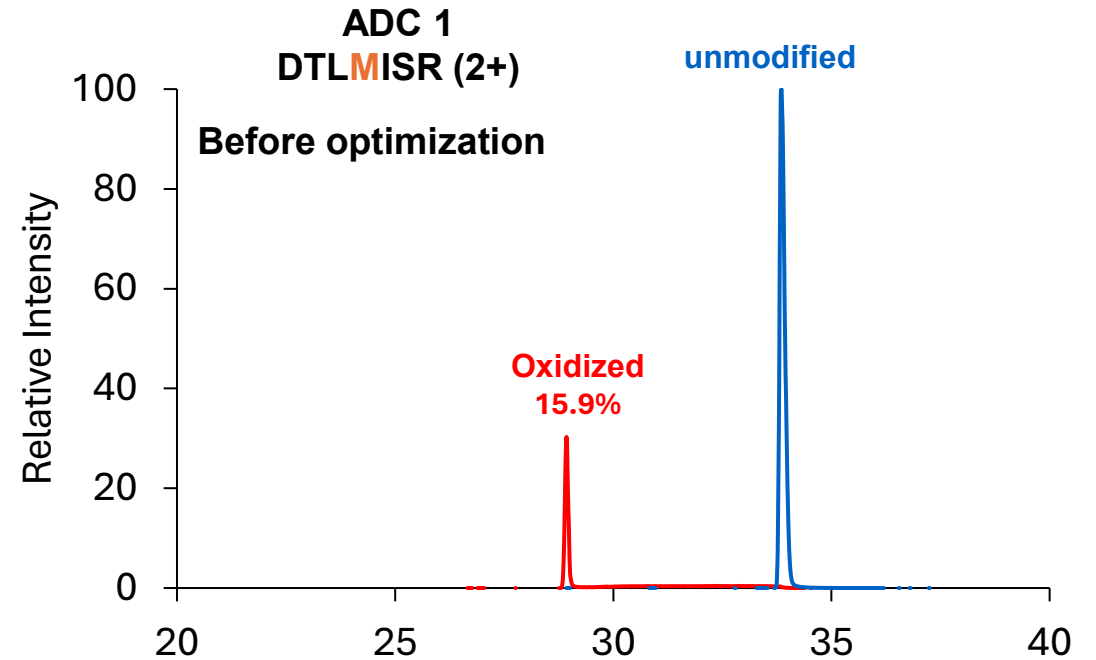
Sample Prep Oxidation

**On Column Oxidation / Post
Column Oxidation**

7-Year Longitudinal QC Study

Sample Prep Associated Oxidation

- “In particular, peptide mapping protocols may influence correct evaluation of PTM values **due to introduction of artificially induced modifications** as a result of the numerous steps and excessive sample handling involved.” (Bones 2020)¹
- “Manual preparation of samples for MAM, however, is labor intensive and produces nontrivial variations across analysts and laboratories.” (Albarghouthi 2021)²
- “The addition of 20 mM of methionine was also evaluated and retrieved better results, maintaining lower levels of oxidation” (Carvalho 2022)³



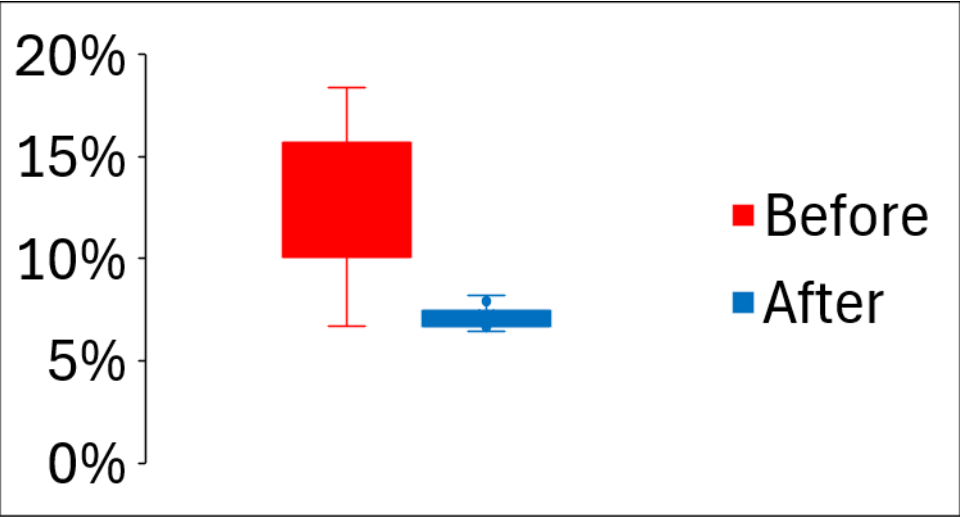
¹ doi.org/10.1007/s00216-020-02809-z

² doi.org/10.1016/j.jpba.2021.113988

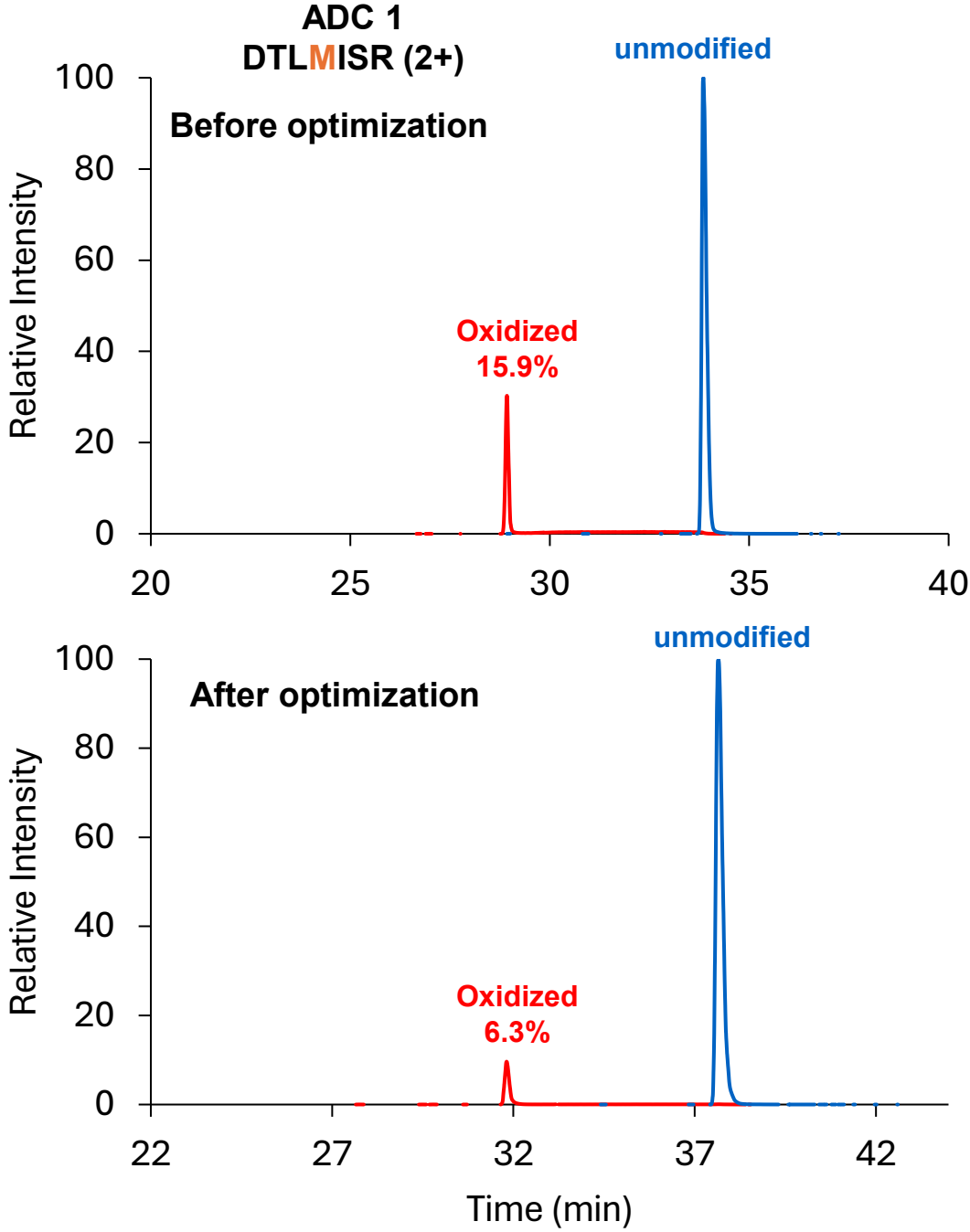
³ doi.org/10.1371/journal.pone.0262711

Sample Prep Associated Oxidation

Parameter	Before	After
Denaturation	Extended DTT duration + GdnCl	DTT GdnCl + 20 mM Met
Desalt	Zebaspin	NAP-5 (Gravity)
Digest	Tris	Tris + 20 mM Met



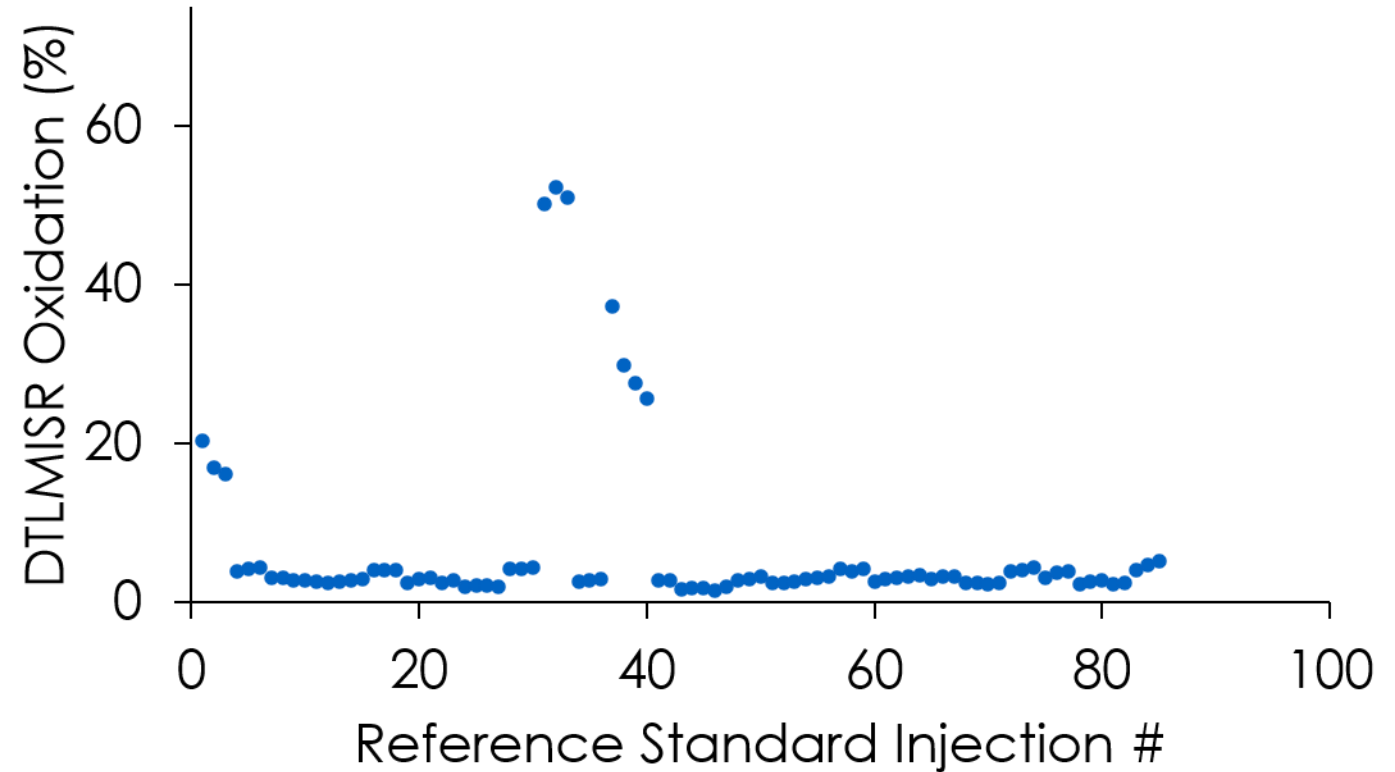
Method optimization focused on reducing the concentration of potential oxidizing species and adding L-methionine to act as a scavenger.



Abrupt and Transient Artifacts Persist Even After Optimization

- Optimized prep conditions are still prone to spontaneous and unpredictable outlier results

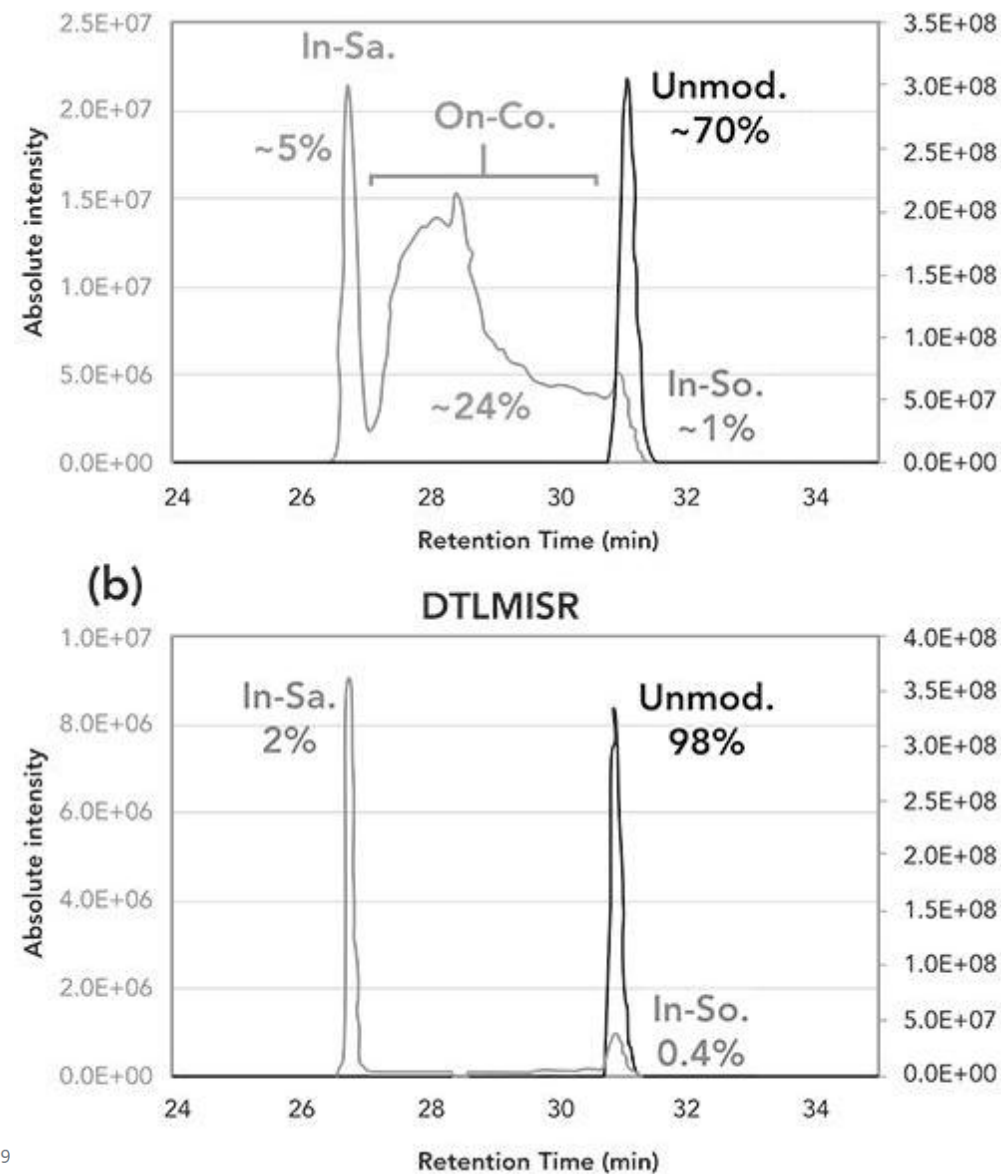
	Total	Nominal
Avg	6.4	3.1
Stdev	10.5	1.1



REFERENCE STANDARD CONTROL CHARTS OFFER ESSENTIAL LONGITUDINAL TRENDING

On Column Oxidation is a Primary Source of Artificial Oxidation

- Native\unmodified peptide spontaneously oxidizes on-column
- Rate is unpredictable, but some peptide sequences are more susceptible than others
- Root cause is not identified but this tends to correlate with column age*



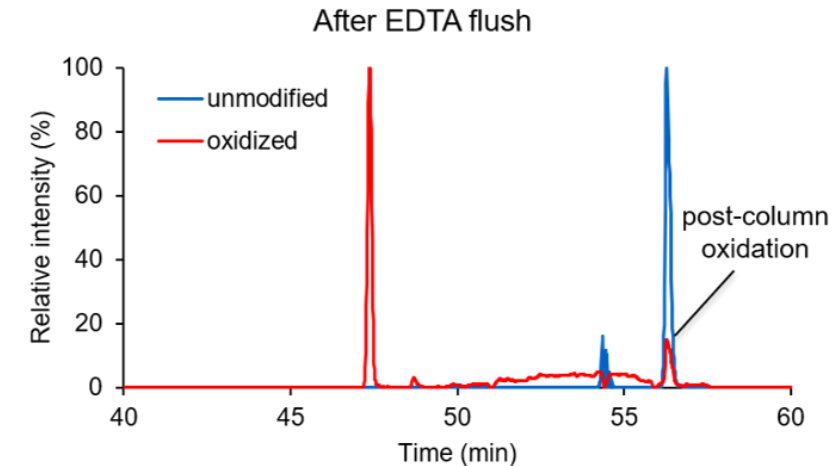
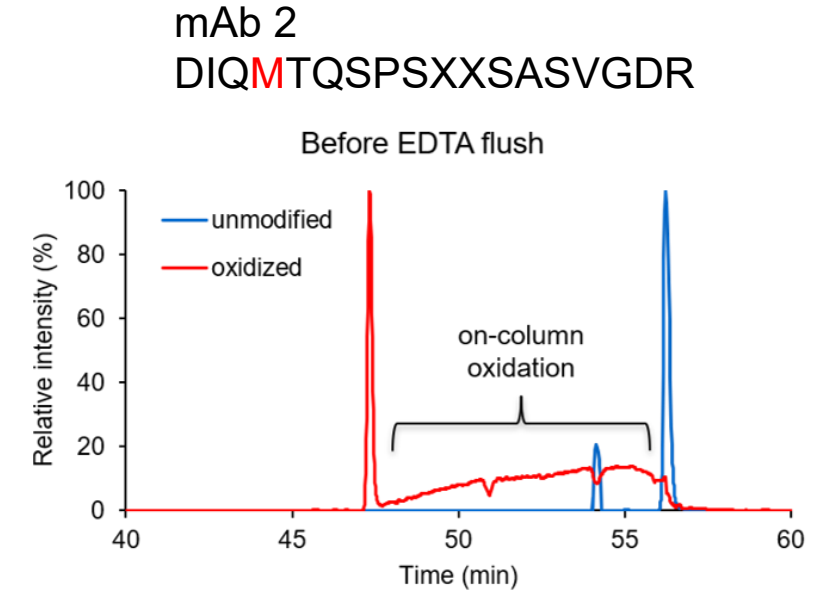
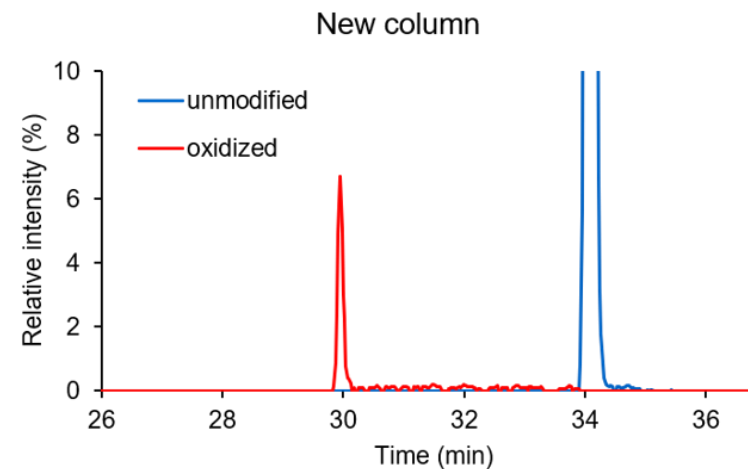
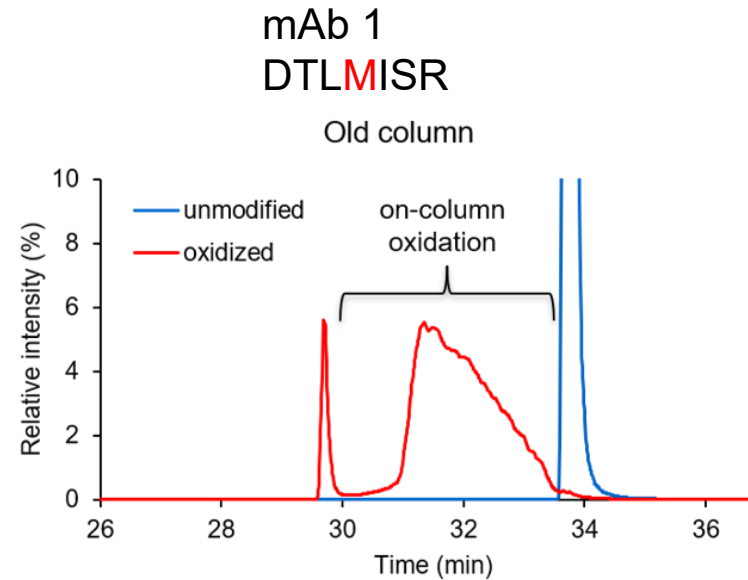
| [Articles](#) | November 1, 2019
LCGC Supplements
Special Issues-11-01-2019 | Volume 37 | Issue 11

Monitoring of On-column Methionine Oxidation as Part of a System Suitability Test During UHPLC-MS/MS Peptide Mapping

Author(s) Björn Mautz, Maximiliane König, Vincent Larraillet

On Column / Post Column Oxidation

- On column oxidation is significantly reduced by EDTA flush
- Post column oxidation is reduced by bypassing UV detector, and by optimizing ESI conditions



Recent Mitigation for On Column Oxidation

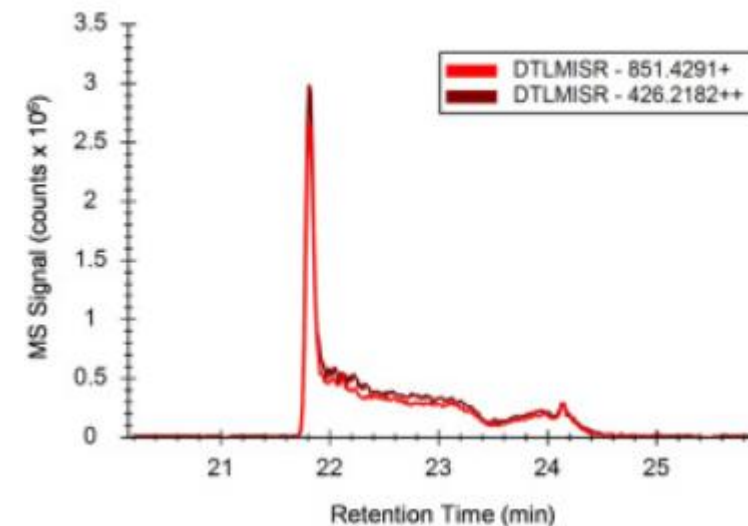
Drug Discovery–Development Interface

Eliminating liquid chromatography-induced methionine oxidation of monoclonal antibodies using high-concentration TCEP injections: The “anTCEPtic” column cleaning method

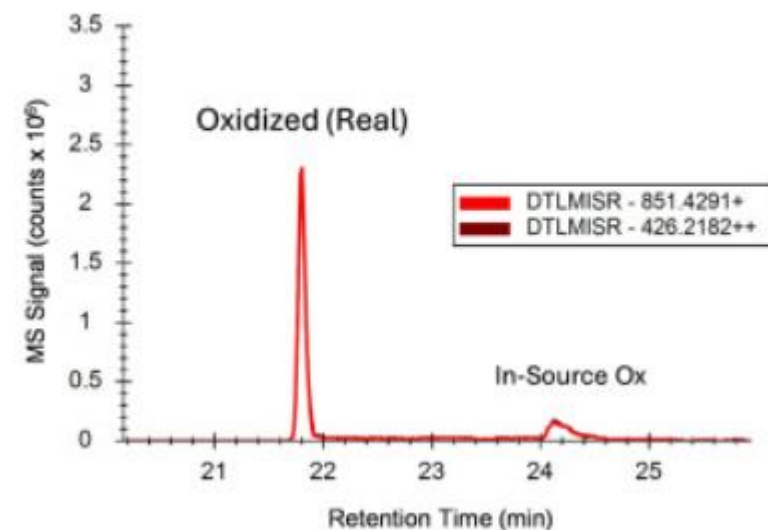
Andrew Kleinberg  , Joy Yoon, Yuan Mao  , Ning Li

Received 2 January 2026, Revised 20 April 2026, Accepted 20 April 2026, Available online 23 April 2026, Version of Record 30 April 2026.

Before TCEP Injection



After TCEP Injection

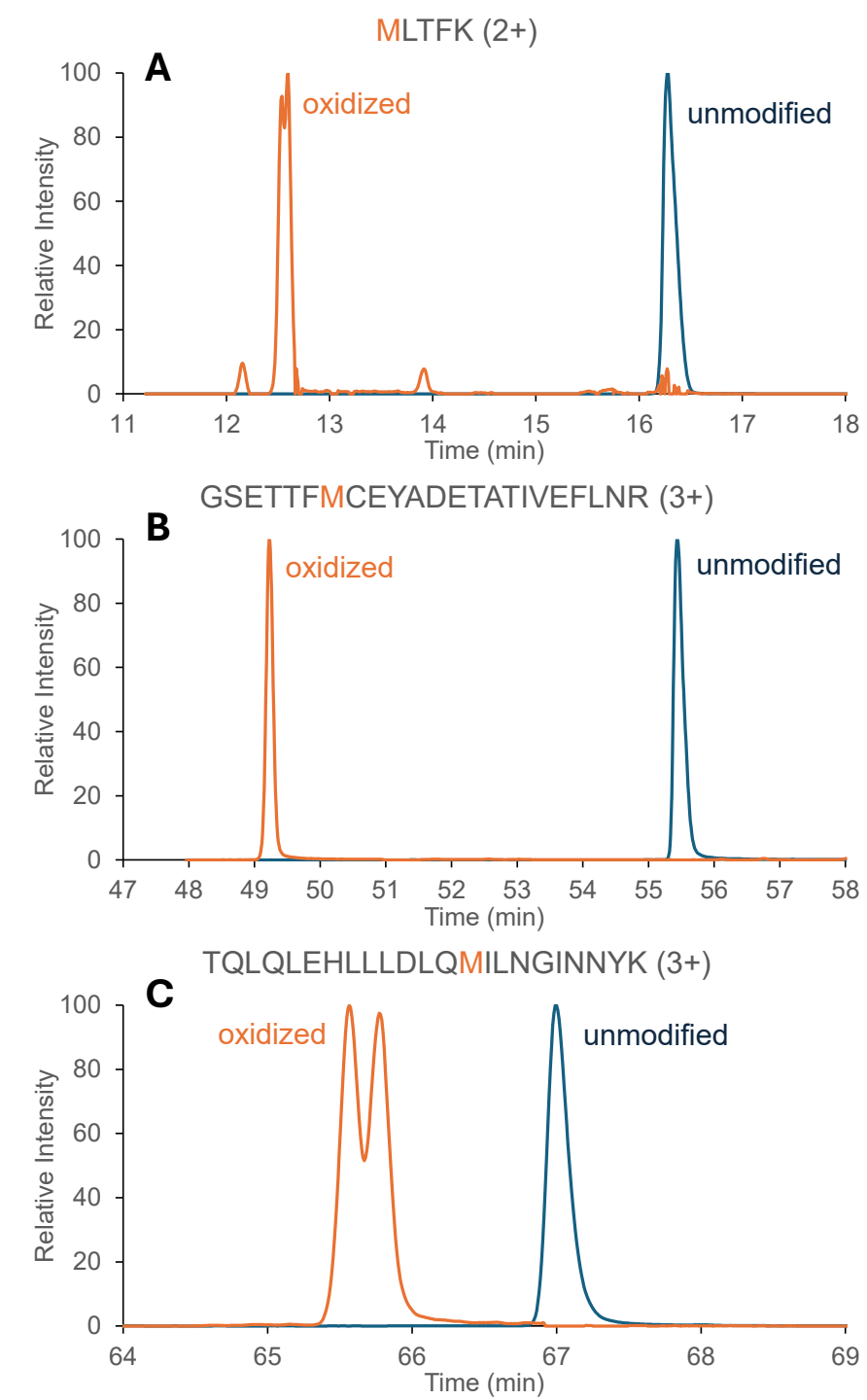


7-Year Longitudinal Study Within QC

MAM Met Ox

- Tryptic digestion and high-resolution LC-MS analysis.¹
- Oxidation of three methionine sites in the Fc-fusion protein were monitored by MAM.
- Attributes were monitored over a 7-year period during clinical development of the Fc-fusion protein.
- A frozen reference standard stored at -70 °C was analyzed in parallel with every run for system suitability and longitudinal trending.

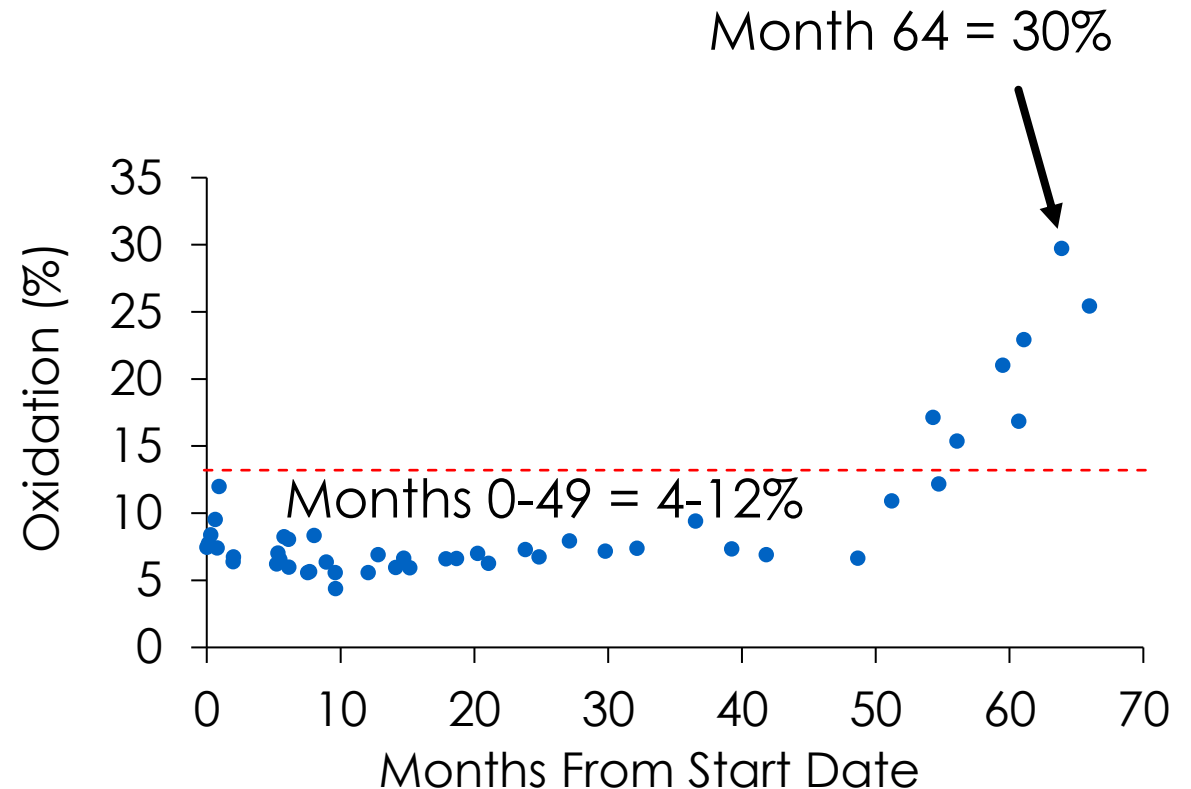
¹ Guan, X.; Eris, T.; Zhang, L.; Ren, D.; Ricci, M.S.; Thiel, T.; Goudar, C.T. A high-resolution multi-attribute method for product characterization, process characterization, and quality control of therapeutic proteins. *Analytical Biochemistry* **2022**, 643, 114575.



Oxidation Abruptly Increased After 4+ Years

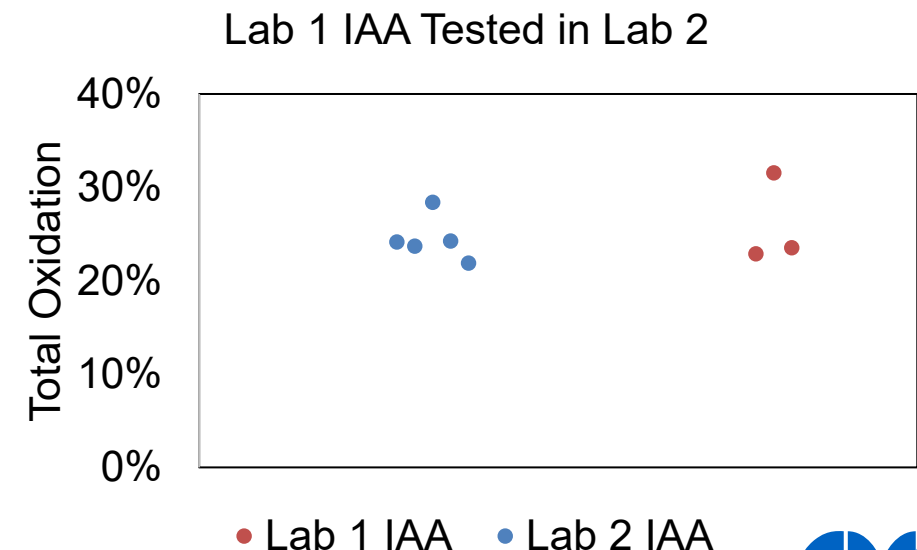
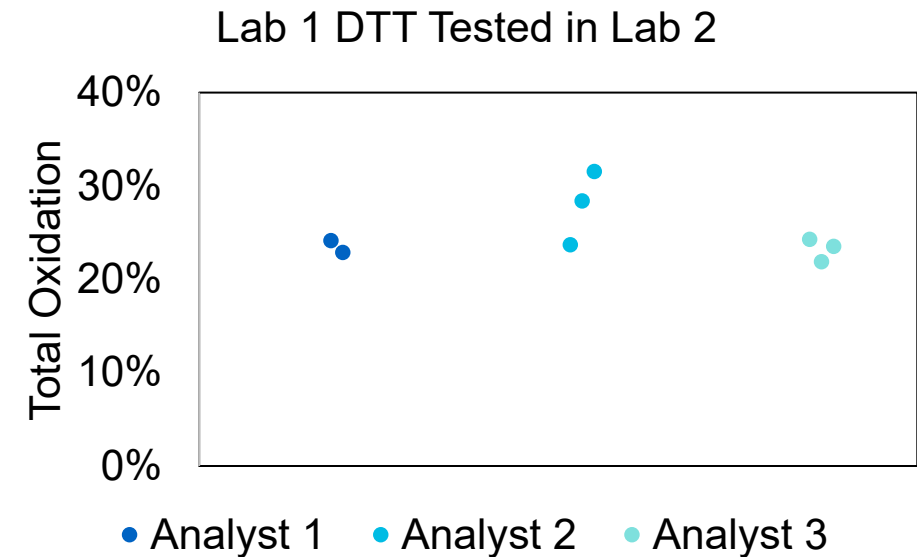
- Total oxidation remained flat for 4+ years before abruptly increasing
- No sign of on column oxidation or other instrument issues
- All impacted sample sets are identified by simple system suitability control, eg:

RS average $\pm$ 3 Stdev



Extensive Root Cause Investigation

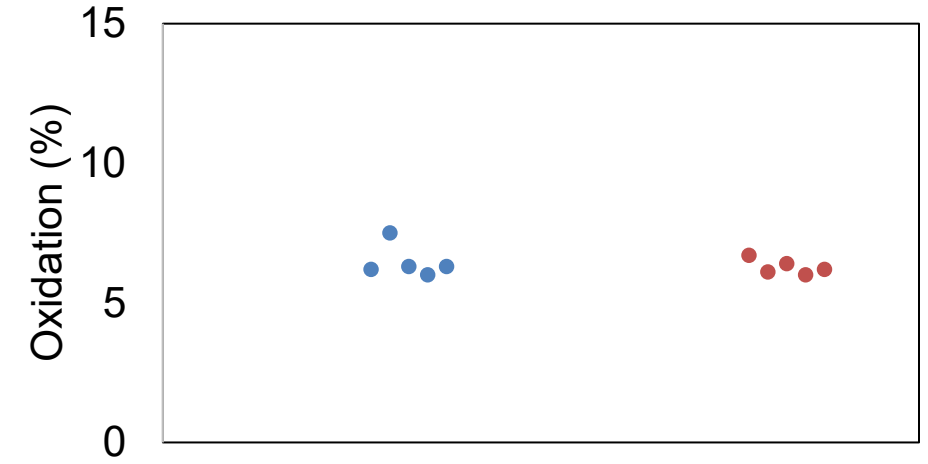
- Team investigated usual suspects
 - Integration issues
 - On column oxidation
 - Source Oxidation
 - Reduction reagents
 - Alkylation reagent
- Supervised analyst sample prep
- Analyst from Lab 2 performed successful preps in Lab 1



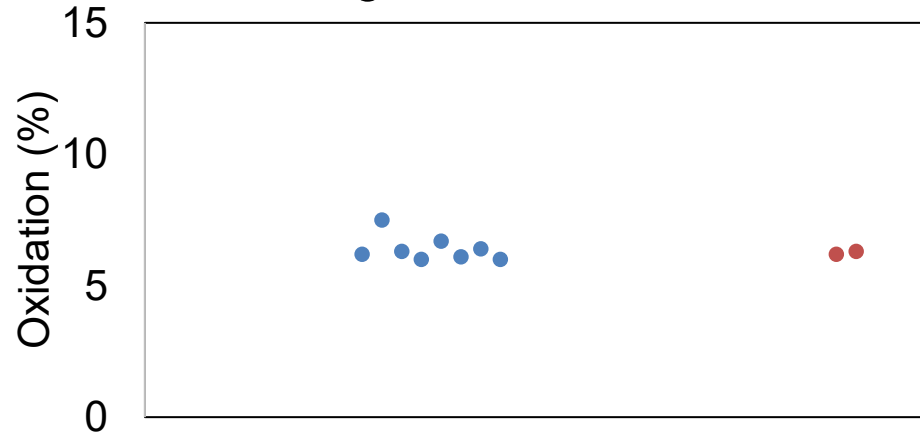
Extensive Root Cause Investigation

- Team investigated unusual suspects
 - Water source
 - Pipettors
 - Pipette tips
 - Heat bath / incubators
 - Shakers

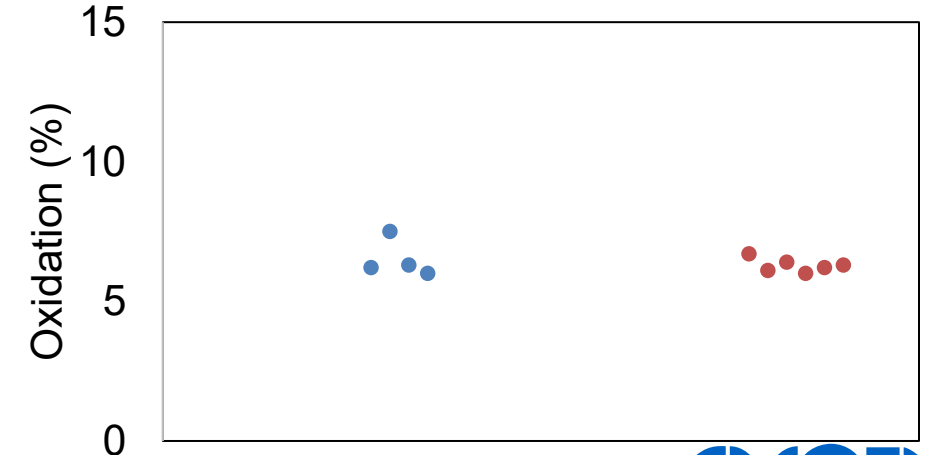
Pipette/Tip



Digestion buffer

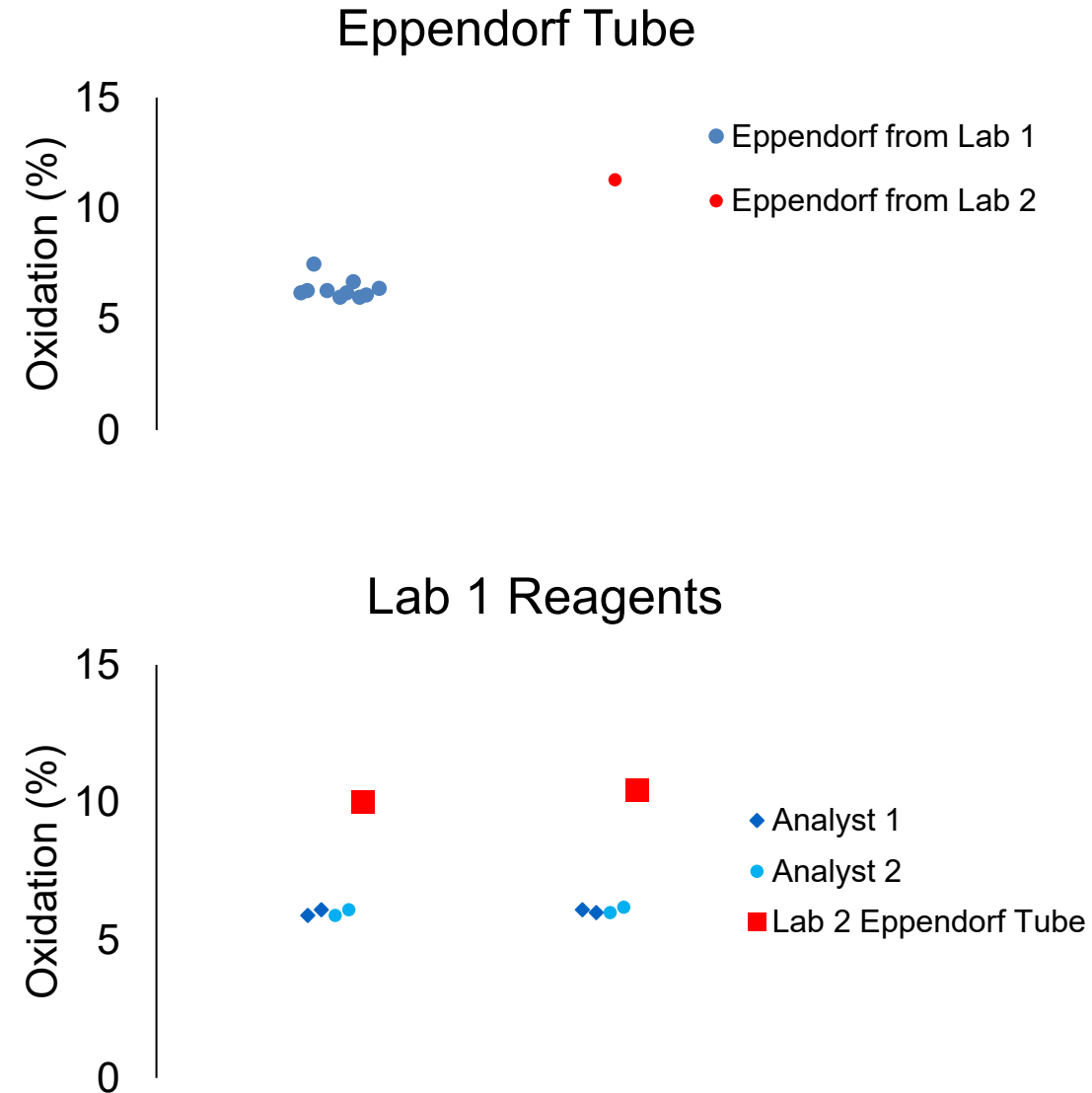


Denaturing buffer

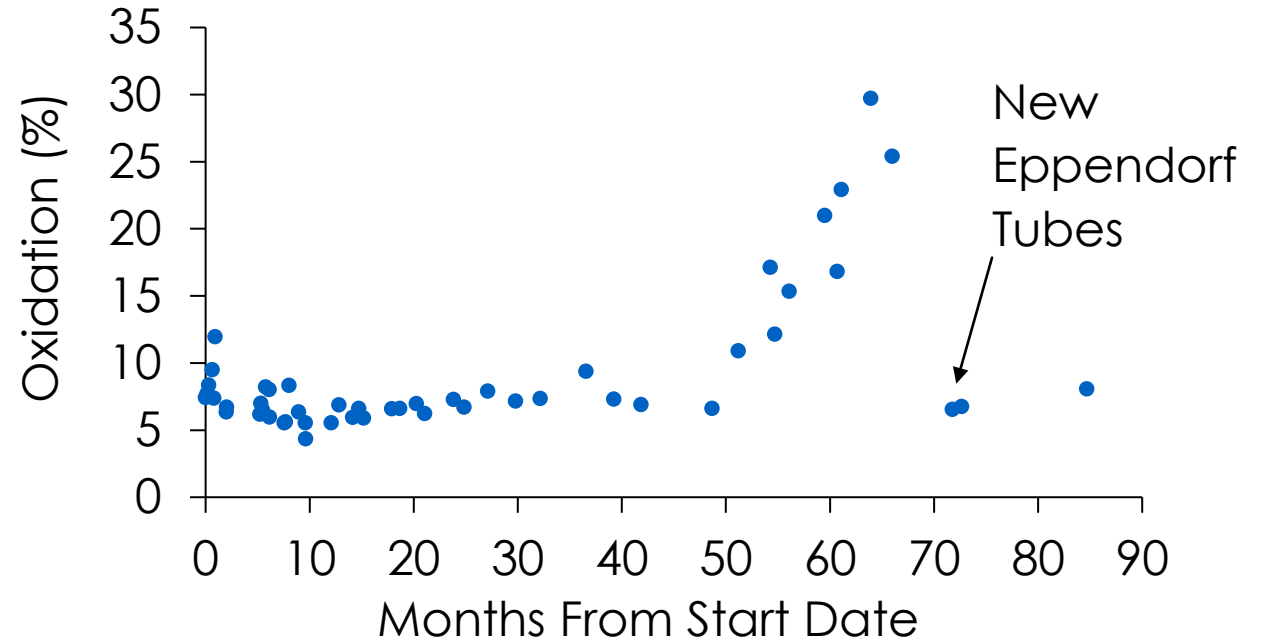
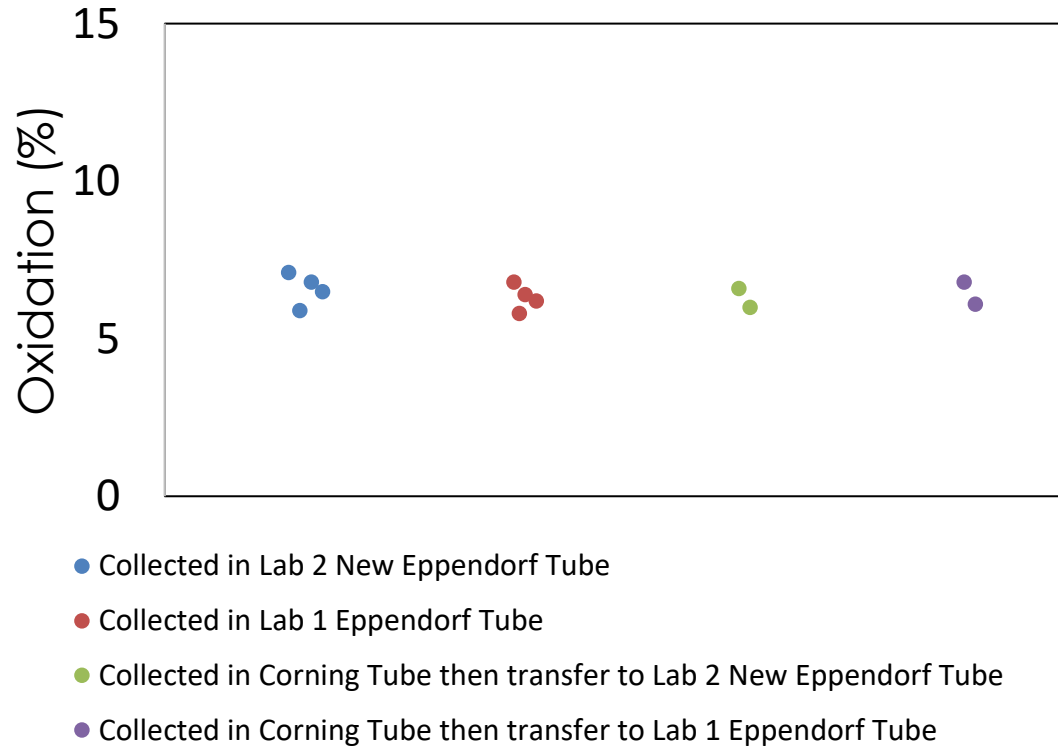


Culprit Finally Identified

- Team observed elevated oxidation in a side-by-side experiment with a single Eppendorf from Lab 2
- Replicate experiments reproduced high oxidation using Lab 2 Eppendorf tubes, regardless of testing lab or other reagents



New Eppendorf Tubes Restored Method Performance



Root Cause Remains Speculative

- A single microcentrifuge tube bag was found to induce high levels of Methionine oxidation.
 - The bag was sequestered within the lab, set aside for MAM
 - The cardboard box had been discarded, and the bag was stored on the top shelf, exposed to ambient lab light
- We hypothesize that the polypropylene became photo-oxidized, producing a reactive compound (ie hydroperoxide^{1,2})

1 [doi.org/10.1016/0141-3910\(91\)90086-7](https://doi.org/10.1016/0141-3910(91)90086-7)

2 doi.org/10.1016/j.polymdegradstab.2012.10.004

Conclusions

- Modern advances continue to address many MAM challenges, however artificial oxidation remains unpredictable.
- Met Ox constitutes a major obstacle to robust implementation in regulated QC environments.
- There is a continued need for enhanced control strategies and further research to ensure reliable therapeutic protein analysis.
 - Consumable qualification, change controls
 - RS trending and system suitability statistics
 - Mitigating exposure to oxidizing surfaces and reagents (iron free LC flow path, reduction of trifluoroacetic acid (TFA) exposure, improved desalting)

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Amanda Helms

Carl Ly

Kevin Millea

Xingwen Li

The Amgen logo, consisting of the word "AMGEN" in a bold, white, sans-serif font, is positioned in the bottom right corner of the slide. The background of the slide is a solid blue color with a decorative pattern of various shades of blue and white geometric shapes (circles, rectangles, and ovals) along the bottom edge.