Application of MAM during Drug Development:

Overcoming Technical Challenges While Advancing Product and Process Knowledge

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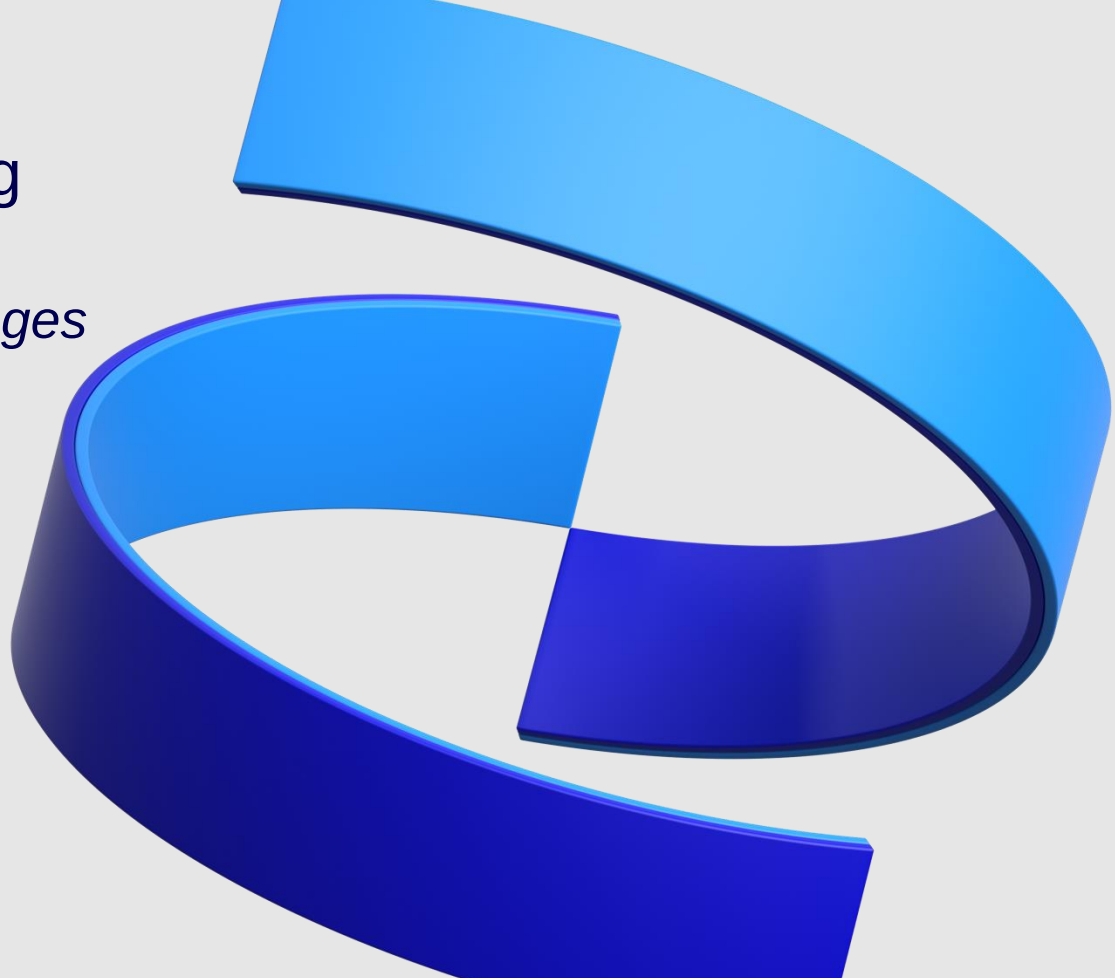
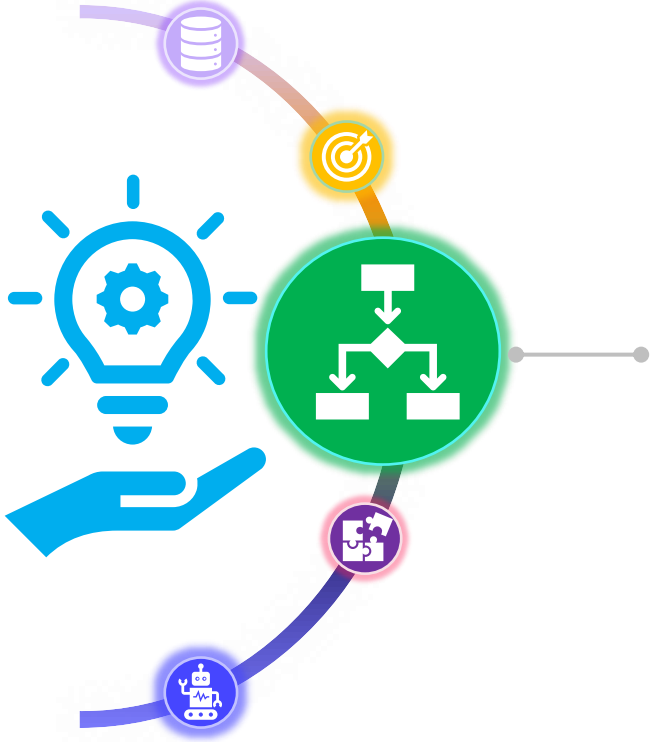


Outline

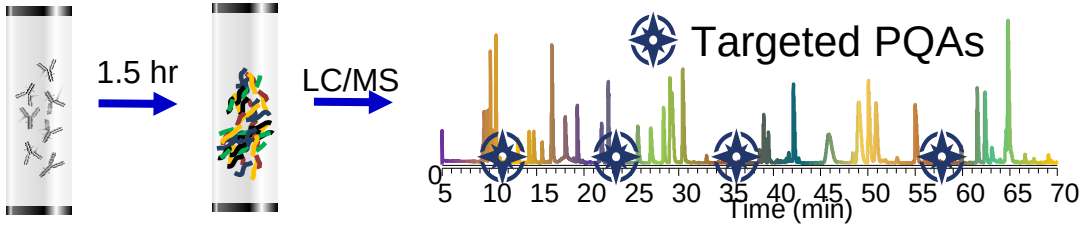
- Outlining Key Benefits of MAM
- Preview of Pfizer's MAM Strategy
- Tackling Unique Challenges to Enable the Application of MAM
- Case Studies on how MAM is Applied within Pfizer
- Conclusions and Future Directions

Innovating Analytics through MAM



Multi-Attribute Method

The multi-attribute method encompasses liquid chromatography-mass spectrometry (LC-MS) peptide mapping and automation principles to simultaneously detect and quantitate multiple product quality attributes.



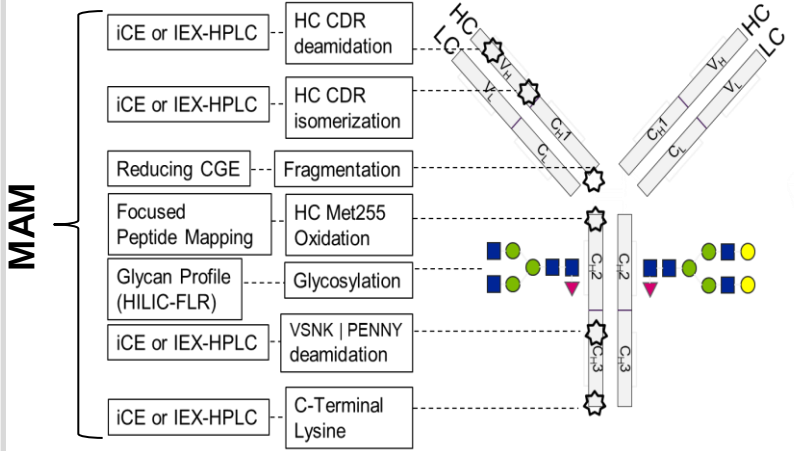
Innovation

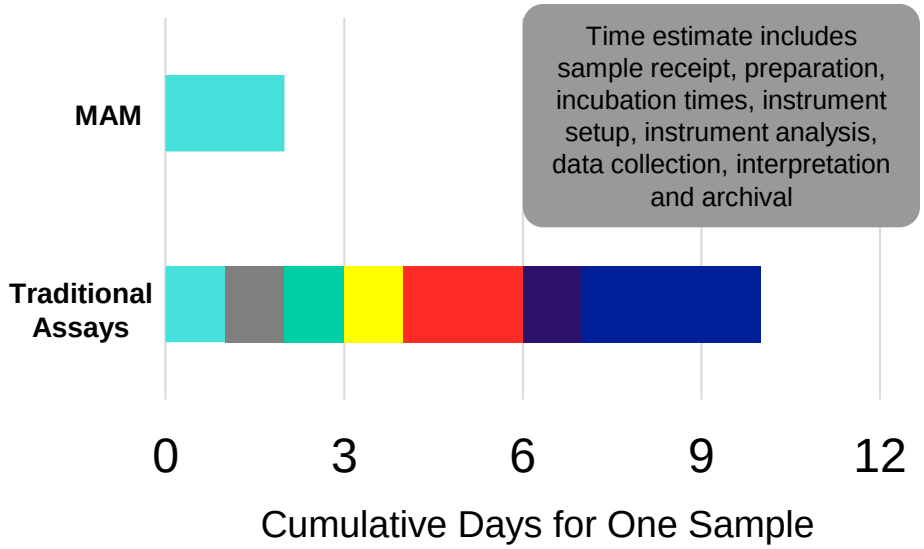
- Rapid digestion = minimal preparation artifacts
- Specific = targeted analysis of program-dependent hotspots
- Automated analysis = increase efficiency and harmonization

Innovative Applications of MAM – Rapid Results

MAM for mAbs/Biotherapeutics

Current Release Test



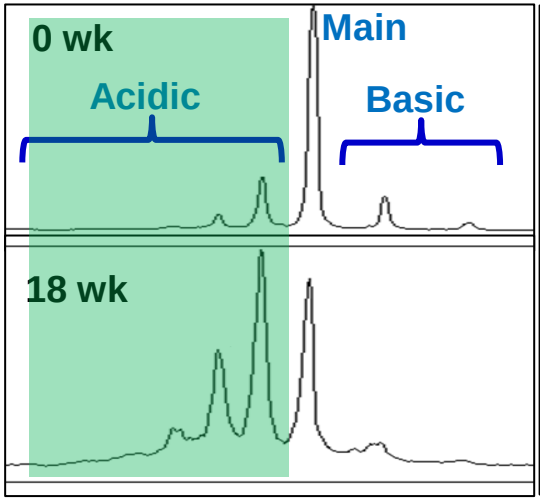


MAM has the potential to significantly expedite analytical testing

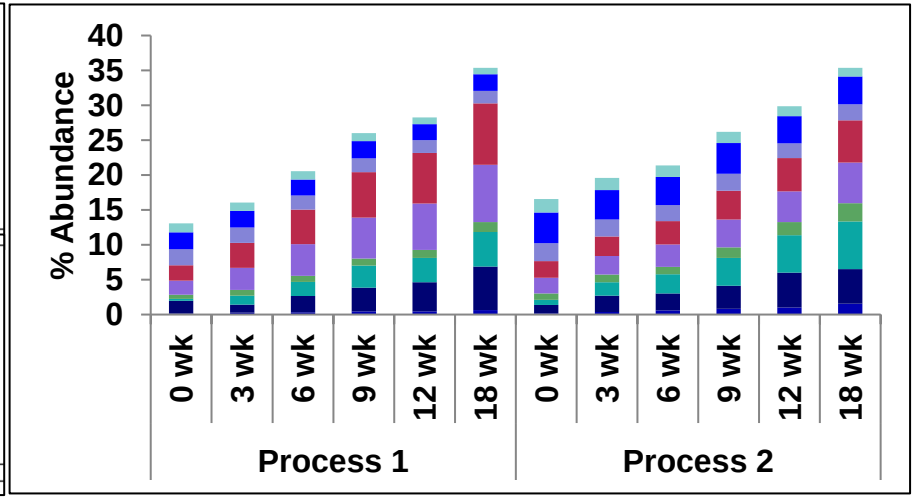
Innovative Applications of MAM – Enhanced Knowledge

Drug Substance Batches Subjected to 40° C Stress for 18 Weeks

iCE Profile



Acidic Species by MAM



- Identification of charge variants/modifications and localization to specific sites
- Site-specific knowledge of glycan occupancy and glycoform compositions at specific sites
- Location of individual clipping sites and much more…

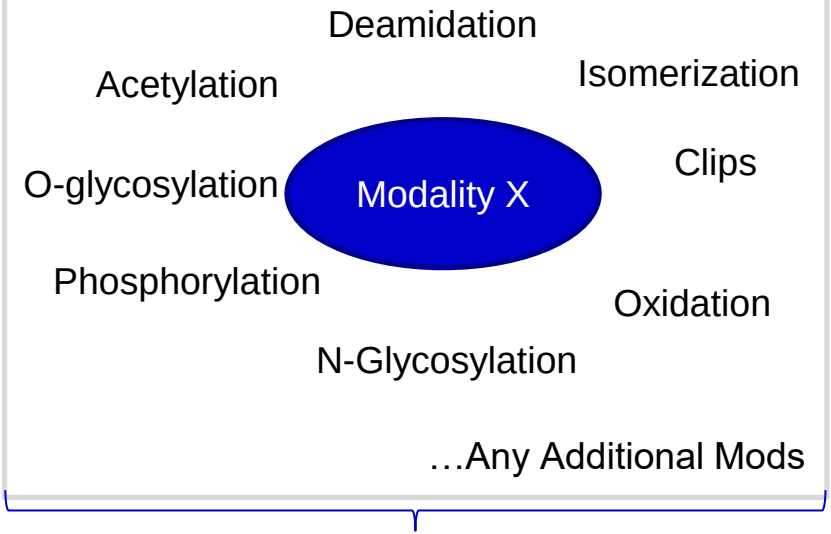
Innovative Applications of MAM – Unprecedented Knowledge

MAM Testing Results Can Fill an Analytical Void

- For new modalities, PQAs may be poorly understood and release tests to monitor PQAs may not be available
- MAM can be leveraged to relay critical information to partner lines and enable understanding of new modalities

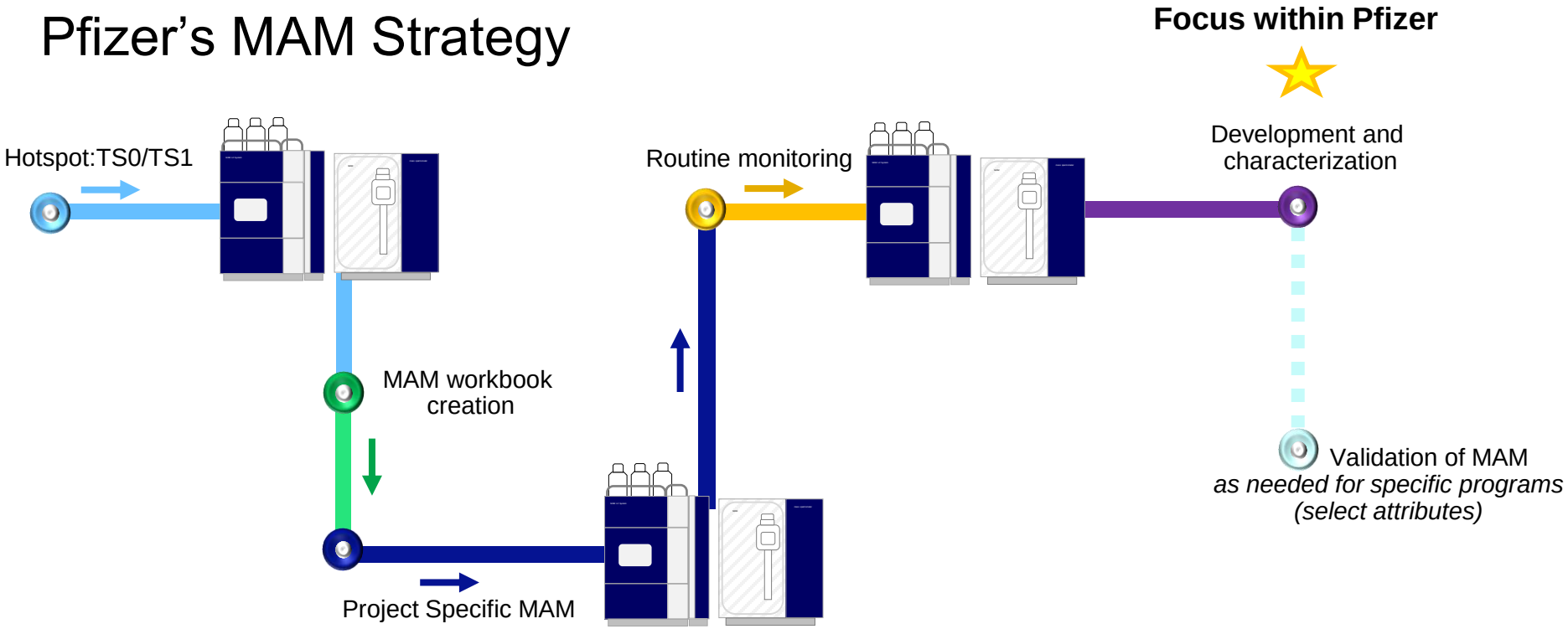
For new modalities, MAM supplies pivotal information that is not captured otherwise

MAM for New Modalities



Release tests may be unavailable at project initiation

Pfizer's MAM Strategy



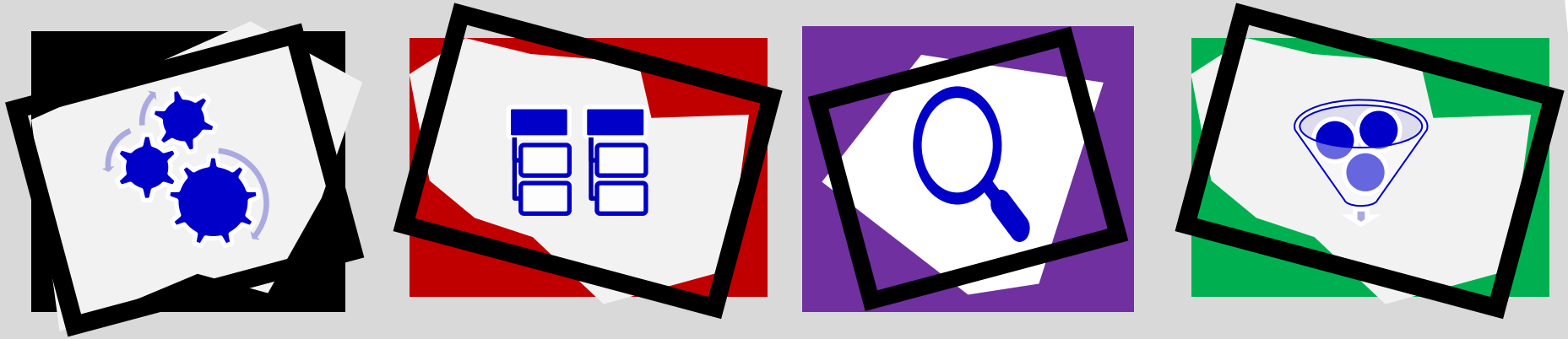
Challenges Associated with Broad Implementation of MAM

Harmonization and Throughput

Workbook Creation

Data Quality

Disseminating Information



Challenge #1: Harmonization and Throughput

Sample Pr
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MABS
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Enhancing the multi-attribute method through an automated and high-throughput sample preparation

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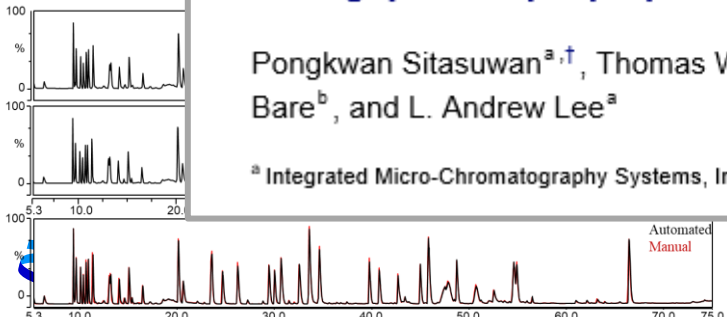


Challenge #1: Harmonization and Throughput

Sample Preparation Automation: Hamilton Robot

MAM Data Collection and Automated Processing/Reporting

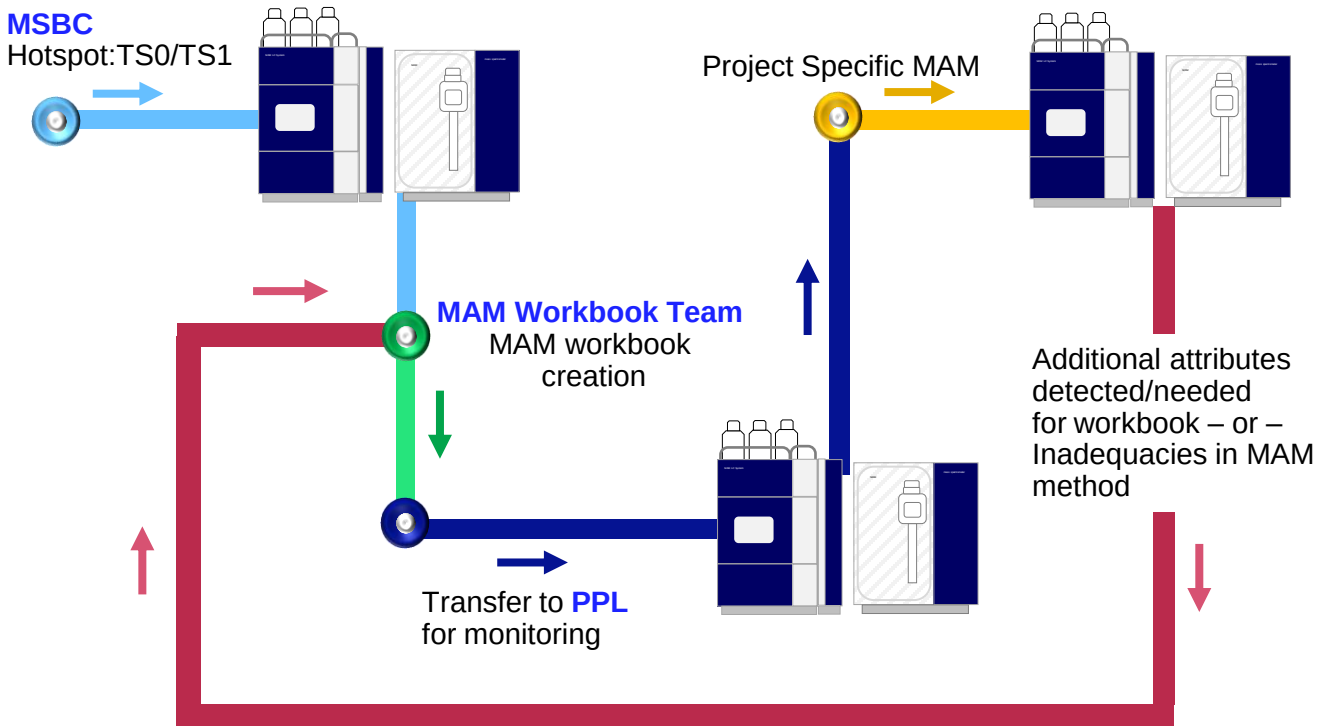
Harmonization of Pfizer instruments and methods provided insights on method variability and inter-lab method transfer.

Pfizer has been a leader in inter-laboratory harmonization within the MAM Consortium.

3 Thermo E+ Systems
1 Thermo QE+ System
Harmonized Vanquish UHPLC

All data processing on consistent versions of Chromeleon software

Challenge #2: Workbook Creation (Bottleneck)

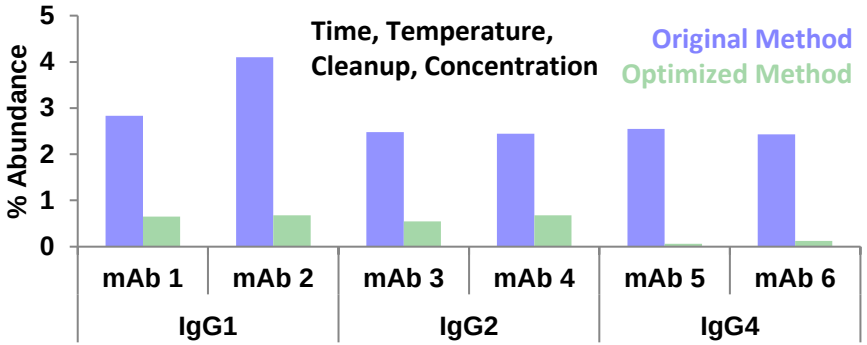


Innovation

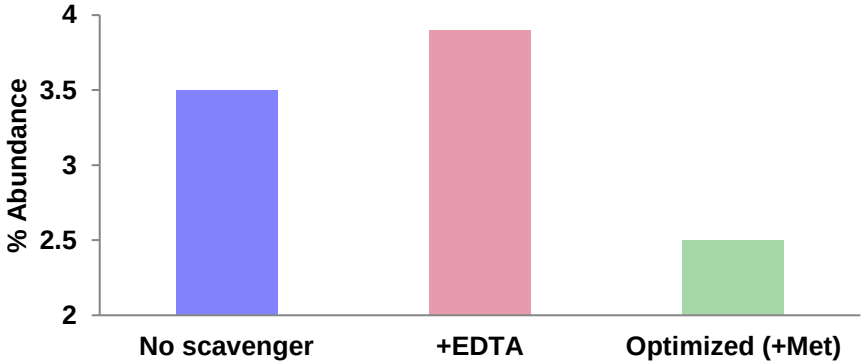
- Defined MS characterization roadmap for hotspot analysis
- Dedicated team capable of making processing methods
- Pfizer specific “Generic Report” to eliminate manual intervention
- Excellent communication and handoff strategy

Challenge #3: Data Quality (Prep)

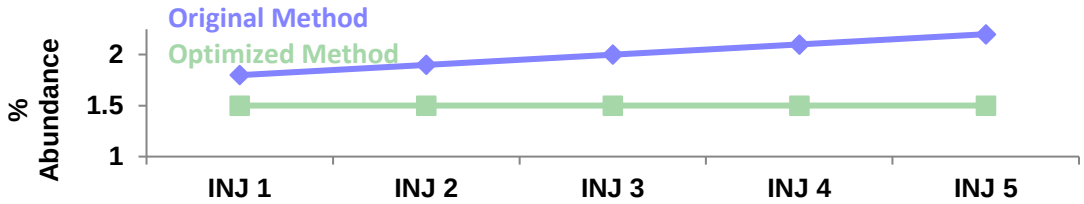
Reduction of Missed Cleavages



Reduction of Method Artifacts



Improved Autosampler Stability



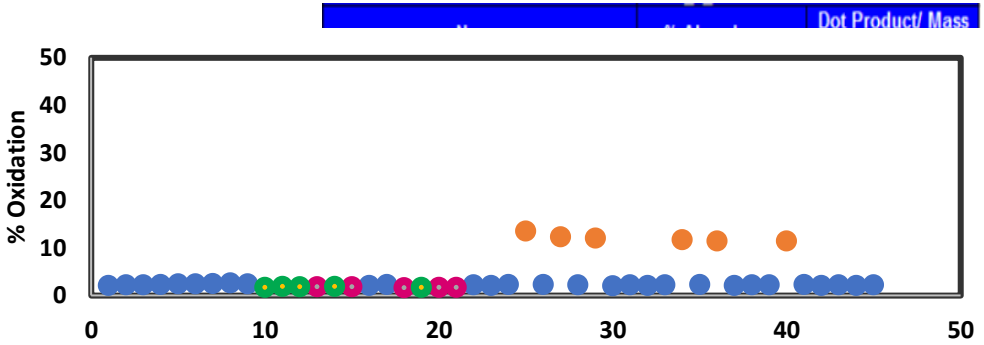
- Superior sample preparation method ensures accurate data

Challenge #3: Data Quality (Report)

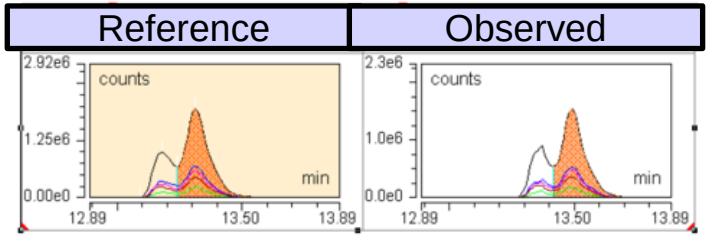
Name		Operator	Lower Rang	Upper Ran	Result
Mass Accuracy - DT	-2	between	-5	5	Passed
Mass Accuracy - DI	-1	between	-5	5	Passed
Mass Accuracy - TV	-2	between	-5	5	Passed
Mass Accuracy - VD	-4	between	-5	5	Passed
Mass Accuracy - DS	-1	between	-5	5	Passed
Component Area - D	1433207199	>=	930000000	n.a.	Passed
Component Area - D	1742509258	>=	730000000	n.a.	Passed
Component Area - T	1535474418	>=	1.1E+009	n.a.	Passed
Component Area - V	1752311243	>=	480000000	n.a.	Passed
Component Area - D	2113953031	>=	630000000	n.a.	Passed
Retention Time - DT	40.07	between	37.97	40.7	Passed
Retention Time - DI	23.81	between	22.24	24.17	Passed
Retention Time - TV	47.87	between	45.96	48.53	Passed
Retention Time - VD	11.50	between	10.42	11.89	Passed
Retention Time - DS	30.18	between	28.45	30.84	Passed
Percent Oxidation D	2.1	between	1.6	2.8	Passed
% Fragmentation	1.3	between	1.2	1.9	Passed
TIC Signal	1095486800	>=	320000000	n.a.	Passed

Product specific system suitability, assay acceptance, and sample suitability criteria

Generic Report



Demonstration of Integrations

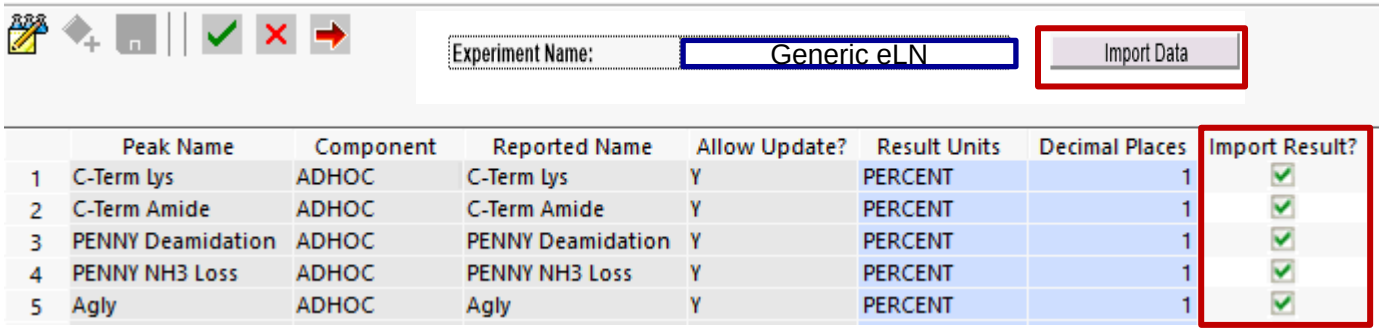


All data in PDF for easy data review

Challenge #4: Disseminating Information

Sample Info	Sample 1	Sample 2
Sample Number	1	2
LIMs ID	XXXXXXX	XXXXXXX
Program	XYZ	XYZ
Injection Time	30Sep21, 1:43:55 AM	30Sep21, 3:4:50 AM
Mod Info		
C-Term Lys	6.3	6.3
C-Term Amide	0.4	0.4
PENNY Deamidation	4.0	4.1
PENNY NH3 Loss	2.2	2.2
Agly	0.1	0.1

Injection results exported and compiled into an excel file



	Peak Name	Component	Reported Name	Allow Update?	Result Units	Decimal Places	Import Result?
1	C-Term Lys	ADHOC	C-Term Lys	Y	PERCENT	1	☑
2	C-Term Amide	ADHOC	C-Term Amide	Y	PERCENT	1	☑
3	PENNY Deamidation	ADHOC	PENNY Deamidation	Y	PERCENT	1	☑
4	PENNY NH3 Loss	ADHOC	PENNY NH3 Loss	Y	PERCENT	1	☑
5	Agly	ADHOC	Agly	Y	PERCENT	1	☑

Excel file is compatible with generic LIMS import

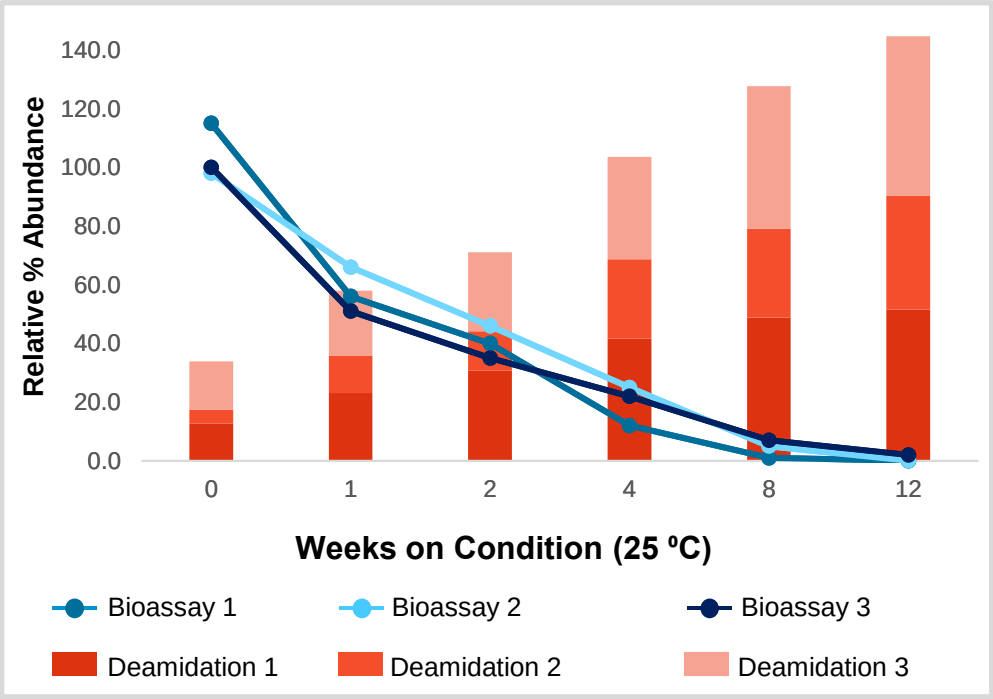
Approach compatible with any number of attributes reported

MAM data are accessible to ARD and partner lines

Case Studies

- Monitoring Deamidation for New Modalities
- LPQ/PPQ Support
- Stability and Degradation Profiles
- Comparability
- Investigating Clips
- Formulation Support

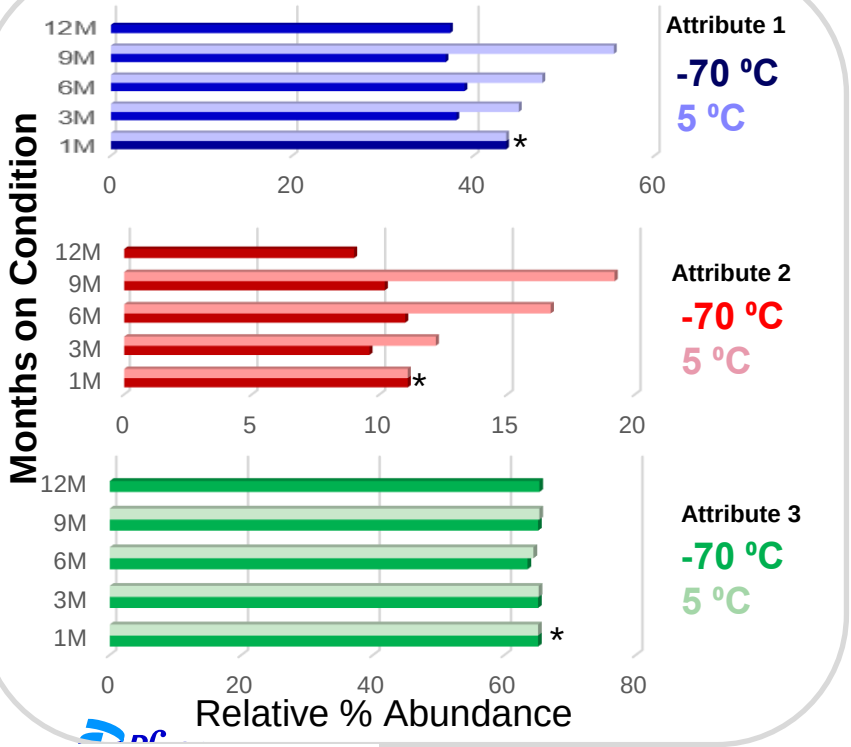
Linking Deamidation to Bioassay Data for a New Modality



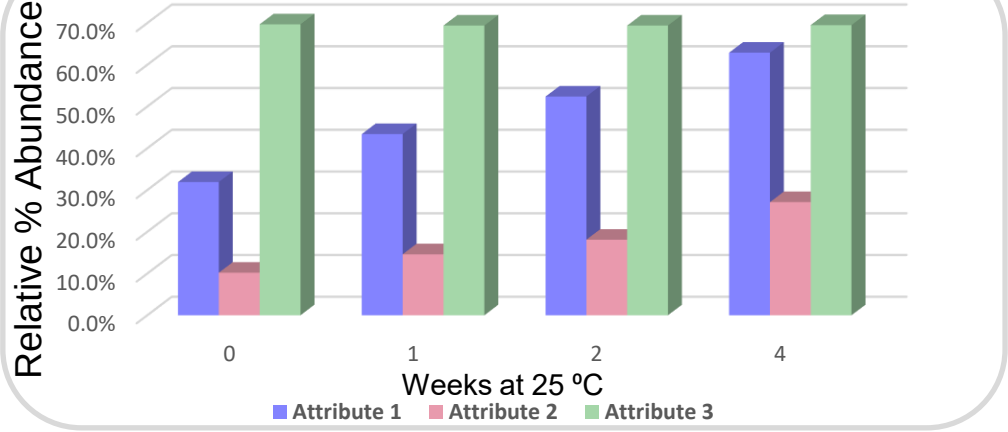
- For a new modality, a potency drop was observed in forced degraded samples with no correlating change in any other release test method
- MS characterization identified several sites of deamidation that increased and were shown to cause potency drop
- A MAM method was developed to monitor deamidation (and other attributes)
- MAM has been used for this modality throughout product development

Application of MAM to Stability and Forced Degradation Samples

Real-Time Stability



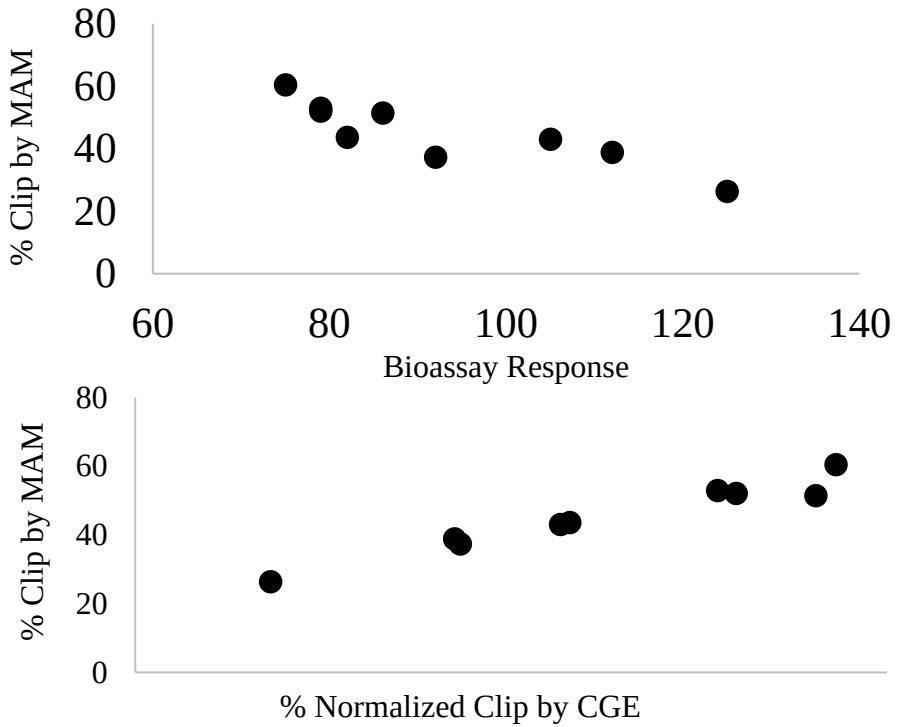
Forced Degradation



- MAM has been used to support real-time stability studies and forced degradation studies
- MAM could be leveraged in analytical comparability studies of forced degraded material to assess rates of changes

* Processing Method Change

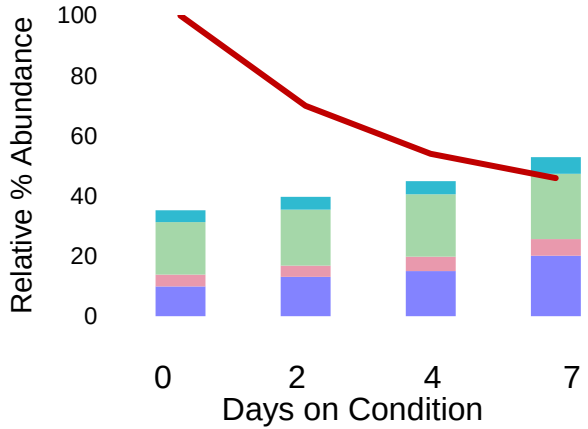
Leveraging MAM to Support Orthogonal Release Method



- A drop in potency was catalogued throughout product development
- CGE, the release test method for purity was implemented, but data were not intuitive to interpret
- MAM was leveraged to bolster the confidence in the CGE assay results
- CGE release method was optimized to report the clip in a similar approach used by MAM
- MAM was routinely used in development to enhance the bioprocess by eliminating clips and improving product efficacy

MAM in LPQ and PPQ Studies

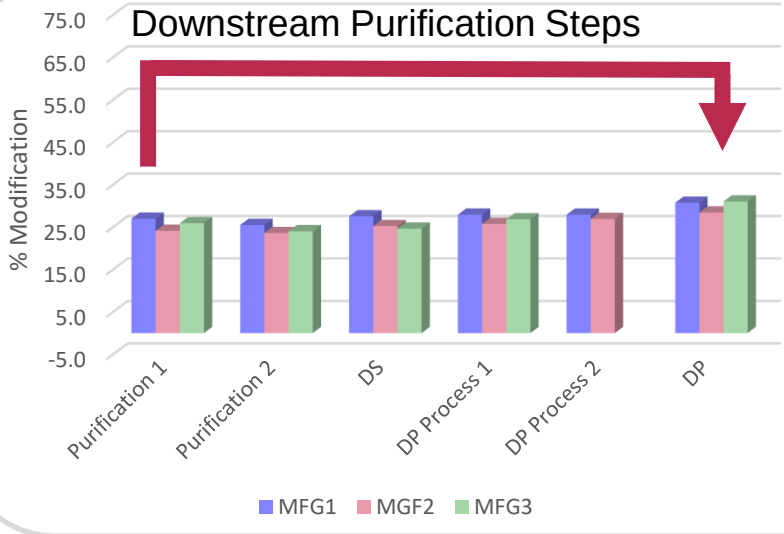
MAM for Understanding Process Changes



Bioassay Response

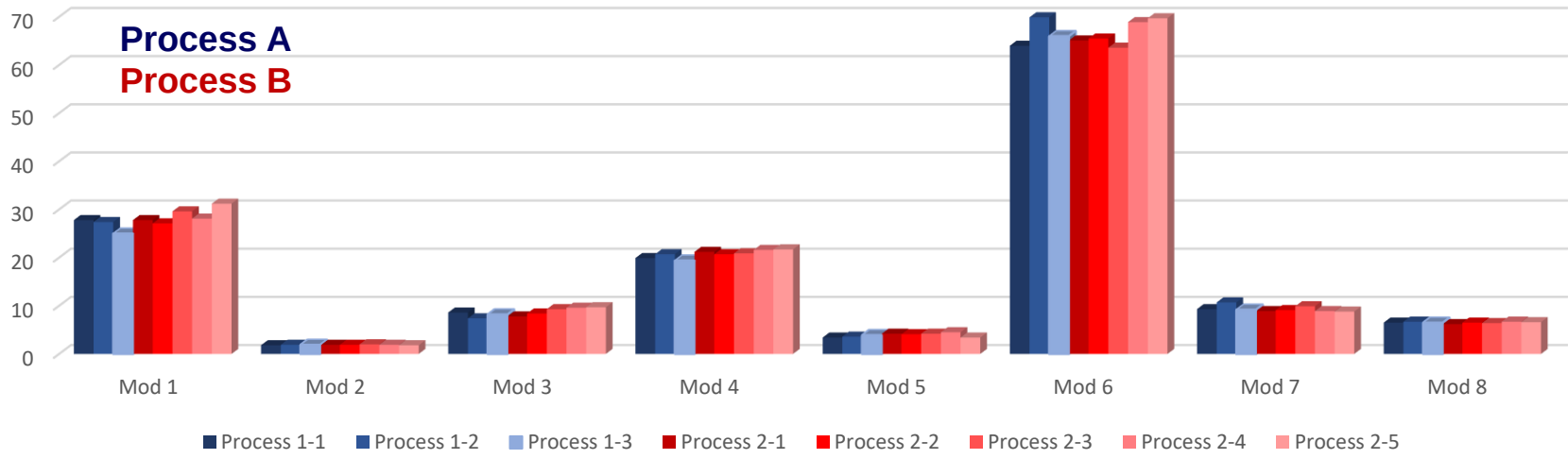
Modification 1
Modification 2
Modification 3
Modification 4

MAM in Process Validation



- MAM has been used to identify critical process parameters (CPP) that impact CQAs
- MAM has supported process validation studies

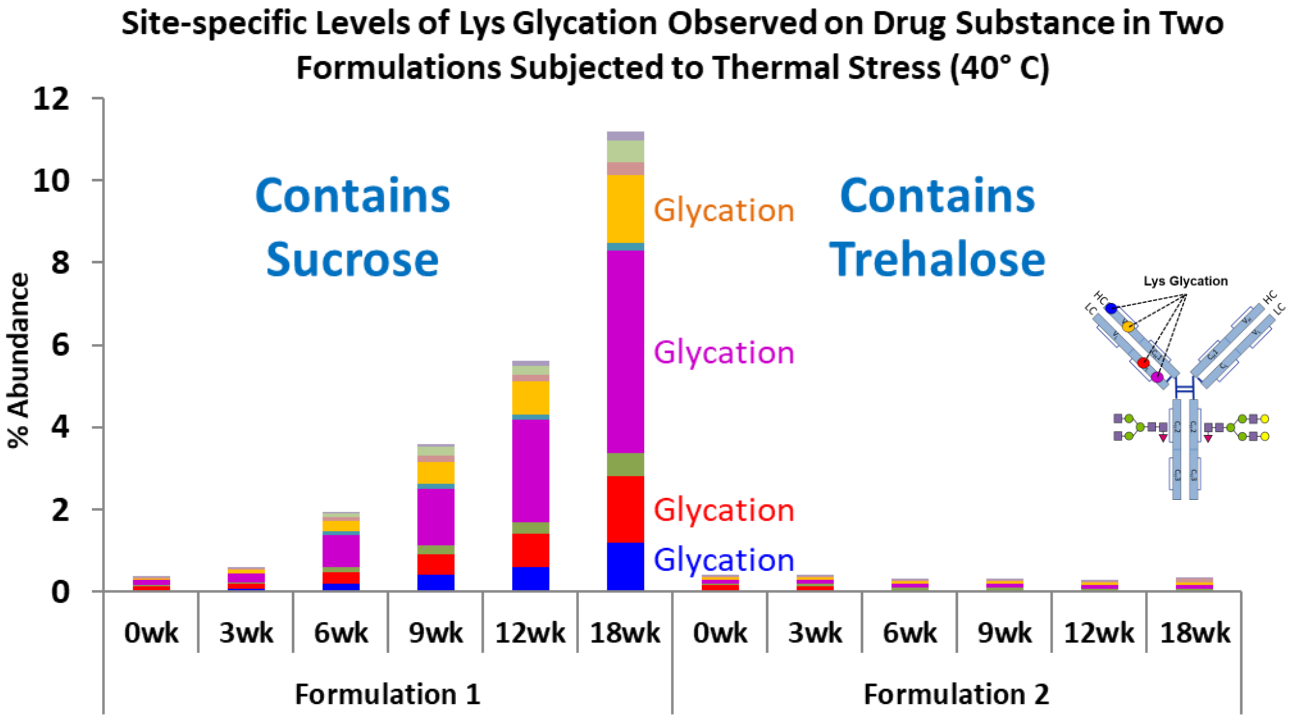
MAM in Analytical Comparability Assessments



- Two manufacturing processes were assessed for analytical comparability
- A panel of attributes were monitored by MAM and were consistent across processes
- MAM data coincides with LC-MS/MS peptide mapping data sets

MAM in Formulation Support

- Long-term product stability in final formulation is used to establish product expiration
- MAM revealed that drug substance in Formulation 1 is at a much higher risk for Lys glycation
- MAM also enabled simultaneous detection of other PQAs

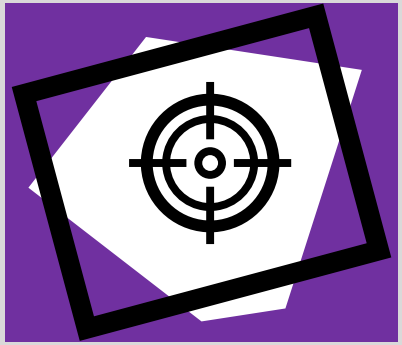


Continued Areas of Improvement for MAM

New Peak Detection

Bioinformatics

Qualification



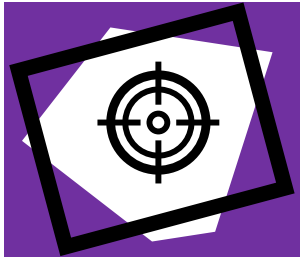
GMP MAM

Continued Area of Focus for MAM

New Peak Detection

- Attribute monitoring is targeted. Without NPD, critical information can be missed
- False positives need to be eliminated

Qualification



- Finalizing qualification approach with efforts ongoing
- Work ensures confidence in method and enhances performance understanding

Bioinformatics

- MAM generates a wealth of information
- Tools to associate MAM data with other attributes are needed

GMP MAM

- Strategy discussions for cGMP MAM ongoing
- Consistently evaluating instrumentation and software to remain current

Conclusions

- MAM is a valuable tool within Pfizer during product development due to the technology's unprecedented speed and information
- Pfizer has overcome challenges to enable a more seamless implementation of MAM
- MAM data are making significant impacts in Pfizer's portfolio
- Pfizer looks forward to continue to innovate and develop MAM for routine support in product development

Acknowledgements

2021 MAM Team Members

- Andrew Dawdy – MSBC
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- Keith Lutke – QC
- Nataliya Parahuz – PPL2
- Bradley Bare – MSBC
- Natalia Kozlova – MSBC
- Beth McCoy – MSBC
- Jay Kang – PPL3
- Qing Yu – PPL3
- Vamsi Kandhi – PPL3
- Daniel Ryan – MSBC
- Anji Trujillo – MSBC
- Zhenjiu Liu – PPL2

+ Pfizer Team Leadership

- Carly Daniels – Team Co-lead, PPL2
- Keith Johnson – Team Co-lead, PPL3
- James Carroll– Team Sponsor, PPL1
- Jason Rouse – Team Sponsor, MSBC
- Justin Sperry – VP BTxPS T&I
- Meg Ruesch – VP ARD

- Herb Runnels – BIT
- Phoebe Baldus – BIT
- Vess Mitaksov – VATT
- Amanda Werle – PPL1
- Leah Wang - MSBC

Thermo Team

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- Brie Gervin
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- Haichuan Liu
- Royston Quintyn
- Kai Scheffler
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- Betty Woo
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