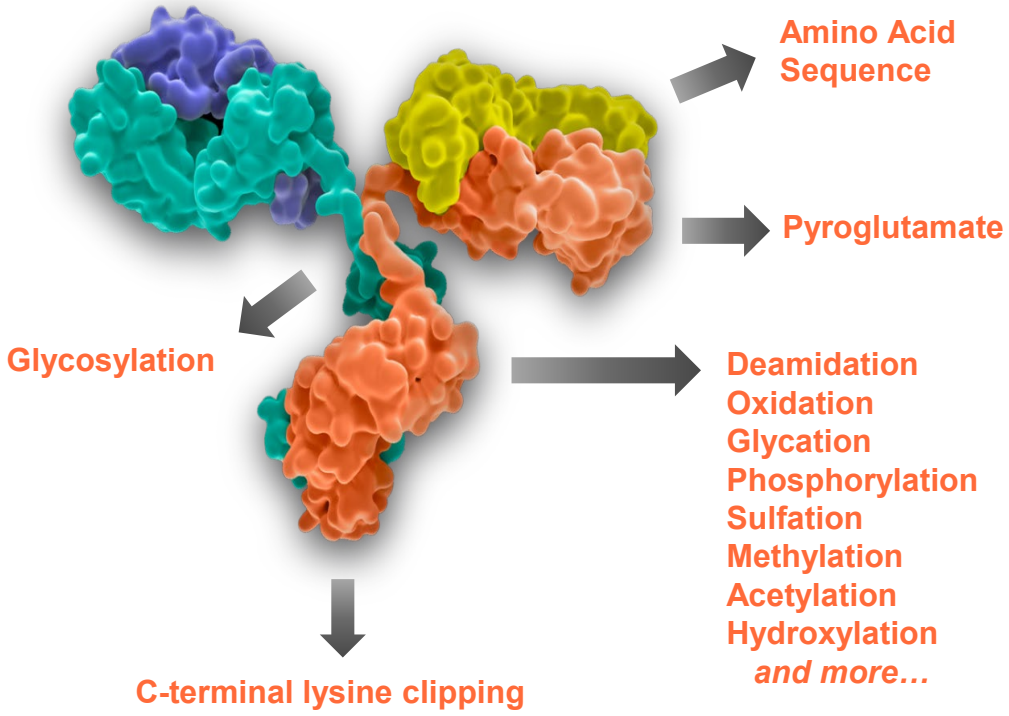
Assessment of the performance of the multi-attribute method (MAM) vs conventional QC methods for evaluation of Product Quality Attributes of adalimumab and etanercept

Diane McCarthy, PhD
October 16, 2023

* Award FAIN No.# U01FD007762 is fully funded by the Food and Drug Administration (FDA) in the amount of $1,530,721

Introduction to Multi-attribute Methods

- Monoclonal antibodies and other protein therapeutics are susceptible to many modifications during and after production
  - Some modifications impact function
  - Others are stability indicating
- A multi-attribute method could use any technology that allows a scientist to investigate multiple quality attributes at the same time
  - Mass spectrometry has emerged as the most mature and widely used platform for MAM



Comparison of MAM to Conventional Methods

Product Quality Attribute		MAM	Conventional Method				
		Pep Map LC-MS	SEC	IEX/cIEF	rCE-SDS	nrCE-SDS	Glycan by HILIC
Identity		+	-	+/-	-	-	-
Soluble aggregates		-	+	-	-	+/-	-
Fragments/Clips		+	+/-	-	+	+	-
Amino Acid Mutation/Mis-incorporation		+	-	-	-	-	-
Cys related modifications	Unpaired Cys	+	-	+/-	-	-	-
	Disulfide Isoform	+	-	-	-	-	-
	Thioether	+	-	-	-	-	-
Glycosylation	N-linked Glycosylation	+	-	+/-	-	-	+
	Non-Glycosylated	+	-	-	+	-	-
	O-Linked Glycan (Ser, Thr)	+	-	+/-	-	-	-
Isomerization (Asp)		+	-	+/-	-	-	-
Oxidation (Met, Trp, Cys)		+	-	-	-	-	-
Hydroxylysine		+	-	-	-	-	-
Charge variants	Deamidation (Asn, Gln)	+	-	+	-	-	-
	Glycation	+	-	+	-	-	-
N-Terminal modifications	Signal peptide	+	-	-	-	-	-
	N-Terminal pyroGlutamate	+	-	+	-	-	-
C-Terminal modifications	Lys deletion	+	-	+	-	-	-
	Amidation	+	-	+	-	-	-

Key

"+" application can be used

"-" application not commonly used

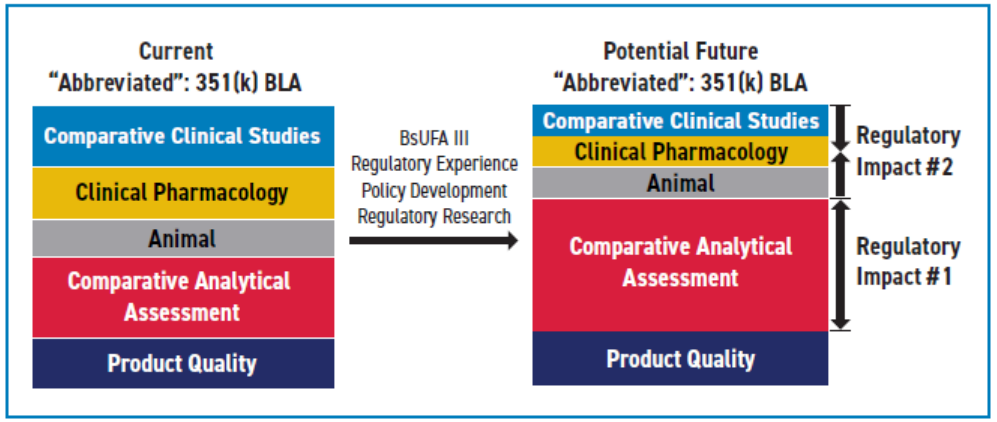
"+/-" application may be used

- MAM offers several potential advantages
  - Improved efficiency by replacing multiple technologies
  - More specific information on site of modification
  - Alignment with Quality by Design (QbD) concepts

Adapted from USP Proposed General Chapter *<1060> Mass Spectrometry Based Multi-Attribute Method for Therapeutic Proteins*

Relevance to BsUFA Regulatory Research Pilot Program

- While some large biopharma companies are implementing MAM in QC, MAM is not as commonly used in biosimilar companies
- Although MAM has been most commonly applied to mAbs, it is applicable to other therapeutics modalities, including therapeutic proteins, vaccines, and gene therapies
- Addresses goals of the BsUFA Regulatory Research Pilot Program by improving on new analytical techniques (goal 1c)
  - More widespread and consistent implementation of MAM would support the Potential Future "Abbreviated" pathway
  - MAM can provide more comprehensive comparative analytical assessment
  - Implementation of MAM can support more efficient analysis of product quality attributes (PQAs)



from BsUFA III Regulatory Research Pilot Program: Research Roadmap

Project Background and Objectives

- A 2019 publication by FDA staff* outlined 4 considerations for adoption of MAM in QC:
  1) risk assessment
  2) method validation
  3) new peak detection capability and specificity
  4) performance vs. conventional methods

- This project addresses #4: the performance of MAM vs conventional methods
  - Collecting data to support bridging from traditional techniques to MAM is a significant investment that can prevent or delay development of biosimilars
- Objectives
  - Support transitioning from conventional techniques to MAM by creating a knowledge base that can lower the barrier of entry to enable wider adoption of MAM by biosimilar manufacturers

* S Rogstad *et al Analytical Chemistry* **2019** *91* (22), 14170-14177 DOI: 10.1021/acs.analchem.9b03808

Overall Study Design

- Selected Adalimumab and Etanercept as model systems for mAbs and Fc fusion proteins due to availability of biosimilar and research grade products
- Assess and compare the ability of conventional QC methods and MAM-based methods to identify product quality attributes (PQAs)
- Correlate changes in those PQAs upon forced degradation with function (bioactivity, binding affinity, and structure)

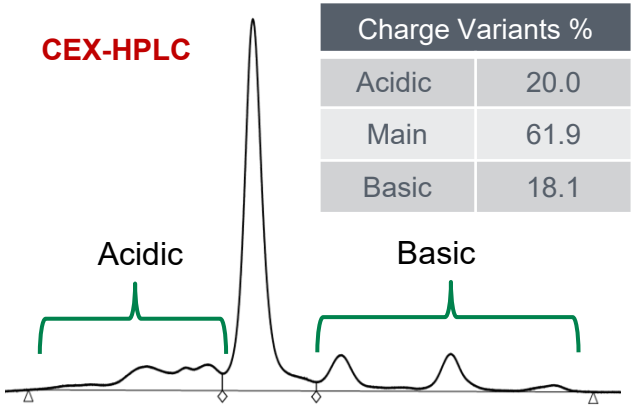
Comparison of Conventional and MAM Methods: Charge Variants

- Used USP mAb 001 Reference Standards as a model system and for establishing System Suitability

Conventional Methods

CEX-HPLC

Charge Variants %	
Acidic	20.0
Main	61.9
Basic	18.1



Location and specific modifications cannot be identified using conventional methods

Multi-attribute Method

Heavy chain:

QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYNMHWVKQT PGRGLEWIGA IYPGNGDTSY
NQKFKGKATL TADKSSSTAY MQLSSLTSED SAVYYCARST YYGGDWYFNV WGAGTTVTVS
AASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS
SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKKAE PKSCDKTHTC PPCPAPELLG
GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD
ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR
WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K

Light chain:

QIVLSQSPAI LSASPGEKVT MTCRASSSVS YIHWFQQKPG SSPKPWIYAT SNLASGVPVR
FSGSGSGTSY SLTISRVEAE DAATYYCQQW TSNPPTFGGG TKLEIKRTVA APSVFIFPPS
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

x 8 sites

Amino Acid	% Deamidation
HC N55	8.7%
HC N61	2.0%
HC N319	10.4%
HC N365	1.8%
HC N388	2.7%
HC N438	2.4%
LC N136	0.9%
LC Q198	0.7%

Chain	% Lys Clipping
Heavy Chain	93.3%

Chain	% Pyroglutamate
Heavy Chain	96.9%
Light Chain	96.4%

Glycan	% of Total Glycan
A2G2F	5.9%
A2G1F	41.0%
A1G0F	9.0%
A2G0F	44.1%

Amino Acid	% Oxidation
HC M34	2.6%
HC M256	6.4%
HC M432	2.9%

Timeline and Project Progress

01

Forced degradation of biotherapeutics from multiple sources

- Obtaining materials for evaluation
- Forced degradation

03

Identification and relative quantification of modifications using a MAM workflow

- MAM method development/optimization for characterization
- Analysis of samples from Aim #1

02

Evaluation of PQAs including charge variants and glycosylation, using conventional methods

- Evaluation of charge variants
- Evaluation of glycosylation
- Assessment of size variants

04

Assessment of bioactivity and structure analysis of biotherapeutic products and stressed samples

- Replicate forced degradation study & analysis in 2nd lab
- Bioactivity assessment
- Assessment of binding
- Structural analysis

Expected Outcome

- Comparison of conventional methods vs. MAM
  - Sensitivity of detection and quantification of variants that impact Product Quality Attributes
  - Correlation of MAM results vs. conventional techniques
  - Association of modifications with differences in structure and biological function
- Roadmap for implementation of MAM
  - Relevant method comparisons
  - Sources of variability across labs
  - Approaches for establishing system suitability
- Publicly available dataset