Development and Characterization of Reference Standards to Support Analysis of Charge Variants

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USP Biologics, Rockville Maryland

Outline

- Introduction to USP
- Collaborative study of USP mAb charge variants using cIEF and icIEF
- Charge variants during real-time stability and forced degradation
- icIEF characterization of ‘co-formulated’ USP mAbs
- Ongoing characterization by CE-MS and MAM

Mission

To improve global health through public standards and related programs that help ensure the quality, safety and benefit of medicines and foods

Collaborating to achieve our mission

Partnering with our expert volunteers

Access to quality vaccines and therapeutics

Partnering with global regulators, including U.S. and international food and drug authorities

Partnering with stakeholders, including industry, practitioners, and academia

USP staff and volunteers have expertise across the supply chain

Biologics

Biologics Monographs 1–Peptides & Oligonucleotides
Michael De Felippis

Biologics Monographs 2–Proteins
Wendy Saffell-Clemmer

Biologics Monographs 3–Complex Biologics & Vaccines
Earl Zablackis

Biologics Monographs 4–Antibiotics
Matthew Borer

Biologics Monographs 5–Advanced Therapies
Mehrshid Alai

Small Molecules

Small Molecules 1
Mary Seibel

Small Molecules 2
Justin Pennington

Small Molecules 3
Eric Kesslen

Small Molecules 4
Kim Huynh-Ba

Small Molecules 5
Amy Karren

Over-the-Counter (OTC) Methods & Approaches
Raphael Ornaf

Excipients

Simple Excipients
Eric Munson

Complex Excipients
Otilia Koo

Excipients Test Methods
Chris Moreton

General Chapters

General Chapters–Dosage Forms
Martin Coffey

General Chapters–Chemical Analysis
Nancy Lewen

General Chapters–Microbiology
Donald Singer

General Chapters–Packaging & Distribution
Renaud Janssen

General Chapters–Measurement & Data Quality
Jane Weitzel

General Chapters–Statistics
Charles Tan

General Chapters–Physical Analysis
Xiaorong He

Healthcare Quality & Safety

Nomenclature & Labeling
Stephanie Crawford

Healthcare Safety & Quality
Melody Ryan

Compounding
Brenda Jensen

Healthcare Information & Technology
Jeanne Tuttle

Dietary Supplements & Herbal Medicines, Food Ingredients

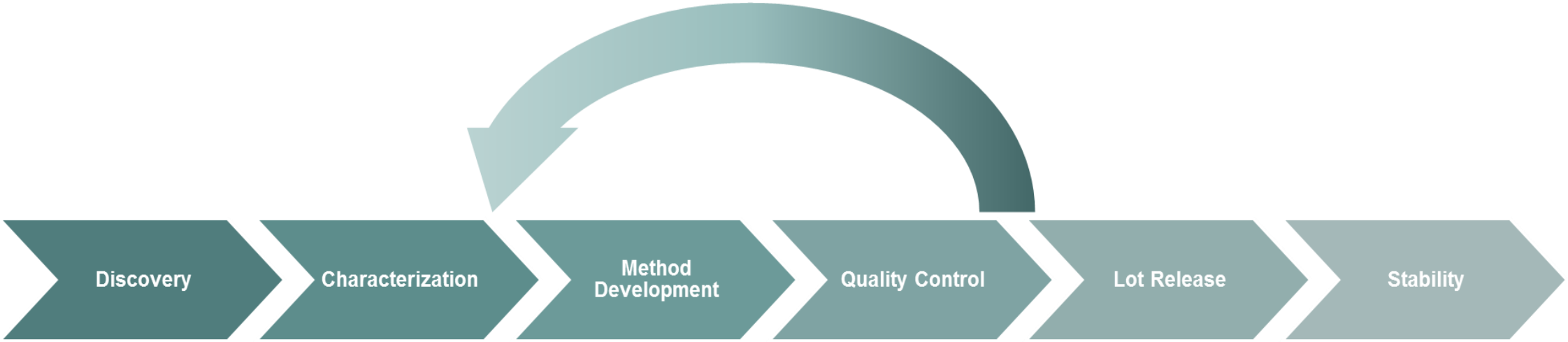
Botanical Dietary Supplements & Herbal Medicines
Robin Marles

Non-botanical Dietary Supplements
Guido F Pauli

Dietary Supplements Admission Evaluation & Labeling
Tieraona Low Dog

Food Ingredients
Jon DeVries

Evolving approaches: Enabling a culture of quality through early stakeholder engagement



- USP Biologics is expanding standards development to cover quality testing **throughout the overall biopharmaceutical product lifecycle**.
  - Early **engagement with stakeholders** to identify common bottlenecks and solutions
  - Focus on **analytical tools** and **performance standards** to support quality assessment
  - Support for **raw materials** qualification and advanced biomanufacturing
  - Standards to support development and testing of **emerging therapeutic modalities**

Collaborative study of USP mAb standards using cIEF and icIEF

- Charge variants impact antigen and FcR binding, immunogenicity and stability
- Isoelectric point (pI) values for identity
- Charge profile for identity
- Quantitation for purity (quantitative or semi-quantitative)

Collaborative characterization of mAbs

	USP mAb 001, monoclonal IgG1	USP mAb 002, monoclonal IgG1	USP mAb 003, monoclonal IgG1
USP Catalog #	1445539	1445547	1445595
CAS #	174722-31-7	216974-75-3	912628-39-8
MW	~147,000 Da	~150,000 Da	~146,000 Da
Package size	200 µl solution (2 mg protein content)	200 µl solution (2 mg protein content)	200 µl solution (2 mg protein content)

- Released in 2020 following characterization in 4 laboratory collaborative study
- “Performance standards” with no compendial use or reference in USP-NF
- USP’s compendial monoclonal standard to be used in method chapter <129> is *USP Monoclonal IgG System Suitability RS*

Certificate values

- SEC-HPLC chromatogram, average values
- cIEF method and electropherogram, average values
- icIEF method and electropherogram , average values
- CE-SDS (reduced and non-reduced) electropherogram, average values
- Glycan CE-LIF electropherogram
- Glycan LC-FLR-MS chromatogram
- Intact mass analysis deconvoluted spectrum, theoretical mass

Charge variant collaborative study

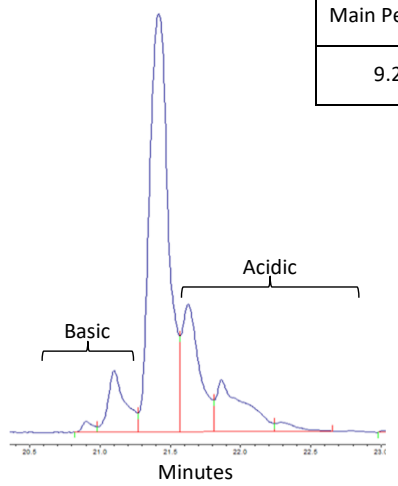
- Total of five participating laboratories
  - Three for cIEF, all using PA800 Plus
  - Three for icIEF, using iCE3 and Maurice
- USP optimized methods based on manufacture's recommendations
- Certificates include method summary, electropherograms, and average values
- Technical note with discussion and more information

  https://www.usp.org/sites/default/files/usp/document/our-work/biologics/cief-icief-tech-note-v6-final.pdf

Typical Electropherogram

USP mAb 001, Monoclonal IgG1 RS

Catalog Number: 1445539
Lot: F11920
Test: Capillary Isoelectric Focusing (cIEF)
Instrument: SciEx, PA800 Plus **Method**: Focus Period 1: 15 minutes, 25,000 V; Focus Period 2: 25 minutes, 30,000 V Sample Load Duration: 150 seconds
Detector: UV280
Capillary: AB SciEx, Neutral capillary
pI Standards: pI 7.0 and pI 10.0
Carrier ampholyte: Pharmalyte 3-10

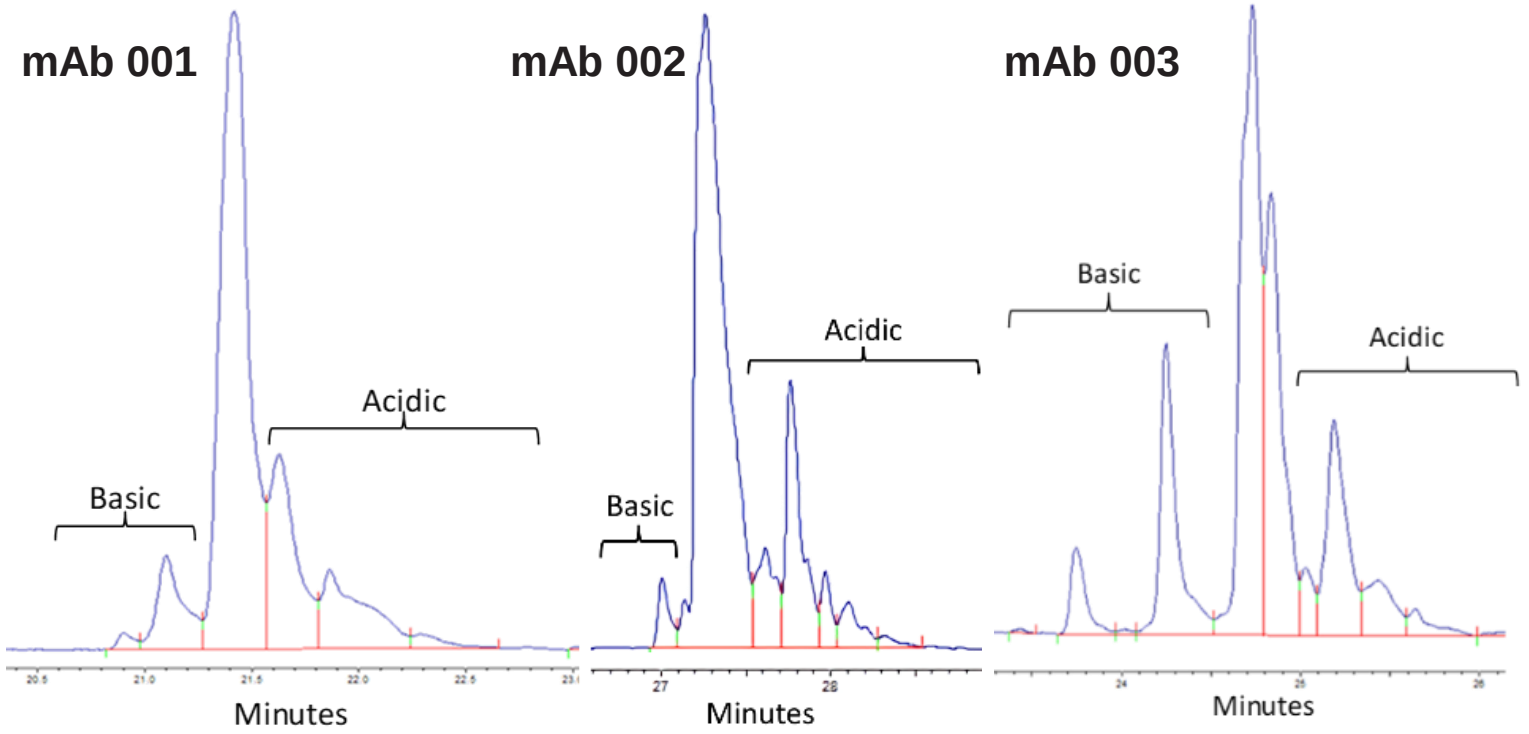


Main Peak pI	Main	Acidic	Basic
9.2	60%	32%	8%

Note: Main peak pI and % species vary based on capillary condition, reagents, instrument, method, and integration parameters.

This electropherogram is supplied for information only, unless otherwise specified in an applicable monograph or general chapter.

Charge variants determined by cIEF

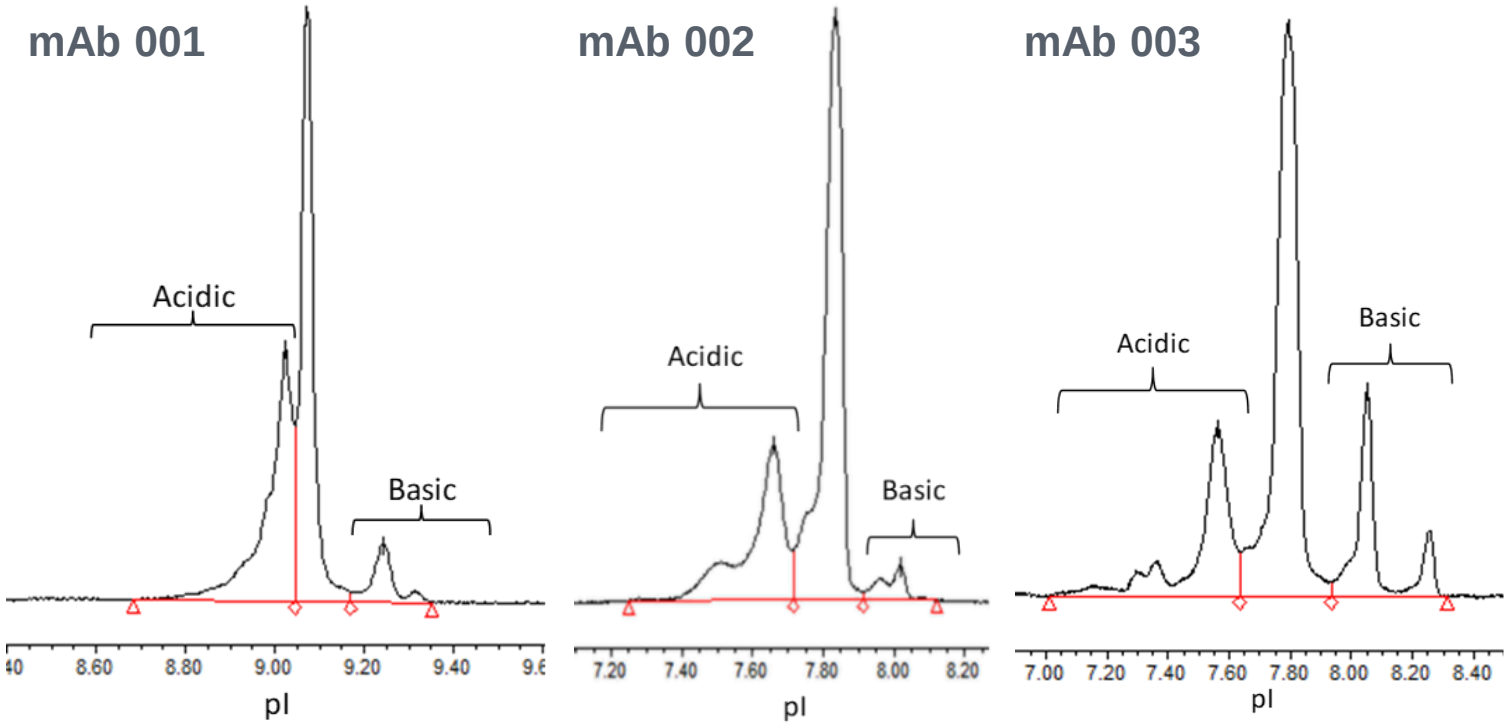


- Similar charge profiles between labs
- Very consistent inter-lab pI
- Inter-lab standard deviation of species measurements less than ~5% (less than ~ 20% RSD)

Reference Standard	pI			Acidic			Main			Basic		
	Average	Std Dev	%RSD	Average	Std Dev	%RSD	Average	Std Dev	%RSD	Average	Std Dev	%RSD
mAb 001	9.2	0.04	0.5%	32%	2.08%	6.5%	60%	1.34%	2.2%	8%	1.31%	16.5%
mAb 002	7.8	0.03	0.4%	31%	3.09%	10.0%	65%	2.51%	3.9%	4%	0.62%	15.8%
mAb 003	7.7	0.02	0.3%	25%	5.02%	20.1%	55%	4.92%	9.0%	20%	0.71%	3.5%

Note: Main peak pI and % species vary based on capillary condition, reagents, instrument, method, and integration parameters. Values are the average from three labs.

Charge variants determined by icIEF



- Similar charge profiles between labs
- Very consistent inter-lab pI
- Inter-lab standard deviation of species measurements less than ~6% (less than ~20% RSD)

Reference Standard	pI			Acidic			Main			Basic		
	Average	Std Dev	%RSD	Average	Std Dev	%RSD	Average	Std Dev	%RSD	Average	Std Dev	%RSD
mAb 001	9.2	0.10	1.1%	38%	2.72	7.1%	54%	3.04	5.7%	8%	1.36	17.0%
mAb 002	7.9	0.08	1.0%	29%	6.09	20.8%	66%	5.98	9.0%	4%	0.31	7.1%
mAb 003	7.9	0.08	1.1%	20%	2.62	13.2%	62%	2.33	3.8%	18%	0.65	3.6%

Note: Main peak pI and % species vary based on capillary condition, reagents, instrument, method, and integration parameters. Values are from three labs and two instrument models.

Comparison between cIEF and icIEF

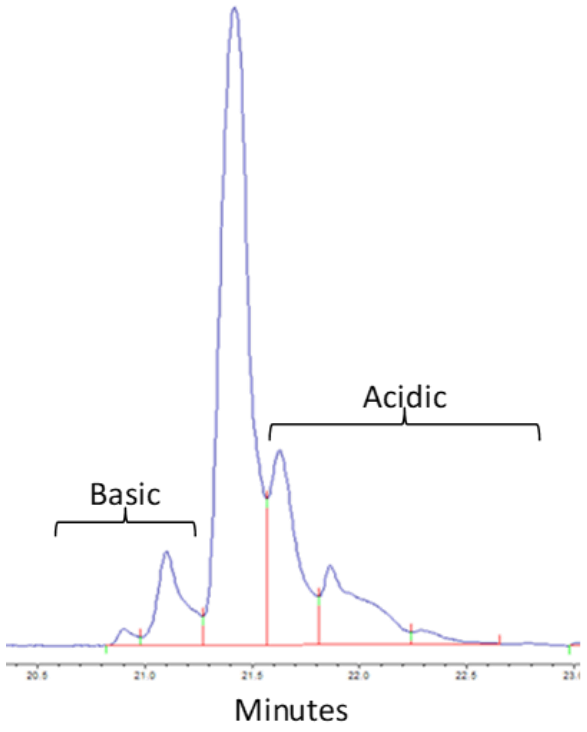
Reference Standard	Method	pI	% Main	% Acidic	% Basic
mAb 001	cIEF	9.2	60%	32%	8%
	icIEF	9.2	54%	38%	8%
	Difference	0	6%	-6%	0%
mAb 002	cIEF	7.8	65%	31%	4%
	icIEF	7.9	66%	29%	4%
	Difference	-0.1	-1%	2%	0%
mAb 003	cIEF	7.7	55%	25%	20%
	icIEF	7.9	62%	20%	18%
	Difference	-0.2	-7%	5%	2%

Inter-method precision

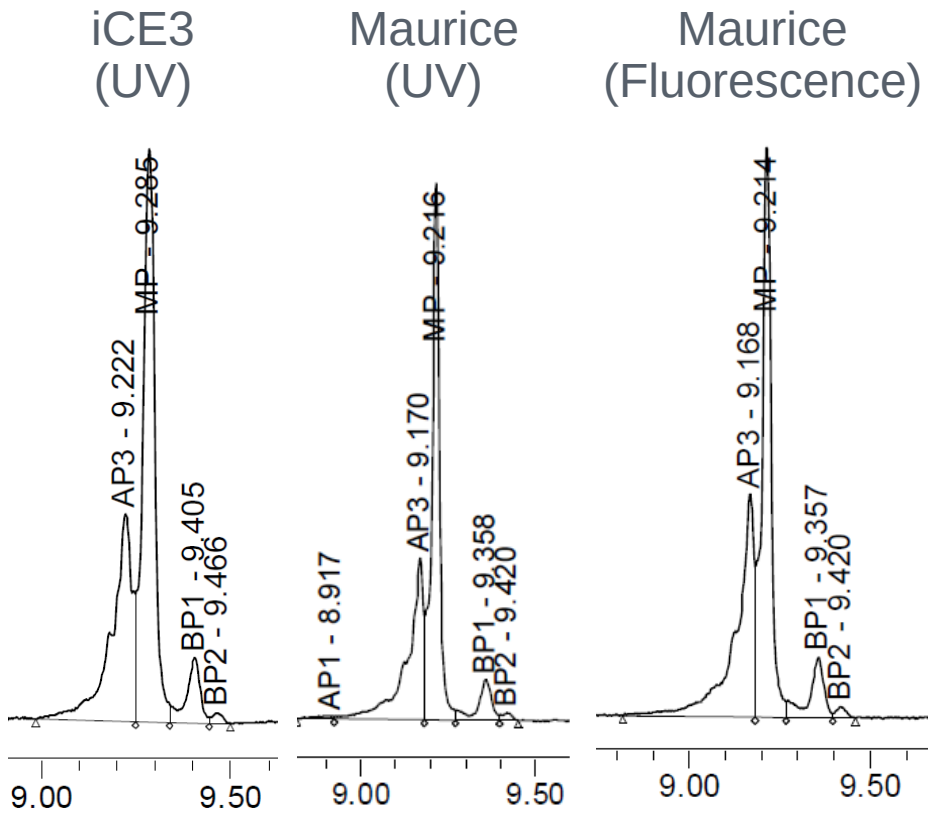
- pI difference ≤ 0.2
- % Group differences ≤ 7%

mAb 001

cIEF



icIEF



Charge variants during real-time stability and forced degradation

- Real-time stability study under slightly stressed conditions to predict future stability and stability during typical use.
- Forced degradation study to understand the evolution of charge variants as stability indicating attributes.

Real-time stability study

- Real-time stability conditions chosen to reflect typical customer storage and use cases
- Maximum of 6 months

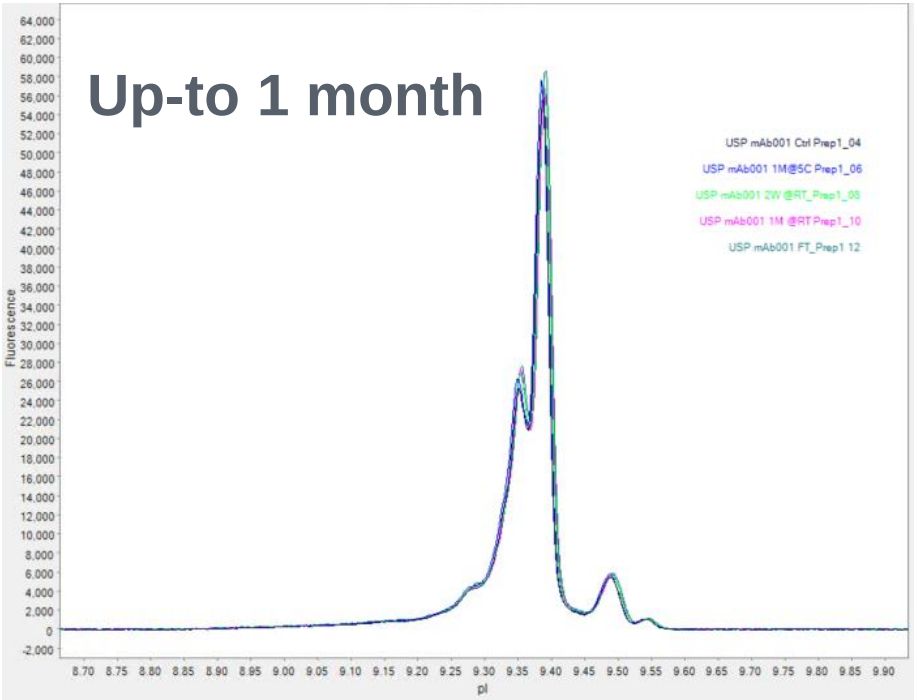
Study design

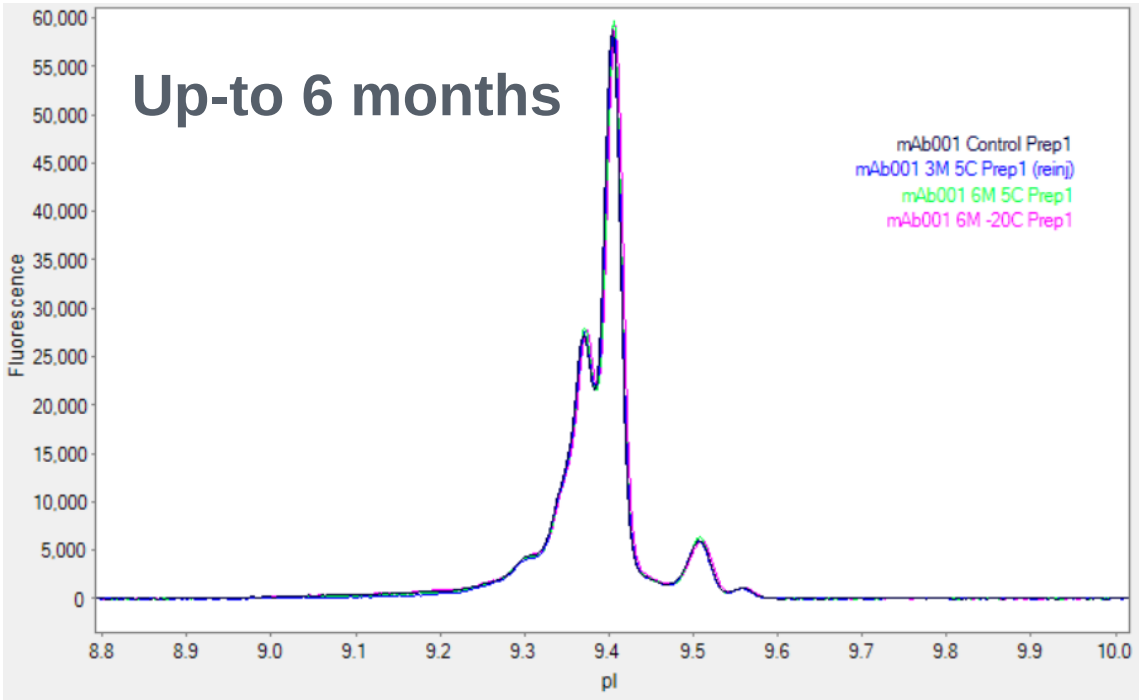
Stability conditions	2 cycles of freeze and thaw	2 week	1 month	3 month	6 month
-70° (control)			X		X
-20°					X
5°			X	X	X
Ambient		X	X		
2 cycles of freeze-thaw	X				

Outcomes

- mAb 001, 002, 003
  - Similar stability profiles
- SEC-HPLC from <129>
  - Change in impurities below limit of quantitation
- CE-SDS Nonreducing from <129>
  - Change in impurities below limit of quantitation
- icIEF for charge variants

Real-time stability: mAb 001



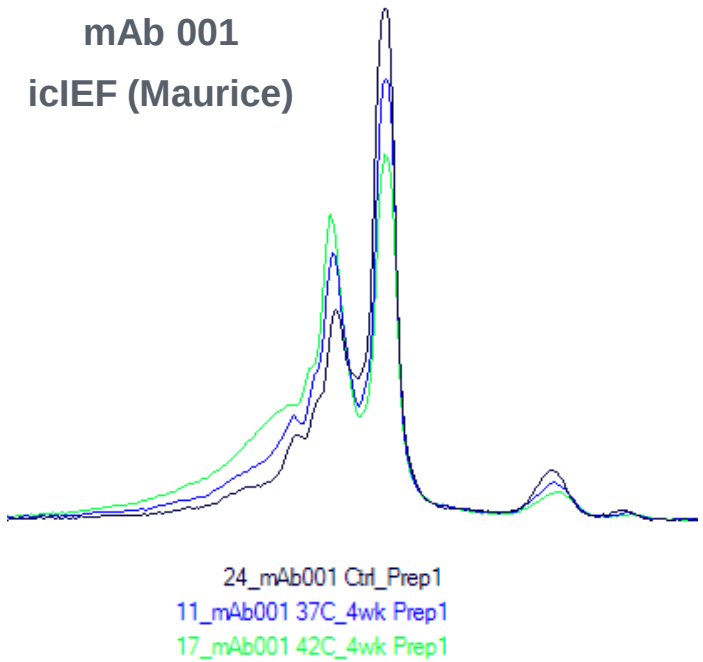


Treatment	Main peak pI	% Acidic	%Main	%Basic
Control (<-70°C)	9.4	44.1	47.8	8.1
1M @ 5°C	9.4	43.0	49.0	8.0
2W @ Room Temp	9.4	44.1	47.8	8.1
1M @ Room Temp	9.4	44.0	48.2	7.9
2X Freeze Thaw	9.4	43.1	48.9	8.0

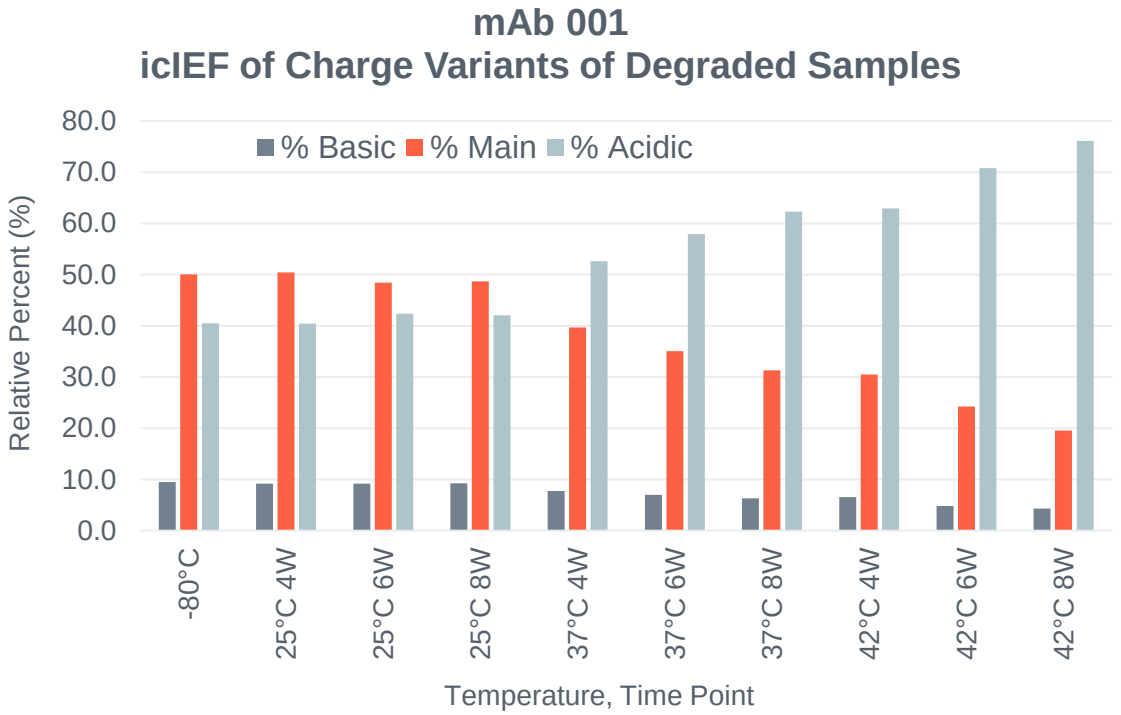
Treatment	Main peak pI	% Acidic	%Main	%Basic
Control (<-70°C)	9.4	42.6	49.4	8.0
3M @ 5°C	9.4	44.0	47.9	8.1
6M @ 5°C	9.4	44.1	47.9	8.0
6M @ -20°C	9.4	42.9	49.1	8.1

Forced degradation study

- A forced degradation study was performed to evaluate the charge variants produced by thermal degradation and if the resulting material had potential as a Performance Standard.
- Samples of USP mAb 001 and USP mAb 002 were held at 25°C, 37°C, and 42°C for 4, 6 and 8 Weeks and analyzed by icIEF (Maurice)



icIEF overlays of degraded USP mAb 001 at -80°C, 37°C, and 42°C for 4 weeks.



icIEF relative percent of Acidic, Basic, and Main species of degraded USP mAb 001 at -80°C, 37°C, and 42°C for 4, 6, and 8 weeks.

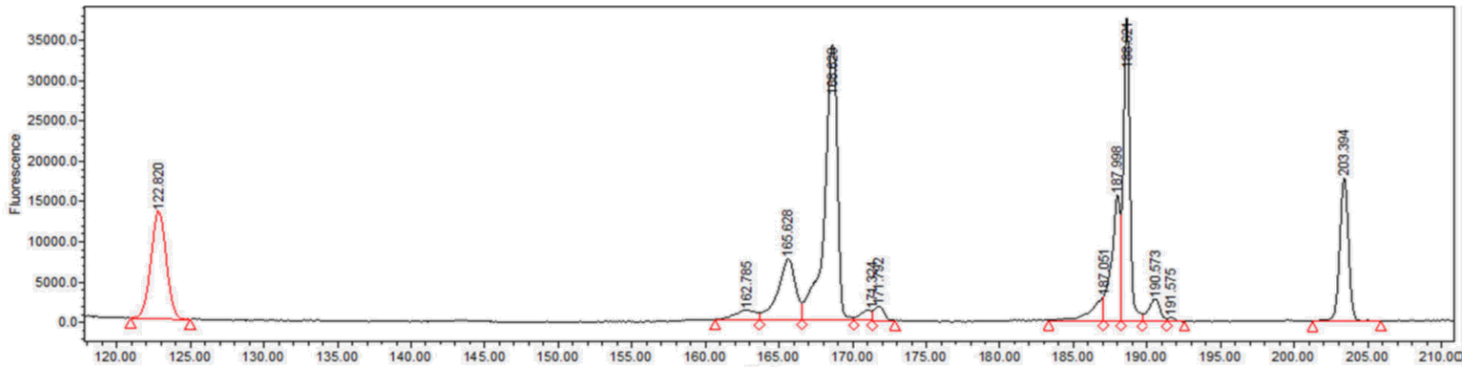
3 icIEF characterization of mixture of USP mAbs

- USP mAbs were used to create surrogate co-formulations and the USP method was used for separation
- Evaluated: Repeatability, Reproducibility, Accuracy, Linearity

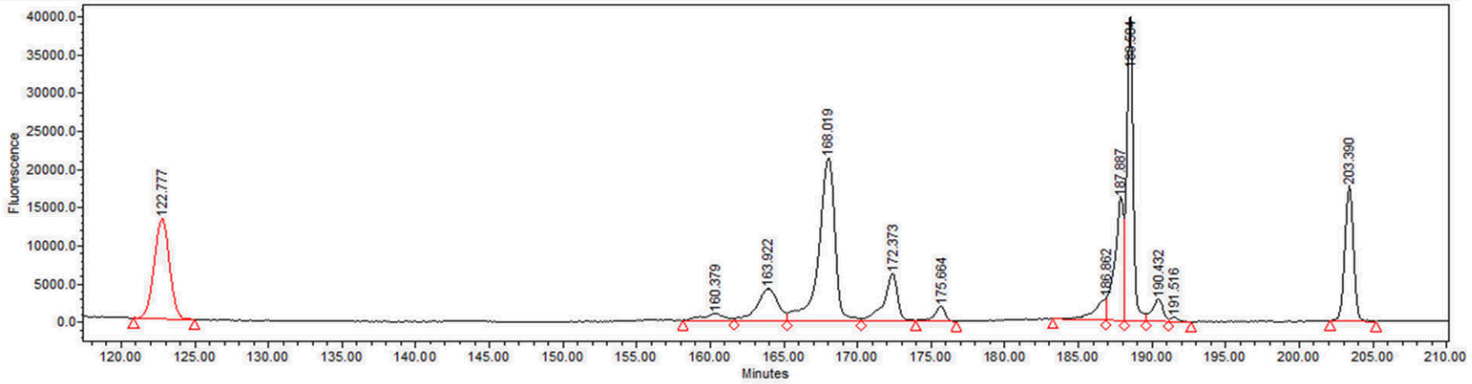
icIEF characterization of mixed USP mAb

- Several co-formulated mAbs are under development
- Several examples of cIEF methods to determine charge heterogeneity and antibody ratio for co-formulated mAbs have been reported[1, 2]
- USP mAbs were used to create surrogate co-formulations (mixtures) and evaluated with the USP method
  - mAb 001 pI 9.2
  - mAb 002 pI 7.9
  - mAb 003 pI 7.9

mAb 001 + mAb 002



mAb 001 + mAb 003



1. CEPharm 2021 Poster: Development and Qualification of a cIEF Method to Determine Charge Heterogeneity and Antibody Ratio for Co-Formulated mAbs by Weichen Xu, BioPharmaceuticals Development, R&D, AstraZeneca, Gaithersburg, US
2. Charge variants characterization and release assay development for co-formulated antibodies as a combination therapy, M. Cao et.al., MABS 2019

Evaluation of icIEF on mixed USP mAbs

- **1:1 mixture** (mg/mL) of mAbs analyzed by collaborative study method
  - pI, Relative %, and Ratio by total peak area
- Standard curve normalized to 1 mg/mL total protein for Linearity

Parameter	Experimental Design	Results (% RSD)	
		pI	Acidic, Main, Basic %
Repeatability	n=6 injections	< 0.1%	< 7%
Reproducibility	6 injections, 3 runs, n=18	< 0.1%	< 5%

Parameter	Experimental Design	Results
Accuracy (mAb ratio)	7 levels, ratios from 0.7 to 1.65	Recovery 98.1 to 100.7%
Linearity	Theoretical vs Experimental ratio of total peak area	R^2 = 0.9987 (Absorbance)

- **Detection bias** (absorbance vs fluorescence)
  - Linearity and Accuracy showed mAb specific bias
  - Ratio corrected area mAb001/mAb002
    - 0.97 Absorbance, 0.66 Fluorescence
  - Ratio corrected area mAb001/mAb003
    - 1.15 Absorbance, 0.84 Fluorescence

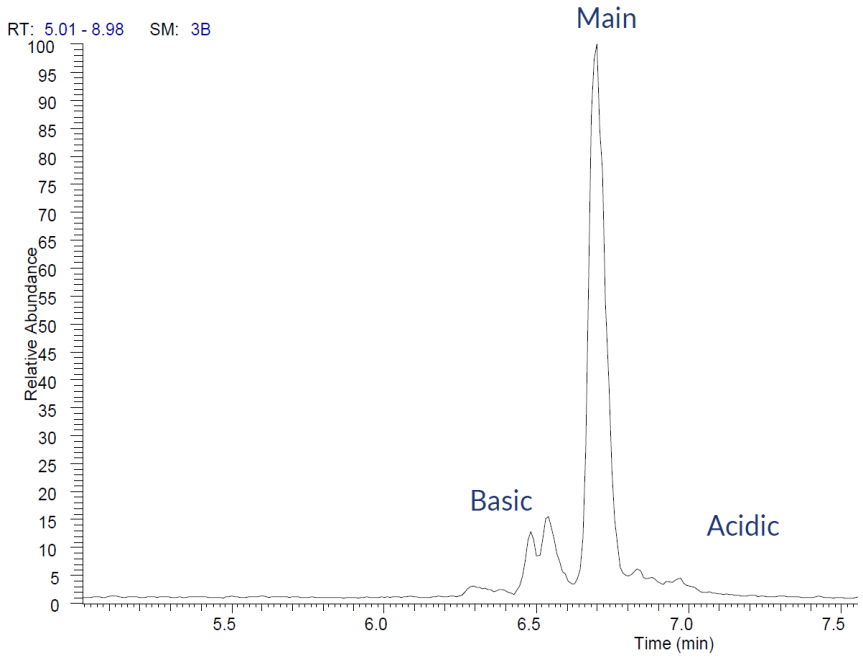
Ongoing characterization by CE-MS and MAM

- Characterization of USP mAbs by CE-MS
  - Summary of charge variant data
- Characterization of USP mAbs using MAM
  - Preliminary charge variant data
  - Deamidation results were method dependent

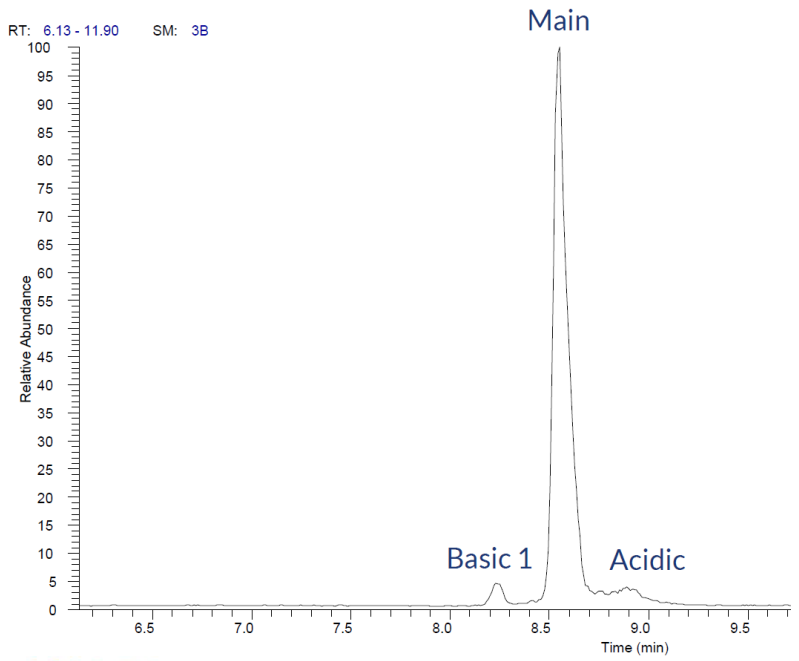
CE-MS characterization of USP mAbs

Native Antibody Analysis (ZipChip by 908 Devices)

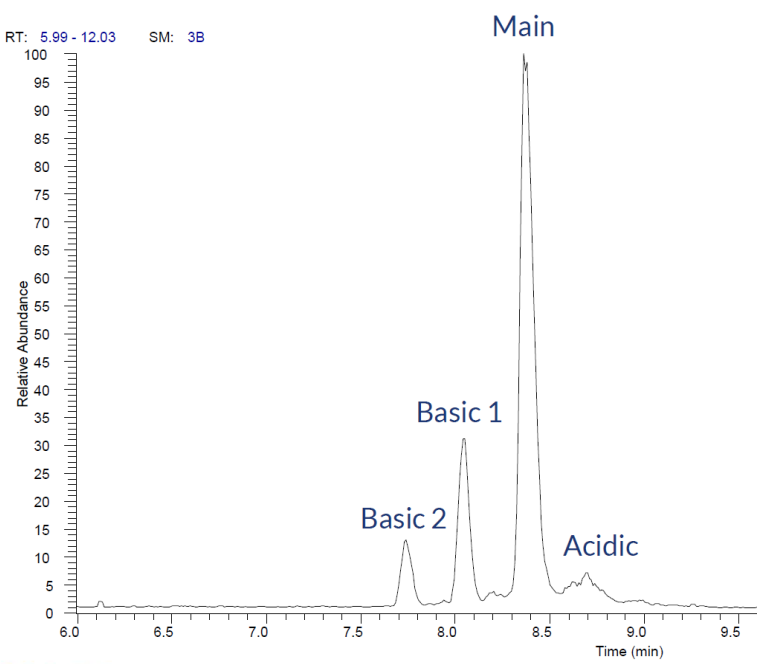
USP mAb 001



USP mAb 002



USP mAb 003



- The ZipChip Native Antibodies Kit with HRN (high resolution) chip
  - Protocol: *Boosting Sensitivity for Intact Antibody Charge Variant Analysis*
- Thermo Exactive Plus EMR Orbitrap Mass Spectrometer

Charge variant summary

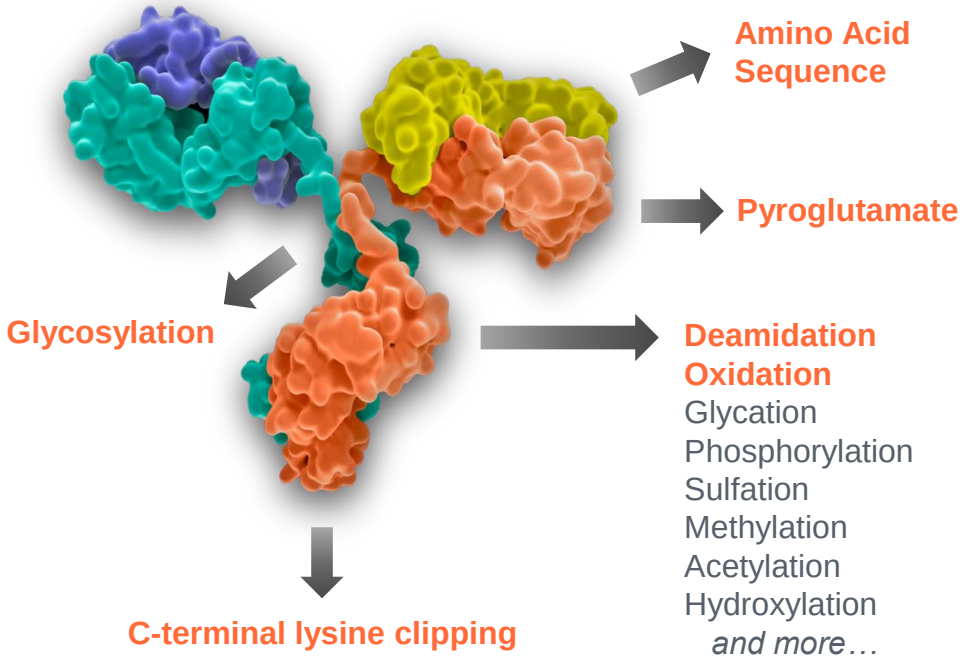
Native Antibody Analysis (ZipChip by 908 Devices)

	mAb 001	mAb 002	mAb 003
	mass in m/z		
Main	147,237.00	149,189.02	145,737.70
Basic			
+1 Lys	147,364.97	149,323.94	145,865.89
+2 Lys	147,490.67		145,993.44
+16 Da Variant	147,253.02		
Acidic			
Deamidation	147,239.95	149,199.22	145,741.08
	147,240.97		
Sialic acid	147,693.64		
	147,853.13		

- mAb 001 - Variants in the acidic region mainly appear to be deamidation, sialic acid species, and additional glycoforms that could be more complex branching structures
- mAb 002 – one basic variant and one low abundance acidic variant with mass shift of ~1 Da indicative of deamidation
- mAb 003 G0F/G1F is the most abundant glycoform in the main variant, but G0F/G0F is most abundant in the basic variants.

Multi-Attribute Methods (MAM)

- MAM leverages the specificity of mass spectrometry
  - Can assess multiple quality attributes
  - Has been used in place of traditional methods
    - Capillary electrophoresis, cation exchange chromatography, peptide mapping, and glycan analysis



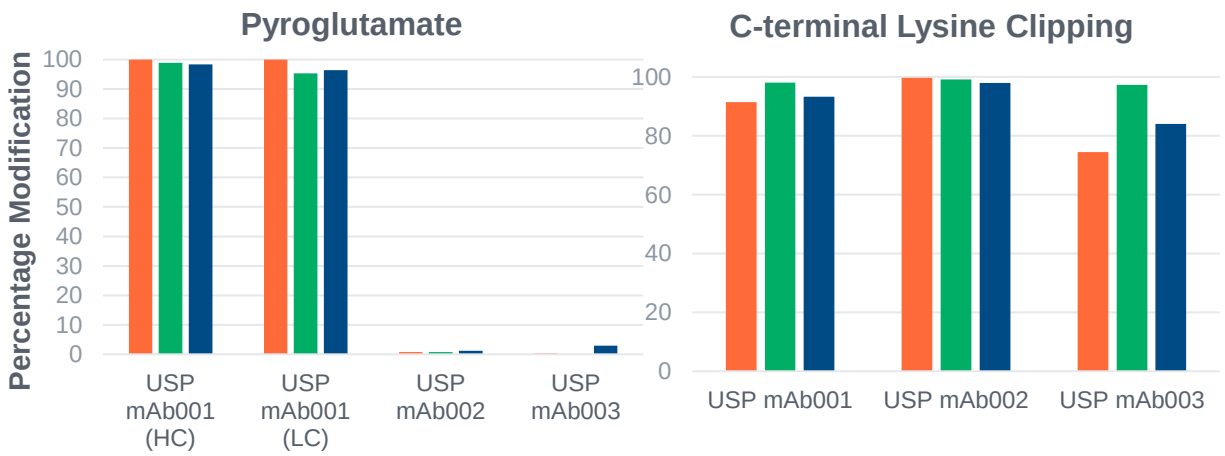
USP Efforts

- 2020 Stakeholder Forum on MAM
- MAM Expert Panel
  - Writing chapter on best practices
- Collaborations with Universities to evaluate utility of MAM
- Initiated development of pre-digested mAb standards
- USP MAM Exchange Community
  - Join at mam.usp.org

Preliminary MAM results for USP mAbs

Charge variants detected by MAM

- Compared data obtained from multiple labs and using multiple digestion methods
- Most results were consistent across labs and conditions
  - Lysine clipping
  - Pyroglutamate
  - Glycosylation
  - Oxidation



Lab B, Lab C, Lab D

- Differences in percent of deamidation ranged from undetectable to over 40% depending on reduction/alkylation and digestion conditions

Peptide	Modification	Relative % of Modification (USP mAb 001)		
		Lab A	Lab B Method 1	Lab B Method 2
Peptide 1		---	---	
	Oxidation	9.60%	9.80%	5.60%
Peptide 2		---	---	
	Deamidation	14.50%	6.60%	ND
	Oxidation	ND*	0.10%	0.20%
Peptide 3		---	---	
	Deamidation	41.80%	28.70%	ND
	Oxidation		0.04%	ND
Peptide 4		---	---	
	Deamidation	ND	9.10%	ND
Peptide 5		---	---	
	Deamidation	36.20%	10.40%	2.80%
Peptide 6		---	---	
	Deamidation	9.40%	8.20%	ND
	Oxidation	ND	1.90%	1.70%

Summary and Next Steps

- cIEF/icIEF introduced as new uses for USP mAb 001, 002, and 003 standards
- Real-time stability study completed (6M)
- Forced degradation studies on USP mAbs show increases in acidic variants and decreases in basic forms with time and temperature
- Demonstration of quantitation of forms in mock co-formulation
- Initial characterization of charge variants by CE-MS and MAM
- Next Steps
  - Further characterization of charge variants by CE-MS (ZipChip)
  - Evaluation of lab-to-lab variability for CE-MS
  - Expansion of mAb portfolio to include other isotypes and pIs

Questions

Empowering a healthy tomorrow