Applications and case studies of USP mAb standards

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Agenda

- Where are the needs
- Applications and case studies
- mAb Related Resources

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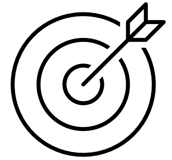
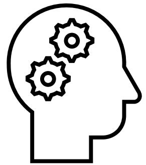
Where are the needs?

The Need: CMC analytical testing of product structure and function

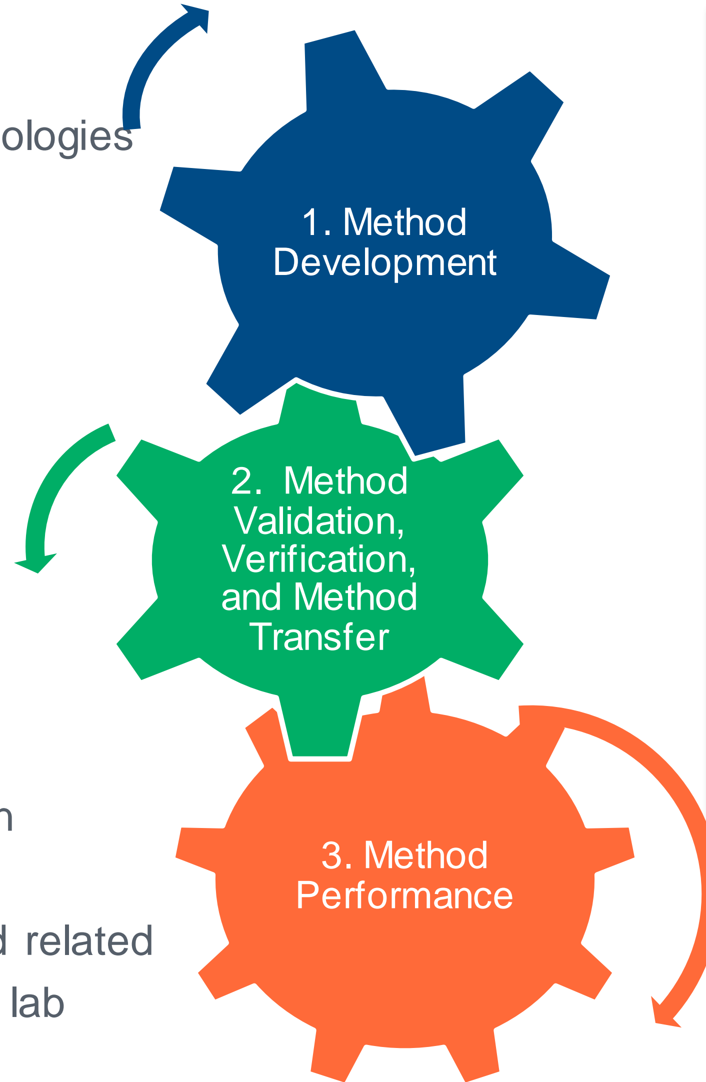


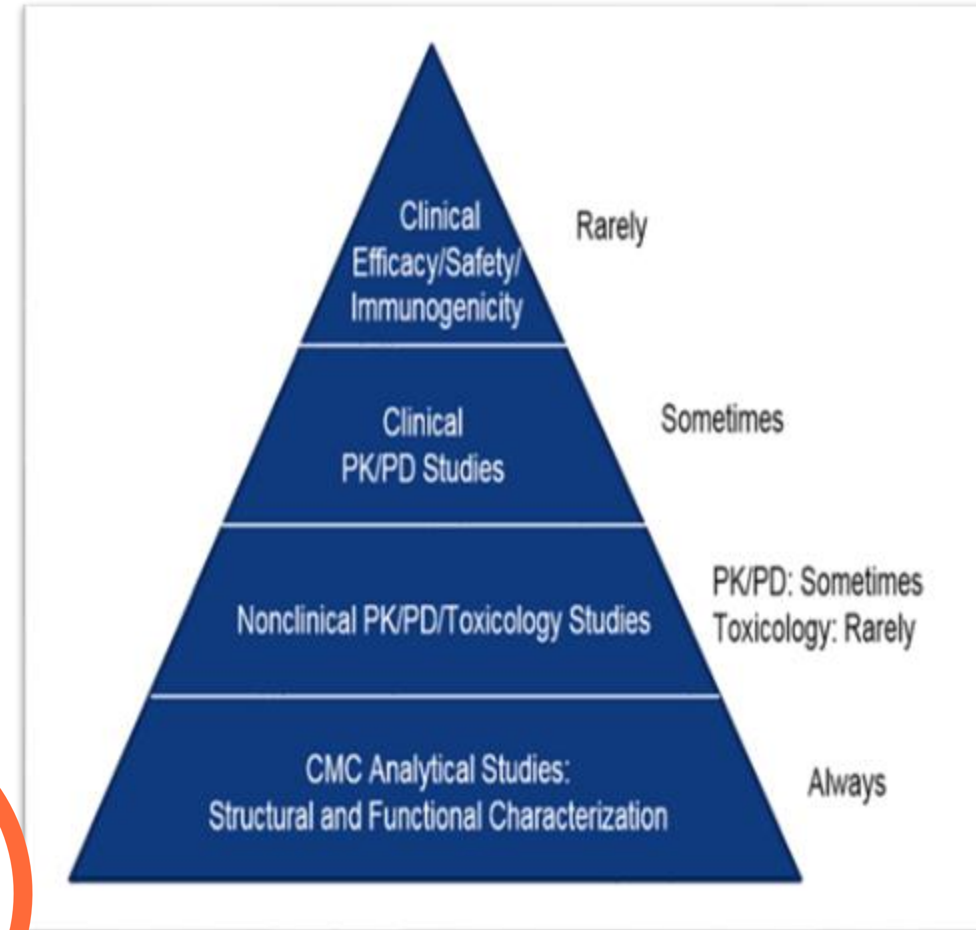
- Qualify changing test methods
- Can be applied to multiple technologies
- Optimize platform technologies
- Method prequalification



- Evaluate instrument/method
- Minimize variability

- External Control
  - Purity or identity indication
- Differentiate factors:
  - product-related vs method related
- Ensure consistency across the lab





- The foundation for comparability

The AAPS Journal (2018) 20: 109 DOI: 10.1208/s12248-018-0268-8

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Why USP mAbs Reference Standard?

USP mAb standards are **physical reference standard** that supports the product lifecycle.

The application of reference standards assists in monitoring **method performance, reducing analytical variance, and evaluating different stages** of research, process development, or production.

As opposed to simply being relevant to a particular drug substance or drug product, these standards are **applicable to product families or classes as a whole.**

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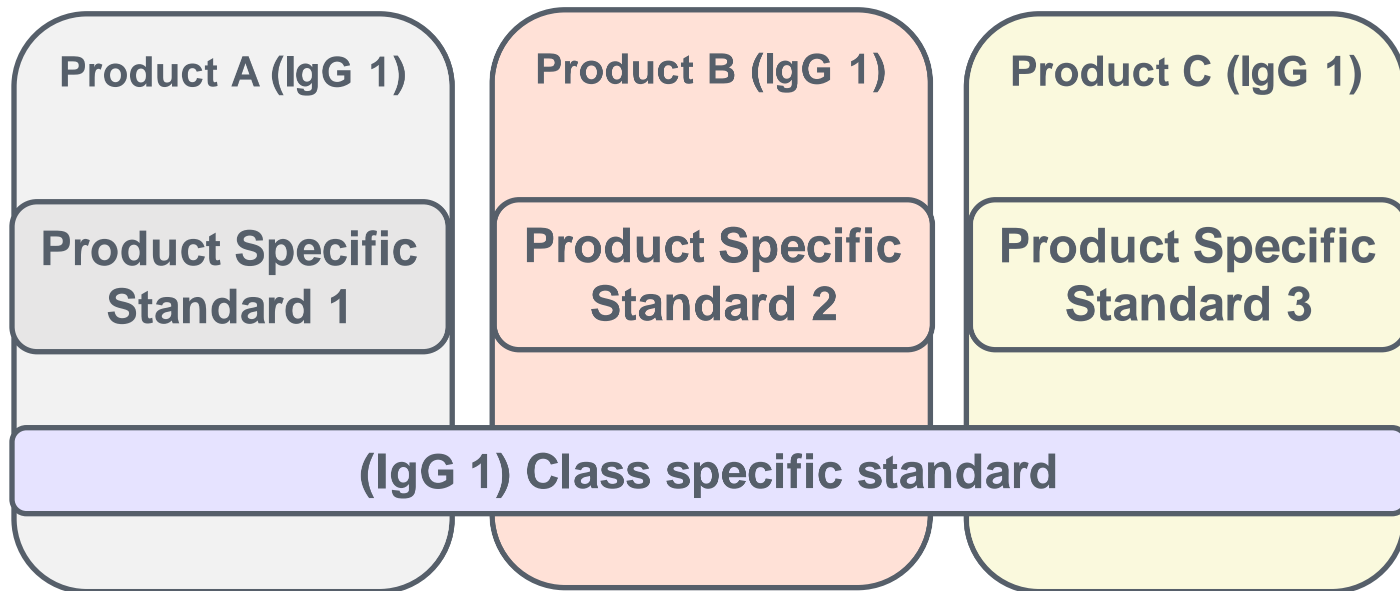
USP Biologics strategy and evolving approaches—enabling a culture of quality

Standards development to cover quality testing throughout the overall biopharmaceutical product lifecycle



- Ensure and demonstrate methods and process performance, system suitability and assays

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Evolving Approaches for USP standards

Product Specific Standards

Product specific quality attributes:

Peptide mapping, Impurity Analysis, potency

E.g. Filgrastim system suitability & Bioassay RS, Insulin RS

General Chapters

General Chapters >1000
Informational chapters
E.g.,<1132> Host Cell Protein analysis

General Chapters <1000
validated methods that users can verify are suitable for their use E.g. *<509>Residual DNA; <89> Endotoxin*

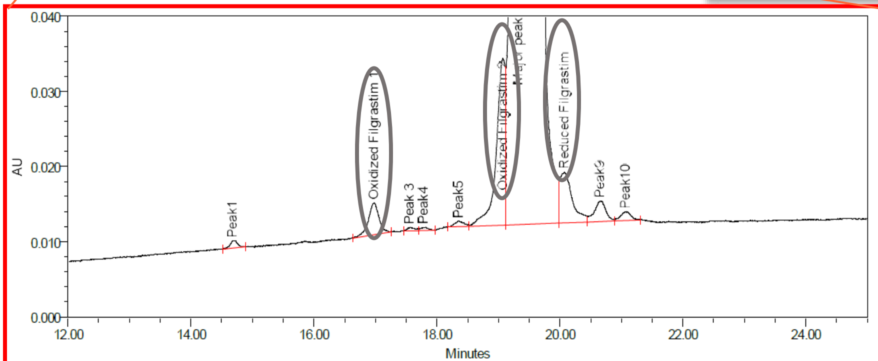
Reference Standards

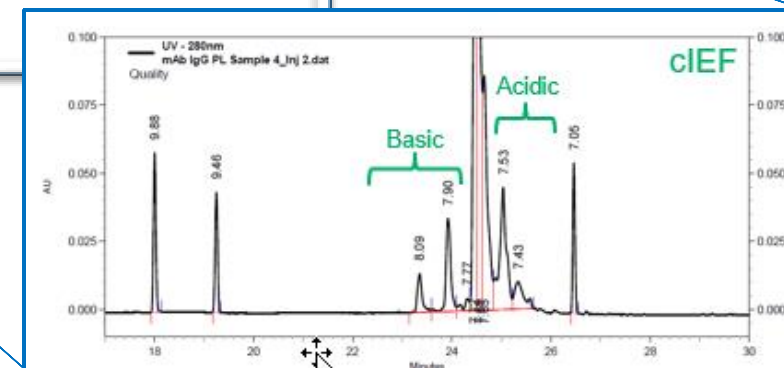
Stand-alone reference standards – not associated with a monograph/ chapter

Broadly applicable across product families or classes.

Support analytical testing throughout the product lifecycle.

Include characterization package . Eg. mAb 001, 002, 003





USP mAb Reference Standards

<Previous>

USP monoclonal IgG SS

(<129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies)

Size Variants - Aggregates
- SEC

Size Variants - Fragments
- CE-SDS

	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
Theoretical pI*	8.7	8.1	8.1
Package size	200 μl solution (2 mg protein content)	200 μl solution (2 mg protein content)	200 μl solution (2 mg protein content)

* Calculated using ProtParam (ExPASy) without glycosylation

<New>

USP 3 mAb RSs

Glycosylation
- HPLC
- LC-MS

Size Variants - Aggregates
- SEC

Molecular Weight
- Mass spectrometry (MS)
- SEC-MALS

Size Variants - Fragments
- CE-SDS

Charge Variants
- Cief (Added)
- icIEF (Added)
- CEX Chromatography

Column Qualification
- CEX
- SEC

Target (Fc) Binding
- Surface plasmon resonance
- Functional bioassays

Multi-attribute Methods (MAM)
- Deamidation, oxidation, other post translational modifications
- Clipping
- Pyroglutamate
- Glycosylation

*** Purple:** Applications included in initial release

Reference Data included in CoA

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usp

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Applications and case studies

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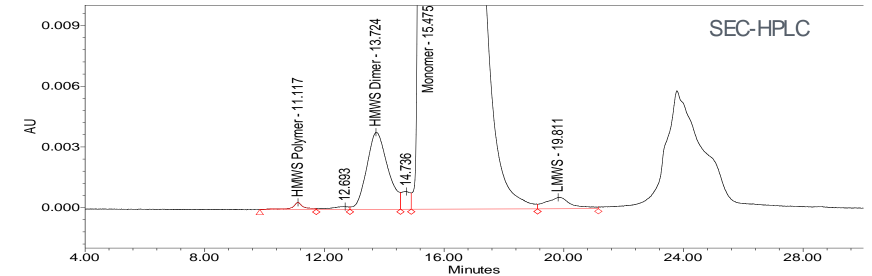
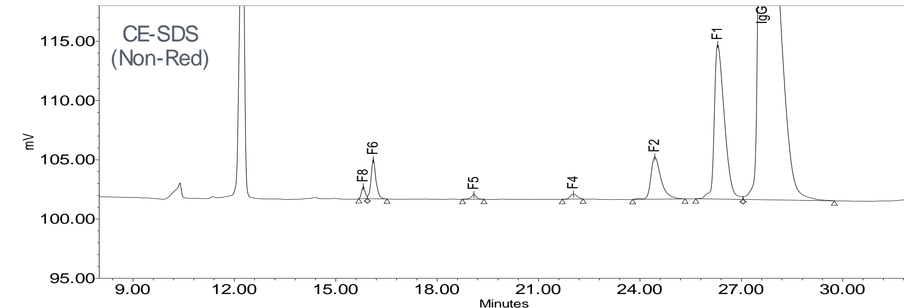
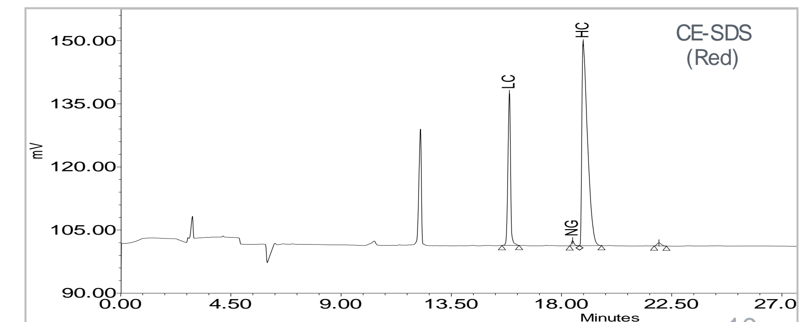
Product Related Impurities

Assessment requires a combination of orthogonal methods

Type of Impurity	Aim	Analytical Technique Used
Structure/Post translational modifications	Deamidation/oxidation N/C terminal modifications	Peptide mapping-HPLC/UPLC and/or MS
	Assessment of Disulfides	
	Intact, LC/HC, HMW/LMW	CE-SDS under reduced or Non reduced conditions, HPLC/UPLC
Product related substances and impurities	Aggregates	SE-HPLC/UPLC,
	Charge Variants-associated with deamidation/ isomerization	Ion exchange HPLC, cIEF, icIEF







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Case study I: Size-Exclusion Chromatography(SEC) column

- Product-related **low molecular weight (LMW) and high molecular weight (HMW) species** are critical quality attributes (CQAs) in mAb products.
  - **Consistent monitoring these species is crucial across the** entire drug production process for controlling mAb product quality during production and storage.
  - HMW protein aggregates and LMW protein fragments are common degradation pathways.
    - **HMW species** often result from non-covalent associations or photo-induced cross-linking.
    - **LMW species** arise from enzymatic or nonenzymatic (chemical) clipping, incomplete formation, or scrambling of disulfide bonds.
    - **SEC and CE-SDS** provide **orthogonal** information for analysis of mAb fragments and aggregates.
- SEC columns need to be optimized to have a high resolution of a mAb **monomer** from a dimer and higher order aggregates, as well as from fragments.

Challenges:

- Diversity of Size Heterogeneity
- Size range: Nanometers to hundreds of micrometers.
- Formed during manufacturing & storage, persisting after extensive purification.
- Can impact on potency and immunogenicity.

Atis, C., *Separation of Monoclonal Antibodies by Analytical Size Exclusion Chromatography*, in *Antibody Engineering*, B. Thomas, Editor. 2018, IntechOpen: Rijeka. p. Ch. 7.

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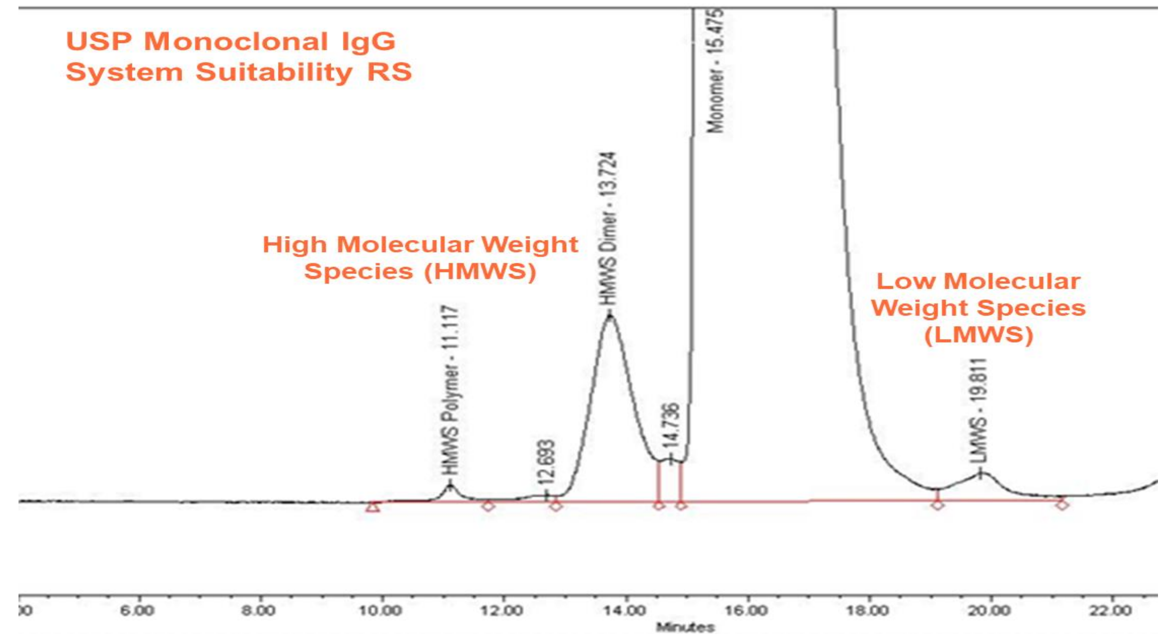
Example 1:
SEC for quantitation of HMW and LMW impurities

Method	Size exclusion chromatography (SEC), Capillary electrophoresis-sodium dodecyl sulfate (CE-SDS)
USP RS	• IgG System Suitability RS
USP GC	<129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies

System Suitability Criteria	▪ Chromatographic similarity
	▪ Consistency of chromatogram in bracketing injection
	▪ % peak area in the bracketing injections
	  ▪ Main peak 99.1%–99.6%.
	  ▪ HMWS: 0.4% – 0.67%
	  ▪ LMWS: NMT 0.2%



- SEC can be used to measure LMW variants, monomer, and high-molecular weight (HMW) variants in the same analysis yielding a measure of monomeric purity.
- Robustly differentiate between Monomer, HMWS (aggregates) and LMWS (fragments).
- User can establish the system suitability criteria for their method.

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Example 2:
Optimize SEC conditions for reproducibility

Background

Key factors for purification

- Increased resolution between monomer peak and other impurities
- Stability during storage: monitor with Size Exclusion Chromatography (SEC)
- Removal of high molecular weight (HMW) and low molecular weight (LMW)

Factors influencing aggregation

- can happen at any stage during expression and purification
- Temperature, pH, Ionic strength, Concentration

Reproducibility

- Challenge in protein aggregation -> immunogenic
- >99% purity is crucial
- Monitor pure monomer

Separation of Monoclonal Antibodies by Analytical Size Exclusion Chromatography: http://dx.doi.org/10.5772/intechopen.73321

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Reproducibility is important in SEC analysis of mAbs

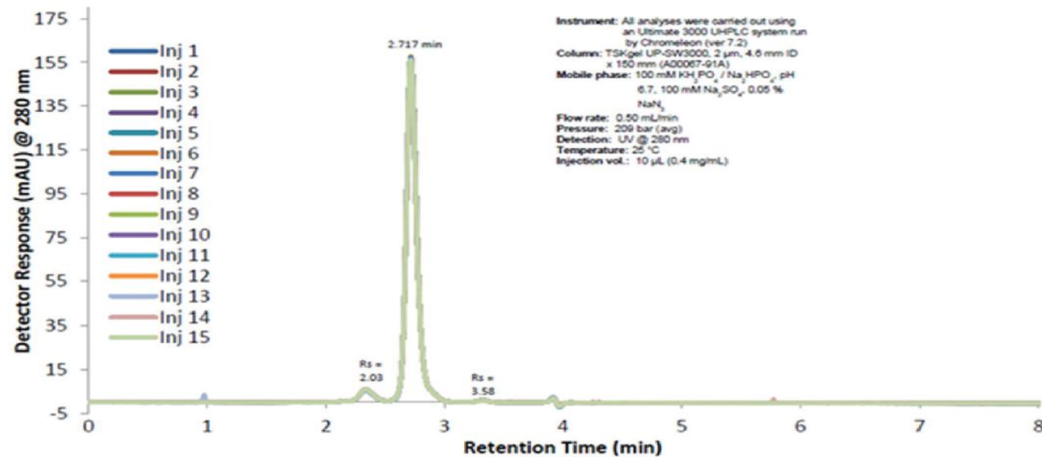

Figure 10. Reproducibility of 15 consecutive analytical injections of a USP mAb using a 15-cm TSKgel UP-SW3000 column at phosphate buffer pH 6.7. The overlay of 15 injections is shown.

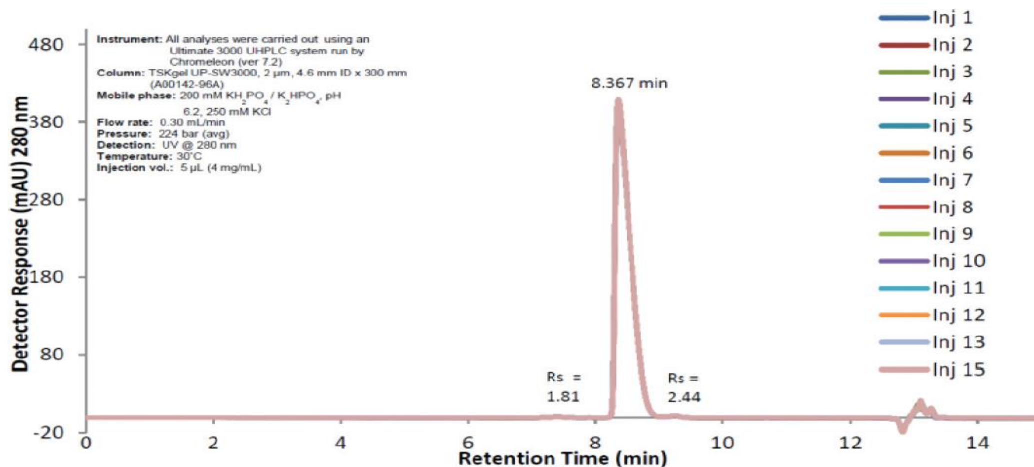

Figure 11. Reproducibility of 15 consecutive analytical injections of a USP mAb using a 30-cm TSKgel UP-SW3000 column at pH 6.2. The overlay of 15 injections is shown.

Key parameters

Low %RSD

- Retention time
- Peak area
- Peak asymmetry

Column health

- Defined QC criteria

Column quality and lifetime over a number of injections

- Same mAb (internal std)

Separation of Monoclonal Antibodies by Analytical Size Exclusion Chromatography: http://dx.doi.org/10.5772/intechopen.73321

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Example 3: Customer experience
Biopharmaceutical Characterization Laboratory from Bangkok

- Customer needs
  - Validate their SEC method for ISO/IEC 17025 certification using HPLC.
- Materials used
  - USP Monoclonal IgG System Suitability RS
  - USP Chapter <129>
- Approach taken: Investigated HPLC Condition optimization using USP materials
  - Examination of the column performance
    - Resolution of peaks, The number of injections, Reagents and solvents
- Challenge Faced: Column didn't pass the SSS criteria as per GC <129>.
- USP Collaborated and provided the necessary information and technical support.
- The reason identified was the sub-optimal column performance. System suitability criteria were successfully met with the new column.
  - This implies a need for additional attention and fine-tuning to optimize the instrument's performance.

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Example 4: Transferring method SEC method from HPLC to UHPLC

Background: advantages of UHPLC as compared to HPLC cover the trend

- UHPLC is an advanced separation technique that allows for shorter run times, less amount of sample, better chromatographic separation, and increased throughput as compared to traditional HPLC. This is made possible by the higher pressure limits in a UHPLC, which allows for the use of columns with lower particle sizes.
- USP published a revision of General Chapter <129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies for public comment in the Pharmacopeial Forum (PF) 49(3) that now includes SE-UHPLC to align with industry trends.
  - USP mAb 001, 002, and 003 were tested using SE-HPLC and SE-UHPLC methods from General Chapter <129>, PF 49(3).
  - The study aimed to show comparability between SE-HPLC and SE-UHPLC using USP mAb RS.

Application Note

Aggregation Analysis Using SE-HPLC and SE-UHPLC Methods in *USP* General Chapter <129> *Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies*

USP Biologics™

Monoclonal antibodies (mAbs) represent one of the fastest growing drug markets worldwide. Since the FDA first approved an antibody therapy (Muromonab CD3) in 1986, over 100 mAbs have been granted U.S. FDA approval.

Manufacturing processes or degradation upon storage can alter the physiochemical properties of these proteins leading to formation of aggregates higher molecular weight species (HMWS). In addition to its impact on quality, the loss of product, and potential batch failure, protein aggregation has been shown to increase immunogenicity and the risk of an unintended harmful immune response, therefore making it a critical quality attribute (CQA) [1].

The biophysical characterization of mAbs is critical for approval and life cycle management. Because of the complex nature of biologics, analytical tests are often challenging and time consuming. USP has developed three analytical Reference Standards (RS) that can be used to overcome some of these challenges by providing users with well characterized standards. Additionally, USP offers a system suitability Standard that allows an analyst to easily assess system suitability criteria described in USP General Chapter <129>.

Purity assessment by size exclusion chromatography (SEC) is most commonly used to assess aggregation. This CQA is a stability-indicating attribute that is assessed throughout the lifecycle of every commercial mAb. Traditionally, size-exclusion ultra high performance liquid chromatography (SE-HPLC) has been used for measuring monomer, high-molecular, and low-molecular weight species; however, size-exclusion ultra high performance liquid chromatography (SE-UHPLC) is becoming the preferred method due to the advantages of UHPLC as compared to HPLC. UHPLC is an advanced separation technique that allows for shorter run times, better chromatographic separation, and increased throughput as compared to traditional HPLC. This is made possible by the higher pressure limits in a UHPLC, which allows for the use of columns with lower particles sizes. Recently, USP published a revision of General Chapter <129> *Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies* for public comment in the *Pharmacopeial Forum* (PF) *49(3)* that now includes SE-UHPLC to align with industry trends [2].

Table 1. General information for the three non-compendial USP mAb Reference Standards

	USP mAb 001,	USP mAb 002,	USP mAb 003,
USP Catalog #	1445539	1445547	1445595
MW	~147,000 Da	~150,000 Da	~146,000 Da
HMWS (%)[1]	0.9	0.8	0.4
Monomer (%)[1]	99.1	99.2	99.6
LMWS (%)[1]	<0.1	<0.1	<0.1
Package size	200 µl solution (2 mg protein content)	200 µl solution (2 mg protein content)	200 µl solution (2 mg protein content)

[1] *Purity data as reported on each USP mAb certificate [3 – 5]. All data were generated using SE-HPLC method.*

USP Biologics. Application note. Aggregation Analysis Using SE-HPLC and SE-UHPLC Methods in USP General Chapter <129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies : https://go.usp.org/aggregation-analysis-app-note?_gl=1*8sirx3*_gcl_au*OTg2NDI5MDg5LjE3MDY2ODcxNDE.*_ga*ODA4ODA5MDI5LjE2NTM0NjgwMjM.*_ga_DTGQ04CR27*MTcwNzIyMjU5NS4yMTAuMC4xNzA3MjIyNTk5LjAuMC4w

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Results

USP experimental

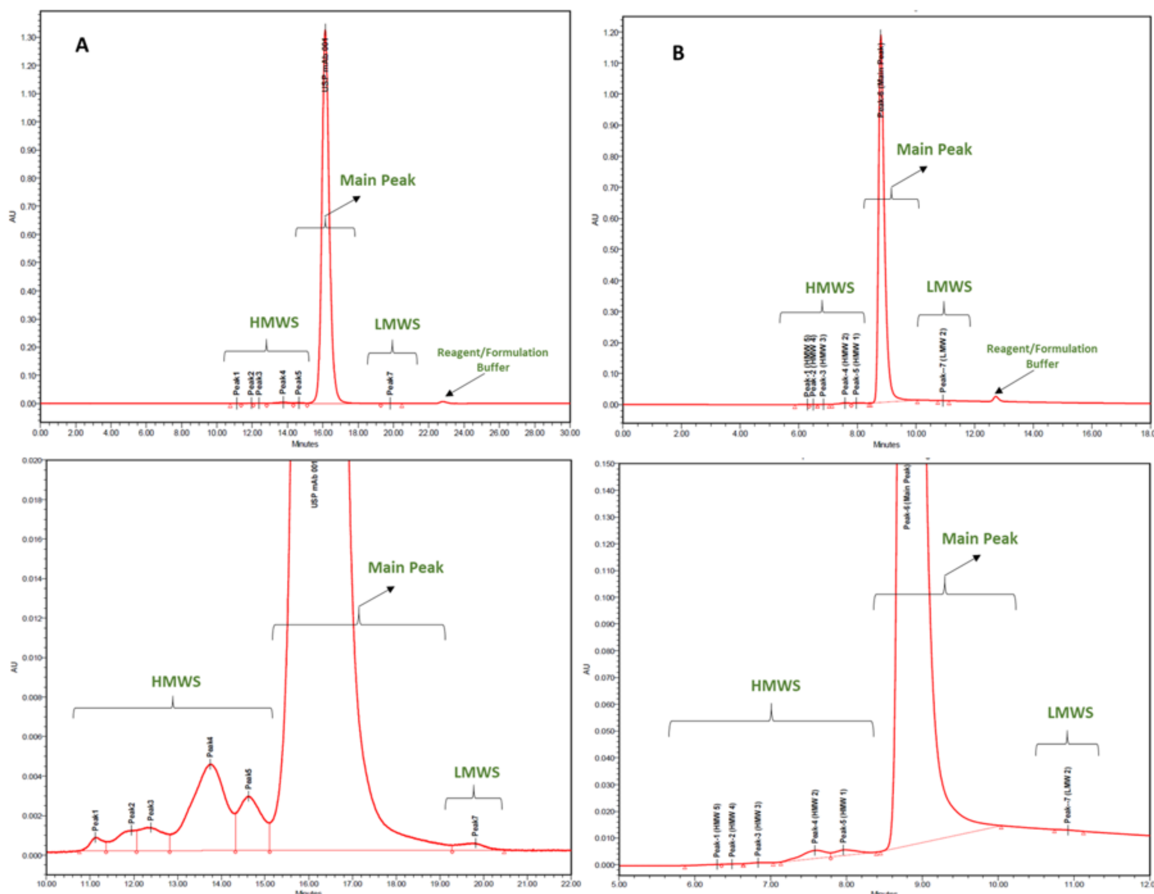

Figure 2: Representative Size Exclusion Chromatograms for USP mAb 001 Monoclonal IgG1 (A) SE-HPLC (B) SE-UHPLC. Top images show full spectra while bottom images show zoomed spectra.

Table 4: SE-HPLC and SE-UHPLC Results

Method	Average %Area	USP mAb 001	USP mAb 002	USP mAb 003
SE-HPLC	HMWS	1.1	0.7	0.4
	Main Peak	98.9	99.2	99.5
	LMWS	<0.1	<0.1	0.1
SE-UHPLC	HMWS	0.7	0.9	0.4
	Main Peak	99.3	99.1	99.6
	LMWS	<0.1	<0.1	<0.1

- Main peak, HMWS, and LMWS peak percentages for each mAb in both SE-HPLC and SE-UHPLC were within ±0.2% of their certificate values.
- Profiles of all three USP mAb samples showed similarity when comparing chromatograms between SE-HPLC and SE-UHPLC.

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Case study I Summary

USP standards can be used in SEC-HPLC for following applications:

- **Purity assessment**:
  - Documentary standard
    - USP chapter <129> ANALYTICAL PROCEDURES FOR RECOMBINANT THERAPEUTIC MONOCLONAL ANTIBODIES
  - Reference standard
    - IgG System Suitability RS
- **Column Qualification**: Monitoring column health
  - Analyze a protein standard mixture with low %RSD in peak parameters (retention time, peak area, asymmetry, theoretical plates), meeting defined QC criteria.
- **Routine Monitoring**: Routine users practice
  - Use the same mAb as an internal standard for consistent SEC column assessment.
  - Ensures reliable results across multiple injections.
- **Optimize Instrument Performance**

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Case Study I Summary (Continue)

Implementation of an Advanced Separation Technique

- Increased interest in transitioning to UHPLC methods.
  - Revised General Chapter <129> with advanced technological applications.
- SE-UHPLC method offers higher throughput, requiring less sample.
- Study confirms comparable performance of SE-HPLC and SE-UHPLC for USP mAb 001, 002, or 003.
- Implementation of the SE-UHPLC purity determination method in quality control requires a verification study per USP Chapter <1226>.

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Case study II: CE-SDS Method Development

- CE-SDS: commonly used for Analyzing protein size and purity
  - The **suitability of such methods must be measured against compendial requirements.**
  - USP <129> describes analytical procedures, namely size-exclusion chromatography (SEC) and capillary electrophoresis sodium dodecyl sulfate (CE-SDS), to assess therapeutic purity.
  - Generally used under reducing and non reducing conditions to analyze non glycosylated heavy chain and LMWs or degradation products.
  - Can be used to demonstrate CE-SDS method with the USP<129> protocol.
    - the **comparability** between different instruments
    - Ease of **method transfer** between different labs
    - **Method performance**
    - **Reproducibility**
    - **Protocol optimization**

Biotechne, Application note. Get USP <129> Equivalent Data with Maurice CE-SDS

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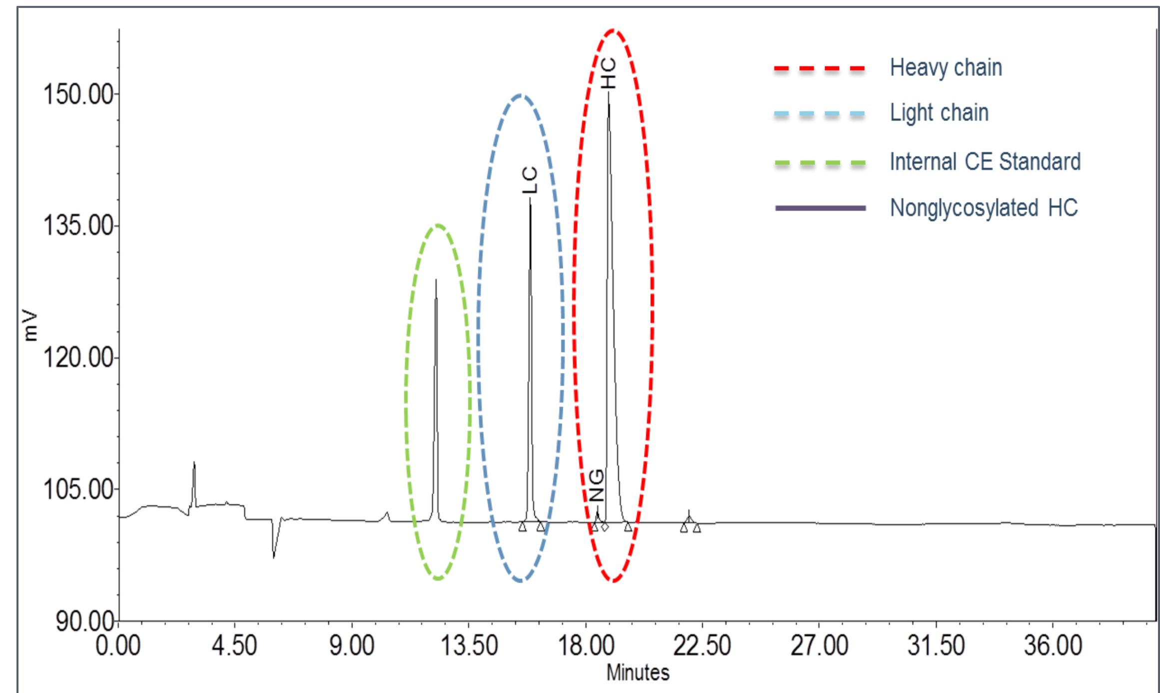
Example 1-1:
Under Reducing Conditions for Non glycosylated heavy chain

Method	Capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) under reducing conditions
USP RS	• IgG System Suitability RS
USP GC	<129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies
System Suitability Criteria	▪ The main peak of the heavy chain and the peak of the nonglycosylated heavy chain (NG) can be clearly identified. ▪ The resolution between the nonglycosylated heavy chain and the intact heavy chain is NLT 1.2. ▪ The ratio of **nonglycosylated** to total heavy chain in the **system suitability solution** should be within the limits of 0.75%–1.34%.



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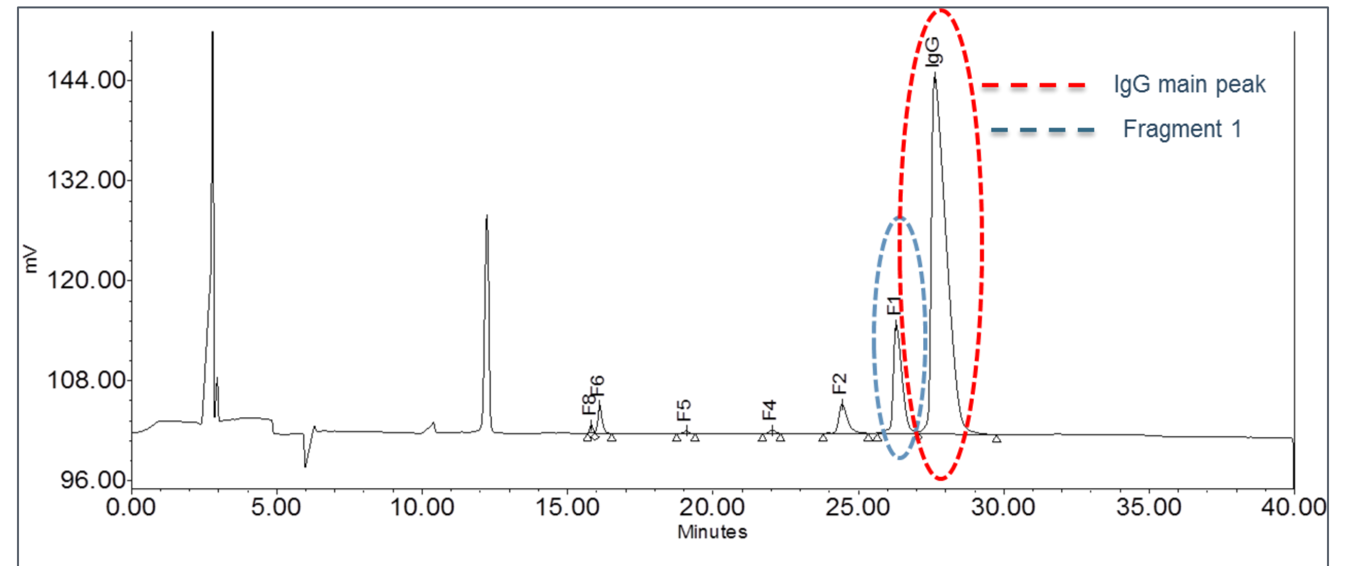
Example 1-2:
Under Non-Reducing Conditions for LMWs Study

Method	Capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) under non-reducing conditions
USP RS	• IgG System Suitability RS
USP GC	<129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies
System Suitability Criteria	▪ The IgG main peak can be clearly identified. The resolution between the IgG main peak and Fragment 1 (F1) is NLT 1.3. ▪ The relative amount of the main IgG peak of the System suitability solution should be within the limits of 61.4%–86.4%.



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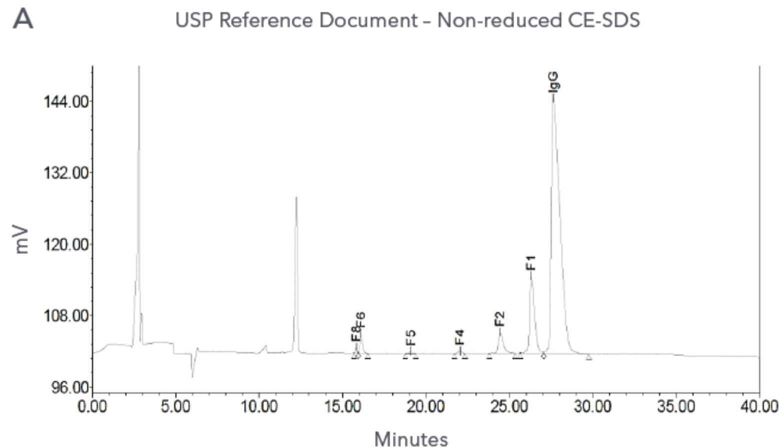
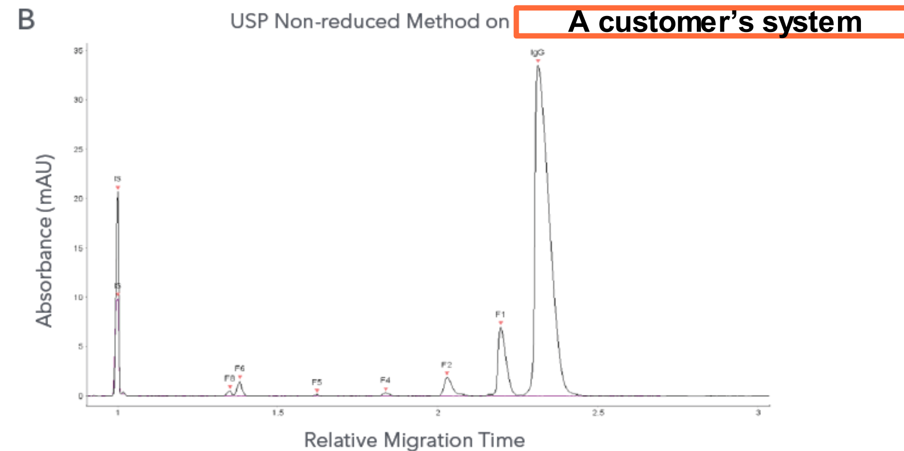
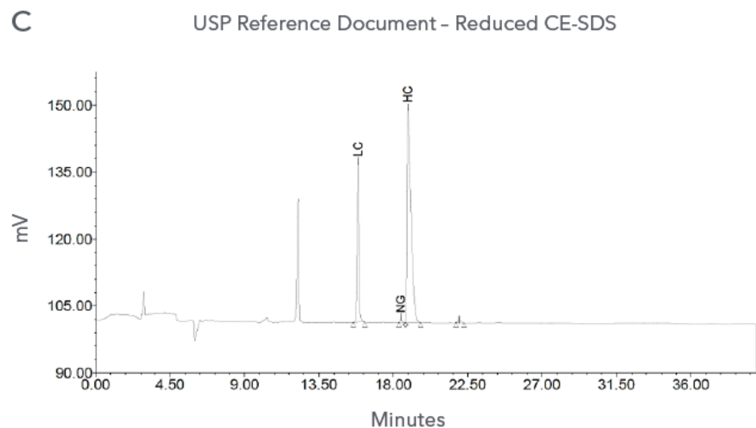
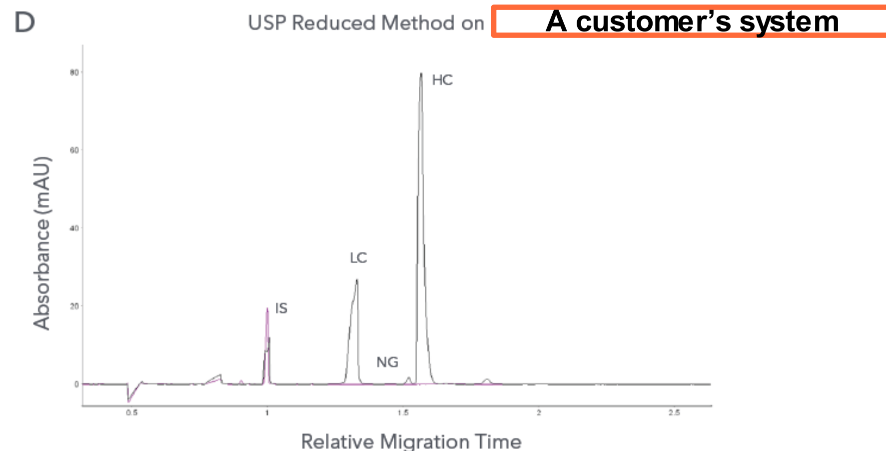
Example 2:
Setting up the Instrument performance as per USP <129> protocol

Running the USP <129> protocol on a customer's system using the IgG System Suitability RS









- The USP<129> method with IgG SSS is used to assess the suitability of analytical methods.
- It serves as a benchmark for comparing the performance and results obtained from different platforms.
  - Non-reduced and reduced methods both yielded expected peaks.

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Setting up the Instrument performance as per USP <129> protocol

Different parameters were investigated during the optimization process

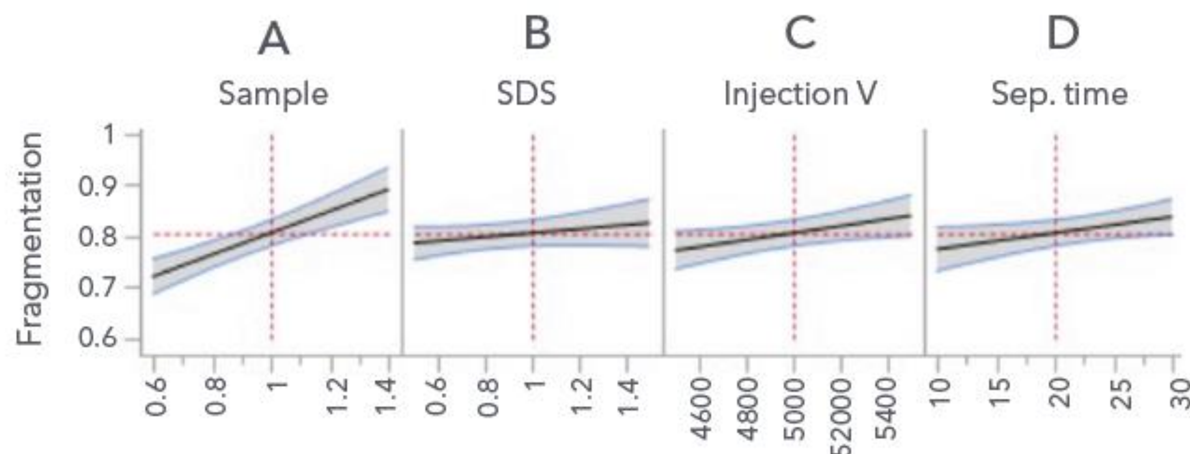

FIGURE 2. Evaluation of varying sample and instrument conditions.

Method optimization: Different sample and instrument conditions
Sample Concentration
Sample buffer concentration
Separation time
Injection voltage

- Varying sample and instrument conditions were linearly correlated with fragmentation.
  - Confirmed the robustness of the method.
  - Demonstrate the comparability of a company's method with USP <129> protocol
- The method was compared with the USP-reduced method.

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Further Method Optimization: reduced/non-reduced conditions

Comparison of the USP and a customer's in-house methods under reduced/non-reduced conditions

Method optimization: reduced CD-SDS
β-ME(β-Mercaptoethanol)
Denaturation time
Denaturation temperature

- Findings from reduced CE-SDS method optimization.
  - Concentration of β-ME nor increasing denaturation time affected IgG profile.
  - Detection of all expected peaks, including heavy chain, light chain, and non-glycosylated heavy chain

Method optimization: non-reduced CD-SDS
alkylating agent (IAM)
Denaturation time
Denaturation temperature

- Finding from non-reduced CE-SDS method optimization.
  - Higher concentration of IAM correlated with lower fragmentation.
  - Shorter denaturation times at lower temperatures were also found to cause lesser fragmentation.

- Conducted thorough investigation of parameters to optimize customer's in-house method.
- Compared optimized method with USP standards to ensure suitability for product requirements.
- Overall, the customer's in-house method provided reliable and comparable results to the USP <129> protocol in both reduced and non-reduced conditions.
- Acknowledged potential need for further optimization based on specific customer requirements.

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Case study II Summary

Method development, transfer, and optimization

- Achieve Optimal Excellence: elevate your quality standards by leveraging USP materials.
- Ensuring Consistent Precision: eliminate variations across instruments in testing.
- Strive for Comparability: Align your results with the USP <129> standard for comprehensive comparability.
- Enhance Reproducibility and Sensitivity: define the sensitivity of your product by developing optimized methods, benchmarked against the USP method.
- Pushing Boundaries: perform beyond standards by exploring and implementing optimized methods tailored to your unique requirements.

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Case study III: cIEF/icIEF
Monitoring Assay Performance and Use in Quality Control

- cIEF/icIEF: Charge variants are typically a CQA and are **stability-indicating**
  - Can impact the quality, stability, and potency of a mAb
  - Charge profile analysis is commonly part of stability characterization studies and monitoring strategies
  - Charge variants arise from
    - C-terminal lysine clipping, Cyclization of Glu/Gln to form pyro-Glu, Deamidation, Amino acid substitutions

- Methods:
    - Standard analysis methods
      - Capillary electrophoresis: cIEF or icIEF, cIEF SCIEX platform (PA 800 Plus), iCIEF (Bio-Techne's Protein Simple Maurice and iCE3 platforms)
      - Cation exchange chromatography: Eluted using pH or salt gradient
    - Emerging methods: CE-MS, Multi-attribute monitoring (MAM)

- Can be used to demonstrate cIEF/icIEF method with the USP mAb 001, mAb 002, and mAb 003.
    - Support **method development and system suitability** for charge variant characterization, release testing, and stability testing.

Biotechne, Application note. Get USP <129> Equivalent Data w ith Maurice CE-SDS

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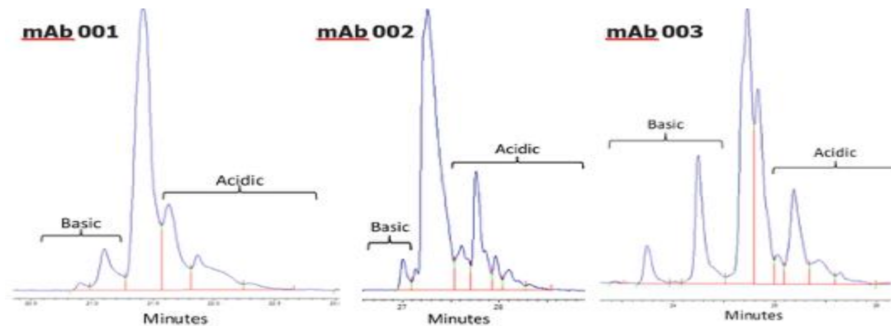
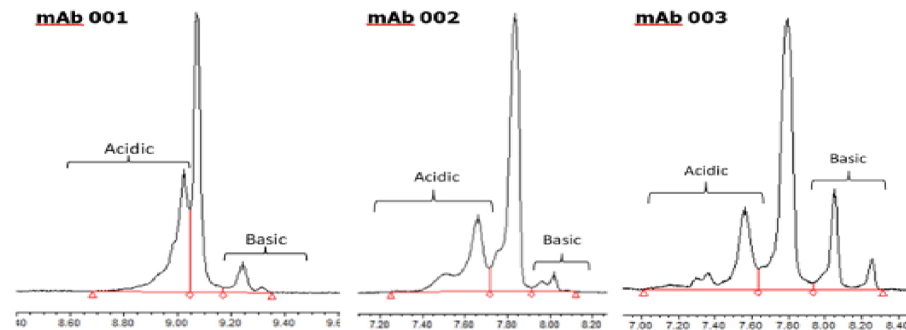
Example 1:
USP mAb RS for Charge variant



cIEF SCIEX platform



Maurice icIEF

Table 4. Theoretical and Experimental pI values of main charge variants determined by cIEF

Reference Standard	Theoretical pI*	Experimental pI (cIEF)	Main (cIEF)	Acidic (cIEF)	Basic (cIEF)
mAb 001	8.7	9.2	60%	32%	8%
mAb 002	8.1	7.8	65%	31%	4%
mAb 003	8.1	7.7	55%	25%	20%

Table 5. Theoretical and Experimental pI values of main charge variants determined by icIEF

Reference Standard	Theoretical pI*	Experimental pI (icIEF)	Main (icIEF)	Acidic (icIEF)	Basic (icIEF)
mAb 001	8.7	9.2	54%	38%	8%
mAb 002	8.1	7.9	66%	29%	4%
mAb 003	8.1	7.9	62%	20%	18%

Note: Main peak pI and % species vary based on capillary condition, reagents, instrument, method, and integration parameters. Values are the average from three labs.
** Calculated using ProtParam (ExPASy) without glycosylation*

- cIEF/icIEF provides:
  - Isoelectric point (pI) values and charge profile to support identity
  - Quantitation for purity (quantitative or semi-quantitative)
  - Relative percent of acidic group, basic group, the main peak, and individual species of interest

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Example 2: Customer experience. Large CMO based in South Korea

- Objective:
  - Development and validation of icIEF assay.
  - Determine their product's isoelectric point (pI).
- Challenge:
  - Despite the effectiveness of the existing method, transitioning to a new model becomes unavoidable.
  - Necessity for method validation
    - Adoption of USP standards provides a recognized and standardized framework
      - Ensures reliability of the validated method
      - Enhances comparability with industry benchmarks
- Solution:
  - USP standards met their needs for antibody characterization.

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Customer feedback (mAbs)

Do you see any value in compendial methods vs. other commercial multiple proprietary technologies (e.g., from proteinsimple, Agilent)?

"Yes. for example, iCE from proteinsimple is a great technology for measuring charge profile of proteins however **it is sometimes troublesome when the current equipment model discontinues**. Generally, most of clients do not want to change their validated method especially for a commercial product but it is inevitable to validate the method using a new model although current method is very well working."

Anonymous scientist

Case study III Summary

Leveraging USP Standards to enhance mAb production with cIEF/icIEF

- Capabilities of cIEF/icIEF:
  - Provides pI values and charge profile for identity.
  - Enables purity assessment quantitation.
  - Necessitates thorough analysis due to the stability-indicating nature of charge variants.
- Challenge and Solution:
  - Existing method is effective but requires a transition to a new model.
  - Adoption of USP standards ensures a standardized framework, enhancing reliability and comparability.
- Application with USP Standards:
  - Utilizes USP mAbs (001, 002, and 003) for method demonstration.
  - Supports method development, system suitability, charge variant characterization, release testing, and stability testing.

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3

mAb Related Resources

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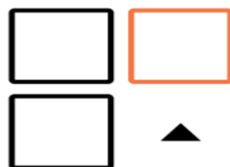
Products

- *USP-NF*
  - General Chapters
  - Monographs
- Reference Standards
- Analytical Reference Materials
- Educational Courses



Categories

- Antibiotics
- Carbohydrates
- Cell, Gene and Tissue products
- Genomic DNA (ATCC ®) – coming soon
- Heparins
- Impurities
- Microbiology
- Monoclonal antibodies
- Peptides
- Oligonucleotides
- Raw materials
- Vaccines

Knowledge Sharing

- FAQs
- Regional Scientific Advisory Panels
- Roundtables
- User Forums
- Webinars
- Workshops

Resources

- Application notes
- Analytical Guidelines
- Infographics
- mAb Analytical Guide
- MAM Exchange
- White papers
- Toolkits

Effective 19 JUL 2023

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mAb Analytical Guide

mAb
Analytical Guide

Release & Stability | Extended Characterization | In-Process Testing | Contact

Release & Stability

- Compendial Tests
- Size Variants
- Charge Variants
- Glycosylation
- Potency & Biological Activity
- Process Related Impurities
- Stability
- Safety
- Excipients
- Concentration

N-Linked Glycan Profiling

Monosaccharide Analysis

Mass Spectrometry

Reference Standards

1478210: Oligosaccharide System Suitability Mixture A
1478221: Oligosaccharide System Suitability Mixture B
1478232 Oligosaccharide System Suitability Mixture C
1478243 Oligosaccharide System Suitability Mixture D

Documentary Standards (USP-NF)

<129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies
<212> Oligosaccharide Analysis
<1084> Glycoprotein and Glycan Analysis-General Considerations

Other Resources

Manuscript: USP mAb Ref Stds: Tools to Verify Glycan Structure
Manuscript: Comprehensive Stds for Monitoring Glycosylation
Manuscript: Macro- and Micro-heterogeneity of Glycosylation
Poster: USP mAb Ref Stds, High Resolution Mass Spectroscopy

https://www.usp.org/mab-analytical-guide

Brochures

Technical Note

USP Monoclonal Antibody Reference Standards

USP Biologics

Table 1. General information for the three non-compendial USP mAb Reference Standards

	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
Theoretical pI*	8.7	8.1	8.1
Experimental pI (cIEF)**	9.2	7.8	7.7
Experimental pI (icIEF)**	9.2	7.9	7.8
Package size	200 µl solution (2 mg protein content)	200 µl solution (2 mg protein content)	200 µl solution (2 mg protein content)

The mAb RSs can be used in a broad range of applications, serving as:

- Internal assay control
- Independent control material for method development
- Standardization of physicochemical testing, such as intact mass, charge heterogeneity, size variants, purity and glycan analyses across laboratories
- Development of platform technologies

Tech note- General

Technical Note

Charge Variant Analysis of USP Monoclonal Antibody Reference Standards

USP Biologics

Deamidation of asparagine

cIEF/icIEF provides:

- Isoelectric point (pI) values and charge profile to support identity
- Quantitation for purity (quantitative or semi-quantitative)
- Relative percent of acidic group, basic group, the main peak, and individual species of interest

Trimming of C-terminal lysine

Table 1. General information for the three non-compendial USP mAb Reference Standards

	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

Tech note- cIEF, icIEF

Technical Note

Higher-Order Structure (HOS) Characterization of USP Monoclonal Antibody Reference Standards

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INTRODUCTION

MATERIALS AND METHODS

Jasco J-815 Spectropolarimeter

Applied Photophysics Chirascan Q100

RedShift BioAnalytics AQS³pro

RESULTS AND DISCUSSION

Contact Us

Tech note- HOS

USP FDA Biosimilars Infographic

Biosimilars: Are they the same quality?

What are biologics?

Biologics (also called biological products) include a wide range of products such as vaccines, monoclonal antibodies, blood components, allergenics, gene therapy, tissues, and proteins.

Biologics are medicines that generally come from living organisms, which can include animal cells and microorganisms, such as yeast and bacteria[1].

They are used to treat a variety of diseases and conditions, such as cancer, kidney diseases, and autoimmune diseases.

What are biosimilars?

A biosimilar is a biologic that is highly similar to another biologic that's already FDA-approved, called a reference product. Biosimilars have no clinically meaningful differences from their reference product in terms of safety, purity, and potency.

Biosimilars have the same:

Route of administration to patients

Strength and dosage form

Potential side effects

Biosimilars are approved for many biologic reference products[2], including:

- Avastin
- Humira
- Lucentis
- Remicade
- Epogen/Procrit
- Herceptin
- Neulasta
- Rituxan
- Enbrel
- Lantus
- Neupogen

https://www.usp.org/biologics/mabs

https://www.usp.org/sites/default/files/usp/document/about/convention-membership/usp-fda-biosimilars-infographic.pdf

USP Resources for mAb characterization

- USP's public quality standards are critical tools for ensuring the quality and safety of monoclonal antibodies.
- mAb performance standards support method performance optimization, minimize analytical variations and evaluation of system suitability throughout product life cycle.
- Quality Parameters like Glycosylation, Charge variants, product and process related impurities can be efficiently and confidently monitored using these standards.

https://www.usp.org/sites/default/files/usp/document/our-work/biologics/pmrc-1256-00-2023august-mab-infographic.pdf

Ensuring quality in monoclonal antibody therapeutics with USP standards

1 CELL LINE DEVELOPMENT & EXPANSION

Cell Line Selection
<1048>

Clone & Banking
<1042>* <1050> <1237>

Expansion & Cryopreservation
<1044>

ANTIBODY CHARACTERIZATION

Identity & Structure
<736> Mass Spectrometry
<1736> Intact & Reduced Mass
<1055> Peptide Mapping
<210> <212> <1084> Glycan Profiling RS 1
<1052> Amino Acid Analysis
<1054> IEF (pI determination)
<1102> <1103> Identity ELISA
<1105> Surface Plasmon Resonance
<891> Thermal Analysis
<761> NMR Spectroscopy
<1853> Fluorescence Spectroscopy
<1430.3> Dynamic Light Scattering

General Properties
<507> <1057> Protein Concentration
<785> Osmolality
<791> pH
<631> Color/Clarity
<790> Appearance

Product-Related Impurities
<129> SEC HPLC, CEX/cIEF, CE SDS, Aggregation & Degradation RS 2
<621> Chromatography
<1053> Capillary Electrophoresis

Process-Related Impurities
<1132> Host Cell Proteins (HCP)
<509> Host Cell Residual DNA (HCD) RS 3
<1130> Residual DNA Testing

Potency
<111> Design & Analysis of Biological Products
<1032> <1033> <1034> Potency Bioassay
<1108> FcRn

Contaminants
<85> Endotoxins RS 4
<63> Mycoplasma
<1237> Virus Testing
<61> <62> <1229.3> Bioburden

2 UPSTREAM PRODUCTION

Bioreactors
<665>* <1665>*

Raw Material & Cell Substrate Qualification
<1043> <1050> <1050.1>

3 DOWNSTREAM PRODUCTION

Purification
Protein A
IEX Chromatography

Purification
Virus Inactivation
Filtration
<1050> <1050.1>

4 FORMULATION & FILL FINISH

Formulation
<1059> <1231>

Sterile Filtration & Filling
<1> <71> <787> <788> <1116> <1211> <1229.4> <1787>

Stability
<1049> <1049.1> *Proposed*

5 PACKAGING & DISTRIBUTION

Packaging & Distribution
<659> <1079> <1079.2> <1207>

Reference Standards

RS 1 Glycan Profiling
N-Acetylneuraminic Acid (Cat # 1612619)
N-Glycolylneuraminic Acid (Cat # 1294284)
Oligosaccharide System Suitability Mixture A (Cat # 1478210)
Oligosaccharide System Suitability Mixture B (Cat # 1478221)
Oligosaccharide System Suitability Mixture C (Cat # 1478232)
Oligosaccharide System Suitability Mixture D (Cat # 1478243)

RS 2 Monoclonal Antibodies
https://www.usp.org/biologics/reference-standards/igg
Monoclonal IgG Suitability (Cat # 1445550)
Monoclonal IgG1, mAb 001 RS (Cat # 1445539)
Monoclonal IgG1, mAb 002 RS (Cat # 1445547)
Monoclonal IgG1, mAb 003 RS (Cat # 1445595)

RS 3 Impurities http://go.usp.org/l/323321/2018-10-19/y96kz
CHO Genomic DNA (Cat # 1130710)
E. coli Genomic DNA (Cat # 1231557)

RS 4 Adventitious Agents
Endotoxin (Cat # 1235503)

Download the Complimentary Chapter <129>
go.usp.org/l/323321/2018-10-16/xxzsl

For more details contact:
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* In-Process Revision

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Thank You

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The standard of trust