Welcome



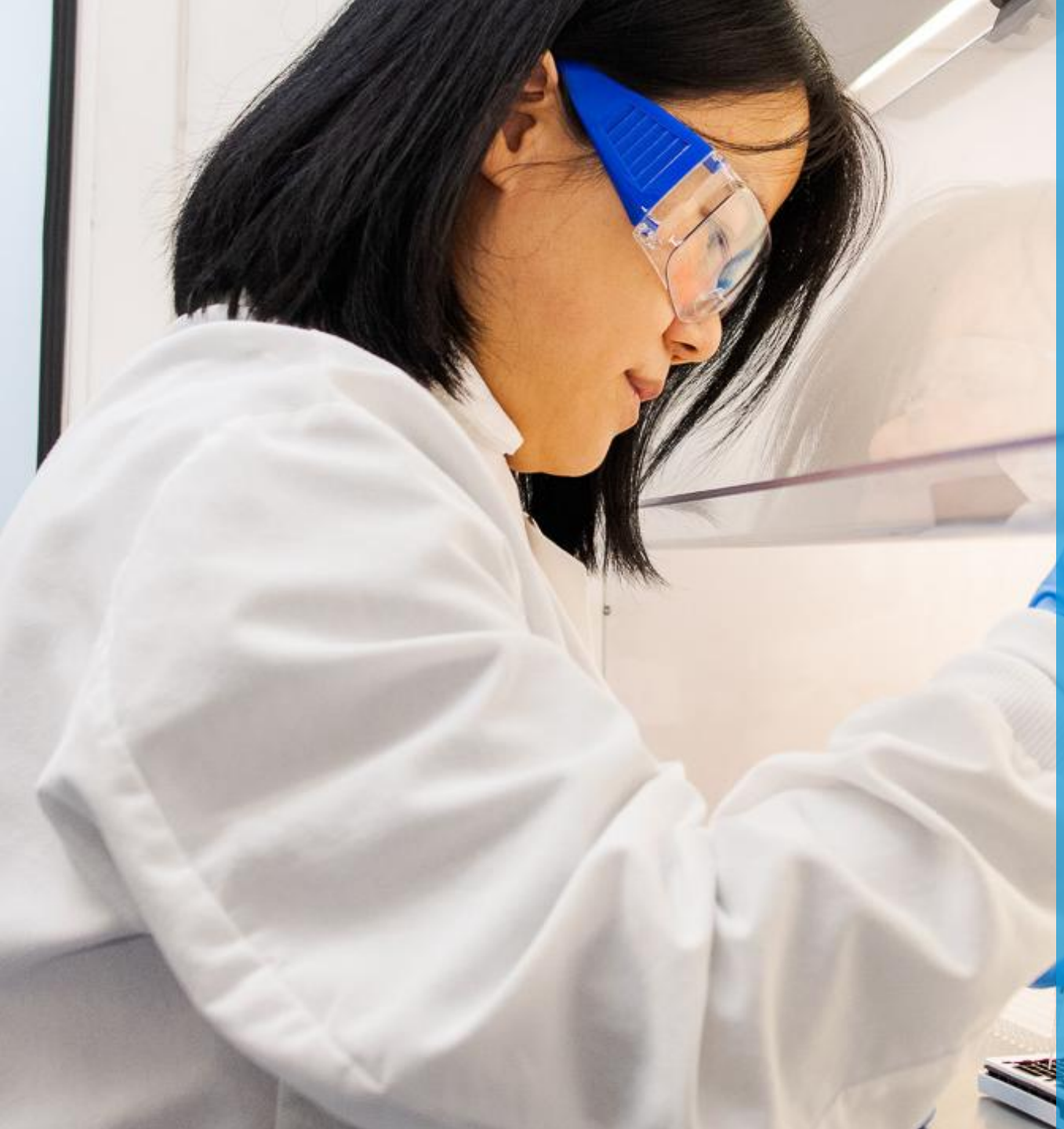
The standard of trust

Evolving USP ⟨129⟩: Evaluation of Fluorescent and Modernized-Buffer CE-SDS Methods for Biologics

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USP Global Biologics

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Outline

- Introduction to USP and Compendia
- USP <129>, CE-SDS
  - Need for Modernization
- CE-SDS by modified method with UV detector
  - Comparison of <129> CE-SDS to modified method
- CE-SDS with Fluorescence detection
  - Comparison of CE-SDS-FL with UV
- Summary and Next Steps



Introduction to USP

The USP Mission

To improve people's health through public standards and solutions that help ensure the quality of medicines, dietary supplements and food ingredients



Engagement Drives USP's Solutions

USP Expert Volunteers



Quality Standards & Solutions



Scientific & healthcare stakeholder communities, including industry, associations, & academia

Global regulatory entities and other pharmacopeias



USP approaches to advancing biologics quality across modalities

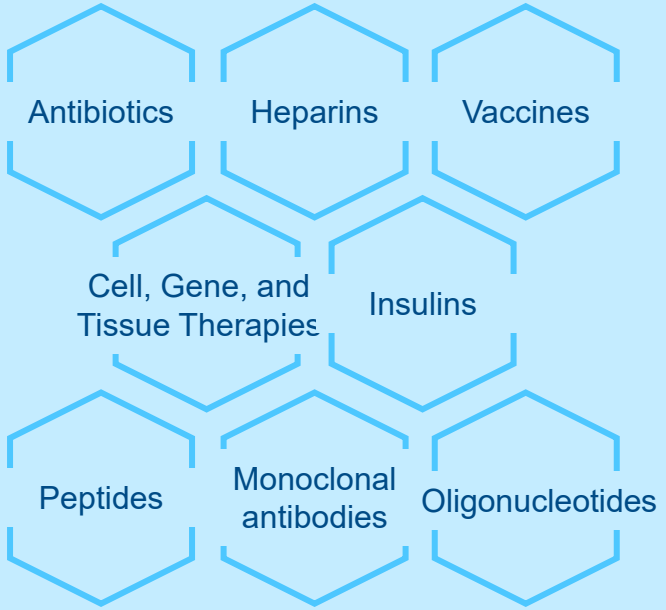
Drug Development and Manufacturing Elements

Common Critical Quality Factors that Influence the Safety, Efficacy, and Consistency of a Biological Drug

- Raw Materials
- Excipients
- Impurities
- Microbiology
- Quality Attributes

Therapeutic Modalities

Classes of Drugs or Living Elements used to Treat a Disease or Condition



USP Standards



- **Documentary Standards**
  - **Monographs** provide specifications for articles, including pharmaceutical articles
  - **General chapters**
    - Numbered under 1000
      - Validated methods
      - Made applicable to an article through reference in General Notices, a monograph or another general chapter below 1000
    - Numbered 1000 to 1999
      - General information and best practices
      - Intended for informational use only



- **Physical Reference Materials**
  - **Reference Standards** provide traceable standards to demonstrate broad-based acceptability of procedures and are often associated with a general chapter <1000
  - **Analytical Reference Materials (ARM)** are non-compendial and well characterized
    - For research and development use in a variety of analytical applications
    - Used for method development, troubleshooting or investigations throughout product lifecycle

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USP <129> and CE-SDS

Chapter contents and need for modernization

General Chapter <129> and IgG System Suitability

<129> Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies

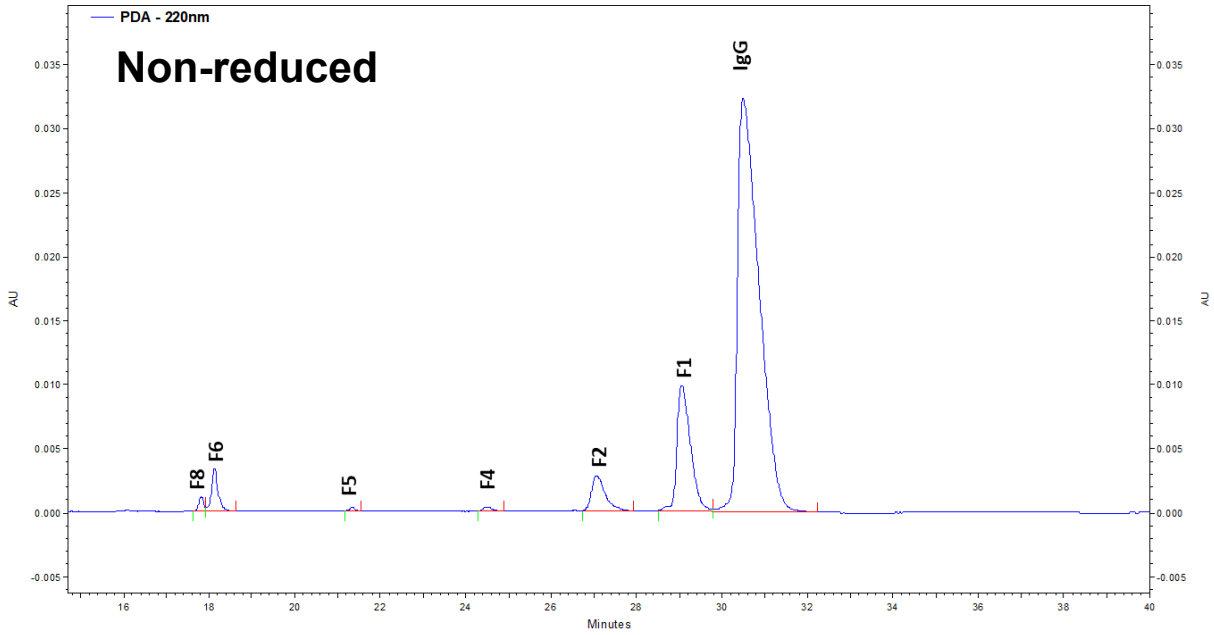
- **Analytical procedures for murine, chimeric, humanized, and fully human IgG**
- **Validated compendial procedures with established system suitability criteria**
  - Size–Exclusion Chromatography (HPLC- and UHPLC)
  - Capillary SDS Electrophoresis (reduced (R) and non-reduced NR))
  - Oligosaccharide Analysis
    - N-Linked Oligosaccharides
  - Monosaccharide Analysis
    - Sialic Acid

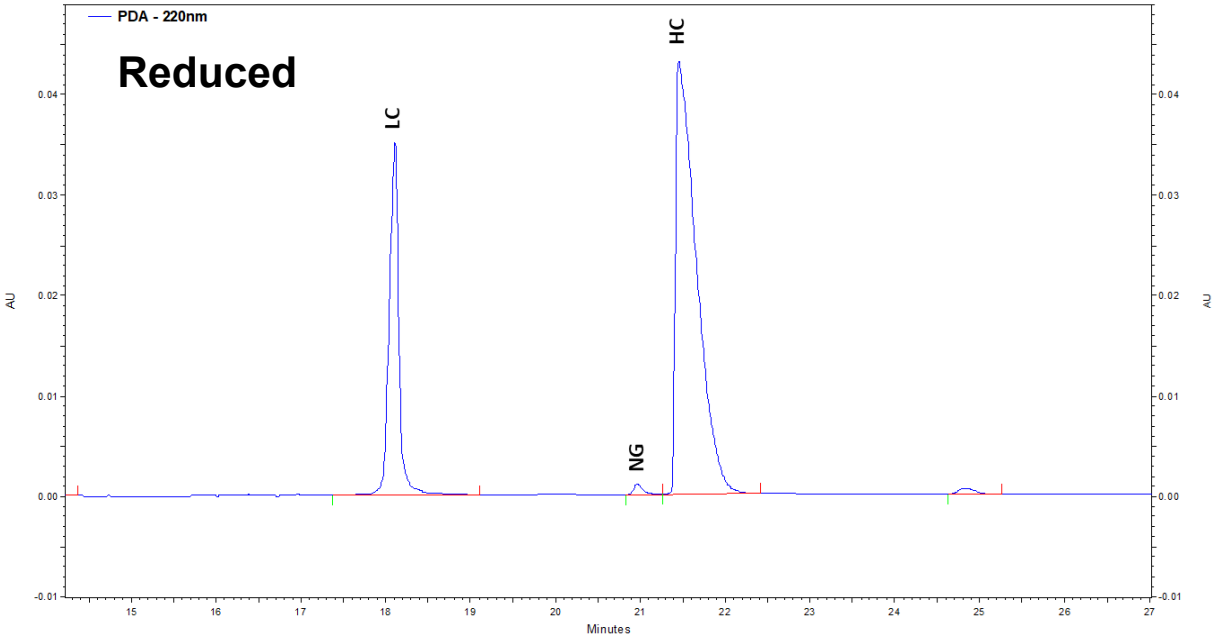


- **IgG1, expressed in CHO**
- **2 mg/vial lyophilized**
- **Meets System Suitability requirements given in <129>**
  - Results confirmed by Multi-lab Collaborative Study
  - Size Exclusion Chromatography
  - CE-SDS (reduced and non-reduced)

nrCE-SDS and rCE-SDS by <129>

Representative Data, Monoclonal IgG System Suitability RS





	Relative Amount of Main Peak
Result	78.7%
Acceptance Criteria	61.4% - 86.4%

	Ratio of NG to Total Heavy Chain
Result	1.06%
Acceptance Criteria	0.75% - 1.34%

Modernization of USP <129>

Responding to Industry needs

- **Addition of SE-UHPLC** method to <129> official April 2025
- **Revisions in progress**
  - **Expand options for CE-SDS workflow (UV detection)**
    - Alternative CE-SDS sample buffer and sample preparation
      - Extended heating time may result in fragmentation for some mAbs
    - Alternative capillaries
      - Shorten analysis time while retaining resolution
  - **Add CE-SDS workflow (Fluorescence detection)**
    - Provides an alternative method (e.g., situations where UV response may be insufficient)
- **Increasing compendial flexibility**
  - Adding validated options allows laboratories to select a fit-for-purpose approach while preserving USP reference standard as a common benchmark for establishing system suitability .

Overview of Chapter Revision

Process for a Revision of a General Chapter

New Method for Addition
- Donation
- USP Developed

Validation
- Donor Validated
- USP Validated

Revised Chapter Drafted
- USP Staff

Review by Expert Committee
- Feedback
- Endorsement to publish in PF

Publication in Pharmacopieal Forum
- Public Comments (90 days)
- Reconciliation of Comments by USP

Approval by Expert Committee

Publication in USP-NF
- Chapter typically official 6 months after publication*

Access Pharmacopeial Forum here.

*Official dates can be delayed.



Proposed CE-SDS with UV Detection

Modified sample buffer and system parameters

Proposed CE-SDS with UV Detection

What's different? What's the same?

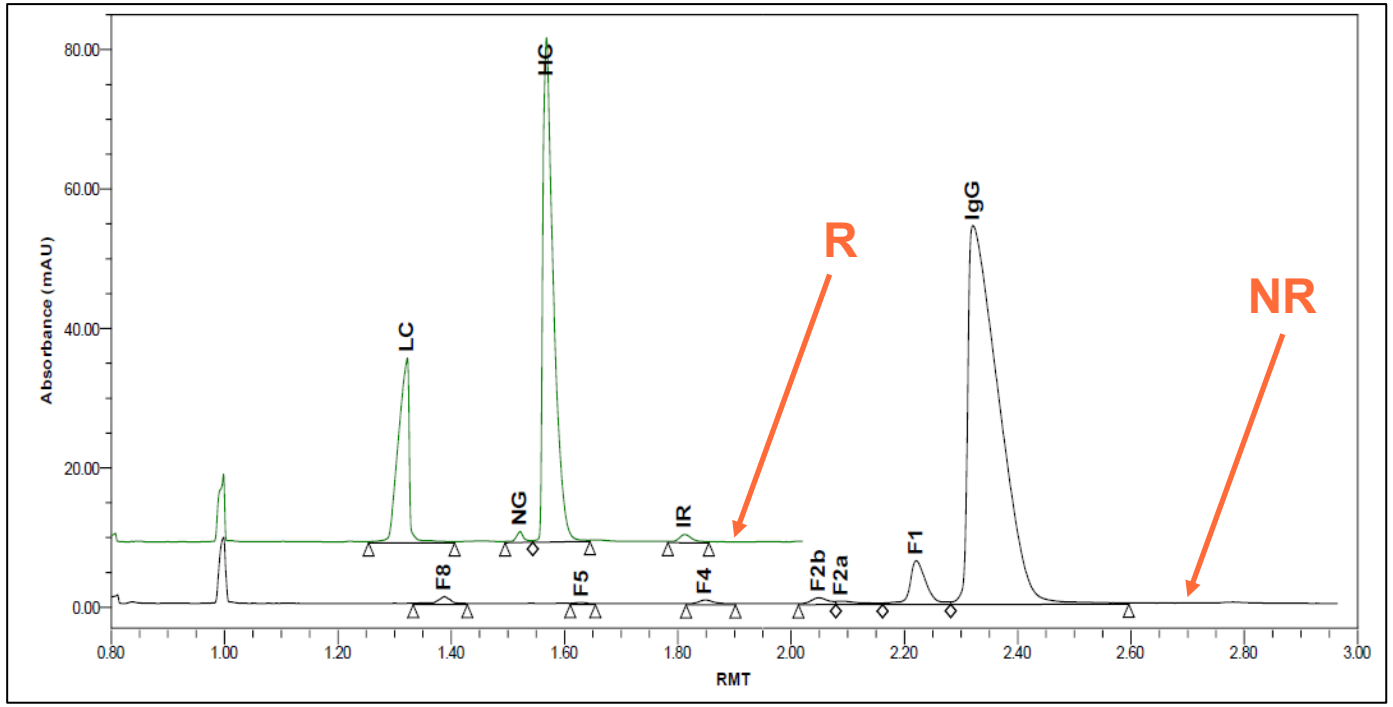
- **Materials and Reagents:**
  - Shorter capillary
  - Shorter run time
  - Modified Sample buffer
    - Non-reducing buffer with alkylating agent
    - Reducing buffer with reducing agent

- **Workflow**
  - Same as <129>
  - Two separation modes
    - Option 1, 35 minutes NR, 25 minutes R
    - Option 2, 8 minutes NR, 5.5 minutes R
  - System Suitability parameters remain the same



Proposed CE-SDS with UV Detection

USP Monoclonal IgG System Suitability RS, Reduced and Non-reduced



- System Suitability criteria met
  - Amount of Main Peak (61.4% - 86.4%) will need to be updated for IgG System Suitability mAb

Non-reduced

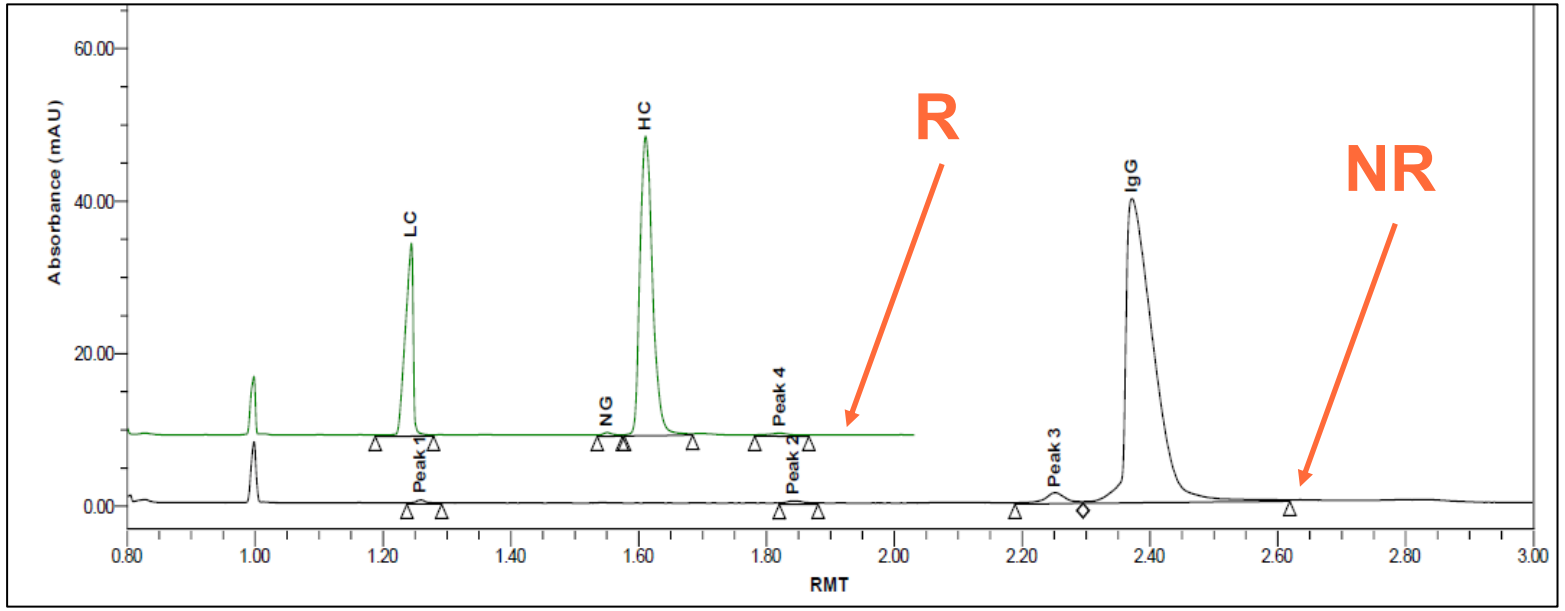
Sample	F8	F6	F5	F4	F2	F1	IgG	Resolution of IgG main peak and Fragment 1 (F1)
USP IgG SS RS_<129>	0.7	3.0	0.2	0.4	3.2	13.8	78.7	1.9
USP IgG SS RS_M-buffer	ND	0.6	0.1	0.4	1.0	5.0	92.9	2.2

Reduced

Sample	LC	NgHC	HC	Resolution of NGHC and HC	Ratio of NG to Total heavy chains (%)
USP IgG SS RS_<129>	30.7	0.7	68.0	1.4	1.06
USP IgG SS RS_M-buffer	26.4	0.9	71.7	2.2	1.18

Proposed CE-SDS with UV Detection

USP mAb 001, Reduced and Non-reduced



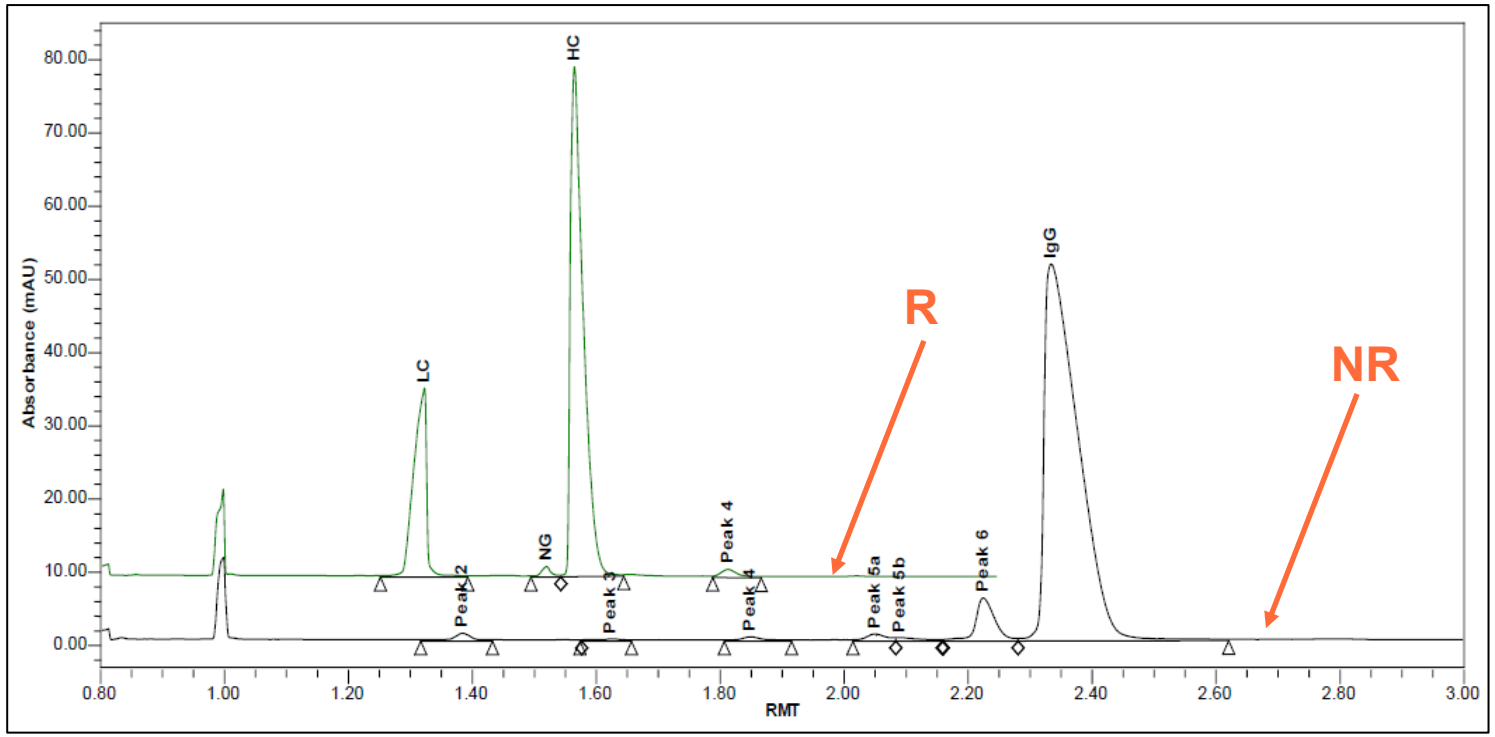
For mAbs like USP mAb001, results are comparable

Non-reduced				
Sample	**Peak 1**	**Peak 2**	**Peak 3**	**IgG**
USP mAb-001_<129>	1.1	0.5	2.5	95.9
USP mAb-001_M-buffer	0.3	0.4	2.1	97.2

Reduced				
Sample	**LC**	**NgHC**	**HC**	**Peak 4**
USP mAb-001_<129>	35.7	0.3	63.8	0.2
USP mAb-001_M-buffer	28.3	0.3	70.9	0.5

Proposed CE-SDS with UV Detection

USP mAb 003, Reduced and Non-reduced



For mAbs sensitive to sample preparation conditions (e.g. USP mAb003), fragmentation is decreased

Non-reduced							
Sample	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**	**Peak 6**	**IgG**
USP mAb-001_<129>	0.6	5.6	0.7	0.4	6.7	17.1	69.0
USP mAb-001_M-buffer	ND	0.7	0.1	0.4	1.0	5.4	92.4

Reduced				
Sample	**LC**	**NgHC**	**HC**	**Peak 4**
USP mAb-001_<129>	30.6	0.7	68.2	0.6
USP mAb-001_M-buffer	26.1	0.8	72.0	1.0

Observations

Comparison of <129> method to modified method with UV detection

- Modified buffer along with updated system parameters
  - System Suitability criteria were met
    - Criteria for percent of main peak will need to be updated for IgG System Suitability mAb
  - For mAbs not sensitive to fragmentation, **profiles and results are comparable**
  - For **sensitive mAbs, fragmentation was reduced**, main peak area increased
    - IgG SS- 79% → 93%
    - mAb 003, 69% → 92%
  - **Shortened run time** versus the current compendial method
    - Two options for running parameters in modified method planned
      - 40 minutes in <129>, 35/25 minutes for one option, 8/5.5 minutes for the second
- USP evaluation shows the modified buffer with updated system parameters is a **viable additional option to the existing method**



CE-SDS with Fluorescence Detection

CE-SDS with Fluorescence Detection

Method Overview

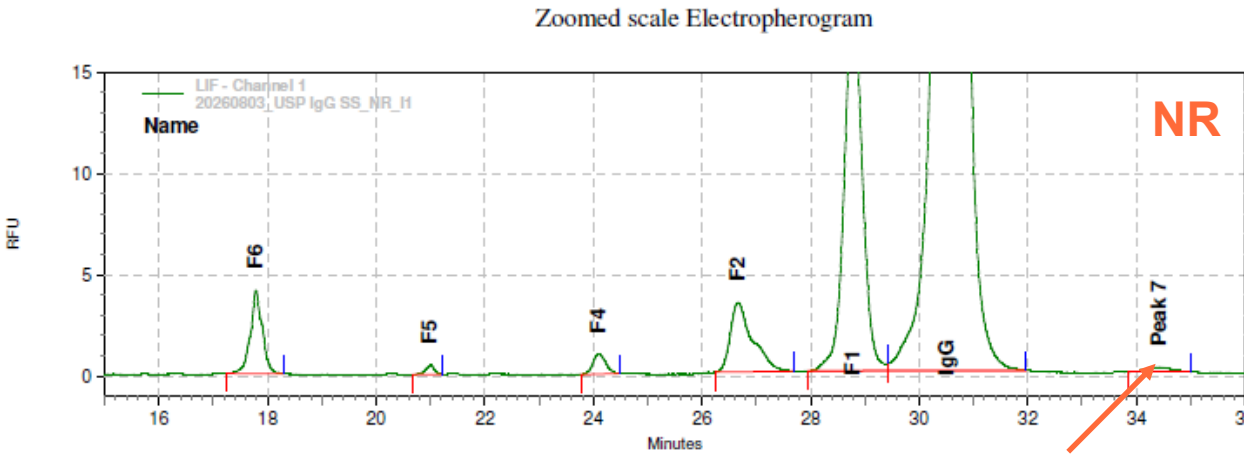
- **Key Reagents:**
  - Chromeo P503 dye
  - Labeling buffer
  - Internal marker

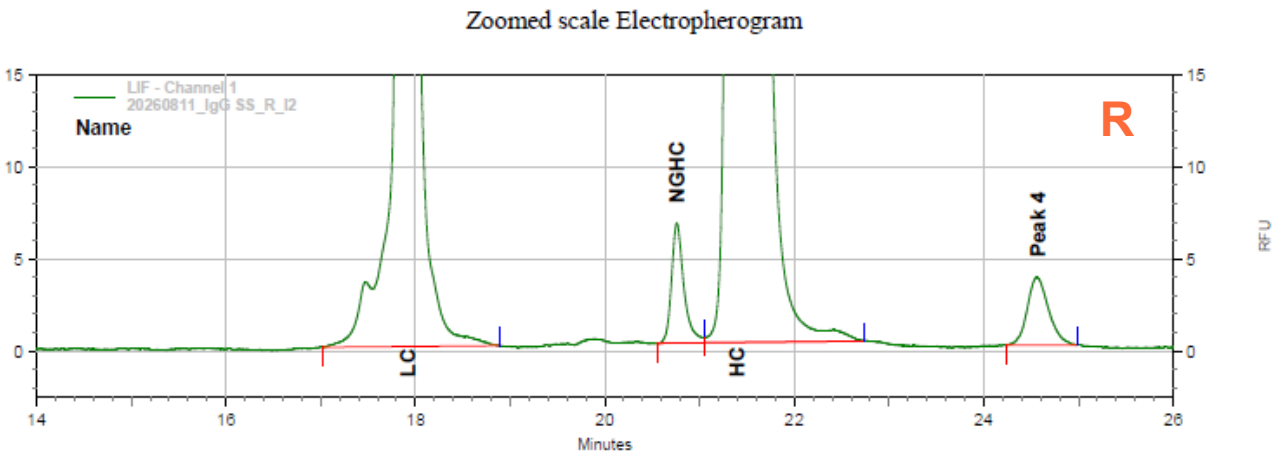
- **Workflow**
  - Similar to <129>
  - Reduced and non-reduced follow the same labeling and thermal treatment
  - System Suitability parameters remain the same



Proposed CE-SDS with Fluorescence Detection

USP Monoclonal IgG System Suitability RS, Reduced and Non-reduced





Observed additional peak post IgG main peak

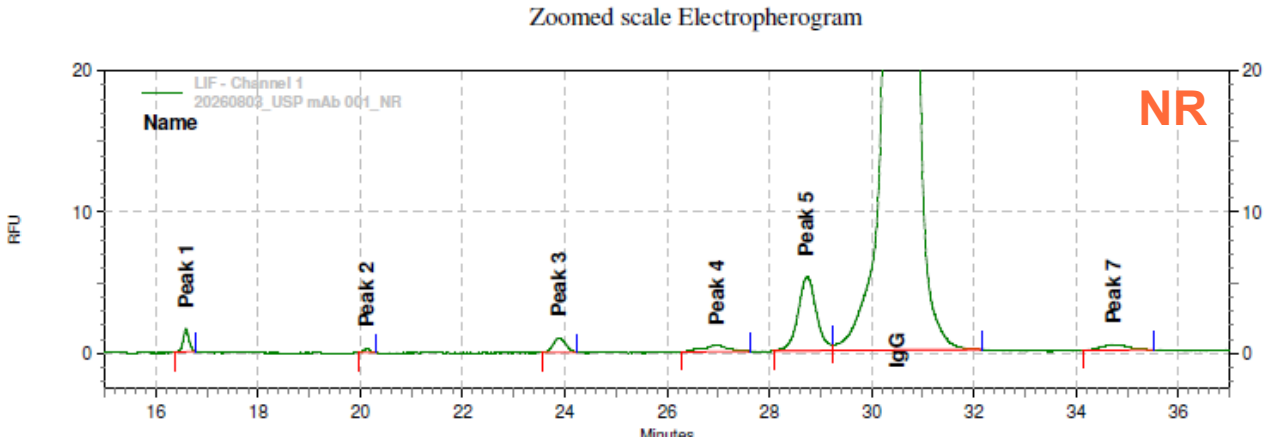
Sample	F8	F6	F5	F4	F2	F1	IgG	Peak 7	Resolution of IgG main peak and Fragment 1 (F1)
USP IgG System Suitability RS_<129>	0.7	3.0	0.2	0.4	3.2	13.8	78.7	ND	1.9
USP IgG System Suitability RS_FLR	ND	2.1	0.1	0.4	2.1	8.6	86.5	0.1	2.8

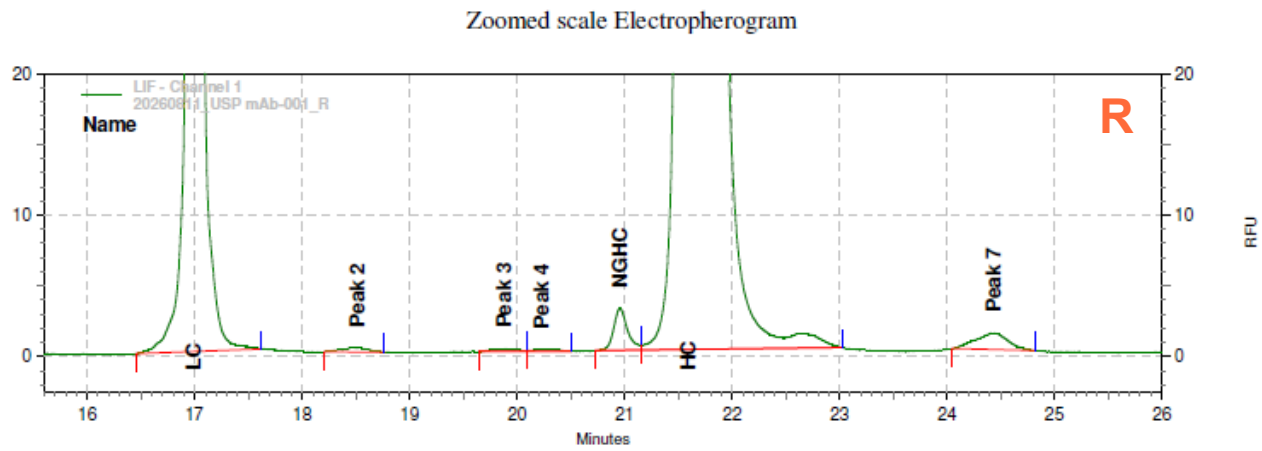
Sample	LC	NGHC	HC	Peak 4	Resolution of NGHC and HC	Ratio of NG to Total heavy chains (%)
USP IgG SS RS_<129>	30.7	0.7	68.0	0.6	1.4	1.06
USP IgG SS RS_FLR	23.8	0.8	74.8	0.7	2.1	1.06

- System Suitability criteria met
  - Amount of Main Peak (61.4% - 86.4%) will need to be updated on IgG System Suitability mAb Certificate

Proposed CE-SDS with Fluorescence Detection

USP mAb 001, Reduced and Non-reduced





Sample	Peak 1	Peak 2	Peak 3	IgG
USP mAb 001_<129>	1.1	0.5	2.5	95.9

Sample	LC	NGHC	HC	Peak 4
USP mAb 001_<129>	35.7	0.3	63.8	0.2

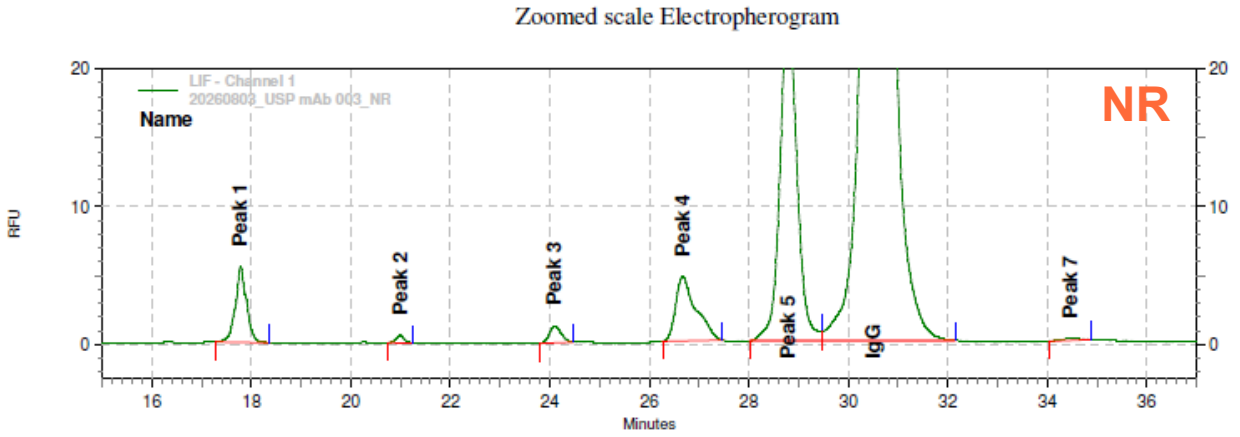
Sample	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	IgG	Peak 7
USP mAb 001_FLR	0.4	0.1	0.4	0.3	2.2	96.5	0.2

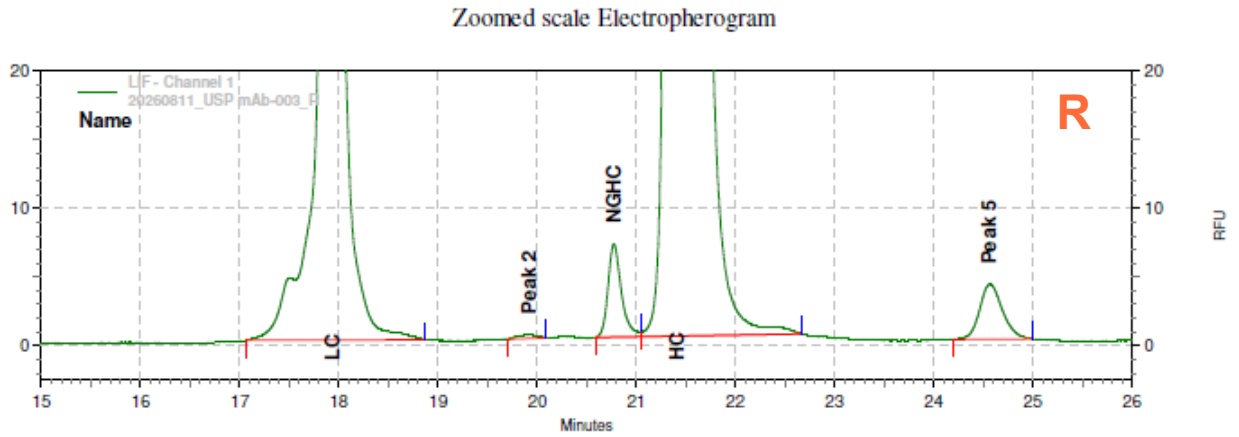
Sample	LC	Peak 2	NGHC	HC	Peak 7
USP mAb 001_FLR	24.7	0.1	0.3	74.5	0.3

Additional impurity peaks detected with fluorescence

Proposed CE-SDS with Fluorescence Detection

USP mAb 003, Reduced and Non-reduced





Sample	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	IgG
USP mAb 003_<129>	0.6	5.6	0.7	0.4	6.7	17.1	69.0

Sample	LC	NGHC	HC	Peak 4	Peak 5
USP mAb 003_<129>	30.6	0.7	68.2	0.6	ND

Sample	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	IgG	Peak 7
USP mAb 003_FLR	2.2	0.1	0.4	2.2	9.0	86.0	0.1

Sample	LC	Peak 2	NGHC	HC	Peak 5
USP mAb 003_FLR	23.3	0.05	0.8	75.2	0.7

Additional impurity peaks detected with fluorescence

Observations

Comparison of fluorescence method to <129> UV detection

- Fluorescence method
  - System Suitability criteria were met
    - Criteria for main peak area percentage will need to be updated for IgG System Suitability mAb
  - **Greater sensitivity** was demonstrated by fluorescence detection
    - Method could enable analysis of low concentration samples
    - Enables measurement of **impurities present at low concentrations**
      - Several distinct supplementary peaks were detected
  - Analysis of Reference Standards by both methods
    - Suggest that both techniques yield **comparable size-variant profiles and results**
- USP evaluation shows the fluorescence method is a **viable additional option to the existing method**

Comparison of CE-SDS Methods

Results from current <129> CE-SDS, proposed UV, proposed Fluorescence

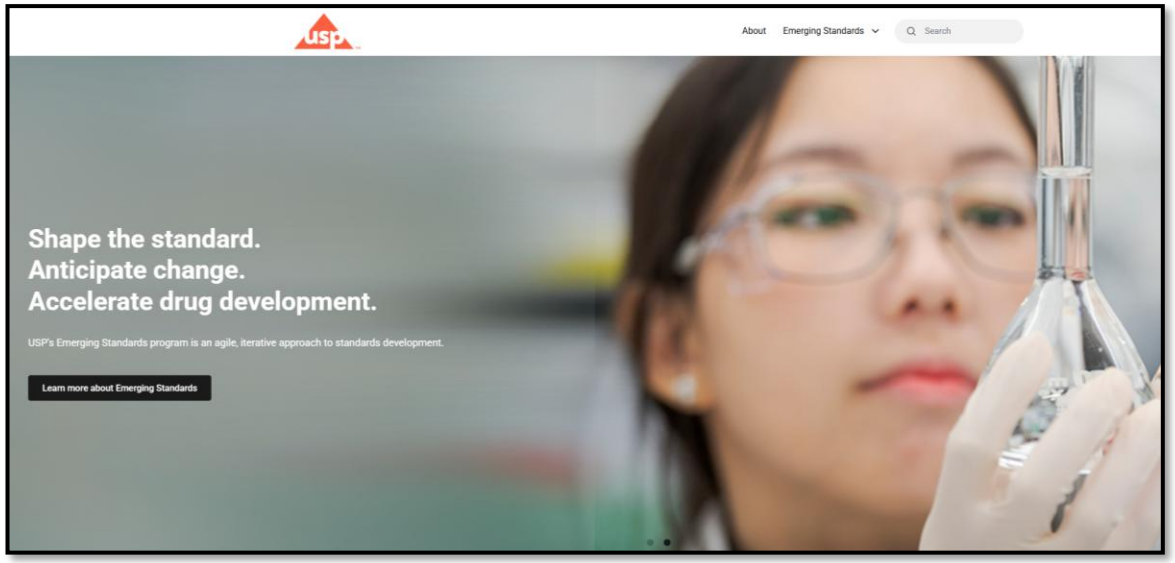
Sample	IgG main peak (%)		
	<129>	UV Proposed	FLR Proposed
IgG System Suitability RS	78.7	92.9	86.5
USP mAb 001	95.9	97.2	96.5
USP mAb 002	93.2	94.0	91.8
USP mAb 003	69.0	92.4	86.0

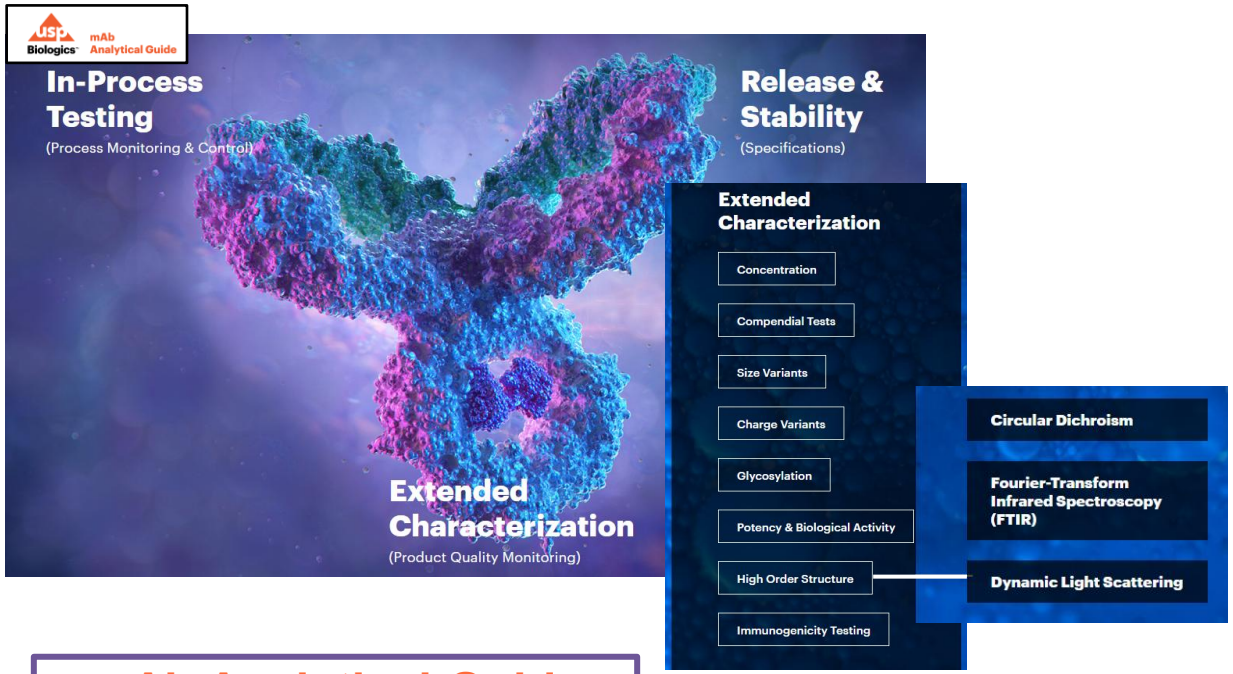
Sample	<129>			UV Proposed			FLR Proposed		
	LC (%)	NGHC (%)	HC (%)	LC (%)	NGHC (%)	HC (%)	LC (%)	NGHC (%)	HC (%)
IgG System Suitability RS	30.7	0.7	68.0	26.4	0.9	71.7	23.8	0.8	74.8
USP mAb 001	35.7	0.3	63.8	28.3	0.3	70.9	24.7	0.3	74.5
USP mAb 002	32.2	0.6	66.8	25.8	0.7	72.8	25.8	0.6	73.0
USP mAb 003	30.6	0.7	68.2	26.1	0.8	72.0	23.3	0.8	75.2

Summary and Next Steps

- A new proposed **CE-SDS method with UV detection** as well as a new method with **fluorescence detection** have been evaluated by USP for **inclusion in <129>**
  - Both methods have been **validated by the donors** and **tested by USP** laboratories
    - These methods deliver similar purity as the current UV method while improving sensitivity, reducing method-induced fragmentation, and shortening analysis time
  - Both methods **provide additional compendial options**, rather than replacing the existing UV method
- Both methods are being evaluated by an Expert Committee
  - Publication in USP Pharmacopeial Forum (PF) is projected for **PF 53 (4) in July 2027**
- Other potential future revisions
  - **Revise System Suitability mAb certificate** for modified UV and fluorescence methods
  - **Remove glycan methods** from <129>, revise/modernize and consolidate with other glycan chapters *e.g.* <210> *Monosaccharide Analysis,* <212> *Oligosaccharide Analysis*
  - **Requesting feedback on proposed and alternative methods**

Resources for mAbs and Therapeutic Proteins





Other USP Resources

- Reference Standards
- Documentary Standards
- USP Educational Contents
- Other Resources

www.usp.org/biologics

mAb Analytical Guide

Questions

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The standard of trust