Horizontal standards for mABs: Recent experiences @MAB WP

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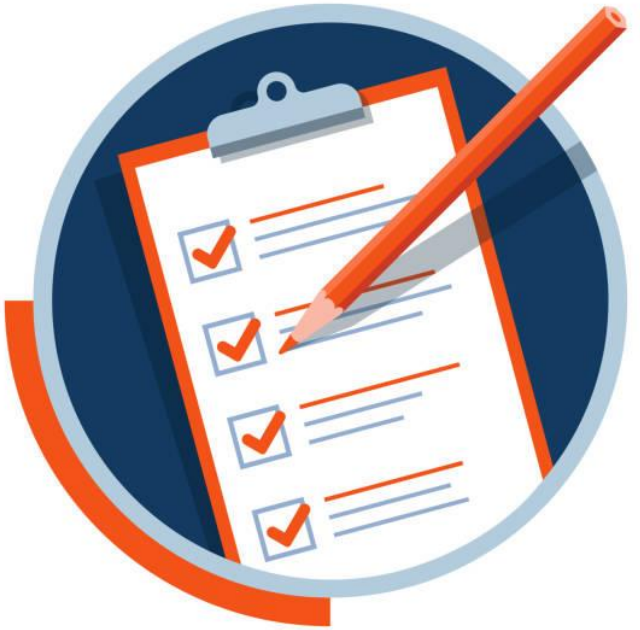
Disclaimer

The content of the following presentation represents the speaker's view and does not necessarily reflect an official point of view

Presentation outline

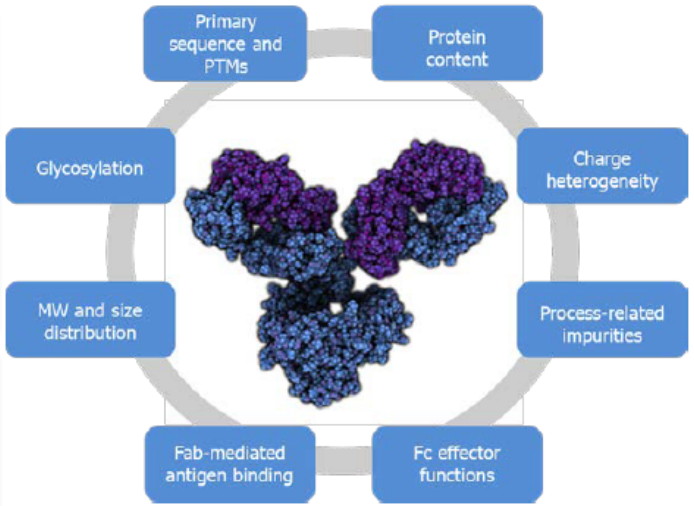
- Ph.Eur. and MAB WP approaches in developing horizontal standards
- cIEF study:
  - “Comparability” between cIEF and icIEF
  - Pitfalls, approaches and perspectives
  - The text: recommendations
- New general texts: recent developments
- Concluding remarks



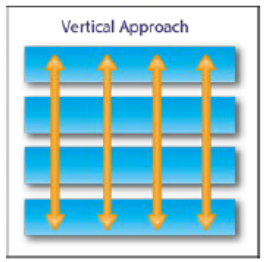
Ph. Eur. Approaches to Public Standard-Setting

Monographs

- Ustekinumab (3165)*
- Adalimumab (3147)*
- Golimumab concentrated solution (3103)*
- Infliximab concentrated solution (2928)

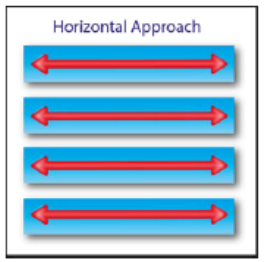


Definition
Production
Identification
Tests
Assay/Content
Assay/Potency



Horizontal standards (general chapters)

- Cell-based assays for potency determination of TNF-alpha antagonists (2.7.26)**
- Size-exclusion chromatography for recombinant therapeutic monoclonal antibodies (2.5.43)*
- Capillary isoelectric focusing for recombinant therapeutic monoclonal antibodies (2.5.44)*



*under elaboration
** to be published in Ph. Eur. Supplement 11.1 (Oct. 2022)

Courtesy of M.Buda - EDQM

Horizontal Standard Development: the first step

- New general chapter Cell-based assay for potency determination of TNF-alpha antagonists (2.7.26) adopted by the European Pharmacopoeia (Ph. Eur.) Commission at its 172nd session in March 2022
- First of three planned new horizontal standards for monoclonal antibodies (mAbs)
- Text published in Ph. Eur. Supplement 11.1 along with updated monographs on TNF-alpha antagonists to create link to the new text (TNF-alpha bioassay package)
- Implemented on 1 April 2023

Horizontal Standard Development Beyond Product Class

2.5.43 Size exclusion chromatography for recombinant therapeutic monoclonal antibodies:

- widely used methodology for determination of size variants (monomer, HMWS); quantitation of LMWS can be highly variable depending on the mAb analysed
- SE-HPLC and SE-UPLC procedures, widely applicable to mAbs, given as examples
- suitability demonstrated by collaborative study

2.5.44 Capillary isoelectric focusing for recombinant therapeutic monoclonal antibodies:

- (i)cIEF procedures for analysis of charge heterogeneity of mAbs, to monitor identity, quality, production consistency
- based on data generated in multi-laboratory verification study
- guidance on the aspects to consider for product-specific application (validation)

Well-defined analytical procedures and tools to control performance (including reference materials) and facilitate analytical assessment of key quality attributes of mAbs

“Horizontal” texts: indicating a direction

- Well-defined analytical procedures and tools
- Evaluation by mean of collaborative studies involving more labs
- Based on validated methods on a single molecule, and extended to a class
- Basic principles collected – e.g.:
  - System suitability criteria
  - Minimal validation performance criteria
  - Indications for resolution and integration approach
  - Identification of appropriate control and reference materials

- Not mandatory
- Starting point for the development and validation of molecule-specific methods

Charge heterogeneity as a CQA

- The major sources of charge-related heterogeneity of therapeutic IgG include differences in glycosylation and degradation pathways
- Potential impact on safety and efficacy
- One of the most used methods is capillary Isoelectric focusing (cIEF) used for:
  - Characterization
  - DS and DP release specifications (identity and purity) → comparison to a reference standard

Major chemical degradation pathways	Effect	Species formed
Sialylation	COOH addition	Acidic
Deamidation	COOH formation	Acidic
C-terminal lysine cleavage	Loss of NH2	Acidic
Adduct formation	COOH formation or loss of NH2	Acidic
Succinimide formation	Loss of COOH	Basic
Methionine, cysteine, lysine, histidine, tryptophan oxidation	Conformational change	Basic
Disulfide-mediated	Conformational change	Basic
Asialylation (terminal Galactose)	Loss of COOH	Basic
C-terminal lysine and glycine amidation	NH2 formation or loss of COOH	Basic

cIEF as batch release assay

Table 4. A typical list of batch release assays for mAb drug substance.

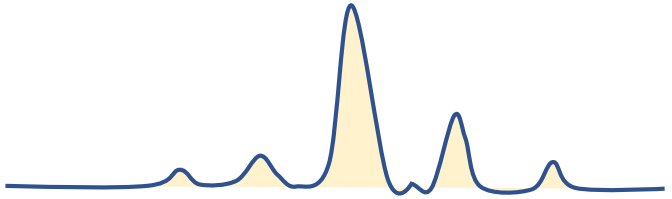
Attributes	Methods
Safety	Bioburden
Safety	Endotoxin
General	Appearance (color and clarity)
General	pH
General	Concentration
Identity	Peptide mapping (LC-UV)
Purity	SDS-PAGE/CE-SDS (non-Reducing and reducing)
Purity	SEC-HPLC
Potency	Antigen binding
Potency	Cell-based assay
Potency	Effector functions*
Charge/identity	IEX-HPLC/IEF/cIEF/CZE
Glycosylation	N-glycan profiling by NP-HPLC of labeled glycans
Impurities	HCPs
Impurities	Host cell DNA
Impurities	Residual protein A

*If involved in the mechanism of action.

One of the most used methods is capillary Isoelectric Focusing (cIEF) used for:

- Characterization
- DS and DP release specifications (identity and purity) → comparison to a reference standard

cIEF collaborative study

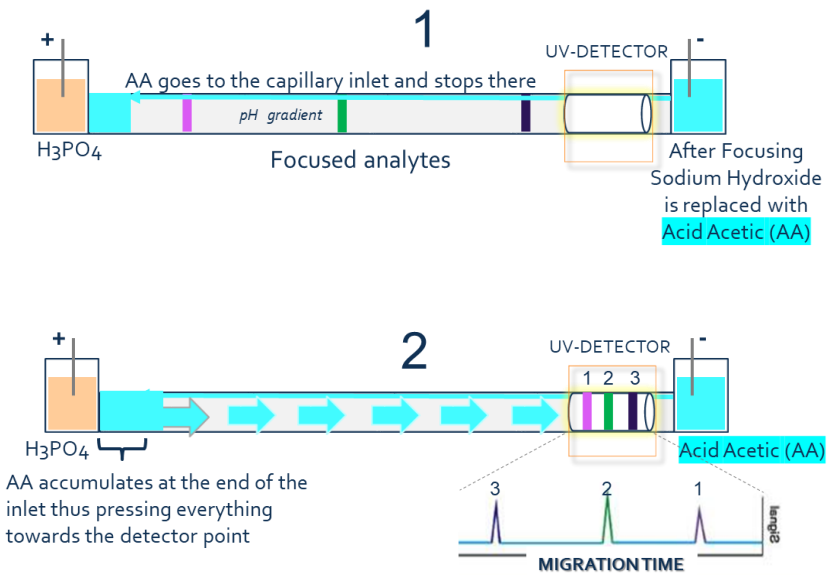


- Experimental verification of a capillary isoelectric focusing (cIEF) procedure used to determine the distribution of charge isoforms of therapeutic mAbs
- The study aims to assess the suitability of a specific cIEF procedure to be applied as a generic method to monitor charge heterogeneity on a broad range of mAbs, as well as to discriminate between closely related mAbs.
- Several laboratories involved, including OMCLs,
- 7 different IgG1 mAbs tested

cIEF : different systems available

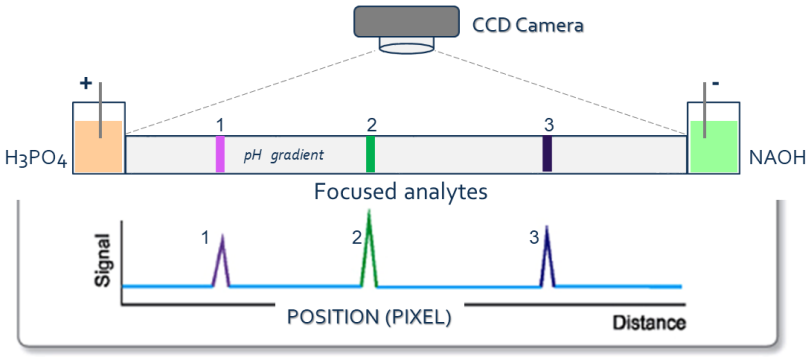
cIEF
(mobilization phase necessary)

The molecular species focused inside the capillary are forced to move through the detector



icIEF
(real-time monitoring of the process)

Detection is performed by a charge coupled device (CCD) camera all along the capillary (5 cm)



The content of the entire capillary is detected at close time intervals during focusing.
This allows the detection of the real-time focusing process with sequential images until the final focusing and separation of the analytes.

Study steps

- “classical” cIEF method and protocol defined, on the basis of a validated (and approved) method from a mAb manufacturer
- Only broad range ampholyte used (pH 3-10)
- Method run on both cIEF and i-cIEF platforms
  - Same reagents and sample concentration, operating conditions, where possible (buffers, temperature, samples composition, etc…)
  - Adaptation to i-cIEF specific requirements
- Satisfactory performance on cIEF system
- Discrepant results on icIEF vs cIEF system

Reagents and materials

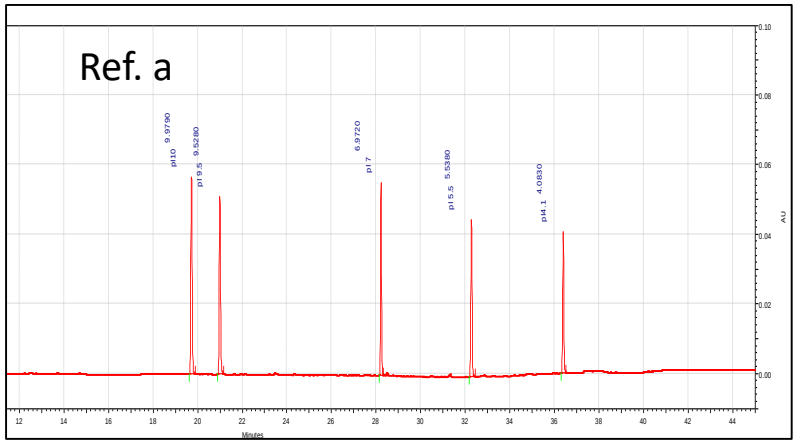
	cIEF	icIEF
Anolyte solution	200 mM Phosphoric acid	80 mM Phosphoric acid * in 0.1 % methyl cellulose
Catholyte solution	300 mM NaOH	100 mM NaOH * in 0.1 % methyl cellulose
Mobilising Solution	350 mM acetic acid	Not Necessary (imaged cIEF system)
Cathodic stabiliser	500 mM L-Arginin	500 mM L-Arginin
Anodic stabilizer	200 mM iminodiacetic acid	200 mM iminodiacetic acid
Urea solution	4.3 M	4.3 M
cIEF GEL	Urea-cIEF Gel 3 M	Not Necessary Cartridge ready to use
TRIS-Buffer	20 mM (pH 8.0)	20 mM (pH 8.0)

Operating volumes and sample solution

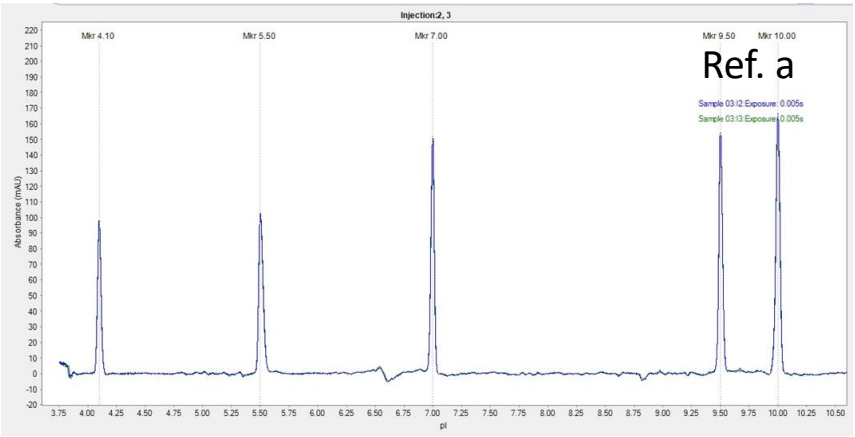
		cIEF		icIEF	
Ref. Sol.	Type	Composition	Final Volume µL	Composition	Final Volume µL
a	pI Marker-Mix (System Suitability)	Urea-cIEF-Gel, Pharm.3-10, stabilisers, pI Markers (all), *water R*.	250	MC1%, Urea, Pharm.3-10, stabilisers, pI Markers (all),Tris B, *water R*	200
b	Sample Pre-Mix	Urea-cIEF-Gel, Pharm.3-10, Stabilisers, pI Markers (4.1, 9.5, 10)	240	MC1%, Urea, Pharm. 3-10, Stabilisers pI Markers (4.1, 9.5, 10)	166
c	Blank	Ref. Sol.b +Tris B	250	Ref. Sol.b +Tris B+ Water R	200
d	Infliximab (CRS)	Ref. Sol.b+CRS+pI M 7+Tris B	250	Ref. Sol.b+CRS+pI M 7+Tris B+Water R	200
TS	Test Solution (A,B,C,D,E,F)	Ref. Sol.b+Desalted Sample	250	Ref. Sol. b+Desalted Sample+Water R	200

Performance at comparable experimental conditions

cIEF



icIEF

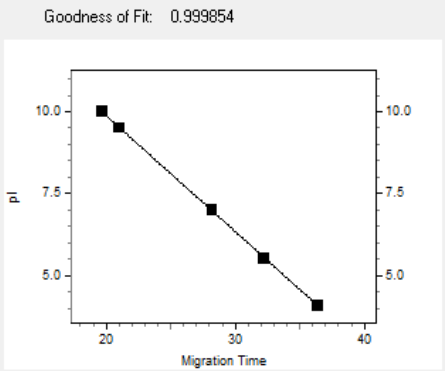


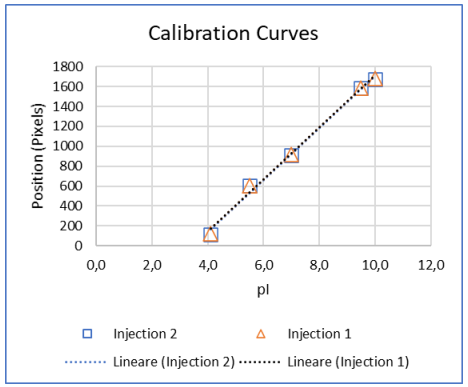
	pI	Migration Time
1	10	19.717000
2	9.5	20.992000
3	7	28.225000
4	5.5	32.283000
5	4.1	36.400000
6		

Fit type: Linear

Reference Peak Time (min): 0

Reference Window %: 0



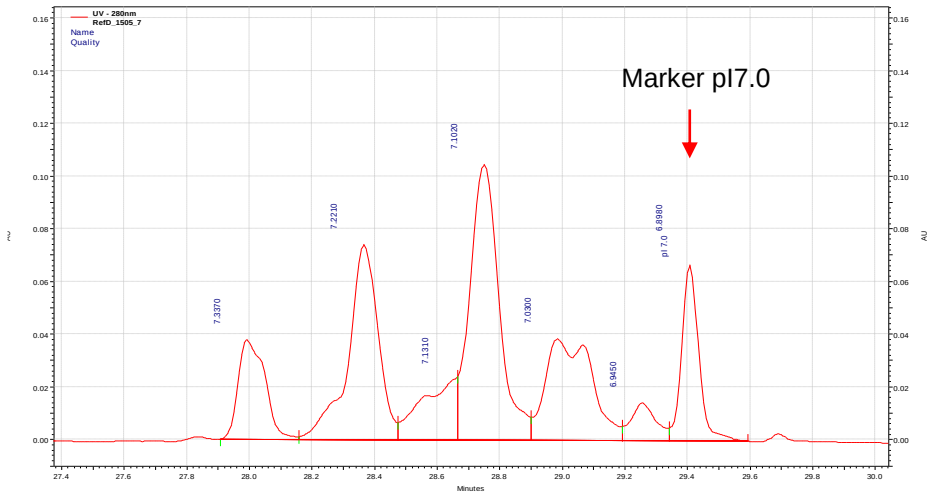


Determination (R^2)	
injection1	injection2
0,9950	0,9951

Linear Fit Parameters (y=m*+q)		
	injection1	injection2
M	0,004	0,004
Q	3,458	3,481

Performance at comparable experimental conditions

cIEF



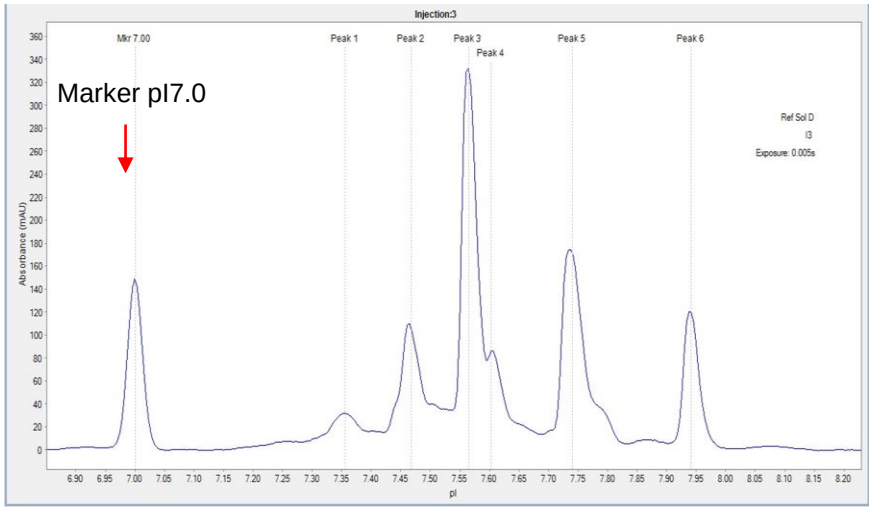
pH range

7.3 — 6.9

Migration time Range (min)

28.0 — 29.5

icIEF



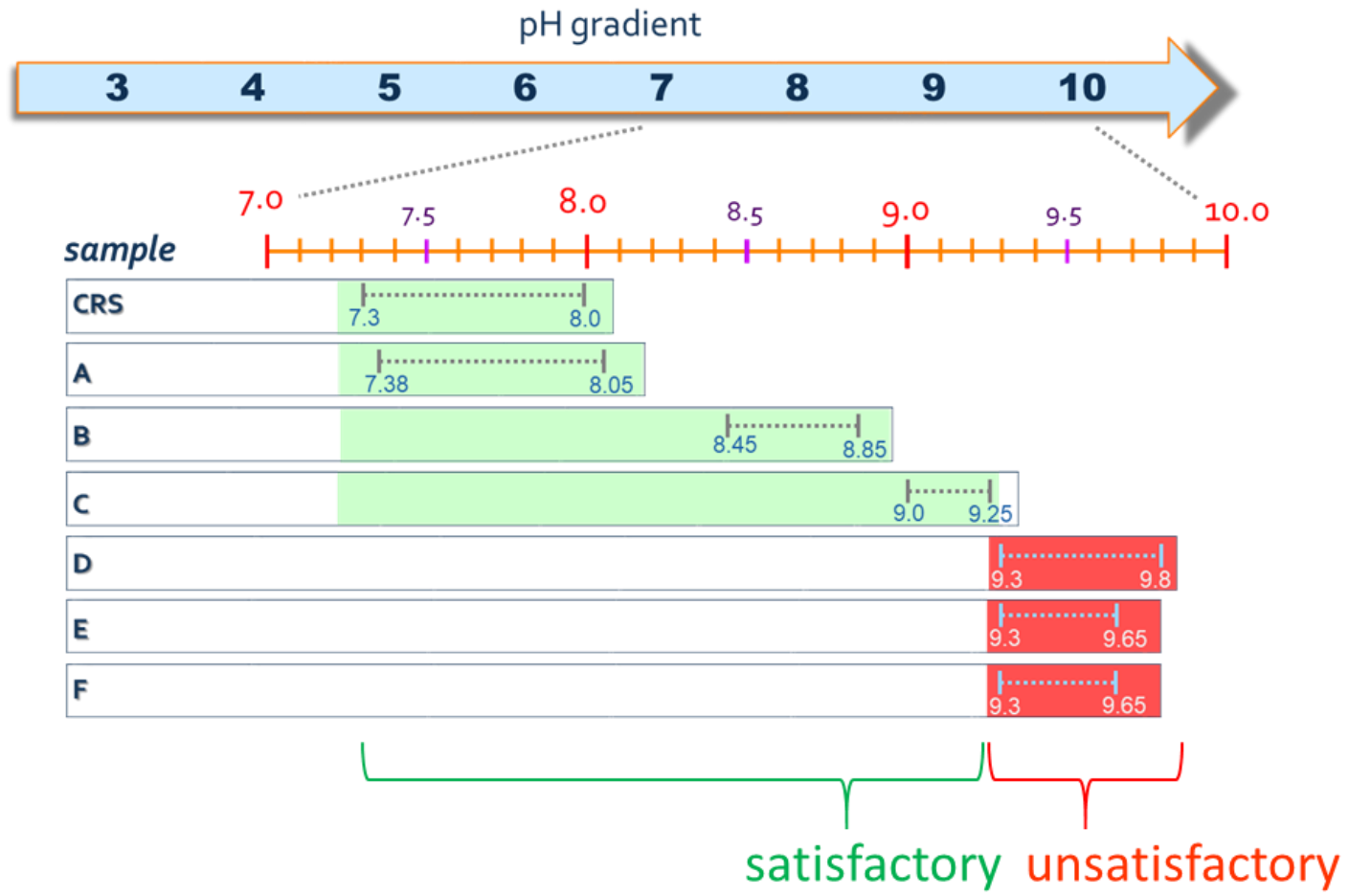
pH range

7.3 — 8.0

Position Range (Pixel)

995 — 1147

mAbs pI range and resolution



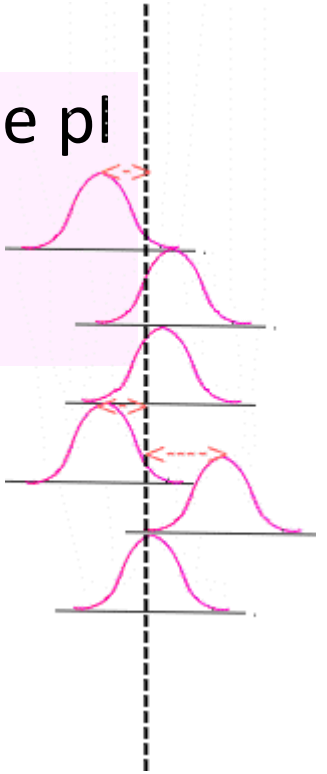
	Calculated (Vector NTI)	Calculated (MassLynx)
Adalimumab	8.7	8.9
Atezolizumab.	8.6	8.8
Belimumab	NA	NA
Bevacizumab	8.5	8.7
Cetuximab	8.7	8.9
Dalotuzumab	9.0	9.1
Denosumab	8.8	9.0
Eculizumab	6.0	6.4
Elotuzumab	8.3	8.0
Infliximab	7.1	7.4
Ipilimumab	8.9	9.1
Ixekizumab	NA	NA
Natalizumab	8.0	7.8
NISTmab	8.8	9.0
Nivolumab	8.0	8.3
Obinutuzumab	8.7	8.8
Ofatumumab	8.8	9.0
Palivizumab	9.0	9.3
Panitumumab	6.8	7.1
Pembrolizumab	7.4	7.8
Pertuzumab	8.7	8.9
Ramucirumab	8.9	8.6
Reslizumab	NA	NA
Rituximab	9.1	9.3
Trastuzumab	8.8	9.0

Isoelectric-points of 25 mAbs determined by icIEF and their theoretical calculations based on amino acids sequences.
NA: not applicable when the light chains and heavy chains sequences were not available.

(A. Goyon et al. Journal of Chromatography B 1065–1066 (2017) 119–128)

Comparison of cIEF and icIEF results

- Inconsistent profiles (different resolution power, variable across the pI scale)
- Different pIs of the variants identified



Variability sources ?

- Different capillary length
- Additional mobilization phase
- Different software calibration approaches

Possible solutions

careful re-evaluation of the factors known to have a significant impact on resolution, e.g. focusing time, ampholytes concentration and range, pH stabilisers (L-arginine/iminodiacetic Acid) concentration, urea concentration

- Further adjustment of experimental conditions of the protocol to be run on icIEF system

- Definition of an imaged-cIEF-specific protocol (from a MAH method validated and approved from a registered mAbs\ dossier)

  - Good resolution
  - Reduced measured pI values inconsistencies
  - Focus on reduced pI window

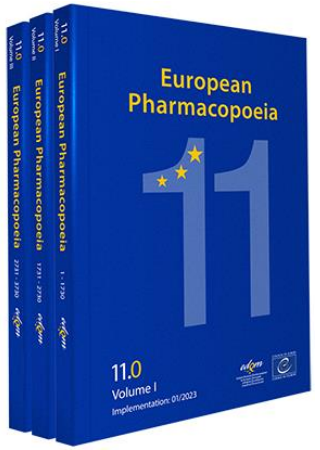
Where are we now

- icIEF method verified by 3 labs
- Evaluation of results ongoing:
  - Comparable resolution
  - “Closer” pIs
- Open questions:
  - Is measured pI an objective parameter?
    - Not to be used as a SST criterion
  - Is identification by cIEF possible only by comparison to a Reference standard?

(i)cIEF general chapter 2.5.44

Draft general chapter on *Capillary isoelectric focusing for recombinant therapeutic monoclonal antibodies (2.5.44)* [version agreed at the last MAB WP meeting in March 2023];

- Timelines draft chapter:
  - publication for comments in Pharmeuropa 35.4 (October-December 2023) [of note: public deadline: 31/12/2023; NPA deadline: 29/02/2024]
  - review of comments by the MAB WP (March 2024)
  - *submission for adoption by the Ph. Eur. Commission.*



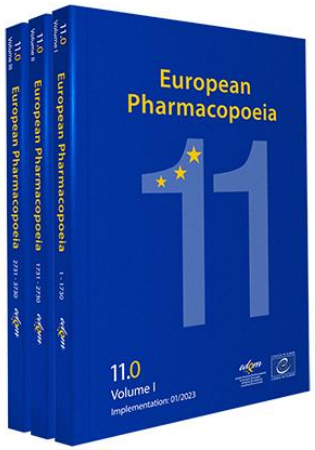
(i)cIEF general chapter 2.5.44 – Structure

- Procedure A (two step cIEF) and Procedure B (imaged cIEF)
- Distinguished sections:
  - Materials/samples/reference solutions
  - Operating conditions
  - System performance
  - System suitability
  - Assay acceptance criteria
- Common sections:
  - Introduction & Scope, Principle
  - Data analysis
  - Results
  - General recommendations

Points to consider in analytical procedure development; general considerations on validation

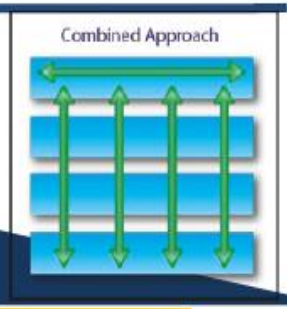
Text recommendations

- Starting conditions for the development of a cIEF or imaged cIEF procedure for a specific mAb
- The extent of the analytical procedure development [..] should be determined based on suitability for a specific product (case by case)
- The measured pI values are affected by the testing environment
- The shape of the pH gradient [..] changes along with a change in the ampholytes used in the analysis. Therefore, careful consideration should be given to selection of ampholytes
- Optimisation may be needed to reach the desired resolution
- Validation needed for each mAb, to demonstrate the suitability of the analytical procedure for the intended use (release/stability)



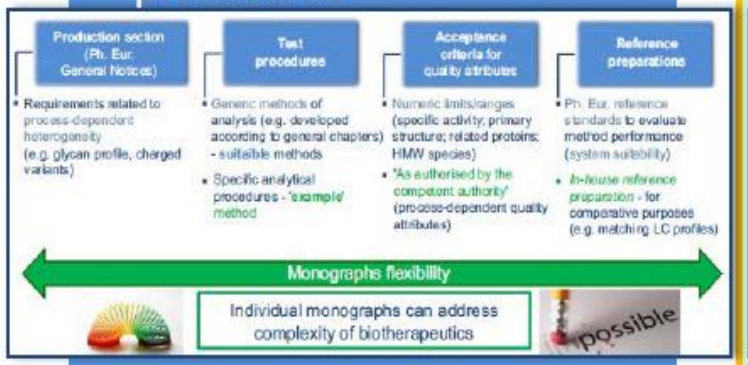
Ph. Eur. Standards for mAbs: Summary

Ph. Eur. Standards for Therapeutic Monoclonal Antibodies: Development Approaches



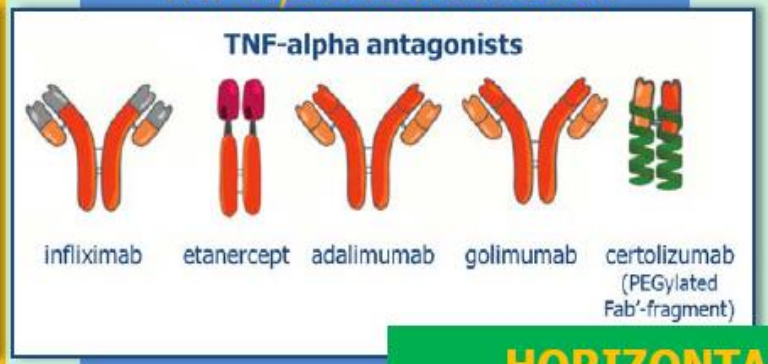
MONOGRAPHS*

- built-in ***flexibility***
- ***examples*** of suitable procedures



PRODUCT CLASS - BASED STANDARDS

- product classes/sub-classes quality attributes
- *TNF-alpha neutralisation*



PERFORMANCE-BASED STANDARDS

- *platform* methodologies
- performance characteristics
- reference standards

HORIZONTAL STANDARDS

PRODUCT KNOWLEDGE, CASE STUDIES, COLLABORATIVE TESTING

*Buda M., Kolaj-Robin O., Charton E. *Biotherapeutic Products in the European Pharmacopoeia: Have all Challenges Been Tackled?* Generics and Biosimilars Initiative Journal. 2022;11(1)

Conclusions

- Flexible concepts of standardisation
- Key quality attributes and associated testing strategies
- Provide common expectations and general methodologies applicable to wide range/classes of mAbs
- Provide guidance on aspects to consider when an analytical procedure is suitable for its intended purpose
- Contribute to standardisation of therapeutic monoclonal antibodies through rationalisation of methodologies and common functionalities
- Increase the knowledge and lead to technical standardization improvement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