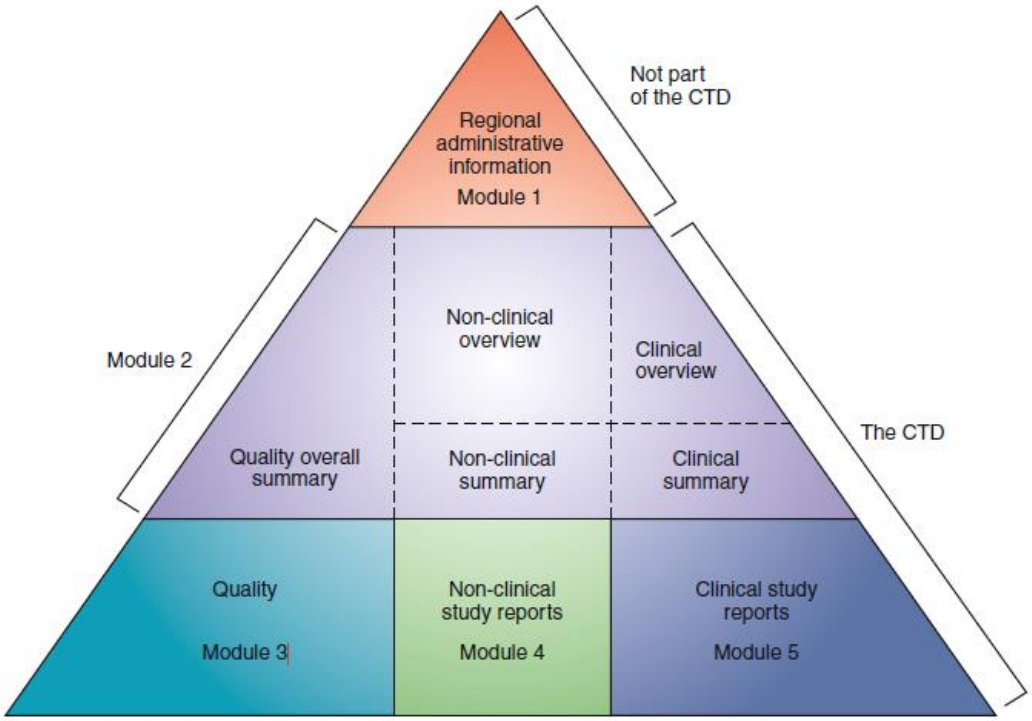
BIOTECHNOLOGY

Analytical method development

Analytical method validation and transfer

Schäfer Tamás
Biotechnológiai analitikai osztály - osztályvezető

The major components of the Common Technical Document (*CTD*)



Module 1: Administrative Information and Prescribing Information (region specific)

Module 2: CTD Module Summaries

Module 3: Quality (CMC Sections)

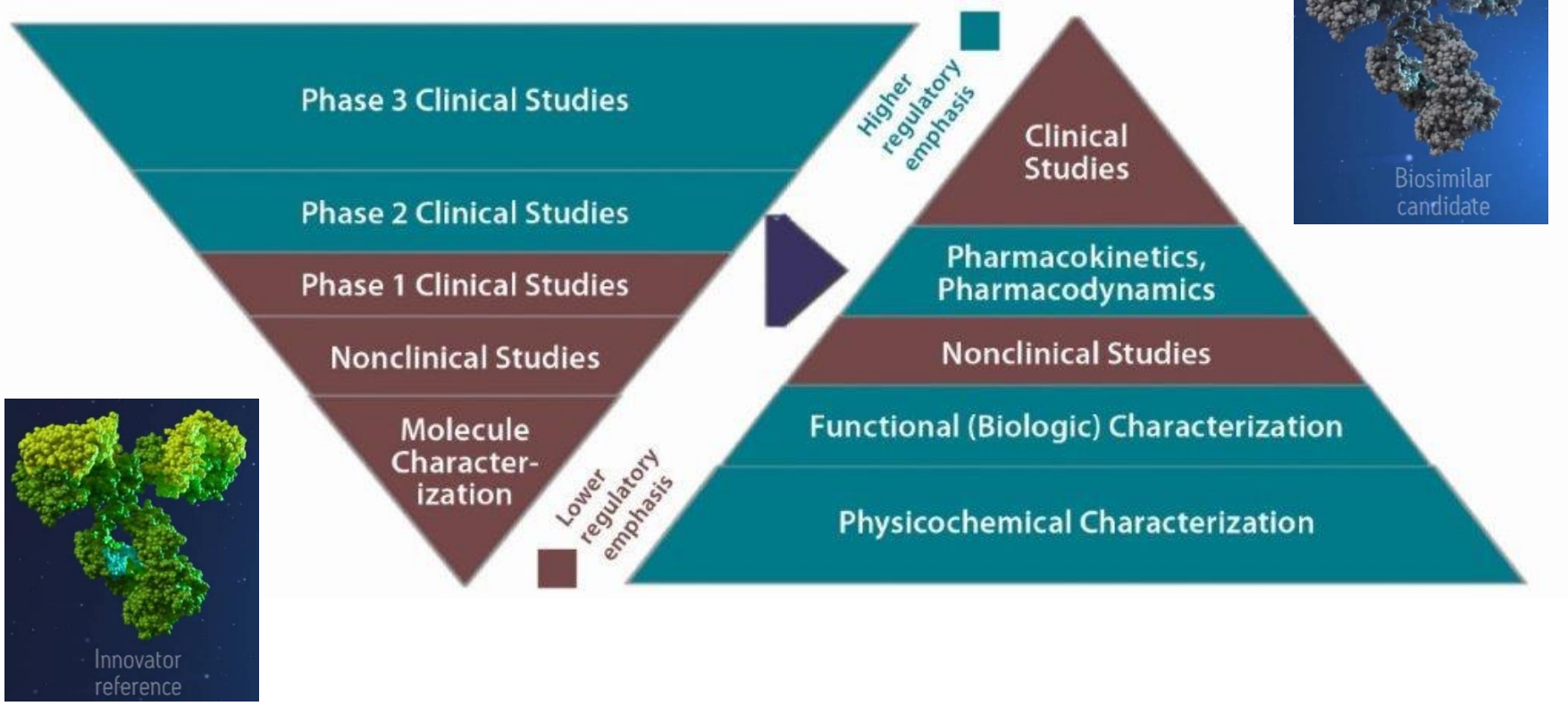
Module 4: Nonclinical Study Reports

Module 5: Clinical Study Reports

https://link.springer.com/content/pdf/10.1007%2F978-1-4471-4920-0.pdf

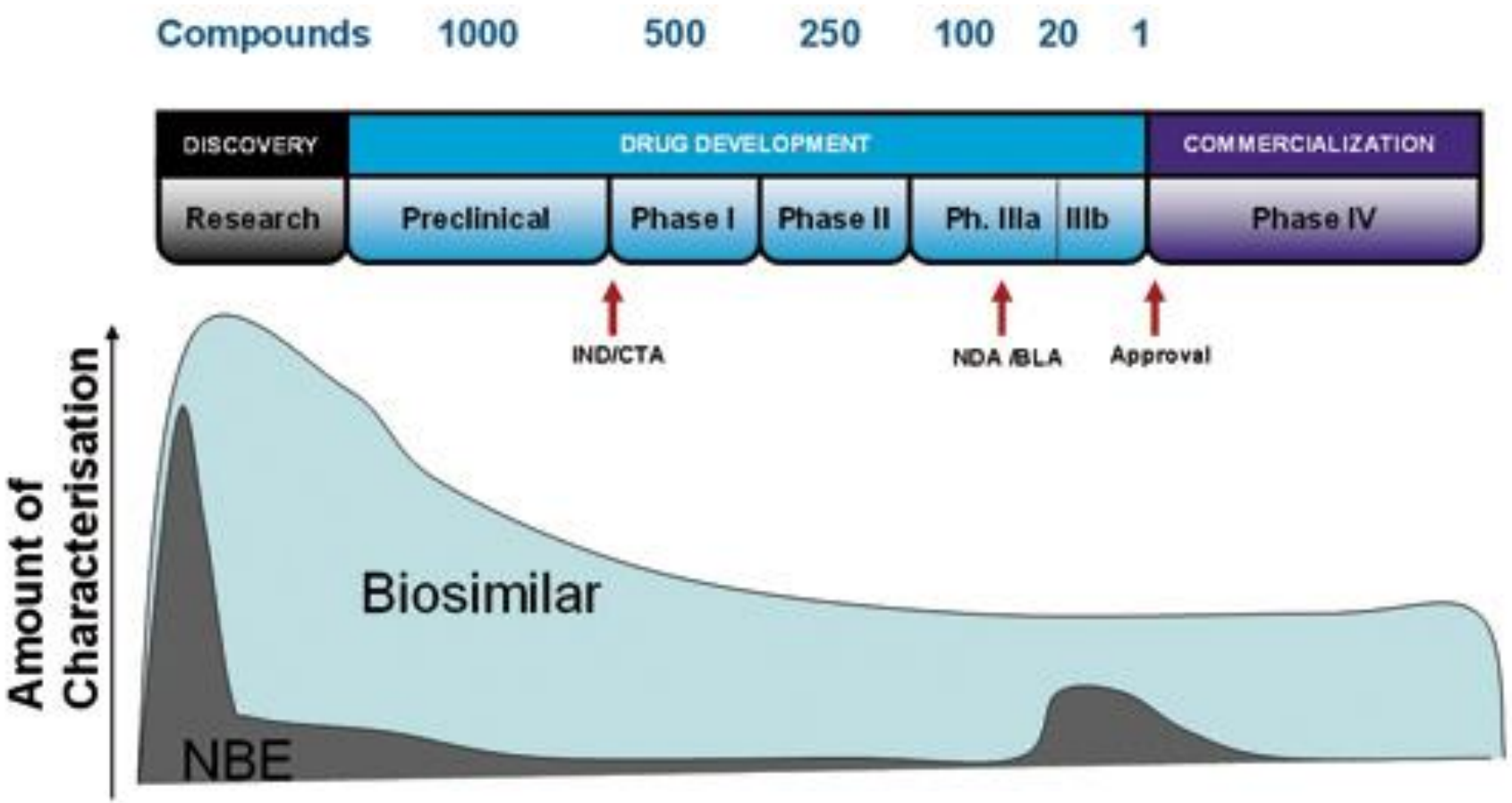
Regulatory Considerations for Early Clinical Development of Drugs for Diabetes, Obesity, and Cardiometabolic Disorders
ICH Guidance M4Q(R1) (September 2002)
ICH M4Q Q&A (July 2003)

Originális biologikumok vs. bioszimilárisok fejlesztése



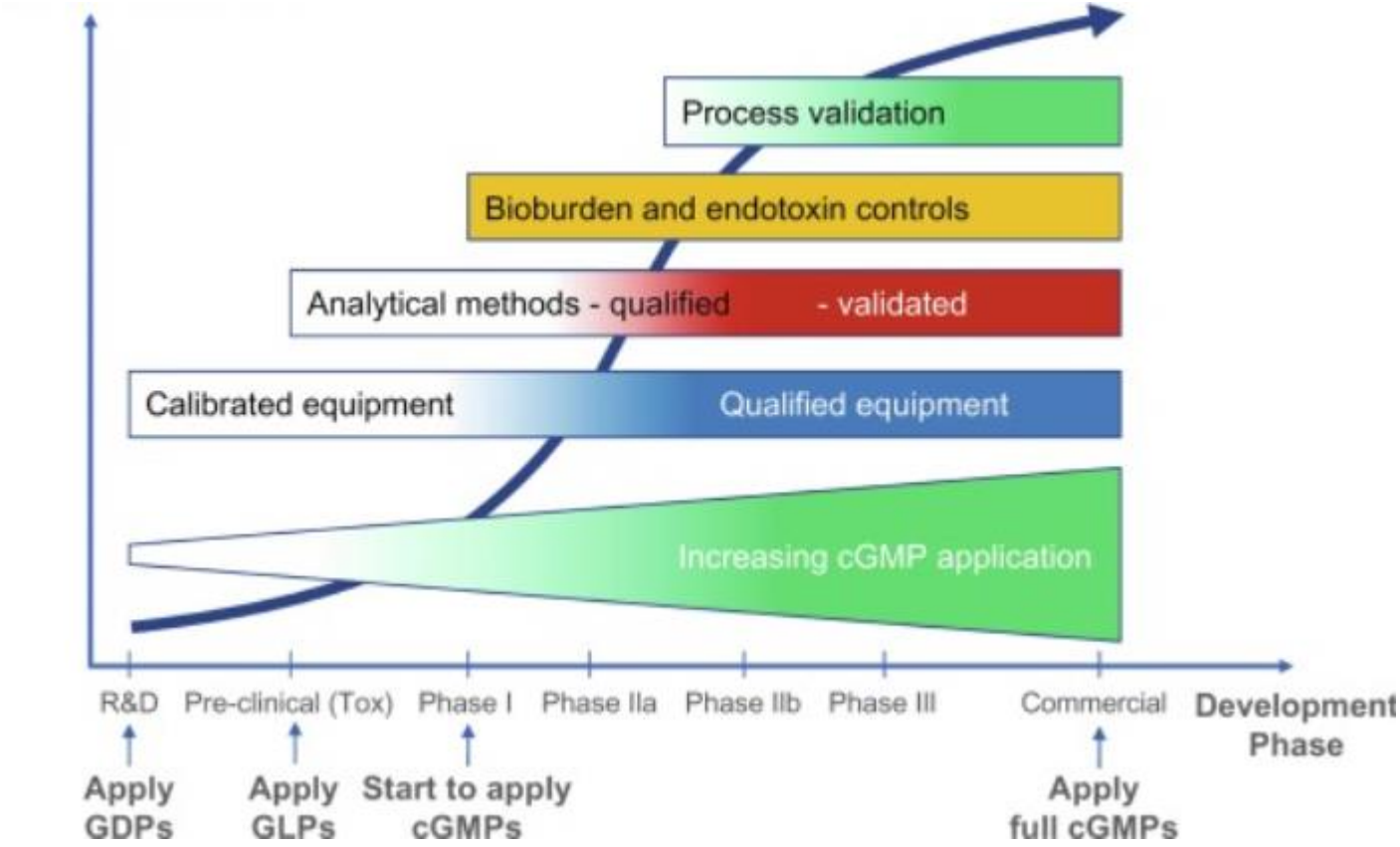
L. Calabrese et al., Biosimilars Part 2: Regulatory and Current Status. Biologic Therapies VI: Optimizing Therapies

Bioszimilárisok fejlesztése – analitika szerepe



BioPharm International, Volume 25, Issue 10, 2012

Transition of GMP requirements from R&D to commercialization



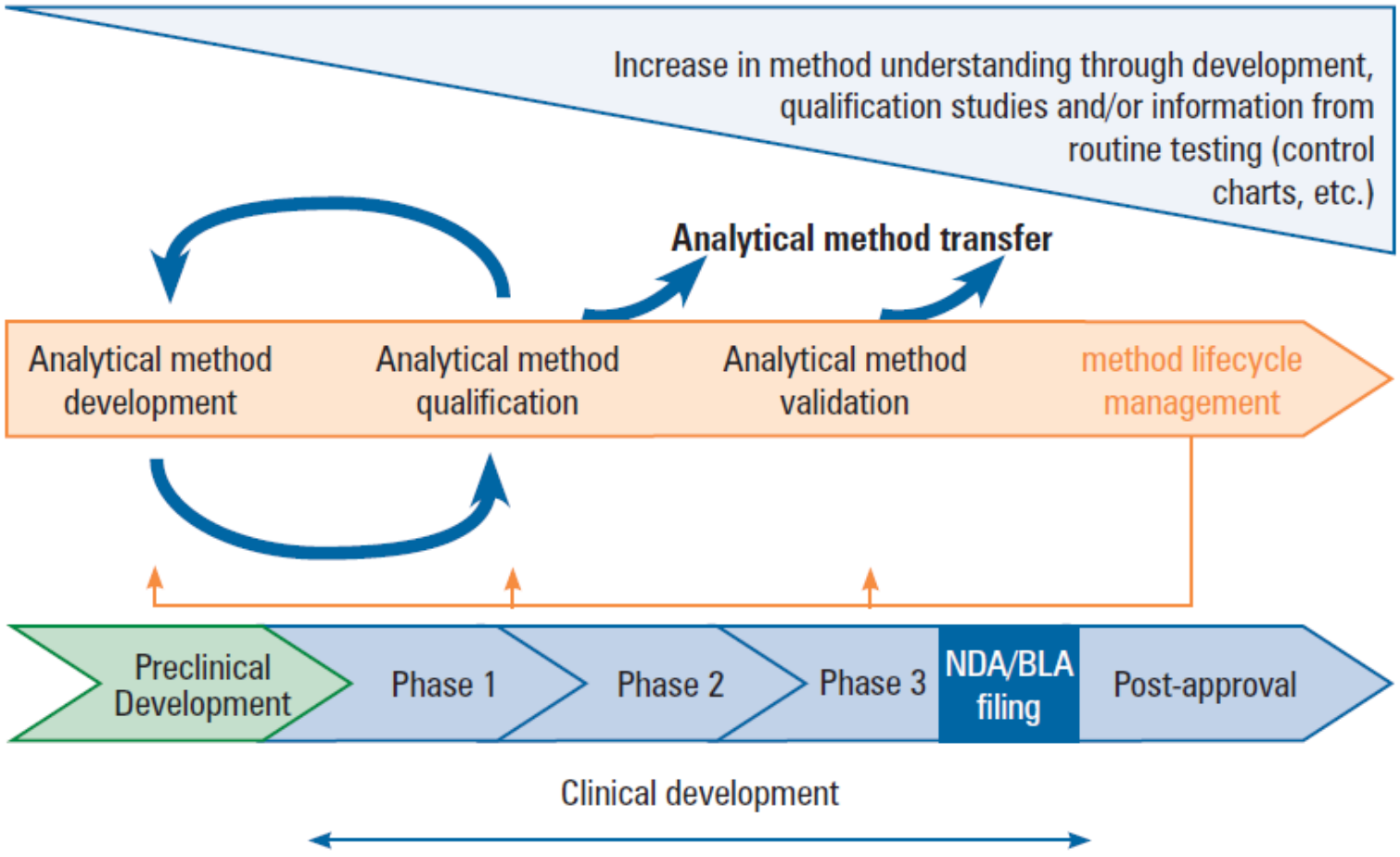
https://www.sciencedirect.com/science/article/pii/B9780081006238000049

Ten Principles of GMP

- **Design and construct the facilities and equipments properly**
- **Follow written procedures and Instructions**
- **Document work**
- **Validate work**
- **Monitor facilities and equipment**
- **Write step by step operating procedures and work instructions**
- **Design, develop and demonstrate job competence**
- **Protect against contamination**
- **Control components and product related processes**
- **Conduct planned and periodic audits**

Method lifecycle and its links to Product Development

Technical Report No. 57-2
Analytical Method Development and Qualification for Biotechnology Products

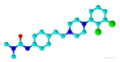


Miért kihívás a biologikumok karakterizálása

MÉRET/KOMPLEXITÁS/HETEROGENITÁS

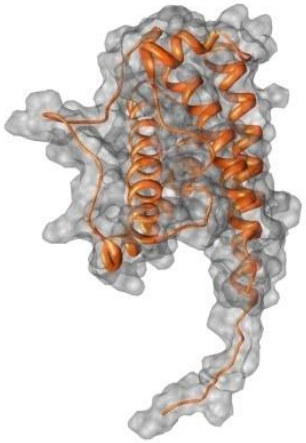
SZERKEZETI VARIÁNSOK

- **kénhidak**
- **pegiláció**
- **glikoformák**
- **N-terminális piroglutamináció**
- **C-terminális lizinvariánsok**

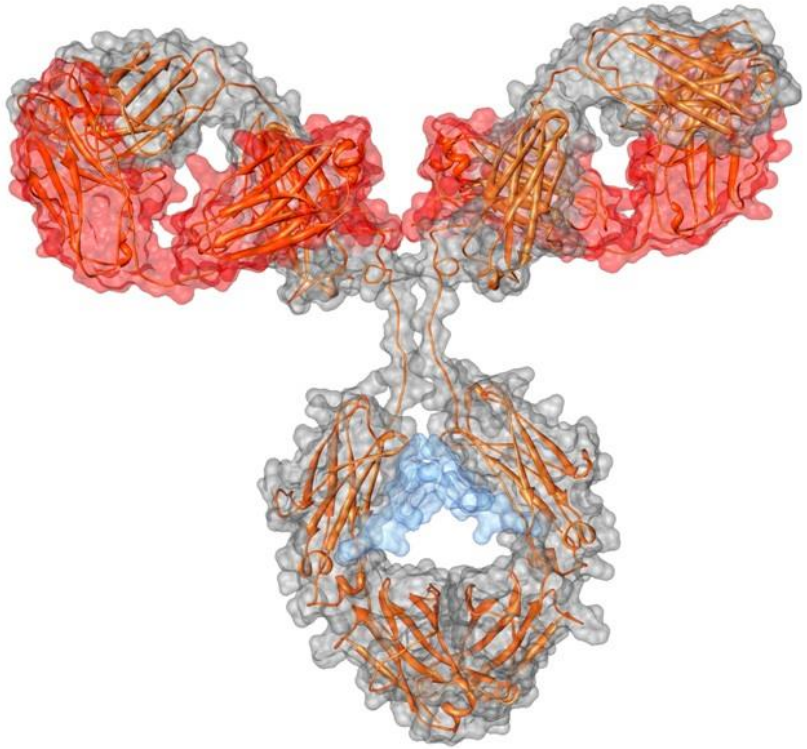


Kismolekula
~400 Da

Peptid
~4 kDa

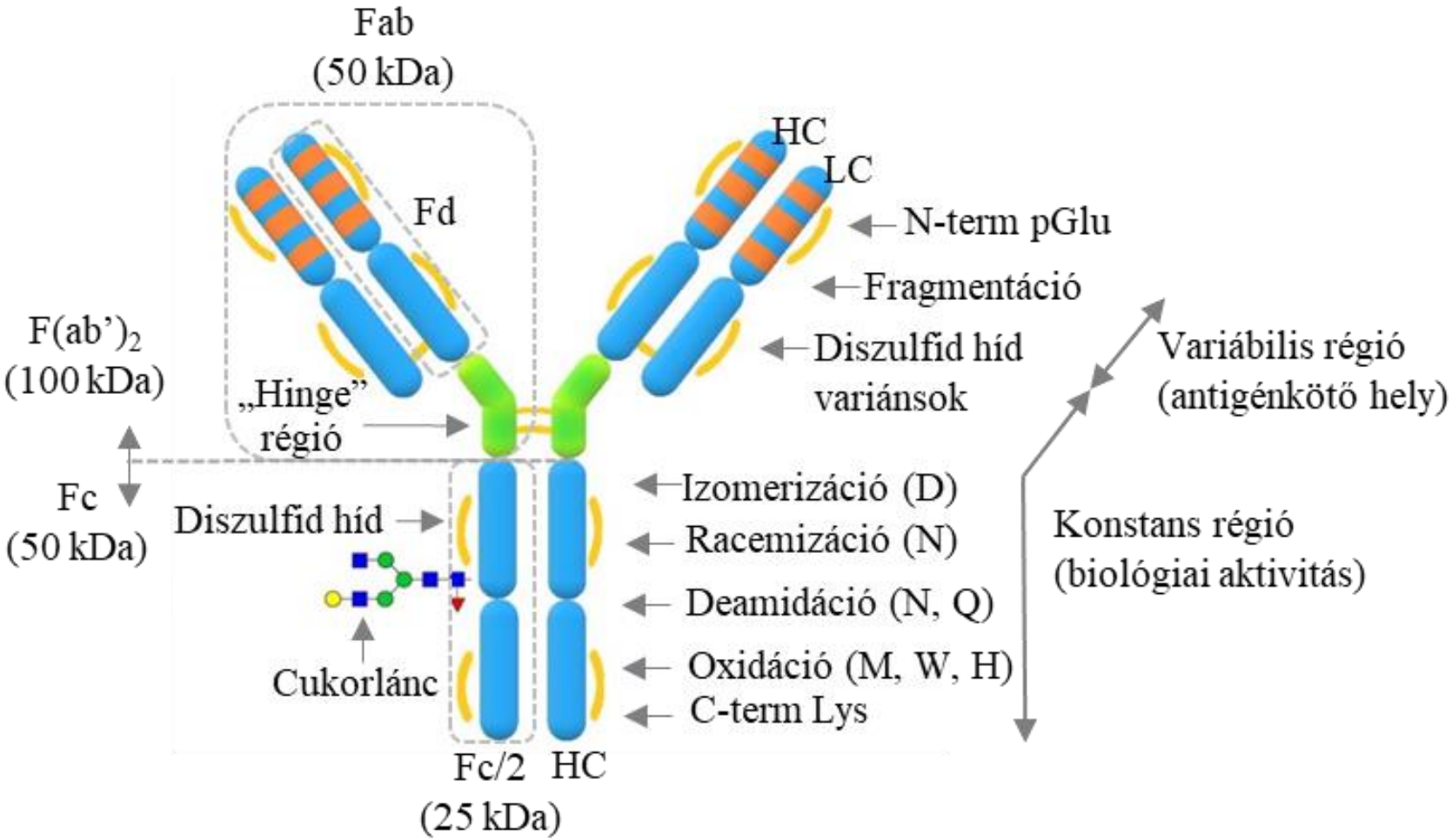


Fehérje
~40 kDa

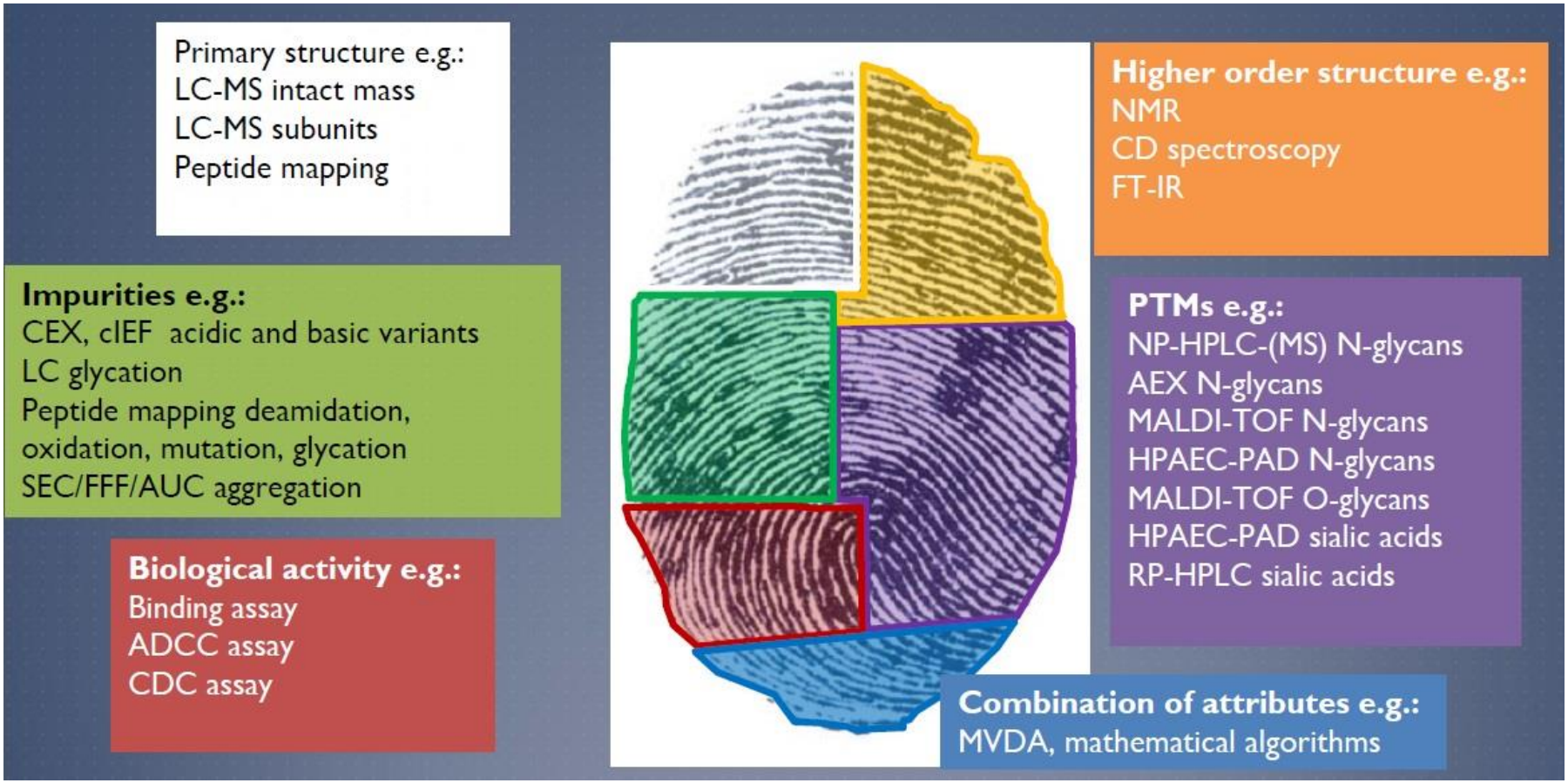


Fehérje/mAb
~150 kDa

mAb-ok (IgG1) szerkezeti felépítése és fontosabb módosításaik



Bioszimilárisok karakterizálása – holisztikus szemlélet



Hatósági elvárás: „...to the extent possible.”

http://www.fda.gov/downloads

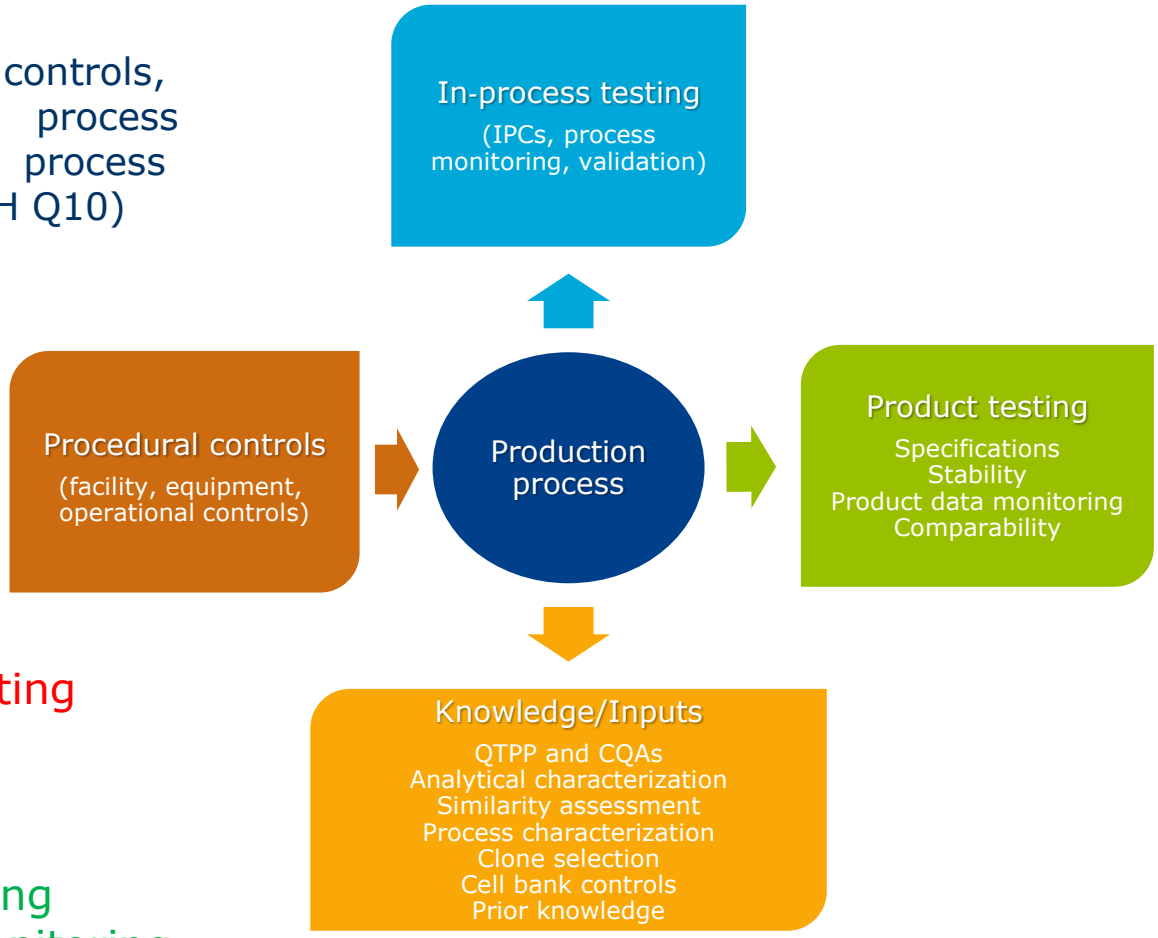
Fehérjék fizikai-kémiai karakterizálása

Paraméter	Attribútum	Módszer
Elsődleges szerkezet	Könnyű és nehézlánc, valamint intakt tömeg	LC-ESI-MS
	Aminosav sorrend (elsődleges szerkezet)	Ortogonális peptidtérkép, nagyfelbontású MS és MS/MS detektálással kombinálva
	Diszulfid hidak	Peptidtérkép (redukcióval és anélkül)
	Szabad cisztein	Peptidtérkép , Ellman reagens
Magasabb rendű szerkezet	Másodlagos és harmadlagos szerkezet	CD, DSC, DSF, HDX-MS, FT-IR, Röntgenkrisztallográfia, HDX-MS, IMS
Glikoziláció	Oligoszacharidok	HILIC, MS (glikopeptidek, jelölt oligoszacharidok), NP-HPLC, HPAEC, Exoglikozidáz enzimek alkalmazása
	Sziálsavak	RP-HPLC (származékolt sziálsavak), HPAEC, NP-HPLC
	Glikozilálatlan mAb	CGE, Peptidtérkép
Heterogenitás	Oxidáció	RP-HPLC, Papain-HIC, Peptidtérkép
	Deamidáció	CEX, Papain-IEX, Peptidtérkép
	Aggregáció	SEC, FFF, MALS, DLS, AUC, SVP
	C- és N-terminális variabilitás	CEX, Papain-IEX, Peptidtérkép, RP-HPLC
	Glikáció	Boronát affinitás kromatográfia, LC-MS, Peptidtérkép
	Fragmentáció (diszulfid hidak)	CGE, SDS-PAGE, SEC, RP-HPLC
	Töltésvariánsok	CEX, cIEF, Peptidtérkép, CZE

Biológiai aktivitás

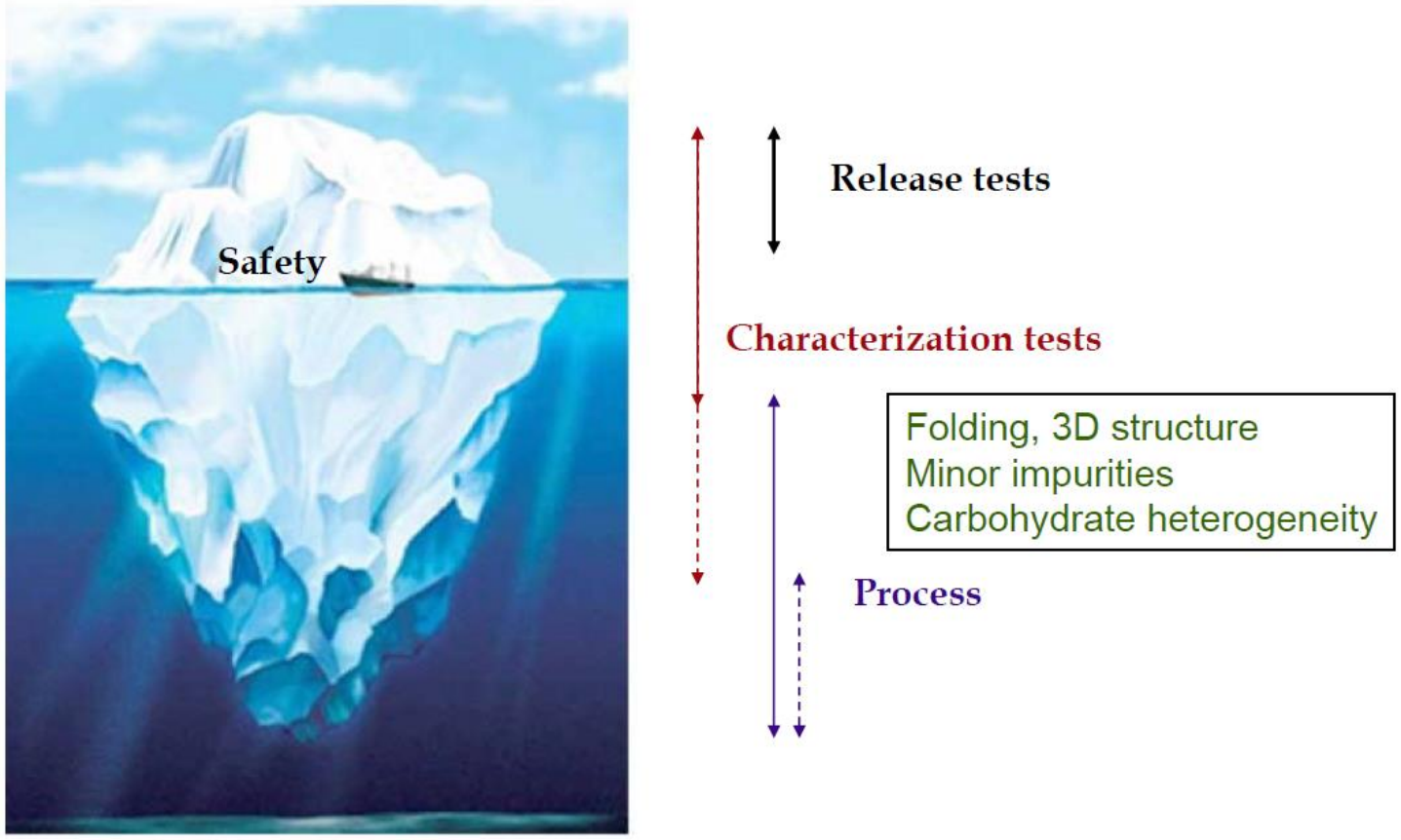
A Strong Integrated Control Strategy is Based on thorough understanding of Process, Product and Prior Knowledge

A control strategy is a planned set of controls, derived from current product and process understanding, that assures process performance and product quality (ICH Q10)



- Raw material controls
- Procedural controls
- Process validation
- In-process control (IPCs) testing
- Release specification testing
- Stability testing
- Characterization testing
- Comparability/similarity testing
- Process and product data monitoring

Protein Characterization: the tip of the iceberg?



Emily Shacter, Ph.D.
https://c.ymcdn.com/sites/casss.site-ym.com/resource/resmgr/Mass_Spec_Speaker_Slides/2008_MS_SchacterEmily.pdf

Specification –Structure

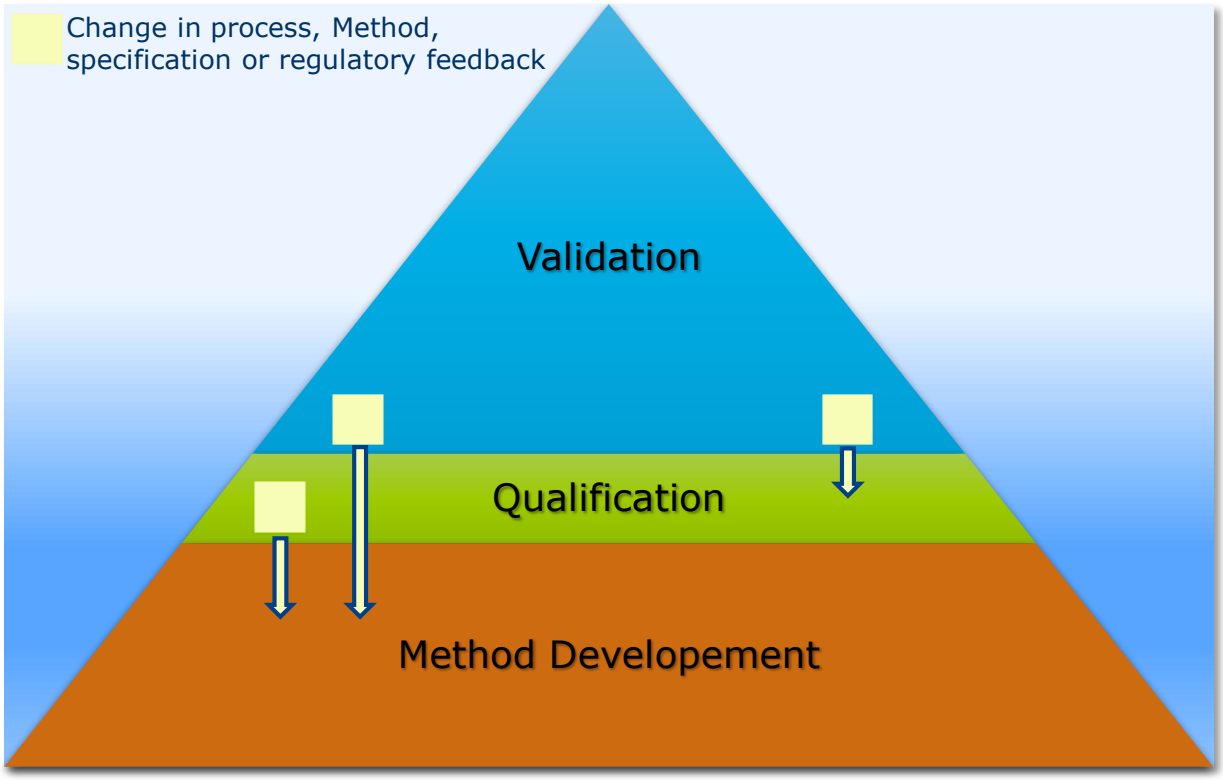
Table 3 Specification for mAb Drug Substance (IND table S.4.1-1)		
Quality Attribute	Analytical Procedure[5]	Acceptance Criteria
Identity	List out method type (e.g. Peptide Map, IEX, ICE, ELISA)	Identification confirmed e.g. "Conforms to reference material"
Quantity Assay[1] (e.g. Protein Content)	UV	Not less than xx.x mg/mL or specify range (see discussion section)
Potency [1]	List out method type (e.g. Binding ELISA)	Not less than 50% and not more than 150% potency relative to potency of reference standard
Monomer Purity[1]	SEC	Not less than 90.0%
Total Aggregates/High Molecular Weight Species[1]	SEC	Not more than 5.0 %
Purity (Reduced) [1]	Reduced CE-SDS	Not less than 90.0%
Total Fragments[1]	Non-Reduced/Reduced CE-SDS	Report result or not more than x.x% [6]
Purity (Non-Reduced) [1]	Non-Reduced CE-SDS	Not less than 90.0%
Residual DNA[3]	qPCR	Not more than xx ppb (value based on WHO limit of 10 ng/dose)[2]
Residual Protein A[3]	ELISA	Not more than x ppm
Residual Host Cell Proteins[3]	ELISA	Not more than x ppm
Additional process related impurity (if applicable)[3]	List method type	Not more than x ppm
Appearance/ Description[1, 4]	Visual	Provide description (e.g. colorless to slightly brown/yellow solution)
Charge Heterogeneity[1] Main Peak Total Acidic Variants Total Basic Variants	List method type (e.g. IEX, ICE)	Compares to reference Report results Report results Report results
Bacterial Endotoxins[1]	USP <85>	Not more than x.x (or x.xx) EU/mg
Total Microbial Count[1]	USP <61>	Not more than x CFU/x mL
pH[1]	USP <791>	Not less than x.x and not more than x.x

[1] Test should be evaluated for inclusion in stability studies. Microbial testing not recommended for frozen drug substance or samples held at accelerated conditions.
[2] Limit = 10 ng/dose ÷ (maximum clinical dose (X mg/kg) × patient mass (kg).
[3] Strategy of testing process residuals as in-process controls or performing a risk assessment may be considered (see discussion/justification section)
[4] For early stage products a simple description is sufficient, however, if color and clarity are assessed then an additional description is not necessary
[5] USP compendia tests are listed as examples. Alternate compendia methods may be considered suitable.
[6] Multiple approaches can be used to define limit, see discussion/justification section.

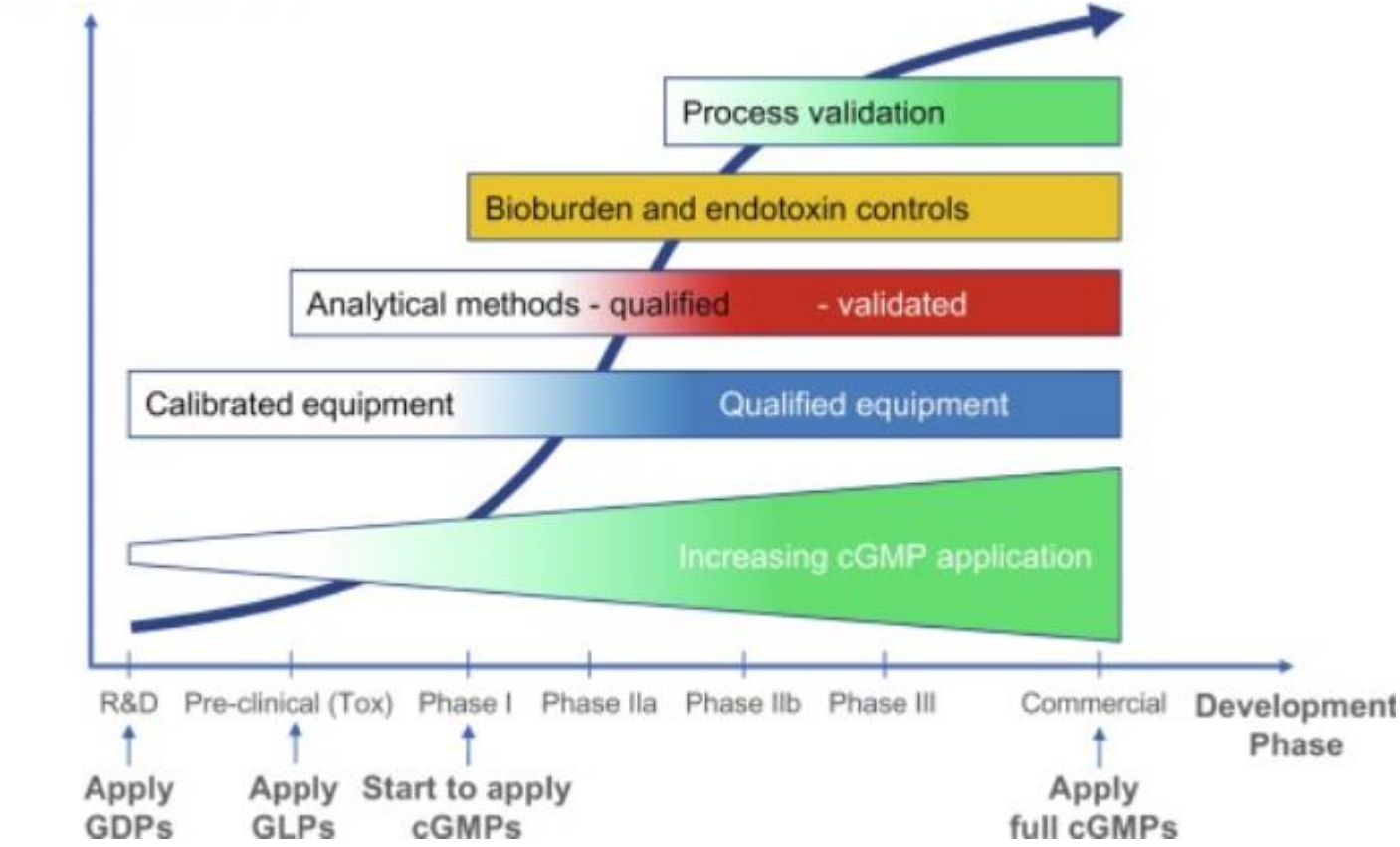
Method Qualification/validation Strategy I.

All analytical test methods

Routine test methods (IPC + release test methods)



Transition of GMP Requirements from Phase 1 to Phase 3 and the Interface to Development Work

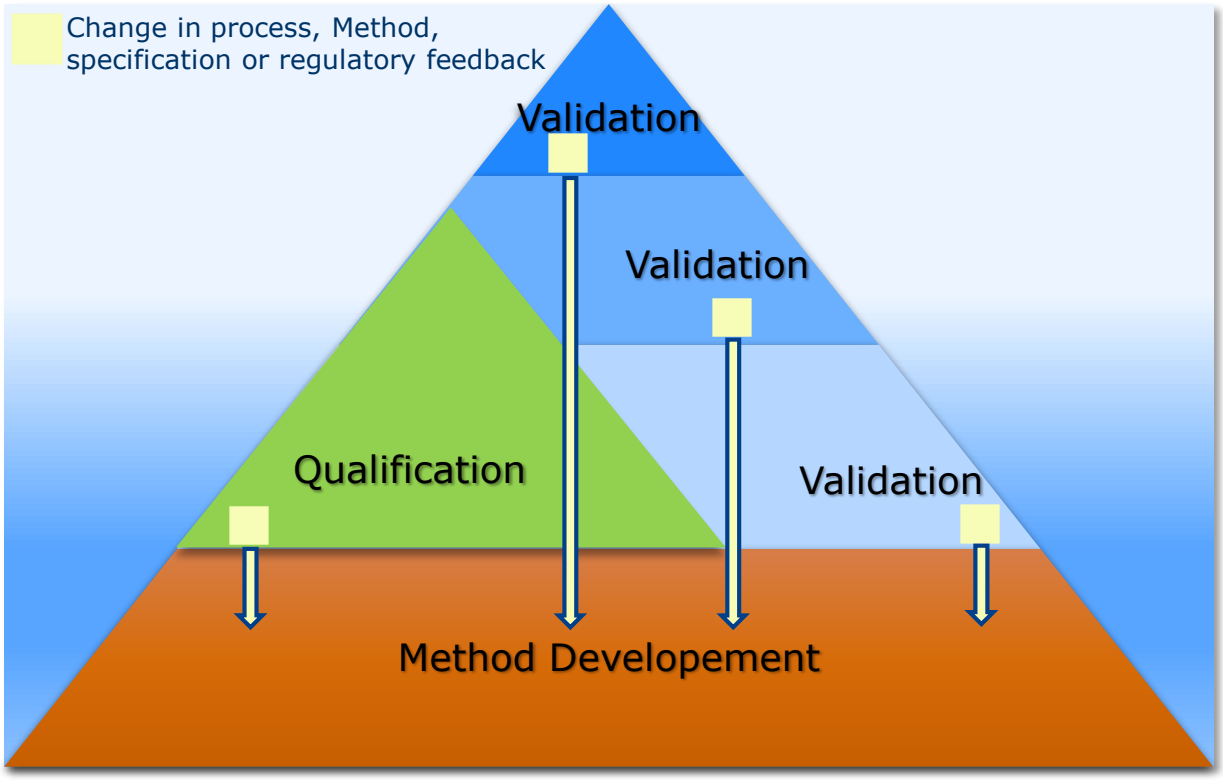


https://www.sciencedirect.com/science/article/pii/B9780081006238000049

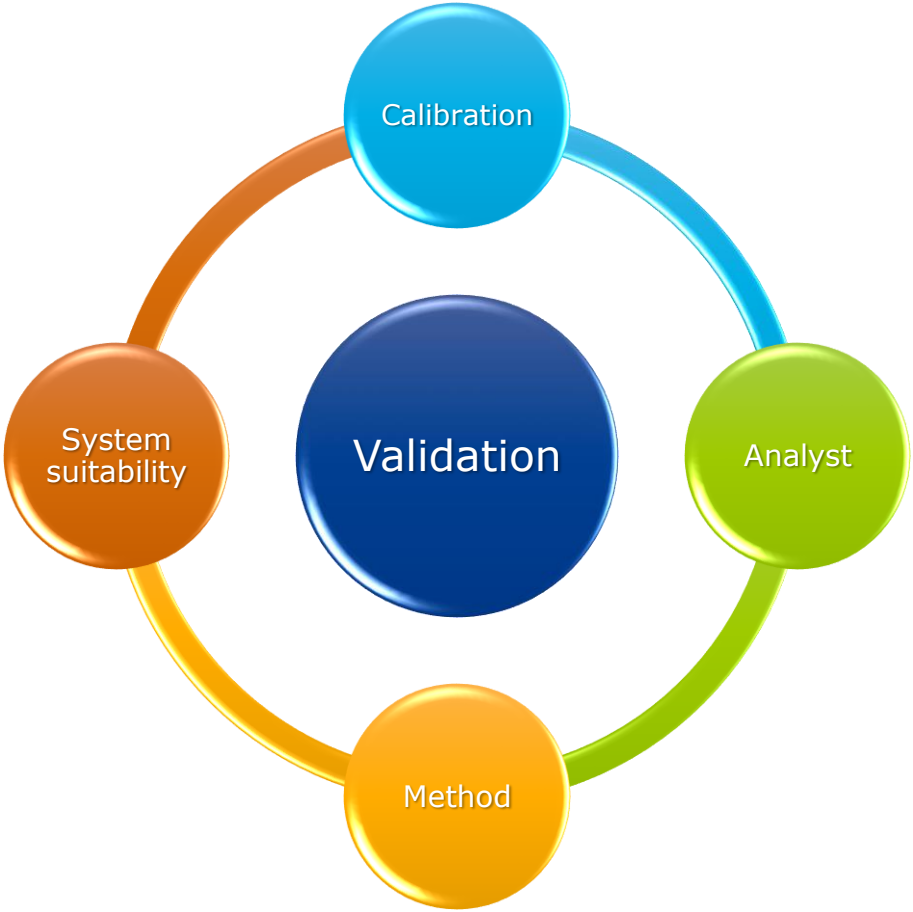
Method Qualification/Validation Strategy II.
‚Phase Appropriate Method validation'

Supportive methods

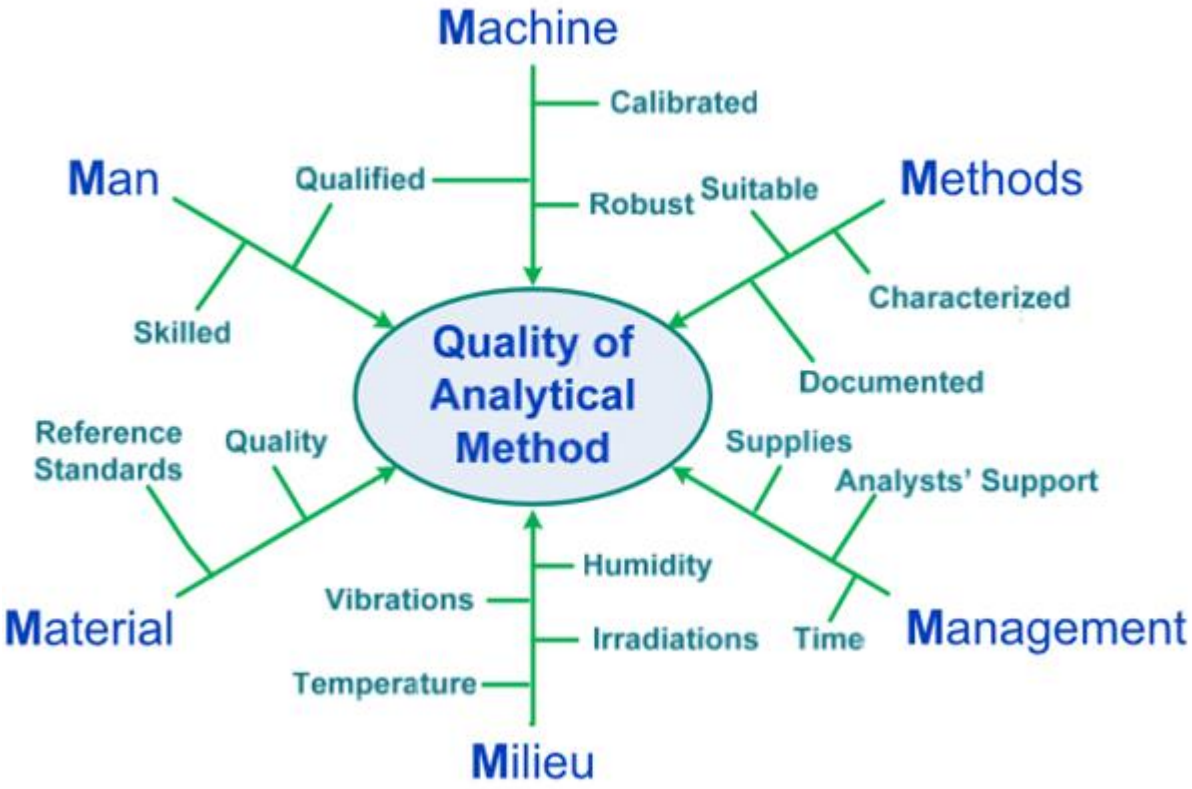
Routine test methods



Validation of analytical method

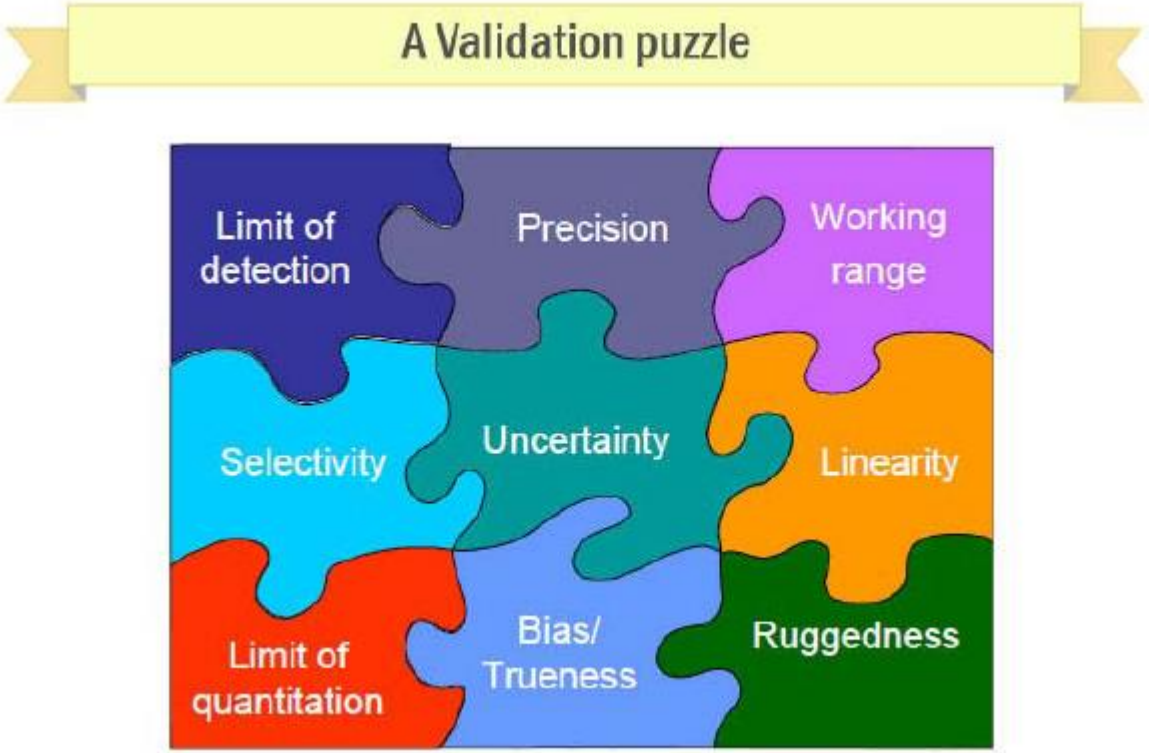


Method validation



Aryo Nikopour, Phase Appropriate Method Validation

Validation puzzle



Aryo Nikopour, Phase Appropriate Method Validation

ICH Validation Study

Experimentally demonstrates that a test method can meet its predetermined specifications for performance of parameters such as:

- Specificity
- Linearity/Range
- LOD/LOQ
- Accuracy
- Precision
- Intermediate Precision
- Robustness

What is method validation?

"The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." *ICH Q2 (R1)*

"Establishing through documented evidence a high degree of assurance that an analytical method will consistently yield results that accurately reflect the quality characteristics of the material tested."
Proposed for 21 CFR 211.222 (not adopted) (yet…)

- Validation is procedure dependent.
- Validation, "Proves" the procedure works as described.
- Validation is product specific.
- Procedures are instrument dependent.

Compendial and Non-compendial methods

Compendial Method: An analytical method published in a Pharmacopoeia.

- General Methods = general procedures for chemical, physical, biological, microbiological, immunological methods applicable to several products
- Product Monographs = product-specific specifications for release, label claims, and (for some older biologics) storage conditions
-

USE OF COMPENDIAL METHODS in GMP LABS

- **All methods (start to finish) still need written SOPs for compliance to GMP**
- **Compendial methods be followed exactly as written to remain validated**

- **Modifications to compendial methods may be acceptable if:**
  - They perform as good or better than the existing method
  - Modifications to methods, or alternative methods, are (re)validated

Non-compendial methods

Non-Compendial Method: An analytical method developed by (or for) the sponsor for a specific product

USE OF NON-COMPENDIAL METHODS

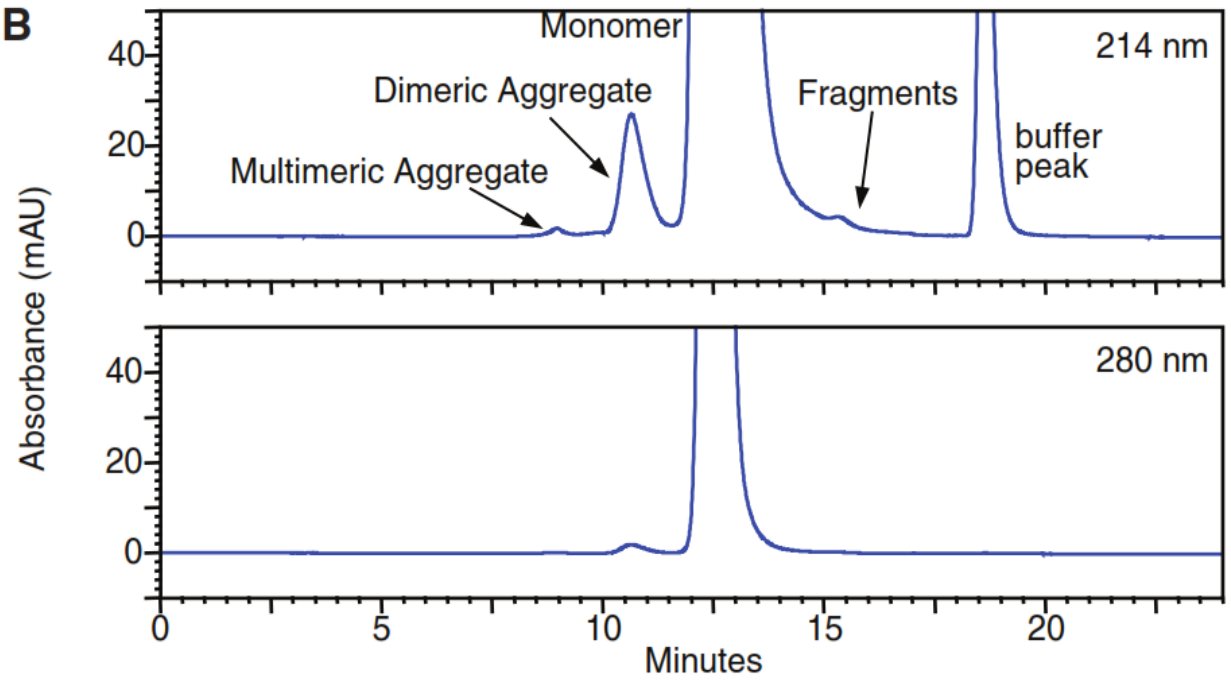
- **Methods should be sufficiently developed and optimized before qualification or validation; DOE studies are most powerful for robust method optimization**
- **The method SOP should be sufficiently detailed to describe all necessary steps starting with the preparation of samples, standards and assay controls and ending with the calculation of reportable results**
- **Methods should be qualified for intended use in generating analytical characterization, comparability or similarity data**
- **Methods used to generate QC release and stability data for commercial product must be fully validated for cGMP compliance**

ICH Qualification/Validation Parameters Per Intended Use

Parameter	Identity	Impurity Detection	Impurity Quantitation	Purity or Potency
Accuracy	**NO**	**NO**	**YES**	**YES**
Precision – Repeatability	**NO**	**NO**	**YES**	**YES**
Precision - Intermediate	**NO**	**NO**	**YES**	**YES**
Specificity	**YES**	**YES**	**YES**	**YES**
Detection Limit	**NO**	**YES**	**YES**	**NO**
Quantitation Limit	**NO**	**NO**	**YES**	**NO**
Linearity	**NO**	**NO**	**YES**	**YES**
Range	**NO**	**NO**	**YES**	**YES**
Robustness	***YES***	***YES***	***YES***	***YES***

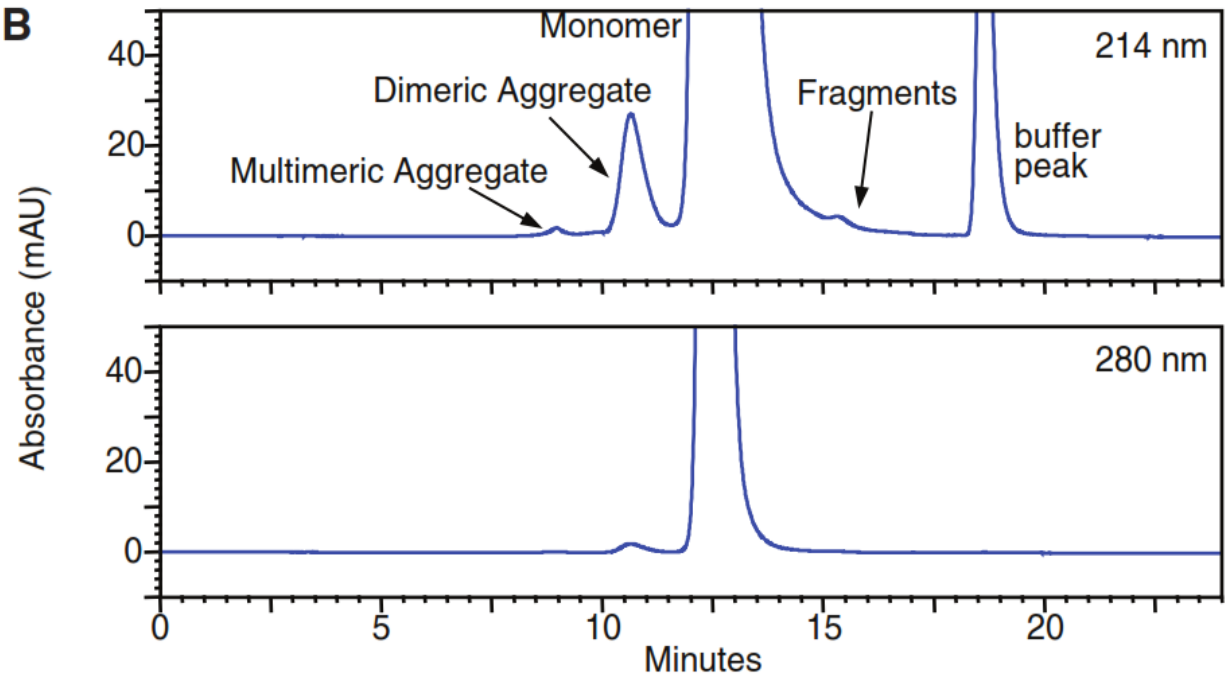
Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.



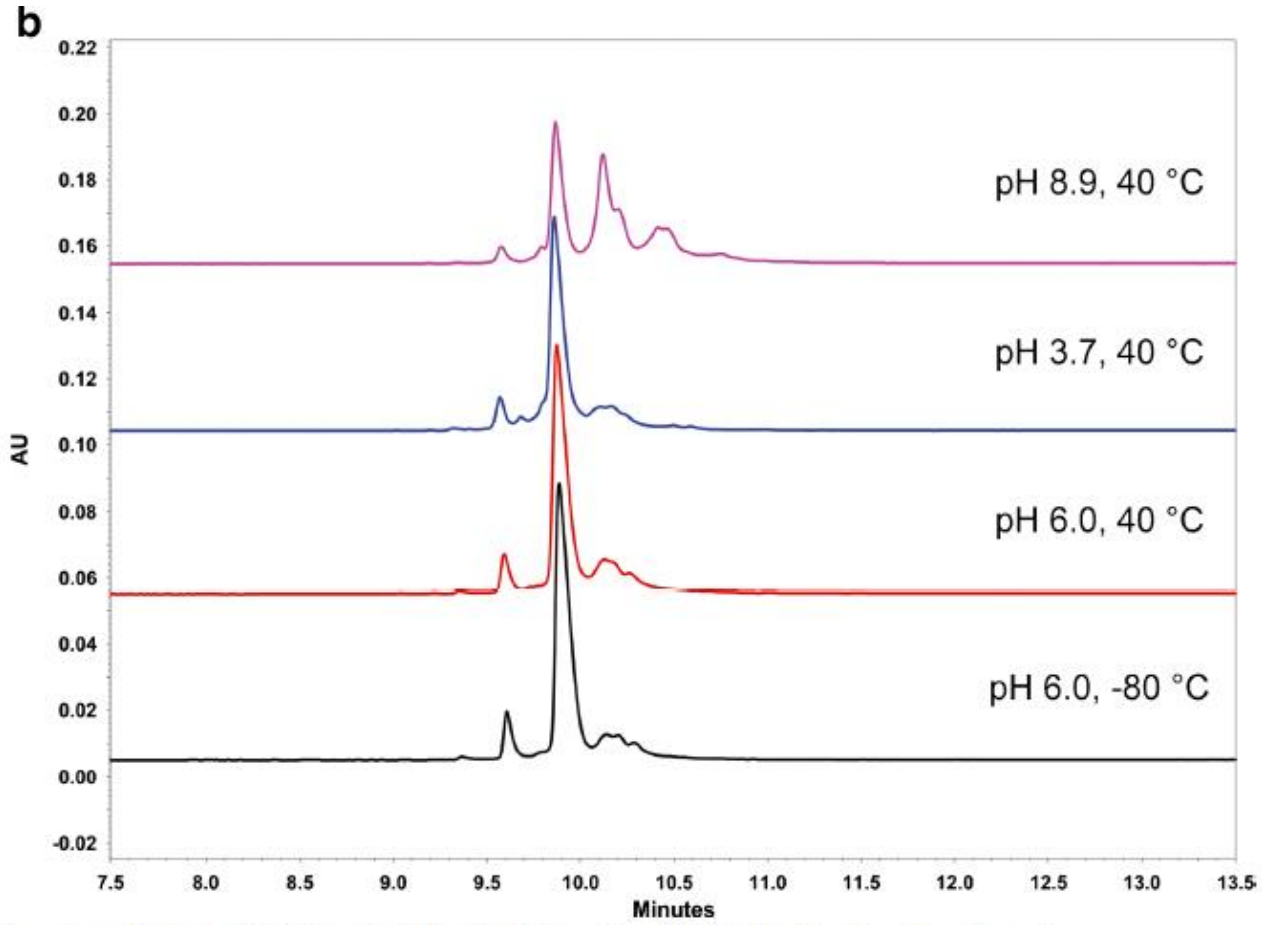
M. D. BOND, M. E. PANEK, Z. ZHANG, D. WANG, P. MEHNDIRATTA, H. ZHAO, K. GUNTON, A. NI, M. L. NEDVED, S. BURMAN, D. B. VOLKIN, JOURNAL OF PHARMACEUTICAL SCIENCES, VOL. 99, NO. 6, JUNE 2010

Specificity SEC chromatogram



M. D. BOND, M. E. PANEK, Z. ZHANG, D. WANG, P. MEHNDIRATTA, H. ZHAO, K. GUNTON, A. NI, M. L. NEDVED, S. BURMAN, D. B. VOLKIN, JOURNAL OF PHARMACEUTICAL SCIENCES, VOL. 99, NO. 6, JUNE 2010

Specificity – stress conditions CZE



Comparison of optimized CIEF (a) and optimized CZE (b) profiles of NISTmAb subjected to pH and thermal stress

Accuracy

***Accuracy:* The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.**

Several methods of determining accuracy are available:

a) application of an analytical procedure to an analyte of known purity (e.g. reference material);

b) comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined

c) accuracy may be inferred once precision, linearity and specificity have been established.

Linearity/Range

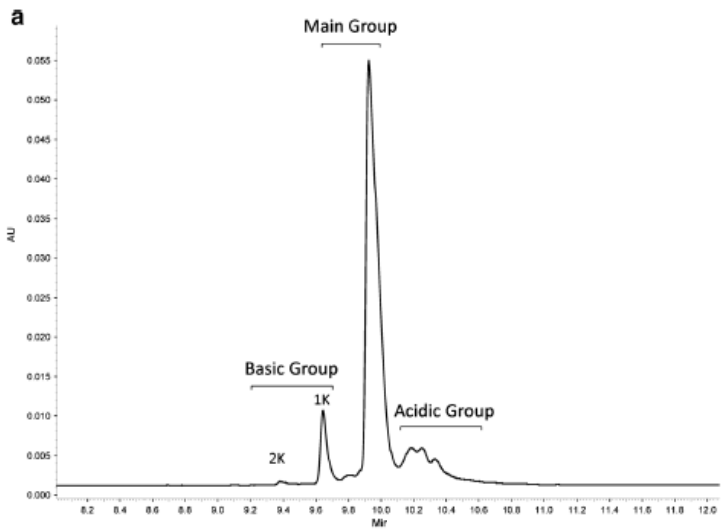
Linearity: *The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.*

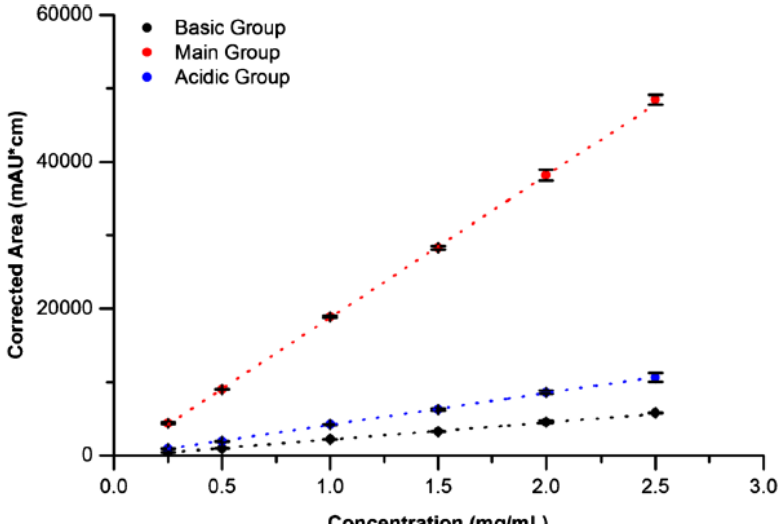
Range: *The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.*

The assay DYNAMIC range is the measurement capability of the method.

The sample WORKING range is the part of the dynamic range in which the intended test samples will be measured.

Linearity/Range





Parameter	Mean (SD)[a]	
Limit of Detection [b]	0.044 (0.012) ng	0.2 (0.1) % RA at Target
Limit of Quantification [b]	0.150 (0.039) ng	0.7 (0.2) % RA at Target
Linear Range (Main Peak) [b]	0.25 to 2.5 mg/mL	17 to 170% of Target
Resolution (1 K:Main) [b]	0.9 (0.001)	
Theoretical Plates (Main Peak) [b]	6×10^4	
Sample Consumption	20 ng	
Run Time per Sample	25 min	

[a] *SD = standard deviation;* [b] *n = 3*

Abigal Turner & John E. Schiel,
Analytical and Bioanalytical Chemistry (2018) 410:2079–2093

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Abigal Turner & John E. Schiel,
Analytical and Bioanalytical Chemistry (2018) 410:2079–2093

Precision

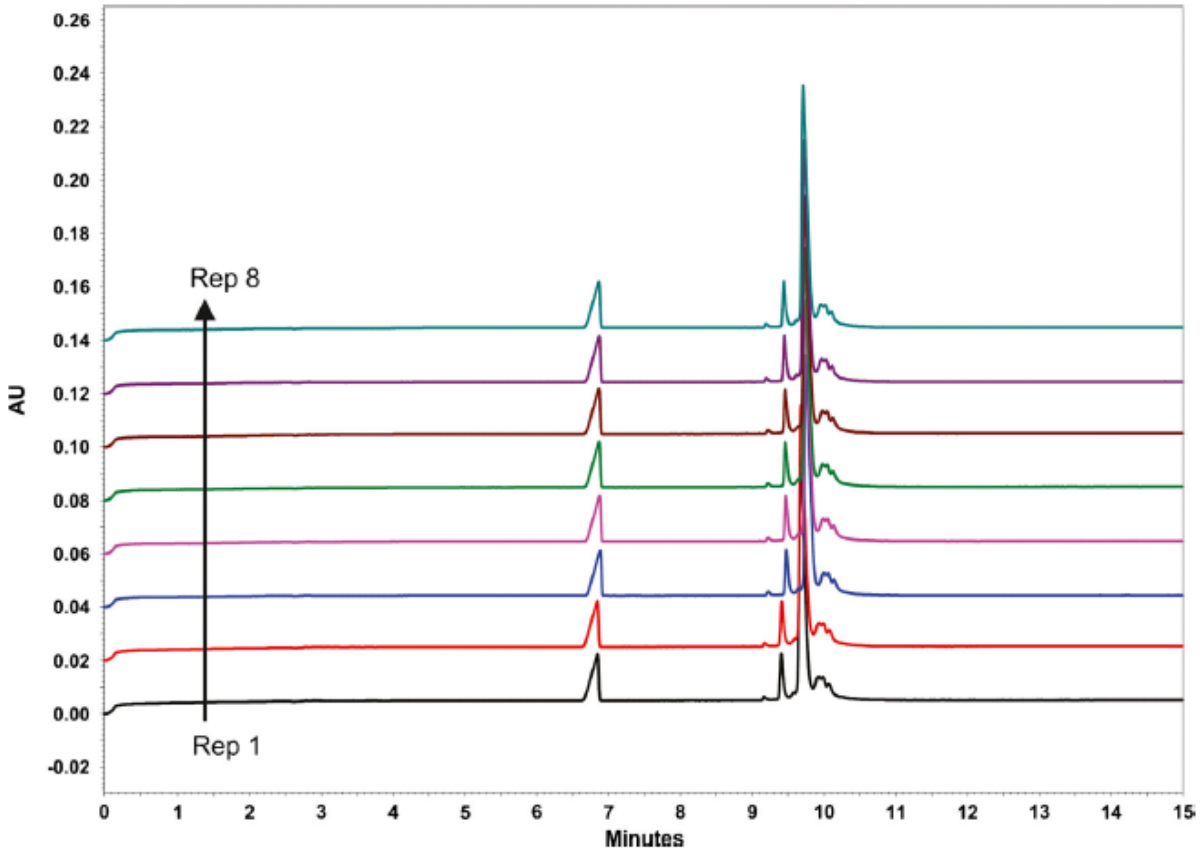
Repeatability *(Intra-assay precision)*

Variation under the same operating conditions over a short interval of time. Repeatability should be assessed using: a) a minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each); or b) a minimum of 6 determinations at 100% of the test concentration.

Intermediate precision *(Inter-assay / intra-laboratory precision)*

Within-laboratory variations on different days, with different analysts, different
instruments. The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used.

Precision



Replicate CZE analyses of Primary Sample 8670 using 400 mmol/L EACA, 2 mmol/L TETA (pH 5.7), 0.03% (w/v) Tween™ 20

Abigal Turner & John E. Schiel,
Analytical and Bioanalytical Chemistry (2018) 410:2079–2093

Precision

Table 5 Intermediate Precision of Optimized CZE Method

Parameter	Mean ± u_c [a]	CV
Instrument Qualification Standard (IQ)		
IQ Standard Migration Time (min)	5.79 ± 0.06	1.0%
PS 8670		
Main Peak Migration Time (min)	9.67 ± 0.17	1.8%
Main Group RA (%)	74.7 ± 0.3	0.5%
Acidic Group RA (%)	16.8 ± 0.4	2.4%
Basic Group RA (%)	8.5 ± 0.3	3.3%

[a] *Stated uncertainty represents the intermediate precision reported as a combined standard uncertainty, at a level of one standard deviation, based on ANOVA analysis as described in* ESM *(n = 54)*

Abigal Turner & John E. Schiel,
Analytical and Bioanalytical Chemistry (2018) 410:2079–2093eterogeneity control assays

Robustness

A: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

B: The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure.

One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.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THANK YOU FOR YOUR ATTENTION!