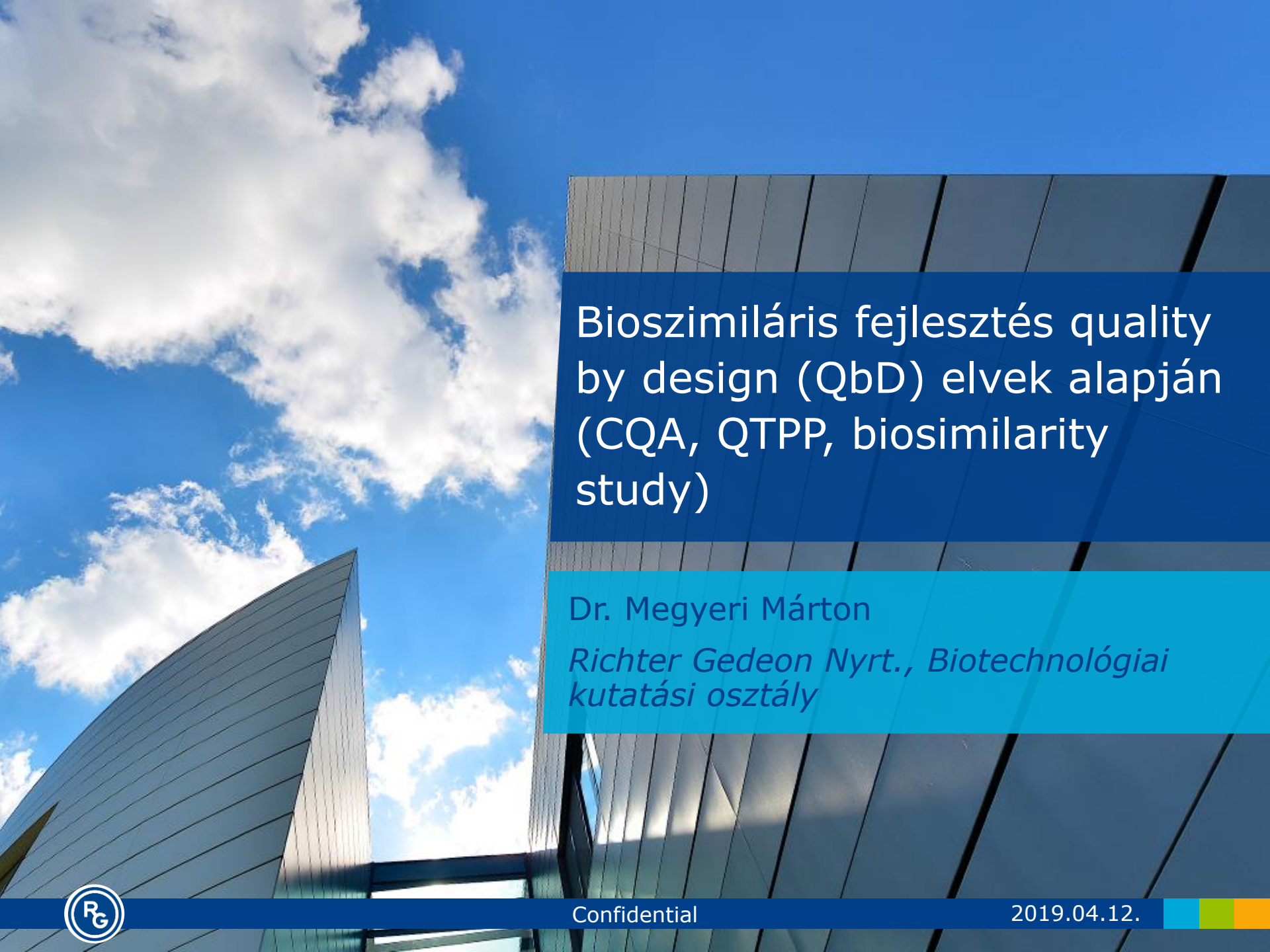


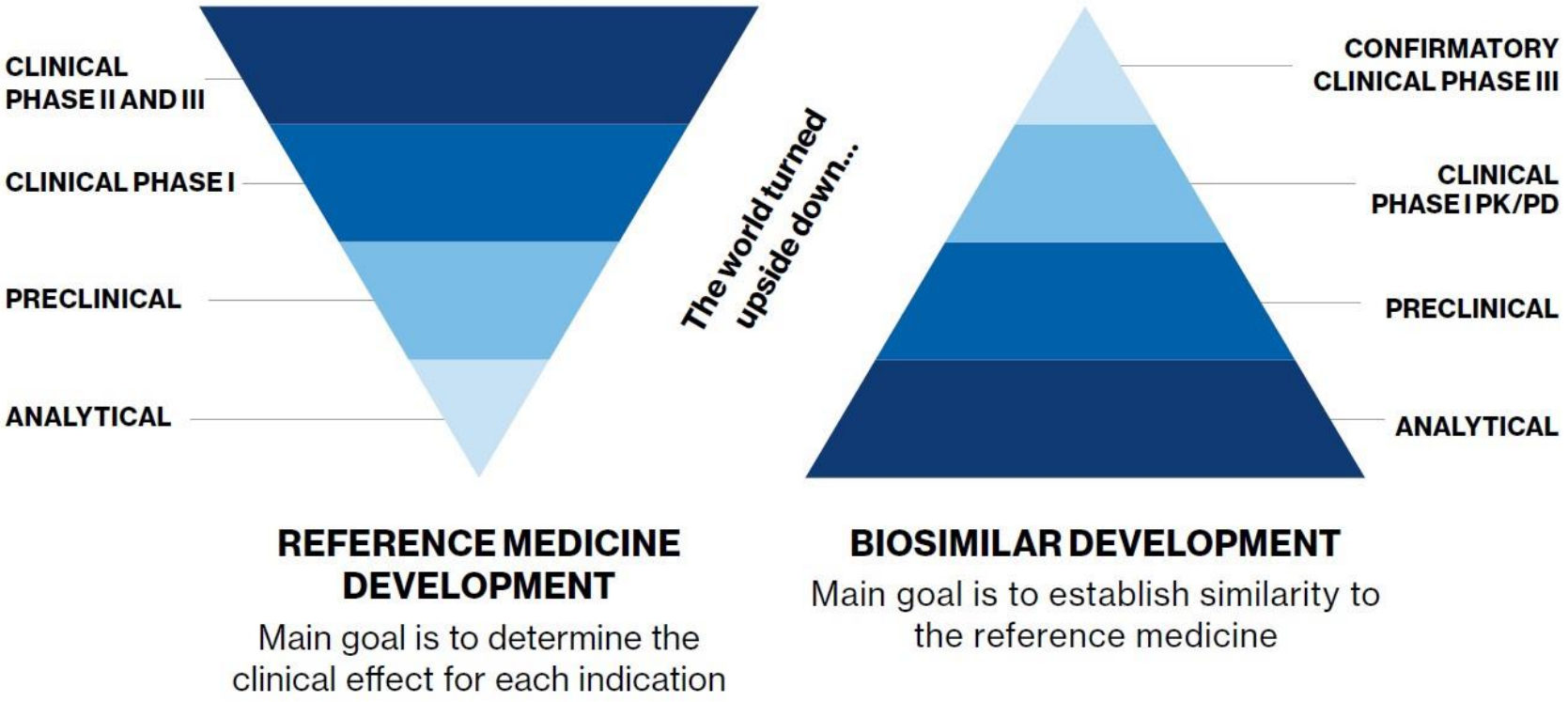
Bioszimiláris fejlesztés quality by design (QbD) elvek alapján (CQA, QTPP, biosimilarity study)

Dr. Megyeri Márton

Richter Gedeon Nyrt., Biotechnológiai kutatási osztály

Bioszimilaritás?
Almát az almával?

A világ a feje tetejére áll - Az originális és bioszimiláris fejlesztés összehasonlítása



McCamish, M and Woollett, G. Clin Pharmacol Ther. 2012; 91(3):405-417

Az analitika a lényeg – de milyen tulajdonságokat mérjünk meg vele?

Forrás: wikipedia

Attribútum, a vallásban olyan tárgy vagy jelvény, amely a szentek, istenek, személyek, embercsoportok, erények jelölésére szolgál és ***elválaszthatatlanul hozzájuk tartozik.***

Forrás: wikipedia

Ábrahám (Kr. e. 2000 k.-) – kés, kos
Adalbert (957 k.-997) – mitra, pásztorbot, evező, könyv, kopja, gerely, pallium, bunkó, sas, megszállott
Adelaide (931-999) – kenyér, hajó
Ágnes (290/293–305) – bárány, pálmaág
Ágnes, Prágai (1205-1282) – szerzetesi öltözék, korona, templommodell, liliom
Ágoston, Hippói (354-430) – galamb, gyermek, kagyló, toll, könyv, lángoló szív
Ágota (-251) – olló nyelvei, lepel, harang, mellek a tálcán, galamb a szájában gyűrűvel
Ambrus (339-397) – méhek, méhkaptár, galamb, ökör, toll
András, Corsini (1302-1373) - bárány, farkas
Angéla, Merici (1474-1540) – ferences öltözék, létra, kereszt, rózsafüzér, lépcső, gyermekek, könyv, feszület, liliom

Forrás: wikipedia

Quality Attributes (QA) and Critical Quality Attributes (CQA)

A CQA is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. CQAs are generally associated with the drug substance, excipients, intermediates (in-process materials), and drug product.

Potential drug product CQAs derived from the quality target product profile and/or prior knowledge are used to guide the product and process development. The list of potential CQAs can be modified when the formulation and manufacturing process are selected and as product knowledge and process understanding increase. Quality risk management can be used to prioritize the list of potential CQAs for subsequent evaluation. Relevant CQAs can be identified by an iterative process of quality risk management and experimentation that assesses the extent to which their variation can have an impact on the quality of the drug product.

Identification of CQAs

Biologicals 44 (2016) 291–305

Contents lists available at ScienceDirect

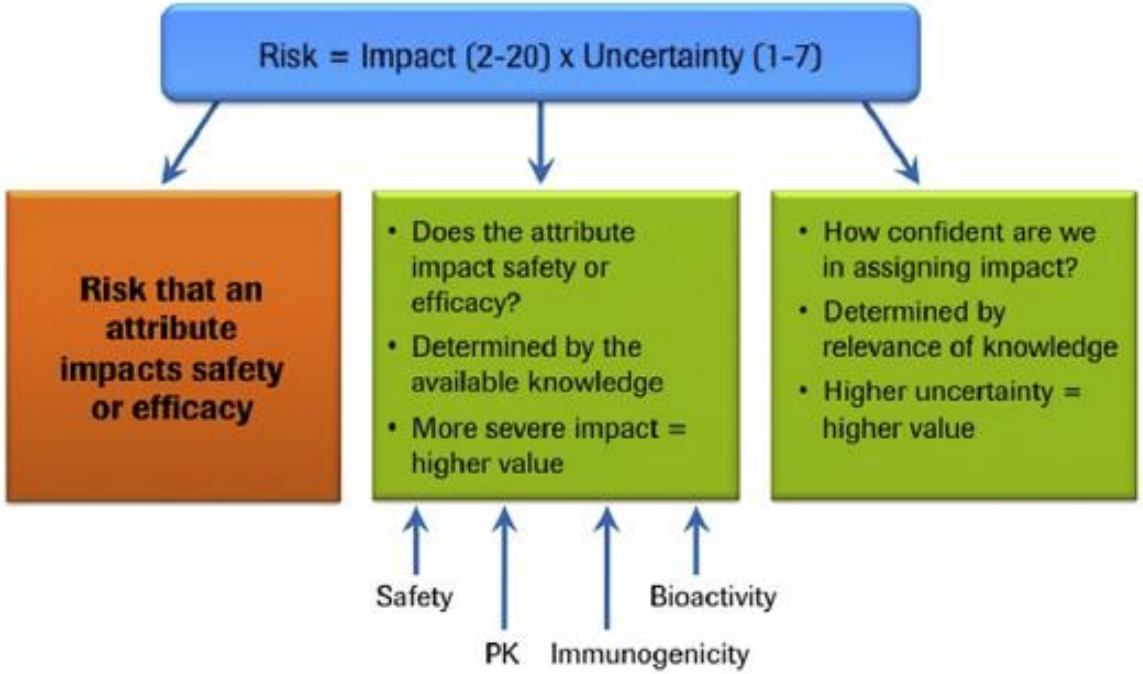
Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Determination of critical quality attributes for monoclonal antibodies using quality by design principles

Nadja Alt [a, *], Taylor Y. Zhang [d], Paul Motchnik [e], Ron Taticek [d], Valerie Quarmby [f], Tilman Schlothauer [b], Hermann Beck [c], Thomas Emrich [b], Reed J. Harris [d]

[a] Pharma Technical Development, Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg, Germany
[b] Pharma Research and Early Development, Roche Innovation Center Munich, Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg, Germany
[c] Pharma Technical Development Biotech Europe, F. Hoffmann-La Roche Ltd, 4070 Basel, Switzerland
[d] Pharma Technical Development, Genentech, South San Francisco, CA 94080, USA
[e] Biologics Quality Control, Genentech, South San Francisco, CA 94080, USA
[f] Research and Early Development, Genentech, South San Francisco, CA 94080 USA


Risk = Impact (2-20) x Uncertainty (1-7)
Risk that an attribute impacts safety or efficacy
• Does the attribute impact safety or efficacy?
• Determined by the available knowledge
• More severe impact = higher value
• How confident are we in assigning impact?
• Determined by relevance of knowledge
• Higher uncertainty = higher value
Safety
PK
Immunogenicity
Bioactivity

A potenciális kritikus attributumok (pCQA-k) értékelése

A klinikumban mérhető teljesítményre gyakorolt hatás alapján osztályozzuk a pCQA-kat

Milyen hatással van az adott attributum:

- **Hatásosságra**
- **Farmakokinetikára**
- **Biztonságosságra**
- **Immunogenicitás**

Az előbbi információ mennyire bizonytalan?

- **Saját klinikai adat**
- **Saját adat hasonló molekulával**

Forrás: Alt et al, 2016

Impact score for the risk ranking and filtering tool.

Impact and rating	Biological Activity[a]	PK[b]	Immunogenicity[c,d]	Safety[d]
Very High (20)	>100% change	>40% change on PK	ATAs detected that may be life threatening	Irreversible or life-threatening AEs
High[e] (16)	40%–100% change	20%–40% change with impact on PD	ATAs detected that may be associated with non-life-threatening loss of efficacy	Reversible AEs that are not life threatening
Moderate (12)	20%–40% change	20%–40% change with no impact on PD	ATA detected with effect that can be managed by clinical treatment (i.e., dose titration, medication, etc.)	AEs that can be managed by clinical treatment (e.g., dose titration, medication)
Low (4)	<20% change	<20% change with no impact on PD	ATAs detected with effect on PK or PD, but no effect on safety or efficacy	Safety effect with minimal clinical significance
None (2)	No change	No impact on PK or PD	ATAs not detected or ATAs detected with no effect on PK, PD, safety, or efficacy	No effect on safety

Uncertainty scale for the risk ranking and filtering tool.

Rank	Uncertainty	Description (product variants & host-cell-derived impurities)
7	Very High	No information (new variant)
5	High	Published external literature for variant in related molecule
3	Moderate	Non-clinical or *in vitro* data with this molecule. Data (nonclinical, *in vitro* or clinical) from a similar class of molecule
2	Low	Variant has been present in material used in clinical trials.[a]
1	Very Low	Impact of specific form established in clinical studies

Impact[b] \ Uncertainty[a]	1 (Very Low)	2 (Low)	3 (Moderate)	5 (High)	7 (Very High)
20 (Very High)	20	40	60	100	
16 (High)	16	32	48	80	112
12 (Moderate)	12 [c]	24	36	60	
4 (Low)	4	8	12	20	
2 (None)	2	4	6	10	

Obligatory CQAs

Protein Content

Osmolality

pH

Appearance (Color, Opalescence, Clarity)

Buffer Content

Excipient Content

Surfactant Content

- Subvisible Particles
- Visible Particles
- Extractable Volume
- Sterility

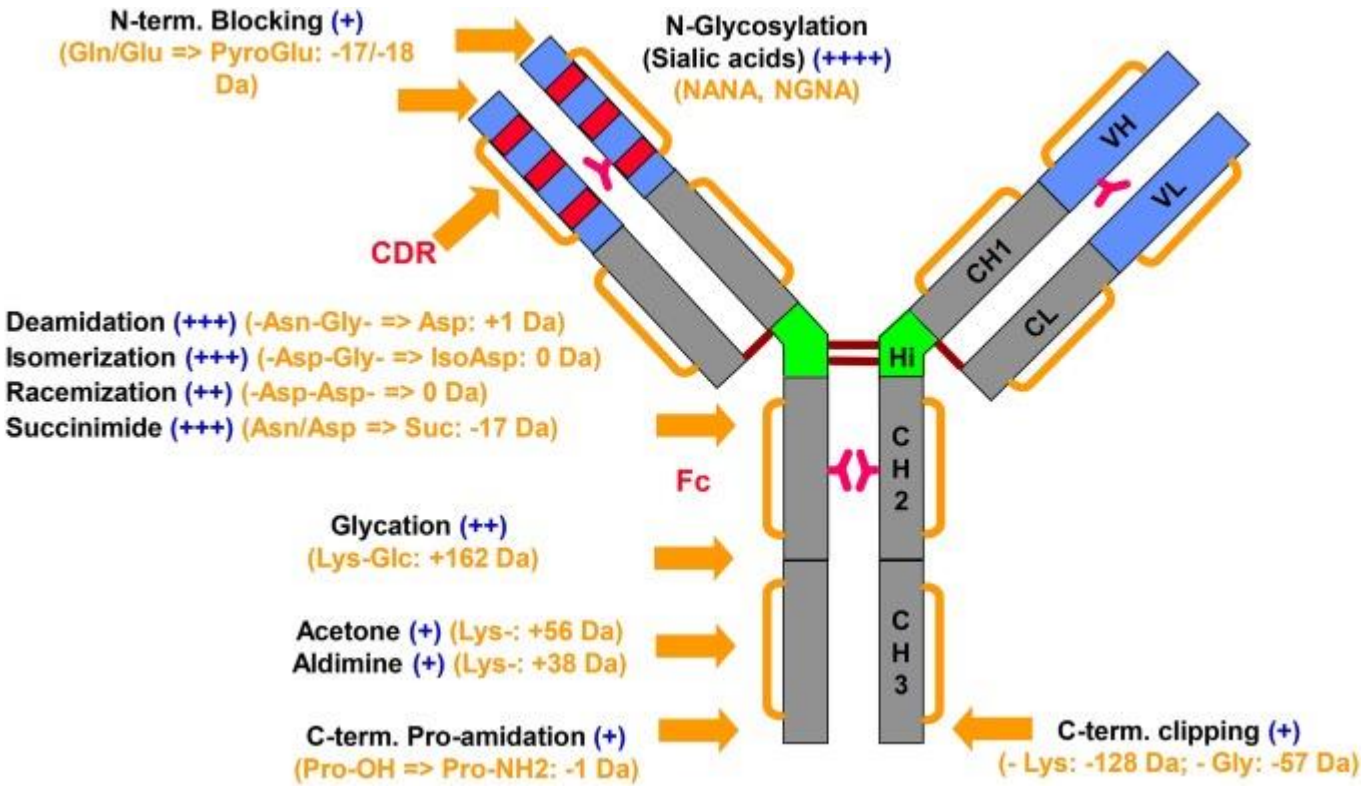
- Viruses
- Microbiological impurities (Bacteria, Mycoplasma)
- Bacterial endotoxins

List of molecular variant pCQAs for a monoclonal antibody.

Category	Quality attribute[a]
Size-related Variants	High Molecular Weight Species (HMWS)
	Low Molecular Weight Species (LMWS)
Charge-related Variants (Acidic)	Deamidation in CDR
	Deamidation in Non-CDR
	Glycation in CDR
	Glycation in Non-CDR
Charge-related Variants (Basic)	Aspartic Acid Isomerization in CDR
	Aspartic Acid Isomerization in Non-CDR
	N-Terminal Leader Sequence (may be molecule specific)
	N-Terminal Pyroglutamic Acid
	C-Terminal Lysine
	C-Terminal Proline (IgG1) or Leu (IgG4) Amidation
Oxidation-related Variants	Oxidation in CDR (Met, Trp)
	Oxidation in Non-CDR (Met, homo-variant)
	Oxidation in Non-CDR (Met, hetero-variant)
Fc Glycosylation	Afucosylation
	Galactosylation
	High-Mannose
	Sialylation (NANA, NGNA)
	Non-Glycosylated Heavy Chain
Structural Variants	Cysteine Forms
	Sequence Variants
	Protein Structure

[a] Certain low abundance variants may need to be added to the list of general known variants such as advanced glycation end-products, hydroxylysine, or oxidative carbonylation.

Monoklonális ellenanyag



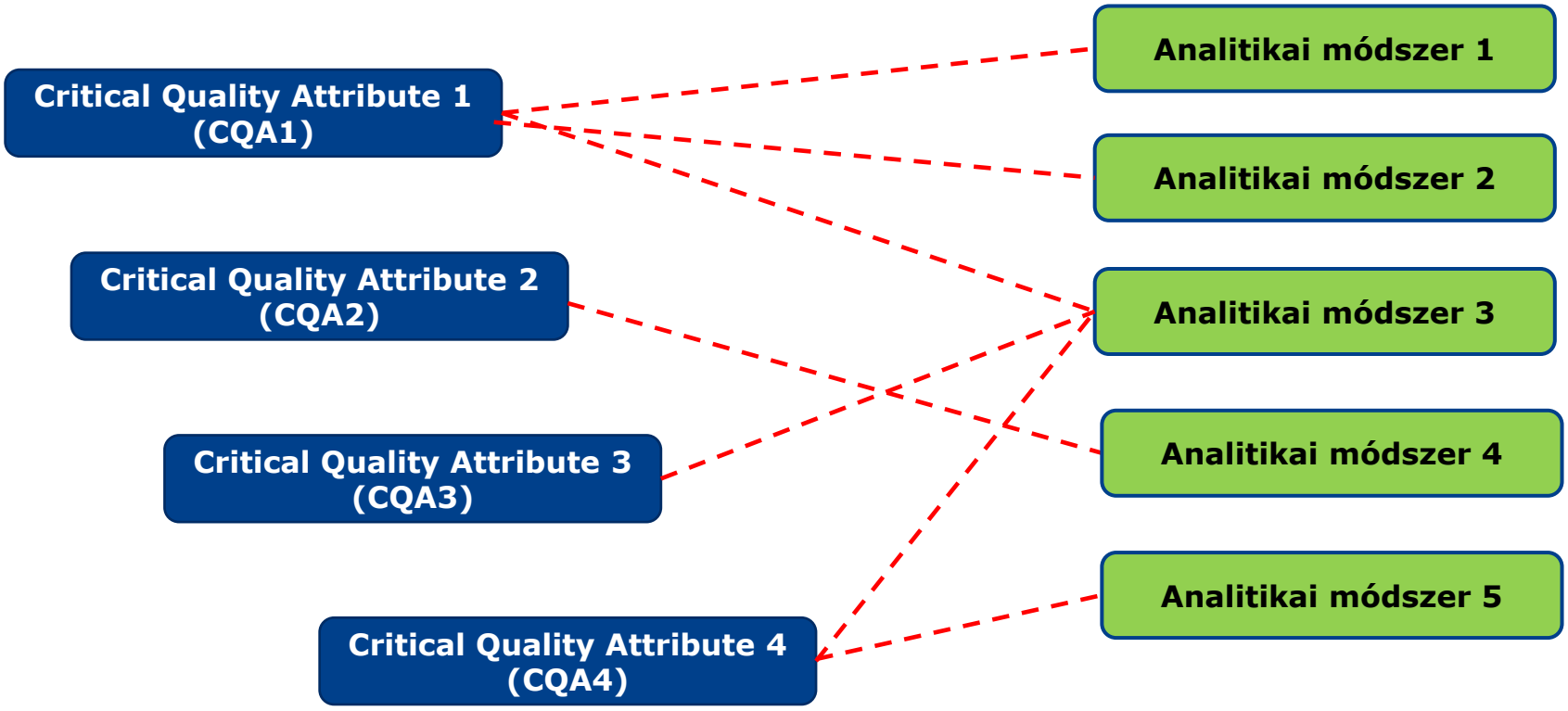
Forrás: Wagner-Rousset et al, 2017

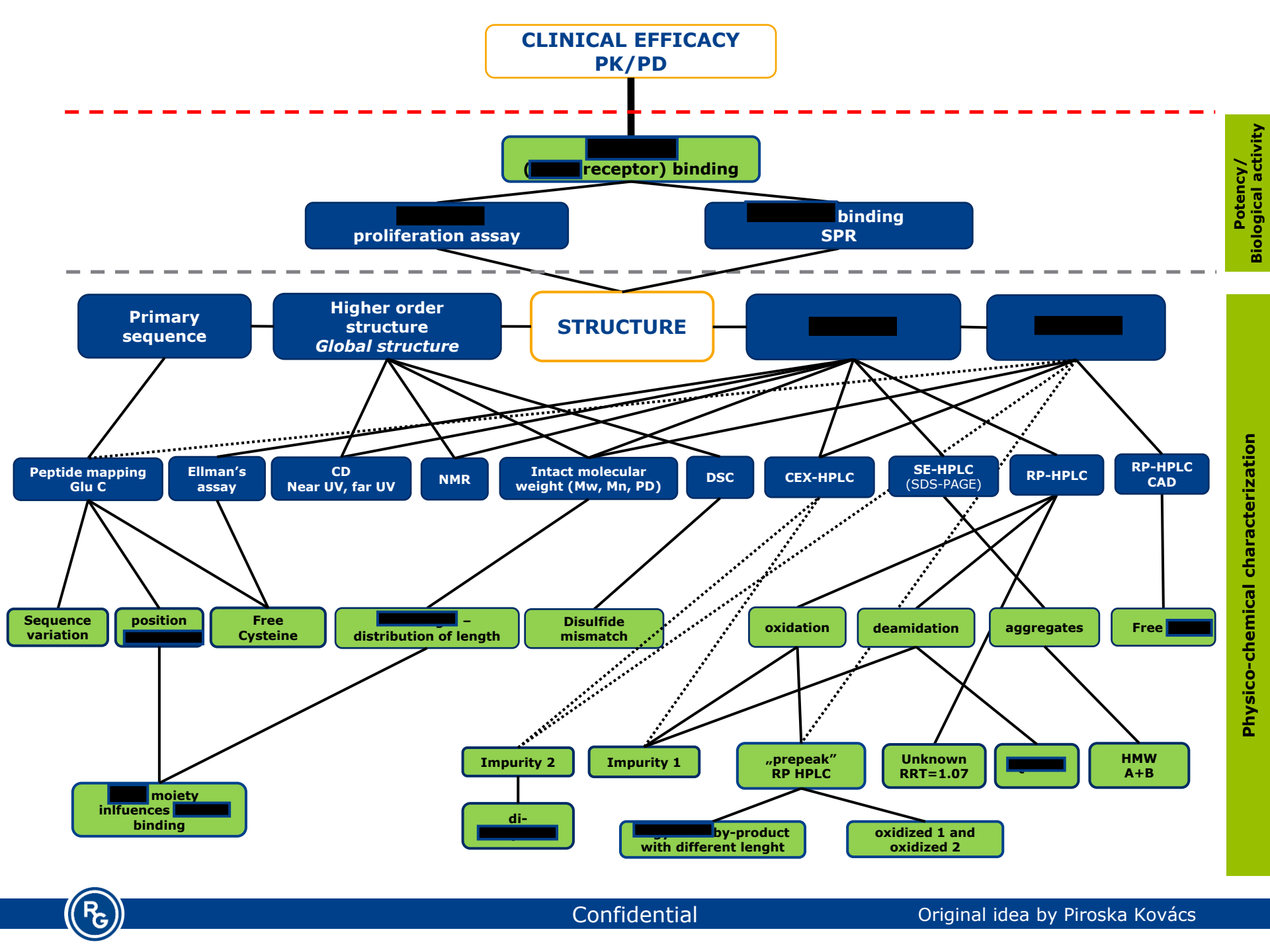
CQA-k meghatározása – klinikai hatás és a bizonytalanság alapján

Purity	Product related variants	Quality Attribnes		CQA	Methods	Efficacy/PD		PK		Immuno-genicity		Safety		E/PD	PK	I	Safe-ty	Risk
						I	U	I	U	I	U	I	U					
		Size-related variants	Aggregates, HMW species (A+B) (SEC, RRT=0.88-0.90)	yes	SE-HPLC, DLS	16	2	4	2	16	2	12	2	32	8	32	24	32
			LMW Fragments	yes	SE-HPLC	16	2	12	2	2	2	2	2	32	24	4	4	32
			[redacted]	yes	SE-HPLC	16	2	12	2	2	2	2	2	32	24	4	4	32
		Charge-related variants	[redacted] product (Impurity 1 IEX RRT:0.85)	yes	IEX;SEC; conversion RP-HPLC	16	2	12	2	2	2	2	2	32	24	4	4	32
			Impurity 2 (IEX RRT: 0.96)	yes	IEX	12	2	12	2	2	2	2	2	24	24	4	4	24
		Related proteins (separation based on hydrophobicity)	Oxidized 1	yes	RP-HPLC	12	2	4	2	4	2	4	2	24	8	8	8	24
			"Pre-peak"	yes	RP-HPLC	12	2	4	2	4	2	4	2	24	8	8	8	24
			Oxidized 2	yes	RP-HPLC	12	2	4	2	4	2	4	2	24	8	8	8	24
			Unknown RRT ~1,07 (RP-HPLC)	yes	RP-HPLC	4	2	12	2	2	2	2	2	8	24	4	4	24
			Deamidated variant [redacted]	yes	RP-HPLC	16	2	4	2	4	2	4	2	32	8	8	8	32
		Impurity of non-protein origin	[redacted]	yes	RP-HPLC CAD	16	2	4	2	2	2	2	2	32	8	4	4	32
	Process related impurities originated from host cell and expression system		Bacterial endotoxins	obligatory	*USP*													
			Microbiological purity/ Sterility	obligatory	*USP*													
			Host cell proteins	yes	ELISA	2	2	2	2	16	2	4	2	4	4	32	8	32
			Host cell DNA	yes	ELISA	2	2	2	2	16	2	4	2	4	4	32	8	32

A CQA-k manifesztációja egyszerre többféle analitikai módszerrel is lemérhető

A CQA-k és analitikai módszerek kapcsolata NEM egy kölcsönösen egyértelmű leképezés (függvény).

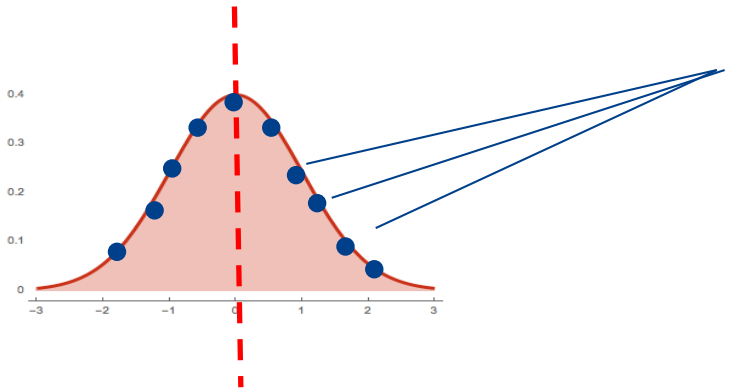



CLINICAL EFFICACY
PK/PD
[redacted] (receptor) binding
[redacted] proliferation assay
[redacted] binding SPR
Potency/ Biological activity
Primary sequence
Higher order structure
Global structure
STRUCTURE
[redacted]
[redacted]
Physico-chemical characterization
Peptide mapping Glu C
Ellman's assay
CD
Near UV, far UV
NMR
Intact molecular weight (Mw, Mn, PD)
DSC
CEX-HPLC
SE-HPLC
(SDS-PAGE)
RP-HPLC
RP-HPLC
CAD
Sequence variation
position [redacted]
Free Cysteine
[redacted] – distribution of length
Disulfide mismatch
oxidation
deamidation
aggregates
Free [redacted]
[redacted] moiety inlfuences [redacted] binding
Impurity 2
Impurity 1
„prepeak" RP HPLC
Unknown RRT=1.07
[redacted]
HMW A+B
di- [redacted]
[redacted] by-product with different lenght
oxidized 1 and oxidized 2

Az analitikai módszerlista biologikumok esetén nagyon hosszú

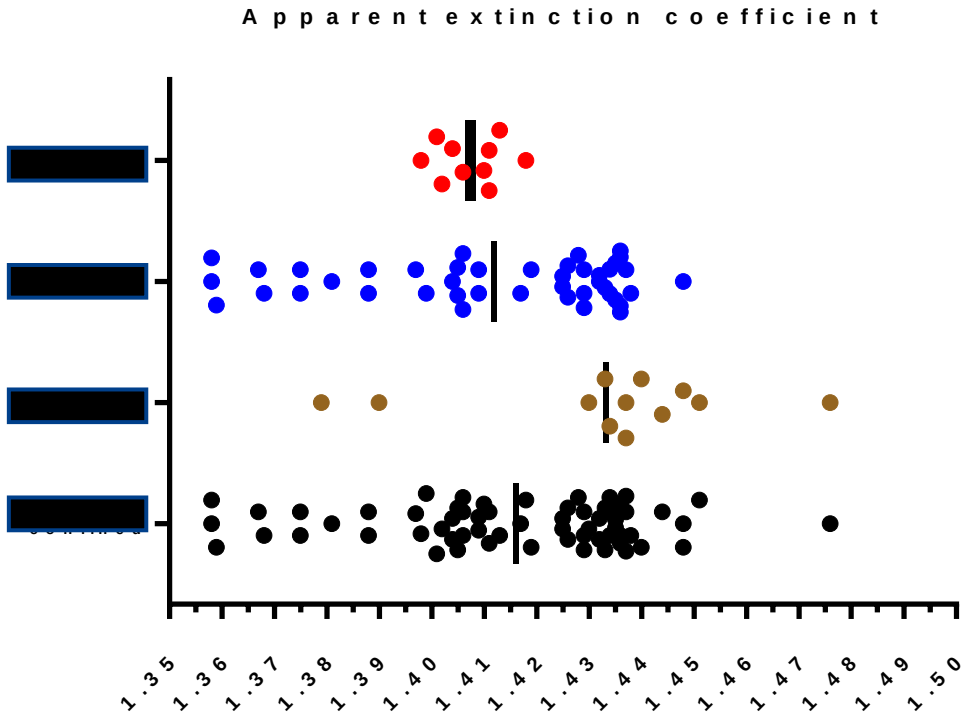
Category	Quality attribute	Method
Composition and strength		
	API content	Amino acid analysis, UV
	API content	UV
	Appearance	Ph.Eur
	Osmolality	Ph.Eur
	pH	Ph.Eur
	Clarity/Opalescence	Ph.Eur
	Colour	Ph.Eur
	Density	Ph.Eur
	Conductivity	Ph.Eur
Buffer content		
	Benzoic	HPLC-UV
	Sorbitol	HPLC-CAD
Surfactant content		
	Polysorbate 20	HPLC-CAD
Adventitious agents and process related impurities and Leachable compounds		
	Viruses	TEM
	Microbiological impurities (Bacteria, Mycoplasma)	Ph.Eur. (2.6.12), (2.6.13)
	Bacterial endotoxins	Ph.Eur. (2.6.14.)
	Host cell protein (HCP)	ELISA / HCP
	Host cell DNA (HCDNA)	Real-time PCR
	Protein A	ELISA
Cell culture medium components		
	Antifoam agent (Simeticone)	ICP-OES
	Pluronic F-68	HPLC-CAD
	2-DFF	No method available
	p-DADMAC	picogreen/fluorescence spectr.
	Metal ions	ICP-OES
	Vitamins	?
	Amino acids	?
	Glucose, lactate, ammonia	Cedex Bio HT
	O_2, CO_2	Blood gas analyzer
Drug product specific QAs		
	Subvisible particles	LO (Ph.Eur)
		MFI
		Archimedes
	Visible particles	Ph.Eur
	Extractable volume	Ph.Eur
	Sterility	Ph.Eur
Product variants		
Size-related Variants	High Molecular Weight Species (HMWS)	SE-HPLC
		AFA
		AUC
		SEC-MALLS
	Low Molecular Weight Species (LMWS)	SE-HPLC
		Non-reduced capillary gel electrophoresis
		Reduced capillary gel electrophoresis
Charge-related Variants (Acidic)	Isoelectric point	cIEF
	Deamidation in CDR	Peptide Mapping Analysis LC-MS
		IEX-HPLC
	Deamidation in non-CDR	Peptide Mapping Analysis LC-MS
		IEX-HPLC
	Glycation in CDR	Peptide Mapping Analysis LC-MS
		IEX-HPLC
	Glycation in non-CDR	Peptide Mapping Analysis LC-MS
		IEX-HPLC
Charge-related Variants (Basic)	Isoelectric point	cIEF
	Asp Isomerisation in CDR (Asp50, CDR2)	Peptide Mapping Analysis LC-MS
		IEX-HPLC
	Asp Isomerisation in non-CDR	Peptide Mapping Analysis LC-MS
		IEX-HPLC
	N-term/Leader Sequence	Peptide Mapping Analysis LC-MS
		Molecular weight determination LC-MS
	N-terminal Pyroglutamic Acid	IEX-HPLC
		Peptide Mapping Analysis LC-MS
		Molecular weight determination LC-MS
	C-term Pro amidation	IEX-HPLC
		Peptide Mapping Analysis LC-MS
		Molecular weight determination LC-MS
	C-terminal lysine	cIEF
		IEX-HPLC (native)
		Peptide Mapping Analysis LC-MS
		Molecular weight determination LC-MS
		IEX-HPLC (CPB digested)
Oxidation-related variants	Oxidation in CDR	IEX-HPLC
		RP-HPLC UV (focused peptide map)
		Peptide Mapping Analysis LC-MS
		hle5-HILIC
	Oxidation in non-CDR (Met 253, home-variant)	IEX-HPLC
		RP-HPLC UV (focused peptide map)
		Peptide Mapping Analysis LC-MS
		hle5-HILIC
	Oxidation in non-CDR (Met	Peptide Mapping Analysis LC-MS
	Oxidation in non-CDR (Met	Peptide Mapping Analysis LC-MS
	Oxidation in non-CDR (Met	Peptide Mapping Analysis LC-MS

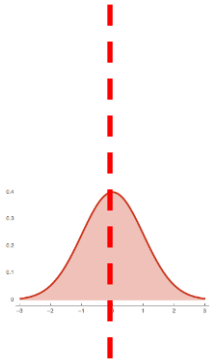
Az originátoron végzett analitikai mérések eredményei egy eloszlást adnak biotechnológiai termékek esetén



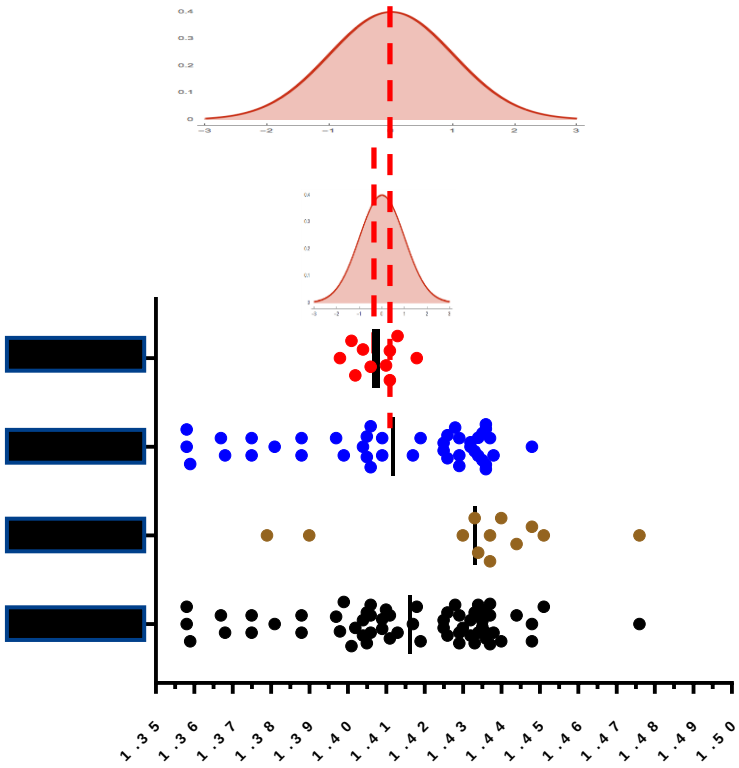
- Különböző lot-számú sarzsok
- A biotechnológiai folyamat élő rendszerekkel dolgozik, ezért természeténél fogva változékony
- Eloszlást ad

A gyűjtött adatok eloszlást adnak



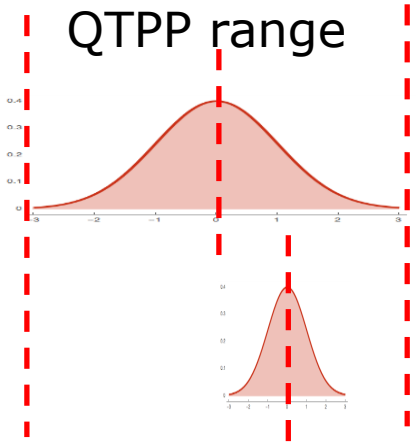


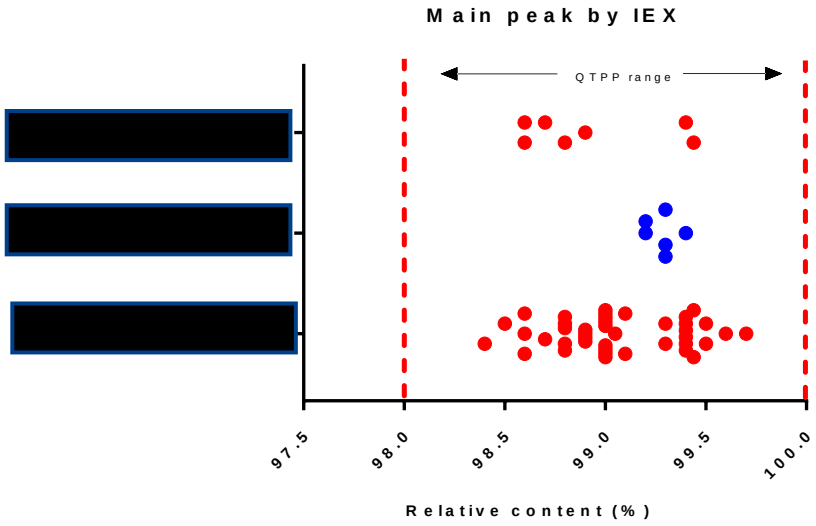
A gyűjtött adatok eloszlást adnak



Az eloszlások statisztikai eszközökkel értékelhetők

A Quality Target Porduct Profile (QTPP) - minőségi termékprofil célpont - az alapja a bioszimilaritás beállításának és vizsgálatának





- Minőségi termékprofil célpont (QTPP)
- Ez NEM a specifikáció, de alapját képezi a specifikációnak
- Saját méréseken kell, hogy alapuljon
- A bioszimilaritást a törzskönyvben a „quality comparability study” keretein belül értékeljük

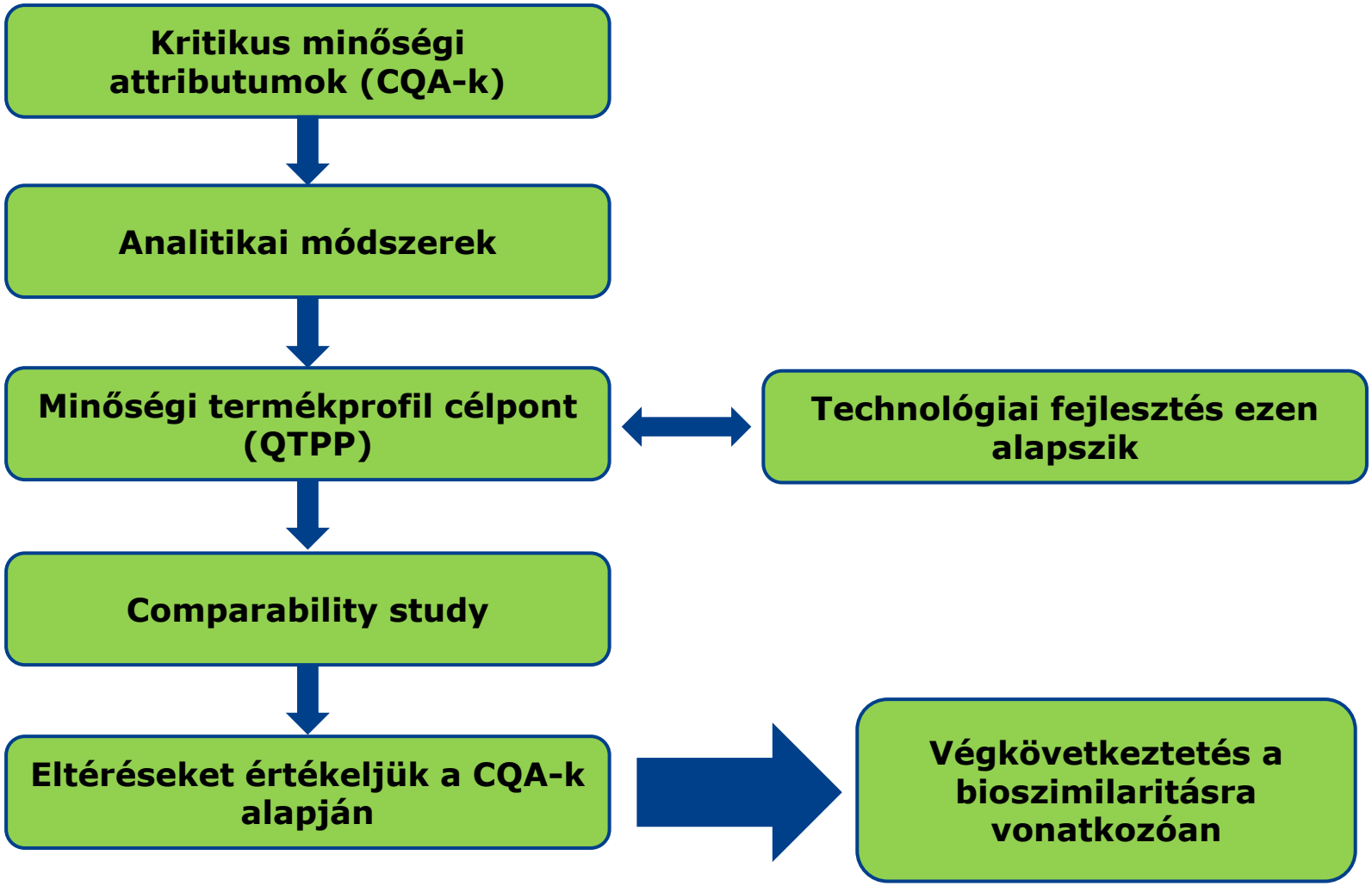
Quality attribute	Product	Min/max ranges	Bar diagram
G0F-GlcNac	RBP A SBP candidate 2	0.05 – 0.3 % 0.00 – 0.2 %	
G0	RBP A SBP candidate 2	1.2 – 2.1 % 1.5 – 2.0 %	
G0F	RBP A SBP candidate 2	20.3 – 32.1 % 25.3 – 33.0 %	
Man5	RBP A SBP candidate 2	0.0 – 0.9 % 0.2 – 0.5 %	
(1,6)G1F	RBP A SBP candidate 2	2.5 – 4.1 % 3.0 – 3.9 %	
(1,3)G1F	RBP A SBP candidate 2	1.6 – 2.3 % 1.7 – 2.2 %	
G2F	RBP A SBP candidate 2	10.5 – 25.7 % 11.0 – 24.4 %	
G2FS1	RBP A SBP candidate 2	35.3 – 39.1 % 36.2 – 38.5 %	
G2FS2	RBP A SBP candidate 2	11.4 – 13.2 % 11.4 – 13.0 %	
NGNA	RBP A SBP candidate 2	3.3 – 5.5 % 1.1 – 2.0 %	
Deamidation	RBP A SBP candidate 2	0.9 – 2.4 % 0.3 – 1.5 %	
Oxidation	RBP A SBP candidate 2	1.2 – 4.3 % 1.0 – 4.1 %	
Dimer	RBP A SBP candidate 2	0.0 – 2.1 % 0.0 – 0.8 %	
Higher aggregates	RBP A SBP candidate 2	0.0 – 0.8 % 0.0 – 0.2%	
Binding assay	RBP A SBP candidate 2	91 – 108 % 93 – 105 %	
CDA activity	RBP A SBP candidate 2	84 – 110 % 90 – 111 %	
ADCC activity	RBP A SBP candidate 2	75 – 132 % 82 – 115 %	

Schiestl et al, Biologicals (2013) 42(2)

Quality attribute	Product	Min/max ranges	Bar diagram
G0F-GlcNac	RBP A SBP candidate 2	0.05 – 0.3 % 0.00 – 0.2 %	
G0	RBP A SBP candidate 2	0.4 – 2.1 % 9.2 – 12.0 %	
G0F	RBP A SBP candidate 2	20.3 – 32.1 % 25.3 – 33.0 %	
Man5	RBP A SBP candidate 2	0.0 – 0.9 % 0.2 – 0.5 %	
(1,6)G1F	RBP A SBP candidate 2	2.5 – 4.1 % 3.0 – 4.2 %	
(1,3)G1F	RBP A SBP candidate 2	1.6 – 2.3 % 1.5 – 2.0 %	
G2F	RBP A SBP candidate 2	10.5 – 25.7 % 11.0 – 24.4 %	
G2FS1	RBP A SBP candidate 2	35.3 – 39.1 % 36.2 – 38.5 %	
G2FS2	RBP A SBP candidate 2	11.4 – 13.2 % 11.4 – 13.0 %	
NGNA	RBP A SBP candidate 2	3.3 – 5.5 % 3.4 – 5.8 %	
Deamidation	RBP A SBP candidate 2	0.9 – 2.4 % 0.3 – 1.5 %	
Oxidation	RBP A SBP candidate 2	1.2 – 4.3 % 1.0 – 4.1 %	
Dimer	RBP A SBP candidate 2	0.0 – 2.1 % 0.0 – 0.8 %	
Higher aggregates	RBP A SBP candidate 2	0.0 – 0.8 % 0.0 – 0.2%	
Binding assay	RBP A SBP candidate 2	91 – 108 % 93 – 105 %	
CDA activity	RBP A SBP candidate 2	84 – 110 % 90 – 111 %	
ADCC activity	RBP A SBP candidate 2	82 – 118 % 380 – 475 %	

Schiestl et al, Biologicals (2013) 42(2)

Összefoglaló: hogyan érjük el a bioszimilíritást, és azt hogyan értékeljük?

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Kérdések? Kapcsolatfelvétel:

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