Strategie zur umfassenden Darstellung der „biosimilarity“ von generischen Proteinwirkstoffen

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Recent Events

EC approves first biosimilar monoclonal Antibody

EU Approves Inflectra For Rheumatoid Arthritis: First 'Biosimilar' Antibody Drug Clears Path For Greater Competition

By Matthew Mientka | Sep 10, 2013 04:23 PM EDT

Hospira kündigt EC-Zustimmung von Inflectra, Europas erste biosimilar mAb-Therapie an

Published on September 10, 2013 at 7:54 AM · No Comments

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What are “biosimilar” medicines?

Questions and answers on biosimilar medicines (similar biological medicinal products)

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What is a biosimilar medicine?

A biosimilar medicine is a biological medicine that is developed to be similar to an existing biological medicine (the ‘reference medicine’). Biosimilars are not the same as generics, which have simpler chemical structures and are considered to be identical to their reference medicines.

The active substance of a biosimilar and its reference medicine is essentially the same biological substance, though there may be minor differences due to their complex nature and production methods. Like the reference medicine, the biosimilar has a degree of natural variability. When approved, its variability and any differences between it and its reference medicine will have been shown not to affect safety or effectiveness.

An authorised biosimilar is generally used at the same dose to treat the same conditions. If there are specific precautions to be considered when taking the reference medicine, the same will generally apply to the biosimilar.

Biosimilars are usually authorised several years after the approval of the reference medicine. This is because the reference medicine benefits from a period of exclusivity, during which biosimilars cannot be authorised.

An agency of the European Union

Why similar and not identical?

Questions and answers on biosimilar medicines (similar biological medicinal products)

EUROPEAN MEDICINES AGENCY
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How are biosimilar medicines evaluated in the EU?

Because the reference medicine has been authorised in the EU for several years and its clinical benefit is established, some studies carried out with the reference medicine may not need to be reproduced. Since 2003, a new EU pathway for approving biosimilar medicines has been in place. The main part of the evaluation is a comparison of the biosimilar with its reference medicine to show that there are no significant differences between them.

The relevant regulatory authority applies stringent criteria in their evaluation of the studies comparing the quality, safety and effectiveness of the two medicines. The studies on quality include comprehensive comparisons of the structure and biological activity of their active substances, while the studies on safety and effectiveness should show that there are no significant differences in their benefits and risks, including the risk of immune reactions.

Biosimilar medicines are manufactured following the same standards as for other medicines, and regulatory authorities perform periodic inspections of the manufacturing sites.

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Question of interchangability?

Questions and answers on biosimilar medicines (similar biological medicinal products)

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➡ **New question which was introduced in the 2012 released Q&A**

Can a biosimilar medicine and its reference medicine be used interchangeably?

The EMA evaluates biosimilar medicines for authorisation purposes. The Agency's evaluations do not include recommendations on whether a biosimilar should be used interchangeably with its reference medicine. For questions related to switching from one biological medicine to another, patients should speak to their doctor and pharmacist.

- Responsibility on interchangeably lies with healthcare professionals!
- Will we see patients on originator medication switching to biosimilars?

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Guidelines for similar biological products

Quality issues

Non-clinical and clinical issues

London, 22 February 2006

EMEA/CHMP/BWP/49348/2005

GUIDELINE ON SIMILAR BIOLOGICAL MEDICINAL PRODUCTS CONTAINING BIOTECHNOLOGY-DERIVED PROTEINS AS ACTIVE SUBSTANCE: QUALITY ISSUES

➡ Currently under revision

London, 22 February 2006

EMEA/CHMP/BWP/42832/2005

GUIDELINE ON SIMILAR BIOLOGICAL MEDICINAL PRODUCTS CONTAINING BIOTECHNOLOGY-DERIVED PROTEINS AS ACTIVE SUBSTANCE: NON-CLINICAL AND CLINICAL ISSUES

London, 20 May 2012

EMA/CHMP/BMWP/403543/2010

Guideline on similar biological products containing monoclonal antibodies – non-clinical and clinical issues

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Guidelines for similar biological products

London, 20 May 2012

EMA/CHMP/BMWP/403543/2010

Guideline on similar biological products containing monoclonal antibodies – non-clinical and clinical issues

Non-clinical and clinical issues

effector function, and binding to Fc receptors. Various assays have been established in the past years that allow for more in-depth characterisation of complex proteins, both on a physicochemical and a functional level, e.g. with potency assays, and there is experience in the assessment of minor quality differences due to changes in manufacturing processes for monoclonal antibodies. However, it may at the current stage of knowledge be difficult to interpret the relevance of minor quality differences in the physicochemical and biological characterization when comparing a biosimilar mAb to a reference mAb.

- In future a reduced non-clinical/clinical testing regime may be feasible?
- Biosimilars become more attractive from a return of investment point of view!

Guidelines for similar biological products

Quality issues

London, 22 February 2006

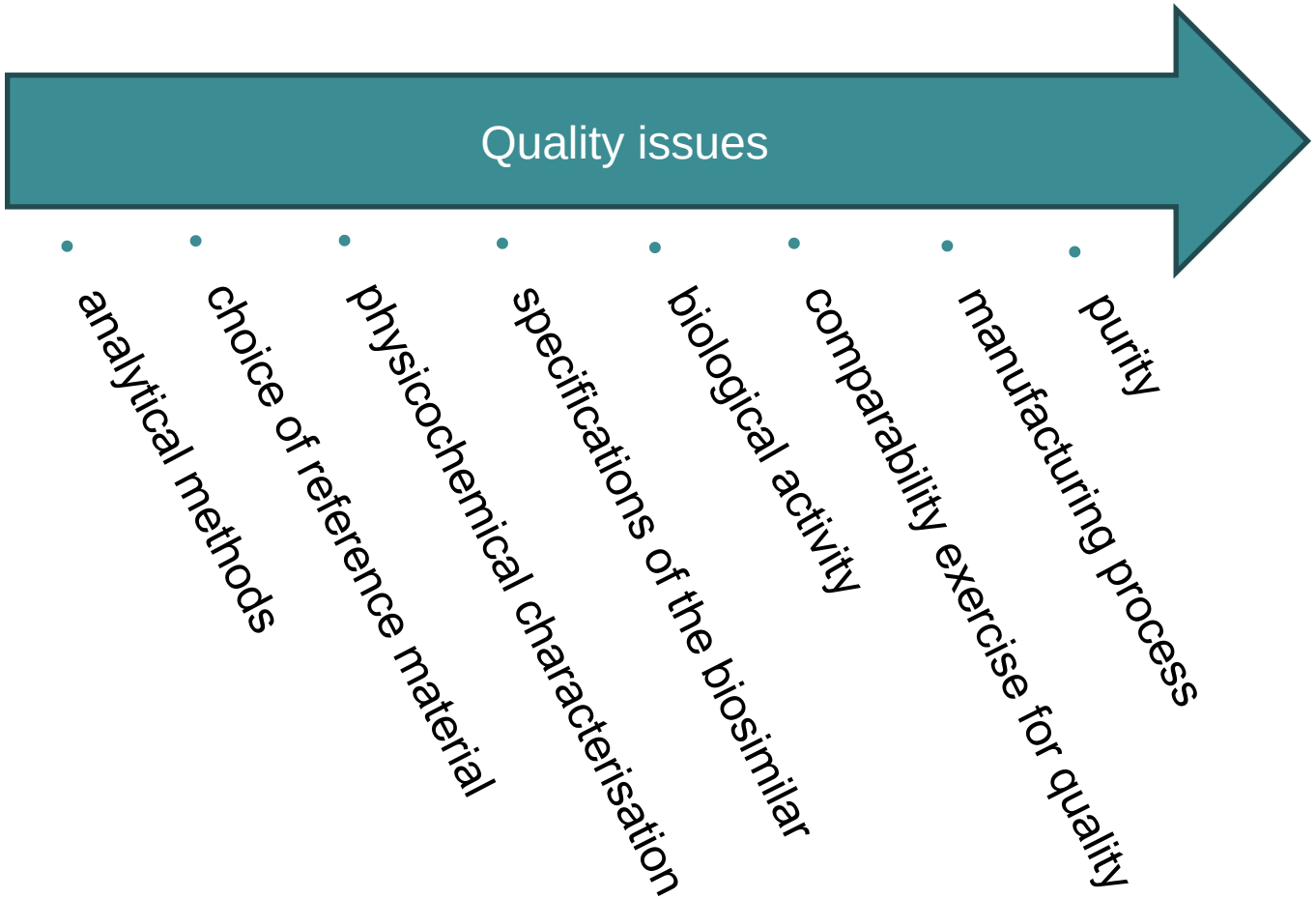
EMEA/CHMP/BWP/49348/2005

GUIDELINE ON SIMILAR BIOLOGICAL MEDICINAL PRODUCTS CONTAINING BIOTECHNOLOGY-DERIVED PROTEINS AS ACTIVE SUBSTANCE: QUALITY ISSUES

The guideline addresses:

- manufacturing process
- comparability exercise for quality
- choice of reference material
- analytical methods
- physicochemical characterisation
- biological activity
- purity
- specifications of the biosimilar

Approach to display biosimilarity



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Analytical methods

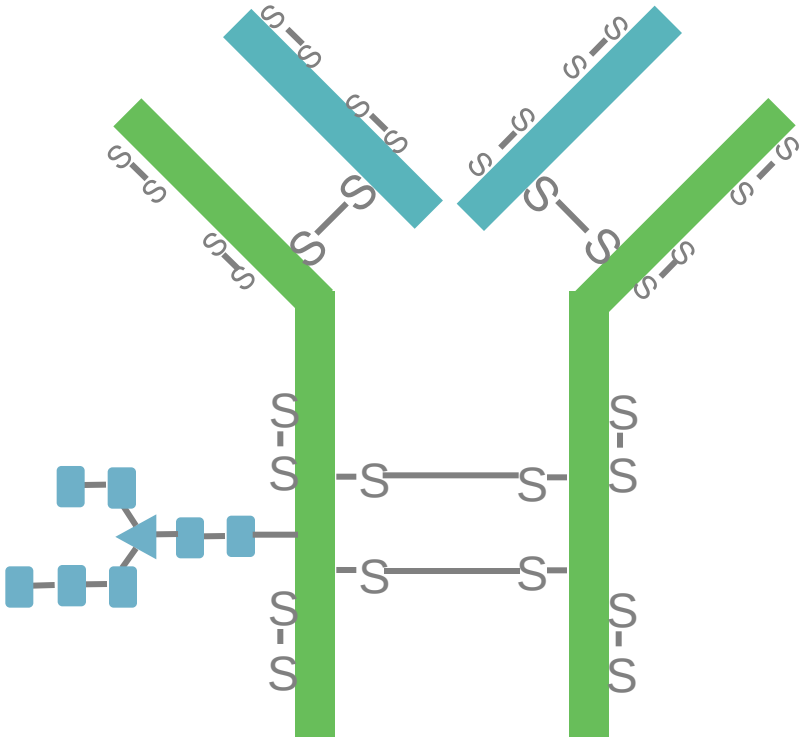
- Suitability of available analytical methods

Given the complexity of the molecule and its inherent heterogeneity, the set of analytical techniques should represent the state-of-the-art. It is the duty of the manufacturer to demonstrate that the selected methods used in the comparability exercise would be able to detect slight differences in all aspects pertinent to the evaluation of quality.

- **Mass spectrometry** → detailed product characterisation.
- **Capillary electrophoresis** → product quality, purity (charge variants, molecular weight, heterogeneity).
- **HPLC** → product quality, purity (charge variants, molecular weight, heterogeneity).
- **ELISA** → characterisation of biological activity, product quality.
- **Cell-bases assay** → characterisation of biological activity

Structure of monoclonal antibodies

The amino acid sequence (primary structure) should be invariant!



Cell type, clone specific & fermentation

- N- and C-terminal processing variants
- Glycosylation and variants

Fermentation

- Degradation by enzymatic hydrolysis

Downstream processing

- Oxidation
- Deamidation
- Disulphide bond formation and shuffling
- Glycation

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In-depth characterisation of the originator product

- Characterisation of the originator.
  → identification of all modifications, characterisation of sugar composition… .

- Identification of quality determining attributes (QDA).
  → modifications that can be controlled during down-stream processing.

- Definition of critical-quality-attributes (CQA).
  → modifications that are produces by the cell line/clone used.
  → modifications that are difficult or impossible to influence by the down-stream process.

Specifications

The specifications are set by the originator!

CQA (e.g. sialylation of glyco-structure)

Variability batches 1 & 2. — Specification

Variability batches 3 & 4. — Specification

QDA (e.g. Methionine oxidation)

Variability batches 1 & 2. — Specification

Variability batches 3 & 4. — Specification

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Specifications

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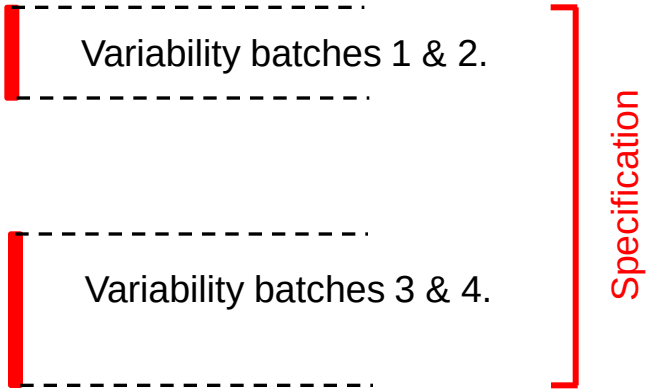
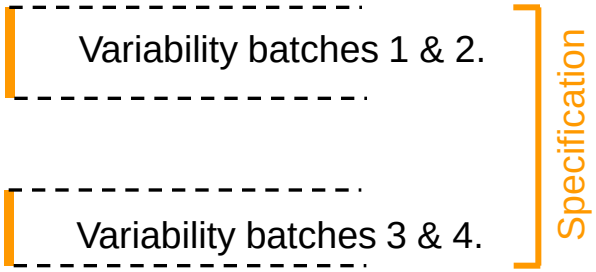
CQA (e.g. sialylation of glyco-structure)



QDA (e.g. Methionine oxidation)



- The more originator batches tested the more precise the specification.
- Only batches which are approved in the EU can be used!
- Organisation of sufficient batches may be difficult.

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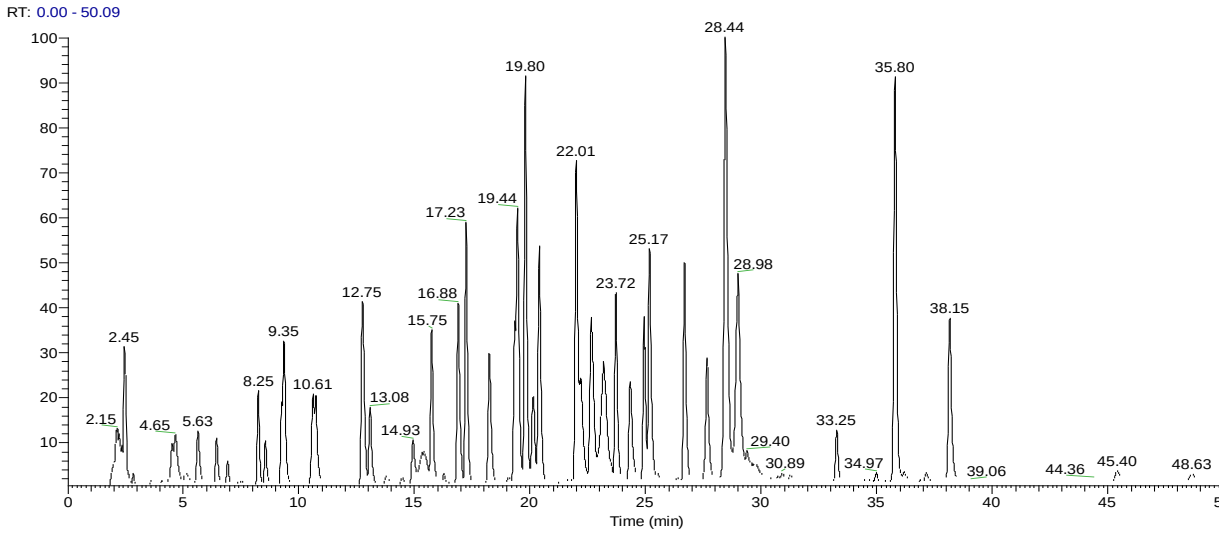
Characterisation of the originator

Analysis of the originator product:



Peptide mass map of a mAb:

- file size: ~500.000 KB
- obtained mass spectra: ~6.000
- amount of tryptic peptides: ~60 (without modifications)

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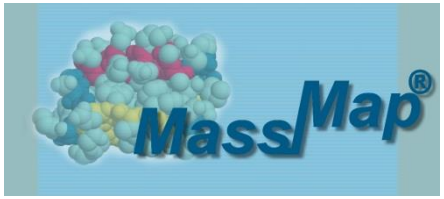
LC-MS data evaluation

We use the Mass Map software to perform an extensive characterisation of the originator.



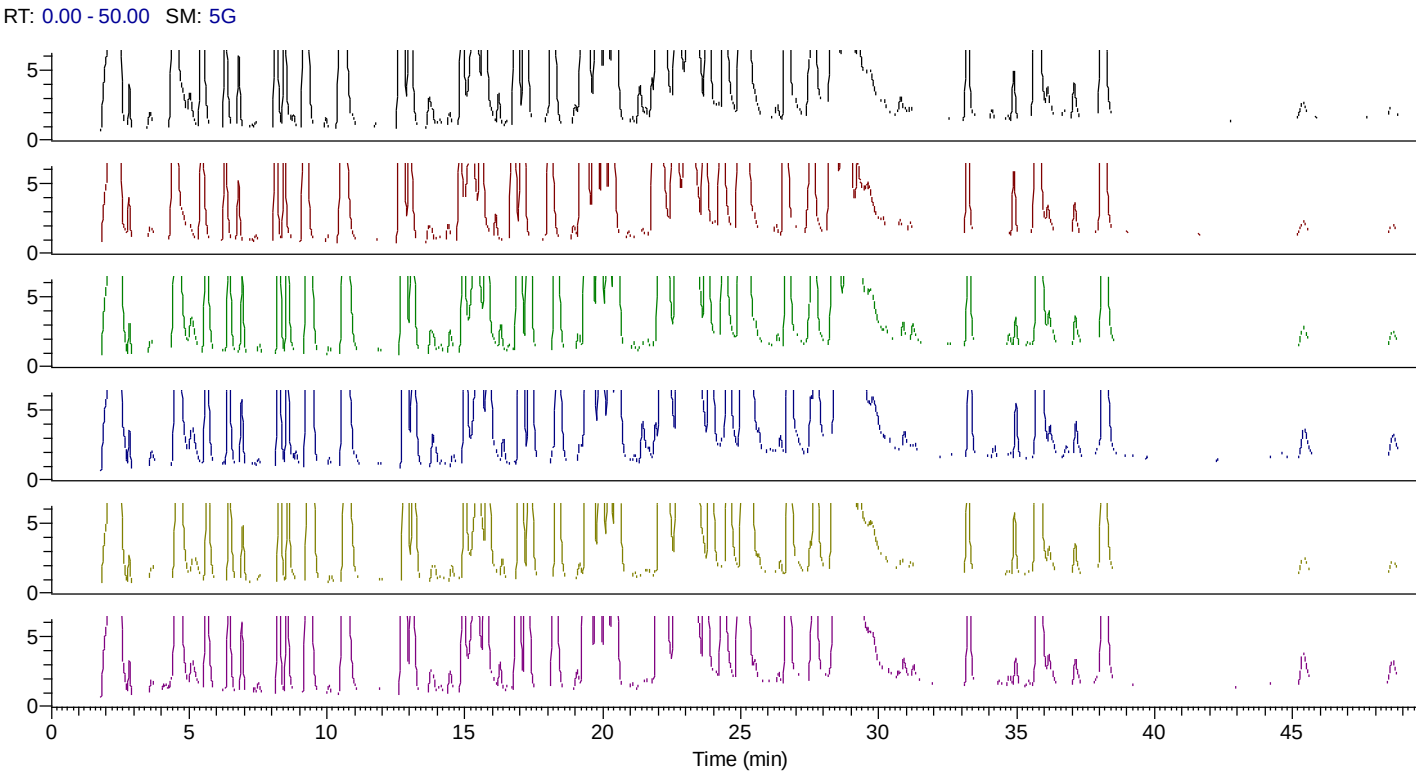
- Data reduction by deleting background ions.
- Set an identification threshold (usually >1%).
- Peptides are identified according to mass and isotopic distribution.

  User has to accept the generated results!
- Signals of identified peptides are removed from the file.
- Signals that are still >1% have to be assigned manually.
- Result table is generated:

  retention time, mass, mass error, isotopic pattern, peak area…

Analysis of 6 originator batches

The result table is used to evaluate further batches:



Total ion chromatogram zoomed to 5% intensity

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Statistical evaluation of all peptides and modifications

Example: statistical evaluation of methionine oxidation:

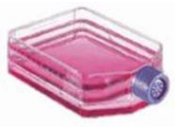
Datensatz-Nr.	Kennung	Gruppe 1: Referenz	HC_SF_20-38	HC_SF_20-38_ox	HC_SF_247-253	HC_SF_247-253_ox	HC_SF_415-437	HC_SF_415-437_ox	HC_SF_98-119	HC_SF_98-119_ox	LC_SF_36-46
1	REF1	X	25,71	0,07	5,93	0,23	40,44	0,27	9,67	0,18	16,98
2	REF2	X	27,08	0,08	5,69	0,18	41,31	0,28	9,74	0,18	14,97
3	REF3	X	26,21	0,09	5,75	0,20	41,61	0,30	9,65	0,18	15,51
4	REF4	X	26,06	0,07	6,12	0,21	40,35	0,27	10,40	0,19	15,83
5	REF5	X	26,88	0,11	5,57	0,21	41,85	0,33	9,48	0,18	14,91
6	AK5	|	27,04	0,09	5,82	0,25	40,41	0,56	9,68	0,20	15,48
7	REF	X	26,04	0,13	5,71	0,22	42,07	0,35	9,76	0,20	15,03
8	AK40	|	28,25	0,13	5,68	0,38	39,95	0,80	7,96	0,36	16,00
		MW 1	26,33	0,09	5,80	0,21	41,27	0,30	9,78	0,18	15,54
		Stabw 1	0,53	0,03	0,20	0,02	0,73	0,03	0,32	0,01	0,79
		relStabw 1 [%]	2,03	28,60	3,44	8,52	1,76	10,74	3,24	5,00	5,08

Mean value and standard deviation can be used to define acceptance criteria.

When to start with the comparability exercise?

Best to start with the optimal clone!

- optimal not meaning highest yield!!!
- *optimal in terms of product characteristics!*



clone selection
- select for best product characteristics

scale up
- optimise culture conditions

validation/production batches
- optimisation of fill-and-finish
- final comparability exercise

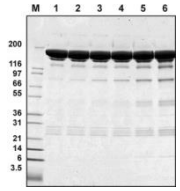
Clone selection

>100 clones

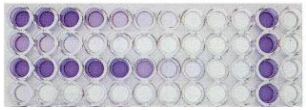
1. Step: identify a sub-set of suitable clones.

- Production of desired product → molecular weight, structure
- Production efficiency → semi quantitative screening
- Biological activity → e.g., binds to the desired epitope

Capillary electrophoresis



Gel electrophoresis



ELISA

<20 clones

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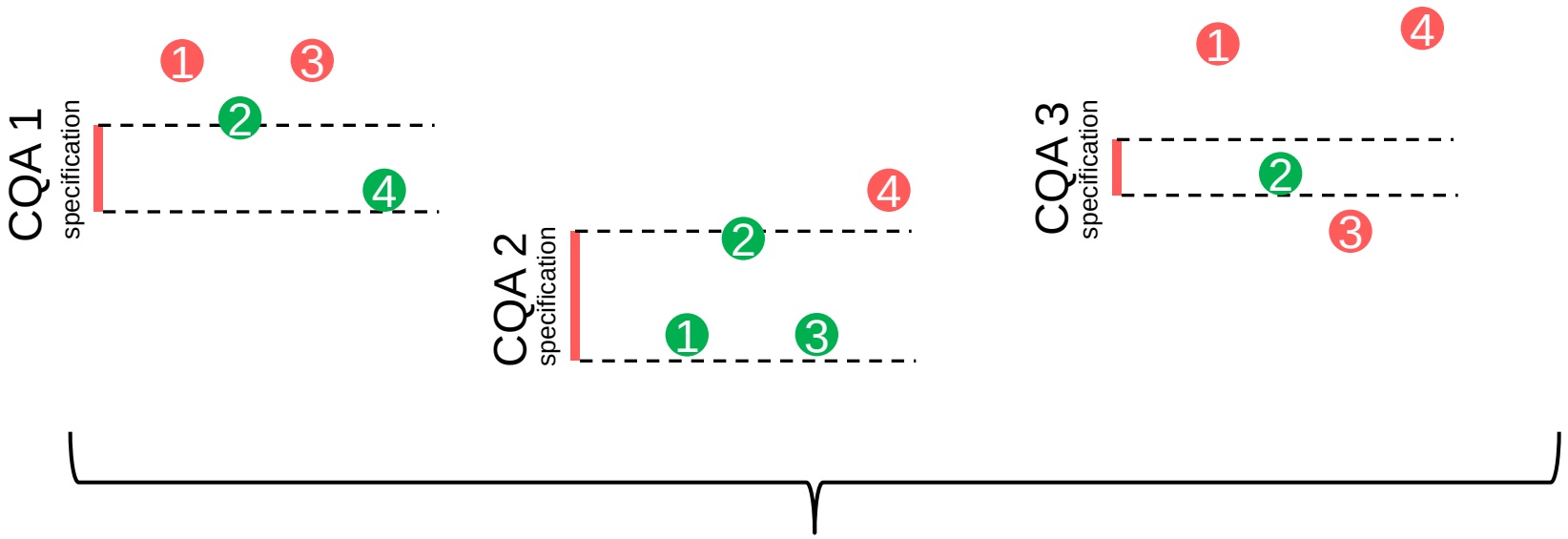
Clone selection

<20 clones

2. Step: identify clones that fit the defined critical quality attributes

- Compare CQA to the originator specification → reduced characterisation by MS



~5 clones

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Comparison of the originator to a biosimilar producing clone

Simple example: oxidised methionines

5 methionine containing peptides → 2 degrees of freedom

	Biosimilar Anteil DS [%]	mean orig. Anteil MW(SG) [%]	mean orig. Anteil StAbw(SG) [%]	Anteil DS-MW(SG) [%]
Pep1 Met	28,25	26,33	0,53	1,92
Pep1 Met-ox.	0,13	0,09	0,03	0,04
Pep2 Met	5,68	5,80	0,20	-0,11
Pep2 Met-ox.	0,38	0,21	0,02	0,17
Pep3 Met	39,95	41,27	0,73	-1,32
Pep3 Met-ox.	0,80	0,30	0,03	0,50
Pep4 Met	7,96	9,78	0,32	-1,83
Pep4 Met-ox.	0,36	0,18	0,01	0,17
Pep5 Met	16,00	15,54	0,79	0,46
Pep5 Met-ox.	0,48	0,49	0,02	-0,01

➡ easy for “simple” modifications.

➡ easy if you are only comparing 1 clone to the originator.

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Comparison of the originator to a biosimilar producing clone

Complex example: N-glycosylation

1 peptide → 13 (glyco-structures) degrees of freedom

Signal	Ant. P [%]	Ant. R [%]	P-R [%]
YNS_A2G0	0,24	0,26	-0,02
YNS_A2G0-GlcNAc	n.b.	n.b.	n.b.
YNS_A2FG0	44,96	47,63	-2,67
YNS_A2FG0-GlcNAc	11,55	9,80	1,74
YNS_A2FG1	24,41	24,47	-0,06
YNS_A2FG1-GlcNAc	5,42	4,88	0,54
YNS_A2FG2	3,14	3,25	-0,11
YNS_A2FG2S1	n.b.	n.b.	n.b.
YNS_A2FG2S2	n.b.	n.b.	n.b.
YNS_Man5	3,20	5,69	-2,49
YNS_Man6	0,92	1,79	-0,87
YNS_deglyc	5,93	0,00	5,93
YNS_nonglyc	0,23	2,22	-1,99

Biosimilar

mean orig.

- complex modification.
- difficult to compare.
- *Data reduction by calculating classification figures!*

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Calculation classification figures

Definition of the classification figures:

#	Name der Kennzahl	YNS_A2G0	YNS_A2G0-GlcNAc	YNS_A2FG0	YNS_A2FG0-GlcNAc	YNS_A2FG1	YNS_A2FG1-GlcNAc	YNS_A2FG2	YNS_A2FG2S1	YNS_A2FG2S2	YNS_Man5	YNS_Man6	YNS_deglyc	YNS_nonglyc
1	Ohne Glycosylierung	0|1	0|1	0|1	0|1	0|1	0|1	0|1	0|1	0|1	0|1	0|1	0|1	1|1
2	Deglyosyliert/desamidiert	0|1	0|1	0|1	0|1	0|1	0|1	0|1	0|1	0|1	0|1	0|1	1|1	0|1
3	Galactosylierung	0|2	0|2	0|2	0|2	1|2	1|2	2|2	2|2	2|2	–	–	–	–
4	Fucosylierung	0|1	0|1	1|1	1|1	1|1	1|1	1|1	1|1	1|1	–	–	–	–
5	High Mannose	0|1	0|1	0|1	0|1	0|1	0|1	0|1	0|1	0|1	1|1	1|1	–	–

Comparison of calculated classification figures:

#	Name der Kennzahl	Wert P [%]	Wert R [%]	P-R [%]	(P-R)/R [%]
1	Ohne Glycosylierung	0,23	2,22	-1,99	-89,59
2	Deglyosyliert/desamidiert	5,93	0,00	5,93	n.b.
3	Galactosylierung	20,12	19,85	0,27	1,36
4	Fucosylierung	99,73	99,71	0,02	0,02
5	High Mannose	4,39	7,65	-3,26	-42,59

The closer these values are to 0 the more similar are both proteins!

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Example pharmaceutical equivalence study of a natural source product

"Worst case scenario"…

- only a few known actives
- many unknown actives
- quality dependent in natural source and processing

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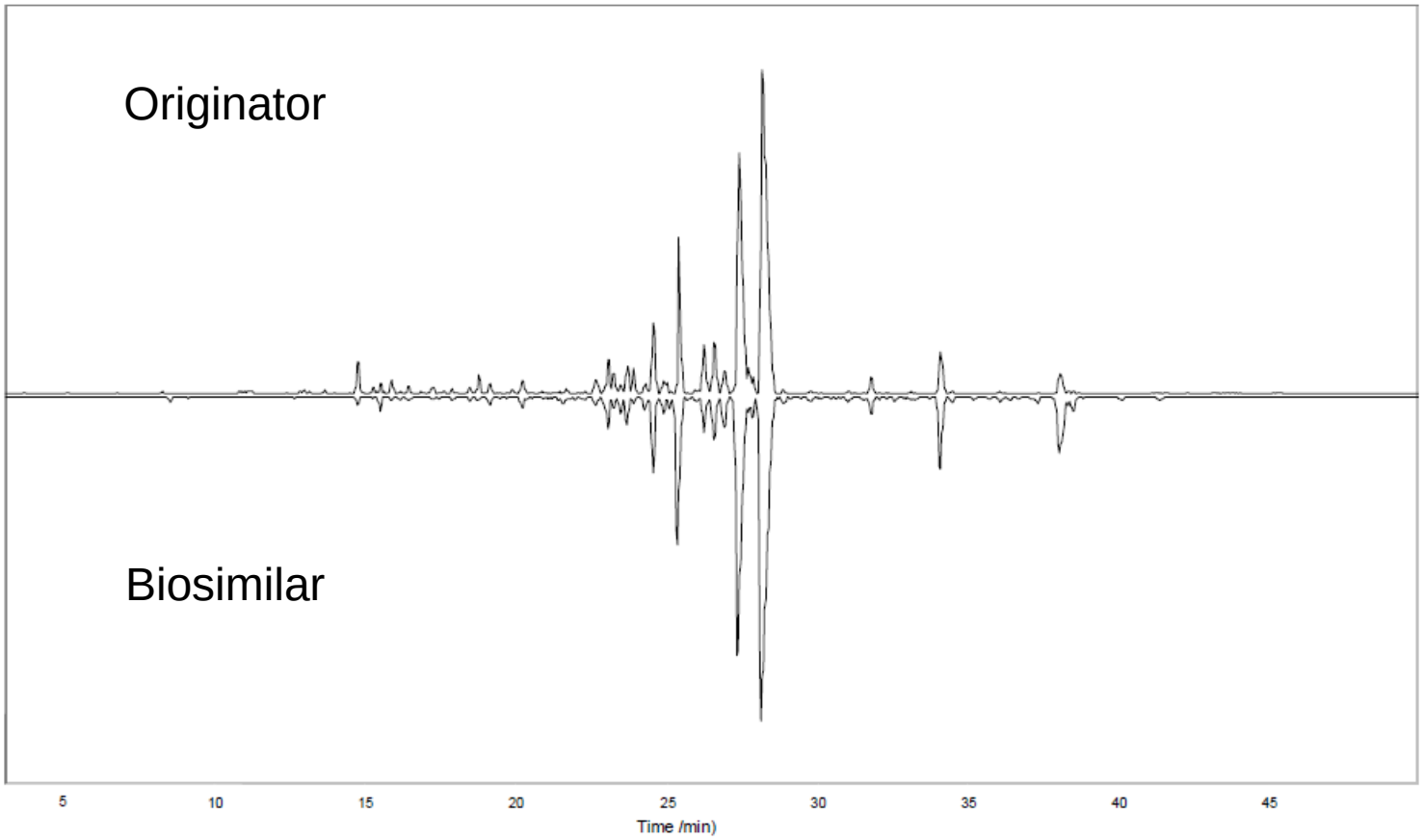
Subtractive display of LC-MS Data:



- *In total 884 peaks were detected!*
- *Do we need to consider all peaks?*

Qualitative pharmaceutical equivalence

Consideration threshold for qualitative PE is >0.1% peak intensity

total number of detected peaks:	884
peaks corresponding to the placebo or formulation:	-185
Ions smaller that the core structure of the active:	-20
peaks that are not always > LOD:	-454
peaks that are not always > 0.1% intensity threshold:	-116
peaks detected in all 9 originator and biosimilar batches:	109
peaks excluded by MS/MS data evaluation:	-3
peaks that have to be evaluated:	106

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Evaluation of the qualitative pharmaceutical equivalence

106 peaks analysed

68% → 72 Peaks always >0.1% in both products

24% → 25 Peaks always >0.1% in one and >LOD in the other product

5% → 6 Peaks always >0.1% in one and at least once >LOD in the other product

3% → 3 Peaks always >0.1% present in only one of the products

- 2 peaks are only present in the originator. → efficacy concern?
- 1 peak is only present in the biosimilar. → safety concern

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Quantitative pharmaceutical equivalence

For the quantitative PE peaks with >1% have to be considered:

- qualitative pharmaceutical equivalence (>0.1%): 106 peaks
- quantitative pharmaceutical equivalence (>1%): 23 peaks

CG-FID and LC-MS methods were validated for all 23 peaks:

- spiking experiments with standards for known compounds: 10 peaks
- experiments with different product concentrations for unknowns: 13 peaks

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Acceptance criteria

Acceptance criteria from USP for the known compounds in the drug product:

Compound:	% related to the sum of three		mid point:
	USP limit for the API	proposed equivalence criteria for the drug product	
1	2.5 – 9.5	2.3 – 10.3	
2	0 – 2.25	0 – 2.4	1.2
3	13.5 – 19.5	12.4 – 21.1	
4	0.5 – 4.0	0.5 – 4.3	2.4
5	0 – 3.25	0 – 3.5	1.75
6	0 – 2.75	0 – 3.0	1.5
7	52.5 – 61.5	48.3 – 66.4	
8	22.5 – 30.5	20.7 – 32.9	
9	0 – 6.25	0 – 6.8	3.4
10	0 – 5.5	0 – 5.9	2.95

The acceptance criteria (ranges) of the minor compounds were take to define criteria for the unknown peaks.

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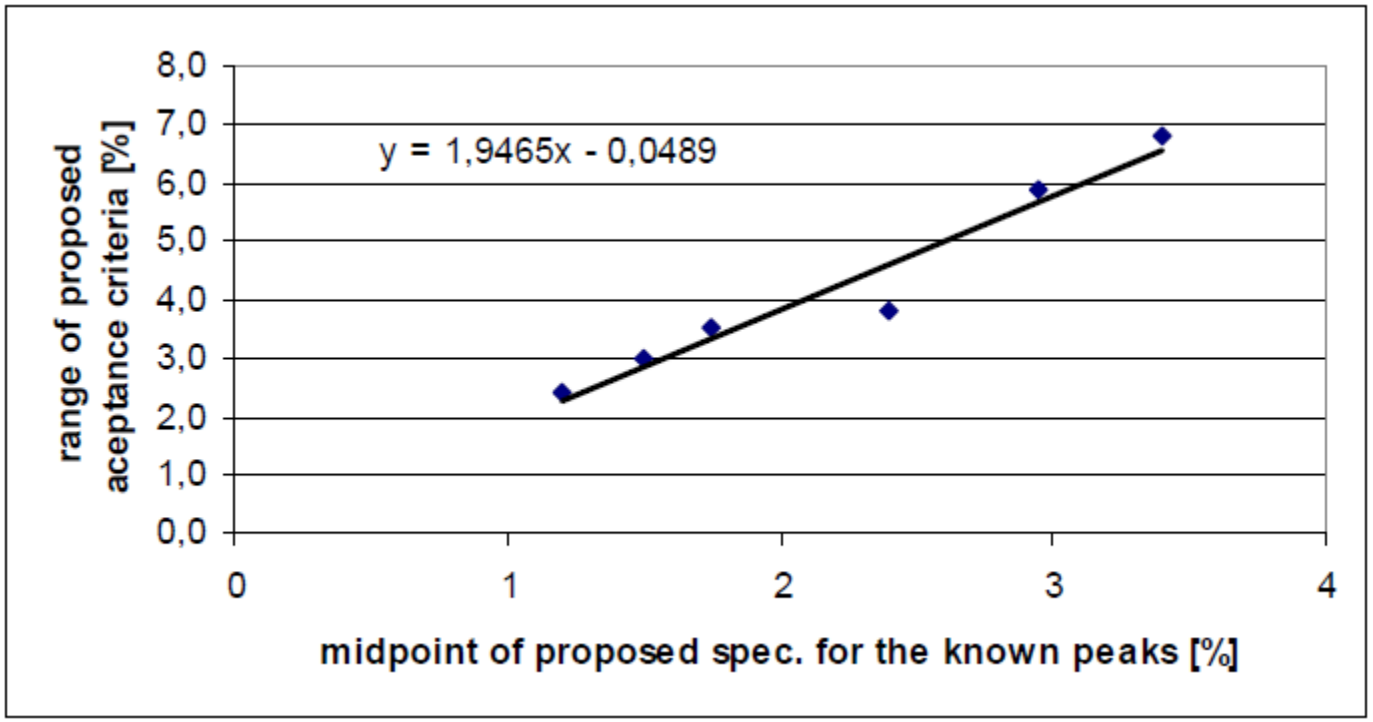
Correlation of range and mid point



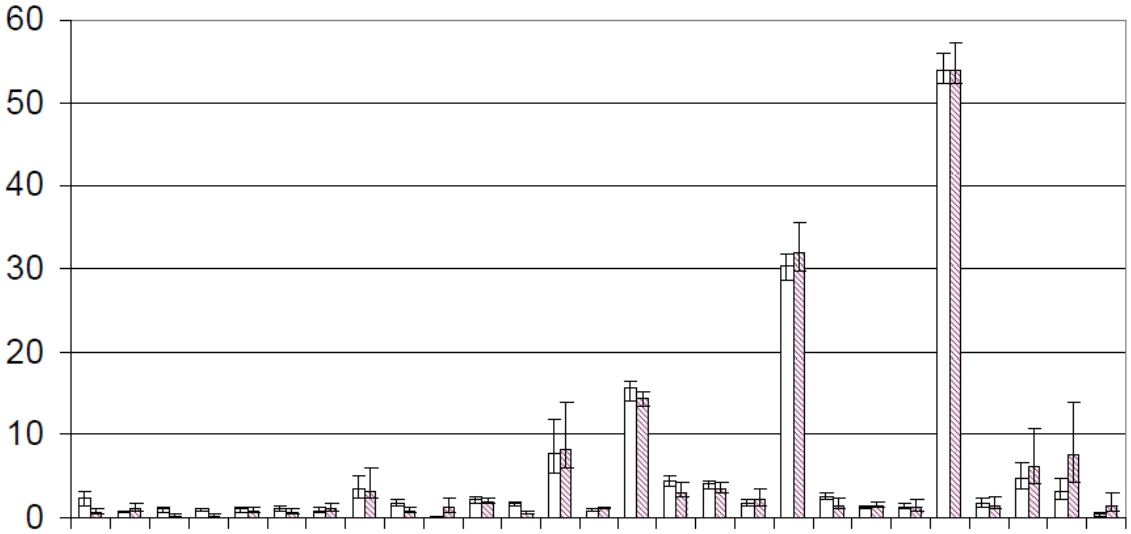
The graph will be used to define the ranges (acceptance criteria) for the minor unknown compounds.

Calculation of the ranges and acceptance criteria for unknown compounds

The mean intensities of the 13 unknown components were established in the 9 testes originator batches

mean intensity in originator	**calculated range**	**calculated equivalence criteria**
1.3	2.4	0.1 – 2.5
3.6	6.9	0.1 – 7.0
1.6	3.0	0.1 – 3.1
1.3	2.4	0.1 – 2.5
3.4	6.5	0.1 – 6.7
1.0	2.0	0.1 – 2.0
1.8	3.5	0.1 – 3.6
1.2	2.4	0.1 – 2.4
0.9	1.7	0.0 – 1.7

Evaluation of the quantitative pharmaceutical equivalence



▨ mean area % of the originator

□ mean area % of the biosimilar

➡ All but 3 peaks show quantitative pharmaceutical equivalence!

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Conclusion

- It is possible to conduct a comparability study even with very complex products.
- Mass spectrometry is the best technique to perform comparability exercises.
- Sophisticated software tools are needed to handle high-content data.
- In future we will see more mAb biosimilars entering the market.

- Uncertain profitability of mAb biosimilars due to clinical test.
- Acceptance with medical professionals and patients (interchangeability).

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