BioEquality

A Platform for the Comprehensive Analysis of Data from Stability Studies and Market Approval of Biosimilars

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A&M STABTEST GmbH

# The Impact of Biopharmaceuticals on the Pharmaceutical Market

- In 1982 Eli Lilly introduced Humulin, the first biotechnologically produced drug substance

- In 1992 Epogen (epoetin alfa) made Amgen the first Fortune 500 biotech company

- In 2005 the per capita spending for biopharmaceuticals was 119 USD (12.9%)

- It is estimated that by 2016 eight of the top ten drugs marketed world wide will be a biopharmaceutical
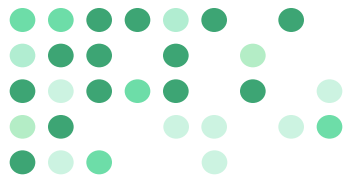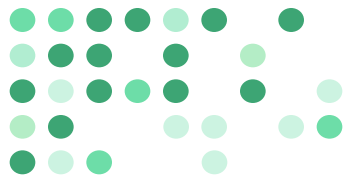
The Efficacy of a Biopharmaceutical is Structure-Dependent

- 3D structure (protein folding, oligomerisation)

- Amino acid sequence

- Post translational modifications

- Binding to co-factors

- Artificial drug-conjugates

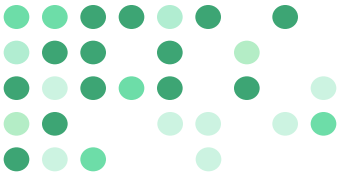 ➡ Efficacy
  ➡ Quality
  ➡ Safety
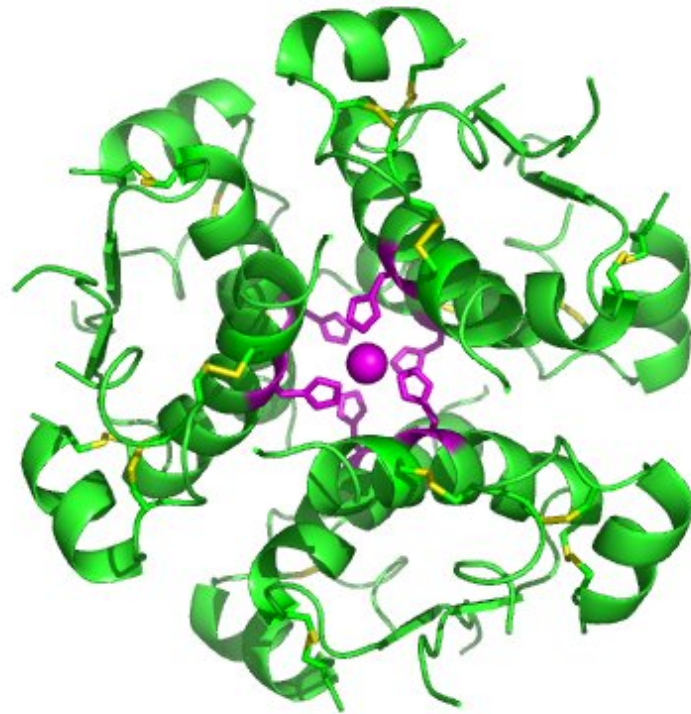
Insulin hexamer (inactive storage form)

Guidelines Regarding Biopharmaceuticals

- Guideline on the Development, Production, Characterisation and Specifications for Monoclonal Antibodies and Related Products. (EMEA/CHMP/BWP/157653/2007)

- Guideline on Requirements for the Quality Documentation Concerning Biological Investigational Medicinal Products in Clinical Trial (Draft EMA/CHMP/BWP/534898/2008)

- Comparability of Biotechnological/Biological Products - ICH Topic Q 5 E (CPMP/ICH/5721/03)

- Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products - ICH Topic Q 5 C (CPMP/ICH/138/95)

- Specifications: Test Procedure and Acceptance Criteria for Biotechnological/Biological Products - ICH Topic Q 6 B (CPMP/ICH/365/96)

➡ All guidelines demand an in-depth characterisation of the product?

What Techniques are the Quality of Biopharmaceuticals?
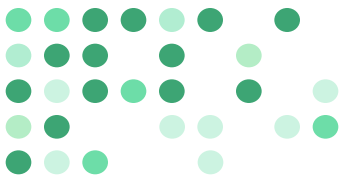
Structural Testing:

- Oligomerisation: SEC-HPLC, native gel electrophoresis

- Protein folding: SEC- and CEX-HPLC, IEF-CE, IEF-GE

- Protein sequence & modifications: RP-HPLC and peptide maps

➡ BUT what information can be obtained from mass spectrometry?

Structural & Efficacy Testing:
- Protein binding: Bioassays (ELISA)

Mass Spectrometry

Mass spectrometry has become a widely used tool in analytical sciences. Modern mass spectrometers are robust and sensitive instruments that can be used in routine analysis.

➡ LTQ-Orbitrap is an ideal instrument for protein characterisation:

- Low resolution spectra for molecular weight determination

- High resolution and accurate mass spectra for

    - Peptide mass maps
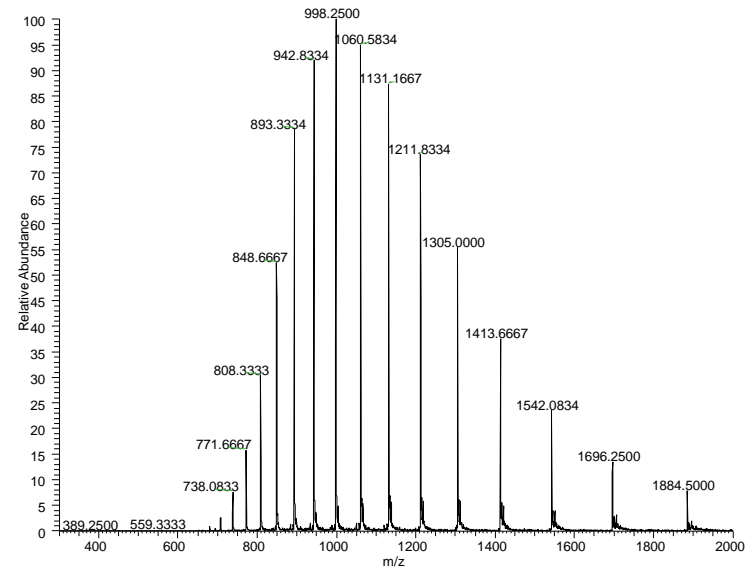
    - Identification and localisation of modifications

Both approaches are needed to get a full picture of the protein

→ Top-Down approach:

- Molecular weight

- *N*- and *C*-terminal processing

- Degradation

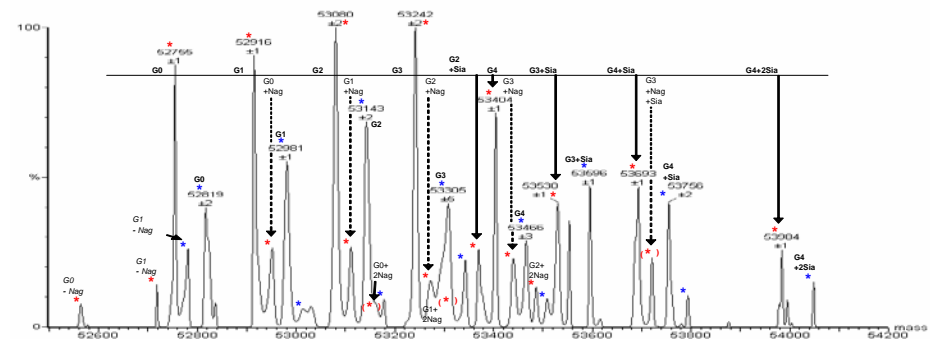- Glycosylation / glycation

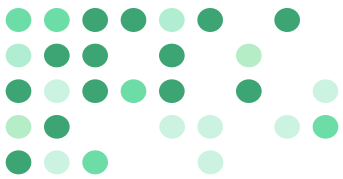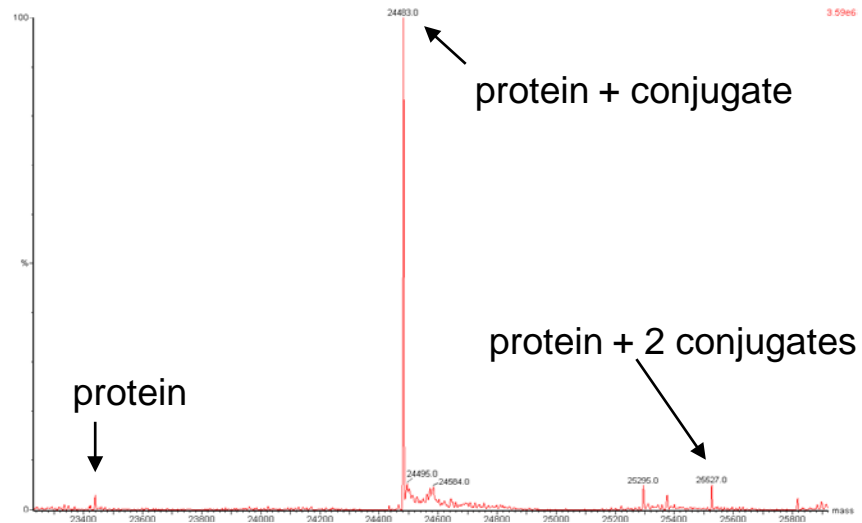- Degree of conjugation

Full MS of Myoglobin

➡ Top-Down approach:

- Molecular weight

- *N*- and *C*-terminal processing

- Degradation

- Glycosylation / glycation

- Degree of conjugation

Deconvoluted spectrum of FAB fragment

➡ Top-Down approach:

- Molecular weight

- *N*- and *C*-terminal processing

- Degradation

- Glycosylation / glycation

- Degree of conjugation

Deconvoluted spectrum of a conjugated protein

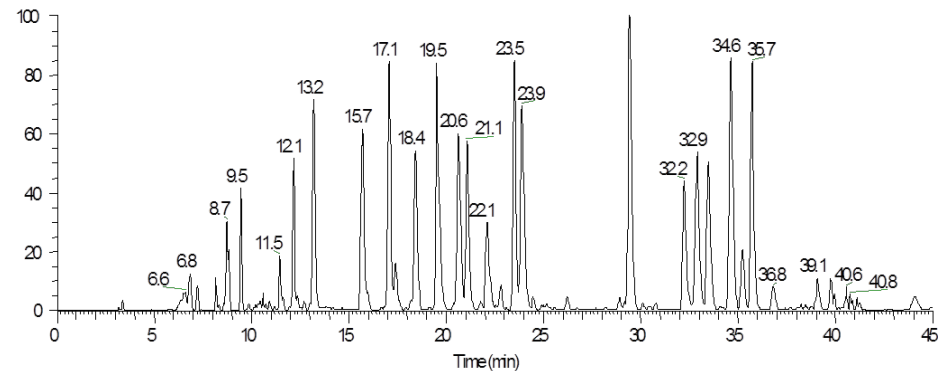➡ No need for accurate mass and high resolution!

Both approaches are needed to get a full picture of the protein

➡ Bottom-Up approach:

- *N*- and *C*-terminal processing

- Location of glycosylation

- Conjugation sites

- Disulfide bonds

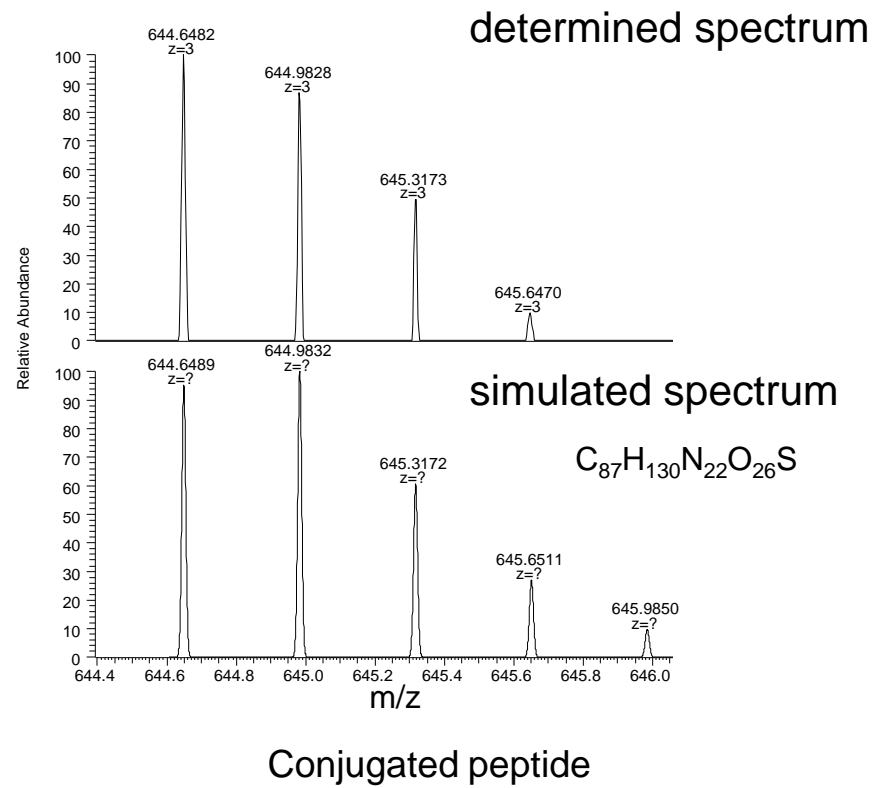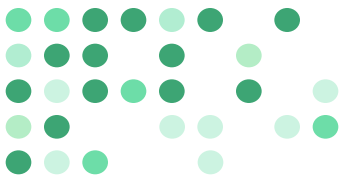- Small modifications

Peptide mass map

Both approaches are needed to get a full picture of the protein

➡ Bottom-Up approach:

- *N*- and *C*-terminal processing

- Location of glycosylation

- Conjugation sites

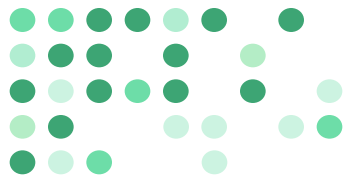- Disulfide bonds

- Small modifications

determined spectrum

644.6482
z=3

644.9828
z=3

645.3173
z=3

645.6470
z=3

simulated spectrum

$C_{87}H_{130}N_{22}O_{26}S$

644.6489
z=?

644.9832
z=?

645.3172
z=?

645.6511
z=?

645.9850
z=?

Relative Abundance

m/z

Conjugated peptide

➡ Bottom-Up approach:

- *N-* and *C*-terminal processing

- Location of glycosylation

- Conjugation sites

- Disulfide bonds

- Small modifications

Peptide 1

Peptide 1+H_2O

Peptide 1-TH

Peptide 1-TH+H_2O

Time (min)
70 71 72 73 74 75 76 77
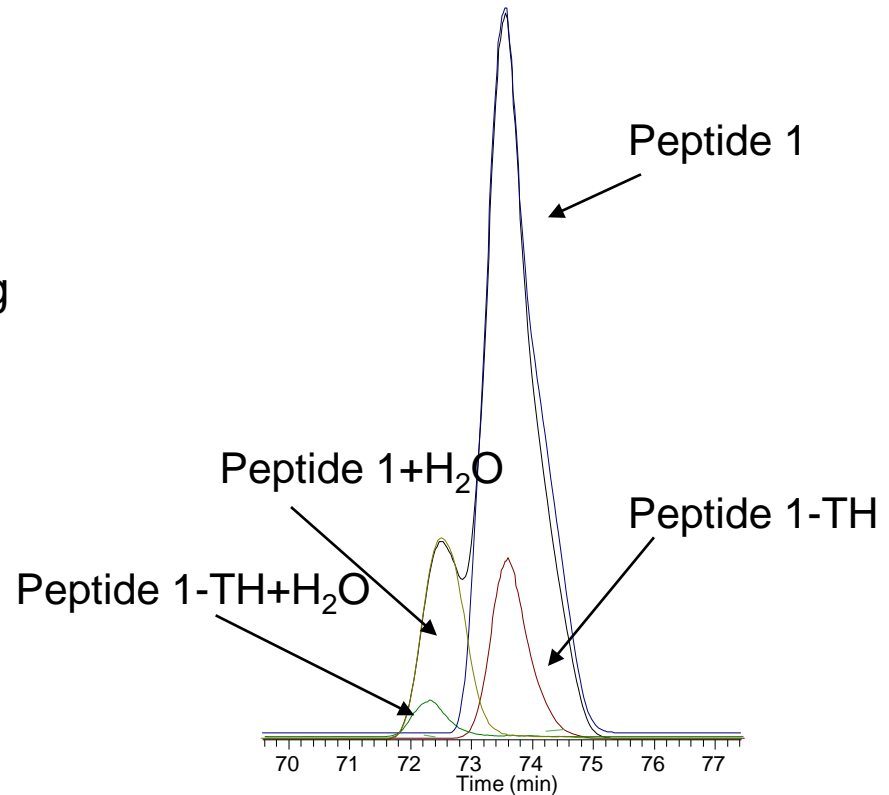
Smaller modifications in a conjugated peptide

➡ Accurate mass and high resolution are helpful!

molecular weight

conjugation degree

disulfide bonds

small modifications

glycostructure

protein degradation

glycosylations sites
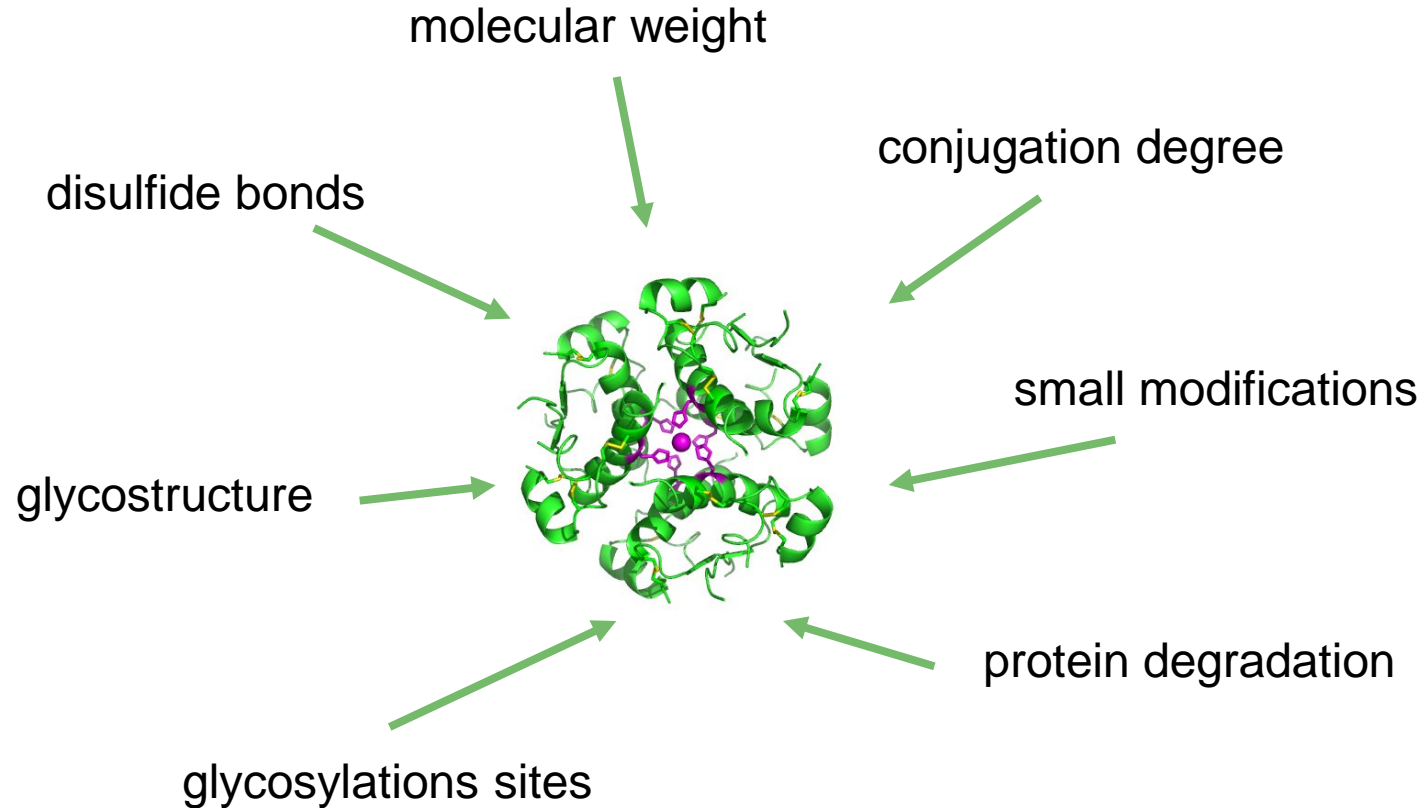
Each peak in the peptide map can contain several signals of different peptides and their modified versions

Software solutions are needed which evaluate the chromatogram and the spectra
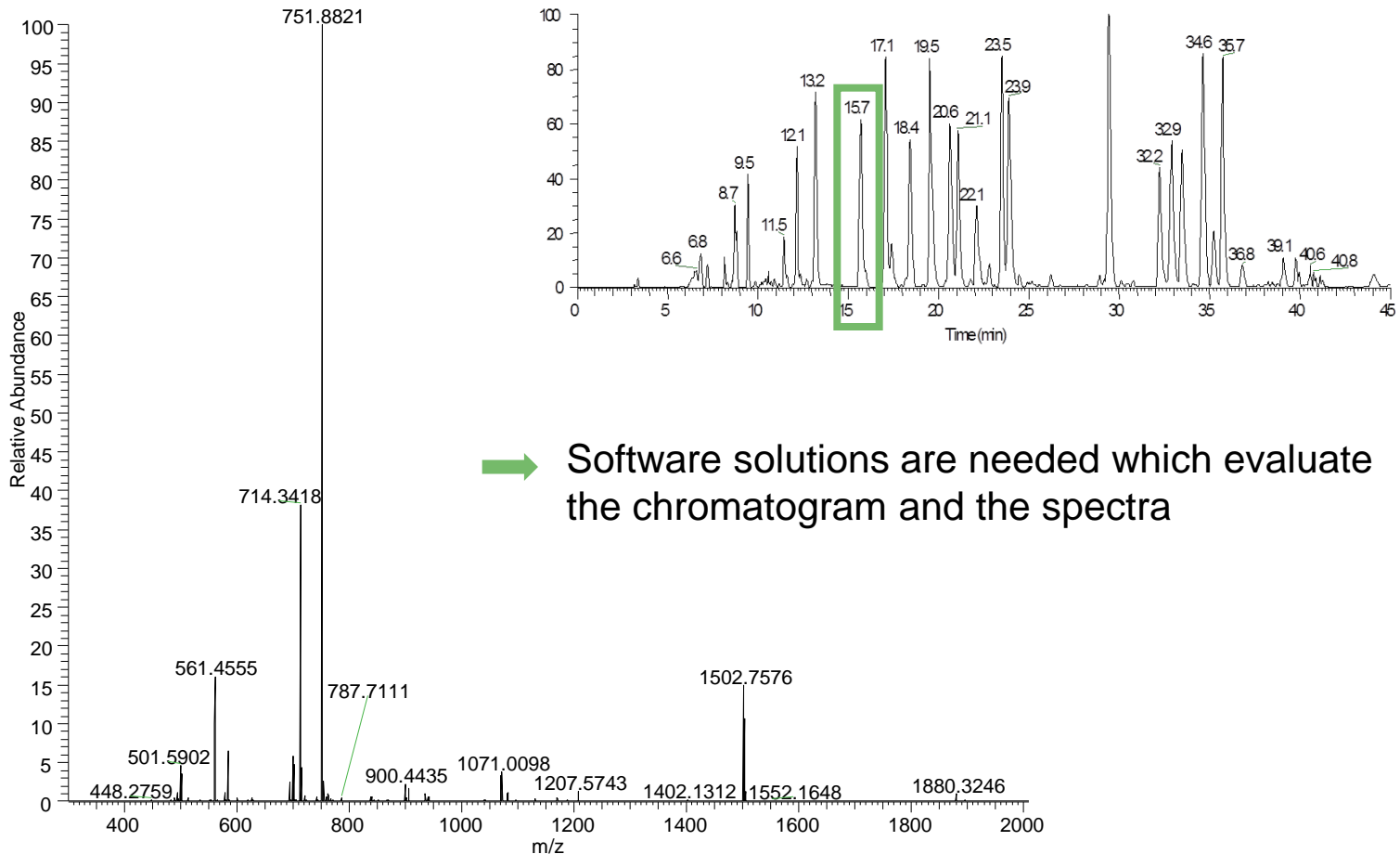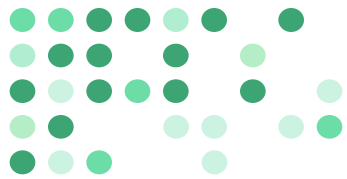
MassMap® is such a Software Solution

The MassMap® software was designed by Prof. Dr. Wozny to analyse peptide mass maps in the pharmaceutical environment
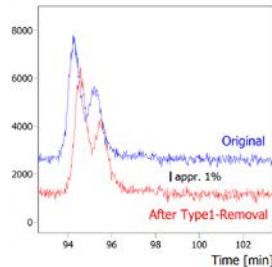
- Reduces electronical and chemical noise (reduces file size)

- Analyses signals down to the 1% level (compared to base peak)

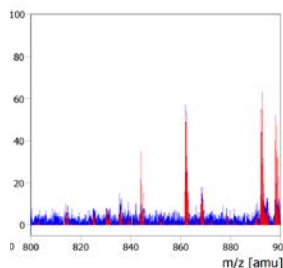- Is fully 21 CFR Part 11 compliant
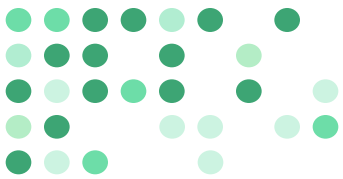
The first step is to reduce the data by removing unspecific signals:
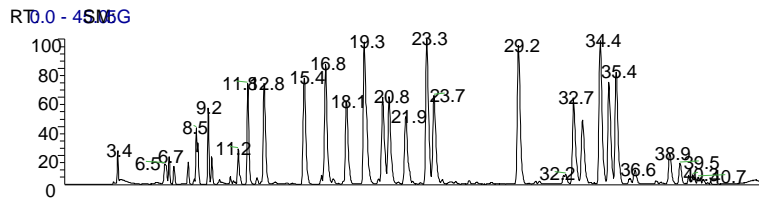
Type-I filtering

Type-II filtering

<u>Original PepMap Data:</u>
- Enormous file size (e.g. 2 GB)
- Difficult to handle

<u>Removal of type-I signals:</u>
- Chemical noise
- Ions that appear statistically over the whole run
- No chromatographic peaks
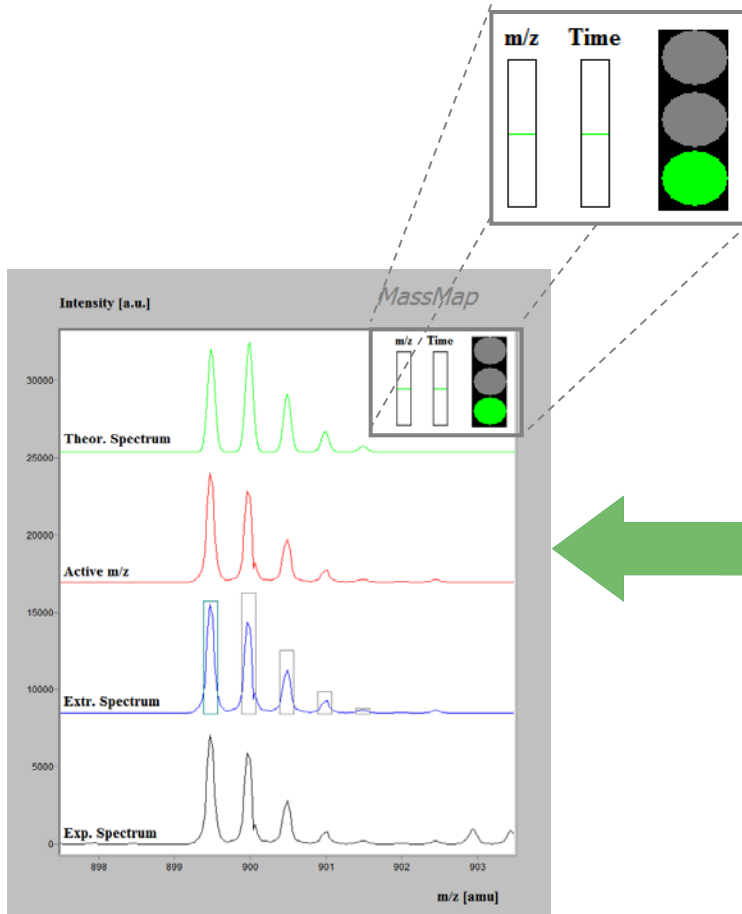- Improvement of s/n ratio

<u>Removal of type-II signals:</u>
- Ions that do not have isotopic peak patterns
- Improvement of s/n ratio
→ Three-fold reduction of data size (~ 700 MB)
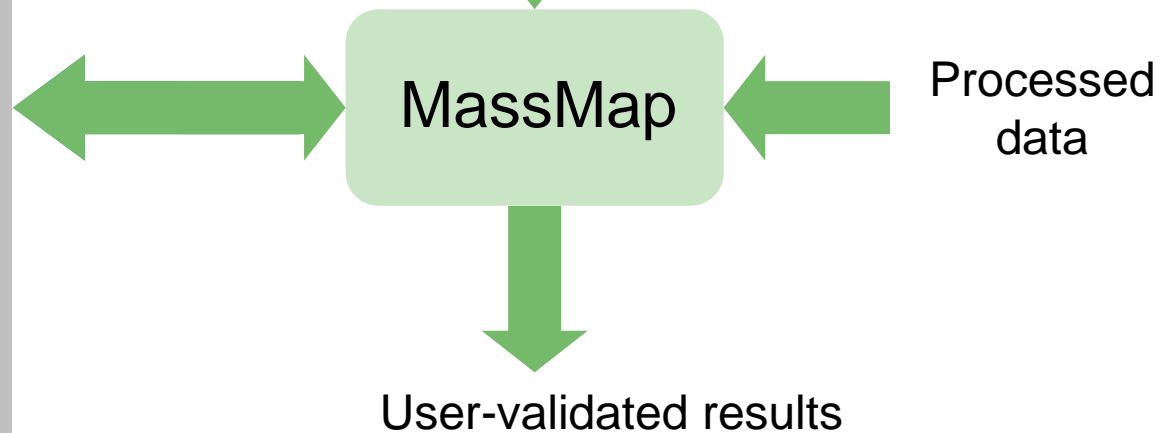
Identification of Known Signals

Evaluation of automated identification:

Amino acid sequence:

Calculation of peptides and modifications

...
HC02,   2323.991834,   2325.6075,   LFSFYSCDASGAYWTYMVR
HC02_oxM,   2339.986734,   2341.6069,   LFSFYSCDASGAYWTYMVR
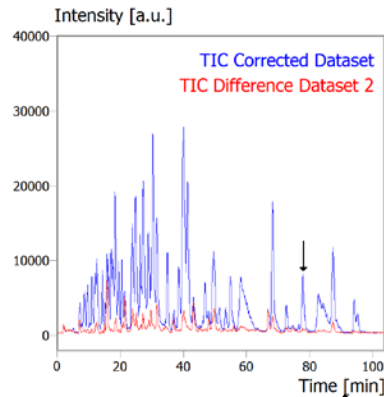...

MassMap

Processed data

User-validated results

Identification of Unexpected Signals

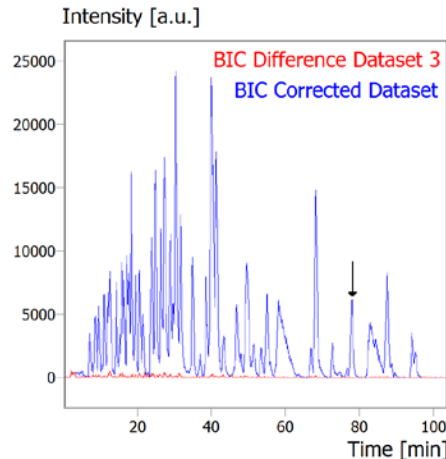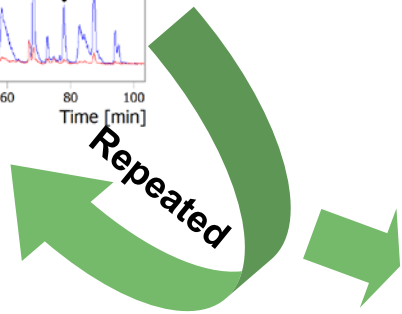Problem: Unexpected signals often have low intensities

User-validated results

Identified peptides and their modifications are removed

Background subtracted ion chromatogram (BIC):
- Subtraction removes all identified peaks
- Ions with low intensities are now visible

Repeated

Sequential analysis:
From most intensive ion to low intense unexpected ions e.g. impurities

# Result: A list of Identified Peptides with Modifications and Unknowns

➡ List of identified peptides

Signal	Lage [min]	Intens. [a.u.]	Anteil [%]
HC22_A2FG0	18,22	635333577	47,68
HC22_A2FG0-GlcNAc	18,43	33841885	2,54
HC22_A2FG1	18,05	396289612	29,74
HC22_A2FG1-GlcNAc	18,32	13836232	1,04
HC22_A2FG1S1	n.b.	n.b.	n.b.
HC22_A2FG2	17,94	47228329	3,54
HC22_A2G0	18,54	50348887	3,78
HC22_A2G0-GlcNAc	18,66	40000810	3,00
HC22_A2G1	18,43	21931273	1,65
HC22_A2G1-GlcNAc	18,60	18168890	1,36
HC22_A2G1S1	n.b.	n.b.	n.b.
HC22_A2G2	n.b.	n.b.	n.b.
HC22_A2G2S1	18,91	36990166	2,78
HC22_A2G2S2	n.b.	n.b.	n.b.
HC22_Man5	18,43	38047543	2,86
HC22_Man6	18,43	470342	0,04
HC22_deglyc	n.b.	n.b.	n.b.
...	...	...	...

➡ Chromatogram with remaining signals
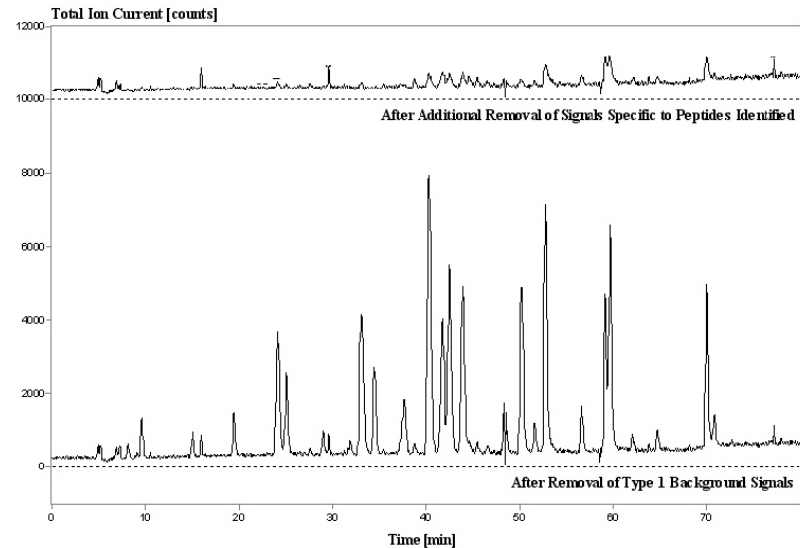
- Peptides with unknown modifications
- Impurities (not protein related)
- Impurities (HCP)
- Degradation products
- Digestion artifacts
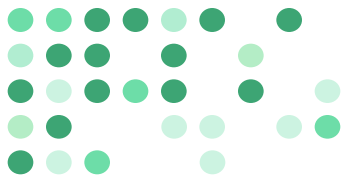
Comparative Analysis of Peptide Mass Maps

- Guideline on Requirements for the Quality Documentation Concerning Biological Investigational Medicinal Products in Clinical Trial (Draft EMA/CHMP/BWP/534898/2008)

  ➡ Comparability of batches used in clinical trials

- Comparability of Biotechnological/Biological Products - ICH Topic Q 5 E (CPMP/ICH/5721/03)

  ➡ Comparability after changes in the manufacturing process

Biosimilars (generic versions of biopharmaceuticals)

By the year 2015 biopharmaceuticals with a market share of 64 billion USD will lose their patent protection!

- Guideline on Similar Biological Medicinal Products Containing Biotechnology-Derived Proteins as Active Substance: Quality Issues (EMEA/CHMP/BWP/49348/2005)

  ➡ Comparability to the market product (originator)
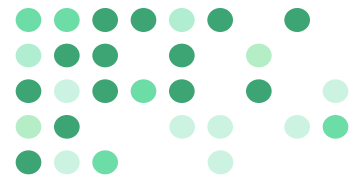
Semi-Quantitative View on PepMaps

MassMap® also has the capability to give relative-quantitative pep map results:
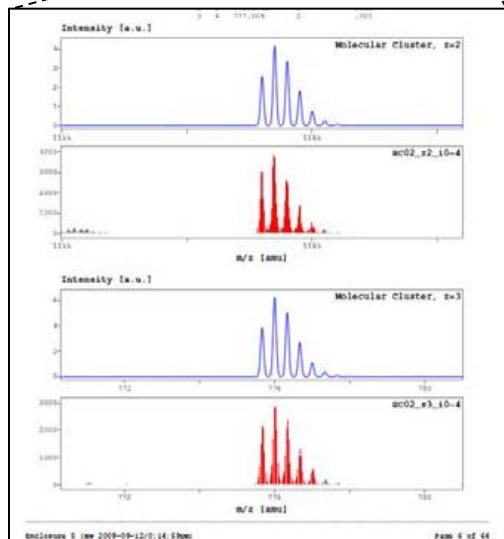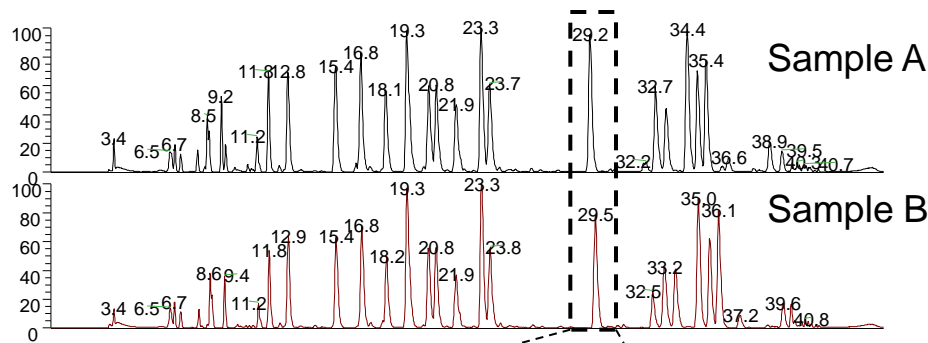
Specific Ion Current chromatogram (SIC):
- Analysis of complete isotope patterns
- Only takes matching isotope patterns (simulated vs. measured)
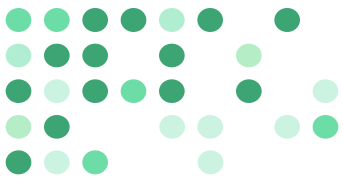- Achieves higher selectivity that normal EIC´s
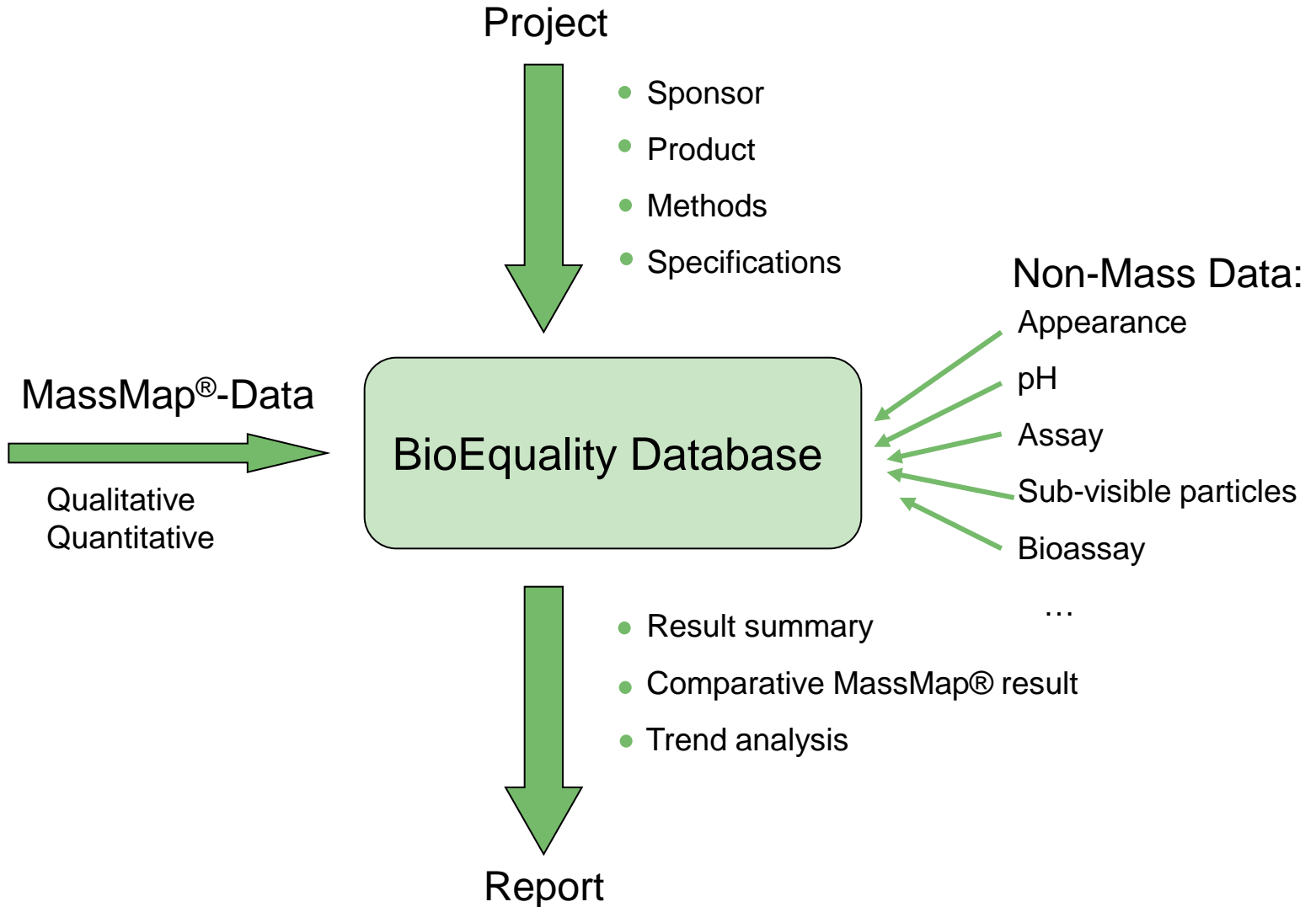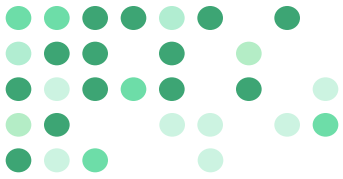
Quantitative report

What if one wants to …

   …compare different batches of an originator to the biosimilar?

   …compare not only the drug substance but the whole product?

   …see a trend in comparative data over the complete product history or
     during stability studies?

The BioEquality Data Base

Project

- Sponsor
- Product
- Methods
- Specifications

Non-Mass Data:

Appearance

pH

Assay

Sub-visible particles

Bioassay

…

MassMap®-Data

BioEquality Database

Qualitative
Quantitative

- Result summary
- Comparative MassMap® result
- Trend analysis

Report

A&M
LABOR FÜR ANALYTIK

Generating a new Project

Project Overview

Projects [ST11-000 A&M Pille]

Study Number	ST11-000
Project Type	Stability
Status	Draft
Study Director	Diane Kleinjohann
Sponsor	A&M STABTEST
Drug Product	A&M Pille
Drug Substance	
Packaging	Fertigspritze
Dosage Form	Injektionslösung
Comment	
Author	Jana Spura
Created at	2011-09-13 14:10 by Jana Spura
Last Updated	2011-09-13 14:17 by Jana Spura
Batches	• 12345

The new project has not been reviewed

- One cannot review their own data

- Incorrect submissions are not checked and commented

- The initial author will see the incorrect data and the reviewer's comment

- An overview of the storage condition 25°C/60% r.h.

- Display of pulling points

- Summary of the tests to be performed per pulling point
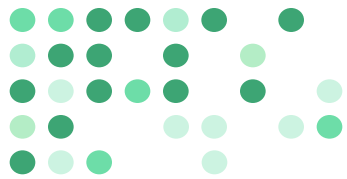
Pepmap Results

The report contains a summary of the most important results:

BioEquality

Projects [ST10-065 Creon 3000] » PepMap Result Report

Overview   Batches   Parameters   Result Report   **PepMap Result Report**   AuditTrail

| Batch	Testing Point	Run #	Molecule	Specification	Theoretical Monoisotop. Mass [amu]	Delta Corr Abs [u]	Delta Corr Rel [ppm]	Intensity	Relative Intensity [%]	Ret. Area [min]
Storage Condition: 40°C / 75% rel. F.										
810484	month 1	1	BE_1	area < 6.0 %	372.1974	-0.0131		16657695	99.1463	24.9
			BE_1							
			BE_2_1	area < 3.0 %	342.2734	-0.02		73300	0.4362	25.0
			BE_2_1							
			BE_2_2							
			BE_2_2	area < 6.0 %	342.2734	-0.0393		70122	0.4173	25.0
	month 1	2	BE_1	area < 6.0 %						
			BE_1		372.1974	-0.0131		16657695	99.1463	24.9
			BE_2_1	area < 3.0 %						
			BE_2_1		342.2734	-0.02		73300	0.4362	25.0
			BE_2_2		342.2734	-0.0393		70122	0.4173	25.0
			BE_2_2	area < 6.0 %						

Trend Analysis (still to come)

A trend analysis for all parameters with specifications will be available for all tests conducted in the project.

For Pepmap-data this will include a trend for all identified peptides
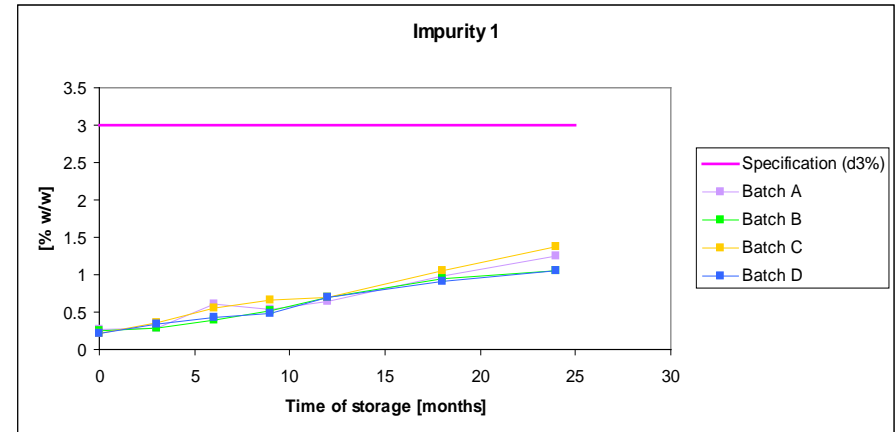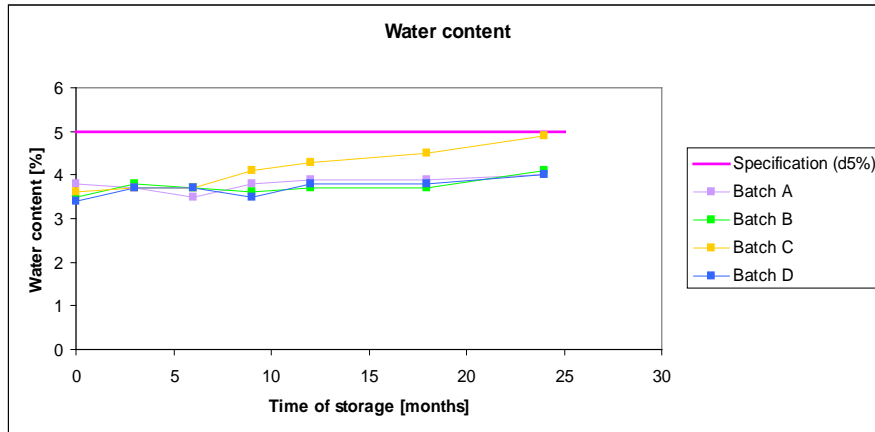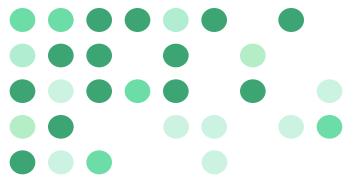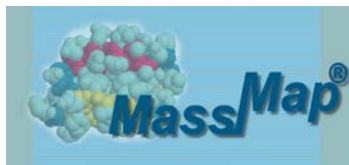
The People Involved in the BioEquality Project

Mass Spectrometry & Stability Studies

- Dr. Lejon Martens
- Dr. Jana Spura
- Diane Kleinjohann

Programming of the BioEquality Software

- Christian Ebeling
- Angela Rumpl
- Stephan Springstubbe

Support with MassMap® Data Import

- Prof. Dr. Manfred Wozny