THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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2024 EDQM virtual training programme:

Independent modules on European Pharmacopoeia texts related to Biologicals and on Microbiology chapters

(Live Webinars)
Date: 30 January 2024 – 01 February 2024

Module 3
Ph. Eur. Reference standards for biologicals

31 January 2024

Module 3: Ph. Eur. Reference standards for biologicals

31 JANUARY 2024 - 14:30-16:00 (CET, FRANCE)

Ph. Eur. Reference Standards for biotherapeutics: intended use in physico-chemical tests

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Head of the Biology Section
Laboratory Department

Introduction

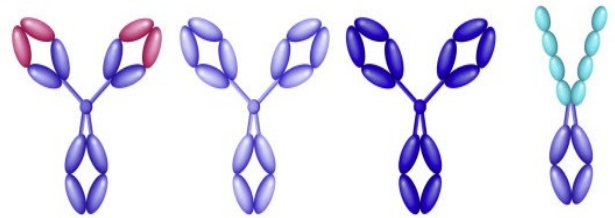
Background information:

→ Reference standards – General aspects (Andrea Lodi)

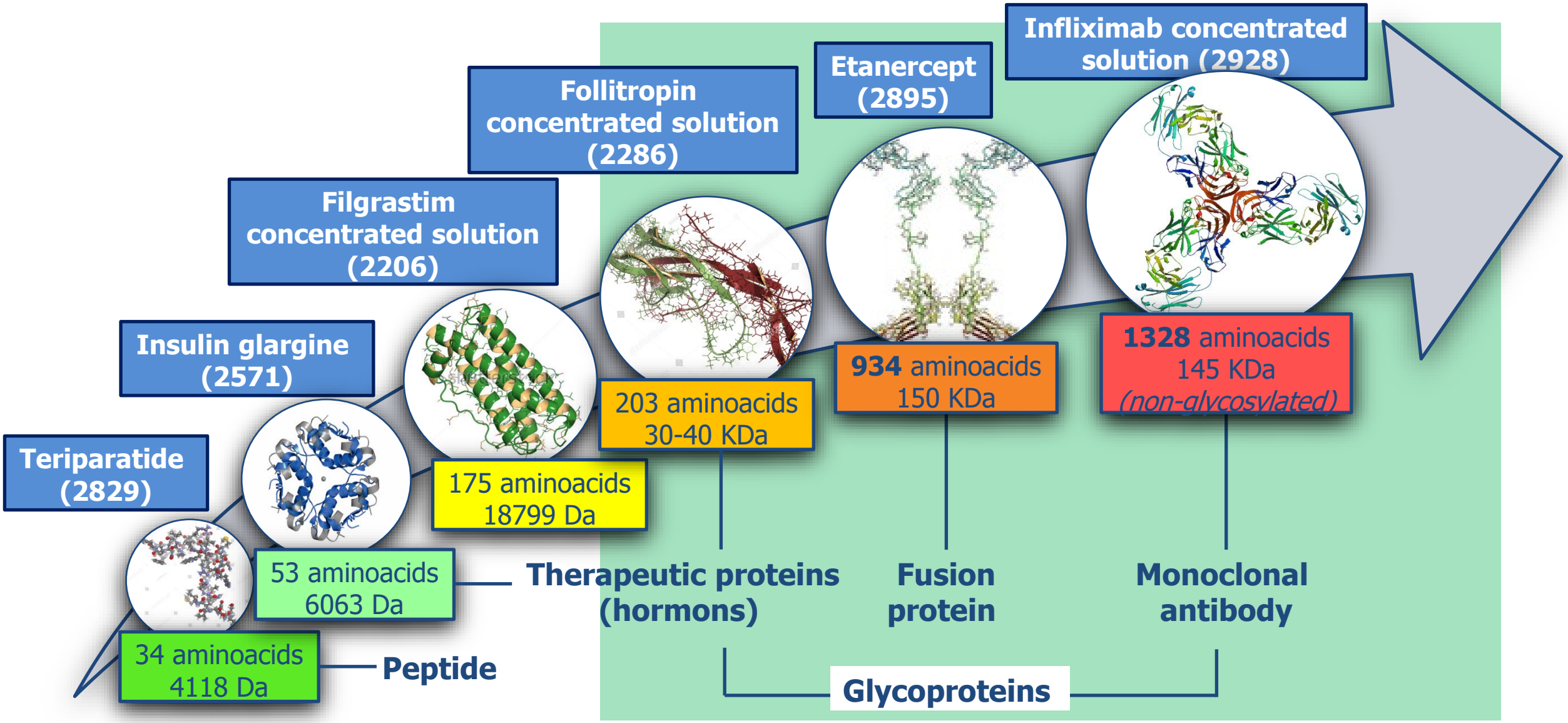
→ Ph. Eur. general chapter 5.12.

Outline:

- Intended use of CRS in individual monograph for biotherapeutics – Examples
  - Qualitative
  - Quantitative
- What's next?
- Take home messages

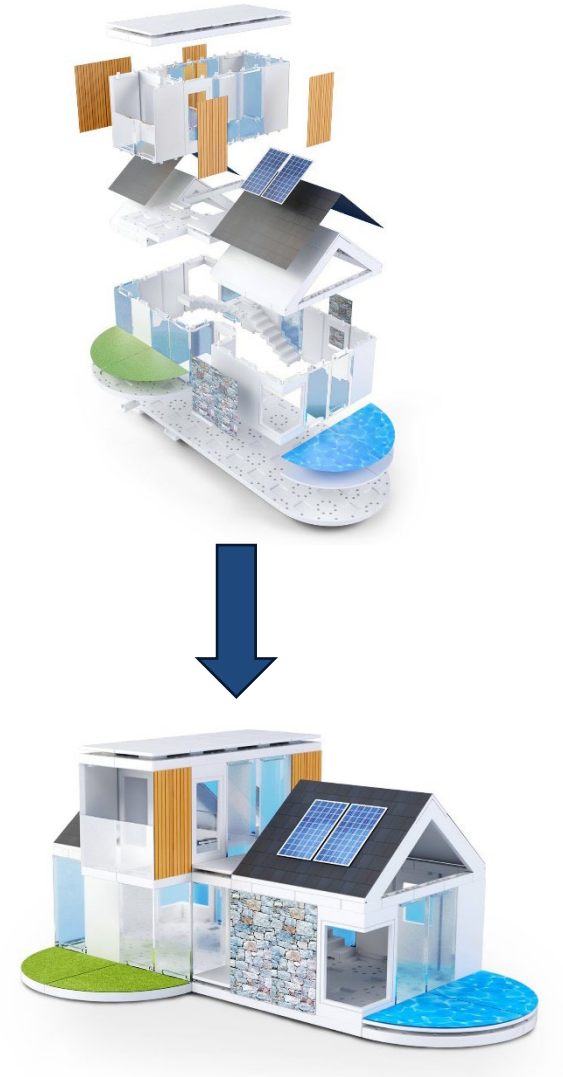


Ph. Eur. monographs for Biotherapeutics

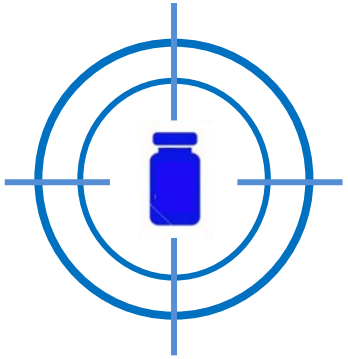


Ph. Eur. monographs for Biotherapeutics

Quality attribute	Ph. Eur. Reference standard
Host-cell-derived proteins	✗
Host-cell- and vector-derived DNA	✗
Residual protein A	✗
Glycan analysis	✓
Charged variants (e.g. capillary IEF)	✓
Peptide mapping (primary structure)	✓
pH	✗
Related proteins	✓
HMM and LMM species (e.g. SEC)	✓
Protein content (e.g. UV)	✗
Potency, biological activity	✓



Ph. Eur. RS classification by intended use



- **Qualitative purpose**
  - **identification** of the substance subject of a monograph
  - **identification** of impurities
  - **system suitability**

    to verify that a measurement system is operated within the boundaries of its validation scope

- **Quantitative use**
  - quantitative determination of the substance subject of the monograph
  - **assigned content**

- **the intended purpose(s) of a CRS is described in a Ph. Eur. monograph**
- **CRS are not intended to be used as reference (comparator) products in the context of applications for biosimilars**

Qualitative use - Examples

Identification of the substance subject of a monograph

Ex. 1

- The elucidation of structure, which involves extensive characterisation of the substance using for ex. mass spectrometry is part of the regulatory filing, not part of testing in a monograph

- **Ph. Eur. general notices**: the tests given in the Identification section are:
  - not designed to give full confirmation of the chemical structure or composition of the product
  - intended to give confirmation, with an acceptable degree of assurance, that the article conforms to the description on the label

  -> Identification is not structure elucidation

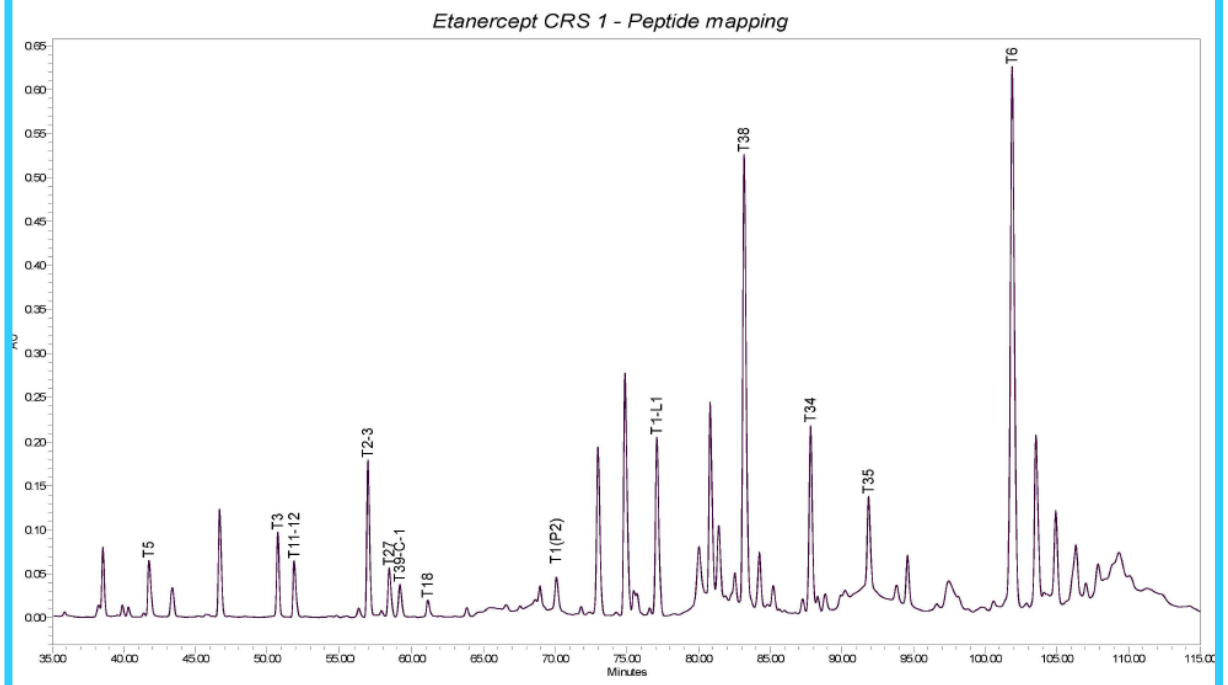
Ph. Eur. CRS for peptide mapping

Peptide mapping:

- involves enzymatic or chemical treatment to form peptide fragments (at specific cleavage sites) that are separated (e.g. by LC) and identified
- fingerprint of a protein
- comparative procedure with CRS: by comparing the info obtained with a CRS treated similarly, the primary structure (sequence) of the protein can be confirmed and alterations can be detected

Etanercept CRS - Peptide mapping

Means: CRS for system suitability and peak identification

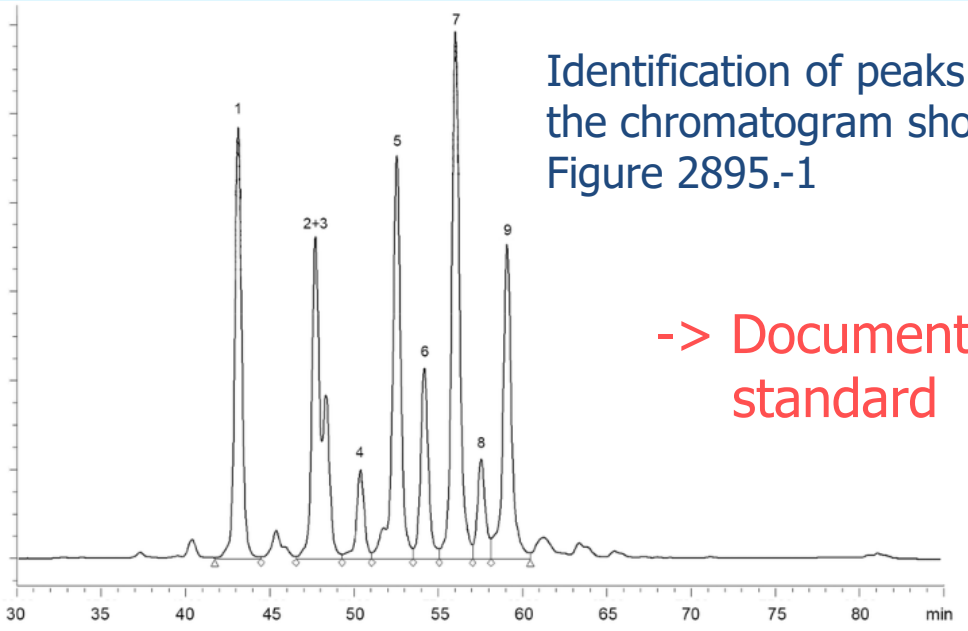
Etanercept monograph – Peptide mapping	Etanercept CRS
• System suitability: - the chromatogram **obtained** with Etanercept CRS is qualitatively similar to the chromatogram **supplied** with Etanercept CRS • Results: the profile obtained with the test solution corresponds (in retention times, peak responses, number of peaks, overall elution pattern) to that obtained with the reference solution	

Confirmation of identity of the analytical target

Ex. 2

Means: chromatogram included in the monograph

Etanercept monograph 2895 – Glycan mapping



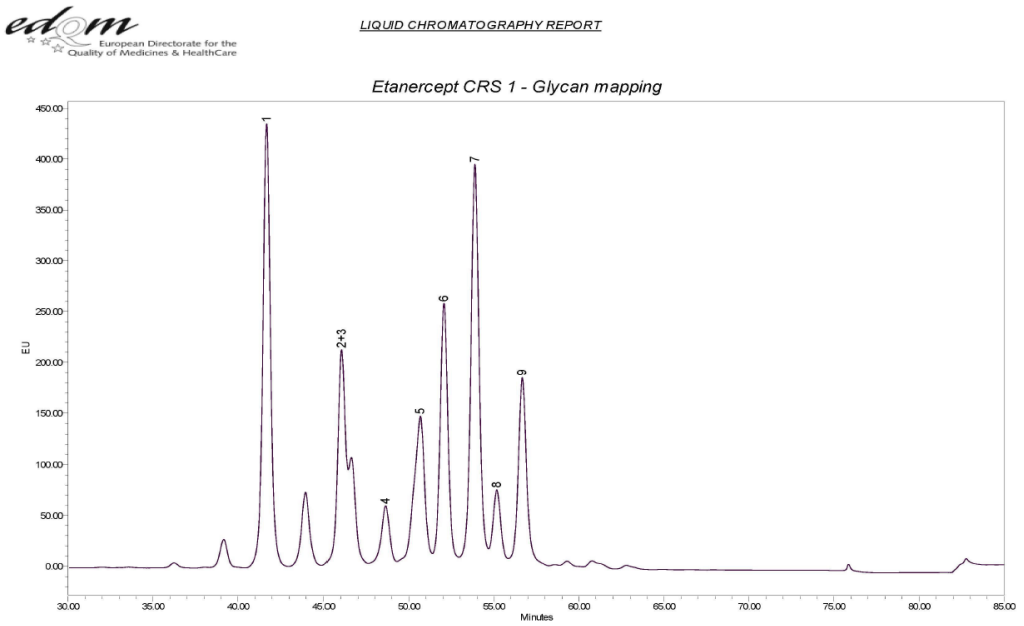
Identification of peaks: use the chromatogram shown in Figure 2895.-1

-> Documentary standard

Peak	Charged	Structure	Peak	Charged	Structure	Peak	Charged	Structure	Peak	Charged	Structure
1.	No	Asialo-, agalacto-, biantennary, core-fucosylated	4.	No	Asialo-, galactosylated biantennary	6.	Yes	Monosialylated-, galactosylated biantennary	8.	Yes	Disialylated, galactosylated biantennary
2+3.	No	Asialo-, mono-galactosylated biantennary, core-fucosylated	5.	No	Asialo-, galactosylated biantennary, core-fucosylated	7.	Yes	Monosialylated-, galactosylated biantennary, core-fucosylated	9.	Yes	Disialylated-, galactosylated biantennary, core-fucosylated

Figure 2895.-1. – *Chromatogram for N-glycan analysis of etanercept*

Etanercept CRS



-> Material standard

Compliance with qualitative requirements

Ex. 3

Means: CRS for system suitability and in-house reference preparation

Infliximab monograph – Glycan mapping

- System suitability:

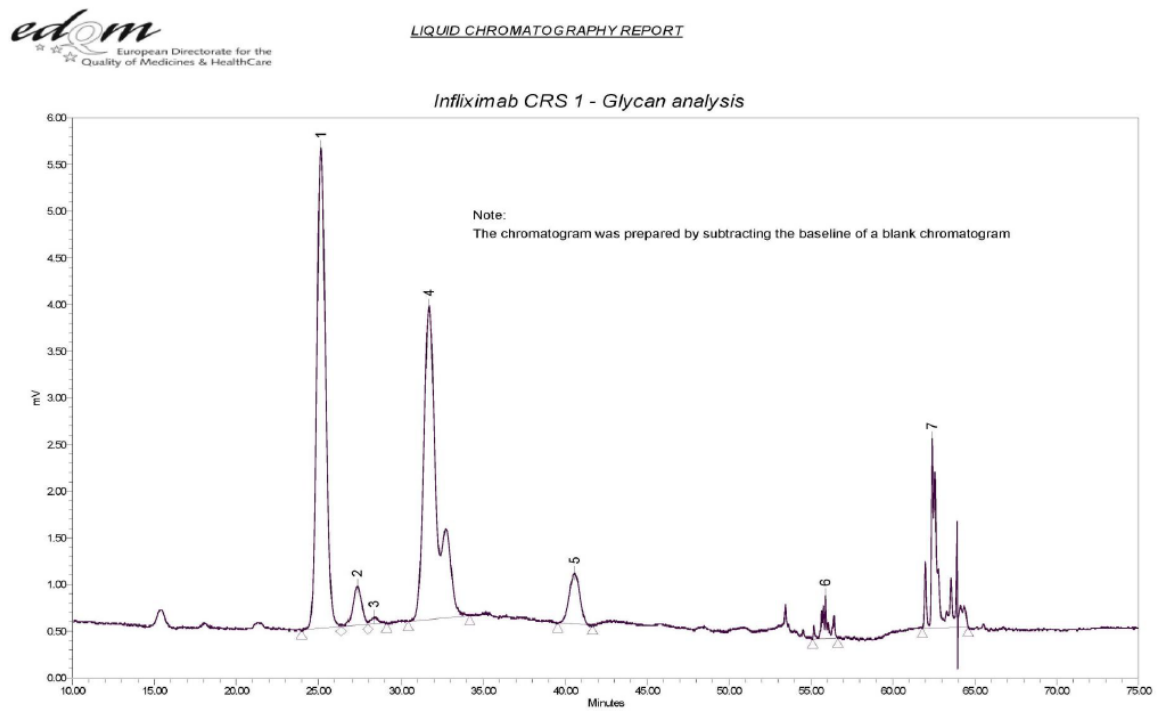
- the chromatogram **obtained** with infliximab CRS is qualitatively similar to the chromatogram **supplied** with infliximab CRS and peaks 1 to 7 are clearly visible

- Results:

- the profile of the chromatogram and the retention times of the peaks obtained with the test solution corresponds to that obtained with the chromatogram obtained with a suitable infliximab in-house reference preparation

-> Consistency of production using a production process specific reference standard

Infliximab CRS



Ph. Eur. CRS Mixtures for rDNA proteins

Ex. 4

Purpose

To assess the system suitability test of chromatographic method (resolution, peak-to-valley ratio)

➡ Complex pattern of related proteins:

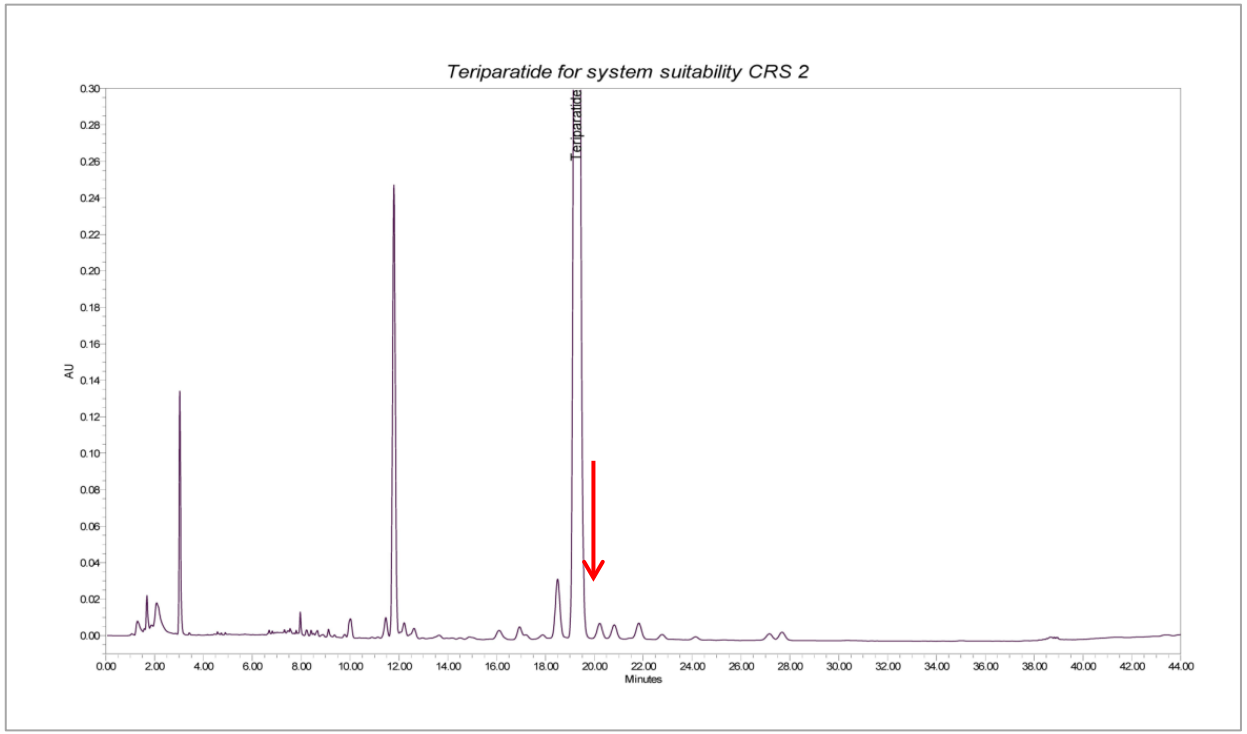
- Deamidation, oxidation, aggregation products:
  - can alter immunogenicity, potency, safety and efficacy of the substance
  - such impurities may be present at low levels in drug substance
- System suitability: need for stressed samples with increased amount of related proteins
- **Ready to use CRS for resolution solutions** are a more robust option than *in situ* degradation solutions prepared by users. The latter may be variable and not necessarily reproducible

CRS Mixtures for rDNA proteins

1) Test for oxidised and deamidated forms

- Teriparatide (2829)

  Resolution solution: incubation of the substance to be examined at 50 °C for 9 days
  -> replaced by *Teriparatide for system suitability CRS*



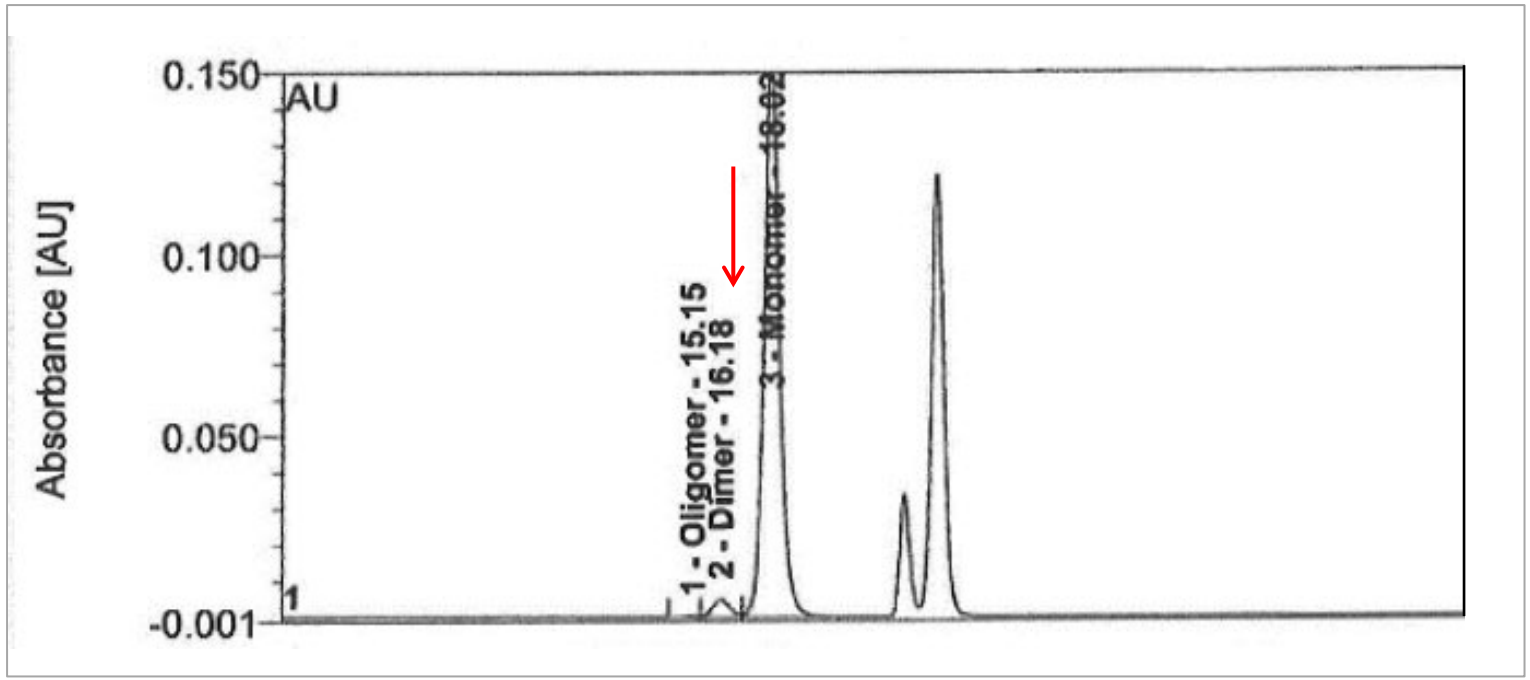
- Other examples: *Somatropin/desamidosomatropin resolution mixture CRS, Interferon gamma-1b for system suitability CRS* with increased deamidated and oxidised forms

CRS Mixtures for rDNA proteins

2) Test for aggregates

- Erythropoietin concentrated solution (1316)

  Introduction of a resolution solution prepared with *Erythropoietin for SEC system suitability CRS* with an increased and defined dimer content



-> impurities present in sufficient amount for peak detection / identification

-> system suitability assessment

SLIDO

- **Where can I find a chromatogram for a CRS?**

  ✓In the leaflet supplied with reference standard
  ✓In the Knowledge database
  ✓by asking in helpdesk

Correct answer in green!

- in leaflets supplied with reference standards if required for their correct use as prescribed in a monograph. Leaflets can be downloaded from the reference standards database
- in the Knowledge database, you may find chromatogram not specific to a CRS. When available and especially when they may prove helpful for interpreting a monograph (e.g. for difficult separations)
- if a chromatogram is not available from one of these 2 sources, the EDQM does not provide it

INFLIXIMAB CONCENTRATED SOLUTION

Infliximabum solutio concentrata

Related proteins. Capillary electrophoresis (*2.2.47*) under both reducing and non-reducing conditions.

Reference solution. Dissolve the contents of a vial of *infliximab CRS* in *water R* to obtain a concentration of 2 mg/mL. Mix 27 µL of the solution and 30 µL of sample buffer. Proceed at the same time and in the same manner as for the test solution.

System suitability: reference solution:

– *reducing conditions*: the electropherogram obtained is qualitatively similar to the electropherogram supplied with *infliximab CRS*;

SLIDO

- **I just ordered Etanercept CRS 1 (Y0001969). It comes in freeze dried product. I would like to know how to dissolve it (solvent, concentration, etc).**

  ✓In water
  ✓In the mobile phase
  ✓As described in the leaflet
  ✓As described in the monograph

Correct answer in green!

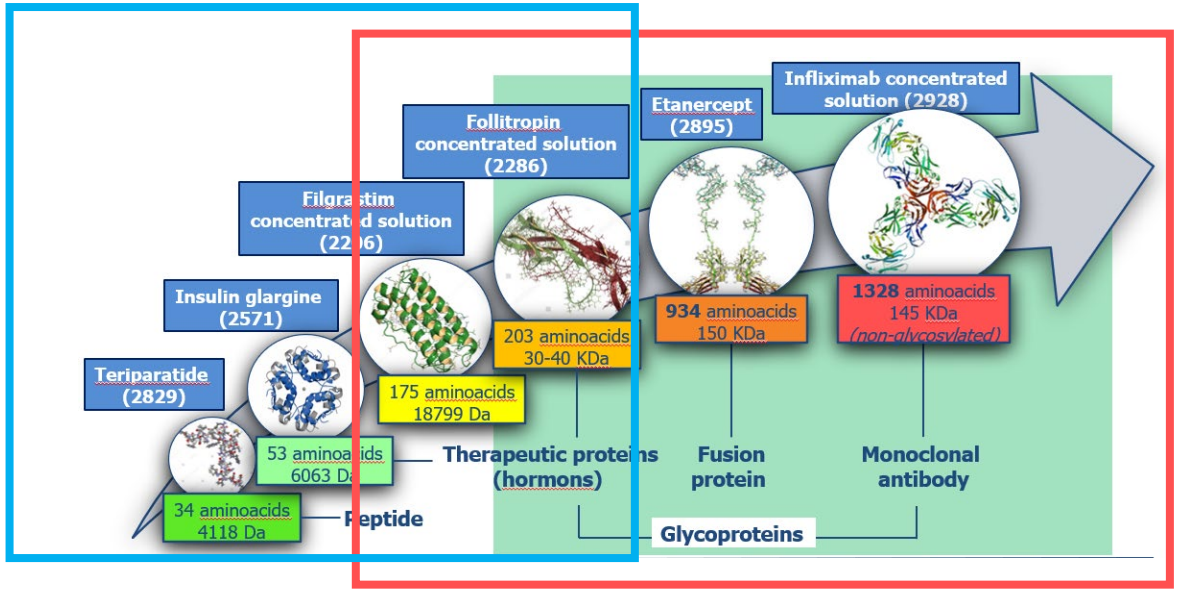
Etanercept CRS is used in the following tests of the corresponding Ph. Eur. monograph n°2895:

N-Linked oligosaccharides mapping, Peptide mapping, Sialic acid, Related proteins by Hydrophobic Interaction Chromatography, Impurities with molecular masses greater than that of etanercept by SEC, Impurities with molecular masses differing from that of etanercept by SDS-PAGE and Protein content.

-> Depending on the test you want to carry out, the solvent to be used and the concentration to reach are described in the monograph 2895.

Quantitative use Assay CRS

Reference standard for biologicals: assignment of content



The procedures for assigning a content to a RS depends on the type of unit of measurement:

- **Physico-chemical assay**: the CRS content :
  - is expressed in mg of peptide/protein per vial
  - is usually assigned based on the **"mass balance"** approach
- **Bioassay**: International Units refer to WHO International standard. BRP are established by the EDQM via the Biological Standardisation Programme (BSP)

For the establishment of a physico-chemical assay CRS:
- the **extent** of testing is greater than when a CRS is used for other purposes *(Ph. Eur. chapter 5.12.)*
- **inter-laboratory study**

Assigned content – Where to find the information?

Example: Follitropin **Leaflet**

Monograph Assay section:

Protein. Size-exclusion chromatography (2.2.30). *Calculate the content of follitropin taking into account the assigned content of follitropin CRS.*

INFORMATION LEAFLET Ph. Eur. Reference Standard

Follitropin CRS batch 3

1. **Identification**

   Catalogue code: Y0001629

2. **Scientific Information**

   2.1 Intended use

   Reference Standard for laboratory tests as prescribed in the European Pharmacopoeia only.
   Established for use with the monograph(s): 2285, 2286.

   2.2 Analytical information related to intended use, when applicable

   Electropherogram : See annex 1
   Chromatograms : See annexes 2, 3, 4, 5
   The "as is" content is : **0.22 mg/vial**

Leaflet - Where to find the information?

Reference substances database

Example: Follitropin

Catalogue Code	Y0001629
Name	Follitropin CRS
Current batch number	3
Unit quantity per vial	0.2 mg
Number of vials per sales unit	1
Used in monograph(s)	2285, 2286
Assigned content	See leaflet
Additional information	
Leaflet	click to download the leaflet
Chemical hazard	none identified
Biological hazard	none identified
SDS Product Code	
CAS Registry Number	N/A
Presentation	
Origin	click to download Origin Of Goods.pdf
Proposed Import HS code	293719
EDQM long term storage conditions	-20°C+/-5°C
Dispatching conditions	Dry-ice
UN Code	Not classified
Shipping group	D1A
Price*	79 EUR
Availability	Available
Sales restriction	No

Batches

batch 3 is valid at this date

Print BVS

RS webshop

Reference Standards

CATALOGUE

- Consult our complete list of standards:
  - European Pharmacopoeia Reference Standards
  - Who International Standards for Antibiotics (ISA)
  - Who International Chemical Reference Substances (ICRS)

ORDER / QUOTATION

- Place your order
- Ask for a quotation

No expiry date is given: see Batch Validity Statement
All across the RS lifetime, regular testing is performed in order to assure the continuous "fitness for use" of the CRS

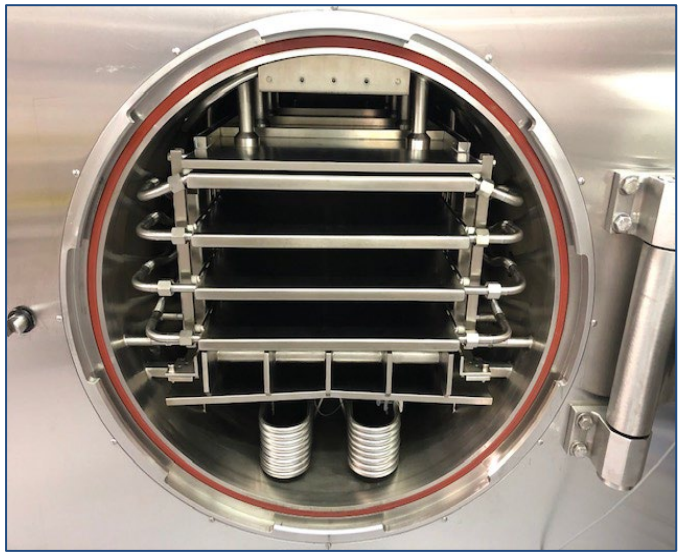
RS Processing

Reference standards processing aims at **minimising the risk of decomposition or degradation**

Whenever possible, the following presentation is selected:

- material in solid form
- packaged in **containers for single use (i.e. glass vials, ampoules**)

CRS for synthetic peptides and rDNA proteins are usually presented as freeze- dried materials to be reconstituted at the time of use



SLIDO

- **I want to run the potency assay described in the Follitropin monograph (2286).In order to run the assay, I want to know the potency in IU/mL. From the leaflet, I can only see the "as is" content = 0.22 mg/vial. How do I know the potency in IU/mL?**

  ✓I convert mg in IU
  ✓I ask EDQM
  ✓I cannot

Correct answer in green!

The Follitropin monograph (2286) describes the use of Follitropin CRS for the following purposes:

- Identification section: isoelectric focusing
- Tests section:
  - Follitropin oligomers by SEC
  - Free subunits by SDS-PAGE
  - Oxidised forms by LC
- **Assay section: Protein content by SEC**

In the assay section, for the potency test, the Follitropin CRS is not described since it has not been established for this purpose and has an assigned content in mg/vial and not in International Unit (IU).

As mentioned in the monograph, the biological activity is assessed based on a reference preparation calibrated in International Units. In the absence of Ph. Eur. reference standard calibrated in IU for this purpose, you can use the WHO IS.

If you want to express your result in IU/mg, you will then need the Follitropin CRS in addition to run the protein assay by Size-exclusion chromatography and determine the protein content.

Ph. Eur. CRS for biotherapeutics: what's next?

Individual monographs:

- Golimumab concentrated solution (3103):

COMMISSION SESSION	EDITION/ SUPPLEMENT	PUBLICATION SCHEDULE	IMPLEMENTATION DATE
177 / November 2023	11.6	July 2024	1 January 2025

- Golimumab for injection (3187): under elaboration

-> 1 CRS for all physico-chemical tests: Golimumab CRS!

Ph. Eur. CRS for biotherapeutics: what's next?

General tests:

- "Capillary isoelectric focusing for recombinant therapeutic monoclonal antibodies" (2.5.44.):

  Pharmeuropa 35.4

  Public deadline: 2023-12-31

  NPA deadline: 2024-02-29

➔1 CRS: "monoclonal antibody for system performance CRS"

- "Size-exclusion chromatography for recombinant therapeutic monoclonal antibodies" (2.5.43.): under elaboration

Take home messages (1)

Ph. Eur. CRS

- Ph. Eur. policy on reference standard is reflected in general chapter 5.12.
- official, legally binding standards, an essential part of Ph. Eur. monographs
- established and guaranteed for their intended use(s)
  - not necessarily suitable for other purposes
  - if a reference standard is to be used for any purpose other than that for which it has been established, its suitability for the new use has to be fully demonstrated by the user

Take home messages (2)

Ph. Eur. CRS

- Relevant:
  - to control the performance of the method
  - to assess acceptance criteria (qualitative, quantitative)
  - to allow independent testing
- Ph. Eur. CRS is just "a" material:
  - not necessarily related to the reference product
  - not necessarily related to the monograph specifications
  - is fit for the intended purpose

EDQM provides RS information (leaflet) and assistance (Helpdesk)

Module 3: Ph. Eur. Reference standards for biologicals

31 JANUARY 2024 - 14:30-16:00 (CET, FRANCE)

Ph. Eur. Biological Reference Preparations (BRP) for Biological Assays and Tests

Sébastien Jouette / Christina Göngrich
EDQM, Council of Europe

31 January 2024

Overview

1. Short introduction to Biological Reference Preparations (BRP) and the Biological Standardisation Programme (BSP)

2. Examples of different types of BRPs, their establishment and use in the context of the Ph. Eur.

3. Possibilities to contribute to the establishment of BRPs

Biological Reference Preparations (BRPs) (1)

Definition (Ph. Eur. General Text 5.12. Reference Standards):

"European Pharmacopoeia biological reference preparation (BRP). A substance or mixture of substances intended for use as stated in a monograph or general chapter of the European Pharmacopoeia.

BRPs are either secondary standards calibrated in International Units or primary standards, which may be used to define a European Pharmacopoeia Unit (Ph. Eur. U.). Other assigned contents may also be used, for example, virus titre or number of bacteria."

Biological Standardisation Programme (BSP)

- Co-funded by the Council of Europe and the EU Commission since 1994 – run by EDQM
- For establishment of Reference Standards and methods for QC testing of biologicals for human and veterinary use in the context of the Ph. Eur.
- Detailed presentation in ‘Suggested Viewing’

Funded
by the European Union
and the Council of Europe

EUROPEAN UNION

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CONSEIL DE L’EUROPE

Implemented
by the Council of Europe

How are BRPs established?

Calibration / Qualification of cBRP in large international collaborative study (in comparison to WHO IS if available)

→

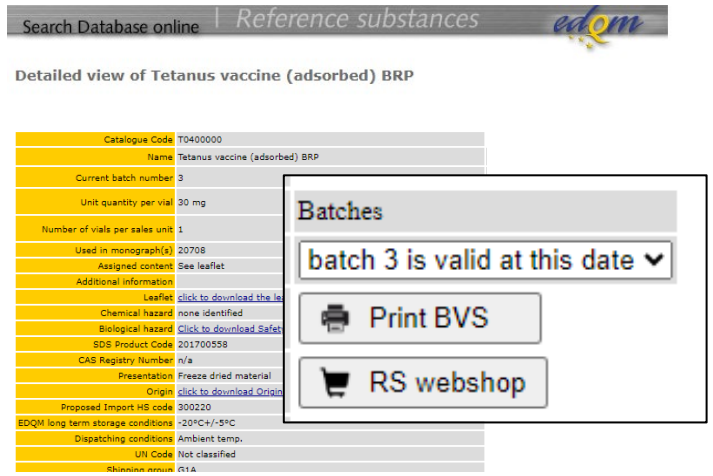
Review of the study results by **participants**, **BSP SC** and relevant **Ph. Eur. Groups of Experts**

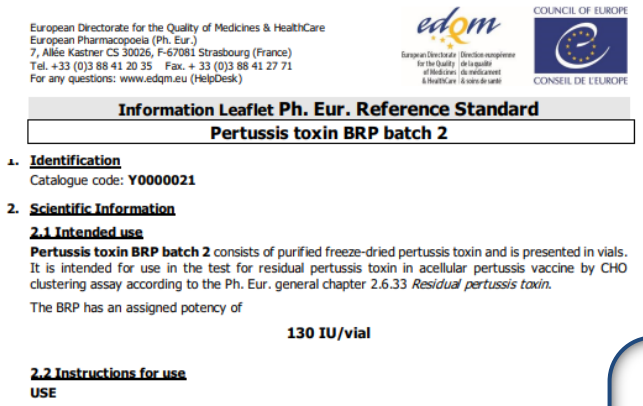
→

Adoption of the BRP by Ph. Eur. Commission

→

BRP established and available for use




European Directorate for the Quality of Medicines & HealthCare
European Pharmacopoeia (Ph. Eur.)
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Tel. +33 (0)3 88 41 20 35 Fax. + 33 (0)3 88 41 27 71
For any questions: www.edqm.eu (HelpDesk)

Information Leaflet Ph. Eur. Reference Standard
Pertussis toxin BRP batch 2

1. **Identification**
Catalogue code: **Y0000021**

2. **Scientific Information**

2.1 Intended use

Pertussis toxin BRP batch 2 consists of purified freeze-dried pertussis toxin and is presented in vials. It is intended for use in the test for residual pertussis toxin in acellular pertussis vaccine by CHO clustering assay according to the Ph. Eur. general chapter 2.6.33 *Residual pertussis toxin.*

The BRP has an assigned potency of

130 IU/vial

2.2 Instructions for use
USE

Publication of the study results in Pharmeuropa Bio & Scientifc Notes

Monitoring programs in place to ensure continued fitness for purpose

Use of Reference Standards for Biologicals in the Ph. Eur. (1)

Physico-chemical tests

- CRS or BRP
- Content in mg/vial
- Chromatogram(s)/spectrum

→ Covered in dedicated presentation

Biological assays / tests

- BRP/BRR
- Content in IU/ampoule or vial
- In specific units e.g. cfu/mL, Lf/mL …
- In arbitrary Ph. Eur. units if no WHO International Standard available

→Determination of biological activity

→Evaluation of system suitability, limits or level of contaminants

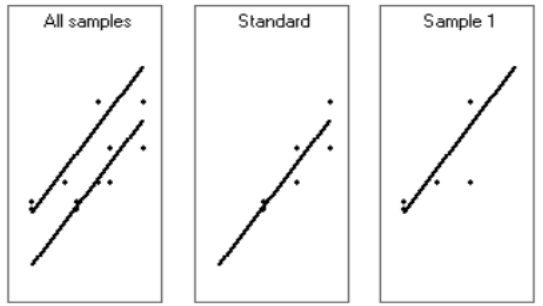
- Validity criteria or acceptable values are stated in the monographs.

Use of Reference Standards for Biologicals in the Ph. Eur. (2)

→ Determination of biological activity

- Standards for potency assays
  (e.g. Diphtheria Vaccine (adsorbed) BRP, Erythropoeitin BRP, ...)
  Statistical analysis compares biological activity of the test sample to the activity of the BRP to express relative potency in standardised units
- Reference sera
  (e.g. Bordetella pertussis antiserum BRP, C. tetani antiserum BRP…)
  Vaccine potency is expressed based on the value assigned to the reference serum

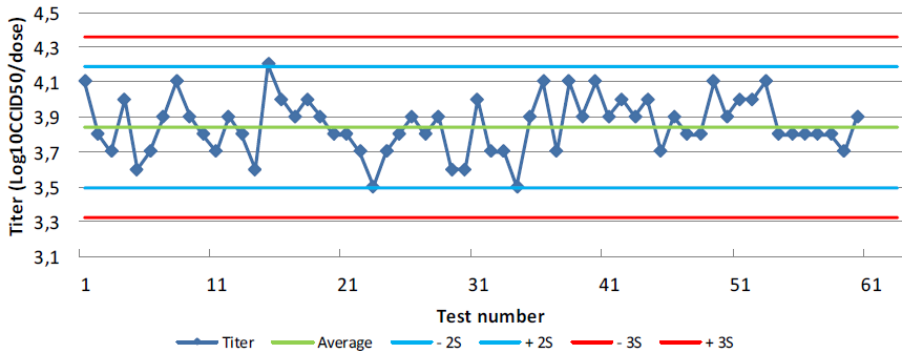
Sample 1			
(IU/dose)	Lower limit	Estimate	Upper limit
Potency	16.6014	22.1352	29.2553
Rel. to Ass.	174.8%	233.0%	308.0%
Rel. to Est.	75.0%	100.0%	132.2%



using CombiStats™

→ For system suitability, limits, level of contaminants
(e.g. Human immunoglobulin for ACA BRP, Diphtheria toxin BRP…)

- Use for monitoring of the method performance
- Use as positive or negative control
- Use for quantitation of contaminants



SLIDO

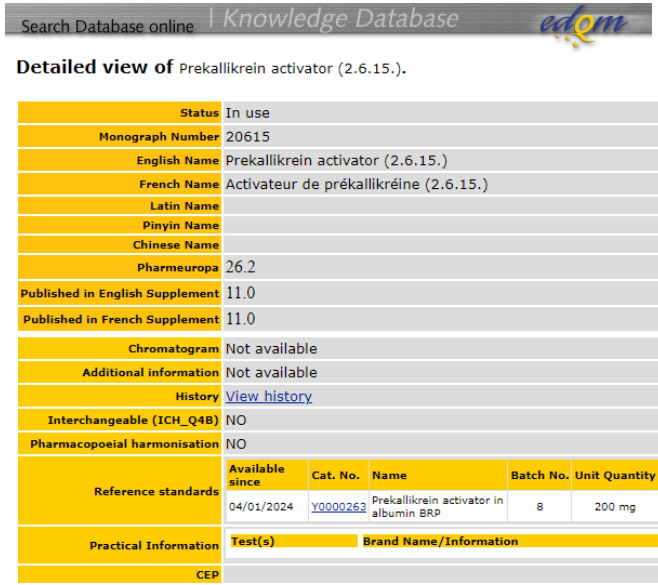
Where can we find information on a BRP and its use ?

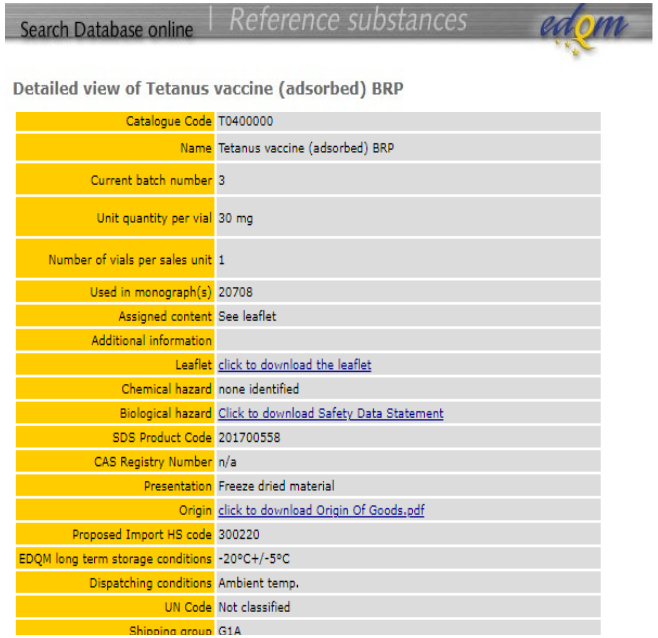
- ✓Specific Leaflet
- ✓Ph. Eur monographs /chapters
- ✓PharmeuropaBio & Scientific Notes / Pubmed
- ✓EDQM website
- ✓EDQM FAQs
- ✓EDQM Helpdesk

Correct answers in green!

Where can we find information on a BRP and its use ?

- Specific Leaflet via Reference substance database
- Ph. Eur monographs /chapters & Knowledge database
- PharmeuropaBio & Scientific Notes publications
- Pubmed





Where can we find information on a BRP and its use ?

- EDQM website
- EDQM FAQs on reference standards
- EDQM Helpdesk

BRP for System Suitability: Diphtheria Toxin BRP(1)

Ph. Eur. Reference Standard - LEAFLET

Diphtheria Toxin BRP batch 1

Diphtheria toxin BRP batch 1 consists of a solution of diphtheria toxin from Corynebacterium diphtheriae Massachusetts 8, Park Williams in 0.75% peptone buffer, filled in glass ampoules. Ampoules contain 1 ml of solution. It is intended for use in the test "Absence of toxin and irreversibility of toxoid" in Vero cells, as described in the Ph. Eur. monograph *Diphtheria vaccine (adsorbed) (0443)*.

Nominal concentration : 1 Lf/mL

BRP for System Suitability: Diphtheria Toxin BRP(2)

ESTABLISHMENT:

BSP054, Project leader D. Sesardic, NIBSC, UK

- Candidate diphtheria toxin donated by a European vaccines manufacturer
- Pretesting of candidate material carried out by the project leader
  - Formulation and stability testing
  - Preliminary characterisation of the specific toxicity
- Collaborative study run to characterise the cBRP and to qualify it for its intended use

→ Candidate material adopted as Diphtheria toxin BRP by the Ph. Eur. Commission in March 2003

D. Sesardic, C. Prior, A. Daas, K.H. Buchheit. Collaborative Study for Establishment of the European Pharmacopoeia BRP Batch 1 for Diphtheria Toxin. Pharmeuropa Bio & Scientific Notes, 2003(1): 5-21

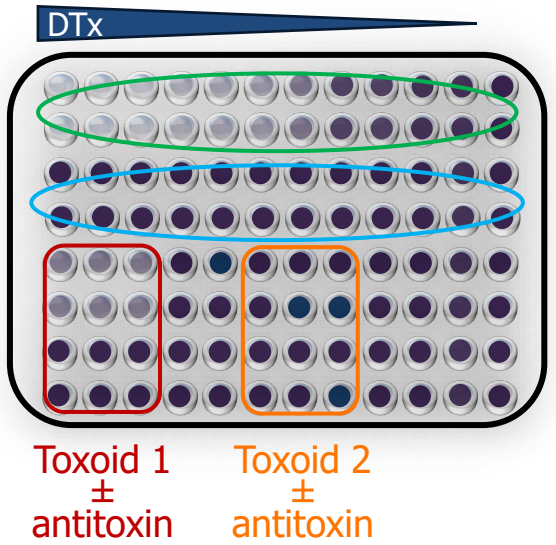
BRP for System Suitability: Diphtheria Toxin BRP(3)

USE: Test for "Absence of diphtheria toxin and irreversibility of toxoid" (0443)

- *In vitro* test taking advantage of the cytotoxic effect of diphtheria toxin on Vero cells.
- Carried out on each batch of bulk purified toxoid to ensure its complete and stable detoxification; test set-up described in detail in the monograph.
- Cells are incubated with

  → serial two fold dilutions of reference toxin in a suitable toxoid, with and without diphtheria antitoxin

  → a defined amount of test toxoid (100 Lf/mL), with and without diphtheria antitoxin



BRP for System Suitability: Diphtheria Toxin BRP(4)

Validity criteria:

- Test invalid if 5 x 10^{-5} Lf/mL of reference toxin do not have cytotoxic effect

→ Diphtheria toxin BRP serves as a system suitability control

- Cytotoxic effect needs to be neutralisable by diphtheria antitoxin

Acceptance criteria:

The toxoid cannot induce a cytotoxic effect that is neutralisable by the antitoxin

0443

"Confirm cytopathic effect by microscopic examination or suitable staining such as MTT dye. The test is invalid if 5 × 10^{-5} Lf/mL of reference diphtheria toxin in 100 Lf/mL toxoid has no cytotoxic effect on Vero cells or if the cytotoxic effect of this amount of toxin is not neutralised in the wells containing diphtheria antitoxin. The bulk purified toxoid complies with the test if no toxicity neutralisable by antitoxin is found in either sample."

Upcoming Study:

- Establishment of Diphtheria Toxin BRP batch 2 (BSP117)

BSP167: Replacement batches of PKA in Albumin BRP (relative quantitation)

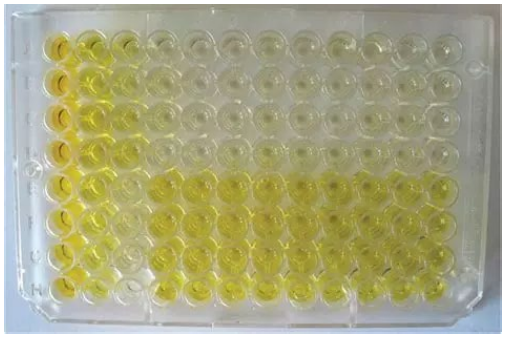
Prekallikrein activator is a pharmacopoeia test (2.6.15) described in the Human Albumin monograph (0255) and also a Batch Release test for Human Albumin solutions, with a 35 IU/ml maximum level indicated in the monograph.

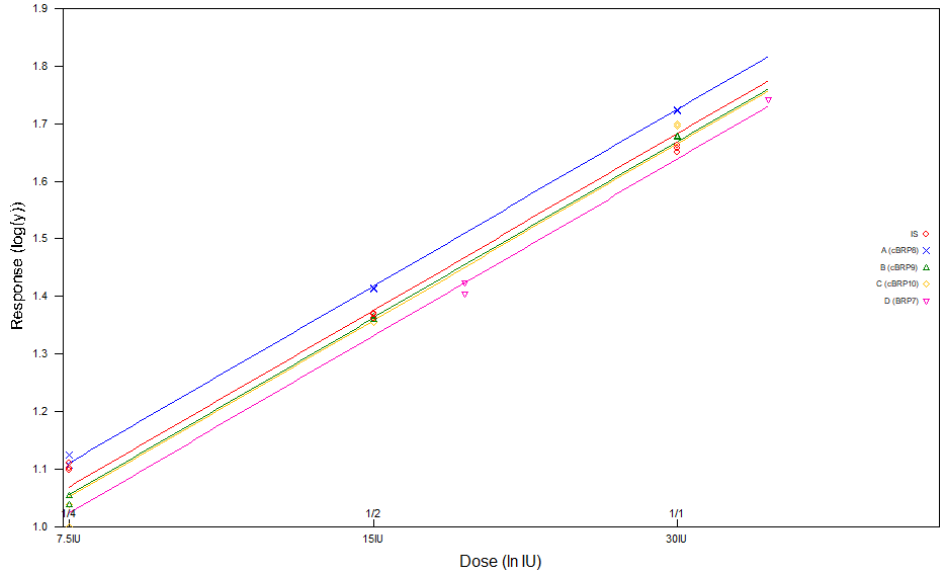
The BRP used in Ph.Eur. 2.6.15, consists of a freeze-dried preparation of albumin in vials with an assigned content of 37 IU/vial

This BRP is used for the determination of the PKA content in albumin products released in EU

The assay may be carried out using an automated enzyme analyser or a suitable microtitre plate system allowing kinetic measurements, with appropriate software for calculation of results. Standards, samples and prekallikrein substrate may be diluted as necessary using buffer B.

Incubate diluted standards or samples with prekallikrein substrate for 10 min such that the volume of the undiluted sample does not exceed 1/10 of the total volume of the incubation mixture to avoid errors caused by variation in ionic strength and pH in the incubation mixture. Incubate the mixture or a part thereof with at least an equal volume of a solution of a suitable synthetic chromogenic substrate, known to be specific for kallikrein (for example, *N-benzoyl-L-prolyl-L-phenylalanyl-L-arginine 4-nitroanilide acetate R* or *D-prolyl-L-phenylalanyl-L-arginine 4-nitroanilide dihydrochloride R*), dissolved in buffer B. Record the rate of change in absorbance per minute for 2-10 min at the wavelength specific for the substrate used. Prepare a blank for each mixture of sample or standard using buffer B instead of prekallikrein substrate.





e.g. quantification by using parallel line analysis in CombiStats™

BSP167: Replacement batches of PKA in Albumin BRP

Phase 1- Preparation with Project Leader: K. Kefeder, AGES/BASG

- Procure candidate starting material (Albumin, PKA concentrate)
- Test the starting materials (SEC, PKA content, etc), formulation
- Produce several pilot batches and assess freeze drying effect
- Produce 3 batches of candidate standard
- Pre-test in EDQM lab and project leader lab
- Accelerated stability studies
- Elaborate study protocol in interaction with statistician: D. Le Tallec
- Invite participant laboratories: 24 participants in EU, North America, Asia, Oceania
  - OMCLs, manufacturers

BSP167: Replacement batches of PKA in Albumin BRP

Phase 2 – Collaborative study with all participants

- Distribute common samples
  - calibrants : 3rd WHO IS and Ph. Eur. Prekallikrein activator in albumin BRP batch 7
  - 3 candidate replacement batches
- Distribute common study protocol
  - materials and methods in line with Ph. Eur 2.6.15 & 0255 (~ May 2023)
  - reporting sheets
- Return of results (31 July 2023) and central analysis
  - Assignment of a unitage to the candidate
  - Continuity with IS and previous BRP
- Draft study report - anonymised data sets (~Oct 2023)

BSP167: Replacement batches of PKA in Albumin BRP

Centrally calculated means and Coefficient of Variations (%) against the WHO 3rd IS or BRP7

	WHO 3rd IS as standard				Sample D (BRP7) as standard		
	A (cBRP8)	B (cBRP9)	C (cBRP10)	D (BRP7)	A (cBRP8)	B (cBRP9)	C (cBRP10)
Valid assay results only							
Overall mean	**37.3**	**33.1**	**34.4**	**37.3**	**37.2**	**33.0**	**34.2**
CV	4.5	4.7	4.7	3.5	5.0	5.9	5.8
Robust mean	**37.3**	**32.9**	**34.2**	**37.3**	**36.9**	**32.7**	**33.9**
CV	4.8	4.1	2.9	3.2	2.5	4.2	2.9
Including invalid assay results*							
Overall mean	**37.3**	**33.0**	**34.4**	**37.3**	**37.2**	**33.0**	**34.3**
CV	4.7	5.4	4.9	3.7	4.7	5.7	5.4
Robust mean	**37.2**	**32.9**	**34.2**	**37.3**	**37.0**	**32.7**	**34.0**
CV	4.6	4.1	3.3	3.5	2.9	4.1	3.4

* Due to a technical issue or declared as invalid following the statistical analysis (i.e. significant deviation from linearity or parallelism of regression lines).

3 new replacement batches adopted by Ph. Eur Commission end of December. BRP 8 available since January 2024

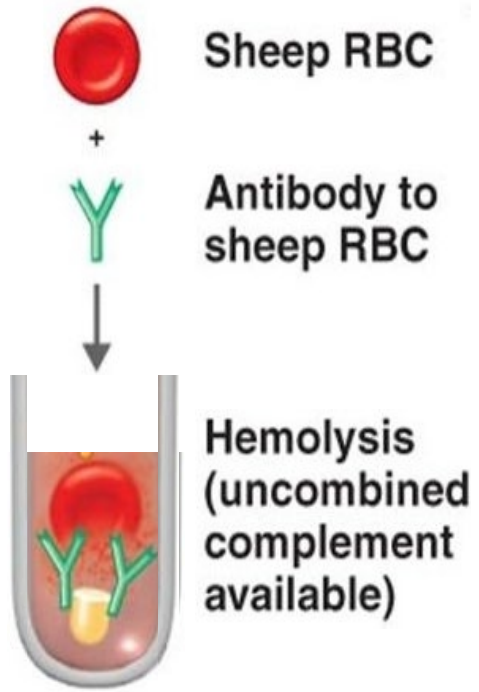
C. Kefeder, D. Le Tallec and S. Jouette. Collaborative study for the establishment of the European Pharmacopoeia Prekallikrein activator in albumin BRP batches 8, 9 and 10. Pharmeuropa Bio & Sci Notes, article in preparation.

BSP155: Replacement batches of Human immunoglobulin for anticomplementary activity BRP (used as negative & positive controls)

Ph. Eur. monograph 0918: Human normal immunoglobulin for intravenous administration

→ *control for anticomplementary activity* • *Test 2.6.17*

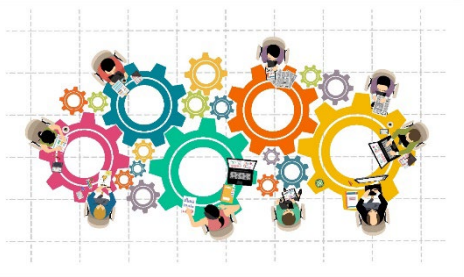
- Measurement of anticomplementary activity (ACA) of Ig
- Determined by measurement of haemolysis of sheep red blood cell by residual complement activity (absorbance, OD_{541nm})
- Human immunoglobulin for anticomplementary activity BRP used as internal control (negative & positive control)



BSP155: Replacement batches of Human immunoglobulin for ACA BRP

Phase 1- Preparation with Project Leaders: *L. Miller and Z. Waibler, PEI*

- Procure candidate starting materials and MTA
- Test different Human IgG for ACA
- Determination of 4 suitable (freeze-dried human Ig) batches of candidate standard
- Stability studies on aliquots made from the initial container
- Elaborate study protocol in interaction with statistician: E. Regourd
- Invite participant laboratories: 6 participants in EU
  - OMCL and manufacturers



BSP155: Replacement batches of Human immunoglobulin for ACA BRP

Phase 2 – Collaborative study with all participants

- Distribute common samples
  - calibrants : Human immunoglobulin for anticomplementary activity BRP batch 2
  - 4 candidate replacement batches
- Distribute common study protocol
  - materials and methods in line with Ph. Eur. chapter 2.6.17 (June 2020)
  - reporting sheets
- Return of results (31 October 2020) and central analysis
  - Assignment of ACA % to the candidate for the negative and positive controls
  - Consider continuity with previous BRP
- Draft study report - anonymised data sets (April 2021)

BSP155: Replacement batches of Human immunoglobulin for ACA BRP

Results of the ACA test, 6 Labs- 4 independent assays per Lab- overall ACA mean values

		BRP batch-2		Sample A		Sample B		Sample C		Sample D	
		Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
		% Act	% Act	% Act	% Act	% Act	% Act	% Act	% Act	% Act	% Act
Including all tests	N	6	6	6	6	6	6	6	6	6	6
	mean	24.6	88.2	23.6	86.7	23.4	86.7	21.8	82.3	21.6	85.2
	SD	4.1	6.0	5.6	7.4	5.9	7.7	3.9	6.3	4.8	5.6
Excluding invalid tests	N	6	6	6	6	6	6	6	6	6	6
	mean	24.6	88.2	23.6	86.7	23.4	86.7	21.8	82.3	21.0	85.2
	SD	4.1	6.0	5.6	7.4	5.9	7.7	3.9	6.3	4.0	5.6

The overall means (excluding invalid assays), for the BRP: 24.6 % for the negative controls and 88.2 % positive controls

For the 4 candidate materials: 21.0 %-23.6 % for the negative controls and 82.3 %-86.7 % for the positive controls

4 new replacement batches adopted by Ph.Eur Commission 170th in June 2021

L. Miller, Z. Waibler, E. Regourd and S. Jouette. Collaborative study for the establishment of the European Pharmacopoeia Human immunoglobulin for anticomplementary activity BRP batches 3, 4, 5 and 6. Pharmeuropa Bio & Sci Notes 2022 (2) 10-21.

The CombiStats software

- Statistical analysis of data of biological dilution assays **in accordance with Ph. Eur. Chapter 5.3: Statistical analysis of results of biological assays and tests** , examples

EP document	Excerpt
Monoclonal antibodies for human use	**design** of the assay and **calculation** of the results are made according to the **usual principles** (for example, 5.3)
Immunonephelometry for vaccine component assay	the **parallel line method** (see general chapter 5.3) or a calibration curve is used for the calculations
Etanercept	calculate the potency using the **four-parameter logistic curve model** (5.3)
Alternative methods for control of microbiological quality	**deviation from linearity** is non-significant (see general chapter 5.3)
Poliomyelitis vaccine (oral)	data obtained from valid assays only are **combined by the usual statistical methods** (for example, 5.3)

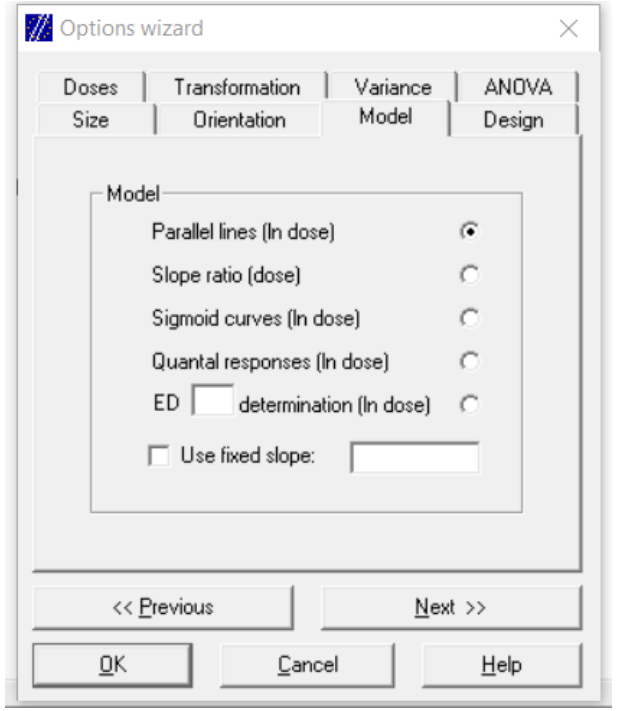
User-friendly interface, well-documented application

Raw data tables

Standard		
Id.	S	
Ass. pot.	1 unit/mg	
Doses	0.25 unit	1.0 unit
(1)	300	289
(2)	310	221
(3)	330	267
(4)	290	236

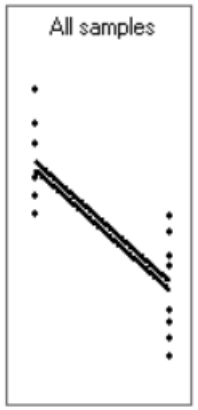
Sample 1		
Id.	T	
Ass. pot.	1 unit/mg	
Doses	0.25 unit	1.0 unit
(1)	310	230
(2)	290	210
(3)	360	280
(4)	341	261

Analysis options

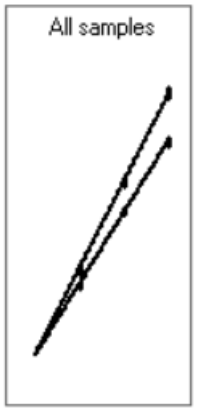


Various regression models (for qualitative and quantitative results)

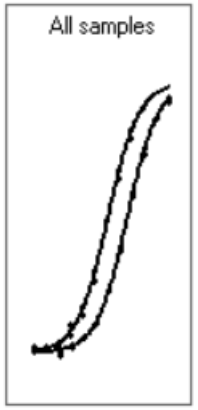
Parallel line analysis



Slope ratio analysis



Sigmoid models



Outputs: assay validity criteria and **potency estimates** of test preparations

Sample 1			
Id.	T		
(unit/mg)	Lower limit	Estimate	Upper limit
Potency	0.430313	0.904222	1.80135
Rel. to Ass.	43.0%	90.4%	180.1%
Rel. to Est.	47.6%	100.0%	199.2%

EDQM Website

https://www.edqm.eu/en/combistats-tm

CombiStats™

- Statistical analysis of results using CombiStats
- How do I pay EDQM invoices?
- How do I complain to the EDQM about an order?
- What's new in version 7?
- Have a question about CombiStats?
- General terms and conditions for the use of CombiStats

The manual provides users with a comprehensive guide to the CombiStats™ software and its many features

How to Participate in a BSP Project?

As participant

Laboratory from manufacturer / OMCL
- if you perform the test
- if you use the Ph. Eur. RS

As project leader

Expert in the field
- with access to a laboratory
- with wide knowledge of products / methods
- if you developed a new method

As donator of starting material

Manufacturer

→ Check the EDQM website for ongoing / future studies (BSP work programme)
→ Contact the EDQM/DBO/BSP (via HelpDesk on the EDQM website or direct contact to a BSP team member)

Many Thanks to All Supporters

Past and Present

BSP Steering Committee Members
Project Leaders
Project Participants
Donators of Material
International Collaborators

… and EDQM collaborators
DLab, DRSL, DAF, ITPD, CED …

EDQM DBO Team

Scientific Project Administrators	Katarina Bacevic, Marie-Emmanuelle Behr-Gross, Angèle Costanzo, Christina Göngrich, Sébastien Jouette, (Chiara Lonigro), Eriko Terao
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Statisticians	David Le Tallec, Elena Regourd
Head of Section	Catherine Milne
Head of Division	Michael Wierer
Head of Department	Laurent Mallet

Thank you for your attention

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