European Pharmacopoeia – recent developments in the field of biopharmaceuticals

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European Pharmacopoeia

- Protecting public health – one common compulsory standard
- Official pharmacopoeia in Europe (complemented by national pharmacopoeias)
- Legally binding quality standards for ALL medicinal products i.e. raw materials, preparations, dosage forms, containers...
- Mandatory at the same date for all Members

- 39 Members (38 Member States & EU)
- 30 Observers (6 European, 22 non-European countries, TFDA, WHO)
- Suppl. 9.8: 2406 monographs, 365 general texts, 2730 reagents
Structure of the Ph. Eur.

- Apply to ALL texts
- Provide basic information, rules and conventional expressions
- Address general issues
- Essential reading

- Classes of substances and dosage forms
- Mandatory to all substances/preparations within the definition scope
- Not cross-referenced in individual monographs

General notices

General chapters

General monographs

Individual monographs

Reference standards
- Established specifically and exclusively for use in Ph. Eur. texts
- 5.12 Reference standards

General methods & texts
- Editorial convenience
- Not mandatory “per se”
- Part of the standard when referred to in a monograph
- Can be used when there is no monograph → may need validation

Specific
- Not stand-alone
- Based on approved specification(s) backed up by batch data
- Validated analytical procedures

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Monographs elaboration procedures

**Multi-source**

- Group of Experts or Working Parties
- On request, data handled confidentially

**Single-source**

- Direct cooperation with innovator
- Working parties of regulators
- Data handled confidentially by EDQM

**MONOGRAPH**

- valid for A, B, C & D
- applicable to B, C, D ....

Experimental verification
Monograph elaboration – experimental verification

- **Robustness** and **transferability** of the methods to be introduced in the monograph
- **Method performance**
- **Sometimes** methods are **out-of-date** or not robust enough

- Specific instructions added
- Strengthen verification of method performance (*e.g.* resolution solution for SST)
- Reference to existing pharmacopoeial methods/general chapters or to monographs on closely related substances
- For certain tests experimental verification may go beyond the monograph itself (*e.g.* peptide mapping by LC-MS to confirm marker peaks in complex peptide maps)
- Validation needed for implementation of alternative methods

**Multiple exchanges with the manufacturers**

**Consumes significant resources**

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Elaboration procedures – monograph examples for biotherapeutics

1990

- Insulin human (0838)
- IFNα-2 (1110)
- IFNβ-1a (1639)
- Follitropin (2285, 2286)
- Human coagulation factor VIII rDNA (1643)
- Filgrastim (2206)
- Somatropin soln. for injection (2370)
- EPO (1316)

2010

- Insulin glargine (2571)
- Human coagulation factor IX (rDNA) DS (2522) and DP (2994)
- Human coagulation factor VIIa (rDNA) (2534)
- Etanercept (2895)
- Teriparatide (2829)

2019

- Insulin glargine (2571)
- Human coagulation factor IX (rDNA) DS (2522) and DP (2994)
- Etanercept (2895)
- Teriparatide (2829)
Evolution of Ph. Eur. monographs and biological complexity

- **Infliximab** concentrated solution (2928)
- **Filgrastim** concentrated solution (2206)
- **Follitropin** concentrated solution (2286)
- **Etanercept** (2895)
- **Insulin glargine** (2571)
- **Teriparatide** (2829)
- **Glycoproteins**
  - **Follitropin**
  - **Fusion protein**
  - **Monoclonal antibody**
- **Therapeutic proteins (hormones)**
  - **Peptide**
  - **203 aminoacids 30-40 KDa**
  - **175 aminoacids 18799 Da**
  - **53 aminoacids 6063 Da**
  - **34 aminoacids 4118 Da**
- **934 aminoacids 150 KDa**
- **1328 aminoacids 145 KDa (non-glycosylated)**

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To find the **appropriate equilibrium** between:

- **flexibility** of expectations, so that they apply to a large variety of products
- **detailed** (prescriptive) requirements so that the respective analytical procedures can be performed successfully in a control laboratory
Too much flexibility leads to a meaningless standard

Ph. Eur. General monograph Monoclonal antibodies for human use (2031)

‘Purity. Tests for process- and product-related impurities are carried out by suitable validated methods.’

‘ASSAY. Carry out a suitable biological assay compared to the reference preparation.’
allowed if lead to the same pass/fail results; subject to the agreement of the competent authority

Possibility to omit tests (since 1997)

if assured that a product is of Pharmacopoeia quality on the basis of its design, together with its control strategy and relevant data

DEMONSTRATION OF COMPLIANCE ≠ TESTING

Enhanced approaches (since 2013)

Use of PAT and/or real-time release testing (including parametric release) as alternatives to end-product testing acknowledged
Ph. Eur. flexibility #2: Production Section

➢ Introduced in 1991 in monographs for biological preparations (discussions in the 80s).

➢ Draws attention to particular aspects of the manufacturing process; not necessary comprehensive.

➢ Mandatory requirements for manufacturers (unless otherwise stated).

➢ Includes statements on e.g. source materials, process, validation and control, in-process testing or tests to be carried out on the final article prior to release.

➢ Cannot necessary be verified by an independent analyst on the final article.
**FILGRASTIM CONCENTRATED SOLUTION**

FILGRASTIM CONCENTRATED SOLUTION

*Filgrastimi solutio concentrata*

(...)

**PRODUCTION**

Filgrastim concentrated solution is produced by a method based on recombinant DNA (rDNA) technology, using bacteria as host cells.

*Prior to release, the following tests are carried out on each batch of the final bulk product, unless exemption has been granted by the competent authority.*

**Host-cell-derived proteins.** The limit is approved by the competent authority.

**Host-cell- or vector-derived DNA.** The limit is approved by the competent authority.

(...)

**INSULIN GLARGINE**

INSULIN GLARGINE

*Insulinum glarginum*

(...)

**PRODUCTION**

Insulin glargine is produced by a method based on recombinant DNA (rDNA) technology under conditions designed to minimise the degree of microbial contamination.

*Prior to release, the following tests are carried out on each batch of the final bulk product, unless exemption has been granted by the competent authority.*

**Host-cell-derived proteins.** The limit is approved by the competent authority.

**Host-cell-derived proteins.** The limit is approved by the competent authority.

**Single-chain precursor.** The limit is approved by the competent authority. Use a suitably sensitive method.
**Ph. Eur. flexibility #3: additional flexibility for complex biotherapeutics - example methods**

**SUITABLE METHOD**

- **general indications** on the test procedure (main steps to be carried out, type of method, readout, cells, reagents...)

- The term “suitable” is a conventional term: ‘In certain monographs [...], the terms ‘suitable’ and ‘appropriate’ are used to describe a reagent, microorganism, test method etc.; if criteria for suitability are not described in the monograph, suitability is demonstrated to the satisfaction of the competent authority.’ (General Notices)

**EXAMPLE METHOD**

- **specific instructions**, quantities, concentrations, compositions of reagents/buffers, chromatographic conditions etc. together with **system suitability criteria**; method may be used as such but any other suitable validated procedure may be used without demonstrating its equivalence to the ‘example’ method (subject to approval by the competent authority);

- *The following procedure is given as an example.*

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### Ph. Eur. flexibility #3: additional flexibility for complex biotherapeutics – acceptance criteria

#### Quality attribute

<table>
<thead>
<tr>
<th>Quality attribute</th>
<th>Flexibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potency (specific activity)</td>
<td>✗</td>
</tr>
<tr>
<td>Protein concentration</td>
<td>✓</td>
</tr>
<tr>
<td>Host-cell-derived proteins</td>
<td>✓</td>
</tr>
<tr>
<td>Host-cell-derived DNA</td>
<td>✓</td>
</tr>
<tr>
<td>Primary structure (peptide mapping)</td>
<td>✗</td>
</tr>
<tr>
<td>Glycan profile</td>
<td>✓</td>
</tr>
<tr>
<td>Isoforms/charge variants</td>
<td>✓</td>
</tr>
<tr>
<td>Product-related impurities (e.g. HMW, LMW by SEC)</td>
<td>✗</td>
</tr>
<tr>
<td>Related proteins</td>
<td>✗</td>
</tr>
</tbody>
</table>

- • numerical limits/ranges
- • ‘as authorised by the competent authority’
**PRODUCTION section:**

- **general requirements for consistency of production:**

<table>
<thead>
<tr>
<th><strong>ETANERCEPT</strong></th>
<th>07/2019:2895</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etanerceptum</td>
<td></td>
</tr>
</tbody>
</table>

**PRODUCTION**

Etanercept is produced in a suitable mammalian cell expression system by a method based on recombinant DNA (rDNA) technology. During the course of product development, it must be demonstrated that the manufacturing process consistently produces a product with the expected O-glycan occupancy using a suitably qualified assay.

<table>
<thead>
<tr>
<th><strong>INFLIXIMAB CONCENTRATED SOLUTION</strong></th>
<th>01/2019:2928</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infliximabum solutio concentrata</td>
<td></td>
</tr>
</tbody>
</table>

**PRODUCTION**

Infliximab is produced in a suitable mammalian cell expression system by a method based on recombinant DNA (rDNA) technology. During the course of product development, it must be demonstrated that the manufacturing process consistently produces a product with the expected N-glycan occupancy and Fc-effector functions (antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC)) using suitably qualified assay(s).
Ph. Eur. flexibility #3: additional flexibility for complex biotherapeutics – example

PRODUCTION section:

- Specific requirements related to process-dependent heterogeneity set in a flexible way:
  - Generic method of analysis

**N-Glycan analysis.** Use a suitable method developed according to general chapter 2.2.59. *Glycan analysis of glycoproteins*, section 2-3:

- release the glycans using one of the agents described in Table 2.2.59.-1, for example peptide N-glycosidase F (PNGase F);
- label the released glycans with one of the fluorescent labelling agents described in Table 2.2.59.-2, for example 2-aminobenzamide;
- analyse the labelled glycans by liquid chromatography (2.2.29) using fluorescence detection.

(...)

ETANERCEPT
Etanerceptum

07/2019:2895
PRODUCTION section:

- Specific requirements related to process-dependent heterogeneity set in a flexible way:
  
  - Generic method of analysis
  - Specific analytical procedure as example

- detailed instructions

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**Test solution.** To 4 µL of the preparation to be examined (about 25 mg/mL) add 21 µL of water R, 3 µL of 0.25 M sodium phosphate buffer solution pH 7.5 R and 2 µL of a 500 000 U/mL solution of peptide N-glycosidase F R. Mix and incubate at 37 °C for 20-24 h. Label the released glycans with 2-aminobenzamide using a suitable procedure. The procedure employs a combination of reagents optimised and validated for the efficient labelling of glycans, and for the subsequent extraction and recovery of the labelled glycans from the reaction. Resuspend or dilute the labelled glycans in 100 µL of water R.

**Reference solution (a).** Dissolve the contents of a vial of etanercept CRS in water R to obtain a concentration of about 25 mg/mL. Carry out the release and labelling of glycans in the same manner as for the test solution. Resuspend or dilute the labelled glycans in 100 µL of water R.

**Reference solution (b).** Use a suitable etanercept in-house reference preparation shown to be representative of batches tested clinically and batches used to demonstrate consistency of production. Dilute, if necessary, with water R to obtain a concentration of about 25 mg/mL. Carry out the release and labelling of glycans in the same manner as for the test solution. Resuspend or dilute the labelled glycans in 100 µL of water R.

**Blank solution.** Prepare at the same time and in the same manner as for the test solution but using water R instead of the preparation to be examined.

**N-Glycan analysis.**

The following procedure is given as an example.

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**Analyse the labelled glycans by liquid chromatography (2.2.29).**

**Column:**
- size: l = 0.25 m, Ø = 4.6 mm;
- stationary phase: an amide derivative of silica gel for chromatography R (5 µm);
- temperature: 35 °C.

**Mobile phase:**
- mobile phase A: mix 9.8 mL of anhydrous formic acid R and 500 mL of water for chromatography R, adjust to pH 4.0 with ammonia R and dilute to 1000 mL with water for chromatography R;
- mobile phase B: acetonitrile R.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent V/V)</th>
<th>Mobile phase B (per cent V/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 2</td>
<td>20 → 30</td>
<td>80 → 70</td>
</tr>
<tr>
<td>2 - 67.0</td>
<td>30 → 52</td>
<td>70 → 48</td>
</tr>
<tr>
<td>67.0 - 67.1</td>
<td>52 → 80</td>
<td>48 → 20</td>
</tr>
<tr>
<td>67.1 - 73.0</td>
<td>80 → 20</td>
<td>20 → 20</td>
</tr>
</tbody>
</table>

**Flow rate:** 0.4 mL/min.

**Detection:** fluorimeter at 330 nm for excitation and 420 nm for emission.

**Autosampler:** set at 2-8 °C.

**Injection:** 10 µL.
PRODUCTION section:

- Specific requirements related to process-dependent heterogeneity set in a flexible way:
  - Generic method of analysis
  - Specific analytical procedure as example
  - Detailed instructions
  - Method performance (system suitability) criteria

- ETANERCEPT 07/2019:2895
  - Etanerceptum
  - N-Glycan analysis.

System suitability:
- the chromatogram obtained with reference solution (a) is qualitatively similar to the chromatogram supplied with etanercept CRS and peaks 1 to 9 are clearly visible;
- no significant peaks are observed in the chromatogram obtained with the blank solution.
PRODUCTION section:

- Specific requirements related to process-dependent heterogeneity set in a flexible way:

  • Generic method of analysis
  • Specific analytical procedure as example

- Detailed instructions
- Method performance (system suitability) criteria

**System suitability:**
- The chromatogram obtained with reference solution (a) is qualitatively similar to the chromatogram supplied with *etanercept CRS* and peaks 1 to 9 are clearly visible;
- No significant peaks are observed in the chromatogram obtained with the blank solution.
Ph. Eur. flexibility #3: additional flexibility for complex biotherapeutics – example

PRODUCTION section:

➢ Specific requirements related to process-dependent heterogeneity set in a flexible way:

• Generic method of analysis
• Specific analytical procedure as example

- detailed instructions
- method performance (system suitability) criteria

- Results – comparison with an in-house standard

ETANERCEPT
Etanerceptum

07/2019:2895

N-Glycan analysis.

(...)

Results:

- the profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with reference solution (b);
- the retention times of the peaks in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with reference solution (b);
- no additional peaks are observed in the chromatogram obtained with the test solution in comparison with the chromatogram obtained with reference solution (b).
Ph. Eur. flexibility #3: additional flexibility for complex biotherapeutics – example

PRODUCTION section:

➢ Specific requirements related to process-dependent heterogeneity set in a flexible way:

- Generic method of analysis
- Specific analytical procedure as example
- Detailed instructions
- Method performance (system suitability) criteria
- Results – comparison with an in-house standard

- Acceptance criteria – as approved by the competent authority

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</tr>
<tr>
<td>(...</td>
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<tr>
<td>(...)</td>
</tr>
</tbody>
</table>

Limits:
- percentage of neutral N-glycans: as approved by the competent authority;
- percentage of sialylated N-glycans: as approved by the competent authority.
Monograph for Biotherapeutics: Additional Flexibility

- **PRODUCTION section**
  - requirements related to process-dependent heterogeneity

- **Test procedures**
  - complex (multi-step) analytical procedure(s) given as example(s)

- **Reference preparations**
  - Ph. Eur. CRS to demonstrate method performance (system suitability)
  - in-house reference preparation – matching profiles

- **Acceptance criteria**
  - limits to be set in agreement with the competent authority

- **MONOGRAPH FLEXIBILITY**

- **Means of enhancing monograph flexibility under well-defined conditions**
  - Compatible with development of biosimilars
  - Address complexity

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Biotherapeutics – dedicated section on EDQM Website

https://www.edqm.eu/en/biotherapeutics

Topics covered

- Biosimilars
- P4-Bio Pilot Phase
- MAB Pilot Phase
- Flexibility in Ph. Eur. monographs

In addition to general and individual monographs, the Ph. Eur. contains a number of general chapters, of which Glycan analysis of glycoproteins (2.2.59) and Host-cell protein assays (2.6.94) could be of particular interest in reference to biotherapeutics.
Acknowledgements

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Emmanuelle Charton

Thank you for your attention!