A guide through individual monographs

European Pharmacopoeia Training Session on Biologicals
4-5 February 2020
Olga Kolaj-Robin, PhD
Basis for monograph elaboration

- Products of proven safety, evaluated and approved by competent authorities of Member States
- Impurities profiles for existing, approved synthetic routes/defined host
- Use of robust, validated analytical methods

Complementarity of specific and general monographs
Synthetic peptides and BTPs Ph. Eur. monograph portfolios

**Synthetic peptides – Ph. Eur. monograph portfolio**

- Buserelin (1077)
- Calcitonin (salmon) (0471)
- Desmopressin (0712)
- Felypressin (1634)
- Gonadorelin acetate (0827)
- Goserelin (1636)
- Leuprorelin (1442)
- Octreotide (2414)
- Oxytocin (0780)
- Oxytocin concentrated solution (0779)
- Protirelin (1144)
- Somatostatin (0949)
- Terlipressin (2646)
- Tetracosactide (0644)

**New monographs in preparation**

- Atosiban (3054)
- Glatiramer (3057)
- Glatiramer injection (3104)*
- Lanreotide (3056)
- Triptorelin (3055)

* finished product monographs; § under revision

**Biotherapeutics – Ph. Eur. monograph portfolio**

- Alteplase for injection (1170)*
- Calcitonin salmon (0471)
- Erythropoietin concentrated solution (1316)
- Etanercept (2895)
- Filgrastim concentrated solution (2206)
- Filgrastim injection (2848)*
- Follitropin (2285)
- Follitropin concentrated solution (2286)
- Glucagon, human (1635)
- Human coagulation factor IX (rDNA) powder for solution for injection (2994)*
- Human coagulation factor IX (rDNA) concentrated solution (2522)
- Human coagulation factor VIII (rDNA) concentrated solution (2534)
- Human coagulation factor VIII (rDNA), B-domain deleted, concentrated solution (3105)
- Human coagulation factor VIII (rDNA), B-domain deleted, powder for injection (3106)*
- Insulin aspart (2084)
- Insulin glargine (2571)
- Insulin lispro (2085)
- Insulin preparations injectable (0854)*
- Insulin, human (0855)
- Interferon alfa-2 concentrated solution (1110)
- Interferon gamma-1b concentrated solution (1440)
- Molgramostim concentrated solution (1641)

* finished product monographs; § under revision

**A guide through sections of Ph. Eur. monographs**

**Synthetic peptide**

- OCTREOTIDE
  - Octreotide
  - Formula
  - Molecular and graphic
  - Relative mass
  - Glycosylated protein except for complex glycoproteins
  - CAS number

**MONOGRAPH SECTION**

- Version date
- Title
- INN
- Formula
- rDNA product
- Molecular and graphic
- Relative mass
- Glycosylated protein except for complex glycoproteins
- CAS number
A guide through sections of Ph. Eur. monographs

**Synthetic peptide**

**OCTREOTIDE**
Octreotide

**MONOGRAPH SECTION**

**DEFINITION**
- chemical nomenclature
- identity and biological activity
- physical form, salt form
- additives (*e.g.* oxytocin conc. sln.)*
- assay limits:
  - content (mass/volume or mass/mass)
  - potency (IU/mg) (synthetic peptides: by convention if present *e.g.* oxytocin, tetracosactide, calcitonin; rDNA proteins: *e.g.* somatropin, insulin)

*Substances for Pharmaceutical Use (2034)*: "A monograph is applicable to a substance processed with an excipient only where such processing is mentioned in the definition section of the monograph."

**rDNA product**

**FILGRASTIM CONCENTRATED SOLUTION**
Filgrastim solutio concentrata

**DEFINITION**
Solution of a protein having the primary structure of the 174-amino-acid isoform of human granulocyte colony-stimulating factor (h-hCSF) plus L Additional amino acids at the N-terminal methionine. The protein is produced as a recombinant protein. The product is not glycosylated. Sol CSF is produced and secreted by endothelial cells, monocytes and other immune cells. The protein stimulates the differentiation and proliferation of monocyte stem cells into mature granulocytes.

Content: minimum 0.9 mg of protein per milliliter.

Potency: minimum 0.9 IU per milligram of protein.

**MONOGRAPH SECTION**

**Production**
- absent for synthetic peptides;
- extensive for vaccines;
- may be present for chemicals;
- may contain specific tests for rDNA products;
- source materials, manufacturing process, validation, control, in-process testing;
- mandatory for manufacturers;
- independent verification difficult;
- compliance: competent authorities

**Characters***
- Appearance, hygroscopicity, crystallinity, solubility
- useful info for analyst
- not analytical requirement

---

*See also 5.11 Characters section in monographs*
Synthetic peptide

**MONOGRAPH SECTION**

**Tests**

- Purity/impurity assessment
- Limits based on specifications and batch data for approved products
- Bacterial endotoxins – covered by 2034; may not be repeated
- Residual solvents – covered by 2034
- Inorganic impurities e.g. sulphated ash
- Optical rotation (or chiral chromatography)
- Absorbance – if appropriate
- Related peptides/substances
- Acetic acid
- Water

Developed on basis of protein size, charge and hydrophobicity

**Identification**

- No second identification
- Often cross-references to Tests and Assay

**Verification of the molecule’s**

- Size
- Sequence
- Isoelectric profile
- Chromatographic properties
- Correct functional configuration
- Specific to product (e.g. glycan analysis)

**Impurities with molecular masses higher than that of filgrastim**

- Size-exclusion chromatography (2.2.39)
- Use the normalisation procedure.

**Impurities with molecular masses differing from that of filgrastim**

- Polyacrylamide gel electrophoresis (2.2.31) under both reducing and non-reducing conditions.

**Impurities with charges differing from that of filgrastim**

- Isoelectric focusing (2.2.34)

**Related proteins**

- Liquid chromatography (2.2.39)
- Use the normalisation procedure.

**Bacterial endotoxins**

- 2.8 mg/l from the volume containing 1.0 mg of protein.
Synthetic peptides  Related peptides – main impurity test

**Identification of impurities and SST**

→ Octreotide impurity mixture CRS

For information!

Relative retention with respect to octreotide (retention time = about 20 min): impurity A = about 0.78; impurity B = about 0.80; impurity C = about 0.94; impurity D = about 1.13; impurity E = about 1.26; impurity G = about 1.33; impurity H = about 1.66.

For interpretation: resolution between the peaks due to impurities F and G.

---

**Impurity limits**

- each specified impurity (sometimes sum)
- unspecified impurities (identification threshold)
- total impurities
- reporting threshold

**Table 2034.2 – Reporting, identification and qualification of organic impurities in peptides obtained by chemical synthesis**

<table>
<thead>
<tr>
<th>Reporting threshold</th>
<th>Identification threshold</th>
<th>Qualification threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 0.1 per cent</td>
<td>&gt; 0.5 per cent</td>
<td>&gt; 1.0 per cent</td>
</tr>
</tbody>
</table>

---

**Synthetic peptides  Related peptides – main impurity test**

**OCTREOTIDE**

Octreotide

(...)

Related substances. Liquid chromatography (2.2.29): use the normalisation procedure.

Limits:
- unspecified impurities: for each impurity, maximum 0.5 per cent;
- total: maximum 2.0 per cent;
- reporting threshold: 0.1 per cent.

---

**GONADORELIN ACETATE**

Gonadorelin acetate

(...)

Related substances. Liquid chromatography (2.2.29).

Limits:
- impurity E: maximum 2.0 per cent;
- sum of impurities F and G: maximum 2.5 per cent;
- sum of impurities C and D: maximum 1.0 per cent;
- unspecified impurities: for each impurity, maximum 0.5 per cent;
- total: maximum 5.0 per cent;
- reporting threshold: 0.1 per cent.
**rDNA products**

**Tests**

- **Impurities with molecular masses higher than that of filgrastim**: Use size-exclusion chromatography (2.2.30) to determine the molecular mass distribution.
  - Retention time: Filgrastim monomer + 17 min to 20 min; dimer + 3 min to 5 min. The molecular mass of filgrastim is known to be in the range of 25-30 kDa.
  - The retention time of the filgrastim monomer is 28 min. The retention time of the filgrastim dimer is 31 min.
  - Impurities with molecular masses higher than that of filgrastim are present. The total of the impurities (as a percentage of the area) is not more than 2 per cent. The impurities are shown in the chromatogram.

- **Impurities with charges differing from that of filgrastim**:
  - In the electropherogram obtained with reference solution (a), the relevant impurity peaks are determined by considering the retention time of filgrastim (5.7 to 5.8 min).
  - Any impurity present in the filgrastim (5.7 to 5.8 min) is less than 0.5 per cent.

- **Related proteins**:
  - Use liquid chromatography (2.2.29) to assess the purity of the product.
  - Resolution: minimum 1.5 between the peaks due to filgrastim and related contaminants.
  - Symmetry factor: maximum 1.4 for the peak due to filgrastim.
  - Any impurity: for each impurity, maximum 1.0 per cent.
  - Total: maximum 2.0 per cent.

---

**A guide through sections of Ph. Eur. monographs**

**Synthetic peptide**

- **OCTREOTIDE**: Oktreotidum (01/2020-2414)

**MONOGRAPH SECTION**

- **Transparency section**
  - Present in synthetic peptide monographs.
  - Controlled by related substances test (synthetic peptides).
  - Not necessary exhaustive – impurities known to and shown to be detected by the test – based on information obtained and verified during monograph elaboration/revision.
Related proteins test - teriparatide

Test solution: 0.7 mg/mL
Column: 150 x 4.6 mm; 3µm; 300Å
Autosampler: 2-8 °C
Column temperature: 40 °C
Detection: UV 214 nm
Flow rate: 1.0 mL/min
Injection volume: 20 µL

Results obtained with 0.7 mg/mL solution of synthetic teriparatide

<table>
<thead>
<tr>
<th>Impurity</th>
<th>% Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>MetO8,MetO18 teriparatide</td>
<td>0.17</td>
</tr>
<tr>
<td>MetO8 teriparatide</td>
<td>0.51</td>
</tr>
<tr>
<td>MetO18 teriparatide</td>
<td>0.82</td>
</tr>
<tr>
<td>X</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Results obtained with 0.7 mg/mL solution of synthetic teriparatide

<table>
<thead>
<tr>
<th>Impurity</th>
<th>% Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>MetO8,MetO18 teriparatide</td>
<td>0.17</td>
</tr>
<tr>
<td>MetO8 teriparatide</td>
<td>0.51</td>
</tr>
<tr>
<td>MetO18 teriparatide</td>
<td>0.82</td>
</tr>
<tr>
<td>X</td>
<td>0.40</td>
</tr>
</tbody>
</table>
Related proteins test - teriparatide

Test solution: 0.7 mg/mL
Column: 150 x 4.6 mm; 3µm; 300Å
Autosampler: 2-8 °C
Column temperature: 40 °C
Detection: UV 214 nm
Flow rate: 1.0 mL/min
Injection volume: 20 µL

Results obtained with 0.7 mg/mL solution of synthetic teriparatide

<table>
<thead>
<tr>
<th>Impurity</th>
<th>% area</th>
</tr>
</thead>
<tbody>
<tr>
<td>MetO8,MetO18 teriparatide</td>
<td>0.17</td>
</tr>
<tr>
<td>MetO8 teriparatide</td>
<td>0.51</td>
</tr>
<tr>
<td>MetO18 teriparatide</td>
<td>0.82</td>
</tr>
<tr>
<td>X</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Impurities with molecular masses greater than that of teriparatide

Test solution: 1 mg/mL
Column: 8.0 x 300 mm; 5µm
Autosampler: 2-8 °C
Column temperature: ambient
Detection: UV 214 nm
Flow rate: 0.4 mL/min
Mobile phase: 0.10 % TFA, 25.0% ACN in water
Injection volume: 20 µL

<table>
<thead>
<tr>
<th>Peak (Impurity)</th>
<th>Retention time (min)</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (aggregates)</td>
<td>14.431</td>
<td></td>
</tr>
<tr>
<td>2 (dimer)</td>
<td>16.505</td>
<td></td>
</tr>
<tr>
<td>3 (monomer)</td>
<td>18.761</td>
<td>2.27</td>
</tr>
</tbody>
</table>
### Interpretation of monographs – case study #2a

**Impurities with molecular masses greater than that of teriparatide**

**Test solution:** 1 mg/mL  
**Column:** 8.0 x 300 mm; 5µm  
**Autosampler:** 2-8 °C  
**Column temperature:** ambient  
**Detection:** UV 214 nm  
**Flow rate:** 0.4 mL/min  
**Mobile phase:** 0.10 % TFA, 25.0% ACN in water  
**Injection volume:** 20 µL

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention time (min)</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (aggregates)</td>
<td>14.431</td>
<td></td>
</tr>
<tr>
<td>2 (dimer)</td>
<td>16.505</td>
<td></td>
</tr>
<tr>
<td>3 (monomer)</td>
<td>18.761 2.27</td>
<td></td>
</tr>
</tbody>
</table>

**Reference solution (b)**

**Test solution:** 1 mg/mL  
**Column:** 8.0 x 300 mm; 5µm  
**Autosampler:** 2-8 °C  
**Column temperature:** ambient  
**Detection:** UV 214 nm  
**Flow rate:** 0.4 mL/min  
**Mobile phase:** 0.10 % TFA, 25.0% ACN in water  
**Injection volume:** 20 µL

For information!
**Interpretation of monographs – case study #2b**

**Impurities with molecular masses greater than that of filgrastim**

**Test solution:** 0.4 mg/mL  
**Column:** 7.8 x 150 mm; 5 µm  
**Column temperature:** 30 °C  
**Detection:** UV 215 nm  
**Flow rate:** 0.3 mL/min  
**Mobile phase:** 4 g/L NH₄HCO₃ in water, pH 7.0  
**Injection volume:** 20 µL

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention time (min)</th>
<th>Relative retention (with ref. to monomer)</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (aggregates)</td>
<td>11.693</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>2 (oligomer 1)</td>
<td>13.214</td>
<td>0.81</td>
<td>3.24</td>
</tr>
<tr>
<td>3 (dimer)</td>
<td>14.032</td>
<td>0.86</td>
<td>1.23</td>
</tr>
<tr>
<td>4 (monomer)</td>
<td>16.238</td>
<td></td>
<td>3.02</td>
</tr>
<tr>
<td>5</td>
<td>18.956</td>
<td></td>
<td>4.47</td>
</tr>
</tbody>
</table>

**Peak Retention time (min) | Relative retention (with ref. to monomer) | Resolution**

1 (aggregates) | 11.693 | 0.72 |
2 (oligomer 1) | 13.214 | 0.81 | 3.24
3 (dimer)     | 14.032 | 0.86 | 1.23
4 (monomer)   | 16.238 |          | 3.02
5             | 18.956 |        | 4.47
Interpretation of monographs – case study #2b

Impurities with molecular masses greater than that of filgrastim

Test solution: 0.4 mg/mL
Column: 7.8 x 150 mm; 5 µm
Column temperature: 30 °C
Detection: UV 215 nm
Flow rate: 0.3 mL/min
Mobile phase: 4 g/L NH₄HCO₃ in water, pH 7.0
Injection volume: 20 µL

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention time (min)</th>
<th>Relative retention (with ref. to monomer)</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (aggregates)</td>
<td>11.693</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>2 (oligomer 1)</td>
<td>13.214</td>
<td>0.81</td>
<td>3.24</td>
</tr>
<tr>
<td>3 (dimer)</td>
<td>14.032</td>
<td>0.86</td>
<td>1.23</td>
</tr>
<tr>
<td>4 (monomer)</td>
<td>16.238</td>
<td>3.02</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>18.956</td>
<td>4.47</td>
<td></td>
</tr>
</tbody>
</table>

A guide through sections of Ph. Eur. monographs

Synthetic peptide

**OCTREOTIDE**
Oxtretidum

**DEFINITION**
Content: 95.0 per cent to 100.0 per cent (anhydrous and acetic acid-free substance).

**ASSAY**
- comparative chromatographic procedures using defined CRS as a standard
- content: anhydrous, acetic acid-free basis
- typically asymmetric limits
- protein content (comparative LC, UV spectroscopy)
- bioassay with ref. to WHO IS or Ph. Eur. standard calibrated in IU
- Exceptionally: in vivo tests; physicochemical tests only; example methods

rDNA product

**FILGRASTIM CONCENTRATED SOLUTION**
Filgrastimi solutio concentrata

**ASSAY**
- Protein: Liquid chromatography (2.2.29) as described in the test for related substances with the following modifications.
- Inject: test solution and reference solution.
- Calculate the content of filgrastim (C₃₇H₃₄N₁₄O₁₄S₄) taking into account the assigned content of C₃₇H₃₄N₁₄O₁₄S₄ in octreotide CRS.

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organization. (…)

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organization. (…)

__For information!__
### Interpretation of monographs – case study #3

**Assay:** filgrastim

<table>
<thead>
<tr>
<th>Lot</th>
<th>Stated potency</th>
<th>Estimated potency</th>
<th>Protein content</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0 x 10⁸ IU/ml</td>
<td>0.8 x 10⁸ IU/ml</td>
<td>1.0 mg/mL</td>
<td>0.8 x 10⁸ IU/mg</td>
</tr>
<tr>
<td>2</td>
<td>1.0 x 10⁸ IU/ml</td>
<td>1.25 x 10⁸ IU/ml</td>
<td>1.25 mg/mL</td>
<td>1.0 x 10⁸ IU/mg</td>
</tr>
<tr>
<td>3</td>
<td>1.0 x 10⁸ IU/ml</td>
<td>1.0 x 10⁸ IU/ml</td>
<td>1.25 mg/mL</td>
<td>0.8 x 10⁸ IU/mg</td>
</tr>
</tbody>
</table>
### Storage
- not mandatory
- decided by competent authority (may decide to make it mandatory)
- storage → to ensure compliance with the monographs
- Conventional expressions (e.g. *in an airtight container*) defined in General Notices

### Labelling
- covered by national and international regulations
- not comprehensive
- only statements necessary to demonstrate (non-) compliance are mandatory (e.g. nominal value for excipients)
- Label → container, package, leaflet, CoA

**General Notices (1) apply to all monographs and other texts**
See the information section on general monographs (cover page)
Knowledge Database – additional source of information

Ongoing revision
- scope
- state of work
- last issue of Pharmeuropa where the draft was published

Associated Reference Standards

Practical Info (e.g. column brand)

CEP holders

Summary

Individual monographs and relevant general monographs and chapters are complementary

Referenced general chapters become mandatory

Monograph sections
- Title
- Formula & CAS
- Mass
- Definition
- Production
- Characters
- Identification
- Tests
- Assay
- Storage
- Labelling
- Impurities

Consult Knowledge Database for additional monograph-associated information

Non mandatory
Thank you for your attention

Stay connected with the EDQM

EDQM Newsletter: https://go.edqm.eu/Newsletter
LinkedIn: https://www.linkedin.com/company/edqm/
Twitter: @edqm_news
Facebook: @EDQMCouncilofEurope