Ph. Eur. Reference Standards for Physico-Chemical tests of Biologicals

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Outline

• Introduction
  • Ph. Eur. RS in the European Pharmacopoeia
  • Classification of Ph. Eur. RS
  • Ph. Eur. CRS life cycle
• CRS for qualitative use
  • Case studies: purpose, examples, establishment
• CRS for quantitative use
  • Case studies: purpose, examples, establishment
• Take home messages
Ph. Eur. Reference standards in the European Pharmacopoeia

**General notices**

**Ph. Eur RS:**
- established under the aegis of and adopted by the European Pharmacopoeia Commission
- alone authoritative in case of arbitration

**General Chapter 5.12. 4/2015 (chapter for information)**
- the term “Reference standard” is used as a general term covering reference substances, preparations and spectra
- RS are used to achieve adequate quality control of substances for pharmaceutical use and pharmaceutical preparations
- Terminology, use, establishment, processing, labelling, storage and distribution, re-test programme

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European Pharmacopoeia: link between texts and reference standards
Ph. Eur. RS for biologicals

About 130 Reference Standards for Biologicals (CRS and BRP)

Bioassay
- **International Standards (WHO)**
  - Primary standards
  - Value assigned in International Units
- **BRP: Ph. Eur. Biological Reference Preparations**
  - Secondary standards calibrated in International Units

Physico-chemical tests
- **CRS: Ph. Eur. Chemical Reference Substances**
  - Normally established as primary standards
Ph. Eur. RS classification by type

- Main substance (identification) 45%
- Mixtures for SST/peak ID. 16%
- Impurities (quantitative) 11%
- Impurities (qualitative) 11%
- Main substance (assay) 17%

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

**Golden rules:**
- the intended purpose 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
Identification – Main substance

Purpose

Identification of substance subject of a monograph, e.g. by NMR, LC (cross-reference to assay section), peptide mapping, ...

Example: synthetic peptide (<1300 Da)

Other examples:
- Buserelin for NMR identification CRS
- Terlipressin for NMR identification CRS
- Heparin Ca/Na for NMR identification CRS

Identification – Main substance

Purpose

Identification of substance subject of a monograph, e.g. by NMR, LC (cross-reference to assay section), peptide mapping, ...

Example: rDNA protein

Other examples:
- Teriparatide CRS
- Follitropin for peptide mapping and glycan analysis CRS
- Human coagulation factor IX (rDNA) CRS...
Identification – Main substance

Establishment

Key quality attribute = identity

- The material selected complies with the relevant requirements of the monograph
- In addition, the characterisation goes further and the structure is elucidated applying a variety of techniques, including NMR (1H, COSY, TOCSY) and mass spectrometry

Remark: the CRS may also be used for additional purposes in the same or other monograph(s)

Identification – Impurity

Purpose

Identification of impurities of the substance subject of a monograph, often in a test for related substances using liquid chromatography method (LC), because of: specific limit for impurity

System suitability test of chromatographic method: selectivity: resolution, peak-to-valley ratio

Example: synthetic peptide

<table>
<thead>
<tr>
<th>BUSERELIN</th>
<th>Basorelin</th>
<th>LC</th>
<th>LOC 139</th>
</tr>
</thead>
</table>

Related substances. Liquid chromatography (2.2.29).

Reference solution (a): Dissolve a suitable amount of n-Busorelin CRS (impurity A) in the mobile phase, dilute an appropriate volume of this solution in the mobile phase to obtain a final concentration of 1 mg/mL. Add 1.0 mL of the test solution to 1.0 mL of this solution.

Identification of impurities: use the chromatogram obtained with reference solution (a) to identify the peak due to impurity A.

System suitability: reference solution (a):
- resolution: minimum 3.5 between the peaks due to impurity A and busorelin.
- Limits:
  - impurity A: maximum 2.5 per cent.

![Image of NMR and MS TIC](COSY/TOCSY – Goserelin for NMR identification CRS)
Identification – Impurity

Establishment
Key quality attribute = identity
In general, the material is further characterised:
- chromatographic purity using method of intended use
- the intended use is verified
- the structure is elucidated by NMR

Important: only the information necessary for the intended use(s) is provided; no additional information e.g. purity, etc. is provided

Mixtures for synthetic peptides

Purpose
Identification of impurities of the monograph substance, often in a test for related substances using a chromatographic method (LC), because of:

specific limit for impurity

System suitability test of chromatographic method:
selectivity: resolution, peak-to-valley ratio

Composition (see monograph): several impurities with/without main compound

“for system suitability CRS”, “for peak identification CRS”, “validation mixture CRS”

Examples: Buserelin for peak identification CRS
Oxytocin/desmopressin validation mixture CRS
Mixtures for rDNA proteins

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 latters may be variable and not necessarily reproducible

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
2) Test for aggregates

- Erythropoietin concentrated solution (1316)
  Reference solution: 2% dilution of the test solution for system suitability purposes
  -> will be replaced by Erythropoietin for system suitability CRS with a defined dimer content

Mixtures

Establishment
Key quality attributes:

Identity of impurities:
- normally confirmed by spiking with individual impurity samples

Fitness for purpose:
- established using the method of intended use
- impurities present in sufficient amount for peak detection / identification
- system suitability assessment

Homogeneity:
- important, especially in case of stressed/degraded samples
Mixtures

Information provided

Often a chromatogram in the CRS leaflet -> explicitly mentioned in the monograph
No additional information e.g. about amount of impurities etc. is provided.

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:

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

the extent of testing is greater than when a CRS is used for other purposes *(Ph. Eur. chapter 5.12.)*
Characterisation for RS processing

Peptides and proteins are often hygroscopic substances
- Sorption-desorption study (SDS) to establish appropriate handling conditions for the bulk material

Uptake of 2.8% of mass after 18 minutes at 40%RH

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
Assay CRS – establishment

- **1st step: characterisation of the bulk material**
  - Verification of compliance with the monograph
  - Confirmation of identity by orthogonal methods (NMR, TOF-MS)
  - Assignment of a content to the bulk material based on a **mass balance approach** taking into account **water content, acetate** (or any other ion) and **related peptides**
  - Confirmation of purity by orthogonal methods (qNMR)

- **2nd step: content assignment**
  - Determination of homogeneity
  - Determination of mg of peptide or protein/vial by LC assay in the CRS candidate against the bulk material as external standard
  - Assigned value checked by orthogonal techniques (qNMR)

Inter-laboratory study (usually n=5 laboratories)

Assigned content – Where to find the information?

**Example: Teriparatide Leaflet**

**Assay section:**
*Calculate the percentage content of teriparatide* \((C_{181}H_{291}N_{55}O_{51}S_{2})\) *taking into account the assigned content of teriparatide CRS*

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**INFORMATION LEAFLET**

Ph. Eur. Reference Standard

**Teriparatide CRS batch 1**

1. **Identification**
   - Catalogue code: Y0001916
   - Unit Quantity: ca 1 mg

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): 2829.

   2.2 **Analytical information related to intended use**
   - Chromatogram(s)/spectrum: Identification by peptide mapping (annex 1)
   - Test for impurities with molecular masses higher than that of teriparatide (SEC) (annex 2)
   - The "as is" content is **0.91 mg per vial**.
**Monitoring (retest-programme)**

**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
- The frequency depends on the intended use and the stability information (12, 24, 36 or 60 months)
- The properties retested are those that might change in the life cycle of a CRS, e.g.:
  - Related proteins by LC

### References substances database

<table>
<thead>
<tr>
<th>Catalogue Code (V)</th>
<th>Name</th>
<th>Current batch number</th>
<th>Unit quantity</th>
<th>Sale unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>V0001914</td>
<td>Teriparatide</td>
<td>1</td>
<td>1 mg</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

**ADDITIONAL INFORMATION**

- Related proteins by LC
- No expiry date is given: see batch validity statement
- Used in monograph/2829
- Assigned content: see leaflet
- CAS Registry Number: 52222-67-9
- Presentation: vial
- Origin: v/a
- Proposed import EUDMD code: 2017/155
- EUDMD long term storage conditions: 2-8°C ± 5°C
- Dispatching conditions: 1°C - 10°C
- UPI Code: Not classified
- Shipping group: Class I
- Price: 79 EUR
- Availability: Available
- Sales restriction: N
Take home messages (1)

**Ph. Eur. CRS**

- 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
- Sustainability of supply must be ensured
- Drift between consecutive batches must be avoided
- EDQM provides RS information (leaflet) and assistance (Helpdesk)
- Ph. Eur. policy on reference standard is reflected in general chapter 5.12. (last revision in 2015)
Thank you for your attention