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

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European Pharmacopoeia training session on Biologicals
4-5 February 2020
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


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., 7/2018 corrected 10.0 (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 medicinal products and their components
• terminology, use, establishment, processing, labelling, storage and distribution, re-test programme
Ph. Eur.: link between texts and reference standards

Reference Standards

Methods

Specifications

Ph. Eur. RS for biologicals

About 140 Reference Standards for Biologicals (CRS and BRP)

Distribution unit of RS for rDNA proteins

Increasing need

Index base 100: 1995
Ph. Eur. RS classification

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 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
Ph. Eur. RS classification by type

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

CRS lifecycle

- Procurement
  - Processing Packaging
    - Quality Control
  - Adoption
    - Release Distribution
    - Information leaflet
  - Characterization Establishment
- Monitoring
- Replacement Batch needed
- Help Desk
- USERS
- Validity statement
- Ph. Eur. need
Qualitative use

CRS for identification – Main substance

**Purpose**

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

**Example:** synthetic peptide (<1300 Da)

Other examples:
- Buserelin for NMR identification CRS
- Terlipressin for NMR identification CRS
- Octreotide for NMR identification CRS
- Heparin Ca/Na for NMR identification CRS
CRS for peak identification of the main substance

**Purpose**

Identification of fragments of substance subject of a monograph, e.g. by peptide mapping, ...

**Example:** rDNA protein

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**CRS for 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** ($^1$H, COSY, TOCSY) and **mass spectrometry**

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Other examples:
- Teriparatide CRS
- Follitropin for peptide mapping and glycan analysis CRS
- Human coagulation factor IX (rDNA) CRS, Etanercept CRS, Infliximab CRS
CRS for 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

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**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
CRS 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”, “impurity mixture CRS”

**Examples:**
- Buserelin for peak identification CRS
- Oxytocin/desmopressin validation mixture CRS
- Terlipressin impurity mixture CRS
- Octreotide impurity mixture CRS

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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
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

2) Test for aggregates

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

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**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.)*
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

![Image of INFORMATION LEAFLET Ph. Eur. Reference Standard](image)

Leaflet - Where to find the information?

References substances database

Example: Teriparatide

<table>
<thead>
<tr>
<th>Catalogue Code</th>
<th>Name</th>
<th>Batches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y0001916</td>
<td>Teriparatide CRS</td>
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<tr>
<td>Current batch number</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Unit quantity per vial</td>
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<td></td>
</tr>
<tr>
<td>Number of vials per unit</td>
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<td></td>
</tr>
<tr>
<td>Lined or non-lined</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Assigned content</td>
<td>See Leaflet</td>
<td></td>
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<tr>
<td>Additional information</td>
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<td>Link to download the leaflet</td>
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<td>CRS Registry Number</td>
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<td></td>
</tr>
<tr>
<td>Presentation</td>
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</tr>
</tbody>
</table>

[Link to download Origin Of Goods pdf]
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

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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.

Thank you for your attention

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