Ph. Eur. Reference Standards for Synthetic Peptides

Dr Sylvie Jorajuria
Laboratory Department

European Pharmacopoeia training session on Biologicals
4-5 February 2020
CRS for synthetic peptides: intended purpose

- **Qualitative use:**
  - CRS for NMR identification
  - Impurity CRS (peak ID, SST)
  - Impurity mixture CRS (peak ID, SST)
  - CRS containing the API and one or more impurities

- **Quantitative use:**
  CRS for use in the LC assay

Case study: Terlipressin

- synthetic dodecapeptide with a cyclic structure (S-S bridge)
- analogue of the natural hormone vasopressin
- used as a vasoactive drug in the management of hypotension
Reference standards in Terlipressin monograph

<table>
<thead>
<tr>
<th>3 tests involve use of a CRS</th>
<th>3 monograph sections</th>
<th>4 types of purposes</th>
<th>3 CRS</th>
<th>Unit quantity/vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear magnetic resonance spectrometry (NMR, 2.2.64.)</td>
<td>Identification</td>
<td>Qualitative: - for identification of the main substance</td>
<td>Terlipressin for NMR identification CRS</td>
<td>ca 2.9 mg</td>
</tr>
<tr>
<td>Related substances (LC) (2.2.29.)</td>
<td>Test</td>
<td>Qualitative: - for peak identification - for method evaluation</td>
<td>Terlipressin impurity mixture CRS</td>
<td>ca 0.03 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Terlipressin CRS</td>
<td>ca 1 mg</td>
</tr>
<tr>
<td>LC assay (2.2.29.)</td>
<td>Assay</td>
<td>Quantitative</td>
<td>Terlipressin CRS</td>
<td>ca 1 mg</td>
</tr>
</tbody>
</table>

-> Terlipressin is hygroscopic: the 3 CRS are freeze dried materials

Terlipressin for NMR identification CRS

**Purpose**

**Identification** of substance subject of a monograph

**Nuclear magnetic resonance spectrometry (2.2.64.)**

- Comparison: dissolve the contents of a vial of terlipressin for NMR identification CRS in deuterium oxide R containing 20 μg/mL of deuterated sodium trimethylsilylpropionate R, adjusted to pH 4 with deuterated acetic acid R, to obtain a concentration of 2.9 mg/mL.

- Results: The $^1$H NMR spectrum obtained is qualitatively similar to the $^1$H NMR spectrum obtained with terlipressin for NMR identification CRS.

-> the CRS is used for sample compliance testing
The material selected complies with the relevant requirements of the monograph.

In addition, the characterisation goes further and the structure is elucidated applying:

**Mass spectrometry:**
- exact mass measurement (qTOF-MS) +
- amino acid sequencing (qTOF-MS-MS)

**NMR:**
- ^1^H NMR, additional 2D NMR (HSQC, COSY, TOCSY, NOESY, HMBC)

-> the CRS spectrum is not given in the leaflet as it is not needed
Terlipressin impurity mixture CRS

**Purpose**

*Identification* of impurities of the monograph substance in the test for related proteins by LC because of:

**specific limit for impurity**

**System suitability** test of chromatographic method:

**selectivity**: resolution

**Related substances.** Liquid chromatography (2.2.29).

Solution A. Dissolve the contents of a vial of terlipressin impurity mixture CRS (containing impurities A, D and L) in 1.0 mL of a 9 g/L solution of sodium chloride R.

Identification of impurities: use the chromatogram supplied with terlipressin impurity mixture CRS and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, D and L.

**System suitability:**

- **resolution**: minimum 1.4 between the peak due to impurity A and the principal peak in the chromatogram obtained with reference solution (c); the peaks due to impurities L and D are separated as shown in the chromatogram supplied with terlipressin impurity mixture CRS.

**Limits:**

- **impurity D**: maximum 0.6 per cent;
- unspecified impurities: for each impurity, maximum 0.5 per cent;
- **total**: maximum 1.5 per cent;
- **reporting threshold**: 0.1 per cent.

---

Terlipressin impurity mixture CRS

**Establishment**

Key quality attributes:

**Identity of impurities:**

- TOF-MS identification of individual impurity A, D and L samples
- confirmed in the mixture by spiking with individual authentic samples of impurity A, D and L

**Fitness for purpose:**

- established using the LC method described in the monograph
- impurities present in sufficient amount for peak detection / identification
- system suitability assessment
Terlipressin impurity mixture CRS

-> the CRS chromatogram is provided in the leaflet as it is needed and explicitly mentioned in the monograph
-> no additional information e.g. about amount of impurities is provided

Terlipressin CRS

• **Purpose**
  Qualitative (system suitability in test for related substances)
  **Quantitative** (assay)

• **Assay**
  LC (2.2.29) as described in the test for related substances with the following modification:
  Injection: 200 µL of the test solution and reference solution (a)
  Calculate the percentage content of terlipressin (C_{52}H_{74}N_{16}O_{15}S_{2}) taking into account the **assigned content** of C_{52}H_{74}N_{16}O_{15}S_{2} in terlipressin CRS

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

- **1st step: characterisation of the bulk material**
  - Verification of compliance with the monograph
  - Confirmation of identity by orthogonal methods: NMR, qTOF-MS, qTOF-MS-MS
  - Assignment of a content to the bulk material based on a mass balance approach taking into account water content, acetic acid and related substances
  - Confirmation of purity by orthogonal methods (qNMR)

- **2nd step: content assignment**
  - Determination of between-vial homogeneity
  - Determination of mg of peptide/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 (n=5 laboratories)**

---

**Terlipressin CRS**

**Content is expressed in mg of peptide/vial ("as is")**

<table>
<thead>
<tr>
<th>INFORMATION LEAFLET Ph. Eur. Reference Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terlipressin CRS batch 1</td>
</tr>
</tbody>
</table>

1. **Identification**
   - Catalogue code: Y0001897
   - 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): 2646.

   2.2 **Analytical information related to intended use, when applicable**
      - The "as is" content is: **1.00 mg of C52H74N16O15S2 per vial**

A **value** assigned to a reference standard is valid for the intended use and not necessarily for other uses [Ph.Eur. Chapter 5.12.]
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

©2020 EDQM, Council of Europe. All rights reserved.