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Ph. Eur. Reference Standards for Synthetic Peptides

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European Pharmacopoeia training session on Biologicals
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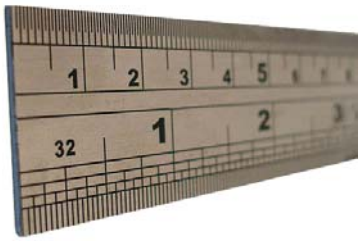
CRS for synthetic peptides: intended purpose

- **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



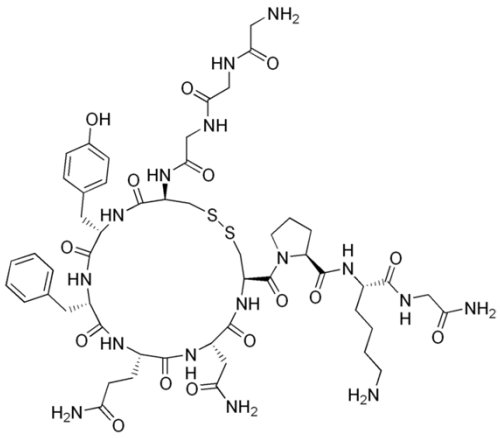
Case study: Terlipressin

TERLIPRESSIN

Terlipressinum

H·Gly·Gly·Gly·Cys·Tyr·Phe·Gln·Asn·Cys·Pro·Lys·Gly·NH_2

$C_{52}H_{74}N_{16}O_{15}S_2$ M_r 1227
[14636-12-5]



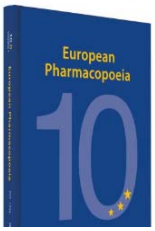
- synthetic dodecapeptide with a cyclic structure (S-S bridge)
- analogue of the natural hormone vasopressin
- used as a vasoactive drug in the management of hypotension

Reference standards in Terlipressin monograph

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

-> 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

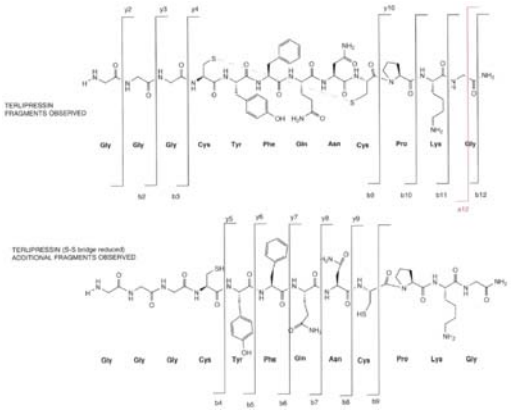
Terlipressin for NMR identification CRS

Establishment

Key quality attribute = **identity**

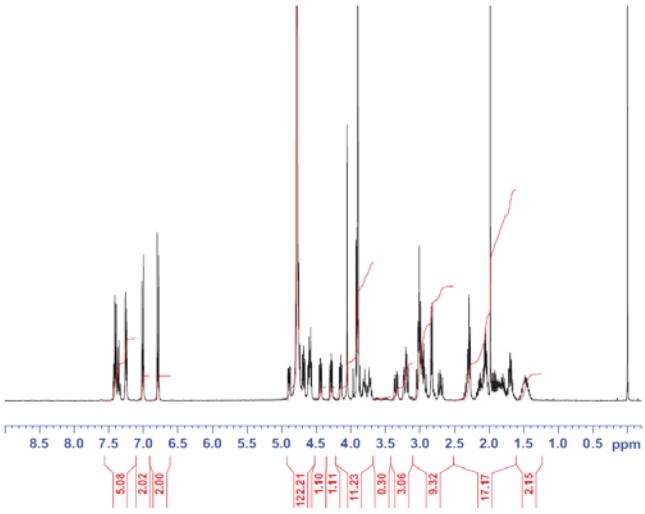
- The material selected complies with the relevant requirements of the monograph
- In addition, the characterisation goes further and the structure is elucidated applying:

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



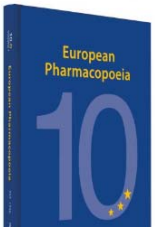
Terlipressin for NMR identification CRS

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:

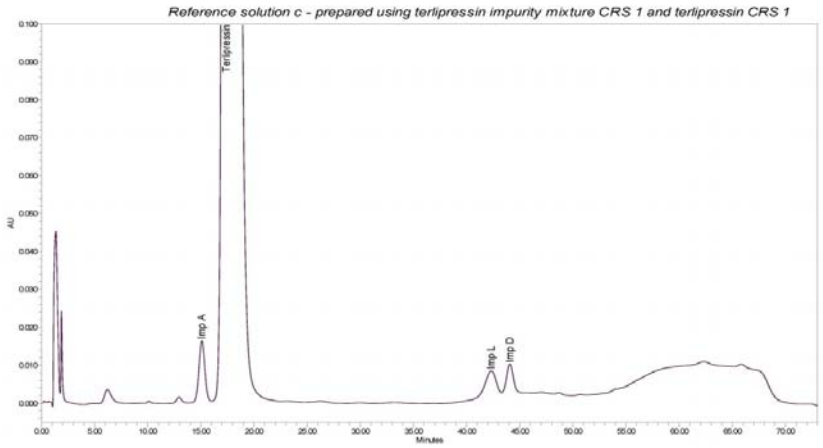
Identity of impurities:

- 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

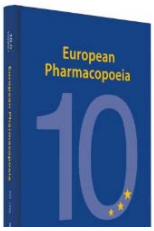
Terlipressin impurity mixture CRS



-> 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.)***

Terlipressin CRS

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")

INFORMATION LEAFLET Ph. Eur. Reference Standard

Terlipressin CRS batch 1

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.]*

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