THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

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

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European Pharmacopoeia training session on 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 Ph. Eur.

General notices

Ph. Eur RS:

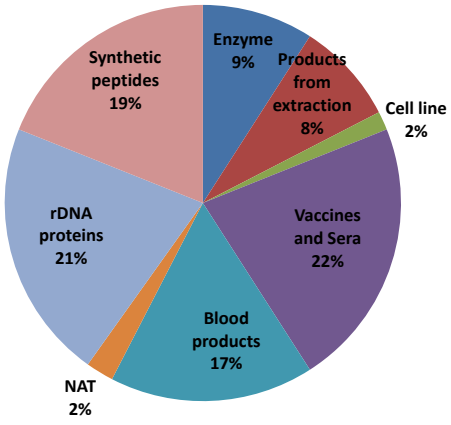
- established under the aegis of and adopted by the European Pharmacopoeia Commission
- alone authoritative in case of arbitration

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

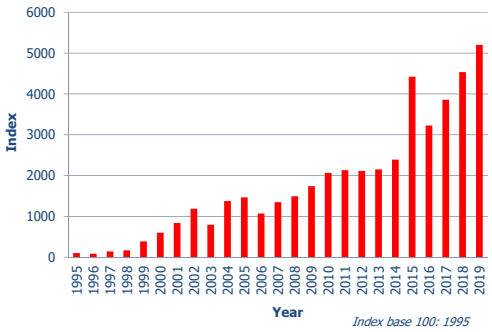
Ph. Eur.: link between texts and reference standards

Ph. Eur. RS for biologicals



About 140 Reference Standards for Biologicals (CRS and BRP)

Distribution unit of RS for rDNA proteins



Increasing need

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

http://www.who.int/biologicals/reference_preparations/en/

Physico-chemical tests

- **CRS: Ph. Eur. Chemical Reference Substances**
  - Normally established as primary standards

https://www.edqm.eu/en/ph-eur-reference-standards-orders-catalogue

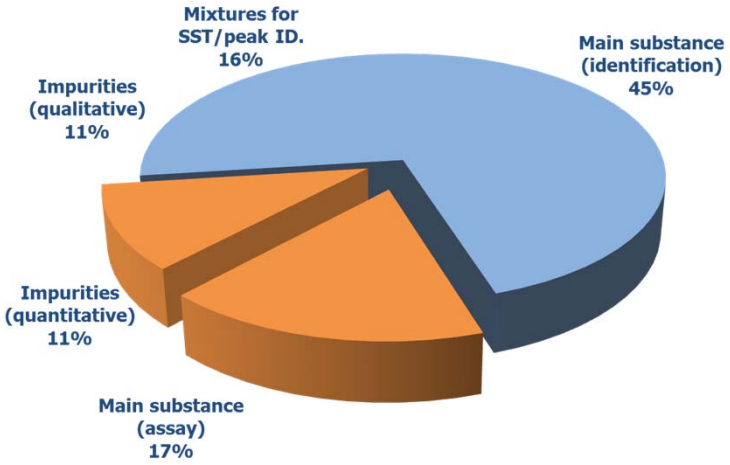
Ph. Eur. RS classification by intended use

- **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**

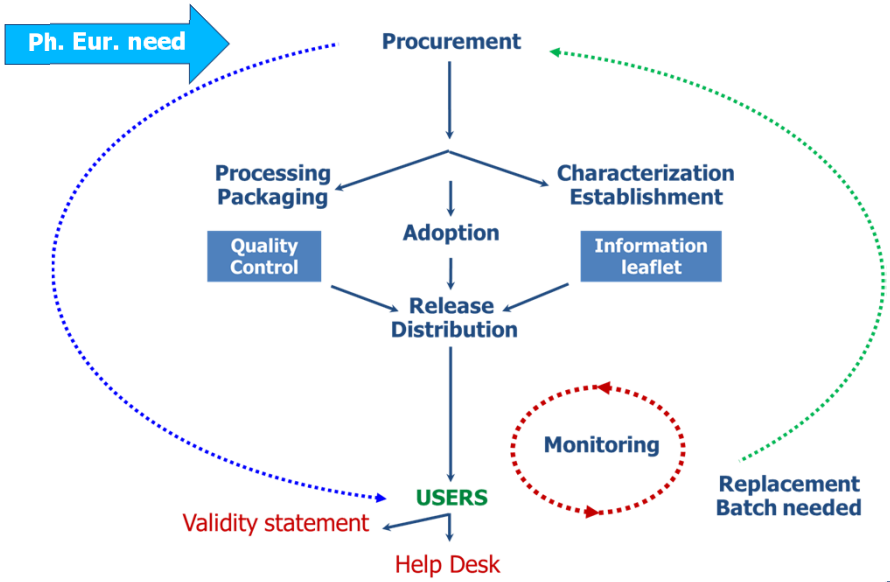
Golden rules:

- **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



CRS lifecycle



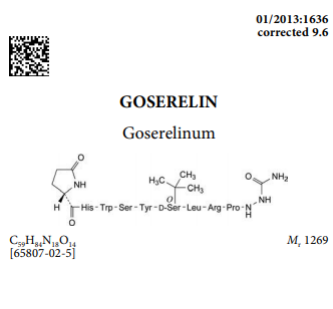
Qualitative use

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)



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GOSERELIN

Goserelinum

$C_{59}H_{84}N_{18}O_{14}$
[65807-02-5]

M_r 1269

IDENTIFICATION

Carry out either tests A and B or tests B and C.

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

Preparation: 13 mg/mL solution in *0.2 M deuterated sodium phosphate buffer solution pH 5.0 R* containing 20 µg/mL of *deuterated sodium trimethylsilylpropionate R*.

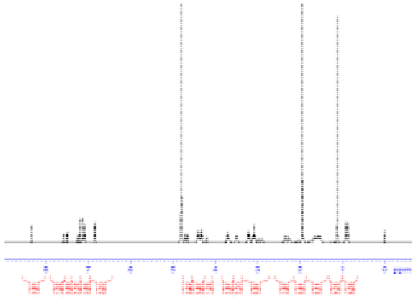
Comparison: 13 mg/mL solution of *goserelin for NMR identification CRS* in *0.2 M deuterated sodium phosphate buffer solution pH 5.0 R* containing 20 µg/mL of *deuterated sodium trimethylsilylpropionate R* (dissolve the contents of a vial of *goserelin for NMR identification CRS* in this solvent to obtain the desired concentration).

Operating conditions:

– *field strength*: minimum 300 MHz;

– *temperature*: 25 °C.

Results: examine the ^{1}H NMR spectrum from 0 ppm to 9 ppm; the ^{1}H NMR spectrum obtained is qualitatively similar to the ^{1}H NMR spectrum obtained with *goserelin for NMR identification CRS*.



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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INSULIN GLARGINE

Insulinum glarginum

$C_{267}H_{404}N_{72}O_{78}S_6$ M_r 6063

IDENTIFICATION

B. Peptide mapping (*2.2.55*).

Reference solution. Prepare at the same time and in the same manner as for the test solution but using *insulin glargine CRS* instead of the substance to be examined.

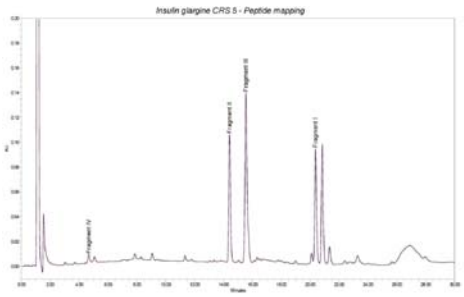
System suitability:

- the chromatogram obtained with the reference solution is qualitatively similar to the chromatogram of insulin glargine digest supplied with *insulin glargine CRS*;
- in the chromatogram obtained with the reference solution, identify the peaks due to digest fragments II and III:

symmetry factor: maximum 1.5 for the peaks due to fragments II and III;

resolution: minimum 3.4 between the peaks due to fragments II and III.

Results: the profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.



Other examples:

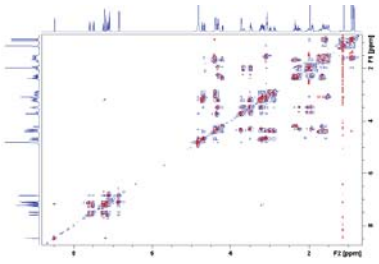
- Teriparatide CRS
- Follitropin for peptide mapping and glycan analysis CRS
- Human coagulation factor IX (rDNA) CRS, Etanercept CRS, Infliximab CRS

CRS for identification – Main substance

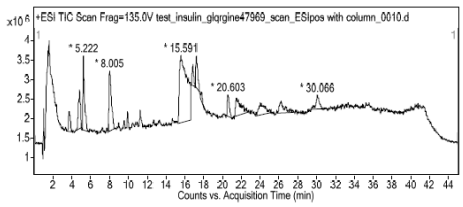
Establishment

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**



COSY/TOCSY – Goserelin for NMR identification CRS



MS TIC (Total Ion Chromatogram) – Insulin glargine CRS

CRS for identification – Impurity

Purpose

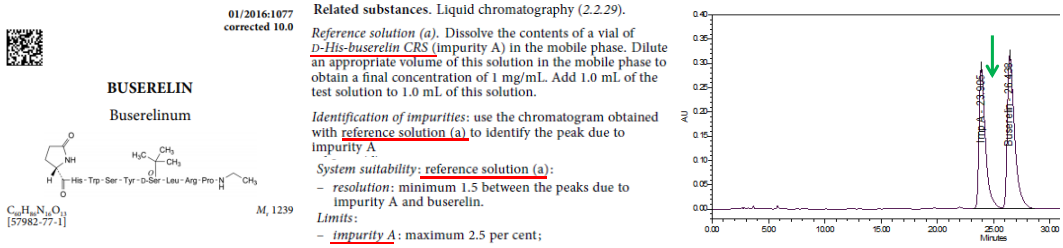
Identification of impurities of the substance subject of a monograph, often in a test for related substances using liquid chromatography method (LC), because of:

specific limit for impurity

System suitability test of chromatographic method:

selectivity: resolution, peak-to-valley ratio

Example: synthetic peptide



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BUSERELIN

Buserelinum

His · Trp · Ser · Tyr · D-Ser · Leu · Arg · Pro · NH-CH3

$C_{60}H_{86}N_{16}O_{13}$ M_r 1239
[57982-77-1]

Related substances. Liquid chromatography (*2.2.29*).

Reference solution (a). Dissolve the contents of a vial of *D-His-buserelin 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 1.5 between the peaks due to impurity A and buserelin.

Limits:

– *impurity A*: maximum 2.5 per cent;

CRS for 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

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

CRS Mixtures for rDNA proteins

Purpose

To assess the system suitability test of chromatographic method (resolution, peak-to-valley ratio)

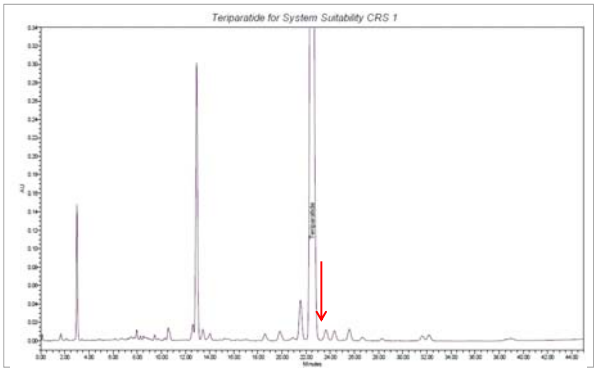
➡ Complex pattern of related proteins:

- 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*

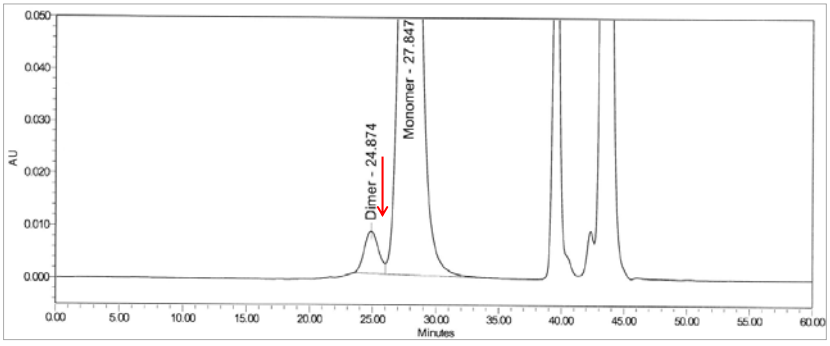


- Other examples: *Somatropin/desamidosomatropin resolution mixture CRS, Interferon gamma-1b for system suitability CRS* with increased deamidated and oxidised forms

CRS Mixtures for rDNA proteins

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



CRS 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

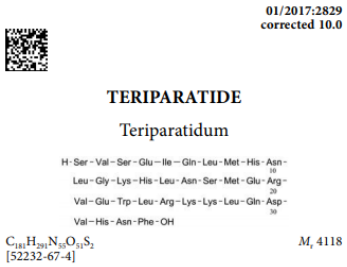
Homogeneity:

- important, especially in case of stressed/degraded samples

CRS 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



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TERIPARATIDE

Teriparatidum

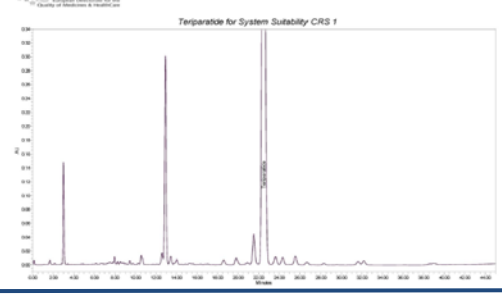
H-Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-
Leu-Gly-Lys-His-Leu-Asn-Ser-Met-Glu-Arg-
Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-
Val-His-Asn-Phe-OH

$C_{181}H_{291}N_{55}O_{51}S_2$ [52232-67-4] M_r 4118

Related proteins. Liquid chromatography (*2.2.29*)

System suitability: resolution solution:

– the chromatogram obtained is similar to the chromatogram supplied with *teriparatide for system suitability CRS*;



Quantitative use
Assay CRS

Reference standard for biologicals: assignment of content

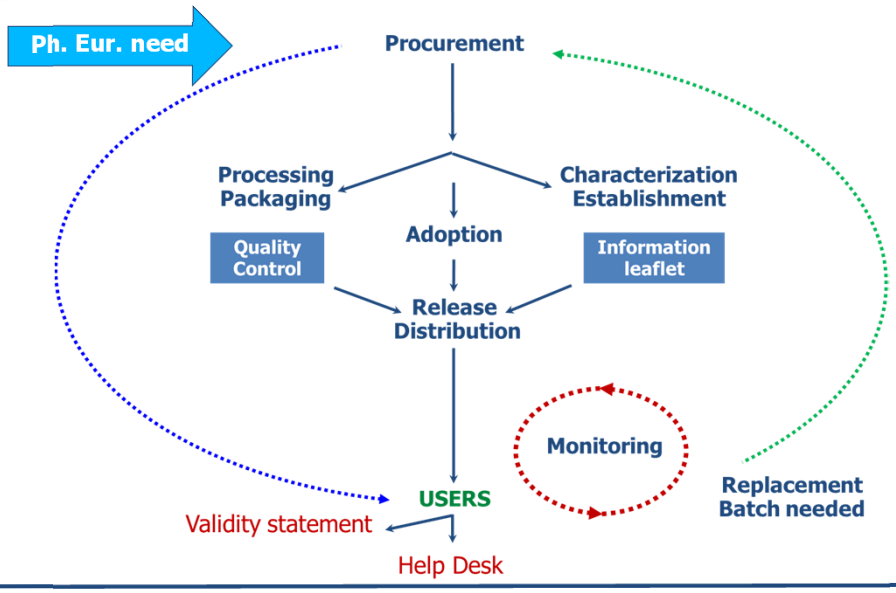
The procedures for assigning a content to a RS depends on the type of unit of measurement:

- **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.)***

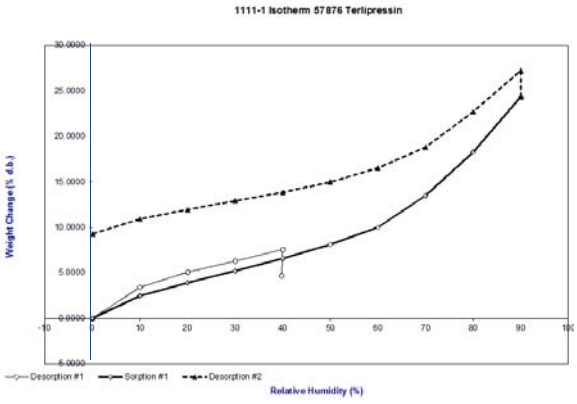
CRS lifecycle



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

INFORMATION LEAFLET Ph. Eur. Reference Standard

Teriparatide CRS batch 2

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 : **0.95 mg of C181H291N55O51S2 per vial**

Leaflet - Where to find the information?

References substances database

Search European Pharmacopoeia Reference Standards

Example: Teriparatide

Catalogue Code	Y0001916
Name	Teriparatide CRS
Current batch number	2
Unit quantity per vial	1 mg
Number of vials per sales unit	1
Used in monograph(s)	2829
Assigned content	see leaflet
Additional information	
Leaflet	click to download the leaflet
Chemical hazard	none identified
Biological hazard	none identified
SDS Product Code	
CAS Registry Number	52232-67-4
Presentation	
Origin	click to download Origin Of Goods.pdf
Proposed Import HS code	293719
EDQM long term storage conditions	-20°C ± 5°C
Dispatching conditions	Ice -20°C
UN Code	Not classified
Shipping group	C1a
Price*	79 EUR
Availability	Available
Sales restriction	No

Batches: batch 2 is valid at this date

Print BVS

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

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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