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
CONSEIL DE L'EUROPE

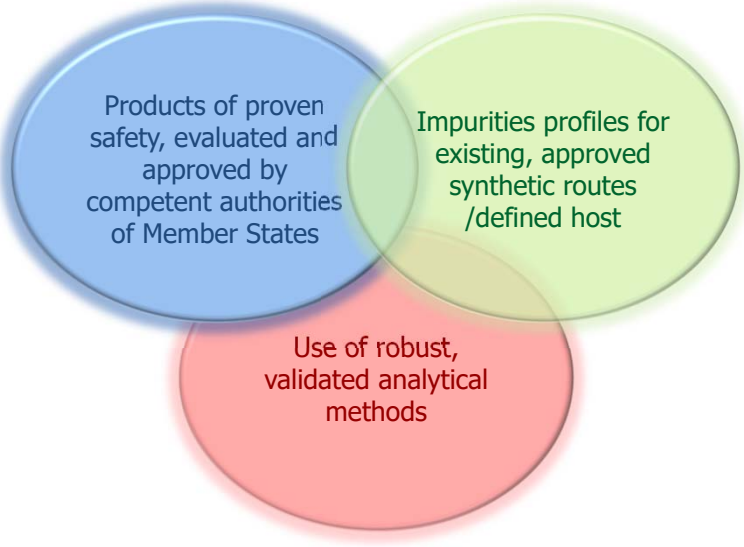
A guide through individual monographs

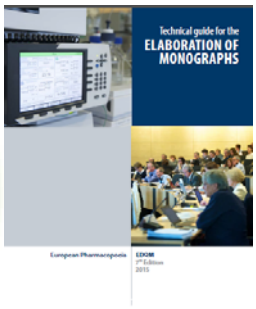
European Pharmacopoeia Training Session on Biologicals

4-5 February 2020

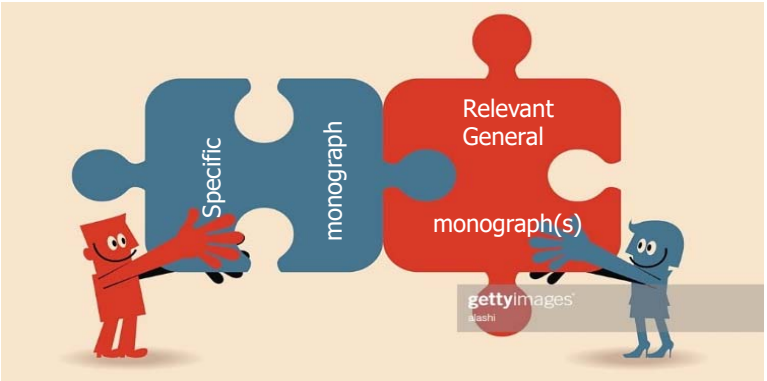
Olga Kolaj-Robin, PhD

Basis for monograph elaboration





Complementarity of specific and general monographs



Synthetic peptides and BTPs Ph. Eur. monograph portfolios

Synthetic peptides – – Ph. Eur. monograph portfolio

- Buserelin (1077)
- Calcitonin (salmon) (0471)
- Desmopressin (0712)
- Felypressin (1634)
- Gonadorelin acetate (0827)
- Goserelin (1636)
- Leuprorelin (1442)
- Octreotide (2414)
- Oxytocin (0780) §
- Oxytocin concentrated solution (0779)
- Protirelin (1144)
- Somatostatin (0949)§
- Terlipressin (2646)
- Tetracosactide (0644)§

New monographs in preparation

- *Atosiban (3054)*
- *Glatiramer (3057)*
- *Glatiramer injection (3104)**
- *Lanreotide (3056)*
- *Triptorelin (3055)*

** finished product monographs; § under revision*

Biotherapeutics – Ph. Eur. monograph portfolio

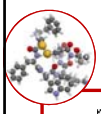
- Alteplase for injection (1170)*
- Calcitonin salmon (0471)
- Erythropoietin concentrated solution (1316)§
- Etanercept (2895)
- Filgrastim concentrated solution (2206)
- Filgrastim injection (2848)*
- Follitropin (2285)
- Follitropin concentrated solution (2286)
- Glucagon, human (1635)
- Human coagulation factor IX (rDNA) powder for solution for injection (2994)*
- Human coagulation factor IX rDNA concentrated solution (2522)
- Human coagulation factor VIIa rDNA concentrated solution (2534)
- Human coagulation factor VIII rDNA (1643)*§
- Infliximab concentrated solution (2928)
- Insulin aspart (2084)
- Insulin glargine (2571)
- Insulin lispro (2085)
- Insulin preparations injectable (0854)*
- Insulin, human (0838)
- Interferon alfa-2 concentrated solution (1110)
- Interferon gamma-1b concentrated solution (1440)
- Molgramostim concentrated solution (1641)
- Somatropin (0951) §
- Somatropin concentrated solution (0950) §
- Somatropin for injection (0952)*§
- Somatropin solution for injection (2370)*§
- Teriparatide (2829)

New monographs in preparation

- *Pegfilgrastim (2889)*
- *Darbepoetin alfa (3009)*
- *Golimumab (3103)*
- *Human coagulation factor VIII (rDNA concentrated solution (3105)*
- *Human coagulation factor VIII (rDNA) powder for injection (3106)**
- *Human coagulation factor VIII (rDNA), B-domain deleted, concentrated solution (3107)*
- *Human coagulation factor VIII (rDNA), B-domain deleted, powder for injection (3108)**
- *Insulin glargine injection (3129)**
- *Teriparatide injection (3130)**

** finished product monographs; § under revision*

A guide through sections of Ph. Eur. monographs



Synthetic peptide

MONOGRAPH SECTION

rDNA product

01/2020:2414

Version date

07/2019:2206
corrected 10.0

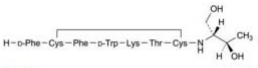
OCTREOTIDE

Octreotidum

Title
INN

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata



$C_{49}H_{66}N_{10}O_{10}S_2$
[83150-76-9]

M_r 1019

Formula
molecular and graphic
Relative mass
glycosylated protein except for complex glycoproteins
CAS number

$C_{845}H_{1339}N_{225}O_{245}S_9$
[121181-53-1]

M_r 18 799

A guide through sections of Ph. Eur. monographs

Synthetic peptide

01/2020:2414

OCTREOTIDE

Octreotidum

DEFINITION

D-Phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-L-cysteinyl-L-threoninol cyclic (2→7)-disulfide.

Synthetic octapeptide analogue of the natural hormone somatostatin. It is available as an acetate.

Content: 95.0 per cent to 103.0 per cent (anhydrous and acetic acid-free substance).

MONOGRAPH SECTION

Definition

- chemical nomenclature
- identity and biological activity
- physical form, salt form
- additives (*e.g.* oxytocin conc. sln.)*
- assay limits:

- content (mass/volume or mass/mass)
- potency (IU/mg) (synthetic peptides: by convention if present *e.g.* oxytocin, tetracosactide, calcitonin; rDNA proteins: *e.g.* somatropin, insulin)

rDNA product

07/2019:2206
corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

DEFINITION

Solution of a protein having the primary structure of the 174-amino-acid isoform of human granulocyte colony-stimulating factor (huG-CSF) plus 1 additional amino acid, an *N*-terminal methionine. In contrast to its natural counterpart, the protein is not glycosylated. huG-CSF is produced and secreted by endothelial cells, monocytes and other immune cells. The protein stimulates the differentiation and proliferation of leucocyte stem cells into mature granulocytes.

Content: minimum 0.9 mg of protein per millilitre.

Potency: minimum 0.9×10^8 IU per milligram of protein.

* ***Substances for Pharmaceutical Use (2034) :*** "A monograph is applicable to a substance **processed with an excipient** only where such processing is mentioned in the **definition section** of the monograph."

A guide through sections of Ph. Eur. monographs

Synthetic peptide

01/2020:2414

OCTREOTIDE

Octreotidum

(...)

MONOGRAPH SECTION

Production

- absent for synthetic peptides;
- extensive for vaccines;
- may be present for chemicals;
- may contain specific tests for rDNA products;
- source materials, manufacturing process, validation, control, in-process testing;
- mandatory for manufacturers;
- independent verification difficult
- compliance: competent authorities

Characters*

- Appearance, hygroscopicity, crystallinity, solubility
- useful info for analyst
- not analytical requirement

CHARACTERS

Appearance: white or almost white powder, hygroscopic.

Solubility: freely soluble in water, in acetic acid and in methanol.

CHARACTERS

Appearance: clear, colourless or slightly yellowish liquid.

rDNA product

07/2019:2206
corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(...)

PRODUCTION

Filgrastim concentrated solution is produced by a method based on recombinant DNA (rDNA) technology, using bacteria as host cells.

Prior to release, the following tests are carried out on each batch of filgrastim concentrated solution, unless exemption has been granted by the competent authority.

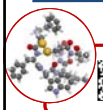
Host-cell-derived proteins. The limit is approved by the competent authority.

Host-cell- or vector-derived DNA. The limit is approved by the competent authority.

* See also 5.11 Characters section in monographs

A guide through sections of Ph. Eur. monographs

Synthetic peptide



OCTREOTIDE 01/2020:2414

Octreotidum

(…)

IDENTIFICATION

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

A. Examine the chromatograms obtained in the assay.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

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

Preparation: 2 mg/mL solution in a mixture of 10 volumes of *deuterated acetic acid R* and 90 volumes of *deuterium oxide R* containing 30 µg/mL of *deuterated sodium trimethylsilylpropionate R*.

Comparison: 2 mg/mL solution of *octreotide for NMR identification CRS* in a mixture of 10 volumes of *deuterated acetic acid R* and 90 volumes of *deuterium oxide R* containing 30 µg/mL of *deuterated sodium trimethylsilylpropionate R*.

Operating conditions:

– *field strength*: minimum 300 MHz;

– *temperature*: 25 °C.

Results: examine the ^{1}H NMR spectrum from 0 to 8 ppm. The ^{1}H NMR spectrum obtained is qualitatively similar to the ^{1}H NMR spectrum obtained with *octreotide for NMR identification CRS*.

C. Amino acid analysis (*2.2.56*). Method 1 for hydrolysis and method 1 for analysis are suitable.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids taking 1/4 of the sum of the number of moles of phenylalanine, threonine and lysine as equal to 1. The values fall within the following limits: threonine: 0.7 to 1.1; threoninol: 0.7 to 1.2; lysine: 0.9 to 1.3; half-cystine: 1.0 to 2.2; phenylalanine: 1.8 to 2.2. Not more than traces of other amino acids are present.

MONOGRAPH SECTION

Identification

- no second identification
- often cross-references to *Tests* and *Assay*

- LC + AAAs
- or LC + NMR (up to ~15aa; dedicated CRS)

Verification of the molecule's:

- size,
- sequence,
- isoelectric profile,
- chromatographic properties,
- correct functional configuration
- specific to product (*e.g.* glycan analysis)

rDNA product

07/2019:2206 corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(…)

IDENTIFICATION

A. It shows the expected biological activity (see Assay).

B. Examine the electropherograms obtained in the test for impurities with charges differing from that of filgrastim.

Results: the principal band in the electropherogram obtained with the test solution is similar in position to the principal band in the electropherogram obtained with reference solution (a).

C. Examine the chromatograms obtained in the test for impurities with molecular masses higher than that of filgrastim.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

D. Examine the electropherograms obtained under both reducing and non-reducing conditions in the test for impurities with molecular masses differing from that of filgrastim.

Results: the principal band in the electropherogram obtained with test solution (a) is similar in position to the principal band in the electropherogram obtained with reference solution (b).

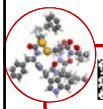
E. Examine the chromatograms obtained in the test for related proteins.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and shape to the principal peak in the chromatogram obtained with the reference solution.

F. Peptide mapping (*2.2.55*).

A guide through sections of Ph. Eur. monographs

Synthetic peptide



OCTREOTIDE 01/2020:2414

Octreotidum

(…)

Specific optical rotation (*2.2.7*): – 18.5 to – 14.5 (anhydrous and acetic acid-free substance).

Dissolve the substance to be examined in a 1 per cent *V/V* solution of *glacial acetic acid R* to obtain a concentration of 2.0 mg/mL.

Related substances. Liquid chromatography (*2.2.29*): use the normalisation procedure.

(…)

Acetic acid (*2.5.34*): 5.0 per cent to 12.8 per cent.

Test solution. Dissolve 10.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 mL with the same mixture of mobile phases.

Water (*2.5.32*): maximum 10.0 per cent, determined on 20.0 mg.

MONOGRAPH SECTION

Tests

- purity/impurity assessment
- limits based on specifications and batch data for approved products
- bacterial endotoxins – covered by 2034; may not be repeated)
- Residual solvents – covered by 2034
- Inorganic impurities e.g. sulphated ash

- optical rotation (or chiral chromatography)
- absorbance – if appropriate
- related peptides/substances
- acetic acid
- water

- Developed on basis of protein size, charge and hydrophobicity
- specific procedures for detection and quantification of specific impurities if necessary

rDNA product

07/2019:2206 corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(…)

Impurities with molecular masses higher than that of filgrastim. Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

(…)

Impurities with molecular masses differing from that of filgrastim. Polyacrylamide gel electrophoresis (*2.2.31*) under both reducing and non-reducing conditions.

(…)

Impurities with charges differing from that of filgrastim. Isoelectric focusing (*2.2.54*).

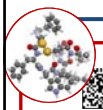
(…)

Related proteins. Liquid chromatography (*2.2.29*): use the normalisation procedure.

(…)

Bacterial endotoxins (*2.6.14*): less than 2 IU in the volume that contains 1.0 mg of protein.

Synthetic peptides Related peptides – main impurity test



01/2020:2414

OCTREOTIDE

Octreotidum

(...)

For information!

Related substances. Liquid chromatography (*2.2.29*): use the normalisation procedure.

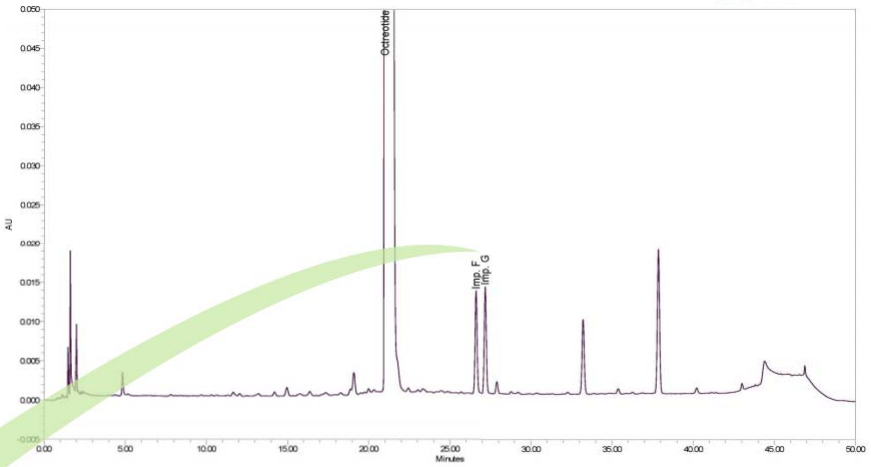
(...)

Identification of impurities: use the chromatogram supplied with *octreotide impurity mixture CRS* and the chromatogram obtained with the resolution solution to identify the peaks due to impurities F and G.

Relative retention with reference to octreotide (retention time = about 20 min): impurity A = about 0.76; impurity B = about 0.89; impurity C = about 0.94; impurity E = about 1.13; impurity F = about 1.30; impurity G = about 1.33; impurity H = about 1.66; impurity I = about 1.88.

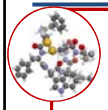
System suitability: resolution solution:

- *resolution*: minimum 2.0 between the peaks due to impurities F and G.



Identification of impurities and SST → *Octreotide impurity mixture CRS*

Synthetic peptides Related peptides – main impurity test



01/2020:2414

OCTREOTIDE

Octreotidum

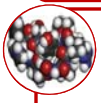
(...)

Related substances. Liquid chromatography (*2.2.29*): use the normalisation procedure.

(...)

Limits:

- *unspecified impurities*: for each impurity, maximum 0.5 per cent;
- *total*: maximum 2.0 per cent;
- *reporting threshold*: 0.1 per cent.



04/2018:0827
corrected 10.0

GONADORELIN ACETATE

Gonadorelini acetas

(...)

Related substances. Liquid chromatography (*2.2.29*).

Limits:

(...)

- *impurity E*: maximum 2.0 per cent;
- *sum of impurities F and G*: maximum 1.5 per cent;
- *sum of impurities C and D*: maximum 1.0 per cent;
- *unspecified impurities*: for each impurity, maximum 0.5 per cent;
- *total*: maximum 5.0 per cent;
- *reporting threshold*: 0.1 per cent.

Impurity limits

- each specified impurity (sometimes sum)
- unspecified impurities (identification threshold)
- total impurities
- reporting threshold

Substances for Pharmaceutical Use (2034) :

Table 2034.-2. – *Reporting, identification and qualification of organic impurities in peptides obtained by chemical synthesis*

Reporting threshold	Identification threshold	Qualification threshold
> 0.1 per cent	> 0.5 per cent	> 1.0 per cent

rDNA products Tests

07/2019:2206
corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

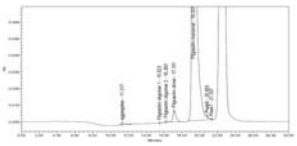
Filgrastimi solutio concentrata

Impurities with molecular masses higher than that of filgrastim. Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

System suitability: resolution solution:
- *retention time*: filgrastim monomer = 17 min to 20 min;
- *resolution*: minimum 3 between the peaks due to the filgrastim dimer and the filgrastim monomer.

Calculate the percentage content of the dimer, oligomers and aggregates.

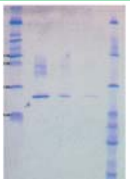
Limits:
- *impurities with molecular masses higher than that of filgrastim, other than the dimer*: maximum 0.5 per cent;
- *total of impurities with molecular masses higher than that of filgrastim*: maximum 2 per cent.

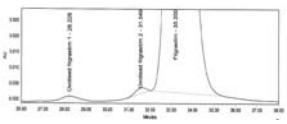


Impurities with charges differing from that of filgrastim. Isoelectric focusing (*2.2.54*).

System suitability:
- in the electropherogram obtained with reference solution (c), the relevant isoelectric point markers are distributed along the entire length of the gel;
- in the electropherogram obtained with reference solution (a), the pI of the principal band is 5.7 to 6.3.

Limit:
- *any impurity*: no band is more intense than the principal band in the electropherogram obtained with reference solution (b) (10 per cent).

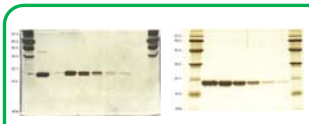




Related proteins. Liquid chromatography (*2.2.29*): use the normalisation procedure.

System suitability: reference solution (c):
- *resolution*: minimum 1.5 between the peaks due to filgrastim and reduced filgrastim;
- *symmetry factor*: maximum 1.8 for the peak due to filgrastim.

Limits:
- *any impurity*: for each impurity, maximum 1.0 per cent;
- *total*: maximum 2.0 per cent.



Impurities with molecular masses differing from that of filgrastim. Polyacrylamide gel electrophoresis (*2.2.31*) under both reducing and non-reducing conditions.

System suitability:
- reference solution (a): the validation criteria are met;
- a band is seen in the electropherogram obtained with test solution (e);
- a gradation of intensity of staining is seen in the electropherograms obtained with test solutions (a) to (e).

Limit: test solution (a):
- *impurities with molecular masses lower or higher than that of filgrastim*: no band is more intense than the principal band in the electropherogram obtained with test solution (d) (2.0 per cent).

A guide through sections of Ph. Eur. monographs

Synthetic peptide

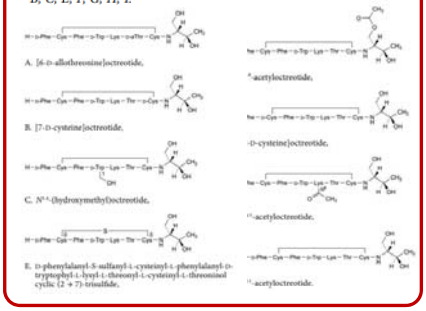
OCTREOTIDE 01/2020:2414

Octreotidum

(...)

IMPURITIES

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *A, B, C, E, F, G, H, I.*



A. [6-D-allothreonine]octreotide,

B. [7-D-cysteine]octreotide,

C. $N^{\alpha 1}$-(hydroxymethyl)octreotide,

E. D-phenylalanyl-*S*-sulfanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-L-cysteinyl-L-threonine cyclic (2 → 7)-trisulfide,

[1-acetyloctreotide,

acetyloctreotide,

-D-cysteine]octreotide,

[1-acetyloctreotide.

MONOGRAPH SECTION

Transparency section

- present in synthetic peptide monographs
- controlled by related substances test (synthetic peptides)
- not necessary exhaustive – impurities **known** to and **shown** to be detected by the test - based on information obtained and verified during monograph elaboration/revision

rDNA product

07/2019:2206
corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(...)

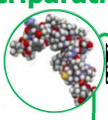
Interpretation of monographs – case study#1

Related proteins test - teriparatide

Test solution: 0.7 mg/mL
Column: 150 x 4.6 mm; 3µm; 300Å
Autosampler: 2-8 °C
Column temperature: 40 °C
Detection: UV 214 nm
Flow rate: 1.0 mL/min
Injection volume: 20 µL

Results obtained with 0.7 mg/mL solution of synthetic teriparatide

impurity	% area
MetO⁸,MetO¹⁸ teriparatide	0.17
MetO⁸ teriparatide	0.51
MetO¹⁸ teriparatide	0.82
X	0.40



TERIPARATIDE 01/2017:2829 corrected 10.0

Teriparatidum

(...)

PRODUCTION

Teriparatide is produced by a method based on recombinant DNA (rDNA) technology. During the course of product development it must be demonstrated that the manufacturing process produces a biologically active protein using a suitable bioassay as approved by the competent authority.

SUBSTANCES FOR PHARMACEUTICAL USE 01/2018:2034

Corpora ad usum pharmaceuticum

(...)

Table 2034.-2. – *Reporting, identification and qualification of organic impurities in peptides obtained by chemical synthesis*

Reporting threshold	Identification threshold	Qualification threshold
> 0.1 per cent	> 0.5 per cent	> 1.0 per cent

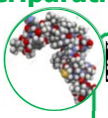
Interpretation of monographs – case study#1

Related proteins test - teriparatide

Test solution: 0.7 mg/mL
Column: 150 x 4.6 mm; 3µm; 300Å
Autosampler: 2-8 °C
Column temperature: 40 °C
Detection: UV 214 nm
Flow rate: 1.0 mL/min
Injection volume: 20 µL

Results obtained with 0.7 mg/mL solution of ~~synthetic~~ teriparatide

impurity	% area
MetO⁸,MetO¹⁸ teriparatide	0.17
MetO⁸ teriparatide	0.51
MetO¹⁸ teriparatide	0.82
X	0.40



TERIPARATIDE 01/2017:2829 corrected 10.0

Teriparatidum

(...)

Related proteins. Liquid chromatography (*2.2.29*): use the normalisation procedure. *Store the solutions at 2-8 °C and use them within 48 h.*

(...)

Column:
– *size*: *l* = 0.15 m, Ø = 4.6 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3.5 µm) with a pore size of 30 nm;
– *temperature*: 40 °C.

(...)

Flow rate: 1.0 mL/min.
Detection: spectrophotometer at 214 nm.
Autosampler: set at 2-8 °C.
Injection: 20 µL.

Interpretation of monographs – case study#1

Related proteins test - teriparatide

Test solution: 0.7 mg/mL
Column: 150 x 4.6 mm; 3µm; 300Å
Autosampler: 2-8 °C
Column temperature: 40 °C
Detection: UV 214 nm
Flow rate: 1.0 mL/min
Injection volume: 20 µL

Results obtained with 0.7 mg/mL solution of ~~synthetic~~ teriparatide

impurity	% area
MetO^{8},MetO^{18} teriparatide	0.17
MetO^{8} teriparatide	0.51
MetO^{18} teriparatide	0.82
X	0.40

TERIPARATIDE 01/2017:2829 corrected 10.0

Teriparatidum

(...)

Related proteins. Liquid chromatography (*2.2.29*): use the normalisation procedure. *Store the solutions at 2-8 °C and use them within 48 h.*

(...)

Column:
- *size*: *l* = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3.5 µm) with a pore size of 30 nm;

07/2016:20246 corrected 9.2

2.2.46. CHROMATOGRAPHIC SEPARATION TECHNIQUES

(...)

ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

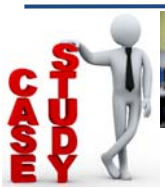
(...)

Liquid chromatography: gradient elution

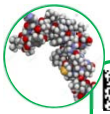
(...)

Column parameters
Stationary phase:
- no change of the identity of the substituent of the stationary phase permitted (e.g. no replacement of C18 by C8);
- *particle size*: no adjustment permitted.

Interpretation of monographs – case study#2a

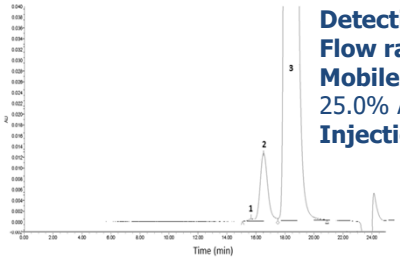


Impurities with molecular masses greater than that of teriparatide



Test solution: 1 mg/mL
Column: 8.0 x 300 mm; 5µm
Autosampler: 2-8 °C
Column temperature: ambient
Detection: UV 214 nm
Flow rate: 0.4 mL/min
Mobile phase: 0.10 % TFA, 25.0% ACN in water
Injection volume: 20 µL

Reference solution (b)



Peak	Retention time (min)	Resolution
1 (aggregates)	14.431	
2 (dimer)	16.505	
3 (monomer)	18.761	2.27

TERIPARATIDE 01/2017:2829 corrected 10.0

Teriparatidum

(...)

Impurities with molecular masses greater than that of teriparatide. Size-exclusion chromatography (*2.2.30*): use the normalisation procedure. *Store the solutions at 2-8 °C and use them within 72 h.*

Test solution. Dissolve the substance to be examined in *water R* to obtain a concentration of 1 mg/mL.

(...)

Column:
- *size*: *l* = 0.30 m, Ø = 7.8 mm;

(...)

Mobile phase: add 1 mL of *trifluoroacetic acid R* to 750 mL of *water R*, mix with 250 mL of *acetonitrile for chromatography R* and degas.

Flow rate: 0.5 mL/min.

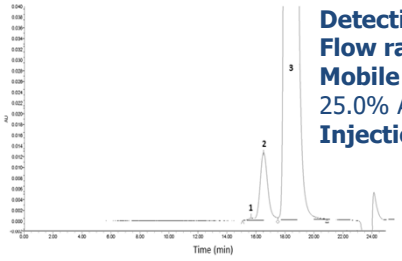
Detection: spectrophotometer at 214 nm.
Autosampler: set at 2-8 °C.
Injection: 20 µL.

Interpretation of monographs – case study#2a

Impurities with molecular masses greater than that of teriparatide

Test solution: 1 mg/mL
Column: 8.0 x 300 mm; 5µm
Autosampler: 2-8 °C
Column temperature: ambient
Detection: UV 214 nm
Flow rate: 0.4 mL/min
Mobile phase: 0.10 % TFA, 25.0% ACN in water
Injection volume: 20 µL

Reference solution (b)



Peak	Retention time (min)	Resolution
1 (aggregates)	14.431	
2 (dimer)	16.505	
3 (monomer)	18.761	2.27

TERIPARATIDE 01/2017:2829 corrected 10.0

Teriparatidum

(...)

Impurities with molecular masses greater than that of teriparatide. Size-exclusion chromatography (*2.2.30*): use the normalisation procedure. *Store the solutions at 2-8 °C and use them within 72 h.*

(...)

Column:
– *size*: *l* = 0.30 m, Ø = 7.8 mm;

(...)

Flow rate: 0.5 mL/min.

07/2016:20246 corrected 9.2

2.2.46. CHROMATOGRAPHIC SEPARATION TECHNIQUES

(...)

ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

(...)

Liquid chromatography : isocratic elution

(...)

Flow rate: ± 50 per cent; a larger adjustment is acceptable when changing the column dimensions (see the formula below).

(...)

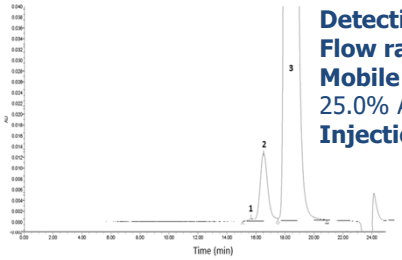
Column dimensions:
– *length*: ± 70 per cent;
– *internal diameter*: ± 25 per cent.

Interpretation of monographs – case study#2a

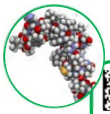
Impurities with molecular masses greater than that of teriparatide

Test solution: 1 mg/mL
Column: 8.0 x 300 mm; 5µm
Autosampler: 2-8 °C
Column temperature: ambient
Detection: UV 214 nm
Flow rate: 0.4 mL/min
Mobile phase: 0.10 % TFA, 25.0% ACN in water
Injection volume: 20 µL

Reference solution (b)



Peak	Retention time (min)	Resolution
1 (aggregates)	14.431	
2 (dimer)	16.505	
3 (monomer)	18.761	2.27



TERIPARATIDE 01/2017:2829 corrected 10.0

Teriparatidum

(...)

Impurities with molecular masses greater than that of teriparatide. Size-exclusion chromatography (*2.2.30*): use the normalisation procedure. *Store the solutions at 2-8 °C and use them within 72 h.*

(...)

Retention time: teriparatide monomer = about 17 min.

System suitability:

(...)

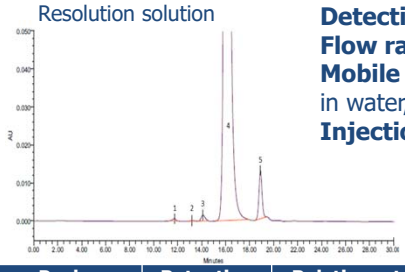
– *resolution*: minimum 2.0 between the peaks due to teriparatide dimer and monomer in the chromatogram obtained with the resolution solution.

For information!

Interpretation of monographs – case study#2b

Impurities with molecular masses greater than that of filgrastim

Resolution solution



Test solution: 0.4 mg/mL
Column: 7.8 x 150 mm; 5 µm
Column temperature: 30 °C
Detection: UV 215 nm
Flow rate: 0.3 mL/min
Mobile phase: 4 g/L NH_4HCO_3 in water, pH 7.0
Injection volume: 20 µL

Peak	Retention time (min)	Relative retention (with ref. to monomer)	Resolution
1 (aggregates)	11.693	0.72	
2 (oligomer 1)	13.214	0.81	3.24
3 (dimer)	14.032	0.86	1.23
4 (monomer)	16.238		3.02
5	18.956		4.47

07/2019:2206
corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(...)

Impurities with molecular masses higher than that of filgrastim. Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

(...)

Test solution. Dilute the preparation to be examined with solution A to obtain a concentration of 0.4 mg/mL.

(...)

Column:
– *size*: *l* = 0.3 m, Ø = 7.8 mm;

(...)

– *temperature*: 30 °C.

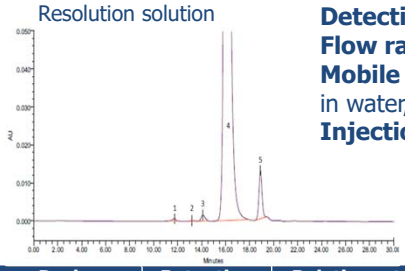
Mobile phase: dissolve 7.9 g of *ammonium hydrogen carbonate R* in 1000 mL of *water for chromatography R* and adjust to pH 7.0 with *phosphoric acid R*; dilute to 2000 mL with *water for chromatography R*.

Flow rate: 0.5 mL/min.

Detection: spectrophotometer at 215 nm.
Injection: 20 µL.

Impurities with molecular masses greater than that of filgrastim

Resolution solution



Test solution: 0.4 mg/mL
Column: 7.8 x 150 mm; 5 µm
Column temperature: 30 °C
Detection: UV 215 nm
Flow rate: 0.3 mL/min
Mobile phase: 4 g/L NH_4HCO_3 in water, pH 7.0
Injection volume: 20 µL

Peak	Retention time (min)	Relative retention (with ref. to monomer)	Resolution
1 (aggregates)	11.693	0.72	
2 (oligomer 1)	13.214	0.81	3.24
3 (dimer)	14.032	0.86	1.23
4 (monomer)	16.238		3.02
5	18.956		4.47

07/2019:2206
corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(...)

Impurities with molecular masses higher than that of filgrastim. Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

(...)

Column:
– *size*: *l* = 0.3 m, Ø = 7.8 mm;

(...)

Flow rate: 0.5 mL/min.

07/2016:20246
corrected 9.2

2.2.46. CHROMATOGRAPHIC SEPARATION TECHNIQUES

(...)

ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

(...)

Liquid chromatography: isocratic elution

(...)

Flow rate: ± 50 per cent; a larger adjustment is acceptable when changing the column dimensions (see the formula below).

(...)

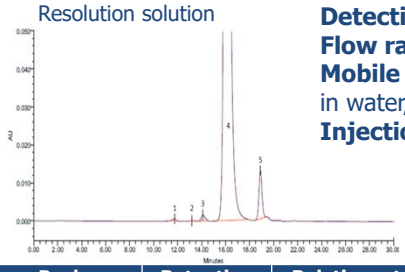
Column dimensions:
– *length*: ± 70 per cent;

Interpretation of monographs – case study#2b

Impurities with molecular masses greater than that of filgrastim

Test solution: 0.4 mg/mL
Column: 7.8 x 150 mm; 5 µm
Column temperature: 30 °C
Detection: UV 215 nm
Flow rate: 0.3 mL/min
Mobile phase: 4 g/L NH_4HCO_3 in water, pH 7.0
Injection volume: 20 µL

Resolution solution



Peak	Retention time (min)	Relative retention (with ref. to monomer)	Resolution
1 (aggregates)	11.693	0.72	
2 (oligomer 1)	13.214	0.81	3.24
3 (dimer)	14.032	0.86	1.23
4 (monomer)	16.238		3.02
5	18.956		4.47

07/2019:2206
corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(...)

Impurities with molecular masses higher than that of filgrastim. Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

(...)

Relative retention with reference to the filgrastim monomer (retention time = about 19 min): aggregates = about 0.60; filgrastim oligomer 1 = about 0.75; filgrastim oligomer 2 = about 0.80; filgrastim dimer = about 0.85.

System suitability: resolution solution:
– *retention time*: filgrastim monomer = 17 min to 20 min;

– *resolution*: minimum 3 between the peaks due to the filgrastim dimer and the filgrastim monomer.

For information!

A guide through sections of Ph. Eur. monographs

Synthetic peptide

OCTREOTIDE 01/2020:2414

Octreotidum

DEFINITION

(...)

Content: 95.0 per cent to 103.0 per cent (anhydrous and acetic acid-free substance).

(...)

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution.

Calculate the percentage content of octreotide ($C_{49}H_{66}N_{10}O_{10}S_2$) taking into account the assigned content of $C_{49}H_{66}N_{10}O_{10}S_2$ in *octreotide CRS*.

MONOGRAPH SECTION

Assay

- comparative chromatographic procedures using defined CRS as a standard
- content: anhydrous, acetic acid-free basis
- typically asymmetric limits

- protein content (comparative LC, UV spectroscopy)
- bioassay with ref. to WHO IS or Ph. Eur. standard calibrated in IU
- Exceptionally: *in vivo* tests; physicochemical tests only; example methods

rDNA product

07/2019:2206
corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(...)

ASSAY

Protein. Liquid chromatography (*2.2.29*) as described in the test for related proteins with the following modification.

Injection: test solution and reference solution (a).

Calculate the content of filgrastim ($C_{845}H_{1339}N_{223}O_{243}S_9$) taking into account the assigned content of $C_{845}H_{1339}N_{223}O_{243}S_9$ in *filgrastim CRS*.

Potency. The potency of the preparation to be examined is determined by comparison of the dilutions of the test preparation with the dilutions of the International Standard of filgrastim or with a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organization.

(...)

Calculate the potency of the preparation to be examined using a suitable statistical method, for example the parallel line assay (*5.3*).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 74 per cent and not more than 136 per cent of the estimated potency.

Interpretation of monographs – case study#3

Assay: filgrastim

07/2019:2206
corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(...)

DEFINITION

(...)

Content: minimum 0.9 mg of protein per millilitre.

Potency: minimum 0.9×10^8 IU per milligram of protein.

(...)

ASSAY

Protein. Liquid chromatography (*2.2.29*) as described in the test for related proteins with the following modification.

Injection: test solution and reference solution (a).

Calculate the content of filgrastim ($C_{845}H_{1339}N_{223}O_{243}S_9$) taking into account the assigned content of $C_{845}H_{1339}N_{223}O_{243}S_9$ in *filgrastim CRS*.

Potency.

(...)

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 74 per cent and not more than 136 per cent of the estimated potency.

Lot	Stated potency	Estimated potency	Protein content
1	1.0 x 10^8 IU/ml	0.8 x 10^8 IU/ml ✓	1.0 mg/mL ✓
2	1.0 x 10^8 IU/ml	1.25 x 10^8 IU/ml ✓	1.25 mg/mL ✓
3	1.0 x 10^8 IU/ml	1.0 x 10^8 IU/ml ✓	1.25 mg/mL ✓

Interpretation of monographs – case study#3

Assay: filgrastim

07/2019:2206
corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(...)

DEFINITION

(...)

Content: minimum 0.9 mg of protein per millilitre.

Potency: minimum 0.9×10^8 IU per milligram of protein.

(...)

ASSAY

Protein. Liquid chromatography (*2.2.29*) as described in the test for related proteins with the following modification.

Injection: test solution and reference solution (a).

Calculate the content of filgrastim ($C_{845}H_{1339}N_{223}O_{243}S_9$) taking into account the assigned content of $C_{845}H_{1339}N_{223}O_{243}S_9$ in *filgrastim CRS*.

Potency.

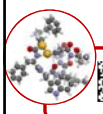
(...)

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits ($P = 0.95$) are not less than 74 per cent and not more than 136 per cent of the estimated potency.

Lot	Stated potency	Estimated potency	Protein content	Potency
1	1.0 x 10^8 IU/ml	0.8 x 10^8 IU/ml ✓	1.0 mg/mL ✓	0.8 x 10^8 IU/mg ✗
2	1.0 x 10^8 IU/ml	1.25 x 10^8 IU/ml ✓	1.25 mg/mL ✓	1.0 x 10^8 IU/mg ✓
3	1.0 x 10^8 IU/ml	1.0 x 10^8 IU/ml ✓	1.25 mg/mL ✓	0.8 x 10^8 IU/mg ✗

A guide through sections of Ph. Eur. monographs

Synthetic peptide



01/2020:2414

OCTREOTIDE

Octreotidum

(...)

MONOGRAPH SECTION

rDNA product

07/2019:2206
corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(...)

STORAGE

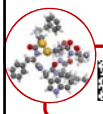
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C.

Storage

- not mandatory
- decided by competent authority (may decide to make it mandatory)
- storage → to ensure compliance with the monographs
- Conventional expressions (e.g. *in an airtight container*) defined in General Notices

A guide through sections of Ph. Eur. monographs

Synthetic peptide



01/2020:2414

OCTREOTIDE

Octreotidum

(...)

MONOGRAPH SECTION

rDNA product

07/2019:2206
corrected 10.0

FILGRASTIM CONCENTRATED SOLUTION

Filgrastimi solutio concentrata

(...)

LABELLING

The label states the octreotide content ($C_{49}H_{66}N_{10}O_{10}S_2$).

Labelling

- covered by national and international regulations
- not comprehensive
- only statements necessary to demonstrate (non-) compliance are mandatory (e.g. nominal value for excipients)
- Label → container, package, leaflet, CoA

LABELLING

The label states:

- the content, in milligrams of protein per millilitre;
- the potency, in International Units per milligram of protein.

General Notices (1) apply to all monographs and other texts *See the information section on general monographs (cover pages)*

Knowledge Database – additional source of information

Status	In use
Monograph Number	00949
English Name	Somatostatin
French Name	Somatostatine
Latin Name	Somatostatinum
Pinyin Name	
Chinese Name	
Pharmeuropa	30.4
Published in English Supplement	10.0
Published in French Supplement	8.1
On-going	Revision
State of work	3 - COM
Pharmeuropa	30.4
Description	Update of related substances test
Chromatogram	Not available
Additional information	Not available
History	View history
Interchangeable (ICH_Q4B)	NO
Pharmacopoeial harmonisation	NO

Reference standards

Available since	Cat. No.	Name	Batch No.	Unit Quantity	Price	SDS Product Code
	S0945000	Somatostatin CRS	6	2.09 mg	90 EUR	

Practical Information

Test(s)	Brand Name/Information
Related substances	From supplement 8.1: Symmetry C18 from Waters is suitable.

CEP

Substance Number	Substance	Certificate Holder	Certificate Number	Issue Date	Status	End date	Type
949	Somatostatin	HEMMO PHARMACEUTICALS PVT. LTD. IN 400 613 Mumbai	R1-CEP 2011-008-Rev 00	11/01/2017	VALID		Chemistry
949	Somatostatin	BCN PEPTIDES S.A. ES 08777 St Quinti De Mediona	R1-CEP 1999-035-Rev 03	05/11/2008	VALID		Chemistry
949	Somatostatin	Merck Biosciences AG CH 4448 Laufelfingen	R2-CEP 1995-033-Rev 01	29/09/2006	WITHDRAWN BY HOLDER	30/06/2014	Chemistry
949	Somatostatin	PolyPeptide Laboratories Inc. US 90503 Torrance	R1-CEP 2007-232-Rev 00	05/08/2014	EXPIRED	21/10/2014	Chemistry
949	Somatostatin	Bachem AG CH 4416 Bubendorf	R1-CEP 2005-245-Rev 01	19/01/2018	VALID		Chemistry
949	Somatostatin F	PolyPeptide Laboratories (Sweden) AB SE 216 13 Limhamn	R1-CEP 2010-169-Rev 01	21/12/2017	VALID		Chemistry

Ongoing revision
- scope
- state of work
- last issue of Pharmeuropa where the draft was published

Associated Reference Standards

Practical Info (e.g. column brand)

CEP holders

Summary

Individual monographs and relevant general monographs and chapters are complementary

Referenced general chapters become mandatory

Monograph sections

- Title
- Formula & CAS
- Mass
- Definition
- Production
- Characters
- Identification
- Tests
- Assay
- Storage
- Labelling
- Impurities

Non mandatory

Consult Knowledge Database for additional monograph-associated information

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**