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Calcitonin (Salmon) Injection

General Notices

Action and use

Hormone.

DEFINITION

Calcitonin (Salmon) Injection is a sterile solution of Calcitonin (Salmon) in Water for Injections.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of calcitonin (salmon), $C_{145}H_{240}N_{44}O_{48}S_2$

90.0 to 115.0% of the stated amount of the peptide.

CHARACTERISTICS

A colourless solution.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.9 to 4.5, Appendix V L.

Calcitonin C

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the injection, if necessary, with a 0.1M solution of *sodium dihydrogen orthophosphate* adjusted to pH 4.0 with *orthophosphoric acid* to give a final concentration of 10 µg of calcitonin (salmon) per mL.
(2) 10 µg of *calcitonin (salmon) EPCRS* per mL in a 0.1M solution of *sodium dihydrogen orthophosphate* adjusted to pH 4.0 with *orthophosphoric acid*.
(3) Heat the injection at 75° for 15 hours and, if necessary, dilute as described for solution (1).

CHROMATOGRAPHIC CONDITIONS

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(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Vydac C18, 300 Å wide pore column for proteins and peptides is suitable).
(b) Use gradient elution and the mobile phases described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 220 nm.
(f) Inject 200 µL of each solution.

MOBILE PHASE

Mobile phase A 100 volumes of a 0.363% w/v solution of *tetramethylammonium hydroxide pentahydrate* and 150 volumes of *acetonitrile* adjusted to pH 2.5 with *orthophosphoric acid*.

Mobile phase B 50 volumes of *acetonitrile* and 450 volumes of a 0.402% w/v solution of *tetramethyl-ammonium hydroxide pentahydrate* adjusted to pH 2.5 with *orthophosphoric acid*.

Use the following gradient.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-21	35→57	65→43	linear gradient
21-22	57→35	43→65	linear gradient
22-30	35	65	re-equilibration

In the chromatogram obtained with solution (3) the peak due to calcitonin C is the largest peak to elute after the injection buffer salts and before the principal peak with a relative retention to that of calcitonin (salmon) of between 0.5 and 0.6.

SYSTEM SUITABILITY

The test is not valid unless the *resolution factor* between the peaks due to calcitonin C and calcitonin (salmon) in the chromatogram obtained with solution (3) is at least 3.0.

LIMITS

In the chromatogram obtained with solution (1) the area of any peak corresponding to calcitonin C is not greater than 7% by *normalisation*.

Related peptides

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dilute the injection, if necessary, with mobile phase A to give a final concentration of 10 µg of calcitonin (salmon) per mL.
(2) Dissolve the contents of a vial of N-*acetyl-cys*[1] *calcitonin EPCRS* in 0.4 mL of mobile phase A, dilute to 40 mL with mobile phase A and add 0.1 mL of solution (1).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm).
(b) Use gradient elution and the mobile phases described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 65°.
(e) Use a detection wavelength of 220 nm.
(f) Inject 200 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 3.26 g of *tetramethylammonium hydroxide pentahydrate* in 900 mL of *water*, adjust the pH to 2.5 with *orthophosphoric acid* and mix with 100 mL of *acetonitrile for chromatography*.

Mobile phase B Dissolve 1.45 g of *tetramethylammonium hydroxide pentahydrate* in 400 mL of *water*, adjust the pH to 2.5 with *orthophosphoric acid* and mix with 600 mL of *acetonitrile for chromatography*.

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Use the following gradient.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-30	72→48	28→52	linear gradient
30-32	48→72	52→28	linear gradient
32-55	72	28	re-equilibration

When the chromatogram for solution (2) is recorded in the prescribed conditions, the relative retention time of *N*-acetyl-cys[1] calcitonin is about 1.15 relative to the principal peak.

SYSTEM SUITABILITY

The test is not valid unless the *resolution factor* between the peaks corresponding to calcitonin and *N*-acetyl-cys[1] calcitonin is at least 5.0 and the *symmetry factor* for the *N*-acetyl-cys[1] calcitonin peak is not greater than 2.5. If necessary, adjust the initial ratio of A:B in the mobile phase.

LIMITS

In the chromatogram obtained with solution (1):

the area of any *secondary peak* is not greater than 3% of the total area of all the peaks;

the sum of the areas of any such peaks is not greater than 5% of the total area of all the peaks by *normalisation*;

disregard any peak with an area less than 0.1 % of that of the principal peak.

ASSAY

Carry out the method described under the test for Calcitonin C.

Calculate the content of calcitonin (salmon) from the areas of the peak due to calcitonin (salmon) and that of any peak due to calcitonin C, using the declared content of $C_{145}H_{240}N_{44}O_{48}S_2$ in *calcitonin (salmon) EPCRS*.

STORAGE

Calcitonin (Salmon) Injection should be protected from light and stored at a temperature of 2° to 8°.

LABELLING

The label states the strength as the number of IU (Units) per mL. The label also states the equivalent number of micrograms of the peptide per mL.

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