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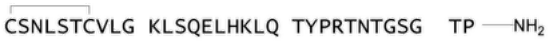
Calcitonin Salmon



$C_{145}H_{240}N_{44}O_{48}S_2$ 3432 daltons CAS RN®: 47931-85-1; UNII: 7SFC6U2VI5.

DEFINITION

Calcitonin Salmon is a polypeptide that has the same sequence as that of the hormone that regulates calcium metabolism and is secreted by the ultimobranchial gland of salmon. It is produced from either synthetic processes or microbial processes using recombinant DNA (rDNA) technology. The host cell-derived protein content and the host cell- or vector-derived DNA content of Calcitonin Salmon produced from an rDNA process are determined by validated methods. It contains NLT 90.0% and NMT 105.0% of calcitonin salmon, calculated on an acetic acid-free and anhydrous basis. [NOTE—1 mg of acetic acid-free, anhydrous Calcitonin Salmon is equivalent to 6000 USP Calcitonin Salmon Units. 1 USP Calcitonin Salmon Unit = 1 Calcitonin IU.]

IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the *Assay*.

ASSAY

• **PROCEDURE**

Solution A: Dissolve 3.26 g of tetramethylammonium hydroxide pentahydrate in 900 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 100 mL of acetonitrile, and mix.

Solution B: Dissolve 1.45 g of tetramethylammonium hydroxide pentahydrate in 400 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 600 mL of acetonitrile, and mix.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	72	28
30	48	52
32	72	28
55	72	28

Standard solution: 1.0 mg/mL of USP Calcitonin Salmon RS in *Solution A*

System suitability solution: Prepare a solution in *Solution A* containing about 0.2 mg/mL of USP Calcitonin Salmon Related Compound A RS and 0.2 mg/mL of USP Calcitonin Salmon RS.

Sample solution: 1.0 mg/mL of Calcitonin Salmon in *Solution A*

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 65°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for calcitonin salmon and calcitonin salmon related compound A are 1.0 and 1.15, respectively.]

Suitability requirements

Resolution: NLT 3 between calcitonin salmon and calcitonin salmon related compound A

Tailing factor: NMT 2.5 for calcitonin salmon

Relative standard deviation: NMT 3%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of calcitonin salmon ($C_{145}H_{240}N_{44}O_{48}S_2$) in the portion of Calcitonin Salmon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of calcitonin salmon from the *Sample solution*

r_S = peak response of calcitonin salmon from the *Standard solution*

C_S = concentration of USP Calcitonin Salmon RS in the *Standard solution* (corrected for water and acetic acid content) (mg/mL)

C_U = concentration of the *Sample solution* (corrected for water and acetic acid content) (mg/mL)

Acceptance criteria: 90.0%–105.0% on an acetic acid-free and anhydrous basis

OTHER COMPONENTS

• **Acetic Acid Content** ⟨503⟩

Sample solution: 1 mg/mL of Calcitonin Salmon in *Diluent*, prepared as directed in the chapter

Acceptance criteria: 4%–15%

IMPURITIES

Change to read:

• **Procedure: Related Peptides and Other Related Substances**

Test 1

[Note—This test is performed on material produced by both chemical synthesis processes and rDNA processes.]

Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Calcitonin Salmon taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area response of each impurity from the *Sample solution*

r_T = sum of the area responses of all the peaks from the *Sample solution*

Acceptance criteria

Individual impurities: NMT 3.0% of the total area of all peaks

Total impurities: NMT 5.0% of the sum of the areas of all the peaks including the principal peak

[Note—Disregard any peaks due to the solvent and any peaks whose area is less than 0.1% of the principal peak.]

Test 2

[Note—This test needs to be performed only on material produced using rDNA technology.]

Buffer A: Dissolve 2.72 g of monobasic potassium phosphate in 1000 mL of water.

Buffer B: Dissolve 2.72 g of monobasic potassium phosphate and 29.2 g of sodium chloride in 1000 mL of water.

Buffer C (pH 3.0 citrate buffer)**:** Dissolve 4.8 g of citric acid in 80 mL of water. Adjust with 1 M sodium hydroxide to a pH of 3.0, and dilute with water to 100.0 mL.

Solution A: Acetonitrile and *Buffer A* (15:85). Adjust with 45% (w/w) potassium hydroxide to a pH of 5.0.

Solution B: Acetonitrile and *Buffer B* (15:85). Adjust with 45% (w/w) potassium hydroxide to a pH of 4.6.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	0	100
15	0	100

Time (min)	Solution A (%)	Solution B (%)
15.1	100	0
22.1	100	0

▲**System suitability solution:**▲ (ERR 1-Oct-2024) Prepare a solution in water containing about 0.5 mg/mL each of USP Calcitonin Salmon RS and USP Calcitonin Salmon Related Compound B RS. To 1 mL of this solution add 100 µL of pH 3.0 citrate buffer.

Sample solution: To 1 mL of a 0.5-mg/mL solution of Calcitonin Salmon add ▲100 µL▲ (ERR 1-Oct-2024) of *Buffer C*.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 20-cm; packing L9

Flow rate: 1.2 mL/min

Injection volume: 50 µL

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for [1,7-bis(3-sulfo-L-alanine)] calcitonin salmon-glycine, [1,7-bis(3-sulfo-L-alanine)] calcitonin salmon, and calcitonin salmon related compound B (calcitonin salmon-glycine) are 0.4, 0.6, and 0.9, respectively; and the retention time for calcitonin salmon is about 9 min.]

Suitability requirements

Resolution: NLT 3.0 between calcitonin salmon and calcitonin salmon related compound B

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Calcitonin Salmon taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response for each impurity

r_T = sum of the responses of all the peaks

Acceptance criteria

Individual impurities: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria NMT (%)
[1,7-Bis(3-sulfo-L-alanine)] calcitonin salmon-glycine	0.4	0.2
[1,7-Bis(3-sulfo-L-alanine)] calcitonin salmon	0.6	0.2
Calcitonin salmon related compound B	0.9	0.6

SPECIFIC TESTS

• **AMINO ACID PROFILE**

(See *Biotechnology-Derived Articles—Amino Acid Analysis ⟨1052⟩*.)

[NOTE—This test needs to be performed only on material of synthetic origin. The concentration of amino acids in the *Internal standard solution*,*Standard stock solution,* and *Standard solution* and the amount of material used to prepare the *Sample solution* can be adjusted depending on the method used for amino acid analysis. The concentrations given are based on analysis using *Method 1*.]

Internal standard solution: 1 mM solution of γ-aminobutyric acid

Standard stock solution: Prepare a mixture containing equimolar amounts of ammonia and the L form of lysine, histidine, arginine, aspartic acid, threonine, serine, proline, valine, glutamic acid, glycine, leucine, and tyrosine, together with half the equimolar amount of L-cystine, in 0.1 M hydrochloric acid. The final concentration is about 2.5 mM for each amino acid.

Standard solution: Transfer 5 mL of the *Internal standard solution* and 2 mL of the *Standard stock solution* into a 50-mL volumetric flask, and dilute with 0.1 M hydrochloric acid to volume.

Sample solution: Place 1.5 mg of Calcitonin Salmon into a heavy-wall ignition tube. Add 1.0 mL of 6 N hydrochloric acid, and allow to cool. Immerse the lower half of the tube in a freezing mixture until the contents are frozen, then evacuate to approximately 10 µm of Hg, purge with nitrogen (repeat the evacuation and nitrogen purge three times), and seal the tube while it is under a 10 µm of Hg vacuum. Heat for 16 h at 110°–115° in an air oven. Cool, open the tube, dry in a vacuum desiccator, remove the contents, and allow to cool to room temperature. Dissolve in 0.1 M hydrochloric acid. Transfer to a 10-mL volumetric flask, add 1 mL of *Internal standard solution*, and dilute with 0.1 M hydrochloric acid to volume.

Analysis

Samples: *Standard solution* and *Sample solution*

Standardize the amino acid analyzer using the *Standard solution*. Inject the *Sample solution* into the amino acid analyzer, and determine the relative proportion of amino acids.

Express the content of each amino acid in moles using an internal standard calibration technique. Calculate the relative proportions of the amino acids by taking as equivalent to one the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, valine, leucine, histidine, arginine, and lysine divided by 20.

Acceptance criteria: The requirements are met if the values fall within the limits in *Table 4*.

Table 4

Name	Acceptance Criteria
Aspartic acid	1.8–2.2
Glutamic acid	2.7–3.3
Proline	1.7–2.3
Glycine	2.7–3.3
Valine	0.9–1.1
Leucine	4.5–5.3
Histidine	0.9–1.1
Arginine	0.9–1.1
Lysine	1.8–2.2
Serine	3.2–4.2
Threonine	4.2–5.2
Tyrosine	0.7–1.1
Half cystine	1.4–2.1

• **Peptide Mapping**

(See *Biotechnology-Derived Articles—Peptide Mapping* ⟨1055⟩.)

[Note—This test needs to be performed only on material produced using rDNA technology.]

Solution A: Water and trifluoroacetic acid (1000:1)

Solution B: Acetonitrile, water, and trifluoroacetic acid (800:200:0.85)

Mobile phase: See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	100	0
50	65	35

Time (min)	Solution A (%)	Solution B (%)
60	40	60
60.1	0	100
65.1	0	100
65.2	100	0
80.2	100	0

Trypsin solution: Freshly prepare a solution containing 0.1 mg/mL of trypsin (previously treated with L-1-tosylamido-2-phenylethyl chloromethyl ketone [TPCK] to remove chymotrypsin activity) in water.

Tris buffer: 1 M tris(hydroxymethyl) aminomethane and 10 mM calcium chloride. Adjust with hydrochloric acid to a pH of 8.0.

Stopping solution: Water and trifluoroacetic acid (1:1)

Standard solution: Prepare a 1.0-mg/mL solution of USP Calcitonin Salmon RS. Transfer 1 mL of this solution to a clean vial. Add 100 µL of *Tris buffer* and 50 µL of *Trypsin solution*. Mix, and incubate at 2°–8° for 16–20 h. Quench the digestion by adding 10 µL of *Stopping solution*.

Sample solution: 1.0 mg/mL of Calcitonin Salmon. Transfer 1 mL of this solution to a clean vial. Add 100 µL of *Tris buffer* and 50 µL of *Trypsin solution*. Mix, and incubate at 2°–8° for 16–20 h. Quench the digestion by adding 10 µL of *Stopping solution*.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 20 µL

Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—Condition the chromatographic system by running a blank gradient program before injecting the digests.]

Acceptance criteria: The chromatographic profile of the *Sample solution* is similar to that of the *Standard solution*.

Change to read:

• **BIOIDENTITY**

RPMI 1640 with L-glutamine: Prepare a mixture of the ingredients in the quantities shown in *Table 6* in sufficient water to obtain 1 L of *RPMI 1640 with L-glutamine* solution, and sterilize by filtration.

Table 6

Calcium nitrate	100.00 mg
Potassium chloride	400.00 mg
Magnesium sulfate, anhydrous	48.84 mg
Potassium chloride	400 mg
Sodium chloride	6000 mg
Sodium phosphate, dibasic, anhydrous	800 mg
Sodium bicarbonate	2000 mg
Glucose	2000 mg
Glycine	10 mg
L-Arginine	200 mg
L-Asparagine	50 mg
L-Aspartic acid	20 mg

L-Cystine dihydrochloride	65 mg
L-Glutamic acid	20 mg
L-Glutamine	300 mg
L-Histidine	15 mg
L-Hydroxyproline	20 mg
L-Isoleucine	50 mg
L-Leucine	50 mg
L-Lysine hydrochloride	40 mg
L-Methionine	15 mg
L-Phenylalanine	15 mg
L-Proline	20 mg
L-Serine	30 mg
L-Threonine	20 mg
L-Tryptophan	5 mg
L-Tyrosine disodium salt dihydrate	29 mg
L-Valine	20 mg
Biotin	0.2 mg
Choline chloride	3 mg
D-Calcium pantothenate	0.25 mg
Folic acid	1 mg
i-Inositol	35 mg
Niacinamide	1 mg
para-Aminobenzoic acid	1 mg
Pyridoxine hydrochloride	1 mg
Riboflavin	0.2 mg
Thiamine hydrochloride	1 mg
Vitamin B_{12}	0.005 mg

Medium A (growth medium)**:** Using aseptic technique prepare the following tissue culture medium.

Table 7

RPMI 1640 with L-glutamine	500 mL
Fetal bovine serum	50 mL
1 M HEPES	5 mL

Penicillin/streptomycin solution (10,000 IU/mL per 10 mg/mL)	5 mL
Human insulin	10 IU
Hydrocortisone	0.5 mg

Medium B (stimulation medium)**:** Dissolve 5 g of bovine serum albumin (BSA) in 500 mL of ▲▲ (ERR 1-Oct-2024) *RPMI 1640 with L-glutamine*.

Solution A (0.2% BSA solution)**:** Dissolve 50 mg of BSA in 25 mL of water. [Note—Use within one day.]

Solution B (formic acid/BSA solution)**:** Add 25 mL of 0.1 M formic acid and 5 mL of *Solution A* to a 50-mL volumetric flask, dilute with water to volume, and mix. [Note—Use within two days.]

Solution C (trypsin/EDTA solution)**:** Prepare a sterile filtered solution containing 0.25% trypsin and 0.53 mM EDTA (tetrasodium ethylenediaminetetraacetate).

Solution D (Dulbecco's phosphate buffered saline)**:** Dissolve 8 g of sodium chloride, 1.15 g of dibasic sodium phosphate, 0.2 g of monobasic potassium phosphate, 0.2 g of potassium chloride, 0.1 g of calcium chloride, and 0.1 g of magnesium chloride in 1 L of water.

Standard stock solution: 20 µg/mL of USP Calcitonin Salmon RS in *Solution B*

Positive control solution: Quantitatively dilute the *Standard stock solution* in *Medium B* to obtain a 1-ng/mL solution of USP Calcitonin Salmon RS.

Negative control solution: *Medium B*

[Note—Prior analysis should be performed to identify the linear portion of the dose-response curve. For example, the *Standard solutions* and *Sample solutions* given below.]

Standard solution A: 0.8 ng/mL of USP Calcitonin Salmon RS from the *Standard stock solution* in *Medium B*

Standard solution B: 0.4 ng/mL of USP Calcitonin Salmon RS from *Standard solution A* in *Medium B*

Standard solution C: 0.2 ng/mL of USP Calcitonin Salmon RS from *Standard solution B* in *Medium B*

Standard solution D: 0.1 ng/mL of USP Calcitonin Salmon RS from *Standard solution C* in *Medium B*

Sample stock solution: 20 µg/mL of Calcitonin Salmon in *Solution B*

Sample solution A: 0.8 ng/mL of Calcitonin Salmon from the *Sample stock solution* in *Medium B*

Sample solution B: 0.4 ng/mL of Calcitonin Salmon from *Sample solution A* in *Medium B*

Sample solution C: 0.2 ng/mL of Calcitonin Salmon from *Sample solution B* in *Medium B*

Sample solution D: 0.1 ng/mL of Calcitonin Salmon from *Sample solution C* in *Medium B*

Cell culture preparation: Prepare a cell culture of the human mammary tumor cell line T-47D. Cells are propagated using *Medium A* at 37° and 5% carbon dioxide. The medium is changed every two days, and cells are passaged every 5–9 days using *Solution C* with a 1:4 subculture.

Cell suspension: For the test use a cell culture that is 5–9 days old. Remove the cell culture medium from the flask by aspiration, add 10 mL of *Solution D,* and rock the culture flask to rinse the entire monolayer. Remove the liquid by aspiration, add 2 mL of *Solution C*, spread over the entire monolayer, allow to stand for 3–5 min, and add 10 mL of *Medium A.* Homogenize the cell suspension using a pipet, transfer to a 15-mL polypropylene tube, centrifuge at about 220 × *g* for 5 min, pour off the supernatant, and resuspend the cell pellet in 10 mL of *Medium A.* Count the cells, and adjust the cell density through dilution using *Medium A* to 2.5×10^4 cells/mL.

Procedure: Place 200 µL of the *Cell suspension* into each well of a 96-well culture plate (the tissue culture plate), and incubate for 18–24 h at 37° and 5% carbon dioxide. Fill each well of an empty, round-bottomed, 96-well culture plate (the prepared plate) with 150 µL of one of the following solutions: *Positive control solution, Negative control solution, Standard solutions A–D,* and *Sample solutions A–D,* so that each solution fills at least five wells on the prepared plate. After incubation remove the culture medium from the tissue culture plate. Using an 8-channel or 12-channel pipet, rapidly transfer 100 µL of solution from each well of the prepared plate to each well of the tissue culture plate. Incubate for 15 min at ambient temperature, remove the solution from each well, stop stimulation by immediately adding an appropriate cell-lysis buffer, and quantitate cAMP produced within the cells, using a validated kit. Perform the test three times, using three different 96-well culture plates. [Note—Some kits include a cell-lysis reagent and a sequestering agent for the cell-lysis reagent. The range of the test kit is between 0.05 and 10 ng/mL of cAMP. The number of cells used in the assay may vary, depending on the validated kit used to quantitate cAMP.]

Analysis: Potency is determined by a 3-dose, 6-point parallel-line assay, using standard statistical methods. The calculation is carried out using both the lower three concentrations and the upper three concentrations. For the assay to be valid, the requirements for regression, parallelism, and difference of quadratics must be met. If the requirements for validity are met to the same extent in both assessments (the lower and the higher assessments) the final result is determined from the concentration range that shows the higher value when the common slope is divided by the root mean square error.

Acceptance criteria: Combine the three potency values by using an unweighted mean on the log scale. Determine a 95% confidence interval in the log scale using standard statistical methods. Convert the average and confidence interval to the potency scale using antilogs to obtain a geometric mean and its confidence interval. The potency levels determined from all three performances of the test are valid if the data analysis indicates the three determinations to be homogeneous, and the confidence interval is fully contained within 64% and 156% of the geometric mean. If the confidence interval requirement is not met, additional assays may be performed to increase the number of assays and make the confidence interval narrower. The determination of whether it meets the identity requirement should be done only after the confidence interval requirement is met. The acceptance criterion for identity is that the geometric mean is within 80% and 125% of the *Assay* value.

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩**

Sample: 25 mg

Acceptance criteria: The total aerobic microbial count is NMT 10^2 cfu/g, and the total combined molds and yeasts count is NMT 10^2 cfu/g.

• **Sterility Tests ⟨71⟩:** Where the label states that Calcitonin Salmon is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **Water Determination, *Method Ic* ⟨921⟩:** NMT 10%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Store in a refrigerator or maintain in a frozen state, protected from light.

• **Labeling:** The labeling states that the material is synthetic or of recombinant DNA origin.

• **USP Reference Standards ⟨11⟩**

USP Calcitonin Salmon RS $C_{145}H_{240}N_{44}O_{48}S_2$ 3432 daltons

USP Calcitonin Salmon Related Compound A RS

N-Acetyl-cys[1]-calcitonin salmon.

USP Calcitonin Salmon Related Compound B RS (calcitonin salmon-glycine)

Calcitonin salmon-glycine.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
CALCITONIN SALMON	Kishan Chandra Senior Scientist I, Documentary Standards	BIO12020 Biologics Monographs 1 - Peptides

Chromatographic Database Information: Chromatographic Database

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