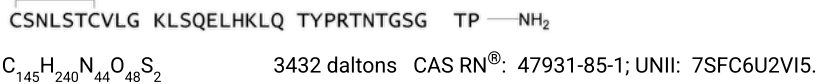


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# Calcitonin Salmon



**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<br>(min) | Solution A<br>(%) | Solution B<br>(%) |
|---------------|-------------------|-------------------|
| 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:

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.