Chloride, previously dried, and dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.170 mg of $C_{10}H_{16}ClNO$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Edrophonium Chloride Injection

塩化エドロホニウム注射液

Edrophonium Chloride Injection is an aqueous solution for injection. It contains not less than 95% and not more than 105% of the labeled amount of edrophonium chloride ($C_{10}H_{16}ClNO$: 201.69).

Method of preparation Prepare as directed under Injections, with Edrophonium Chloride.

Description Edrophonium Chloride Injection is a clear and colorless liquid.

Identification **(1)** To a volume of Edrophonium Chloride Injection, equivalent to 0.04 g of Edrophonium Chloride according to the labeled amount, add 4 mL of barium nitrate TS, shake, and filter. Proceed with the filtrate as directed in the Identification (1) under Edrophonium Chloride.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under the Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 272 nm and 276 nm.

pH 6.5 - 8.0

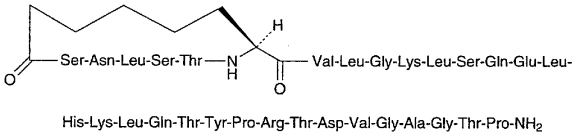
Assay Conduct this procedure without exposure to daylight, using light-resistant containers. Measure exactly a volume of Edrophonium Chloride Injection, equivalent to about 0.05 g of edrophonium chloride ($C_{10}H_{16}ClNO$), place in a chromatographic column prepared by pouring 10 mL of weakly basic DEAE-bridged dextran anion exchanger (Cl type) (50 to 150 μm in particle diameter) into a chromatographic tube about 2 cm in inside diameter and about 10 cm in length, add 25 mL of water, and elute at the flow rate of 1 to 2 mL per minute. Wash the column with two 25-mL portions of water at the flow rate of 1 to 2 mL per minute. Combine the washings with above effluent solutions, and add water to make exactly 100 mL. Measure exactly 10 mL of this solution, and add 10 mL of phosphate buffer solution, pH 8.0, and 5 g of sodium chloride. Wash this solution with four 20-mL portions of a mixture of diethyl ether and hexane (1:1), collect the water layer, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.05 g of Edrophonium Chloride Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 3 hours, and dissolve in water to make exactly 100 mL. Measure exactly 10 mL of this solution, and prepare the standard solution in the same manner as the sample solution. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution at 273 nm as directed under the Ultraviolet-visible Spectrophotometry.

Amount (mg) of edrophonium chloride ($C_{10}H_{16}ClNO$)
= amount (mg) of Edrophonium Chloride Reference Standard
$\times \frac{A_T}{A_S}$

Containers and storage Containers—Hermetic containers, and colored containers may be used.
Storage—Light-resistant.

Elcatonin

エルカトニン



His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asp-Val-Gly-Ala-Gly-Thr-Pro-NH_2

$C_{148}H_{244}N_{42}O_{47}$: 3363.77
[60731-46-6]

Elcatonin contains not less than 5000 Elcatonin Units and not more than 7000 Elcatonin Units per 1 mg of peptide, calculated on the dehydrated and deacetic acid basis.

Description Elcatonin is a white powder.
It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in acetonitrile.
It is hygroscopic.
The pH of its solution (1 in 500) is between 4.5 and 7.0.

Identification Dissolve 5 mg of Elcatonin in 5 mL of water. Determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Constituent amino acids Put about 1 mg of Elcatonin in a test tube for hydrolysis, add phenol-hydrochloric acid TS to dissolve, replace the air inside with Nitrogen, seal the tube under reduced pressure, and heat at 110 ± 2°C for 24 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, dissolve the residue in 1 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh exactly 1.33 mg of L-aspartic acid, 1.19 mg of L-threonine, 1.05 mg of L-serine, 1.47 mg of L-glutamic acid, 1.15 mg of L-proline, 0.75 mg of glycine, 0.89 mg of L-alanine, 1.17 mg of L-valine, 1.89 mg of L-2-aminosuberic acid, 1.31 mg of L-leucine, 1.81 mg of L-tyrosine, 1.83 mg of L-lysine hydrochloride, 2.10 mg of L-histidine hydrochloride monohydrate and 2.11 mg of L-arginine hydrochloride, dissolve them in 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chro-

matography according to the following conditions: 14 peaks of amino acids appear on the chromatogram obtained from the sample solution, and their respective molar ratios against alanine are 1.7 - 2.2 for aspartic acid, 3.5 - 4.2 for threonine, 2.4 - 3.0 for serine, 2.7 - 3.2 for glutamic acid, 1.7 - 2.2 for proline, 2.7 - 3.2 for glycine, 1.6 - 2.2 for valine, 0.8 - 1.2 for 2-aminosuberic acid, 4.5 - 5.2 for leucine, 0.7 - 1.2 for tyrosine, 1.7 - 2.2 for lysine, 0.8 - 1.2 for histidine and 0.7 - 1.2 for arginine.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography composed with a sulfonated styrene-divinylbenzene copolymer (3 μm in particle diameter).

Column temperature: Varied between 50°C and 65°C.

Chemical reaction vessel temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Buffer solutions A, B, C and D, with sodium ion concentrations of 0.10 mol/L, 0.135 mol/L, 1.26 mol/L and 0.20 mol/L, respectively. The ion concentration of the mobile phase is changed stepwise from 0.10 mol/L to 1.26 mol/L by using these buffer solutions.

	Components of buffer solutions			
Buffer solution:	A	B	C	D
Citric acid	8.85 g	7.72 g	6.10 g	—
Sodium citrate	3.87 g	10.05 g	26.67 g	—
Sodium hydroxide	—	—	2.50 g	8.00 g
Sodium chloride	3.54 g	1.87 g	54.35 g	—
Ethanol	60.0 mL	—	—	60.0 mL
Thiodiglycol	5.0 mL	5.0 mL	—	—
Purified water	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount
Total amount	1000 mL	1000 mL	1000 mL	1000 mL

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for about 20 minutes while passing Nitrogen, and use this solution as solution A. Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for about 20 minutes while passing Nitrogen, and use this solution as solution B. Mix solution A and solution B before use.

Flow rate of mobile phase: Adjust the flow rate so that the retention time of arginine is about 75 minutes.

Flow rate of reaction reagent: About 0.2 mL per minute.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use a column from which aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, 2-aminosuberic acid, leucine, tyrosine, lysine, histidine and arginine are eluted in this order, with complete separation of each peak.

Purity **(1)** Acetic acid—Weigh accurately 3 - 6 mg of Elcatonin quickly under conditions of 25 ± 2°C and 50 ± 5% relative humidity, add exactly 1 mL of the internal standard solution to dissolve it, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of acetic acid (100), and add the internal standard solution to make exactly 100 mL. Pipet 5 mL of this solution, add the internal standard solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of acetic acid to that of the internal standard: the amount of acetic acid is not more than 7.0%.

$$\text{Amount (\%) of acetic acid } (CH_3COOH) = \frac{Q_T}{Q_S} \times \frac{W_{ST}}{W_{SA}} \times 50$$

W_{ST}: Amount (g) of acetic acid (100) taken

W_{SA}: Amount (mg) of sample taken

Internal standard solution—A solution of citric acid (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 13.2 g of diammonium hydrogenphosphate in 900 mL of water, add phosphoric acid to adjust the pH to 2.5, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 4 minutes.

Selection of column: Proceed with 20 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column from which acetic acid and citric acid are eluted in this order with the resolution between their peaks being not less than 2.0.

(2) Related substances—Dissolve 1.0 mg of Elcatonin in 1 mL of a mixture of trifluoroacetic acid TS and acetonitrile (2:1), and use this solution as the sample solution. Take exactly 0.3 mL of the sample solution, add a mixture of trifluoroacetic acid TS and acetonitrile (2:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine each peak area by the automatic integration method: the total of the peak areas other than the peak of elcatonin of the sample solution is not larger than the peak area of elcatonin of the standard solution, and each peak area other than the peak of elcatonin of the sample solution is not larger than 1/3 of the peak area of elcatonin of the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of trifluoroacetic acid TS and

acetonitrile (change the ratio linearly from 85:15 to 55:45 in 30 minutes).

Flow rate: Adjust the flow rate so that the retention time of elcatonin is about 25 minutes.

Selection of column: Dissolve 2 mg of Elcatonin in 200 μL of trypsin TS for test of elcatonin, warm at 37°C for 1 hour, then add 1 drop of acetic acid (100), and heat at 95°C for 1 minute. To 10 μL of this solution add 50 μL of the sample solution, and mix. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column such that the resolution between the peak of elcatonin and the peak which appears immediately before the peak of elcatonin is not less than 2.0, and the retention time of elcatonin is about 25 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of elcatonin from 10 μL of the standard solution is between 50 mm and 200 mm.

Time span of measurement: Continue measurement until the regularly changing base-line of the chromatogram disappears, after the solvent peak.

Water Weigh accurately 1 - 3 mg of Elcatonin quickly under conditions of 25 ± 2°C and 50 ± 5% relative humidity, and perform the test as directed in 2. Coulometric titration under the Water Determination: not more than 8.0%.

Nitrogen content Weigh accurately 0.015 - 0.02 g of Elcatonin quickly under conditions of 25 ± 2°C and 50 ± 5% relative humidity, and perform the test as directed under the Nitrogen Determination: it contains not less than 16.1% and not more than 18.7% of nitrogen (N: 14.007) in the peptide, calculated on the dehydrated and de-acetic acid basis.

Assay (i) Animals: Select healthy male Sprague-Dawley rats each weighing between 90 g and 110 g. Keep the rats for not less than 3 days before use, providing an appropriate uniform diet and water.

(ii) Diluent for elcatonin: Dissolve 2.72 g of sodium acetate in water to make 200 mL, add 0.2 g of bovine serum albumin, and adjust the pH to 6.0 with acetic acid (100). Prepare before use.

(iii) Standard solution: Dissolve Elcatonin Reference Standard in the diluent for elcatonin to make two standard solutions, one to contain exactly 0.075 Unit in each mL which is designated as the high-dose standard solution, S_H, and the other to contain exactly 0.0375 Unit in each mL which is designated as the low-dose standard solution, S_L.

(iv) Sample solution: Weigh accurately 0.5 - 2.0 mg of Elcatonin quickly under conditions of 25 ± 2°C and 50 ± 5% relative humidity, and dissolve in the diluent for elcatonin to make two sample solutions, the high-dose sample solution, T_H, which contains the Units per mL equivalent to S_H and the low-dose sample solution, T_L, which contains the Units per mL equivalent to S_L.

(v) Deproteinizing solution for elcatonin: Dissolve 160 g of trichloroacetic acid and 30.6 g of strontium chloride in water to make 3600 mL.

(vi) Procedure: Divide the animals into 4 equal groups of not less than 10 animals each. Withhold all food, but not water, for 18 to 24 hours before the injections, and withhold water during the assay until the final blood sample is taken. Handle the animals with care in order to avoid undue excitement.

Inject exactly 0.2 mL each of the standard solutions and the sample solutions into the tail vein of each animal as indicated in the following design:

First group	S_H	Third group	T_H
Second group	S_L	Fourth group	T_L

At 1 hour after the injection, take a sufficient blood sample to perform the test from the carotid artery and vein of each animal under ether anesthesia, centrifuge the blood samples to separate serum, and determine the serum calcium according to the following (vii).

(vii) Serum calcium determination: Take exactly 0.3 mL of the serum, add the deproteinizing solution for elcatonin to make exactly 3 mL, mix well, centrifuge, and use the supernatant liquid as the sample solution for calcium determination. Separately, pipet 1 mL of Standard Calcium Solution for Atomic Absorption Spectrophotometry, and add a solution of sodium chloride (17 in 2000) to make exactly 10 mL. Pipet 5 mL of this solution, add the deproteinizing solution for elcatonin to make exactly 50 mL, and use this solution as the standard solution for calcium determination. Determine the absorbances, A_T and A_S, of the sample solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions. Determine the absorbance, A_0, of a solution obtained in the same manner used for preparation of the standard solution, but with 1 mL of water instead of the standard solution.

Amount (mg) of calcium (Ca) in 100 mL of the serum

$$= 0.01 \times \frac{A_T - A_0}{A_S - A_0} \times 10 \times 100$$

Gas: Combustible gas—Acetylene
Supporting gas—Air
Lamp: Calcium hollow-cathode lamp
Wavelength: 422.7 nm

(viii) Calculation: Amounts of calcium in 100 mL of the serum obtained with S_H, S_L, T_H and T_L in (vii) are symbolized as y_1, y_2, y_3 and y_4, respectively. Sum up individual y_1, y_2, y_3 and y_4 to obtain Y_1, Y_2, Y_3 and Y_4, respectively.

Units per mg of peptide, calculated on the dehydrated and de-acetic acid basis

$$= \text{antilog } M \times (\text{units per mL of the high-dose standard solution}) \times \frac{b}{a}$$

$$M = 0.3010 \times \frac{Y_a}{Y_b}$$

$$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$$
$$Y_b = Y_1 - Y_2 + Y_3 - Y_4$$

a: Amount (mg) of the sample

$$\times \frac{100 - [\text{water content (\%)} + \text{acetic acid content (\%)}]}{100}$$

b: Total volume (mL) of the high-dose sample solution prepared by dissolving the sample with diluent for elcatonin.

F' computed by the following equation should be smaller than F shown in the table against n with which s^2 is calculated. Calculate L (P=0.95) by use of the following equation: L should be not more than 0.20. If F' exceeds F, or if L exceeds 0.20, repeat the test, increasing the number of animals or arranging the assay conditions so that F' is not more than F and L is not more than 0.20.

$$F' = \frac{(-Y_1 + Y_2 + Y_3 - Y_4)^2}{4fs^2}$$

f: Number of the animals of each group.

$$s^2 = \frac{\Sigma y^2 - \frac{Y}{f}}{n}$$

Σy^2: The sum of squares of y_1, y_2, y_3 and y_4 in each group.

$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$

$n = 4(f - 1)$

$$L = 2\sqrt{(C - 1)(CM^2 + 0.09062)}$$

$$C = \frac{Y_b^2}{Y_b^2 - 4fs^2t^2}$$

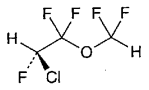
t^2: Value shown in the following table against n used to calculate s^2.

n	$t^2=F$	n	$t^2=F$	n	$t^2=F$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Tight containers.
Storage—Not exceeding 8°C.

Enflurane

エンフルラン



and enantiomer

$C_3H_2ClF_5O$: 184.49
(*RS*)-2-Chloro-1,1,2-trifluoroethyl difluoromethyl ether
[*13838-16-9*]

Description Enflurane is a clear, colorless liquid.
It is slightly soluble in water.
It is miscible with ethanol (95) and with diethyl ether.
It is a volatile, and not an inflammable.
It shows no optical rotation.
Boiling point: 54 - 57°C

Identification **(1)** Take 0.05 mL of Enflurane, and prepare the test solution as directed to the Oxygen Flask Combustion Method using 40 mL of water as the absorbing liquid. The test solution responds to the Qualitative Tests for chloride and fluoride.

(2) Determine the infrared absorption spectrum of Enflurane as directed in the liquid film method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index n_D^{20}: 1.302 - 1.304

Specific gravity d_{20}^{20}: 1.520 - 1.540

Purity **(1)** Acid or alkali—To 60 mL of Enflurane add 60 mL of freshly boiled and cooled water, shake for 3 minutes, separate the water later, and use the layer as the sample solution. To 20 mL of the sample solution add one drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide TS: the color of the solution is purple. To 20 mL of the sample solution add one drop of bromocresol purple TS and 0.06 mL of 0.01 mol/L hydrochloric acid TS: the color of the solution is yellow.

(2) Chloride—To 20 g of Enflurane add 20 mL of water, shake well, and separate the water layer. Take 10 mL of the water layer add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid (not more than 0.001%).

(3) Nonvolatile residue—Evaporate exactly 65 mL of Enflurane on a water bath to dryness, and dry the residue at 105°C for 1 hour: the of the residue is not more than 1.0 mg.

(4) Related substances—Proceed the test with 5 μL of Enflurane as directed under the Gas chromatography according to the following conditions. Determine each peak area other than the peak of air which appears soon after the injection of the sample by the automatic integration method, and calculate the amount of each peak by the area percentage method: the amount of the substances other than enflurane is not more than 0.10%.

Operating conditions—

Detector: A thermal conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μm in particle diameter, coated with diethylene glycol succinate ester for gas chromatography in the ratio of 20%.

Column temperature: A constant temperature of about 80°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of enflurane is about 3 minutes.

Selection of column: Mix 5 mL of Enflurane and 5 mL of 1,2-dichloroethane. Proceed with 1 μL of this mixture under the above operating conditions, and calculate the resolution. Use a column giving elution of enflurane and 1,2-dichloroethane in this order with the resolution between these peaks being not less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of enflurane from 5 μL of Enflurane is not less than 50% of the full scale.

Time span of measurement: About three times as long as the retention time of enflurane.

Water Not more than 0.10% (10 g, direct titration).

Containers and storage Containers—Tight containers.
Storage—Not exceeding 30°C.