

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Ecothiopate Iodide in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals—To 1.0 g of Ecothiopate Iodide in a Kjeldahl flask add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add 2 mL of nitric acid, and heat. Repeat this procedure twice, add several 2-mL portions of hydrogen peroxide (30), and heat until the solution becomes colorless, and white fumes are evolved. After cooling, transfer the solution together with a small quantity of water to a Nessler tube, and add water to make about 20 mL. Adjust the solution with ammonia solution (28) and ammonia TS to a pH between 3.0 and 3.5, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: proceed in the same manner as the preparation of the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Ecothiopate Iodide in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μ L each of the sample solution and the standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 50°C, 3 hours).

Assay Weigh accurately about 0.125 g of Ecothiopate Iodide, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add 30 mL of water, then add exactly 10 mL of phosphate buffer solution, pH 12, stopper the container, and allow to stand at $25 \pm 3^\circ\text{C}$ for 20 minutes. To this solution add quickly 2 mL of acetic acid (100), and titrate with 0.002 mol/L iodine VS (potentiometric titration). Perform the test in the same manner without phosphate buffer solution, pH 12, and make any necessary correction.

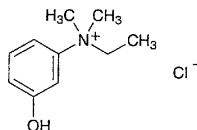
Each mL of 0.002 mol/L iodine VS
= 1.533 mg of $\text{C}_9\text{H}_{23}\text{INO}_3\text{PS}$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 0°C.

Edrophonium Chloride

塩化エドロホニウム



$\text{C}_{10}\text{H}_{16}\text{ClNO}$: 201.69

N-Ethyl-*N*-(3-hydroxyphenyl)-*N,N*-dimethylammonium chloride [116-38-1]

Edrophonium Chloride, when dried, contains not less than 98.0% of $\text{C}_{10}\text{H}_{16}\text{ClNO}$.

Description Edrophonium Chloride occurs as white crystals or crystalline powder. It is odorless.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

It is hygroscopic.

It is gradually colored by light.

Identification (1) To 5 mL of a solution of Edrophonium Chloride (1 in 100) add 1 drop of iron (III) chloride TS: a light red-purple color develops.

(2) Determine the absorption spectrum of a solution of Edrophonium Chloride in 0.1 mol/L hydrochloric acid TS (1 in 20,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Edrophonium Chloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Edrophonium Chloride (1 in 50) responds to the Qualitative Tests for chloride.

pH Dissolve 1.0 g of Edrophonium Chloride in 10 mL of water: the pH of this solution is between 3.5 and 5.0.

Melting point 166 – 171°C (with decomposition).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Edrophonium Chloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals—Proceed with 1.0 g of Edrophonium Chloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Edrophonium Chloride according to Method 1, and perform the test using Apparatus B (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Edrophonium Chloride in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 100 mL. Pipet 3 mL of this solution, add ethanol (95) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μ L each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonia solution (28) (16:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying Not more than 0.20% (1 g, in vacuum, phosphorus (V) oxide, 3 hours)

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.2 g of Edrophonium

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 μ m 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 sam-

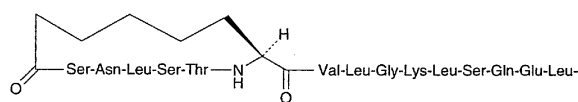
ple solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 273 nm as directed under the Ultraviolet-visible Spectrophotometry.

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-NH₂

$C_{148}H_{244}N_{42}O_{47}$; 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 $110 \pm 2^\circ C$ 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 μ L each of the sample solution and the standard solution as directed under the Liquid Chro-