(3) Determine the absorption spectrum of a solution of Epirizole in 0.1 mol/L hydrochloric acid TS (1 in 200,000) 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.

Melting point 88 - 91°C

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Epirizole in 20 mL of water: the solution is clear and colorless.

(2) Chloride—Add 0.5 g of Epirizole to a ground mixture of 0.7 g of potassium nitrate and 1.2 g of anhydrous sodium carbonate, mix well, transfer little by little to a platinum crucible, previously heated, and heat until the reaction is completed. After cooling, add 15 mL of dilute sulfuric acid and 5 mL of water to the residue, boil for 5 minutes, filter, wash the insoluble matter with 10 mL of water, and add 6 mL of dilute nitric acid and water to the combined filtrate and washings to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: proceed with the same quantities of the same reagents as directed for the preparation of the test solution, and add 0.25 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL (not more than 0.018%).

(3) Heavy metals—Proceed with 2.0 g of Epirizole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

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

(5) Related substances—Dissolve 1.0 g of Epirizole in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol 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 2 μL 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 isopropyl diethyl ether, ethanol (95) and water (23:10:2) 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. Place this plate in a chamber filled with iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(6) Readily carbonizable substances—Perform the test with 0.10 g of Epirizole: the solution has no more color than Matching Fluid A.

Loss on drying Not more than 0.5% (1 g, silica gel, 4 hours).

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

Assay Weigh accurately about 0.5 g of Epirizole, previously dried, dissolve in 40 mL of acetic acid (100) and titrate with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS) until the color of the solution changes from purple through blue-green to green. Perform a blank determination, and make any necessary correction.

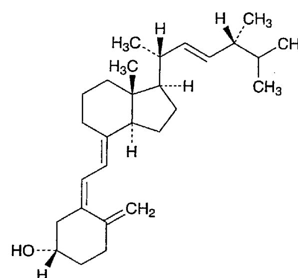
Each mL of 0.1 mol/L perchloric acid VS
= 23.426 mg of $C_{11}H_{14}N_4O_2$

Containers and storage Containers—Well-closed containers.

Ergocalciferol

Calciferol
Vitamin D_2

エルゴカルシフェロール



$C_{28}H_{44}O$: 396.65
(3*S*,5*Z*,7*E*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol [*50-14-6*]

Ergocalciferol contains not less than 97.0% and not more than 103.0% of $C_{28}H_{44}O$.

Description Ergocalciferol occurs as white crystals. It is odorless, or has a faint, characteristic odor.

It is freely soluble in ethanol (95), in diethyl ether and in chloroform, sparingly soluble in isooctane, and practically insoluble in water.

It is affected by air and by light.

Melting point: 115 - 118°C Transfer Ergocalciferol to a capillary tube, and dry for 3 hours in a desiccator (in vacuum at a pressure not exceeding 2.67 kPa). Immediately fire-seal the capillary tube, put it in a bath fluid, previously heated to a temperature about 10°C below the expected melting point, and heat at a rate of rise of about 3°C per minute, and read the melting point.

Identification (1) Dissolve 0.5 mg of Ergocalciferol in 5 mL of chloroform, add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and shake: a red color is produced, and rapidly changes through purple and blue to green.

(2) Determine the infrared absorption spectrum of Ergocalciferol as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Ergocalciferol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance $E^{1\%}_{1\,\mathrm{cm}}$ (265 nm): 445 - 485 (0.01 g, ethanol (95), 100 mL).

Optical rotation $[\alpha]^{20}_{D}$: +102 - +107° (0.3 g, ethanol (95),

20 mL, 100 mm). Prepare the solution of Ergocalciferol within 30 minutes after the container has been opened, and determine the rotation within 30 minutes after the solution has been prepared.

Purity Ergosterol—Dissolve 0.010 g of Ergocalciferol in 2.0 mL of diluted ethanol (95) (9 in 10), add a solution of 0.020 g of digitonin in 2.0 mL of diluted ethanol (95) (9 in 10), and allow the mixture to stand for 18 hours: no precipitate is formed.

Assay Weigh accurately about 0.03 g each of Ergocalciferol and Ergocalciferol Reference Standard, and dissolve each in isooctane to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 3 mL each of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 to 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 ergocalciferol to that of the internal standard. Perform the procedure rapidly avoiding contact with air or other oxidizing agents and using light-resistant containers.

$$\text{Amount (mg) of } C_{28}H_{44}O = \text{amount (mg) of Ergocalciferol Reference Standard} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of dimethyl phthalate in isooctane (1 in 100).
Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 10 to 30 cm in length, packed with a silica gel for liquid chromatography (5 to 10 μm particle diameter).

Column temperature: Ordinary temperature.

Mobile phase: A mixture of hexane and *n*-amylalcohol (997:3).

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

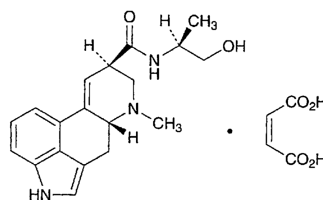
Selection of column: Dissolve 0.015 g of Ergocalciferol Reference Standard in 25 mL of isooctane. Transfer this solution to a flask, heat in an oil bath under a reflux condenser for 2 hours, and cool immediately to room temperature. Transfer the solution to a quartz test tube, and irradiate with a short-wave lamp (main wavelength: 254 nm) and a long-wave lamp (main wavelength: 365 nm) for 3 hours. To 10 mL of this solution add the mobile phase to make 50 mL. Proceed with 10 μL of this solution under the above operating conditions. Use a column with the ratios of the retention time of previtamin D_2, trans-vitamin D_2 and tachysterol$_2$ to that of ergocalciferol being about 0.5, about 0.6 and about 1.1, respectively, and with resolution between previtamin D_2 and trans-vitamin D_2 being not less than 0.7, and that between ergocalciferol and tachysterol$_2$ being not less than 1.0.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, under Nitrogen atmosphere, and in a cold place.

Ergometrine Maleate

マレイン酸エルゴメトリン



$C_{19}H_{23}N_3O_2.C_4H_4O_4$: 441.48
(8*S*)-9,10-Didehydro-*N*-[(1*S*)-2-hydroxy-1-methylethyl]-6-methylergoline-8-carboxamide monomaleate [*129-51-1*]

Ergometrine Maleate, when dried, contains not less than 98.0% of $C_{19}H_{23}N_3O_2.C_4H_4O_4$.

Description Ergometrine Maleate occurs as a white to pale yellow, crystalline powder. It is odorless.

It is sparingly soluble in water, slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 185°C (with decomposition).

It gradually changes to yellow in color on exposure to light.

Identification (1) Prepare a solution of Ergometrine Maleate (1 in 50): the solution shows a blue fluorescence.

(2) Dissolve 1 mg of Ergometrine Maleate in 5 mL of water. To 1 mL of this solution add 2 mL of 4-dimethylaminobenzaldehyde-ferric chloride TS, shake, and allow to stand for 5 to 10 minutes: a deep blue color develops.

(3) To 5 mL of a solution of Ergometrine Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the solution disappears immediately.

Optical rotation $[\alpha]_D^{20}$ +48 – +57° (after drying 0.25 g, water, 25 mL, 100 mm).

pH Dissolve 0.10 g of Ergometrine Maleate in 10 mL of water. The pH of the solution is between 3.0 and 5.0.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Ergometrine Maleate in 10 mL of water: the solution is clear and colorless to light yellow.

(2) Ergotamine and ergotoxine—To 0.02 g of Ergometrine Maleate add 2 mL of a solution of sodium hydroxide (1 in 10), and heat to boiling: the gas evolved does not change moistened red litmus paper to blue.

(3) Related substances—Dissolve 5 mg each of Ergometrine Maleate and Ergometrine Maleate Reference Standard in 1.0 mL of methanol, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate, prepared with silica gel for thin-layer chromatography and dilute sodium hydroxide TS. Develop the plate with a mixture of chloroform and methanol (4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: the spots obtained from the sample solution and the standard solution show a red-purple color