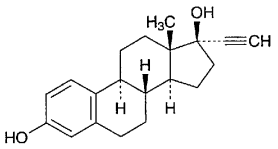
Storage—Without fill up, light-resistant, remote from fire, and not exceeding 25°C.

Ethinylestradiol

エチニルエストラジオール



$C_{20}H_{24}O_2$: 296.40
17α-Ethynylestra-1,3,5(10)-triene-3,17β-diol [*57-63-6*]

Ethinylestradiol, when dried, contains not less than 98.0% of $C_{20}H_{24}O_2$.

Description Ethinylestradiol occurs as white to pale yellow crystals or crystalline powder. It is odorless.

It is freely soluble in pyridine and in tetrahydrofuran, soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 2 mg of Ethinylestradiol in 1 mL of a mixture of ethanol (95) and sulfuric acid (1:1): a purplish red color develops with a yellow-green fluorescence. Add carefully 2 mL of water to this solution: the color of the solution changes to red-purple.

(2) Transfer 0.02 g of Ethinylestradiol to a glass-stoppered test tube, dissolve in 10 mL of a solution of potassium hydroxide (1 in 20), add 0.1 g of benzoyl chloride, and shake. Collect the resulting precipitate, recrystallize from methanol, and dry in a desiccator (in vacuum, phosphorus (V) oxide): the precipitate melts between 200°C and 202°C.

Optical rotation $[\alpha]_D^{20}$: −26 – −31° (after drying, 0.1 g, pyridine, 25 mL, 200 mm).

Melting point 180 – 186°C or 142 – 146°C

Purity Estrone—Dissolve 5 mg of Ethinylestradiol in 0.5 mL of ethanol (95), and add 0.05 g of 1,3-dinitrobenzene. Add 0.5 mL of freshly prepared dilute potassium hydroxide-ethanol TS, allow to stand in a dark place for 1 hour, and add 10 mL of ethanol (95): the solution has no more color than the following control solution.

Control solution: Proceed in the same manner as mentioned above, omitting Ethinylestradiol.

Loss on drying Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.2 g of Ethinylestradiol, previously dried, and dissolve in 40 mL of tetrahydrofuran. Add 10 mL of a solution of silver nitrate (1 in 20), and titrate with 0.1 mol/L sodium hydroxide VS (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.641 mg of $C_{20}H_{24}O_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ethinylestradiol Tablets

エチニルエストラジオール錠

Ethinylestradiol Tablets contain not less than 90% and not more than 110% of the labeled amount of ethinylestradiol ($C_{20}H_{24}O_2$: 296.40).

Method of preparation Prepare as directed under Tablets, with Ethinylestradiol.

Identification (1) Evaporate to dryness 5 mL of the sample solution obtained in Assay, and add 2 mL of a mixture of sulfuric acid and ethanol (95) (2:1) to the residue: a light red color with a yellow fluorescence develops. To the solution add carefully 4 mL of water: the color of the solution changes to red-purple.

(2) Evaporate to dryness 10 mL of the sample solution obtained in Assay, add 0.2 mL of acetic acid (31) and 2 mL of phosphoric acid to the residue, and heat on a water bath for 5 minutes: a red color with a yellow-green fluorescence develops.

Content uniformity Place 1 tablet of Ethinylestradiol Tablets in a separator, add 10 mL of the 2nd fluid of test fluids under the Disintegration Test, and shake until the tablet is disintegrated. Add 10 mL of dilute sulfuric acid and 20 mL of chloroform, shake vigorously for 5 minutes, and filter the chloroform layer into a conical flask through filter paper on which 5 g of anhydrous sodium sulfate is placed. Extract the aqueous layer with two 20-mL portions of chloroform, proceed with the extracts in the same manner as before, and combine the filtrates with the previous one. Evaporate gently the combined filtrate on a water bath with the aid of a current of nitrogen, dissolve the residue in exactly 100 mL of methanol, and centrifuge, if necessary. Pipet x mL of the supernatant liquid, add methanol to make exactly V mL of a solution containing about 0.04 μg of ethinylestradiol ($C_{20}H_{24}O_2$) per mL, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g of Ethinylestradiol Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, dissolve in methanol, dilute to a volume containing about 0.04 μg of ethinylestradiol ($C_{20}H_{24}O_2$) per mL, and use this solution as the standard solution. Pipet 4 mL each of sulfuric acid-methanol TS into three glass-stoppered test tubes, T, S and B, cool in ice, to each tube add exactly 1 mL each of the sample solution, the standard solution and methanol, shake immediately, and allow to stand in a water bath at 30°C for 40 minutes, then allow to stand in a water bath at 20°C for 5 minutes. Perform the test with these solutions as directed under the Fluorometry. Determine the fluorescence intensities, F_T, F_S and F_B, of these solutions using the fluorophotometer, at about 460 nm of the excitation and at about 493 nm of the fluorescence.

Amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$)
= amount (mg) of Ethinylestradiol Reference Standard

$$\times \frac{F_T - F_B}{F_S - F_B} \times \frac{V}{2500} \times \frac{1}{x}$$

Assay (i) Chromatographic tube: Pack a pledget of glass wool in the bottom of a tube 25 mm in inside diameter and 300 mm in length, and place 5 g of anhydrous sodium sulfate on the glass wool.

(ii) Chromatographic column: Place 5 g of siliceous earth for chromatography in a 200-mL beaker, soak well in 4 mL of 1 mol/L hydrochloric acid TS, and mix uniformly. Put the siliceous earth into the chromatographic tube in small portions to make 60 to 80 mm in height in proper hardness with a tamping rod.

(iii) Standard solution: Weigh accurately about 0.01 g of Ethinylestradiol Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in chloroform to make exactly 100 mL. Pipet 5 mL of this solution, and add chloroform to make exactly 100 mL.

(iv) Sample: Weigh accurately not less than 20 Ethinylestradiol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.5 mg of ethinylestradiol ($C_{20}H_{24}O_2$), place in a 50-mL beaker, add 2 mL of water, shake well, add 3 mL of chloroform, and shake well again. Add 4 g of siliceous earth for chromatography, mix well until the contents do not stick to the inner wall of the beaker, and use the substance as the sample.

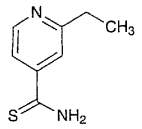
(v) Procedure: To the chromatographic column add the sample with a funnel, and pack in proper hardness. Mix well the sample sticking to the beaker with 0.5 g of siliceous earth for chromatography, and place in the chromatographic tube. Wipe off the sample solution sticking to the beaker and the tamping rod with glass wool, and place it in the chromatographic tube. Push down the sample, and press lightly on the chromatographic column to make the height of the column 110 mm to 130 mm. Take 70 mL of chloroform, rinse the inner wall of the chromatographic tube with a portion of the chloroform, and transfer the remaining portion to the chromatographic tube. Collect the effluent solution at a flow rate not more than 0.8 mL per minute. After completing the elution, rinse the lower end of the chromatographic tube with a small quantity of chloroform, add chloroform to make exactly 100 mL, and use this solution as the sample solution. Transfer 6 mL each of the sample solution and the standard solution to each separators, and add 20 mL each of isooctane. Add exactly 10 mL of a mixture of sulfuric acid and methanol (7:3), shake vigorously for 5 minutes, allow to stand in a dark place for 15 minutes, and centrifuge. Perform the test with the resulting color solutions as directed under the Ultraviolet-visible Spectrophotometry, using a solution, prepared with 6 mL of chloroform in the same manner, as the blank. Determine the absorbances, A_T and A_S, of the subsequent solutions obtained from the sample solution and the standard solution at 540 nm, respectively.

Amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$)
= amount (mg) of Ethinylestradiol Reference Standard
$\times \frac{A_T}{A_S} \times \frac{1}{20}$

Containers and storage Containers—Well-closed containers.

Ethionamide

エチオナミド



$C_8H_{10}N_2S$: 166.24
2-Ethylpyridine-4-carbothioamide [*536-33-4*]

Ethionamide, when dried, contains not less than 98.0% of $C_8H_{10}N_2S$.

Description Ethionamide occurs as yellow crystals or crystalline powder, having a characteristic odor and taste.

It is soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid and in dilute sulfuric acid.

Identification **(1)** To 0.05 g of Ethionamide add 0.1 g of 1-chloro-2,4-dinitrobenzene, and mix. Transfer 0.01 g of the mixture to a test tube, and heat over a small flame for a few seconds to melt. Cool, and add 3 mL of potassium hydroxide-ethanol TS: a red to orange color is produced.

(2) Place 0.8 g of Ethionamide in a 100-mL beaker, and dissolve in 20 mL of sodium hydroxide TS by heating while shaking occasionally: the gas evolved turns a moistened red litmus paper to blue. Boil gently, and evaporate the solution to 3 to 5 mL. After cooling, add gradually 20 mL of acetic acid (100), and heat on a water bath: the gas evolved darkens moistened lead (II) acetate paper. Evaporate the solution on a water bath to 3 to 5 mL with the aid of a current of air, cool, add 10 mL of water, and shake. Filter the crystals by suction, recrystallize from water immediately, and dry in a desiccator (in vacuum, silica gel) for 6 hours: the crystals melt between 233°C and 237°C (with decomposition).

Melting point 161 - 165°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Ethionamide in 30 mL of ethanol (95): the solution is clear, and shows a yellow color.

(2) Acid—Dissolve 3.0 g of Ethionamide in 30 mL of methanol by warming, add 90 mL of water, allow to stand in ice water for 1 hour, and filter. To 80 mL of the filtrate add 0.8 mL of cresol red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

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

(4) Arsenic—Prepare the test solution with 1.0 g of Ethionamide according to Method 3, and perform the test using Apparatus B. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(5) Related substances—Proceed with the test avoiding sunlight. Dissolve 0.50 g of Ethionamide in 20 mL of methanol, and use this solution as the sample solution.