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.

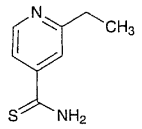
(v) Procedure: To the chromatographic column add the sample with a funnel, and pack in proper hardness. Mix well the sample sticking to the beaker with 0.5 g of siliceous earth for chromatography, and place in the chromatographic tube. Wipe off the sample solution sticking to the beaker and the tamping 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.

Pipet 1 mL of this 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 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 chloroform and methanol (9:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate 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 0.5% (1 g, 105°C, 3 hours).

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

Assay Weigh accurately about 0.3 g of Ethionamide, previously dried, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-red to dark orange-brown (indicator: 2 mL of *p*-naphtholbenzein TS). Perform a blank determination.

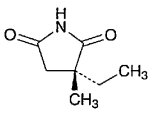
Each mL of 0.1 mol/L perchloric acid VS
= 16.625 mg of $C_8H_{10}N_2S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Ethosuximide

エトスクシミド



and enantiomer

$C_7H_{11}NO_2$: 141.17
(*RS*)-2-Ethyl-2-methylsuccinimide [*77-67-8*]

Ethosuximide contains not less than 98.5% of $C_7H_{11}NO_2$ calculated on the anhydrous basis.

Description Ethosuximide occurs as a white, paraffin-like solid or powder. It is odorless or has a slight, characteristic odor.

It is very soluble in methanol, in ethanol (95), in diethyl ether, and in *N,N*-dimethylformamide, and freely soluble in water.

Melting point: about 48°C

Identification (1) To 0.2 g of Ethosuximide add 10 mL of sodium hydroxide TS, and boil: the gas evolved turns a moistened red litmus paper blue.

(2) Dissolve 0.05 g of Ethosuximide in 1 mL of ethanol (95), add 3 drops of a solution of copper (II) acetate monohydrate (1 in 100), warm slightly, and add 1 to 2 drops of sodium hydroxide TS: a purple color is produced.

(3) Determine the absorption spectrum of a solution of Ethosuximide in ethanol (95) (1 in 2000) 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.

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

(2) Chloride—With 1.0 g of Ethosuximide, perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Heavy metals—Proceed with 1.0 g of Ethosuximide 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).

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

(5) Acid anhydride—Dissolve 0.50 g of Ethosuximide in 1 mL of ethanol (95), add 1 mL of hydroxylammonium chloride-iron (III) chloride TS, and allow to stand for 5 minutes. Add 3 mL of water, mix, and allow to stand for 5 minutes: the red to red-purple color of this solution is not more intense than that of the following control solution.

Control solution: Dissolve 0.070 g of succinic anhydride in ethanol (95) to make exactly 100 mL. To 1.0 mL of this solution add 1 mL of hydroxylammonium chloride-iron (III) chloride TS, and proceed in the same manner.

(6) Cyanide—Dissolve 1.0 g of Ethosuximide in 10 mL of ethanol (95), and add 3 drops of iron (II) sulfate TS, 1 mL of sodium hydroxide TS and 2 to 3 drops of iron (III) chloride TS. Warm gently, and acidify with dilute sulfuric acid: not a blue precipitate and a blue color are produced within 15 minutes.

Water Not more than 0.5% (2 g, direct titration).

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

Assay Weigh accurately about 0.2 g of Ethosuximide, dissolve in 20 mL of *N,N*-dimethylformamide, and titrate with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination.

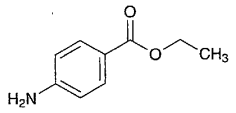
Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 14.117 mg of $C_7H_{11}NO_2$

Containers and storage Containers—Tight containers.

Ethyl Aminobenzoate

Anesthamine
Benzocaine

アミノ安息香酸エチル



$C_9H_{11}NO_2$: 165.19
Ethyl 4-aminobenzoate [*94-09-7*]

Ethyl Aminobenzoate, when dried, contains not less than 99.0% of $C_9H_{11}NO_2$.