Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 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: A mixture of water and methanol (51:49).

Flow rate: Adjust the flow rate so that the retention time of estriol is about 10 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of estriol and the internal standard in this order with the resolution between these peaks being not less than 8.

Containers and storage Containers—Tight containers.

Estriol Injection (Aqueous Suspension)

エストリオール水性懸濁注射液

Estriol Injection (Aqueous Suspension) is an aqueous suspension for injection. It contains not less than 90% and not more than 110% of the labeled amount of estriol ($C_{18}H_{24}O_3$: 288.38).

Method of preparation Prepare as directed under Injections, with Estriol.

Description Shake Estriol Injection (Aqueous Suspension): a white turbidity is produced.

Identification **(1)** Shake well, take a volume of Estriol Injection (Aqueous Suspension), equivalent to 2 mg of Estriol according to the labeled amount, add ethanol (95) to make 20 mL, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Estriol.

(2) Determine the absorption spectrum of the sample solution obrtained in (1) as directed under the Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 279 nm and 283 nm.

Assay Shake well, pipet a volume of Estriol Injection (Aqueous Suspension), equivalent to about 5 mg of estriol ($C_{18}H_{24}O_3$), and dissolve in methanol to make exactly 20 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.025 g of Estriol Reference Standard, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Estriol.

$$\text{Amount (mg) of estriol } (C_{18}H_{24}O_3) = \text{amount (mg) of Estriol Reference Standard} \times \frac{Q_T}{Q_S} \times \frac{1}{5}$$

Internal standard solution—A solution of methyl benzoate for estriol limit test in ethanol (95) (1 in 5000).

Containers and storage Containers—Hermetic containers.

Estriol Tablets

エストリオール錠

Estriol Tablets contain not less than 90% and not more than 110% of the labeled amount of estriol ($C_{18}H_{24}O_3$: 288.38).

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

Identification **(1)** Weigh a portion of powdered Estriol Tablets, equivalent to 2 mg of Estriol according to the labeled amount, add ethanol (95) to make 20 mL, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Estriol.

(2) Determine the absorption spectrum of the sample solution obtained in (1) as directed under the Ultraviolet-visible Spectrophotometry: it exhibits a maximum between 279 nm and 283 nm.

Content uniformity To one tablet of Estriol Tablets add exactly 5 mL of water, disperse the fine particles with ultrasonic wave, add exactly 15 mL of methanol, and shake for 15 minutes. Centrifuge this solution for 10 minutes, pipet a definite amount of the supernatant liquid, and add methanol to make exactly a definite amount of solution so that each ml of the solution contains about 5 μg of estriol ($C_{18}H_{24}O_3$). Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Proceed with 20 μL of the sample solution as directed in the Assay under Estriol. Use a solution of methyl benzoate in methanol (1 in 40,000) as the internal standard solution. Calculate the mean value from each ratio of peak areas of 10 samples: the samples conform to the requirements if the deviation (%) of the mean value and each ratio of peak areas is within 15%. If the deviation (%) exceeds 15%, and 1 sample shows deviation within 25%, repeat the test with 20 samples. Calculate the deviation (%) of the mean value from each ratio of peak areas of the 30 samples used in the 2 tests and each ratio of peak areas: the samples conform to the requirements if the deviation exceeds 15%, not more than 1 sample shows deviation within 25%, and no sample shows deviation exceeding 25%.

Dissolution test Perform the test with 1 tablet of Estriol Tablets at 50 revolutions per minute according to Method 2 under the Dissolution Test, using 900 mL of water as the test solution. Take 20 mL or more of the dissolved solution 30 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL so that each mL contains about 0.1 μg of estriol ($C_{18}H_{24}O_3$) according to the labeled

amount, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g of Estriol Reference Standard, previously dried at 105°C for 3 hours, dissolve in methanol to make exactly 100 mL, then pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 100 μL each of the sample solution and the standard solution according to the operating conditions as directed in the Assay under Estriol, and determine the peak areas of estriol, A_T and A_S, from these solutions.

The dissolution rate of Estriol Tablets in 30 minutes is not less than 80%.

$$\text{Dissolution rate (\%) with respect to the labeled amount of estriol } (C_{18}H_{24}O_3) = W_S \times \frac{A_T}{A_S} \times \frac{V'}{V} \times \frac{1}{C} \times \frac{9}{10}$$

W_S: Amount (mg) of Estriol Reference Standard.
C: Labeled amount (mg) of estriol ($C_{18}H_{24}O_3$) in 1 tablet.

Assay Weigh accurately and powder not less than 20 Estriol Tablets. Weigh accurately a portion of the powder, equivalent to about 1 mg of estriol ($C_{18}H_{24}O_3$), add exactly 5 mL of water, disperse the fine particles with ultrasonic wave, shake with 25 mL of methanol for 10 minutes, centrifuge, and take the supernatant liquid. Add 25 mL of methanol, repeat the above procedure twice, combine the supernatant liquid, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.025 g of Estriol Reference Standard, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the standard solution. Proceed with 20 μL each of the sample solution and the standard solution as directed in the Assay under Estriol.

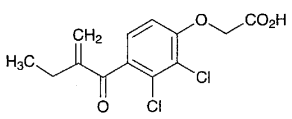
$$\text{Amount (mg) of estriol } (C_{18}H_{24}O_3) = \text{amount (mg) of Estriol Reference Standard} \times \frac{Q_T}{Q_S} \times \frac{1}{25}$$

Internal standard solution—A solution of methyl benzoate for estriol limit test in methanol (1 in 5000).

Containers and storage Containers—Tight containers.

Etacrynic Acid

エタクリン酸



$C_{13}H_{12}Cl_2O_4$: 303.14
[2,3-Dichloro-4-(2-ethylacryloyl)phenoxy]acetic acid
[*58-54-8*]

Etacrynic Acid, when dried, contains not less than 98.0% of $C_{13}H_{12}Cl_2O_4$.

Description Etacrynic Acid occurs as a white, crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in methanol, freely soluble in ethanol (95), in acetic acid (100) and in diethyl ether, and very slightly soluble in water.

Identification **(1)** Dissolve 0.2 g of Etacrynic Acid in 10 mL of acetic acid (100), and to 5 mL of this solution add 0.1 mL of bromine TS: the color of the test solution disappears. To the remaining 5 mL of the solution add 0.1 mL of potassium permanganate TS: the color of the test solution changes to light orange immediately.

(2) To 0.01 g of Etacrynic Acid add 1 mL of sodium hydroxide TS, and heat in a water bath for 3 minutes. After cooling, add 1 mL of disodium chlomotropate TS, and heat in a water bath for 10 minutes: a deep purple color develops.

(3) Determine the absorption spectrum of a solution of Etacrynic Acid in methanol (1 in 20,000) 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.

(4) Perform the test with Etacrynic Acid as directed under the Flame Coloration Test (2): a green color appears.

Melting point 121 - 125°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Etacrynic Acid in 10 mL of methanol: the solution is clear and colorless.

(2) Heavy metals—Proceed with 1.0 g of Etacrynic Acid according to Method 4, 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 Etacrynic Acid 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).

(4) Related substances—Dissolve 0.20 g of Etacrynic Acid in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add ethanol (95) 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, ethyl acetate and acetic acid (100) (6:5:2) to a distance of about 15 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 0.25% (1 g, in vacuum, 60°C, 2 hours).

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

Assay Weigh accurately about 0.1 g of Etacrynic Acid, previously dried, place in an iodine bottle, dissolve in 20 mL of acetic acid (100), and add exactly 20 mL of 0.05 mol/L bromine VS. To this solution add 3 mL of hydrochloric