ing conditions, and calculate the ratios, Q_T and Q_S, of the peak area of estradiol benzoate to that of the internal standard.

$$\text{Amount (mg) of } C_{25}H_{28}O_3 = \text{amount (mg) of Estradiol Benzoate Reference Standard} \times \frac{Q_T}{Q_S}$$

Internal standard solution—A solution of progesterone in methanol (13 in 80,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 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 35°C.

Mobile phase: A mixture of acetonitrile and water (7:3).

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

Selection of column: Proceed with 5 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and estradiol benzoate in this order with the resolution between these peaks being not less than 9.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Estradiol Benzoate Injection

安息香酸エストラジオール注射液

Estradiol Benzoate Injection is an oily solution for injection. It contains not less than 90% and not more than 110% of the labeled amount of estradiol benzoate ($C_{25}H_{28}O_3$: 376.49).

Method of preparation Prepare as directed under Injections, with Estradiol Benzoate.

Description Estradiol Benzoate Injection is a clear, oily liquid.

Identification To a volume of Estradiol Benzoate Injection, equivalent to 1 mg of Estradiol Benzoate according to the labeled amount, add chloroform to make 5 mL, and use this solution as the sample solution. Separately dissolve 1 mg of Estradiol Benzoate Reference Standard in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 50 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 dichloromethane to a distance of about 15 cm, and air-dry the plate. Then develop the plate with a mixture of chloroform and methanol (99:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same *Rf* value.

Assay Transfer an exactly measured volume of Estradiol Benzoate Injection, equivalent to about 10 mg of estradiol benzoate ($C_{25}H_{28}O_3$), to a separator, add 30 mL of hexane saturated with diluted methanol (9 in 10), and extract with five 15-mL portions of dilute methanol (9 in 10) saturated with hexane. Filter the extract through a filter paper washed with 10 mL of diluted methanol (9 in 10), to the filtrate add methanol to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.025 g of Estradiol Benzoate Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Transfer 2 mL each of the sample solution and the standard solution, exactly measured, to light-resistant 20-mL volumetric flasks, and evaporate to dryness on a water bath with the aid of a current of air. Dissolve the residue in 1 mL of methanol, add 10 mL of boric acid-methanol buffer solution, shake, and boil under a reflux condenser for 30 minutes. Cool, add 5 mL of boric acid-methanol buffer solution, shake, and cool with ice. To each solution add 2 mL of ice-cold diazo TS quickly, shake vigorously, add 2 mL of sodium hydroxide TS, then add water to make 20 mL, and filter after shaking. Discard the first 3 mL of the filtrate, and perform the test with the subsequent filtrate as directed under the Ultraviolet-visible Spectrophotometry using a solution, prepared with 2 mL of methanol 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 in a 4-cm cell at 490 nm, respectively.

$$\text{Amount (mg) of estradiol benzoate } (C_{25}H_{28}O_3) = \text{amount (mg) of Estradiol Benzoate Reference Standard} \times \frac{A_T}{A_S} \times \frac{2}{5}$$

Containers and storage Containers—Hermetic containers.

Estradiol Benzoate Injection (Aqueous Suspension)

安息香酸エストラジオール水性懸濁注射液

Estradiol Benzoate 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 estradiol benzoate ($C_{25}H_{28}O_3$: 376.49).

Method of preparation Prepare as directed under Injection, with Estradiol Benzoate.

Description Estradiol Benzoate Injection (Aqueous Suspension) produces a white turbidity on shaking.

Identification Extract a volume of Estradiol Benzoate Injection (Aqueous Suspension), equivalent to 1 mg of Estradiol Benzoate according to the labeled amount, with 5 mL of chloroform, and use this extract as the sample solution. Separately, dissolve 1 mg of Estradiol Benzoate Refer-

ence Standard in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 50 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 (99:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same Rf value.

Assay Measure exactly a volume of well-mixed Estradiol Benzoate Injection (Aqueous Suspension), equivalent to about 2 mg of estradiol benzoate ($C_{25}H_{28}O_3$), dissolve the crystals with an appropriate quantity of methanol, and add methanol to make exactly 20 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g of Estradiol Benzoate Reference Standard, previously dried in desiccator (reduced pressure, phosphorus (V) oxide) for 4 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the standard solution. Proceed with these solutions as directed in the Assay under Estradiol Benzoate.

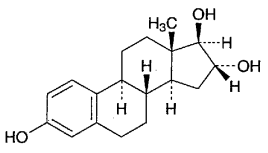
Amount (mg) of estradiol benzoate ($C_{25}H_{28}O_3$)
= amount (mg) of Estradiol Benzoate Reference Standard
$\times \frac{Q_T}{Q_S} \times \frac{1}{5}$

Internal standard solution—A solution of progesterone in methanol (13 in 100,000).

Containers and storage Containers—Hermetic containers.

Estriol

エストリオール



$C_{18}H_{24}O_3$: 288.38
Estra-1,3,5(10)-triene-3,16α,17β-triol [*50-27-1*]

Estriol, when dried, contains not less than 97.0% and not more than 102.0% of $C_{18}H_{24}O_3$.

Description Estriol occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in methanol, slightly soluble in ethanol (95) and in 1,4-dioxane, and practically insoluble in water and in diethyl ether.

Identification (1) Dissolve 0.01 g of Estriol in 100 mL of ethanol (95) by warming, and use this solution as the sample solution. Evaporate 1 mL of this solution on a water bath to dryness, add 5 mL of a solution of sodium *p*-phenolsulfonate in diluted phosphoric acid (1 in 50), heat at 150°C for 10 minutes, and cool: a red-purple color develops.

(2) Determine the absorption spectrum of the sample solution obtained in (1) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Estriol Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

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

Optical rotation $[\alpha]_D^{20}$: +54 – +62° (after drying, 0.04 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point 281 – 286°C

Purity (1) Heavy metals—Proceed with 1.0 g of Estriol 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).

(2) Other steroids—Dissolve 0.040 g of Estriol in 10 mL of ethanol (95) by warming, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and acetic acid (100) (18:1:1:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate, and heat at 105°C for 15 minutes: 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% (0.5 g, 105°C, 3 hours).

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

Assay Weigh accurately about 0.025 g each of Estriol and Estriol Reference Standard, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 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 estriol to that of the internal standard, respectively.

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

Internal standard solution—A solution of methyl benzoate for estriol limit test in methanol (1 in 1000).