

minutes, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Identification under Glycyrrhiza.

Purity Insoluble matter—Dissolve 2.0 g of Glycyrrhiza Extract in 18 mL of water, and filter. To 10 mL of the filtrate add 5 mL of ethanol (95): a clear solution results.

Assay Weigh accurately about 0.15 g of Glycyrrhiza Extract, place in a glass-stoppered, centrifuge tube, add 25 mL of dilute ethanol, and heat at 50°C for 30 minutes with occasional shaking. Cool, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of dilute ethanol, and proceed in the same manner. Combine the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.02 g of Glycyrrhizic Acid Reference Standard (separately determine the water content), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Glycyrrhiza.

$$\begin{aligned} & \text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ &= \text{amount (mg) of Glycyrrhizic Acid Reference} \\ & \quad \text{Standard, calculated on the anhydrous basis} \\ & \quad \times \frac{A_T}{A_S} \end{aligned}$$

Containers and storage Containers—Tight containers.

Crude Glycyrrhiza Extract

カンゾウ粗エキス

Glycyrrhiza Extract contains not less than 6.0% of glycyrrhizic acid (C₄₂H₆₂O₁₆: 822.93).

Method of preparation Boil coarse powder of Glycyrrhiza or the root and stolon of *Glycyrrhiza glabra* Linné (*Leguminosae*) which meets the requirement of Glycyrrhiza with Water or Purified Water, filter the solution under pressure, and evaporate the filtrate.

Description Crude Glycyrrhiza Extract occurs as lustrous, dark yellow-red to blackish brown plates, rods or masses. It is comparatively brittle when cold, and the fractured surface is dark yellow-red, shell-like, and lustrous. It softens when warmed.

It has a characteristic odor and a sweet taste.

It dissolves in water with turbidity.

Identification To 0.6 g of Crude Glycyrrhiza Extract add 10 mL of a mixture of ethanol (95) and water (7:3), dissolve by warming if necessary, cool, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Identification under Glycyrrhiza.

Purity (1) Water-insoluble substances—Boil 5.0 g of pulverized Crude Glycyrrhiza Extract with 100 mL of water. After cooling, filter the mixture through tared filter paper, wash with water, and dry the residue at 105°C for 5 hours: the mass of the residue is not more than 1.25 g.

(2) Foreign matter—The filtrate obtained in (1) does not have a strong bitter taste.

(3) Starch—To about 1 g of pulverized Crude Glycyrrhi-

za Extract add water to make 20 mL, shake the mixture thoroughly, and filter. Examine the insoluble substance on the filter paper under a microscope: the residue contains no starch grains.

Total ash Not more than 12.0% (1 g, proceed as directed in the Total ash under Crude Drugs).

Assay Weigh accurately about 0.15 g of Crude Glycyrrhiza Extract, place in a glass-stoppered, centrifuge tube, add 25 mL of dilute ethanol, and heat at 50°C for 30 minutes with occasional shaking. Cool, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of dilute ethanol, and proceed in the same manner. Combine the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.02 g of Glycyrrhizic Acid Reference Standard (separately determine the water content), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Glycyrrhiza.

$$\begin{aligned} & \text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ &= \text{amount (mg) of Glycyrrhizic Acid Reference} \\ & \quad \text{Standard, calculated on the anhydrous basis} \\ & \quad \times \frac{A_T}{A_S} \end{aligned}$$

Containers and storage Containers—Tight containers.

Chorionic Gonadotrophin

胎盤性性腺刺激ホルモン

Chorionic Gonadotrophin is a dried preparation of gonad-stimulating hormone obtained from the urine of pregnant women or from the placenta. It contains not less than 1500 chorionic gonadotrophin Units per mg.

It contains not less than 80% and not more than 125% of the labeled Units of chorionic gonadotrophin.

Description Chorionic Gonadotrophin occurs as a white to light yellow-brown powder. It is odorless.

It is freely soluble in water and practically insoluble in diethyl ether.

Identification Calculate *b* by the following equation, using *Y*₃ and *Y*₄ obtained in the Assay: *b* is not less than 120.

$$\begin{aligned} b &= \frac{E}{I} \\ E &= \frac{Y_3 - Y_4}{f} \end{aligned}$$

f: Number of test animals per group.

$$I = \log \frac{T_H}{T_L}$$

Purity (1) Clarity and color of solution—Dissolve 0.05 g of Chorionic Gonadotrophin in 5 mL of isotonic sodium chloride solution: the solution is clear and colorless or light yellow.

(2) Estrogen—Inject subcutaneously into each of three female albino rats or albino mice ovariectomized at least two

weeks before the test, single dose of 100 units according to the labeled Units dissolved in 0.5 mL of isotonic sodium chloride solution. Take vaginal smear twice daily, on the third, fourth and fifth day. Place the smear thinly on a slide glass, dry, stain with Giemsa's TS, wash with water, and again dry: no estrus figure is shown microscopically.

Loss on drying Not more than 5.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Toxicity Dissolve Chorionic Gonadotrophin to prepare a solution containing 2000 units per mL according to the labeled Units, and use this solution as the sample solution. Inject intravenously 0.5 mL of the sample solution to each of five well-fed, healthy albino mice weighing about 20 g: no mouse dies within 48 hours after injection. If any mouse dies within 48 hours, repeat the test using 10 albino mice weighing 19.5 to 20.5 g: all the animals survive for 48 hours.

Pyrogen Dissolve Chorionic Gonadotrophin in isotonic sodium chloride solution to prepare a solution containing 1000 units per mL according to the labeled Units, inject 1.0 mL of this solution per kg of body mass of rabbit, and perform the test: it meets the requirements of the Pyrogen Test.

Assay (i) Test animals—Select healthy female albino rats weighing about 45 g.

(ii) Standard solution—Dissolve a quantity of Chorionic Gonadotrophin Reference Standard in bovine serum albumin-isotonic sodium chloride solution to prepare four kinds of solutions, having 7.5, 15, 30 and 60 Units per 2.5 mL, respectively. Inject these solutions into four groups consisting of five test animals each, and weigh their ovaries, as directed in procedure of (iv). Inject bovine serum albumin-isotonic sodium chloride solution to another group, and use this group as the control group. According to the result of this test, designate the concentration of the reference standard which will increase the masses of the ovaries about 2.5 times the mass of the ovaries of the control group as a low-dose concentration of the standard solution, and the concentration 1.5 to 2.0 times the low-dose concentration as a high-dose concentration. Dissolve a quantity of Chorionic Gonadotrophin Reference Standard, in bovine serum albumin-isotonic sodium chloride solution, and prepare a high-dose standard solution S_H and a low-dose standard solution S_L whose concentrations are equal to those determined by the above test.

(iii) Sample solution—According to the labeled units, weigh accurately a suitable quantity of Chorionic Gonadotrophin, dissolve in bovine serum albumin-isotonic sodium chloride solution, and prepare a high-dose sample solution T_H and a low-dose sample solution T_L having Units equal to the standard solutions in equal volumes, respectively.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously 0.5 mL of S_H , S_L , T_H and T_L in each group for 5 days. On the sixth day, excise the ovaries, remove the fat and other unwonted tissues attached to the ovaries, and remove the adhering water by lightly pressing between filter paper, and immediately weigh the ovaries.

(v) Calculation—Designate the mass of ovaries by S_H , S_L , T_H and T_L as y_1 , y_2 , y_3 and y_4 , respectively. Sum up y_1 , y_2 , y_3 and y_4 on each set to obtain Y_1 , Y_2 , Y_3 and Y_4 .

Units per mg of Chorionic Gonadotrophin

$$= \text{antilog } M \times \left(\frac{\text{units per mL of the high dose}}{\text{of the standard solution}} \right) \times \frac{b}{a}$$

$$M = \frac{IY_a}{Y_b}$$

$$I = \log \frac{S_H}{S_L} = \log \frac{T_H}{T_L}$$

$$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$$

$$Y_b = Y_1 - Y_2 + Y_3 - Y_4$$

a: Mass (mg) of sample.

b: Total volume (mL) of the high dose of the test solution prepared by diluting with bovine serum albumin-isotonic sodium chloride solution.

F' computed by the following equation should be smaller than F_1 against n when s^2 is calculated. And compute L ($P = 0.95$) by the following equation: L should be not more than 0.3. If F' exceeds F_1 , or if L exceeds 0.3, repeat the test increasing the number of the test animals or arranging the assay method in a better way until F' is smaller than F_1 or L is not more than 0.3.

$$F' = \frac{(Y_1 - Y_2 - Y_3 + Y_4)^2}{4fs^2}$$

f: Number of test animals per group.

$$s^2 = \frac{\Sigma y^2 - \frac{Y^2}{n}}{n}$$

Σy^2 : The sum of the squares of each y_1 , y_2 , y_3 and y_4 .

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

$$n = 4(f - 1)$$

$$L = 2\sqrt{(C - 1)(CM^2 + P)}$$

$$C = \frac{Y_b^2}{Y_b^2 - 4fs^2t^2}$$

t^2 : Value shown in the following table against n used to calculate s^2 .

<i>n</i>	$t^2 = F_1$	<i>n</i>	$t^2 = F_1$	<i>n</i>	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Tight containers.

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